Diversity and Distributions, (Diversity Distrib.) (2010) 1–11

A Journal of Conservation Biogeography
Diversity and Distributions

BIODIVERSITY RESEARCH

Geographical linkages between threats and imperilment in freshwater fish in the Mediterranean Basin

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ABSTRACT

Aim The level of imperilment of mediterranean freshwater fish is among the highest recorded for any group of organisms evaluated to date. Here, we describe the geographical patterns in the incidence of threats affecting mediterranean freshwater fish and test whether the effects of specific threats are spatially related to the degree of imperilment of fish faunas.

Location The Mediterranean Basin Biome.

Methods From the IUCN Red List, we recorded the six main threats to 232 endemic freshwater fish species. We used data on fish distributions from IUCN to characterize the spatial patterns in the incidence of threats (as percentage of species affected) through multivariate statistics. We studied the relationships between threat incidence and two estimators of imperilment (proportion of species threatened and an index of extinction risk) at two spatial scales (10×10 km and basins) using partial least squares regressions (PLSR) that incorporated the effects of species richness and mean range size.

Results The main axis of variation in the incidence of threats to freshwater fish split areas mainly affected by invasive species from those areas where species are threatened by pollution and agriculture. Wherever invasive species and water extraction were predominant threats, fish assemblages consistently tended to be more imperilled.

Main conclusions As far as we know, this is the first large-scale analysis on the spatial relationships between the incidence of threats and level of imperilment of any taxonomic group. Our results highlight the primary role of invasive species and water extraction as drivers of native fish declines in the Mediterranean Basin. Large-scale patterns described here should be generated by local-scale impacts of both threats on fish biodiversity, widely reported in Mediterranean areas. Because all the species under concern are endemic, control of invasive species and reducing overexploitation of freshwater resources should be conservation priorities for mediterranean freshwater systems.

Keywords
Biological invasions, fish conservation, mediterranean rivers, overexploitation, spatial gradients, water resources.

INTRODUCTION

With the current massive loss of biodiversity, understanding the geographical patterns in the distribution of extinction risk and its underlying causes is crucial (Orme *et al.*, 2005; Schipper *et al.*, 2008). Population and species declines can be the result of different threats or combinations of threats, such as habitat destruction, harvesting or the impacts of invasive species, whose incidence can vary spatially. Therefore, determining whether the effects of any specific threat is related to the level of imperilment of biotas is essential for designing effective conservation and management strategies aimed at maximizing biodiversity preservation. The spatial relationships between extinction risk, human impacts and environmental

DOI: 10.1111/j.1472-4642.2010.00680.x
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variables have been analysed for several terrestrial groups (e.g. mammals, birds and plants; Forester & Machlis, 1996; Davies *et al.*, 2006). Typically, these analyses use indirect variables for quantifying human pressures (e.g., population density, monetary wealth or land cover variables) as explanatory variables for extinction risk (Davies *et al.*, 2006; Price & Gittleman, 2007). These variables are often used as surrogates for the processes that directly drive species imperilment, such as habitat destruction, pollution or impacts of invasive species, which are mostly recorded on a species-by-species basis (e.g. Hayward, 2010). Combining the information on the impacts of different extinction drivers affecting species (instead of estimating them through indirect surrogates) with that on species distributions allows a more straightforward geographical interpretation of the spatial distribution of the relative importance of those extinction drivers (see Schipper *et al.*, 2008). The spatial co-occurrence of the impacts of extinction drivers and extinction risk can then be analysed to search for linkages between the incidence of given threats and the degree of imperilment of biotas.

Freshwater ecosystems are among the most heavily disturbed around the world (Dynesius & Nilsson, 1994; Levin *et al.*, 2009). Thereby, their biodiversity suffers in many cases more intense declines than those of terrestrial or marine environments (Ricciardi & Rasmussen, 1999; Darwall *et al.*, 2009), and freshwater fish are one of the most highly threatened vertebrate groups (Duncan & Lockwood, 2001). The Mediterranean Basin is a global biodiversity hotspot (Kark *et al.*, 2009), bearing an important freshwater biodiversity that includes over 250 endemic fish species (Smith & Darwall, 2006; Reyjol *et al.*, 2007). However, rivers and streams in the Mediterranean Basin have a long history of intense alterations owing to the high water demand in these densely populated areas and owing to the unpredictable availability of water resources (Hamdy *et al.*, 1995; Gasith & Resh, 1999). On the basis of a recent IUCN regional assessment on freshwater fish endemic to the Mediterranean area (Smith & Darwall, 2006), 70% of the species for which adequate data were available (i.e. data sufficient species) are currently threatened with extinction or already extinct. This level of imperilment is among the highest recorded globally for any taxonomic group (e.g. Vié *et al.*, 2009).

In this work, we analyse the drivers of species declines (henceforth threats) affecting freshwater fish endemic to the Mediterranean Basin species with two main aims: (1) to describe the main geographical patterns in the incidence of threats; and (2) to examine the spatial covariation between threat incidence and the level of imperilment of endemic fish assemblages. Regarding the first aim, we used direct gradient multivariate statistics to summarize the spatial variation in the incidence of different threats in a reduced number of continuous gradients, accounting for the influences of human-related and natural environmental variables. With respect to the second aim, we analysed the relationships between threat incidence and two estimators of the degree of imperilment of fish faunas using a partial least squares regression approach. Our null hypothesis here was that imperilment would not be linked to the incidence of any specific threat or combination of threats. If this hypothesis was rejected, we would be able to identify direct threats with a prominent role in the decline of mediterranean freshwater fish fauna.

METHODS

Study area and fish database

We used the data compiled by the International Union for the Conservation of Nature (IUCN) on the distribution range of 232 freshwater fish species endemic to the Mediterranean Basin, including both extant and extinct ($n = 8$) species (Smith & Darwall, 2006). We did not include northern African species, because of concern of the 'apparent lack of data' on freshwater fish distribution and status from this area (after Smith & Darwall, 2006). The boundary of the study area was established on the basis of the distribution of the Mediterranean Biome as defined by the World Wildlife Fund (Olson *et al.*, 2001; Kark *et al.*, 2009). Species distributions were initially depicted as generalized polygons of their range (downloadable from http://www.uicnmed.org/web2007/cd_fwfish/index.html), which encompass original species records. We projected distribution polygons into the Albers Equal Area Projection at a spatial resolution of 10 × 10 km. Since the original polygons are coarse approximations to the distribution of species, their projection on 10 × 10 km cells produces an unknown amount of false presences and overestimates the real area of occupancy of species, which may affect biodiversity (e.g. species richness) mapping (see Supplementary Material in Schipper *et al.*, 2008). Thus, we used species richness as a control variable in the statistical models rather than simply analysing richness patterns. To overcome other potential biases in the IUCN distribution data, main statistical analyses (see below) were run at two spatial scales (10 × 10 km cells and river basins) to check the consistency of observed patterns.

At each 10 × 10 km cell, we calculated the total species richness and the mean range size of the species present in the cell. Range size for cell i was calculated as the mean number of 10 × 10 km cells occupied by species recorded in i (i.e. cells including species with restricted distributions would have low-range size values) (Schipper *et al.*, 2008). All statistical analyses were based on 10 × 10 km cells that fulfilled three conditions: (1) having at least three data-sufficient mediterranean endemic fish species (i.e. those whose conservation status could be accurately assessed); (2) having at least three species with information on threats; and (3) being included in a single river basin (see Fig. 1). The application of these restrictions resulted in a final dataset of 7996 – 10 × 10 km cells.

We compiled information on extrinsic threats affecting the 232 fish species from the IUCN Red List of Threatened Species (henceforth the Red List, available at http://www.iucnredlist.org). This information had been collected through a regional assessment on freshwater fish endemic to the Mediterranean Basin with the participation of some 20 experts on fish biology and conservation from most mediterranean countries (see

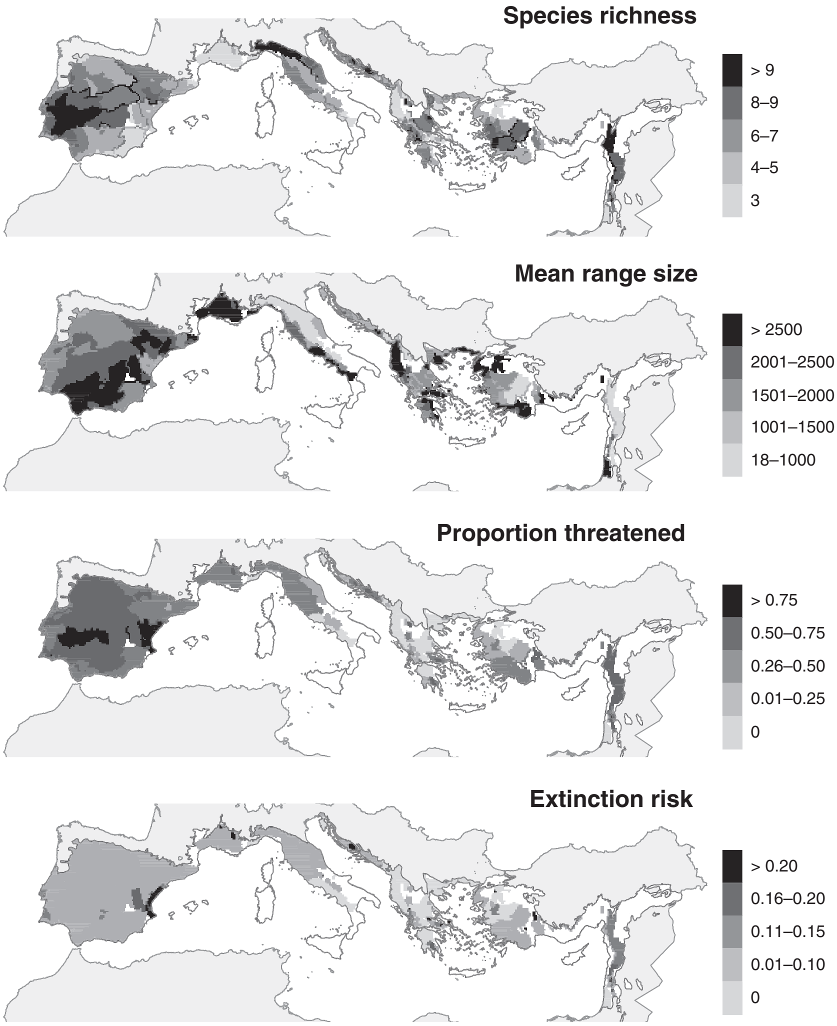

Figure 1 Spatial patterns of species richness, mean range size and degree of imperilment of endemic freshwater fish in the Mediterranean Basin. Darker grey colours indicate higher values of any of the four variables. White areas are 10 × 10 km cells within the Mediterranean Biome with (1) less than three data-sufficient species; or (2) less than three species with available information on threats, which were not included in our analyses (see Methods). Note that cells included in more than one basin are represented here (i.e. they are clearly highlighted in the species richness map), although they were excluded from statistical analyses (e.g. see Fig. 2).

Smith & Darwall, 2006 for details). The Red List provides detailed accounts on the incidence of main threats affecting the species assessed. Note that this information is available for both threatened and non-threatened species (*sensu* IUCN Red List), since a species may not be considered threatened and still be affected by a particular threat. Moreover, these data are available even for many data-deficient species (DD; i.e. those whose conservation status could not be accurately assessed). We simplified the IUCN threat classification to six main extrinsic threat categories: (1) reservoirs and channel construction (including other infrastructures such as interbasin connections); (2) agriculture (leading to agricultural water pollution); (3) water extraction; (4) invasive species (predation, competition and hybridization effects); (5) overfishing (including accidental mortality); and (6) pollution (see Table 1). This classification of threats included only direct human-driven processes, thus excluding natural or large-scale global-change-driven phenomena (e.g. droughts) and species' intrinsic factors leading to imperilment (e.g. restricted range or limited dispersal). The citation of any of the six threats in the Red List species' factsheet was translated to a presence–absence matrix in which fish species were rows and the six threats were columns. In this matrix, every species could be affected by more than one threat (mean number of categories cited for each fish species was 3.3 ± 1.0 SD).

Table 1 Threats to endemic mediterranean freshwater fish. Numbers shown are the percentages of species and 10 × 10 km cells affected by each threat type (in the case of cells, at least one species affected in a cell) and mean percentage of species affected by each threat type in cells, calculated from the average values of the 7996 cells in the dataset.

	Species (n = 212)	10 × 10 km cells (n = 7996)	
Threats	Occurrence (%)	Occurrence (%)	Mean % species affected
Pollution	88.7	100.0	86.8
Water extraction	84.9	100.0	86.5
Invasive species	59.9	99.9	72.6
Reservoirs	46.2	96.9	61.2
Agriculture	33.0	84.6	29.0
Overfishing	15.6	52.6	13.0

The final matrix included information on threats affecting 212 fish species (six of them extinct), since in 20 cases, no information was available on specific threats. These data were then combined with that on species distributions to obtain a geographical dataset of the occurrence of threats. At each 10×10 km cell, we recorded the number of species affected by each of the six threats listed in Table 1 and calculated their percentages in relation to species richness, previously excluding those species for which there was no available information on specific threats (i.e. the 20 cases cited earlier). Note that these percentages are not interdependent variables, since they are not calculated in relation to each other (i.e. they do not sum to 100), and thus, theoretically all six threats could be simultaneously affecting all fish species within a certain 10×10 km cell. The percentage of species affected by a specific threat in a cell was taken as a measure of its relative importance there (Schipper *et al.*, 2008).

At each 10×10 km cell, we compiled information on four human pressure and three natural climatic environmental variables. The list of environmental variables and other details are given Appendix S1 in supporting information.

Geographical gradients in threats

We used redundancy analysis (RDA) to summarize the geographical variation in the threats dataset in relation to environmental variables (i.e. human impacts and energy-related climatic variables). Redundancy analysis is a direct gradient ordination analysis that is the equivalent of a canonical correspondence analysis (CCA) but being specifically designed for datasets believed to have linear relationships between the species and environmental matrices (ter Braak & Smilauer, 1998). The ordination produced by the RDA is constrained by the environmental variables, addressing the question of how 10×10 km cells and threats distribute along environmental gradients. Redundancy analysis is the appropriate constrained ordination technique for a dataset if the standard deviation of species turnover (in SD units) on the first axis of a detrended correspondence analysis (DCA) is < 2, and, in our case, it was 1.24. The significances of the first canonical axis and of all resulting canonical axes together were examined through comparison with random ordinations using Monte Carlo permutation tests, with 1000 permutations. We used Canoco 4.5 to run both detrended correspondence and redundancy analyses (ter Braak & Smilauer, 1998).

Imperilment and threats

We calculated two variables describing the level of imperilment of freshwater fish. On the one hand, we recorded the proportion of threatened species (including extinct ones) in each cell, calculated as the sum of extinct (EX, including extinct in wild, EW), critically endangered (CR), endangered (EN) and vulnerable (VU) species, divided by the number of species recorded in a cell, excluding DD species. The proportion of threatened species is a simple and clear measure to assess the taxonomic or geographical distribution of imperilment (e.g. Owens & Bennett, 2000). This measure, however, does not take into account the fact that extinction risk varies among the different threat categories defined by the IUCN. For example, a hypothetical cell with all species classified as CR would have a much higher extinction risk than another cell with all species classified as VU, even though both areas would have the same proportion of threatened species. Therefore, after Clavero *et al.* (2009), we calculated for each cell an index of extinction risk using the following formula:

$$\text{Extinction risk cell } i = \frac{\text{EX}_i + 0.75 \times \text{CR}_i + 0.2 \times \text{EN}_i + 0.02 \times \text{VU}_i}{\text{N}'_i}$$

where EX_i, CR_i, EN_i and VU_i are, respectively, the number of species classified as extinct (including EW species), CR, EN and VU within cell i and N'_i is the number of mediterranean endemic species in the cell, either threatened or not, excluding DD species. The weights given to CR, EN and VU are based on the quantitative assessment of species' extinction risk within a given time frame used by the IUCN's Red List criterion E (Mace *et al.*, 2008). This criterion establishes extinction probability thresholds to assign species to threat categories, which for CR species is a 50% probability of extinction in 10 years, for EN a 20% in 20 years and for VU a 10% in 100 years. The thresholds associated with CR and VU categories were recalculated for a period of 20 years (producing the weights given in the equation above) to obtain probability values that could be compared across categories (Clavero *et al.*, 2009).

The relationships between threats incidence and the level of freshwater fish imperilment were analysed at two spatial scales: 10×10 km cells and basins. Basins were derived from the HYDRO1k database (available at http://eros.usgs.gov/). Basin values of the different variables were calculated through averaging the values of 10×10 km cells included within each basin limits. Basins encompassing less than five cells fulfilling the conditions noted earlier were deleted from further analyses. At each spatial scale, the proportion of threatened species and extinction risk was used as dependent variables in partial least squares regressions (PLSR) analyses. Independent variables used in PLSR analyses were (1) total species richness; (2) mean species range size; and (3) the proportion of species affected by each of the six main direct threats listed in Table 1. Through these PLSRs, we aimed to assess the relationships between threats and freshwater fish imperilment while accounting for the effects of richness and mean range size. PLSR is a statistical technique that combines features of multiple regression and principal components analyses (PCA) (Abdi, 2003). PLSR is especially suitable when predictors are highly correlated, a frequent feature of ecological datasets. This was the case of threat incidence data, since, both at the cell and at basin scales, 6 out of the 15 possible pairwise correlations had coefficients larger than 0.4 (in absolute values). PLSR searches for a set of components (called latent vectors) that maximize the covariation between the predictor dataset and the dependent variable and that, as in a PCA, are interpreted through the weights of the original variables (Abdi 2003). The strength of the relationships between each original predictor and the dependent variable can also be evaluated through standardized coefficients (β). Although widely used in other research fields, the use of PLSR in ecological studies is still quite infrequent (Carrascal *et al.*, 2009). In spite of this, PLSR is useful in many ecological analyses, which often include large amounts of predictors, and it has been shown to provide more reliable results than multiple regression or principal component regression (i.e. running a PCA followed by multiple regression) (Carrascal *et al.*, 2009). We performed 4 PLSR analyses (two imperilment-related dependent variables × two spatial scales), always keeping the first two extracted vectors to homogenize the interpretation of results. PLSRs were run using STATISTICA 6 software (StatSoft Inc., Tulsa, OK, USA). We checked for the concordance between the main spatial gradients of variation in threats incidence extracted by the RDA and the vectors extracted by the PLSR using Pearson correlation analyses.

RESULTS

On average, almost half (0.43 ± 0.25 SD) of fish species 10 × 10 km cells were threatened or extinct, while extinction risk estimates averaged 0.04 (±0.05 SD). Around 15% of the cells (n = 1157) did not bear any threatened species, while all species were threatened in 178 cells (2.2%). Mean fish species richness was 6.1, ranging from 3 to 17 (note that cells with less than three species were not included in this study). Average range size was 2042.1 – 10 × 10 km cells (range 18.0–4012.7) (Fig. 1).

Water pollution and water extraction were the most widespread threats, affecting 89% and 85% of the 212 species, respectively. Both of these threats were ubiquitous across the Mediterranean basin and affected on average around 85% of the species occurring in 10 × 10 km (Table 1). Invasive species were the third main cause of fish species' declines, affecting 60% of the species and occurring as a threat in almost all cells, with a mean frequency of occurrence of over 70%. Impacts derived from reservoirs and other water infrastructures were a threat for about half of the species, while those resulting from agriculture practices were a threat for one-third of them. Overfishing was as a relatively minor threat (Table 1).

The first two axes of the redundancy analysis (RDA1 and RDA2) accounted for 17.5% of the variation in the relative occurrence of threats among 10 × 10 km cells while resuming 84.1% of their relationship with environmental variables. Both RDA1 and the whole ordination proved to perform better than random ordinations, as shown by the Monte Carlo tests ($P < 0.001$ in both cases). RDA1 had, to its negative end, cells with a high incidence of threats posed by invasive species and, secondarily, reservoirs, while agriculture and pollution were the main threats towards its positive extreme (Fig. 2). This gradient was positively related to precipitation seasonality, population density and average annual precipitation. Areas of high incidence of invasive species were concentrated in the Iberian Peninsula, central Italy and areas in north-western Turkey, whereas in large areas of the southern Balkan Peninsula and the eastern mediterranean freshwater fish were mainly threatened by agriculture and pollution. RDA2 was mainly related to the impacts of overfishing and water extraction, both of which increased to the negative extreme, and to those of reservoirs, which were more important to the gradient's positive end. RDA2 was positively related to annual precipitation and negatively to temperature, largely varying along a south–north axis (Fig. 2).

The first vector extracted in all four PLSR analyses strongly resembled RDA1, being positively related to the incidence of invasive species and water extraction and negatively to the impacts of agriculture and pollution (Table 2). Range size had weak relationships with the proportion of threatened species, but both cells and basins having species with narrower average distribution ranges tended to have higher extinction risk values. The incidence of water extraction was positively related to both estimators of freshwater fish imperilment at both scales, but especially when analysing basin-scale data. Threats posed by invasive species showed consistently positive relationships with both imperilment estimators and at both spatial scales under analysis. The relationships between the impacts of invasive species and water extraction and the level of fish imperilment were reflected in the positive associations between the first vector of PLSR analyses and imperilment estimators (Fig. 3). Thus, areas within the Mediterranean Basin where invasive species and water extraction are major threats to native endemic fishes tend to hold the most imperilled fish assemblages (Table 2).

DISCUSSION

We detected clear and consistent relationships between the incidence of threats and the level of imperilment of fish faunas, which suggest that invasive species and water extraction are prominent drivers of declines of freshwater fish endemic to the Mediterranean Basin. Our analyses involved the combination of two different data sources: (1) the distribution and threat status of species and (2) the threats affecting those species. This approach has some shortcomings that must be noted. First, the account of threats affecting a certain species is unique for its whole distribution range, so possible geographical variations within ranges are not considered. Second, since occurrence of threats was recorded in a presence–absence matrix, the relative importance of each particular threat for the different species could not be assessed. However, IUCN assessments produce the most complete available large-scale information in relation to direct threats affecting species and have been previously used for mapping the incidence distribution of different threats (Schipper *et al.*, 2008). Data on each endemic mediterranean freshwater fish species were compiled after reaching consensuses in specific workshops participated by numerous expert ichthyologists with knowledge on both fish taxonomy and threats' impacts (Smith & Darwall, 2006), which is the usual working procedure in IUCN regional or taxonomic assessments (e.g. Vié *et al.*, 2009). Thus, in spite of probably missing

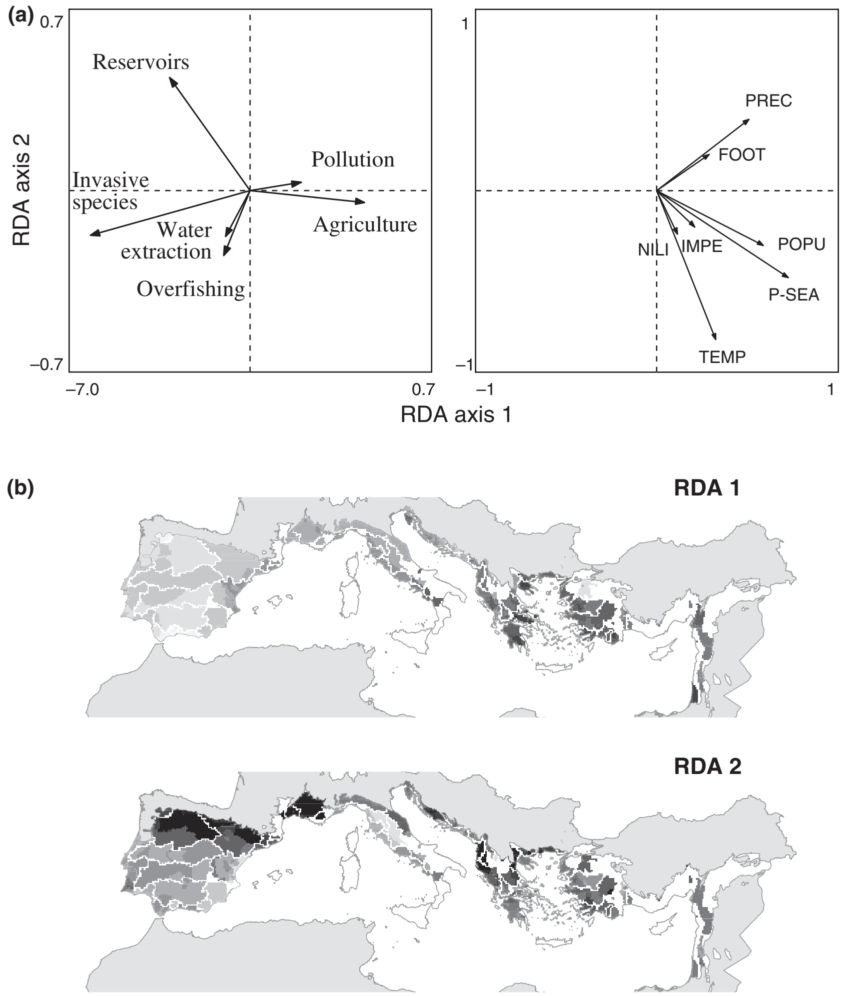

Figure 2 Results of the redundancy analysis (RDA) resuming the geographical variation in the incidence threats affecting mediterranean freshwater fish at its relationships with environmental variables. (a) Biplot diagrams showing the relationships of threat types (left) and environmental variables (right) with the first two axes resulting from the RDA ordination. Codes for environmental variables are FOOT, human footprint; IMPE, percentage impervious areas; NILI, night lights; POPU, population density; PREC, precipitation; P-SEA, precipitation seasonality; TEM, temperature (see Appendix S1 for details). (b) Spatial representation of RDA axes. Darker grey colours indicate higher values of threat gradients.

particular details, this approach gives the best possible account of the large-scale geographical variation in the incidence of direct threats driving species' imperilment.

The main spatial gradient of variation in the incidence of different threats (i.e. RDA1) separated areas where fish are mainly affected by water pollution and agriculture from those where invasive species are the most important threats. It had been previously suggested that species invasions should be favoured in degraded, human-altered ecosystems (e.g. Moyle & Light, 1996; Taylor & Irwin, 2004), and a global analysis of freshwater fish introductions by Leprieur *et al.* (2008) showed that human-related variables were the ones that better explained the interbasin variation in non-native species richness. However, our RDA analysis associated the impacts of invasive species with low-populated, low-income areas. These apparently contradictory patterns could result from different scales of analysis [Leprieur *et al.* (2008) used information on non-native fish distribution at a whole basin scale] and form the fact that the Mediterranean itself is a heavily invaded and populated area where patterns arising at larger scales (and including less invaded, more natural areas) might not be detected. Notwithstanding, the main difference between our work and those relating invasions to human-related variables is that we have analysed the variations in the direct impacts of different threats (including those of invasive species) on native species and not the variation of factors originating those threats (e.g. number of invasive species present, but also population density or the proportion of transformed land). In our approach, a high incidence of threats posed by invasive species in an area does not necessarily mean that it bears more invasive species, and the same can be said about other threats analysed. The RDA ordination implies that the general trend in the Mediterranean Basin is that freshwater fish are mainly threatened either by a combination of agriculture and pollution or by invasive species. Intensive agriculture and high pollution levels are positively associated with human population, and, thus, threats posed by invasive species would tend to be dominant in less populated areas. In fact, invasive species can be themselves affected by human alterations of freshwater systems (e.g. Didham *et al.*, 2007). Leprieur *et al.* (2006) gave an example of how certain human-derived habitat disturbances (in that case, water abstraction) could benefit a native fish species by having a negative effect on an invasive, predaceous one.

Table 2 Results of four partial least squares regressions (PLSR) analyses run for the two estimators of imperilment at the two spatial scales under analysis. The table shows the weights of the original variables for the two first latent vectors extracted by the each one of the PLSR analyses as well as the standardized regression coefficients (β). β-values larger than 0.20 (in absolute value) are highlighted in bold. Bottom rows show (1) the proportion of the variance in the Y variable accounted for by the extracted vectors; (2) the proportion of the variance in the original X dataset (i.e. predictors) accounted for by the extracted vectors; (3) Pearson correlation coefficients (r) between redundancy analysis (RDA1) (see Fig. 2) and the first vector extracted by each PLSR; and (4) Pearson correlation coefficients (r) between RDA2 and the second vector extracted by each PLSR.

	Proportion species threatened						Extinction risk					
	10 × 10 km cells			Basins			10 × 10 km cells			Basins		
	v1	v2	β	v1	v2	β	v1	v2	β	v1	v2	β
Pollution	−0.36	0.41	−0.06	−0.38	0.45	−0.03	−0.20	0.19	−0.10	−0.21	0.42	0.10
Water extraction	0.39	0.15	**0.20**	0.46	0.59	**0.43**	0.18	−0.05	0.16	0.43	0.28	**0.43**
Invasive species	0.53	0.36	**0.37**	0.41	0.23	**0.24**	0.43	0.15	**0.31**	0.33	−0.04	0.14
Reservoirs	0.14	−0.32	0.04	0.20	−0.26	−0.10	−0.20	−0.46	−0.18	0.04	−0.34	**−0.22**
Agriculture	−0.34	0.48	−0.02	−0.41	0.30	−0.09	−0.14	0.45	0.11	−0.30	0.34	0.04
Overfishing	0.44	0.21	0.18	0.34	−0.22	0.08	−0.02	−0.53	−0.19	0.23	−0.23	0.07
Richness	0.30	0.36	0.13	0.26	0.35	0.14	0.43	−0.11	0.11	0.39	0.19	0.04
Range size	−0.18	−0.42	−0.14	−0.29	−0.26	−0.09	−0.70	−0.47	**−0.31**	−0.61	−0.65	**−0.39**
R^2 of Y	0.43	0.03		0.42	0.03		0.23	0.03		0.28	0.06	
R^2 of X	0.31	0.17		0.37	0.13		0.22	0.22		0.34	0.19	
r RDA1	−0.88	–		−0.89	–		−0.49	–		−0.77	–	
r RDA2	–	−0.53		–	−0.04		–	−0.13		–	−0.02	

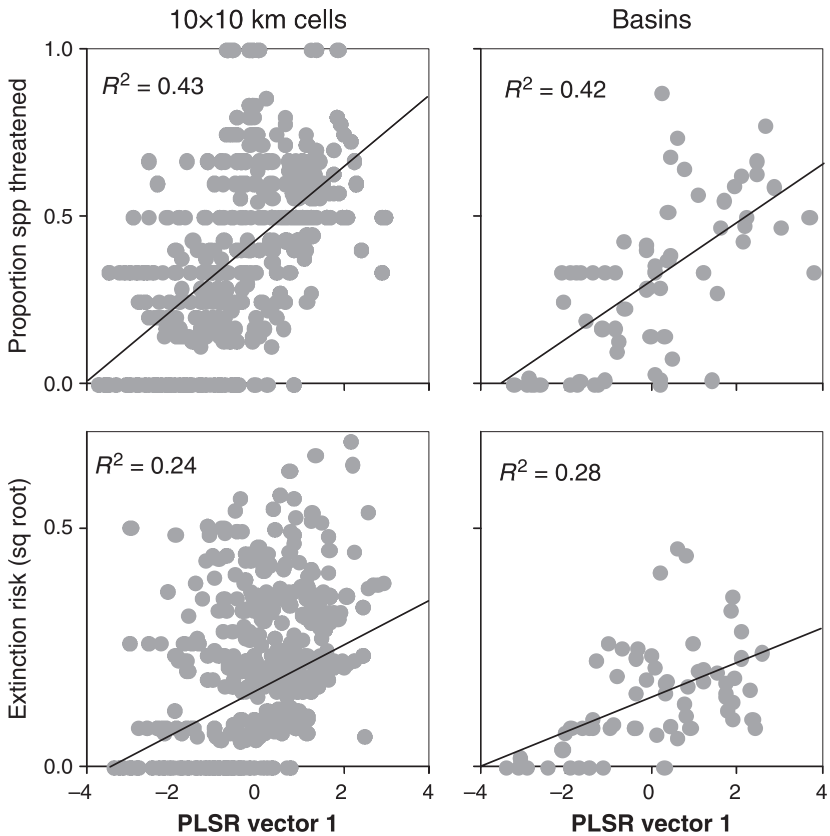

Figure 3 Linear relationships between the first latent vectors extracted by the partial least squares regressions analyses and the two variables related to the level of imperilment of endemic freshwater fish faunas in the Mediterranean Basin, represented at the two spatial scales under analysis. See Table 2 for the interpretation of latent vectors. Coefficients of determination (R^2) are also provided.

The most consistent pattern of covariation between threats and imperilment of endemic freshwater fish faunas was the higher level of imperilment wherever invasive species and water extraction were more important threats, a pattern that was consistent across spatial scales and using different quantifications of imperilment (Fig. 3).

Regarding the impacts of invasive species, Leprieur *et al.* (2008) analysed the invasion level across numerous river basins around the world and found that basins with larger proportions of alien freshwater species also had higher proportion of threatened species. Such large-scale analyses show the covariation between threats (or community features like invasion level) and extinction risk, but the processes generating such patterns must be analysed at smaller spatial scales. The identification of such processes is often controversial (e.g. Gozlan, 2008; Vitule *et al.*, 2009), since, in most occasions, multiple threats co-occur in those areas where native biotas have declined and their relative impact may vary across regions or taxa (e.g. Didham *et al.*, 2005). For example, few invaded systems are free from some kind of habitat degradation, leading to possible confounding effects when trying to isolate each factor's impact.

The important role of invasive species in native declines has been, however, repeatedly highlighted in mediterranean freshwater systems (Marr *et al.*, 2010). All five mediterranean-climate areas in the world are hotspots of freshwater fish invasions, with non-native species constituting over one-fourth of total fish richness in most basins (Leprieur *et al.*, 2008; Marr *et al.*, 2010). In the particular case of the Iberian Peninsula, which has a highly threatened fish fauna (see Fig. 1), the figures are even worse, with the proportion of non-native species most often surpassing 50% (Clavero & García-Berthou, 2006). Light & Marchetti (2007) used path analyses to show that the imperilment of California's native fish at the watershed scale was best explained by the direct effect of invasive species. A similar analytical approach was followed by Hermoso *et al.* (2010) to show that the conservation status and native species richness of fish communities in a large Iberian basin (Guadiana River) was negatively associated with the abundance of invasive species, while, on the other hand, biodiversity loss could not be explained by habitat degradation alone. In the same basin, Hermoso *et al.* (2009) found that most native fish species showed a strong negative response to invasive species, while the responses to other sources of perturbation were neither that clear nor that generalized. Local extinctions of whole groups of native species have been recorded in Mediterranean areas after the introduction of invasive species although there were no apparent habitat degradation (García-Berthou & Moreno-Amich, 2000), and predation by piscivorous species was identified as the main cause driving to extinction two species endemic to the Mediterranean Basin (*Telestes ukliva*, in Croatia, and *Alburnus akili*, in Turkey) (see http://www.iucnredlist.org).

The impacts of water extraction, the second most frequent threat in the area, were positively linked to imperilment at both scales of analysis, although its effects were stronger when analysing basin data. Water extraction has been described as one of the main threats to freshwater ecosystems worldwide (e.g. Xenopoulos *et al.*, 2005), being an especially intense problem in arid and semiarid regions, such as Mediterranean ones (Gasith & Resh, 1999). In fact, the proportion of fish species affected by water extraction in the Mediterranean basin is higher than in any other region in which fish conservation status has been analysed to date (Darwall *et al.*, 2009). A striking example of the impacts of water exploitation is the Tablas de Daimiel, an inland wetland National Park in Spain, which was completely dried up as a result of the overexploitation of groundwater resources in the upper Guadiana River basin (Bromley *et al.*, 2001). Moreover, the impacts of freshwater extraction will likely increase in the future, because of warmer and drier climatic conditions predicted for the area under climate change scenarios. For example, projections compiled by Bates *et al.* (2008) predict a reduction in mean run-off between 6 and 36% in Mediterranean Europe by 2070s, a drastic reduction in summer low flow (up to 80%) and increases in drought frequencies.

Our results show that areas with a higher proportion of fish species threatened by water extraction tended to have more imperilled fish assemblages. Most mediterranean freshwater fish are able to come through summer droughts by surviving in the remaining pools, where the abiotic environment can be extremely harsh and biotic interactions get more intense (e.g. Magalhães *et al.*, 2002). However, water extraction for human uses can aggravate the effects of summer droughts, often leading to the complete, unnatural desiccation of water courses. For example, Benejam *et al.* (2010) found that in an Iberian basin, those sites impacted by water extraction had lower fish abundances and tended to lack intolerant species. In spite of this, Hermoso *et al.* (2010) noted that impacts of water extraction on fish communities may be difficult to quantify, and thus, they can often be underestimated in field-based studies.

Mean range size was the main predictor of extinction risk values at both scales of analysis while having no apparent effect on the proportion of invasive species. The weighing of the different categories of threat (EX, CR, EN or VU) to construct the extinction risk index (see Methods) largely explains these patterns. Species with small distribution ranges tend to be at higher risk than those with large ranges. In fact, the size of the distribution range is one of the criteria followed by the IUCN to assign a species into a category of threat (e.g. Mace *et al.*, 2008), and, hence, our measure of extinction risk at any territorial unit cannot be taken as independent from mean range size of species living there. Finally, the number of endemic fish species tended to be positively related to the level of imperilment, i.e. the more species, the more threatened fish assemblages. This pattern can be related to the nested distribution patterns of freshwater fish along the Mediterranean, with poorer fish communities tending to be composed by widespread species and richer communities incorporating rarer, often threatened ones. In fact, richness and range size were negatively correlated both at the cell ($r = -0.44$; $P < 0.001$) and at the basin ($r = -0.54$; $P < 0.001$) scales. Moreover, while over 60% of the species included in our analyses ($n = 212$) were threatened, the mean percentage of threatened species within cells was lower than 40%, further showing that non-threatened species tend to be widespread in the study area.

In summary, our results show clear and consistent large-scale spatial relationships between the impacts of both invasive species and water extraction and the level of imperilment of freshwater fish endemic to the Mediterranean Basin. To our knowledge, this is the first large-scale study explicitly relating the incidence of direct threats and extinction risk. Results are in general agreement with smaller scale, field-based studies showing negative relationships between native fish conservation status and invasive species and overexploitation of water resources reported throughout mediterranean-climate areas. Together with the maintenance or recuperation of fluvial systems' naturalness (including runoff regimes) and reductions in water pollution, the control of both invasive species and the exploitation of water resources should be first-order conservation priorities for mediterranean freshwater systems (e.g. Marr *et al.*, 2010). Moreover, the impacts of both threats are likely to increase in the future because of the continuous introductions of exotic species and the expansion of already established ones (e.g. Clavero & García-Berthou, 2006) and the increased pressure on freshwater resources suggested by future climatic scenarios (Bates *et al.*, 2008).

ACKNOWLEDGEMENTS

We acknowledge the work made by the people at IUCN, and in particular, at its Centre for Mediterranean Cooperation, for putting together and making freely accessible data on threats and conservation status of freshwater fish endemic to the Mediterranean Basin. L. Faggiano provided us statistical advice, and discussions with L. Brotons, M. de Cáceres and the members of the Ecology Centre at the University of Queensland, as well as comments by three anonymous referees, helped us to improve the manuscript. M.C. benefited from a Juan de la Cierva postdoctoral contract granted by the Spanish Ministry of Education and Science.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1 List and details on environmental variables used in the analyses.

Table S1 List of the seven variables used to characterize natural characteristics and human pressures in 7996 – 10 × 10 km cells throughout the Mediterranean Basin.

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Author contributions: All four authors conceived and discussed initial ideas and gathered data; M.C. and V.H. analysed the data; M.C. led the writing, which was actively participated by the rest of authors.

Editor: Anthony Ricciardi