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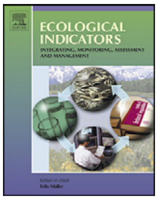

A new biological indicator to assess the ecological status of Mediterranean trout type streams

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ABSTRACT

Fuelled by the generalized degradation of freshwater ecosystems, the development of tools to assess their ecological status has been the focus of intensive research in the last decades. Although fish are one of the key biological quality elements used to describe the ecological status of rivers, fish metrics that accurately respond to disturbances in Mediterranean trout type streams are still lacking. In these systems, multimetric indices are not optimal indicators because of their low species richness and abundances, thus alternative approaches are needed. Since carrying capacity defines the potential maximum abundance of fish that can be sustained by a river, its relationship with actual density (D/K ratio) could be an accurate indicator of population conservation status and consequently of the ecological status of the river. Based on this rationale, we modeled carrying capacity dynamics for 37 brown trout populations during a 12-year study period. We analyzed the response of the D/K ratio to a gradient of increasing environmental harshness and degradation in order to assess its suitability to accurately measure brown trout conservation status. Our results showed that the D/K ratio was highly sensitive to temporal and spatial variations in environmental conditions and the levels of human-induced environmental degradation. Variations in the environmental and human degradation factors included in the best explaining regression models developed for the whole population and by age classes accounted for between 58 and 81% of the variation in the D/K ratio. Likewise, the D/K ratio was sensitive to both general and life stage specific disturbance factors. Further analyses helped identify the factors limiting population abundance. Therefore, the D/K ratio could be an interesting indicator to consider when defining objective management plans and corrective actions in degraded rivers and streams subject to Mediterranean climatic conditions.

1. Introduction

Freshwater ecosystems are one of the most endangered of the world, having been suggested that decline in biodiversity may be greater in freshwaters than in any other ecosystem type (Dudgeon et al., 2006). In particular, Mediterranean rivers have been historically subjected to a strong human pressure, showing the highest anthropogenic contribution of any climatic zone (Maceda-Veiga and Sostoa, 2011). In addition, ongoing climate change is projected to worsen the naturally harsh conditions (high temperatures and drought) in a region already vulnerable to climate variability, where freshwater ecosystems are susceptible to undergo the major alterations (Giorgi and Lionello, 2008; IPCC, 2007; Lionello et al., 2008). Consequently, impacts resulting from projected changes in climatic conditions will add to the increasing pressure of human activities on Mediterranean freshwaters (Freni et al., in press).

Fuelled by the generalized degradation of freshwater ecosystems, the development of tools to assess their ecological status has been the focus of intensive research in the last decades. Legal regulations as the Clean Water Act in the United States or the European Water Framework Directive have been introduced in an attempt to address these problems, requiring the protection and restoration of biological integrity as part of water quality standards (Hermoso et al., 2010). So in this overall context, conditions of freshwater ecosystems are increasingly assessed by their biological properties with fish being one of the key biological quality elements used to describe the ecological status of rivers (*e.g.*, Cowx et al., 2009; Logez and Pont, 2011; Vehanen et al., 2010). Fish are suitable as biological indicators (Meador et al., 2008), since their relative longevity in comparison to other biological elements allows them to better integrate long-term impacts (Maceda-Veiga and Sostoa, 2011).

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Profuse efforts have been committed to develop efficient tools to measure the ecological status of freshwaters based on fish. Since their first proposal, multimetric indices of biotic integrity (IBIs; Karr, 1981; Karr et al., 1986) have been widely used in fisheries research and management as ecological indicators to assess and define the health of stream systems. In fact, the use of indices based on fish communities has been extensive not only from a local scale perspective but also at larger spatial scales, to the point that assessment methods, such as the European Fish Index (EFI; FAME, 2004), have been developed at the European spatial scale. However, the EFI general index does not work well in European Mediterranean rivers and streams (Benejam et al., 2008), whose fish communities share common problems and characteristics with streams under similar climatic conditions in other regions that make it difficult to develop traditional IBIs either (see Hermoso et al., 2010 for detailed discussion on the question). Further complications arise in mountain trout streams where the assessment of the fish community is typically not an optimal ecological indicator because of the naturally low species richness (Jurajda et al., 2010), particularly in peripheral trout populations that naturally occur at low densities as well. As discussed by Benejam et al. (2008), considering only richness and compositional metrics in Mediterranean mountain trout streams mostly evaluates trout abundance so that it is likely to result in redundant metrics and in the fact that we would be actually analyzing taxonomic composition rather than ecological structure, which is supposed to be the advantage of multimetric indices. What with these constraints, identifying fish metrics that respond to disturbances in Mediterranean trout type streams is ever more necessary for assessing their ecological status.

Carrying capacity defines the potential maximum abundance of fish that can be sustained by a stream. This ecological parameter provides then a basis for evaluating the conservation status of a population and for assessing the changes in its dynamics resulting from habitat alteration or other anthropogenic impacts. In this way, in mountain trout streams where brown trout is the key dominant and often the only fish species and top-predator, the conservation status of the different life stages structuring the brown trout population could be an indicator of the quality of the ecosystem functioning. Hence the stream carrying capacity and its relationship with observed fish abundance could be useful metrics to employ when assessing the ecological status of Mediterranean trout type streams, especially in systems where indices based on fish communities have not been validated. The specific objectives of this study were (1) to determine the stream carrying capacity for brown trout populations with the aim of evaluating their conservation status through the analysis of the density-carrying capacity ratio; (2) to analyze the response of the density-carrying capacity ratio to a gradient of increasing environmental harshness and degradation in order to assess its suitability to accurately measure brown trout conservation status; and (3) to identify the major environmental and human-induced degradation factors driving the spatial variation of brown trout conservation status.

2. Methods

2.1. Study area

Brown trout conservation status was analysed in 37 study sites located in 22 rivers from three major basins (Aragón, Arga and Ega river basins) belonging to the Ebro river basin, a Mediterranean drainage (Fig. 1). The study area was situated between latitudes 42°29′ and 43°03′N and longitudes 0°43′ and 2°20′W. Selected sites were chosen to cover the existing variability of environmental and geo-morphological conditions within the area, which are fully described in Ayllón et al. (2010a). Sampling sites corresponded to first to fourth-order rivers and were located at an altitude ranging from 460 to 895 m. Median summer discharge ranged from 0.03 to 1.01 $m^3 s^{-1}$ and mean daily summer water temperature ranged between 11.6 and 16.6 °C. Moreover, selected sites are differentially subject to a wide range of human pressures.

2.2. Fish assessment

Brown trout populations were sampled by electrofishing every summer from 1993 to 2004. Trout were anaesthetized with tricaine methane-sulphonate (MS-222) and individuals were measured (fork length, to the nearest mm) and weighed (to the nearest g). Scales were taken for age determination. Fish density (trout ha^{-1}) with variance was estimated separately for each sampling site by applying the maximum likelihood method (Zippin, 1956) and the corresponding solution proposed by Seber (1982) for three removals assuming constant-capture effort. Population estimates were carried out separately for each year class.

A qualitative assessment of the abundance of fish species other than brown trout was simultaneously performed at each site. During every brown trout sampling, abundance of non-trout fish species was categorized and ranked as non-present, rare, frequent, abundant and very abundant. The presence of a fish species was considered to be stable when it was found in the site most of the years of the study period, otherwise it was regarded as occasional. Brown trout naturally occurred in allopatry and was the prevailing fish species in all sites. It was the only fish species existing in one site (AR13), but for the occasional presence of the Pyrenean minnow *Phoxinus bigerri*. This last species was the only one present in all the remaining sites. In fact, it was the only species stably found in 15 sites. In 28 out of the 37 sites, there were three or less fish species stably accompanying brown trout. Apart from the Pyrenean minnow, these species were essentially the Ebro nase *Parachondrostoma miegii* and the Pyrenean stone loach *Barbatula quignardi*, and less frequently the Ebro barbel *Barbus graellsii*, the Iberian red fin barbel *Barbus haasi* and the Pyrenean gudgeon *Gobio lozanoi*. The occasional presence of European eel *Anguilla anguilla*, bermejuela *Achondrostoma arcasii* and introduced rainbow trout *Onchorhynchus mykiss* was also recorded. The maximum number of species stably found across years was five in the lower reaches of the rivers from the Aragón basin and four in the lower reaches of the rivers from the Arga and Ega basins. The number of occasional species recorded was higher in the Arga and Ega basins, though.

2.3. Carrying capacity and conservation status

In salmonids, it has been suggested that spatio-temporal variations in population density are typically related to changes in physical habitat conditions (Klemetsen et al., 2003; Milner et al., 2003). Therefore, physical habitat was considered the main environmental factor limiting trout population size, and carrying capacity was defined as the maximum density of fish a river can naturally support during the period of minimum available habitat. The dynamics of stream physical habitat was modeled by means of the Physical Habitat Simulation system (PHABSIM; Milhous et al., 1989). PHABSIM simulations determine the potentially available habitat for an aquatic species and its life stages as a function of discharge by coupling a hydraulic model with a biological model of habitat selection (the habitat suitability criteria, HSC). The standard output of PHABSIM simulations is the curve that relates the Weighted Usable Area (WUA; m^2 WUA ha^{-1}, an index combining quality and quantity of available habitat) with stream discharge.

Topographic, hydraulic and channel structure data required to perform PHABSIM simulations were collected at each study site during the summer of 2004. The data collection procedures are fully described elsewhere (Ayllón et al., 2010b, in press).

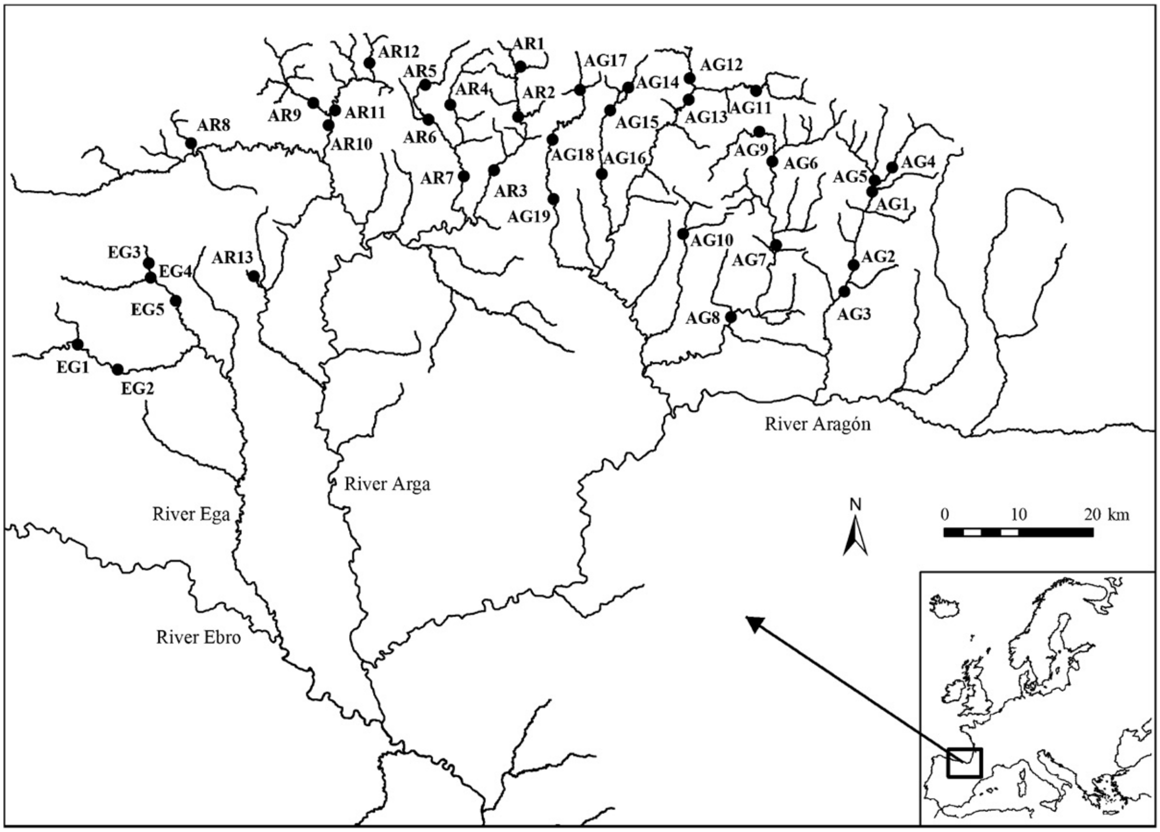

Fig. 1. Map of the study area showing the sampling sites.

Average length of study sites was 100.8 ± 23.4 m, and average assessed area of study sites was 816.2 ± 381.7 m^2. To model brown trout habitat selection, reach-type specific HSC for YOY (0+), juvenile (1+) and adult (>1+) life stages were developed (see Ayllón et al., 2010a).

Hydraulic data were calibrated in PHABSIM following procedures in Waddle (2001). Habitat competition analyses were performed using the HABEF program within PHABSIM system to model spatial segregation of cohorts due to competition and, hence, to avoid an overestimation of potential available habitat for each age class. For further details on intercohort competition analyses check Ayllón et al. (2010b) or Parra et al. (2011). Historical time series of mean summer discharge for the 12-year study period (1993–2004) were provided at each study site by the closest gauging stations. Then, summer habitat time series for each age class were obtained by coupling WUA curves as a function of discharge with discharge time series.

However, the carrying capacity of an environment is not only determined by available resources but also by how individuals compete for its use. In territorial species as brown trout, the size of the defended territories will set the maximum number of individuals that a given habitat can sustain. Territory size provides then the link between available habitat and carrying capacity. Territory size was estimated as a function of body size by means of an allometric territory size relationship specifically developed for brown trout (Ayllón et al., 2010b). Consequently, to calculate territory size, length at age *i* was determined for every age class, year, and site. Finally, carrying capacity was estimated for every age class (0+, 1+ and >1+), year, and site through the following ratio: $K_i = WUA_i/T_i$ where K_i is the carrying capacity of age-class *i* (trout ha^{-1}), WUA_i is the mean summer WUA of age class *i* ($m^2\ ha^{-1}$) and T_i is the area of the territory used by an individual of average body size of age-class *i* ($m^2\ trout^{-1}$).

Afterwards, estimated carrying capacities were compared to recorded densities so that conservation status was measured through this relationship, the D/K ratio.

2.4. Determinants of conservation status

We tried to determine the environmental and degradation factors driving the conservation status of the studied populations. To do this, we measured a total of 33 metrics to characterize different aspects of the thermal and flow regimes and quantify the degradation levels derived from different human activities operating at diverse spatial scales (Table 1). Three approaches were used in this characterization: (1) *in situ* measures to describe characteristics at each sampling site; (2) extrapolated data from historical climatic datasets from permanent stations; and (3) remotely collected data, using geographic information systems (GIS) to describe attributes at larger spatial scales. Hence, degradation metrics measured at the watershed and segment spatial scales were calculated by means of ArcGis 9.2 software (ESRI Inc., Redlands, CA) from digital data provided by regional agencies. Land use metrics were measured at two spatial scales, local and watershed, and within the whole catchment area and restricted to the riparian zone (defined as a 100 m buffer at each river side). Water quality data were obtained from seasonal monitory surveys carried out annually by regional agencies and the River Ebro Hydrographic Confederation. Angling statistics were calculated following Almodóvar and Nicola (2004) from data derived from creel-surveys conducted by regional agencies during the fishing season (March–August) along the whole study period. The fish species richness index, which combines abundance and number of species, was reckoned from the qualitative data collected during fish sampling. Water temperature was measured with data loggers permanently placed at each site between July 2004 and October 2005. We then fitted simple linear regression models

Table 1
Environmental and human-induced degradation metrics used to characterize the sampling sites.

Spatial scale	Variable	Description (units)
Watershed	Urban_N	% of urban land use within the riparian zone along the complete river network upstream the study site
	Agricultural_N	% of agricultural land use within the riparian zone along the complete river network upstream the study site
	Urban_W	% of urban land use within the watershed upstream the study site
	Agricultural_W	% of agricultural land use within the watershed upstream the study site
	Quarries	Number of quarries within the watershed upstream the study site (n 1000 ha^{-1})
	Mines	Number of mines within the watershed upstream the study site (n 1000 ha^{-1})
	Industrial premises	Number of industrial premises within the watershed upstream the study site (n 1000 ha^{-1})
	Upstream dams	Number of dams upstream the study site
	Impassable upstream dams	Number of impassable dams upstream the study site
	Downstream dams	Number of dams downstream the study site
Segment	Urban_L	% of urban land use within the riparian zone along the study segment
	Agricultural_L	% of agricultural land use within the riparian zone along the study segment
	Altered flow	% of the study segment presenting an altered flow regime
Site	Dissolved oxygen	Concentration (O_2, mg l^{-1})
	pH	pH value
	Nitrite	Concentration (NO_2^-, mg l^{-1})
	Ammonia	Concentration (NH_4^+, mg l^{-1})
	Phosphate	Concentration (PO_4^{3-}, mg l^{-1})
	Annual harvest	Angling annual harvest (Trout ha^{-1} year^{-1})
	Exploitation rate	Angling exploitation rate (%)
	Fish species richness	Index combining number and abundance of fish species present at the study site along the study period
	Temp max	Annual maximum water temperature (°C)
	Temp 7-d max	Annual maximum mean water temperature during seven consecutive days (°C)
	Temp 30-d max	Annual maximum mean water temperature during 30 consecutive days (°C)
	Low pulse count	Number of low flow pulses within each water year
	Low pulse length	Mean duration of low flow pulses (days)
	High pulse count	Number of high flow pulses within each water year
	High pulse length	Mean duration of high flow pulses (days)
	Reversals	Number of hydrologic reversals
	7-d MAX Flow oct–dec	7-Day maximum flow during October–December/Annual median flow
	7-d MAX Flow jan–feb	7-Day maximum flow during January–February/Annual median flow
	7-d MAX Flow emergence	7-Day maximum flow during March–April/Annual median flow
	7-d MAX Flow annual	7-Day maximum annual flow/Annual median flow

with daily recorded water temperature as the dependent variable, and daily air temperature data provided by the closest meteorological stations as the independent variable. Finally, we calculated back historical time series of water temperature at each site based on historical air temperature time series. Historical discharge time series were provided at each study site by the closest gauging stations and were analyzed by means of IHA V7 software (The Nature Conservancy, Olympia, WA).

2.5. Data analyses

Significant deviance of observed densities from estimated carrying capacities was tested for each age-class and site by means of *t*-tests. We also explored the existence of spatial differences in the D/K ratio among sites through one-way analysis of variance (ANOVA) and subsequent Tukey's test.

A principal component analysis (PCA) was performed to summarize the spatial heterogeneity in environmental conditions and degradation levels across study sites. Afterwards, we carried out a general regression model (GRM) with the extracted principal components as continuous predictors, basin as a categorical factor, all interaction terms, and the D/K ratio as the dependent variable.

Finally, we performed correlation analyses (Pearson r) and stepwise multiple regressions to determine the factors driving spatial variations in the D/K ratio within each major basin. We first performed correlation analyses between all metrics listed in Table 1 and the D/K ratio. Metrics that were not significantly related to D/K ratio were excluded from the regression analyses. We subsequently calculated the matrix of correlations for pairs of the remaining metrics. When metrics measuring similar pressures were highly correlated ($|r| > 0.7$), only the variable most correlated with the D/K ratio was used in the regression analyses to avoid multicollinearity. These analyses were carried out to identify the major environmental and human-induced degradation factors specifically affecting the ecological status of study systems within each basin. Since river basin is the basic unit of river management, this downscaling approach may help plan acute conservation and restoration measures within each freshwater system. Data from Arga and Ega river basins were pooled for these analyses. For all analyses, significance level was set at $\alpha = 0.05$.

3. Results

The PCA revealed three main axes accounting for 49.5% of the total variance of environmental and disturbance characteristics among study sites (Table 2). The first factor was highly and positively correlated to metrics measuring the level of environmental degradation due to human activities (degradation factor). Therefore, increasing factor scores indicated increasing degradation. Metrics measuring the relative magnitude of extreme flows at different periods of time along the water year were strongly and negatively related to the second factor (flow factor). Finally, the third factor loaded heavily and negatively on water temperature metrics (temperature factor).

There were significant differences in the D/K ratio among sites (ANOVA, $F_{36,381} = 4.3$, $p < 0.001$). *Post hoc* Tukey's tests revealed the existence of four homogeneous groups (Table 3). The first group comprised 17 sites showing high D/K ratios (>90%, mean 97.8%) and not significant differences between density and carrying capacity (*t*-test, $p > 0.3$). This group had all age classes at carrying capacity at all sites but >1+ age class at two out of the 17 sites (*t*-test, $p < 0.01$). The second group of 8 sites had medium D/K ratios (71–90%, mean 79.3%) and a total density not significantly different from carrying capacity (*t*-test, $p > 0.09$), but not all age classes were at carrying capacity at all sites (*t*-test, $p < 0.05$). The 5 sites of the third group

Table 2
Factor loadings (unrotated) for the first three principal components (PCs; DF = degradation, FF = flow, TF = temperature) from principal components analysis of variation in environmental and disturbance characteristics of sampling sites in the study area. Only loadings from metrics significantly contributing to extracted factors are shown. Loadings in bold were significant ($p < 0.05$).

Variable	DF	FF	TF
Nitrite	**0.59**	0.35	−0.20
Ammonia	**0.73**	−0.12	0.08
Urban$_L$	**0.54**	−0.15	0.30
Agricultural$_L$	**0.56**	−0.22	0.35
Urban$_N$	**0.64**	−0.27	0.25
Agricultural$_N$	**0.67**	−0.16	0.40
Urban$_W$	**0.73**	−0.05	0.40
Agricultural$_W$	**0.57**	−0.28	0.43
Mines	**0.72**	0.12	−0.05
Industrial premises	**0.67**	0.16	0.06
Upstream dams	**0.82**	−0.08	−0.30
Impassable upstream dams	**0.79**	−0.02	−0.13
Fish species richness	**0.65**	−0.13	−0.41
Temp max	0.26	−0.29	**−0.82**
Temp 7−d max	0.36	−0.32	**−0.77**
Temp 30-d max	0.43	−0.36	**−0.68**
7-d MAX Flow oct–dec	−0.26	**−0.88**	−0.06
7-d MAX Flow jan–feb	−0.32	**−0.85**	−0.09
7-d MAX Flow emergence	−0.32	**−0.84**	−0.18
7-d MAX Flow annual	−0.28	**−0.89**	−0.05
Variance explained (%)	22.0	14.6	12.9

presented low D/K ratios (50–71%, mean 64.2%) and a total density significantly lower than carrying capacity (*t*-test, $p < 0.05$), though not all age classes were below carrying capacity (*t*-test, $p > 0.07$). The fourth group encompassed 7 sites with a very low D/K ratio (<50%, mean 32.2%), being all age classes and so total density significantly below carrying capacity (*t*-test, $p < 0.001$).

The conservation status of populations significantly differed between basins (χ^2 test, $p < 0.05$). The Aragón river basin exhibited the best conservation status since 63% of sites showed a high D/K ratio (Table 3). Meanwhile, most sites (50%) in Arga and Ega basins displayed a low to very low D/K ratio.

General regression models showed that the D/K ratio of all age classes and thus total D/K ratio increased with increasing values of the temperature factor and decreasing scores of the degradation factor (Table 4). That is, increasing levels of environmental degradation and increasing extreme water temperatures caused a decrease in the D/K ratio of all age classes, the effect of temperature being higher at the Aragón River basin (Table 4). Moreover, the D/K ratio of YOY trout significantly depended on the flow factor, D/K ratio decreasing with increasing intensity of extreme flows, the effect again being higher at the Aragón River basin (Table 4). Because of this latter effect of flow conditions on recruitment performance, total D/K ratio was also significantly related to the flow factor.

Stepwise multiple regressions showed that the effect of studied variables on the D/K ratio varied across basins and age classes (Table 5). Within the Aragón River basin, both environmental (extreme water temperatures and flow conditions during

Table 3
Mean (±standard deviation) density-carrying capacity ratio (total and by age classes) and conservation status category of sampling sites.

Site	Conservation status	Total D/K	D/K 0+	D/K 1+	D/K >1+
AG17	High	104.2 ± 23.3	111.3 ± 47.5	84.2 ± 30.3	104.4 ± 40.6
AR1	High	103.9 ± 27.3	100.5 ± 41.6	108.5 ± 30.3	97.1 ± 35.5
AR8	High	101.7 ± 75.9	104.4 ± 100.4	93.8 ± 53.1	85.1 ± 54.2
AR12	High	100.7 ± 21.8	121.0 ± 77.9	96.1 ± 24.2	98.8 ± 27.5
AG14	High	100.6 ± 18.8	91.7 ± 35.1	117.4 ± 34.2	114.0 ± 56.4
AG1	High	99.4 ± 28.5	81.2 ± 49.4	99.8 ± 49.1	95.3 ± 37.1
AG6	High	99.3 ± 53.8	113.1 ± 128.5	78.6 ± 34.7	110.9 ± 34.7
AG12	High	98.5 ± 37.6	97.1 ± 50.6	99.5 ± 59.7	89.6 ± 35.0
AG15	High	98.3 ± 19.3	97.7 ± 61.0	109.6 ± 38.4	73.2 ± 28.6**
AR13	High	98.2 ± 56.3	102.1 ± 85.9	103.0 ± 55.6	75.9 ± 27.6
AG11	High	96.7 ± 52.3	98.1 ± 64.8	89.3 ± 62.2	103.4 ± 55.2
AR2	High	95.3 ± 27.6	95.6 ± 45.2	98.9 ± 38.3	79.4 ± 42.7
AG5	High	93.8 ± 33.9	115.0 ± 114.6	79.9 ± 58.8	85.6 ± 28.6
AG9	High	93.6 ± 40.6	80.8 ± 62.4	101.0 ± 22.8	83.4 ± 32.4
AG16	High	93.3 ± 34.1	95.0 ± 70.0	99.5 ± 31.3	76.8 ± 38.9
AG13	High	93.1 ± 30.3	109.2 ± 94.4	96.7 ± 46.0	70.0 ± 23.3**
AG18	High	92.4 ± 50.2	74.2 ± 63.2	112.3 ± 82.4	103.3 ± 51.3
AG4	Medium	89.7 ± 46.2	98.6 ± 82.3	77.9 ± 41.5	87.0 ± 43.3
AG19	Medium	86.9 ± 40.6	93.4 ± 59.9	78.6 ± 50.0	72.2 ± 32.9*
AR11	Medium	85.5 ± 26.8	78.0 ± 39.0	95.1 ± 41.8	86.9 ± 33.4
EG3	Medium	77.4 ± 23.3	81.3 ± 53.3	89.9 ± 60.1	74.2 ± 31.9*
EG4	Medium	76.9 ± 54.9	83.7 ± 96.0	65.2 ± 70.4*	81.2 ± 51.7
AG2	Medium	76.8 ± 67.5	62.8 ± 27.1*	95.2 ± 38.5	105.8 ± 76.2
AR9	Medium	76.3 ± 23.0	76.6 ± 25.3	78.3 ± 52.1	87.6 ± 31.8
EG5	Medium	74.9 ± 22.7	71.7 ± 61.1*	75.2 ± 70.4	75.9 ± 37.5
AR3	Low	71.7 ± 22.5*	48.0 ± 29.1*	56.2 ± 33.2*	104.7 ± 41.5
AR4	Low	70.8 ± 29.3*	64.4 ± 44.1*	75.2 ± 55.4	58.5 ± 24.6***
AG10	Low	61.1 ± 33.0***	48.7 ± 38.5***	57.3 ± 63.7*	75.8 ± 28.1*
AR5	Low	60.8 ± 36.2*	39.9 ± 43.2*	80.6 ± 45.2	95.1 ± 54.1
AG3	Low	56.6 ± 47.3**	67.9 ± 77.4*	59.7 ± 38.9*	22.2 ± 14.0***
EG2	Very low	46.7 ± 22.9***	53.1 ± 38.5**	30.9 ± 31.8***	69.7 ± 41.4**
AR7	Very low	42.0 ± 30.7***	42.2 ± 39.5***	36.9 ± 48.7***	50.2 ± 44.1***
EG1	Very low	34.8 ± 27.4***	32.1 ± 28.7***	56.9 ± 63.6**	69.3 ± 107.1**
AG7	Very low	34.2 ± 21.8***	45.8 ± 40.2***	34.6 ± 18.5***	17.3 ± 20.9***
AR6	Very low	32.7 ± 16.3***	17.9 ± 22.8***	49.4 ± 42.0**	50.5 ± 19.0***
AR10	Very low	30.2 ± 24.5***	36.6 ± 63.9**	32.5 ± 25.0***	21.9 ± 16.6***
AG8	Very low	4.4 ± 4.1***	5.8 ± 6.7***	3.2 ± 4.2***	3.5 ± 6.4***

* $p < 0.05$, significant deviance of density from carrying capacity with its probability.
** $p < 0.01$, significant deviance of density from carrying capacity with its probability.
*** $p < 0.001$, significant deviance of density from carrying capacity with its probability.

Table 4
Summary of the best general regression models explaining spatial variation in density-carrying capacity ratio (total and by age classes). DF, FF and TF refer to the degradation, flow and temperature factors, respectively, extracted from the PC analysis.

Dependent variable	Independent variables	Coefficient	R^2	F	P
Total D/K ratio	Intercept	81.83	0.64	15.33	<0.001
	DF	−17.10			
	FF	8.07			
	TF	6.19			
	Basin × FF (Aragón)	5.41			
	Basin × TF (Aragón)	10.55			
0+ D/K ratio	Intercept	83.02	0.70	15.66	<0.001
	DF	−21.34			
	FF	12.18			
	TF	6.80			
	Basin × FF (Aragón)	8.65			
	Basin × TF (Aragón)	9.52			
1+ D/K ratio	Intercept	81.50	0.63	14.51	<0.001
	DF	−17.23			
	TF	8.89			
	Basin × TF (Aragón)	8.12			
>1+ D/K ratio	Intercept	80.87	0.53	9.50	<0.001
	DF	−12.33			
	TF	10.26			
	Basin × TF (Aragón)	11.45			

emergence) and degradation (number of upstream dams) factors were included in the final models (Table 5). In contrast, spatial variations in the D/K ratio within the Arga-Ega basins were explained only by variations in the levels of environmental degradation, although the explanatory variables retained in the best explaining models differed among age classes (Table 5). The variance of D/K ratio explained by either regional or basin specific fitted models was highest for YOY and lowest for adult trout (Tables 4 and 5).

4. Discussion

After analyzing the relationship between density and carrying capacity in 37 sites during 12 years across an environmentally heterogeneous area and through a gradient of human degradation, results showed that D/K ratio was an accurate indicator of population conservation status. Variations in the environmental and human degradation factors included in the best explaining regression models developed for the whole population and by age classes accounted for between 58 and 81% of the variation in the D/K ratio. We also observed that the factors driving such variations differed across life stages and river basins.

Results suggested that the effects of extreme environmental events may be overridden by impacts derived from high levels of human degradation. It was evidenced by the fact that in spite of water temperature being a main driver of conservation status at the regional spatial scale, it was a major determinant only in the Aragón River basin. This basin presented the lowest levels of human degradation and thus the best conservation status. Consequently, spatial variations in the D/K ratio were principally determined by changes in temperature and flow conditions. In contrast, in the rest of studied basins increasing deviations of population density from maximum potential were better explained by increasing intensity of human degradation rather than by environmental spatial variability. This remained true despite the fact that sampling sites were distributed along a wide gradient of water temperature and flow regimes. It suggests that high levels of human degradation can drive trout populations to such a poor conservation status that the regulatory effects of extreme environmental events are no longer detectable.

According to correlation analyses (data not shown) and regression models, the presence of upstream dams was the main anthropogenic factor impairing studied populations, affecting all life stages at all basins. Dams transform natural systems through water and sediment flow alteration, water temperature modification and river connectivity disruption (Poff and Hart, 2002). Therefore, the damming of a river is usually regarded as a cataclysmic event in the life of a riverine system (Ligon et al., 1995) and their cumulative ecological effects over a watershed to be profound (Poff and Hart, 2002). The impacts of damming on fish communities are well documented, especially for salmonids (*e.g.*, Almodóvar and Nicola, 1999; Gosset et al., 2006; Murchie et al., 2008). What is more, mathematical models predict that extinction risk dramatically increases in salmonid populations fragmented by damming, particularly when carrying capacity is low (Charles et al., 2000; Hilderbrand, 2003; Morita and Yokota, 2002). Further, ongoing global warming magnifies the environmental constraints faced by trout populations persisting at low-latitude margins, and the presence of dams severely limits the capacity for adaptation and migration to a changing climate of stream-dwelling trout populations that live in landlocked linear aquatic habitats (Almodóvar et al., in press).

Landscape transformations due to human activities were also a major determinant of population decline in most altered basins. In the Arga and Ega river basins, population impairment was mostly caused by changes from natural towards agricultural land uses. The final impacts of increasing intensity of agricultural activities on river systems are exerted by diverse mechanisms and pathways (reviewed by Allan, 2004). At any rate, impairment of conservation status of studied populations was best predicted by modifications of land use within the riparian zone through the whole river network. Removal of riparian vegetation typically entails higher water temperatures in summer, decreased water and habitat quality, fewer energy inputs and a markedly decline in the amount of woody debris entering the stream (Allan, 2004). Importantly, streams whose riparian forests have been removed or overgrazed will have reduced input of terrestrial invertebrate prey with likely consequences for salmonid populations (Gustafsson et al., 2010; Kawaguchi and Nakano, 2001; Saunders and Fausch, 2007). However, since concentrations of ammonia and especially nitrite (included in the best explaining model) were negatively correlated with conservation status in the Arga and Ega river basins, the impact of agricultural activities is bound to have been mainly *via* water pollution. Finally, water temperature responds to shading over of thousands of meters, and energy inputs as well as inputs of

Table 5
Results of stepwise multiple regression analyses testing the effect of environmental and human-induced degradation factors on density-carrying capacity ratio (total and by age classes) within each basin.

Dependent variable	Independent variables	Coefficient	R^2	F	P
Aragón					
Total D/K ratio	Intercept	348.05	0.81	36.88	<0.001
	Upstream dams	−3.33			
	Temp max	−13.06			
0+ D/K ratio	Intercept	419.40	0.77	17.47	<0.001
	Temp max	−15.26			
	Upstream dams	−2.49			
	7-d MAX Flow emergence	−2.75			
1+ D/K ratio	Intercept	367.26	0.68	17.71	<0.001
	Upstream dams	−8.65			
	Temp max	−14.45			
>1+ D/K ratio	Intercept	381.24	0.66	16.26	<0.001
	Upstream dams	−3.09			
	Temp max	−15.12			
Arga-Ega					
Total D/K ratio	Intercept	112.63	0.79	29.97	<0.001
	Agricultural_N	−1.21			
	Nitrite	−367.43			
0+ D/K ratio	Intercept	116.92	0.79	29.97	<0.001
	Agricultural_N	−1.52			
	Nitrite	−344.90			
1+ D/K ratio	Intercept	97.46	0.61	12.46	<0.001
	Upstream dams	−1.38			
	Agricultural_N	−0.68			
>1+ D/K ratio	Intercept	97.48	0.58	10.52	<0.01
	Agricultural_N	−0.82			
	Upstream dams	−0.98			

nutrients, sediments or pollutants are local but subject to downstream transport over long distances and so may be influenced by riparian conditions along a stream's entire length (Allan, 2004).

Although it was not included in the best explaining models, the fish species richness index was negatively correlated with the D/K ratio of all life stages. In almost all sites showing a high conservation status, the Pyrenean minnow was the only accompanying fish species, and there were generally no more than two stably frequent fish species occurring with brown trout. In the Aragón river basin, the richest fish communities were associated with lower reaches, where temperature was limiting and more suitable to warm water fish species. In the Arga and Ega basins the highest species richness was associated with the worst conserved populations living in highly degraded reaches where water temperature was also limiting. Therefore, the D/K ratio was capable of explaining both the species replacement patterns along altitudinal gradients and the increasing presence of species more tolerant to anthopogenic stressors in degraded reaches.

Interestingly, the response of the D/K ratio to diverse environmental and degradation factors varied among life stages. Different life stages of the same species may not respond in the same way to a given disturbance or show independent responses to different types and degree of degradations or climatic events. So the D/K ratio performed well in that sense, being highly sensitive to age specific disturbances. For example, the regression model for the Aragón River basin showed that flow conditions during emergence was one of the main predictors of YOY conservation status, meanwhile it was not even correlated to adult D/K ratio. Since swimming performance increases with age (Videler, 1993), YOY and especially newly emerged recruits, are subject to a high mortality during flooding events while adults have a higher capacity to survive such extreme events (Cattanéo et al., 2002; Daufresne et al., 2005; Nicola et al., 2009). In the Arga and Ega river basins, YOY conservation status was highly sensitive to eutrophication, probably as a result of higher susceptibility to pollution or affections to reproduction patterns (Chaumot et al., 2003), while older and more mobile life stages were more affected by damming.

Environmental legislation typically mandates managers to define thresholds below which biotic condition is unacceptable and restoration is needed (Groffman et al., 2006). Some recent studies suggest critical boundary values for biotic metrics corresponding to acceptable quality can be set solely by biotic criteria (*e.g.*, Aroviita et al., 2010). In that respect, it is clear that when trout populations are significantly below carrying capacity (low and very low conservation status) restoration actions are needed. Nevertheless, what happens when a population is already weakened but not significantly below its carrying capacity (medium conservation status)? In this case, a D/K ratio value over 90% seems an accurate threshold to define good conservation status of a trout population and so of the studied stream. It is worth noting that a population D/K ratio fell below 90% when at least two life stages had a D/K ratio below that threshold. So as a rule of thumb, rehabilitation measures might be necessary when two or more life stages are already weakened. However, discriminating least from most disturbed sites is not a sufficient management target. When managers must face the recovery of declining populations is crucial to know if such decline is due to habitat or non-habitat factors in order to allocate properly restoration efforts. The analysis of the density-carrying capacity relationship may help identify which factors are limiting population abundance since the D/K ratio was sensitive to both general and life stage specific disturbance factors. Therefore, the D/K ratio could be an interesting indicator to consider when defining objective management plans and corrective actions in degraded trout rivers and streams under Mediterranean climatic conditions.

Concerns about the feasibility of monitoring the D/K ratio over a large spatial scale may arise, however, since collecting habitat data to model carrying capacity may require considerable field effort. In our approach, as long as channel morphology and structure remain stable, habitat surveys must take place just once so that habitat availability is simulated thereafter as a function of river discharge. Hence, contrarily to other quality parameters that must be monitored at short time intervals, it is not necessary to sample habitat along time, but only to monitor discharge. Regarding the biological criteria, as habitat selection patterns do not change across stream reaches presenting similar local and catchment-scale physical attributes (Ayllón et al., 2010a), suitability curves can be developed by reach typology with no need of building site-specific ones. Further, habitat values could be predicted throughout a river system from a low number of modeled sites based on average reach characteristics and simple hydraulic geometry relationships (Lamouroux and Capra, 2002; Lamouroux and Jowett, 2005; Rosenfeld et al., 2007), which would both reduce the cost of site-specific habitat studies and facilitate their generalization at larger spatial scales.

5. Conclusions

There is an increasing need to find simple tools that allow managers to assess the ecological status of rivers based on their biological properties. For this reason, fish are increasingly being used as biological indicators, though fish metrics that accurately respond to disturbances in Mediterranean trout type streams are still lacking. In the present study, we showed that the D/K ratio was highly sensitive to temporal and spatial variations in environmental conditions and the levels of human-induced environmental degradation. Likewise, the D/K ratio was sensitive to both general and life stage specific disturbance factors. Based on these results, we concluded that the D/K ratio was an accurate indicator of brown trout population conservation status and consequently of the ecological status of the studied trout type streams. Given the simplicity of the index, we expect it to be applicable to other Mediterranean trout basins.

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