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FISH AS ECOLOGICAL INDICATORS IN MEDITERRANEAN FRESHWATER ECOSYSTEMS

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Contents

Summary

Summary

Although fish have been used as ecological indicators for more than 20 years in North America, few studies have been done in Europe, and particularly in the Mediterranean basin. The Water Framework Directive includes fish fauna as one of the biological elements, jointly with aquatic flora and benthic invertebrates, to assess and monitor water and habitat quality. Successful implementation of the Directive depends in part on the development of reliable, science-based tools to directly assess biological conditions. The aim of this thesis is to contribute to the use of freshwater fish as ecological indicators in the Mediterranean basin.

In the **first article** data for eight biotic and abiotic indices for four Catalan river basins were compiled in order to analyse the relationships among indices. Although most biological indices were correlated, fish reflected different ecological aspects due to their particular features (e.g., higher mobility and longevity). Brown trout (*Salmo trutta*) was proposed to be considered as of uncertain native status in the Tordera basin (i.e. considered not native nor introduced), to compute metrics such as percentage of native species or of native individuals, because although genetic information points to an introduction, there is a very old record (by 1845) and further information is needed to clarify its status. This status has profound effects on the results of fish metrics; therefore it is very important to know the status for each species in each basin.

In the **second article** the stream flow regimes (real and predicted by the Sacramento model, based on temperature and rainfall) of six Mediterranean basins were studied. A decrease of streamflow and aquifer levels was detected, despite no observed decrease of rainfall precipitation. The number of days that the river is dry has increased and the stream flow regularly observed is well below the naturalized flow predicted by the model. Meanwhile, the numbers of inhabitants and industries and the quantity of water abstracted has increased in the river basin. During six years the fish assemblage of Tordera river was studied at impacted and unimpacted sites to know the effect of these droughts, provoked by water abstraction. Of the thirty metrics tested to detect sites impacted by water abstraction, we detected four significant fish metrics: catch per unit effort, number of benthic species, number of intolerant species, and proportion of intolerant individuals. Furthermore, the role of biotic indices when the river is artificially dry is discussed. The detailed characterization of the natural flow regime (and hence drought events) should precede the application of biotic indices in streams severely affected by water abstraction, and in cases of artificially dry rivers,

biotic indices should be given the worst score (instead of the customary missing value that biases the overall ecological assessment).

In the **third article** the effects of a prescribed partial drawdown on fish assemblage in autumn 2005 in Sau, a eutrophic reservoir, were studied. Species composition in the pelagic zone varied significantly during the drawdown with higher proportion of bleak (*Alburnus alburnus*) during the days of worst water quality, confirming some previous studies that have shown that bleak is more tolerant than roach (*Rutilus rutilus*) to poor water quality and thus a potential good indicator of water pollution. The weight–length relationship (i.e. condition) of roach and bleak also varied significantly during the drawdown following the same pattern in both species. The close relationship found between water quality and fish condition demonstrates that fish condition can be a good metric of the well being of fish, even for extreme short-term changes.

In the **fourth article** the condition and fecundity of freshwater fishes were assessed in Flix reservoir (where there are around 200,000-360,000 tons of industrial pollutants) and also downstream until the Delta and at Riba-roja reservoir (as a control) in order to detect a possible impact on fish biology. The responses to the pollutants were species-specific and common carp (*Cyprinus carpio*) was the species with the clearest effects on fitness-related traits at the impacted area, despite also being among the most resistant to pollution. There were some metrics clearly affected in several fish species: DELT anomalies, presence of ectoparasites, eviscerated weight and liver weight. DELT anomalies and ectoparasites were more abundant at the impacted area than at control sites. Eviscerated and liver weights had the highest values at the control area. Although the fecundities observed in our study were within the ranges of other studies for the same species, significantly lower values at the impacted area for carp and pumpkinseed (*Lepomis gibbosus*) were detected.

This thesis has shown that inland fish of the Mediterranean basin can be valuable ecological indicators, as in other regions of the world. Furthermore, the results of this thesis also illustrate some pressures that freshwater ecosystems suffer in this region. We have shown that the abusive withdrawal of water is causing significant streamflow reduction, artificial droughts in Mediterranean streams and problems to manage reservoirs during these drought periods. We have also shown the negative effects on the fish biota of a massive accumulation of pollutants in a reservoir.

Resum

Resum

Tot i que a Estats Units els peixos continentals fa més de 20 anys que són utilitzats per avaluar l'estat de conservació dels ecosistemes aquàtics, a Europa i més concretament a la conca Mediterrània, hi ha molt pocs treballs al respecte. La Directiva Marc de l'Aigua de la Unió Europea proposa els peixos com un dels elements biològics a tenir en compte alhora de monitoritzar les masses d'aigua. Perquè això sigui possible i es pugui implementar amb èxit la directiva, s'han de desenvolupar metodologies i eines científiques fiables. L'objectiu d'aquesta tesi és contribuir a l'ús dels peixos continentals com a indicadors de l'estat ecològic a la conca Mediterrània.

En un primer treball, es van comparar diferents índex biològics i fisicoquímics a quatre conques catalanes i es va detectar que encara que tots els índex biològics estaven correlacionats significativament, els peixos integren i expressen els estressos de manera i a una escala diferents, aportant una informació complementària als altres índexs. Es proposa que l'estatus natiu/introduït de la truita (*Salmo trutta*) a la conca de la Tordera sigui considerat incert, ja que tot i que la genètica indica un origen introduït, hi ha una citació molt antiga (primera meitat del XIX) i manquen més dades per confirmar el seu estatus. El fet de considerar la truita com a espècie autòctona o exòtica té un efecte molt elevat en el resultat dels índexs, pel que es recalca la importància de conèixer l'estatus de cada espècie a les diferents conques.

També es van estudiar els cabals (els *reals* i els predits pel model Sacramento, basat en temperatura i precipitació) de sis conques catalanes. S'ha mostrat que els règims hídrics estan alterats amb una tendència a la disminució del cabal i del nivell dels aqüífers. Paral·lelament no s'ha detectat una disminució de les precipitacions però sí un augment de la zona urbanitzada, de la població i de l'extracció d'aigua a la conca de la Tordera. S'ha estudiat durant sis anys la comunitat de peixos de la Tordera, una conca molt afectada per l'extracció d'aigua, i s'ha detectat quatre mètriques (captures per unitat d'esforç, nombre d'espècies bentòniques, nombre d'espècies intolerants i proporció d'individus intolerants) que es troben significativament afectades en zones més castigades per manca d'aigua degut a l'activitat humana. Es discuteix la necessitat de conèixer en profunditat el règim hídric de cada conca abans d'utilitzar els índex biològics. Així mateix es proposa que si el punt de mostreig està sec per efectes humans es posi el valor menor de l'índex, enlloc de no posar valor com es fa fins ara.

Es va estudiar els efectes del buidat parcial de l'embassament de Sau l'any 2005 sobre la comunitat de peixos. La composició d'espècies capturades va variar significativament al llarg del buidat amb un augment de l'alburn (*A. alburnus*) a les aigües pelàgiques durant els dies de pitjor qualitat de l'aigua, cosa que concorda amb què l'alburn sembla més tolerant a la mala qualitat de l'aigua que la madrilleta vera (*R. rutilus*) i suggereix un potencial com a bioindicador. La condició de l'alburn i la madrilleta vera varen canviar significativament al llarg del buidat. L'estreta relació trobada entre la condició dels peixos i la qualitat de l'aigua demostra que la condició pot ser una bona mètrica, fins i tot per canvis a curt termini.

Es va estudiar la biologia dels peixos de l'embassament de Flix (on hi ha una gran quantitat de contaminants acumulats), i també aigües avall fins al delta i a l'embassament de Riba-roja (com a control) per tal de detectar un possible impacte a nivell individual i poblacional. Les respostes van ser diferents per cada espècie i la carpa va ser la que va mostrar més clarament els impactes, probablement pel seu ús de l'hàbitat i ecologia alimentària. Pel conjunt de les espècies hi ha quatre variables que van respondre clarament a l'impacte de Flix: DELT (deformitats, aletes erosionades, lesions o tumors), presència de paràsits, pes eviscerat i pes del fetge. El percentatge de DELT i la presència de paràsits externs era major en la zona impactada que no en els punts de control (Riba-roja). Els valors més alts de pes eviscerat i pes del fetge estaven als punts de control.

Aquesta tesi ha mostrat que els peixos continentals de la conca Mediterrània, igual que en altres regions el món, poden ser bons indicadors de l'estat ecològic. A més, els resultants d'aquesta tesi han mostrat algunes de les presions que pateixen els ecosistemes d'aigua dolça d'aquesta regió. Hem mostrat com l'ús abusiu de l'aigua està causant una reducció dels cabals, provocant sequeres artificials als rius mediterranis i causant problemes de gestió als embassaments. També hem mostrat els efectes negatius a la biologia dels peixos degut a l'alta acomulació de contaminants al medi.

Introduction

Introduction

Freshwater ecosystems are a hotspot of diversity because they contain 2.4% of all known species despite only occupying 0.8% of the terrestrial surface and only representing 0.3% of the water of the planet (Margalef 1983; McAllister *et al.* 1997). They are also among the most altered ecosystems worldwide due to human activities (Malmqvist & Rundle 2002). Freshwater ecosystems have been profoundly altered with industrial, agriculture and urban pollution, water abstraction and regulation, introduction of exotic species, and alteration of riparian habitat and natural hydromorphology (Baron *et al.* 2002; Nilsson *et al.* 2005; Xenopoulos & Lodge 2006). Although humans only exploit about 10-15% of current total runoff, water abstraction mostly for irrigation, is very severe in many arid or semiarid regions, where it can account for more than 100% of water availability (Vörösmarty & Sahagian 2000). Mediterranean-climate streams (e.g. those surrounding the Mediterranean Sea or in coastal California) are particularly affected by water abstraction (either directly or via groundwater withdrawal), because they combine a deficit of water resources with higher population densities and agriculture development than more arid regions (Gasith & Resh 1999). In wetter Mediterranean regions, water abstraction may change a perennial stream to an intermittent one, intensifying the duration and extent of droughts (Gasith & Resh 1999). According to the World Conservation Union (IUCN), water pollution and water abstraction (before other threats like invasive species and the construction of dams) are identified as the two greatest current threats and are perceived to be the main future threats for freshwater fish in the Mediterranean basin (Smith & Darwall 2006). Despite the present efforts for pollution abatement in aquatic ecosystems, there are still some cases of severe accumulation of industrial pollutants due to past activities (e.g., Huuskonen *et al.* 1998; Farkas *et al.* 2000; Durrieu *et al.* 2005). Furthermore, agricultural runoff, urban wastewater and bad operation of some treatment plants provoke an important contribution of pollutants and nutrients in the aquatic systems (Carpenter *et al.* 1998; Lavado *et al.* 2004).

Many wetlands, meanders, lagoons and oxbows have been eliminated for the sake of "sanitation"; water is being transferred between basins and natural streamflow is broken due to dam constructions (Sabater 2008). In the world, a total of 52% of the surface area occupied by large river systems (discharge over 350 m^3/s) is strongly modified, Europe containing the lowest fraction of unaltered segments (Sabater 2008). Overall, dumping of pollutants and nutrients (both diffuse and punctual), the construction of dams, water abstraction, the introduction of exotic species and alteration of riparian habitats provoke a very important

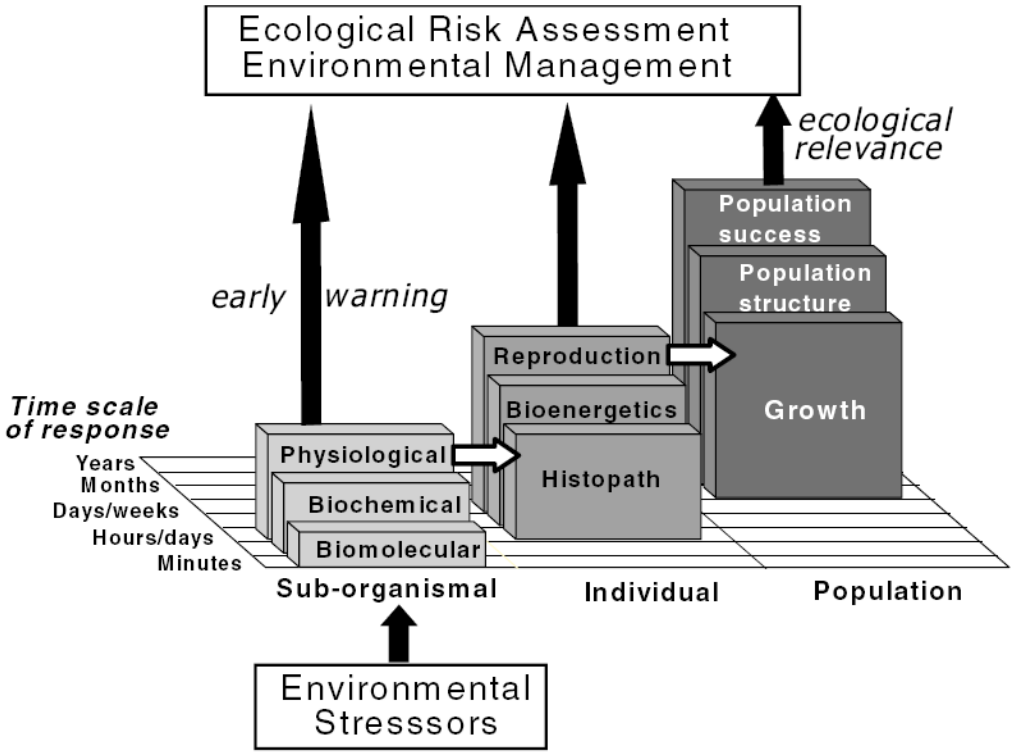
impact in the aquatic systems and associated biotic communities (Pires *et al.* 1999; Nilsson *et al.* 2005, Xenopoulos & Lodge 2006). Economic growth is an ultimate cause of resource collapse and biodiversity loss (Brown 2000; Czech *et al.* 2000; Angermeier 2005).

The recently issued European Water Framework Directive (WFD) mandates the restoration and maintenance of healthy aquatic ecosystems, where 'health' is assessed on the basis of hydromorphological, chemical, and biological attributes. This directive reflects a significant shift in the emphasis placed on biotic measures of water quality relative to former approaches that focused narrowly on water chemistry. The current view of aquatic ecosystem health emulates the notion of ecological integrity, which has increasingly guided the management of water resources in the United States over the past two decades (Karr & Dudley 1981; Karr 1981). A critical and often limiting step in assessing ecosystem health is the development of tools to directly measure biotic condition. In the WFD one of the biological elements, jointly with aquatic flora and benthic invertebrate fauna, is fish fauna. Successful implementation of the directive depends, in part, on development of reliable, science-based tools to directly assess biological conditions (Angermeier & Davideanu 2004).

Fish as ecological indicators

It is widely known that long-term exposure to environmental stressors such as pollution or low oxygen causes detrimental effects on important fish features such as metabolism, growth, resistance to diseases, reproductive potential, and, ultimately, the health, condition, and survival of fish (Barton *et al.* 2002). These negative effects may be transferred to the population or community levels (Fig. 1). The effects at the individual and population levels depend on the intensity and duration of stress exposure and species-specific features (Adams & Greeley 2000). The knowledge, for each species, of their functional attributes, range of tolerance and responses in front of different kinds of stress will permit to use freshwater fish as ecological indicators. The biological indicators complement the traditional physicochemical indicators, facilitating a better assessment and management of freshwater ecosystems.

Figure 1. Hierarchical response of organisms to environmental stressors illustrating the more sensitive early warning indicators at the lower levels of biological organization and the slower-responding but more ecologically relevant indicators at the higher levels of organization (from Adams & Greeley 2000).

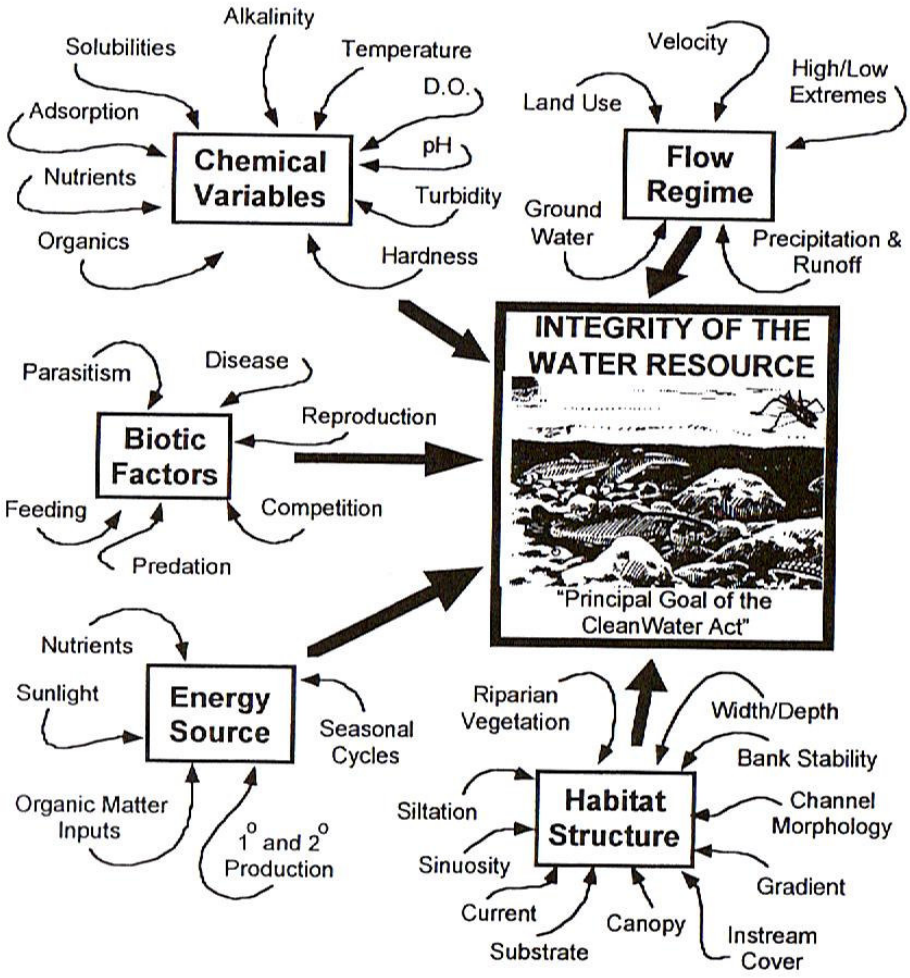


Fish have some particular features and advantages as indicators of the health of freshwater ecosystems (see e.g., Simon 1999). Fish continually inhabit the receiving water and integrate the chemical, physical, and biological histories of the waters. Most fish species have long lifespans (about 2-10 years) and can both reflect long-term and current water quality. The sampling frequency needed for trend assessment is less than for short-lived organisms and taxonomy of fishes is well established, enabling professional biologists the ability to reduce laboratory time by identifying most specimens in the field. Fish have large ranges and are less affected by natural microhabitat differences than smaller organisms, making them extremely useful for assessing regional and macrohabitat differences. Fish are highly visible and valuable components of the aquatic community to the public, making communication easier.

Ecotoxicologists generally assess the impact of pollutants on freshwater fish using standard toxicity tests at the laboratory. Although these types of controlled laboratory studies provide important information on how organisms respond to environmental stressors, they also have some limitations (Cairns 1983; Lagadic *et al.* 1994; Adams & Greeley 2000). First, ecotoxicological tests mainly inform about acute and not sublethal or chronic effects (Hela *et al.* 2005). Moreover, although polluted sites in nature generally consist of a mixture of pollutants, most ecotoxicological studies focus on exposure and effects of single compounds (Yang 1994). Another limitation of laboratory tests relates to the bioavailability of toxicants: unlike the concentrations of toxicants of controlled toxicity tests, physico-chemical properties such as waterflow or pH of natural ecosystems may mediate bioavailability (De Zwart *et al.* 2005). In general, physico-chemical processes (e.g., ionization, dissolution, precipitation, complexation, sorption, and partitioning) reduce the concentration of toxicants that is actually experienced by the biota. These processes depend on individual properties of the toxicants and on abiotic characteristics of the ecosystem (De Zwart *et al.* 2005). Therefore, although laboratory studies provide invaluable preliminary information on the effects of environmental stressors, further studies in natural situations are needed to increase ecological realism.

The knowledge, for each fish species, of their functional attributes, ranges of tolerance and responses in front of different kinds of stress is the needed background to use fish as biological indicators. Mainly in the United States some metrics and biotic indices with fish have been developed to measure relative ecosystem health (e.g., rivers, reservoirs). Although the study of fish as ecological indicators started at the beginning of 20th century (Simon 1999), it is not until the year 1981 that James Karr proposed (Karr 1981) the first biological index based on fish, namely the Index of Biotic Integrity (IBI). James Karr defined biological integrity as "the ability to support and maintain a balanced, integrated, and adaptive community of organisms having a species composition, diversity and functional organization comparable to those of natural habitats within a region" (Karr & Dudley 1981). Different factors (both biotic and abiotic) may affect this biotic integrity (Fig. 2).

Figure 2. The five principal factors, with some of their important chemical, physical, and biological components that influence and determine the integrity of surface water resources (from Simon 1999).

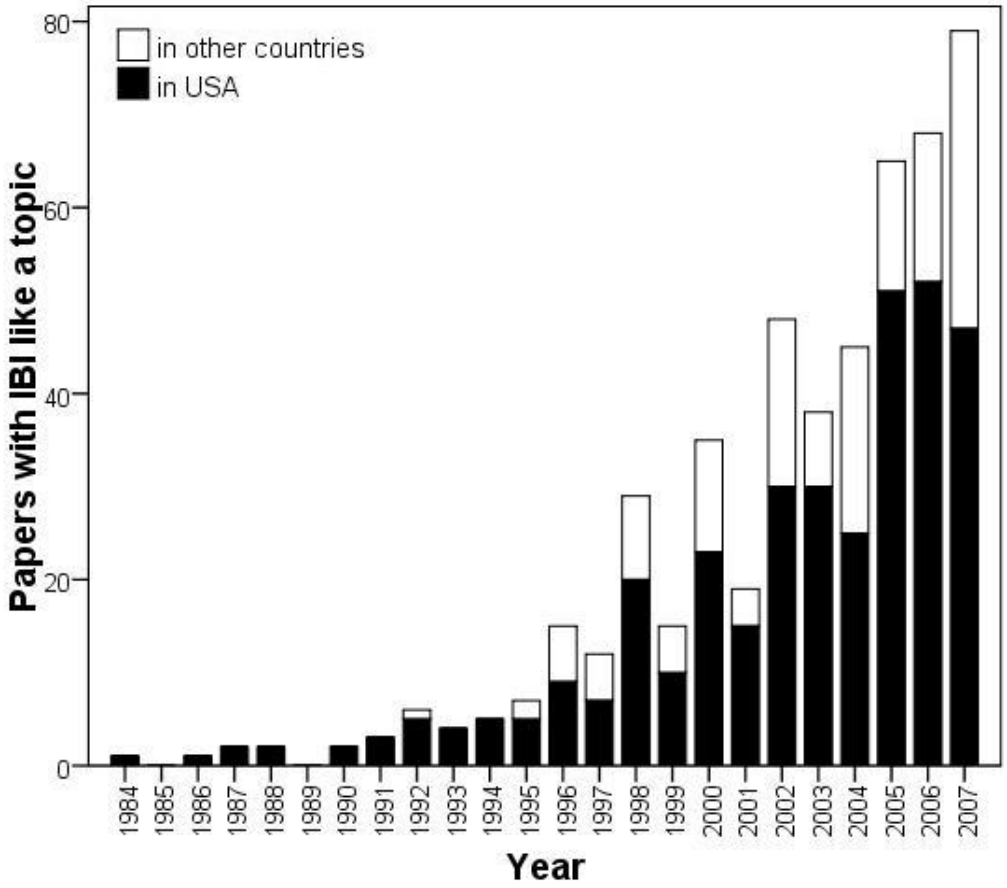


The original IBI consists of twelve fish-assemblage attributes (called metrics) that reflect predominant anthropogenic effects on streams. Each metric describes a particular taxonomic, trophic, reproductive, or tolerance feature of the assemblage (Table 1). An IBI score represents comparisons between metric values at a sampling site and those expected under conditions least affected by anthropogenic disturbance. The original version of the IBI has been modified in numerous ways for application in many different regions and habitat types of United States (Fausch *et al.* 1984; Karr *et al.* 1986; Miller *et al.* 1988; Lyons *et al.* 1996; Simon 1999; Angermeier *et al.* 2000). These new versions maintain a multimetric structure but they incorporate different typologies, number of metrics and values. The utilization of the fish as bioindicators is spreading all over the planet and every year more papers are published developing new IBIs in other regions (Figure 3).

Table 1. The 12 metrics of the original index of biotic integrity (Karr 1981).

Species richness and composition metrics
Total number of fish species (total taxa)
Number of Catostomidae species (suckers)
Number of darter species
Number of sunfish species
Indicator species metrics
Number of intolerant or sensitive species
Percent of individuals that are *Lepomis cyanellus* (Centrarchidae)
Trophic function metrics
Percent of individuals that are omnivores
Percent of individuals that are insectivorous Cyprinidae
Percent of individuals that are top carnivores or piscivores
Reproductive function metrics
Percent of individuals that are hybrids
Abundance and condition metrics
Abundance or catch per effort of fish
Percent of individuals that are diseased, deformed, or have eroded fins, lesions, or tumors (DELTs)

Figure 3. Number of papers published on IBIs along the years. "Index of Biotic Integrity" was searched at ISI Web of Science and papers from USA and other countries were identified.



The utilization of fish as biological indicators in Europe is less widespread than in the United States (Fig. 3), until recently only in France, Belgium and Romania (Hughes & Oberdorff 1999; Oberdorff *et al.* 2002; Kestemont *et al.* 2000; Angermeier & Davideanu 2004). A European project (FAME: Fish-based Assessment Method for the Ecological Status of European Rivers) has recently tried to develop new tools using fish assemblages (Pont *et al.* 2006; Pont *et al.* 2007). However, there are few works which specifically study the Mediterranean basin, where fish assemblages have particular characteristics: few native species, poor knowledge of their ecological requirements, high number of endemisms with wide range of tolerance to environmental variations, and many exotic species (Moyle & Marchetti 1999; Smith & Darwall 2006). Moreover, the climate and streamflow regime in the Mediterranean basin are very different than in central and northern Europe, therefore works that study specifically the use of the fish as bioindicators in the Mediterranean basin are urgently needed. Until now, only four works have been published on fish indices in the Iberian Peninsula: Sostoa *et al.* (2003), Ferreira *et al.* (2007a, b) and Magalhães *et al.* (2008). Sostoa *et al.* elaborated a first IBI for Catalan rivers, as a project for the Catalan Water Agency. In that study, they classified Catalan rivers in five typologies and overall they selected twelve fish metrics (Table 2). Ferreira *et al.* published two works within the FAME project about the Iberian Peninsula (2007a) and Mediterranean basin (2007b). Although in these papers they tested many fish metrics they did not proposed an IBI; they only studied which fish metrics detected human disturbance. In the recent published paper of Magalhães *et al.* (2008) they selected five fish metrics to make an IBI for the Guadiana river. In all of these works the authors emphasized the difficulties to elaborate an IBI in the Mediterranean basin, because the particularities of the fish assemblages that made difficult to select fish metrics and to make an IBI with sufficient metrics to function correctly.

Therefore, although in the United States the use of inland fish as ecological indicators has been widely developed and it is widely established at government agencies, in Europe and specifically in the Mediterranean basin, it is still at an early stage. Furthermore, due to the particularities and difficulties of Mediterranean fish assemblages explained above, it is urgently needed to deepen the knowledge to develop the tools needed to use Mediterranean inland fish as ecological indicators.

Table 2. The fish metrics, for each river typology, selected for the first Catalan IBI (Sostoa *et al.* 2003).

Low Mediterranean mountain
Number of native species
Number of insectivore natives
Intolerant natives (ind/ha)
Humid Mediterranean mountain
Proportion of native species
Proportion of intolerant
Littoral streams
Proportion of native species
Proportion of insectivore species
Number of natives / Number of original distribution natives
Ebro main rivers
Number of tolerant natives
Long lived species (ind/ha)
Number of lithophilic exotic species
High mountain
Total density natives (ind/ha)

Study areas

Mediterranean rivers

Mediterranean streams have flow patterns strongly seasonal: low flow in the hot summer drought and flash floods during autumn and spring storms (Gasith & Resh 1999). During the summer some parts of the stream can remain reduced as a series of pools. Interannual variability in precipitation is high while lengthy periods of drought are common (Gasith & Resh 1999). This hydrological variability of Mediterranean-type regions profoundly determines the life forms and life cycles of aquatic organisms, as well as ecological processes (Ferreira *et al.* 2007a). Besides these natural factors, the water resources of the Mediterranean basin suffer a high human pressure because it is a highly populated area with great population, urban and industrial growths, especially in the last 50 years (Sala & Bar 1992; Aparicio *et al.* 2000; Araus 2004). The pressure on water availability is obviously higher in dry countries, such as the Mediterranean basin. For example although the average of European water consumption per capita is 207 m^3/year, the Mediterranean countries have higher annual consumption, e.g., 530 m^3 in Spain, 523 m^3 in Italy, and 334 m^3 in Greece (Vergés 2002).

Figure 4. A & B: Tordera and Fluvià streams have a flow regime strongly seasonal. C & D: Field work in the Tordera river basin. E & F: Two common species in Tordera basin: Mediterranean barbel (*Barbus meridionalis*) (E) and brown trout (*Salmo trutta*). Photos by: E. García-Berthou (B) and L. Zamora (C, D, F).

In Catalonia, with the exception of the Ebro, rivers have a basin area of intermediate dimensions (from 312 km^2 of Foix to 4,948 km^2 of Llobregat) and average streamflows that oscillate between 1.5 m^3/s of Francolí to 20 m^3/s of Llobregat. One of the main studied rivers in this thesis is the Tordera stream (Figure 4). The Tordera rises in coastal mountains, drains an area of ca. 895 km^2, and has highly variable flow, with episodic floods. Mean annual rainfall in the basin ranges from 1000 mm near the summit to 600 mm on the coast; mean annual water yield is 170.4 hm^3/year and mean discharge is ca. 4 m^3/s. Apart from the direct pressure on the water resources, Catalan rivers, like other in the Mediterranean basin, also

suffer from alterations in natural hydromorphology and riparian vegetation (Mas-Pla *et al.* 1999; Munné *et al.* 2003). Although the pollution for industrial and urban waste has in general decreased thanks to entry in operation of many treatment plants (Prat & Rieradevall 2006), there are some remarkable exceptions such as the large amount of industrial pollutants (mainly organochlorides and heavy metals) in the Flix reservoir, in the Ebro river (Grimalt *et al.* 2003).

Nowadays all Catalan rivers are being studied in order to fulfil the Water Framework Directive, and also fish assemblages are studied to improve the IBICAT index (Sostoa *et al.* 2003). For these reasons it is necessary more research focusing on the relationship between water quality and fish responses. Information of these aspects will help to analyse, monitor and manage Mediterranean rivers in general. As indicated by the WFD, these studies should not be limited to rivers, because reservoirs have great strategic importance as water supply.

Reservoirs

Nowadays, 15% (40,000 km^3 y^{-1}) of the world's total runoff is retained in 45,000 large dams (higher than 15 m height) (Nilsson *et al.* 2005). The purpose of damming includes irrigation, hydroelectric power generation, flood control, and public water supply (Han *et al.* 2000; Poff & Hart 2002; Nilsson *et al.* 2005). The construction of artificial reservoirs destroys terrestrial ecosystems, alters the downstream flux of water and sediment causing extensive modification of aquatic communities, and obstructs the dispersal and migration of organisms (Poff & Hart 2002; Nilsson *et al.* 2005; Villanueva *et al.* 2006). Although the reservoirs share some features with lakes, they have their own characteristics. Reservoirs have larger inputs of nutrients and particulate matter and stronger water-level fluctuation than natural lakes, which lead to eutrophication and stress to their ecological communities (Carol *et al.* 2006). In Mediterranean countries, with few natural lowland lakes, reservoirs are an attraction for anglers and consequently a site where many exotic species are first introduced (Benejam *et al.* 2005; Carol *et al.* 2006; Clavero & García-Berthou 2006). Catalan reservoirs were constructed mainly around the fifties and they have 93 hm^3 of average capacity. Foix, Sau and Susqueda are probably the most eutrophic reservoirs (for more details Table 3). Sau is one of the main reservoirs studied in this thesis (Figure 5 and 6). We studied in this reservoir the effects of a partial drawdown on fish condition. Flix and Riba-roja reservoirs have been also studied in this thesis in order to know the effects of the large accumulation of pollutans on fish biology.

Table 3. Physical and limnological features of the most importan Catalan reservoirs (Carol *et al.* 2006).

Reservoir name	Altitude (m)	Basin surface (km^2)	Reservoir surface (ha)	Capacity (hm^3)	Conductivity (μS/cm)	Alkalinity (meq/l)	Total *P* (μM)	Integrated chlorophyll 0–8 m ($\mu g/m^2$)
Baells	630	532	367	115.4	517	3.25	1.12	7.29
Boadella	159	182	364	62.0	312	2.79	0.48	24.22
Camarasa	336	2850	624	113.0	246	1.80	1.72	12.39
Escales	821	152	400	154.0	213	1.77	0.41	4.09
Flix	41	82246	320	11.0	687	3.26	1.51	2.99
Foix	101	290	71	3.7	1270	4.63	10.70	443.34
Riba-roja	70	79177	2152	210.0	684	3.26	1.64	11.57
Riudecanyes	118	31	30	5.3	476	2.72	0.77	19.81
Sant Antoni	501	2070	927	205.0	222	1.71	1.51	6.22
Sant Ponç	530	318	139	24.4	431	3.00	0.34	15.17
Santa Fe	1080	5	6	1.0	38	0.39	0.89	110.21
Sau	425	1564	570	168.5	592	3.02	3.51	82.93
Susqueda	351	1850	466	233.0	491	2.60	2.33	21.76
Terradets	372	2620	330	23.0	236	1.79	1.46	9.14

Due to the artificial nature of reservoirs, reference conditions to develop a fish index do not exist. For this reason different authors (e.g., Jennings *et al.* 1995; McDonough & Hickman, 1999) elaborated an alternative method to derive the "reference" conditions for reservoirs based on the observation that, being an artificial system, they lack natural reference sites to assess the deviation from unaffected conditions. These authors adapted the original fish metrics of Karr *et al.* (1986) and proposed the RFAI (Reservoir Fish Assemblage Index) (Table 4).

Figure 5. G & H: Professional fishermen working with a purse seine in the pelagic zone during the drawdown in Sau reservoir (October 2005).

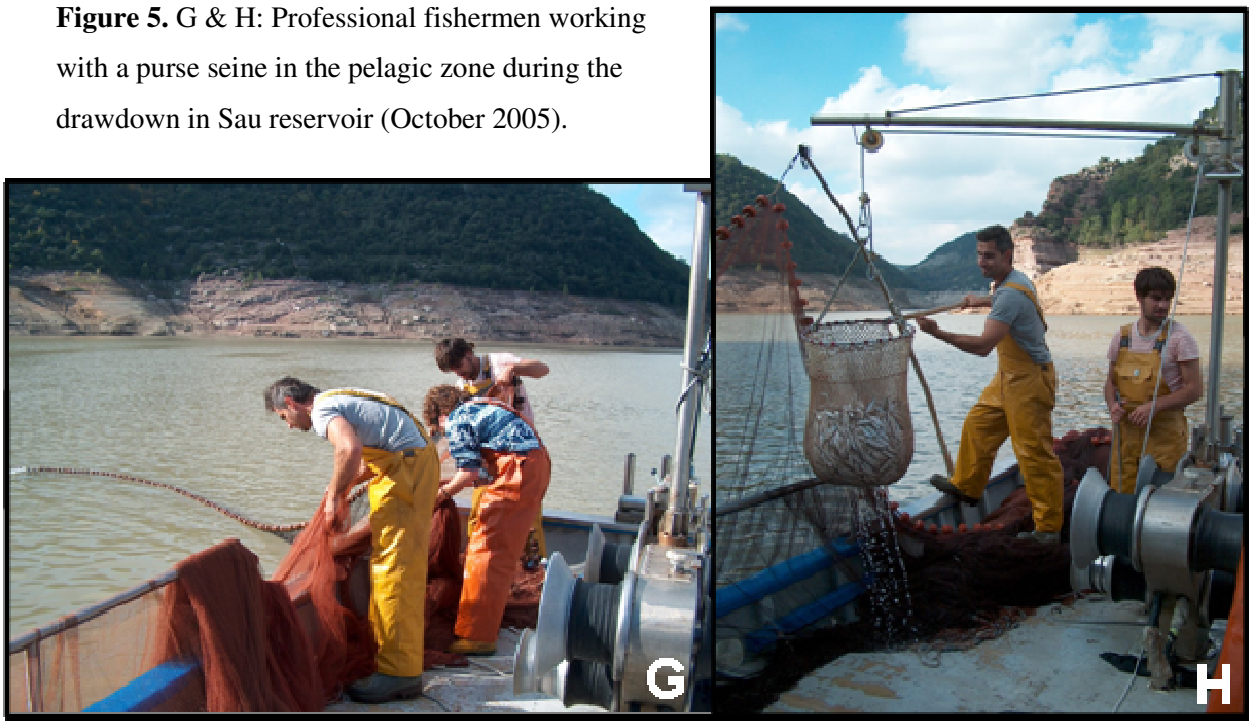


Table 4. Fish metrics in the Reservoir Fish Assemblage Index (RFAI) (from http://www.tva.gov/environment/reports/tellicoeis/pdf/appendixesc2-6.pdf).

Species Richness and Composition
1. **Total number of species**—Greater numbers of species are considered representative of healthier aquatic ecosystems. As conditions degrade, numbers of species at a site decline. 2. **Number of piscivore species**—Higher diversity of piscivores is indicative of better quality environment. 3. **Number of sunfish species**—Lepomid sunfish (excludes black basses, crappies, and rock bass) are basically insectivores, and high diversity of this group is indicative of reduced siltation and suitable sediment quality in littoral areas. 4. **Number of sucker species**—Suckers are also insectivores but inhabit the pelagic and more riverine sections of reservoirs. 5. **Number of intolerant species**—This group is made up of species that are particularly intolerant of habitat degradation. Higher densities of intolerant individuals represent better environmental quality. 6. **Percentage of tolerant individuals** (excluding Young-of-Year)—This metric signifies poorer quality with increasing proportions of individuals tolerant of degraded conditions. 7. **Percentage dominance by one species**—Ecological quality is considered reduced if one species dominates the resident fish community.
Trophic Composition
8. **Percentage of individuals as omnivores**—Omnivores are less sensitive to environmental stresses due to their ability to vary their diets. As trophic links are disrupted due to degraded conditions, specialist species such as insectivores decline while opportunistic omnivorous species increase in relative abundance. 9. **Percentage of individuals as insectivores**—Due to the special dietary requirements of this group of species and the limitations of their food source in degraded environments, proportion of insectivores increases with environmental quality.
Reproductive Composition
10. **Number of lithophilic spawning species**—Lithophilic broadcast spawners spawn over rocky substrate and do not provide parental care. This guild is expected to be sensitive to siltation. Numbers of lithophilic spawning species increase in reservoirs providing suitable conditions reflective of good environmental quality.
Abundance
11. **Total catch per unit effort** (number of individuals)—This metric is based upon the assumption that high quality fish assemblages support large numbers of individuals.
Fish Health
12. **Percentage individuals with anomalies**—Incidence of diseases, lesions, tumors, external parasites, deformities, blindness, and natural hybridization are noted for all fish measured, with higher incidence indicating poor environmental conditions.

Objectives

The overall objective of this thesis is contribute to the use of freshwater fish as ecological indicators in the Mediterranean basin.

Article I. Assessing fish metrics and biotic indices in a Mediterranean stream: effects of uncertain native status of fish. The fish assemblage and limnological features of the Tordera stream were sampled to evaluate the usefulness of several fish metrics and to compare habitat quality and biotic indices currently in use. Data for eight biotic and abiotic indices for this and three other Catalan river basins were also compiled in order to analyse the relationships among indices. Furthermore, we briefly discuss the role of introduced species, particularly in headwater streams, in the development of fish indices.

Article II. Assessing effects of water abstraction on fish assemblages in Mediterranean streams. The stream flow regimes of six Mediterranean basins were studied to know the alterations suffered during the 20th century. The effects of human-caused droughts in the Tordera river, one of these six river basins, was studied in order to evaluate the utility of fish metrics in detecting the effects of water abstraction in Mediterranean streams. Furthermore, the role of biotic indices when the river is artificially dry is discussed.

Article III. Short-term effects of a partial drawdown on fish condition in a eutrophic reservoir. Due to the need of optimizing water quality in Sau reservoir, a partial drawdown was prescribed in autumn 2005. During the partial drawndown there was a decrease of 12 meters in the water level in only 40 days, with an obvious impairment of the water quality. The aims of this chapter are to investigate the effects of this drawdown on the fish assemblage and condition (weight-length relationship) of roach (*Rutilus rutilus*) and bleak (*Alburnus alburnus*).

Article V. Decreases in condition and fecundity of freshwater fishes in a highly polluted reservoir. In Flix reservoir (reservoir of the Ebro river basin), there are around 200,000-360,000 tons of industrial pollutants. This exceptional amount of pollutants provides a good opportunity to analyse their effects on fish populations in natural conditions, which is rarely available to ecotoxicologists. The objectives of this chapter are to test whether the reproductive traits and prevalence of diseases and parasites at this impacted area are different

from those in a neighbouring upstream reservoir unaffected by the pollution (control) and also from downstream sites.

Figure 6. I & J: Electrofishing boat. K: Setting gillnets. L & M: Catching and measuring fishes. N: Flix reservoir. Photos by: S. Saura-Mas.

ARTICLE I

L. Benejam, E. Aparicio, M. J. Vargas, A. Vila-Gispert and E. García-Berthou. "Assessing fish metrics and biotic indices in a Mediterranean stream: effects of uncertain native satus of fish". *Hydrobiologia*. Vol. 603, issue 1 (May 2008) : p. 197-210

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Abstract

Implementation of the Water Framework Directive requires tools for measuring and monitoring the ecological status of aquatic ecosystems. Several indices are in use in the Iberian Peninsula, although there has been little comparison among them. We sampled the fish assemblage and limnological features of the Tordera stream (NE Spain) quarterly from September 2001 to May 2003 to evaluate the usefulness of several fish metrics and to compare habitat quality and biotic indices currently in use. Data for eight biotic and abiotic indices for this and three other Catalan river basins were also compiled in order to analyse the relationships among indices. In the Tordera stream, fish abundance and richness increased with stream order except in the last sampling site that had the lowest fish abundance owing to the effects of drought and water abstraction. Although most indices were positively correlated, some displayed low or null correlations particularly for the Tordera basin which is more affected by water abstraction and less by pollution; a commonly used physico-chemical index (ISQA) was the least correlated. In a regional fish index (IBICAT) under development, the brown trout (*Salmo trutta*) has been previously considered as introduced in the Tordera basin. Here, we report an old published record that demonstrates that trout was present before 1845 and we argue that its status should be considered as uncertain given the current information available. Whether brown trout is treated as native or introduced to this river basin has profound effects on the results of fish metrics because of its dominance in the upper reaches. We briefly discuss the role of introduced species, particularly in headwater streams, in the development of fish indices. Our study exemplifies the need for careful, basin-specific assessment of native/introduced status in the development of fish metrics.

Keywords: Water Framework Directive - Biotic integrity - Introduced species - Brown trout - *Salmo trutta*

ARTICLE II

Ll. Benejam, P. Angermeier, A. Munné i E. García-Berthou. “Assessing effects of water abstraction on fish assemblages in Mediterranean streams”. (En premsa)

Abstract

Freshwater abstraction strongly affects streams in arid and semiarid ecosystems, particularly those in Mediterrean-climate regions. Excessive abstraction reduces the availability of water for other human uses and impairs the capacity of streams to support native biota. We investigated the flow regime and related variables in six river basins of the Iberian Peninsula and show that they have been strongly altered, with negative trends for flow and aquifer levels during the 20th century. We observed lower flows and higher frequency of stream-drying than those predicted by the official hydrological model used in this region. We also observed dry conditions for some streams predicted by the model to be permanently flowing. Meanwhile, there has been no decrease in precipitation. We investigated the fish assemblage of a stream in one of these river basins (Tordera) for six years and show that sites more affected by water abstraction display significant differences in four fish metrics (catch per unit effort, number of benthic species, number of intolerant species and proportional abundance of intolerant individuals). We discuss the utility of these metrics in assessing impacts of water abstraction and point out the need for detailed characterization of the natural flow regime (and hence drought events) prior to the application of biotic indices in streams severely affected by water abstraction. In particular, in cases of artificially dry streams, it is more appropriate for regulatory agencies to assign index scores that reflect impairment than to assign “missing” scores, as is customary for Iberian streams.

Keywords: water abstraction, drought, natural flow regime, streamflow, fish metrics, Mediterranean streams.

ARTICLE III

Ll. Benejam, J. Benito, J. Ordóñez, J. Armengol and E. García-Berthou. “Short-term effects of a partial drawdown on fish condition in a eutrophic reservoir”. *Water, Air & Soil Pollution.* Vol. 190, number 1-4 : p. 3-11.

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Abstract

Prescribed or natural drawdowns occur frequently in reservoirs but their effects on fish populations have been barely studied. As a consequence of a severe drought and the need to optimize water quality, a partial drawdown was prescribed in autumn 2005 to a eutrophic reservoir that provides water supply to a large metropolitan area (Barcelona, Spain). In order to avoid a potential massive fish kill given the reduced oxygen availability and high fish abundance, preventive purse seine fisheries were performed to reduce the fish stock. The fisheries had little effect on the fish assemblage because final population size structure and species composition did not change significantly. The species composition of the purse seine catches varied significantly during the drawdown with higher proportion of bleak (*Alburnus alburnus*) in pelagic water during the days of worst water quality, confirming that bleak is more tolerant than roach (*Rutilus rutilus*) to poor water quality and a potential good indicator of water pollution. The weight–length relationship (i.e. condition) of roach and bleak also varied significantly during the drawdown following the same tendency in both species, losing and recovering their weight (4.99% in roach and 5.96% in bleak) in only 16 days. The close relationship found between water quality and fish condition demonstrates that fish condition can be a good metric of the well being of fish, even for extreme short-term changes.

Keywords: Water quality - Ecological indicators - Iberian Peninsula - Roach *Rutilus rutilus* - Bleak *Alburnus alburnus*

ARTICLE IV

Ll. Benejam, J. Benito i E. García-Berthou. “Decreases in condition and fecundity of freshwater fishes in a highly polluted reservoir”. (En premsa).

Abstract

Despite many efforts for pollution abatement in aquatic ecosystems, there are still some cases of high accumulation of industrial pollutants due to past activities. In Flix reservoir (Ebro river basin, Spain), there are around 200,000-360,000 tons of industrial pollutants with a high concentration of heavy metals and organochlorides, due to the activity of an organochlorine industry during more than half century. This exceptional amount of pollutants provides a good opportunity to analyse their effects on fish populations in natural conditions that are rarely available to ecotoxicologists. We compared the reproductive traits and prevalence of diseases and parasites at this impacted area with a neighbouring upstream reservoir unaffected by the pollution (control) and also to downstream sites. DELT (deformities, eroded fins, lesions, and tumors) anomalies and ectoparasites were clearly more frequent at the impacted area for several fish species (common carp, roach and pumpkinseed). A significant negative impact of Flix reservoir on condition (eviscerated and liver weight, acounting for fish size) and reproductive traits (gonadal weight and number of mature eggs) was also detected for several fish species. The responses to the pollutants were species-specific and common carp (Cyprinus carpio) was the species with the clearest effects on fitness-related traits at the impacted area, despite also being among the most resistant to pollution.

Keywords: heavy metals, organochlorides, common carp Cyprinus carpio, Flix reservoir

General discussion

General discussion

Human impacts in Mediterranean fresh waters

The results of this thesis, besides helping to use freshwater fish as ecological indicators in the Mediterranean basin, also illustrate some pressures that freshwater ecosystems suffer in this region. We have shown that the abusive use of water is causing significant streamflow reduction, artificial droughts in Mediterranean streams (**Article 2**) and problems to manage reservoirs during these drought periods (**Article 3**). We have also investigated the negative effects on the fish biota (**Article 4**) of a massive accumulation of pollutants in a reservoir (**Article 4**).

The increase of human population in Mediterranean basin has not stopped during the last century, together with industrial activities and urbanization and the consequent increase of water demand (Sala & Bar 1992; Xenopoulos & Lodge 2006; Smith & Darwall 2006; Magalhães *et al.* 2007). The high pressure on water resources, as **Article 2** has shown, is provoking in Catalan rivers and other regions in the Mediterranean basin a decrease of streamflows and an increase of drought periods and its severity, losing in this way its natural character. Natural drought is a seasonally predictable event in Mediterranean-type ecosystems (Gasith & Resh 1999), to which native species have been adapted through evolutionary history (Moyle 1995; Poff 1997). However, this equilibrium is fragile and an increase in frequency and severity of drought events due to water abstraction may provoke serious problems for the conservation of Mediterranean and semiarid native fish assemblages (Magalhães et al. 2003, 2007). Many authors suggest that restoration or preservation of native stream biota will require maintenance or restoration of natural flow regimes (Power *et al.* 1996; Stanford *et al.* 1996; Poff *et al.* 1997). Therefore, in order to preserve the native biota it is necessary not only the absence of pollution in the water but also that rivers have a natural discharge and natural flow regime again. Consequently, comprehensive hydrological studies for each river basin are needed and it is also urgent to apply the policies, strategies and managements to respect the natural stream flow regime, only in this way we will be able to preserve the integrity of the ecosystem functioning of Mediterranean streams and their biota.

Beside the negative consequence on the river and their biota, the increase of water consumption also causes a higher use of the water in reservoirs. This growing demand of water resource may provoke periods of difficult management in Mediterranean reservoirs, such as **Article 3** has shown and has also been the case during the 2007-2008 drought in Catalonia. Furthermore, in the present scenarios of climate change different studies suggest

that extreme conditions will rise in frequency in the Mediterranean basin, with increases of temperatures and decreases of rain (Gibelin & Déqué 2003; Milly *et al.* 2005). This context of continuous increase of demand and reduction of water availaibility may cause an increase of dam construction and water abstraction in the Mediterranean basin, with associated problems such as the alterations of habitat and natural flow regime (Prat & Ibáñez 1995; Poff & Hart 2002; Nilsson *et al.* 2005; Villanueva *et al.* 2006). These alterations on freshwater ecosystems also facilitate the introduction and establishment of exotic species (Clavero & García-Berthou 2006; Carol *et al.* 2006). Different authors have shown that the exotic species have more facility to establish in a new area if their habitat and communities has been altered (Moyle & Leidly 1992; Scott & Helfman 2001). Therefore, freshwater ecosystems of the Mediterranean basin are highly susceptible to be invaded by exotic species. Besides imposing huge economic costs, invasive species cause extinction of native species, reduction of genetic diversity, and biotic homogenization through a variety of mechanisms (García-Berthou 2007).

In Flix reservoir, as it has been analyzed in **Article 4**, there is a large amount of pollutants with negative effects on biota. Despite the present efforts for pollution abatement in aquatic ecosystems there are still some cases of high accumulation of industrial pollutants due to past activities, for example in Lake Balaton, Hungary (Farkas *et al.* 2000), in Gironde estuary - France (Durrieu *et al.* 2005) or Lake Kernaala, Finland (Huuskonen *et al.* 1998). It will be necessary to restore these natural areas and to take out the pollutants. Furthermore, it is desirable that current legislation prevents that similar cases could be repeated.

Therefore, this thesis has provided more evidence of the high degree of alteration and human impacts that the Mediterranean basin is suffering. Concretely, it has demonstrated that the high consumption of water provokes a reduction of streamflow and an increase of artificial droughts in the Mediterranean streams (**Article 2**), as well as complicates reservoir management (**Article 3**). Furthermore, this thesis has shown that the existence of a high accumulation of industrial pollutants in some freshwater ecosystems provokes negative effects on their biota (**Article 4**).

Fish as ecological indicators

Utility of fish as ecological indicators in Mediterranean freshwater ecosystems

This thesis has shown that inland fish of the Mediterranean basin can be valuable ecological indicators, as in other regions of the world.

Article 1 showed that fish provided different information than more traditional physicochemical or other biological indices. Although most biological indices were correlated, due to the particular features of fish (e.g., higher mobility and longevity) they reflected different ecological aspects. Our results also suggest that a physicochemical index (ISQA) used routinely by the Catalan Government should not be generally used because it does not capture disturbances that emerge with the other indices. In this article, brown trout (*Salmo trutta*) was proposed to be considered as of uncertain native status in the Tordera basin (i.e. considered not native nor introduced), to compute metrics such as percentage of native species or of native individuals. Despite no paleoecological information in this river basin, there is an old published record in the basin (Madoz 1845), which is older than most known trout introductions. Whether brown trout is treated as native or introduced to this river basin has profound effects on the results of fish metrics because of its dominance in the upper reaches, similar situation occur in California's streams (Moyle & Marchetti 1999). For these reason, in this article we recommend for headwater sites, or sites with low richness, to assess metrics based on age or size structure, fish individual state, or other taxa such as amphibians (Moyle & Marchetti 1999) to compensate for low fish richness. Furthermore we propose that metrics that penalize for the presence or abundance of introduced fishes (to measure historic biotic integrity) and metrics that profit from their indicator value could be combined. Miller *et al.* (1988) suggest that although an IBI with less than 12 metrics may work, it may be less responsive to a broad spectrum of degradation. Therefore we propose that IBIs in headwater sites, or sites with low richness, should not be used unless a sufficient number of nonredundant metrics is identified.

In the **second article**, we tested thirty fish metrics to detect sites impacted by water abstraction and we detected four significant metrics: catch per unit effort, number of benthic species, number of intolerant species, and proportion of intolerant individuals. Although most of these four metrics are part of different IBIs (e.g., Miller *et al.* 1988; Angermeier & Davideanu 2004), in our study we have shown that they may be useful in the Mediterranean basin. Low abundance of fish (catch per unit effort) should be expected in a site affected by water abstraction, given that drought affects fish survival, reproduction, and promotes

emigration (Matthews & Marsh-Matthews 2003; Keaton *et al.* 2005; Davey & Kelly 2007). The drying progress causes a deterioration of water quality (e.g., higher temperature, less dissolved oxygen) (Danehy *et al.* 1998; Lake 2003), causing a decrease in the richness and relative abundance of intolerant species. In agreement with the well known effect of drought on species composition (Magoulick 2000; Marchetti & Moyle 2001; Matthews & Marsh-Matthews 2003), we detected low values of benthic species at impacted sites, because in these areas the Mediterranean barbel *Barbus meridionalis* (a native species) decreased in abundance in contrast to the minnow *Phoxinus phoxinus* (an introduced species to the basin). Therefore, low values of these four metrics may be useful to detect effects of water abstraction. These fish metrics may be used for monitoring rivers with problems of water abstraction. Some of these fish metrics are already used in existing IBIs, so our results help to understand their behaviour.

In the **third article,** fish condition was studied during a partial drawdown in a eutrophic reservoir. Although fish condition is a relatively well studied aspect (Lloret & Rätz 2000; Vila-Gispert *et al.* 2000; Oliva-Paterna *et al.* 2003), very few studies have analyzed it in reservoirs. This article develops its potential as a tool to monitor and assess water quality. Species composition in the pelagic zone varied significantly during the drawdown with higher proportion of bleak (*Alburnus alburnus*) during the days of worst water quality, confirming some previous studies (Irz *et al.* 2002; Dray *et al.* 2003) that have shown that bleak is more tolerant than roach (*Rutilus rutilus*) to poor water quality and thus a potential good indicator of water pollution. The condition of roach and bleak varied significantly and quickly during the drawdown closely following water quality, with lowest values short after the worst water quality days. Two points may be highlighted from these results: i) condition was simply measured with total weight; and ii) the short-term effects of drawndown on condition. Fish condition can be assessed by different methods: morphometric, physiological and biochemical (Barton *et al.* 2002; Lloret *et al.* 2002). When morphometric (weight-length) methods are used it is recommended to measure eviscerated weight in order to avoid the influence of viscera and gonads (Barton *et al.* 2002). Our study was realized in autumn, out of reproduction period, because of this the total weight was sufficient to detect the change on condition without the need to dissect. On the other hand, although seasonal variation in condition is routinely studied (e.g. Jamet 1995; Hartman & Margraf 2006), there are few examples of such short-term variation in fish condition. Although fish condition changes seasonally and for each species, our results show that it could be used as a tool to assess water

quality. For example, during the last drought in Catalonia (year 2007-2008) this metric was successfully used to assess the variation of fish health in Sau and Susqueda reservoirs.

In the **fourth article** the effects of high accumulation of pollutants in the condition and fecundity of freshwater fishes was assessed. Although all the species studied showed some significant variation among sampling sites, the responses were species-specific and common carp was the species that showed more fitness-related traits with significant effects at the impacted area. Carp is probably more exposed to the contaminants than other species because it is benthic and has more physical proximity with the pollutants than pelagic species. However, there are some metrics that clearly affected different fish species: DELT anomalies, presence of ectoparasites, eviscerated weight and liver weight. DELT anomalies were more abundant at the impacted area than at control sites (Riba-roja). The prevalence of DELT anomalies was already proposed as one of the twelve metrics of the original index of biotic integrity (IBI) (Karr 1981) and has since become an increasingly accepted indicator of water quality and fish health (Plumb 1994; Yoder & Rankin, 1999). The occurrence of ectoparasites followed a pattern similar to that of DELT anomalies, with highest prevalence (of copepods and leeches) at the impacted area. Therefore this article shows that DELT anomalies and ectoparasites could be good bioindicators. Eviscerated or liver weights, after accounting for the correlation with length, are common measures of condition of fish (Adams 2002; Lloret *et al.* 2002). Both variables had the highest values in control area. Similar results of lower condition in ecosystems with poor water quality have been reported for a number of species, including roach and bleak (Laflamme *et al.* 2000; Vila-Gispert *et al.* 2000). Fish condition is a measure of the physical and biological circumstances of a fish and is affected by interactions among food availability, physical factors and environmental conditions (Lloret & Rätz, 2000; Vila-Gispert *et al.* 2000; Oliva-Paterna *et al.* 2003); consequently a low condition is indicating a poor quality of environment. Although the fecundities observed in our study were within the ranges of other studies for the same species (Hardy 1978; Crivelli 1981; Vila-Gispert 1996), we detected significantly low values at the impacted area for carp and pumpkinseed. These low values are consistent with documented effects of contaminants on fecundity because the pollutants may affect fish reproduction in several ways (Ma *et al.* 1995; Bieniarz *et al.* 1997). Although eviscerated, liver and gonadal weights are easily measured and consequently are suitable to assess and monitor, fecundity is more difficult to measure and thus only adequate for more in-depth studies.

Problems and possible solutions in the Mediterranean basin

The fish assemblages of the Mediterranean basin, similarly to other Mediterranean areas such as California, have particular characteristics that hamper IBIs' development: few native species, poor knowledge of their ecological requirements, high number of endemisms with wide range of tolerance to environmental variations and many exotic species (Moyle & Marchetti 1999; Smith & Darwall 2006). The Index of Biotic Integrity has been developed in areas with complex fish communities: many native species with different trophic levels. The IBIs characterize by having many metrics (normally around twelve), independent among them (metrics with redundant information should be avoided) and of different levels of organization (individual, population, community, ecosystem and landscape) (Miller *et al.* 1988; Karr & Chu 1999). In order to correctly detect different kinds of ecosystem alterations, Karr & Chu (1999) emphasized that IBIs should have metrics for each organization level (Table 1).

Table 1. Types of metrics, suggested number of metrics for each type, and corresponding levels in the biological hierarchy. Well-constructed multimetric indexes contain the suggested number of metrics from each type and therefore reflect multiple dimensions of biological systems (Karr & Chu 1999)

	LEVEL					
Metric type	**Number of metrics**	**Individual**	**Population**	**Community**	**Ecosystem**	**Landscape**
Taxa richness	3-5	X	X	X	X	
Tolerance, intolerance	2-3		X	X		
Trophic structure	2-4			X	X	X
Individual health	1-2	X				
Other ecological attributes	2-3	X	X	X	X	X

Due to low fish richness is difficult to develop enough metrics for IBIs in the Mediterarnean basin (Ferreira *et al.* 2007b). Miller *et al.* (1988) suggested that although an IBI with less than 12 metrics may work, it may be less responsive to a broad spectrum of degradation. The low fish richness in the Mediterranean basin hinders the use of very common fish metrics such as: diversity of species, trophic specialization and reproductive strategies. This low richness is especially problematic in headwater sites, where often there are only one or two species and sometimes one is an introduced or translocated salmonid (Moyle & Marchetti 1999). The first article of this thesis showed the problem to compute IBIs in these cases.

This low richness could be compensated assessing metrics based on age or size structure, fish individual state or including other aquatic biota. For instance, there are IBIs that combine fish metrics with benthic invertebrates (Kerans & Karr 1994) and both adults and tadpoles of amphibians (Moyle & Marchetti 1999). With regard to individual health, although DELT anomalies (assessed in **Article 4**) are incorporated in many IBIs (Sanders *et al.* 1999), the presence of ectoparasites or fish condition (studied in the third and fourth articles) are not. These fish metrics could help to increase the number of metrics in Mediterranean IBIs, concretely at individual health level (Karr & Chu 1999), because they have responded significantly in front of habitat degradation and poor quality of water.

Introduced species are a serious environmental problem (Clavero & García-Berthou 2005). Some authors suggest that exotic species should not be included in absolute richness metrics of IBIs (Karr *et al.* 1986; Scott & Helfman 2001) but could be a reliable indicator of poor river health (Kennard *et al*. 2005). Moreover, other authors indicate that although exotic species are a loss of biotic integrity they might provide a great deal of information about water and habitat quality (Moyle & Marchetti 1999). This represents a conflict between using an IBI to measure diversity and abundance of native organisms versus using an IBI to measure water and habitat quality. Exotic species have been incorporated in metrics of different IBIs of the Mediterranean basin (Sostoa *et al.* 2003; Ferreira *et al.* 2007a; Ferreira *et al*. 2007b; Magalhães *et al.* 2008) (Table 2). Only Ferreira *et al.* (2007b) included exotic species in the metric of absolute richness; in the other works where this metric was present (Sostoa *et al.* 2003; Ferreira *et al.* 2007a), only native species were considered. Although Sostoa *et al.* (2003) and Magalhães *et al.* (2008) suggest some metrics exclusively for native species (e.g., number of native insectivores), in general exotic and native species are pooled in metrics of trophic and reproduction function. Although to take the information about water and habitat quality that exotic species provide could be a solution in front of the problem of low native species in Mediterranean basin, we think that to value positively the abundance and richness of exotics is counterproductive.

Table 2. Different works of IBIs and metrics in the Mediterranean basin and their treatment of exotic species. *These works tested many fish metrics but they did not make an IBI. =, metrics with pooled both native and exotic species, they have the same treatment. -, metrics exclusively for native species, exotic species are excluded. +, metrics exclusively for exotic species valuing positively the presence of them.

Reference	Region of application	Nº of metrics	Treatment of exotic species
Sostoa *et al.* 2003	Catalonia	1-3	= , -
Pont *et al.* 2006	Europe	10	=
Ferreira *et al.* 2007*a**	Iberian Peninsula	20	= , -
Ferreira *et al.* 2007*b**	Mediterranean Europe	31	= , -, +
Magalhães *et al.* 2008	Guadiana basin	5	= , -

Hydrological variability of Mediterranean-type regions profoundly determines the life forms and life cycles of aquatic organisms, as well as ecological processes (Blondel & Aronson 1999; Gasith & Resh 1999). Fish fauna from these heterogeneous ecosystems must frequently survive under alternating scenarios of too much or too little water with a few intermediate but crucial periods of investment in recruitment and growth (Ferreira *et al.* 2007b). Under these conditions, fishes tend to have short life spans, rapid growth rates, high fecundity and early sexual maturity and spawning, as well as generalist and opportunistic feeding strategies (e.g. Granado-Lorencio 1996; Vila-Gispert & Moreno-Amich 2002). Consequently, Mediterranean fish have a wide tolerance to abiotic variability (Ferreira *et al.* 2007b). The poor knowledge of their ecological requirements hampers the development of IBI and in some cases it provokes the existence of contradictory references regarding the categorization of some species (e.g., tolerant/intolerant). The solution of this gap of information is to increase the basic studies of their ecological requirements. Long term studies monitoring both fish assemblages and physicochemical parameters could be invaluable with this regard.

The problem of the reference condition

Another of the great problems to develop metrics and IBIs in the Mediterranean basin is the lack of reference areas to test the metrics. The aim of metrics and IBIs is to assess the ecological status. Ecological status, according to the WFD, "is an expression of the quality of the structure and functioning of aquatic ecosystems associated with surface waters". In order to know the ecological status the current condition has to be compared to natural conditions (structure, composition, function, diversity) in the absence of human disturbance or alteration (reference condition) (Stoddard *et al.* 2006). Chovarec *et al.* (2000) suggest that "reference condition is the state that has existed before the human interferences, or at least without human influences that have altered significantly their natural characteristics". Owen *et al.* (2001) considers that the "reference condition is when physical-chemical, hydromorphologic and biological values corresponding to the area without human alteration". In multimetric biological assessment, reference condition equates with biological integrity, defined as "the condition at sites able to support and maintain a balanced, integrated, and adaptive biological system having the full range of elements and processes expected for a region" (Karr & Chu 1999).

The concept of reference condition is widely known and used. For example the EPA (US Environmental Protection Agency) in United States (Davis & Simon 1995), the "National River Health Program" in Australia (Parsons & Norris 1996), the "River Health Programme" in South Africa (Eekhout *et al.* 1996) and the "Water Frame Directive" in Europe use the concept of reference condition to assess the ecological status and to develop fish metrics and indices.

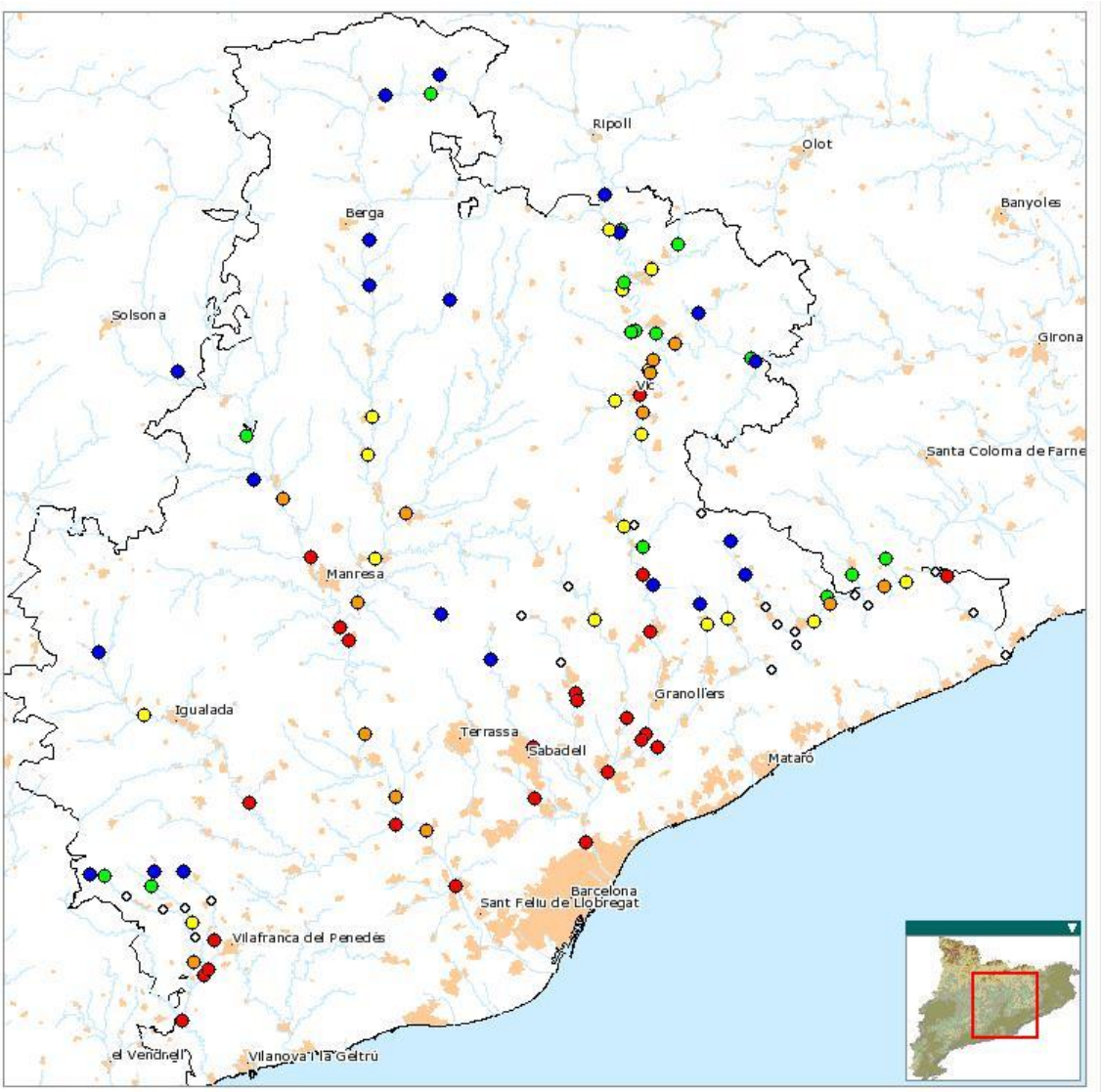
In some regions, biologists can actually find and sample from sites that have not been influenced, or have been influenced only minimally, by humans (Karr & Chu 1999). In other regions, where pristine sites are unavailable, biologists have to use different methods to select the reference conditions (Hughes 1995; Johnson 1993; Reynoldson *et al.* 1997). One of the methods is the "expert criterion", which is easy but requires an exhaustive validation (Owen *et al.* 2001). In other cases, researchers may use predictable models, paleolimnology information or in some cases must rely on historical data, collected when human activity was low, to define reference condition (Reynoldson *et al.* 1997; Karr & Chu 1999; Bennion et al. 2004).

The problem in many regions in the world, and especially in Mediterranean basin, is that pristine sites are unavailable due to an intensive human activity during many centuries (Prat 1993; Bonada 2002). Consequently, in areas as river mouths it can be difficult to know the original biota composition and also the natural flow regime, because reservoirs and water

abstraction have profoundly altered them. The **second article** of this thesis showed that intermediate and lower reaches of some rivers were often dry while they never should be according to a widely used hydrological model. This fact, for example, complicates the interpretation of droughts because very accurate analysis could be necessary to know if the sites found dry usually, for example in the Foix, Besòs and Tordera (Fig.1), are due to human or natural causes. In this **second article** we suggested that the detailed characterization of the natural flow regime should precede the application of biotic indices and that in cases such as these (artificially dried rivers), the lowest possible score for biotic indices or ecological status (but specifying that the river was artifically dry) should be applied. Otherwise, having good scores in other occasions will overestimate the average ecological status of the river. In the highly impacted areas it will be necessary to apply detailed studies and use predictable models, paleolimnological information and historical data to establish the reference conditions (Chovarec *et al.* 2000; Owen *et al.* 2001). As a last resort the "expert criterion" also could be used (Bonada 2002).

One extreme case of the problem of reference conditions is the case of reservoirs. Due to their artificial nature natural reference conditions do not exist for reservoirs. For this reason different authors have adapted the original fish metrics of Karr *et al.* (1986) and suggested to name it the RFAI (Reservoir Fish Assemblage Index) (Jennings *et al.* 1995; McDonough & Hickman, 1999). The WFD mandates the restoration of the good surface water status (near the reference conditions) before 2015 for all surface water bodies. WFD considers reservoirs as artificial water bodies or heavily modified water body, therefore in these cases the aim is obtain the good ecological potential. In the WFD the good ecological potential of one artificial water body is defined as the nearest values to the most similar natural water body.

Figure 1. Map of the Ecostrimed project (http://www.ecostrimed.net) with the results of ecological quality for 2006. The empty circles (○) are the sampling sites "dry or without data".



Research prospects

Until nowadays inland fish in the Mediterranean basin have been barely used as ecological indicators and there are few studies on this topic. Therefore it is necessary and urgent to follow research on this subject. Long term studies monitoring both fish communities and physicochemical parameters provide invaluable information. The WFD mandates to sample fish assemblages every three years in all water bodies. Although these punctual samples could give some management information, in the Mediterranean basin we need more sampling effort to improve the available information of inland fish. With only one sample every three years we cannot obtain the needed information to improve existing indices and tools. More intensive and extensive studies are needed to know important aspects like population

dynamics and their responses to natural and anthropogenic perturbations. Although some countries like the UK or France started over 20 years ago to extensively sample fish populations every year, the first extensive project in Catalan rivers was in 2003 by Sostoa *et al.* when they studied 333 sampling sites to develop IBICAT. In the same year a similar project in the Catalan reservoirs was developed (Carol *et al.* 2006); there are almost no previous available data so the temporal variation and reference situations are largely unknown.

The ecological requirements of native species are also poorly known. Sometimes some species are not categorized for some attribute (e.g. tolerant/intolerant) or there is contradictory information. Consequently basic studies are also needed. On the other hand, exotic species are every year more abundant in the Mediterranean basin, and the study of their impact in native biota and ecosystems is required. All of these aims may be achieved with long term studies of fish assemblage, parallel to physicochemical and hydrological surveys.

Until the 1970s and 1980s, Catalan rivers were heavily pollution due to urban and industrial waste. From the 1990s river pollution has in general decreased thanks to entry in operation of many water treatment plants (Prat & Rieradevall 2006). Now, in the twenty-one century, we have overcome the extreme contamination situation and therefore it is the hour to go on to make a new step. Clean water is not enough; now it is necessary that river ecosystem properties recover to their natural condition. It is necessary that rivers have a natural discharge again with a typical Mediterranean flow regime. Obviously these must be compatible with water available for population and the security for cities near the rivers. River habitats must be restored and fauna and flora must be properly managed. This new challenge is not only the duty of fulfillment of a directive. This new challenge has to go beyond the necessary implication of the public administration and has to imply all the society. As some authors suggest (e.g., Kemptom *et al.* 1995; Ladd & Bowman 1995), it will be crucial that a majority of our societies acquire new attitudes and values about healthy biota and ecosystems, because sustainable use of ecosystems will require fundamental change in the interactions between the economies of humans and nature (Angermeier 2007). Fish may be the best tool to achieve this challenge by facilitating the communication of river ecological health to the society.

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Conclusions

Conclusions

1. The inland fish of the Mediterranean basin, as in other regions of the world, can be good ecological indicators. Although most biological indices were correlated, fish reflected different quality aspects due to their particular features (e.g., higher mobility and longevity). Our results suggest that a physicochemical index (ISQA) routinely used by the Catalan Government should not be generally used because it does not capture disturbances that emerge with many other indices.

2. Brown trout (*Salmo trutta*) in the Tordera basin is proposed to be considered as of uncertain native status, because although genetic information points to an introduction, there is a very old record (by 1845) and further information is needed to clarify its status. This status has profound effects on the results of fish metrics.

3. We propose that in headwater sites (with low fish richness), metrics which penalize for the presence or abundance of introduced fishes (to measure historic biotic integrity) and metrics that profit from their indicator value might be combined. In order to compensate for the low fish richness we recommend to assess metrics based on age or size structure, fish individual state, or other taxa such as amphibians. Indices of biotic integrity (IBI) in headwater sites, or sites with low richness, should not be used unless several and sufficient nonredundant metrics are identified.

4. The streamflow regimes (real and predicted by models) of six Mediterranean basins were studied. A significant decrease of the streamflow and aquifer levels was detected, despite no observed decrease of rainfall precipitation. The number of days that the river is dry has increased and the streamflow regularly observed is well below the naturalized flow predicted by the SAC-SMA model. Meanwhile, the numbers of inhabitants and industries and the quantity of water abstracted has increased in the river basin.

5. During six years the fish assemblage of Tordera river was studied at impacted and less impacted sites to know the effect of these droughts, caused by water abstraction. Of the 30 tested metrics to detect sites impacted by water abstraction, we detected four significant fish metrics: catch per unit effort, number of benthic species, number of intolerant species and proportion of intolerant individuals.

6. The detailed characterization of the natural flow regime (and hence drought events) should precede the application of biotic indices in streams severely affected by water abstraction, and in cases of artificially dry rivers, biotic indices should be given the worst score (instead of the customary missing value that biases the overall ecological assessment).

7. The effects of a prescribed partial drawdown on fish assemblage in autumn 2005 in Sau, a eutrophic reservoir, were studied. The species composition of the purse seine catches varied significantly during the drawdown with higher proportion of bleak (*Alburnus alburnus*) in pelagic water during the days of worst water quality, confirming that bleak is more tolerant than roach (*Rutilus rutilus*) to poor water quality and a potential good indicator of water pollution.

8. The weight–length relationship (i.e. condition) of roach and bleak also varied significantly during the drawdown following the same pattern in both species, losing and recovering their weight (4.99% in roach and 5.96% in bleak) in only 16 days. The close relationship found between water quality and fish condition demonstrates that fish condition can be a good metric of the well being of fish, even for extreme short-term changes.

9. The effects of the large amount of industrial pollutants of Flix reservoir in condition and fecundity of freshwater fishes were assessed. Although all the species studied showed some significant effects of the presence of pollutants, the responses were species-specific and common carp (*Cyprinus carpio*) was the species that showed more fitness-related traits with significant decreases at the impacted area. Common carp is a benthic omnivore that stirs the bottom to feed; therefore, carp is probably more exposed to the contaminants, either by physical proximity or ingestion, and for this reason despite being a species very tolerant to pollution, it shows stronger effects of pollution than other cohabiting fish species.

10. There are some metrics clearly affected in several fish species: DELT anomalies, presence of ectoparasites, eviscerated weight and liver weight. DELT anomalies and ectoparasites were more abundant at the impacted area than in control sites (Riba-roja reservoir). Eviscerated and liver weights had the highest values at the control area. Although the fecundities observed in our study were within the ranges of other studies for the same species, significantly low values at the impacted area for carp and pumpkinseed (*Lepomis gibbosus*) were detected.

Conclusions

(en català)

Conclusions

1. Els peixos continentals mediterranis, igual que el d'altres bioregions del món, poden ser bons indicadors de la qualitat de l'ecosistema. Encara que tots els índex biològics tenen una certa correlació entre ells, els peixos expressen els estressos d'una manera diferent degut a les seves particulars característiques biològiques (e.g., més longetivitat, més capacitat de moviment). Els nostres resultats suggereixen que l'índex fisicoquímic utilitzat de manera rutinària per l'administració catalana (ISQA) és insuficient ja que no captura les degradacions ambiental que detecten molts altres índexs.

2. Es proposa que l'estatus de la truita (*Salmo trutta*) a la conca de la Tordera sigui considerat incert, ja que tot i que la informació genètica indica que seria d'origen introduït, existeix una cita molt antiga a la conca (al voltant de 1845) i per tant serà necessària més informació per aclarir el seu estatus. L'estatus de l'espècie té un efecte molt gran en els resultats de les mètriques.

3. Es proposa que a les parts altes dels rius, amb poques espècies, es combinin mètriques que puntuïn negativament el fet que les espècies siguin introduïdes (per mesurar la integritat biòtica) amb d'altres mètriques que aprofitin el seu caràcter bioindicador. Amb l'objectiu de compensar la baixa riquesa d'espècies proposem incorporar mètriques de la demografia de la població, estat de salut dels peixos i variables d'altre biota aquàtica. Els IBIs a les parts altes de rius, o en llocs amb baixa riquesa, no s'haurien d'utilitzar si no s'obtenen les suficients mètriques per construir l'índex.

4. S'han estudiat els cabals (els *reals* i els *esperats* predits per un model) de sis conques catalanes. S'ha detectat una tendència a la disminució del cabal i del nivell dels aqüífers encara que no s'ha observat una disminució en les precipitacions. Així mateix, s'han detectat uns cabals menors i una freqüència més alta de dies secs que els predits a partir dels models hidrològics. Paral·lelament el nombre d'habitants, d'indústries i la quantitat d'aigua extreta ha augmentat.

5. S'ha estudiat durant sis anys la comunitat de peixos de la Tordera, en zones molt impactades i altres menys impactades per conèixer l'efecte de les sequeres produïdes per l'extracció d'aigua. De les 30 mètriques testades per detectar les zones impactades per l'extracció de l'aigua, se n'han detectat quatre de significatives: captures per unitat d'esforç, nombre d'espècies bentòniques, nombre d'espècies intolerants i proporció d'individus intolerants.

6. Una detallada caracterització del regim hídric natural (i dels períodes de sequera) ha de precedir l'aplicació dels índex biològics en els rius fortament afectats per l'extracció d'aigua, i en els casos de sequeres artificials (provocades per l'extracció d'aigua), els índex biològics haurien de tenir el menor valor (enlloc de no posar valor com s'ha fet fins al moment).

7. Es va estudiar els efectes del buidat parcial de l'embassament de Sau l'any 2005 sobre la comunitat de peixos. La composició d'espècies capturades va variar significativament al llarg del buidat amb un augment de l'alburn (*A. alburnus*) a les aigües pelàgiques durant els dies de pitjor qualitat de l'aigua. Es confirma que l'alburn és més tolerant a la qualitat pobre de l'aigua que la madrilleta vera (*R. rutilus*) i el seu potencial com a bioindicador.

8. La relació pes-longitud (i.e. condició) de l'alburn i la madrilleta vera varen canviar significativament al llarg del buidat seguint la mateixa tendència, perdent i recuperant bona part del seu pes (4.99% a la madrilleta vera i 5.96% a l'alburn) amb només 16 dies. L'estreta relació trobada entre la condició dels peixos i la qualitat de l'aigua demostra que la condició pot ser una bona mètrica, fins i tot per canvis a curt termini.

9. Es van estudiar els efectes de l'acumulació de contaminants de l'embassament de Flix en la condició i fecunditat dels peixos. Encara que totes les especies estudiades mostraven algun impacte significatiu degut a la presència dels contaminants, les respostes van ser diferents per cada espècie i la carpa (*Cyprinus carpio*) va ser la que va mostrar més clarament els impactes amb uns valors menors a l'àrea impactada. La carpa és una espècie bentònica que remou el fons per menjar, per tant, probablement està més exposada als contaminants, per proximitat física i ingestió directa, que les espècies pelàgiques. Per aquesta raó, encara que la carpa sigui una espècie tolerant a la contaminació mostra més clarament els efectes dels contaminants que altres espècies de la zona.

10. Hi ha algunes mètriques clarament afectades a diferents espècies: DELT (deformitats, aletes erosionades, lesions o tumors), presència de paràsits, pes evicerat i pes del fetge. El percentatge de DELT i la presència de paràsits externs era major en la zona impactada que no en els punts de control (Riba-roja). Els valors més alts de pes evicerat i pes del fetge estaven als punts de control. Tot i que les fecunditats observades van ser semblants a d'altres estudis, es van detectar valors baixos d'ous madurs per carpa i peix sol (*Lepomis gibbosus*) a l'àrea impactada.