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Decreases in Condition and Fecundity of Freshwater Fishes in a Highly Polluted Reservoir

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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, 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 a century. This exceptional amount of pollutants provides a good opportunity (and need) to analyse their effects on fish populations under natural conditions, which is 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 (reference sites) and also to downstream sites. Deformity, eroded fin, lesion and tumour (DELT) 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 weights, adjusted for fish size with analysis of covariance) and reproductive traits (gonadal weight and number of mature eggs, adjusted for fish size) 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 tolerant to pollution.

Keywords Heavy metals · Organochlorides · Common carp · *Cyprinus carpio* · Flix reservoir

1 Introduction

Aquatic ecosystems have been profoundly altered by human activities, including dam construction, alteration of riparian habitat, water abstraction and pollution with urban, agricultural and industrial wastes. 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 (e.g. Farkas et al. 2000; Durrieu et al. 2005). Most heavy metals are toxic to fish at low concentrations and harm them in many ways, both physically and physiologically (Hoole et al. 2001). Chemical pollutants act as biological stressors to fish, and it is widely known that long-term exposure to environmental stressors causes detrimental effects on important features such as metabolism, growth, reproduction and, ultimately, the condition and survival of fish (Barton et al. 2002; Benejam et al. 2008). The exposure to environmental stressors such as contaminants can also predispose fish to infectious diseases because the immune system is a

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sensitive target to environmental pollutants (Rice 2001).

Ecotoxicologists generally assess the impact of pollutants on freshwater fish using standard toxicity tests in the laboratory. Although this type of controlled laboratory studies provides invaluable preliminary information on the effects of environmental stressors, further studies under natural conditions are needed to increase ecological realism due to a number of reasons, such bioavailability of toxicants and interaction among several pollutants (Cairns 1983; Adams and Greeley 2000; de Zwart and Posthuma 2005). Although the body burden of many pollutants has been described for a number of feral fish populations (e.g. Has-Schön et al. 2006; Mendil and Uluozlu 2007), the ultimate toxicological effects on individual fitness (e.g. condition and reproduction) in the wild have been barely investigated, almost exclusively with regard to sexual alterations (e.g. Jobling et al. 2002; Toft et al. 2004).

Flix reservoir (Ebro River, Spain), is a highly polluted reservoir that provides a unique opportunity to assess the effects of contaminants on natural freshwater fish populations. The Ebro is the river in the Iberian Peninsula with the highest waterflow (annual mean, 255–424 m^3/s; drainage area, 85,820 km^2) and discharges into the Mediterranean Sea originating a delta of more than 30,000 ha (Fig. 1). An organochlorine industry has been operating since the beginning of the twentieth century at the shore of Flix reservoir, dumping wastes in the river. This resulted in deposition of 200,000–360,000 tons of industrial wastes in the riverbed, nowadays occupying an area of 700 m of length and 60 m of width (Grimalt et al. 2003; Carrasco et al. 2008; Quirós et al. 2008). This mixture of industrial pollutants is composed of high concentrations of heavy metals (mainly mercury), organochlorides (hexachlorobenzene, pentachlorobenzene, DDTs, polychlorobiphenyls, polychloronaphthalenes and polychlorostyrenes) and radioactive ^{210}Pb. For example, these sediments have average concentrations of 49 μg/g of mercury, 19 μg/g of hexachlorobenzene and 39 μg/g of total polychlorophenyls (PCBs), and concentrations of DDT, polychlorostyrene and polychloronaphthalene reach 1,300, 360 and 1,100 ng/g, respectively (see Grimalt et al. 2003 for further details). Furthermore, several studies have found high concentrations of PCBs, DDTs and heavy metals in tissues of Flix reservoir fish (Lavado et al. 2004; Carrasco et al. 2008; Eljarrat et al. 2008; Navarro et al. 2009). Pollutants originating from this site are also known to be transported downstream, mainly mobilised during floods (Grimalt et al. unpublished data), ending up in the Ebro delta, 90 km away from the reservoir (Grimalt et al. 1988; Amaral et al. 1996; Quirós et al. 2008).

The objectives of this study are (1) to assess the condition and reproductive traits of the freshwater fishes that inhabit this highly polluted reservoir by comparing them with upstream and downstream populations and (2) to compare the effects of the pollutants in several exposed fish species and different life-history traits. A reservoir (Riba-roja) only 10 km upstream of Flix dam, very similar in

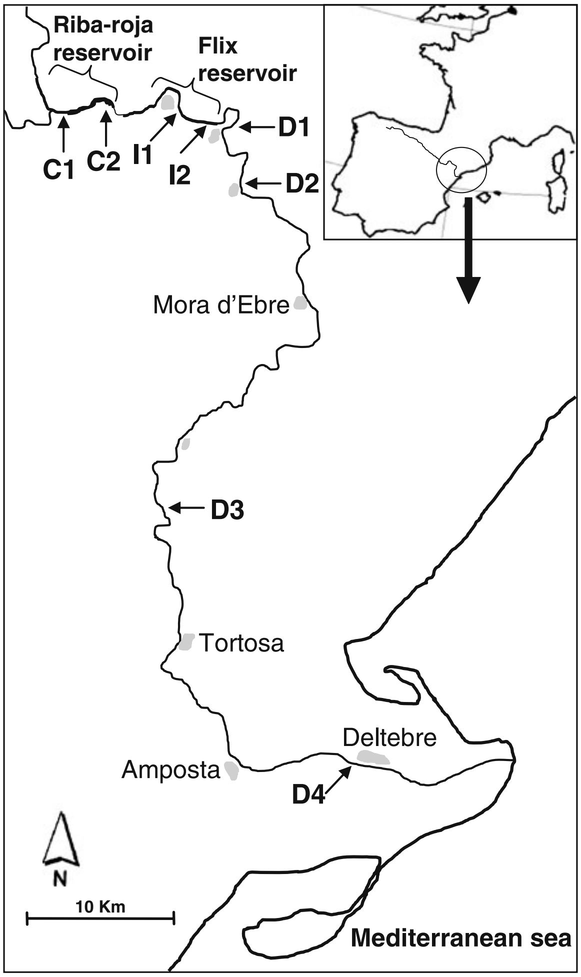

Fig. 1 Map of the study area with the eight sampling sites along the Ebro River: two controls at the Riba-roja reservoir (*C1* and *C2*), two sites at the polluted area of Flix reservoir (*I1* and *I2*) and four downstream (*D1–D4*). The main towns (*grey areas*) are also shown

ecological features but not affected by the polluted wastes, provides an invaluable reference site to examine the effects of contaminants on fish populations in natural conditions.

2 Methods

2.1 Study Area

The condition and reproductive traits of freshwater fish were studied from eight sampling sites along the Ebro River (Fig. 1). The industrial pollutants are situated at Flix reservoir (site I2 in Fig. 1). We sampled two sites in Riba-roja reservoir to serve as control or reference sites (C1 and C2), two sites in Flix reservoir as the most impacted area (I1 and I2) and four sites downstream (D1, D2, D3 and D4). All sampling sites were at least 3 km apart from each other and had similar physical and limnological features (e.g. pH range of 7.8–8.8 and conductivity of 1,231–1,491 μS/cm). In particular, sampling sites C2 and D1 were, respectively, only 9 and 3 km apart from the polluted area (I2) and were separated by the two reservoir dams (60 and 26 m high, respectively), so their environmental features were very similar except for the pollution. The littoral area of all these sites presented a poor diversity of substratum types characterised by smooth, weakly sloped banks, with abundant vegetation especially in summer (mainly *Chara* sp. and *Potamogeton* spp.). The fish assemblage is dominated by cyprinids not native to the Iberian Peninsula but widespread and abundant throughout Europe, such as common carp (*Cyprinus carpio*), rudd (*Scardinus erythrophthalmus*), bleak (*Alburnus alburnus*), roach (*Rutilus rutilus*) and also some other exotic species such as pumpkinseed sunfish (*Lepomis gibbosus*) and European catfish (*Silurus glanis*; see Carol et al. 2006 for further details on Riba-roja and Flix reservoirs).

2.2 Field and Laboratory Methods

Fish were sampled in May, July, October 2006 and January 2007 during daylight by boat electrofishing in the littoral zone at all sampling sites, except sites D3 and D4 that were only sampled in October 2006. The electrofishing boat was equipped with a 5.0-GPP Smith-Root Inc. engine (Vancouver, WA, USA), providing up to 1,000 V and 16 A. Captured fish were preserved on ice and transported to the laboratory, where fork length and total, eviscerated, liver and gonadal weights were measured to the nearest millimetre and milligramme, respectively. Fish were also examined for DELT anomalies (external deformities, eroded fins, lesions and tumours; Sanders et al. 1999) and ectoparasites (only copepod crustaceans and leeches were found).

Fish fecundity is usually estimated as the number of mature oocytes present in the ovary immediately before spawning. Fecundity of common carp, rudd and pumpkinseed was estimated from gonadal samples within their reproductive period (May), using gravimetric subsampling (Bagenal and Braum 1999). The oocytes were separated by submerging samples of ovaries into Gilson's solution. The eggs of two subsamples (about 1 g each) were filtered through 15 sieves with diameters ranging from 1.6 to 0.1 mm, and matured eggs were counted in each sieve to estimate its diameter. Fecundity was obtained by multiplying the total weight of ovaries by the average number of mature eggs per gramme ovary. Navarro et al. (2009) report high mercury concentrations and response of biochemical biomarkers for a subsample of the same carp that we studied.

2.3 Statistical Analyses

The percentage of individuals with presence of ectoparasites and DELT were compared among populations with a *G* test (likelihood ratio statistic) of independence (Sokal and Rohlf 1995). Analysis of covariance (ANCOVA) was used to compare the condition (eviscerated weight–length relationship) and the liver weight–length relationship among sites along the Ebro River taking into account fish size (covariate). ANCOVA has several advantages over condition factors (e.g. weight length^{-3}) and similar indices (see review in García-Berthou and Moreno-Amich 1993). The adjusted or predicted means in ANCOVA are the means of values of the response variable adjusted for effects of covariates, typically length (García-Berthou and Moreno-Amich 1993); these adjusted means thus allow comparing groups or treatments for the response variable, after accounting for covariates such as fish size. Male and female pumpkinseed and rudd were analysed separately for eviscerated weight to account for significant sex-related effects for these species. The liver weight was only measured for common carp and European catfish, and the sex effects were not significant for these species, so males and females were analysed together. ANCOVA (fork length as covariate) was also applied to analyse the variation of gonadal weight, number of mature eggs and the average diameter of mature eggs among sampling sites. Because the number of mature eggs was correlated with gonadal weight independently of fork length, gonadal weight was also used as a covariate to analyse the number of mature eggs. All factors were considered as of fixed effects. We also used partial η^2 (partial eta squared) as a measure of effect size (i.e. importance of factors). Similarly to r^2, partial η^2 is the proportion of variation explained for a certain effect (effect SS/(effect SS + error SS)). Partial η^2 has an advantage over η^2 (effect SS/total SS) in that it does not depend on the number of source variation in the ANOVA design used because it does not use the total sum of squares (SS) as the denominator (Tabachnick and Fidell 2001). Because of the large number of species and variables involved, we only report inferential and descriptive statistics where significant ($P<0.05$) site effects or interactions were found.

All the quantitative variables (except the number of mature eggs and the average diameter of mature eggs of carp) were log-transformed for the analyses because linearity and homoscedasticity were clearly improved. All data analyses were performed with SPSS 15.

3 Results

3.1 Common Carp

Common carp (*C. carpio*) was the species with highest frequency of ectoparasites (8.7% of the 772 carp individuals) and DELT anomalies (23.2%). The percentage of carp individuals with ectoparasites was significantly different among the eight sampling sites ($G=43.9$, $df=7$, $P<0.0005$), with Flix reservoir (I1) having the highest prevalence (Fig. 2). The percent-

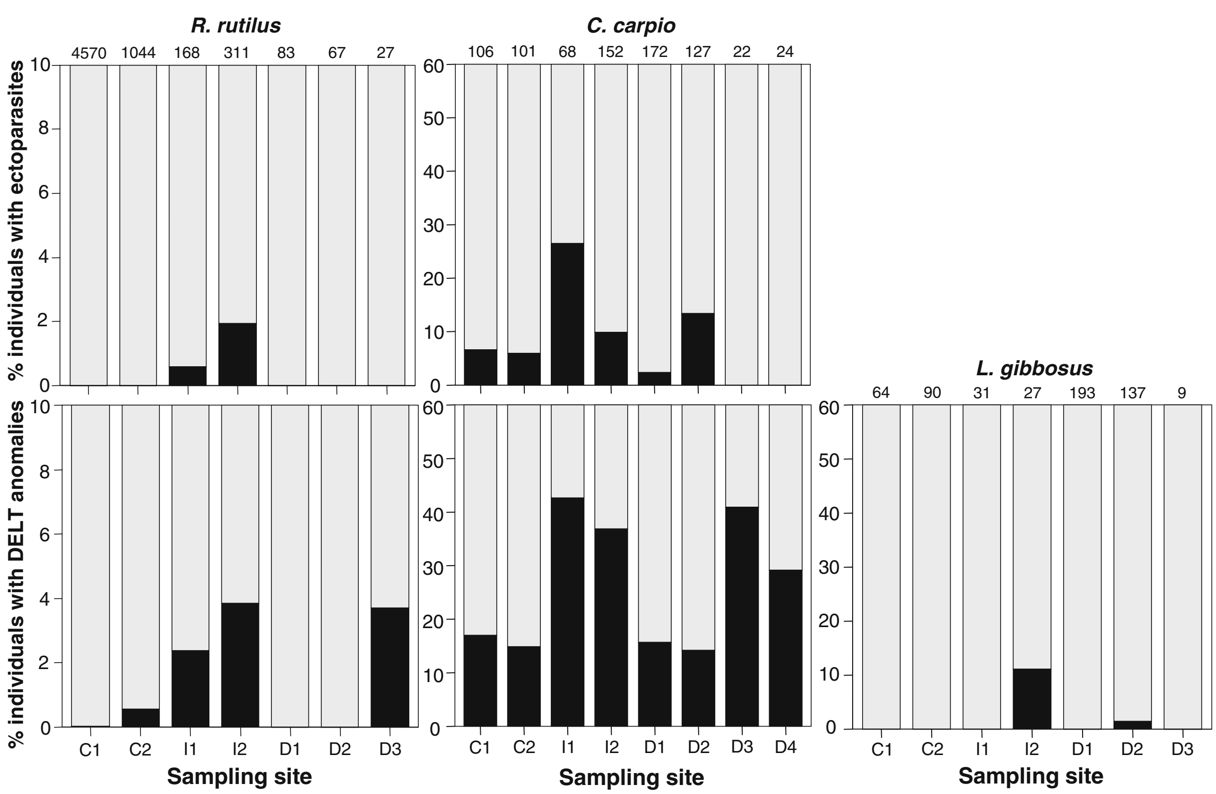

Fig. 2 Proportion of roach (*R. rutilus*), common carp (*C. carpio*) and pumpkinseed sunfish (*L. gibbosus*) individuals with DELT anomalies (*bottom*) and ectoparasites (*top*) along the Ebro River: presence (*black bar*) and absence (*grey bar*). The figures above the *bars* are total number of fish. See Fig. 1 for sites codes

age of carp individuals with DELT anomalies also varied significantly among sampling sites (G=49.9, df=7, P<0.0005). The impacted area (I1 and I2) and the two farthest downstream sites (D3, D4) had the highest percentages of individuals with DELT anomalies (Fig. 2).

The eviscerated and liver weights of common carp (pooling males and females) significantly varied among sampling sites (Table 1). In spring, the eviscerated weight of carp (ANCOVA-adjusted for fish length) clearly decreased from the control sites (C1 and C2) to Flix reservoir (I1, I2) (Fig. 3). In summer, carp

Table 1 ANCOVAs of the eviscerated (EW) and liver (LW) weights of different species with sampling site and season (factors) and fork length (covariate)

	SS	*df*	*P* value	η^2	SS	*df*	*P* value	η^2
	EW				LW			
C. carpio								
Fork length	11.2039	1	<0.0005	0.94	0.7570	1	<0.0005	0.38
Site	0.0921	7	<0.0005	0.11	0.2930	6	0.006	0.19
Season	0.0109	2	0.120	0.01	0.0099	1	0.414	0.01
Site × season	0.1110	5	<0.0005	0.13	0.0933	3	0.106	0.07
Error	0.7523	294			1.2281	83		
S. glanis								
Fork length	11.5578	1	<0.0005	0.99	1.7234	1	<0.0005	0.71
Site	0.0220	6	0.500	0.11	0.7806	6	0.002	0.52
Season	0.0101	2	0.296	0.06	0.0069	1	0.618	0.01
Site × season	0.0019	2	0.785	0.01	0.0015	1	0.812	0
Error	0.1698	42			0.7113	26		
	EW male				EW female			
L. gibbosus								
Fork length	10.7646	1	<0.0005	0.99	7.2654	1	<0.0005	0.98
Site	0.0162	5	0.001	0.11	0.0083	5	0.077	0.06
Season	0.0007	1	0.315	0.01	0.0001	1	0.881	0.00
Site × season	0.0045	5	0.318	0.04	0.0251	5	<0.0005	0.17
Error	0.1258	163			0.1204	147		
S. erythrophthalmus								
Fork length	7.9977	1	<0.0005	0.99	5.1759	1	<0.0005	0.97
Site	0.0067	3	0.102	0.10	0.0084	4	0.747	0.05
Season	0.0007	1	0.418	0.01	0.0285	1	0.014	0.14
Error	0.0580	56			0.1694	39		
	EW							
R. rutilus								
Fork length	85.5080	1	<0.0005	0.98				
Site	0.2293	5	<0.0005	0.15				
Season	0.0437	1	<0.0005	0.03				
Site × season	0.1816	5	<0.0005	0.12				
Error	1.3254	510						
A. alburnus								
Fork length	2.1881	1	<0.0005	0.95				
Site	0.0127	3	0.015	0.10				
Season	0.0001	1	0.939	0.00				
Site × season	0.0109	2	0.011	0.08				
Error	0.1180	101						

Partial η^2 (partial eta squared), a measure of the importance of factors, are also given (see text for interpretation). All quantitative variables were $\log_{10}$ transformed condition was worse in the site closest to the pollutants (I2) than in the other Flix reservoir site (I1). Liver weight showed minima at the impacted area (I2) and near the river mouth (D3), with values well below those of the control sites. The gonadal weight of male carp was also significantly different among sampling sites (Table 2), decreasing from the control sites to the impacted area particularly in spring (Fig. 3). The number of mature eggs was also significantly different among sampling sites, using ANCOVA with two covariates (ANCOVA with fork length and gonadal weight as covariates, Table 3), decreasing at the impacted site (I2) and recovering downstream (Fig. 3). The mean diameter of mature eggs varied significantly among sites after accounting (as covariates) for fork length and number of mature eggs (Table 4); the lowest values were at the impacted area and downstream (Fig. 3). The fact that the number of eggs is significant as a covariate indicates a trade-off between egg size and fecundity. At the control sites, there was a normal situation of pre-spawning period with low numbers of large mature eggs (C1) or high number of mature eggs with intermediate diameter (C2). In contrast, at the impacted area and downstream, fecundities were low (I2 and D1) or high but with very small eggs (I1 and D2).

3.2 Other Fish Species

Among the other fish species, significant variation among sites was only found for ectoparasite (G=37.7, df=6, P<0.0005) and DELT (G=74.3, df=6, P<

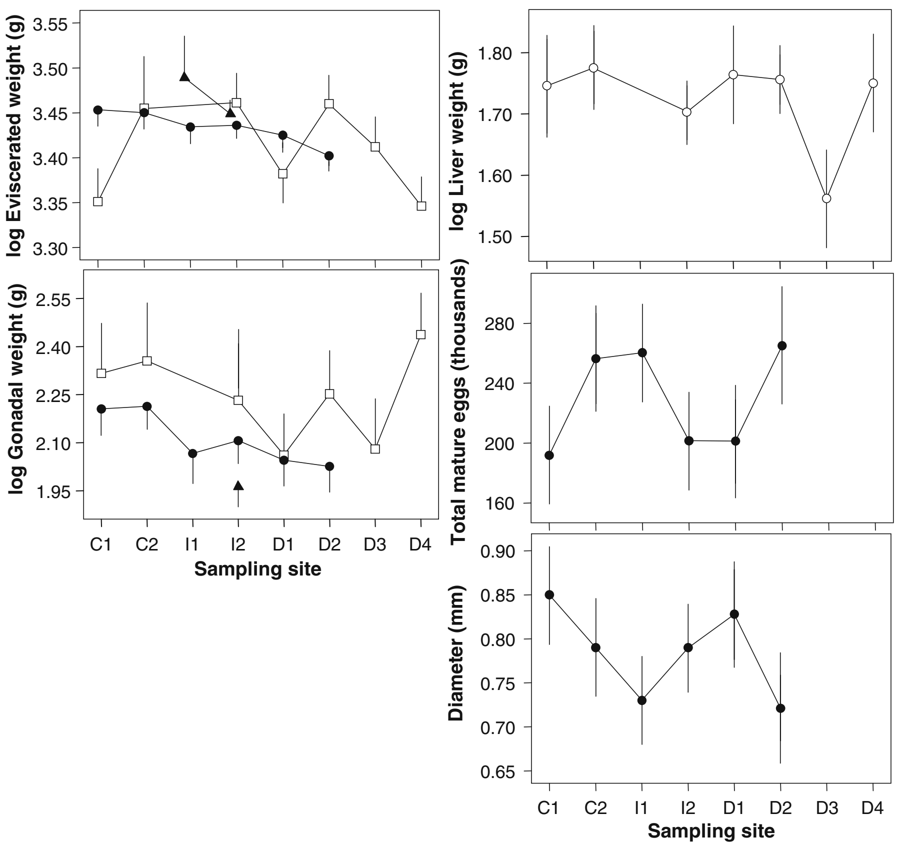

Fig. 3 Size-adjusted means (ANCOVA with fish length as covariate and population and season as factors) of eviscerated, liver and gonadal weights of common carp at the different sampling sites (see Fig. 1 for site codes). The 95% confidence interval for the adjusted means is also shown; only the *upper* or *lower bar* is shown in some cases to improve readability. The omitted means correspond to site and season combinations in which the species was not captured. The total number of mature eggs was adjusted for fish length and gonadal weight (covariates). The diameter of mature eggs with fish length and number of mature eggs as covariates is also shown. Spring (*filled circle*), summer (*filled triangle*), autumn (*empty square*) and, when season was not significant, pooled data (*empty circle*)

0.0005) prevalences in roach (*R. rutilus*) and for DELT prevalence (G=17.3, df=6, P=0.008) in pumpkinseed (*L. gibbosus*); in all cases, the highest prevalence was at the impacted area and particularly the site closest to the wastes (I2) (Fig. 2). The eviscerated weights of roach, bleak (*A. alburnus*) and male pumpkinseeds significantly varied among sampling sites (Table 1). The eviscerated weights of pumpkinseed and bleak dropped from Riba-roja (C2) to Flix (I2) in contrast to roach (Fig. 3). The liver weight of European catfish (*S. glanis*) had the highest values at the control sites and decreased at the impacted area and downstream (Table 2 and Fig. 4). The gonadal weights of male and female pumpkinseeds increased along the river but less so at the impacted area (Table 2 and Fig. 4). The number of mature eggs of pumpkinseed was marginally significant (Table 3) and was minimal at the site closest to the wastes (I2) and just downstream (Fig. 4). The variation of egg diameter among sites was not significant (Table 4).

4 Discussion

4.1 Comparison of Life History Traits

The prevalence of ectoparasites and DELT anomalies was significantly different among sampling sites and

Table 2 ANCOVAs of the gonadal weight (GW) of five fish species with sampling site and season (factors) and fork length (covariate)

	GW male				GW female			
	SS	*df*	*P* value	η^2	SS	*df*	*P* value	η^2
C. carpio								
Fork length	1.6475	1	<0.0005	0.33	2.6549	1	<0.0005	0.19
Site	0.8333	7	<0.0005	0.20	0.4501	7	0.625	0.04
Season	0.3302	2	0.002	0.09	1.5806	2	<0.0005	0.12
Site × season	0.0917	4	0.453	0.03	0.1343	3	0.665	0.01
Error	3.4118	137			11.3978	134		
S. glanis								
Fork length	0.5885	1	0.081	0.25	3.0619	1	0.001	0.66
Site	0.9224	4	0.283	0.34	0.1838	2	0.551	0.10
Season	0.0083	2	0.974	0.00	0.2680	2	0.428	0.14
Site × season	0.0023	1	0.905	0.00	0.0013	1	0.926	0.00
Error	1.7529	11			1.6058	11		
L. gibbosus								
Fork length	0.3136	1	<0.0005	0.55	6.0112	1	<0.0005	0.76
Site	0.0218	5	0.023	0.08	0.1693	5	0.030	0.08
Season	0.0151	1	0.003	0.05	0.0001	1	0.977	0.00
Site × season	0.0042	5	0.755	0.02	0.0242	5	0.871	0.01
Error	0.2617	161			1.9096	144		
S. erythrophthalmus								
Fork length	4.4588	1	<0.0005	0.86	3.8122	1	<0.0005	0.70
Site	0.0431	3	0.356	0.06	0.0293	4	0.951	0.02
Season	0.3451	1	<0.0005	0.32	0.1925	1	0.039	0.10
Error	0.7177	55			1.6503	39		
R. rutilus								
Fork length	1.0660	1	<0.0005	0.75	0.8597	1	<0.0005	0.81
Site	0.0142	4	0.482	0.04	0.0123	5	0.613	0.06
Season	0.0000	1	0.921	0.00	0.0078	1	0.138	0.04
Site × season	–				0.0018	1	0.471	0.01
Error	0.3571	88			0.2029	59		

All quantitative variables were $\log_{10}$ transformed highest at the impacted area (Flix reservoir, sites I1 and I2), namely for common carp and roach. The prevalence of DELT anomalies was already included as one of the 12 metrics of the original index of biotic integrity (Karr et al. 1986) and has become an increasingly accepted indicator of water quality and fish health (Simon 1999 and references therein). There are many studies that show low percentages of DELT anomalies at more pristine sites and high percentages at sites affected by industrial and sewage pollution (e.g. Fournie et al. 1996; Sanders et al. 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. The presence of these parasites on the skin surface can affect the epidermal cells to proliferate, resulting in cell sloughing and scale loss (Hoole et al. 2001). Lesions and haemorrhaging at sites of attachment typically develop due to secondary infections from opportunist fungi and bacteria. Moreover, ectoparasites can be a vector of fish diseases (Prenter et al. 2004). Therefore, our results support that the frequency of DELT anomalies and ectoparasites are good indicators of poor water quality and suggest that the enormous amount of polluted wastes at Flix reservoir seem to increase these prevalences. The facts that these prevalences increased from the neighbouring control sites to the impacted sites and soon after decreased at the first downstream site (D1) and that several species behaved similarly support this interpretation.

Table 3 ANCOVAs of the number of mature eggs of three fish species with sampling site (factor) and fork length and gonadal weight (covariates)

	Number of mature eggs			
	SS	*df*	*P* value	η^2
C. carpio				
Fork length	8.4×10^{10}	1	0.026	0.06
Site	1.7×10^{11}	5	0.073	0.12
Fork length × site	1.7×10^{11}	5	0.078	0.12
Error	1.3×10^{12}	79		
L. gibbosus				
Fork length	5.8493	1	<0.0005	0.72
Site	0.2006	5	0.410	0.08
Error	2.3038	59		
S. erythrophthalmus				
Fork length	7.2863	1	<0.0005	0.79
Site	0.2704	4	0.541	0.09
Error	1.6943	32		
C. carpio				
Fork length	6.4×10^{11}	1	<0.0005	0.15
Gonadal weight	1.1×10^{11}	1	<0.0005	0.74
Site	8.4×10^{11}	5	0.004	0.18
Error	3.7×10^{11}	83		
L. gibbosus				
Fork length	0.0285	1	0.229	0.02
Gonadal weight	1.1835	1	<0.0005	0.51
Site	0.1624	5	0.153	0.13
Error	1.1202	58		
S. erythrophthalmus				
Fork length	0.0157	1	0.474	0.02
Gonadal weight	1.0364	1	<0.0005	0.53
Site	0.0321	4	0.896	0.03
Error	0.9283	31		

Quantitative variables were $\log_{10}$ transformed only for pumpkinseed and rudd (because improved linearity). Interactions were removed from the model if they were not significant ($P>0.10$)

In our study, we also evaluated the effects at Flix reservoir on different life-history traits such as eviscerated, liver and gonadal weights and fecundity. The eviscerated and liver weights of common carp (adjusted for fish length with ANCOVA) significantly varied among sampling sites, and all had minima at the impacted area or downstream of this reservoir. Navarro et al. (2009) have recently showed that mercury concentration and response of biochemical biomarkers in a subsample of the same carp individuals that we studied were actually higher downstream of Flix reservoir because of historical transport of pollutants. The eviscerated weight of roach, bleak and male pumpkinseed also varied significantly among sampling sites and, in general, decreased markedly from the control sites to the neighbouring impacted site. The condition of fish is often measured as the total, eviscerated or liver weights after accounting for the correlation with length (Barton et al. 2002; Benejam et al. 2008). Similar results of lower condition (measured as size-adjusted eviscerated and total weights) in ecosystems with poor water quality have been reported for a number of species, including roach and bleak (Laflamme et al. 2000; Benejam et al. 2008). On the other side, the liver serves as a major storage site for glycogen, thus providing an indication of the nutritional state of fish (Adams and Greeley 2000). Environmental

Table 4 ANCOVAs of the average diameter of mature eggs of three fish species with sampling site (factor) and fork length (covariate)

	Model 1				Model 2			
	SS	*df*	*P* value	η^2	SS	*df*	*P* value	η^2
C. carpio								
Fork length	0.0724	1	0.059	0.04	0.2670	1	<0.0005	0.22
Number of mature eggs	–				0.7403	1	<0.0005	0.44
Site	0.1894	5	0.101	0.10	0.1784	5	0.011	0.16
Error	1.6666	84			0.9263	83		
L. gibbosus								
Fork length	0.0002	1	0.345	0.02	0.0022	1	0.003	0.146
Number of mature eggs	–				0.0043	1	<0.0005	0.251
Site	0.0001	5	0.990	0.01	0.0003	5	0.898	0.027
Error	0.0172	59			0.0129	58		
S. erythrophthalmus								
Fork length	0.0019	1	0.005	0.22	0.0003	1	0.157	0.064
Number of mature eggs	–				0.0019	1	0.002	0.274
Site	0.0019	4	0.085	0.22	0.0013	4	0.125	0.202
Error	0.0069	32			0.0050	31		

Model 2 also includes the number of mature eggs as a second covariate (see text for interpretation). Quantitative variables were $\log_{10}$ transformed only for pumpkinseed and rudd (because linearity was improved)

stress may affect liver size through energy consumption, supplied by glycogen and fat stores from the liver, resulting in decreases of liver size (Barton et al. 2002). Although some studies have shown increases of liver size when fish have been exposed to certain types of contaminants, particularly petroleum hydrocarbons and organic chemicals (Chuiko et al. 2007; Yeom et al. 2007), other studies have found smaller liver sizes in areas polluted with heavy metals and PCBs (Hinck et al. 2007; Roussel et al. 2007) in agreement with our results. Raldúa et al. (2007) reported more prevalence of liver pathologies in fish at sites with higher whole-body mercury concentrations. Our results support that the morphometric measurement of fish condition is a simple cost-effective measure of its well being, particularly because condition generally affects growth, reproduction and survival (Adams 1999; Marshall and Frank 1999).

Although the fecundities observed in our study were within the ranges of other studies for the same species (Crivelli 1981; Vila-Gispert and Moreno-Amich 2000), we detected significantly low values at the impacted area for carp and pumpkinseed. Furthermore, the results for common carp indicate a trade-off along the sampling sites between the number of mature eggs and their diameter, with lower fecundities and smaller eggs at the impacted area and downstream. Egg trade-offs along environmental gradients are frequent in fish (e.g. Power et al. 2005). These low fecundities at the impacted area for carp and pumpkinseed and the trade-off for common carp are consistent with documented effects of contaminants on fecundity because the pollutants may affect different levels of fish reproduction. For example, gonadotropins are hormones that regulate fish reproduction (e.g. gonadal development and the production of reproductive steroids), and their levels have been shown to be affected by in vivo exposure to metals and pesticides (Ma et al. 1995; Bieniarz et al. 1997). Alterations in gonadal weight and fecundity have been also demonstrated in field studies with exposure to aromatic hydrocarbons, PCBs, and other chlorinated compounds (Adams et al. 1992; Johnson et al. 1997).

4.2 Species-Specific Responses

Although all the species studied showed some significant effects of the presence of pollutants, the responses were species-specific. Common carp was the species that showed more fitness-related traits with effects of the impacted area: Seven out of eight variables were significant, including higher prevalences of ectoparasites and DELT anomalies. In an extensive project on freshwater fish assemblages in Ohio, the prevalence of DELT anomalies also varied among species and was highest for common carp (Sanders et al. 1999). This

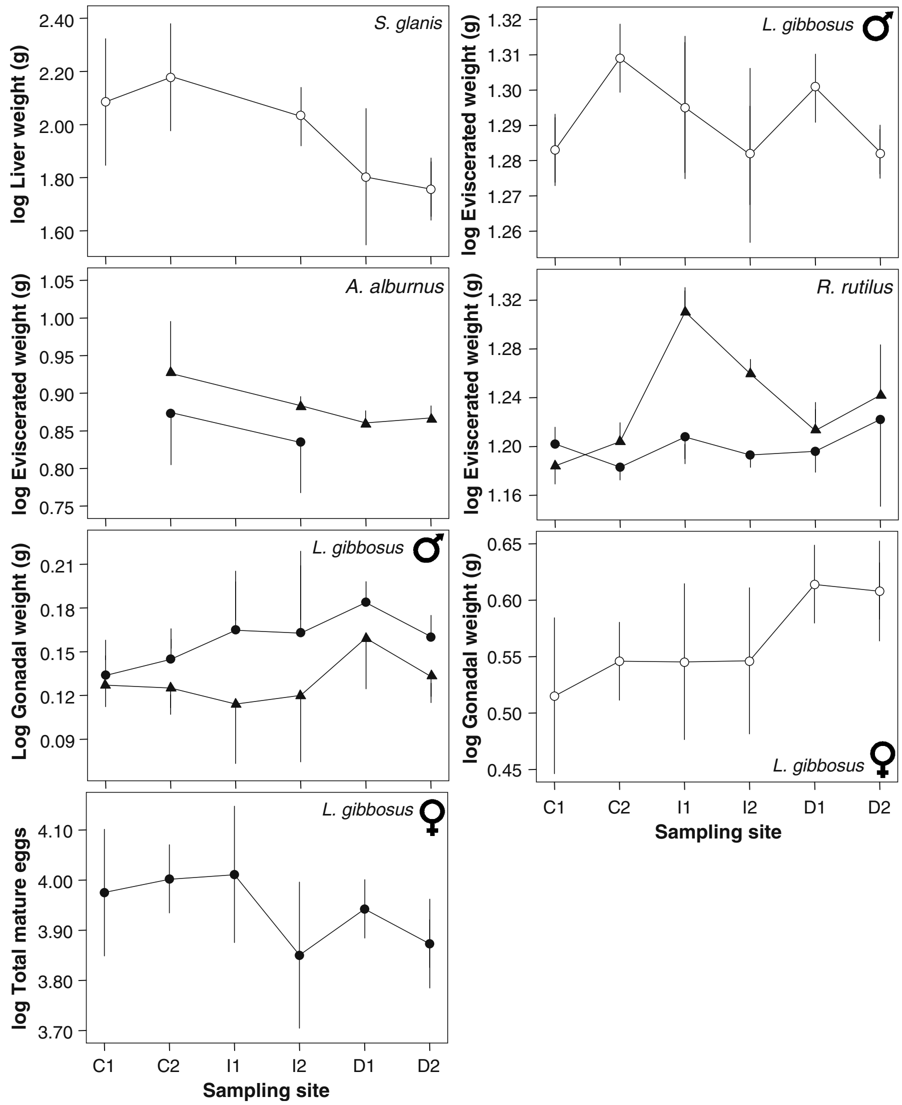

Fig. 4 Size-adjusted means (ANCOVA with fish length as covariate and population and season as factors) of eviscerated, liver and gonadal weights of different species. The 95% confidence interval for the adjusted means is also shown; only the *upper* or *lower bar* is shown in some cases to improve readability. The total number of mature eggs of pumpkinseed was adjusted for fish length and gonadal weight (covariates). Spring (*filled circle*), summer (filled triangle) and, when season was not significant, pooled data (*empty circle*)

might seem paradoxical because common carp is a species that has been introduced worldwide and is extremely tolerant to poor water quality and degraded habitat and can inhabit polluted sites that many other species do not tolerate (Carol et al. 2006). However, the paradox might be explained by the habits and ecology of this species. Common carp is a benthic omnivore that stirs the bottom to feed (García-Berthou 2001) in contrast to other fishes in the Ebro River, such as roach, rudd, bleak and pumpkinseed, which have a more pelagic or littoral habitat and feed at the water column or on littoral invertebrates (e.g. García-Berthou and Moreno-Amich 2000 and references therein). Similarly, catfish in Flix reservoir rests at shallow reed beds during the day (Carol et al. 2007) and mostly preys on red swamp (*Procambarus clarkii*; presumably largely littoral; Carol et al. 2009). Therefore, carp is probably more exposed to the contaminants, either by physical proximity or by ingestion, and despite being very tolerant to pollution, thus shows stronger effects of pollution than other cohabiting fish species.

5 Conclusion

Morphological anomalies and ectoparasites were significantly more prevalent at the impacted area (Flix reservoir) for several fish species (common carp, roach and pumpkinseed). We also detected a significant negative decrease in condition (eviscerated and liver weights, adjusted for fish size with ANCOVA) and reproductive traits (gonadal weight and number of mature eggs, adjusted for fish size) at the polluted area for several fish species. Compared with upstream control sites, low values of fitness-related traits were also observed far downstream of the polluted reservoir, suggesting physical or biological transport of the pollutants. The responses to the pollutants were species-specific, and common carp (*C. carpio*) was the species with the clearest effects on fitness-related traits at the impacted area, despite also being among the most tolerant to pollution.

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