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Evaluation of PCBs and DDTs in endemic Iberian barbel *Barbus bocagei* (Steindachner, 1864) populations

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HIGHLIGHTS

- PCB and DDT concentrations were detected in endemic Iberian barbel from Jarama River, in Spain, at ng/g concentrations.
- High PCB and DDT concentrations found in barbel from Jarama River are likely contributing to a reduction of barbel fitness.
- A higher incidence of abnormalities and ectoparasites was found in individuals exhibiting the highest PCB and DDT levels.
- The ratio of p,p'-DDE/DDT lower than 1 suggests barbel individuals from HI have been more recently exposed to DDT.

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ABSTRACT

PCB and DDT levels were evaluated in populations of endemic Iberian barbel (*Barbus bocagei*) in the Jarama River in Spain via a pollution gradient from well-preserved areas upstream to contaminated downstream areas. Age structure, abundance, recruitment and levels of morphological abnormalities and ectoparasites were assessed. Upstream to downstream PCB concentrations ranged from 3.4 to 101.4 ng/g (ww) and from 0.9 to 19.6 ng/g ww for DDTs. The PCB pattern was dominated by the PCB 153, 138 and 180 congeners, and the less chlorinated ones had a relatively high contribution upstream.

Barbels exposed to low PCB and DDT levels had a well-balanced population with a predominant cohort of young fish, indicating good recruitment. The most contaminated sites displayed a disrupted age distribution, where the proportion of young fish was clearly under-represented. Recruitment and total density of barbel populations decreased downstream where the highest PCB and DDT levels were found. In addition, a higher incidence of abnormalities and ectoparasites was observed at these sites. High concentrations of PCBs and DDTs most likely contribute to the reduction of Iberian barbel reproductive performance in the most contaminated sites, as shown by the disrupted age-distribution found in our study.

1. Introduction

The presence of persistent organic pollutants (POPs) in freshwater ecosystems leads to continuous exposure of fish to sublethal levels that could produce various adverse physiological effects, especially in reproduction (Viganó et al., 2000, 2001; Rogers-Gray et al., 2001; Barnthouse et al., 2003). Freshwater fish are useful indicators of environmental degradation because they cover a range of trophic levels, allowing for a comprehensive view of the aquatic conditions (Lydy et al., 2000). In addition, these fish are sensitive to a variety of anthropogenic impacts (Pont et al., 2006), which can affect them directly or through other components of the aquatic ecosystem (Fausch et al., 1990).

Depending on their trophic position, fish are vulnerable to bioaccumulated lipophilic organochlorine contaminants (OCs), which has been well documented worldwide (Viganó et al., 2000; Orrego et al., 2005). In addition, several studies have shown the direct relationship between environmental stress caused by contamination and the increase in abnormalities (Lemly, 2002) and parasites in fishes (Landsberg et al., 1998; Schwaiger, 2001; Almeida et al., 2008) because development in poor environmental conditions reduces the efficiency of the immune system (Valtonen et al., 2003; Almodóvar et al., 2004). Therefore, a variety of xenobiotics can induce diverse biological responses in fish, affecting the organisms from the biochemical level to the population-community level (Hugla et al., 1995; Porter and Janz, 2003).

Although the impacts of chemical contaminants have been well documented in fish from the molecular level to the whole organism level, few studies have focused on the higher levels of biological organization (Siligato and Böhmer, 2002; Arnot and Gobas, 2004). Some authors have reported that polychlorinated biphenyls (PCBs) have

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contributed to declines in wild fish populations (Colborn and Thayer, 2000). However, even in the best-documented cases, conclusions regarding the ecological risks of these chemicals have been based primarily on inferences from laboratory studies rather than on direct observations of effects on populations (Barnthouse et al., 2003). Therefore, intermediate-level effects occurring between the short-term early warning responses and the more ecologically long-term relevant changes at the population or community levels must be examined to obtain a realistic view of the ecological health status of a given ecosystem (Mayon et al., 2006). Therefore, accurate analysis of the hypothesis that chemical exposures are reducing the reproductive success of a fish population requires long-term monitoring of an adult size population, the exposures of these adults to the chemicals of interest over a wide range of exposure conditions and the number of surviving young produced.

The use of fish populations as indicators of river health is legislated and mandatory in Europe due to the introduction of the Water Framework Directive (Directive 2000/60/EC, 2000). Since 2000 when the WFD was established, many efforts in European countries have been focused on developing efficient tools to measure the ecological status of freshwater based on fish (Ayllón et al., 2012). Smith and Darwall (2006) identified water pollution as the major threat to Mediterranean endemic freshwater fish. However, little is known about the POP contamination and their potentially negative effects on endemic fish populations. In particular, barbels have been previously used for ecotoxicological studies due to their reported sensitivity for particular biomarkers (Hugla and Thomé, 1999; Viganó et al., 2000). Bottom-dwelling fish, such as barbels, tend to accumulate lipophilic contaminants, such as organochlorine compounds, in their tissues directly from water and sediment as well as through their diet, enabling the assessment of the transfer of pollutants through the trophic web.

The aim of this study is to identify potential changes in the population dynamics caused by selected organochlorine compounds, such as PCBs and DDTs, in populations of Iberian barbel (*Barbus bocagei*, Steindachner, 1864) inhabiting the Jarama River, which is a Spanish tributary of the Tajo River that drains through a highly industrialized and urbanized area. The Jarama River has suffered serious degradation due to the urban, industrial, and agricultural activities in the surrounding area (Fernández et al., 2000). The Tajo basin has been identified as regionally important for fish endemism and as a center of threatened species within the Mediterranean (Smith and Darwall, 2006).

Iberian barbel was the monitoring species selected because it is found along the Jarama River and has already been used as a bioindicator species in other studies (Viganó et al., 2000; Christoforidis et al., 2008; Raldúa et al., 2008). Iberian barbels are a cyprinid species endemic to the Iberian Peninsula, and they mostly inhabit middle and lower reaches of streams and feed on detritus and benthic invertebrates. The species is abundant in many freshwater ecosystems, and it is also exploited for angling because it is part of the human diet. Therefore, the study of the impact of contaminants on their populations is important not only from an ecological point of view but also as a matter of concern for human health.

2. Material and methods

2.1. Study area

This study was performed in 15 sampling sites from the Jarama River in the region of Madrid, Spain, and the distance between sampling sites was approximately 5 km. The study area is located between 530 and 890 m above sea level. The sampling sites were selected to evaluate the effects of different types of environmental deterioration primarily from industrial effluents and agricultural activities on fish populations.

The different water uses provided a defined pollution gradient from well preserved areas of high ecological value upstream to contaminated areas downstream, which were delimited by the confluence of tributaries (Henares and Manzanares rivers) draining highly industrialized and urbanized areas. The 15 sampling sites were grouped into three areas based on different impacts from human activities as follows: low impact (LI), only affected by agricultural activities; moderate impact (MI), from agricultural and industrial activities; and heavy impact (HI), from industrial and urban effluents.

2.2. Fish population assessment

At the 15 sampling points, fish were sampled during summer and autumn from 2006 to 2009 by electrofishing using a 2200 W DC generator since it is considered the most effective and benign technique to capture freshwater fish (Cowx and Lamarque, 1990). Electrical output settings were adjusted to achieve an optimum combination of efficient fish capture and fish welfare under the range of environmental conditions within the study area. Electrofishing followed the standardized procedures described in the European Committee for Standardization (CEN) directives EN 14962: 2006 and EN 14011: 2003, which specify the methods that should be used for sampling fish according to the WFD.

Individuals were anesthetized with MS-222 (tricaine methanesulfonate), measured (fork length, FL, to the nearest mm), weighed (W, to the nearest g) and scales were taken for age determination. All of the fish were examined for external lesions, morphological abnormalities (e.g., hypertrophy of the mouth, occurrence of nodules in different parts of the body and spinal deformities) and ectoparasites, which could be associated with the presence of contaminants in the water. Morphological abnormalities were recorded in each individual according to the methodology described in Almeida et al. (2008). Then, the fish were released to the sampling sites except for specimens collected for residue analyses. Fish densities with variance were estimated separately for each sampling site by applying the maximum likelihood method (Zippin, 1956) and the corresponding solution proposed by Seber (1982) for three removals assuming a constant-capture effort. Biomass was calculated following Mahon et al. (1979). Population estimates were performed separately for each year class.

Within each sampling area defined in the study (LI, MI, HI), five specimens of Iberian barbel were captured with a total of 15 samples available for residue analysis. The competent authorities only allowed for the capture of 15 barbels (i.e., five per sampling area) because the Spanish laws on ethical conduct in the use of nonhuman animals in scientific research are very restrictive, especially for endemic species.

To minimize the effects of age on fish contamination, individuals were selected as homogeneously as possible. Each fish was packed in a plastic bag and frozen immediately after capture on dry ice. Samples were maintained at −80 °C prior to residue analysis.

To determine if the relationship between length and weight differed between sampling sites, we tested the null hypothesis that the slopes were equal by an analysis of covariance. To analyze whether the age structure of the populations varied between stream reaches, we performed a log–linear analysis. The assumption of normality of distributions was verified through a Kolmogorov–Smirnov test. Data were $\log_{10}$ transformed before the analyses were performed when they did not meet the assumption of normality of distributions. The significance level for all of the statistical tests was set to $\alpha = 0.05$.

2.3. Organochlorine compound analysis

Quantities ranging between 1.4 and 2.6 g of lyophilized samples of muscle were used for residue analysis. The following organochlorine compounds were analyzed: PCB congeners #28, 52, 95, 101, 105, 114, 118, 123, 132, 138, 149, 153, 156, 157, 167, 170, 180, 183, 189 and 194 as well as pp′DDD, pp′DDE and pp′DDT. Sample treatment involved three steps, as described previously by Merino et al. (2005). Briefly, the sample treatment involved three steps. First, the extraction step was performed using a solid-phase matrix dispersion procedure. An additional cleanup step was based on the use of a multilayer silica column containing neutral silica, silica modified with sulfuric acid, and silica modified with potassium hydroxide. A final fractionation step was performed using Supelclean Supelco ENVI-Carb tubes (Bellefonte, PA, USA). The fraction containing the majority of PCBs and DDTs was used for instrumental analysis.

The separation and quantification of PCBs and DDTs were performed using gas chromatography (GC) on a Hewlett Packard 6890 gas chromatograph equipped with a 63Ni μ-electron capture detector (Palo Alto, CA, USA). A DB-5 (J&W Scientific, Folsom, CA, USA) fused silica capillary column (60 m × 0.25 mm i.d. and 0.25 μm film thickness) was used. The carrier gas was nitrogen at a head pressure of 192.2 kPa. The detector and injector temperatures were 300 °C and 270 °C, respectively. Further details on the determination and quantification of PCBs and DDTs are available in Roscales et al. (2010).

The quality assurance criteria were based on the application of quality control and quality assurance measures, which included the analysis of a blank with each batch of three samples covering the complete analytical procedure. For comparative purposes, the concentrations were expressed on a wet weight (ww) basis. The ratio dry/wet basis ratio ranged between 0.21 and 0.28.

3. Results and discussion

3.1. Organochlorine compounds

Most of PCB and DDT congeners analyzed in this study were detected in all of the samples (Table SI 1a and 1b). The total PCB concentrations found in barbel notably increased when moving downstream with average concentrations ranging from 3.4 ng/g (ww) in LI areas upstream to 101.4 ng/g (ww) in HI areas downstream. In barbel populations, the bulk of the individuals is typically sedentary and resides within restricted home ranges not higher than 800 m. Only a small fraction moves within a maximum average distance of 2 km (Penaz et al., 2002; Aparicio and de Sostoa, 2005; Vilizzi et al., 2006). The limited extent of movement makes barbels particularly vulnerable to continuous exposure to local levels of organochlorine contaminants. Mazet et al. (2005) also found an increasing contamination pattern downstream in the Drôme River, France, with PCB concentrations ranging from 7.8 to 56.9 ng g^{-1} ww. In addition, Viganó et al. (2000) observed that the total PCB content of downstream cyprinids ranged from five to more than eightfold higher than the corresponding upstream samples.

PCB levels in the barbel samples from the Jarama River are within the range found in Iberian (Bordajandi et al., 2003) and European freshwater fish (Mazet et al., 2005) as well as in other freshwater fish species worldwide (Braune et al., 1999). In particular, PCB concentrations from LI (ranging from 1.3 to 5.6 ng g^{-1}) and MI (ranging from 2.6 to 12.1 ng g^{-1}) sites were similar to that found in other cyprinid species from Europe. For example, average concentrations found by Erdogrul et al. (2005) in kalashpa (*Acanthobrama marmid*) from the Sir Dam Lake, Turkey, were 3.0, 0.9 and 0.4 ng/g (ww). Yamacuchi et al. (2003) reported average PCB levels of 1.8 and 3.3 ng/g (ww) in perch (*Perca fluviatilis*) and roach (*Rutilus rutilus*), respectively, from the Thames River, United Kingdom. In the Nestos River, Greece, Christoforidis et al. (2008) observed mean concentrations of approximately 5 ng/g (ww) in muscle samples of barbel (*Barbus cyclolepis*) and European chub (*Leuciscus cephalus*). Accordingly, the average concentration determined by Svobodova et al. (1995) in carp (*Cyprinus carpio*) from the Czech Republic was 3 ng/g (ww), and Covaci et al. (2006) found PCB levels ranging from 3.5 to 18 ng/g ww in the same species from the Danube Delta.

However, PCB concentrations in HI sites downstream of the Jarama River (ranging from 38.7 to 269.7 ng/g) are comparable to the levels (i.e., near 65 ng/g (ww)) found by Mayon et al. (2006) in muscle samples of chub (*Leuciscus cephalus*) from a Walloon hydrographical network.

However, the PCB levels found in barbel from HI sites in the Jarama River were significantly lower than values reported for other fish species living in areas with a high industrial impact, such as Berlin rivers (i.e., 1049 ng/g (ww)) (Fromme et al., 1999), moderately polluted areas, such as the Vanajavesi River in Finland (852–1742 ng/g ww; Tulonen and Vuorinen, 1996) or in the Great Lakes region, USA (504.1 ± 48.8 ng/g ww; Henry et al., 1998).

The PCB pattern (Fig. 1) was dominated by the most persistent congeners including PCB #153, 138 and 180. In general, PCB #153 is one of the main contributors to the total PCB content in freshwater fish species worldwide due to its long half-life (Chevreuil et al., 1995; González Sagrario et al., 2002; Manirakiza et al., 2002; Bordajandi et al., 2003; Mazet et al., 2005; Moon et al., 2006). For less chlorinated congeners (PCB #28 and 52), which are more readily metabolized and eliminated than the highly chlorinated ones (McFarland and Clarke, 1989), there was a relatively higher contribution in the more pristine areas upstream. The PCB pattern found in our study was similar to that found by Viganó et al. (2000, 2008) in a contaminated gradient within the

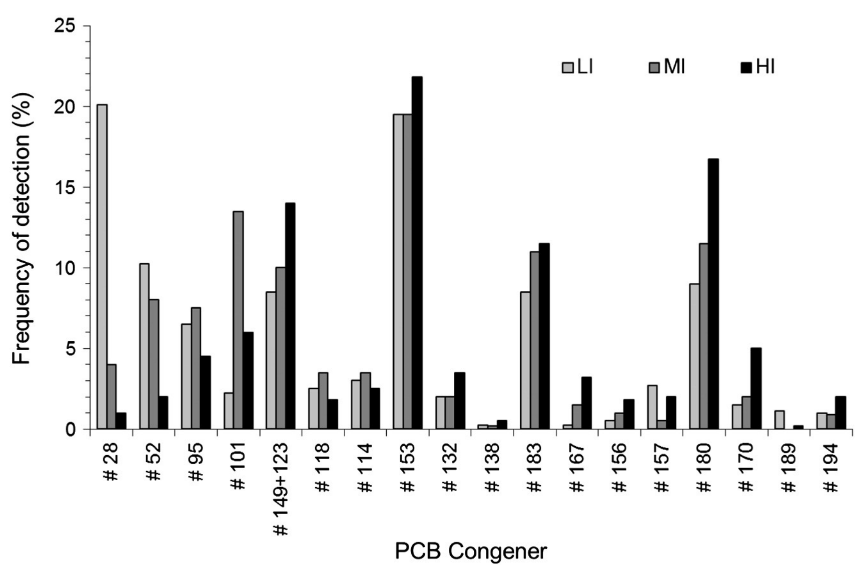

Fig. 1. Relative contribution of PCB congeners to total PCB content in Iberian barbel from three sampling areas along the pollution gradient in Jarama River, Spain (LI = low impact, MI = moderate impact, HI = heavy impact).

Po River, Italy, for barbel (*Barbus plebejus*), chub (*L. cephalus*) and nase (*Chondrostoma söetta*).

Although PCBs are regulated under the Stockholm Convention and have not been in use for years, their high persistence in the environment may result in their accumulation in the Jarama River after flowing through urban and industrial areas where these chemicals are transferred to the aquatic ecosystem.

Total DDT concentrations also increased when moving downstream from 0.9 ng/g (ww) in LI areas (range from 0.2 to 1.8 ng/g) to 20 ng/g (ww) in HI areas (range from 8.2 to 51.1 ng/g). Christoforidis et al. (2008) reported average levels of approximately 0.4 ng/g (ww) in barbel from the Nestos River in Greece. However, the average levels found in HI localities were similar to concentrations found in salmonids from high mountain areas (19 ng/g ww) by Vives et al. (2005) but significantly lower than values reported for cyprinids elsewhere. For example, Erdogrul et al. (2005) found higher mean values ranging from 14 to 77 ng/g ww in cyprinids from Turkey, and De la Cal et al. (2008) found that muscle samples of barbel (*Barbus graellsii*) and bleak (*Alburnus alburnus*) in the Cinca River in Spain contained 11 to 997 ng/g ww and 5 to 840 ng/g ww, respectively.

The p,p′-DDE/DDT ratio has been used to discriminate between recent and past uses of DDT. Low p,p′-DDE/DDT ratios (<1) indicate the recent use of DDT (Muñoz-Arnanz and Jiménez, 2011). The data obtained in the current study indicated that the p,p′-DDE/DDT ratio in LI reaches and MI reaches was higher than 1, while in HI areas, this value was under 1. This information suggests that barbel individuals from HI have been more recently exposed to this pesticide compared to the other sampled areas.

In 1977, DDT use was banned in Spain. Therefore, our results could indicate the existence of currently unknown sources of DDT in our study area. This result is supported by previous studies in the same study area. For example, Fernández et al. (2000) found high levels of DDT in surface and ground waters, and Merino et al. (2005) detected DDT concentrations in eggs from peregrine falcon and its main prey. In addition, the most recent study conducted on agricultural soils from southwestern Spain reported an unexpected, recent use of DDT (Muñoz-Arnanz and Jiménez, 2011).

The ratio between DDT and PCB concentrations indicates the degree to which agricultural sources contribute to pollution when compared to industrial sources. The DDT/PCB ratio was 0.38, 0.15 and 0.22 for the LI, MI and HI areas, respectively. In the three study areas, industrial sources of pollution were more important, as indicated by the higher content of PCBs compared to DDTs.

3.2. Fish population analysis

Barbel populations in the Jarama River are long-lived with a maximum longevity of 13 years. The population age structure was significantly different between stream reaches ($\chi^2 = 34119.64$, $p < 0.001$). Barbel in LI and MI areas had a well-balanced population with a predominant cohort of young fish, which indicated good recruitment, and older individuals from different age classes (Fig. 2). The most contaminated sites (HI) revealed a disrupted size distribution where the proportion of young fish was clearly underrepresented, which indicated recruitment deterioration.

In addition, the density and biomass of the 0+ and 1+ age classes were much higher in LI (Table 1) and MI stream reaches than in the HI sites. Similarly, the total density of barbel populations decreased downstream where the highest PCB and DDT concentrations were found. Therefore, the densities in less polluted reaches were far greater than in the most contaminated HI area.

To evaluate fish condition, linear regression models of body mass as function of fork length were fitted in each locality (Table 1). The comparison of slopes from the equations in Table 1 revealed significant differences in body condition between LI and MI reaches compared with HI reaches (interaction stream reach × fork length, ANCOVA, $F_{2,278} = 12{,}65$, $p < 0.001$) with the best condition in the less polluted areas. The frequency of individuals with external lesions, morphological abnormalities and ectoparasites was much higher in HI areas compared with LI and MI sites. The negative influence of industrial and urban effluents on barbel condition has been previously reported in Spanish basins (Maceda-Veiga et al., 2010) and in other cyprinids (Liney et al., 2006).

Organochlorine compounds are known endocrine disruptor chemicals because they mimic or antagonize the action of steroid hormones (Kelce et al., 1995; Jobling et al., 1998; Barnhoorn et al., 2009). Their effects include reduced egg production and decreased viability of eggs and larvae, which may lead to changes in the reproduction process that could alter the overall population dynamics of the Iberian barbel inhabiting the most contaminated sites, as shown by the disruption in the size distribution. For example, Hugla and Thomé (1999) experimentally determined that PCBs had a dramatic effect on barbel (*Barbus barbus*) reproduction in the Meuse River in France. These pollutants reduced the fecundity and hatching rate of the species. Similarly, Mayon et al. (2006) observed that chub females from PCB contaminated rivers in Belgium displayed a significantly higher proportion of preovulatory atretic follicles compared to reference sites.

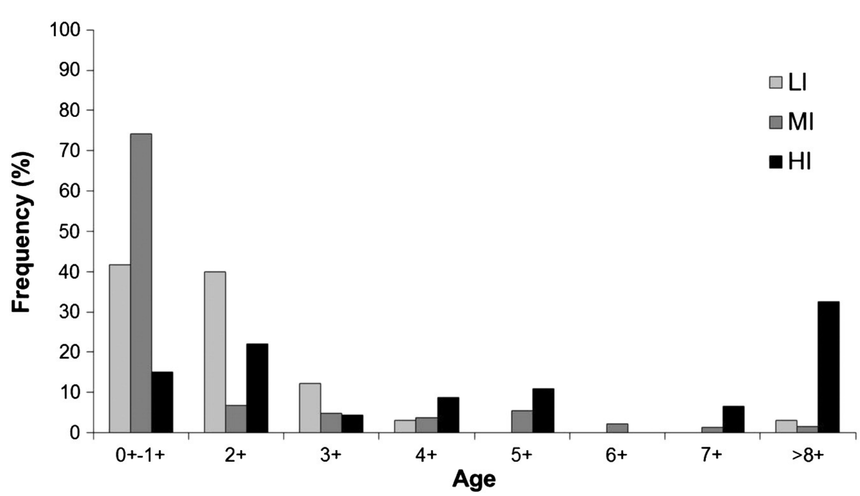

Fig. 2. Age structure of Iberian barbel in the three sampling areas along the pollution gradient in Jarama River, Spain (LI = low impact, MI = moderate impact, HI = heavy impact).

Table 1
Mean total and recruitment (0+ and 1+ age-classes) density (fish $ha^{-1} \pm SD$), mean total and recruitment biomass (kg $ha^{-1} \pm SD$), fork length–weight relationship (FLWR) parameters and proportion of external lesions, morphological abnormalities or ectoparasites (LAEs) in barbel populations from three sampling areas along the pollution gradient in Jarama River, Spain (LI = low impact, MI = moderate impact, HI = heavy impact).

	LI	MI	HI
Total density	3145.2 ± 234.4	2906.0 ± 98.6	1591.0 ± 129.5
Total biomass	257.0 ± 7.9	114.8 ± 9.8	465.3 ± 49.7
Recruitment density	1308.3 ± 130.3	2153.8 ± 27.0	240.8 ± 36.3
Recruitment biomass	19.5 ± 2.1	6.0 ± 0.5	2.8 ± 0.7
FLWR	$\log_{10}W = -4.86 + 3.01 \log_{10}FL$ ($R^2 = 0.99$, d.f. = 1,72, $p < 0.001$)	$\log_{10}W = -4.86 + 3.02 \log_{10}FL$ ($R^2 = 0.99$, d.f. = 1,16, $p < 0.001$)	$\log_{10}W = -4.51 + 2.85 \log_{10}FL$ ($R^2 = 0.99$, d.f. = 1,43, $p < 0.001$)
LAE	1.32%	5.74%	17.39%

4. Conclusions

The abundance of barbel populations in the Jarama River decreased downstream where the highest PCB and DDT levels were found in fish samples. These fish also exhibited a higher incidence of abnormalities and ectoparasites at these sites. High concentrations of PCBs and DDTs are likely contributing to a reduction in barbel fitness in the Jarama River because the effects of these pollutants reduce egg production and decrease the viability of eggs and larvae. These effects may lead to changes in the reproduction process that could affect the overall population dynamics of the Iberian barbel inhabiting the most contaminated sites, as shown by the disrupted age distribution.

Chronic effects of PCBs and DDTs could represent a serious element of risk for the barbel populations in the Jarama River, which adds to other conservation problems that have already been reported, such as habitat destruction or invasive predators. This threat could be extended to other endemic cyprinids, which are also exposed to these POPs within the study area. Therefore, further investigations should be undertaken to determine if organochlorine contamination is present in other species and what the potential health effects are concerning individuals. In addition, local people consume fish from rivers; therefore, the possible health risk associated with PCBs and DDTs should be evaluated because fish consumption is an important route of exposure.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2014.01.013.

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