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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