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Influence of flow diversions on giant freshwater pearl mussel populations in the Ebro River, Spain

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ABSTRACT

1. Freshwater mussels, historically a component of freshwater benthic invertebrate biomass, are one of the most imperilled animal groups on the planet. *Margaritifera auricularia* was once a common freshwater mussel inhabiting large rivers throughout Western Europe. It was believed to be extinct until 1996 when a few small populations were found in Spain and France.
2. Currently *M. auricularia* is one of the most endangered species in the world. The current status of this mollusc in the Ebro River was surveyed, finding a few adult specimens at only two localities, and many old, empty shells throughout the river.
3. Using a simple analysis of historical hydrological data, dramatic changes in water flow might have led to localized extirpation of this freshwater mussel.
4. Other factors contributing to the slow extirpation of this long-lived invertebrate from other areas of the Ebro Basin include the continuous reduction of water levels during the reproductive season, overharvesting for nacre, construction of impoundments, extinction of fish hosts, and impaired water quality.

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KEY WORDS: *Margaritifera auricularia*; catchment; biodiversity; endangered species; invertebrates; drought; hydropower

INTRODUCTION

Freshwater mussels are one of the most imperilled animal groups on the planet, and play an extremely important role in the ecology of freshwater ecosystems as a main component of the freshwater biomass in some systems (Vaughn and Hakenkamp, 2001; Strayer *et al.,* 2004). The dramatic changes taking place in freshwater ecosystems during the last century have played a part in the large-scale disappearance of these animals, although the causal factors are largely unknown (Harding *et al.,* 1998; Lydeard *et al.,* 2004).

The greatest diversity of freshwater mussels, also known as unionoids, exists in North America, with nearly 300 different species. Of these, 37 are extinct, 73 critically imperilled, 42 imperilled, 50 vulnerable, 42 secure and 44 apparently secure (Strayer *et al.,* 2004). In Europe, where many mussel populations are in clear decline, there are only 16 native

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species, belonging to six genera (Araujo and de Jong, 2015). European *Margaritifera margaritifera* populations have declined by 95% during the 20th century (Young *et al.,* 2001; Geist, 2010), whereas *Margaritifera auricularia* survives only in a single Mediterranean basin (the Ebro River in Spain; Araujo and Ramos, 2000, 2001) and in four Atlantic basins in France (Adour, Garonne-Dordogne, Charente and Loire; Prie *et al.,* 2010). All of these basins are threatened habitats and mussel populations do not appear to be actively recruiting (Prie *et al.,* 2010). By contrast, at the end of the 19th century a single fisherman could harvest 500 kg of *M. auricularia* for nacre (the nacreous layer is the main part of the freshwater mussel's shell) on a single day in the Ebro River near

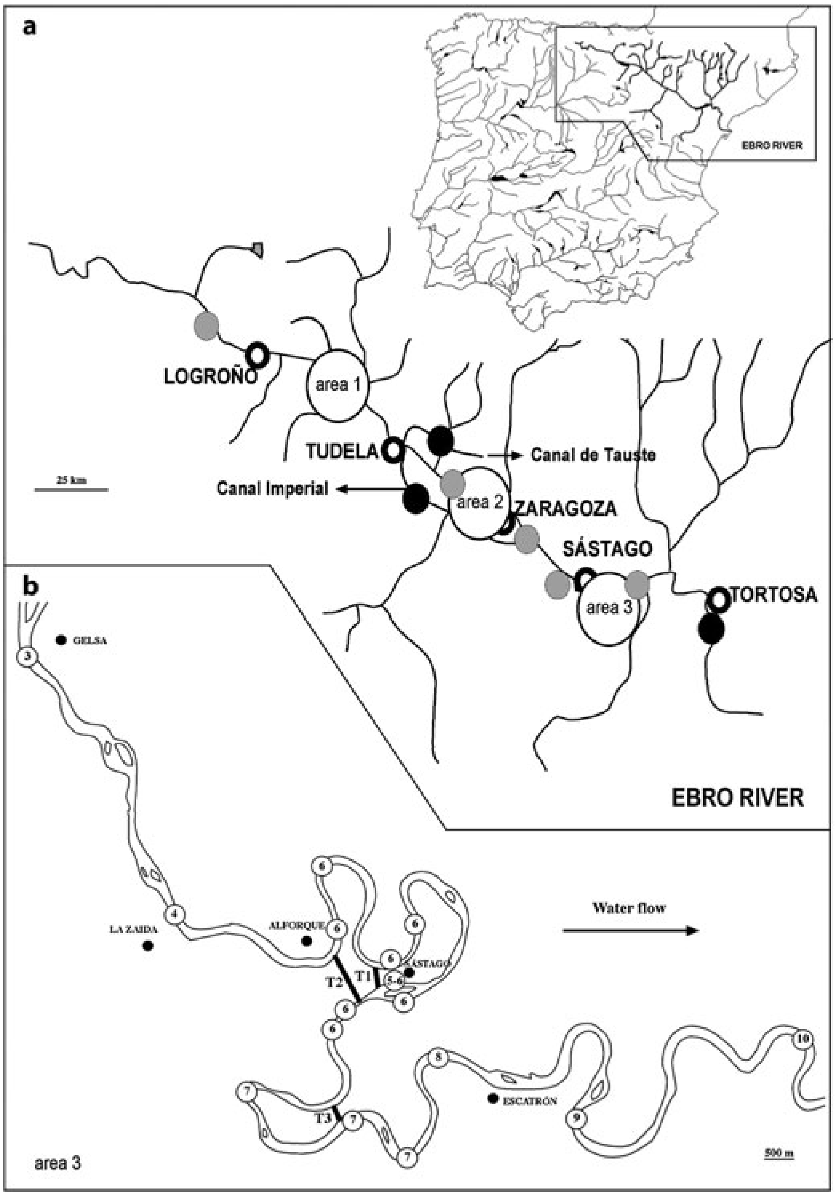

Figure 1. (a) Distribution of *M. auricularia* in the Ebro River (Spain). Black dots indicate locations of living *M. auricularia* specimens (Gómez and Araujo 2008; Araujo *et al.*, 2009), grey dots indicate historic sites of *M. auricularia,* and dots with white centre indicate town names. Large circles indicate areas of the Middle Ebro surveyed that are described in this paper. See Table 1 and Figure 1(b) for details. Area 1: surveys 1 to 4. Area 2: surveys 5 and 6. Area 3: surveys 7 to 14. (b) Area 3 of the Middle Ebro River. Numbers in circles indicate surveys (Table 1). Only one live *M. auricularia* specimen was found in survey 5. Black bars (T1, T2 and T3) show the location of tunnels for water diversion to power stations. Black dots indicate town names.

Sástago (Zaragoza) (Figure 1(A)). This is equivalent to 1300–4000 individuals, depending on whether empty or complete specimens were weighed. By 1915, mussel collecting dramatically decreased, with fishermen requiring one and a half months to collect 50 kg (Haas, 1916, 1917). There are three known populations of *M. auricularia* in the Ebro basin (Figure 1(A)): the Canal Imperial de Aragón (*c.* 5000 individuals), the Canal de Tauste (80 individuals) and the Lower Ebro (70 individuals) (Araujo and Ramos, 2001; Gómez and Araujo, 2008).

Freshwater mussels have a temporary but obligatory parasitic stage in which the larvae (glochidia) attach to the external surface or gill filaments of their vertebrate hosts (mainly fish) before metamorphosis to the free-living juvenile stage. The biology and life cycle of *M. auricularia* were recently described (Araujo and Ramos, 1998; Grande *et al.,* 2001; Araujo *et al.,* 2002, 2003). There are two known native host fish of *M. auricularia* glochidia: Atlantic sturgeon, *Acipenser sturio* (Preece *et al.,* 1983; Araujo and Ramos, 2000) and blenny, *Salaria fluviatilis* (Araujo *et al.,* 2001). Of these, only the blenny was historically common in the Ebro and probably increased *M. auricularia* recruitment; however, in recent years the Spanish blenny populations have decreased by 50% (Doadrio, 2001). It is already known that the distribution and abundance of host fish might be an important factor in limiting unionoid populations (Strayer, 2008).

Numerous factors have probably contributed to the drastic decline of *M. auricularia* populations, including the decline of host fishes, pollution, climate change and commercial exploitation (Preece *et al.,* 1983; Altaba, 1990; Araujo and Ramos, 2001). The goal of this paper is to demonstrate how the human alteration of freshwater ecosystems has contributed to *M. auricularia* extirpation in the Ebro River (NE Spain). These results contribute to extending knowledge of the anthropogenic effects on freshwater mussels, which is essential for river restoration projects that include freshwater mussel conservation programmes (Cooksley *et al.*, 2012; Varandas *et al.*, 2013; Moorkens and Killeen, 2014; Quinlan *et al.*, 2015). The species is currently listed in Appendix IV of the European Habitats Directive and in Appendix II of the Bern Convention, the two main documents outlining European biodiversity conservation regulations. *Margaritifera auricularia* is also listed as being in critical danger by the International Union for Conservation of Nature (Cuttelod *et al.,* 2011) and considered threatened with extinction by inclusion on the Spanish National Endangered Species List (Araujo and Ramos, 2001).

MATERIAL AND METHODS

Study area and mussel survey

Fourteen sampling surveys were undertaken between September 2000 and June 2006 in the Middle Ebro River to determine the status of *M. auricularia*. The stretch of river surveyed measured 25 km (Table 1; Figure 1). Some surveys were carried out in areas with an impending threat of habitat modification (e.g. dredging, crossing gas pipelines, installation of refrigeration circuits for energy plants or water pumps), while others were in habitats with historical records of *M. auricularia*. Half of the surveys were in the three meanders of the Ebro River around the locality of Sástago, downstream of Zaragoza (Aragón), where a huge population was present at the beginning of the 20th century.

The sampling design was developed so as to locate freshwater mussel beds, such as those historically cited in the Ebro. Given the zero visibility on the river bed, divers worked by touch. The sections studied were prospected by transects, closer together in areas with increased likelihood of mussels (appropriate substrates). Depending on these and other variables (e.g. river discharge), the type of transect used was decided using two methods: transverse and/or longitudinal linear (TLL) and transverse and/or longitudinal diagonal (TLD). The former was the method generally used for convenience and ease of use. The transect was formed by a 50 m long submerged rope, weighted at the centre and at each end, and marked with floating buoys. The rope was traversed along its whole length by two divers, one on each side, so that the corridor under consideration was about 4 m. This system was used in cases of strong current or unfavourable substrate for mussels (e.g. mud). Transects were traversed to reach the opposite side of the river, in the case of the transverse one, or parallel to

Table 1. Results of mussel surveys conducted between 2000 and 2006 in the Ebro River at sites where *Margaritifera auricularia* was historically present (Figure 1)

Survey	Date	Length surveyed (in metres)	Type of sampling	*Margaritifera auricularia*	*Unio mancus*	*Potomida littoralis*	*Anodonta anatina*
1. Arrúbal*	2-6 June 2003	1000	1	5 dead specimens and 3 old fragments	7 live and valves	many valves and fragments	2 live and fragments
2. La Rioja	18-22 July 2005	1700	2	5 dead specimens and 4 old fragments	valves and fragments	9 live and valves	16 live and fragments
3. Navarra	12-16 Sept. 2005	3100	2	-	18 live and valves	44 live and valves	48 live and fragments
4. Navarra*	5-8 June 2006	1000	1	-	1 live and fragments	4 live	16 live and fragments
5. Ribera Alta	8-11 Oct. 2002	3000	1, 2	38 live	20 live and valves	70 live and valves	24 live and valves
6. Zaragoza	4-15 July 2005	6000	1, 2	1 fragment	179 live and many valves	619 live and many valves	27 live and fragments
7. Gelsa*	18-22 Sept. 2000	500	1, 2, 3	-	4 live and valves	45 live and valves	7 live and fragments
8. La Zaida*	25-29 Sept. 2000	700	1, 2, 3	-	2 live and valves	11 live and valves	5 live and fragments
9. Meanders 1, 2	12-14 July 2000	Punctual inspections	1, 2	1 live and several old shells	10 live and many shells	10 live and many shells	59 live and fragments
10. Meanders 1, 2	23 Sept -8 Oct. 2002	12 400	1, 2	1 recent shell and many old fragments	5 live and many valves	6 live and many valves	5 live and valves
11. Meander 3 (Menuza)*	17-21 Sept. 2001	1800	1	4 old fragments	1 live and valves	7 live and valves	4 live and valves
12. Escatrón*	9-12 June 2003	1600	1, 2	1 recent valve and 1 old valve	valves	2 live and valves	valves and fragments
13. Gotor*	24-28 Sept. 2001	1900	1, 2	15 old fragments	many valves and fragments	many valves and fragments	1 live and valves
14. Mequinenza*	23-27 July 2001	1000	1	-	-	valves and fragments	valves and fragments

*Areas with impending habitat modification. Type of sampling: (1) diving using transects; (2) wading; (3) substrate extraction.

the shore line, in the case of the longitudinal one. Transects were moved by motorboats.

The TLD method enabled comprehensive information to be collected across a width of 10 m per transect. This was costly in effort and time, used in areas with appropriate substrate for mussels (e.g. settled gravel), but not in areas of high current. The submerged weighted rope was 10 m long. In the transverse-diagonal case, the rope was parallel to the river bank and was traversed in two directions by divers; on completing the first sub-10 m transect, one diver moved the rope making zig-zag sub-transects until reaching the opposite bank. In the case of longitudinal-diagonal transects, the same operation was performed but followed the shore line. In areas with high densities of mussels, divers picked up bottom samples along transects, subsequently washing them on the shore with a water pump and a sieve (2 × 2 cm). In shallow areas, where it was not possible to use scuba equipment, the survey was conducted by wading using tactile searches and waterscopes. All live mussels found were collected, identified, measured and returned to their original location.

Discharge analysis

Like many freshwater mussels, *M. auricularia* is strongly dependent upon continuous water flow for food, reproduction and fish vectors (Strayer, 2008; Moorkens and Killeen, 2014). Therefore, the discharge in environments harbouring *M. auricularia* is likely to be one of the key variables for its continued occurrence. In this way, the long-term flow datasets in the areas where the species was historically recorded in the Ebro River were studied for an area where the species reached high numbers in the early 20th century.

The sampling reach comprised three meanders and was one of the first in Spain to have hydropower stations installed (Figure 1(B)). The first tunnel (T1, maximum capacity 50 m^3 s^{-1}), diverted water from the second meander and was built in 1908 to provide energy to a calcium carbide factory. A second tunnel (T2, maximum capacity 200 m^3 s^{-1}), connecting the first two meanders opened in 1929, while a third tunnel (T3, maximum capacity 252 m^3 s^{-1}), which diverted water from the third meander, was built in the 1950s (Guillén and Ríos, 1994).

Discharge data were gathered from the Ebro Water Authority, which recorded daily discharge at Zaragoza from 1913–1933 and 1943–2010 at a site 64 km upstream from the study reach; Zaragoza is the closest discharge station to Sástago where long-term data have been recorded. Unfortunately, no discharge records were available between 1933 and 1943. There are no significant tributaries between the Zaragoza gauge station and Sástago. Only temporary streams may occasionally increase Ebro discharge, albeit in small quantities, but they are offset by frequent illegal pumping that diverts some water upstream of Sástago. Minor water diversions have existed between the discharge recording location and the mussel sampling sites in this catchment since 1913, but they are negligible.

Monthly discharge was computed to make analysis easier by multiplying daily discharge times by the number of days in each month. The historical daily flow data at the meanders and after T1 and T2 between 1999 and 2002 were used to calculate how often discharge was negligible in these meanders owing to the diversion of flow to these hydropower stations. We hypothesized that periods of channel dryness had the largest impact on mussel population recruitment (i.e. periods coinciding with release of juveniles, usually between April and May) (Araujo *et al.,* 2001, 2003). Using monthly discharge data, the years with periods of channel dryness during the first five months of juvenile life were determined to know if nil discharge could be deleterious for the long-term survival of *M. auricularia*.

Age assessment

One valve of two recently dead specimens of *M. auricularia* from the Ebro River (length 153 cm) and the Canal Imperial (length 149 cm) were sectioned to study their age, based on the number of growth rings (Clark, 1980; Neves and Moyer, 1988). The specimens were sectioned in an oblique direction from the umbo towards the postero-ventral region to cover the longest distance. These thin sections were then polished using carborundum and observed under optical and binocular microscopes (Araujo *et al.*, 2014). Each growth ring, or intra-shell periostracum layer (Checa, 2000), was considered as one year. The approximate age of living specimens of *M. auricularia* was inferred by comparing shell lengths with the sectioned specimens.

RESULTS

Records of *M. auricularia* in the Ebro basin

Living *M. auricularia* were found in only two localities (Table 1), both of which were altered 'flow refuge' habitats (Strayer *et al.,* 2004) reminiscent of the historic natural dynamics of the Ebro River (Ollero, 1996): the braided channels of the Ribera Alta (area 2 in Figure 1), with 38 individuals found in a large mud layer and one individual from the second meander near Sástago (area 3 in Figure 1). In the two sectioned shells the number of growth rings do not mark the exact number of years, because during the first 10 years no periostracum layers are included in the shell. Nevertheless, the shells analysed by thin section had more than 50 marked growth rings. All living specimens collected were larger than 150 mm and hence older than 50 years.

Effects of discharge on *M. auricularia* survival

Using data from the historical daily flow records at the meanders (from 1913–1933 and 1943–2010) and daily water turbinated for T1 and T2 between 1999 and 2002 (data not shown), the potential impact of these two power stations on *M. auricularia* populations was calculated. The second meander was dry for at least five consecutive months each year, probably having a lethal impact on mussel populations in this reach (Figure 2, 3). Throughout the study period, discharge was infrequently higher than 240 m^3 s^{-1} during the five months of each year when the release of *M. auricularia* juveniles is more likely. Surplus water, only present after the amount of discharge had been diverted to the power plants, reached the extent of a meander in eight years only (1953, 1961, 1962, 1963, 1972, 1977, 1992 and 1993). The consecutive transformation of meanders into long pools between dams would have affected the early months of life for juveniles, which are usually released between April and May (Araujo *et al.,* 2001, 2003), thus having a potentially large impact on species extirpation. Between 1908 and 1929, when only T1 was functional, flow impacts were presumably lower.

DISCUSSION

Results suggest that the management of a hydropower plant during the first half of the 20th century resulted in the extirpation of the last large population of *M. auricularia* in the Ebro Basin and the Iberian Peninsula. Nevertheless, extirpation of *M. auricularia* is not the result of a single cause. Other necessary

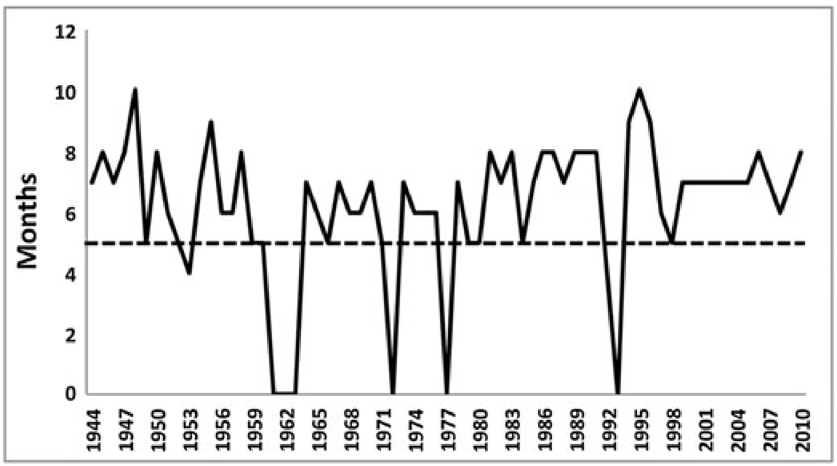

Figure 2. Discharge diversion at power station T2 in the Ebro River from 1943 to 2010. A lethal impact on the *M. auricularia* population might have occurred when T2 diverted water flow (between 25 and 240 m^3 s^{-1}) for more than five consecutive months (dashed line).

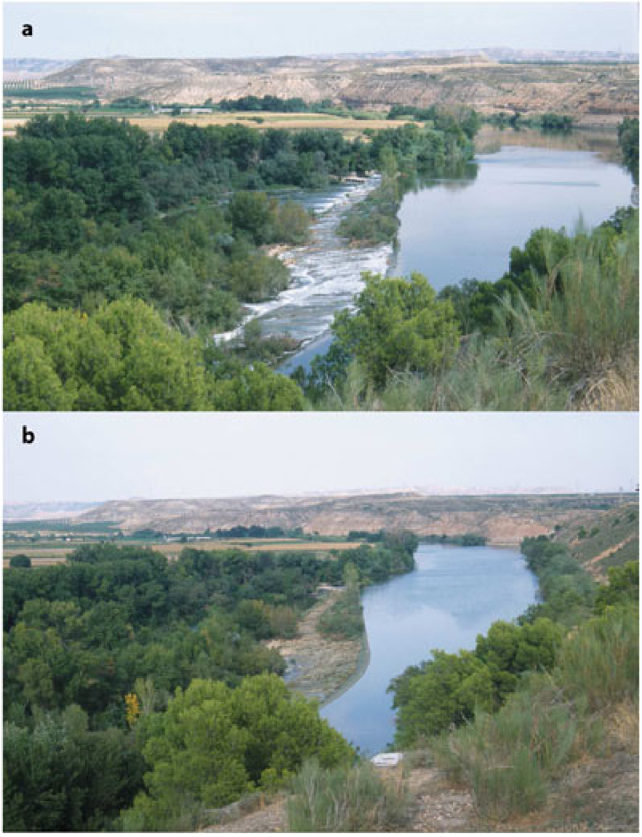

Figure 3. Area of the Ebro River at the meanders with the power station tunnel closed (a) and open (b).

conditions for *M. auricularia* recovery are clean water and uncontaminated sediment, which depend on natural water flow through river meanders (Moorkens and Killeen, 2014). Over the course of time, the synergistic action of mussel overfishing, discharge flows from two chemical factories, absence of native host fishes, pig farms, increase of agricultural plots and irrigation in 'isolated bypass reaches', probably contributed also to the demise of this species (Vaughn and Taylor, 2000; Strayer, 2008; Quinlan *et al.*, 2015). Similar synergistic actions are likely to have modified mussel habitats including the braided channels of the Upper Ebro River at the Ribera Alta (area 2 in Figure 1), where mussel populations were buried under layers of silt. The conditions necessary for supporting healthy *M. auricularia* populations in the Ebro have disappeared and been replaced by those favouring human development (Barrera, 1999), thus leading to impoverished biodiversity. For instance, the irrigated area in the basin rose from 420 000 ha in 1926 to 783 948 ha 75 years later, and the discharge of the Ebro River has diminished concurrently. Other habitat modifications, which were extensive during the second half of the 20th century, included the 40% decrease in the area covered by autochthonous vegetation (Ollero, 1996) and the building of thousands of water control structures (e.g. small dams, breakwaters and jetties). At present 46% of the Middle Ebro shores (318 km) are flanked by these structures (Ollero, 1996; Barrera, 1999). In addition, 65 impoundments were constructed to regulate the modern Ebro River basin, the first one located at the mouth of the river (maximum volume 540 hm^3), followed by the construction of the large (1534 hm^3) Mequinenza impoundment during the 1960s.

There are growing concerns about the impacts of fragmentation and flow regulation structures on the health of freshwater mussel populations (Layzer *et al.,* 1993; Layzer and Madison, 1995; Vaughn and Taylor, 1999; Hardison and Layzer, 2000). Furthermore, more recent work demonstrates that at least *M. margaritifera* is adapted to a combination of stable substrate conditions that are kept clean through high water velocities with low fine sediment infiltration (Cooksley *et al.*, 2012; Moorkens and Killeen, 2014; Quinlan *et al.*, 2015). The impact of these structures is most damaging to rarer species and species with narrow requirements for host fish (e.g. *M. auricularia*), as they act as fish dispersal barriers, increase fine sediment levels and create unfavourable interstitial water chemistry (Strayer, 2008; Moorkens and Killeen, 2014). Several studies have also reported the lethal effect of un-ionized ammonia from industrial, municipal and agricultural wastewaters on juvenile freshwater mussels (Newton, 2003; Strayer and Malcom, 2012). All these environmental changes have occurred at the same time as a dramatic modification of the Ebro fish fauna. Of the 46 taxa in the basin, 27 are indigenous and 19 introduced (Sostoa and Lobón, 1989; Franch *et al.,* 2008; Miranda *et al.,* 2010; Doadrio, pers. comm.). Of the two known native host species for *M. auricularia* historically present in the Ebro, the Atlantic sturgeon (*Acipenser sturio*) is currently extirpated (Sostoa and Lobón, 1989) and blenny (*Salaria fluviatilis*) populations have decreased by 50% in recent years (Doadrio, 2001), making mussel recruitment unlikely. Living specimens of the three other freshwater mussels native to the Ebro were found in nearly all the localities surveyed (Table 1), but at lower abundance than expected, especially in areas that maintain braided channels. The poor survival of these species, which were previously abundant (Haas, 1916; 1917), could be due to their shorter life-cycle and a greater number of potential host fishes (Araujo *et al.,* 2005).

At the basin scale, many factors difficult to define drive the distribution of mussel populations (Strayer, 2008). However, the diversion of water from the Sástago meanders may be directly responsible for *M. auricularia* extirpation at this site. Populations in these meanders probably represent one of the few source populations (Strayer *et al.,* 2004; Strayer, 2008) in the Ebro Basin. This is a paradigmatic example of an invertebrate undergoing slow steady extirpation for > 70 years ('extinction debt') owing to the interaction between man-made changes in habitat quality and the unique biology of these animals (i.e. slow growth rate, complex life history and long lifespan). Although these relict populations persist under harsh environmental conditions, they may take several decades to disappear following habitat changes because of their long lifespan (Dynesius and Nilsson, 1994; Harding *et al.,* 1998; Rowe, 2008). Artificial structures in rivers are integral to the social and economic landscape, but existing structures (i.e. hydroelectric power stations) should not be considered as fixed components of the catchment hydrology (Cooksley *et al.*, 2012; Varandas *et al.*, 2013). The results reported on the changes in water flow that probably led to the extirpation of *M. auricularia* in the Ebro pave new research paths, which will be important for whole-catchment restoration projects, including freshwater mussel conservation programmes.

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