Water mill canals as habitat for *Margaritifera margaritifera*: Stable refuge or an ecological trap?

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ABSTRACT

Anthropogenic habitats may serve as a refuge for aquatic species, including freshwater mussels (Bivalvia, Unionida). Evaluating the role of anthropogenic habitats is a fundamental, but still ignored, conservation issue given the pace that humans have been converting natural ecosystems. In this study, possible differences in abundance, size and condition index of the freshwater pearl mussel *Margaritifera margaritifera* colonizing anthropogenic (water mill canals) and natural (Tuela River) habitats were assessed. No differences were found in the abundance of freshwater pearl mussels colonizing both habitats, but individuals present in the water mill canals have a significantly higher condition index and size. Water mill canals seem to provide stable conditions for the settlement, growth and survival of freshwater pearl mussels. However, the occurrence of an exceptional drought during the late summer of 2017 was responsible for an almost 100% mortality in one of the two water mill canals surveyed in this study. Therefore, and during extreme climatic events, these anthropogenic structures may function as an ecological trap for freshwater pearl mussels. This study can be used by managers to promote future actions that enhance freshwater pearl mussel protection and guarantee their survival, including on anthropogenic habitats.

1. Introduction

Human activities have dramatically changed aquatic ecosystems resulting in loss and fragmentation of habitats, pollution, over-exploitation of resources, introduction of invasive species and climate change (Dudgeon et al., 2006; Reid et al., in press). This situation, in one hand, leads to biodiversity loss at an unprecedented rate while on the other hand is responsible for a growing interest in conserving the remnant areas with low human disturbance (Pimm et al., 2018). Traditionally, research and management target natural ecosystems almost ignoring the possible role of anthropogenic infrastructures in the conservation of biodiversity (Lindenmayer et al., 2008). However, anthropogenic habitats may serve as a refuge for biodiversity and over the last years a growing number of studies emphasize their potential importance in the conservation of terrestrial and aquatic ecosystems (Kowarik, 2011; Chester and Robson, 2013; Martínez-Abraín and Jiménez, 2016). For example, Lundholm and Richardson (2010) provided several case studies of endemic species that have naturally colonised anthropogenic habitats offering physical structures analogous to their natural environments. These authors also provided examples of rare and threatened species that have appeared on post-industrial sites, showing that particular environmental conditions can provide effective refuges. In many cases this occurs because artificial habitats in some way mimic the local-scale attributes of natural habitats required by particular species, but this situation may also be a response to the growing human disturbance resulting in a decrease in natural habitat cover due to agricultural or urban land use. Therefore, evaluating the role of anthropogenic habitats on biodiversity is a fundamental conservation issue given the pace that humans have been converting natural ecosystems (Elphick, 2000).

In freshwater ecosystems Chester and Robson (2013) identified several anthropogenic structures that may function as an important habitat for some species and include, for example, irrigation and transport canals, agricultural wetlands and ponds, rural and urban...
drainage ditches, rice fields, fire dams, golf course lakes, stormwater ponds, aquaculture ponds, and gravel pits. Another example of these anthropogenic structures as habitat for aquatic biodiversity are water mill canals. Water as a source power for mills were widespread in Europe and North America until the XIX century and many of these old structures, or at least their remnants, still exist today and have a high historical importance (Munro, 2002). In addition, water mill canals might be used as habitat for several aquatic species, although their role has been rarely assessed. In contrast, this type of infrastructure is also responsible for negative effects on biodiversity because the water-courses are fragmented (e.g. due to the construction of weirs and small dams that divert the water to the mills), channelized and their substrate disturbed.

Examples of aquatic species that are positively affected by these anthropogenic structures include fish, amphibians, invertebrates, among others (Brand and Snodgrass, 2010; Chester and Robson, 2013; Halliday et al., 2015). However, these structures may also function as an ecological trap (i.e. when animals prefer habitats where their fitness is lower than in other available options; Schlaepfer et al., 2002). Examples of ecological traps in freshwater ecosystems include some amphibian species that may be attracted by stormwater ponds in urban areas where embryos and larvae are exposed to higher concentrations of pollutants or are stranded during fluctuations in water level (Brand and Snodgrass, 2010) and aquatic insects that are attracted by polarized light of polished black gravestones (similar as water surface) laying their eggs in these artificial structures and resulting in high mortalities (Horváth et al., 2007). Ecological traps can lead to reductions in population size, and in some extreme cases can increase the risk of species extinction. Therefore, how organisms respond to ecological traps and how they can be mitigated are important conservation topics in an era of environmental change (Hale and Swearer, 2016). This dichotomy of anthropogenic structures functioning as stable refuges or ecological traps for biodiversity may be highly context dependent and given the low number (and highly biased to terrestrial ecosystems and bird species) of studies on this topic there is a great need to enlarge the ecosystem type and taxonomic spectrum of research (Hale et al., 2015; Hale and Swearer, 2016).

In this study, we used the freshwater pearl mussel Margaritifera margaritifera (Linnaeus, 1758) as model organism to assess if anthropogenic habitats (i.e. water mill canals) may function as possible refuges or ecological traps. This species is classified as Endangered by the IUCN and over the last years many studies have been performed to address their distribution, abundance, genetic diversity and population structure in several European countries (Geist, 2010; Sousa et al., 2015; Hastie et al., 2000; Lois et al., 2014; Popov and Ostrovsky, 2014; Lopes-Lima et al., 2017, 2018; Sousa et al., 2013; Santos et al., 2015). However, and as far as we know, no study has ever evaluated the possible presence and physiological condition of this species in anthropogenic habitats and how this situation may positively or negatively affect individuals colonizing these structures in comparison to natural habitats. Therefore, the aims of this study were to: i) assess possible differences in abundance, size structure and physiological condition between freshwater pearl mussels colonising anthropogenic and natural habitats and ii) evaluate if these anthropogenic habitats function as a stable refuge or as an ecological trap for freshwater pearl mussels. We hypothesize that freshwater pearl mussels will have a lower abundance and physiological condition and a distinct size structure in water mill canals (when compared to natural habitats), since this species is considered a habitat specialist being dependent on high quality substratum, and nutrient poor and highly oxygenated waters.

2. Material and methods

2.1. Study area

The Tuela River is a tributary of Tua River (Douro basin, Iberian Peninsula) and has a length of 102 km. The climate is typically temperate and precipitation have high seasonal and inter-annual variability (Oliveira et al., 2012). Floods may occur during winter/early spring, with a gradual decline in river flow throughout the year, reaching minimal values in the late summer/early autumn (Sousa et al., 2012, 2018). The Tuela basin is under low human pressure and is mostly forested (Ferreira et al., 2007). The upstream areas are inside the Montesinho Natural Park, which support a high terrestrial and aquatic biodiversity. In these upstream areas, a high abundance of freshwater pearl mussels is found, turning this river the second most important (after Rabaçal River) for the conservation of this species in Portugal (Sousa et al., 2015, 2018). Although with a very low human pressure, the upstream area of the Tuela River present some old weirs connected to water mill canals.
2.2. Sampling strategy and data analysis

Sampling was performed in four sites: two in water mill canals (Canal 1 – Melro and Canal 2 – Fresulfe) and another two in adjacent areas to the water mill canals but under natural conditions (River 1 and River 2) in July 2017 (Figs. 1 and 2). The two water mill canals are located upstream of the small Trutas dam and have a total length of near 200 m being connected upstream to the Tuela River by small weirs (Fig. 2). These two water mills are currently deactivated.

In each of the four sampling sites, temperature, conductivity, dissolved oxygen and pH were measured using a YSI EXO 2 multi-parameter probe. Water samples were also collected to determine the total suspended solids (TSS) and total suspended organic matter (OSS). For this, 1 L of water was filtered using GFC filters, which were posteriorly dried at 60 °C for 48 h and then brought to a muffle at 550 °C for 8 h. TSS and OSS were determined by weight difference (following Zieritz et al., 2016, 2018). In addition, sediments were also collected in each site to determine the mean size granulometry and organic matter content (following Sousa et al., 2007).

Regarding biological characterization, a stretch with a total length of 200 m in the water mill canals or river natural conditions was visually surveyed using snorkelling to determine abundance and size structure of M. margaritifera. These surveys were always performed by two people and six to seven-five-minutes replicates were made in each site, with a total of 13 replicates for each habitat type. Abundance of M. margaritifera is shown as the total individuals found in 5 min per person and represented by the number of individuals per catch per unit of effort (ind. CPUE). Mussel dimensions (shell length, height and width) were measured to the nearest 0.1 mm with a Vernier calliper. All specimens were carefully returned to the water mill canal or river in their original position. Physiological condition was determined after randomly selecting a total of 100 individuals collected in both habitats (25 for each site, totaling 50 individuals from the water mill canals and 50 individuals from river). All individuals were measured (length, width and height) and their wet biomass determined using a scale. With this data, the non-lethal condition index (CI) was determined and compared in both habitats, as described in Anacleto et al. (2013):

\[ \text{CI} = \frac{[\text{Wetweight}]}{[\text{Shelllength}] \times [\text{Shellheight}] \times [\text{Shellwidth}]} \]

This CI was chosen in order to minimize possible stress to the animals since this is a protected species by European law. Differences in average abundance and condition index of freshwater pearl mussels between the two (i.e. anthropogenic vs. natural) habitats were assessed by a t-test. Differences in average length of freshwater pearl mussels between the two habitats were assessed by a Wilcoxon test since data depart from normality even after several transformations. All tests were preceded by a Shapiro–Wilk test to check if the residuals of the models had a Gaussian distribution, and a Fligner test to check for homoscedasticity (Zar, 2009).

All statistical tests were performed with RStudio (version 1.1.463).

3. Results

Abiotic conditions measured in the four sites were very similar (Table 1).

<table>
<thead>
<tr>
<th></th>
<th>River 1</th>
<th>Canal 1</th>
<th>River 2</th>
<th>Canal 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature (°C)</td>
<td>22.30</td>
<td>22.50</td>
<td>21.60</td>
<td>21.70</td>
</tr>
<tr>
<td>Conductivity</td>
<td>90.60</td>
<td>92.00</td>
<td>83.40</td>
<td>81.50</td>
</tr>
<tr>
<td>Oxygen (%)</td>
<td>101.50</td>
<td>100.00</td>
<td>90.30</td>
<td>92.50</td>
</tr>
<tr>
<td>Oxygen (mg L⁻¹)</td>
<td>7.83</td>
<td>7.78</td>
<td>7.24</td>
<td>7.67</td>
</tr>
<tr>
<td>pH</td>
<td>8.10</td>
<td>8.08</td>
<td>7.92</td>
<td>7.96</td>
</tr>
<tr>
<td>Total Suspended Solids (mg L⁻¹)</td>
<td>0.90</td>
<td>0.80</td>
<td>0.90</td>
<td>0.85</td>
</tr>
<tr>
<td>Organic Suspended Solids (mg L⁻¹)</td>
<td>0.50</td>
<td>0.50</td>
<td>0.50</td>
<td>0.50</td>
</tr>
<tr>
<td>Mean size of sediment (mm)</td>
<td>29.53</td>
<td>29.31</td>
<td>21.41</td>
<td>22.91</td>
</tr>
<tr>
<td>Organic Matter in sediment (%)</td>
<td>1.69</td>
<td>1.67</td>
<td>1.46</td>
<td>1.54</td>
</tr>
</tbody>
</table>

Fig. 2. Melro water mill canal (A) and adjacent natural area in the Tuela River (B); and Fresulfe water mill canal (C) and adjacent natural area in the Tuela River (D).
test = 4.19; $P < 0.01$; $N = 100$): average (±sd) CI in the water mill canals was 6.93 (±0.34) and in the natural conditions was 6.64 (±0.35) (Fig. 3B).

Significant differences were also found in the average length of freshwater pearl mussels colonizing the water mill canals and natural conditions ($W = 27784; P < 0.01; N = 432$). Freshwater pearl mussel length ranged between 38.0 and 90.0 mm (average ± sd = 71.62 ± 8.17 mm) in the water mill canals and between 38.0 and 85.0 mm (average ± sd = 69.11 ± 7.66 mm) in the natural habitat (Fig. 3C).

4. Discussion

In this study, freshwater pearl mussels found in water mill canals have a significantly higher CI and average size than in adjacent natural conditions, but no differences were detected regarding abundance. These anthropogenic habitats seem to offer stable conditions for freshwater pearl mussel settlement, growth and survival. Therefore, future management actions devoted to the conservation of this threatened species in the Tuela River (and elsewhere) should not ignore the importance of water mill canals as suitable habitat. However, one of the two studied water mill canals dried almost completely at the end of August 2017 leading to the die-off of nearly all individuals in the canal, turning this particular site into an ecological trap for freshwater pearl mussels.

The two studied water mill canals were constructed many decades ago and seem to function as a high quality habitat with very similar abiotic characteristics (i.e. temperature, oxygen, conductivity, pH, dissolved solids, and sediment characteristics) to the adjacent natural habitat. These comparable habitat conditions are probably responsible for the similar juvenile settlement and adult survival, resulting in approximate abundances in both type of habitats. Interestingly, the individuals present in the water mill canals had a higher CI and a slightly higher average length. This situation is possibly a response to the more stable conditions in these anthropogenic habitats, given that the water levels and current velocity are buffered by the water mill canal walls and possibly oscillate less than in adjacent natural conditions, therefore contributing to higher physiological fitness and growth rates. In addition, other factors not measured in this study, such as availability and quality of food may be different in both habitats although this situation is less probable since the water flowing in the anthropogenic canals comes from the river.

Although we clearly showed that the anthropogenic habitats seem to present stable conditions for the settlement and survival of freshwater pearl mussels the occurrence of an extreme drought in 2017 turned one of the sampled water mill canals into an ecological trap. In fact, the Melro water mill canal dried almost completely at the end of the 2017 summer causing the mortality of almost all mussels in this artificial habitat (Fig. 4 and for details about the die-offs see Sousa et al., 2018). On the other hand, the other canal and the two sites sampled in the river had a much lower mortality (Sousa et al., 2018). Therefore, the consequences of the 2017 drought were highly context dependent, and the Fresulfe canal still continues to serve as a stable refuge (Sousa, personal observation).

Since the older freshwater pearl mussels colonizing the Melro canal (and the other three surveyed sites) had settled several decades ago, we suppose that this was the first time in decades that this water mill dried completely. Looking to the lengths of freshwater pearl mussels found in the Melro canal (i.e. some specimens with more than 85 mm) and using a growth rate similar to the described for some populations in Portugal it is expected that some of these old specimens have, at least, 40 years (Varandas et al., 2013). Therefore, it is reasonable to think that these canals, including Melro, were stable and with enough water to maintain...
the individuals for at least the last four decades until the summer of 2017. Given the negligible mobility of freshwater pearl mussels they did not accompanied the decrease in the water levels during the 2017 drought and died stranded in their original position in the Melro canal. Although the individuals present in the water mill canals have an overall better physiological condition than in the natural habitat, the occurrence of the extreme drought resulted in high mortality rates, turning spurious all the possible benefits gained in the earlier decades after settlement. It was the occurrence of this extreme event that converted the Melro canal into an ecological trap. If that had not happened, we may have considered that these anthropogenic structures offer similar or even superior (given the higher CI and length measured) conditions than adjacent natural habitats.

This dichotomy of anthropogenic structures functioning as stable refuges or ecological traps have been widely discussed in the literature, including examples with freshwater mussel species (Araujo and Ramos, 2000). In one hand, critically endangered species such as Pseudunio auricularius (= Margaritifera auricularia) and Pseudunio marocanus (= Margaritifera marocana) have been described in anthropogenic structures such as irrigation canals (Gómez and Araujo, 2008; Sousa, personal observation). These structures seem to present stable conditions for the conservation of these species resulting in higher density and physiological condition in anthropogenic habitats in comparison to natural habitats (Gómez and Araujo, 2008; Sousa, personal observation). Conversely, these same studies mentioned that human activities such as regular cleaning and dredging are responsible for massive mortalities of these mussels in these anthropogenic habitats (Araujo and Ramos, 2000; Gómez and Araujo, 2008; Sousa, personal observation).

According to Robertson and Hutto (2006) effects generated by ecological traps can be mitigated by increasing the quality and stability of these habitats. In the particular case of this study, and from a management perspective, a careful monitoring of the water level during extreme droughts could guarantee the survival of many of the freshwater pearl mussels present in the water mill canals. In fact, if the Melro canal had been carefully managed in the summer of 2017, the registered mortality could have been much lower because there was enough water upstream of the weir to maintain the sufficient ecological flow to the canal. In alternative, translocating the freshwater pearl mussels to the adjacent suitable natural areas or even to laboratory facilities and posterior (after water levels return to normal conditions) reintroduction in the canal would guarantee the survival of most specimens. This management measures should ideally be supervised by experienced scientists together with local populations and volunteers in order to maximize the interest of the general public for the conservation of this species.

In addition, and although many of these water mills were constructed many decades ago, their presence may negatively affect several other aquatic organisms, including fish species, due to the fragmentation (i.e. presence of weirs) and disturbance (i.e. change in sediments) of habitats. These effects still persist nowadays even recognizing that many of these old water mills are now deactivated. These possible negative effects generated by the presence of water mills may discourage the re-introduction of freshwater pearl mussels in streams with these or similar anthropogenic infrastructures (see for example Zajac and Zajac, 2014). However, and as shown in this study, in almost pristine rivers where the water mills canals were constructed without destabilizing the sediments, these anthropogenic structures may have high conservation importance and may be used to re-introduce freshwater pearl mussels.

5. Conclusion

Water mill canals may function as a stable refuge for freshwater pearl mussels but if these anthropogenic habitats are not managed carefully and/or if subjected to extreme climatic conditions (droughts) may be transformed into ecological traps. As extreme climatic events are predicted to increase in number and intensity in the future, it will be interesting to assess how these anthropogenic habitats will function as an attractive sink for M. margaritifera. Only future assessments could give a definitive answer to this topic (i.e. anthropogenic structures acting as stable refuges or ecological traps), but given the conservation importance of M. margaritifera, and since the Tuela River is located in the southern edge of the species distribution, the anthropogenic structures in this system should be carefully monitored. In addition, the mortality occurred during the 2017 drought in the Melro Canal opens a unique opportunity to assess the possible re-colonization process by freshwater pearl mussels.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecolind.2019.105469.

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