Translocation as an ultimate conservation measure for the long-term survival of a critically endangered freshwater mussel

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Abstract Pseudunio auricularius (Spengler, 1793) is one of the most threatened unionid species worldwide. Translocation is considered one of the ultimate actions that can save this species from extinction in the Iberian Peninsula. Since 2013, massive mortalities have been recorded in the Canal Imperial de Aragón (CIA), an anthropogenic habitat where the highest density of P. auricularius had been recorded in Spain. An adequacy habitat index was calculated assigning scores to different environmental variables to select the most suitable river stretches receiving the translocated specimens. A total of 638 specimens have been translocated: 291 in 2017, 291 in 2018, and 56 in 2019. The first-year survival in the group of individuals translocated in 2017 was 41.6%. The next year, 95% of these specimens were found alive, suggesting a successful initial establishment. Specimens translocated in 2018 and 2019 showed a survival of c. 69% and 49%, respectively. In contrast, the control group left in CIA in 2017 showed a much lower survival rate of 19.7% after one year, which remained equally low during the next two years. Currently, the conditions in the Ebro River seem to allow a higher survival rate for P. auricularius than those in the CIA; nevertheless, future monitoring should confirm their long-term success.

Keywords Canal Imperial de Aragón · Ebro River basin · Extinction risk · Margaritiferidae · Pseudunio auricularius

Introduction

Humans have exerted increasing pressure on natural resources, which has a direct negative impact on wildlife (Hunter, 2007; IPBES, 2019). Freshwater ecosystems are not an exception, suffering intense degradation due to pollution, habitat destruction and fragmentation, overexploitation, climate change, and introduction of invasive species (Reid et al., 2019;
Birk et al., 2020), resulting in a high rate of biodiversity loss (Dudgeon 2019; Tickner et al. 2020). Freshwater mussels (Bivalvia, Unionida) are among the animal groups that have been most negatively affected by humans, their current situation being of major concern (Haag & Williams, 2014; Lopes Lima et al., 2014, 2017, 2018; Ferreira-Rodríguez et al., 2019; Böhm et al., 2021).

At present, the application of conservation measures to mitigate the loss of biodiversity due to anthropogenic impacts is common practice. One of these measures is the translocation of specimens, an increasingly applied practice during the last 30 years (Armstrong & Seddon, 2008; McMurray & Roe, 2017; Jourdan et al., 2019). The translocation of individuals has been used not only in order to save specimens at risk but also as a methodology to reintroduce species in locations where they had become extinct or to reinforce dwindling populations (Haag & Williams, 2014). According to the IUCN guidelines (IUCN/SSC 2013), the translocation of individuals for conservation purposes is “the intentional movement and release of a living organism where the primary objective is a conservation benefit.” As a concept, translocation seems a relatively straightforward task to carry out but, in fact, it needs a rigorous planning of all the steps involved in the process, including appropriate collection, handling and transport methods, assessment of habitat stability and suitable environmental and biological conditions in the recipient site, among others (Cope et al., 2003; Dunn et al., 2000; Luzier & Miller 2009; Moorkens 2017; Pires et al., 2020). In addition, any translocation plan should include a careful evaluation of the tradeoffs between conservation benefits and the costs and risks for the target species, so as for other species present in the recipient community (Cope & Waller, 1995; IUCN/SSC, 2013; Tsakiris et al., 2017; Brian et al., 2021). Finally, it is also essential to establish a long-term monitoring plan to assess the success or failure of the action (Luzier & Miller, 2009; Germano et al., 2015; Jourdan et al., 2019).

A recent review dealing with reintroduction attempts of aquatic macroinvertebrates concluded that these types of interventions are not as common as they are in terrestrial organisms, and that their viability as a conservation strategy has not been fully evaluated (Jourdan et al., 2019). These authors show that one important factor that may contribute to the failure of translocations is the complex life cycle of the target aquatic organisms. Freshwater mussels have a partly parasitic life cycle. Fertilized mussels release the larvae (glochidia) into the water and these attach to the gills or other body parts of specific host fish species. Once attached to the fish, they undergo a metamorphosis to become small juvenile mussels (see Modesto et al., 2018 for a revision). Therefore, for the specific case of freshwater mussels, any planned translocation always needs to take into account the presence of suitable host fish species to be able to succeed.

The translocation success of freshwater mussels is difficult to assess, and, in some cases, the methodology has been criticized as ineffective (Cope & Waller, 1995; Haag & Williams, 2014; Germano et al., 2015; Jourdan et al., 2019). For example, Dunn et al. (2000) reported an increase in the mortality of freshwater mussels when translocated at low temperatures. Stodola et al. (2017) reported variable survival depending on species and site. In other cases, high mortalities have been related to predation by raccoons or to specimens being washed away by large floods (Sousa et al., 2012; Stodola et al., 2017; Zając et al., 2019; Hart et al., 2021). Besides survival, sub-lethal effects have also been reported, including losing body condition, which may indicate poor adaptation to the new habitat (Hart et al., 2021).

Other authors obtained positive results when translocating freshwater mussels. For example, Tsakiris et al. (2017) obtained high survival rates for Quadrula houstonensis (Lea, 1859) and Amblema plicata (Say, 1817) (85 and 99%, respectively), emphasizing that site selection was a key element for success. The work of Valovirta et al. (1998) reports substantial differences in the survival of Margaritifera margaritifera (Linnaeus, 1758) when comparing translocations within the same river (survival above 90%) or between different rivers (50% or lower), which reinforces the importance of environmental and biological characteristics of donor and recipient localities (Jourdan et al., 2019).

The Giant Freshwater Pearl Mussel, previously known as Margaritifera auricularia (Spengler, 1793) and recently renamed as Pseudunio auricularius (Lopes Lima et al., 2018), is one of the most threatened freshwater mussel species worldwide (Prié et al., 2018; Soler et al., 2018), classified as Critically Endangered in the IUCN Red List (Prié, 2021).
Extinct in a large part of its ancient distribution area (Altaba, 1990; Araujo & Moreno, 1999; Araujo & Ramos, 2000), this species is nowadays present in five hydrographic basins: Ebro River (Spain) and Garonne, Vienne-Creuse, Adour, and Charente Rivers (France) (Prié et al., 2018; Soler et al., 2018).

In Spain, *P. auricularius* has a restricted distribution, being only present in the Ebro River basin (Altaba, 1990; Altaba et al., 2001; Araujo & Ramos, 2000; Altaba & López, 2001). The Canal Imperial de Aragón (CIA, Ebro River basin, Spain, for more details see Gómez & Araujo, 2008) was known to harbor around 6000 tagged specimens. Since 2013, the CIA population has suffered a severe and rapid decline estimated as approximately 80% of individuals lost in 2019 (Nakamura et al., 2018a; Guerrero et al., 2021). The causes of this mortality are still under study and may encompass (1) absence of host fish due to the extinction of the original host, the common sturgeon (*Acipenser sturio* Linnaeus, 1758) in the Ebro Basin (Araujo et al., 2001, 2003; López & Altaba, 2005) and decline of the river blenny (*Salarias fluviatilis* (Asso, 1801)), the only recognized native host fish still present in the Ebro basin for *P. auricularius* (Araujo et al., 2001, 2003), reaching the point of being cataloged as endangered species in the Aragón region (Abad Ibañez & Ginés Llorens, 2020); (2) artificial regulation of river flow due to the construction of dams for hydroelectric power plants and water withdrawals (Araujo & Álvarez-Cobelas, 2016), causing high mortalities; (3) pollution causing lethal or sub-lethal effects in freshwater mussels (Nakamura et al., 2021); (4) possible diseases or parasites (Guerrero et al. 2021); and (5) invasion by non-native species competing for the same resources than native mussels (Gimeno et al., 2017), including the zebra mussel (*Dreissena polymorpha* (Pallas 1771)) and the Asian clam *Corbicula fluminea* (Müller, 1774), and by fish such as *Silurus glanis* Linnaeus, 1758, *Ameiurus melas* ( Rafinesque, 1820), and *Gambusia holbrooki* Girard, 1859 that can compete with or prey on the river blenny (Araujo et al., 2003; López & Altaba, 2005; Araujo & Álvarez-Cobelas, 2016).

Taking into account the high risk of extinction of this important population, the Regional Government of Aragón implemented an emergency action plan for the conservation of *P. auricularius*, discussing its pros and cons with scientific experts, managers, companies, and NGOs working with freshwater mussels. The results of these discussions determined that the best option to preserve the last alive specimens in the CIA was the translocation of adult specimens from this anthropogenic habitat to the natural environment of the Ebro River. Although this river was in the past the main habitat for the species and the source of specimens colonizing the CIA (Haas, 1916a, b, 1917; Azpeitia, 1933), only a few dozen specimens were known to occur currently in the Ebro River (Regional Government of Aragón, unpublished data). Nowadays, environmental conditions in the Ebro River, unlike in the CIA, allow the presence of freshwater mussels [*Potomida littoralis* (Cuvier, 1798), *Anodonta anatina* (Linnaeus, 1758), and *Unio mancus*, Lamarck, 1819], including juvenile specimens, indicating recent recruitment (Nakamura and Guerrero pers. observ.). Although some stretches of the Ebro River are heavily disturbed, it is still possible to find localities that maintain good conditions for freshwater mussels and therefore for *P. auricularius*.

Considering this adverse conservation scenario and the unique opportunity provided by the implementation of an emergency action plan developed by the Regional Government of Aragón, this study assessed the survival probability of *P. auricularius* translocated from CIA to selected localities in the Ebro River, with the final goal of increasing its long-term survival.

**Materials and methods**

Selection of donor and recipient localities

During 2016 a first evaluation was carried out at the CIA to choose the localities from which the specimens would be translocated. We reviewed the census information on *P. auricularius* along the entire canal and choose a section from kilometer 32 to 86, where the density was higher. Then we selected the specific donor localities based on the abundance of alive *P. auricularius* and easy access to the canal. Basic autecological data, including number of specimens and biometric measurements (length, width, height and weight), plus water depth and physical and chemical variables, including pH (± 0.01), conductivity (± 0.01 μS/cm), dissolved oxygen (± 0.1%DO), and temperature (± 0.1 °C), were measured using
a multiparametric Thermo Scientific™ ORION portable meter. Nitrite (± 0.05 mg/L), ammonium (± 0.1 mg/L), and phosphate (± 0.1 mg/L) concentrations were estimated using colorimetric kits (Visocolor® ECO). These variables were measured when the specimens were extracted from the canal and again when they were introduced to the new localities in the river.

In the Ebro River, a preselection of possible recipient localities was started during 2016 and continued in the summer of each following year, until 2019. Twelve localities were initially selected based on 1) aerial photographs taken during previous years (1996–2016, www.ign.es/web/comparador_pnoa/index.html) to assess the stability of the river stretches and avoid localities subjected to strong sedimentation or erosion and 2) technical reports and previous studies (Gómez & Araujo, 2008; Araujo et al., 2009; Araujo & Álvarez-Cobelas, 2016) where the presence of freshwater mussels (Unionids) such as *P. littoralis*, *U. mancus*, or *P. auricularius* had been confirmed. Only one evaluated locality was outside the main channel of the Ebro River: its tributary, the Vero River, near Castillazuelo village (not shown on the map, Fig. 1). It is a Pyrenean stream located to the North of the main study area, 90 km away from the main course of the Ebro River, with the presence of high densities of *P. littoralis* (Regional Government of Aragón, unpublished data), and which has no major pollution problems as is the case in the Ebro River main channel and its close connected canals.

An adequacy index was designed to evaluate the preselected localities. The aim was to integrate as many variables as possible that could affect the survival of *P. auricularius*. Localities with previous presence of the species or at least the presence of other living mussel species were especially taken

![Fig. 1 Ebro River and Canal Imperial sections where recipient and donor localities were selected, respectively (Aragón region, Spain)](image-url)
into consideration. The range of values for each variable included in the index was based on available literature about the optimum habitat conditions for the species (Altaba, 1990, 2001; Araujo & Ramos, 2000; Araujo & Álvarez-Cobelas, 2016; Soler et al., 2018) and on 20 years of experience gathered in several projects aiming at conserving the species, both in the natural environment and in captivity (Wantzen & Araujo, 2018; Nakamura et al. 2018b, 2019, 2021). In each recipient locality, a plot (surface area 8–20 m²) was chosen to assess ten physical and chemical variables, plus four biological and five anthropogenic variables (Table 1). First, the presence of mussels and especially P. auricularius was confirmed in the plot. The variable “Gravel substrate cover (%)” was visually estimated. We placed a 30 × 30-cm square (replicated 3 times) onto the bottom of the river and registered the substrate percentage cover inside each square looking through an aquascope and before inspecting the substrate to sample the mussels. We used an adapted sediment classification described in Gibson et al. (1998): boulders (64–256 mm), gravel (2–64 mm), sand (0.06–2 mm), and silt (<0.06 mm). The percentage of macrophyte cover and dark sediments (as a proxy for anoxia) on the substrate surface was also visually assessed for each plot. A 0.5-m-wide transect, in the middle of the plot and across its length, was used to assess the density of native unionid mussels. All specimens, alive or dead, were extracted from the transect by palpating the entire surface and, once extracted, they were identified to species level and their density was estimated taking into account the transect area (length of the plot×0.5 m wide). Living specimens were left back in the transect, naturally

### Table 1 Limiting environmental values of P. auricularius used for the selection of recipient localities in the Ebro River. Based on Altaba (1990, 2001), Araujo and Ramos (2000), Araujo et al. (2001, 2003); Araujo & Álvarez-Cobelas (2016), Nakamura et al. (2018b, 2019), Soler et al. (2018), and Wantzen and Araujo (2018)

| Variable                                      | Variable code | Less appropriate (Score = 1) | Appropriate (Score = 3) | Optimum (Score = 5) |
|-----------------------------------------------|---------------|-------------------------------|-------------------------|---------------------|
| **Physical and chemical variables**           |               |                               |                         |                     |
| pH                                            | pH            | < 7.7 or > 8.5                | 7.7–7.9 or 8.2–8.5      | 7.9–8.2             |
| Conductivity (µs/cm)                          | EC            | < 400 or > 2200              | 400–1000 or 1400–2200   | 1000–1400           |
| Dissolved oxygen (%)                          | DO            | < 70                         | 70–90                   | > 90                |
| Nitrate (mg/L)                                | NO3           | > 10                         | 5–10                    | < 5                 |
| Nitrite (mg/L)                                | NO2           | > 0.5                        | 0.05–0.5                | < 0.05              |
| Ammonium (mg/L)                               | NH4           | > 0.5                        | 0.3–0.5                 | < 0.3               |
| Phosphates (mg/L)                             | PO4           | > 0.5                        | 0.3–0.5                 | < 0.3               |
| Depth in summer (cm)                          | DIS           | < 40                         | 40–60                   | > 60                |
| Gravel substrate cover (%)                    | GSC           | < 40                         | 40–60                   | > 60                |
| Dark sediment (%)                             | DS            | > 50                         | < 50                    | 0                   |
| **Biological variables**                      |               |                               |                         |                     |
| Freshwater mussels density (ind/m²)           | FWMd          | < 1                          | 1–2                     | > 2                 |
| Macrophyte cover (%)                          | MC            | 50–100                       | 10–50                   | < 10                |
| Alive Asian clam density (ind/m²)             | ACd           | > 500                        | 200–500                 | < 200               |
| Previous P. auricularius density (ind/m²)     | Pa_d          | 0–0.1                        | 0.2–0.5                 | > 0.5               |
| **Anthropogenic variables**                   |               |                               |                         |                     |
| Distance to nearby agricultural activity (km)  | DAA           | < 0.5                        | 0.5–1                   | > 1                 |
| Distance to nearby urban treatment plant outlets (km) | DURB       | < 0.5                        | 0.5–1                   | > 1                 |
| Distance to nearby villages (km)              | DV            | < 0.5                        | 0.5–1                   | > 1                 |
| Fishermen presence (nº encounters)            | FP            | > 3                          | 1–3                     | 0                   |
| Accessibility to the point                    | AP            | Difficult                    | Medium                  | Easy                |
buried in the substrate. In addition, three samples were extracted (initial, middle, and end of the transect; variable length in each plot) using 30×30-cm squares to assess the density of *C. fluminea*. Once the samples were taken, living and dead specimens were separated to estimate their mean density along the transect. Anthropogenic variables were also taken into account and included the following: nearby human activities such as agriculture and outlets from treatment plants and nearby villages, measuring in both cases the distance in kilometers from the plot to the agricultural field or to the village/city on an orthophoto. Fishermen presence was considered as a threat to the translocation process, first because the location of the new mussels could be noticed and consequently the mussels could suffer acts of vandalism and second because fishermen are expected to enter to the plot to fish and inadvertently disturb the specimens. So, the higher the presence of fishermen, the lower the score that was given to a locality. Furthermore, the variable “accessibility to the point” was assessed, providing a positive value for the general evaluation score, i.e., the easier the access, the higher the score. Estimating the access to a sampling site as “easy” did not necessarily mean direct access to the shore through a path (which could be used by fishermen), but rather implied that there were no obstacles (trunks, trees, large stones) or very deep areas, concluding that the site was easily wadable for translocation works. Values of each selected variable were classified into three ranges with an associated score: less appropriate (1 point), appropriate (3 points), and optimum (5 points) conditions (Table 1). The sum of values for all variables resulted in a global score for each locality, which allowed us to choose those with higher scores. Although all the variables were evaluated in the same way to avoid biases, a single variable could be decisive in discarding a locality if it was totally out of range. For example, the presence of sediments with signs of anoxia or the absence of alive mussels were decisive factors to discard a locality.

The preselected localities (Fig. 1) were evaluated during three different years: L1–L9 in 2016, L10 in 2017, and L11–L12 in 2018. They were usually evaluated one year before proceeding with the translocation of specimens.

Translocation procedure

Translocations were carried out in autumn, between October and December each year, to avoid extreme air or water temperatures, as well as the most sensitive reproductive period of the species, which in *P. auricularius* occurs in February–March in Spain (Grande et al. 2001). For the procedure we followed these steps: (1) Physical and chemical variables were measured in both, the donor and recipient localities the same day of the translocation, in order to confirm that all the variables were within the optimal range for the species. (2) In the CIA, we chose preferentially those localities that harbored a large number of specimens, in order to leave a control group in that locality. Specimens to be translocated were mostly extracted on foot by direct searching of the bottom of the canal and using an aquascope, while on other occasions they were collected by divers due to high water levels. Taking into account the low number of remaining specimens and the high mortality in the CIA, the option of leaving a control group for each year of extraction was not considered, so that a single control group was set in the first year of the translocation (2017). (3) Specimens were selected randomly and verifying that they presented good external conditions (without bumps or breaks in the shell and being well-buried in the substrate). Each group of specimens that was extracted from the canal was distributed in more than one locality to avoid losing the survival information of the entire group in case of any local stochastic event. (4) Collected specimens were marked as quickly as possible using two identical numbered tags, one on each side of the shell. The labels were glued to the flattest part of the shell below the umbo with cyanoacrylate glue. This procedure was done for both groups of specimens: the translocated ones and those that remained as control group. In addition, we fixed an external PIT-tag (Passive Integrated Transponder, FDX-B 12×2.12 mm, Loligo Systems®) on the translocated specimens. This PIT-tag was glued onto the posterior end of the shell to facilitate its reading (due to the position adopted by the adult mussel, half of the shell buried in the substrate and leaving the posterior area out of the sediment). We used cyanoacrylate glue during the first-year campaign, but the following years we used a two-component epoxy adhesive (Superite®) instead (see Results).
was used to record the PIT-tag number and associate it to the plastic tag number that had been previously assigned to each specimen. During the first year we used an antenna that was only 0.6 m long, but later on we changed to using a 1.5-m antenna, allowing to sweep a larger and deeper area during field surveys, although the detection sensitivity was the same (15 cm). In addition, before translocation, a small permanent mark was made to each specimen with a Dremel® directly on the shell and using a different symbol for each CIA donor locality, which would allow us in future to assess the survival in the river in relation to a particular donor locality. Biometric data from each specimen were collected using a manual caliper (±0.05 mm), including shell length, height, and width, so as wet weight with a field scale (Nahita Blue 5171, ±0.1 g). (5) The collected specimens were kept moist using towels soaked in CIA water and ice to cool the box in which they were transported, but avoiding direct contact with it. The number of specimens in the boxes were kept low to avoid shell damage. Transportation was done as quickly as possible. (6) In the recipient localities, the exact final location of the translocated specimens was previously marked with wooden stakes forming a square plot (8–20 m²). (7) The specimens were placed in their natural position partially buried in the substrate with the help of a garden shovel to open a hole in the substrate. Once the process had finished, location information (UTM coordinates plus identification of landmarks) was taken.

Post-translocation actions

Previous studies recommended permanent monitoring of translocated specimens (Dunn et al., 2000; Jourdan et al., 2019; Luzier & Miller, 2009; IUCN/SSC, 2013). In large rivers, such as the Ebro River (Spain), this is a difficult task due to the harsh environmental conditions (high depth, poor visibility, and winter floods), an issue that can be even more complicated for species that live buried, such as freshwater mussels (Prié et al., 2018; Hernández et al., 2021). In our case, it was not possible to monitor the specimens during high water levels. Therefore, monitoring was carried out once a year during the river’s driest period (July to September). At the same time, annual assessment of control localities (from CIA) were undertaken during October–November, taking advantage of maintenance works and consequent lower water levels in the CIA. Physical and chemical variables (as described above) were measured at every visit (at least twice a year). Mussel monitoring was carried out by direct observation of the specimens with the help of an aquascope to determine if they were alive or not. For this reason, we waited for the time of the year with the best conditions: low flow, no wind, and no rain that could increase water turbidity through sediment input. Each detected specimen was marked with a small red flag placed next to it to indicate its position and facilitate its identification by means of distance reading its electronic PIT-tag, so avoiding manipulation of specimens. In some plots with a high percentage of silty substrate that was easily resuspended, we used two ropes placed perpendicularly in the center of the plot to divide it into four squares. In this way, we were able to search each square carefully to prevent any visible specimen from going unnoticed. Once the PIT-tag was read, the flag was removed, thus avoiding potentially reading the same specimen several times. If the specimen was dead (with an open shell) it was removed from the plot and recorded. The number of not detected specimens was calculated by summing the numbers of living and dead specimens found and subtracting this from the total number of initially translocated specimens.

All recipient localities were surveyed every year, accounting for a maximum period of three years for those translocated in 2017 and a minimum of one year for those translocated in 2019. The exception was locality L3 that could not be surveyed in 2020, due to high water level. So, we excluded this locality from the calculation of survival for that year. Survival (and mortality) percentages were estimated as the sum of all living (or dead) specimens found that corresponded to a particular year of translocation, divided by the total number of individuals translocated that year and multiplied by 100. The percentages of not detected (ND) individuals were correspondingly calculated as the number of missing individuals (i.e., total minus the sum of dead and living individuals found) that had been translocated a particular year, divided by the total number of individuals translocated that year and multiplied by 100. Chi-square ($\chi^2$) tests were used to check for differences in the frequency between live and dead specimens translocated
to the river in 2017 versus the control specimens that remained in the canal the same year, by means of the software SPSS Statistics v.23.

Given the peculiar life cycle of *P. auricularius*, in which the glochidium larvae need to parasitise a suitable fish host (Araujo et al. 2001; López & Altaba 2005; Modesto et al. 2018), particular attention was devoted to this factor. In order to increase future recruitment of *P. auricularius*, we also translocated its known fish host in the Ebro River, the freshwater river blenny (*Salaria fluviatilis*) (Araujo et al. 2001) to four recipient localities, with individuals coming from the Canal de Monegros. Maintenance works are performed annually in this canal located in Huesca province, north of the study area. In this canal, a high density of the river blenny had been reported (Abad Ibañez & Ginés Llorens, 2020) and in October 2019 specimens were collected using hand nets in some shallow stretches of the canal. Specimens were collected in the morning, keeping them in several tanks with water from the same canal and aeration and in the afternoon, they were transported directly to the recipient localities, where the specimens of *P. auricularius* had already been translocated. The fish were released after a process of acclimatization, for at least two hours, in water from the recipient locality.

Results

Selection of donor and recipient localities

The best localities for freshwater mussel translocation, according to our index score, were located in the middle Ebro River, upstream from the city of Zaragoza up to Novillas (Fig. 1). The selected six localities were L1, L2, L3, L10, L11, and L12 according to their higher scores (Table 2).

Out of the six selected localities, those that obtained the best results were L2, L1, and L10 (71, 69, and 69 points, respectively) (Table 2). We chose five of the six localities with the highest scores, with the exception of the selected site L3, which had a lower score (55 points). We decided to include L3, despite having 90% macrophyte cover and a moderate-high density of Asian clam (837 ind/m²), because it was the locality in which the highest number of young *P. auricularius* had been found (this information not being known previously). We found three new specimens in 2017 when we evaluated the plot and two more in 2018, with shell lengths ranging between 10 and 13 cm. On the other hand, L9 was the only locality discarded with a high score (75 points), due to its location in a tributary and not in the main channel of the Ebro River and also because, historically, it was not within the range of the natural distribution of the species. Despite this situation, we evaluated this locality since it might serve as a backup locality in the case the Ebro River would not work during the first year and mortality in the CIA increased to the point of having to save the last living specimens. Finally, we discarded all other localities because either they suffered desiccation in summer (L8), had 100% macrophyte cover (L4 and L5), sediments presented signs of anoxia (L7), or had very high densities of the invasive *C. fluminea* (L6, L7, and L8) (Table 2).

Results of physical and chemical variables were similar between donor and recipient localities, with the exception of phosphates in L12 (0.7 mg/l) and a relatively high value of conductivity in L3 (2676 µs/cm). The substrate of the selected recipient localities was dominated by gravel and sand, and only in L1, L3 and L10 small patches of finer sediment (silt and clay) were observed, especially near the margins where tree logs were present.

Freshwater mussel densities in the selected localities varied between 0.8 and 7.0 ind/m², with maximum values in L11 (7.0 ind/m²) and L1 (4.9 ind/m²), with the presence of *P. littoralis* and *U. mancus*, and L2 (4.5 ind/m²) with only *P. littoralis*. Before the translocation, *P. auricularius* specimens were detected in L1, L3, and L10 with densities of 0.15, 0.25, and 0.07 ind/m², respectively. Live Asian clam densities were also highly variable, reaching maximum values of 1215 ind/m² in L1 and minimum values of 241 ind/m² in L12 (Table 2).

All localities were affected by nearby agricultural activities. L3 and L12 were also subjected to nearby sewage discharge, but always downstream from the selected translocation plot. The proximity to villages or to the city of Zaragoza was also evaluated and only localities L3 and L12 were less than 1 km away. The presence of fishermen was common along the river, especially in sites L2, L3, and L11.
Table 2 Mean values and scores of the characterization values of limiting variables and other characteristics in CIA control localities and in the Ebro River recipient localities (Score results between brackets, selected localities in **bold**, NA: not available). Variable codes as in Table 1

| Variables | CIA | Controls | L1 | L2 | L3 | L4 | L5 | L6 | L7 | L8 | L9 | L10 | L11 | L12 |
|-----------|-----|----------|----|----|----|----|----|----|----|----|----|-----|-----|-----|
| **Physical and chemical** |     |          |    |    |    |    |    |    |    |    |    |     |     |     |
| pH        | 8.20 (5) | 8.03(5) | 8.06 (3) | 7.84 (3) | 7.99 (5) | 8.12 (5) | 8.26 (3) | 7.95 (5) | 7.99 (5) | 8.16 (5) | 7.72 (3) | 7.77 (3) | 7.93 (5) |
| EC        | 1829 (3) | 1948 (3) | 2060.5 (3) | 2676 (1) | 1719(3) | 2135(3) | 1681(3) | 1657(3) | 1510(3) | 605.2 (3) | 1977.5 (3) | 2177.5 (3) | 1930.5 (3) |
| DO        | 117 (5) | 99.8 (5) | 105 (5) | 108 (5) | 107 (5) | 109(5) | 134.8 (5) | 105 (5) | 94.8(5) | 90(5) | 103.1 (5) | 111.5 (5) | 100.2 (5) |
| NO3       | 0 (5) | 10 (3) | 0 (5) | 5 (3) | NA | 10(3) | 5 (3) | 5 (3) | 5 (3) | 1 (5) | 0 (5) | 5 (3) | 0 (5) |
| NO2       | 0.03 (5) | 0.02 (5) | 0.04 (5) | 0.05 (3) | 0.10 (3) | 0.03 (3) | 0.05 (3) | 0.03 (5) | 0 (5) | 0.01 (5) | 0.03 (5) | 0.06 (3) | 0.02 (5) |
| NH4       | 0 (5) | 0 (5) | 0 (5) | 0 (5) | 0 (5) | 0.2 (5) | 0.2 (5) | 0 (5) | 0.1 (5) | 0.15 (5) | 0.10 (5) | 0.08 (5) |
| PO4       | 0.10 (5) | 0.10 (5) | 0 (5) | 0 (5) | 0 (5) | 0 (5) | 0 (5) | 0.7 (1) | 0 (5) | 0.2 (5) | 0 (5) | 0 (5) | 0.7 (1) |
| DIS       | 300–400 (5) | 44 (3) | 51 (3) | 47 (3) | 39 (1) | 50 (3) | 58 (3) | 30 (1) | 37 (1) | 30 (1) | 62 (5) | 45 (3) | 62 (5) |
| GSC       | 60 (3) | 40 (3) | 40 (3) | 45 (3) | 70 (5) | 35 (1) | 50 (3) | 40 (3) | 30 (1) | 15 (1) | 26 (1) | 21 (1) | 75 (5) |
| DS        | 0 (5) | <50 (3) | 0 (5) | <50 (3) | <50 (3) | <50 (3) | >50 (1) | <50 (3) | <50 (3) | <50 (3) | 0 (5) | 0 (5) | 0 (5) |
| **Subtotal** | 46 | **40** | **44** | **34** | **36** | **36** | **32** | **36** | **38** | **40** | **36** | **44** |    |
| **Biological** |     |          |    |    |    |    |    |    |    |    |    |     |     |     |
| FWMd      | 0.02 (1) | 4.9 (5) | 4.5 (5) | 1.4 (3) | 2 (3) | 2 (3) | 3.6 (5) | 4.9 (5) | 1.4 (3) | 5 (5) | 0.8 (1) | 7 (5) | 0.9 (1) |
| MC        | 10 (5) | 80 (1) | 90 (1) | 90 (1) | 100 (1) | 100 (1) | 70 (1) | 40 (1) | 30 (3) | 0 (5) | 10 (5) | 30 (3) | 60 (1) |
| ACd       | 1017 (1) | 1214.8 (1) | 585.2 (1) | 837 (1) | 74 (5) | 20.7 (5) | 2450 (1) | 2722.2 (1) | 2855.5 (1) | 0 (5) | 766.8 (1) | 359.3 (3) | 240.7 (3) |
| Pa_d      | 0.08 (1) | 0.15 (3) | 0 (1) | 0.25 (3) | 0 (1) | 0 (1) | 0 (1) | 0.04 (1) | 0 (1) | 0.07 (1) | 0 (1) | 0 (1) | 0 (1) |
| **Subtotal** | 8 | **10** | **8** | **8** | **10** | **10** | **8** | **8** | **8** | **16** | **8** | **12** | **6** |
| **Anthropogenic** |     |          |    |    |    |    |    |    |    |    |    |     |     |     |
| DAA       | <0.5 (1) | 0.06 (1) | 0.09 (1) | 0.28 (1) | 0.1 (1) | 0.1 (1) | 0.32 (1) | 0.09 (1) | 0.02 (1) | 0.03 (1) | 0.05 (1) | 0.1 (1) | 0.05 (1) |
| DURB      | >2 (5) | >1 (5) | >1 (5) | 0.1 (1) | >1 (5) | 1.5 (5) | 0.34 (1) | 0.05 (1) | 0.88 (3) | >1 (5) | >1 (5) | >1 (5) | 0.1 (1) |
| DV        | >2 (5) | 3 (5) | 1.8 (5) | 1.3 (5) | 4 (5) | 1.4 (5) | 3.4 (5) | 0.4 (1) | 1.1 (5) | 1.2 (5) | 1.6 (5) | 1.8 (5) | 0.3 (1) |
| FP        | >3 (1) | 1 (3) | 2 (3) | 2 (3) | 0 (5) | 1 (3) | 1 (3) | 1 (3) | 0 (5) | 0 (5) | 2 (3) | 0 (5) |    |
| AP        | Easy (5) | Easy (5) | Easy (5) | Medium (3) | Difficult (1) | Medium (3) | Easy (5) | Easy (5) | Easy (5) | Easy (5) | Easy (5) | Easy (5) | Easy (5) |
| **Subtotal** | 17 | **19** | **19** | **13** | **17** | **17** | **15** | **11** | **17** | **21** | **21** | **19** | **13** |
| **Total scores** | 71 | **69** | **71** | **55** | **62** | **63** | **59** | **51** | **61** | **75** | **69** | **67** | **63** |
Translocation results

A total of 638 adult specimens (mean shell length ± SD for 2017 = 153.0 ± 7.3 mm, 2018 = 152.7 ± 7.1 mm, 2019 = 151.6 ± 9.1 mm) were randomly translocated during the three years to the 6 selected localities: 291 specimens in 2017; 291 in 2018; and 56 specimens in 2019. In the CIA, six control groups (203 specimens) were established in 2017 and their survival assessed during the following three years.

In the group translocated in 2017 we found a significant loss of PIT-tags (approximately 50%) in two of the three selected localities. This situation was possibly related to either the extraordinary winter flood occurred in April 2018 (2037 m³/s) (Fig. 2) or to the initial method of PIT-tag attachment, as we stuck them with cyanoacrylate glue. However, in 2018 and 2019 we used epoxy glue instead for the transponders, obtaining almost zero loss during the next floodings. No loss of both plastic labels at the same time was detected.

The mean recovery rate of translocated specimens after the first year was 64.5%, but variable between years of translocation; 48% for specimens translocated in 2017 (and the same for control groups in the canal), 91% for those translocated in 2018, and 54% for individuals translocated in 2019. On the other hand, the mean percentage of specimens not being recovered within the three years and after one-year post-translocation was 35.5%, but with high differences between translocation years (52% for 2017, 8.66% for 2018, and 46% for 2019.

For the specimens translocated in 2017, the overall minimal survival (“minimal” because not all the translocated specimens were found, so we may still expect some undetected specimens to be alive) after one year was 41.6% considering all individuals from different localities together. Separately for each locality, it was 60.2% (n = 118) for L1, 7.5% (n = 109) for L2, and 31.3% for L3 (n = 64). For the specimens translocated in 2018, overall survival was 68.7%, with local survival values of 61% for L1 (n = 218), 96.4% in L2 (n = 28), and 88.9% in L10 (n = 45). After the

![Fig. 2](Fig. 2) Ebro River flow (m³/seg) during the study years (2016–2020) (blue arrows: translocation actions; red arrows: field survey of the previous year(s) translocation(s); stars: evaluation and selection of the plots)
second year, 95% and 87% of those specimens surviving the first year were again found alive for individuals translocated in 2017 and 2018, respectively. The survival of specimens translocated in 2019, after one year, was 48.7% in L10 (n = 37) (Fig. 3), excluding data from locality L3 (n = 19, not accessed due to the high water level; see above). Accumulated survival during the second and third years for specimens translocated in 2017, slightly decreased (39.9% in 2019 and 37.1% in 2020). The percentage of specimens that were not detected (ND) was higher than 50% during the three years for this group of specimens and their (accumulated) mortality values reached 11% during the third year. For the specimens translocated in 2018, survival decreased from 68.7% the first year to 60.4% the second year. The percentage of not detected specimens rose from 8.6% the first year to 15.6% the second year. Mortality slightly increased (first year = 22.7%, second year = 24.0%), compared to the initial mortality value. Specimens translocated in 2019 (for which only one locality could be evaluated) showed a survival rate very similar to the proportion of not detected specimens (alive = 48.7%, ND = 46.0%) and only 5.4% of the specimens were found dead. If we only consider the recovered individuals, the estimated average mortality rate was 18% during the first year of translocation.

The control groups presented a very low survival (Fig. 3). A survival of only 19.7% was recorded after the first year, with a mortality rate of 27.6% and more than 50% of specimens not detected (ND = 52.7%). The second year, survival slightly increased to 23%, but there was also an increase in mortality, rising up to 40%. In the third year, survival remained at 19% and mortality increased to almost 50% and 34% of specimens were not detected. In the group translocated in 2017, survival was significantly higher in the three studied years when compared with the control specimens left in the canal the same year (n\textsubscript{2017} = 494; first year: $\chi^2 = 26.049$, $P < 0.001$, second year: $\chi^2 = 6.140$, $P < 0.001$, third year: $\chi^2 = 8.138$, $P < 0.001$).
A total of 500 specimens of river blenny were captured in Canal de Monegros and released in four localities: L1 (196 ind), L2 (112 ind), L10 (80 ind), and L11 (112 ind).

Discussion

Mitigation translocation is defined by Bradley et al. (2020) as a type of conservation translocation which has the immediate objective of relocating specimens threatened with death. Armstrong and Seddon (2008) reported a substantial increase in publications related to reintroductions and analysis of their effectiveness through subsequent monitoring using different types of organisms. Also Bradley et al. (2020) reviewed 59 examples of studies that had been carried out to assess the best management options to improve these techniques in future. Clearly, there is a growing scientific interest in translocations, despite its controversy. One of the reasons for such controversy is the scarcity of information on translocation results, due to the lack of long-term monitoring for most of the completed translocation actions (Cosgrove & Hastie, 2001; Haag & Williams, 2014; Germano et al., 2015; Tsakiris et al., 2017; Jourdan et al., 2019).

Translocation of P. auricularius was assumed by the Aragón governmental authorities as an emergency task in order to save the last living specimens found in the CIA. This, in addition to captive breeding (Nakamura et al. 2019), is probably one of the few remaining options to avoid the extinction of the species in Spain. In the CIA, the protection of P. auricularius and other mussel species is a conservation issue with conflicting interests. On one hand, there is the utilitarian purpose of the canal used to provide water mainly for agriculture and to small villages and the city of Zaragoza. On the other hand, this canal supports a high biodiversity of organisms and their survival is not fully compatible with regular maintenance works. Reaching a balance between the two sides is complex and, at present, the CIA has reached a point of being unsustainable for mussel survival (Guerrero et al. 2021).

Even with a high mortality rate, P. auricularius has become the only species of freshwater mussel that still inhabits the CIA, since P. littoralis, A. anatina, and U. mancus have all disappeared (Guerrero et al. 2021). All the described disturbances may have turned the canal into an ecological trap for freshwater mussel species (Sousa et al. 2021). The CIA is nowadays an habitat invaded and modified by the Asian clam and the zebra mussel, as well as by a non-native fish directly affecting the mussels reproductive cycle. Wels catfish (Silurus glanis) and pike-perch [Sander lucioperca (Linnaeus, 1758)] are some of the non-native fish present in the canal that compete and prey on native hosts of the freshwater mussels (Soler et al. 2019), such as the river blenny and barbels (Barbus graellsii Steindachner, 1866, B. haasi Mertens, 1925).

We obtained better survival results than expected for the translocated specimens, considering the undergoing process of rapid mortality of P. auricularius in the canal. The ecophysiological condition of the translocated specimens, although not assessed, was assumed to be weak (Fig. 4). In addition, the results of the control groups that were left in the canal confirmed that mortality had not stopped there, showing an increasing mortality reaching almost 50% after three years. Cope & Waller (1995) did an extensive review of translocations with freshwater mussels carried out in the USA in the 1980s and early 1990s, reporting an average rate of recovered specimens of 43% and an average mortality rate (estimated considering only the recovered individuals) of 49%. In comparison, our results showed a higher recovery rate (65%) and a much lower mortality rate (18%) in the translocated habitats and, consequently, we may conclude that the decision to translocate the specimens probably was the best conservation option at this time.

Translocation should be, in all cases, one of the last options to consider for the protection of species that suffer a critical situation. However, where local population declines have the potential to put in danger the persistence of the species, as is the case in P. auricularius in the CIA, translocation should be planned and performed (Fig. 4; Hart et al., 2021).

Management implications and risk analysis

The success of a translocation action depends on many factors and one of the hardest is the selection of the recipient localities. The adequacy habitat index implemented herein, using what we considered important variables to maximize the establishment and survival of P. auricularius and based on previous information and expert knowledge on its habitat
preferences, seems to have worked adequately. Nevertheless, more tests using this tool would be needed to confirm its applicability in other contexts or for other species. Furthermore, there are more variables that could be added for future evaluations, e.g., hydrodynamics and hydromorphology (Geist, 2010; Holmgren, 2022), or the previous presence of the host fish in the area (Araujo et al., 2001; López & Altaba, 2005). In addition, the riparian cover should also be evaluated, as it may affect the growth of macrophytes and filamentous algae that negatively impact on mussel survival (Wilson et al., 2011). Finally, the presence of predators such as the red swamp crayfish [Procambarus clarkii (Girard 1852)] (Meira et al. 2019), beavers (Castor fiber Linnaeus 1758) (Rudzīte, 2005), or fish, such as carp (Cyprinus carpio Linnaeus 1758) (Feo et al., 2017), can provide extra information related to mussel mortality.

The percentage of not detected specimens is usually high in translocation monitoring (Cope & Waller, 1995; Fischer & Lindenmayer, 2000; Tsakiris et al., 2017; Zajac et al., 2019) and our study was no exception. The high percentage of not detected specimens may be masking part of the survival-mortality results, even though the specimens were marked with PIT-tags in order to facilitate its subsequent location in the river (Kurt et al., 2007). Prié et al. (2018) report for P. auricularius a detection of 75% in the Charente River using diving. Our study reported slightly lower values (62%), but using a different methodology (i.e., aquascope surveys). Besides the possible influence of the method used, we have identified two other possible causes of this higher percentage of non-detection: some of the specimens may have been outside the plot (washed away during floods) and/or they were totally buried at the time of monitoring. Hernández et al. (2021), in their study on detectability, state that the variables that most affect the movement behavior of mussels are temperature and the type of substrate. In the Ebro River we had access to the plots only in summer, when water temperature was higher and the mussels were more active; therefore it is quite possible that many specimens were outside the plot going unnoticed. Consequently, only long-term monitoring including also areas outside the plots will allow a more accurate evaluation of the recover rate.

When selecting localities for translocation, it is important to take into account the effects of large flood events (Fischer & Lindenmayer, 2000; Jourdan et al., 2019; Hart et al., 2021). The extraordinary flood that occurred in the Ebro River in spring (April 2018, Fig. 2) just following the initial translocation a few months earlier caused that 72% and 58% of the specimens were lost in localities L2 and L3.
respectively, without the possibility of finding them the following summer, even when a great effort was made to search them some kilometers downstream from the translocation point. It was even noted that the plot was mostly free of Asian clams, which surely were also affected by the flood. Zająć et al. (2019) studied the dispersal and mortality of translocated thick-shelled river mussels *Unio crassus* Philippsen, 1788 after a flood, and reported low mortality (<20%). Furthermore, among living specimens, 15% were buried, 20% were not visible, and 17% were not found. Therefore, it is possible that specimens of *P. auricularius* could be found downstream from the plots for years to come.

There are a multitude of risks when doing a translocation and they are well summarized in the IUCN translocation guide (IUCN/SSSC, 2013; Miller & Payne, 2006). For example, there is a risk of co-dispersal of bacteria, viruses, fungi, or other pathogens with the translocated specimens. Brian et al. (2021) mention that parasites and diseases in freshwater mussels are highly prevalent and may contribute to some of the massive mortalities that have been reported in recent years. Since 2013, when the high mortality of *P. auricularius* was detected in the CIA, the Aragón Government began to investigate the possible causes. From analysis of water samples, sediment, and tissue, in search of heavy metals, herbicides, fungicides, and pesticides (Nakamura et al., 2021), and histological sections of dead specimens of *P. auricularius* in search of parasites, bacteria, and fungi, overall results were negative or inconclusive. As Jourdan et al. (2019) stated, selecting a donor population within the same catchment as the recipient site is a simple rule to reduce the risk of transferring allochthonous pathogens or parasites. We followed this recommendation and our translocation actions were planned to take place within the same river basin, and the two systems involved—canal and river—share the same water origin.

In the same way, the inclusion of the host fish *Salaria fluviatilis* in the translocation process may be an important measure to increase the probability of future recruitment and survival of *P. auricularius* in the Ebro River. It is a fish species with a marked territorial behavior which uses shelters for the female to place the eggs (Vinyoles et al., 2002; Vinyoles & Sostoa, 2007). Taking this into account, bricks and flat stones were introduced in the plots near the mussels before the fish were released. In future, it would be interesting to check if there has been an interaction between fish and mussels, either by checking the infestation of fish in the area (checking their gills looking for glochidium larvae) or by assessing the presence of juvenile freshwater mussels in plots without previous presence of *P. auricularius*.

**Conclusion**

*Pseudunio auricularius* is facing a serious risk of extinction worldwide (Prié et al., 2018) and the situation in Spain is especially dramatic. The low number of recorded specimens and the disappearance and decline of the two native host fishes in the Ebro basin (sturgeon and river blenny) may impair their recruitment and future survival. Although the environmental characteristics of the river are quite different from those of the canal (higher current velocity, greater annual flow changes, higher macrophyte and algal cover, among others), the results reported here indicate that the translocated specimens have a higher survival in the Ebro River than in CIA. In addition, translocating specimens to various localities in different years can decrease the risk of mortality due to catastrophic events and maximize the probability of survival and establishment of new sub-populations. However, improvements in the methodology and in the index score used to evaluate potential recipient localities should be pursued and future monitoring should assess not only the survival of *P. auricularius* but also its reproduction and recruitment.

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Data availability The datasets generated during and/or analyzed during the current study are not publically available, to secure the protection of the target species of this study in their natural habitat, but are available from the first author on reasonable request.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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