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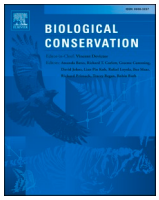

Short communication

The silent extinction of freshwater mussels in Portugal

Manuel Lopes-Lima [a,b,*], Joaquim Reis [c], Maria G. Alvarez [c], Pedro M. Anastácio [d], Filipe Banha [d], Pedro Beja [a,e], Paulo Castro [f], Mafalda Gama [d], Maria G. Gil [c], André Gomes-dos-Santos [g,h], Fernando Miranda [i], Joana Garrido Nogueira [a], Ronaldo Sousa [f], Amílcar Teixeira [i], Simone Varandas [a,j], Elsa Froufe [g]

[a] BIOPOLIS Program in Genomics, Biodiversity and Ecosystems, CIBIO, Research Centre in Biodiversity and Genetic Resources, InBIO Research Network in Biodiversity and Evolutionary Biology, Associated Laboratory, Campus de Vairão, Universidade do Porto, 4485-661 Vairão, Portugal
[b] EBM, Estação Biológica de Mértola, Rua Professor Batista da Graça nº 1, 7750-329 Mértola, Portugal
[c] MARE - Marine and Environmental Sciences Centre/ARNET-Aquatic Research Network, Faculdade de Ciências da Universidade de Lisboa, Campo Grande, 1749-016 Lisbon, Portugal
[d] MARE - Marine and Environmental Sciences Centre, ARNET - Aquatic Research Network, Institute for Research and Advanced Training (IIFA), University of Évora, 7002-554 Évora, Portugal
[e] CIBIO, Research Centre in Biodiversity and Genetic Resources, InBIO Research Network in Biodiversity and Evolutionary Biology, Associated Laboratory, Instituto Superior de Agronomia, Universidade de Lisboa, 1349-017 Lisboa, Portugal
[f] CBMA - Centre of Molecular and Environmental Biology, Department of Biology, University of Minho, Campus Gualtar, 4710-057 Braga, Portugal
[g] CIIMAR, Interdisciplinary Centre of Marine and Environmental Research, Terminal de Cruzeiros de Leixões, Matosinhos, Portugal
[h] Department of Biology, Faculty of Sciences, University of Porto, Rua do Campo Alegre 1021/1055, 4169-007, Porto, Portugal
[i] Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal
[j] CITAB-UTAD - Centre for Research and Technology of Agro-Environment and Biological Sciences, University of Trás-os-Montes and Alto Douro, Forestry Department, Vila Real, Portugal

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ABSTRACT

Freshwater mussels are one of the most threatened animal groups in the world. In the European Union, threatened and protected mussel species are not adequately monitored, while species considered to be common and widespread receive even less attention. This is particularly worrying in the Mediterranean region, where species endemism is high and freshwater habitats are severely affected by water scarcity. In the absence of hard data on population trends, we report here a long-term comparison of freshwater mussel assemblages at 132 sites covering 15 different hydrological basins in Portugal. This study reveals a widespread decline of 60 % in the number of sites and 67 % in the overall abundance of freshwater mussels across Portugal over the last 20 years, indicating that all species are rapidly declining and threatened with extinction. These results show that current legislation and conservation measures are largely ineffective and highlight the importance of updating the Habitats Directive to enforce standard monitoring protocols for threatened species in the European Union and to extend monitoring to other freshwater species thought to be common and widespread. Efficient water management, restrictions on irrigation expansion in important biodiversity areas, mitigation of hydrological changes and loss of aquatic habitat connectivity caused by physical alterations are urgently needed to reverse these declining population trends. For the severely endangered species *Margaritifera margaritifera*, *Potomida littoralis*, and *Unio tumidiformis*, where populations are now critically low, more urgent action is needed, such as ex-situ conservation, protection of remaining populations and large-scale habitat restoration.

1. Introduction

Biodiversity is declining at an unprecedented rate, but terrestrial vertebrates, especially mammals and birds, still receive most of the scientific and cultural attention (Ceballos et al., 2015, 2017; Dirzo et al., 2014; Mammola et al., 2020). Much less information is available for

* Corresponding author at: Applied Ecology Group, CIBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, Campus de Vairão, Universidade do Porto, 4485-661 Vairão, Portugal.
E-mail address: manuelpmlopeslima@gmail.com (M. Lopes-Lima).

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invertebrates, although recent reports draw attention to a similarly dire situation in some taxonomic groups, such as the so-called 'insect Armageddon' (Hallmann et al., 2017; Sánchez-Bayo and Wyckhuys, 2019; Wagner, 2020). These reported declines are mainly associated with a "death by a thousand cuts" or a slow, continuous accumulation of multiple threats, such as land-use and climate change, intensive agriculture, introduced species, eutrophication, and pollution, among others (Wagner et al., 2021). However, the few available studies of insect decline have been conducted in highly humanised and well-studied regions, such as Germany and central California (Wagner et al., 2021). In more remote regions, and for other invertebrate groups, we know very little about population trends, and potential declines and extirpations may remain off the scientific and public radar (Cowie et al., 2022; Czaja et al., 2023).

This is true for many groups of molluscs in terrestrial and freshwater ecosystems, including freshwater mussels (Bivalvia: Unionida), which, despite many warnings about their poor conservation status, still suffer from a lack of systematic monitoring and targeted conservation efforts (Lopes-Lima et al., 2018, 2021), with local and regional exceptions in North America and Europe. As a result, many species and populations thought to be stable may be quietly disappearing at an alarming rate, particularly in understudied regions where the understanding of the decline of these organisms, is one of the key issues in freshwater mussel conservation (Aldridge et al., 2023).

Freshwater mussels are a group of bivalves with more than 900 species found in many rivers and lakes on all continents except Antarctica (Lopes-Lima et al., 2014). These mussels were originally very abundant in many ecosystems around the world, where they dominated both in abundance and biomass (Strayer, 2014). They play an important role in ecosystems by improving water and substrate quality and are important ecosystem engineers that provide critical habitat for other species (Ilarri et al., 2018; Zieritz et al., 2022). Freshwater mussels tend to be long-lived, wide-ranging and highly sensitive to habitat disturbance, making them good indicators of the ecological integrity of freshwater ecosystems (Lopes-Lima et al., 2020). This sensitivity makes them highly vulnerable to habitat degradation caused by human activities. Several species, especially those that are less abundant and have restricted ranges, are now extinct or highly threatened (Lopes-Lima et al., 2018). Many others with widespread distributions are thought to be abundant, stable, and of no conservation concern. However, there is a lack of systematic and comprehensive monitoring of freshwater mussels, making quantitative assessment of their status very difficult and unreliable (Lopes-Lima et al., 2021).

Several freshwater mussel species are included in the European Union's main nature conservation policy, the Habitats Directive. Although regular monitoring is mandatory for species included in this Directive, no guidance is given to countries on how these monitoring schemes should be applied. As a result, many countries provide only anecdotal reports on the conservation status of species, mostly based on expert opinion and with very limited quantitative data, which can lead to a complete mismatch between the reports and the true conservation status of species (Riccardi et al., 2021). Furthermore, the vast majority of species are still ignored in European and national conservation legislation, with almost no systematic monitoring and no hard data on the status of their populations. However, strong declines of widespread freshwater mussel species have recently been reported in the UK and Spain, but in surveys limited to a few sites in the Thames and Ebro basins (Nakamura et al., 2023; Ollard and Aldridge, 2023). Both studies call for regular population surveys of key species as an essential tool to maintain an informed picture of ecosystem health and guide conservation management.

The situation is similar in Portugal, Spain, and most of the Mediterranean region, where long-term monitoring data are lacking for most populations (but see Nakamura et al., 2023). Most freshwater habitats are increasingly threatened by water scarcity due to the intensification of agricultural practices and the shift and expansion of traditional rainfed crops to irrigated cultivation in recent decades (Casas et al., 2015). The overexploitation of surface and groundwater, coupled with global warming and the increased frequency and intensity of extreme weather events such as heatwaves and droughts, has led to massive wetland loss and degradation, increased pollution, nitrate contamination, saltwater intrusion into aquifers and areas upstream of estuaries, and created conditions for the establishment of non-native species resistant to these stresses (García-Ruiz et al., 2011; Anastácio et al., 2019).

There are six freshwater mussel species in Portugal (Reis, 2006). Two of them are listed as threatened in Europe in the IUCN Red List (*Margaritifera margaritifera* as Critically Endangered and *Unio tumidiformis* as Vulnerable) (Cuttelod et al., 2011), and both are protected at the European and national level. Three species are considered widespread in the country, two of which (*Unio delphinus* and *Potomida littoralis*) are listed as Near Threatened in Europe based on anecdotal reports of decline, and one (*Anodonta anatina*) as Least Concern (Cuttelod et al., 2011). The remaining species (*Anodonta cygnea*) is restricted to two coastal lagoons and was therefore not included in our analyses. Prior to the current study, apart from the extensive work carried out in Europe on the most charismatic species *Margaritifera margaritifera*, (e.g. Sousa et al., 2013, 2015), no quantitative information on population trends of the remaining species was available for Portugal and Europe in general. Given the lack of long-term monitoring programmes and the scarcity of hard data on the decline of freshwater mussels on a larger scale, the aim of this study was to estimate population trends of all freshwater mussel species in Portugal, based on a 20-year comparison of their diversity and abundance at 132 sites across the country (Fig. 1).

2. Methods

This study was conducted in the summer of 2022 at the same 132 sites where an earlier thorough survey of over 450 sites in Portugal between 2002 and 2004 had revealed the presence of mussels (Reis, 2006). Each site had previously been registered with precise GPS coordinates (Reis, 2006) and the same mussel survey methodology was used as in the current study. At each site, a 100 m stretch of river was measured with a tape measure around the mid-point of the site from the previous survey and extensively searched for mussels by experienced researchers (2–5 per site) using snorkelling or wading with aquascopes for a total of at least one man-hour (Fig. 1). Searches were systematic, with each operator zigzagging from bank to bank along the entire length of the defined river section. These surveys were supplemented by dives to depths of up to 11 m in deeper areas, especially in the reservoirs of the dams, using the same minimum effort. If the abundance of a species reported as present in the previous survey was less than 10 individuals, the search was extended to 200 m of river length (85 sites in total) around the central point. At each site, mussels were morphologically identified, counted and returned to their original location. To estimate habitat changes at each site between sampling events, we used satellite images available on Google Earth between 2002 and 2022. Depending on the site, between 10 and 20 images were available and examined for each site. Particular attention was paid to assessing the presence of water in the river channel, as lack of water inevitably leads to the death of the entire mussel population.

3. Results

We found live mussels only in 40 % (53 out of 132) of the surveyed sites where mussels had previously been recorded (Fig. 1). Dramatic declines in occurrence were observed for all species (Figs. 1 & 2), ranging from 50 % (15 out of 30 for *M. margaritifera*) to 82 % (3 out of 18 for *U. tumidiformis*) (Sup. Figs. 1–5). Declines in abundance were also observed in the sites currently occupied by each species, ranging from 57 % (*M. margaritifera*) to 71 % (*U. delphinus*), and with an overall decline for all species of 67 % (Fig. 2). In contrast to native mussels,

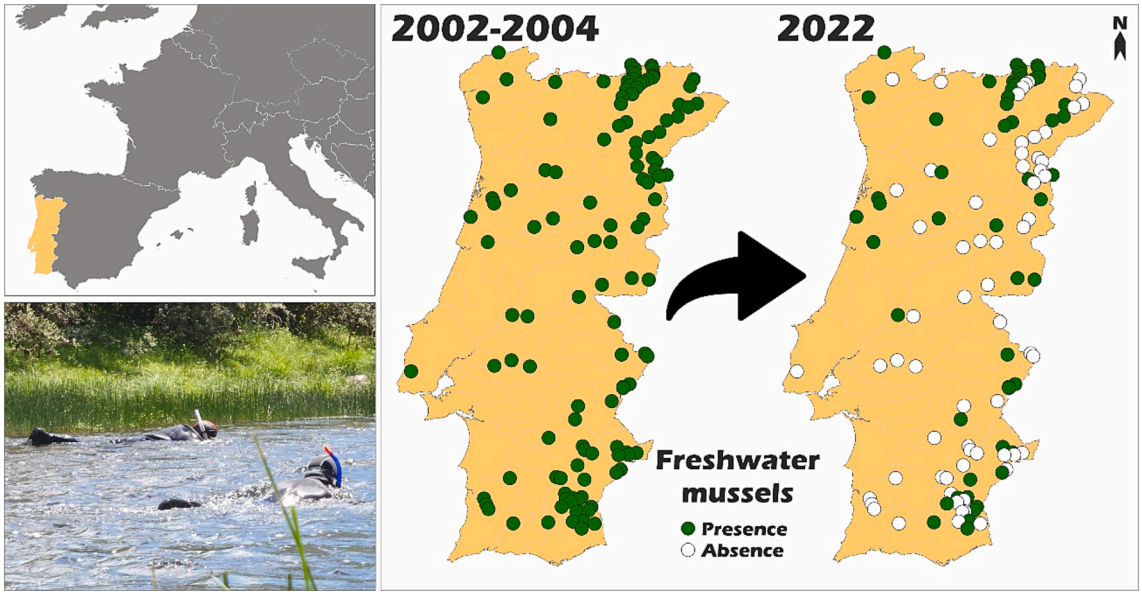

Fig. 1. Maps of Portugal representing the sites sampled in the 2002–2004 and 2022 surveys showing the presence and absence of freshwater mussels.

occurrence of the invasive freshwater bivalve *Corbicula fluminea*, increased from 27 to 59 sites (118 % increase) between surveys (Figs. 2 and 3). Of the 79 sites where mussels had been extirpated, eight were completely dry at the time of our revisit, while satellite imagery showed that a further 17 sites had dried up at least once in the last 20 years (Fig. 3). Eighteen additional sites had been converted from lotic to lentic habitats and are now located in reservoirs (Fig. 3).

4. Discussion

The present study reports massive declines for all native species surveyed, both in number of sites and total abundance (Fig. 3). There are almost no studies on population trends of freshwater mussels across Europe, but two recent studies have reported massive declines at the local level. One of these studies reports a decline in freshwater mussel density of over 90 % in a large stretch of the River Thames since 1964 (Ollard and Aldridge, 2023). The other includes a series of surveys over the last 15 years on two major channels in the Ebro River basin in Spain and reports the extirpation of three species that were once considered locally common and abundant (Nakamura et al., 2023). Our results and these studies highlight the urgent need to survey freshwater mussel populations in Europe to understand current population trends, not only of the more rare and threatened species, but also of others thought to be common and widespread.

Although the causes of the reported declines were not investigated in the present study, drought was the most likely culprit at 32 % of the 79 sites where mussels were extirpated, as each site was dry at least once in the last 20 years (Fig. 3). Another major impact on freshwater mussels (23 % of the sites with no mussels) was the alteration of riverine lotic habitat by dams (Fig. 3). This is supported by the fact that no native

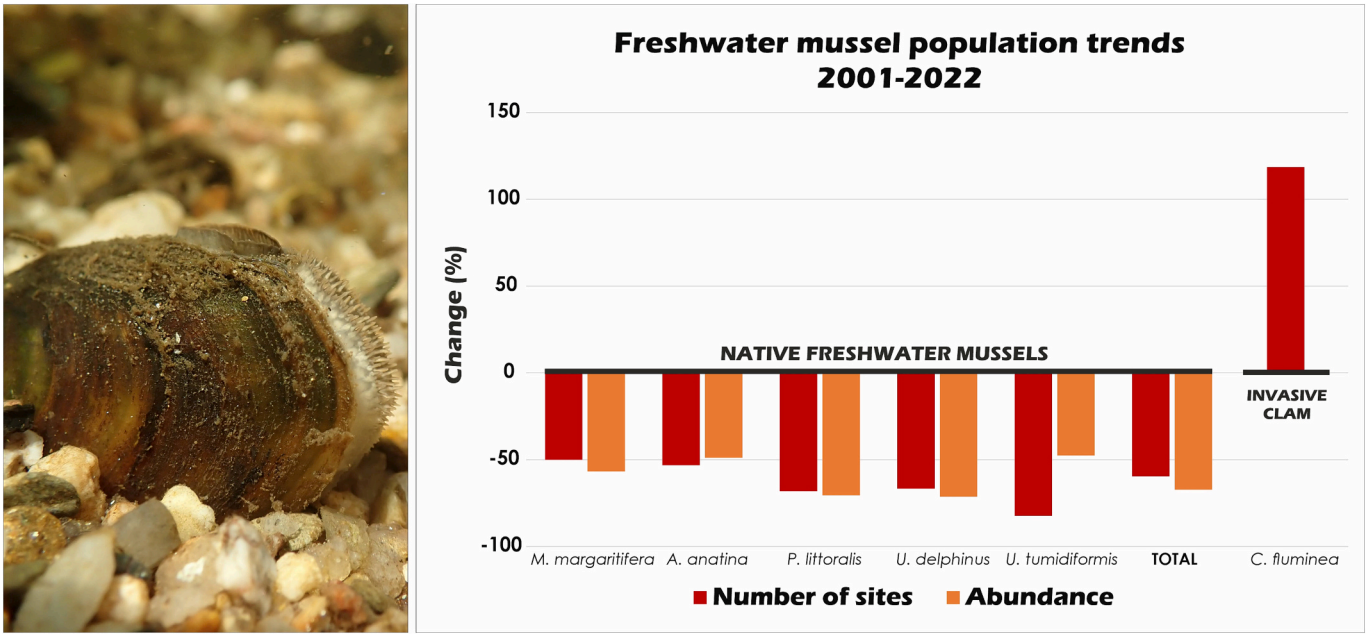

Fig. 2. Left: A specimen of *Unio delphinus* in its natural habitat; **Right**: Changes (%) in the overall presence in the number of sites and abundance of freshwater mussels and the invasive freshwater bivalve *Corbicula fluminea*.

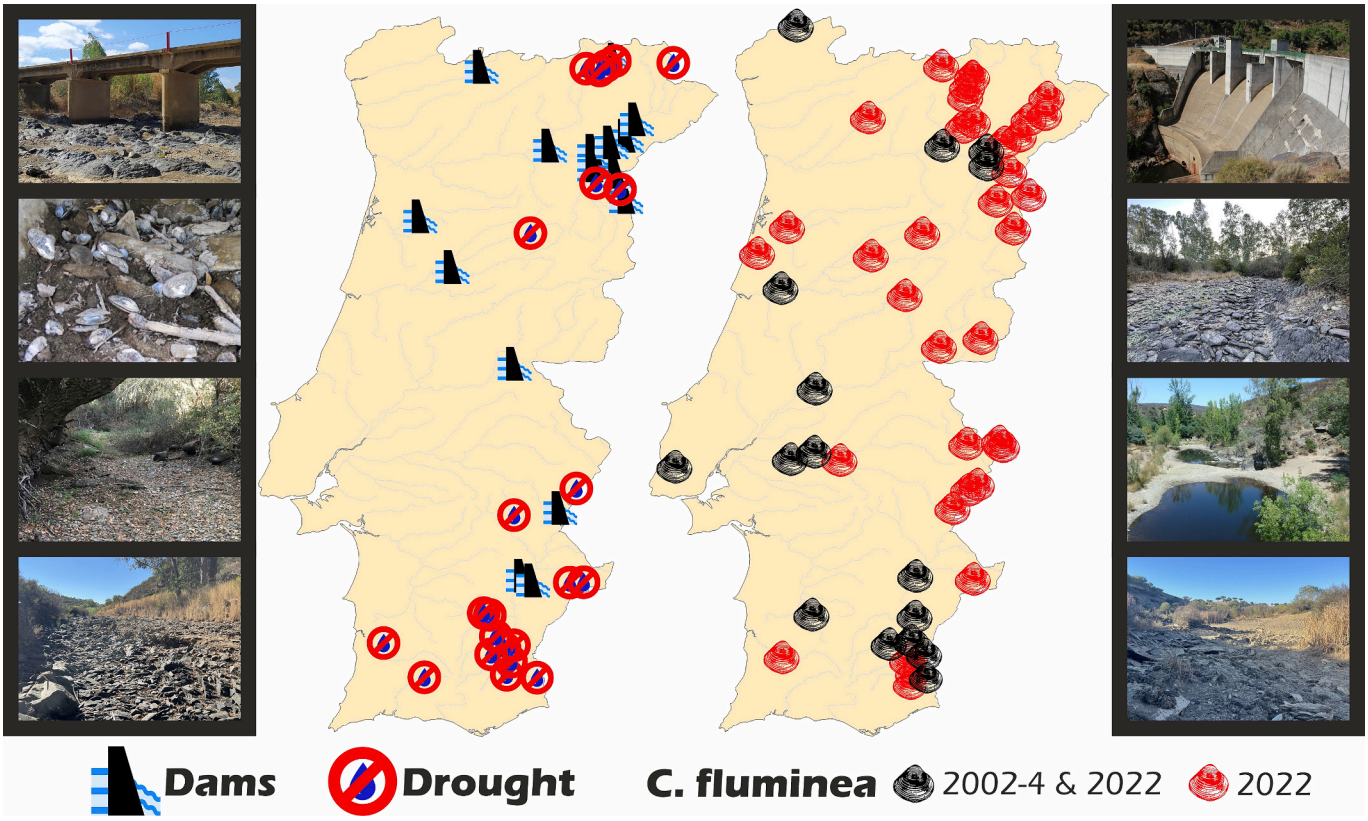

Fig. 3. Maps of Portugal representing on the **left** - sites under the influence of dams, sites that were dry at least once over the last 20 years; and on the **right** – sites with the presence of the invasive bivalve *Corbicula fluminea* in the 2002–4 and 2022 surveys. Images on either side of the maps show sampled locations affected by drought or dams.

freshwater mussels were found in the 18 reservoirs surveyed, apart from a single living and old specimen of *U. delphinus*. The apparent disappearance of freshwater mussels from the remaining sites is most likely related to the continuous increase of multiple threats over the last decades, possibly acting in synergy. The continuing decline was already reported in the first survey (Reis, 2003, 2006) for sites previously known to support freshwater mussels in the first half of the 20th century (Nobre, 1941). Water overuse due to the intensification of irrigated agriculture and poor flow management by dams and embankments are leading to water depletion in Mediterranean freshwater habitats (Sousa et al., 2020). These effects are exacerbated by the ongoing aridification of the Iberian Peninsula (Cresswell-Clay et al., 2022). Other factors, such as point source, but especially diffuse pollution and nutrient enrichment from agricultural, industrial, and urban runoff, also contribute to the continuous degradation of Mediterranean riverine habitats (Lassaletta et al., 2009). Finally, the systematic introduction of non-native species in the Iberian Peninsula (Anastácio et al., 2019), such as fish, crayfish and the bivalve *C. fluminea*, has been shown to severely affect native freshwater mussels through predation, competition and impaired recruitment (Douda et al., 2013; Ferreira-Rodriguez et al., 2018; Meira et al., 2019; Ilarri and Sousa, 2012; Modesto et al., 2021). The increase in *C. fluminea* has been suggested as the main cause of the disappearance of three common freshwater mussel species in two large channels in Spain (Nakamura et al., 2023), but the mechanism for this effect is still not well understood.

The Mediterranean is one of the most threatened biomes in the world, and this is compounded by the predicted negative impacts of future climate change (Boithias et al., 2014; Martín-Vélez and Abellán, 2022). Water scarcity is likely to be one of the greatest challenges for human populations and the vast and unique biodiversity of the Mediterranean (Sabater and Barceló, 2010 and references therein). Efficient water management is therefore needed to reduce the conflict between human water needs and the maintenance of biodiversity and ecological processes in freshwater habitats (Iglesias et al., 2007). Agricultural policies should support traditional rainfed crops in at least some areas of higher biodiversity value, and better and more efficient water use practices elsewhere (Molle and Sanchis-Ibor, 2019). Additional efforts should be made to mitigate hydrological changes and loss of connectivity caused by physical alterations such as dams, weirs and canals, pollution, siltation and the introduction of non-native species.

The inadequacy of the current network of protected areas to represent and protect freshwater biodiversity in the Mediterranean has been demonstrated (Hermoso et al., 2015; Nogueira et al., 2021a, 2021b) and should be addressed through a reassessment of the priority species and habitats listed in the Habitats Directive, which serve as the baseline for the Natura 2000 network. This should be integrated into the EU Biodiversity Strategy for 2030, which proposes, among other targets, a 30 % coverage of protected areas and the restoration of 25,000 km of rivers (Sousa et al., 2023).

Effective legislation to implement and enforce the collection of baseline information in data-poor areas, followed by efficient monitoring programmes for freshwater species, especially those of conservation concern, is critical and urgent. These measures should include the establishment and enforcement of standard monitoring protocols for Habitats Directive species, such as freshwater mussels.

This study will also allow for a more thorough assessment of the conservation status of these species at a national level, which should consider all species as threatened according to the IUCN extinction risk criteria, based on the decline trends presented. In addition, the species *Margaritifera margaritifera*, *Potomida littoralis* and *Unio tumidiformis* (Sup. Figs. 1, 3 and 5) are now highly localised and therefore require special conservation attention. We strongly recommend that these species and

their remaining populations should be protected, and that individual catchments be identified for large-scale restoration, together with the implementation of long-term breeding to reverse their decline.

5. Conclusions

The animal world is dominated by invertebrates, both in terms of biodiversity and biomass. These organisms also possess unparalleled morphological, functional, and phylogenetic diversity and are responsible for many ecosystem functions and vital services. Conserving their diversity and ecological role in ecosystems should therefore be a top priority for future research and policy. The alarming declines reported here for Portugal are most likely occurring for many freshwater taxa throughout the Mediterranean hotspot, and possibly in Europe, where dramatic declines have recently been reported, and are a consequence of the accumulation and synergy of multiple pressures on freshwater ecosystems. These include a combination of climate change, habitat loss and degradation, and the spread of invasive species, all of which are leading to large-scale defaunation of Mediterranean ecosystems. To reverse the current overall decline trends of these and other freshwater species in Portugal, there is an urgent need to increase the efficiency of water management, limit the expansion of irrigated crops in areas with water scarcity, and mitigate hydrological changes and loss of aquatic habitat connectivity caused by physical alterations. It is also essential to implement measures to control or eradicate invasive aquatic species. These measures should be implemented at the Iberian scale, as most of the river basins are transboundary. Given the failure of key biodiversity conservation policies to protect these species at the European level, we argue that the Habitats Directive should be re-evaluated to 1) include more neglected species of urgent conservation concern, such as some freshwater mussels, and 2) establish standardised and sustained regular monitoring of Habitats Directive species at a large scale to detect drastic population changes from going unnoticed, ultimately allowing rapid mobilisation of conservation action to prevent the extinction of these species.

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CRediT authorship contribution statement

Manuel Lopes-Lima: Conceptualization, Formal analysis, Investigation, Writing – original draft, Visualization, Supervision, Project administration. **Joaquim Reis:** Conceptualization, Investigation, Writing – review & editing. **Maria G. Alvarez:** Investigation, Writing – review & editing. **Pedro M. Anastácio:** Investigation, Writing – review & editing. **Filipe Banha:** Investigation, Writing – review & editing. **Pedro Beja:** Formal analysis, Writing – review & editing. **Paulo Castro:** Investigation, Writing – review & editing. **Mafalda Gama:** Investigation, Writing – review & editing. **Maria G. Gil:** Investigation, Writing – review & editing. **André Gomes-dos-Santos:** Investigation, Writing – review & editing. **Fernando Miranda:** Investigation, Writing – review & editing. **Joana Garrido Nogueira:** Investigation, Writing – review & editing. **Ronaldo Sousa:** Investigation, Writing – review & editing. **Amílcar Teixeira:** Investigation, Writing – review & editing. **Simone Varandas:** Investigation, Writing – review & editing. **Elsa Froufe:** Conceptualization, Investigation, Writing – original draft, Visualization, Supervision, Project administration.

Declaration of competing interest

Nothing to report.

Data availability

Data will be made available on request.

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References

Aldridge, D.C., Ollard, I.S., Bespalaya, Y.V., Bolotov, I.N., Douda, K., et al., 2023. Freshwater mussel conservation: a global horizon scan of emerging threats and opportunities. Glob. Chang. Biol. 29, 575–589.
Anastácio, P.M., Ribeiro, F., Capinha, C., Banha, F., Gama, M., et al., 2019. Non-native freshwater fauna in Portugal: a review. Sci. Total Environ. 650, 1923–1934.
Boithias, L., Acuña, V., Vergoñós, L., Ziv, G., Marcé, R., Sabater, S., 2014. Assessment of the water supply: demand ratios in a Mediterranean basin under different global change scenarios and mitigation alternatives. Sci. Total Environ. 470, 567–577.
Casas, J.J., Bonachela, S., Moyano, F.J., Fenoy, E., Hernández, J., 2015. Agricultural practices in the mediterranean: a case study in southern Spain. In: Preedy, V.R., Watson, R.R. (Eds.), The Mediterranean Diet: An Evidence-Based Approach. Academic Press, pp. 23–36.
Ceballos, G., Ehrlich, P.R., Barnosky, A.D., García, A., Pringle, R.M., Palmer, T.M., 2015. Accelerated modern human–induced species losses: entering the sixth mass extinction. Sci. Adv. 1, e1400253.
Ceballos, G., Ehrlich, P.R., Dirzo, R., 2017. Biological annihilation via the ongoing sixth mass extinction signaled by vertebrate population losses and declines. Proc. Natl. Acad. Sci. 114, E6089–E6096.
Cowie, R.H., Bouchet, P., Fontaine, B., 2022. The sixth mass extinction: fact, fiction or speculation? Biol. Rev. 97, 640–663.
Cresswell-Clay, N., Ummenhofer, C.C., Thatcher, D.L., Wanamaker, A.D., Denniston, R. F., Asmerom, Y., Polyak, V.J., 2022. Twentieth-century Azores High expansion unprecedented in the past 1,200 years. Nat. Geosci. 15 (7), 548–553.
Cuttelod, A., Seddon, M., Neubert, E., 2011. European red list of non-marine molluscs. Publications Office of the European Union, Luxembourg, p. 97.
Czaja, A., Covich, A.P., Becerra-López, J.L., Cordero-Torres, D.G., Estrada-Rodríguez, J. L., 2023. The freshwater mollusks of Mexico: can we still prevent their silent extinction?. In: Mexican Fauna in the Anthropocene. Springer International Publishing, Cham, pp. 81–103 (Jan 31).
Dirzo, R., Young, H.S., Galetti, M., Ceballos, G., Isaac, N.J.B., Collen, B., 2014. Defaunation in the Anthropocene. Science 345, 401–406.
Douda, K., Lopes-Lima, M., Hinzmann, M., Machado, J., Varandas, S., Teixeira, A., Sousa, R., 2013. Biotic homogenization as a threat to native affiliate species: fish introductions dilute freshwater mussels' resources. Divers. Distrib. 19, 933–942.
Ferreira-Rodriguez, N., Sousa, R., Pardo, I., 2018. Negative effects of *Corbicula fluminea* over native freshwater mussels. Hydrobiologia 810, 85–95.
García-Ruiz, J.M., López-Moreno, J.I., Vicente-Serrano, S.M., Lasanta-Martínez, T., Beguería, S., 2011. Mediterranean water resources in a global change scenario. Earth Sci. Rev. 105, 121–139.
Hallmann, C.A., Sorg, M., Jongejans, E., Siepel, H., Hofland, N., et al., 2017. More than 75 percent decline over 27 years in total flying insect biomass in protected areas. PLoS One 12, e0185809.
Hermoso, V., Filipe, A.F., Segurado, P., Beja, P., 2015. Effectiveness of a large reserve network in protecting freshwater biodiversity: a test for the Iberian Peninsula. Freshw. Biol. 60, 698–710.
Iglesias, A., Garrote, L., Flores, F., Moneo, M., 2007. Challenges to manage the risk of water scarcity and climate change in the Mediterranean. Water Resour. Manag. 21, 775–788.
Ilarri, M., Sousa, R., 2012. *Corbicula fluminea* Müller (Asian clam). In: Francis, R.A. (Ed.), A Handbook of Global Freshwater Invasive Species. Routledge, London, UK, pp. 173–183.
Ilarri, M., Amorim, L., Souza, A.T., Sousa, R., 2018. Physical legacy of freshwater bivalves: effects of habitat complexity on the taxonomical and functional diversity of invertebrates. Sci. Total Environ. 634, 1398–1405.
Lopes-Lima, M., Teixeira, A., Froufe, E., Lopes, A., Varandas, S., Sousa, R., 2014. Biology and conservation of freshwater bivalves: past, present and future perspectives. Hydrobiologia 735, 1–13.
Lassaletta, L., García-Gómez, H., Gimeno, B.S., Rovira, J.V., 2009. Agriculture-induced increase in nitrate concentrations in stream waters of a large Mediterranean catchment over 25 years (1981–2005). Sci. Total Environ. 407 (23), 6034–6043.
Lopes-Lima, M., Burlakova, L.E., Karatayev, A.Y., Mehler, K., Seddon, M., Sousa, R., 2018. Conservation of freshwater bivalves at the global scale: diversity, threats and research needs. Hydrobiologia 810, 1–14.

Lopes-Lima, M., Hinzmann, M., Varandas, S., Froufe, E., Reis, J., et al., 2020. Setting the stage for new ecological indicator species: a holistic case study on the Iberian dolphin freshwater mussel *Unio delphinus* Spengler, 1793. Ecol. Indic. 111, 105987.
Lopes-Lima, M., Riccardi, N., Urbanska, M., Köhler, F., Vinarski, M., et al., 2021. Major shortfalls impairing knowledge and conservation of freshwater molluscs. Hydrobiologia 848, 2831–2867.
Mammola, S., Riccardi, N., Prié, V., Correia, R., Cardoso, P., et al., 2020. Towards a taxonomically unbiased European Union biodiversity strategy for 2030. Proc. R. Soc. B 287, 20202166.
Martín-Vélez, V., Abellán, P., 2022. Effects of climate change on the distribution of threatened invertebrates in a Mediterranean hotspot. Insect Conserv. Divers. 15, 370–379.
Meira, A., Lopes-Lima, M., Varandas, S., Teixeira, A., Arenas, F., Sousa, R., 2019. Invasive crayfishes as a threat to freshwater bivalves: interspecific differences and conservation implications. Sci. Total Environ. 649, 938–948.
Modesto, V., Dias, E., Ilarri, M., Lopes-Lima, M., Teixeira, A., et al., 2021. Trophic niche overlap between native freshwater mussels (order: Unionida) and the invasive *Corbicula fluminea*. Aquat. Conserv. Mar. Freshwat. Ecosyst. 31, 2058–2071.
Molle, F., Sanchis-Ibor, C., 2019. Irrigation policies in the Mediterranean: trends and challenges. In: Molle, F., Sanchis-Ibor, C., Avellà-Reus, L. (Eds.), Irrigation in the Mediterranean. Global Issues in Water Policy, vol. 22. Springer, Cham, pp. 279–313.
Nakamura, K., Sousa, R., Mesquita-Joanes, F., 2023. Collapse of native freshwater mussel populations: prospects of a long-term study. Biol. Conserv. 279, 109931.
Nobre, A., 1941. Fauna malacológica de Portugal II. Moluscos terrestres e fluviais. Mem. Estud. Museu Zool. Univ. Coimbra 124, 1–277.
Nogueira, J.G., Sousa, R., Benaissa, H., De Knijf, G., Ferreira, S., et al., 2021a. Alarming decline of freshwater trigger species in western Mediterranean key biodiversity areas. Conserv. Biol. 35, 1367–1379.
Nogueira, J.G., Teixeira, A., Lopes-Lima, M., Varandas, S., Sousa, R., 2021b. Assessment of terrestrial protected areas for the conservation of freshwater biodiversity. Aquat. Conserv. Mar. Freshwat. Ecosyst. 31, 520–530.
Ollard, I., Aldridge, D.C., 2023. Declines in freshwater mussel density, size and productivity in the River Thames over the past half century. J. Anim. Ecol. 92, 112–123.
Reis, J., 2003. The freshwater pearl mussel [*Margaritifera margaritifera* (L.)](Bivalvia, Unionoida) rediscovered in Portugal and threats to its survival. Biol. Conserv. 114, 447–452.
Reis, J., 2006. Atlas dos bivalves de água doce em Portugal continental. Instituto da Conservação da Natureza, Lisboa, Portugal.
Riccardi, N., Bo, T., Bogan, A.E., Douda, K., Froufe, E., et al., 2021. *Microcondylaea bonellii*, a testimonial for neglected endangered species. In: Imperiled: The Encyclopedia of Conservation, vol. 1-3. Elsevier Science, pp. 436–446.
Sabater, S., Barceló, D. (Eds.), 2010. Water Scarcity in the Mediterranean: Perspectives Under Global Change, vol. 8. Springer Science & Business Media.
Sánchez-Bayo, F., Wyckhuys, K.A., 2019. Worldwide decline of the entomofauna: a review of its drivers. Biol. Conserv. 232, 8–27.
Sousa, R., Amorim, Â., Sobral, C., Froufe, E., Varandas, S., Teixeira, A., Lopes-Lima, M., 2013. Ecological status of a Margaritifera margaritifera (Linnaeus, 1758) population at the southern edge of its distribution (River Paiva, Portugal). Environ. Manag. 52, 1230–1238.
Sousa, R., Amorim, Â., Froufe, E., Varandas, S., Teixeira, A., Lopes-Lima, M., 2015. Conservation status of the freshwater pearl mussel Margaritifera margaritifera in Portugal. Limnologica 50, 4–10.
Sousa, R., Ferreira, A., Carvalho, F., Lopes-Lima, M., Varandas, S., et al., 2020. Small hydropower plants as a threat to the endangered pearl mussel *Margaritifera margaritifera*. Sci. Total Environ. 719, 137361.
Sousa, R., Zając, T., Halabowski, D., Aksenova, O.V., Bespalaya, Y.V., et al., 2023. A roadmap for the conservation of freshwater mussels in Europe. Conserv. Biol. 37, e13994.
Strayer, D.L., 2014. Understanding how nutrient cycles and freshwater mussels (Unionoida) affect one another. Hydrobiologia 735, 277–292.
Wagner, D.L., 2020. Insect declines in the Anthropocene. Annu. Rev. Entomol. 65, 457–480.
Wagner, D.L., Grames, E.M., Forister, M.L., Berenbaum, M.R., Stopak, D., 2021. Insect decline in the Anthropocene: death by a thousand cuts. Proc. Natl. Acad. Sci. 118, e2023989118.
Zieritz, A., Sousa, R., Aldridge, D.C., Douda, K., Esteves, E., et al., 2022. A global synthesis of ecosystem services provided and disrupted by freshwater bivalve molluscs. Biol. Rev. 97, 1967–1998.