Behavioural responses of freshwater mussels to drought and dewatering

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2023

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This thesis was supported by the Portuguese Foundation for Science and Technology (FCT) under the project EdgeOmics - Freshwater Bivalves at the Edge: Adaptation genomics under climate-change scenarios (PTDC/CTAAMB/3065/2020) funded by FCT through national funds.

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You will always be in the shimmering horizons of memory!

Aos meus avós (Silvina e Serafim, Maria dos Prazeres e António) e à minha mãe Maria José.

Sworn Statement

I, Henrique Manuel Lopes Pinto Caldas Rodrigues, enrolled in the Master Degree Recursos Biológicos Aquáticos at the Faculty of Sciences of the University of Porto hereby declare, in accordance with the provisions of paragraph a) of Article 14 of the Code of Ethical Conduct of the University of Porto, that the content of this dissertation reflects perspectives, research work and my own interpretations at the time of its submission.

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Agradecimentos

Em primeiro lugar agradecer à Faculdade de Ciências da Universidade do Porto (FCUP), pela oportunidade de realizar o Mestrado em Recursos Biológicos Aquáticos, ao Centro Interdisciplinar de Investigação Marinha e Ambiental (CIIMAR), por mais uma vez me acolher e ao Instituto Politécnico de Bragança (IPB) onde fui muito bem recebido.

Agradecimentos especiais:

À minha orientadora, Doutora Elsa Froufe, por me ter dado a conhecer os bivalves de água doce, desconhecidos da maioria e subestimados à primeira vista, mas tão importantes para a regulação dos ecossistemas. Por desde o início se ter mostrado disponível para em tudo me esclarecer no mundo da ciência, por todas as horas perdidas na ajuda e revisão da escrita da minha dissertação. A paciência para os meus erros, complicações, questões, por aquelas repentinas viradas de computador para si, enquanto trabalhava, e claro, pela maneira como me integrou na sua fantástica equipa de investigação.

Ao meu coorientador, Professor Doutor Amílcar Teixeira, agradecer pela maneira como me recebeu no IPB, pela organização dos trabalhos de campo e laboratoriais e pelo entusiasmo com que me levou a conhecer parte do Parque Natural de Montesinho. Pelas horas na revisão, análise e correções desta dissertação, pela sua simpatia, paciência, conselhos científicos, e me ter mostrado um dos pontos fortes transmontanos a sua gastronomia.

Ao ICNF - Instituto de Conservação da Natureza e das Florestas, nomeadamente à Diretora Regional Norte, Arqª Sandra Sarmento, pelas condições criadas para o desenvolvimento do estudo, nomeadamente nas infraestruturas do Posto Aquícola de Castrelos. Um agradecimento especial à Engª Cristina Camilo e aos técnicos José Veiga e Manuel Borges pelo apoio na realização das experiências no Posto Aquícola.

Um agradecimento ao Engº Fernando Teixeira e ao Dr. Fernando Miranda do IPB pela colaboração no desenvolvimento das experiências laboratoriais e de campo da minha tese e ainda pela oportunidade de participar em algumas atividades de conservação no rio com o fim de avaliar o estado da fauna piscícola local.

Ao Paulo Castro, estudante de doutoramento do Centro de Biologia Molecular e Ambiental da Universidade do Minho, pela ajuda com o software R e pela partilha do seu conhecimento de estatística nas abordagens metodológicas e análise de dados.

Ao Professor Doutor Filipe Castro, pelos conselhos que me deu em relação a algumas abordagens da tese, e pela sua disponibilidade.

Ao Doutor Francisco Arenas pela sua simpatia e por me ter fornecido material para fazer algumas fotografias.

Aos membros da equipa de investigação do CIIMAR da minha orientadora, Doutora Elsa Froufe, (Aquatic Ecology and Evolution), e do Professor Doutor Filipe Castro (Animal Genetics and Evolution):

À Ana e ao André Santos, a quem tive o privilégio de assistir às suas defesas de doutoramento, pela simpatia, conversas e disposição a ajudar sempre que os solicitei.

Ao André Machado, sempre disposto a trazer boa disposição, humor e energia à equipa.

À Beatriz, minha colega de dissertação de mestrado, pelas conversas, companhia nas viagens, pela entreajuda e simpatia.

Ao David, pela simpatia, conversas, cultura geral, por ser um verdadeiro contador de histórias e curiosidades, tal como por ser uma das pessoas que melhor conhece o repertório de J.R.R. Tolkien com quem falei.

Ao Diogo pelas conversas e boa disposição.

Ao Duarte, pelos diversos conselhos para a tese, pelas histórias das suas viagens pelo mundo, pelas curiosidades e criatividade.

À Elza, pelo seu sentido de humor, pela ajuda na revisão de estatística, conselhos que me deu de estatística, e por estar sempre atenta e pronta a dar ideias à equipa.

À Giulia, pela sua delicadeza, simpatia e conversas.

Ao João Miguel Cordeiro, pela sua sempre boa disposição, simpatia e iguarias.

Ao Manu, pela simpatia, sempre boa-disposição, pelas conversas e cordialidade.

Ao Manuel Lopes-Lima, por me ter ajudado e aconselhado na revisão da minha primeira apresentação no CIIMAR.

À Raquel pelo entusiasmo com que nos transmite conhecimento dentro da Biologia, pela sua simpatia e pelas conversas.

Ao Rui, pelas nossas conversas, partilha do gosto por automóveis e curiosidades científicas, tal como os crónicos últimos a acabar o almoço.

Aos meus colegas e amigos de Mestrado da F.C.U.P., nomeadamente ao António, à Cheyenne, à Inês, à Joana, ao João e ao Pedro. Pela amizade, pelo estudo em conjunto e entreajuda nos trabalhos.

Às minhas amigas e amigos, de fora da faculdade, pelas conversas, partilha, lazer, ajuda, e motivação em momentos mais delicados, em especial ao Mário e à Mónica.

À minha família:

Em primeiro lugar à minha avó, Maria dos Prazeres, que ainda acompanhou parte desta dissertação, e que foi uma segunda mãe e conselheira.

À minha tia Paula e aos meus primos (João, Joana, Pedro e Paulo), com uma dedicação especial à minha pequena prima Zara, filha do meu primo João, nascida no passado ano.

Ao Constâncio, viúvo da minha mãe, por ser um amigo para a vida e não só isso como parte da minha família, tal como pela amizade do seu filho e nora (Ari e Isabel).

Por último, mas não menos importante:

Ao meu pai, Manuel, que tornou possível a minha frequência académica, me dando sempre liberdade para realizar as minhas escolhas. Apesar da sua personalidade sui generis, é uma pessoa que muitas vezes não o querendo demonstrar, quando é preciso está presente, e se necessário, dá a sua camisa por aqueles que lhe são importantes.

A todos o meu Obrigado!

Resumo

Os mexilhões de água doce são um dos grupos faunísticos mais ameaçados à escala mundial. Nas zonas mediterrânicas, consideradas hotspots de biodiversidade, os impactos de origem natural e antrópica como a poluição e eutrofização da água, a degradação e fragmentação de habitats, a introdução de espécies invasoras e subsequente declínio da ictiofauna nativa hospedeira têm vindo a provocar o declínio e extinção de muitas populações nativas de mexilhões de água doce. Mais recentemente, as alterações climáticas estão na origem de fenómenos hidrológicos extremos, com períodos de seca prolongados alternando com precipitações intensas de curta duração, responsáveis por condições ambientais muito severas nos ambientes aquáticos, em especial para os organismos sésseis ou de diminuta mobilidade. Por tal motivo, é fundamental compreender os efeitos das alterações dos níveis de água nos sistemas aquáticos e, em particular, predizer as respostas comportamentais das espécies mais vulneráveis, de modo a encontrar a melhor gestão dos regimes de caudais e o potencial restauro de condições ambientais adequadas. Neste contexto, no presente estudo foram avaliadas as respostas comportamentais, através do movimento e uso do habitat, de 4 espécies nativas de mexilhões de água doce, i.e., *Anodonta anatina, Potomida littoralis, Unio delphinus* e *Unio tumidiformis*, considerando por cada espécie 2 populações originárias do Norte e Sul de Portugal (*U. tumidiformis* está presente apenas no Sul), perante cenários de condições ambientais adversas. Foram desenvolvidas experiências em: **1) laboratório**, com o recurso a rampas, simulando o desnível das margens para o centro de sistemas naturais, com a consequente manipulação do nível de água através de três tratamentos / taxas de diminuição do fluxo de água, i.e., a) baixa: 4 cm/dia; b) moderada: 8 cm/dia, c) rápida: 4 cm/h durante 10h ou ~96 cm/dia) para além do tratamento/controlo, sem diminuição do nível de água; e **2) no campo**, através da monitorização diária e sazonal do movimento e uso do habitat de exemplares marcados (PIT-Tags e outras marcas) em rios do Norte e Sul de Portugal. Os resultados obtidos mostraram, para as experiências realizadas em laboratório, diferenças significativas nos movimentos horizontais entre espécies, onde *A. anatina* (máximo de 213 cm em 10h) e *U. delphinus* se deslocaram mais ativamente do que *P. littoralis* (muitos exemplares ficaram encalhados e não conseguiram acompanhar o desnível de água) e entre taxas de diminuição do fluxo de água, com deslocações mais expressivas no tratamento rápido. Relativamente aos movimentos verticais, foi encontrado um padrão similar aos deslocamentos horizontais, com a deteção de diferenças significativas entre espécies, embora *A. anatina* tenha demonstrado uma menor capacidade para se enterrar no substrato, e entre tratamentos / taxas de diminuição do fluxo de água, tendo sido registadas as percentagens superiores de enterramento precisamente no tratamento controlo (i.e. > 50%, com exceção de *P. littoralis*, originária do Norte). Importa assinalar que não foram observadas diferenças significativas nos movimentos horizontais e verticais entre populações da mesma espécie, mas de origem diferente, i.e., Norte vs. Sul de Portugal, embora a percentagem de indivíduos encalhados tenha sido significativamente superior nas populações do Sul, com exceção de *A. anatina*. Em *P. littoralis*, sugere-se uma maior adaptação dos exemplares provenientes de rios de carácter mais temporário, típicos do Sul de Portugal, com tendência para a mobilidade para o centro do canal, sempre que o nível da água decresce no período estival. Em *U. delphinus*, nas populações do Norte houve uma maior tendência para os indivíduos seguirem continuamente o nível da água, enquanto nas populações do Sul registou-se um comportamento mais séssil e de enterramento no substrato, o que sugere uma maior tolerância à dessecação. As experiências laboratoriais são corroboradas pelas experiências de campo, nomeadamente na monitorização contínua (intervalos de 20 minutos) diária, onde se detetaram movimentos imediatos, da margem para zonas vizinhas mais profundas, de maior magnitude para *U. delphinus* e *A. anatina* e menos de *P. littoralis*. As experiências sazonais, ainda que realizadas para apenas *U. delphinus*, mostraram que as condições ambientais e em particular o microhabitat disponível interferem com o movimento dos mexilhões. Com efeito, foram detetadas diferenças significativas entre as populações dos 3 rios amostrados no Norte de Portugal, com padrões distintos de variação sazonal e inclusive nos movimentos longitudinal e lateral, que parecem indicar uma adaptação às condições locais, embora maioritariamente junto à margem dos rios.

Admite-se que os recentes fenómenos hidrológicos extremos na Ibéria, com ocorrência de secas prolongadas, possam implicar uma diminuição drástica do nível de água nos rios e limitar o movimento e/ou a sobrevivência da maioria das populações de mexilhões. Consequentemente, a gestão eficiente da água, nomeadamente na agricultura e outros usos (abastecimento, turismo, indústria, produção de energia), e a proteção/restauro dos ecossistemas aquáticos e ribeirinhos é um dos desafios futuros mais importantes para a estratégia nacional, europeia e mundial de conservação da natureza e da biodiversidade.

Palavras-chave: comportamento, Unionidae, movimento, uso do habitat, alterações de caudal

Abstract

Freshwater mussels are one of the most endangered faunal groups worldwide. In Mediterranean areas, considered biodiversity hotspots, natural and anthropic impacts such as water pollution and eutrophication, habitat degradation and fragmentation, the introduction of invasive species and the subsequent decline of host native ichthyofauna have been causing the decline and extinction of many native populations of freshwater mussels. More recently, climate change and the extreme hydrological phenomena, with prolonged periods of drought alternating with intense precipitation of short duration, are responsible for harsh environmental conditions in aquatic environments, especially for sessile or low-mobility organisms. For this reason, it is essential to understand the effects of water level alterations on aquatic systems and to predict the behavioural responses of the most vulnerable species, to find the best management of flow regimes and the potential restoration of environmental conditions. In this context, the present study evaluated the behavioural responses, through movement and habitat use, of 4 native species of freshwater mussels, i.e., *Anodonta anatina, Potomida littoralis, Unio delphinus* and *Unio tumidiformis*, considering 2 populations for each species from the North and South of Portugal (*U. tumidiformis* is present only in the South), to face adverse environmental conditions. Experiments were carried out in **1) in the laboratory,** using ramps, simulating the slope of the banks towards the centre of natural systems, with the consequent manipulation of the water level through 3 treatments /rates of dewatering, i.e., a) slow: 4 cm/day; b) moderate: 8 cm/day; and c) fast: 4 cm/h for 10h or ~96 cm/day) beyond treatment/control, where no decrease in water level occurred; 2**) in situ**, through daily and seasonal monitoring of the movement and habitat use of tagged specimens (PIT-Tags and other marks) in rivers of the North and South of Portugal. The results obtained showed, for the experiments carried out in the laboratory, significant differences in the horizontal movements between species, where *A. anatina* (maximum of 213 cm in 10h) and *U. delphinus* moved more actively than *P. littoralis* (many specimens were stranded) and between rates of dewatering, with more expressive displacements in the fast treatment. Regarding vertical movements, a similar pattern to horizontal displacements was found, with the detection of significant differences between species, although *A. anatina* showed a lower capacity to burrow in the substrate, and between treatments /rates of dewatering, where the highest percentages have been recorded precisely in the control treatment (i.e., > 50%, except for *P. littoralis*, from the North of Portugal). It should be noted that no significant differences were observed in horizontal and vertical movements between populations of the same species and different origins, i.e., North vs. southern Portugal, although the percentage of stranded individuals was significantly higher in southern populations, except for *A. anatina*. For *P. littoralis* is suggested a greater adaptation of the specimens living in temporary rivers, typical of southern Portugal, with a tendency to move towards the centre of the channel, whenever the water level decreases in the summer period. For *U. delphinus*, in northern populations there was a tendency for the individuals to continuously follow the water level, while in southern populations a more sessile and burrowing behaviour in the substrate, which fact suggests higher desiccation tolerances from southern individuals. Laboratory experiments are corroborated by field experiments, namely in daily monitoring (20 minutes intervals), where immediate movements were detected, from the margin to deeper neighboring areas, of greater magnitude for *U. delphinus* and *A. anatina* and less for *P. littoralis*. Seasonal experiments, even performed for only *U. delphinus*, showed that environmental conditions and in particular the available microhabitat interfere with mussel movement. Indeed, significant differences were detected between the populations of the 3 rivers sampled in northern Portugal, with distinct patterns of seasonal variation and even longitudinal and lateral movements, which seem to indicate an adaptation to local conditions, although most individuals remained near the banks of the rivers.

The recent extreme hydrological phenomena detected in Iberia, with the occurrence of prolonged droughts, may imply a drastic decrease in the water level in rivers and limit the movement and/or survival of most mussel populations. Consequently, the efficient management of water, namely in agriculture and other uses (urban supply, tourism, industry, energy production), and the protection/restoration of aquatic and riparian ecosystems is one of the most important future challenges for the National, European and World strategies for the nature conservation and biodiversity.

Keywords: behaviour, Unionidae, movement, habitat use, flow alterations

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List of Abbreviations

AA	*Anodonta anatina*
*Aa*N	*Anodonta anatina* NORTH
*Aa*S	*Anodonta anatina* SOUTH
ANOVA	ANALYSIS OF VARIANCE
APA	AGÊNCIA PORTUGUESA DO AMBIENTE
APHA	AMERICAN PUBLIC HEALTH ASSOCIATION
BMAA	BETA-METHYLAMINO-L-ALANINE
CIIMAR	INTERDISCIPLINARY CENTRE OF MARINE AND ENVIRONMENTAL INVESTIGATION
DO	DISSOLVED OXYGEN
EC25	ELECTRIC CONDUCTIVITY
FCT	FUNDAÇÃO PARA A CIÊNCIA E TECNOLOGIA
FCUP	FACULTY OF SCIENCES OF THE UNIVERSITY OF PORTO
GPS	GLOBAL POSITIONING SYSTEM
HAB	HARMFUL ALGAL BLOOMS
HDX	HALF DUPLEX
ICNF	INSTITUTO DA CONSERVAÇÃO DA NATUREZA E FLORESTAS
INAG	INSTITUTO DA ÁGUA
IPB	INSTITUTO POLITÉCNICO DE BRAGANÇA
IPMA	INSTITUTO PORTUGUÊS DO MAR E DA ATMOSFERA
IUCN	INTERNATION UNION FOR CONSERVATION OF NATURE
KW-H	KRUSKAL WALLIS
MNFI	MICHIGAN NATURAL FEATURES INVENTORY
N	NORTH
NASA	NATIONAL AERONAUTICS AND SPACE ADMINISTRATION
NE	NORTHEAST
PIT TAG	PASSIVE INTEGRATED TRANSPONDER
Pl	*Potomida littoralis*
*Pl*N	*Potomida littoralis* NORTH
*Pl*S	*Potomida littoralis* SOUTH
S	SOUTH
SE	STANDARD ERROR
TDS	TOTAL DISSOLVED SOLIDS

Ud	*Unio delphinus*
*Ud*N	*Unio delphinus* NORTH
*Ud*S	*Unio delphinus* SOUTH
UNEP	UNITED NATIONS ENVIRONMENT PROGRAMME
UP	UNIVERSITY OF PORTO
US	UNITED STATES
USA	UNITED STATES OF AMERICA
Ut	*Unio tumidiformis*

1. Introduction

1.1. Freshwater ecosystems

Aquatic ecosystems are of fundamental importance for the biosphere since they are not only inextricably linked to the origin of life, its development on our planet and metabolic processes, but they also contain a significant part of the existing prokaryotic, eukaryotic, and viruses as regulators (Mora et al., 2011; Sime-Ngando, 2014; Van As et al., 2012).

About 71% of the Earth's surface is covered by water, of which 97.2% is salt water, and the remaining 2.8% is freshwater (Nace, 1967; Alexander et al., 1983). Much of this freshwater is concentrated in glaciers and other forms of ice (2.15%), and of the total water on the planet, only 0.3% can be used by humans, with 98% of all liquid freshwater being groundwater and the remaining surface water (Nace, 1967; Alexander et al., 1983). Although freshwater ecosystems cover less than 1% of the Earth's surface, they are home to more than 10% of all species, around one-third of vertebrate species, and 51% of fish species (Pittock, 2015; Almond et al., 2020). Preservation of freshwater ecosystems is a central issue for society because they provide many basic ecosystem services. Freshwater is indispensable for humanity. People not only drink freshwater to survive and directly obtain food from rivers and lakes but also use it to produce energy, food in agriculture and livestock (Figure 1), as well as for many industrial production activities (Reynaud and Lanzanova, 2017; Nyingi et al., 2013). Freshwater ecosystems have a regulating effect on climate through various ecosystem services such as carbon sequestration, groundwater recharge, flood regulation, water purification, stabilization of coastlines, the concentration of nutrients in wetlands by controlling soil erosion, and retention of sediments (Aldous et al., 2011; Nyingi et al., 2013). Freshwater ecosystems are also important for the tourism and cultural sectors as they can be used for recreation activities like swimming, boating, water sports, bird watching, shoreline activities and diving (Reynaud and Lanzanova, 2017; Nyingi et al., 2013; Ansari et al., 2014). Rivers and lakes can provide important habitats for many aquatic plant and animal species, many migratory, as well as valuable activities such as wildlife observation (Wantzen et al., 2016; Vari et al., 2021). These activities can serve to raise awareness to spread the importance of conservation and citizen science. Freshwater ecosystems are among the most affected ecosystems, not only because of their pollution and overexploitation but also because of the increasing impacts of climate change (Negm et al., 2020). All taxonomic groups of freshwater organisms are at much greater risk of extinction than terrestrial organisms, with nearly one-third of their species in danger (Collen et al., 2014). Statistically, monitored freshwater populations have declined by 83% since 1970, representing a loss of 4% of species per year, with two of the main reasons being barriers to migration routes and habitat loss (Dudgeon et al., 2006; Reid et al., 2019; Almond et al, 2020). Not to mention that about 84% of freshwater megafauna occurs outside of recognized protected areas, which calls for an urgent review of policies (Carrizo et al., 2017).

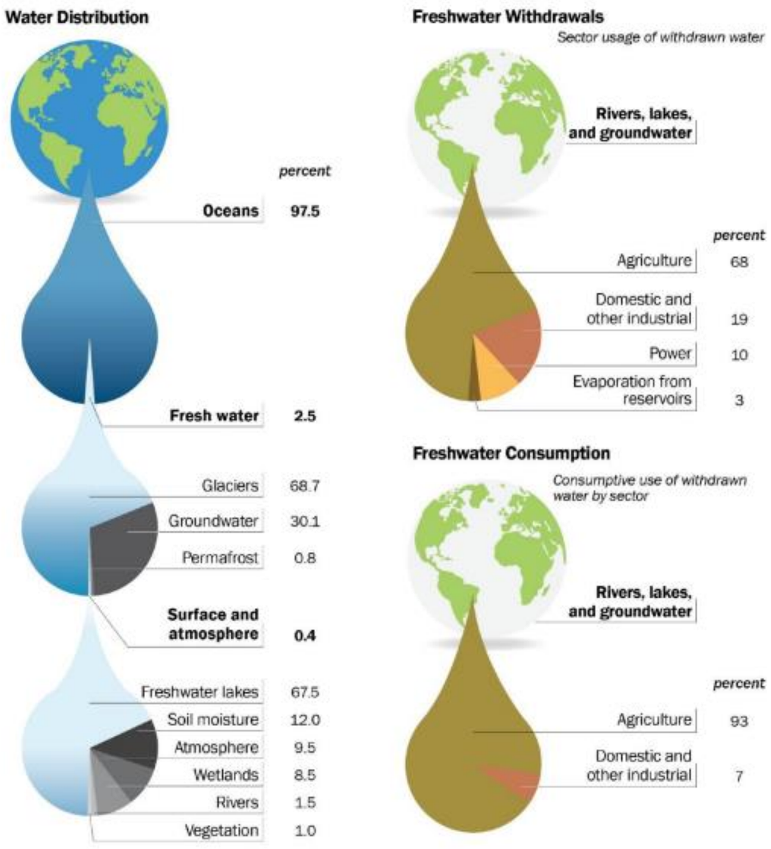


Figure 1 Water distribution and current human use, Office of the Director of National Intelligence (USA) quoted by World Bank, 2010.

1.2. Anthropogenic impacts and overexploitation

The demographic explosion that led to the quadrupling of the world population, during the 20th century, which has continued to gallop in the present century, has severe consequences (Mehta and Kumar, 2002). Factors such as population growth, new consumption patterns, and socioeconomic development have led to an increase in freshwater consumption of 1% per year since 1980 (United Nations, 2019). At this rate, freshwater consumption is predicted to increase by 20-30% from current levels by 2050 (United Nations, 2019). This situation has an immediate negative impact on the stability of ecosystems, especially aquatic ones since the main consequences of these phenomena are pollution, overexploitation of aquatic biological resources, changes in water flows, habitat destruction and the introduction of invasive species (Okello et al., 2015; Ormerod et al., 2010; Dudgeon et al., 2006). In this context, the activities of agriculture, livestock and industry are among the greatest antagonists when it comes to the preservation of freshwater ecosystems (Horrigan et al., 2002). Not only do they use vast amounts of water to produce their resources, but also pesticides and fertilizers, which have high solubility, eutrophicate rivers and infiltrate into important groundwater reservoirs, the aquifers (Horrigan et al., 2002). The construction of dams and weirs and the diversion of watercourses for irrigation in agriculture, industry, and the public sector have negative consequences for aquatic organisms (Dudgeon et al., 2006; Diez et al., 2012). Associated with these infrastructures remains habitat destruction, which not only alters river dynamics (Dudgeon et al., 2006) but also deforestation leads to the destruction and/or disruption of river margins, in addition to river pollution through the leaching effects of clay soiling, which settles in the water mix (Pereira-Silva et al., 2011). As a result, aquatic organisms inevitably suffer inestimable losses of key species, not to mention the effects of overfishing associated with the introduction of exotic species (Arthington et al., 2016; Dudgeon et al., 2006). This is due to recreational purposes, plague control, mistakenly increasing diversity in local rivers or even accidental release, which is complicated by the fact that many of these species are highly adaptable and resilient (Pyke, 2008; Hulme et al., 2015; Mills et al., 1993).

1.3. Climate change effects and Mediterranean Basin rivers

Freshwater ecosystems have an uncertain future. The overexploitation of their natural resources combined with the severe effects of climate change brings unprecedented challenges to their stability and the regulation of inhabiting species populations and the food chain.

The increase in water demand in agriculture, industry, and recreation sectors (Figure 2), especially in Mediterranean basin countries such as Greece, Italy, Portugal and Spain, is leading to a dramatic decline and loss of endemic freshwater biodiversity (Benejam et al., 2010). In Europe, 77% of threatened species are at risk of losing 90% of their distribution area, with 50% of mussels not yet having a protected area in their estimated range (Markovic et al., 2014). In parallel, many terrestrial protected areas do not protect freshwater biodiversity, and even in freshwater-protected areas, there is a risk of pollution from upstream waters (Nel et al., 2008, 2011; Abell et al., 2008). To complicate matters, these regions have greater interannual variability in precipitation and river flows, with more extreme and frequent climatic events, with wet locations becoming wetter and dry areas becoming drier (Millan et al., 2005; Huntington, 2006). These increases in more frequent and intense extreme thermal phenomena with irregularities in the precipitation index caused by the impacts of climate change means that freshwater ecosystems, and in particular the organisms living in them are among the most affected (Vaquer-Sunyer et al., 2011; Diamond et al., 2018; Stillman, 2019). Moreover, increasing seasonal temperatures have a rising effect on evapotranspiration and evaporation rates which reduces river discharge and the number of suspended particles in the water column (Gordon et al., 2004; Döll et al., 2010). Increased water temperatures and CO_2 concentrations can lead to hypoxic effects that attenuate the growth of eukaryotic algae, which promotes threatening cyanobacterial blooms to become prevalent (Visser et al., 2016). On the other hand, drastic water temperature reductions seem to have a negative impact on fish, leading to mass mortality (Szekeres et al., 2016). Increases in dissolved humic matter from terrestrial, wetland sources and macrophytes cause “brownification”, where the water takes on a yellow-brown colour because of high concentrations of dissolved organic iron and carbon (Graneli, 2012). Water “brownification” has dangerous impacts on ecosystem regulation, freshwater organisms, and people (Solomon et al., 2015; Van Dorst et al., 2018). This may lead to a selection of species capable of utilising inorganic-organic matrices containing phosphorus and other elements that favour H.A.B. of dominant algal strains of cyanobacteria that produce toxins such as microcystins or BMAA (Kritzberg et al., 2012; Erkvall et al., 2013; Banack et al., 2015).

Climate change is raising sea levels and altering hydroclimate, particularly in coastal areas, with socioeconomic and environmental impacts that directly affect freshwater species and people (Reid et al., 2019), resulting in freshwater salinization. Today freshwater salinization appears to be occurring on an unprecedented scale with lowland aquifers affected by hydrostatic pressure, coastal freshwater ecosystems affected by sea level rise, and irrigation of agricultural lands (Herbert et al., 2015; Reid et al., 2019). In warmer and drier climates, such as the southern Mediterranean basin, evaporation rates may increase with climate change, and greater amounts of water must be applied to prevent crop death from desiccation (Vorosmarty et al., 2010), leading to a vicious cycle of freshwater waste. In snowy areas, it is also common to use salt to melt or de-ice frost roads, which has dramatic effects on soil ecosystems due to salinization and could also contaminate aquifers or surface freshwater ecosystems (Findlay et al., 2011).

Ecological processes of many freshwater ecosystems have a gradual effect from genes to their populations, resulting in changes in phenology, reduction in body size, the spread of algal blooms, shifts in distribution, and changes in interspecific interactions

(Scheffers et al., 2016). Another threat to native species is that invasive species also carry diseases and parasites that affect endemic freshwater populations (Hoffman 1990; Holdich et al., 1991). Climate changes have direct and indirect impacts on global health, with critical and unprecedented impacts on wildlife and human health, carrying emergent contaminants and diseases to other latitudes (Daszak et al., 2000; De Laender et al., 2011).

Critical actions to reduce the impacts of climate change on freshwater ecosystems include reducing greenhouse gas emissions, expanding freshwater-protected areas, and restoring habitats that provide refugia for species to thermally adapt (Pittock et al., 2008; Heino et al., 2009). Beyond all the threats described that affect freshwater ecosystems and are potentiated by the effects of climate change, other stressors need to be explored in more detail. In Portugal, Agência Portuguesa do Ambiente (APA), within the Water Framework Directive, has been fundamental for the analysis of water quality and its pressures to mitigate the impacts of climate change stressors (Lopes-Lima et al., 2016). An increasing number of scientists working on these problems and parallel dissemination in media and academia brought more information about better-detailed targets at a larger scale (Lopes-Lima et al., 2016).

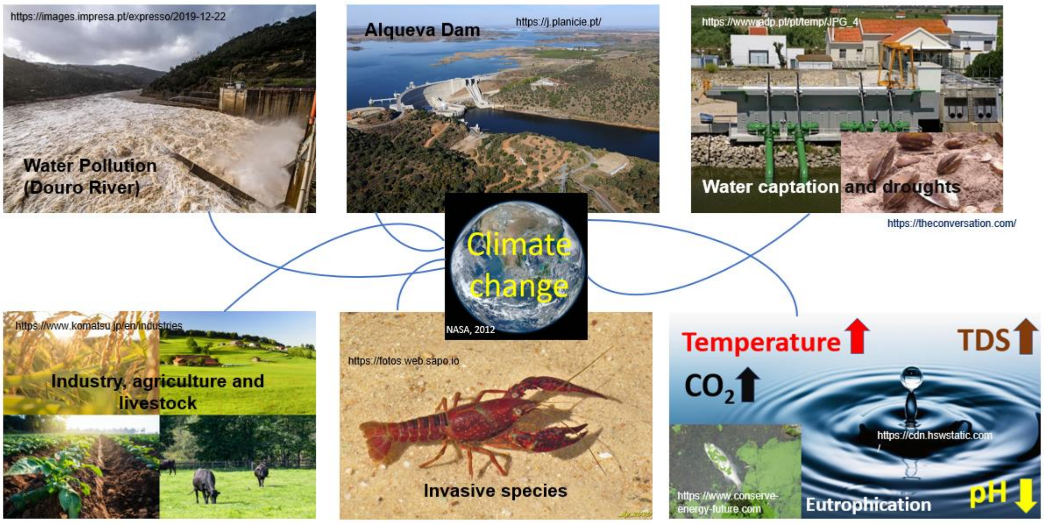
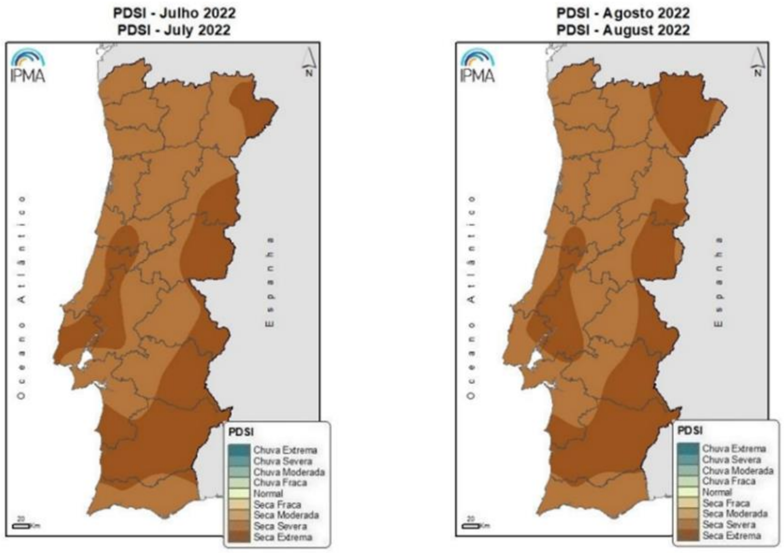


Figure 2 Everything is binded! Scheme of threats to freshwater ecosystems in a climate change scenario.

1.4. Iberian rivers

The Iberian Peninsula is a very heterogeneous region, ranging from the humid to sub-humid and higher mountainous North to the drier, arid, and semi-desert South (UNEP, 1992). The main rivers of the Iberian Peninsula are Douro, Tejo, Guadiana, and Guadalquivir, which flow into the Atlantic Ocean, and Ebro into the Mediterranean Sea (Sabater et al., 2009). The northern rivers have less temperature variation and have lower temperatures throughout the year, between 3 and 31ºC (Jesus et al., 2015). Southern rivers, on the other hand, have higher temperatures between 4 and 38ºC with irregular breaks between droughts and floods and less oxygenated water (Jesus et al., 2013). Southern rivers are also more susceptible to prolonged drought and extreme temperatures (Füssel et al., 2012) because they are in warmer regions and generally carry less water than northern rivers, due to great discrepancies in precipitation index (Sabater et al., 2009), which contributes to these factors (Figures 3 and 4). Portugal and Spain are characterized by a strong interannual variation in the precipitation index, which can be as high as 40% in the Southeast of the peninsula (Barry et al., 1998; Vide et al., 2001). This has parallel effects on the flow regime of rivers over the years with large disparities between wetter and drier years (Daveau, 1988). There are significant discrepancies between the annual precipitation index between the North and South, with the first ranging from 1000-2000 mm/year, while the semi-desert areas in the South have 300-350 mm/year (Sabater et al., 2009). This region is one of the most affected by the effects of climate change, and with more intense and prolonged periods of anomalies in the precipitation index, the vulnerability to drought and dewatering effects on many streams is a concern (Figures 3 and 4).



PDSI - Julho 2022
PDSI - July 2022

PDSI - Agosto 2022
PDSI - August 2022

Figure 3 Spatial distribution of meteorological drought index from 31st July to 31st August in Portugal, IPMA 2022.

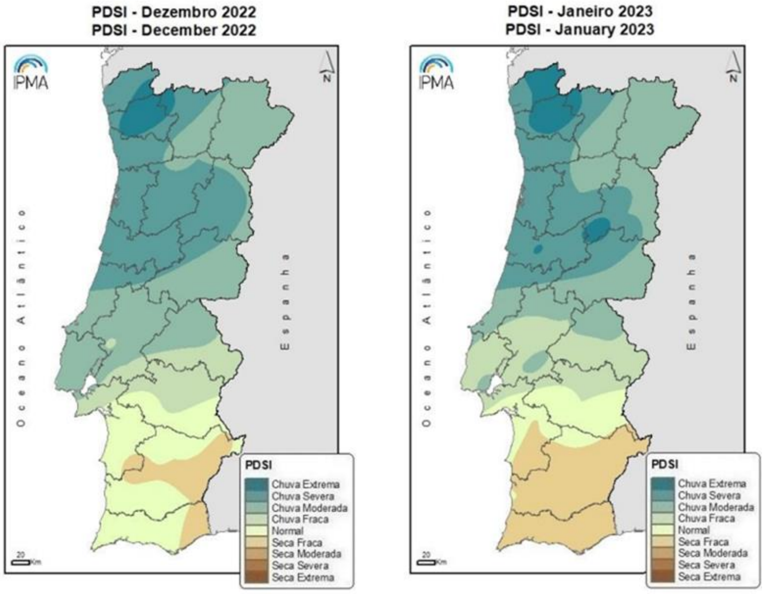


Figure 4 Spatial distribution of meteorological drought index from 31st December to 31st January in Portugal, IPMA 2023.

Riparian woodlands are located between the aquatic and terrestrial realms and are responsible for many ecosystem services and diverse functions that are fundamental to longitudinal, vertical, and lateral biomass and energy fluxes (Naiman et al., 1997). These areas also influence the ecological processes of vegetation in terms of productivity, biogeochemical regulation, and biodiversity (Aguiar et al., 2011). Riparian woodlands agglutinate the land and preserve water quality from pollution caused by surface runoff, sedimentation, and erosion (Pusey et al., 2003; Gregory et al., 1991). Especially through agricultural activities where the riparian line can serve as an interceptor for contaminants that can be lixiviated such as nitrates and phosphates (Pusey et al., 2003; Gregory et al., 1991). Riparian woodlands regulate the transfer of solar energy to freshwater ecosystems, where shade produced by vegetation protects water and biodiversity from heat (Pusey et al., 2003). These woodlands are natural refuges, ecological corridors, and habitats, but when they are destroyed and fragmented, the likelihood of riparian zone invasion and terrestrialization increases (Schuft et al., 1999; Aguiar et al., 2011). Some Iberian Mediterranean rivers are examples of riparian woodlands in poor ecological condition, with less complex vegetation with small, isolated, and homogeneous riparian patches with low intersection of the patch distribution (Fernandes et al., 2011). Patches are very peculiar homogeneous areas with specific processes that can be identified within the riparian zone (Apan et al., 2002). Three characteristics are fundamental to assessing the condition of riparian zones, i.e. side length, structure, and composition (Moser et al., 2000).

1.5. Freshwater mussels

1.5.1. Characterization and ecological roles

Several groups of animals play key roles in maintaining freshwater ecosystems, one of which is freshwater mussels from the order Unionida (Lopes-Lima et al., 2016). The family Unionidae is a very diverse group with about 700 species (Lopes-Lima et al., 2020). The European Unionida are composed of 16 species, 2 of each belonging to the family Margaritiferidae and 14 to the family Unionidae (Lopes-Lima et al., 2016). This group probably originated in the Early Jurassic period, throughout Southeast and East Asia and, afterwards expanded to other landmasses (Bieler et al., 2014; Bolotov et al., 2017). These animals are characterized by a slow growth rate, considerable life expectancy (7 years in *U. tumidiformis* over 100 years in *M. margaritifera*) (Bauer, 1992; Reis et al., 2016), and two-hinged shells, the latter being a common feature among the class Bivalvia (Hochwald, 2001; Gosling, 2003; Ramos, 2011). The maximum growth rate of Unionidae occurs in the first year, when they grow about 20mm, with a decrease in subsequent years, with shells growing at least 5mm per year for the first 10 years (Aldridge, 1998). After reaching asymptotic length, shell length becomes a less effective indicator of age, with shell rings providing a much more reliable method (Aldridge, 1998). In terms of sexual dimorphism, there are no significant differences between both sexes, but in some cases, female shells appear to be more rounded and enlarged both laterally and ventrally, likely due to enlarged gills (marsupium) containing glochidia (Zieritz et al., 2011). Freshwater mussels are found in rivers, lakes, and streams worldwide where they inhabit the sediment or gravel and are among the most endangered groups of organisms (Piper, 2007; Moore et al., 2018). These invertebrates can be considered ecosystem engineers (Figure 5) due to their filtration and water clearance abilities, influence in regulating biogeochemical cycles and in the primary and secondary productivity of freshwater environments (Strayer et al., 1999; Gutiérrez et al., 2003; Vaughn et al., 2018), sometimes making up >90% of benthic biomass of rivers (Negus, 1966). Freshwater mussels also act as biofilters due to their tolerance to pollutants and ability to bioaccumulate, so they can be used to remove water contaminants and as bioindicators to monitor water quality (Lopes-Lima et al., 2014; Vaughn et al., 2018). Their shells serve as biofilms with which algal and microbial communities are associated contributing to nitrogen, phosphorus, and carbon cycling (Hoellein et al., 2017). They also serve as a refuge from predators, not only for the bivalve but also for other taxonomic groups, depending on whether they are empty or not (Gutiérrez et al., 2003). Unionoids are fundamental to the economy as a food source, shells are used for nacre production, jewelry (pearls) and trading, mainly in Southeast Asia (Cummings et al., 1992; Zieritz et al., 2018). They can be used in aquaculture, not only because of their filtration abilities but also because their shells are rich in CaCO3, which provides feedback to acidification (Hamester, et al., 2012). Freshwater mussels also play an important role in bioturbation by disrupting the redox effects of sediment layers through their oxygenation and influencing the nitrogen cycle, which communities are hotspots for a variety of organisms with complex interactions (Welsh, 2003; Vaughn et al., 2018).

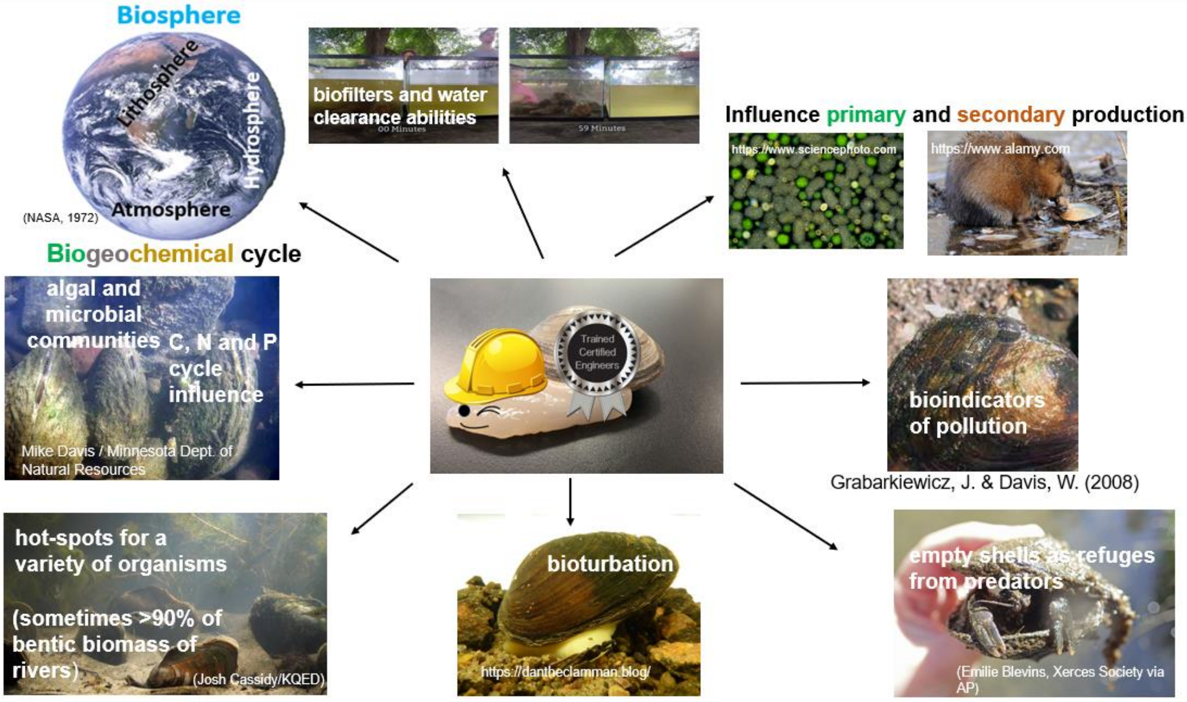


Figure 5 Scheme of freshwater mussels as ecosystem engineers

1.5.2. Life cycle

Freshwater mussels have a very characteristic and unique life cycle when brooding the larvae, and must live parasitically to metamorphose into juveniles and reach the adult stage (Lopes-Lima et al., 2016; Lopes-Lima et al., 2014). This ectoparasitic larval stage is called glochidia and is between 0.2 to 0.5 mm in size (Piper, 2007; Lima et al., 2012). The internal fertilization process first occurs through the gill surface (Figure 6), with sperm entering the mantle cavity through the inhalant flow and female eggs are brooded in a specialized gill, named the marsupium (Hinzmann et al., 2015; Lopes-Lima et al., 2016). After fertilization, the eggs develop into bivalve larvae (glochidia), and the respective brooding duration, in the marsupium, varies between species, with short-term brooders lasting about 2-6 weeks and long-term up to 8 months (Jacobson et al., 1997;

Garner et al., 1999; Haag, 2013). Following this process glochidia are released by the female after maturation and must parasitize a vertebrate host, usually fish, in which they encyst/encapsulate and metamorphose (Reis et al., 2009; Haag, 2013; Lopes-Lima et al., 2016), in the formation of cysts glochidia are exclusively surrounded by host tissue (Hoggarth et al., 1988; Reis et al., 2014).

Normally, glochidia attach mainly to the gills, but also to the fins or skin of the host fish (Kat, 1984). This behaviour could favour the dispersal of bivalves and nutrient extraction, which, in addition to their low mobility as sessile animals, could explain their wide distribution (Kat, 1984; Denic et al., 2015). Regularly parasitism lasts between 10 days to several months in some species such as *M. margaritifera*, until metamorphosis to the juvenile form (pediveliger) is completed (Lima et al., 2006; Treasurer & Turnbull, 2000). The teleosts targeted by the mussel vary from species to species (Lopes-Lima et al., 2016), with some parasitizing different groups and others specifically choosing a particular group of fish such as salmonids in the case of *M. margaritifera* (Taeubert et al., 2012; Karlsson et al., 2012). Nevertheless, host information on mussel species, host populations, and host variation due to local abiotic adaptations needs to be further investigated (Taeubert et al., 2012; Lopes-Lima et al., 2016). The success rate of glochidia in finding a suitable host is extremely low (0.01%), so many larvae must be produced to ensure reproductive success (Young and Williams, 1984; Barnhart, 2000; Modesto et al., 2018). In addition, the rate of metamorphosis from glochidia to juveniles is low due to the suitability of the fish host, failure of attachment or encystment, host immune responses, fish blood composition, and water temperature (Lima et al., 2012; Amyot et al., 1997). Usually, annual fecundity is >200000, but can exceed 10000000, there are also some species with relatively lower values such as *U.tumidiformis*, which fecundity does not surpass 15000 per reproductive phase (McMahon & Bogan, 2001; Reis, 2010; Haag, 2013). When the metamorphosis process is complete, pediveligers detach from the host and must drop into a suitable substrate and begin pedal feeding on finely divided organic matter, to survive to the adult stage (Neves et al., 1987). When these juveniles are dropped, they immediately begin to grow with a very active behaviour, and after a few hours, a small shell growth ring appears (Reis et al., 2014). At this stage, young mussels are much more sensitive to pollutants and changes caused by non-native macrophytes than in the adult stage (Lasee, 1991; Yeager et al., 1994; Moore et al., 2018).

The reproductive potential of Unionidae is related to the maximum size and age of the bivalves, as larger individuals have larger gills containing more glochidia (Aldridge, 1998). Thus, long-lived and larger individuals reproduce more frequently, produce more individuals per time, and have higher lifetime fecundity than short-lived ones (Aldridge, 1998). In addition, short-lived species may be subject to greater population fluctuations than longer-lived species (Aldridge, 1998).

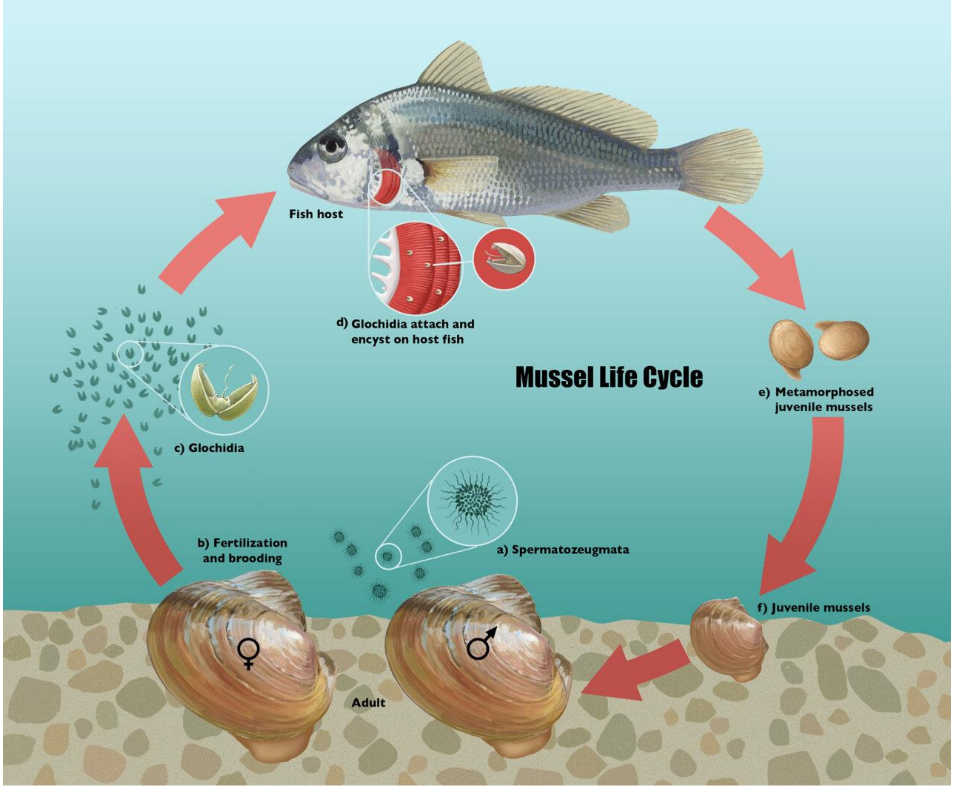


Figure 6 Freshwater mussel life cycle, in Hewitt et al., 2021.

1.5.3. Threats to freshwater mussels

In Europe, extreme floods and droughts have led to massive die-offs of freshwater mussels, which are becoming more intense and frequent (Mouthon et al., 2006; Sousa et al., 2012). Freshwater mussels from Mediterranean basin rivers are among the species most affected by these climatic stresses mainly because they are restricted to small rivers, of which southern Iberia is a good example of that (Benejam et al., 2010). Not only do they have a low dispersion capacity, but they also must cope with high temperatures, drought, pollution, habitat destruction, invasive species, parasites, water irrigation and deprivation, all at the same time as climate change impact (Dudgeon et al., 2006; Strayer & Dudgeon, 2010; Lopes-Lima et al., 2016). On the other hand, high flows can increase the total of suspended particles, which leads to an increase in metabolic rates in freshwater mussels, reducing growth and reproduction (Luck, K., et al., 2022).

Water temperature, CO_2 concentration, and water acidification are affecting freshwater mussels. In this context, even small temperature fluctuations seem to drastically affect the success of glochidial metamorphosis of Unionidae, which has a crucial impact on recruitment success (Taeubert et al., 2014; Sousa et al., 2013, 2015).

Greenhouse gases such as CO_2 appear to have effects on the shell growth of juvenile freshwater mussels and at high concentrations can have narcotization effects on the bivalves where the animal appears to have an extended foot and a gaping shell (Waller et al., 2016). This gas also appears to erode the periostracum and shell, and CO_2 concentrations shouldn´t exceed 76 mg/l to prevent juvenile mussel mortality and shell damage (Waller et al., 2016). This can lead to severe acidosis as H^+ ions enter the animals, whereupon they lose ions from the hemolymph and tissues (Pynnönen, 1990). Mature glochidia may also be more sensitive to acidification than developing glochidia, due to direct contact with the water (Silverman et al., 1987; Pynnönen, 1990). In this context, acidification of the water can lead to the death of the fish as the gills produce too much mucus (Pynnönen, 1990), which leads to a reduction in the rate of encystment in potential host fish. Water salinization also appears to be affecting the ability of glochidia to encyst on fish hosts and impair their viability (Beggel et al., 2015). Subsequently leading to declines in freshwater mussel populations, not to mention that climate change potentially threatens almost 50% of the world’s freshwater fish on which these invertebrates depend (Darwall et al., 2015). Some freshwater mussel species are better adapted to the effects of climate change than others, some of them are invasive like *D. polymorpha*, and the overlap with native mussel species can have serious consequences (Lopes-Lima et al., 2016). Not only some freshwater mussels are already threatened with extinction, but they also have specific hosts and will find fewer refuges (Lopes-Lima et al., 2016).

The introduction of alien species is one of the greatest threats to freshwater diversity in the Iberian Peninsula (Sala et al., 2000). The red swamp crayfish *Procambarus clarkii* (Girard 1852) first introduced in Spain and later in Portugal, has promptly spread in many Iberian freshwater ecosystems (Pérez-Bote et al., 2000; Gutierréz-Yurrita et al., 1999; Pérez-Bote, 2004). A study by (Meira et al., 2019) revealed that this crayfish species feeds on several Iberian freshwater mussel species such as *A. anatina*, *P. littoralis,* and *U. delphinus*, with the former species being the most predated. *U. tumidiformis,* a vulnerable and strictly localised Iberian freshwater mussel species (Araujo et al., 2009), could potentially be affected by these invasive crayfish, which should be better studied.

Anthropic activities associated with climate change are reducing the distribution area of freshwater mussels, leading to a decline in the role of freshwater mussels, a loss of genetic variability within species, an increase in distances, and a dispersal reduction, all of which increase the species risk of extinction (Strayer, 2008). As ecosystem engineers, the decline of freshwater mussel populations has led to changes in ecosystem processes, and this group is very sensitive to biodegradable pollution, leading to the conclusion that unionids are the first mollusks to disappear by pollution (Mouthon, 1996). Moreover, the climate change effects have a direct impact on the behaviour of freshwater mussels, affecting their population structures and spatial distributions (Lopes-Lima et al., 2014). Some of these factors are water temperature, turbidity, O_2 concentration, current velocity, altitude, nutrients as abiotic factors, and competition, parasitism, disease, and predation as biotic factors (Lopes-Lima et al., 2014; Luck et al., 2022).

One factor of concern to scientists that requires more attention is that even in theoretically healthy freshwater ecosystems with well-stabilized communities and trophic interactions, there has been a massive decline in mussel species in recent decades (Lopes-Lima et al., 2016; Hermoso et al., 2011). In parallel, there is a great need for research on the biomass, secondary production, growth, and density of freshwater mussels, as well as on fish hosts, food, antagonists, and habitat fragmentation, the main factors affecting spatial distribution (Strayer, 2008). Fortunately, concern about these problems in Europe has led to major improvements in water quality in recent decades with the reintroduction of mussels into rivers, lakes and ponds (Lopes-Lima et al., 2016).

1.5.4. Physiology

The ability to acquire nutrients is the basic requirement for the growth of a bivalve species (Hawkins et al., 1999). Freshwater mussels may feed on suspended particles through filtration, sediment during pedal feeding, or a mixture of phytoplankton, fine organic detritus, and bacteria (Frischer et al., 2000; Vaughn et al., 2018). During the filtration process freshwater mussels produce upstream mechanisms to strike, retain, and transport particles on the ctenidium (Ward et al., 2004). In this process, a current is created that flows through the interfilament spaces of the ctenidia into the suprabranchial cavity and out the exhalant aperture of the siphon (Ward et al., 2004). Particles are transported through the mouth via marginal pathways, either in a cohesive mucus filament (ventral) or in a less cohesive mucus water slurry (dorsal) toward labial palps and stomach (Ward et al., 1993; Ward, 1996; Ward et al., 2004). Mussels have behaviour changes in filtration rate due to their preferences and pre-ingestion that are influenced by determinate factors like the shape and size of particles, their composition and chemistry (Vaughn et al., 2018). Particles that don’t meet the needs of the mussels are excreted as pseudofeces encapsulated in mucus, less nutritious materials are moved for rapid egestion as feces, while the higher quality products are completely digested in diverticula (Ward et al., 2004; Vaughn et al., 2018). The conjunction of faeces and pseudofeces is considered biodeposits because they repackage nutrients (Vaughn et al., 2018). Particle-feeding mussels profoundly impact pelagic and benthic processes by depositing faeces and pseudofeces to enrich benthic substrate, removing phytoplankton, dissolved nutrients cycling and can turn complex molecules into inorganic form (Ward et al., 2004). Freshwater mussels are also a food source for many predators (Figure 7), as they can be eaten as juveniles by platyhelminths, crayfish and insect larvae, and as adults by various species of fish, turtles, mammals such mustelids, raccoons, muskrats, feral pigs, skunks, and even opportunistic birds (Neves et al., 1989; Vaughn, et al., 2018; Van Ee et al., 2020).

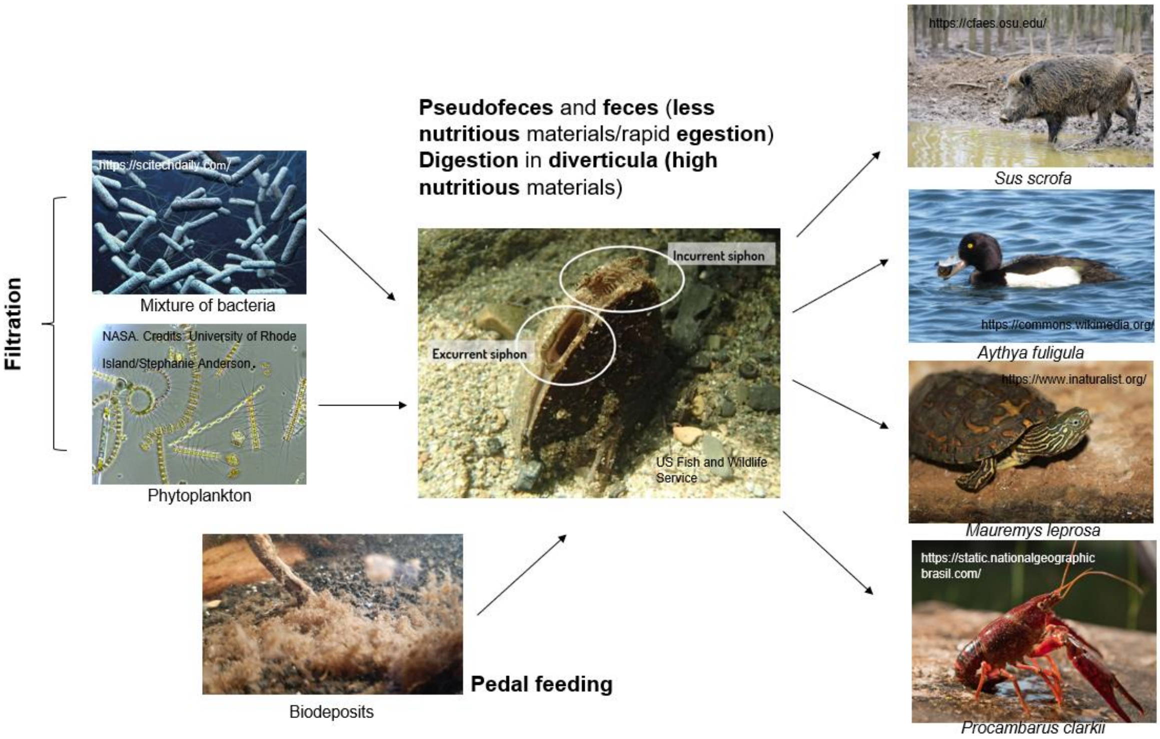


Figure 7 Scheme of freshwater mussels' physiology and predators.

1.5.5. Drought, dewatering and species behaviour

Freshwater mussels, as sessile organisms, are among the animals most sensitive to extreme drought and dewatering. Escaping these events as sessile entities is challenging for mussels, and direct mortality associated with stranding, as well as indirect effects of high temperatures, low O_2 and predation, are common (Galbraith et al., 2015).

Prolongation and severity of drought events, smaller water flows and several human activities demanding water combined with climate change scenarios have dramatically changed the seasonality of water availability (Gleick, 1998; Poff et al., 1997). Most studies focused on the long-term effects of flow changes (Poff et al., 2010). On the other hand, more emphasis should be placed on the acute effects of short-term flows, which can greatly affect ecosystem communities (Poff et al., 2010). Dewatering is a short-term effect that agitates scientists due to negative impacts on numerous streams, especially in water harvesting and management (Galbraith et al., 2010, 2012, 2015). Water harvesting activities can significantly amplify the intensity, duration, and regularity of natural droughts, even in ecosystems where they have rarely been observed, making it difficult for fauna to adapt (Galbraith et al., 2010, 2012). The acute nature of the dewatering rate is important because even very adaptable organisms could suffer drastic effects such as stranding due to fast dewatering (Perry et al., 1986; Bradford et al., 1995; Halleraker et al., 2003).

Adaptations for survival can be categorized as the ability to tolerate desiccation effects, avoidance behaviours, and life history adaptations such as dormancy (Williams, 2006). The capacity to survive these conditions depends on local environmental conditions, duration of the perturbation event, species, and habitat characteristics (Lake, 2000; Magoulick et al., 2003). Highly mobile organisms may shift the drought-affected area to another, through their avoidance behaviour, while low mobility and sessile organisms have developed physiological adaptations (Galbraith et al., 2015; Wilbur et al., 1973).

Freshwater mussels may exhibit different dewatering behaviours depending on the species and abiotic features in which they are found (Galbraith et al, 2015). The behaviour of freshwater mussels may be influenced by physiological tolerances and their life history strategies (Gough et al., 2012; Mitchell et al., 2018). Some species burrow vertically and can survive for extended periods during drought (Isely, 1914). The majority of species move short distances horizontally in response to lowering water levels (Galbraith et al., 2015). Mussels that burrow when stranded may have the greatest desiccation tolerance, and less tolerant species move more toward the water’s edge (Mitchell et al., 2018). Freshwater mussels can be divided, based on life-history traits, into three different categories, the equilibrium, the periodic and the opportunistic species (Haag et al., 2012). These categories are influenced by life-history strategies such as body size, age at maturation and lifespan, fecundity (Grime, 2001; Haag, 2012), and the type of habitat in which they reside (Haag et al., 2012). Equilibrium species have long lifespans, low to moderate growth rates, can seal their shells, likely making them probably more tolerant of desiccation, and burrow deeper than the other groups (Haag et al., 2012; Mitchell et al., 2018). These species have the lowest mobility and highest desiccation tolerance and likely live in stable ecosystems (Haag et al., 2012; Mitchell et al., 2018). Periodic species are midway between equilibrium and opportunistic species with intermediate desiccation tolerance, inhabit ecosystems with greater water level fluctuations, and have the highest mobility among these three groups (Haag et al., 2012; Mitchell et al., 2018). Opportunistic species have low desiccation tolerance, early reproduction, higher growth rates, inhabit highly disturbed ecosystems, and have intermediate mobility (Haag et al., 2012; Mitchell et al., 2018).

A better understanding of stream dewatering could provide inestimable knowledge for maintaining and restoring healthy mussel populations allowing to answer several pertinent questions such as: 1) Do populations differ in their behaviour toward dewatering? 2) Do different dewatering rates result in different responses? 3) Do strand populations differ in their tolerance to dewatering?

1.6. Freshwater mussel studied species

Four native freshwater mussel species were used in this study: *Anodonta anatina*, *Potomida littoralis* and *Unio delphinus,* relatively widespread in Portugal, and *Unio tumidiformis*, only present in southern Portugal (Figure 8).

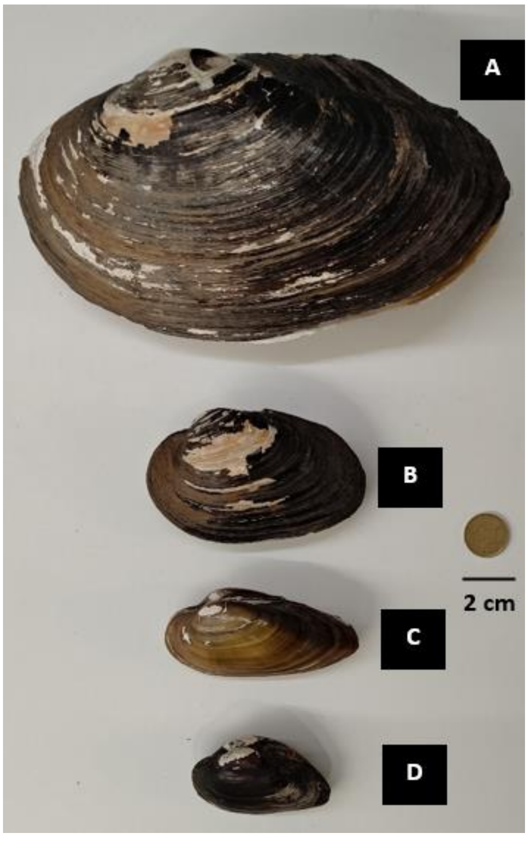


Figure 8 Freshwater mussel species relative sizes comparison: *Anodonta anatina* (A), *Potomida littoralis* (B), *Unio delphinus* (C) and *Unio tumidiformis* (D).

1.6.1. *Anodonta anatina* (Linnaeus, 1758)

These freshwater mussels from the sub-family Unioninae, and tribe Anodontini, are a widespread species in Europe (Hinzmann et al., 2013; Lopes-Lima et al., 2016), whose conservation status is classified as “least concern” and whose populations are declining (Lopes-Lima et al., 2014). The species range extends from the Iberian Peninsula, Morocco, Central Europe, Great Britain, and Scandinavia to eastern Russia, with the species threatened in Germany and decreasing in Iberia (Froufe et al., 2014; Froufe et al., 2017; Lyubas et al., 2023). Confusion often occurs between the Anodontini species *A. anatina* and *A. cygnea*, but genetic analysis can objectively confirm the two distinct species, with 9% of genetic distance between them and 1% variability between *A. anatina* individuals (Froufe et al., 2014). Additionally, *A. anatina* seems to prefer flowing waters with more dioecious individuals, while *A. cygnea* prefers stranded or slow-flowing waters with higher hermaphrodite rates (Hinzmann et al., 2013). The study by Froufe et al., (2014 and 2017) and Lyubas et al., 2023 revealed several genetic lineages in the haplotype dataset with four notoriously diverse genetic groups, with the highest diversity in the European lineage (non-Iberian and non-Italian) and the lowest in the Italian. *A. anatina* species exhibit striking intra-specific variation with periostracum with green-yellow-brown, yellow-brown and black-brown colouration (Araújo et al., 2009; Klishko et al., 2018). The species' shell morphology varies from more ovate-oblong, more ovate-square, or rectangular, from very flattened shells to convex (Araújo et al., 2009; Klishko et al., 2018).

The reproductive cycle of *A. anatina* is completed in about 10 months. (Hinzmann et al., 2013). Gametogenesis is synchronized and occurs primarily between January and May after larvae are accumulated in the outer gills of females from September to March, and the release of mature glochidia begins in March for 2-3 weeks (Hinzmann et al., 2013). Previous studies (Kat, 1983; Bauer, 1988) have shown that *A. anatina* was strictly dioecious, with occasional hermaphroditism. However, the study by Hinzmann et al. (2013) showed that there are high rates of hermaphroditism in stranded waters, while they are mainly dioecious in flowing rivers and streams. The life expectancy of this species is about 10 years, usually, they start reproducing after 4-5 years, but negative conditions can accelerate the age of maturity to 2 years and drastically reduce lifespan to less than 4 years (Økland, 1963; Zieritz et al., 2011; Heard, 1975). The species can grow up to 15 cm with water extraction and agricultural pollution being two of the major threats to this mussel, but under the right conditions, the lifespan of species can exceed 15 years (Araujo et al., 2009; Tomilova et al., 2020; Anders et al., 1993).

1.6.2. *Potomida littoralis* (Cuvier, 1798)

This species of the sub-family Gonideinae has a western Mediterranean distribution, occurring in North of Africa, Portugal, Spain and France (Froufe et al., 2016). *P. littoralis* is classified as endangered, and in recent decades populations have declined in Iberia (Lopes-Lima et al., 2014; Pérez-Quintero, 2006; Barea-Azcón et al., 2008). A massive 75% decline has been observed in recorded populations of this species in Europe (Froufe et al., 2016; Lopes-Lima et al., 2014). *P. littoralis* has two mitochondrial lineages, one exclusively in Europe and another with a majority in North Africa and some regions of Europe (Froufe et al., 2016). This species has a solid shell, not very long, curved, high and rough, ranging from oval elliptical to quadrangular (Araujo et al., 2009; Ilarri et al., 2018). The periostracum is black-greenish with yellow radial lines and is usually highly eroded in umbo with a contrasting white colouration (Araujo et al., 2009; Ilarri et al., 2018).

This freshwater mussel species inhabits slow-flowing waters without tidal influence, constituted by a subtract with fine sediments and gravel, where sedimentation predominates to erosion (Pérez-Quintero, 2006). The species prefers larger rivers but can be found in great lakes and even in secondary rivers with a rapid flow (Araujo et al., 2009). In some Iberian rivers, *P. littoralis* may find refuge from highly modified main channels in secondary channels, river affluents, lakes, and rice ditches (Gómez et al., 2008). This species can be burrowed from lighter to bulky substrate and can live in the deeper central zones of rivers from 6-10m (Haas, 1917; Reis, 2006).

The gametogenesis of *P. littoralis* begins in January with maximum gonadosomatic production in May and the final spawn in June (Şereflişan et al., 2013). In Ebro River, pregnant females were observed in July and August and the juveniles hatched in September, this species can be considered summer breeders (Haas, 1917; Nagel, 1988). With this seasonal proximity between pregnancy and juvenile forms, they probably don’t have synchronized reproduction (Nagel, 2004). *P. littoralis* reaches sexual maturity at 4-5 years and can grow up to 9 cm, with average sizes between 6-8 cm (Haas, 1941; Nagel, 2004).

1.6.3. *Unio delphinus*, Spengler 1793

U. delphinus, has a restricted area in the Atlantic region of the Iberian Peninsula (Lopes-Lima et al., 2020b). The species can be found in the Guadalquivir, Guadiana, Tejo, Douro, and Minho rivers (Lopes-Lima et al., 2020b). These mussels were once considered a subspecies of the congener and more widespread *U. pictorum* but are now recognized as defined species (Araujo et al., 2009). *U. delphinus* is listed as Near-threatened, with populations South of Tejo particularly endangered due to water exploitation, invasive fish species, habitat degradation, and fragmentation, aggregated with the seasonal highly extreme conditions of menacing droughts and dewatering observed in this region (Sousa et al., 2014; Gomes-dos-Santos et al., 2019; Lopes-Lima et al., 2020). These effects, in just 50 years, have led to a decline of 20-30% in the abundance of the species populations (Araujo, 2011). The species have a solid-round, yellow-green oval, or rectangular shell, the anterior part of which is short and curved and the posterior part is elongated (Araujo et al., 2009). The conductivity of water and the pH also influence the shape of the umbo section (Araujo et al., 2009).

U. delphinus is a strictly dioecious species without hermaphroditism and the sex ratio is balanced in this species (Lopes-Lima et al., 2020b). Gametogenesis in females is continuous, year-round with cells at all developing stages, in males, the cycle is continuous and semiannual, but cell development is subject to seasonal variation (Lopes-Lima et al., 2020b). In females, oocyte maturation occurs from January to May and in October, and in males, from March to May and October to November, as spawning progresses (Lopes-Lima et al., 2020b). Eggs are produced from April to July and glochidia from May to August, brooding is short-termed (2-3 weeks) (Lopes-Lima et al., 2020b).

This species is fast-growing and short-lived, related to other unionids, with sexual maturity of 2 years and a lifespan of 11 years (Haag et al., 2011; Lopes-Lima et al., 2020b). *U. delphinus* grows fast up to 2 cm in the first year, mitigating growth in the following ones (Lopes-Lima et al., 2020b). Size varies between lentic and lotic ecosystems, 6-8 cm in lagoons and more than 10 in rivers (Lopes-Lima et al., 2020b). The lifespan of the species is also influenced by the ecosystem: 11 years in large rivers (Douro), 9 years in lagoons, and only 7-8 years in small rivers (Lopes-Lima et al., 2020b). Species have a North-South tendency with populations from higher latitudes having a longer life expectancy and slower growth rate (Lopes-Lima et al., 2020b).

1.6.4. *Unio tumidiformis*, Castro 1885

U. tumidiformis is restricted to 3 locals in the Atlantic rivers of the Southwest of Iberia, Guadiana, Mira and Sado (Reis et al., 2009; Araujo et al., 2009). Previous studies suggested that the species inhabits other rivers, with the Guadalquivir being a possibility, but Tejo and Mondego are implausible (Araujo et al., 2009). *U. tumidiformis* is listed as Vulnerable with scattered and isolated populations, especially in the Guadiana and Mira rivers (Araujo et al., 2009). The species inhabits riverbanks, sandy and silt slopes, with low vegetation in the shade of trees, and may occur in rivers (Araujo et al., 2009). During droughts, which are one of the greatest menaces to the species, *U. tumidiformis* populations can find refugia in river puddles alongside fish and can survive buried in sand or silt (Araujo et al., 2009). They are highly adaptable to changes in flow regimes, but water exploitation, droughts, and the effects of climate change are causing severe population declines (Reis et al., 2016). *U. tumidiformis* has a very large, oval shell that is elongated but not as much as that of *U. delphinus*, with the anterior section being very short and rounded and the posterior enlarged and high (Araujo et al., 2009). The colour of the shell ranges from yellow-green to dark brown with yellow, green, or reddish radial rays, and the umbo section is curved and prominent (Araujo et al., 2009).

Follicular development of the species proceeds from March to June, the development of gametes from July to November, and maturation from December to February (Reis et al., 2016). Glochidia can be founded from March to August (Araujo et al., 2009). *U. tumidiformis* has a very low fecundity compared to other Unionidae with 1500-15000 glochidia produced, with synchronization between its spawn and higher population groups of *Squalius*, its main host (Reis et al., 2016). The metamorphosis from glochidia to juveniles occurs very rapidly in *Squalius* species hosts, from just a few days to two weeks (Reis et al., 2014).

The species reaches sexual maturity at 2 years and its lifespan generally never exceeds 7 years, with populations in the Vascão River having the lowest life expectancy (Reis et al., 2016). The rapid growth and adaptability to different climatic conditions of this species reflect its life-history adaptation to highly degraded environments, where eutrophication stress also promotes rapid growth (Reis et al., 2016).

1.7. Aims of the work

The main goal of this thesis was to evaluate the behavioural responses of four unionid species, *Anodonta anatina, Potomida littoralis, U. delphinus and U. tumidiformis*, considering 2 populations for each species from the North and South of Portugal (*U. tumidiformis* only in the South), to adverse environmental conditions, through:

1) Laboratory experiments carried out in ramps under three dewatering flow rates/treatments: a) slow: 4 cm/day; b) moderate: 8 cm/day, c) fast: 4 cm/h for 10h or ~96 cm/day) and control: no decrease in water level, to:

- Identify differences in horizontal and vertical (burrowing) movements among species and between North and South populations.

- Understand which populations are more prone to become stranded and how the dewatering rate affects this behaviour.

2) Field experiments based on daily and seasonal monitoring of the movement and habitat use of tagged specimens (PIT-Tags and other marks) in rivers of North and South of Portugal, to:

- Evaluate daily displacement among species and between populations from the North and South of Portugal.
- Determine seasonal movements, i.e. longitudinal (upstream vs downstream) and lateral movement (centre vs bank) and microhabitat used by *U. delphinus*.

2. Material and Methods

2.1. Locations (North vs. South of Portugal)

Several watercourses were selected considering the presence of native freshwater mussel populations and the differences in environmental conditions between the North and South regions in Portugal (Figure 9). The selected watercourses were Rabaçal (Figure 10), Tua and Sabor rivers, which are in Northeast Portugal, belonging to the Douro basin, and Guadiana River and their tributaries, Vidigão and Oeiras streams (Figure 11) belonging to the Guadiana basin, located in Southeast Portugal.

Figure 9 Location of sampling sites in Rabaçal, Tua and Sabor rivers (Douro basin) and in Guadiana River and Oeiras and Vidigão streams (Guadiana basin).

Figure 10 Sampling site in Rabaçal River (Douro basin), near Miradeses (Northeastern Portugal).

Figure 11 Sampling site in Oeiras Stream (Guadiana basin), near Mértola (Southeastern Portugal).

2.2. Abiotic characterization

To monitor water quality, several physical and chemical parameters were measured during the experiments. Water temperature was continuously recorded (hourly), with a datalogger. Dissolved oxygen (D.O.) (mg $O_2.L^{-1}$), total dissolved solids (T.D.S.) (mg.L^{-1}), electrical conductivity EC25 (µS.cm^{-1}), and pH were seasonally recorded, using portable probes (Figure 12). All procedures were performed and determined according to the APHA (2005) procedures.

Figure 12 Measuring, in situ, physical and chemical parameters (Rabaçal River, North of Portugal).

2.3. Laboratory experiments

For each species and population (North and South), 24 individuals were collected from the watercourses, through snorkeling or using an Aquascope (Figure 13).

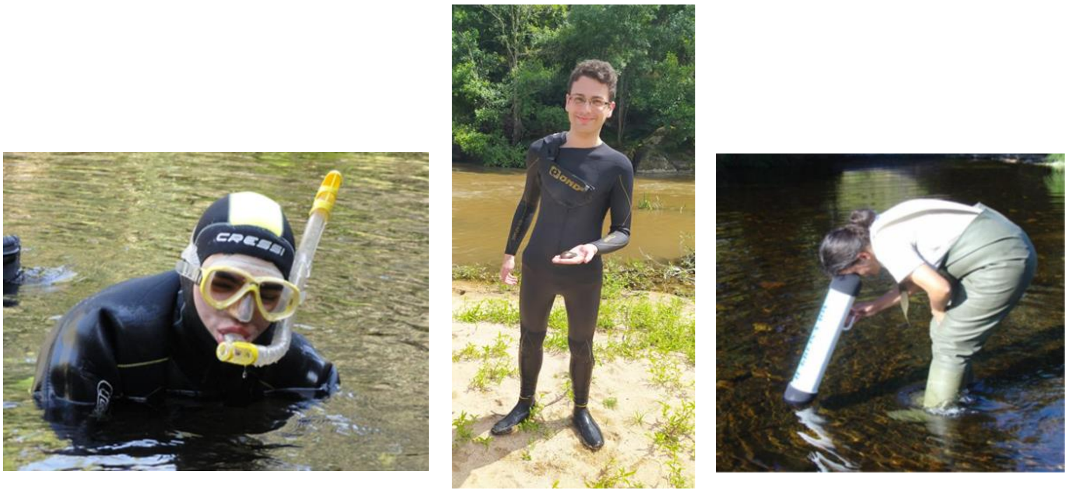


Figure 13 Collecting mussels, through snorkelling (left and centre) and using an Aquascope (right) (Rabaçal River (Douro basin), near Miradeses village, Northeastern Portugal)).

Mussels were kept alive with water from the river and dissolved oxygen supply used, during transportation. At Posto Aquícola de Castrelos (Bragança, NE Portugal) the animals were kept for 8 days for acclimation, in raceway tanks and fed daily with microalgae. The water temperature was maintained at 20ºC. All individuals were measured using a caliper and three dimensions were registered (Table 1).

Table 1 Dimensions (mean ± standard error, cm) per species and populations (North vs. South).

Species	**Population**	**Lenght**	**Height**	**Width**
A. anatina	North	9.53 ± 0.16	5.58 ± 0.09	3.33 ± 0.06
	South	7.04 ± 0.15	4.18 ± 0.08	2.36 ± 0.05
P. littoralis	North	7.78 ± 0.08	4.66 ± 0.05	3.27 ± 0.07
	South	7.58 ± 0.15	5.01 ± 0.10	2.73 ± 0.05
U. delphinus	North	6.42 ± 0.17	3.11 ± 0.08	2.20 ± 0.06
	South	5.88 ± 0.09	3.13 ± 0.05	2.22 ± 0.03
U. tumidiformis	South	6.65 ± 0.07	3.79 ± 0.06	2.72 ± 0.04

The dewatering experiments were performed in the laboratory at Posto Aquícola de Castrelos and intended to mimic dewatering rates that could be experienced by the native freshwater mussel populations, from North and South of Portugal, facing harsh environmental conditions, due to climate changes but also from water abstraction for irrigation and dam operation purposes.

2.3.1. Dewatering experiment

Four 10 m long raceway tanks were used in this experiment, each with three wooden ramps, constructed with a slope (21.8º), covered with a uniform layer of sand, as a natural substrate, with 10 cm in height (Figure 14). The upstream and downstream ends of each tank, at full volume, were 10 and 50 cm, respectively. The experimental and tank designs were similar to the ones used by Galbraith et al. (2015).

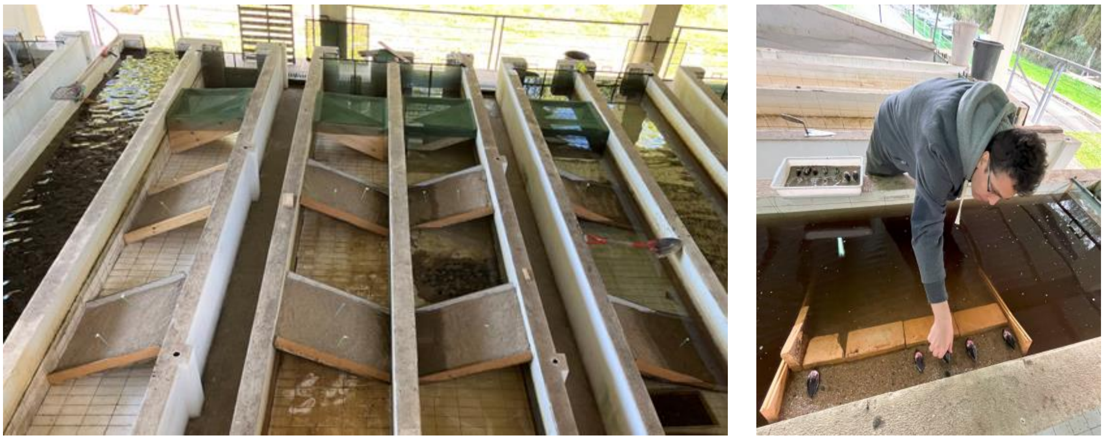


Figure 14 Four tanks, with three ramps, corresponding to control, slow, moderate, and fast dewatering rates.

The trial consisted of three ramps for each of the following treatments: 1) no dewatering (Tank 1), and three dewatering manipulations: 2) slow dewatering tank: 4 cm of water removal/day (Tank 2); 3) moderate dewatering tank: 8 cm of water removal/day (Tank 3); and 4) fast dewatering tank: 4 cm of water removal/hour (Tank 4), over 10 hours (Figure 15).

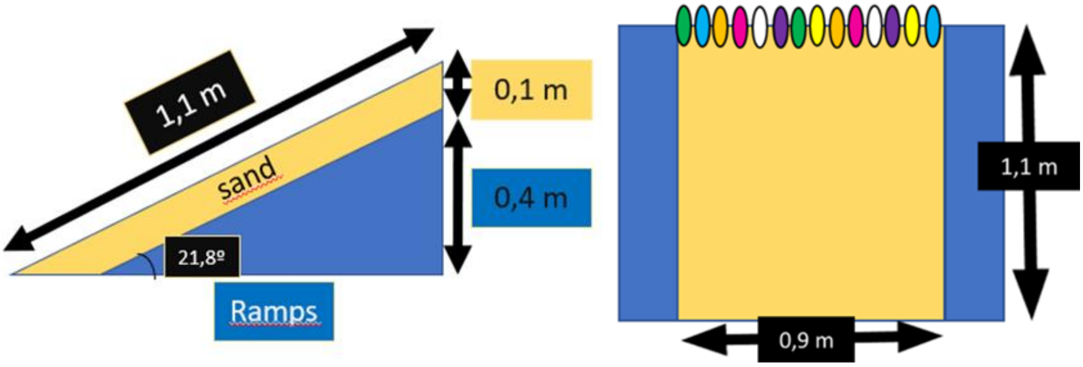


Figure 15 Ramp dimensions and structure (left) and schematic ramp upper view with dimensions and mussel species placement (right).

All treatments of the dewatering trial started at the same time (26th of July 2023). Control and slow treatments were performed for 9 days with 2 observations per day (9 am and 5 pm). The moderate treatment, for 5 days with 2 observations per day (9 am and 5 pm). Finally, the fast treatment consisted of 10 observations from 8:30 am to 5:30 pm (10 hours). The mussels were placed in the ramps 24 h before each treatment for acclimation. On each ramp, 4 mussels from each species (North (N) + South (S)) were placed, except *U. tumidiformis*, only (S) n=2, and a total of 14 individuals per ramp. All mussels were buried equally (30% of shell), in the same position, with the ventral region facing the ramp.

The freshwater mussel behaviour was analysed through the measurement of the following variables:

1. **Horizontal movement -** a metric tape was used to determine the difference between the final and the initial position. Flags were used to mark the position and change with individual movement, following the rails.
2. **Vertical movement –** a metric tape was used to measure the shell exposure and then calculate the percentage of the unburied part of the individual.
3. **Stranded individuals -** defined when the mussel was completely out of the water (emersed) and showed no response for at least 1 hour. If the mussel was stranded with a gaping shell for more than 48 hours, they were presumed dead. To avoid mortality, they were returned to another container with water.

All individuals from each population, for all species, were painted with different nail polish colour marks (Table 2) to promote easier identification during the experiment.

Table 2 Species, total number of individuals (n), river (populations), region North (N) and South (S), and respective colour for both experiments.

Species	n	Population	River	Colour
A. anatina	24	North	Rabaçal	Pink
	24	South	Guadiana	White
P. littoralis	24	North	Rabaçal	Purple
	24	South	Guadiana	Yellow
U. delphinus	24	North	Rabaçal	Green
	24	South	Oeiras	Orange
U. tumidiformis	24	South	Vidigão	Blue

Different coloured flags, corresponding to the colour of the mussel population, were used to track the species' movements (Figure 16).

Figure 16 Painted mussels and coloured flags for tracking the individual movement of mussel populations.

The temperature was held relatively constant (~20 ºC) and continuously recorded (hourly) with a datalogger (Newshift ©). The variables of dissolved oxygen (mg $O_2.L^{-1}$; % of saturation), total dissolved solids (mg.L^{-1}), electrical conductivity EC25 (µS.cm^{-1}), and pH were daily (control, slow and moderate treatments) or hourly (fast treatment) measured, using a portable probe (HACH 40d ©).

2.4. Field experiments

Two field experiments were done: 1) daily movement, monitoring hourly the displacement in one North (Rabaçal River) and one South (Oeiras Stream) river of Portugal of 2 individuals of *U. delphinus, P. littoralis, A. anatina* and *U. tumidiformis* (only in the South), and 2) seasonal movement, monitoring at least one time per annual season, in three northern rivers (Rabaçal, Tua and Sabor), the movement and microhabitat used by tagged *U. delphinus* populations (n=100).

2.4.1. Daily movement

Freshwater mussel species were collected, considering 2 individuals per species, with similar lengths. For easier identification, each animal was marked with colour nail polish in the upper part of the shell. Each species was also marked with a different colour. Moreover, an individual PIT tag (OREGON ©) was glued in the shell of each mussel for monitoring in more deep and turbid zones, using a portable antenna (Figure 17).

Figure 17 Marking mussels with nail polish (left) and PIT tag (right) for individual monitoring.

The experiment was done in July 2022. The movement was observed for 8 hours in the Rabaçal River and 3 hours in the Oeiras Stream with intervals of 20 minutes. In the Rabaçal River, the mussels were also monitored for 4 consecutive days. Mussels were identified and placed 30 cm on the edge of the bank in a sandbar zone and spaced 20 cm each other (Figure 18).

Figure 18 Identification of individuals using a PIT reader (left), mussel's displacement (centre) and mussel's movement (right).

The horizontal distance from their respective initial release point, the direction of the movement toward or away from the water and the behaviour of the mussels were recorded using flags and a handheld PIT-antenna to monitor the movement (Figure 19).

Figure 19 Identification of hourly movements of tagged and visually marked mussels using flags.

2.4.2. Seasonal movement and microhabitat use

The experiment was performed from spring 2021 to autumn 2022. For each of the 3 selected watercourses (Rabaçal, Tua and Sabor rivers), 100 mussels from the species *U. delphinus,* with a size range of 50-72 mm, were collected and measured and individually PIT-tagged (OREGON ©, HDX + PIT tag, dimensions 12.0 mm x 2.12 mm and weight 0.1 g) (Figure 20) using super glue to attach to the valve in a process of two minutes of duration to avoid stress to the animal. Five groups of 20 individuals were immediately positioned near the bank in a sandy substrate, defining a five-meter transect, and each group was placed 1 m far apart. The selected PIT tags have reduced dimensions and weight to create the minimal impact possible on the behaviour of the animals and follow the practical rule for fish that the tracking devices should never surpass 2% of the animal's body weight.

Figure 20 PIT tag model used in the experiment.

The detection device was a Mobile Reader Kit, which is a mobile device constituted of a battery, one portable antenna and one receptor/reader transported in a bag. The basic operation for the acquisition of data starts as the 1st operator moves from downstream to upstream direction, through the riverbed and scans inside a physical square test. Every time the operator moves, the physical square (with weigh fixed in the bottom of the river) synchronically moves, with the antenna only prospecting inside the square. All detected mussels were recorded in 7 seasonal surveys by measuring the distance to three reference points (coordinate system) and the distance to the initial release point (Figure 21).

Figure 21 Mobile Reader kit contents (left) and 1st and 2nd operators monitoring in the river (right).

Every time there is a mussel detection, a sonorous signal is emitted, and the alphanumerical PIT tag code is saved in the memory card inside the receptor/reader in the interior of the bag. Simultaneously, the code is transmitted by Bluetooth from the receptor/reader to a mobile device from the 2nd operator. The 1st operator does the georeferentiation and indicates to the 2nd operator of the complementary information of the place about microhabitat variables (i.e., dominant substrate, flow velocity, depth, and shading in the square device). The receptor/reader of the Mobile Reader Kit can simultaneously make the transmission of the GPS coordinates of each detection. Nevertheless, as the spacing between detections can be very small, the error associated with these GPS detections impossibilities its use.

2.5. Data analysis

2.5.1. Laboratorial experiments

Tanks were used as the experimental units for all statistical analysis (n = 2 control tanks and n = 2 treatment tanks). The dewatering rate manipulation was: 1) slow - 4 cm/day: 10 days of duration; 2) moderate - 8 cm/day: 5 days duration, and 3) fast - 4 cm/hour: 10 hours duration.

Response variables for each tank were obtained considering:

- Horizontal movement: mean total horizontal movement individual^{-1}.d^{-1};
- Vertical movement: mean vertical movement (cm) and burrowing %;
- Stranded Individuals: mean % individuals stranded;

The mussel horizontal movement performance was also analysed considering their locomotive capacities to reach the bottom of the ramp, since most of them showed a particular behaviour of burrowing and/or moving in the deepest zone, from one side to another of the ramp. Very few individuals return to the upper position in the ramp.

Finally, mussels' displacement was considered to evaluate the positioning at the end of each treatment to briefly evaluate if mussels tended to aggregate or disperse through the ramps, between more stable or stressful conditions.

Previously to the statistical treatments, horizontal movement values were square root transformed and vertical movement was normalized with a logit transformation. When response variables met the requirements of normality and homogeneity of variance, an Analysis of Variance (ANOVA) was applied to the data, since is more robust to minor deviations from these assumptions (Zar, 1999). A 2-way ANOVA was used to evaluate the overall effects of populations, dewatering rate, and populations × dewatering rate interaction for the measured variables of movement and vertical movement. When the main effects were significant 1-way ANOVA followed by Tukey's post hoc tests was also used to compare separately differences in movement among treatments for each population and differences among populations at each dewatering rate (including controls).

Stranded behaviour was calculated by the mean % of individuals for each population stranded at the end (last observation) of each treatment.

2.5.2. Field experiments

Non-parametric Mann-Whitney U tests were performed to detect statistical differences in water quality variables between northern and southern watercourses, i.e., Rabaçal River and Oeiras Stream.

The Kruskall-Wallis (KW-H) tests were used as a nonparametric analysis of variance to detect significant differences in daily and seasonal movements, such as for the mean movement per season, and longitudinal and lateral movements among the tagged mussels of the three sampled rivers.

The available and used microhabitat for *U. delphinus* in the 3 sampled rivers were seasonally determined for the following variables: A) dominant substrate composition, classified according to the categories: 1) organic detritus; 2) sand (< 2.0 cm); 3) gravel (2.1-7.5 cm); 4) pebble (7.6-15.0 cm); 5) boulder (> 15.0 cm); 6) bedrock; B) total depth, measured directly with a stick meter, C) water velocity (m.s-1) using a Valeport © flowmeter, and D) shading, by the estimation of cover percentage.

Preference curves for the dominant substrate of microhabitat used by *U. delphinus* in three rivers, during summer and winter seasons were established. The preference was corrected according to habitat availability by calculating for each class of a given variable the use/availability proportion followed by standardization to obtain a range from 0.0 (unsuitable) to 1.0 (optimal). These curves were fitted to the data using polynomial regressions and the best model was selected. All statistical analyses were performed using the STATISTICA 7.0 package (STATSOFT, 2004).

3. Results

3.1. Laboratorial dewatering experiment

During the experiment, the physical and chemical water variables maintained a good quality in all tanks, ranging from 1) temperature between 19.3 and 21.2 ºC; 2) dissolved oxygen levels between 7.0 and 8.5 mgO_2.L (and 80.2 - 94.3 % saturation); 3) electrical conductivity EC25 between 42.1 and 68.2 $\mu S.cm^{-1}$; and 4) pH between 7.1 and 7.3.

3.1.1. Horizontal movement

Horizontal movement distances had great discrepancies, from no movement to a maximum of 213 cm registered, in 10 h, by one *A. anatina* individual (from the South population), in the fast-dewatering treatment. The 2-way ANOVA showed that total mean horizontal movement was significantly affected by both main effects, i.e. mussel populations ($F = 8.76$, $p < 0.001$) and dewatering rates ($F = 8.83$, $p < 0.001$). However, no significant differences were detected in the horizontal movement between mussel populations of the same species (N vs. S). The only exception was identified in the Control treatment, namely for *A. anatina* (N vs. S) where significant differences ($p = 0.027$) were found. When evaluating different species populations, significant differences were detected in the horizontal movements between *P. littoralis* N and *A. anatina* S ($p = 0.0014$), and *P. littoralis* S and *A. anatina* S ($p=0.022$).

For dewatering manipulation, i.e., Slow, Moderate, and Fast treatments, differences between species were not significant ($p > 0.05$). *A. anatina* S had the highest mean horizontal movement per day in Control (Mean: 26.89 cm; SE: ± 4.66 cm) and *U. delphinus* N had the highest mean horizontal movement in Slow (34.64 ± 6.07), Moderate (35.5 ± 10.16) and Fast treatments (149.2 ± 33.65). On the other hand, P. *littoralis* was the species with the lowest horizontal movement in all treatments. *P. littoralis* N had the lowest mean horizontal movement per day in Control (6.03 ± 0.93) and Slow (10.0 ± 2.79), and *P. littoralis* S in Moderate (7.9 ± 5.66) and Fast (46.9 ± 28.75).

The dewatering rate was the most influential variable with Fast treatment registering much higher mean horizontal movements. In ascending order of movement rate, Control, Slow and Moderate treatments, registered slight differences betwixt (Figures 22 and 23). The 1-way ANOVA tests allowed to identify significant differences between species/populations for control ($F=4.88$; $p=0.0029$), slow ($F=4.59$; $p = 0.0040$) and moderate ($F = 3.09$; $p = 0.0249$) treatments, but not for fast treatment ($F = 1.737$; $p = 0.1620$).

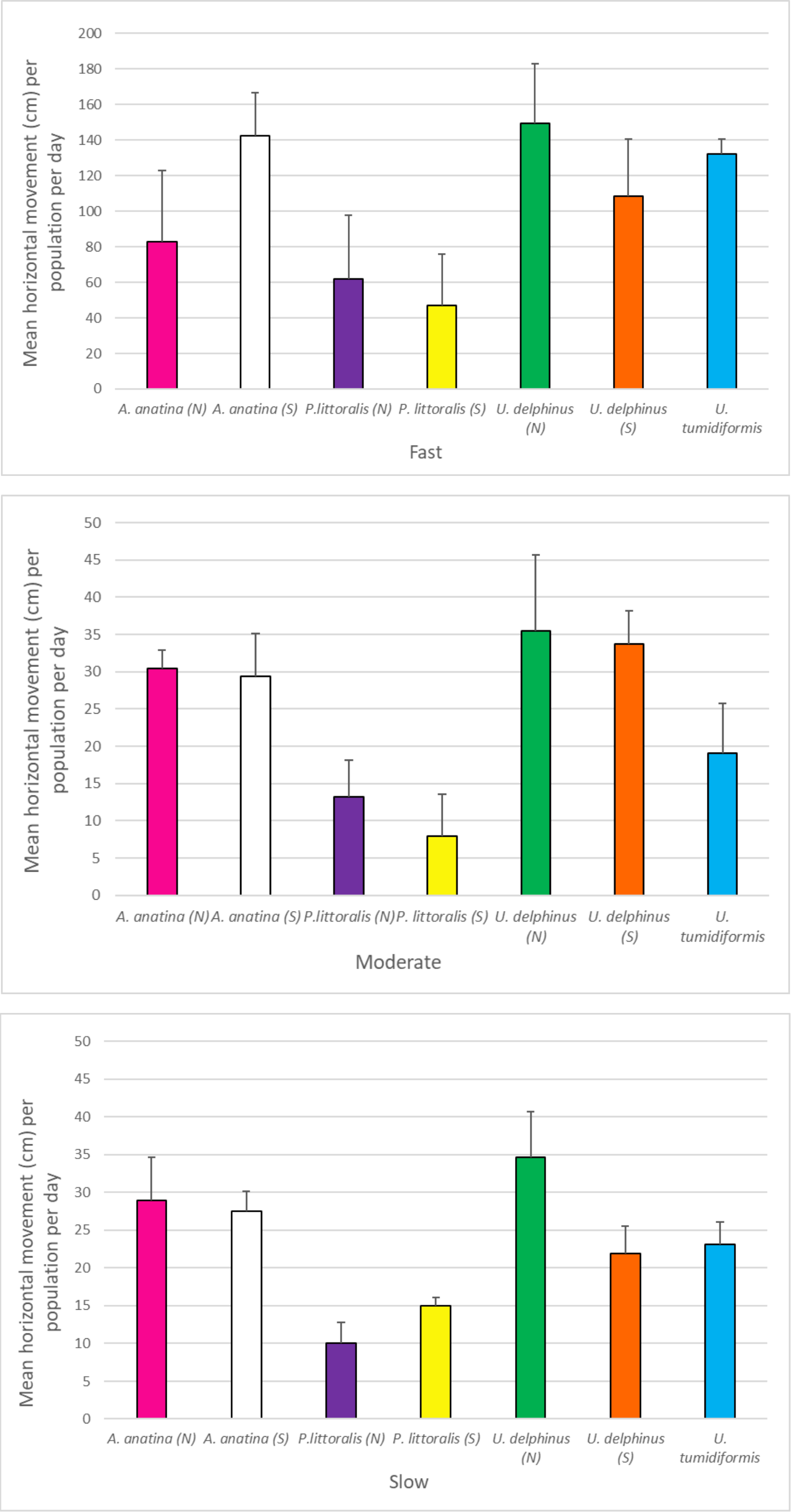


Figure 22 Total horizontal movement (Mean ± SE) per population (n=6 on each treatment) per day in dewatered treatments.

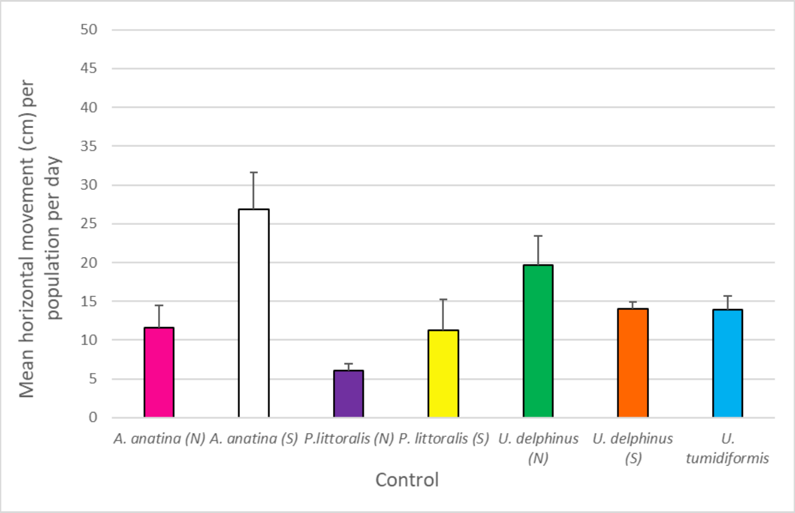


Figure 23 Total horizontal movement (Mean ± SE) per population (n=6) per day in the control treatment.

The mussels' horizontal movement performance was also analysed considering the movement and time needed to reach the bottom of the ramp since most individuals only showed displacement in the bottom position (Table 3).

Considering each treatment and the total locomotive capacities of the populations, in the control ramps, for *A. anatina* S all individuals reach the bottom of the ramp with 3 individuals (50%) on the first day and the remaining 3 on more than one day, being the best performers between all populations in control ramps. Contrarily, for *P. littoralis* N, no individuals reach the bottom of the ramp, being the most sessile populations in the control. In the slow treatment, *A. anatina* S individuals were again the best performers among populations in control ramps with 3 individuals (50%) reaching the bottom of the ramp on the first day and the other 3 in more than one day. On the other hand, *P. littoralis* N and *P. littoralis* S had the subtlest performance with 5 individuals, for each population, (83%) reaching the bottom of the ramp in more than one day and 2 individuals, for each population, (17%) never reaching the bottom of the ramp.

In the moderate treatment, *A. anatina* S individuals continued to be the fastest to reach the bottom of the ramp with 4 individuals (67%) reaching the bottom of the ramp on the first day and 2 individuals on more than one day. Conversely, *P. littoralis* N and *P. littoralis* S had the lowest results with 1 individual, for each population, (17%) reaching the bottom of the ramp and 5 individuals, for each population, never reaching the bottom of the ramp.

In the fast treatment, *A. anatina* S was confirmed as the quickest species with 2 individuals (33%) reaching the bottom of the ramp on the first day, 3 (50%) in more than one day and 1 individual (17%) never reaching the bottom of the ramp. *P. littoralis* S (n=6) had the worst performance with 1 individual (17%) reaching the bottom of the ramp in more than one day and 5 individuals (83%) never reaching the bottom of the ramp.

Table 3 Horizontal movement performance (%) of mussel populations (n=6 on each treatment) for control and dewatering treatments, considering the locomotive capabilities of populations during 3 different periods of the experiment.

Treatment	Period	*Aa*N	*Aa*S	*Pl*N	*Pl*S	*Ud*N	*Ud*S	*Ut*
Control	**≤ 1 day**	**0%**	**50%**	**0%**	**0%**	**33%**	**17%**	**33%**
	> 1 day	**83%**	**50%**	**0%**	**33%**	**33%**	**83%**	**50%**
	never	**17%**	**0%**	**100%**	**67%**	**33%**	**0%**	**17%**
Slow	**≤ 1 day**	**17%**	**50%**	**0%**	**0%**	**17%**	**50%**	**33%**
	> 1 day	**83%**	**50%**	**83%**	**83%**	**83%**	**33%**	**67%**
	never	**0%**	**0%**	**17%**	**17%**	**0%**	**17%**	**0%**
Moderate	**≤ 1 day**	**17%**	**67%**	**0%**	**0%**	**17%**	**33%**	**33%**
	> 1 day	**83%**	**33%**	**17%**	**17%**	**66%**	**67%**	**50%**
	never	**0%**	**0%**	**83%**	**83%**	**17%**	**0%**	**17%**
Fast	**≤ 3 hours**	**0%**	**33%**	**0%**	**0%**	**17%**	**17%**	**0%**
	> 3 hours	**67%**	**50%**	**50%**	**17%**	**66%**	**50%**	**83%**
	never	**33%**	**17%**	**50%**	**83%**	**17%**	**33%**	**17%**

Symbols: *Aa*N - *Anodonta anatina* North; *Aa*S - *Anodonta anatina* South; *Pl*N – *Potomida littoralis* North; *Pl*S – *Potomida littoralis* South; *Ud*N – *Unio delphinus* North; *Ud*S – *Unio delphinus* South; *Ut* – *Unio tumidiformis*

There was a tendency for the generality of the individuals to be found aggregated at the end of each experiment.

In control and slow there was a general tendency for the species to aggregate through the centre of the ramp, with a slightly different, but similar tendency for *U. tumidiformis* to aggregate in the upper centre of the ramp. In moderate and fast treatment more diversity of displacement for all species was found with individuals found in the centre, upper centre and near origin (upper position of the ramp).

3.1.2. Vertical movement (burrowing)

Vertical burrowing ranged between 0 and 100%. The 2-way ANOVA showed that vertical movement was, similarly to the horizontal movement, significantly affected by mussel populations ($F = 9.40$, $p < 0.001$) and dewatering rates ($F = 3.67$, $p = 0.03$). Mean burrowing rates were higher in populations from the South for all treatments. *U. delphinus* S was the population with the highest and most uniform mean burrowing with a maximum of 78.22%; SE: ± 9.64 in control tanks. The lowest mean burrowing rate was from *A. anatina* N with 9.83% ± 4,74 in the fast treatment (Figure 24).

The 1-way ANOVA tests conducted for the burrowing variable and each dewatering treatment showed significant differences between populations in slow (F=8.18; p < 0.001), moderate (F=3.25; p=0.02) and fast treatment (F=4.25; p=0.006).

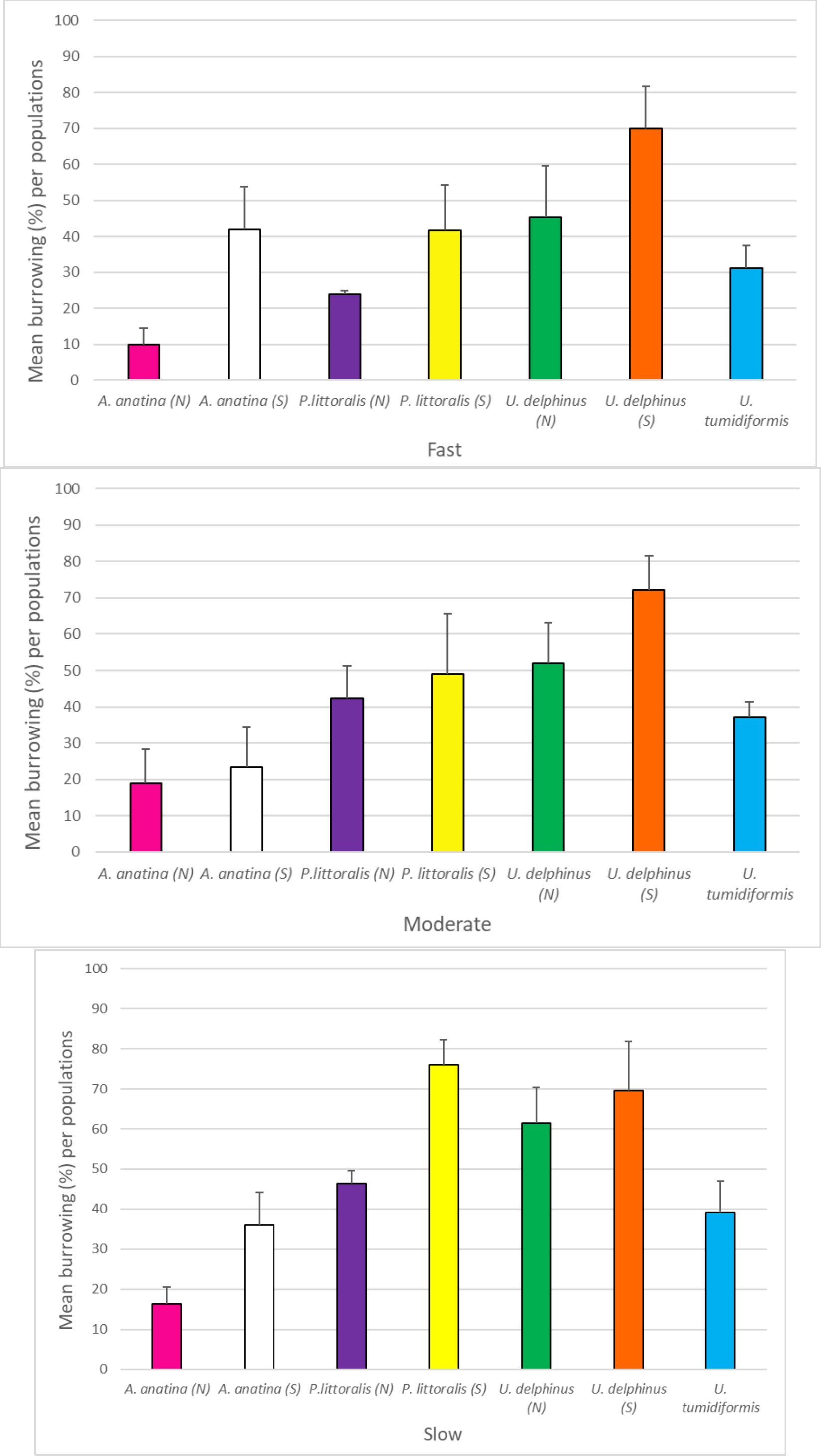


Figure 24 Total vertical movement (Mean ± SE) per population (n=6 on each treatment) in dewatered treatments.

However, for the control treatment (Figure 25), no differences among populations were found (F = 2.53; p = 0.053). Mean burrowing did not differ significantly between correlated populations with *Aa*S-*Aa*N (p = 0.55), *Pl*S-*Pl*N (p=0.18) and *Ud*S-*Ud*N (p = 0.64), except *Ut*-*Ud*S (p = 0.023). Evaluating different species populations, significant differences were detected between *P. littoralis* S and *A. anatina* N (p < 0.001), *P. littoralis* S and *A. anatina* S (p = 0.049), *U. delphinus* N and *A. anatina* N (p < 0.001), *U. delphinus* S and *A. anatina* S (p < 0.001), *U. delphinus* S and *P. littoralis* N (p = 0.003), *U. tumidiformis* and *A. anatina* N (p = 0.046).

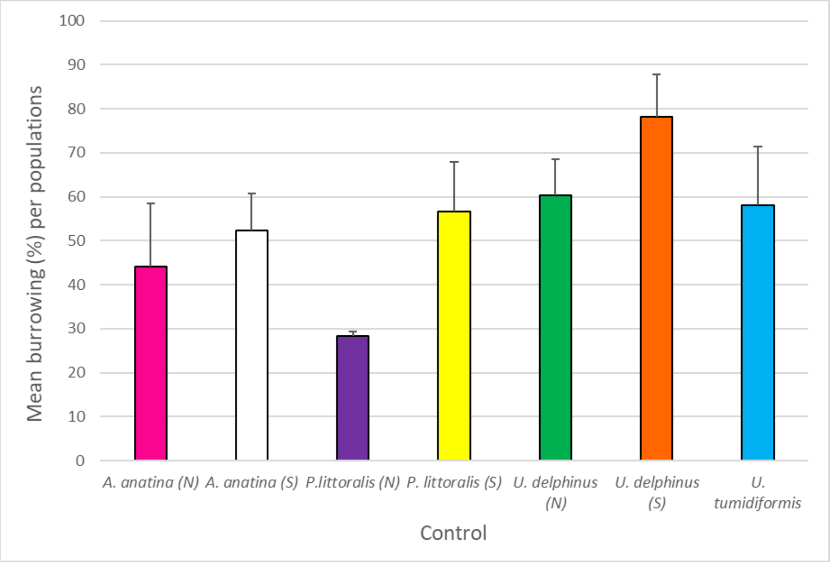


Figure 25 Total vertical movement (Mean ± SE) per population (n=6) in the control treatment.

Finally, notably the most significant value was between *U. delphinus* S and *A. anatina* N (p < 0.001). Comparing treatments, mean burrowing differed significantly in Moderate-Control (p = 0.025), but not significantly in Slow-Control (p = 0.54) and in Moderate-Slow (p = 0.24).

3.1.3. Stranded behaviour

Stranded behaviour was considered as the condition of each individual at the final of the experiment for all treatments. Following the already described parameters, if the mussel had no movement and is completely out of the water (emersed) for at least one hour, it will be considered stranded.

No individuals became stranded in the control, whereby only slow, moderate and fast treatments were considered in the statistical tests.

In fast dewatering tanks, for *A. anatina* N 33% of the individuals became stranded and in *A. anatina* S no individuals became stranded. For *P. littoralis* N 50% of the individuals became stranded and for *P. littoralis* S 67% of the individuals became stranded. For *U. delphinus* N 17% of the individuals became stranded and in *U. delphinus* S 33% of the individuals became stranded. Finally, *U. tumidiformis* had 17% of the individuals stranded (Figure 26).

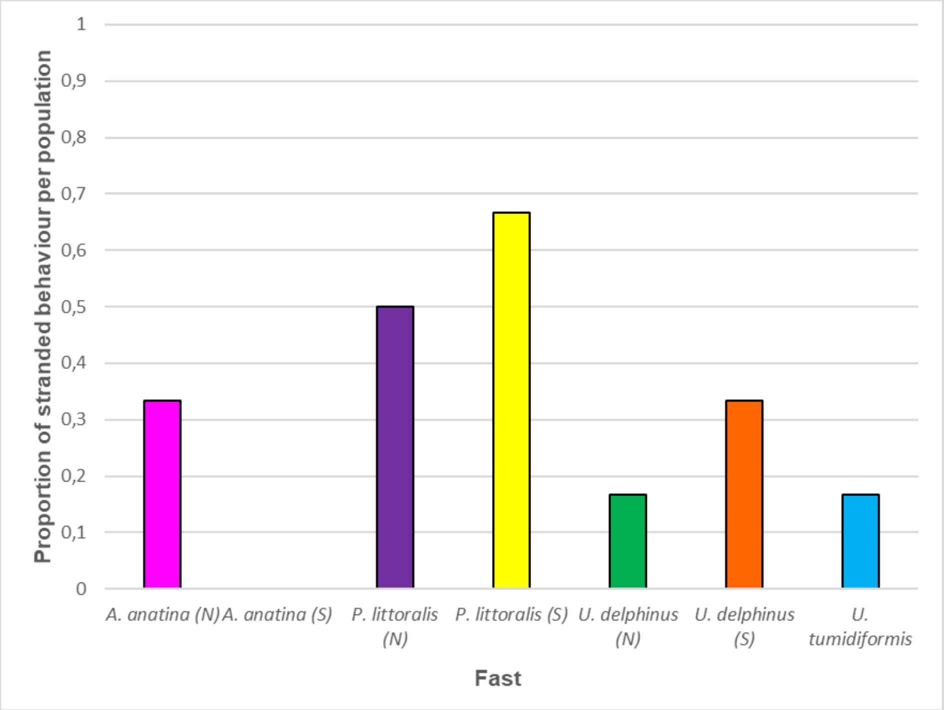


Figure 26 Proportion of stranded behaviour per population (n=6) at the end of fast treatment.

In moderate dewatering tanks, for *A. anatina* N 33% of the individuals became stranded and in *A. anatina* S 17% of the individuals became stranded. For *P. littoralis* N 50% of the individuals became stranded and in *P. littoralis* S 83% of the individuals became stranded. For *U. delphinus* N 0% of the individuals became stranded and in *U. delphinus* S 50% of the individuals became stranded. Finally, *U. tumidiformis* had 50% of the individuals stranded (Figure 27).

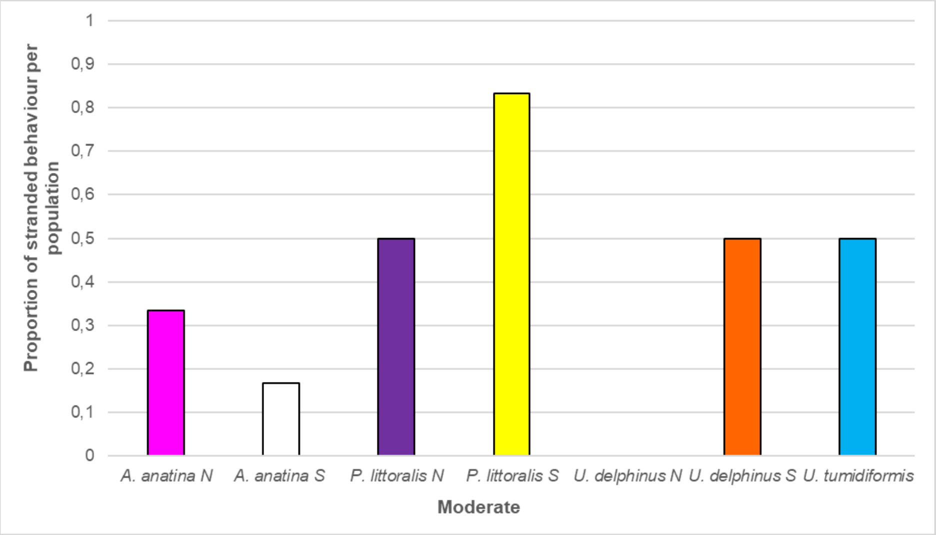


Figure 27 Proportion of stranded behaviour per population (n=6) at the end of moderate treatment.

In slow dewatering tanks, for *A. anatina* N 67% of the individuals became stranded and in *A. anatina* S 67% of the individuals became stranded. For *P. littoralis* N 67% of the individuals became stranded and in *P. littoralis* S 33% of the individuals became stranded. For *U. delphinus* N 50% of the individuals became stranded and in *U. delphinus* S 67% of the individuals became stranded. Finally, *U. tumidiformis* had 67% of the individuals stranded (Figure 28).

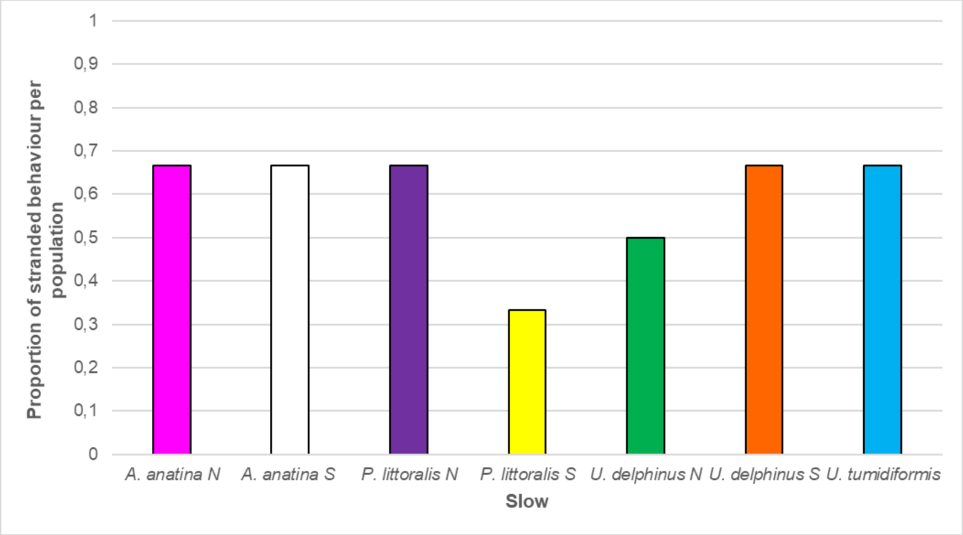


Figure 28 Proportion of stranded behaviour per population (n=6) at the end of slow treatment.

In control tanks, no mussels had become stranded.

3.2. Field experiments

3.2.1. Abiotic characterization

The variation of water temperature for different observation periods in two watercourses located in the North, i.e., Rabaçal River and South, i.e., Oeiras Stream can be visualized in Table 4.

Table 4 Variation of water temperature TºC (mean ± standard error) for different observation periods (maximum and minimum, mean and mean of maximum during August).

Location (River/Stream)	Observation period	Water Temperature ºC			
		Max. T ºC	Min. T ºC	Mean T ºC	Mean max. TºC (August)
Rabaçal	22/04/2022_18/05/2023	34.61	6.07	16.78 ± 0.07	32.3 ± 0.32
Oeiras	29/05/2021_05/05/2022	38.07	0	18.44 ± 0.08	30.8 ± 0.53
Rabaçal	29/05_16/09/2022	34.61	18.11	25.25 ± 0.07	32.3 ± 0.32
Oeiras	29/05_16/09/2021	38.07	14.08	25.01 ± 0.08	30.8 ± 0.53

Differences in the water temperature patterns between northern and southern rivers can be visualized in Figures 29 and 30. Water temperature, continuously recorded (hourly), ranged between 6.1ºC to 35 ºC for the Rabaçal River, and from 0ºC to 38 ºC for the Oeiras Stream.

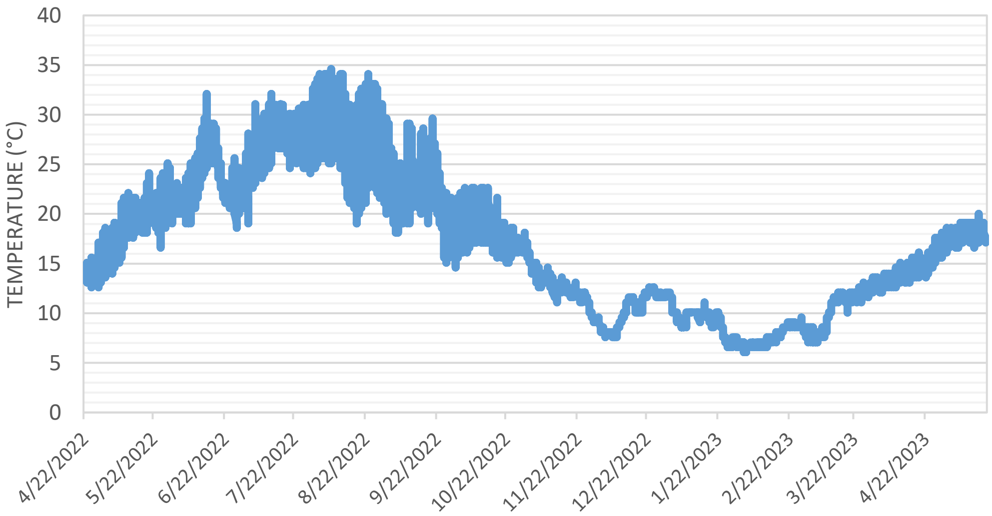


Figure 29 Water temperature in Rabaçal River (Northeastern Portugal) from April to May 2023.

In the Rabaçal River, between the 29th of July and the 12th of August, the daily highest temperatures were over 32 ºC, and on the 12th of June a singular peak of 32 ºC was registered.

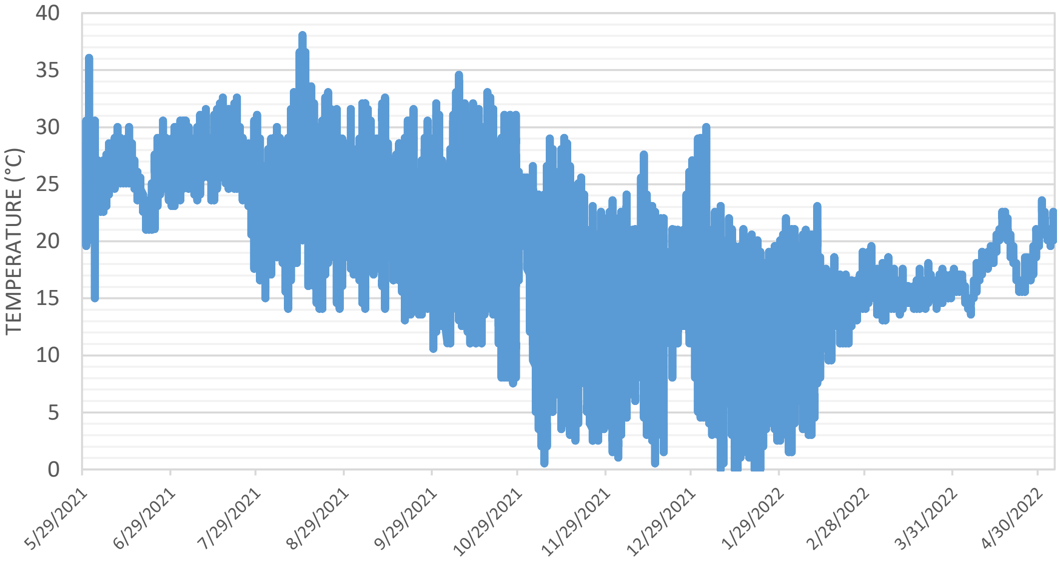


Figure 30 Water temperature in Oeiras Stream (Southeastern Portugal) from May 2021 to April 2022.

In the Rabaçal River, the highest temperature of almost 35ºC was registered on the 7th of August 2022 (Figure 31).

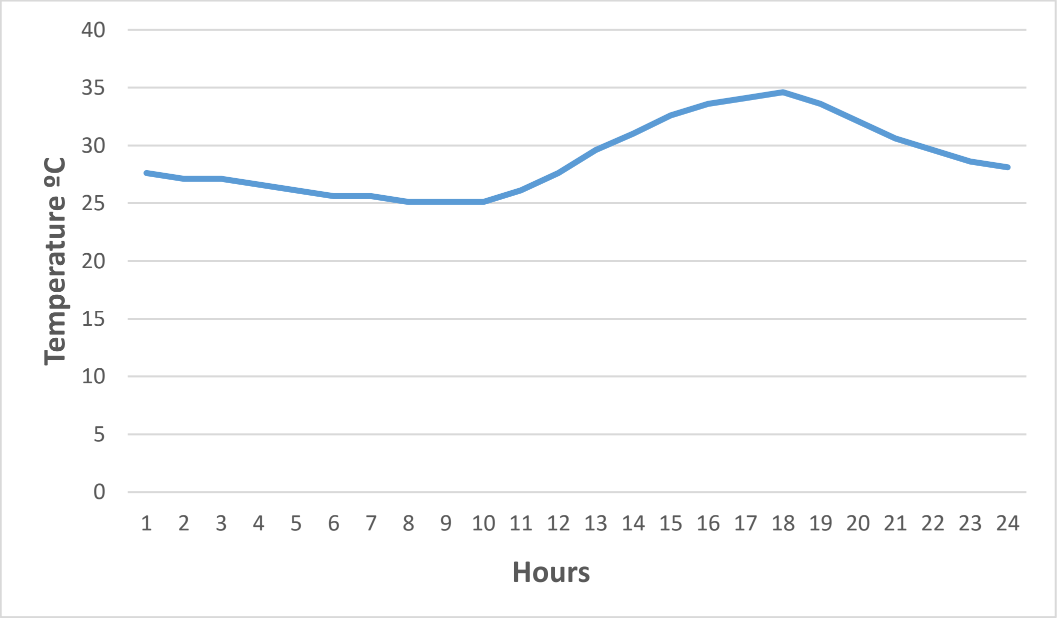


Figure 31 Water temperature peak in Rabaçal River (Northeastern Portugal) on the 7th of August 2022.

In the Oeiras Stream, between the 11th and 18th of August, and the days 22nd and 23rd, the maximum water temperature was over 32 ºC, with a peak of 38 ºC on the 13th, 14th (Figure 32), and 15th of August.

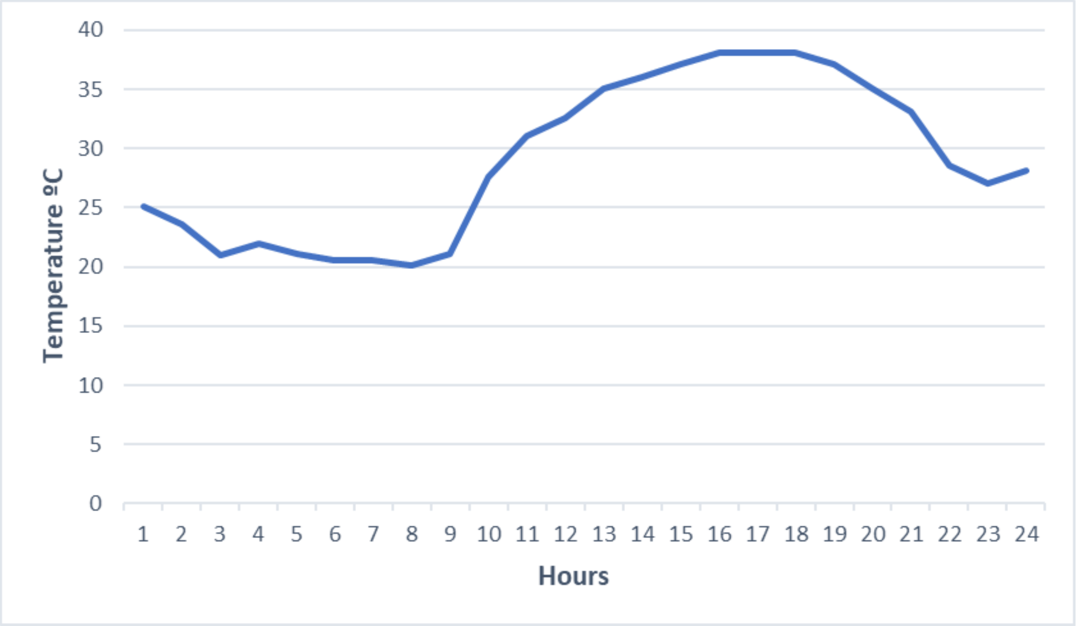


Figure 32 Water temperature peak in Oeiras Stream (Southeastern Portugal) on 14th August 2021.

The results of other abiotic parameters, such as dissolved oxygen (DO), total dissolved solids (TDS), Electrical Conductivity (EC25), and pH are presented the Tables 5 and 6. Significant differences ($p < 0.05$, Mann-Whitney U Test) were found for water quality variables between northern and southern watercourses, namely for the electrical conductivity EC25 ($\mu S.cm^{-1}$), pH, TDS ($mg. L^{-1}$) and dissolved oxygen ($mg\ O_2\ L^{-1}$).

Table 5 Abiotic parameters (air temperature, Conductivity, pH, TDS, and O_2) for Rabaçal River, near Miradeses (year 2022).

Date	Hour	Air Temp. (ºC)	Cond. EC25 ($\mu S.cm^{-1}$)	pH	TDS ($mg. L^{-1}$)	O_2 ($mg\ O_2. L^{-1}$)	O_2 (% sat.)
26/07	**10:20**	**20.9**	**78.8**	**6.75**	**45.9**	**6.10**	**71.9**
	16:44	**28.0**	**83.5**	**6.77**	**42.2**	**6.69**	**85.1**
27/07	**09:10**	**19.0**	**77.3**	**6.73**	**42.2**	**5.79**	**65.7**
	15:26	**23.3**	**84.3**	**6.74**	**42.1**	**7.68**	**96.8**
28/07	**09:19**	**19.4**	**78.5**	**6.69**	**42.4**	**5.67**	**65.5**
	14:55	**24.0**	**83.0**	**6.79**	**43.1**	**7.87**	**95.2**

Table 6 Abiotic parameters (air temperature, Conductivity, pH, TDS, and O_2) for Oeiras Stream and Vascão River (Guadiana) (year 2022).

Date	**Hour**	**River**	**Location**	**Air T. (ºC)**	**Cond. EC25 (µS.cm^{-1})**	**pH**	**TDS (mg.L^{-1})**	**O_2 (mg/l^{-1})**	**O_2 (% sat.)**
26/06	**14:15**	**Oeiras**	**Somincor**	**24.0**	**551.0**	**7.79**	**268.0**	**8.40**	**98.7**
	18:30		**Morena**	**24.5**	**1188.0**	**7.76**	**581.0**	**8.78**	**106.5**
27/06	**11:00**	**Vascão**	**Pêgo do Quim**	**20.0**	**294.0**	**7.70**	**581.0**	**8.70**	**101.4**
	15:00		**Corvos**	**21.0**	**333.0**	**7.71**	**581.0**	**8.96**	**107.1**
	16:00		**Vidigão**	**25.2**	**549.0**	**7.75**	**262.0**	**9.21**	**113.5**
28/06	**15:20**	**Oeiras**	**Morena**	**29.5**	**1280.0**	**7.77**	**585.0**	**8.01**	**103.9**

Some environmental conditions between North and South regions, can be observed in Table 7, based on the typology defined for rivers of Portugal (INAG, 2008).

Table 7 Descriptive statistics (mean ± standard deviation) of environmental variables for the typology of sampled watercourses (adapted from Water Framework Directive, INAG 2008)

Watercourses	Sabor River	Rabaçal and Tua rivers	Oeiras Stream
Variable	N1 > 100 Km^2	N2	S1 > 100 Km^2
Mean Annual Temperature (ºC)	12.6 ± 1.2	13.1 ± 1.0	15.8 ± 0.9
Mean Annual Precipitation (mm)	1196.4 ± 347.3	597.7 ± 81.1	587.1 ± 83.9
Altitude (m)	274.1 ± 204.6	299.8 ± 141.4	136.9 ± 67.6
Drainage Area Dimension (Km^2)	548.6 ± 657.0	960.5 ± 1115.4	438.5 ± 579.2
Thermal air temperature (ºC)	10.2 ± 1.2	11.6 ± 0.6	11.4 ± 1.2
Flow regime: Min-Max (mm)	100 - 2200	25 - 400	25 - 400

Symbology: N1 > 100 Km^2 – northern rivers of Median-Great Dimension; N2 – Alto Douro rivers of Median-Great Dimension; S1 > 100 Km^2 - southern rivers of Median-Great Dimension.

3.2.2. Daily movement

The daily monitoring surveys allowed to verify significant different movement patterns (K-W H = 26.32; $p < 0.05$) among the tagged mussel species. In fact, for the Rabaçal River, *Unio delphinus* showed a higher movement (i.e., displacement of 470 and 789 cm, during the observation period) from the bank to deeper zones, towards the centre of the channel, comparatively with the other mussel species, i.e., *A. anatina* (93 and 334 cm) and *P. littoralis* (0 and 73 cm). Moreover, the displacement of mussels was more effective during the first 3 hours, changing their behaviour after this period, stabilizing and most of them burrowing in the sandy substrate (Figure 33).

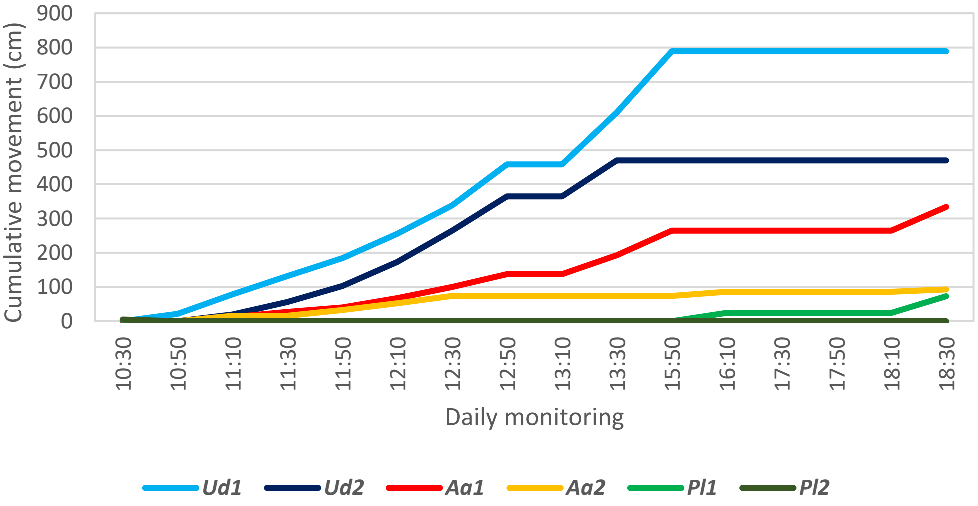


Figure 33 Total (cumulative) movement (cm) of 2 individuals per each of the 3 native mussel species, i.e., *U. delphinus* (*Ud*), *A. anatina* (*Aa*) and *P. littoralis* (*Pl*), in the Rabaçal River, during a daily monitoring period (25th July 2022).

After the initial displacement of mussels, the movement rate decreased substantially, and most individuals maintained the position in the 3 following days (Figure 34).

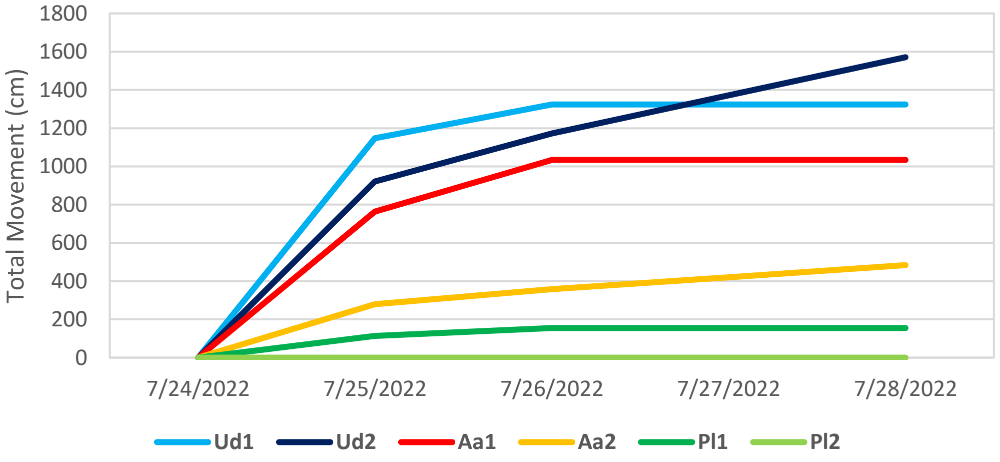


Figure 34 Total (cumulative) movement (cm) of 2 individuals per each of the 3 native mussel species, i.e., *U. delphinus* (*Ud*), *A. anatina* (*Aa*) and *P. littoralis* (*Pl*), in the Rabaçal River, during a 4-day monitoring period (July 2022).

The movement of mussels in the Oeiras Stream followed a similar pattern, and significant differences were found (K-W H = 7.184); $p < 0.05$) with *U. delphinus*, displaying higher movements, when compared with the other species (Figure 35).

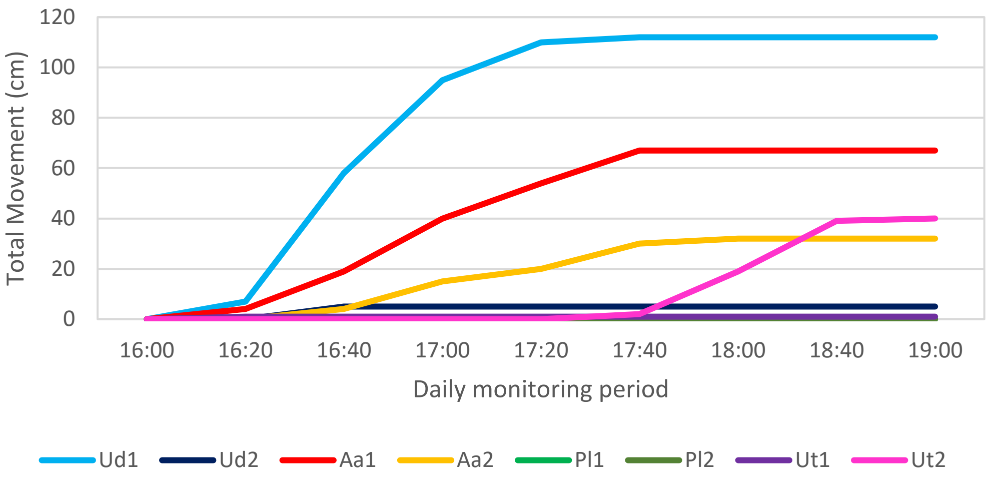


Figure 35 Total movement (cm) of 2 individuals per each of the 4 native mussel species, i.e., *U. delphinus* (*Ud*), *A. anatina* (*Aa*), *P. littoralis* (*Pl*), and *U. tumidiformis* (*Ut*) in the Oeiras Stream, during a daily monitoring period (June 2022).

3.2.3. Seasonal movement and microhabitat use

Significant differences (KW-H = 7.833; $p < 0.05$) were found for the mean movement per season among the three *U. delphinus* populations, based on the detected tagged mussels (Figure 36).

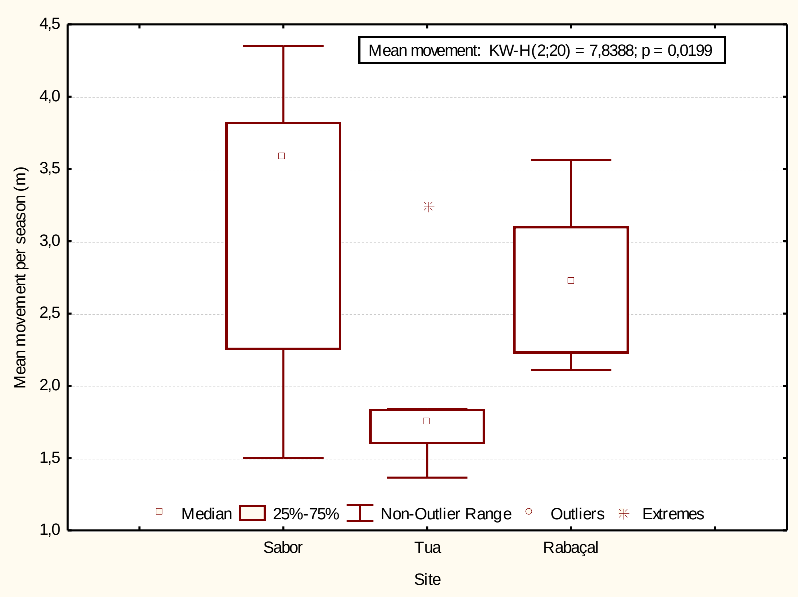


Figure 36 Boxplots showing the seasonal movement for *Unio delphinus* (n=100 on each river) in the Sabor, Tua, and Rabaçal rivers between the years 2021 and 2022. Boxplots show median values (central line), the range from the 25th to 75th percentile (box) and the largest and lowest value within 1.5 times interquartile range below and above the 25th and 75th percentile (whiskers) and dots represent extreme values.

A detailed analysis of the Sabor mussel populations shows a higher movement rate (Mean = 4.35 m ± Standard deviation = 2.28) in opposition to the lower movement rate (1.37 m ± 0.68) observed in the Tua River (Figures 36 and 37).

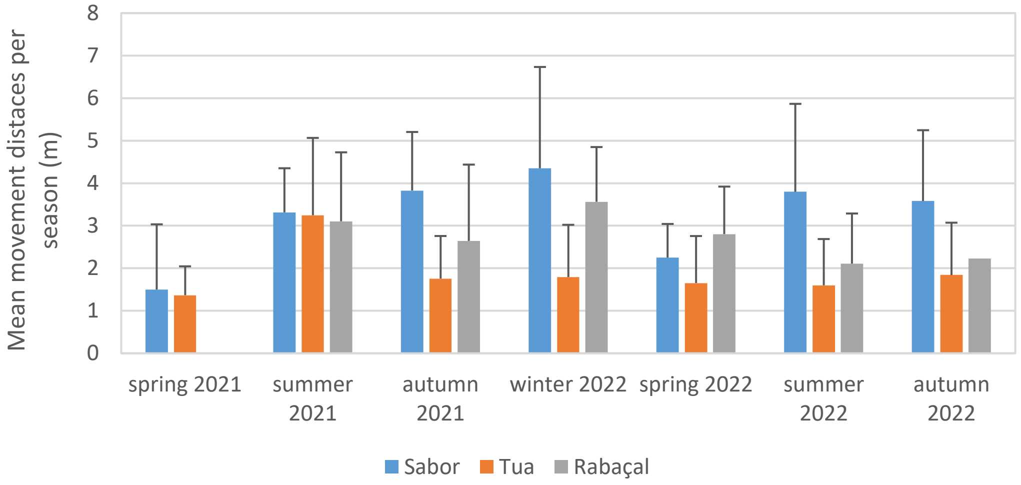


Figure 37 Movement distances per season (mean ± SD) for *Unio delphinus* (n=100 on each river) and the three sampled zones in the Sabor, Tua, and Rabaçal rivers, between the years 2021 and 2022.

There was a superior displacement of the mean movement distance per season of the mussels in the Sabor River compared to the Tua and Rabaçal rivers, probably related to the river typology and associated environmental conditions, such as the different flow regime, i.e. N1 > 100 km^2 - *versus* N2 typology (Figure 38).

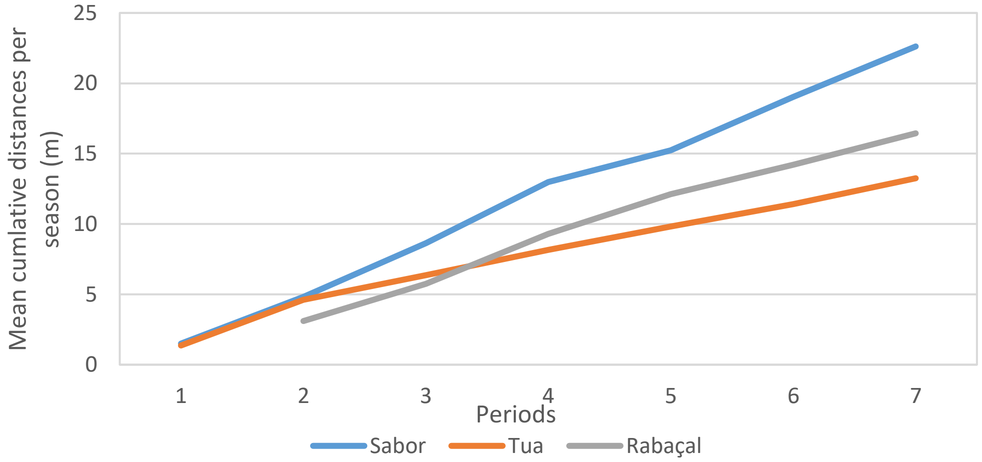


Figure 38 Mean cumulative movement distances per season for *Unio delphinus* (n=100 on each river) and the three sampled zones in the Sabor, Tua, and Rabaçal rivers, between the years 2021 and 2022.

Other analyses allowed to clarify the longitudinal (upstream vs. downstream) (Figures 39 and 40) and lateral (centre vs. bank) (Figures 41 and 42) movements of each mussel population in the three experimental zones of Sabor, Tua, and Rabaçal rivers.

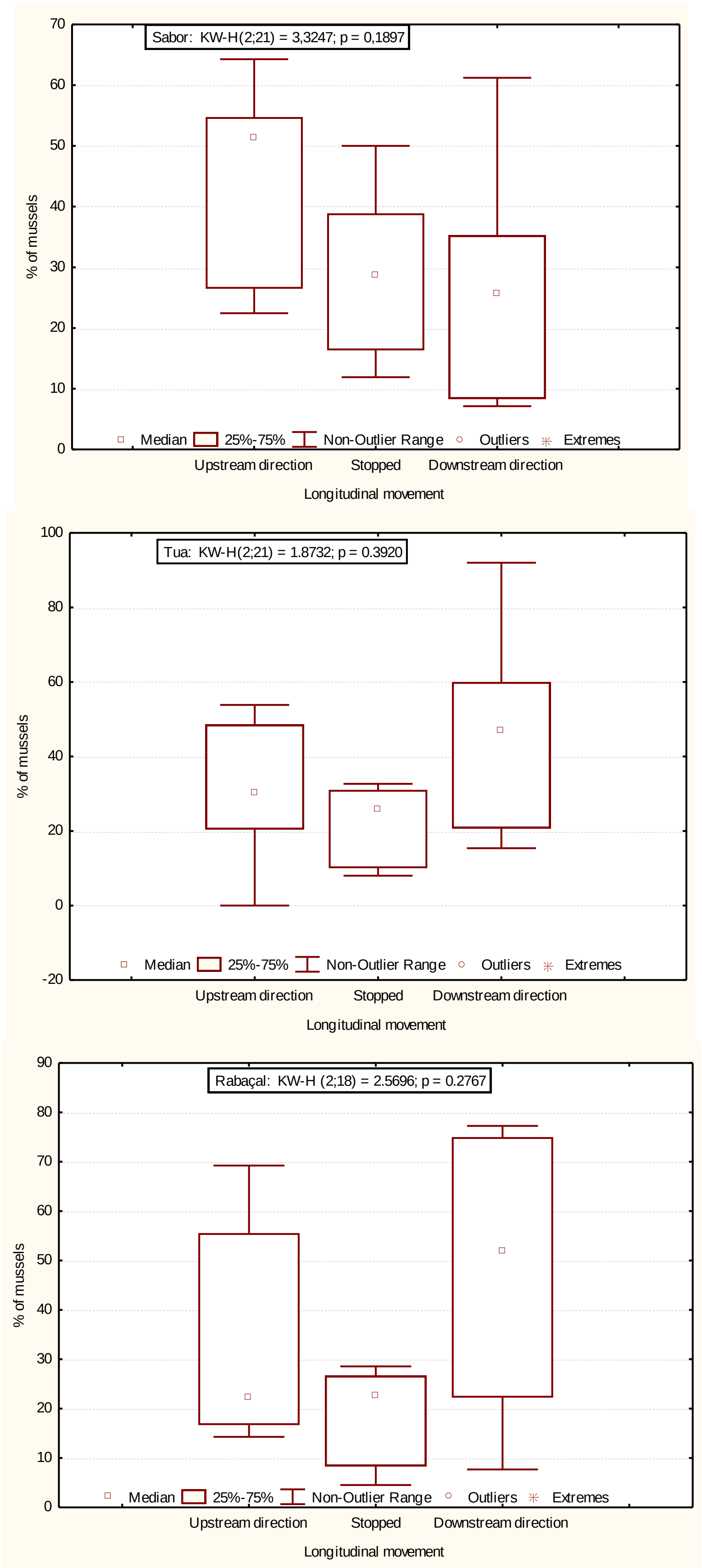


Figure 39 Boxplots showing the longitudinal movement for *Unio delphinus* (n=100 on each river) in the Sabor, Tua, and Rabaçal rivers between the years 2021 and 2022. Boxplots show median values (central line), the range from the 25th to 75th percentile (box) and the largest and lowest value within 1.5 times interquartile range below and above the 25th and 75th percentile (whiskers) and dots represent extreme values.

No significant differences were found (KW-H tests; $p > 0.05$) for longitudinal movements. However, the main displacement in Rabaçal and Tua rivers was in the downstream direction, while for Sabor River was in the upstream direction. The seasonal pattern can be observed in Figure 40.

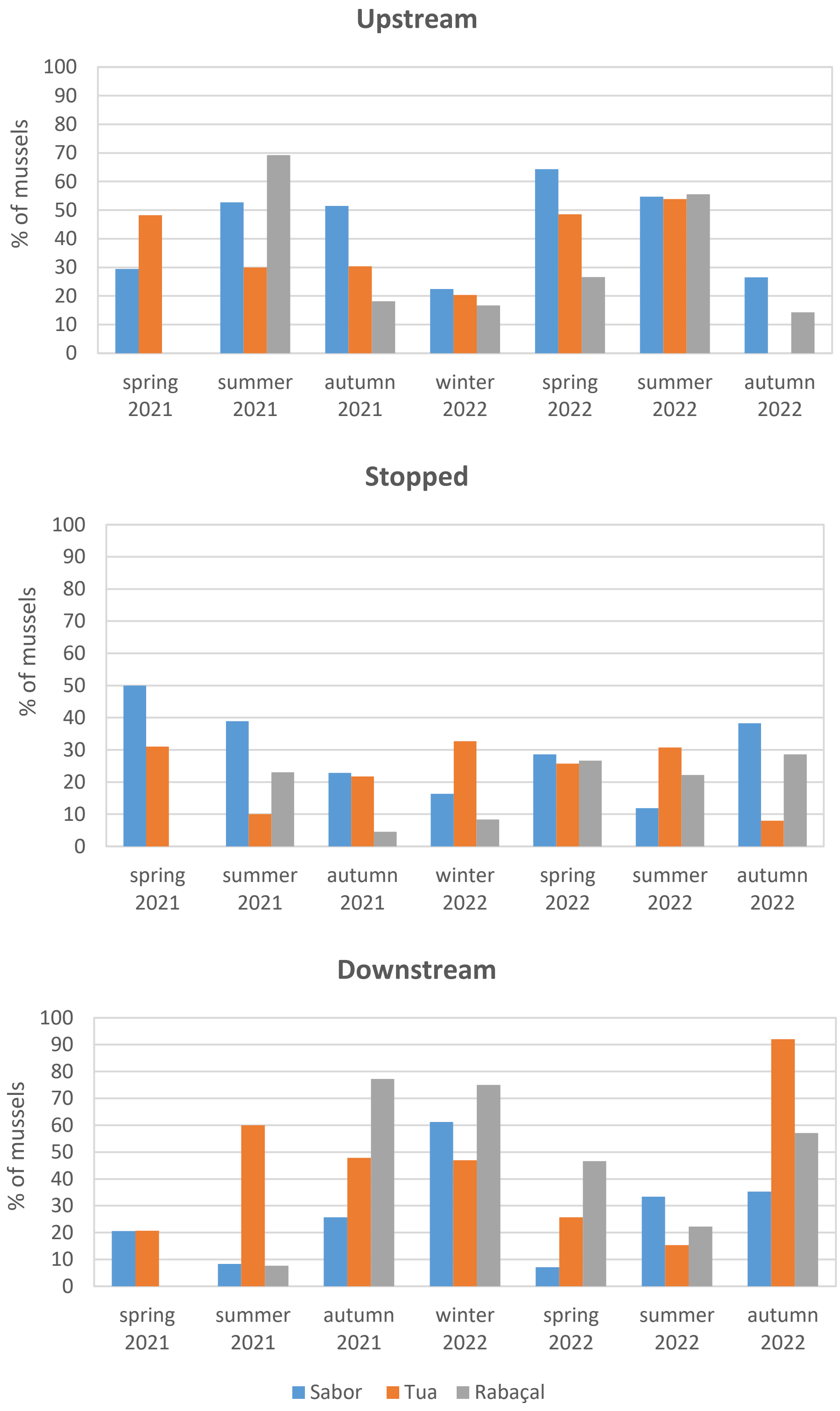


Figure 40 Longitudinal movement per season for *Unio delphinus* (n=100 on each river) in the three sampled zones in the Sabor, Tua, and Rabaçal rivers, between the years 2021 and 2022.

The analysis of the lateral movements revealed significant differences but only for the Sabor River (KW-H = 6.48; $p < 0.05$). For Sabor and Tua rivers the main displacement was in the direction of the centre of the channel, while for the Rabaçal River was for the bank (Figure 41).

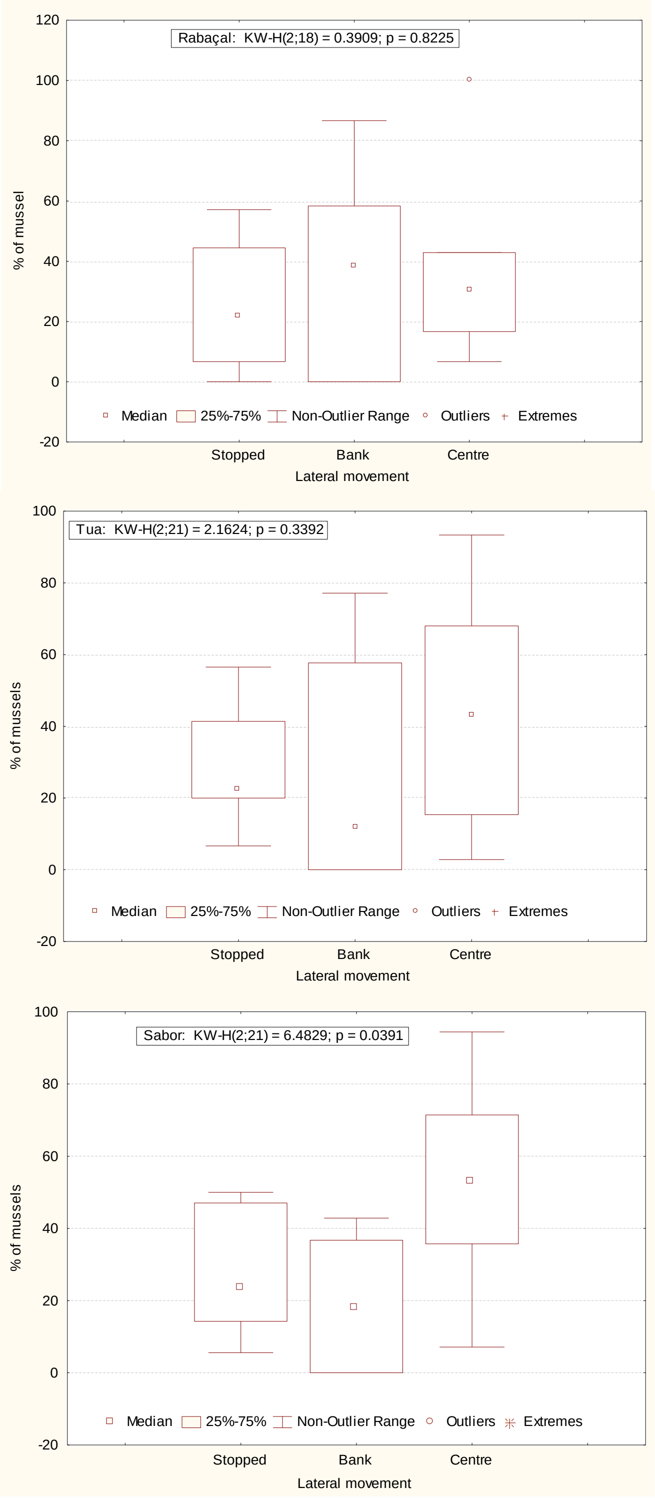


Figure 41 Boxplots showing the lateral movement for *Unio delphinus* (n=100 on each river) in the Rabaçal, Tua, and Sabor rivers between the years 2021 and 2022. Boxplots show median values (central line), the range from the 25th to 75th percentile (box) and the largest and lowest value within 1.5 times interquartile range below and above the 25th and 75th percentile (whiskers) and dots represent extreme values.

The detailed analysis per season showed a more pronounced movement in direction to the bank precisely during spring and winter seasons, probably due to the higher flow and adverse conditions far away from the banks of the river (Figure 42).

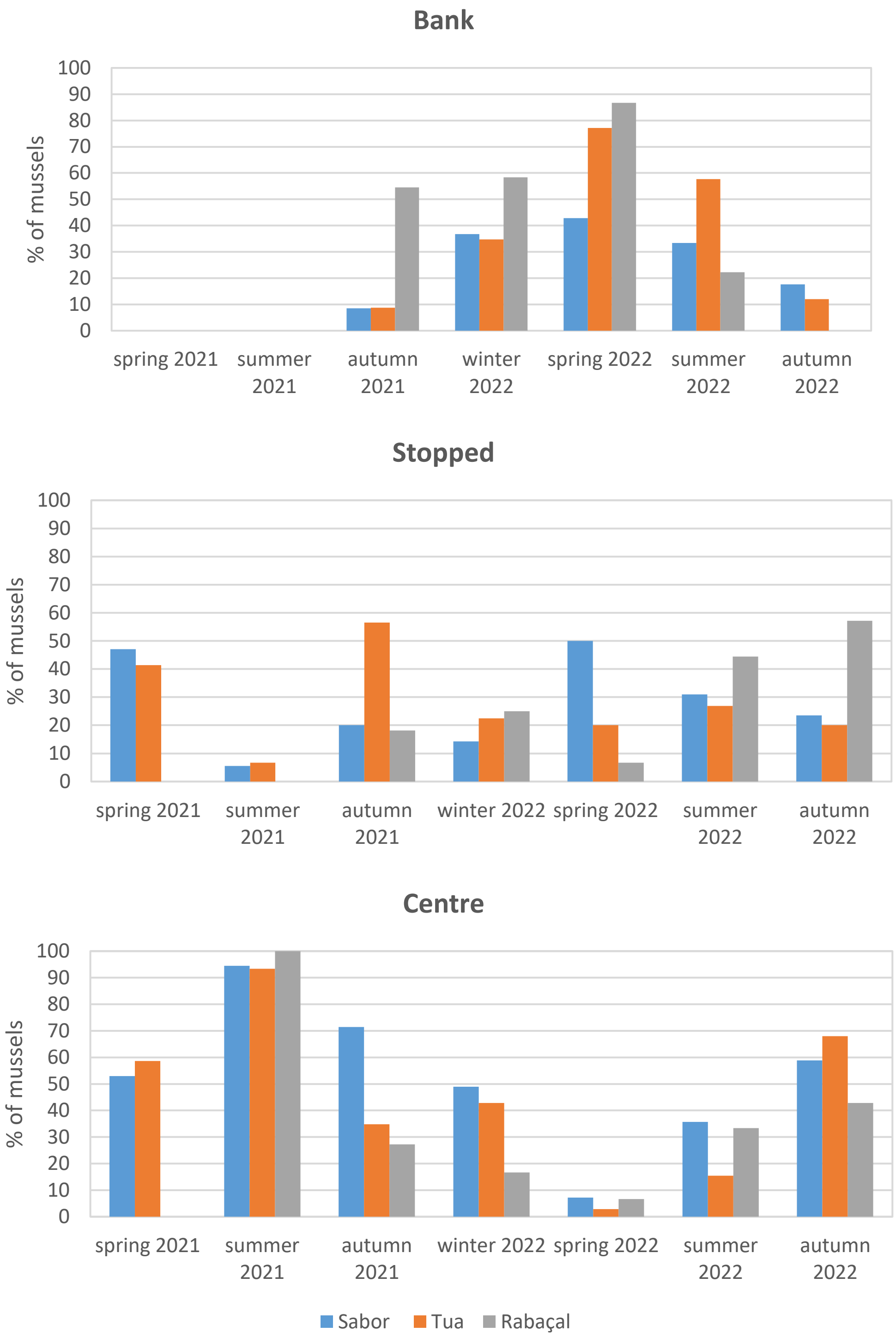


Figure 42 Lateral movement per season for *Unio delphinus* (n=100 on each river) in the three sampled zones in the Sabor, Tua, and Rabaçal rivers, between the years 2021 and 2022.

The microhabitat used by *U. delphinus* was mainly composed of sand and gravel materials, deposited near the banks of the rivers. However, differences in the available microhabitat among rivers and seasons were detected (Table 8). In fact, substantial changes can be found near the bank of sampled mussel populations among rivers and seasons, namely between summer and winter periods for the microhabitat variables of water level (depth), water current, shading (canopy varied with tree phenology) and inclusively substrate, as a response of sedimentation and erosion dynamic processes.

Table 8 Seasonal microhabitat available and used by *Unio delphinus* in the 3 sampled rivers (Sabor, Tua and Rabaçal rivers) of the Douro basin).

River	Season	Available / Use	Depth (cm)	Water velocity (class)	Dominant substrate (class)	Shading (%)
Sabor	Summer	Available	70.6 (30.5)	1 (1-2)	3 (2-6)	80 (0-100)
		Use	43.9 (7.4)	1	2	70
	Autumn	Available	90.3 (25.6)	2 (1-3)	4 (1-6)	30 (0-50)
		Use	74.0 (16.0)	1	3	40
	Winter	Available	110.4 (51.0)	4 (1-5)	4 (1-6)	10 (0-30)
		Use	88.0 (22.0)	2	3	30
	Spring	Available	104.1 (25.0)	3 (1-5)	3 (2-6)	70 (0-100)
		Use	72.5 (12.5)	2	4	50
Tua	Summer	Available	80.2 (20.3)	2 (1-2)	3 (1-6)	30 (0-50)
		Use	39.0 (12.1)	1	2	30
	Autumn	Available	103.4 (21.3)	2 (1-3)	3 (1-6)	10 (0-40)
		Use	66.2 (7.4)	1	2	20
	Winter	Available	110.2 (22.4)	4 (1-5)	4 (1-6)	10 (0-20)
		Use	68.4 (16.2)	2	3	10
	Spring	Available	98.5 (12.3)	4 (1-5)	4 (1-6)	30 (0-50)
		Use	67.1 (14.2)	2	3	30
Rabaçal	Summer	Available	50.5 (11.2)	1 (1-2)	3 (1-6)	10 (0-20)
		Use	43.0 (19.1)	1	2	5
	Autumn	Available	83.6 (22.3)	2 (1-3)	3 (1-6)	0 (0-10)
		Use	80.1 (10.2)	1	2	5
	Winter	Available	100.2 (20.3)	4 (1-5)	4 (1-6)	0 (0-10)
		Use	80.2 (12.2)	2	3	10
	Spring	Available	90.5 (32.1)	4 (1-5)	4 (1-6)	10 (0-20)
		Use	65.2 (21.7)	2	3	5

Note: Mean values are given for depth and followed by the standard deviation (in parentheses), while median values (with the range given in parentheses) are given for dominant substratum size (1 - organic detritus; 2 - sand; 3 - gravel; 4 - pebble; 5 - boulder 6 - bedrock), water velocity (1 - no current; 2- low; 3 – moderate; 4 – high; 5 - very high) and cover (in 10% increments).

The behaviour of *U. delphinus* in the use of the microhabitat varied according to the environmental conditions of each river. Thus, in the Rabaçal River, most of the detected specimens were found in the sand substrate during the year, while in the Tua River mainly in the gravel substrate. However, the microhabitat used in the Sabor River varied substantially among seasons, probably due to the higher flow variation and the greater availability of coarse substrate to supply cover to the buried mussels (Figure 43).

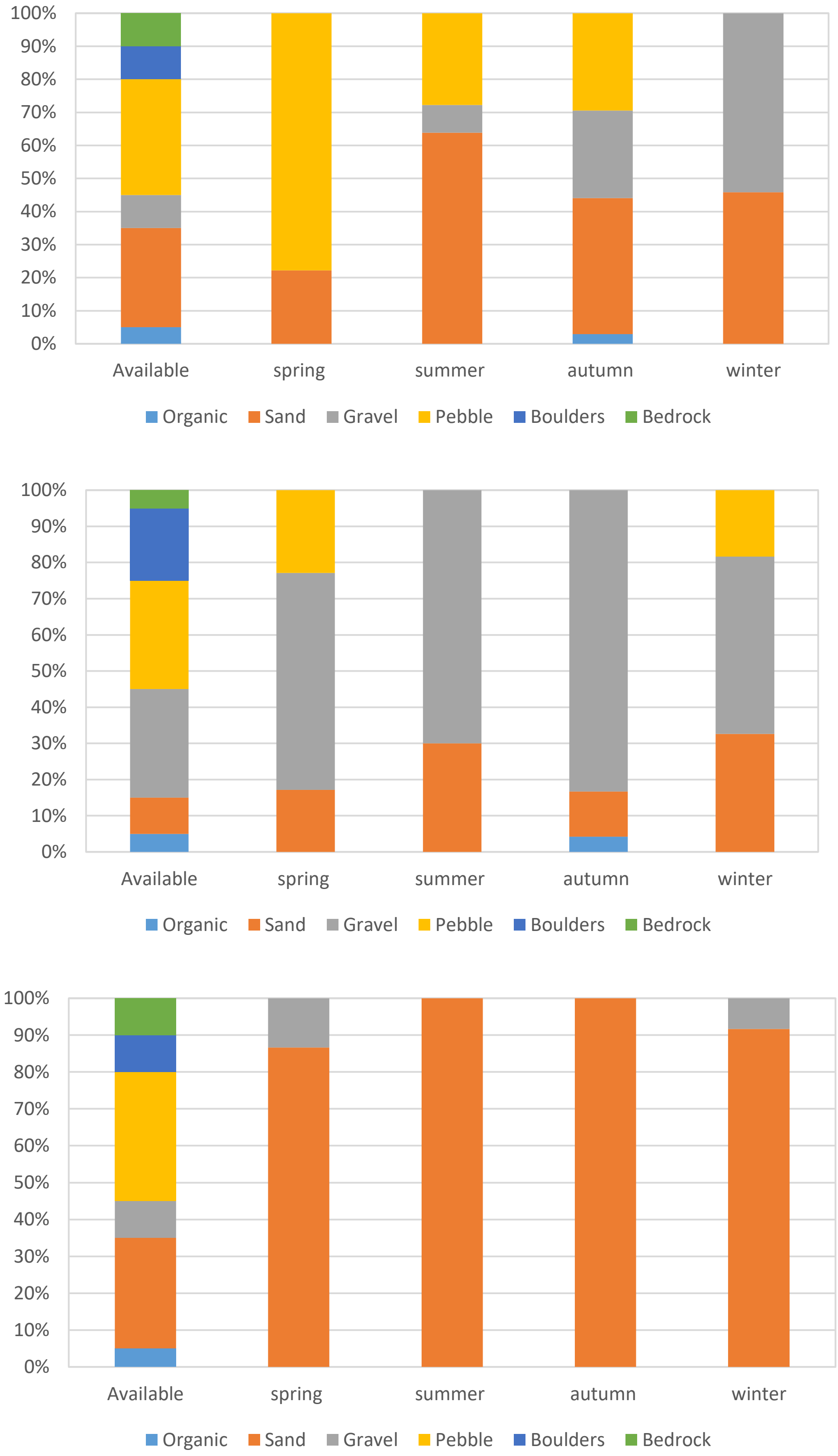
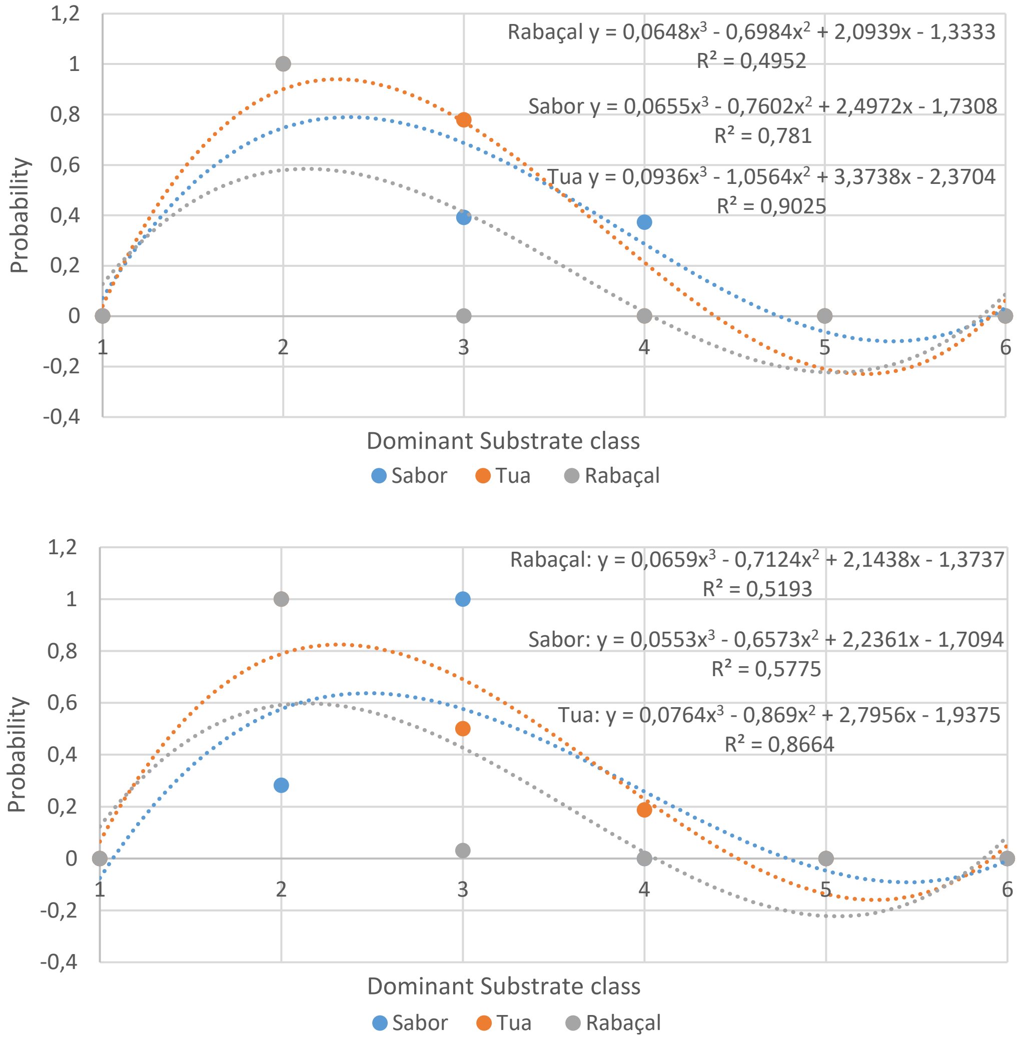


Figure 43 Available and used dominant substrate by *U. delphinus* (n=100 on each river) in the Sabor (upper position), Tua (intermediate) and Rabaçal (lower position) rivers.

Despite the variation in the colonized dominant substrate, the constructed preference curves showed a similar tendency of mussels to find sandy and gravelly areas, near the banks of the rivers, inclusively for different flow conditions, i.e., for summer (low flow) or winter (high flow) seasons (Figure 44).



Note: Dominant substratum size (1, organic detritus; 2, sand; 3, gravel; 4, pebble; 5, boulder 6, bedrock),

Figure 44 Preference curves for the variable of dominant substrate for *U. delphinus* (n=100 on each river) in three rivers, during summer (upper position) and winter (lower position) seasons.

Other microhabitat variables also contributed to the movement and the different microhabitat use observed along the seasons. The depth and water current changed clearly between dry and wet periods, i.e., summer versus winter seasons. In the Tua River, minimum and maximum values were registered, during sampling events, of 10 cm (summer) and 120 cm (winter), for the depth variable, and water current ranging between 0 and 3 m/s. Also, for shading promoted by the canopy of alder, ash, and willow trees the values ranged between 0 and 100%. This pattern was detected for all sampled rivers (Table 8).

The detectability of PIT-tagged mussels was less than 50% for Sabor and Tua rivers for all sampled seasons, while for the Rabaçal River higher than 50% but only for the initial periods of summer and autumn seasons (Figure 45). From the total, 88%, 82% and 92.5% of PIT-tagged mussels were detected, at least once, in Sabor, Tua and Rabaçal rivers, respectively, ranging the individual detections between 1 and 6 times. Mussel visibility changed during the surveys and 5 dead mussels were collected after the final surveys and were eliminated from the analyses.

Figure 45 Percentage of PIT-tagged *Unio delphinus* (n=100 on each river) detected in the three sampled zones in the Sabor, Tua, and Rabaçal rivers, along the experimental period of 2021 and 2022.

4. Discussion

4.1. Laboratory dewatering experiments

The **horizontal mussel movement** tended to slightly increase from the control, no dewatering, to moderate dewatering treatment. However, in the fast-dewatering treatment, a substantial increase in the movement was detected, suggesting that dewatering has a huge stress effect on the behaviour of the mussel species in the experiment. The horizontal movement among species in all treatments was generally consistent, being *A. anatina* and *U. delphinus* the species with the highest movement rates, which can be associated with their opportunistic behaviour, as described in Mitchell et al. (2018). This type of species has high colonization potential, due to its considerable locomotive capacity, high fecundity, and fast growth which favors the capacity to adapt to hydrological changes in the ecosystems (Mitchell et al., 2018). Moreover, the shell dimension and shape (more elongated) can also favor, particularly in *Unio delphinus*, horizontal movement through the sand, with less attrition and lower energetic wastes (Gough et al., 2012). In opposition, *P. littoralis* showed a more typical burrowing behaviour and the lowest horizontal movement rate. This factor might be explained by its bioecological characteristics, since this species tends to colonize current zones (riffle and run habitats) with coarse substrate, holding a more stable position and avoiding displacement. In fact, the shell morphology of *P. littoralis*, thicker and more robust than the other mussel species (Ilarri et al., 2015) allows a higher resistance in more turbulent zones, during higher flow periods, and higher desiccation tolerance, during dry periods, displaying a predominance behaviour to remain burrowed.

No significant differences were found between mussel populations of the same species from North and South Portugal. *U. delphinus* N was the population that had the highest movement rate in all dewatering treatments, suggesting a relatively adaptable and movable species in cases of drastic dewatering. However, more complex behaviours were detected. For instance, *A. anatina* N registered higher movement rates in slow and moderate dewatering and, contrarily, *A. anatina* S moved more in fast treatment, which suggests that southern species may be better adapted to faster dewatering scenarios. This behaviour can be expected from species of southern Iberia with drastic hydrological variations and persistent droughts due to extreme thermal phenomena (Araujo et al., 2009; Hirschi et al., 2011; Russo et al., 2019; Ribeiro et al., 2020).

The greatest number of individuals that reach the ramp's bottom was observed in the slow dewatering treatment, in opposition to the fast dewatering treatment where the fewest number of individuals reach the bottom of the ramp. This might be related to a stimulus of the mussels in slow treatment to induce movement, following the slow reduction in water level. On the other hand, the critical dewatering rates of fast treatment might lead the individuals to a more pronounced defensive and burrowing behaviour since most of them cannot accompany the dewatering rate. These variations might also be associated with the individual health conditions at the beginning of the experiment.

In terms of mussels' displacement, no significative differences were observed between different species in all treatments, with a general propensity for individuals to be found aggregated. In the control and slow treatments, there was a tendency for all mussel species to aggregate through the centre of the ramp, with a slight difference, but a similar tendency of *U. tumidiformis* to aggregate in the upper centre of the ramp. In natural habitats, mussel beds are associated with environmental stability during flows (Morales et al., 2006). The positioning of mussels in the centre or lower centre of the bed can have energetic advantages, e.g., reduce energetic wastes and conserve more energy for reproduction (Moles & Layzer, 2008). Lower levels of flow and depth are positively correlated with freshwater mussels' concentrations, increasing substrate viability, and giving more stability to the animals (Goodding et al., 2019; Martinsky & Woolnough, 2023).

There was more diversity of placement in moderate and fast treatments, with individuals being displaced between the centre, the upper centre and near origin (upper position of the ramp). These factors indicate that dewatering has stress effects on mussel's placement as suggested that mussels generally are found aggregated in stable habitat conditions, or stable substrate (Strayer, 1999).

Freshwater mussel movement is not linear, and they might inevitably **move vertically/burrow** simultaneously during horizontal movement. *U. delphinus* was the species with the highest mean burrowing rate in control, and *P. littoralis* in slow, moderate, and fast treatments. *A. anatina* was the species with the lowest mean burrowing rates, which potentially could be related to low desiccation tolerance, but also by the experimental lab conditions, as suggested by Galbraith et al. (2015). *A. anatina* is the largest species in the experiment, with some individuals surpassing 10 cm in length. Since the substrate depth on each ramp was only 10 cm, this situation can diminish the burrowing capacity of larger individuals to completely burrow.

Focusing on mussels' populations, *U. delphinus* S registered the highest mean burrowing rate in control, moderate and fast treatments. On the other hand, *U. delphinus* N moved more horizontally suggesting that southern mussels showed higher burrowing capacities. Control tanks which maintained, during the experiment, the highest (total) depth may indicate that all tested species, with no dewatering stress, tend to burrow to not lose placement and avoid dislodging effects (Poznańska-Kakareko et al., 2021; Martinsky et al., 2023), probably simulating their natural behaviour in the wilderness.

The percentage of **stranded individuals** has diminished with increasing dewatering rates, i.e., from slow to fast treatments, contrary to the experiments developed by Galbraith et al. (2015) and Mitchell et al. (2018). Our results might indicate that the Iberian freshwater mussel populations can display life-history adaptations to fast dewatering events. It seems to be the case for the most sessile species of the study, i.e. *P. littoralis*, showing stranded rates under 70% in fast treatment for South populations and 50% for North populations**.** Nevertheless, regularly the species with higher movement rates and lower burrowing are less tolerant to desiccation than the species that burrow more, which tend to become stranded. Southern populations tend to become more stranded than northern populations, except for *A. anatina*.

Mussels which can seal the shell with a thicker structure are more tolerant to emersion (Byrne & McMahon, 1994; Mitchell et al., 2018). The effects of sealing the shell may increase the level of toxic products and respiratory acidosis (Byrne & McMahon, 1994; Galbraith et al., 2015) in filtration feeders such as freshwater bivalves which normally have high concentrations of toxic products, due to their high capacity to bioaccumulate. Some studies suggested that shell size can influence unionid desiccation tolerance (Güler, 2020). As suggested by Bartsch et al. (2000) increasing volume rates and decreasing surface area (larger species) might mitigate drought effects in mussels. A study by Nakano (2018) identified that mussels with higher body weight and shell length have longer survival rates, in 2 of 6 species studies. Nevertheless, Nakano (2018) also identified the same pattern for lighter individuals. Additionally, in Güler (2020) the 2 species studied did not differ in emersion tolerance in proportion to size, which factor should be better studied. The study by Nakano (2018) also didn’t detect higher tolerances depending on the shell shape as suggested by Bartsch et al. (2000) that species with more elliptical shells will be more susceptible to desiccation and retain higher temperatures than species with more rounded shells. The abiotic seasonality has a direct impact on the mussel movement, especially temperature (Engel, 1990), dissolved O_2 (Galbraith et al., 2015) and nutrient availability (Bovbjerg, 1957), influencing the behaviour of freshwater mussels.

The instability of the water level and the risk of emersion lead mussels to move and follow the water level. The differences between species with higher mobility, normally less tolerant to drought, to burrowers and more sessile, generally drought tolerant species, might have also contributed to these variations, not forgetting the individual conditions of each mussel. This last statement was explored at population level in a study by Zieritz et al., (2014) indicating mussel distribution tendencies can be associated with population health.

Mussel beds can be associated with higher productivity ecosystems and mussel reproduction. As Vaughn (2017) suggested these mussel aggregations are high-productivity ecosystems with increases in benthic algae through the excretion of nutrients and biodeposits, which attracts macroinvertebrates, and subsequently fish. The attraction of fish to these highly productive benthic communities can naturally increase the chances of mussels glochidia finding a suitable host, and the potential to disperse and colonize new habitats (Vaughn, 2012).

4.2. Field experiments

The **daily monitoring surveys** showed significant differences between species movements, corroborating some of the observations in laboratory experiments. Notorious tendencies in the horizontal movements for some species and more sessile behaviours for other species were detected. A general propensity was observed for individuals moving more during the first hours or days of the experiments and then stabilising. This factor suggests that initially, mussels had positional/manipulation stress which impelled the individuals to move towards the river; after finding a suitable refugium most of them burrowed in the substrate or moved more slightly through the centre deep of the river.

The 1st experiment in the Rabaçal River (North Portugal) showed a gradual increase in the horizontal movements of *P. littoralis*, *A. anatina* and *U. delphinus*. Comparing field results with the laboratory observations, similar tendencies were observed, but the most notorious differences were related to the increases in the horizontal movements in the field, especially in *U. delphinus.* Evaluating reaction time towards the water, *U. delphinus* was the faster to react, followed by *A. anatina* and by far the slowest was *P. littoralis* with only one of the individuals reacting after more than 5 hours and the other not moving until the final of the experiment. It can be hypothesized that species with higher locomotive capacities have underestimated movement in lab experiments due to the dimensions of the ramps. On the other hand, more sessile species might have overestimated movement due to the laboratory conditions of the substrate that might cause stress and promote movement (Newton et al., 2015), not forgetting the dewatering effects. The 2nd experiment in Rabaçal River confirmed *U. delphinus* as the most movable species and *P. littoralis* as the lowest**.** All individuals that moved had higher movement rates on the first day, gradually decreasing and, in three cases, with no movement after the 3rd day. Finally, in the Oeiras Stream (South Portugal) similar tendencies among the species were noticed. *U. delphinus* was the most movable species and *P. littoralis* with the most sessile behaviour. When compared with southern species used in the laboratory, the only population that had similar values to laboratory registers in movement rate was one individual of *U. delphinus*. All the other individuals from the species *U. delphinus*, *A. anatina*, *U. tumidiformis* and *P. littoralis* moved significantly more in lab conditions than in the field. This corroborates the suggested typical more sessile behaviour and with higher drought tolerance of species prevenient from the South. The different environmental conditions of the Rabaçal River and the Oeiras Stream didn't decisively affect the mussel behaviours. The recent summer seasons in Northeastern and Southeastern Portugal were very similar with very dry and prolonged periods. However, southern regions are more prone to intermittent flow and small pool formation, due to low precipitation, high temperatures and anthropic activities. On some occasions, pools are the only refuge for individuals, forcing mussels to live in these precarious habitats (Gough et al., 2012). When in pools, mussels might burrow in the sand substrate due to high temperatures which also might explain the lower movement rates registered by southern individuals.

Significant differences were found in horizontal movements, considering the **seasonal monitoring surveys**, of the 3 PIT-tagged *U. delphinus* populations (n=100) monitored in the field experiments. Noticeably, higher movements were detected in the Sabor River. The great variations in substrate selection among seasons and the highest flow regime, typical of median-sized northern rivers of Portugal (N1 > 100 km^2), can contribute to explaining the higher rates of movements which potentially were caused by downstream dislodge of mussels and the stressful conditions in finding a more suitable microhabitat. Winter was the season with higher movement rates, in Sabor and Rabaçal rivers, probably due to the erosion processes in the river bottom and the subsequent downstream entrainment of benthic fauna, including freshwater mussels. Tua River was the watercourse with the lowest movement per season compared with Rabaçal River which has a similar flow variation (Table 7). The majority of mussels were found in gravel in Tua and sand in Rabaçal, the latter with a lower granulometry substrate which is more suitable for mussel movement and gravel a more stable substrate for mussels to burrow. In terms of longitudinal movements (upstream vs downstream), no significant differences were found. Nevertheless, mussels' displacement tended to be in the upstream direction in Sabor River where the flow regime was higher and in the downstream direction in the other 2 rivers. The tendency of upstream movements among the three rivers was more notorious during the summer when the flow is moderate and is easier to locomote against the current. In winter general upstream movements were minimum suggesting burrowing and more sessile behaviour from the mussels avoiding being dragged downstream. The mussel's downstream movement tended to be more pronounced in winter and autumn, which is also facilitated by the higher river flow when the movement is considered voluntary. However, could also be overestimated by some involuntary downstream movement by dislodgment due to the intensity of the flow. Upstream and downstream dispersal can be influenced by a diversity of factors as suggested by Kappes and Haase (2012), namely abiotic factors such as the quantity of nutrients, water temperature, flow and sediment characteristics, or mussels' sex, length and breeding season. In terms of lateral movement (centre vs bank) there was a clear tendency from mussels to move to the riverbank during the winter and spring, probably due to the higher flow and adverse conditions far away from the banks of the river which can lead mussels downstream. On the other hand, during summer and autumn when the river flow tends to be more stable, there was a tendency for mussels to migrate to the centre of the river. The movement to the centre of the river might be explained by mussels searching for deeper waters, with lower temperatures and better food supplies (Bovbjerg, 1957), furthermore burrowing or reproductive behaviour.

Regarding microhabitat use, freshwater mussels tended to colonize riverbanks in particular areas mainly with a sandy substrate, low water velocities, and highly shaded by the canopy of riparian trees. In the present study sand was the dominant substrate where mussels were detected in all sampled rivers. The small grain sizes facilitate the movement activity of mussels to find a suitable habitat (Poznańska-Kakareko et al., 2021; Eissenhaeur, 2023) or even because of the higher borrowing rates is only possible in this fine substrate (Hernández et al., 2021). However, habitat availability is continuously changing as a result of the different hydrological conditions, namely during extreme events of floods and dry periods, leading to the movement of mussels to adapt to the best conditions and develop their life-cycle phases. For instance, during winter and spring seasons the selection of coarse granulometry substrate (MNFI, 2020; Martinsky & Woolnough, 2023) as gravel and pebbles accoupled with sand, might be to avoid downstream movement and possible dislodgment due to great instability of substrate caused by high flow regimes (Strayer, 1999; Steuer et al., 2008; Poznańska-Kakareko et al., 2021). Tua and Rabaçal rivers had slighter variations in flow regime compared with the Sabor River, due to a lower mean precipitation index and a larger drainage area dimension (Table 7). In the Tua River, the main substrate composition was gravel and pebble (30% each) and fine organic matter (20%). The most used substrate in all seasons was gravel (more than 50%), followed by sand and pebbles in spring and winter, and just sand in summer (26%). The higher utilization of gravel was not a surprise, being one of the most available substrates and conferring more stability to mussels during high flow regime variations than lower granulometry substrates (e.g., sand). On the other hand, in the Rabaçal River substrate was dominated by pebbles (35%) and sand (28%), moreover among seasons was the watercourse where *U. delphinus* individuals were more selective in microhabitat use with over 80% of individuals found in the sand among all seasons. Less than 20% of the individuals were found in gravel substrate. Analysing all rivers, the anecdotal use (majority <1%) of a larger substrate (e.g., boulders and bedrock) suggests that were short-stop places which mussels use to emigrate to more suitable habitats or to find refugia during high flow seasons in the substrate nearby (Eissenhauer, 2023). The constructed preference curves confirmed, for all sampled rivers, the mussel tendency to find both sandy and gravelly areas, namely during the low flow conditions of the summer season. The *U. delphinus* preference for near bank zones can be considered as a strategy to avoid the higher flowing and more unstable centre of the river which potentially might “wash” mussels into inadequate downstream zones (Poznańska-Kakareko et al., 2021; Eissenhauer, 2023). Indeed, the observed variation in the microhabitat use by mussel populations seems to be a behavioural response to the global environmental changes that occurred in rivers, adjusting through small movements their position to find the optimal condition. The differences in flow regimes between drier and wetter seasons were notorious in the three selected rivers which can explain some of the selected substrates and positions among seasons, as a response to the shadow, water depth and current variations, that influenced the microhabitat selection.

The monitoring technology used seemed to be appropriate for the *in-situ* monitoring surveys. In fact, the detected pit-tagged mussels ranged from 88% in Sabor, 82% in Tua and 92,5% in Rabaçal rivers. However, for each sampled season the detectability was low with less than 50% in all sampled seasons, except for Rabaçal with over 50% in summer and autumn. Several causes for the non-detection of pit-tagged mussels can be formulated, e.g. as dragged down, manipulation from predators (e.g., birds, fish, crayfish), the limited range of detection from the antenna, human error and the unlikely hypothesis of problems with the pit-tag. The fact that 5 of *U. delphinus* were collected with empty shells (and thus, removed from the data) shows the probability of predation, manipulation that could affect the mussel detection and other factors, such as high-water flow, turbidity, and noise. Some authors have suggested that pit-tag can interfere with mussels burrowing rates reducing their capacity (Wilson et al., 2011; Martinsky and Woolnough, 2023). Manipulation and cleaning the mussel shells might also cause stressful effects on the mussels burrowing behaviour (Eissenhauer, 2023).

5. Conclusion

The results obtained in this study showed significant differences between species responses to dewatering effects especially notorious in the fast-dewatering treatment, where the drastic reduction in water level forced the freshwater mussels to respond. *A. anatina* and *U. delphinus* had the highest movement rates and *P. littoralis* the lowest. The field daily experiments, corroborated the results obtained in the laboratory, as the species had similar behaviours in terms of horizontal movement, i.e., *A. anatina* and *U. delphinus* with the highest movement rates and *P. littoralis* the lowest. The results obtained suggest that the higher movable species can track small decreases in water level, but the lower desiccation tolerance can make them more vulnerable to complete droughts or barriers that prevent mussels from reaching other pools from the same river The least mobile species, *P. littoralis*, is prone to becoming stranded even at moderate dewatering levels, nevertheless is the species in the study with the highest tolerance to desiccation in theory, which can seal the shell and survive for long periods burrowed. The other species have shown a general capacity to track the water level even in fast treatment varying from 33% in *A. anatina* N to 0% stranded in *A. anatina* S. The results obtained in the field daily experiment suggest that if the experimental ramps were larger, the differences in horizontal movement could be more significative and the percentage of stranded individuals could increase.

The vertical movement had similar tendencies to horizontal movement with significant differences among species. *A. anatina*, except for control treatment, showed a lower capacity to burrow in the substrate, and *P. littoralis* and *U. delphinus* had the highest burrowing rates, especially regarding the Southern populations. Burrowing rates were higher in control than in the three dewatering treatments, except for *P. littoralis* N. Dewatering seemed to have high-stress impacts on the behavior of the mussel since burrowing rates tended to decline as dewatering rates increased. The differences in horizontal and vertical movement among individuals from the same species and populations didn’t differ significantly. On the other hand, different populations of the same species were significantly different. These results suggest that freshwater mussels might have intraspecific life-history adaptations between North and South populations. The laboratory experiments suggest that *U. tumidiformis* has high adaptability to drainage events, registering considering movement rates and burrowing capacities. Nevertheless, a vulnerable species restricted to southern Iberia, it is a case of concern for future conservation as this region experiences increasing intensity of droughts, extreme thermal phenomena, and drastic changes in flow regimes.

The seasonal experiments, which used a single population of *U. delphinus* in each of the three rivers (Rabaçal, Tua, and Sabor), revealed various patterns in seasonal microhabitat variation as well as in longitudinal and lateral movements, which were influenced by the various seasonal characteristics of each watercourse, such as depth, flow, and substrate availability. The results suggest that the individuals are adapted to the local conditions but tended to aggregate near the banks of the rivers. This behavior is concerning, especially during lengthy droughts or rapid water drainage events when there is a danger that freshwater mussels could become stranded on the banks of rivers and be unable to move to the river's center or, in the case of southern rivers, to the residual pools.

Finally, this study provides for the first time, insights into the movement responses of two populations (North and South) of four species, *A. anatina*, *P. littoralis*, *U. delphinus*, and *U. tumidiformis*, to different intensity drainage events. The laboratory trial results were validated by the movement displacement capacities of mussels in situ between the North (Rabaçal River) and the South (Oeiras Stream) as well as by the seasonal longitudinal and lateral movements and microhabitat use preferences of *U. delphinus* in three different watercourses in the North (Rabaçal, Tua and Sabor).

Overall, the findings of this study could be applied in future conservation strategies, considering the behaviour and microhabitat preferences of individual species, to improve the success of local conservation and restoration of populations of freshwater ecosystems at both national and international levels.

6. References

Abell, R.; Thieme, M. L.; Revenga, C. et al. (2008) Freshwater ecoregions of the world: a new map of biogeographic units for freshwater biodiversity conservation. BioScience, 58, 403–414.

Aguiar, F. C.; Fernandes, M. R.; Ferreira, M. T. (2011). Riparian vegetation metrics as tools for guiding ecological restoration in riverscapes. Knowl. Managt. Aquatic Ecosyst. 402 https://doi.org/10.1051/kmae/2011074

Aldous, A.; Fitzsimons, J.; Richter, B.; Bach, L. (2011). Droughts, floods and freshwater ecosystems: Evaluating climate change impacts and developing adaptation strategies. Marine & Freshwater Research 62: 223. https://doi.org/10.1071/MF09285.

Aldridge, D. C. (1998). The Morphology, Growth and Reproduction of Unionidae (BIVALVIA) in a Fenland Waterway. Journal Molluscan Studies, 65 (1), 47-60. doi:10.1093/mollus/65.1.47

Alexander, R. B.; Alley, W. M.; Alt, D. F.; Aronson, D. A.; Averett, R. C.; Bennett, G. D.; Bertoldi, G. L.; Bradford, W. L.; Bredehoeft, J. D.; Chancy, T. H.; Chase, E. B.; de la Torre, A. C.; Cragwall, J. S.; Cragwall Jr., J. S.; Davidson, E. S.; Edelen Jr., G. W.; Feltz, H. R; Fischer, J. N.; Foxworthy, B. L.; Glysson, G. D.; Greeson, P. E.; Grubb, H. F.; Hampton, E. R.; Haupt, C. A.; Heath, R. C.; Hirsch, R. M.; James II, I. C.; Johnston, R. H.; Jorgensen, D. G.; Kammerer, J. C.; Kilpatrick, F. A.; LaBaugh, J. W.; Laird, L. B.; Laney, R. L.; Lang, S. M.; Lindholm, G. F.; Lins, H. F.; Luckey, R. R.; Luoma, S. N.; Mallard, G. E.; Mann IV, W. B.; McWreath III, H. C.; Meade, R. H.; Meisler, H.; Meyer, G.; Moody, D. W.; Moore, J. E.; Newton, J. G.; Novitze, R. P.; Putnam, A. L.; Reilly, T. E.; Riggs, H. C.; Rosenshein, J. S.; Saboe, C. W.; Schefter, J. E.; Schoen, R.; Smith, R. A.; Solley, W. B.; Spieker, A. M.; Turk, J. T.; Vecchioli, J.; Wait, R. L.; Weeks, J. B.; Weiss, M.; Woll, P. W.; Young, H. L. (1983). National Water Summary 1983- Hydrologic Events and Issues by U.S. geological Survey

Almond, R. E. A.; Grooten M.; Petersen, T. (Eds). WWF, Gland, Switzerland. (2020) Living Planet Report 2020. Bending the curve of biodiversity loss: a deep dive into freshwater.

Amyot, J. P., & Downing, J. A. (1997). Seasonal variation in vertical and horizontal movement of the freshwater bivalve *Elliptio complanata* (Mollusca: Unionidae). Freshwater Biology, 37, 345–354. https://doi. org/10.1046/j.1365-2427.1997.00158.x

Anders, K.; Wiese, V. (1993). Glochidia of the freshwater mussel, *Anodonta anatina*, affecting the anadromous European smelt (*Osmerus eperlanus*) from the Eider estuary, Germany., 42(3), 411–419. doi:10.1111/j.1095-8649.1993.tb00344.x

Ansari, Abid A.; Gill, S. Singh (2014). Eutrophication: Causes, Consequences and Control || Environmental Impacts of Tourism on Lakes., 10.1007/978-94-007-7814-6(Chapter 7), 81–88. doi:10.1007/978-94-007-7814-6_7

APA (Agência Portuguesa do Ambiente) (Diretiva 2000/60/CE do Parlamento Europeu e do Conselho, de 23 de Outubro de 2000)

Apan, A. A.; Raine, S. R.; Paterson, M. S. (2002). Mapping an analysis of changes in the riparian landscape structure of Lockyer Valley catchment Queensland, Australia. Landscape Urban Plan., 59, 43–57.

Araujo, R.; Reis, J.; Machordom, A., et al. (2009). The naiades of the Iberian Peninsula. Iberus 27:7–72.

Araujo, R. (2011). *Unio delphinus*. IUCN red list of threatened species. Version 2015.2. Available at http://www.iucnredlist.org/. Accessed 30.06.2015.

Arthington, A. H.; Dulvy, N. K.; Gladstone, W.; Winfield, I. J. (2016). Fish conservation in freshwater and marine realms: status, threats and management. Aquatic Conservation: Marine and Freshwater Ecosystems, 26(5), 838–857. doi:10.1002/aqc.2712

Banack, S.; Caller, T.; Henegan, P.; Haney, J.; Murby A.; Metcalf, J.; Powell, J.; Cox, P.; Stommel, E.; Banack, S. A.; Caller, T.; Henegan, P.; Haney, J.; Murby, A.; Metcalf, J. S.; Powell, J.; Cox, P. A.; Stommel, E. (2015). Detection of cyanotoxins, β-N-methylamino-L-alanine and microcystins, from a lake surrounded by cases of amyotrophic lateral sclerosis. Toxins 7, 322–336.

Barea-Azcón, J. M.; Ballesteros-Duperón, E.; Moreno, D. (2008). Libro Rojo de los Invertebrados de Andalucía. Consejería de Medio Ambiente, Junta de Andalucía: Sevilla.

Barnhart, C. (2000). A pilot project for restoration of the Neosho mucket, *Lampsilis rafinesqueana*. Department of Biology Southwest Missouri State University 901 S. National Springfield, Missouri 65804"

Barry, R. G.; Chorley, R. J. (1998). Atmosphere, Weather and Climate. Routledge: London and New York.

Bartsch, M. R.; Waller, D. L.; Cope, W. G. & Gutreuter, S. (2000). Emersion and thermal tolerances of three species of unionid mussels. Journal of Shellfish Research 19: 233–240.

Bauer, G. (1988). Threats to the freshwater pearl mussel *Margaritifera margaritifera* in central Europe. Biol. Conserv. 45:239–253.

Bauer, G. (1992). Variation in the life span and size of the freshwater pearl mussel. J. Anim. Ecol. 61: 425.

Beggel, S.; Geist, J. (2015). Acute effects of salinity expose on glochidia viability and host infection of *A. anatina* (Linnaeus, 1758) Science of the Total Environment 502, 659-665

Benejam, L.; Angermeier, P. L.; Munne, A; García-Berthou, E. (2010). Assessing effects of water abstraction on fish assemblages in Mediterranean streams. Freshwater Biology 55, 628–642.

Bieler, R.; Mikkelsen, P. M.; Collins, T. M.; Glover, E. A.; González, V. L.; Graf, D. L.; Harper, E. M.; Healy, J.; Kawauchi, G. Y.; Sharma, P. P.; Staubach, S.; Strong, E. E.; Taylor, J. D.; Tëmkin, I.; Zardus, J. D.; Clark, S.; Guzmán, A.; McIntyre, E.; Sharp, P.; Giribet, G. (2014). Investigating the Bivalve Tree of Life - an exemplar-based approach combining molecular and novel morphological characters. http://dx.doi.org/10.1071/IS13010

Bolotov, I. N. et al. (2017). Ancient river inference explains exceptional Oriental freshwater mussel radiations. Scientifc Reports 7, 2135, https://doi.org/10.1038/s41598-017-02312-z

Bovbjerg, R. V., (1957). Feeding related to mussel activity. Proceedings of the Iowa Academy of Science 64: 650

Bradford, M. J.; Taylor, G. C.; Allan, J. A.; Higgins, P. S. (1995). An experimental study of the stranding of juvenile coho salmon and rainbow trout during rapid flow decreases under winter conditions. North American Journal of Fisheries Management 15:473–479.

Carrizo, S. F.; Jahnig, S. C.; Bremerich, V.; Freyhof, J.; Harrison, I.; He, F.; Langhans, S. D.; Tockner, K.; Zarfl, C; Darwall, W. (2017). Freshwater megafauna: flagships for freshwater biodiversity under threat. BioScience 67, 919–927.

Collen, B.; Whitton, F.; Dyer, E. E.; Baillie, J. E. M.; Cumberlidge, N.; Darwall, W. R. T.; Pollock, C.; Richman, N. I.; Soulsby, A.-M.; Böhm, M. (2014). Global patterns of freshwater species diversity, threat and endemism. Global Ecology and Biogeography, 23(1), 40–51. doi:10.1111/geb.12096

Cummings, K. S.; Mayer, C. A. (1992). Field Guide of Freshwater mussels of the Midwest. Illinois Natural History Survey Manual 5. 194 pp.

Darwall, W. R.; Freyhof, J. (2015). Lost fishes, who is counting? The extent of the threat to freshwater fish biodiversity. In Conservation of Freshwater Fishes (eds G. P. Closs, M. Krkosek and J. D. Olden), pp. 1–35. Cambridge University Press, Cambridge.

Daszak, P.; Cunningham, A. A.; Hyatt, A. D. (2000). Emerging infectious diseases of wildlife — threats to biodiversity and human health. Science 287, 443–449.

Daveau, S. (1988). Geografia de Portugal: O Ritmo Climático e a Paisagem, 2nd Vol. João Sá da Costa: Lisboa.

De Laender, F.; Hammer, J.; Hendriks, A.J.; Soetaert, K.; Janssen, C.R. (2011). Combining Monitoring Data and Modeling Identifies PAHs as Emerging Contaminants in the Arctic. Environmental Science & Technology, 45(20), 9024–9029. doi:10.1021/es202423f

Denic, M., Taeubert, J.-E. & Geist, J. (2015). Trophic relationships between the larvae of two freshwater mussels and their fish hosts. Invertebrate Biology 134, 129–135.

Diamond, S.E.; Chick, L. D.; Perez, A.; Strickler, S.A.; Zhao, C. (2018). Evolution of plasticity in the city: urban acorn ants can better tolerate more rapid increases in environmental temperature. Conserv Physiol 6: coy030. doi:10.1093/conphys/ coy030.

Döll, P.; Zhang, J., (2010). Impact of climate change on freshwater ecosystems: a global-scale analysis of ecologically relevant river flow alterations. Hydrol. Earth Syst. Sci. Discuss. 7, 1305–1342. https://doi.org/10.5194/hess-14-783-2010.

Dudgeon, D.; Arthington, A. H.; Gessner, M. O.; Kawabata, Z. I.; Knowler, D. J.; Lévêque, C.; Naiman, R. J.; Richard, A. H. P.; Soto, D.; Stiassny, M. L. J.; Sullivan, C. A. (2006). Freshwater biodiversity: importance, threats, status and conservation challenges., 81(2), 163–0. doi:10.1017/s1464793105006950

Eissenhauer, F.; Grunicke, F.; Wagner, A.; Linke, D.; Kneis, D.; Weitere, M.; Berendonk, T. U. (2023). Active movement to coarse grained sediments by globally endangered freshwater pearl mussels (*Margaritifera margaritifera).* https://doi.org/10.1007/s10750-023-05138-1

Ekvall, M. K.; de la Calle Martin, J.; Faassen, E. J.; Gustafsson, S.; Lurling, M.;Hansson, L.-A. (2013). Synergistic and species-specific effects of climate change and water colour on cyanobacterial toxicity and bloom formation. Freshwater Biology 58, 2414–2422.

Engel, H. H. B. J., 1990. Untersuchungen zur Autökologie von *Unio crassus* in Norddeutschland. PhD Dissertation, University of Hannover. Hannover

Fernandes, M. R.; Aguiar, F. C.; Ferreira, M. T. (2011). Assessing riparian vegetation structure and the influence of land-use using landscape metrics and geostatistical tools. Landscape Urban Plan., 99, 166–177.

Findlay, S. E. G. & Kelly, V. R. (2011). Emerging indirect and long-term road salt effects on ecosystems. Annals of the New York Academy of Sciences 1223, 58–68.

Frischer, M.E.; Nierzwicki-Bauer, S.A.; Parsons, R.H.; Vathanodorn, K.; Waitkus, K. R. (2000) Interactions between zebra mussels (*Dreissena polymorpha*) and microbial communities. Can J Fish Aquat Sci 57(3):591–599

Froufe, E.; Sobral, C.; Teixeira, A.; Lopes, A.; Sousa, R.; Varandas, S.; Lopes-Lima, M. (2013). Development and multiplexing of microsatellite loci for the near threatened freshwater mussel *Potomida littoralis* (Cuvier, 1798) using 454 sequencing.

Froufe, E.; Sobral, C.; Teixeira, A.; Sousa, R.; Varandas, S.; Aldridge, D. C.; Lopes-Lima, M. (2014). Genetic diversity of the pan-European freshwater mussel *Anodonta anatina* (Bivalvia: Unionoida) based on CO1: new phylogenetic insights and implications for conservation." *Aquatic Conservation: Marine and Freshwater Ecosystems* 24.4. 561-574. DOI: 10.1002/aqc.2456

Froufe, E.; Prié, V.; Faria, J.; Ghamizi, M.; Gonçalves, D. V.; Gürlek, M. E.; Karaouzas, I.; Kebapçi, Ü.; Şereflişan, H.; Sobral, C.; Sousa, R.; Teixeira, A.; Varandas, S.; Zogaris, S.; Lopes-Lima, M. (2016). Phylogeny, phylogeography, and evolution in the Mediterranean region: news from a freshwater mussel (*Potomida*, Unionida). Molecular Phylogenetics and Evolution, (), S1055790316300641–. doi: 10.1016/j.ympev.2016.04.030

Froufe E; Lopes-Lima M.; Riccardi N.; Zaccara S.; Vanetti I.; Lajtner J.; Teixeira A.; Varandas S.; Prié V.; Zieritz A.; Sousa R. Lifting the curtain on the freshwater mussel diversity of the Italian Peninsula and Croatian Adriatic coast. Biodiversity and Conservation. (2017) Dec; 26:3255-74.

Füssel, H.; Jol, A.; Kurnik, B.; Hemming, D. (2012). Climate Change, Impacts and Vulnerability in Europe 2012: An Indicator-Based Report, EEA Rep 304.

Galbraith, H. S.; Spooner, D. E.; Vaughn, C. C. (2010). Synergistic effects of regional climate patterns and local water management on freshwater mussel communities

Galbraith, H. S.; Blakeslee, C. J.; Lellis, W. A. (2012). Recent thermal history influences thermal tolerance in freshwater mussel species (Bivalvia:Unionoida). Freshwater Science, 31(1), 83–92. doi:10.1899/11-025.1

Galbraith, H. S.; Blakeslee, C. J.; Lellis, W. A. (2015). Behavioral responses of freshwater mussels to experimental dewatering. Society of the Freshwater Science, 34(1):42-52 DOI: 10.1086/679446

Garner, J. T.; Haggerty, T. M; Modlin, R. F. (1999). Reproductive cycle of *Quadrula metanevra* (Bivalvia: Unionidae) in the Pickwick Dam tailwater of the Tennessee River. American Midland Naturalist 141, 277–283

Gleick, P. H. (1998). Water in crisis: paths to sustainable water use. Ecological Applications 8:571–579.

Gomes-dos-Santos A, Froufe E, Gonçalves DV, Sousa R, Prié V, Ghamizi M, Benaissa H, Varandas S, Teixeira A, Lopes-Lima M. Freshwater conservation assessments in (semi-) arid regions: Testing River intermittence and buffer strategies using freshwater mussels (Bivalvia, Unionida) in Morocco. Biological Conservation. (2019). Aug 1; 236:420-34.

Gomez, I.; Araujo, R. (2008). Channels and ditches as the last shelter for freshwater mussels: the case of *Margaritifera auricularia* and other naiads inhabiting the mid Ebro River Basin, Spain. Aquatic Conservation: Marine and Freshwater Ecosystems 18, 658–670.

Goodding, D. D.; Williams, M. G.; Ford, D. F.; Williams L. R. & Ford, N. B. (2019). Associations between substrate and hydraulic variables and the distributions of a sculptured and an unsculptured unionid mussel. Freshwater Science 38: 543–553. DOI:10.1086/704795

Gordon, N.D., McMahon, T.A., Finlayson, B.L., Gippel, C.J., Nathan, R.J., 2004. Stream Hydrology: An Introduction for Ecologists. John Wiley and Son.

Gosling, E. M. (2003). Bivalve Molluscs: Biology, Ecology, and Culture. Oxford, UK: Blackwell

Gough, H. M.; Gashco Landis, A. M.; Stoeckel, J. A. (2012). Behaviour and physiology are linked in the responses of freshwater mussels to drought. Freshwater Biology, 57(11), 2356–2366. https://doi.org/10. 1111/fwb.12015

Grabarkiewicz, J. D. & Davis, W. S. (2008). An Introduction to Freshwater Mussels as Biological Indicators Including Accounts of Interior Basin, Cumberlandian, and Atlantic Slope Species. EPA-260-R-08-015

Graneli, W. (2012). Brownification of Lakes. In: Bengtsson, L., Herschy, R.W., Fairbridge, R.W. (eds) Encyclopedia of Lakes and Reservoirs. Encyclopedia of Earth Sciences Series. Springer, Dordrecht. https://doi.org/10.1007/978-1-4020-4410-6_256

Gregory, S. V.; Swanson, F. J.; McKee, W. A.; Cummins, K. W. (1991). An ecosystem perspective of riparian zones. BioScience 41, 540-51.

Grime, J. P. (2001). Plant strategies, vegetation processes, and ecosystem properties. Chichester, UK: John Wiley & Sons.

Güler, M. (2020). Survival durations and behavioural adjustments of two freshwater bivalves (*Unio terminalis*, Unionida and *Corbicula fluminea*, Venerida) under two emersion conditions. *Ann. Limnol. – Int. J. Lim. 56: 29*

Gutierrez, J. L.; Jones, C. G.; Strayer, D. L.; Iribarne, O. O. (2003). Mollusks as ecosystem engineers: the role of shell production in aquatic habitats. Oikos 101, 79–90.

Gutiérrez-Yurrita, P. J.; Martínez, J. M.; ILHÉU, M.; BRAVO, M. A.; BERNARDO, J. M.; MONTES, C. (1999). The status of crayfish populations in Spain and Portugal. In: F. Gherardi & D. M. Holdich (eds.), Crayfish in Europe as alien species. How to make the best of a bad situation. Crustacean Issues, 11: 161-192. (A.A. Balkema, Rotterdam).

Haag, W. R.; Rypel, A. L. (2011). Growth and longevity in freshwater mussels: Evolutionary and conservation implications. Biol. Rev. 86, 225–247. https://doi.org/10.1111/j. 1469-185X.2010.00146.x.

Haag, W. R. (2012). North American freshwater mussels: Natural history, ecology, and conservation. Cambridge, UK: Cambridge University Press. https://doi.org/10.1017/CBO9781139048217

Haag, W. R. (2013). The role of fecundity and reproductive effort in defining life-history strategies of North American freshwater mussels

Haas, F. (1917). Estudio sobre las náyades del Ebro. Boletín de la Sociedad Aragonesa de Ciencias Naturales, 16: 71-82.

Haas, F. (1941). Records of large fresh-water mussels. Field Museum of Natural History, Zoological Series, 24: 259-27

Halleraker, J. H.; Saltveit, S. J.; Harby, A.; Arnekleiv, J. V.; Fjeldstad, H. P.; Kohler, B. (2003). Factors influencing stranding of wild juvenile brown trout (*Salmo trutta*) during rapid and frequent flow decreases in an artificial stream. River Research and Applications 19:589–603.

Hamester, M. R. R.; Balzer, P. S.; Becker, D. (2012). Characterization of calcium carbonate obtained from oyster and mussel shells and incorporation in polypropylene. Materials Research, 15(2), 204–208. doi:10.1590/S1516-14392012005000014

Hawkins, A. J. S.; James, M. R.; Hickman, R. W.; Hatton, S.; Weatherhead, M. (1999). Modelling of suspension - feeding and growth in the green - lipped mussel *Perna canaliculus* exposed to natural and experimental variations of seston availability in the Marlborough Sounds, New Zealand. Marine Ecology Progress Series 191 : 217 – 232

Heard, W. H. (1975). Sexuality and other aspects of reproduction in *Anodonta* (Pelecypoda: Unionidae). Malacologia 15:81–103.

Heino, J.; Virkkala, R.; Toivonen, H. (2009). Climate change and freshwater biodiversity: detected patterns, future trends and adaptations in northern regions. Biological Reviews 84, 39–54

Herbert, E. R.; Boon, P.; Burgin, A. J.; Neubauer, S. C.; Franklin, R. B.; Ardon, M.; Hopfensperger, K. N.; Lamers, L. P. M.; Gell, P. (2015). A global perspective on wetland salinization: ecological consequences of a growing threat to freshwater wetlands. Ecosphere 6, art206.

Hermoso, V.; Clavero, M.; Blanco-Garrido, F.; Prenda, J. (2011). Invasive species and habitat degradation in Iberian streams: an analysis of their role in freshwater fish diversity loss.

Hernández, B. A.; Mitchell, Z. A.; Robertson, C. R. & Schwalb, A. N. (2021). Burrowing behaviour of unionid mussels in subtropical rivers: Implications for survey guidelines. Aquatic Conservation: Marine and Freshwater Ecosystems 31: 903–915. https://doi.org/10.1002/aqc.3525.

Hewitt, T. L., Haponski, A. E.; Foighil, D. Ó. (2021). Evolution of diverse host infection mechanisms delineates an adaptive radiation of lampsiline freshwater mussels centered on their larval ecology. PeerJ 9: e12287 https://doi.org/10.7717/peerj.12287

Hinzmann, M.; Lopes-Lima, M.; Teixeira, A.; Varandas, S.; Sousa, R.; Lopes, A.; Froufe, E.; Machado, J. (2013). Reproductive Cycle and Strategy of *Anodonta anatina* (L., 1758): Notes on Hermaphroditism

Hinzmann, M.; Lopes-Lima, M.; Bobos, I.; Ferreira, J.; Domingues, B.; Machado, J. (2015). Morphological and Chemical Characterizationof Mineral Concretions in the Freshwater Bivalve *Anodonta cygnea* (Unionidae).

Hochwald, S. (2001). Plasticity of life-history traits in *Unio crassus*. In: Ecology and Evolutionary Biology of the Freshwater mussels Unionoidea.

Hoellein, T. J.; Zarnoch, C. B.; Bruesewitz, D. A.; DeMartini, J. (2017). Contributions of freshwater mussels (Unionidae) to nutrient cycling in an urban river: filtration, recycling, storage, and removal

Hoffman, G. L. (1990). *Myxobolus cerebralis*, a worldwide cause of salmonid whirling disease. Journal of Aquatic Animal Health 2, 30–37.

Hoggarth, M. A.; Gaunt, A. S. (1988): Mechanics of glochidial attachment (Mollusca: Bivalvia: Unionidae). J. Morphol. 198: 71–81

Holdich, D. M. & Reeve, I. D. (1991). Distribution of freshwater crayfish in the British Isles, with particular reference to crayfish plague, alien introductions and water quality. Aquatic Conservation: Marine and Freshwater Ecosystems 1, 139–158.

Horrigan, L.; Lawrence, R. S.; Walker, P. (2002). How sustainable agriculture can address the environmental and human health harms of industrial agriculture Environmental

Health Perspectives, 110(5), 445–456. doi:10.1289/ehp.02110445

Hulme, P. E. (2015). Invasion pathways at a crossroad: policy and research challenges for managing alien species introductions. J. Appl. Ecol. 52, 1418–1424

Hirschi, M., Seneviratne, S.I., Alexandrov, V., Boberg, F., Boroneant, C., Christensen, O. B., Formayer, H., Orlowsky, B., Stepanek, P., (2011). Observational evidence for soil moisture impact on hot extremes in southeastern Europe. Nat. Geosci. 4 (1), 17–21. https://doi.org/10.1038/ngeo1032.

Huntington, T. G. (2006). Evidence for intensification of the global water cycle: review and synthesis. Journal of Hydrology 319, 83–95.

Ilarri, M. I.; Souza, A. T.; Sousa, R. (2015). Contrasting decay rates of freshwater bivalves' shells: Aquatic versus terrestrial habitats

Ilarri, M. I.; 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. Science of The Total Environment, 634(), 1398–1405. doi:10.1016/j.scitotenv.2018.04.070

Isely, F. B. (1914). Experimental study of the growth and migration of fresh-water mussels. Appendix III to the Report of the U.S. Commissioner of Fisheries for 1913. [Also issued as US Bureau of Fisheries Document No. 792]. US Bureau of Fisheries, Washington, DC.

Jacobson, P. J.; Neves, R. J.; Cherry, D. S.; Farris, J. L. (1997). Sensitivity of glochidial stages of freshwater mussels (Bivalvia: Unionidae) to copper.

Jesus, T. F.; Inácio, A.; Coelho, M. M. (2013). Different levels of hsp70 and hsc70 mRNA expression in Iberian fish exposed to distinct river conditions. Genetics and Molecular Biology, 36(1), 061–069. doi:10.1590/S1415-47572013000100009

Jesus, T. F.; Grosso, A. R.; Almeida-Val, V. M. F.; Coelho, M. M. (2015). Transcriptome profiling of two Iberian freshwater fish exposed to thermal stress

Kappes, H. & P. Haase, (2012). Slow, but steady: dispersal of freshwater molluscs. Aquatic Sciences 74: 1–14. https:// doi.org/10.1007/s00027-011-0187-6.

Karlsson, S.; Larsen, B. M.; Hindar, K. (2012). Host-dependent genetic variation in freshwater pearl mussel (*Margaritifera margaritifera* L.)

Kat, P. W. (1983). Sexual selection and simultaneous hermaphroditism among the Unionidae (Bivalvia: Mollusca). J Zool 201:395–416.

Kat, P. W. (1984). Parasitism and the Unionacea. Biological Review 59: 189–207.

Klishko, O. K.; Lopes-Lima, M.; Bogan, A. E.; Matafonov, D. V.; Froufe, E. (2018). Morphological and molecular analyses of Anodontinae species (Bivalvia, Unionidae) of Lake Baikal and Transbaikalia

Kritzberg, E. S.; Ekstrom, S. M. (2012). Increasing iron concentrations in surface waters - a factor behind brownification? Biogeosciences 9, 1465–1478.

Lake, P. S. (2000). Disturbance, patchiness, and diversity in streams. Journal of the North American Benthological Society, 19(4), 573–592. https://doi.org/10.2307/1468118

Lasee, B. A. (1991). Histological and ultrastructural studies of larval and juvenile *Lampsilis* (Bivalvia) from the upper Mississippi River. Ph.D. thesis. Iowa State University, Ames, IA, USA.

Lima, P.; Kovitvadhi, U.; Kovitvadhi, S.; Machado, J. (2006). In vitro culture of glochidia from the freshwater mussel *Anodonta cygnea*, 125(1), 34–44. doi:10.1111/j.1744-7410.2006. 00037.x

Lima, P.; Lopes-Lima, M.; Kovitvadhi, U.; Kovitvadhi, S.; Owen, C.; Machado, J. (2012). A review on the ‘‘in vitro’’ culture of freshwater mussels (Unionoida)

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), 1–13. doi:10.1007/s10750-014-1902-9

Lopes-Lima, M.; Sousa, R.; Geist, J.; Aldridge, D. C.; Araujo, R.; Bergengren, J.; Bespalaya, Y.; Bódis, E.; Burlakova, L.; Van Damme, D. Douda, K.; Froufe, E.; Georgiev, D.; Gumpinger, C.; Karatayev, A.; Kebapçi, Ü.; Killeen, I.; Lajtner, J.; Larsen, B. M.; Lauceri, R.; Legakis, A.; Lois, S.; Lundberg, S.; Moorkens, E.; Motte, G.; Nagel, K. O.; Ondina, P.; Outeiro, A.; Paunovic, M.; Prié, V.; von Proschwitz, T.; Riccardi, N.; Rudzīte, M.; Rudzītis, M.; Scheder, C.; Seddon, M.; Şereflişan, H.; Simić, V.; Sokolova, S.; Stoeckl, K.; Taskinen, J.; Teixeira, A.; Thielen, F.; Trichkova, T.; Varandas, S.; Vicentini, H.; Zajac, K.; Zajac, T.; Zogaris, S. (2016). Conservation status of freshwater mussels in Europe: state of the art and future challenges. Biological Reviews, (), n/a–n/a. doi:10.1111/brv.12244

Lopes-Lima, M.; Hattori, A.; Kondo, T.; Lee, J. H.; Kim, S. K.; Shirai, A.; Hayashi, H.; Usui, T.; Sakuma, K.; Toriya, T.; Sunamura, Y.; Ishikawa, H.; Hoshino, N.; Kusano, Y.; Kumaki, H.; Utsugi, Y.; Yabe, S.; Yoshinari, Y.; Hiruma, H.; Tanaka, A.; Sao, K.; Ueda, T.; Sano, I.; Miyazaki, I.; Gonçalves, D. V.; Klishko, O. K.; Konopleva, E. S.; Vikhrev, I. V.; Kondakov, A. V.; Gofarov, M. Y.; Bolotov, I. N.; Sayenko, E. M.; Soroka, M.; Zieritz, A.; Bogan, A. E.; Froufe, E. (2020). Freshwater mussels (Bivalvia: Unionidae) from the rising sun (Far East Asia): phylogeny, systematics, and distribution

Lopes-Lima, M; Hinzmann, M.; Varandas, S.; Froufe, E.; Reis, J.; Moreira, C.; Araújo, S.; Miranda, F.; Gonçalves, D. V.; Beja, P.; Sousa, R.; Teixeira, A. (2020b). Setting the stage for new ecological indicator species: A holistic case study on the Iberian dolphin freshwater mussel *Unio delphinus* Spengler, 1793. Ecological Indicators, 111(), 105987–doi: 10.1016/j.ecolind.2019.105987

Luck, K.; Ackerman, J. D. (2022). Threats to freshwater mussels: The interactions of water temperature, velocity and total suspended solids on ecophysiology and growth-.doi.org/10.1016/j.scitotenv.2022.153101

Lyubas AA, Tomilova AA, Kondakov AV, Konopleva ES, Vikhrev IV, Gofarov MY, Eliseeva TA, Aksenova OV, Bovykina GV, Kryuk DV, Gorbunova TL. Phylogeography and Genetic Diversity of Duck Mussel *Anodonta anatina* (Bivalvia: Unionidae) in Eurasia. Diversity. (2023) Feb 12;15(2):260.

Magoulick, D. D.; Kobza, R. M. (2003). The role of refugia for fishes during drought: A review and synthesis. Freshwater Biology, 48(7), 1186–1198. https://doi.org/10.1046/j.1365-2427.2003.01089.x

Markovic, D.; Carrizo, S.; Freyhof, J.; Cid, N.; Lengyel, S.; Scholz, M.; Kasperdius, H.; Darwall, W. (2014). Europe's freshwater biodiversity under climate change: distribution shifts and conservation needs, pp 1097-1107

Martinsky, M. E.; Woolnough, D. A. (2023). Movement and surrounding community of the understudied and endangered *Ligumia recta* (Mollusca, Unionidae). https://doi. org/10.1007/s10750-023-05145-2

McMahon, R. F. & Bogan, A. E. (2001). Mollusca: Bivalvia. In Ecology and Classification of North American Freshwater Invertebrates (eds J. H. Thorp and A. P. Covich), pp. 321–429. Academic Press, San Diego.

Mehta, P. Kumar (2002). Greening of the Concrete Industry for Sustainable Development

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. Science of the Total Environment 649, 938-948

Millan, M. M.; Estrela, M. J.; Miró, J. (2005). Rainfall components: variability and spatial distribution in a Mediterranean area (Valencia Region). Journal of Climate 18, 2682–2705.

Mills, E. L.; Leach, J. H.; Carlton, J. T.; Secor, C. L. (1993). Exotic Species in the Great Lakes: A History of Biotic Crises and Anthropogenic Introductions. Journal of Great Lakes Research, 19(1), 1–54. doi:10.1016/S0380-1330(93)71197-1

Mitchell, Z. A.; McGuire, J.; Abel, J.; Hernández, B. A.; Schwalb, A. N. (2018). Move on or take the heat: Can life history strategies of freshwater mussels predict their physiological and behavioural responses to drought and dewatering? Freshwater Biology, (), –. doi:10.1111/fwb.13187

MNFI (Michigan Natural Features Inventory), (2020). *Ligumia recta* (Black Sandshell). Michigan State Status Assessment. https://mnf. anr.msu.edu/species/description/12376/Ligumia-recta.

Modesto, V.; Ilarri, M.; Souza, A. T.; Lopes-Lima, M.; Douda, K.; Clavero, M.; Sousa, R. (2018). Fish and mussels: Importance of fish for freshwater mussel conservation.

Moles, R. K. & Layzer, J. B. (2008). Reproductive ecology of *Actinonaias ligamentina* (Bivalvia:Unionidae) in a regulated river. Journal of the North American Benthological Society, Vol. 27, No. 1 (Mar. 2008), pp. 212-222. DOI: 10:1899/07-006.1

Moore, T. P.; Collier, K. J.; Duggan, I. C. (2018). Interactions between Unionida and non-native species: A global meta-analysis

Mora, C.; Tittensor, D. P.; Adl, S.; Simpson, A. G. B.; Worm, B.; Mace, G. M. (2011). How Many Species Are There on Earth and in the Ocean? PLoS Biology, 9(8), e1001127–. doi: 10.1371/journal.pbio.1001127

Morales, Y.; Weber, L. J.; Mynett, A. E.; Newton, T. J. (2006). Effects of substrate and hydrodynamic conditions on the formation of mussel beds in a large river. Journal of the North American Benthological Society, 25 (3): 664-676. doi.org/10.1899/0887- 3593

Moser, T. J.; Lindeman, D. R.; Wigington, P. J.; Schuft, M.; Van Sickle, J. (2000). Methods for multi-spatial scale characterization riparian corridors, U.S. Environmental Protection Agency, Office of Research and Development, National Health and Environmental Effects Laboratory, 7 p.

Mouthon, J. (1996). Molluscs and biodegradable pollution in rivers: proposal for a scale sensivity of species. Hydrobiologia 317:221-229.

Mouthon, J.; Daufresne, M. (2006). Effects of the 2003 heatwave and climatic warming on mollusc communities of the Saone: a large lowland river and of its two ˆ main tributaries (France). Global Change Biology 12, 441–449.

Nace, R. L. (1967). Are We Running Out of Water?

Nagel, K. O. (1988). Anatomische, morphologische und biochemische Untersuchungen zur Taxonomie und Systematik der europäischen Unionacea (Mollusca: Bivalvia). Ph. D. thesis, University of Kassel (Germany). ii + 101 pp

Nagel, K. O. (2004). Observations on the reproductive period of the freshwater mussel *Potomida littoralis* (Unionidae)

Naiman, R. J.; Décamps, H. (1997). The ecology of interfaces: riparian zones. Annu. Ver. Ecol. Syst., 28, 621–658.

Nakano, M. (2018). Survival duration of six unionid mussel species under experimental emersion. Hydrobiologia 809: 111–120. https://doi.org/10.1007/s10750-017-3453-3

Negm, Abdelazim M.; Romanescu, Gheorghe; Zeleňáková, Martina (2020). [Springer Water] Water Resources Management in Romania ||., 10.1007/978-3-030-22320-5(), –. doi:10.1007/978-3-030-22320-5

Negus, C. L. (1966). A quantitative study of growth and production of unionid mussels in the River Thames at Reading. Journal of Animal Ecology 35, 513–532.

Nel, J. L.; Roux, D. J.; Abell, R.; Ashton, P. J.; Cowling, R. M.; Higgin, J. V.; Thieme, M.; Vier, J. H. (2008) Progress and challenges in freshwater conservation planning. Aquatic Conservation: Marine and Freshwater Ecosystems, 19, 474– 485.

Nel, J. L.; Reyers, B.; Roux, D. J.; Impson, D. N.; Cowling, R. M. (2011) Designing a conservation area network that supports the representation and persistence of freshwater biodiversity. Freshwater Biology, 56, 106–124.

Neves, R. J.; Widlak, J. (1987). Habitat ecology of juvenile freshwater mussels (Bivalvia: Unionidae) in a headwater stream in Virginia. American Malacological Bulletin 5: 1–7.

Neves, R. J.; Odom, M. C. (1989). Muskrat Predation on Endangered Freshwater Mussels in Virginia. The Journal of Wildlife Management, 53(4), 934– . doi:10.2307/3809591

Newton, T. J.; Zigler, S. J.; Gray, B. R. (2015). Mortality, movement and behaviour of native mussels during a planned water-level drawdown in the Upper Mississippi River. doi:10.1111/fwb.12461

Nyingi, D. W.; Gichuki, N.; Mordecai, O. O. (2013). [Developments in Earth Surface Processes] Kenya: A Natural Outlook - Geo-Environmental Resources and Hazards Volume 16 || Freshwater Ecology of Kenyan Highlands and Lowlands., (), 199–218. doi:10.1016/b978-0-444-59559-1.00016-5

Okello, C.; Tomasello, B.; Greggio, N.; Wambiji, N.; Antonellini, M. (2015). Impact of Population Growth and Climate Change on the Freshwater Resources of Lamu Island, Kenya. Water, 7(12), 1264–1290. doi:10.3390/w7031264

Økland, J. (1963). Notes on population density, age distribution, growth and habitat of *Anodonta piscinalis* Nilss. (Moll. Lamellibr.) in a eutrophic Norwegian lake. Nytt Magasin for Zoologi 11:19–43.

Ormerod, S. J.; Dobson, M.; Hildrew, A. G.; Townsend, C.R.: Multiple stressors in freshwater ecosystems. Freshwater Biol (2010), 55(Suppl 1):1-4.

Pereira-Silva, E. F. L.; Pires, J. S.; Hardt, E.; Santos, J. E.; Ferreira, W. A. (2011) Avaliação da qualidade da água em microbacias hidrográficas de uma Unidade de Conservação do Nordeste do estado de São Paulo, Brasil

Pérez-Bote, J. L.; Pula, H. J.; Cascos, G. (2000). Distribución del cangrejo rojo *Procambarus clarkii* Girard, 1859 (Decapoda, Cambaridae) en Extremadura. Graellsia, 56: 71-78.

Pérez-Bote, J. L. (2004). Feeding Ecology of the Exotic Red Swamp Crayfish, *Procambarus clarkii* (Girard, 1852) in the Guadiana River (Sw Iberian Peninsula). Crustaceana, 77(11), 1375–1387. doi:10.1163/1568540043166001

Pérez-Quintero, J. C. (2006). Diversity, habitat use and conservation of freshwater molluscs in the lower Guadiana River basin (SW Iberian Peninsula). Aquatic Conservation: Marine and Freshwater Ecosystems17: 485–501.

Piper, R. (2007). Extraordinary Animals: An Encyclopedia of Curious and Unusual Animals

Pittock, J.; Hansen, L. J; Abell, R. (2008). Running dry: freshwater biodiversity, protected areas and climate change. Biodiversity 9, 30–38.

Pittock, J. (2015). Biodiversity and the climate, energy and water nexus. Pages 283-302 in Pittock J.; Hussey, K.; and Dovers, S., editors. Climate, Energy and Water. Cambridge University Press, New York, USA

Poff, N. L.; Allan, J. D.; Bain, M. B.; Karr, J. R.; Prestegaard, K. L.; Richter, B. D.; Sparks, R. E.; Stromberg, J. C. (1997). The natural flow regime: a paradigm for river conservation and restoration. BioScience 47:769–784.

Poff, N. L.; Zimmerman, J. K. H. (2010). Ecological responses to altered flow regimes: a literature review to inform the science and management of environmental flows. Freshwater Biology 55:194–205.

Poznańska-Kakareko, M.; Wiśniewski, K.; Szarmach, D.; Witkowska, A.; Kakareko, T.; Jermacz, Ł.; Kobak, J. (2021). Importance of substratum quality for potential competitive niche overlap between native and invasive unionid mussels in Europe. Science Environment 799: 149345. doi.org/10.1016/j.scitotenv.2021.149345

Pusey, B. J.; Arthington, A. H. (2003). Marine and Freshwater Research, 54(1), 1–. doi:10.1071/MF02041

Pyke, G. H. (2008). Plague minnow or mosquito fish? A review of the biology and impacts of introduced *Gambusia* species. Annual Review of Ecology, Evolution, and Systematics 39: 171–191.

Pynnönen, K. (1990). Physiological responses to severe acid stress in four species of freshwater clams (Unionidae)., 19(4), 471–478. doi:10.1007/bf01059064

Ramos, P. I. A. (2011). (Master thesis) Estudo das populações de bivalves (Unionidae) de rios do Norte de Portugal: Importância da qualidade ambiental na conservação de espécies ameaçadas

Reid, A. J.; Carlson, A. K.; Creed, I. F.; Eliason, E. J.; Gell, P. A.; Johnson, P. T. J.; Kidd, K. A.; MacCormack, T. J.; Olden, J. D.; Ormerod, S. J.; Smol, J. P.; Taylor, W. W.; Tockner, K.; Vermaire, J. C.; Dudgeon, D.; Cooke, S. J. (2019). Emerging threats and persistent conservation challenges for freshwater biodiversity. Biological Reviews, (), –. doi:10.1111/brv.12480

Reis, J. (coord.) 2006. Atlas dos bivalves de água doce em Portugal continental. Instituto da Conservação da Natureza, Lisboa. 130 pp.

Reis, J.; Araujo, R. (2009). Redescription of *Unio tumidiformis* Castro, 1885 (Bivalvia, Unionidae), an endemism from the south-western Iberian Peninsula. J. Nat. Hist. 43: 1929–1945.

Reis, J. (2010). (PhD thesis) Systematics, Biology and Conservation of *Unio tumidiformis* Castro, 1885 (UNIONIDAE:BIVALVIA) in the South-west of the Iberian Peninsula

Reis, J.; Collares-Pereira, M. J.; Araujo, R. (2014). Host specificity and metamorphosis of the glochidium of the freshwater mussel *Unio tumidiformis* (Bivalvia: Unionidae)

Reis, J.; Araujo, R. (2016). Life history of the freshwater mussel *Unio tumidiformis* (Bivalvia: Unionidae) in a temporary Mediterranean-type stream. Invertebrate Biology, 135(1), 31–45. doi:10.1111/ivb.12114

Reynaud, A., and Lanzanova, D. (2017). A global meta-analysis of the value of ecosystem services provided by lakes. Ecological Economics 137: 184–194. https://doi.org/10.1016/j.ecolecon. 2017.03.001.

Ribeiro, A. F. S.; Russo, A.; Gouveia, C. M.; Pires, C. A. L. (2020). Drought-related hot summers: A joint probability analysis in the Iberian Peninsula. https://doi.org/10.1016/j.wace.2020.100279

Russo, A.; Gouveia, C.M.; Dutra, E.; Soares, P.M.M.; Trigo, R.M. (2019). The synergy between drought and extremely hot summers in the Mediterranean. Environ. Res. Lett. 14 (1), 014011 https://doi.org/10.1088/1748-9326/aaf09e.

Sabater, S.; Muñoz, I.; Feio, M. J.; Romaní, A. M.; Graça, M. A. S. (2009). The Iberian rivers (Chapter 4)

Sala, O. E.; Chapin, F. S.; Armesto, J. J.; Berlow, E.; Bloomfield, J.; Dirzo, R.; Hubert-Sannwald, E.; Huenneke, L.; Jackson, R. B.; Kizing, A.; Leemans, R.; Lodge, D. M.;

Mooney, H. A.; Oesterheld, M.; Proff, N. L.; Sykes, M. T.; Walker, B. H.; Walker, M.; Wall, D. H. (2000). Biodiversity scenarios for the year 2100. Science, New York, 287: 1770-1774. doi: 10.1126/science.287.5459.1770.

Scheffers, B. R.; De Meester, L.; Bridge, T. C. L.; Hoffmann, A. A.; Pandolfi, J. M.; Corlett, R. T.; Butchart, S. H. M.; Pearce-Kelly, P.; Kovacs, K. M.; Dudgeon, D.; Pacifici, M.; Rondinini, C.; Foden, W. B.; Martin, T. G.; Mora, C., et al. (2016). The broad footprint of climate change from genes to biomes to people. Science 354, aaf7671.

Schuft M. J.; Moser T. J.; Wigington P. J.; Stevens D. L.; McAllister L. S.; Chapman S. S; Ernst T. L. (1999). Development of landscape metrics for characterizing riparian-stream networks. Photogramm. Eng. Rem. S., 65, 1157–1167.

Şereflişan, H.; Çek, Ş.; Şereflişan, M. (2013). The Reproductive Cycle of *Potomida littoralis* (Cuvier, 1798) (Bivalvia: Unionidae) in Lake Gölbaşi, Turkey.

Silverman, H.; McNeil, J.W.; Dietz, T.H. (1987). Interaction of trace metals Zn, Cd, and Mn, with Ca concretions in the gills of freshwater unionid mussels. Can J Zool 65:828-832

Sime-Ngando, T. (2014). Environmental bacteriophages: viruses of microbes in aquatic ecosystems.

Simeone, D.; Beasley, C. R.; Carmo-Reis, S. (2023). Experimental evidence associates burrowing behavior of *Castalia ambigua* Lamarck 1819 (Bivalvia: Hyriidae) with shell shape and density

Solomon, C. T.; Jones, S. E.; Weidel, B. C.; Buffam, I.; Fork, M. L.; Karlsson, J.; Larsen, S.; Lennon, J.T. et al. (2015). Ecosystem consequences of changing inputs of terrestrial dissolved organic matter to lakes: Current knowledge and future challenges. Ecosystems 18: 376–389.

Sousa, R.; Varandas, S.; Cortes, R.; Teixeira, A.; Lopes-Lima, M.; Machado, J.; Guilhermino, L. (2012). Massive die-offs of freshwater bivalves as resource pulses. Annales de Limnologie - International Journal of Limnology, 48(1), 105–112. doi:10.1051/limn/2012003

Sousa, R.; Amorim, A.; 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). Environmental Management 52, 1230–1238.

Sousa, R.; Novais, A.; Costa, R.; Strayer, D. L. (2014). Invasive bivalves in fresh waters: impacts from individuals to ecosystems and possible control strategies. Hydrobiologia 735, 233–251. https://doi.org/10.1007/s10750-012-1409-1.

Sousa, R.; Amorim, A.; 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.

Steuer, J. J.; T. J. Newton & S. J. Zigler, (2008). Use of complex hydraulic variables to predict the distribution and density of Unionids in a side channel of the Upper Mississippi River. Hydrobiologia 610: 67–82. doi:10.1007/s10750-008-9423-z

Stillman, J. H. (2019). Heat Waves, the New Normal: Summertime Temperature Extremes Will Impact Animals, Ecosystems, and Human Communities. Physiology, 34(2), 86–100. doi:10.1152/physiol.00040.2018

Strayer, D. L., 1999. Use of flow refuges by unionid mussels in rivers. Journal of the North American Benthological Society 18: 468–476. doi:10.2307/1468379

Strayer, D. L.; Caraco, N. F.; Cole, J. J.; Findlay, S.; Pace, M. L. (1999). Transformation of freshwater ecosystems by bivalves. BioScience 49, 19–27. Strayer, D. L., Caraco, N. F., Cole, J. J., Findlay, S. & Pace, M. L. (1999).

Strayer, D. L. (2008). Freshwater mussel ecology: a multifactor approach to distribution and abundance. Freshwater Ecology Series, volume 1. University of California Press, Berkeley and Los Angeles, California.

Strayer, D. L.; Dudgeon, D. (2010). Freshwater biodiversity conservation: recent progress and future challenges. J. North Am. Benthol. Soc. 29:344–58

Szekeres, P.; Eliason, E.; Lapointe, D.; Donaldson, M.; Brownscombe, J; Cooke, S. (2016). On the neglected cold side of climate change and what it means to fish. Climate Research 69, 239–245.

Taeubert, J.-E.; Martinez, A. M. P.; Gum, B.; Geist, J. (2012). The relationship between endangered thick-shelled river mussel (*Unio crassus*) and its host fishes. Biological Conservation, 155(), 94–103. doi:10.1016/j.biocon.2012.06.005

Taeubert, J. E.; El-Nobi, G.; Geist, J. (2014). Effects of water temperature on the larval parasitic stage of the thick-shelled river mussel (*Unio crassus*). Aquatic Conservation: Marine and Freshwater Ecosystems 24, 231–237.

Tomilova, A. A.; Lyubas, A. A.; Kondakov, A. V.; Konopleva, E. S.; Vikhrev, I. V.; Gofarov, M. Y.; Ozcan, T.; Altun, A.; Ozcan, G.; Garlek, M. E.; ÅerefliÅan, H.; Kebapasi, A.; Froufe, E.; Lopes-Lima, M.; Bolotov, I. N. (2020). An endemic freshwater mussel species from the Orontes River basin in Turkey and Syria represents duck mussel's intraspecific lineage: Implications for conservation. Limnologica, (), 125811–.doi:10.1016/j.limno.2020.125811

Treasurer, J. W. & Turnbull, T. (2000). The pathology and seawater performance of farmed Atlantic salmon infected with glochidia of *Margaritifera margaritifera*

The IUCN Red List of Threatened Species

UNEP. World Atlas of Desertification. Edward Arnold, London (1992)

United Nations World Water Development Report (2019).

United States Fish and Wildlife Service (U.S. Fish and Wildlife Service)

Van As, J.G; Du Preez, J. The Story of Life & the Environment: An African Perspective (2012)

Van Dorst, R. M.; Gårdmark, A.; Svanbäck, R.; Beier, U.; Weyhenmeyer, G.A.; Huss. M. (2018). Warmer and browner waters decrease fish biomass production. Global Change Biology. https://doi.org/10.1111/gcb.14551.

Van Ee, B. C.; Nickerson, Z. L.; Atkinson, C. L. (2020). Picky Pigs Prefer Pigtoes: Evidence for Species-Selective Feral Pig Predation on Freshwater Mussels

Vaquer-Sunyer, R., Duarte, C.M. (2011). Temperature effects on oxygen thresholds for hypoxia in marine benthic organisms. Glob. Change Biol. 17 (5), 1788–1797.

Vari, A.; Podschun, S. A.; Erős, T.; Hein, T.; Pataki, B.; Iojă, I. C.; Adamescu, C. M.; Gerhardt, A.; Gruber, T.; Dedić, A.; Ćirić, M. ; Gavrilović, B.; Báldi, A. (2021). Freshwater systems and ecosystem services: Challenges and chances for cross-fertilization of disciplines. Ambio, (), –. doi:10.1007/s13280-021-01556-4

Vaughn, C. C. (2012). Life history traits and abundance can predict local colonisation and extinction rates of freshwater mussels. Freshwater Biology (2012) 57, 982–992. doi:10.1111/j.1365-2427.2012.02759.x

Vaughn, C. C. (2017). Ecosystem services provided by freshwater mussels. Hydrobiologia, (), –. doi:10.1007/s10750-017-3139-x

Vaughn, C. C.; Hoellein, T. J. (2018). Bivalve Impacts in Freshwater and Marine Ecosystems. Annual Review of Ecology, Evolution, and Systematics, 49(1), annurev-ecolsys-110617-062703–. doi:10.1146/annurev-ecolsys-110617-062703

Vide, J. M.; Cantos, J. O. (2001). Climas y Tiempos de Espana/Weather and Climates in Spain (Vol. 43). Alianza Editorial.

Visser, P. M.; Verspagen, J. M. H.; Sandrini, G.; Stal, L. J.; Matthijs, H. C. P.; Paerl, H. W.; Huisman, J. (2016). How rising CO2 and global warming may stimulate harmful cyanobacterial blooms. Harmful Algae 54, 145–159.

Vorosmarty, C. J.; McIntyre, P. B.; Gessner, M. O.; Dudgeon, D.; Prusevich, A.; Green, P.; Glidden, S.; Bunn, S. E.; Sullivan, C. A.; Liermann, C. R; Davies, P. M. (2010). Global threats to human water security and river biodiversity. Nature 467, 555–561.

Waller, D. L.; Bartsch, M. R.; Fredricks, K. T.; Bartsch, L. A.; Schleis, S. M.; Lee, S. H. (2016). Effects of carbon dioxide on juveniles of the freshwater mussel (*Lampsilis siliquoidea* [Unionidae])

Wantzen, K.M.; Ballouche, A.; Longuet, I.; Bao, I.; Bocoum, H.; Cisse´, L.; Chauhan, M.; Girard, P. et al. 2016. River culture: An eco-social approach to mitigate the biological and cultural diversity crisis in riverscapes. Ecohydrology & Hydrobiology 16: 7–18. https://doi.org/10.1016/j.ecohyd.2015.12.003.

Ward, J. E., MacDonald, B. A., Thompson, R. J., Beninger, P. G. (1993). Mechanisms of suspension feeding in bivalves: resolution of current controversies by means of endoscopy. Limnol. Oceanogr. 38, 265 – 272.

Ward, J. E. (1996). Biodynamics of suspension-feeding in adult bivalve molluscs: particle capture, processing and fate. Invertebr. Biol. 115, 218 – 231.

Ward, J. E.; Shumway, S. E. (2004). Separating the grain from the haff: particle selection in suspension- and deposit-feeding bivalves

Welsh, David T. (2003). It's a dirty job but someone has to do it: The role of marine benthic macrofauna in organic matter turnover and nutrient recycling to the water column. Chemistry and Ecology, 19(5), 321–342. doi:10.1080/0275754031000155474

Wilbur, H. M.; Collins, J. P. (1973). Ecological aspects of amphibian metamorphosis. Science, 182(4119), 1305–1314. https://doi.org/10. 1126/science.182.4119.1305

Williams, D. D. (2006). The biology of temporary waters. Oxford: Oxford University Press.

Wilson, C. D., G. Arnott, N. Reid & D. Roberts, 2011. The pitfall with PIT tags: marking freshwater bivalves for translocation induces short-term behavioral costs. Animal Behavior 81: 341–346. https://doi.org/10.1016/j.anbehav.2010.10.003

Yeager, M.; Cherry, D.; Neves, R. (1994). Feeding and burrowing behaviors of juvenile rainbow mussels, *Villosa iris* (Bivalvia: Unionidae). Journal of the North American Benthological Society, 13, 217–222. https://doi.org/10.2307/1467240

Young, M. & Williams, J. (1984). The reproductive biology of the freshwater pearl mussel *Margaritifera margaritifera* in Scotland. I. Field studies. Archiv fur Hydrobiologie 99: 405–422.

Zar, J. H. (1999). Biostatistical analysis. 4th edition. Prentice– Hall, Upper Saddle River, New Jersey.

Zieritz, A.; Aldridge, D. C. (2011). Sexual, Habitat-Constrained and Parasite-Induced Dimorphism in the Shell of a Freshwater Mussel (*Anodonta anatina*, Unionidae)

Zieritz, A.; Geist, J.; Gum, B. (2014). Spatio-temporal distribution patterns of three stream dwelling freshwater mussel species: towards a strategy for representative surveys. Hydrobiologia (2014) 735:123–136. DOI 10.1007/s10750-014-1897-2

Zieritz, A.; Bogan, A. E; Froufe, E.; Klishko, O.; Kondo, T. et al., (2018). Diversity, biogeography, and conservation of freshwater mussels (Bivalvia: Unionida) in east and Southeast Asia. Hydrobiologia 810:29–44. https://doi.org/10.1007/s10750-017-3104-8