GO WITH THE FLOW: MANAGING RIVERS FOR MIGRATORY FISH FACING FRAGMENTATION AND CLIMATE CHANGE

Daniel Chagas Roquette Mameri

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Ph.D José Maria Santos

Ph.D Maria Teresa Ferreira

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THESIS PRESENTED TO OBTAIN THE DOCTOR DEGREE

IN RIVER RESTORATION AND MANAGEMENT

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JURY:

President:

- Doutora Manuela Rodrigues Branco Simões, Professora Associada com Agregação do Instituto Superior de Agronomia da Universidade de Lisboa.

Members:

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- Doutor José Maria Horta e Costa Silva Santos, Professor Auxiliar do Instituto Superior de Agronomia da Universidade de Lisboa.

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Resumo

Os rios albergam cerca de 1100 espécies de peixes migradores a nível global, que enfrentam barreiras ao movimento, sendo estas físicas (barragens e açudes) ou determinadas por condições ambientais desfavoráveis. Esta tese teve como principal objetivo avaliar o efeito de flutuações de caudal e temperatura, causadas pela regularização de caudais, na transponibilidade de barreiras e comportamento natatório de peixes migradores nativos, considerando ainda o impacto que as alterações climáticas poderão ter nos ecossistemas ribeirinhos. A tese é constituída por um capítulo introdutório (Capítulo 1) e quatro trabalhos, incluindo: (1) dois casos de estudo, nos quais é determinada a transponibilidade de barreiras para várias espécies nativas em função de diferentes regimes de caudais e variáveis ambientais (Capítulo 2) e em cenários climáticos distintos (Capítulo 3); e ainda (2) dois ensaios experimentais, em que é avaliado o comportamento natatório dos peixes em resposta a ondas de calor (Capítulo 4) e rápidas flutuações de caudal resultantes da produção de energia hidroelétrica (*hydropeaking*) com descidas abruptas de temperatura – *cold thermopeaking* (Capítulo 5). Os primeiros dois capítulos sugerem que a eficácia das passagens para peixes está fortemente dependente não apenas de um regime de caudais adequado (podendo os caudais ecológicos mínimos ser insuficientes em contexto de seca), mas também de condições ambientais favoráveis, nomeadamente a temperatura dos rios. Nos ensaios realizados, a atividade e ousadia de juvenis de *Luciobarbus bocagei* foram menores após exposição a uma onda de calor; já a deriva de larvas de *Chondrostoma nasus* aumentou com o *cold thermopeaking*. Os resultados obtidos realçam a importância de manter regimes térmicos adequados nos rios, passando pela proteção de habitats ripícolas que atuem como refúgios térmicos para as fases mais vulneráveis dos peixes migradores, e pela monitorização das libertações de caudal nas barragens, por forma a não afetarem significativamente a temperatura dos rios a jusante.

Palavras-chave: peixes migradores; transponibilidade; caudais; temperatura; adequabilidade de habitat

Abstract

Rivers harbour approximately 1100 migratory fish species, facing barriers to free movement, whether they are physical (dams and weirs) or determined by unfavourable habitat conditions. The main goal of this thesis was to evaluate the effect of flow and thermal changes in rivers caused by flow regulation, while also considering the ongoing climate change, to fish passability and swimming behaviour. This thesis is structured in a total of six chapters, with an introduction to the theme (Chapter 1) and four original works, including: (1) two case studies where fish passability through barriers equipped with a fishway was evaluated, in relation to different flows and other environmental variables (Chapter 2) and assuming different discharges under distinct climate change predictions (Chapter 3); and (2) two experimental studies, where swimming behaviour and activity were assessed in response to previous exposure to an experimental heatwave (Chapter 4) and rapid flow fluctuations as a consequence of hydropower generation (hydropeaking), coupled with abrupt thermal variations – cold thermopeaking (Chapter 5). Results from the first two chapters suggest that fishway efficacy is strongly dependant not just on a suitable flow regime (minimum ecological flows may be insufficient under drought scenarios), but also on other favourable habitat conditions such as water temperature, triggering upstream migration. In the experimental designs that were conducted, activity and boldness in juvenile barbel *Luciobarbus bocagei* were lower after exposure to a heatwave under laboratory conditions; for the other experimental study, the drift of nase *Chondrostoma nasus* larvae increased with cold thermopeaking. These results highlight the importance of ensuring suitable thermal regimes in rivers, namely by protecting sites that can act as thermal refugia for the most vulnerable life stages of migratory fish, and by monitoring and adjusting flow releases in dams to ensure that water temperature downstream does not change significantly.

Keywords: migratory fish; passability; flows; temperature; habitat suitability

Resumo alargado

Os ecossistemas de água doce albergam uma biodiversidade considerável, com cerca de 10% das espécies de vertebrados a nível mundial, apesar de cobrirem menos de 3% da superfície do planeta. Dentro dos vertebrados, os peixes de água doce e migradores são o grupo mais representado, estimando-se um total de 17,800 espécies segundo a IUCN, 1100 das quais migradoras, incluindo espécies potamódromas (que migram ao longo do rio) e diádromas (que migram entre o rio e o meio marinho). As comunidades piscícolas reagem fortemente às condições do ambiente que as rodeiam, estando o sucesso destes movimentos migratórios fortemente dependente da existência de condições ambientais favoráveis. Esta tese teve como principal objetivo avaliar o efeito das flutuações de caudal e temperatura da água causadas pela regularização de caudais em barreiras (barragens e açudes), considerando ainda o impacto que as alterações climáticas poderão ter nos ecossistemas ribeirinhos e na adequabilidade de habitats para as espécies piscícolas nativas.

No Capítulo 1, é apresentado o estado da arte, com uma introdução aos ecossistemas ribeirinhos e às espécies de peixes migradores, que dependem de regimes de caudais e regimes térmicos adequados à persistência destas populações, sobretudo tendo em consideração que estas espécies efetuam migrações e alternam entre habitats ao longo do seu ciclo de vida na procura de locais para refúgio, alimentação e reprodução. Foi também apresentada a problemática da regularização de caudais por parte de barreiras (barragens e açudes) que, em conjunto com as alterações climáticas, constituem obstáculos à migração dos peixes. No final do Capítulo 1, é apresentada a estrutura da tese, que inclui dois casos de estudo e dois estudos experimentais, nos quais são avaliados: a transponibilidade de obstáculos (Capítulos 2 e 3), o comportamento natatório (Capítulo 4) e a deriva (Capítulo 5), em função de diferentes regimes de caudais e variações térmicas, provocados pela regulação de caudais em barreiras e/ou alterações climáticas.

No Capítulo 2, foi avaliada a eficácia de passagem de três espécies potamódromas (barbo-comum *Luciobarbus bocagei*, boga-do-Norte *Pseudochondrostoma duriense* e truta-de-rio, *Salmo truta fario*, num ascensor para peixes na barragem do Touvedo, no rio Lima, em função de diferentes variáveis ambientais (incluindo variáveis de caudal e temperatura da água) e modos de funcionamento da respetiva central hidroelétrica (caudais turbinados e caudal ecológico). As monitorizações das passagens no ascensor demonstraram que a temperatura da água foi uma das variáveis ambientais mais fortemente associadas à utilização do ascensor por parte do barbo e da boga, enquanto que o caudal médio diário foi a única variável significativa no modelo de passagem do ascensor pela truta. A boga registou mais passagens no ascensor na ausência de caudais turbinados (havendo libertação de caudal ecológico), enquanto que o barbo e a truta registaram mais passagens com caudais turbinados, com uma turbina (potência de produção de energia hidroelétrica a metade) e duas turbinas (potência máxima) em funcionamento, respetivamente. Este trabalho permitiu comprovar a importância das variáveis ambientais

associadas ao caudal e temperatura dos rios na migração para montante de peixes potamódromos, as quais foram exploradas nos capítulos seguintes.

No Capítulo 3, foi estimado o potencial de transponibilidade do açude do Pego (equipado com uma rampa para peixes), no rio Tejo, por parte de seis espécies migradoras, considerando os caudais médios diários entre 1991 e 2005 (período de referência), e ainda dois regimes de caudais assumindo dois cenários de alterações climáticas, baseados na emissão de gases de efeito de estufa (cenário “moderado” RCP 4.5 e “extremo” RCP 8.5). Para avaliar o potencial de passagem, foram construídas curvas de preferência de habitat (velocidade da água e profundidade), com base na avaliação de peritos, e modelada a adequabilidade de habitat com o modelo hidráulico River2D, para seis espécies no rio Tejo, incluindo: (1) três espécies anádromas (o sável *Alosa alosa*, a savelha *Alosa fallax* e a lampreia-marinha *Petromyzon marinus*); (2) duas espécies catádromas (a enguia-europeia *Anguilla anguilla* e a taínha *Chelon ramada*); e (3) uma espécie potamódroma (o barbo-comum *Luciobarbus bocagei*). O modelo River2D revelou que um caudal mínimo de 3 $m^3 s^{-1}$ é necessário para assegurar a passagem de todas as espécies pelo açude, sendo a passagem assegurada pela rampa para peixes. Espera-se, de acordo com os cenários climáticos RCP 4.5 e RCP 8.5, que, na ocorrência de caudais baixos (Q90, situados no percentil 10), grande parte do açude, incluindo a rampa para peixes, não esteja submersa, impossibilitando a migração destas espécies para montante. Uma das soluções passaria por aumentar os caudais libertados a montante do açude, pela barragem de Belver. No entanto, considerando que o açude já não se encontra a servir o seu propósito inicial (por encerramento e reconversão prevista da Central Termoelétrica do Pego, que utilizava o açude para captação de água para arrefecimento das turbinas), uma solução mais viável passaria pela respetiva remoção.

No Capítulo 4, foram realizados ensaios experimentais para averiguar o efeito das ondas de calor no comportamento natatório de juvenis de barbo-comum. Uma onda de calor pode ser definida, segundo a *World Meteorological Organization*, como um aumento de 5ºC na temperatura do ar durante um período de seis ou mais dias consecutivos, sendo que se prevê que estes fenómenos se venham a tornar cada vez mais frequentes (e com maior magnitude) com as alterações climáticas. Considerando a forte correlação entre a temperatura do ar e da água dos rios, foi simulada uma onda de calor em ambiente laboratorial, com peixes colocados em tanques com água a 30ºC, 5ºC acima da temperatura da água nos grupos de controlo (25ºC). Após o período de exposição, os peixes foram transferidos para os mesocosmos do *campus* do Instituto Superior de Agronomia, e o seu comportamento natatório foi avaliado em ensaios de uma hora à temperatura dos grupos de controlo. Verificou-se que os peixes previamente expostos à onda de calor apresentaram menores índices de atividade e ousadia, realizando menos tentativas para transpor um obstáculo presente nos canais dos mesocosmos, em comparação com os grupos de controlo. Uma vez que os peixes de água doce e migradores nativos poderão estar expostos a condições semelhantes durante o período estival, em pequenas massas de água desconectadas entre si (pegos), com tendência a atingir temperaturas mais elevadas do que os rios em situação de conectividade fluvial, torna-se importante proteger e restaurar a vegetação ribeirinha autóctone, que promova ensombramento e refúgio térmico para estas espécies.

Finalmente, no Capítulo 5, foi avaliado o impacto do *hydropeaking* (rápidas flutuações de caudal a jusante da libertação de caudais turbinados) e das rápidas descidas de temperatura (5,5ºC) associadas (*cold thermopeaking*) na deriva de larvas do cipriniforme *Chondrostoma nasus*. Para o efeito, foram realizados ensaios numa estação experimental ao ar livre localizada em Lunz am See, na Baixa Áustria, equipada com dois canais de 40 metros de comprimento que mimetizam troços de rio. Verificou-se que o número de larvas que sofreram deriva foi superior no tratamento de *cold thermopeaking*, em comparação com o controlo (*hydropeaking*). Este aumento na deriva das larvas esteve também associado à magnitude da descida da temperatura quando a libertação de caudal era mais elevada (quanto mais fria a água, maior a deriva), sugerindo que as larvas de cipriniformes poderão reagir mais fortemente a rápidas alterações de temperatura do que a variações de caudal. Deste modo, é necessário assegurar um regime térmico adequado a jusante das centrais hidroelétricas, por forma a precaver a deriva de fases larvares e salvaguardando a migração para montante destas espécies.

No Capítulo 6, é apresentada uma discussão geral dos resultados e são enunciadas as principais conclusões desta tese, que procurou combinar casos de estudo, à escala do mesohabitat (com um foco na transponibilidade de barreiras e adequabilidade de habitat em troços com barreiras) com ensaios experimentais (avaliando o impacto das ondas de calor e *cold thermopeaking* no comportamento natatório e deriva dos peixes). Ao nível da transponibilidade de barreiras, a implementação de um regime de caudais ecológicos (já prevista e iniciada, no âmbito da Diretiva Quadro de Água) em rios regulados permitiria não só assegurar um caudal mínimo a jusante das barragens, aumentando a área submersa junto dos obstáculos, mas também facilitar a passagem de espécies e fases de desenvolvimento com menor capacidade natatória, nomeadamente larvas e juvenis. Não obstante, o potencial de passagem está dependente não apenas da existência de caudais mínimos (que, em contexto de seca, poderão ser insuficientes), mas também de outras condições ambientais favoráveis à migração dos peixes, nomeadamente variáveis associadas à temperatura da água nos rios. Os resultados obtidos nos ensaios experimentais em resposta a ondas de calor e *cold thermopeaking*, em que o comportamento natatório e a deriva de juvenis e larvas, respetivamente, foram afetados por alterações na temperatura da água, realçam a necessidade de manter regimes térmicos adequados nos rios. Reforça-se, por isso, a importância de proteger, num futuro próximo, habitats ripícolas que atuem como refúgios para as fases mais vulneráveis dos peixes migradores, nomeadamente através da criação de zonas de abrigo junto a barreiras para evitarem caudais elevados, e ainda de refúgios térmicos durante o período estival, evitando o stress térmico causado por fenómenos meteorológicos extremos como as ondas de calor.

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CHAPTER 1: Introduction – the rivers of today and tomorrow

1.1. River flow and fish migration

Rivers have long been recognized as being amongst the most valuable ecosystems worldwide, sustaining biodiversity and providing a wide range of ecosystem services to human populations (Lynch *et al.* 2016; Thieme *et al.* 2021). Together with lakes and reservoirs, rivers are estimated to cover only 2.3% of global land surface area (excluding ice sheets), yet they host almost 10% of the described animal species, including one third of vertebrates (Reid *et al.* 2019). Among vertebrates, fish are estimated to be the most representative group, with around 17,800 species described so far and a high proportion of threatened species (Collen *et al.* 2014; IUCN 2021; Kottelat and Freyhof 2007).

Of the total freshwater fish species that can be found in rivers, 1,100 migrate in some way to complete their life cycles (WFMF 2022). Migration can be defined as a synchronized movement of a substantial proportion of a population between distinct habitats, with a repetitive pattern through time and within or across generations (McIntyre *et al.* 2015). Whether they migrate only in fresh waters (potamodromous) or between the river and sea (diadromous), migratory fish are dependent on suitable environmental conditions to shift between habitats in search for shelter, feeding and breeding sites, or even, at a larger scale, to complete migratory routes (Baras and Lucas 2001; Benitez *et al.* 2016).

Even considering the tremendous diversity of environmental variables that may determine habitat suitability, river flow is consistently regarded as one of the main drivers determining fish habitat and movement patterns throughout its life cycle (Poff *et al.* 1997; Palmer and Ruhi 2019). Fish communities evolve and adapt in direct response to natural changes in river flow, dealing with intra-annual variations and relying on flow variations for migration and spawning. The natural flow regime, a concept first introduced in the late 90s by Poff *et al.* (1997), is composed of five key flow components which interact among them and maintain river ecosystem integrity: magnitude, frequency, duration, timing, and rate of change. The natural flow regime is characterized by strong seasonal patterns, such as the occurrence of high and low flows, which are crucial for most migratory fish, as they synchronize seasonal movements according to these variations in the flow regime (Mittal *et al.* 2015; Poff *et al.* 1997).

Particularly in the Mediterranean region, river flow conditions are usually distinct between the two periods that characterize these regions: the wet season (usually from October to February) and the dry season (from March to September). This seasonal phenomenon is especially pronounced in intermittent rivers, where longitudinal connectivity is partially or totally interrupted during the dry season (Cid *et al.* 2017). Low flows are expected to occur mostly during the dry season, reducing habitat availability (and suitability) for fish species. Instead of freely moving across the river, fish get confined in summer pools acting as refugia during this period of flow intermittency, until river connectivity is re-established at the end of the dry season (Cid *et al.* 2017; Pires *et al.* 2014). Hence, river flow or, more precisely, the components of the natural flow regime (including flow intermittence, low flows, and high flows) play a crucial role in keeping ecosystem integrity for freshwater and migratory fish populations (Poff *et al.* 1997).

1.2. Emerging threats in changing rivers

Despite their recognized value, rivers are also amongst the most endangered ecosystems in the planet (Dudgeon *et al.* 2006; Vörösmarty *et al.* 2010; Reid *et al.* 2019). In Europe, about 60% of inland waters are below Good Status, the goal required by the Water Framework Directive, with negative impacts to freshwater biodiversity (Araújo *et al.* 2022). While water pollution has traditionally been identified as a major cause for habitat degradation, flow modification through dams, but also through smaller barriers such as weirs, has assumed a central role as one of the main stressors leading to habitat loss in rivers in the last decades (Dudgeon *et al.* 2006; Vörösmarty *et al.* 2010; Reid *et al.* 2019). In a recent assessment by Belletti *et al.* (2022), it is shown that more than one million barriers fragment European rivers. This interruption of the free flow of rivers also represents a partial or total loss of longitudinal connectivity, if the ability of fish and other aquatic organisms to overcome these obstacles is compromised (Fullerton *et al.* 2010; Branco *et al.* 2012).

Flow regulation can occur by the presence of the physical barrier itself, but also as a consequence of hydropower (Nilsson *et al.* 2005). In a world of growing energy demand and search for renewable energy sources, hydropower assumes a key role in energy production, representing over 70% of the renewable energy supply as of 2016 (Moran *et al.* 2018). A recent report by Schwarz (2019) reveals that the number of installed hydropower plants in Europe surpasses 20,000 (21,665), with 8,507 more being planned and 278 currently under construction. This development in hydropower energy can be partially explained by the recently launched European Green Deal, which reinforces the need to increase renewable energy sources (EC 2019), despite the numerous studies that have documented the adverse impacts of hydropower production in the ecological condition of rivers, especially in fish populations (Young *et al.* 2011). Specifically, in Portugal, hydropower generates between 40 and 50% of the total electricity consumed (Ferreira and Feio 2019). With a relevant contribution for energy production and consumption, flow regulation by large hydropower plants (LHP) is also included as one of the main negative consequences of dam activity (Mittal *et al.* 2015).

Despite recent attempts to regulate the settlement of new dams, thousands of barriers, mostly for hydropower generation, are already planned or under construction worldwide (**Figure 1**), often lacking consideration for the ecological consequences in rivers and freshwater organisms (Zarfl *et al.* 2015; Winemiller *et al.* 2016). To mitigate such impacts, the construction and improvement of fishways – defined as hydraulic structural solutions to allow fish movements past the barriers, while partially restoring river connectivity (FAO 2002) – has been globally carried out, with a multitude of fishway types and designs considered for different contexts.

One of the most reported effects of dam-induced flow modification is hydropeaking, a process where short-term releases of high quantities of water due to hydropower production leads to a rapid increase of the water level and flow velocity downstream dams (Boavida *et al.* 2015, Schmutz *et al.* 2015). By affecting flow conditions downstream, hydropeaking is regarded as a major threat to aquatic organisms (Hayes *et al.* 2019). Particularly for younger life stages such as larvae and juvenile, which generally present lower swimming performances (Lechner *et al.* 2016), the rapid change of flow conditions downstream dams may substantially reduce habitat suitability, which may unable fish to overcome large barriers, even if a suitable fishway is incorporated. If fish cannot overcome the flow velocities caused by hydropeaking, they may drift downstream, as shown in a study with juvenile salmonid *Thymallus thymallus* by Auer *et al.* (2017). Whether it affects young stages or adult individuals, hydropeaking alters natural flow fluctuations in the flow regime of rivers (Schmutz *et al.* 2015). While fish are well adapted to the seasonal fluctuations of a natural flow regime, they may be unable to cope with artificial fluctuations caused by hydropeaking and flow regulation in general (Boavida *et al.* 2015). This can lead to a decrease or even failure in annual recruitment, affecting migration in the long-term and ultimately compromise population persistence in rivers (Oliveira *et al.* 2017).

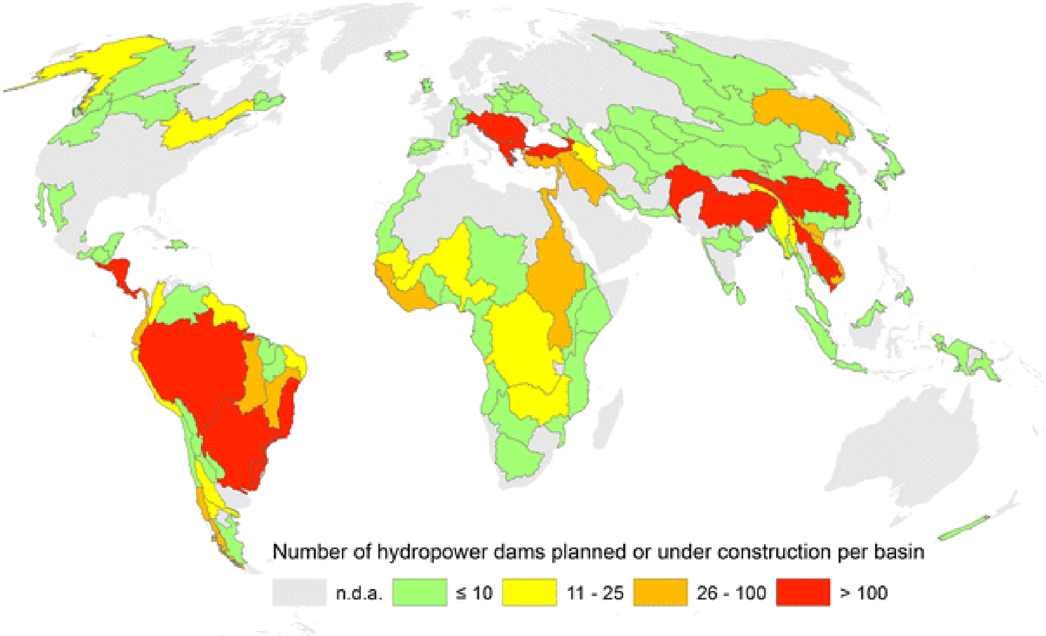
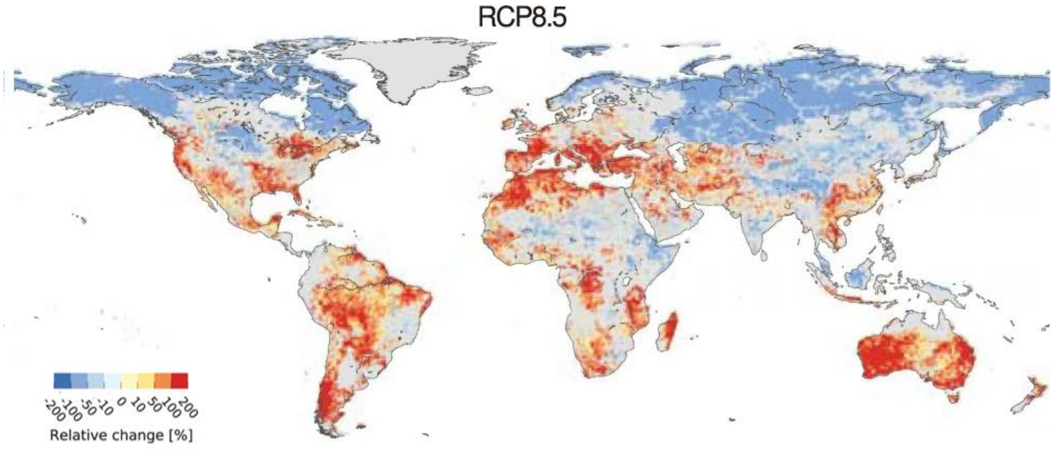


Figure 1. *Global spatial distribution of future hydropower dams, either planned or under construction (note: n.d.a. stands for "no data available"). Retrieved from Zarfl et al. (2015).*

Apart from rapid flow changes downstream hydropower plants, hydropeaking may also induce rapid short-term fluctuations in water temperature, if the water released from the dam is at a different temperature than the one in the downstream reach (Zolezzi *et al.* 2010). This phenomenon can be caused due to water stratification in reservoirs, following a seasonal pattern (Hayes *et al.* 2022). When releases from deeper water layers in stratified reservoirs occur (hypolimnetic discharges), it may lead to a temperature drop in the receiving river and consequently to cold thermopeaking. Contrastingly, epilimnetic discharges with generally warmer surface water can induce warm thermopeaking (Toffolon *et al.* 2010).

In contrast with high discharges resulting from hydropeaking, the release of low flows downstream reservoirs is also an issue for migratory fish, as the wetted area downstream of these barriers may be substantially reduced. Under low flow conditions, fish may be unable to overcome not only dams, but also smaller barriers such as weirs which are, in general, far more numerous than dams and may pose significant threats to successful fish migration (García-Vega *et al.* 2018). Indeed, the permeability of a weir to fish passage (i.e., passability) is not only dependent on its structural properties (such as length, slope, and substrate), but also on the hydraulic conditions (e.g., flow regime). Depending on flow conditions within a given time and area, fish may only surpass these obstacles provided that key hydraulic variables, such as flow velocity and water depth (that structure habitat suitability) are adequate, providing “flow windows” for fish passage (Shaw *et al.* 2016; Santos *et al.* 2018).

Particularly in Mediterranean climate-regions such as the Iberian Peninsula, low flow conditions and river intermittency are expected to be exacerbated with climate change (**Figure 2**), as current predictions for this region indicate a decrease in precipitation in the following decades, coupled with an increase in mean annual temperature and evapotranspiration (Satoh *et al.* 2022). Under these circumstances, river discharge is expected to decrease, increasing both drought frequency and duration and potentiating longitudinal fragmentation (van Vliet *et al.* 2013a; Sellami *et al.* 2016; Lennox *et al.* 2019), reducing the suitable habitat for fish to overcome barriers (Mameri *et al.* 2021).



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Hence, in Mediterranean rivers, the available habitat for riverine fish can be drastically reduced, being limited to isolated (disconnected) pools, generally with poor habitat quality (Cid *et al.* 2017). Understanding fish responses to increasingly drought-stricken rivers is thus of most importance to plan effective conservation and management strategies for freshwater and migratory fish (Lennox *et al.* 2019). In addition to increased flow intermittency and mean temperature increases, extreme weather events such as heatwaves are also expected to become more frequent (IPCC 2022). Although multiple definitions of this phenomena exist, a heatwave can be defined as an increase of at least 5ºC in air temperature for more than five consecutive days, in a specified reference period (WMO 2001). As water temperature is strongly correlated with air temperature (Caissie 2006), heatwaves can disrupt the thermal regime of rivers (Olden and Naiman 2010) and potentially lead to thermal stress in aquatic organisms (van Vliet *et al.* 2013b; Pansch *et al.* 2018). According to the Global Drought Observatory (GDO) of the European Commission's Joint Research Centre, in 2022, many regions in Europe have been facing a severe drought since the beginning of the year, with a worrisome combination of persistent lack of precipitation widely affecting river discharges, plus a sequence of heatwaves, since May 2022 (Toreti *et al.* 2022). Warmer and drier conditions than usual are expected in the Mediterranean region, and particularly in the Iberian Peninsula until November 2022 (Toreti *et al.* 2022).

Given the importance of natural flow and thermal regimes in assuring suitable habitat conditions, when its components are modified through human action, it can have negative effects on fish populations. Hence, the modification of flow and thermal regimes by anthropogenic activities, namely through flow fragmentation and climate change, may have a considerable impact on the suitable habitat for the different fish species, disrupting seasonal movements and threatening critical life stages such as larvae and juveniles, but also breeding adults. The development of suitable strategies to mitigate the impact of these stressors should consider not only seasonal variations in flow and the management of flow conditions downstream barriers, but also predicted reductions in river discharge and changes in thermal conditions in the following decades, following climate change predictions and its impacts on future flow conditions.

1.3. Thesis objectives and structure

River fragmentation, whether it is directly caused by the presence of dams and weirs, or by the flow regulation occurring in these barriers, is expected to increase with climate change, making it essential to assess how flow and thermal conditions near these obstacles can change with climate and alter fish movement and migration within rivers. Hence, the development of successful strategies for the management of migratory fish in rivers would benefit from the assessment of how current stressors, with an impact on key environmental variables such as river flow and water temperature, may affect fish movement at different scales, going from mesohabitat (habitat suitability) to individual assessments (fish behaviour). Furthermore, it should consider how these stressors may interact and change over time and their impact on migratory fish species.

This thesis aims to assess how fish movement can be affected by flow fragmentation and climate change, based on current and future scenarios of modified flow and thermal regimes, in the face of flow regulation (hydropeaking and thermopeaking) and climate change-driven events (heatwaves and water scarcity). Outcomes from this PhD are expected to provide recommendations for the management of fish populations considering the flow and temperature fluctuations occurring in disturbed rivers. This thesis comprises six chapters, organized with the aim of responding to the thesis' specific goals. Chapter 1 provides the essential background for understanding the aims of the thesis and the link between the four studies included in this PhD. Chapters 2 to 5 concern the research carried out to support the discussion and conclusions of this thesis, including two case studies at the mesohabitat scale (Chapters 2 and 3) and two experimental designs where fish swimming behaviour was assessed in response to thermal changes (Chapters 4 and 5).

Specifically, the goals of this thesis are:

(1) to understand the role of current environmental conditions in determining fish passage through barriers, in particular large hydropeaking plants (Chapter 2);
(2) to predict changes in fish passability through a small barrier, based on key environmental drivers, under future climate change scenarios (Chapter 3);
(3) to assess how fish swimming behaviour may be affected by changes in water temperature during a heatwave (Chapter 4);
(4) to evaluate the impact of rapid thermal fluctuations caused by hydropeaking (thermopeaking) in fish movement near barriers (Chapter 5).

Each of these chapters corresponds to a published paper in an ISI Web of Science (WoS) journal, being identical to the published version, with the addition of an abstract in Portuguese, as mentioned in the regulations of Instituto Superior de Agronomia. Finally, Chapter 6 presents a general discussion of the results and the conclusions of this thesis. This final chapter also includes considerations to the current mitigation measures being applied and recommendations for the management of fish populations in rivers in the face of climate change.

Note: *some freshwater fish genus previously belonging to the Cyprinidae family, namely Chondrostoma and Pseudochondrostoma, were reclassified to the Leuciscidae family, following a recent phylognenetic revision by Schönhuth et al. (2018). This reclassification was later recognized in the Portuguese Freshwater and Migratory Fish Guide in 2021 (Collares-Pereira et al. 2021), by the time Chapter 2 (dealing with a former cyprinid species, Pseudochondrostoma duriense) was already published. In Chapter 5, the term cypriniform (concerning the order Cipriniformes) was used to designate the nase Chondrostoma nasus.*

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CHAPTER 2: Passability of Potamodromous Species through a Fish Lift at a Large Hydropower Plant (Touvedo, Portugal)

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CHAPTER 2: Passability of Potamodromous Species through a Fish Lift at a Large Hydropower Plant (Touvedo, Portugal)

Daniel Mameri [1], Rui Rivaes [1], João M. Oliveira [1,2], João Pádua [3], Maria T. Ferreira [1],
José M. Santos [1]

[1] Forest Research Centre (CEF), School of Agriculture, University of Lisbon, Tapada da Ajuda, 1349-017 Lisboa, Portugal; dmameri@isa.ulisboa.pt (D.M.); ruirivaes@isa.ulisboa.pt (R.R.); joliveira@isa.ulisboa.pt (J.M.O.); terferreira@isa.ulisboa.pt (M.T.F.)
[2] Centre for Ecology, Evolution and Environmental Changes (cE3c), Faculty of Sciences, University of Lisbon, 1749-016 Lisboa, Portugal
[3] EDP Labelec – Estudos, Desenvolvimento e Actividades Laboratoriais, S.A., Rua Cidade de Goa, 4, 2685-038 Sacavém, Portugal; Joao.Padua@edp.com

Resumo

As grandes barragens, nomeadamente as que se destinam à produção de energia hidroelétrica, atuam como barreiras ao movimento dos peixes, nomeadamente de espécies potamódromas, que migram ao longo do rio. As passagens para peixes constituem soluções estruturais que, quando bem planeadas, permitem restaurar parcialmente a conectividade longitudinal e mitigar este impacto, sendo que os ascensores para peixes são considerados os mais adequados para grandes barragens em que a altura a vencer é considerável, e nas quais o nível da água a montante e jusante da barragem pode diferir bastante em função dos caudais libertados. Este estudo teve como principal objetivo avaliar a eficácia de um ascensor para peixes incorporado na barragem do Touvedo, no rio Lima, à passagem para montante de três espécies potamódromas nativas: o barbo-comum (*Luciobarbus bocagei*), a boga-do-Norte (*Pseudochondrostoma duriense*) e a truta-de-rio (*Salmo truta fario*). Através de registos de passagem destas espécies com recurso a vídeo-monitorização do ascensor, complementados com amostragens por pesca elétrica a jusante da barragem, verificou-se que quase 80% das passagens ocorreram entre o período de verão e início de outono. A temperatura da água (medida a cada hora) esteve fortemente associada à utilização do ascensor por parte do barbo e da boga, enquanto que para a truta, o caudal médio diário foi a variável mais significativa para a passagem desta espécie. A utilização do ascensor também variou consoante o modo de operação da central hidroelétrica: a boga passou com mais frequência (67,8%) na ausência de caudal turbinado (quando apenas o caudal ecológico estava a ser libertado); o barbo (44,8%) foi mais observado no dispositivo com a barragem a turbinar a metade (50 $m^3 s^{-1}$); já a truta, utilizou mais a passagem (44,2%) durante os caudais turbinados à potência máxima (100 $m^3 s^{-1}$). Este estudo permitiu a avaliação da eficácia do ascensor para peixes do Touvedo, realçando a diferente resposta das espécies aos caudais turbinados e a necessidade de considerar os requisitos específicos de cada espécie no estabelecimento de um regime de caudais adequado, em termos de atratividade da passagem. Os resultados deste trabalho foram discutidos com vista à adoção de estratégias de gestão destas espécies e de estudos futuros.

Palavras-chave: peixes potamódromos, migração, ascensor, energia hidroelétrica, gestão de espécies

2.1. Abstract

River fragmentation by large hydropower plants (LHP) has been recognized as a major threat for potamodromous fish. Fishways have thus been built to partially restore connectivity, with fish lifts representing the most cost-effective type at high head obstacles. This study assessed the effectiveness with which a fish lift in a LHP on the River Lima (Touvedo, Portugal), allows potamodromous fish – Iberian barbel (*Luciobarbus bocagei*), Northern straight-mouth nase (*Pseudochondrostoma duriense*) and brown trout (*Salmo trutta fario*) – to migrate upstream. Most fish (79.5%) used the lift between summer and early-fall. Water temperature was the most significant predictor of both cyprinids' movements, whereas mean daily flow was more important for trout. Movements differed according to peak-flow magnitude: nase (67.8%) made broader use of the lift in the absence of turbined flow, whereas a relevant proportion of barbel (44.8%) and trout (44.2%) passed when the powerhouse was operating at half (50 m^3s^{-1}) and full-load (100 m^3s^{-1}), respectively. Size-selectivity found for barbel and trout could reflect electrofishing bias towards smaller sizes. The comparison of daily abundance patterns in the river with fish lift records allowed the assessment of the lift's efficacy, although biological requirements of target species must be considered. Results are discussed in the context of management strategies, with recommendations for future studies.

Keywords: potamodromous fish; migration; lift; hydropower; species management

2.2. Introduction

Rivers are currently one of the most threatened ecosystems in the world [1,2], with flow regulation and longitudinal fragmentation by dams and weirs being among the main causes of environmental degradation and reduction of available habitat for freshwater fauna [3–5]. Large hydropower plants (LHP) are particularly harmful for fish populations, not only by causing the blockage to their movements, but also by increasing the risk of fish stranding, drifting and dewatering of spawning grounds caused by flow variations, as results of peak-operations in response to energy demands [6–9]. In fact, a myriad of studies have reported significant declines or extinctions of many fish affected by LHP [10–13]. Particularly impacted are potamodromous species, i.e., freshwater species that seasonally undergo upstream migrations along the river, for the purpose of finding suitable habitats for reproduction, which are needed to complete their life-cycles [14–16]. A significant amount of research has therefore been carried out with the goal of restoring longitudinal connectivity in an upstream direction [15,17]. In this context, the development of fishways to transpose barriers stood up as a hydraulic structural solution which facilitates fish movements past the barriers, while partially restoring river connectivity [18–20].

From the different fishway types that have been constructed worldwide to address upstream migration of fish [18,21], fish lifts are the most used and cost-effective at high dams (>15 m; [22]), from the economic and biologic point of view [23,24]. A fish lift consists of a mechanical system which is located at the foot of an obstruction, which attracts (by a guiding flow) the fish into a cage with an inscale (non-return device), raising it and then emptying it in the reservoir upstream, transporting fish over the barrier (for schemes see [18,25]). Although much less studies on fish lifts are available when compared to other fishways, such as pool-type or nature-like facilities [17,26], these structures have nonetheless been monitored in different regions and targeted different fish species [27]. In Europe, fish lift studies have mostly addressed salmonids [23,24,28] and eels [29], but also cyprinids [30,31]. However, most assessments were performed without considering the abundance and size-structure of fish species downstream the dam (often costly due to the human resources and equipment involved) that potentially constitute the migrant population to use the fish lift (but see Discussion below). Such information, in addition to seasonal and daily patterns of fish migration and associated environmental triggers [32], is fundamental to address fish lift selectivity and efficacy, and may be useful to support management decisions.

Studies of the effectiveness of fishways on LHP often focus on high-value economic and recreational species, namely diadromous and salmonids [33,34], whereas studies on potamodromous fish have often been neglected [17,26]. These species, however, are well represented in riverine fish assemblages, particularly in Iberia [9,35,36] and free instream movement is crucial for their survival [37]. Moreover, this is particularly important as potamodromous fish are key components of the lower and middle reaches of temperate rivers [38] and sensitive to river regulation and longitudinal fragmentation [16].

Within the fish community present in the study area, the cyprinids Iberian barbel *Luciobarbus bocagei* (Steindachner, 1864) (hereafter barbel) and Northern straight-mouth nase *Pseudochondrostoma duriense* (Coelho, 1985) (hereafter nase), and the salmonid brown trout *Salmo trutta fario* (Linnaeus, 1758) (hereafter trout) are amongst the most abundant species in northern Iberian rivers [39] and were therefore the focus of this study.

The main goal of this study was therefore to assess the effectiveness with which a fish lift in a large hydropower plant on the River Lima (Portugal), allows potamodromous fish to migrate upstream. For this, we assessed the seasonal and daily use of the lift by the fish population and compared it with (1) the environmental factors that are known to be associated with the triggering of the fish upstream migration; (2) the peak-flow magnitudes at the power plant (0, 50 and 100 m^3s^{-1}, see Study Area); and (3) the size structure (to infer selectivity) and abundance (to calculate a ratio of effectiveness) of the fish population downstream.

We predict that (i) fish counts through the lift would vary between the different months for all three species following patterns outlined in the literature, i.e., cyprinid species movements should mainly occur during the reproductive season, i.e., summer [40,41], and further extending to early fall when species start to search for winter, feeding or thermal refuges [15,42] (with regard to the trout, movements are predicted as well to occur in the reproductive season, in this case, between late fall and early-winter [43]); (ii) daily activity of the studied species would not show marked diurnal/nocturnal preferences due to the absence of natural predators in the River Lima [16,44], which should not restrict movements to take place preferentially during the night, when survival is expected to be maximum [26]; (iii) water temperature and flow, two of the most important environmental factors responsible for triggering migration [35,45], would be the most significant ones for the target species; (iv) large-sized and faster-flowing species would be better able to cope with higher peak-flow magnitudes (50 and 100 m^3s^{-1}) than smaller ones, and hence expected to use the lift during such conditions; and (v) selectivity should be low, although the presence of larger individuals in the lift when compared to the downstream river segment, would be expected to occur as a result of upstream migration of adults to spawning sites [41].

2.3. Material and Methods

2.3.1. Study Area

The River Lima runs for 135 km in the north-west part of Iberian Peninsula, being shared by Spain and Portugal (**Figure 3**). It runs on a NE-SW direction and is characterized by a relatively high run-off, as a result of a mean annual rainfall of about 2000 mm. Geology is mainly granitic and the topography consists of a series of steep and narrow valleys in the upper reaches, contrasting with the lower reaches, with milder gradients and wider valleys dominated by alluvial materials.

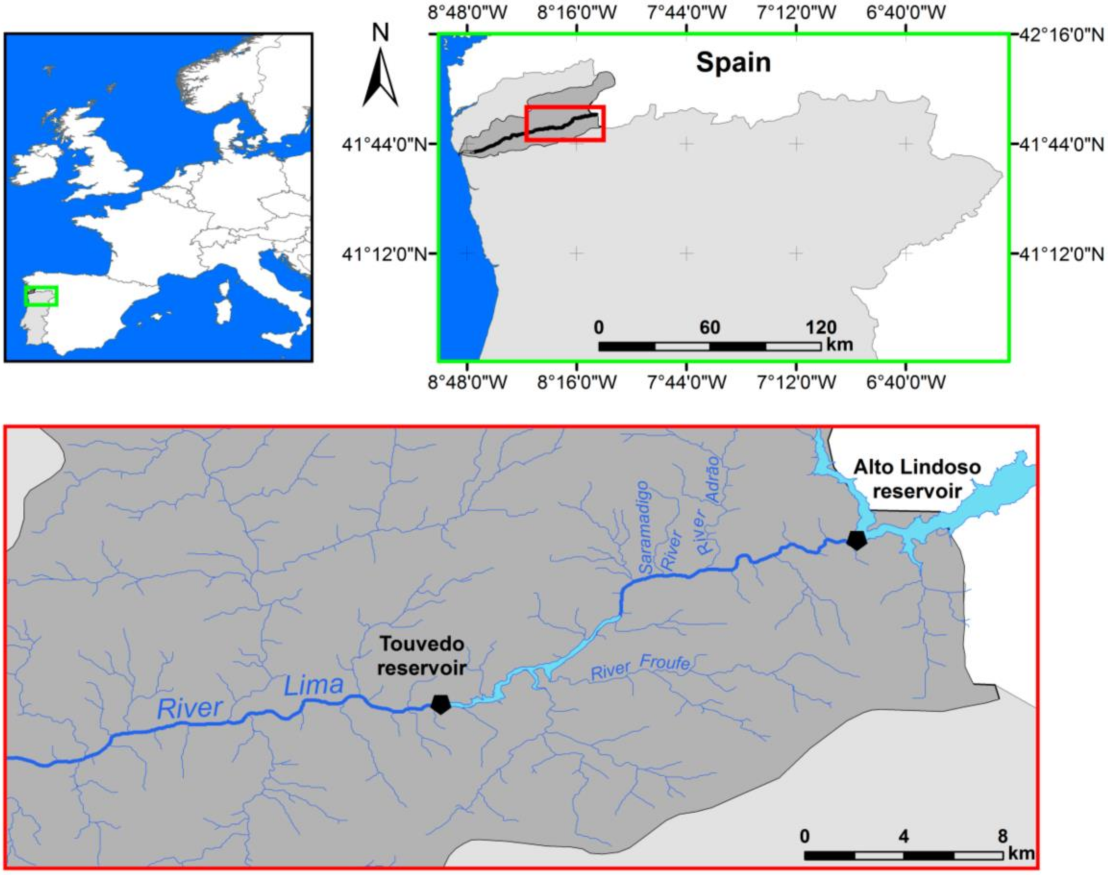

Figure 3. Map of the study area in the River Lima, North Portugal. The black pentagons refer to the dam locations.

The Touvedo LHP stands at 47 km from the river mouth and is the first large instream barrier to upstream fish migration. It is 42 m high and it serves as a tailwater reservoir for the high flows released by the Alto Lindoso Dam, located 16.5 km upstream (250 m^3s^{-1} at full operation), by temporarily storing them, and then returning them to the river, with values not higher than 100 m^3s^{-1}. The dam is equipped with a 22-MW Kaplan turbine and three spillway gates, with a maximum discharge of 3200 m^3s^{-1} when the reservoir reaches the run-off storage limit. Mean number of spillway discharge events is 32/year, which mainly occur (c. 80%) from mid-autumn to early spring [44]. The Touvedo LHP works under three peak-flow magnitudes: (i) 0 m^3s^{-1}, turbine shutdown (i.e., powerhouse off), which is compensated by a 5.5 m^3s^{-1} constant ecological flow (a minimum of 4.0 m^3s^{-1} + 1.5 m^3s^{-1} from the fish lift) to ensure the connectivity of the different habitats and movements of species downstream; (ii) 50 m^3s^{-1}, half-load operation; and (iii) 100 m^3s^{-1}, full-load operation.

A network of spawning, feeding and refuge habitats is available for fish upstream the Touvedo dam. These are mainly located in the Rivers Adrão, Froufe and Saramadigo, which have no man-made obstacles (i.e., all free-flowing) and also no sources of pollution across their watersheds [9].

The dam features a fish lift (2.1 m long, 1.3 m wide and 2.9 m high) which is located on the left bank. It has 3 entrances (two placed above the turbine gates and another one displaced 20 m downstream, to take advantage of the turbined flow) and was initially designed to improve diadromous species movements, such as Atlantic salmon *Salmo salar* and sea trout *Salmo trutta trutta*. A maximum attraction flow of 4.5 m^3s^{-1} is released to promote attraction towards the entrances, which have a mean water velocity varying between 0.21 and 0.55 m s^{-1} (turbine shutdown, powerhouse off) and between 0.68 and 0.91 m s^{-1} (turbine operating, powerhouse on), as previously measured with a SonTek FlowTracker Handheld ADV (model number P4267, Qualitas Instruments Ltd., Madrid, Spain, 2012) at nine points across their width [44]. Inside the attraction circuit, the fish move towards the trapping cage, which is set to raise and empty every 4-h.

2.3.2. Fish Passage through the Lift

To account for seasonal variations in migration patterns, monitoring of fish passage through the lift was made on a monthly basis, from March 2013 until February 2014. Continuous data was acquired through an automatic video-recording system, which included a video camera (Bosch, mod. MR700, Gerlingen, Germany) placed on the top of the fish lift (allowing the collection of trapping cage images during the final period of the cage ascent) and a video recorder (Bosch, mod. LTC455). Target species (barbel, nase and trout) were the most frequent and abundant potamodromous fish species previously recorded in the catchment [30,44]. Following this approach, no fish handling was required, as opposed to other monitoring techniques (e.g., mark-recapture or radio telemetry), thus avoiding causing injury or stress to the fish. The camera was installed on the upper part of the fish lift in order to acquire images of the lift cage during its final period of ascension. The trapping cage was sealed with 20 × 20 cm white quadrats to obtain clearer images for identification and estimates of fish lengths [29]. Collected data included: the timing of fish passage (day and hour), the number of fish per cycle, the identification of each fish to the species level and the estimated total length of individuals (TL, to the nearest cm). For further details on the video-recording system, see [29].

To determine the role of environmental variables on fish movements, six potential predictors were recorded: (1) water temperature, recorded on an hourly basis by a Vemco Minilog-II probe placed in the downstream river segment; (2) mean daily flow, defined as the amount of flow through the turbine, spillway or ecological flow provided by the dam reports on an hourly basis; (3) daily flow fluctuation, i.e., the standard deviation of hourly flow – turbined, spillway or ecological – provided by the dam reports; (4) mean daily rainfall, provided by a nearby weather station (code 03F/01G, managed by the Portuguese Institute for Sea and Atmosphere, I.P.), located 17 km downstream from the Touvedo dam; (5) accumulated rainfall, obtained by combination of the mean daily rainfall that occurred on the three preceding days (as we predicted that fish would move upstream a few days earlier in response to accumulated rainfall; (6) photoperiod, as the time of civil twilight, i.e., the length (in hours) of the daytime period, obtained at http://zenite.nu/ (accessed June 2018); and (7) the proportion of illumination of the moon, based on the ephemeris available at http://www.rodur.ago.net/en/ (accessed June 2018), obtained by dividing the lunar cycle into four phases.

2.3.3. Fish Catches Downstream

To obtain a measure of fish lift efficacy, surveys (n = 9) were performed once every month (unable to sample on March 2013 and January-February 2014, due to adverse weather conditions) in a river segment (total length: 340 m) located immediately downstream the dam, by using a combined wadable and boat electrofishing scheme (DC, 300–700 V, SAREL model WFC7-HV, Electracatch International, Wolverhampton, UK) to obtain the most reliable picture of fish abundance (unit effort = 1 fishing day – 4h of effective sampling – along with ratios of fish-lift records to downstream catches; for further details on the sampling procedure, see [29]). Fish were then identified and measured for TL (nearest cm); native specimens were then returned to the river alive, whereas non-natives were sacrificed in accordance with Portuguese legislation. Fish surveys were not performed in March 2013 and January–February 2014 due to adverse weather conditions (high flow events) that prevented secure access to the river.

2.3.4. Data Analyses

Monthly fish counts recorded in the lift were initially plotted on a line chart to examine seasonal activity and search for migration periods. Next, to search for eventual daily patterns of passage through the lift, two periods were considered: 06:00–18:00 h (day) and 18:00–06:00 h (night) [41]. For both data, the chi-square test of goodness of fit was conducted to account for differences in the relative abundance of fish passing through the lift in each month and between day and night periods, respectively.

The relative influence of environmental variables on the fish counts in the lift was also evaluated through generalized linear models (GLM) following a Poisson distribution. For this purpose, a forward stepwise approach was conducted, based on the Akaike Information Criterion (AIC) for each fitted model, selecting only the variables leading to the most adequate model (i.e., lowest AIC). In each model, variable significance was set at $\alpha = 0.05$. To improve data distribution, we applied a log (x + 1) transformation to all environmental variables before fitting them into the GLM, with the exception of the proportion of illumination of the moon, which was arcsin-transformed. Durbin–Watson statistics for each model were also calculated to detect possible autocorrelation between residuals (values ranging from 1 to 2 are considered to be acceptable). To search for significant differences in species movements according to the different peak-flow magnitudes (0 m^3s^{-1}, powerhouse off; 50 m^3s^{-1}, powerhouse at half-load; 100 m^3s^{-1}, powerhouse at full-load), the chi-square test of goodness of fit was employed. Size selectiveness in the fish lift was assessed by comparing the population size structure of each species recorded in the lift with the one obtained downstream the dam, using Fisher's exact test. Size-classes were partitioned in 5-cm intervals, to allow a more detailed effect of selectivity [29].

Literature has outlined the absence of a standardized procedure to evaluate fish passage efficacy [17,46], a qualitative concept consisting of checking if the fishway is capable of allowing the target species to pass. This concept differs from efficiency, which focuses on its quantitative performance, defined as the percentage of marked fish that enter and successfully negotiate the fishway out of the total fish previously marked [47]. A ratio of fish lift efficacy was therefore calculated by dividing the number of fish observed ascending the lift (number day^{-1}) by the total number of fish captured below the dam (unit effort = number in 1 fishing day), being considered as a proxy of the lift's efficacy [48].

All analyses were conducted in R version 3.5.2, [49], using the packages *stats* (implemented in R) and *MASS* [50].

2.4. Results

2.4.1. Seasonal Fish Counts in the Lift

Fish counts through the lift varied significantly between the different months for all three species (barbel: $\chi^2 = 57.828$, df = 11, $p < 0.001$; nase: $\chi^2 = 232.440$, df = 11, $p < 0.001$; trout: $\chi^2 = 66.315$, df = 11, $p < 0.001$) (**Figure 4**). A total of 548 barbel, 1801 nase and 63 trout were recorded passing the fish lift within the study period, with most of the fish being observed between summer and early fall (79.5%), i.e., August and October. Among the three species, nase was the most abundant (74.7% of the total fish counts), with the highest counts being recorded in August (699) and October (491). Barbel (22.8%) was more abundant in October (125), while trout (2.6%), the least abundant species, peaked a maximum of 13 individuals in both April and October.

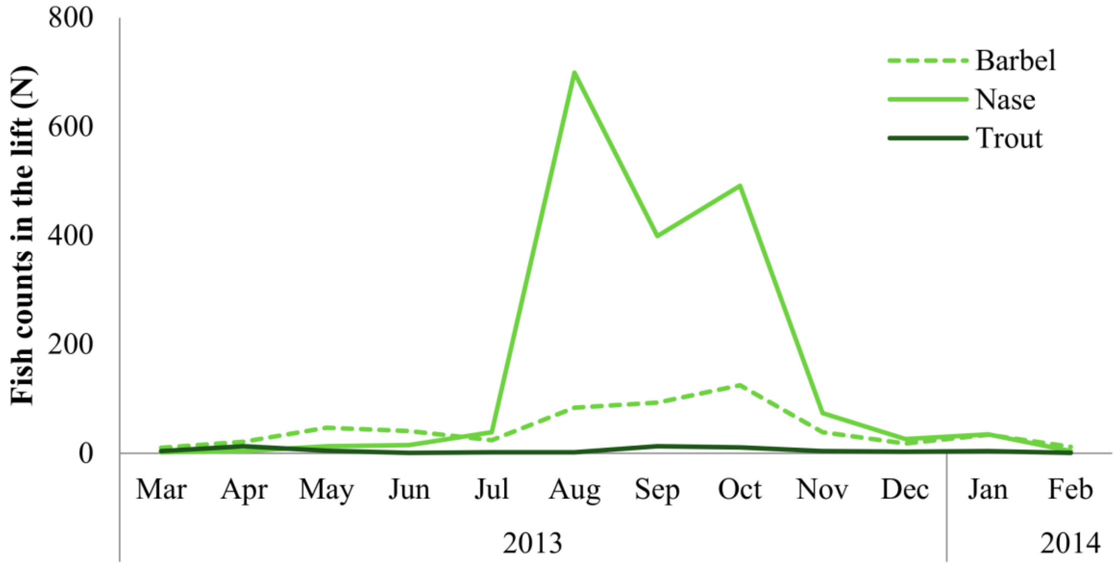

Figure 4. *Fish counts for barbel (dotted green line), nase (green line) and trout (dark green line) in the fish lift, between March 2013 and February 2014.*

2.4.2. Daily Patterns of Fish Passage

Fish recordings in the lift did not vary significantly with the time of day for barbel (χ^2 = 0.006, df = 1, p = 0.936), nase (χ^2 = 0.810, df = 1, p = 0.368); and trout (χ^2 = 3.028, df = 1, p = 0.082). Among the three species, the highest difference in percentage of fish passing between two periods belonged to trout (58.7% of the fish counts recorded during the night period) (**Figure 5**).

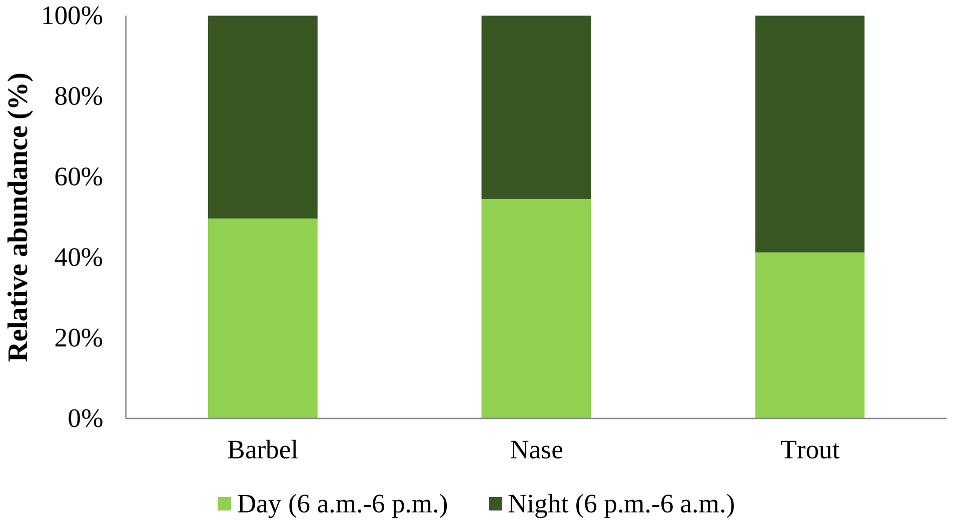

Figure 5. *Abundance of barbel, nase and trout recorded in the fish lift during the day (06:00–18:00) and night (18:00–06:00).*

2.4.3. Effects of Environmental Variables on Fish Passability

Water temperature (F = 22.425, p < 0.001) and daily flow fluctuation (F = 4.894, p < 0.001) were positively associated with nase passability. The fitted model for this species included a third significant variable positively associated – photoperiod (F = 3.955, p = 0.049) (**Table 1**). Water temperature was also included in the model for barbel passability (F = 7.138, p = 0.008), together with mean daily flow (F = 7.733, p = 0.006) and accumulated rainfall (F = 12.818, p < 0.001) as significant variables, all with positive associations (Table 1). For trout, only the mean daily flow (positively associated) was retained in the final model (F = 3.941, p = 0.049).

Table 1. *Variables entered in the GLMs explaining species abundance in the Touvedo fish lift. Seven different factors were analyzed, but only those included in the final models are presented. For each species, significance of each variable in the final model was calculated through the F-test. Beta coefficients (ß) and Durbin–Watson statistics (D) for each model are also presented.*

Variable	**ß**	***F*-test**	**p-value**	***D***
P. duriense				1.127
Water temperature	0.466	22.425	<0.001	
Flow variation	0.228	4.894	0.029	
Photoperiod	0.198	3.955	0.049	
L. bocagei				1.812
Water temperature	0.167	7.138	0.008	
Mean daily flow	0.155	7.733	0.006	
Acumulated rainfall	0.276	12.818	<0.001	
S. trutta fario				1.996
Mean daily flow	0.151	3.941	0.049	

2.4.4. Fish Passage in Relation to Peak-Flow Magnitudes

Fish lift use varied according to peak-flow magnitudes, with nase showing significant differences in passability (χ^2 = 55.460, df = 2, p < 0.001). Accordingly, passability of this species was the highest (67.8%) when the powerhouse was off (0 m^3s^{-1}, **Figure 6**). Contrastingly, passability of larger species, i.e., barbel (χ^2 = 6.480, df = 2, p = 0.039) and trout (χ^2 = 5.631, df = 2, p = 0.060) occurred mainly when the powerhouse was operating, being the highest for the barbel (44.8%) upon operation at half-load (50 m^3s^{-1}), whereas for the trout, the largest portion of individuals (44.2%) migrating occurred when the powerhouse was operating at full-load, though differences in the last species were not significant (100 m^3s^{-1}; χ^2 = 5.631, df = 2, p = 0.060).

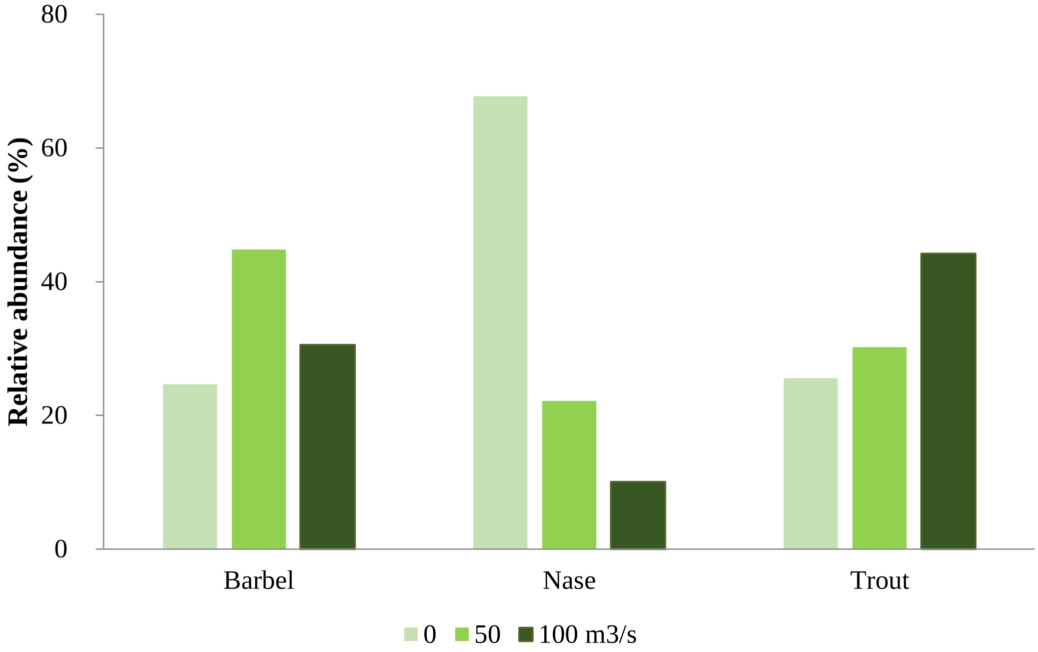

Figure 6. *Relative abundance (%) of barbel, nase and trout recorded in the lift for different peak-flow magnitudes: 0 m^3s^{-1} (pale green; powerhouse off), 50 m^3s^{-1} (light green; powerhouse at half-load) and 100 m^3s^{-1} (dark green; powerhouse at full-load).*

2.4.5. Fish Lift Selectivity

Differences in population size structure were found when comparing the proportions of fish counts in the lift and captures downstream of the Touvedo dam for all three species (Fisher's exact test, $p < 0.05$), though these differences were more pronounced in barbel and trout (Fisher's exact test, $p < 0.001$), with some selectiveness being observed (**Figure 7**). For both species, the proportions of individuals observed in the lift (barbel: mean ± SD = 22.4 ± 6.9 cm; trout: 23.5 ± 4.2 cm) were generally larger than the ones captured in the river segment downstream (barbel: 18.1 ± 4.5; trout: 16.5 ± 4.5 cm). Nonetheless, for barbel, both lift recordings and river surveys revealed 15–20 cm individuals as the most abundant size class (Figure 5). For nase, despite differences in size-class distributions were found (Fisher's exact test, $p = 0.016$; mean size ± SD = 13.0 ± 3.6 in the lift and 13.3 ± 2.9 in caught fish), the same size classes were represented in the lift and river surveys, but with a larger proportion of the smallest size individuals (TL ≤ 10 cm) occurring in the fish lift: 24.3% (10.1% in the river downstream) (**Figure 7**).

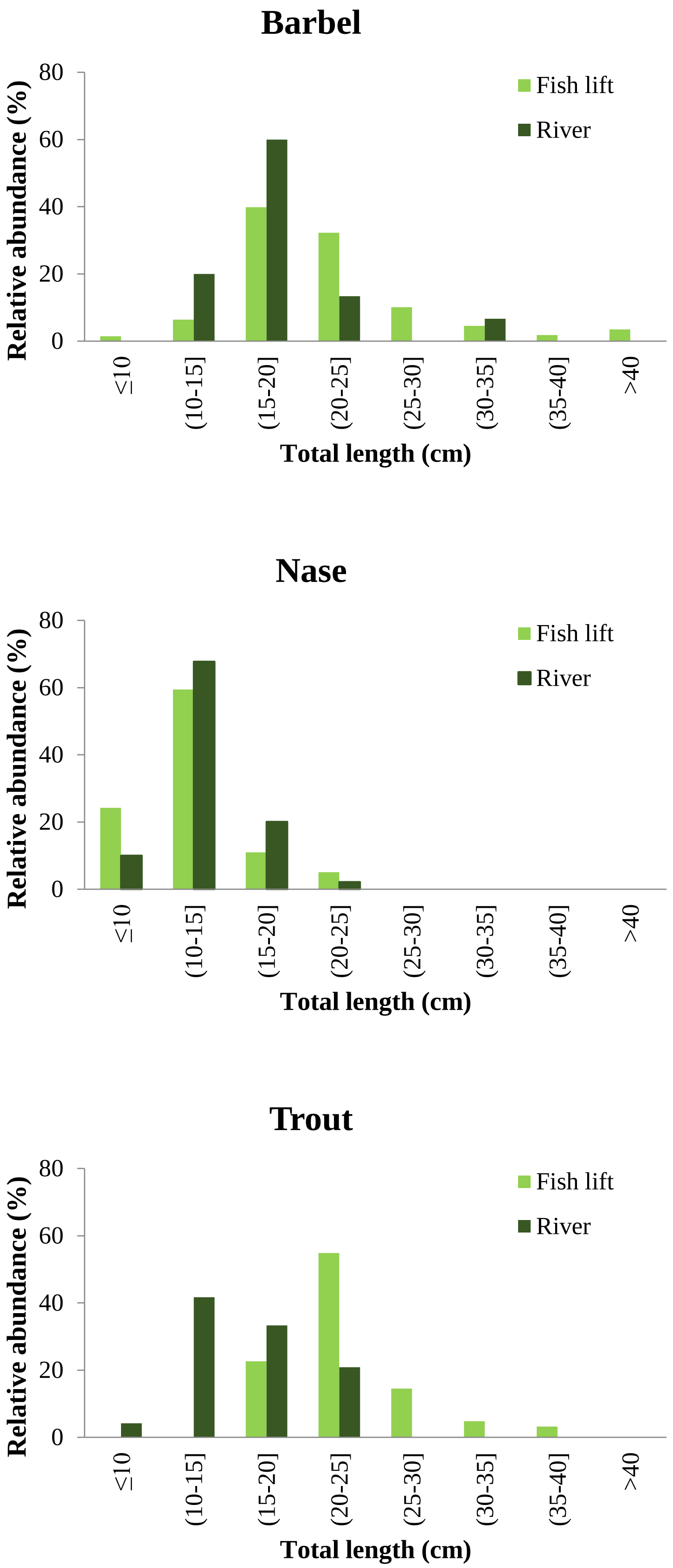

Figure 7. *Size class (cm) distributions of barbel, nase and trout recorded in the lift (light green) and captured downstream of the Touvedo dam (dark green).*

2.4.6. Fish Lift Efficacy

The passage-to-catch ratio was generally higher in barbel (mean ± SD = 1.36 ± 1.06) than in nase (mean ± SD = 0.26 ± 0.25) and trout (mean ± SD = 0.10 ± 0.10). Average monthly ratios for the barbel were superior to the unit in half of the study period, attaining a maximum value of 3.10 in September (**Table 2.2**), when this species recorded higher counts in the lift (**Figure 2.2**). Ratios for nase in its most active period (August-October; Figure 2) varied between 0.43 and 0.75, being considerably higher than the ratios obtained in the remaining months (≤0.10; **Table 2.2**). For trout, ratios were generally low throughout the year, despite an increase was observed in September–October, when mean values were superior to the overall mean obtained for this species (0.10).

Table 2. *Mean daily number of barbel, nase and trout migrating through the Touvedo fish lift and captured downstream by electrofishing in 2013 (unit effort = 1 fishing day (4h of effective sampling)), along with ratios of fish-lift records to downstream catches. (a) Undetermined ratio due to the absence of caught individuals by electrofishing, despite being observed in the fish lift.*

	Barbel			Nase			Trout		
Month	Fish lift N day^{-1}	Electr. catch N unit effort^{-1}	Ratio	Fish lift N day^{-1}	Electr. catch N unit effort^{-1}	Ratio	Fish lift N day^{-1}	Electr. catch N unit effort^{-1}	Ratio
Apr	0.70	0	a)	0.13	5	0.03	0.43	5	0.09
May	1.52	0	a)	0.42	1	0.42	0.16	0	a)
Jun	1.36	2	0.68	0.50	18	0.03	0.03	1	0.03
Jul	0.77	0	a)	1.26	13	0.10	0.06	1	0.06
Aug	2.80	0	a)	23.30	30	0.75	0.07	2	0.03
Sep	3.21	1	3.10	13.76	31	0.43	0.45	3	0.14
Oct	4.03	12	0.34	4.03	34	0.47	0.35	1	0.35
Nov	1.34	1	1.30	1.34	28	0.09	0.14	2	0.07
Dec	0.62	0	a)	0.62	29	0.03	0.10	9	0.01
Mean	1.82	1.78	1.36	5.04	21.00	0.26	0.20	2.67	0.10

2.5. Discussion

This study assessed the effectiveness with which a fish lift in a LHP allows native potamodromous fish to migrate upstream. To accomplish such goal, we assessed the seasonal and daily use of the lift by fish and compared it with the environmental factors that are known to be associated with the triggering of the fish upstream migration, the peak-flow magnitudes at the power plant and the size structure and abundance of the fish population downstream. This continuous monitoring of fish stocks downstream the dam, though time-consuming and enclosing inherent technical difficulties of sampling a large river [51], has seldom been used in fish lift evaluation studies (e.g., [23,30]) and provided a useful proxy of the efficacy of the fish lift that, together with the seasonality of fish movements, can be used by managers to better plan fish lift operations and shutdowns.

As it was expected, fish counts through the lift varied significantly between the different months for all three species. Overall, the highest number of fish counts was attained in late summer–early fall, when almost 80% of the individuals used the fish lift, with nase being the most abundant species (74.7% of the total fish counts), followed by barbel (22.7%). The large proportions of both species observed in late summer is consistent with previous reports on these rheophilic cyprinids [52,53]. Similarly, in a work by De Leeuw and Winter [53] in the lowland rivers Meuse and Rhine in the Netherlands, the authors reported more movements of the common barbel *Barbus barbus* and common nase *Chondrostoma nasus* during both summer-early fall (July–October) and spring (spawning season for both species). It is highly likely that such activity observed in the lift is related to reproductive migrations, which takes place during these periods, particularly in summer [40,41], when these potamodromous species migrate upstream to seek areas for reproduction, typically in gravel and pebble beds located in upstream tributaries [39]. These species also showed movements outside their usual spawning period, displaying a second peak in early-fall (September–October), a result that is also consistent with other studies, in which "out of season" movements may reflect a search for winter, feeding or thermal refuges, as it has been observed in other potamodromous cyprinids [15,54]. The presence of trout in the fish lift was residual (only 2.6% of the total fish counts), with movements occurring throughout the year and not only restricted to the spawning period, which typically occurs during late fall and winter [43]. The similarity of observed seasonal fish counts in the lift, with the species migratory ecology, provides therefore an indication that the lift is not disrupting the seasonality of fish movements, serving therefore its purpose.

No significant differences were found in daily patterns of fish passage ascending the fish lift, which is in accordance with our expectations. Though some studies suggest that cyprinids are more active during the night to avoid predation (e.g., [37,41]), such patterns can be quite species-specific [16]. In a recent study conducted in the Meuse river basin (Belgium), Benitez *et al.* [16] found that the common barbel *Barbus barbus* did not show any differences in daily activity when passing through the existing fishways, contrarily to the trout, which was more active during the day, a result that was also supported by some authors (e.g., [43]), but not others (e.g., [42]). In a series of surveys conducted in the Zêzere River (Tagus river basin), Santos *et al.* [52] also did not find differences in daily activity for the Iberian straight-mouth nase (*Pseudochondrosoma polylepis*), a sister species of the present *P. duriense*. Such findings, as well those of the present study, reflect the absence of predators in the sampled river segment downstream of the Touvedo dam [44], which does not constrain the activity of native species to take place during night-time periods when survival would be expected to be maximum [26,41].

Water temperature was found to be the most significant predictor of the abundance of both cyprinids (barbel and nase), which is consistent with our expectations and with previous findings on the migratory ecology of these species, where increasing water temperature acts as an environmental trigger for the upstream movements of these species [30,55]. Flow variables (mean daily flow and daily flow fluctuations) were also important to explain upstream movements of both cyprinids, as it was previously expected: daily flow fluctuation, which can act as an environmental trigger for fish migration [45,56], also had a positive effect on the observed nase in the lift, whereas barbel abundance in the fish lift was positively associated with increasing mean daily flow. Moreover, accumulated rainfall, which also has a direct influence in river flow [57], was also present in the model for barbel passability. Taken together, these results corroborate the ones in the literature in which flow (in addition to water temperature) is also one of the most important variables in triggering fish migration [45,58], particularly under conditions of high-water level fluctuation [59] as in the Touvedo LHP. Photoperiod was also positively associated with barbel passability, which seems to indicate a higher activity during the day in the lack of predators [60], as previously mentioned. Mean daily flow was the only variable selected by the model to explain the number of trout individuals that migrated through the fish lift. This result is in agreement with some studies [61,62], but not with others [30,41] that pointed out water temperature as the most important factor in the upstream migration of this species. It should be noted, however, that the number of individuals recorded was considerably lower compared to the remaining potamodromous species, which may have reduced the statistical power of our analyses. On the other hand, it is possible that a different hierarchy of environmental factors stimulated the same behaviour in different years [45]. Hence, long-term studies could provide a broader understanding of the interaction between environmental variability and potamodromous fish movements, in order to clarify trends over long time series (>10 years), while also providing important data for scientists and ecosystem managers.

Fish passage in relation to the different peak-flow magnitudes differed in two of the three species, with the largest proportion of nase (67.8% of total abundance) using the fish lift in the absence of turbined flow (powerhouse off). Contrarily, barbel made broader use of the fish lift when the powerhouse was operating at half-load (50 m^3s^{-1}). As for trout, it should be noted that while differences were not significant, the largest proportion of movements occurred when the powerhouse was operating at full-load (100 m^3s^{-1}). It is our conviction that nase made larger use of the fish lift when the powerhouse was off (i.e., with turbines shutdown) due to the lower water velocities (0.21–0.55 m s^{-1}) that occur at the lift entrances upon this scenario [44]. Though nase is a medium-sized cyprinid [63], for which adults can cope critical swimming speeds up to 0.78 m s^{-1} [64] and therefore theoretically being able to negotiate such a range of velocities, individuals found in both river segment downstream and lift were mainly juveniles, small-sized fish (mean: 13.0 cm TL), for which swimming performance is typically lower than larger conspecifics [65,66]. It is thus important to ensure that water velocities that nase will face within the entrances that lead to the lift are sufficiently attractive—not too low (<0.20 m s^{-1}) to hinder attraction [29], nor too high, above their critical swimming speed (>0.78 m s^{-1}, [64])—for appropriate entrance and passage, particularly during summer and early fall when most of the individuals (74.7%) used the fish lift.

It is tempting to suggest that managers should try to implement management strategies, such as periodic turbine shutdown [67], that best balance the trade-off between energy production and the potential for upstream fish migration [68], at least during the critical migratory periods. However, this is often difficult to achieve and dependent on the characteristics of the national network of hydropower schemes as well as on the specificities of the energy market.

Nevertheless, the specific requirements of the different species migrating, namely swimming performance, should be taken into account when planning for a mitigation flow scheme such as ecological flow releases. Both barbel (mean TL: 22.4 cm) and trout (mean TL: 23.5 cm), the two largest species that used the lift, have rheophilic habits during part (barbel) or the whole (trout) life-cycle [36], which, combined with their greater ability to withstand higher velocities during short time periods (adult barbel: $U_{crit} = 0.81$ m s^{-1}, [69]; trout: $U_{crit} = 0.65$ m s^{-1} and U_{max} varying between 0.94–1.26 m s^{-1}, depending on water temperature, [70]), may have determined the larger proportion of individuals of both species using the fish lift under the half- load (50 m^{3}s^{-1}) and full-load (100 m^{3}s^{-1}) conditions, respectively.

It should be noted, however, that the willingness to enter and use the fish lift cannot be explained solely by the water velocity at the entrances nor the size of individuals. Such motivation can also be driven by other internal (such as the physiological condition or fatigue level) or external (such as turbidity or turbulence) factors not accounted for in the present study. For example, some recent studies on fish passage have pointed out the importance of turbulence in determining the success and timing of potamodromous fish migration upstream [71,72]. It is clear that future studies should focus on experimental controlled conditions, where the variables of interest (e.g., water velocity and associated turbulence parameters) can be manipulated while controlling for potential confounding factors (e.g., temperature), which provide an excellent opportunity disentangle the effect that multiple factors have on fish attraction and passage through fish lifts.

The comparison of species size-structure between observed fish in the lift and those captured downstream, which gives an indicator of fishway selectivity, showed some differences for all three species, particularly for barbel and trout, with the occurrence of larger individuals in the fish lift relatively to the river downstream, as it was previously predicted (see Introduction). Such selectivity could have also arisen as a result of sampling the fish with electrofishing in specific habitats, such as deep pools, where typically the larger fish, like barbel and trout [44], dwell, and where capture efficiency is often lower than in shallower (up to 1.5 m) habitats [9,63]. On the other hand, the smaller individuals of these species may not display a marked migration stimulus, at least associated with reproduction (e.g., [15]), so their abundance in the fish lift should be lower than that of the larger ones. Another relevant aspect that could partially explain the lower abundance of smaller-sized individuals of these species is related to the potential effect of water velocity in the fish lift attraction circuit (up to 0.90 m s^{-1} when the powerhouse is on), which may have limited the entrance of smaller individuals, for which swimming capacity is typically more limited comparatively to the larger ones [66]. Assessing their swimming capabilities would help clarify if the observed patterns are related with their lower swimming capabilities, or the lack of environmental cues for these smaller fish to perform upstream migrations.

As a proxy of the fish lift’s efficacy, the standardized passage-to catch ratio was used, as there are presently, to the best of our knowledge, no standard methods nor metrics to evaluate efficacy, neither any defined thresholds (e.g., [46,73]). Our results showed that the mean value of this indicator was higher than 1 for the barbel, suggesting that more individuals were using the fish lift compared to those that were available downstream and captured by electrofishing. As outlined above, such results should be analyzed with caution as most barbel, particularly the larger individuals, often dwell in deep pool habitats [9] where electrofishing is clearly less effective [74], and thus their population downstream that is potentially available to migrate could have been under–evaluated. The use of other techniques, such as mark-recapture or passive integrated transponders (PIT) telemetry [75], can be useful to provide more accurate data on barbel stocks arriving at the foot of large–scale barriers. The mean ratio obtained for the nase (0.26), the most abundant species in the fish lift, was higher than those reported by Noonan *et al.* [26], who reviewed worldwide estimates of fish passage efficiency across all types of fishways. In the case of fish lifts and the presence of non–salmonid species, the mean value reported was only 0.10, which makes the present estimate (0.26) quite optimistic in the current context, although their work focuses on efficiency rather than efficacy as in the present study. However, since the concept of efficacy is not defined in terms of minimum standards (e.g., [41,46]), it should be specified with respect to the biological requirements of the species using the fishway, and not as an absolute value [47].

In the Lima basin, cyprinids are the most dominant and abundant species in the main river [9,41]. Consequently, the main goal of the lift, rather than allowing the whole species' population downstream to move upstream the dam (as it would in the case of anadromous or catadromous species), is to prevent fragmentation of potamodromous populations between different river segments [25]. For such species that carry out their life cycles downstream and upstream the dam [30], simple documentation of them passing upstream is sufficient [76], providing enough evidence that a considerable proportion of individuals used the fish lift, assuring a long-term sustainability of fish populations.

It should also be noted that the low ratios observed in the months outside the migratory season (for barbel, nase and trout) do not necessarily represent low lift efficacy, as they may reflect the absence of migratory stimuli and the consequent lack of motivation to overcome the obstacle. Trout was the species that theoretically performed the lowest, as shown by their lowest mean ratio (0.10) when compared to the other two species, as well to the corresponding mean value (0.35) in the literature for salmonids using fish lifts [26]. This is unlikely to reflect a lower performance of trout upon negotiating water velocities to enter the lift, as trouts are typically better swimmers and withstand higher velocities than cyprinids, but instead their natural low abundance in the present cyprinid-type river segment [77]. Future studies should try to associate efficacy to other indicators, namely efficiency and delay, to achieve a broader assessment of fish passage through a fishway [78]. In the particular case of fish lifts, it would be important to try to quantify the two components of attraction efficiency [17]: guidance (i.e., arrival at the entrance) in response to attraction currents, and entry (i.e., decision to enter). In this sense, biotelemetry techniques could be applied to monitor such fine-scale activity.

Finally, it should be pointed out that actions to improve the efficacy of upstream movements of potamodromous species at fish lifts may not always be the best practice. Fish lifts are unidirectional systems, transporting fish from downstream to upstream of dams, but do not operate on the reverse side (i.e., from upstream to downstream), therefore not allowing subsequent downstream migration. When this is coupled with the absence of suitable spawning and growth habitats upstream (even if they are present downstream), fish lifts may act as ecological traps, doing more harm than good to fish populations [79]. Although a unidirectional fishway, the Touvedo fish lift is not likely to be acting as an ecological trap, due to the existence of a network of good quality habitats upstream the Touvedo dam (see Study Area). Taken together, the Touvedo fish lift enables the upstream migration of a “considerable” number of adult potamodromous fish in the proper seasonal timing, which is a positive step towards the maintenance of populations above and below the dam, potentially contributing to their future sustainability. However, different fish species were found to be affected differently by the peak-flow magnitudes (nase preferentially migrating during periods of turbine shutdown, whereas barbel and trout making broader use of the lift when the powerhouse was operating), which points to the need of a proper peak-flow management during the species reproductive season. Future studies should consider determining to what extent fish can safely use the spillway gates or the turbines as a pathway in their descendent routes.

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CHAPTER 3: Climate Change Effects on Fish Passability across a Rock Weir in a Mediterranean River

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CHAPTER 3: Climate Change Effects on Fish Passability across a Rock Weir in a Mediterranean River

Daniel Mameri [1], Rui Rivaes [1] , Maria Teresa Ferreira [1], Stefan Schmutz [2], José Maria Santos [1]

[1] Forest Research Centre (CEF), School of Agriculture, University of Lisbon, Tapada de Ajuda, 1349-017 Lisboa, Portugal; ruirivaes@isa.ulisboa.pt (R.R.); terferreira@isa.ulisboa.pt (M.T.F.); jmsantos@isa.ulisboa.pt (J.M.S.)
[2] Department of Water, Atmosphere and Environment, Institute of Hydrobiology and Aquatic Ecosystem Management, University of Natural Resources and Life Sciences, Vienna, Gregor-Mendel-Straße 33, 1180 Wien, Austria; stefan.schmutz@boku.ac.at

Resumo

As alterações climáticas e as expectáveis reduções de caudal nos rios, em particular no Mediterrâneo, representam um grande desafio para a gestão de populações de peixes nativos, que pode condicionar ainda mais a passagem de peixes por pequenas barreiras como açudes. Com recurso a modelos hidráulicos de adequabilidade de habitat (River2D), foi investigado o impacto que estas reduções de caudal, regulado a montante pela barragem de Belver, poderão ter no potencial de passagem de espécies migradoras nativas pelo açude do Pego, localizado no rio Tejo junto a uma antiga central termoelétrica, e equipado com uma rampa para peixes na margem direita. Foram tomados como referência valores de caudais retirados de estações hidrométricas, no período de 1991 a 2005, sobre os quais se aplicaram reduções de 30% e 60% de caudal, assumindo cenários de emissões moderadas (RCP 4.5) e extremas (RCP 8.5) de gases de efeito de estufa até ao final do século (2070–2100), com base nas previsões para a bacia do Tejo. Os resultados revelaram que é necessário um caudal mínimo de 3 $m^3 s^{-1}$ para assegurar a passagem das espécies nativas (que para baixos caudais ocorre exclusivamente pela rampa) consideradas neste estudo, e que se prevê um acréscimo significativo na ocorrência de caudais inferiores a este valor em ambos os cenários climáticos considerados. Esta previsão de redução do potencial de passagem de pequenas barreiras por parte de espécies nativas realça a importância de considerar não apenas o regime de caudais atual na gestão da ictiofauna aquando da construção de açudes, mas também as alterações de caudal que poderão ocorrer nas próximas décadas. Quando estes obstáculos se tornam obsoletos, a sua remoção deverá ser considerada para um restauro mais efetivo da conectividade fluvial.

Palavras-chave: rampa para peixes, pequenas barreiras, alterações climáticas, caudais baixos, adequabilidade de habitat

3.1. Abstract

Climate change represents a major challenge for the management of native fish communities in Mediterranean rivers, as reductions in discharge may lead to a decrease in passability through small barriers such as weirs, both in temporary and perennial rivers. Through hydraulic modelling, we investigated how discharges from a large hydropower plant in the Tagus River are expected to affect the passability of native freshwater fish species through a rock weir (Pego, Portugal), equipped with a nature-like fish ramp. We considered not only mean daily discharge values retrieved from nearby gauging stations (1991–2005) for our flow datasets, but also predicted discharge values based on climatic projections (RCP) until the end of the century (2071–2100) for the Tagus River. Results showed that a minimum flow of 3 $m^3 s^{-1}$ may be required to ensure the passability of all species through the ramp and that passability was significantly lower in the RCP scenarios than in the historical scenario. This study suggests that climate change may reduce the passability of native fish species in weirs, meaning that the construction of small barriers in rivers should consider the decreases in discharge predicted from global change scenarios for the suitable management of fish populations.

Keywords: fish ramp; small barriers; climate change; low flows; habitat suitability

3.2. Introduction

Rivers have long been among the most endangered ecosystems worldwide, facing multiple threats including the introduction and dispersal of invasive species [1,2], chemical [3] and thermal pollution [4], flow regulation [5,6], longitudinal fragmentation [7] and climate change [8], with the later acting as an enhancer of the previous ones [9,10]. One common consequence of these threats is the gradual loss of suitable habitat, which is particularly worrisome in the case of migratory freshwater fishes, as these migrate along the river (potamodromous) or between the river and sea (diadromous) in different stages of their life cycle to perform critical functions, such as reproduction, feeding and sheltering [11].

Climate change can significantly alter flow regimes [8], leading to the increase of extreme flow events [12–14]. In Mediterranean-climate rivers, facing an annual dry season (usually from March to September, being more pronounced from June onwards), increased droughts may further potentiate river fragmentation and loss of suitable habitat due to increased flow intermittency. [15] This issue is particularly relevant as it encompasses the migratory period of potamodromous and diadromous fish species; therefore, interfering directly with their migrations and recruitment, and lately affecting the sustainability of their populations [16].

Fish movement and migration may be further limited by the presence of small barriers such as weirs, which are generally far more numerous than large dams and clearly represent significant barriers to fish migration [17,18]. These instream structures change the depth and velocity patterns, creating vertical drops that change the hydrodynamics of aquatic systems and may prevent the movement of migratory fish to spawning, feeding and refuge areas [19], and thus their permeability should be assessed for a proper management of these populations [20,21]. The permeability of a weir to fish passage (i.e., passability) will depend not only on its structural properties (e.g., length, slope, substrate), but also on the hydraulic conditions (e.g., flow regime), within a given time and area, providing "flow windows" for fish to pass [22,23]. The passability of a given fish species and life history stage through weirs will also depend on key hydraulic variables, namely flow velocity and water depth [23], that structure habitat suitability [24].

The potential combined effects of weir passability along with the climate change driven increased flow intermittency of rivers is seldom investigated and deserves greater attention. Despite some uncertainty attributed to flow predictions due to different modelling assumptions, studies so far have suggested that in the Mediterranean region, mean monthly flows and annual flow rates are expected to decrease with climate change [25,26]. On the other hand, extreme flow conditions (high flow and low flow magnitudes, and duration) are expected to increase in Mediterranean rivers [27], potentially reducing the suitable habitat area for fish species to be able to overcome barriers.

The use of modelling approaches to determine measures of habitat suitability, such as habitat suitability indexes (HSI), can provide a relative measure of fish passability, based on the available area for fish in specific stages of their life cycle, considering specific flow requirements determined for each species [28]. Habitat suitability curves (HSC) can be developed for this purpose, but often require detailed data at a microhabitat scale, which are typically scarce in large rivers [29]. Recent studies have recommended the use of mesohabitat data (ranging from 10 to 100 m) as the relevant spatial scale of habitat use by fish [30,31] and the use of expert opinion to build HSC based on previous literature and expert knowledge on the species, lowering both the research effort and need for empirical data on habitat use and preference of fishes [32].

For modelling habitat suitability, 2D hydraulic models have the advantage of being more robust and predicting hydraulic conditions more accurately over 1D models, provided that sufficient and good resolution bed topography data is collected, and that model calibration is performed [33–35]. Relative measures of fish passability can be estimated based on the suitable habitat and hydraulic conditions (such as water depth and flow velocity) of small instream obstacles over time and space [24,36,37].

The main goal of this study is to evaluate the passability of the low-head Pego weir, in the Tagus River, Central Portugal, to the different migratory fish species, in relation to historical (1991–2005) and future flow conditions based on two global warming scenarios (RCP 4.5 and RCP 8.5), following the reports of ISI-MIP: Inter-Sectoral Impact Model Intercomparison Project [38]. As current projections for the Mediterranean region suggest a decrease in river discharge in the following decades, it is expected that fish passability will be lower under scenarios of low flow conditions, due to the reduction of the submerged area of the weir. Specifically, we expect passability for all species to be lower under global climate change scenarios.

3.3. Material and Methods

3.3.1. Study Area

This study was conducted in the Pego weir, located in the Tagus River, close to the municipality of Abrantes, Portugal (**Figure 8**). The Tagus River is the longest river in the Iberian Peninsula (1110 km), with a drainage area of 80,630 km^2 and a hydrological regime typical of a Mediterranean-climate river, with lower flow values in summer months and higher values in winter [39]. Data collected from gauging stations shows that it has a relatively high flow variation coefficient (72.15%), contrasting with a relatively low annual coefficient of variation (7.29%), within the period ranging from 1991 to 2005 [40].

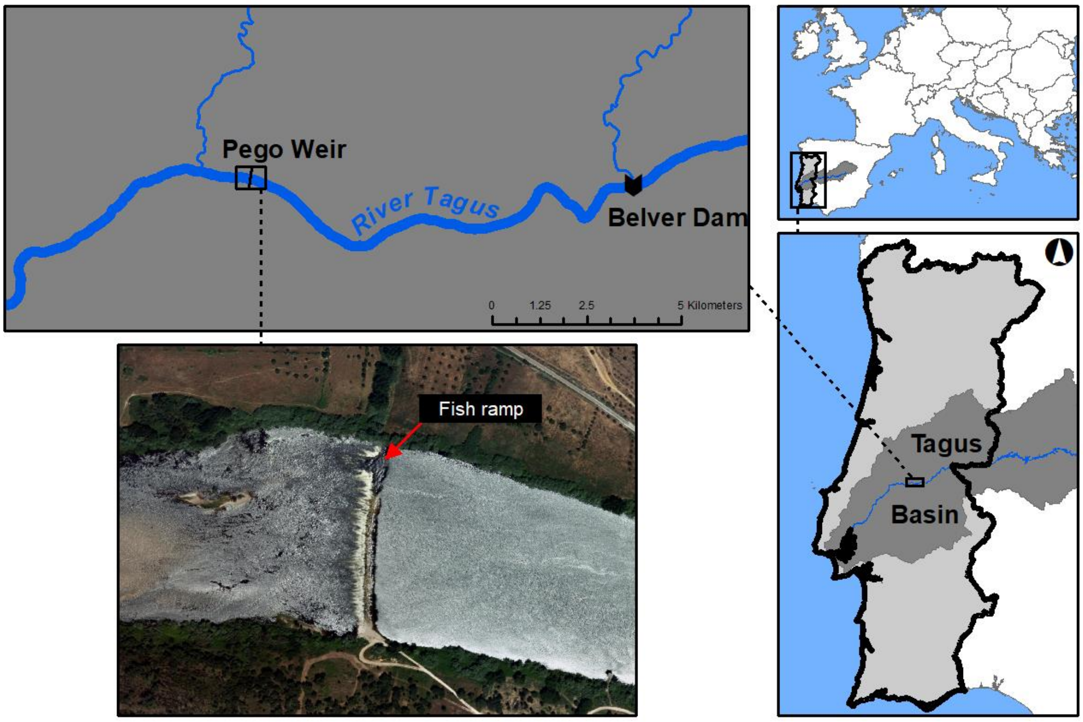

Figure 8. *Pego weir in the Tagus River, approximately 12 km downstream of the Belver dam.*

Within this river basin, the Pego weir, a 250 m wide low-head ramped weir, was built between 1992 and 1995, to allow water collection to cool down the turbines from a nearby coal thermal power plant, which has recently ceased activity (**Figure 8**). The weir features a fish ramp located close to the river's right bank, which is approximately 20 m long and 4 m wide, at an elevation of 23 m, with a longitudinal slope of 2.5% (**Figure 12**). Both the weir and the fish ramp present a substrate dominated by rocks and boulders. This structure was built to ensure the passability of the local native fish species. The closest upstream barrier is the Belver dam, one of the main large hydropower plants in the Tagus River basin, located 12 km upstream of the Pego weir, with an installed power of 80.7 MW and a storage capacity of 7.5 hm^3. Flow data provided by the Portuguese Environmental Agency and local gauging stations reveal that turbined flows do not exceed 800 m^3 s^{-1} [40]. The Belver dam is a run-of-the-river hydropower plant, with low water retention and a reduced thermal stratification in the water column.

3.3.2. Flow Data and Topographic Survey

Flow data was retrieved from the Portuguese national network on water resources (SNIRH), which aggregates data on discharge and other water quality related parameters collected from gauging and meteorological stations nationwide [40]. Mean daily discharge values were retrieved from the available data recorded in the nearby Belver gauging station (station code: 17J/01A), from 1st January 1991 to 31st December 2005, in line with the historical period considered in the Intergovernmental Panel on Climate Change [38] reports on climate projections (1986–2005). Missing values (approximately 15%) were estimated by linear regression ($R^2 = 0.934$) from mean daily flows recorded in the Almourol gauging station (station code: 17G/02A), approximately 28 km downstream from the study area. It should be noted that there are no tributaries between the Pego weir and the Belver dam, and that inflows in the Pego weir are strongly associated with the turbined flows in Belver [41].

For the climate projections, two global warming scenarios (RCP 4.5 and RCP 8.5) were used, following the reports of ISI-MIP: Inter-Sectoral Impact Model Intercomparison Project [38]. The considered scenarios stand for “Representative Concentration Pathways” (RCP), describing general trajectories of greenhouse gases (GHG) emissions, concentrations and land use emissions until the end of the century (2100), according to specific radiative forcing values, namely 4.5 and 8.5 W m^{-2}. RCP 4.5 is described as a more conservative and moderate scenario, while RCP 8.5 is the extreme one. Particularly for the Tagus River basin, decreases in average monthly flows of 30% and 60% in the late century (2071–2100) were previously estimated following RCP 4.5 and RCP 8.5 trajectories, respectively, when integrating reservoirs and water management processes in the hydrological models for river discharge at Almourol, with a strong decrease in hydropower production under both future climate scenarios being expected [26]. These projections consider the regional warming trends through statistical downscaling and bias correction, as an alternative to regional climatic models, with the goal of preserving warming trends [42]. Focusing on low flows (Q90), as these are expected to be more impacted by climate change, we considered two hydrologic scenarios: one with a reduction of 30% on mean daily discharges (RCP 4.5) and another with a 60% (RCP 8.5) reduction, both until the end of the century (2071–2100), regarding the original discharge dataset retrieved from the gauging stations.

3.3.3. Suitability Curves and Habitat Modelling

Fish passability was determined by modelling habitat suitability for the native freshwater fish community using River2D, a two-dimensional depth averaged model which combines the hydraulic conditions close to the weir with HSC for each fish species [43]. The key hydraulic variables for which HSC were developed were water depth (above the weir) and flow velocity, similarly to previous studies assessing fish passability in weirs [44,45]. The boundary conditions considered in this model were the inflow section (in $m^3\ s^{-1}$) and the water level at the outflow section (in meters), making use of a rating curve of the cross-section that was computed with an acoustic Doppler current profiler (ADCP), that took measurements in the cross-section during several different discharges. Model calibration was done by comparing the modeled values for flow velocity and water depth with the values measured in the field. Field measurements took place at the end of the dry season (September) under different flow conditions. The River2D model ran with a spatial mesh of 2 × 2 m in general, refined to 0.5 × 0.5 m in the weir area, similarly to one used for the topographic survey of the 2D model.

The fish community is composed by a multitude of species with different migratory traits, including: (i) anadromous—Allis shad (*Alosa alosa*), twaite shad (*Alosa fallax*) and sea lamprey (*Petromyzon marinus*), (ii) catadromous—European eel (*Anguilla anguilla*) and thlinlip grey mullet (*Chelon ramada*) and (iii) potamodromous species—Iberian barbel (*Luciobarbus bocagei*). Due to the lack of detailed data on HSC for these species, an expert judgment approach [46,47] following a literature review (**Table 3**) was used to build the species HSC, based on flow velocity and water depth (**Figures 13** and **14** in Supplementary Material). Such approaches based on the application of literature or expert opinion-based data, performed in similar conditions – i.e., when empirical data is scarce and difficult to gather (cost limited, lack of time or reference conditions, species with low detectability such as the diadromous ones) – were applied elsewhere [46–50]. A previous assessment of the river topography allowed for the characterization of the hydraulic conditions (flow velocity and water depth) and for the calibration of the River2D model [41].

Table 3. *Literature considered for the construction of the species habitat suitability curves (HSC), based on key variables that structure fish passability at low-head ramped weirs [45]: flow velocity at the ramp weir and water depth above the weir.*

Common Name	Scientific Name	References
Allis shad	*Alosa alosa*	[51,52]
Twait shad	*Alosa fallax*	[51,53]
Sea lamprey	*Petromizon marinus*	[51,54–56]
Thinlip grey mullet	*Chelon ramada*	[51,57,58]
European eel	*Anguilla anguilla*	[51,58–62]
Iberian barbel	*Luciobarbus bocagei*	[51,63–67]

To evaluate passability and determine the minimum flow required for each species to pass the weir, a stepwise approach was followed, modelling in steps of 1 m^3 s^{-1} and assessing the response of the habitat suitability index (HSI) throughout the weir and particularly in the fish ramp. This index was calculated as a product of the separate suitability indices: flow velocity index (VSI), water depth index (DSI) and channel index (CSI, which considers the substrate, dominated by rocks and boulders, constant and evenly distributed within the study area and thus with no significant influence in habitat suitability, assuming a constant value in the formula): HSI = VSI × DSI × CSI [43]. Mean HSI values from each scenario were adjusted to a sigmoid function [68], allowing the estimation of HSI for each discharge value of the datasets by interpolation.

3.3.4. Data Analyses

Quantile distribution of the mean daily discharges from the historical data were analyzed to determine the low flow conditions (Q90, corresponding to the 10th percentile) in the two hydrologic scenarios considered (30% in RCP 4.5 and 60% in RCP 8.5). The comparison of mean daily discharge between each scenario was achieved using the non-parametric Kruskal–Wallis test due to non-normally distributed data (Shapiro–Wilk). A Kruskal–Wallis test (followed by a post hoc Dunn test for pairwise differences) was also performed to investigate differences in HSI between flow scenarios (for each species) and between species (in each scenario). Analyses were conducted in R, version 4.1.0 [69] and Statistica, version 10 [70].

3.4. Results

3.4.1. Flow under Future Climate Change Scenarios

Low flow conditions for the historical period (1991–2005) included discharges from 0 to 11.0 m^3 s^{-1}, with null values being recorded in 316 days, mostly between March and September (71%), which is usually considered the dry season in the Mediterranean climate. Mean daily discharge was significantly different between the three scenarios ($\chi^2 = 28.232$, df = 2, $p < 0.001$), being lowest in RCP 8.5 (mean value: 1.2 m^3 s^{-1}), intermediate in RCP 4.5 (2.1 m^3 s^{-1}) and highest in the historical (3.1 m^3 s^{-1}) scenario (**Figure 9**).

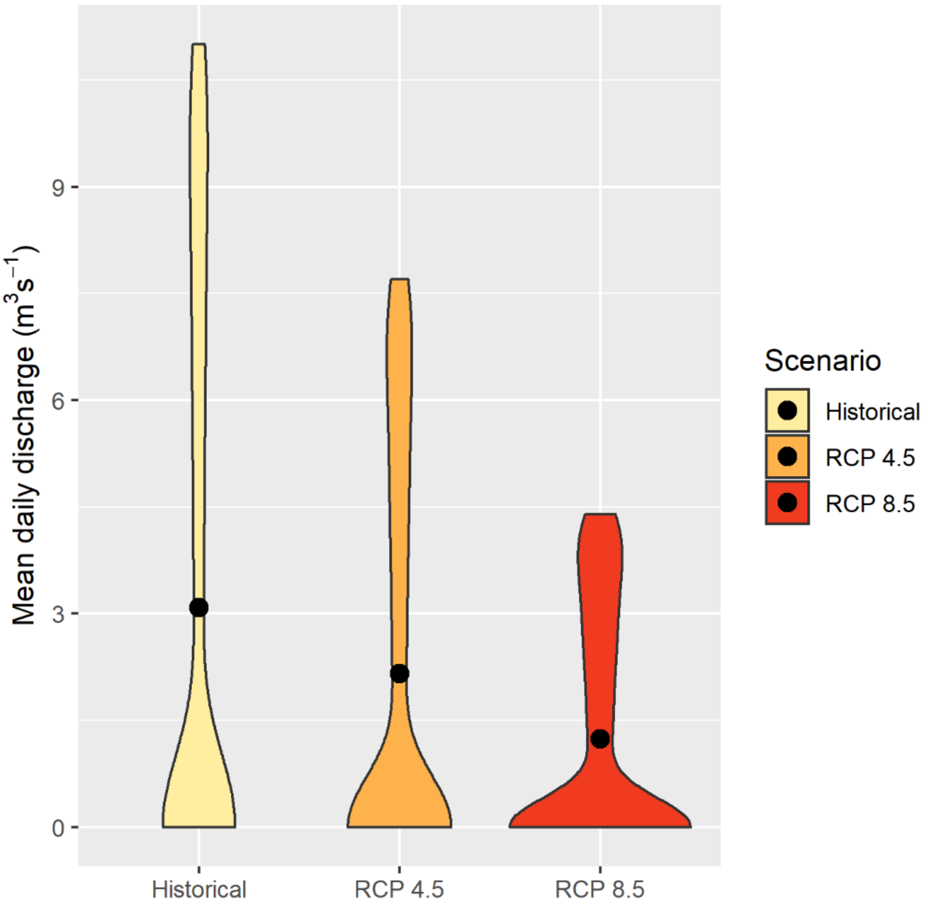

Figure 9. *Density distribution of mean daily discharge (m^3 s^{-1}) for the historical, RCP 4.5 and RCP 8.5.*

3.4.2. Minimum Flow Assessment

River2D modelling revealed that under low flow conditions, more than half of the weir was emersed, with the percentage of submersed area varying from 21% (for inflows of 1 $m^3 s^{-1}$) to 42% (for inflows of 11 $m^3 s^{-1}$), which was reflected in a generally low passability close to the ramp for all species. The suitable area allowing fish passage was only observed at a minimum discharge of 3 $m^3 s^{-1}$ (when the fish ramp became submersed), regardless of the species (**Figure 10**).

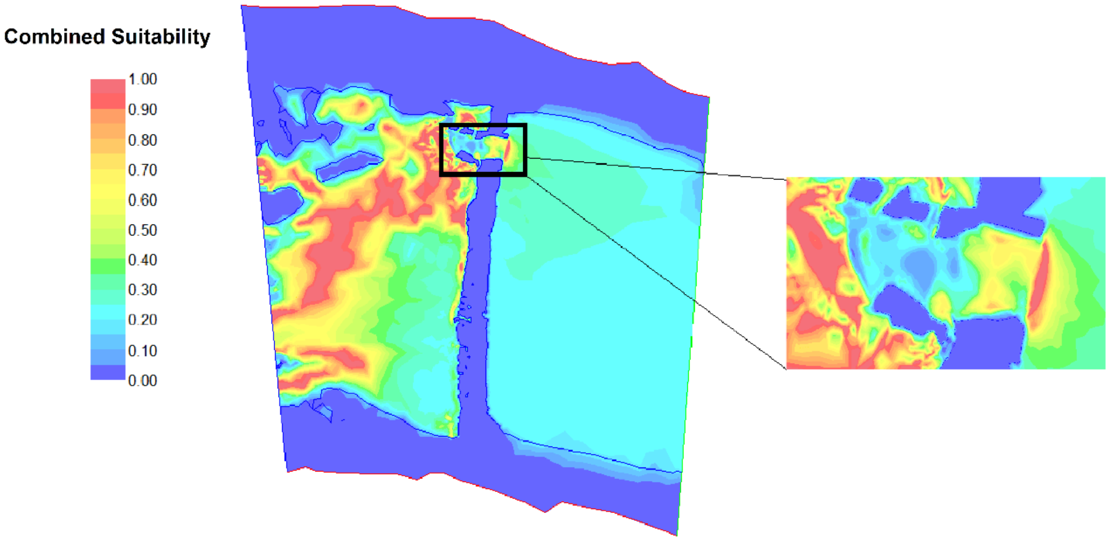

Figure 10. *River2D output habitat suitability index for P. marinus (which displayed generally lower HSI values among the species present) in the Pego weir (left) and specifically at the fish ramp (right), for an inflow of 3* $m^3 s^{-1}$*. Colored scales are presented for each case (HSI varying from 0-blue to 1-red, water depth from 0 to 5.32 m).*

3.4.3. Passability across Different Climate Change Scenarios

Habitat suitability under low flow conditions was significantly different among species (χ^2 = 38.752, df = 5, p < 0.001). Overall, *A. alosa* had the highest mean HSI (ranging from 0.08 ± 0.11 in RCP 8.5 and 0.13 ± 0.16 in the historical scenario), while *P. marinus* scored the lowest (mean HSI: 0.06 ± 0.07 in RCP 8.5 and 0.09 ± 0.11 in historical). Both species had significantly different mean HSI when compared to the remaining species (Dunn post hoc test): *A. anguilla* (mean HSI: 0.05 ± 0.10 RCP 8.5 and 0.09 ± 0.08 in historical), *A. fallax* (mean HSI: 0.05 ± 0.08 RCP 8.5 and 0.10 ± 0.12 historical), *L. bocagei* (mean HSI: 0.05 ± 0.08 RCP 8.5 and 0.10 ± 0.12 historical) and *C. ramada* (mean HSI: 0.06 ± 0.09 RCP 8.5 and 0.10 ± 0.13 historical). Significant differences for HSI between the three scenarios were also observed within each species (χ^2 = 58.794, df = 2, p < 0.001) and for pairwise comparisons, with all species attaining higher scores in the historical scenario (**Figure 11**).

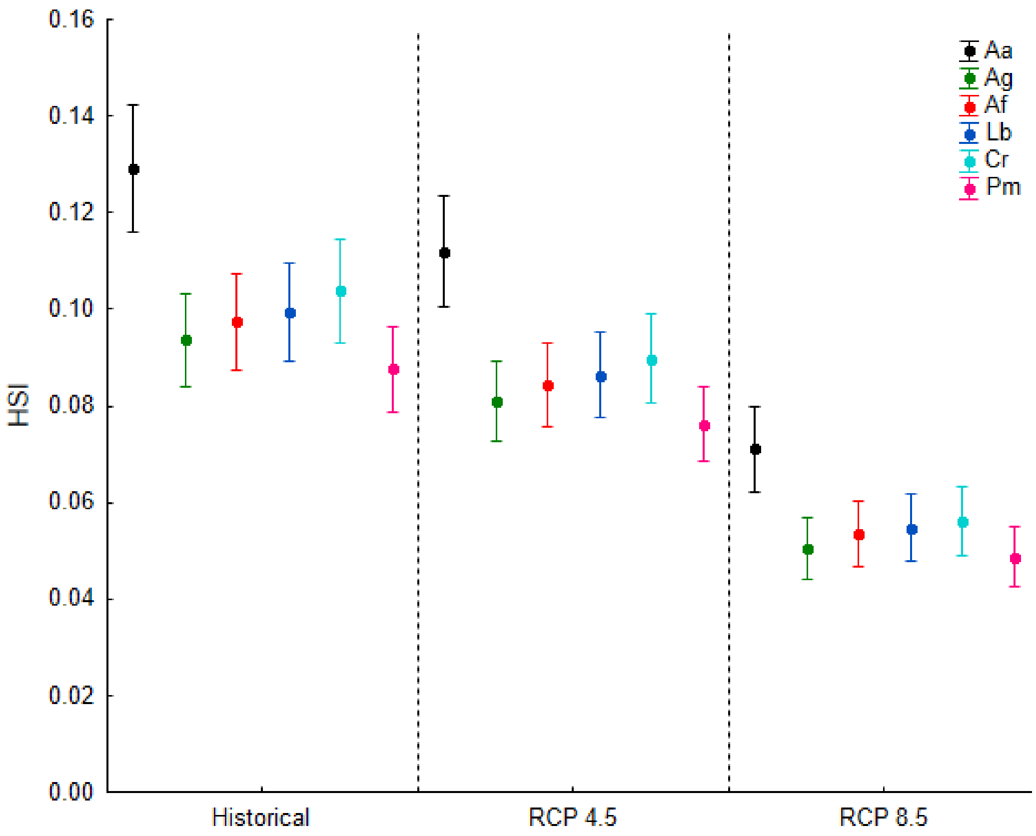

Figure 11. *Habitat suitability index (HSI) in each scenario for each species: Alosa alosa (Aa), Anguilla anguilla (Ag), Alosa fallax (Af), Luciobarbus bocagei (Lb), Chelon ramada (Cl), Petromyzon marinus (Pm). Mean values and 95% confidence intervals are given by dots and whiskers, respectively.*

3.4. Discussion

Changes in hydrology under climate change can lead to shifts in fish habitat suitability and distribution in rivers [71]. For migratory fish species, which shift from different habitats (spawning, feeding, refuge) during their life cycle, this habitat loss is particularly worrisome, particularly in the presence of barriers to fish movement, making it essential to assess how habitat may change in the advent of future flow regimes [16]. However, quantifying the impact of climate change in natural populations is challenging, as different effects are expected depending on the climate model trajectories that are assumed [72], the temporal range (mid or late century) and the effect of climate change on the multiple stressors already acting [73].

In this study, we built flow datasets for the different climatic scenarios – RCP 4.5 and RCP 8.5 – by assuming the predicted changes in monthly discharge described by Lobanova *et al.* [26] for the Tagus River, with reductions of 30 and 60%, respectively, until 2100. Passability for all species occurred at a minimum flow of 3 $m^3 s^{-1}$, and the frequency of null flows and flows lower than the required threshold for fish passage increased in both RCP 4.5 and RCP 8.5 scenarios. This result is particularly relevant as the increase in “zero-flow” day frequency has been reported as a severe threat to hydrological connectivity and species persistence in rivers [74]. By adding the cumulative effect of a small barrier (weir), this means that the decrease in the occurrence of suitable flows for fish populations close to the Pego weir, over the next decades, may lead to an overall decrease in the weir passability to the different fish species.

Passability was found to be significantly lower in the RCP 4.5 and RCP 8.5 scenarios relatively to the historical dataset (1991–2005). Habitat modelling using River2D also revealed that under low flow conditions, passability only occurred in the area covered by the fish ramp, close the right bank. A previous assessment of the Pego weir showed that inflows of at least 30 $m^3 s^{-1}$ are required to allow fish passage across the remaining area of the weir, with all species being able to pass the weir if it was submerged [41]. This further enhances the importance of this fishway for upstream migration, as mean daily discharge will tend to decrease in future climates [8,26], while droughts are expected to increase in frequency and intensity [14,74].

While 2D assessment of fish passability was only performed for low flow conditions, it revealed differences between the different species, which can be explained by their different swimming capacities. Overall, the two clupeid species, *A. alosa* and *A. fallax*, had the highest passabilities in all scenarios, attaining the first and second highest HSI values, respectively. Previous studies on nature-like fishways (such as rock ramps) reported higher passability for *Alosa* species compared to the other ones present in the fish community using the same fishway [75]. Contrastingly, the sea lamprey *P. marinus* had the lowest passability, even though nature-like fishways are suggested as more adequate for allowing lamprey passage when compared to technical fishways such as pool and Denil fishways [76]. Emphasis on improving attraction efficiency under low flow conditions, considering the swimming performance and behavior of the different species composing the migratory fish community, should be put into future fishway adjustments (e.g., boulder arrangement: [77]).

The passability of the Pego weir was modelled for different climate scenarios, considering the key hydraulic variables—flow velocity and water depth—that are known to highly influence fish passage in this type of instream structures. As the difficulty in gathering appropriate data for developing HSC would reveal cost and effort-intensive for a large river such as the Tagus River, an expert judgement approach was followed [32,36,49,50]. Coupled with an extensive characterization of the hydraulic conditions in the study area, a relative measure of passability based on the habitat suitability of each species was obtained, proving that this approach may be a useful alternative to empirical studies for fish population management purposes.

Successful fish passage across an instream obstacle is a more complex phenomenon other than depending solely on flow velocity and water depth above the weir. The willingness to negotiate a barrier is also driven by internal factors – the physiological condition, such as fatigue level, migratory phase, age, and body size [78] – as well as individual predisposition to move upstream, and other external factors not accounted on the present study, such as water temperature [79]. Though our modelling approach enabled us to estimate fish passability for different migratory species across a small weir in a large river, considering the critical hydraulic variables, future validation with empirical studies, such as fish telemetry tracking, is essential if we want to effectively use such tools in river conservation and management plans [80].

3.5. Conclusions

To conclude, this study provided evidence that under low flow conditions, the passability of migratory fish species in the Pego weir is generally low and only occurring through the fish ramp. Results suggest that under future flow regimes, the permeability of the weir to fish passage is likely to further decrease for all species. This highlights the need to account for future impacts of altered flow regimes driven by climate change on fish populations, considering current and future climatic models and flow requirements for each species when modelling habitat suitability and fish passability. Moreover, it is crucial to adapt conditions for obstacle transposition, namely by improving attraction efficiency close to the ramp, followed by monitoring surveys of fishway efficiency. The ongoing transition of fossil fuels to renewable energy occurring in Portugal (and in other countries worldwide), is expected to lead to an adjustment of coal thermal plants (to coal-fired plants using charcoal) or in some cases to a complete shutdown, making the barriers that were built to accommodate its activity obsolete and thus potential targets for removal. This would allow for a more efficient re-establishment of longitudinal connectivity for all fish species.

Supplementary Materials:
The following are available online at https://www.mdpi.com/article/10.3390/w13192758/s1, **Figure 12**: Fish ramp (20 m long, 4 m wide, 23 m elevation, 2.5% longitudinal slope), at the right margin of the Tagus River, incorporated in the Pego weir. Picture retrieved following the study by Ferreira *et al.*; **Figure 13**: Suitability curves for the flow velocity at the ramp weir for each species, based on the literature review; **Figure 14**: Suitability curves for the water depth above the weir for each species, based on the literature review.
Author Contributions: Conceptualization, J.M.S. and M.T.F.; methodology, D.M., J.M.S. and R.R.; software, D.M. and R.R.; formal analysis, D.M., J.M.S. and R.R. and S.S; investigation, D.M., J.M.S. and R.R.; writing—original draft preparation, D.M.; writing—review and editing, J.M.S., M.T.F., R.R. and S.S.; supervision, J.M.S., M.T.F. and S.S.; project administration, M.T.F.; funding acquisition, M.T.F. All authors have read and agreed to the published version of the manuscript.

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3.7. Supplementary Material

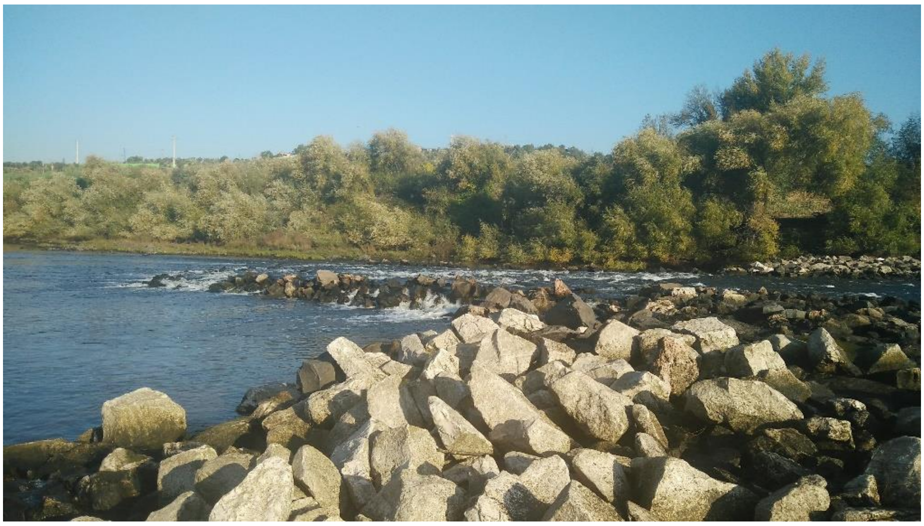

Figure 12. *Fish ramp (20 m long, 4 m wide, 23 m elevation, 2.5% longitudinal slope), at the right margin of the Tagus River, incorporated in the Pego weir. Picture retrieved following the study by Ferreira et al. [41].*

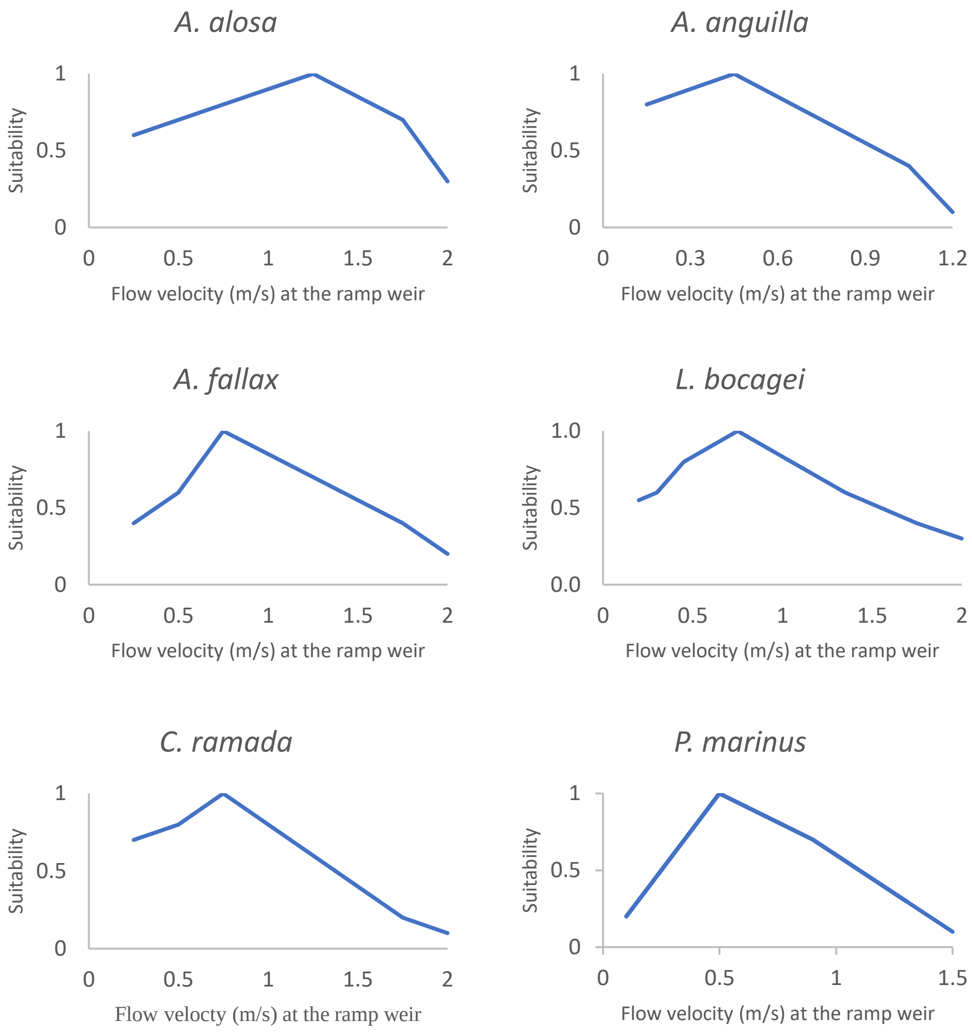

Figure 13. Suitability curves for the flow velocity at the ramp weir for each species, based on expert judgment following a literature review (see **Table 3**).

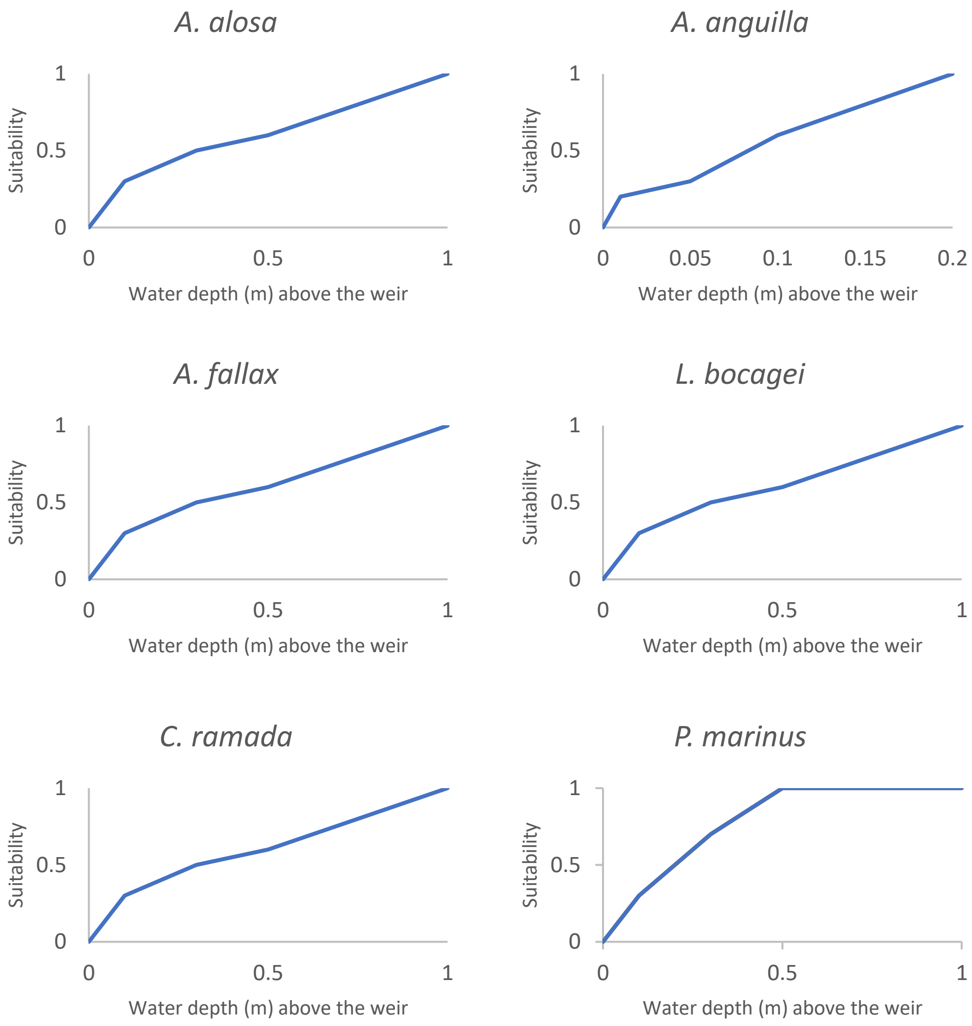

Figure 14. *Suitability curves for the water depth above the weir for each species, based on expert judgment following a literature review (see **Table 3**).*

CHAPTER 4: Heatwave effects on the swimming behaviour of a Mediterranean freshwater fish, the Iberian barbel *Luciobarbus bocagei*

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CHAPTER 4: Heatwave effects on the swimming behaviour of a Mediterranean freshwater fish, the Iberian barbel *Luciobarbus bocagei*

Daniel Mameri, Paulo Branco, Maria Teresa Ferreira, José Maria Santos

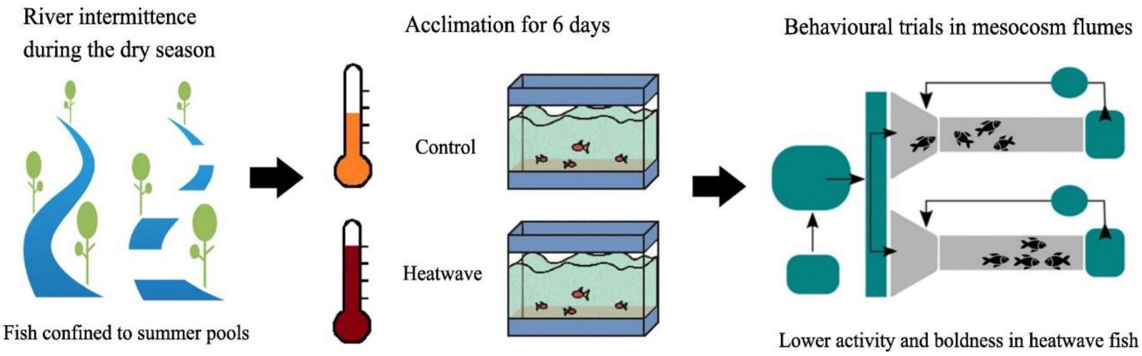
Forest Research Centre (CEF), School of Agriculture, University of Lisbon, Tapada da Ajuda, 1349-017, Lisboa, Portugal

Resumo

Os eventos meteorológicos extremos como as ondas de calor, que podem ser definidas como aumentos de pelo menos 5ºC durante 6 ou mais dias consecutivos, estão a tornar-se mais frequentes com as alterações climáticas. Os organismos aquáticos que estão dependentes da temperatura do ambiente em redor (ectotérmicos), como os peixes, encontram-se por isso mais vulneráveis a estes fenómenos, sobretudo em rios mediterrânicos. Nos meses de verão, vários cursos de água secam parcialmente, ficando os peixes confinados em pegos desconectados entre si e potencialmente sujeitos a stress térmico até a conectividade fluvial ser novamente restabelecida no final da estação seca. Neste estudo, procurou-se avaliar o efeito de uma onda de calor, simulada num ambiente laboratorial, no comportamento natatório de juvenis de uma espécie potamódroma nativa da Península Ibérica, o barbo-comum *Luciobarbus bocagei*, nos canais dos mesocosmos do *campus* do Instituto Superior de Agronomia, em Lisboa. Os comportamentos monitorizados incluíram a atividade (em movimento ou repouso), ousadia (predisposição para transpor um obstáculo) e a coesão de cardume, em situações de baixa velocidade, a 18 cm s^{-1}, mimetizando *riffles* nos rios. Os ensaios comportamentais nos mesocosmos, após exposição prévia à onda de calor (30ºC), revelaram que a atividade e a ousadia dos barbos expostos a este fenómeno foram inferiores comparativamente ao grupo de controlo (25ºC). Este estudo realça a importância de identificar e preservar refúgios térmicos em rios que possam ajudar a mitigar o impacto das ondas de calor em peixes nativos, sobretudo no período estival. Procurar compreender a interação de ondas de calor com outros fatores de stress, nomeadamente a escassez de oxigénio, poderá ser essencial para a elaboração de estratégias de mitigação destes fenómenos em peixes de água doce nativos.

Palavras-chave: alterações climáticas, ondas de calor, stress térmico, comportamento natatório, rios intermitentes

4.1. Graphical Abstract



4.2. Abstract

Heatwaves, which can be defined as increases of at least 5 °C in air temperature for more than five consecutive days for a specified reference period, are expected to become more frequent under the ongoing climate change, with freshwater organisms being particularly vulnerable to high temperature fluctuations. In Mediterranean climate areas, depending on the extent of summer droughts and loss of longitudinal connectivity, river segments may become isolated, maintaining fish populations confined to a series of disconnected pools, with no possibility to move to thermal refugia and thus becoming more prone to thermal stress. In this study, we evaluated the effect of a simulated heatwave on the swimming behaviour of juvenile stages of a potamodromous native cyprinid fish, the Iberian barbel *Luciobarbus bocagei*, under experimental mesocosm conditions. Behavioural traits included fish activity, boldness and shoal cohesion and were continuously measured at a constant flow velocity of 18 cm s^{-1}, which is typical of riffle habitats. Overall, results show that the behaviour of juvenile Iberian barbel is likely to be affected by heatwaves, with fish displaying lower activity and boldness, while no clear difference was observed in shoal cohesion. This study highlights the importance of managing thermal refugia that are crucial for fish to persist in intermittent rivers. Future studies should focus on the interaction of heatwaves with other stressors, such as oxygen depletion, for a broader understanding of the perturbation affecting freshwater fishes under a changing climate.

Keywords: climate change, heatwaves, thermal stress, swimming behaviour, intermittent rivers

4.3. Introduction

Riverine environments are among the most diverse in the world (Dudgeon, 2019), providing vital ecosystem services such as fish production, water supply, nutrient transport, health benefits and recreational value (Green *et al.*, 2015). But they are also among the most vulnerable, as they experience strong anthropogenic pressures from increasing human populations, such as damming, urbanization and nutrient enrichment (Reid *et al.*, 2019; Wen *et al.*, 2017). Climate change leading to global warming has been a major topic of concern and will continue to be in the next decades, as it is expected to potentiate the effect of these pressures and lead to an increase in the frequency and severity of extreme events, such as heatwaves (IPCC, 2014; Perkins *et al.*, 2012; Seneviratne *et al.*, 2014). Although a universally accepted definition is lacking (Xu *et al.*, 2016), heatwaves can be defined as increases of at least 5 °C in air temperature for more than five consecutive days for a specified reference period (WMO, 2001), representing discrete events of potentially high thermal stress (Pansch *et al.*, 2018). In contrast to global warming driven increase in annual mean temperature, heatwaves are suggested to be more significant to natural populations, as high temperatures can have disproportionate effects on individuals' life-history traits (such as development time, fecundity and mortality), behaviour, survival and reproduction (Chiu *et al.*, 2012; Clarke, 2003; Dhillon and Fox, 2007). Heatwaves, which are expected to increase in frequency, duration and/or amplitude (Zampieri *et al.*, 2016), may act as important selective forces shaping species distribution and the spatial and temporal dynamics of their populations (Sandblom *et al.*, 2014; Shultz *et al.*, 2016).

The observed increase in the frequency of heatwaves over the past years, particularly in the Mediterranean region (Fischer and Schär, 2010; Meehl and Tebaldi, 2004; Vautard *et al.*, 2014), has also been associated with an increase in the frequency and severity of summer droughts, affecting evapotranspiration, rainfall and soil dryness, which in turn can act as precursors and amplifiers of heatwaves (Lennox *et al.*, 2019; Vautard *et al.*, 2007; Zampieri *et al.*, 2009). This concern is particularly relevant in Mediterranean rivers, characterized by seasonal and predictable hot and dry summers (Cid *et al.*, 2017; Gasith and Resh, 1999), where the intensity and duration of heatwaves is expected to increase over the next years (IPCC, 2014). These rivers are also characterized by hotspots of biodiversity and harbour highly threatened endemic fish fauna (Hermoso and Clavero, 2011; Smith and Darwall, 2006), which will increasingly face the effects of heatwaves, and therefore their interaction with such phenomena deserves a clear attention.

Several studies have been conducted on the effect of heatwaves on fish species, but these have mostly targeted marine organisms (Kikuchi *et al.*, 2019; Lenanton *et al.*, 2017; Pansch *et al.*, 2018; Vinagre *et al.*, 2018; Wernberg *et al.*, 2012), while studies on freshwater fish still remain relatively scarce (Figueiredo *et al.*, 2019; Troia and Giam, 2019), despite their generally high conservation status (Smith and Darwall, 2006). Furthermore, the vulnerability of freshwater fish to such warming events is even more pronounced in river systems affected by increased connectivity fragmentation due to man-made barriers, which do not allow fish to freely move along the river network to find thermal refugia (Isaak and Rieman, 2013; Kurylyk *et al.*, 2014; Magoulick and Kobza, 2003; Sutton *et al.*, 2007). Longitudinal fragmentation can also occur due to river intermittence (Jaeger *et al.*, 2014), when these systems dry out during the dry season, reducing the available habitat to series of disconnected pools (Cid *et al.*, 2017; Gasith and Resh, 1999), from where fish disperse after river connectivity is re-established (Magalhães *et al.*, 2002; Pires *et al.*, 2014).

Successful re-colonization of river reaches following flow reestablishment is thus critical for the survival of Mediterranean freshwater fish populations, and dependent on the ability of fish to perform movements and migrations along the river. Studies at the individual level, namely through behavioural trials measuring the impact of stressors in fish behaviour and movement (e.g. oxygen depletion: Branco *et al.*, 2016; ashes from wildfires: Gonino *et al.*, 2019) are thus critical to understand how populations will respond (Killen *et al.*, 2013; Lennox *et al.*, 2019). To the best of our knowledge, no information exists on the effects of heatwaves on Mediterranean freshwater fish behaviour and activity, particularly of potamodromous cyprinid fish, that perform seasonal migrations within rivers for reproduction, feeding and to seek refuge against harsh environmental conditions. Experimental work, preferentially performed under controlled conditions where variables of interest can be manipulated while controlling for confounding effects, offers an excellent opportunity to improve knowledge on heatwave-related disturbance on fish and better inform managers for future risk assessments.

Using an experimental mesocosm, the goal of this study was to assess the effect of a simulated heatwave on the swimming behaviour of a Mediterranean potamodromous cyprinid, the Iberian barbel *Luciobarbus bocagei* (Steindachner, 1864). For the purpose of this study and considering that freshwater fish can experience considerable diel variations in river temperatures (higher during the day and lower during the night; Allan and Castillo, 2007), we divided the heatwave treatment in two phases: an initial gradual increase in temperature followed by a stabilization of this value with a 5 °C difference to the control (see more in detail in Section 2.2. Fish sampling and housing and Fig. S1). We hypothesized that previous exposure to a heatwave would: (1) reduce the number of active fish; (2) reduce boldness; and (3) decrease shoaling cohesion. The findings from this study can be used to inform river restoration practices to be considered in Mediterranean landscapes that are particularly prone to such events, especially considering low flows and increases in temperature are expected to become more frequent under the current climate change projections (van Vliet *et al.*, 2013).

4.4. Material and Methods

4.4.1. Compliance with ethical standards

All procedures involving animal manipulation, including capture in their natural environment, housing and behavioural trials, were carried out in strict compliance with European standards (Directive 2010/63/EU) and Portuguese legislation (Decree-Law 113/2013, 7th August, article 35, no. 5, transposing the European Directive for animal experimentation). Permits regarding fish capture, handling, transportation and holding were issued by the Portuguese Institute for Conservation of Nature and Forests (ICNF) (permit numbers 273/2019/CAPT, 274/2019/CAPT and 275/2019/CAPT), which also authorized keeping *L. bocagei* in captivity for 10 days, in accordance with the methodology presented in this study. Fish housing and trials were coordinated by J. M. Santos, who holds a FELASA level C certification (www.felasa.eu) to direct animal experiments. Thus, all the necessary procedures to complete this study were authorized and performed with minimum handling stress. No fish were sacrificed for the purpose of this study.

4.4.2. Fish sampling and housing

Sampling of juvenile Iberian barbel took place in Lizandro, a small Atlantic coastal river in West Portugal, by means of wadable electrofishing (Hans Grassl IG-200, Germany), following the recommendations of the European Committee of Standardization (CEN, 2003). A total of two electrofishing events were performed (one per week), in late June–early July, collecting 20 fish per event. After each fishing event, captured individuals (mean total length (TL) ± standard deviation (SD) = 90.2 ± 10.4 mm, range: 77–118 mm) were transported to the campus of the School of Agriculture, University of Lisbon, in a fish transport box (Hans Grassl, 190 L), filled with river water and featuring a portable aeration device (ELITE, Germany) to reduce transportation stress.

At the campus, fish were maintained for a maximum period of 48 h in an acclimation tank (volume = 700 L), which featured a High Performance Canister Filter FX4 (turnover rate: 2650 L h−1, Fluval, Quebec, Canada), with biological media (Biomax, Fluval, Quebec, Canada), to reduce potential stress prior to the acclimation in the laboratory. The tank featured two U-shape ceramic roof tiles (45 cm long×25 cm wide×10 cm high) to provide shelter (Stammler and Corkum, 2005) and reduce stress. Water quality in the acclimation tank (temperature = 22.5 ± 0.6 °C; pH = 7.5 ± 0.2; dissolved oxygen (DO) = 7.7 ± 0.3 mg L^{-1}) was checked daily with portable meters (HANNA's temperature probe HI98304 and oxygen probe HI98193, Portugal) and aquarium test stripes (for pH; Tetra, Germany). No fish died following transportation and housing in the tank.

Following the acclimation step after each sampling event and period of quarantine, 16 fish were moved to four PVC tanks (65 L each), in shoals of 4 fish per tank, randomly selected. Each tank was covered by a mesh panel on the top to provide visual cover for the fish and was equipped with one filter (turnover rate: 378 L h−1, AquaClear 20, Quebec, Canada) with biological media (Biomax, Fluval, Quebec, Canada). Fish shoals in two tanks acted as controls, whereas the remaining two were subjected to an experimental heatwave treatment. For the purpose of this work, a heatwave was defined as a 5 °C increase in water temperature in relation to the control temperature (Kikuchi *et al.*, 2019), for six consecutive days. Control temperature had a mean value of 24.5 ± 0.7 °C (**Table 4**), which is consistent with the range of stream temperature records in the Lizandro River in summer (18.0–26.1 °C; mean ± SD = 22.1 ± 1.9 °C; data retrieved between 1998 and 2008 in a nearby meteorological station – code 20A/01; APA, 2019). Temperature increase in the heatwave tanks was achieved through the use of thermal heaters (one per tank, Hydor 300 W, Italy), switched on every day at 9:00 h (temperature = 24.9 ± 0.7 °C) and calibrated to 32 °C, allowing an increase in water temperature at a rate of 1.6 °C/ h, until a 5 °C difference to the control temperature was achieved (at 12:00 h, see **Table 4** in Results and **Fig. 18** in Supplementary data for further details). Heaters were then calibrated to 30 °C and kept operating for further six hours, until 18:00 h (temperature = 29.7 ± 0.6 °C), when they were turned off. This assured a gradual and natural temperature decrease overnight, similar to the one that rivers experience during such phenomena (Allan and Castillo, 2007). To observe a minimum 5 °C difference over the controls, water temperature was monitored four times a day (HANNA's probe HI98304), whereas DO (HANNA's probe HI98193), pH, nitrates and nitrites (aquarium test strips; Tetra, Germany) were checked once a day. To prevent higher levels of nitrates and nitrites, 10 L (15%) of the total amount of water in each tank were replaced every two days. Fish feeding (Tetra Pond sticks) stopped 24 h prior to behavioural trials.

4.4.3. Mesocosm facility

The effects of the experimental heatwave on fish behaviour were assessed in the mesocosm facility located at the School of Agriculture campus, University of Lisbon, Portugal. Mesocosms are outdoor experimental systems that examine the natural environment under controlled conditions, where a variable can be manipulated while controlling for confounding factors and incorporating natural variations (such as photoperiod and air temperature), thus providing a link between field surveys and highly controlled laboratory experiments (Calapez *et al.*, 2017; Gonino *et al.*, 2019).

Table 4. *Water quality parameters (mean values and standard deviations, SD) throughout the acclimation step for control and heatwave groups (note: overnight temperatures refer to the minimum temperatures recorded within the period 18:00 h–09:00 h.; DO stands for dissolved oxygen). (*) Significant differences in water temperatures and DO were found between control and heatwave treatments (temperatures: Mann-Whitney; DO: t-test; p < .05).*

Parameter	Control (mean ± SD)	Heatwave (mean ± SD)
Temperature (ºC)		
09:00 h*	24.4 ± 0.7	24.9 ± 0.7
12:00 h*	24.5 ± 0.8	29.3 ± 0.5
15:00 h*	24.5 ± 0.7	29.3 ± 0.6
18:00 h*	24.6 ± 0.7	29.7 ± 0.6
Overnight*	24.4 ± 0.7	24.9 ± 0.5
DO (mg L^{-1})*	7.0 ± 0.3	6.6 ± 0.3
pH	7.2 ± 0.1	7.2 ± 0.1
Nitrates (mg L^{-1})	20.9 ± 6.8	23.0 ± 5.3
Nitrites (mg L^{-1})	0.5±0.5	1.0 ± 1.6

The present mesocosm consisted of a set of 2 tinplate-lined outdoor artificial flume channels (each 0.4 m wide, 4 m long and 0.2 m deep), with the water being supplied from a 3000 L central tank (**Fig. 15**). The water source was an in situ natural spring considered to have good quality (temperature: 19.1 °C; pH = 8.06; conductivity = 0.87 mS cm^{-1}; DO = 9 mg L^{-1}; Leite *et al.*, 2019; Gonino *et al.*, 2019). Water was then distributed to head containers (70 L) located in the uppermost section of each channel (henceforth designated as upstream tanks). Each channel was delimited downstream by a fixed mesh panel (to prevent fish from dropping out of the channel) that allowed water to ran to a downstream tank (70 L) connected to a pump (Kripsol OK-71 B, 0.56 kW), operating in a recirculation flow system towards the upstream tank, thus allowing upholding water conditions independently of the source tank. Water distribution and recirculation was ensured with a PEAD pipe system connected to the pump. Each flume channel (**Fig. 15**, section IV) was separated from the upstream tank (III) by a 47 cm long and 36 cm high ramp, at a slope of 50%. Fish could move freely between the main channel and the upstream tank, provided that they could successfully negotiate the ramp.

4.4.4. Experimental procedure

Following the six-day acclimation period, fish were transported to the mesocosm, to assess their swimming behaviour after exposure to the control and heatwave treatments. Two shoals of fish (each n = 4) – a control and one subjected to the heatwave - were then placed in the lowermost section (60 cm length and 40 cm wide) of each of the two flume channels, delimited upstream by a removable mesh panel for acclimation purposes (see section V in **Fig. 15**). Fish were then acclimatized to the channels' conditions for 10 min (water temperature = 23.1 ± 0.7 °C, pH = 8.6, DO = 8.9 mg L^{-1}, conductivity = 1.0 ms cm^{-1}). The water column was maintained at a depth of 12 cm with an average velocity of 18 cm s^{-1}, within the range of velocities that the Iberian barbel experiences in Mediterranean rivers throughout the year, namely in riffle habitats (Martínez-Capel *et al.*, 2009; Santos *et al.*, 2018). At the start of each trial, the upstream mesh panel was removed to allow fish to swim freely through the whole channel.

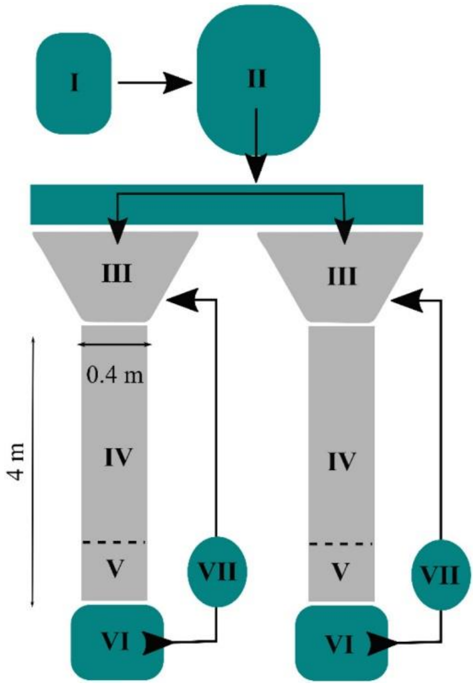


Figure 15. *Schematics of the mesocosm facility: (I) natural spring acting as the water source; (II) 3000 L central tank, retrieving water directly from the spring; (III) upstream tank (70 L capacity, one for each channel); (IV) flume channel (area where fish behaviour was visible and monitored by the observer), including (V) the acclimation zone in the lowermost area; (VI) downstream tank, to collect the water; (VII) pump connected to the downstream tank, redirecting the water through the pipes back again to the upstream tank, creating a recirculating flow.*

Behavioural traits monitored included: (1) activity, given by the total number of active fish (i.e. displaying exploratory behaviour, directional changes or fleeing pattern, and not holding position); (2) boldness, given by the total counts of fish that entered the ramp in the upstream section of the flume and actively attempted to negotiate it, based on the idea that a novel environment is considered dangerous, and that venturing into the uppermost section represents willingness to undertake risk (Laubenstein *et al.*, 2018); (3) shoal cohesion, given by the number of fish in the flume that were within one body length of each other (Gonino *et al.*, 2019). Similar behavioural traits have been used in previous studies to assess fish behaviour due to their ecological relevance (Amaral *et al.*, 2019; Ariyomo and Watt, 2012; Brownscombe *et al.*, 2014; Laubenstein *et al.*, 2018; Leite *et al.*, 2019; Gonino *et al.*, 2019). Both activity and boldness have been positively associated with increased feeding and growth rates (Laubenstein *et al.*, 2018) and with the searching for more favourable areas for refuge and spawning (Biro *et al.*, 2003). Shoaling cohesion may confer increased survivorship to fish living in groups, namely by improving threat detection and reducing stress and individual risk, despite the potential drawback of increased competition for space and food (Tien *et al.*, 2004).

Each of the two experimental treatments (control and heatwave) was replicated four times. Each replicate lasted for 60 min and consisted in the instantaneous sampling of behavioural traits, together with the total number of fish in the main channel of the flume. The observers stood still at an approximate distance of 0.5 m downstream from each channel, with a full view of it, approaching and leaving discreetly the observation points whenever necessary, to obtain a snapshot status of fish behaviour every 3 min (with the exception of boldness, as it was measured continuously, with each attempt to actively negotiate the ramp being assigned to the corresponding 3-min interval). This procedure was tested in previous experiments in the mesocosm facility with the same species and was considered adequate, since no sudden changes in fish behaviour were noted (Gonino *et al.*, 2019). Channel treatment and observer allocations were randomized (i.e. the same treatment was not always housed by the same flume, and observers monitored a different flume in each trial), thus avoiding possible pseudo-replication issues. After each trial, fish were removed from the channel and measured (TL, to the nearest mm). Fish were used only once and were released after trials were concluded. Fish total length (TL) did not differ between treatments (control: 90.2 ± 7.3 mm, heatwave: 91.7 ± 13.1 mm; t-statistics = −0.384, df = 30, p = .704).

4.4.5. Data analysis

Data normality and heteroscedasticity of water quality parameters and fish TL were evaluated through the Shapiro-Wilk and F tests, respectively, and differences between treatments for each parameter were assessed through the Mann-Whitney test (all except DO and TL, for which a t-test was applied). Each of the behavioural traits (activity, boldness and shoal cohesion) was fitted into a generalized linear model (GLM) following a Poisson distribution, with three fixed effects: (1) treatment (control/heatwave); (2) time of sampling (every 3-min interval, when behaviour was recorded); and (3) number of fish present in the main channel of the flume (excluding the upstream tank, thus accounting for a potential bias in the measured behaviour, driven by the number of fish that were not visible to the observer – see Fig. 1). A fourth Poisson GLM was built to assess a possible association between fish activity and shoal cohesion (Bartolini *et al.*, 2014; Pritchard *et al.*, 2001), with the last being the only fixed effect incorporated in the model (see **Fig. 19** in Supplementary data). A significance level (α) of 0.05 was considered and all analyses were performed in R (R Core Team, 2019, version 3.6.2). Model fit was assessed through the deviance goodness-of-fit test, by comparing the deviance of each fitted model with the one of the corresponding null model (function “pchisq” from the R software “stats” package).

4.5. Results

4.5.1. Exposure to experimental heatwave

During the acclimation phase, DO and water temperatures were significantly different between experimental treatments (**Table 4**). Differences in mean temperature during the heatwave period (09:00 h–18:00 h) ranged between 0.5 °C (at 09:00 h, before turning the heaters on) and 5.1 °C (at 18:00 h, before turning the heaters off) − (**Table 4**). Minimum temperatures recorded overnight also differed between experimental groups, contrastingly with pH, nitrates and nitrites, for which no significant differences were found (**Table 4**).

4.5.2. Assessment of fish behaviour

Experiments revealed that fish activity was dependent on the type of treatment (β = −0.279, z = −3.699, p < .001), with heatwave fish displaying lower frequencies of active individuals (59.5% active) relatively to control ones (82.7% active) (**Fig. 16**). As activity depended on the number of fish visible to the observer (average of 3.4 ± 0.6 in control and 3.9 ± 0.6 in heatwave), an effect of the number of fish present in the channel was found (β = −0.325, z = −0.078, p b .001), while the effect of the time of sampling (3-min interval) was not significant (β = −0.004, z = −1.601, p = .109). Boldness was also significantly different (β = −0.549, z = −3.230, p = .001) between the control (98 out of 156 attempts to negotiate the ramp, corresponding to 62.8%) and the heatwave fish (58 out of 156 attempts, 37.2%) (**Fig. 16**). No significant effect on boldness was found when considering the number of fish in the channel (β = 0.014, z = 0.096, p = .924) nor the time of sampling (β = −0.003, z = −0.626, p = .532).

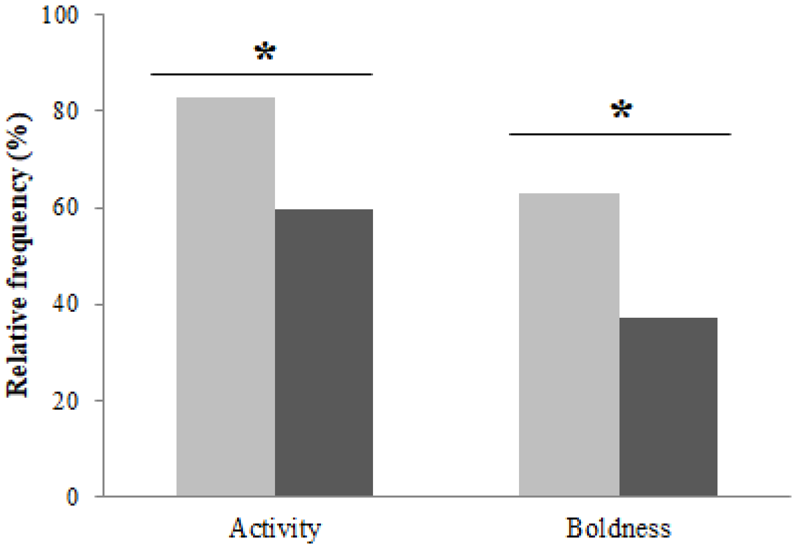


Figure 16. *Relative frequency (%) of active fish (activity) and active attempts to negotiate the ramp (boldness) in each of the two experimental treatments: control (light grey) and heatwave (dark grey). The Poisson Generalized Linear Model revealed significant differences between control and heatwave fish in terms of both activity and boldness (“*”, p < .05).*

Contrastingly with activity and boldness, no clear effect of the treatment was observed on shoal cohesion (β = −0.085, z = −1.796, p = .073). The time of sampling was also a non-significant variable for this behavioural trait (β = −0.001, z = −0.818, p = .413). However, similarly to activity, this behavioural trait was influenced by the number of fish in the channel (β = −0.185, z = −3.963, p < .001). Fish were found to be alone (i.e. with no fish within one body length distance) only once, in the control treatment (**Fig. 17**). Shoal sizes of 2 and 3 fish occurred more frequently in control fish, and shoal sizes of 4 fish were found more often among heatwave fish, but with similar relative frequencies for each shoal size (**Fig. 17**).

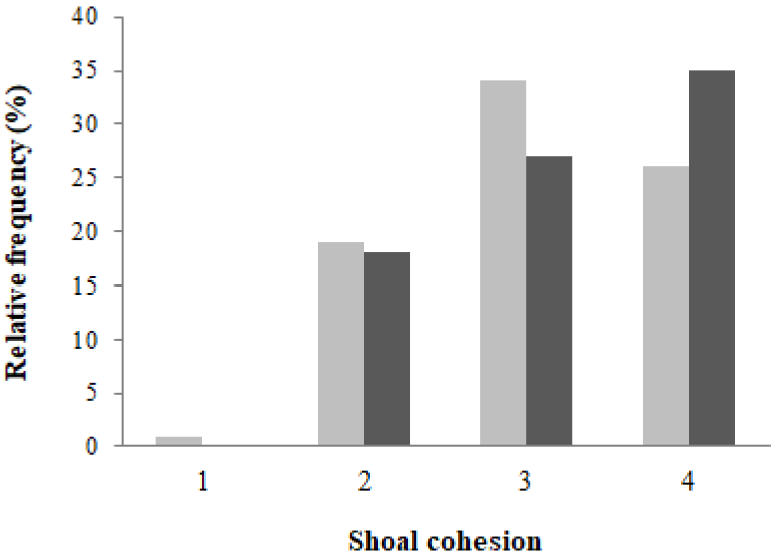


Figure 17. *Relative frequency (%) for each measured value of shoal cohesion (classified as 1, no fish within a body length of each other; 2, two fish within a body length of each other; 3, three fish within a body length of each other; 4, all fish within a body length of each other; 4, all fish within a body length of each other) in each of the two experimental treatments: control (light grey) and heatwave (dark grey). The Poisson Generalized Linear Model did not reveal significant differences in shoal cohesion between treatments (β = −0.085, z = −1.796, p = .073).*

4.6. Discussion

Sequential stressful events with intermittent stress relaxation may have additive, synergistic or antagonistic cumulative effects (Bevelhimer and Bennett, 2000; Gunderson *et al.*, 2016), depending on species identity, stressor identity, the existence of stress memory and the duration of the recovery phase (Gunderson *et al.*, 2016; Walter *et al.*, 2013). In Mediterranean climate regions, fish survival, dispersal and recruitment following flow re-establishment depend on habitat heterogeneity and favourable environmental conditions (Pires *et al.*, 2010; Magalhães *et al.*, 2002), such as water temperatures that are within their thermal tolerance ranges. Changes in thermal regimes, particularly increases in water temperature close to sublethal values following successive heatwaves, can lead to thermal stress and influence freshwater fish condition and survival (Beitinger *et al.*, 2000; Jesus *et al.*, 2016; Zaragoza *et al.*, 2008). This study assessed the effect of a simulated heatwave on the behaviour of juvenile Iberian barbel, contributing to reduce the knowledge gap on the effect of these extreme climate events on the behavioural responses of Mediterranean freshwater fishes. Overall, the heatwave treatment was characterized by a daily gradual temperature increase up to 5 °C, during six consecutive days and by lower levels of DO relatively to the control, but still considerably higher than the ones used in oxygen depletion studies on the Iberian barbel, which ranged from 1.4 mg L^{-1} to 4.4 mg L^{-1} for high and mild oxygen depletion, respectively (Branco *et al.*, 2016; Hayes *et al.*, 2019).

Fish activity under the simulated heatwave was found to be significantly lower than in control one as initially predicted, with this decrease in relation to temperature also being observed in another potamodromous species, the brown trout *Salmo trutta* (Colchen *et al.*, 2017), though it should be noted that the magnitude of temperature treatments was considerably lower (between 0 and 12 °C). Decreases in activity as consequence of other stressors (other than temperature) were also reported by Gonino *et al.* (2019) for the Iberian barbel, with the stressor being the high concentration of ashes in water, following wildfires in Mediterranean landscapes. Some studies concerning thermal stress have shown the opposite effect, i.e., fish became more active when temperature increased (Baduy *et al.*, 2016; Bartolini *et al.*, 2014), but the later focused on tropical species that may experience higher temperature extremes in their natural habitats. It has also been hypothesized that higher temperatures can increase activity until the optimum thermal range of a species (Britton and Pegg, 2011; Vinagre *et al.*, 2018); however, when water temperature approaches the critical thermal maximum (CTM) of that species, individuals may become less active (Baras, 1995). Although CTM was not determined for the Iberian barbel, previous work by Jesus *et al.* (2016) involving transcriptome profiling in Iberian chubs (*Squalius carolitertii* and *Squalius torgalensis*) has shown that temperature increases from 18 °C to 30 °C at a rate of 1 °C/h (in a 12 h treatment) could increase gene expression of Heat Shock Proteins (HSPs), which respond to thermal stress. Furthermore, studies on the common barbel *Barbus barbus* (Linnaeus, 1858) revealed that CTM in juvenile stages varied between 31 and 32 °C (Rutledge and Beitinger, 1989; Souchon and Tissot, 2012), slightly above the mean temperature that fish experienced during the heatwave treatment in this study. During the dry season, Mediterranean fish populations may be exposed to temperatures higher than 30 °C (Jesus *et al.*, 2016; Pires *et al.*, 2010). Depending on the extent of summer droughts and/or loss of longitudinal connectivity, these habitats may become isolated from the river reach upstream and/or downstream, maintaining fish confined in pools (Cid *et al.*, 2017; Gasith and Resh, 1999), with no possibility to move to cooler waters. Further increases in river water temperature during one or subsequent heatwaves, coupled with additional stressors such as oxygen depletion and increased competition, can add further stress to native fish (Magoulick and Kobza, 2003).

Similarly to activity, the number of fish that entered the ramp in the upstream section of the flume and actively attempted to negotiate it (quantified as a measure of boldness) was also lower in heatwave fish than in control ones. Previous studies on different vertebrate species highlight a significant effect of boldness upon an individual's probability to disperse and migrate (Dingemanse *et al.*, 2003; Fraser *et al.*, 2001). Particularly in freshwater fish, it has been shown that the probability of migrating is higher in bolder individuals (e.g. in the roach *Rutilus rutilus*, Chapman *et al.*, 2011; in the mosquito fish *Gambusia affinis*, Cote *et al.*, 2010). Although bolder individuals may be more exposed to predators (Biro and Dingemanse, 2009), this greater willingness to disperse and explore novel environments may be beneficial when the predation risk is low, or when competition for resources is high at the original environment (Chapman *et al.*, 2011). This seems to be the case for some Mediterranean potamodromous species such as the Iberian barbel, as they move along the river throughout their life cycle to search feeding areas, refuge zones or spawning habitats, and where the predation risk is generally considered to be low due to the residual abundance of piscivorous fish (e.g. largemouth bass *Micropterus salmoides*), which seems to be the case of the Lizandro River, where fish sampling for this study took place. It should also be noted that while not being the focus of this study, passage efficiency (i.e. the number of successful attempts to overcome the ramp and move to the upstream tank) was also determined and was found to be relatively low for both treatments (15.3% in control and 10.3% in heatwave), though the features of the obstacle (47 cm long and 36 cm high ramp, at a slope of 50%) are not considered suitable for cyprinids in general (Larinier, 2002).

Shoal cohesion was characterized by assessing shoal size, i.e., the number of fish in the channel that were within one body length of each other, through instantaneous sampling of behaviour every 3 min. Contrastingly with our initial prediction and the other two behavioural traits (activity and boldness), no significant differences in shoal cohesion were found between heatwave and control fish. Previous works have shown that shoal cohesion and coordination among its members tends to decrease with increasing temperature alone (Bartolini *et al.*, 2014; Colchen *et al.*, 2017) or in combination with another stressor (e.g. acidification in meagre *Argyrosomus regius*; Maulvault *et al.*, 2018). Nonetheless, it should be noted that these studies used a different metric to assess shoal cohesion: the nearest neighbour distance (NDD), which consists of the closest distance of a fish to a conspecific (Tien *et al.*, 2004). In this study, distance between fish was only assessed at a precision of one body length to allow the observer in each flume to properly obtain a snapshot picture of three behavioural traits simultaneously (activity, boldness and shoal size) and instantaneously. The lack of significant differences in shoal cohesion may also be partially explained by fish activity, as some authors have suggested that an increase in activity may promote higher shoal cohesion (Pritchard *et al.*, 2001). This trend was observed in this study, with a significant (yet mild) association between these two traits (see **Fig. S4.2** in Supplementary data). Regardless of the relation between fish activity and shoal cohesion, evidence from previous studies suggests that reduced shoal cohesion may lower survival and dispersion (Bartolini *et al.*, 2014; Colchen *et al.*, 2017; Maulvault *et al.*, 2018), making it a suitable trait to assess the response to a stressor.

4.7. Conclusions

Overall, this study shows that the swimming behaviour of Iberian barbel is likely to be affected by the occurrence of heatwaves, displaying lower activity and boldness. This can have negative consequences on both individual and collective performance, affecting seasonal migrations for feeding and reproduction, and ultimately annual recruitment and population persistence. By assessing fish behavioural response at the individual level, we gain valuable information on how its populations may respond to environmentally stressful conditions (Killen *et al.*, 2013; Lennox *et al.*, 2019). It should be noted that these responses may depend not only on the duration and frequency of the heatwave (which in our study lasted six days), but also on the rate of temperature change (or experimental ramping rate, 1.6 °C/h in this work) and on its interaction with critical thermal limits and warming tolerances (Allen *et al.*, 2016) or flow conditions leading to plasticity in behaviour (Alexandre *et al.*, 2014). Such interactions should be considered in future assessments on the effect of heatwaves on freshwater fish. Moreover, other climate-driven stressors may come into play in association with heatwaves, due to a combination of both anthropogenic pressure and climate change, such as drought (Lennox *et al.*, 2019), salinization (Cañedo-Argüelles *et al.*, 2016, 2019) and dispersion of exotic species (Quiroga *et al.*, 2017), for which the distribution range may increase due to generally higher tolerance to temperature increases (see the work on the chanchito *Australoherus facetus*; Baduy *et al.*, 2016).

While this study focused on juvenile stages of a Mediterranean fish species that make use of pools during the dry season, it is expected that other life stages, larvae (Pankhurst and Munday, 2011) and adults (White *et al.*, 2019), as well as other freshwater organisms such as amphibians (Carreira *et al.*, 2016), crayfish (Carreira *et al.*, 2017), snails (Leicht and Seppälä, 2019) and macrophytes (Cao *et al.*, 2015) may also be affected by heatwaves. Potential consequences of increases in water temperature include changes in movement patterns, delays in migration and changes in microhabitat use in search for cooler water patches (Caissie, 2006; Jonsson, 1991; Olden and Naiman, 2010). One important conservation measure to counteract these negative heatwave impacts would be to restore and preserve autochthonous riparian forests (such as willow *Salix* sp., ash *Fraxinus* sp., alder *Alnus* sp. and poplar Populus sp.), that can provide instream habitat and overhanging shade, promoting cooler water patches (Kurylyk *et al.*, 2014; Ormerod, 2009; Torgersen *et al.*, 1999; Trimmel *et al.*, 2018). Recent studies have highlighted the role of cool water patches for fish species such as salmonids (e.g. Fullerton *et al.*, 2017; Hess *et al.*, 2016; Keefer *et al.*, 2019). These patches can act as thermal refugia, avoiding fish having to cope with physiological stress resulting from temperatures above their optimum thermal range (Farless and Brewer, 2017; Magoulick and Kobza, 2003). To conclude, future studies should focus on the interaction of the multiple stressors driven by climate change, for a broader understanding of the perturbation affecting freshwater fishes, and mitigation measures should focus on river restoration and management of thermal refugia.

CRediT authorship contribution statement: Daniel Mameri: Methodology, Investigation, Formal analysis, Data curation, Writing - original draft. Paulo Branco: Methodology, Investigation, Writing - review & editing. Maria Teresa Ferreira: Conceptualization, Writing - review & editing. José Maria Santos: Conceptualization, Supervision, Methodology, Investigation, Writing - review & editing.

Declaration of competing interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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4.9. Supplementary Material

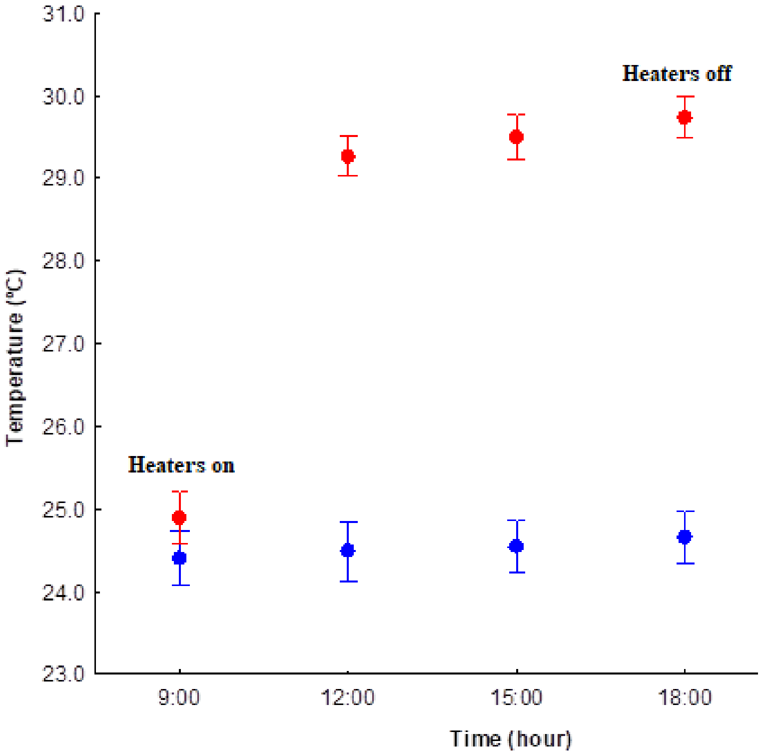

Figure 18. *Water temperature (ºC) in control (blue) and heatwave (red) treatments during the period when turned on (9:00-18:00h). Mean values and 95% confidence intervals for both treatments are represented by dots and arrows, respectively.*

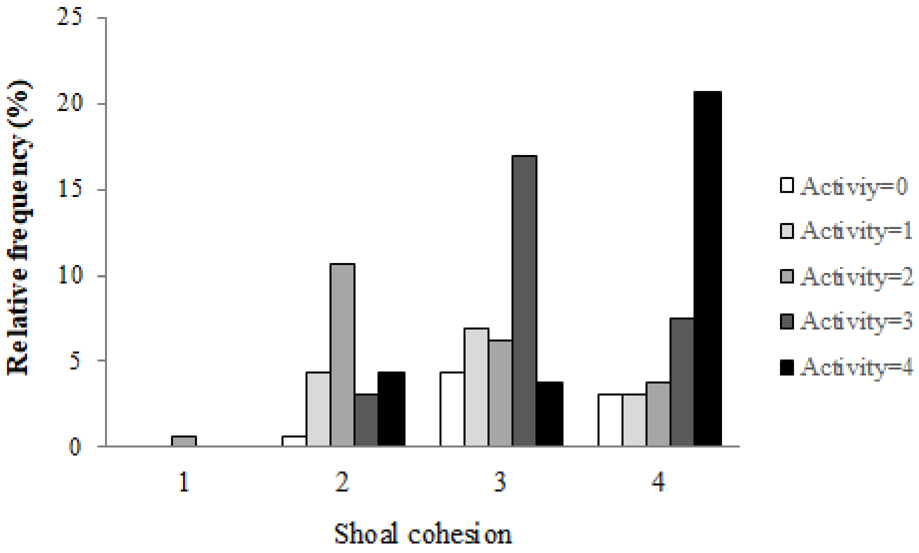

Figure 19. *Shoal cohesion in relation to fish activity (i.e. the number of active fish). A significant association was found between shoal cohesion (classified as 1, no fish within a body length of each other; 2, two fish within a body length of each other; 3, three fish within a body length of each other; 4, all fish within a body length of each other) and fish activity (β=0.057, z=3.175, p=0.002; Poisson Generalized Linear Model with activity as the single fixed effect).*

CHAPTER 5: Cold thermopeaking-induced drift of nase *Chondrostoma nasus* larvae

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CHAPTER 5: Cold thermopeaking-induced drift of nase *Chondrostoma nasus* larvae

D. Mameri [1]; D. S. Hayes [2], S. Führer [2]; E. Fauchery [3]; S. Schmutz [2], A. Monserat [4], T. Hasler [2], D. R. M. Graf [2], J. M. Santos [1]; M. T. Ferreira [1], S. Auer [2]

[1] Forest Research Centre (CEF) and Associate Laboratory TERRA, School of Agriculture, University of Lisbon, Tapada da Ajuda, 1349-017 Lisboa, Portugal
[2] University of Natural Resources and Life Sciences, Vienna, Department of Water, Atmosphere and Environment, Institute of Hydrobiology and Aquatic Ecosystem Management, Gregor-Mendel-Straße 33, 1180 Wien, Austria
[3] Graduate School of Engineering, University of Tours, 64 avenue Jean Portalis, 37200 Tours, France
[4] École Nationale Supérieure de l'Energie, l'Eau et l'Environnement, 21 Avenue des Martyrs, 38031 Grenoble, France

Resumo

A investigação em *hydropeaking*, que consiste na rápida variação da altura e velocidade da água a jusante de centrais hidroelétricas após a libertação de caudais turbinados, tem vindo a expandir-se rapidamente nos últimos anos, com um foco recente no impacto das variações de temperatura associadas (*thermopeaking*). As variações de caudal e temperatura podem afetar os movimentos dos peixes e padrões migratórios em fases críticas do seu ciclo de vida, ao induzir, por exemplo, a deriva catastrófica destas espécies, se não forem capazes de lidar com estas variações ou de procurar refúgio. Neste estudo, foi avaliado o impacto do *cold thermopeaking*, com descidas médias de temperatura de 5,5ºC no pico de caudal de um evento de *hydropeaking*, na deriva de larvas da espécie cipriniforme *Chondrostoma nasus*, em comparação com um tratamento de *hydropeaking* a uma temperatura constante. Os ensaios realizados nos canais da estação hidromorfológica de Lunz am See (*HyTEC – Hydromorphology and Temperature Experimental Channels*), nos meses de verão, revelaram um aumento da deriva das larvas para jusante dos canais com a descida abrupta da temperatura durante a subida (*up-ramping*) e pico de caudal (*peak flow*), estando os dois fenómenos fortemente associados. Também foi encontrada uma correlação entre a temperatura no início dos ensaios e a deriva observada na fase de aclimatação (sendo esta maior quanto mais fria estava a água). Os resultados obtidos sugerem que as larvas de cipriniformes poderão reagir mais fortemente a alterações abruptas de temperatura comparativamente a variações de caudal, e que é necessário manter um regime térmico adequado a jusante das centrais hidroelétricas, por forma a precaver a deriva de fases larvares e salvaguardando a migração para montante destas espécies.

Palavras-chave: temperatura da água, larvas, cipriniformes, energia hidroelétrica, canais experimentais, caudais turbinados

5.1. Abstract

Research on how intermittent water releases from hydropower plants affect the early life stages of fish has advanced in the last years, focusing not only on the direct impacts of rapid flow changes (hydropeaking), but also on the short-term fluctuations in water temperature (thermopeaking). Flow and thermal fluctuations caused by hydropeaking may affect fish movement patterns and migration at critical stages of a species' life cycle, e.g., by inducing passive downstream drift. Using two experimental outdoor channels, we investigated how nase (*Chondrostoma nasus*, Cypriniformes) larvae respond to a rapid drop in water temperature during hydropeaking (simulating a cold thermopeaking event), reaching on average 5.5 °C under peak flow (maximum discharge) conditions, in comparison with a hydropeaking treatment with a constant water temperature regime. Responses of fish larvae were analyzed during acclimation, up-ramping (increase in discharge), peak flow and down-ramping (decrease in discharge) phases. Fish drift increased during peak flow in the cold thermopeaking treatment compared to hydropeaking. Higher drift rates were also negatively associated with pronounced water temperature drops during peak flow conditions. In addition, the starting temperature of the experiment influenced drift during up-ramping. Overall, the results suggest that cold thermopeaking may increase drift in the early life stages of cypriniform fish compared with hydropeaking with stable water temperature. Hence, monitoring and active water temperature adjustments following hydropower releases should be adopted as strategies to mitigate power plant-related impacts on aquatic organisms.

Keywords: thermal fluctuations, young-of-the-year, cyprinids, hydropower, flume experiments, pulsed flows

5.2. Introduction

Throughout their life cycle, fish shift between habitats for feeding, reproduction, and sheltering (Lucas and Baras 2001). Drift, which can be defined as a downstream movement of aquatic organisms, willingly (active) or forced by water velocities exceeding a species' swimming capability (passive), plays a fundamental role in the migration of early life stages of fish such as larvae (Zens *et al.* 2018; Nagel *et al.* 2021). Drift has long been described as an important fish migration process to search for more suitable rearing habitats (Jonsson 1991; Lucas and Baras 2001; Reichard *et al.* 2004; Pavlov *et al.* 2008; Koster *et al.* 2013; Lechner *et al.* 2016). While fish swimming performance can determine the success of habitat shifts, these movements are triggered by changes in environmental conditions. Particularly flow velocity and water temperature are known to be among the main environmental drivers of fish migration in search of more suitable areas for each life stage (Jonsson 1991; Poff *et al.* 1997; Caissie 2006; Rakowitz *et al.* 2008; Webb *et al.* 2008; Olden and Naiman 2010).

Humans impact flow velocity and water temperature through the construction of river regulation infrastructure, of which hydropower plants are one of the most common worldwide (Steel and Lange 2007; Shen and Diplas 2010; Tofolon *et al.* 2010; Jones and Petreman 2014; Couto and Olden 2018; Hayes *et al.* 2018; Song *et al.* 2018). Hydropeaking is one operation mode of hydropower plants, consisting of short-term flow fluctuations downstream of dams caused by the rapid release of water from turbines due to peaks in energy demands (Greimel *et al.* 2018; Hayes *et al.* 2022a). Research on hydropeaking increased significantly in the last decade (Boavida *et al.* 2015; Auer *et al.* 2017; Romão *et al.* 2018; Costa et al. 2019; Amaral et al. 2021; Hayes *et al*. 2021; Führer *et al.* 2022). Particularly for early life stages of fish, such as larvae and juveniles, the impacts of hydropeaking can endanger successful recruitment and, ultimately, their survival, as it may cause fish to become stranded or passively drift, making them unable to reach critical habitats for life cycle requirements (Kupren *et al.* 2011; Rolls *et al.* 2013; Wang *et al.* 2013; Lechner *et al.* 2018).

More recently, research on the ecological impacts of hydropeaking focused not only on the direct impacts of rapid flow changes, but also on the associated short-term fluctuations in water temperature (Carolli *et al.* 2011; Bruno *et al.* 2012; Schülting *et al.* 2016; Choi and Choi 2018; Feng *et al.* 2018; Auer *et al.* 2023), a process known as thermopeaking (Zolezzi *et al.* 2010). Thermopeaking occurs due to water stratification in reservoirs, following a seasonal pattern (McCartney 2009; Tofolon *et al.* 2010; Hayes *et al.* 2022a). When releases from deeper water layers in stratified reservoirs occur (hypolimnetic discharges), it may lead to a temperature drop in the receiving river (cold thermopeaking), particularly during the summer season. In alpine rivers, water temperature during peaking operations can cool down the water temperature downstream the dam up to 6 °C in spring and summer (Zolezzi *et al.* 2010). Contrastingly, in winter, the opposite pattern is observed, with an increase in temperature in the receiving river – warm thermopeaking (Zolezzi *et al.* 2010).

While awareness of the impacts of thermopeaking is growing, its ecological impacts on freshwater populations are still poorly understood. Much of the published literature on the ecological effects of thermopeaking focuses on macroinvertebrate drift (e.g., Carolli *et al.* 2011; Bruno *et al.* 2012; Schülting *et al.* 2016). Fewer studies have assessed the impacts of thermopeaking on fishes (Auer et al. 2023; Casas-Mulet *et al.* 2016). Understanding the impacts of abrupt changes in temperature due to thermopeaking is particularly important, considering that it can lead to involuntary downstream displacement, i.e., passive drift (Young *et al.* 2011; Auer *et al.* 2017, 2023). Such involuntary movements are likely linked to increased hydraulic stress (Fuiman and Batty 1997; von Herbing 2002). Also, fish may seek areas with more optimal temperatures, entailing habitat shifts (Keckeis *et al.* 1997; Schiemer *et al.* 2002; Auer *et al.* 2023).

Therefore, it is crucial to understand how fish cope with rapid changes in flow conditions and associated short-term thermal variations caused by hydropower activity to establish the best mitigation frameworks, including active temperature adjustment of the water released during hydropeaking. In this study, we evaluated the impact of hydropeaking and cold thermopeaking on larvae of nase *Chondrostoma nasus* (L.), a cypriniform species for the conservation of European rivers, whose populations have been declining in the last several decades (Jurajda 1995; Schiemer *et al.* 2002; Hayes *et al.* 2022b). We performed fume experiments in an outdoor semi-natural stream facility to assess whether cold thermopeaking could lead to a higher fish drift than hydropeaking without temperature changes. We predicted that (1) hydropeaking with cold water release (cold thermopeaking) would entail greater fish larvae drift than hydropeaking with constant water temperature, and that (2) the more pronounced the temperature drop, the higher the drift will be, and (3) fish drift will occur not only in higher velocity areas, but also closer to the shoreline (Auer *et al.* 2023).

5.3. Material and Methods

5.3.1. Experimental set-up

This study was conducted in the summer, from July 22nd until August 3rd, 2021. All trials were conducted during daylight, from 8:30 to 18:30, in the absence of rain. We used nase larvae (mean TL = 25.1 mm ± 2.1 SD) from a fish hatchery in Lower Austria that used wild-caught breeders (Auer et al. 2017). Larvae were transferred to the HyTEC (Hydromorphology and Temperature Experimental Channel) facility in Lunz am See (Lower Austria) and reared in circular holding tanks (with an approximate volume of 0.7 m^3 each) 1 month before the start of the experiments. The tanks had a continuous water supply from lake Lunzer See and fish were fed 2–4 times a day with live brine shrimp (*Artemia*) at different hours each day to avoid learning effects (Brodersen *et al.* 2008). Larvae were in the sixth (VI) larval stage of development (Penaz 1974), with a total length ranging from 20.9 to 29.2 mm at the start of the experiments.

The experimental facility consists of two outdoor seminatural channels, 40 m long and 6 m wide, a research station for controlling the water discharge in the two channels, and two pressure pipelines that take the required water from Lunzer See and transport it to the channels (Fig. 1). One pipe is located near the surface and the other is installed deeper, allowing temperature manipulation in both channels and fish tanks. The water is led back into Lunzer Seebach, the lake’s natural run-of, via a height-adjustable dam beam construction at the downstream end of the experimental channels. Channel water temperature, depth, and flow velocity are easily adjusted, guaranteeing controlled and repeatable experimental conditions (Auer *et al.* 2023; Haug *et al.* 2022). The two parallel channels had a longitudinal slope of 0.5% and a lateral slope of 5%, with a fat bank substrate dominated by fine gravel and coarse sand (median grain size d_{50}=2.0 mm, 90th percentile grain size d_{90}=5.1 mm). The experimental area encompassed the most downstream section of both channels (4 m length and 3 m width), with five drift nets installed at the lower end of each channel (**Figure 20**). Flow velocity measurements (Flowtherm NT–Hoentzsch) were conducted immediately before the start of this study and checked on both channels during the experimental period to ensure identical flow velocity gradients. Water depth and flow velocity were measured every 30 cm along a cross section, with up to three measurements alongside the water column in each point coordinate (**Figure 21**).

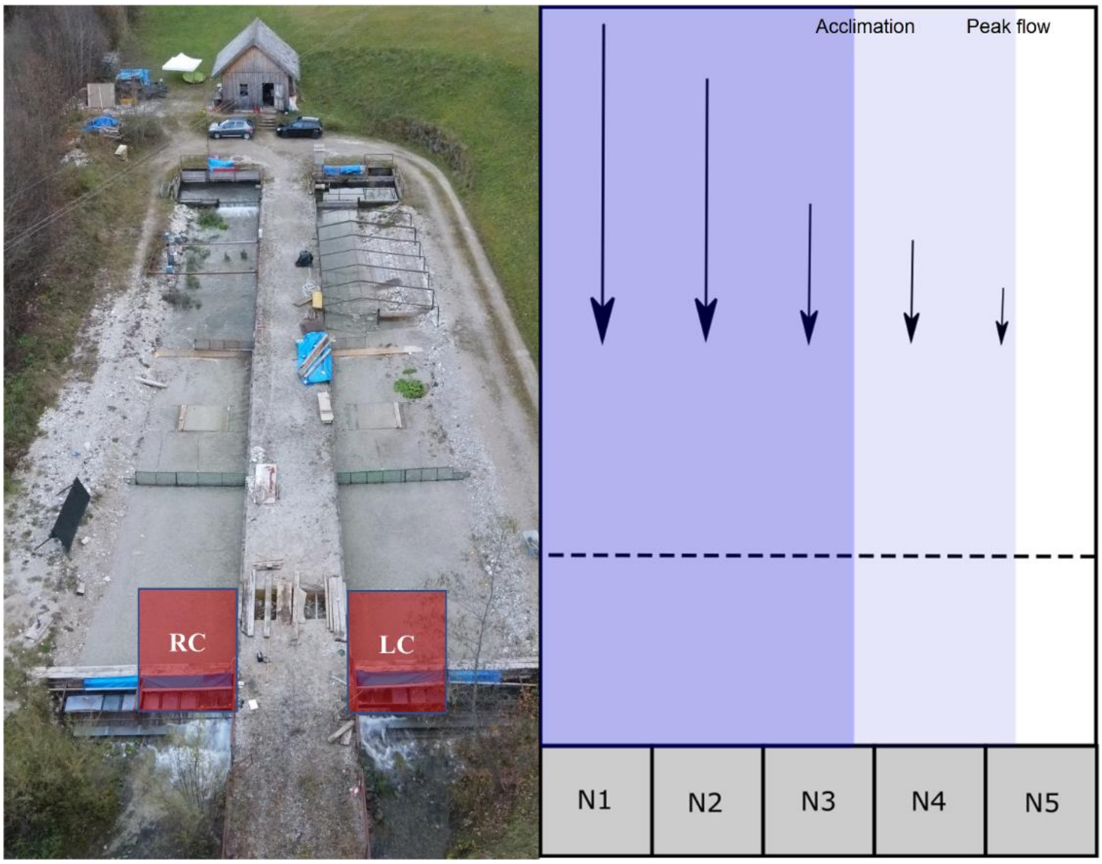

Figure 20. *(Left) Aerial picture of the experimental facility (https://hydropeaking.boku.ac.at). The lower sections of both channels (RC–right channel, LC–left channel, both in red) were used for the trials. (Right) Scheme of the experimental area in LC (symmetrical to the one in RC): flow direction (and velocity) is indicated by the (length of) arrows, and the downstream drift nets (64 × 75 cm) are numbered from the deepest and fastest segment (N1) to the shallowest and slowest segment (N5); the wetted area at acclimation (wetted width: 1.50 m) and peak flow (2.60 m) are represented by the blue and light blue fills, respectively; the dashed line refers to the cross-section where flow velocity was measured (**Figure 21**).*

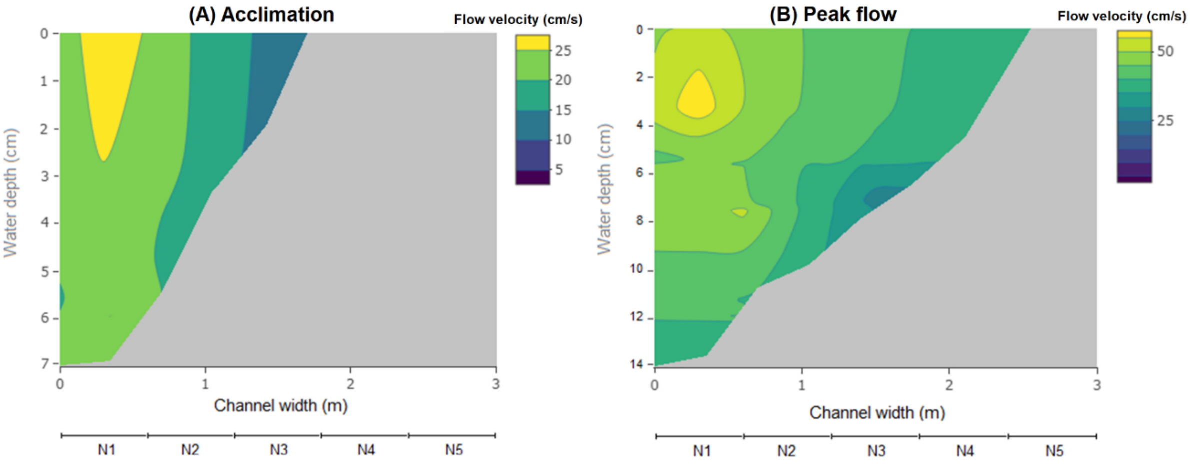

Figure 21. *Channel hydraulics during (A) acclimation (15 L·s^{-1}) and (B) peak flow (80 L·s^{-1}). Water depth (y-axis, in cm) and flow velocity (contour, in cm·s^{-1}) were measured every 30 cm along a cross section (**Figure 20**), from the deepest part of the channel to the shoreline (x-axis), with up to three measurements alongside the water column in each point coordinate. The color legend is relative to each plot. The area without water (including the gravel bank with a lateral slope of 5%) is represented in grey. Nets range from N1 (fastest and deepest segment) to N5 (slowest and shallowest).*

Two hydropeaking treatments were implemented: one with a constant temperature regime ("hydropeaking") and a second one where the temperature dropped rapidly during hydropeaking ("cold thermopeaking"). Each trial lasted 30 min, starting with 50 nase larvae being transported in opaque buckets and stocked in the upstream channel section (3 m ahead from the drift nets and 1 m behind the upper limit of the trial area) at a base flow of 15 L·s^{-1}. The water temperature in the tanks (mean = 20.0 °C ± 1.6 SD) and channels (mean = 19.8 °C ± 1.7 SD) was nearly identical, with an average temperature difference of 0.3 °C. Also, the starting temperature in hydropeaking (mean = 19.8 °C ± 1.8 SD) and cold thermopeaking (mean = 19.8 °C ± 1.7 SD) trials was the same. Each experiment consisted of four phases, simulating a single-peak hydropeaking event in Alpine rivers facing flow regulation by hydropower plants (adapted from Auer *et al.* 2017): (1) a 10-min acclimation period at baseflow, (2) up-ramping, with a discharge increase during 5 min, with a vertical up-ramping rate of 1.5 cm·min^{-1}, (3) peak flow, with a discharge of 80 L·s^{-1} that was maintained for 10 min, and (4) a 5-min down-ramping, resulting in a vertical down-ramping rate of 1.4 cm·min^{-1} (**Figure 22**). In each phase, we counted the fish that drifted into the nets at the downstream end of the channel (**Figure 20**). After each trial, all remaining larvae were cleared from the channel at base flow using hand nets. Overall, nine replicates were performed for each treatment. Drift was compared between the two different treatments (hydropeaking versus cold thermopeaking) for each phase of the trials (acclimation, up-ramping, peak flow, and down-ramping); additionally, comparisons in drift among the different nets (N1–N4) were also performed (Auer et al. 2023). The temperature was recorded on a minute basis using a multiparametric probe (Flowtherm NT–Hoentzsch), complemented with values recorded using pressure probes (Aquitronic ATP05). During cold thermopeaking, water temperature started dropping during up-ramping, reaching its maximum drop during peak flow [mean drop ± SD = 5.5 ± 1.7 °C compared with hydropeaking (**Figure 23**)].

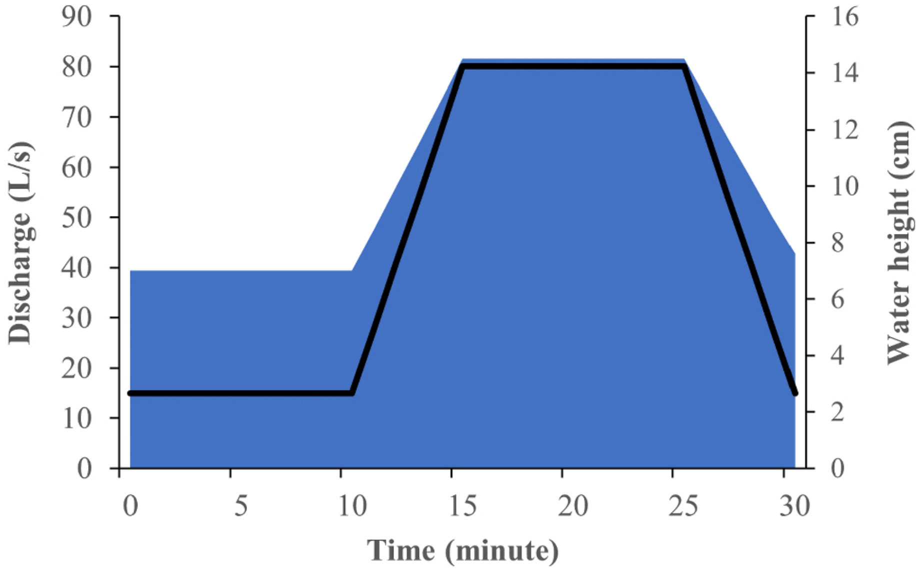

Figure 22. *Overview of the experimental setup: channel discharge, in L·s^{-1} (black line) and water height, in cm (blue area) throughout each trial (total duration of each trial: 30 min).*

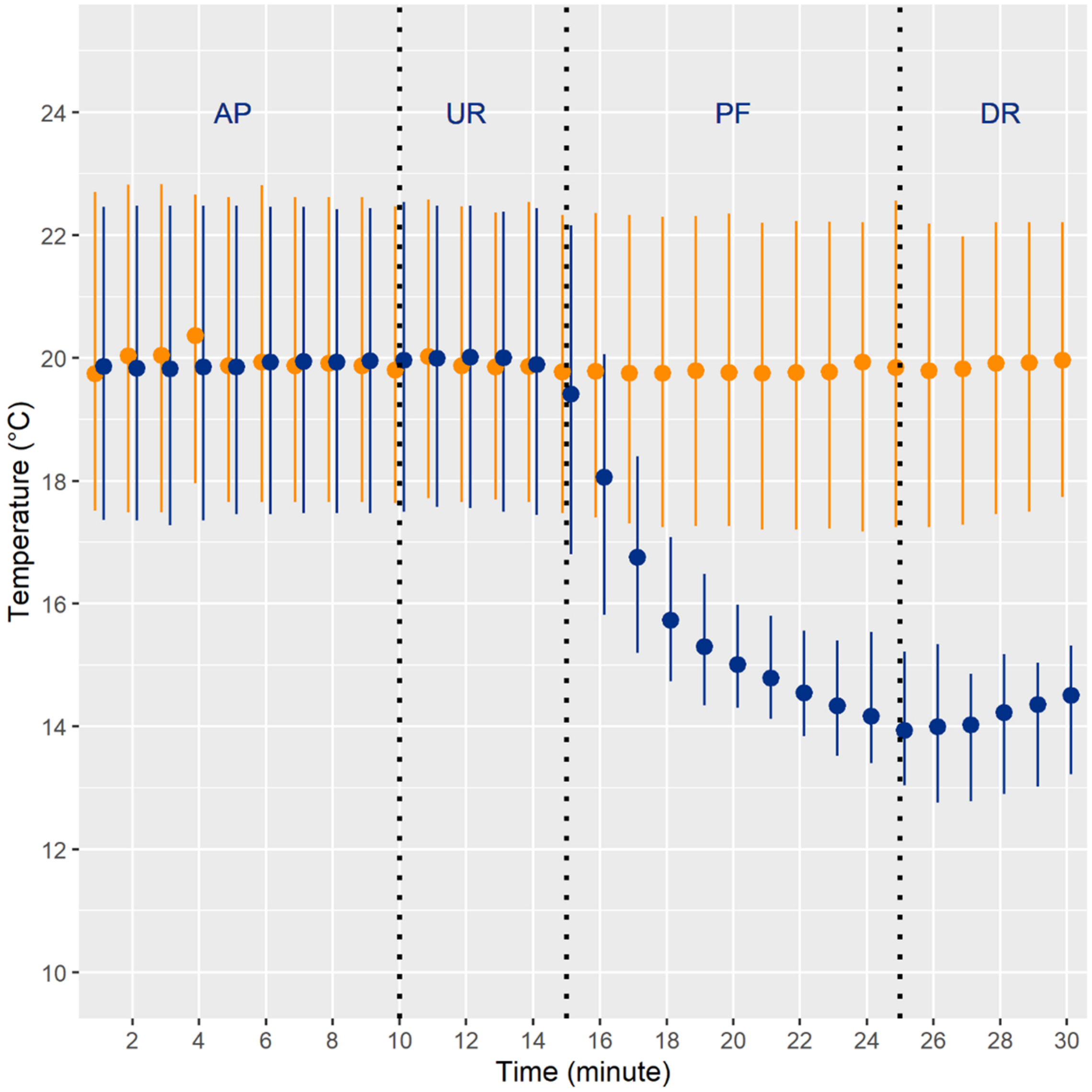

Figure 23. *Temperature measurements during hydropeaking (orange) and cold thermopeaking (blue) experiments (1-min time resolution), for each phase of a trial: acclimation phase (AP), up-ramping (UR), peak flow (PF), and down-ramping (DR). Dots and whiskers represent the mean values and 95% confidence intervals, respectively. At the start of up-ramping (minute 10), there was a water temperature drop in the cold thermopeaking treatment, but not in hydropeaking (where temperature remained constant).*

5.3.2. Data analysis

Larvae drift, expressed as drift rates, was obtained by dividing the absolute frequency of drifted fish (per phase, considering all nets) by the absolute frequency of fish present in the channel at the beginning of each phase (i.e., that did not drift in previous phases) (Auer et al. 2017):

$$Drift\ rate = \frac{frequency\ of\ drifted\ fish}{frequency\ of\ fish\ in\ the\ channel}$$

Data normality and homoscedasticity were assessed using the Shapiro–Wilk and F test of equality of variances, respectively. As both assumptions were not met, comparisons of drift between different experimental treatments (hydropeaking and cold thermopeaking) were performed with the non-parametric Mann–Whitney test. Differences among trial phases and lateral drift distribution [in which net fish were found (**Figure 20**)] were evaluated using the non-parametric Kruskal–Wallis test (followed by Dunn *post hoc* tests for pairwise comparisons). Effects of hydropeaking and thermopeaking on fish drift were further explored through multiple regression models, following a stepwise approach where the starting temperature of each trial and the temperature drop at peak flow were included as predictors for fish drift in each phase, considering all trials pooled together (n=18). The "drop1" function from the "stats" package (R Core Team 2021), which uses the Akaike Information Criterion (AIC), a weight of evidence approach, was used to obtain the most parsimonious model (with the lowest AIC), with the thermal variables most associated with fish drift in each phase. Finally, a chi-squared test was performed to assess if the lateral drift distribution (in which net the fish were found) depended on the treatment (hydropeaking and cold thermopeaking). All analyses were conducted in R (version 4.1.0) at a significance level (α) of 0.05.

5.4. Results

5.4.1. Drift during a hydropeaking event

Drift was significantly different between the phases of the hydropeaking ($\chi^2 = 19.276$, df = 3, $p < 0.001$) and the cold thermopeaking treatments ($\chi^2 = 19.649$, df = 3, $p < 0.001$). Fish drift was highest during acclimation for both treatments, representing around 30% of the total drift observed in all trials (**Figure 24**). For hydropeaking trials, drift during acclimation was significantly higher (mean ± SD = 0.32 ± 0.17) than the ones observed in the subsequent phases (Dunn *post hoc*, $p < 0.05$): up-ramping (mean ± SD = 0.04 ± 0.03), peak flow (mean ± SD = 0.05 ± 0.05), and down-ramping (mean ± SD = 0.03 ± 0.04). For thermopeaking trials, fish drift at acclimation (mean ± SD = 0.29 ± 0.16) and peak flow (mean ± SD = 0.18 ± 0.15) were not significantly different (Dunn post hoc, p = 0.176). However, both were significantly higher than during up-ramping (mean ± SD = 0.05 ± 0.07) and down-ramping (mean ± SD = 0.04 ± 0.07). Comparing the two treatments per phase, results showed that larvae drift in peak flow was higher during cold thermopeaking than during hydropeaking (Mann–Whitney, U = 15, $p = 0.027$). These differences were not observed in the other three phases (Fig. 5): acclimation (U = 44, $p = 0.790$), up-ramping (U = 42, $p = 0.928$), and down-ramping (U = 35, $p = 0.616$).

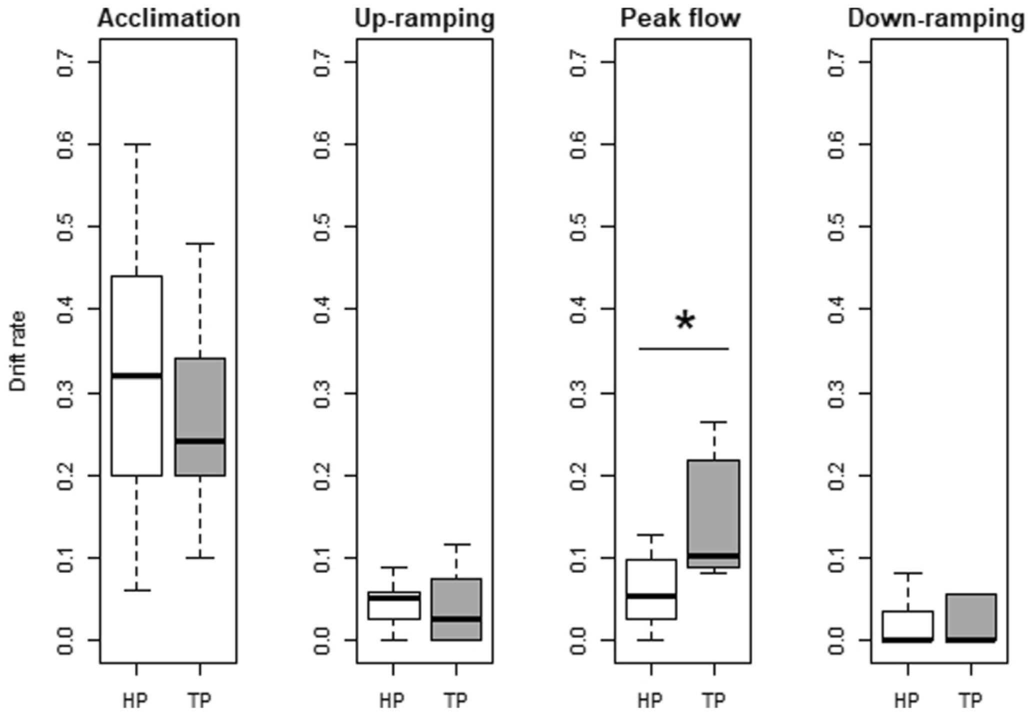

Figure 24. *Drift rates of hydropeaking (HP – white) and cold thermopeaking (TP – dark grey) treatments during each of the four phases: acclimation, up-ramping, peak flow, and down-ramping. Bold lines and whiskers outside the boxes refer to median values and interquartile ranges, respectively. Significant differences (p < 0.05) in drift rates between HP and TP are marked with "*" (peak flow: U = 15, p = 0.027).*

5.4.2. Temperature influence on drift

An effect of the starting temperature and temperature drop was observed at different phases of the trials. The final adjusted models for each phase retained only one variable each: starting temperature for acclimation and up-ramping, and temperature drop magnitude for the peak flow and down-ramping phase (**Table 5**). However, significant regressions were only found for drift during up-ramping (with starting temperature as a predictor) and peak flow (with temperature drop as a predictor). For up-ramping, this association was negative: lower (colder) starting temperatures were associated with higher fish drift during up-ramping ($\beta = -0.469$, $R^2 = 0.176$, $p = 0.047$). On a similar level, during peak flow, higher temperature drops (colder water) were linked to more pronounced fish drift ($\beta = 0.509$, $R^2 = 0.192$, $p = 0.039$).

Table 5. *Summary of the stepwise regression models for drift rates in each experimental phase (acclimation, up-ramping, peak flow, down-ramping), considering all trials pooled together (n=18). The standardized beta coefficient (β), adjusted R^2, and p value of the variables retained in the best-fitting model (lowest AIC - Akaike Information Criterion) are presented for each phase (significance at α=0.05 marked with an asterisk "*").*

Phase	*Acclimation*	*Up-ramping*	*Peak flow*	*Down-ramping*
Starting temperature				
β coefficient	–0.456	–0.469		
Adjusted R^2	0.159	0.176	-	-
p value	0.057	0.047*	-	-
Temperature drop				
β coefficient			0.569	0.374
Adjusted R^2	-	-	0.192	0.137
p value	-	-	0.039*	0.079

5.4.3. Lateral drift distribution

Considering all trials pooled together, the majority (90%) of displaced fish were found in N1 (43%) and N2 (47%), located in mid-channel areas of higher flow velocities, reaching up to 58 cm·s^{-1} during peak flow conditions (**Figure 21**). Contrastingly, few fish drifted into N3 (9%) and N4 (1%). N5 was only partially wetted during the trials (**Figure 20**), even during peak flow, and no fish were found in this net. Significant differences were found in the lateral drift distribution of both treatments, considering all phases ($\chi^2 = 10.1$, df = 7, $p = 0.018$). This difference was mainly caused by the observed drift within the first 1.6 m of the channel (N1–N3) during peak flow, where higher drift rates were found for cold thermopeaking fish than for hydropeaking ones (**Figure 25**).

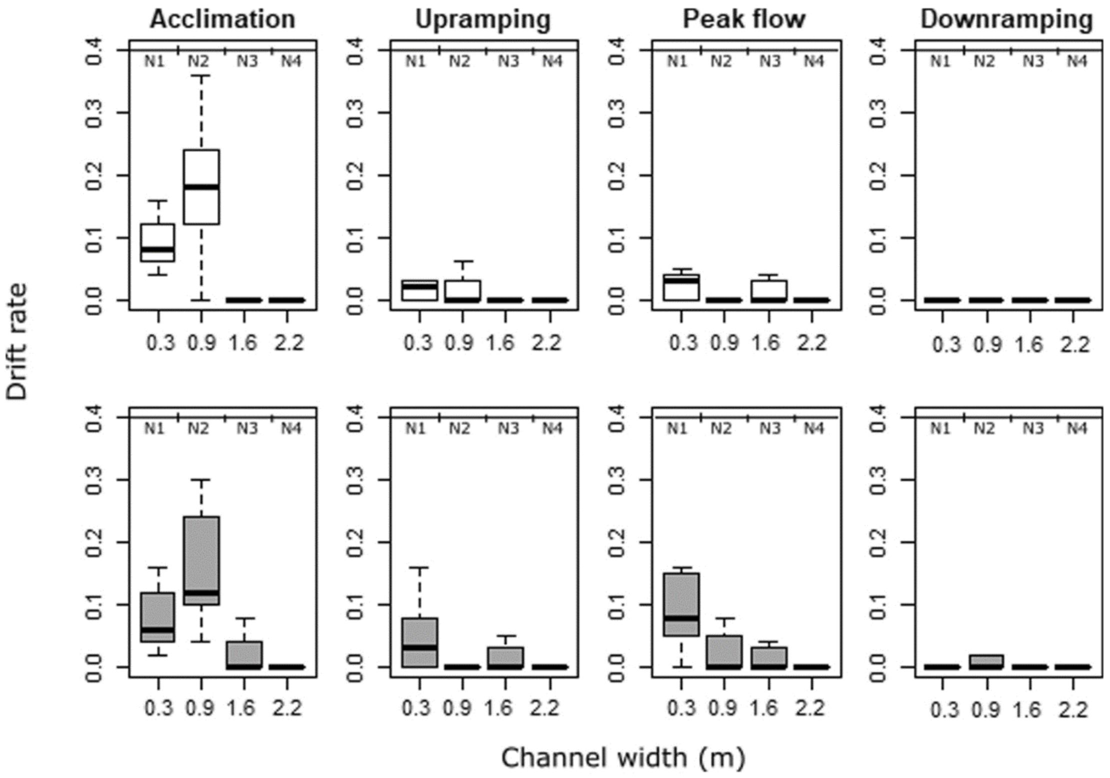

Figure 25. *Drift rates at hydropeaking (HP – white) and cold thermopeaking (TP – dark grey) in relation to the distance from the channel (0.3, 0.9, 1.6, and 2.2 correspond to the mid-points of N1, N2, N3 and N4, respectively; no drift was observed in N5), during each of the four phases: acclimation, up-ramping, peak flow, and down-ramping. Bold lines and whiskers refer to median values and interquartile ranges, respectively.*

5.5. Discussion

5.5.1. Hydropeaking and thermopeaking impact on drift

The survival of early life stages of fish strongly depends on favorable habitat conditions. Adequate flow and thermal conditions can determine recruitment success and, subsequently, population viability (Lucas and Baras 2001; Sonny *et al.* 2006; Kupren *et al.* 2011; Hayes *et al.* 2021). Hence, it is crucial to understand how early life stages of fish are affected by rapid and artificial variations in flow velocity and water temperature caused by hydropeaking power plants. Such process-based knowledge is needed to evaluate the impacts of hydropeaking and thermopeaking, and to develop adequate mitigation strategies (Schmutz *et al.* 2015; Sanz-Ronda *et al.* 2019; Hayes *et al.* 2022a). By conducting this experimental study, we aimed to assess how hydropeaking and cold thermopeaking affect nase larvae drift.

In both hydropeaking and cold thermopeaking treatments, drift was highest during acclimation. This result is consistent with preliminary trials performed with nase larvae, where the drift was also higher in the first minutes after stocking than the rest of the time (**Figure 26**). Hence, this initial period not only allowed fish to acclimate to the experimental setup under controlled conditions, but also served to remove potentially unfit fish before the start of the experiment (Auer *et al.* 2017; Mameri *et al.* 2019). In the hydropeaking treatment, the drift rate did not differ during up-ramping, peak flow, and down-ramping. In contrast, at cold thermopeaking, the drift rate was significantly higher during peak flow (when the temperature change was more pronounced) than during up-ramping and down-ramping. Sudden drops in temperature under rapidly increasing flows, accompanied by increased hydraulic stress, may promote downstream drift, potentially as a behavioral response to maximize successful dispersal (Lechner *et al.* 2013; Zens *et al.* 2018), which may be harmful if there is no suitable habitat downstream (as in the case of this experiment, with the drift nets). The results suggest that nase larvae react more strongly to temperature changes than to changes in hydraulic conditions (Zitek *et al.* 2004; Rolls *et al.* 2013). Indeed, rapid decreases in water temperature can also lead to “cold shock” in fish and reduced swimming performance (Smith and Hubert 2003; Donaldson *et al.* 2008). Looking into each phase, peak flow exhibited the greatest temperature drop of the thermopeak (surpassing 5 °C), and higher drift rates were positively associated with the magnitude of the temperature drops during peak flow (occurring in the cold thermopeaking trials).

Swimming performance depends on water temperature, and when facing suboptimum thermal conditions, a fish’s swimming ability may be compromised due to lower levels of oxygen diffusion in the skeletal muscles (Farrell 2002). This phenomenon may have reduced larvae responsiveness to increased flow conditions (Donaldson *et al.* 2008; Morgan *et al.* 2022), even though the water temperature during the trials was within the thermal tolerance range of the nase (8.0–29.0 °C; Leuven *et al.* 2011). This reduced swimming performance is likely to have, at least partially, led to an overall increase in drift rates in cold thermopeaking during peak flow when compared with hydropeaking. It should be noted, however, that only one temperature drop and one ramping rate (velocity at which the water level increases or decreases) were tested in this experiment. Future studies should assess larvae drift across gradients of temperature and water level variation to understand better the impact of these environmental conditions on fish swimming (Auer *et al.* 2023).

5.5.2. Lateral drift distribution and active drift

Lateral fish distribution across the drift nets revealed that larvae that experienced cold thermopeaking drifted more than hydropeaking fish in higher velocity areas in the channel (N1–N2) and closer to the shoreline (N3), suggesting that fish may be less active when exposed to temperature drops (Martelo *et al.* 2013). The increased drift closer to the shoreline (N3) during cold thermopeaking trials, which has also been observed for juvenile grayling *Thymallus thymallus* (L.) (Auer *et al.* 2023), may indicate that fish were trying to avoid colder temperatures by moving into the shallow zones in search of thermal refugia. In nature, young stages of cypriniform species are also known to shift closer to the shoreline to avoid faster currents (Bodensteiner and Lewis 1994; Copp *et al.* 2002; Reichard and Jurajda 2004; Lechner *et al.* 2013; Greimel *et al.* 2018). However, the flow velocity measurements at base flow conditions were far below the velocity thresholds described for this species. Indeed, the critical flow velocity for nase larvae with 15–25 mm length is estimated to be 4–5 times their corresponding body length (Flore *et al.* 2001). These critical values were only exceeded during peak flow in the mid-channel section, but not during base flow conditions.

It should be noted that we did not distinguish between active and passive drift. However, some studies have highlighted the importance of active larvae drift in downstream dispersal (Robinson *et al.* 1998; Reichard *et al.* 2004; Pavlov *et al.* 2008; Lechner *et al.* 2016). Particularly for the nase, a study in the Danube River revealed that larvae were more active during low flow conditions, thus suggesting that the hydraulic conditions could facilitate active dispersal (Lechner *et al.* 2018). The high drift rates observed during acclimation in both treatments (hydropeaking and cold thermopeaking), as well of base flow trials, therefore, seem to suggest that active drift may have occurred. Further evidence comes from the fact that cold thermopeaking fish drifted more in N3 than in hydropeaking. Behavioral studies in nature-like channels, like the ones presented here, would help to clarify the role of active and passive drift in the total observed drift caused by hydropeaking and thermopeaking.

5.5.3. Management recommendations

Earlier studies suggested that hydropower releases into rivers should be adapted to avoid peaking during key life cycle periods of aquatic species (Jones and Petreman 2014; Hayes *et al.* 2019; Moreira *et al.* 2019). The release of ecological flows, following recent European policies for the Water Framework Directive implementation, can provide an effective mitigation measure to dampen not only flow ramping rates, but also temperature changes during hydropower releases (EU Commission 2015), namely by adapting water releases during hydropeaking that mimic natural temperature fluctuations (Casas-Mulet *et al.* 2016; Heggenes *et al.* 2017; Tonolla *et al.* 2017; Halleraker *et al.* 2022). According to Zolezzi *et al.* (2010), in Alpine rivers, thermopeaking can have the same magnitude as the one simulated in this experiment. This can potentially harm fish by causing increased involuntary downstream displacement, ultimately affecting population viability.

In addition to drift, medium and long-term effects of both cold and warm thermopeaking should also be investigated, such as displacement of larvae and early juveniles from the regulated site, food availability, and spawning success in the following reproductive season. Finally, one aspect which was not targeted in this experiment was stranding caused by the fish's inability to shift from shallow to deeper areas during down-ramping (Führer *et al.* 2022; Hayes *et al.* 2023). Conducting similar thermopeaking experiments with a larger variety of microhabitats may help to investigate how temperature fluctuations can promote habitat shifts and lead to larvae drift and stranding (Auer *et al.* 2017, 2023; Antonetti *et al.* 2023; Hayes *et al.* 2023).

5.5.4. Conclusions

Overall, our results highlight that hydropeaking, combined with cold thermopeaking, increases the drift of sensitive life cycle stages of cypriniform fish populations, if occurring during sensitive life cycle stages. To our knowledge, this was the first experimental study assessing the influence of cold thermopeaking in larvae of a cypriniform species and comparing it with hydropeaking. Hence, ensuring suitable flow and water temperature conditions for early life stages of migratory fish, downstream from hydropower plants, is of utmost importance to avoid involuntary passive drift and ultimately population decline. Monitoring and active adjustment of water temperature following intermittent water releases should be included as main mitigation strategies to establish best-practice hydropower operations.

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Data availability: Supplementary data is provided alongside this manuscript. Generated data from this study is available from the corresponding author upon reasonable request.

Declarations

Conflict of interest: The authors declare that there are no conflicts of interest.

Consent to publish: All authors have read and approved the final version of the manuscript.

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5.7. Supplementary Material

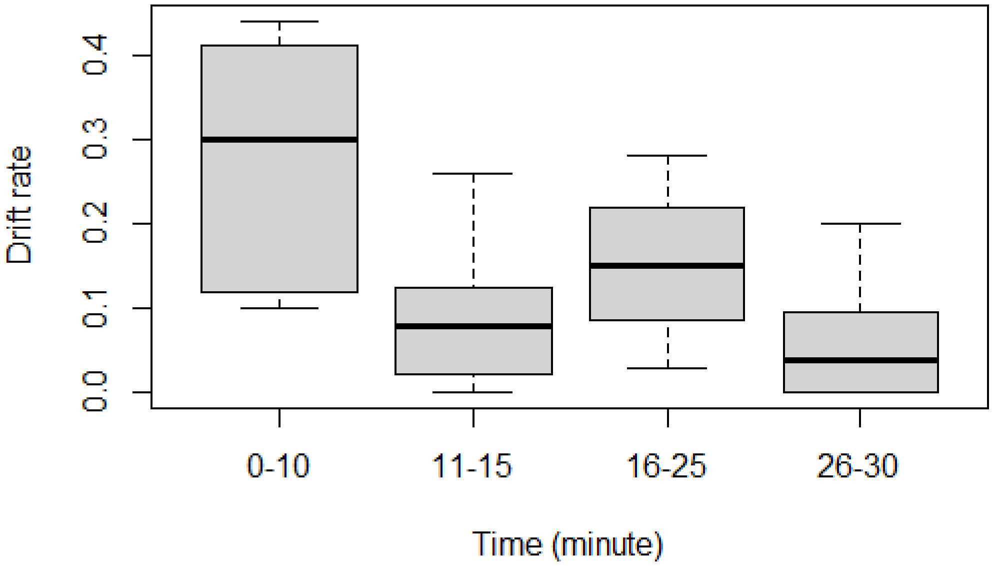

Figure 26. *Drift rates in base flow trials (50 fish stocked in each), with a constant discharge of 15 L s^{-1} for 30 minutes. Drift rates were calculated for the time periods corresponding to each of the experimental phases: acclimation (minutes 0-10), up-ramping (11-15), peak flow (16-25) and down-ramping (26-30). Bold lines and whiskers refer to median values and interquartile ranges, respectively.*

CHAPTER 6: Overall discussion and conclusions – where do we flow now?

6.1. Key-environmental variables for managing migratory fish populations

Managing rivers for aquatic populations involves addressing not only the maintenance of suitable conditions for these species to thrive, but also the remaining ecosystem services they provide, of which energy production frequently comes on top (Thieme *et al.* 2021). In a world of growing energy demands, hydropower assumes a fundamental role in energy production and consumption, with Portugal being no exception (Feio and Ferreira 2019). Climate change adds a further layer of challenges to already disturbed river systems, with the need to account for future environmental conditions in these already dynamic freshwater systems (Reid *et al.* 2019).

Flow and water temperature have long been regarded as key environmental drivers for habitat suitability in fish (Poff *et al.* 1997; Caissie 2006; Olden and Naiman 2010), while also acting as triggers for migration and habitat shifts in general. The Water Framework Directive (WFD) states the need to ensure the good ecological status of water bodies, which partially depends on keeping flow and thermal regimes in rivers as close to their natural regimes as possible, to ensure that critical stages of fish and, consequently, population persistence, is not compromised (Poff *et al.* 1997; Olden and Naiman 2010; Acreman *et al.* 2014; Hayes *et al.* 2022). For the Mediterranean, predictions suggest a decrease in river discharge and precipitation (van Vliet *et al.* 2013; Lobanova *et al.* 2016), and an increase in both mean and maximum temperatures, with the last one being associated to heatwaves (IPCC 2022). Based on expected scenarios on how environmental conditions and river dynamics, namely flow and thermal regimes, will change, one crucial first step is to understand how these changes in key environmental drivers will: (1) affect aquatic organisms in regard to current conditions; (2) whether they can cope and adapt to them; and finally (3) how can we mitigate these impacts, by implementing mitigation measures or adjusting the ones that are currently in place.

The natural dynamics of riverine systems and the species they harbour, with constant changes in environmental conditions, shape the life history strategies of aquatic species. Having to deal with growing anthropogenic stressors, either these species find new mechanisms to adapt to the changing environment, or, in most cases, they are unable to do so (at least fully) and are impacted by these stressors. Measuring the impact of these changes in aquatic organisms is challenging, as they vary across time and space. Evaluating these in migratory fish represents a further layer of complexity, as not only different life stages may react differently to stressors, but also the habitat where they can be found is likely to be different depending on whether they are at a larval, juvenile, or adult stage. Furthermore, impact can be manifested on the short, mid or long-term, and may vary from affecting a reduced number of individuals to an entire population.

In this thesis, focus was given into assessing changes in fish passability through small and large barriers (weirs and dams, respectively) and on swimming behaviour in response to changes in flow and thermal regimes, trying to fill knowledge gaps in the current literature. Regarding fish passability, the temporal range of the impact of flow changes ranged from a mid-term period (passability and fish lift efficacy across seasons, in Chapter 2) to a long-term period (habitat suitability and potential passability until the end of the century, in Chapter 3). As for the swimming behaviour, both experimental designs focused on the short-term effect of thermal changes in river temperature, caused by heatwaves (Chapter 4) and cold thermopeaking (Chapter 5).

6.2. Thesis novelties and take-home messages

The main goal of this thesis was to assess how fish movement could be affected by flow fragmentation and climate change, based on current and future scenarios of modified flow and thermal regimes, considering not only flow regulation and its direct impacts on the short-term (hydropeaking and thermopeaking), with implications to fish movement and fish passage, but also climate change and how environmental conditions are expected to change with it, namely through drought and extreme weather events. To accomplish this main goal, this thesis was structured in four chapters, which included: (1) two case studies dealing with barrier passability through fishways, under hydropeaking schemes (Chapter 2) and low flow conditions (Chapter 3); and (2) two experimental designs focusing on the swimming behaviour and activity after exposure to heatwaves (Chapter 4) and cold thermopeaking (Chapter 5).

In Chapter 2, fish passability was determined in relation to different environmental variables, time of day and year, powerhouse operation (two hydropeaking magnitudes and one ecological flow release, when both turbines were turned off) and fish size. A seasonal component in fish use of the lift was found for all species, with most of these movements being related to the reproductive season of these species, which were also associated with variations in key environmental drivers for fish passage in native potamodromous fish species, including mean flow, flow variation and water temperature. Several studies on fishway efficacy have been published in the past years, but fewer have combined field work with continuous video-monitoring of the fishway in the Mediterranean region (Croze *et al.* 2008; Santos *et al.* 2016). One major finding of this study was the different use of the fish lift by the three potamodromous species in relation to different hydropower release schemes (ecological flow, half-power with one turbine, and full power with two turbines), This result highlights the complexity of adjusting flow releases to the different species composing the fish community downstream barriers. For a more complete assessment, future studies should also consider the attractiveness of the fishway and the suitability of the river stretch downstream the barrier, in terms of guidance to the entrance by attraction currents, and at the entry (determining the fish decision to enter) should also be taken into account in future studies on fishway efficacy, namely by conducting telemetry studies with a sub-sample of the fish community downstream the dam.

One potential non-invasive approach to estimate fish passability is to model habitat suitability, based on the idea that if the habitat is suitable enough for a certain species and if its swimming performance allows it, fish will be more likely to overcome a barrier (Boavida et al 2020). In Chapter 3, habitat suitability in a river stretch of the Tagus River where an obstacle was present (Pego weir) was modelled as a measure of passability for different migratory fish species, in relation to different mean daily discharge scenarios, considering current flow and future flow conditions expected under different greenhouse gas emissions (RCP 4.5 and RCP 8.5). Overall, droughts and low flow conditions are expected to become more frequent considered the ongoing climate change and global warming predictions for the Mediterranean basin, with a persistent combination of lack of precipitation affecting river discharges and a sequence of heatwaves (Toreti et al. 2022; IPCC 2022). As the percentage of submerged area, particularly close to the fishway, is crucial to increase the chances of successful fish passage, ensuring minimum discharges during critical migration periods of these species would be of uttermost importance to allow a successful upstream migration. For the Pego weir, the River2D model revealed that a minimum flow of 3 m^3 s^{-1} is necessary to ensure the passage of all fish species through the fish ramp incorporated in the weir. Although it is a relatively low discharge which could be easily achievable by implementing a minimum ecological flow, the persistence of zero and low flow conditions is expected to increase under the RCP 4.5 and RCP 8.5 greenhouse gas emission scenarios, as revealed by their density distributions of mean daily discharge. Whereas the release of minimum "ecological" flows has been a raising concern in Portugal, and while some dams are currently on the processes of incorporating them, these may not be enough in scenarios of reductions in river discharges until the end of the century, as the different components of the natural flow regime should be incorporated in response to the fish communities present (EC 2015). It should be noted that currently, the thermal powerplant located in Pego is no longer functioning, and it is expected to be re-converted to a charcoal-fuelled thermal powerplant, complemented with renewable energy production (JN 2022). As the Pego weir is no longer serving its original purpose (water retrieval for turbine cool down at the thermal powerplant), the removal of the weir should be considered for a complete restoration of the longitudinal connectivity, following other barrier removals from the Dam Removal Europe initiative, with over 6,000 barriers removed (WFMF 2022) in this stretch of the Tagus River, rather than investing in a structural solution which will require constant monitoring, maintenance and will only partially ensure fish passage. However, in cases where barrier removal is not a possibility, a suitable and dynamic regime of flow releases in dams, particularly during critical migratory periods for fish species, should be adopted.

Focusing on fish swimming behaviour and activity in response to water temperature, the experimental designs in Chapters 4 and 5 showed that short-term exposures to altered thermal regimes can impact early life stages of fish. Specifically, in Chapter 4, the exposure to a heatwave can alter, even if only at short-term, the swimming behaviour of juvenile Iberian barbel, namely in terms of activity and boldness, which may limit its ability to explore more suitable habitats for feeding and growing, for instance. While some literature can be found on the impact of thermal stress on migratory fish species (Colchen *et al.* 2017; Madeira *et al.* 2013) and even heatwave effects on marine fish (Islam *et al.* 2020; Madeira *et al.* 2020), this study was the first published work on the effects of heatwaves in a Mediterranean freshwater fish species, thus filling a knowledge gap on thermal stress research, while considering the ongoing climate change. While not assessed in Chapter 4, the combined effect of temperature increases, together with other water quality parameters, such as lower dissolved oxygen (hypoxic stress) and ammonia and nitrogen derivatives, is also of relevance for fish species to tolerate stress overall, in response to oxygen availability and thermal fluctuations (Filice *et al.* 2021; Magoulick and Kobza 2003). Future experimental studies on fish thermal stress would also benefit from the measurement of physiological parameters after the trials, namely the ones associated with stress, such as enzyme activity and protein (e.g., HSPs – Heat Shock Proteins) metabolism (Jesus *et al.* 2016; Schleger *et al.* 2022). Moreover, the effects of chronic exposure to high and sub-lethal temperatures, as well as stress accumulation during periods of thermal fluctuations, should be investigated (Bevelhimer and Bennett 2000). The impact in a longer temporal range, such as delays in migratory routes, considering not just heatwaves but also the loss of longitudinal connectivity during the dry season, has yet to be assessed, and should be a priority in rivers facing intermittency, namely in the Iberian Peninsula and other Mediterranean-climate regions. These results reinforce the need to ensure suitable thermal habitats for cypriniform fish during the dry season, when several Mediterranean-climate rivers dry partially, forming a series of disconnected pools which will tend to warm during these extreme events (Cid *et al.* 2017). Identifying, protecting, and restoring autochthonous riparian forests, namely willow *Salix* sp., ash *Fraxinus* sp., alder *Alnus* sp., and poplar *Populus* sp., would provide instream habitat and overhanging shade, promoting cooler water patches (Dzara *et al.* 2019; Fullerton *et al.* 2017).

Finally, in Chapter 5, the effect of temperature drops during a hydropeaking event (cold thermopeaking) on larval drift were assessed in the cypriniform nase *Chondrostoma nasus*. This was the first experimental study assessing the influence of cold thermopeaking in larvae of a cypriniform species and comparing it to regular hydropeaking. Drift plays a fundamental role in fish migration, particularly in early life stages such as larvae and juveniles (Nagel *et al.* 2021; Zens *et al.* 2018), with a direct impact on annual recruitment and population persistence (Lechner *et al.* 2018). Research on thermopeaking in Alpine (but also temperate) rivers has been rapidly advancing in the last years and will hopefully be extended to Mediterranean-climate rivers, which are already facing other thermal and drought-related stressors, as previously mentioned. Specifically in Alpine rivers, cold thermopeaking is known to occur in thermally stratified reservoirs during hydropower generation in summer (Antonetti *et al.* 2022; Zolezzi *et al.* 2010), which corresponds to the season when this study took place. Overall, results showed that drift, which was generally low, significantly increased during the peak flow phase of a single hydropeaking event, when the discharge was highest (80 L s^{-1}) and the water colder (average drop of 5.5ºC), compared to hydropeaking at constant temperature. Furthermore, the highest drift rates during peak flow were found to be correlated with the magnitude of the temperature drop in the cold thermopeaking treatments: the colder the water, the higher the drift. One potential mitigation solution would be to adjust dam releases considering the critical migration and reproductive periods of the species moving upstream the reservoirs, namely by installing temperature control devices (Zarri *et al.* 2019). This study highlighted that nase larvae react stronger to sudden changes in temperature rather than flow (i.e., increasing discharge from base to peak flow). Furthermore, the lateral fish distribution observed suggests that a combination of high flow velocities, such as the ones fish experienced during hydropeaking and, more specifically, during peak flow, combined with a sudden temperature drop, is likely to be more impactful in fish larvae rather than the cold water itself, if fish are unable to laterally shift and shelter in lower velocity areas (Führer *et al.* 2022). Similarly to the impact of heatwaves in Chapter 4, consequences of rapid thermal changes on fish physiology (i.e., stress hormones and HSPs; Inoue *et al.* 2008), fitness, swimming performance, upstream migration and reproductive success should also be assessed in future studies. Finally, different magnitudes of thermal variation, not just for temperature drops, but also warm thermopeaking, should be tested to have a broader assessment of the impact of thermopeaking-inducing fish drift.

To conclude: some of the knowledge gaps on the impacts of flow regulation and climate change on migratory fish species will hopefully be filled with the results and conclusions obtained in this thesis, but there are still questions that remain (at least fully) unanswered. For instance: which are the mid and long-term consequences of fish exposure to rapid flow and thermal fluctuations, namely when we investigate their migratory patterns, occurrence and abundance within a river basin, and population persistence in rivers? Moreover, a considerable amount of work is yet to be done when it comes to improving flow management in regulated rivers. A crucial step would be optimizing current monitoring tools on key environmental variables, namely by ensuring a fully functional and widespread network of monitoring sites for assessing flow conditions in river stretches. Combining this monitoring network with the use of automatic devices, such as dataloggers, to measure thermal conditions in rivers and obtain high resolution datasets on water temperature, a significant amount of input data can be collected to model habitat suitability and future thermal conditions for migratory fish in rivers.

6.3. References

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