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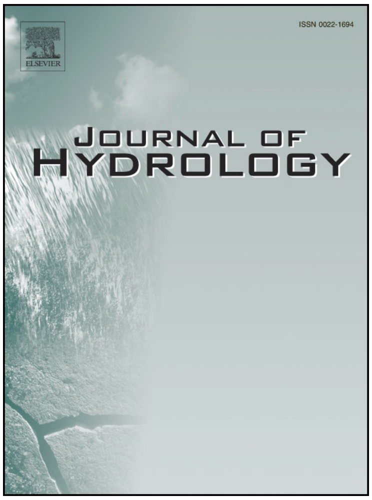

Research papers

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The effect of hydrological variability on stepped fishways

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Abstract

River systems are highly dynamic, affecting all associated structures and their derived uses. This is particularly relevant for applications such as hydropower production and other water abstractions. This dynamic nature also extends to mitigation measures like fishways, which are vital for reducing the impact of river fragmentation on fish populations. Fishways must be designed to balance biological and hydraulic fish requirements, needing adaptability to varying boundary conditions. This study examines the effect of hydrological variability on fish passage through fishways, particularly for the Iberian barbel (*Luciobarbus bocagei*). We hypothesized that hydrological scenarios can significantly affect upstream fish passage. To test this, we conducted laboratory and field studies, assessing fish movement under different boundary conditions. We compared passage rates, time metrics, and their correlations with the evolution of fishway hydraulics, and employed survival analysis to determine biometric limits. Our findings show that hydrological changes markedly influenced fish passage rates and timings, producing fish size selection and highlighting the impact of factors such as maximum velocity and power dissipation in the studied metrics. These insights underline the necessity of incorporating hydrological variability into fishway design and management, enhancing their effectiveness for fish conservation in river ecosystems, particularly under growing climatic uncertainties. This research underscores and discusses the need for comprehensive, long-term hydrodynamic studies in fishway assessment and design, advocating for adaptive management strategies to accommodate environmental changes.

Keywords: Fishway; Non-uniformity; Climatic uncertainty; Hydrological variability; PIT telemetry; upstream migration.

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1. Introduction

Successfully managing the use of natural water resources by the human society, such as hydropower production or other water abstractions and, at the same time, mitigating its potential impacts, requires a comprehensive understanding of the highly dynamic nature of river systems, where many variables, factors, and uncertainties are involved (Poff et al., 1997). With several interests at play, basin managers must balance conflicting targets: society and its dependence on ecosystem services, ecological requirements and compliance with legal directives (e.g. Habitats and Water Framework Directives, EU Biodiversity strategy), and overall ecosystem functioning (DeRolph et al., 2016). Energy security and resilience based on renewable energy is something that is on current political agendas (e.g. Repower Europe) and hydropower is viewed as the best-known solution as it is, at this point, the best energy storage solution. Nonetheless, hydropower generation is directly affected by the river's water levels and flow, which can be altered by changes in climate and precipitation patterns, water usage, land use practices, the presence and distribution of vegetation, and geological factors, all in different time scales (Lobanova et al., 2016; Moran et al., 2018). Any hydraulic solution designed to mitigate the impacts of hydropower generation –or other river uses– will also be subject to the same possible alterations (Yaseen et al., 2019). Therefore, it is crucial to consider these variabilities and uncertainties when designing and implementing mitigation measures.

Hydropower and water abstractions significantly impact river ecosystems primarily through habitat fragmentation caused by hydraulic structures such as dams, which disrupt river connectivity and hinder aquatic organism movement, including migratory fish species (Kuriqi et al., 2021; Nilsson et al., 2005; Richter et al., 1997). This fragmentation leads to cascading effects on the ecosystem, such as changes in species abundance, alterations in sediment dynamics, and the degradation of river landscapes, ultimately threatening biodiversity and ecosystem services (Poff et al., 1997; Power et al., 1996; Pringle, 2003). In Europe "Dams and Water management /use" has been identified by the International Union for the Conservation of Nature the threat that affects more native freshwater dependent fish species (Costa et al., 2021). Globally, it is estimated that only about 23% of large rivers remain free-flowing, and in Europe, over half of the river networks are fragmented, affecting up to 1.7 million kilometers of river habitat (Duarte et al., 2021; Grill et al., 2019).

Addressing this fragmentation is critical for river management and is supported by international regulations such as the EU Water Framework (2000/60/EC) and Habitats (1992/43/ECC) Directives. The EU's 2030 Biodiversity Strategy specifically aims to restore 25,000 km of rivers to a free-flowing state. To mitigate habitat fragmentation, the most commonly used structures are stepped fishways. The term "stepped fishways" encompasses all fishway types characterized by a succession of cross-walls and pools in a stepped pattern, including vertical slot, pool-weir, and step-pool nature-like fishways (Fuentes-Pérez et al., 2017). These structures help species bypass river barriers by providing a series of pools and cross-walls that divide the barrier's total height(H) into manageable steps or water drops (ΔH) (Clay, 1995; FAO/DVWK, 2002).

Fishways are extensively studied structures, guided by well-established design handbooks and guidelines (Clay, 1995; FAO/DVWK, 2002; Larinier et al., 2002; U S Fish and Wildlife Service, 2019). Despite the availability of this resources, designing effective fishways remains challenging, demanding a multidisciplinary effort that intersects hydraulic and civil engineering with biology and river basin management (Fuentes-Pérez et al., 2024; Williams et al., 2012). When the designing team is not multidisciplinary, there is an unintended focus on one aspect over others, creating design choices that create fishway ill-functioning, underscoring the delicate balance required between biological needs and hydraulic functionality. Ensuring this balance and adapting to the dynamic nature of river ecosystems are crucial for the resilience and effectiveness of fishways. The design process must be complemented by proper planning and post-construction assessment and management to enhance the performance and reliability of these structures.

Fishways, similar to hydropower production or other water abstractions, are subject to natural or artificial variability in rivers (Marriner et al., 2016). This variability or alteration can cause modifications to their boundary conditions, such as changes in upstream and downstream water levels. These changes can be triggered by various factors, including natural or artificial variability of river discharge, as well as modifications to the surrounding area during construction or other short, medium, and long-term alterations (e.g., vegetation growth, sedimentation, land use, climatic change, water abstraction) (García-Vega et al., 2018).

Modifications to boundary conditions can cause fishways to deviate from their fixed design working conditions, which are typically uniform scenarios (same depth and drops in all cross-walls, as shown in Figure 1) (Rajaratnam et al., 1986). Instead, fishways are subjected to non-uniform scenarios, i.e., different water levels to those established during design conditions, that are rarely analyzed, even though they are the most probable working conditions of fishways (Fuentes-Pérez et al., 2019) and can directly affect fish passage (Fuentes-Pérez et al., 2018; Sanz-Ronda et al., 2016). Furthermore, any geometrical variation in the fishway resulting from inadequate design, deviations during construction, clogging, or lack of maintenance can also generate non-uniform scenarios inside the fishway that may act as a bottleneck in the fish passage (Fuentes-Pérez et al., 2021; Santos et al., 2012; Valbuena-Castro et al., 2020).

The variation in water levels within fishways directly affects the velocities at the cross-walls and the velocity and turbulence profiles in the pools. Non-uniform profiles generate effects on the distribution of velocities and turbulence within the pools of vertical slot fishways comparable to those observed with a change in slope (Fuentes-Pérez et al., 2019; Liu et al., 2006; Wu et al., 1999), influencing the usage inside these pools (Fuentes-Pérez et al., 2018). Specifically, backwater profiles tend to reduce the velocity magnitudes within the pools and slots, allowing for a more random distribution of fish throughout the pool. In contrast, drawdown profiles increase the overall turbulence levels and velocities in the pools and cross-walls compared to uniform profiles(Fuentes-Pérez et al., 2019; Marriner et al., 2016), potentially affecting fish passage efficiency.

Figure 1. **Fishway hydraulic performance.** Possible water distribution profiles in a fishway and principal hydraulic variables involved. (1 column)

Non-uniformity is a natural aspect of fishway design and behavior, and it will always be present. Therefore, understanding its effect on fish is essential for improving fishway design, optimizing their performance and management during operation, and adapting them to hydrological and climatic uncertainty. This becomes even more crucial in light of climate

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change, which is intensifying the frequency, severity, and duration of extreme weather events, thereby directly influencing river dynamics (Panteli and Mancarella, 2015). Neglecting non-uniformity conditions can lead to inadequate fishway designs and assessments. For example, relying solely on mean performance values or specific scenarios to characterize fishway hydraulics can misattribute changes in performance to flow conditions rather than to actual fishway functionality. Such oversights can lead to significant consequences for fish populations and aquatic ecosystems and thus, finally failing as a mitigation measurement for river fragmentation. Therefore, in this paper, we aim to analyze and assess the possible effects of hydrological variability on the biological performance (specifically in the upstream fish passage) of fishways. Our initial hypotheses are:

1. Different hydrological scenarios, such as uniform and non-uniform, can have a significant impact on the fish passage in fishways. In some cases, non-uniform features may act as a bottleneck and reduce fish passage, while in other cases, they may facilitate or increase passage.
2. The impact of hydrological scenarios on fish passage may be influenced by a range of factors, including fish size, swimming ability, as well as the specific physical features of the fishway.

Our final goal is to demonstrate that the optimization of fishways, as well the assessment of their performance requires careful consideration of the hydrological and physical conditions within the fishway. This is crucial for contributing to the conservation of fish populations in riverine ecosystems and for making more meaningful assessments. The findings have direct implications for fishway design, operation, and assessment workflows, as well as for the implementation of adaptive management strategies for water usage and river systems.

2. Methodology

2.1. Formulation of hydraulic responses to the hydrological variability on fishways

The river dynamics affect the boundary conditions of fishways, leading to alterations in overall water levels within these structures and, thus, hydraulic conditions inside them (Figure 1). This phenomenon has been explored in various studies and guidelines (FAO/DVWK, 2002; Fuentes-Pérez et al., 2014; Krüger et al., 2010; Marriner et al., 2016; Rajaratnam et al., 1986) and a general 1D formulation for all types of stepped fishways was established in Fuentes-Pérez et al. (2017).

In this general formulation, the necessary boundary conditions are first defined. These are (1) the fishway's discharge (Q) or upstream headwater level ($h_{1,1}$) and tailwater level ($h_{2,n}$, where n represents the total number of cross-walls in the fishway), (2) the basic geometrical parameters of fishways, such as the geometrical difference between cross-walls (ΔZ), and (3) specific geometry of cross-walls, like the slot width (b) in case of vertical slot fishways (VSF) and its discharge coefficient. Then, an iterative bottom-up calculation is performed to determine the expected depths in the fishway pools (Fuentes-Pérez et al., 2024).

Discharge equations are crucial components in this workflow, as they must support discharge calculation under varying boundary conditions. Using Poleni's discharge equation (Poleni, 1717), Eq. (1), in conjunction with Villemonte's submergence coefficient (C) (Villemonte,

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1947), Eq. (2), it is possible to precisely predict uniform and non-uniform profiles (Fuentes-Pérez et al., 2017).

$$Q = \frac{2}{3} \cdot C \cdot b \cdot h_1^{1.5} \cdot \sqrt{2 \cdot g} \qquad \textit{Eq. 1}$$

$$C = \beta_0 \cdot \left[1 - \left(\frac{h_2}{h_1}\right)^{1.5}\right]^{\beta_1} \qquad \textit{Eq. 2}$$

In these equations, g represents the acceleration due to gravity (9.81 m^2/s), while β_0 and β_1 are coefficients dependent on the flow control structure's geometry in the cross-wall.

Water levels can be easily transformed in more complex information directly related to fishways' biological responses inside them, such as maximum velocity (V_{max}) in the cross-wall (directly related to ΔH (Eq. 3, Rajaratnam et al., 1986)) or the volumetric power dissipation in the pool (*VPD*, Eq. 4, where ρ is the water density (FAO/DVWK, 2002)). Both variables are of extreme importance in the design and assessment of fishways and have demonstrated a correlation with fish movements along them (Bravo-Córdoba et al., 2021; Larinier, 2002a; Towler et al., 2015). These calculations can be applied to most common stepped fishways and provide a general low-computing-power framework for predicting fishway hydraulic behavior and assessing potential effects on fish passage.

$$V_{max} = \sqrt{2 \cdot g \cdot \Delta H} \qquad \textit{Eq. 3}$$

$$VPD = \frac{Q \cdot \Delta H \cdot g \cdot \rho}{Volume\ of\ the\ pool} \qquad \textit{Eq. 4}$$

Considering this and the basic description of hydraulic performances shown in Figure 1, it is possible to define three types of water level profiles inside fishways: (1) Uniform profiles (U), where ΔH is constant and equal to the topographic difference between cross-walls (ΔZ), same water depth and *VPD* in all pools and same velocity in the cross-walls; (2) M1 profiles, generated by the decrease of headwater or the increase of tailwater levels, producing lower water drops ($\Delta H < \Delta Z$), velocities and *VPD*; and (3) M2 profiles, generated when the headwater level increases or the tailwater level decreases, generating higher water drops ($\Delta H > \Delta Z$), velocities and *VPD*.

It is important to note that, depending on the complexity of the fishway design (e.g., mixed cross-wall connections, varying slopes, or resting pools) or deviations during construction (e.g., different ΔZ between cross-walls or different b between cross-wall connections),

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uniform and non-uniform profiles may appear mixed within the same structure. This is frequently observed in field structures.

2.2. Study sites

To investigate the impact of hydrological variability on fish passage, two experiments were conducted. The first experiment was carried out under laboratory conditions, providing an ideal setting for examining uniform and non-uniform water level conditions. The second experiment took place in the field, serving as a case study that highlights non-uniformity in real-world conditions where geometrical deviations exist, while still showcasing the impact of non-uniformity on fish passage. Due to the distinct test conditions employed, the methodologies utilized in the experiments differ, even though it is expected that the findings and conclusions will converge.

Figure 2. **Fish passage experiments.** Studied fishways and geometrical characteristics. a) Lab experiments. b) Field experiments. (2 Columns)

2.2.1. Lab experiments

Lab experiments were carried out in a full-scale indoor VSF at the Hydraulics and Environment Department of the National Laboratory for Civil Engineering (LNEC) in Lisbon, Portugal. The VSF was constructed within a glass-walled open channel of 10 m in length, 1 m in width, and 1.2 m in height, and it corresponds to design #11, as defined by Rajaratnam et al. (1992). In total, it features six pools (1.875 m long and 1 m wide) separated by five cross-walls (0.105 m-wide slots), with a bottom slope (*S*) of 8.5%. The facility also comprises an upstream chamber (1.5 m long, 1.0 m wide, and 1.2 m high) and a downstream tank (4.0 m long, 3.0 m wide, and 4.0 m high). Further information about the laboratory setup is available in Romão et al., (2017). Lab setup allows the precise control of the boundary conditions of the VSF, adjusting the discharge and the water level in the downstream tank using a gate. Thus, the effects of the three possible water level profiles (U, M1, M2) were studied, conducting five replicates with fish assemblages (groups of five fish) for each water profile. Table 1 summarizes the topographic differences between cross-walls and the water drops at each cross-wall for the studied scenarios.

Table 1. Summary of topographic differences between cross-walls (ΔZ in m) and water drops (ΔH in m) in studied fishways and scenarios.

Cross-wall		**1**	**2**	**3**	**4**	**5**	**6**	**7**
Portugal	**$\Delta Z_{i,i-1}$**	-	0.172				-	-
	ΔH_i Uniform (U)	0.207	0.178	0.172	0.153	0.142	-	-
	ΔH_i Backwater (M1)	0.069	0.074	0.099	0.118	0.128	-	-

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	ΔH_i Drawdown (M2)	0.308	0.211	0.200	0.171	0.173	-	-
Quintana	**$\Delta Z_{i,i-1}$**	-	0.219	0.188	0.210	0.212	0.196	0.301
	ΔH_i Backwater (M1_1)	0.084	0.129	0.160	0.199	0.239	0.156	0.275
	ΔH_i Backwater (M1_2)	0.161	0.164	0.221	0.250	0.230	0.141	0.269

2.2.2. Field experiments

Field experiments were carried out in a VSF at the Quintana del Puente hydropower plant in the Arlanza River (42° 4’ 25.92” N 4° 13’ 7.56” W; Palencia, North-Central Spain). The section of the fishway used for the study consists of nine pools (2.10 m long and 1.60 m wide) separated by eight vertical slots (0.20 m-wide slots), with a bottom slope of 6.5 %. The theoretical water drop between pools is 0.15 m, the mean water depth in design conditions of 0.92 m, and the *VPD* in the pools of approximately 130 W/m^3. There is a gate in the upstream slot to control the flow discharge through the fishway. The hydraulic scenarios on the field setup did not allow the control of the boundary conditions of the VSF with the same precision as in the lab experiments, but it was possible to make some fittings with the flow control gate and by reducing the area of the control section above the resting area to simulate submerged conditions. Thus, the effects of two water level profiles (M1_1, M1_2) were studied, conducting two replicates per profile. Table 1 summarizes the measured topographic differences between cross-walls and the water drops at each cross-wall for the studied scenarios.

2.3. Fish collection, handling, and monitoring

In both experiments, Iberian barbel (*Luciobarbus bocagei*, Steindachner 1864) was used as a model or representative species of medium-sized Mediterranean potamodromous fish following a morpho-ecologic guild approach (Branco et al., 2013). All experiments and procedures were performed following European Union ethical guidelines (Directive 2010/63/UE), Portuguese legislation (Decree-Law 113/2013, 7th August, article 35, n. 5, transposing the European Directive for animal experimentation), and Spanish Acts ECC/566/2015 and RD 118/2021 (by which it is modified RD 53/2013), with the approval of the competent authorities (Instituto de Conservação da Natureza e Florestas and Direção Geral de Alimentação e Veterinária in Portugal and Regional Government on Natural Resources and Water Management Authority in Spain).

2.3.1. Lab experiments

For lab experiments, 75 fish were collected (3 water level profiles x 5 replicates per profile x 5 fish per replicate), with a total fish length range of 0.15-0.28 m. The experiments were conducted between October and November 2016. Fish were collected in the Lizandro River, a

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small coastal river located in central Portugal. The sampling was performed using low-voltage electrofishing gear (Hans Grassl IG-200), the least biased method for sampling stream fish (Cowx, 1989), which follows standard electrofishing procedures adopted by the European Committee for Standardization (Comité Européen de Normalisation, 2003). In the lab, fish were kept in holding tanks (0.7 m^3) equipped with proper life support and filtration systems (Fluval Canister Filter FX5, turnover rate: 2300 L/h). Water quality was controlled using a multiparametric probe (HI 9812–5; HANNA), and fish were acclimated to ambient temperature and natural photoperiod for a minimum of 48 hours before the start of the experiments. Fish were not fed before the experiments and were returned alive to their sampling site after the experiment ended. For each replicate (90 min), prior to the experiment, fish were acclimated to the fishway flow conditions for 30 min at the downstream end of the fishway. The acclimation area was created using two mesh panels placed 1 m apart. After acclimation, the upstream mesh panel was removed, allowing fish to navigate the fishway voluntarily. All tests were conducted using natural light, from 8:30 h to 18:20 h. During the experiment, hand notes of all events (upstream and downstream passages through slots) and their occurrence times were registered, which were later supported by camera recordings (GoPro HERO 3 - 1080p, 60 fps). Due to the low number of fish per replicate and the possibility of passive observation through the glass wall of the channel, no tagging system was used for fish identification. This approach reduced the analysis power but minimized any potential influence of handling and manipulation.

2.3.2. Field Experiments

For field experiments, 90 fish were collected (2 water level profiles x 2 replicates per profile with 15 fish per replicate in M1_1 and 30 fish per replicate in M1_2), with a fork length range of 0.09-0.30 m. The experiments were conducted in July 2022. Fish were collected downstream of the hydropower plant dam one day prior to testing. The sampling was performed by electrofishing (Hans Grassl ELT60II backpack equipment), following the standard electrofishing procedures (EN 14011:2003). Fish were kept in acclimation tanks (inside the fishway). All fish were measured, weighted, and PIT-tagged intraperitoneally (Passive Integrated Transponders (PIT) tags of 12- and 23-mm length; always < 2% of the fish's body weight). No fish died during or after the tagging process. For each replicate (240 min), fish were acclimated to the fishway flow for 30 min at the downstream pool of the fishway. The acclimation area was created using two mesh panels placed in the slots. After acclimation, the upstream mesh panel was removed. All tests were conducted from 9:30 h to 18:30 h. During the experiment, hydraulic and environmental variables were continuously recorded (see next section for more details). Four pass-through PIT-tag antennas were alternately placed in the vertical slots for monitoring fish movements (Figure 2b) (for more details related to the tagging process and the antennas' installation see Bravo-Córdoba et al. (2018)).

2.4. Scenarios and hydraulic variables monitoring

The laboratory environment was well-suited for establishing and defining the different experimental scenarios, as all boundary conditions could be precisely controlled. Thus, three scenarios were defined to represent the three different profiles that could be present in fishways. The boundary conditions used to achieve these water level profiles were: 1) U: Q = 0.081 m^3/s, $h_{2,n}$ = 0.65 m; 2) M1: Q = 0.050 m^3/s, $h_{2,n}$ = 0.79 m; and 3) M2: Q = 0.081 m^3/s, $h_{2,n}$ = 0.43 m (water level profiles can be seen in the results section). A comprehensive hydraulic analysis of these scenarios can be found in Fuentes-Pérez et al., (2019). Water depth was measured with 0.1 cm precision at each cross-wall using rulers installed downstream and on the opposite side of the slots. A camera was used to account for water level oscillations (8 s record at 25 Hz, using a Canon EOS 600D, Tokyo, Japan).

Field experiments were more challenging, as the ability to modify boundary conditions was constrained by the river. Due to field constraints, the initial goal was to achieve two profiles, U and M1. However, the results yielded two M1 profiles of different magnitudes, with the second one closer to uniformity. The boundary conditions were: 1) M1_1: Q = 0.232 m^3/s, $h_{2,n}$ = 0.85 m; 2) M1_2: Q = 0.232 m^3/s, $h_{2,n}$ = 0.61 m. Due to the uncertainty of field conditions (i.e., fluctuations in the river discharge due to hydropower operation), water levels were continuously monitored (every 5 min, 0.5 cm of precision) using a network of ultrasound sensors (Fuentes-Pérez et al., 2021) to make necessary adjustments in case of deviations (±2 cm).

2.5. Data treatment and analysis

All statistical analyses were performed using *R* version 4.0.2 (R Core Team, 2020). In order to account for the hydrodynamic variation among scenarios, a visual representation of the evolution of ΔH and VPD in each scenario was made for both lab and field experiments. This will allow us to show the influence of the variation of the boundary conditions in fishway hydrodynamics.

Analyzed passage metrics were defined as follows:

a) ***Passage proportion to a slot (PP_{SX}) or antenna (PP_{AX}):*** Percentage of fish that reached a specific slot (S) or antenna (A) from the beginning to the end of experiments, considering the entire fish sample. That is to say, Passage Proportion to S1 (PP_{S1}) will count the proportion of fish that passed the first slot, while Passage Proportion to A4 (PP_{A4}) will count for the proportion of fish that reached the fourth antenna.
b) ***Time to a slot or antenna ($T_{SX}|T_{AX}$) and transit time between slots or antennas ($TT_{SX1\text{-}SX2}|TT_{AX1\text{-}AX2}$):*** Time expended by fish from one point to another of the fishways. This can be from the beginning of the experiment to the detection in a slot or antenna (e.g., In lab conditions time to S1 will be T_{S1}, time to S5 will be T_{S5}) or from one antenna/slot to another antenna/slot of the fishway (e.g., In field conditions transit time from A1 to A5 will be $TT_{A1\text{-}A5}$). For analytical purposes of time, only one successful passage per fish was considered, in the case of transit time the one with the minimum time.

In lab conditions, an individual fish tracking system was not utilized. Instead, experimental annotations and camera support were employed to ascertain fish passage through each slot. While it was possible to establish clear slot passage and times in most replicates and scenarios, certain circumstances complicated the tracking. This was particularly noticeable in scenarios with lower velocities and turbulence that prompted increased movement (M1 scenarios), where ensuring the correct tracking of fish in middle cross-walls was challenging due to instances of fish crossing paths (one ascending while another descending). As a conservative measure, we opted not to include the passage proportion at each slot in the analysis (due to the possibility of double/multiple counts of the same fish). Instead, we focused exclusively on the fish arrival events at the last pool that were guaranteed to be successful. This approach

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may have introduced a negative bias in the estimated passage proportion (indicating fewer fish) and a positive bias in time-related metrics (suggesting longer ascent times) in M1 scenarios (Figure 3). In field conditions, the tracking system provided a comprehensive record of fish movement.

Figure 3. Fish counting approach. This illustrates the conservative method applied in processing laboratory data, using an example with three fish. In scenarios lacking an individual tracking system, our analysis could only confidently assert, with 100% certainty, that at least two fish successfully completed the process. (1 Column)

Since there were no significant differences in passage proportions and transit times, nor in the biometric characteristics among replicas for the field conditions, and only minor differences in biometric characteristics under lab conditions (refer to Table S1 in the supplementary material), all replicates for the same scenario were integrated into a single dataset. This allowed us to make the results more meaningful, facilitate interpretation, and broaden the dataset. The Chi-squared (χ^2) test was used to account for differences in passage proportion while the Kruskall-Wallis (KW) test to account for differences in time variables and length among replicas. When these tests were significant, post hoc pairwise tests (χ^2 pairwise test and Dunn's multiple comparison test with Bonferroni correction respectively) were performed for those with three or more groups for comparison (i.e., lab replicates).

After the integration of replicates, passage proportion (*PP*), times (*T*), and transit times (*TT*) were calculated. The *PP* was compared among scenarios and fishway sections over the duration of the experiment using Kaplan-Meier curves and the log-rank test. Differences in times and transit time between scenarios and sections were carried out by the KW test (with the corresponding pairwise test if necessary). This allowed us to identify possible bottlenecks during the upstream passage in each experiment. Additionally, Pearson correlations (ρ) were calculated to find trends for passage metrics with classical hydraulics variables (ΔH -directly related to V_{max}- and *VPD*).

Under field condition scenarios, a broader analysis was conducted to study and quantify the effect of biometric parameters on ascent time. For this purpose, a parametric regression survival model was built to examine the studied scenarios (Castro-Santos, 2005; Haro et al., 2004). In this analysis, the concept of survival time (i.e., time until an event occurs) was applied to time to A4 (T_{A4}) (i.e., time from the beginning until a fish is registered in the last antenna), considering the effect of the biometric parameters (i.e. fork length, condition factor, and weight). Those fish that did not reach the considered antenna 4 during the length of the experiment were included as censored with a T_{A4} of 240 min (i.e., duration of the experiment) (Hosmer and Lemeshow, 1999; Kleinbaum and Klein, 2005). This means that a censored fish may have reached the most upstream antenna if the experiment had been enlarged. Parametric regression models were fitted using the *survival* R package (Therneau and Grambsch, 2000). In order to get the best adjustment, different distributions for the models (exponential, Weibull and log-logistic) were considered, as well as an stepwise procedure (for non explanatory variable elimination (significance level = 0.05)), to obtain the best fitting model according to the Akaike Information Criterion (AIC).

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3. Results

3.1. Hydraulics

Figure 4. **Hydraulic scenarios.** Summary of the studied hydraulic scenarios in lab (a and b) and field conditions (c and d). Additional variables are covered in the supplementary materials (Figure S1). S stands for slot and P for pool. (2 columns)

Figure 4 summarizes the studied scenarios during lab (a and b) and field experiments (c and d). The measured profiles during lab conditions align with the theory outlined in the methodology section. In the scenario classified as uniform, water drops and water level variations were lower (mean values of ΔH: $\Delta H_U = 17.03 \pm 2.50$ cm; $\Delta H_{M1} = 9.76 \pm 2.60$ cm; $\Delta H_{M2} = 21.28 \pm 5.60$ cm), which resulted in uniform velocities and *VPD*s in the cross-walls and pools, respectively (mean values of *VPD*: $VPD_U = 103.05 \pm 20.54$ W/m^3; $VPD_{M1} = 70.19 \pm 30.65$ W/m^3; $VPD_{M2} = 162.05 \pm 83.25$ W/m^3– mean values of *Vmax*: $V_{max,\ U} = 1.82 \pm 0.13$ m/s; $V_{max,\ M1} = 1.37 \pm 0.19$ m/s; $V_{max,\ M2} = 2.03 \pm 0.25$ m/s). In non-uniform scenarios, these variations along the fishway were more pronounced, with lower drops downstream in the fishway during M1 profiles ($\Delta H_{min,\ M1} = 6.90 \pm 0.37$ cm), translating to lower velocities and *VPD*s ($V_{max,\ min,\ M1} = 1.16 \pm 0.03$ m/s; $VPD_{min,\ M1} = 37.31 \pm 2.42$ W/m^3), whereas larger water drops during M2 profiles ($\Delta H_{max,\ M2} = 30.81 \pm 4.45$ cm) and higher *VPD*s and velocities ($V_{max,\ max,\ M2} = 2.46 \pm 0.18$ m/s; $VPD_{max,\ M2} = 307.62 \pm 72.52$ W/m^3). It is worth mentioning that the studied scenarios are just representations of the three general groups defined for water level profiles (Figure 1), but the transition between scenarios is continuous, meaning there are infinite possible hydraulic scenarios.

Regarding field scenarios (Figure 4, c and d), it was possible to see how water drops change when modifying the most downstream water level. An increase in this boundary condition will be propagated upstream, reducing water drops ($\Delta H_{S1,M1\_1} = 8.37 \pm 0.01$ cm , $\Delta H_{S1,M1\_2} = 16.06 \pm 0.02$ cm), velocities ($V_{max,\ S1,M1\_1} = 1.29 \pm 0.11$ m/s, $V_{max,\ S1,\ M1\_2} = 1.77 \pm 0.09$ m/s), and VPDs ($VPD_{P1,M1\_1} = 40.38 \pm 6.62$ W/m^3, $VPD_{P1,M1\_2} = 83.78 \pm 8.19$ W/m^3). However, it was also possible to observe a non-progressive change in drops (in contrast to lab scenarios), especially in the upstream slots (S5-S7), due to geometrical deviations (different topographic levels between slots or deviations in the slot widths) and/or hydraulic influence of initial cross-walls (control gate). Despite this geometrical influence, the same pattern in hydraulic variables observed in laboratory experiments can be seen. The increase of water level downstream, M1_1, reduces the initial drops (i.e. most downstream drops), which translates to lower velocities in slots and lower *VPDs* in the pools when compared to M1_2.

3.2. Passage proportions, times, and transit times

Figure 5 summarizes the *PP* in the different scenarios of both experiments. The monitoring with PIT-tag technology during field experimentation allowed for tracking of individuals through the antennas, while for lab experiments, individual discrimination was only possible in the first and last slot, establishing *T*, *TT* and *PP* with a conservative approach (see materials and methods sections).

During lab experiments, significant differences were observed among different scenarios in both, the *PP* to slot number 1 (S1) (*p*-value < 0.001) and the *PP* to the last slot (S5) (*p*-value < 0.001) (Figure 5 a and b). *PP* exhibited a negative trend with mean drops increase (Pearson

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correlation (ρ), ρ_{PPS1} = -0.84; ρ_{PPS5} = -0.91) and *VPD*s (ρ_{PPS1} = -0.97; ρ_{PPS5} = -0.99) (Figure 4), non-uniform M1 profiles significantly outperformed in terms *PP*, followed by uniform profiles.

Meanwhile, in field conditions, while no significant difference in *PP* was observed between scenarios, the *PP* to A1 and A2 were higher in the M1_1 profile than in the M1_2 profile. This agrees with the lower drops, velocities, and *VPD*s found in the M1_1 profile (Figure 4).

Figure 5. Passage proportion results. Passage proportion (*PP*) for different scenarios in lab and field experiments. a) and b) Evolution of *PP* to the first slot (S1) and to the last slot (S5). c), d), e), and f) Evolution of *PP* to the different installed antennas. (2 columns)

Regarding time to S1 and S5 (T_{S1} and T_{S5}) and transit time from S1 to S5 ($TT_{S1\text{-}S5}$) in lab conditions (Figure 6, a and b), the T_{S1} (also named in specialized literature time to the first attempt) and T_{S5} were found to be significantly higher during the M2 scenario than for M1 (*p*-value (T_{S1}) = 0.0002; *p*-value (T_{S5}) = 0.0005) and Uniform (*p*-value (T_{S1}) = 0.0005 ; *p*-value (T_{S5}) = 0.0045) scenarios. The median value followed the distribution of *VPD* ($\rho > 0.98$ in both cases) and ΔH values ($\rho > 0.86$ in both cases). This is in accordance with the $TT_{S1\text{-}S5}$, where it is also possible to see a distribution that follows the values of hydraulic conditions (higher values of *VPD* ($\rho = 0.90$) and ΔH ($\rho = 0.99$) are in accordance with higher median $TT_{S1\text{-}S5}$), but in this case, without significant differences between $TT_{S1\text{-}S5}$ distributions (Figure 6b).

Figure 6. Distribution of times (*T*) and transit times (*TTs*). a) Time to S1 and S5 during lab experiments. b) Transit time S1-S5 in lab experiments. c) Time to A1 and A4 in field experiments. d) Transit time A1-A4 in field experiments. e) Evolution of transit times between antennas (A1-A2, A2-A3 and A3-A4) in studied scenarios during field experiments. f) Distribution of fork length in fish that arrived at different antennas and did not pass through, grouped by scenario and combined for antennas (A1+A2 and A3+A4). (2 Columns)

Similarly, in field conditions, it was possible to see a lower median value in the T_{A1} for the M1_1 scenario, in line with the magnitude of hydraulic conditions, but without significant differences between scenarios (Figure 6c). On the other hand, fish that successfully arrived to A4 exhibited a median time (T_{A4}) lower for M1_2 scenarios, as well as lower $TT_{A1\text{-}A4}$, with a wider range in both variables; however, there were no significant differences between scenarios. *TT* between antennas showed a similar progress in both scenarios (Figure 6e). It showed a positive trend with the maximum value of hydraulic conditions in the corresponding section between antennas ($\rho_{\Delta H,M1\_1} = 0.73$, $\rho_{\Delta H,M1\_2} = 1.00$, $\rho_{VPD,M1\_1} = 0.79$ and $\rho_{VPD,M1\_2} = 0.98$) (Figure 4), with significant differences only between $TT_{A1\text{-}A2}$ and $TT_{A3\text{-}A4}$ (*p*-value = 0.045) in M1_1 scenarios. Additionally, the individual identification of fish during field experiments allowed for the examination of the influence of hydraulic conditions on them. A selection based on fish size is observed in the M1_2 scenario, distinguishing between fish that reached the uppermost antenna and those that attempted but failed to ascend (*p*-value = 0.011, Figure 6f)

3.3. Influence of biometric parameters

Taking into account the significant differences in fish size detected on different antennas in the field experiments (Figure 6f), a parametric regression survival model was applied to quantify the effect of biometric parameters in the studied scenarios for T_{A4} (Table 2). Among the tested distributions, the exponential distribution had the lower AIC. The only biometric variable of interest was found to be the fork length, which had no significant influence on the passage time during M1_1 scenario, but had a significant influence in M1_2 scenario. The model reveals that during the M1_2 scenarios, the longer the fish, the less time is required for a successful passage (lower time to A4).

Table 2. Summary of the parametric regression survival models field experiments for the time to A4 (min) for scenarios studied in field experiments. AIC stands for Akaike Information Criterion.

Exponential distribution	β	***p*-value**
a) Non-Uniform M1_1		
Intercept	8.446	< 0.0001
Fork Length	-0.070	0.33
Shape	1 (fixed)	-
AIC	85.44	0.35 (model)
b) Non-uniform M1_2		
Intercept	15.636	< 0.0001
Fork Length	-0.470	< 0.0001
Shape	1 (fixed)	-
AIC	131.115	< 0.0001 (model)

4. Discussion

4.1. Effect of hydrological variability on fishways

This study confirms that river hydrology can significantly impact fishway passage proportions as well as time related metrics due to fluctuations in boundary conditions. The research underscores the need for comprehensive analyses of river system dynamics when designing

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or assessing fishways to ensure optimal performance throughout the entire hydrological cycle, thereby mitigating the fragmentation impacts of water usage infrastructures, although partially. It is noteworthy that non-uniform scenarios are the typical operating conditions in fishways, with uniform conditions being exceptional or limited to a specific area of the fishway, representing just one among countless possible scenarios. As such, non-uniform scenarios, being a natural component of fishway performance, should be considered during the design, management, and assessment stages. This research shows that fishways can handle non-uniformity to some extent; however, certain hydrodynamic scenarios negatively affect fish passage, deviating from their primary objective of allowing the free movement of fish.

In general, the results demonstrate that passage proportion is related to the magnitude of classical hydraulic variables used for the characterization of fishways, such as maximum velocity through cross-walls (or ΔH) and *VPD* (FAO/DVWK, 2002; Larinier et al., 2002). Considering the results, the magnitudes suggested by guidelines seem not conservative, but rather absolute limits. In the case of the studied species, Iberian barbel, a *VPD* of 200 W/m^3 (≤ 150 W/m^3 according to FAO/DVWK (2002)) and a velocity in the cross-walls of 2 m/s ($\Delta H \leq$ 0.2 m, max velocity of ≈ 2.0 m/s according to FAO/DVWK (2002)) appear to be values that make fish passage difficult, which aligns with guidelines. However, it is worth mentioning that during non-uniform performances, both variables increase proportionally, and therefore their influence cannot be separated. Indeed, the review by Bravo-Córdoba et al. (2021) suggests that the maximum velocity could reach up to 2.4 m/s ($\Delta H \approx 0.30$ m) without significantly affecting the performance/passage metrics.

When designing a fishway considering the maximum biophysical limits of fish, special attention should be given to those scenarios with the potential of increasing hydraulic variables, in other words, the M2 scenarios. This study shows that scenarios that increase the water drop and *VPD* between pools (M2<U<M1) result in lower passage proportions, longer transit times, and potential selection of fish by size. This aligns with fish swimming ability studies (Castro-Santos et al., 2013; Ruiz-Legazpi et al., 2018; Sanz-Ronda et al., 2015), which have found that fork length is positively related to distance traveled, swimming speed, and fatigue time. This can be attributed to the greater muscular strength (Webb and Weihs, 1986) and a larger anaerobic scope (Ferguson et al., 1993; Goolish, 1989) of larger individuals. Moreover, other fishway assessment studies have corroborated that, given the same hydraulic scenarios, larger fish exhibit shorter transit times (Bravo-Córdoba et al., 2021), supporting our findings. Consequently, non-uniform scenarios offering lower ranges of VPDs, velocities, and turbulence (M1) can enhance or facilitate passage through the fishways, while scenarios that increase these variables (M2) could act as ascent bottlenecks.

Considering the results, one might argue that established biophysical limits for fishway design may be overly conservative, as they allow the passage of a “significant proportion” of the migratory fish, and thus, achieve fish conservation goals. However, defining an appropriate proportion in terms of population viability is complex, dependent on specific circumstances, and without a consensual percentage in the literature (Birnie-Gauvin et al., 2019). Instead, individual targets are often set (O’Connor et al., 2022). Nevertheless, by incorporating hydrological variability into the design or retrofitting of fishways, we could only enhance the specific passage proportion while minimizing transit times (i.e., reducing delays).

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Another important aspect is the time to the first attempt (*T* to S1/A1), which may be considered a measurement of attraction to the slot, also influenced by individual motivation, in each scenario. In both experiments, the scenario with lower drops generated faster responses, with significant differences found among M2, M1, and Uniform scenarios in lab conditions. This might seem counterintuitive, as motivation or attractiveness for fish is usually related to higher velocities in cross-wall (Bravo-Córdoba et al., 2021), and specialized literature agrees, although it establishes a range of values. For example, optimal attraction flow from the river- fishway connection is defined in the range from 1 m/s to 2 m/s (Larinier, 2002b). The maximum velocities during low-drop scenarios were 1.16 m/s (M1) and 1.28 m/s (M1_1) in lab and field scenarios, respectively. However, it is important to consider the potential impact of *VPD* and turbulence at the entrance, especially in M2 profiles during lab experiments. These factors might have made it more challenging for fish to approach the slot compared to a real fishway entrance connected to the river, where often there are no volumetric restrictions to dissipate the energy. Attraction to the fishways or the location of the fishway entrance is generally a larger-scale issue in fishways, which seem mostly affected by the auxiliary discharge that could increase location and attraction to the fishways downstream (Williams et al., 2012) or a correct placement of entrance (Bunt, 2001; Bunt et al., 2012), rather than the water drop in the most downstream cross-wall. Nevertheless, considering the velocity ranges established by the literature and the results found here, both uniform and M1 scenarios seem to be compatible with fish passage and produce a lower delay, while M2 scenarios seem to exceed the swimming ability or preferences of a great proportion of fish.

When considering the studied species, it is important to note that the Iberian barbel is a benthic and rheophilic species. Although it is representative of several species found in circum-Mediterranean regions (Sanz-Ronda et al., 2019), fish communities comprise various species with potentially different behaviors. Therefore, we should expect different responses from other species types. Additionally, the analysis utilized a limited range of fish sizes, and fish size has been shown to be a strong predictor of fishway negotiation success. Consequently, fishway evaluation and design should not overlook smaller individuals, particularly small-sized fish species, which are more likely to be affected by a fishway's limitations and non-uniform performance.

4.2. Incorporating Hydrological Variability in Fishway Design, assessment and management

Results confirm that hydrological variability impacts fishway performance, and therefore, special attention should be given to the hydrological cycle and the evolution of the boundary conditions during the fishway design. Furthermore, considering the expected modifications/divergences during the construction, such as geometrical deviations or alterations to the surrounding area of the fishway, a hydraulic assessment (e.g., Valbuena-Castro et al., (2020)) should be mandatory before removing any machinery from the construction site (reducing costs and enhancing fishway performance). This would ensure that the expected working dynamics (for example evolution of classical parameters such as *ΔH* and *VPD*) are within the projected range or meet the target species' needs and allow for taking measures in case they do not. Today, various tools are available for conducting such analyses quickly and to model fishway adaptations to hydrological variability, ranging from complex 3D tools (e.g., OpenFOAM, Flow 3D, etc. (Fuentes-Pérez et al., 2022)) in combination with

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individual based models (Mawer et al., 2023) to more user-friendly and low computational cost 1D fishway simulation tools (Escalas (Fuentes-Pérez et al., 2024) or Cassiopée (Dorchies et al., 2022)).

To achieve adaptability, special attention should be given to design conditions during the design stage. Considering variability, it must be ensured that the mitigation measure works under the three different possible scenarios, low and high discharge periods, as well as with the discharges during target fish migration season, the latter to ensure that the fishway performs optimally when it is more crucial. To achieve this, the design scenario could be based on the most common discharge during the migration season. Adaptations can be then implemented to handle hydrological variability under more extreme conditions.

Scientific studies and experiments provide valuable insights into innovative solutions for fishway design. For instance, submerged pre-barrages, manual/automatic sill elevation devices to ensure optimum ΔH in the fish entrance and fishway, water exceeding notches or gates for controlling fishway discharge, as well as clogging detection systems, have been document to handle hydrological variability (Fuentes-Pérez et al., 2016; Larinier, 2002b, 2002a). Some solutions are partially covered in design guidelines (*e.g.* FAO/DVWK, 2002), but further effort should be made to integrate these findings into design principles to enhance the effectiveness and adaptability of fishway structures, though they are rarely considered in current design practices.

Similarly, the impact of hydrological variability is often overlooked in most fishway assessment studies. In this field, significant emphasis has been placed on standardizing fish passage evaluation metrics to integrate and compare results (Bravo-Córdoba et al., 2021; Castro-Santos et al., 2009) to understand why most fishway efficiencies remain low (Hershey, 2021). For example, by using survival analysis methods and multistate Markov models, researchers can account for the various ongoing and interacting processes that compete with each other (Silva et al., 2018). However, despite well-performed biological assessments, there is still work to be done in characterizing hydraulics during the assessment process. It is crucial to avoid any hydraulic oversimplifications, which do not take into account the complexity of the problem and could let to erroneous conclusions.

Considering the results presented here and the dynamic nature of rivers, analyzing only one hydrological scenario or delivering average hydraulic variable values seems insufficient for assessing fishway performance over extended periods. Since passage proportion and time related metrics are directly linked to the hydrodynamic scenario, how can we accurately conclude fishway performance without considering the dynamic nature of fishway hydraulics? Long-term analysis is crucial, but so is a precise characterization and monitoring of the hydrodynamic behaviors that might affect standard metrics. While this may appear complex, current advances in digitalization and real-time control (e.g., Smart fishways (Fuentes-Pérez et al., 2021; Quaranta et al., 2023)), can offer feasible solutions for monitoring, assessment, and management. For instance, this can be achieved by continuously monitoring water levels in fishways during the assessment and then calculating simple variables as demonstrated in the present study.

Furthermore, it is more critical than ever to consider hydrological variability in fishway design and assessment, as near-future climate scenarios predict increased stressors on river ecosystems, such as potential alterations in water temperature and changes in the magnitude, intensity, and frequency of rainfall, consequently affecting river flow (Segurado et al., 2016; Solomon et al., 2007). This is especially true in Mediterranean areas, where higher water temperatures and more frequent and prolonged droughts are expected (Hermoso and Clavero, 2011). As a result, migration periods may be altered (García-Vega et al., 2022, 2018), potentially leading to a mismatch between the optimal working conditions of fishways and the peak of migrations. However, by considering hydrological variability in the design process and management, we can adapt fishways to future climatic uncertainty or establish adaptive strategies to maximize passage while making it compatible with other water uses (Birnie-Gauvin et al., 2017).

Many compendium works have identified missing pieces in the complex puzzle of fishway design and performance (Cooke and Hinch, 2013; Silva et al., 2018; Williams et al., 2012), but none have pointed out the variability of fishway hydraulics. Hydrological variability exists (and it is anticipated that it will either increase or at least, uncertainty in this regard will persist), and we now have evidence of its significant effect on fishway performance.

5. Summary and Conclusions

This study is a novel assessment of the effects of hydrological variability on fish passage through vertical slot fishways. It confirms that river hydrology significantly impacts hydraulics inside the fishway, fish passage proportions and passage time related metrics due to fluctuations in boundary conditions. The results highlight the necessity for a comprehensive analysis of river system dynamics in the design, assessment, and management of fishways to ensure optimal performance throughout the entire hydrological cycle. This is particularly critical in stepped fishways, which are characterized by varying water drops and water levels under different river scenarios.

Non-uniform scenarios, which are typical operating conditions in fishways, should be integral to the design, management, and assessment phases. The study reveals that passage proportion is closely related to classical hydraulic variables like water drop or maximum velocity through cross-walls and volumetric power dissipation. The research suggests that non-uniform scenarios offering lower ranges of VPDs and water drops can enhance or facilitate passage through fishways. Conversely, scenarios increasing these variables could create ascent bottlenecks, particularly impacting smaller-sized individuals of the studied species.

Thus, incorporating hydrological variability into fishway design is critical. This involves considering potential modifications during construction, such as geometrical deviations, and performing a hydraulic assessment before finalizing construction. Design conditions should ensure that mitigation measures work under different hydrological scenarios, particularly during target fish migration seasons. This approach involves integrating technical adaptations and potential climatic uncertainties during the design phase.

The study underlines the need for long-term analysis and accurate characterization and monitoring of hydrodynamic behaviors that may influence passage metrics. With current advancements in digitalization, simulation, and real-time control, exemplified by initiatives

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like the Smart Fishways H2020 EU project, practical solutions for the monitoring, assessment, and adaptive management of fishways are now available.

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8. Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this manuscript, the authors used ChatGPT for checking grammar, spelling of certain sentences. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication

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Figure 1. **Fishway hydraulic performance.** Possible water distribution profiles in a fishway and principal hydraulic variables involved. (1 column)

Figure 2. **Fish passage experiments.** Studied fishways and geometrical characteristics. a) Lab experiments. b) Field experiments. (2 Columns)

Figure 3. Fish counting approach. This illustrates the conservative method applied in processing laboratory data, using an example with three fish. In scenarios lacking an individual tracking system, our analysis could only confidently assert, with 100% certainty, that at least two fish successfully completed the process. (1 Column)

Figure 4. **Hydraulic scenarios.** Summary of the studied hydraulic scenarios in lab (a and b) and field conditions (c and d). Additional variables are covered in the supplementary materials (Figure S1). S stands for slot and P for pool. (2 columns)

Figure 5. Passage proportion results. Passage proportion (*PP*) for different scenarios in lab and field experiments. a) and b) Evolution of *PP* to the first slot (S1) and to the last slot (S5). c), d), e), and f) Evolution of *PP* to the different installed antennas. (2 columns)

Figure 6. Distribution of times (*T*) and transit times (*TTs*). a) Time to S1 and S5 during lab experiments. b) Transit time S1-S5 in lab experiments. c) Time to A1 and A4 in field experiments. d) Transit time A1-

A4 in field experiments. e) Evolution of transit times between antennas (A1-A2, A2-A3 and A3-A4) in studied scenarios during field experiments. f) Distribution of fork length in fish that arrived at different antennas and did not pass through, grouped by scenario and combined for antennas (A1+A2 and A3+A4). (2 Columns)

HIGHLIGHTS

- Novel investigation of hydrology's impact on fishway effectiveness.
- Combined lab and real-world independent field tests.
- Focused on Iberian barbel ascent movements, applicable to other species.
- Uncovered vital factors in fish migration and fishway assessment.
- Suggested design, retrofitting, and management updates for climate resilience.

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