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Ascent ability of brown trout, *Salmo trutta*, and two Iberian cyprinids – Iberian barbel, *Luciobarbus bocagei*, and northern straight-mouth nase, *Pseudochondrostoma duriense* – in a vertical slot fishway

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Abstract – Passage performance of brown trout (*Salmo trutta*), Iberian barbel (*Luciobarbus bocagei*), and northern straight-mouth nase (*Pseudochondrostoma duriense*) was investigated in a vertical slot fishway in the Porma River (Duro River basin, Spain) using PIT telemetry. We analysed the effects of different fishway discharges on motivation and passage success. Both cyprinid species ascended the fishway easily, performing better than the trout despite their theoretically weaker swimming performance. Fishway discharge affected fish motivation although it did not clearly influence passage success. Observed results can guide design and operation criteria of vertical slot fishways for native Iberian fish.

Key-words: Iberian fish / vertical slot fishway / fish passage / motivation / transit time

Résumé – Aptitude au passage de la truite, *Salmo trutta*, et de deux cyprinidés ibériques, le barbeau ibérique, *Luciobarbus bocagei*, et le nase à bouche droite, *Pseudochondrostoma duriense* dans une passe à poissons à fentes verticales. La performance de passage de la truite (*Salmo trutta*), du barbeau ibérique (*Luciobarbus bocagei*), et du nase à bouche droite (*Pseudochondrostoma duriense*) a été étudiée dans une passe à fentes verticales dans la rivière Porma (bassin de la rivière Douro, Espagne) en utilisant la technologie PIT. Nous avons analysé les effets de différents débits dans la passe à poissons sur la motivation et la réussite de passage. Les deux espèces de cyprinidés montaient la passe facilement, étaient plus performantes que les truites, malgré leur performance de nage théoriquement plus faible. Le débit dans la passe affectait la motivation des poissons bien qu'il n'ait pas influencé clairement le succès de passage. Les résultats observés peuvent guider dans les critères de conception de passes à fentes verticales pour les poissons indigènes.

Mots-clés : Poissons ibérique / passe à fente verticale / passage des poissons / motivation / temps de transit

1 Introduction

The potamodromous fish fauna of the Iberian Peninsula is mostly composed of rheophylic cyprinids. Among the most common of these are the Iberian barbel (*Luciobarbus bocagei* Steindachner, hereafter referred to as 'barbel'), and Northern straight-mouth nase (*Pseudochondrostoma duriense* Coelho; hereafter referred to as 'nase') and their close relatives (Doadrio, 2001). They occupy a range of riverine habitats, from floodplains to headwaters and often co-occur with brown trout (*Salmo trutta* L.). Like many cyprinids, barbel and nase usually ascend to headwaters to spawn in spring.

In Iberia, many cyprinids are local endemics and have IUCN (International Union for Conservation of Nature) protection status. Iberian barbel is listed as "least concern"; nase is more threatened, being categorized as "vulnerable" and it is mentioned on Annex II of the European Union Habitats Directive (92/43/EEC). Barriers to movement block access to essential habitat and are among the principal threats affecting these communities (Doadrio *et al.*, 2011).

Fishway design recommendations usually distinguish between salmonids and a large group of fish, often called "others", "non salmonids", "weak swimmers", "white fish", or "coarse fish" (Alexandre *et al.*, 2013; Mallen-Cooper, 1999; Mateus *et al.*, 2008).

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Fig. 1. Study site location: Porma River, Castilla y León region (Northwest Spain).

Fish passes for salmonids are designed with higher slope or drop per pool than for other fish (Larinier, 2002a). This is because salmonids are widely assumed to be comparatively strong and motivated swimmers. This assumption is founded on very few data, however, and recent studies suggest that other freshwater species may be as strong or stronger swimmers than salmonids (Castro-Santos, 2005; Pompeu and Martínez 2007; Castro-Santos *et al.*, 2013; Sanz-Ronda *et al.*, 2015).

One of the most popular fishway designs in Spain is the vertical slot fishway. The design was originally developed for salmonid passage (Clay, 1995), but is increasingly favored for a variety of species, including cyprinids (Sanz-Ronda *et al.*, 2010).

Vertical slot fishways were initially designed to accommodate large fluctuations in upstream and downstream water levels while maintaining constant such hydraulic factors as energy dissipation rate and velocity (Rajaratnam *et al.*, 1986; Larinier, 2002b; Rodríguez *et al.*, 2006; Wang *et al.*, 2010). They have other advantages, though, notably that the slots offer passage opportunities throughout the water column, and are thought to be compatible with a broad range of species (Stuart and Berghuis, 2002; Mallen-Cooper and Brand, 2007; White *et al.*, 2010).

Recent compilations of passage performance, however, have raised serious questions about the effectiveness of the design, which ranged from 0%–100% (Bunt *et al.*, 2011; Noonan *et al.*, 2012).

The reason for this variation in performance is poorly understood, and there is a need for rigorous biological evaluation of fish ladders if hydraulic designs are to be improved (Castro-Santos *et al.*, 2009; Bunt *et al.*, 2011; Cooke and Hinch, 2013).

Recent efforts to establish standardized passage metrics based on movement theory have yet to be widely adopted (Castro-Santos *et al.*, 2009; Castro-Santos and Haro, 2010; Castro-Santos and Perry, 2012; Cooke and Hinch, 2013). By explicitly measuring rates of movement and failure as fish ap-

proach, enter, and pass numerous fishways of various designs it will become possible to better understand why and to what extent some fishways perform better than others (Travade and Larinier, 2002; Bunt *et al.*, 2011).

Passage performance results from interactions between the structural and hydraulic features of each fishway, fish behavior, and swimming ability (Makrakis *et al.*, 2010). Behavior and swimming performance are in turn affected by environmental parameters like temperature, turbidity, dissolved oxygen, etc. and biological parameters like species, age, sex or physiological status (James and Johnston, 1998; Plaut, 2002; Clough *et al.*, 2004; Pedersen *et al.*, 2008).

There are few published studies of passage performance of Iberian fishes. Most of the existing work was performed in laboratory settings and focused on the hydraulic preferences of fish within pool-type fish ladders (Silva *et al.*, 2009; Silva *et al.*, 2012; Branco *et al.*, 2013). The relevance of these data to field conditions is not well-established, however, and more work is needed to verify the principles learned in the laboratory (Thiem *et al.*, 2013).

The purpose of this paper is to evaluate the passage performance of native brown trout, Iberian barbel and northern straight-mouth nase ascending a vertical slot fishway in field conditions and under different flow regimes.

2 Methods

2.1 Study site

The experiments were carried out in the Porma River (part of the Duero River watershed), in the village of Vegas del Condado, (province of León in Northwest Spain; 42° 41' 17.40" N; 5° 21' 27.26" W; Figure 1). The river drains a watershed of 1146 km² with mean annual discharge of about 25 m³·s⁻¹. It is strongly regulated for irrigation and during spring and summer discharge is higher and water temperature is lower than under natural conditions.

Fig. 2. (a) Plan view of fishway site location at the dam. (b): Antennas and top closing mesh installation. (c) longitudinal scheme of the experimentation design.

The reach under study belongs to the metarhitron area with an average altitude of about 860 m above sea level. The zone corresponds to a C4 category (gravel-bed stream of moderate sinuosity with a slope of 0.001–0.02 m/m (Rosgen and Silvey, 1996)), and the most abundant fish species are brown trout, Iberian barbel, northern straight-mouth nase, and Northern Iberian chub (*Squalius carolitertii* Doadrio), all of them native and potamodromous fish.

In 1986 a gravity dam (1.80 m high by 35 m wide) was built. To mitigate the habitat fragmentation caused by the dam, a straight vertical-slot fishway was built in 2009 on the right bank of the dam (Figure 2). The length of the fish ladder is 24 m with a mean slope of 7.7% (range: 7.1%–9.4%). The design flow is $0.350 \text{ m}^3 \cdot \text{s}^{-1}$, with 9 pools of 2.4 m long $\times$ 1.6 m wide and an average water depth of 1.1 m. The drop between pools is 0.2 m. The width of the slots is 0.2 m and the water velocity through them is variable depending on the flow and the local bottom slope. The volumetric energy dissipated is between 180 and $190 \text{ W} \cdot \text{m}^{-3}$, depending on river discharge. The bottom of the structure is covered by substrate from the riverbed to increase roughness. The uppermost weir (fishway exit) has a sluice gate that is used to regulate flow within the fishway.

Water temperature (Orpheus Mini, OTT Hydromet GmbH) and water oxygen saturation (PCD-650 portable oximeter®)

were monitored throughout the day at 30 min intervals. Suspended sediments were collected three times daily and analyzed in the laboratory using gravimetric methods.

2.2 Fish testing

We used a passive integrated transponder (PIT)-tag and antenna system to study fish behavior (Castro-Santos *et al.*, 1996; Franklin *et al.*, 2012).

Fish were caught by electrofishing (Erreka model; 2200 W, 5 A) one day prior to testing. They were captured trying to pass a chute, close to the tail of the small reservoir formed by the dam –500 m upstream of the fish ladder– in an apparent status of movement activity. All fish were tagged with PIT-tags, (Half-duplex tags measuring 23 mm long by 3.85 mm diameter, 0.6 g; TIRIS model RI-TRP-WRHP; Texas Instruments, Dallas, Texas, USA). They were anaesthetized with a solution of $0.10 \text{ g} \cdot \text{L}^{-1}$ MS-222 and tagged intraperitoneally by making an incision posterior to the left pectoral fin and gently inserting the tag into the peritoneal cavity (Castro-Santos and Vono, 2013). No fish died during or after the marking process. This method has been shown to have negligible effects on growth, survival, and behavior of many species (Ostrand *et al.*, 2011; Ficke *et al.*, 2012).

Fish were then transferred to the staging area (Figure 1), where they immediately recovered swimming and orientation capacities. Fish were allowed to acclimate to their new environment for 24 h before starting the experiment. The density of fish in this pool was $0.75 \text{ kg}\cdot\text{m}^{-3}$ for trials with trout and $1.22 \text{ kg}\cdot\text{m}^{-3}$ for trials with barbel and nase (both species were combined in the same pool). After the experiments, fish were sacrificed following the ethical guidelines of the EU legislation (Council, 1986).

2.3 Ascent efficiency

Antennas were placed in all except for the middle slot, and each antenna was connected to a dedicated reader (ORFID® Half Duplex reader with antenna Multiplexer, Figure 2), programmed to interrogate the antennas at 14 Hz (3.5 Hz or 0.29 s per antenna). Fish were confined within the study section of the fishway by closing off the lowermost and uppermost slots with wire mesh. Once fish were introduced into the staging area they were allowed to rest for 24 h. A second mesh screen was placed at the upstream end of the staging area to prevent fish from ascending the fishway during this period. Just before each experiment, the flow gate was opened up to achieve the desired water level in and discharge through the fishway. Once desired conditions were established, the mesh at the upstream end of the staging area was removed and fish were allowed to ascend the fishway volitionally.

Brown trout testing was carried out about two weeks before spawning and during a period when this species exhibits strong migratory activity in this area. Similar criteria were used for barbel and nase. Each species was subject to three trials, each lasting 24 h and performed under different flow conditions. Fish were not fed during experiments, although they could find some food in the fishway bottom and drifting in the current.

2.4 Data analysis

2.4.1 Onset of movement

During each trial, fish were able to make several ascents; once a fish reached the upper limit of the fishway or its maximum ascent distance it usually descended again to the resting pool, from where they tried to ascend again, thus accounting for several attempts. Attempts were not considered valid unless the fish was detected at least at Antenna 2, to separate exploratory movements from ascending movements. The last detection at Antenna 1 is considered as the start time of the attempt. Attempts in which fish were detected at the antenna furthest upstream were deemed “successful”; otherwise, they were deemed “failures”.

2.4.2 Motivation analysis

We quantified ascent motivation using three methods, evaluating the effects on each of flow discharge. The three methods

were: (1) whether or not each fish made any attempts – analyzed using the logistic regression and compared in pairs with the chi-square test of independence; (2) the number of attempts staged by those fish that tried to ascend – analyzed using a one-way analysis of variance (ANOVA), with a Tukey’s HSD test and compared with a multiple-range test; and (3) the attempt rate.

Attempt rate was included in the analysis as an indicator of motivation, and was quantified using survival analysis (stratified proportional hazards model (Castro-Santos, 2004)). For those fish that staged at least one attempt, the time between the start of the trial and the first detection constituted the first attempt time, resulting in an uncensored observation. Subsequent attempt times were the time between the start of the attempt and the start of the previous attempt. The time between the start of the last attempt and the end of the trial was a censored observation. Fish that did not stage any attempt were included as censored observations, with attempt time equal to the duration of the trial (Castro-Santos, 2004; Castro-Santos *et al.*, 2013).

2.4.3 Passage analysis

Of those fish that staged attempts, passage performance was analyzed using three additional metrics: (1) success: proportion of ascending fish that reached the uppermost antenna – statistically analyzed with logistic regression; (2) height exceeded: proportion of fish exceeding a specific height, censoring fish that reached the uppermost antenna – height = 1 m; and (3) transit time: minimum time to travel between the lowest and uppermost antenna – analyzed using stratified proportional hazard regression. In each case effect of flow discharge on performance were measured.

All statistical analyses were performed using SAS software (version 9.4).

3 Results

Brown trout trials were conducted between 26-November and 2-December, 2010; barbel and nase were tested together in trials conducted between 31-May and 5-June, 2011 (Table 1). Water temperature fluctuated over the course of each day. During the brown trout experiments it ranged from 2.8–5.6 °C and during the cyprinid experiments it ranged from 7.6–12.0 °C. Oxygen saturation was 100% and pH varied from 7.9 to 8.1. Suspended sediments were always less than $5 \text{ mg}\cdot\text{L}^{-1}$.

Water velocity in the slots was different depending on the fishway flow and the local bottom slope (Table 2). Flow was obtained through dilution gauging, using Rhodamine WT, replicating the hydraulic conditions of the experiment without fish. Maximum velocity always occurred on the sixth slot, where slope exceeded 9%. The lower notches showed slower velocities, because they were influenced by the backwater level in the river, increasing their depth and decreasing the drop between pools.

A total of 20 trout, 17 barbel, and 8 nase were tested in the fishway (Table 3). Nase were slightly larger than barbel, and both cyprinids were slightly larger than trout, but this difference was not significant ($P > 0.127$).

Table 1. Date, time and flow rate of the trials for the species under study. Hours are in solar time (12:00 h coincides with the highest sun elevation).

	First test		Second test		Third test	
	Trout	Barbel & Nase	Trout	Barbel & Nase	Trout	Barbel & Nase
Start date	11/26/2010	05/31/2011	11/29/2010	06/02/2011	12/01/2010	06/04/2011
Start solar time	14:30 h	08:00 h	12:30 h	08:00 h	16:30 h	08:00 h
Discharge flow	Medium ($0.334 \pm 0.006 \text{ m}^3 \cdot \text{s}^{-1}$)		High ($0.402 \pm 0.006 \text{ m}^3 \cdot \text{s}^{-1}$)		Low ($0.245 \pm 0.005 \text{ m}^3 \cdot \text{s}^{-1}$)	

Table 2. Cumulative height, local slope between pools and mean water velocity within the slots under three different flow tests (see Figure 1). Slots are numbered from bottom to top.

Slot (Antenna)	Cumulative height (m)	Slope (%)	Mean water velocity ($\text{m} \cdot \text{s}^{-1}$)		
			Low flow ($0.245 \text{ m}^3 \cdot \text{s}^{-1}$)	Medium flow ($0.334 \text{ m}^3 \cdot \text{s}^{-1}$)	High flow ($0.402 \text{ m}^3 \cdot \text{s}^{-1}$)
3 (1)	0.19	7.14	1.45	1.77	1.81
4 (2)	0.40	8.24	1.59	1.79	1.87
5 (–)	0.58	6.70	1.64	1.89	1.94
6 (3)	0.82	9.41	1.82	1.96	2.08
7 (4)	1.01	6.94	1.60	1.75	1.87

Table 3. Biological and environmental conditions. Sequence indicates the order in which trials were performed. Sample size (n) as total number of attempts and available fish (between parentheses), number of fish that staged attempts within each trial (Attempting), and total number of attempts staged (Attemptstot) for each species–treatment combination. Condition Factor (CF) = $100 \cdot \text{M} \cdot \text{FL}^{-3}$ (M: mass (g); FL: Fork Length (cm)). (mean $\pm$ SD).

Species	Flow rate	Sequence	Attempting	Attemptstot	Fork length –FL– mm		Condition Factor –CF– $\text{g} \cdot \text{cm}^{-3}$	
					Male	Female	Male	Female
Brown trout <i>n</i> = 32 (20)	Low	3	4	5				
	Medium	1	8	11	219.5 $\pm$ 32.8	223.2 $\pm$ 36.5	1.14 $\pm$ 0.08	1.15 $\pm$ 0.11
	High	2	11	16				
Barbel <i>n</i> = 106 (17)	Low	3	9	26				
	Medium	1	10	54	241.8 $\pm$ 58.2	250.3 $\pm$ 46.9	1.50 $\pm$ 0.17	1.42 $\pm$ 0.09
	High	2	9	26				
Nase <i>n</i> = 31 (8)	Low	3	3	8				
	Medium	1	4	17	238.6 $\pm$ 75.0	286.7 $\pm$ 40.7	1.20 $\pm$ 0.16	1.28 $\pm$ 0.11
	High	2	3	6				

3.1 Motivation analysis

Motivation varied by species (Table 4). Barbel and nase appeared to be more motivated than brown trout: they made more attempts ($P < 0.001$) and had a greater proportion attempting, although difference in proportion was not significant ($P = 0.340$). The cyprinids staged attempts sooner than the trout ($P < 0.001$) (Figure 3), and barbel staged their attempts sooner than nase ($P = 0.015$).

Discharge affected motivation of both trout and barbel (Table 5). More trout staged attempts at high flows than at low or medium flows, although significance was marginal: $P = 0.055$ and Logistic regression coefficient $\pm$ SE value for proportion attempting parameter at high flow = 1.235 ± 0.597 ; and $P = 0.096$ and attempt rate hazard model coefficient at high flow = 0.716 ± 0.402 . This means that attempt rate under high flow condition is $\exp(0.716) = 2.04$ -fold greater than

in medium flow. No significant differences were observed between medium and low flow.

Among barbel the number of attempts was greatest at the medium flow condition ($P = 0.004$; mean attempts $\pm$ SE value at medium flow = 5.40 ± 0.58 ; high flow = 2.89 ± 0.61 ; low flow = 2.89 ± 0.48) and the attempt rate was faster ($P = 0.005$; coefficients for high flow = -0.654 ± 0.250 and low flow = -0.690 ± 0.251).

3.2 Passage analysis

3.2.1 Success

Success rates ($n \text{ success} \cdot n \text{ attempts}^{-1}$) were similar among species ($P = 0.989$; trout = 0.70, barbel = 0.71, nase = 0.70). Trout had the lowest success at the highest flows ($P = 0.029$).

Table 4. Comparative results for attempt proportion and attempt number among species. *P*-value (*P*) between parenthesis and significant differences represented by letters. Number of attempts: mean $\pm$ SE.

Species	Attempt – not attempt (% attempting) (<i>P</i> = 0.340)		Number of attempts (<i>n</i> attempts· <i>n</i> fish with attempt ⁻¹) (<i>P</i> < 0.001)	
	%	Tukey's HSD test	Mean $\pm$ SE	Tukey's HSD test
Brown trout	38.8%	a	1.4 $\pm$ 0.1	a
Barbel	58.8%	b	3.8 $\pm$ 0.4	b
Nase	50.0%	a	2.0 $\pm$ 0.3	a

Fig. 3. Survivorship curves showing time to stage the first attempt. Curves are depletion functions, showing the effects of different attempt rates – barbel had the fastest attempt rate and so produced a greater proportion attempting.**Table 5.** *P*-values of the effect of flow discharge on motivation intraspecific metrics.

	Motivation parameter (<i>P</i> -values)		
	Attempt-not attempt	Number of attempts	Attempt rate
Brown trout	0.055	0.844	0.096
Barbel	0.924	0.004	0.005
Nase	0.880	0.528	0.301

3.2.2 Height exceeded

Another index of passage is the proportion of fish ascending to a given height. Although most of the fish that made some attempt reached the upper level, not all of them were successful within 24 h of testing.

There were no significant differences among all three species (*P* = 0.217). The small sample size for nase may be obscuring an effect however: failure rate for trout was greater than for barbel by 71% per meter of elevation, although this result was only marginally significant (*P* = 0.082). Flow rate influenced ascent, but only among trout, which ascended furthest under medium and low discharges (*P* = 0.042).

Fig. 4. Relative frequency of transit time for trout, nase and barbel.

3.2.3 Transit time

Transit time data did not follow a normal distribution. Therefore, the median, and not the mean, was used as a representative information.

Transit time of rheophilic cyprinids were similar (*P* = 0.624) probably due to small sample size (Median of transit time: barbel = 1013 s and nase = 322 s) and both species ascended the fish ladder faster than trout (Figure 4) (*P* = 0.012; median of transit time for trout = 2090 s). Post-hoc tests showed significant differences between trout and barbel (*P* = 0.008) and between trout and nase (*P* = 0.011). Transit time was unaffected by fishway discharge.

4 Discussion

This study has shown that both barbel and nase were better able to pass a vertical slot fishway than brown trout, and that superior performance appeared to be more driven by motivation than by actual ability to ascend the structure once fish passed the first slot. This contradicts a widespread expectation that trout perform better at passing fishways than rheophilic cyprinids. This expectation led to recommendations that fishway designs for barbel and nase be less challenging than those for trout (Puertas et al., 2012).

These expectations and recommendations were founded in part on the belief that cyprinids are weaker swimmers than trout. This has been discredited: sprinting ability of barbel and

nase is very similar to that of trout (Castro-Santos *et al.*, 2013; Sanz-Ronda *et al.*, 2015), and the low expectations of these two species appear to be unjustified.

Having once started the ascent, the three species showed remarkable similarities in their likelihood of passage success. Difference in performance was still evident, however, with the cyprinids passing much more quickly than the trout. It is likely that the reduced motivation and increased transit time exhibited by the trout was related in part to the lower temperatures experienced by that species. Motivation is one of the key factors driving passage performance, although it is rarely measured (Castro-Santos, 2004; Wagner *et al.*, 2012; Cooke and Hinch, 2013). Tagging and handling may also influence motivation. We expect this to produce conservative results, however and do not believe this influences the conclusions of this study.

At lower temperatures metabolic rates and sustained swim speeds are both reduced (Brett, 1964; Beamish, 1978; McKenzie and Claireaux, 2010; Yan *et al.*, 2012). Several species of fish have been shown to exhibit reduced attempt rates at lower temperatures (Castro-Santos, 2002; Castro-Santos, 2004). These studies were performed during the period when all species were undergoing spawning migrations, however, and so the reduced performance of trout holds importance for their management and conservation.

Fishway discharge influenced overall ascent ability. It affected motivation, although it did not influence passage, except by delaying transit time for trout in high flows. Motivation of barbel was higher at medium flows, and for trout it was greater at high flows. Increased motivation in response to elevated velocities and flows has been widely reported in fishway and swimming flume studies (Weaver, 1963; Castro-Santos, 2004; Castro-Santos *et al.*, 2013).

Generally, in vertical slot fishways, the slot velocities are relatively unaffected by discharge (Rodríguez *et al.*, 2006). Nevertheless, we observed changes in water velocity at the slots when flow increased in the fishway – it enlarged about 20% from low to high discharge – and also in the main recirculation areas at pools. It is likely that the changed behavior is a response to these factors.

Finally, a fact that is manifested in this experiment, as well as in similar ones, is the strong variation of the transit time, which was strongly skewed toward longer times, producing a large difference between mean and median (Gowans *et al.*, 1999; White *et al.*, 2010).

This suggests that the transit time depends more on the individuals own behavior than events dependent on factors under study. In addition, the median is the index that best represents the behavior of the sample. The distribution of transit times is consistent with diffusion theory (Castro-Santos *et al.*, 2009), and is one reason that survival analysis techniques typically model the logarithm of time or of the hazard (event rate). Skewed distributions also indicate that movement is not strongly directed, and are characteristic of structures that impart reduced rates of movement, increased tortuosity, or migratory delay (Castro-Santos and Haro, 2003).

As with most fish species, further research is needed on the interplay between swimming abilities, migration habits and behavior inside fish passes of Iberian native species and specifically for cyprinids. If similar studies are performed over a

range of fishway designs and sizes it will become possible to improve both performance and cost-effectiveness of engineered fish passage structures.

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