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RESEARCH ARTICLE

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Do artificial velocity refuges mitigate the physiological and behavioural consequences of hydropeaking on a freshwater Iberian cyprinid?

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Abstract

The rapid flow fluctuations experienced downstream of hydropeaking facilities can alter the river hydromorphology. Given the dependence of riverine fish on physical habitat, those alterations have the potential to change the physiology and behaviour of fish. We assessed whether artificial velocity refuges mitigated the physiological and behavioural consequences of hydropeaking for the Iberian barbel (*Luciobarbus bocagei*). Hydropeaking trials were conducted in an indoor flume equipped with deflectors that created low flow velocity areas to serve as refuges. The FLOW-3D was used to obtain detailed characterizations of the different velocity fields, which facilitated the interpretation of fish responses. Changes in flow magnitude and duration triggered stress responses, demonstrated by the increased blood glucose levels in the single up-ramping event for 60 L s^{-1} and in the step up-ramping event. Fish tended to seek out velocity refuges to avoid higher flow velocities and harsh hydraulic conditions at peak flows, and during the longer events. The movement behaviour frequency increased when fish were subjected to the highest peak flow (60 L s^{-1}), particularly the individual sprints and the drifts. For the base flow (7 L s^{-1}) and the lowest peak flow (20 L s^{-1}) conditions, fish swam freely in the flume, whereas in the harshest hydraulic conditions they showed more difficulty in finding velocity refuges. This research presents a novel approach by combining physiology and behavioural observations with hydraulic modelling to assess the extent to which artificial flow refuges mitigate the consequences of hydropeaking. Our work serves as a model approach for future mitigation studies for fish in hydropeaking rivers.

KEYWORDS

hydropeaking, Iberian barbel, movement behaviour, physiology, pulsed flows, velocity refuges

1 | INTRODUCTION

Flow regime is an intrinsic characteristic of freshwater ecosystems, defining their ecological function and natural dynamics (Poff et al., 1997). Aquatic species have evolved adaptive traits to cope with the inherent predictability of natural flow disturbances (Lytle & Poff, 2004; Pankhurst, 2011). However, the continuous disruption of natural flow regimes has drastically altered the river physical character (Dudgeon et al., 2006), affecting critical life-cycle events of freshwater biota. Given the increasing global demand for energy, it is expected that hydropower production will increase as a result of low production costs, relatively low carbon emissions, and high efficiency to respond to peak demand (Zarfl, Lumsdon, Berlekamp, Tydecks, & Tockner, 2015). The large and rapid flow fluctuations in response to the subdaily or daily changes in hydroelectricity demand, termed as hydropeaking (Cushman, 1985; Young, Cech, &

Thompson, 2011), have the potential to alter the ecological function of freshwater ecosystems.

There has been an increasing effort to determine the ecological impacts of hydropeaking on downstream fish populations and communities including mechanistic research to understand the basis for such changes at the individual level. For example, researchers have studied the effects of rapid changes in flow on metabolic, namely, changes in blood physiology (Flodmark et al., 2002; Krimmer, Paul, Hontela, & Rasmussen, 2011; Taylor, Cook, Hasler, Schmidt, & Cooke, 2012) and swimming costs (Cocherell et al., 2011; Geist et al., 2005), ontogenetic changes, namely, changes in growth (Krimmer et al., 2011; Puffer et al., 2015), reproductive success (Burnett et al., 2014), or survival (Korman, Kaplinski, & Melis, 2011). Furthermore, movement behaviour is crucial to assure the success of life-cycle stages, namely, growth, survival, and reproduction (Kahler, Roni, & Quinn, 2001). In hydropeaking rivers, downstream displacement (Boavida, Harby, Clarke, & Heggenes, 2017), smaller scale (Jones & Petreman, 2015; Krimmer et al., 2011; Taylor et al., 2014; Taylor et al., 2014) to larger scale movements (De Vocht & Baras, 2005), reproductive migration changes (Burnett et al., 2014), and stranding due to dewatering (Saltveit, Halleraker, Arnekleiv, & Harby, 2001; Young et al., 2011) are the most documented examples of consequences for fish. However, contradictory results have emerged while studying the effects of pulsed flows on fish movement behaviour, either there was a notable change (e.g., Boavida et al., 2017; Krimmer et al., 2011), or there was no indication of an altered movement behaviour (e.g., Flodmark, Forseth, L'Abée-Lund, & Vøllestad, 2006; Jones & Petreman, 2015), or an inconsistent effect was found, possibly explained by the availability of velocity refuges that reduced the need to move (e.g., Scruton et al., 2005). These findings suggest that the movement behaviour of fish in rivers affected by hydropeaking strongly depends on river morphology. It would be expected that a heterogeneous river habitat would provide more velocity refuges (Person, Bieri, Peter, & Schleiss, 2014; Vehanen, Bjerket, Heggenes, Huusko, & Mäki-Petäys, 2000). However, higher density of boulders and blocks may increase hydraulic instability and stranding risk under hydropeaking conditions (Auer, Zeiringer, Fuhrer, Tonolla, & Schmutz, 2017; Tuhtan, Noack, & Wieprecht, 2012). This knowledge is necessary to understand which hydraulic conditions influence fish movements.

Fish responses to stress are considered an adaptive mechanism for fish to cope with any external perceived stressors and maintain homeostasis (Pankhurst, 2011). In cases where the stressor persists over time, deleterious effects on overall fish performance (escaping predators, finding refuge, and foraging) and in specific life-cycle stages (reproduction and growth) will likely occur (Barton, 2002; Pankhurst, 2011). Although changes in the blood physiology have occurred in down-ramping conditions (Arnekleiv, Urke, Kristensen, Halleraker, & Flodmark, 2004; Flodmark et al., 2002; Krimmer et al., 2011), the flow conditions which trigger the stress response and the extent to which their severity may cause long-term deleterious effects remain unknown. Studies conducted in the field or in laboratory conditions assert that the absence of a physiological response after a severe flow component fluctuation indicates that fish were seeking out velocity refuges (i.e., flow refuging), although this possibility has not been properly assessed (Arnekleiv et al., 2004; Flodmark et al., 2002; Taylor et al., 2012). In field conditions, there is an additional difficulty in explaining the large variance in the movement behaviour results and in suborganismal responses (e.g., Taylor et al., 2012; Taylor, Hasler, Hinch, et al., 2014), which is not favourable to the design and implementation of effective mitigation measures. It is still difficult to isolate variables and find a mechanistic link between a rapid flow change and a measurable fish response (Costa, Lennox, Katopodis, & Cooke, 2017). The identification of this cause–effect association is essential to determine whether there is a biologically meaningful negative impact and if it is indeed necessary to implement mitigation strategies.

Given this evidence, performing experiments under controlled environmental conditions has been encouraged (Young et al., 2011) as they reduce the uncertainty caused by confounding external variables. Specifically, by enabling visual observations, controlled lab experiments enable the interpretation of suborganismal responses (Arnekleiv et al., 2004; Flodmark et al., 2002; Taylor et al., 2012) and have been demonstrated to be a valid approach in understanding smaller scale behavioural and physiological changes (Auer et al., 2017; Flodmark et al., 2002; Ribi, Boillat, Peter, & Schleiss, 2014). However, very few studies evaluate fish responses to structural habitat mitigation measures (Ribi et al., 2014) and only a handful propose mitigation measures based on hydrodynamic models (e.g., Boavida, Santos, Ferreira, & Pinheiro, 2015; Person et al., 2014;).

The effects of hydropeaking events have been studied mainly for the fast swimming salmonids (Arnekleiv et al., 2004; Flodmark et al., 2002; Krimmer et al., 2011; Taylor et al., 2012). Conversely, the movement behaviour and habitat preferences of cyprinids under rapid flow changes has been scarcely studied (Vilizzi & Copp, 2005). Being more susceptible to downstream displacement due to their low swimming ability (Taylor & Cooke, 2012) and as the most representative group of fish inhabiting Portuguese rivers, mainly consisting of autochthonous species, it seems important to understand the effects of rapid flow changes on cyprinids to encourage the design of successful structural mitigation measures. In the present study, young adults of Iberian barbel (*Luciobarbus bocagei*, Steindachner, 1864), abbreviated as *L. bocagei*, an endemic potamodromous cyprinid of the Iberian Peninsula, were selected. The fingerlings and juveniles are predominantly rheophilic (Martínez-Capel & García de Jalón, 1999), as well as the spawning adults, choosing faster currents to migrate upstream during reproductive season (Rodriguez-Ruiz & Granado-Lorencio, 1992). Outside this period, the adults of *L. bocagei* tend to be limnophilic, preferring lower velocities. We adopted an integrative approach to better understand the biological consequences of simulated hydropeaking conditions on *L. bocagei*, incorporating behavioural metrics, blood physiology, and hydraulic modelling. The main objective of this study was to assess the effects of simulated hydropeaking conditions on the stress physiology and movement behaviour of *L. bocagei* in an experimental indoor flume equipped with lateral velocity refuges. Specifically, the following null hypotheses were tested: (a) flow magnitude and hydropeaking event duration do not cause significant changes in the levels of blood glucose and lactate of *L. bocagei*, nor in the movement behaviour of this species in the flume and (b) *L. bocagei* uses equally the available refuges and remaining flume area when subjected to peak flows and base flows.

2 | MATERIALS AND METHODS

2.1 | Fish sampling, transportation, and acclimation

Fish sampling took place at the Lisandro River (38.900554° N, −9.365715° W), a medium-sized tributary of the Tagus River, central Portugal. This tributary is not influenced by hydropeaking which makes it a suitable source of fish that are naïve to hydropeaking events. Fish were sampled once a week during three nonconsecutive weeks between October and November 2015 using a low-voltage (400 V) electrofishing gear (Hans Grassl IG-200), according to European norms (European Committee for Standardization–CEN [CEN, 2003]) and national guidelines (INAG, 2008). In each sampling occasion no more than 80 fish were captured, resulting in a total of 120 fish (mean total length [TL] ± standard deviation (*SD*) cm; 18.0 ± 3.8 cm; mean total weight [TW] ± *SD* g; 56.3 ± 36.6 g). After electrofishing, fish were transported in a constantly aerated fish transport tank (Linn Thermoport 190 L) to the laboratory. The fishes were equally transferred to two 900-L tanks with ambient temperature and natural photoperiod for a 48–72 hr acclimation period where no food was provided. To eliminate the potential effects of acclimation time, the fishes that were subjected to different acclimation periods were also subjected to different flow events. Each tank was covered with a fine-meshed garden net and contained four clay roof tile refuges. Tank water was biologically filtered and permanently aerated. Water quality parameters (mean ± *SD*) were monitored in a daily basis using a multiparameter probe (YSI 556 MPS) for temperature (22.6 ± 1.6 °C), pH (7.84 ± 0.84), dissolved oxygen (7.62 ± 0.66 mg L^{-1}) and conductivity (287.7 ± 60.2 µs cm^{-1}), and in a weekly basis using photometry (WTW-Spectroflex 6600) for nitrites (0.08 ± 0.09 mg L^{-1}), ammonia (0.002 ± 0.003 mg L^{-1}), and chlorine (0.12 ± 0.12 mg L^{-1}). Partial water changes (15%) were performed every other day. After the acclimation period fish were fed with a commercial diet for benthic species every day at night to avoid additional stress caused by food deprivation. The flume water temperature (24.4 ± 2.1 °C) and dissolved oxygen (7.00 ± 0.74 mg L^{-1}) were monitored twice a day. The water quality parameters were in accordance with the national legislation for water quality standards to protect and improve the aquatic environment according to water use for cyprinid waters (Ministry of the Environment, 1998).

2.2 | Flume experimental setup

2.2.1 | Experimental facility and refuge configuration

The experiments took place between October 5 and November 17 2015, in an indoor artificial flume (Figure 1) located at the Hydraulics and Environmental Laboratory (IST, University of Lisbon, Portugal). The flume has a rectangular cross section (8 m long, 0.7 m wide, and 0.8 m high) and was built on a steel frame with glass panels on both sides. An upstream reservoir controlled by a plane gate enabled rapid variation of flow and a downstream flap gate controlled the water level. The usable area for fish was limited by two perforated metallic panels creating a 6.5-m-long reach usable for fish (Figure 1). The hydropeaking events could be simulated up to 60 L s^{-1}.

To mimic lateral refuges in a river channel, four PVC flashboards (0.30 m × 0.76 m × 0.015 m) herein termed as deflectors (D1–D4) were installed in the PVC false bottom of the flume (Figure 1). The deflectors were installed in a configuration characteristic of a meandering river reach, creating a more heterogeneous flow environment. The upstream deflectors were installed side by side, with a 28° opening angle between the flume wall and the deflector, thus occupying one fourth of the flume width (Figure 1, D1). This configuration allowed increasing water velocities in the area affected by the flashboards, creating a harsher hydraulic environment for fish. The other two deflectors located downstream on opposite walls, with a 45° opening angle, occupied one third of the flume width (Figure 1, D4). The remaining width allowed the fish to swim freely and to fully exhibit their swimming behaviour.

2.2.2 | Hydropeaking events

The hydropeaking events were set according to usual operation procedures of a Portuguese hydropower plant (e.g., Boavida et al., 2015) in

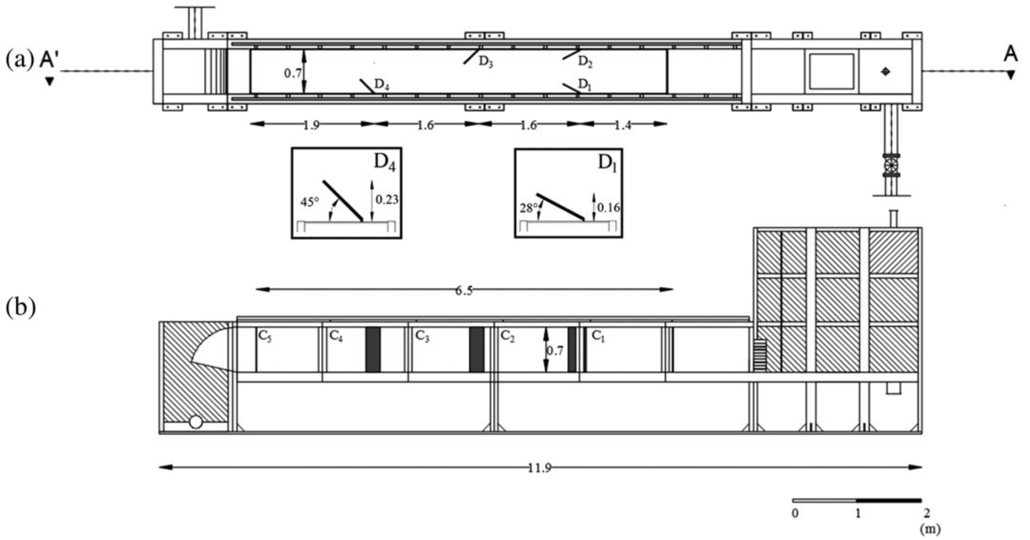

FIGURE 1 (a) Top and (b) lateral view of the indoor experimental flume with representation of the artificial refuges (deflectors: D1 to D4) and behaviour observation areas (C1 to C5). D1 = D2 and D3 = D4 in terms of opening angle relatively to the flume wall

order to test whether the volume of water and the rapid changes over time would induce fish movement behaviour and promote significant changes in the blood physiology. The experimental design consisted of different hydropeaking events that combined flow magnitude and duration. The peak event flow ratio (magnitude) is defined as the maximum flow divided by the minimum flow (Sauterleute & Charmasson, 2014). Given three tested discharges, that is, two peak flows (20 and 60 L s^{-1}) and a base flow (7 L s^{-1}), the flow ratios were 2.9 and 8.6, which are considered moderate to high (Harby & Noack, 2013; Sauterleute & Charmasson, 2014). Duration was tested considering the sum of sequences of flow stimulus (base flow followed by base flow or base flow followed by peak flow) lasting 20 min each, resulting in three different total durations. The combination of flow magnitudes and durations resulted in four hydropeaking events with three total durations, as follows: (a) single up-ramping (SgUR; treatments: Q_{20}, Q_{60}, Q_{base1}; 20 min), (b) single up- and down-ramping (SgUDR; treatments: $Q_{20\text{-}base}$, $Q_{60\text{-}base}$, Q_{base2}; 20 + 20 min), (c) step up-ramping (StUR; treatments: $Q_{20–60}$, Q_{base2}; 20 + 20 min), and (d) repeated up- and down-ramping (RpUDR; treatment: $RQ_{60\text{-}base}$; 20 + 20 + 20 + 20 min; Table 1; Figure 2). Q_{base1} and Q_{base2} were tested in the SgUR, SgUDR, and StUR events to compare the physiology and movement behaviour changes of *L. bocagei* when subjected to hydropeaking events with a reference condition where hydropeaking was absent. The RpUDR event intended to test if doubling and quadrupling the total event duration for the 60 L s^{-1} flow conditions would affect the movement behaviour and blood physiology of *L. bocagei*.

Before starting a hydropeaking event, the flume discharge was set at Q_{base} for 30 min (acclimation period), maintaining the upstream gate open at a 10° angle and the downstream gate at 72°. To simulate a hydropeaking event, the upstream gate was closed while filling up the flume reservoir to its maximum capacity, and at the same time, the discharge was manually controlled until attaining the tested peak flow. Afterwards, the upstream gate was suddenly opened to a maximum of 10° while releasing the tested peak flow. After each flow stimulus (20 min), the discharge was reduced to 7 L s^{-1} or increased to 20 or 60 L s^{-1} according to the tested event. The mean time (±*SD*) for up- and down-ramping was 28.12 ± 1.86′, corresponding to an up-ramping rate of 0.55 cm s^{-1}.

TABLE 1 Description of the tested hydropeaking events. The treatments changed according to the tested discharges: base flow (Q_{base} = 7 L s^{-1}) and peak flow (Q_{20} = 20 L s^{-1} and Q_{60} = 60 L s^{-1}) and according to the sequences of 20 min discharge stimulus and total event duration

Hydropeaking event	Treatment	Discharge sequences (L s^{-1})	Total event duration (min)[a]
SgUR	Q_{20} Q_{60} Q_{base1}	20 60 7	20
SgUDR	$Q_{20\text{-}base}$ $Q_{60\text{-}base}$ Q_{base2}	20–7 60–7 7–7	40
StUR	$Q_{20\text{-}Q60}$ Q_{base2}	20–60 7–7	40
RpUDR	$RQ_{60\text{-}base}$	60–7–60–7	80

Note. Before each hydropeaking event there was an acclimation period where *Luciobarbus bocagei* were subjected to a 7 L s^{-1} discharge for 30 min. RpUDR = repeated up- and down-ramping; SgUDR = single up- and down-ramping; SgUR = single up-ramping; StUR = step up-ramping.

[a] excluding the 30-min acclimation.

Each treatment comprised a group of five *L. bocagei* and was replicated three times. Each fish was tested only once. The selected number of replicates and group size is currently accepted for this type of research (Auer et al., 2017; Branco, Santos, Katopodis, Pinheiro, & Ferreira, 2013). Bigger schools of *L. bocagei* likely occur in nature, particularly during the reproductive season; however, a school of five fish was selected to optimize the observation of fish movement behaviour in the flume, to reproduce a representative schooling behaviour (Weihs, 1973), and to reduce the number of fish to be collected from the wild.

2.2.3 | Hydraulics

A detailed flow characterization was conducted using an acoustic Doppler velocimeter, the Nortek-AS Vectrino 10 MHz, with Vectrino Plus firmware. Three orthogonal velocity components were measured with a four-beam down-looking probe mounted on a fixed stem. This equipment measured the three components of flow velocity (x, y, z). The velocity components were measured at one horizontal plane set at a 5 cm water depth for 7, 20, and 60 L s^{-1}. The reference grid consisted of 128 points with a maximum spacing interval of 10 cm at the deflector area and 15–50 cm at the remaining flume area. This mesh grid allowed a more refined characterization of the deflector area, where the most relevant fish movement behaviour and hydraulic characteristics were expected. The velocity measurements were performed at each point of the defined grid at a 100 Hz sampling rate and for a sampling period of 180 s, which is considered to be adequate for accurate velocity measurements (Buffin-Bélanger & Roy, 2005; Silva, Santos, Ferreira, Pinheiro, & Katopodis, 2011). The velocity magnitude was defined as $u_{mag} = \sqrt{u^2 + v^2 + w^2}$, where u corresponds to the longitudinal (x axis), v the transverse (y axis), and w the vertical (z axis) velocity directions. The velocity measurements were used to calibrate numerical models set-up with the FLOW-3D software (Flow Science Inc., 2012; Figure 3). The numerical simulations allowed us to obtain detailed velocity characterizations for the base flow and for both peak discharges (i.e., 20 and 60 L s^{-1}; Figure 3). The FLOW-3D numerical models were used as an additional tool to better understand the *L. bocagei* deflector approaches and flume movement behaviour according to the tested configurations.

2.3 | Fish responses

2.3.1 | Physiological responses

Blood glucose and lactate concentrations were measured to assess physiological responses to stress. Glucose has been widely used as a secondary physiological indicator of stress in flow variability research (Costa, Lennox, Katopodis & Cooke, 2017). Changes in this physiological indicator generally occur due to endocrine processes associated directly with primary responses to stress (Pankhurst, 2011). Blood lactate is tightly linked with muscle activity. As a metabolic by-product of anaerobic exercise, it will likely increase when

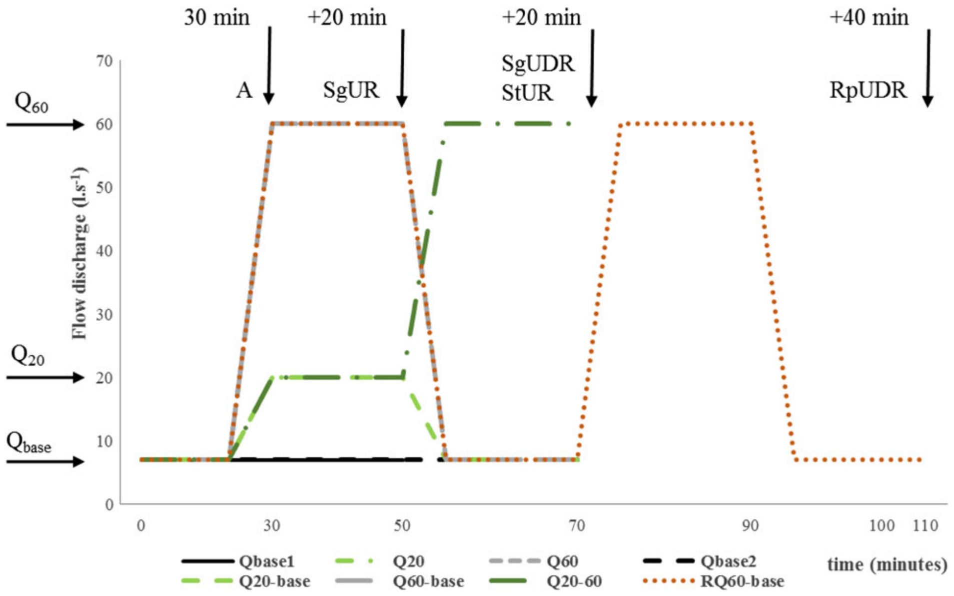

FIGURE 2 Hydropeaking events tested (SgUR, single up-ramping; SgUDR, single up- and down-ramping; StUR, step up-ramping; and RpUDR, repeated up- and down-ramping) with indication of the duration (minutes) for each hydropeaking event (vertical black arrows; [30 + 20] for SgUR, [30 + 20 + 20] for SgUDR and StUR, and [30 + 20 + 20 + 20 + 20] for RpUDR). The horizontal black arrows indicate the discharges tested (Q_{base} = 7 L s^{-1}, Q_{20} = 20 L s^{-1}, and Q_{60} = 60 L s^{-1}). The first 30 min (A) correspond to the acclimation period of *Luciobarbus bocagei* in the flume. The mean time (±*SD*) for up and down ramping was 28.12 ± 1.86′, corresponding to an up-ramping rate of 0.55 cm s^{-1}

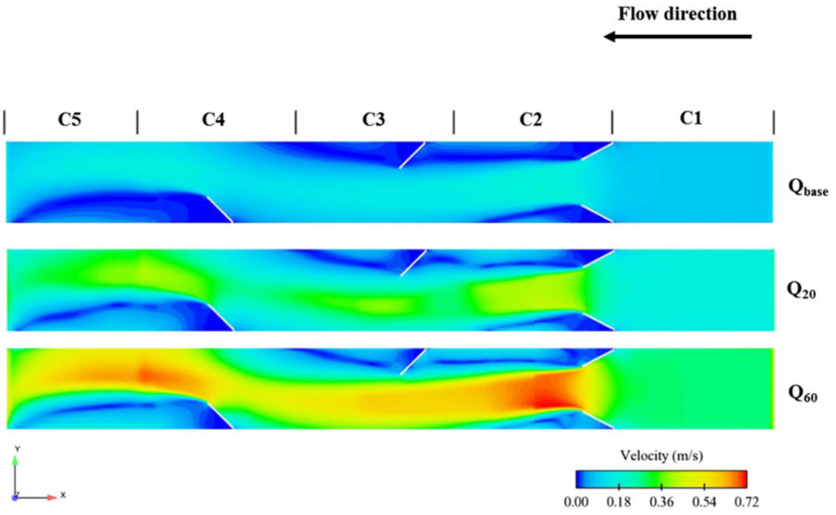

FIGURE 3 Velocity magnitudes for the tested discharges: base flow (7 L s^{-1}) and peak flows (20 and 60 L s^{-1}). C1 to C5 represent the five observation areas (results obtained with FLOW-3D)

aerobic swimming is no longer sufficient to maintain sustained swimming activity (Wendelaar Bonga, 1997).

To quantify these blood parameters, after a treatment, each fish was dip netted from the flume and transferred to a container with permanently oxygenated water and immediately placed in a V-shaped plastic trough in a supine position. Blood samples (0.1–0.5 ml) were collected via caudal puncture using 23 G or 25 G preheparinized needles within 3 min of capture. Laboratory studies have demonstrated that capture-related stress will not significantly influence cortisol concentrations if the sample is taken quickly (Sumpter, 1997). The levels of blood lactate and glucose were immediately measured using the portable meters Lactate Plus (Nova Biomedical UK) and Accu-check Aviva (Roche), respectively. Lactate and glucose portable meters have been confirmed to provide valid results (Beecham, Small, & Minchew, 2006; Stoot et al., 2014; Tanner, Fuller, & Ross, 2010).

2.3.2 | Movement behaviour

The behaviour metrics were divided into two categories: deflector approaches and movement in the flume. The behaviour frequency was measured as the number of occurrences of each behaviour pattern in periods of 20 min, consisting of one period for the SgUR event, two periods for the SgUDR and StUR events, and four periods for the RpUDR event, according to the changes in flow stimulus. During each period, the deflector approaches were counted for each deflector (D1–D4), classified in downstream and upstream approaches, and registered as individual (I) or group (G; i.e. 2 to 5 fish) approaches from downstream (Idown or Gdown) or upstream (Iup or Gup), respectively. Upstream and downstream approaches were directly associated with negative and positive rheotaxis, respectively.

The flume movement metrics were selected for their potential to increase when fish were subjected to the selected hydropeaking events. Thus, the selected metrics were fish sprints, as a sustained swimming activity, lasting a few seconds, characterized by several tail beats; fish drifts defined as voluntary or involuntary downstream fish displacements associated to drag; and jumps as sudden voluntary whole-body leaps outside the water. Sprints and drifts were registered for individuals (Isprint and Idrift) and groups (Gsprint and Gdrift). The behaviour occurrences were observed in five different areas (C1 to C5, Figure 1) and their frequency was registered for each I and G (i.e., 2 to 5 fish) in each area, except for jumps. One behaviour occurrence was only assigned to a specific area (C1 to C5) and only if it started there. The fish movement behaviour observations were visually assessed by two observers who registered the occurrences on an ethogram sheet. One observation area was addressed to each observer: observer one monitored C1 to C3 and observer two C4 to C5. Each observer recorded all behaviour metrics. To avoid fatigue, after each experiment, the observers rested during the acclimation period (30 min). The total observation period per day never exceeded 180 min. To avoid differences in behaviour observation results, the two observers monitored the same area during pilot tests, and the movement behaviour results were considered satisfactory when there was an agreement for the movement behaviour results obtained by the observers.

2.4 | Data analysis

A preliminary data exploration was conducted to assess outliers in the levels of physiological indicators of *L. bocagei* ($n = 5$) for each replicate. If outliers were graphically detected, an interpretation of raw values was performed to verify if they corresponded to real outliers. If the levels of both blood lactate and glucose of *L. bocagei* were extreme in comparison with the rest of the individuals, the values were removed. This approach was used to avoid losing individual information that would not stand out if the raw data were transformed, or mean values were used to follow the assumptions required to the application of the parametric tests. Kruskal–Wallis tests were performed to test for statistical evidence that the levels of blood glucose and lactate between replicates differed. As there was not any statistical evidence that corroborated that hypothesis, the physiological responses of each *L. bocagei* were a true replicate. This preliminary analysis was followed by a Kruskal–Wallis test with a Nemenyi post hoc test for pairwise contrasts, to check for differences in the blood physiology of *L. bocagei* between treatments of each experiment (SgUR, SgUDR, and StUR) (Pohlert, 2015). In case there were ties between mean rank sums, the chi-square approach was used (Pohlert, 2015). The Wilcoxon rank sum test was used to compare the levels of physiological indicators of *L. bocagei* between the StUR event and the respective base-flow treatment and between the hydropeaking events with different durations (20 vs. 40 min) where the 20 L s^{-1} discharge was applied.

A two-way distance-based multivariate analysis of variance based on Euclidean distance (Oksanen, 2015) was performed to test whether there was a treatment effect in the (a) frequency of deflector use and (b) flume movement behaviour metrics of *L. bocagei*. This method does not require the assumptions of parametric tests (Anderson, 2001), handles small samples (Walters & Coen, 2006), and both continuous and factor predictors (Oksanen, 2015). If an effect was detected, a detailed analysis per metric of deflector use and flume movement behaviour was performed using the Kruskal–Wallis test with a Nemenyi post hoc test for pairwise contrasts (Pohlert, 2015). All statistical analysis were performed for $\alpha = .05$ with the R software (R Core Team, 2016).

3 | RESULTS

3.1 | Hydraulics

The maximum flow velocity measured for 7 L s^{-1} was 0.18 m s^{-1} (Table 2), which is in accordance with the suitability curves for juveniles and adults (Martínez-Capel & García de Jalón, 1999) and the sustained swimming ability (Mateus, Quintella, & Almeida, 2008) of *L. bocagei*. For peak flows, velocities were 0.41 and 0.71 m s^{-1} for the 20 and the 60 L s^{-1} discharges, respectively (Table 2). These hydraulic conditions were within the range for estimated critical swimming speeds for *L. bocagei* (Mateus et al., 2008) for young adults and also based on knowledge of velocity refuges in fishway passages (Silva et al., 2011). As expected, the FLOW-3D simulations showed that the lowest and the highest velocity ranges occurred in the 7 and the 60 L s^{-1} discharges, respectively. The immediate area downstream of the deflectors was characterized by the lowest flow velocities for the three simulated discharges (i.e., 7, 20, and 60 L s^{-1}), and this was particularly evident for the 7 L s^{-1} discharge (Figure 3). The harshest hydraulic conditions occurred when the highest peak flow was tested and were prominent in the immediate downstream area between D1 and D2 in C2 and on the right area downstream D4 in C4 and C5 (Figure 3).

3.2 | Physiological responses

There were significant differences in the blood glucose levels of *L. bocagei* in at least one treatment of the SgUR event (χ^2 [2] = 7.821, $p = .020$). Pairwise comparisons evidenced that this indicator differed significantly between Q_{60} and Q_{base1} ($p = .021$). The highest mean levels were observed in Q_{60} (61.1 ± 14.0 mg dl^{-1}) and the lowest in the base flow treatment (Q_{base1}: 45.6 ± 9.1 mg dl^{-1}; Figure 4). Contrarily, the mean blood lactate levels were lower in both the peak flow treatments (Q_{20}: 3.05 ± 1.6 mM; Q_{60}: 3.06 ± 2.0 mM) than in Q_{base1} (4.09 ± 2.2 mM). However, there were no significant differences for this physiological indicator between treatments (χ^2 [2] = 2.089, $p = .352$).

After being subjected to Q_{base2}, the mean blood glucose levels in *L. bocagei* were 55.1 ± 10.8 mg dl^{-1}. The mean levels of this indicator were higher in *L. bocagei* subjected to the same duration peak flow treatments, ranging from 58.5 ± 16.1 mg dl^{-1} in $Q_{60\text{-}base}$, to 64.4 ± 11.4 mg dl^{-1} in Q_{20-60}, and 65.1 ± 15.4 mg dl^{-1} in $Q_{20\text{-}base}$ (Figure 4). For the StUR event, the blood glucose levels were significantly higher in Q_{20-60} than in Q_{base2} ($W = 162$, $p = .039$). For the SgUDR event, blood glucose did not differ significantly between peak flows and Q_{base2} (χ^2 [2] = 4.557, $p = .102$). Blood lactate levels ranged from 3.15 ± 2.1 mM in $Q_{60\text{-}base}$ and 3.42 ± 2.2 mM in Q_{20-60}, to 3.95 ± 2.0 mM in $Q_{20\text{-}base}$. The highest mean level of blood lactate

TABLE 2 ADV results for longitudinal velocity and mean water depth for the tested discharges of 7, 20, and 60 L s^{-1} at 5-cm depth

Discharge (L s^{-1})	Velocity magnitude (m s^{-1})			Water depth (cm)(Mean ± *SD*)	
	Mean ± SD (m s^{-1})	Maximum (m s^{-1})	Minimum (m s^{-1})		
				Upstream	Downstream
7	0.10 ± 0.06	0.18	0.004	11.8	11.8
20	0.21 ± 0.13	0.41	0.008	16.0	15.6
60	0.34 ± 0.22	0.71	0.009	23.5	22.1

Note. The upstream and downstream velocities were collected in the downstream area of C1 and the upstream area of C5, respectively, where the ADV was fixed. The velocity magnitude is defined as $u = \sqrt{\bar{u}^2 + \bar{v}^2 + \bar{w}^2}$, where u corresponds to the longitudinal (x axis), v the transverse (y axis), and w the vertical (z axis) velocity directions. ADV = acoustic Doppler velocimeter.

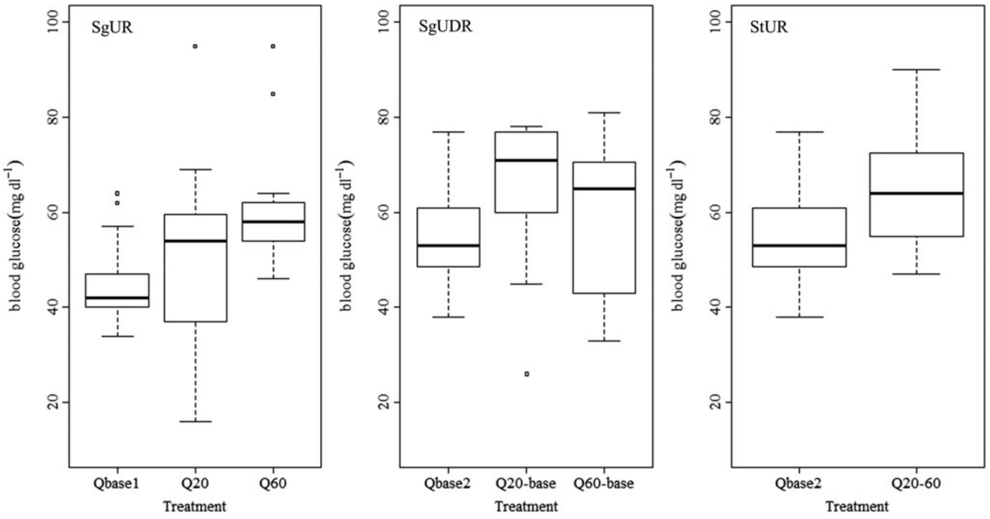

FIGURE 4 Boxplot indicating the variation of blood glucose levels (mg dl^{-1}) between the treatments of the single up-ramping (SgUR), single up- and down-ramping (SgUDR) and step up-ramping (StUR) events

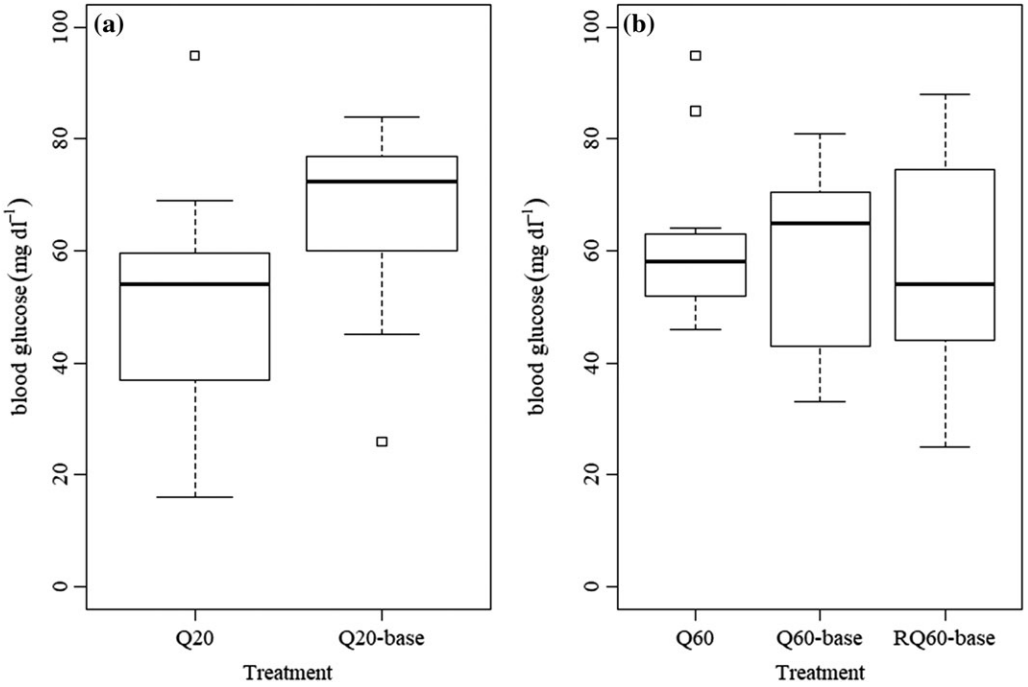

FIGURE 5 Boxplot indicating the variation of blood glucose levels (mg dl^{-1}) for the different hydropeaking event durations: 20 (Q_{20}) versus 40 min ($Q_{20\text{-base}}$) for the 20 L s^{-1} discharge and (b) 20 (Q_{60}) versus 40 ($Q_{60\text{-base}}$) versus 80 min ($RQ_{60\text{-base}}$) for the 60 L s^{-1} discharge

(3.71 ± 2.1 mM) was registered for the same duration base-flow treatment (Q_{base2}). These differences were neither statistically significant between the StUR (W = 96.5, *p* = .519) nor the SgUDR (χ^2 [2] = 1.567, *p* = .457) events.

For the 20 L s^{-1} peak flow, the mean blood glucose levels were 51.0 ± 19.2 mg dl^{-1} after the 20 min treatment (Q_{20}, SgUR) and 65.1 ± 15.4 mg dl^{-1} after the 40 min treatment ($Q_{20\text{-base}}$, SgUDR; Figure 5a,b), and this difference was statistically significant (W = 46.5, *p* = .009). Blood lactate levels did not differ significantly between these two treatments (W = 83, *p* = .342). For the 60 L s^{-1} peak flow, there were no significant differences in the blood glucose levels (χ^2 [2] = 0.175, *p* = .916) of *L. bocagei* between the hydropeaking events that lasted 20 (SgUR: 61.1 ± 14.0 mg dl^{-1}), 40 (SgUDR: 58.5 ± 16.1 mg dl^{-1}), or 80 min (RpUDR: 58.3 ± 18.3 mg dl^{-1}). The same pattern was verified for blood lactate levels (χ^2 [2] = 2.879, *p* = .237), where mean values ranged from 3.06 ± 2.03, 3.15 ± 2.08 to 1.97 ± 1.1 mM, for the SgUR, SgUDR, and RpUDR events, respectively.

3.3 | Movement behaviour

In general, the frequency of downstream deflector approaches was higher than upstream deflector approaches (Figure 6a, 6c, and 6e). The occurrences of sprints and drifts were more frequent when *L. bocagei* were subjected to 60 and 20 L s^{-1} discharge conditions than to base-flow discharge treatments, with increased incidence for individual behaviour (Figure 6b, 6d, and 6f).

The results from the multivariate analysis showed that for the 20 min duration event (SgUR), there was a treatment effect on the deflector use by *L. bocagei* (F = 3.821, *p* = .005). Looking in detail

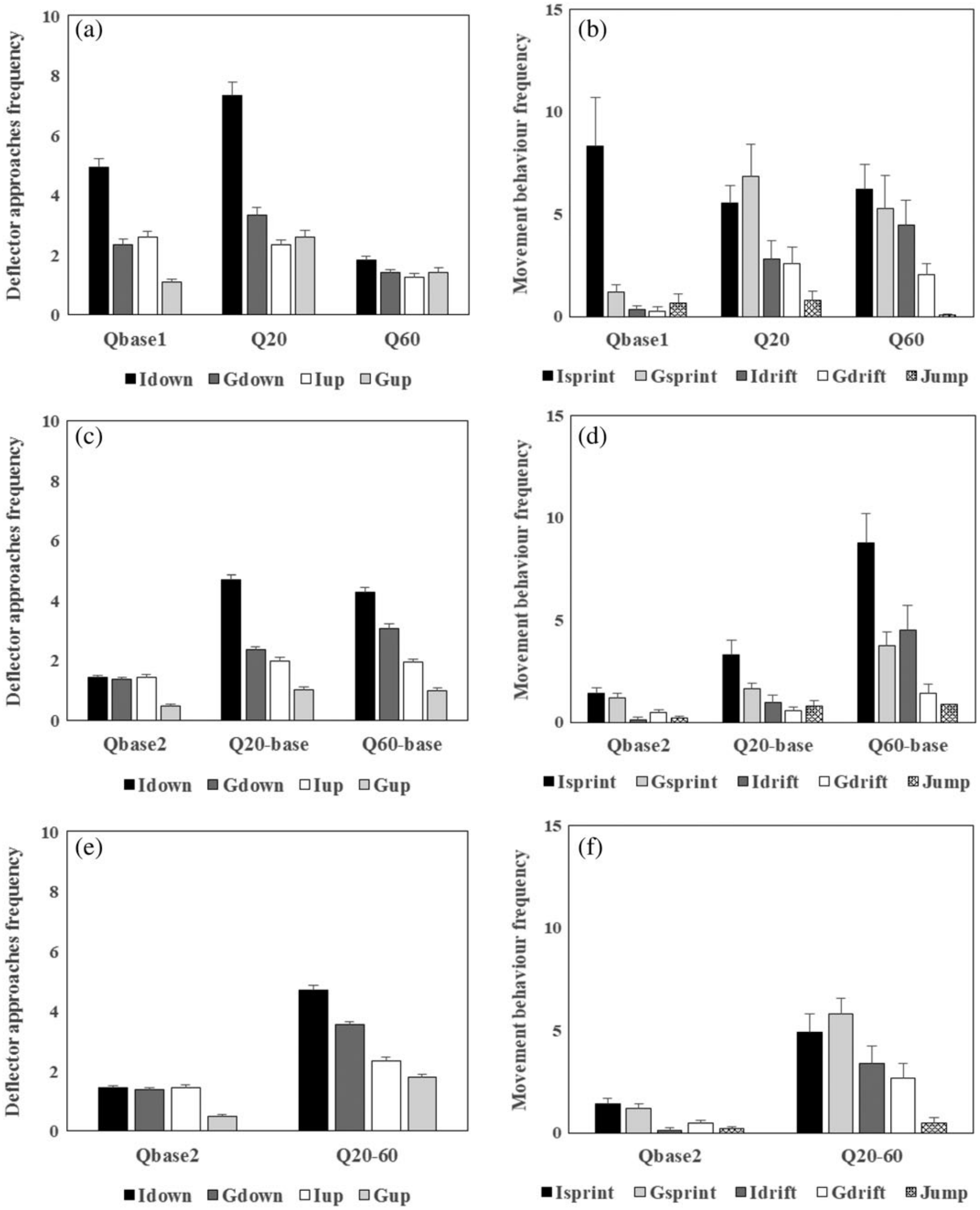

FIGURE 6 Mean (+*SE*) frequency of occurrences for each behaviour metric referring to deflector approaches and movement behaviour for (a and b) single up-ramping (SgUR), (c and d) single up- and down-ramping (SgUDR), and (e and f) step up-ramping (StUR) hydropeaking events

TABLE 3 Single up-ramping results for the pairwise comparisons between peak (Q_{20} = 20 L s^{-1} and Q_{60} = 60 L s^{-1}) and base (Q_{base1} = 7 L s^{-1}) flow treatments, according to deflector approaches and flume movement behaviour, using the Kruskal–Wallis test with a Nemenyi post hoc test for pairwise contrasts

		Deflector approaches				Flume movement behaviour				
		Downstream		Upstream		Sprints		Drifts		Jumps
Pairwise comparisons		Idown	Gdown	Iup	Gup	Isprint	Gsprint	Idrift	Gdrift	Jump
Q_{20} vs. Q_{base1}	χ^2	1.175	0.754	0.00089	1.549	4,877 * 10^{-5}	**13.515**	**6.1899**	**7.2937**	0.0194
	p	.556	.69	.42	.46	1.00	**.001**	**.045**	**.026**	.99
Q_{60} vs. Q_{base1}	χ^2	5.088	0.437	1.732	0.003	1.951 * 10^{-2}	**6.475**	**12.781**	**7.7924**	1.782
	p	.078	.80	.42	1.00	.99	**.039**	**.002**	**.026**	.41
Q_{20} vs.Q_{60}	χ^2	**11.153**	2.341	1.654	1.424	1.760 * 10^{-2}	1.281	1.1818	0.00824	2.174
	p	**.004**	.31	.44	.49	.99	.527	.554	.996	.34

Note. Significant results (α = .05) are indicated in bold.

for each deflector approach metric, Idown differed according to the discharge stimulus (χ^2 [2] = 11.611, p = .003). Idown approaches were more frequent in Q_{20} in comparison with Q_{60} (Figure 6a; Table 3). Deflector use did not differ between treatments for Gdown and both Iup and Gup (Table 3). Overall, deflector use was lower in Q_{60} (Figure 6a). There was also a treatment effect in the frequency of sprints and drifts of *L. bocagei* (F = 3.519, p = .002); Gsprint (χ^2 [2] = 14.18, p < .001), Idrift (χ^2 [2] = 13.435, p = .001), and Gdrift (χ^2 [2] = 10.063, p = .006) differed significantly between the three flow treatments and were predominantly higher in Q_{60} and Q_{20} in comparison with Q_{base1} (Figure 6b; Table 3). There was no statistical evidence for fish jumping more in Q_{60} and Q_{20} in comparison with Q_{base1} (Table 3).

For the 40-min duration hydropeaking events (SgUDR and StUR), there was a treatment effect on the frequency of deflector use (F = 3.389, p = .001). Both Idown and Gdown differed significantly between treatments (χ^2 [3] = 15.679, p = .001, and χ^2 [3] = 12.139, p = .007, respectively). Pairwise comparisons showed that Idown were more frequent in the SgUDR ($Q_{60\text{-base}}$ and $Q_{20\text{-base}}$) and in the StUR ($Q_{20–60}$) events in comparison with Q_{base2} (Figure 6c and 6e, respectively). Gdown were more frequent only in the StUR event in comparison with Q_{base2} (Figure 6c and 6e; Table 4). There was a treatment effect on the frequency of sprints and drifts of *L. bocagei* in the 40-min duration hydropeaking events (F = 11.273, p = .001). Isprint (χ^2 [3] = 29.815, p < .001), Gsprint (χ^2 [3] = 33.336, p < .001), Idrift (χ^2 [3] = 25.652, p < .001), and Gdrift (χ^2 [3] = 11.714, p = .008) differed significantly in at least one treatment. Pairwise comparisons revealed that Isprint increased in the SgUDR ($Q_{60\text{-base}}$) and StUR events in comparison with Q_{base2} (Figure 6d,f, respectively; Table 4), and also in $Q_{60\text{-base}}$ in comparison with $Q_{20\text{-base}}$ (Figure 6d; Table 4). Gsprint were more frequent in the SgUDR event ($Q_{60\text{-base}}$) in comparison with Q_{base2} and in the StUR event in comparison with both the SgUDR event ($Q_{20\text{-base}}$) and Q_{base2} (Figure 6d and 6f, respectively; Table 4). Idrift increased in the SgUDR ($Q_{60\text{-base}}$) and in the StUR events in comparison with Q_{base2} (Figure 6d,f; Table 4). Gdrift were more frequent only in the StUR event in comparison with Q_{base2} (Figure 6f; Table 4). In the SgUDR event for both peak flows tested, flume activity decreased in the last 20 min of the hydropeaking event, where a 7 L s^{-1} discharge was applied (Figure 7b). This difference was particularly evident in $Q_{60\text{-base}}$ for Isprint and Idrift (Figure 7b). Conversely, in the second period of the StUR event (60 L s^{-1}), the frequency of movement behaviour increased, particularly fish drifts (Figure 7b).

For the 20 L s^{-1} peak flow treatments, there was no effect of total event duration on the deflector use by *L. bocagei* (F = 2.466,

TABLE 4 Single up- and down-ramping and step up-ramping results for the pairwise comparisons between peak ($Q_{20\text{-base}}$, $Q_{60\text{-base}}$ and $Q_{20–60}$) and base (Q_{base2}) flow treatments for each behaviour according to deflector approaches and movement behaviour, using the Kruskal–Wallis test with a Nemenyi post hoc test for pairwise contrasts

		Deflector approaches				Flume movement behaviour				
		Downstream		Upstream		Sprints		Drifts		Jumps
Pairwise comparisons		Idown	Gdown	Iup	Gup	Isprint	Gsprint	Idrift	Gdrift	Jump
$Q_{20\text{-base}}$ vs. Q_{base2}	χ^2	**12.032**	3.095	0.217	0.442	2.685	0.981	2.698	0.148	0.664
	p	**.007**	.377	.97	.93	.443	.806	.441	.986	.88
$Q_{60\text{-base}}$ vs. Q_{base2}	χ^2	**8.587**	3.847	1.852	0.458	**27.952**	**8.359**	**18.141**	1.095	0.004
	p	**.035**	.278	.60	.93	**<.001**	**.039**	**<.001**	.778	1.00
$Q_{20–60}$ vs. Q_{base2}	χ^2	**10.304**	**12.068**	0.545	7.052	**8.383**	**28.643**	**17.283**	**9.825**	0.032
	p	**.016**	**.007**	.91	.07	**.039**	**<.001**	**<.001**	**.020**	1.00
$Q_{20\text{-base}}$ vs. $Q_{20–60}$	χ^2	0.0670	2.940	0.074	3.961	1.580	**19.021**	6.324	7.562	0.991
	p	.995	.401	.99	.27	.664	**<.001**	.097	.056	.80
$Q_{60\text{-base}}$ vs. $Q_{20–60}$	χ^2	0.078	2.287	0.387	3.915	5.720	6.055	0.010	4.361	0.014
	p	.994	.515	.94	.27	.126	.109	.999	.225	1.00
$Q_{20\text{-base}}$ vs. $Q_{60\text{-base}}$	χ^2	0.290	0.041	0.800	0.00013	**13.310**	3.612	6.847	0.438	0.771
	p	.962	.998	.85	1.00	**.004**	.306	.077	.932	.86

Note. Significant results (α = .05) are indicated in bold.

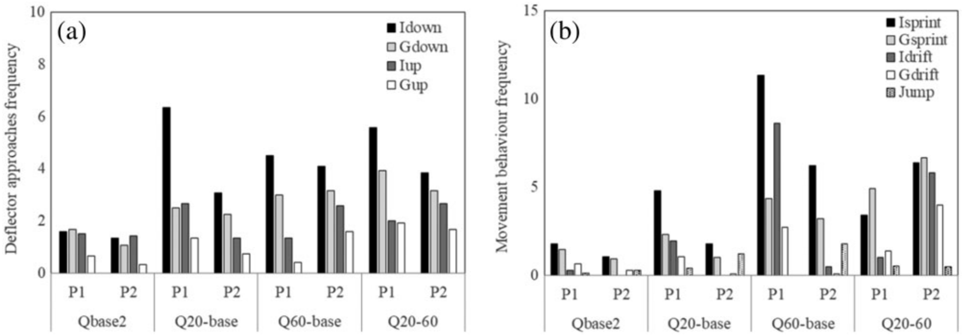

FIGURE 7 Mean frequency of occurrences for each behaviour metric referring to (a) deflector approaches and (b) movement behaviour for each 20-min duration flow stimulus (P1 vs. P2) of the 40-min duration hydropeaking events: single up- and down-ramping ($Q_{20\text{-base}}$, $Q_{60\text{-base}}$) and step up-ramping ($Q_{20–60}$) and respective base-flow treatment (Q_{base2})

TABLE 5 Comparisons between total hydropeaking event duration for 20 and 60 L s^{-1}

Peak discharge (L s^{-1})	Comparisons between treatments		Deflector approaches				Flume movement behaviour				
			Downstream		Upstream		Sprints		Drifts		Jumps
			Idown	Gdown	Iup	Gup	Isprint	Gsprint	Idrift	Gdrift	Jump
20	Q_{20} vs. $Q_{20\text{-base}}$	W	184.5	164	171.5	194	**313.3**	**384.5**	**310**	**311.5**	233
		p	.175	.507	.342	.069	**.027**	**<.001**	**<.001**	**<.001**	.842
60	Q_{60} vs. $Q_{60\text{-base}}$	χ^2	4.679	2.154	1.393	0.320	0.859	0.333	0.765	2.362	0.406
		p	.096	.341	.50	.85	.65	.846	.68	.31	.82
	Q_{60} vs. $RQ_{60\text{-base}}$	χ^2	**8.499**	**7.278**	2.477	0.448	0.411	1.822	2.125	0.099	2.555
		p	**.014**	**.026**	.29	.80	.81	.402	.35	.95	.28
	$Q_{60\text{-base}}$ vs. $RQ_{60\text{-base}}$	χ^2	0.496	1.980	0.131	2.768	4.572	6.548	0.416	3.125	1.353
		p	.780	.371	.94	.25	.10	.038	.81	.21	.51

Note. The comparisons were performed to test for differences between treatments for each deflector approach and movement behaviour metric, using the Kruskal–Wallis test with a Nemenyi post hoc test for pairwise contrasts for the 60 L s^{-1} treatments and with Wilcoxon rank sum test for the 20 L s^{-1} treatments. Significant results (α = .05) are indicated in bold.

p = .065). On the other hand, the flume movement behaviour frequency of *L. bocagei* was affected by total event duration in the flume (F = 1.097, p = .001). Pairwise comparisons evidenced that these differences occurred in Isprint, Gsprint, Idrift, and Gdrift (Table 5), and except for Isprint, they were higher in the shortest duration event. When the flow stimulus was set at 60 L s^{-1}, event duration affected the deflector use (F = 2.789, p = .007). Idown (χ^2 [2] = 8.502, p = .014) and Gdown (χ^2 [2] = 7.819, p = .020) differed significantly in at least one hydropeaking event duration (i.e., 20 [SgUR], 40 [SgUDR], or 80 min [RpUDR]; Table 5). The downstream approaches were higher in the RpUDR event in comparison with the SgUR event for both individuals and groups (Figure 8). On the other hand, there was no statistical evidence that upstream deflector approaches (i.e., Iup and Gup) differed between these hydropeaking events (Table 5). Rank comparisons evidenced that Gsprint differed significantly between the three hydropeaking events (χ^2 [2] = 7.057, p = .029; Table 5), which were higher in the RpUDR event in comparison with the SgUDR. Isprint and Idrift were always higher in the time periods where fish were affected by the 60 L s^{-1} discharge, in opposition to the 7 L s^{-1} discharge (Figure 9), and although group behaviour also

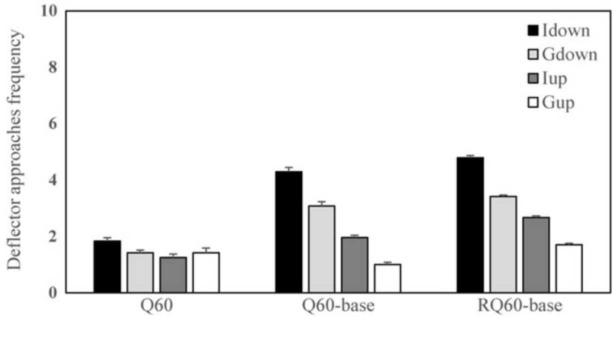

FIGURE 8 Mean (+*SE*) frequency of occurrences for each behaviour metric referring to deflector approaches for the treatments with 60 L s^{-1} peak discharge: Q_{60} (single up-ramping, 20 min), $Q_{60\text{-base}}$ (single up- and down-ramping, 40 min), and $RQ_{60\text{-base}}$ (repeated up- and down- ramping, 80 min)

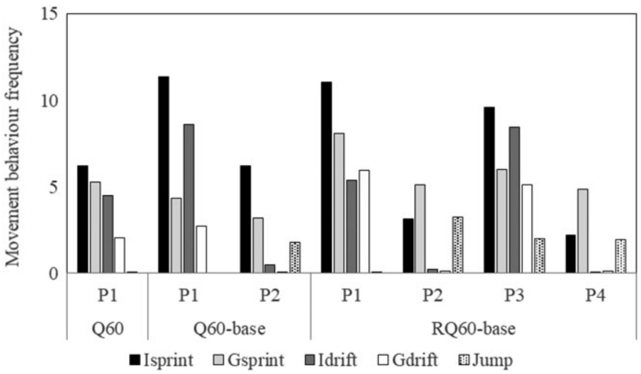

FIGURE 9 Mean frequency of occurrences of each movement behaviour: sprints (Isprint and Gsprint), drifts (Idrift and Gdrift), and jumps (Jump) for each 20-min timespan flow stimulus (P1, P2, P3, and P4) and for the 60 L s^{-1} discharge treatments: Q_{60} (single up-ramping), $Q_{60\text{-base}}$ (single up-and down-ramping), and $RQ_{60\text{-base}}$ (repeated up- and down-ramping)

increased, it was not as evident as individual behaviour. In the RpUDR event in the last two stages there was a decrease in flume activity in comparison with the first two stages, although it was not statistically significant (Figure 9).

4 | DISCUSSION

In the present study, we assessed the physiological and behavioural consequences of simulated hydropeaking events for *L. bocagei* in the presence of velocity refuges. The increased levels of blood glucose observed in fish exposed to the highest peak flow (Q_{60} ;SgUR), and in the event with the longest duration peak flow stimuli ($Q_{20–60}$; StUR), enable us to reject the null hypothesis that flow magnitude and hydropeaking event duration do not affect the blood physiology of *L. bocagei*. However, simulated hydropeaking events failed to elicit significant changes in blood lactate. In general, the velocity refuges were used more frequently during the peak flows in comparison with base flows; downstream approaches were the most frequent, and individual approaches stood out particularly in the 20 L s^{-1} flow stimulus. The available flume area was used differently between peak flow and base flow treatments. Sprints and drifts were more frequent in the peak flow treatments and in particular, during the peak flow stimulus. These results enable us to reject the null hypothesis that the use of velocity refuges and flume movement behaviour are equal under peak flows and base flows.

The more unstable hydraulic conditions and higher flow velocities in the 60 L s^{-1} conditions, obtained with the FLOW-3D numerical model (Figure 3), and the unpredictability of the StUR event with the potential cumulative effect of the two tested peak flows, that is, 20 L s^{-1} followed by 60 L s^{-1}, were not favourable for *L. bocagei* to successfully find the velocity refuge. These factors may explain the increased levels of blood glucose and the lowest overall deflector approaches in Q_{60} (SgUR; Figure 6a). The similar levels of blood glucose between Q_{base2}, $Q_{20\text{-}base}$, and $Q_{60\text{-}base}$, (Figure 4 SgUDR) together with the increased individual downstream deflector approaches when fish were subjected to 20 L s^{-1} in both the SgUR (Figure 6a) and SgUDR (Figure 7) events, indicate that hydropeaking events with short duration peak flow stimulus and moderate flow ratios create advantageous conditions for this species to find a low flow refuge, reducing the chances of an acute physiological response.

The increased levels of blood glucose in $Q_{20\text{-}base}$ in comparison with Q_{20} (Figure 5a) suggest that event duration also affects the blood physiology of *L. bocagei*. After 40-min timespan, there seemed to be a peak in the blood glucose response. However, this trend was not verified in the levels of blood glucose between *L. bocagei* subjected to Q_{60}, $Q_{60\text{-}base}$, and $RQ_{60\text{-}base}$ (Figure 5b). The similar high levels of blood glucose between these treatments suggest that the glucose peak lasted for at least 80 min. The less severe 20 L s^{-1} flow conditions possibly resulted in a slower increase in the blood glucose response, in opposition to the early peak shown after 20 min under the harsher 60 L s^{-1} discharge. Different stressor intensities and combinations seem to result in different physiological responses over time. For example, earlier and more prolonged glucose and lactate responses were observed in European chubs affected by electrofishing in comparison with handling (Bracewell, Cowx, & Uglow, 2004). Higher ranges of glucose levels than those reported here were found in *L. bocagei* that were exposed to a combination of chemical pollution and confinement for 2 hr in the Tajo river (Carballo, Jiménez, de la Torre, Roset, & Muñoz, 2005). In experiments where the effects of handling, acclimation time and hydropeaking (flow reduction) were assessed in juvenile brown trout, peak levels of blood glucose were observed immediately after electrofishing (i.e., capture), levelled off after 72 hr of acclimation, and increased again after dewatering (Arnekleiv et al., 2004). The combination of other stressors (nutritional status, temperature, and fish density) could also have contributed to the sustained blood glucose response 24 hr after flow reduction (Arnekleiv et al., 2004). However, responses to flow are not universal. Juvenile brown trout subjected to flow reduction showed no effect on the blood glucose response (Flodmark et al., 2002). These studies highlight that the stressor severity, the timespan from which it was applied to sampling, and fish condition influence the responses to stress.

The relative stability of blood lactate levels suggests that the flow stimulus was not vigorous enough to promote anaerobic energy production through lactate accumulation or that the timespan from flow stimulus to blood collection was insufficient to observe an elevation in blood lactate (Gleeson, 1996). The stability between the glucose response in the SgUDR treatments, its persistence between the three 60 L s^{-1} duration events, and the low lactate response could denote a suborganismal and behavioural adjustment to conserve energy through compensation. This adjustment was favoured by the short duration 60 L s^{-1} flow stimulus and the low velocity areas inside and in the proximity of the downstream area of the deflectors (Figure 3).

The repeatability of the $RQ_{60\text{-}base}$ resulted in similar glucose levels in comparison with the same peak flow treatments (i.e., Q_{60} and $Q_{60\text{-}base}$; Figure 5b). Longer simulated hydropeaking events could possibly result in habituation, with no physiological or behavioural changes over time. Similar results were obtained by Krimmer et al. (2011) and Flodmark et al. (2002), where the presence of refuges in hydropeaking rivers and in simulated down-ramping indoor experiments, might have alleviated the stressor severity resulting in a less pronounced stress response in brook trout and in brown trout, respectively. These studies were conducted in hydropeaking rivers (Krimmer et al., 2011) and in stream channels (Flodmark et al., 2002) where it was difficult to directly observe fish behaviour, reinforcing the importance of performing indoor flume experiments to validate these explanations. If there was a sustained simultaneous lactate and glucose response, caused by the extreme flow conditions, exhaustion would possibly occur and fish would no longer be able to respond to the flow stimulus (Flodmark et al., 2002) and to regain homeostasis. In nature, these responses would likely become maladaptive with deleterious effects on the fishes overall condition, growth, reproduction, behaviour, and disease resistance and would likely have impacts at the population level (Barton, 2002; Pankhurst, 2011).

The effects of flow magnitude and hydropeaking event duration were more evident in the behavioural aspects of the study (overall deflector approaches and flume activity) than for the physiological metrics we used. Those effects were particularly evident in $Q_{20–60}$

(StUR) and $Q_{60\text{-base}}$ (SgUDR; Figure 6). The deflector approaches were more frequent in the peak flow treatments for both peak flows tested (Figure 6a 6c, and 6e). Similarly, in a simulated pulsed-flow study conducted in an experimental flume, under increasing flow velocities, three juvenile stream fishes (cyprinid, salmonid, and catastomid) swam faster and also hid in the available rocky substrate, presumably to conserve energy (Chun et al., 2011). Under maximum velocities up to 0.46 m s^{-1}, those juveniles were able to maintain their swimming speed and hold position (Chun et al., 2011), whereas in the present study, those activities were possible under velocities up to 0.72 m s^{-1}. Both the presence of deflectors and the shorter duration peak flow stimulus in the present study may have ameliorated the potential negative effects of higher velocities. Exceptionally, there was a low frequency of deflector approaches in Q_{60} (SgUR), explained by the existing critical hydraulic conditions (Figure 3) perceived by *L. bocagei*. Attempting to cope with the flow disturbance and high water velocities (Figure 3), it became challenging for fish to use the deflectors as low flow areas to conserve energy and to recover. These results were in agreement with the increased glucose levels shown in this hydropeaking event, indicating that *L. bocagei* are possibly experiencing stressful flow conditions, which resulted in an early glucose peak after 20 min of exposure. In contrast to the 20 and the 7 L s^{-1} flow conditions, these results indicate that the most favourable hydraulic conditions were exceeded when *L. bocagei* were subjected to a discharge of 60 L s^{-1}, compromising their overall swimming performance. In a study where velocity refuge, water temperature, and season were combined in simulated fluctuating flow conditions, brown trout moved to velocity refuges due to displacement, to avoid the first high flow winter periods (Vehanen et al., 2000); however, the water velocities that the fish experienced were lower than those registered in this study.

In the 40-min events, *L. bocagei* sought the deflectors more actively under peak flows individually and from downstream in comparison with Q_{base2} (Figure 6c and 6e). Similar results have been reported for juvenile brown trout, which preferred to approach lateral refuge configurations from downstream under hydropeaking conditions (Ribi et al., 2014). Generally, fish tend to avoid demanding hydraulic conditions, choosing low-flow areas for flow refuging (Arnekleiv et al., 2004; Flodmark et al., 2002; Taylor et al., 2012; Vehanen et al., 2000). However, when discriminating each flow stimulus period, the difference in the frequency of deflector approaches was less evident for $Q_{60\text{-base}}$ (SgUDR; Figure 7a) and there was a lower deflector use in the last 20 min of $Q_{20\text{–}60}$ (StUR; Figure 7a). These results could be explained by the critical hydraulic conditions created in the vicinity of the deflector area in the 60 L s^{-1} flow stimulus (Figure 3), limiting this cyprinid's ability to seek out velocity refuge inside and downstream of the deflector.

In the natural environment, *L. bocagei* often occurs in schools possibly to benefit from the external hydrodynamic stimuli and to reduce the energetic costs associated to swimming activity (Bleckmann & Zelick, 2009; Liao, 2007). Under the highly modified hydropeaking conditions that were tested, a group behaviour disruption and an increase in the individual activity of *L. bocagei* were expected. The difference between group and individual approaches during the 20 L s^{-1} flow stimulus (Figure 7a) could be explained by the lower energy required to hold station when subjected to this discharge (Scruton et al., 2008) in comparison with the more severe hydraulic conditions experienced during the 60 L s^{-1} flow stimulus. In addition, the higher flow velocities existing under the 20 L s^{-1} discharge in comparison with the 7 l.s^{-1} flow conditions and the smaller size of young adults possibly favoured the relative performance (Mateus et al., 2008) and rheophilic behaviour (Rodriguez-Ruiz & Granado-Lorencio, 1992) of *L. bocagei*. Although this species usually occurs in schools, the flow velocities and hydraulic conditions created (Figure 3) either promoted the disruption of group behaviour and facilitated the individual access to the deflectors as recovery areas, or created hydraulic conditions that favoured these young adults to freely use all available areas in the flume. Changes in social interactions due to fluctuating flows in artificial tanks have been reported for salmonids (Sloman et al., 2002; Sloman, Taylor, Metcalfe, & Gilmour, 2001), resulting in lower growth rates of the less dominant fish (Sloman et al., 2002), whereas in constant flow conditions, the hierarchical structure remained stable. The alteration in social interactions, with consequent reduced growth rates in the less dominant fish (lower ability to access food), and the higher energy costs of holding station to negotiate with the flow disturbance, likely to occur following hydropeaking, may have deleterious consequences for subsequent life-cycle events, namely, reproduction or migration. The reduced availability of low flow habitats in these highly instable flow environments will likely hinder the fishes' capacity to successfully persist under hydropeaking conditions.

The hypothesis that flow magnitude affected the movement behaviour of *L. bocagei* was supported by the increased frequency of sprints and drifts in peak flows in comparison with the base-flow stimulus (Figure 6b, 6d, and 6f) and in the time periods where *L. bocagei* were subjected to the 60 L s^{-1} flow conditions (Figure 7b and Figure 9). The increased individual sprints during the 20 min where *L. bocagei* were subjected to 60 L s^{-1} discharge (Figure 7b) suggest that group behaviour could be disrupted. In nature, this species characteristically occurs in schools, benefiting from the surrounding hydraulic conditions to spend less energy (Liao, 2007; Sfakiotakis, Lane, & Davies, 1999), thus the disruption of social behaviour caused by fluctuating, unpredictable flows could affect diel activity and crucial life-cycle stages. This effect has been demonstrated in fluctuating flow environments in experimental flumes (Sloman et al., 2001, 2002).

The higher frequency of sprints suggests that *L. bocagei* were coping with the perceived flow velocities favouring positive rheotaxis. The increased frequency of drifts (either voluntary or involuntary) suggests that this could be a mechanism for *L. bocagei* to recover from the additional effort necessary for holding station, which is a function of the fish drag multiplied by the water velocity (Webb, 1988), due to the severe hydraulic conditions created, particularly in the 60 L s^{-1} discharge conditions. The more time *L. bocagei* spends coping with the severe flow conditions, for example, sprinting or holding station, the less energy will be available for diel activities, namely, foraging (Flodmark, Vøllestad, & Forseth, 2004) or avoiding predators (Korman & Campana, 2009).

The presence of deflectors in a fluctuating flow environment with a moderate 2.9 flow ratio (Q_{peak}:Q_{base}), is expected to improve the swimming performance of this species at this life stage. This was demonstrated by the lower levels of blood glucose in *L. bocagei* for the base flow and the shorter duration peak flow stimulus events and by the favourable hydraulic conditions created in the 20 L s^{-1}, which allowed *L. bocagei* to swim freely and easily reach velocity refuges. In opposition, the increased levels of blood glucose and the frequency of movement behaviour in the StUR event indicate that hydropeaking events characterized by prolonged peak flow sequences are not beneficial for *L. bocagei*, thus alternating short duration timespan peak flows with base flows is advisable. The increased frequency of deflector approaches in the 60 L s^{-1} treatments and the lower changes in the physiological levels in the $Q_{60\text{-base}}$ and $RQ_{60\text{-base}}$ events suggest that the presence of deflectors does provide refuge from high flows. Nevertheless, the simultaneous lower deflector use in these treatments in comparison with the lower peak flow treatments and the increase in the overall movement behaviour suggest that this flow ratio and the critical hydraulic conditions created in the vicinity of the deflectors (Figure 3) seem to decrease the capacity of *L. bocagei* to find the flow refuges downstream of the deflectors.

This study demonstrated that the duration and the sequences of peak flows together with flow ratio created distinct flow patterns, resulting in varied behavioural responses of fish. Likewise, in rivers affected by hydropeaking, the short-term water level and velocity fluctuations alter fish behaviour in diverse ways. Hence, before defining the dimensions of the deflectors, it is necessary to identify the river hydromorphological changes caused by hydropeaking that will alter channel morphology (Schmutz et al., 2015), and the extent to which the added habitat heterogeneity (deflectors) might create unstable hydraulic conditions for fish (Auer et al., 2017). Hauer, Holzapfel, Leitner, and Graf (2017) used numerical simulations to demonstrate that in river channels influenced by hydropeaking, more heterogeneous habitats with alternating gravel bars created a more unstable flow environment than when compared with reaches that only contained point bars. Therefore, the dimensions, spatial arrangement, and number of the proposed deflectors should be assessed according to the rivers' hydromorphology, biological processes, and societal demands (Woolsey et al., 2007). Although the flume was 0.7-m wide, it was possible to manipulate the dimensions of the deflectors, (opening angle and length) according to the size of young adults of *L. bocagei*. In a natural context, the deflectors would have to be adapted to the river width in order to provide habitat conditions similar to the simulated in this study. If they were simply scaled up, this would result in oversized refuges. According to our findings and to fish passage studies with *L. bocagei* (Santos, Branco, Katopodis, Ferreira, & Pinheiro, 2014), the distance from the river bank to the edge of the deflector (determined by the opening angle of the deflector) should be at least in the same order of magnitude as the fish body length, thus not requiring overly wide angles in relation to the river bank. However, the fact that this species often occurs in schools should also be taken into consideration. In rivers affected by hydropeaking, the proposed opening angle would guide the flow, reducing the deposition of fine sediment in contrast with wider angled structures (Hauer et al., 2017) and clogging associated with accumulated driftwood (Ribi et al., 2014). Peak flows also create turbulent conditions, particularly in the vicinity and in the downstream edge of the deflectors, from which fish can benefit (Liao, 2007; Santos et al., 2014), likely increasing their attraction to the deflectors. To avoid fish stranding during the critical down-ramping phase, it should be guaranteed that the area behind the deflectors would not allow the formation of potential stranding zones or assure a minimum water depth of 0.5 m behind them (Almeida, Boavida, & Pinheiro, 2017; Ribi et al., 2014).

5 | CONCLUSIONS

This study provided new insight on the effects of hydropeaking events on the physiology and movement behaviour of *L. bocagei*. The physiological changes and the increased movement behaviour of *L. bocagei* in the indoor experimental flume demonstrated that this species was affected by flow magnitude and hydropeaking event duration. The presence of deflectors, mimicking natural lateral refuges, increased the flume's morphological heterogeneity and provided alternative velocity options, alleviating the severity of the peak flow conditions. The less harsh hydropeaking events characterized by lower peak flows (20 L s^{-1}), were favourable for maintaining the homeostasis of this species. The unstable hydraulic conditions created during the 60 L s^{-1} flow stimulus appear to have reduced the fishes' ability to seek out the deflectors as velocity refuges. These hydropeaking conditions will affect the downstream populations of *L. bocagei* as the energy available for diel activities (foraging and avoiding predators) and life-cycle stages (growth, reproduction) will be reduced. In addition, as this species naturally occurs in schools, the consequent disruption of social behaviour will also affect the energy budget, as the beneficial hydraulic consequences of group behaviour will decrease. The evident increase in the frequency of individual downstream deflector approaches when *L. bocagei* were subjected to the 20 L s^{-1} discharge indicates that the presence of deflectors favours energy conservation due to the increased availability of velocity refuges and that the hydraulic conditions created during the lower peak flows are favourable for this cyprinid to find low velocity areas. Future experimental research is recommended to understand whether an increase in the severity of hydropeaking events results in habituation or exhaustion. The increasing trend for energy production using hydropower, the continuous alteration of freshwater ecosystems downstream hydropower plants, and the need to develop new strategies to mitigate the impacts of these structures are strong arguments to conduct these types of studies. Finally, the use of an integrated approach strengthened our findings, contributed to an increase in knowledge about the impacts of hydropower in fish and served as a model for future mitigation studies for fish.

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