



Mussel behaviour as a tool to measure the impact of hydrodynamic stressors

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Abstract Freshwater mussels (FMs) are useful bioindicators to detect environmental disturbances. However, studies that evaluate FMs suitability for monitoring the hydrodynamic stressors impact are lacking. Since future climatic scenarios predict an increase in frequency and intensity of extreme events, understanding how flood conditions affect freshwater organisms are crucial for their conservation. In this study, we performed experiments in an artificial flume to evaluate the eligibility for FMs behaviour for developing a tailored biological early warning system (BEWS). For this, we used the valvometric technique (Hall magnetic sensors) to measure the FMs valve gaping behaviour when subjected to

increasing discharges/sediment transport mimicking the onset of floods. After analysing baseline behaviour in non-stressful conditions, we performed experiments in steady and transient conditions to verify the FMs' response and the threshold that prompted it. Under steady conditions, FMs maintained a constant gaping frequency that characterizes their normal behaviour. The FMs promptly reacted to discharge variations with sediment transport, showing a transition from their normal behaviour to higher valve gaping frequencies. We demonstrated that FM transition behaviour is a useful tool to measure hydrodynamic stressors. A future step will be the application of this BEWS on natural ecosystems to assess possible hydrodynamic changes in real-time.

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Introduction

Freshwater mussels (FMs) occur in several types of waterbodies from small streams and ponds to big rivers and lakes, and in certain occasions, account for most of their benthic biomass (Lopes-Lima et al., 2014, 2018, 2021). They have a complex life cycle (i.e. an obligate parasitic larval stage on vertebrate host, usually a fish; Modesto et al., 2018) that, coupled with molecular, cellular, biochemical, physiological, or behavioural features, make FMs one of the most sensitive freshwater organisms to natural and human disturbances (Vaughn et al., 2008; Hartmann et al., 2016; Ladeiro et al., 2017; Premalatha et al., 2020). Due to their fast responsiveness to disturbances, they have the potential to be used as a biological early warning system (BEWS) to detect changes in surface and ground waters, as well as in wastewater effluents and drinking water (Kramer et al., 1989; White et al., 2002; Gerhardt et al., 2006; Bae & Park, 2014).

FMs' behaviour, such as valve gaping, provides a reliable indication of their response to environmental disturbances (Kramer & Foekema, 2001; Hartmann et al., 2016). Besides reflecting normal physiological activities (breathing, feeding, and waste elimination), valve gaping behaviour is a reaction of FMs to environmental (external) stimuli (Krebs & Davies, 1993; Nagai et al., 2006; Robinson, 2009). Valvometry exploits the FMs' capacity to open and close their valves (shells) when exposed to abnormal conditions as an indicator of possible environmental disturbances (Sow et al., 2011). Various types of behaviour can be recorded, like an increased frequency of valve opening-closing activity, the closure of the valves for unusually long periods (avoidance), and the complete valve opening associated with the lack of individual movements, which indicates FM's death (Kramer & Foekema, 2001). Magnetic sensors based on Hall effect (Hall sensor) connected to an Arduino board offer the opportunity to register valvometric measurements with very high resolution in real time (Robson et al., 2009; Sow et al., 2011; Hartmann et al., 2016; Guarini et al., 2020). This improves our ability to detect FMs' behavioural responses to physical and chemical stressors, particularly temperature and chemical pollutants (Kramer et al., 1989; Nagai et al., 2006; Robson et al., 2009; Hartmann et al., 2016). However, we are not aware of any study considering

the application of this technique to changes in hydrological regimes in freshwater ecosystems and how this may translate in alterations in the behaviour of FMs.

This is an important knowledge gap because FMs can be highly affected by climate change, particularly the impact of extreme climatic events such as droughts and floods (Bódis et al., 2014; Sousa et al., 2012, 2018; Nogueira et al., 2021). The frequency and intensity of extreme climatic events associated with climate change are predicted to increase in frequency and intensity in the near future (Milly et al., 2005; Woodward et al., 2010). These extreme events are expected to significantly impact freshwater communities (Döll & Zhang, 2010; Lake, 2011; Tesfaye et al., 2020). For example, major floods can be responsible for important changes in the sediments, and composition of aquatic and riparian vegetation, and can increase the drift of aquatic organisms (Dodds, 2002), with possible ecological effects not only on the aquatic realm but also on adjacent terrestrial ecosystems (Novais et al., 2015, 2017). As benthic organisms, high variation in discharge can deeply affect FMs behaviour by exposing them to different flow velocities and bottom shear stress (Strayer, 1999). Although FMs can be deeply impacted by hydro-morphological modifications (Watters, 1994, 2000; Hastie et al., 2001), an evaluation of the eligibility of behavioural responses to detect hydrodynamic stressors is still lacking.

Since high-flow conditions are known to affect invertebrate communities (e.g. Gibbins et al., 2007), including FMs assemblages (Zigler et al., 2008; Allen & Vaughn, 2010; Hornbach et al., 2010), we decided to assess whether these FMs can be used as a tool to detect the impact of hydrodynamic stressors (i.e. discharge variations and sediment transport). As a model species, we used the most common northern-Italian native species, *Unio elongatulus* Pfeiffer, 1825, a generalist species inhabiting both lotic and lentic habitats (Froufe et al., 2017). To date, no IUCN conservation status assessment exists for this species since this name has only recently been revived for the *Unio* populations in Italy (Prié & Puillandre, 2014). However, *U. elongatulus* is protected under the Habitats Directive (Annex V) and the Bern Convention (Annex III). To identify potential FMs behavioural endpoints we performed manipulative experiments

under artificial flume conditions. The objective of our study was twofold: (i) to investigate if hydrodynamic stressors may influence FMs valve gaping frequency and opening amplitude and (ii) whether these behavioural responses can be leveraged for monitoring the occurrence of hydrodynamic stressors on fluvial environments. Behavioural responses were analysed under varying discharge conditions to assess the influence of (1) steady discharges (keeping constant the velocities and flow depths) without sediment transport, and (2) sharp and rapid increases of discharge without or with sediment transport. We hypothesized that (1) high constant discharges, and (2) wide and rapid variations of discharges with sediment transport may affect FMs' individual valve gaping frequency and opening amplitude.

Materials and methods

Animals' collection and housing

Unio elongatulus specimens (average \pm SD length size of 66.7 ± 5.38 mm) were collected in the mesotrophic Lake Caldonazzo ($46^{\circ}01'N$, $11^{\circ}14'E$), Italy, in June 2020, and immediately transported to the laboratory (Department of Civil, Environmental and Mechanical Engineering, University of Trento, Italy). Animals were acclimated ($15^{\circ}C$, 12:12 h light:dark cycle) for two weeks in recirculated flow-through 500 L aquarium with dechlorinate-aerated

water and gravel-sandy substrate, and fed with a mixed culture of natural algae, mainly *Chlorella* sp. and *Euglena* sp.

Description of the experimental design

Normal behaviour

Two experiments were designed to assess the standard (1) feeding and (2) movement behaviour during 24 h ($n=8$). The first experiment, performed in the acclimation aquarium under standing water conditions, measured the FM behavioural response to food supply (after 5 h of starvation). The second experiment was carried out in an artificial flume (6 m \times 40 cm) with 6 cm of sandy sediment. Individuals were placed at a 20 cm distance from each other to avoid mutual interaction. The movements were measured under non-stressful conditions with low discharge rate (Q1, defined as baseline, Table 1) and registered by taking camera pictures every 10 min.

Hydrodynamic-induced behaviour

After 1 h acclimation into the flume at low discharge (Q1, Table 1), FMs ($n=32$) were exposed to constant flow discharges [Q2, Q3, Q4] during 4 h, followed by a rapid increase of discharge without [from Q2 and Q3 to Q4] and with [from Q2 and Q3 to Q5 and Q6] sediment transport during 1 h and 30 min (Table 1).

Table 1 Exposure scenarios of the hydrodynamic conditions considering: water flow rate Q/B (being B the width of channel and Q the discharge); water depth; the cross-section averaged flow velocity; the specific solid discharge, Qs/B (i.e. per unit of width);

the Shields parameter [$\theta = u^{*2}/(s-\rho)d$ —being u^* the shear velocity and d the median sediment diameter] indicating the incipient sediment motion conditions; and the shear Reynolds number [$Re = u^*d/\nu$ —with ν =water kinematic viscosity]

Discharge	Q/B (m ² s ⁻¹)	Water depth (m)	Velocity (m s ⁻¹)	Qs/B (kg/(ms))	θ	Re^*
Q1	0.005	0.115	0.041	0	0.002	5.6
Q2	0.013	0.135	0.098	0	0.007	10.8
Q3	0.019	0.140	0.134	0	0.011	13.6
Q4	0.029	0.150	0.192	0	0.019	17.8
Q5	0.036	0.085	0.430	0.003	0.056	35.3
Q6	0.055	0.110	0.500	0.032	0.066	40.0

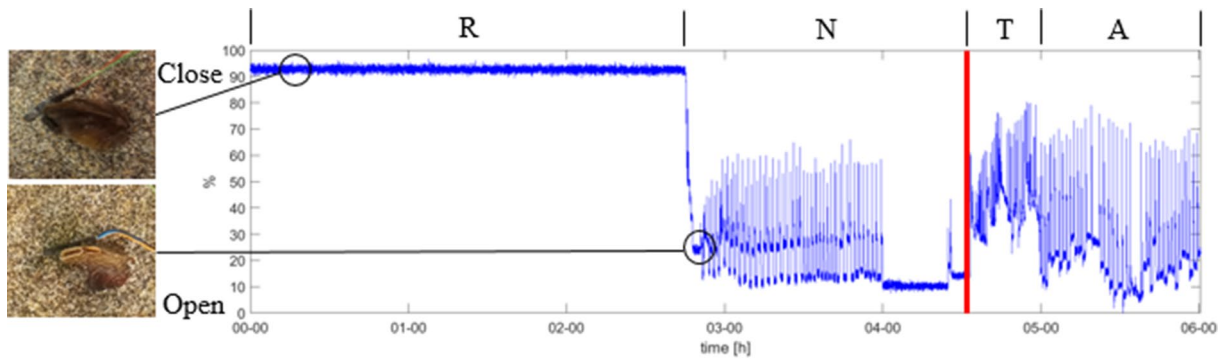


Fig. 1 Example of *Unio elongatulus*' individual behavioural responses. Pictures on the left demonstrate mussels with Hall magnetic sensors attached to their shells: upper represents a closure and lower represents an opening of the valves. The letters R, N, T, and A represent the mussels' behaviour: resting, normal, transition, and adaptation, respectively. The red vertical line indicates the beginning of the response to hydrodynamic stressors

Valve gaping behavioural analysis

Hall sensor technologies and Arduino board were used to record valve movements. The Hall element sensor (Honeywell SS495A1) equipped with a serial cable (FTDI USB-232R-5) was glued by cyanoacrylate adhesive to the edge of the left valve, and the magnet of the right valve directly opposite to the sensor. Arduino hardware equipped with a microcontroller (Badamasi, 2014) was used to measure the valve movements per second (i.e. gap distance between the two sides of the valve). FMs' behaviour was measured by valve gaping frequency (Hz) and opening amplitude (%). The magnetic field data (mV) were normalized in percentage (%), where the minimum value (0%) corresponds to the complete valve opening and the maximum (100%) to the complete closure (Fig. 1). Five behavioural responses (following Hartmann et al., 2016) were identified: normal, resting, transition, adaptation, and avoidance (Fig. 1). During normal behaviour (standard feeding and moving) FMs presented valve gaping, whilst during resting behaviour valves were kept constantly opened for water filtration. In the hydrodynamic-induced behaviour experiments, transition was the sudden increase in valve gaping, adaptation the valve gaping reduction after the transition and avoidance the complete closure of the valves. To interpret the variations in valve gaping frequency and opening amplitude, the valvometric recording was compared with the camera recording. The valve gaping frequency and

opening amplitude of each FM behaviour were calculated using the statistical language Matlab (MATLAB software, 2010).

Data analysis

Differences in valve gaping frequency and opening amplitude ($n=8$) in response to food supply were tested by Wilcoxon Signed Rank Tests ($\alpha=0.05$) using the *wilcox.test* function from the package *dplyr* (Wickham et al., 2020) keeping two feeding periods as fixed factors (Table 2).

The *kruskal.test* function from the package *MASS* (Venables & Ripley, 2002) was used for the Kruskal–Wallis Test (Chi-Square; $\alpha=0.05$) to compare differences in valve gaping frequency and opening amplitude ($n=32$) within and between levels of hydrodynamic stress: (i) keeping discharge rates, periods, and discharge variations \times periods as fixed factors and (ii) between the different types of behaviours (Table 2).

General Additive Models (GAMs) using package *mgcv* (Wood, 2011, 2017) were fitted to the data using the *gam* function to determine if the number of unresponsive individuals influenced the patterns of FMs' behavioural response. Two models were compared (Multiple R-squared R^2 and Akaike Information Criterion AIC) including: (1) all the individuals and (2) only the ones reacting to the stressor, keeping as fixed factor the number of FMs and the discharge variation. For the GAM of transition behaviour, we excluded the individuals

Table 2 Description of fixed factors used on Wilcoxon Signed Rank Tests (Feeding experiments) and Kruskal–Wallis Tests (Hydrodynamic-induced behaviour experiments): discharges and sequential periods

Experiments		Discharge	Period
Normal behaviour	Feeding	Q1	WF (without food) F (with food)
		Hydrodynamic-induced behaviour	Constant
Hydrodynamic-induced behaviour	Small variation (SV)	Q2–Q4	V1 (22.5 min)
		Q3–Q4	V2 (22.5 min)
		Q2–Q5	V3 (22.5 min)
	High variation (HV)	Q2–Q6	V4 (22.5 min)
		Q3–Q5	
		Q3–Q6	

showing normal or resting behaviour, for the GAM of adaptation behaviour only the FMs showing adaptation were included. The relationship between the percentage of reactive FMs and the intensity of the stressor was tested by a third model (R^2) including only the individuals that present transition and adaptation behaviour.

All the analyses were performed using the open-source statistical language R software (R Core Team, 2020).

Results

Normal behaviour

Valve gaping frequency was constant during both food supply periods (0.0136 ± 0.00828 Hz) and not significantly different from the starvation periods (0.0111 ± 0.00374 Hz; Table S1, Fig. 2a). Valve opening amplitude was similar with ($18.9 \pm 3.44\%$) and without ($20.1 \pm 5.53\%$) food supply (Table S1, Fig. 2a). Similar values of gaping frequency (0.0119 ± 0.00463 Hz) and amplitude ($18.3 \pm 6.17\%$) were measured under non-stressful conditions (Table S1, Fig. 2b).

The videos evidenced that each gape (=peak on the recording trace) corresponded to a singular horizontal movement and that the longer and more intense the gaping was, the greater the distance travelled. This behavioural pattern, classified here as normal behaviour, was not common to all individuals.

Some of them (12.5%) displayed no valve movements (peaks) during the whole experiments, and videos suggest that their behaviour resembled a resting phase.

Hydrodynamic-induced behaviour

No significant differences in gaping frequency were observed in constant (0.0132 ± 0.00744 Hz) and small discharge variations without sediment transport (from Q2 and Q3 to Q4; 0.0177 ± 0.00473 Hz), i.e. below 35.3 of shear Reynolds number (Table 1). Above this threshold (0.0256 ± 0.00660 Hz), the increase of discharge prompted the sediment transport (from Q2 and Q3 to Q5 and Q6), and the reaction of FMs passed from the normal behaviour with constant valve gaping frequency to a variable behaviour (example in Fig. 3). Indeed, significant increases in valve gaping frequency (0.0308 ± 0.00428 Hz) were observed immediately after the onset of high discharge rates with sediment transport, i.e. from the period C4 of constant discharges (Q2 and Q3) to the period V1 of high discharges (Q5 and Q6) (Table 2, Figs. 3, 4). Most of valve opening amplitude values did not significantly differ between constant ($15.7 \pm 7.55\%$) and variable discharges ($15.2 \pm 8.78\%$), except for discharges from Q3 to Q5 (Table S1). Opening amplitude significantly decreased from the period C4 of Q2, and significantly increased from the period C4 of Q3, to the first period V1 of high discharges with sediment transport (Q5 and Q6) (Table S1).

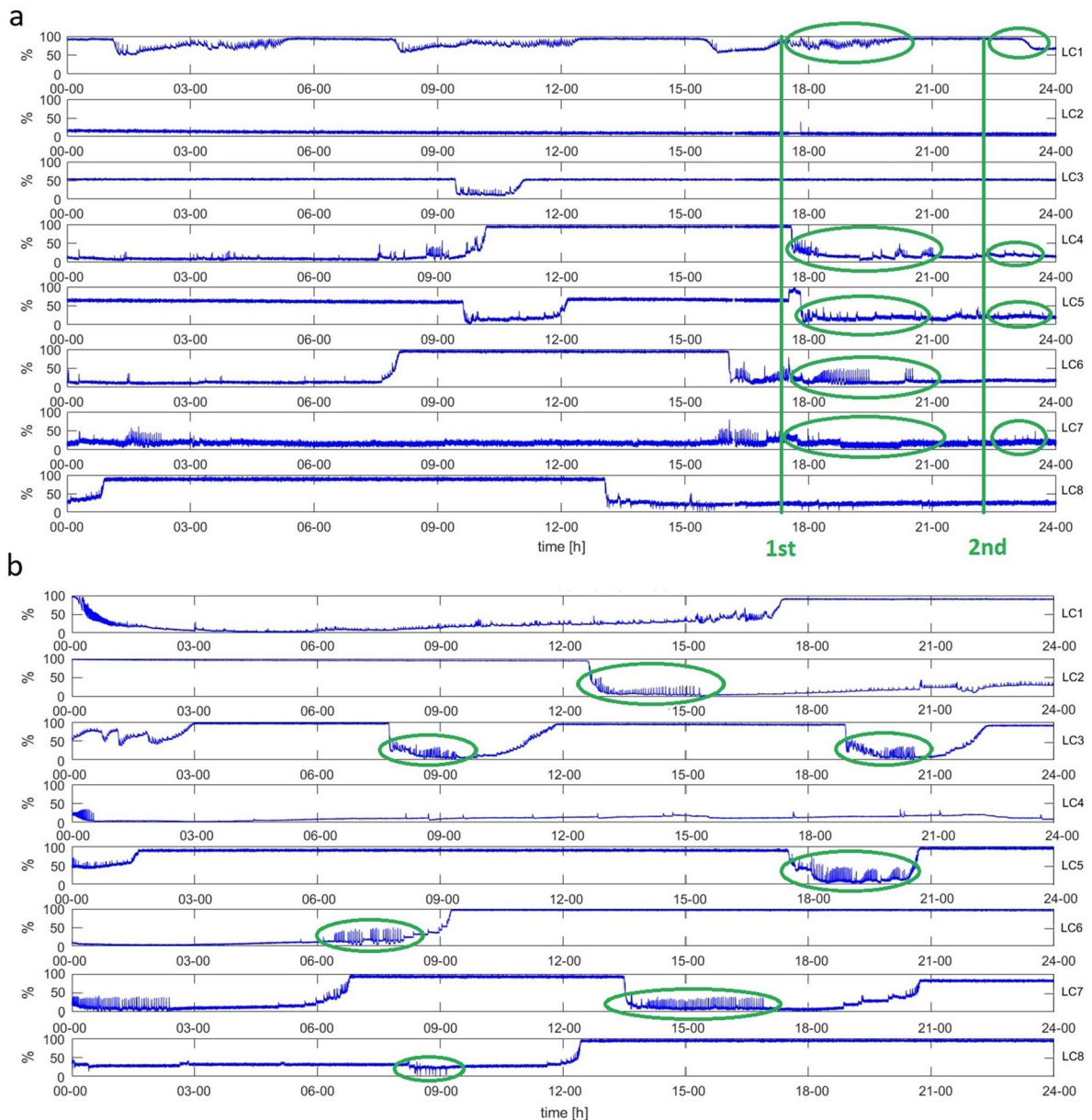


Fig. 2 Examples of *Unio elongatulus*' individual behavioural responses (group LC1-LC8) on normal behaviour experiments of **a** feeding (with and without food, green lines represent the

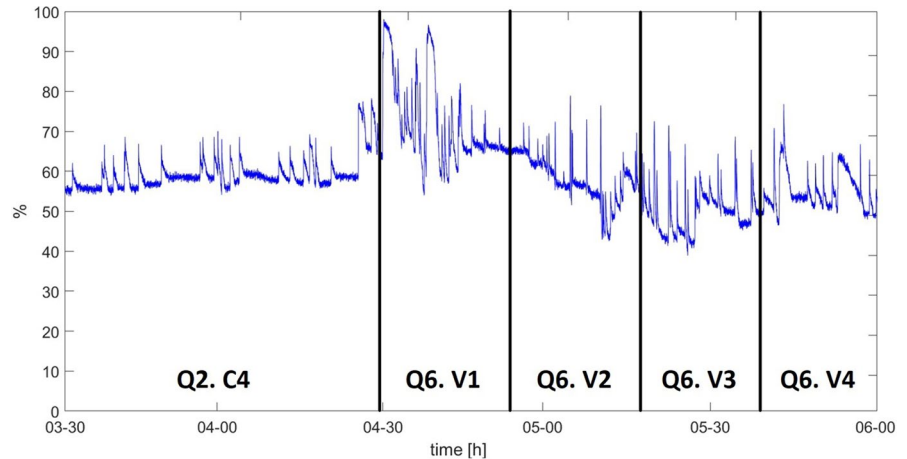
1st and 2nd periods of feeding), and **b** movement. The green circles represent the constant valve gapping frequencies in the feeding and movement

Valve gapping behavioural analysis

The average of all individual signals was used to identify the behavioural patterns (examples in Fig. 5). The transition frequency (0.0252 ± 0.00718 Hz)

significantly differed from the one displayed during normal behaviour periods (0.0078 ± 0.00300 Hz, Table S1). Gapping frequency during normal behaviour also significantly differed from that of adaptation behaviour (0.0140 ± 0.00537 Hz; Table S1).

Fig. 3 Example of original signal of *Unio elongatulus* individual (LC1) showing the behavioural response before (last period C4 of constant discharge Q2) and after the discharge with variation (periods V1, V2, V3, and V4 of Q6)



Conversely, no significant differences were observed in the valve opening amplitude between normal ($13.8 \pm 4.35\%$) and transition ($15.5 \pm 4.06\%$) or adaptation ($14.7 \pm 7.51\%$) behaviour.

Most FMs (88–97%; Table 3) reacted promptly to increased discharge with sediment transport, showing a transition from their normal behaviour to a significantly higher valve gaping frequency (Figs. 4, 5). Some FMs (44–60%; Table 3) presented an adaptation behaviour shown by a gradual decrease in frequency in response to the persistence of stressful conditions (Figs. 4, 5). The remaining ones were apparently inactive, keeping their valves open in the same position. No FM displayed the avoidance behaviour even when extreme discharge variations were applied. The percentage of reactive FMs and the intensity of their reaction significantly increased from the lowest (from Q2 and Q3 to Q4) to the highest stress levels (from Q2 and Q3 to Q5 and Q6). Out of the GAMs, the model including all the data was the best descriptor of the stress-induced reaction (transition behaviour: $R^2=0.209$, $AIC=-1121.33$; adaptation behaviour: $R^2=0.063$, $AIC=-1283.17$; Table S2), suggesting that the number of non-responding FMs did not affect the general patterns of the behavioural response (Table S2). The percentage of FMs reacting increased with the increase of stress intensity as shown by the third model (transition behaviour: $R^2=0.212$; adaptation behaviour: $R^2=0.113$; Table S2).

Discussion

The prompt FMs' response to discharge variations confirm that they are sensitive to physical factors such as hydrodynamic forces and sediment load. Since hydrological changes can be amongst the major causes of FM's mortality (Hastie et al., 2001; Sousa et al., 2012), it is expected a rapid behavioural reaction to discharge variation. Completely unknown is the type and intensity of their response. FMs' response to disturbances spans from valve gaping to valve closure (avoidance behaviour) or movement (escape behaviour) (e.g. Cândido & Romero, 2007; Hartmann et al., 2016). These responses are easily observed in field and laboratory conditions, but the challenge is to quantitatively detect them to describe a behavioural pattern. The development of valvometric technologies significantly improved detection abilities, shifting the main challenge from the description to the interpretation of behavioural patterns (Bae & Park, 2014). To our knowledge, this is the first study addressing and interpreting FMs' behavioural patterns in response to discharge variations. To date, the valvometric technique was mainly used as a real time system to monitor the impact of chemical stressors (e.g. Tran et al., 2003; Hartmann et al., 2016). We just found only one previous research dealing with a physical stressor, i.e. suspended solids (Lummer et al., 2016).

The FMs' behavioural response varied between exposures to steady and transient conditions. Under

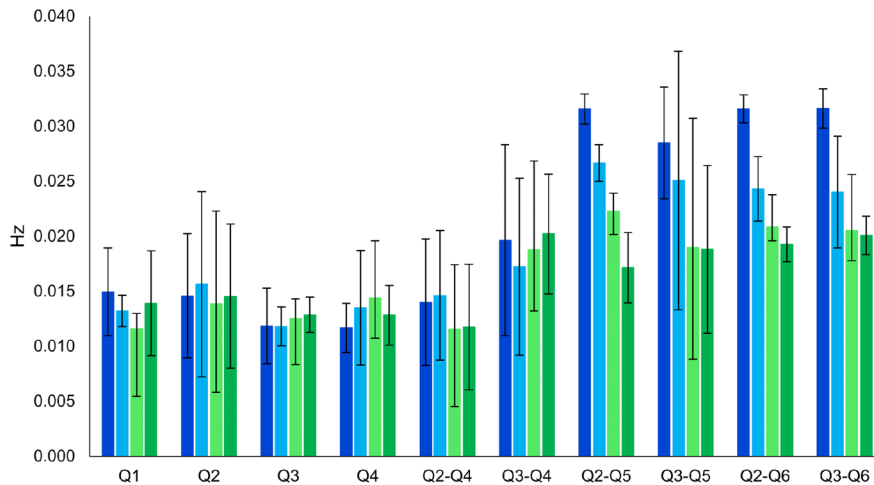


Fig. 4 Mean (\pm SD) valve gaping frequency (Hz) of *Unio elongatulus* individuals (LC1-LC16) in hydrodynamic-induced behaviour experiments with constant discharges (Q1, Q2, Q3, and Q4) and variable discharges (Q2–Q4, Q3–Q4, Q2–Q5, Q3–Q5, Q2–Q6, and Q3–Q6). The different experi-

mental periods C1, C2, C3, and C4 in constant discharges and V1, V2, V3, and V4 in variable discharges are represented by dark blue, light blue, light green, and dark green bars, respectively

constant water discharges without sediment transport, FMs maintained constant valve gaping frequency showing no change into their normal behaviour. This behaviour mimics their daily routines measured during our normal behaviour experiments. Gaping activity of FMs is related to physiological vital processes such as nutrition, breathing, and faeces and pseudo-faeces elimination (Riisgård, 2001; Sow et al., 2011; Addis et al., 2021). Valve gaping is also necessary for the FMs to straighten up, to perform horizontal movements and to burrow (Cândido & Romero, 2007). In fact, even if the FMs have limited mobility, they can perform horizontal and vertical movement by means of their muscular foot (Allen & Vaughn, 2009; Ferreira-Rodríguez, 2019). Video records showed that valves are opened to protrude the foot to anchor in the sediment; then the contraction of the foot muscle to induce displacement causes a reduction in the opening of the valves. Sequences of protraction/dilatation of the foot combined with adduction and opening of the valves are operated when the FMs are moving (Cândido & Romero, 2007). The horizontal movements that we observed might be related to feeding. Since the FMs were not fed during the experiments, these movements could be prompted by the need to find food. Indeed, FMs can move to areas where food is not depleted (Schwalb & Push, 2007) or the

movement itself can promote the food ingestion through a water current into the anterior portion of FMs (McMahon, 1991).

In addition to the valve movements for basic vital functions, FMs can change their gaping behaviour in response to fluctuations in environmental conditions as depth, light, temperature, and particulate matter (Tran et al., 2003; Ropert-Coudert & Wilson, 2004; Robson et al., 2009). Our study adds one factor to this list, i.e. hydrodynamic stressors, because high enough water discharges induced sediment transport and sharp FMs' responses. FMs reacted promptly by switching from the normal behaviour performed under low constant discharges to a transition behaviour characterized by an increase of valve gaping frequency. The duration of this transition behaviour increased with the intensity of water discharges and sediment transport. Accordingly, Hartmann et al. (2016) reported an increase in FMs' transition frequency in response to higher de-icing salt concentration and exposure time. Contrarily, Lummer et al. (2016) observed no significant differences in transition frequency of FMs in response to suspended fine sediments at different concentrations and particle size classes. These differences in the behavioural responses seem to be related to the type of stressors applied: de-icing salt is a soluble substance influencing the ion concentration of FMs'

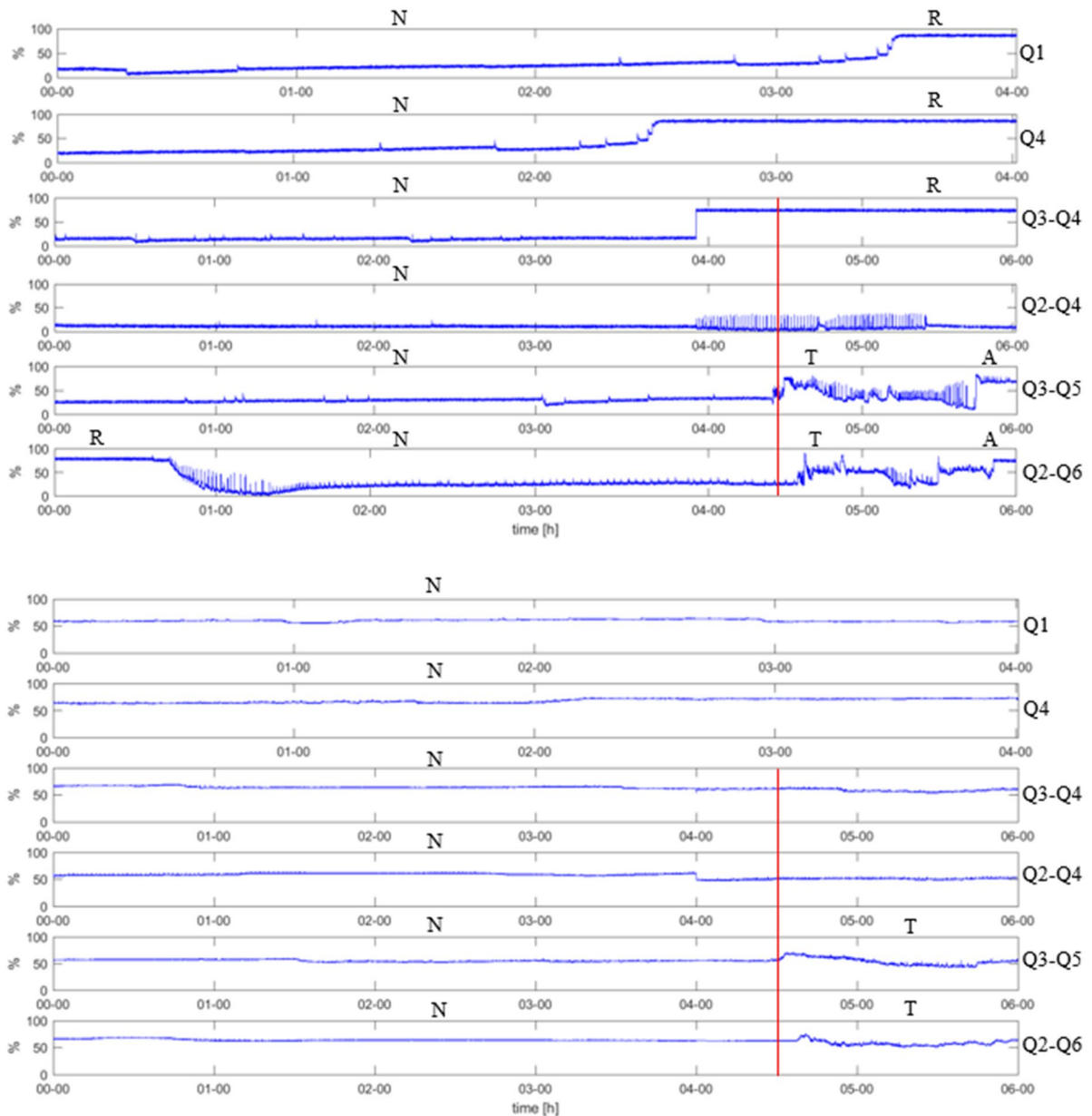


Fig. 5 Example of behavioural responses of **a** one *Unio elongatulus*’ individual (LC13) and **b** the average of 8 individuals (LC9-LC16) to: constant discharge, low (Q1) and high (Q4); discharges with low variation rates without sediment transport (Q3-Q4, Q2-Q4), and high variation rates with sediment trans-

port (Q3-Q5, Q2-Q6). The letters N, R, and T are the mussels’ behaviour: normal, resting, and transition, respectively. The red vertical line indicates the beginning of variation in the discharge rates

tissues, whereas suspended solids do not enter in the tissues (Lummer et al., 2016). Contrarily, we proved that physical factors not entering on body tissues can also induce a behavioural alteration.

Unlike the gaping frequency, the valve opening amplitude did not present a clear pattern of variation between exposures to steady and transient conditions. This is possibly related to the fact that the amplitude is constrained by the length of adductor muscles,

Table 3 Number of mussels used on the General Additive Models (GAMs): (1) total number used in all experiments (N_T); (2) number that presented valve gaping activity (N_A), normal behaviour (N_N); (3) number that presented response to

the hydrodynamic stressors with transition behaviour (N_T) and with adaptation behaviour (N_{T+A}); and (4) the percentage (%) that presented transition behaviour (P_T), and with adaptation behaviour (P_{T+A})

Experiments	Discharge	N_T	N_A	N_N	N_T	N_{T+A}	P_T	P_{T+A}
Feeding	Q1	8	6	6	–	–	–	–
		8	6	6	–	–	–	–
Constant	Q1	32	24	24	–	–	–	–
	Q2	32	22	22	–	–	–	–
	Q3	32	25	25	–	–	–	–
	Q4	32	24	24	–	–	–	–
Small variation (SV)	Q2-Q4	32	26	9	17	9	53	28
	Q3-Q4	32	23	2	22	13	69	41
	Q2-Q5	32	31	0	31	14	97	44
High variation (HV)	Q2-Q6	32	30	0	30	14	94	44
	Q3-Q5	32	29	1	28	19	88	59
	Q3-Q6	32	28	0	28	19	88	59

which varies with size and shell shape within and between species (Nagai et al., 2006; Robson et al., 2009).

The FMs' ability to resist floods depends on substrate stability (related with sediment granulometry) and associated hydrodynamic variables as water depth, flow velocity, and shear stress (Strayer, 1999; Allen & Vaughn, 2010; Zajac & Zajac, 2011). The hydrodynamic tension promoted by the high variation in water discharges, in our experimental artificial flume, exposed FMs to high flow velocities and shear stress (represented by the shear Reynolds number). High water velocities can increase the shear stress over the bed, due to the roughness elements of the bed and/or to the FM shape outcropping the bed (Sansom et al., 2020). When this shear stress overpasses the critical threshold, it prompts sediment transport that in our study resulted to be the stimulus triggering the increase of valve gaping frequency. However, this hydrodynamic stressor is probably related to several factors, such as the spacing, density, and height of exposure of roughness elements (Chow, 1959; Sansom et al., 2020), that were not tested in our study.

The capacity of some FMs to significantly decrease their valve gaping frequency after the transition period, suggests their capacity to adapt to hydrodynamic fluctuations, at least within the limits of our experimental conditions. The ability to resist to flood conditions depends on the position of FMs within the hydrodynamic settings of each habitat.

That is, FMs are thought to be able to bury deeper into the sediment in areas with faster flow or move horizontally to other microhabitats with less intense current (e.g. between tree roots in the banks or under the cover of boulders), when subject to these flood conditions (Hastie et al., 2001; Zajac et al., 2019). During our experiments with high discharge and sediment transport, FMs seemed to be unable (or unwilling) to perform horizontal movements, trying to bury themselves into sediments. This behaviour may be different in other FM species that have a distinct life history strategy, behaviour, and shell sculpture (Strayer, 1999; Allen & Vaughn, 2009; Gooding et al., 2019; Randklev et al., 2019). We tested one generalist species inhabiting both lentic and lotic habitats that is expected to have the ability to adapt to a wide range of hydrodynamic conditions. Less tolerant species could be less ready and quick to show behavioural signs of adaptation. This ability to adapt can also differ between populations of the same species. We tested organisms collected from a lake population, which could be supposed to be more adapted to still waters and more sensitive to flow, than river populations. Moreover, the reduction of the reactive response is expected as the exposure time increases, both due to physiological limits to the maintenance of high rhythms, and because of adaptation. This arises a question on a possible effect of adaptation on the behaviour of FMs' populations living in environments subjected to high hydrodynamic variations. Besides

comparing different species, adaptation to different levels of hydrodynamic stressors should be explored in future studies by comparing populations from different habitats.

Avoidance behaviour did not occur even in response to the high water discharges with sediment transport. Avoidance is typically observed as the main response to the exposure to chemicals (Tran et al., 2003, 2007; Fournier et al., 2004; Andrade et al., 2016; Hartmann et al., 2016), but FMs cannot rely on this strategy to respond to hydrodynamic stressors. The high hydrodynamic tension applied prompted sediment erosion around FMs, forcing them to open their valves and protract their foot fully extended to anchor into the sediment.

Overall, behavioural analysis was challenging because of the individuals' specific responses. Anyway, most of the FMs presented clear patterns of transition in response to hydrodynamic variations. GAM analyses showed that excluding non-responding individuals (with no valve gaping activity or normal behaviour) did not affect the possibility to clearly detect a transition behaviour. The percentage of individuals switching to transition behaviour grew up to 97% as the intensity of the stress increased. Whilst a delay in response testifies the individual variability in stress tolerance, transition behaviour can be considered a useful tool to detect the threshold value of the impact induced by hydrodynamic variables on FMs.

Conclusion

Since hydrodynamic stressors significantly affect habitat conditions, rapid and precise detection and prediction of these impacts is crucial for ecosystem management. However, this can be challenging due to the multifaceted effects of stressors on natural ecosystems. We proved that FMs' transition behaviour can be a useful tool to detect hydrodynamic stressors. FMs high sensitivity, ease of handling, and of behaviour recording, as well as their prompt reactivity to transient discharges, make them preferable over other benthic species for the set-up of biological early warning systems of hydrodynamic impacts. On the other hand, by using this method that is a non-invasive procedure, data with ecological relevance can be collected without affecting

the organisms' physiology, which is of great importance since this species belongs to a highly threatened group. Anyway, more is needed not only to improve ecological knowledge about FMs' behaviour but also to validate the use of this innovative tool in the detection of hydrodynamic stressors in natural ecosystems. Therefore, the next step will be the validation of this methodology in the field. This transition from the laboratory to the field can be highly useful in the monitoring of natural ecosystems with possible pay-offs in the conservation and management of freshwater ecosystems.

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Declarations

Conflict of interest The authors have no conflict of interest.

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