

Identification of hydrodynamic changes in rivers by means of freshwater mussels' behavioural response: An experimental investigation

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Abstract

The present work concerns the interaction between hydraulic processes and biological communities in rivers. In particular, the aim of this study is to investigate the interactions between flow dynamics and the freshwater mussels (FMs) to verify if the mussels' behavioural response to the hydrodynamic stress could be used to monitor natural extreme events in rivers. Although the influence of mussels on the kinematic characteristics of flow at the water-sediment interface was investigated by a certain number of studies, their behavioural response to flow, both in static and dynamic conditions, remains understudied. Laboratory experiments were performed in an artificial flume exposing *Unio elongatulus* to different values of flow discharge, both in steady and in unsteady conditions either with or without sediment transport. Mussels' behavioural responses were detected by using Hall sensor technology to measure gaping frequency, amplitude and duration, both in static conditions and under the effect of hydrodynamic stresses. Five categories of behavioural response were identified: Normal Activity (NA), Resting (Re), Transition (Tr), Adaptation (Ad) and Avoidance (Av). During NA (standard feeding and moving), FMs presented valve gaping, while during Re valves were kept constantly opened for water filtration. After a variation of flow discharge (ΔQ), FMs promptly reacted showing a transition from their normal behaviour, with constant gaping frequency (below 0.01 Hz), to higher valve gaping frequencies. The mean valves' gaping frequency increased as a function of ΔQ , and the highest values were reached in the presence of sediment transport. The mean valve opening amplitude was less sensitive to ΔQ . Its range of variation was very narrow with the highest values corresponding to the protrusion/retraction of the animals' foot to move or anchor to the substrate. The percentage of mussels responding to the discharge variation (Transition behaviour) increases with ΔQ confirming that mussels' behavioural response represents a promising tool for monitoring the occurrence of hydrodynamic stressors in fluvial systems.

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KEYWORDS

biotic communities, experiments, floods, hydrodynamics, rivers

1 | INTRODUCTION

One of the most significant discussions in current research is the effects of climate change on the aquatic ecosystems. The functions of biological communities are influenced by the interaction between biological, physical–chemical and hydraulic processes. Hydrodynamics especially deals with the physical interactions between flow and organisms and thus with the flow-induced forces and reaction forces generated by the organisms (Lopez & Vaughn, 2021). Recent ecologically oriented research (eco-hydrology, eco-hydraulics, eco-geomorphology) investigated such interactions, and significant progress has been made in this field. A growing body of research (e.g. Butler & Sawyer, 2012; Jones et al., 1994; Polvi & Sarneel, 2017) indicates that some filter feeders' species can be considered as 'ecosystem engineers' because they have a significant impact on the aquatic environment. Freshwater mussels (FMs) meet the criteria to be considered as typical 'ecosystem engineers' who can alter the near-bed hydraulic characteristics (Sansom et al., 2018; Wu et al., 2020) and consequently the local hydrodynamic conditions (Lopez & Vaughn, 2021). At the same time, their habitat and therefore their survival are affected and limited by hydrodynamics.

Mussels are relatively sedentary, so they are highly vulnerable to hydrological changes, which may result from both natural process and artificial modifications (Geist, 2010; Lopes-Lima et al., 2016). Numerous studies have shown how FMs could interact with the fluvial environment in passive way by influencing erosion and deposition processes (Daniel & Brown, 2014; Jones et al., 2014; Sansom et al., 2018) and in active way by affecting mixing processes and thus nutrient cycling and the mass transport (Atkinson et al., 2014, 2018; Vaughn et al., 2004). As a result of the exposure to environmental stressors, FMs tend to modify their filtration rate and their syphon orientation (Perles et al., 2003) as well as the patterns of valve closing/opening activity (Hartmann et al., 2016). For these characteristics, mussels are being used as sensitive biosensors of environmental disturbance since several decades (Gerhardt et al., 2006; Kramer et al., 1989; Miller et al., 1999; Slooff et al., 1983). The development of valvometric technologies (Kramer et al., 1989; Robson et al., 2009; Sow et al., 2011) allowed the measure the mussels' valve movements in real time by using Hall sensors. To the date, the valvometric technique was mainly used to monitor the impact of chemical stressors (e.g. Hartmann et al., 2016; Tran et al., 2003) or suspended solids (Lummer et al., 2016) on mussels.

Despite mussels' habitat requirements and their role in habitat modification were addressed by several studies (e.g. Haag, 2012; Vaughn, 2017; Vaughn et al., 2015), little is known about the interactions between mussels and hydrodynamic variables (Lopez & Vaughn, 2021) and even less about their behavioural reactions to hydraulic stressors. The present study is part of a large research

programme aimed to verify if the mussels' behavioural response could be used as a tool for an early warning system for flow variations in rivers, also during floods, which is especially important in a context of a climate change. As a model species, we used the most common northern-Italian native species, *Unio elongatulus* (Pfeiffer, 1825), a generalist species occurring in both lotic and lentic habitats (Froufe et al., 2017). Since *U. elongatulus* is in dramatic decline particularly in rivers (Riccardi, Bo, et al., 2022), we tested a lake population collected from Lake Maggiore (Italy). To analyse how the variations of flow discharge and sediment transport might influence mussels' valve gaping, we performed laboratory experiments, especially focusing on the hydrodynamic aspects and the mussels' behavioural response. The present work complements another one (Modesto et al., 2023), performed on a different mussel population, collected in Lake Caldonazzo (Italy). The specific objectives of the present work are (i) to analyse and identify the mussels' response in terms of valve gaping frequency, amplitude and duration for increasing values of flow discharge, both in steady and in unsteady conditions either in the absence or in the presence of sediment transport; and (ii) to identify and characterize the different mussels' behavioural response to the different hydrodynamic conditions examined. The paper is organized as follows: Section 2 describes the experimental apparatus and the data analysis methodology; Section 3 presents the results; the discussion is driven in Section 4 and finally conclusions in Section 5.

2 | MATERIAL AND METHODS

2.1 | Experimental apparatus and acquisition methods

Experimental tests have been conducted at the Hydraulic Laboratory (Department of Engineering) of the University of Palermo (Italy) to analyse the response of *U. elongatulus* mussels to different hydrodynamic conditions.

In the laboratory, mussels were kept in a 400-L aquarium supplied with dechlorinate-aerated water, with a layer of 10-cm sandy bed (diameter 0.7–1.3 mm) over 5 cm of gravel substrate. The mussels were fed three times per week with a mixed culture of natural algae (mostly *Chlorella* sp).

In analogy to Modesto et al. (2023), two series of runs were performed to monitor the valves' movements of two groups consisted of eight mussels (in total 16 mussels). Each run was repeated twice so that a data set of 32 recorded values for each examined condition was available. The first series [hereon referred to as 'normal behaviour'—NB series] was conducted in order to analyse the mussels' behaviour during their usual activities, such as feeding or moving. The second series of runs [hereon referred to as 'hydrodynamic-induced

behaviour'—H-IB series] was aimed to identify the mussels' behavioural response to different hydrodynamic stresses. Thus, the NB runs have been considered as a reference for the analysis of the H-IB runs.

The mussels' valve opening/closing were recorded during all runs by using Hall sensor technology (Hartmann et al., 2016; Modesto et al., 2023; Robson et al., 2009; Sow et al., 2011; Wilson et al., 2005) and the Arduino platform, which included the Arduino board with ATMEGA2560-16AU microcontroller (16 MHz 256 Kb 8-bit), the SD memory card and the Arduino software. A Hall sensor (Honeywell SS495A1, 13 × 10.5 mm, 1.1 g weight) and a magnet (12 × 10 mm, 1.8 g weight) were glued to the opposite valves of each mussel, and the valves' opening/closing were recorded as a change in the voltage (mV) outputs acquired every 0.33 s and stored on the SD card connected to the Arduino board. A thermal sensor DS18B20 (accuracy $\pm 0.5^\circ\text{C}$) was also used during the experiments to measure the water temperature.

The NB series included two experimental tests to assess the feeding ('feeding' test) and movement ('movement' test) behaviours. For the 'feeding' test, the mussels' valve opening/closing movements were recorded for a time period of 25 h while they were kept in the laboratory aquarium. During this test, the mussels were fed two times: first time after 17 h of starvation period and the second time after a further time interval of 6 h. The food supply was almost instantaneous. The time intervals were sufficient to record the animals' response. The 'movement' test was conducted in a straight laboratory flume 40 cm wide, 11 m long and with a bed of quartz sand ($d_{50} = 0.65$ mm, $d_{90} = 0.9$ mm) of longitudinal slope, S , around equal to 0.1% (see Figure 1a), which is similar to that used in Modesto

et al. (2023). In the movement test, the mussels' response, without feeding but under non-stressful conditions with a low water discharge flowing (i.e. flow discharge Q_1 in Table 1), was monitored for a time period of 24 h, which is comparable with the total observation time of the feeding test. The mussels were arranged on the bed at a mutual distance of around 20 cm in the longitudinal direction and 15 cm in the transversal direction (Figure 1b). A gate located at the end of the flume was used to guarantee the uniform water depth along the monitored channel reach. A video camera (30 fps) was used to record the mussels' movement.

The H-IB series were also conducted in the straight channel shown in Figure 1a with the mussels arranged in the same configuration as that used in the movement test. Experimental runs were carried out by exposing the mussels to different hydrodynamic conditions. Table 1 lists the values of the flow discharge (Q), the specific flow rate (Q/B , B = width of the channel), the water depth (h), the mean flow velocity (V), the specific sediment discharge (Q_s/B), the Shields parameter ($\theta = u_*^2/[\gamma_s - \gamma]d$ with $u_* = [gRS]^{0.5}$ = shear velocity, g = gravity acceleration, R = hydraulic radius, $d = d_{50}$ = median sediment diameter, γ_s = specific weight of sediment = 2650 kg/m^3 , γ = specific weight of water) and the shear Reynolds number ($Re = u_*d/\nu$, with ν = water kinematic viscosity) considered for the experimental runs. In Table 1, the values of the mean temperature measured during the aforementioned runs have been also reported. In steady flow conditions, the runs were conducted by maintaining a constant value of flow discharge for a duration of 6 h (i.e. the same time interval of the NB series of runs), always below the threshold for the sediment incipient motion (i.e. Q_1 , Q_2 , Q_3 and Q_4 of Table 1). These runs were followed by the T-runs of Table 2 characterized by

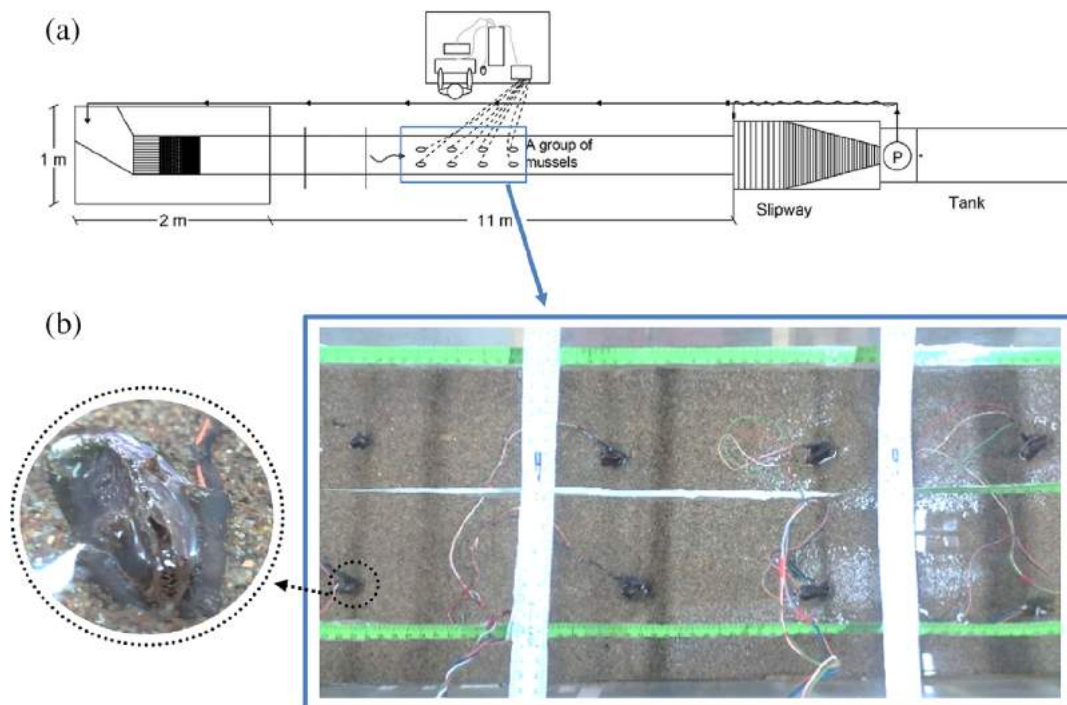


FIGURE 1 (a) Scheme of the experimental apparatus used for the experiments; (b) Hall element sensor (left) and row of mussels in the flume (right).

TABLE 1 Hydraulic conditions considered for the experimental runs.

Symbol	Q(m ³ /s)	Q/B(m ² /s)	h(m)	V(m/s)	Qs/B(kg/ms)	θ	Re*	Temperature(°C)
Q1	0.0021	0.005	0.040	0.131	0	0.020	30.35	17.13
Q2	0.003	0.008	0.053	0.141	0	0.025	34.03	17.88
Q3	0.006	0.015	0.067	0.222	0	0.030	37.35	17.94
Q4	0.008	0.020	0.088	0.227	0	0.039	40.61	16.75
Q5	0.095	0.024	0.092	0.258	0.005	0.051	51.02	-
Q6	0.011	0.028	0.100	0.275	0.017	0.055	52.47	-

	Run	Flow discharge increase	ΔQ (m ³ /s)	Temperature(°C)
T-runs	T1	$\Delta Q1 = Q3 - Q4$	0.002	17.94
	T2	$\Delta Q2 = Q3 - Q5$	0.0035	17.19
	T3	$\Delta Q3 = Q3 - Q6$	0.005	17.88
	T4	$\Delta Q4 = Q2 - Q4$	0.005	17.63
	T5	$\Delta Q5 = Q2 - Q5$	0.0065	17.31
	T6	$\Delta Q6 = Q2 - Q6$	0.008	17.94

TABLE 2 Scheme of T-runs conducted.

an almost instantaneous increase of the flow discharge (unsteady flow conditions). All the H-IB runs were conducted during the day, the mussels were never fed during the run, and the data recording started after a mussels' 2 h of acclimation. The T-runs consisted of a first steady stage (6 h duration), followed by an almost instantaneous increase of flow discharge, which was then maintained constant during a second steady stage (1 h 30 min duration). In this last stage, a shorter time interval was sufficient to detect animals' response after the increase of the flow discharge. The first steady stage was without sediment transport (i.e. with flow discharge equal either to Q2 or to Q3 of Tables 1). In the second steady stage, the flow discharge was set to a value lower (Q4, Table 1; runs T1 and T4, Table 2) or greater (Q5 and Q6, Table 1; runs T2, T3, T5 and T6, Table 2) than the threshold for sediment motion.

2.2 | Data analysis

For each run, the mussels' behavioural response was examined in terms of valve opening amplitude and valve opening/closure frequency and duration. To this aim, the data collected in terms of voltage [mV] outputs were elaborated through a Matlab code appositely developed. 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 (Modesto et al., 2023). Through preliminary tests over a group of eight mussels, a threshold for the application of low-pass filtering, useful to highlight the mussels' gaping, has been determined to analyse the recorded time series. The mussels' behavioural response was classified both on the base of the preliminary tests and following Hartmann et al. (2016).

Two categories of behavioural response (i.e. Normal Activity and Resting) were identified in the first steady stage (i.e. before the flow discharge increment). In the second steady stage (i.e. after the flow

discharge increment), four categories of behavioural response (i.e. Normal Activity, Transition, Adaptation, Avoidance) were identified (Figure 2). The Normal Activity (NA) behaviour was associated with the valves' opening/closure during the mussel's physiological activities, such as filtering and/or movement. Based on the preliminary analyses this behaviour corresponded to values of normalized valves' gaping $\leq 80\%$. Resting (Re) behaviour was associated to the absence of valves' opening/closing activity with normalized valves' gaping greater than 80%. Transition (Tr) behaviour was the mussel's response to the instantaneous (e.g. at instant Tt) increase of flow discharge either in the absence or in the presence of sediment transport. Adaptation (Ad) behaviour reflected the animal's adapting to the new hydrodynamic condition and corresponded to values of the normalized valves' gaping lower than 100%. Avoidance (Av) behaviour was associated with mussel's valves completely closed and thus to values of normalized valves' gaping equal to 100%. Thus, Avoidance behaviour represents the mussel's evasive response to the stress, and, according to Hartmann et al. (2016), it is distinctly different to the 'undisturbed' Resting mussels' behaviour.

To better interpret the mussel's behavioural responses during the H-IB runs, data analysis was also conducted by dividing each stage into smaller time intervals (sub-periods). The first steady stage (i.e. before the instant Tt) was divided into four sub-periods of the duration of 60 min, and the second steady stage (i.e. after the instant Tt) was divided into four sub-periods of the duration of 22 min.

3 | RESULTS

3.1 | NB series of runs

The NB series consisted of two tests: the 'feeding' and the 'movement' tests. As an example, the 'feeding' test individual responses of mussels M2, M4 and M6 are presented in Figure 3. During the

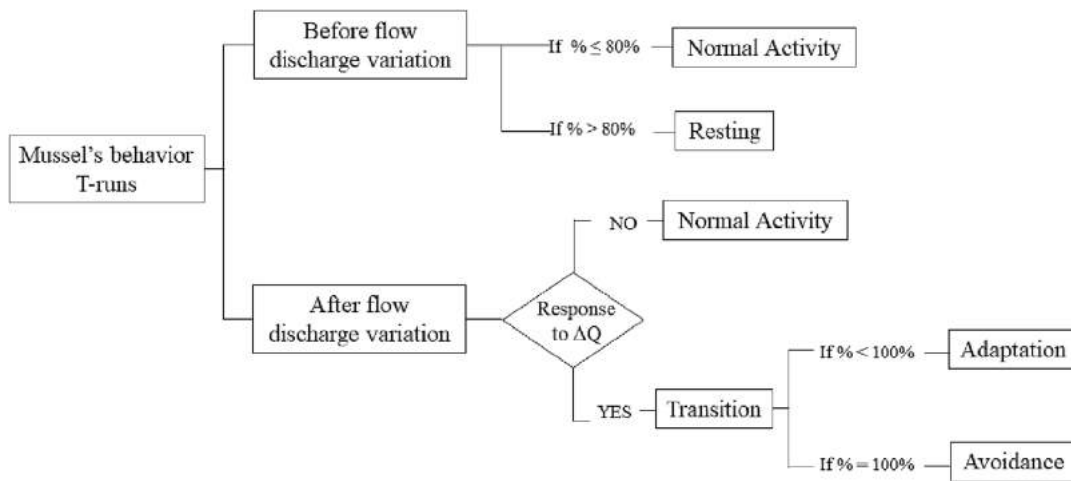


FIGURE 2 Flowchart of mussel's behaviour identification.

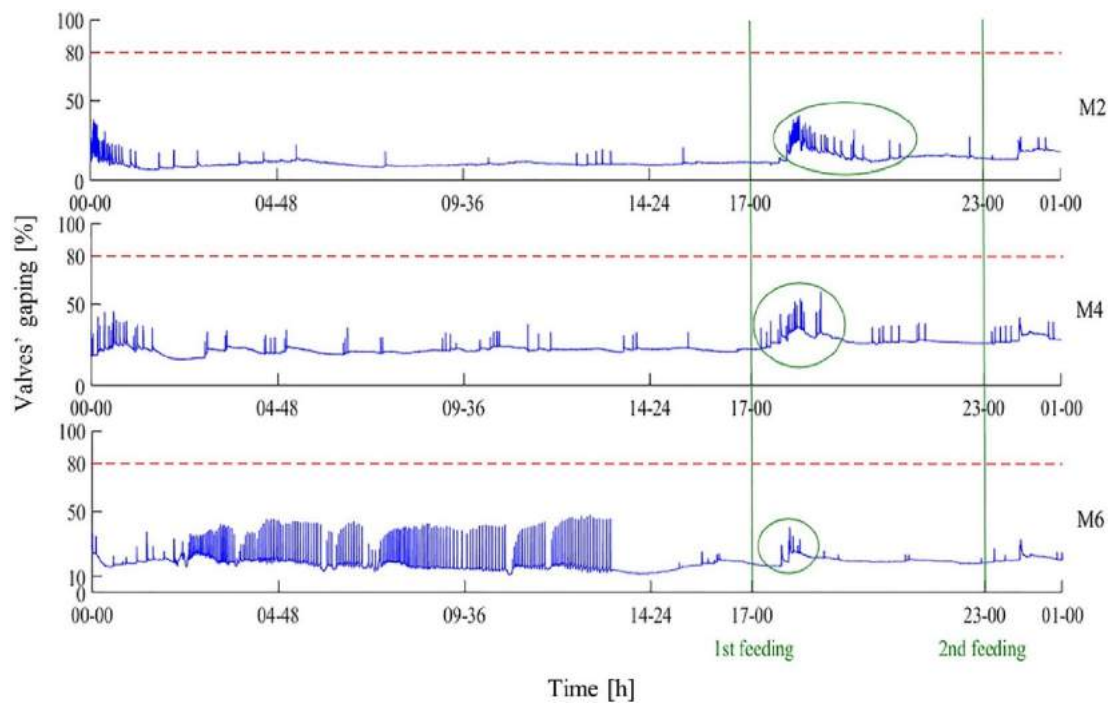


FIGURE 3 Individual recorded signal of mussels M2, M4 and M6 during the feeding test. The green vertical lines indicate the food introduction.

starvation period, the mussels showed NA behaviour, but after food introduction, they increased their gaping frequency. This reaction was observed in 81% of the mussels, while the remaining 19% did not show any response to food introduction.

The mean gaping frequency and the mean amplitude were similar during the starvation (0.075 ± 0.0041 Hz and 10.28%) and feeding (0.0072 ± 0.002 Hz and 10.98%) (Figure 4). Video-camera registration showed that sequences of rapid valves' gaping observed during starvation (e.g. mussel M6 in Figure 3) corresponded to mussel's horizontal displacement. During the 'movement' test, Re behaviour was more frequent than NA (Figure 5a). The gaping sequences occurred when

mussels were moving (e.g. Figure 5b shows the position of mussels M3–M8 at the end of the run). The mussel M8 changed position during the long sequences of the valves' gaping shown in the recorded signal of Figure 5a, the mussel M7 moved in the time periods corresponding to the shorter sequences of valves' gaping of Figure 5a, and very short displacements have been observed for mussels M5 and M6.

In the 'movement' test, the mean gaping frequency assumes a value of 0.006 ± 0.003 Hz, that is, slightly lower than that observed both in the starvation and in the feeding stages (Figure 4). The mean amplitude assumes a value slightly above 20% and thus a value higher than that in the starvation and the feeding stages of the test.

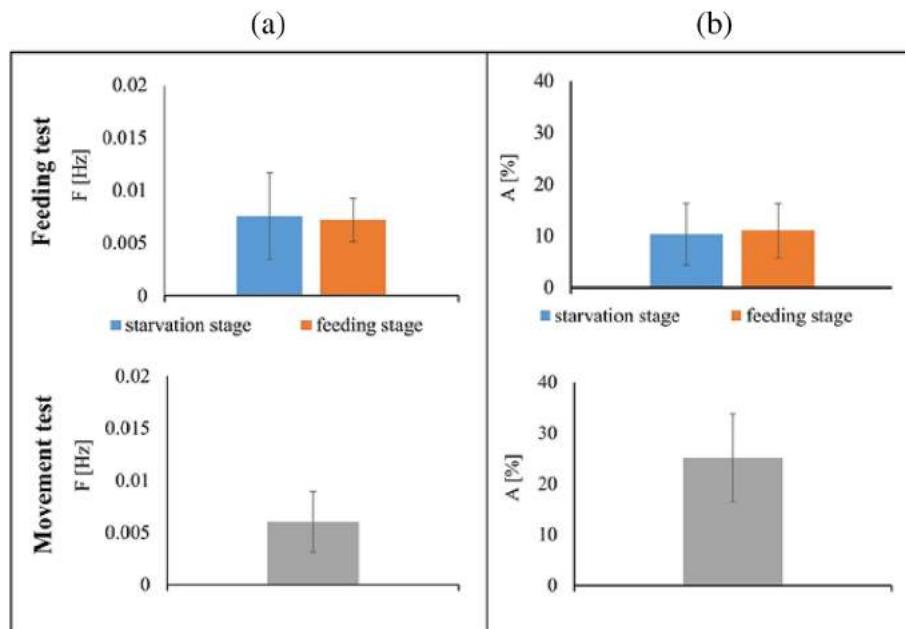


FIGURE 4 'Feeding' test and 'movement' test: (a) mean mussels' valves gapping frequency, F; (b) mean mussels' valves gapping amplitude, A.

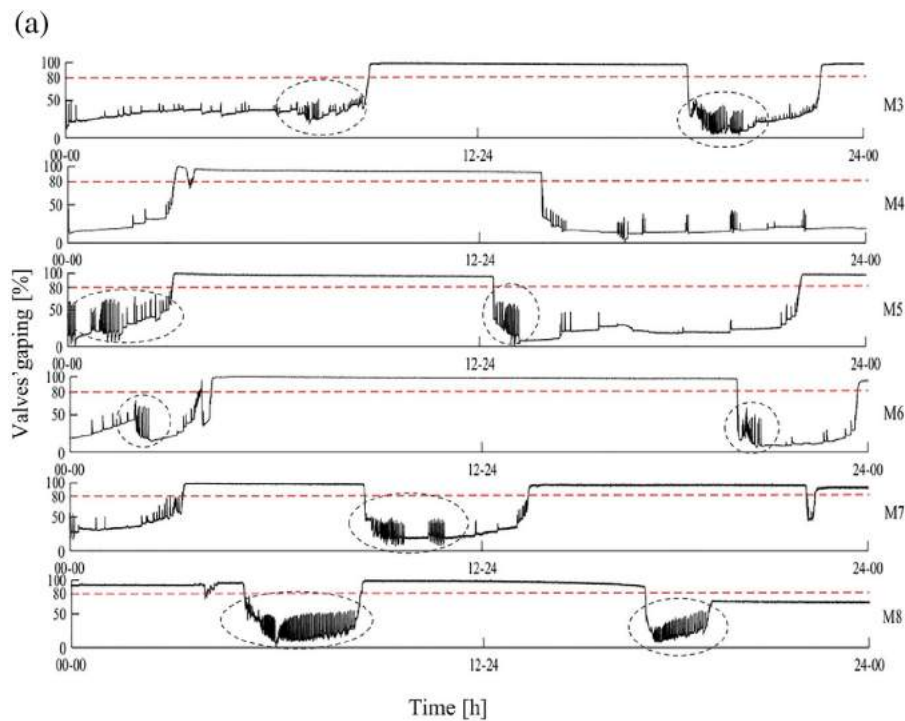
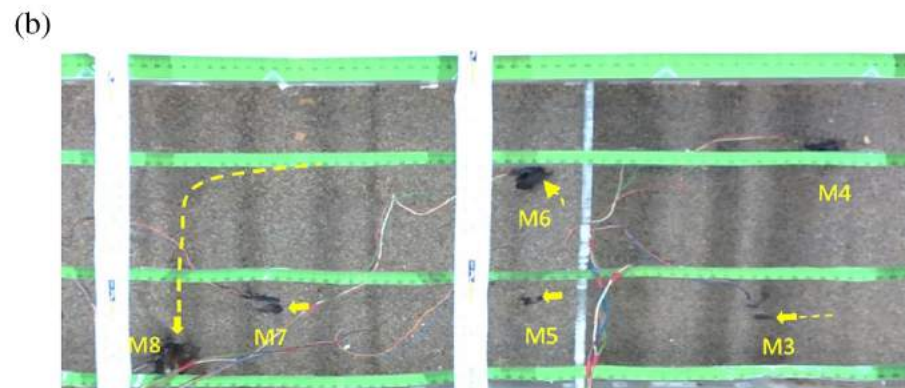


FIGURE 5 'Movement' test: (a) individual signals recorded for mussels M3, M4, M5, M6, M7 and M8; (b) recorded image at the end of the run.



3.2 | H-IB series of runs

Figure 6 shows the average of the recorded signal of the eight mussels both without sediment transport (T1 and T4) and with low (T2 and T5) and high (T3 and T6) sediment transport. During the steady stage preceding the increase of flow discharge (T_t), the NA behavioural response occurred. After the instant T_t , mussels reacted by increasing the gaping frequency, and the signal becomes rapidly

irregular (Figure 6). This effect became more evident as ΔQ increased, and the sediment transport was present. As an example, see in Figure 6g the response of the mussel M2 in the last 1 h 30 min of the T6 run. Before T_t , the mean frequency increased from 0.0066 ± 0.0006 Hz to 0.0091 ± 0.0017 Hz as flow discharge increased from Q1 to Q4 (Figure 7a). After T_t , the mean frequency increased with ΔQ , ranging from 0.0098 ± 0.0046 Hz to 0.0103 ± 0.003 Hz without sediment transport (T1, T4), from 0.0105 ± 0.0025 Hz to 0.0123 Hz

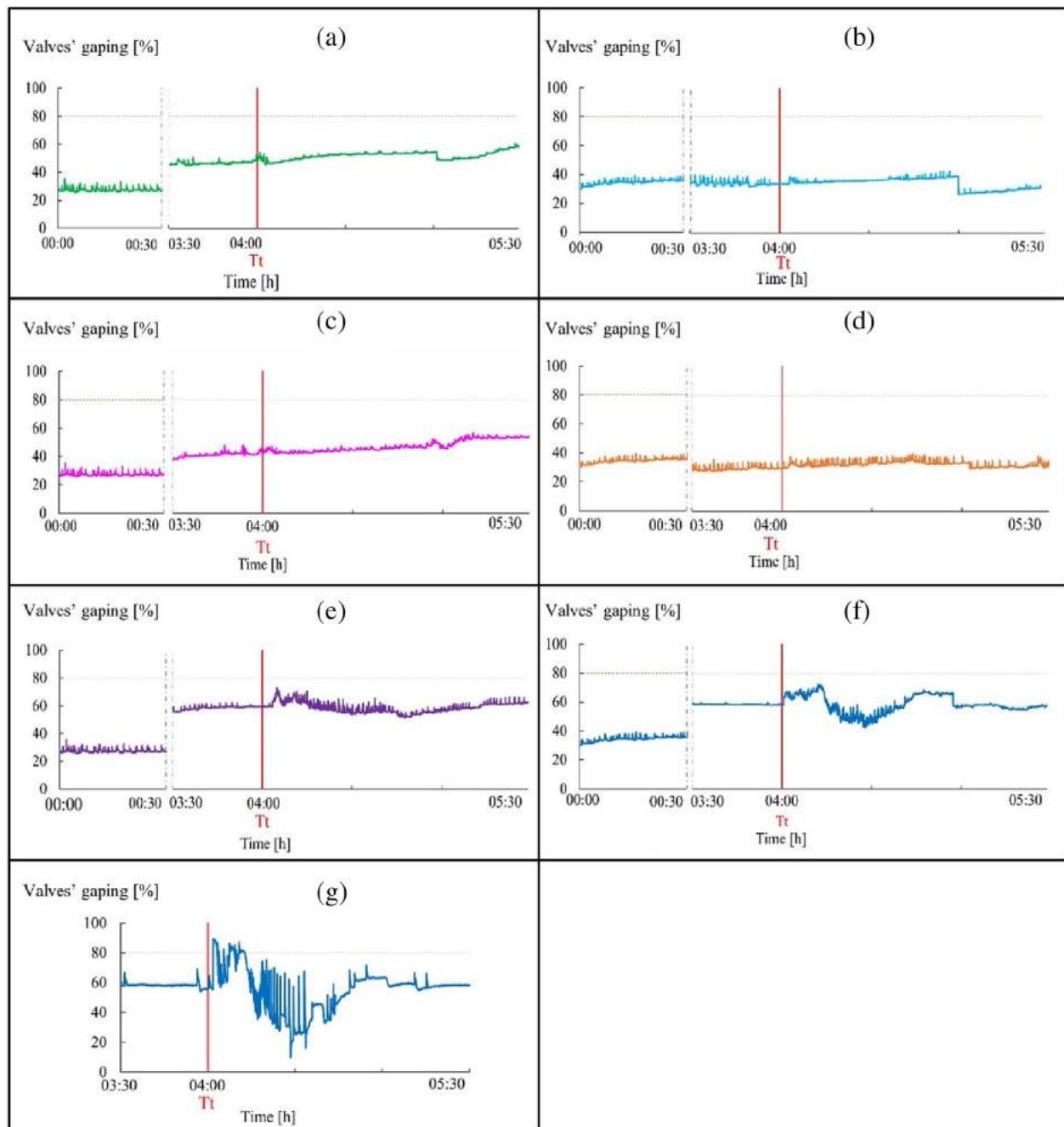


FIGURE 6 H-IB runs: (a) run T1 (average response: ΔQ_1 , without sediment transport); (b) run T4 (average response: ΔQ_4 , without sediment transport); (c) run T2 (average response: ΔQ_2 , with low sediment transport); (d) run T5 (average response: ΔQ_5 , with low sediment transport); (e) run T3 (average response: ΔQ_3 , with high sediment transport); (f) run T6 (average response: ΔQ_6 , with high sediment transport); (g) individual response of mussel M2 in run T6. The symbol T_t indicates the instant corresponding to the increase of flow discharge.

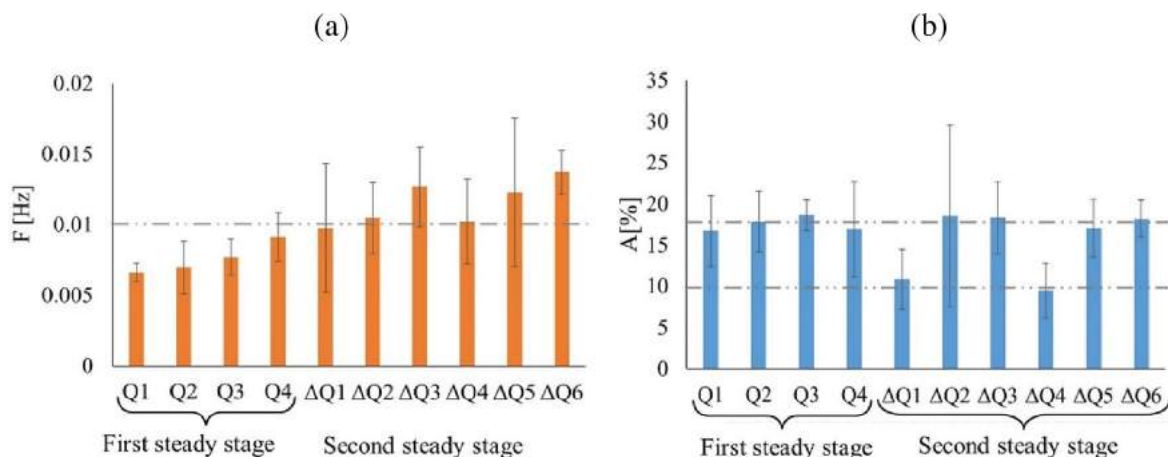


FIGURE 7 H-IB runs - entire period of observation: (a) mussels' mean valves gaping frequency, F ; (b) mussels' mean valves gaping amplitude, A .

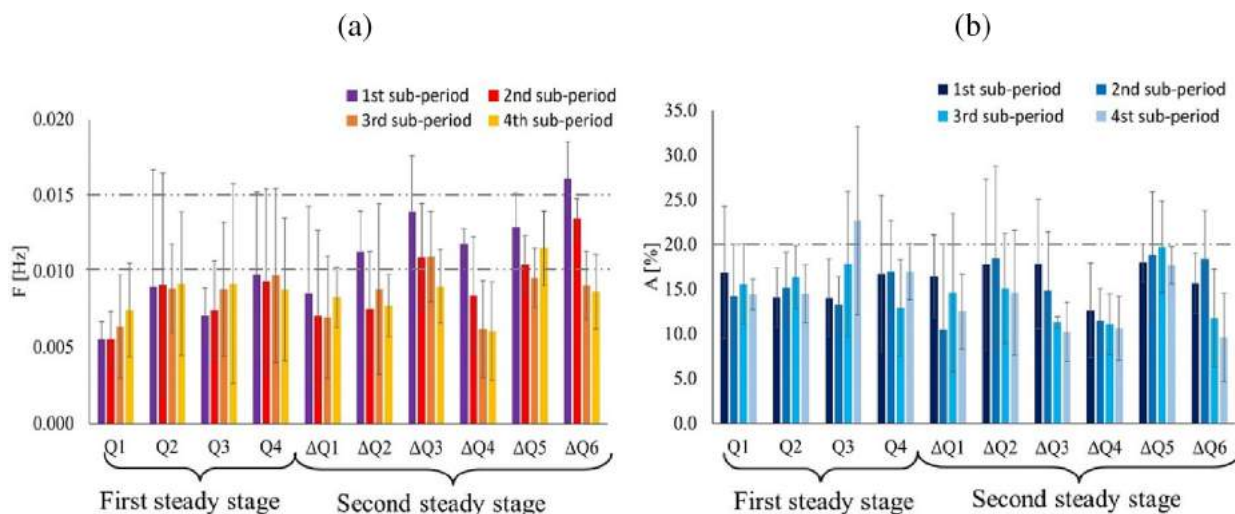


FIGURE 8 H-IB runs—four sub-periods in the entire period of observation: (a) mussels' mean valves gaping frequency, F ; (b) mussels' mean valves gaping amplitude, A .

± 0.0053 Hz with low sediment transport (T2, T5) and from 0.0127 ± 0.0028 Hz to 0.0137 ± 0.0016 Hz with high sediment transport (T3, T6) (Figure 7a).

Before T_t , the mean amplitude slightly increases with flow discharge, ranging between $16.73 \pm 4.3\%$ and $18.64\% \pm 1.88\%$. After T_t , the mean amplitude averaged 18%, except for T1 and T4 (without sediment transport) that showed average values ($10.83 \pm 3.67\%$ and of $9.46\% \pm 3.28\%$, respectively) similar to the starvation period.

The mean frequency and the mean amplitude estimated in each sub-period of the two steady stages as shown in Figure 8. Before T_t , the mean gaping frequency is always below 0.01 Hz with no significant difference between the four sub-periods; after T_t , it was highest in the first sub-period; then it decreased. In the first sub-period, the mean gaping frequency increased with ΔQ (up to 0.0085 ± 0.0057 Hz for the lower ΔQ , run T1, and up to 0.016 ± 0.002 Hz for the higher ΔQ , run T6). After the first sub-period, it decreased more for the

lower ΔQ values (0.0072 ± 0.0036 Hz without or with low sediment transport) than for the higher ones (even slightly exceeding 0.01 Hz, runs T3, T5 and T6).

The mean amplitude is similar in the four sub-periods of each run, except in the fourth period of the Q3, due to the horizontal movement of some mussels. The mean amplitude was always below 20%, with higher values in the first and in the second sub-periods.

3.3 | Behavioural responses

Behavioural responses Normal activity (NA) and Resting (Re) have been detected before T_t , while Transition (Tr), Adaptation (Ad) and Avoidance (Av) have been detected, besides NA, after T_t .

While the percentage of mussels presenting NA behaviour increased with the increase of flow discharge, the percentage of

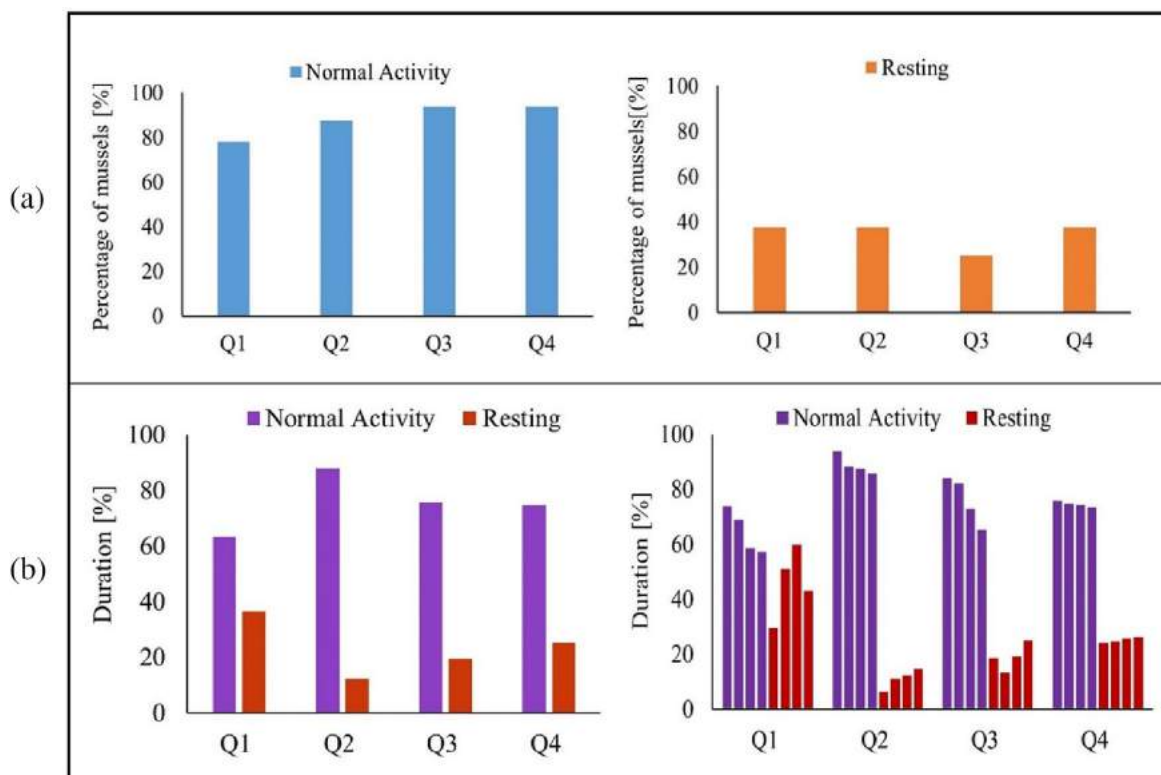


FIGURE 9 H-IB runs—first steady stage: (a) percentage of mussels having either Normal Activity (NA) or Resting (Re) behaviours; (b) duration of Normal Activity (NA) and Resting (re) behaviours: analysis in the entire period of observation and in the four sub-periods of the entire period of observation.

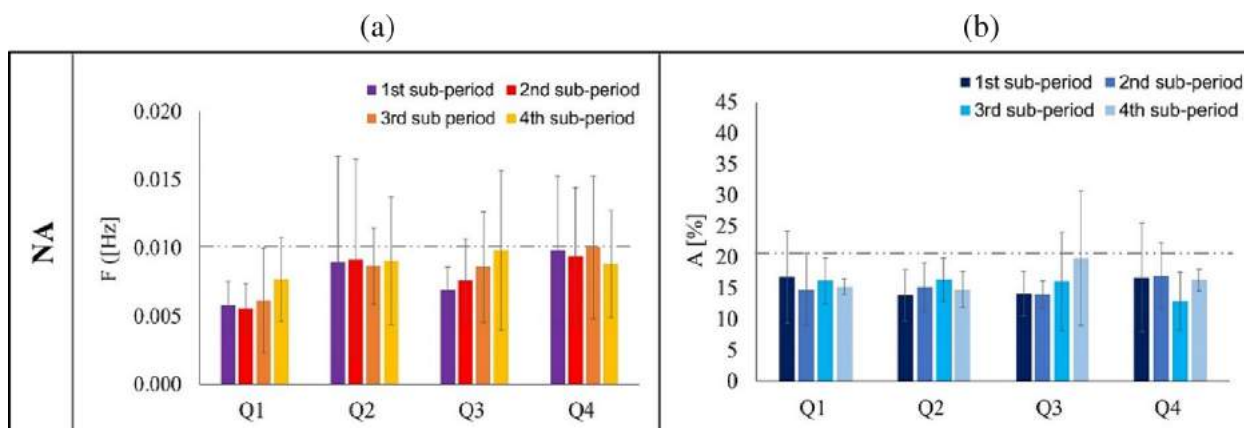


FIGURE 10 H-IB runs—first steady stage: analysis of Normal Activity (NA) behaviour in each of the four sub-periods: (a) mussels' mean valves gaping frequency, F ; (b) mussels' mean valves gaping amplitude, A .

mussels showing Re behaviour remained almost constant (Figure 9a). NA behaviour lasted for 76% of the run duration showing no evident variation with the increase of the flow discharge (Figure 9b); Re behaviour occurred for 24% of the run duration with the highest value ($\sim 38\%$) at the lowest flow discharge, Q1. The mean gaping frequency of the mussels with NA behaviour increased with the flow discharge but was always under 0.01 Hz. The mean amplitude remained almost constant below 20% (Figure 10).

After the sudden increase of flow discharge (T_t), the percentage of mussels presenting NA, T_r , A_d and A_v varied with ΔQ (Figure 11a). The percentage of mussels showing NA behaviour decreased with ΔQ , from 62.5% (T_1 , $\Delta Q = \Delta Q_1 = 0.002 \text{ m}^3/\text{s}$) to 50% (T_4 , $\Delta Q = \Delta Q_4 = 0.005 \text{ m}^3/\text{s}$), without sediment transport, and from 68.75% (T_2 , $\Delta Q = \Delta Q_2 = 0.0035 \text{ m}^3/\text{s}$) to 37.5% (T_5 , $\Delta Q = \Delta Q_5 = 0.0065 \text{ m}^3/\text{s}$), with low sediment transport. Conversely, with high sediment transport, such a percentage increased with ΔQ

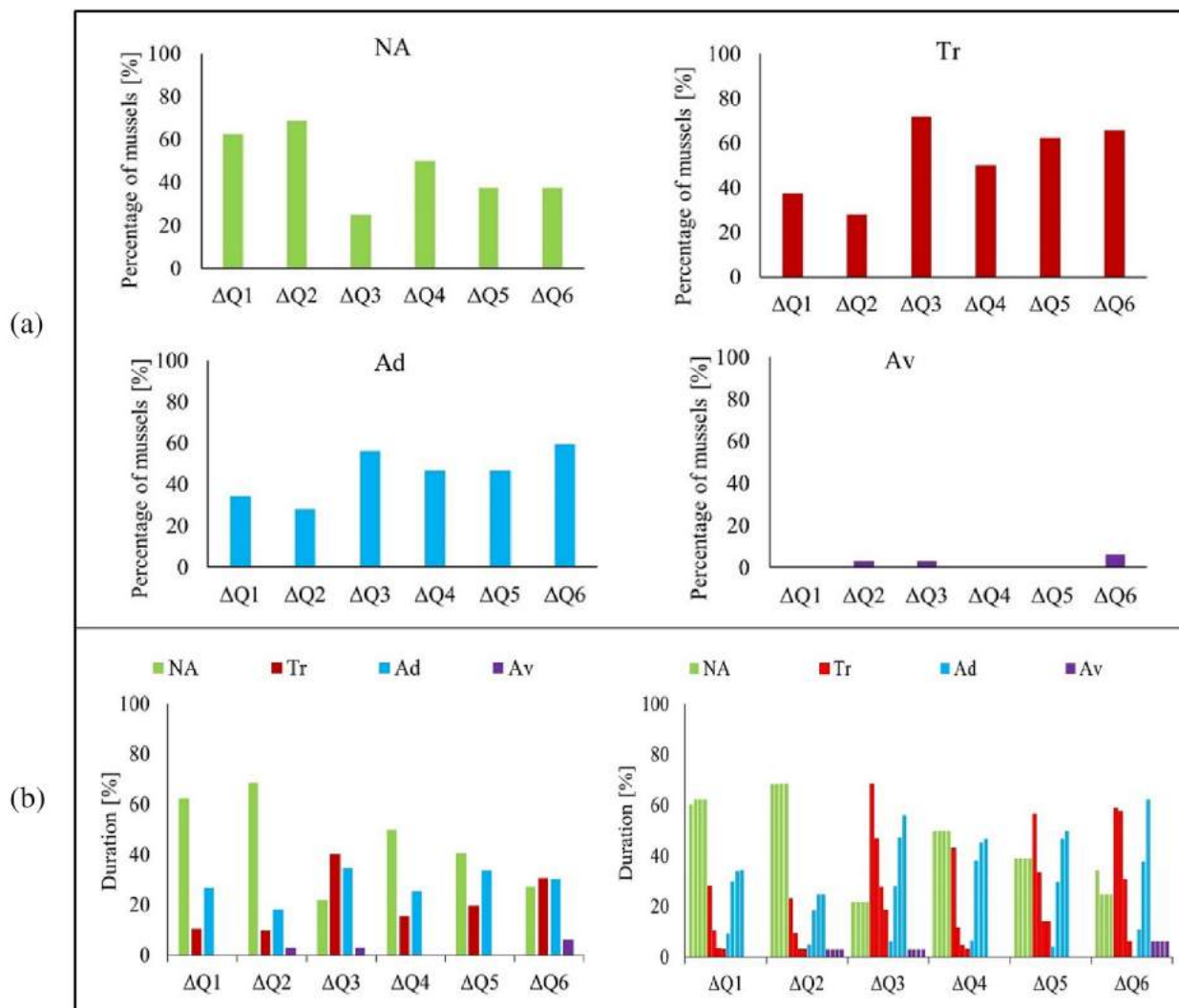


FIGURE 11 H-IB runs—second steady stage: (a) percentage of mussels having Normal Activity (NA), Transition (Tr), Adaptation (Ad) and Avoidance (Av) behaviours; (b) duration of Normal Activity (NA), Transition (Tr), Adaptation (Ad) and Avoidance (Av) behaviours: analysis in the entire period of observation and in the four sub-periods of the entire period of observation.

from 25% (T3, $\Delta Q = \Delta Q3 = 0.005 \text{ m}^3/\text{s}$) to 37.5% (T6, $\Delta Q = \Delta Q6 = 0.008 \text{ m}^3/\text{s}$). The percentage of mussels with Tr behaviour followed the opposite trend increasing from 38% (T1, $\Delta Q = \Delta Q1 = 0.002 \text{ m}^3/\text{s}$) to 50% (T4, $\Delta Q = \Delta Q4 = 0.005 \text{ m}^3/\text{s}$) without sediment transport and from 28% (T2, $\Delta Q = \Delta Q2 = 0.0035 \text{ m}^3/\text{s}$) to 62% (T5, $\Delta Q = \Delta Q5 = 0.0065 \text{ m}^3/\text{s}$) with low sediment transport. As sediment transport increased (T3, T6), the percentage of mussels reacting with Tr behaviour decreased from 72% (T3, $\Delta Q = \Delta Q3 = 0.005 \text{ m}^3/\text{s}$) to 68% (T6, $\Delta Q = \Delta Q6 = 0.008 \text{ m}^3/\text{s}$). Therefore, the percentage of mussels responding to low flow discharge variation (T1), even in the presence of low sediment transport (T2), was lower than at higher values of ΔQ both without (T4) and with low (T5) or high (T3 and T6) sediment transport.

The percentage of mussels having Ad behavioural response showed the same trend observed for the Tr behaviour. The Av

behaviour occurred only in 6.25% of mussels at the highest ΔQ (T6) and in 3.125% at lower ΔQ (T2 and T3).

The duration of the Tr behaviour increased with ΔQ and sediment transport, that is, the higher the intensity of the stress, the longer the behavioural Tr reactions, especially in the sub-period following the stress. The duration of the Ad behaviour followed the same trend as the Tr behaviour. The Tr behaviour lasted about half as long as the Ad behaviour in the runs without or with low sediment transport (T1, T4, T2, T5), while it exceeded the duration of the Ad behaviour with high sediment transport (T3, T6) (Figure 11b). The duration of NA behaviour was almost constant within the four sub-periods, but it decreased with ΔQ and sediment transport. Finally, the Av behaviour occurred, for a short time, only in the presence of sediment transport.

Figure 12 presents the mean gapping frequency and the mean amplitude by considering only the mussels presenting the NA, Tr and Ad behaviours. The mean gapping frequency of the NA and Ad

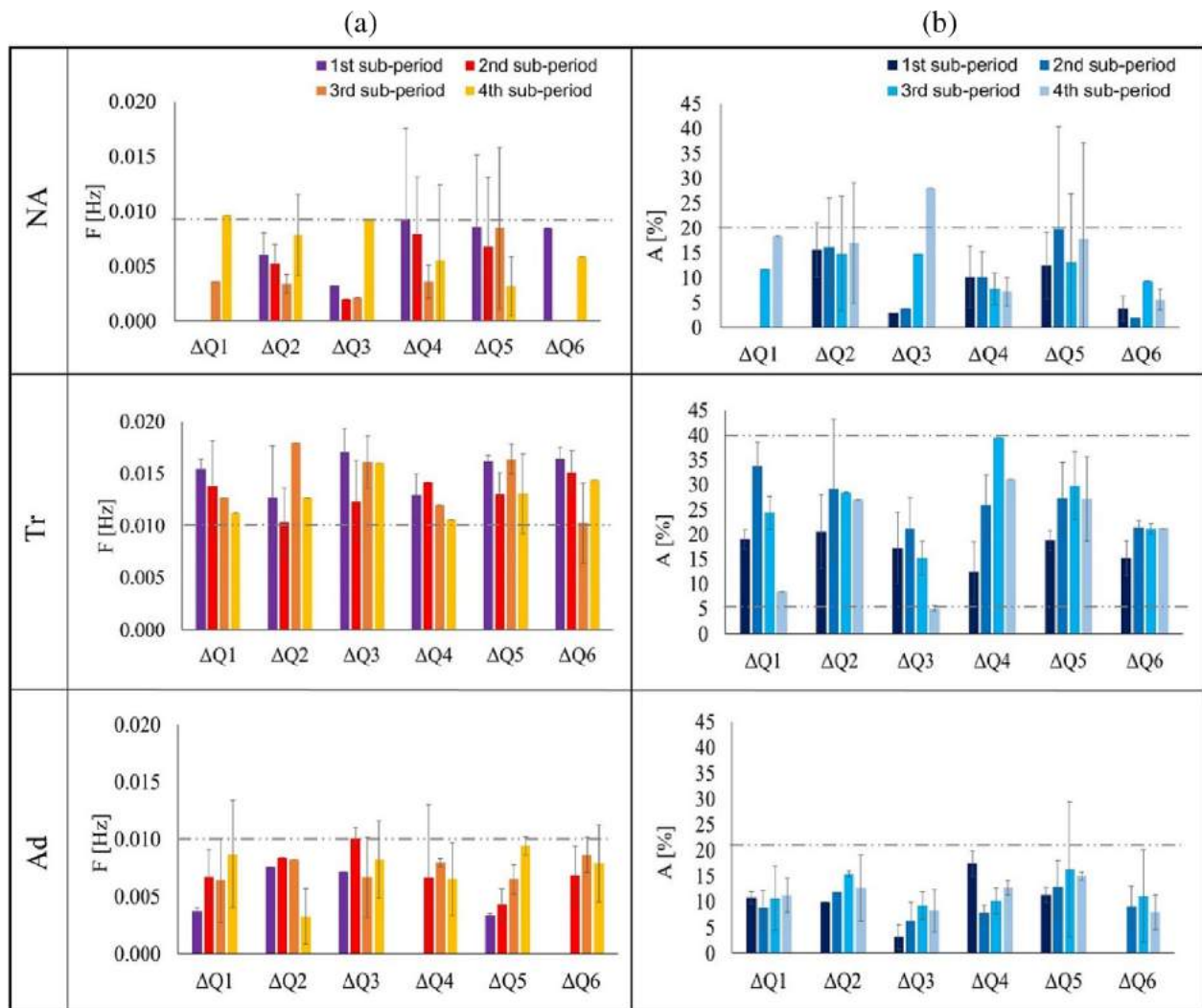


FIGURE 12 H-IB runs—second steady stage: analysis of Normal Activity (NA), Transition (Tr) and Adaptation (Ad) behaviours in each of the four sub-periods of the entire period of observation: (a) mussel's mean valves gaping frequency, F; (b) mussel's mean valves gaping amplitude, A.

behaviours was always below 0.01 Hz, and the mean amplitude below 20% except for the fourth sub-period of ΔQ3 due to mussel's horizontal movement. The mean gaping frequency of the Tr behaviour was higher than 0.01 Hz across all the four sub-periods, and the highest values occur in the presence of sediment transport.

The mean amplitude ranges between 5% and 40% with low values in the first sub-period and the highest values in the second or in the third sub-periods.

4 | DISCUSSION

The mussel's response to discharge variations confirms that they are highly sensitive to the hydrodynamic stress. The mussel's gaping frequency and amplitude during feeding or moving activities in the NB runs (under non-stressful conditions) was considered as a reference for the analysis of the mussel's response to the hydrodynamic stress. During the feeding period, the mean valve gaping frequency was always lower

than 0.01 Hz, and the mean amplitude was around 10%. Starved mussel's showed lower gaping frequency and greater mean gaping amplitude due to the horizontal displacement, as confirmed by video-camera records. To move the mussel must open their shells enough to protrude the foot, which is inserted into the substrate and subsequently contracted to drag the rest of the body (Candido & Romero, 2007).

The horizontal movements that we observed could be prompted by the need to find food (Schwalb & Pusch, 2007) or the movement itself can promote the food ingestion through a water current into the posterior portion of mussel's (McMahon, 1991). These results match those obtained in similar experimental conditions using a different mussel population (Modesto et al., 2023).

Mussel's behaviour under low water discharge (24-h movement test) was substantially similar to that observed during the starvation period (17 h), with a mean gaping frequency slightly lower than in the feeding period. Instead, the mean amplitude under low water discharge was slightly higher than during the feeding and the starvation periods, confirming that it is related to mussel's displacements. The

starvation period showed the increase in food-seeking movements, which was reflected by the increase in the average valve's amplitude. This indicates that the value of valves' opening amplitude can give an indication on the animal's activity. Before Tt, the mean gaping frequency increased with the flow discharge. At the lowest discharge (Q1), gaping frequency values never exceeded 0.01 Hz, like in the movement test. The increment of flow discharge (i.e. after Tt) prompted a gaping response with an increase of the mean gaping frequency above 0.01 Hz. As already observed by Modesto et al. (2023), the mussel's response does not depend on the value of the flow discharge before Tt, but on magnitude of variation of flow discharge (ΔQ) and on the consequent sediment transport. Clear evidence of this comes from the fact that, for the same value of ΔQ (runs T3 and T4), a stronger mussels' behavioural reaction occurred in the presence of sediment transport. Contrarily, the mean amplitude showed no clear trend of variation. A behavioural reaction to sediment transport is justified by the fact that freshwater mussels survive if the substrate offers sufficient stability to avoid mortality by dislodgement (Lopez & Vaughn, 2021). This is corroborated by the increase in the percentage of mussels shifting from NA or Re behaviour to Tr or Av behaviour with ΔQ , especially in the presence of sediment transport.

The capacity of some mussels to decrease their valve gaping frequency after the transition period suggests their adaptation to the persisting hydrodynamic stress. In fact, the adaptation of sensory systems to a steady stimulus (Torre et al., 1995) reduces responsiveness to ambient stimulation levels through the adjustment of sensitivity (Brenner et al., 2000).

Since the degree of adaptation increases as the intensity and frequency of the stimulus increases (e.g. Capraro et al., 1979; Hollins et al., 1990), we can expect the stimulus–response relation to be species and populations specific. The species that we tested occurs in both lacustrine and riverine habitats spanning a wide range of hydrodynamic conditions. However, in rivers, it occupies slow-flowing or sheltered areas outside of the main current where it is protected from high scouring flows. Therefore, it can be expected to be highly sensitive and responsive to hydrological variations compared to species that are morphologically and physiologically adapted to withstand stronger hydrological forces in the main river channel (Randklev et al., 2019). Furthermore, the lacustrine population that we tested is supposedly adapted to still waters and therefore predictably more sensitive to flow variations than a river one. However, all these admittedly speculative hypotheses can only be verified by a comparison between different species and between populations from different habitats. Unfortunately, the extirpation of most mussels' populations from Italian rivers (Riccardi, Bo, et al., 2022), including the only Italian species living in fast flowing rivers, *Microcondylaea bonellii* (Férussac, 1827; Riccardi, Aksenova, et al., 2022), hampers this comparison.

Despite uncertainties about the influence of adaptation, the results of this study confirm and validate those obtained from another *U. elongatulus* population (Modesto et al., 2023), suggesting that mussels' behaviour could valuably complement current river monitoring methodologies, in conditions far from the schematic and controlled laboratory ones.

5 | CONCLUSION

The main conclusions of the present study can be synthesized as follows:

1. The NB runs have highlighted that during the mussels' activities, such as feeding or moving, the mean valve gaping frequency is always less than 0.01 Hz and the mean amplitude assumes values ranging from 10% to 20% depending on their activities. Mussels slightly increase gaping activity in response to feeding and reduce the horizontal displacements, thus keeping mean amplitude at 10%. Mussels during the starvation period reduce their gaping frequency but when they are moving, in order to search for food.
2. The results obtained in the H-IB runs have highlighted that before the increment of flow discharge, always without sediment transport, the mean frequency gaping increases with the flow discharge (although never above 0.01 Hz), while the mean amplitude is almost constant (maintaining values below 20%).
3. A sharp increase of flow discharge (i.e. after the transition instant Tt) prompts a gaping reaction, and the frequency increases (exceeding 0.01 Hz) with ΔQ , both without and with sediment transport. The mean amplitude maintains almost constant values around 20% with sediment transport, except without sediment transport with values similar to those obtained in the starvation period.
4. The percentage of mussels showing a gaping reaction (Tr behaviour) increases with the discharge variation ΔQ , especially in the presence of sediment transport. The Av (Avoidance) behaviour is rare and only occurs in the presence of sediment transport.
5. The results of this study are consistent with those obtained using other population of the same species tested by Modesto et al. (2023), suggesting that different mussel populations respond similarly to the hydrodynamic changes.
6. Since behavioural responses to stresses may vary as a function of adaptation to ambient stimulation levels, for practical applications, the analysis of the responses must be standardized on the species/population-specific levels of responsiveness.

In summary, the results of this study show that the mussels' behavioural response can be considered a useful tool to evaluate variations of flow discharge with or without sediment transport. Thus, these findings are encouraging to support the use of mussels as biosensor in their natural habitat (rivers or lakes) to monitor the occurrence of the events involving hydro-morphological variations, such as floods. Field validation is in progress to confirm if this methodology is a suitable and reliable tool for early warning system of hydrodynamic variations, especially important in a context of a climate change. Future research has been planned to investigate the influence of other environmental factors (e.g. substrate composition, water turbidity, mussels' density), which could affect mussels' response to hydrodynamic stressors.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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