Water Resources Research

RESEARCH ARTICLE

10.1029/2025WR041499

A Dedicated Alarm Criterion by Using Freshwater Mussels' Valve Movement as Biological Early-Warning System to Identify Impacts of Flow Discharge Variations in Fluvial Ecosystem

Donatella Termini[1,2], **Nina Benistati**[1], **Ashkan Pilbala**[3], **Vanessa Modesto**[4], **Luigi Fraccarollo**[5], **Dario Manca**[4], **Sebastiano Piccolroaz**[5], **and Tommaso Moramarco**[6]

[1]Department of Engineering, Viale delle Scienze, University of Palermo, Palermo, Italy, [2]NBFC, National Biodiversity Future Center, Palermo, Italy, [3]Department of Civil, Environmental and Architectural Engineering, University of Padova, Padua, Italy, [4]Aquatic Systems Biology Unit, TUM School of Life Sciences, Technical University of Munich, Freising, Germany, [5]Department of Civil, Environmental and Mechanical Engineering, University of Trento, Trento, Italy, [6]The National Research Council (CNR) - Research Institute for Geo Hydrological Protection (IRPI), Perugia, Italy

Key Points:

- Valvometry technique is used to investigate the freshwater mussels' response to river's hydrodynamic disturbances
- The analysis is conducted through laboratory-based and field-based experiments
- A threshold condition and a benchmark graph for ecosystem alarm criterion in clear water are defined

Supporting Information:

Supporting Information may be found in the online version of this article.

Correspondence to:

D. Termini,
donatella.termini@unipa.it

Citation:

Termini, D., Benistati, N., Pilbala, A., Modesto, V., Fraccarollo, L., Manca, D., et al. (2025). A dedicated alarm criterion by using freshwater mussels' valve movement as biological early-warning system to identify impacts of flow discharge variations in fluvial ecosystem. *Water Resources Research*, *61*, e2025WR041499. https://doi.org/10.1029/2025WR041499

Received 7 JUL 2025
Accepted 27 SEP 2025

Author Contributions:

Conceptualization: Donatella Termini, Nina Benistati, Luigi Fraccarollo, Tommaso Moramarco
Data curation: Donatella Termini, Nina Benistati, Ashkan Pilbala, Luigi Fraccarollo, Dario Manca, Sebastiano Piccolroaz
Formal analysis: Donatella Termini, Nina Benistati
Funding acquisition: Donatella Termini, Luigi Fraccarollo, Tommaso Moramarco
Investigation: Donatella Termini, Nina Benistati, Ashkan Pilbala, Vanessa Modesto, Luigi Fraccarollo, Sebastiano Piccolroaz

Abstract Understanding the impact of hydrodynamic variations, induced by climate change or anthropogenic pressures on aquatic habitats is necessary for effective freshwater conservation. In this work, an ecosystem-impact approach is applied by using freshwater mussels (FMs) as biological indicators of perturbations of aquatic environment. The valvometry technique is used to investigate FMs' response in different substrate compositions and environments. Laboratory flume experiments were performed with *Unio elongatulus* over two substrates (gravel and sand), and in situ pilot installation with stuck *Unio mancus* was realized in Paglia river (Umbria, Italy). FMs' response to flow discharge variation was analyzed in terms of average gaping frequency (*Fa*) and gaping amplitude (*Aa*). For the field case, the analysis concerned valvometry data recorded during a moderate flood on 31 March 2022. Both laboratory and field-based experiments showed that FMs promptly react to flow perturbation increasing values of *Fa* and decreasing values of *Aa* as the percentage of flow discharge variation, $\Delta Q/Q_{bp}$ (being ΔQ the flow discharge variation and Q_{bp} the flow discharge before the perturbation), increased. Based on the data, a threshold condition was derived which can be used as an ecosystem alarm criterion based on mussel behavior. This could help practitioners, academic ecologists and controlling agencies in decision-making processes. In this view, the paper also presents, to our knowledge for the first time, a $[Fa, \Delta Q/Q_{bp}]$ benchmark graph which could be helpful in guiding the selection of the ecosystem alarm criterion in clear water, constituting a base for future development in natural conditions with suspended materials.

1. Introduction

Identifying the effects of climate change on the aquatic environment is paramount to defining mitigation and/or adaptation strategies which are crucial to keeping ecological, economic, and social damage within limits (IPCC, 2012, 2014).

Several studies (Allan & Castillo, 2007; Moyle & Mount, 2007; Stähly et al., 2019; Wohl et al., 2015) indicate that the degradation of aquatic ecosystem is strongly triggered by hydrodynamic variations and the consequent sediment transport changes, which modify the aquatic habitats and biotic community's conditions. Freshwater ecosystem is particularly susceptible to climate changes (Hassan et al., 2020; Poff et al., 1997; Singh et al., 2021; Strayer & Dudgeon, 2010) and the impacts on freshwater species are especially evident compared to those on terrestrial or marine ones (Bódis et al., 2014; Döll & Zhang, 2010; Nogueira et al., 2021; Tesfaye et al., 2020; Woodward et al., 2010). Flow and sediment dynamics are important abiotic drivers of fluvial ecosystems' functioning (Gibbins et al., 2007; Wallace & Webster, 1996). Flood events could determine significant changes in sediments' characteristics (Eaton & Lapointe, 2001; Mao, 2018) and in the composition and distribution of riparian vegetation (Olokeogun et al., 2020; Termini, 2021) with possible ecological effects on the aquatic environment (Novais et al., 2017).

In this context, as predictions define an increase in the frequency and intensity of extreme events (IPCC, 2012), rapid and precise detection of hydrological impacts on the freshwater ecosystem would be crucial for maintaining a sustainable condition.

Methodology: Donatella Termini, Nina Benistati, Tommaso Moramarco
Project administration: Tommaso Moramarco
Resources: Vanessa Modesto
Writing – original draft: Donatella Termini
Writing – review & editing: Donatella Termini, Nina Benistati, Ashkan Pilbala, Vanessa Modesto, Luigi Fraccarollo, Sebastiano Piccolroaz, Tommaso Moramarco

A risk–based approach has been generally adopted to assess the impacts of hydrodynamic river changes due to climate change. Climate change models are used for simulating large-scale patterns of seasonal variations (see as an example Randall et al., 2007) and eco-hydrodynamic models (Akstinas et al., 2022; Jodhani et al., 2023; Vanzo et al., 2016), together with in-field monitoring of the hydrodynamic variables (i.e., water level and flow velocity), are used to define the stress conditions in rivers (Alimenti et al., 2021; Dolcetti et al., 2022; Simpson, 2001; Sukodolov, 2015).

In the last decades, an ecosystem-impact approach, which is instead based on monitoring the behavior or physiology of living organisms, has attracted the interest of some authors in identifying the effects of disturbances in aquatic habitats (Pivato et al., 2019; Quadroni et al., 2017). Bivalve mollusks, such as freshwater mussels (FMs), match the requirements that should be considered for monitoring organisms (Vereycken & Aldridge, 2022). FMs are ideal indicators of biological integrity and water quality conditions in aquatic ecosystems (Borcherding, 2006; Hartmann et al., 2016; Ladeiro et al., 2017; Micu, 2020; Premalatha et al., 2020; Vaughn et al., 2015) and thus they are considered target species for identifying habitat quality conditions (Geist, 2011). At the same time, FMs populations are declining dramatically (e.g., Lopes-Lima et al., 2017; Priè et al., 2014; Sousa et al., 2008). Freshwater Unionids, which are considered in the present work, are more sensitive to human and natural disturbances than other taxa (Lopes-Lima et al., 2017; Riccardi et al., 2022). In this way, efforts in defining conservation strategies to sustain populations and their functions in aquatic ecosystems are urgently needed (Sousa et al., 2023).

FMs are especially vulnerable to habitat alterations (Hartmann et al., 2016; Silverman et al., 1995; Strayer, 1999; Strayer et al., 1999; Vaughn & Hakenkamp, 2008). They are filter-feeders with reduced mobility, living at the sediment-water interface in rivers, which their survival and reproduction also depend on the variations of river discharge, and thus of hydrodynamics and mixing processes, and on the substratum characteristics, which determine habitat modifications and expose them to varying feeding rates and predators risks (Engelhardt et al., 2004; Perles et al., 2003; Robson et al., 2010). In turn, they may modify the kinematic characteristics of flow close to streambed (Lopez & Vaughn, 2021; Sansom et al., 2018; Wu et al., 2020), influencing bed stability and the substrate configuration or composition (Hassan et al., 2020; Janetski et al., 2009). High-flow conditions and flow discharge variability can significantly influence the behavior of FMs by subjecting them to varying flow velocities and bottom shear stress (Allen & Vaughn, 2010; Hornbach et al., 2010; Singh et al., 2021; Zigler et al., 2008). Since FMs possess a lifespan of several decades, they can adapt to extreme flow conditions (Lopes-Lima et al., 2017). Given the severity and increasing frequency of hydrological changes in the context of climate changes (among others Lamond & Penning-Rowsell, 2014), there is a strong need to define monitoring methods suitable to detect real-time responses, such as those provided by the behavioral reactions of mussels.

Bivalves display a defensive reaction to external stimuli by opening and closing their valves, modifying their filtering flow rate and adjusting their degree of body exposure to the flow (Hartmann et al., 2016; Perles et al., 2003). Thus, FMs' valves' gaping activity reflects their physiological responses and could serve as a reliable indicator of their reaction to disturbances (Hartmann et al., 2016; Kramer & Foekema, 2001). The development of a Biological Early Warning Systems (BEWS) based on the physiology and behavior of organisms is crucial for the continuous monitoring of the status of the aquatic environment (Butterworth et al., 2001; Jeffrey & Madden, 1991).

Although FMs' valve gaping activity (i.e., valvometry) has become a well-established measure of environmental variations (Garcia-March et al., 2016; Miller et al., 1999; Tran et al., 2003) and biomonitoring (among others Kramer et al., 1989; Gerhardt et al., 2006; Bae & Park, 2014; Hartmann et al., 2016) in aquatic environments, more systematic research should be conducted to understand their behavioral reactions to flow discharge variations, which are increasingly determined by climate changes, in freshwater environments. Much knowledge remains to be discovered, especially about characterizing FMs' response to continuously changing in flow conditions and the influence of other factors, such as the bottom substratum. Substratum granulometric composition may affect their ability to move or to remain burrowed during flood events (Hoffman et al., 2006; Lara & Parada, 2009; Poznańska-Kakareko et al., 2021). Compared to sand beds, gravel beds enhance bed stability, the near-bed turbulence levels in the incoming flow, and water mixing, which are critical aspects of mussel habitats (Lazzarin et al., 2023; Lopez & Vaughn, 2021).

In this context, the present work is part of a broader research program, "Enterprising" PRIN2017 (funded by the Ministry of Education, University and Research of Italy), aimed at identifying the potential of FMs' valvometry as

BEWS of impacts of hydrological disturbances in rivers. Specifically, climbing over the local effects determined by FMs on bed roughness modification, flow velocity and turbulence fields (e.g., Lazzarin et al., 2023; Zhang et al., 2025), this work specifically focuses on the FMs' response to variations of flow discharge and sediment transport in rivers. Modesto et al. (2023) and Termini et al. (2023) conducted laboratory experiments, in sand-bed flumes but with different *U. elongatulus* populations, that verified the hypothesis that mussels' behavioral response, in terms of valves gaping, correlate with flow discharge variations and sediment transport intensity. Modesto et al. (2023) focused more on the classification of mussels' behavioral response to the variations examined. Termini et al. (2023) expanded this classification and focused on the relation with hydrodynamic variations. Laboratory studies by Termini et al. (2023) and Modesto et al. (2023) informed the field work described by Pilbala et al. (2024) where they explored the possibility of using FMs valvometry to detect hydrological disturbances in actual riverine conditions by applying a continuous wavelet transform-based signal processing approach. Although results shown in these previous works have allowed significant progress in understanding how to detect FMs' behavioral response in terms of valves gaping to flow discharge variations and sediment transport intensity, further steps are needed to exploit such response as BEWS. To fill this gap, our present work advances the findings from the aforementioned studies by performing comparative analyses of the FMs' responses under different dynamic and environmental conditions, with the aim to show the similarities in the FMs' response.

For clarity, the present work has in common with the previous ones the same valvometry technique and measurement approach. With respect the previous works, the new aspects of the present paper are: (a) to expose FMs to higher values of flow discharge and sediment transport; (b) to compare FMs' responses using different substrate composition (sand and gravel); (c) to compare FMs' responses in controlled laboratory and uncontrolled field conditions); (d) to identify, on the basis of our data, a threshold of FMs' response for alerting with respect the entity of the disturbance, defined as a function of the flow discharge variation. Therefore, here we illustrate results obtained using FMs' valvometry data collected both in laboratory- and field-based experiments. The controlled laboratory environment allowed us to conduct basic, repeatable experiments, and they are used as a kind of benchmark data to investigate the variability of FMs' response in a natural uncontrolled environment. To identify a generalizable approach, a statistical analysis is conducted to compare the FMs' responses, obtained for the different substrates and the different environments and species, to evaluate the possible statistical differences. Thus, the specific objectives of the present work are: (a) to identify the variability ranges of FMs' response, in terms of average gaping frequency (Fa) and amplitude (Aa), for different flow discharge variations by considering two different substrate compositions (gravel and sand) and conditions (laboratory and field); (b) to investigate the statistical difference of FMs' response between the two different substrates and between environments; (c) to identify, based on the collected valvometric data, an appropriate threshold condition on Fa and/or Aa for defining an alert criterion that could be used by academic ecologists and by local controlling and management agencies.

The manuscript is organized as follows: Section 2 describes the data used and the analysis methodology; Section 3 presents the results; the discussion is reported in Section 4 and conclusion in Section 5.

2. Material and Methods

2.1. Laboratory and Field Experiments

Three series of experiments were performed, two in a laboratory flume and one in situ.

2.1.1. Laboratory Experiments

The laboratory experiments were conducted at the Hydraulic laboratory of the Department of Engineering - University of Palermo (Italy), in a recirculating rectilinear flume 40 cm wide, 11 m long, and with a longitudinal bed slope approximately equal to 0.1%, with a submersible pump (SULZER AS0641) in the downstream tank. A sediment collector at the end of the flume allowed to measure the sediment transported as bed load. For further details on the experimental apparatus, please refer to Termini et al. (2023). *Unio elongatulus* (Pfeiffer, 1825) specimens collected from Lake Maggiore (Italy–46°05′53″N 08°42′53″E) were used for these experiments.

Freshwater mussels are often encountered in sand and gravel bed rivers that provide more favorable habitat (Howard & Cufey, 2006; Lazzarin et al., 2023). Different substratum characteristics may determine habitat modifications (Engelhardt et al., 2004) and may affect the FMs' behavioral responses. Thus, for the specific

Table 1

Hydraulic Conditions Considered for the Laboratory Experiments: Q_{bp} = Flow Discharge Before the Perturbation; Q_{ap} = Flow Discharge After the Perturbation; ΔQ = Increase in Flow Discharge, $\Delta Q/Q_{bp}$ = Percentage of Discharge Increase With Respect the Value Before the Perturbation, Q_s/B = Specific Sediment Transport Rate (B = Channel Width); s.t. = Abbreviation of "Sediment Transport"; H_{gravel}, V_{gravel} = Water Depth and Mean Velocity in Gravel-Bed Runs; h_{sand}, V_{sand} = Water Depth and Mean Velocity in Sand-Bed Runs

	Q_{bp}, Q_{ap}					
Run	Symbol	Value (m^3/s)	ΔQ (m^3/s)	$100 \times \Delta Q/Q_{bp}$ (%)	Q_s/B (kg/ms)	s.t. classes (over sand substrate)
P-run	Q1, Q1	0.002, 0.002	\	\	\	No
B-run1	Q2, Q2	0.003, 0.003	\	\	\	No
B-run2	Q3, Q3	0.006, 0.006	\	\	\	No
E-run1	Q3, Q4	0.0060, 0.0080	0.00	33.33	0.00	No
E-run2	Q3, Q5	0.0060, 0.0095	0.00	58.33	0.01	Low
E-run3	Q3, Q6	0.0060, 0.0110	0.01	83.33	0.02	Medium
E-run4	Q3, Q7	0.0060, 0.0145	0.01	141.67	0.02	High
E-run5	Q3, Q8	0.0060, 0.0220	0.02	266.67	0.04	Very high
E-run6	Q2, Q4	0.0030, 0.0080	0.01	166.67	0.00	No
E-run7	Q2, Q5	0.0030, 0.0095	0.01	216.67	0.01	Low
E-run8	Q2, Q6	0.0030, 0.0110	0.01	266.67	0.02	Medium
E-run9	Q2, Q7	0.0030, 0.0145	0.01	383.33	0.02	High
E-run10	Q2, Q8	0.0030, 0.0220	0.02	633.33	0.04	Very high

Symbol	Q (m^3/s)	h_{gravel} (m)	h_{sand} (m)	V_{gravel} (m/s)	V_{sand} (m/s)
Q1	0.002	0.036	0.040	0.146	0.131
Q2	0.003	0.045	0.053	0.167	0.142
Q3	0.006	0.060	0.067	0.250	0.224
Q4	0.008	0.078	0.088	0.256	0.227
Q5	0.010	0.086	0.092	0.276	0.258
Q6	0.011	0.092	0.100	0.299	0.275
Q7	0.015	0.135	0.088	0.269	0.414
Q8	0.022	0.167	0.093	0.329	0.595

purposes of the present work, two series of runs were conducted, respectively, with FMs arranged over a gravel substrate ($d_{50} \cong 6$ cm and $d_{84} \cong 8.4$ cm, with d_x = diameter for which x% by weight is finer) and with FMs arranged over a quartz-sand substrate (with $d_{50} \cong 0.65$ cm and $d_{84} \cong 0.9$ cm). The experimental conditions were defined in such a way that the tests conducted over sand substrate allowed us to analyze the FMs' response to the variations of both flow discharge and sediment transport, while those over the gravel bed to isolate their response to the flow discharge variations, in the absence of sediment transport. Furthermore, over the sand substrate FMs performed horizontal movements by their valve gaping, but not over the gravel substrate. In fact, although FMs have limited mobility, they could perform horizontal and vertical movement by means of their muscular foot (e.g., Ferreira-Rodríguez, 2019). Each series of runs included a preliminary run (hereon indicated as P-run), aimed to identify FMs' behavior during their usual activity under very shallow flowing, two "baseline" runs (hereon indicated as B-runs), aimed to determine the FMs' response under different values of constant water discharge (i.e., in steady flow conditions) and in the absence of sediment transport, and ten "exposure" runs (hereon indicated as E-runs), aimed to investigate the FMs' response to flow discharge variations as during a flood event (Table 1). All runs were conducted in clear water conditions (i.e., without suspended material) and without feeding the mussels. This was in analogy to previous works (Modesto et al., 2023; Termini et al., 2023). For the P-run a group of 8 mussels, arranged on the bed at a mutual distance of 20 cm in the longitudinal direction and of 15 cm in the transversal one (see Figure 1), was considered. During this run, FMs were exposed to a very low constant water discharge (Q1 in Table 1) for 24 hr.

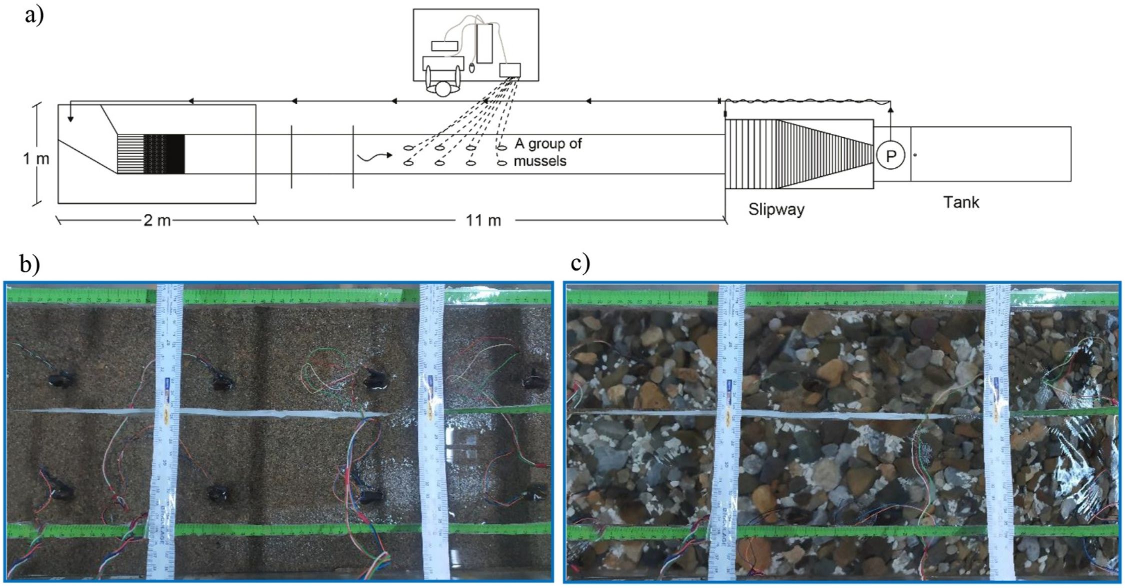

Figure 1. (a) Plane-view of the laboratory flume; (b) FMs arrangement over the sand substrate; (c) FMs arrangement over the gravel substrate. This figure is adapted from Figure 1 of Termini et al. (2023).

The B-runs (see Table 1) were conducted during the day by exposing two groups of 8 mussels (i.e., 16 mussels), arranged as shown in Figure 1, to a constant water discharge for 6 hr, including 2 hr of acclimatization. Two values of flow discharge, both below the value corresponding to the sediment incipient motion, were considered. The critical shear stress was estimated using the critical mobility number suggested by Yalin (1992) for both sand and gravel beds.

The E-runs were also conducted during the day considering the same FMs' number and arrangement as in the B-runs. In the E-runs the FMs were first exposed to a constant water discharge (Q_{bp}, in Table 1), as in the B-runs, followed by an almost instantaneous (i.e., at the instant Tt) increase of flow discharge (Q_{ap}, Table 1), which was then kept constant for 1 hr and 30 min.

Sediment transport was always absent in the E-runs conducted over the gravel substrate, while it occurred over the sand substrate when the flow discharge exceeded the value corresponding to the sediment incipient motion.

The B-runs and the E-runs were repeated twice so that 32 mussels' recorded time series were available for each run. During all the runs, the FMs' valve opening/closing activity was continuously monitored and recorded as a voltage (mV) signal at a frequency of 3 Hz. 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 FM's valves. The voltage signals were stored on an SD card connected to an Arduino board (with ATMEGA2560-16AU microcontroller 16 MHz 256 Kb 8-bit). The FMs' response of the Hall effect sensor in millivolts (mV), and then, by knowing the minimum and maximum values of the valves' gaping 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 (Termini et al., 2023).

2.1.2. Field Experiments

The in situ pilot installation was realized under the Adunata Bridge in the Paglia River (42°43′30.01″N 12° 9′ 24.32″E), a significant tributary of the Tiber River valley (Figure 2a) in Umbria region, Italy. A gauging station (Orvieto Scalo) was also available close to the pilot installation site, thus having information about the water level and the flow discharge variation every 30 min. For the in situ experiment (see also Pilbala et al., 2024), we used the native species previously reported in this river catchment (Froufe et al., 2017). Thus, thirteen *Unio mancus* (Lamarck, 1815) specimens, collected from the natural reserve of Montepulciano Lake (Siena, Tuscany- Italy; 43° 5′34.94″N 11°55′34.50″E), were stuck and caged as shown in Figure 2b.

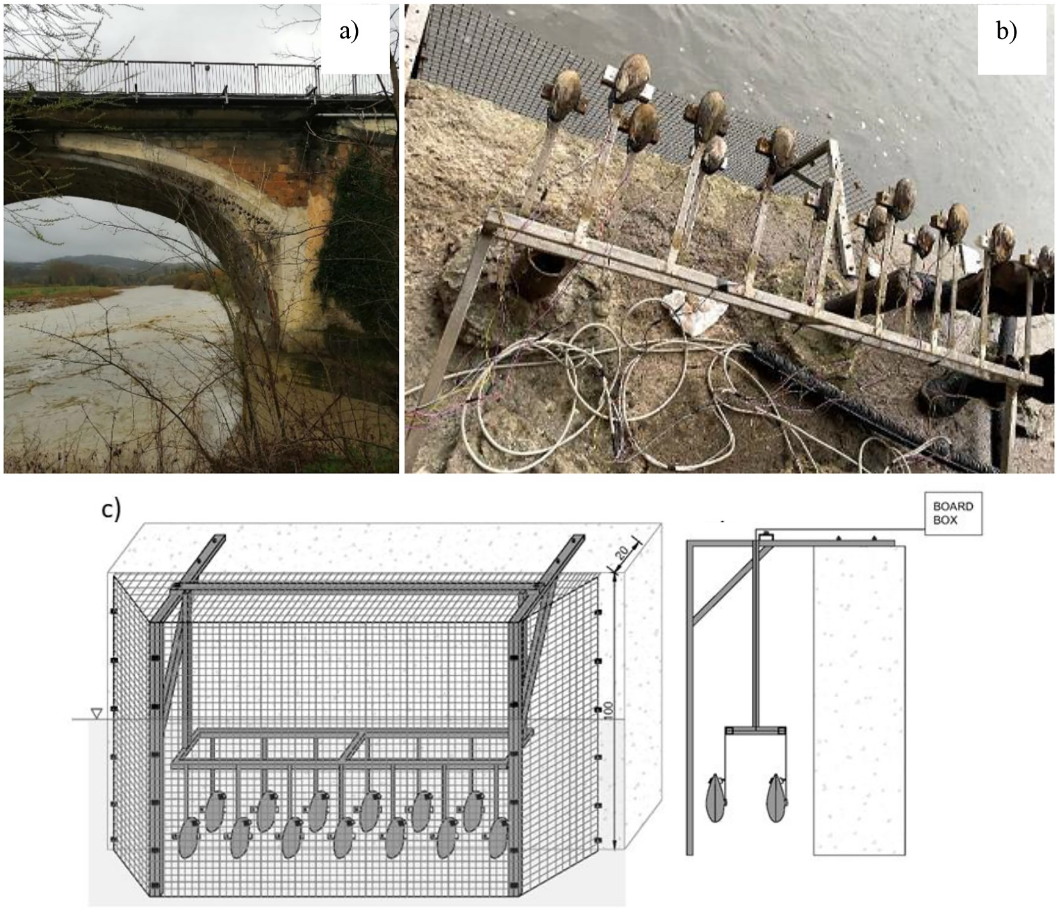

Figure 2. In situ pilot installation: (a) installation site: Adunata Bridge in the Paglia River; (b) *U. mancus* FMs stuck on steel rods; (c) design of the frontal and lateral views of anchored steel rods of the stuck FMs (dimensions are in cm). This figure is adapted from Figure 3 of Pilbala et al. (2024).

FMs were glued on steel vertical rods anchored on the concrete right bank of the considered cross-section (Figure 2); the glued mussels were protected by a steel grid (Figure 2c) to protect them during flood events. The height of the vertical rods was defined on the basis of average yearly recorded water level data to ensure that the mussels were always submerged. Laboratory studies conducted by Pilbala et al. (2024) demonstrated that, in the presence of hydrodynamic disturbances, stuck mussels exhibit a behavior that is consistent with that of freely moving mussels and allow sharper event detection and easier signal interpretation due to their movement hindrance, making them suitable for field applications. In the field, the valve gaping activity was continuously monitored, using a system like that used in the laboratory experiments, and recorded as voltage signal (mV) at a frequency of 2 Hz. The collected data were daily uploaded to a cloud-based system via modem technology. For the present work, analysis concerns the valvometry data recorded during the flood occurring on 31 March 2022 (hereon indicated as F-run).

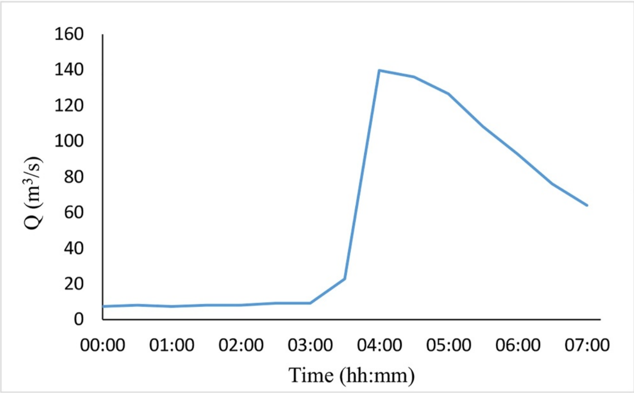

Figure 3. Hydrograph of flood occurring on 31 March 2022.

The first low increase of flow discharge (i.e., passing from a value of flow discharge of 9.14 m^3/s to a value of 22.75 m^3/s) was recorded at 03:30 a.m. and the flood peak, equal to about 140 m^3/s, was recorded at 04:00 a.m., then the flow discharge decreased (Figure 3). The flood hydrograph was schematized by considering subsequent flow discharge variations ΔQ corresponding to recording time steps of 30 min (Table 2). For the analysis, particular attention has been given to the percentage of flow discharge variation corresponding to the flood peak $100 \times \Delta Q/Q_{bp} = 514$ (i.e., that corresponding to F-run,$_{ap1}$ in Table 2).

Table 2
Field Experiment–Data of Flood on 31 March 2022 (F-Run): Q_{bp} = Flow Discharge Before the Flood Peak; Q_{ap} = Flow Discharge After the Flood Peak; ΔQ = Increase in Flow Discharge, $100 \times \Delta Q/Q_{bp}$ = Percentage of Discharge Variation With Respect to the Value Before the Flood Peak

Time (hr)	F-run	Q_{bp}, Q_{ap} (m^3/s)	ΔQ (m^3/s)	$100 \times \Delta Q/Q_{bp}$ (%)
02:30–03:00	F-run,$_{bp1}$	9.14, 9.14	0.00	0.00
03:00–03:30	F-run,$_{bp2}$	9.14, 22.75	13.61	148.96
03:30–04:00	F-run,$_{ap1}$	22.75, 139.70	116.95	514.05
04:00–04:30	F-run,$_{ap2}$	139.70, 136.08	−3.62	−2.59

2.2. Data Treatment and Statistical Tests

As indicated in Section 2.1, by scanning the recorded voltage (mV) data sets, the minimum and the maximum values for each mussel were identified, and all collected voltage data were normalized, setting the complete valve opening and closure equal to 0% and 100%, respectively. This normalization allowed us to compare the valve activity of different animals. Preliminarily, tests were conducted to define a threshold for applying high-pass filtering for possible outliers in the recorded voltage data sets (Termini et al., 2023).

FMs' reaction to hydrodynamic variations was analyzed in terms of average gaping frequency (*Fa*) and normalized average valve opening amplitude (*Aa*). To better identify FMs' behavior immediately after the perturbation (i.e., after the instant Tt, representing either the instant corresponding to the induced flow discharge variation in E-runs or the instant of the flood peak occurrence in F-run), the entire duration (hereon indicated as "period") of data recording before and after the instant Tt was divided into smaller time intervals (hereon indicated as "sub-periods"). For the E-runs, in accordance with Termini et al. (2023) and based on preliminary tests, sub-periods of the duration of 22 min were considered; for the F-run the sub-periods had a duration of 30 min according to the recording time step of flow discharge at the gauging station. Given that we focus on FMs' reaction suddenly after the perturbation, only two sub-periods after Tt have been considered in the following analysis.

First, the values of *Fa* and *Aa* have been estimated for all the runs by considering both the entire period and the two sub-periods after Tt. Then, to explore the statistical difference in FMs' response both between the two different substrates and between the laboratory and the field conditions, statistical tests were performed among the *Fa* and *Aa* distributions using the open-source R platform. The Shapiro–Wilk test (Mishra et al., 2019) was applied to the *Fa* and *Aa* data sets to verify if/when they followed the normal distribution. Both the Wilcoxon Test and the T-Test were used for normally distributed data sets; only the Wilcoxon Test was applied otherwise. The statistical difference between the two data groups was considered significant for a p-value < 0.05.

The comparison between the *Fa* and *Aa* data sets of the two E-runs series allowed us to verify the existence of a statistical difference in FMs' response to flow discharge variation and/or to the sediment transport for the different substrates. The comparison between the laboratory-based and the field-based *Fa* and *Aa* data sets allowed us to identify the existence of a difference in FMs' response to flow discharge variation in controlled and uncontrolled environmental conditions. Due to the different order of magnitude of flow discharge values in the laboratory and the field, the comparison was performed by considering the ratio $\Delta Q/Q_{bp}$ (i.e., the percentage of flow discharge variation, ΔQ, on the value of flow discharge, Q_{bp}, either before the perturbation—for the E-runs—or before the flood peak—for the F-run).

3. Results

3.1. Average Valves Activity

The values of *Fa* and *Aa* on sand and gravel substrate, over the entire period and the two sub-periods after Tt are summarized in Figures 4 and 5, respectively.

Regardless of the substrate typology, *Fa* is consistently below 0.01 Hz in the absence of flow discharge variations (B-runs) and increases as the flow discharge increases (Figure 4). For the same flow discharge value, *Fa* is higher on the sand substrate than the gravel substrate. Under variable flow discharge (E-runs), *Fa* increases with $\Delta Q/Q_{bp}$, attaining its highest values immediately after the perturbation (first sub-period, Figure 4b). On the gravel substrate, *Fa* is lower than 0.01 Hz for flow discharge variation $\Delta Q \leq 0.0065$ m^3/s and flow discharge after the perturbation $Q_{ap} \leq Q5$ (see Table 1). In comparison, it exceeds 0.01 Hz when flow discharge variation exceeds 0.0065 m^3/s and $Q_{ap} > Q5$ (Table 1). *Fa* is lower than 0.01 Hz on the sand substrate for the lowest flow discharge

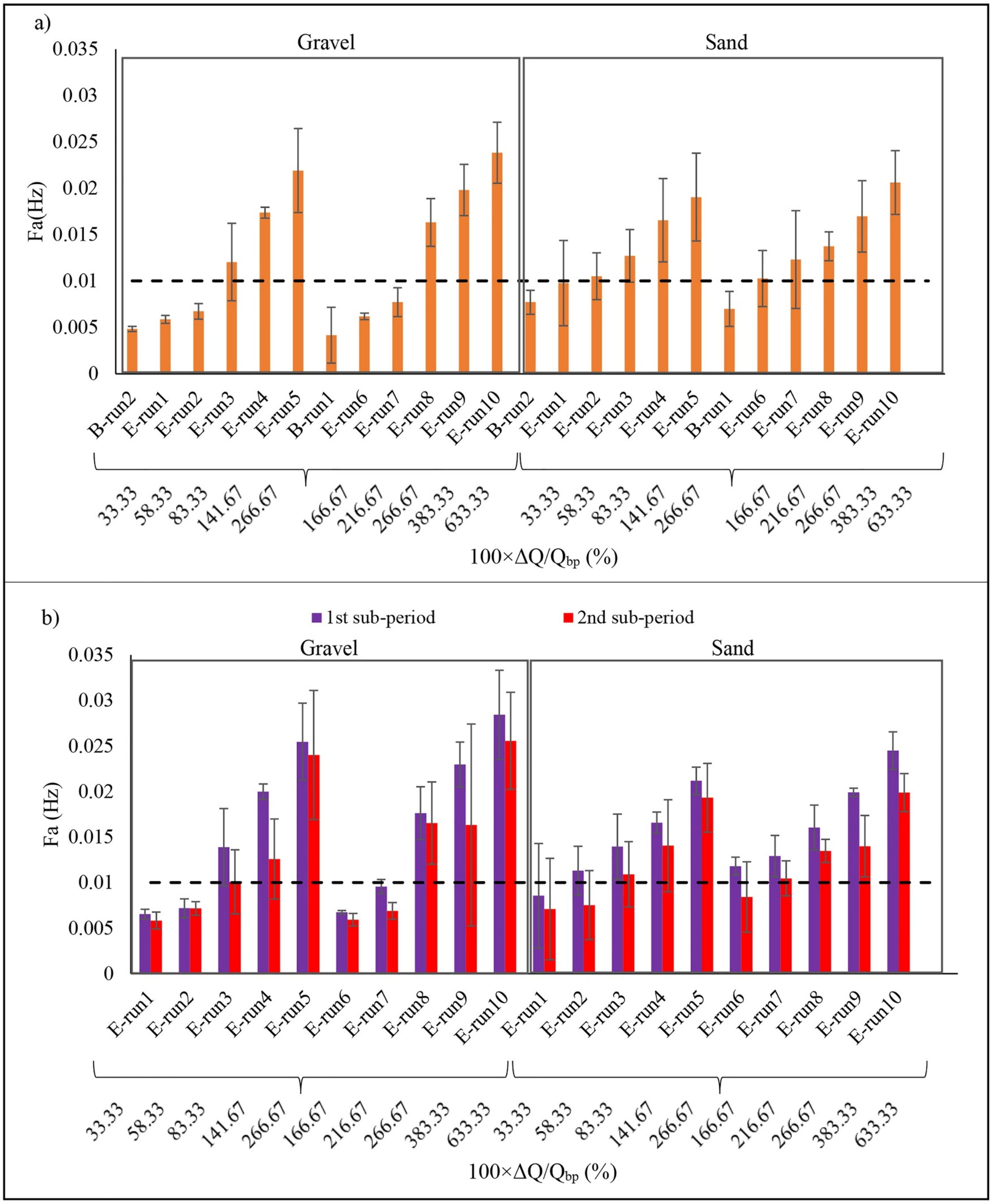

Figure 4. Histogram of the average valves gaping frequency (*Fa*): (a) in B-runs and in E-runs over the entire period; (b) in E-runs over the two sub-periods after Tt.

variation (E-run1, Table 1), without sediment transport. The values of *Fa* are higher on the sand than on the gravel substrate for the same $\Delta Q/Q_{bp}$ and in the absence of sediment transport.

In the case of the sand substrate, the comparison between E-run6 and E-run3, that is for the same ΔQ, highlights that *Fa* is slightly greater in E-run3, which is characterized by higher values of $\Delta Q/Q_{bp}$ and Q_{ap} and in the presence of sediment transport. The effect of different sediment transport rates on FMs' response can be observed by comparing E-run5 and E-run8, which have the same percentage of flow rate variation (i.e., $100 \times \Delta Q/Q_{bp} = 266.66$): *Fa* has greater values in E-run5 characterized by a higher value of Q_{ap} which determines a higher sediment transport rate.

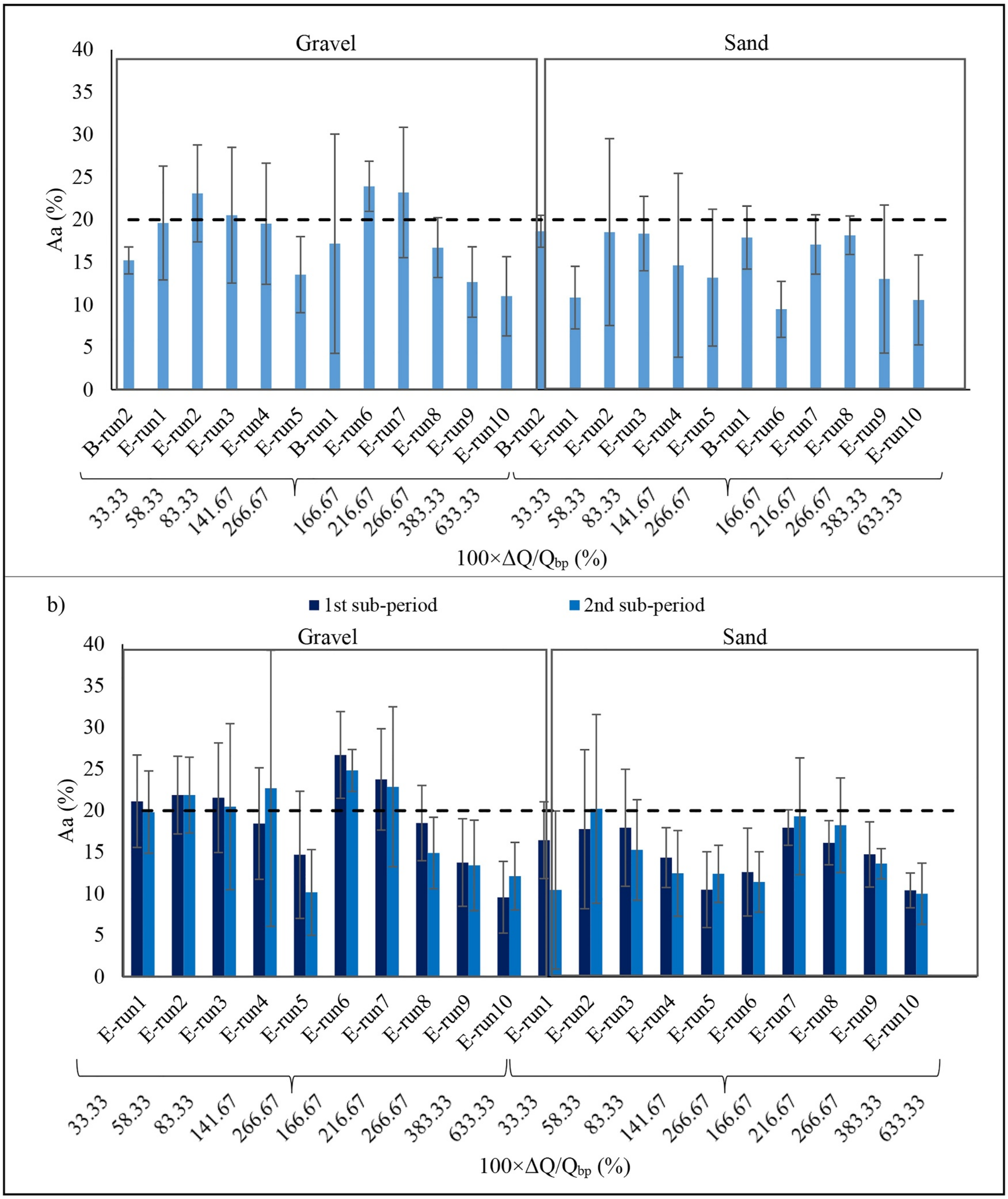

Figure 5. Histogram of the average valves gaping amplitude (*Aa*): (a) in B-runs and in E-runs over the entire period; (b) in E-runs over the two sub-periods after Tt.

However, it should be noted that over the gravel substrate, and thus in the absence of sediment transport, the comparison between E-run5 and E-run8 also indicates that *Fa* assumes greater values for greater Q_{ap} (i.e., in E-run5). Still, these values of *Fa* are greater than those obtained in the same run over the sand substrate.

The average opening amplitude (*Aa*) over the gravel substrate (Figure 5) is lower than 20%, except for E-run6 and E-run7. Under constant discharge (B-runs), the *Aa* values are similar regardless of the value of flow discharge (Figure 4a) and the substrate typology. In the E-runs, the values of *Aa* tend to decrease as $\Delta Q/Q_{bp}$ increases, especially over the gravel substrate. Higher values of *Aa* are generally observed in the first sub-period (Figure 4b) and for lower values of Q_{ap} (E-run8) for both the substrates, as well as for lower values of sediment transport rate over the sand substrate.

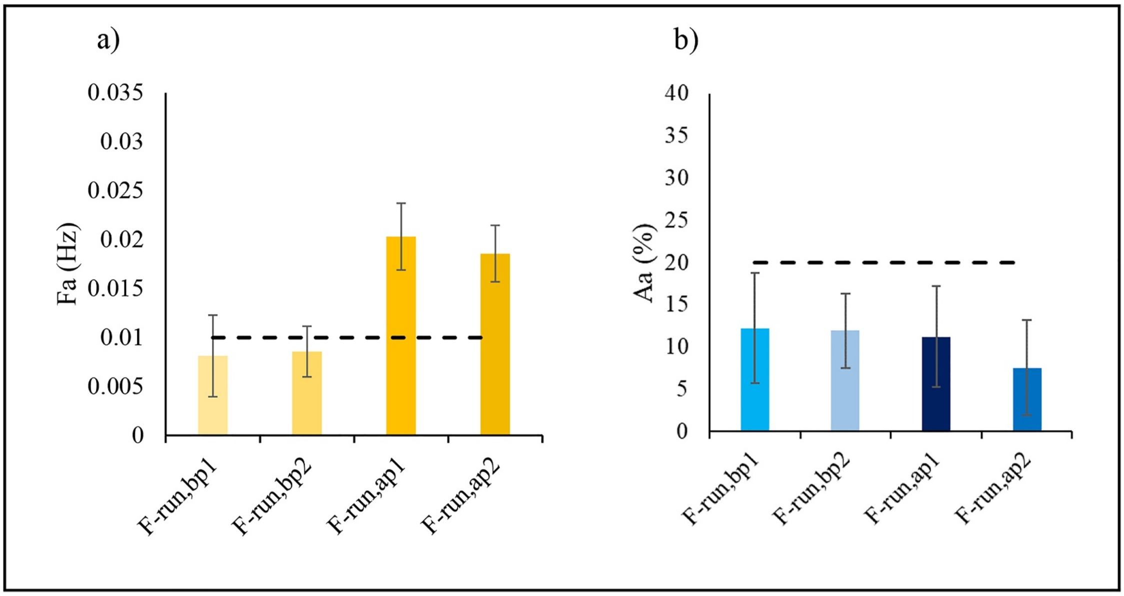

Figure 6. Histogram of FMs response of the field-based data during flood on 31 March 2022 (F-run) over the two sub-periods before and after the flood peak: (a) in terms of average valves gaping frequency (*Fa*); (b) in terms of average valves gaping amplitude (*Aa*).

Fa and *Aa* average values measured in field-based experiments (F-run) before and after the flood peak are schematized in Figure 6. *Fa* values were lower than 0.01 Hz before the flood peak and higher after the peak. The values of the average opening amplitude, *Aa*, obtained in the F-run, decrease as $\Delta Q/Q_{bp}$ increases, assuming values below 20% (Figure 6b).

3.2. Statistical Comparison Tests

The results of the statistical comparison tests between the two different substrate compositions (gravel and sand) in the E-runs for *Fa* and *Aa* data sets are reported in Table S1 in Supporting Information S1.

FMs' response in terms of *Fa* is statistically not different over the two substrates (Table S1a in Supporting Information S1). However, a difference occurs in the first sub-period after the instant Tt in E-run1, E-run6, and E-run7, characterized by low flow discharge variation (i.e., $\Delta Q \leq 0.0065$ m^3/s) either in the absence (E-run1 and E-run6) or in the presence of low sediment transport over the sand substrate (E-run7). In the second sub-period, the FMs' response in terms of *Fa* differs only in E-run1, that is, for very low flow discharge variation ($\Delta Q = 0.002$ m^3/s, $100 \times \Delta Q/Q_{bp} = 33.33$). The FMs' *Aa* (Table S1b in Supporting Information S1) is not statistically different over the two substrates. However, a difference occurs in both sub-periods in E-run2 and E-run6 (in the second sub-period a difference also occurs in E-run1), that is with low flow discharge variation ($\Delta Q \leq 0.005$ m^3/s) and/or with low sediment transport.

The comparison of the FMs' response between E-run6 and E-run3, characterized by the same ΔQ but a different $\Delta Q/Q_{bp}$ and Q_{ap} indicates that, over the gravel substrate (i.e., in the absence of sediment transport) (Table in Supporting Information S1), the FMs' response is statistically different in terms of *Fa* and not significantly different in terms of *Aa*, confirming that FMs' gaping frequency increases with the flow discharge variation. Over the sand substrate (Table S3 in Supporting Information S1), on the contrary, the analogous comparison presents no significant difference in both *Fa* and *Aa* in the two sub-periods after Tt.

The results of the comparison tests for *Fa* and *Aa* data sets between the F-run and the E-runs conducted over the gravel substrate are summarized in Table S4 in Supporting Information S1, while those between the F-run and the E-runs conducted over the sand substrate are summarized in Table S5 in Supporting Information S1. The FMs' *Fa* response in the field is statistically different from that obtained in the E-runs with exceptions, over the gravel substrate, in both sub-periods of E-run1, E-run2, and of E-run6 and in the second sub-period of E-run3 and E-run7 (Table S4a in Supporting Information S1) and, over the sand substrate, in the second sub-period of E-run1, E-run2, E-run6, and E-run7 (Table S5a in Supporting Information S1).

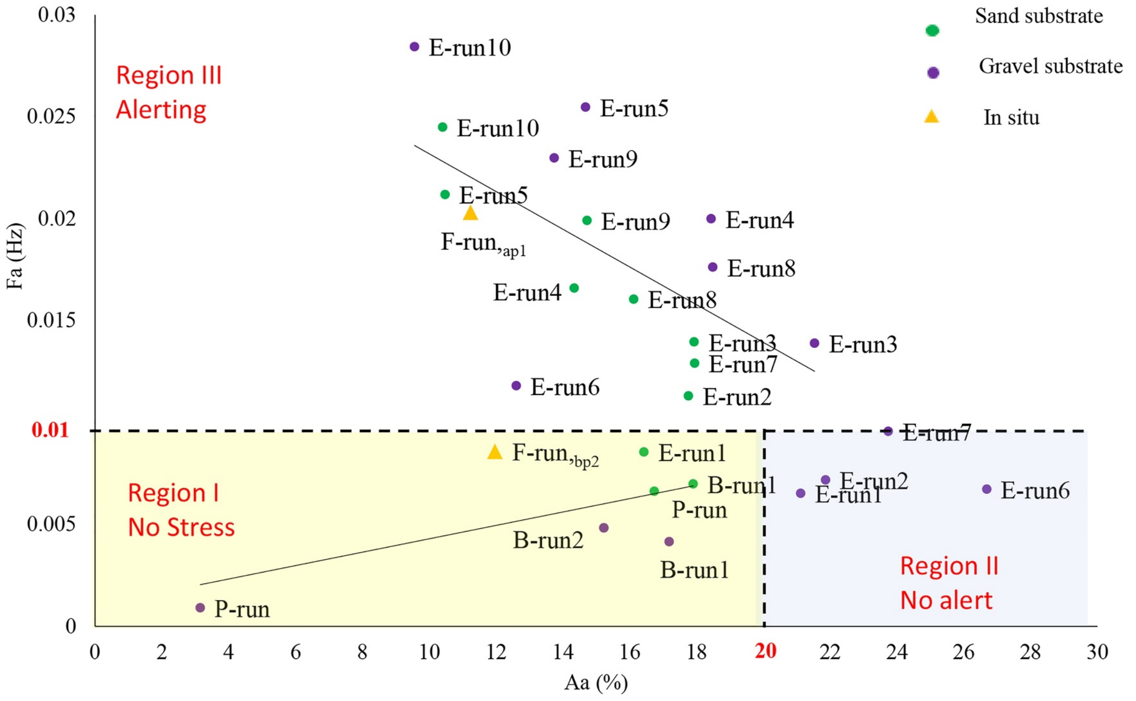

Figure 7. Threshold [*Fa, Aa*] plane based on laboratory experiments.

The *Aa* response of F-run is not statistically different from that obtained in the E-runs except in the first sub-period of E-run7 and E-run 10 over the gravel substrate (Table S4b in Supporting Information S1) and in the first sub-period of E-run5 and E-run10, and in the second sub-period of E-run1, E-run4, E-run6, and E-run10 over the sand substrate (Table S5b in Supporting Information S1).

3.3. Threshold Plane and Benchmark Graph for Ecosystem Alarm Criterion

The achieved results suggest that FMs' response to the hydrodynamic stress immediately after the perturbation is eligible for developing a procedure for an FMs-Based Early Warning System. This requires identifying and delimitating a threshold indicative of reaching the alarm level. Figure 7 reports the pair of values [*Fa, Aa*] of the first sub-period after Tt in the P-, B-and E-runs. On the [*Fa, Aa*] plane, three regions can be identified: region I, characterized by $Fa < 0.01$ Hz and $Aa < 20\%$, with an increasing trend of *Aa* as *Fa* increases; region II, characterized by $Fa < 0.01$ Hz and $Aa > 20\%$; region III, characterized by $Fa > 0.01$ Hz and *Aa* generally <20%, with a decreasing trend of *Aa* as *Fa* increases. Region I of the figure includes pairs of [*Fa, Aa*] obtained in P-run and B-runs, which identify the FMs' usual movement activity and the FMs' response, respectively, under a constant water discharge and in the absence of sediment transport. The pair of [*Fa, Aa*] values corresponding to FMs' response recorded before the flood peak (F-run,$_{bp2}$) also falls in this region. Region II includes pairs of [*Fa, Aa*] values corresponding to the E-runs on the gravel substrate (i.e., in the absence of sediment transport) and characterized by low $\Delta Q/Q_{bp}$ and Q_{ap} (i.e., $Q_{ap} \le Q5$, E-run1, E-run2, E-run6, E-run7). Finally, region III includes pairs of [*Fa, Aa*] values obtained in all the remaining E-runs over both substrates, identifying FMs' response to high values of $\Delta Q/Q_{bp}$, in runs with or without sediment transport. The pair of [*Fa, Aa*] relevant to FMs' response after the flood peak (F-run,$_{ap1}$) also falls in this region, as well.

In Figure 8 the *Fa* values of the E-runs, grouped for similar *Aa* values, are plotted against the percentage of the flow discharge variation $\Delta Q/Q_{bp}$. Three different ranges of *Fa*-values can be identified and, within each range, the [*Fa*-$\Delta Q/Q_{bp}$] points can be interpolated by a logarithmic function having the value of the mean gaping amplitude, *Aa,$_m$*, as a parameter. Each range includes [*Fa*-$\Delta Q/Q_{bp}$] points corresponding to a different level of stress condition: the first level (low level) includes data-points corresponding to the E-runs on the gravel substrate, in the absence of sediment transport, and low $\Delta Q/Q_{bp}$; the second level (medium level) includes data-points

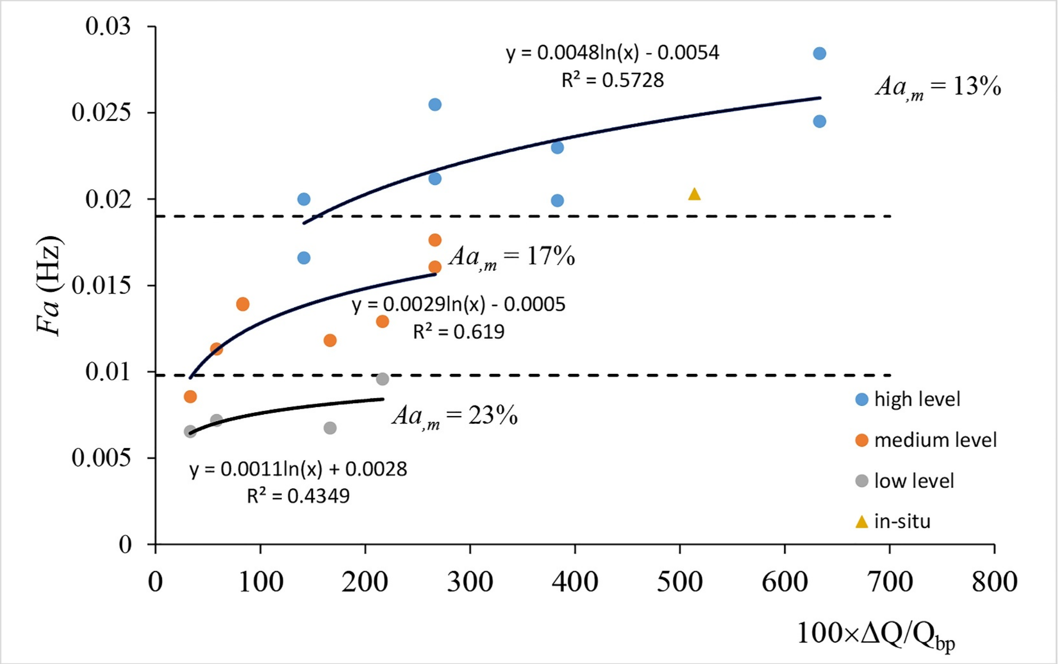

Figure 8. Benchmark graph (clear-water conditions).

characterized by percentage of flow discharge variation $100 \times \Delta Q/Q_{bp} < 100$ over both substrates and, thus, either without sediment transport or with low or medium sediment transport (in case of sand substrate—Table 1); the third level includes data-points obtained over both substrates characterized by high values of $\Delta Q/Q_{bp}$ and sediment transport. The value of the parameter $Aa_{,m}$ decreases as the Fa-values increase. The first range, that is for Fa-values less than 0.01 Hz and $Aa_{,m}$-value equal to 23% (i.e., greater than 20%), corresponds to low $\Delta Q/Q_{bp}$ (low-flow perturbation conditions), as well as the points falling in region II of the threshold plane. The second range, that is for $0.01 \text{ Hz} \le Fa < 0.02$ Hz and $Aa_{,m}$-value of 17% (i.e., lower than 20%), corresponds to low-flow perturbations ($100 \times \Delta Q/Q_{bp} < 300$) and low values of Q_{ap} (≤Q5—see Table 1). The third range, that is for $Fa \ge 0.02$ Hz and $Aa_{,m}$ value of 13% (i.e., lower than 17%) corresponds to high-flow perturbations ($100 \times \Delta Q/Q_{bp} > 300$) and high values of Q_{ap} (>Q5). Just by way of example in Figure 8, the [Fa-$\Delta Q/Q_{bp}$] pair corresponding to *U. mancus*'s response in the field after the flood peak (e.g., F-run,$_{ap1}$) has been also reported ($Aa = 11.5\%$). It can be noted that it falls within the third range of Fa-values below the interpolating curve with $Aa_{,m}$ value of 13%.

The concept inside this graph is that laboratory data obtained in clear water condition may be used as a benchmark to evaluate the potential impact of disturbances on the ecosystem by observing the displacement between the expected $Aa_{,m}$ and the measured gaping amplitude, Aa. Under this umbrella, the following gaping index could be considered:

$$Ig = 1 - \left| \frac{Aa_{,m} - Aa}{Aa_{,m}} \right|$$

$Ig = [0,1]$ indicates that if the measured gaping amplitude, Aa, is equal to the expected value obtained in clear water conditions, $Aa_{,m}$, Ig is equal to 1; when Aa differs from the expected value $Aa_{,m}$, the relative number representing the index Ig is less than 1. A high distance from the $Aa_{,m}$ means that something is changing from the clear water conditions. For the in situ case after the flood peak, being $Aa = 11.5\%$ and $Aa_{,m} = 13$, it is $Ig = 0.88 \cong 0.9$. In other words, the graph can be used as a proxy of alerts for the fluvial ecosystem. It is worth of stressing that this finding was obtained into an environment totally different from the laboratory one and with a FMs species different.

4. Discussion

As it is clear from the Introduction, although FMs' valve movements have been used as a well-established measure of the impacts of chemical stressors in aquatic habitats, this technique appears to be underutilized in investigating the impacts due to hydrodynamic changes. The present study, as part of the national research program PRIN2017, demonstrates that the valvometric technique could be used as an ecosystem alarm criterion based on mussel behavior. By using the same FMs' response detecting methodology as that illustrated in previous works (Modesto et al., 2023; Termini et al., 2023), this paper presents a more general approach, extending the analysis of FMs' behavior for different substrates and different environments for identifying a preliminary method for defining the ecosystem alarm criterion in clear water condition.

The analysis is performed using valvometry data collected during both laboratory- and field-based experiments. The laboratory experiments were especially appropriate for investigating the influence determined by the substrate typology on the FMs' (*U. elongatulus*) response. The field experiment, conducted at Adunata Bridge along the Paglia River (Umbria, Italy), allowed us to validate lab-based results and to investigate the different FMs' (*U. mancus*) response in a natural uncontrolled environment.

The laboratory-based results have indicated that, in the absence of flow discharge variation (e.g., in steady flow condition), regardless of the substrate typology, the average valves' gaping frequency, *Fa,* is always below 0.01 Hz, assuming higher values for greater values of flow discharge. The average valve's opening amplitude, *Aa* takes almost similar values, ranging between 15% and 18% (i.e., consistently below 20%), for different values of flow discharge. For the same value of flow discharge, *Fa* assumes higher values over the sand substrate than those obtained over the gravel substrate. This difference could be due to the possible mussels' horizontal movement in the sand substrate, as previously verified in Termini et al. (2023). By isolating the signal determined by the mussels' horizontal movement (i.e., in the so-called “movement test”) Termini et al. (2023) verified that it was different, in terms both of shape and of mean frequency, to that observed under a flow discharge variation. This is consistent with findings obtained by Pilbala et al. (2024), which compared the behavior of stuck and free *U. mancus* specimens in a laboratory flume, highlighting that the signal of stuck mussels shows less complexity because of their horizontal movement limitation. Over the gravel substrate, the FMs cannot bury and/or horizontally move, and their response is primarily limited to gaping associated with flow discharge variations. As well as over the gravel bed, the stuck mussels observed by Pilbala et al. (2024) cannot bury and/or horizontally move and, because of this, their response to flow discharge variations is simpler than that of free mussels.

When an abrupt variation of flow discharge occurs, regardless of the substrate typology, the FMs' average valve gaping frequency, *Fa,* increases as the percentage of flow discharge variation, $\Delta Q/Q_{bp}$, increases. For a given value of $\Delta Q/Q_{bp}$, the highest value of *Fa* is obtained suddenly after the flow perturbation (i.e., in the first subperiod). This demonstrates the quick FMs' response to the hydrodynamic stress, regardless of the substrate typology. Results also show that while in the presence of a low-flow perturbation (i.e., low values of $\Delta Q/Q_{bp}$) the values of *Fa* over the gravel substrate are lower than those over the sand substrate, as well as in steady flow condition, the opposite happens in the presence of a high-flow perturbation (i.e., high values of $\Delta Q/Q_{bp}$). While for low-flow perturbations over the gravel substrate, *Fa* is always lower than 0.01 Hz, over the sand substrate, *Fa* is lower than 0.01 Hz only for very low flow discharge variation. In the presence of high-flow perturbations (i.e., high values of $\Delta Q/Q_{bp}$) *Fa* always exceeds 0.01 Hz over both substrates, with higher values over the gravel substrate for the same $\Delta Q/Q_{bp}$. This is consistent with Pilbala et al.’ (2024) findings, obtained during laboratory experiments with stuck (i.e., without possible horizontal movement, as it is over the gravel substrate) mussels, demonstrating that they experience a stimulus stronger than free mussels due to the impossibility of actively searching for shelter at the event time. The lower *Fa*-values obtained over the sand substrate are likely because FMs can bury in the substrate to find shelter from water flow and sediment transport. On the contrary, in both substrates the *Aa* values tend to decrease as $\Delta Q/Q_{bp}$ increases, generally assuming values lower than 20%. For the same $\Delta Q/Q_{bp}$, greater *Aa* values are obtained for a lower flow rate after the perturbation, Q_{ap}.

The comparison tests performed for the same flow discharge variation (ΔQ) but different $\Delta Q/Q_{bp}$ and Q_{ap} values, indicate that over the sand substrate the FMs' response is not different, because FMs may move or find a shelter in the substrate; on the contrary, over the gravel substrate, where FMs' horizontal movement is hindered, the FMs' response is different in terms of *Fa*.

In the field experiment, the average valves' gaping frequency, *Fa*, of the stuck FMs assumed values lower than 0.01 Hz before the flood peak and greater than 0.01 Hz after it, as observed in the laboratory experiments. Just after the flood peak, *Fa* values were like those measured in the laboratory, especially over the gravel substrate. In the field, *Aa* decreased as the flow discharge increased, always assuming values below 20%, as well as in the laboratory tests.

The comparison between the laboratory-based and the field-based data sets indicated that the response of the stuck FMs in the field is not significantly different from that obtained in the laboratory over the gravel substrate (where the FMs' movement was also hindered) in terms of *Aa;* in terms of *Fa,* it was not significantly different only for values of the percentage of discharge variations lower than those obtained during the flood event. This could be related to the fact that while the laboratory-based data sets were collected in clear water conditions, suspended materials were present in the field (Brouwer et al., 2023).

The achieved results have suggested it could be appropriate to construct a dedicated procedure for using FMs' response for an early warning system. Based on the pairs of *U. elongatulus* [*Fa, Aa*] values of all the laboratory experiments (i.e., in clear water conditions), three different conditions could be identified: the so-called “no stress” condition (region I) including [*Fa, Aa*] pairs corresponding both to FMs' standard activity or under steady flow conditions; the so-called “no alert” condition (region II) corresponding to [*Fa, Aa*] pairs obtained for low-flow perturbations, $\Delta Q/Q_{bp}$, and in the absence of sediment transport; the so-called “alerting condition” (region III) including (*Fa, Aa*) pairs corresponding to FMs' response under high-flow perturbations, $\Delta Q/Q_{bp}$, both in the absence and the presence of sediment transport. It is interesting to note that the field-based *U. mancus* [*Fa, Aa*] pairs confirmed the conditions identified on the above-mentioned [*Fa, Aa*] threshold plane, considering that the [*Fa, Aa*] pair obtained before the flood peak of 31 March 2022 fell within region I and the [*Fa, Aa*] pair obtained after the flood peak fell within region III.

Grouping the *Fa*-values for similar *Aa* values (Figure 8), a [*Fa,* $\Delta Q/Q_{bp}$] benchmark graph was defined. In this graph, the *Fa* values are related to the percentage of the flow discharge variation, $\Delta Q/Q_{bp}$, through logarithmic curves whose parameter is given by the mean gaping amplitude, $Aa_{,m}$. It should be noted that this benchmark graph, which is based on laboratory data, is limited to clear water conditions. Although we considered *U. mancus* for field data, to show the concept beneath the analysis, it has been verified that the pair [*Fa*-$\Delta Q/Q_{bp}$] corresponding to *U. mancus*'s response, after the flood peak in field-experiment, falls close, and precisely below, to the interpolating curve with parameter $Aa_{,m} = 13\%$. This point is found below the interpolating curve. This could be because in the field the water was not clear and suspended materials were present. It should also be considered that two different species have been used in the laboratory and the field with a possible different response to the hydraulic variations. However, the obtained benchmark graph could be helpful in guiding the selection of the ecosystem alarm criterion in clear water conditions through the identification of the gaping index. Thus, this analysis paves the way for further developing a BEWS even for more complex situations, such as natural rivers. The possibility of defining a benchmark graph exerts a crucial role in the continuous monitoring of the status of the aquatic environment, facilitating the direct and uninterrupted detection of dangerous flow variation conditions based on the FMs' behavior.

5. Conclusion

Based on the above results, the main conclusions can be drawn:

- FMs' response, both in terms of average gaping frequency (*Fa*) and in terms of average gaping amplitude (*Aa*) to the hydrodynamic variations, is generally not different for different conditions of the bed substrate;
- the average gaping frequency, *Fa,* increases as the percentage discharge variation, $\Delta Q/Q_{bp}$, increases, demonstrating the quick FMs' response to the hydrodynamic stress, regardless of the substrate typology. Because of the possible FMs' horizontal movement over the sand substrate, the values of *Fa* are higher for low flow discharge variations than those obtained over the gravel substrate. For high values of the percentage discharge variations, the opposite behavior occurs because of the presence of sediment transport.
- the response of the stuck FMs recorded in the field-based experiment during the flood of 31 March 2022 compares well to that obtained in the laboratory over the gravel substrate especially in terms of *Aa* and in terms of *Fa.* To understand these outcomes, we must remember that in the experiments the values of the percentage discharge variations, $\Delta Q/Q_{bp}$, were lower than those relevant to the flood and the presence of suspended materials only in the field;

- both the laboratory-based and field-based results indicate that: (a) before the occurrence of the hydrodynamic perturbation and in the absence of sediment transport, *Fa* increases as the flow discharge increases, but it maintains values always below 0.01 Hz; the *Aa* assumes almost similar values, below 20%, for different values of flow discharge; (b) after the occurrence of the hydrodynamic perturbation, both in the absence and the presence of sediment transport, *Fa* increases as the percentage of flow discharge variation increases, assuming values greater than 0.01 Hz; *Aa* tends to decrease as the percentage of flow discharge variation increases but maintains values consistently below 20%.
- a [*Fa, Aa*] "so-called" threshold plane related to *U. elongatulus* reaction to the hydrodynamic perturbation in laboratory-based experiments is defined. Three regions corresponding respectively to the "no stress" condition, "no alerting" and "alerting" conditions are identified in this plane. After the flood event, the *U. mancus* response obtained in the field-based experiment validates the regions' identification on this plane.
- a preliminary [Fa, $\Delta Q/Q_{bp}$] benchmark graph has been defined on the basis of laboratory [Fa, Aa] data. This graph could be a guide for a first setting of the ecosystem alarm criterion, by considering that each curve identifies a range of level of alarm condition in clear water of the examined system so that the greater the distance from the curve within each range, the greater the probability that for such a condition the water is not clear. This graph constitutes a base for future development in more turbid water, such as in natural rivers.

In summary, the present study further confirms that FMs are efficient bio-indicators for detecting the impacts of hydrodynamic variations in rivers. The benchmark graph presented in this work identifies for the first time a dedicated alarm criterion associated to FMs reaction. This confirms the possibility to use of FMs as real-time BEWS for identifying potential threats to the aquatic ecosystem.

Thus, the present work paves the way to get a BEWS for the ecosystem's disturbance by investigating FMs's behavior. But, future works should be conducted to extend the results obtained in other contexts, especially with unclear water conditions. Specifically, laboratory tests aiming to combine hydrodynamic stressors and other types of stressors (such as pollutants, suspended materials, etc.) impacting the ecosystems quality, must be carried out in-depth. A more comprehensive analysis will be also conducted for a deeper examination of the feedbacks between the local hydrodynamic conditions and mussels, along with the verification into whether/how a single species can reflect the overall ecosystem response.

Conflict of Interest

The authors declare no conflicts of interest relevant to this study.

Data Availability Statement

The data can be accessed online (Termini, 2024).

Acknowledgments
This work has been supported by the Italian PRIN 2017 project Enterprising. Termini D.'s contribution was also partially supported by Project "National Biodiversity Future Center–NBFC" CUP B73C22000790001, under the National Recovery and Resilience Plan (NRRP), Mission 4 Component 2 Investment 1.4–Call for tender No. 3138 of 16 December 2021, rectified by Decree n.3175 of 18 December 2021 of Italian Ministry of University and Research funded by the European Union–Next Generation EU. The authors thank the technical staff (Massimo Mannino and Giovanni Arcidiacono) of the University of Palermo for their help in setting up the laboratory experiments. The fieldwork was conducted with the help and technical support of Augusto Benigni and Cristiano Corradini from CNR-IRPI. Authors thank Nicoletta Riccardi for her contributions and suggestions during the experimental research. Open access publishing facilitated by Universita degli Studi di Palermo, as part of the Wiley - CRUI-CARE agreement.

References

Akstinas, V., Kriščiūnas, A., Šidlauskas, A., Čalnerytė, D., Meilutytė-Lukauskienė, D., Jakimavičius, D., et al. (2022). Determination of river hydromorphological features in low-land rivers from aerial imagery and direct measurements using machine learning algorithms. *Water*, *14*(24), 4114. https://doi.org/10.3390/w14244114

Alimenti, F., Bonafoni, S., Gallo, E., Palazzi, V., Vincenti, G. R., Mezzanotte, P., et al. (2021). Non-contact measurement of river surface velocity and discharge estimation with a low-cost doppler radar sensor. *IEEE Transactions on Geoscience and Remote Sensing*, *58*(7), 5195–5207. https://doi.org/10.1109/tgrs.2020.2974185

Allan, J. D., & Castillo, M. M. (2007). *Stream ecology: Structure and function of running waters* (2nd ed.). Springer Netherlands. https://doi.org/10.1007/978-1-4020-5583-6

Allen, D. C., & Vaughn, C. C. (2010). Complex hydraulic and substrate variables limit freshwater mussel species richness and abundance. *Journal of the North American Benthological Society*, *29*(2), 383–394. https://doi.org/10.1899/09-024.1

Bae, M. J., & Park, Y. S. (2014). Biological early warning system based on the responses of aquatic organisms to disturbances: A review. *Science of the Total Environment*, *466–467*, 635–649. https://doi.org/10.1016/j.scitotenv.2013.07.075

Bódis, E., Tóth, B., & Sousa, R. (2014). Massive mortality of invasive bivalves as a potential resource subsidy for the adjacent terrestrial food web. *Hydrobiologia*, *735*(1), 253–262. https://doi.org/10.1007/s10750-013-1445-5

Borcherding, J. (2006). Ten years of practical experience with the Dreissena-Monitor, a biological early warning system for continuous water quality monitoring. *Hydrobiologia*, *556*(1), 417–426. https://doi.org/10.1007/s10750-005-1203-4

Brouwer, S., Humphries, P., Holland, A., & McCasker, N. (2023). Effect of suspended sediment concentration on the clearance and biodeposition rates of an Australian freshwater mussel (Hyriidae: Alathyria jacksoni). *Freshwater Biology*, *68*(8), 1413–1427. https://doi.org/10.1111/fwb.14137

Butterworth, F. M., Villalobos-Pietrini, R., & Gonsebatt, M. E. (2001). Introduction. In F. M. Butterworth, A. Gunatilaka, & M. E. Gonsebatt (Eds.), *Biomonitors and biomarkers as indicators of environmental change 2*, *Environmental science research* (Vol. 56, pp. 1–8). Springer. https://doi.org/10.1007/978-1-4615-1305-6_1

Dolcetti, G., Hortobágyi, B., Perks, M., Tait, S. J., & Dervilis, N. (2022). Using noncontact measurement of water surface dynamics to estimate river discharge. *Water Resources Research*, *58*(9), e2022WR032829. https://doi.org/10.1029/2022WR032829

Döll, P., & Zhang, J. (2010). Impact of climate change on freshwater ecosystems: A global-scale analysis of ecologically relevant river flow alterations. *Hydrology and Earth System Sciences*, *14*(5), 783–799. https://doi.org/10.5194/hess-14-783-2010

Eaton, B. C., & Lapointe, M. F. (2001). Effects of large floods on sediment transport and reach morphology in the cobble-bed Sainte Marguerite River. *Geomorphology*, *40*(40), 291–309. https://doi.org/10.1016/s0169-555x(01)00056-3

Engelhardt, C., Krüger, A., Sukhodolov, A., & Nicklisch, A. (2004). A study of phytoplankton spatial distributions, flow structure and characteristics of mixing in a river reach with groynes. *Journal of Plankton Research*, *26*(11), 1351–1366. https://doi.org/10.1093/plankt/fbh125

Ferreira-Rodríguez, N. (2019). Spatial aggregation of native with non-native freshwater bivalves and activity depletion under summer heat waves: 'dangerous liaisons' in a climate change context. *Hydrobiologia*, *834*(1), 75–85. https://doi.org/10.1007/s10750-019-3910-2

Froufe, E., Lopes-Lima, M., Riccardi, N., Zaccara, S., Vanetti, I., Lajtner, J., 2017). Lifting the curtain on the freshwater mussel diversity of the Italian Peninsula and Croatian Adriatic coast. *Biodiversity and Conservation*, *26*, 3255–3274.

Garcia-March, J. R., Jiménez, S., Sanchis, M. A., Monleon, S., Lees, J., Surge, D., & Tena-Medialdea, J. (2016). In situ biomonitoring shows seasonal patterns and environmentally mediated gaping activity in the bivalve, *Pinna nobilis*. *Marine Biology*, *163*(2), 29. https://doi.org/10.1007/s00227-016-2812-3

Geist, J. (2011). Integrative freshwater ecology and biodiversity conservation. *Ecological Indicators*, *11*(6), 1507–1516. https://doi.org/10.1016/j.ecolind.2011.04.002

Gerhardt, A., Ingram, M. K., Kang, I. J., & Ulitzur, S. (2006). In situ on-line toxicity biomonitoring in water: Recent developments. *Environmental Toxicology and Chemistry*, *25*(9), 2263–2271. https://doi.org/10.1897/05-486R1.1

Gibbins, C. S., Vericat, D., & Batalla, R. J. (2007). When is stream invertebrate drift catastrophic? The role of hydraulics and sediment transport in initiating drift during flood events. *Freshwater Biology*, *52*(12), 2369–2384. https://doi.org/10.1111/j.1365-2427.2007.01858.x

Hartmann, J. T., Beggel, S., Auerswald, K., Stoeckle, B. C., & Geist, J. (2016). Establishing mussel behaviour as a biomarker in ecotoxicology. *Aquatic Toxicology*, *170*, 279–288. https://doi.org/10.1016/j.aquatox.2015.06.014

Hassan, B., Qadri, H., Ali, M. N., Khan, N. A., & Yatoo, A. M. (2020). Impact of climate change on freshwater ecosystem and its sustainable management. In H. Qadri, R. Bhat, M. Mehmood, & G. Dar (Eds.), *Fresh water pollution dynamics and remediation* (pp. 105–121). Springer Nature Singapore Pte Ltd. Book chapter. https://doi.org/10.1007/978-981-13-8277-2_7

Hoffman, A. L., Olden, J. D., Monroe, J. B., LeRoy Poff, N., Wellnitz, T., & Wiens, J. A. (2006). Current velocity and habitat patchiness shape stream herbivore movement. *Oikos*, *115*(2), 358–368. https://doi.org/10.1111/j.2006.0030-1299.14675.x

Hornbach, D. J., Kurth, V. J., & Hove, M. C. (2010). Variation in freshwater mussel shell sculpture and shape along a river gradient. *The American Midland Naturalist*, *164*(1), 22–36. https://doi.org/10.1674/0003-0031-164.1.22

Howard, J. K., & Cufey, K. M. (2006). The functional role of native freshwater mussels in the fluvial benthic environment. *Freshwater Biology*, *51*(3), 460–474. https://doi.org/10.1111/j.1365-2427.2005.01507.x

IPCC. (2012). *Managing the risks of extreme events and disasters to advance climate change adaptation a special report of working groups I and II of the intergovernmental panel on climate change Ed. CB Field et al (Cambridge)* (p. 582). Cambridge University Press.

IPCC. (2014). Summary for policymakers In: climate change 2014: Impacts, adaptation, and vulnerability. In *Part A: Global and sectoral aspects. Contribution of working group II to the fifth assessment report of the intergovernmental panel on climate change ed CB Field et al. (Cambridge)* (pp. 1–32). Cambridge University Press.

Janetski, D. J., Chaloner, D. T., Tiegs, S. D., & Lamberti, G. A. (2009). Pacific salmon effects on stream ecosystems: A quantitative synthesis. *Oecologia*, *159*(3), 583–595. https://doi.org/10.1007/s00442-008-1249-x

Jeffrey, D. W. & Madden, B. (Eds.) (1991)., *Bioindicators and environmental management*. Academic Press.

Jodhani, K. H., Patel, D., & Madhavan, N. (2023). A review on analysis of flood modelling using different numerical models. *Materials Today: Proceedings*, *80*, 3867–3876. https://doi.org/10.1016/j.matpr.2021.07.405

Kramer, K. J. M., & Foekema, E. M. (2001). The "Musselmonitor®" as biological early warning system. In F. M. Butterworth, A. Gunatilaka, & M. E. Gonsebatt (Eds.), *Biomonitors and biomarkers as indicators of environmental change 2*, *Environmental science research* (Vol. 56, pp. 59–87). Springer. https://doi.org/10.1007/978-1-4615-1305-6_4

Kramer, K. J. M., Jenner, H. A., & de Zwart, D. (1989). The valve movement response of mussels: A tool in biological monitoring. *Hydrobiologia*, *188*(1), 433–443. https://doi.org/10.1007/bf00027811

Ladeiro, M. P., Barjhoux, I., Bigot-Clivot, A., Bonnard, M., David, E., Dedourge-Geffard, O., et al. (2017). Mussel as a tool to define continental watershed quality. In *Chapter 13 from the book organismal and molecular malacology*. Retrieved from http://www.intechopen.com/books/organismal-and-molecularmalacology

Lamarck, J.-B. P. A. D. M. (1815). Histoire naturelle des animaux sans vertèbres … précédée d'une introduction offrant la determination des caractères essentiels de l'animal, sa distinction du vegetal et des autres corps naturels, enfin, l'exposition des principes fondamentaux de la zoologi (Vol. *t.1*). published by the author Retrieved from https://www.biodiversitylibrary.org/item/47694

Lamond, J., & Penning-Rowsell, E. (2014). The robustness of flood insurance regimes given changing risks resulting from climate change. *Climate Risk Management*, *2*(2014), 1–10. https://doi.org/10.1016/j.crm.2014.03.001

Lara, G., & Parada, E. (2009). Substrate selection by the freshwater mussel *Diplodon chilensis* (Gray, 1828): Field and laboratory experiments. *Journal of Molluscan Studies*, *75*(2), 153–157. https://doi.org/10.1093/mollus/eyp006

Lazzarin, T., Constantinescu, G., Di Micco, L., Wu, H., Lavignani, F., Lo, B. M., et al. (2023). Influence of bed roughness on flow and turbulence structure around a partially-buried, isolated freshwater mussel. *Water Resources Research*, *59*(4), e2022WR034151. https://doi.org/10.1029/2022WR034151

Lopes-Lima, M., Sousa, R., Geist, J., Aldridge, D. C., Araujo, R., Bergengren, J., et al. (2017). Conservation status of freshwater mussels in Europe: State of the art and future challenges. *Biological Reviews*, *92*(1), 572–607. https://doi.org/10.1111/brv.12244

Lopez, J. W., & Vaughn, C. C. (2021). A review and evaluation of the effects of hydrodynamic variables on freshwater mussel communities. *Freshwater Biology*, *66*(9), 1665–1679. https://doi.org/10.1111/fwb.13784

Mao, L. (2018). The effects of flood history on sediment transport in gravel-bed rivers. *Geomorphology*, *322*, 196–205. https://doi.org/10.1016/j.geomorph.2018.08.046

Micu, A. (2020). In Poznan, Poland, eight clams get to decide if people in the city get water or not. *ZME Science*. https://www.zmescience.com/science/poznan-mussel-water-plants-892524/

Miller, A. C., Payne, B. S., & Shaffer, L. R. (1999). A shell gape monitor to study effects of physical disturbance on freshwater mussels. *Journal of Freshwater Ecology*, *14*(2), 241–247. https://doi.org/10.1080/02705060.1999.9663675

Mishra, P., Pandey, C. M., Singh, U., Gupta, A., Sahu, C., & Keshri, A. (2019). Descriptive statistics and normality tests for statistical data. *Annals of Cardiac Anaesthesia*, *22*(1), 67–72. https://doi.org/10.4103/aca.ACA_157_18

Modesto, V., Tosato, L., Pilbala, A., Benistati, N., Fraccarollo, L., Termini, D., et al. (2023). Mussel behaviour as a tool to measure the impact of hydraulic stressors. *Hydrobiologia*, *850*(4), 1–14. https://doi.org/10.1007/s10750-022-05126-x

Moyle, P. B., & Mount, J. F. (2007). Homogenous rivers, homogenous faunas. *Proceedings of the National Academy of Sciences*, *104*(14), 5711–5712. https://doi.org/10.1073/pnas.0701457104

Nogueira, J. G., Lopes-Lima, M., Varandas, S., Teixeira, A., & Sousa, R. (2021). Effects of an extreme drought on the endangered pearl mussel Margaritifera margaritifera: A before/after assessment. *Hydrobiologia*, *848*(12–13), 3003–3013. https://doi.org/10.1007/s10750-019-04103-1

Novais, A., Pascoal, C., & Sousa, R. (2017). Effects of invasive aquatic carrion on soil chemistry and terrestrial microbial communities. *Biological Invasions*, *19*(8), 2491–2502. https://doi.org/10.1007/s10530-017-1459-4

Olokeogun, O. S., Ayanlade, A., & Popoola, O. O. (2020). Assessment of riparian zone dynamics and its flood-related implications in Eleyele area of Ibadan, Nigeria. *Environmental Systems Research*, *9*(1), 6. https://doi.org/10.1186/s40068-020-00167-4

Perles, S. J., Christian, A. D., & Berg, D. J. (2003). Vertical migration, orientation, aggregation, and fecundity of the freshwater mussel *Lampsilis siliquoide*. *Ohio Journal of Science*, *103*(4), 73–78.

Pfeiffer, C. (1825). Naturgesch. Dtsch. Land-Süßw. Moll. 2. Retrieved from https://www.biodiversitylibrary.org/page/52682547#page/175/mode/1up

Pilbala, A., Riccardi, N., Benistati, N., Modesto, V., Termini, D., Manca, D., et al. (2024). Real-time biological early warning system based on freshwater mussels' valvometry data. *HESS*, *28*(10), 2297–2311. https://doi.org/10.5194/hess-28-2297-2024

Pivato, M., Carniello, L., Moro, I., & D'Odorico, P. (2019). On the feedback between water turbidity and microphytobenthos growth in shallow tidal environments. *Earth Surface Processes and Landforms*, *44*(5), 1192–1206. https://doi.org/10.1002/esp.4567

Poff, N. L., Allan, J. D., Bain, M. B., Karr, J. R., Prestegaard, K. L., Richter, B. D., et al. (1997). The natural flow regime. *BioScience*, *47*(11), 769–784. https://doi.org/10.2307/1313099

Poznańska-Kakareko, M., Wiśniewski, K., Szarmach, D., Witkowska, A., Kakareko, T., Jermacz, Ł., & Kobak, J. (2021). Importance of substratum quality for potential competitive niche overlap. *Science of the Total Environment*, *799*, 10. https://doi.org/10.1016/j.scitotenv.2021.149345

Premalatha, P., Saravanan, K., & Karuppannan, P. (2020). Freshwater Mussels (Bivalvia: Unionoida) as a biological and water quality indicator: A review. *Ecology Environment and Conservation*, *26*(2), 809–812.

Prie, V., Molina, Q., & Gamboa, B. (2014). French naiad (Bivalvia: Margaritiferidae,unionidae) species distribution models: Prediction maps as tools for conservation. *Hydrobiology*, *735*(1), 81–94. https://doi.org/10.1007/s10750-013-1597-3

Quadroni, S., Crosa, G., Gentili, G., & Espa, P. (2017). Response of stream benthic macroinvertebrates to current water management in Alpine catchments massively developed for hydropower. *Science of the Total Environment*, *609*, 484–496. https://doi.org/10.1016/j.scitotenv.2017.07.099

Randall, D. A., Wood, R. A., Bony, S., Colman, R., Fichefet, T., Fyfe, J., et al. (2007). Climate models and their evaluation. In S. Solomon, D. Qin, M. Manning, Z. Chen, M. Marquis, K. B. Averyt, et al. (Eds.), *Climate change 2007: The physical science basis. Contribution of working group I to the fourth assessment report of the intergovernmental panel on climate change*. Cambridge University Press.

Riccardi, N., Aksenova, O., Bo, T., Bogan, A. E., Haag, W. R., Konopleva, E., et al. (2022). Bad taxonomy and poor detectability has hindered Italian *Unio* conservation. In *The case of* Unio elongatulus *and* Unio mancus*: What is their real conservation status? Tentacle* (Vol. 30).

Robson, A. A., Garcia, D. L. C., Wilson, R. P., & Halsey, L. G. (2010). Behavioural adaptations of mussels to varying levels of food availability and predation risk. *Journal of Molluscan Studies*, *76*(4), 348–353. https://doi.org/10.1093/mollus/eyq025

Sansom, B. J., Atkinson, J. F., & Bennett, S. J. (2018). Modulation of nearbed hydrodynamics by freshwater mussels in an experimental channel. *Hydrobiologia*, *810*(1), 449–463. https://doi.org/10.1007/s10750-017-3172-9

Silverman, H. E. C., Achberger, J. W. L., & Dietz, T. H. (1995). Filtration and utilization of laboratory-cultured bacteria by Dreissena polymorpha, Corbicula fluminea, and Caranculina texasensis. *Biology Bulletin*, *189*, 308–319.

Simpson, M. R. (2001). *Discharge measurements using a broad-band acoustic doppler current profiler*. US Geological Survey.

Singh, G., Singh, A., Singh, P., & Mishra, V. K. (2021). Impact of climate change on freshwater ecosystem. In *Water conservation in the era of global climate change – Chapter 4* (pp. 73–98). Elsevier.

Sousa, R., Dias, S. C., Guilhermino, L., & Antunes, C. (2008). Minho River tidal freshwater wetlands: Threats to faunal biodiversity. *Aquatic Biology*, *3*, 237–250. https://doi.org/10.3354/ab00077

Sousa, R., Zając, T., Halabowski, D., Aksenova, O. V., Bespalaya, Y. V., Carvalho, F., et al. (2023). A roadmap for the conservation of freshwater mussels in Europe. *Conservation Biology*, *37*(2), e13994. https://doi.org/10.1111/cobi.13994

Stähly, S., Franca, M. J., Robinson, C. T., & Schleiss, A. J. (2019). Sediment replenishment combined with an artificial flood improves river habitats downstream of a dam. *Scientific Reports*, *9*(1), 5176. https://doi.org/10.1038/s41598-019-41575-6

Strayer, D. L. (1999). Effects of alien species on freshwater mollusks in North America. *Journal of the North American Benthological Society*, *18*(1), 74–98. https://doi.org/10.2307/1468010

Strayer, D. L., Caraco, N. F., Cole, J. J., Findlay, S., & Pace, M. L. (1999). Transformation of freshwater ecosystems by bivalves: A case study of zebra mussels in the Hudson River. *BioScience*, *49*(1), 19–27. https://doi.org/10.2307/1313490

Strayer, D. L., & Dudgeon, D. (2010). Freshwater biodiversity conservation: Recent progress and future challenges. *Journal of the North American Benthological Society*, *29*(1), 344–358. https://doi.org/10.1899/08-171.1

Sukhodolov, A. N. (2015). Field-based research in fluvial hydraulics: Potential, paradigms and challenges. *Journal of Hydraulic Research*, *53*(1), 1–19. https://doi.org/10.1080/00221686.2015.1012126

Termini, D. (2021). Investigation of a Gravel-Bed River's pattern changes: Insights from satellite images. *Applied Sciences*, *11*(5), 2103. https://doi.org/10.3390/app11052103

Termini, D. (2024). Data figures 3,4,5,6,7,8 [Dataset]. *Zenodo*. https://doi.org/10.5281/zenodo.14330718

Termini, D., Benistati, N., Tosato, L., Pilbala, A., Modesto, V., Fraccarollo, L., et al. (2023). Identification of hydrodynamic changes in rivers by means of freshwater mussels' behavioural response: An experimental investigation. *Ecohydrology*, *17*(4), e2544. https://doi.org/10.1002/eco.2544

Tesfaye, T. W., Dhanya, C. T., & Gosain, A. K. (2020). Modeling the impact of climate change on the environmental flow indicators over Omo-Gibe basin. *Ethiopia Modeling Earth Systems and Environment*, *6*(4), 2063–2089. https://doi.org/10.1007/s40808-020-00813-x

Tran, D., Ciret, P., Ciutat, A., Durrieu, G., & Massabuau, J. C. (2003). Estimation of potential and limits of bivalve closure response to detect contaminants: Application to cadmium. *Environmental Toxicology and Chemistry*, *22*(4), 914–920. https://doi.org/10.1002/etc.5620220432

Vanzo, D., Zolezzi, G., & Siviglia, A. (2016). Eco-hydraulic modelling of the interactions between hydropeaking and river morphology. *Ecohydrology*, *9*(3), 421–437. https://doi.org/10.1002/eco.1647

Vaughn, C. C., Atkinson, C. L., & Julian, J. P. (2015). Drought-induced changes in flow regimes lead to long-term losses in mussel-provided ecosystem services. *Ecology and Evolution*, 1–14.

Vaughn, C. C., & Hakenkamp, C. C. (2008). The functional role of burrowing bivalves in freshwater ecosystems. *Freshwater Biology*, *46*(11), 1431–1446. https://doi.org/10.1046/j.1365-2427.2001.00771.x

Vereycken, J. E., & Aldridge, D. C. (2022). Bivalve molluscs as biosensors of water quality: State of the art and future directions. *Hydrobiologia*, *850*(2), 231–256. https://doi.org/10.1007/s10750-022-05057-7

Wallace, J. B., & Webster, J. R. (1996). The role of macroinvertebrates in stream ecosystem function. *Annual Review of Entomology*, *41*(1), 115–139. https://doi.org/10.1146/annurev.en.41.010196.000555

Wohl, E., Bledsoe, B. P., Jacobson, R. B., Poff, N. L., Rathburn, S. L., Walters, D. M., & Wilcox, A. C. (2015). The natural sediment regime in rivers: Broadening the foundation for ecosystem management. *BioScience*, *65*(4), 358–371. https://doi.org/10.1093/biosci/biv002

Woodward, G., Perkins, D. M., & Brown, L. E. (2010). Climate change and freshwater ecosystems: Impacts across multiple levels of organization. *Philosophical Transactions of the Royal Society B*, *365*(1549), 2093–2106. https://doi.org/10.1098/rstb.2010.0055

Wu, H., Costantinescu, G., & Zeng, J. (2020). Flow entrainment mechanics around a freshwater mussel aligned with the incoming flow. *Water Resources Research*, *56*, 1–24. https://doi.org/10.1029/2020WR027983

Yalin, M. S. (1992). *River mechanics*. Pergamon Press.

Zhang, J., Xu, M., Huber, B., Grunzner, M., & Blanckaert, K. (2025). Roughness and energy losses induced by mussel growth on the walls of hydraulic structures and application to a water transfer project. *Water Resources Research*, *61*(1), e2023WR036503. https://doi.org/10.1029/2023WR036503

Zigler, S. J., Newton, T. J., Steuer, J. J., Bartsch, M. R., & Sauer, J. S. (2008). Importance of physical and hydraulic characteristics to unionid mussels: A retrospective analysis in a reach of large river. *Hydrobiologia*, *598*(1), 343–360. https://doi.org/10.1007/s10750-007-9167-1