Marine Environmental Research 213 (2026) 107641

Contents lists available at ScienceDirect

Marine Environmental Research

journal homepage: www.elsevier.com/locate/marenvrev

"The hidden Filter": Quantifying the ecological role of *Lithophaga lithophaga* (Linnaeus, 1758) in Mediterranean benthic-pelagic processes

T. Marrocco [a], A. Petrucciani [a,b,*], B. Calcinai [a], S. Puce [a], C. Cerrano [a,c,d,e], A. Norici [a]

[a] Department of Life and Environmental Sciences, Università Politecnica delle Marche, Via Brecce Bianche s.n.c., 60131, Ancona, Italy
[b] Interuniversity Consortium on Chemical Reactivity and Catalysis (CIRCC), Via Celso Ulpiani, 27, 70126, Bari, Italy
[c] National Biodiversity Future Center (NBFC), Piazza Marina 61, 90133, Palermo, Italy
[d] Stazione Zoologica di Napoli Anton Dohrn, Villa Comunale, 80122, Napoli, Italy
[e] CoNISMa, Piazzale Flaminia 9, 00196, Roma, Italy

A B S T R A C T

The current impact of global warming in marine habitats is rapidly altering ecosystem functioning at different levels. Marine heat wave and massive mortality events are compromising the survivor of active and passive filter feeders. Nevertheless, the scarcity of data on the filtration rate and selectivity of ecologically crucial understudied filter feeders is compromising our understanding of the consequences. The endolithic bivalve *Lithophaga lithophaga* has been largely overlooked in this context. Because of this, we experimentally assessed its feeding behaviour by measuring clearance rates with two diatom species (*Chaetoceros muelleri* and *Conticribra weissflogii*) and a control microalga (*Isochrysis galbana*) under different concentrations (high $1.1{\cdot}10^6$ cells/ml and low $2.5{\cdot}10^5$ cells/ml) and across two *L lithophaga* size classes. On average, commonly sized individuals (~8 g) exhibited clearance rates of 0.24 L $h^{-1}{\cdot}g^{-1}$, suggesting that dense populations (>100 ind·m^{-2}) could filter up to 192 L $h^{-1}{\cdot}m^{-2}$. Notably, we observed for the first time the production of pseudofeces by *L. lithophaga* when fed diatoms, especially at high concentrations, whereas *I. galbana* was almost entirely ingested, indicating pre-ingestive selection of particles. Moreover, pseudofeces analysis revealed enrichment in silica and carbohydrates, pointing to active rejection of frustule-bearing cells and involvement in the biogenic silica cycle in Mediterranean ecosystems. These findings confirm the ecological role of *L. lithophaga* as a relevant filter feeder, contributing to particulate organic matter flux and nutrient cycling. This underscores the importance to broad *L. lithophaga* future conservation and restoration actions in a changing ocean, since its crucial involvement in ecosystem functioning.

1. Introduction

Benthic-pelagic coupling plays a prominent role in aquatic ecosystems, allowing the exchange of energy, mass or nutrients from the pelagic to the benthic domain. This process is particularly important for shallow marine ecosystems, where substantial amounts of energy are accumulated on the substrate (Griffiths et al., 2017; Kiljunen et al., 2020). Here, organic matter is consumed by demersal and benthic organisms, contributing to the biogeochemical cycles of carbon, nitrogen, phosphorus and silicon, while strongly modulating species and community composition (Schnack-Schiel and Isla, 2005; Soetaert et al., 2000). Nutrient release from the benthos and sediment-water interactions subsequently recycle energy back to the pelagic domain, structuring trophic networks and ultimately regulating ecosystem stability (Kopp et al., 2015; Ricci et al., 2022). In fact, the physical and biological processes regulating the transfer of organic matter between benthic and pelagic habitats are influenced by particle sinking rates and aggregation processes that facilitate exchange between domains and promote the transfer of high-quality organic matter to benthic areas (Griffiths et al., 2017; Schnack-Schiel and Isla, 2005). Filter-feeding organisms such as poriferans, cnidarians, molluscs, polychaetes, and ascidians significantly influence the surrounding water column and modify the quality of material reaching the seafloor through biodeposition. Bivalves, in particular, demonstrate remarkable filtration rates of up to 19 l h^{-1} and produce biodeposits called pseudofeces, providing benthic habitats with particles containing phosphorus, nitrogen, and silica of biogenic origin (Riisgård, 2001; Van et al., 2015). Climate change is accelerating biodiversity loss, with global warming driving an increase in mass mortality events (MMEs) among both active and passive filter feeders (Garrabou et al., 2022). These events, defined as the rapid catastrophic die off of organisms (Fey et al., 2019), threaten the survival of key species in aquatic ecosystems and may disrupt essential ecosystem functions (Fey et al., 2019; Jones et al., 2017). However, predicting their cascading effects remains challenging due to a lack of quantitative data on the filtering capacity of many ecologically important, yet understudied, filter feeding bivalves (Jones et al., 2017). Indeed the Mediterranean Sea hosts more than 350 species of bivalves (Renda et al., 2022); the role of some of them in the functional coastal

* Correspondent author. Department of Life and Environmental Sciences, Università Politecnica delle Marche, Via Brecce Bianche s.n.c., 60131, Ancona, Italy.
E-mail address: a.petrucciani@staff.univpm.it (A. Petrucciani).

https://doi.org/10.1016/j.marenvres.2025.107641
Received 11 August 2025; Received in revised form 7 October 2025; Accepted 19 October 2025
Available online 21 October 2025

benthic ecosystems have long been recognized (e.g. mussels and clams) providing data on different filter-feeding behaviors and mechanisms such as the formation of acid mucopolysaccharide tubes (AMPS) (Van et al., 2015; Beninger et al., 1999). Nonetheless, some species remain relatively understudied despite their ecological importance. Among these overlooked species, *Lithophaga lithophaga* (Linnaeus, 1758) is widely distributed along the entire Mediterranean coastline, yet remains relatively understudied despite being observed since ancient times (Fraschetti et al., 2001; Devescovi and Iveša, 2008). This species exhibits an endolithic lifestyle, excavating cavities into calcareous rocky surfaces (Kleemann, 1974). Although the erosive activity of boring bivalves has been described as capable of creating spatial heterogeneity exploited by multiple organisms (e.g., Polychaeta, Malacostraca, Maxillopoda, Bivalvia, Actinopterygii) (Fraschetti et al., 2001; Bagur et al., 2019; Colletti et al., 2020; Fanelli et al., 1994), the role of *L. lithophaga* playing part in benthic-pelagic processes through its feeding behaviour has largely been overlooked. Given the multiple anthropogenic threats to this protected species, ranging from illegal fishing to climate change, deepening our understanding of their filter-feeding behaviour is essential to clarify their ecological role within the food web and to assess the potential consequences of their decline on ecosystem functioning (Colletti et al., 2020). Such knowledge is critical for developing more effective conservation strategies and management plans also for the associated community. In the assessment of filtering capacity, primary food source for filter feeders must be included; diatoms, foundational components of aquatic ecosystem are well known in bivalve diet (Fanelli et al., 1994). As major primary producers in both coastal and open ocean ecosystems, diatoms play a critical role in the transfer of energy, nutrients, and organic matter between the water column and sediments, helping sustain both pelagic and benthic food webs Their ability to exploit silicic acid to build their silica shell (frustule) combined with their tendency to form aggregates, contributes to their rapid sinking and makes them key agents in the export of both silicon and carbon to the ocean floor (Tréguer et al., 2018).

The aim of this study was to evaluate the clearance rate of *L. lithophaga*, by measuring the reduction in the total number of provided diatom cells - specifically *Chaetoceros muelleri* (Lemmermann, 1898) and *Conticribra weissflogii* (Grunow) (K. Stachura-Suchoples & D. M. Williams, 2009), which are representative of the phytoplankton community in the water column (Totti et al., 2019; Siokou-Frangou et al., 2010). The choice of testing the clearance rate was mainly made to avoid animal sacrifice, since the clearance rate only considers the decrease in particles in the water column, rather than the particles that have been effectively metabolized, as is the case with the filtration rate (Riisgård, 2001). By complementing the experiment with an analysis of the biodeposits produced by the animals, results can provide valuable insights into how *L. lithophaga* may influence the availability and vertical distribution of organic matter within the marine environment. These aspects have been studied for many freshwater and marine bivalve species, but no data are available for *L. lithophaga* (Prins et al., 1994; Riisgård et al., 2003). Three younger (smaller) organisms and three older (larger) ones were selected to compare clearance activity between age groups. Moreover, two different concentrations of diatom suspensions were compared to a known commercial microalgal species used for bivalve feeding *Isochrysis galbana,* Parke, 1949 (Camacho-Rodríguez et al., 2020), to test differences in feeding behaviour based on *L. lithophaga* food selection.

2. Materials and methods

2.1. Sampling method

The Conero Promontory, where the sampling was conducted, represents an important naturalistic emergency in the upper Adriatic Sea, as it is characterised by a hard substrate in an otherwise sandy coastline (Calcinai et al., 2011; Pulido Mantas et al., 2022). The calcareous, rocky substrate that characterises the Promontory is subject to the continuous action of boring organisms which can increase the biodiversity of the area (Calcinai et al., 2011). These boring organisms mainly include sponges of the genus *Cliona*, polychaetes of the genus *Polydora* and numerous bivalves including *Lithophaga lithophaga* (Calcinai et al., 2011; Pulido Mantas et al., 2022). Due to recent evidence of illegal fishing of *L. lithophaga* in the area, which may undermine the health status of the local population, and the benthic assemblages' integrity of the Conero Promontory, only exposed or partially exposed shells were collected to avoid further damage to the natural environment (permission n. 0019895 by Italian Ministry of the Environment) (Pulido Mantas et al., 2023). The samples were collected, from the same population, with hands by SCUBA diving operators in Passetto area (43°37′01.0"N, 13°32′06.5"E; Ancona, Italy), and kept in the aquarium for acclimation period of 2 weeks before the start of the experiment (Thompson et al., 2012). The permit granted by the Ministry of the Environment authorized the collection of only a very limited number of individuals. For this reason, we collected the minimum number necessary to ensure valid statistical analysis.

2.2. Experimental set up

To assess the clearance rate of *L. lithophaga*, the feeding behaviour of the animals under controlled laboratory condition was evaluated. Three younger individuals (~30 mm in length, smaller) and three older ones (between 40 and 50 mm in length, larger) were selected based on shell length (Peharda et al., 2015). Each specimen was kept in a separate beaker filled with 1.5L of 0.22 μm filtered seawater (20 C°, 35.193 PSU, pH 8.4) with an oxygen aerator. Individuals were starved for 3 days prior the experiment to ensure complete elimination of fecal pellets and deposits related to prior feeding (Kuehr et al., 2021; De et al., 1976). On the 3rd day, each sample was fed with a fixed amount of microalgal suspension; since the filtration rate of mussels varies with the concentration of algae (Riisgård et al., 2003), the treatment was carried out with a final cell suspension ranging from $1.5{\cdot}10^5$ to $1{\cdot}10^6$ cells/ml for the high concentration treatment and from $5{\cdot}10^4$ to $2.5{\cdot}10^5$ cells/ml for the low concentration treatment. Results were compared to control beaker in which no animals were present; the scheme of the experiment was resumed in Fig. 1.

Microalgae selected for the experiment were two planktonic diatom species (to avoid short retaining time in the water column), *Chaetoceros muelleri* (CCAP 1010/3) and *Conticribra weissflogii* (DCG 0320), and one haptophyte, *Isochrysis galbana* (RCC 1353). *L. lithophaga* is able to filter objects with peculiar size based on the gills' morphology and previous studies on Mytilidae, efficiently retaining particles from >4 μm an up to 37 μm (AkşiT et al., 2014; Evan Ward and Shumway, 2004). For this reason, all the selected species has a size bigger than 4 μm to maximise the percentage of filtered microalgae. Diatom species were selected due to their ecological predominance in the coastal ecosystems while *I. galbana* due to its relevance as commercial feed ingredient (Totti et al., 2019; Siokou-Frangou et al., 2010; Camacho-Rodríguez et al., 2020).

2.3. Clearance rate determination

To assess the clearance rate, the decrease in the total amount of microalgal cells in the water column was calculated. Triplicate samples of water from each beaker were taken immediately after the microalgae addition (T0) and after 0.25–0.5 – 1–2 – 3–4 – 5 h; the last samples were taken after 16 h from the initial supply. Cell number in the water samples was measured using a CASY® TT Cell Counter (Innovatis AG, Reutlingen, Germany) following the procedure illustrated by Petrucciani et al. (2022). Aliquots of 80 μl of seawater were diluted in 8 ml of an electrolyte solution (CASY® TON; Innovatis AG). Cells were pumped into the cell counter through a 150 μm capillary at a constant flow, and the number of cells was determined through the enumeration of events measured as change in conductivity. The same instrument was

Fig. 1. Experimental set-up. A total of 6 specimens of *L. lithophaga* were selected to have three younger organisms and three older ones. All of them were fed with *Chaetoceros muelleri*, *Conticribra weissflogii* and *Isochrysis galbana* at two different concentrations, giving a total of 6 independent treatments.

also used to measure the cellular size as the volume of electrolyte solution displaced by the passage of cells through a measuring pore (Palmucci et al., 2011). The numbers obtained from the cell counts were then fitted using a non-linear regression equation called exponential decay **(1):**

$$N(t) = N_0 \cdot e^{-kt} \quad (1)$$

where N(t) is the resulting number of cells at time t, N_0 is the initial number of cells and K is the resulting decay constant expressed as $\frac{1}{hours}$. K decay constants were then used to calculate clearance rate among the analysed conditions (Table S1): these values were multiplied per the total amount of seawater in the beakers (1.5L) and divided per grams of each specimen. Weight of animals were estimated from shell length according to Devescovi (2009) (Table S2) avoiding the animal sacrifice. Thus, clearance rate efficiency was defined as the ratio between the rate of filtered water per gram of animal (Gosling, 2003).

2.4. Pseudofeces and feces analysis

At the end of the experiment (after 24 h), the feces and pseudofeces produced by the animals were collected and analysed once they had been correctly distinguished. Pseudofeces are light, cloud-like, deposits, whereas feces are densely packed (Galimany et al., 2018). Visible pseudofeces were transferred with a plastic Pasteur pipette to a falcon tube and all the seawater of the beakers was filtered with a mesh size of 30 μm to collect all the fecal pellets. Once obtained, the material was washed twice with a 0.5M solution of ammonium formate to remove the seawater salts and used for quantification. A fixed amount of fecal/-pseudofecal suspension was dried in pre-weighted tubes at 80 °C till a stable dry weight was attained.

Pseudofeces deriving from animals fed with diatoms were further analysed to assess their organic and inorganic composition. Since a negligible amount of pseudofeces were produced when the animals were fed with *I. galbana,* this group was excluded from the analyses. Aliquots of the suspensions were transferred to a silicon window and dried at 80 °C to acquire FTIR spectra with a Tensor 27 FTIR spectrometer (Bruker Optics, Ettlingen, Germany). Spectra were normalized on the common peak of amide I (~1,650 cm^{-1}) and the relative abundances of lipids, carbohydrates, proteins, and silica were observed after bands assignment to cellular pools (Palmucci et al., 2011). The absolute abundance of silicon in pseudofeces was measured using a total reflection X-ray fluorescence spectrometer (S2 PICOFOX®, Bruker AXS® Microanalysis GmbH, Berlin, Germany) and compared to those in living diatoms following the procedure described by Petrucciani and coauthors in 2022 (Petrucciani et al., 2022). Both sampled diatoms and pseudofeces were washed twice with an ammonium formate solution isosmotic to the culturing media and resuspended in 250 μl of dH_2O. A solution of 0.1 g l^{-1} Ga (Sigma-Aldrich, St. Luis, MO, USA) in 5 % HNO_3 was added as internal standard to a final concentration of 0.5 ml l^{-1}. The suspension was carefully vortexed, and an aliquot of 10 μl was deposed on a plastic sample holder, dried on a heating plate, and measured for 1,000

s. Spectral deconvolution and quantification of elemental abundances were performed by the Spectra 6.1 software (Bruker AXS Microanalysis GmbH, Berlin, Germany).

2.5. Statistical analysis

A two-way analysis of variance (ANOVA) was applied to look for significant differences among the means of dependent variables (i.e. clearance rate and Si content in pseudofeces) in different concentration of algal suspension (independent variable) and for each *L. lithophaga* size (independent variable). The analysis was followed by Tukey's *post-hoc* test and the level of significance was set at 0.05. GraphPad prism 8.0.2.263 was used to perform the tests (GraphPad Software, San Diego, CA, United States). Multivariate permutational analyses of variance (PERMANOVA) (Anderson et al., 2008), tested hypothesis about species- and concentration-selection of particles and *L. lithophaga* size affecting clearance rate. PERMANOVA was run setting 9,999 permutations with Bray–Curtis dissimilarity matrices that were produced on untransformed matrices and used (i) concentration of nutrients (high; low), (ii) *L. lithophaga* size (large; small) and (iii) microalgal species (*C. mullerei; C. weissflogii; I. galbana*) as fixed factors. Additionally, data were visualized using non-metric multi-dimensional scaling (nMDS) on the distances among relevant centroids using 'bubble' plot option.

3. Results

3.1. Clearance rate determination

The clearance rate of *L. lithophaga* was determined across different species and concentrations of microalgal suspensions. The study revealed an average clearance rate of 0.24 ± 0.18 L $h^{-1}{\cdot}g^{-1,}$ with the highest rate observed in smaller individuals fed with a low concentration of *C. muelleri*, reaching 1.2 L $h^{-1}{\cdot}g^{-1}$ (Fig. 2, Fig. S1, Table S1).

Results highlighted smaller bivalves showing a higher clearance rate as compared to larger ones, significantly evident when specimens were fed with low concentration of *I. galbana* ($p = 0.01$) (Fig. 2). Although not statistically significant, animals fed with *C. muelleri* showed higher clearance rate when lower concentration of suspension was supplied (Fig. 2). In contrast to the other species, treatments with *C. weissflogii* resulted in more consistent clearance rate values.

3.2. Pseudofeces and feces analysis

The amount of pseudofeces released by *L. lithophaga* varied depending on the species and concentration of microalgae provided. However, the size of the animals did not appear to influence the proportion of particles expelled. When fed with *C. muelleri* at high concentration, both smaller and larger organisms produced greater amount of pseudofeces, releasing ~70 % of filtered cells as biodeposits (indicated by black segment of the rings in Fig. 3). In contrast, when C. *muelleri* was supplied at low concentration, the production of pseudofeces dropped significantly, amounting to only about ~5 %, 1/5 of that released at high concentration. In the case of the other diatom *C. weissflogii*, the quantity of biodeposits remained consistent regardless of supplied concentration, with both low and high treatments resulting in the release of approximately 30 % of the filtered cells as pseudofeces. Notably, when *L. lithophaga* was fed with *I. galbana* at both concentrations, pseudofeces production was negligible in comparison to the two diatoms examined (Fig. 3). Indeed, at high concentration, only about 2 % of the filtered cells were expelled as biodeposits, and this value dropped to 0 % when the algal suspension was supplied at a lower concentration (Fig. 3).

The non-metric multi-dimensional scale (nMDS) plots (Fig. 4) pictured the spatial distribution of clearance rate and pseudofeces produced data based on Bray–Curtis similarity among treatments with different microalgal species. Moreover, in Fig. 4B the area of the dots is proportional to both these dependent variables; thus, wider is the area, higher is the clearance rate and/or pseudofeces weight produced. Data points clearly clustered according to microalgal species provided: in particular, specimens fed with *I. galbana* formed a distinct group that was statistically different from that fed with diatoms at both high (H) and low (L) concentrations (Fig. 4A, Bray–Curtis dissimilarity, $p < 0.05$). Specimens fed with *C. muelleri* did not form a homogeneous group; instead, a separation was observed between high and low concentration treatments. Notably, data from animals fed with high concentrations of *C. muelleri* clustered with those fed with *C. weissflogii* (Fig. 4A, Bray–Curtis dissimilarity, $p < 0.05$; Table S3); in particular, bubble blot size indicates a high production of pseudofeces with relative low clearance rate (Fig. 4B). On the other hand, data related to organisms fed with *C. muelleri* at low concentration and *I. galbana* at high and low concentration showed wider clearance rate with lower pseudofeces produced, indicating that these specific combinations of food type and concentration promoted efficient clearance (Fig. 4B).

Since pseudofeces production in diatoms was abundant, FTIR analysis was conducted on these biodeposits and compared to spectra from the cultured microalgal suspension. All spectra were normalized to a common peak at 1,650 cm^{-1}, associated with the protein pool, to facilitate comparison of other spectral features. Notably, both red lines representing pseudofeces exhibited increased peak intensity in the 900–1200 cm^{-1} range (Fig. 5), relative to the blue lines representing the microalgal suspension. This region is typically attributed to the carbohydrate pool (Palmucci et al., 2011). However, in diatoms, absorbance

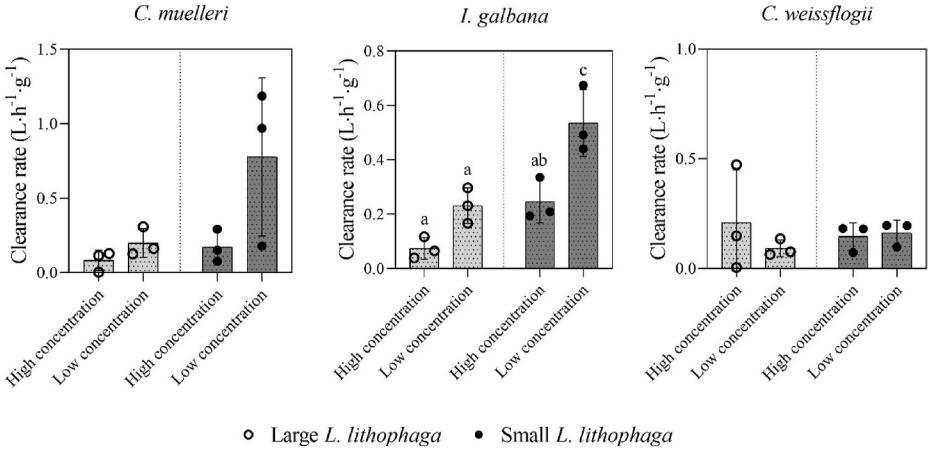

Fig. 2. Bar plot showing clearance rates, reported as liters per hour per gram of animal. Larger specimens are represented with white dots and light grey bars while smaller specimens are represented with black dots dark grey bars. Letters represent significant differences among the treatments (Two-way ANOVA).

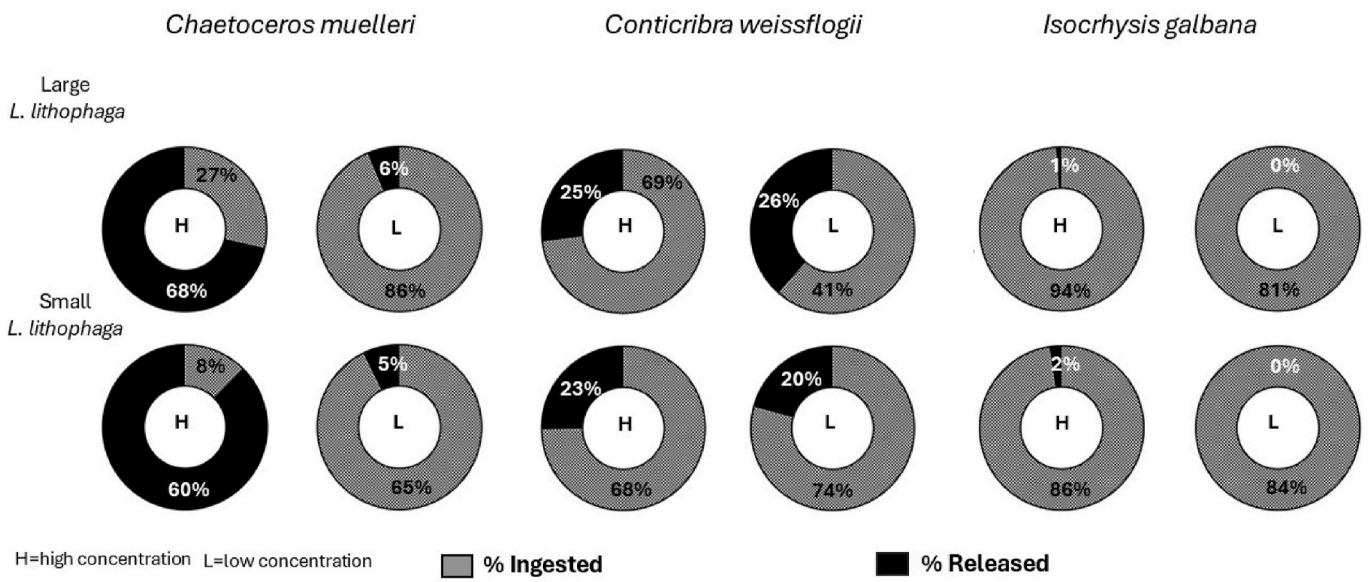

Fig. 3. Ring chart showing the total amount of filtered microalgae in grey and the total amount of released cells in black. (H) represent high concentration treatment while (L) low concentration treatment.

from silica also occurs around 1,075 cm^{-1}, within this same range. Accordingly, the peak at this wavelength in *C. muelleri* pseudofeces (Fig. 5A) is markedly higher than that observed in the cultured suspension, likely reflecting a combined contribution from both carbohydrates and silica (Palmucci et al., 2011).

The silicon content in pseudofeces, expressed as milligrams of Si per milligram of pseudofeces, is shown in Fig. 6. Interestingly, the amount of silicon in the pseudofeces appeared to be independent from animal size, as no significant differences were observed across samples produced by animals of varying sizes for either diatom species ($p > 0.05$). Additionally, the concentration of the algal suspension did not appear to affect silicon content. However, a slight reduction in Si levels was noted when *C. weissflogii* was provided, compared to the other diatom species.

Another important type of biodeposit produced by these animals is fecal pellets. During the experiment, it was observed that *L. lithophaga* produced a negligible quantity of fecal pellets when fed with diatoms, possibly due to the higher production of pseudofeces recorded under these conditions (Figs. 3 and 4). In contrast, feeding with *I. galbana* resulted in substantial fecal pellet production without pseudofeces releasing, highlighting an inverse correspondence of this two biodeposits (Fig. 7). A comparison of pellet weights revealed a positive relation between algal cell concentration and feces production (Table 1), a trend not consistently observed for pseudofeces, particularly in the case of *C. weissflogii* (Figs. 3 and 4). Again, unlike pseudofeces production, which appeared independent of animal size (Figs. 3 and 4), fecal pellet production showed that larger *L. lithophaga* individuals excreted approximately twice the fecal mass compared to smaller ones at both algal concentrations.

4. Discussions

Given the limited information on the ecology of *L. lithophaga*, our results offer the first insights into its feeding preferences and ecological role, particularly considering the importance of filter feeders in influencing the surrounding water column and altering the quality of material reaching the seafloor. In particular, experiments in controlled laboratory environment allowed to observe the feeding behaviour of *L. lithophaga*. For the first time in this species, the expulsion of pseudofeces was observed, a phenomenon well documented in other Mytilidae (Riisgård et al., 2003; Galimany et al., 2018). This bivalve produced a loosely packed, cloud-like deposit (Fig. 7), particularly when fed with diatoms. Pseudofeces production was inversely related to fecal pellet expulsion, the latter consisting of well-packed, metabolized algal cells that were primarily observed when *I. galbana* was supplied.

4.1. Clearance performance

Results of laboratory experiments revealed that the concentration of microalgal suspension deeply affected *L. lithophaga* feeding behaviour, with the maximum value of clearance rate (1.2 L h^{-1} g^{-1}) performed by one of the smaller *L. lithophaga* when fed with low concentration of *C. muelleri* (Fig. 2), and the highest percentage of released cells when specimens were supplied with high concentration of the same species (Fig. 3). In general, the decline in clearance rate at higher algal concentrations may reflect a physiological response to digestive system saturation, as observed in other bivalves (Gosling, 2003). Conversely, at lower concentrations, more representative of natural Adriatic phytoplankton levels (Totti et al., 2000), clearance efficiency increased due to the longer time needed to reach satiation. This behaviour aligns with previous findings by Gosling (2003), who reported an 85 % reduction in filtration rate in mussels fed with high concentrations of *C. weissflogii*, and a 56 % reduction with *I. galbana*, highlighting particle concentration as a key regulator of ingestion.

Animal size also affected clearance rate and fecal pellet production (Fig. 2 and Table 1). Smaller and younger individuals exhibited higher clearance rates and lower fecal output when fed with *I. galbana*. (Fig. 2 and Table 1). This observation could be attributed to a higher metabolic activity per unit mass in juveniles, requiring greater particle intake to meet higher energy demands, as supported by previous studies on other Mytilidae (Denis, 1999; Widdows, 1978). Indeed, as body size decreases, the filtration rate increases in relation to body weight, leading to a greater intake of food particles with lower production of feces per unit of body mass (Peharda et al., 2015; Lorrain et al., 2004).

Besides the notable differences linked to food concentration and animal size, the species supplied emerged as the most influential variable in the experimental outcomes. *L. lithophaga* exhibited extensive particle rejection when fed with both diatom species, while almost no particles were expelled when fed *I. galbana* (Figs. 3 and 4). This indicates a capacity for pre-ingestion selection, defined as the ability to collect and transport particles toward the mouth and eliminate unwanted material from the pallial cavity, was observed in *L. lithophaga* (Ward and Shumway, 2004). Such selection may result from specific interactions

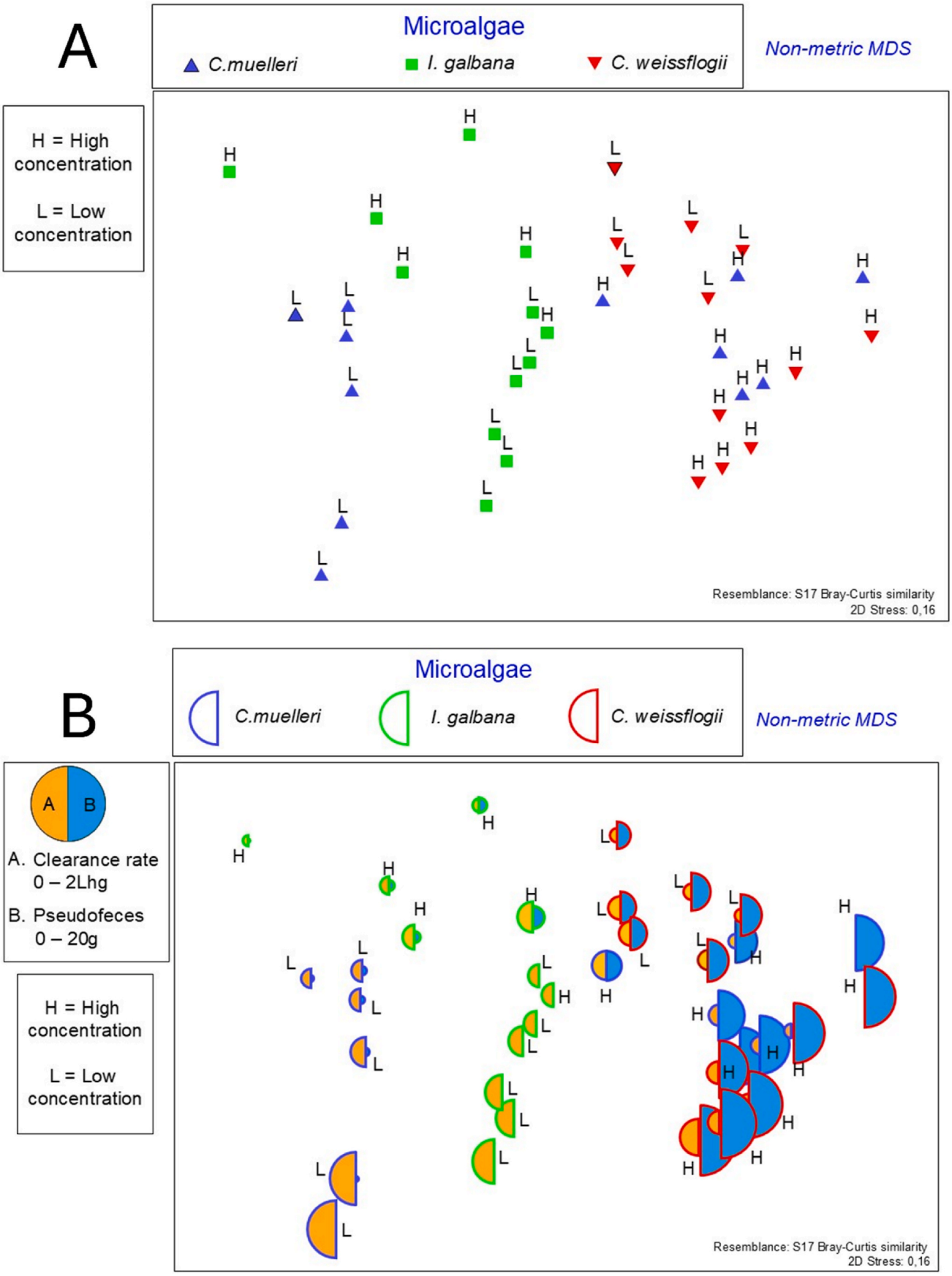

Fig. 4. Non-metric MDS plot highlighting data distribution based on Bray-Curtis similarity. Three colours represent three microalgal species: *C. muelleri* (blue), *I. galbana* (green), *C. weissflogii* (red). High and low concentrations are represented with H and L letters respectively.

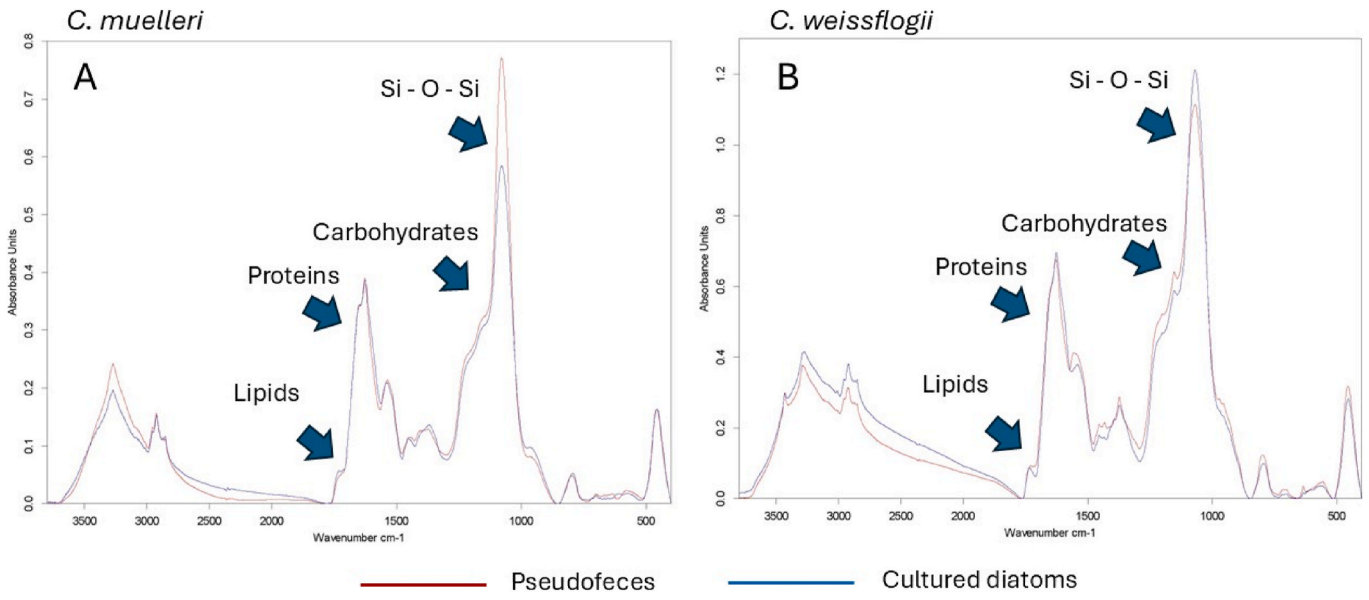

Fig. 5. FTIR spectra of *C. muelleri* (A) and *C. weissflogii* (B) pseudofeces (red line) compared to that of the cultured algae (blue line).

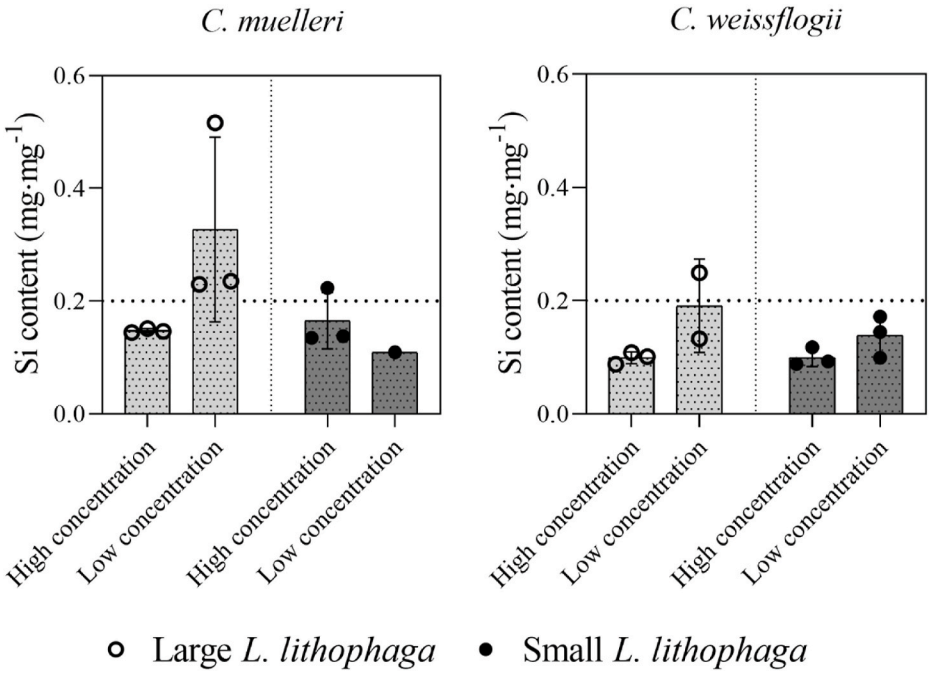

Fig. 6. Bar plot representing the quantified silica abundance in pseudofeces (expressed as milligrams of silica per milligrams of pseudofeces). In large animals, higher standard deviation resulted from an outlier specimen with a general greater clearance rate and pseudofeces production.

between cell surface polysaccharides and cilia and mucus of bivalves ctenidia (Ward and Shumway, 2004; Miranda-et al., 2022). Nevertheless, given the frequent rejection of diatoms, we cannot rule out that frustule presence could be connected to the elevated proportion of particles discharge. Indeed, bivalve's feeding behaviour could be directed towards actively excluding silica from its digestion, confirming previous studies on other bivalves (Gosling, 2003; Ward and Shumway, 2004; Jørgensen, 1996). The composition of pseudofeces (Fig. 5) supports this hypothesis: they were enriched in carbohydrates and silica, indicating the presence of carbohydrate-rich mucopolysaccharides, possibly similar to acid mucopolysaccharides (AMPS), which aid in particle handling (Beninger et al., 1999), and a selective avoidance of silica, confirming a low preference for diatoms. In contrast, when fed with *I. galbana*, pseudofeces production ceased, especially at low algal concentrations, implying complete ingestion and digestion of this preferred food source. Results thus highlighted the importance of diet quality, in the sorting and elimination processes of the mucus-bound particles, indicating the ability of *L. lithophaga* to select between different phytoplankton species, presumably depending on microalgal cell composition (Miranda-Arizmendi, 2022). However, although *L. lithophaga* rejected diatoms under high-concentration and in controlled laboratory experiments, its natural exposure to low concentrations of *Chaetoceros* species may indicate an adaptive capacity to utilize certain diatoms with thinner frustules (Totti et al., 2019; Waite et al., 1995; Moschino and Marin, 2006). Reduced pseudofeces production at low concentrations of *C. muelleri* (Fig. 3) supports the

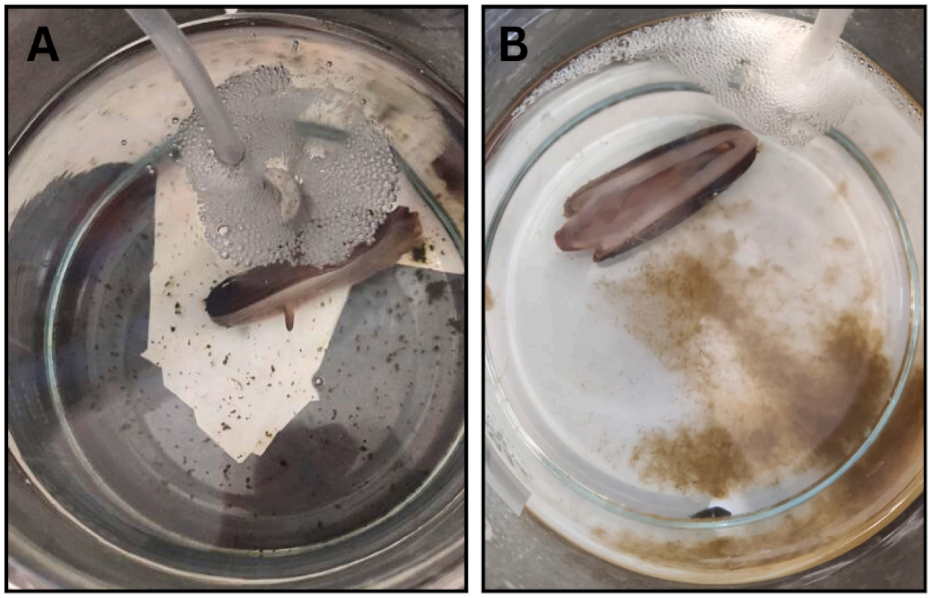

Fig. 7. Pictures of *L. lithophaga* (large individuals) fed with high concentration of (**A**) *I. galbana* and (**B**) *C. muelleri* (24 h after algal supply). In (**A**) is possible to observe in the bottom of the beaker fecal pellet, well packed biodeposits formed by metabolized algal cells; in (**B**) a loosely packed, cloud-like deposit was found (pseudofeces).

Table 1
Fecal pellet in milligrams (mg) excreted by larger and smaller *L. lithophaga* after fed with *Isochrysis galbana*, at high and low concentrations.

	Isochrysis galbana feces (mg)	
	High concentration	Low concentration
Large specimens	1.19 ± 0.62	0.61 ± 0.42
Small specimens	0.57 ± 0.27	0.36 ± 0.12

hypothesis that *L. lithophaga* may selectively filter diatoms based on frustule ultrastructure.

Despite broad similarities with other mytilids, *Lithophaga lithophaga* exhibited a distinct feeding-related behaviour in our trials. In mytilids, starved individuals typically open the valves, extend siphons and rapidly increase clearance rate immediately after algal addition (Widdows, 1978; Riisgård, 1991; Kamermans, 1993). In contrast, in our experiments *L. lithophaga* individuals that had been handled or approached by operators, delayed valve opening by several minutes after the disturbance (data not shown). Moreover, upon the algal pulse they first closed the valves briefly before resuming filtration, and this response was independent of body size (data not shown). We interpret this heightened reactivity as consistent with the species' endolithic niche (living burrowed within shaded rock crevices) where individuals are less accustomed to abrupt environmental change and show strong responses to mechanical/light stimuli (Devescovi and Iveša, 2008; Colletti et al., 2020). Documenting these behavioural responses is important because valve-gape dynamics and siphon extension provide a rapid, non-destructive proxy of physiological state, offering an indirect qualitative metric of health and experimental viability in the lab. Comparable behavioural indicators are widely used in bivalves (valvometry as a stress and performance proxy) and, by analogy, in other benthic invertebrates such as sponges (osculum contraction/pumping arrests) and cnidarians (polyp expansion–retraction) to flag sublethal stress and ensure data quality (Escobar-Calderon et al., 2022; Kumala et al., 2017; Gregorin et al., 2025).

4.2. Ecological implications of *L. lithophaga* feeding behaviour

Beside the well described role of *L. lithophaga* as habitat former (Fraschetti et al., 2001; Colletti et al., 2020), our results provide insights in their role in supplying nutrients to the benthic compartment. Despite the limited sample size and the potential influence of biotic and abiotic factors, which were not accounted for due to the controlled laboratory conditions, the findings of this study provide valuable insights into the ecological role of this species. Indeed, the high percentage of algal particles processed and compacted by our experimental specimens (Figs. 3 and 4) and found at the bottom of the beakers suggested that such aggregated particles can sink rapidly and provide benthos with organic matter. The role of other filter feeders in nutrient recycling is well represented in literature as force in modulating community composition, structuring the trophic network and influencing ecosystem stability (Kopp et al., 2015; Giordani et al., 2002). These organisms cycle large amounts of particulate matter (PM), actively pumping carbon, nitrogen and also biogenic silica into the water column until to the benthic compartment, potentially increasing PM flux fourfold compared to passive sinking (Griffiths et al., 2017; Chauvaud et al., 2000). Indeed, different species rely on the organic PM flux, starting from zooplankton to suprabenthos, benthic suspension feeders, surface deposit feeders, and a cluster of fishes, cnidarians and polychaetes, also organised in different trophic groups (Kopp et al., 2015; Giordani et al., 2002). Furthermore, considering that *L. lithophaga* abundance can reach more than 100 individuals per square metre (Pulido Mantas et al., 2023), multiplying the average clearance rate recorded (0.24 L g^{-1} h^{-1}) of the most common individuals (almost 8 g of weight, Peharda et al., 2015), is possible to obtain 192 L h^{-1}·m^{-2} of clearance rate per unit of area. This value further underscores the role of *L. lithophaga* as an important nutrient recycler, particularly considering that each kilogram of organisms can exhibit a clearance capacity of approximately 240 L h^{-1}. Extrapolating this data enables a quantitative assessment of the species' contribution to key ecological processes such as benthic-pelagic coupling, that regulate energy flow in marine ecosystems and support local biodiversity. Consequently, a decline in population, increasingly driven by climate change, could lead to the collapse not only of the habitat-associated community but also of the broader food web sustained by its filter-feeding activity (Fraschetti et al., 2001; Fanelli et al., 1994).

Moreover, the high rejection rate of diatoms observed in these experiments (Fig. 3), along with the corresponding high silicon content found in pseudofeces (Fig. 6), underlines the potential role of this filter

feeder in the biogenic silica cycle. Given the significant contribution of diatoms to global silicon fluxes, producing approximately 240 Tmol Si per year, with an estimated export of 78.8 Tmol Si per year to sediments and 26.2 Tmol Si per year to the deep sea (Tréguer et al., 2021), *L. lithophaga* and its demonstrated feeding preferences may influence the spatial distribution of silicon in the Mediterranean Sea. Consequently, the increased availability of biogenic silica in the benthic compartment may contribute to ecosystem biodiversity by providing a valuable resource for the formation of sponge spicules (Maldonado et al., 2005), Conversely, a collapse of the *L. lithophaga* population would likely disrupt this process, further impacting benthic community structure and function.

Finally, understanding the feeding preferences and behaviour of *L. lithophaga*, along with the ecosystem services it provides, such as water filtration, facilitation of chemical and organic matter fluxes, and the restoration of water quality, can support assessments of the growth and development of natural populations. These processes depend not only on the availability of suitable substrate for habitation but also on the availability of food sources (Griffiths et al., 2017; Kleemann, 1974). Therefore, recognizing and protecting populations of *L. lithophaga* is crucial not only for biodiversity conservation but also for maintaining critical ecosystem functions and nutrient cycles, ultimately supporting the resilience of Mediterranean benthic ecosystems and including this information in future restoration projects (Bekkby et al., 2020).

CRediT authorship contribution statement

T. Marrocco: Writing – review & editing, Writing – original draft, Investigation, Formal analysis, Data curation. **A. Petrucciani:** Writing – review & editing, Writing – original draft, Investigation, Formal analysis, Data curation. **B. Calcinai:** Writing – review & editing, Validation, Supervision, Project administration, Methodology, Conceptualization. **S. Puce:** Writing – review & editing, Validation, Supervision, Project administration, Methodology, Funding acquisition. **C. Cerrano:** Writing – review & editing, Validation, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization. **A. Norici:** Writing – review & editing, Writing – original draft, Validation, Supervision, Methodology, Conceptualization.

Permissions

Permission n. 0019895 by Italian Ministry of the Environment provided, to collect *L. lithophaga.*

Funding

This work is funded by the PRIN project “Corals and other benthic species HIdden LIfe histories. The tools of Behavioral ecology and Stable isotope analysis - CHILI”, financed by European Union – Next Generation EU, Mission 4, CUP I53D23003300006, ref. code 2022NRM7NX and by the PADI Foundation (grant number 74956). The projects coordinated by CIRCC (Progetti Competitivi 2023/CMPT231981, MIUR, European Union’s Horizon research and innovation actions programme DESIRED, 101083355) provided financial support for the research.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marenvres.2025.107641.

Data availability

Data will be made available on request.

References

AkşiT, D., Falakali Mutaf, B., 2014. The gill morphology of the date mussel *Lithophaga lithophaga* (Bivalvia: mytilidae). Turk. J. Zool. 38, 61–67.
Anderson, M., Gorley, R.N., Clarke, K., 2008. PERMANOVA+ for Primer: Guide to Software and Statistical Methods. Primer-E. Plymouth.
Bagur, M., Gutiérrez, J.L., Arribas, L.P., Palomo, M.G., 2019. Vacant Bivalve boreholes increase invertebrate species richness in a physically harsh, low intertidal platform. Diversity 11, 39.
Bekkby, T., et al., 2020. Habitat features and their influence on the restoration potential of marine habitats in Europe. Front. Mar. Sci. 7.
Beninger, P., Veniot, A., Poussart, Y., 1999. Principles of pseudofeces rejection on the bivalve mantle:integration in particle processing. Mar. Ecol. Prog. Ser. 178, 259–269.
Calcinai, B., Bavestrello, G., Cuttone, G., Cerrano, C., 2011. Excavating sponges from the Adriatic Sea: description of *Cliona adriatica* sp. Nov. (Demospongiae: clionaidae) and estimation of its boring activity. J. Mar. Biol. Assoc. U. K. 91, 339–346.
Camacho-Rodríguez, J., et al., 2020. Use of continuous culture to develop an economical medium for the mass production of *Isochrysis galbana* for aquaculture. J. Appl. Phycol. 32, 851–863.
Chauvaud, L., Jean, F., Ragueneau, O., Thouzeau, G., 2000. Long-term variation of the Bay of Brest ecosystem:benthic-pelagic coupling revisited. Mar. Ecol. Prog. Ser. 200, 35–48.
Colletti, A., et al., 2020. The date mussel *Lithophaga lithophaga*: biology, ecology and the multiple impacts of its illegal fishery. Sci. Total Environ. 744, 140866.
De Zwaan, A., Wijsman, T.C.M., 1976. Anaerobic metabolism in bivalvia (Mollusca) characteristics of anaerobic metabolism. Comparative biochemistry and physiology part B. Comp. Biochem. 54 (3), 313–323.
Denis, L., 1999. Clearance rate responses of Mediterranean mussels, *Mytilus galloprovincialis*, to variations in the flow, water temperature, food quality and quantity. Aquat. Living Resour. 12, 279–288.
Devescovi, M., 2009. Biometric differences between date mussels *Lithophaga lithophaga* colonizing artificial and natural structures. Acta Adriat. 50 (2), 129–138.
Devescovi, M., Iveša, L., 2008. Colonization patterns of the date mussel *Lithophaga lithophaga* (L., 1758) on limestone breakwater boulders of a marina. Period. Biol. 110, 339–345.
Escobar-Calderon, J.F., Chmist-Sikorska, J., Douda, K., 2022. High-frequency video analysis extends beyond the capabilities of valvometry in acute behavioral disturbance detection in bivalves. Ecol. Indic. 136, 108656.
Evan Ward, J., Shumway, S.E., 2004. Separating the grain from the chaff: particle selection in suspension- and deposit-feeding bivalves. J. Exp. Mar. Biol. Ecol. 300, 83–130.
Fanelli, G., Piraino, S., Belmonte, G., Geraci, S., Boero, F., 1994. Human predation along Apulian rocky coasts (SE Italy): desertification caused by *Lithophaga lithophaga* (Mollusca) fisheries. Mar. Ecol. Prog. Ser. 110, 1–8.
Fey, S.B., Gibert, J.P., Siepielski, A.M., 2019. The consequences of mass mortality events for the structure and dynamics of biological communities. Oikos 128, 1679–1690.
Fraschetti, S., et al., 2001. Spatial variability and human disturbance in shallow subtidal hard substrate assemblages: a regional approach. Mar. Ecol. Prog. Ser. 212, 1–12.
Galimany, E., et al., 2018. Design and use of an apparatus for quantifying bivalve suspension feeding at Sea. J. Vis. Exp. JoVE 58213. https://doi.org/10.3791/58213.
Garrabou, J., et al., 2022. Marine heatwaves drive recurrent mass mortalities in the Mediterranean Sea. Glob. Change Biol. 28, 5708–5725.
Giordani, P., et al., 2002. Gradients of benthic–pelagic coupling and carbon budgets in the Adriatic and Northern Ionian Sea. J. Mar. Syst. 33 (34), 365–387.
Gosling, E.M., 2003. Bivalve Molluscs: Biology, Ecology, and Culture. Fishing News Books, Oxford ; Malden, MA.
Gregorin, C., Domenichelli, F., Gridelli, S., Vega fernÁndez, T., Puce, S., Musco, L., 2025. The detection of specific prey cues triggers distinct predatory behaviour in Aurelia coerulea polyps (Cnidaria: scyphozoa). Eur. Zool. J. 92 (1), 906–924.
Griffiths, J.R., et al., 2017. The importance of benthic-pelagic coupling for marine ecosystem functioning in a changing world. Glob. Change Biol. 23, 2179–2196.
Jones, H.F.E., Pilditch, C.A., Hamilton, D.P., Bryan, K.R., 2017. Impacts of a bivalve mass mortality event on an estuarine food web and bivalve grazing pressure. N. Z. J. Mar. Freshw. Res. 51, 370–392.
Jørgensen, C.B., 1996. Bivalve filter feeding revisited. Mar. Ecol. Prog. Ser. 142, 287–302.
Kamermans, P., 1993. Food limitation in cockles (*Cerastoderma edule* (L.): influences of location on tidal flat and of nearby presence of mussel beds. Neth. J. Sea Res. 31, 71–81.
Kiljunen, M., et al., 2020. Benthic-pelagic coupling and trophic relationships in northern Baltic Sea food webs. Limnol. Oceanogr. 65, 1706–1722.
Kleemann, K.H., 1974. Raumkonkurrenz bei ätzmuscheln. Mar. Biol. 26 (4), 361–364.
Kopp, D., Lefebvre, S., Cachera, M., Villanueva, M.C., Ernande, B., 2015. Reorganization of a marine trophic network along an inshore–offshore gradient due to stronger pelagic–benthic coupling in coastal areas. Prog. Oceanogr. 130, 157–171.
Kuehr, S., Diehle, N., Kaegi, R., Schlechtriem, C., 2021. Ingestion of bivalve droppings by benthic invertebrates may lead to the transfer of nanomaterials in the aquatic food chain. Environ. Sci. Eur. 33 (1), 35.

Kumala, L., Riisgård, H.U., Canfield, D.E., 2017. Osculum dynamics and filtration activity in small single-osculum explants of the demosponge *Halichondria panicea*. Mar. Ecol. Prog. Ser. 572, 117–128.
Lorrain, A., et al., 2004. δ13C variation in scallop shells: increasing metabolic carbon contribution with body size? Geochem. Cosmochim. Acta 68, 3509–3519.
Maldonado, M., et al., 2005. Siliceous sponges as a silicon sink: an overlooked aspect of benthopelagic coupling in the marine silicon cycle. Limnol. Oceanogr. 50, 799–809.
Miranda-Arizmendi, V., et al., 2022. Sulfated polysaccharides from *Chaetoceros muelleri*: macromolecular characteristics and bioactive properties. Biology 11, 1476.
Moschino, V., Marin, M.G., 2006. Seasonal changes in physiological responses and evaluation of "well-being" in the Venus clam *Chamelea gallina* from the Northern Adriatic Sea. Comp. Biochem. Physiol. A. Mol. Integr. Physiol. 145, 433–440.
Palmucci, M., Ratti, S., Giordano, M., 2011. Ecological and evolutionary implications of carbon allocation in Marine Phytoplankton as a function of nitrogen availability: a fourier transform Infrared Spectroscopy approach. J. Phycol. 47, 313–323.
Peharda, M., et al., 2015. Growth and longevity of *Lithophaga lithophaga* : what can we learn from shell structure and stable isotope composition? Mar. Biol. 162, 1531–1540.
Petrucciani, A., Knoll, A.H., Norici, A., 2022. Si decline and diatom evolution: insights from physiological experiments. Front. Mar. Sci. 9, 924452.
Prins', T.C., Smaal, A.C., 1994. The Role of the Blue Mussel *Mytilus edulis* in the Cycling of Nutrients in the Oosterschelde Estuary (The Netherlands).
Pulido Mantas, T., Pola, L., Cerrano, C., Gambi, M.C., Calcinai, B., 2022. Bioerosion features of boring polydorid polychaetes in the North Adriatic Sea. Hydrobiologia 849, 1969–1980.
Pulido Mantas, T., et al., 2023. Photogrammetry as a promising tool to unveil marine caves' benthic assemblages. Sci. Rep. 13, 7587.
Renda, W., et al., 2022. The new checklist of the Italian Fauna: marine Mollusca. Biogeogr. – J. Integr. Biogeogr. 37.
Ricci, P., et al., 2022. Contribution of intermediate and high trophic level species to benthic-pelagic coupling: insights from modelling analysis. Front. Mar. Sci. 9.
Riisgård, H.U., 1991. Filtration rate and growth in the blue mussel, *Mytilus edulis* Linneaus, 1758: dependence on algal concentration. J. Shellfish Res. 10, 29–35.
Riisgård, H.U., 2001. On measurement of filtration rates in bivalves—the stony road to reliable data: review and interpretation. Mar. Ecol. Prog. Ser. 211, 275–291.
Riisgård, H.U., Kittner, C., Seerup, D.F., 2003. Regulation of opening state and filtration rate in filter-feeding bivalves (*Cardium edule, Mytilus edulis, Mya arenaria*) in response to low algal concentration. J. Exp. Mar. Biol. Ecol. 284, 105–127.
Schnack-Schiel, S.B., Isla, E., 2005. The role of zooplankton in the pelagic-benthic coupling of the Southern Ocean. Sci. Mar. 69, 39–55.
Siokou-Frangou, I., et al., 2010. Plankton in the open Mediterranean Sea: a review. Biogeosciences 7, 1543–1586.
Soetaert, K., Middelburg, J.J., Herman, P.M.J., Buis, K., 2000. On the coupling of benthic and pelagic biogeochemical models. Earth Sci. Rev. 51, 173–201.
Thompson, E.L., et al., 2012. Optimal acclimation periods for oysters in laboratory-based experiments. J. Molluscan Stud. 78, 304–307.
Totti, C., et al., 2000. Seasonal variability of phytoplankton populations in the middle Adriatic sub-basin. J. Plankton Res. 22, 1735–1756.
Totti, C., et al., 2019. Phytoplankton communities in the northwestern Adriatic Sea: interdecadal variability over a 30-years period (1988–2016) and relationships with meteoclimatic drivers. J. Mar. Syst. 193, 137–153.
Tréguer, P., et al., 2018. Influence of diatom diversity on the ocean biological carbon pump. Nat. Geosci. 11, 27–37.
Tréguer, P.J., et al., 2021. Reviews and syntheses: the biogeochemical cycle of silicon in the modern ocean. Biogeosciences 18, 1269–1289.
Van Broekhoven, W., et al., 2015. Nutrient regeneration from feces and pseudofeces of mussel *Mytilus edulis* spat. Mar. Ecol. Prog. Ser. 534, 107–120.
Waite, A.M., Olson, R.J., Dam, H.G., Passow, U., 1995. Sugar-containing compounds on the cell surfaces of marine diatoms measured using concanavalin a and flow cytometry. J. Phycol. 31, 925–933.
Ward, J.E., Shumway, S.E., 2004. Separating the grain from the chaff: particle selection in suspension- and deposit-feeding bivalves. J. Exp. Mar. Biol. Ecol. 300, 83–130.
Widdows, J., 1978. Combined effects of body size, food concentration and season on the physiology of *Mytilus edulis*. J. Mar. Biol. Assoc. U. K. 58, 109–124.