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How sediment granulometry affects feeding behaviour in sea cucumbers: a case study on *Holothuria sanctori*

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The sea cucumber *Holothuria sanctori* is an Atlanto-Mediterranean deposit feeder that contributes to sediment processing in coastal areas. However, the feeding ecology of this species, including its sediment processing capacity, remains largely unexplored under controlled conditions. This study investigates the effect of sediment granulometry on the feeding behaviour of *H. sanctori*, focusing on organic matter (OM) selectivity and sediment ingestion. Specimens were tested under five experimental treatments, each containing sediments with particle sizes ranging from 0 to 1000 µm, with standardized OM availability across treatments. A natural simulation with a hard, sediment-free substrate was also included. Data on defecation rate and faecal OM content was collected daily to evaluate sediment processing capacity. Results indicate that *H. sanctori* optimally selects OM (average OM: 4.2%) and processes sediment (average defecation rate: 1.5 g/day per 100 g biomass WW) in fine particle treatments (< 250 µm). Additionally, the species exhibits an extended sediment processing capacity across a wide range of sediment sizes, including particles up to 1000 µm. The study further revealed that the sea cucumber increases sediment ingestion (by one order, on average) to compensate for reduced OM availability (by 0.15 order, on average), maintaining a constant OM intake. The results suggest that sediment granulometry might not be the primary factor in determining the species' microhabitat preferences in the natural environment due to its extended capacity to process different sediment granulometries. These findings enhance our understanding of *H. sanctori*'s feeding behaviour and highlight its potential in Integrated Multi-Trophic Aquaculture applications.

Keywords Bioturbation, Deposit-feeding, Holothuroids, Integrated Multi-Trophic Aquaculture (IMTA), Food selectivity, Organic matter, Sediment reworking

Holothuroids, commonly known as sea cucumbers, are predominantly benthic invertebrates belonging to the phylum Echinodermata, with more than 1800 species[1]. Among the various holothuroid species, deposit-feeding sea cucumbers are particularly important in maintaining healthy ecosystems[2]. They ingest sediment and associated organic matter (OM) content, including inhabiting microorganisms (microalgae, bacteria, and infauna)[3–7]. Their feeding activities enhance sediment functionality by displacing, ingesting, and defecating processes. This drives bioturbation, which promotes oxygenation and accelerates benthic nutrient cycling[3,6,8–10].

Feeding behaviour in deposit-feeding sea cucumbers involves selecting, ingesting, and processing sediment particles, with potential preferences for certain characteristics, such as OM content and sediment granulometry[4,9,11–13]. Such preferences are often driven by the aim of optimizing nutrient acquisition, favouring particles enriched in OM or having specific sizes that typically provide greater OM availability[14–16]. Species like *Holothuria scabra, Parastichopus regalis,* and *Australostichopus mollis* have been found to preferentially inhabit sediment patches abundant in OM or composed of finer particles, generally less than 0.5 mm in diameter[14,15,17–19]. The properties of sediment, including OM availability and granulometry, play a significant

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role in shaping the species' microhabitat preferences[4], which subsequently influence their ecological distribution and feeding behaviour[20–22].

Deposit-feeding sea cucumbers exhibit diverse microhabitat preferences, with some species favouring superficial (epibenthic) feeding, while others prefer burrowing into deeper (endobenthic) sediment layers[12,17,23,24]. These preferences can also vary based on sediment type, with some species favouring fine-grained sediments and others preferring coarser ones[4,11]. Additionally, microhabitats and substrate type preferences can vary across developmental stages, seasons, and geographical locations[4,18,25–28]. For instance, *H. scabra* juveniles are typically found in mud and seagrass beds with higher OM, while adults favour sandy substrates with lower organic matter content[29]. Moreover, species such as *Holothuria atra, Holothuria hawaiiensis* and *Bohadschia vitiensis* exhibit substrate preference variations depending on the developmental stage and reproductive cycle, generally favouring coarser substrates but shifting to finer sediments during reproduction[26].

In the Mediterranean, some deposit-feeding sea cucumbers, including *Holothuria polii, H. tubulosa,* and *H. sanctori*, co-exist within coastal ecosystems such as seagrass and algal beds[11,30]. These species display distinct feeding behaviours, particularly in their selectivity for particle sizes and OM[11,31–33]. The variability likely enables these species to occupy different microhabitats, potentially reducing interspecific competition and promoting co-existence[12,13]. Previous studies suggest that sediment granulometry plays a more significant role in microhabitat selection than trophic differentiation in these species[34,35]. However, it remains uncertain whether their feeding preferences result from natural associations with particular microhabitats or from dietary adaptations to specific sediment granulometries.

In this context, *Holothuria sanctori* is of particular interest for investigating this question due to its distinctive feeding behaviour, marked by a high selectivity for OM[11]. This makes it an ideal model organism to explore how sediment granulometry, influences feeding behaviour, including sediment ingestion and OM selectivity. *Holothuria sanctori* is an Atlanto-Mediterranean deposit-feeding sea cucumber, that primarily inhabits hard substrates in natural environments, where it mainly ingests fine particles ranging from 60–200 µm[11]. The species' specialized mouth tentacles (peltate shape) are adapted for ingesting surface sediments and settled organic particles[4,5,36].

This study aims to assess the role of sediment granulometry in shaping *H. sanctori*'s feeding behaviour by testing different sediment sizes under standardized OM availability. Additionally, it aims to assess whether fine particle ingestion in this species is driven by trophic preferences and sediment-processing adaptations or by the species' microhabitat associations.

We hypothesize that *H. sanctori* has an exclusive ability to process sediments within a specific granulometric range, irrespective of consistent organic matter content. If confirmed, sediment granulometry may have a primary role in shaping feeding behaviour, microhabitat preferences, and ecological segregation between *H. sanctori* and other sympatric species.

Materials and methods

Collection of wild sea cucumbers

A total of 30 *H. sanctori* specimens with a mean weight of 85.22 ± 15.42 g (Mean ± SD) were collected from a rocky bottom at Santa Marinella, central Tyrrhenian Sea, Italy (42°02′59.59" N, 11°49′06.14" E). The specimens were manually collected at depths ranging from two to six metres by Scuba divers in February 2024. They were then placed in seawater-filled buckets for transport to the Laboratory of Experimental Ecology and Aquaculture (LESA) at the University of Rome Tor Vergara. Upon arrival, the specimens were transferred to two free-substrate maintenance tanks, each with a capacity of 400L (15 specimens per tank), to allow for gut content evacuation through defecation. The maintenance tanks were filled with seawater (salinity: 35 PSU; temperature: 20 ± 0.5 °C) and equipped with aeration and filtration systems in a naturally illuminated room. The animals were kept in these conditions for two weeks to acclimatise to indoor conditions before starting the experiment.

Particle size categorization

The faecal pellets of the wild specimens evacuated in the maintenance tanks were collected and dried at 60 °C until reaching a stable dry weight. After complete drying, the faecal matter was sieved using a sieve shaker (IG/3/EXPORT, GIULIANI, Italy) to determine the size-frequency distribution of faecal particles[37] into five size categories [I(0–100 µm); II(100–250 µm); III(250–500 µm); IV(500–1000 µm); V(> 1000 µm)]. According to the distribution percentage of each size, the first four size categories, prevalent in the wild faeces were selected as experimental treatments in this study (Table 1).

Size class (µm)	Average ± SD (%)
> 1000	2.9 ± 0.4
500–1000	6.8 ± 0.9
250–500	16.8 ± 1.7
100–250	38.8 ± 1.2
< 100	34.7 ± 1.8
Sum	100

Table 1. Size-frequency distribution (%) of sediment particles in wild *H. sanctori* faeces (N = 30).

Experimental substrate and OM content

The experimental substrate used in this study consisted of natural sediment, collected from the sampling area in February 2024. In order to ensure that sediment was free of organic material to be used as an experimental substrate, the collected sediment underwent a bleach treatment (HCl 15%; CARLO ERBA) following the procedures of Hammond[38]. It was immersed in the sodium hypochlorite solution for 24 h, then rinsed thoroughly with fresh water several times and subsequently dried in an oven (60 °C; 48 h). After complete drying, sediment samples (n = 6; 50 ± 0.5 g each) were taken and subjected to inspection of the absence of organic matter using the loss-on-ignition technique[39]. This involved the incineration of samples in a muffle furnace (550 °C) for a 6 h duration with subsequent verification of the stability of sample weights. The organic matter content was determined using the formula:

$$\text{OM} = [(\text{W}_{\text{i}} - \text{W}_{\text{g}} / \text{W}_{\text{i}})] \times 100$$

where OM is the organic matter content (%). W_i is the initial sample weight (g). W_g is the sample weight after ignition (g).

To minimize the influence of structural water weight that can affect the estimation of organic matter in very fine (clay) experimental sediment when using the loss-on-ignition method[40,41], also a total organic carbon (TOC) analysis was performed to address any flaw in the gravimetric measurement using an elemental analyser (Thermo Flash EA 1112).

After confirming the negligible organic matter content (OM= 0.02%), the experimental sediment was sieved and sorted according to the pre-defined particle size categories: (0–100 µm); (100–250 µm); (250–500 µm); (500–1000 µm); (> 1000 µm). The largest size category (> 1000 µm) was excluded, while the remaining categories were labelled and stored separately for subsequent use.

The organic matter source provided to the sea cucumbers in this study was aquaculture feed powder (*Skretting* Classic K-3P; OM%= 87.5 ± 0.6). The total organic matter content in the feed sample was estimated using the previously mentioned loss-on-ignition method[39].

Experimental setup

In this study, the influence of sediment granulometry on *H. sanctori* sediment ingestion and OM selectivity was investigated. Different four sediment-containing treatments were tested, including treatment (A) with a particle size range of 0–100 µm; treatment (B) with a particle size range of 100–250 µm; treatment (C) with a particle size range of 250–500 µm; treatment (D) with a particle size range of 500–1000 µm. In addition, treatment (E) with a hard substrate (tank bottom) and an absence of soft sediment (sediment-free), was used to emulate the natural hard-bottom conditions typically inhabited by *H. sanctori*.

The trial was conducted in April 2024 over a period of 15 consecutive days at the LESA facility. It was carried out in a Recirculating Aquaculture System (RAS) composed of 15 experimental tanks, each with a capacity of 30 L. Each treatment was represented by three replicates (tanks), with each tank measuring (0.5 × 0.3 × 0.2) m. The seawater exiting the system underwent mechanical and biological filtration including sand filtering and protein skimming processes. The filtered water was further sterilized by a UV unit and then conditioned by a refrigeration unit to maintain a constant temperature of 20 °C ± 0.5, before being returned to the experimental tanks (Fig. 1).

Throughout the experiment, system functionality was checked daily, with water quality parameters maintained within convenient ranges for the sea cucumbers with salinity: 35 ± 0.5 PSU; DO: 6.0 ± 0.7 ppm; pH: 8.3 ± 0.1; alkalinity: 150 ± 10 ppm; total ammoniacal nitrogen: 1.1 ± 0.2 ppm; nitrite: < 0.2 ppm; nitrate: < 20 ppm.

Before introducing the sea cucumbers into the system, the experimental substrates were added to the respective tanks according to their assigned particle size range under investigation. Approximately, two centimetres of sediment (1200 g) was added to the bottom of each sediment-containing tank and the system was allowed to operate for two weeks before the actual start of the experiment. The sea cucumber specimens were randomly assigned to the tanks at a stocking density of two specimens per tank. This resulted in a mean biomass of 170.43 g (± 23.50 SD) of sea cucumber (wet weight, 57) per replicate, equivalent to 1200 g/m^2.

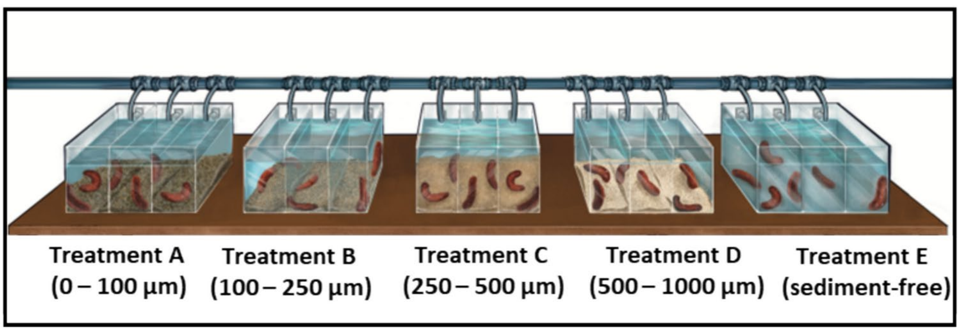

Fig. 1. Schematic diagram of the experimental treatments within rearing tanks of the recirculating aquaculture system (RAS).

Meanwhile, aquaculture feed powder (*Skretting* Classic K-3P; OM%= 87.5 ± 0.6) was added to each tank at the start of the experiment (5 g per tank) as a food source. After adding the feed, the sediment was gently raked to ensure a homogeneous distribution of organic matter within the sediment.

Sampling and data collection

Holothuria sanctori faeces were collected on a daily basis to assess the defecation rate and faecal organic matter content throughout the study. The faeces were collected using a modified bulb pipette to aspirate the faecal thread[33]. To overcome the limited quantity of faeces produced daily in some treatments, faeces produced every three days were gathered and the corresponding data was consolidated and treated as distinct sampling pinpoints over the course of the experiment as shown in Tables 1 and 2 (Supplementary data). The defecation rate was estimated based on the mass of the faecal pellets production in each tank. The collected faeces were dried at 60 °C until reaching a stable dry weight, allowing estimation of the defecation rate using the formula:

$$\text{DR} = (\text{W}_\text{f}/\ \text{W}_\text{b}) \times 100$$

where DR is the defecation rate (g/day) per 100 g of sea cucumber biomass, W_f is the dry weight of faeces produced daily (g) and W_b is the wet weight of sea cucumber biomass (g).

In order to estimate the faecal OM content, the dried faeces were burnt in a muffle furnace (550 °C, 6 h) using the previously described loss-on-ignition method[39]. Additionally, the availability of OM in the sediment was assessed by measuring the OM content of sediment samples collected at five consecutive time points throughout the experiment (Days: 0, 4, 8, 12, and 15). Three sediment samples were taken from each tank replicate, resulting in a total of 9 samples per treatment. The sediment samples were collected from the uppermost superficial layer, dried and then incinerated to estimate the OM content.

Statistical data analysis

The data on the defecation rate and faecal organic matter content were statistically analysed using IBM *SPSS* Statistics software (version 22.0). Initially, the data were examined for normality of distribution and homogeneity of variance by applying Levene's and Kolmogorov–Smirnov tests, respectively. Multivariate analysis of variance (MANOVA) was then executed to test the potential effect of sediment particle size as an independent factor on defecation rate and OM concentration as two dependent factors. When a significant difference was indicated by the Wilks' Lambda *p*-value ($p < 0.05$), post hoc analyses were performed to further understand these differences. In such cases, Tukey's Honestly Significant Difference (HSD) test was employed to identify specific differences between the different particle size treatments. Additionally, to compare changes in OM concentration over time for each treatment, the Friedman Test was applied, as the data were not normally distributed. This non-parametric test allowed for the assessment of significant differences in OM concentration across the different sampling days.

To further explore relationships between variables, a multiple regression analysis was conducted to assess the effects of OM availability and defecation rate as independent variables on OM selectivity as the dependent variable. The model also included categorical variables to account for treatment and sampling effects. The regression coefficients and *p*-values ($p < 0.05$) were used to evaluate the significance and direction of the relationships between the variables.

Results

Throughout the experiment, the sea cucumber specimens showed no stress signs or disease manifestations and were feeding and defecating actively. During active feeding, the specimens were never observed to completely detach from the tank walls (hard substrate). They used their oral tentacles to collect sediment particles, exclusively from the superficial layer of the soft substrate (experimental mud and sand particles), while the rest of the body, or at minimum one-third of the body length, consistently remained attached to the tank walls via tube feet (Fig. 2). At the end of the experiment, the sea cucumber survival rate was 100%.

Particle size categorization

Analysis of wild *H. sanctori* faeces to determine the distribution frequency for each size class is shown in Table 1. Fine sediment particles less than 250 µm were found to be predominant, constituting approximately 75% of the total ingested particles.

Defecation rate

At the end of the experiment, the highest average defecation rate was observed in treatment B (100–250 µm), with 1.602 ± 0.204 (g/day) per 100 g biomass. This rate was significantly higher (MANOVA; N = 15; $p < 0.05$) compared to treatments C, D, and E, but not significantly different from treatment A. Treatment A (0–100 µm) followed treatment B, with an average defecation rate of 1.410 ± 0.169 (g/day) per 100 g biomass, which was significantly higher than treatments D and E. Treatments C (250–500 µm) and D (500–1000 µm) exhibited lower rates of 1.177 ± 0.167 and 0.784 ± 0.195 (g/day) per 100 g biomass, respectively, both of which were significantly higher than treatment E. The lowest defecation rate was observed in treatment E (MANOVA; N = 15; $p < 0.05$), where the specimens were directly fed onto the hard tank bottom, with an average defecation rate of 0.108 ± 0.01 (g/day) per 100 g biomass (Fig. 3).

Throughout the trial, successive sampling revealed a steadily increasing trend in defecation rate over time across all the experimental treatments (Repeated measures ANOVA; N = 3; $p < 0.05$). At the end of the experiment, the defecation rates increased significantly, with ratios of 6.71, 5.13, 20.08, 19.78, and 2.71 times the initial rates for treatments A, B, C, D, and E, respectively (Table 1, Supplementary data). The defecation rate was always highest throughout the experiment in treatment B (MANOVA; N = 15; $p < 0.05$), followed by treatments A, C, and D and finally, the hard substrate treatment E.

Faecal OM

Interestingly, all experimental treatments showed a relatively constant concentration of OM (Repeated measures ANOVA; N = 3; $p > 0.05$) in the faeces over the 15-day trial (Table 2, Supplementary data). However, there were remarkable differences in the OM concentration in faeces across different treatments (Fig. 4). The highest OM concentration (MANOVA; N = 15; $p < 0.05$) was found in the sediment-free treatment (E), with an average value of 47.84 ± 1.80%. In sediment-containing treatments, the highest OM content was found in small particle size treatments B and A at 4.66 ± 0.38 and 3.67 ± 0.52, respectively, with significant differences with treatments C, D, and E (MANOVA; N = 15; $p < 0.05$). In the large particle size treatments, the OM content was relatively low, averaging 2.63 ± 0.29% in treatment C and 3.17. ± 0.32 in treatment D, with no observed significant difference between the two treatments (MANOVA; N = 15; $p > 0.05$).

OM availability in the foraging substrate

The average organic matter concentration across all treatments was approximately 1% in all sediment-containing treatments (A, B, C, and D). Over the course of subsequent sediment sampling, organic matter levels decreased steadily across all treatments from approximately 1.41% in sediment-containing treatments at the beginning of the experiment to approximately 0.75% in treatments A, C, and D at the end of the experiment (Friedman Test; N = 3; $p < 0.05$). In treatment B, the organic matter concentration was lower than in the other treatments, at approximately 0.41% (Table 2). In treatment E, where food was provided directly above the hard tank bottom, the organic matter content decreased consistently over time, starting from 87.5 ± 0.62% at the beginning of the experiment and reaching 28.84 ± 1.06% at the end (Friedman Test; N = 3; $p < 0.05$).

The regression analysis revealed that OM availability had a statistically significant negative effect on the defecation rate (coefficient = − 12.05, $p = 0.01$), indicating that higher OM availability is associated with decreased defecation rates. Additionally, OM availability showed a statistically significant positive effect on OM selectivity (coefficient = 0.059, $p = 0.01$), suggesting that increased OM availability enables organisms to exhibit higher selectivity for organic matter. The correlation between defecation rate and OM selectivity was negative (coefficient = − 0.293) but not statistically significant ($p = 0.59$).

Discussion

This study investigates the feeding behaviour of the sea cucumber *H. sanctori*, analysing the potential influence of substrate granulometry on sediment reworking including OM selectivity and defecation rate. The obtained total survival, active feeding and defecation in this experiment, and the consistency of observations on this species' feeding behaviour confirm the reliability of the experimental rearing conditions. This experiment highlighted the preference of *H. sanctori* for association with hard substrates, even in the absence of hydrodynamism and predation susceptibility, suggesting a species adaptation to this posture as a survival and feeding trait. Moreover, this species demonstrated a high ability to process a wide range of sediment sizes to extract the OM content, maintaining its selectivity in all the experimental treatments.

The very high selectivity of *H. sanctori* was demonstrated in this study, with specimens being able to select and accumulate approximately three times more OM in their faeces than the OM available in their foraging substrate. This behaviour was consistently demonstrated across all the experimental treatments, including the hard substrate treatment (E), where the OM levels in the sea cucumber faecal matter exceeded that of the available diet itself. This finding strongly supports previous claims that this sea cucumber is highly selective for organic matter, as noted by Mezali and Soualili[11] and Navarro et al.[33]. Further evidence from[11,42], which compared OM selectivity among sympatric Mediterranean holothuroids, highlighted the remarkable selectivity of *H. sanctori*. These studies found that *H. sanctori* displays OM selectivity of up to 4.5 times the OM available in the substrate, significantly higher than that reported in *H. forskali* (2.8 times), and *H. tubulosa* (1.5 times). In contrast, *H. polii* showed a remarkable low selectivity, with OM content in its gut comparable to that of the substrate.

The ability of *H. sanctori* to process a broad range of sediment particle sizes (0–1000 μm) suggests that sediment granulometry may influence feeding behaviour but is not the primary factor driving its distribution and microhabitat selection. Observations of the species predominantly ingesting fine particles (< 250 μm) likely reflect its presence in specific microhabitats rather than a strict preference. Thus, other factors such as hydrodynamism, substrate complexity, or local trophic conditions may have a greater influence on its microhabitat preferences[43–45].

The selectivity towards OM-rich particles has been reported in many wild deposit-feeding holothurians such as *H. forskali*, *H. atra*, *H. marmorata* and *H. hawaiiensis*[11,26,46] and highlighted in the review by[4]. The exact mechanism of selectivity in sea cucumbers remains debated. However, factors such as particle size, specific gravity, surface texture, and the presence of organic biofilm are proposed to play significant roles in distinguishing organic from inorganic particles[47,48]. The species *H. sanctori*, is an epibenthic species with peltate-shaped mouth tentacles, which explains the enhanced ability to select OM from the superficial sediment layer. This behaviour may reflect a feeding adaptation that allows the species to capitalize on the high organic load found in that layer, including detritus, microphytobenthos, and microfaunal communities. These organic components could serve as preferred food items in sea cucumber diets[4], contributing to higher organic content in both ingested particles and faces. Nevertheless, the exact mechanism employed by this species remains uncertain, whether it is due to active selection by the mouth tentacles or a result of enhanced digestion and assimilation processes along the digestive tract.

The selectivity towards OM in deposit-feeding sea cucumbers is usually accompanied by a preference for a certain particle size[4]. The efficiency of *H. sanctori* in sediment intake and OM extraction from the fine particles

Sampling	Treatment A (0.0–100 µm)	Treatment B (100–250 µm)	Treatment C (250–500 µm)	Treatment D (500–1000 µm)	Treatment E (sediment-free)
Day-0	1.41 ± 0.14	1.41 ± 0.02	1.41 ± 0.03	1.41 ± 0.08	87.5 ± 0.62
Day-4	1.49 ± 0.20	1.57 ± 0.71	1.00 ± 0.11	1.13 ± 0.07	46.38 ± 5.74
Day-8	0.82 ± 0.13	0.93 ± 0.22	1.16 ± 0.28	1.05 ± 0.20	39.71 ± 3.48
Day-12	0.51 ± 0.09	0.49 ± 0.40	0.59 ± 0.12	0.56 ± 0.07	38.63 ± 3.86
Day-15	0.73 ± 0.07	0.41 ± 0.11	0.78 ± 0.15	0.71 ± 0.13	28.84 ± 1.06
Average (%)	0.99 ± 0.47	0.96 ± 0.52	0.99 ± 0.31	0.97 ± 0.27	48.21 ± 22.84

Table 2. Mean (± SD) organic matter concentration (%) in *Holothuria sanctori* experimental substrates over the experimental period. Day-x: timing of sediment sampling in days relative to the start day (Day-0) of the experiment. Each sampling event represents the average OM percentage in each treatment. The OM was estimated by percentage (%) based on the differences in dry weight of samples before and after muffle burning. SD: standard deviation.

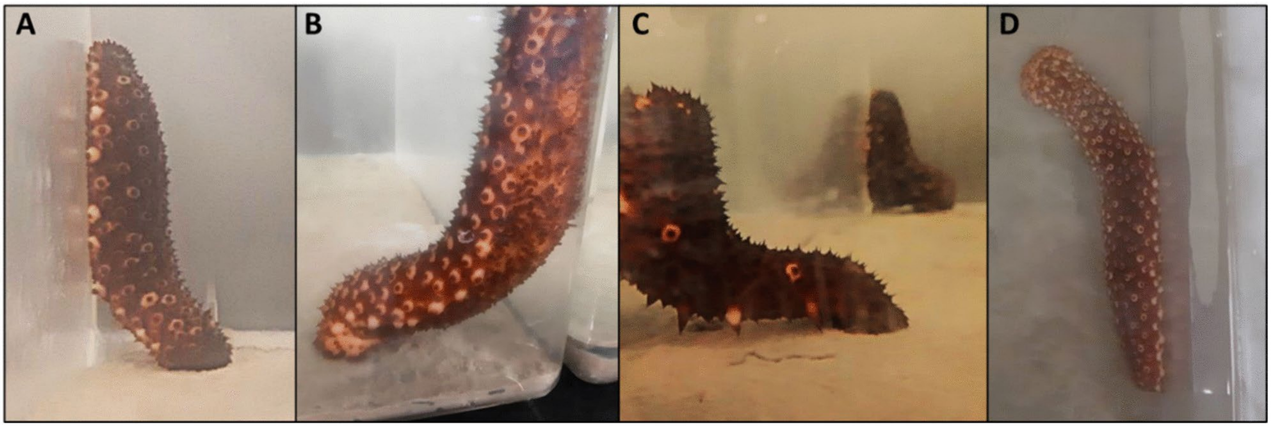

Fig. 2. *Holothuria sanctori* specimens adhered to the experimental hard substrate (tank walls) during active feeding on the soft substrate. (**A**–**D**) Sea cucumbers with their bodies partially attached to the tank wall, while the anterior section, including the mouth, extends toward and feeds on the soft substrate. Note that (**A**–**C**) are side views, whereas (**D**) is an upper view, showing the sea cucumber attached to the right side of the tank as it feeds on the soft substrate (100–250 µm).

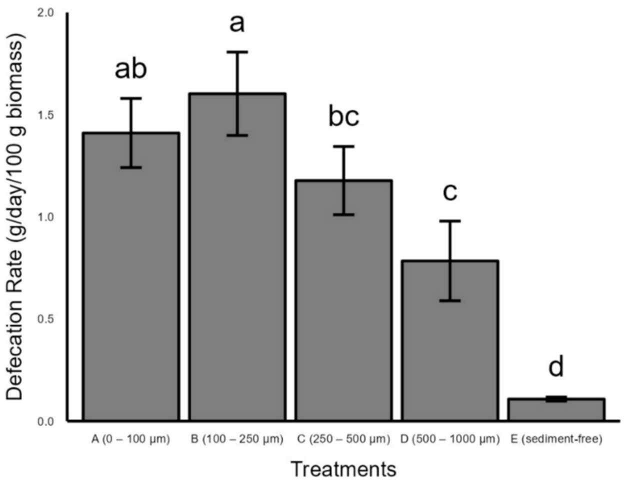

Fig. 3. Mean (± SE) defecation rates (g/day) per 100 g (WW) of *Holothuria sanctori* across experimental treatments. (SE: standard error. WW: wet weight. Different superscripts indicate significant differences (MANOVA; N = 15; $p < 0.05$) between treatments).

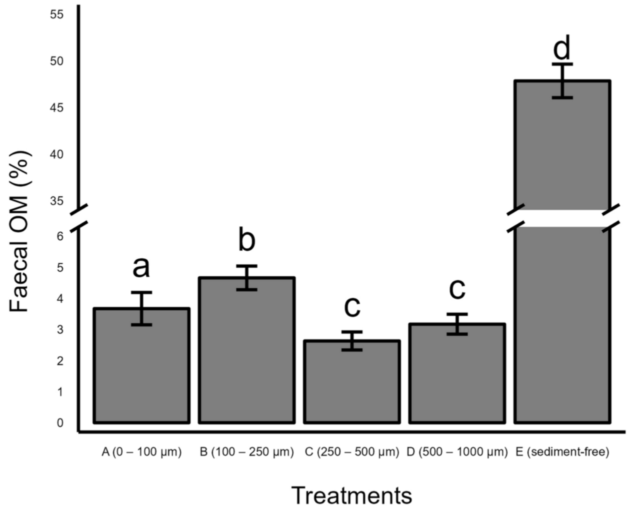

Fig. 4. Organic matter concentration (mean ± SD; %) in *Holothuria sanctori* faeces across different experimental treatments. (SD: standard deviation. Different superscripts indicate significant differences (MANOVA; N = 15; $p < 0.05$) between treatments. The broken axis graph was used to enhance the visualization of the data due to the significant difference in the OM values between treatments).

(< 250 µm) may be due to physiological adaptation that enables the species to ingest and process particles within this small size range. *Holothuria sanctori* has a long, thin gut, which is likely adapted to process fine enriched particles settling on hard bottoms[30]. The association of some deposit-feeding sea cucumbers with small particles has been linked to the high nutritional value of fine substrates[16,22]. A higher surface area to volume ratio in small sediment particles leads to greater colonization by microflora such as bacteria and microalgae[49,50], which can enhance the energy output, taking into account the cost/benefit energy from the ingestion, digestion and assimilation processes.

The species' behaviour in different trophic conditions is a species-specific trait and is regulated by the feeding strategy employed by the sea cucumber species as reported in several studies[16,17,51–55]. The species *H. polii* and *H. tubulosa* co-habitants of *H. sanctori* display a distinct feeding strategy, showing less particle selectivity[31], while ingesting greater quantities of sediment to meet their energy requirements[30]. Conversely, the deposit-feeder *H. forskali*, shows a similar feeding strategy to that of *H. sanctori*, being a highly selective species for fine sediment particles and OM[11,31]. Consequently, both species are generally segregated in different habitats, with *H. sanctori* occurring in shallow hard substrates (up to 30 m), while *H. forskali* is also found in deeper waters (up to 150 m), associated with both soft and hard substrates[30,31].

By combining data from the current study on I. species defecation rate, II. OM selectivity, and III. OM availability in the substrate, surprisingly, *H. sanctori* could maintain a relatively constant OM concentration in their faeces throughout the experiment in all the treatments. As the availability of OM decreases in the substrate over time, the ingestion rate increases accordingly to compensate for reduced resource quality and fulfil energy requirements. This compensatory feeding behaviour likely explains the observed increase in sediment processing (defecation rate) with decreasing organic matter availability. Regardless of the nutritional conditions of the substrate, this behaviour appears to help maintain a relatively constant intake of organic matter, thus stabilizing its concentration in faeces. This relationship can be visualized as a hexasterisk (✱), where each of the three variables: defecation rate, OM selectivity, and OM availability, forms a line radiating from the centre. As OM availability decreases (one axis), the defecation rate increases (second axis), while OM selectivity (third axis) adjusts to maintain equilibrium, ensuring stable OM intake. To our knowledge, this steady selection ability of OM has not been reported in other deposit-feeding species. However, the trade-off between ingestion rate and organic content is acquainted[22]. When there is a limitation in the OM availability in the substrate, sea cucumbers tend to increase their food intake or enhance their food assimilation to meet their nutritional requirements[5,22,48]. The inverse correlation between organic and sediment intake was demonstrated in this study, especially in large particle-size groups (PS: 250–1000 µm). In treatment C, the ingestion rate exceeded that of treatment D but the selectivity (faecal OM content) in treatment D was higher than in treatment C. This could be seen as a trade-off pattern among these two treatments to obtain the required energy. However, in fine particle size groups (PS: PS < 250 µm), this trade-off pattern was less pronounced, as specimens seem to behave ideally in terms of their food intake and OM selection abilities, as discussed earlier.

In the hard substrate group (treatment E), it is possible that the high availability of OM in the foraging substrate resulted in enhanced OM selectivity. In this sediment-free group (treatment E), there was a very high OM availability (pure feed), which potentially facilitated the food assimilation at the lowest energy cost and the highest OM return to meet the nutritional requirements. Therefore, the food intake ratio was very low in this treatment compared to the other treatments, taking into account the absence of inorganic particles. These findings lead us to hypothesize that *H. sanctori* can adjust its food intake ratio according to the OM selective capability from the substrate which can be affected by the OM availability (content). When sufficient OM level is present in the substrate, this species selects the organic particles, minimizing the sediment ingestion ratio. Otherwise, when OM becomes scarce, *H. sanctori* increases its food ingestion while maintaining its OM selectivity until the nutritional requirements are fulfilled.

This study represents a significant advancement in understanding the feeding behaviour of *H. sanctori*, highlighting its capacity to process organic matter across various particle sizes. The species' high OM selectivity and adaptability to different granulometries increase its potential for diverse applications, including Integrated Multi-Trophic Aquaculture (IMTA), where it can serve as an extractive species to process waste from higher trophic level species, such as finfish and shellfish. Deposit-feeding sea cucumbers are increasingly recognized as promising candidates for such roles[56–58]. Recent studies have explored the potential for co-culture and bioremediation with Mediterranean sea cucumbers[57,59–64], emphasizing the importance of understanding interspecific capabilities when selecting the ideal extractive species. Critical factors such as habitat preference, OM processing capacity, and energy flows within aquaculture systems are essential for this selection. As a highly selective species, *H. sanctori* may be particularly suited to processing aquaculture waste, typically rich in OM. However, to fully assess its potential in these systems, further research is needed on its tolerance to aquaculture-enriched conditions, bioremediation capabilities, and growth performance. Additionally, the presence of hard substrates appears to be essential for the survival and feeding of *H. sanctori*, reflecting its suitability for integration into land-based IMTA systems, where hard substrates like concrete ponds or tank walls are available. In offshore IMTA systems, artificial hard structures or biogenic substrates, such as hard dead shells beneath cultivated shellfish, could also be utilized.

Lastly, further investigation into the feeding strategies and preferences of low-trophic extractive species is crucial for advancing sustainable aquaculture applications. Understanding species-specific capabilities and requirements will help diversify candidate species and optimize their ecological and practical roles in these systems.

Conclusion

In conclusion, this study demonstrates the significant role of sediment granulometry in shaping the feeding behaviour of *H. sanctori*, particularly its sediment ingestion and organic matter selection capacity. The species exhibits a high sediment reworking efficiency, especially in fine particulate sediments between 100 µm and 250 µm in diameter, while also processing a broader range of particle sizes from 0 to 1000 µm. These findings suggest that microhabitat conditions primarily determine the size of ingested particles in natural environments. Additionally, *H. sanctori* displays a remarkable ability to selectively extract OM, balancing organic matter availability with sediment ingestion to meet its energy requirements.

Data availability

Data is provided within the manuscript and the supplementary information file.

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Author contributions
MM: Conceptualization, Methodology, Investigation, Formal analysis, Data curation, Writing—original draft; LG: Methodology, Writing—review and editing; DP: Writing—review and editing; LC: Writing—review and editing; AF: Writing—review and editing; SV: Writing—review and editing, Supervision; SC: Supervision, Writing—review and editing, Funding acquisition; AR: Conceptualization, Methodology, Funding acquisition, Writing—review and editing, Supervision, Project administration. All authors reviewed the manuscript.

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Declarations

Competing interests
The authors declare no competing interests.

Human and/or animal participation
All experimental specimens were handled in accordance with ethical guidelines for animal welfare. The water quality parameters were maintained under optimal conditions during transportation, maintenance, and experimental periods. The number of sampled specimens was limited to the number specified in the experimental design and in compliance with the local authority permission delegated by Ministero dell'Agricoltura, della Sovranità Alimentare e delle Foreste (MASAF). All the collected specimens were used for scientific and experimental purposes only.

Additional information
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