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Use of Sediment Geochemistry and Video Analysis for habitat description of the sea cucumber *Holothuria (Roweothuria) poli*

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Abstract

The increasing commercial value of holothurians has triggered considerable scientific interest. The aim of the study was to investigate *Holothuria (Roweothuria) poli* populations and sediment characteristics in two contrasting coastal areas in the same geographic region. The first (S1) consists of an exposed sandy, low organic material and well-oxygenated seabed. The other (S2) is sheltered, with a silty sediment, high labile organic matter, and negative redox values. *H. poli* populations were studied monthly by means of ROV survey while sediment samples were collected seasonally. At S2, holothurians were significantly more abundant and with higher biomass. In both areas, seasonal patterns were observed, with S1 presenting a peak of density and biomass during the summer months, while in S2 the peak was recorded in spring. The *H. poli* distribution seemed to be determined by environmental and biological factors, including availability of food resources, redox regime in the sediments and spawning-related behaviour.

Keywords: Holothuroidea; benthic habitat; sediment variables; ROV; population density.

Introduction

Sea cucumbers (Echinodermata; Holothuroidea), are widespread marine benthic invertebrates that have been recognized for both their ecological and economic importance for decades (Hickman *et al.*, 2006; Purcell *et al.*, 2016). There are around 170 known species of *Holothuriidae* globally, and are found in both shallow and deep seas (Hamel *et al.*, 2022). Currently, the great demand for and the high market price of sea cucumber impact its significant exploitation and fishery expansion (Purcell *et al.*, 2013; Toral-Granda, 2008; Yang *et al.*, 2015). Their sedentary nature aids the ease of collection, and the lack of effective fishery management has also contributed to the rapid collapse of endemic and commercial natural populations, especially in Indo-Pacific Asia (Gianasi *et al.*, 2021; Toral-Granda, 2008). Because of the demanding global market, sea cucumber fisheries have expanded since the 1990s, even in the Mediterranean Sea (González-Wangüemert *et al.*, 2015). At present, sea cucumber fishery in the Mediterranean region, which has become a profitable and important fishery component in recent years, is exported mainly to the Asian markets (Aydin, 2017; Boncagni *et al.*, 2019; González-Wangüemert *et al.*, 2019).

Sea cucumbers are sediment feeders, their diet consisting of diatoms, bacteria and particulate organic matter (Uthicke & Karez, 1999). *Holothuria tubulosa* and *Holothuria poli*, the two most abundant and commercially exploited holothurian species in the Mediterranean Sea (Gonzalez-Wangüemert *et al.* 2019) inhabit areas with soft substrate, mainly in areas close to seagrass meadows, due to accumulation of organic material (Aydin, 2019). Also, sea cucumbers are active bioturbators and can significantly affect both the composition and the oxygenation of the sediments, therefore preventing anoxic conditions (Chatzivasileiou *et al.*, 2024). Also, their feeding behaviour contributes to the resuspension of nutrients to the water column and enhancing microbial activity (Tsikopoulou *et al.*, 2023). In addition, they are considered capable of mitigating the effects of climate change on the sediment, especially in coral reef areas, by contributing to the calcium carbonate cycle (Buccheri *et al.*, 2019).

Holothuria poli, one of the 37 sea cucumber species widely distributed in the Mediterranean Sea (Aydin, 2017; Rakaj *et al.*, 2018), is one of the most targeted and commercially exploited species in the global seafood trade (Friedman *et al.*, 2011; González-Wangüemert *et al.*, 2019). This species lives predominantly in soft bottom sediments and muddy substrates in shallow to deep waters, not under 40m depth, particularly in areas where seagrass detritus accumulates intensively —in the North Atlantic Ocean, Red Sea, Mediterranean Sea, and Biscay Bay (Aydin, 2019; Boncagni *et al.*, 2019). Juveniles tend to dwell in shallow waters while the heaviest and largest individuals were observed in unfished and deeper areas (González-Wangüemert *et al.*, 2019). The sea cucumber *H. poli* is listed under species of the least concern (LC) in the IUCN (International Union for the Conservation of Nature) Red List of Endangered Species, however its populations have been subject to continuous fishing in recent years. The majority of the commercial exploitation of sea cucumbers has been carried out in Turkey since 1996 (Aydin, 2008), but processing of *H. poli* began only in 2011, which contributed to the increase in total sea cucumber production in the country (Aydin, 2017). It has been estimated that around 80% of the 7.2×10^5 to 1.08×10^6 individuals harvested daily in Turkey is of the species *H.* poli is exported (González-Wangüemert *et al.*, 2019). This significant exploitation of sea cucumbers, particularly this species, has raised global concerns given its important ecological roles in bioturbation, bioremediation, nutrient recycling, local buffering, and symbiotic associations in the trophic chain (Boncagni *et al.*, 2019; MacTavish *et al.*, 2012; Purcell *et al.*, 2016; Zamora *et al.*, 2018). Furthermore, the unregulated harvesting of the species with insufficient management initiatives poses a risk to its natural stocks, similar to what has happened in several fishing grounds in Asia (Rakaj *et al.*, 2018; Sellem *et al.*, 2019).

Despite its ecological and economic values, little is known about *H. poli* ecology and habitat preferences in the Mediterranean basin as there are few studies on its natural populations (Mezali *et al.*, 2014). This knowledge gap on species ecology, particularly in relation to sediment geochemical properties, should be filled for the effective management of the species in nature. The scarce information on the biology and ecology of the target species hinders the stock management initiatives needed for its conservation and sustainability (González-Wangüemert *et al.*, 2019).

Meanwhile, technological advancement has facilitated various types of assessments that provide a wide array of research opportunities for ecological studies. To fill the information gaps mentioned above, the present study aims to describe temporal variability in sediment characteristics and population density of *H. poli* through the use of sediment geochemistry and underwater video analysis in two sites with distinct topographical characteristics in Agios Nikolaos, Crete, Greece. Our study is the first attempt to compare and elucidate environmental conditions relative to *H. poli* populations in the mentioned sites, which is a fundamental component for establishing fishery management practices, species conservation, restocking, and facilitating polyculture perspectives in the Mediterranean aquaculture industry.

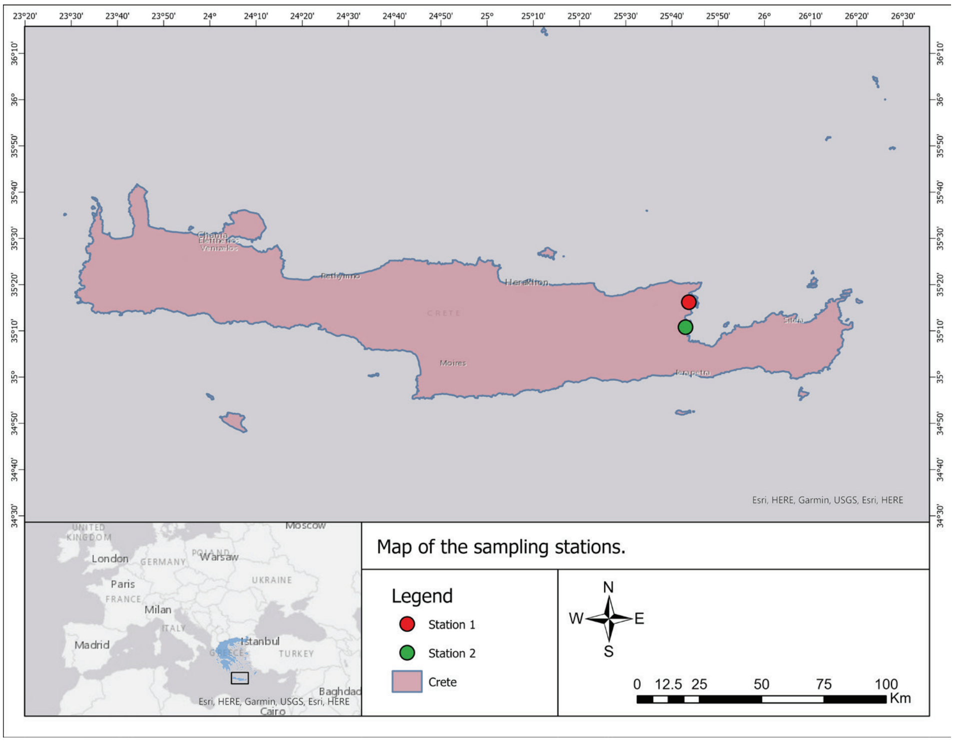

Fig. 1: Sampling stations of the two Holothurians population in the coast of the Agios Nikolaos in Crete, Greece.

Materials and Methods

Study areas and sampling

The current study was conducted in the Agios Nikolaos region, a coastal area in the eastern part of Mirabello Bay, Cretan Sea, Greece (Fig. 1). Mirabello Bay is situated in the South Aegean Sea, Eastern Mediterranean Sea (EMS), an oligotrophic system considered less productive due to its arid climate (Psarra *et al.*, 2000). Field sampling was conducted on a monthly basis for the year 2021. Every sampling started at 11.00 am and ended no later than 14.00. Samples taken each month for the population and sediment quality variables at the two stations were analyzed, as shown in Table 1. The selection of the two sampling stations was based on their different environmental characteristics (sediment type, hydrological parameters and position). Exact coordinates of the sampling stations are not disclosed according to the permission terms granted by the Agios Nikolaos local authorities, since the area is of archaeological significance and any activities including diving and fishing are not permitted.

Station 1 (S1) (Fig. 2a) was situated on an open shore 50 m from an uphill rocky coast and 1 km from a river inlet; adjacent to a touristic area with popular beaches, it has a salinity range of 36-46psu, measured during every sampling event by means of a hand refractometer (A. Krüss Optronic GmbH), and a sandy bottom substrate inhabited by the seagrass *Cymodocea nodosa*, which has a wide and random distribution at shallow water depths (4-4.5 m). Station 2 (S2) was situated 20m from the coast in an enclosed bay with numerous small islands separated by narrow passages. The bottom substrate is muddy, and is also dominated by the seagrass *C. nodosa* with random distribution at 4-4.5m depth. In addition, S2 (Fig. 2b) is a prehistoric archaeological site where fishing is banned. Hence, a special permit was obtained from the Archeology Authorities of Lassithi to carry out the study in this area. Salinity in S2 (measured as above) ranged from 40 to 44 psu. The map of the sampling stations was generated using QGIS software version 3.14 and Google

Table 1. Experimental measurements calendar.

Month	**Population variables**			**Sediment variables**		
	Station	**Population Density**	**Size measu-rement**	**Granulo-metry**	**Labile OM%**	**Redox potential**
Jan	S1/S2	✓/✓	✓/-	-/-	-/-	-/-
Feb	S1/S2	✓/✓	✓/✓	✓/✓	✓/✓	-/-
Mar	S1/S2	✓/-	✓/-	✓/-	✓/-	✓/-
Apr	S1/S2	✓/✓	✓/✓	-/-	-/-	-/-
May	S1/S2	✓/✓	✓/✓	✓/✓	✓/✓	-/-
Jun	S1/S2	✓/✓	✓/✓	✓/✓	✓/✓	✓/✓
Jul	S1/S2	✓/✓	✓/✓	-/-	-/-	-/-
Aug	S1/S2	✓/✓	✓/✓	-/-	✓/✓	✓/✓
Sep	S1/S2	✓/✓	✓/✓	-/-	-/-	-/-
Oct	S1/S2	✓/✓	✓/✓	✓/✓	✓/✓	✓/✓
Nov	S1/S2	✓/✓	✓/✓	-/-	-/-	-/-
Dec	S1/S2	✓/✓	✓/✓	-/-	-/-	-/-

Fig. 2: 4 Underwater photos from 4 snapshots at Station 1 (A). Each transect was divided into four sub-sections of equal length and each snapshot is a moment of these sub-sections. The same at Station 2 (B), that have been taken with a Parallenz camera on the ROV.

earthPro. As may be seen in Figure 2, the benthic habitat in S1 is more heterogenous (including patches of hard substrate, sandy and silty bed, seagrasses) than S2 which is uniformly silty with sparse cover by *Cymodocea*.

Sediment quality parameters

SCUBA divers collected sediment samples by inserting corer samplers (4.5 cm internal diameter) up to 10 cm deep in the sediment. Analysis of the vertical redox potential profile (Eh) was carried out using a *Unisense Field Microprofiler* as described in Chatzivasileiou *et al.*, (2024). This instrument uses a 200 μm microelectrode to provide a detailed vertical profile by taking measurements every 0.5 cm in the first 5 cm of the sediment samples. Three replicated corers were measured at each station.

Similarly, for sediment geochemistry, three replicated samples were collected at each station. For organic matter content analysis measured through the loss on ignition (LOI) method (Loh, 2005), sediment samples were separated into four layers (0–1, 1–3, 3–5, and 5–10 cm). Sectioned samples were placed into zip-lock bags and freeze-dried at -54°C. Dried sediments were homogenized, and aliquots of 0.5 g sediments were combusted in a muffle furnace, cooled, and reweighed. The bioavailable organic matter (labile OM%) was calculated by the weight loss of the dried sample after initial combustion at 250° C for 16 hours, and refractory organic matter (refractory OM%) after further combustion for 16 hours at 500° C.

Sediment characterization was carried out through the granulometric analysis method (Gray & Elliott, 2009). Two replicates of sediment samples were collected from both stations. Sampled sediment samples were weighed, washed, and filtered through a 63 μm mesh to separate the silt and clay fraction from the sand. Separated sections of sediments were dried in the oven at a temperature of 70° C for 2 days. The dried sand fraction was then sieved using stratified stainless sieves of decreasing geometric scale (Wentworth classification scale). Measurements were then used for the determination of median particle diameter (MD), sorting coefficients, and silt-clay and sand fractions percentage.

Population parameters

The population density of *H. poli* in the sampling stations was analyzed mainly through underwater video techniques using a mini remotely operated vehicle (ROV) (Blue Robotics ROV2) equipped with a 150 m cable, gamepad controller, and two video cameras (thHe HD ROV camera and a Parallenz dive 4k resolution camera). In each sampling event, individuals from the population were collected and identified as *H. poli* from their morphology. As described in Moussa & Wirawati (2018), *H. poli* has cylindrical bodies tapering anteriorly, brown coloration with lighter ventral side. Also, the scattered white dorsal papillae with dark brown bases are distinct for this species (Moussa & Wirawati, 2018).

The ROV was operated through QGround Control, an open-source application with configuration settings that act as a user interface providing a live video stream, compass, movement speed, and dive time. The ROV also provides live measurements of depth and water temperature. The accurate establishment of video transects was done through the deployment of the ROV with an attached floating GPS, able to take precise locations (±5 m). The starting and ending coordinates of each transect were fixed and recorded every time with the floating GPS. Upon reaching the sea bottom, the ROV started moving a few inches from the substrate, alongside the coast at a 4 to 4.5m depth, at a maintained velocity of 0.1-0.2 m/s, with a constant heading for a distance of 50 m.

Video outputs were then analyzed for the quantification of all individuals in every video transect. The Parallenz field view is known to be 150 ^{o}, from the camera specifications, however extrapolation to area was avoided due to field view distortions. Therefore, population density was expressed as number of individuals per transect (50 m) similarly to the methodology described by Raoult *et.al.*, (2020). The distribution pattern of the population was assessed by dividing each transect into four equal-length sub-sections. In more detail, since the transect length and speed was constant each time, the recordings were divided into fousr equal parts (codenamed a,b,c,d), corresponding to four equal spatial segments.

The need for considerable information regarding population parameters and characteristics cannot be met solely through video techniques; therefore, complementary observation is recommended (Mallet & Pelletier, 2014). Characteristics of the body size measurements of *H. poli* species in the area were determined by collecting at least *n*= 10–15 individuals via SCUBA diving in each station during every sampling event. After the collection, the animals were left out on the ground for a few minutes to lose the excess water, and then were measured using a two-decimal weighing balance for the weight (g) and a calliper for the length (cm). At the end of each sampling, individuals were released back gently to the sea.

Statistical analyses

The transcription of data and creation of graphical images were achieved through Microsoft Excel and GraphPad_Prism version 8.0 software. Data regarding sediment variables and population parameters between months for each station were presented as mean ± standard deviation (SD). Variability between stations and months on the variables recorded was analyzed using two-way analysis of variance (ANOVA) after variables were checked for assumptions of normality using Shapiro-Wilk test and Levene's test. In case of significant interaction between the factors "station" and "months", a between-subjects effects analysis was conducted. Significant differences were further analyzed through pairwise comparisons using *post-hoc* analysis (Türkiye's test). The above-mentioned statistical analyses were performed using SPSS v26.

Results

Sediment variables

Granulometry

The granulometry results of sediment samples taken from S1 (Table 2) for four months showed an average percentage sand fraction of 94.33%; the remaining 5.66% was attributed to silt & clay. For median grain size (MD) analysis, the particle size of an average of 0.13±0.04 mm was recorded, while the sorting coefficient (σ1), which represents the degree of mixing of sediments through wave and current action, varied between months but had an average value of 0.87±0.30.

Granulometry results in S2 showed an average silt-clay fraction of 73.5%; the remaining 26.5% was attributed to sand. The median grain size (MD) of sampled sediments had an average size of 0.04±0.02, and the sorting coefficient (σ1) had an average value of 1.27±0.26 (Table 2).

For sediment quality variables, there was an interaction between the two factors (Station and Months) for Eh and labile OM% data (Table 4), indicating differences within months. Pairwise comparison of Eh and labile OM% showed that geochemical variables were different at each sampling month for most sediment layers (Table 3), except for Eh in the first cm layer in July and November.

Redox potential

Redox values met the assumption of normality (Shap-

Table 2. Granulometry indicators for sediments taken in S1 and S2 in each sampling month.

Granulometry	Stations	Feb	Mar	May	Jun	Nov	Mean±SD
MD	1	0.19	0.15	0.11	0.11	0.11	0.13±0.04
	2	0.02		0.05	0.05	0.03	0.04±0.02
σ1	1	0.91	1.1	0.57	0.55	0.37	0.87±0.30
	2	0.93		1.20	1.44	1.50	1.27±0.26
Silt & clay (%)	1	5.34	7.79	3.67	5.01	6.5	5.66±1.39
	2	83.96		69.70	68.39	71.94	73.49±7.13

Table 3. Two-way ANOVA and pairwise comparison of biogeochemical variables that were taken in two stations in different months.

Eh		***Comparisons for factor: S1 vs. S2 within month***				
Station* Month interaction	**	***Feb***	***May***	***Jun***	***Aug***	***Nov***
0cm		-	-	*ns*	*	*ns*
-0.5cm		-	-	**	*	*ns*
-1cm		-	-	**	**	*ns*
-1.5cm		-	-	**	**	**
-2cm		-	-	**	**	**
-2.5cm		-	-	**	*	**
-3cm		-	-	**	**	**
-3.5cm		-	-	**	*	**
-4cm		-	-	**	*	**
-4.5cm		-	-	**	*	**
-5cm		-	-	**	**	**
Labile OM%						
Station* Month interaction	**					
0 to-1cm		**	**	**	**	**
-1 to -3cm		**	**	**	**	**
-3 to -5cm		**	**	*	**	**
-5 to -10cm		**	**	**	*	*

*: $p < 0.05$, **: $p < 0.01$, ns: non-significant

iro- Wilk Stat= 0.949, p>0.05) and homogeneity of variance (Levene's test: F= 1.502, p>0.05). Sediments in S1 were well oxidized in most sampling months; in contrast, S2 had negative Eh values below the top 2 cm. Despite that difference, Eh varied significantly seasonally in both stations, with the lowest Eh values in August in both areas (Table 3). Comparison of Eh per different sediment layers between the two stations (ANOVA results at Table 3) showed that S2 had consistently lower values than S1 during all sampling periods investigated.

In S1, for most months, the Eh values for the top 5cm were positive, meaning that the surface layer sediments were well oxidized (Fig. 3a). However, during August, S1 sediment had negative Eh values at sediment layers beneath 1.5 cm, indicating anoxic conditions. Analysis of variance between months showed that Eh values changed seasonally ($p < 0.05$) at all depths (0 to -5 cm), with the highest positive Eh values in March. The apparent decrease with depth in recorded Eh values was observed in the first months, from March, June, and August, but then started increasing again in November.

Results for sediment quality variables in S2 are shown in Figure 3b. Redox profiles generally showed a decreasing trend with sediment depth (0 to -5 cm depth). However, a low redox potential was recorded in S2, creating a hypoxic-anoxic condition in sediments (Fig. 3b). Higher Eh values in the first centimetre were recorded in June, while the lowest were found in August. Also, redox potential was observed to decrease from June to August and then increase in November in S2.

Organic matter

Organic matter values met the assumption of normality (Shapiro- Wilk: Stat = 0.987, p>0.05) and homogeneity of variance (Levene's test: F= 1.612, p>0.05). A comparison of labile OM% values between two stations (S1 vs. S2) in each sampling month showed that the two stations had different ($p < 0.001$) amounts of labile OM, which also varied seasonally (Table 3). In S2, the sediment was organically enriched, with the OM values being almost four times higher than that of S1 (Fig. 4).

The highest mean value of labile OM % in S1 from 0 to -10 cm was recorded during March at 0.66±0.16, followed by August at 0.61±0.09, and the lowest in October at 0.19±0.06. More specifically, the labile OM% in S1 showed differences [F_{stat}=4.22, $p < 0.019$], from -1 to -3 cm depth in March vs October and August vs October, and at -3 to -5 cm depth [F_{stat}=5.03, p=0.010] in August vs February, respectively. However, labile OM% in S2 had the highest value in February (4.14±0.31) and the lowest in June (2.88±0.40) (Fig. 4b).

Population parameters

The between-subjects effects analysis indicated a significant interaction between the "Station" and "Month" factors ($F_{stat} = 4.5$, $p < 0.01$), meaning that there were differences within subjects. For each month, between the two stations (S1 vs S2) population density was different from February to June and again in November. Also, comparison of weight between stations at each month showed that in April, June and July, holothurian biomass from S1 was higher than that in S2 (Table 4 & Fig. 5).

Comparison of the mean values of population density between months showed a statistically significant difference [$F_{stat} = 12.61$, $p < 0.001$], with the highest population density recorded in August (0.26 ind. m^{-2}) and the lowest found in October (Fig. 5). However, in S2, population density also seemed to change with time [$F_{stat} = 12.88$, $p < 0.05$], with the highest density recorded in April (4.17±0.88 ind. m^{-2}) and the lowest in September (Fig. 5).

Moreover, the distribution of *H. poli* in S1 was patchy, with a higher occurrence of the population at the last section of the transect (part d), (Fig. 6a), presenting an increasing pattern from the first section (part a) for most of the months. On the other hand, in S2, the observed population was randomly distributed across the transect

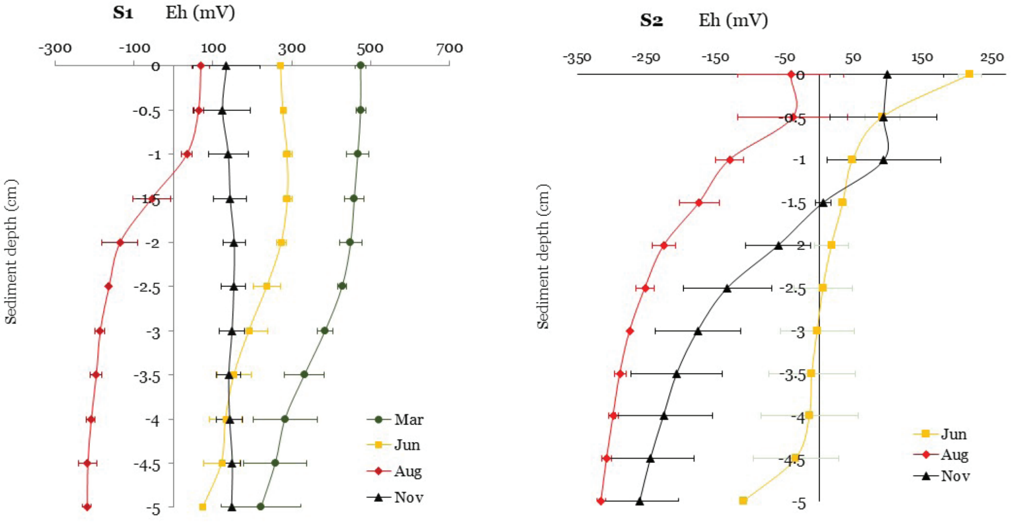

Fig. 3: Plots of redox potential (Eh) measured in the top 5 cm of sediments in stations S1 (A) and in S2 (B) during various sampling months.

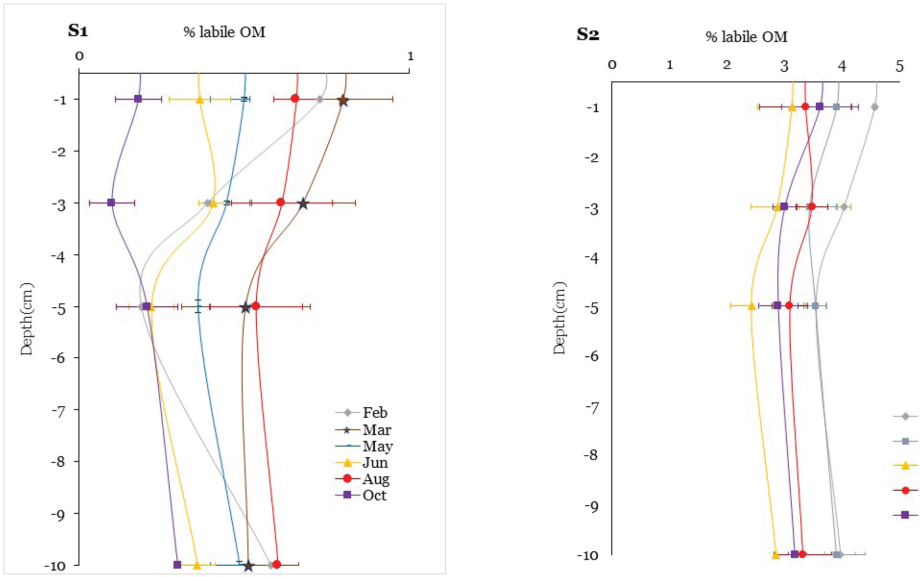

Fig. 4: Plots of labile organic matter (Labile OM %) measured in the 10 cm of sediments in Station 1 (A) and Station 2 (B) during sampling months.

Table 4. Pairwise Comparison of holothurian population parameters between the two stations for each sampling month.

	Station *Months interaction	Comparisons for factor: S1 vs. S2 within month										
Population parameters		Jan	Feb	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Pop. density (ind. m^{-2})	**	-	S1 < S2	S1 < S2	S1 < S2	S1 < S2	S1 < S2	ns	ns	-	S1 < S2	-
Weight (g)	*	ns	ns	S1 > S2	ns	S1 >S2	S1 > S2	ns	ns	ns	ns	ns

*: $p < 0.05$, **: $p < 0.01$, ns: non-significant

Table 5. Pairwise comparison of holothurians weight from S1 between months.

	Jan	***Feb***	***March***	***Apr***	***May***	***Jun***	***Jul***	***Aug***	***Sep***	***Oct***	***Nov***
Feb	ns										
March	*	*									
Apr	*	*	ns								
May	ns	*	*	*							
Jun	*	*	ns	ns	*						
Jul	*	*	ns	ns	*	ns					
Aug	*	*	*	ns	*	*	ns				
Sep	ns	*	*	*	ns	*	*	ns			
Oct	ns	ns	*	*	ns	*	*	*	ns		
Nov	*	*	*	ns	ns	*	ns	ns	ns	ns	
Dec	ns	ns	*	*	ns	*	*	*	ns	ns	*

* : $p < 0.05$, ns : non-significant

locations (Fig. 6b).

Regarding holothurian size, in S1 the average length of sampled individuals was 7.9±1.5 cm, and the mean weight recorded was 58.8±19 g throughout the sampling duration (Fig. 7a). ANOVA showed a significant difference in weight recorded in some months ($p < 0.001$), with the heaviest individuals recorded in June (78.1±16 g), and the lowest weight in February (38.8 g). S2 individuals had a mean weight of 53.3±14 g and an average length of 7.9±1 cm. Heavier individuals were recorded in the month of August (63.3±1 g) and lighter individuals in May (43.9±0.99 g) (Fig. 7b). Comparison between sam-

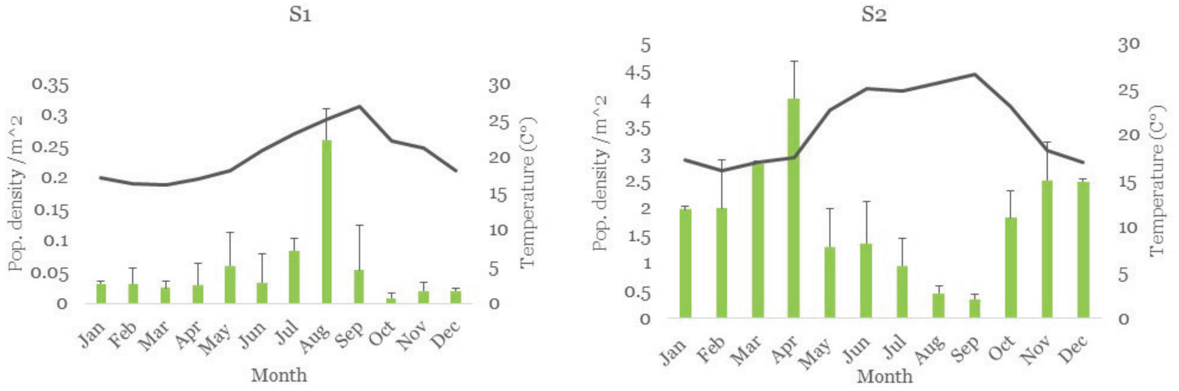

Fig. 5: Population density (bars) and water temperature (line) measured per month at S1 and S2 respectively. Please note difference in scale for the population density axes among the two graphs.

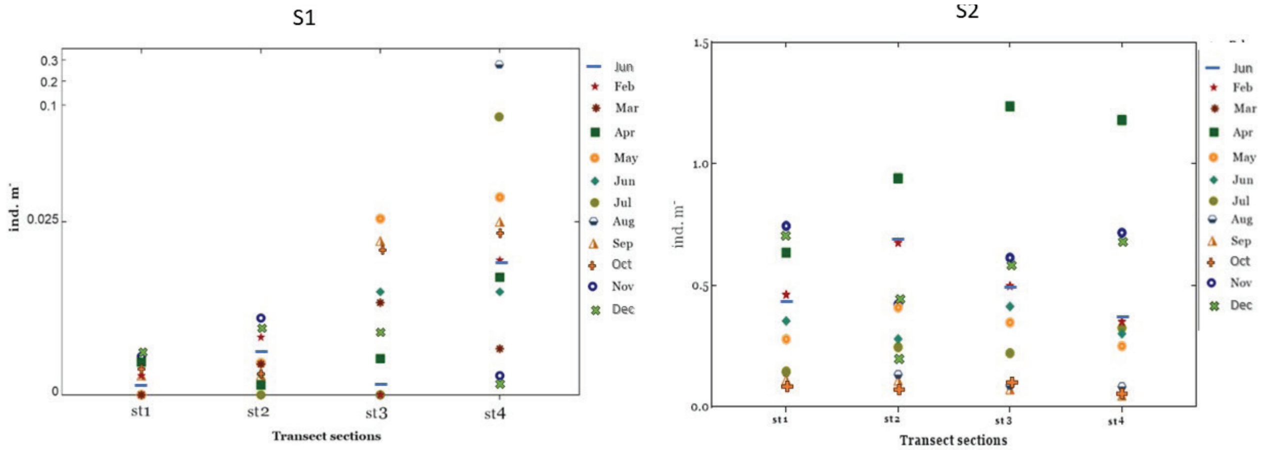

Fig. 6: Population distribution per transect section (a-d) recorded every month in S1 (A) and in S2 (B).

pled months in S1 showed that in summer months, the holothurians' weight increased (Fig. 7a).

Discussion

Knowledge of holothurian's habitat characteristics and their influence on benthic communities is considered to be important for ecosystem-based fisheries management (EBFM) (Purcell *et al.*, 2016). Videos collected using ROV surveys are becoming increasingly important in marine research (Grane-Feliu *et al.*, 2019), but have mainly focused on fish species. In this study we employed well established sediment geochemical techniques along with non-destructive seafloor ROV videos in order to couple complementary sources of information. Our results showed that even in a relatively small distance from each other, two areas populated by *H. poli* showed remarkable variation in environmental attributes, population densities and behavior of the animals in the field.

Behavioral aspects of organisms within the population are modified by their surroundings and are very often related to interactions with food resources (Lohrer *et al.*, 2004), but also holothurians can significantly impact the benthic trophic conditions by altering the biochemical composition and nutritional quality of organic matter

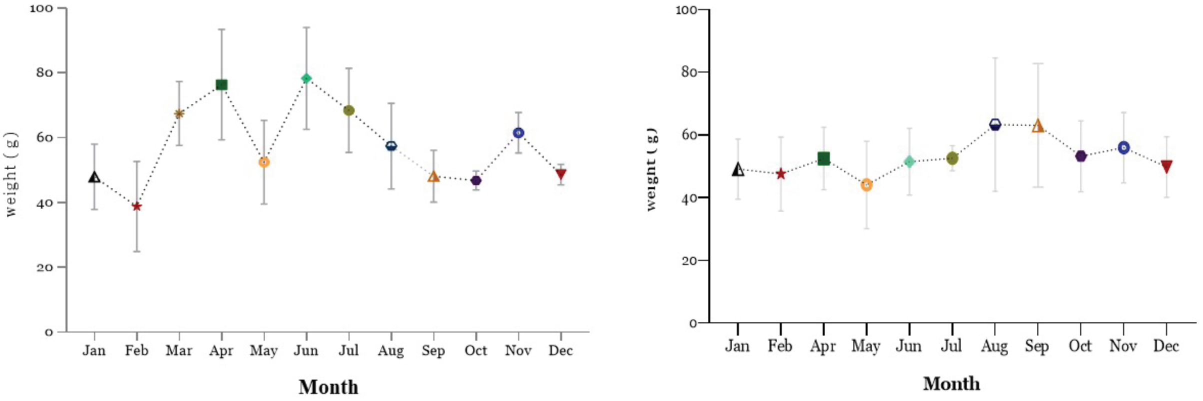

Fig. 7: Average Weight of holothurians (g ind-1, ± SD) recorded monthly over a year period in S1 (A) and in S2 (B).

(Pasquini *et al.*, 2023). Thus, sediment features are the most important factors characterizing holothurians habitat and affecting species ecology (Lohrer *et al.*, 2004). Several studies have been conducted demonstrating the increase of organic matter decomposition and oxygenation of the sediment from sea cucumbers (MacTavish *et al.*, 2012; Slater & Jeffs, 2010; Zamora *et al.*, 2018; Chatzivasileiou *et al.*, 2024). Evaluations of population structure and densities of *H. poli* have been described in previous studies (Mezali *et al.*, 2014; Sellem *et al.*, 2019), but little emphasis has been given to sediment characteristics. Our findings are compatible with those of (Mezali *et al.*, 2014), who compared the digestive tract contents of holothurians with the ambient sediment and found that *H. poli* selects fine sediment fraction. They attributed this preference to the higher percentage of organic matter that coats fine sediment particles. Feces of holothurians were organically enriched when compared with the ambient sediments, indicating the holothuroids' ability to extract essential nutrients from the sediment, contributing to their overall nutritional intake (Ennas *et. al.*, 2024). Holothurians may selectively consume finer particles to maximize their energy efficiency and optimize their feeding strategies.

Varius holothurian species have been associated with different types of sediment (Purcell *et al.*, 2016), mostly sandy, coral reef lagoons and seagrass beds, whereas a few have been shown to colonize silty substrates. In our present study, the silty sediment site (S2) was found to have a significantly higher abundance and biomass of *H. poli* specimens (Fig. 5). It is known that some sea cucumbers totally or partly bury beneath the sediment surface (Altamirano *et al.*, 2017; Mercier *et al.*, 2000). Animal-sediment relations are interactive, i.e. the holothurian activity pushes sand and silt aside, favoring mixing surface and subsurface sediment layers (Purcell *et al.*, 2016), but sea cucumbers are also attracted to fine organic-rich sediments, ingesting and defaecating remarkably high quantities of sediment (9-82 kg ind^{-1} y^{-1} according to (Purcell *et al.*, 2016).

According to our findings, most individuals in the less organically enriched site (S1) clustered in specific zones, with only a small number being found in seagrass meadows. This site had a population density significantly lower than that of S2. The population of S1 aggregated around rocks. These rock formations (crevices, holes, etc.) were covered by macroalgae and created protected areas where organic matters, composed mostly of seagrass detritus, accumulated. In contrast, in the sheltered S2, *H. poli* individuals were scattered widely throughout the extent of the area. *H. poli* preference seemed to be a soft-muddy substrate (Aydın & Erkan, 2015) with a high amount of labile OM%. In both areas sampled, holothurians were found in sparce seagrass meadows, indicating a potential ecological relationship. According to (Coulon & Jangoux, 1993), *H. poli* is very often completely lined with a thin layer of sand and *Posidonia* parts, which makes the animal almost invisible. A mucous film secreted by the skin attaches sand and leaves to the body. A study by (Boncagni *et al.*, 2019) showed *H. poli* prefer to consume seagrass detritus, which constitutes 63% to 74% of its diet composition, although it has been reported that sea cucumbers are unable to utilize either seagrasses or macroalgae and therefore do not compete with macroherbivores (Robinson *et al.*, 2015). Seagrass detrital pathways attribute significantly to the life of this species. In this context, as a deposit feeder, (Purcell *et al.*, 2016; Zamora *et al.*, 2018) *H. poli* facilitates labile OM% decomposition and algal productivity; however, its grazing on microphytes has been reported to have a negative impact on the biomass of primary producers (MacTavish *et al.*, 2012).

High labile OM% concentration is associated with food resources for deposit-feeders like *H. poli*. In a previous study on the feeding activities of five Mediterranean sea cucumber species, *H. poli* was observed to have the least selectivity with regards to organic matter ingestion (Mezali *et al.*, 2022); perhaps this is one of the underlying reasons driving the abundant population observed in S2. Moreover, in the study of (MacTavish *et al.*, 2012), increased efflux in the presence of the sea cucumber *Australostichopus mollis* was found to facilitate algal productivity, which highlights the activities of the species influencing the bacterial abundance intended for remineralization, which, in turn, provides a mechanistic explanation of redox variation. In addition, as a bioturbator, *H. poli* also affects several biogeochemical properties, which include the stimulation of bacterial population and recycling and the mixing of sediments (MacTavish *et al.*, 2012; Zamora *et al.*, 2018).

Regarding seasonal variation, a decrease of labile OM% during summer and an increase during winter were observed in both stations. The decrease in summer could be explained by the labile OM% decomposition rate and direct temperature relationship, where an increase in temperature drives several chemical processes and multiple enzymatic reactions from billions of microorganisms, thus increasing sediments' metabolic processes (Arndt *et al.*, 2013; Fazi *et al.*, 2005). Another potential explanation could be that the reproduction activities of *H. poli* during summer and perhaps labile OM% consumption was greater for gonad development. Moreover, the mobility of these benthic organisms over seasons might also transport nutrients via excretion and feeding (Hou *et al.*, 2018).

The population dynamics of benthic communities are generally influenced by two parameters: food availability and larval dispersal (Bachelet, 1990). Dispersion of the larvae presumably would take farther places in the open shore area, like in S1, lowering the recruitment of the existing population (Mercier & Hamel, 2009). The highest monthly variation in population density was observed during summer in S1; more specifically in August. Also, most of the holothurians had gained weight in the summer months, probably because it was the beginning of the breeding season. Sea cucumbers are gonochoristic species with an annual reproductive cycle (Leite-Castro *et al.*, 2016a). Their reproductive cycle is synchronized with the annual sea temperature cycle, with spawning occurring between July and September (Kazanidis *et* *al.*, 2014). *H. poli*, as broadcast spawners, mates should be proximate for the successful fertilization of gametes (Mercier & Hamel, 2009). So, a few days before spawning, holothurians tend to aggregate in high frequencies to help synchronize gametogenesis and fertilization (Leite-Castro *et al.*, 2016b). In other words, holothurians throughout the year should be dispersed as possible to exploit organic content of a particular sediment area, but during the reproduction/spawning period, they should aggregate in high densities to enhance reproductive success. This rule is compatible with the pattern observed in S1. However, in S2, the environment is quite different i.e. enclosed, with limited turbulence and oxygenation, high productivity and sediment organic content, all contributing to extremely low Redox values (negative even at the sediment-water interface). Under these conditions, the dense population of holothurians in S2 should probably avoid too high levels of concentration or at least lower than the ones they can sustain during cooler months of the year. As shown in Figure 5, the densities at S2 during August -September are the minimal ones unlike the pattern observed at S1. However, it should be noted that during summer months, the minimum density at S2 is still higher (more than double) than the corresponding one at S1 (e.g., for August S1 42 and S2 90, for September S1 25 and S2 62 ind/transect).

It is unclear if the high population density in S2 (during April) is also be related to reproduction behavior and larval dispersion. In Mezali *et al.* (2014) study on *Holothuria sanctori,* a high intensity of spawning was recorded in a nutrient-rich environment like S2. Holothurians release their gametes in the water column, where fertilization takes place (Kazanidis *et al.*, 2014), and for few weeks, holothurian larvae remain in the planktonic phase (Rakaj *et al.*, 2018). Therefore, in an enclosed, slow-water-renewal regime and low energy area such as S2, larvae would settle relatively close by and thus increase the recruitment capacity in the established population. The stability of a species' population is influenced by the species' recruitment capacity; thus, successful recruitment enhances the population integrity.

The variation in redox values recorded between different months could be attributed to complex factors, including temperature, linked to labile OM% decomposition by bacterial communities (Karakassis *et al.*, 1998), mobility of organisms, feeding, and excretion activities of *H. poli* (Levinton*, 1972)*. High densities of sea cucumbers influence the O_2 concentration, even at elevated temperature levels (Lee *et al.*, 2017). Sea cucumbers as bioturbators are known to be capable of oxygenating sediments and enhancing productivity in benthic ecosystems (Purcell *et al.*, 2016; Chatzivasileiou *et al.*, 2024). However, in S2, redox was very low, especially in August, inducing a hypoxic-anoxic condition, which could explain the low density recorded during August and September. *H. poli* feeding and bioturbation efficiency may not be enough to prevent these anoxic conditions in S2. On the contrary conditions in S1 were constantly oxic, while the increase in water temperature was delayed and occurred in August.

Holothurians in the Mediterranean have the ability to modify the composition and nutritional quality of sedimentary organic matter across different trophic conditions and temperatures, including high-temperature regimes (Ennas *et al.*, 2024). Holothuroids' feces consistently show organic enrichment, even under extreme sea temperature levels (26–29 °C), like the ones in Agios Nikolaos region, suggesting that they can continue feeding and potentially function as bioreactors during marine heatwaves (Ennas *et al.*, 2024). Holothuroids could be an effective means for mitigating benthic eutrophication, even in warming seas, though their efficiency may depend on seasonal variations in metabolism and sediment quality (Wang, *et al.*, 2008; Günay *et al.*, 2015; Chatzivasileiou *et al.*, 2024).

In summary, our study found that there were distinct differences between the two *H. poli* populations, which reflect the differences in sediment characteristics at those two stations. From the results, it seems that sea cucumbers in a muddy and organically enriched area (S2) were more evenly distributed and with a high population density. In contrast, in an open shore area (S1) with low labile OM%, the population density was low and the distribution spatially aggregated. However, despite the differences, holothurians seemed to respond to seasonal changes with the similar behavioral patterns.

Conclusion and recommendation

The spatiotemporal distribution of *H. poli* seemed to be determined by a combination of environmental and biological factors, mainly:

a. availability of food resources: In both cases, the organic material showed maximum concentrations during winter and early spring, which gradually decreased towards summer months after the consumption by benthic organisms, including *H. poli*;
b. Redox regime: This was determined by the availability of organic material, benthic bioturbation and resuspension by wave action, which was considerably higher at the S1 site and less so in the sheltered/confined S2. *H. poli* were able to tolerate rather low redox conditions but were apparently stressed when negative values reached the sediment surface. They are known to dig into the sediment, although not deeply;
c. Seasonal population density changes: It is known that *H. poli* and several other holothurian species are known to spawn during summer months, and during this period they develop their gonads, which later in the year pass to a resting stage. During the pre-spawning period, the individuals use aggregative behaviour to maximize reproductive success and synchronize with phytoplankton bloom to optimize larval feeding. Studying the preferences for holothurian species will provide very useful information on these bioturbators, which significantly affect seabed geochemical properties, but will also help the technical design of Integrated Multitrophic Aquaculture systems.

As mentioned before, *H. poli* is currently listed as a species of *Least Concern* on the IUCN Red List of Threatened Species. However, its population and habitat should be regularly studied to obtain accurate data on fishing pressure and to detect early signs of overfishing. The use of ROV methods could provide an easy and accurate tool for collecting data on the population's exploitation level. Furthermore, future research should focus on spatial distribution and seasonal variability of holothurian species to assess their habitat preferences and behavioral responses. Further investigation is needed to address the influence of other environmental variables —such as temperature, hydrodynamism, substrate heterogeneity, and anthropogenic pressures, on the reproductive behavior of the species, providing insight into population structure and dynamics. Expanding the geographic scope of studies will also be crucial for capturing broader ecological gradients and for assessing regional consistency or variability in observed patterns. Such efforts will contribute to a more holistic understanding of ecosystem functioning and resilience.

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