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BIOLOGIA MARINA

Cambiamenti degli ecosistemi marini profondi nel mar Mediterraneo orientale: un confronto di 30 anni (1989 vs 2023)

Ecosystem shifts in the deep Eastern Mediterranean Sea: a comparison across 30 years (1989 vs 2023)

Tesi di Laurea Magistrale

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Riassunto

L'oceano profondo è un ambiente unico ed estremo che supporta un grande range di organismi marini. Al contrario di quanto si credeva, è vulnerabile ai cambiamenti climatici. L'aumento delle temperature, l'acidificazione degli oceani e i cambiamenti nella circolazione oceanica possono alterare la distribuzione e la diversità delle specie, con potenziali conseguenze per l'intera catena alimentare.

Il presente studio vuole investigare i cambiamenti nella meiofauna negli ambienti marini profondi del Mediterraneo orientale rispetto a 30 anni prima, in relazione al contenuto di materia organica. Lo studio confronta dati ottenuti nel 2023 con i risultati di precedenti ricerche condotte nel 1989 per esaminare i cambiamenti nella biomassa, abbondanza e biodiversità della meiofauna.

I risultati evidenziano un aumento della materia organica labile presente nei sedimenti che ha portato a un leggero aumento dell'abbondanza della meiofauna, ma a un cambiamento significativo nella struttura della comunità, con i nematodi che sono diventati dominanti e i policheti che sono diminuiti.

Sono comunque necessari ulteriori studi per comprendere meglio le cause di questi cambiamenti e il loro impatto a lungo termine sugli ecosistemi marini profondi nel Mar Egeo.

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1. Introduction

The deep sea is defined as the ocean lying below 200 m-deep, generally beyond the depth of continental shelves and no primary production by photosynthesis, and it is the largest ecosystem on Earth. The deep sea may be viewed as an extreme environment for life, with an absence of sunlight below 1,000 m in depth, low temperature and high pressure (average 400 atmospheres) (IUCN, 2019). Deep-sea ecosystems (excluding hydrothermal vents) are thought to be extremely stable compared with coastal environments since temperature and salinity remain almost constant with increasing water depth (Danovaro et al., 2001).

Organisms inhabiting this extreme environment have developed various adaptations to overcome challenges, such as biochemical constraints caused by high pressure and low temperature. Brown and Thatje (2014) found that deep- sea benthic organisms have altered enzyme structures to cope with these difficulties. However, these enzymes that are resistant to high pressure make slower the overall metabolism of the animals (Macdonald, 2021). The implications of a slower metabolism may pose issues when faced with disturbances, such as those resulting from human activities that alter the environment. (Danovaro et al., 2017).

The deep ocean is less affected by anthropogenic greenhouse gas emissions than the surface ocean, as it is relatively isolated from changes in temperature, ocean acidification, and mixed layer depth. However, recent research has shown that there are direct and rapid connections between the surface and deep ocean. The main source of food for deep-sea organisms is organic matter sinking from the surface due to productivity levels. The volume of this export productivity is influenced by different factors (temperature, upwelling areas, nutrient supply, mixed layer depth, size, and type of organic matter), all of which are impacted by climate change. The amount and type of export productivity play a crucial role in determining the abundance and diversity of benthic species (Corliss et al., 2009). The arrival of detritus in the deep sea varies depending on latitude, with higher latitudes experiencing episodic influx events that bring large amounts of resources, while lower latitudes have a more constant flux due to steady surface temperatures and low primary production (Billett et al., 1983). For instance, in regions with seasonal organic carbon input, opportunistic species can thrive, leading to lower diversity. Conversely, tropical areas with consistent organic carbon input have higher species diversity and a more even distribution of species (Corliss et al., 2009).

1.1 Ecosystem shifts in the deep sea

The deep sea faces two main challenges: indirect effects of anthropogenic climate change, such as ocean currents shifts, acidification, and warming, and direct human impacts like increased fishing, oil drilling, and deep-sea mining (Glover and Smith, 2003). Climate change poses the biggest threat, impacting ocean environments globally. While the deep sea is not immune to these effects (O' Brien et al., 2021), limited long-term data makes it difficult to assess ecosystem changes. Even so, research suggests deep-sea ecosystems are vulnerable to climate-induced species shifts (Brito-Morales et al., 2020), challenging the assumption that the deep sea is less susceptible to climate change threats. Available projections suggest that by 2100, temperatures at abyssal depths (3000–6000 m) could increase by 1°C and contribute to reductions in water column oxygen concentrations (Sweetman et al., 2017).

Temperature plays a crucial role in determining the distribution of marine organisms, a positive correlation between temperature and species richness has been documented in shallow waters (Trittensor et al., 2010), while in the deep sea, a complex relationship between temperature and diversity has been observed (Yasuhara and Danovaro, 2016). At low temperatures (<∼5°C), diversity tends to increase, while at high temperatures (>∼10–15°C),

diversity decreases. This relationship follows a unimodal pattern when considering a wide temperature range. Nevertheless, temperature is likely to have a substantial impact on diversity at temperatures above (>∼10–15°C) and below (<∼5°C), while showing less influence at intermediate temperatures.

Deep-sea organisms are more vulnerable to changes in temperature compared to those in coastal areas, as deep-sea temperatures tend to stay consistent over time. For instance, it has been observed in the deep Mediterranean Sea that even slight shifts in temperature as small as 0.1°C can have a significant impact on the biodiversity and composition of deep-sea nematode assemblages, as it was observed a positive linear relationship between the magnitude of temperature shifts and changes in species richness (Danovaro et al., 2004). Nematodes account for more than 90% of the benthic organisms in deep-sea marine sediments. They have a short lifespan of only a few weeks and do not go through a planktonic larval stage. Because of this, nematodes are not exposed to temperature fluctuations as much as longer-lived organisms, but they are still greatly affected by changes in temperature (Lambshead, 2004), (Danovaro et al., 2009). This is because they are ectothermic organisms, meaning they rely on the surrounding environmental temperature to regulate their own body temperature (Glauser, 2022).

So, changes in ocean temperature are expected to directly affect the distribution and diversity of deep-sea species. Furthermore, there may be indirect effects on deep-sea ecosystems, such as through changes in primary production (Rogers, 2015).

In fact, recent model projections indicate that global primary production and export of POC are expected to decrease in the future (Rogers, 2015). This suggests that in the deep sea, there may be a decrease in POC flux at low to mid-latitudes, while high latitudes could see an increase in flux along with changes in seasonality. These projections align with models suggesting that export flux to the deep ocean may increase in polar regions but decline elsewhere, potentially impacting deep-sea benthic biomass, which, consequently, are likely to alter the size structure of deep-sea communities (Rogers, 2015). With the projected effects of climate change, including increased CO2 emissions, it is likely that surface primary production patterns will be altered, leading to significant changes in deep-sea biota (Smith et al., 2013). The warming of the ocean is linked to widespread ocean hypoxia or anoxia in deep waters, as well as ocean acidification and the release of hydrogen sulfide (H2S) into shallow waters. (Rogers, 2015).

Climate change has two primary physical effects on oxygen levels in the oceans. Firstly, warmer water holds less oxygen, and secondly, stratification

inhibits the mixing of surface and deeper waters, decreasing oxygen flow throughout the ocean. Additionally, the reduced nutrient injection from deep waters to the euphotic zone due to stratification can limit primary production and the sinking of particulate organic carbon below the euphotic zone. (Rogers, 2015). Another issue to consider is the expansion of eOMZs, which leads to an increase in the water volume where microbial denitrification and anaerobic ammonium oxidation occur. These processes convert nitrogen in the form of ammonium or nitrates to nitrogen gas and nitrous oxide (N2O), a potent greenhouse gas. This can create a positive feedback loop in terms of global warming. On the other hand, the decrease in biologically available nitrogen, specifically nitrates, may also reduce primary production, leading to lower microbial oxygen consumption and potentially causing hypoxia in the water column (Rogers, 2015). Additionally, the decrease in primary production contributes to a positive feedback loop that accelerates the rate of CO2 increase by reducing the uptake of CO2 through the biological carbon pump. Numerous studies have been conducted in the deep sea of the Pacific, revealing that El Niño events in the eastern Pacific lead to rising sea surface temperatures which deepen the mixed layer, decrease nutrient concentrations in surface waters, and reduce primary production and organic material export to the seabed. Consequently, deep-sea benthic ecosystems are likely to experience more oligotrophic conditions during these warming events due to

changes in plankton food-web structure. Recent research in the North Pacific has found that since the late 1970s, increased sea surface temperatures (1.5–3°C) have impacted surface productivity, resulting in a decrease in organic matter reaching sediments. These studies suggest that climate change has a more significant impact on benthic oxygen levels than changes in ocean circulation patterns (Danovaro et al., 2001).

In regards of the Mediterranean Sea, there has been a general warming trend observed in water temperatures over the past 30 years, with an increase of approximately 0.12°C likely due to greenhouse gas-induced global warming. On the other hand, in the nineties, the eastern Mediterranean experienced the opposite effect, as a response to regional variability in atmospheric forcing. Changes in deep waters in this region occurred in two phases: the first phase, between 1987 and 1992, saw the formation of dense, relatively warm water in the south Aegean (known as the Cretan Deep Water) due to increased salinity. The second phase, from 1992 to 1994, saw a drop in deep-water temperature of around 0.4°C, resulting in even denser deep-water formation. As a result, the previous eastern Mediterranean Deep and Bottom Waters were raised by a few hundred meters, creating a separate nutrient-rich intermediate-water layer known as the Transitional Mediterranean Water. This water layer, influenced by cyclonic circulation, moved closer to the surface at depths of 100-150 meters, near the euphotic zone.

These alterations have the potential to greatly impact the development and survival of deep-sea organisms, and, at the end, inhibit the recovery of deep-sea communities. Finally, the anticipated rise in temperature and decline in oxygen and pH levels in the deep ocean due to current climate change trends could exacerbate the negative effects on the metabolism of deep-sea organisms, especially because they seem to be more susceptible to shifts in environmental conditions compared to shallow-water counterparts (Danovaro et al., 2017). Because of its nature, the eastern Mediterranean Sea is used as a model for how oceanic circulation can be affected in similar environments (Danovaro et al., 2001).

1.2 Benthic response to ecosystem shifts in the deep sea

The benthic response to climate change depends on local surface ocean conditions so it can differ depending on location (O'Brien et al., 2021). Despite the lack of detailed information, it is generally accepted that temperature plays a role in influencing the biogeography of deep-sea communities at large scales. Some studies suggest that there may be a relationship between species diversity in benthic ecosystems and temperature, with diversity peaking at certain temperatures. This relationship is weaker at higher temperatures, with changes in diversity only being

observed at lower temperatures around 15°C. Moreover, thermal energy has a strong impact on biological communities at microscopic scales, while available chemical energy has a significant influence on processes at macroscopic scales. Smaller organisms are generally more resilient to temperature changes than larger organisms, which may explain why small species are able to persist through extinction events (Danovaro et al., 2017). But, for example, nematodes, which are the most abundant metazoans in deep-sea ecosystems, are particularly sensitive to temperature shifts (Danovaro et al., 2017). Indeed, although temperature changes in the deep sea occur slowly, even gradual warming can have a negative impact on biodiversity and a decrease of 0.4°C in temperature was found to affect nematode communities by increasing biodiversity and decreasing abundance. (Danovaro et al., 2004).

Another factor that can bring changes in benthic communities is the shifting in the quality of phytodetritus reaching the deep seabed. The composition of phytoplankton in an ocean basin may change even if primary productivity remains constant and this phenomenon, observed in various regions such as the North Sea, is linked to rising sea surface temperatures. These changes can affect the balance between primary production and community respiration, as well as influence trophic interactions within ecosystems.

In regards of changes in oxygen levels, studies have shown that decreased oxygen levels in benthic communities typically result in lower species diversity. Additionally, protozoans and meiofauna tend to thrive in oxygen minimum zones (OMZs) due to the abundance of organic material and possible protection from predators, leading to higher population densities.

As for the Mediterranean Sea, while our knowledge of the Eastern basin remains limited, Danovaro et al. (2001) found that a decrease in meiofaunal abundances was observed following a climate-driven cooling of the water. Whereas studies in the Western Mediterranean have shown that temperature changes can have a significant impact on deep-sea benthic fauna. In this region, macrofauna populations include reproductively sterile pseudo populations that rely on larval inflow from colder deep Atlantic Ocean populations. The high temperature and salinity of the Mediterranean bottom waters make it difficult for species from colder oceans to successfully establish themselves. Similar temperature-induced changes have been observed in other parts of the Mediterranean, such as the Ligurian and Tyrrhenian Seas. These changes include the emergence of new macrobenthic pseudo populations and the extinction or introduction of certain species, all of which have been attributed to climate influences.

2. Aims of the study

The scope of the present study is to investigate if there are any changes occurring in the Eastern Mediterranean deep sea in terms of meiofauna biomass, abundance, and biodiversity comparing the values observed 30 years ago, in relation to organic matter content. This study compares data on the biochemical composition of organic matter and meiofaunal assemblages collected in 2023 with the results reported in "Meiofauna of the deep Eastern Mediterranean Sea: distribution and abundance in relation to bacterial biomass, organic matter composition and other environmental factors" (Danovaro et al., 1995).

3. Materials and methods

3.1 Study area: the Eastern Mediterranean Sea

The Eastern Mediterranean region is outlined by the Ionian Sea, the Libyan and Levantine Seas and the Aegean Sea (Otero, 2022). Its deep-sea floor has various unique geological features such as continental slopes, submarine canyons and landslides, seamounts, cold seepages, hydrothermal vents, bathyal or basin plains with abundant deposits of muds and the deep-hypersaline anoxic basins (Otero, 2022). Additionally, the Eastern

Mediterranean experiences the formation of intermediate water during winter in the Levantine basin, and during summer stratification, a thermocline forms around 50m, with a consistent temperature below 100m ranging from 13-14.5°C.

The present study was carried out in the Eastern part of the Ionian Sea and southwestern part of the Cretan Sea (South Aegean, Eastern Mediterranean) (Figure 1). The Cretan Sea occupies a significant portion of the Aegean Sea and is situated between the islands of the Cyclades plateau and the Cretan arc. This area is known for being extremely oligotrophic, with primary production in the Aegean Sea estimated at only 20.3 gC m^{-2} y^{-1} (Dugdale and Wilkerson, 1988), and likely even lower in the South Aegean. (Tselepides and Eleftheriou, 1992).

3.2 Sampling strategy and sediment sampling

In April 2023 sediment samples were collected on board of the *R/V Aegaeo* in 11 stations (Table 1) selected along an eastern-western transect located North of Crete at a depth ranging 500-2500 m (Figure 1).

Table 1- Stations, coordinates and depths

Station	Latitude (N)	Longitude (E)	Depth (m)
A22	35° 52.1878'	25° 07.2311'	1878
A21	35° 51.0872'	24° 50.2085'	1531
A20	35° 56.0398'	24° 35.9534'	1075
A18	35° 55.4503'	24° 14.1755'	1007
A 16	36° 06.3051'	23° 43.4051'	593
A14	36° 02.7339'	23° 25.2083'	1280
A12	36° 01.0548'	23° 16.0727'	660
A7	35° 55.4945'	22° 56.2560'	570
A6	35° 55.2275'	22° 48.8064'	966
A4	35° 52.2190'	22° 52.1396'	1315
A5	35° 53.6187'	22° 36.5885'	2454

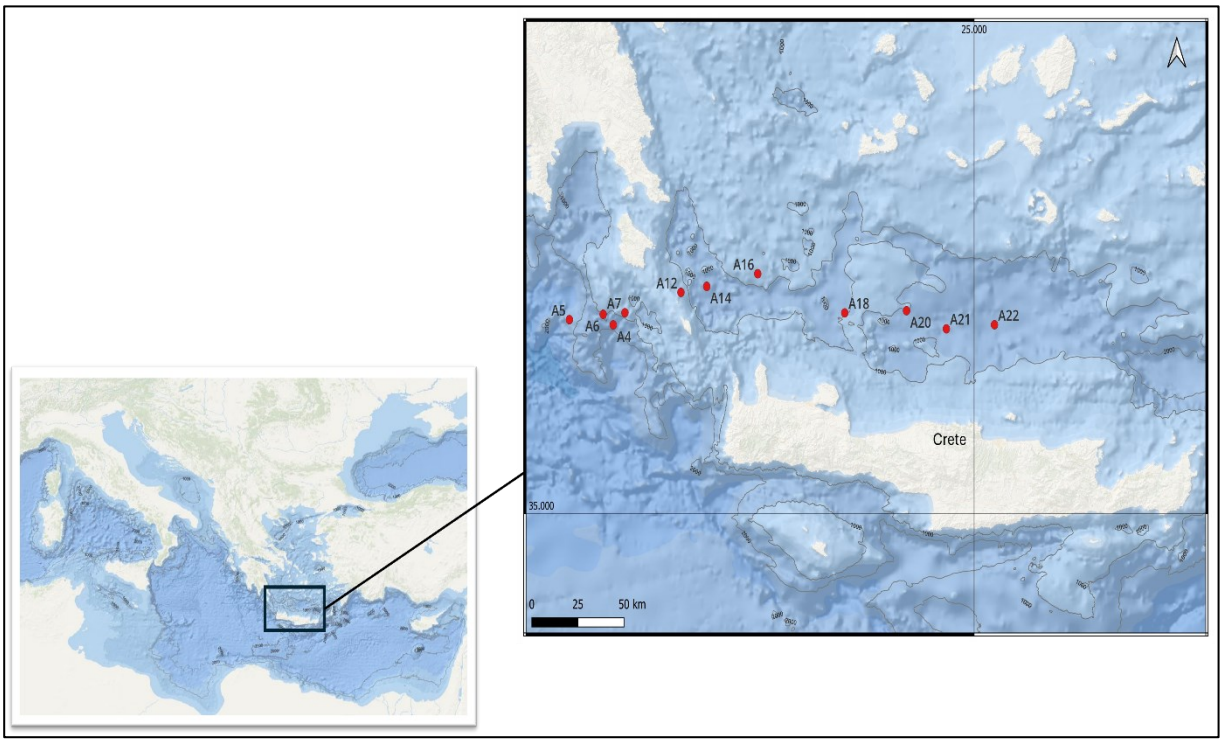

Figure 1- Map of the study area. Reported is the location of the stations along the eastern-western transect off Crete.

Samples were collected using a box corer (Figure 2), except for station A18 where a multicorer was used.

Figure 2- Box corer sampler before the deployment in situ

The box-corer, hermetically closed, penetrated approximately 40cm into the seafloor and retrieved undisturbed samples with clear water on top of the sediment surface. For each station three replicates were collected.

3.3 Environmental variables

Data on water potential temperature and salinity were collected close to the sediment through the CTD. Additional data on surface chlorophyll-a concentrations and potential bottom water temperature were obtained from datasets provided by Copernicus, by selecting the area of study. These datasets were chosen for analysis due to the absence of data from 1989 in the study area, allowing for a comprehensive comparison of environmental factors. Comparison of temperature data from both the CTD and Copernicus datasets revealed negligible differences. Despite this, it was determined that the data from Copernicus would be utilized for consistency in the study.

3.4 Phytopigments and biochemical composition of organic matter

A sub core (a plexiglass tube with a 5 cm inner diameter and 20 cm length) was taken from the centre of each box corer and then horizontally sectioned in layers (0-1cm, 1-3cm, 3-5cm, 5-10cm, 10-15cm) obtaining a total of 160 samples that were put in plastic containers of 60 ml and 120 ml and frozen at -4°C until analysis in the laboratory.

Photosynthetic Pigments

Chlorophyll-a and phaeopigment were analysed according to Lorenzen and Jeffrey (1980): the pigments were extracted from surface sediments (0-3cm) using 5 ml of 90% acetone solution. The extracts were then examined fluorometrically to assess the chlorophyll-a and, after acidification with 200 µl of 0.1 N HCl, to measure phaeopigments.

Sediment Proteins

The content of proteins was determined following the methodology proposed by Hartree (1972). The sediment was placed in test tubes to which were added three reagents.

To start, 0.9 ml of Reagent A were added to the test tubes. The tubes were then placed in a hot water bath at 50°C for ten minutes. Next, 0.1 ml of Reagent B was added to the tubes, which were then left to rest for another ten minutes. Finally, 3 ml of Reagent C were added to the tubes, followed by another ten minutes in a hot water bath at 50°C.

After the incubation steps, the test tubes were centrifuged for 15 minutes at 800 x g. Once the centrifugation was complete, the absorbance of the samples was measured at 650 nm using a spectrophotometer.

Sediment Carbohydrates

Total carbohydrates concentrations were determined following the method proposed by Gerchacov and Hatcher (1972): after the sediment was dried and weighted, 1 ml of reagent water was added to the test tubes and vortexed. Afterwards, 1 ml of 5 % distilled phenol was poured and in a second moment another 5 ml of sulphuric acid. At the end of the treatment, the test tubes were centrifugated for 30 minutes at 800 x g. Finally, the absorbance was measured at 485 and 600 nm using a spectrophotometer.

Sediment Lipids

Lipids content was determined using the Blight and Dyer (1959) and Marsh and Weinstein (1966) methodologies. After treating the sediment samples with 1 ml of reagent water and exposing them to ultrasound treatment, 1.25 ml of chloroform and 2.5 ml of methyl alcohol were added, and the samples were left to rest for 10 minutes at 4°C. After centrifugating the test tubes for another 10 minutes at 800 x g, the supernatant was transferred in Pyrex tubes and 1.25 ml of chloroform together with 1.25 ml of reagent water were poured. The tubes were centrifugated another time for 5 minutes and, after removing the supernatant, they were placed in a hot dry bath (100°C) till evaporation. In a second moment, 2 ml of sulfuric acid were added, and the tubes were placed again in a hot dry bath (200°C) for 15 minutes. After

cooling down, 3 ml of reagent water were added to the carbonized samples. Finally, the absorbance was measured spectrophotometrically at 375 nm.

Labile Organic Matter (LOM)

The LOM was defined as the sum of all sugars, carbohydrates, proteins and lipids, expressed in mg/g.

Biopolymeric Carbon (BPC)

The obtained concentrations of carbohydrates, proteins and lipids were transformed into carbon equivalents using the coefficients 0.40, 0.49 and 0.75 μg C μg-1 respectively (Fabiano et al., 1995).

Furthermore, the percentage of total phytopigments to BPC was determined by calculating the algal contribution, after converting phytopigments into carbon equivalents using a mean value of 40 μg C μg-1 phytopigment.

3.5 Meiofauna

A sub core (a plexiglass tube with a 2.5 cm inner diameter and 20 cm length) was taken from the centre of each box corer and then horizontally sectioned in layers (0-1cm, 1-3cm, 3-5cm, 5-10cm, 10-15cm) obtaining a total of 160 samples that were put in plastic containers of 60ml and 120ml and frozen at -4°C until analysis in the laboratory.

Meiofauna was extracted following the protocol by DANOVARO (2009): the samples were sonicated three times for one minute and sieved through a 500 µm mesh size into a Backer. The content of the backer was then filtered through 40 µm and 20 µm mesh size sieves. The remaining fraction of the sediment was put in a 50 ml falcon mixed with Ludox TM and centrifugated three times for 10 minutes at 3000 rpm (800 x g). After each centrifugation the supernatant was sieved again through the sieve. At the end of the three centrifugations, the material collected on the sieve was fixed in a 50 ml tube with 70% ethanol together with few drops of Bengal Rose colouring to stain the meiofaunal organisms.

Meiofauna identification to the order level was carried out by sieving the content of each falcon through a sieve and the residues were placed inside of a cuvette made of 20 columns and 10 rows. All the present organisms were counted and identified under a Leica stereomicroscope (3.5X).

In 1989, meiofaunal abundances were measured over a 6 cm sediment depth. To align with this method, 10% of the total abundances obtained in 2023 over a depth of 10 cm were deducted. This decision was influenced by findings from Danovaro et al. (1995), which suggested that meiofaunal abundance typically constitutes around 10% of the total abundances beyond 6 cm.

The meiofaunal biomass was derived by using the volumetric method, which consists in extrapolating organism dry weight from volumetric estimates. These can be obtained from body length (L) and width (W) of each individual, using the equation proposed by Warwick and Price (1979):

$$V = C\ L\ W^2$$

Where C is the conversion factor specific for each taxon.

As for nematodes, they were first moved and mounted on slides after formalin–ethanol–glycerol treatment to prevent dehydration (Danovaro et al., 2009), and then measured following the same process as above but under an optical microscope (40X) (Fig. 3) and following the formula proposed by Andrassy (1956):

$$V = L\ W2\ 0.063 \times 10^{-5}$$

V is expressed in nl (10^{-9} l) while W and L are expressed in µm.

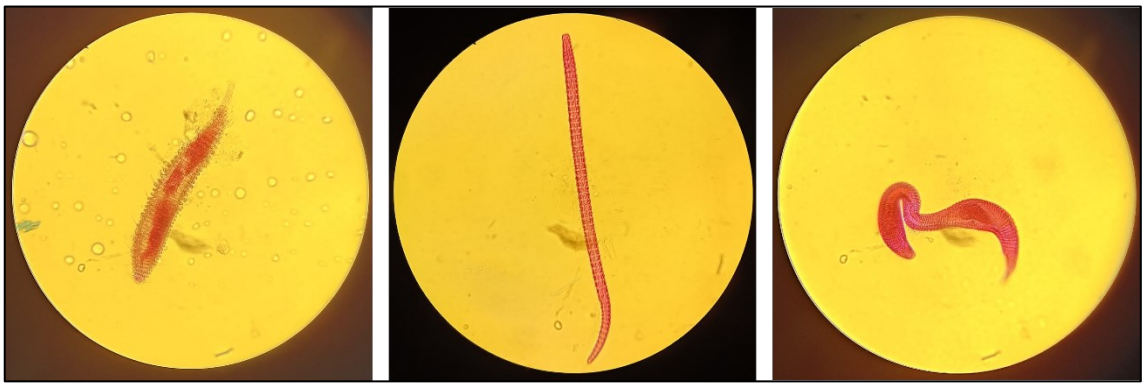

Figure 3- examples of nematodes seen under the optical microscope

The total biomass in 2023 was calculated by integrating over a depth of 10 cm. In order to compare the 1989 results accurately, similarly to the abundance, also in this case, a 10% deduction was applied to the total biomass. This deduction was based on observations by Danovaro et al. (1995) indicating that meiobenthic biomass accounted for approximately 10% of the total biomass beyond a depth of 6 cm.

3.6 Statistical analyses

To test for differences in the biochemical composition of organic matter, meiofaunal abundance, biomass and composition among stations and between 1989 and 2023, a two-way distance-based permutational analyses of variance (PERMANOVA) was used. Additionally, to identify the potential drivers (primary productivity expressed as the concentration of cholorophyll-a in the surface water, potential bottom temperature, organic matter components) of meiofaunal variables (abundance, biomass, composition) a Redundancy Analysis (RDA) was carried out, based on Bray-Curtis similarity index. All the analysis were performed using PRIMER-e v.7 computer programme.

4. Results

4.1 Environmental parameters

The bottom potential temperature taken from Copernicus databases appears to be increased on average about 0.28°C from 1989 to 2023. When looking at specific coordinates of the stations studied, the average value in 1989 was 13.88°C, while in 2023 it was 14.16°C during the sampling periods. When considering larger spatial and temporal scales, that are the broader Aegean Sea area and the entire year, the same pattern is observed as there is a clear trend of increasing temperatures throughout the years. Indeed, in 2023, the minimum and maximum values were 14.3°C and 14.6°C, respectively, compared to 14.0°C and 14.3°C in 1989 (Figure 4). The average annual bottom potential temperature for the Aegean Sea was 14.41°C in 2023, compared to 14.16°C in 1989, indicating an average increase of 0.25°C over the past 30 years.

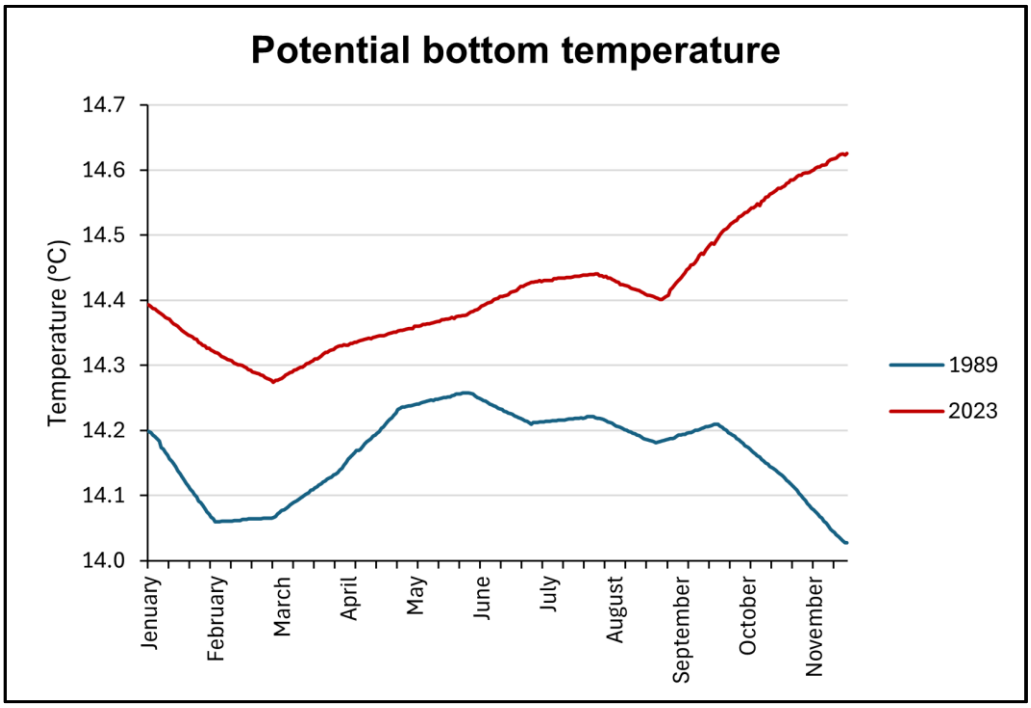

Figure 4- Bottom potential temperature annual trends in 1989 and 2023

Copernicus datasets only go back as far as 1997 when it comes to sea surface chlorophyll-a concentrations. Despite this limitation, from an analysis of the trend in the Mediterranean Sea, it can be observed that in the Aegean Sea area there is no significant trend over the past 25 years considered (Fig.5).

Moreover, focusing on the past six years (2018-2023), the data from Copernicus show that there has been no significant trend, with annual concentrations fluctuating year after year (Table 3).

Table 3-Average chl-a concentrations throughout the years 2018-2023 in the Aegean Sea area

	2018	2019	2020	2021	2022	2023
Chl-a (mg m-3 y)	0.076	0.094	0.095	0.074	0.160	0.140

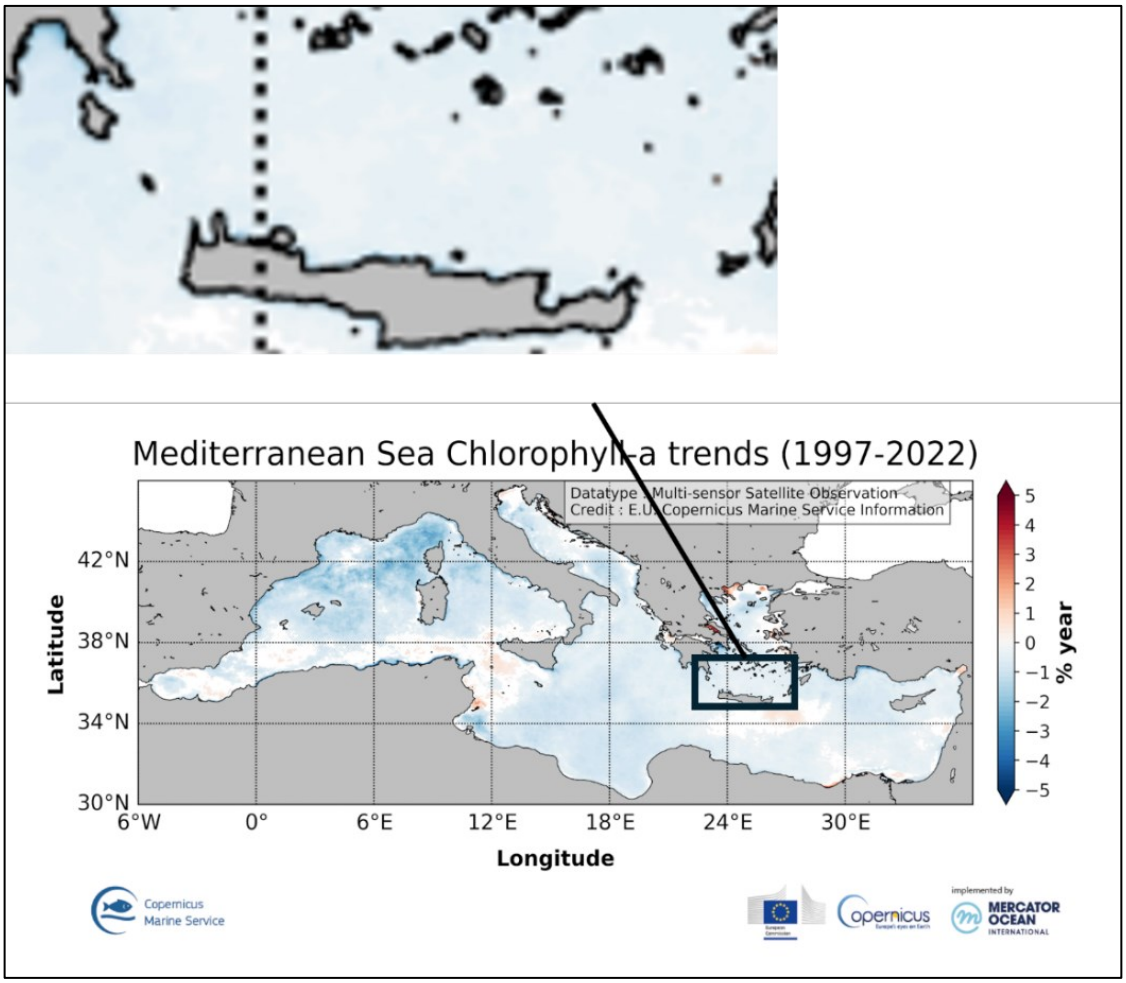

Figure 5-- Mediterranean Sea Chlorophyll trends 1997-2022 with a focus on the Aegean Sea area

4.2 Biochemical composition of organic matter

The analysis carried out showed that overall, the organic matter in the superficial (0-2cm) sediment layers has increased compared to 1989. Specifically, the LOM and BPC levels have risen by 34.48% and 31.48% respectively. Since they depend on the variables considered, it was observed

that carbohydrates and proteins have also experienced significant increases by 25.73% and 129.28% respectively. However, lipids did not show a significant change actually, on average, they decreased by 9.09% (Fig.6). The results of PERMANOVA analyses (Supplement Table 1) confirmed the significant differences observed in proteins concentrations between 1989 and 2023 with ***$P < 0.001$.

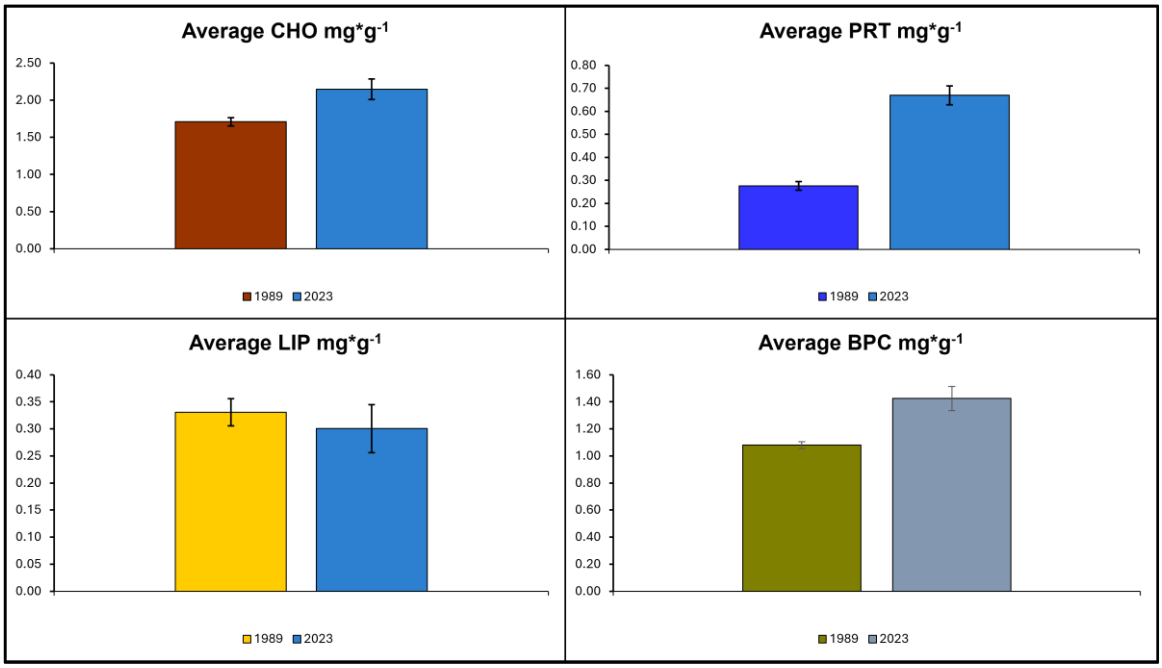

Figure 6- Comparison of average values of CHO, PRT, LIP and BPC

In terms of organic matter composition, carbohydrates were still the dominant category, although their average contribution was lower in 2023 compared to 1989 (68.86% in 2023 and 73.81% in 1989). Proteins showed an increase in contribution from 11.91% in 1989 to 21.48% in 2023, while

the contribution of lipids decreased too with an average of 14.28% in 1989 and 9.64% in 2023.

There has been a notable decrease of 90.19% in the CPE, with values in 2023 ranging between 0.24 and 0.76 µg g^{-1}. In contrast, in 1989 the values ranged between 1.45 and 4.84 µg g^{-1} reaching an unusual peak of 23.20 µg g^{-1} at station A14 (Fig.7).

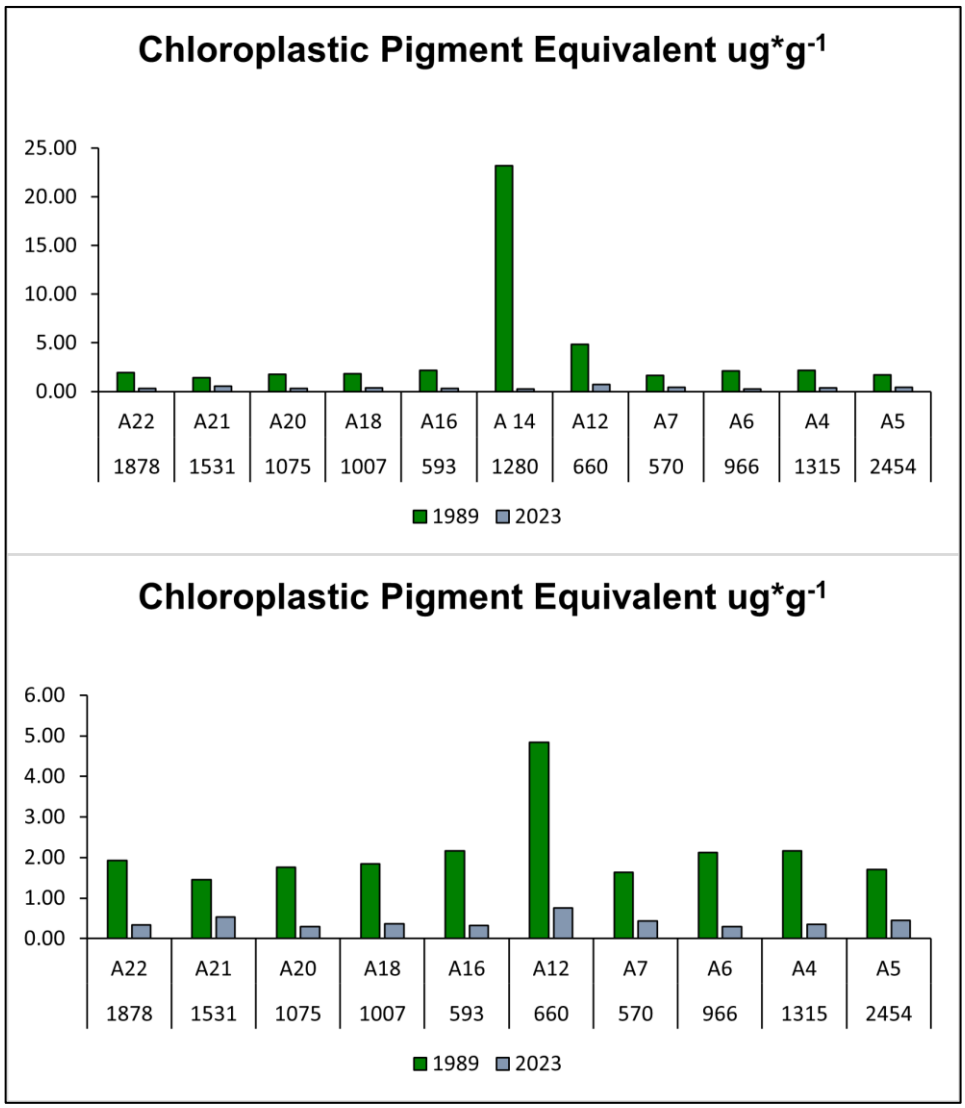

Figure 7- Comparison of the CPE values 1989 and in 2023, with and without considering the station A14

In addition, when comparing the contribution of the CPE to the BPC, it is important to note that the average value in 2023 was 1.40%, significantly lower than the 17.61% average value in 1989 (Fig.8).

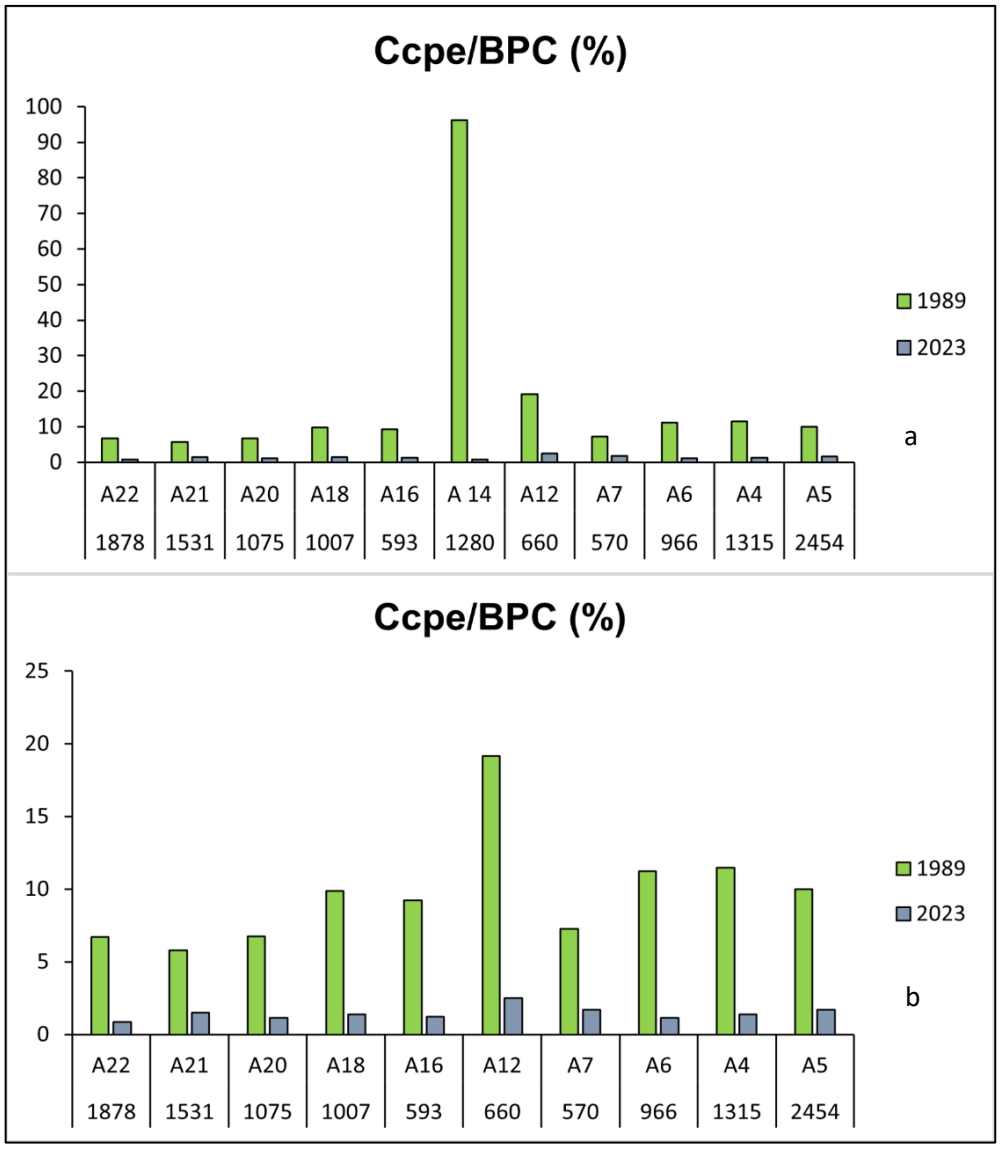

Figure 8- Comparison of the Ccpe/BPC at all stations in 1989 and 2023, with (a) and withou (b) considering the station A14

4.3 Meiofaunal abundance and biomass

The average abundance of meiofauna has slightly increased by 7.82% overall compared to numbers from 1989 (Fig.9). However, when looking at individual stations, there has been a decrease at stations A22 (1878 m), A21 (1531 m), A12 (660 m) and A20 (1075 m) (Fig.10). Station A20, in particular, was the most diverse in terms of abundance, with an unusual peak in turbellarian density in 1989 not observed in 2023 (196 ind. 10 cm^{-2}) (Fig.11). On contrast, a significant increment of 1655% has been noted at station A4 (1315 m) shifting from 4 ind. 10 cm^{-2} in 1989 to 70 ind. 10 cm^{-2} in 2023. Nematodes remained the most abundant taxon in both years, representing an average of 93% of total meiofauna in 2023 compared to 68% in 1989 (Fig.11). Furthermore, the station A12 (660 m) appeared to have the highest concentration of nematodes in both years (262 ind cm^{-2} in 2023 and 245 ind $cm^{-2.}$ in 1989).

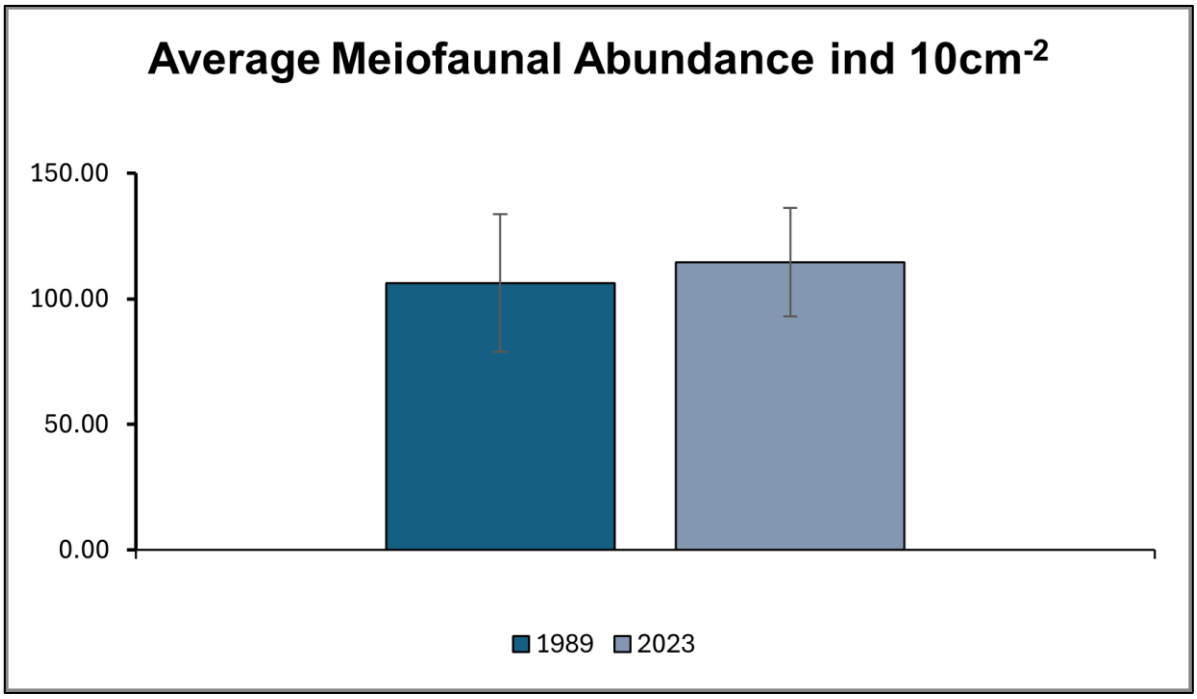

Figure 9- Comparison of the overall average meiofaunal abundance between 1989 and 2023

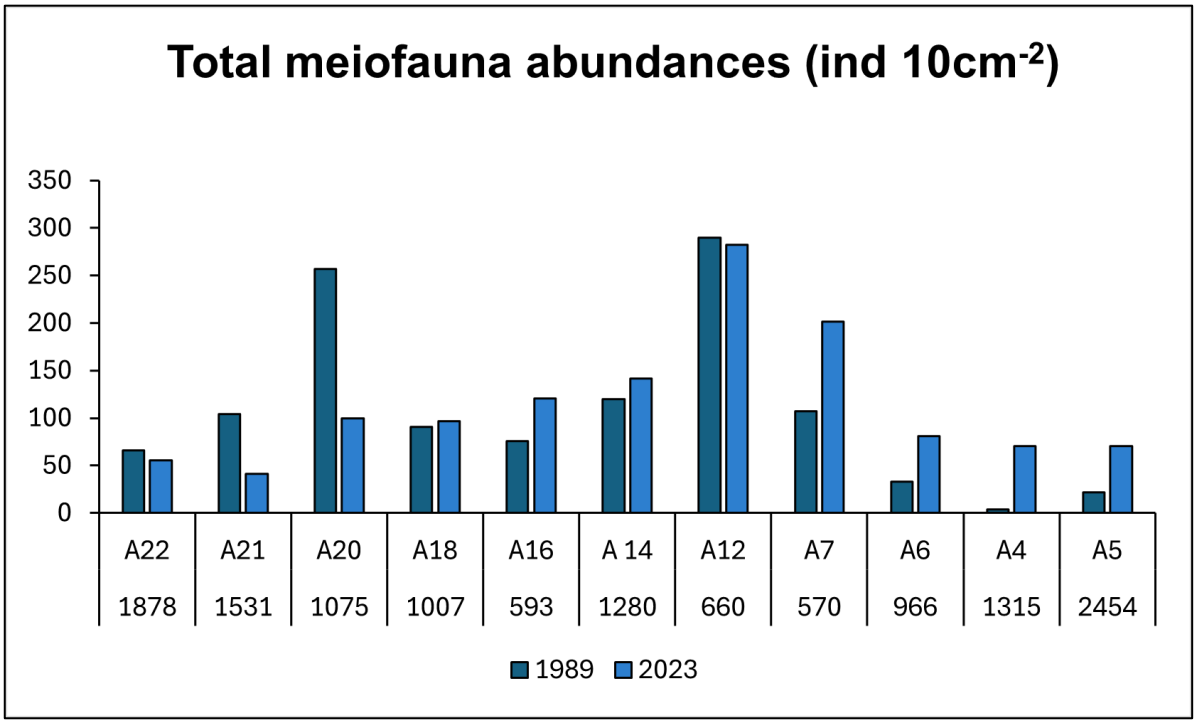

Figure 10- Comparison of the total meiofaunal abundances at each station between 1989 and 2023

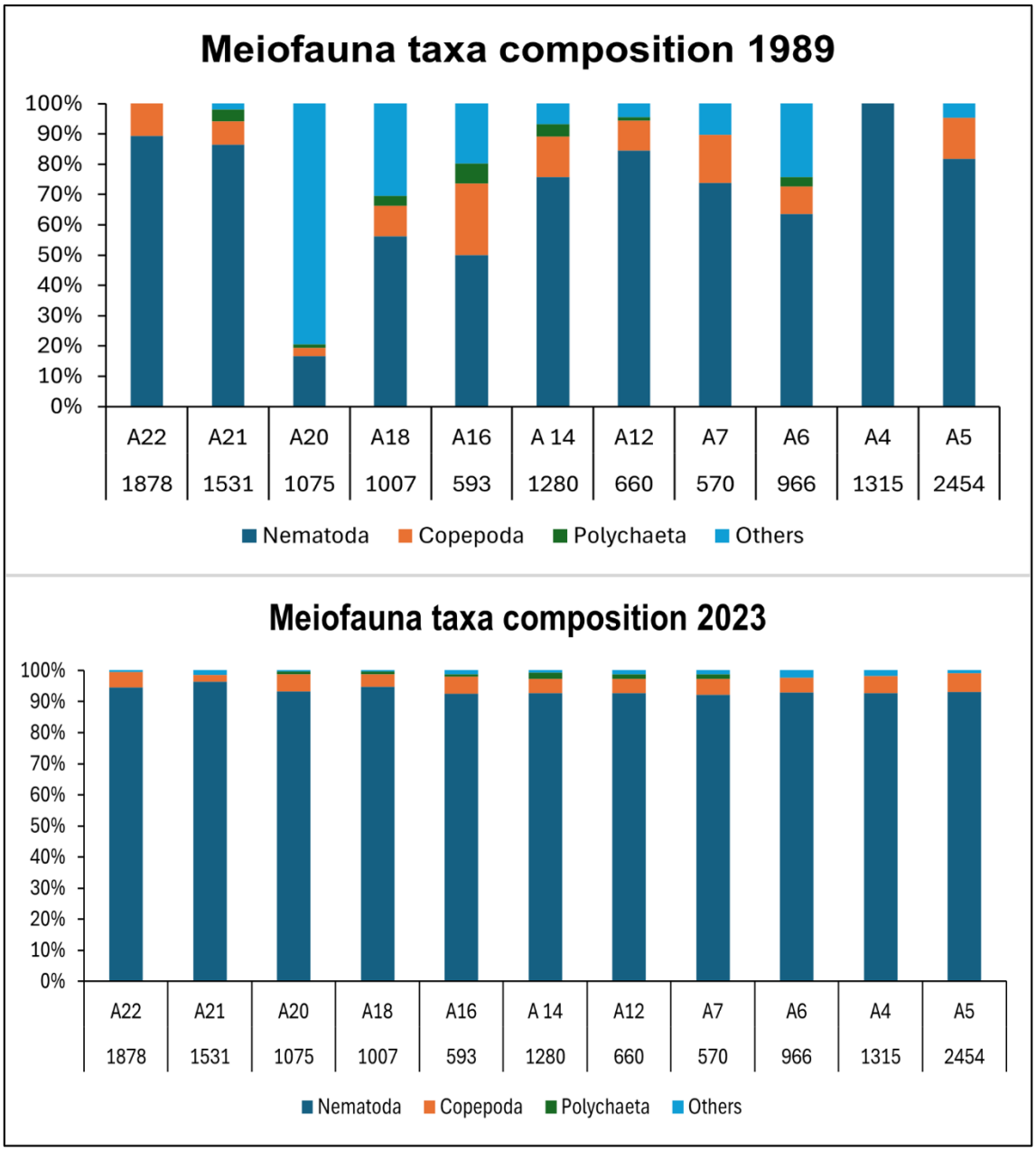

Figure 11-- Comparison of the community structure in terms of taxa at each station between 1989 and 2023

In terms of biomass, there was a significant decrease in values from 1989 to 2023. The highest biomass value observed in 1989 at station A20 was 585.06 µg/g, compared to the highest value of 13.74 µg/g at station A12 in 2023. Station A4 was the only one that did not experience a notable change in

biomass values. It had a biomass value of 2.78 µg/g in 1989, which increased slightly to 2.90 µg/g in 2023. (Fig.12). The average biomass value in 1989 was 246.45 ug/g, which decreased to 4.65 µg/g in 2023 (Fig.13). The results from the PERMANOVA test (Supplement Table 1) showed confirmed statistically these results with ***P < 0.001. This drastic shift can be attributed to changes in the taxa composition. In 1989, polychaetes were the dominant taxon contributing to biomass values (71.5%), followed by nematodes (13.8%) and copepodes (11.7%). However, in 2023, nematodes were the primary contributors to biomass (56.6%), followed by copepodes (26.7%) and polychaetes (12.6%) (Fig.14). This is also the reason why station A4 had such a low biomass in 1989, with only 4 nematodes present.

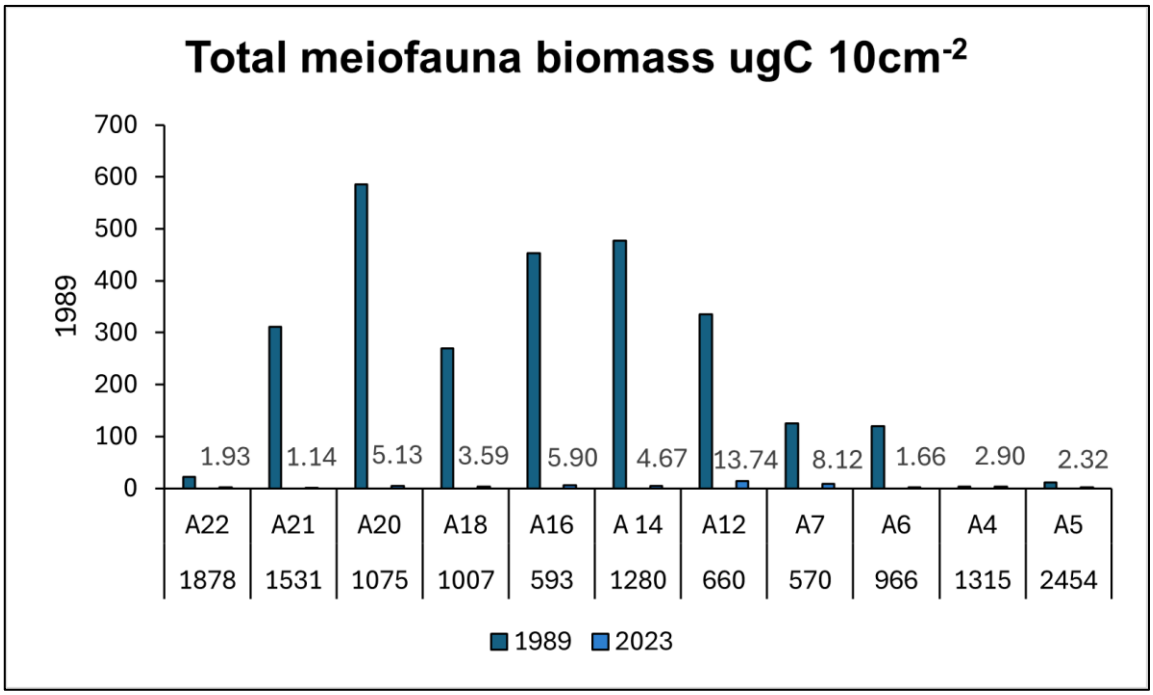

Figure 12- Comparison of meiofaunal biomass at each station between 1989 and 2023

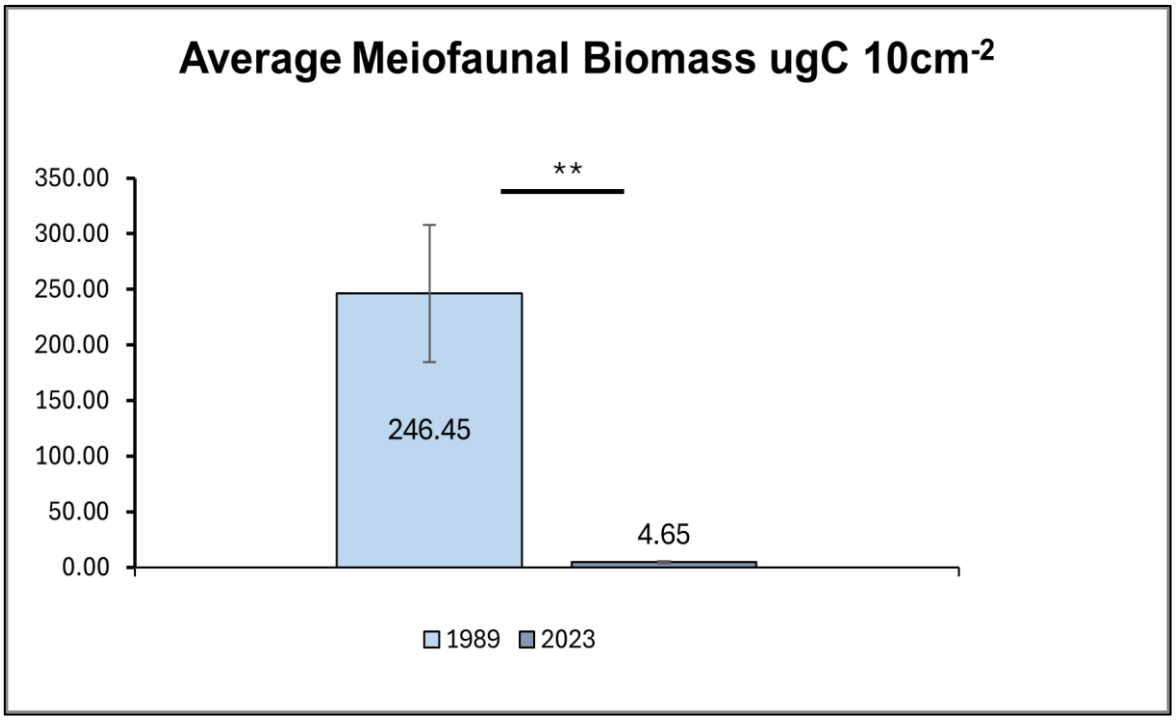

Figure 13- Comparison of average biomass values between 1989 and 2023

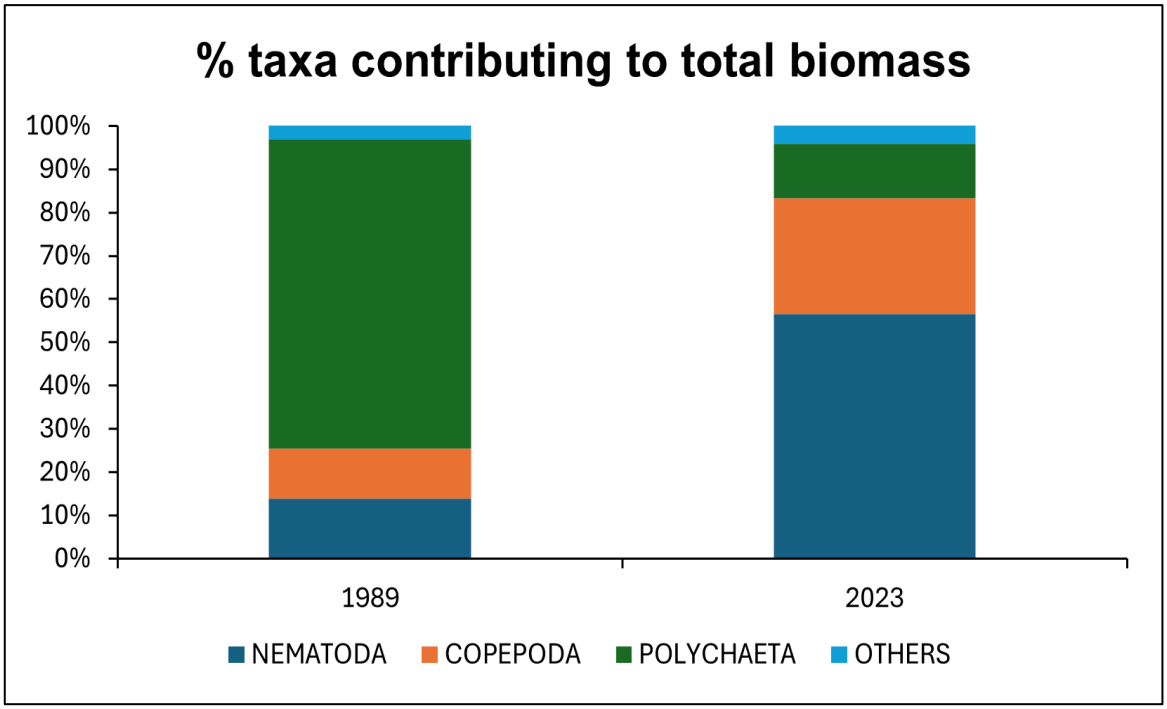

Figure 14- Comparison of the meiofaunal taxa contributing to the total biomass in 1989 and in 2023

Furthermore, the results from the Redundancy Analysis showed that considering all the abiotic factors as predictor variables, the variance response of the meiofaunal assemblage composition can be mostly accounted to the temperature (19%) and the proteins contribution (Fig.15).

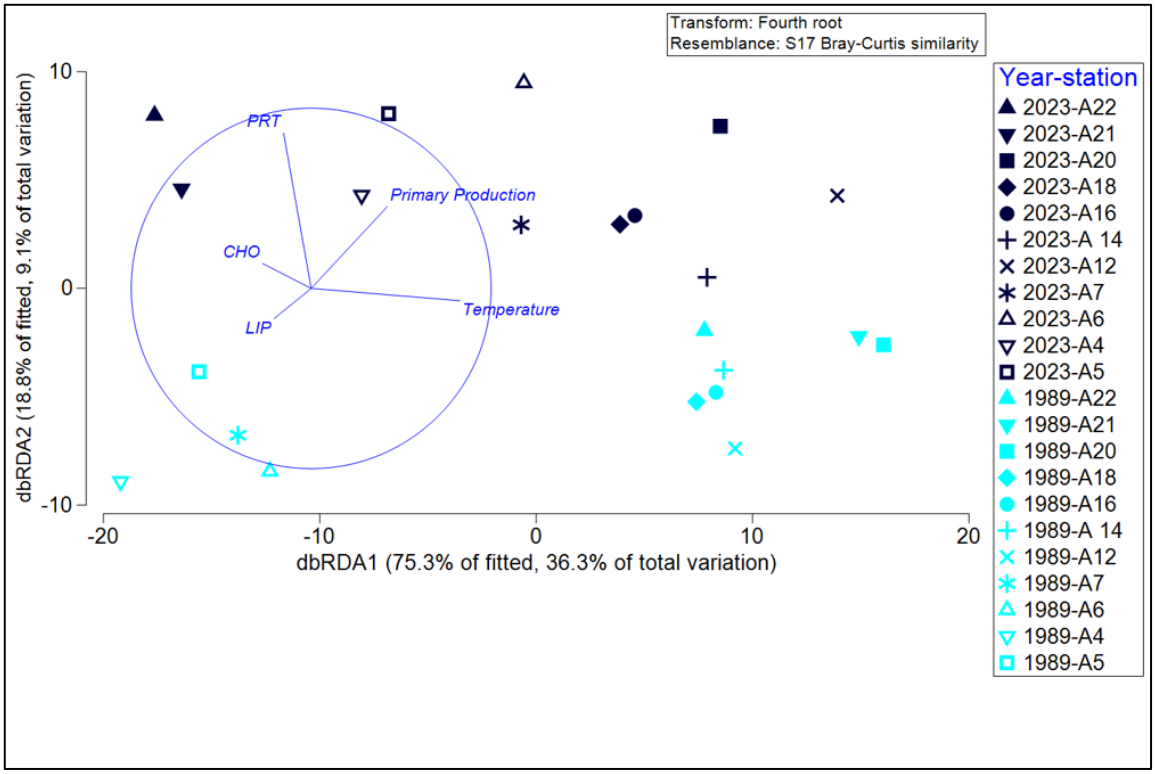

Figure 15- results from the Redundancy Analysis for each station in 1989 and in 2023

5. Discussion

The Aegean Sea is one of the areas characterized by the lowest values of primary production (PP worldwide (Tselepides and Elefhteriou, 1992). It is certainly among the least productive areas within the Mediterranean that shows a clear gradient of conditions from the western to the eastern Mediterranean and from north to south, depending on both the arrival of greater fluvial and continental inputs and the contribution of more productive waters from the Atlantic. The values of primary production in the wide area of our study are around 20 g C m^{-2} y^{-1}, which are also five times lower than the average values in the Mediterranean (Azov, 1991). The aim of our study was to understand if significant changes have occurred in over 30 years in this area, which has also been characterized by peculiar oceanographic dynamics such as the so-called Aegean transient, a temporary sinking of waters due to strong evaporation and therefore an increase in density that, sinking towards deeper layers, led to the rise of deeper and nutrient-rich waters that, coming into contact with the euphotic zone, triggered production processes (Psarra et al., 2000). So, a particular dynamic of a temperature variation combined with a variation in productivity that, as demonstrated by Danovaro et al., (2001, 2004), also led to significant changes in the biodiversity and functioning of deep-sea marine ecosystems. However, the

analysis presented in this study allow us, with all the limitations resulting from the fact that we only compare two sampling periods, to see if pictures taken 30 years apart show a significant change in the structure and function of deep-sea marine ecosystems. First of all, it is important to confirm that not only the same stations were selected, but also the sampling methods were consistent, using the same devices, sieves size, length of cores taken for analysis, protocols used for previous analyses conducted with samples collected in 1989. An analysis conducted, evident from both temperature data and potential temperature databases provided by Copernicus, shows the presence of significant changes in bottom potential temperature, with values that appear to be generally increasing over the last 30 years. A temperature increasing that, although on average of 0.2°C, is consistent with trends already observed in the deep Mediterranean up to almost 2000 m depth and expected in light of the progressive warming of the water masses and the trend of increasing surface temperatures. Since the organisms present at the bottom are poikilotherms, which means their metabolism depends entirely on temperature conditions (Lee and Atkinson, 1976), they can be particularly sensitive to a temperature increase that would result in an increase in their metabolism, this is also true for the microbial component and enzymatic activities that could lead to higher rates of organic matter degradation. From this point of view, it is useful to also compare the observed changes in terms

of primary production. In this case, data from Copernicus were used, where, by selecting exactly the intervention area, it was observed that comparing between 1989 and 2023, there was no significant change in chlorophyll concentration. This was confirmed also by Peters et al., (2022) who additionally focused on a five years' time series from 2013 to 2017 observing that there is in fact a consistent decrease in chlorophyll-a concentrations throughout the entire Mediterranean Sea, but in the Aegean Sea it is lower compared to other mediterranean subregions. In our study, we could observe from the values of concentration of chloroplast pigments equivalent on the sediment surface that there has been a tendency towards a decrease in primary production. This measurement actually allows us to evaluate the contribution of fresh material of primary origin, that is derived from phytoplanktonic primary production at the bottom. In this case, it can be noticed that the average values are lower compared to those observed 30 years ago. The interesting fact is that, despite a significant decrease in the production of chloroplast pigments, consistently observed at all sampled stations, this study has highlighted an apparently opposite behaviour in terms of concentrations of labile organic matter present in the sediments. Labile organic matter is defined as that biochemical pool of protein, lipids, and carbohydrates that can be more or less rapidly metabolized, used as a nutritional source for benthic organisms, particularly detritivores at the

bottom, and for the microbial component. However, not all components are directly assimilated. Carbohydrates, for example, are known to have a typically refractory and conservative composition. Since the overall amount of phytoplankton input has decreased and there have been no significant variations over time, as determined by Copernicus in primary production in the area over the past 30 years, this increase in organic matter at the bottom could be attributed to other reasons. One possible scenario could be that there is increased production happening at greater depths compared to those that are typically detected by satellites. Another explanation for the observed increase in the organic matter is the result of a depositional phenomenon of organic matter accumulation due to lateral input of continental origin. Several hypotheses can be formulated regarding this, one example is that intensive trawling activities on continental shelves up to 300-500 m depth could result in resuspension of these organic loads with deposition in deeper areas, which are the focus of our study and range from about 1000 m to about 2000 m, where no trawling activities occur. Therefore, human activities in sediment displacement may have led to the transfer of organic sediment loads to deeper environments. However, these data require further investigation and remain at a hypothesis level. The increase in organic matter has also led to changes in sediment composition, with carbohydrates becoming less dominant and proteins more prevalent. This shift may have significant

implications for meiofaunal communities, as different taxonomic groups may be better adapted to different environments.

Knowing that benthic components are highly dependent on food availability, an increase in organic matter could lead to an increase in the abundance of meiofaunal components. This has been observed in terms of average values to a very limited extent, indicating no statistically significant differences but at the same time, a trend towards an approximately 8% increase in meiofauna abundance. Additionally, a significant difference in terms of biomass has been observed, determined by a significant change in community structure that saw polychaetes and turbellarians abundant in 1989. In particular, the polychaetes were responsible for about 70% of the total biomass. This data may seem somewhat anomalous but was consistent with the community structure observed at that time. The most relevant finding, in the face of these changes, observed in deep-sea benthic ecosystems seems to be the change in community structure in 2023, where we see a significant decrease in the contribution of polychaetes and an increase in nematodes and harpacticoid copepods, which also contribute to the biomass by about 25%. The changes recorded in this comparison, are changes that manifest themselves in the community structure that shows a dominant presence of nematodes in 2023 and the disappearance of some components that were significantly observed, such as in stations A20, A18, A16, which also included turbellarians and

polychaetes among others. Many of these components were characterized by predatory behaviours and belonged to predatory feeding groups (Martens and Shockaert 1986, Fauchald & Jumars, 1979) that, in some cases, compensated for the absence of larger predators at such depths. It is known that this peculiarity of the deep eastern Mediterranean is due to the low concentration of food availability and the low abundance of potential prey, making the presence of large predators very difficult. The increase in carbohydrates and proteins in the sediments can explain the increase in the meiofaunal abundance as the nutrient availability increased, but at the same time can lead to competition for the resources where stronger competitors may outcompete weaker ones, leading to a decrease in diversity.

6. Conclusions

The Aegean Sea is one of the most oligotrophic areas within the Mediterranean, with primary production values significantly lower than the average in the region. The study shows that there have been significant changes in the deep-sea marine ecosystems over the past 30 years, with an increase in bottom potential temperature and a decrease in primary production. However, there has been an increase in labile organic matter present in the sediments, likely due to lateral input of continental origin. This

increase in organic matter has led to a slight increase in meiofauna abundance, but a significant change in community structure, with nematodes becoming dominant and polychaetes decreasing in abundance. These changes in meiofaunal assemblage composition have implications for the benthic trophic webs in the area. Further research is needed to better understand the causes of these changes and their long-term impacts on deep-sea marine ecosystems in the Aegean Sea.

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