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How Does Seasonality and Environmental Parameters Influence Meiofaunal Communities Associated with *Cystoseira* sp. Macroalgae on Rocky Substrates of Rimel, Bizerte (Tunisia)?

ABSTRACT

The current study investigated the seasonal variations of meiofauna communities (i.e., nematodes, copepods, oligochaetes, polychaetes, and larvae of crustaceans, respectively) associated with ***Cystoseira*** sp. macroalgae on the rocky substrates of Rimel, Bizerte (Tunisia). Sampling campaigns took place monthly for a full year and environmental parameters, such as temperature, pH, dissolved oxygen, and suspended matter were measured *in situ*. The results underscore the significance of several abiotic factors in shaping meiofaunal communities over time. Copepods showed a positive correlation with pH ($r = 0.592$, $p = 0.0426$), which achieved a peak in abundance in alkaline conditions. The nematodes were highly abundant during early summer, but declined during high summer temperatures, highlighting their sensitivity to thermal stress. The larvae of crustaceans, in contrast, preferred the more stable winter conditions, reaching maximum abundance in February. Multivariate analyses showed distinct communities in time (ANOSIM (Analysis of Similarities), $R = 0.745$, $p = 0.001$) and seasonal clustering, with spring months characterized by more homogenous conditions as opposed to summer-autumn months. The findings of the current survey underscore the complex interplay between meiofauna and environmental factors, offering valuable insights into the ecological functionality of rocky intertidal habitats. Future studies should explore the role of additional factors, such as organic matter quality and trophic interactions in shaping meiofauna dynamics dwelling on rocky substrate.

KEYWORDS: Meiofaunal taxa; Meiofaunal abundance; physicochemical parameters; seasonal variation; rocky substrates; Rimel cost; Tunisia.

INTRODUCTION

Meiofauna is a size-based artificial group, defined by organisms that pass through a 1 mm mesh sieve but are retained by a 40 µm mesh sieve. It includes small benthic invertebrates such as nematodes, copepods, oligochaetes, and polychaetes (Semprucci & Balsamo, 2012; Carriço et al., 2013). These taxa play crucial roles in ecosystem functioning and serve as bioindicators of environmental changes. Nematodes dominate meiofaunal communities and indicate sediment contamination, organic matter levels, and trophic interactions (Santos et al., 2018).

This Copepods contribute to sediment oxygenation and are highly sensitive to pollutants, making them reliable indicators of water quality and bioaccumulation (Hussain et al., 2020). Oligochaetes thrive in organically enriched and hypoxic environments, serving as markers of eutrophication and pollution from sewage or industrial discharge (Chapman et al., 1982). Polychaetes play a key role in sediment mixing and nutrient cycling, with species composition changes reflecting sediment disturbances and chemical contamination (Hutchings, 1998; Mandario et al., 2019). However, most studies with a focus on meiofauna took place on soft substrates, such as sandy or muddy sediments; studies focused on hard substrate dwelling meiofauna are few (Danovaro & Fraschetti., 2002).

Meiofaunal communities dwelling on rocky substrates exhibit distinct characteristics shaped by key factors, such as algal cover, substrate structural complexity, and hydrodynamic conditions (Coull et al., 1983; Danovaro et al., 2000). Compared to soft substrates meiofauna, which are normally dominated by nematodes, rocky substrates support often more evenly distributed communities, but dominated by copepods and amphipods, due to their affinity for micro-refuges offered by macroalgae and fissures (Beckley 1982; Coull et al., 1983; Danovaro & Fraschetti, 2002). These microhabitats host also various species of macroalgae, such as *Cystoseira* sp., a genus of brown macroalgae that plays a crucial ecological role in coastal marine ecosystems, particularly along the rocky shores of the Mediterranean Sea. *Cystoseira* sp. supports diverse meiofauna communities due its remarkable structural complexity, fostering greater abundance and diversity of these organisms (Ape et al., 2018a; Ape et al., 2020). This genus follows a seasonal life cycle characterized by fluctuations in growth and reproductive stages throughout the year. Its presence and morphology vary seasonally, reflecting distinct developmental phases rather than a constant form throughout the year (Sales & Ballesteros, 2012).

Despite their ecological importance, long-term data linking meiofauna and environmental key-factors that drive their existence remain scarce. The current study aimed to fill this knowledge gap based on a 12-month survey of the abundance and composition of meiofauna communities that inhabits the rocky substrates of Rimel, Bizerte (Tunisia). Furthermore, the current study also explored the preferences of meiofauna with macroalgal cover, particularly the complex species of the genus *Cystoseira* sp.

MATERIALS AND METHODS

Collection site and environmental parameters

Meiofauna samples were collected monthly, from March 2021 to February 2022, from *Cystoseira* sp. algae located on the rocky shoreline of the Rimel site (37°15'10.4"N 9°56'39.4"E) in Bizerte, Tunisia (Figure 1). The Rimel coast faces multiple environmental challenges, including pollution from petroleum, microplastics, and metals (Boufahja et al., 2012; Martins et al., 2015; Abidli et al., 2019), making it an ideal site for year-round biomonitoring and assessing the impact of these pollutants on marine meiofauna.

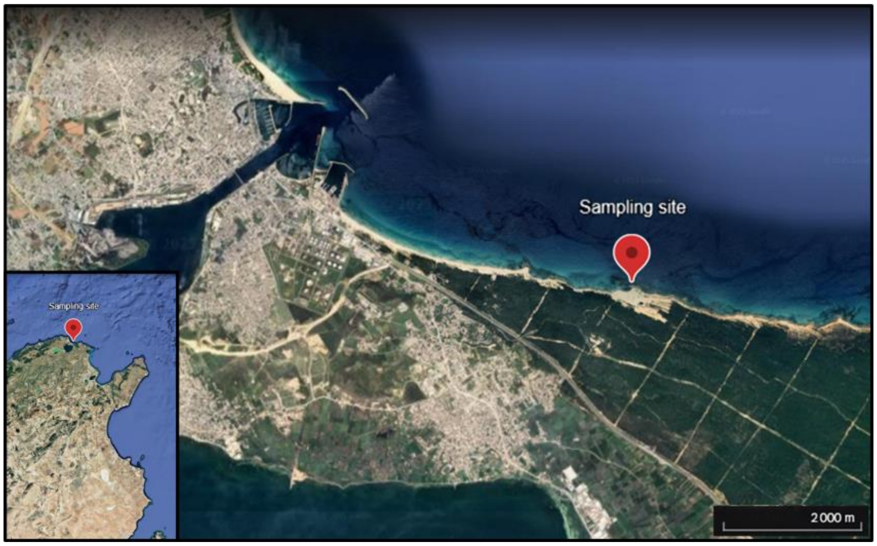

Figure 1. Location of the Sampling Site on the Rocky Shoreline of Rimel, Bizerte (37°15'10.4"N, 9°56'39.4"E), Tunisia

Dissolved oxygen at water-sediment interface was measured with and oximeter (WTW OXI 330/SET, WTW, Weilheim, Germany). The water temperature and salinity were recorded with a thermosalinometer (WTW LF 196, Weilheim, Germany), and pH with a pH meter (WTW pH 330/SET-1, Germany).

Meiofauna sampling and taxonomic analysis

Meiofauna sampling on rocky substrates associated with various *Cystoseira* species was done with a modified manual hand corer, according to Beckley and McLachlan (1979). The hand corer, with an inner diameter of 8.5 cm, had one open end fitted with a flexible rubber ring (1 cm thick) to adapt to rough surfaces, whereas the other end was sealed with a securely attached plastic bag. During sampling, at 50 cm depth (up to 1 m depth), where these species form dense populations on hard substrata in the upper sublittoral zone (Bellissimo et al., 2014), with the collection bag securely positioned upwards.

A lateral window, located 2 cm above the corer opening, allowed the insertion of a spatula to scrape the rock surface, effectively dislodging debris, whereas simultaneously guiding meiofauna and associated particles into the collection bag. Once the sampling was complete, the bag was carefully sealed to ensure watertightness.

In the laboratory, meiofauna were separated from macrobenthos and macroalgae by sieving through 1-mm mesh size sieve and retaining it on a second sieve of 40-µm mesh size (Danovaro et al., 2002). The collected specimens were fixed in 4% unbuffered formaldehyde solution and stained with Rose Bengal (0.2 $g.L^{-1}$) to facilitate counting. Meiofauna was individually counted on Delfus plate with a gridded bottom (200 squares of 5 mm^2) under a dissecting microscope (Model WildHeerbrugg M5A), at 50× magnification (Elarbaoui et al., 2015).

Statistical analysis

In order to perform the statistical analyses, the data were first tested for normality (i.e. Kolmogorov-Smirnov test) and equality of variance (i.e. Bartlett test) (Clarke, 1993 ; Clarke and Gorley, 2001). The software GraphPad Prism 8 was used to analyze the monthly monitoring data (Cass, 2000). One-way analysis of variance (ANOVA) and Tukey's HSD (Honestly Significant Difference) test were applied to raw data in order to test for the significant differences ($p < 0.05$) between compartments. Densities of different taxa were compared, and Pearson's correlation coefficient (r) was calculated to illustrate relationships between biotic and abiotic variables, providing a clear visualization of interdependencies. Primer v.5 software was employed to perform non-metric multidimensional scaling (nMDS) ordination based on square root-transformed densities of meiofaunal taxa, using Bray-Curtis similarity indices (Bray & Curtis, 1957). Additionally, the ANOSIM (Analysis of Similarities) analysis was applied to determine significant differences among compartments, followed by SIMPER (Similarity Percentage) analysis (Clarke, 1993) was conducted with the same software to assess the contribution of individual taxa to the average dissimilarity among sampling months.

RESULTS

Physico-chemical parameters

In the current study, the water temperature gradually increased from March (16.2 °C), reaching a peak in August (29.3 °C) before gradually decreasing to a minimum in February (14.3 °C). Salinity fluctuated monthly, ranging from 35.8 PSU in January to 38 PSU in August. Dissolved oxygen remained relatively stable throughout the year, in average 10 mg.L^{-1}, except in May, when it peaked at 12.46 mg. L^{-1}. pH also varied in time, with a maximum of 8.67 in May and a minimum of 8.17 in June. Finally, suspended particulate matter (SPM) ranged from a maximum of 28.2 mg. L^{-1} in May to a minimum of 19 mg. L^{-1} in September (Table 1).

Table1. Monthly variation of physicochemical parameters. SPM: suspended particulate matter

Month	Temperature (°C)	Salinity (PSU)	Dissolved oxygen (mg. L^{-1})	pH	SPM (mg. L^{-1})
March	16.2	37.7	9.85	8.25	18.9
April	21.4	36	10.23	8.49	32.5
May	26.6	37.2	12.46	8.67	28.2
June	27.6	37.7	10.2	8.17	22.78
July	29.3	38	9.6	8.36	21.79
August	26.3	37.33	10.05	8.38	19
September	23.1	37.7	9.51	8.24	26.5
October	21.6	36.8	10.05	8.35	27.8
November	18.2	36.6	10.01	8.24	29.7
December	15.2	35.8	9.95	8.31	27.3
January	14.3	36.1	10.1	8.24	24.27
February	15.4	36.9	10.4	8.26	23

Pearson's correlation analysis did not reveal any significant relationship among environmental parameters and meiofauna abundance. However, a significant positive correlation was observed between pH and copepod abundance ($r = 0.592$, $p < 0.05$), explaining 35% of the variance ($R^2 = 0.3504$). The 95% confidence interval for the correlation coefficient ranged from 0.02733 to 0.8702, indicating moderate to strong correlations among variables.

Meiofaunal abundance

Nematodes

The nematode abundance fluctuated significantly over the monitoring period, peaking in June (414.66 ± 86.18 ind. per $10cm^2$) and dropping sharply in July (85.33 ± 8.73 ind. per $10cm^2$). One-way ANOVA confirmed significant variations among sampling months (Levene's test: $F = 22.62$, $p = 0.05$ and Tukey-HSD post-hoc tests, $p < 0.05$). The most notable differences were observed between March *vs.* April, May *vs.* June, June *vs.* July, and December *vs.* January (Figure 2).

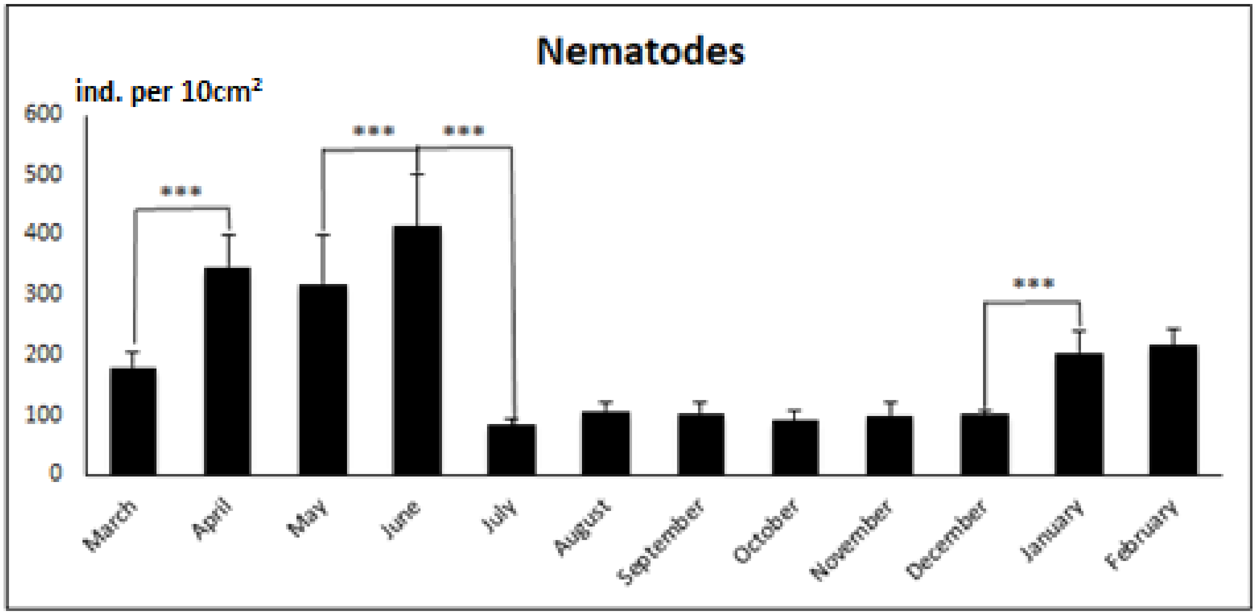

Figure 2. Monthly distribution of nematode density. Significant differences ($p < 0.05$) according to Tukey's HSD test are indicated by *** = $p < 0.001$.

Copepods

The copepods exhibited marked seasonal dynamics, reaching maximum abundance in April (325 ± 47.31 ind. per $10cm^2$) and minimum in December (82 ± 16.52 ind. per $10cm^2$). One-way ANOVA revealed significant differences among sampling months (Levene's test: $F = 14.082$, $p = 0.05$). Significant variations were noted between March *vs.* April, April *vs.* May, May *vs.* June, and June *vs.* July (Tukey-HSD post-hoc tests, $p < 0.05$, see Figure 3).

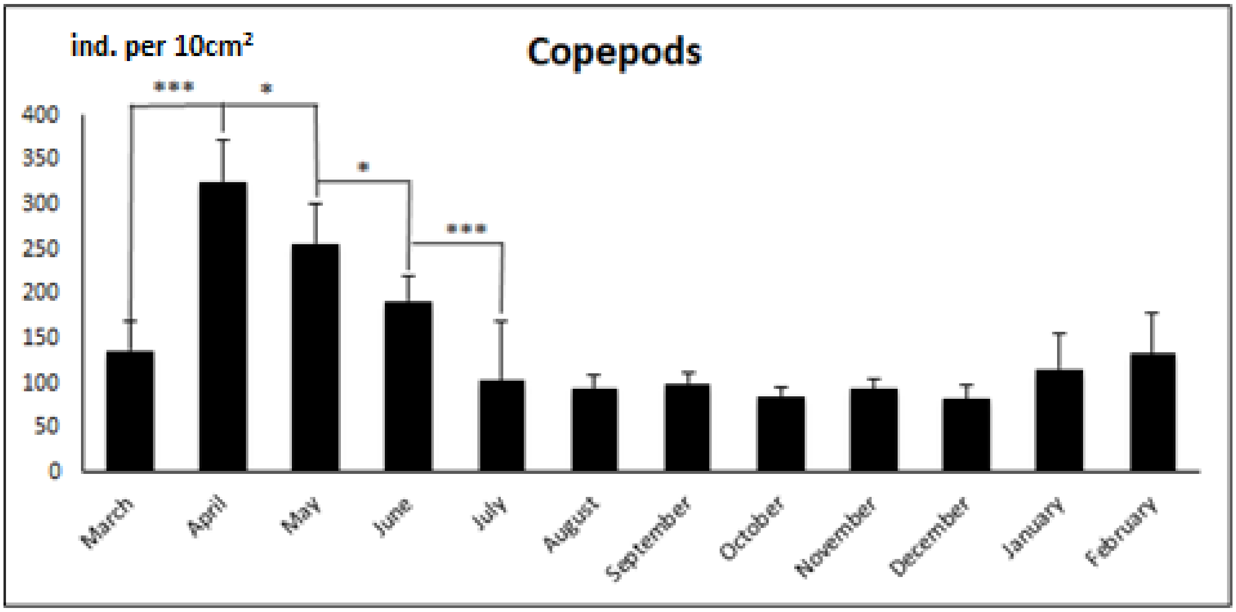

Figure 3. Monthly distribution of copepods density. Significant differences (p < 0.05) according to Tukey's HSD test are indicated by * = p < 0.05; *** = p < 0.001.

Polychaetes

The polychaetes reached maximum abundance in January (57.66 ± 3.06 ind. per 10cm2) and minimum during the summer (12.66 ± 2.08 ind. per 10cm²). One-way ANOVA (Levene's test: F = 8.71, p = 0.05) confirmed significant monthly variations, with a single notable difference observed between June *vs*. July, according to the Tukey-HSD test (p < 0.05) (Figure 4).

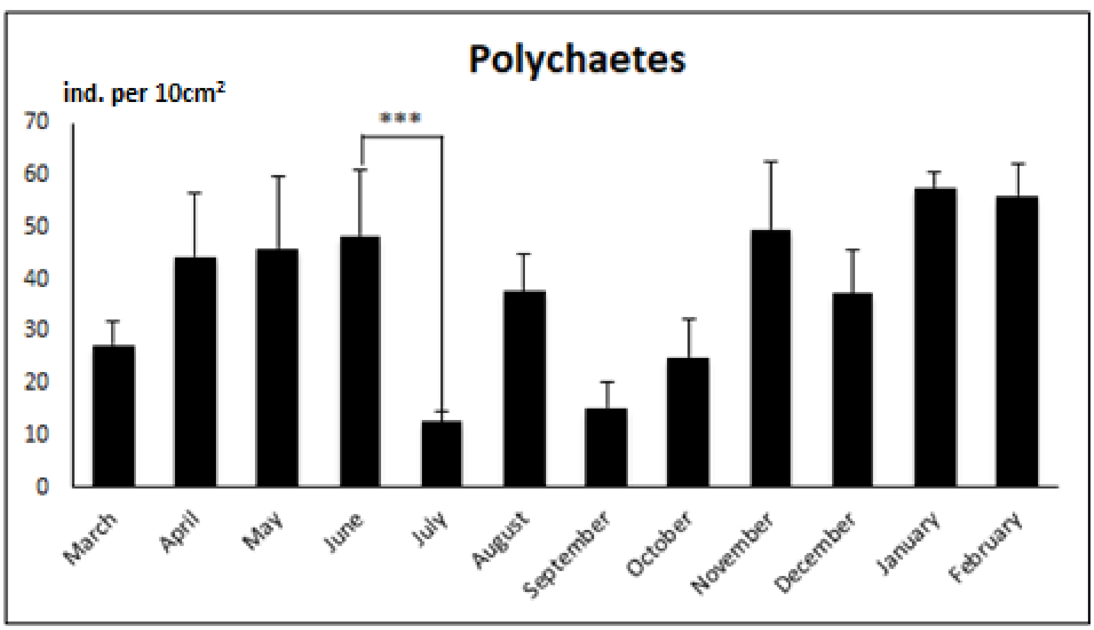

Figure 4. Monthly distribution of polychaetes density. Significant differences (p < 0.05) according to Tukey's HSD test are indicated by *** = p < 0.001.

Oligochaetes

The oligochaete abundance varied significantly over a full year, reaching a peak in April (13.66 ± 6.11 ind. per 10cm²) and minimum values in September, October, and December (2.66 ± 1.52 to 2.66 ± 0.57 ind. per 10cm², respectively). One-way ANOVA test identified significant differences (Levene's test: $F = 4.06$, $p = 0.05$) over the sampling period, with significant differences between March *vs*. April, as indicated by the Tukey-HSD test ($p < 0.05$) (Figure 5).

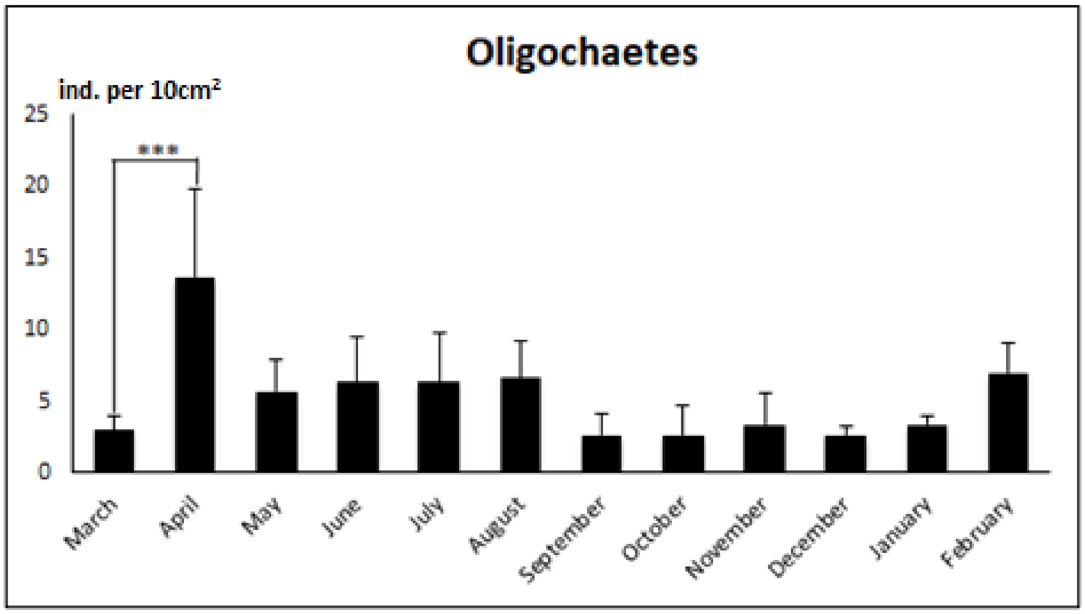

Figure 5. Monthly distribution of oligochaetes density. Significant differences ($p < 0.05$) according to Tukey's HSD test are indicated by *** = $p < 0.001$.

Crustacean Larvae

The larvae of crustaceans displayed seasonal preference for the winter season, with a peak in density in February (43.66 ± 5.03 ind. per 10cm²) and minimum values recorded in September (10.66 ± 2.08 ind. per 10cm²). One-way ANOVA indicated significant differences (Levene's test: $F = 11.94$, $p = 0.05$), supported by the Tukey-HSD post-hoc test ($p < 0.05$). The most significant differences were observed between March *vs*. April, June *vs*. July, and December *vs*. January (Figure 6).

Multivariate Analysis

ANOSIM analysis revealed significant differences within meiofauna communities in time (global $R = 0.745$, $p = 0.001$), indicating important seasonal variations in density. High R values between distant months, such as March *vs*. June (i.e. $R = 0.926$), reflected the impact of seasonal variations on the community structure. However, closely spaced periods, such as April *vs*. May (i.e. $R = 0.037$), exhibited less marked differences, suggesting similar environmental conditions with low impact on the meiofauna assemblages.

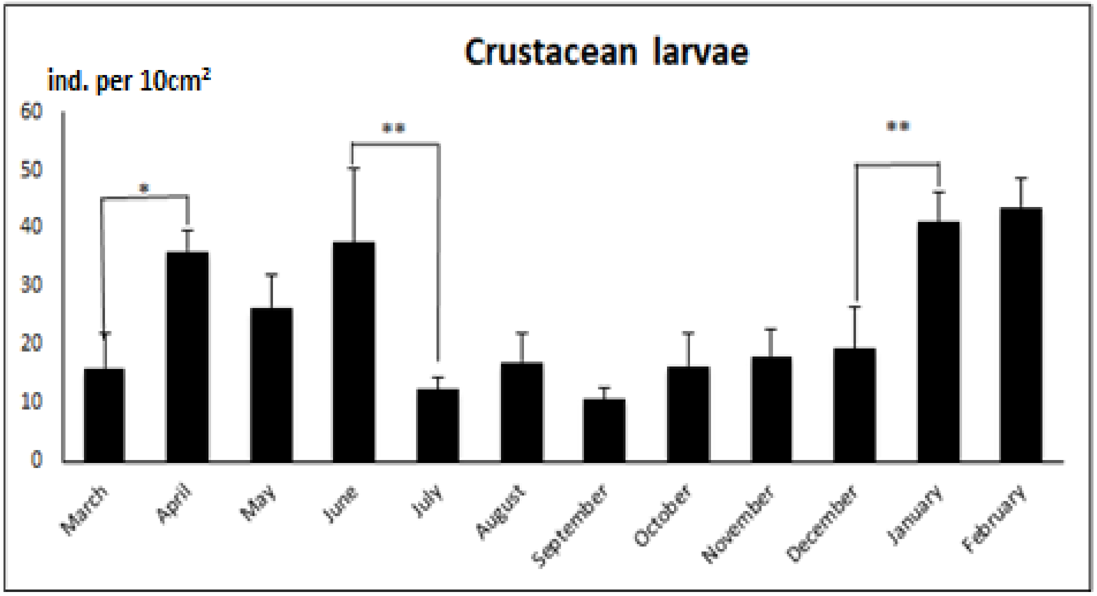

Figure 6. Monthly distribution of crustacean larvae density. Significant differences ($p < 0.05$) according to Tukey's HSD test are indicated by * = $p < 0.05$; ** = $p < 0.01$.

The nMDS ordination results highlighted an important effect of season variation on meiofauna distribution, with a stress value of 0.07, which indicates good representation quality (Figure 7). Samples from spring months (i.e. April to June) clustered on the left side of the graph, reflecting relatively homogeneous meiofaunal composition during this period. In contrast, summer, autumn, and winter months (i.e. June to December) were scattered towards the right side of the ordination panel, indicating greater heterogeneity, which was likely driven by increased environmental variability. Finally, the samples from January, February, and March were mostly clustered in the center on the graph, suggesting relatively homogeneous meiofauna communities in these months (Figure 6).

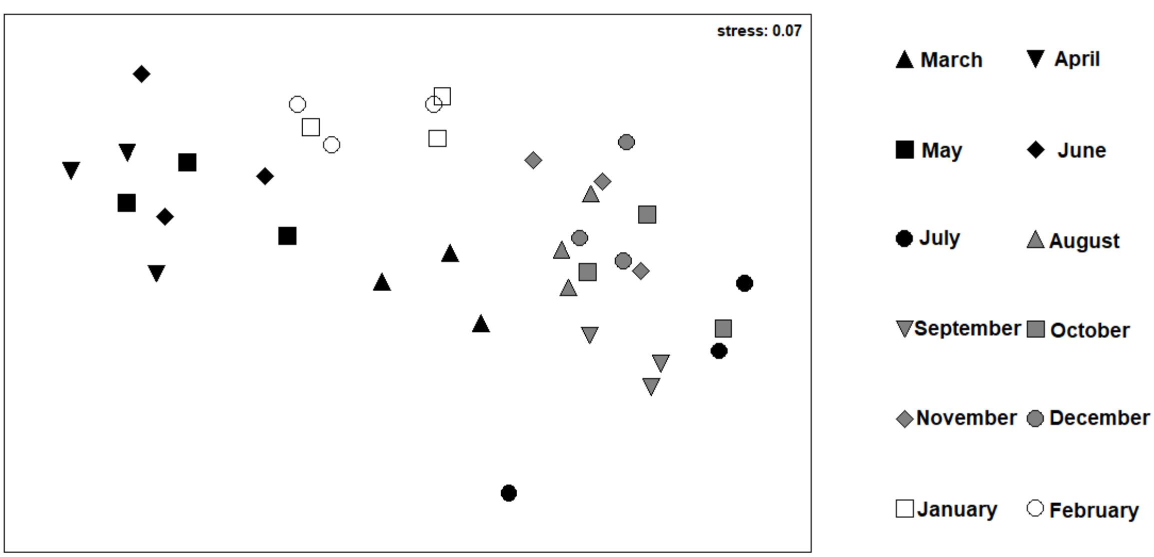

Figure 7. Non-metric multidimensional scaling (nMDS) 2D plot based on Monthly Distribution of meiofauna density

DISCUSSION

The current study was, to the best of our knowledge, the first to explore seasonal changes in meiofaunal communities associated with *Cystoseira* sp. macroalgae on rocky substrates in Tunisia. Using a combined approach of statistical analyses, physicochemical measurements, and multidimensional ordination, this research highlighted the temporal dynamics of these meiofaunal communities. Previous studies examining the physicochemical parameters of water and sediments in the Rimel area revealed that while certain regions exhibited relatively high concentrations of toxic metals, the overall environmental conditions remained favorable, supporting diverse marine communities and high biodiversity (Boufahja et al., 2010 ; Martins et al., 2015 ; Abidli et al., 2019).

The physicochemical parameters showed pronounced seasonal variations, which likely influenced the distribution of meiofaunal communities. Water temperature increased from March (16.2 °C) to a peak in August (29.3 °C) before declining in December (15.2 °C). In this context, nematode density peaked in June (414.66 ± 86.18 ind. per $10cm^2$) during a period of high temperatures, followed by a sharp decline in July (85.33 ± 8.73 ind. per $10cm^2$). This pattern suggests increased sensitivity to summer conditions and aligns with previous findings by Ape et al. (2018b), who reported that higher temperatures were associated with lower meiofaunal densities in intertidal zones.

pH levels ranged from 8.17 to 8.67, while suspended particulate matter fluctuated between 18.9 $mg.L^{-1}$ and 32.5 $mg.L^{-1}$. These environmental parameters likely influenced copepod density, which reached its highest density (325 ± 47.31 ind. per $10cm^2$) during periods of elevated pH and suspended matter levels. A significant positive correlation between pH and copepod density ($r = 0.592$, $p = 0.0426$) suggests that these microcrustaceans thrive in slightly alkaline conditions. This result partially contrasts with the findings of Lee et al. (2017), who demonstrated that a decrease in pH negatively affects the density and species richness of meiofaunal communities. The results of this study indicate that the effects of pH may vary depending on specific thresholds, taxa of interest, and interactions with other abiotic factors. Our findings highlight a distinct response of copepods to pH variations, suggesting both adaptability and potential benefits in alkaline conditions.

The density of oligochaetes and polychaetes remained relatively low throughout the year, with modest peaks in April and January, respectively. These fluctuations may be attributed to variations in trophic resource availability or changes in salinity (Amei et al., 2021; Sowa & Krodkiewska, 2020; Warwick & Clarke, 1994; Bagheri & McLusky, 1982). Crustacean larvae displayed a strong preference for winter conditions, peaking in February (43.66 ± 5.03 ind. per $10cm^2$). This period was characterized by low temperatures (14.3 °C) and stable dissolved oxygen concentrations, averaging 10 $mg.L^{-1}$. These findings align with previous observations by Cronin et al. (2017), who reported that seasonal occurrences of planktonic larvae from benthic taxa were closely correlated with winter seabed and surface water temperatures. This synchronization suggests an adaptive response of larvae to seasonal environmental variations, enhancing their survival and development in stable, cold environments.

The ANOSIM analysis revealed significant differences between sampling months (global $R = 0.745$, $p = 0.001$), particularly between March and June ($R = 0.926$). These findings are further supported by the nMDS ordination (stress = 0.07), which shows that spring months (April to June) clustered together due to homogeneous conditions, whereas summer and autumn samples were more dispersed, reflecting increased environmental heterogeneity. These observations are supported by studies suggesting that spring months provide more homogeneous environmental conditions, leading to higher meiofaunal abundance. In contrast, summer and autumn months exhibit greater environmental heterogeneity, influenced by factors such as organic matter, salinity, pollution, and temperature, which drive variations in meiofaunal diversity and community structure (Stein & Kreft, 2015; Stark et al., 2020; Ghosh & Mandal, 2021).

These observations indicate, first, the good condition of the *Cystoseira* sp. cover, whose decline has been associated with a significant reduction in meiofaunal biodiversity (Bianchelli & Danovaro, 2020). Second, the findings further support the notion that the impact of environmental parameters on meiofauna is complex and taxon-specific, as evidenced by previous studies (França et al., 2024; Baldrighi et al., 2019; Kim et al., 2019). This highlights the necessity of a nuanced approach to accurately assess meiofaunal community dynamics and their responses to environmental fluctuations. Additionally, factors such as organic matter availability and trophic interactions may also play important roles in shaping meiofaunal distribution, although they were not explicitly examined in this study. These results underscore the importance of an integrative approach, combining statistical and multivariate analyses, to better understand the complex interactions driving seasonal variations in meiofaunal density and community structure. Future research should further investigate these mechanisms, particularly the effects of organic matter quality and trophic interactions on meiofaunal dynamics.

CONCLUSIONS

The current study explored the seasonal variations of meiofauna communities dwelling on rocky substrates in Tunisia and revealed marked temporal structuring, dictated by key abiotic factors, such as temperature, pH, dissolved oxygen and suspended matter. The results showed that nematodes and copepods, the dominant meiofauna groups, were extremely variable in density over a year. The nematodes reached maximum in density in June, followed by significant drop in July, reflecting potential sensitivity to high summer temperatures. The copepods, in turn, showed positive correlations with pH, reaching maximum density during times of slightly alkaline conditions, which contrasts with generally reported trends in literature. Multivariate analysis also highlighted significant distinctions in variation among sampling months, as well as the importance of seasonal factors in structuring meiofauna communities.

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Conflict of interest statement

We declare that we have no conflict of interest.

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