Ecological Studies of Epiphytic Microalgae and Epiphytic Zooplankton on Seaweeds of the Eastern Harbor, Alexandria, Egypt

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Abstract: A qualitative and quantitative study on the epiphytic microalgae and epiphytic zooplankton were conducted in order to follow up their community structures on seaweeds in relation to some physico-chemical variables in the coastal waters of the Eastern Harbor of Alexandria. Water and macroalgal samples were collected seasonally during two years successively: Winter, summer, autumn (2012) and spring (2013). The collected seaweeds were Ulva fasciata (green alga), Corallina mediterranea, Corallina officinalis, Gelidium sp., Pterocladiella capillacea, Hypnea musciformis and Grateloupia doryphora (red algae). The studied water quality parameters were pH, salinity, dissolved oxygen, oxidizable organic matter and nutrient salts. The abundance of epiphytes were significantly different between morphotypes (two-way ANOVA), p≤0.05; ranking the branched thalli as the first preference for microalgal epiphytes, sheet-like thalli with a smooth surface as the second one, while the lowest rank was for the mucilaginous species. The same result was found for epiphytic zooplankton. The results of the statistics revealed insignificant seasonal variations in the epiphytic microalgae and very weak correlations between the abundance of microalgae and the physico-chemical parameters. In contrast, there were significant differences for epiphytic zooplanktonic seasonal variations. Whereas, the zooplankton count was correlated preferably with environmental water salinity, followed by nutrients.

Keywords: Epiphytic Microalage, Epiphytic Zooplankton, Seaweeds, Water Quality, Alexandria

Introduction

Epiphytism is a widespread phenomenon in marine benthic communities, especially in the rocky intertidal zone (Kraberg and Norton, 2007). Although most epiphytic algae are essentially facultative and are not specifically associated with a host species (Wahl and Mark, 1999), some are known as specific and obligate epibionts on certain hosts (Pearson and Evans, 1990). Actually, algal epiphytes play an important role in coastal benthic communities. They provide potential for mutualistic interspecific associations (Stachowicz and Whitlatch, 2005), as well as food and habitats for animals and they are considered as primary producers in the food chain (Danilov and Ekelund, 2000). They enter the second level of the food chain when they are grazed by invertebrates (Navarro, 1987). Moncreiff et al. (1992) reported that the production of epiphytic algae often exceeded that of macroalgae and seagrass itself.

On the other hand, epiphytes growing on the seaweeds cover the photosynthetic area of the seaweeds blade and therefore reduce the photosynthetic capabilities of the host algae. Another disadvantage of epiphytes is that; they decrease the host growth rates and result in loss of biomass through direct competition with the host algae for space, nutrients and inorganic carbon from the water column (Buschmann and Gómez, 1993). The rapid growth of
epiphytes, such as diatoms, which are considered the most important structural elements of the epiphyton on seaweeds or seagrass (Jacobs and Noten, 1980), is usually the result of high nutrients. These high nutrient salts levels are usually caused by human activities in the area (Lobban and Harrison, 1994).

In fact, environmental parameters highly affect the population and growth rate of epiphytes. Temperature and light intensity have a significant effect on the growth of the epiphytic organisms. There is indirect evidence from seasonal studies which show that the epiphyte biomass increases with increasing light and temperature (Lepoint et al., 1999). Epiphyte growth rate is highly correlated with dissolved oxygen and inorganic carbon. Observational and experimental studies have, indeed, reported an increase in epiphyte biomass in response to nutrient loading (Tomasko and Lapointe, 1991). While others have demonstrated shifts in composition of epiphytes under nutrient loading (Wear et al., 1999). This led implicitly to the assumption that epiphytes could be useful indicators of environmental conditions and, in particular, of nutrient concentrations (Lü and YongJian, 2009).

Currently, many land-based activities such as industrial and agricultural processes have contributed to the contamination of water systems in many coastal areas, increasing nutrient loads, leading to eutrophication and causing in turn adverse effects on aquatic biota and human health. These effects and their consequences have attracted recently the attention of researchers all over the world (David et al., 1999). Considering little literature was concerned about the distribution and effects of epiphytic populations on macroalgal hosts and their species composition on macroalgal tissues and the relationships between them and their hosts. In fact, these literatures focused on the epiphytic macroalgal species (Rindi and Guiry, 2004; Muñoz and Fotedar, 2009; Kersen et al., 2011), whereas very few literatures dealt with epiphytic microalgae and/or epifauna. Tanaka et al. (1984) conducted a study on epiphytic microalgal communities on Sargassum piluliferum and artificial seagrasses in Sargassum bed. Totti et al. (2009) conducted a survey on epiphytic diatom communities on ten species of seaweeds belonging to brown, red and green macroalgae from Iceland coastal waters. Gestoso et al. (2010) investigated the variability of epifaunal assemblages associated with native and invasive macroalgae.

Noticeably, many areas in Egypt have been reported to be polluted principally due to the discharge of untreated wastes into the coastal areas by many land based sources. Pollution in the Eastern Harbor is attributed to discharge of untreated domestic waste water through several submerged minor sewage outfalls distributed along the harbor coast. Along with, various small industries such as: Car cleaning and repairing, some foodstuff plants, gas stations, small dairy plants and some foundries. Also, the harbor water receives additional waste effluents from fishing ships and the shipyard situated on its western side. Other wastewater discharges occur through two marine outfalls lying at the outer sides of the harbor, the Qayet Bay and Silsila marine outfalls (Abdallah, 2007). Accordingly, the Eastern harbor was classified in the previous research as a very high eutrophic estuary (EEAA, 2008). In view of the enormous discharge of mixed effluents into this Harbor, it becomes imperative to investigate the extent of pollution in the Harbor and its consequences on biota.

However, the studies conducted on epiphytic microalgae are scarce in Egypt, focusing on one or two classes only, without studying the effect of ecological conditions on these organisms, thus lacking much information, especially on associated fauna which was not studied before. Madkour and El-Shoubaky (2007) investigated the epiphytic diatoms growing on seaweeds inhabiting the Port Said coast (Mediterranean Sea) seasonally (2004-2005). El-Zayat (2012) carried out a study on epiphytic harmful microalgae along Alexandria Coast; whereas Ismael (2012) and Hegazy (2013) focused only on the benthic bloom of Cyanobacteria associated with macroalgae in Alexandria waters.

The objective of this study was to conduct a qualitative and quantitative study on the epiphytic microalgae and epiphytic zooplankton on seaweeds, which is the first one in consideration of epiphytic zooplankton assemblages and their relationship with epiphytic microalgae and both of them in relation to their macroalgal host. This study, also attempts to emphasize the effect of the quality of coastal waters of the Eastern Harbor on these biota and to assess condition index for macroalgae and epiphytes.

**Materials and Methods**

**Study Area**

Samples were performed seasonally during two years successively: Winter, summer, autumn (2012) and spring (2013), covering the study area, the Eastern Harbor (E.H.), which is a shallow semi-enclosed basin receiving sewage effluents coming from several minor sewers in addition to shipping activities, fisheries and other sources of pollution (Abdallah, 2007) (Fig. 1). However, the site sheltered four or five common seaweeds, which were recorded previously (Shams El-Din et al., 2007) and were found along the Egyptian Mediterranean coast.
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Fig. 1. The study area and sampling station during 2012-2013

Water Samples

Surface water samples were collected seasonally, from the same areas and during the same time of sampling of algae. Water pH was measured in situ using Metrohm (827 pH) pH electrode calibrated with TRIS buffer on total scale (pHT) following Dickson et al. (2007). The water samples were collected directly then brought to laboratory for determinations of all studied parameters: Salinity, oxidizable organic matter (O.M.), chlorophyll-a and nutrient salts. Dissolved oxygen was fixed in field and measured according to the modified Winkler’s method according to Strickland and Parsons (1972) taking all the required precautions. Measurements of salinity was done using a Bekman Induction Salinometer (Model RS-7C), which is standardized with Standard Seawater Copenhagen, Denmark, of chlorinity 39.377 g l⁻¹. The conductivity ratio was measured to the nearest 1×10⁻⁵ and converted to salinity, up to the nearest 0.01 g l⁻¹ using the tables of salinity/conductivity conversions. Nutrient salts (nitrite, nitrate, ammonium, phosphate and silicate) were measured spectrophotometrically in filtered samples, using 6800UV/VIS Spectrophotometer (JENWAY) according to Grasshof et al. (1975). Chlorophyll was measured to illustrate water productivity according to Strickland and Parsons (1972). Oxidizable organic matter was measured according to the method of Carlberg (1972). The readings of temperature were obtained by personal communication (Tarek El Gezery).

Collection of Macroalgae

Each host algal species was collected from the study area at the sublittoral zone at a 0.5-1 m depth, representing the algal groups present in this area. Thereafter, algal samples were separated in the field and put in polyethylene plastic bags, then kept in ice-box. In the laboratory, Herbarium sheets with a preliminary identification of separated host species were done and/or preserved in 4% formalin. Microscopic identification of the investigated host algae was carried out according to Aleem (1993) and Braune (2008). The collected algae were Ulva fasciata, Corallina mediterranea, Corallina officinalis, Gelidium sp., Pterocladiella capillacea, Hypnea musciformis and Grateloupia doryphora.

Separation of Epiphytes

Separation of Epiphytic Microalgae

Three replica of each algal host were prepared for microalgae estimation. About 5 gm of host algae were weighed for each replica and then were put in plastic bottles containing about 30 ml filtered seawater; they were then, vigorously shook and finally washed with little filtered seawater. The shaking procedure was repeated several times to ensure that most of the attached organisms were separated. This method was used by previous researchers to remove microepiphytic species (Mabrouk et al., 2011). Thereafter, the samples were passed through 100 µm mesh sieves to remove host
algae and large particles and were completed to 50 ml to obtain a concentrate of microalgae, which were finally preserved with 4% neutralized formalin. An estimation of the microalgal count was carried out in a counting cell (Sedgewick Rafter Cell) and was expressed as units per gm fresh weight of macroalgae (units comprised of cells, colonies and filaments), using a binocular microscope. The reported results are the means of triplicate count for epiphytic microalgae on each host for each sampling period. The identification of microalgal taxa followed Peragallo and Peragallo (1897-1908), Cupp (1943), Heurk (1962), Hendey (1964), Sournia (1968; 1986), Dodge and Hart-Jones (1982) and Mizuno (1990). The phytoplankton species are updated according to the taxonomic database sites, like algaebase.com (ab), World Register of Marine Species (WoRMS), Canadian Register of Marine Species (CaRMS), Nordic microalgae and aquatic protozoa (NOD) and Integrated Taxonomic Information System (ITIS).

Separation of Associated Zooplankton

The associated zooplankton was also estimated in the same samples used for estimation of epiphytic microalgae and was expressed as organisms per gm fresh weight of macroalgae. The reported results are the means of triplicate count for epiphytic zooplankton on each macroalgal species for each sampling period. The identification of epiphytic zooplankton taxa followed Jorgensen (1924), Rose (1933) and Tregouboff and Rose (1957).

The zooplankton species are updated according to the taxonomic database sites, like World Data base for Protozoa, IOMS and integrated Marine Observing System for Copepoda.

Statistical Analysis

Correlation coefficient of total count on each host, total count of each class, total count of dominant species of epiphytic microalgae and epiphytic zooplankton separately with physico-chemical parameters (temperature, pH, salinity, dissolved oxygen, nitrite, nitrate, reactive soluble phosphate, silicate, oxidizable organic matter and chlorophyll-a) were calculated at confidence limit 95% (p≤0.05), using Excel program 2007. Also the correlation between the epiphytic microalgae and the epiphytic zooplankton was calculated at confidence limit 95%.

Macroalgal thalli were subdivided in three groups and defined on the basis of both thallus morphology and surface characteristics as follows: Branched thalli, sheet-like thalli with smooth surface and flat thalli with mucilaginous surface. Differences between seaweed morphotypes coupled with the abundance of epiphytes seasonally were assessed through a two-way Analysis Of Variance (ANOVA) separately for epiphytic microalgae and epiphytic zooplankton groups to examine the effect of seasonal variations for each host species (4 levels) and different morphotypes of host species (3 levels), using Excel program 2007.

Results

Water Quality

The measured water quality of surface sea water samples collected from the Eastern Harbor during 2012-2013 is as shown in Table 1. During the study period, surface water temperature varied following the variation of air temperature. It fluctuated between 22.76°C in autumn to 28.85°C in summer.

The recorded pH was the highest during winter (8.48) and the lowest during summer (7.93). The readings of salinity were typical to that of the Mediterranean Sea during the three seasons, ranging from 38.25‰ during spring 2013 to 39.23‰ during summer 2012. Sea water in E.H. showed dissolved oxygen content of a moderate values during all seasons except the autumn (2.59 mg O₂ l⁻¹), with an annual average of 3.92±0.90 mgO₂ l⁻¹.

Chlorophyll-a attained a maximum value of 5.06 µg l⁻¹ during spring (2013), indicating the high photosynthesis activity during this season and which synergized with a high oxygen content. On the other hand the lowest chlorophyll content was recorded during winter (2012) (2.86 µg l⁻¹). The oxidizable organic matter ranged between (0.80 mgO₂ l⁻¹) during winter and (4.80 mgO₂ l⁻¹) during summer. The annual average oxidizable organic matter was of 3.58±1.87 mgO₂ l⁻¹.

As far as nutrients are concerned, nitrite and nitrate showed the maximum content during summer (1.75 and 3.28 µmol l⁻¹), respectively; whereas, the minimum content was recorded during winter (0.45 and 0.93 µmol l⁻¹), with an annual average of 1.27±0.58 and 2.31±1.04 µmole l⁻¹, respectively. Inorganic phosphate concentration was minimal during summer and autumn (0.04 µmole l⁻¹) and maximal during winter (0.26 µmole l⁻¹), with an annual average of 0.11±0.10 µmol l⁻¹. On the other hand, inorganic silicate’s annual average was (5.41±2.62 µmol l⁻¹) and its concentration ranged between (3.02 µmol l⁻¹) during winter and (8.69 µmol l⁻¹) during autumn.

Species Composition of Seaweeds

A total of 63 host algal samples were collected during (2012-2013) at the depths (0.5-1 m), representing 7 species belonging to two algal classes. Chlorophyceae was represented by the order Ulvales comprising of one family; Ulvaceae which was, represented by one species; Ulva fasciata Delile, whereas the second class, Rhodophyceae was represented by four orders (Corallinales, Gelidiales,
Gigartinales and Haliymeniales) comprising of one family each (Corallinaeae, Gelidiaceae, Hypneaceae and Haliymeniales). The family Corallinaeae was represented by two species *Corallina mediterranea* and *Corallina officinalis* Linnaeus whereas, the family Gelidiaceae was represented by two species *Gelidium sp.* and *Pierocladia capillacea* (S.G.Gmelin) Santelices and Hommersand. The family Hypneaceae was represented by one species *Hypnea musciformis* (Wulffen) Lamouroux and the family Haliymeniales was represented by the species *Grateloupia doryphora* (Montage) M.A. Howe, which is introduced in the Mediterranean Sea and considered as invasive species (Gavio and Fredricq, 2002). *G. doryphora* was introduced recently in the Egyptian Mediterranean Sea (personal communication with Nihal Shams El-Din).

**Epiphytic Microalgae**

**Community Composition**

During the study period, a total of 152 microalgal taxa belonging to 68 genera were identified to the genus or species level (Table 2). These taxa included 68 freshwater and brackish forms and 84 marine forms. Five algal groups were represented in the Harbor; namely Bacillariophyceae (88 species), Chlorophyceae (24 species), Dinophyceae (18 species), Cyanophyceae (15 species) and Euglenophyceae (7 species). There were no marked differences among the host species during the same season in respect to species number. In contrast, the species number differed seasonally in respect to each host species (Fig. 2). The maximum number of species was determined on the species *C. officinalis* during summer (2012) (45 species) and on *C. mediterranea* during spring (2013) (42 species) (Table 2). However, the typical freshwater forms contributed by a very low number to the total number of species attached on the surface of each host. This number did not exceed 7 species on all the host algae, except on *G. doryphora* during winter, *C. mediterranea*, *C. officinalis*, *P. capillacea* during summer and on *U. fasciata* during autumn (Fig. 2). However, only few species were recorded on all host algal species (*Chroococcus sp.*, *Actinoptychus undulatus*, *Gramatophora oceania*, *Licmophora dalmatica*, *licmophora gracilis*, *Navicula cryptocephala* and *Nitzschia kützingiana*). In contrast, there were epiphytic species that showed selectivity and were specific to a definite host (Table 2).

**Relative Abundance**

There is a great difference in the relative abundance of the epiphytic microalgal groups temporally and specifically. However, the host species *U. fasciata* recorded high percentage of diatoms during all the seasons except during autumn, where the Cyanophyceae was dominant (61.90%). The two groups, Euglenophyceae and Dinophyceae were scarcely present on *U. fasciata* and all the other host species during the four seasons, except one case of high dinoflagellates contribution (Fig. 3). The two corallinates *C. mediterranea* and *C. officinalis* showed similar patterns of groups' distribution, since during winter (2012) the diatoms were dominant contributing 96.58% on the former species and 98.52% on the latter. The diatoms lead to the succession of Cyanophyceae during summer (2012), contributing 92.23% on *C. mediterranea* and 88.22% on *C. officinalis*. During autumn, Chlorophyceae was dominant on *C. officinalis* (75.58%), whereas, *C. mediterranea* was not found. The spring season was characterized by the contribution of the different groups with different percentages on the surface of both host algae (Fig. 3). *Gelidinium sp.* was recorded only during winter (2012), where Chlorophyceae was co-dominant with Bacillariophyceae. However, diatoms prevailed during the study period. They were dominant on *P. capillacea* during winter, (86.25%), summer (70.53%) and autumn (83.55%), against dinoflagellates, which attained for the first time (87.92%) during spring (2013). The rhodophyte *H. musciformis* showed different patterns during the three seasons, where Cyanophyceae predominated during summer (85.49%), during autumn (78.96%) and co-shared the dominance with diatoms during spring (2013) (48.40 and 49.76%), respectively. The last species during this study, *G. doryphora* was found only during winter (2012) and spring (2013), where the different groups of epiphytic microalgae co-shared with different percentages (Fig. 3).

**Table 1. The water quality of sea water samples collected from the macroalgal ambiance in the Eastern Harbor during (2012-2013)**

|                | T (°C) | pH      | Salinity (%) | D.O. mg O2/l | NO3 | NO2 | PO4 | SiO2 | OM mg O2/l | Chlorophyll (µg l⁻¹) |
|----------------|--------|---------|--------------|--------------|-----|-----|-----|------|------------|----------------------|
| Winter (2012)  | -      | 8.48    | 38.35        | 4.41         | 0.45| 0.93| 0.26| 3.02 | 0.80       | 2.86                  |
| Summer (2012)  | 28.85  | 7.93    | 39.23        | 4.49         | 1.75| 3.28| 0.04| 6.31 | 4.80       | 4.50                  |
| Autumn (2012)  | 22.76  | 8.09    | -            | 2.59         | 1.60| 2.13| 0.04| 8.69 | 4.48       | 2.96                  |
| Spring (2013)  | -      | 8.29    | 38.25        | 4.20         | 1.26| 2.90| 0.10| 3.60 | 4.25       | 5.06                  |
| max            | 28.85  | 8.48    | 39.23        | 4.49         | 1.75| 3.28| 0.26| 8.69 | 4.80       | 5.06                  |
| min            | 22.76  | 7.93    | 38.25        | 2.59         | 0.45| 0.93| 0.04| 3.02 | 0.80       | 2.86                  |
| average        | 25.81  | 8.20    | 38.61        | 3.92         | 1.27| 2.31| 0.11| 5.41 | 3.58       | 3.84                  |
| SD             | -      | 0.24    | 0.54         | 0.90         | 0.58| 1.04| 0.10| 2.62 | 1.87       | 1.10                  |
Table 2. Check list of the epiphytic microalgae during the study period (2012-2013)

| Bacillariophyceae | Cyanophyceae | Euglenophyceae |
|------------------|-------------|---------------|
| *Cocconeis*      | *Coccomyxa*  | *Euglena*     |
| *Cyclotella*      | *Cyanobacteria* | *Euglena*     |
| *Cylindrotheca*   | *Cyanobacteria* | *Euglena*     |
| *Diatoma*        | *Cyanobacteria* | *Euglena*     |
| *Dinobryon*      | *Cyanobacteria* | *Euglena*     |
| *Euglena*        | *Cyanobacteria* | *Euglena*     |
| *Fragilaria*     | *Cyanobacteria* | *Euglena*     |
| *Gymnodium*      | *Cyanobacteria* | *Euglena*     |
| *Pediastrum*     | *Cyanobacteria* | *Euglena*     |
| *Phytothrix*     | *Cyanobacteria* | *Euglena*     |
| *Pinnularia*     | *Cyanobacteria* | *Euglena*     |
| *Spirulina*      | *Cyanobacteria* | *Euglena*     |
| *Symploca*       | *Cyanobacteria* | *Euglena*     |

| U. fasciata | C. mediterranea | C. officinalis | Gelidium sp. | P. capillacea | H. muscipiformis | G. diphtherorhabdus |
|-------------|----------------|---------------|--------------|--------------|----------------|---------------------|
| w s a P     | w s a P        | w s a P       | w s a P      | w s a P      | w s a P         | w s a P             |

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Standing Crop

The total count of the epiphytic microalgae displayed wide variations among host species during each season. The three host species *U. fasciata*, *G. doryphora* and *Gelidium sp.* maintained relatively low count of microalgae during the seasons in which they were recorded. The highest epiphytic count on *U. fasciata* was recorded during autumn (4694 units/gm fresh weight) (Table 3), where the dominant species were both cyanophycean *O. limosa* (33.51%) and *Chroococcus sp.* (19.28%) (Fig. 4). The maximum number of epiphytes on *G. doryphora* (7558 units/gm f.w.) was recorded during spring. The dominant species were the chlorophyte *Crucigenia quadrata* (77.83%) and the cyanophyte *Chroococcus sp.* (21.03%). Whereas, *Gelidium sp.* appeared only during winter and was dominated by four epiphytic
species with different percentages (Fig. 4). The rhodophyte *C. mediterranea* showed a peak during summer, where the total count attained a maximum of $5.414 \times 10^5$ units/gm f.w., so that *Chroococcus sp.* overwhelmed the other epiphytes and recorded 89.28% (Table 3 and Fig. 4). The species *C. officinalis* recorded two peaks of epiphytic microalgae. The summer peak was amounted to be $(6.202 \times 10^5$ units/gm f.w.) due to the prevalence of *Chroococcus sp.* (87.02%) and the autumn peak $(6.659 \times 10^5$ units/gm f.w.) due to the dominance of *Stigeoclonium sp.* (75.51%). The two last host species *H. musciformis* and *P. capillacea* recorded their peaks during summer attaining a maximum of $7.992 \times 10^4$ and $2.170 \times 10^4$ units/gm f.w., respectively (Table 3). Again, the cyanophyte *Chroococcus sp.* was dominant on *H. musciformis* (80.35%) but retrograded on *P. capillacea* (12.00%) and co-dominated with four diatoms to form the main bulk of epiphytic community (Fig. 4).
Fig. 3. The distribution of different classes of epiphytic microalgae during the study period (2012-2013)

Actually, the summer season was almost the most productive one. Whereas, the cyanophytes especially *Chroococcus* sp. and chlorophytes played an important role in the main bulk during all seasons. On the other hand, there were some harmful epiphytes, which were recorded during this study on different host species (Table 4). Many of them contributed very low percentages and appeared occasionally, except the cyanophytes *Oscillatoria limosa*, *O. tenuis* and *Planktothrix aghardhii*, which appeared frequently and during different seasons (Table 2 and Fig. 4).
Fig. 4. The Distribution of dominant species of epiphytic microalgae during the study period (2012-2013)

**Epiphytic zooplankton**

**Community Composition**

During the study period, in total, 50 zooplankton taxa including 32 genera were identified. Most of them were Protozoa (33 species: 17 Tintinnida, 14 Foraminifera and 2 non tintinnid ciliates), Copepoda (7 species) in addition to its larval stages and Rotifera (6 species). Acantharia, Radiolaria and Tardigrada were represented only by one species each besides free living nematodes. Other groups (polychaetes, amphipods, decapods, cirripedes, echinoderms, isopods and lamillibranchs) were represented by their larval stages (Table 5). The maximum number of species were determined on the species *C. officinalis* during all seasons except winter (2012) (only 7 species) and on *U. fasciata* during winter and autumn (Table 5). However, only few taxa were recorded on most host algal species (*Spirillina vivipora, Euterpa acutifrons*, free living nematodes and amphipods).
Table 3. The total count (units/gm f.w.) of epiphytic microalgae on the different macroalgal species during (2012-2013)

| Chlorophyceae | Winter (2012) | Summer (2012) | Autumn (2012) | Spring (2013) |
|---------------|---------------|---------------|---------------|---------------|
| Order: Ulvales | Ulva fasciata  | 1769±478      | 2908±2903     | 4694±3463     | 735±472       |

Rhodophyceae

Order: Corallinales

Family: Corallinaceae

Corallina mediterranea 5608±3299 5414±23383 * 6346±3144

Corallina officinalis 7984±116412 6204±976778 66590±130443 2602±2158

Order: Gelidiaceae

Family: Gelidiaceae

Gelidium sp. 2308±665 * * *

Pterocladiella capillacea 15608±9228 21710±17817 * 7992±94037 28143±30928 3602±1471

Note: The sign (*) means that the species was not found.

Table 4. Potentially harmful epiphytic microalgae species recorded during the study period (2012-2013)

| Species | Trophic state | Toxin | Harmful effect | References |
|---------|---------------|-------|----------------|------------|
| Cyanophyceae | Oscillatoria limosa | autotrophic | microcystins | Newcombe (2009) |
| Oscillatoria tenuis | autotrophic | microcystins | | |
| Phormidium tenue | autotrophic | - | | |
| Planktothrix aghardhii | autotrophic | microcystins, anatoxin | non-toxic or toxic | Sivonen et al. (1989) |
| Planktothrix formosa | autotrophic | Microcystins, homoanatoxin-a | neurotoxic | Steffensen et al. (2001) |
| Planktothrix planctonica | autotrophic | microcystins | | |
| Bacillariophyceae | Asterionellopsis glacialis | autotrophic | - | Bloom-forming | Ismail (2014) |
| Cyclotella menghniiana | autotrophic | - | Bloom-forming | |
| Odontella aurita | autotrophic | - | Bloom-forming | Özmen-Say and Balkis (2012) |
| Proboscia alata | autotrophic | - | Bloom-forming | |
| Pseudo-nitzschia pungens | autotrophic | domoic acid | ASP | IOC, Casteleyen et al. (2008) |
| Pseudo-nitzschia seriata | autotrophic | domoic acid | ASP | IOC, Feuling et al. (2004) |
| Thalassionema nitzschioides | autotrophic | - | Bloom-forming | Özmen-Say and Balkis (2012) |
| Thalassiosira estuariensis | autotrophic | - | fish gill lesions and mortality | Yao et al. (2006) |
| Dinophyceae | Dinophysis caudata | autotrophic | DSP toxin, ichthyotoxins | Bloom-forming | Özmen-Say and Balkis (2012) |
| Neoeucotrichium furca | Mixotrophic | - | Bloom-forming, fish-killing, anoxia | |
| Neoseratium fusus | Mixotrophic | - | Fish-killing | |
| Prorocentrum micans | autotrophic | - | non-toxic or toxic, DSP | Tilstone et al. (2010) |
| Prorocentrum triestinum | autotrophic | - | Bloom-forming, fish-killing | Özmen-Say and Balkis (2012) |

Relative Abundance

There is a great difference in the relative abundance of the epiphytic zooplankton groups temporally and specifically. However, the host species U. fasciata was characterized by the contribution of the different groups with different percentages during winter and summer (Fig. 5). During autumn Protozoa was dominant (43%) which lead to the succession of Copepoda during spring (50%). The two corallinates C. mediterranea and C. officinalis showed different pattern of groups' distribution, where C. mediterranea recorded a high percentage of Protozoa during winter (55%). The two groups, Copepoda and nematodes co-shared the dominance during summer (2012) and spring (2013). While on the surface of C. officinalis, the Protozoa was dominant during winter (51%), autumn (41%) and co-shared the dominance with Copepoda during spring (2013) (35 and 36% respectively), against free living nematodes, which attained (62%) during summer (2012). Gelidium sp. was recorded only during winter (2012), where Protozoa (42%) was co-dominant.
with Copepoda (32%). The host species *P. capillacea* recorded a high percentage of free living nematodes during all the seasons except during spring (2013), where Copepoda was co-dominant with Protozoa. The rhodophyte *H. musciformis* showed different patterns during the three seasons, where free living nematodes predominated during summer (55%) and autumn (74%); Copepoda co-shared the dominance with Rotifera and nematodes (41, 27 and 21%), respectively during spring. The last species during this study, *G. doryphora* was found only during winter (2012) and spring (2013), where Prorozoa predominated during winter and co-shared the dominance with Copepoda and nematodes which attained (26 and 13%), respectively, during spring. Rotifera was scarcely present on all other host species during all seasons.

![Graph showing distribution of different classes of epiphytic zooplankton during the study period (2012-2013)](image)

Fig. 5. The distribution of different classes of epiphytic zooplankton during the study period (2012-2013)
Standing Crop

The total count of the epiphytic zooplankton displayed wide variations among host species during each season. The four host species *U. fasciata*, *G. doryphora*, *H. musciformis* and *Gelidium sp.* maintained relatively low count of epiphytic zooplankton during the seasons in which they were recorded. The highest zooplankton count on *U. fasciata* was recorded during autumn (358 organisms/gm fresh weight) (Table 6), where the dominant species were the two tintinnid *Undella dilatata* (18%) and *Acanthostomella elongata* (12%) beside the free living nematodes (25%) (Fig. 6). The
maximum number of zooplankton on *G. doryphora* (131 organisms/gm f.w.) was recorded during winter. The dominant species was the tintinnid *U. hyalina* (21%). Whereas, *Gelidium sp.* appeared only during winter and was dominated by the foraminiferan *Spirillina vivipora* (31%) (Fig. 6). The two corallinates, *C. mediterranea* and *C. officinalis* recorded their peaks during summer attaining a maximum of 2097 and 1497 organisms/gm f.w. respectively. Free living nematodes were dominant on *C. officinalis* (62%) but retrograded on *C. mediterranea* (30%) and co-dominated with the copepod *Euterpina acutifrons* and its larvae to form the main bulk of epiphytic zooplankton community (Table 6 and Fig. 6). The species *H. musciformis* showed a peak during summer (317 organisms/gm f.w.) due to the dominance of nematodes (55%). The last host species *P. capillacea* recorded the highest peak during winter attaining a maximum of 4158 organisms/gm f.w due to the prevalence of free living nematodes (93%) (Table 6 and Fig. 6).

Fig. 6. The distribution of dominant species of epiphytic zooplankton during the study period (2012-2013)
Table 6. The total count (organisms/gm f.w.) of epiphytic zooplankton on the different macroalgal species during (2012-2013)

|                          | Winter (2012) | Summer (2012) | Autumn (2012) | Spring (2013) |
|--------------------------|---------------|---------------|---------------|---------------|
| Chlorophyceae            |               |               |               |               |
| Order: Ulvales           |               |               |               |               |
| Family: Ulvaceae         |               |               |               |               |
| Ulva fasciata            | 305±56        | 139±61        | 358±45        | 94±39         |
| Rhodophyceae             |               |               |               |               |
| Order: Corallinaceae     |               |               |               |               |
| Family: Corallinaceae    |               |               |               |               |
| Corallina mediterranea   | 691±207       | 2097±1299     | *             | 1270±153      |
| Corallina officinalis    | 1044±603      | 1497±537      | 1289±461      | 380±72        |
| Order: Gelidiales        |               |               |               |               |
| Family: Gelidiaceae      |               |               |               |               |
| Gelidium sp.             | 310±53        | *             | *             | *             |
| Pterocladiella capillacea| 4158±1345     | 375±67        | 534±74        | 457±146       |
| Order: Gigartinales      |               |               |               |               |
| Family: Hypneaceae       |               |               |               |               |
| Hypnea musciformis       | *             | 317±11        | 309±138       | 251±82        |
| Order: Halymeniales      |               |               |               |               |
| Family: Halymenaceae     |               |               |               |               |
| Grateloupa doryphora     | 131±51        | *             | *             | 23±2          |

Note: The sign (*) means that the species was not found

Table 7. Two way Analysis of variance (ANOVA) carried out on the morphotypes of the host species and the seasonal variations of epiphytic microalgae groups

| Source of Variation       | Difference effect | df   | MS        | F         | P-value | F crit |
|---------------------------|------------------|------|-----------|-----------|---------|--------|
| Sample (Morphotypes)      | 2>1>3 In (A>B>C>D>E) | 2    | 2815306828.0 | 2.859 | 0.068   | 3.204  |
| Sample (Groups)           | -                | 4    | 804379824.2 | 0.817 | 0.521   | 2.579  |
| Columns (Seasons)         | -                | 8    | 745793180.9 | 0.757 | 0.641   | 2.152  |
| Within                    | -                | 45   | 984692427.7 |         |         |        |
| Total                     |                  | 59   |           |           |         |        |

Note: The morphotypes no.1 = sheet-like with smooth surface, no. 2 = branched thalli, no. 3 = mucilaginous surface. In the branched group: A = C. officinalis, B = C. mediterranea, C = H. musciformis, D = P. capillacea, E = Gelidium sp.; n = 12, p>0.05

Table 8. Two way analysis of variance (ANOVA) carried out on the morphotypes of the host species and the seasonal variations of epiphytic zooplankton groups

| Source of Variation       | Difference effect | df   | MS        | F         | P-value | F crit |
|---------------------------|------------------|------|-----------|-----------|---------|--------|
| Sample (Morphotypes)      | 2>1>3 In (D>A>B>C>D>E) | 2    | 45243.500  | 12.700    | 0.000   | 3.080  |
| Sample (Groups)           | -                | 11   | 20532.050  | 5.767     | 0.000   | 1.878  |
| Columns (seasons)         | w>s>a>p          | 22   | 14489.640  | 4.067     | 0.000   | 1.642  |
| Within                    | -                | 108  | 3562.432   |           |         |        |
| Total                     |                  | 143  |           |           |         |        |

Note: The morphotypes no.1 = sheet-like with smooth surface, no.2 = branched thalli, no. 3 = mucilaginous surface. In the branched group: A = C. officinalis, B = C. mediterranea, C = H. musciformis, D = P. capillacea, E = Gelidium sp. W = winter, s = summer, a = autumn, p = spring; n = 12, p>0.05

Statistical Analysis

The abundance of epiphytes were significantly different between morphotypes (two-way ANOVA), p≤ 0.05; ranking the branched thalli (C. mediterranea, C. officinalis, Gelidium sp., H. musciformis and P. capillacea) as the first preference for microalgal epiphytes, sheet-like thalli with smooth surface (U. fasciata) as the second one, while the lowest rank was for the mucilaginous species G. doryphora, which was based on the average of the total count of each group. Among the branched species, the order of preference of epiphytic microalgae was as follows: C. officinalis > C. mediterranea > H. musciformis > P. capillacea > Gelidium sp., based on their average of total count (Table 7). The same result was found for epiphytic zooplankton except that the order of preference among branched group was as follows: P. capillacea > C. officinalis > C. mediterranea > H. musciformis > Gelidium sp. (Table 8). On the other hand, no
significant differences were observed for epiphytic microalgal seasonal variations. In contrast, there were significant differences for epiphytic zooplanktonic seasonal variations (Table 8).

**Discussion**

Epiphytic algae has significant role in energy and nutrient transformation as well as being a bio-indicator of pollution (Davies, 2009). Various factors affect the distribution of epiphyte loads across both seasonal and regional scales. Structural factors of macroalgae affect the space and time signal because of their importance for epiphyte colonization and growth. Physico-chemical factors affecting epiphyte loads include nutrient availability, temperature, salinity, water quality and current and light availability. Epiphyte abundance has been experimentally demonstrated to be the function of nutrient availability (Frankovich and Fourqurear, 1997).

pH measurements in the Eastern Harbor (2012-2013) showed seasonal variation revealing the effect of discharging water to the harbor. Low pH value during summer is attributed to the mineralization of organic matter and release of CO$_2$ which causes a decrease in pH value. pH showed a highly significant negative correlation with salinity ($r = -0.91$), nitrate ($r = -0.95$) and organic matter ($r = -0.86$). All of these correlations confirm that the seasonal variation in pH is resulted from the microbial activity via organic matter mineralization processes. On the other hand, pH showed a high positive correlation with phosphate ($r = 0.91$) indicating that discharged waste water is another factor controlling water pH in such polluted harbor.

Temperature is an important physical factor influencing the growth of planktonic plants as well as its productivity and its community structure and species abundance. Water temperature during the current study showed seasonal pattern that is exactly similar to predominating in the Egyptian coastal water which is directly affected by the air temperature seasonal pattern and solar radiation. The annual average of sea water temperature is approximately similar to the recorded by (El-Geziry and Mayiya, 2006) in the Eastern Harbor that was 22.10°C with amplitude of 14.9°C.

The salinity variations are of particular significance as they reflect changes caused by the mixing of both fresh and sea water. During the investigation period the readings of salinity were typical to that of the Mediterranean Sea during the three seasons, ranging from 38.25% during spring 2013 to 39.23% during summer 2012 with an annual average 38.61±0.54. According to Said et al. (2007) salinity in the upper layer for the whole Egyptian Mediterranean Sea water ranged from 38.8 to 39.2%.

Dissolved Oxygen concentration (DO) in natural water is generally changeable and represents a momentum balance between the rate of supply and consumption. In the biologically active coastal zone the net concentration of dissolved oxygen is highly variable depending on this balance. During the study period in Eastern Harbor, dissolved oxygen concentration showed a wide seasonal variation with approximately low annual average 3.92±0.9 mgO$_2$ l$^{-1}$. Although dissolved oxygen is supposed to be high in relation to the intensive growth of epiphytic microalgae, surprisingly it recorded content less than <4.5 mgO$_2$ l$^{-1}$. The saturation state of the recorded dissolved oxygen in Eastern Harbor ranged from 30 to 58.25%. These recorded contents were less than the range of dissolved oxygen in open sea water (0-5.6 mgO$_2$ l$^{-1}$) (Talley et al., 2011) and much less than the recorded by Abdel-Halim and Khairy (2007) (7.58 mgO$_2$ l$^{-1}$) in Eastern Harbor. This could be attributed to the increase in the biochemical oxygen demand to break down organic matter brought to the bay by the sewage (Owili, 2003).

According to Satpathy et al. (2010), the distribution and behavior of nutrients in the coastal environment exhibit considerable variations due to rainfall, tidal incursion, quantum of fresh water inflow and biological activities. This agrees with the present study where the lowest nutrient recorded was during winter as a result of strong mixing, low residence time, low fresh water discharge and low biological activities during this season. Nitrite plays an intermediate role in several biological processes such as ammonium oxidation, assimilation and excretion of nitrite by phytoplankton and bacteria (Satpathy et al., 2010). The nitrite concentration in the study area indicated that it’s not biogenic; they were mainly produced via the biological processes. This was verified by the strongly negative correlation with phosphate ($r = -0.99$), while the positive correlation with nitrate ($r = 0.85$) indicates that there is oxidation of ammonia to nitrite and then nitrate by nitrifying bacteria in addition to releasing from biodegradable organic matter via mineralization.

Phosphorus is a very important factor for the growth and reproduction of both plant and micro organisms. In the E.H. phosphate concentrations were usually below 1 µmol l$^{-1}$ (Abdel-Halim and Khairy, 2007). Phosphorus sustained relatively low concentrations in the present study (0.04-0.26 µmol l$^{-1}$). The lowest phosphate content 0.04 µmol l$^{-1}$ was during summer coincided with the highest sum of total epiphytic count (1266219 units/gm f.w.) (Table 3) confirming the phosphorous preferentially uptake in photosynthetic process. The
biological activity during winter was the lowest coupled with the highest phosphate concentration due to low photosynthetic uptake. This is supported by the measurements of the lowest sum of total epiphytic microalgae (106595 units/gm f.w.) (Table 3). The annual average content of phosphate 0.11±0.1 µmol l$^{-1}$ is much lower than that recorded by Abdel-Halim and Khairy (2007) in Eastern Harbor which was 0.51 µmol l$^{-1}$. The strongly negative correlations between inorganic phosphate and oxidizable organic matter ($r = -0.98$) revealed the phosphorous replacement as a result of microbial decomposition of organic matter (Enoksson et al., 1996).

Silicate concentration in the study area showed variations (3.02-8.69 µmol l$^{-1}$) with an annual average 5.4 µmol l$^{-1}$ that is higher than the recorded in the study of Abdel-Halim and Khairy (2007). Inorganic silicate in Eastern harbor during the study period showed a significant correlation with salinity ($r = 0.91$) indicating the anthropogenic source of silicate.

Marine macroalgae are conspicuous and dominant features of temperate intertidal and subtidal ecosystems and have important direct and indirect effects on coexisting species (Schmidt and Scheibling, 2006; Gestoso et al., 2010). Macroalgae are considered ecosystem engineers because they add spatial complexity to the substratum, modulating availability of resources and can affect assemblages of associated epibionts (Schmidt and Scheibling, 2006; Gestoso et al., 2010).

In the present study, the epiphytic microalgae were highly diversified in the study area, indicating the richness of associated epiphytic communities and the important role of the host algae, which offer a highly suitable natural substratum to these organisms (Romagnoli et al., 2007). However, the number of typical freshwater forms was relatively low compared to marine forms and only few of them were dominant and formed the epiphytic peaks. These species were the widespread cyanophyte *Chroococcus sp.* and the chlorophyte *Stigeoclonium sp.* The latter species is usually found as attached tufts or mats on submerged rocks or aquatic plants. It is sometimes abundant in polluted waters, such as the outflow of sewage treatment plants and is tolerant of heavy metals (Person, 1989). However, Palmer (1969) has shown that *Stigeoclonium* and other genera, which are recorded during this study like Oscillatoria, Euglena, Scenedesmus, Chlamydomonas, Navicula, Nitzschia and Ankistrodesmus are the species found in organically polluted waters. These results are supported by Jafari and Gunale (2006). In the present study, *Chroococcus sp.* was not influenced by any of the physico-chemical parameters, whereas, *Stigeoclonium sp.* was negatively correlated with dissolved oxygen ($r = -0.98$, $p\leq0.05$).

Although, the cyanophytes and chlorophytes were responsible for epiphytic peaks, the diatoms were the dominant group and prevailed during the study period. Sullivan and Currin (2000) reported that epiphytic diatoms are ubiquitous in shallow water environments and may be the most taxonomically diverse group of organisms in estuarine ecosystems. Vegetated ecosystems are ideal habitats for benthic diatoms and other epiphytes (Frankovich et al., 2006). Algal thalli may represent an order of magnitude greater surface area relative to sediments (Zieman et al., 1989) for the colonization and growth of diatoms. However, the successful behavior of diatoms to attach themselves to the algal host is the main factor to this mutualistic relationship (Romagnoli et al., 2007). This may be attributed to those pennate diatoms attaching themselves to seaweeds by mucilage stalks and sheaths or gelatinous pads such as Achnanthes, Gramatophora, Licmophora, Tabellaria and Synebra or by the attachment of the cell along its entire valve face such as Navicula and Pleurosigma. The centric forms like Bididdulphia, Cosinodiscus and Cyclotella, were often trapped by the thallus of seaweeds or held in the tangle of attached forms. This is in agreement with many authors (Madkour and El-Shoubaky, 2007; Totti et al., 2009). Noticeably, the other factors that affect the occurrence of diatoms or other groups are the environmental conditions such as light availability, hydrodynamic regime, salinity and nutrients (Frankovich et al., 2006) and biological processes such as grazing (Hillebrand et al., 2000), chemical interactions with the host (Amsler et al., 2005) and physiological responses (Ruesink, 1998), which may explain the succession of other groups on each algal host during the study period. In the present study, the measured physico-chemical parameters were not limiting factors to all the epiphytic groups except Chlorophyceae, which was negatively correlated with D.O. ($r = -0.98$, $p\leq0.05$).

On the other hand, the total count was relatively lower on *U. fasciata* and *G. doryphora* for epiphytes than the other hosts namely; corallinates, *H. musciformis* and *P. capillacea*; whereas Gelidium sp., is a branched thallus, which was recorded only during winter, harbored relatively higher total count than epiphytes growing on smooth and mucilaginous hosts during the same season. Our results were supported by the significant correlation between total count of epiphytic microalgae and morphotypes of hosts (two-way ANOVA), $p\leq0.05$, giving the order of preference first to the branched thalli, followed by smooth surface and then the mucilaginous one, with the order of preference among...
the branched thalli as follows: C. officinalis > C. mediterranea > H. musciformis > P. capillacea > Geldi sp., which may be due to the increase of surface area of the algal host in this direction. These findings agreed with Totti et al. (2009), who reported that host algae with a filamentous or branched structure usually present a high degree of structural complexity and might therefore increase their suitability as habitats for epibionts. In this trend, Madkour and El-Shoubaky (2007) found that there was a relationship between the architecture of seaweeds and the associated epiphytes. Most of the foliose and flattened macroalgal thalli was nearly not preferred as a substrate. By contrast, the fine and sometimes branched thallus hosted many epiphytic diatoms. Whereas, Wuchter et al. (2008) reported that smooth and slimy surfaces offer little protection against grazing.

Considering the species composition of epiphytic microalgae, the enumerated species showed different patterns of preference. Some epiphytes showed host specificity giving unique epiphytic flora on few hosts. Other ones did not reflect the previous preference pattern and existed on the majority of the macroalgae indicating the absence of selectivity. Our results agreed with the findings of Madkour and El-Shoubaky (2007). In fact, there is no rule for the selectivity of epiphytes and there is a great debate about the reason of the preference of one species to a definite host. Many authors reported different results. For instance, Patrick et al. (1968) found variation in species composition as a function of substrate indicating the presence of selectivity between epiphytic algal species and the respect substrate, whereas Fontaine and Nigh (1983) and Sullivan (1984) found similarities in epiphytic communities, demonstrating non selectivity between epiphytes and the hosts. Ismael (2012) found that in general Oscillatoriales preferred green algae to red algae and on the species level they showed selectivity for each host of green algae. In this trend, El-Zayat (2012), found that the abundance of Oscillatoria spp. was most important on green algae than red algae. He concluded that no macroalgae appeared to be overall “best” or “worst” host, indicating that host specificity does not depend on the morphology of the algae but may involve specific requirements between epiphytic and host either by site or by host.

Regarding the harmful algae, all the recorded species in the present study were previously recorded as epiphytes in the Eastern Harbor (El-Zayat, 2012; Ismael, 2012) and/or as planktonic species (Ismael and Halim, 2000; Ismael et al., 2005; Ismael, 2014). Noticeably, many species of them were recently included in the list of Harmful Algae in North Africa (HANA) endorsed by the Intergovernmental Oceanographic Commission of UNESCO like Dinophysis caudata, Prorocentrum micans, Prorocentrum triestinum, Pseudo-nitzschia pungens and Pseudo-nitzschia spp. In addition to Oscillatoria spp. and Planktothrix spp. which were newly separated as two genera according to Anagnostidis and Komárek (1988). The other species were recently included in the list of harmful algae over the world Phormidium tenue (Mohamed et al., 2006), Odontella aurita, Proboscia alata, Thalassionema nitzschoide, Neoceratium furca, Neoceratium fusus (Özman-Say and Balkis, 2012) and finally, Thalassiosira aestivalis (Yao et al., 2006). Although, they all contributed by a very low count to the main bulk, except Oscillatoria limosa, Oscillatoria tenuis and Planktothrich aghardhii, their occurrence as epiphytic or even planctonic forms threatened the Eastern Harbor and may have a drastic effects on biota. These species are potentially harmful and can flourish at suitable environmental conditions and secrete their toxins that can cause fish mortality (Ismael, 2012). In the present study, correlation coefficient was conducted between the three frequent harmful species (Oscillatoria limosa, Oscillatoria tenuis and Planktothrich aghardhii) and the physico-chemical parameters to determine the limiting factors for their flourishing in the Harbor. Oscillatoria limosa and Planktothrich aghardhii was only correlated with oxygen (r = -0.98, p≤ 0.05), Oscillatoria tenuis was positively correlated with temperature and nitrate (r = 0.99, 0.96) at p≤0.05, respectively.

However, the results of statistics of the present study revealed insignificant seasonal variations in the epiphytic abundance and very weak correlations between the epiphytic microalgae and the measured physico-chemical parameters on the groups and species level. Moreover, almost insignificant correlation resulted between the total epiphytic count on each algal host and these parameters, suggesting that there are other factors, which can be the limiting ones rather than water quality such as irradiance, desiccation, wave exposure and water motion and substratum (Molina-Montenegro et al., 2005). In this trend Michelutti et al. (2003) found that the impact of substrate type on species assemblages is more effective than water chemistry.

Marine macroalgae harbor abundant and diverse assemblages of epiphytic zooplankton. Patterns of distribution and abundance of epiphytic zooplankton, which are often variable in space and time, differ markedly among macroalgae species (Viejo, 1999). Epiphytic zooplankton densities were relatively low on U. fascicata; the smoothly surface host and G. doryphora; the slimy surface one compared to the branched thallus hosts whose harbored higher densities. Our results was
supported by the significant correlation between total count of epiphytic microalgae and morphotypes of the hosts (two-way ANOVA), p≤0.05, giving the order of preference first to the branched thalli, followed by smooth surface and then the mucilaginous one, with the order of preference among the branched thalli as follows: P. capillacea > C. officinalis > C. mediterranea > H. musciformis > Gelidium sp. A possible explanation for the increase in epiphytic zooplankton densities may be that the leaf surface area becomes progressively more attractive for epiphytic zooplankton.

In general, the composition of epiphytic zooplankton assemblages differed between habitats in terms of abundance rather than composition of species. None of the epiphytic zooplankton was exclusively found in the different habitats, apart from the nematodes, which was associated with all hosts and numerically dominated in most of them with different percentages. Moreover, some species showed specificity to few hosts. Although the epiphytic zooplankton assemblages differed between habitats, there were no clear host-plant specialists. However, the differences in the distribution of abundances of epiphytic zooplankton between the habitats suggest the presence of some mechanisms of host selection. This assumption has to be made with caution; however, further experimental work would be necessary to test hypotheses related to preferences, specificity and choice of habitats (Olabarria et al., 2002). Several studies have reported that low specificity of epifauna to the host plant is quite common in marine systems dominated by macroalgae, presumably because few marine epifaunal organisms live and feed directly on host tissues (Arrontes, 1999; Wikstrom and Kautsky, 2004; Prado and Thibaut, 2008). In contrast, some studies have reported strong host specificity, likely determined by specific chemical, structural and morphological characteristics of the algal species (Hay et al., 1987; Edgar and Klumpp, 2003; Schmidt and Scheibling, 2006).

Complexity has been reported as an important factor influencing the composition and structure of epifaunal assemblages associated with different macroalgae (Viejo, 1999; Buschbaum et al., 2006). In fact, one mechanism by which macroalgae might influence their associated epifaunal assemblages is through the provision of complex habitats (Taylor and Cole, 1994; Buschbaum et al., 2006). Species diversity has been correlated with habitat complexity in a variety of systems and increased epiphytic zooplankton densities have often been related to the presence of seaweeds in many marine seagrass meadows (Orth 1992; Parker et al., 2001). In our study, diversity did not vary between the habitats, except the branched thallus, C. officinalis which harbored high diversity during most seasons.

Numerical fluctuations of zooplankton did not always coincide with the same for microalgae (Rickett and Watson, 1992). However, occasionally hosts of the maximum epiphytic zooplankton abundance didn’t coincide with those of the highest epiphytic microalgae concentration, as occurred during winter, 2012 on P. capillacea; this contrast might be due to zooplankton predation. In particular, the epiphytic zooplankton consists mainly of nematodes. At the same time, a good coupling occurred between epiphytic microalgae and zooplankton suggested by higher abundances of occurring during summer, 2012 (r = 0.95) and the lowest of both in spring, 2013 (r = 0.39) at p≤0.05 and n = 4. This coupling occurs also at hosts scale during autumn, 2012 (r = 0.90, p≤0.05 and n = 4) with the highest epiphytic microalgae and zooplankton occurring on C. officinalis and the lowest on Gelidium sp. during winter, 2012. Perhaps some of the zooplankton were feeding on larger suspended particulars or small nauplii or were preying on each other for some time.

The statistical relationships between the abundance of epiphytic zooplankton on each algal host and the physicochemical environment variables were analyzed. The environmental variables that best correlated with the zooplankton count were water salinity, followed by nutrients. Specifically, copepods responded positively to temperature, while protozoa and nematodes responded positively to salinity. Some species were spatially controlled by these variables. For instance, Spirillina vivipora responded negatively to temperature and positively to salinity (r = -0.92, p<0.05 and r = 0.96, p<0.05, respectively).

Conclusion

Marine macroalgae harbor abundant and diverse assemblages of epiphytic microalgae. The morphotypes of algal hosts were more effective for the abundance of microalgae epiphytes rather than water quality in the study area. Whereas, the epiphytic zooplankton were less diversified than microalgae and were affected by both, the morphotypes of the algal hosts and the water quality. On the other hand, the hosts harbored some harmful algae. Although most of them are relatively low in count, the Harbor should continuously be put under control to predict any bloom of these harmful species or other ones that may appear in the Harbor.

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Author’s Contributions

All authors equally contributed in this work.

Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and no ethical issues involved.

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