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# Phytoplankton biochemical contents and zooplankton composition in vegetated and non-vegetated regions in Bardawil Lagoon, North Sinai, Egypt

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## Abstract

Zooplankton community and biochemical contents of phytoplankton were compared between non-vegetated and vegetated regions among two plants communities, *Halophila stipulacea* (Forsskål) Ascherson and *Cymodocea nodosa* (Ucria) Ascherson, in Bardawil Lagoon. There were slight differences in water properties between the three studied sectors in Bardawil Lagoon. The results illustrated a strong allelopathic effect of *H. stipulacea* plant on the protein contents of phytoplankton. Non- vegetated area of the lake recorded the maximum contents of carbohydrate, thus the two plants had negative chemical interference on phytoplankton carbohydrates. *Halophila stipulacea* had a slight stimulating effect on phytoplankton lipid contents. While there was no variation in lipids at the area covered with *C. nodosa* and non-vegetated area. The three studied sectors were characterized by a similar taxonomic composition of zooplankton communities and considerable variations in densities of various zooplankton groups. All classes were collected in greater numbers in vegetation, Copepoda and Pteropoda are highly associated with *H. stipulacea*, while *C. nodosa* gathering rotifer, protozoa and meroplankton, which could indicate there was a stimulating allelopathic effect of macrophytes on their density.

**Keywords:** Bardawil Lagoon, vegetated area, phytoplankton, zooplankton, biochemical contents.

## 1. Introduction

Lake Bardawil is the only oligotrophic hypersaline lake along the Mediterranean coast of Egypt [1]. The lake had suffered from many problems, which might lead to environmental degradation, shortage in fish catch and substantial changes in its ecosystem. Among which, aquatic macrophytes have been pointed as a factor causing variation on the trophic cascades [2]. In shallow temperate lakes, these plants can influence the spatial distribution of fish [3], zooplankton [4] and phytoplankton [5].

*Ruppia cirrhosa* (dominates the eastern coast of the lake), *Cymodocea nodosa* (dominant on the western coast of the lake) and *Halodule uninervis* (an associated species near the lake-sea connection) were detected among submerged seagrasses of Bardawil Lagoon [6]. *Ruppia cirrhosa* and *Cymodocea nodosa* were recorded in Bardawil Lagoon from spring 2003 to winter 2004 [7]. *Cymodocea nodosa* is widely distributed through the Mediterranean Sea and in the Eastern Atlantic Ocean from the coast of Senegal to the south of Portugal [8-10]. This plant can colonies different types of environment, such as open coastal waters, costal lagoons and estuaries [11]. *Halophila stipulacea* a tropical seagrass entered the Mediterranean Sea from the Red Sea after the opening of the Suez Canal in 1869 [12]. *Halophila stipulacea* is included in the 100 worst invasive alien species in the Mediterranean list due to its potential to cause serious negative impacts on biodiversity [13]. The genetic signatures and some biological activities of *H. stipulacea* were studied from the eastern part of Bardawil Lagoon [14].

Allelopathy, defined as biochemical interactions between aquatic primary producers, as direct or indirect effect (stimulatory or inhibitory) of one species, including microorganisms, on adjacent organisms, through the production of chemical compounds generally implies a negative interaction has always been intriguing as a process explaining the dominance of certain plant or algal species over others [15, 16]. Inhibition of phytoplankton by allelochemicals released by macrophytes is supposed to be one of the mechanisms that contribute to stabilization of clear water states in shallow lakes [17]. Macrophytes with allelopathic potential may play an important role in the restoration of eutrophic lakes [18]. The role of allelopathy in aquatic systems has received increasing attention as a potential means of controlling algal blooms [19]. The aim of the study is to compare the zooplankton community structure and biochemical contents of phytoplankton between vegetated and non-vegetated regions and among two plants communities, *Halophila stipulacea* and *Cymodocea nodosa*, in Bardawil Lagoon.

## 2. Material and Methods

Bardawil is a costal lagoon located along the Mediterranean shore of the Sinai region (31°09 N, 33°08 E), Egypt. The lagoon extends for about 76.37 km length and has a maximal width of 16.65 km; with area of approximately 519 km. Bardawil Lagoon is extremely shallow; the average water depth is of 1.2 m. The whole water supply comes from the Mediterranean Sea, which flows constantly through two openings [20]. The sampling program takes place bimonthly during the period from October 2011 to September 2012, samples were collected from three sectors of Lake Bardawil, Sector I at the eastern side with plant cover *Halophila stipulacea*, Sector II at water circulated area in the northern middle and with plant cover *Cymodocea nodosa* and Sector III with no plant cover (Fig.1).

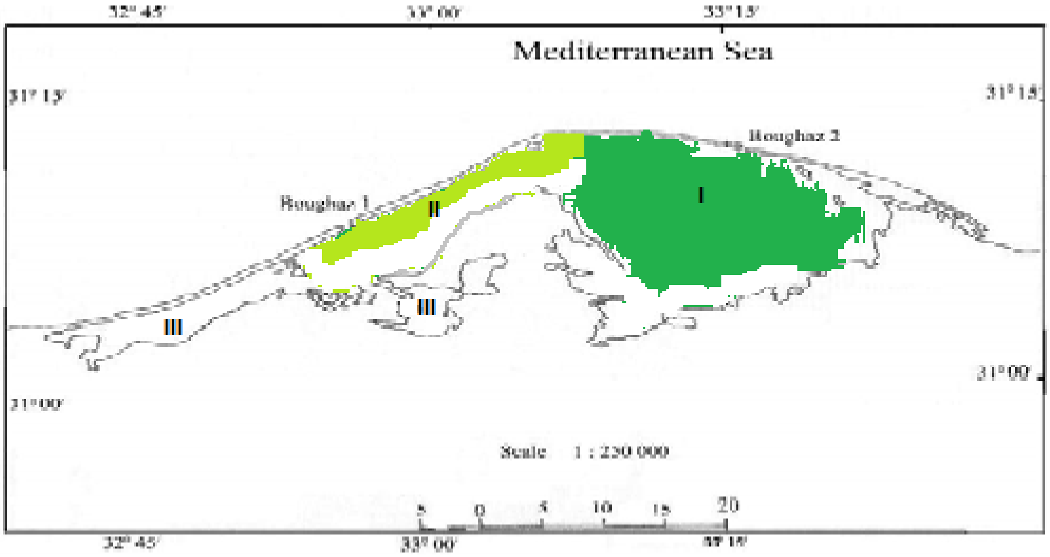

**Fig 1:** Map of Bardawil Lagoon showing the three studied sectors.

Physicochemical parameters including temperature, pH, Electric conductivity (EC), dissolved oxygen (DO) and nutrient salts were estimated by using the standard methods of APHA (2005) [21].

At each location water samples were collected by plastic bottles, then sieved and filtered through zooplankton net (100 µm mesh size) to separate macrozooplankton. Constant volume of filtered water was refiltered on Whatman GF/F (0.7 µm pore diameter) fiber circles and then transferred to the laboratory in ice tanks to determine the biochemical contents of the separated phytoplankton. Total protein contents were determined by Biuret method [22], total lipid contents were analyzed by the Sulpho-phospho-vanillin procedure [23] and carbohydrate contents were measured according to Phenol-sulphuric acid method [24].

Zooplankton samples were collected from each sampling site by filtering 50 liters from surface water through a zooplankton net of 55 µm mesh diameter. Collected samples were kept in 100 ml plastic bottles with some lake water to which 4% formalin was added as a preservative. Samples were studied under the compound microscope and the specimens were identified at the species level when possible. Zooplankton numbers were expressed as number of organisms per cubic meters.

Measuring of some biological quality parameters for zooplankton, including zooplankton density, number of species (s), species richness (d), evenness (J´) and diversity index (H´), were carried out by using Primer 5 (2001). Principal component analysis (PCA) between different zooplankton assemblages and phytoplankton biochemical contents relative to environmental variable at three different studied sectors was performed using XLSTAT 2012.

## 3. Results and Discussion

It is observed that temperature, pH, electric conductively (EC) and dissolved oxygen (DO) did not exhibit remarked differences in the three studied sectors of Bardawil Lagoon, but nutrients showed distinct variations at different regions (Fig.2). The variations in water temperature were detected between seasons with highest values in July (30.5 °C) and the lowest in December and March. Alkaline pH from 8.1 to 8.4 and low electric conductivity (61.6-75.5 µS cm$^{-1}$) was detected. Dissolved oxygen attained the lowest value of 4.83 mg L$^{-1}$ in September and highest of 11.72 mg L$^{-1}$ in December. No effect of free-floating plants was found, except for the lower transparency, on the physico-chemical variables in macrophyte banks [25]. The relative importance of zooplankton, macrophytes, and fish on water-quality characteristics at the ecosystem scale was clear in wetlands of the Prairie Pothole Region (PPR) of North America [26]. Nitrites were depleted completely in some stations during the study periods and attained the highest value of 14.5 µg L$^{-1}$ in vegetated area of *C. nodosa* in July. At *H. stipulacea* the nitrate concentration, elevated most of time with a mean of 89 µg L$^{-1}$. Average value of phosphorous was high in the non-vegetated area (9.7 µg L$^{-1}$) and reduced to 8.2 and 8.8 µg L$^{-1}$ at *C. nodosa* and *H. stipulacea*, although it sometimes increased at vegetated regions during the study period (Fig. 2). It was observed that nitrite, dissolved oxygen, temperature and ammonium did not exhibit a regular variation in vegetated and unvegetated areas of three lakes located in the Eastern Mediterranean Region of Turkey [27]. Macrophytes in East Taihu Lake, China adsorb dissolved nutrients from the water [28].

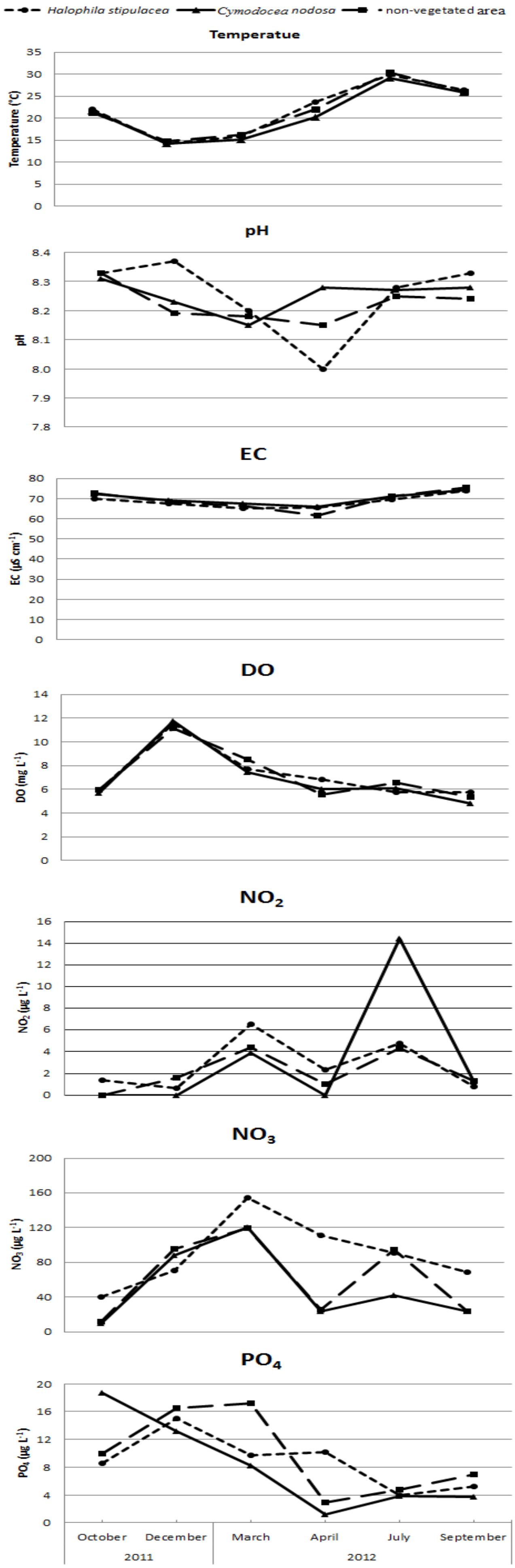

**Fig 2:** Fluctuations in water quality parameters at the three sectors in Bardawil Lagoon.

Protein concentrations fluctuated during the study, it attained the highest value of 41.6 g/l at *C. nodosa* and 30.55 g/l at non-vegetated area in December (Fig. 3), which accompanied with The highest value of $NO_3$ (88.6 and 95.2 µg/l). Phytoplankton accumulates nitrate in the form of intracellular nitrogen such as amino-acids and proteins [29]. Bacillariophyceae and Dinophyceae were the two main classes of phytoplankton in Bardawil Lagoon, constituted together 96% of the total phytoplankton cells. The most abundant species of diatoms belonged to the following genera: *Chaetoceros*, *Bacteriastrum*, *Leptocylindrus*, *Skeletonema* and *Thalassionema*. The dominant dinoflagellates belonged to *Prorocentrum*, *Exuviella*, *Diplopsalis*, *Ceratium*, *Hermesium* and *Peridinium* [30]. An increase in the protein fraction of *Phaeodactylum tricornutum* diatom was detected at low temperatures [31]. There was a sharp increase in carbohydrates yield at the three studied regions in October. Also, the peaks of lipid were detected at *H. stipulacea* in October (20.48 mg/l) and in April at the three sectors. Carbohydrates constitute the major part of the biochemical contents in epiphytic microalgae at Bardawil Lagoon [32], while proteins were the main algal constitute during the present study. High incorporation of carbon into proteins and low incorporation into lipids were a characteristic pattern of the photosynthetic allocations of phytoplankton throughout the euphotic zone; this indicates that phytoplankton had no nitrogen limitation and physiologically healthy conditions [33].

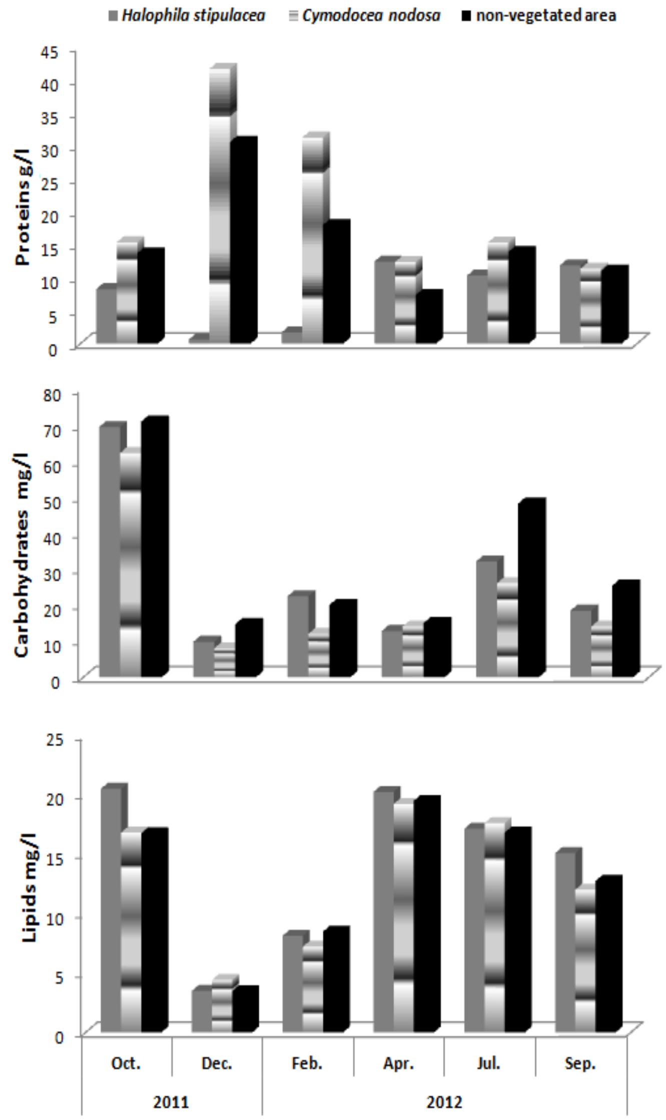

**Fig 3:** Differentiation in phytoplankton biochemical contents at Bardawil Lagoon.

The results illustrated strong allelopathic inhibition effect of *H. stipulacea* plant on the protein contents of phytoplankton (7.87 g/l), it elevated to 21.18 g/l at area covered with *C. nodosa* and to 15.73 g/l at non-vegetated area away from the two plants effect (Table, 1). *H. stipulacea* collected from Bardawil Lagoon release allelochemicals in the form of phenolic compound extract (0.523 mg tannic acid equivalent/g) into the water environment [14]. Phenolic allelochemicals from *Onobrychis sativa* weed can inhibit amino acid transport and protein synthesis and the subsequent growth of treated plants [34]. Phenolic compounds are one major class of identified allelochemicals present in many submerged macrophytes [35]. In our field survey, lower concentrations of $NO_2$ with averages of 2.7 µg/l at *H. stipulacea* area, while at *C. nodosa* $NO_2$ reached to 3.26 µg/l, which illustrated the elevation in phytoplankton protein level at the second sector, where nutrient limitation of target [36] and donor [37] species are often mentioned as important factors influencing the extent of allelopathic effects [38]. In *Cladophora* sp. alga an increase in phosphorus concentration was accompanied by increase in protein contents [39].

**Table 1:** Variations in biochemical contents of phytoplankton at selected sectors in Bardawil Lagoon.

| | ***Halophila stipulacea*** | ***Cymodocea nodosa*** | **non-vegetated area** |
|---|---|---|---|
| Proteins (g/l) | 7.78 | 21.18 | 15.73 |
| Carbohydrates (mg/l) | 27.43 | 22.67 | 32.28 |
| Lipids (mg/l) | 14.08 | 12.87 | 12.94 |

Phytoplankton carbohydrates consider the most important component for metabolism as it supplies the energy needed for respiration and other metabolic processes [40]. The study showed that area with no plant cover recorded the maximum contents of carbohydrate (32.28 mg/l), thus the two plants had negative chemical interference on phytoplankton carbohydrates, where its value declined at *C. nodosa* to 22.67 mg/l and at *H. stipulacea* to 27.43 mg/l. The production of antimicrobial activities from the seagrasses isolated from Bardawil Lagoon, *C. nodosa, Ruppia cirrhosa* and *H. stipulacea* was considered to be an indicator of these seagrasses to synthesize bioactive secondary metabolites with moderate antioxidant activity [41, 14]. Diatoms, the main phytoplankton group in Bardawil Lagoon during the present study (Personal communication), was found extremely sensitive by allelochemicals of submerged macrophytes [42, 43].

*H. stipulacea* had a slight stimulating effect on lipid contents (14.08 mg/l), while there was no variation in lipids at the area covered with *C. nodosa* and non-vegetated area (Table, 1). Target organisms may be stimulated by the allelochemicals [44, 45]. Antialgal allelochemicals isolated from macrophyte *Phragmites communis* Tris were increased the concentration of unsaturated lipid fatty acids in cell membrane of green alga *Chlorella pyrenoidosa* and blue green alga *Microcystis aeruginosa* [46].

Zooplankton community in Bardawil Lagoon was mainly represented by five groups, Protozoa, Rotifera, Copepoda, Pteropoda and meroplankton. Zooplankton seemed to prefer the vegetation lake regions as the highest mean densities of about 217 and 200 x $10^3$ Ind. were recorded in *H. stipulacea* and *C. nodosa* regions. Mean density in non-vegetated area decreased to 118 x $10^3$ Ind., the abundance and diversity of aquatic invertebrate populations are directly influenced by the amount and species of aquatic plant present [47]. The increase of zooplankton in macrophytes habitats may be due to creation a refuge for zooplankton which reduces the predatory effect of planktivorous fish [48]. Zooplankton crop in the present study were higher in the vegetated regions which could indicate there was a stimulating allelopathic effect of macrophytes on their density. Submerged plants and these with floating leave protect bigger zooplankton organisms from predator fish [49] and other studies explain why zooplankton prefer vegetated areas as it have more periphyton and bacteria which form a food resource for several zooplanktons [50].

In September high average standing crop of zooplankton was counted from *H. stipulacea* (625 x $10^3$ Ind. m$^{-3}$) and non-vegetated regions (311 x $10^3$ Ind. m$^{-3}$), while the highest abundance from *C. nodosa* was recorded in April (390 x $10^3$ Ind. m$^3$). Low zooplankton density was recorded in February for the three habitats (Table, 2). In Bardawil Lagoon, spring and summer were seasons of highest abundance of zooplankton, while a severe depletion in its density were occurred in winter and autumn [51]. The greatest number of zooplankton species was recorded at vegetated regions (37) and consequently the highest richness (2.931) were detected among *C. nodosa* in July. The evenness and diversity index also showed the highest values at the *C. nodosa* in February (Table, 2). During the study non-vegetated area attained the low species richness and number of species with maximum of 23 species in July. The low species richness under eutrophication conditions and the abundance of few species in a particular biotope result from their tolerance of the environmental variability and their capability for optimum exploitation of food resources [51]. The species richness of Rotifera, Cladocera and Copepoda were higher in the vegetated areas than unvegetated areas of three reservoirs in Northeastern Mediterranean Region, Turkey [27]. Despite the seasonal changes, the highest zooplankton diversity always characterised the plant habitats and the lowest at the open water zone [52].

**Table 2:** Quantitative and diversity measures of zooplankton community at different studied sectors in Bardawil Lagoon.

| | **2011** | | **2012** | | | |
|---|---|---|---|---|---|---|
| | **October** | **December** | **February** | **April** | **July** | **September** |
| ***Halophila stipulacea*** | | | | | | |
| Zooplankton total abundance | 152,800 | 160,200 | 29,400 | 234,400 | 97,900 | 625,400 |
| No. of species (s) | 23 | 28 | 20 | 27 | 26 | 24 |
| Species richness (d) | 1.843 | 2.253 | 1.847 | 2.103 | 2.175 | 1.723 |
| Evenness (J´) | 0.529 | 0.406 | 0.672 | 0.582 | 0.508 | 0.512 |
| Diversity index (H´) | 1.657 | 1.354 | 2.014 | 1.919 | 1.654 | 1.627 |
| ***Cymodocea nodosa*** | | | | | | |
| Zooplankton total abundance | 197,000 | 93,000 | 27,500 | 389,500 | 216,250 | 285,500 |
| No. of species (s) | 17 | 21 | 17 | 19 | 37 | 18 |
| Species richness (d) | 1.312 | 1.748 | 1.565 | 1.398 | 2.931 | 1.353 |
| Evenness (J´) | 0.633 | 0.662 | 0.755 | 0.660 | 0.587 | 0.557 |
| Diversity index (H´) | 1.794 | 2.016 | 2.139 | 1.943 | 2.119 | 1.609 |
| **Non-vegetated area** | | | | | | |
| Zooplankton total abundance | 39,330 | 38,330 | 47,670 | 158,170 | 112,000 | 310,670 |
| No. of species (s) | 12 | 15 | 17 | 17 | 23 | 19 |
| Species richness (d) | 1.040 | 1.327 | 1.485 | 1.337 | 1.892 | 1.423 |
| Evenness (J´) | 0.567 | 0.483 | 0.508 | 0.608 | 0.535 | 0.584 |
| Diversity index (H´) | 1.408 | 1.309 | 1.440 | 1.722 | 1.676 | 1.719 |

The three studied regions were characterized by a similar taxonomic composition of zooplankton communities and considerable variations in its densities. The copepods form the bulk of the zooplankton population in the three habitats and they ranged from 47-58% of the total zooplankton density and its nauplii were the commonly dominant zooplankton during all the study period (Fig. 4). Analysis of zooplankton stands at the three studied sectors, to describe the composition and distribution of copepods that were highly detected in September and found in small numbers in February. Diatoms, the dominant phytoplankton group in Bardawil Lagoon, were better food for copepods as indicated by shorter development time, higher survival and its high fatty acids content [53]. *Oithona nana* was the most common and dominant adult copepod during the study is a widely distributed neritic species in the world ocean [54] and it dominated in the plankton throughout the year from Bardawil Lagoon [51, 55] and other temperate regions such as the Mediterranean Sea [56]. Copepoda (Nauplius and adult) were recorded high abundance in vegetated region with highest density at *H. stipulacea* (Fig.4). Several studies recorded high crustacean density in macrophytes dominated water bodies [57, 58]. Others found that microcrustacean densities were frequently higher in the plants which could indicate there was no chemical effect (allelopathy) of macrophytes on their spatial distribution [25].

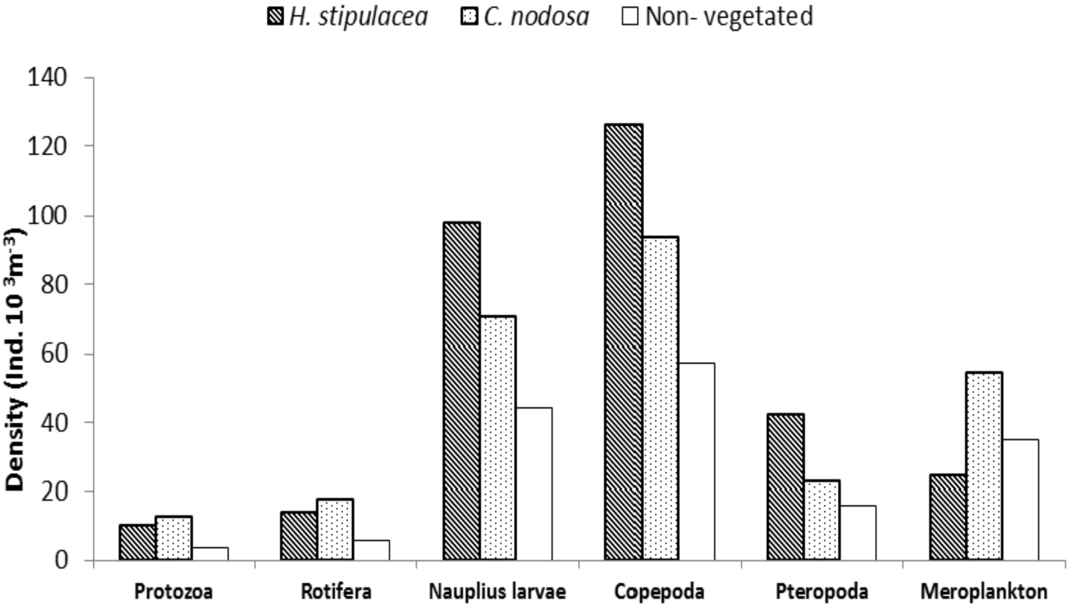

**Fig 4:** Fluctuations of different zooplankton groups at the three studied sectors in Bardawil Lagoon.

In this study Protozoa and Rotifers were poorly represented, Rotifera presented mean densities varied from17,700 Ind. m$^{-3}$ in *C. nodosa* to 5860 Ind. m$^{-3}$ in non-vegetated area. *Syncheata Clava* was the only species of rotifers registered in the samples during all the sampling dates, except in October where genus *Keratella* sporadic at *H. stipulacea* area and in July members of *Keratella* and *Brachious* were detected mainly at *C. nodosa*. Rotifers seemed to prefer the vegetated region in Lake Bardawil coinciding with the study in a shallow Argentinean lake which detected that, the presence of macrophytes favored increases in both planktonic rotifer densities and species richness, mainly through the possibilities of refuge plus the incorporation of plant-associated families and food diversification [59]. In Lake Rotomanuka (New Zealand) distribution of macrophytes plays an essential role in rotifer distribution under various water quality parameters and seasonal conditions [60]. The maximum population density of Protozoa reported in April with major peaks of 43,500 Ind. m$^{-3}$at *C. nodosa* and 25,400 Ind. m$^{-3}$ among *H. stipulacea*, its density decreased to12,300 Ind. m$^{-3}$ at non-vegetated area. Protozoa sustained lowest numbers at the other study periods, tintinnids was of the most common and diverse protozoans in Bardawil Lagoon.

*Limacina inflata* was the unique species of Pteropoda detected in the samples, its density increased in vegetated sectors with highest average of 42,200 Ind. m$^{-3}$ at area with *H. stipulacea* cover (Fig. 4). Mollusca larvae were the most dominant meroplankton in Bardawil Lagoon, it homogenously distributed in different sectors during all the sampling dates. It showed higher densities among *C. nodosa* in October and April, rarely detected in winter season (December and February) and increased at non- vegetated area in July and September. Larvae of Polychaeta was the second important meroplankton, it dominated meroplankton at vegetated sectors in April and decreased during the rest of the study. Generally meroplankton was higher at sectors with plant cover. Aquatic vegetation appears to have a greater influence upon benthic macroinvertebrates than upon zooplankton in Orange Lake as all classes of benthic macroinvertebrates were collected in significantly greater numbers from vegetation [61].

Principal component analysis (PCA) characterized patterns of variation in zooplankton assemblages and phytoplankton biochemical contents relative to environmental variable at three different vegetated and non-vegetated lake areas. It allowed discrimination of a group surrounding the F1 and F2 component axes (Fig. 5), thus explaining 100% of the variance. Axis I, explaining 56.24% of the variance in species scores was well correlated with water temperature, EC, DO and $NO_3$. The eigenvalue of axis I was 10.12, *Othiona nana,* meroplankton and *Limacina inflate,* phytoplankton proteins and lipids had the highest score on axis I, while Protozoa and Rotifera revealed the lowest. Axis II explained 43.76% of the variation. It was well correlated with pH, $NO_2$, $PO_4$, Protozoa, Rotifera, nauplius larvae, Copepoda and phytoplankton carbohydrates. Protozoa revealed a strong positive correlation with pH and $NO_2$, while their presence was inversely proportional with water temperature and $PO_4$. Conversely, *O. nana* and *L. inflate* were directly correlated with water temperature and $NO_3$. Protozoa, rotifers and meroplankton can withstand the lack of oxygen. Copepoda, *O. nana*, nauplius larvae*, L. inflate* and phytoplankton lipid contents are highly associated with *H. stipulacea*, while *C. nodosa* gathering

Rotifera, Protozoa and meroplankton (Fig. 5). The presence of aquatic plants (emergent, floating-leaved, and submerged) has no effect on the nutrient availability in the subtropical shallow lakes from Florida [62]. Phytoplankton and nutrient concentrations in prairie wetlands are strongly influenced by submersed macrophytes, although influences may depend on plant community composition [26].

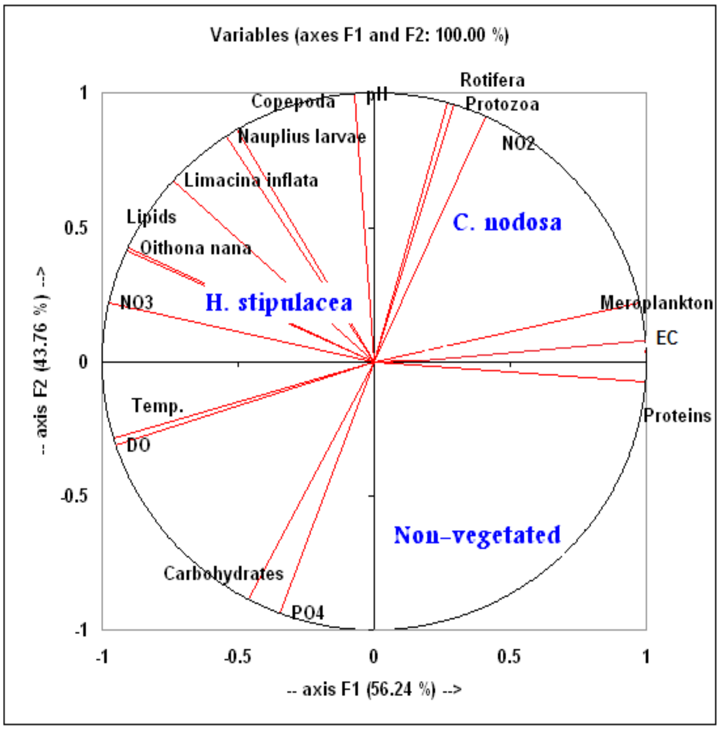

**Fig 5:** Principal component analysis (PCA) between environmental and biological variables in the different vegetated and non-vegetated regions of Bardawil Lagoon. Water Temperature (Temp.), Dissolved Oxygen (DO), Electrical Conductivity (EC), Nitrite ($NO_2$), Nitrate ($NO_3$) and Phosphate ($PO_4$).

## 4. Conclusion

The study revealed negative allelopathic effect of *H. stipulacea* and *C. nodosa* in Bardawil Lagoon on protein and carbohydrate contents of phytoplankton and has a positive effect on zooplankton density. In field study it is often difficult to distinguish allelopathic effects from other competitive interactions. So, the study recommends further investigations on the role of allelopathy under natural conditions and to determine the importance of this factor in the complex ecosystem.

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