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Key Drivers for Copepod Assemblages in a Eutrophic Coastal Brackish Lake

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Abstract: The copepod assemblages and abiotic parameters were investigated at 11 stations in a large coastal lake (Lake Manzalah, Nile Delta) from 2009–2010 in order to verify any impacts of eutrophication and salinity on the copepod species composition. The environmental conditions and the copepod assemblages appeared to have changed in comparison with previous studies, possibly because of increasing eutrophication and invasions of non-indigenous species (NIS). The aim of the present study was the identification of species which can be used as ecological indicators of high trophic status. Among the nine copepod species of Lake Manzalah, *Acartia tonsa*, *Mesocyclops ogunnus*, and *Apocyclops panamensis* were reported for the first time. *Acartia tonsa*, a well-known NIS for the Mediterranean, numerically dominated the copepod assemblages in some portions of the lake. The distribution of *Acanthocyclops trajani* and *Thermocyclops consimilis* was insensible to eutrophication because they can stand high levels of nutrients and hypoxia. Compared with previous reports, the copepod assemblage of Lake Manzalah was richer in species. The invasions of NIS, in addition to the heterogeneous progress of eutrophication in the lake, created an environmental mosaic with many species in total, but with single areas suitable for only a small number of them.

Keywords: ecological indicators; brackish waters; Copepoda; Nile Delta; hypertrophic state

1. Introduction

Eutrophication is recognized as a global environmental issue and one of the most severe hazards for aquatic ecosystems [1,2] where it leads to significant changes in the community structure and ecosystem functioning [3,4]. It is also responsible for large increases in the biomass of primary producers, severe reductions in diversity, and declines in water quality [5,6].

Eutrophication risk is higher in confined or enclosed coastal ecosystems with long water-residence times and low remixing. Confined coastal water bodies are especially vulnerable to eutrophication due to their restricted water exchange with the adjacent open sea [7,8]. In many Mediterranean coastal lakes, the eutrophication level is enhanced by the general absence of tide excursions, other than by anthropogenic pressures such as those arising from intensive use of fertilizers in the surrounding fields [9]. Coastal lakes are also susceptible to invasive non-indigenous species (NIS) whose settlement is generally favored in polluted and/or physically degraded situations [10,11]. Among the most commonly known planktonic invaders are copepods and cladocerans [12].

Zooplankton have been proposed as biological indicators for pollution, water quality, and eutrophication of coastal lakes and lagoons [13–17]. In warm-temperate waters affected by domestic and industrial waste, higher abundance of zooplankton and a shift towards an opportunist-dominated community have been documented [18]. Among the zooplankton, copepod assemblages are well-known to be affected by eutrophication and pollution [17–19]. Eutrophication has been found to contribute to the reduction of copepod diversity [20] and often results in the replacement of large-sized copepods with smaller ones [21–24]. The general shift to small-sized species with increasing eutrophication has been attributed to a change in the food quality, from large diatoms to small flagellates—the preferred prey of smaller species [19].

The structure and composition of zooplankton assemblages are significantly modified by increases of primary productivity caused by nutrient enrichment [25,26], and eutrophication affects zooplankton species diversity and their succession along physical and chemical gradients [27,28]. Certain Copepoda are not negatively affected by high trophic levels [19], making them successful species in eutrophic ecosystems [29]. According to Reference [30], some copepod species in eutrophic conditions produce large populations supported by huge phytoplankton biomass.

Lake Manzalah is the largest and the most productive coastal lake in Northern Egypt [31] and it can be taken as a paradigmatic example of how the ongoing disturbance deriving from a growing human pressure can affect the species composition. The amount of Nile-derived water arriving in Lake Manzalah was reduced from 6692 million [32] to about 4000 million $m^3 \cdot y^{-1}$ after construction of the El-Salam Canal [33]. This fact has been considered responsible for important changes in the sediment and benthos of Lake Manzalah [34]. Furthermore, a trend of increased phytoplankton biomass has been observed, with a clear shift in species composition towards the dominance of small-sized species [35].

Zooplankton community changes correlated with declining water quality include a simplification of the species composition and an increased abundance of individuals, such as for rotifers studied by Reference [36]. In the present study, copepod abundance and distribution were considered with the aim of finding bio-indicators of the environmental status of Lake Manzalah that has been considered as progressively deteriorating in the past 50 years.

2. Materials and Methods

2.1. Study Site

Lake Manzalah (formerly known as Lake Tanis, 31°00′–31°30′N, 31°50′–32°20′E) is the largest of the North African Mediterranean wetlands. It is located at the north-eastern corner of the Nile Delta (Figure 1), bordered by the Suez Canal to the east and the Damietta branch of the Nile to the west. It is separated from the Mediterranean Sea by a sandbar, interrupted at a level of three channels locally known as Bughaz (El-Gamil 1, El-Boughdady, and El-Gamil 2). Lake Manzalah is approximately rectangular in shape, about 60 km long and 40 km wide, and has an average depth of 1.3 m [37]. Over 1000 islands of different sizes are scattered throughout the lake, some of them inhabited. Large areas in the north-west of the lake have been turned into fish farms, while much of the southern part of the lake has been divided into large plots, drained, and converted to agricultural use [38]. The overall area of the lake has declined from more than 1400 km^2 in the early 1970s to less than 700 km^2 in 2003 [39]. It has been reported that the maximum mean value of salinity during winter in the lake is 9.56 ± 14.31 and it gradually declines during summer (9.40 ± 13.19) through spring (8.06 ± 10.50), and reached its minimum value during autumn (7.54 ± 7.25) [40]. Lake water temperature maximum mean value is reported to reach 30.7 ± 1.57 °C during summer, and the minimum was observed during winter (15.6 ± 0.97 °C), while the average values of water temperature during spring and autumn, however, were nearly similar; being 22.6 ± 0.7 and 23.3 ± 1.49 °C, respectively [40].

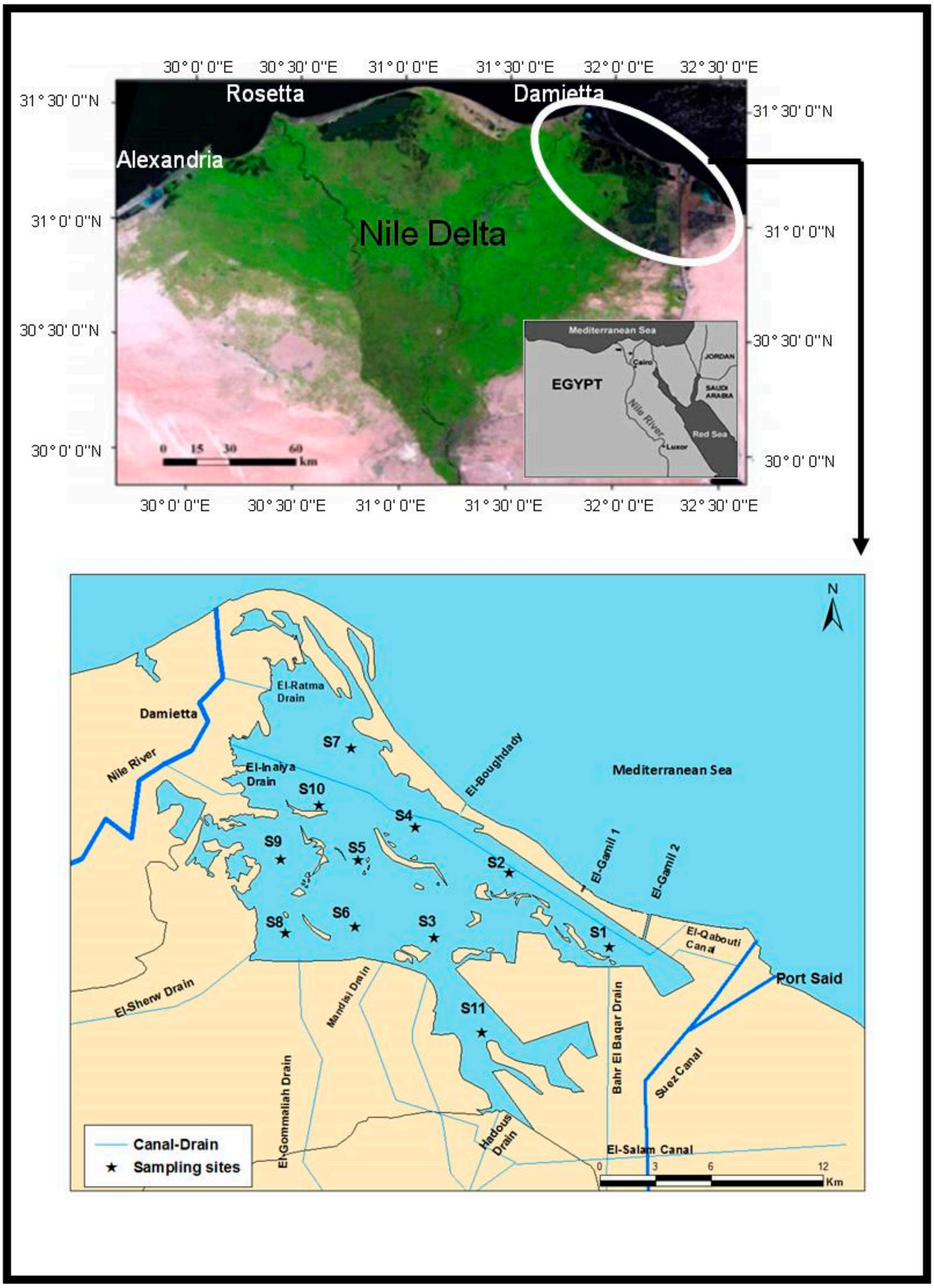

Figure 1. Location of the study area (Lake Manzalah) and of the sampling stations.

2.2. Sampling and Laboratory Analyses

Sample collections were carried out on four dates (18 July 2009, 18 October 2009, 18 January 2010, and 18 April 2010) at 11 stations (Figure 1). The four dates represented four seasons and were useful for the study of seasonal variability, other than for comparisons with previous studies. The northern stations (S2, S4, and S7) were more influenced by sea while S1, S3, S6, S8, S9, and S11 were in the south and they are the most influenced by the inland drainage waters. Station 5 and S10 were in the centre of the lake. The abiotic parameters were measured following the methods of the American Public Health

Association (APHA 1992). Water temperature was measured using an ordinary centigrade thermometer with a precision of 0.1 °C. Electrical conductivity and salinity were measured by conductivity meter (YSI. SCT-33). The pH was measured using the Hydrolab probe WTW Multiset 430i. Dissolved oxygen concentration was measured according to the modified Winkler method. Nutrients were measured using the following methods: phante method for ammonium, colorimetric for nitrite, cadmium reduction of nitrate. Reactive silicate, orthophosphate, and total phosphorus were measured using the molybdate and the ascorbic acid methods, respectively. All measures and sampling were conducted in the top 50 cm of the water column.

The trophic state index (TSI_{TP}) of single stations of Lake Manzalah was estimated on the basis of the classical Carlson's Trophic State Index (TSI_{TP}) [41], developed for freshwater lakes and proposed by the United States' Environmental Protection Agency [42] for classifying lakes and reservoirs. It was calculated using total phosphorus (TP) values. Generally, TSI_{TP} values < 40 correspond to oligotrophy, 40–60 to mesotrophy, 60–80 to eutrophy, and >80 to hypertrophy [43].

Zooplankton organisms were collected by filtering 40 litres (collected with a 10 L plastic container from the first 50 cm of surface layer) of lake water through a 55 µm mesh plankton net. Zooplankton samples were immediately preserved in 4% neutral (pH 7.3) formalin. At least 3 sub-samples (3 mL) were drawn from each sample, with a wide mouthed pipette, after careful mixing, and poured into a counting cell. Copepods nauplii were considered as a single group; copepodids were counted and grouped according to the order (Calanoida, Cyclopoida, Harpacticoida), and adults were grouped according to the species. Species were identified according to keys and References [44,45]. Abundances were expressed as individuals per cubic metre ($ind \cdot m^{-3}$). Shannon's index [46], expressed as $H' = \sum Pi \; Log_2 Pi$, where Pi = dominance of species i, computed as $Pi = ni/N$, ni = number of species i in sample, and N = number of samples.

2.3. Statistics

Means and standard deviations were reported when appropriate. Two-way ANOVA analysis, followed by a post-hoc comparison using Duncan's test, was used to identify significant differences in physicochemical and biological parameters among sampling stations and seasons. The ANOVA tests were performed using Excel-Stat software. Prior to the ANOVA tests, the normality of all variables was checked by means of the Shapiro–Wilk test and log-transform was used when necessary. Homogeneity of variance of all variables among sites was assessed using Levene's test. Pearson's correlation analysis was performed to evaluate potential relationships between copepod abundance and abiotic and biotic variables. In order to obtain one composite measure of eutrophication status condition and another of salinity, a principal components analysis (PCA) was carried out on the physicochemical parameters and the most dominant copepod taxa.

3. Results

3.1. Physicochemical Parameters and Trophic State

Significant differences in physicochemical parameters among stations and seasons were shown (Table 1) (Figure 2). Water temperature showed a typical annual profile with a maximum of 29.8 °C during summer, and a minimum of 13.4 °C during winter (in both cases, averages of 11 stations). The annual average temperature of different stations differed by more than 3 °C, from 16.6 ± 6.7 to 19.7 ± 7.3 °C (Table 1). The average salinity exhibited marked differences between summer and winter (Table 1) and were also sensibly different in different stations. Lowest salinity values (annual average < 3.2) were measured at S1, S3, S5, S6, S8, S9, and S11 (Table 1, Figure 2), and highest salinity values (annual average > 3.2) were measured at S2, S4, S7, and S10, with summer values reaching 20 in S7 (Figure 2). The annual average of pH did not vary with season, but with stations (Table 1), reaching 9.20 at S9 during summer and 7.44 at S1 during spring (Figure 2).

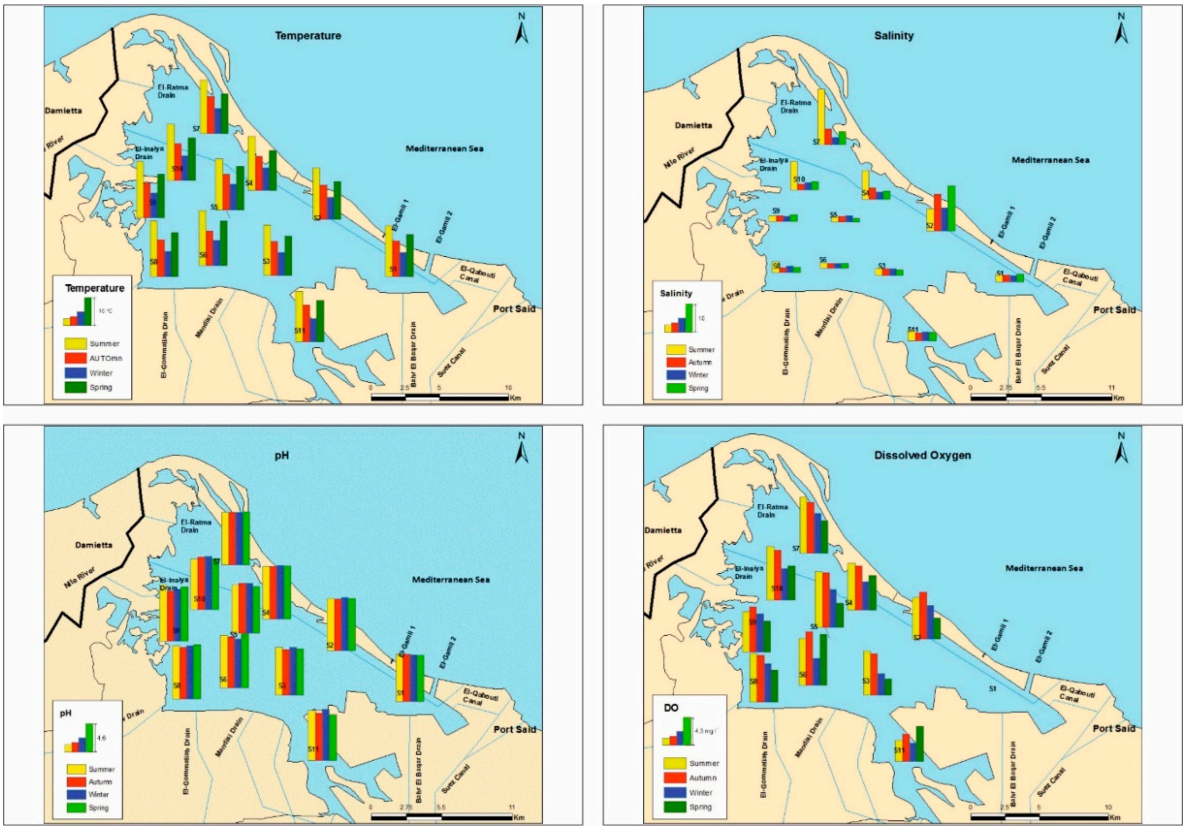

Figure 2. Spatiotemporal variations of physicochemical variables in Lake Manzalah.

Dissolved oxygen concentration was generally low over the whole lake (average annual values, 0.0–6.9 mg·l^{-1}) and anoxia prevailed at S1 (Table 1, Figure 2). Oxygen concentrations in the other ten sampling stations ranged between 2.5 (at S3, spring) and 8.6 mg·l^{-1} (at S5 and S7, summer). Ammonium (Table 1, Figure 3) was the dominant nitrogen form at S1, S3, S4, S7, S9, and S11, where it accounted for 97.4, 85.4, 60.1, 53.3, 49.0, 52.2, and 92.2 % of total dissolved inorganic nitrogen (DIN) (DIN = NH_4^+ + NO_3 + NO_2), respectively.

Nitrates ranged from 2.28 μg·l^{-1} at S5 during spring to 302.16 μg·l^{-1} at S1 during summer (Figure 3), showing significant differences between stations and seasons (Table 1). Nitrates accounted for up to 50% of total DIN at sampling stations S2, S5, S6, S7, and S8. Nitrites varied widely among sampling stations, with significantly higher values at S1 (Table 1, Figure 3). Ammonium concentration was exceptionally high at S1 (Table 1, Figure 3). Phosphates varied among sampling stations and seasons (Table 1) fluctuating from 12.10 μg·l^{-1} to 389.62 μg·l^{-1} at S7 and S1, respectively (Figure 3). The N/P DIN to DIP (DIP = PO_4^{3-}) ratio, indicator of nutrient limitation ranged from 1.8 at S2 and 9.9 at S7, being below that optimal for phytoplankton growth (the so-called Redfield ratio = 16) and suggesting potential N limitation. Only at S1 the N/P ratio was 46.1 and suggests a phosphorous limitation. Silicates varied among sampling stations (Table 1), with higher values at S11 (>3.8 μg·l^{-1}) (Figure 3). The correlation coefficients are presented in the form of matrix for the physicochemical parameters (Table 2).

Table 1. Physicochemical parameters observed in Lake Manzalah. Annual mean ± SD at each station and seasonal mean ± SD in the whole lake are shown. *F*-values were determined by two-way ANOVA test, *p*-values for differences among stations and seasons within each parameter. Values in each line that share the same letter (a, b, c, d) are not significantly different ($p > 0.05$). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

	Physicochemical Parameters									
	Temperature (°C)	**EC mS·cm^{-1}**	**Salinity**	**pH**	**Oxygen (mg·l^{-1})**	**Ammonium (µg·l^{-1})**	**Nitrates (µg·l^{-1})**	**Nitrites (µg·l^{-1})**	**Phosphates (µg·l^{-1})**	**Silicates (µg·l^{-1})**
Stations										
S1	17.0 ± 6.4 ^{abc}	5.0 ± 0.3 ^{a}	2.6 ± 0.3 ^{a}	7.7 ± 0.1 ^{a}	0.0 ± 0.0 ^{a}	17264 ± 16874 ^{b}	237.4 ± 59.9 ^{c}	210.7 ± 62.8 ^{c}	383.9 ± 41.2 ^{e}	2.0 ± 0.2 ^{ab}
S2	16.6 ± 6.7 ^{a}	19.3 ± 6.3 ^{c}	11.4 ± 4.0 ^{c}	8.6 ± 0.1 ^{b}	5.5 ± 1.7 ^{bcd}	56.5 ± 9.1 ^{a}	70.0 ± 20.31 ^{ab}	4.4 ± 0.6 ^{a}	72.9 ± 14.2 ^{c}	1.5 ± 0.1 ^{a}
S3	17.0 ± 6.5 ^{a}	5.4 ± 1.5 ^{a}	2.4 ± 0.2 ^{a}	7.7 ± 0.2 ^{a}	4.7 ± 2.2 ^{bc}	399.6 ± 9.7 ^{a}	16.5 ± 3.3 ^{a}	51.8 ± 12.3 ^{b}	160.6 ± 42.6 ^{e}	2.5 ± 0.2 ^{bc}
S4	17.0 ± 7.2 ^{abc}	8.9 ± 5.8 ^{ab}	5.0 ± 3.6 ^{ab}	8.7 ± 0.1 ^{b}	5.9 ± 1.3 ^{cd}	96.6 ± 11.1 ^{a}	59.9 ± 15.7 ^{ab}	4.4 ± 0.9 ^{a}	65.4 ± 16.7 ^{be}	2.9 ± 0.3 ^{c}
S5	18.5 ± 5.9 ^{bcde}	3.9 ± 0.7 ^{a}	1.9 ± 0.4 ^{a}	7.9 ± 0.2 ^{a}	6.6 ± 2. 3 ^{d}	75.4 ± 20.7 ^{a}	83.7 ± 11.9 ^{ab}	6.3 ± 2.9 ^{a}	84.5 ± 10.4 ^{abc}	3.1 ± 0.4 ^{cd}
S6	19.2 ± 6.9 ^{e}	3.3 ± 0.1 ^{a}	1.7 ± 0.1 ^{a}	8.4 ± 0.3 ^{b}	6.8 ± 1.9 ^{d}	72.8 ± 4.8 ^{a}	88.8 ± 4.9 ^{b}	4.7 ± 0.5 ^{a}	32.6 ± 4.8 ^{ab}	2.6 ± 0.4 ^{bc}
S7	18.4 ± 6.6 ^{abcd}	13.8± 12.4 ^{bc}	8.4 ± 7.9 ^{bc}	8.6 ± 0.1 ^{b}	6.9 ± 1.6 ^{d}	82.3 ± 7.9 ^{a}	80.9 ± 39.6 ^{ab}	4.7 ± 1.5 ^{a}	16.9 ± 3.4 ^{a}	3.7 ± 0.5 ^{de}
S8	19.6 ± 7.2 ^{e}	4.6 ± 1.5 ^{a}	2.4 ± 0.9 ^{a}	8.7 ± 0.2 ^{b}	6.4 ± 1.2 ^{cd}	75.5 ± 7.8 ^{a}	81.7 ± 28. 8 ^{ab}	5.5 ± 1.3 ^{a}	49.3 ± 27.5 ^{abc}	3.8 ± 0.9 ^{ef}
S9	19.7 ± 7.3 ^{de}	4.1 ± 0.4 ^{a}	2.1 ± 0.8 ^{a}	8.7 ± 0.4 ^{b}	5.9 ± 0.9 ^{cd}	85.6 ± 19.5 ^{a}	68.9 ± 34.3 ^{ab}	5.9 ± 2.3 ^{a}	23.9 ± 7.4 ^{a}	2.9 ± 0.2 ^{c}
S10	19.7 ± 7.2 ^{cde}	7.9 ± 6.1 ^{ab}	4.5 ± 3.8 ^{ab}	8.5 ± 0.2 ^{b}	6.4 ± 1.7 ^{cd}	84.1 ± 11.1 ^{a}	72.4 ± 34.9 ^{ab}	4.8 ± 0.8 ^{a}	42.7 ± 13.7 ^{abc}	2.9 ± 0.2 ^{c}
S11	18.0 ± 6.3 ^{ab}	5.9 ± 0.6 ^{a}	3.1 ± 0.4 ^{a}	8.9 ± 0.4 ^{a}	3.9 ± 1.2 ^{b}	787.8 ± 234 ^{a}	24.0 ± 7.8 ^{ab}	42.6 ± 10.5 ^{b}	162.1 ± 49.2 ^{d}	4.4 ± 0.5 ^{f}
F value (df)	4.9 (10) ***	4.4 (10) **	4.4 (10) **	10.6 (10) ***	12.9 (10) ***	4.1 (10) **	19. 9 (10) ***	38.9 (10) ***	91.7 (10) ***	13.1 (10) ***
Seasons										
Summer	29.6 ± 1.3 ^{d}	6.2 ± 5.9	10.5 ± 8.4	8.4 ± 0.4	6.3 ± 2.4 ^{b}	477.6 ± 885.1	61.9 ± 49.1 ^{a}	28.6 ± 54.3	117.1 ± 123.2 ^{b}	3.2 ± 1.1
Autumn	19.8 ± 0.6 ^{b}	3.7 ± 3.3	6.9 ± 5.2	8.2 ± 0.4	6.4 ± 2.3 ^{b}	2778.7 ± 8281.3	100.4 ± 87.5 ^{b}	28.6 ± 54.3	94.4 ± 97.6 ^{a}	2.8 ± 0.9
Winter	13.4 ± 0.6 ^{a}	2.9 ± 1.8	5.6 ± 2.9	8.4 ± 0.4	4.4 ± 1.7 ^{a}	3295.7 ± 9878.1	80.8 ± 49.9 ^{ab}	29.4 ± 52.6	81.0 ± 88.4 ^{a}	3.0 ± 0.8
Spring	23.2 ± 1.2 ^{c}	3.9 ± 4.0	6.9 ± 6.4	8.3 ± 0.6	4.4 ± 1.9 ^{a}	386.7 ± 685.4	78.4 ± 51.2 ^{ab}	28.4 ± 46.7	92.6 ± 104.5 ^{b}	2.6 ± 0.6
F-value (df)	969.1 (3) ***	-	-	-	12.0 (3) ***	-	4.2 (3) *	-	4.9 (3) *	-

Table 2. Pearson's correlation matrix for the physicochemical parameters.

	Temperature	**EC**	**Salinity**	**pH**	**Oxygen**	**Silicates**	**Phosphates**	**Ammonium**	**Nitrates**	**Nitrites**
Temperature	1									
EC	−0.480 *	1								
Salinity	−0.447 *	0.998 **	1							
pH	0.459 *	0.350	0.379	1						
Oxygen	0.508 **	0.141	0.158	0.680 **	1					
Silicates	0.627 **	−0.328	−0.306	0.110	0.278	1				
Phosphates	−0.545 **	−0.184	−0.204	−0.757 **	−0.985 **	−0.282	1			
Ammonium	−0.400 *	−0.174	−0.176	−0.530 **	−0.907 **	−0.358	0.904 **	1		
Nitrates	−0.207	−0.117	−0.100	−0.218	−0.637 **	−0.398	0.629 **	0.894 **	1	
Nitrites	−0.461 *	−0.223	−0.234	−0.681 **	−0.961 **	−0.309	0.971 **	0.971 **	0.769 **	1

* Correlation is significant at the level of 0.05, ** Correlation is significant at the level of 0.01.

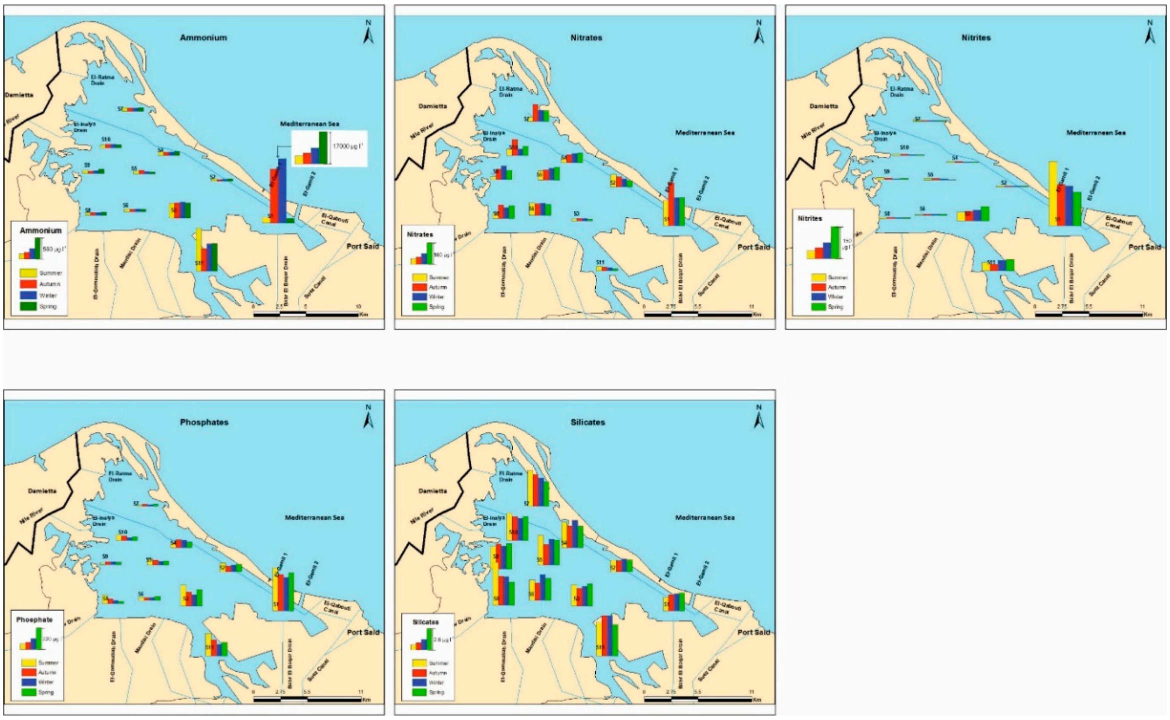

Figure 3. Spatiotemporal variations of nutrients in Lake Manzalah.

The Carlson's Trophic State Index (TSI_{TP}) for each station in Lake Manzalah, ranged between 63 (summer, S9) and 117 (winter, S1), corresponding to lightly eutrophic and heavily hypertrophic conditions (Figure 4). Stations were not similar, and highest TSI_{TP} values were observed at S1, S3, and S11 (Figure 4). Winter was characterised by the highest TSI_{TP} values at all the stations (Figure 4).

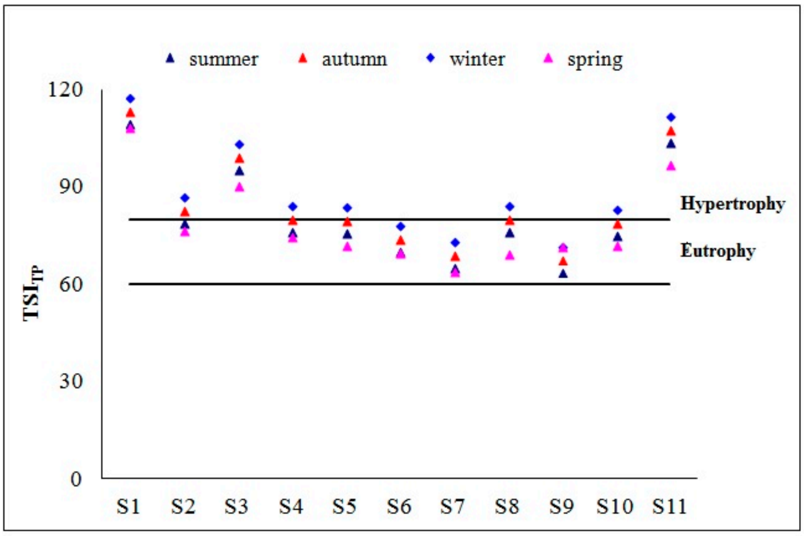

Figure 4. Seasonal trend of trophic state index (TSI_{TP}) determined with total phosphorus (TP). Eutrophy is recognized for TSI_{TP} values between 60–80, and hypertrophy for TSI_{TP} values above 80.

3.2. Copepod Species Composition, Distribution, and Abundance

Nine copepod species belonging to three orders, Cyclopoida, Calanoida, and Harpacticoida, were found in Lake Manzalah (Table 3). With five species, Cyclopoida was the richest in species.

Table 3. Density of adult copepods observed in Lake Manzalah. Annual mean ± SD at each station and seasonal mean ± SD in the whole lake are shown. *F*-values (with indication of degrees of freedom, DFs) were determined by two-way ANOVA test, *p*-values for differences among stations and seasons within each species. Values in each line that share the same letter (a, b, c, d) are not significantly different ($p > 0.05$). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Order	**Cyclopoida (× 10^3 ind·m^{-3})**					**Calanoida (× 10^3 ind·m^{-3})**	**Harpacticoida (× 10^3 ind·m^{-3})**			
Species	***Acathocyclops trajani***	***Thermocyclops consimilis***	***Mesocyclops ogunnus***	***Apocyclops panamensis***	***Oithona nana***	***Acartia tonsa***	***Nitocra lacustris***	***Onychocamptus mohammed***	***Euterpina acutifrons***	***H′***
Stations										
S1	1.5 ± 2.6 ^{a}	0.5 ± 0.9 ^{ab}	- ^{a}	-	-	- ^{a}	-	-	-	0.6 ^{a}
S2	1.0 ± 1.0 ^{a}	- ^{a}	- ^{a}	0.5 ± 0.9	0.5 ± 0.9	3.0 ± 3.0 ^{bc}	-	0.5 ± 0.9	-	1.3 ^{b}
S3	2.7 ± 2.8 ^{ab}	1.2 ± 1.3 ^{ab}	0.2 ± 0.4 ^{ab}	-	-	- ^{a}	0.2 ± 0.4	-	-	1.0 ^{a}
S4	6.5 ± 8.9 ^{ab}	- ^{a}	- ^{a}	-	-	1.5 ± 1.6 ^{abc}	-	-	-	0.5 ^{a}
S5	0.5 ± 0.9 ^{a}	1.0 ± 1.7 ^{ab}	- ^{a}	-	-	- ^{a}	-	0.5 ± 0.9	-	1.0 ^{a}
S6	1.5 ± 1.6 ^{a}	0.5 ± 0.9 ^{ab}	- ^{a}	-	-	- ^{a}	1.5 ± 1.6	-	-	1.0 ^{a}
S7	0.5 ± 0.9 ^{a}	0.5 ± 0.9 ^{ab}	0.5 ± 0.9 ^{ab}	-	-	37.5 ± 59.2 ^{c}	0.5 ± 0.9	-	-	0.2 ^{c}
S8	2.0 ± 1.4 ^{ab}	0.5 ± 0.9 ^{ab}	- ^{a}	-	-	- ^{a}	-	-	-	0.5 ^{a}
S9	2.0 ± 2.5 ^{a}	1.1 ± 0.9 ^{ab}	1.7 ± 2.1 ^{b}	-	-	0.5 ± 0.9 ^{ab}	2.5 ± 3.8	-	-	1.5 ^{b}
S10	1.0 ± 1.0 ^{a}	0.7 ± 1.3 ^{ab}	1.5 ± 2.6 ^{ab}	0.5 ± 0.9	-	2.5 ± 0.9 ^{c}	-	-	0.5 ± 0.9	1.6 ^{b}
S11	6.2 ± 5.1 ^{b}	3.5 ± 2.6 ^{b}	0.7 ± 1.3 ^{ab}	-	-	- ^{a}	0.5 ± 0.9	0.5 ± 0.9	-	1.1 ^{b}
F (df)	3.3 (10) **	2.6 (10) *	2.5 (10) *	-	-	6.9 (10) ***	-	-	-	6.6 (10) ***
Seasons										
Summer	0.2 ± 0.6 ^{a}	0.4 ± 0.8	- ^{a}	-	0.2 ± 0.6	0.4 ± 1.1 ^{a}	0.2 ± 0.6	-	-	
Autumn	0.9 ± 1.3 ^{ab}	1.1 ± 1.7	- ^{a}	-	-	14.0 ± 39.9 ^{b}	0.3 ± 0.6	-	0.2 ± 0.6	
Winter	2.7 ± 4.1 ^{b}	1.6 ± 1.9	1.5 ± 2.1 ^{b}	-	-	0.5 ± 1.2 ^{a}	0.2 ± 0.6	-	-	
Spring	5.4 ± 5.5 ^{c}	0.4 ± 0.8	0.2 ± 0.6 ^{a}	0.4 ± 0.8	-	1.4 ± 2.3 ^{ab}	1.3 ± 2.4	0.5 ± 0.9	-	
F (df)	16.1 (3) ***	-	6.5 (3) **	-	-	3.8 (3) *	-		-	

The populations of *Acanthocyclops trajani*, *Mesocyclops ogunnus* (Cyclopoida), and *Acartia tonsa* (Calanoida) showed significant abundance differences between stations and seasons (Table 3). The abundances of *Thermocyclops consimilis* (Cyclopoida) and *Onychocamptus mohammed* (Harpacticoida) were significantly different between stations and seasons, respectively (Table 3). The highest mean copepod abundance was seen at S7 where *A. tonsa* dominated the adult assemblages (Table 3).

Abundances of nauplii ranged between 4 ind·m^{-3} (S1, winter) and 160,000 ind·m^{-3} (S6, summer) (Figure 5). Numbers of nauplii correlated positively with temperature ($r = 0.620$, $p < 0.01$). The highest nauplii average abundances were observed at S9 (mean ± SD = 73,000 ± 21,000 ind·m^{-3}) and S6 (mean ± SD = 56,500 ± 60,200 ind·m^{-3}). The average annual nauplii contribution on the total copepod abundances was 67.4%, with the maximum of 92.8% at S5 during autumn. Copepodids abundances were significantly different among stations, ranged from 1 (S1, winter) to 64,000 ind·m^{-3} (S7, autumn) (Figure 5) and were correlated positively with salinity ($r = 0.629$, $p < 0.01$).

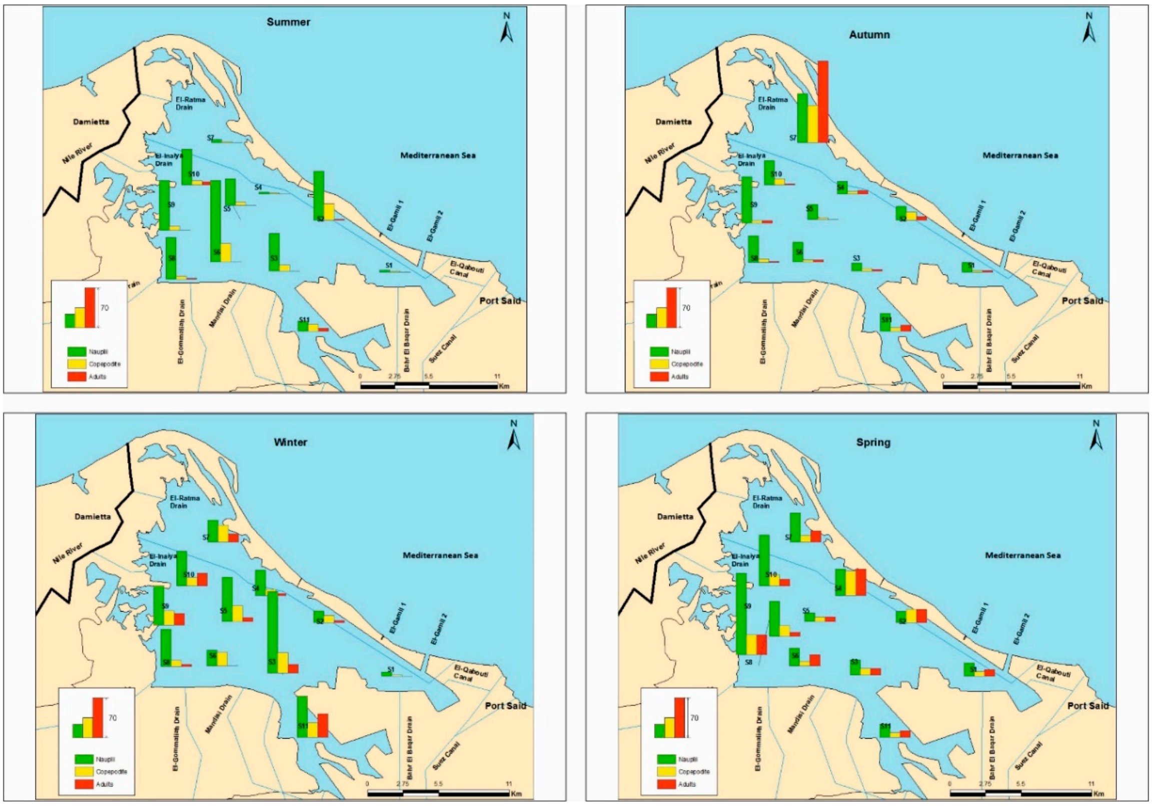

Figure 5. Spatiotemporal variations of copepod demographic class abundance: nauplii, copepodids, and adults in Lake Manzalah.

The abundance of adult copepods showed significant differences between stations and seasons. It varied from 0 during summer (S1, S3, S4, S5, S6, S7, and S9), autumn (S5), and winter (S1 and S6) to 140,000 ind·m^{-3} during autumn at S7 (Figure 5). The seasonal distribution of adults is shown in Figure 6 and Table 2. In detail, *Oithona nana* (Cyclopoida) and *Euterpina acutifrons* (Harpacticoida) reached 2,000 ind·m^{-3} at S2 and S10, respectively (Figure 6). Abundances of *O. nana* correlated positively with temperature ($r = 0.780$, $p < 0.01$). *Acartia tonsa* represented 47.7% of the total adults (Table 2), thus being deeply responsible for adult copepods' abundances ($r = 0.962$, $p < 0.01$). However, *A. tonsa* was found only at five stations (S2, S4, S7, S9, and S10) (Figure 6). This species showed an autumnal abundance, being the only adults at S2, S7, and S10 (Figure 6). Adults of *A. trajani* and *T. consimilis* were the most abundant Cyclopoida during the study period, accounting for 27 and 10.9% of total adults, respectively (Table 3). *Acanthocyclops trajani* was the only species with adults at S8 during autumn, winter, and spring. *Acanthocyclops trajani* adults were also dominant at S2 and S4 during winter and at S1 and S3 in spring (Figure 6). *Thermocyclops consimilis* accounted for 100% of total adult copepods in summer at S8, in autumn at S1, and in winter at S5 (Figure 6). *Nitocra lacustris* was the most abundant Harpacticoida accounting for up to 44.4% of adults at S9 during spring (Figure 6). Seasonal averages of diversity (H') ranged from 0.2 (S7) to 1.6 (S10) (Table 3).

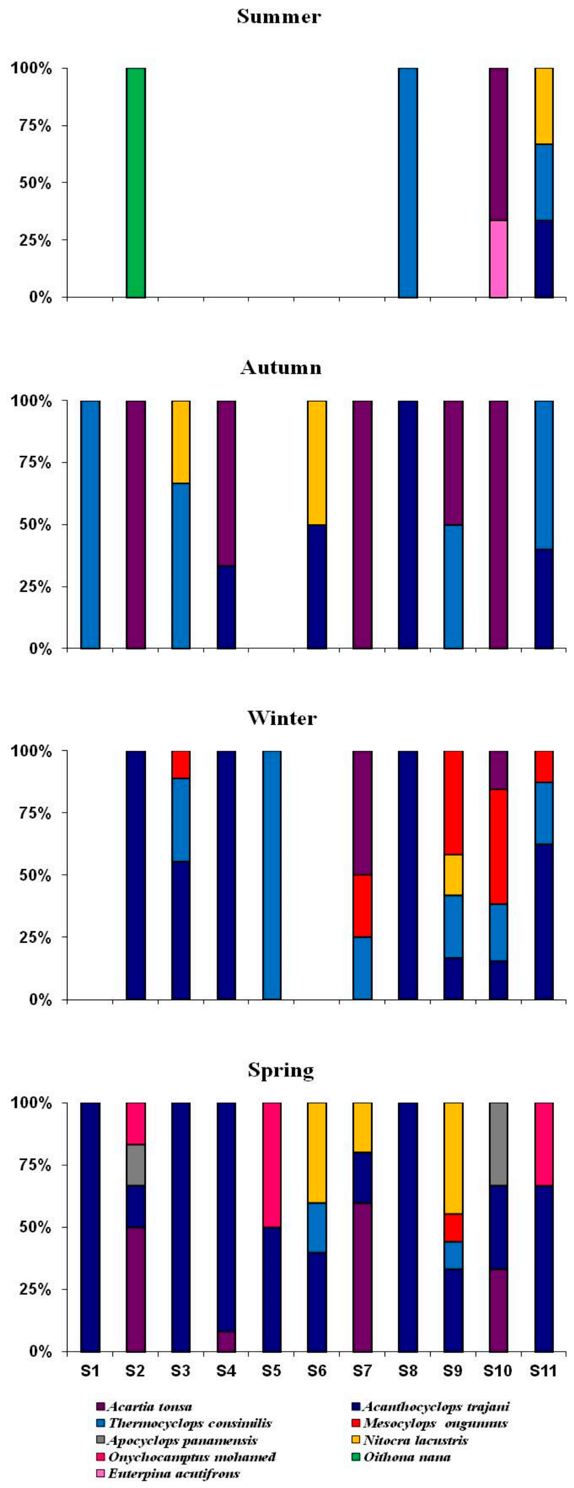

Figure 6. Spatiotemporal variations of adult copepod percentages in Lake Manzalah.

The distribution of adults of the most common taxa *A. tonsa*, *A. trajani*, *T. consimilis*, *N. lacustris*, and *M. ougunus* with respect to salinity and nutrients (DIN and DIP = dissolved inorganic nitrogen and phosphorus, respectively) is shown in Figure 7. *Acartia tonsa* and *A. trajani* were the most tolerant to the salinity variations in the Lake. *Acanthocyclops trajani*, *T. consimilis*, and *N. lacustris* were the most tolerant to variations of DIN and DIP (Figure 7). In addition, *A. trajani* and *T. consimilis* were the only adults observed in anoxic waters.

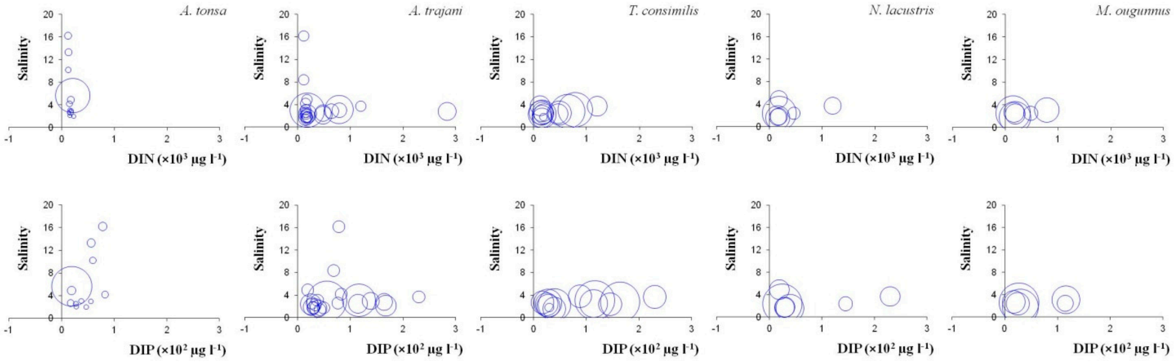

Figure 7. Abundances of *Acartia tonsa*, *Acanthocyclops trajani*, *Thermocyclops consimilis*, *Nitocra lacustris*, and *Mespcyclops ogunnus* (these five species represent 96.3% of total adult copepods population) in function of values of salinity, DIN, and DIP at Lake Manzalah. Circles diameters are correlated with abundances. Only adults have been considered.

3.3. Copepod Assemblages and Environmental Conditions

In order to highlight the impact of physicochemical parameters and the most dominant copepod taxa, a PCA was performed (Figure 8). Component axes PC1 and PC2 explained 49.1% of the variance (F1 = 31.8%). The 1st Principal Component (PC1) axis represents the eutrophication gradient (EG) and shows the negative correlation of nutrients: ammonium, nitrates, nitrites, phosphates and TSI with dissolved oxygen and pH (G2). This EG is negatively correlated with juvenile stages of copepods: nauplii densities ($r = -0.41$, $p < 0.01$), copepodids ($r = -0.36$, $p < 0.05$).

The 2nd Principal Component (PC2), which represents the salinity gradient (SG), was positively correlated with *A. tonsa* and. *A. trajani* (high salinity, HS, taxa), and negatively correlated with *T. consimilis*, *M. ogunnus*, and *N. lacustris* as well as overall adults (low salinity, LS, taxa).

In summary, adults, in general, avoid hypoxic situations and their distribution is more affected by salinity than by nutrients.

The distribution of sites indicates: S1 is isolated and characterized by the highest concentrations of nutrients, and anoxic conditions; hypertrophic conditions were also detected in S11 and S3. Station S7 showed the highest annual salinity averages and hosted the HS taxa; the other sampling sites are typically colonized by LS taxa. Winter seem to have the most drastic conditions of eutrophication.

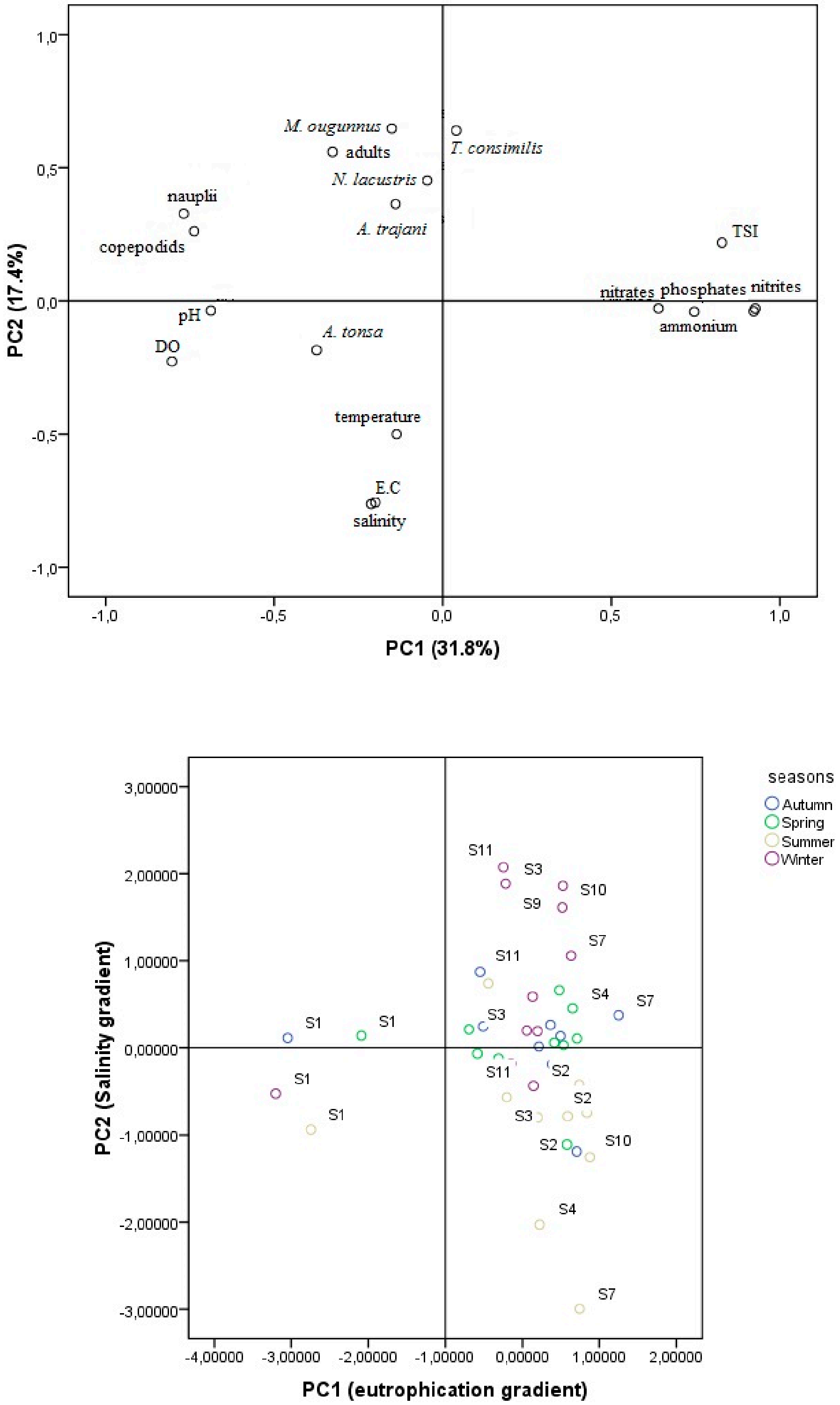

Figure 8. Principal components analysis (PCA): ordination model, with rotation of varimax axes, of variables (above) and stations and seasons (below), in plane of first two principal components.

4. Discussion

Lake Manzalah appeared as roughly separable into two opposite parts, the brackish north part (S2, S4, S7, S10) influenced by the sea, and the mostly freshwater south part (S1, S3, S8, S9, S11) confined and more affected by eutrophication. The two opposite portions are separated by an intermediate environment, whose transitional role is probably facilitated by isles scattered in any direction. The water circulation, in addition, is responsible of anomalous characteristics of stations [47]. For example, S1 (close to a channel of lake–sea communication) showed mostly freshwater, and anoxic conditions. From this point of view, Lake Manzalah is a highly heterogeneous and dynamic ecosystem [48,49]. In fact, the lake receives about 4000 million $m^3 \cdot y^{-1}$ of untreated industrial, domestic, and agricultural drainage waters [50] through several inputs in its southern part. The input from Bahr El-Baqer discharges domestic and industrial sewage, while Hadous, Mandisi, El- Sherw, and El-Gammaliah discharge agricultural effluent [51]. Highly productive fresh waters from the Nile enrich the lake through canals from the Damietta Branch, El-Inaniya, and El-Ratama.

The high concentrations of nutrients recorded in the present study identify all the stations at a high trophic level, according to References [52,53]. Indeed, since the completion of the Aswan High Dam in 1970, which drastically reduced the seasonality of the Nile flow, the growing population in the Nile delta has increased the discharge of municipal, agricultural, and industrial wastes into Lake Manzalah [53]. In addition, because of the shallowness of the lake, there is no thermal stratification to impede the resuspension of nutrients from sediments [54], which have been shown to store high concentrations of such elements. Nitrates were the main form of dissolved nitrogen at S2, S5, S6, and S8. This nitrogen form is a major component of agricultural soil fertilizers, and its concentrations in transitional waters are usually linked to runoff discharge, which lixiviates the excess from plant uptake [55,56]. Ammonium is a typical characteristic of eutrophic waters [57]; it was the main form of dissolved nitrogen at S3, S4, S9, S10, and S11, but it was highly abundant in S1. Reference [57] hypothesized that the main source of anthropogenic nitrogen is mainly the non-treated wastewaters. Reference [58] suggested that the identity of the primary growth-limiting nutrient in the Mediterranean coastal systems may shift from phosphate in oligotrophic lakes towards nitrogen as eutrophication proceeds. So, it can be concluded that the high P availability in Lake Manzalah confirms its eutrophic state.

The copepod assemblage of the Lake Manzalah shares some species with other Nile Delta lakes [59,60]. During the present study, three species (*Acartia tonsa*, *Apocyclops panamensis*, and *Mesocyclops ogunnus*) were reported for the first time, being absent from the 2003–2004 data of References [34] and [36]. The three new records for Lake Manzalah cannot simply be added to the previous assemblages, because some species recorded by Reference [36] are absent in the present study. *Acartia tonsa*, a species typical of coastal embayments, is a well-known invasive non-indigenous species (NIS) in the Mediterranean area [10,61]. It is the first time that this species is reported from the African side of the Mediterranean basin. This species has recently entered the Ponto-Mediterranean Province [62] from the Baltic Sea via the Black Sea [62,63], probably due to passive transport of resting eggs with aquaculture species and equipment in Mediterranean coastal lakes. Typically, the species is considered an invasive ecological element because it forms stable and dominating populations with evidence of exclusion of other species historically present in the same coastal lakes [10,62]. The settlement of this species in coastal brackish waters appears favoured by stress conditions which, evidently, give rise to problems to the indigenous species depressing their competitiveness [10]. *Acartia tonsa* progressively settled in many Mediterranean coastal brackish waters, and its invasion is typically southward directed [62,63]. The species is particularly interesting from this point of view, because it seems to not be affected by the ongoing climate warming, thus suggesting that human alterations of ecological equilibriums in coastal brackish waters are locally more effective than climate change in spreading species geographic ranges. However, eutrophication and warming often have synergistic effects on plankton [64–66].

The cyclopoid *A. panamensis* is found in many fresh and brackish water environments [67]. According to Reference [60], it is a recent NIS in the Nile delta lakes. *Mesocyclops ogunnus* is widespread in Nile–delta lakes [68] and is described as abundant in Lake Nasser [69]. However, the present study is the first record in Lake Manzalah. *Onychocamptus mohammed* is distributed in fresh and saline inland waters worldwide, with a range including the South China Sea, all the coasts of Europe, Northern and

Central Africa, the Caribbean Sea, and Brazil [70]. This species was previously recorded in Egypt at Wadi El-Rayan (Fayoum Province) and Edku Delta Lake [71,72].

The comparison of copepods species composition in Lake Manzalah with previous studies is given in Table 4. Based on a recent work [73], *A. americanus* corresponds to *A. trajani*. According to Reference [60], it invaded the lakes in the course of the second half of the twentieth century. *Acanthocyclops trajani* tolerates high eutrophy levels [74,75] and it has become the dominant pelagic cyclopoid in all the eutrophic Nile–delta lakes [60]. *Acanthocyclops trajani*, as *A. tonsa*, is considered to be a northern biogeographic element in the fauna of the Nile–delta lakes [60,61].

Table 4. Occurrence of copepod species in Lake Manzalah in comparison with previous studies (references are indicated). The bold lettering evidences the species of the present study.

Copepod Species	References
Acanthocyclops trajani (= A. americanus)	[34,36,76,77]
Thermocyclops consimilis	[77]
Mesocyclops ougunnus	
Apocyclops panamensis	
Oithona nana	[34,36]
Thermocyclops sp.	[34,36]
Nitochra lacustris	[34,36]
Onychocamptus mohammed	
Euterpina acutifrons	[34,36]
Harpacticus sp.	[77,78]
Mesochra holdeti	[34,36]
Acartia tonsa	
Paracalanus parvus	[34,36]
Paracartia latisetosa	[76]

Due to the settlement of NIS species, and of eutrophic conditions, we expected to register a simplification of species composition, with success of only the few adapted to the stressed situation. The surprising abundance of Copepoda species in Lake Manzalah, at this point, has to be justified on the basis of its environmental diversification. The lake appeared as a mosaic of habitats, rather than a unique brackish water environment. The ecological simplification acted in restricted areas and under different constraints (NIS at the brackish part, hypertrophy at the fresh part). The relative isolation of these two extremes is favoured by the existence of about 1000 islands which somehow physically fragment the whole lake. Such a partitioning also facilitates the formation of different micro-habitats in the lake, and/or an increase in the number of benthic macrophyte communities, from where also zooplankton composition could be affected. The last consideration is supported by Reference [79], who recorded an increase in macrophyte communities in Lake Manzalah, with 11 dominant communities, whose number increased with decreasing salinity and increasing eutrophication near the points of discharge into the western and southern parts of the lake. Copepod diversity, like that of macrophytes, is probably explained by the increased spatial variability of the ecosystem. The lake spatial variability (due to about 1000 islands scattered in it) has been enhanced by the non-homogeneous subtraction of peripheral parts of the lake for agricultural purposes in the last 40 years [38,39], with the formation of deep inlets in the southern shoreline. The species richness is effective only if the whole lake, and the whole period are considered. Single stations showed significantly lower number of species (from 2 to 6 in the whole considered period).

Reference [19] suggested that copepods are capable of acclimating/adapting to eutrophication stressors, and that population levels may eventually return to pre-stress levels. Indeed, only a small part of this species assemblage can be considered as indicators of eutrophication. *Acartia tonsa* is observed in large numbers in brackish eutrophic waters [10], where it is considered resistant to the frequently-occurring hypoxia (also related to high temperatures) [80]. It is considered to be a euryhaline species [81], which does not suffer seasonality of the Mediterranean area [62]. Its euryhalinity, however, does not enable the species to flourish in the southern part of Lake Manzalah (with more diluted and hypertrophic waters). In the present study, *A. tonsa* was limited to the stations with higher average salinity values, i.e., S2, S4, S7, and S10. The hypertrophic and diluted waters of the rest of the lake are suitable for the settlement of *A. trajani*, a typical indicator of such a kind of water.

The historical modification of the copepod assemblage in Lake Manzalah can thus be attributed to the progressive partitioning of the environment and the consequent modification in the food spectrum [82]. The dominance of Cyclopoida in the majority of the sampling stations in Lake Manzalah is consistent with their hypothesized ability to better survive under adverse conditions because they are considered less specialised than other copepods [83]. Reference [84] suggested that the lower metabolic requirements of Cyclopoida compared to Calanoida may explain the high abundance of the former group in eutrophic waters. Several species of the genus *Thermocyclops* have been considered to be indicators of eutrophic waters [85,86]. The present study considered *T. consimilis* and *A. trajani* the two best indicators of eutrophic waters in Lake Manzalah, since they tolerate high levels of nutrients and hypoxia and marked spatial variations in salinity.

Lake Manzalah is not a unique environment, but a mosaic of habitats, which significantly differ in salinity, pH, dissolved oxygen, and nutrients. Copepod species varied in their degree of tolerance, thus being indicators of different situations.

Given that Lake Manzalah is subjected to cumulative regional sewage input, and thanks to the already available historical data, long-term environmental and biological monitoring will be particularly useful and interesting from an ecological point of view. Wider planktonic spectrum studies and research into zooplankton physiological responses, by means of bioassays and in situ experiments on sewage pollutants, should provide additional information about the environment characteristics and processes of hypertrophic lakes.

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