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Tintinnida (Ciliophora) as bio-indicator for certain pollutants at al-max area, Alexandria, Egypt

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Abstract

Tintinnids community was studied seasonally at Al-Max area during the period from spring 2014 to winter 2015. This study was aimed to throw light on the occurrence of tintinnid species that considered as bio-indicators for certain environmental factors. Fifty three species of tintinnids belong to 21 genera were recorded. From them eight species viz. *Eutintinnus pingius, Proplectella angistior, Favella ehrenbergii, Ascampbelliiella sp, Helicostomella subulata, Tintinnopsis nudicauda, Favella panamensis* and *Tintinnopsis campanula* are considered as bio-indicators for dissolved oxygen, salinity and pH. While *Favella panamensis, Favella ehrenbergii* and *Tintinnopsis campanula* showed that for nitrites and *Favella panamensis, Favella ehrenbergii* and *Tintinnopsis campanula* for nitrates. However, *Tintinnopsis nudicauda* is positively correlated for temperature and the other seven species are negatively correlated with temperature. Furthermore, *Tintinnopsis radix*, *Tintinnopsis campanula*, *Tintinnopsis nudicauda* and *Favella adriatica* are considered as euryhaline species.

Keywords: Tintinnids, bio-indicator, Al-Max Bay, Pollution & Environmental conditions

1. Introduction

Tintinnids are unicellular organisms. They form a group of loricate ciliates (Montagnes, 2013) [25]. They inhabit marine and freshwaters (McManus and Santoferrara, 2013) [24] and play a vital role in aquatic food chains which feed on phytoplankton and bacteria, in turn they act as food for larger organisms such as copepods and fish larvae (Stoecker, 2013) [35].

Tintinnids are part of the microzooplankton that have many advantages as a favorable bio-indicator to evaluate environmental stress and anthropogenic impacts in aquatic ecosystems (Jiang *et al*., 2011, 2013a, 2013b, Xu *et al*., 2011a, b, c, 2014) [19, 18, 20, 39, 40, 41, 42] and to monitor sea water quality (Wu *et al*. 2016) [38]. Owing to their short life cycle and delicate pellicles, they may respond more quickly to environmental changes than any other metazoans (Coppellotti and Matarazzo, 2000, Ismael and Dorgham, 2003) [8, 17]. Many ciliated microbiota can tolerate extreme of environmental hazards than macrofauna (Xu *et al*. 2011a, b) [39, 40].

Tintinnids are important components of the aquatic ecosystem and play a crucial role in transferring elements and energy from low trophic levels (pico- and nano-phytoplankton) to high one such as copepods (Crawford *et al*. 1997 and Corliss, 2002) [10, 9].

This study aims to:

- Investigate distribution and abundance of tintinnids at Al-Max area.
- Determine the correlation coefficient between tintinnid density and certain environmental parameters.
- Clear the differentiation between different sites using diversity indices and similarity.
- Show the tintinnid species that considered as bio-indicators.

Materials and Methods

Study area: This study was carried out on tintinnid assemblages in Al-Max area during the period from spring 2014 to winter 2015. Four sites were selected at the study area to represent different habitats. The coordinates and local names are shown in Table (1) and Figure (1).

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Table (1): Show position of the selected sites at Al-Max area during the present study.

9		North	East
I	Al - Umoum Drain	31° 8' 56"	29° 50' 42"
II	Al - Umoum Drain outlet	31° 9' 7.18"	29° 50' 27.04"
III	Al-Max Bay (~ 1.2 Km far of the outlet)	31° 9' 22.56"	29° 49' 55.00"
IV	Al-Max Bay (~ 4 Km far of the outlet)	31°10' 39.34"	29° 48' 52.71"

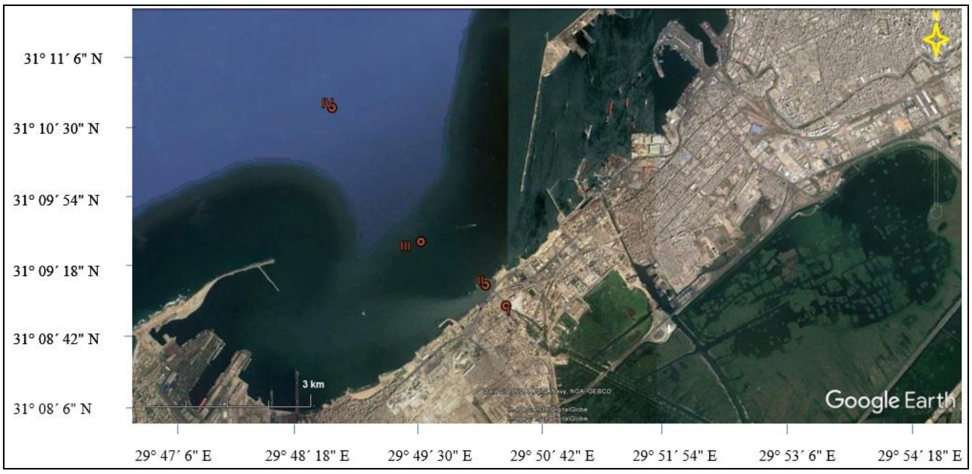

Fig1: Map shows sites of collection at Al-Max area during the study period.

Values of both water temperature and dissolved oxygen were measured using TRANS digital dissolved oxygen meter model HD3030. Seawater salinity was determined by using ADWA digital Salinometer model AD 410, while pH values were measured using ADWA pH digital meter model AD 11. Dissolved nitrites, phosphates and COD were measured according to APHA (1995) [7]. Dissolved nitrates were measured according to Mullin and Riley (1956) [26].

Escherichia coli samples were collected by filling 10 ml sterilized glass bottles directly from the water followed by rapidly and tightly closing. They were kept in an ice box, and then estimated (as colonies per ml) through 48 h. in the center laboratory of drinking water Company at Abu Hommos, El-Behaira Governorate, Egypt.

Sample collection and treatment

Plankton samples were collected by filtering known volumes of water, using 55µm-mesh plankton net. These samples were preserved in 5% buffer formalin solution. In the laboratory, the sample volume has adjusted to 100 ml. Sub-samples of 0.5–1 ml were transferred to a counting chamber and then examined under a compound binocular and trinocular microscopes. For identification, many taxonomic guides have been used such as Tregouboff and Rose (1957) [36], Marchall (1969) [23], Paulier (1997) [28] and WORMS database [37].

The tintinnids standing crop was calculated as individuals per cubic meter according to Santhanam and Srinivasan (1994) [32] formula as following:

$N = n (v/V)*1000$

Where, N= Total number of individuals per cubic meter; n= Average number of individuals in one ml of the sample; v= Volume of zooplankton concentrate (ml) and V= Volume of total water filtered (L).

Statistical analyses

Correlation analyses were carried out by computer Excel program and MINITAB 14 statistical program, while diversity indices similarity were done by PRIMER 5 statistical program.

Results and Discussion

1. Environmental conditions

Figure (2) exhibits that all variables showed distinct spatial differences except temperature. These results clarified that, water temperature ranged between 17.58±0.08 ºC at site IV during winter and 29.80±0.70 ºC at site I during summer. This pattern agrees with that noticed by Aboul Ezz *et al*. (2014) [5] and Shreadah *et al*. (2014) [34] at Al-Max Bay.

Salinity increases gradually from site I to site IV. It ranged between 2.47±0.09‰ at site I in spring and 27.65±0.78‰ at site IV in winter (Figure 2). This remarkable wide range in salinity may be due to the effects of huge volume of the discharged fresh water from Al-Umoum Drain into the bay and the evaporation by high temperature. These findings coincide with that showed by Nessim *et al*. (2010) [27], Aboul Ezz *et al*. (2014) [5], Abou Zaid *et al*. (2014) [3], and Shreadah *et al*. (2014) [34].

Values of pH ranged between 7.50±0.0 at site I during summer and 9.30±0.0 at site III during spring (Figure 2). Then pH occurred on alkaline side, this is related to high photosynthetic activity (Hammer, 1971 and Hegab, 2015) [14, 15].

Dissolved oxygen levels increased remarkably from 1.26±0.16 mg/l at site II to 9.9±0.17mg/l at sites III and IV in spring (Figure 2). This is related to the effect of direct draining especially sewage at the former sites, and is in agreement with Heneash *et al*. (2015) [16].

The lowest value of COD was 5.47±5.10 mg/l at site IV in winter, increased sharply into the highest average of 22.93±5.10 mg/l at the same site in spring and 22.67±0.92 mg/l at site II in winter (Figure 2). These values were greatly varied from site to another but their annual averages exhibited the maxima at sites II and I. The present results indicated that, the high level of COD at sites I and II is attributed to the high content of organic matters and wastes discharged from the surrounding factories within these two sites. The present findings are similar to that noticed by Aboul-Ezz *et al* (2014) [5] at Al-Max Bay.

The minimum concentrations of nitrite were 15.27±2.93 and 15.48±1.87 µg/l, at site II in spring and site I in summer, respectively, increased to the maximum levels being 411.61±58.77µg/l at site I in autumn. The highest annual average was 124.79±95.89 µg/l at site I while the lowest one was 66.19±22.78 µg/l at site IV (Figure 2). These results are similar to that recorded by Shreadah *et al.* (2014) [34]. They reported that, nitrites ranged from 107.52 µg/l at Al-Umoum Drain outlet to 35 µg/l at the highest offshore stations. Also, Nessim *et al.* (2010) [27] recorded nitrites from 25.2 to 158.2 µg/l at Al-Max Bay. These changes are due to the increasing of agricultural wastes and fertilizers in the drain and high rate of nitrate reduction and ammonia oxidation forming nitrite as intermediate state by the action of denitrifying bacteria.

Nitrate values ranged between 0 at both sites III and IV in spring and 480.09±45.51µg/l at site II in autumn (Figure 2). This pattern of results coincides with Shreadah *et al.* (2014) [34] at Al-Max Bay. Furthermore, the biological consumption, particularly the phytoplankton uptake could be low leading to increase nitrate content in the medium (Satpathy *et al.* 2010a) [33].

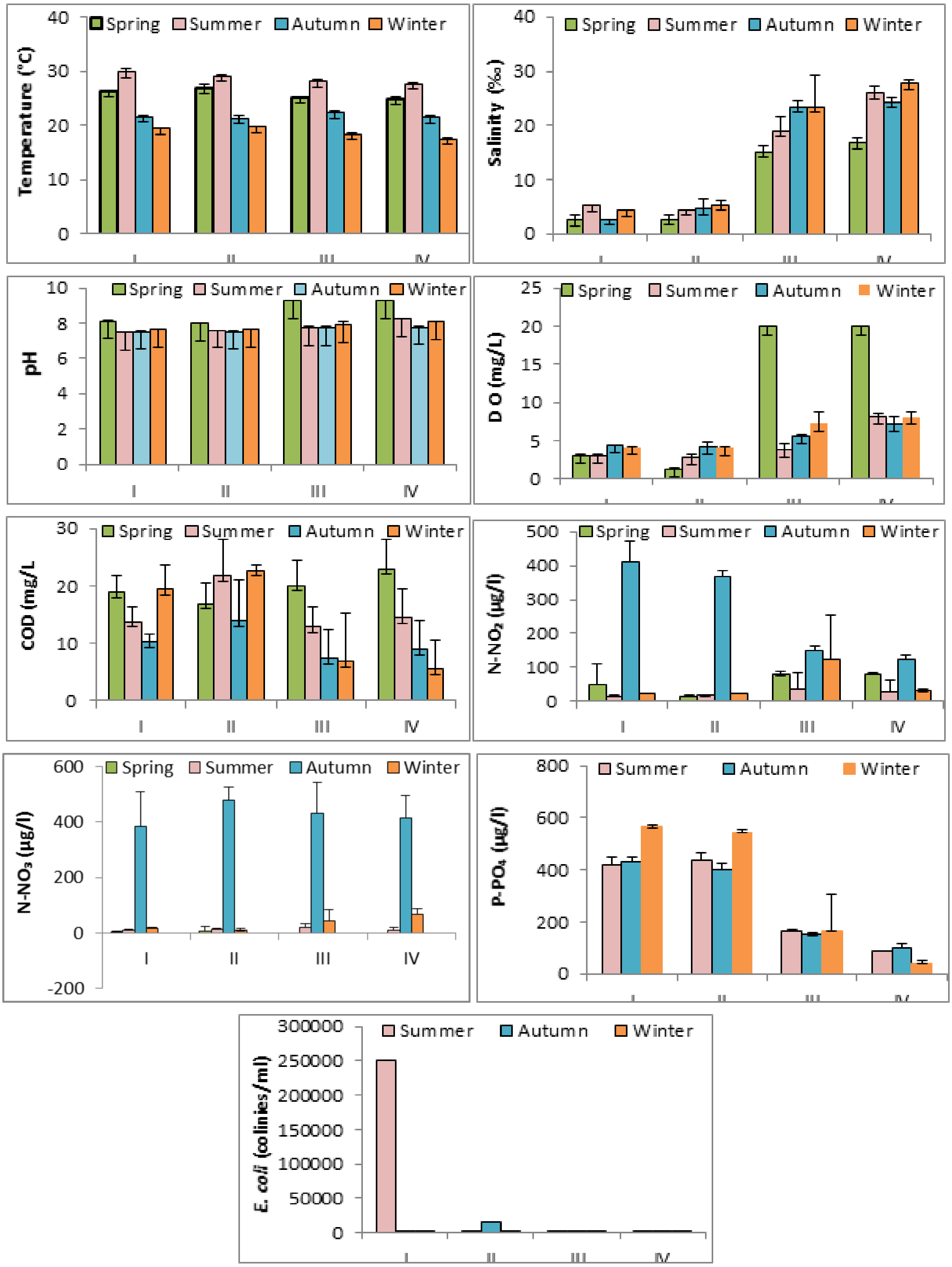

Fig 2: Spatial and temporal variations in the environmental factors at Al-Max area selected sites from spring 2014 to winter 2015.

Phosphate concentrations were measured during summer, autumn and winter only. The average values of phosphates were highly fluctuated between 38.33±00 and 564.99±00 µg/l at sites IV and I, respectively during winter. Usually, seawater serves as the main source of phosphates in estuarine and coastal waters except those receive fresh water loaded with domestic wastes contaminated by detergent runoff from fields rich with phosphate-phosphorous fertilizer (Satpathy *et al*. 2010a) [33].

In general, both sites I and II exhibited higher nutrient content, compared with the other sites. The enrichment is due to agricultural runoff, sewage effluents and untreated drainage.

Escherichia coli bacteria were counted during summer, autumn and winter only, their numbers at sites I and II were higher than that at sites III and IV. The values of this bacteria fluctuated sharply between 160 colonies/ml at site IV and 250000 colonies/ml at site I in summer (Figure 2)**.** It is worth to mention that, *Escherichia coli* are found in the lowest intestine of warm-blooded organisms, so they were chosen to indicate the sewage pollution. The highest increase at Al-Umoum Drain especially in summer mostly was attributed to direct discharge from surrounding houses in addition to daily discharging semi and untreated sewage effluents.

2. Tintinnid faunal composition

During this work, fifty three (53) species in addition to cysts of tintinnids were recorded belonging to 11 families and 21 genera. Tintinnid species were divided into agglutinated and non-agglutinated (hyaline) forms on the basis of morphological characteristics. The agglutinated genus *Tintinnopsis* was the dominant one with a maximum 11 species forming 20.8% of total tintinnid species number and 67.66% (5300 ind./m^3) of total tintinnid densities. These results are similar to that postulated for genus *Tintinnopsis* by previous authors as Abdel-Aziz (2004) [1] who listed 10 species, Dorgham *et al*. (2009) [11] who collected 14 species, Abou Zaid and Hellal (2012) [4] who recorded 12 species, Abo-Taleb *et al*. (2016) [2] who listed 17 species and Rakshit *et al*. (2017) [29] who collected 13 species. But these results are higher than that reported by Aboul Ezz *et al*. (1990) [6] and Dorgham *et al*. (2013) [12] recorded 3 species and 4 species, respectively.

Tintinnopsis followed by genera *coxliella* with 5 species, *Codonellopsis*, *Eutintinnus*, *Favella* (4 species for each), *Helicostomella* and *Undella* (3 species for each), *Epiplocylis*, *Liprotintinnus*, *Metacylis*, *Parafavella* and *Proplectella* (2 species for each). The remaining nine genera were represented by a single species for each one.

The dominance of *Tintinnopsis* in estuarine and coastal waters during the present work agrees with that reported by Feng *et al*. (2015) [13] and Jiang *et al*. (2011) [19]. This may be due to their unique flexible adaptive strategies by which they reached their maximum abundance; or to the eurythermal and euryhaline nature of *Tintinnopsis* which can sustain in different aquatic environments. These data coincide with that observed by Krinsic (1987) [22] and Reynolds (1997) [30]. In contrast, non-agglutinated genera, such as *Favella*, *Metacylis*, *Eutintinnus*, *Amphorellopsis* and *Helicostomella* were recorded in low density (about 32% of the total tintinnid community).

3. Spatial distribution and abundance

Results in Table (2) show that, the tintinnid communities in the studied sites were varied greatly between sites. Sites IV and III recorded the highest species number (42 and 39 species, respectively), with high density forming 15175 and 11327 inds./m^3 respectively. On the other hand, the lowest number of species was recorded at sites I and II, listed 1 and 5 species, respectively, forming 139 and 4695 inds./m^3 respectively. The tintinnid biodiversity in this region has been largely affected by anthropogenic activities as well as natural catastrophic events. It is assumed that the prevalent site-specific variations in biodiversity might be related to the environmental conditions (Ismael and Dorgham, 2003) [17] and in other sites as mentioned by Coppellotti and Matarazzo (2000) [8].

Tintinnopsis nudicauda was the most dominant species at sites II, III & IV, represented 76.92, 51.98 and 50.18 %, respectively. While *Favella panamensis* dominated at sites III and IV amounted 15.61 and 13.63%, respectively. At the same time, *Favella ehrenbergii* dominated at site IV only, and had a low density attained 12.96%. However, only one species, *Tintinnopsis radix* was reported at all sites (Table 2***)***. The other collected species considered rare. This refers to their high tolerance to different environmental parameters and in congruence with that noticed by Abo-Taleb *et al*. (2016) [2] who listed the last three species (*Favella panamensis*, *Favella ehrenbergii* and *Tintinnopsis radix*) within the most dominant species.

Table 2: Spatial distribution, average density (ind./ m^3) and relative abundance (RA) of the recorded species at Al-Max area during the study period from spring 2014 to winter 2015**.**

Sites and density		I		II		III		IV	
Species		**D±SE**	**RA**	**D±SE**	**RA**	**D±SE**	**RA**	**D±SE**	**RA**
	Family: Codonellidae (Kent, 1882)								
1	*Tintinnopsis beroidea* Stein, 1867	0	0.00	0	0.00	186±139	1.64	204±139	1.34
2	*Tintinnopsis campanula* (Ehrenberg, 1840)	0	0.00	139±139	2.96	1011±684	8.92	534±311	3.52
3	*Tintinnopsis cylindrica* Daday,1887	0	0.00	0	0.00	0	0.00	18±18	0.12
4	*Tintinnopsis gracilis* Kofoid & Campbell, 1929	0	0.00	0	0.00	26±21	0.23	11±11	0.07
5	*Tintinnopsis karajacensis* Brandt, 1896	0	0.00	0	0.00	211±211	1.86	179±179	1.18
6	*Tintinnopsis levigata*Kofoid& Campbell, 1929	0	0.00	0	0.00	0	0.00	11±11	0.07
7	*Tintinnopsis mortenseni* Schmidt, 1901	0	0.00	0	0.00	44±44	0.39	28±28	0.19
8	*Tintinnopsis nana* Lohmann, 1908	0	0.00	0	0.00	13±13	0.11	46±46	0.30
9	*Tintinnopsis nudicauda* Paulmier, 1997	0	0.00	3611±3611	76.92	5888±5831	51.98	7615±7391	50.18
10	*Tintinnopsis radix* Brandt, 1907	139±139	100.00	278±278	5.92	652±325	5.76	281±129	1.85
11	*Tintinnopsis sp*	0	0.00	0	0.00	14±14	0.12	62±25	0.41
12	*Codonella sp*	0	0.00	0	0.00	4±4	0.04	0	0.00
	Codonellopsis indica Kofoid & Campbell, 1929								

13		0	0.00	0	0.00	146±146	1.29	47±47	0.31
14	*Codonellopsis americana* Kofoid&Campbell, 1929	0	0.00	0	0.00	0	0.00	298±298	1.97
15	*Codonellopsis sp*	0	0.00	0	0.00	0	0.00	37±37	0.25
16	*Codonellopsis sp*	0	0.00	0	0.00	61±52	0.54	141±141	0.93
17	Family: Epiplocylididae *Epiplocylis atlantica* Kofoid &Campbell, 1929	0	0.00	0	0.00	18±13	0.16	8±8	0.05
18	*Epiplocylis sp*	0	0.00	0	0.00	0	0.00	11±11	0.07
19	Family: Metacylididae *Climacocylis scalaria* (Brandt, 1906)	0	0.00	0	0.00	4±4	0.04	0	0.00
20	*Metacylis lucasensis*	0	0.00	0	0.00	0	0.00	8±8	0.05
21	*Metacylis annulifera* (Ostenfeld&Schmidt, 1901)	0	0.00	0	0.00	28±28	0.25	94±56	0.62
22	*Coxliella annulata* (Daday, 1886)	0	0.00	0	0.00	0	0.00	11±11	0.07
23	*Coxliella bolivari* Osorio Tafall, 1941	0	0.00	0	0.00	15±15	0.13	0	0.00
24	*Coxliella fasciata* (Kofoid,1905)	0	0.00	0	0.00	4±4	0.04	9±9	0.06
25	*Coxliella laciniosa (*Brandt, 1906)	0	0.00	0	0.00	123±105	1.09	0	0.00
26	*Coxliella sp*	0	0.00	0	0.00	4	0.03	19±19	0.12
27	*Helicostomella edentate* (Fauré-Frémiet, 1908)	0	0.00	0	0.00	4±4	0.04	0	0.00
28	*Helicostomella kilensis* (Laackmann, 1906)	0	0.00	0	0.00	4±4	0.03	25±15	0.16
29	*Helicostomella subulata* (Ehrenberg, 1833)	0	0.00	0	0.00	68±45	0.60	276±172	1.82
30	Family: Ascampbelliellidae *Ascampbelliiella sp*	0	0.00	278±278	5.92	0	0.00	0	0.00
31	Family: Ptychocylididae *Favella adriatica* Jörgensen, 1924	0	0.00	389±389	8.29	25±14	0.22	42±42	0.28
32	*Favella companula* (Schmidt, 1901)	0	0.00	0	0.00	35±29	0.30	179±179	1.18
33	*Favella ehrenbergii* (Claparéde&Lachmann, 1858)	0	0.00	0	0.00	631±316	5.57	1967±1222	12.96
34	*Favella panamensis* Kofoid&Campbell, 1929	0	0.00	0	0.00	1768±1170	15.61	2069±1524	13.63
35	Family: Rhabdonellidae *Rhabdonellopsis longicaulis*Kofoid&Campbell, 1929	0	0.00	0	0.00	0	0.00	11±11	0.07
36	Family: Tintinnidae *Amphorelopsis tetragona* (Jörgensen, 1924)	0	0.00	0	0.00	15±15	0.13	60±50	0.39
37	*Eutentinnus tergescens* (Kofoid&Campbell, 1929)	0	0.00	0	0.00	0	0.00	8±8	0.05
38	*Eutintinnus elegens* Kofoid &Campbell, 1929	0	0.00	0	0.00	11±11	0.10	22±22	0.15
39	*Eutintinnus lusus-undae* (Entz, 1885)	0	0.00	0	0.00	20±20	0.17	25±15	0.16
40	*Eutintinnus pingius* (Kofoid & Campbell, 1929)	0	0.00	0	0.00	83±83	0.73	397±261	2.61
41	*Liprotintinnus bottnicus* (nordqvist, 1890)	0	0.00	0	0.00	36±22	0.32	0	0.00
42	*Liprotintinnus nordqvisti* (Brandt, 1906)	0	0.00	0	0.00	44±44	0.39	40±27	0.26
43	Family: Undellidae *Undella clevi* Jörgensen, 1924	0	0.00	0	0.00	0	0.00	8±8	0.05
44	*Undella hemisphaerica* Laackmann, 1909	0	0.00	0	0.00	4±4	0.03	0	0.00
45	*Undella ostenfeldi* Kofoid&Campbell, 1929	0	0.00	0	0.00	4±4	0.04	0	0.00
46	*Undellopsis sp*	0	0.00	0	0.00	18±13	0.16	0	0.00
47	*Proplectella angistior* (Jörgensen, 1924)	0	0.00	0	0.00	79±79	0.70	234±234	1.54
48	*Proplectella sp*	0	0.00	0	0.00	0	0.00	19±19	0.12
49	Family: Xystonellidae *Parafavella cylindrica* (Jörgensen, 1899)	0	0.00	0	0.00	0	0.00	11±11	0.07
50	*Parafavella sp*	0	0.00	0	0.00	4±4	0.04	23±23	0.15
51	*Parundella messinensis* (Brandt, 1906)	0	0.00	0	0.00	13±13	0.11	0	0.00
52	Family: Petalotrichidae *Petalotricha ampulla* (Fol, 1881)	0	0.00	0	0.00	0	0.00	72±72	0.47
53	*Cymatocylis calyciformis* (Laackmann, 1909)	0	0.00	0	0.00	0	0.00	14±14	0.09
54	*Cysts of tintinnids*	0	0.00	0	0.00	12±12	0.11	0	0.00
Total No. of individuals		139	100.00	4695	100.00	11327	100.00	15174	100.00
Total No. of species		1		5		38		42	

4. Temporal Distribution and Abundance

The highest number of species (32 species) was recorded in summer, from them five species dominated the others viz, *Eutintinnus pingius*, *Proplectella angistior*, *Favella ehrenbergii*, *Ascampbelliiella sp* and *Helicostomella subulata*. These species were represented by 17.38, 15.16, 14.04, 13.49 and 10.94%, respectively. In autumn, *Tintinnopsis nudicauda* was the only dominant species (88.21%). While in winter *Favella panamensis*, *Favella ehrenbergii* and *Tintinnopsis campanula* were dominated by 36.73, 22.21 and 14.88% of total individuals, respectively. During spring, the same three species (*F. panamensis*, *F. ehrenbergii* and *T. campanula*) mentioned above during winter were dominated by 41.76, 23.15 and 22.31%, respectively. At the same time, *Favella ehrenbergii* and *Tintinnopsis radix* appeared all the year round. However, *Favella panamensis*, *Tintinnopsis beroidea* and *Tintinnopsis campanula* appeared at all seasons except autumn, while *Tintinnopsis gracilis* and *Tintinnopsis nudicauda* occurred all the year round except winter. While *Amphorelopsis tetragona* was recorded during all seasons except spring. In addition *Tintinnopsis sp.* disappeared only in summer. These results showed that, 16 species occurred only in two seasons, eight of them were listed in summer and autumn. Regarding the rest 29 species, all of them were noticed only during one season (Table 3).

Favella panamensis and *Favella ehrenbergii* were abundant especially during winter, forming 58.94% of the total individuals during this study. In addition to their frequency during spring, summer and winter for the former and all the year round for the later. These species were noticed only at offshore sites (III & IV). This refers to their less tolerance to fresh water and other pollutants, and coincides with that reported by Dorgham *et al.* (2013) [12] where they recorded *Favella ehrenbergii* at less stressed area.

Table (3): Temporal average density (inds./m^3) and relative abundance (RA) of the recorded species at Al-Max area during the study period from spring 2014 to winter 2015.

Seasons		Spring		Summer		Autumn		Winter		An. AV	An. RA
Species		D±SE	RA	D±SE	RA	D±SE	RA	D±SE	RA		
1	*Tintinnopsis beroidea*	79±46	3.35	12±7	0.56	0	0.00	300±173	3.86	97	1.24
2	*Tintinnopsis campanula*	525±304	22.31	4±4	0.19	0	0.00	1155±630	14.88	421	5.37
3	*Tintinnopsis cylindrica*	0	0.00	0	0.00	0	0.00	18±18	0.24	5	0.06
4	*Tintinnopsis gracilis*	11±11	0.48	4±4	0.19	22±22	0.11	0	0.00	9	0.11
5	*Tintinnopsis karajacensis*	0	0.00	0	0.00	0	0.00	390±226	5.02	97	1.24
6	*Tintinnopsis levigata*	11±11	0.48	0	0.00	0	0.00	0	0.00	3	0.04
7	*Tintinnopsi smortenseni*	0	0.00	0	0.00	72±43	0.38	0	0.00	18	0.23
8	*Tintinnopsis nana*	0	0.00	0	0.00	0	0.00	59±44	0.75	15	0.19
9	*Tintinnopsis nudicauda*	42±42	1.79	170±120	8.26	16902±6453	88.21	0±0	0.00	4279	54.62
10	*Tintinnopsis radix*	34±34	1.43	71±57	3.44	787±227	4.10	458±290	5.90	337	4.30
11	*Tintinnopsis sp*	11±11	0.48	0	0.00	24±24	0.12	41±26	0.53	19	0.24
12	*Codonella sp*	0	0.00	4±4	0.19	0	0.00	0	0	1	0.01
13	*Codonellopsis indica*	0	0.00	0	0.00	194±138	1.01	0	0	48	0.61
14	*Codonellopsis americana*	0	0.00	0	0.00	298±298	1.56	0	0	75	0.96
15	*Codonellopsis sp*	0	0.00	0	0.00	37±37	0.20	0	0	9	0.11
16	*Codonellopsis sp*	0	0.00	8±8	0.38	194±133	1.01	0	0	51	0.65
17	*Epiplocylis atlantica*	0	0.00	12±7	0.57	0	0.00	14±14	0.18	6	0.08
18	*Epiplocylis sp*	11±11	0.48	0	0.00	0	0.00	0	0.00	3	0.04
19	*Clymacocylis scalaria*	0	0.00	4±4	0.19	0	0.00	0	0	1	0.01
20	*Metacylis lucasensis*	0	0.00	8±8	0.38	0	0.00	0	0.00	2	0.03
21	*Metacylis annulifera*	0	0.00	66±39	3.23	56±56	0.29	0	0.00	31	0.40
22	*Coxliella annulata*	11±11	0.48	0	0.00	0	0.00	0	0	3	0.04
23	*Coxliella bolivari*	0	0.00	0	0.00	0	0.00	15±15	0.2	4	0.05
24	*Coxliella fasciata*	0	0.00	4±4	0.19	9±9	0.05	0	0.00	3	0.04
25	*Coxliella laciniosa*	0	0.00	109±109	5.29	0	0.00	14±14	0.18	31	0.40
26	*Coxliella sp*	0	0.00	4±4	0.19	19±19	0.10	0±0	0.00	6	0.08
27	*Helicostomella edentate*	0	0.00	4±4	0.19	0	0.00	0	0.00	1	0.01
28	*Helicostomella kilensis*	0	0.00	19±15	0.94	9±9	0.05	0	0.00	7	0.09
29	*Helicostomella subulata*	0	0.00	225±169	10.94	119±93	0.62	0	0.00	86	1.10
30	*Ascampbelliiella sp*	0	0.00	278±278	13.49	0	0.00	0	0	69	0.88
31	*Favella adriatica*	0	0.00	0	0.00	11±11	0.06	445±372	5.73	114	1.46
32	*Favella companula*	0	0.00	4±4	0.19	0	0.00	210±171	2.7	53	0.68
33	*Favella ehrenbergii*	544±317	23.15	289±253	14.04	39±23	0.21	1724±1316	22.21	649	8.28
34	*Favella panamensis*	982±570	41.76	4±4	0.19	0	0.00	2851±1675	36.73	959	12.24
35	*Rhabdonellopsis longicaulis*	11±11	0.48	0	0.00	0	0.00	0	0.00	3	0.04
36	*Amphorelopsis tetragona*	0	0.00	8±8	0.38	52±52	0.27	15±15	0.2	19	0.24
37	*Eutentinnus tergescens*	0	0.00	8±8	0.38	0	0.00	0	0.00	2	0.03
38	*Eutintinnus elegens*	34±21	1.43	0	0.00	0	0.00	0	0.00	8	0.10
39	*Eutintinnus lusus-undae*	0	0.00	35±21	1.71	9±9	0.05	0	0.00	11	0.14
40	*Eutintinnus pingius*	0	0.00	358±259	17.38	122±122	0.64	0	0.00	120	1.53
41	*Liprotintinnus bottnicus*	0	0.00	0	0.00	22±22	0.12	14±14	0.18	9	0.11
42	*Liprotintinnus nordqvisti*	11±11	0.48	0	0.00	73±44	0.38	0	0.00	21	0.27
43	*Undella clevi*	0	0.00	8±8	0.38	0	0.00	0	0.00	2	0.03
44	*Undella hemisphaerica*	0	0.00	4±4	0.19	0	0.00	0	0.00	1	0.01
45	*Undella ostenfeldi*	0	0.00	4±4	0.19	0	0.00	0	0.00	1	0.01
46	*Undellopsis sp*	0	0.00	4±4	0.19	0	0.00	14±14	0.18	5	0.06
47	*Proplectella angistior*	0	0.00	312±220	15.16	0	0.00	0	0.00	78	1.00
48	*Proplectella sp*	0	0.00	0	0.00	19±19	0.10	0	0.00	5	0.06
49	*Parafavella cylindrica*	11±11	0.48	0	0.00	0	0.00	0	0.00	3	0.04
50	*Parafavella sp*	23±23	0.96	4±4	0.19	0	0.00	0	0.00	7	0.09
51	*Parundella messinensis*	0	0.00	0	0.00	0	0.00	13±13	0.16	3	0.04
52	*Petalotricha ampulla*	0	0.00	0	0.00	72±72	0.38	0	0.00	18	0.23
53	*Cymatocylis calyciformis*	0	0.00	0	0.00	0	0.00	14±14	0.18	3	0.04
54	*Cysts of tintinnids*	0	0.00	12±12	0.58	0	0.00	0±0	0.00	3	0.04
Total individuals		2352	100.00	2060	100.00	19161	100.00	7763	100.00	8414	100.00
No. of species		16		32		23		19		54	

5. Correlation Coefficients

Table (4) exhibits tintinnid densities which have significant positive correlation coefficients all the year round with salinity (r = 0.88, 0.68, 0.78 and 0.84), pH (r = 0.88, 0.83, 0.78and 0.84) and dissolved oxygen (r = 0.90, 0.78, 0.65 and 0.76) in spring, summer, autumn and winter respectively. This means that the abundant tintinnid species during these seasons viz, *Eutintinnus pingius*, *Proplectella angistior*, *Favella ehrenbergii*, *Ascampbelliiella sp Helicostomella subulata*, *Tintinnopsis nudicauda, Favella panamensis* and *Tintinnopsis campanula* can tolerate high values of salinity, pH and DO, and then they prefer alkaline and oxygenated water. This agrees with that mentioned by Heneash *et al*. (2015) [16].

At the same time, it significantly correlated positively with nitrites in spring (r = 0.59) which shows that the abundant tintinnid species in this season viz, *Favella panamensis*, *Favella ehrenbergii* and *Tintinnopsis campanula* can tolerate high nitrite concentration. Also tintinnid species were correlated significantly in positive pattern with nitrates in winter (r = 0.78), which indicates that the abundant tintinnid species in winter viz, *Favella panamensis*, *Favella ehrenbergii* and *Tintinnopsis campanula* can tolerate high nitrate concentration. This is related to the feeding habits of tintinnids which consume algae that need nutrients through their growing (Kamiyama and Arima, 2001, Rosetta and McManus, 2003 and Montagnes, 2013) [21, 31, 25].

At the same time, it is revealed that, tintinnid species had significantly positive correlation with temperature in autumn (r = 0.59). On the other hand, negative correlation was signified with phosphates during all the three studied seasons (r = -0.68, -0.79 and -0.84) in summer, autumn and winter, respectively. This means that more concentrations of phosphates affect negatively tintinnid densities.

Table 4: Multiple correlation coefficient values (r) between tintinnid densities and physic-chemical parameters at Al-Max area during the study period from spring 2014 to winter 2015.

Seasons	Parameters	r	p-Value	Seasons	Parameters	r	p-Value
Spring	Tem.	-0.73	0.007	Autumn	Tem.	0.59	0.04
	Salinity	0.88	0.0001		Salinity	0.78	0.003
	pH	0.87	0.0002		pH	0.78	0.003
	DO	0.90	0.00006		DO	0.65	0.02
	COD	0.32	0.31		COD	-0.34	0.28
	Nitrites	0.59	0.045		Nitrites	-0.79	0.002
	Nitrates	-0.33	0.29		Nitrates	0.09	0.79
	Phosphates	…..	……		Phosphates	-0.79	0.002
	E. coli	…...	……		*E. coli*	-0.24	0.756
Summer	Tem.	-0.72	0.009	Winter	Tem.	-0.85	0.0007
	Salinity	0.68	0.01		Salinity	0.84	0.001
	pH	0.83	0.0009		pH	0.84	0.001
	DO	0.78	0.003		DO	0.76	0.004
	COD	-0.10	0.75		COD	-0.65	0.02
	Nitrites	0.04	0.90		Nitrites	0.2	0.52
	Nitrates	0.01	0.98		Nitrates	0.78	0.003
	Phosphates	-0.68	0.02		Phosphates	-0.84	0.001
	E. coli	-0.65	0.354		*E. coli*	-0.62	0.379

6. Similarity index

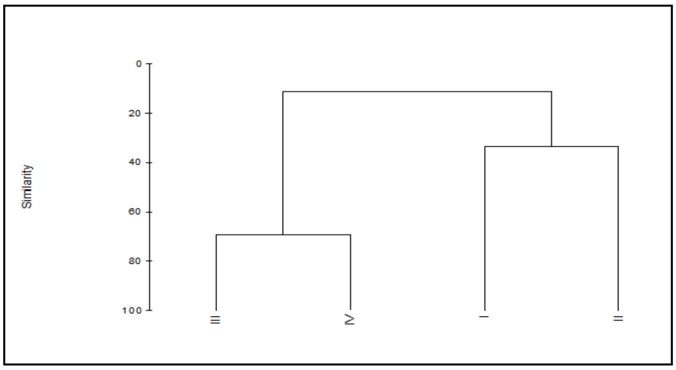

Fig 3: Cluster diagram showing similarity between different sites at Al-Max area during the study period from spring 2014 to winter 2015.

7. Biodiversity measures

Figure (4) shows that species richness has the lower values 0 and 0.47 at site I and II, respectively. This reflects the high pollution at those sites. On contrast, the highest richness values were 4.3 and 4.1, recorded at sites IV and III. These results mean that sites III and IV may be subjected to moderate pollution. However, Shannon diversity exhibited the lowest values 1.16 $X10^{-15}$ and 0.8 at sites I and II, respectively. This shows that these sites are polluted while the highest values were 1.9 and 1.8 at sites IV and III respectively, which mean moderate pollution. Furthermore, evenness index revealed that there is no evenness value at site I due to presence of *Tintinnopsis radix* only, while amounted 0.5 at the other three sites, which means equitability at those sites.

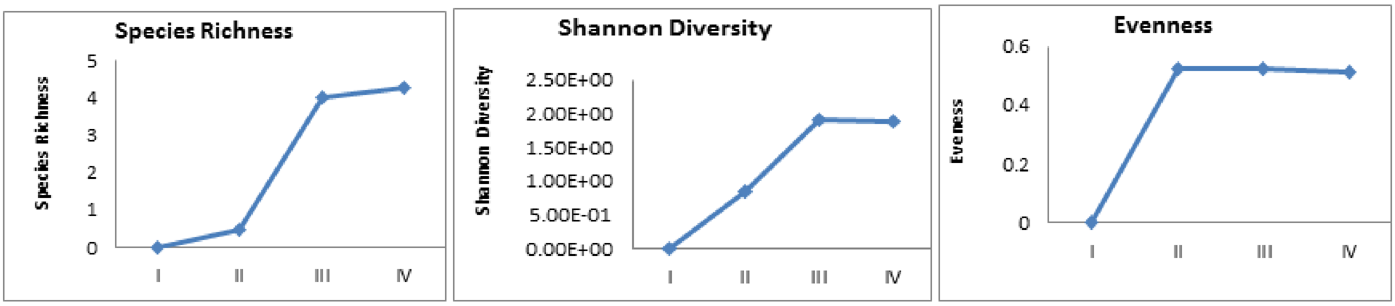

Fig 4: Spatial variations in species richness, evenness and Shannon diversity of tintinnids at Al-Max area sites during the study period from spring 2014 to winter 2015.

Conclusion

1. -Fifty three species of tintinnids were recorded during this work; they were dominated by genus *Tintinnopsis*, from them some species are considered as bio-indicator for some physico-chemical parameters as the following:

a. *Eutintinnus pingius*, *Proplectella angistior*, *Favella ehrenbergii*, *Ascampbelliiella sp*, *Helicostomella subulata*, *Tintinnopsis nudicauda*, *Favella panamensis* and *Tintinnopsis campanula* for dissolved oxygen, salinity and pH.

b. *Favella panamensis*, *Favella ehrenbergii* and *Tintinnopsis campanula* for nitrite.

c. *Favella panamensis*, *Favella ehrenbergii* and *Tintinnopsis campanula* for nitrate.

d. *Tintinnopsis nudicauda* for high temperature while all above species except *Tintinnopsis nudicauda* are considered as bio-indicator for low temperature.

2. *Tintinnopsis radix*, *Tintinnopsis campanula*, *Tintinnopsis nudicauda* and *Favella adriatica* considered as euryhaline species.

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