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Original Article

Influence of chemical parameters on *Artemia* sp. (Crustacea: Anostraca) population in Al Wathba Lake in the Abu Dhabi Emirate, UAE

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Abstract: Long term monitoring programme on Brine shrimp (*Artemia* sp.) is being carried out by the Environment Agency, Abu Dhabi, United Arab Emirates (EAD) with the prime purpose of understanding the population dynamics, ecology and habitat requirements of *Artemia* at Al Wathba Lake, situated within Al Wathba Wetland Reserve, which is an artificial wetland near Abu Dhabi City. The present study, being a component of this programme, intends to understand the influence of chemical parameters such as dissolved oxygen, nitrate, nitrite, phosphate, ammonia and total organic carbon on *Artemia* biomass and cyst production at different sites of the Al Wathba Lake. The study was carried out by sampling lake water quarterly for a period of 5 years from 2010 to 2014. The *Artemia* population was found to have direct impact of the above mentioned parameters on its abundance. The abundance was highest during the year 2010. Further, temperature, dissolved oxygen, and nitrate were found to be the most crucial parameters for production of *Artemia*. Furthermore, this study aimed to determine the significant relationship between physico-chemical parameters and *Artemia* sp. population dynamics and cyst production.

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Introduction

The brine shrimp, *Artemia* which is a micro-crustacean (Crustacea: Anostraca), are typical inhabitants of hypersaline habitats and are well-adapted to thrive in the extreme environments. Lenz (1987) observed that zooplankton dynamics are influenced by various biotic and abiotic factors of their habitat (e.g. food availability, temperature, salinity, etc.) as well as the predictability and seasonality of their environment. A monitoring study conducted to assess the influence of physico-chemical parameters on the distribution and cyst production of *Artemia franciscana* revealed a significant interaction among salinity, dissolved oxygen and nitrates on cyst production (Krishnakumar et al., 2014). Contrary to this, some studies have found no significant relationships between physico-chemical variables and *Artemia* population structure and density (e.g. Ben Naceur et al., 2011).

The aim of this study was to identify physico-chemical variables that influence the *Artemia* biomass and cyst production across the sampling sites in the Al Wathba Lake. Therefore, an attempt was made to evaluate the impact of various physico-chemical variables on the distribution and cyst production potential of *Artemia*, confined to Al Wathba Lake. Previous study showed that Artemia population inhabiting Al Wathba Lake is mostly affected by water temperature, salinity and pH (Al Dhaheri, 2004). Also, in a further study (Al Dhaheri and Saji, 2013), it was found that salinity is correlated with *Artemia* occurrences in sampling locations. In the wild, *Artemia* generally is found in salinities from 10-300 ppt (Sorgeloos, 1980), but are more rarely found in waters lower than 45 ppt and above 300 ppt. Salinity is one of the crucial factors that control growth and survival of *Artemia* (Van

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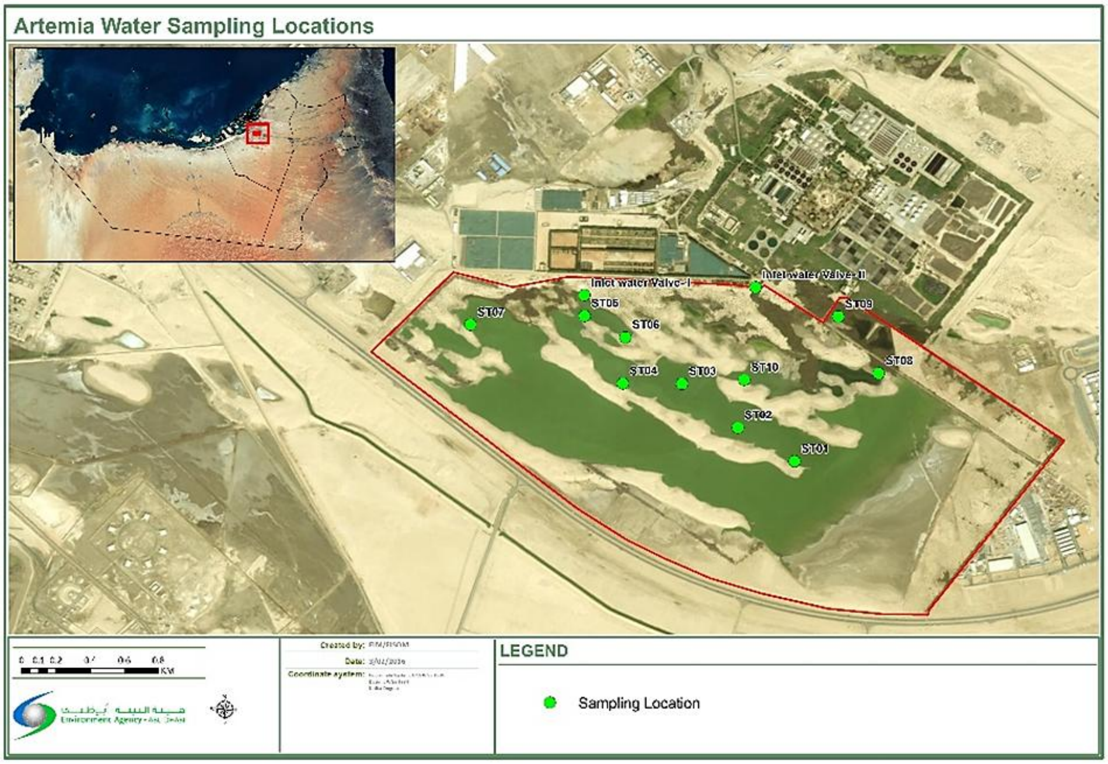

Figure 1. Map of Al Wathba Wetland Reserve with locations of sampling points

Stappen, 2002). In its natural environment, temperature, feeding and salinity are the most important factors influencing *Artemia* populations (Wear and Huslett, 1987).

Materials and Methods

Sampling site and sampling period: The present study was conducted in the Al Wathba Lake at Al Wathba Wetland Reserve (AWWR) (N: 24.25812, E: 54.60282) which is located 40 km east of Abu Dhabi City (Fig. 1). The water body extends for approximately 1.5 km in length and 0.5 km in width a maximum depth of almost two metres (Brook et al., 2004). The water in Al Wathba Lake is supplemented by brackish water from the nearby Al Wathba camel race track as well as discharge of treated sewage effluent from the adjacent Mafraq Wastewater Treatment Plant. A set of water samples from all sites was collected for analysis of the following chemical parameters: pH (*test method* APHA 4500 H^+), salinity (*test method* APHA2520 (B), dissolved O_2, Nitrate (NO_3), Nitrite (NO_2), Phosphate (PO_4), Ammonia (NH_3), Total Organic Carbon (TOC), Copper (Cu), Cadmium (Cd), and Iron (Fe). The chemical analysis was conducted at the laboratories of Arab Center for Engineering studies, Abu Dhabi, UAE (ACES).

Sampling techniques: Extensive field study was carried out for five years from 2010 to 2014. Sample collection was carried out on quarterly (Q1, Q2, Q3 and Q4) basis at day time. The sampling sites for Artemia monitoring were chosen on a 100 m grid overlaid on a geo-corrected digital satellite image of the AWWR (Al Dhaheri, 2004). Altogether, nine sampling locations were selected on the basis of their representation of the physical-chemical characteristics of Al Wathba waters. In addition to these nine sampling sites of the lake, water samples from two fresh water inlet valves was also included to understand the water quality of tertiary treated water from Al Mafraq Sewage Treatment Plant (MSTP). Water samples were collected using a horizontal sampling device (Van-dorn water sampler) deployed for surface sample collection. Temperature and pH (*in situ*) was measured using WTW handheld Multi-Parameter instrument

Table 1. Physico-chemical parameters (mean ± SD) for different sites at Al Wathba Lake.

		Temperature (°C)	pH	Salinity(mg L^{-1})	Dissolved Oxygen (mg L^{-1})
2010	March	26.83 ± 1.65	8.13 ± 0.08	121.18 ± 001.19	6.73 ± 0.39
	June	34.31 ± 1.03	7.01 ± 2.64	180.88 ± 073.25	7.92 ± 0.41
	September	35.47 ± 2.66	7.81 ± 0.49	249.08 ± 119.15	4.64 ± 2.49
	December	23.72 ± 1.34	7.62 ± 0.18	106.13 ± 002.31	4.94 ± 0.86
2011	March	25.10 ± 1.64	8.07 ± 0.26	137.28 ± 027.04	4.84 ± 2.23
	June	33.84 ± 1.69	7.86 ± 0.22	213.86 ± 054.09	4.18 ± 1.76
	September	35.75 ± 2.39	7.17 ± 0.48	295.68 ± 115.09	5.20 ± 2.24
	December	22.82 ± 1.95	7.50 ± 0.18	126.78 ± 025.28	4.87 ± 1.51
2012	March	24.02 ± 1.00	7.64 ± 0.27	194.82 ± 050.49	3.67 ± 1.27
	June	27.69 ± 1.38	7.53 ± 0.24	061.79 ± 073.78	6.67 ± 1.67
	September	35.36 ± 3.08	7.83 ± 0.25	135.08 ± 114.14	6.82 ± 1.22
	December	24.91 ± 0.41	8.05 ± 0.15	127.09 ± 022.87	7.05 ± 0.89
2013	March	24.64 ± 0.64	8.64 ± 0.19	213.66 ± 021.23	5.34 ± 1.79
	June	33.37 ± 1.76	7.99 ± 0.49	313.26 ± 029.52	4.75 ± 1.68
	September	34.96 ± 1.44	7.83 ± 0.45	272.04 ± 050.91	6.88 ± 0.84
	December	25.68 ± 2.14	7.72 ± 0.32	152.45 ± 024.14	6.27 ± 0.79
2014	March	25.68 ± 0.91	7.92 ± 0.35	124.63 ± 034.86	7.54 ± 0.34
	June	33.57 ± 1.69	8.00 ± 0.66	297.61 ± 091.37	6.56 ± 1.47
	September	35.89 ± 1.53	7.45 ± 0.37	357.06 ± 017.07	7.64 ± 0.61
	December	24.17 ± 1.99	8.14 ± 0.27	085.22 ± 019.75	7.39 ± 0.31

(Multi/350i/SET). The time of sampling and depth of each sampling site was also documented.

Counting of Artemia: *Artemia* were counted on a grid under stereo-microscope after filtration of the water samples with plankton mesh size 20 µm and euthanized with sparkling water (*Artemia* were killed by the CO_2 contained in the sparkling water). Cyst density calculated in 1 litre of the sample (total cyst/1 litre = ((total cyst/6 ml) x 1000) (Al Dhaheri, 2004).

Data Analysis: Descriptive statistics such as the mean, standard error, standard deviation, minimum and maximum values of each physico-chemical factor were calculated. One-factor analysis of variance (ANOVA) used for each parameters (temperature, salinity, dissolved oxygen, Nitrate (NO_3), Nitrite (NO_2), Phosphate (PO_4), Ammonia (NH_3), Total Organic Carbon (TOC), Copper (Cu), Cadmium (Cd), and Iron (Fe)). Pearson's correlation coefficient (r) was calculated for the statistical interpretation of the influence of the physico-chemical variables on *Artemia* abundance. All the above mentioned statistical analyses were performed using IBM SPSS statistical software version 21.0. (IBM Corp. Released 2012). Limits of significance for all critical ranges were set at $P<0.05$ two-tailed.

Results

The salinity varied during the study period and ranged between 173.61-219.47 ppt and was found to be increased with the increase of temperature. Over the whole period of the study, the lowest recorded value for salinity was in the month of June (Q2) 2012 and the highest value in the month of September (Q3) 2014, 61.79 and 357 ppt, respectively (Table 1). The overall temperature varied between 22.82-35.89°C across the years. The lowest temperature value recorded was in the month of December (Q4)

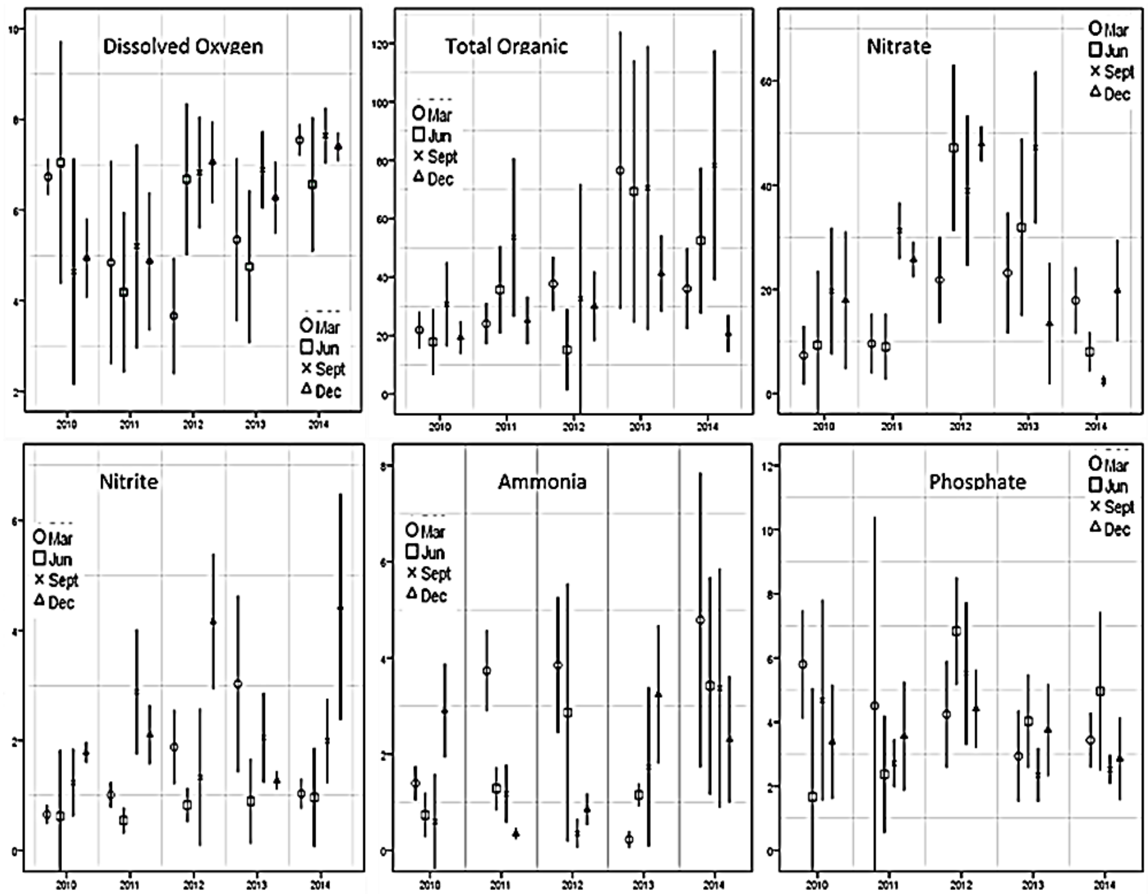

Figure 2. Mean (± SD) concentration (mg L^{-1}) values of physico-chemical parameters recorded across different months at nine sampling sites at Al Wathba Lake.

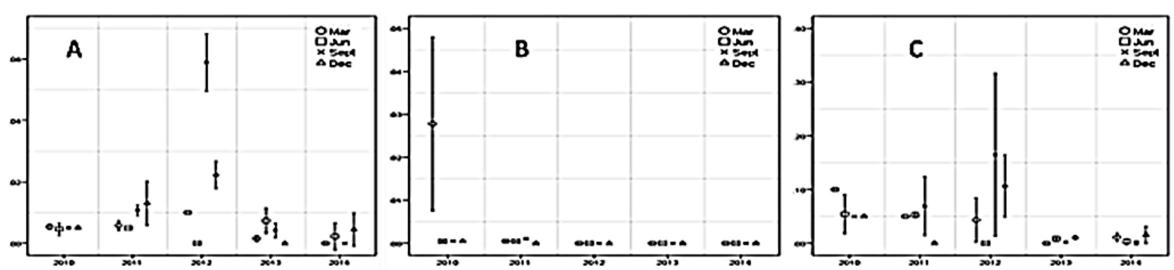

Figure 3. Mean (± SD) concentration (mg L^{-1}) values of heavy metals (A-Copper; B-Cadmium; C- Iron) recorded across different months at nine sampling sites at Al Wathba Lake.

2011 and highest value recorded in the month of September (Q3) 2014.

The surface water pH ranged between 7.0 (June (Q2)) 2010) and 8.6 (March (Q1) 2013). High seasonal variations of dissolved oxygen were observed during the month of June (Q2) 2010 and in the month of March (Q1) 2012. The average values of oxygen concentration varied from 3.6-7.9 mg L^{-1}.

The results of the nutrient analysis (Table 1 and Figs. 2 and 3) showed that the mean values of Nitrate (NO_3) ranged between 2.24 mg L^{-1} in September (Q3) 2014 and 45.13 in June (Q2) 2012. The mean value of Nitrite (NO_2) ranged between 0.19 mg L^{-1} in June (Q2) 2010 and 4.28 in December (Q4) 2014. The minimum Ammonia concentration (0.21 mg L^{-1}) was recorded in March (Q1), 2013 and the maximum (4.43 mg L^{-1}) in March (Q1) 2014. The minimum Phosphate concentration (1.31 mg L^{-1}) recorded was in June (Q2) 2010 and the maximum concentration (6.24 mg L^{-1}) in June (Q2) 2012. The mean values of Total Organic Carbon (TOC) ranged

Table 2. Correlation between *Artemia* and Cyst density and water physico-chemical parameters.

	Temperature	pH	Salinity	DO	Nitrates	Nitrites	Ammonia	Phosphates	Total Organic Carbon	Cu	Cd	Fe
Cysts	**-0.293****	-0.025	-0.063	**-0.187***	-0.099	-0.002	0.064	-0.106	-0.080	0.028	-0.025	-0.007
Juveniles	**-0.324****	0.098	-0.040	-0.100	**-0.185***	-0.048	0.012	0.007	0.072	-0.097	**0.382****	0.021
Adults	**-0.288****	0.036	-0.063	**-0.161***	**-0.199****	-0.102	0.091	0.055	-0.141	-0.052	**0.433****	0.115
Total	**-0.311****	-0.018	-0.065	**-0.193****	-0.112	-0.008	0.067	-0.102	-0.080	0.021	0.008	-0.002

Correlations is significant at the 0.05 level (2-tailed). **. Correlation is significant at the 0.01 level (2-tailed).

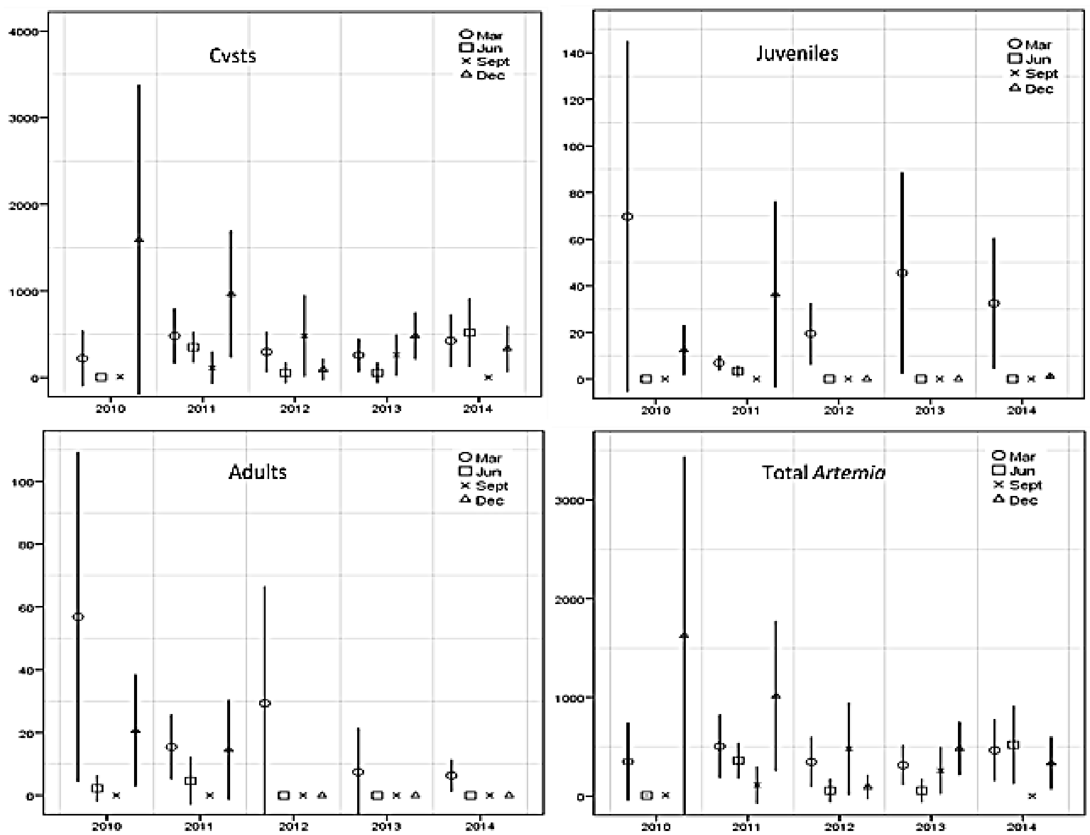

Figure 4. Fluctuation in *Artemia* cyst, juvenile and adult density during the period 2010 to 2014 at Al Wathba Lake.

between 15.85 mg L^{-1} in June (Q2) 2012 and 85.85 mg L^{-1} in September (Q3) 2014. TOC was the most frequently abundant nutrient and NO_3, NO_2, PO_4 and NH_4 were moderately recorded during the study period. The inorganic heavy metals had generally very low concentration values except for a couple times (e.g. in 2011 and 2012). Cd showed presence at a very high concentration only during March 2010, which was unexplainable as to how and why it had such high concentration value. Based on test results, Cu and Cd concentrations are not exceeding the standard limits (EHSMS, 2003). We conclude that brine shrimps are not currently at risk from heavy metals (Cu and Cd) and Iron (Fe) as the concentrations of heavy metals and Iron were recorded below the critical limit of lake water quality standards.

In Al Wathba Lake, the average *Artemia* adult density was measured between 0-64 individuals L^{-1} and juveniles between 0-78 individuals L^{-1}. The maximum *Artemia* adult and juvenile densities were observed in March (Q1) 2010. The average number of individuals collected during the study period was lowest in the months of June (Q2), September (Q3) and December (Q4) 2012, 2013 and 2014. Whereas the maximum cyst density was observed in the year 2011 (335 individuals L^{-1}) and the minimum density was observed in the year 2010 (251 individuals L^{-1}). Cyst density was highest when temperature, pH, salinity, dissolved oxygen, nitrate, nitrite, ammonia, phosphate and total organic carbon contents recorded were 29.3°C, pH 7.65, 4.77 mg L^{-1}, 16.63 mg L^{-1}, 1.51 mg L^{-1}, 1.42 mg L^{-1}, 2.73 mg L^{-1} and 38.09 mg L^{-1}, respectively. For the population composition, cysts made up a very high fraction of the samples during the month of December (Q4) 2010 (Table 2 and Fig. 4).

Overall, temperature was found to have a significantly negative effect ($P<0.01$; Table 2) on all the *Artemia* reproductive stages alike, while salinity was not found to play a significant effect on the *Artemia* stages. Similarly, dissolved oxygen also had a negative effect on the *Artemia* populations except for the juveniles, are better able to cope with fluctuations in oxygen levels due to physiological adaptions in their life cycle stage, suggesting it survives in sites where dissolved oxygen is least in concentration. The present study showed that hatching and survival of cysts occur in two periods March (Q1) and December (Q4) and maximum adult density occurred in March (Q1) in almost all sampling periods. Amongst chemicals, nitrates were the only group that affects the juveniles and adults alike negatively. Similarly, Cd was the only hard metal affecting negatively the juveniles and adults alike in the Al Wathba Lake, However, throughout the sampling years Cd concentrations were negligible but its very high concentration at the start of the sampling cannot be explained.

Discussion

Artemia population was abundant when the average concentration of salinity, temperature and pH, dissolved oxygen, nitrite, nitrate, ammonia, phosphate and total organic carbon were 121 ppt, 26.83°C, 8.13, 6.73 mg L^{-1}, 4.96 mg L^{-1}, 0.55 mg L^{-1}, 1.27 mg L^{-1}, 4.68 mg L^{-1}, 21.76 mg L^{-1}, respectively. Previous study on the influence of physico-chemical variables on the distribution of the invader *Artemia franciscana* in the salterns of Kelambakkam, Southeast coast of India showed that *Artemia* population was abundant when salinity, temperature and dissolved oxygen were from 70 to 200 ppt, 24.5 to 35.4°C and 0.8 to 6.1 mg L^{-1}, respectively (Krishnakumar et al., 2014). In the current study, it is evident that temperature, dissolved oxygen, nitrate are the most crucial parameters for production of Artemia at Al Wathba Lake.

In natural environments, temperature, feeding conditions, and salinity are important factors influencing *Artemia* populations (Wear and Haslett, 1987; Van Stappen et al., 2001; Torrentera and Dodson, 2004). In the current study, the maximum cysts were observed during the month of December (Q4) 2010 when the temperature recorded low for the whole study period. Also in a study of the correlations between environmental parameters and *Artemia* populations in Sabkhet El Adhibet in Tunisia (Ben Naceur et al., 2011), it was found that physico-chemical parameters present significant connections with the reproductive mode and offspring output. Conversely, Camargo et al. (2004) state that the variation in physico-chemical conditions of some Thalassohaline (marine) sites in the Colombian Caribbean did not influence by environmental factors, principally oxygen, salinity, and temperature. Torrentera and Dodson (2004), who studied the ecology of the brine shrimp *Artemia* in the Yucatan (Mexico) salterns, showed that the *Artemia* population dynamics and abundance are highly influenced by environmental factors, principally oxygen, salinity, and temperature. In addition, Camargo et al. (2004) reported that the reproductive experiment (mean cyst production per female) does not entirely agree with the estimated cyst production potential but may be due to a combination of certain parameters (i.e. salinity, oxygen concentration, low nitrate, and starvation of the adult *Artemia* population after reaching a high density). In the current study, the maximum *Artemia* adult and juvenile densities were observed in March

(Q1) 2010 and during this period maximum level of dissolved oxygen recorded. On the other hand, Lenz (1987) observed that zooplankton population dynamics are influenced by abiotic factors (salinity, temperature, and nutrient concentration) and by biological interactions (predation, competition, and grazers). Sorgeloos et al. (1986) reported that the best conditions for *Artemia* biomass production are at the lower salinity levels (100 ppt) and under conditions of very regular food availability.

According to a previous study (Gaevskaya, 1916; Gilchrist, 1960; Amat, 1980; Litvinenko et al., 2007), salinity and salt composition are the most important ecological characteristics affecting morphological and biometrical *Artemia* parameters. Similar study showed that different schedules in shrimp abundance and reproductive strategy correlated with habitat differences, principally oxygen, salinity and temperature (Torrentera and Dodson, 2004). The field data clearly showed lower abundance in all the populations at the highest salinities and highest peak of cyst production at the highest salinities during the dry season (Torrentera and Dodson, 2004). Studies on the combined effect of temperature and salinity on the survival of *Artemia* of various geographical origins (with two *Artemia* salina populations from Spain and Cyprus) showed that temperature is the most important factor that affected *Artemia* survival, and that the *A. salina* and *A. parthenogenetica* strains did not survive at temperature exceeding 30°C and *A. franciscana* at 34°C (Ben Naceur et al., 2009). In our study, the variation in water temperature showed a difference during the study period; the maximum surface water temperature in the lake recorded during the year 2010 was 29.9°C and the minimum salinity recorded during the year 2011 with a temperature value 27.9°C. Vanheacke and Sorgeloos (1989) reported that the maximum growth and biomass production occur in the range of 20-27°C. But the impact of temperature on growth varies from one strain to another, but for most strains, growth is limited below 15°C temperature (Ben Naceur et al., 2009). This is evident to *Artemia* population at Al Wathba Lake, the season for optimal density is confined to December-March. Triantaphyllidis et al. (1995) showed that the salinity effects on survival, maturity, growth, biometrics, and reproductive and lifespan characteristics of bisexual and a parthenogenetic population of Artemia.

Vos (1979) found nauplii growth decreases and the overall appearance of adults deteriorates with pH values below 7.0 and they concluded that the optimum pH for Artemia growth ranges from 8.0 to 8.5. Stao (1967) determined that cysts hatching efficiency was greatly compromised at pH below 8. In this study, the maximum *Artemia* adult and juvenile densities were observed in March (Q1) 2010 when the pH value observed 8.6 which is the maximum during the study period. However, several studies have been performed to estimate the various environmental and genetic components on the life span and reproductive traits of *Artemia* (Vanhaecke et al., 1984; Wear et al., 1986; Triantaphyllidis et al., 1995, Browne et al., 2002; Abatzopoulos et al., 2003; Ben Naceur et al., 2009). Most of these researchers have focused on temperature and salinity as the most important physical parameters affecting the life history of *Artemia*. The average number of individuals collected during the study period was lowest in the months of June (Q2), September (Q3) and December (Q4) 2012, 2013 and 2014 and during these period highest salinity, temperature and also the levels of other parameters like nitrate, nitrite, ammonia, phosphate and total organic carbon remained high. The cyst production showed a decreasing trend along with increase of temperature.

Generally, brine shrimp (*Artemia* spp.) have not been considered particularly sensitive to metals. Numerous researchers have demonstrated that relative to other aquatic organisms, brine shrimp range from moderately sensitive to insensitive to a wide range of metals (Brix et al., 2006). Only Cd at concentrations between 1.0 and 33 mg L^{-1} significantly suppressed growth rate and reproduction. Cadmium was recorded highest in Mach 2010 which is unusual phenomena with respect to its value as compared to rest of years.

The present study clearly indicated that Fe and Cu are not influencing the *Artemia* population due to its lower concentrations in the Al Wathba Lake (EHSMS, 2003). However, high levels of Cd, Cu, and Pb, and Fe can act as ecological toxins in aquatic and terrestrial ecosystems (Balsberg-Pahlsson, 1989; Guilizzoni, 1991; Joshi et al., 2002).

To summarize, the results indicate a strong correlation between some of the physico-chemical parameters of water and *Artemia* life cycle stages in the Al Wathba Lake. The *Artemia* population was found to be significantly negatively affected by physico-chemical parameters like temperature, dissolved oxygen and nitrate, which are the most crucial parameters for production of *Artemia*. The concentration of dissolved oxygen is not influencing the survival of juveniles but affected cyst production as its number decreases with lower dissolved oxygen.

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