DISCUSSION
DISCUSSION

In pond systems, the success of *Artemia* cyst and biomass production relies on the favourable growth of the *Artemia* population after inoculation. This growth is, amongst others, significantly influenced not only by abiotic factors (temperature, salinity etc.) but also by the availability of suitable food in culture ponds. As *Artemia* is a non-selective filter feeder they graze small food particles and their main food sources are microalgae, bacteria and organic detritus. The availability of food resources in turn also influences the fatty acid content of the on grown adult and nauplii. All these parameters if managed properly will lead to increased production of *Artemia* and high-quality cyst production.

The salinity varied between 110 and 120.5 ppt in both the ponds during Season I and in Season II it varied from 100 to 120 ppt during the present investigation. Ahmed *et al* (2000) reported a salinity variation of 85 to 180 ppt in different *Artemia* production ponds treated with different fertilizers.

Post and Youssef (1977) reported that, brine shrimp could survive in supersaturated brines of salinity 340‰. Sorgeloos (1980) reported brine shrimp was seldom found in waters of salinity lower than 45‰. He also reported that, the lowest salinity in which *Artemia* is found in nature varies from place to place and is determined by the upper salinity tolerance level of the local predator. Salinity plays an important role in the life cycle of brine shrimp.

Amat (1982) observed a direct relationship between salinity and temperature and that determined the mode of reproduction of the female population. Generally this animal releases nauplii in suitable low saline condition and promotes the formation of cysts.
Castro et al. (2000) reported a salinity of 100 – 300 g/litre at one of the Artemia pond sites in Mexico. Del Castilo (1997) recorded a salinity of 290 g/litre at La Salina site in Mexico. Torreterera and Dodson (2004) reported a salinity of 185g/litre at Celestum site in Mexico. Gajardo (1993) has reported a salinity of 102 g/litre in Salar de Sunire Artemia site of Chile. Zuniga et al (1999) has recorded 167 g/litre salinity in another Artemia site of Chile.

Abatzopoulos et al. (2006) studied Artemia populations in 17 geographical locations and reported that, salinity increase has caused a dramatic reduction of population sizes in several hypersaline settings in Iran. They also reported a salinity of 60 to 250g/l in Bakhtegan lake, 150g/l from Mighan lake and 200g/l in Qom salt lake. Rodrigues et al. (2009) reported a salinity of 22.7 PSU in the salt pan of the Gulf of Kutch, India. Abraham and Saramma (2010) reported that the work in the different stations of Thane salt pan during 1991 showed a salinity range of 36 to 324‰ in May and 35 to 172‰ during January to May. This result was akin to the results obtained in the present study where salinity ranged between 110‰ and 120.5‰ in Season I (January to May).

Artemia cyst will start to develop when the salinity of the medium drops below a certain threshold value which is strain dependent. In the present study a positive correlation was found between cyst production and salinity in season I. Generally Artemia release nauplii in suitable low saline conditions directly and if the salinity increases, the adverse condition promotes the formation of cysts (Gilchrist, 1960). A similar direct and close relation between salinity level and cyst production was observed by Ahmed et al. (2000). A similar finding and
relationship was also reported by Lenz (1987), Amat et al. (1987) and Gilchrist (1960). Sumithra (1992) in the study of Singach salterns found that when the sea water salinity of 35 ppt was pumped into the reservoir and circulated by gravity flow through a series of condenser pans and finally evaporated to obtain salt, *Artemia* could survive in 180 ppt of solar ponds.

Kuruppu and Ekaratne (1995) reported that, changes that occurred in salinities and water depth influenced the *Artemia* population size. They also noted that, in salinity ranging from 20 to 200 ppt, *A. parthenogenetica* were unable to tolerate higher salinities and survived only for a short span of time. These observations were identical to that of the present study.

*Artemia* have developed a very efficient ecological defense mechanism by their physiological adaptation to media with very high salinity, where their predators cannot survive. To achieve this they possess the best osmoregulation system known in the animal kingdom; in addition they are able to synthesize very efficient respiratory pigments or haemoglobins to cope with the low oxygen levels that prevail at high salinity. (Sorgeloos, 1980).

The maximum level of water pH of 8.3 was observed in season I in the month of June in Pond I. The same was recorded in Pond II also. In season II maximum level of water pH of 8.5 was observed in both Ponds I and II. Similar range of pH was recorded in Bhayandar (8.2) and Thane (8.3) salt pans which favoured the growth of Zooplankton community, which was reported by Mustafa et al. (1999). Tarnchalanukit and Wongrat (1987) reported that the pH range 7.0 – 7.8 was suitable for *Artemia* production. Ahmed et al. (2000) reported that the pH
range of 7.0 to 7.8 showed no significant variation among the four treatments of *Artemia* production ponds.

According to Sundararaj *et al.* (2006), pH values ranged from 7.5 to 8.9 in Vedaranyam and Kelambakkam Solar salt works. Shirodkar and Prasad (1999) reported that, pH of 7.2 was found in Goa salt works. Although in nature brine shrimps are found in neutral to alkaline waters, very little is known about the influence of pH on juveniles or adults. In their natural habitat *Artemia* are mostly found in a pH range of 7.8 to 8.2. In California, USA, some *Artemia* populations can be found in alkaline lakes, having a pH range of 9 to 10. It is important to note that the hatching efficiency of the cysts decreases when the pH drops below 8.0 (Sato, 1967). The values of pH reported by the above workers were in accordance with that recorded in the present study which supported the growth and survival of *Artemia* species.

In the present study water temperature varied between 26 and 33.5°C in the culture ponds during the two seasons. This was suitable for *Artemia* culture. This could be supported with the following references.

Persoone and Sorgeloos (1980) reported water temperature in the range from 25°C to 35°C in *Artemia* culture ponds. The maximum temperature that *Artemia* populations can tolerate has been reported to be close to 35°C. Brine shrimp from Macau (Brazil) survived for many weeks at temperatures around 40°C. (Vos and Tansutapanit, 1979). This tolerance threshold is however, strain-dependent. In Bhandyar salt pan temperature fluctuated between 23 and 44°C (Mustafa, 1995).
Castro et al. (2000) when studying the water temperature in the Mexican 
Artemia site documented a temperature of 27°C and a pH of 7.7. Torrentera and 
Dodson (2004) have reported a temperature of 29.1°C and a pH of 8.1 in the 
Celestum site of Mexico. Castro et al. (1995) has reported a temperature of 28°C 
and a pH of 8 at Los Colorados of Mexico. Castro et al (1998) has reported a 
temperature of 23.2°C and a pH of 8.9 at one of the Mexican Artemia site. Asri et 
al. (2002), studied Artemia populations and reported that, the temperature in the 
Gaav Khooni lake region in Iran ranged from 26.6 to 37.4°C.

Recent investigations point out temperature is a critical factor in 
determining the biogeography distribution and competitive ability of sexual and 
parthenogenetic Artemia (Browne et al., 1995). The suitable salinity level in most 
of Artemia production systems is greater than 80 to 120 ppt and the temperature of 
24 to 26°C is required to support a good reproduction (Hoa, 2003).

Recently, Rafael et al. (2009) have compared two pure zygogenetic strains 
of A.franciscana from a San Francisco Bay and an Artemia species from a natural 
population in Mexico. They have observed a significantly lower propagation of 
males or a significantly higher propagation of females in the presence of 
parthenogenetic individuals upon incubation below 33°C.

Ahmed et al. (2000) observed a temperature range of 23 to 28°C during the 
culture of Artemia and this offered a maximum yield with the strain from GSL 
origin which they used for inoculation. Similar observations was reported by 
Vanhaecke and Sorgeloos (1989).
There existed a significant relationship (p<0.02) between the water temperature and cyst production in pond I and pond II during two seasons of the present study. Ahmed et al. (2000) observed a direct relationship between cyst production and water temperature and the highest production of 15.63 Kg/ha was obtained from the ponds with high water temperature. It is also reported that neither temperature nor salinity directly controls the cyst production (Vanhaecke and Sorgeloos 1989), but several other influencing factors like physiological (Persoone and Sorgeloos, 1980; Lenz and Dana, 1987) and physical factors (Versichale, 1983) which are indirectly related to environmental temperature and metabolism are also equally responsible.

In the present study, dissolved oxygen varied between 4.0 ml/l and 4.6 ml/l. During the peak periods of monsoonal rains the recorded values of dissolved oxygen was higher in both the ponds. Similar values for oxygen were recorded by other authors also. Vallejo et al. (1980) reported that oxygen was found to be a critical factor for the resumption of development while temperature appeared as the second critical factor for hatching. Marichamy (1986) reported that, the dissolved oxygen content varied from 3.59 to 3.79 ml/l in Veppalodai salt pan (Tuticorin) during 1986 post summer season.

Ahmed et al. (2000) observed a dissolved oxygen (6 to 10 ppm) in the course of culture period. Gopalakrishnan et al. (1994) reported a dissolved oxygen content of 3.3 to 4 ppm in the Uppodai river in the vicinity of Tuticorin coast receiving salt pan effluents. Sujata Mishra and Panigrahya (1999) reported that, the dissolved oxygen contents ranged from 3.51 – 5.62 ml/l during 1991 and 1.22 to
5.74 ml/l during 1992 in Bahuda estuary salt lands of Orissa. No definite pattern of annual cycle was discernible with respect to dissolved oxygen. Further more, dissolved oxygen level in the summer was less than winter which was related to increasing *Artemia* biomass in summer (Ahmadi, 2005).

In the present study phosphate level varied between 0.01 and 0.02 mg/l in pond I. Similar values were noted in Pond II during the first season. During the second season there was not much difference between the two ponds and values, very similar to the first season were recorded.

Pirastru (1994), reported that, with the increase in the concentration of phosphates and manganese and the successive increase in phosphates and iron, the algal biomass increased correspondingly.

In the present study calcium was found to vary between 0.78 mg/l and 4.8 mg/l recorded during the culture period in both the ponds. The highest magnesium content recorded in the two ponds was 1.1 mg/l and lowest was 0.76 mg/l in Pond I and 0.89 mg/l in Pond II. During season II the values ranged between 0.91 and 1.1 mg/l in Pond II.

Shirodkar *et al.* (1999) reported that, the calcium of the brines ranged from 5.0 to 14.1 mg/l where as the Magnesium recorded the concentration of 8.0 to 156.5 mg/l. The lowest calcium and magnesium of 5.0 and 8.0 mg/l respectively were observed in Goa salters. Generally all the brines are enriched in Sodium, Calcium, Magnesium. Bensam and Marichamy (1975) reported that the calcium of the *Artemia* culture pond of Veppalodai salt pan, Tuticorin ranged from 0.20 to 0.01 ppm. Cole and Brown (1967) stated that, high concentrations of certain compounds like carbonate, bicarbonate and potassium were lethal to *Artemia*. 
The ranges of concentrations of Ca$^{2+}$, SO$_4^{2-}$, Mg$^{2+}$, Cl$^-$ and TDS in the northern part of the Great Salt Lake for a 30 year period were 0.2 – 0.5, 6 – 20, 3 – 10, 45 – 100 and 90 – 270 g.l$^{-1}$ respectively (Gwynn, 2002). This observation was similar to the results obtained in the present study. Average concentrations of Mg$^{2+}$ and Ca$^{2+}$ in Urmia Lake was reported to be 4.8g.l$^{-1}$ and 4.5 g.l$^{-1}$, respectively (Alipour, 2006).

The microalgae living in every solar saltwork constitute a biological system, which are able to aid or harm salt production. Systems aiding salt production maintain stable species composition and concentrations, they produce and accumulate sufficient matter to power the biota, prevent leakages (Jones et al., 1981) and also increase the solar energy absorption (Coleman and White, 1992). The microalgae produce a thick, many layered mat on the bottom of the ponds which prevents leakage of the brine. The quality and quantity of salt production in a solar salt work is determined by the hydrobiological activity (Sorgeloos et al., 1983) Dunaliella whose cells accumulate Carotenoids, when grown in high salt concentrations appear red. These coloured micro organisms increase the absorption of solar energy which raises brine temperature and enhances evaporation. Light energy absorbed is converted into heat thus increasing the heat accumulation of the water and thereby improves the salt colour in salterns (Sorgeloos, 1983).

Hence the distribution of the phyto and zooplankton in the chosen solar salt pans were studied. The following species of phytoplankton such as Rhizosolenia retgera, Oscillatoria sp., Nostoc sp., Fabrea salina, Diatoms, Nitzschia lorgissima, Dunaliella, Bacillaria paradoxa, Procentrum maximum, Cocconeis, Chlorella sp.,
Cheatoceros cibenii, Navicula sp., Spirulina sp. etc. were recorded. These belong to the following five families such as Chlorophyceae, Bacillariophceae, Cyanophyceae, Rhodophyceae and Haptophyceae. Phytoplankton biomass in the Pond I ranged between $0.13 \times 10^5$ Nos / m$^3$ and $2.9 \times 10^5$ Nos / m$^3$. In pond II it ranged between $0.12 \times 10^5$ Nos / m$^3$ and $2.9 \times 10^5$ Nos / m$^3$.

Sundaraj et al. (2006) studied the phytoplankton population in two stations namely Vedaranyam and Kelambakkam solar salt works in Tamil Nadu. They reported a total of 31 species comprising of 25 genera. The phytoplankton community consisted of Cyanophyceae, Chlorophyceae, Rhodophyceae and Bacillariophyceae. The presence of Oscillatoria salina, Xenococus sp. was reported at station I. The dominant species at station II were Xenococeus acervatus, Coccochloris elabens, Spirulina platensis and Oscillatoria salina. They also reported that a study of phytoplankton population revealed that the density and diversity at Kelambakkam were very poor when compared to Vedaranyam.

Reginald and Banu (2009) studied the distribution of phytoplankton in the crystallizer ponds in Tamaraikulam solar salt pans. They reported only two species of phytoplankton namely Coccochloris sp. and Dunaliela salina belonging to Bacillariophyta and Chlorophyta, during the four seasons studied.

Abdul Rahman (2006) studied the plankton community in the salterns of Kelambakkam, Chennai. A total of 26 species of phytoplanktons belonging to 19 genera were identified. Out of the total 26 species, 6 belong to Cyanophyceae, 3 to Chlorophyceae, 16 to Bacillariophyceae and 1 to Dianophyceae. The species identified were Coccochlins elabens, Dunaliella salina, Spirulina platensis,
Oscillatoria salina, Navicula spp., Nitzchia sp. and Chaetocerus sp. Members of Cyanophyceae were found to be the major components followed by Chlorophyceae, Bacillariophyceae and Dianophyceae.

Anh (2009) identified four phyla of algae consisting of 67 species in salt ponds in Vietnam. Among them Bacillariophyta (diatoms) were predominant (60-63%) followed by Cyanophyta (15-16%) Dinophyta (13-15%) and Chlorophyta (8-10%). Mohebbi et al. (2006) studied the composition, abundance and fluctuation of phytoplankton during different months in hypersaline Urmia lake of Iran. He reported that 10 genera belonged to Bacillariophyceae (diatoms) 2 genera to Chlorophycea (green algae) and 2 genera to Cyanophycea.

Artemia is a non selective voracious phytoplankton feeder and its density is largely determined by the availability of phytoplankton. (Abdul Rahman and Jeyalakshmi, 2009). Evagelopoulos et al. (2009) studied the phytoplankton and macrofauna communities in the Kalloni solar saltworks in Greece. They identified different species belonging to 5 classes Bacillariophyceae, Dyanophyceae, Cryptophyceae, Cyanophyceae, Dictyophyceae. Most of the species belonged to Bacillariophyceae and Dyanophyceae.

Esmaeili Dahesht et al., (2010) reported that, fourteen phytoplankton genera including Bacillariophyceae (10 genera), Chlorophyceae (2 genera) and Cyanophyceae (2 genera) were identified during the sampling period. (July 2005 – February 2006).

Nedumaran and Manokaran (2009) reported the cyanobacterial flora in some salt pans of Pudukottai District, Tamilnadu of which 11 belonged to family
Oscillatoriaceae, 6 to Chroococcaceae, 5 Nostocaceae and 3 each of Scytonemataceae, Rivulariaceae and Stigonemataceae.

The growth and multiplication of phytoplankton are primarily dependent on temperature, solar illumination and the availability of certain essential nutrients such as nitrates and phosphates (Pillai, 1986). It was observed that pH' nutrients and predominance of selective phytoplankton as food like Dunaliella salina were some of the important factors controlling the distribution of Artemia in a saltpan ecosystem (Mustafa, 1995).

Britten and Johnson (1987) found that diatoms constituted the bulk of benthic algal biomass in low salinity, but did not occur in salinities above 130 ppt. This observation is in agreement with the results of the present study where phytoplankton & zooplankton distributes was observed in salinities between 90-130 ppt. The planktonic diatoms can be used as indicators of time integrated environmental pond condition in solar saltworks (Campbel and Davis (2000)).

In laboratory experiments, several species of algae have been considered as a appropriate food for Artemia such as Phaeodactylum tricornutum and Tetraselmis suecica (Fabregas et al., 1996a, 1996b; 1998), Isochrysis sp. (Evjemo and Olsen, 1999), Chaetoceros sp. (Naegel, 1999; Toi et al., 2006); Dunaliella, Isochrysis sp. and Chaetoceros muelleri (Lora-Vilchis et al., 2004).

In the present study, the distribution of the planktonic species did not follow any definite pattern. In the monsoon season Bacillariophyceae was high while Chlorophyceae was low. In the premonsoon Rhodophyceae was found to be abundant where as in summer Chlorophyceae and Erythrophyceae dominated.
Sundararaj et al. (2006), reported about the plankton biomass in two typical solar saltworks of Tamilnadu namely Vedaranyam & Kelambakkam. The total no. of cells /ml reported by them varied from 1060-2230 in the two different season studied. In general, the peak values were recorded during November & December and lowest values in March & August in all the stations studied. The higher concentration of planktons \(2.6 \times 10^5\) no/m3 were observed during March and April. This was higher than all the previously reported data and the reason may be due to the frequent application of urea used as dressing during the course of the study. The smallest average density of phytoplankton 97249 L\(^{-1}\) was observed in December 2005 and the greatest average density of 481983 L\(^{-1}\) was registered in August 2005. In this *Dunaliella sp.* composed 92.1% of the lake’s phytoplankton. Statistical analysis of phytoplankton fluctuations showed a significant difference among different months (\(P<0.05\)) in North West of Iran. (Esmaelli Dashesht et al., 2010).

Davis (1980) also reported that decrease in *Artemia* population when the phytoplankton population reached its maximum may be related to the appearance of predators and competitors like amphipods, copepods and the presence of migratory birds including flamingos. The same condition was also observed in the present study during summer. But during the monsoon the phytoplankton was found to decrease when the *Artemia* population was at the highest level.

Even though the algal blooms are beneficial to salt farms their decomposed products act as chemical traps and finally present as early precipitate of gypsum. This contaminates the NaCl in the crystallizer and thus the salt quality is reduced
(Sorgeloos, 1986). The hazardous effect of phytoplankton blooming on quantity and quality of salt are also well documented. (Tackaert and Sorgeloos, 1992).

But the present study to revealed that the saltworks chosen for the study can be grouped as a biologically managed one. Even though the zooplankton and microalgae were encountered, their blooming was checked by the grazing of *Artemia* which multiplied in large numbers. This helped in the production of quality salt which is substantiated when the quality of the salt was compared with that of the previous year (when *Artemia* was not cultured). This is supported by Sorgeloos and Tackaert (1990) who offered a following typical standard to be adopted by the developing countries with regard to salt purity: 99.5% for Grade 1 industrial salt, 98.5% for Grade II 96% for edible common salt and 97% for table salt.

In the present study Zooplankton count was recorded in both the ponds. During the present investigation in both the experimental ponds the documented count of Zooplankton ranged from $0.06 \times 10^5$ Nos/m$^3$ to $1.4 \times 10^5$ Nos/m$^3$ in Season I. The same was recorded in Season II also. The Zooplankton comprised of *Holoplankton, Meroplankton, Protozoans, Coelenterate, Arthropoda* and *Chaetognatha*.

Similar occurrence and dominance was observed by Abdul Rahman (2006) who reported a total of 12 Zooplankton groups from Kelambakkam area. The species identified were *Favella sp.*, *Brachionous sp. Medusa, Polychaete, calanoid copepods and larvae of molluscs*. More number of species were identified during presummer, early summer and post summer seasons. In the present study,
similar reports of Zooplankton abundance was noted in premonsoon and summer seasons. These observations corroborated the fact that the occurrence of zooplankton and phytoplankton has a telling effect on *Artemia* growth and production.

In practice, the success of culture relies on the favourable growth of the *Artemia* population following inoculation as it is influenced not only by abiotic factors (temperature and salinity) but also by the availability of suitable food in the culture ponds. (Baert *et al*., 1996; Hoa *et al*., 2007). As *Artemia* is a non-selective filter feeder, they graze small food particles ranging in size from 1 to 50µm and their main food sources are microalgae, bacteria and organic detritus (Van Stappen, 1996a; Fernandez, 2001; Dhont and Sorgeloos, 2002).

Bacteria may range from obligate aerobes to photoautotrophs to anaerobic sulfate – reducers bacteria. So, despite the high salt concentrations, microbial diversity is common to solar salt works even in the crystallizers (Litchfield *et al*., 1998). Given the apparently harsh environment of hypersaline lakes and salterns, it may seem surprising that a diverse and interesting group, including both prokaryotic and eukaryotic microorganisms, could form complex functioning communities in these waters. The survival strategies for these microorganisms required biochemical adaptations to their proteins, lipids and nucleic acids.

Halophilic archael proteins have been modified to contain high concentrations of acidic amino acids ie. glutamic acid and aspartic acid (Lanyi, 1974). The proteins of bacteria growing in hypersaline environments also tend to have higher concentrations of the acidic amino acids than similar types of proteins from nonsalt – tolerant eubacteria. (Qua *et al*., 1981)
Oren et al. (1996) studied the microbial population from the salterns in Eilat, Israel, Santa pola, Spain and Coastal Australia (Anton et al., 1999, 2000, Burns et al., 2004) and recorded 1.3 x 10^7, 1 x 10^7 to 5 x 10^7 cells/ml respectively, while hyper saline environment in Delta del Ebro (Spain) harbored upto 10^8 cells/ml (Guiza – Boixareu et al., 1996) and solar salterns in La Palma (Canary Islands Spain) contained more than 10^8 cells/ml (Ochsenreiter et al., 2002). These high numbers of cells in many hypersaline environments are attributed to lack of predation and high nutrient levels (Oren, 2002). The low levels of cells could be related to the organic matter content in the feeding water as expected, much lower in spring water than in concentrated coastal marine waters.

Ragavan and Furato (2004) reported a relatively low average count of 7 to 5 x 10^3 of extreme halophiles in offshore sediments. Pigmented halophilic archaea and micro algae absorb light energy in saltern ponds, thereby raising the water temperature increasing the rate of evaporation and hastening the deposition of salt.

In the present study the microbial species identified were Vibrio sp. and Micrococcus sp in the different ponds. Garcia et al. (1987) isolated a total of 54 moderately halophytic Vibrios from several salterns located in different areas of Spain. Litchfield et al. (2009) reported that the greatest diversity occurred at the inlet and lower salinity ponds as the salt is known to select for halo tolerant and halophilic microbes. They opined that the solar salterns, world wide are an excellent reservoir of untapped microbial diversity which must be preserved.
Dripolin (2009) studied the microbial population in the Urani salt pan in Tuticorin. She reported maximum of \(8 \times 10^5\) cfu/ml in water and \(11 \times 10^5\) cfu/gm in the sediments samples collected. She identified *Vibrio sp.* and *Micrococcus sp.* in the different ponds. Lenin *et al.* (2006) studied the microbial diversity in Maras salterns and reported that the total count per ml in the ponds were \(2 \times 10^6\) to \(3 \times 10^6\) cells / ml. They also reported the *Halo bacterium, Pseudomonas sp* and Strain GSB P66, P65 etc.

Cyst diameter is an important parameter to characterize *Artemia* for aquaculture use. In fact cyst biometry assists in the determination of number of cysts / g. Generally 1g from strains that produce small cysts contain more cysts/g, thus usually can produce more nauplii / g (Camargo *et al.*, 2005).

In the present study the cyst diameter ranged from 0.2 to 0.31 mm in the two ponds. Castro *et al.* (2006a) has reported the cyst and nauplii characteristics of *A.fransiscana* populations in different sites of Mexico. They recorded, the cyst diameter of 0.21 - 0.27 mm and a nauplii size of 0.37 – 0.42 mm in the 13 different sites studied. They also reported a cyst diameter of \(2.26 \pm .91\) mm in *A.franciscana* species of nauplii size of 4.51 mm. They studied the species in different sites of Chile. The nauplii size of 4.13 mm and the cyst diameter of 2.54 mm were recorded in *A.persimilis* species in the same site. These observations were similar to the report of the present work.

Naceur *et al.* (2011) documented a cyst diameter of 246.8 µm in the Sabkhet Boujmal cysts. Similar reports on cyst size (249.8 µm) were recorded for
Artemia franciscana from Salina Cero (Camargo et al., 2005) and from Great Salt lake Utah (Sorgeloos et al., 1986).

The nutritional effectiveness of a food organism is in the first place determined by its ingestibility and as a consequence by its size and form (Agh and Sorgeloos, 2005). Naupliar length was different from one geographical source of Artemia to another. Vanhaecke and Sorgeloos (1980) studied the nauplii size of 11 Artemia strains and they reported a variation of this criterion from 429.0 to 517 µm.

In the present study the recorded mean length of the nauplii was 2.22 mm (222 µm). Naceur et al. (2008) reported a nauplii length of 236.7 µm from Sahline salt work while Van Baller et al. (1987) documented a size of 258.8 and 251.6 µm from Megrine and Betralta salt works respectively. Naceur et al. (2011) reported a naupliar length of 457.5 µm from Sabkhet Boujmal.

Triantaphyllidis et al. (1996) has reported a cyst diameter of 0.23 mm and an average nauplii length of 4.3 mm. The cyst weight was 3.4 µg and diameter ranged from 0.2 to 0.3 mm. The length of the nauplii was measured as 2.22 mm and its weight was 1.82 µg. Castro and Gajardo (2006) proposed that in Mexico Artemia strain the average cyst diameter was 237 ± 14 µm, while the average nauplii length was 431 ± 23 µm.

Leger et al. (1986) mentioned that the adequate size of nauplii (<400 µm) to feed fish larvae will solve feeding and ingestion problems and will allow for a better selection of the cysts to be used in diets in which live feed is used for fish species.
Legar and Sorgeloos (1992) further reported the naupliar size of 223 µm obtained from the cysts proves that it is highly feasible to use these for feeding fish fry and crustaceans. The cyst size of 200 µm is also a suitable feed. This is similar to the Mexican nauplii studied by Castro et al. (2006 a, b) and Artemia franciscana species located throughout the American continent (Vanhaecke and Sorgeloos, 1980, Alvarez and Sanchez, 1994; Castro et al., 2000).

Christopher et al. (2005) studied the Artemia population found in the salt pan of Tamarajkulam for its hatching characteristics. He reported a hatching percentage of 69.2, hatching efficiency of 230600 nauplii /g of cyst, a hatching output of 661mg/g and a hatching rate in which T₀ was 18 and T₁₀ was 24. Vanhaecke and Sorgeloos (1989) reported the hatching efficiency of 60000 nauplii /g cyst in his work in Canada. A hatching efficiency of 20480 and 203590 nauplii /cyst a hatching percentage of 80.5 – 86.9, a hatching output of 369714 – 375811 mg/g and a hatching rate T₀ – 16 and T₁₀ – 24 has been reported in the present study.

The biochemical composition of Artemia sp., in particular fatty acid profiles of cysts and nauplii has been extensively studied because of the use of nauplii as live food in aquaculture. (Bengtson et al., 1991). Lian et al. (2003) has revealed that the fatty acid compositions in terms of 20:5 (n-3) eicosapentaenoic acid, EPA 22:6 (n-3) (docosahexaenoic acid, DHA), total (n-3) HUFA and total fatty acid methyl ester (FAME) of dried cysts and brine cysts, are higher than those in Artemia nauplii and moina. Naceur et al. (2011) observed the fatty acid composition of Artemia salina cysts collected in Sabkhat Borjmal, Tunisia. The
predominant fatty acids were palmitic acid (4.67%) palmitoleic acid (3.45%) oleic acid (6.46%) and low quantities of DHA, ARA, EPA were observed.

Naceur et al. (2010) analysed the fatty acid profile of cysts of invasive Artemia franciscana in Tunisia and observed low levels of linoleic acid and DHA and high levels of EPA. It is known that the fatty acid composition of Artemia nauplii can vary among strains and also from one batch to another within the same strain (Leger et al. 1986). This led Watanabe et al. (1978) to classify Artemia into two groups: freshwater Artemia, with n-3 unsaturated fatty acids such as linoleic acid (18 : 3n – 3) but lacking eicosapentanoic acid (20:5 n-3) producing good survival and growth among freshwater animals and marine type Artemia whose lipids contain 20 : 5n – 3 (EPA) thereby making them suitable for feeding marine animals.

The inoculated GSL cysts showed a high content eicosapentanoic acid (EPA) (20 : 5) and docosahexanoic acid DHA, (22 : 6n – 3) and arachidonic acid (ARA 20 : 4n – 6). The harvested cysts revealed a high composition of Hexadecanoic acid, Tetradecanoic acid, oleic acid and octadecanoic acid.

Based on the above said classification Artemia cysts harvested in the present study can be considered as freshwater type, thus suitable for feeding freshwater animals. The comparison of the fatty acid profile of the harvested F1 cysts and inoculated GSL cysts showed a change in the composition. The F1 cyst revealed a high concentration of glycerine, but lacked EPA and ARA, whereas GSL showed a high level of Eicosapentanoic acid. The difference in the fatty acids profile established, in this work can be considered as an adaptation of this species.
in their new environment. The same was reported by Naceur et al. (2010) where he found a difference in Fatty acid profile between the invasive *Artemia franciscana* from HM and those reported in the bibliography. Several authors reported that Fatty acid composition of *Artemia* is considered to be more environmentally than genetically determined and that *Artemia* adults and the cysts they produce strongly reflect the fatty acid profile of their diet (Bengtson *et al*., 1991; Navarro *et al*., 1993). In fact some fatty acids are preferentially associated with certain classes of algae, and can therefore be useful as biomarkers for these algal classes, whose diversity depends on the characteristics of the medium. (Ruiz *et al*., 2007). However Navarro and Amat (1992) brought attention to a possible genotypic influence on the fatty acid profile of *Artemia* given the presence and proportion of some fatty acids in cysts irrespective of dietary levels available to parental population.

In the present study the fatty acids profile of the adult *Artemia* was also studied. This showed a profile different from the cysts produced by them in the F1 generation. So the Fatty acid content in the F1 cysts could be influenced both environmentally by the availability of algae for the parent and also due to genotypic changes in the offspring. This can be substantiated by the fact that the DNA sequencing also revealed a change in the F1 cysts compared to adult and the inoculated cysts (GSL).

During the present investigation, production of *Artemia* cysts and biomass were recorded for every season. Production of 3.844 Kg/ha of cysts was recorded in Season I in both the ponds and 2.246 Kg/ha in Season II. In the F1 generation the
production was 5.98 Kg/ha. Various studies have been taken up to improve the quality and quantity of *Artemia* cysts and biomass production.

The increasing demand of good quality cysts and adequate sized nauplii to serve as feed for the first life stages of cultured species has led several countries to search for wild *Artemia* populations or to perform inoculation programmes at diverse sites (Camara, 2004; Dhont and Sorgeloos, 2002).

Ahmed *et al.* (2000) investigated the effects of various fertilization treatments on the production performance of *Artemia* cyst and biomass. They found out that there was significant higher production of *Artemia* cyst (15.23 Kg/ha) and a biomass of 196.10 Kg/has was obtained when the ponds were treated with urea (25 Kg) and 250 Kg of chicken manure.

During the present investigation a positive correlation was observed to prevail between cyst production and salinity during the first season. A similar direct and close relationship between salinity and cyst production was observed by Ahmed *et al.* (2000). A similar finding and relationship was also reported by Lenz (1987), Amat *et al.* (1987) and Gilchrist (1960).

Sultana *et al.* (2011) has recorded a cyst production of 1.68 to 3.58 Kg/ha in the three ponds studied in the coastal area of Ghorabari, Pakistan. They suggested that in addition to temperature and salinity, the most important and critical parameter for the cyst production was the quantity and quality of micro algal species which exert effect on the length of life cycle and number of cyst in the brood pouch which in turn determines the cyst yield.
Ovoviviparous reproduction is mostly dominant at low salinity levels, whereas cysts mostly are produced at salinity beyond 150 ppt (Sorgeloos, 1980). Dutrieu (1968) and Okazaki and Hedgecock (1987) reported that, hypoxia might trigger cyst production, but the studies by Vanden et al. (1978), Heip et al. (1978) and Lavens and Sorgeloos (1987) revealed that the specific haemoglobin alone induced cyst production in *Artemia*.

It has been reported that the production of cysts and biomass is directly related to the environmental temperature and salinity of the culture media. (Ahmadi, 2005) and these two factors are also said to be responsible for the quality of the product also. Royan et al. (1990) reported that, the biomass production amounted to 6.3 Kg/m$^3$ in the GSL strain; 3.78 and 4.35 Kg/m$^3$ in the Indian and Sri Lankan strains respectively.

It was further observed in the present study, that there was no significant variation when the cyst production was compared in both seasons. Similarly, there was no significant variation in the biomass of *Artemia* produced during the two seasons. This shows that the seasonal changes of temperature and salinity did not influence either the production of cyst or biomass. So the conditions prevailing in Tuticorin salterns favour the production of biomass and cyst throughout the year. In the present investigation the factors like dissolved oxygen (4 to 4.6 ml/l), pH (7.1 to 8.5), temperature (26 to 33.5°C) and salinity (110 ppt to 125 ppt) were found suitable for *Artemia* production as described by Tarnchalancikit and Wongrat (1987). So the present environmental conditions of *Artemia* production ponds in salt – *artemia* culture system can be considered as suitable for production of *Artemia* cysts and biomass.
Artemia has a general tendency to form and release cysts with the increase in salinity level and worsening environmental conditions. But release of nauplii in high saline environment upto 150 ppt as observed in the present study is also not unlikely. As reported by Olenykcova and Pleskacheuskaya (1979) fully grown Artemia could have both oviparous and ovoviviparous mode of reproduction. So the increased amount of biomass as well as cyst production with the consequent increase in salinity level in the production ponds as observed in the present study was not unlikely as also reported by Ahmed et al. (2000).

To establish the quality and marketing price of Artemia, the quality and size of cyst and nauplii are taken into consideration. This has, together with the hatching and nutritional qualities, provide a higher or lower demand of the production. (Anh, 2009).

In the present investigation it has been proved that the quality of cyst and nauplii produced are high based on the above said parameters. Further the environmental conditions prevailing in Tuticorin salterns make the area suitable for Artemia culture. Further the availability of cysts throughout the year during the four seasons (Summer, Premonsoon, Monsoon and Postmonsoon) is another plus point. So the production of Artemia biomass and cysts can be taken up as a profitable enterprise along with salt production. Further the performance of GSL strain has been found suitable under climatic / environmental conditions and has good potential for commercial production in the coastal areas of Tuticorin.

The inoculated GSL strain was also well adapted to the local environmental conditions. Vos and Tansutapanit (1979) showed that Artemia may adapt to higher
temperatures with every following generation under the same circumstances. Adaptation of a newly inoculated strain may result in phenotypical and genotypical variations in the existing stocks, eventually yielding a new *Artemia* genotype (Vanhacke and Sorgeloos, 1989). This was substantiated in the present study where there was a genetic variation among the cysts of original USA strain (GSL) of *A. franciscana* and its offsprings – adult and F1 cyst were analysed by comparing mtDNA analysis using RAPD and RFLP profiling. Moreover, the 16SrRNA has also been partially sequenced to analyze their homology with the stored sequences in NCBI. The BLAST searches done also show how it differs from other strains like *Artemia franciscana* strain TCN, *Artemia franciscana* strain VDM, and *Artemia franciscana* strain TKM. There were differences between the DNA sequences obtained in the present study and those stored. Based on this they were submitted to GENBANK and were successfully accepted by NCBI and are assigned Accession Number JN572920, JN572921, JN572922.

This shows the emergence of a new strain (*Artemia* sp. DR 2011) in the F1 generation adapted to the conditions prevailing in Tuticorin salterns when compared to the inoculated GSL strain. This may be due to mutations due to conditional stress, since our geographical and climate conditions vary widely from USA. Another possibility for genetic variability can also be attributed to hybridization of the original GSL strain with the local or indigenous Tuticorin strain, since the birds act as carriers of cysts from place to place or different geographical locations.