Do environmental factors influence the morphotypes in *Artemia franciscana* Kellogg, 1906 (Crustacea: Anostraca)?

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Abstract

The present study investigates the impact of environmental factors on the occurrence of morphotypes in *Artemia franciscana* (Article 4; ICZN, 2000) encountered from different salterns of Tamilnadu, southeast coast India. Samplings of brine shrimp were done at four different sites (Kelambakkam, Vedaranyam, Tuticorin and Nagarcoil) on a seasonal basis from December, 2014 to August, 2016. Variations of physicochemical factors (temperature, pH, salinity, DO, nitrate, nitrite, ammonia, phosphate and silicate) were recorded in water samples at all the sampling sites. Intensive survey indicated the occurrence of morphotypes M1, M3, M4 in males and F1, F2, F3 in females from all sampling sites. Principal component analysis, Pearson's correlation and regression analysis indicated that all physicochemical factors are not significantly correlated with the occurrence of morphotypes. From these studies, it is evident that morphotypes are not seemed to be influenced by physicochemical factors prevailing at the salterns. However, the variation in water temperature seems to influence the abundance of morphotypes M1 and M4 in males and F1 and F2 in females from all the sampling sites, which was further confirmed by cluster analysis.

Keywords Brine shrimp · *Artemia* · Morphotypes · Salterns

1 Introduction

Artemia is a hypersaline crustacean which inhabits in salterns with salinity ranging from 80 to 340 g L⁻¹ [1]. Environmental impact on growth and reproduction has been previously reported in *Artemia* populations [2–4]. Several studies have focused on salinity, temperature, pH, DO and also other environmental factors on the metabolic and physiological changes in the aquatic organisms [5]. Increasing temperature can increase higher level of salinity which had shown variations in abundance and size differences in the *Artemia* populations [4, 6, 7].

Furthermore, environmental factors also shown to play an important role in *Artemia* populations for their evolutionary adaptation to the newer habitats [8, 9]. These environmental factors may influence the distribution patterns of *Artemia* all over the world except Antarctica [10]. The

genus *Artemia* has composed of complex bisexual as well as parthenogenetic populations. Taking into the account of earlier studies, more bisexual species have been documented in all hypersaline environments around the world. These studies reported different phenotypes in *Artemia* based on their morphological and morphometric characters [11–14]. These phenotypic variations among *Artemia* populations, also reported as strain, confined to their locality. These phenotypes were also genetically confirmed by Maniatsi et al. [15, 16] using second and third intron gene (p26) in new world species.

Recent survey on *Artemia* diversity indicated the complete extinction of native population *A. parthenogenetica* due to introduction of the exotic *A. franciscana* in salterns of South India. Therefore, *A. parthenogenetica* dominated in all the salterns of South India until the late 1990s has been replaced by *A. franciscana* [17, 18]. The occurrence

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of the invasive species in the salterns of Kelambakkam (Tamil Nadu, India) and also other salterns in South India has been genetically proven as *A. franciscana* [17–19]. This population has shown different morphotypes (M1, M2, M3, M4 in males and F1, F2, F3 in females) as evidenced through morphological characters of the population encountered in Kelambakkam saltern, Tamilnadu [20]. However, morphotype M2 reported by Krishnakumar and Munuswamy [20] was not encountered during our sampling in all salterns. Interestingly, the earlier report clearly documented the existence of all morphotypes in the same saltern of Kelambakkam, irrespective of seasonality [20].

Therefore, the present study was made to augment whether the environmental factors influence various morphotypes in *A. franciscana* population colonized in various salterns of southeast coast of India.

2 Materials and methods

2.1 Collection sites

For this study, four different salterns were selected such as Kelambakkam (12°45'26.62"N; 80°12'43.80"S), Vedaranyam (10°20'108"N; 079°50'191"E), Tuticorin (08°58'343"N; 078°12'132"E) and Nagarcoil (08°58'227 62"N; 78°11'326"E). Field samplings were carried out on the seasonal basis from December 2014 to August 2016. According to the heterogeneity of niche, three samples were collected within a single (pond) saltern during each field visit. The study period was divided into four seasons, viz summer (April–June); premonsoon (July–September); monsoon (October–December) and postmonsoon (January–March). The surface water samples were collected seasonally from all sampling areas (station 1 to 4) during the study period.

2.2 Physiochemical analysis

For this analysis, water samples were collected in polyethylene containers in volumes of 1L under the water surface about 20–30 cm after punning the container with water samples; the samples were kept in a refrigerator at –20 °C, while water samples for dissolved oxygen (DO) were collected in sterile dark Winkler bottles using the modified methodology of [21]. Air and water temperatures were measured in the field with a mercury thermometer (0–100 °C) graduated up to .1 intervals, at the depth of 20 cm. The pH values were measured directly in the field by using a portable pH meter as well as properly calibrated digital pH meter [ECPHTUTOR-S, (Accuracy ± .01)] in the laboratory. Other water quality parameters (nitrate, nitrite, ammonia, phosphate and

silicate) were determined separately for all the samples using a spectrophotometer (Model UV 160A, Shimadzu, Japan) by following standard methods [22].

2.3 Assessment of different morphotypes in *Artemia franciscana*

Artemia sp. was collected using a net with 200-μm-size mesh. For morphological study, the samples were fixed in 4% neutral buffer formalin (NBF). Morphotypes were separated based on primary and secondary sexual characters, such as the structure of the second antennae in males and shape ovisac in females as described by Krishnakumar and Munuswamy; [20] after fixation, the samples were counted on a grid under stereo-microscope (Leica RSM-9). As many as 600 individuals from each sampling site collected during various seasons were observed to study the distribution patterns of morphotypes. Observation was done with as many as three times to confirm the distribution patterns throughout the sampling periods of *Artemia* (A total number of 9600 individual morphotypes were counted) and classified with respective morphotypes.

2.4 Culturing of *Artemia* sp

Based on the field observations, *Artemia* cysts were collected from different sites of Tamil Nadu. These cysts were hatched out with standard hatching conditions following the methodology of Sorgeloos [23]. Accurately, 1 gL⁻¹ of cysts were incubated (24 h) in autoclaved seawater (adjusted psu 35, and temperature 25 ± 1 °C) under optimum light intensity (1000 lux) with strong aerations. After hatching, these nauplii were transferred into the fiber-reinforced culture tanks (3000 L volume, 120 psu salinity and 32.33 ± 1.86 °C temperature). Initially, 35 psu was used and gradually it was increased to 120 psu with commercial salts under mild aeration condition in a 12D:12L photoperiodic condition. Before inoculation of *Artemia*, saline water was treated with chlorine and then neutralized with sodium thiosulphate to eliminate the chlorine effect in the culture tanks. Initially, stocking density was maintained at 100 nauplii/liter and fed with *Chlorella vulgaris* (3.4 × 10⁴ cells/mL) as a larval feed [23]. The physical parameters like salinity, temperature and pH of the water samples of the culture tanks were estimated. After the 28th day, nauplii were removed once in every 3 days and adults only were maintained in the culture. At the end of the 60th day of culture, the density of each morphotype was determined. The laboratory culture experiment was carried out three times, and the obtained morphotypes were counted.

2.5 Statistical analysis

Data on morphotypes occurrence and different physico-chemical factors were subjected to principal components analysis (PCA) and cluster analysis using Ward's method [24]. In addition, results obtained with environmental factors versus occurrence of morphotypes were subjected to Pearson's correlation and regression analysis was performed with SPSS (Ver. 21) for Windows.

3 Results

3.1 Physicochemical factors of the salterns

Data obtained on physicochemical factors recorded at different stations showed significant variation (Table 1). Results indicated highest values of air and water temperature, pH and salinity during summer at all sampling sites. However, the dissolved oxygen was relatively lower during summer and higher during postmonsoon. With reference to chemical factors, maximum level of ammonia and nitrate were recorded during monsoon and minimum during summer. In contrast, maximum values of nitrite, phosphate and silicate were recorded during summer and lowest during monsoon and premonsoon.

3.2 Description of morphotypes

Morphotypes are categorized into six types such as M1, M3, M4 in males and F1, F2, F3 in females. In male morphotype, M1 hind antennae are pot-shaped, curved basal width and pointed tip region. Whereas, the M3 has a straight and pointed tip region with valley-shaped basal width. M4 resembled that of M1 males, but without a pointed tip region and U-shaped basal width. In females, F1 ovisac is laterally triangular and pointed with spines. F2 ovisac is umbrella-shaped without spines, and F3 ovisac is convex in shape without spines. Morphological studies performed with male and female populations from all the sampling sites showed similar structure and shapes. Samples collected from the sampling sites showed that M1 and M4 in males and F1, F2 in females were abundant in all the four seasons during the study. Maximum occurrence of M1 and M4 morphotypes was recorded during postmonsoon and summer at all the sampling sites. In female morphotypes, F1 and F2 were recorded maximum during postmonsoon and summer at sampling sites 1, 3 and 4. The minimum occurrence of F3 in female and M3 in male was recorded at all sampling sites during premonsoon and summer period (Figs. 1, 2).

3.3 Statistical interpretation on morphotypes versus environmental factors

Principal components analysis (PCA) was performed for six different morphotypes, and ten environmental factors indicated that at sampling site I, the component 1 of the PCA explained 50.61% (eigenvalue 5.956) of the variance (water temperature) positively correlated with all the morphotypes (M1, M4 in male and F1, F2 in female). Component 2 explained 26.62% (eigenvalue 2.893) of the variance (air temperature); it was positively correlated with F3 and M3 morphotype (Fig. 3). In sampling site II, the component 1 explained 58.15% (eigenvalue 6.412) of variance (air temperature) positively correlated with morphotypes M1, M4 and F1, F3. The component 2 explained 31.44% (eigenvalue 4.109) of variance (water temperature) and positive correlation with M3, F2 (Fig. 4). At sampling site III, component 1 explained 47.71% (eigenvalue 6.164) morphotypes M3, M4 and F3 positively correlated with water temperature. Component 2 explained 40.73% (eigenvalue 4.41) of morphotype M1, F1 and F2 was positively correlated with air temperature (Fig. 5). In sampling site IV, component 1 explained 48.8% (eigenvalue 6.267) of morphotypes (M1, M3, M4 and F1, F2, F3) positively correlated with air and water temperatures. Component 2 explained 29.5% (eigenvalue 3.389) of chemical factors (nitrate, nitrite, ammonia and phosphate, silicate) and negatively correlated with all the morphotypes abundance (Fig. 6). Based on PCA data, chemical factors were negatively correlated with morphotypes abundance in all the sampling sites. Morphotypes abundance was positively influenced by air and water temperature and negatively with other environmental factors. Further, permutation test carried out on morphotypes occurrence with various environment factors indicated 42.75% and 64.84% of the total variance in site I and II, respectively. Similarly, 60.63% and 49.6% in site III and IV, respectively ($p < .05$; Monte Carlo permutation test).

Karl Pearson's correlation indicated that most of environmental factors are not significantly correlated with the type of morphotypes for details refer to Table 2.

Regression analysis ($n = 32$) on relationship between the morphotype occurrence and physicochemical factors showed a positive relationship with physical factors (temperature, pH, salinity and DO) and, however, negative relationships with chemical factors such as nitrate, nitrite, ammonia, phosphate and silicate (Table 3). When considering the seasonal surveys separately ($n = 8$), most of the preceding relationships were not significant. Particularly, silicate had most negative relationship. In contrast, in some cases, salinity and pH had a negative relationship (Tuticorin and Nagarcoil). Dissolved oxygen showed negative relationship with Vedaranyam and

Table 1 Variations in the physicochemical variates of surface water at sampling sites of Tamil Nadu

Sampling sites		Site I	Site II	Site III	Site IV
Parameters		Kelambakkam	Vedaranyam	Tuticorin	Nagarcoil
Air temperature (°C)	Min	29.6	28.67	29.67	30.17
	Max	32.66	34.33	33.33	32.33
	Range	3.06	5.67	3.67	2.17
	Mean ± SD	30.94 ± 1.03	31.10 ± 1.87	31.17 ± 1.34	31.15 ± .72
Water temperature (°C)	Min	29.33	29.67	30.33	29.67
	Max	33.00	33.33	33.67	32.83
	Range	3.67	3.67	3.33	3.17
	Mean ± SD	31.16 ± 1.03	31.42 ± 1.16	31.58 ± 1.16	31.06 ± 1.22
Salinity(psu)	Min	114.67	102.67	115.67	103.33
	Max	159.67	160.67	162.33	153.67
	Range	45.00	58.00	46.67	50.33
	Mean ± SD	138.08 ± 17.01	137.42 ± 21.74	142.46 ± 14.79	130.71 ± 17.67
pH	Min	8.00	7.97	7.93	7.97
	Max	8.63	8.67	8.67	8.97
	Range	.63	.70	.73	1.00
	Mean ± SD	8.26 ± .23	8.25 ± .26	8.38 ± .26	8.31 ± .36
DO (ml/L)	Min	2.77	3.21	3.39	3.48
	Max	4.82	4.88	4.83	4.96
	Range	2.05	1.67	1.44	1.48
	Mean ± SD	3.86 ± .78	3.98 ± .63	4.27 ± .55	4.42 ± .47
Nitrate(mg/L)	Min	1.25	1.15	1.03	1.25
	Max	2.84	2.82	2.83	3.05
	Range	1.60	1.67	1.80	1.80
	Mean ± SD	2.13 ± .50	2.09 ± .53	1.89 ± .61	2.30 ± .56
Nitrite(mg/L)	Min	1.28	.77	.80	.98
	Max	2.25	2.17	2.03	2.14
	Range	.97	1.39	1.23	1.16
	Mean ± SD	1.82 ± .29	1.44 ± .49	1.59 ± .39	1.54 ± .44
Ammonia(mg/L)	Min	.79	1.08	1.21	.61
	Max	2.22	2.08	2.34	2.67
	Range	1.43	1.00	1.13	2.07
	Mean ± SD	1.87 ± .46	1.52 ± .32	1.82 ± .37	1.66 ± .62
Phosphate (mg/L)	Min	.94	1.13	1.23	.99
	Max	1.68	1.71	1.70	1.68
	Range	.74	.58	.48	.68
	Mean ± SD	1.46 ± .27	1.51 ± .18	1.53 ± .17	1.46 ± .22
Silica (mg/L)	Min	1.41	1.41	1.50	1.54
	Max	2.55	2.53	2.52	2.25
	Range	1.15	1.10	1.02	.71
	Mean ± SD	2.09 ± .43	1.99 ± .41	2.03 ± .35	1.98 ± .28

Nagarcoil. Finally, regression analysis elucidated that local abiotic factors, not more significant with distribution patterns of morphotypes, but had an effect on the abundance of populations. Results indicated that all air and water temperatures supported the abundance of morphotypes but did not influence any morphotypical variations from the sampling sites. All the morphotypes

were predominantly found in all the sampling sites irrespective of environmental conditions.

The Euclidean distance usually provides the similarity and distance between the factors. The cluster analysis further conformed environmental factors and their interaction with morphotype occurrence (Wards method [24]). The Euclidean distance measurement

Fig. 1 Occurrence of morphotype in different seasons at different sites during 2014–2015. **A** Kelambakkam; **B** Vedaranyam; **C** Tuticorin; **D** Nagarcoil

Fig. 2 Occurrence of morphotype in different seasons at different sites during 2015–2016. **A** Kelambakkam; **B** Vedaranyam; **C** Tuticorin; **D** Nagarcoil

Fig. 3 Two-dimensional plot showing relationship between the environmental factors and morphotypes occurrence from Kelambakkam (for details refer to text) (POM—Postmonsoon, SUM—Summer, PRM—Premonsoon, MON—Monsoon)

Fig. 4 Two-dimensional plot showing relationship between the environmental factors and morphotypes occurrence from Vedaranyam (POM—Postmonsoon, SUM—Summer, PRM—Premonsoon, MON—Monsoon)

Fig. 5 Two-dimensional plot showing relationship between the environmental factors and morphotypes occurrence from Tuticorin (POM—Postmonsoon, SUM—Summer, PRM—Premonsoon, MON—Monsoon)

Fig. 6 Two-dimensional plot showing relationship between the environmental factors and morphotypes occurrence from Nagarcoil (POM—Postmonsoon, SUM—Summer, PRM—Premonsoon, MON—Monsoon)

optimized three groups in all sampling sites. The first group (cluster I) only with salinity; the second group (cluster II) involved pH, DO, nitrate, nitrite, silicate, ammonia and phosphate; and the third group (cluster III) included air temperature, water temperature and

different morphotypes (M1, M3, M4, F1, F2, and F3). Results indicated that the third cluster of sampling site I, II and III showed that air and water temperatures are grouped with all the encountered morphotypes (M1, M3, M4 in male and F1, F2, F3 in female) (Fig. 7a–d).

Table 2 The correlation coefficient among studied water parameters with morphotype occurrence

	Air temperature	Water temperature	Salinity	pH	DO	Nitrate	Nitrite	Ammonia	Phosphate	Silicate	M1	M3	M4	F1	F2	F3
Air temperature	1															
Water temperature	.058	1														
Salinity	.518**	.361*	1													
pH	-.035	.139	.386*	1												
DO	-.586**	-.339	-.698**	-.145	1											
Nitrate	.515**	.082	.504**	.486**	-.455*	1										
Nitrite	.150	.080	.451**	.484**	-.244	.446*	1									
Ammonia	-.275	-.274	-.395*	-.064	.477*	-.392*	.322	1								
Phosphate	.506**	.259	.524**	.080	-.540**	.458**	.017	-.535**	1							
Silicate	.648**	.167	.527**	.102	-.651**	.525**	.196	-.337	.836**	1						
M1	.130	.065	.160	-.056	-.282	-.006	.535**	.141	-.093	.013	1					
M3	.187	.047	-.161	.376*	-.024	.114	.033	-.013	-.289	-.249	-.117	1				
M4	.191	.074	.114	-.146	.125	-.028	-.200	-.201	.262	.244	-.449**	-.672**	1			
F1	.070	.255	-.081	.064	-.077	.012	.093	.023	-.224	.003	.040	.015	.265	1		
F2	.243	.032	-.018	.216	.012	.435*	.373*	-.015	.140	.239	-.022	.125	.060	.099	1	
F3	.059	.129	.062	-.167	.001	-.353*	-.303	-.061	.048	-.131	.005	-.173	-.031	-.443*	-.798**	1

*Correlation is significant at the .01 level (2-tailed)

*Correlation is significant at the .05 level (2-tailed)

Table 3 Equation parameters of the multiple linear regression models

	Grouped data (n=32)	p value	Kelambakkam (n=8)	p value	Vedaranyam (n=8)	p value	Tuticorin (n=8)	p value	Nagarcoil (n=8)	p value
Air temperature	R ² = .186 Intercept = 32.085 ± 6.695; p = .000064	.047	R ² = .909 Intercept = 25.102 ± 7.155; p = .176	.029	R ² = .998 Intercept = 35.853 ± 3.827; p = .067	.075	R ² = .917 Intercept = 84.381 ± 208.97; p = .755	.085	R ² = .952 Intercept = 24.734 ± 26.326; p = .519	.039
Water temperature	R ² = .201 Intercept = 23.23 ± 6.136; p = .000857	.041	R ² = .952 Intercept = 26.138 ± 6.301; p = .150	.0397	R ² = .971 Intercept = 23.295 ± 9.988; p = .257	.312	R ² = .635 Intercept = 11.644 ± 381.16; p = .980	.086	R ² = .975 Inter- cept = 139.602 ± 32.069; p = .143	.088
Salinity	R ² = .125 Intercept = 42.840 ± 97.495; p = .664137	.730	R ² = .860 Intercept = 40.781 ± 146.59; p = .827	.638	R ² = .723 Inter- cept = 508.046 ± 576.65; p = .540	.819	R ² = .946 Intercept = .863 34.31 ± 53.602; p = .421	.418	R ² = .588 Inter- cept = -192.53 ± 138.41; p = .641	
pH	R ² = .199 Intercept = 6.494 ± 1.472; p = .000171	.422	R ² = .907 Intercept = 8.225 ± 1.620; p = .123	.535	R ² = .512 Intercept = 13.019 ± 9.439; p = .399	.945	R ² = .863 Intercept = 68.774 ± 53.602; p = .421	.632	R ² = .795 Intercept = -9.381 ± 27.710; p = .792	.738
DO	R ² = .0923 Intercept = 7.141 ± 3.557; p = .055	.855	R ² = .983 Intercept = 11.410 ± 2.306; p = .127	.237	R ² = .882 Intercept = -2.310 ± 10.933; p = .867	.595	R ² = .902 Intercept = 31.930 ± 93.340; p = .790	.549	R ² = .964 Inter- cept = -10.399 ± 15.055; p = .615	.344
Nitrate	R ² = .1948 Intercept = -1.298 ± 2.914; p = .659	.442	R ² = .347 Intercept = -.775 ± 9.316; p = .947	.984	R ² = .687 Intercept = 10.088 ± 15.140; p = .625	.850	R ² = .997 Inter- cept = -102.457 ± 15.658; p = .0965	.087	R ² = .589 Intercept = -28.803 ± .718; p = .913	.913
Nitrite	R ² = .452 Inter- cept = -2.16228 ± 1.835; p = .249	.529	R ² = .859 Intercept = -.712 ± 2.559; p = .827	.639	R ² = .582 Intercept = -3.247 ± 16.278; p = .874	.916	R ² = .963 Inter- cept = -30.406 ± 40.774; p = .592	.347	R ² = .981 Inter- cept = -6.775 ± 10.084; p = .623	.522
Ammonia	R ² = .137 Intercept = -5.104 ± 2.528; p = .054	.677	R ² = .999 Intercept = -6.628 ± .293; p = .0281	.521	R ² = .968 Intercept = -3.631 ± 2.874; p = .426	.325	R ² = .948 Intercept = -9.381 ± 46.353; p = .872	.409	R ² = .712 Inter- cept = 12.871 ± 56.198; p = .856	.829
Phosphate	R ² = .201 Intercept = -1.451 ± 1.101; p = .199	.518	R ² = .990 Intercept = -2.013 ± .600; p = .184	.179	R ² = .809 Intercept = 1.017 ± 4.057; p = .843	.720	R ² = .964 Intercept = -4.320 ± 17.758; p = .848	.342	R ² = .534 Inter- cept = -12.565 ± 25.848; p = .711	.937
Silicate	R ² = .145 Intercept = -1.136 ± 1.968; p = .568	.643	R ² = .850 Intercept = -3.006 ± 3.860; p = .578	.655	R ² = .964 Intercept = -3.726 ± 3.908; p = .515	.347	R ² = .846 Inter- cept = -47.753 ± 76.142; p = .643	.663	R ² = .867 Inter- cept = -25.498 ± 17.701; p = .386	.624

Multiple linear regression models using forward stepwise method explaining the variations of the main morphotypes occurrence. Significant p values (p < .05) are in bold characters

Fig. 7 Cluster analysis of similarity between physicochemical factors and morphotypes occurrences during December 2014–August 2016. **A** Kelambakkam, **B** Vedaranyam, **C** Tuticorin **D** Nagarcoil

Other environmental factors are grouped into two different clusters, first cluster was formed with salinity and second cluster indicated that all the chemical variances were grouped together.

Similarly, at sampling site, four first clusters were involved only with salinity. Whereas the second cluster included both air and water temperatures grouped with all the morphotypes. The third cluster had shown that different environmental factors such as pH, DO, nitrate, nitrite, silicate, ammonia and phosphate are grouped in a single cluster.

Data subjected to cluster analysis clearly indicated that air and water temperatures are closely related to the abundance of morphotypes from all the sampling sites, however, did not show any impact on occurrence of morphotypes in all different sampling sites.

3.4 Observation of laboratory raised morphotypes

Artemia was cultured in the laboratory by maintaining appropriate environmental conditions, (Viz) salinity (120 psu), pH (7.9 to 8.2) and temperature (31 to 32 °C). During the study periods, the maximum *Artemia* density was observed with Kelambakkam populations compared with others. Similarly, maximum lifespan was observed in Kelambakkam and minimum in Nagarcoil. Laboratory raised *Artemia* culture showed presence of six morphotypes in the same culture conditions (Table 4). After the 10th and 11th instar, specific characteristic of each morphotype was observed. Morphotypes thus segregated, cultured individually and the specific characteristic of each morphotype retained throughout its life time.

Fig. 7 (continued)

Table 4 Frequency of occurrence morphotypes by hatching cysts from various collection sites

Name of culture	M1	M3	M4	F1	F2	F3
Kelambakkam	90.00 ± 3.87	32.50 ± 13.96	88.33 ± 1.29	95.00 ± 23.23	71.66 ± 10.32	23.33 ± 5.1
Vedaranyam	82.5 ± 9.74	15.83 ± 13.10	83.33 ± 14.37	70.83 ± 17.07	84.33 ± 19.52	31.66 ± 7.60
Tuticorin	108.33 ± 14.37	15.83 ± 7.85	81.66 ± 9.03	91.66 ± 9.30	62.66 ± 3.61	33.33 ± 9.89
Nagarcoil	83.33 ± 32.96	9.33 ± 1.03	60.00 ± 26.83	68.33 ± 19.27	33.33 ± 5.16	21.66 ± 1.29

Number of morphotypes present in 500 nauplii inoculated

4 Discussion

Earlier studies on the invasive species reported that climate change can influence many adaptive characters to new environmental conditions and construct their

hotspot of living among the native populations [25]. Sometimes these invasive species were eliminated due to extreme environmental conditions to the nonnative environment [26]. Earlier, studies documented that the invasive *A. franciscana* are well adapted to the newer environmental conditions in India [17, 18]. Many aquatic

organisms can change their morphology (pleomorphic character) according to different environmental conditions. These pleomorphisms were reported in Antarctic *Pyramimonas gelidicola* and *Daphnia* sp [27]. Earlier studies on South Indian *Artemia* populations showed morphological variations [20]. Besides, various populations encountered at different locations are named to different strains namely, Kelambakkam, Tuticorin, Vedaranyam and Nagarcoil. Recent research reported that *A. franciscana* colonized in many salterns in India and it occupied all the salterns of South India [18]. Later, it was proven by molecular markers and it showed that all belong to the same species *A. franciscana* [19, 20, 28, 29]. These invader populations also showed many phenotypes within a population [20]. In the present study, six different morphotypes were observed in all the sampling sites, except the M2 morphotype described earlier [20]. The present investigation focused whether environmental factors influence the morphotypes of the brine shrimp in the different salterns of South India. Usually, environmental parameters impact development, growth, adsorption–desorption, oxidation–reduction and physiology of populations [30, 31]. The present study indicated that the air and water temperatures slightly influence the abundance of different morphotypes in *A. franciscana*. In general, temperature ranging from 28.67 to 34.33 °C seemed to be favorable for growth of brine shrimp. This was greatly supported by Van Stappen [32] who reported that brine shrimp inhabits water bodies wherein usually temperature fluctuates between 26 and 35 °C.

Morphotypes abundance was seen in summer months probably due to increase in temperature, pH and salinity. Similarly phosphate, nitrite-nitrogen and nitrate-nitrogen content registered maximum during summer, in all sampling sites [33–36] and silicate during the postmonsoon [37]. These nutrients properly regulate the phytoplankton production and form source of feed for *Artemia* population in the brine systems [30].

Previous studies reported that physicochemical factors may influence the size differences in aquatic populations in relation to changing environmental conditions [38]. However, present study did not show any such influence of physicochemical factors on morphotypes in *A. franciscana*. Whereas [39] and [40] reported that different environmental conditions had impact on the size differences of *Artemia* populations. It may occur due to the environmental pressures and adaptations of different geographical areas [41]. Interestingly, different morphotypes were encountered in *A. franciscana* and their occurrence was recorded in all sampling sites [42–45]. In general, the morphotype was categorized based on structure of second antenna in male and ovisac shapes in females [20]. These morphological changes driven by genetic alterations at genomic level

in particular environmental conditions are also known to alter the phenotypes [46–48].

However, the data obtained on the physicochemical factors clearly indicated no influence on morphotypes except air and water temperatures [49–51]. Earlier studies explained that temperature had its impact only on the size differences and not induced any permanent phenotypic variation in aquatic organisms [52, 53]. Whereas, air and water temperatures may influence the morphotype abundance but not morphological variation in different morphotypes [54–57].

The present study also indicated that air and water temperatures contribute to the abundance of morphotypes in different sampling sites as evident from scatter plot analysis [58]. Similarly, these two factors had the strongest effect in driving morphotypes abundance in an aquatic system [59]. Cluster analysis indicated existence of three different clusters, and it indicated that each cluster shared different parameters with their specific lineage. Further correlation and regression analysis indicated that most of the factors had negative correlation with morphotypes occurrence except air and water temperatures. Similarly Bowersox et al. [60] have reported that distribution of *Lepidodora kindtii* positively correlated with temperature and negatively correlated with other physicochemical factors. These are the most crucial parameters for production of more nutrients for *Artemia* populations [61]. Thus, the present study documented that morphological variations in morphotype are not influenced by environmental parameters although it had shown impact on distribution of *A. franciscana*. However, molecular characterization of each morphotype would certainly provide the authentication of these morphotypes.

Authors contribution ST—designed and performed the experiments; ST—analyzed data; NM—supervised and written the manuscript.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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