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Production of cysts and biomass of the exotic species of brine shrimp *Artemia franciscana* (Kellog) in out-door culture system

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ABSTRACT

The production of cysts and biomass of the exotic species of brine shrimp *Artemia franciscana* was studied for a period of 90 days in out- door culture system at a salinity range of 38 to 83 ppt. From the initial stocking density of 231 to 294 nauplii / 700 l of brine the population density steadily increased and reached the maximum of 389.7 to 565.3 numbers / l on 38th day of culture. The total quantity of cysts and biomass harvested from the culture system ranged from 26.450 g to 33.860 g and from 813.6 g to 1226.7 g respectively. The first maturity and first spawning were recorded at the age of 7-8 and 12-13 days respectively and the maximum density of 59.3 riding pairs / l was recorded on 59th day of culture . The prospect of the culture of the exotic species is discussed in this paper.

Introduction

The sexual species of brine shrimp *Artemia franciscana* is widely distributed with its biotopes extending from America to Australia (Vanhaecke *et al*., 1987). In Indian sub-continent the occurrence of this species was not known till 1998 when its inhabitation was noticed for the first time from the salt pans of Karapad, south of Tuticorin (Rajamani *et al*., 1998). Subsequent survey in the salt pans and salt water pools revealed that this species occurred in the salt pans of Alangarathittu region, east of Tuticorin while in the salt pans of Veppalodai north of Tuticorin only the native species *A. parthenogenetica* were found to occur (Rajamani *et al*., 2001). Further survey carried out during the later period showed that *A. parthenogenetica* was totally absent in the ecosystems at Karapad, Alangarathittu and Veppalodai regions and instead the exotic species *A. franciscana* was found to flourish (Rajamani *et al*., 2003).

Tuticorin has a place in the world geographical map on the distribution of brine shrimp due to the wide-spread and dense occurrence of the asexual species of brine shrimp, *A. parthenogenetica* in several salt pan and salt water pools in and around Tuticorin (Achari, 1971., Royan, 1980 and Vanhaecke *et al*., 1987). However, the gradual elimination of the native species from the natural ecosystem in and around Tuticorin during the recent past suggests that the exotic species may spread to other

biotopes also in the Indian sub-continent sooner or later. The present paper reports on some aspects of the production potential of the exotic species in out-door culture systems carried out with the cysts obtained from a natural ecosystem at Tuticorin during the year 2001.

Materials and methods

Stocking particulars

The I instar nauplii obtained from the hatching of wild cysts of *Artemia franciscana* were reared at 90 ppt and the riding pairs obtained from this population were subjected to different salinity stress. All the I instar nauplii produced by the individual female in a single spawning from the above salinity stress experiments viz. 238 from 90 ppt., 231 from 110 ppt. and 294 from 50 ppt. were stocked in three 1 t FRP rectangular tanks maintained under open sun light i.e. Tank No.1 (Exp. No. 33a) ; Tank No.2 (Exp. No. 33b) and Tank No.3 (Exp. No. 33c) filled with 700 l of brine with a salinity of 40 – 41 ppt. in order to assess the quantities of cysts and biomass that can be produced by a single female subjected to salinity stress at the time of egg formation. As the entire series of experiments for mass production of biomass and cysts of the brine shrimp were conducted with the I instar nauplii obtained from a single female as shown in the following flow-chart. The experiments were not conducted in duplicate and the inherent variations in the total number of nauplii obtained and stocked in the three different tanks were ignored.

Hatching of wild cysts (at 25 ppt)

↓

Time taken for first hatching: 14 hrs and 10 mins

Production of I instar nauplii

↓

Rearing at 90 ppt for 12 days

Production of riding pairs

↓

Isolation of three riding pairs (one for each salinity)

↓

↓	↓	↓
90 ppt (Control)	110 ppt (Hyper-saline stress)	50 ppt (Low-saline stress)

Total number of nauplii produced by one female in a single spawning

↓	↓	↓
238*	231*	294*

(*Stocked in three 1 t FRP tanks for the production of biomass and cysts)

Hydrographical conditions and feed given

Although the initial salinity in the culture tank at the time of stocking was only 40-41 ppt., on day 12 and 13 brine was gradually added in order to increase the salinity to over 60 ppt. so as to eliminate the filamentous algal growth. Subsequently, on day 20 and day 21 also brine was gradually added to increase the salinity around 70 ppt in order to arrest copepod proliferation . Initially the tanks were fertilized with inorganic fertilizer (Urea 10 g, ammonium sulphate 1g and super phosphate 1g per 700 liter) for algal production. Initial algal bloom was sufficient to serve as feed only up to day 7 and hence from day 8 onwards rice bran was given as supplimentary feed following the procedure given by Sorgeloos and Kulasekarapandian (1986). The quantity of rice bran was gradually increased as the culture progressed (Table 1).

TABLE 1. *Hydrographical conditions in the experimental tanks and quantity of feed given*

Week	Salinity (ppt)		Temperature (°C)		p H		Algal cells/ml	
	Min	Max	Min	Max	Min	Max	Min	Max
I Week	40.0	44.0	25.0	30.5	8.2	8.9	8,000	27,500
							8,000	12,500
							Rice bran (g)	
II Week	38.0	60.0	23.5	29.5	8.2	8.7	6.3	
III Week	55.0	68.0	23.5	30.5	8.2	8.6	18.0	
IV Week	64.0	72.0	22.0	25.5	7.9	8.6	33.3	
V Week	64.0	68.0	22.0	27.0	8.0	8.4	45.0	
VI Week	66.0	70.0	24.0	27.5	7.8	8.2	45.9	
VII Week	68.0	73.0	22.5	29.0	7.1	8.1	44.1	
VIII Week	70.0	76.0	23.5	30.5	7.1	7.5	61.2	
IX Week	73.0	81.0	23.5	31.5	7.1	7.4	75.6	
X Week	68.0	83.0	22.0	31.0	7.1	7.6	41.4	
XI Week	65.0	75.0	22.0	27.5	7.2	7.7	38.7	
XII Week	72.0	80.0	23.5	31.0	7.3	7.9	52.2	
XIII Week	76.0	80.0	26.0	31.0	7.7	7.9	61.2	

Aeration was given at four corners of the tank in order to aerate the water and to accumulate the faecal matters at the center of the tank.

Monitoring of the population growth

The conditions of the *Artemia* in the tanks were observed daily. The changes in the population density and composition of various stages viz. cyst, nauplius, juvenile, adult and riding pair were monitored at weekly intervals by taking random samples and counting the numbers present in the sample and then relating it to the total volume. The hydrographical conditions of the culture tank viz. ambient temperature , salinity and pH were recorded daily. The concentration of the algal cells in the culture tank was counted with the help of a haemocytometer. Bottom sediments were cleaned and 10 % water exchange was given at irregular intervals of 6 to 13 days depending upon the accumulation of the sediments in the tank.

Harvesting of cysts and biomass

Two partial harvests were done with 800 m strainer to harvest riding pairs and adults on day 38 and on day 66. Final harvest was made on day 90. The total number of animals harvested during partial and final harvests was estimated by random sampling. The wet and dry weight (dried at 60 ^{0}C for 12 hours in an oven and kept in a desiccator for 1 hour) of animals harvested was recorded. During partial and final harvest, cysts produced in the culture system were collected , processed and stored in plastic containers. Cyst quality was evaluated in terms of T_0 (Time taken for first

hatching), hatching percentage at 24 hrs. and 48 hrs.

Statistical analysis

The results on the population density in the three culture tanks on the day of two partial harvests made on 38th and 66th days and also at time of final harvest made on 90th day of the culture were subjected to ANOVA test in order to find out whether the production potential of the female subjected to salinity stress at the time of egg formation is significantly affected in the culture systems.

Results and discussion

Progressive changes in population density

A steady increase was recorded in the population density in all the three experimental tanks from the time of stocking with marginal variation between the three tanks. The maximum numbers recorded on 38th day of culture was 565.3 Nos. / l in tank No. 1 whereas it was 389.7 and 512 Nos./ l in tank No. 2 and 3 respectively (Figs. 1a –1c). The biomass produced in the culture system was harvested partially in all the three tanks on 38th day of culture. After the partial harvest the remaining females released either nauplii or cysts and the density of the population increased steadily and reached the maximum of 588.3 and 448 Nos. / l on 66th day in tank No.1 and 2 respectively whereas in tank No. 3 the maximum of 510.3 Nos. / l was recorded on 59th day i.e. in the previous sampling. The final harvest was made on 90th day in all the three tanks when the population densities were in the order of 456.3, 457.7 and 469.0 Nos. / l in tank Nos. 1, 2 and 3. The variations in the population density of the animals between the three tanks on 38th, 66 and 90th days of culture were subjected to

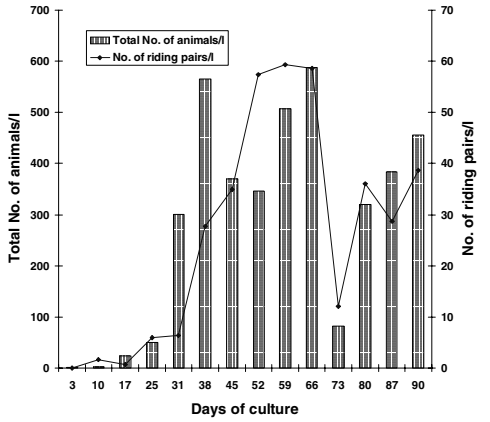

Fig. 1 a. Progressive changes in the density of total population and riding pairs of *A.franciscana* in Tank No. 1 (Exp. No. 33 a)

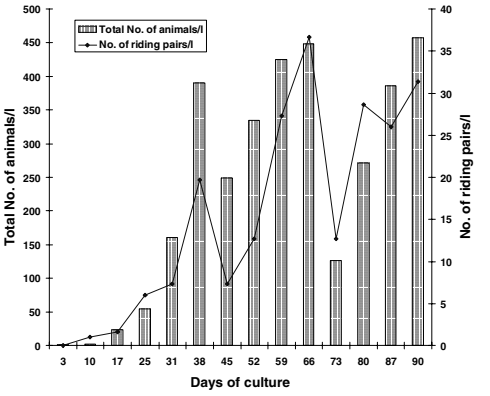

Fig. 1 b. Progressive changes in the density of total population and riding pairs of *A.franciscana* in Tank No. 2 (Exp. No. 33 b)

ANOVA test and it was found that the variation was not significant at 5 % level ('F' Values being 3.33 (P: 0.14) and 0.72 (P: 0.54) for the effect of salinity and duration of culture respectively) indicating clearly that when females undergo salinity stress in the range of 50 and 110 ppt during egg formation it will not affect their production potential.

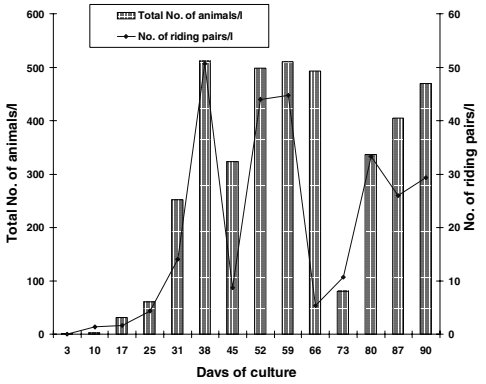

Fig. 1 c. Progressive changes in the density of total population and riding pairs of *A.franciscana* in Tank No. 3 (Exp. No. 33 c)

Age at first pairing and first spawning

The age of *A. franciscana* at the time of first pairing was found to be 8 days in tank No. 1 and 7 days in tank No. 2 and 3. The age at first spawning was found to be 13 days in tank Nos. 1 and 2 whereas it was 12 days in tank No. 3. The duration of incubation of eggs in the brood pouch of the brine shrimp was from 5 to 6 days (Fig. 2). The first off spring produced in all the three tanks were nauplii. The intensity of the nauplii produced in the three tanks increased steadily from 17th day onwards reaching the maximum of 252.7 ,194.7 and 167.3 Nos. / l on 38th day i.e. at the time of partial harvest.

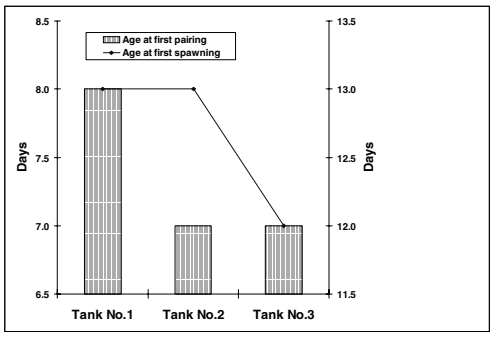

Fig. 2. Age of *A.franciscana* at first pairing and first spawning in the three culture tanks

Composition of various stages

The composition of various stages of the animals viz. cysts, nauplii, juveniles, adults , riding pairs and dead animals in the population was studied every week and the results obtained on the 38th and 66th days of culture i.e on the day of first and second partial harvests and also on the 90th day of culture i.e. at the time of final harvest are given in Figs. 3a to 3c. It can be seen from the figures that the composition of cysts in the three tanks varied between 27.2 and 35.6 % on 38th day of culture i.e. at the time of first partial harvest. The composition of cyst was high ranging between 51.7 and 55.7 % at the time of second harvest carried out on 66th day of culture. At the time of final harvest carried out on 90th day of culture the composition of cysts fluctuated between 54.6 and 65.5 %.

The density of nauplii was high only during the initial period with its composition in the three tanks ranging between 32.7 and 50 % which came down to values ranging between 6.2 and 10.7 % on 68th day of culture. However, during the later part of the experiment the composition increased and reached the value ranging from 15.2 to 34 %. The composition of Juveniles and adults in the population was less in all the three tanks at the time of the two partial harvests carried out on 38th and 66th days and at the time of final harvest carried out on 90th day of culture. The composition of dead animals in the population constituted only negligible proportions.

Composition of riding pairs

The intensity of the occurrence of riding pairs in the population is very important as it primarily determines the population growth. The number of riding

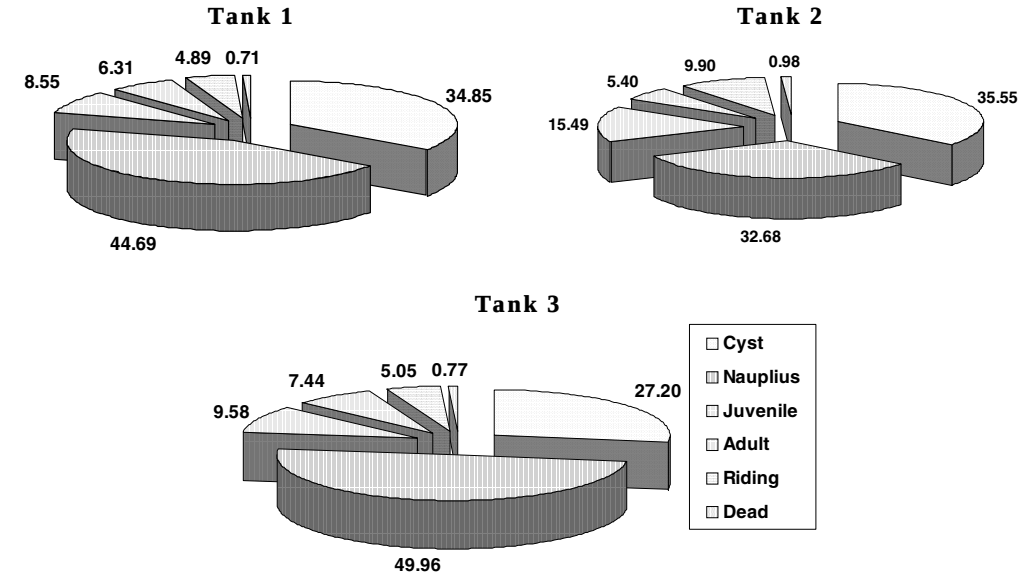

Fig. 3 a. Composition of various stages of *A.franciscana* at the time of first harvest on 38th day of culture

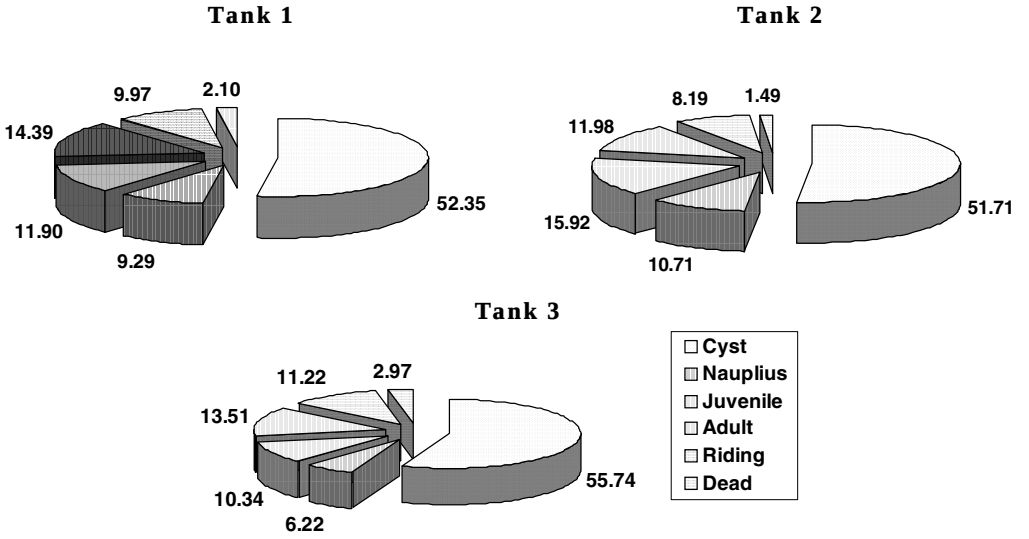

Fig. 3 b. Composition of various stages of *A.franciscana* at the time of second harvest on 66th day of culture

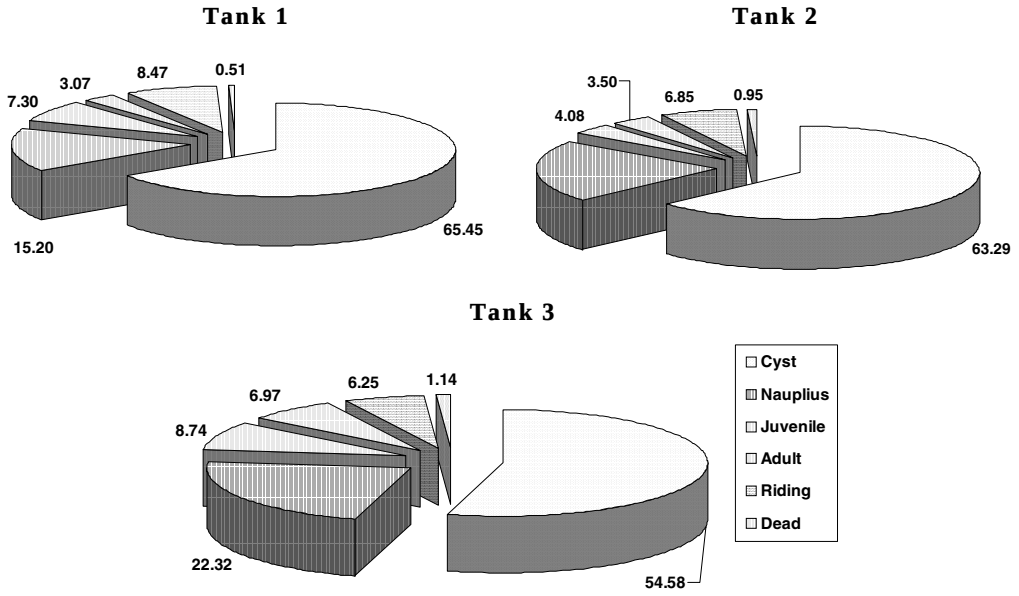

Fig. 3 c. Composition of various stages of *A.franciscana* at the time of final harvest on 90th day of culture

pairs recorded in the three tanks showed a gradual increase in all the three tanks indicating that the prevailing environmental conditions were favourable for the proliferation of the animals. At the time of first partial harvest the number of riding pairs recorded in the three tanks were in the order of 27.7, 19.7 and 50.7 pairs / l whereas at the time of second partial harvest the number of riding pairs recorded were in the order of 58.7, 36.7 and 5.3 pairs / l. Although the number of riding pairs recorded in tank No.3 was less on the date of second partial harvest, during the previous samplings the number of riding pairs recorded were 44.0 and 44.7 pairs / l indicating that the females were in active reproductive stage at the time of the partial harvest (Figs. 1a- 1c). At the time of final harvest also the number of riding pairs recorded in the three tanks was high, ranging between 29.3 and 38.7 pairs / l which suggest that the culture can be prolonged still further and higher production of both cysts and biomass can be achieved even beyond 90 days.

Production of cysts and their quality

The total quantities of cysts produced were in the order of 33.860, 26.450 and 27.870g in tank Nos. 1, 2 and 3 (Tables 2a & b and Fig. 4). The first production of cyst was noticed on 22nd , 23rd and 21st day respectively in tank Nos. 1, 2 and 3. The size of the cysts produced in the three tanks did not show much variation. The overall size of the cysts produced in the three tanks varied between 236 ± 10 m and 248 ± 10 m. The quality of the cysts produced in the culture systems were assessed in terms of T_0 (Time taken for first hatching), hatching percentage at 24 hrs. and 48 hrs. The time taken for first hatching showed only marginal variation ranging between 13 hrs. 10 mins. to 14 hrs. 10

TABLE 2 a *Quality evaluation of cysts produced in experiment Nos. 33 a, b and c - Month wise*

Parameter	December			January			February		
	33 a	33 b	33 c	33 a	33 b	33 c	33 a	33 b	33 c
Cyst size (mm)	0.246+0.01	0.248+0.01	0.242+0.004	0.240+0.01	0.242+0.01	0.242+.004	0.236+0.01	0.240+0.01	0.242+.01
I Instar size (mm)	0.482+0.004	0.482+0.005	0.482+0.004	0.484+0.005	0.484+0.005	0.482+0.004	0.484+0.005	0.482+0.004	0.484+0.005
T0(Hrs)	14.10	14.10	14.10	13.30	13.10	13.30	13.20	13.20	13.20
Hatching % (24 Hrs.)	43.00%	47.00%	46.00%	48.00%	43.00%	52.00%	51.00%	54.00%	53.00%
Hatching % (48 Hrs.)	69.00%	72.00%	70.00%	71.00%	69.00%	74.00%	74.00%	78.00%	78.00%
Quantity of cyst produced (g)	2.620	1.280	2.400	13.830	8.910	11.350	17.410	16.260	14.120

TABLE 2 b *Quality evaluation of cysts produced in experiment nos. 33 a, b and c - Over all*

Parameter	33a		33a		33a	
	Min	Max	Min	Max	Min	Max
Cyst size (mm)	0.236+0.01	0.246+0.01	0.240+0.01	0.248+0.01	0.246+0.01	0.248+0.01
I Instar size (mm)	0.482+0.004	0.484+0.005	0.482+0.004	0.484+0.005	0.482+0.004	0.484+0.005
T0(Hrs)	13.20	14.10	13.10	14.10	13.20	14.10
Hatching % (24 Hrs.)	43.00%	51.00%	43.00%	54.00%	46.00%	53.00%
Hatching % (48 Hrs.)	69.00%	74.00%	69.00%	78.00%	70.00%	78.00%
Quantity of cyst produced (g)	33.860		26.450		27.870	

mins. Similarly the hatching percentages recorded at 24 hrs and 48 hrs also did not show much variation between the cysts collected from the three tanks with their values ranging from 43 to 54 % and from 69 to 78 % respectively. The size of the I instar was found to range between 482 and 484 µ only in all the three tanks (Tables 2a and 2b).

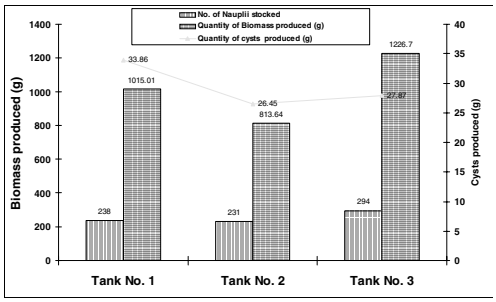

Fig. 4. Quantities of cysts and biomass of *A. franciscana* produced in the three culture tanks

Production of biomass

The quantities of biomass (wet weight) produced in the tanks were 1015.0, 813.6 and 1226.7 g respectively in tank Nos. 1 , 2 and 3 (Fig. 4). In terms of dry weight the quantities of biomass produced were in the order of 99.19 , 74.64 and 117.26 g. Dry matter constituted less than 10 % with its value ranging between 9.17 and 9.77 %, the minimum and maximum being in the brine shrimp harvested from tank Nos. 2 and 3, respectively. As the nauplii stocked in the three tanks were obtained from a single brood of three different females collected from the same population and maintained at three different salinities viz. from 90 ppt, 110 ppt, and 50 ppt the results on the rate of production recorded in the three tanks suggests that the biomass production potential of a single female is within the range of 813.7 - 1226.7 g / 90 days under the given environmental conditions.

The results obtained in the present investigation on the population density, composition of riding pairs and production of biomass and cysts in the culture system from the initial stocking density of less than 300 I instar nauplii / 700 l of brine clearly suggests that this species is well adapted to the prevailing environmental conditions. The high density of active riding pairs recorded in the culture system throughout the culture period further strengthens this view.

Bossuyt and Sorgeloos (1980), James *et al*., (1987), Lavens *et al*., (1987) and Dhert *et al*., (1992) have reported higher production of biomass of *A. franciscana* in the culture systems. It has been reported that the production of biomass of the brine shrimp varies widely from 5 to 25 kg per cubic metre of tank volume and this difference is attributed mainly to the initial stocking density. According to Dhont *et al*. (1993) the number of nauplii stocked in 100 – 500 l rectangular polyethylene tanks ranged from 5 to 50 per ml which is obviously much higher than what has been stocked in the present investigation. As stated earlier, the objective of the present study was to find out the production potential of a single female when it is subjected to stress conditions during the time of egg formation. The results of the investigation suggests that the production potential of *A.franciscana* is high although insignificant variation is noticed in the production potential between the nauplii obtained from the females subjected to salinity stress at 50 and 110 ppt. at the time of brood development. Thus the present investigation clearly indicates that there is a good prospect for the culture of the

exotic species, *A. franciscana* in miniature out-door culture systems, which may to a very great extent meet the feed requirements of the various larval stages as well as brood stock of shrimps maintained by small-scale shrimp hatcheries.

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