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Production of brine shrimp, *Artemia salina* biomass and cyst in indoor tank using crude salt

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Abstract: Live feed (e.g. Brine shrimp) culture is an integral component in successful fish and shellfish hatcheries. This contribution aims to develop the easiest and the cheapest technology for the production of *Artemia salina* biomass and cyst in indoor installations using crude salt where brine is unavailable. Collection of biomass of the cultured *Artemia salina* continued from their age of day 6 to day 30, and the cyst was collected from their age of day 20 to day 30. The highest rate of biomass production was observed at day 22 to 24 while the highest rate of cyst production was observed at their age of day 24 to 26. In the first observation (at day 6) 373.248 gm wet weight of *Artemia salina* were collected from the experimental tank and the average weight of each *Artemia salina* was found 0.00072 gm. At the end of the experiment (at day 30), 2964.218 gm wet weight of *Artemia salina* were collected from each replicate tank and the average weight of each *Artemia salina* was found .0074 gm. Average size of each *Artemia salina* was found 1.1cm at their age of 30. The highest density (800 ind. /L) but the lowest biomass (0.00072 gm/ind.) was recorded in the first observation. A sharp growth rate of *Artemia salina* was observed at day 14 and continued to day 22. Rate of cyst production was the highest at day 26 and then it decreased

gradually as the age of *Artemia salina* was increased. At the initial stage, salinity was maintained at 5 ppt while it was increased gradually at 120 ppt at the end of the experiment. Statistical analysis revealed that no significance differences were found between three replications in case of population density, biomass and cyst production of *Artemia salina.* The developed production method was found feasible and profitable which can be integrated in marine and shellfish hatcheries where brine is unavailable.

Key words: Crude salt, saline water, *Artemia salina*, Shrimp hatchery, Oviparous.

INTRODUCTION

Artemia salina, popularly known as brine shrimp, are small brachiopod crustaceans found in natural salt lakes or man-made salterns scattered throughout the tropical, subtropical and temperate climatic zones [1]. They are euryhaline organisms, capable of living and reproducing in a salinity range of 5 to 200 ppt [2]. In addition, the organisms have evolved with two modes of reproduction: ovoviviparous mode, producing free swimming nauplius larvae released by the mother from fertilized eggs when habitat conditions are optimal; and the oviparous mode, producing dormant cysts in diapause when conditions are extreme or unfavorable[2]. The unique dormant cysts which can be dried transported and hatched on demand [3], which have made this organism as an excellent food source for larviculture of fish and shellfish in the hatchery. Among the live diets used in fish and shellfish hatchery, *Artemia* constitute the most widely used food item. Around 2000 metric tons of *Artemia* cysts were marketed worldwide annually and over 90% of all marketed cysts were originated from the Great Salt Lake, USA [2].

As high salinity is the common feature for the occurrence of *Artemia*, it is not naturally available in Bangladesh coastal area. But with the expansion of aquaculture production in Bangladesh, the demand for *Artemia salina* cysts has increased exponentially since 1990. Until now, the shell fish hatcheries of the country are heavily dependent on imported cyst from foreign countries. By realizing this fact, Mahmood *et al.*[4], and Mahmood *et al.* [5] worked and reported on the *Artemia salina* cyst production in the coastal saltpans in Bangladesh. These studies were followed by three successful trials by the teachers and graduates of the Institute of Marine Sciences and Fisheries[5,6] (Bangladesh) in 1992 and 1994 . Although they developed a technique to produce *Artemia salina* cyst and biomass, no further work on improving the technique took place. As salinity in open salterns and salt lakes falls below the optimum level during monsoon (June-November), it is not possible or economically viable to carry out the outdoor culture of *Artemia salina* during that time. However, the introduction of controlled culture of *Artemia salina* in indoor tanks, where salinity can be maintained by adding crude salt from the salt farms, could be used to continue production of *Artemia salina* biomass and cyst. Carrying on the culture of organisms in covered tanks or ponds, during the monsoon would also be economical and practical, as hatchery owners and poor, subsistence salt-farmers can procure materials locally at affordable prices and can carry out construction of the shelters and tanks themselves, or find cheap professional help locally. In Bangladesh, a number of endemic finfish and crustacean species seem to have aquaculture potential, but live food availability is one of the major constraints for developing economically viable culture practices for these species[4,5]. The purpose of the present study, therefore, was to produce *Artemia salina* cyst in indoor tanks using crude salt and indigenous materials, to make a continuous, year-round supply of *Artemia salina* live feed available to the hatcheries throughout the country.

MATERIALS AND METHODS

Collection and Processing of crude salt: Crude salt was procured from the local market in Chittagong city, Bangladesh, and stored in plastic containers. Debris from the salt was removed by washing and sieving it with water repeatedly. Saline water of different concentrations was prepared by mixing crude salt with water in plastic containers, and then allowing the containers to remain undisturbed to let the suspended solids and other impurities to settle to the bottom.

Experimental set up: A simplified design and a slight modification of the experiment followed by Dhert *et al.*[7] was adopted in this study. An experimental earthen tank (1.8m x 1.8m x o.3m) was constructed and set up in the open yard of the Institute of Marine Sciences and Fisheries building. The tank was filled with 648 litre of saline water free from impurities. A temporary roof was constructed over the tank to shield it from direct sunlight and rainfall. Mechanical aeration of the water in the tank was carried out by agitating the water on alternate days to maintain an optimum level of dissolved oxygen in the tank. A reservoir tank with a small layer (up to 6 inch) of pebbles was set up and filled with saline water for recirculation of the water in the experimental tank and the reduction of organic load in it. The filtered water from the reservoir tank was discharged into the experimental tank at a rate of 75L/day to recycle 150 liters of saline water every two days and a similar amount discharged from it to maintain a continuous water flow in and out of the experimental tank. One gram of *Artemia salina* cyst was added to the tank and cultured for up to 30 days to get biomass and cyst. Total biomass and weight of cyst was measured by using microbalance (Electrical Analytical Balance, OSK 11325A). Three replicates (R-1, R-2 & R-3) were conducted for the present research.

Determination of water parameters: Temperature of the water in the tank was recorded by a centigrade laboratory thermometer with a measuring range from 0^{o}C to 110^{o}C. Dissolved oxygen (DO) in the water was determined following the Azid modification of Winkler Method[8]. Digital pH meter (pH by HANNA instruments) was used to determine hydrogen ion concentration of water, and the water salinity was determined by Refractometer (TANAKA, New S-100, Salinity, and Japan).

Hatching of Artemia salina cyst: A V-shaped plastic container capable of holding 5 litres of saline water (5 ppt) was used for hatching dried cyst of *Artemia salina* purchased from the ADB hatchery at Cox's Bazar, Bangladesh. One gram of *Artemia salina* cyst was added to the hatching container that received continuous and vigorous aeration for 48 hours by which time the cysts hatched into nauplii. After hatching of the nauplii, the air flow into the tank was turned off to let the culture settle for ten minutes. The nauplii, attracted by light, concentrated at the bottom of the V-shaped container where sufficient light was ensured for this reason. To harvest, nauplii were siphoned out from the bottom of the container, and transferred to the rearing tanks, previously enriched with organic matter, bacteria and algae.

Application of food in the rearing tank: After hatching, *Artemia salina* nauplii were separated and inoculated in to the rearing tanks in which salinity was gradually increased from 5 ppt initially to 120 ppt at the end of the experiment. Before inoculation, water in the tank was enriched with organic matter, principally the decomposing rice bran, and moderately aerated for three days to grow algae and bacteria. This type of food was utilized by *Artemia salina* as primary feed until reaching the pre-adult stage, 5-6 days after hatching. At this stage, a mixture of egg yolk, rice bran, poultry excreta and oil cake was supplied as food. Feeding schedule was determined on the basis of water transparency, and supply of food to the

culture tanks continued until water became turbid. Moderate aeration of the tank continued to keep the food in suspension and to maintain supply of O_2 to the growing *Artemia salina*.

Measurement of Artemia salina biomass and size: Before samples were taken, a homogenous distribution of *Artemia salina* in water of the tank was ensured by aerating it vigorously. Using the random sampling technique, a 100ml water sample was taken from each replicate tank to estimate the density and biomass of *Artemia salina.* The biomass of *Artemia salina* was measured with microbalance (Electrical Analytical Balance, OSK 11325A) on wet weight basis, and the size of the *Artemia salina* was measured on a centimeter scale.

Measurement of Artemia salina cyst: *Artemia salina* became adult and engaged in mating at the age of 15-16 days. Just after mating, *Artemia salina* released naupli as well as cyst, but as the salinity increased gradually in the culture tank, *Artemia salina*, at age 17-18 days, started to release cyst only. A thin layer of cyst was observed on the surface water of the tank that accumulated at the periphery of the tank due to wind action. The accumulated *Artemia salina* cyst was collected from the tank by the siphoning technique. The collected cyst was mixed with freshwater in buckets to separate waste material from the cyst which fell to the bottom. The cyst was siphoned up from the bottom of the collection bucket, and subsequently mixed with high saline water (200 ppt) to let the heavier substances like sand and mud to settle to the bottom. Most of the cyst floated over the water surface and was collected in fresh condition. At the end of this stage, cyst was washed with freshwater and then again with 20 ppt saline water before letting it dry. The dried cyst was stored at room temperature and its hatching performance tested after a certain period.

Cyst and biomass collection: The collection of the biomass of cultured shrimp started on day 6, and continued till day 30. The cyst collection started from day 20 and continued until day 30. Biomass and cyst were collected in the morning on alternate days. The collected cyst was hatched and reared in the same experimental tank ten months after, in order to evaluate the cyst quality of the offspring (F_1 generation) by measuring the hatching percentage, population density (ind./L) and biomass production. Environmental variables were recorded throughout the experiment in the same way as the previous experiment.

Statistical analysis: The data were analyzed statistically with a one-way analysis of variance (ANOVA) to find an overall difference in the replications. Tukeys HSD (SPSS V10.0) test was performed on the data to test whether differences between the replications (R_1, R_2 & R_3) were significant for *Artemia salina* in terms of population density, biomass and cyst production at a significance level of $p < 0.05$.

RESULTS AND DISCUSSION

In the present study, the biomass and cyst production were low at the early and final life stages of *Artemia* (Table 1). The highest rate of biomass production was observed on days 21 and 22, while the highest rate of cyst production was observed on days 25 and 26. Adult *Artemia salina* was harvested from the age of day 6 to day 30 after the inoculation of naupli in the rearing tank. The density and biomass of *Artemia salina* were recorded from day 6 of the experiment when harvesting of adult shrimp began. A total of 373.248 grams of *Artemia salina*, with an average density of 800 individuals/L, the highest recorded density during the experiment, *were* collected from the experimental tank on day 6, and the average weight of each *Artemia salina* was found to be 0.00072 gm, the lowest record during the experiment.

Table 1: Values (mean ± S.D; n=3) of biomass and cyst production of *Artemia salina* during 30 days of experiment.

Age (day)	Weight/ind. (gm)	Population density (ind./L)	Biomass (gm)/L	Total biomass (gm) in the whole tank	Weight of cyst (gm)/150L water exchange	Total weight of cyst (gm) of whole tank
1-5	-	-	-	-		
6	0.00072	800±24.25	0.576±0.03	373.248±16.35	-	-
8	0.00075	769±7.94	0.577±0.01	373.708±3.27	-	
10	0.00081	751±11.50	0.611±0.02	395.904±15.43	-	-
12	0.00135	742±9.17	1.001±0.06	648.894±38.86	-	-
14	0.00230	718±6.24	1.656±0.07	1073.172±47.36	-	-
16	0.00453	663±20.21	3.007±0.08	1948.417±53.45	-	-
18	0.00539	651±13.65	3.511±0.10	2275.169±65.90	-	-
20	0.00647	672±3.00	4.350±0.10	2819.007±64.88	1.873±0.108	8.093±0.466
22	0.00801	730±8.74	5.850±0.03	3790.502±19.45	2.185±0.112	9.439±0.483
24	0.00803	720±8.74	5.781±0.01	3745.967±4.93	2.602±0.147	11.239±0.637
26	0.00806	713±8.00	5.747±0.05	3723.792±32.54	2.982±0.115	12.882±0.496
28	0.00802	685±6.66	5.499±0.07	3563.028±42.87	2.468±0.161	10.663±0.695
30	0.00739	619±7.94	4.574±0.07	2964.218±42.34	2.123±0.165	9.171±0.711

"-" indicates the data were not measured.

At the end of the experiment on day 30, a combined total of 2964.218 grams of *Artemia salina* was collected from the three replicate tanks, and the average weight of individual *Artemia salina* was found to be 0.0074 gm. The average size of individual *Artemia salina* was found to be 1.1 cm at the end of the experiment on day 30, with the females noticeably bigger in size than the males. A sharp increase in the growth rate of *Artemia salina* biomass was observed on day 14 which continued until day 22 (Fig. 1 and 2).

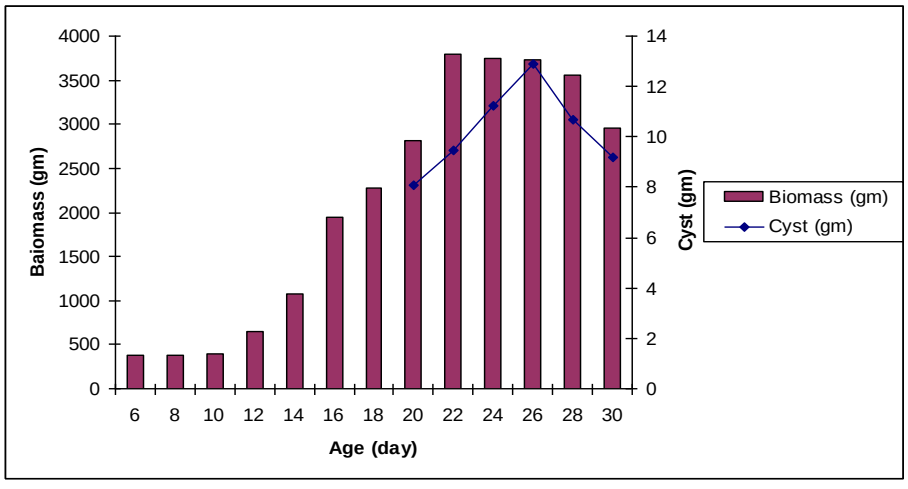

Figure 1: Relationship between age, biomass and cyst production of *Artemia salina.*

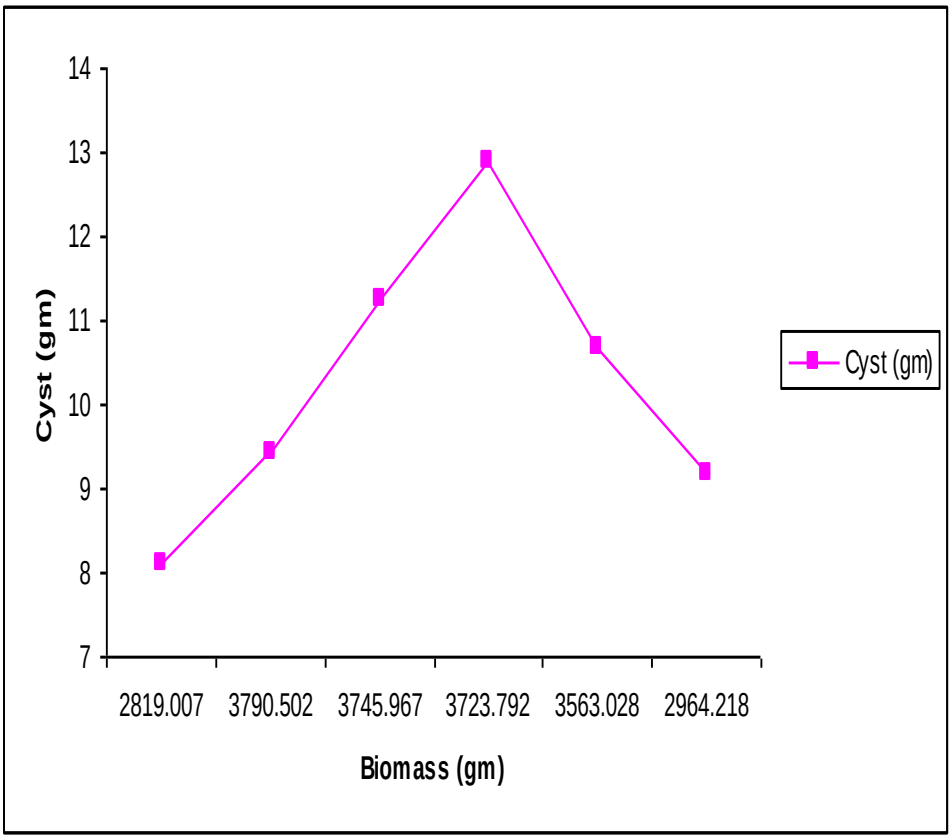

Figure 2: Relationship between biomass and cyst production of *Artemia salina.*

The rate of cyst production was the highest on day 26 but it decreased as the age of *Artemia salina* increased. At the initial stage of the experiment, the rearing tanks were filled with water of 5 ppt salinity which was increased gradually to 120 ppt by the end of the experiment (Table 2). The water temperatures (^{0}C) recorded were 26.75, 29.13 and 30.45 in the replicates R-1, R-2 and R-3 respectively, and the dissolved oxygen levels (ml/L) recorded were 4.06, 3.84, 4.01 respectively. The pH values of the water in the three replicate tanks were 7.67, 7.66, and 7.73. One-way ANOVA indicated there were no significant differences ($p>0.05$) among the replications (R_1, R_2 & R_3) with regard to the population density observed, and the biomass and cyst produced by *Artemia salina* during the experiment (Table 3).

Table- 3: ANOVA for comparing the outputs from the three replications.

Factor	F	P-value
Population density	0.032	0.968
Biomass (Wet Weight)	0.001	0.999
Amount of Cyst	0.069	0.933

Table- 2: Values of water quality parameters during 30 days of experiment of the *Artemia salina* biomass and cyst production tanks.

Day	Salinity (ppt).	Water Temperature (°C)	Dissolved Oxygen (ml/L)	pH
1	5	28.00	5.13	7.30
2	5	28.33	4.71	7.30
3	20	28.33	4.29	7.60
4	30	28.17	3.94	7.60
5	30	28.17	3.74	7.87
6	30	28.50	3.38	7.70
7	35	29.00	3.06	7.67
8	40	28.83	3.05	7.87
9	45	28.83	3.35	7.93
10	55	28.50	3.54	7.87
11	60	28.67	4.33	7.87
12	70	28.83	4.24	7.93
13	80	29.00	4.34	7.80
14	90	28.50	3.75	7.90
15	90	28.67	3.60	7.93
16	100	28.50	3.91	7.23
17	105	28.83	3.85	7.67
18	110	28.67	4.37	7.73
19	110	29.00	4.78	7.77
20	120	29.00	4.83	7.73
21	120	28.67	4.16	7.67
22	120	28.83	3.85	7.80
23	120	28.67	3.86	7.50
24	120	29.00	3.20	7.40
25	120	28.83	4.13	7.73
26	120	29.33	3.82	7.83
27	120	29.33	4.06	7.93
28	120	29.00	3.84	7.87
29	120	29.67	3.82	7.27
30	120	29.67	4.07	7.33
Mean ± S.D.	0.0	28.78 ± 0.40	3.97 ± 0.50	7.87 ±0.22

The water temperatures (^{0}C) recorded were 26.75, 29.13 and 30.45 in the replicates R-1, R-2 and R-3 respectively, and the dissolved oxygen levels (ml/L) recorded were 4.06, 3.84, 4.01 respectively. The pH values of the water in the three replicate tanks were 7.67, 7.66, and 7.73.

One-way ANOVA indicated there were no significant differences ($p>0.05$) among the replications (R_1, R_2 & R_3) with regard to the population density observed, and the biomass and cyst produced by *Artemia salina* during the experiment (Table 3). A second set of three replicate experiments was conducted after ten months to evaluate the cyst quality by measuring hatching percentage, biomass production and population density of the offspring (F_1 generation) from the cyst obtained above. In each replicate, salinity was

gradually raised from 5 to 120 ppt as in the previous experiment. The recorded average water temperatures (°C), dissolved oxygen levels (ml/L) and the pH were 26.25, 4.28 & 6.99 respectively. The recorded hatching percentage was 78.3. At the end of the experiment for F_1 generation, on day 30, 2628.774 gm of *Artemia salina* were collected from the replicate tanks, and the average weight of individual Artemia *salina* was found to be 0.00675 gm. Clegg and Trotman[9] found that *Artemia salina* tends to reproduce oviparously under adverse environmental conditions, while as ovoviviparity seems to be favored under favorable conditions. They concluded that candidates for adverse environmental conditions in their study were high salinity, high temperatures, low oxygen levels, and deprivation of food.

Dhont and Lavens[10] suggested that water quality parameters should be maintained within an optimal range, recommending salinity between 32-65 ppt, oxygen above 2 mg/l, temperature between 19-25°C, and the pH between 6.5-8 during the production of different *Artemia salina* in controlled conditions. These essential water quality parameters (salinity, water temperature, dissolved oxygen & pH) were measured throughout the experimental period in this study. Salinity is one of the important parameters to control the growth and survival of *Artemia salina.* Though it is euryhaline in nature, it thrives when exposed to optimum salinity. Better growth and survival of *Artemia salina* was observed in seawater (34-55 ppt) by Soundarapandian and Saravanakumar[11] when they cultured *Artemia salina* in three salinities (freshwater, brackishwater and seawater) for the biomass production. Naegel & Rodriguez[12] found that *Artemia salina* individuals show lower size values in higher salinity, and concluded that the main reason for the decrease in the size of the adults at high salinity levels (200–250 ppt) was due to the food becoming a limiting factor.

The salinity of the water in the rearing tanks during the present study was raised gradually from 5 ppt at the beginning to 120 ppt at the end of the experiment, as the age of *Artemia salina* increased. The *Artemia salina* reached adult stage in 16-19 days when cultured in a salt pond [13]. It attained adult stage in freshwater (2-4ppt) on the 20th day, in brackishwater (28-33ppt) on the 17th day, and in seawater (34-55 ppt) on the 14th day [11]. They recorded the maximum size of the adult *Artemia salina* (1.2cm) in seawater and the minimum size in the freshwater (0.4cm). The culture was performed outdoor, and the water in the tanks during the study was exchanged once every two days. In the present study, *Artemia salina* attained the adult stage in 13-15 days while the salinity was maintained at 80-90 ppt in each replicate. Water was also exchanged at the same rate as the experiment mentioned above. Triantaphyllidis *et al.* [14] reported that a parthenogenitic population did well at salinities of 60 ppt and 100 ppt while at 35, 40 and 80 ppt the survival was less than 50% after 27 days of culture. Soundarapandian and Saravanakumar[11] report that adult *Artemia salina* size in their study was around 1cm.

A number of studies of different *Artemia salina* strains showed a substantial difference in size between sexes [12,15,16] , which was also observed during the present study. The females were observed to be larger than the males, and the average size of the male and female *Artemia salina* was found to be 1.1 cm. Water temperature is probably the most important environmental variable in culturing *Artemia salina* because it directly affects metabolism, oxygen consumption, growth, moulting and overall survival. Soundarapandian and Saravanakumar[11], studying the effect of salinity on *Artemia salina* biomass production maintained the water temperature at 29-32°C as lowering the temperature would result in slow growth and cause mortality if it exceeded 30°C. In the present study, water temperature (°C) in the three replicates, R-1, R-2 and R-3, was recorded as 26.75, 29.13and 30.45 respectively, which is regarded as optimum for *Artemia salina* growth. With regard to oxygen, only low concentrations (less than 2 mg O_2/L) will limit the production of biomass. For optimal production, however, O_2 concentrations higher than 2.5 mg O_2/L are recommended.

Oxygen levels higher than 5 mg/L resulted in the production of pale animals (low in the respiratory pigment, haemoglobin), with a lower individual dry weight[10]. The observed dissolved oxygen levels in the water in rearing tanks in the present study did not undergo wide variations in any of the replicates, and continued to be within the optimum range (4.06, 3.84, and 4.01 respectively in R1, R2 and R3).

pH is one of the vital environmental characteristics, which impacts the survival, and growth of *Artemia salina* under culture conditions. It also affects the metabolism and other physiological processes of *Artemia salina.* pH range of the rearing medium was 7.8-8.5, 8.2-8.9 and 8.5-9.3 respectively for the freshwater, brackish water and seawater in the study reported by Soundarapandian and Saravanakumar[11]. Camargo *et al.* [17] found *Artemia salina* living in nature in neutral to alkaline waters, at temperatures generally below 34°C, and at rather low O_2 levels, and Dhont and Lavens[10] report that it is generally accepted that the pH tolerance for *Artemia salina* ranges from 6.5 to 8.

The average pH values recorded in the present study were 7.67, 7.66, 7.73 for replicates R-1, R-2 and R-3 respectively, which conforms to the findings of the studies mentioned above. Since *Artemia salina* are non-selective filter feeders, a wide range of food has been successfully used in culturing various strains of the species. According to Asil *et al.*[3], the criteria for food selection should be based on particle size, digestibility, and solubility. Feeds that have been used include live microalgae such as nanochloropsis and a wide variety of inert foods, which are far more practical for aquarists to use. Inert feeds include yeasts, both active and inactive, micronized rice bran, whey, wheat flour, soybean powder, fish meal, egg yolk, and homogenized liver.

In the present study, agricultural byproducts, such as rice bran, oil-cake and small portion cod liver oil were used as cheap food sources for the culture of *Artemia salina* which have been recommended by [10]Dhont and Lavens[10] as a cost-effective alternative. In the present study, the best growth and higher rate of survival of *Artemia salina* was found in the tanks pre-enriched with decomposed organic particles, micro-algae and bacteria. Dry feeds, consisting of fragments and irregular particles, cannot be counted in the culture tank, therefore, the feed concentration in a culture tank is recommended to be determined by measuring the transparency of the water with a simplified Secchi-disc[10]. During the present study, the food, consisting of egg yolk, rice bran, chicken manure, oil cake and cod liver oil, was added to the experimental tank until the water became turbid which yielded satisfactory results.

CONCLUSIONS

The present study revealed that production of *Artemia salina* cyst using crude salt, where brine is unavailable, is possible throughout the country in controlled environment in both the monsoon and non-monsoon seasons. Dissemination of knowledge about the proposed technique to raise brine shrimp as live feed can enhance the aquaculture production in the country and make possible exploitation of other species of fish and crustacea for both the subsistence farmers and the country's wider fisheries industry. It can also save a significant amount of foreign currency for the country as Bangladesh is heavily dependent on the import of *Artemia salina* cyst.

Conflicts of interest: The authors declare there are no conflicts of interest.

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REFERENCES

1. S.M. Asil, A.E. Fereidouni, H. Ouraji, & K.J. Khalili,. Effects of different light intensities on growth, survival, reproductive and life span characteristics of *Artemia salina* urmiana (Günther 1890). *Aquaculture Research*, 2013, 44 (4): 554-566. [DOI: 10.1111/j.1365-2109.2011.03058.x]
2. W.N.Camargo,J.S. Ely,G.C. Duran and P.Sorgeloos Influence of some physicochemical parameters on Artemia salina biomass and cyst production in some thalassohaline aquatic environments from the Colombian Caribbean. *J. World. Aqua. Soc.*, 2004, 35: 296-305. [DOI: 10.1111/j.1749-7345.2004.tb01085]
3. J.S. Clegg and Trotman, C.N.A., 2002. Physiological and biochemical aspects of Artemia salina ecology. In: Abatzopoulos TJ, Beardmore JA, Clegg JS, Sorgeloos P, editors. *Artemia salina*: Basic and applied Ichthiology. *Biology of aquatic organisms*, 1: 129-170.
4. M.De Donato & C.Graziani, Determinacion de las caracterısticas biometricas para la evaluacion de tres razas de *Artemia salina* en Venezuela. Saber, Ano 6, 1993. 5, 20–25.
5. E.Del Castillo-Arias & B.C. Farfan, Hydrobiology of a salt pan from the Peninsula of Baja California, Mexico. *International Journal of Salt Lake Research*., 6, 1997, 233–248.
6. P. Dhert, R.B. Bombeo, P. Lavens and P. Sorgeloos,. A simple semi flow-through culture technique for the controlled super-intensive production of *Artemia salina* juveniles and adults. *Aquacultural Engineering*. 11, 1992, 107-119. [DOI: 10.1016/0144-8609(92)90013-N]
7. J. Dhont And P. Lavens, Tank production and use of on grown *Artemia salina*. Laboratory of Aquaculture & *Artemia salina* Reference Center, University of Gent, Belgium. FAO Fisheries Technical Paper. No. 361. Rome, FAO, 1996, p32.
8. A.R.A. Gobino, Z. Carlos, M. Roberto and M.M.M. Alenjandro,. Ecological and biological notes on the brine shrimp *Artemia salina* (Crustacea: Branchiopoda: Anostraca) from Carmen Island, Baja California Sur, Mexico. *Hydrobiologia*, 560, 2006, 417–423. [Doi10.1007/s10750-005-1562x]
9. S. Kulasekarapandian and P. Ravichandran, *Artemia salina* cyst production at Kelambakkam near Chennai. *J. Mar. Biol. Ass. India*. 45, 2003, 166-177.
10. N. Mahmood, S.M.B. Haider and S.Q. Saikat, Study of production performances of Artemia cysts of different geographical strains in the coastal saltpans of Bangladesh. Final report, BARC Contract Research Scheme; Institute of Marine Sciences and Fisheries, University of Chittagong, Bangladesh. 1992, p.32.
11. N. Mahmood, S.M.B. Haider and S.Q. Saikat, Successful inoculation of *Artemia salina* and production of cysts in the coastal saltpans of Bangladesh. *Pakistan J. Mar. Sci.*, 2, 1993, 23-31.
12. N. Mahmood, M.A. Islam and S.Q. Saikat, *Artemia salina* biomass and cyst production trial in a coastal saltpan of Bangladesh. Abs. No. 63. 1994. 9th Nat. Zool. Conf. Zool. Soc. Bangladesh. Dhaka.
13. L.C.A. Naegel & Rodriguez, Ecological observations and biomass proximate composition of the brine shrimp *Artemia salina* (Crustacea: Anostraca) from Pichilingue, Baja California Sur, Mexico. In Maeda-

Martinez, A. M., B. V. Timms, D. C. Rogers, F. A. Abreu-Grobois & G. Murugan(eds.), Studies on Large Branchiopod Biology, 4. *Hydrobiologia*, 486, 2002, 185–190.

14. L. Ronald, G. Stappen, N. Hoa, &P. Sorgeloos; Effect of carbon/nitrogen ratio manipulation in feed supplements on *Artemia salina* production and water quality in solar salt ponds in the Mekong Delta, Vietnam. *Aquaculture Research. 2013,* [DOI: 10.1111/are.12135]
15. P. Soundarapandian and G. Saravanakumar, Effect of Different Salinities on the Survival and Growth of *Artemia salina*. *Current Research Journal of Biological Sciences*, 1, 2009, 20-22.
16. G. Triantaphyllidis,V. Poulopoulou, T.I. Abatzopoulos, A.A.P. Perez and P. Sorgeloos,. International study on *Artemia salina* XLIX. Salinity effects on survival, maturity, growth, biometrics, reproductive and lifespan characteristic of a bisexual and a parthenogenitc population of *Artemia salina* .*Hydrobiologia*, 302, 1995, 215-227.
17. G.Van Stappen, Introduction, biology and ecology of *Artemia salina*. In Lavens, P. & P. Sorgeloos (eds), Manual on the Production and Use of Live Food for Aquaculture. FAO Fisheries Technical Paper, pp. 199679–130.

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