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Production of *Artemia* Biomass in Indoor Culture Tank in Bangladesh

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Abstract

Artemia known as brine shrimp that rich in essential amino acids. An excellent food for newly hatched fish and shrimp larvae gained a unique position in the aquaculture system and is given as live feed. The study was carried out to develop an easiest and cheapest technology for the production of *Artemia* biomass and cyst in the coastal area. Crude salt collected from the salt pan was used to biomass culture of *Artemia* species. Agricultural by-products (e.g. rice bran, oil-cake, and cod liver oil) were used as food. Adult *Artemia* sp. was harvested at the age of 15 days after the inoculation of cyst in a hatching tank. From hatching tank, newly hatched nauplii were transferred to larval rearing tank. 63.06 gm (wet weight) of *Artemia* sp. were collected from each tank and the weight of each *Artemia* was found 0.0036 g. At the end of the experiment, the survival rate was found 46.12% based on first observation. A sharp growth rate of *Artemia* sp. was observed on day 11 and it was continued to day 13. At day 14 and onwards the growth of *Artemia* sp. became almost uniform.

Keywords: *Artemia*; Biomass; Production; Crude salt; Aquaculture.

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1. Introduction

In Bangladesh, aquaculture activities are currently expanding. Some endemic finfish and crustacean species seem to have aquaculture potential, but live food availability is one of the major constraints for the cultural development of these species. Studies on *Artemia* populations represent an alternative for the exploitation of natural resources and promoting the development of the local aquaculture industry. Potentially, *Artemia* sp. is an excellent food source, which could provide quality feed for fish and crustaceans [1,2] in sufficient amounts and at the proper times for the growing aquaculture industry of this country.

Artemia, popularly known as brine shrimp, are small brachiopod crustaceans found in natural salt lakes or man-made salterns [3]. *Artemia* has unique biology, producing

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dormant cysts that can be dried, transported and hatched on demand. *Artemia* sp. is the most popular live larval food currently used in the aquaculture industry. A single location, the Great Salt Lake in Utah (USA), supplies approximately 90% of the world's commercial *Artemia* sp. cyst production. *Artemia* sp. is euryhaline organisms, capable of living and reproducing at a salinity range of 5 to 200 (ppt), the widest range of salinity of any multicellular organism. However, they are only commonly found at salinities greater than 70 ppt, where their aquatic predators cannot survive [4]. *Artemia* sp. also synthesizes very efficient respiratory pigments which allow them to survive in low oxygen levels found in high salinities [4]. In addition, they present two modes of reproduction: ovoviviparous (producing free-swimming nauplius larvae released by the mother from fertilized eggs when conditions are stable) and oviparous (producing dormant cysts in diapause when conditions are extreme or unfavorable).

The most interesting feature of this crustacean in respect of aquaculture is that it serves as an important food for early stages of shrimp and other organisms in aquaria and small ponds having no natural foods. Being live food, the larvae of *Artemia* are readily taken by aquatic animals under nursery conditions without fouling the aquarium water. As such the brine shrimp, *Artemia* is widely used by aquaculturist as an excellent live food for crustacean and fish [5]. It constitutes the principal ration and frequently the only food for the larvae and juveniles of many cultured species, such as freshwater prawns (*Macrobrachium* sp.), shrimp (penaeids), lobsters (*Homarus* sp.), crabs and various finfish [2,6,7]. Adult *Artemia* may be preferred over nauplii while they are fed by larger fish and invertebrates.

Various systems have been developed for the controlled culture of *Artemia*. Intensive culture systems, such as semi flow-through systems for indoor use, and extensive culture systems, such as high salinity, static outdoor culture ponds, have been used to culture *Artemia* with rice bran and organic wastes [8-11]. Another option for *Artemia* production is the use of artificial or man-made high-salinity ecosystems, such as permanent solar salt operations or seasonal (artisanal) salt ponds. However, a common problem observed in these culture systems is the decline of cyst production over time. A recently developed alternative, the "multi-cycle system", has been successful in maintaining elevated yields of cyst production in small Salinas in Vietnam and Thailand [12,13]. In this system, culture cycles only last for 5-6 weeks. At the end of each cycle, the culture ponds are drained, the remaining *Artemia* are killed and the pond is re-inoculated [14].

In 1976, at the Kyoto Food and Agriculture Organization (Technical Conference on Aquaculture), the supply of *Artemia* cysts was seen as a restrictive factor in the development of world aquaculture. Intensive research has been conducted since then to attempt to switch live larval foods by artificial diets, but *Artemia* cysts are still the larval food of choice for over 80% of the aquatic species cultured so far [11]. This bottleneck in the expansion of world aquaculture has led researchers to look for alternative production sites.

In Bangladesh, Karim [15], Mahmood and Begum [16] initiated laboratory scale research work on *Artemia*. Mahmood [17] studied the growth of *Artemia* in the coastal

saltpans for the first time. From January 1988 to December 1989 *Artemia* cyst production in the coastal saltpans in Bangladesh came up successful [18-20]. Three more successful trials were made by the teachers and graduates of the Institute of Marine Sciences and Fisheries in 1992 and 1994 [21-23]. Although, they have developed a technology to produce *Artemia* cyst and biomass further progress on such type of research was not continued.

The present study was carried out to develop an easiest and cheapest technology for the production of *Artemia* biomass and cyst in the coastal area as well as other parts of the country using crude salt where brine is not available. As salinity drastically falls below the required level during monsoon (June-November), it is not possible or economically viable to carry out the outdoor culture of *Artemia*. In fact, such a gap can be filled with the introduction of the controlled culture of *Artemia* in indoor tank providing pre-stock of crude salt from the salt farms. This culture system has the viability in case of economic and cultural point of view both for the hatchery owners and subsistence level poor salt-farmers because the culture set-up can easily be constructed with the locally available cheapest instruments.

2. Materials and Methods

2.1. *Study area*

The present research was carried out in the Institute of Marine Sciences and Fisheries, University of Chittagong, Chittagong (Fig. 1).

2.2. *Sample collection*

The crude salt was collected from the salt producing fields of Banskhali, Chittagong by using bags made of plastic material. Crude salt was stored in a plastic container and thus it allowed to mix with water. Mud and debris of the collected salt were isolated after washing it with water repeatedly. Saline water of different gradation was prepared from crude salt and it was allowed to remain in static condition for settling the suspended solids and other debris in the bottom of the plastic container. *Artemia* cysts used in the present experiment were San Francisco Bay Brand that was collected from the ADB hatchery, Cox's Bazar. This brand seemed to be a better quality rather those the brands of China, The Philippines, Singapore, and Thailand [21,24].

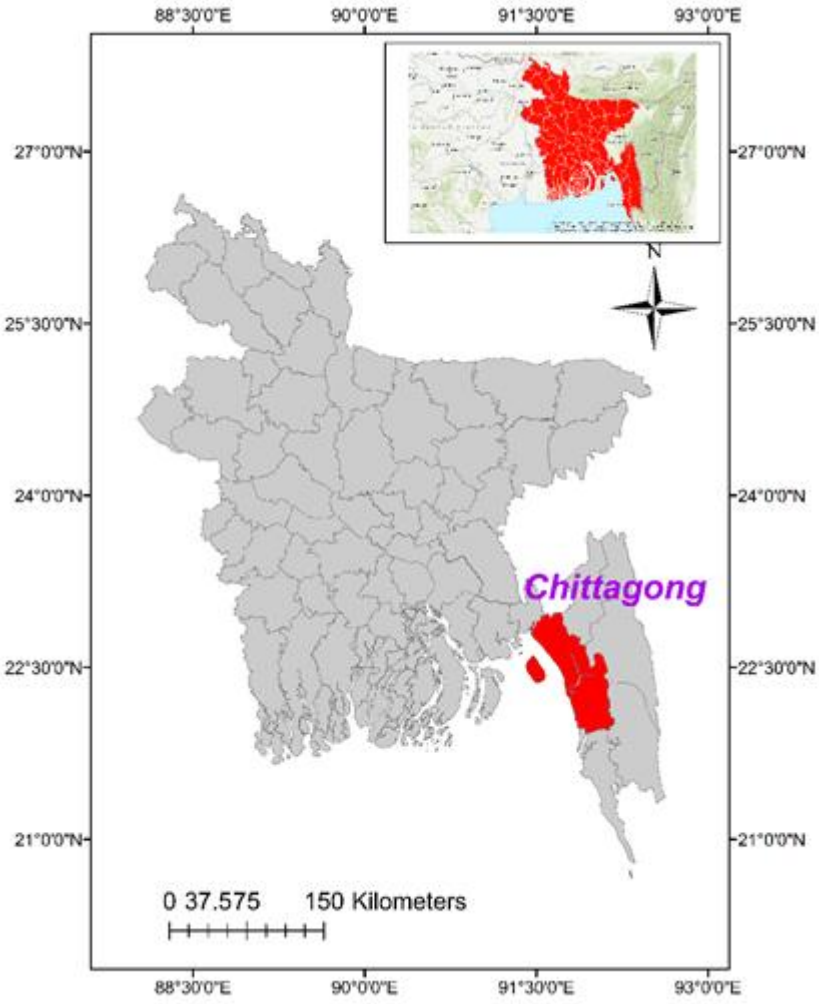

Fig. 1. Map showing the experimental sites of the study area (map created by ArcGIS 10.3).

2.3. *Experimental setup*

Three tanks (30"×18"×16") made of plastic material were set up in the laboratory. Before starting the experiment, all the tanks were washed thoroughly with clean water. Necessary equipment (aerator, refractometer, thermometer, dissolved oxygen (DO) bottle, Hanna pH meter (HI 9124 HI 9125, China), microbalance, burette, pipette etc.) were collected and set in the laboratory for measuring the water parameters and biomass of *Artemia* at different concentrations. The tanks were kept at a position of the laboratory where sunlight could not bring any deleterious effects. Mechanical aeration was provided for maintaining the optimum level of dissolved oxygen. Each tank was filled with 30 L of

saline water and 0.75 g of *Artemia* cyst/tank was provided and cultured in the tank up to 15 days for getting biomass. Total biomass was measured by using microbalance (Electrical Analytical Balance, OSK 11325A).

2.4. *Determination of water parameters*

Water quality variables such as temperature, DO, hydrogen ions concentration and salinity were measured periodically. DO was determined following the Azide modification of Winkler Methods [25]. A centigrade laboratory thermometer, an electrical pH meter, and a refractometer were used to measure water temperature, pH and salinity respectively.

2.5. *Hatching*

Three 'V' shaped plastic containers containing 1.5 L of saline water (35 ppt) were used for hatching *Artemia* cyst that was collected from the local market. *Artemia* cyst (0.75 grams/container) was provided to each tank with continuous vigorous aeration. After hatching nauplii were harvested by turning off the air flow of the aerator and let the culture settling for ten min. Since nauplii were attracted by light, they were concentrated at the bottom of the 'V' shaped container where sufficient light was ensured. Nauplii were collected from the bottom of each container through siphon out and transferred to the rearing tanks previously enriched with organic matter, bacteria and algae at the subsequent stage.

2.6. *Inoculation and feeding*

Artemia nauplii were separated and inoculated in the rearing tanks after hatching. The water of the tanks was previously enriched with organic matter by decomposing rice bran with moderate aeration for one week to grow algae and bacteria. This type of food was utilized by *Artemia* as primary feed. Naupli became pre-adult after 5-6 days of hatching. At this stage, a mixture of egg yolk, cod liver oil, rice bran, and oil cake was supplied as food at a rate of 0.5 mL/L. Feeding schedule was determined on the basis of water transparency. Broadcasting of food in the culture tanks continued until the water became turbid. Moderate and continuous aeration was provided to keep the food in suspension and supply O_2 to *Artemia*.

2.7. *Measuring technique of Artemia biomass*

A homogenous distribution of *Artemia* in water of the tanks was ensured by giving vigorous aeration before sampling. The 100 mL water sample was taken from each tank to estimate the density and biomass of *Artemia* by using random sampling. Biomass of *Artemia* was measured with digital electrical balance (Electrical Analytical Balance, OSK 11325A).

3. Results and Discussion

For an adequate production of *Artemia* in controlled conditions, water quality parameters should be maintained within an optimal range (salinity between 32-65 ppt, oxygen above 2 mg/L, temperature between 19-25 °C, and pH between 6.5-8.0). In this study water quality was considered optimal since no wide variations were observed for any of the variables [26]. *Artemia* (especially *Artemia salina)* individuals showed lower size values in higher salinity [27].

Lowering the temperature would result in a slow growth rate and a temperature above 30 °C would cause mortality. Hence in the present study water temperature was maintained in optimum level for *Artemia* growth. The temperatures in three tanks A, B, and C were 25.83333 ±0.859125 °C, 25.76667 ±0.883715 °C and 25.8 ±0.840918 °C, respectively.

In the present study, salinity increased from 35 to 65 ppt with time to control the degradation of excess feed and bacterial growth. Water pH was maintained 7.8 ±0.173205, 7.766667 ±0.154303 and 7.76 ±0.140408 in the three tanks A, B and C respectively. The level of Dissolved Oxygen was also maintained 3.12-6.8 mL/L. Recorded water parameters of *Artemia* rearing tank have been presented in Table 1.

Table 1. Water parameters of the *Artemia* biomass production tank.

Date	Salinity (ppt).			Water Temperature (°C)			Dissolved Oxygen (mL/L)			pH		
	Tank			Tank			Tank			Tank		
	A	B	C	A	B	C	A	B	C	A	B	C
25.03.07	35	35	35	24	24	24	6.4	6.8	6.4	7.6	7.6	7.6
26.03.07	35	35	35	24	24	24	6.2	6.4	6.0	7.6	7.6	7.6
27.03.07	35	35	35	25	25	25	5.56	5.56	5.56	7.6	7.6	7.6
28.03.07	35	35	35	25.5	25	25.5	5.42	5.22	5.42	7.7	7.6	7.7
29.03.07	40	40	40	26	26	26	4.82	4.54	4.54	7.8	7.8	7.8
30.03.07	40	40	40	26	26	26	5.22	5.56	4.82	7.6	7.6	7.6
31.03.07	40	40	40	26	26	26	4.54	4.54	4.24	7.6	7.6	7.6
01.04.07	45	45	45	26	26	26	4.24	4.7	4.7	8	7.8	7.8
02.04.07	50	50	50	26.5	26.5	26.5	3.47	3.47	4.22	7.9	7.9	7.9
03.04.07	55	55	55	26.5	26	26.5	3.47	3.47	3.47	7.8	7.8	7.8
04.04.07	60	60	60	26	26	26	4.16	4.12	4.20	7.8	7.8	7.8
05.04.07	65	65	65	26.5	26	26	3.12	3.50	3.50	8	7.9	7.8
06.04.07	65	65	65	26.5	26.5	26.5	3.40	3.12	3.47	8	7.9	7.8
07.04.07	65	65	65	26.5	26.5	26.5	3.12	3.12	3.12	8	8	8
08.04.07	65	65	65	26.5	27	26.5	4.24	3.47	3.40	8	8	8
Mean ±S.D.	48.67± 12.60	48.67± 12.60	48.67± 12.60	25.83 ± 0.86	25.77± 0.88	25.8± 0.84	4.49 ±1.1	4.51± 1.19	4.471± 1.02	7.8± 0.17	7.77± 0.15	7.76± 0.14

Artemia are non-selective filter feeders (meaning they don't care what they pick out of the water), a wide range of food has been successfully used. The criteria for food selection should be based on particle size, digestibility, and solubility (powdered milk won't work). 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. Inert feeds include yeasts, both active and inactive (a brewers supply is the best source, bread yeast is expensive) micronsized 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 intensive culture of *Artemia* up to the adult stage as a cost-effective alternative following Dhont and Lavens [26].

Ansari [28] reported that nutritional requirement of *Artemia* was very important and was successfully reared on a mono diet like pig dung, chicken dung, rice bran, wheat bran, yeast and diatom like *Nitzchia*, *Navicula* and *Chlorella* with simple technology. The *Artemia* grew almost equally in the three media such as chicken dung, yeast and rice bran [24]. Luxuriant growth and biomass of *Artemia* were found by Islam [23] and Mahmood [18] while they fertilized with chicken manure, rice bran, urea, TSP of the culture pond.

Tunsutapanich [29] reported that *Artemia* is a filter feeder and therefore it is omnivorous in food habit. *Artemia* can be directly fed with chicken feed, rice bran, minced fish and chopped chicken dung. In addition, when these foods become rotten, they turn into fertilizers which can serve to produce natural food for *Artemia.* The result of food habit coincides with the present result in that the *Artemia* grew more or less satisfactorily in the media such as rice bran, oil cake, and cod liver oil.

As the *Artemia* is filter feeder and omnivorous in food habit it can take decomposed food particle. In addition, its food consists of bacteria and other microorganisms [29]. Therefore the best growth and higher rate of survival were found in the pre-enrich tanks with decomposed organic particles, micro-algae, and bacteria in the present research.

Although some preliminary success have been attained through this experiment, a lot of more remains to be done on performances of different geographical strains of *Artemia* in our climate and soil, the nutritional value of local cysts, cyst collection and preservation; and transfer of technology to the farmer level.

Artemia became adult and began to reproduce after two weeks of hatching. Density and biomass of *Artemia* were recorded from the age of 6 days to 15 days after the inoculation of nauplii in the rearing tank. In the 1st observation (at day 6) 5.76 gm of *Artemia* (wet weight) were recorded from each tank and the weight of each *Artemia* was found 0.00015 g. At the end of the experiment (at day 15), 63.06 g of *Artemia* (wet weight) were collected from each tank and the weight of each *Artemia* was found 0.0036 gm. At the end of the experiment, the survival rate was found 46% based on first observation. Lavens and Sorgeloss [30] recorded 15.5 g *Artemia* biomass on day 8 and 10 g *Artemia* biomass on day 15 in the Great Salt Lake. Islam *et al.* [31] reported 0.576 g/L of *Artemia* biomass on day 6 and 3.007 g/L of *Artemia* biomass on day 16 in an indoor tank.

Table 2. Biomass and density of *Artemia* of three experimental tanks.

Age (day)	Tank	Weight/individuals (ind.) (g)	Density (ind./L)	Total Biomass (g)	Survival rate (%)
Day 1-5		-	-	-	
Day 6	A	0.000150	1280	5.76	-
	B	0.000150	1237	5.57	-
	C	0.000145	1314	5.71	-
	Mean	0.000148	1277	5.67	-
Day 7	A	0.00020	1152	6.91	90.00
	B	0.00021	1139	7.18	92.08
	C	0.00019	1160	6.61	88.28
	Mean	0.0002	1150	6.90	90.05
Day 8	A	0.00030	1000	9.00	78.13
	B	0.00029	976	8.49	78.90
	C	0.00027	1011	8.19	76.94
	Mean	0.000287	996	8.57	78.00
Day 9	A	0.00038	896	10.21	70.00
	B	0.00040	888	10.66	71.79
	C	0.00038	912	10.40	69.41
	Mean	0.000387	899	10.43	70.40
Day 10	A	0.0006	680	12.24	53.13
	B	0.0006	659	11.87	53.27
	C	0.0006	725	13.05	55.18
	Mean	0.0006	688	12.38	53.88
Day 11	A	0.0010	640	19.20	50.00
	B	0.0009	621	16.76	50.20
	C	0.0012	672	24.19	51.14
	Mean	0.00103	644	19.91	50.43
Day 12	A	0.0024	624	44.93	48.75
	B	0.0020	592	35.52	47.86
	C	0.0025	659	49.44	50.15
	Mean	0.0023	625	43.13	48.94
Day 13	A	0.0032	608	58.37	47.50
	B	0.0034	579	59.08	46.81
	C	0.0031	637	59.22	48.48
	Mean	0.00323	608	58.92	47.61
Day 14	A	0.0035	600	63.00	46.88
	B	0.0034	566	57.77	45.76
	C	0.0034	621	63.32	47.26
	Mean	0.00343	596	61.30	46.67
Day 15	A	0.0036	595	64.28	46.48
	B	0.0036	560	60.48	45.27
	C	0.0035	611	64.18	46.50
	Mean	0.00357	589	63.06	46.12

Highest density (1314 individuals/L) but the lowest biomass (0.00015 g/individual) was recorded in the 1st observation. The density of *Artemia* gradually decreased with the increase of biomass (Table 2). A sharp growth rate of *Artemia* was observed on day 11 and it was continued to day 13 (Fig. 2). Islam *et al.* [31] recorded the highest density (800 individuals/L) at the first observation and the lowest density (619 individuals/L) was recorded on day 30.

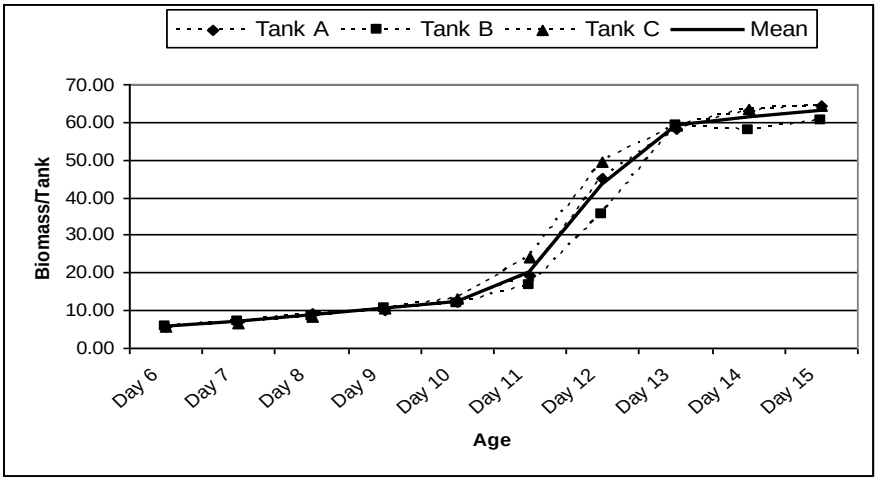

Fig. 2. The growth rate of *Artemia* biomass (g).

4. Conclusion

In comparison with the other research findings, the present findings showed a sharp growth rate of *Artemia* sp. at day 11 and it continued to day 13. At day 14 and onwards growth of *Artemia* sp. became almost unchanging. This result indicates a good growth rate of *Artemia* under control condition. Further research is needed to make the findings more reliable.

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