

EFFECT OF DIFFERENT FEEDS AND WATER SALINITIES ON THE CYST PRODUCTION OF BRINE SHRIMP, *ARTEMIA* SP.

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ABSTRACT

Effect of different feeds and salinities on cyst production in *Artemia* sp. was studied. Among the 12 different feeds used, *ragi* powder suspension showed the best growth and cyst production. A wide range of salinity from 90 to 210 ‰ was used to study its effect on cyst production. Among all these treatments, 130 ‰ salinity was found suitable for induction of oviparity and cyst production in the San Francisco Bay strain of *Artemia* sp.

Keywords : *Artemia* sp., oviparity, cyst, feeds, reproductive capacity

INTRODUCTION

The special characters of *Artemia* spp., namely capabilities to grow at high density, high nutritive value and higher assimilation by culture organisms, make it an ideal live-feed in aquaculture. All the life stages of *Artemia* spp. are used as feed in aquaculture operation according to the feed size requirement of predator. The decapsulated cysts are used as direct food source for crustaceans like *Penaeus monodon* (Wilkenfeld *et al.*, 1984) and *Macrobrachium rosenbergii* (Lavina and Figuerosa, 1980). The freshly hatched nauplii of brine shrimp have been used as live feed for the larvae of *M. rosenbergii* (Ling, 1969; Raje and Joshi, 1992), groupers and sea bass (Tookwinas, 1989). The adult *Artemia* spp. have also been recorded to be an ideal live-feed for stage-8 larvae of *M. rosenbergii* (Aquacop, 1977).

A more promising approach to understand the control of reproduction mode in *Artemia* spp. may lie in investigation into the common physiological pathways whereby diverse environmental factors induce a switch from oviparity (Berthelemy-Okazaki and Hedgecock, 1987). This mode of reproduction in *Artemia* spp. is greatly affected by the environmental conditions in nature, but which factor exactly controls the mode of reproduction is still unknown. However, no single environmental factor is clearly responsible for controlling reproductive mode; moreover, interactions among several factors appear to be significant.

In view of the vital importance of *Artemia* spp. in hatchery operations, the cultivation of brine shrimp to produce cyst

is being attempted in all parts of the world, but the quality of such cysts is seldom good. During cyst production of *Artemia* spp., appropriate feed becomes the most important pre-requisite. Salinity of water is another important parameter that contributes to the oviparity and cyst production in *Artemia* spp. The impact of salinity on cyst production has been studied by some workers, to name a few, De Los Santos *et al.* (1980), Lavens and Sorgeloos (1984), and Joshi and Mehta (1994). However, the impact of feed quality on cyst production in different salinities is not clearly known. The effect of different feeds on the growth of *Artemia* spp. has also been reported by some workers, namely De Los Santos *et al.* (1980), Johnson (1980), Coutteau *et al.* (1990); Kadri *et al.* (1992) and Rahman and Rathinasamy (1997). But, information on the effect of salinity variation on growth is meagre.

Thus, the impact of water salinity and feed on growth and cyst production of *Artemia* spp. together has not been investigated and reported so far. Therefore, the present piece of research was attempted.

MATERIAL AND METHODS

1. Cyst hatching and rearing containers:

The San Francisco Bay bisexual strain cysts were used in all the experiments. White plastic funnels of 2 l Capacities were used as hatching containers. The container was filled with 2 l of saline water (35 ‰) and aerated vigorously. One gramme of cysts was hatched in this water. Majority of the cysts hatched within 25 hours, after

which aeration was discontinued. A powerful beam of light was focused on the stem of the funnel so as to concentrate *Artemia* spp. nauplii which were siphoned out easily.

White coloured enamel trays of 8 l capacity (35 x 50 cm) were used for mass culture of the nauplii till maturity. Mature females along with males were taken out and placed individually in plastic bottles (25 ml capacity), along with an adult male each. After a brood had taken place, all the cysts or nauplii were removed carefully from the rearing bottle. For rearing of the animals, the water of desired salinity was prepared by dissolving appropriate quantity of crude salt in fresh water.

2. Feed preparation

a) Algal feeds

The pure unicellular algal cultures required during the experiment were procured from the culture laboratory of algal feeds at the College of Fisheries, Ratnagiri.

b) Other feeds

The dried or powdered feeds like groundnut oil cake, rice bran; wheat bran, etc. were soaked in saline water for one hour and then ground in a kitchen blender. This mixture was then passed through sieves of different mesh sizes so as to get feed particles appropriate to animals of different age as given below:

Age (d)	Feed particle size (µm)
1 to 5	25 to 30
6 to 10	30 to 40
11 onwards	40 to 50

It was difficult to decide the optimal quantity of food to be distributed; hence turbidity stick (8 cm long) was used for trays. Turbidity stick was first submerged; then feed was added in trays until the bottom plate was still just visible. However, when adult individual pairs were reared in the plastic bottle, a measured quantity of feed at 0.05 mg per animal was used.

3. Experiments conducted

The different experiments conducted during the study are shown in the flow-chart given below:

Flow-chart showing different experiments	
Experiment	Duration (d)
1. Screening test for feeds	30
↓ Suitable feed selected.	
2. Effect of broad range of salinities on growth and cyst yield of <i>Artemia</i> sp.	30
↓ Optimum salinity range found out.	
3. Effect of narrow range of salinities on growth and cyst yield of <i>Artemia</i> sp.	45
↓ Optimum salinity found out.	

4. Abiotic factors during the experiments

Water salinity varied during different experiments because it was one of the parameters to be studied. All the experiments were carried out at ambient temperature. The water pH in all the experiments was maintained at 8.1 to 8.2. The water salinity was estimated by titrimetric method (Strickland and Parsons, 1972). Water pH was recorded with a pH meter (American Marine INC; range 4.0 to 9.2).

5. Statistical analysis

All the experiments were carried out in triplicate and the results were analysed by standard single factor ANOVA.

RESULTS AND DISCUSSION

Experiment - 1

Screening test for feeds

In this experiment, twelve different feeds were fed to nauplii of *Artemia* sp. to study their acceptability by the animal and also their effect on growth and cyst production (Table 1). Salinity of the culture media was maintained at 50 ‰ for the first five days and then it was increased to 100‰. It was retained at this level till the termination of the experiment. With groundnut oil cake, animals grew to adults of 11.00 mm in length in a period of 15 days. While using other feeds, namely rice bran, ragi powder and *Tetraselmis* spp., a size of 9.5 mm was obtained in identical period. The feeds, namely wheat flour, fishmeal and rice powder, caused mortality and therefore, were found to be unsuitable. When the cyst yield per female was considered, it was found to be the best with ragi followed by baker's yeast. Significant difference ($P < 0.05$) was observed in cyst production using different feeds. *Artemia* sp. fed with *Spirulina* sp., *Tetraselmis* spp., wheat bran and rice bran produced 109, 97, 96 and 91 cysts, respectively. *Spirulina* sp., baker's yeast and *Tetraselmis* spp. even though showed better results were rejected due to the higher cost involved.

A number of reports are available on the use of different feeds on the growth of

Table 1: Feed acceptability test for the growth and cyst yield of *Artemia* sp.

Sr. no.	Feed used	Growth (mm)					Cyst yield per female (15 days)	Remarks
		Day						
		3	6	9	12	15		
1	<i>Cheatoceros</i> spp.	2.40	4.50	6.00	7.50	8.00	56.00±7.23	Feed availability poor
2	<i>Tetraselmis</i> spp.	2.40	4.30	6.10	7.70	9.50	97.00±23.58	Feed availability poor
3	<i>Spirulina</i> spp.	2.30	4.40	6.20	8.00	9.00	109.00±8.14	Costly feed
4	Rice bran	2.40	4.50	6.10	8.00	9.50	91.00±10.21	Feed satisfactory
5	Baker's yeast	2.50	4.40	6.00	7.50	9.00	132.00±14.25	Costly feed
6	Soybean meal	2.40	4.00	5.90	7.00	8.20	-	Water quality bad
7	Ragi powder	2.50	4.50	6.20	9.00	9.50	156.00±26.64	Feed satisfactory
8	Groundnut oil cake	2.30	4.50	6.00	8.50	11.00	91.00±8.76	Feed satisfactory
9	Wheat flour	2.00	-	-	-	-	-	Total mortality on 4 th day
10	Fish meal	2.05	-	-	-	-	-	Total mortality on 6 th day
11	Rice powder	2.0	4.10	5.90	-	-	-	Total mortality on 10 th day
12	Wheat bran	2.0	3.90	6.00	7.60	9.00	96.00±12.49	Feed satisfactory

Artemia spp. Bossuyt and Sorgeloos (1980) used a suspension of rice bran in saturated brine for batch culturing of *Artemia* sp. at high densities. Douillet (1987) used five different diets at three different concentrations on the San Francisco Bay strain of *Artemia* sp. He could not find any relationship between feeds used and growth obtained. Johnson (1980) evaluated the growth and survival of the Brazil strain of *Artemia* spp. by using five different types of diets and concluded that lipid in diet is essential for continued fertility of *Artemia* sp. George *et al.* (1995) reported that the survival, growth rate, fecundity and nutritional quality of *Artemia* sp. cultured using live algae were the best. Rehman and Rathinasamy (1997) used rice bran as feed and reported that the number of eggs per brood pouch ranged between 41 and 71. Thus, reports regarding use of different diets for growing *Artemia* sp. biomass are available in plenty. However, reports regarding the effect of feed on either oviparity or cyst production of the animal are scanty. Dwivedi *et al.* (1980) found that using yeast as feed, 13% females became oviparous, while with algal diet, it was 23%. Versichele and Sorgeloos (1980) reported that a diet containing *Spirulina* sp. and rice bran induces oviparity in *Artemia* spp. (SFB strain) while the algae *Scenedesmus* spp. and *Dunaliella* spp. proved to be less efficient in inducing oviparity.

Among the twelve feeds used in this experiment, *ragi* powder was the feed that led to the best growth of the animal, induced oviparity and yielded the highest number of cysts in 15 days. Hence, *ragi* powder was adjudged as the best feed and used in subsequent experiments.

Experiment - 2

Effect of different salinities (broad range) on growth and cyst production of *Artemia* sp.

In this experiment effect of different salinities (broad range) on growth and cyst yield of *Artemia* sp. was worked out. The growing animals were noted to be most healthy and active in 120 and 150 ‰ saline waters. The animals attained the highest mean length of 11.5 mm in 120 ‰ salinity. At 120 and 150 ‰ salinity, pairing was observed on the 11th day, while in 90 and 180 ‰ this was noted on the 13th day. *Artemia* sp. reared in 210 ‰ rearing media were sluggish and the growth was slow from the beginning. Total mortality was observed on the 4th day of rearing in 210 ‰ rearing media.

Reproductive capacity is the capacity to produce cyst. The reproductive capacity of females as indicated by the colour of the females, showed that on the 15th day, the animals were light pink with red spots (Table 2) in 120 and 150 ‰ rearing salinity. On the 18th day, females in the same rearing media were dark-red and started producing cysts. In 130 ‰ the females produced cysts only on 15th day. Conversely, at 90 ‰ the first brood produced nauplii on the 18th day.

Thus in the present work, red females or light-pink females with red spots produced nauplii in the first brood, in 90, 120, 150 and 180 ‰ while dark-red females always produced cysts in different salinities. From the 21st day onwards, females in 180 ‰ were red, while females in 90, 120, and 150 ‰ salinity were dark-red in colour and produced cysts exclusively. When the

Table 2: Effect of salinity (broad range) on growth and cyst yield of *Artemia* sp.

Sr. no.	Rearing salinity (‰)	Growth (mm)					Reproductive capacity			Cyst yield per female (15 days)	Remarks
		Day					Day				
		3	6	9	12	15	15	18	21		
1	90	2.70	4.50	6.80	9.00	9.40	++	+++ (N)	++++ (C)	87.00±2.920	Pair formation on 13 th day
2	120	2.50	5.50	7.05	10.00	11.50	++	++++ (C)	++++ (C)	193.00±4.371	Pair formation on 11 th day
3	150	2.40	4.50	6.20	9.00	9.50	++	++++ (C)	++++ (C)	126.00±5.229	Pair formation on 11 th day
4	180	2.20	4.30	5.70	8.00	8.00	+	++ (C)	+++ (C)	57.00±4.580	Pair formation on 13 th day
5	210	2.20	-	-	-	-	-	-	-	-	Total mortality on 4 th day

+ = Light pink, ++ = Light pink with few red spots, +++ = Red, ++++ = Dark red
(N) = Nauplii produced; (C) = Cyst produced

Table 3 A: Effect of different salinities (narrow range) on growth, survival and oviparity of *Artemia* sp.

Salinity (‰)	Growth (mm) after 15 th day	Survival (%) after 15 th day	Oviparity (%) after days						
			15	20	25	30	35	40	45
120	10.70±0.61	68.00±5.50	1.00±0.57	10.00±2.08	61.66±10.17	84.00±4.16	91.33±2.84	91.00±5.56	79.66±4.05
130	10.60±0.35	66.00±8.02	1.00±0.57	9.00±4.58	64.66±6.43	80.66±3.52	92.00±2.08	90.00±2.64	82.33±3.48
140	9.80±0.58	52.00±5.29	0.33±0.33	6.33±1.20	43.33±6.88	71.00±9.45	65.00±9.33	68.00±4.72	61.33±1.45
150	9.00±0.83	49.00±7.55	0.66±0.33	4.66±1.45	52.00±7.93	62.66±6.93	52.33±8.35	45.33±2.66	38.33±11.92

Table 3 B: Yield of *Artemia* sp. cyst during 30 days from different salinity (narrow range) combinations

Replicate	Salinity (‰)			
	120	130	140	150
1	272	335	286	211
2	244	300	192	106
3	170	371	169	202
Mean	229	335	216	173

average cyst production by single female in different salinities was observed, it was recorded to be the highest in 120 ‰ salinity (193 cysts/15 days). In 150 ‰ salinity, 126 cysts were produced in the same period. At 90 and 180 ‰ salinity, the rate of cyst production slowed down. Significant difference ($P > 0.05$) was not observed in cyst production due to different salinities.

Experiment - 3

Effect of narrow range salinity on growth and cyst production of *Artemia* sp.

In this experiment, the effect of rearing salinity in the narrow range of 120-150 ‰ was studied using *ragi* as feed to find out growth and survival in 15 days period and cyst production for 30 days period. The effect of salinity on growth and survival is presented in table 3 A. The growth after 15 days was noted to be the best in 120 ‰ rearing media (10.7 mm) followed by 130 ‰ (10.6 mm), while in 150 ‰ the animal grew to 9.0 mm size in the same period. The survival was also almost the same in 120 and 130 ‰ (68 and 66%, respectively). The animals were observed to be most healthy and active in 120 and 130 ‰ waters. Oviparity studies were undertaken on the basis of colour of brood pouch of the female

in case of ovoviviparous females. The percentage of oviparous females in different salinities clearly indicated that in all the salinity treatments, the percentage of oviparous females was found to increase from the 15th to 35th day. On the 15th day in 120 and 130 ‰ salinity, 1.00% of females were oviparous, which increased to 91.33 and 92.00% on the 35th day in 120 and 130 ‰ waters, respectively. However, on the 35th day in 140 and 150 ‰ salinity 65.00 and 52.33% females, respectively were recorded to be oviparous. From the 40th day onwards, the percentage of oviparous females decreased slightly. This decrease continued till the 45th day. The yield of *Artemia* sp. cyst (Table 3 B) during the 30-d period in varying salinities, namely 120, 130, 140 and 150 ‰ showed that the mean cyst production is the highest in 130 ‰ (335 numbers) followed by 120 ‰ (229 numbers). The ANOVA reveals significant difference ($P < 0.05$) in cyst production using different salinities.

Quite a few reports are available on growth and its relationship with salinity and the reports are contradictory. While working on a parthenogenetic population of *Artemia* spp., George *et al.* (1995) reported that the bisexual strain showed the best growth performance at 35 ‰ while no

striking difference in growth was noted at 100, 140 and 150 ‰. A better growth and survival was reported by Dwivedi *et al.* (1980) at 45, 60 and 75 ‰ salinity than at 30 ‰. Majumdar (1995) reported that 75-80 ‰ is the salinity suitable for cyst production while Berthelemy-Okazaki *et al.* (1987) found better cyst production at 120 ‰ salinity. Douillet (1987) while working on the SFB strain of *Artemia* sp. studied the effect of four different salinities namely 30, 60, 90 and 120 ‰ and concluded that rearing at 60 ‰ results in the best growth. He further reported that the brine shrimp fed with *Spirulina* spp. at 60 ‰ reached a greater length than when fed on other diets. Working on a similar line, Vanhaecke *et al.* (1984) found that the optimal conditions for the SFB strain were 62 ‰ salinity and 26.6⁰ C temperature. Baid (1963) reported that *A. salina* reared in 115 ‰ salinity matured in 22 days. He further reported that *A. salina* reared at 125‰ salinity have a higher growth rate compared to 65 ‰ salinity.

The present relationship between the colour of animals and oviparity is supported by earlier reports of Versichele and Sorgeloos (1980). In the present work, it was recorded that females with white shell glands produced nauplii and those with brown shell glands deposited cysts. Baker (1966) reported the presence of iron in culture medium to be responsible for cyst production. *Ragi* powder, which was used as a feed for *Artemia* spp. in the present work, is reported to be rich in iron content at 5.4 mg/kg (Anon, 1952). This high content of iron might have stimulated oviparity in females. Bowen *et al.* (1969) stated that the iron content in feed or algae

is the most important component to induce oviparity of *A. salina*. Working on an identical line. Versichele and Sorgeloos (1980) reported cyclic oxygen stresses and the presence of chelated iron acting in concert to stimulate cyst production by 20 and 30%, respectively, compared to their single effect. However, Chow (1968) stated that animal colouration is only a crude way to estimate the haemoglobin concentration and therefore according to him, the proposed correlation between animal colouration and mode of reproduction should be reconsidered. However, in the present work, it has been found very clearly that red and dark-red animals invariably produced cysts only.

Like the feed, salinity also plays an important role in the induction of oviparity in *Artemia* spp. Dutrieu (1960) suggested that under low oxygen concentration, excretion of hematin via shell gland situated in the ovisac induces cyst dormancy. According to the theory put forth by D'Agostino (1980) and Berthelemy-Okazaki *et al.* (1987), the first brood of *Artemia* spp. is invariably ovoviviparous. This statement was found to be true in the present work only up to a limited extent as in 90 and 180 ‰ rearing media the first brood produced was nauplii while in 120 and 150 ‰ the first brood produced was only cyst.

In the present experiment, mean cyst production in 30 days was found to be 229, 335, 216 and 173 numbers in 120, 130, 140, and 150 ‰ respectively. It shows that cyst production decreases as salinity is raised to 140 and 150 ‰. However, contradicting with this observation, Berthelemy-Okazaki *et al.* (1987) reported that water salinity

above 120 ‰ inhibits cyst production. Thus, the present experiment, where the effect of different water salinities, on the growth, oviparity and cyst production of the animals using ragi powder as a feed was studied, it was revealed that under the present experimental conditions, 130 ‰ may be the suitable water salinity for getting better cyst production.

CONCLUSION

An appropriate feed and a specific salinity were found to positively influence the cyst production in the San Francisco Bay strain of *Artemia* sp. Among the twelve different feeds used, the locally available ragi powder suspension was found to be the most suitable feed for cyst production. A wide range of salinity from 90 to 210 ‰ was used for rearing of *Artemia* sp. to study the effect of varying salinities on cyst production. Among all these salinities, 130 ‰ was noted to be the optimal one. When animals were reared in 130 ‰ salinity and fed with appropriate dose of ragi powder suspension, the cyst production was at its highest.

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