

Laboratory and field studies on an Indian strain of the brine shrimp *Artemia*

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Abstract

The Tuticorin strain of the brine shrimp *Artemia* was studied under both laboratory and field conditions. Studies on the survival of the nauplii at different temperatures and salinities revealed that the nauplii preferred a salinity of 35 ‰ and a temperature of 27 °C. Biochemical changes during the larval stages showed a decreasing trend in protein, lipid and caloric contents. The effects of different inert feeds such as *Spirulina*, rice bran and yeast on the growth of *Artemia* are also discussed.

Introduction

In recent years, there has been a great demand for quality brine shrimp cysts in aquaculture, with the demand exceeding the supply. This has necessitated scientists from all over the world to concentrate on the culture of brine shrimp in salt pans and saline lakes. India, having a coastline of 5 700 km and extensive areas of saline ponds, lakes, and salt pans (Fig. 1) offers unique opportunities for the mass culture of *Artemia*. There are many geographical strains of brine shrimp reported from India (Kulkarni, 1953 ; Baid, 1958 ; Royan *et al.*, 1970 ; Achari, 1971 ; Royan, 1979), but none of the strains has yet been completely studied. The present paper summarises the results of some laboratory experiments and a few observations made on natural populations of one of the Indian strains (Tuticorin) of brine shrimp.

Materials and methods

For the laboratory studies, the cysts collected from a natural population of *Artemia* (Tuticorin) were used.

Experiments on the survival of the nauplii in different salinities and at different temperatures were carried out following the methods described by Sorgeloos *et al.* (1976). A *Spirulina* suspension was given as food ; the experiments lasted for 96 hr. Fig. 2 gives the mean percentage of survival of nauplii at different salinities and temperatures.

Biochemical composition and caloric contents of cysts, decapsulated cysts, nauplii-instar I, II, III, and metanauplii were determined by ash-, carbohydrate-, protein-, lipid-, and energy analysis. Ash content was determined by combusting the dry samples in a muffle furnace at

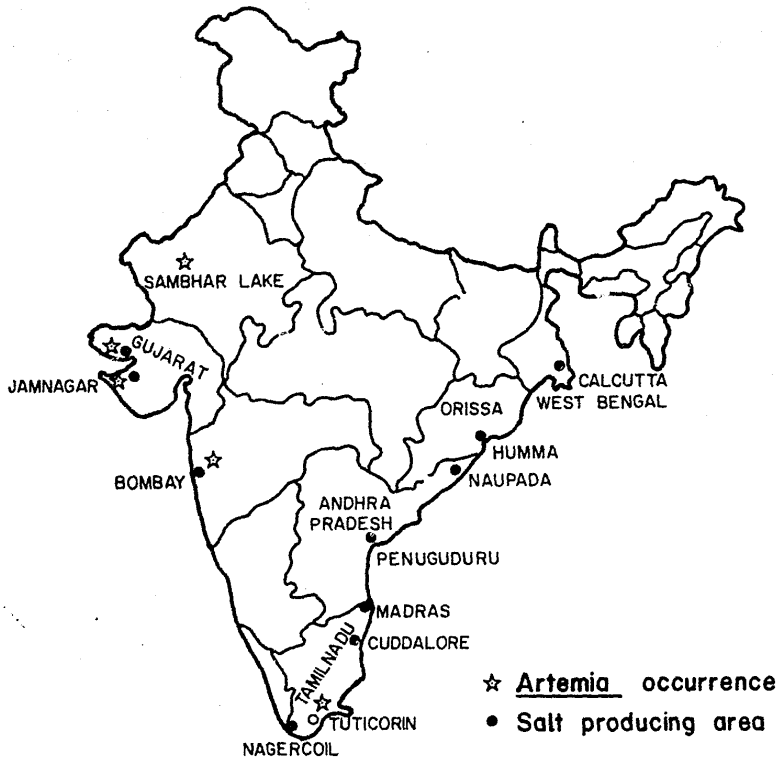


FIG. 1. Salt producing areas along the coastline of India and locations of *Artemia* occurrence.

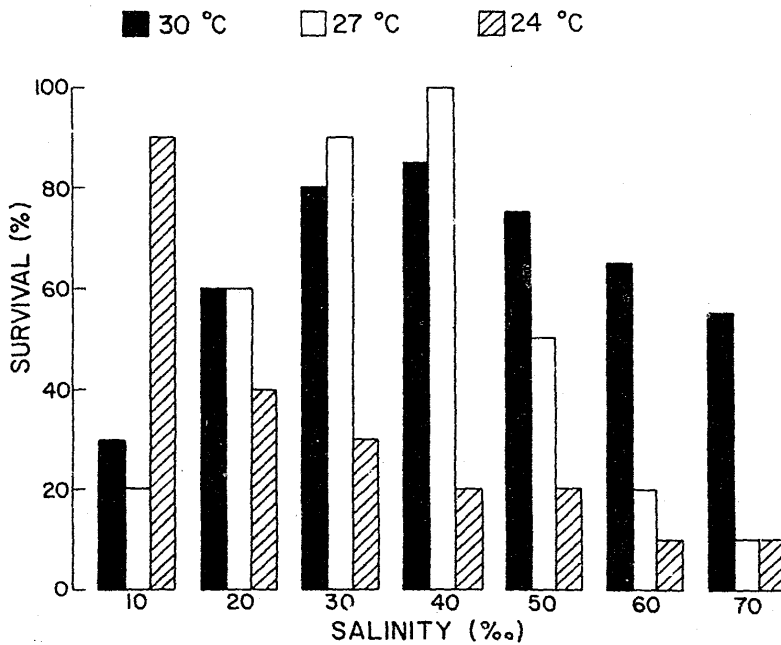


FIG. 2. Percentage survival of nauplii at different salinities and temperatures.

500 °C for 4-5 hr. Biochemical constituents such as carbohydrate, total protein, and lipid were estimated following the procedures of Dubois *et al.* (1956) Raymont *et al.* (1964) and Folch and Stanley (1956) respectively. The caloric content was determined in an adiabatic bomb calorimeter (Parr 1200). Table I shows the variations in biochemical constituents and energy contents at different stages of development.

TABLE I
Changes in chemical composition of eggs, decapsulated eggs,
different naupliar instars and metanauplius of *Artemia*
(all values are based on dry weights)

	Egg	Decapsulated Egg	Instar			Metanauplius
			I	II	III	
Number/mg	262	296	357	400	714	909
Protein (%)	58.00	61.14	58.00	56.80	51.98	49.91
Lipid (%)	25.64	25.57	23.30	21.00	20.90	18.00
Carbohydrate (%)	7.76	11.05	12.80	10.41	11.21	6.30
Ash (%)	6.23	4.15	5.74	8.30	12.00	20.83
Energy (cal/g ash-free dry wt)	4 924	5 448	5 241	4 915	4 821	3 827

The effect of different types of food on the growth rate of this strain was studied under laboratory conditions. 3 500 nauplii of a length of 0.6 ± 0.05 mm were transferred to plastic troughs containing 7 l of seawater (35 ‰ salinity and 30 °C temperature). Three types of feeds, namely dried *Spirulina*, rice bran, and baker's yeast, were given at a daily ration of 100 mg/100 animals/day during the first week ; during the later part of the experiment, the ration was increased to 400 mg/100 animals/day. Food was given twice daily and the water was changed every second day. Fig. 3 shows the average length (mm) of the animals at different moments for different feeds. Animals were observed daily under a dissection microscope for the formation of an egg pouch and also to find out the exact day of formation of the eggs. The number of eggs per female was counted until the adults attained the maximum body length.

In addition to growth studies in small plastic troughs, large scale rearing experiments were carried out by growing *Artemia* in a large concrete tank (approximately 6 m × 3 m × 0.7 m). The salinity was 35 ‰ at the time of inoculation of the nauplii and 140 ‰ towards the end. The tank was enriched with nitrogen and phosphorus salts in the ratio of 15:1 in order to stimulate growth of a natural population of algae. As the salinity increased due to evaporation, the algae began to die and to sediment. No food was added during the experiment since the decomposing algae provided a good source of nourishment. The *Artemia* population in the tank was about 500 nauplii/l. Fig. 3 shows the average length (mm) observed at different moments. A maximal length of 10.2 mm was obtained at the end of the experimental period.

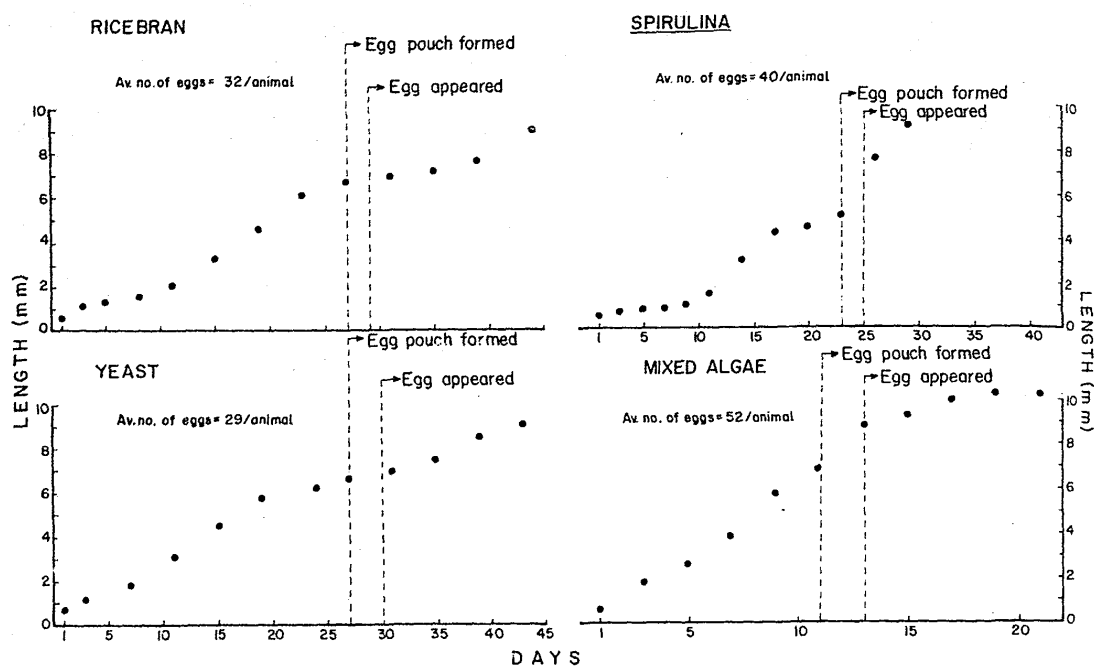


FIG. 3. Average length of the animals at different moments for various feeds.

Results and discussion

It was already reported (Royan, 1976ab) that the Indian strain (Tuticorin) of *Artemia* has a maximum hatching at 35 ‰ salinity and an optimum temperature of 30 °C. Sorgeloos and Persoone (1975) while reviewing the literature showed that different geographical strains of *Artemia* have different salinity and temperature optima for hatching. Similar observations had been reported earlier by Boone and Bazz-Becking (1931), Urbani (1959) and Von Hentig (1971).

Experiments on the influence of light on hatching of the cysts showed that variation in light intensities had little influence on the hatching, whereas in the dark the hatching was very low (23%) (Royan, 1976b). This corroborates the finding of Sorgeloos (1973) who reported that light triggered the hatching mechanism in the dormant cysts irrespective (beyond a minimum threshold) of the light intensity. He also stated that darkness decreased the percentage of hatching to a large extent.

Though *Artemia* has been reported to be euryhaline and eurythermal (Bayly, 1972), Sorgeloos *et al.*, (1976) showed that this was not a general rule; the latter authors indeed observed that certain strains such as the one of the San Francisco Bay to be euryhaline, while some others (Bulgaria) were stenohaline and stenothermal. The optimal survival of the Indian strain seems to be situated in the salinity range 30 to 40 ‰. However, with a decrease in temperature, the optimal salinity also decreased. The observed preferential temperature was 27 °C, a temperature usually met within peninsular Indian waters.

Benijts *et al.* (1976) reported that in the San Francisco Bay strain the individual caloric content (ash-free) decreased by 27% from the instar I to the instar II stage. Further, as also shown in the present work, they reported a similar increase in ash weight and drop in lipid content with growth. In the present study, the protein content was observed to decrease during growth from instar I to metanauplii. Claus *et al.* (1978) working with Utah and San Francisco Bay strains observed that both in fed and starved conditions, the percentage of protein in the larvae increased during growth in the first 48 hr. They also observed marked differences in the protein synthesis between these two strains. They further reported a decrease in lipid and carbohydrate content during the same time period, pointing to variations of certain biochemical constituents from strain to strain during growth. Variations in temperature and salinity conditions may have a marked influence on the biochemical composition of different stages. Von Hentig (1971) reported a marked difference in protein, lipid, and carbohydrate contents in the Utah strain hatched under different temperature and salinity conditions. Paffenhöfer (1967) reported that the Utah strain from Great Salt Lake showed marked changes in its biochemical composition after a starvation period of 96 hr at 31 °C. Thus, the variations in the biochemical composition reported in the present study could be an unique feature of this Indian strain coupled to the influence of temperature (hatching and rearing of the nauplii at 30 °C).

The growth observed with the three different foods showed marked differences. Of the three types of food, *Spirulina* seemed to provide the best growth, as well as the shortest time needed for formation of the egg pouch and eggs. The average number of eggs was also high in comparison to that of the other two sets of animals fed different diets. Animals fed rice bran and yeast showed egg pouch formation on the 27th day, the *Spirulina* fed animals on the 23rd day. Irrespective of the types of food, all the animals showed formation of eggs within 3 days from the date of formation of the egg pouch.

Much work has already been done on the growth of *Artemia* (different geographical strains) fed with different types of food. Ivleva (1969) reported that a Russian strain (food not mentioned) took 35 days to attain the maximum size. Sorgeloos and Persoone (1975) observed that *Artemia* grown on a diet of live algae attained the adult stage within 2 weeks. Teramoto and Kinoshita (1961) even reported that *Artemia* grown on baker's yeast or acetone butanol fermentation waste, grew to the adult size within 6 to 7 days and that spawning took place within 11 days. Reeve (1963) on the other hand reported that the Utah strain fed with live algal cells needed 54 days for the completion of growth under laboratory conditions. Baid (1963) reported that an Indian strain (Rajasthan) fed with yeast took 32 days to become sexually mature.

It thus seems evident that the time taken for the completion of growth and maturity largely depends on the nature of the strain under study, the type of food, the ration and the culture conditions. Our studies showed that *Spirulina* could be preferentially used for growing the Indian strain both under laboratory conditions and for mass culture. Sorgeloos and Persoone (1975) have already recommended the use of dried *Spirulina* as food for brine shrimp.

The results obtained by growing *Artemia* nauplii in a bigger tank were somewhat different from those obtained in plastic troughs. A maximum length of 10.2 mm was attained at the end of the 19th day. Egg pouch and egg formation were observed on the 11th and 13th day respectively. The average number of eggs observed was 52/animal. Thus, a faster growth was observed in the animals reared in the tank.

Different types of feeds have so far been tried out to mass culture brine shrimps. Nimura (1967) reported that the use of any artificial feed in mass culturing resulted in higher mortality in the larval stages as compared to a live algal diet. Sorgeloos and Persoone (1975) have recommended the use of *Scenedesmus* or *Spirulina* for mass culturing purposes.

Recently it has been demonstrated that rice bran as well as other agricultural wastes can be considered as a suitable food for brine shrimp (Sorgeloos, 1978).

In the tank experiments, the food material provided was nothing but settled decomposing mixed natural phytoplankton. The potential food value of mixed phytoplankton had been shown by many workers (Provasoli, 1969).

Table II gives the percentage of assimilation efficiency, gross growth efficiency and net growth efficiency in the shrimp *Metapenaeus monoceros* (Fabricius) fed with different stages of brine shrimp including decapsulated cysts (Royan, 1980). These results prove that the decapsulated cysts could be used profitably in all aquaculture hatcheries. This could be attributed to the high caloric value (Table I) of the decapsulated eggs. Sumitrá-Vijayaraghavan *et al.* (1978) have shown that, of the 16 different types of feed tried for the shrimp *M. monoceros*, maximum conversion efficiency was found when the shrimps were fed adult *Artemia*. Table II shows that decapsulated eggs are even superior to adult *Artemia*. The advantages of using decapsulated eggs in aquaculture have recently been summarized by Bruggeman *et al.* (1979).

TABLE II
Percentage values of assimilation efficiency (A/C), gross growth efficiency (P/C or K_1), net growth efficiency (P/A or K_2), relative growth rate (RGR), and relative consumption (RC) in *Metapenaeus monoceros* fed different stages of *Artemia*

Food	A/C	K_1	K_2	RGR (mg/ \bar{w} /d)	RC (mg/ \bar{w} /d)
Decapsulated eggs	88.15	45.90	52.07	62.30	135.7
Freshly hatched nauplii	92.28	37.09	40.19	80.30	216.6
Juveniles	89.32	26.67	29.86	62.60	234.7
Adults	79.10	30.00	37.90	39.00	240.0

Finally some observations were made in an earlier study on a natural population of brine shrimp in a salt pan near Tuticorin. The population was traced in different condenser pans and information was obtained on the percentage occurrence of oviparous versus ovoviviparous adults in the population, the density of the animals in different condenser pans, the total cyst production per hectare, *etc.* (Royan *et al.*, 1978).

Summary

From the present study, certain general conclusions can be drawn on the biology of the Indian strain of *Artemia*. Under both laboratory and field conditions reproduction was found to be parthenogenetic with predominant oviparity. For the survival of the nauplii, a salinity of

35‰ and a tropical water temperature of 27 °C were close to optimal. Under laboratory conditions, *Spirulina* was found to be the most suitable of the various feeds tried. An experiment in a larger container with a greater depth of the water column, and with decomposing natural phytoplankton as food gave an even faster growth than in the laboratory trials. Decapsulated eggs and first instar nauplii gave a better conversion efficiency than adult *Artemia* in feeding the shrimp *Metapenaeus monoceros*.

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