

Life history of the brine shrimp *Artemia parthenogenetica* Sri Lanka (Crustacea: Branchiopoda: Anostraca: Artemiidae)

M.M. Kuruppu¹ & S.U.K. Ekaratne²

Abstract

Development and life cycle of Sri Lankan *Artemia* were studied in sea water of 35 ppt salinity under constant aeration at 29°C. Cysts from Mahalewaya saltern were incubated and hatched according to standard methods. Rupturing of cyst shell began with the appearance of a fissure in the cyst wall. This fissure was clearly visible after 9 to 10 hours of incubation and after 12 hours, the embryo began to protrude through the fissure. After 21 hours, hatching was completed with the release of an orange-coloured instar I nauplius which had a body length of $475.4 \pm 1.62 \mu\text{m}$ (mean \pm s.e.m.). This instar I nauplius possessed three pairs of appendages, but there was no gut as it was non-feeding.

Eight hours after hatching, the instar II nauplius stage was reached, having a pale orange colour, possessing a gut and feeding on algal cells. The instar III carried a bifurcate gnathobase, larval mandibles and a labrum. Paired eyes were visible in instar IV and the development of thoracopods commenced as lobular protrusions. Thoracopods developed, one pair per day, until there were 11 pairs. *Artemia* reached the pre-adult stage, complete with eight abdominal segments, on day 12. The broodpouch and a pair of ovaries were visible on day 13 and on day 14, there were ova within the broodpouch. Free-swimming nauplii were released on the 15th day. Ovoviviparous reproduction occurred every 3 to 4 days thereafter.

Oviparous reproduction took place at 132 ppt salinity on the 46th day, when salinities were increased at the rate of 4 ppt day⁻¹. Ovoviviparity occurred at 68 nauplii parent⁻¹ while oviparity was at 37 cysts parent⁻¹.

The study showed that *Artemia parthenogenetica* Sri Lanka was a parthenogenetic strain, able to develop and reproduce ovoviviparously within a period of 15 days after hatching. No males were observed during the experiment.

KEY WORDS: Crustacea, *Artemia*, brine-shrimp, life history, nauplii, cysts, saltern, hypersaline.

Introduction

The genus *Artemia* inhabits hypersaline environments and has been reported from many parts of the world (Persoone & Sorgeloos, 1980). *Artemia* in Sri Lanka was first reported in 1981 (Anon., 1981). In the updated list reporting the worldwide distribution of *Artemia*, Sri Lankan populations were recorded as occurring in Bundala, Hambantota, Puttalam and Palavi and were ascribed to the sibling species *Artemia parthenogenetica*, showing a parthenogenetic reproductive mode (Sorgeloos et al., 1986).

The first descriptions of *Artemia* were with reference to bisexual strains which were classified as *Artemia salina*, *A. Tunisiana*, *A. franciscana*, *A. Persimilis*, *A. urmiana* and *A. monica* (Sorgeloos et al., 1986). Barrigozzi (1980) recommended

¹ National Aquatic Resources Agency, Crow Island, Colombo 15, Sri Lanka.

² Department of Zoology, University of Colombo, Colombo 3, Sri Lanka.

that all parthenogenetic *Artemia* be referred to *Artemia parthenogenetica* followed by the vernacular or Latin name of the locality of origin. Although life histories of bisexual *Artemia* have been described (eg. Heath, 1924; Sorgeloos, 1980a; Schrehardt, 1987; Lenz & Dana, 1987), the parthenogenetic species has not received such attention. We describe here, for the first time, the life history of *Artemia parthenogenetica* Sri Lanka, based on laboratory-reared animals derived from natural populations occurring in Hambantota.

Materials and methods

Artemia cysts collected from the Mahalewaya salterns (6°08'N; 81°07'E) were hydrated in natural sea water of salinity 35 ppt and incubated under continuous aeration and illumination at a pH of 8.2 and a room temperature of 29±1 °C. The cysts under incubation were examined microscopically from time to time and samples were fixed in 5% formalin solution for making of drawings and preparation of photographic plates of different stages observed. The instar I nauplii emerging from cysts were separated from hatching debris and 50 nauplii were introduced into a conical flask containing a litre of water of 35 ppt salinity and cultured under constant aeration and natural photoperiod at 29±1 °C and a pH of 8.0±0.1. The *Artemia* nauplii were fed with the unicellular green alga *Tetraselmis suecica*. The *Artemia* culture was maintained in three replicates. Ten animals from each flask were examined microscopically daily and the features of development were recorded. The various stages were measured using a calibrated eye-piece micrometer and 3-4 animals of each developmental stage were preserved for preparation of photographs. Reproduction, through nauplii production, was studied using isolated adults held in individual culture vessels at 35 ppt salinity. Another set of individually held adults were used for cyst production studies, where salinities were gradually increased from 35 ppt (using common salt, at a rate of 4 ppt day⁻¹) until cyst production took place.

Algal cultures were maintained at 25 °C in a temperature controlled room and grown in Walne medium (Walne, 1979) in sterilized natural sea water of 35 ppt salinity with continuous aeration.

Abbreviations in figures: a, biconcave cyst; a1, first antenna; a2, second antenna; ab, abdomen; as, abdominal segmentation; b, spherical cyst; e, embryo; f, fissure; g, gut; h, hatching membrane; l, labrum; le, developing lateral eye; m, mandible; n, gnathobase; s, shell; t, thoracopods; u, anus; y, eye.

Results

Prior to hydration, *Artemia* cysts were dark brown and biconcave in shape. Within about an hour of hydration, the cysts gradually acquired a spherical shape (Figs. 1 and 4.2). After 9 to 10 hours of incubation, the embryo became visible through a fissure that appeared in the cyst shell (Figs. 2 and 4.3). After 12 hours, the embryo protruded through the fissure as an orange-coloured mass. The embryo developed into an oval shape covered by a thin membrane through which it was attached to the cyst shell (Figs. 3 and 4.5). A pair of lateral appendages could be seen on the embryo as lateral bulges. The eye was visible as a red spot. After 18 hours, slight movements could be observed within the membrane. After 21 hours of incubation, the embryonic membrane ruptured and the embryo, deep orange in colour, hatched out as the instar I nauplius. This sequence of stages in the hatching of *Artemia* is illustrated in Fig. 4.1-4.6

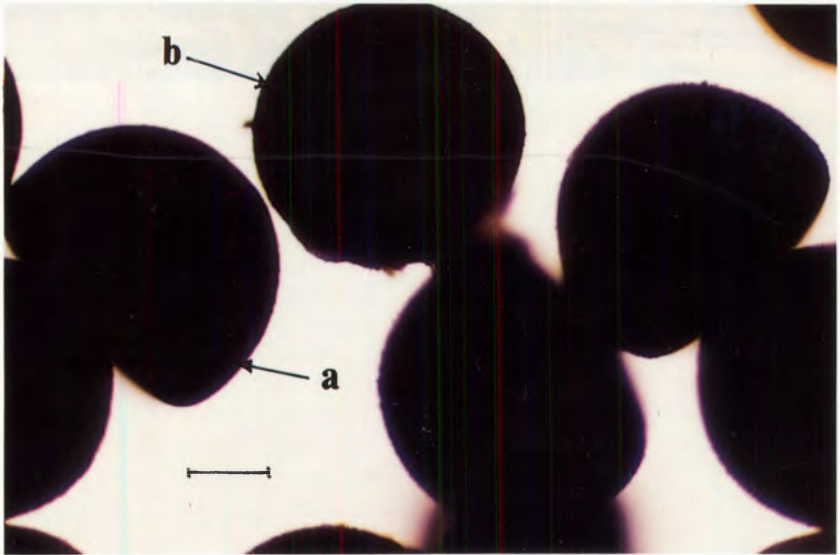


Figure 1. Cysts of *Artemia parthenogenetica* Sri Lanka showing biconcave nature before hydration (a) and change over to a spherical shape following one hour of hydration (b). Scale bar = 76µm. (All stages are in lateral view, unless specified otherwise.)

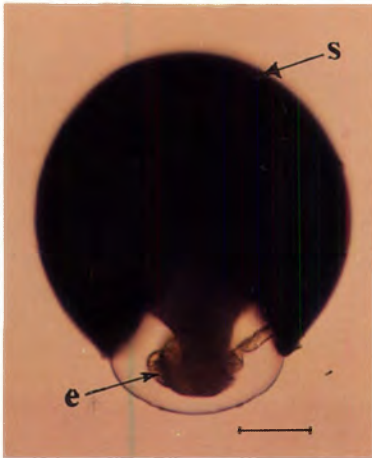


Figure 2. Cysts of *Artemia parthenogenetica* Sri Lanka showing embryo (e) beginning to protrude through fissure in cyst shell (s). Scale bar = 76µm.

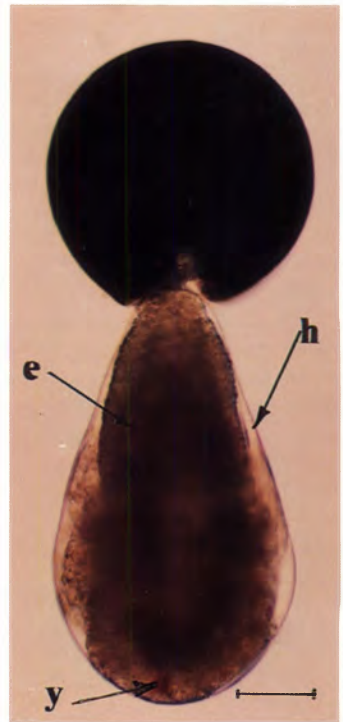


Figure 3. Oval-shaped pre-nauplius stage embryo (e) of *Artemia parthenogenetica* Sri Lanka showing the red eye (y) and the thin hatching membrane (h) that covers the embryo and continues to attach the embryo to the shell. Scale bar = 76µm.

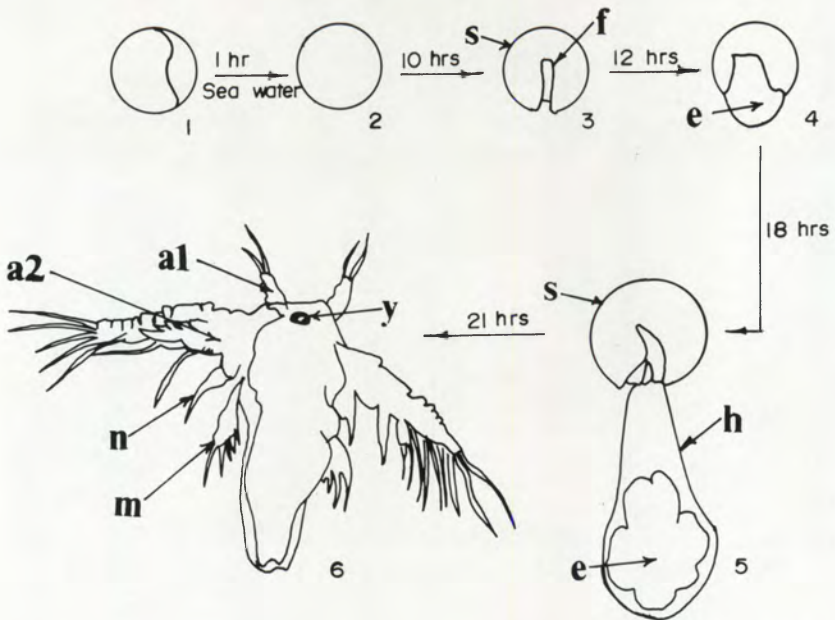


Figure 4. The sequence of stages in the hatching of *Artemia parthenogenetica* Sri Lanka. 4.1, biconcave cyst; 4.2, hydrated spherical cyst; 4.3, initiation of cyst shell breaking through a fissure (f) in shell (s); 4.4, embryo (e) beginning to protrude through fissure; 4.5, pre-nauplius stage embryo which has protruded completely from the shell (s) while being invested by the hatching membrane (h) by which it continues to attach to the shell; 4.6, instar I nauplius stage after release from hatching membrane, in dorso-lateral view showing median eye (y), first antenna (a1), second antenna (a2) and mandible (m). The gnathobase (n) is curved and non-bifurcate at this stage.

Instar I nauplius stage. The instar I nauplius was a non-feeding stage with a mean body length of $475.4 \pm 1.62 \mu\text{m}$. It was triangular in shape, the anterior region being broader than the posterior end and possessed three pairs of appendages, namely, the antennules, the antennae and the mandibles and an anteriorly placed single median eye (Figs. 4.6 and 5). The antennule was small and unjointed. Each antenna was made of three sections, the protopodite placed nearer the attachment to the body, the endopodite bearing three setae and the exopodite carrying ten setae. Situated adjacent to the body on the pair of antennae, there was a short gnathobase (Fig. 6.I) which possessed a single slightly curved spine. Development of the gut took place gradually in an antero-posterior direction. There was no anal opening. The nauplius moved by lashing the pair of antennae in an antero-posterior direction.

Instar II nauplius (metanauplius I) stage. Eight hours after the hatching of the first instar, it developed into the instar II stage having a mean length of $743 \pm 6.6 \mu\text{m}$. The body was pale orange in colour and lighter than in the instar I stage. Feeding commenced from this developmental stage, which possessed an open anus as well as a clearly defined digestive tract (Fig. 7). The gnathobase carrying small spines was long and pointed and was slightly curved towards the body

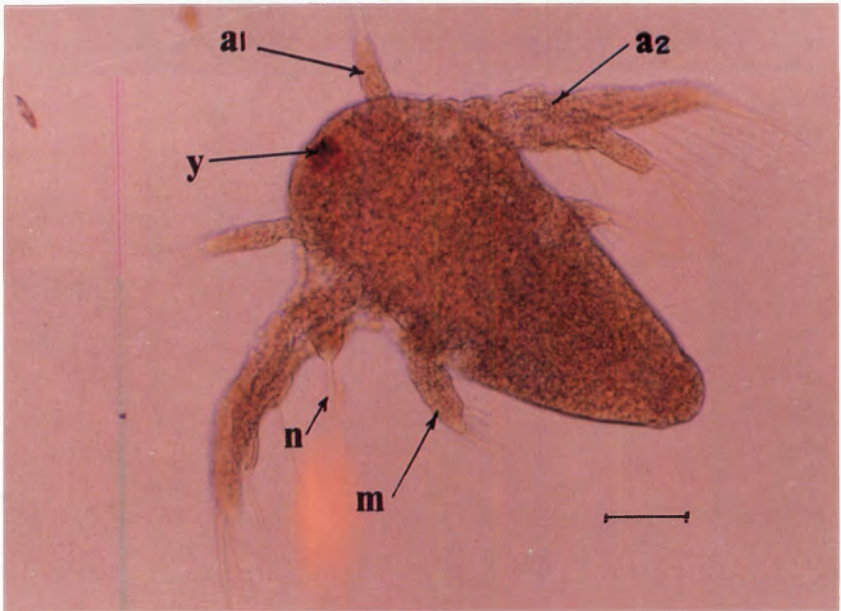


Figure 5. Instar I nauplius stage of *Artemia parthenogenetica* Sri Lanka after release from hatching membrane (h), in dorso-lateral view. For key see Figure 4.6. Scale bar = 76 μ m.

(Fig. 6.II). The median naupliar eye was pigmented and prominent (Fig. 7). The dorsal neck organ and the ventrally placed labrum on the head region were also visible.

Movements of the larva were accomplished by quick lashing movements of the antennae. The setae and spines on appendages assist in filtering algal cells from the surrounding environment and directing them towards the mouth. Ingested algal cells moved down the digestive tract of the trunk region.

Instar III nauplius (metanauplius II) stage. The instar III stage developed within 15 to 18 hours after hatching. This third stage of development possessed a bifurcate gnathobase (Fig 6.III) and had a mean body length of 892 \pm 5.0 μ m. The median eye was deeply pigmented. The larval mandibles and the labrum were seen clearly. The antennular setae were longer than in instar II. The trunk region of the body was slender and more elongated than in instar II (Fig. 8).

Instar IV nauplius (metanauplius III) stage. The instar IV stage developed one day after hatching. The mean body length was 1008 \pm 4.2 μ m and slight brown markings of paired eyes could be observed postero-laterally to the median eye. There were four thoracomeres each separated by intersegmental furrows and two pairs of lobular protrusions representing the swimming appendages or the thoracopods (Fig. 9). The antenna measured 50 μ m in total length. The first and second pairs of thoracopods were developing from the first two thoracomeres. The anterior portion of the intestine could be observed clearly and intestinal matter was observed moving towards the posterior with regular intestinal contractions. Anal contractions and ejection of faecal matter through the anus were



Figure 6. Structural development of the gnathobase (n) in second antenna in the developmental stages of *Artemia parthenogenetica* Sri Lanka. (I) in instar I stage; (II) in instar II stage; (III) in instar III stage.

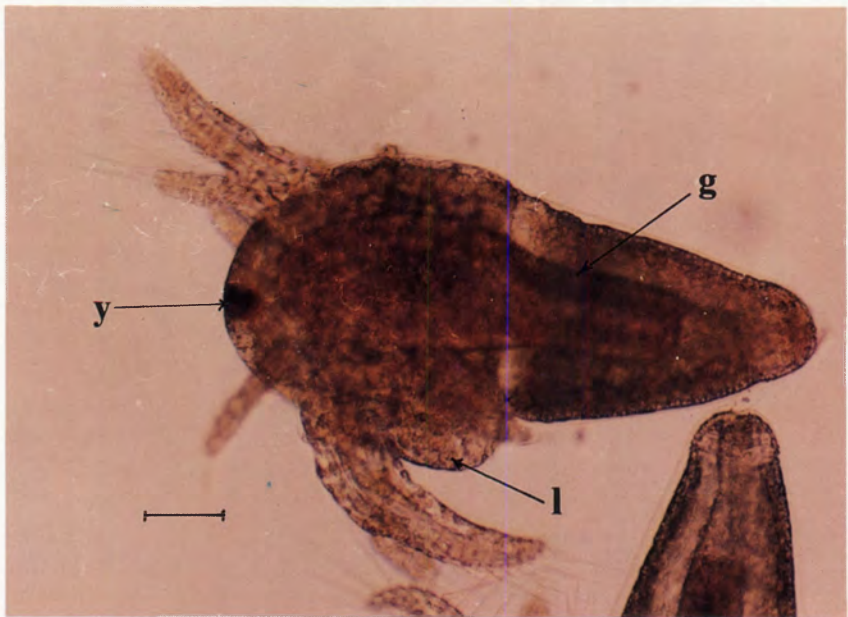


Figure 7. Instar II nauplius stage of *Artemia parthenogenetica* Sri Lanka, which is the first feeding stage, showing developed gut (g), median naupliar eye (y) and ventral labrum (l). Scale bar = 96µm.

observed. The abdominal region became demarcated with the development of thoracopods in the thoracic region. These thoracopods first appeared in the anterior thoracomeres. The abdomen was short at this stage and horizontal markings were seen in this region, indicating the first appearance of abdominal segmentation.

Further larval stages. At each larval stage, beginning with metanauplius II, a pair of thoracopods developed per day in each thoracomere, until there were 11 pairs. Each thoracopod became differentiated into three lobular parts carrying setae. Together with the pair of second antennae these thoracopods produced

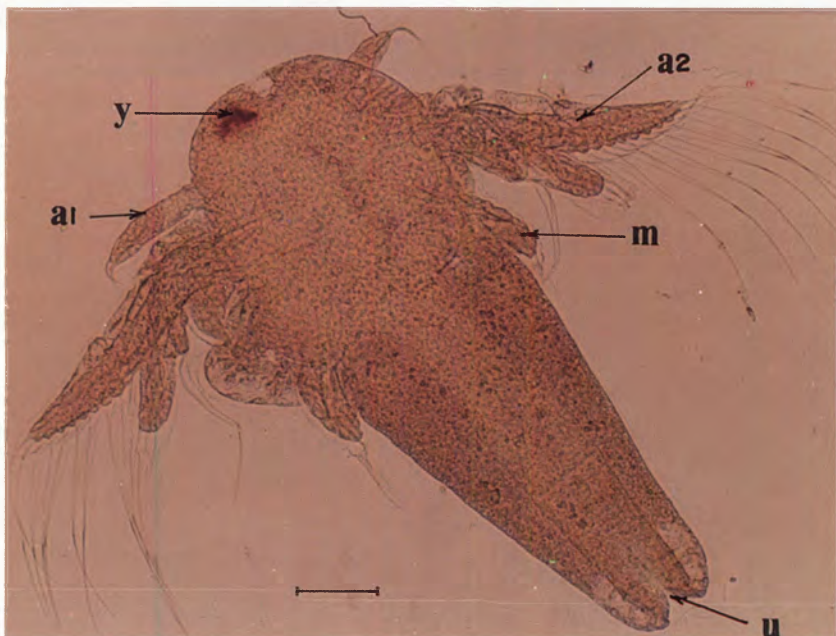


Figure 8. Instar III nauplius stage of *Artemia parthenogenetica* Sri Lanka in dorso-lateral view showing median eye (y), first antenna (a1), second antenna (a2), mandible (m) and anus (u). Scale bar = 100 μ m.



Figure 9. Instar IV nauplius stage of *A. parthenogenetica* Sri Lanka showing first antenna (a1), second antenna (a2), developing lateral eye (le), labrum (l), developing thoracopods (t), short abdomen (ab) and initial abdominal segmentation (as). Scale bar = 100 μ m.

propulsive strokes. At metanauplius IV stage, the paired lateral eyes became more prominent developing as brown markings. On the 7th day after hatching, the lateral eyes were slightly protruding out of the head. Segmentation became clear in the abdominal region. With further development the naupliar eye decreased in size and the second antennae became less setose and reduced in size. The lateral eye lobes began protruding out of the body with the development of the eye stalk.

Preadult stage. *Artemia* reached the pre-adult stage on the 12th day with the development of eight abdominal segments and 11 thoracopod pairs in the thoracic region. The last abdominal segment carried 3 pairs of spicules on the anal furca. One pair was short and curved towards the anus while the median pair was longer than the other spicules.

On the 12th day, the anterior region of the abdomen had become broader than the posterior region indicating the development of the broodpouch posterior to the last pair of thoracopods. Each anal furca carried 11 setae bearing bristles. The naupliar eye was reduced to a small dark spot.

Adult stage. On the 13th day, with the development of a well defined broodpouch, the adult stage was reached. The broodpouch protruded laterally from the abdomen and possessed a spine. The adults, 11.86 ± 0.09 mm in length, carried a pair of stalked eyes, a reduced pair of antennules and 11 pairs of thoracopods. A pair of ovaries was visible on the 13th day as elongated streaks along the sides of the abdomen. Traces of oviducts and slight demarcations of ova were seen within ovaries. The following day contraction and relaxation movements could be observed within the ovaries. The oviducts carried a linearly placed batch of 20 ova on either side of the abdomen ($n=1$). On the 14th day the ova formed a cluster within the broodpouch moving towards the posterior end of the broodpouch with alternate contraction and relaxation. At this time another batch of ova were developing within the oviducts. These ova were oval in shape and darker in colour than those in the broodpouch. Ovoviviparous reproduction occurred on the 15th day and every 3 to 4 days thereafter. The mean number of nauplii for the first brood was 68 ± 15.4 nauplii parent⁻¹ ($n=5$). With increase in salinity, oviparity commenced at a salinity of 132 ppt, with cysts being produced at 37 ± 1.3 cysts parent⁻¹ ($n=5$). No males were observed during the experiment.

Discussion

The development and life cycle of the bisexual strain of *Artemia* have been studied in detail and over a long period of time (e.g. Heath, 1924; Kinne, 1977; Sorgeloos, 1980a; Schrehardt, 1987). Larval development of the bisexual strain is broadly similar to that of the parthenogenetic strain. With regard to life history characteristics, the bisexual strain has both functional males and females, whereas in the parthenogenetic strain reproductively functional males are absent. In certain parthenogenetic strains, however, adult animals that are morphologically similar to males of the bisexual strain are present, though they have not been observed to participate in sexual reproduction (Lal Mohan, 1980; Ahmadi, 1987).

The capability of *Artemia* strains to reproduce both ovoviviparously and oviparously has attracted much interest (Sorgeloos, 1980b; Lavens & Sorgeloos,

1987; Berthelemy-Okazaki and Hedgecock, 1987). In natural *Artemia* habitats, ovoviviparity occurs at lower salinities. As solar evaporation drives water salinities upwards, the same ovoviparously reproducing individual would switch to oviparity, which was found to occur at 132 ppt in our laboratory studies. Oviparity is an adaptation to produce dormant cysts to withstand the inhospitable conditions such as desiccation and extreme temperatures, even though the triggering mechanisms for the induction of the state of dormancy is not yet known (Lavens & Sorgeloos, 1987).

These resistant cysts are able to retain viability over a long period and are, therefore, utilized as an off-the-shelf live feed in aquaculture hatcheries. For this reason, the major interest and studies on *Artemia* have centred round their reproduction and egg formation. Parthenogenetic *Artemia* populations are able to produce a higher percentage of viviparous offspring than cysts (Browne et al., 1984). In *Artemia parthenogenetica* Sri Lanka viviparous offspring amounted to 64.8 % of total offspring.

Development in *Artemia* varies depending on strain, type of food, ration and culture conditions (Sorgeloos et al., 1986). We found that eggpouch formation in *Artemia parthenogenetica* Sri Lanka required 13 days at 29°C, whereas *Artemia parthenogenetica* from Tuticorin (India) required 23 days (when laboratory reared and fed with *Spirulina*; Royan, 1980) and that *Artemia* from Rajasthan (India) required 32 days (when fed with yeast; Baid, 1963). However, egg formation took a shorter period, similar to that reported by us for *Artemia parthenogenetica* Sri Lanka, when the Tuticorin strain was reared in large tanks and fed with a natural population of algae (Royan, 1980).

With regard to fecundity characteristics in the life cycle of parthenogenetic and bisexual strains, the bisexual populations appear to have a higher fecundity (Vu & Nguyen, 1987; Amat Domenech, 1980). The bisexual strain is naturally distributed in subtropical and temperate regions (Sorgeloos et al., 1986) whereas the parthenogenetic strains are more tropical in distribution. The higher cyst fecundity in bisexual strains could be of adaptive significance when related to the more rigorous climatic conditions of their temperate/subtropical habitat.

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