

Influence of salinity on the reproductive potential of a laboratory cultured harpacticoid copepod, *Amphiascoides subdebilis* Willey, 1935

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With 6 Figures and 1 Table

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Abstract

Laboratory studies were carried out to assess the influence of salinity on the adult life-span and reproductive activity of *Amphiascoides subdebilis* Willey, 1935, a brackish water harpacticoid copepod. An aqueous solution of *Phaseolus aconitifolius* (Yellow gram) + *Triticum durum* (Wheat) at a concentration of 4 ppt⁻¹ in combination with cultured green algae (*Chlorobium* sp.), was found to be the best unit feeding condition. The range of salinity tolerance was 11–45‰ S and reproduction occurred in the range of 17–40‰ S. Water media of 30‰ S was optimum for reproduction and survival, promoting minimum maturation time (13.3 days), highest longevity (114 days) and mean maximum fecundity (128 eggs per female). The survival period was inversely affected by changing the salinity beyond the optimum. The copepod has the ability to retain its reproduction for several days under reduced food conditions.

Introduction

Environmental parameters such as seasonal temperature variations, photoperiod, lunar periodicity, salinity of seawater and food availability have been regarded as important parameters in regulating the reproductive cycle of marine invertebrates (GIESE & PEARSE 1974, XU & BARKER 1990). Temperature has a marked influence on development rate, time of maturity, individual growth rate, fecundity and survival ship in temperate-zone ectotherms (LAUDIEN 1973). Whereas in tropical estuarine environment, salinity vary in relation to monsoonal rains (ALONGI 1990) and may profoundly affect the distribution and abundance of estuarine species. Benthic animals from estuarine intertidal habitat exhibit a variety of behavioural responses to the salinity changes (DAVENPORT et al. 1980). Therefore, salinity could be an important determinant of the growth, survival and reproductive activity of estuarine benthic harpacticoid species.

In this laboratory study, I have explored the probable effect of salinity changes

on the survival and reproductive activities of *Amphiascoidea subdebilis*, Willey 1935, a commonly occurring, euryhaline benthic harpacticoid copepod in coastal waters of India. Although it is known to occur in diverse ecological habitats (INGOLE 1982, MURALIKRISHNA MURTHY 1983, KONDALARAO & MURTY 1988, INGOLE et al. 1990), very little information is available on its biology (INGOLE 1982) and nothing is known about the environmental requirements of this species. The genus *Amphiascoidea* play a significant role in the benthic food web (KONDALARAO & MURTY 1988, INGOLE et al. 1990) and has been regarded as a potential alternative food source in brackishwater fish farming (WALKER 1979, KAHAN 1979, SHIRGUR 1989). Considering the importance of this species as a potential live food source for aquaculture, investigations were carried out on its reproductive behaviour, including maturation, reproductive period and maximum egg production at optimum salinity and super-abundant food conditions. This data is not only regarded as the basic environmental requirements for mass propagation but also as a necessary prerequisite in planning a much wider field programme to study the biology and ecology of this important species.

Material and Methods

Specimens of *A. subdebilis*, including males and egg bearing females, were collected originally from the seawater circulating system of the Marine Biological Research Station (M.B.R.S.), Ratnagiri, India, during November, 1979. The seawater was drawn from the intertidal zone of the Bhate creek (17°02' N; 73°20' E). The organisms were initially maintained in small glass beakers in water with a salinity of 25‰ (the same as the circulating seawater) and fed a aqueous solution of de-oiled groundnut oil cake (*Arachis hypogoea*).

Experimental procedure: Three experiments were carried out under constant laboratory conditions (12h light: 12h dark photoperiod and $24 \pm 1^\circ\text{C}$ temperature).

Salinity tolerance: Experiments were conducted in small glass test-tube, containing 30 ml water of various salinities. Different salinity ranges from 10 to 47‰ S were prepared with fresh seawater which was passed through a Whatman filter paper and sterilized by heating for 10 min. just below boiling point (the method outlined in WALKER 1979) and it was diluted with distilled water. Higher salinity ($\geq 34\text{‰ S}$) was made by evaporating the seawater in natural sunlight. During the earlier studies INGOLE (1982) have reported that *A. subdebilis* could not survive in salinities $\leq 11\text{‰ S}$, therefore, salinity levels below 10‰ were not investigated in the present study.

Three replicates were kept for each salinity grade. Four pairs of newly matured specimens (a pair consisted of young adult male and female) were harvested from the stock cultures, washed by serial transfer in a sterile culture media of 25‰ and inoculated in each test-tube containing water of various salinities. All the experimental animals were fed with an aqueous solution (4 ppt⁻¹) of *Phaseolus aconitifolius* (Yellow gram) + *Triticum durum* (Wheat) and a cultured green algae (*Chlorobium* sp.) was added to the above solution proportionally to make a unit (4 ppt) feeding condition. The combination of *Phaseolus* (P) + *Triticum* (T) + Algae (A) was observed to provide the best unit feeding condition (UFC) for culturing *A. subdebilis* (INGOLE 1982, SHIRGUR 1989). Aqueous solution of *Phaseolus* and *Triticum* was freshly prepared prior to the feeding, whereas the green algae (*Chlorobium* sp.) was harvested from the outdoor tank cultures.

All eggs and juveniles were carefully removed from each replicate daily, using a pointed dropper, and counted to evaluate individual fecundity. Fecal pellets and other debris were removed from the experimental test-tube daily, or when necessary. Mortality was recorded and 25%, 50%, 75% and 100% mortality rate calculated. Survival and fecundity were estimated, as an average from

three replicates. Age from hatching to adult (VIth copepodite) stage was estimated as per INGOLE (1982) and was further included to observe survival period of an individual specimen to estimate the longevity.

Growth and maturation under the optimum condition: As deduced from the pilot experiments, under constant room temperature of $24 \pm 1^\circ\text{C}$, water media around 30‰ salinity was the optimum for better survival of *A. subdebilis*. Accordingly, experiments in the present study were conducted in 10 glass petri-dishes (8.5 × 15 cm) under this condition as described above. The nauplii hatched (from clutches) in experiment no. 1 were isolated within 2–3 h of hatching, their total length measured and 10 nauplii were inoculated in each petri-dish containing 30 ml of culture media.

Growth (in length) measurements (from anterior end of the cephalon to the posterior end of the caudal rami) were made daily until the C6 (VI copepodite) or till they were sexually mature. Length measurements after C6 were taken every 7 days until the growth become static. The animals were carefully placed on a hollow glass slide with 30‰ S water and a small drop of MgCl_2 (aqueous solution) was added. This treatment was sufficient to anesthetize the specimen. Length measurement to the nearest of 10 μm was done under a binocular microscope fitted with a squared eye-piece micrometer. Five measurements were taken for each specimen and the average was considered for later calculations. After the length measurement, all animals were washed in 30‰ S water and immediately transferred to respective petri-dishes. The development time of various life stages was calculated for a group of specimen hatched from a single clutch. The mean length attained by individual animal was calculated and specific growth rate (G) for each 3 days derived by:

$$G = (\text{Log } L_2 - \text{Log } L_1) / (T_2 - T_1) \times 100 \text{ (BAL \& JONES 1960)}$$

where L_2 and L_1 are the length at time T_2 and T_1 , respectively.

Pairing behaviour: To study the pairing behaviour and egg laying process with and without the male partner, females bearing first batch of eggs were maintained individually in 30‰ S water. 10 young pairs (male and female) were also maintained individually as a control. All the specimens were fed with P + T + A feeding combination. Number of egg sacs produced by an individual female was calculated taking an average of 10 females.

Effect of starvation on egg laying: To study the possible effect of starvation on egg production, a series of experiments were conducted in 10 glass petri-dishes with 30‰ S water with 10 controls. Two mature specimens (male and female) were inoculated in each petri-dish. The control specimens were fed with P + T + A food ration at the normal rate. The experiment lasted for a period of 30 days.

Results

Salinity tolerance: *A. subdebilis* withstands a wide range of salinity from 11–45‰ S. The maximum survival period as well as the incidence of 25%, 50% and 75% mortality is shown in Fig. 1. The period of survival increased with increasing salinity from 12–31‰ S and decreased from 31‰ S. Maximum survival was at 31‰ S. In the salinity range of 42 to 44‰, 50% of the animals died within 4 days. The remaining animals survived for 30 days. In higher salinities (≥ 45) the animals moved very fast, more vigorously than at 33–39‰ S and mortality occurred within 1–10 h. *A. subdebilis* survived longer than 100 days in the salinity range of 28 to 35‰ S. The mean maximum survival of 114 days was observed at the intermediate value of 30‰ S.

Growth of *A. subdebilis* under the optimal conditions: *Amphiascoidea subdebilis* grew to a maximum of 1.03 mm under constant laboratory conditions. Growth from hatching (1st nauplius, N1) to maximum was achieved in 25 days. The values of per-

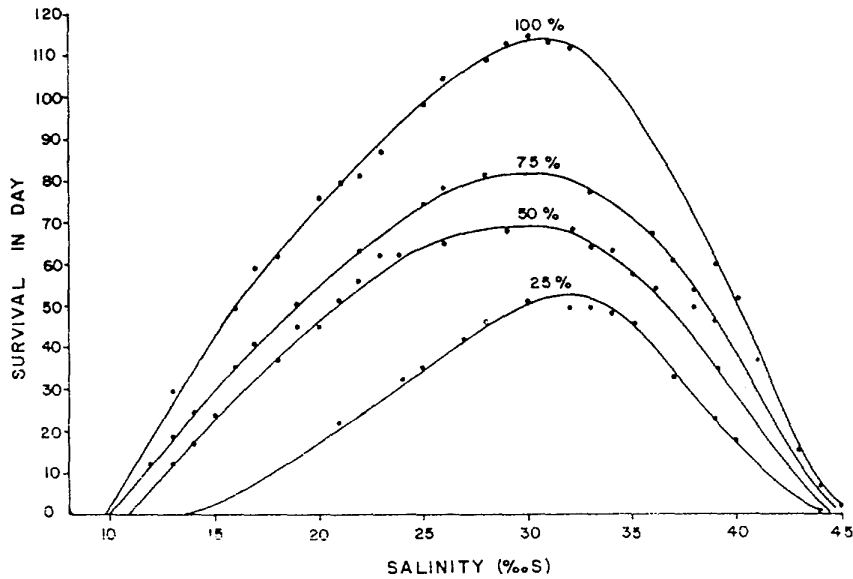


Fig. 1. Salinity tolerance in *A. subdebilis* with 25%, 50%, 75% and 100% mortality (mean of 3 replicates were considered while fitting the curves)

cent increase in length, absolute length and growth rates are plotted against age (in days) and are shown in Fig. 2. The growth rate was similar in both the sexes during initial stages but decreased in females after 7 days (i.e., after C3). The rate of $280 \mu\text{m}^{-\text{d}}$ was recorded at the age of 3 days (N3) which decreased upto 200 and $50 \mu\text{m}^{-\text{d}}$ in C5 and C6, respectively. Relative growth also decreased from 47% to 0.4% as length increased. Maximum size (1.03 mm) was achieved by male in 15–35 days. The length become almost constant unless senility and natural mortality.

Maturation and Pairing behaviour: The mating or pairing in *A. subdebilis* occurs after the C6 stage. Although a C5 male was observed to mate with an adult (C6) female, it is not known whether a C5 male can produce spermatophores. A C6 female was observed to produce egg-sacs within 2 days after mating with an adult male. Maturity in *A. subdebilis* was therefore attainment of C6 stage. Experiments conducted in different salinity indicated that earliest maturation at given conditions (optimal food and constant temperature) occurred in the salinity range of 28–31‰ S. An increase or decrease in the salinity beyond this range resulted in an increase of maturation time (Fig. 3).

Females maintained individually could not produce their second batch of egg-sac in the absence of males. However, females, in the control, reproduce normally producing their second, third and subsequent egg batches. After a deprivation period of one week, young adult males were introduced individually in each of the

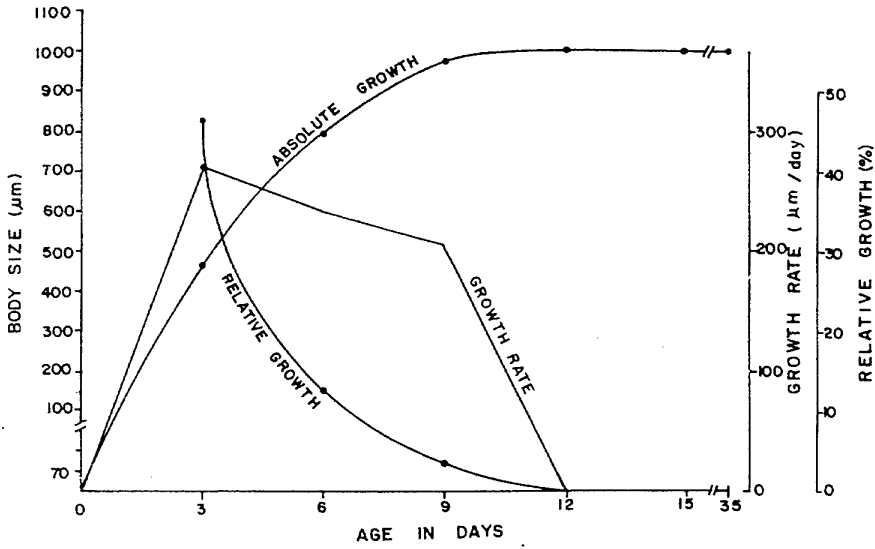


Fig. 2. Growth pattern in *A. subdebilis* (unsexed data)

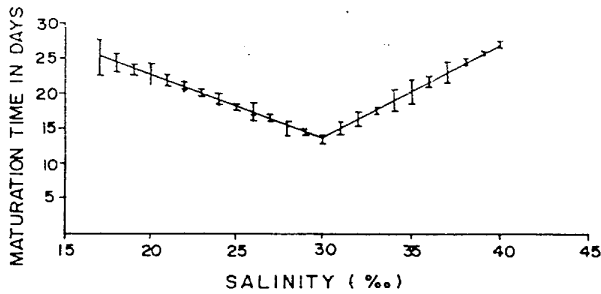


Fig. 3. Correlation of Maturation time (90% confidence limit) with salinity at controlled temperature

Table 1. Release of egg-sacs under the male deprived condition

| Petri-dish Number | First week | | Second week | |
|----------------------|-----------------------------|---------------------|--------------------------|---------------------|
| | experimental ♀ without ♂ | control ♀ with ♂ | experimental ♀ with ♂ | control ♀ with ♂ |
| 1 | 1 | 1 | 2 | 2 |
| 2 | 1 | 2 | 3 | 3 |
| 3 | 1 | 3 | 1 | 2 |
| 4 | 1 | 3 | 2 | 3 |
| 5 | 1 | 3 | 1 | 3 |
| 6 | 1 | 2 | 0 | 2 |
| 7 | 1 | 1 | 2 | 2 |
| 8 | 1 | 1 | 0 | 2 |
| 9 | 1 | 3 | 3 | 1 |
| 10 | 1 | 2 | 0 | 3 |
| Mean ± sd | 1 ± 0 | 2.1 ± 0.9 | 1.4 ± 1.2 | 2.3 ± 0.7 |

experimental petri-dish. As shown in Table 1, 7 out of 10 experimental females produced their second batch of egg-sac within 2–3 days and mean number of egg sacs produced by experimental female was 1.4 ± 1.2 as against 2.3 ± 0.7 eggs-sacs per female of the control specimens.

Life cycle: Under the optimum laboratory conditions, 4 nauplius stage at daily interval brought the nauplius to the copepodite stage and the larval development was completed with further 6 copepodite moults. Mating occurs any time after the last copepodite moult and formation of egg-sac takes place within 2 days of mating. *A. subdebilis* produced maximum of 128 eggs which were released in 6–7 clutches. Number of eggs in each egg-sac varied from 4–14. The mean maximum fecundity (126 eggs per female) and survival (114 days) was observed at 30‰ salinity. The fertile period is much shorter than the mean longevity and on an average only 67% of the total survival is devoted to reproduction.

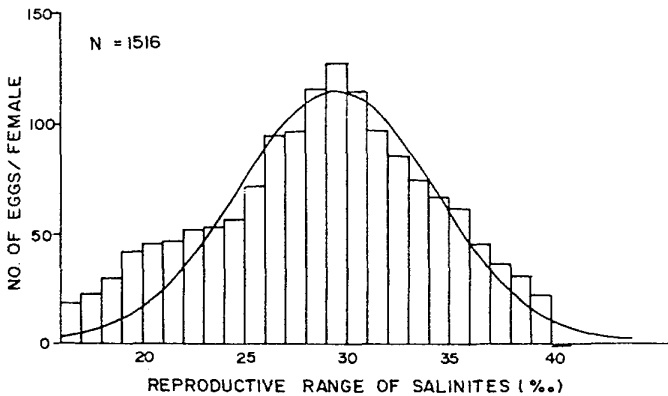


Fig. 4. Correlation of fecundity (no. of eggs/female) with salinity ($n = 3$, for each salinity grade, curve fitted according to Taylor's (1965) method)

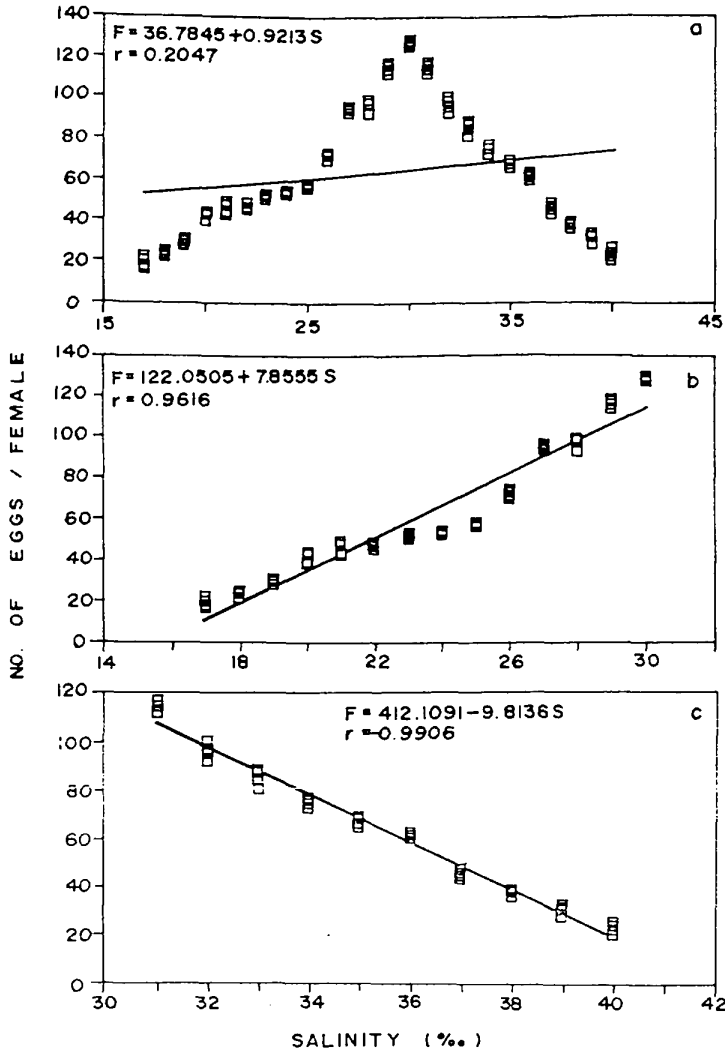


Fig. 5. Regression equations between salinity and fecundity (b and c: data sub-divided at transition points)

Effect of salinity on egg production: The results shown in Fig. 4 and 5 were obtained from the first experimental series. Breeding in *A. subdebilis*, occurred in the salinity range of 17–40‰ S. Although the specimens could survive for ≥ 40 days in higher salinities (41–44‰ S), they did not reproduce. Duration of the reproductive phase varied from 15 to 76 days. The maximum duration of reproductive phase of

76 days was observed in the salinity range of 28–31‰ S. Mean fertile period was much shorter than the mean longevity. The observed mean life-span of *A. subdebilis* could be subdivided theoretically as, early developmental phase (11.8%), the fertile reproductive phase (66.7%) and finally the sterile phase (21.5%).

The female *A. subdebilis* produced a maximum of 7 pairs of egg-sacs in a total reproductive duration of 76 days. In general, the egg production was directly related to the salinity of the culture media. A good correlation was found between lower salinities (17–30) and fecundity ($F = 122.0505 + 7.8555 S$, $r = 0.9616$, $P \geq 0.001$, $n = 42$, Fig. 5b), whereas, egg production was negatively correlated with higher salinity (≥ 31 , $F = 412.1091 - 9.8136 S$, $r = -0.9906$, $P \geq 0.001$, $n = 30$, Fig. 5c). Females produced 14–24 eggs at a time and an individual specimen could produce a maximum of 128 eggs in their total life-span.

Effect of starvation on egg laying: The mean of all 10 experiments and results of the control experiments were pooled and average data is presented in Fig. 6. On an average, the experimental female was observed to produce only one pair of egg-sac, which was released in three different clutches. First clutch was comprised of 14 ± 2.7 sd egg, second of 4.2 ± 2.2 sd eggs and third of 2.1 ± 1.5 sd eggs. The clutch-size decreased considerably in experimental specimens as compared to the control. Specimens in the control produced the second and third batch of egg-sacs but the experimental specimens failed to produce the second batch of eggs for 14 days. Egg production stopped completely during the starvation. Food (P + T + A) was added to all experimental petridishes on 15th day. 80% of the experimental females re-entered the reproductive phase and produced a second batch of eggs within 5 days of renewed food supply.

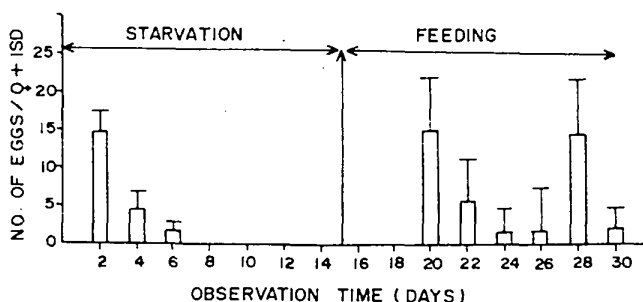


Fig. 6. Effect of starvation on egg production in *A. subdebilis*

Discussion

The species specific population dynamics of harpacticoids have been studied in a number of places, but rarely in response to environmental parameters other than temperature (HICKS & COUL 1983). Results of the present study suggest that a widely distributed, estuarine harpacticoid, *A. subdebilis* survived longer than 100 days in the salinity range of 28–35‰ S with the maximum survival of 114 days recorded in

30‰ S. The survival period was 43% higher at a lower temperature (24 °C) than the reported survival of 80 days obtained at relatively higher (27–30 °C, SHIRGUR 1989) temperatures. The enhanced survival up to 114 days indicates that lower water temperature is conducive for survival of *A. subdebilis*. Although it survives and reproduces in lower salinities, the reproductive ratio, (number of eggs per female) was two magnitude higher in a polyhaline condition (28–31‰ S; Fig. 4) showing that salinity can substantially influence the longevity and fecundity of the estuarine harpacticoid species.

Water-temperature, salinity and available food conditions are regarded as the principal environmental factors affecting the growth of harpacticoids (GAUDY et al. 1982) and egg production is a complex response to both environmental and physiological conditions (JONASDOTTIR 1989). Female *A. subdebilis* did not produce egg-sacs in the absence of male partner. Even when a male partner was provided after deprivation for one week, only 70% females released egg-sacs normally. Remaining 30% appeared to have lost their fertility due to the deprivation of males. Repeated mating is therefore necessary for every successive egg-sac production and since the fertile period is much shorter than the total longevity, mating at an appropriate maturity stage is crucial. Therefore, fecundity in a female dominated population could be less than that of a 1:1 sex ratio population.

Similarly, when regular food supply was resumed, about 80% females produced the second batch of egg-sacs. Thus, early recovery of reproductive phase occurred due to availability of food after a prolonged starvation. Reporting similar studies on other copepod species, JONASDOTTIR (1989) and ISHII (1990) suggested that a fresh food supply actually triggers the release of ovisacs. The genus *Amphiascoides* is known to feed on its own faeces under the deprivation of food (WALKER 1979). As in *Centropages hamatus* (LAMPITT et al. 1990), the females of *A. subdebilis* may begin to perform coprophagy on their own faeces under the starvation, by removing the peritrophic membrane with its attached bacterial flora and this may then be ingested. Thus deprivation of food may not lead to absolute starvation in the initial stages. However, it is obvious that a regular food supply is necessary to ensure continuous egg-sacs production.

The survival of *A. subdebilis* was 43% higher at a lower temperature and the fecundity was 40% higher in an optimal salinity range (28–31‰ S). The females provided with males and abundant food seems to spend considerable period in sterile old age, whereas females deprived of food for limited periods seem to have prolonged reproductive period. Thus recruitment and distribution of this species in the natural environment could be related to the temperature regimes, salinity variations, and food availability. This species has an ability to delay its reproductive potential for several days, which enables it to survive prolonged starvation.

Finally, the data presented here clearly show that the brackishwater species, *A. subdebilis*, is an ideal animal for mass culture. It has a rapid life cycle, longer survival period, flexible feeding habits, wide environmental tolerance and good resistance to the experimental conditions. Also the methodology adapted here will certainly aid to future the large scale mass propagation required for aquaculture practices.

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