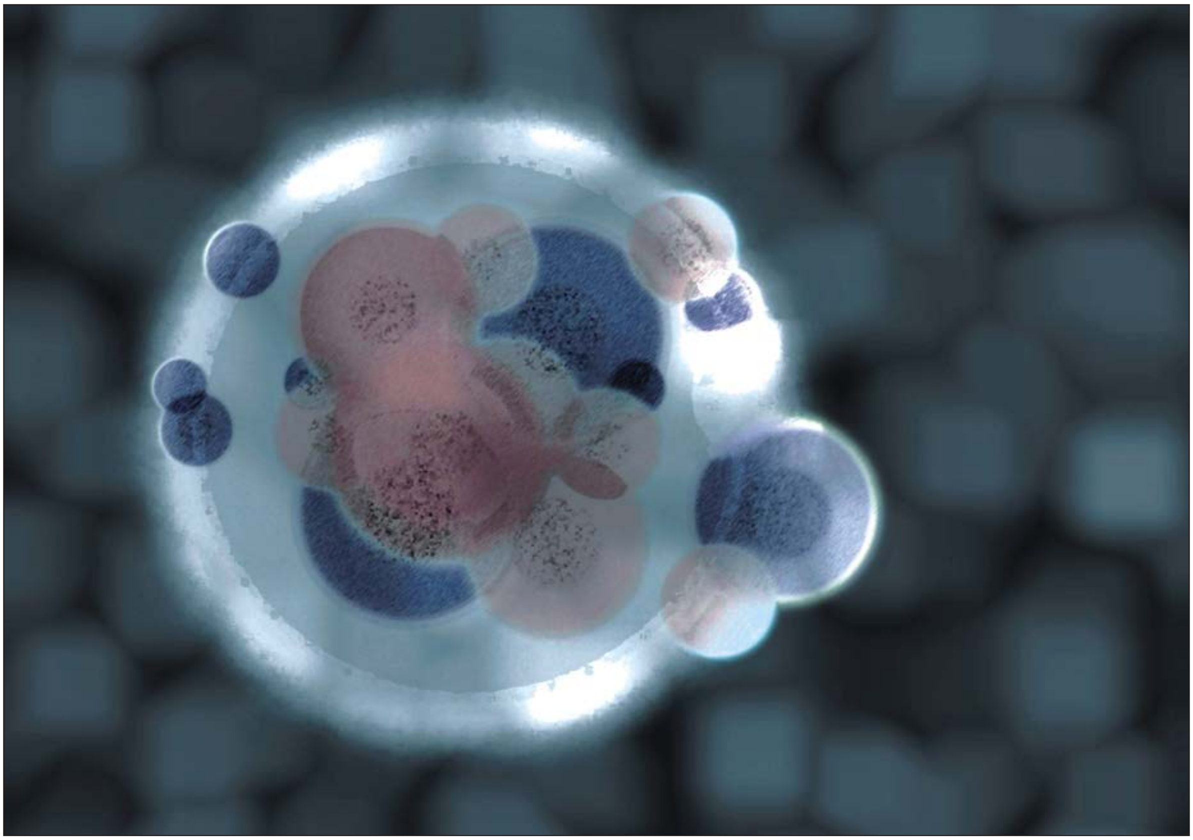

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Effect of Salinity on Larval Development and Survival of the Caspian Sea Barnacle, *Balanus improvisus* Darwin (1854)

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Abstract: The effect of salinity on larval stage and survival of *B. improvisus* was evaluated under controlled laboratory conditions. This study is carried out for the first time on the Caspian Sea *B. improvisus* population. Larvae were provided using egg lamella at later development stage. Hatch nauplii were mass reared in 0.6 L glass beakers(1 larva per 2 mL) on a different salinity and daily monoalgal diet (*Chaetoceros calcitrans*) at 21×10^5 cells per mL, under continuous light at 25°C (±1). The majority of nauplius II larvae in 8-25‰ range, completed the life cycle to cypris stage after 7 days. But larval size was decreased with increase salinity. Larvae that held in beakers at 7 and 28‰, completed life cycle after 8 days and about 36‰, was gained 9 days. With increase salinity from 12 due to 36‰, survival was decreased. Larval survival was highest in 12‰ (66%) and lowest in 36‰ (14%).

Key words: Salinity, larval development, survival, *Balanus improvisus*, Caspian sea

INTRODUCTION

Being a wide spread species in many region of the temperate subtropical zones of the world oceans, *Balanus improvisus* is believe to have spread from Atlantic coast of America to Europe and then to the west of Africa, the Mediterranean and Red seas, Australia, Japan, Hawaii and the pacific coast of north and south America. In the Soviet Union, the species inhabits the Baltic, Black and Caspian seas and the sea of Azov (Zevina and Gorin, 1971; Korn, 1991).

There are two species of barnacle namely Balanus *improvisus* and *B. eburneus* in the Caspian Sea (Promishlonost, 1968). But only the former species has been found in the south Caspian Sea (Rahmani, 2001).

Salinity stress has been shown to effect the growth and survival of a wide range of marine invertebrate larvae and, in turn, subsequent metamorphic competence and juvenile survival (Pechenik, 1987; Pechenik *et al*., 1998).

In coastal and estuarine environments, crustacean larvae face temporal and spatial variability in salinity, experiencing osmotic stress that may reduce growth and survival. This effect should vary depending on physiological adaptations such as the capability of osmoregulation in early developmental life-history stages (Charmantier, 1998). In species where the larvae are osmoconformer until metamorphosis (e.g., *Libinia marginata, Chionoecetes opilio*; Charmantier, 1998), only a narrow range of salinity variation is tolerated. Osmoregulating species, whose larvae show the adult type of osmoregulation already from the beginning of larval development (e.g., *Macrobrachium petersi, Palaemonetes argentinus*), are found in freshwater habitats or in environments with highly variable or extreme salinities; they have a wide range of salinity tolerance (Charmantier, 1998). A third pattern is found in species like *Penaeus japonicus, Cancer irroratus* (Charmantier, 1998) and *Homarus gammarus* (Charmantier *et al*., 2001), where the larvae are osmoconformers while the adults are osmoregulators. However, even with these physiological mechanisms most euryhaline animals are not immune from the effects of salinity change; documented sublethal effects of altered salinity include reduced rates of growth and development (Tietjen and Lee, 1972; Lambert *et al*.,1994; Qiu and Qian, 1997; Anger *et al*., 1998; Rosas *et al*., 1999; Specker *et al*., 1999) and reduced fecundity (Morritt and Stevenson, 1993; Cheung, 1997; Kinne, 1971; Remane and Schlieper, 1971). However, relatively few studies have directly assessed the effects of salinity on the development and survival of the barnacle larvae (Harms, 1986; Dineen and Hines, 1992, 1994; Anil *et al*., 1995; Qiu and Qian, 1997; Walker and Lester, 1998; Qiu and Qian, 1999; Thiyagarajan *et al*., 2003).

The objectives of the current study were to examine the effect of salinity on the survival and development of the nauplii of *B. improvisus*.

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MATERIALS AND METHODS

Rearing of *Balanus improvisus*: Adult individuals of *B. improvisus* were collected from Noor coast, South Caspian Sea and then transferred to the laboratory in department of Biology, University of Tehran. Specimens were placed in an aquarium kept in constant temperature room (25°C±1) under continuous light. Hatched nauplii were mass reared in 0.6 L glass beakers (1 larvae per 2 mL), using filtered seawater (salinity = 12-13‰, 1 μm filtered and UV-treated) on a different salinities include 7, 12, 15, 18, 25, 28 and 36‰ and on a daily monoloalgal diet (*Chaetoceros calcitrans*) at 2×10^5 cells per mL, under continuous light (2500-3000 Lx) at 25°C (±1).

Measurement of the larvae: Larvae were observed under light microscope (ZISS KF2) once a day to check with larval stage and also measurement. Size of the nauplii was recorded at stage V using an ocular micrometer. Total length of the nauplii was measured from the frontal margin to the tip of the dorsal thoracic spine. At least 20 larvae at each stage were measured.

Alga culture: Alga was cultured using filtered and autoclaved sea water by adding adequate nutrients (Conwey medium) adopted from Walne (1970). The monoalgal (*Chaetoceros calcitrans*) culture was stirred manually once a day to prevent settlement of alga on glass. The algal concentration was kept constant throughout the experiment by daily checking, using a haemocytometer.

Statistical analysis: The normality and homogeneity of variance were checked with Shapiro-Wilks and Cochrans test, respectively (Zar, 1999). The effects of salinity on size (length), mortality and larval duration of nauplii were evaluated using one-way ANOVA and then means were compared with Duncans test.

RESULTS

Effect of salinity on larval survival: Despite of some variation on percent survival in naupliar stages, >50% of nauplii successfully developed into cyprid, irrespective of all experiment of salinity (Fig. 1). Larvae reared at 12‰ showed higher survivorship (>80%) than those cultured at other salinities. Larval survival was decreased from 12‰ due to 36‰ thus lowest larval survival was observed at 36 (Fig. 1).

One-way ANOVA indicated highly significant differences among the mean survival rates of the larvae reared at different salinities (Table 1). The results of Duncan test showed that mean percent survival of the nauplii was differed in all salinity treatment (Fig. 1). The relationship between survival and salinity indicated a decrease in survival with increasing salinity at all experiments, except at 7‰.

Table 1: One way ANOVA of the influence of the salinity on total length, survival and larval duration of the nauplii

Factor	SS	MS	F	p<	df
Length	146409	24401.4	34525.5	0.0005	6
Survival	23659	3943.2	8143.6	0.0005	6
Larval duration	74.2	12.3	82.3	0.0005	6

df = Degree of Freedom; SS = Sum of Squares; MS = Mean of Squares; F = Fischer Constant

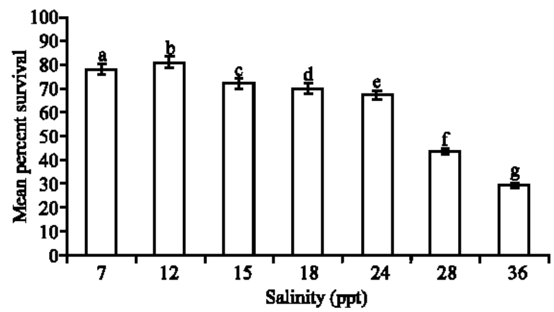

Fig. 1: Effect of salinity on survival of *B. improvisus* larvae, Data are plotted as the mean (±SD), n = 5, Each replicate consisted of 20 larvae

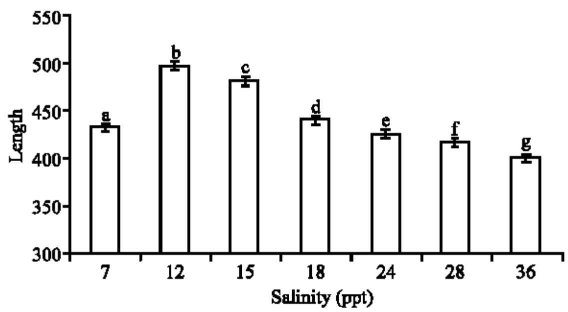

Fig. 2: Effect of salinity on length (satage V) of *B. improvisus* larvae. Data are plotted as the mean (±SD), n = 5. Each replicate consisted of 20 larvae

Effect of salinity on the size of nauplii: Salinity had a significant effect on the length of the nauplii (Table 1). Also, there were differences between mean length of all salinity treatment regarding to Duncan's results (Fig. 2).

Maximum and minimum lengths of the nauplii were observed at 12 and 36‰, respectively (Fig. 2). Length of the larvae was decreased with increasing the salinity from 12‰ due to 36‰, regularly (Fig. 2). At 7‰ this process has not been followed. The relationship between larval length and salinity indicated a decrease in the length of larvae with increasing salinity at all experiments, except at 7‰.

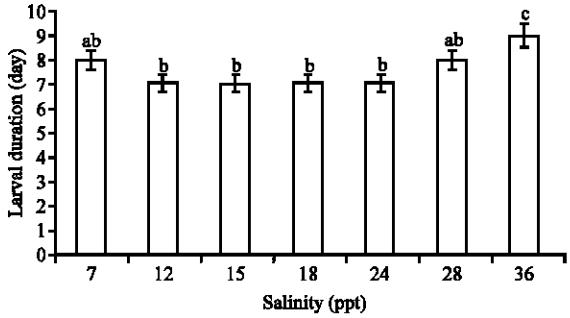

Fig. 3: Effect of salinity on larval duration of *B. improvisus* larvae. Data are plotted as the mean (±SD), n = 5. Each replicate consisted of 20 larvae. Treatment results with the same letter above the error bar are not significantly different at $p<0.05$

Effect of salinity on larval duration of the nauplii: Differences in salinity showed a significant effect on the total naupliar duration at all treatment (Table 1). The majority of nauplius II larvae completed the life cycle to sypris stage after 7‰ days. Larvae that held in 7 and 28‰, completed the life cycle after 8 day and about 36‰ was gained 9 days (Fig. 3).

Duncan's results revealed that there were no differences between mean larval duration at salinities of 12, 15, 18 and 24‰. The same situation was observed between 7 and 28‰. But, there were differences between two former groups and later group (36‰). These results shown in Fig. 3.

DISCUSSION

Two of the most potent abiotic factors in the life of marine and brackish water organisms are temperature and salinity (Kinne, 1963). Stress factors such as temperature and salinity may have a significant effect on the amount of stored energy reserves in larvae due to increased metabolic activity (Fraser, 1989). Salinity can affect yolk utilization and larval growth and survival by influencing the amount of energy needed for osmoregulation (Howell *et al.*, 1998). In many marine invertebrates species salinity affects the survival of their larvae (O'Connor and Epifanio, 1985; Anger, 1991; Luppi *et al.*, 2003) and often also the duration of their development, which is generally prolonged under osmotic stress (O'Connor and Epifanio, 1985; Gonc¸alves *et al.*, 1995; Anger, 1996; Luppi *et al.*, 2003).

Euryhaline animals cope with fluctuating salinity either through morphological and behavioral mechanisms that enable them to avoid exposure to the osmotic stress or through physiological mechanisms (osmoregulation, volume regulation) that enable them to maintain constant cell and body volume (Kinne, 1971; Withers, 1992). But, yet with these physiological mechanisms nearly all euryhaline animals are not protected from the consequence of salinity change.

A reduction in salinity not only leads to an increase in metabolism and other physiological activities (Anger and Dawirs, 1981) but also to some physiological adjustment in order to cope with osmotic stress (Pechenik *et al.*, 2000). In this study, reduction in salinity led to more larval duration from optimum, also caused to low length in the nauplii. Thus, present results coincide with above findings.

Salinity in the Caspian Sea is about 13‰ and therefore, this salinity is optimum for animals which inhabit on this sea. Higher and significant mean survival and growth rate was observed in larvae reared at 12‰ than at the other salinity. This indicates that water salinity would be optimal for the normal growth and development of larvae of *B. improvisus*.

Although the majority of nauplii of *B. improvisus* successfully developed into cypris stage in all salinity experiment, but, salinity had a significant effect on the instar duration.

At high salinity (36‰), the total naupliar duration was 9 days; however, at low salinity (7‰), larval duration was substantially shorter (8 days). During optimal salinity rearing conditions for this species in the Caspian Sea (12-15‰), majority of nauplius II developed into cyprids on Day 7. These discrepancies might have originated from adaptation rates of this species to salinity and its tolerance to change of salinity.

Result of this study showed that *B. improvisus* can be characterized as a strong osmoregulator, because it adapt rapidly to the new salinities when exposed to different salinities.

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