

Reproduction of the ctenophore, *Beroe ovata*, in the Caspian Sea water

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Abstract: The experiments with *Beroe ovata* showed that this ctenophore can survive and reproduce in the Caspian Sea water, though at far lower rate than in the Black Sea; larval growth in the Caspian Sea water was also slower and mortality higher. Maximum fecundity of 2210 and 240 eggs recorded in laboratories of Turkey and Iran, respectively. About 34 to 100% of eggs in the Caspian Sea water could not develop and hatch. Larvae were at different stages of development, usually with size between 1.2 to 2mm. The highest number of eggs and larvae were obtained in tanks where *Beroe* individuals were together with *Mnemiopsis leidyi*. The poor reproduction of *B. ovata* in the Caspian Sea water could be due to both the acclimation stress to low salinity and possible damage of individuals during the transportation.

Keywords: *Beroe ovata*, Reproduction, Caspian Sea, Iran

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Introduction

Appearance and mass development of the ctenophore, *Mnemiopsis leidyi*, in the Black Sea in 1980s resulted in spreading this species over other seas of the Mediterranean basin. First introduced into the Caspian Sea in 1999 through ballast water, *M. leidyi* has since spread throughout the sea where salinity is above 4.3 ‰ (Esmaeili *et al.*, 1999; Ivanov *et al.*, 2000; Shiganova *et al.*, 2001). Its population erupted, particularly in the southern regions of the Caspian Sea, reaching a mean biomass of about 1 kg.m⁻² (55g/m⁻³) in 2001 and an abundance in excess of the highest ever recorded in the Black Sea (Shiganova *et al.*, 2003; Shiganova *et al.*, unpublished).

At an international meeting held in Baku, Azerbaijan, 2001, under the aegis of the Caspian Environment Program, *Beroe ovata* was proposed as the best candidate to control *M. leidyi* population and, therefore, prevent the catastrophic change in the ecosystem of the Caspian Sea. Two specific features of this species allow thinking so. Firstly, its feeding specificity so developed that even larval stages of the *B. ovata* feed exclusively on other species of ctenophores. Secondly, its reproductive rate and fecundity are almost as great as that of *M. leidyi* or even more (Finenko *et al.*, 2006).

Similar to *M. leidyi* and most other ctenophores, *B. ovata* is a hermaphrodite (Harbison & Miller, 1986). Gonads are located along meridional channels in such a way that ovaries and android glands appear on different sides of channel (Zaika & Revkov, 1994; Arashkevich *et al.*, 2001). Like the majority of ctenophores, in *B. ovata* the sperm and eggs are discharged into the seawater where they are fertilized (Macginite & Macginite, 1968). There is no clear notion yet how egg makes its way outside (Arashkevich *et al.*, 2001).

When kept at a normal daylight rhythm, animals seem to shed gametes without any predictable periodicity about once a day or every 2 days for up to 2 weeks. If they are kept in the dark for long periods (48-72h), spawning can be induced in most mature animals within 2-3h of exposure to bright sunlight ((Zaika & Revkov, 1994).

The peak of *B. ovata* breeding in the Black Sea was observed in October, while in general its reproductive period was found to last from the middle of September to the middle of November (Arashkevich *et al.*, 2001). Large mature specimens were eliminated after reproduction, while a small fraction of the population composed of specimens of a new generation, apparently, descended to the near-bottom and spent winter in low-mobile condition until the next burst of *M. leidy* (Shiganova *et al.*, 2003)

The pattern of distribution indicates that breeding of *B. ovata* takes place within shallow near-shore areas; subsequently, the eggs and larvae are supplied to the open sea (Arashkevich *et al.* 2001).

In 2001-2002, the propagation experiments of *B. ovata* with the aim of its mass culture in the Caspian Sea water were initiated in the laboratory, but the results were unsuccessful. Both the spawning and hatching rates were very low and none of the larvae developed into adults (Kideys *et al.*, 2002, 2004).

In order to understand the feasibility of *B. ovata* introduction into the Caspian Sea, as an effective predator on *M. leidy*, experiments on survival and reproduction of *B. ovata* in the Caspian Sea water (12.6 ‰) were performed in three laboratories: 1) University of Ondokuz Mayıs, Sinop, Turkey; 2) Inland Water Aquaculture Research Center, Iran, and 3) Caspian Sea Ecological Research Center, Iran.

Material and Methods

All the experiments near the Black Sea (Sinop, Turkey) were conducted with freshly collected *B. ovata* ranging in size from 40 to 65mm. The specimens were firstly acclimated for 24 hours to different salinities and then placed individually into separate 4-5 l jars. Seawater salinity in the jars differed from 18‰ for the Black Sea water (6 replicates) to 15‰ for the Caspian plus the Black Sea water (Mixed seawater, 10 replicates) and to 12‰ for the Caspian Sea water (9 replicates).

The seawater in the jars was daily examined for eggs and larvae via filtering the water through 55µm mesh. The jars with eggs were stored without any handling to

let the eggs hatch into larvae. The hatching success was checked by stereomicroscope in 2-4 days after the eggs spawning.

About 130 adult *B. ovata* (70 specimens in the Black Sea water and 60 specimens acclimated to the Caspian Sea water) were conveyed in 9 and 20 l tanks from Sinop (Turkey) to Iran within a day. Part of the animal was transported to Inland Water Aquaculture Research Center (Bandar Anzali, Guilan province), others (95 specimens) were maintained in aquaria conditions in Caspian Sea Ecological Research Center (Sari, Mazandaran province). Mortality of the ctenophores due to the transportation was about 50% in both cases, as a result only 15 and 48 adult *B. ovata* were later used for the experiments in Bandar Anzali and Sari, respectively.

Preliminary acclimated to the Caspian Sea water, *B. ovata* in Bandar Anzali were placed individually in separate 3 l jars. The ctenophores were daily fed with *M. leidy* in excess, and seawater in the jars was checked daily for one week to see the availability of eggs and larvae. In Sari 48 specimens of *B. ovata* were maintained in 3 l aquaria where they were fed and examined twice a day for two days. Then 42 individuals from this experiment were removed and used for the mesocosm experiments (Javanshir *et al.*, 2003). Salinity in the mesocosm tanks was kept between 11.3-12.0‰ and the main focus of mesocosm was to find out the possibility of *Beroe* individuals shift in feeding habit other than *M. leidy*. When the experiment was over, each tank was checked for the presence of eggs and larvae for 5 days. The reproduction study was also continued daily with other (3 l) aquaria containing adult *Beroe* specimens. The observed eggs and larvae were immediately transferred into the 0.3 l glass jars containing fresh Caspian Sea water. The water was permanently aerated and small pieces of *M. leidy* were added into it as a food for the larvae.

In addition to these larvae, 600 larvae *B. ovata* were brought in the Black Sea water from Sinop to Bandar Anzali. They also were kept in 0.3 l glass containers and after 3 days acclimation were treated under three different salinities in four replicates.

As a control we used one *Beroe* specimen (length 30 mm) in the Black Sea water during experiments in Bandar Anzali and Sari.

Results

Our experiments showed that *Beroe ovata* could produce eggs and the eggs could develop and hatch into larvae in the Caspian Sea water (S 12‰), however, with much lower rates than in the Black Sea water (S 18‰) and mixed seawater (S 15‰).

The highest number of eggs were obtained at the salinity of 15‰ (mixed water) for the largest specimens in the experiments, although these results were statistically insignificant ($P > 0.05$) (Table 1).

Table 1: Some indicators of reproduction of *Beroe ovata* in the Caspian, the Black, and mixed seawaters (Sinop, Turkey)

Conditions	Number of spawning adults	Length of adults	Number of eggs		Number of Larvae	
		mean \pm SD (mm)	mean \pm SD	Max	mean \pm SD	Max
The Black Sea water	6	43 \pm 5	720 \pm 1170	3030	1.8 \pm 3.6	9
Mixed water	10	51 \pm 8	2940 \pm 2750	8900	1.0 \pm 1.7	5
The Caspian Sea water	9	45 \pm 4	350 \pm 710	2210	1.3 \pm 2.4	6

The lowest salinity (Caspian water) brought about the lowest number of viable eggs (66%) as compared with mixed water (97.2%) and the Black Sea water (100%). Besides, only 57% of eggs in the Caspian water were developed into larval stage in the next 2 days (Table 2), which was far lower than the eggs in the Black Sea water (97%), but higher than mixed water (31%). The survival of the larvae during the first 4-days of cultivation was also the highest in the Black Sea water.

Table 2: Survival of early stages of *Beroe ovata* in the Caspian and Black Sea waters (Sinop, Turkey)

Conditions	Initial numbers of eggs	Number of eggs detected at 24 hours	Number of larvae detected in 2 days	Eggs developed in 2 days, %	larval survival in 4 days(%)
		Mean \pm SD	Mean \pm SD		
The Black Sea water	300	204 \pm 119	292 \pm 107	97	1
Mixed water	300	108 \pm 40	92 \pm 17	31	0.7
The Caspian Sea water	300	200 \pm 45	172 \pm 40	57	0.3

Reproduction of *B. ovata* in the Caspian Sea in the course of the Iranian experiments in Bandar Anzali and Sari occurred to be less intensive than in Sinop (Turkey), possibly due to damages incurred to the adult specimens in the course of transportation. In Bandar Anzali for the first two days we obtained only 324 eggs and 2 larvae from 15 specimens. Then, during the following 7-days period, we obtained another 335 eggs and 40 larvae (Fig. 1). About 47% of eggs and 40% of larvae died at various stages of development.

In Sari *Beroe* reproduction rate in the Caspian Sea water was especially low during all period of observation. In sum, for 16 adult *Beroe* we counted about 100 eggs at the first stage of development. All the eggs numbering 1 to 9 for each mature individual were obtained only for the largest specimens (Fig. 1); the specimens less than 48mm did not spawn in these experiments.

After 4-days of cultivation in the Caspian water, only 2.7% of larvae survived and a single specimen grew to 5 mm in the length while most of them were 1-2 mm; in the Black Sea water the larval survival rate ranged from 29% to 71% in different replicates. Fecundity was also higher in the Black Sea water, numbering 20-590 and 30-1880 eggs in Sari and Bandar Anzali, respectively (Fig. 2).

The results of mesocosm experiment are given in Table 3. Altogether about 860 eggs and 190 larvae were obtained from adult *Beroe* in these tanks. Most eggs (79%) and larvae (68%) were observed in the tanks where *Beroe* were kept with *M. leidy*. Much lower number (21% of eggs and 32% of larvae) was found in the

tanks where adults *Beroe* neighbored with zooplankton. The ctenophore reproduced in each tank no more than twice. Many larvae in the tanks were in bad condition and grew slowly.

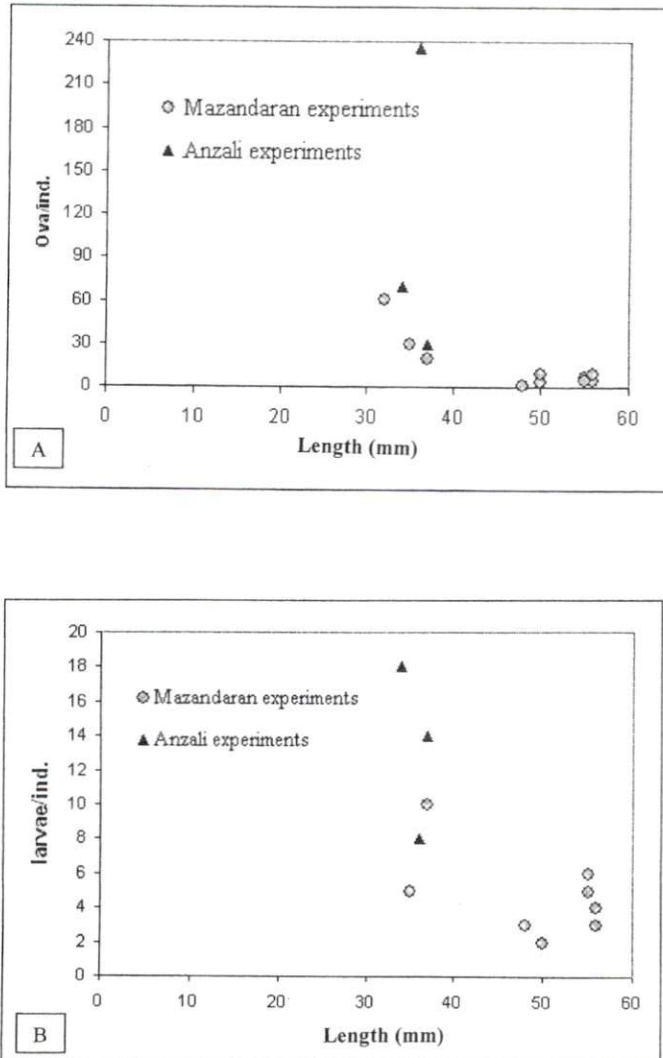


Figure 1: Egg production (A) and hatching out (B) of *Beroe ovata* in the Caspian Sea water (Iranian experiments)

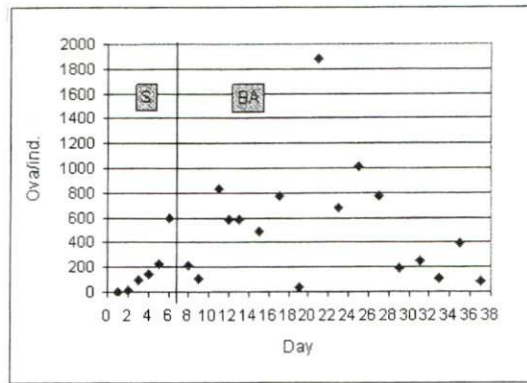


Figure 2: Fecundity of *Beroe ovata* in the Black Sea water in Sari (S) and Bandar Anzali (BA).

Table 3: Reproduction of *Beroe ovata* in mesocosm experiments

Tank	Composition	Initial number of <i>Beroe</i> **	Initial size of <i>Beroe</i> , (mm)	Date									
				22.09.03		24.09.03		25.09.03		26.09.03		27.09.03	
				O*	L*	O*	L*	O*	L*	O*	L*	O*	L*
1	Zooplankton + <i>Beroe</i>	7	36±8	-	-	90	0	0	0	0	0	0	0
2	Zooplankton + <i>Mnemiopsis</i> + <i>Beroe</i>	7	38±8	263	38	120	0	0	90	60	0	60	0
5	Zooplankton + <i>Beroe</i>	7	31±9	-	-	30	30	0	0	0	0	-	-
6	Zooplankton + <i>Beroe</i>	7	34±6	-	-	30	0	30	-	0	0	-	-
9	Zooplankton + <i>Beroe</i>	7	29±13	-	-	0	0	0	30	0	0	0	0
10	Zooplankton + <i>Mnemiopsis</i> + <i>Beroe</i>	7	39±7	-	-	180	0	0	0	0	0	0	0

O* Number of eggs; L* Number of larvae

Discussion

Our data confirmed earlier observation that the adults *Beroe* could reproduce in the Caspian Sea water. Feeding on *M. leidy*, some larvae of *Beroe* grew at least to 5mm. These experiments were more successful than previous ones in Iran (Kideys *et al.* 2002, 2004). In former studies only few eggs (<20) in the Caspian Sea water were developed and hatched into larvae. Besides, the larvae almost never grew in

the course of the experiments. The experiments with Marmara Sea water was better and gave more eggs, but more than 90% of them did not develop; only 7 larvae from 138 eggs of *B. ovata* hatched out and no one of them survived after 24h of cultivation (Kideys *et al.*, 2004).

Many factors could have affected the results of these and the former experiments. The reproduction of *B. ovata* usually starts at the body length of 30-35mm and increases with size (Arashkevich *et al.*, 2001). The ctenophores with the body size about 40mm did almost not reproduce even in the Black Sea water (Shiganova *et al.*, 2003). All the specimens used in our tests were on the stage of maturation (30-50mm) while the biggest fecundity was usually observed for the largest specimens. Reproduction of the ctenophore in the Caspian Sea water could probably be better if larger specimens were used for the experiments. In another study (Finenko *et al.*, 2003), the survival rate of the smallest size group of adult specimens (10-19mm) was, however, found to be the highest (91%) at salinity 12‰.

Hatching and larval survival are both key processes for recruitment of populations of many organisms. In this experiments, hatching and larval growth rate of *B. ovata* were very low in the Caspian and the Black Sea waters. Similar results were obtained nearby the Black Sea in 2003 (Finenko *et al.*, unpublished). According to our data, although *B. ovata* was able to reproduce in the Caspian Sea water even without adaptation to low salinity and about 10% of eggs could develop to larvae, the hatching success was much higher (83-87%) in the Black Sea water. Besides, larval survival and growth rate in the Caspian water (20-30%) were lower than those in the Black Sea water (80%). A better result on the *B. ovata* reproduction in the Caspian Sea salinities was obtained during another experiment in Gelendzhik (Shiganova, unpublished), in which the average hatching rates of $28 \pm 26\%$ and $80 \pm 14\%$ were recorded for 11.4 and 12.2 ‰ salinities, respectively.

Although poorly studied, a lot of external and internal factors could affect fertilization, egg development, hatching and larval survival of ctenophores. Our experiments showed that *B. ovata* larvae could grow in the Caspian Sea water, although the growth rate was slow and mortality was high. This could partly be due to impact of lower salinity and partly stress incurred during the course of acclimation. The salinity tolerance of this species is, probably, lower than *M. leidy*. But, even *Mnemiopsis* has two times lower fecundity in the Caspian Sea than in the Black Sea water (Shiganova, unpublished). *Beroe* larvae were found to be more sensitive to decrease of seawater salinity than the adult specimens, and the

juvenile specimens more resistant to low salinity than the adult ones. Therefore, longer preliminary acclimation of juvenile stages (10-20mm) could provide better resistance to low salinity for adults and larvae, which could shift the survival range of this species towards lower salinity (Finenko *et al.*, 2003).

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