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REPRODUCTION CHARACTERISTICS AND GROWTH RATE OF CTENOPHORE *BEROE OVATA* LARVAE IN THE CASPIAN AND BLACK SEA WATERS

Reproduction characteristics, survival and growth rate of ctenophore *Beroe ovata* larvae were studied in the Caspian and the Black Sea water at salinity about 12 and 18 ‰ accordingly. Both larvae placed in the Caspian water with and without acclimation showed the lower values of reproduction indices as compared with those in the Black Sea water. Nevertheless, adult *Beroe ovata* could reproduce in the Caspian water even placed there without any salinity acclimation and some share of eggs (about 10 – 15 % of total number of the laid eggs) developed into larvae. In the Black Sea water the hatching success was much higher and was equal to 83 – 87 %. *Beroe* larvae grew in the Caspian Sea water though their growth rate was rather slow and survival was not as high as in the Black Sea.

Key words: *Beroe ovata*, hatching success, survival, growth rate.

The Black Sea is an impressive example of damaging impact of invasive species upon the ecosystem on the whole and on the planktonic community in particular. The introduction and subsequent explosion of the ctenophore *Mnemiopsis leidyi* in the late 80's resulted in dramatic decrease not only the abundance of some species but also temporary poverty in biodiversity of zooplankton community [13, 17]. *M. leidyi* competition with planktonic fish for zooplankton as a food brought to remarkable decline in the fish stock registered those years [8].

Appearance of new alien ctenophore *Beroe ovata* in the Black Sea in the late 90-s appeared to be very effective in controlling high levels of *M. leidyi* and reduced its population explosion to the events of short duration. If in the years of *M. leidyi* bloom it was regularly found in abundance in plankton during 7 months, later after *B .ovata* introduction *M. leidyi* predominated only for 1 – 2 months a year [2, 3]. With the decimation of *M. leidyi* levels, zooplankton biomass and hence fish recruitment has been restored. No species appear to have been lost from the Black Sea fauna during the recovery process even if, at the height of the *M. leidyi* outbreak, many fell to levels as low as to make their observation impossible.

A warning that *Mnemiopsis leidyi* might also invade the Caspian Sea had been voiced as early as 1995 [1, 6]. Unfortunately, at the end of the 1990s the invasion of *M. leidyi* in the Caspian Sea was reported [7].

Investigations in the Caspian Sea in 2000 – 2002 showed that the effect of new alien *Mnemiopsis leidyi* on zooplankton community during the first years of the invasion was the most tremendous as compared to all other seas [4]. In the southern Caspian (Iranian waters) it was the highest in summer due to high water temperature and population size structure when juvenile ctenophores with mean length of 2 – 5 mm made up the most of the population. These ctenophores could consume the available stock of zooplankton during 3 – 8 days in winter-spring months and practically during one day in summer. The computed critical ctenophore biomass that does not affect (decrease) abundance of mesozooplankton in the Caspian Sea is to be about 4 g m^{-3} or 120 g m^{-2} if the most of ctenophores occur in the upper 30 m layer [12]. As it is clear from the monitoring data, the *M. leidyi* biomass in summer in different regions of the Caspian Sea is far in excess of this value [9, 12, 14, 15].

Such high pressure of ctenophore resulted in dramatic decrease of non-gelatinous zooplankton biomass, loss of some species and as a consequence further drops in pelagic fish stocks. Catches of the main of them, particularly kilka (*Clupeonella* spp.) for some riparian countries was reported to have decreased. Within two years (2000 – 2001) almost a 50 % decrease in the kilka catches of Iranian fishermen has occurred, with a minimum of 15 million US dollars economic loss [9].

During the First International Workshop on “The Invasion of the Caspian Sea by the Comb Jelly *Mnemiopsis leidyi* – Problems, Perspectives, Needs for Action”, organized by the Caspian Ecological Program (CEP) in April 2001, it was concluded that *Beroe ovata* is the best candidate to control *Mnemiopsis* population in the Caspian Sea.

Special experimental studies performed in Mazandaran Fisheries Research Center of Iran in 2001 – 2002 confirmed that *B.ovata* could live and grow intensively in the Caspian Sea water with a salinity of around 12 ‰ [10, 11]. However the reproduction was very low and only a few larvae could hatch, which died in 1 – 2 days.

More detail and intensive study of reproduction of *B. ovata* in the Caspian Sea water should have been carried out to clarify the possibility and feasibility of *Beroe* introduction in the Caspian Sea.

Therefore, in September 2003 such experiments were conducted in Sinop, southern Black Sea by a team of scientists from Ukraine, Russia, Turkey and Iran. These experiments were aimed to study 1) reproduction characteristics (proportion of animals spawned, size of egg clutch, hatching success) of *Beroe ovata* transferred to the Caspian Sea water from the Black Sea and 2) the growth rate of *Beroe* larva in the Caspian water by comparison with those in the Black Sea water.

Materials and Methods. Reproduction characteristics of *B. ovata* in the Caspian and the Black Sea water were studied in four sets of experiments (September, from 12 to 26) with ctenophores fresh collected in the Sinop Bay or with previously acclimated to lower salinity during 12 hours.

The Caspian Sea water was delivered by plane from Iran, Mazandaran and before the experiments was kept in 20-l containers in Aquaculture Department of Sinop University. In the experiments without previous acclimation freshly collected *Beroe ovata*, 40 – 50 mm in size, were placed individually in 4 – 5 l jars filled with seawater filtered through 180-μm mesh. In each set six experimental jars were filled with the Caspian Sea water and six jars – with the Black Sea water. The temperature was 22°C. Animals were kept in the experimental jars for 24 hr, and then were carefully removed and measured. The jars were left without any handling for the time necessary for larva hatching, for approximately 24 hr. Then the water was concentrated to a 100-ml volume and number of larvae and undeveloped eggs was estimated under a dissecting microscope.

In other set of experiments *Beroe* were acclimated to lower salinity being transferred into 16 ‰ water (mixed the Black and Caspian Sea water 1:1) for 24 hr. Six jars with the animals were left at salinity of 16‰ and *Beroe* from other six jars were transferred into Caspian Sea water. Six containers with ctenophores in the Black Sea water were left as controls. The number of eggs and larvae were examined daily until *Beroe* stopped reproduction in the Black Sea, mixed and Caspian Sea water. The conditions of the experiments (temperature, volume and number of animals) were the same as in the first set of the experiments.

One hundred fifty – three hundred fresh laid eggs were put into 50 – 150 ml glasses to examine the hatching success.

The experiments on survival and growth of *Beroe* larva were performed during the period from 4 to 26 September. In the first series of experiments for study the effect of lower salinity on survival, new-hatched *Beroe* larvae were previously adapted to lower salinity being transferred into 16 ‰ water (mixed the Black and Caspian Sea water 1:1) for 24 hr. Four jars were left at salinity of 16 ‰ and the larvae from other 4 jars were transferred into Caspian Sea water. The jars with the Black Sea water served as a control. The larvae were kept at 26^0C in 100-ml jars at initial density of 40 individuals per jar without any food.

In other set of experiments 160 newly hatched *B. ovata* larvae were placed in the Black Sea water, 160 adapted to the lower salinity larvae, which were previously put in water of 16‰ salinity for 1 day, were transferred to the Caspian water. The water was filtered through 0.45-µm glass fiber filter. The temperature in the experiment was 22^oC. The larvae were cultivated in 100-ml glass jars, 40 – 60 specimens per jar. *Mnemiopsis leidyi* tissue cut to small (1 – 2 mm) pieces was used as food for larvae.

Additionally the experiments on *Beroe* larvae growth in the Caspian Sea water were conducted using *M. leidyi* larvae as a food. Twenty larvae placed in 100-ml jars in three replicates were fed with freshly hatched *M. leidyi* larvae with size of 250 – 300 µm at initial concentration of 30 individuals per jar. In subsequent days the *M. leidyi* larvae density was increased to 50 – 60 specimens per jar.

The larvae were enumerated, measured, transferred into new filtered water, and fresh food was added daily. Wet weight of larvae was calculated from formula: $W = V\rho$, where W is the wet body weight (mg), $V = 1/6\ L\ 3.14\ D^2$ is the volume of ellipsoid, mm^3, L is the length and D is the diameter of ellipsoid (larva width), mm, ρ is the body density which is closed to sea water density of 1.012 g/cm^3 for Caspian sea water [16]. Sixty *Beroe* larvae were kept in the Caspian water and in the Black Sea water without any food and served as a control. All used larvae belonged to the same brood.

Results and discussion. In the period of our work, September 12 – 26, the proportion of reproduced *Beroe* decreased both in the Caspian and Black Sea waters from 80 to 0% and from 100 to 50 % of all adult ctenophore, correspondingly (Table 1).

Size of their egg clutches also decreased dramatically from 544 to 0 eggs in the Caspian water and from 4498 to 57 eggs in the Black Sea water. It was connected with the end of reproduction period in the sea. Both *Beroe* placed in the Caspian water with and without acclimation showed the lower values of reproduction indices as compared with those in the Black Sea water. Nevertheless, *B. ovata* could reproduce in the Caspian water even placed there without any acclimation and some share of eggs (about 10 – 15 % of total number of the laid eggs) developed into larvae (Fig. 1). In the Black Sea water the hatching success was much higher and was equal to 83 – 87 %.

The new laid eggs that were transferred from the experimental jars to other ones to study hatching success did not develop at all; they either stopped their development or disintegrated in both experimental treatments.

The number of laid eggs examined during 4 days in *Beroe* at different salinities decreased but the most dramatic decreasing was observed in the Caspian Sea water (Fig. 2).

Survival of *Beroe* larvae under starvation and feeding both with larvae and pieces of *Mnemiopsis* during 6 – 8 days in the Caspian and Black Sea waters at 22^0C differed significantly (Fig. 3).

At the end of experiment, in the Caspian water only 20% of starveling and 30 – 40 % of feeding larvae were alive.

Table 1 Reproduction characteristics of *Beroe ovata* in the Caspian and Black Sea waters
Табл. 1 Репродукционные характеристики *Beroe ovata* в каспийской и черноморской воде

Reproduction characteristics	Date	The Caspian Sea water	The Black Sea water
Proportion of *Beroe* spawned, %		80	100
Clutch size (mean±SD),	12 – 14 September	544±928	4498±2652
Hatching success, %		15±3	96±4
Proportion of *Beroe* spawned, %	18 – 20 Sept	71	86
Clutch size (mean±SD)		409±754	1684±543
Hatching success, %		10±3	95±1
Proportion of *Beroe* spawned, %		67	67
Clutch size (mean±SD)	21 – 23 Sept	55±43	525±386
Hatching success, %		10±5	87±15
Proportion of *Beroe* spawned, %		0	50
Clutch size (mean±SD)	24 – 26 Sept	0	57±17
Hatching success, %		0	83±10
Proportion of *Beroe* spawned, %	12 – 14 Sept	80	83
Clutch size (mean±SD)	Acclimated *Beroe*	103±142	2156±576

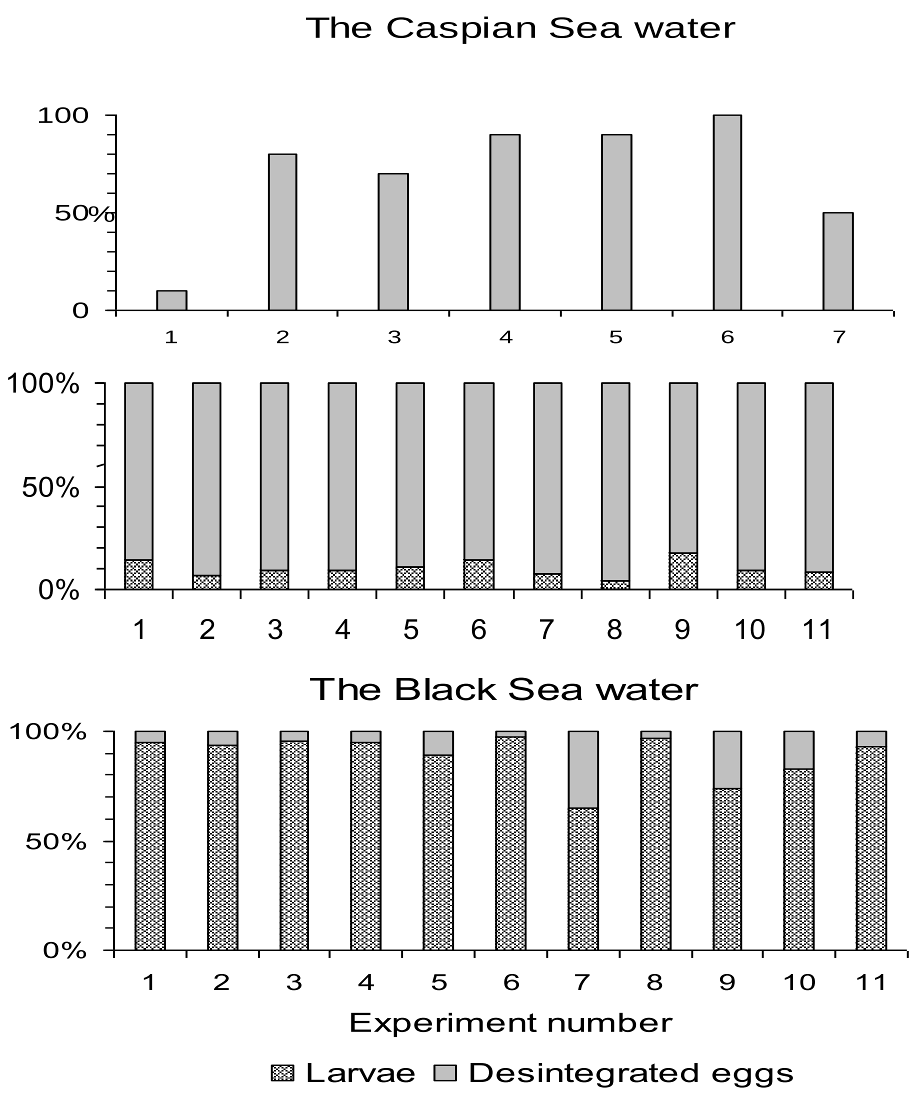

Fig.1 Percentage of disintegrated eggs and hatched larvae in *B*eroe *ovata* in the Caspian and Black Sea water: A – 12 – 14 September;
B, C – 18 – 20 September 2003
Рис. 1 Процент разрушенных яиц и вылупившихся личинок у *Beroe ovata* в каспийской и черноморской воде:
A – 12 – 14 сентября; B, C – 18 – 20 сентября 2003 г.

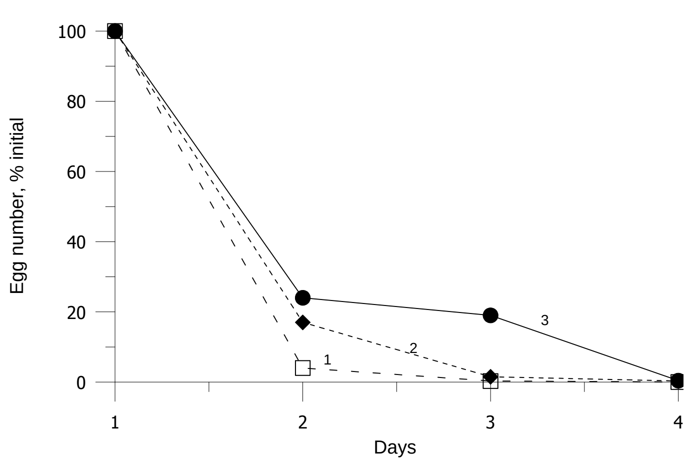

Fig. 2 Dynamics of egg number in *Beroe ovata* at different salinity: 1 – Caspian water (12 ‰); 2 – mixed water (Black Sea: Caspian Sea water – 1:1, 16 ‰); 3 – Black Sea water

Рис. 2 Динамика количества яиц у *Beroe ovata* при разной солёности: 1 – каспийская вода (12 ‰); 2 – смешанная (черноморская: каспийская – 1:1, 16 ‰); 3 – черноморская (18 ‰)

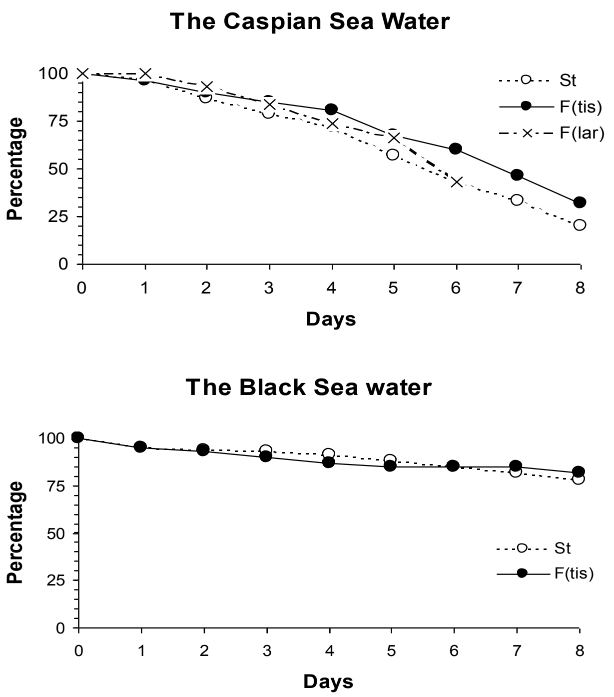

Fig. 3 Survival of *Beroe* larvae in the Caspian and Black Sea water: St is starveling; F(tis) – feeding on *M. leidyi* tissue; F (lar) – feeding on *M. leidyi* larvae

Рис. 3 Выживаемость личинок *Beroe* в каспийской и черноморской воде: St – голодавшие; F(tis) – питавшиеся тканями *M. leidyi*; F (lar) – питавшиеся личинками *M. leidyi*

Time for 50 % survival of the *Beroe* larvae in the Caspian Sea water was about 6 and 7 days for starveling and feeding larvae correspondingly. In the Black Sea water about 80% of larvae survived both under starvation and feeding. The significant difference in survival of starveling larvae in the Caspian and Back Sea water was also observed at 26^{0}C, but survival rate was much lower than at 22^{0}C and time for 50 % survival ranged from 1.5 to 2.5 days for all treatments respectively (Fig. 4).

Probably low survival rate in these experiments, besides the high temperature, could be result of some special condition of the parents that is an important factor determining survival and development of larvae.

The mean size (length) of *Beroe* larvae cultivated in the Caspian water increased insignificantly during 6 days of both sets of feeding experiments (Fig. 5, Table 2). The insignificant growth of *Beroe* larvae feeding on *Mnemiopsis* larvae probably was result of lack of food (there was not enough *M. leidyi* larvae because it almost stopped reproduction that time). The average daily ration was about 2 ind/ ind/day. However, the maximal growth observed for some larvae was noticeable, from 0.5 to 0.75 mm. There was a great variability in growth rate of larvae, while some of them increased in size, another part stopped their growth and even decreased in size.

We divided all larvae feeding on *Mnemiopsis* larvae into two groups: the first one are larvae that grew during experiment (their weight was higher than initial weight) and second group are larvae that had lower or equal of initial weight at the end of the experiment.

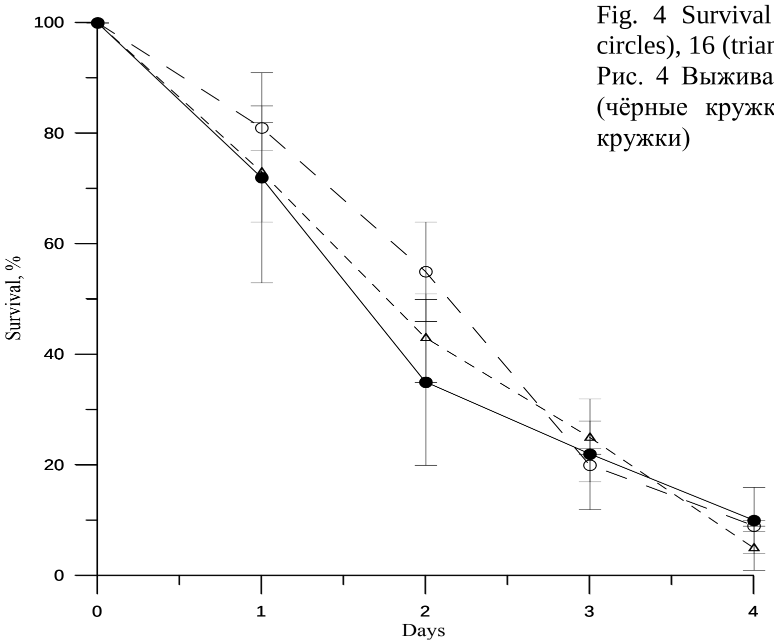

Fig. 4 Survival of *Beroe* larvae at salinity of 12 (black circles), 16 (triangles) and 18 ‰ (open circles) at 26 ^{0}C
Рис. 4 Выживаемость личинок *Beroe* при солёности 12 (чёрные кружки), 16 (треугольники) и 18 ‰ (белые кружки)

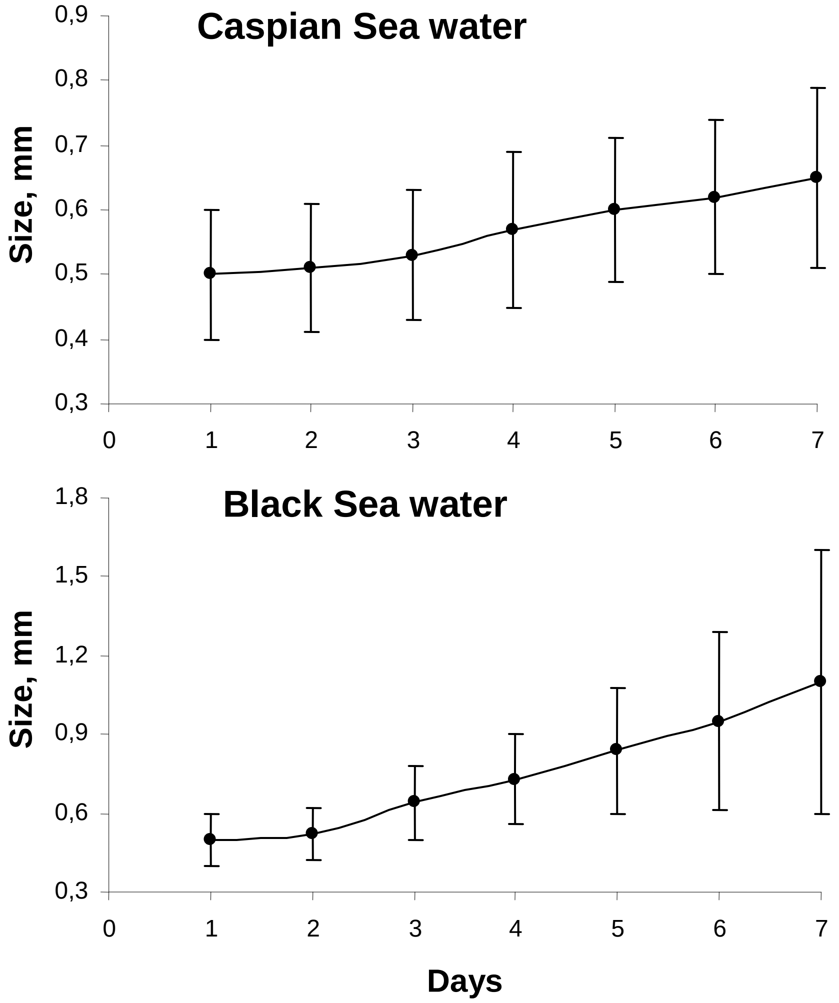

Fig. 5 Growth of *Beroe ovata* larvae in the Caspian and Black Sea water
Рис. 5 Рост личинок *Beroe ovata* в каспийской и черноморской воде

Table 2 Size of *Beroe ovata* larvae at the end of the feeding experiments (food is *Mnemiopsis. leidyi* larvae): L is length, D is width, W is wet weight, N is a number of observations. Duration of the experiment is 6 days.
Табл. 2 Размер личинок *Beroe ovata* в конце пищевых экспериментов (пища – личинки *Mnemiopsis leidyi*)*:* L – длина, D – ширина, W – сырая масса, N – количество наблюдений. Продолжительность эксперимента 6 дней.

	L, µm	D, µm	L/D	W mkg	N
Initial	440±76	310 ±77	1.48±0 34	24.6±12.0	20
1	478±115	394±138	1.29±0.27		9
2	450±93	413±106	1.15±0.31		8
3	470±140	373±129	1.38±0.58		15
average ± SD	467±120	389±124	1.30±0.45	28.5±25.2	32

It was found that the average weight of larvae in the first group was 47.5±2.2 mkg, in the second one it was 10.0±0.7 mkg against 24.6 ±12 mkg of initial weight. It is worth noting that in the Caspian Sea water larvae changed their body form and were more round than in the Black Sea (Table 2). Their growth in width was higher than it was in the length. The growth rate of larvae cultivated in the Black Sea water was higher than that in the Caspian water (Fig.5). But there was the same tendency for a part of larvae to stop development.

Thus, we can conclude that *Beroe* larvae can grow in the Caspian Sea water though their grow rate is slow and mortality is high.

It is well known that the narrowest limits of tolerance for salinity are typical for initial ontogenetic stages. In our experiments *Beroe* eggs appeared to be the most sensitive to lower salinity.

During ontogenesis the salinity range is getting wider. According to our previous results larvae *Beroe* were more sensitive to salinity decreasing than adult ctenophores [5]. Meantime juvenile individuals were more resistant to reduced salinity from adult *Beroe.* It seems possible to suppose that if to introduce *Beroe* to the Caspian Sea at the stages of early larvae the only small part of them could survive. But rather long preliminary acclimatization of juvenile animals (preferably with size of 10 – 20 mm) can affect upon the salinity resistance of both adult and embryos and larvae produced by them. As a result the survival range can be shifted towards lower salinity.

Conclusion. Our experiments showed that *B. ovata* can reproduce in the Caspian Sea water even without previous acclimation and some share of eggs laid (10 – 15 % of total number) can develop into larvae. It enables to suppose that *Beroe* could survive in the Caspian Sea (in southern Caspian with salinity about 12 ‰) if it is introduced there occasionally like it was in case of *M. leidyi.*

Beroe larvae can grow in the Caspian Sea water though their growth rate is rather slow and survival is not as high as in the Black Sea. The south region is the most suitable for *Beroe* introduction because of its highest salinity in the Caspian Sea. Spreading of *Beroe* from the South to the North will occur as far as acclimation of ctenophore to lower salinity takes place.

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Розмноження і швидкість росту личинок *Beroe ovata* у воді Каспійського і Чорного морів. Г. А. Фіненко, А. Г. Арашкевич, А. Е. Кідейс, А. Рухі, А. Мірзаяні, С. Багері, З. Бірінчі, Ф. Устюн, Х. Сатілміс, Ф. Сахін, Л. Бат. Швидкості розмноження, виживаності та росту личинок реброплава *Beroe ovata* були вивчені в каспійській і чорноморській воді з солоністю 12 і 18 ‰ відповідно. У личинок, які були поміщені в каспійську воду як без попередньої аклімації так і з аклімацією, репродуктивні показники були нижчі порівняно з личинками в чорноморській воді. Проте, дорослі реброплави, які були поміщені в каспійську воду навіть без попередньої аклімації, могли розмножуватися і деяка частина (10 – 15 % загальної кількості відкладених яєць) розвивалася в личинки. В чорноморській воді ця частка була значно вища (83 – 87 %). Личинки *Beroe* були здатні рости в каспійській воді, хоча їх виживаність і швидкість росту були значно нижчі, ніж в чорноморській.

Ключові слова: *Beroe ovata*, відсоток личинок, що вилупилися, виживаність, швидкість росту

Размножение и скорость роста личинок *Beroe ovata* в воде Каспийского и Чёрного морей. Г. А. Финенко Е. Г. Арашкевич, А. Е. Кидейс, А. Рухи, А. Мирзаяни, С. Багери, З. Биринчи, Ф. Устюн, Х. Сатилмис, Ф. Сахин, Л. Бат. Скорости размножения, выживаемости и роста личинок гребневика *Beroe ovata* были изучены в каспийской и черноморской воде с соленостью 12 и 18 ‰ соответственно. У личинок, помещённых в каспийскую воду, как без предварительной акклимации, так и с акклимацией, репродуктивные показатели были ниже по сравнению с личинками в черноморской воде. Тем не менее, взрослые гребневики, помещённые в каспийскую воду даже без предварительной акклимации, могли размножаться и некоторая часть (10 – 15 % общего количества отложенных яиц) развивалась в личинки. В черноморской воде эта доля была значительно выше (83 – 87 %). Личинки *Beroe* были способны расти в каспийской воде, хотя их выживаемость и скорость роста были значительно ниже, чем в черноморской.

Ключевые слова: *Beroe ovata*, процент вылупившихся личинок, выживаемость, скорость роста

ЗАМЕТКА

Amphorellopsis acuta **(Ciliophora: Spirotrichea: Tintinnida) – новый вид тинтиннид в Чёрном море *[Amphorellopsis acuta*** **(Ciliophora: Spirotrichea: Tintinnida) – новий вид тинтиннід у Чорному морі. *Amphorellopsis acuta*** **(Ciliophora: Spirotrichea: Tintinnida) – new species in the Black Sea].** К настоящему времени в Чёрном море известно 7 новых видов раковинных инфузорий, относящихся к 3 родам*: Eutintinnus lusus-undae Entz., 1885; E. tubulosus (Ostenfeld) Kof. & Campb., 1939; E.apertus Kof. & Campb., 1929; Salpingella decurlata Jorgensen, 1924; Tintinnopsis directa Hada, 1934; T. nudicauda Paulmer,* 1995 (Гаврилова, 2010); *T. tocantinensis Kof. & Campb., 1929* (Селифонова, 2011). Чужеродные виды тинтиннид ежегодно встречаются в акваториях крупных портовых городов Новороссийска, Севастополя и Туапсе. Некоторые из них появляются эпизодически, а другие, как, например, *E. lusus-undae, E .tubulosus и T. directa,* в отдельные годы достигают высокой численности, вытесняя местные виды. Наибольшее количество чужеродных видов (4) отмечено в Новороссийской бухте, где доля тинтиннид в летне-осеннее время может достигать 40 % суммарного количества инфузорий (Селифонова, 2011). В октябре 2010 г. в портовой акватории Новороссийской бухты обнаружена тинтиннида *Amphorellopsis acuta* (Schmidt, 1901) (рис. 1). Её численность в поверхностных водах (температура 19.5°C) составляла 5500 экз м$^{-3}$. Данный вид широко распространён в неритической зоне Индийского океана, Южной Атлантике, Мексиканском заливе, Средиземном и Японском морях, но в Чёрном море отмечен впервые (Gavrilova, Dolan, 2007). Лорика *A. acuta* прозрачная, гиалиновая, двухслойная; соотношение длины и орального диаметра 2.3 – 2.8. Оральный конец цилиндрический, расширенный, воронкообразный, аборальная область треугольная с острым концом, от которой отходят 3 ребра (~ 0.6 длины лорики). Длина лорики 85–115 µm; диаметр орального отверстия 33 – 42 *µm* (Fernandes, 2004; режим доступа: http://www.nies.go.jp/chiiki1/protoz/morpho/amphorel.htm). Форма и размеры тинтиннид по результатам промеров 8 экз. раковинок схожи с описанными в литературе. Длина лорики составляет 105 – 120 µm, диаметр орального отверстия 40 – 45 µm. Наиболее вероятно, что тинтиннида попала в Новороссийскую бухту с балластными водами коммерческих судов (Селифонова, 2009).

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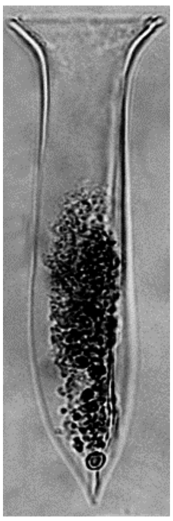

Рис. 1 Общий вид *Amphorellopsis acuta*
Fig. 1 General view of tintinnid *Amphorellopsis acuta*