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The Influence of Salinity on Early Ontogeny of the Mussel *Mytilus edulis* and the Starfish *Asterias rubens* from the White Sea

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Abstract—The influence of increased and decreased salinity on early larvae of the mussel *Mytilus edulis* L. and the starfish *Asterias rubens* from the White Sea is studied. Fertilized egg cells, embryos, larvae, and yearlings of the two species were compared. Salinity tolerance changes with the stage of development of mussels and starfish. Morphological changes occurring in eggs and embryos exposed to extremely high and low salinities are reported.

Keywords: salinity, tolerance, mussel, starfish, White Sea

The mussel *Mytilus edulis* L. is an amphiboreal species widely distributed in the seas of the Atlantic and Arctic oceans. It is the most common mollusk species of the intertidal and upper subtidal zones of the White Sea and inhabits the same biotopes as its predator, the starfish *Asterias rubens* L. The distribution range of the starfish is almost as extensive as that of the mussel: in the North Atlantic, it is restricted to Iceland and northern Norway, and in the south, to Senegal [2]. Owing to their eurytopic nature, these species are able to live under a wide range of temperature and salinity conditions. *M. edulis* has a wider range of tolerance to varying salinities, which enables it to survive in areas where it co-occurs with starfish. Therefore, it would be of interest to elucidate the differences in salinity tolerance in early ontogeny. The present study is a continuation of a comparative investigation of the tolerance of *M. edulis* and *A. rubens* to changing salinities [10, 11].

MATERIALS AND METHODS

This work was done at the A.O. Scarlato Biological Station of the Zoological Institute, Russian Academy of Sciences (Chupa Inlet, Kandalaksha Bay, White Sea). Experiments with *A. rubens* were conducted in the summer and fall of 1985, and with *M. edulis*, in the summer of 1996 in isothermic rooms at a constant temperature (12°C) and illumination.

Most gametes, embryos, and larvae at different stages were obtained in the laboratory. Brachiolarias and pediveligers were collected directly from plankton. Artificial fertilization was carried out as follows. Unfertilized egg cells were placed in seawater of reduced or increased salinity (experiment) or of the normal salinity for the White Sea (25‰, control). One to two drops of a thick sperm suspension were added per 250 ml of water. Gametes were taken from several individuals. To obtain a control larval culture, fertilized eggs were placed in 5-l continuously aerated aquariums, and, later on, larvae at definite developmental stages were sampled for experiments. The larvae were fed a mixture of the planktonic algae *Monochrysis* sp. and *Dunaliella* sp. Five to ten embryos or larvae were maintained in 10- to 15-ml vials at different salinities; the water in the vials was replaced with aerated water at regular intervals.

Survival of the fertilized egg cells and embryos of the two species was determined by counting the proportion of live and dead egg cells, embryos, and deformed embryos in a microscopic field. For the later developmental stages, the number of embryos and larvae in the samples was counted. In all cases, the number of live embryos and larvae is given in percent of embryos and larvae in the controls. Experiments were performed in several replicates. Where possible, morphological changes produced by exposure to high and low salinities were analyzed in detail and compared with development in the normal range of salinity. Photographs were taken using an MFN-14 photo attachment.

The duration of the experiments varied with the developmental stage. Experiments with starfish embryos and larvae lasted 2–5 days, and with mussel embryos and larvae, 7 days because they survive better in culture. For comparison, we also present original data on the salinity tolerance of yearling mussels and starfishes (see table). Both starfishes and mussels were collected in the subtidal zone, from rafts in mussel farming grounds where their habitat conditions are fairly uniform.

RESULTS

Mytilus edulis

Egg cell development. Fertilized egg cells and dividing embryos were 55–60 μm in diameter (Fig. 1a,

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Age, size, exposure time, and salinity tolerance of *Mytilus edulis* and *Asterias rubens* at different developmental stages

Stage	Age	Length, μm	Exposure, days	100% survival salinity range (‰)
		Mytilus edulis		
Egg cell development	–	55–60	7	22–26
Conchostoma	12 h	65–70	7	20–26
Veliger	4 days	75–250	7	5–45
Pediveliger	More than 30 days	250–400	7	5–45
Young mussel	1 year	17–35 mm	7(30)	10–45(40)
		Asterias rubens		
Egg cell development	–	180–220	2	24–26
Blastomere stage	8 h	180–220	4	18–26
Gastrula	2–3 days	160–170	2	18–26
Bipinnaria	10 days	320–370	5(10)	12(14)–28
Brachiolaria	More than 20 days	2.2–3.0 mm	5	12–28
Young starfish	1 year	10–40 mm (radius)	7	16–34

2a). At 22–26‰, fertilization was almost 100%, and embryos normally developed to veligers within a week. Various pathological changes in the egg cells and embryos occurred both at increased (Fig. 1b, c) and reduced (Fig. 2b, c) salinities. At a salinity below 8 and over 45‰, fertilization did not occur. In water of reduced salinity, egg cells frequently ruptured. At 10‰, 40–45% of the egg cells were fertilized and developed until the polar body appeared, perishing within the next several hours. At 12‰, the proportion of fertilized egg cells was 55% and some of them reached the 16–32 blastomere stage. They were unable to undergo gastrulation and perished within three days. Presumably, this is related to deformation resulting from the formation of an internal cavity (Fig. 2b). At 14‰, fertilization occurred in up to 90% of the egg cells; many of them went through cleavage, but not gastrulation; some survived for a week devoid of any archenteron, mouth, and oesophagus. At 16 and 18‰, the percent fertilization was also high, at about 90%; within three days, the eggs underwent gastrulation and transformed into ugly conchostomas with highly deformed shell glands. These deformed larvae were incapable of turning the shell gland inside out and, therefore, of forming a normal shell. At 20‰, embryos and larvae barely differed from those that developed normally at 22 to 26‰; however, their shell was weakly developed or had deformed margins. These deformities were sufficient for the larvae to be unable to metamorphose.

At 28–30‰, 100% fertilization occurred, as at optimal salinity (22–26‰). The larvae reached the late trochophore and early veliger stages at 28‰ and the late trochophore stage at 30‰; in external appearance, they were barely distinguishable from normal larvae, but they died at the age of 3 days.

At 32‰, there were few unfertilized eggs cells. The larvae developed to the trochophore stage; they had various deformities and died within 3 days. At higher salinities (34 to 40‰), the proportion of fertilized egg cells gradually decreased, and at 38 and 40‰, there were only a few of them; development only progressed until the first polar body appeared. They perished within the first day. At 34‰, about 20% of the larvae reached the sterroblastula stage, but they only survived

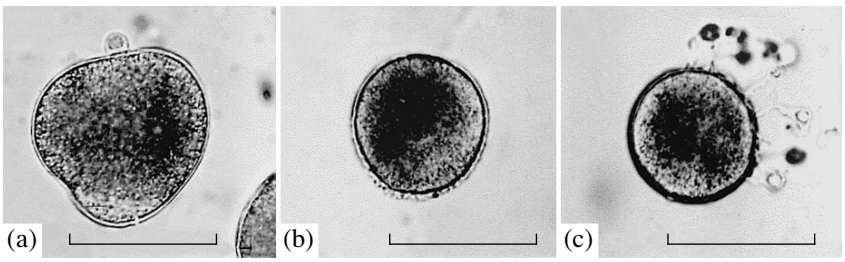

Fig. 1. Early embryonic development of the mussel *Mytilus edulis* (a) at normal and (b, c) extremely high salinity. (a) A fertilized egg cell forming the first polar lobe; (b) an egg cell not capable of fertilization; (c) an egg cell losing its membrane. Scale 50 μm.

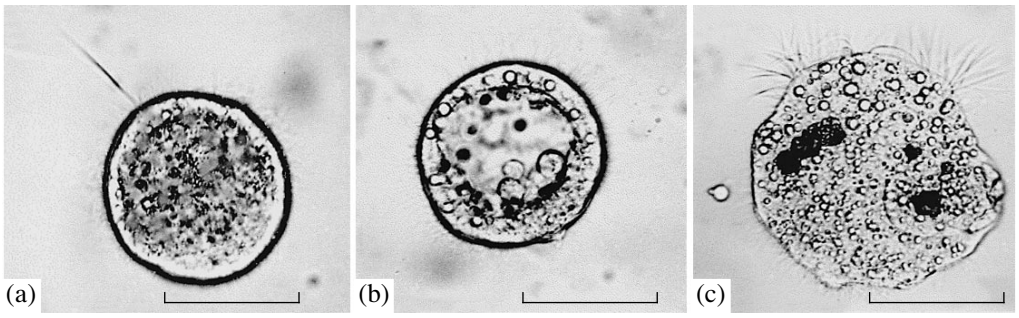

Fig. 2. Embryonic development of the mussel *Mytilus edulis* at (a) normal and (b, c) low salinity. (a) A normally developing blastula; (b) a mussel blastula with an abnormal inner cavity; (c) a deformed blastula. Scale 50 μm.

1 or 2 days. At 36‰, only about 10% of the embryos reached the 8–16 blastomere stage, and they also died within the first day.

Conchostoma. The conchostoma measured 65–70 μm. A characteristic feature of this stage was the presence of the archenteron and the shell gland. Normal larval development was observed in the salinity range from 20 to 26‰. At 0–5‰, the larvae perished within a few hours, and at 8–10‰, within 3 days. At 12–16‰, the larvae survived for a week, but they had a deformed shell and other nonspecific abnormalities and, as a consequence, were unable to metamorphose. Such deviations also occurred at 18‰, when there were few normal larvae.

Few viable larvae were observed after a week in the experiment with high-salinity water of 28‰. At higher salinities, even the surviving larvae had various deformities that were sufficient to render metamorphosis impossible.

Veliger. As the larvae developed, they ranged in size from 75 to 250 μm. For the experiment, we used 4-day-old veligers of 100 μm. This stage was distinguished by the fact that the velum had a shell. With salinity changing over a broad range from 5 to 45‰, larvae at this stage remained alive for more than a week. Observations prolonged after the experiment ended (1 week) showed that, at 5‰, the larvae perished within 10 days. At 8 and 45‰, up to 50% of the larvae died within 2 weeks. In the salinity range of 10 to 40‰, all the larvae survived for 2 weeks. In fresh water, the larvae died within 5 h. At high salinities (50 and 55‰), they survived for up to 3 days.

Pediveliger. The size of the pediveliger varied with age from 250 to 350–400 μm. A characteristic feature of this stage was the presence of the foot and the posterior adductor. As for the veligers, 100% of the pediveligers survived for at least a week at a salinity ranging from 5 to 45‰. At 50‰, up to 50% of the larvae perished within a week; at 55‰, larval mortality over the same time period was 100%.

Asterias rubens

Egg cell development. The egg was 180–220 μm in length. Our observations showed that 100% fertilization and normal development to the gastrula stage occurred within 2 days at a salinity of 24–26‰. At 20 and 28‰, unfertilized egg cells were found, and development of the embryos was slightly retarded. Thus, within 2 days, all fertilized egg cells turned into gastrulas in the salinity range from 24 to 26‰, while, at 20 and 28‰, gastrulas accounted for no more than 60% of the eggs, the rest being blastulas. At 16‰ and below, as well as at 34‰ and above, fertilization did not occur and defects of the egg membrane and the nucleus were clearly visible. Only some of the eggs (20%) reached the 16-blastomere stage at 18 and 30‰, after which they perished. At 32‰, only very few egg cells were fertilized; these developed to the 4-blastomere stage and then perished.

Thirty-two blastomeres–blastula. Like the egg cells, the embryos varied in size from 180 to 220 μm. A 100% rate of survival and normal development of embryos to the gastrula stage occurred within 4 days at salinities from 18 to 26‰. Development completely stopped and the embryos rapidly died at 10‰. At 12‰, about 20% of the embryos developed to the blastula stage, but they all died within a day. At 30–34‰, up to 20% of the embryos also turned into blastulas, but these also perished within the same period of time.

The starfish embryo is liberated from the egg membrane at the transition from the blastula to the gastrula stage. Normally, 100% hatching of blastulas from the egg membrane and the beginning of gastrulation occurs a day after fertilization. At a reduced salinity, this process was delayed for 1 day and took place only in some of the embryos. Thus, 20% of the blastulas at 14‰ and 50% at 16‰ had been released from the egg membrane two days after fertilization. Within three days at 14 and 16‰, 100% embryo mortality was observed.

Gastrula. The gastrula was up to 170 μm in length and 2–3 days of age. Well-formed larvae had a gastral invagination and clearly discernible mesenchymal cells. A 100% larval survival rate and normal development was observed in the first two days at a salinity

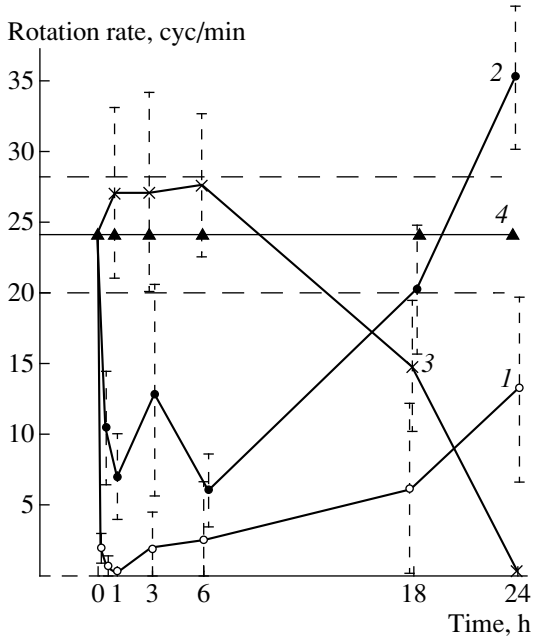

Fig. 3. Changes in the rotation activity of starfish *Asterias rubens* gastrulas at varying salinities (1-day exposure): (*1*) 16‰, (*2*) 18‰, (*3*) 28‰, (*4*) control (after [9], with modifications).

range of 18 to 26‰. Larvae at this stage moved actively, spiraling forward. The condition of the larvae at various salinities was examined by determining the speed of rotation about their axes (Fig. 3). These observations showed that, at 18‰, rotation speed decreased by more than half compared to the control within the first 6 h. However, a day later, it was already higher than that of the control. At 16‰, within the first hour, the rotation velocity of the larvae dropped to zero, but then increased. A day later, it was still lower than that of the control larvae, suggesting inhibition of the activity. At 28‰, the speed of movement did not differ from that of the control within the first 6 h, but it subsequently decreased (Fig. 3). After 24 h, almost half of the larvae perished; the rest were quiescent.

Bipinnaria. Starfish bipinnarias were 320–370 μm in length at the age of 10 days, with easily discernible body parts and intestines. At 14–28‰, test larvae survived for up to 10 days. They survived for 5 to 10 days at 12‰. At 10‰, all the larvae perished during the first day; at 30‰ and above, mortality occurred in some of the larvae during the experiment.

Brachiolaria. The larvae were 2.2–3.0 μm long. At 12–28‰, the larvae retained their activity for 5 days; in the salinity range of 18 to 28‰, up to 50% of the larvae metamorphosed to young starfish. At 14–16‰, their movement was slightly slower and differed little, if at all, from that of the control. At 12‰, the slowing down of motion was more marked. At 10‰, all larvae perished within the first two days.

DISCUSSION

Salinity and temperature are known to be the most important abiotic factors influencing the geographical distribution and reproductive processes of marine invertebrates [8, 19–21, 23]. The White Sea has a unique hydrological regime, due to the two-layered structure of the water [1], where the upper layer is characterized by lower-than-oceanic salinity (24–26‰) and a considerable magnitude of seasonal temperature fluctuations. Moreover, the salinity of the upper 15-m water layer, in which *A. rubens* and *M. edulis* occur, varies substantially during the year, reaching a minimum when the ice melts. The adaptive responses of mussels and starfish to changing salinity have been extensively studied; however, the effect of salinity as a factor limiting the reproductive processes and early ontogeny of the two species has not been adequately studied.

The fertilization and development of mussels from the White Sea under reduced salinity has been described in detail only in the work of Yaroslavtseva *et al*. [14]. The results of our observations differ slightly from those reported in the above paper, perhaps because of some methodical differences, as well as the individual properties of the mussels. Thus, according to Yaroslavtseva *et al*., marked deviations in larval development occur at 12‰, but larvae are able to reach the veliger stage and survive for a week even under these conditions. In our experiments, the proportion of fertilized eggs was about 50%; they were unable to undergo gastrulation and perished within three days.

Yaroslavtseva *et al*. report that egg cells undergo normal fertilization during the first week of development in a salinity range from 14 (with retardation) to 22‰ (higher salinities were not tested). In our experiments, fertilization also occurred when the salinity was reduced to 14‰ (in 90% of the egg cells), and many of the larvae survived for a week. At 16 and 18‰, they underwent gastrulation and transformed into ugly conchostomas; at 20‰, they had only slight deviations in shell formation. However, they were later unable to metamorphose. Therefore, we believe that, for the development of mussel embryos and larvae to follow the normal pattern, the salinity must be no lower than 22‰ at the time of fertilization. Similarly, there are differences in the lower limits of salinity tolerated by the trochophore (or conchostoma, according to later accepted terminology), which is 8‰ according to Yaroslavtseva *et al*. and 20‰ according to our data.

It is likely that variations in salinity tolerance limits are also due to the physiological heterogeneity of the material used in the two series of experiments. The experiments of Yaroslavtseva were begun in June, i.e., soon after the spring drop in salinity; we conducted our experiments in July and August. This probably accounts for the lower salinity tolerated by the egg cells and embryos in the former case. A similar salinity acclimation effect in spawning individuals has been noted for bivalve mollusks: a change in ambient salinity dur-

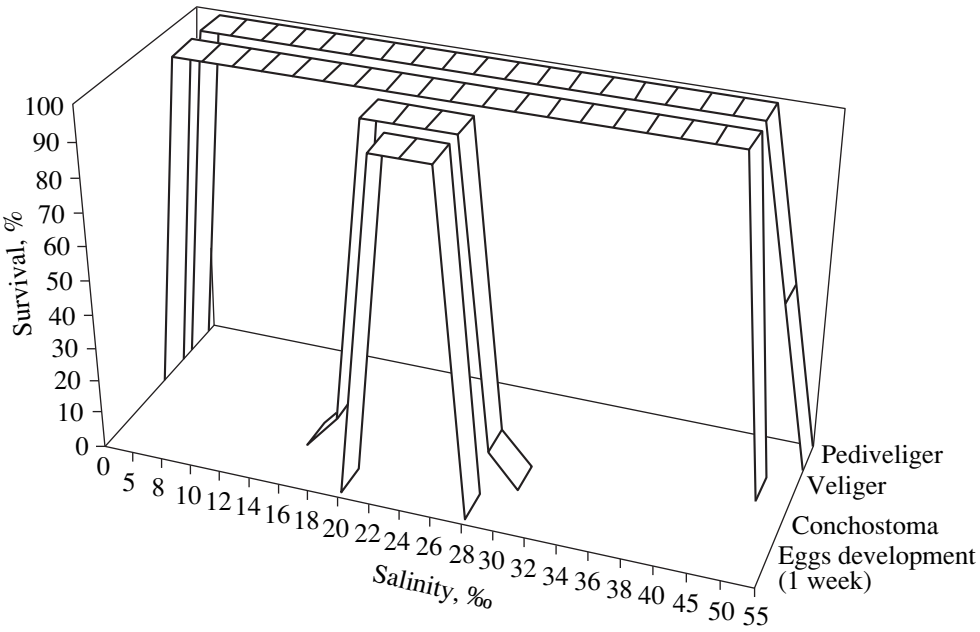

Fig. 4. Response to salinity of the mussel *Mytilus edulis* from the White Sea at different developmental stages (7-day exposure).

ing gamete maturation in females leads to adaptive changes that facilitate the normal course of embryonic development in offspring [17]. This is confirmed by observations on a chiton indicating that preliminary acclimation to reduced salinity conditions before spawning lowers the salinity limit at the release of the trochophores [4]. When adult *Apostichopus japonicus* sea cucumbers are acclimated to a reduced salinity of 24–26‰, the salinity range in which fertilization and subsequent embryonic and larval development follow the normal pattern is also lower than that of the offspring from sea cucumbers maintained before spawning at a normal salinity of 32‰ [5]. It is evident that individual differences in the salinity tolerance of mollusks may be quite significant, as, for example, in the oyster *Crassostrea gigas* [15].

Both the results of Yaroslavtseva *et al*. [14] and our findings suggest that the lower limit of salinity survived by adult mussels from the White Sea is 10‰.

We now focus on the details of abnormal processes resulting in egg cell death in the lethal salinity zone. At increased salinities, fertilization in mollusks is impeded or virtually impossible. One of the most obvious reasons for this is the detachment of the egg membrane (Fig. 1b); as a result, the egg cell shrinks, releasing water into the environment because of its increased osmolarity. This process closely resembles the cortical reaction that occurs in bivalves after fertilization and is a natural form of protection against polyspermy. In our case, the egg membrane separates without the participation of the sperm, and the egg cell cannot be fertilized. Subsequently, the unfertilized egg cell completely loses the egg membrane and perishes (Fig. 1c). Under normal salinity conditions, the embryo has begun the first cleavage division by this time.

At reduced salinities, the egg cells often explode because of increased osmotic pressure, and we need not speak of fertilization. However, when development does proceed under such conditions, the embryo cannot develop into a sterroblastula characteristic of the mussel because a cavity appears inside the blastula (Fig. 2b). Such an embryo can be considered a coeloblastula. It is interesting to note that the coeloblastula is a typical stage of freshwater mollusks and presumably performs an osmoregulatory function [3].

In water of increased or reduced salinity, starfish egg cells are subject to similar deformities. The salinity ranges within which successful egg fertilization can occur and normal embryonic development can proceed are similar for mussels and starfish, at 22–26 and 24–26‰, respectively. This range is narrow, a peculiarity that characterizes fertilization as the most sensitive period in the ontogeny of the two species. This range corresponds to the salinity regime typical of the White Sea. Thus, the salinity of the neighboring Barents Sea is 32–34‰; it is close to oceanic salinity but probably does not match the salinity range tolerated by White Sea mussels and starfish at the most sensitive period of their ontogeny.

In mussels as well as starfish, the ranges of salinity tolerance successively widen from the eggs to the embryos and larvae. Salinity tolerance increases in the following way: fertilized egg cells $\longrightarrow$ embryos $\longrightarrow$ early larvae $\longrightarrow$ late larvae. This observation is in agreement with the well-known Pelsener–Shelford ecological principle, which indicates that tolerance to

environmental variations increases with ontogenetic development in various organisms.

We now expand on how the range of salinity tolerance increases in the early ontogeny of mussels and starfish. Some differences between mussels and starfish appear by the embryonic level. Starfish embryos developing at 14–16‰ perish in the transition from the blastula to the gastrula stage. Mussel larvae at the conchostoma stage, which is analogous to the gastrula stage, are subject to various abnormalities, but they do not die. This may be because the *A. rubens* embryo completely loses its egg membrane in this period, while the *M. edulis* embryo retains it for a long time [12]. When comparing the larval stages of mussels and starfish, differences are also found in the pattern of increase of salinity tolerance at transitional stages. Thus, a marked change in salinity tolerance occurs in mussel larvae at the transition from the conchostoma to the veliger stage (Fig. 4). The range of salinity tolerance expands from 20–26‰ in the conchostoma to 5–45‰ in the veliger. This is probably related to the ability of the veliger to isolate itself from the environment by forming a true shell.

In the early ontogeny of the starfish, the range of salinity tolerance changes more smoothly: it gradually widens from the most vulnerable egg cell stage (24–26‰) to the late larval brachiolaria stage (12–28‰) (see table). Starfish larvae are not capable of isolating themselves from the environment, as mussel larvae do with the help of the shell, and the ranges of salinity tolerance of larval starfish are narrower than those of mussels (see table). Unlike mussels, which are able to adapt to changes in salinity at an early larval stage at the organismal level, the range of salinity tolerance of larval and adult starfish is a reflection of the adaptive capabilities of its tissues.

It is noteworthy that, in the ontogeny of starfish and mussels, a decrease in the lower extreme of salinity occurs at the late larval stages, 12‰ in bipinnaria and brachiolaria relative to 16‰ in adult starfish (see table). This may be attributed to the fact that these larvae occur in the near-surface water, where they experience reduced salinities. The bipinnarias of the starfish *A. amurensis* are more tolerant to low salinities and survive at 13‰ [22], while adults are only able to tolerate a salinity of 18‰ [7]. A similar adaptive plasticity also characterizes mussels; their veligers and pediveligers are able to withstand a salinity as low as 5‰, while yearling mussels tolerate only 10‰ (see table; [14]). Similar results have been reported for the edible mussel from the Sea of Japan [13]. Increased tolerance of low salinity has been demonstrated for the larvae of other marine invertebrates, such as the crustacean *Carcinus maenas* [16], *Gammarus duebeni* [18], the scyphoid medusa *Aurelia aurita*, and *Cyanea capillata* [6]. It is conceivable that this is a peculiarity of a number of marine invertebrates that have free-swimming larvae, which promote the spread of the species and allow for new areas to be colonized.

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