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Adaptability of the Amphipod *Pontoporeia affinis* (Crustacea: Amphipoda) to Salinity Changes

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Abstract—Salinity tolerance of the amphipod *Pontoporeia affinis* from the Baltic Sea was examined after acclimation to increased and decreased salinity. There were adaptive shifts in tolerance related to ambient salinity. A sharp change in salinity tolerance of amphipods occurred after acclimation to extremely low salinity.

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The salinity of the Baltic Sea is markedly lower than normal oceanic salinity; therefore, many freshwater species form stable populations here. The amphipod **Pontoporeia affinis** is a relic freshwater species, which occurs widely in lakes of Northern Europe and estuaries of rivers flowing into the seas of the Arctic Ocean [9]. This amphipod is also a common inhabitant of the Baltic Sea, where it occurs at salinities from 3.4 to 10.6‰ [7].

In brackish-water ecosystems, salinity often plays a key role for the development of many species of aquatic invertebrates. One of the major indices reflecting the response of organisms to environmental factors is potential salinity tolerance [3, 5, 6]. The assessment of the potential tolerance range is important in theoretical and practical aspects, particularly in solving problems connected with the introduction of new species or the evaluation of possible responses of populations to environmental changes. To date, the ranges of potential tolerance to an environmental factor have been determined for very few species of aquatic invertebrates. Many aspects of salinity adaptation and ionic regulation in aquatic organisms [1, 2] have been studied fairly adequately; however, how tolerance changes in relation to environmental conditions has been investigated in a limited number of widely distributed forms. Hence, study of the tolerance of organisms in relation to habitat conditions, as well as the assessment of potential tolerance, is very important.

The present study examines the change in the salinity tolerance range of the amphipod *P. affinis* as a result of acclimation of this species to different salinities.

MATERIALS AND METHODS

Experiments were carried out in May 1994 at the Asko field station of Stockholm University (Baltic Sea, Asko Island). Amphipods (7–10 mm in body length) were collected in the vicinity of the station from the 10 m depth with a bottom trawl. At sampling places, salinity was 6.2‰. Prior to experiments, amphipods were kept for 1 day at the initial salinity to remove injured or impaired individuals and then placed in aquaria with a salinity of 0.9, 1.3, 1.9, 2.6, 4.1, 6, 8.9, 13.6, 20.4, and 30‰ for subsequent acclimation (300 specimens in each salinity).

Acclimation was carried out for 2.5 weeks in an isothermal room (temperature 13°C) with constant light and aeration. Each aquarium with amphipods contained about 20 liters of water and a 1.5- to 2-cm layer of sand. The water and special food of test animals was not changed throughout the acclimation period.

After 2.5 weeks in water of different salinities, the salinity tolerance range of amphipods was determined. To this end, animals were removed from the acclimation aquaria and placed in groups of 10 individuals in vessels with water of the tested salinities (in two replicas). Tests were conducted in 0.8-l vessels without a substrate and aeration at a temperature of 20°C. After 2 days of exposure, the number of active animals (%) in test vessels was calculated. The data were averaged for each of the test salinities and formalized by means of a sigmoid dose–response curve:

$$Y = a + \frac{(b - a)}{1 + 10^{(c - x)k}},$$

where a is the minimum value of the response function (in our case 0%); b, the maximum value of the response function (in our case 100%); c, the logarithm of a 50%

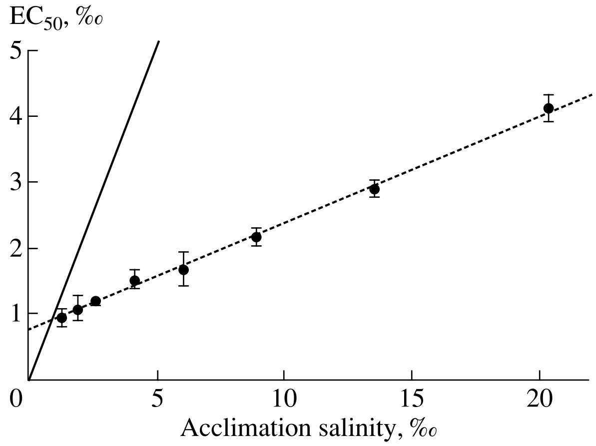

Fig. 1. The relationship between lower tolerance limit of the amphipod *Pontoporeia affinis* (EC_{50}) and acclimation salinity. Dashed line—regression line, solid line—isoosmoticity line, vertical bars—95% confidence intervals.

response ($\log EC_{50}$); and k, the coefficient of inclination. Salinity corresponding to 50% survival of individuals (EC_{50}) was used to calculate salinity tolerance of amphipods. Calculations were made using "GraphPad Prism 3.0″ computer programs.

Water for the experiments was obtained from seawater with a salinity of 6‰ collected at Asko Island. Water of reduced salinity was prepared by diluting seawater with distilled water; water of increased salinity was achieved by the addition of commercial sea salt.

RESULTS AND DISCUSSION

By the end of the acclimation period, living *Pontoporeia affinis* were found in the salinity range of 0.9 to 20.4‰. In almost all aquaria, amphipod survival was fairly high (over 60%). The exception was 6‰, at which amphipod survival was only 40%. At 30‰, there were no live individuals by the end of acclimation.

For amphipods acclimated at 6‰ (close to the initial environmental conditions), EC_{50} (2-day exposure at a temperature 20°C) was 1.7‰. After acclimation to decreased and increased salinity, correspondingly, there was a shift in tolerance limits of the test organisms. EC_{50} was 0.9‰ for amphipods acclimated at 1.3 and 4‰ for those acclimated at 20.4‰. In the salinity range from 1.3 to 20.4‰, the relation of the tolerance limits to ambient salinity was clearly linear (Fig. 1). The change in tolerance was proportional to the salinity of acclimation; but it was less marked: with an ambient salinity change by a factor of 15, EC_{50} correspondingly changed by a factor of 4.5.

A group of amphipods acclimated at 0.9‰ showed paradoxical results: their response to test salinity was inverted. Usually, in the course of experiments, mortality increased with lowering salinity; however, in amphipods acclimated at 0.9‰, mortality generally decreased

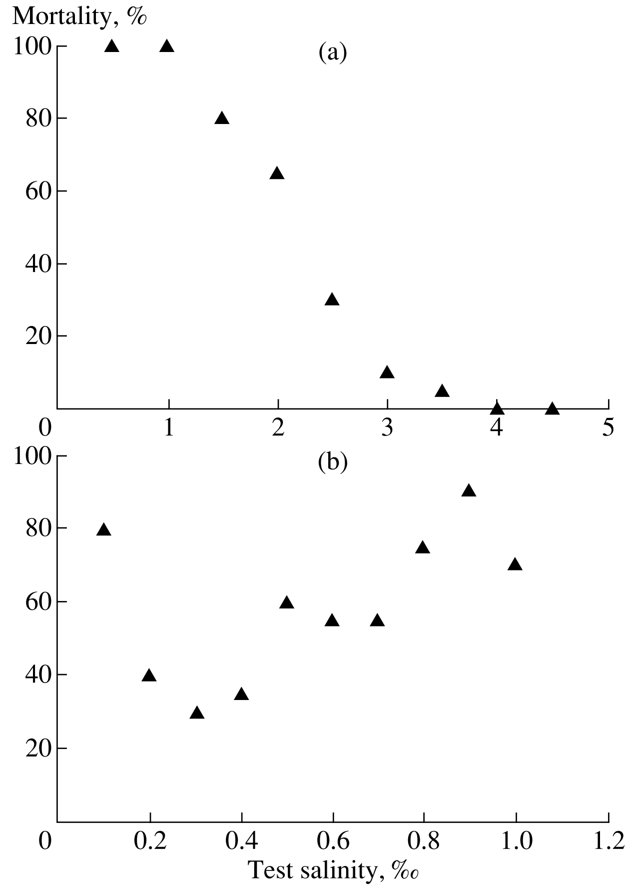

Fig. 2. The relationship between mortality of the amphipod *Pontoporeia affinis* and salinity of water in the course of tests. a—typical relation (after acclimation to 8.9‰), b—inverted relation (after acclimation to 0.9‰).

with a decrease in salinity. Mortality was highest at salinity corresponding to that of acclimation, and minimal at 0.3‰ (Fig. 2).

Based on the results from acclimation of *P. affinis* in the salinity range of 1.5 to 20.4‰, we determine the limits of potential salinity tolerance of this species. The regression line for the change in the tolerance limit crossed the isoosmoticity line at a point of 0.8‰ (Fig. 1). Following the previously suggested approach [4], the resultant value, at first approximation, can be taken as the lower limit of the potential tolerance range of the *P. affinis* population.

It is not clear why *P. affinis*, which forms large concentrations in the Baltic Sea, on the one hand, and is common in fresh waters, on the other, was unable to withstand the low salinity of Baltic waters. Amphipods from the initial salinity (6‰), as well as specimens acclimated to low salinity (1.3‰), perished in fresh water (<0.5‰). It is not improbable that the *P. affinis* population in the investigated region has became incapable of due hyperosmotic regulation that is needed for existence in fresh water. This phenomenon, when, along with the original euryhaline forms able to live in waters of a wide range of salinity, there are predominantly fresh water or predominantly marine forms, is known as “genetic triads” [8].

Further, very smooth lowering of salinity and more time may be needed for the adjustment of osmoregulatory mechanisms during freshwater exposure. This is probably supported by the fact that, after prolonged acclimation to 0.9‰, the amphipods showed an inverted response to salinity and were able to quite well endure fresh water (0.3‰). It is not inconceivable that the paradoxical results with this group of amphipods are due to the synergism of extremely low salinity and increased water temperature during the test. In any case, the question needs further study.

Generally, the results of our experiments and literature data suggest that *P. affinis* is a euryhaline species, which is able to withstand sharp variations of water salinity within a wide range (for the investigated population, at least from 1 to 20‰). This ability accounts for the wide distribution of this species in wild and artificial biotopes and for its significant role in the bottom communities.

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