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Niche overlap in mud snails (Hydrobiidae): freezing tolerance

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Abstract

Tolerance to freezing was measured in four species of hydrobiid snails exposed to temperatures from 0° to −8 °C and salinities from 0 to 30‰ S for up to 7 d. Experiments showed the following increasing order of tolerance to freezing: *Potamopyrgus jenkinsi* (Smith), *Hydrobia neglecta* Muus, *H. ulvae* (Pennant) and *H. ventrosa* (Montagu). Survival decreased with increasing salinity in *P. jenkinsi,* while the opposite was found in *Hydrobia* species. *H. ulvae* survived only slightly better than *H. neglecta,* while *H. ventrosa* was very tolerant to freezing even at low salinities in contrast to the other species of *Hydrobia. H. neglecta* suffered higher winter mortality than *H. ventrosa* during the severe winter of 1985 in an estuary.

Introduction

Hydrobiid mud snails are very common in shallow marine and brackish environments. In Denmark it is hardly possible to find a single square meter of sea bed where they are missing, except on black, stinking mud and exposed sandy beaches (Muus, 1967; Hylleberg, 1986). The population density of these small snails (2 to 5 mm) is often in the range of 20 000 to 40 000 m^{-2}. The snails are deposit feeders and able to digest a variety of the microorganisms associated with sediment particles (Kofoed, 1975; Lopez and Levinton, 1978). Diatoms are the most important food source (Muus, 1967; Fenchel and Kofoed, 1976; Jensen and Siegismund, 1980). Three species from the genus *Hydrobia* are found: *H. ulvae, H. neglecta,* and *H. ventrosa;* in addition, *Potamopyrgus jenkinsi* can be common.

The species may coexist in the same patch of sea bed, but rarely in equal abundance (Muus, 1967; Fenchel, 1975; Hylleberg, 1986). Hydrobiids display exploitative interspecific competition in laboratory experiments (Fenchel and Kofoed, 1976). Since the snails obviously compete for important food items, it is of interest to estimate as many parameters of the fundamental niches as possible in order to judge the integrated responses to environmental variations. Some parameters of the fundamental niches of hydrobiids have previously been reported in *Hydrobia* species (Hylleberg, 1975, 1976; Lassen and Kristensen, 1978). In nature, hydrobiids are subjected to a variety of environmental changes which determine the response of the snails. Both salinity and temperature change seasonally. Therefore, the distribution group of interacting species may be restricted severely during a certain season. This may alter the importance of biological interactions. The purpose of the present study is to show tolerance to freezing in combination with salinity. Such data were necessary for the interpretation of the distribution of the four species of mud snails in an estuary (Siegismund and Hylleberg, 1987).

Materials and methods

Experimental determination of freezing tolerances

Three experiments were carried out: In the first, *Hydrobia neglecta* Muus and *H. ventrosa* (Montagu) were collected from Kysing Fjord (Siegismund and Hylleberg, 1987), *H. ulvae* (Pennant) from the Wadden Sea (Site 8 in Hylleberg, 1986) and *Potamopyrgus jenkinsi* (Smith) from the river Mattrup Å. The snails were collected in April 1979 after a severe winter, stored at 3 °C at 20‰ S (*Hydrobia*) or fresh water (*P. jenkinsi*), and provided with surface sediment as food source until used in experiments one month later. Batches of ten individuals were transferred directly from storage tanks to 80 test tubes with 20‰ S sea water (*Hydrobia* spp.) and fresh water (*P. jenkinsi*). The tubes were placed in a −7 °C water bath containing anti-freeze fluid, a crystal of ice was added, and the tubes were sealed with parafilm. The 10-ml test tube water immediately froze solid. At 12- to 24-h intervals the tubes were transferred to 3 °C for 1 d; surviving snails were then counted at room temperature (ca 18° to 20 °C). The criterion for survival was the ability to contract musculature upon mechanical stimulation with a needle.

The second experiment was carried out as above, but with *Hydrobia ulvae* collected in Limfjorden (St.F in Hylleberg, 1986) and *Potamopyrgus jenkinsi* from the river Bjørnsholm Å. The snails were collected in December 1983 before the habitats froze solid in the field. They were stored at 2 °C without food until the experiments were carried out in January/February 1984. In the storage room, the snails were acclimated for one week in water with different test salinities. We considered one week to be sufficient time for salinity acclimation since our main interest was comparisons among the species. Samples of 50 snails were transferred to 72 test tubes and frozen as above. Separate experiments were made at temperatures ranging from 0° to −8 °C at 2 C° intervals. A single test was made at −12 °C, but all the snails died. The snails were exposed to experimental temperatures for periods of 2, 4 and 7 d, thawed at room temperature (18° to 20 °C), and survival counted after one day with the same criterion as in the 1979 experiment.

The third experiment was also carried out as above. The *Hydrobia* species were collected from Kysing Fjord, with additional samples of *H. neglecta* and *H. ventrosa* from Kalø (Site 130 in Hylleberg, 1986). The samples were collected in April 1985 after a severe winter. They were used in experiments one month later. Only the tolerance to freezing at −5 °C was tested. Survival was counted daily for one week. The criterion for survival was active crawling at room temperature. Control snails transferred from storage at 2 °C directly to room temperature had no mortality.

Estimation of winter mortality

On April 16, 1985 samples were collected from the head to the mouth of Kysing Fjord (St. 1 to 7, Siegismund and Hylleberg, 1987). Surface sediment was passed through a 1-mm sieve in the field. The total residue was stored at 2 °C in sea water from the locality and sorted within 2 d into actively crawling snails and recently dead specimens. Criteria for the latter category were: remains of soft parts, operculum present, black deposits and white sulphur bacteria at the shell aperture, or glossy periostracum. Old shells, i.e. snails dead before onset of the winter, were white, calcareous and lacked periostracum. Such shells were discarded. There is pronounced variation in hydrobiid shell morphology (Muus, 1967), but dead shells can be identified by comparison with live snails from the locality, after some experience.

Results

Experimental determination of freezing tolerance

The first experiment on freezing tolerance was carried out at −7 °C and a fixed salinity of 20‰ S. All *Hydrobia neglecta* were dead after 5 d, all *H. ulvae* after 11 d, however, some *H. ventrosa* were still alive after 13 d. LD_{50} values were estimated graphically from the percentage survival plotted against time. The data displayed only a small scatter in *H. neglecta* and *H. ulvae* and somewhat more in *H. ventrosa*. The LD_{50} values were 2.5, 5.5 and 11 d, respectively. Additional tests were made at −5° and −3 °C. LD_{50} values were higher, but the same order of tolerance to freezing was obtained at all temperatures. *Potamopyrgus jenkinsi* did not tolerate freezing in fresh water at any of the temperatures. It survived only a few hours at the highest test temperature. Since this species is widespread in mixohaline water, a second experiment was carried out in order to test the effect of salinity on tolerance to freezing at a given temperature.

Combined effects of salinity and temperature

In the second experiment the pattern of survival was identical after 2, 4 and 7 d at combinations of temperature from 0° to −8 °C and salinities from 5 to 30‰ S. However, survival was enhanced, and lower temperatures were tolerated with decreasing time of exposure to freezing. The survival after the maximum incubation time (7 d) is shown in Figs. 1 to 4.

The most striking difference among the species was observed between *Potamopyrgus jenkinsi* and the *Hydrobia* species as in the first experiment. Only a small fraction of *P. jenkinsi* survived at −2 °C for 7 d (Fig. 1). They only survived at the lower salinities (5 and 10‰ S), whereas *Hydrobia* species' survival was enhanced at higher salinities (Figs. 2 to 4). After 2 and 4 d of freezing, the same tendency was observed. A further difference between *P. jenkinsi* and *Hydrobia* species was observed at 0 °C where *P. jenkinsi* had almost 100% survival at all salinities while survival decreased at the lowest salinities in *Hydrobia* species.

Among the *Hydrobia* species, the sequence of increasing tolerance to freezing was *H. neglecta, H. ulvae* and *H. ventrosa*. In *H. neglecta,* no survival was recorded below −6 °C after 7 d (Fig. 2). Further, the survival decreased drastically below 15‰ S and was negligible at 5‰ S. A similar picture was found in *H. ulvae* (Fig. 3), although the decrease in survival was smaller at 10‰ S. An additional point that shows the higher tolerance of *H. ulvae* was the survival at −6 °C after 2 d of freezing. At this combination, survivors were only recorded among *H. neglecta* at the two highest salinities (average 6%), whereas survivors were found at all salinities in *H. ulvae* (average 36% survival).

Hydrobia ventrosa tolerated more freezing and lower salinities than any of the other mud snails. It survived freezing at −6 °C for 7 d (Fig.4). At temperatures ranging from 0° to −4 °C, it survived almost with 100% down to 10‰ S and was the only species with a relatively high survival at 5‰ S. As in the first experiment, *H. ventrosa* displayed more scatter of data than the other mud snails.

The experiment was repeated in 1985 in order to observe if full crawling activity would be obtained by snails

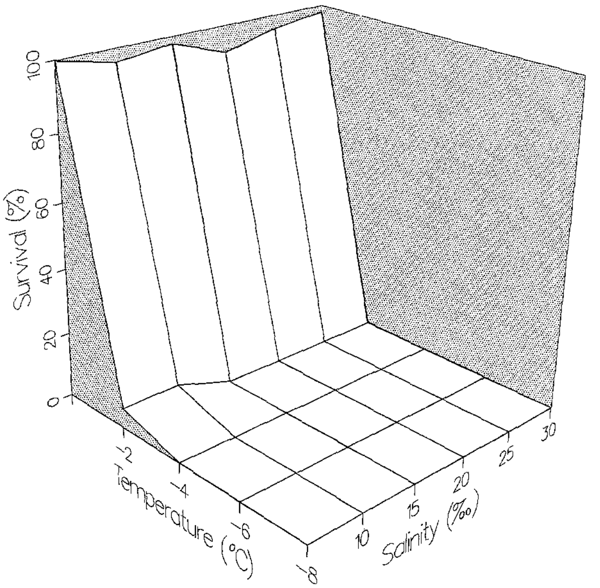

Fig. 1. *Potamopyrgus jenkinsi*. Percentage survival after 7 d of exposure to combinations of temperature and salinity as indicated

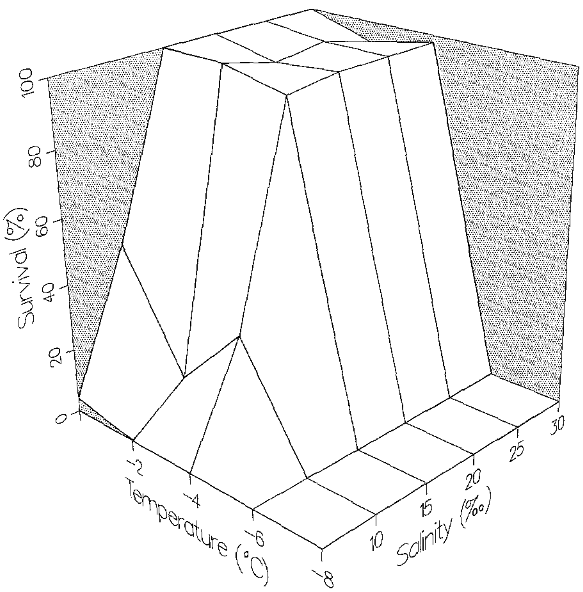

Fig. 3. *Hydrobia ulvae*. Percentage survival after 7 d of exposure to combinations of temperature and salinity as indicated

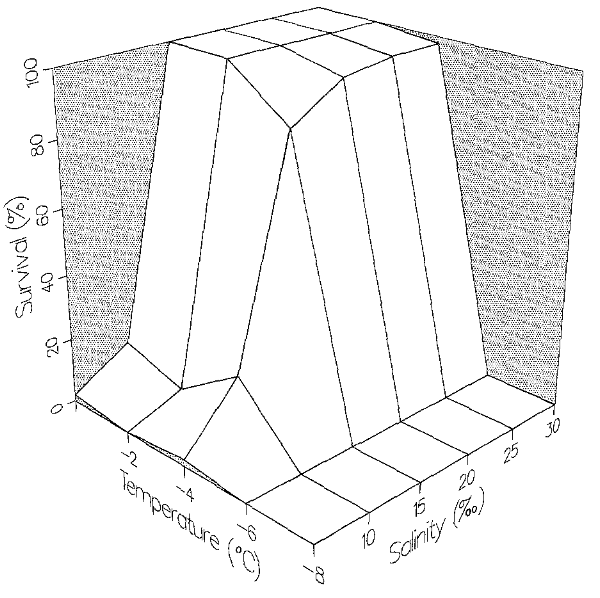

Fig. 2. *Hydrobia neglecta*. Percentage survival after 7 d of exposure to combinations of temperature and salinity as indicated

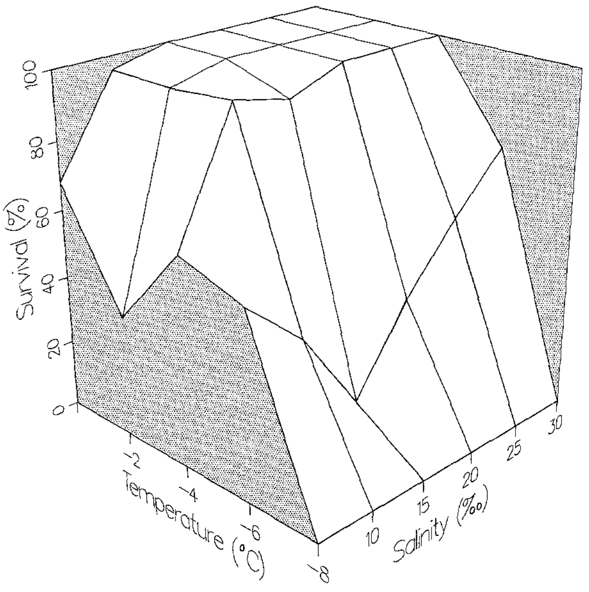

Fig. 4. *Hydrobia ventrosa*. Percentage survival after 7 d of exposure to combinations of temperature and salinity as indicated

exposed to freezing. The absolute number of snails judged alive after freezing was lower than in previous experiments but the order of species tolerating freezing at combinations of salinity was unchanged. Only at high salinity, 25 to 30‰ S, did *Hydrobia neglecta* and *H. ulvae* attain full crawling activity after 2 d at –5 °C (3 and 14%, respectively). Lower temperatures and longer incubation time resulted in 100% mortality. *H. ventrosa* survived –5 °C for 7 d; best at high salinities. Seventy percent of the individuals were fully active in water of 30‰ S. Complete survival decreased to 2% at 5‰ S. At 5 to 10‰ S, many shells were damaged by expansion of the ice during incubation. The soft snail body protruded through holes in the broken shell. Yet, such snails could crawl on the first days after thawing. However, they all died before observations were terminated after one week. The criteria of survival differed in the 1984 and 1985 experiments. This may explain why survival was rated higher in the former experiments, especially at low salinities.

Field evidence of winter mortality

The estuary Kysing Fjord has a well developed salinity gradient most of the year (Muus, 1967; Siegismund and Hylleberg, 1987). The estuary is shallow and may freeze

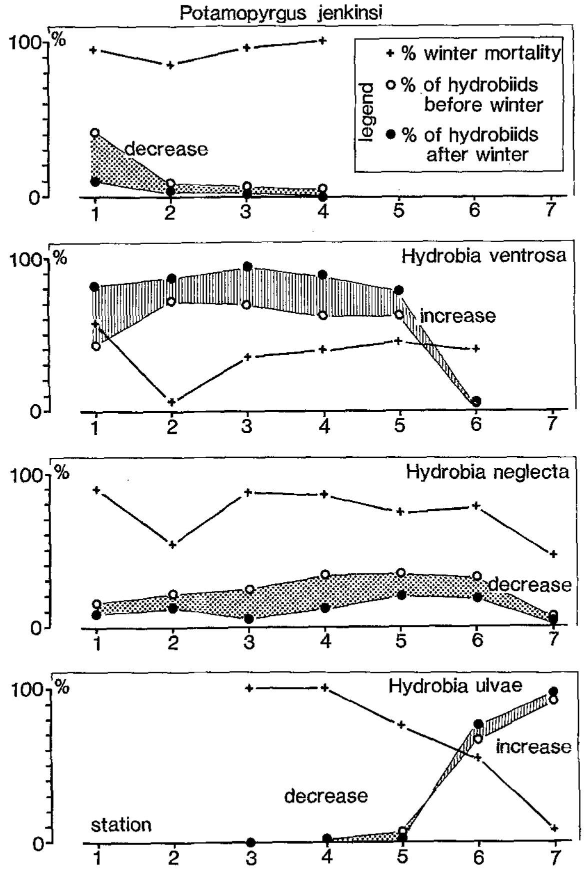

Fig. 5. *Potamopyrgus jenkinsi, Hydrobia ventrosa, H. neglecta* and *H. ulvae.* Relative winter mortality calculated by difference between number of snails before and after winter at St. 1 to 7 in the estuary Kysing Fjord. St. 1 is at the head of the estuary and St. 7 is at the mouth of the estuary. The difference between relative proportions of the four species shows whether a species has decreased or increased its abundance relative to other species at each station

solid during severe winters. In order to assess the natural winter mortality, samples were collected along the salinity gradient (St. 1 to 7 in Siegismund and Hylleberg, 1987). From the number of living and recently dead snails, the relative frequency of each species before and after the winter could be determined, as well as the mortality of each species (Fig. 5).

Before the estuary was frozen solid, and in accordance with other records (Siegismund and Hylleberg, 1987), *Potamopyrgus jenkinsi* and *Hydrobia ventrosa* were equally abundant and *H. neglecta* was present at low salinities at St. 1. During the winter *P. jenkinsi* and *H. neglecta* suffered higher mortalities than *H. ventrosa,* which became the dominant species at the beginning of the period of reproduction. Sampling through the estuary showed extinction of *P. jenkinsi* at higher salinities and a decrease of *H. neglecta* and *H. ulvae* at lower salinities, thus leading to a clear dominance of *H. ventrosa.* Only at the high salinity at the mouth of the estuary did *H. ulvae* show a small winter mortality and the species became relatively more dominant than before the onset of winter (St. 6 and 7). These findings agree with casual observations from other estuaries as reported by Muus (1967).

It is concluded that our experiments on freezing tolerance agree well with estimates of natural winter mortality. Survival of *Potamopyrgus jenkinsi* decreased rapidly with increasing salinity at sub-zero temperatures. The *Hydrobia* species showed the opposite characteristic. The total range of temperature tolerated in the state of active life is large in *H. ventrosa,* smaller in the more marine *H. ulvae* and least in *H. neglecta.* In the latter two species, survival is significantly promoted by high salinity.

Discussion

It is obvious that the experiments presented here cannot be regarded as replications of natural winter conditions experienced by mud snails living at high salinity in the North Sea and low salinity in the Baltic Sea. It is, however, important to note that identical orders of survival were obtained with hydrobiids exposed to freezing under a variety of experimental conditions. The sequence of tolerance to freezing is very robust and indicates that sub-zero temperatures in nature can cause species-specific mortality in a variety of biotopes. We believe that the sequence of tolerance in the studied population is representative for all species' populations in Denmark, but we hesitate to extrapolate our results, for example, to populations from the Baltic Sea where *Potamopyrgus jenkinsi, Hydrobia ventrosa* and *H. ulvae* live constantly at low salinities. Within the same species of mud snail, the detrimental effects of freezing may be different at different salinities and in genetically different populations. We have not assessed this matter directly since the experiments were carried out on batches of snails. However, in accordance with previous studies *H. ventrosa* yielded more scatter of the response data than any of the other mud snails (Hylleberg, 1975; Lassen and Kristensen, 1978). This indicates a more pronounced variation among individuals that can be of ecological significance, especially in populations under physiological stress in fluctuating environments. Individual variation provides a basis for selection and population survival.

Previous studies on niche dimensions have shown that, in terms of tolerance to desiccation and high temperature, the order of decreasing tolerance was *Hydrobia ulvae, H. neglecta* and *H. ventrosa.* The last was also the least tolerant species to anoxia and hydrogen sulphide (Muus, 1967; Lassen and Kristensen, 1978). This order was changed with respect to tolerance to freezing: *H. ventrosa, H. ulvae,* and *H. neglecta. H. ventrosa* survived well at all salinity-temperature combinations, whereas *H. ulvae* and *H. neglecta* were significantly hampered by low salinity. This pattern resembles the combined effects of temperature and salinity on feeding activity. Hylleberg (1975) found that *H. ventrosa* was only slightly affected by salinity and temperature variations within the range normally experienced by this species. However, summer temperatures (20 °C) resulted in maximum feeding activity at 30, 25 and 20‰ S, respectively in *H. ulvae, H. neglecta* and *H. ventrosa.*

The present laboratory experiments and field study show that freezing must be extremely severe in order to wipe out one population, say *Hydrobia neglecta,* and not the coexisting *H. ventrosa.* In open systems with a depth gradient similar to the Kysing Fjord estuary, this is very unlikely to happen. Along the lines of Reise (1985) we are dealing with a hierarchy of promoting and repressive processes in the inner part of Kysing Fjord, i.e. recurrent fluctuating stress situations where competition has a diminished effect on species composition and relative abundances. Tolerances to abiotic conditions become important distributing factors. In the case of tolerance to freezing, the present evidence indicates that a severe winter can reset the clock of competition within, as well as between species, striving for the same resources. In the presence of such environmental impact, it is unlikely that the density of competitors can reach a high level over a longer period so that one or more species become eliminated from the assemblage due to competitive processes. On the other hand, in the deeper parts of Kysing Fjord near the mouth of the estuary, the environment fluctuates less and provides conditions for potential biotic interactions. Tolerance to freezing cannot explain why *H. ventrosa* is absent from that part of the estuary, nor the coexistence of *H. neglecta* and *H. ulvae.* The first species performs well at high salinity and temperatures present at the mouth (Hylleberg, 1975; Lassen and Kristensen, 1978; Lassen and Clark, 1979). The latter two species have similar tolerances to freezing and both survive best at high salinity. In this case, other factors, such as way of reproduction and migration, become important factors in the understanding of the pattern of occurrence as discussed in Siegismund and Hylleberg (1987).

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