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Effect of season on the acute thermal tolerance and thermal inactivation of membrane (Mg^{2+} + Na^{+} + K^{+})- and Mg^{2+}-adenosine triphosphatase activity of the Baltic Sea amphipods, *Monoporeia affinis* and *Gammarus* spp.

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This paper presents a study of the acute upper thermal tolerance and thermal inactivation (IA50) of the membrane-bound (Mg^{2+} + Na^{+} + K^{+})- and Mg^{2+}-adenosine triphosphatases of the Baltic amphipods, *Monoporeia affinis*, inhabiting a stable cold environment (ca. 3.5 °C), and *Gammarus* spp., living in annually fluctuating temperatures (0–20 °C). For *M. affinis* the lethal temperatures (LT50) in a continuously rising temperature were 29.8 °C in winter, 29.5 °C in spring and 30.2 °C in summer. For *Gammarus* spp. the LT50 was 31.0 °C in spring, 32.8 °C in summer and 30.2 °C in autumn. Seasonality in the LT50 values was observed in *Gammarus* spp., but in *M. affinis* only the summer value differed from the other seasons. The LT50 of *M. affinis* did not change after temperature acclimation of the animals at 13.5 °C. The thermal inactivation values (IA50) of the ATPases of *M. affinis* (44.3–47.4 °C) were higher than those of *Gammarus* spp. (40.3–45.2 °C). In *Gammarus* differences in the IA50 values between the seasons were found while in *M. affinis* only between spring and summer.

Introduction

The northern Baltic Sea represents a climate regime in which marine poikilotherms are constantly influenced by a strong variation of environmental parameters, e.g. temperature, photoperiod and biological production. In the water column, water temperatures below 6 °C prevail, but on the surface and in shallow water summer temperatures can reach 20 °C (Haapala and Alenius 1994). The sea area is often ice-covered between January and April. Baltic poikilotherms are therefore presumed to show adaptation (acute/seasonal/evolutionary) to compensate for the effects of low temperatures. Poikilothermal animals have been classified according to their living temperatures as either eury- (wide range) or stenothermal (narrow range) species. The amphipods *Gammarus* spp. and *Monoporeia affinis* are common crustacean species in the ecosystem of the northern Baltic Sea and represent eury- and stenothermal species, respectively. *M. affinis* has a circumpolar distribution in northern brackish waters and lakes below the highest coast line. It is classified as a "glacial relict" in the Baltic Sea and a cold-stenothermal crustacean (Segerstråle 1937, Väinölä and Varvio 1989). *Gammarus* spp. also have a wide geographical distribution in littoral zones from the Arctic region in the north to the east coast of North America in the west (Segerstråle 1959). As eurythermal and euryhaline species, *Gammarus* spp. tolerate changes in temperature and salinity, as one would expect considering their wide geographical distribution. *Gammarus oceanicus* from the Gulf of Finland displays an elevated temperature tolerance in summer compared to that in winter (Suomalainen 1958).

M. affinis is the dominant benthic crustacean species in soft bottom habitats in the northern Baltic Sea. The temperature preference and tolerance of the amphipod families *Pontoporeiidae* inhabiting brackish and freshwater environments have been a subject of interest since the beginning of the 20th century, e.g. in the lakes of northern Germany (Samter and Weltner 1904), in Scandinavian lakes (Ekman 1915, Valle 1927) and in the Gulf of Finland (Segerstråle 1937, 1978). From these early studies it was concluded that *M. affinis* avoids higher temperatures but is able to live in brackish water at temperatures near 20 °C and can also be found in the surface layers of lakes during summer months. Its reproduction is restricted to cold water, either in the cold season or during the warm season in cold deep water. However, past studies were primarily focused on ecological research, not on the temperature biology. In the southern Baltic Sea, large numbers of experiments have been conducted on *Gammaridae* (Kinne 1970 and references therein, Furch 1972).

A hypothesis for the cause of heat death, as presented by Gladwell *et al.* (1975) and Bowler (1987), includes a sequence of irreversible events starting from the perturbation of the plasma membrane and loss of ion gradient and ending in the death of cells. In this connection the activities of membrane-bound ATPases are often determined because of the role of these enzyme systems in ion transport through cell membranes and in thermal death. The general argument is that the cost of the cold adaptation of enzymes is a decrease in thermal tolerance (Cossins *et al.* 1981).

The purpose of this study was to find out if there are intra- and interspecific seasonal differences in the acute upper thermal tolerance of *Gammarus* spp. and *Monoporeia affinis* inhabiting different thermal environments. In addition, the thermal inactivation of the membrane-bound (Mg^{2+} + Na^{+} + K^{+})- and Mg^{2+}-adenosine triphosphatases (ATPases) of these species was determined. When measuring the upper thermal tolerance of the species, an exposure at a constant temperature or an exposure to a continuously rising temperature was applied (Precht 1973, Bowler 1987, Kivivuori and Lahdes 1996).

Material and methods

Sampling and material

Monoporeia affinis was collected at station SR5 (61°05.00´N, 19°36.00´E, depth 125 m) in the southern Gulf of Bothnia, the Baltic Sea. Sampling took place in winter (February–March), spring (June) and summer (August) in 1994. The sampling procedure was the same as described by Lehtonen (1996). The animals were stored in a holding aquarium with a thin layer of sedi-

ment on the bottom at an *in situ* temperature of 3.5–4 °C for at least 24 hours before the exposure tests. Sediment was added to prevent extra mortality (Smith 1972). Egg-carrying females and the age class 0+ were not selected for the exposures. The age distribution of the animals used in the experiments was determined after the heat exposures. The animals mainly represented the age classes 1+ and 2+ (length: 4–9.5 mm, wet wt: 1.0–10.5 mg), namely 96.2% in winter, 86.5% in spring and 89.4% in summer, the rest being in the age class of 3+. Adult males were not found among the collected animals.

Gammarus spp. were collected at the station of Seili (60°15.25´N, 21°57.30´E) near the Archipelago Research Institute, in the Archipelago Sea, the Baltic Sea, in spring (May), summer (August) and autumn (November–early December) in 1994 as described in Lahdes *et al*. (2000a). Prior to the exposure tests the amphipods were stored for at least 24 h in aerated plastic containers at *in situ* temperatures of 8 °C in spring, 20 °C in summer and 5.5 °C in autumn. The species distribution was the same as described in Lahdes *et al*. (2000a). Species (*G. oceanicus, G. zaddachi, G. locusta*) and sexes were not differentiated since the purpose was to examine variation between the seasons in existing communities, not between individuals.

Sea water used in the experiments was collected at the sampling sites SR5 and Seili at the time of samplings. In the following text, the term "holding temperature" refers to the *in situ* temperature.

Tests of the upper lethal temperature

The tests with *M. affinis* were performed on board r/v *Aranda*, while tests with *Gammarus* spp. took place in the laboratories of the Archipelago Research Institute and the Laboratory of Animal Physiology, University of Turku.

The tests in the present study are denoted as Test I, IIa and IIb referring respectively to Methods I and II recommended by Precht (1973):

Test I: A fixed-time exposure at constant temperatures.

Test IIa: Exposure to continuously rising temperature (0.2 °C min^{-1}). At given temperatures a group of animals is removed from the bath and replaced in the holding temperature. The mortality is determined after a given period of recovery.

Test IIb: Exposure to continuously rising temperature (0.2 °C min^{-1}). During the heating the death temperature of one animal at a time is determined both immediately and after a given period of recovery.

Test I was applied to winter collected *Monoporeia affinis*. Test IIa to both *M. affinis* and *Gammarus* spp. and Test IIb to *Gammarus* spp. Test IIa was also used in the acclimation study to determine the LC50 in *M. affinis*.

Test I

Test I included exposures of *M. affinis* for seven days at a constant temperature of 13.5 °C or three days at 19.6 °C. Animals were taken from the holding aquarium and placed in 50 ml of water at the same temperature. They were then poured directly into glass jars (1 or 2 litres). with sediment on the bottom containing water preheated to the testing temperature or the holding temperature. The density of the animals in the jars was ten specimens/l. Control groups were kept at the holding temperature. The number of parallel jars (both exposed group and control group) was five at 13.5 ° and two at 19.6 °C. The total number of tested animals in both the exposed and control groups was 59 specimens at 13.5 °C and 45 specimens at 19.6 °C, respectively. Mortality was determined daily immediately without a recovery time. The animals were carefully removed from the water using a net spoon. Animals that displayed no movement in the net within 10–15 seconds were considered dead.

Test IIa

Monoporeia affinis. The animals were placed in five glass jars, 10–12 specimens in each jar, containing 60–80 ml of water at the holding temperature. The jars were placed in a water bath thermostated to the same temperature as the holding one. The control jars were kept at the holding temperature. The water bath temperature

was increased at a rate of 0.2 °C min^{-1} (Lauda RCS 20D controlled by a PC programme). When temperature had risen to 28, 29, 30, 31and 32 °C, one jar at a time was removed from the water bath and replaced in the holding temperature. The exposure times were 2, 2.08, 2.17, 2.25 and 2.33 hours, respectively. After 12 hours of recovery, the mortality of treated and control animals was estimated individually as in Test I.

Gammarus spp. The animals were placed in five glass jars, 2–6 specimens (depending on their size) in each jar, containing 60–80 ml of water at the holding temperature. The jars were placed in a water bath thermostated to the same temperature. The water bath temperature was increased at a rate of 0.2 °C min^{-1} (Lauda MS). The control jars were kept at the holding temperature. The jars were removed from the water bath one by one at fixed temperatures of 30, 31, 32, 33 and 34 °C, and replaced in the holding temperature for 12 hours, after which the mortality of the treated and control groups was determined as for *M. affinis* in Test I. The corresponding exposure times were 1.8, 1.9, 2, 2.1 and 2.2 hours in spring, 0.83, 0.92, 1, 1.08 and 1.17 hours in summer and 2.04, 2.12, 2.2, 2.3 and 2.4 hours in autumn.

Test IIb

Gammarus spp. The experimental procedure was the same as in the Test IIa with the exception that the heat death temperatures for individuals were established using two animals in each jar and determining the heat death temperature of an individual *Gammarus* specimen during heating at a rate of 0.2 °C min^{-1}. After the heat test, each animal was replaced in the holding temperature for 12 hours in order to check whether any recovery had occurred.

Effect of temperature acclimation on temperature tolerance of *M. affinis*

The field value (Day 0) for the thermal tolerance of the animals was tested directly after the sampling using Test IIa. The field value represents Day 0 both for the exposed and control groups. The number of the tested animals was 64. The experimental animals were then acclimated at 13.5 °C and control animals at the holding temperature. After the acclimation of one, three and seven days at the respective temperatures both acclimated and control animals were tested for thermal tolerance using the Test IIa. The total number of exposed animals were 69, 60 and 67 on day one, three and seven, respectively. The corresponding numbers of control animals were 66, 66 and 72. The acclimation response ratio (ARR) was calculated by dividing the change in the thermal tolerance by the change in field (4 °C) and the acclimation (13.5 °C) temperatures. This ratio is used to describe the efficiency of acclimation/acclimatisation (Layne *et al*. 1987).

The thermal inactivation of (Mg^{2+} + Na^{+} + K^{+})- and Mg^{2+}-adenosine triphosphatases (3.6.1.3) in Fraction 1 membranes

In the ATPase measurements of the present study membranes prepared from the same pool of animals as in the studies of the membrane fluidity were used (Lahdes *et al*. 2000a). Fraction 1 is the fraction designated as plasma fraction collected at the interface of 1.0 M/1.25 M on the discontinuous sucrose gradient after the centrifugation of 50 000× g for 2 hours.

The activity of ATPases was measured according to the method used by Gladwell (1975), with the modification that the reaction was stopped by adding 4 ml of a mixture of 1% Lubrol and 1% ammonium molybdate in 1.8 N H_2SO_4 (Atkinson *et al*. 1973). Membrane samples were preincubated in test tubes for 15 minutes at temperatures of 25, 35, 40, 45 and 50 °C. After 15 minutes, the tubes were removed, dipped into an ice bath for 10 seconds and then put into a 25 °C water bath. The residual activities were measured in incubation media containing either (a) 3 mM $MgCl_2$ + 3 mM Tris-ATP for Mg^{2+}-ATPase or (b) 3 mM $MgCl_2$ + 20 mM KCl + 100 mM NaCl + 3mM Tris-ATP for total ATPase.

The residual activity of the total and Mg-ATPases in each series is presented as a percentage of the activity measured at 25 °C. The use

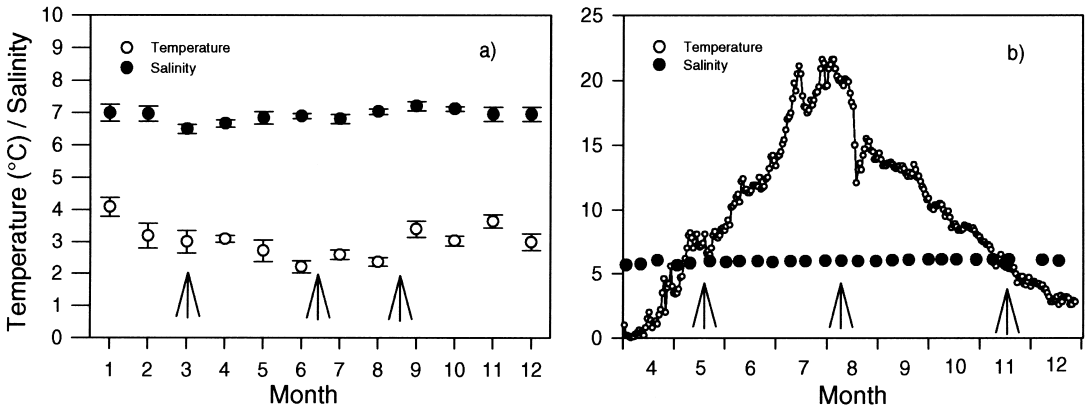

Fig. 1. The annual temperature and salinity variation at the sampling stations of (**a**) SR5 in the Gulf of Bothnia ± SE between 1962–1995 in the near bottom water (120–125 m), symbols: temperature (○), salinity (●), and of (**b**) Seili in the Archipelago Sea in the experimental year 1994 (surface water), symbols: temperature (line with circles), salinity (●). The arrows indicate the time of the samplings. Data provided by the Finnish Institute of Marine Research.

of residual activity was also recommended by Cossins *et al*. (1981).

Statistics

The temperature at which 50% of the animals died (LT50) and the temperature at which 50% of the enzyme activity was inactivated (IA50), with 95% confidence limits, were calculated with the PROBIT analysis according to Finney (1971) using a computer program from the National Swedish Environmental Agency. The significance of the differences in the thermal tolerance and thermal sensitivity of ATPases among seasons was tested with Tukey's HSD test (parametric one-way analysis of variance for comparisons of pairs) using a computer programme STATISTICA for Windows. The level of significance used was $p < 0.05$.

Results

Environmental conditions

On station SR5 (125 m) the variation of temperature in the bottom water was minimal (Fig. 1a). The water temperature below 40 metres remains more or less constant (< 5 °C) throughout the year. Although vertical migrations have been described (Segerstråle 1937, Marzolf 1965, Donner *et al*. 1987), it is likely that the bottom-resident *M. affinis* only seldom experience the higher temperatures prevailing in the water layers 85 metres above. Salinity values also remained at a constant level during the study period. Figure 1b illustrates the temperature and salinity conditions in the surface water during the experimental year at the sampling station for the *Gammarus* spp. situated in the Archipelago Sea. The temperature differences were 12 °C between the spring and summer samplings and 15 °C between the summer and autumn samplings (data obtained from the Data Register of the Finnish Institute of Marine Research).

Acute upper lethal temperature

Test I: The mortality of *Monoporeia affinis* at 13.5 °C after seven days was 5.2% in the experimental group was and 7.0% in the control group. The exposure for three days at 19.6 °C resulted in the mortalities of 22.2% and 8.3% in the exposed and control groups, respectively.

Test IIa. *Monoporeia affinis*: The range of LT50 was from 29.5 °C in spring to 30.2 °C in summer animals (Table 1). The differences in lethal temperatures between winter and spring and winter and summer were not significant, but between spring and summer it was significant, $p = 0.05$ (groups = 3, df = 34).

The behaviour of the animals during the

heating proceeded according to the following sequence: At a temperature of about 10 °C the animals increased their activity compared to the control animals. At about 16 °C the animals spent most of their time at the bottom of the test jar, with occasional sudden bursts of swimming. Starting from about 21 °C, the animals were mostly inactive, but started swimming when agitated. At a temperature higher than 23 °C they were passive and at 25 °C floating on the surface with the legs still moving. At these experimental temperatures, the point at which control of coordination was lost can be regarded as the critical thermal maximum (CTMax) of *M. affinis* (Nelson and Hooper 1982). At the experimental temperature of 28 °C, which was the lowest test temperature, the animals remained motionless and death could only have been confirmed under a microscope. When transferred immediately to the holding temperature, some recovery within 12 hours was observed at up to 30 °C. The mortality at 32 °C was always 100%.

Test IIa and b. *Gammarus* spp. There was only a minor difference (p = 0.26) in the data obtained from the heat death values of a group of animals (Test IIa) or an individual animal (Test IIb). In the calculation of the LT50 values the combined data were used. The LT50 values (Table 2) correlated with the environmental water temperature, being highest in summer and lowest in autumn. The differences in the lethal temperatures among all three seasons were statistically significant (Table 2, groups = 3, df = 75). In summer the temperatures of heat deaths were very close to each other once an experimental temperature of 32 °C was reached and no confidence limits were obtained. In the spring and autumn samples, deaths occurred more evenly while the temperature was rising during the test.

The effect of the rising temperature on the behaviour of the animals in the experiments could be observed when the experimental water temperature reached about 25 °C in spring and autumn, and 29–30 °C in the summer. The animals became passive, lying curved at the bottom of the jar with only their legs moving. Co-ordination of movement was also impaired. This point could be suggested as the critical thermal maximum (CTMax) of these animals, using the same criteria as with *M. affinis*. The difference in LT50 values for *M. affinis* and *Gammarus* spp. is 1.5 °C in spring and 3 °C in summer. In *Gammarus* spp. the values for autumn were approximately the same as those in *M. affinis* in winter (Tables 1 and 2).

The effect of a temperature acclimation on thermal tolerance of *M. affinis*

The LT50 of *M. affinis* acclimated at 13.5 °C for seven days increased from 29.6 °C on Day 0 to 30.6 °C on Day 7. At the same time the LT50

Table 1. The LT50 values (°C) of *Monoporeia affinis* from Test IIa and 95% lower and upper confidence limits. *N* is the total number of animals used in the experiments. The number of replicate experiments in is given parentheses.

Season	*N*	LT50	Lower	Upper
Winter	296 (3)	29.8	29.3	29.9
Spring	185 (3)	29.5	29.2	29.8
Summer	90 (2)	30.2	29.7	30.7
Winter–Spring	n.s.			
Winter–Summer	n.s.			
Spring–Summer	p = 0.05			
Acclimation test (7d)				
Day 0 (field value)	64 (1)	29.6	29.2	29.8
Controls, Day 7	72 (1)	29.8	29.4	30.3
Exposed, Day 7	67 (1)	30.6	30.1	31.1

Table 2. The LT50 values (°C) of *Gammarus* spp. from Test II, 95% lower and upper confidence limits and the statistical significance of the differences in the lethal temperatures among the seasons. *N* is the total number of animals used in the experiments. The number of replicate experiments is given in parentheses.

Season	*N*	LT50	Lower	Upper
Spring	27 (4)	31.0	30.8	31.2
Summer	88 (4)	32.8	–	–
Autumn	44 (4)	30.2	30.1	30.3
Spring–Summer	$p < 0.001$			
Spring–Autumn	$p = 0.020$			
Summer–Autumn	$p < 0.001$			

values of the control animals kept at 4 °C also increased slightly to 29.8 °C (Table 1). The difference between control and exposed groups on Day 7 was not significant ($p = 0.13$, groups = 2, df = 8). The acclimation response ratio (ARR) for *M. affinis* in this experiment was 0.08.

Inactivation of membrane ATPases at elevated temperatures

The temperature dependence of enzyme inactivation is shown for *Gammarus* spp. in Fig. 2a and b and for *M. affinis* in Fig. 2c and d. The thermal inactivation values (IA50) for Mg^{2+}-ATPase were lower than those for total ATPase (Table 3). For *Gammarus* spp. the most responsive period for both enzymes was the summer and for *M. affinis* the spring. The IA50 values for both analysed ATPases were higher for *M. affinis* than for *Gammarus* spp. For *Gammarus* spp., the difference in thermal sensitivity among all the seasons, except between spring and autumn in total ATPase, were either significant or very significant for both enzymes (groups = 3, df = 33). For *M. affinis*, statistically significant difference was observed only between spring and summer (groups = 3, df = 27).

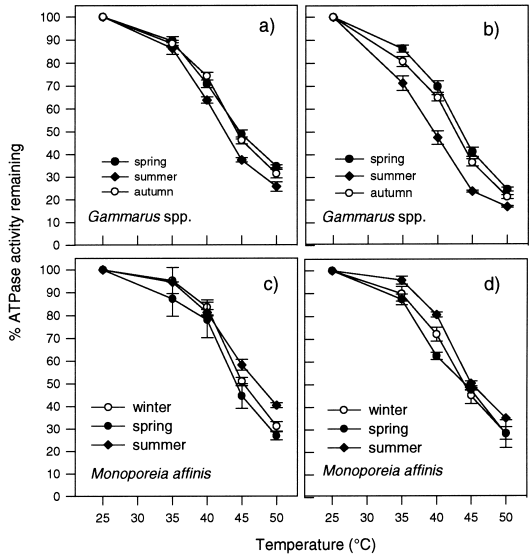

Fig. 2. The temperature inactivation of adenosine triphosphatases of the Fraction 1 membranes presented as residual activity (%) of the activity measured at 25 °C after pretreatment at different temperatures: *Gammarus* spp. (**a**) (Mg^{2+} + Na^+ + K^+)-ATPase and (**b**) Mg^{2+}-ATPase, symbols: (●) spring, (◆) summer, (○) autumn, (mean ± SE of triplicate measurements). *Monoporeia affinis* (**c**) (Mg^{2+} + Na^+ + K^+)-ATPase and (**d**) Mg^{2+}-ATPase, symbols: (○) winter, (●) spring, (◆) summer, (mean ± SE of duplicate measurements).

Discussion

Upper thermal tolerance

This study was designed to follow the seasonal variation of the short-term heat tolerance of the amphipods without acclimation. This is justified because the acclimation to a constant temperature in the laboratory may erase seasonal effects, e.g., Spraque (1963) on *Asellus intermedius* and Lagerspetz and Bowler (1993) on *Asellus aquaticus*. On the other hand, Layne *et al.* (1987) found persisting seasonality in the CTMax of the crayfish and Cuculescu *et al.* (1998) in two marine crab species, although the actual CTMax values for laboratory-acclimated and naturally acclimatised samples differed considerably.

A measurement of the acute thermal tolerance gives results predicting the relative thermal resistance of the animals (e.g. Bradley 1991), not necessarily the absolute tolerance values in nature. In general, exposure at a constant temperature gives lower values than exposure at a continuously rising temperature (Kivivuori and Lahdes 1996). An overall view illustrating the entire temperature range of species and their living functions (= tolerance polygons) are only available for a few species, probably due to the complex acclimation procedures required under laboratory conditions (Cossins and Bowler 1987).

In this study the LT50 values of deep-water *M. affinis* were relatively high, and seasonal differences were slight (Table 1). The thermal tolerance was highest in the animals collected in late summer, coinciding with the time of good physiological condition of the animals after sedimentation of the spring bloom (Lehtonen 1996). Because the environmental temperature did not change, it was nutrition that most likely had a positive effect on the thermal tolerance (Precht 1973). Segerstråle (1978) noted *M. affinis* in

high densities in shallow water at summer temperatures exceeding 18 °C, and in the laboratory this amphipod showed signs of disturbance at temperatures higher than 22 °C only (Segerstråle 1937). The sequence of the symptoms of disturbance resembled the behaviour of the animals in our experiments. In this study the 3-day mortality at 19.6 °C was already 22% and may indicate that deep-water animals inhabiting a stable cold environment are less heat-tolerant than shallow-water animals. In natural waters temperature changes only slowly with season and this provides time for aquatic organisms to adapt to new conditions, which is in agreement with the observations presented by Segerstråle (1937, 1978).

Measurements of the temperature tolerance of a related species, freshwater *Diporeia hoyi* (earlier *Pontoporeia affinis*), collected in Lake Superior in November, resulted in a 24-h LT50 of 12 °C (Smith 1972) at constant temperature. This LT50 value is considerably lower than the values found in a brackish water environment by Segerstråle (1937, 1978) as well as those in this study. Temperature tolerance is also related to the salinity of the water, whereby in fresh water the tolerance of various invertebrate species is lower than in saline water (Kinne 1970). Consequently, the values of the temperature tolerance of freshwater species are not directly comparable with those of species living in the Baltic Sea.

The isopod *Saduria entomon*, coexisting with *M. affinis*, is another "glacial relict" in the Baltic Sea. In an earlier study, Kivivuori and Lagerspetz (1990) measured the acute lethal temperature of 30.8 °C in cold-acclimatised *S. entomon* which is at same level as that in *M. affinis* shown in this study. A 7-day acclimation at a temperature approximately 10 °C higher than the environmental temperatures resulted in low acclimation response ratios (ARR) 0.08 in LT50 for *M. affinis* and 0.12 in CTMax for *S. entomon*. It may demonstrate features of cold stenothermy in *M. affinis* and, as stated by Kivivuori and Lagerspetz (1990), in *S. entomon*.

The corresponding shift in the thermal tolerance (LT50 and CTMax) is clear in eurythermal crustaceans like the freshwater isopod *Asellus aquaticus*, where 7-day acclimation at 10 °C and 20 °C resulted in a shift of 4–5 °C in CTMax and a corresponding ARR of 0.50 (Lagerspetz and Bowler 1993). In the crayfish *Orconectes rusticus,* the shift was also about 4 °C when acclimated in the laboratory to 5 °C and 25 °C, and the response could already be observed after 1 day (Claussen 1980).

In the Archipelago Sea, *Gammarus* spp. can

Table 3. Thermal inactivation of total (Mg^{2+} + Na^+ + K^+)- and Mg^{2+}-adenosine triphosphatases of *Gammarus* spp. and *Monoporeia affinis* as IA50 values (°C), 95% lower and upper confidence limits and the statistical significance of the differences in thermal sensitivity among the seasons. IA50 is the temperature at which 50% of the enzyme activity is inactivated.

		Total ATPase			Mg^{2+}-ATPase		
Species	Season	IA50	Lower	Upper	IA50	Lower	Upper
Gammarus spp.	Spring	45.6	44.9	46.3	44.1	43.6	44.7
	Summer	43.6	42.4	44.9	40.3	39.2	41.3
	Autumn	45.2	44.5	45.9	42.8	42.2	43.5
	Spring–Summer	$p < 0.001$			$p = 0.001$		
	Spring–Autumn	n.s.			$p = 0.030$		
	Summer–Autumn	$p = 0.001$			$p < 0.001$		
M. affinis	Winter	46.1	44.9	47.5	44.8	44.0	45.6
	Spring	44.9	43.3	46.9	44.3	43.5	45.2
	Summer	47.4	46.6	48.5	46.3	45.6	47.2
	Winter–Spring	n.s.			n.s.		
	Winter–Summer	n.s.			n.s.		
	Spring–Summer	$p = 0.002$			$p = 0.003$		

experience annual temperature variations of ca. 20 °C. In our study, changes in the LT50 values closely followed the changes in natural water temperature (Table 2), and are consistent with the observed heat death temperatures of 29 °C in summer and 23 °C in winter in *Gammarus oceanicus* from the Gulf of Finland (Suomalainen 1958). The LT50 values for *Gammarus salinus* from the Kiel Bay, using the same method as in this study, were 32.7 °C and 34.5 °C for animals acclimated to 8 °C and 20 °C, respectively (Furch 1972). As mentioned before (Kinne 1970), the higher salinity in the Kiel Bight, compared with the northern Baltic, might somewhat increase the heat tolerance of *Gammarus salinus*.

In the present study the differences in LT50 values between the two amphipod species are small, except for the summer values. In cold-acclimatised *Gammarus* specimens and *M. affinis* both the LT50 and estimated CTMax temperatures were approximately at the same level, but in summer-*Gammarus*, loss of coordination and heat death were very close to each other. Despite the similarity in LT50 values in cold water conditions, the pronounced ability of *Gammarus* spp. and poor ability of *M. affinis* to adapt to higher temperatures shows the difference in thermal behaviour of these amphipod species (eury- *vs.* stenosthermy). On the other hand, the tolerance values of these Baltic crustaceans are at a distinctly higher level compared with those of Arctic and Antarctic species (e.g. Hirche 1990, Lahdes *et al.* 1993).

Inactivation of (Mg^{2+} + Na^{+} + K^{+}) ATPases

In connection with thermal tolerance studies, the activities of membrane-bound ATPases are often determined because of the role of these enzyme systems in ion transport through cell membranes and in thermal death.

In this study the ATPases of summer-acclimatised *Gammarus* spp. were the most sensitive to elevated temperatures, unlike the heat death of the animals in the same season. In *M. affinis* the inactivation temperatures of ATPases correlated positively with the heat death value and were higher than in *Gammarus* spp. The lower IA50 values for Mg^{2+}-ATPase than those for the total ATPase in both species (Table 3) are in accordance with the study by Gladwell (1975). The magnitude of the IA50 values lie in about the same range as those for the synaptic and renal microsomal membranes of goldfish (Cossins *et al.* 1981, Schwarzbaum *et al.* 1992), but are considerably higher than those for the crustacean *Austropotamobius pallipes* (Gladwell 1975). Some earlier studies have shown that the warm acclimation increased the thermal resistance of Mg^{2+}-ATPase in freshwater crayfish (*Austropotamobius pallipes*), (Mg^{2+} + Na^{+} + K^{+})-ATPase in goldfish (*Carassius carassius*) as well as the temperature tolerance of the animal (Gladwell 1975, Cossins *et al.* 1981). It seems, however, that a lot of inconsistencies can be found concerning the membrane response between different species caused by thermal stress (Cossins and Bowler 1976, Lagerspetz 1985).

Membrane structure and the functioning of ATPases are closely related (Gennis 1989 and references therein). It is already known that the composition of membrane phospholipids can have different effects on the thermostability of the ATPases (Volmer and Veltel 1985). The thermal behaviour of these enzymes, especially those of *Gammarus* spp. cannot be fully explained without observing the concurrent fluidity and membrane structure. When the IA50 value of *Gammarus* spp. was at the lowest in summer, the share of non-bilayer forming plasmalogens in the membranes was higher but the membranes were more ordered than in cold-water conditions. (Lahdes *et al.* 2000a, 2000b). The role of non-bilayer lipids on protein-lipid interactions, in general, is poorly understood so far (Lee 1998, Simidjiev *et al.* 2000). Instead, in *M. affinis* ordered membranes, but high IA50 and LT50 values occurred simultaneously (Lahdes *et al.* 2000a). The phospholipids of *M. affinis* contained branched chain fatty acids, but their role in the enzyme functions is not established. Analyses on the more detailed phospholipid composition of *Monoporeia affinis* are still in progress (E. Lahdes and T. Farkas unpubl.). The results may elucidate the behaviour the ATPases of both studied taxa.

In general, in cold conditions dissipative ion fluxes increase (Pörtner *et al.* 1998). It seems that the adaptive regulation of the

mechanism ensuring ion transport under temperature changes can proceed by adjusting the ATPase activity and/or the membrane order. This scenario was observed for example in two temperature-acclimated freshwater fish species. In the eurythermal roach (*Rutilus rutilus*) there was a change in the ATPase activity and IA50, while the membrane order remained constant. In the cold-stenothermal arctic char (*Salvelinus alpinus*), on the other hand, ATPase activity and IA50 remained constant, but the membrane order was adjusted according to the temperature acclimation (Schwarzbaum *et al*. 1992). It is possible that in *Gammarus* spp. the temperature adaptation of membranes is also arranged by adjusting the membrane fluidity rather than the properties of ATPases, which is then reflected in their thermostability. However, trying to interpret the contradictions in the present work would be speculation, because the interactions of enzyme proteins and the physico-chemical characteristics of membranes are still inadequately known.

Conclusions

M. affinis, collected from a stable low temperature environment in different seasons, showed higher short-term heat tolerance values than expected, and the seasonal variation in heat resistance was minimal. The warm acclimation of *M. affinis* did not change the thermal resistance, supporting the idea of the cold-stenothermal nature of this animal. In *Gammarus* spp. seasonal changes in thermal tolerance correlated with the sea water temperature. The LT50 values of *M. affinis* and cold-acclimatised *Gammarus* spp. had a narrow range. However, the response of the latter species and the poor potential of *M. affinis* for acclimation to elevated water temperatures demonstrate the difference in their thermal biology (eury- *vs*. stenosthermy). The heat tolerance values of both crustacean species examined were closely in accordance with values obtained from other brackish water species. The thermal inactivation of ATPases correlated positively with the thermal tolerance in *M. affinis* but not in *Gammarus* spp. Contrary to expectations, the IA50 values of the ATPases of *M. affinis* were higher than those of *Gammarus* spp. It is possible that the behaviour of the thermal resistance of the ATPases in *Gammarus* spp. is related to the lipid-protein interactions in the cellular membranes of these animals containing a great amount non-bilayer forming plasmalogens. It is also possible that in *Gammarus* spp. the temperature adaptation of membranes is also arranged by adjusting the membrane fluidity rather than the properties of ATPases, which is then reflected in their thermostability.

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