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I. M. Sokolova · A. I. Granovitch
V. Ja. Berger · K. Johannesson

Intraspecific physiological variability of the gastropod *Littorina saxatilis* related to the vertical shore gradient in the White and North Seas

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Abstract Physiological responses to desiccation and temperature stress as well as behavioural responses to fast and abrupt environmental changes were investigated in high- and low-shore *Littorina saxatilis* (Olivi) from several populations from the White and North Seas. Variations in evaporation rates, resistance to air exposure and to acute and chronic temperature stress between animals from different shore levels were similar in White and North Sea periwinkles, consistent with the adaptive nature of these variations. High-shore snails were found to be able to conserve body water reserves better, to resist higher temperatures and to survive longer under conditions of combined temperature and desiccation stress than their low-shore counterparts. In a temperature range of 25 to 35 °C, the rate of evaporative water loss was positively correlated with temperature in low-shore snails while being largely temperature-independent in high-shore snails. Median lethal time during air exposure in *L. saxatilis* was negatively but not linearly related to the temperature of exposure. In a temperature range of 30 to 38 °C, the resistance to heat exposure in air was only slightly dependent on the temperature, with $Q_{10} = 1.4$ for the median lethal time; the heat resistance dropped drastically at temperatures above 38 °C, with $Q_{10} = 593.8$. This suggests different mechanisms of temperature resistance in different parts of the studied temperature range. In contrast, behavioural response to extreme salinity fluctuations was not uniform in the high- and low-shore periwinkles from the White and North Seas, which may reflect specific environmental conditions at different shore levels in the two areas studied. Observed physiological and behavioural variations are discussed from the viewpoint of different adaptive strategies employed by eulittoral and eulittoral-fringe animals within populations of a single species.

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I. M. Sokolova (✉) · V. Ja. Berger
White Sea Biological Station,
Zoological Institute of the Russian Academy of Sciences,
Universitetskaya nab., 1, 199034 St. Petersburg, Russia

A. I. Granovitch
Department of Invertebrate Zoology,
Faculty of Biology and Soil Science,
St. Petersburg State University, Universitetskaya nab.,
7/9, 199034 St. Petersburg, Russia

K. Johannesson
Göteborg University, Department of Marine Ecology,
Tjärnö Marine Biological Laboratory,
45296 Strömstad, Sweden

Present address:
I. M. Sokolova
Alfred-Wegener-Institute for Polar and Marine Research,
Lab. Ecophysiology, Columbusstr. 30,
27568 Bremerhaven, Germany

e-mail: isokolova@awi-bremerhaven.de
Fax: +49-0471-48311159; Tel.: +49-0471-48311311

Introduction

The intertidal zone is a specific habitat where periods of immersion and air exposure regularly alternate. In typical tidal areas, diurnal and lunar tidal cycles result in a well-defined vertical gradient of environmental conditions in the intertidal zone. Along the vertical gradient of the littoral, several important environmental characteristics (especially temperature and degree of aerial exposure) change concordantly and drastically. Temperature and desiccation stresses are much more extreme in the upper part of the intertidal zone than in the lower part, especially when the supratidal zone, which is only irregularly wetted by occasional splash, is taken into account (Newell 1979; Johannesson and Johannesson 1989).

Distribution of intertidal animals, including molluscs, is usually stratified along the vertical gradient of the littoral (reviews: Newell 1979; Underwood 1979). It has been suggested that mollusc species living at different shore levels pursue different adaptive strategies (McMahon and Russel-Hunter 1977; McMahon 1988, 1990). Eulittoral animals living below the mean high tide water level are regularly and predictably immersed during each high tide. They possess a relatively high capacity for aerial respiration, and can maintain activity and regulate body temperature by evaporation at low tide. In contrast, molluscs inhabiting the eulittoral fringe zone (above mean high tide water level) experience prolonged and often unpredictable periods of emergence between spring tides and/or occasional splash due to wave action (Johannesson and Johannesson 1989; McMahon 1990). While exposed at low tide, they usually cease activity and isolate themselves inside the shell in order to avoid contact with a heated substrate and to minimise evaporative water loss. In general, inhabitants of the eulittoral fringe can conserve water better during air exposure, and are hence incapable of evaporative thermoregulation. They also possess higher thermal resistance than the species inhabiting the eulittoral zone, and have morphological peculiarities which are believed to be of adaptive value, increasing extracorporal water stores, reducing evaporation and/or increasing irradiative heat loss (McQuaid and Scherman 1988; Britton and McMahon 1990; McMahon 1990; Britton 1995). However, the relationship between morphology and physiology seems to be complex and variable both among and within species (Chapman 1997).

The two adaptive strategies outlined above were based on comparisons between different, sometimes distantly related, species inhabiting eulittoral and eulittoral fringe zones (McMahon 1990). However, there is a major intrinsic danger in the interpreting of physiological or morphological differences between such species as adaptive to specific life conditions, because, in this case, adaptations to the environment are confounded by evolutionary constraints on potentially adaptive variation. There are two ways to avoid this problem. One way would be to use a wide spectrum of related species and to map the physiological or morphological variation in their phylogeny in order to distinguish between similarities due to shared evolutionary history and those due to convergent evolution. However, such a study is possible to perform only in some groups with well-known phylogeny. Another, simpler and less extensive approach is to analyse adaptations within a genetically, morphologically and ecologically cohesive system such as a single species or even a single population of a certain species. This was the approach we used in the present study. The intertidal gastropod *Littorina saxatilis* (Olivi) (Prosobranchia: Littorinidae) was chosen as a model species because of its wide vertical distribution – from the upper subtidal zone to the splash zones. Obviously, this species must possess efficient adaptations to be able to invade different intertidal zones including the environmentally harsh and unpredictable eulittoral fringe.

Our study aimed to investigate physiological variation in populations of *Littorina saxatilis* along a vertical shore gradient, with emphasis on those physiological parameters that may be adaptive under the extreme environmental conditions of the eulittoral fringe, including evaporation rate, thermal resistance in water and air and behavioural responses to extreme environmental fluctuations. To test whether the observed patterns of intrapopulational physiological variation along the vertical shore gradient are consistent in different populations of *L. saxatilis*, we compared snails from high- and low-shore levels in several populations from two areas differing greatly in climatic and tidal regimes: the practically atidal, temperate Swedish coast of the North Sea and the tidal, subarctic shores of the White Sea.

Materials and methods

Collection sites

Animals were collected in July and August in 1997 and 1998 in the North Sea, near Tjärnö Marine Biological Laboratory (58°52′N; 11°10′E), Sweden, and in the White Sea, near the Biological Station of the Zoological Institute of the Russian Academy of Sciences (66°22′N; 33°39′E). The two regions are separated by nearly 10° latitude. In each region, *Littorina saxatilis* from several populations were sampled.

In the North Sea, samples were taken on the northern part of the Swedish west coast, on three islands: Saltö, Ursholmen and Jutholmen, separated by a distance of 1 to 7 km. All three sites are rather steep granite rocks exposed to strong (Saltö) or very strong (Jutholmen and Ursholmen) wave action. While the tidal range in the area is negligible (max. 0.3 m), the distance between the low and high water marks is >1.5 m; this variation is due to combination of varying air pressures and wind directions. Adult snails were collected in, or slightly below, the low-shore zone of *Semibalanus balanoides* and at the top of the splash zone, which is also the top of the distribution of *Littorina saxatilis*. The low-shore zone is mostly submerged at least for a couple of hours each day. The high-shore zone, on the other hand, is irregularly wetted, mainly by splash on windy days. Distances between low- and high-shore samples were approximately 5 m (Ursholmen), 3 m (Jutholmen) and 1.5 m (Saltö).

In the White Sea, four populations were sampled: (1) near Cape Kartesh, (2) at Ivanov-navolok, (3) on Keret Island and (4) on Fettakh Island. Sampling sites were separated by a distance of 300 m to 5 km and, except at Sites 1 and 2, also by a water barrier. The latter two sites were >3 km apart along the coastline. Sampling sites were situated on sheltered (Kartesh, Fettakh Isl.) and moderately exposed (Keret Isl., Ivanov-navolok) rocky shores. Tidal range in this area is 1.8 to 2.2 m. Adult snails were collected on the low shore, from small stones and gravel patches in the low intertidal zone, within the brown macrophyte belt (*Ascophyllum nodosum* and *Fucus vesiculosus*) and on the high shore, in the narrow belt (30 to 50 cm) along the upper limit of the *Littorina saxatilis* distribution on the rock. Our observations showed that the high-shore levels at the White Sea sites were emerged for 18 to 22 h d^{-1} during summer spring tides and for 24 h d^{-1} during summer neap tides, whereas corresponding values for the low-shore levels were 8 to 10 and 2 to 4 h d^{-1} during spring and neap tides, respectively. Distance between the high and low shore varied from 2–3 to 8–10 m depending on the steepness of the rock.

At one of the White Sea sites (Fettakh Isl.), the air, water and substratum temperatures as well as the body temperature of the periwinkles were measured during low tides on 7 and 12 July 1997. Temperature was measured to the nearest 0.5 °C using a semiconductive microthermistor MT-54 (Karmanovs Construction, Russia) with a probe diameter of ca. 1 mm. Body temperature of the adult periwinkles (5 to 7 mm shell diameter) was measured in situ by inserting the probe under the operculum. Each measurement took 2 to 3 s. During measurements of substratum temperature, the probe was shaded against the wind but not against the sun. Air temperature was measured in close proximity to snails, 5 to 10 mm above the substratum.

Laboratory maintenance

Snails were transferred to the laboratory within 1 h after collection and placed in aquaria with well-aerated natural sea water for 3 to 7 d prior to experimentation. This short acclimation period was necessary to equilibrate the body water content in high- and low-shore snails. The specimens were continuously immersed, but some space was provided to allow them to crawl out onto the damp aquarium walls. Water temperature and salinity approximated the ambient values at the collection sites (North Sea: 15 to 19 °C and 20 to 22‰; White Sea: 10 to 12 °C and 25 to 27‰). No food was allowed. Only adult animals (5 to 8 mm shell diameter) were used.

Experimental methods and statistics

Experiment 1

Evaporative water loss was measured according to a method modified from McMahon and Britton (1985). Snails of similar shell size (5.5 to 6.3 mm shell diameter) were taken out of the aquaria and blotted with tissue paper to remove excess water from the shell surface and opercular area. The snails were weighed to the nearest 0.025 mg and placed individually in the cells of 40-cell wells in thermostats with a constant temperature of 25, 27, 30 or 35 °C (±0.25 °C) for the White Sea specimens and 27 °C (±0.25 °C) for North Sea specimens. A desiccant (indicator silica gel) was added in excess to ensure dry conditions in experimental chambers. After the specified exposure periods (12 or 36 h) snails were taken out of the thermostat chambers and weighed. They were then placed in sea water, allowed to recover for 2 h and scored for mortality. After scoring, the snails were dried at 100 °C for 24 h to a constant weight, allowed to cool down in the presence of desiccant and weighed. For each shore level in each population, 30 to 55 animals were initially included in the experiment. However, the number of individuals used in the final analysis varied, as only survivors were used. For comparison of the evaporative water loss in animals from different shore levels, populations and seas, snails were air-exposed for 36 h at 27 °C. However, to assess the impact of environmental temperature on the rate of water loss, a shorter exposure period (12 h) was chosen to assure adequate survivors at each experimental temperature.

Water loss was determined as percent of the total (corporal + extracorporal) body water:

$$\mathrm{WL} = \frac{W_{\mathrm{in}} - W_{\mathrm{exp}}}{W_{\mathrm{in}} - W_{\mathrm{dry}}} \times 100\% \ ,$$

where WL is water loss (%), W_{in}, W_{exp} and W_{dry} are initial weight, weight after a given exposure period and final dry weight of a snail (mg), respectively. Initial amount of body water (calculated as $W_{\mathrm{in}} - W_{\mathrm{dry}}$) was similar in high- and low-shore *Littorina saxatilis* within each studied population (Student's *t*-test, $p > 0.05$; data are not presented).

Water loss was compared in the periwinkles from different shore levels, populations and seas using two- and three-factor mixed model analysis of variance (ANOVA) (Sokal and Rohlf 1995; Underwood 1997). *Shore level* and *Sea* were treated as fixed factors, and *Population* as a random factor. In the three-way ANOVA, *Population* was nested in *Sea*. Prior to the analysis, the data on water loss were log-transformed, and assumptions of normal distribution and homogeneity of variances were tested using the chi-squared or the Kolmogorov–Smirnov test and Cochran's test, respectively . Both assumptions were found to be true at the 5% significance level. The Student–Newman–Keuls' (SNK) test was used for post hoc comparisons (Sokal and Rohlf 1995). For comparisons of two independent samples, Student's *t*-test was employed.

Experiment 2

The chronic upper thermal limit in air was estimated by the survival rate of the periwinkles under conditions of air exposure at elevated temperature. Adult snails (5 to 8 mm shell diameter) were briefly blotted with tissue paper, put in batches of ca. 100 individuals in Petri dishes and placed into thermostats with constant temperatures of 30, 35, 38, 40, 42 or 44 °C (±0.25 °C). A dessicant (indicator silica gel) was used to control humidity. After specified exposure periods, 15 to 30 (North Sea) or 30 to 80 (White Sea) snails were taken out of the thermostats, allowed to recover for 2 h in seawater and scored for mortality.

Probit regressions were computed and compared following the rationale outlined in Bliss (1935a, b). The regressions were used for calculation of a median lethal time (LT_{50}) and of 95% confidence limits of LT_{50}.

Experiment 3

Acute upper thermal limits in water and air were determined as the upper critical temperature at which death occurred on exposure to temperatures rising at 1 °C per 5 min (McMahon 1990). Subsamples of 10 to 15 (North Sea) or 30 to 40 (White Sea) adults (5 to 7 mm shell diameter) from the high- and low-shore levels were randomly placed into perforated plastic cages. All snails were initially held at room temperature (20 to 25 °C). Cages were placed in a jar with natural sea water with a salinity of 20 to 22‰ (North Sea) or 25 to 27‰ (White Sea); the jar was mounted in a water bath with a manually controlled heater. The temperature was then raised in steps of 1 °C per 5 min (±0.25 min and ±0.1 °C). The water in the jar was vigorously aerated to maintain temperature uniformity, and the temperature was continuously monitored by a thermometer inserted into the experimental jar. Two cages (one for each shore level) were withdrawn at subsequent 0.5 to 1 °C (White Sea) or 0.3 to 0.5 °C (North Sea) temperature gains between 42 and 50 °C immediately after the desired temperature was reached. Snails were transferred into plastic jars with sea water at room temperature and allowed to recover for 2 h before scoring of mortality.

A dry bath (Techne Dry Block, DB-30, Cambridge Ltd.) was used to determine acute thermal limits in air. Molluscs were blotted with tissue paper and placed on the aluminium block of the dry bath, which served as a heat transfer medium. The inner space of the bath with the cube was covered by a plastic cover to provide temperature uniformity. Indicator silica gel was placed under the cover. The temperature regime was the same as described above for the water bath. Two samples (one for each shore level) were withdrawn in the same range and at the same temperature increases as mentioned for the water bath experiments.

Calculation of the probit regressions according to the methodology described in Bliss (1935a) was, in many cases, impossible due to the limited number of points between 0 and 100% mortality. Hence, acute thermal resistance of the snails from different shore levels within each population was compared using log-linear models of analysis of contingency tables (Sokal and Rohlf 1995). Between-population comparisons were impossible due to different temperature points at which samples were withdrawn. Median acute lethal temperatures (considered the median lethal dose, LD_{50}) were inferred to the nearest 0.1 °C from the graphic representation of the probit regressions calculated by an iterative quasi-Newtonian procedure with the maximum likelihood estimate of the model parameters (Sokal and Rohlf 1995). A maximum of 20 iterations was required for convergence of parameters.

Experiment 4

Behavioural responses to extreme environmental fluctuations were measured as the rate of closing and opening of the operculum upon transfer from sea to fresh water and vice versa, respectively. This extreme osmotic stress was chosen because it evokes a rapid non-specific isolation response in shelled gastropods (reviewed in: Kinne 1971; Berger 1986). To measure the time taken to open the shell, animals were placed individually in small cylindrical vials (10 to 15 ml vol.) with fresh water at room temperature (20 to 25 °C) and kept for 30 to 40 min until they were fully withdrawn into the shell. Fresh water was then decanted without disturbing snails, and vials were gently filled with natural sea water of the same temperature. The time between first contact with sea water and opening of the shell was measured by stop-watch to the nearest 1 s. A shell was considered open when a narrow slit appeared between the outer aperture lip and the operculum.

To measure the time taken to close the operculum, snails were gently transferred from the sea water to fresh water with their aperture upright and the foot fully extended, and the time between first contact with fresh water and withdrawal into the shell until a slit between the outer aperture lip and the operculum disappears, was measured with a stop-watch to the nearest 1 s. A few animals were discarded which responded to the mechanical disturbance by closing the operculum during transfer from sea to fresh water.

Times to close and to open the shell were compared in the *Littorina saxatilis* from different shore levels, populations and seas using two- and three-factor mixed-model ANOVAs (Sokal and Rohlf 1995; Underwood 1997). *Shore level* and *Sea* were treated as fixed factors, and *Population* as a random factor. In the three-way ANOVA, *Population* was nested within *Sea*. Prior to analysis, assumptions of normal distribution and homogeneity of variance were tested. If any of the assumptions were violated, the data were transformed until a normally distributed data set with homogeneous variances was obtained. Time to close the aperture was found to conform to the normal distribution, and the variances across the studied groups were homogeneous ($p > 0.05$). In contrast, between-group variances for time to open the aperture were heterogeneous. Therefore, we applied $\log_{10}$-transformation which was successful in improving homogeneity of variance in the White Sea samples, but not in those from the North Sea. None of the attempted transformations were successful in the latter case, so for the analysis of the effect of shore level on time to open in North Sea periwinkles, the non-parametric Kruskal–Wallis median test was employed (Sokal and Rohlf 1995).

Results

In situ body temperature of *Littorina saxatilis* during low tide

Body temperature of *L. saxatilis* measured during low tide on two sunny days in July significantly differed in animals from the high- and low-shore levels of Fettakh Isl. (Student's *t*-test, 7 July 1997: $t = 9.65$, $df = 111$, $p < 0.0001$; 12 July 1997: $t = 15.49$, $df = 86$, $p < 0.0001$). Not only means, but also temperature ranges experienced by the snails were different on high and low shores (Fig. 1). The highest temperatures measured in periwinkles were 45 to 48 °C in some high-shore specimens during low tide, while average values of body temperature were 30 to 35 °C. On the low shore, the maximum recorded body temperature of *L. saxatilis* was 35 °C, whereas average values were 18 to 28 °C.

Experiment 1: evaporative water loss in the high- and low-shore periwinkles

Three-way mixed-model ANOVA showed that both position in the intertidal zone and population of origin had significant influence on the evaporative water loss by the periwinkles after 36 h of air exposure at 27 °C (Table 1). On average, the low-shore periwinkles lost relatively more water during air exposure (Fig. 2). The North Sea *Littorina saxatilis* appeared to retain water better during air exposure at 27 °C than the White Sea periwinkles (Fig. 2), though differences in the rate of water loss between specimens from the two seas were only marginally significant ($0.05 < p < 0.10$, Table 1).

Comparison of the water loss in White Sea periwinkles after 12 h of air exposure at different temperatures provides more evidence in favour of the hypothesis that the high-shore periwinkles are better able to retain water while emerged. In all the studied populations, water in the low-shore periwinkles tended to evaporate faster than that in their high-shore counterparts at every experimental temperature (Table 2). As was shown by a series of two-way ANOVAs, the effect of shore level was significant at all experimental temperatures except 27 °C (Table 2). At 25 and 35 °C significant interpopulational variation in evaporative water loss was also detected, while at 27 °C, *Level* × *Population* interaction was

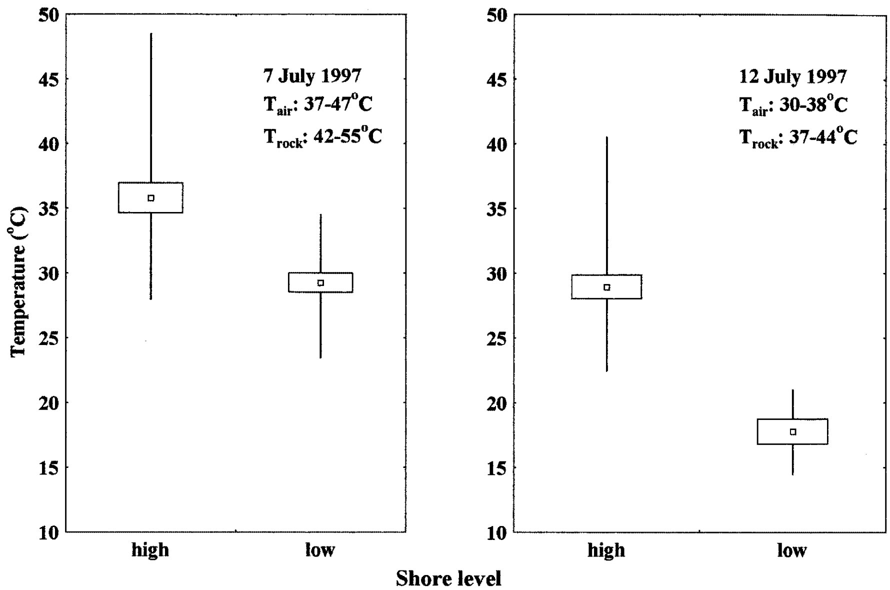

Fig. 1 *Littorina saxatilis*. Body temperature of White Sea *L. saxatilis* measured in situ during low tide (Fettakh Isl., July 1997). Means (*open squares*), 95% confidence intervals (*boxes*) and range of temperature variation (*whiskers*) are shown. Only periwinkles found on the open rock surface subject to direct sunlight were included. Substrate was smooth black rock; air temperature was measured near the snails, 5 to 10 mm above the substratum

Table 1 *Littorina saxatilis*. ANOVA of the log-transformed evaporative water loss after 36 h of air exposure at 27 °C (Experiment 1) [Factors: *Level* position on the shore (fixed, two levels – high and low); *Sea* (fixed, two levels – White Sea and North Sea); *Population* population of origin (random, nested in *Sea*, two levels)]. Interactions including factors *Sea* and *Population* (*Sea* × *Population* and *Sea* × *Population* × *Level*) were impossible to estimate due to nesting (*df* degrees of freedom; *MS* mean squares)

Factors	*df*, effect	MS, factorial	*df*, error	Ms, error	*F*-ratio	*p*-level
Level	1	0.677	2	0.023	28.93	0.033
Sea	1	3.987	2	0.312	12.78	0.070
Population	2	0.312	144	0.027	11.44	<0.001
Level × *Sea*	1	0.043	2	0.023	1.85	0.306
Level × *Population*	2	0.023	144	0.027	0.86	0.426

significant. This indicates a differential response of the high- and low-shore periwinkles in the two populations studied. An explanation for this deviation may be that the water loss in the samples from Fettakh Isl. was, statistically, significantly greater in the low-shore periwinkles than in their high-shore counterparts (SNK test, $p < 0.05$), whereas in the sample from Kartesh, differences in evaporation rate between the high- and low-shore animals were not significant.

Temperature during exposure – by itself – influenced the evaporation rate of *Littorina saxatilis* in air (Table 2). In the samples from Fettakh Isl., tested in a range of temperatures from 25 to 35 °C, mean water loss increased with increasing temperature in the low-shore periwinkles ($r = 0.968$, $n = 4$, $p < 0.05$) but not significantly so in the high-shore specimens ($r = 0.676$, $n = 4$, $p > 0.05$). A tendency of increase in evaporative water loss with increasing temperature can also be seen in the samples from Ivanov-navolok and Keret Isl., tested at 30 and 35 °C (Table 2), however, the influence of temperature on evaporation rate was only significant in the samples from Ivanov-navolok (Student's t-test, low-shore snails: $t = 5.12$, $df = 47$, $p = 0.0006$; high-shore snails: $t = 3.50$, $df = 52$, $p = 0.001$) but not in those from Keret Isl. (low-shore snails: $t = 1.10$, $df = 40$, $p = 0.28$; high-shore snails: $t = 0.65$, $df = 52$, $p = 0.52$).

Experiment 2: mortality under conditions of air exposure at elevated temperature (chronic upper temperature limits)

Probit analysis detected significant differences in the air exposure–mortality relationships between the high-shore and low-shore periwinkles in most populations studied (Table 3). As a rule, regression lines had similar slopes and differed only in their vertical position, with the exception of samples from Keret Isl. at 35 °C and Ivanov-navolok at 40 °C, where the slopes of the exposure–mortality relationships were significantly different. Differences in mortality rates between the high- and low-shore snails were not statistically significant in the samples from Saltö Isl. at 40 °C or 44 °C, and were marginally significant in the samples from Ivanov-navolok at 35 °C and Ursholmen Isl. at 44 °C

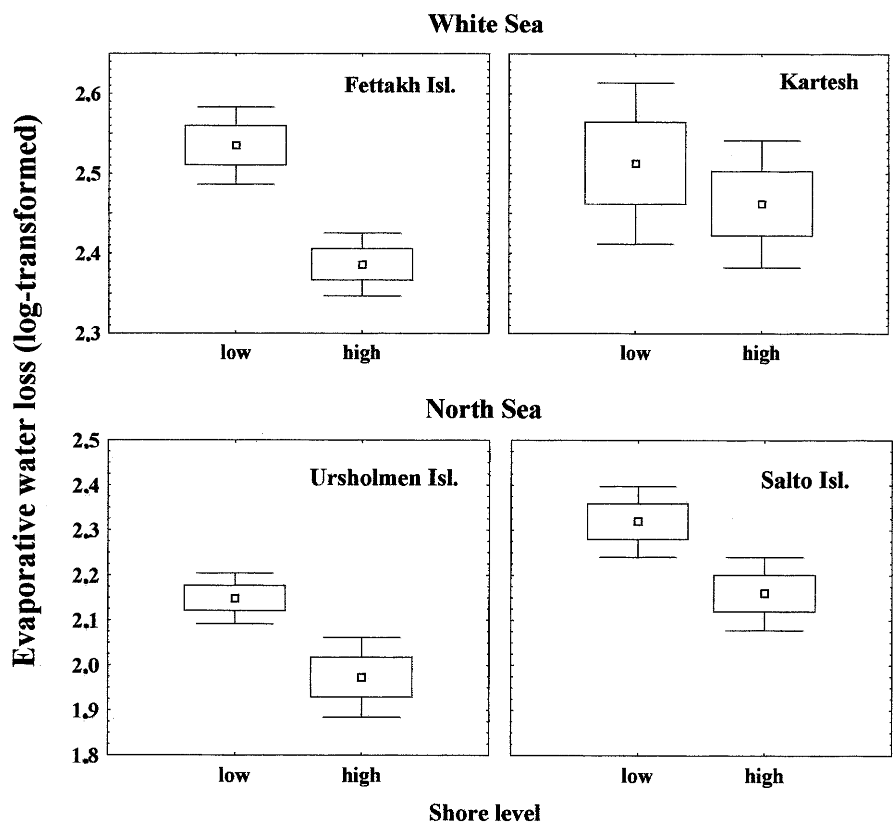

Fig. 2 *Littorina saxatilis*. Evaporative water loss after 36 h of air exposure at 27 °C in White and North Sea *L. saxatilis* (Experiment 1). Mean values (*open squares*), standard errors (*boxes*) and 95% confidence intervals (*whiskers*) of water loss (percent of total body water, $\log_{10}$-transformed) are shown; $N = 19$ for each population and shore level

Table 2 *Littorina saxatilis*. Mean water loss (percent total body water) after 12 h of air exposure at different temperatures in White Sea *L. saxatilis* collected at different intertidal levels (Experiment 1). Mean values of evaporative water loss (%), standard errors and *N* (in parentheses) are given (–, no data available). ANOVAs were performed on $\log_{10}$-transformed values of water loss. Asterisks mark the average water loss in the low-shore snails that is significantly different from the respective value for the high-shore counterparts by *t*-test (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$)

Population	Level	Temperature (°C)			
		25	27	30	35
Fettakh	Low	17.0 ± 0.91 (55)	21.4 ± 2.13 (30)***	21.9 ± 1.89 (16)	31.2 ± 3.83 (6)**
	High	14.6 ± 1.31 (54)	11.2 ± 0.74 (30)	18.2 ± 1.40 (20)	17.8 ± 1.73 (6)
Kartesh	Low	20.9 ± 1.03 (55)**	19.6 ± 1.87 (30)	–	–
	High	17.0 ± 0.85 (55)	15.4 ± 1.12 (30)	–	–
Ivanov-navolok	Low	–	–	21.9 ± 1.42 (21)*	33.1 ± 1.87 (28)***
	High	–	–	17.1 ± 1.19 (24)	22.5 ± 1.15 (30)
Keret	Low	–	–	22.9 ± 1.25 (21)**	26.4 ± 2.01 (21)***
	High	–	–	16.7 ± 1.25 (21)	18.0 ± 1.31 (25)
ANOVA					
Factors					
Level (fixed)		$F_{1,1} = 862.99$ $p = 0.022$	$F_{1,1} = 3.73$ $p = 0.304$	$F_{1,2} = 41.82$ $p = 0.023$	$F_{1,2} = 92.50$ $p = 0.011$
Population (random)		$F_{1,215} = 14.95$ $p = 0.0001$	$F_{1,116} = 1.34$ $p = 0.250$	$F_{2,125} = 0.03$ $p = 0.969$	$F_{2,110} = 8.05$ $p = 0.0005$
Level × *Population*		$F_{1,215} = 0.02$ $p = 0.899$	$F_{1,116} = 5.81$ $p = 0.018$	$F_{2,125} = 0.51$ $p = 0.603$	$F_{2,110} = 0.38$ $p = 0.684$

Table 3 *Littorina saxatilis*. Analysis of the time course of mortality during air exposure at elevated temperatures in high- and low-shore *L. saxatilis* (Experiment 2). Intercepts (*a*) and slopes (*b*) of the respective probit regressions (mortality rates transformed to probits versus $\log_{10}$-transformed values of exposure duration) are given. Values of chi-squared test statistics ($df = 2$) for comparison of intercepts (χ^2_a) and slopes (χ^2_b) between the regression lines for high- and low-shore periwinkles are shown. *N* is the number of time points (exposures) with which the regression line was constructed (**$p < 0.01$, *$p < 0.05$). Estimation and comparisons of the parameters of probit regression followed the methodology described in Bliss (1935a, b)

Temp. (°C)	Sea	Population	High shore			Low shore			χ^2_a	χ^2_b
			a	*b*	*N*	*a*	*b*	*N*		
30	White	Ivanov-navolok	−2.97	4.47	9	−2.71	4.76	10	37.3**	0.12
30	White	Keret Isl.	−4.25	5.72	8	−2.60	5.34	8	78.2**	0.14
35	White	Fettakh Isl.	−3.09	6.41	10	−4.56	7.97	10	19.8**	3.5
35	White	Ivanov-navolok	−5.15	7.90	6	−6.07	8.83	6	5.7	0.50
35	White	Keret Isl.	−3.24	5.08	11	1.51	2.73	13	181.8**	7.0*
35	North	Ursholmen Isl.	−8.89	8.36	6	−2.61	5.21	5	14.1**	2.2
38	North	Saltö Isl.	−2.88	6.85	8	0.24	4.65	9	9.9**	3.2
38	North	Ursholmen Isl.	−1.79	5.14	11	−0.87	4.70	11	6.8*	0.10
40	White	Ivanov-navolok	2.19	2.45	8	−3.11	8.16	7	2.8	28.7**
40	White	Keret Isl.	−3.04	7.01	9	0.05	4.92	9	41.3**	2.6
40	North	Saltö Isl.	1.22	4.08	7	−0.27	6.04	6	3.2	2.4
40	North	Ursholmen Isl.	−3.65	7.73	10	1.11	4.40	10	36.9**	5.5
42	North	Saltö Isl.	1.05	5.31	8	2.14	6.03	9	43.0**	0.13
42	North	Ursholmen Isl.	0.37	8.08	8	1.69	8.14	8	22.0**	0.00
44	North	Saltö Isl.	5.52	2.82	7	6.01	3.46	7	4.0	0.77
44	North	Ursholmen Isl.	4.47	5.40	6	5.23	3.68	6	5.4	1.9

($0.05 < p < 0.10$ for the intercepts). Intercepts and, in some cases, slopes of the regression lines differed significantly between most of the studied populations at a specific shore level (results of the pairwise comparisons not shown), though no consistent between-site or between-sea patterns appeared in this variation.

In general, median lethal time of the periwinkles rapidly decreased with increasing temperature of exposure (Fig. 3), from 16–63 h at 30–35 °C to 0.5–1 h at 44 °C. In all studied populations, the high-shore periwinkles tended to be more resistant to air exposure at elevated temperatures than their low-shore counterparts (Table 4). Within-population differences in LT_{50} between the animals from different shore levels were impressive and ranged from ca. 16–25 h at 30–35 °C, 1–6 h at 38–42 °C to 10–30 min at 44 °C. Relative difference in median lethal time (percentage of the mean LT_{50}) between snails from high- and low-shore levels averaged 41.5 ± 6.02% (range: 6.2 to 88.7%, $N = 16$) and was not correlated with the temperature during exposure ($R = 0.068$, $N = 16$, $p > 0.05$).

Experiment 3: acute high thermal limits in water and air

Acute high thermal limits tested under conditions of fast temperature increase (1 °C per 5 min) in water were

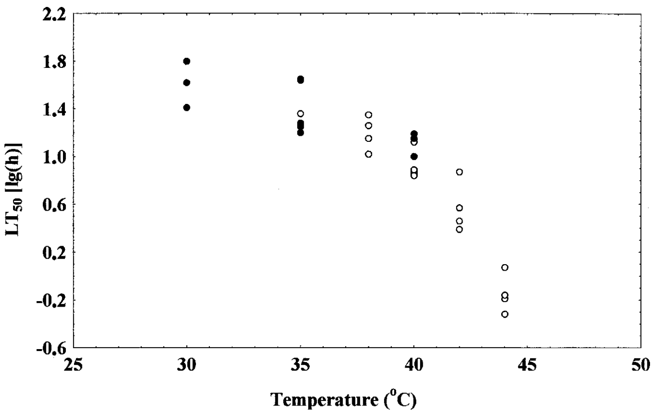

Fig. 3 *Littorina saxatilis*. Temperature-dependent changes in median lethal time (LT_{50}) for White and North Sea *L. saxatilis* when exposed to air (Experiment 2). (*closed circles* White Sea populations; *open circles* North Sea samples)

similar in samples of *Littorina saxatilis* from the four populations studied (Fig. 4). The median lethal temperature was 45.3 to 46.4 °C and 46.4 to 47.4 °C in the studied White Sea and North Sea populations, respectively. In general, when exposed to air, the median lethal temperature measured for snails from Saltö Isl. also fell within this range (45.5 to 46.1 °C) though LD_{50} in the air was nearly 1 °C lower than LD_{50} in water in animals from the same site (Fig. 5). The high-shore periwinkles were more resistant to elevated temperatures under conditions of acute temperature increase in both water and air than were their low-shore counterparts (Figs. 4, 5). Log-linear analysis showed that these differences in mortality were statistically significant in all populations studied (Table 5).

Experiment 4: behavioural response to extreme environmental fluctuations

To investigate the rate of behavioural response to fast and abrupt environmental change, we compared rates of the opening and closing of the shell aperture upon transfer from fresh to seawater and vice versa.

Time to open

Mixed-model three-way ANOVA [fixed factor *Level*, random factor *Population* nested in *Sea* (fixed)] showed a significant effect of *Sea* × *Level* interaction ($F_{1,4} = 38.39$, $p = 0.003$) on log-transformed data on the time taken to open the aperture upon transfer from fresh to sea water. Because of this, we performed separate two-way ANOVAs (with a fixed factor *Level* and a random factor *Population*) for the samples from each sea. It was found that in both cases only the factor *Level* had a significant effect on time to open ($F_{1,2} = 54.06$, $p = 0.018$ and $F_{1,2} = 756.61$, $p = 0.001$ for the North and White Sea animals, respectively). It should be noted that, unlike the White Sea periwinkles, for the North Sea animals, the distribution of the variable studied significantly deviated from normal ($p < 0.05$) and variances were heterogeneous ($p < 0.001$) even after appropriate data transformation. This indicates the need for caution when the results of the analysis on the North Sea snails are discussed. Therefore, we performed non-parametric Kruskal–Wallis median tests for each of the studied North Sea populations, which confirmed a significant effect of the factor *Shore level* on time to open (Kruskal–Wallis test statistics for $df = 1$: $H = 83.36$, $N = 376$; $H = 48.29$, $N = 220$ and $H = 19.51$, $N = 140$ for the samples from Saltö, Ursholmen and Jutholmen Islands, respectively; in all cases $p < 0.0001$). In the North Sea, the low-shore animals were faster to open upon transfer from fresh to sea water, whereas the reverse situation was observed in the White Sea periwinkles (Fig. 6A, B).

Time to close

Upon transfer to fresh water the time taken to close was compared in animals from two White Sea populations (Ivanov-navolok and Fettakh Isl.). Mixed-model

Table 4 *Littorina saxatilis*. Chronic upper temperature limits of the high- and low-shore *L. saxatilis* during air exposure at elevated temperatures (Experiment 2). Table shows values of $\log_{10}$-transformed median lethal time (LT_{50}) and 95% confidence intervals of LT_{50}, calculated according to Bliss (1935a, b) (–, no data available)

Sea	Population	Level	Temperature (°C)					
			30	35	38	40	42	44
White	Fettakh	Low	–	1.20 ± 0.02	–	–	–	–
		High	–	1.26 ± 0.03	–	–	–	–
	Ivanov-navolok	Low	1.62 ± 0.04	1.25 ± 0.04	–	1.00 ± 0.03	–	–
		High	1.80 ± 0.07	1.28 ± 0.04	–	1.19 ± 0.14	–	–
	Keret	Low	1.41 ± 0.06	1.27 ± 0.06	–	1.00 ± 0.04	–	–
		High	1.62 ± 0.04	1.65 ± 0.08	–	1.15 ± 0.04	–	–
North	Ursholmen	Low	–	1.36 ± 0.21	1.26 ± 0.06	0.84 ± 0.12	0.39 ± 0.07	−0.16 ± 0.22
		High	–	1.64 ± 0.07	1.35 ± 0.07	1.12 ± 0.04	0.57 ± 0.06	0.07 ± 0.10
	Saltö	Low	–	–	1.02 ± 0.07	0.86 ± 0.08	0.46 ± 0.08	−0.32 ± 0.14
		High	–	–	1.15 ± 0.06	0.89 ± 0.13	0.87 ± 0.19	−0.19 ± 0.13

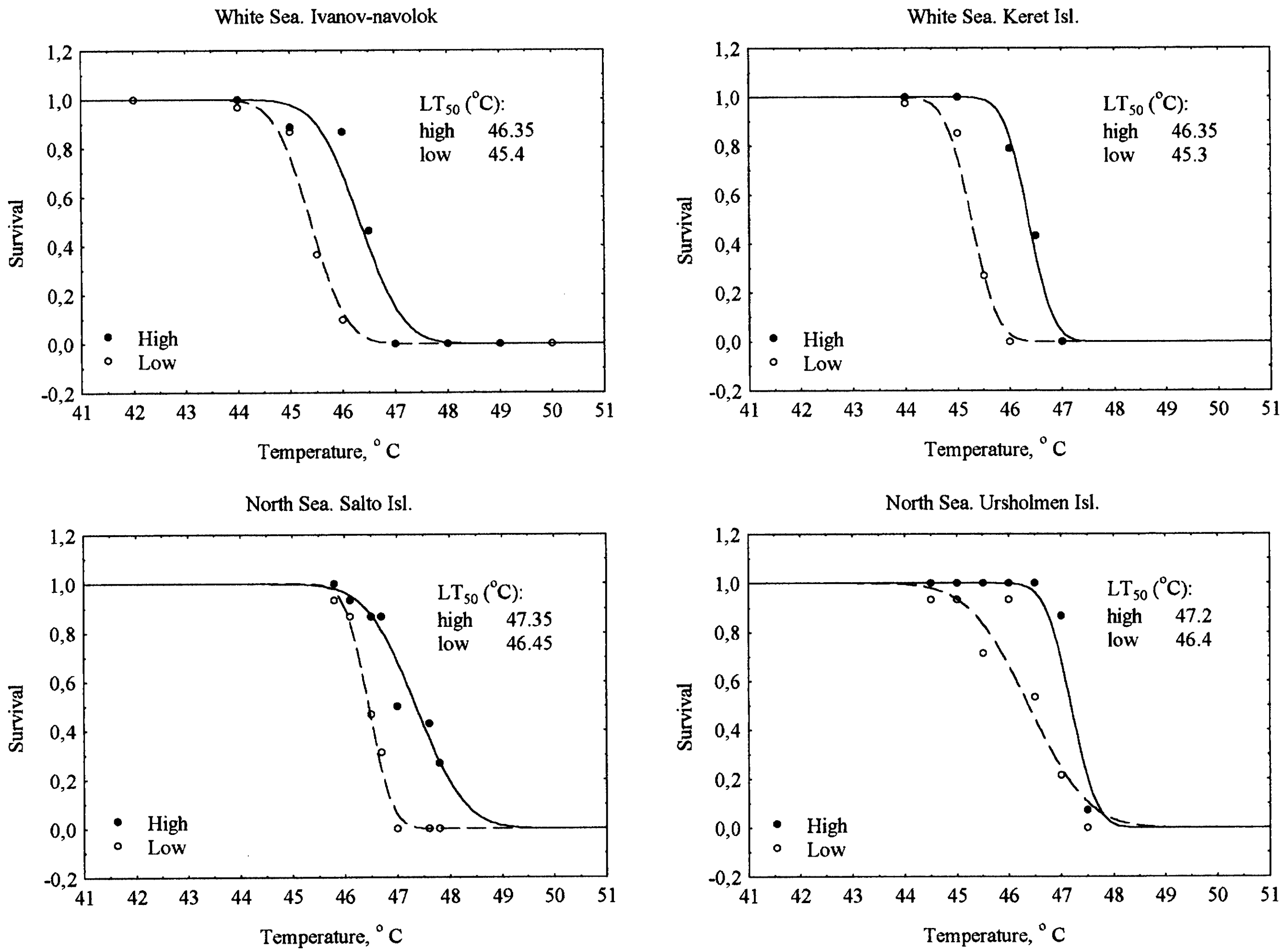

Fig. 4 *Littorina saxatilis*. Acute high temperature limits (LD_{50}) of White Sea and North Sea *L. saxatilis* in sea water (Experiment 3). Regression lines were constructed by an iterative, quasi-Newtonian procedure with maximum likelihood loss function

two-way ANOVA (with a fixed factor *Level* and a random factor *Population*) showed that the factor interaction *Level* × *Population* was significant ($F_{1,38} = 7.77$, $p = 0.008$). Hence, the effects of shore level were further analysed in each of the two populations. Time to close the operculum upon transfer to fresh water tended to be shorter in the high-shore periwinkles (Fig. 6C); however, the difference was only statistically significant in the snails from Fettakh Isl. (Student's *t*-test, Fettakh Isl.: $t = 4.88$, $df = 20$, $p < 0.001$; Ivanov-navolok: $t = 0.78$, $df = 18$, $p = 0.44$).

Discussion

Physiological functions of *Littorina saxatilis* during low tide are compromised by several interacting factors which have synergistic negative effects on performance. Among these, the desiccation, extreme temperatures and oxygen deprivation experienced by the snails at low tide are recognised as the most important ones (Newell 1979; McMahon 1990). As indicated by our data, during summer neap tides the White Sea periwinkles may spend up to 5–7 d out of the water in an isolated state on the

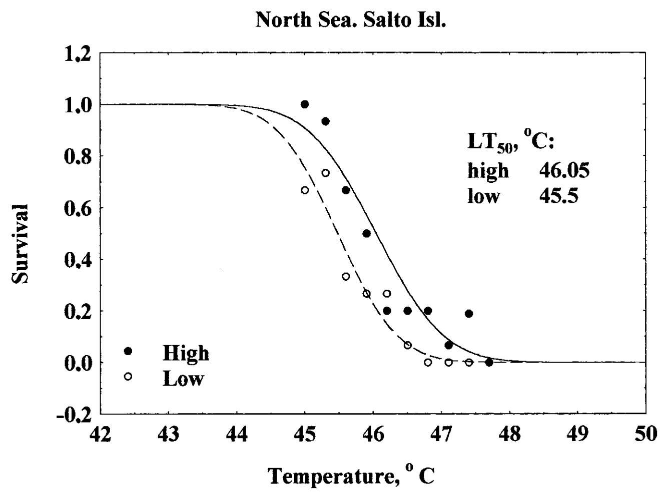

Fig. 5 *Littorina saxatilis*. Acute high temperature limits (LD_{50}) of *L. saxatilis* from Saltö Isl. (North Sea) in air (Experiment 3). Regression lines were constructed by an iterative, quasi-Newtonian procedure with maximum likelihood loss function

Table 5 *Littorina saxatilis*. Log-linear comparisons of acute high temperature limits for high- and low-shore *L. saxatilis* in water (*w*) and air (*a*) (Experiment 3). Degrees of freedom (*df*) and chi-squared values for partial (χ^2_{par}) and marginal association (χ^2_{mar}) are given (** p < 0.001, * p < 0.01). Note highly significant interactions *Shore level* × *Survival* and *Temperature* × *Survival*, which can be interpreted as a significant effect of either shore level or experimental temperature on survival of the periwinkles

Population	*Shore level* × *Survival*			*Temperatuure* × *Survival*			*Shore level* × *Temperature*		
	df	χ^2_{par}	χ^2_{mar}	*df*	χ^2_{par}	χ^2_{mar}	*df*	χ^2_{par}	χ^2_{mar}
Ivanov-navolok (w)	1	25.8**	7.5*	4	271.5**	253.2**	4	18.4*	0.12
Keret Isl. (w)	1	63.1**	20.5**	4	304.1**	261.6**	4	42.6**	0.06
Ursholmen Isl. (w)	1	26.4**	14.6**	6	108.3**	96.5**	6	11.8	0.09
Saltö Isl. (w)	1	32.5**	22.8**	6	98.6**	88.9**	6	11.3	1.7
Saltö Isl. (a)	1	13.7**	10.2**	9	129.4**	125.9**	9	5.1	1.6

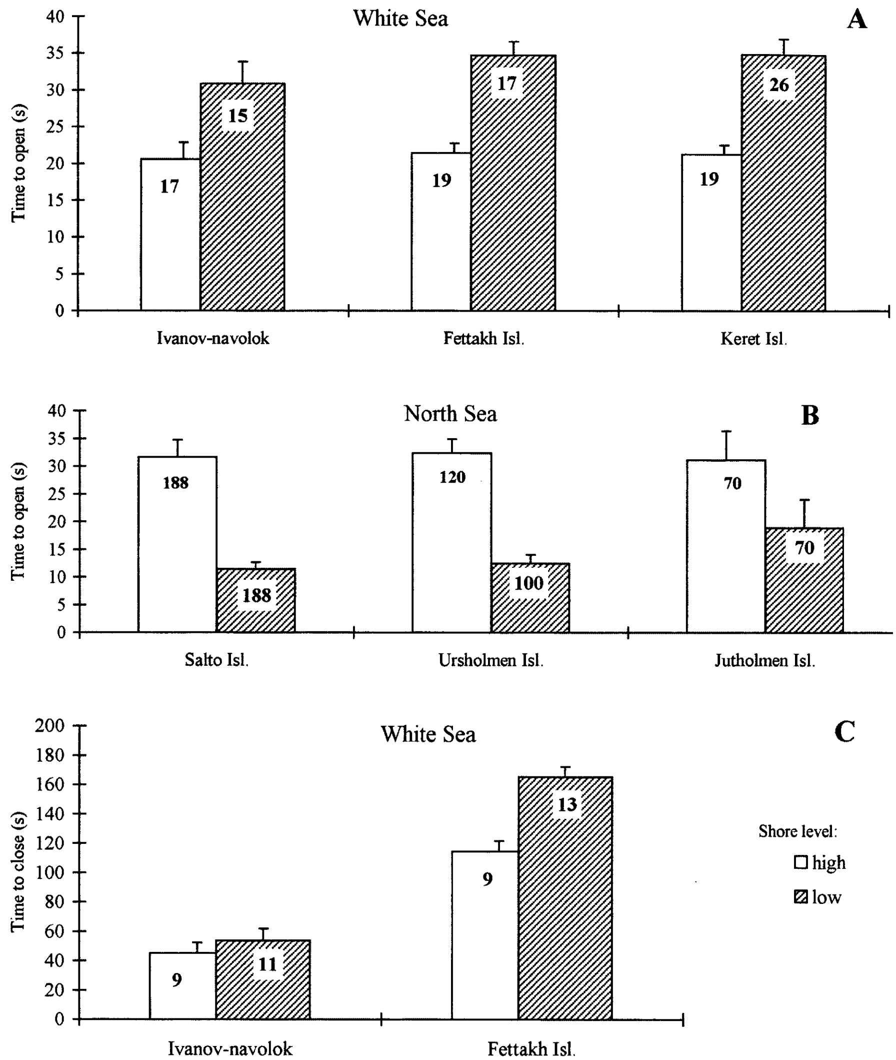

Fig. 6 *Littorina saxatilis*. Rates of behavioural response to acute salinity changes in the high- and low-shore *L. saxatilis* from the White and North Seas (Experiment 4). Average time to open (**A**, **B**) or to close (**C**) the shell aperture upon transfer from fresh to sea water and vice versa, respectively; standard errors of the mean (*error bars*) are shown; *N* is given in each bar

high shore, experiencing daily temperature extremes of up to 40–48 °C. Thermal and desiccation stress experienced by the low-shore animals is shorter and not as extreme. The difference in mean body temperatures between the high- and the low-shore periwinkles may reach 6 to 10 °C during summer low tides. On the practically atidal Swedish coast of the North Sea one may expect an even more pronounced contrast in the thermal and desiccation conditions of the high- and low-shore environments, as the high-shore *L. saxatilis* spend most of their time out of the water, relying heavily on the water supply from occasional splash, while in general the low-shore periwinkles are continuously immersed. Although the high- versus low-shore contrasts may be modified to a certain extent by the microenvironmental features of the substrate (like the presence of shady places, deep crevices, macroalgal cover, etc.) (Berger et al. 1995), these effects are relatively small on topographically simple substrates like the open rock surfaces examined in the present study.

The between-level pattern of variation in water economy was similar to that previously described for various gastropod species inhabiting contrasting intertidal horizons (Broekhuysen 1940; Evans 1948; Southward 1958; Brown 1960; Wolcott 1973; Cleland and McMahon 1986; McMahon 1990). Within all studied *Littorina saxatilis* populations, the rate of evaporative water loss was consistently lower in the high-shore snails as compared to their low-shore counterparts at all studied temperatures in a range of 25 to 35 °C. Surprisingly, evaporative water loss (as measured after 12 h of desiccation) increased with the temperature of exposure only in the low-shore periwinkles. This suggests that the high-shore snails were not only better able to conserve the water during air exposure but also seemed to have a better ability to regulate water loss during temperature fluctuations.

For methodological reasons it was difficult to determine exactly the lethal limits of dehydration, because the evaporative water loss could only reliably be quantified in survivors. However, the onset of mass mortality was detected in our experiments after 12 h of air exposure at 35 °C, which suggests that the water loss of 26 to 33% and 18 to 22% in high- and low-shore periwinkles, respectively, may be close to the lethal limits. This may indicate that the ability to tolerate desiccation is higher in the upper-shore periwinkles than in the low-shore ones. Similarly, Davies (1969) has shown that upper-shore *Patella vulgata* were able to survive a 60% loss of body weight, while lower-shore limpets tolerated only a 44% loss of body weight. Evaporative water loss for 50% mortality ranged between 16% of wet tissue weight in the eulittoral gastropod *Oxystele sinensis* and 33 to 38% of wet weight in the eulittoral-fringe *Littorina knysnaensis* and *Oxystele variegata* (Brown 1960). Thus, the magnitude of the differences in desiccation tolerance between high- and low-shore subpopulations in some species (e.g. Davies 1969; present study) may be quite comparable to the degree of interspecific variation in this parameter.

In addition to the more efficient water economy and increased desiccation tolerance, the upper-shore *Littorina saxatilis* demonstrated significantly higher resistance to elevated temperatures, either under conditions of acute temperature rise or during chronic exposure to elevated temperatures. In the high-shore *L. saxatilis*, acute high temperature limits (LD_{50}) were ca. 1 and 0.5 °C higher in water and air, respectively, as compared to the limits in low-shore periwinkles from the same population. Even more pronounced differences in survival at elevated temperatures were found under more realistic conditions of chronic exposure in air. At the highest test temperature (44 °C), 50% of the high-shore snails survived 0.2 to 0.5 h longer than the low-shore ones, while at the lowest tested temperature (30 °C), the difference comprised 16 to 21 h. At 30 °C, 50% of the periwinkles were able to survive for more than 40 h in air, irrespective of shore level. This indicates a rather high potential for heat-stress resistance in *L. saxatilis* populations in general. However, at 42 and 44 °C the resistance was dramatically reduced with LT_{50} comprising 2.5 to 7 h and < 1.5 h, respectively. It is worth noting that extreme heating (up to 40 °C and more, see Fig. 1) may persist during summer low tides in the White Sea intertidal zone for at least several hours (authors' personal observations), which suggests that resistance to elevated air temperatures may be of selective value at the high-shore level. Moreover, under the natural conditions of the high intertidal zone, heat resistance would be compromised by the negative cumulative effects of prolonged anaerobiosis, which may last for 5 to 7 d during summer neap tides. During such periods the actual heat resistance may be greatly decreased, even at relatively low temperatures (around 30 °C). The potential impact of anaerobic end-product accumulation and changes in energy status on the survival of high- and low-shore periwinkles during air exposure at elevated temperature is currently under investigation (Sokolova in preparation).

Survival time of *Littorina saxatilis* in air depends crucially on the temperature during exposure. This phenomenon is well known for many other marine invertebrates, including molluscs (reviewed in: Newell 1979). However, in contrast to the results of previous studies in intertidal gastropods and barnacles (Newell 1979), for *L. saxatilis* the dependence of LT_{50} on the exposure temperature was not linear over the studied temperature range, with the breakdown point situated around 38 °C. Between 30 and 38 °C mortality rates were only slightly dependent on the temperature, with Q_{10} = 1.4 for LT_{50}. We hypothesise that survival time in air may depend primarily on the rates of anaerobic metabolism and on the consequent negative changes in intracellular acid-base and energy status in the temperature range of 30 to 38 °C. The weak correlation between temperature and survival rates suggests that *L. saxatilis* has a good capacity for metabolic regulation at these temperatures, which may in turn be a consequence of the metabolically arrested state that *Littorina* spp. enter during anaerobiosis (Sokolova et al. 2000). In contrast, when the temperature exceeded the threshold level of 38 °C, the resistance to heat exposure in air dropped drastically, with a Q_{10} of 539.8 for LT_{50} values. This cannot be explained by variations in the metabolic rate alone and implies the effect of additional adverse factors (like protein denaturation due to extreme heat shock, metabolic dysfunction, etc.). Validation of the hypothesis concerning different sources of mortality during air exposure at different temperatures in *L. saxatilis* would be a promising subject for future investigations.

In contrast to the physiological parameters studied, the behavioural response to extreme environmental challenge was not uniform in the animals from the White and the North Seas. In the White Sea populations, the high-shore periwinkles were fastest to respond to extreme environmental changes (transfer to fresh water and back to water of normal salinity). On the contrary, the high-shore *Littorina saxatilis* from the North Sea resumed their activity upon transfer from the fresh to sea water much later than their low-shore counterparts. Possibly, this discrepancy in the rate of response to extreme environmental changes reflects different environmental conditions (from the viewpoint of the predictability of exposure duration) in the White and the North Sea populations. On the White Sea shores, *L. saxatilis* is regularly and predictably immersed during spring tides, while on the high-shore levels of the atidal North Sea coast the animals are wetted unpredictably, depending on the weather conditions. Hence, it may be important for these animals to respond to a relatively durable stimulus only. Otherwise, precocious opening upon short-term exposure to an occasional stimulus may lead to a large loss of water without a subsequent opportunity to rehydrate. Neither White Sea nor low-shore North Sea *L. saxatilis* normally run this risk.

All the considered parameters of physiology and behaviour varied considerably between the populations of *Littorina saxatilis* studied, with variability between shore levels usually exceeding the variation among populations from the two geographically distant areas of the North and White Seas. This may indicate the predominance of a local adaptation determining physiological differentiation in a species such as *L. saxatilis* with a low dispersal ability. A similar situation has been previously described for genetic variation in growth rates and allozyme frequencies in *L. saxatilis* (Behrens Yamada 1987, 1989; Johannesson and Johannesson 1990). Alternatively, this may suggest that environmental contrasts (and a resulting differential selective pressure) between high- and low-shore levels exceed the differences in the relevant environmental conditions between different populations and even between the two seas.

As is clearly shown by our data, differences in evaporation rates, survival during prolonged exposure to air at elevated temperatures and acute thermal resistance of the high- and low-shore *Littorina saxatilis* are consistent with the predictions of McMahon's (1990) hypothesis based on between-species comparisons. Microenvironmental differences between the high- and low-shore levels obviously exert strong selective forces on *L. saxatilis* populations, which probably lead to the observed adaptations in water conservation as well as desiccation and thermal resistance in the high-shore periwinkles. Besides this, *L. saxatilis* subpopulations inhabiting different shore levels demonstrate a high degree of physiological and genetic differentiation in growth rates, shell shape and colouration (Johannesson et al. 1993, 1995b, 1997), allozyme frequencies (Johannesson and Johannesson 1989; Johannesson et al. 1995a), heat stability and activity of enzymes (Hull et al. 1999). The complex of physiological and biochemical adaptations to the environmental extremes of the high intertidal zone, which are at least partly genetically determined, allow *L. saxatilis* populations to benefit from low competition and to use the underexploited resources of this zone. However, a population has a high price to pay for this. Temperature and desiccation stresses on the high shore have been repeatedly shown to cause severe mortality in intertidal inhabitants (Hodgkin 1959; Frank 1965; Sutherland 1970). During our study we also detected high mortality (up to 36%) during a summer neap tide in the high-shore *L. saxatilis* population on Fettakh Isl. (authors' unpublished data). This suggests a trade-off between the potential benefits and encountered risks of life in the high intertidal zone, which controls the upper limits of the *L. saxatilis* distribution.

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