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VERTICAL ZONATION AND HEAT TOLERANCE OF THREE LITTORINID GASTROPODS ON A ROCKY SHORE AT TANJUNG CHEK JAWA, SINGAPORE

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ABSTRACT. – Three common rocky shore littorinid taxa, i.e., *Littoraria* spp. (a collective term for *L. strigata* and *L. articulata*), *Echinolittorina malaccana* and *E. vidua* occupied different tidal heights based on field observations carried out at Tanjung [=Cape] Chek Jawa, Pulau [=Island] Ubin, Singapore in 2002. *Littoraria* spp. were consistently observed at a lower level on the shore than *E. malaccana* and *E. vidua*. Manually translocated littorinids returned to their preferred zones in the field, i.e., *E. malaccana* returned to the region above the MHWS level of 2.7 m while *Littoraria* spp. remained below the region occupied by *E. malaccana*. Further, *E. malaccana* individuals with their shell nodules removed by filing did not occupy a lower zone than intact conspecifics in the field. Although significant temperature differences (TD) were observed between rock and shell surfaces in the laboratory ($TD_{Littoraria} \approx TD_{\text{filed } E.\ malaccana} < TD_{E.\ malaccana}$), preference for a particular height on the shore is probably governed more by physiological factors (e.g., heat tolerance) than by morphological adaptation (e.g., shell morphology). As the upper shore is exposed to the sun for longer periods of time, it is expected that the heat tolerance of inhabitants of the higher shore would be greater than those occupying the lower regions. Results of a 1-hour lethal temperature experiment support this hypothesis: the temperature at which 50% mortality (1h LT_{50}) was observed was highest for *E. malaccana* (50.4°C) compared to *E. vidua*, (48.1 °C) and *Littoraria* spp. (47.5°C), possibly reflecting their relative tidal positions on the shore. Enzyme stability may also account for high heat tolerance of *E. malaccana*. Incubation of the enzyme, glutamate oxaloacetate transaminase (GOT) at 55°C showed that *E. malaccana* sustained high GOT activity compared to those of *Littoraria* spp. and *E. vidua* which decreased sharply at the same temperature, again suggesting inherent physiological adaptation in *E. malaccana*.

KEY WORDS. – Zonation; heat tolerance; enzyme stability; *Littoraria* sp.; *Echinolittorina malaccana*; *Echinolittorina vidua*.

INTRODUCTION

The intertidal rocky shore is universally divided into zones according to characteristic intertidal flora and fauna (Raffaelli & Hawkins, 1996): the supralittoral, midlittoral and sublittoral zones. Such zonation is due to the stresses of varying intensities along the height of the shore. Higher levels of the shore (e.g., supralittoral zone) are exposed to the air and sun for a longer period of time when compared with lower shore levels (Newell, 1979; Cleland & McMahon, 1989). Despite the extreme physical conditions in the supralittoral zone, many gastropod species have adapted successfully to life in this harsh zone. Gastropods living above the high tide mark are especially vulnerable to desiccation and thermal stress, as they typically experience prolonged aerial exposure spanning many days (McMahon, 1990). Desiccation may be overcome by behavioural adaptations such as the withdrawal of the foot and closure of the shell opening with an operculum to reduce evaporation. Attachment to the substratum using a mucous sheet – the mucous holdfast (Bingham, 1972; Lim, 2008), further ensures that they will not be dislodged easily or maintain minimal contact with the heated substratum when out of water. Survival in prolonged aerial exposure also depends on physiological adaptations such as the reduction of metabolic rates, e.g., in *Littorina saxatilis* (see McMahon & Russell-Hunter, 1977) and elevated thermal tolerance, e.g., in *Nodilittorina pyramidalis*, *N. granularis* and *Littorina brevicula* (see Fraenkel, 1966) as well as *Austrolittorina unifasciata* (= *N. unifasciata*; see Reid, 2007) (see McMahon, 1990).

Common littorinids found on the rocky shores of Singapore include *Littoraria articulata* (Philippi, 1846), *L. strigata* (Philippi, 1846) (often as *Littorina undulata* in previous literature) and *Echinolittorina malaccana* (Philippi, 1847) (also referred to in the literature variously as *Nodilittorina pyramidalis or N. trochoides*; see Reid, 2007). A less common rough periwinkle, *Echinolittorina vidua* (Gould, 1859) (previously *Nodilittorina vidua*; see Reid, 2007) is also found in certain rocky areas in Singapore.

Two previous littorinid distribution studies have been conducted in Singapore on the south-eastern coast of Singapore; one at a man-made breakwater (see Lam, 1980) and the other at a monsoon drain (see Tan, 1988). To date, there is no published literature with quantitative data on the vertical distribution of *Littoraria* spp., *Echinolittorina malaccana* and *E. vidua* at natural rocky shores in Singapore. The purpose of the first part of this study was to determine the zonation pattern of these littorinids at the rocky shores of Tanjung [=Cape] Chek Jawa, Pulau [=Island] Ubin, Singapore, through a distribution survey.

A translocation experiment involving *Littoraria* spp. and *E. malaccana* was also carried out to verify the zonation pattern observed. Dislodged littorinids are able to crawl upwards to their original zone, e.g., *N. exigua* (see Ohgaki, 1988a), *Littorina littorea* (see Petraitis, 1982) and *Littorina unifasciata* (see Chapman, 1999). It is hypothesized that displaced *Littoraria* spp. and *E. malaccana* individuals in this study would return to their preferred zones.

Shell sculpturing was reported by Vermeij (1973) to reduce the relative effective areas for absorption, i.e., area in a plane that is perpendicular to incident sun's rays. This implies that there is greater heat loss by reflection of solar radiation by an uneven surface, thereby reducing heat absorption. The shell of *E. malaccana* is nodulated while that of *Littoraria* spp. individuals is smooth. Since *E. malaccana* occupies a higher region of the shore, the nodulation may be an advantage for survival under prolonged aerial exposure. The increased surface area of the shell due to the presence of nodules could facilitate convection and heat loss from the shell. To test this hypothesis, the nodulated shells of *E. malaccana* individuals were filed down and included in the translocation study to elucidate the possible role of nodulation in zonation. The effect of nodulation on heat reflection was tested both in the field (for *E. malaccana* and *Littoraria* spp.) and in the laboratory (for *E. malaccana*, both intact and filed, and *Littoraria* spp.). The temperature difference between rock and shell surfaces was used as the response variable.

Two laboratory experiments were conducted to establish possible physiology-related reasons for the zonation pattern: (1) a heat tolerance test, represented by 1 hour lethal temperatures (1h LT_{50}), of the littorinids, and (2) a heat stability test of the enzyme, glutamate oxaloacetate transaminase (GOT), extracted from these littorinids. The enzyme GOT, also known as aspartate aminotransferase, was chosen because it is one of the most active enzymes in the cell (Cooper & Meister, 1985). The metabolic importance of GOT is that it brings about a free exchange of amino groups between glutamate and aspartate; both of which are required for separate but essential steps in the urea cycle for ammonia detoxication and nitrogen excretion. The free movement of nitrogen between the glutamate and aspartate pools is an important balancing process that is vital for normal cell metabolism (Cooper & Meister, 1985).

MATERIAL AND METHODS

Distribution studies

Two distribution studies were carried out at the rocky shore of Tanjung Chek Jawa on Pulau Ubin (approx. 1° 24'N, 103° 59'E), an island off the north-eastern coast of Singapore. The mean high water spring tide level (MHWS) at the study site is 2.7 m (Hydrographic Department, 2001). These studies were made at (i) a vertical rock and (ii) a sloping rock (angle of inclination ≈ 35.6°). The vertical rock is located further inland (i.e., more up-shore in a little 'cove-like' recess) as compared to the sloping rock located at a small spit jutting out seawards. Hence, the vertical rock surface studied is exposed earlier by the receding tide and inundated later by the incoming tide when compared to the sloping rock. Both rocks have sparse algal cover and a fair number of crevices.

Reid (1986) reported that the two closely related species *Littoraria articulata* and *L. strigata* possess shells with similar shape, colour and banding pattern, and without examination of internal anatomical features, it was difficult to ascertain their identities in the field. Thus, these two littorinid species are collectively referred to as *Littoraria* spp. in this study as dissection of every individual encountered was neither feasible nor possible. Data from the vertical rock were collected during three sampling sessions: June/July, September and December 2002, with a total of 10 transect belts laid at each session. The distribution surveys were all carried out during three to five days of consecutive spring tides. Accessibility to the study site was only possible when the tides were suitably low. Each belt transect consisted of a transparent 10 cm-wide plastic strip with a measuring tape attached. The position of each *Littoraria* spp. (collectively *Littoraria*) and *Echinolittorina malaccana* (*E. malaccana*) individual encountered beneath the strip was recorded to the nearest 0.5 cm. Distribution of *Littoraria*, *E. malaccana* and *E. vidua* was similarly determined at the sloping rock on 28 and 29 August 2002. A region covered by a total of 18 belt transects, each 15 cm in width, was surveyed. The tide level (TL) at the top of the vertical rock is 0.1 m above MHWS.

Translocation experiment

A translocation experiment was conducted in December 2002. Thirty individuals of *Littoraria* and 60 *E. malaccana* individuals were collected and transported to the laboratory in a container (with seawater from the study site) for colour-coding with nail polish. The nodules of 30 *E. malaccana* adult individuals were filed off (henceforth termed "filed *E. malaccana*") using an ordinary machinist's file. All experimental snails were returned to the study site within two days to minimize stress and ensure their survival during the translocation experiment. Based on the distribution patterns observed from previous sampling periods, the MHWS level (2.7 m) was found to approximately separate the regions occupied by *Littoraria* and *E. malaccana*. Thus, the marked littorinids were returned to the 2.7 m TL with the MHWS level clearly marked at the study site. Crowe & Underwood (1999) suggested that a snail's behaviour changed by merely dislodging it from its original position. This may affect the results of the translocation study. However, Chapman (1999) found that although translocation affected the distance moved by the snails, it did not result in any change in the direction at which the snails dispersed.

During the next four consecutive days (16 to 20 December 2002), the distance between the locations of every marked individual from the MHWS level was measured to the nearest 0.1 cm. The mean tidal level occupied by each species on each of the four days was calculated. Crowe & Underwood (1999) cited that upon disturbance, littorinids are likely to require two days before resuming their natural ways. Thus, four days would be sufficient for the duration of the translocation study. Data collected were analysed with one-way Analysis of Variance (ANOVA) tests to ascertain whether the preferred zones occupied by the two species differed significantly. The Tukey's multiple comparison test was applied when ANOVA results were significant.

Effect of shell nodulation of *E. malaccana* on heat reflection

Collection of data was carried out on two occasions: at Tanjung Chek Jawa and in the laboratory. Temperature measured by a Raynger® ST™ infrared thermometer (Raytek®) was taken as an indication of the amount of heat energy given off by the littorinid. The thermometer detects emitted energy (e.g., radiated heat energy from within the snail/gastropod) and reflected heat energy (from an external source e.g., the sun). At Tanjung Chek Jawa, the average temperature of the shell of the rough periwinkle, *E. malaccana*, was measured using the average-function of the infrared thermometer. The average temperature of the rock surface, i.e., at the perimeter of the animal, was then measured. Likewise, the temperatures of the shell surface of the smooth periwinkle, *Littoraria*, and the surrounding surface were measured under direct sunlight. A total of 55 pairs of temperature readings were recorded for individuals of various sizes in each species. All individuals, whose shell temperatures were measured, were selected such that they were found on relatively smooth surfaces and not in any sort of pit or crevice. This was to ensure that measurements would not be confounded by other factors, e.g., shade or reflection of heat from adjacent surfaces.

The temperature difference (TD) between rock surface and that of the shell surface (i.e., $TD = T_{rock\ surface} - T_{shell\ surface}$) for *E. malaccana* and *Littoraria* were tested separately for significance using *t*-tests. $TD_{Littoraria}$ and $TD_{E.\ malaccana}$ were then compared using the Mann-Whitney test statistic, as there was non-normality in the data set. In the laboratory, 25 *E. malaccana*, 25 filed *E. malaccana* and 25 *Littoraria* were used in an experiment involving heat treatment. Five *E. malaccana*, five filed *E. malaccana* and five *Littoraria* were placed on a piece of relatively smooth rock that most resembled that at the field site. The rock was then placed approximately 20 cm beneath a lamp and left to warm up for 5 minutes. Pairs of temperature readings were then measured: the temperature of the shell surface and the average temperature of the substrate surface at the perimeter of the snail. The procedure was repeated until all 75 animals were used. A one-way ANOVA was used to test for significant differences among $TD_{Littoraria}$, $TD_{E.\ malaccana}$ and $TD_{filed\ E.\ malaccana}$. In order to maintain approximately equal variances, two obvious outliers were removed prior to ANOVA analyses. The distance of the infrared thermometer from the surface was kept as constant as possible by maintaining the laser spot at approximately the same size during each measurement.

Lethal temperatures study

Littoraria, *E. malaccana* and *E. vidua* individuals with shell lengths between 7–11 mm were collected from the sloping rock at Tanjung Chek Jawa in November 2002. Snails were used within 24 hours of collection. The experiment involved 12 temperature treatments between 44 and 52°C (see below). For each temperature treatment, 30 littorinid individuals (i.e., 10 *Littoraria*, 10 *E. malaccana* and 10 *E. vidua*) were placed in an aluminium can containing 100ml of artificial seawater. Heat treatment of the snails submerged in seawater served to eliminate the effect of desiccation (see Evans, 1948). A plastic cover was placed over the can to prevent water loss due to evaporation during heating. The seawater was brought to the required temperature in a water bath from room temperature (22–23°C). The temperature of the seawater was measured with "K" type thermocouple probe connected to an AI 104 humidity temperature meter (Azex Instruments (S) Pte. Ltd.). The probe was inserted through a hole in the plastic cover. Once the required temperature was attained, the time was noted and the seawater was maintained at a relatively constant temperature for an hour (the temperature was monitored and recorded at 15 minute intervals). This length of time was used by Fraenkel (1966) in a heat stress experiment conducted on 12 species of intertidal prosobranchs in south-western Japan. The time taken for the seawater to reach the required temperature ranged between 20 and 30 minutes. Monitoring of seawater temperatures indicated that the seawater could not be assumed to be at the temperature shown by the water bath as the temperature probe that was inserted into the experimental container registered some fluctuations. Hence, the temperature of each heat treatment was taken to be the average temperature of the temperatures recorded during the hour. The resultant 12 temperature treatments were 44.1°C, 44.7°C, 45.6°C, 47.0°C, 47.4°C, 48.0°C, 48.4°C, 48.7°C, 49.5°C, 50.3°C, 50.8°C and 51.8°C. The temperature range selected was based on temperatures observed in the field.

After heat treatment, the littorinids were then transferred to the centre of a holding tank containing artificial seawater and an algae-covered rock. The tank was left in a normal external environment (i.e., out of the air-conditioned laboratory). At the end of 24 hours in the holding tank, the number of live individuals was recorded. An individual was considered to be alive if it displayed foot attachment, had scaled up the sides of the tank, moved onto the rock or responded to gentle prodding of the foot. The percentage mortalities obtained were converted into probit values and graphed against temperature. The 1-hour lethal temperature (1h LT_{50}) of each group was taken to be the temperature at which there was 50% mortality. As it was not feasible to have a large sample size, statistical analyses of the 1h LT_{50}s was not carried out.

Heat stability of GOT

Six samples of tissues (each consisting approximately 0.5 g fresh weight of pooled flesh from a few individuals) of each of the three littorinid taxa were frozen using liquid nitrogen and stored at -80°C. The frozen samples were pounded to powder in liquid nitrogen, reweighed and homogenised with thrice their weight of cold 0.05M Tris-HCl buffer (from Sigma), pH 7.0. Homogenisation was done using a Polytron PT1300D homogeniser for a duration of 3 × 20 s with 20 s intervals. The homogenates were sonicated with a Heat Systems sonicator at 40% output power for one minute. The mixtures were centrifuged at 9000 rpm, 4 °C for 10 min in a Beckman J2-MC refrigerated centrifuge. The supernatants were pipetted into clean test tubes, which were kept in ice. Enzyme analyses were conducted within a few of hours of sample preparation using the GOT optimized aspartate-aminotransferase EC 2.6.1.1 UV-test kit (Sigma). The sample used for enzyme analyses was a 50× dilution of the extract (0.03 ml of the supernatant mixed with 1.47 ml of Tris-HCl buffer, pH 7.0). Prior to enzyme analyses, Reagent A (260 mmol/L aspartate, 104 mmol/L phosphate buffer pH 7.4, 0.234 mmol/L NADH, 780 U/L MDH and 1560 U/L LDH) and the samples were incubated separately at 25°C, 45°C, 50°C and 55°C for 30 minutes. After the 30 min incubation, 0.1 ml of sample was added to 1.3 ml of Reagent A and mixed by inversion. After a minute of incubation in a temperature controlled cuvette compartment of a UV-1601 spectrophotometer with a CPS temperature controller (Shimadzu), 0.125 ml of Reagent B (156 mmol/L 2-oxoglutarate) was added and mixed. The absorbance of the mixture was recorded eight times at 25 s intervals and the mean absorbance change per minute (ΔA/min) was calculated. Glutamate oxaloacetate transaminase (GOT) activity (µmol NADH/min/g wet weight) was calculated according to the following formula:

$$GOT = \frac{(\Delta A/\text{min})\ (\text{total volume})\ (\text{volume of buffer} + \text{wet weight of tissue}) \times 50}{6.22 \times \text{sample volume} \times \text{wet weight of tissue}}$$

There were six replicates for each of the four temperature treatments for all three littorinid taxa. GOT activity was converted to a percentage value based on the results of previous temperatures, as an indication of change in activity due to increase in temperature. Data were arcsine-transformed and analysed with one-way ANOVA. This was performed only on data for 50°C and 55°C, as most of the data for 25°C and 45°C were greater than 100% and could not be arcsine-transformed. All statistical analyses in this study were carried out using MINITAB (Release 12.21, 1998).

RESULTS

Distribution studies

At the vertical rock study site, *Littoraria* and *E. malaccana* were found in two distinct regions of the high intertidal zone in June/July (Fig. 1a): *Littoraria* occupied the lower region and *E. malaccana*, the higher region. The boundary between the two regions was located approximately at 2.6 m (0.1 m below MHWS). The mean tide level (mean TL) occupied by *E. malaccana* was 2.8 ± 0.2 m (range = 2.4 m to 3.6 m) while that of *Littoraria* was 2.0 ± 0.2 m (range = 1.8 m to 3.1 m) (Fig. 1a). In September 2002, the mean TL occupied by *E. malaccana* increased to 3.0 m ± 0.3 m (range = 2.1 m to 3.5 m), while that of *Littoraria* also increased to 2.3 ± 0.2 m (range = 1.9 m to 3.2 m) (Fig. 1b). The band of *E. malaccana* had a 0.6 m overlap with of the *Littoraria* band. In December 2002, the overlapping region decreased to 0.3 m in width (Fig. 1c). *E. malaccana* was found between TL of 2.5 m to 3.6 m (mean TL = 3.2 ± 0.3 m) and *Littoraria* was found between TL of 2.0 m to 3.5 m (mean TL = 2.4 ± 0.2 m) (Fig. 1c). At the sloping rock, *E. malaccana* also occupied the highest region of the rock (range = 2.6m to 2.8 m) while *E. vidua* individuals occupied a region that overlapped with that of *Littoraria* (Fig. 2). The distribution of *E. vidua* extended slightly higher than the *Littoraria* zone but was distinctly below that of *E. malaccana*.

Translocation experiment

The mean TL occupied by *Littoraria* on the four consecutive days was slightly lower than the MHWS level of 2.7 m while *E. malaccana* and filed *E. malaccana* were found at least 0.3 m above the MHWS level (Table 1). One-way ANOVA results showed that on all four days there was a significant difference ($P < 0.05$) among the mean TLs occupied by the three species of littorinids (Table 2). Tukey's multiple

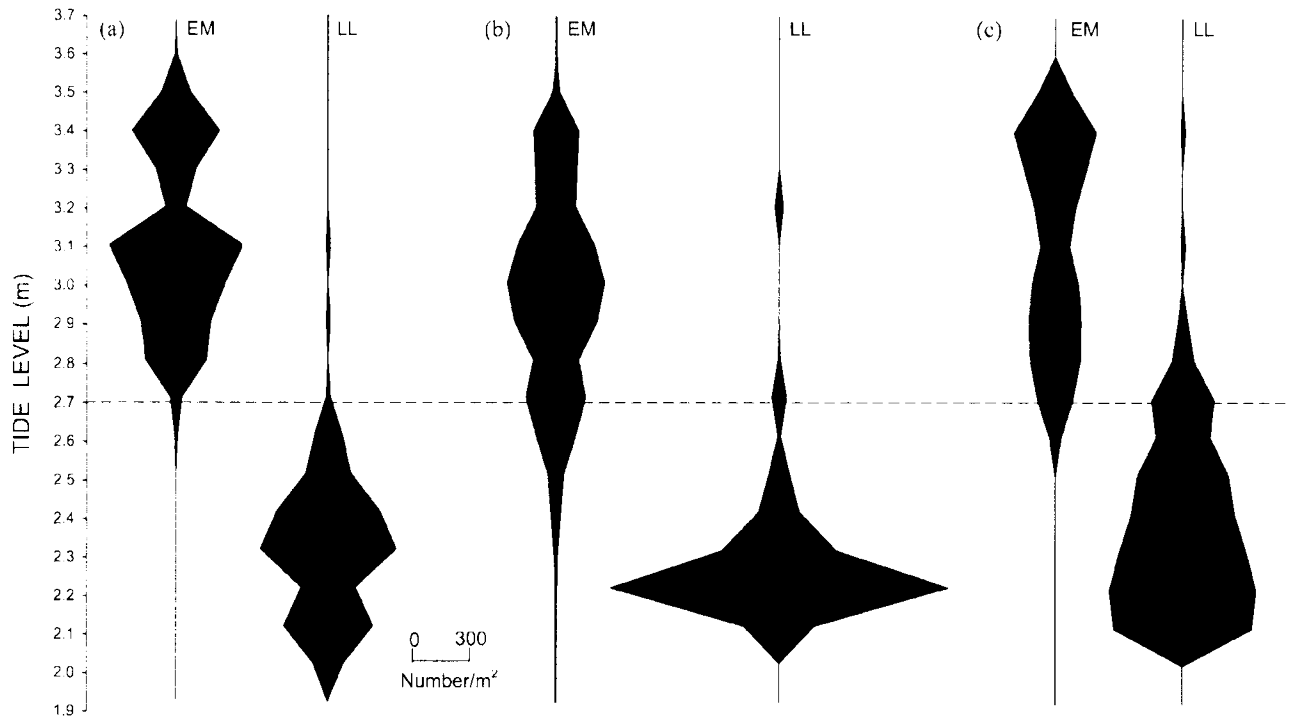

Fig. 1. Kite diagrams showing the distributions of *Littoraria* spp. (LL) and *Echinolittorina malaccana* (EM) on a vertical rock at Tanjung Chek Jawa in (a) June/July, (b) September, and (c) December 2002. (MHWS is represented by dashed line).

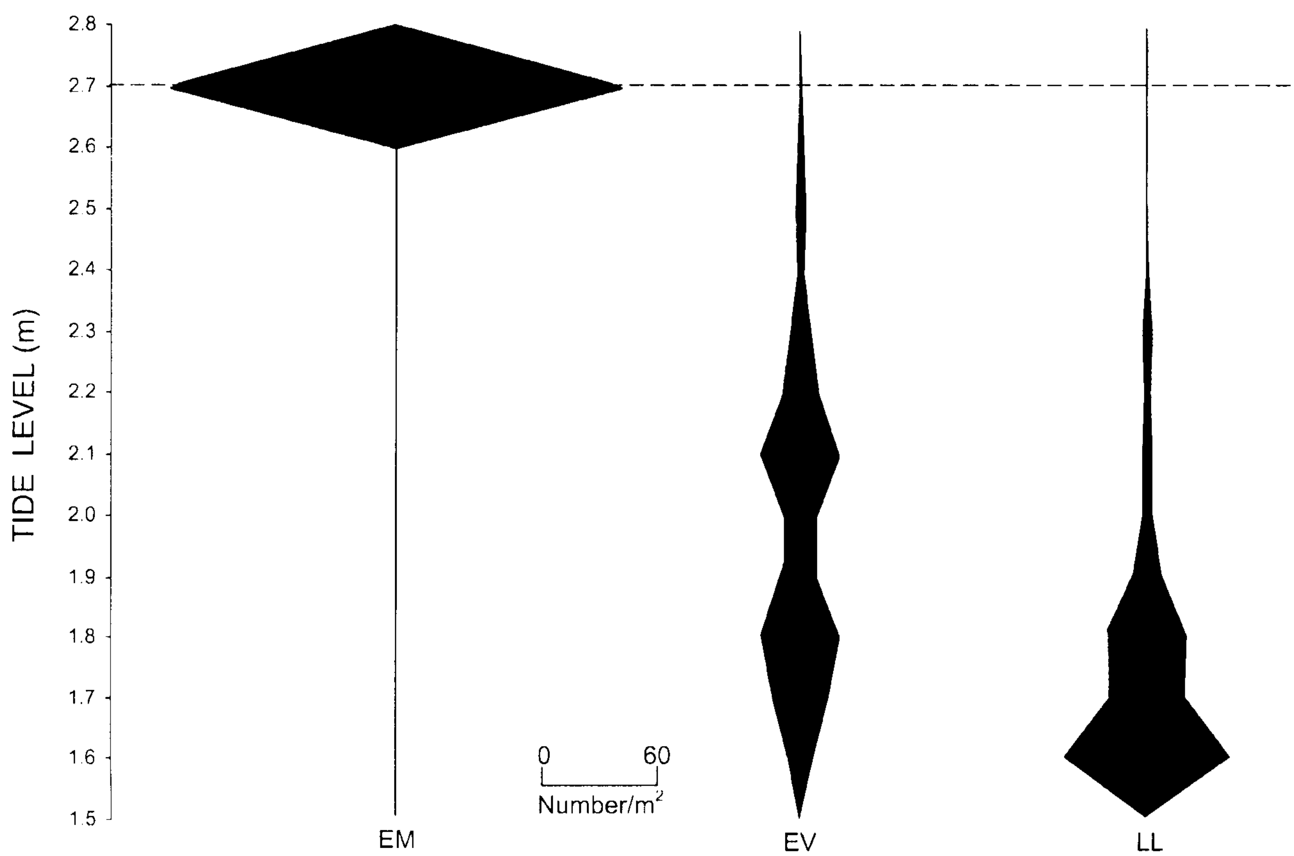

Fig. 2. Kite diagrams showing the distributions of *Littoraria* spp. (LL), *Echinolittorina malaccana* (EM) and *E. vidua* (EV) on a sloping rock (angle of elevation of approximately 35.6°) at Tanjung Chek Jawa. (MHWS is represented by dashed line).

Table 1. Mean tide levels occupied by marked *Littoraria* spp. (*Littoraria*), *Echinolittorina malaccana* (*E. malaccana*) and filed *E. malaccana* (filed *E. malaccana*) on four consecutive days (16 – 20 Dec.2002) at Tanjung Chek Jawa, Singapore.

	Mean tide level ± S.D. (m)		
	Littoraria	*E. malaccana*	Filed *E. malaccana*
Day 1	2.69 ± 0.20	3.11 ± 0.23	3.03 ± 0.23
Day 2	2.54 ± 0.24	3.11 ± 0.20	3.04 ± 0.23
Day 3	2.59 ± 0.25	3.14 ± 0.19	3.11 ± 0.26
Day 4	2.67 ± 0.21	3.16 ± 0.21	3.09 ± 0.21

Table 2. Results of one way ANOVA and Tukey's multiple comparison for tide levels that were occupied by marked *Littoraria* spp. (*Littoraria*), *Echinolittorina malaccana* (*E. malaccana*) and filed *E. malaccana* (filed *E. malaccana*) on four consecutive days (16 – 20 December 2002) at Tanjung Chek Jawa, Singapore. (*: significant).

	ANOVA			Tukey's test
	F	df_1, df_2	*P*	
Day 1	29.99	2, 87	0.00 *	*Littoraria* < filed *E. malaccana* ≈ *E. malaccana*
Day 2	54.23	2, 87	0.00 *	*Littoraria* < filed *E. malaccana* ≈ *E. malaccana*
Day 3	52.41	2, 87	0.00 *	*Littoraria* < filed *E. malaccana* ≈ *E. malaccana*
Day 4	33.84	2, 87	0.00 *	*Littoraria* < filed *E. malaccana* ≈ *E. malaccana*

comparison tests showed that there was no significant difference between the TLs occupied by filed *E. malaccana* and *E. malaccana* on the four days ($P > 0.05$) (Table 2). Both *E. malaccana* and filed *E. malaccana* were found at a significantly higher TL ($P < 0.05$) than *Littoraria* (i.e., *Littoraria* < filed *E. malaccana* ≈ *E. malaccana*) (Table 2).

Effect of shell nodulation of EM on heat reflection

Results from *t*-tests showed that $TD_{Littoraria}$ and $TD_{E.\ malaccana}$ recorded at Tanjung Chek Jawa were both significantly greater than 0°C ($t = 3.36$ and $t = 7.61$ respectively, $P < 0.05$ for both). The mean TD between rock surface and shell surface for *E. malaccana* and *Littoraria* were 0.55 ± 0.54°C and 0.15 ± 0.34°C respectively. $TD_{E.\ malaccana}$ was significantly greater than $TD_{Littoraria}$ (W = 3942.5, $P < 0.05$).

The ANOVA results of the laboratory heat experiment showed that mean TD varied significantly with the treatment group of littorinids in question (Table 3, $P < 0.05$). Tukey's multiple comparison test showed that there was no significant difference between $TD_{Littoraria}$ and $TD_{filed\ E.\ malaccana}$ but $TD_{E.\ malaccana}$ was significantly greater than both $TD_{Littoraria}$ and $TD_{filed\ E.\ malaccana}$ ($TD_{Littoraria} \approx TD_{filed\ E.\ malaccana} < TD_{E.\ malaccana}$) (Table 3).

Lethal temperature study

Littoraria recorded the lowest 1h LT_{50} of approximately 47.5°C, and *E. malaccana*, the highest, at approximately 50.4°C (Fig. 3). *Echinolittorina vidua* had an intermediate 1 h LT_{50} of approximately 48.1°C (Fig. 3).

Heat stability of GOT

Incubation of GOT at 45°C resulted in increased GOT activity for all three species of littorinids with greatest increase in GOT activity seen in *E. malaccana* (236.48 ± 6.11%, Table 4). The maximum GOT activity for *E. vidua* occurred after incubation at 50°C while those of *Littoraria* and *E. malaccana* were at 55°C (Fig. 4). ANOVA results showed that after incubation at 50°C, the mean percentage GOT activity remaining for *E. malaccana* was significantly greater than that of *E. vidua* but not significantly different from that of *Littoraria* ($P < 0.05$, *E. vidua* < *Littoraria* < *E. malaccana*). After incubation at 55°C, mean percentage GOT activity remaining for *E. malaccana* was significantly greater than those from *E. vidua* and *Littoraria*, and that of *Littoraria* significantly greater than that of *E. vidua* ($P < 0.05$, *E. vidua* < *Littoraria* < *E. malaccana*).

DISCUSSION

During all three sampling periods, *Littoraria* (i.e., *L. articulata* and *L. strigata*) was generally found below the MHWS level of 2.7 m. In consultation with the Singapore Tide Tables and Port Information (Hydrographic Department, 2001), it was estimated that the MHWS level could be left dry for at least eight days during a two-week tidal cycle before the next high tide wetted the region. *Littoraria* individuals may not be able to cope with the physical stresses beyond the MHWS level during the

Table 3. Results of one-way ANOVA and Tukey's multiple comparison of the variable, temperature difference (TD) between rock surface and shell surface of *Littoraria* spp. (*Littoraria*), *Echinolittorina malaccana* (*E. malaccana*) and filed *E. malaccana* in the laboratory. (*: significant).

Variable	Mean ± S.D. (°C)	ANOVA			Tukey's test
		F	df_1, df_2	*P*	
$TD_{Littoraria}$	0.128 ± 0.177				
$TD_{E.\ malaccana}$	0.300 ± 0.204	5.85	2, 72	0.004 *	$TD_{Littoraria} \approx TD_{filed\ E.\ malaccana} < TD_{E.\ malaccana}$
$TD_{filed\ E.\ malaccana}$	0.163 ± 0.174				

aerial exposure. At the sloping rock, *E. vidua* was found to occupy a region distinctly below that of *E. malaccana* and it overlapped with the upper region in which *Littoraria* were found.

Echinolittorina malaccana was generally found above and at the MHWS level. Lam (1980) also reported that *E. malaccana* (as *Tectarius malaccensis*) inhabited the splash zone above MHWS on a man-made breakwater. A similar distribution pattern was observed by Ohgaki (1988b) for another rough periwinkle, *Nodilittorina exigua*, in Shirahama, Japan. Ohgaki (1988b) suggested that *N. exigua* remained above and around the MHWS level to escape harsh conditions (e.g., strong wave action) that exist lower down the shore. In addition, these nodulated littorinids are adapted for survival under prolonged aerial exposure. Among *E. malaccana* (as *T. malaccensis*), *E. vidua* (as

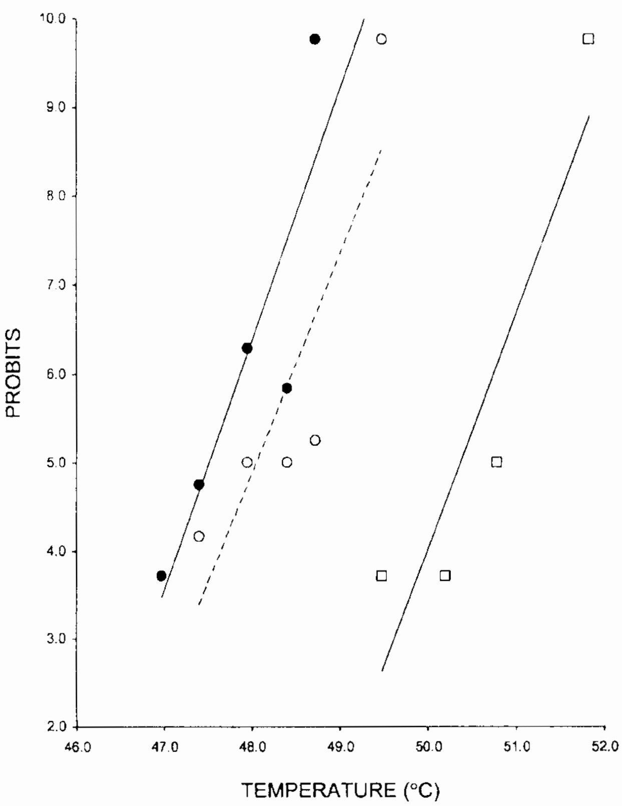

Fig. 3. Probit response curves of *Littoraria* sp. (●), *Echinolittorina vidua* (O) and *E. malaccana* (□), from which the 1h LT_{50} values are obtained.

Littorina granularis) and *Littoraria* (as *L. undulata*), Lam (1980) found that *E. malaccana* was most tolerant to desiccation at 38 ± 1°C, a trait that partly accounts for its successful survival above the MHWS level.

The distributions of *Littoraria* and *E. malaccana* were generally distinct from each other, with the former found at a lower level than the latter. This zonation observed at Tanjung Chek Jawa was verified by the translocation study, whereby displaced individuals of *Littoraria* and *E. malaccana* returned to their preferred regions on the shore. Such movements were also observed in *Austrolittorina unifasciata* (as *L. unifasciata*) and *Nodilittorina pyramidalis* in New South Wales, Australia by Chapman (1999). In addition, physical removal of shell nodules in *E. malaccana* did not result in a change of preference for a tide level, i.e., the filed *E. malaccana* did not move to a level significantly lower or higher than that of their unfiled counterparts. However, results from the laboratory experiment showed that filed *Echinolittorina* shells and smooth *Littoraria* shells were both warmer than nodulated shells, as evidenced by the mean $TD_{E.\ malaccana}$ being significantly greater than both $TD_{filed\ E.\ malaccana}$ and $TD_{Littoraria}$. This suggests that although shell ornamentation helps to reduce shell temperature, it is probably not a major factor in determining the choice of tidal level at which the species remained.

Rough, nodulated shells reflect more heat energy (Nybakken, 2001). The higher TD of the rough periwinkle, *E. malaccana*, observed in this study may be due to this reason, as the infrared thermometer senses reflected heat energy (refer to Raynger® ST™ manual). The significant difference between $TD_{Littoraria}$ and $TD_{E.\ malaccana}$ at Tanjung Chek Jawa suggested that the nodulation on *E. malaccana* helped in reducing the amount of heat transmitted into the body of the littorinid. This is because the more heat energy is reflected, less is absorbed and transmitted (Jones, 2000). Another factor that may account for the difference in TDs is colour, which affects absorption and reflection of heat. According to Vermeij (1973), a light-coloured surface absorbs less radiation from visible light. Many high shore gastropods (e.g., *Nerita peleronta, Tectarius muricatus* and *Littorina unifasciata antipodium*) are light coloured while gastropods at lower shores have darker shells (Nyabakken, 2001; Vermeij, 1973). The nodules on *E. malaccana* are of a much lighter colour (pale yellow) than the rest of the shell (dark brown). This colouration may further enhance

Table 4. Mean percentage glutamate oxaloacetate transaminase (GOT) activity remaining for *Littoraria* sp. (*Littoraria*), *Echinolittorina vidua* and *E. malaccana* after incubation at 45°C, 50°C and 55°C.

Species	Mean percentage GOT activity ± S.D. (%)			
	25°C	45°C	50°C	55°C
Littoraria	100.00 ± 0.00	176.32 ± 69.78	112.27 ± 56.57	67.87 ± 7.95
E. vidua	100.00 ± 0.00	191.99 ± 10.39	98.41 ± 9.39	47.27 ± 8.73
E. malaccana	100.00 ± 0.00	236.48 ± 6.11	101.94 ± 22.97	97.59 ± 33.69

heat reflection by the nodules. Filed shells were much darker in colour than intact conspecifics. McQuaid and Schermann (1988) reported that the pale-shelled *L. a. africana* had a lower body temperature than the dark brown morph *L. a. knysnaensis*. They also found that there was a higher mortality rate among individuals of the two sub-species that were painted black.

The preference for a higher or lower shore level may likely be due to physiological adaptations. McMahon (2001) hypothesized that mid-shore gastropods had modified respiratory systems for aerial and aquatic gas exchange, which disabled them from surviving in aquatic environments. Likewise, *E. malaccana* may have adapted to an almost terrestrial life above and about MHWS such that it is unable to cope with prolonged immersion. Interspecific competition between *Littoraria* and *E. malaccana* could also influence the zonation pattern. However, more studies would have to be conducted to elucidate the interaction between the two genera at the study site.

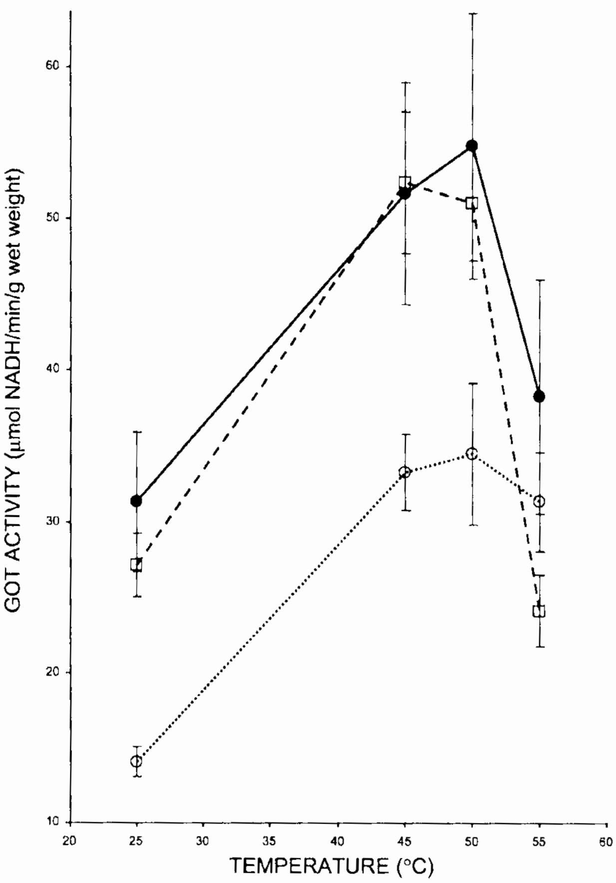

Fig. 4. Mean glutamate oxaloacetate transaminase (GOT) activities (μmol NADH/min/g wet weight) of *Littoraria* sp., *Echinolittorina malaccana* and *E. vidua* from Tanjung Chek Jawa at four temperatures: 25 °C, 45 °C, 50 °C and 55 °C. (Vertical bars represent standard errors). *Littoraria* sp. ●——●; *Echinolittorina malaccana*, O·······O; *E. vidua* □- - -□.

The 1h LT_{50}s of the three taxa of littorinids in this study increased with their relative position on the shore. *Littoraria* found lowest on the shore had the lowest 1h LT_{50} of 47.5°C while the inhabitant of the highest level on the shore, *E. malaccana*, had the highest 1h LT_{50} of 50.4°C. The 1h LT_{50} of *E. vidua* (48.1°C) reflected its position on the shore relative to *E. malaccana* and *Littoraria*, i.e., between the two genera. The increase in thermal tolerance with increase in position on the shore is consistent with findings of other researchers. Southward (1958) found that the high-shore top-shells *Monodonta lineata* and *Gibbula umbilicalis* tolerated higher temperatures that *G. cineraria* and *Callipstoma zizyphinum*, which inhabited low water. Fraenkel (1966) observed that *N. pyramidalis* at the upper part of the intertidal zone was most heat resistant, while *Chlorostoma argyrostoma lischkei* collected from the water's edge was least resistant. In addition, Wolcott (1973) reported that two littoral fringe limpets (*Acmaea digitalis* and *A. scabra*) maintained higher tissue temperatures that two other species (*A. pelta* and *A. testudinalis scutum*) present in the eulittoral zone.

The 1h LT_{50} value for *N. pyramidalis* in Japan was determined by Fraenkel (1966) to be 48.5°C, which was lower than the 1h LT_{50} of 50.4°C obtained in this study. His samples were heated in air instead of seawater, which may have resulted in some degree of desiccation (see Evans, 1948) thus affecting the thermal tolerance of the littorinid. Wolcott (1973) reported that removal of body water by desiccation reduced the thermal tolerance of the limpet *A. digitalis* by about 3°C.

In addition, the different 1h LT_{50} may be due to the geographical location in which the species inhabits. Thermal tolerance is reportedly negatively correlated to latitude (Fraenkel, 1968; McMahon, 2001). This may be a result of the higher level of solar radiation all year round in the tropics when compared with that of temperate areas (Moore, 1972). Southward (1958) reported that *Littorina neritoides* found in North Africa was more heat tolerant than *L. saxatilis* found all around Britain. Of the 60 gastropods collected from various localities (e.g., Australia, England, Hong Kong, Jamaica, Texas), McMahon (2001) found that *Nodilittorina ziczac* from Jamaica (18°N) had the highest mean heat coma temperature (HCT) of 46.9°C. *Littorina mariae* found in Robin Hoods Bay, England (54°N) had the lowest mean HCT of 26.4°C. Similarly, *N. pyramidalis* at Wakayama, Japan (34°N) had a slightly lower thermal tolerance than *E. malaccana* found Tanjung Chek Jawa, Singapore (approx. 1°N). Tropical *E. malaccana* possibly adapted to higher temperatures near the equator. This species is found on rocks that may be heated to temperatures of 45°C to 50°C (Lim & Fahmy, 2008). McMahon (2001) reported a HCT of 46.3°C for *E. malaccana* in Hong Kong (22°N) (c.f. 1h LT_{50} 50.4°C recorded in this study). This is further evidence for the resilience of this species under high temperatures.

Enzyme stability, such as that observed for GOT in *E. malaccana*, may account for the relatively high heat tolerance of this littorinid in the current study. Glutamate oxaloacetate transaminase is required for the regulation of carbon and nitrogen flow in various biochemical pathways (Cooper & Meister, 1985). An enzyme's function is highly dependent on its structure. The stability of this structure is temperature-sensitive (Somero, 1978), denaturing at high temperatures. Among *Littoraria* sp., *E. malaccana* and *E. vidua*, the percentage of GOT activity remaining after incubation at 55 °C was highest for *E. malaccana*. This indicated that GOT from *E. malaccana* was most stable, even at a temperature above its 1h LT_{50} (50.4°C). Somero (1978) reported that enzymes denature at temperatures that are usually higher than the upper lethal temperature of the species. After incubation at this temperature, the activity of GOT from *Littoraria* and *E. vidua* fell sharply, a likely result of denaturation of the enzymes. Hull et al. (1999) also found that enzymes of high shore animals (e.g., high shore *L. saxatilis* and *L. arcana*) were more stable, i.e., had higher activity rates at 56°C, than those of low shore groups (e.g., *L. obtusata*, *L. neglecta* and *L. littorea*). This trend of increased stability with increased height on the shore was also evident within a single species, *L. saxatilis*, found at high and mid-shore levels.

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