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# Life on a hot dry beach: behavioural, physiological, and ultrastructural adaptations of the littorinid gastropod *Cenchritis (Tectarius) muricatus*

**Abstract** The distribution and abundance of the littorinid gastropod *Cenchritis (Tectarius) muricatus* were determined on an exposed shore at Lee Stocking Island, Bahamas in July/August 1995 and June 1999. Activity patterns were observed on two occasions shortly before a hurricane passed near the island in 1995. *C. muricatus* extended from the low water mark to 3.6 m vertical height above it. Highest densities ( $20/\text{m}^2$ ) were found just above the high tide mark but the animal remained relatively abundant ( $8/\text{m}^2$ ) at the highest point. Small animals were only found at high shore levels. With the exception of animals very near to the water's edge *C. muricatus* was inactive by day and on dry nights. Many individuals of the high shore populations became active at night during periods of high humidity and rainfall, moving distances of up to 4 m overnight. At lower levels, extensive pairing and copulation was associated with these periods of rainfall. Abundance changed dramatically after the passage of the hurricane. At the level of maximum abundance numbers were reduced from  $20/\text{m}^2$  to  $2.8/\text{m}^2$ . Long-term experimental studies of water loss rate and haemolymph concentration showed that both water loss rate and increase in

haemolymph concentration are greatest in the early period of desiccation. The concentration of the blood after 20 weeks is 250% of the original and the water lost is of the order of 22.5%. After an initial increase there appears to be evidence of regulation of haemolymph concentration possibly by exchange with free water in the shell itself. The considerable reserve capacity in tolerance is no doubt in part due to the structure and functioning of the kidney. Adaptive features of the excretory system identified from ultrastructural examination include the replacement of podocyte-containing filtration chambers in the auricle by extracellular tubules permeating the walls of both auricle and ventricle, which suggests a reduction in the rate of primary urine formation. This is also indicated by a reduced surface area of the nephridial gland, which resorbs organic solutes from the urine. Excretory cells contain single large vacuoles in which arise large multi-layered concretions composed of phospholipids and calcium salts typical of prosobranchs in which excretory products may be stored for long periods.

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## Introduction

The littorinid snail *Cenchritis muricatus* is a familiar component of the littoral fauna in the Caribbean, Bahamas and southern Florida, often abundant from the waterline to well beyond the highest parts of the splash zone (Clench and Abbott 1942; Abbott 1954; Lewis 1960; Borkowski 1971, 1974; Bandel 1974; Bandel and Wedler 1987; Britton 1992; Lang et al. 1998). Many individuals near the upper limit of distribution are, in dry periods, attached by a mucous film to maritime vegetation, often as much as 0.5 m above the adjacent rock surface where they are fully exposed to the harsh conditions characteristic of this environment. Such individuals appear totally inactive in fine weather both by day and night and previous authors (e.g. Bandel 1974) have assumed that they

may remain inactive until conditions are suitable for activity.

A tropical storm that became Hurricane Erin passed close to Lee Stocking Island in July/August 1995 and caused heavy rainfall followed by extremely strong winds that generated unusually heavy wave action at the study site. We were able to establish base-line data about the population before the approach of severe weather; in addition we were also able both to observe the effects of this unusual weather on the behaviour of *C. muricatus* and to determine the effects of wave action on the population.

Although specimens of *C. muricatus* removed from the field are capable of surviving several months of desiccation (Rosewater 1963; Britton 1992) there are no blood osmolality data available for this species. Neither is there any information as to whether kidney structure reflects the fact that these animals suffer frequent periods of prolonged dehydration in their natural habitat. An opportunity to re-visit the island in June 1999 allowed us to re-survey the population. We also collected animals for an experiment with the objective of ascertaining how blood osmolality is affected by dehydration and for subsequent fixation with the aim of studying the adaptive features of the kidney.

## Materials and methods

### Ecological and behavioural observations

The main distribution and behavioural studies were carried out over the period 27 July–1 August 1995 on the northeast coast of Lee Stocking Island, Bahamas (23°46.5'N, 76°5.5'W). The study site was a small promontory that sloped upwards at an angle of 30° and had a total height of 3.6 m from the low water mark (LWM) to the ridge crest.

A vertical transect was surveyed 3 days before the passage of Hurricane Erin and also on the day following the passage of the hurricane (1 August 1995). A line of contiguous 1 m<sup>2</sup> quadrats was marked with a tape from the LWM to the crest of the ridge. The number of *C. muricatus* in each quadrat was recorded, as were the vegetation types, the nature of the rock surface, and whether the snails were on the rock or the vegetation. Crevices and the underside of loose stones were closely examined for small individuals. A line level was used to determine the contours of the slope. Relative humidity was measured with a hand-held digital thermohygrometer (AZ Instrument Co., 8711) and air temperature was measured 5 cm above the rock surface.

Small animals were conspicuously under-represented in this survey. It was felt this might be artefactual and, therefore, rigorous searches were carried out along horizontal transects at three selected heights adjacent to the original transect. The first 100 individuals encountered at each level were measured and the maximum shell length recorded.

At a separate but adjacent site on the promontory, 8 animals attached to a bush (*Rhachichallis americana*) were individually marked with small numbered labels and their exact positions recorded. The site was revisited during the evening of the same day, following a period of heavy rain, to note any changes in the positions of the marked animals. The following day, 11 inactive animals on rocks in the mid-intertidal were labelled in similar fashion, and their positions on the rock were marked with fingernail polish. The changes in position of these animals were noted the following morning, after a heavy rain storm.

When we returned to the shore after this night of heavy rainfall (29 July 1995) many animals were found to be copulating. We undertook a survey relating incidence of association/copulation to height above sea level at six different heights ranging from 0.2 to 2.8 m above low water. At each height, we laid out a 30-m tape horizontally, at right angles to the original transect line, then examined 0.5×1 m areas contiguously along the tape, for a distance of 15 m except in a few cases where the end of the transect traversed sand. We counted the number of animals/m<sup>2</sup> and noted the number of animals found singly, in pairs, or associated in larger numbers. Periodic (arbitrary) checks of pairs were carried out to confirm that paired animals were copulating. A similar survey was carried out to determine abundance and numbers copulating after the wave action generated by the tropical storm/hurricane had subsided.

The transect described above was re-surveyed in June 1999, size-frequency data obtained for the same levels as in the previous survey, and association levels also recorded.

### Physiological experiments

#### *Desiccation and weight loss*

Inactive animals were individually numbered, weighed to the nearest milligram with a microbalance (Mettler AE166), and then re-hydrated by immersion in fully aerated artificial seawater (Tropic Marine; 34‰, 1,000 mosmol kg<sup>-1</sup>) for 1 h. All became active during this period. Following immersion, animals were dried with tissue paper to remove adherent water, re-weighed, and then placed in a sealed plastic aquarium with three beakers holding saturated MgCl<sub>2</sub> solution covered with mesh. Relative humidity was maintained at 40%. Relative humidity was monitored with a hair hygrometer that was checked regularly against a calibrated electronic temperature/humidity probe (Vaisala HMP31) connected to a data logger/meter (Squirrel 1250 series). The aquarium was placed in a thermostatically controlled incubator at 35°C. At a series of time intervals between 0 h (post-immersion) and the termination of the experiment, 10 snails were removed, weighed, and their haemolymph concentration determined. In parallel with the main experiment a separate batch of 25 animals was used to monitor rate of water loss, by weighing at 7-day intervals throughout the experiment. At the end of the experiment the viability of all 25 snails was checked; 2 snails were dead and these were thus excluded from the subsequent data analysis.

All water loss data were ultimately expressed as a percentage of the initial post-immersion wet weight. These data were transformed (arcsine of the square root of the proportion; Underwood 1997) prior to analysis. All osmolality and water loss data were tested for normality and homogeneity of variances prior to analysis. Data were analysed using either parametric or non-parametric analysis of variance as appropriate (SPSS ver. 9.0).

#### Haemolymph sampling procedure

Individual snails were removed from the experimental chamber, weighed, and the shell opened using a small vice to apply the minimum force necessary to crack the shell sufficiently to expose the soft tissue of the mollusc. The mantle of the snail was carefully peeled back posteriorly to expose the head and pericardial region. A sample of haemolymph was taken directly from the heart using a siliconised fine glass capillary and the sample collected over ice in an Eppendorf tube. The osmolality of an 8-µl sub-sample of haemolymph was then measured with a vapour pressure osmometer (Wescor 5200, Wescor Inc., Utah, USA).

#### Ultrastructure

Experimental snails were dissected in 3% glutaraldehyde in Sorensen's phosphate buffer and 14% sucrose; pieces of kidney tissue were fixed in the same solution for at least 1 h. They were rinsed in

buffer and post-fixed in 1% aqueous osmium tetroxide. Some were decalcified in 5% EDTA before dehydration in ethanol, whilst the remainder were untreated. Both batches were embedded in Spurr resin. Thin sections were stained in aqueous uranyl acetate and lead citrate and examined in a Hitachi H600 transmission electron microscope. Material for scanning electron microscopy was critical point dried and sputter coated after fixation and dehydration as described above. It was examined in a Hitachi S3100 SEM.

## Results

### Habitat characteristics

The low intertidal was composed of deeply pitted limestone with cavities of all sizes that higher on the shore graded into a less pitted, cracked limestone pavement with many gaps and cavities and some loose material. The maritime vascular plants *Rhachichallis americana*, *Strumpfia maritima*, and *Borrichia arborescens* (Cornell and Cornell 1982) were found on the upper two-thirds of the transect. The highest daytime temperatures and lowest relative humidities (RHs) recorded were 33.6°C and 60% RH on an open unshaded rocky surface and 30.5°C and 71% in shade.

### Pre-hurricane distribution and abundance of *C. muricatus*

Before the severe weather *C. muricatus* were found over the whole length of the transect, those specimens found at the top of the ridge, 19 m from the sea, being more than 3.5 m vertically above the LWM (Fig. 1). *C. muricatus* achieved highest abundance approximately 1 m above the LWM (Fig. 1). Densities were considerably lower at higher levels of the shore but remained similar throughout the upper half of the transect. Where plants were present they were clearly the preferred habitat and at some levels, snails were only found on the vegetation (Fig. 2). Often the snails resided in the highest parts of the vegetation.

During the initial survey, at which time temperature near the surface ranged from 30.5°C to 33.6°C over the length of the transect and relative humidity only showed minor variation, from 69% to 72%, *C. muricatus* was inactive and no copulating pairs were evident.

The size-frequency distributions of the snail populations found by rigorous searching at three shore levels are shown in Fig. 3. The 0.64-m, 1.8-m, and 3.0-m levels were chosen as representative. The mean size (shell length) at the lower two levels was virtually identical (0.64 m: 18.85 mm; 1.8 m: 18.56 mm) and the range of sizes was very similar. However, the mean size was considerably lower at the highest site (15.84 mm), and the range of sizes was greater than at the lower stations. The results of the survey undertaken in June 1999 show a very similar pattern of distribution, abundance and size frequency with, once again, the only small animals found occurring high up on the shore.

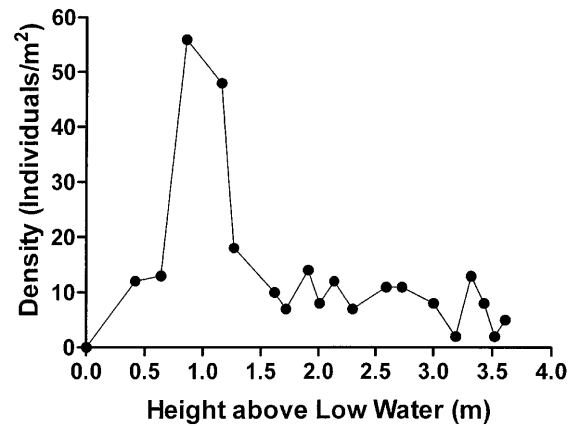


Fig. 1 Density of *Cenchritys muricatus* along a transect of horizontal length 20 m running from low water level to a height of 3.6 m above low water

### Activity patterns

Inspection at night during dry weather (27 July 1995) revealed no activity over most of the transect, although a relative humidity of 78% was recorded. Only animals actually being splashed by the sea were extended from the shell and in some cases actually moving.

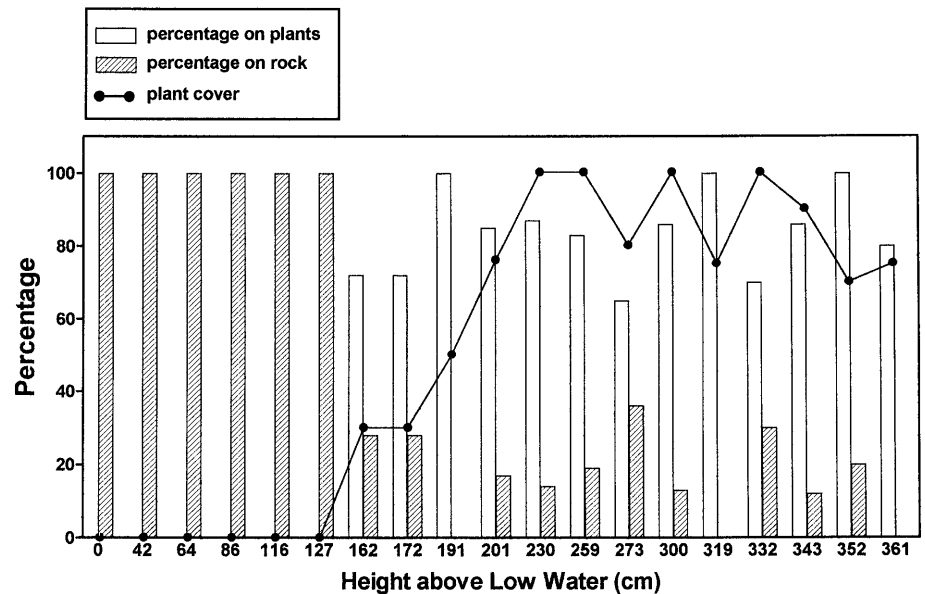
The study site was also visited during the evening of the day on which the animals were marked (28 July 1995), as it had rained during the intervening period. Three of the original eight marked animals remained on the bush and all had moved. The remaining five could not be located and are presumed to have moved from the bush. The minimum distances the three marked individuals on the bush could have moved were 13, 30, and 54 cm, respectively. Five unmarked animals had immigrated onto the bush and must have moved at least 1 m. Of the ten animals marked lower down the shore, only four were found. Two were up-shore of the nearest possible point of origin and two were down-shore. The distance from the nearest point of origin ranged from 45 cm to 3.5 m.

### Association in groups and sexual activity

The association survey demonstrated that after the night of heavy rain (29 July 1995) a large proportion of the lower shore individuals were associated in pairs or groups. Figure 4 shows that at low shore levels (0.2 m above LWM) a high proportion (84%) were associated together in groups of up to seven individuals. At 0.64 m above LWM pairs and groups represented 54% of the whole, 36% at 1.05 m above LWM, and only two instances of association were seen at 1.50 m above LWM. None were found associated above this level. All pairs inspected were, without exception, copulating.

We confirmed that this aggregative behaviour was not merely a local effect by brief examination of a population on the south side of the island on the same

**Fig. 2** Substratum preferences shown by *C. muricatus* along the main transect



day. Snails in this population were also aggregated and copulating. Our second visit to the island followed an episode of heavy rainfall at Lee Stocking and our data show *C. muricatus* aggregating at the same shore levels as in the previous survey.

#### Post-hurricane distribution and abundance

As Hurricane Erin passed near Lee Stocking, the low shore sustained structural damage. There was evidence that pieces of limestone had been broken off from low water level. Considerable quantities of sand had been thrown up onto the area of the beach used for the survey of association and copulation intensity.

The density of *C. muricatus* on the lower part of the shore was significantly lower after the storm (Fig. 5). The number of *C. muricatus* present at the 0.64-m level was reduced by 89% and at 1.05 m above the LWM by 45%. Above this level the population appeared to be unaffected. None of the few *C. muricatus* found in pairs were copulating (Fig. 4). A few individuals were, however, found both on the damp, newly deposited sand and on exposed rocky surfaces on the low shore. Several of the latter individuals appeared to be moving upwards away from the sea during daylight.

#### Physiological measurements

##### *Preliminary results*

Preliminary measurements of haemolymph concentration of snails collected from the Bahamas and kept in dry conditions for 3 months in the laboratory in London gave values of  $1,997.6 \pm 165.1$  mosmol  $\text{kg}^{-1}$  to  $2,217.8 \pm 313.2$  mosmol  $\text{kg}^{-1}$ . All the measured values were therefore approximately double the strength of sea

water (approximately  $1,000$  mosmol  $\text{kg}^{-1}$ ), suggesting well-developed tissue tolerance to elevated solute concentrations in the haemolymph.

##### *Main experiment*

The animals for this experiment were collected from three different shore levels at Lee Stocking, kept separately before the experiment, and kept separate in the experiment. There was, however, no difference between the haemolymph osmolality for snails originally collected from different levels on the shore at any sampling interval (Kruskal–Wallis:  $H = 2.5\text{--}5.2$ ,  $df = 2$ ,  $P > 0.05$  in every case). Thus data for snails from all three levels were pooled for subsequent data analysis. Both the concentration of snail haemolymph and the percentage weight loss are plotted against time in Fig. 6. Mean haemolymph concentrations increased significantly from  $993 \pm 13$  mosmol  $\text{kg}^{-1}$  immediately post-immersion to  $2,573 \pm 342$  mosmol  $\text{kg}^{-1}$  after 140 days (20 weeks) of desiccation stress (Kruskal–Wallis:  $H = 82.9$ ,  $df = 8$ ,  $P < 0.001$ ). Similarly weight loss showed a significant increase with time from 0% to  $21.3 \pm 1.9\%$  of post-immersion wet weight (Kruskal–Wallis:  $H = 83.8$ ,  $df = 8$ ,  $P < 0.001$ ). Water loss data for the population of *C. muricatus* monitored separately at seven-daily intervals shows a similar pattern with weight loss increasing from 0% to  $22.8 \pm 2.9\%$  over the same period. What is clear is that the period of rapid water loss is in the first 7 days of desiccation after which the rate begins to level off, water being lost at a more or less constant rate for the last 84 days (12 weeks) of the experiment (Fig. 6). Interestingly after an initial increase in haemolymph osmolality over the first week of the experiment the concentration stabilises up until 56 days (see Fig. 6). The samples taken after 56 days (8 weeks) indicate a significant increase in the haemolymph osmolality. Taken together these data

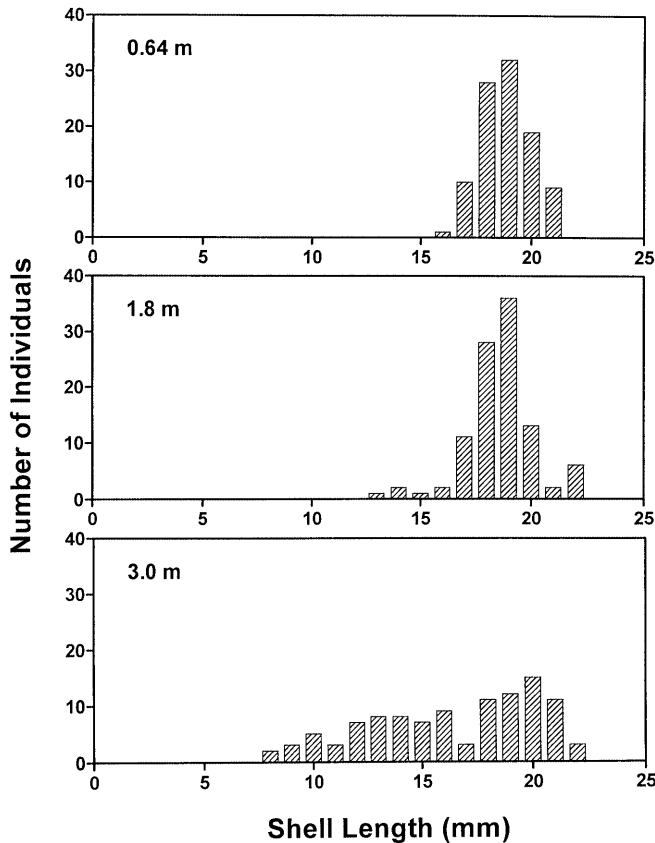


Fig. 3 Size-frequency histograms of the population of *C. muricatus* at selected height levels along the main transect

may indicate that for a period of approximately 2 months the snails are capable of regulating haemolymph concentration, possibly by exchange with free water within the shell. After this time, possibly because all free water within the shell has been lost by evaporation, haemolymph concentration increases and water is lost from the tissues at a more or less constant rate.

#### General organisation of excretory system

The excretory system conforms to the same pattern as in other prosobranchs in that the heart appears to be the site of primary urine formation, and the pericardial cavity is linked to the kidney by a reno-pericardial canal (Fretter and Graham 1994). The large kidney extends over the underlying viscera, and the vascularised excretory folds on the dorsal wall are hypertrophied (Fig. 7A), extending onto the anterior wall to the left of the kidney opening and forming a sponge-like mass almost filling the lumen. Large numbers of white excretory spherules usually fill the lumen (Figs. 7D, E; 8A, B, E). The nephridial gland, the main site of organic solute resorption, occupies its typical position along the left (pericardial) side of the dorsal wall, and it also spreads onto the anterior wall, but it is not thrown into tubular evaginations as it is in *Littorina* (Andrews and Taylor 1988).

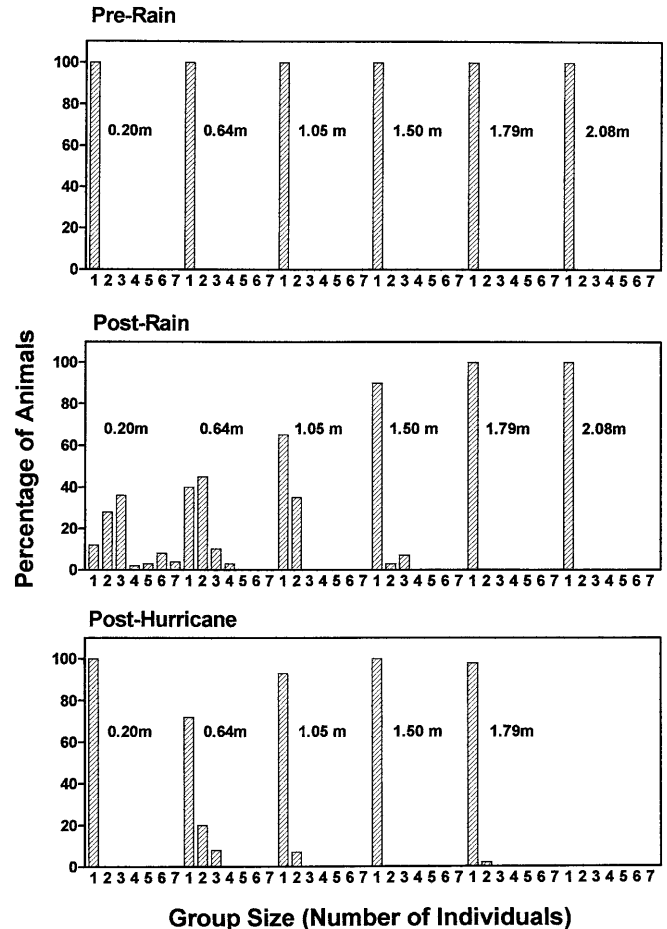
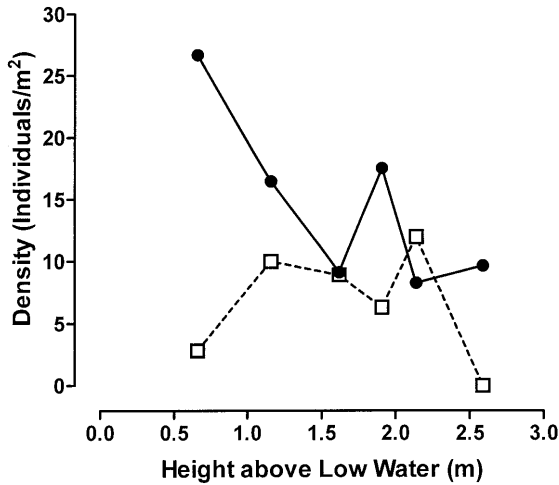


Fig. 4 Incidence of pairing and larger groupings of *C. muricatus* before and after rain and before and after Hurricane Erin

#### Ultrastructure

Examination of the heart wall showed that the filtration chambers lined with podocytes that typify the auricle of *Littorina littorea* and other aquatic and intertidal prosobranchs (Andrews 1988) are absent. The epicardium of both auricle and ventricle is, however, composed of cells with thin margins permeated by extracellular tubules that extend from the basal lamina to the pericardial cavity (Fig. 7B, C). These tubules are believed to carry a filtrate of blood into the pericardial cavity and thence to the kidney. The epithelium over the dorsal folds of the kidney is composed of the two cell types typical of this area in prosobranchs. These are the excretory and re-sorptive cells, the latter being essentially the same as the epithelium of the nephridial gland. These resorptive cells are ciliated and were active in all snails examined. They bear coated vesicles on the apical membranes and have a conspicuous endocytotic canal system. The cytoplasm is filled with lysosomes at different stages and residual bodies. Resorptive cells have reserves of glycogen even in snails emerged for 140 days (20 weeks). The excretory cells are far more numerous (Figs. 7D, E; 8A–E) and differ from those of aquatic and mid-littoral species by

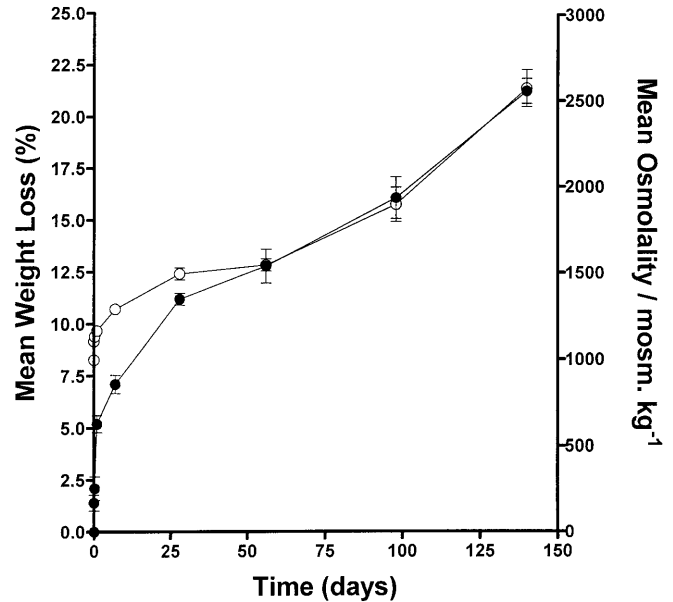


**Fig. 5** Population densities of *C. muricatus* at six shore levels before (solid circles) and after (open squares) Hurricane Erin. (Figures obtained with the association data)

having a single large excretory vacuole containing one layered concretion (spherule) rather than the many smaller vacuoles with diffuse granular contents typical of mid-littoral temperate water species such as *L. littorea* (Figs. 7D, E; 8A, B, E). The cytoplasm is unusual in storing large quantities of glycogen and containing numerous deposits of phospholipid. In the hydrated state the smooth endoplasmic reticulum of the excretory cells forms conspicuous whorls in the cytoplasm, but in snails dehydrated for 20 weeks the whorls are few or absent. The apical cell membrane bears long microvilli in young cells (Fig. 8A, D) but they become progressively shorter and sparser as the cells age and their vacuoles enlarge. In old cells laden with a large concretion their domed apices are smooth and convex (Fig. 8B, E). Basally, fine processes push the thin basal lamina into the underlying blood spaces, and occasionally vesicles can be seen on the cell membrane. The excretory vacuoles are almost entirely filled in some cells and empty in others in the same specimen. Excretory or storage material is released into the vacuole by rupture of the membrane (Fig. 8A, C) from sites all around its margins. Most of it is phospholipid that forms an electron-dense coating over a previously laid layer of finely granular calcium salts material. Successive layers of these two types of material are added until the concretion fills the vacuole and the cytoplasm forms only a thin margin around it. The apical membrane then ruptures, shedding all the cell content. Both resorptive and excretory cells have reserves of glycogen even after 17 weeks (insert in Fig. 8C).

## Discussion

It is well known that *Cenchritis muricatus* has considerable ability to tolerate desiccation (Clench and Abbott 1942; Rosewater 1963; Borkowski 1971, 1974; Bandel

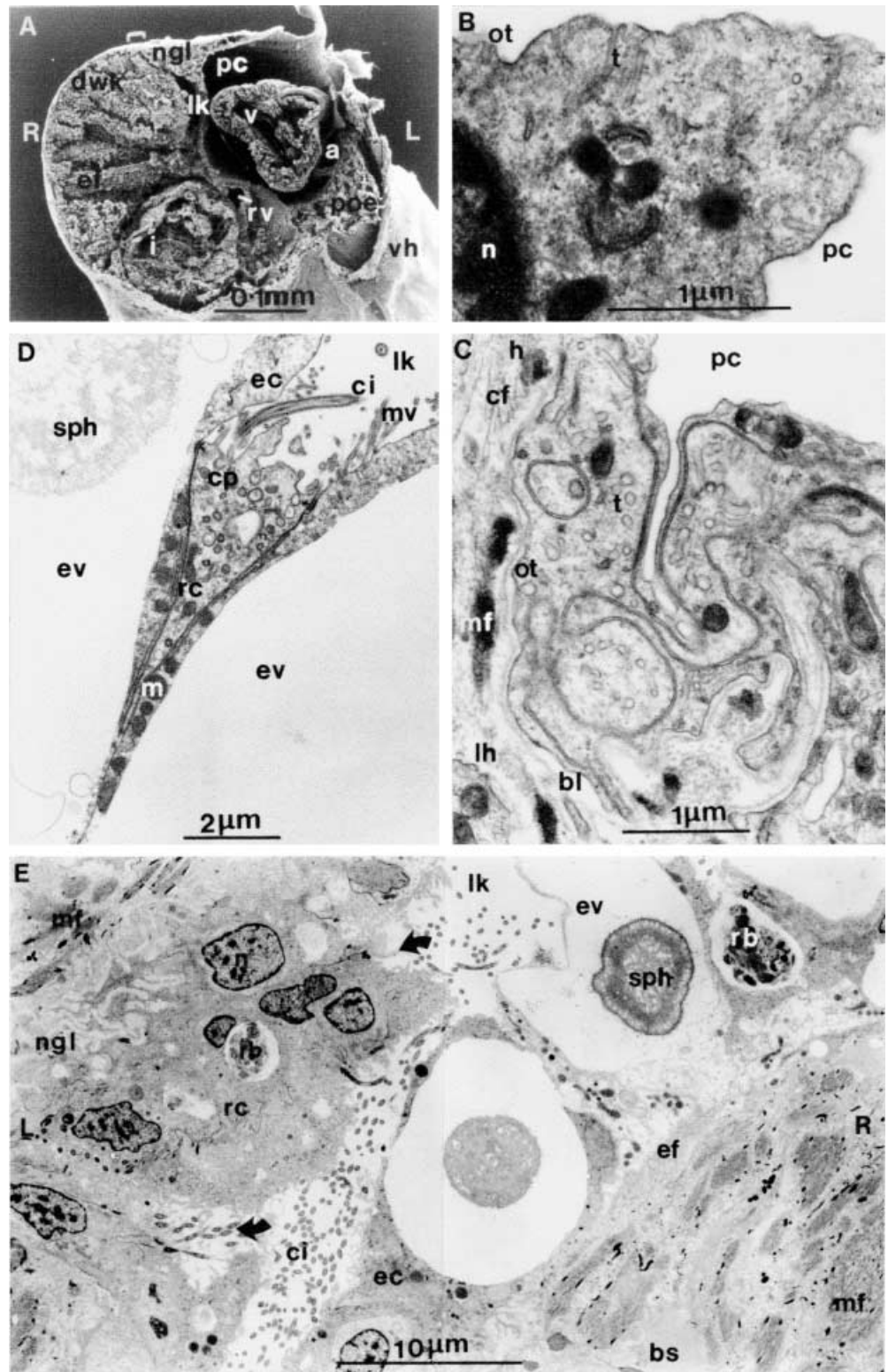


**Fig. 6** Weight loss (solid circles) and increase in haemolymph osmolality (open circles) for *C. muricatus* maintained at 35°C and 40% relative humidity for up to 20 weeks (140 days)

1974; Bandel and Wedler 1987; Britton 1992; Lang et al. 1998). Rosewater (1963) demonstrated that specimens kept in dry conditions for up to 18 months become active when placed in seawater. Our results confirm that the species can live considerable distances from the sea where high temperatures and dry conditions are the norm. *C. muricatus* climbs up on vegetation where this is available. This behaviour, although potentially exposing the animal to greater desiccation stress, may allow the animal to avoid high temperatures at the rock surface. The distribution patterns observed are similar to those of Lang et al. (1998) although our population extends higher into the maritime supralittoral area. Abundance at comparable heights above sea level appears similar.

It is noteworthy that abundance declines very little over the upper half of our transect. Surprisingly, the size-frequency data show small animals to be few in number and confined to the upper part of the transect. A similar pattern has been reported by Borkowski (1974) and by Lang et al. (1998). This pattern may be the result of variable recruitment success of *C. muricatus* from year to year. *C. muricatus* must recruit onto the tidal part of the shore, as it reproduces by producing pelagic egg capsules (Borkowski 1971) from which swimming veliger larvae emerge (Lewis 1960). The absence of small individuals in the lower part of the shore may indicate that recruitment is unpredictable and has not occurred recently. Small individuals high on the shore may be relatively old animals that have had little opportunity for feeding, rather than recent recruits. This suggestion would accord with the findings of Clench and Abbott (1942), who found larger individuals in conditions where spray was more common and attributed this larger size to greater feeding opportunity. It is also possible, as

**Fig. 7A–E** The excretory system of *C. muricatus*. **A** Scanning electron micrograph (SEM) of a section through the base of the visceral hump of *C. muricatus* showing the heart and kidney. Short white bar (top) indicates position of section **E**. **B** Transmission micrograph (TEM) of part of an epicardial cell showing openings of tubules into the pericardial cavity. **C** TEM of peripheral regions of epicardial cells showing tubules originating basally and ramifying through the cytoplasm. **D** TEM of the apical part of a ciliated resorptive cell and adjacent excretory cells. **E** TEM of a horizontal section through the left side of the dorsal wall of the kidney (indicated by a white bar in **A**) showing part of the nephridial gland on the left and part of an excretory fold on the right. Arrows indicate vestigial tubular outpushings of nephridial gland. Abbreviations: *a* aorta; *bl* basal lamina; *bs* blood space; *cf* collagen fibres; *ci* cilia; *cp* coated pit; *dwk* dorsal wall of kidney; *ec* excretory cell; *ef* excretory fold; *ev* excretory vacuole; *h* haemocyanin; *lh* lumen of heart; *lk* lumen of kidney; *m* mitochondrion; *mf* muscle fibre; *mv* microvilli; *n* nucleus; *ngl* nephridial gland; *ot* opening of tubule; *pc* pericardial gland; *poe* posterior oesophagus; *R* right; *rb* residual body; *rc* ciliated resorptive cell; *rv* afferent renal vein; *sph* excretory spherule; *t* tubule; *v* ventricle; *vh* visceral hump



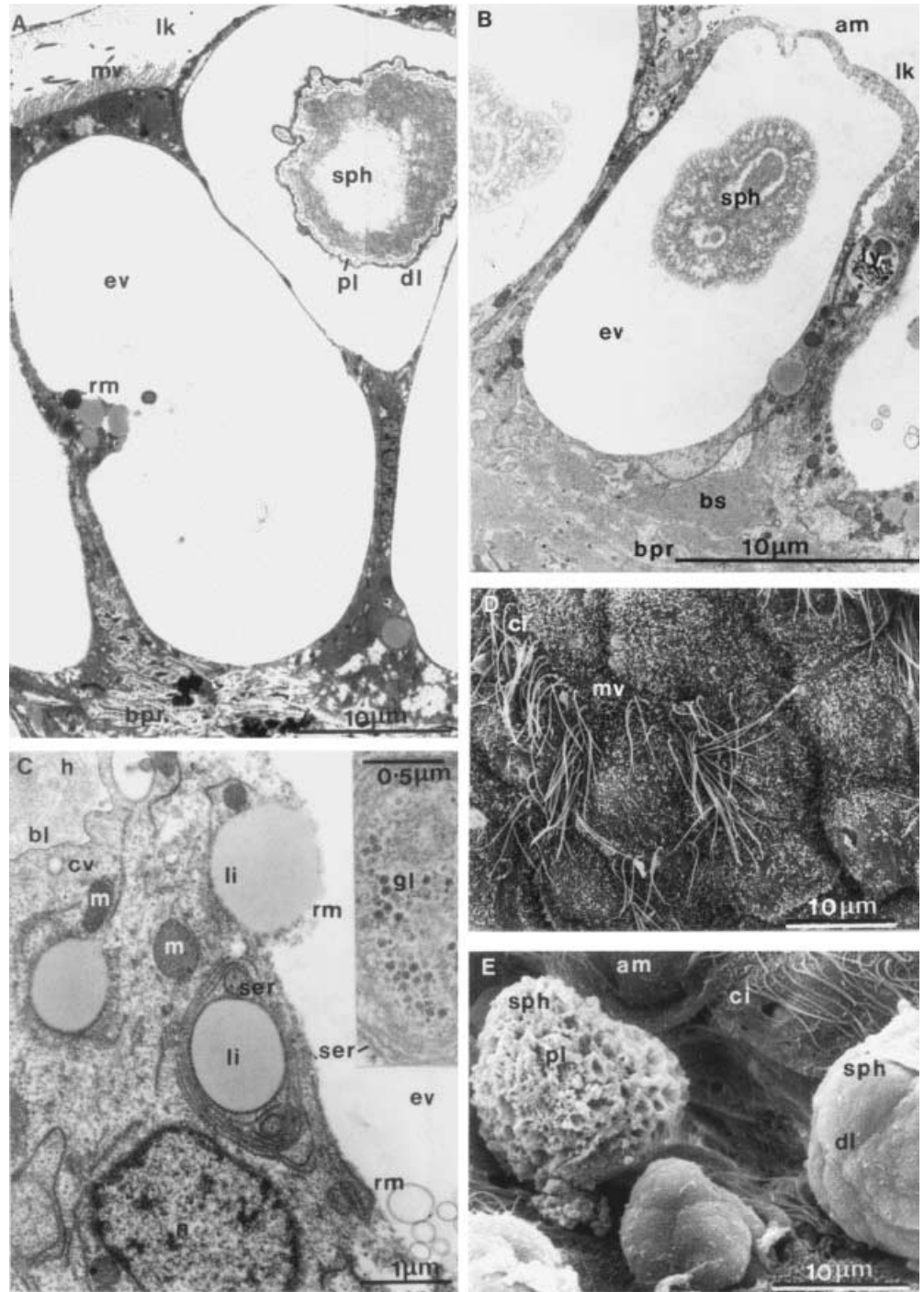
Lang et al. (1998) suggest, that the absence of small individuals from these shores in June and August and from Jamaican sites in March and July is because recruitment is in late summer/autumn. The low growth rates measured by Borkowski (1974) do not seem to support this suggestion but his observations were on

large animals. Only direct observation at the appropriate season will clarify the matter.

Our results unequivocally demonstrate that rainfall induces activity in high and mid-shore individuals. Animals migrated both onto and off the experimental bush and up and down shore from their original lower



**Fig. 8A–E** Detail of excretory cells of *C. muricatus*. **A** TEM of young excretory cell with well-developed apical microvilli and almost empty vacuole, with part of an adjacent older cell and large spherule. **B** TEM of mature excretory cell, apical membrane devoid of microvilli, with underlying blood space invaded by basal processes. **C** TEM detail of cytoplasm and vacuolar membrane of excretory cell. *Inset*: high magnification showing glycogen rosettes. **D** SEM of young excretory cells interspersed with cilia of resorptive cells. **E** SEM detail of excretory spherules showing smooth (dense) and reticular (pale) layers respectively, shed from underlying older excretory cells with smooth apical membranes. Abbreviations: *am* smooth apical membrane of older excretory cell; *bl* basal lamina; *bpr* basal processes of excretory cells; *bs* blood space; *ci* cilia; *cv* coated vesicle; *dl* dense layer of excretory spherule; *ev* excretory vacuole; *gl* glycogen; *h* haemocyanin; *li* lipid; *lk* lumen of kidney; *ly* lysosome; *m* mitochondrion; *mv* microvilli; *n* nucleus; *pl* diffuse 'pale' layer of excretory spherule; *rm* ruptured vacuolar membrane; *ser* smooth endoplasmic reticulum; *sph* excretory spherule



shore locations. It is plain that individuals can move significant distances during favourable conditions. Whether conditions allow downwards migration as far as the sea to breed every year is not known, but the presence of large individuals at the highest level during the peak of the known breeding season (Borkowski 1971) suggests that some individuals may not breed every year.

One interpretation of the high level of association and copulation following rainfall is that rainfall has stimulated the latter activity. Copulatory activity may only be

possible in moist conditions. Perhaps rain is a proximate cue that synchronises copulatory activity during the breeding season in this species. Since spawning and migration down-shore to spawn have been shown to be lunar related in other molluscs (Berry 1986), it is possible that the rainfall coincided with the phase of the moon at which *C. muricatus* migrates down-shore and is reproductively active and the real stimulus was lunar in nature.

The violent conditions caused by the passage of Hurricane Erin near the island had a dramatic negative

effect on the low shore populations of *C. muricatus*, removing a high proportion of the population at the level of maximum abundance. However, the rains preceding the storm apparently enhanced reproductive opportunity by producing conditions suitable for copulation. This is an unexpected positive effect of the hurricane.

The adaptations that allow littorinids to live successfully in the eu-littoral fringe have been investigated by a number of authors, most recently by McMahon (1990), Britton (1992), and Lang et al. (1998). These include considerable temperature tolerance, low water loss during emersion, the ability to aestivate, and the ability to minimise water loss through utilising uric acid as a waste product. Britton (1992), in a comparative study of the adaptation of Jamaican littoral fringe littorinids, found that *C. muricatus* lost 27.3% of its weight in 28 days when kept at ambient temperature (19.5–23.6°C) and humidity in the laboratory. Most water was lost in the first 8 days of dehydration. Our results resemble those of Britton (1992) in terms of the pattern of water loss in that most of the water loss is in the first 7 days of desiccation after which the rate begins to level off, water being lost at a more or less constant rate over the last 12 weeks (84 days) of the experimental period of dehydration. The apparent discrepancy in the actual values of percentage water loss compared with our data is due to difference in expression of the water loss data: Britton's study expressed water loss in terms of percentage of total body water whereas the present data is expressed in terms of percentage of post-immersion wet weight. Hence the percentages reported here are less than those reported by Britton (1992) despite apparently more stressful conditions.

Interestingly after an initial increase in haemolymph osmolality over the first 7 days of the experiment the concentration stabilises up until 8 weeks (see Fig. 6). The samples taken after 56 days show a significant increase in the haemolymph osmolality. This may indicate that for a period of approximately 2 months the snails are capable of regulating haemolymph concentration, possibly by exchange with free water within the shell (cf. *Acmaea limatula* Segal and Dehnel 1962). This capacity to tolerate elevated haemolymph osmolality (including a degree of regulation) is far in excess of the likely requirements in the field situation, a phenomenon often described for intertidal molluscs (e.g. Wolcott 1973 for acmaeid limpets). After 56 days, possibly because all free water within the shell has been lost by evaporation, haemolymph concentration increases and water is lost from the tissues at a more or less constant rate. The wide variance in values evidenced by the error bars in Fig. 6 may indicate the variation between individuals in the storage of, and subsequent exchange with, this free water.

An ability to tolerate an increase in the osmolality of both haemolymph and extracorporeal water associated with desiccation (or immersion in hypersaline water) has been noted in many species of prosobranch and pulmonate limpets (see Williams and Morrill 1995 for review). For example, Wolcott (1973) found that limpets

of the genus *Acmaea* had a phenomenal tolerance of increased haemolymph osmolality; the eu-littoral fringe species *A. scabra* and *A. digitalis* tolerated desiccation levels of approximately 70% water loss resulting in the concentration of chloride in the extracorporeal water being approximately 300% seawater. During aestivation in the amphibious, freshwater prosobranchs *Pomacea lineata* and *P. depressa* (lasting up to 400 days and initiated by the loss of 20% of the body water), the haemolymph osmotic pressure doubled, attaining levels of  $120 \text{ mM l}^{-1} \text{ Na}$  (equivalent to approximately  $200 \text{ mosmol kg}^{-1}$ ) in some individuals (Little 1968). There was, however, evidence that *Pomacea* spp. were capable of 'osmoregulation' in that the rise in haemolymph osmotic pressure was less than theoretical values calculated from water loss data. Mediterranean populations of the salt-marsh pulmonate *Ovatella myosotis* are remarkable in that individuals can tolerate external seawater concentrations of up to 90‰ with the blood being iso-osmotic with the external medium in this range (Seeleman 1968). Similarly the saltmarsh assimineid snail *Assiminea grayana* can tolerate external concentrations up to 200% seawater, maintaining the osmotic pressure of the haemolymph slightly above ( $32\text{--}67 \text{ mM l}^{-1} \text{ NaCl}$ ) that of the external medium over the range 50–200% ( $275\text{--}1,100 \text{ mM l}^{-1} \text{ NaCl}$ ) seawater (Little and Andrews 1977). The tolerance of elevated haemolymph concentration described here for *C. muricatus* is similar to these values with some individuals, which had survived 20 weeks desiccation at 35°C and 40% RH, having haemolymph concentrations in excess of  $2,500 \text{ mosmol kg}^{-1}$  (approximately equivalent to 250% seawater or  $1,650 \text{ mM l}^{-1} \text{ NaCl}$ ). To our knowledge there are no comparable measurements of blood osmotic pressures for other high-shore littorinids exposed to desiccation stress but it is known that *Littorina littorea* typically exhibits passive tolerance to large changes in cell volume. The haemolymph is iso-osmotic with the medium over a limited range, with NaCl moving between the environment and extracellular fluid, whilst water tends to move between the extracellular and intracellular compartments to dilute or concentrate intracellular osmolytes such as potassium and free amino acids (Taylor and Andrews 1988).

The structure of the excretory system differs from the general aquatic prosobranch pattern, having major modifications for coping with long periods of desiccation. Thus auricular filtration chambers and podocytes are missing and reduced filtration takes place through tubules permeating the epicardial cells. This resembles the pattern found in the terrestrial littorinacean *Pomatias elegans* and the littoral fringe littorinid *Melaraphe neritoides* (Andrews 1988). This is believed to reflect an emphasis on uricotelic rather than an ammonotelic excretion and a reduction of water loss in primary urine formation. The nephridial gland has a reduced surface area that is consistent with production of a smaller volume of primary urine from which the gland resorbs organic solutes (Andrews and Taylor 1988). The excre-

tory cells themselves, unlike in such species as *L. saxatilis*, characteristically contain a single large vacuole rather than many small ones. The vacuole accretes multi-layered concretions that contain excretory material that is discharged by rupture of the cell membrane when conditions are appropriate. Similar concretions are known from the amphibious pilids and the terrestrial assimineid *Pseudocyclattus* (Little and Andrews 1977) in which they have been demonstrated to contain water. In every respect it seems *C. muricatus* can be held as an example of an animal superbly adapted to an exacting habitat.

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## References

- Abbott RT (1954) Review of the Atlantic periwinkles, *Nodilittorina*, *Echininus*, and *Tectarius*. Proc U S Natl Mus 103(3328):449–464
- Andrews EB (1988) Excretory systems of molluscs. In: Trueman ER, Clark M (eds) The Mollusca, vol 11. Form and function. Academic Press, Orlando, Fla., pp 381–448
- Andrews EB, Taylor PM (1988) Fine structure, mechanism of heart function and haemodynamics in the prosobranch gastropod molluscs *Littorina littorea* (L.). J Comp Physiol [B] 158:247–262
- Bandel K (1974) Studies on the Littorinidae from the Atlantic. Veliger 17:92–114
- Bandel K, Wedler E (1987) Hydroid, amphineuran and gastropod zonation in the littoral of the Caribbean Sea, Colombia. Sencenb Mar 181/182:1–129
- Berry AJ (1986) Semilunar and lunar spawning periodicity in some tropical littorinid gastropods. J Molluscan Stud 52:144–149
- Borkowski TV (1971) Reproduction and reproductive periodicities of south Floridian Littorinidae (Gastropoda: Littorinidae). Bull Mar Sci 21:826–840
- Borkowski TV (1974) Growth, mortality and productivity of south Floridian Littorinidae (Gastropoda; Prosobranchia). Bull Mar Sci 24:409–438
- Britton JC (1992) Evaporative water loss, behaviour during emersion, and upper thermal tolerance limits in seven species of eulittoral-fringe Littorinidae (Mollusca; Gastropoda) from Jamaica. Proc Third Int Symp Littorinid Biol 3:69–82
- Clench WJ, Abbott R (1942) The genera *Tectarius* and *Echininus* in the western Atlantic. Johnsonia 4:1–100
- Cornell DS, Cornell HB (1982) Flora of the Bahamas Archipelago. Cramer, Vaduz, Liechtenstein
- Fretter V, Graham A (1994) British prosobranch molluscs. Ray Society, London
- Lang RC, Britton JC, Metz T (1998) What to do when there is nothing to do: the ecology of Jamaican intertidal Littorinidae (Gastropoda; Prosobranchia) in repose. Hydrobiologia 378:161–185
- Lewis JB (1960) The fauna of the rocky shores of Barbados. Can J Zool 38:391–435
- Little C (1968) Aestivation and ionic regulation in two species of *Pomacea* (Gastropoda, Prosobranchia). J Exp Biol 48:569–585
- Little C, Andrews EB (1977) Some aspects of excretion and osmoregulation in assimineid snails. J Moll Stud 43:263–285
- McMahon R (1990) Thermal tolerance, evaporative water loss, air-water oxygen consumption and zonation of intertidal prosobranchs: a new synthesis. Hydrobiologia 193:241–260
- Rosewater J (1963) Resistance to desiccation in dormancy by *Tectarius muricatus*. Nautilus 76:111
- Seeleman U (1968) Zur Überwindung der biologischen Grenze Meer – Land durch Molluskan I. Untersuchungen an *Alderia modesta* (Opisth.) und *Ovatella myosotis* (Pulmonata). Oecologia 1:130–15
- Segal E, Dehnel PA (1962) Osmotic behaviour in an intertidal limpet, *Acmaea limatula*. Biol Bull 122:417–430
- Taylor PM, Andrews EB (1988) Osmoregulation in the intertidal gastropod *Littorina littorea*. J Exp Mar Biol Ecol 122:35–46
- Underwood AJ (1997) Experiments in ecology: their logical design and interpretation using analysis of variance. Cambridge University Press, Cambridge
- Williams GA, Morrill D (1995) Habitat partitioning and thermal tolerance in a tropical limpet, *Cellana grata*. Mar Ecol Prog Ser 124:89–103
- Wolcott TG (1973) Physiological ecology and intertidal zonation in limpets (*Acmaea*): a critical look at ‘limiting’ factors. Biol Bull 145:389–432