Crabs, waves, and snails: testing for adaptations in the dangerous benthos

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Introduction

Gastropods of the genus *Lavigeria* form a species flock in Lake Tanganyika where many of the approximately 45 species live sympatrically or microallopatrically (West et al., 2003). *Lavigeria grandis* and *L. nassa* are conspicuous grazers in the rocky benthic zone, but have somewhat offset depth distributions at Jakobsen's Beach; *L. grandis* is the most common gastropod from 1-2m whereas *L. nassa* densities peak from 5-10m (Sekandende, 1999). These closely related species may have adaptively radiated in this area to occupy different niches. One major factor in this radiation may be the primary predator of these snails- the crab *Platythelphusa armata*. *Platythelphus armata* lives at depths below 5m and devours its snail prey by crushing or peeling back its shell (Marijnissen, personal communication). Therefore, it may be beneficial for snails living sympatrically with this crab to expend energy in producing a thick shell, though it may not be worth the expense where the predator is not a significant threat. *Lavigeria grandis* have thinner shells than do *L. nassa* (West et al., 2003), and we suspect that these two species have diverged to adapt to the shallow predator-free and deep predator-present areas respectively. But the predator-free shallows have a trade-off; shallower water is subject to increased wave action and is often a limiting factor in determining gastropod distributions (Prowse and Pile, 2005). To test this prediction we carried out five studies that address feeding preference of the predator, morphology of the snail, and the environments in which they live.

We tested crab preference for *L. grandis* or *L. nassa* through a transplant experiment where both snail species were placed in predator habitat, and also with a lab choice experiment where each crab was given a choice of *L. grandis* and *L. nassa* of roughly equal size. We also analyzed the proportion of shell and soft tissue material of the two species to see if one is a more rewarding food item for the crabs. To quantify wave energy we placed clod cards at depths varying through the snails' ranges, and to test the ability of the snail to live in a high wave environment we measured the foot area of individuals from both species. Together these studies give support to the hypothesis that these two snail species have adapted to differing predation pressure and varying environments.

Methods

Collection and Snail Measurements

All snails were collected from the south coast of Jakobsen's (Mwamahunga) Beach (4° 54.64' S, 29° 35.92' E), near Kigoma, Tanzania in July of 2007 using snorkel or SCUBA. Jakobsen's Bay is surrounded by undeveloped shoreline and is relatively pristine. Measurements, taken using digital calipers, included height along the coiling axis, width perpendicular to height, and lip thickness. Lip thickness was measured by inserting the calipers 2mm into the aperture. We calculated a generalized size metric of $\sqrt{}H*W$.

Transplant

We ran six sets of paired trials of control and experimental treatments. Treatments were 40x10cm circular cages constructed with 5mm wire mesh (Fig. 1). We weighted cages down by sewing fabric tubes filled with sand to the bottom perimeter. Experimental treatments had four doors 17cm wide and 8cm high to allow crab access, whereas control cages had no doors in order to exclude crabs and test for caging effects. We tethered ten snails (five *L. grandis* and five *L. nassa*) to each cage on 12cm long tethers. We glued snails to nylon thread with SuperGlue™ and tied them to numbered paperclips that were attached evenly around the inside of the cages. All snails measured 15-20mm in height. We selected this size class because it showed a moderate level of predation in both *L. grandis* and *L. nassa* that allowed us to look at differences in predation pressure between species (Rosales 2002). We placed the cages at 10m depth at Jakobsen's Beach using SCUBA equipment, and after seven days we counted each snail as attached or not attached to the cage. We analyzed differences between species and control vs. experimental treatment using a Chi-squared analysis.

Choice

We ran three choice trials between July 21st and July 31st, 2007. Each trial consisted of twelve containers, twelve *L. grandis*, twelve *L. nassa*, and twelve *P. armata*. Crabs were collected from Jakobsen's Beach and starved for 24-48 hours prior to use in a trial. To begin the trial, crabs were placed individually into aerated clear plastic 2.4 L containers containing fresh lake water. Paired snails within 0.5 mm —one *L. grandis* and one *L. nassa*—were placed into each container. All snails were 15-20 mm in height (as discussed above). Visual barriers were erected around each chamber for the entire length of the trial. Each trial lasted for 18 hours—13 dark and five light (Socci, 2001, Rosales, 2002). At the end of the trial, we removed snails and/or shell fragments from each container and recorded the condition of each snail as one of three categories: undamaged (no damage to any part of shell), attempted predation (parts of shell were missing due to crab interaction, but snail was still alive), or predation (snail was consumed by crab). Larger shell fragments were vouchered in plastic bags.

Crabs were subject to five measurements based on those of Cumberlidge et al (1999), Socci (2001), and Rosales (2002). All crab measurements were taken after trails as not to cause additional stress to the crab. We measured maximum carapace length and width, major chela diagonal (maximum diagonal distance between the lower propodus-carpus articulation and the dactyl pivot point articulation), major chela length (maximum distance between the lower propodus-carpus articulation and the tip of the pollex), and the major chela height (maximum distance of the propodus measured perpendicular to the chela length) (Fig. 2). We also scored the dentition of the major chela into three classes based on the amount of wear: 1) lacking pronounced molariform teeth; 2) partially worn molariform teeth; and 3) fully differentiated, not worn down molariform teeth (Rosales, 2002). Categorical data were recorded for the sex of the crab, side of major chela, molt, and (for females) brooding or non-brooding. After completion of a trial, crabs were marked on the posterior part of the carapace with red fingernail polish to avoid the use of any individual in multiple trials and were then released at the site of capture. We analyzed the results with a three-outcome Chi-squared test with the outcomes being *L. grandis* and *L. nassa* of each trial being in the same predation category, *L. grandis* being in a more affected predation category, and *L. nassa* being in a more affected predation category.

Mass

We measured the height, width, and lip thickness of 50 *L. grandis* and 50 *L. nassa*, then scrubbed them with a toothbrush to completely remove algae. We crushed the snails using a table vice and separated shell from soft-tissue material. The samples were dried to a constant mass at 60°C. We weighed the samples and analyzed differences in soft-tissue mass to shell mass between species using standard linear model Tukey Tests.

Clod Cards

We quantified wave energy using Doty's (1971) clod card technique. We mixed 400 grams of Plaster of Paris with 270 mL of water to produce a watery paste. The paste was poured into a flexible plastic ice cube tray and allowed to set for approximately thirty minutes. The plaster cubes were cured for a minimum of four days in the lab, the corners rounded so that all cubes were within two grams of each other, and weighed individually. Each clod card was attached to a clean petri dish with clear silicone adhesive, cured for 24 hours and reweighed.

Clod cards were placed in the same location as our transplant experiment at depths of 1, 2, 5, and 10 m. We attached each petri dish to a rock using rubber bands and left them in the field for 24 hours. We ran control cards by placing them in aquaria in the lab containing lake water for 24 hours. Cards were dried to constant mass. We used a logarithmic regression to test for an association between depth and mass loss.

Foot Area

We collected 53 *L. grandis* from approximately 1-2 m depth and 70 *L. nassa* (53 from above wave base- approximately 1-2m and 17 below wave base- approximately 10 m). We measured height and width and placed snails in water in clear, aerated Plexiglas containers until they emerged from their shells and were mobile. We photographed the foot of each snail through the Plexiglas and calculated foot area from the photographs using tpsDig, version 1.40 (Rohlf, 2004). We compared foot area to size between species and between deep and shallow *L. nassa* using a standard linear model Tukey Test.

Results

Transplant

We recovered 59 of the 60 control snails and 47 of the 60 experimental snails at the end of the seven-day trial (one *L. grandis* missing from the control trials, and ten *L. grandis* and three *L. nassa* were missing from the experimental trials). The Chi-squared results did not show any significant differences between species.

Choice

Of the 36 choice trials, 15 *L. grandis* and six *L. nassa* were predated by full crushing or peeling, ten *L. grandis* and ten *L. nassa* experienced attempted predation, and 11 *L. grandis* and 20 *L. nassa* were undamaged (Fig. 3). In 21 of the 36 trails, the paired snails were in the same category of predation, and in 15 trials *L. grandis* was in a more affected category than *L. nassa* (Fig. 4). Difference in lip thickness between the two snails in a trial did not predict whether *L. grandis* was more affected than *L. nassa* (Fig.5). Our Chi-squared test indicated that *L. grandis* was significantly more affected by the crab predator than *L. nassa* (Pearson=11.03, d.f.=2, p=0.004).

Mass

A general linear model Tukey Test of species showed that *L. grandis* has more tissue mass than *L. nassa* when accounting for shell mass with a p-value of <0.05.

Clod Cards

We recovered 41 of the 52 clod cards that were set out. Change in mass was negatively logarithmically correlated with depth with $R^2=0.8921$ (Fig. 6). The greatest mass was lost at the shallowest depths and tapered off as depth increased. Cards at all depths lost more mass than control cards.

Foot Area

We found that *L. grandis* had significantly larger foot area than *L. nassa* when corrected for size (p- <0.05) using a general linear model Tukey Test. We did not find a difference in foot area to size between *L. nassa* that were collected in deep water and those that were collected in shallow water.

Discussion

The results of our five studies indicate that *L. grandis* and *L. nassa* have adapted differently to meet selection pressures from different depths, including predation pressure and wave action.

Our transplant experiment suggested that if *L. grandis* were present at 10m where *P. armata* is abundant, the crabs will eat it, but our samples sizes were not sufficient to support the suggestion that it is significantly preferred to *L. nassa*. We are confident that the methods used to tether the snails did keep them securely in place as we only lost one of the 60 snails in the control cages. We are also fairly confident that the losses in the experimental cages were due to crab predation because we found several of the tethers still glued to shell fragments that appear to be the remains of crab crushing events. However, the weak trend that we found in the field was strengthened by choice trials. In all 36 choice trials *L. grandis* was as affected or more affected by the crab predator than *L. nassa* even though paired individuals were within 0.5 mm length of each other. This indicates that there is something about *L. grandis* other than size that is driving the crabs to choose it over *L. nassa*. We did not see that lip thickness was driving this trend (Fig. 5), so there is some other difference between these species that results in this preference.

We did find a species difference in shell and soft-tissue that may explain the preference. We found that *L. grandis* has more soft tissue for the mass of shell than *L. nassa*. A greater mass of shell probably means greater foraging costs for the crab. Our results indicate that *L. grandis* has a larger reward of soft-tissue for the cost of getting through its shell; this makes *L. grandis* a more energetically beneficial food item for the crab. These three studies demonstrated why *L. grandis* does not occupy the same space as its predator *P. armata*, but what are the tradeoffs of living in shallow water?

Lake Tanganyika experiences very windy afternoons in the dry season that result in extensive wave action, and this action is more intense near the surface. The snails spend their time grazing on rocks, and if waves knock them off they are more exposed to predators and to the elements. Our clod card experiment indicated that wave action decreases with depth throughout the ranges of these snails; *L. grandis* lives in a wavier environment than *L. nassa*. To determine if *L. grandis* was better adapted to the waves, we should measure both the foot area as well as the tenacity of the foot (Prowse and Pile, 2005). However, lack of resources prevented us from being able to measure the latter. Foot area alone has been shown to be a good predictor of ability to hang on in wave action (Trussel, 1997), and it has been shown to be phenotypically plastic in some gastropod species (Trussel et al., 1993). We found that *L. grandis* does have a larger foot area than *L. nassa* for its size, suggesting that *L. grandis* may have a greater ability to adhere to the rocks in shallow, wavy water. We did not find a difference in foot area for size between shallow and deep *L. nassa* indicating that this species may not be phenotypicly plastic for this trait.

These studies suggest that *L. grandis* may be better adapted to living the wavy, predator-free shallows whereas *L. nassa* is better adapted to deep water containing the predator, *P. armata*. It is likely that this story is much more complicated than covered by the scope of this study and many further studies should be conducted to look at this question. One observation that we made in the choice trials was that when *L. nassa* had attempted predation events there were generally small chips taken out of the lip, whereas when *L. grandis* was attempted it was generally missing large amounts of its last whorl such that it needed to retract far into its shell for soft tissue not to be damaged. This may indicate that there is something about shell composition that makes *L. grandis* more easily crushed. We suggest conducting a shell strength experiment to test how much force it takes to crush the shells of these two species as well as looking at the percent organic matter in the shells of the two species to see if there is a difference in shell composition. It would also be beneficial to conduct experiments on the tenacity of the foot of the two species through both foot strength trials and chemical analysis of the mucus to see if something more than foot area plays into their ability to hang on to rocks.

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Figure 1 Cages for transplant experiment. Twelve cages were constructed out of wire mesh and sand-filled fabric tubes--six control cages and six experimental cages. Control cages had no door and therefore excluded crab predators, whereas experimental cages had four cut-out doors that allowed crab access.

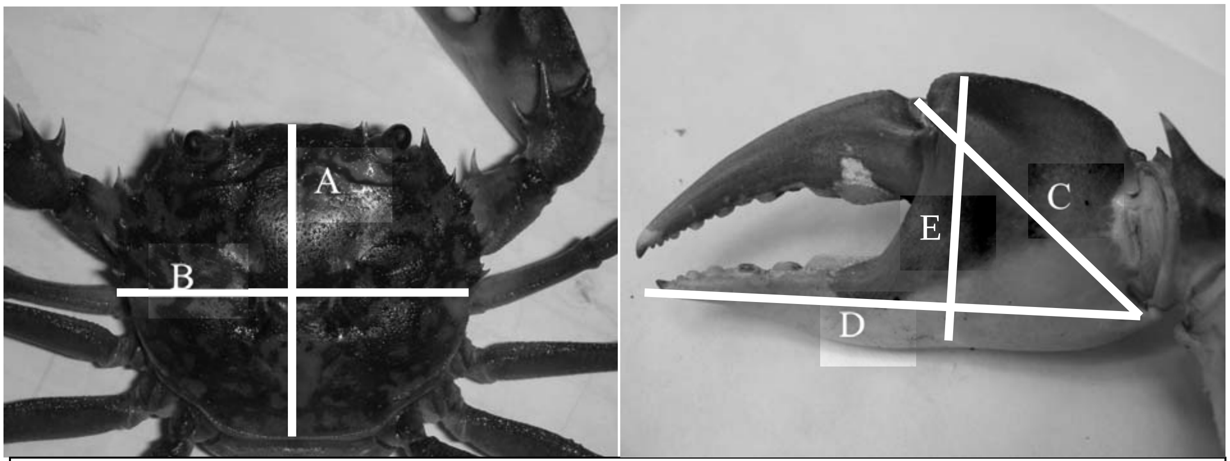

Figure 2 Crab measurements for choice experiment. A-maximum height of carapace, B-maximum width of carapace, C-maximum diagonal length of major chela, D-maximum length of major chela, E-maximum height of major chela. .

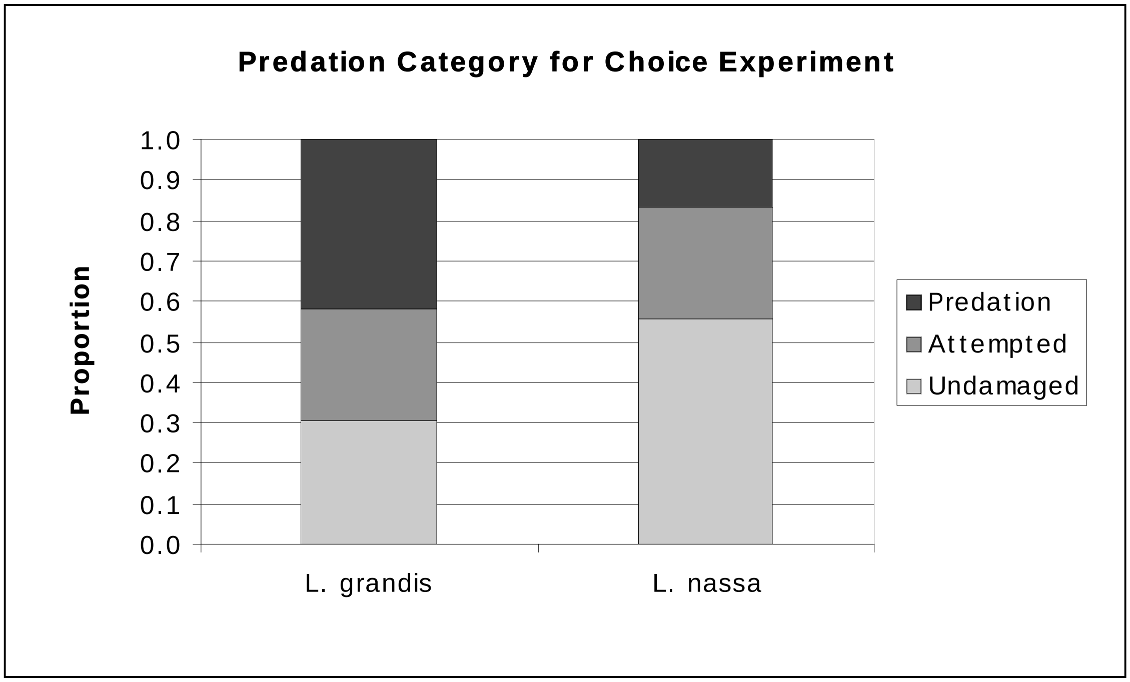

Figure 3 Proportion of trials in which the two snail species were predated upon, attempted, and undamaged. This graph does not take in to account the pair design of this study. *L. grandis* and *L. nassa* were attempted the same number of times, but *L. grandis* was predated upon more than *L. nassa*, and *L. nassa* was undamaged more than *L. nassa*.

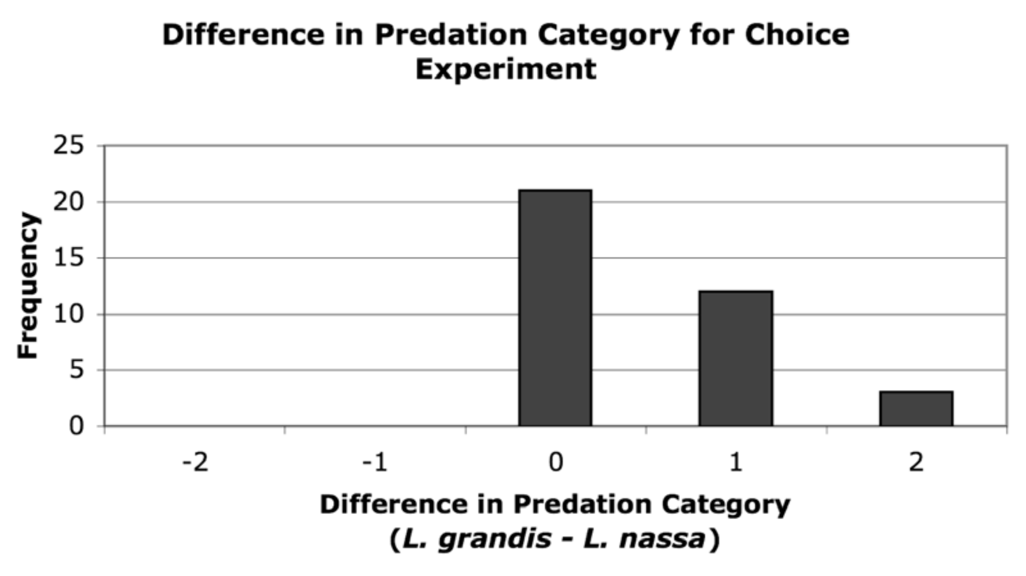

Figure 4 Differences between species in predation category for the choice experiment. The categories of predation were scored as undamaged=0, attempted=1, and predated=2. We then subtracted the score for *L. nassa* from *L. grandis* to obtain a measure of the difference between snails within a paired trial. This helps control for differences between crabs. On this graph, a 0 indicates that both snails in a trial experienced the same category of predation from the crab that they were paired with, a negative number indicates greater predation of *L. nassa* than *L. grandsi*, and a positive number indicates greater predation on *L. grandis* than *L. nassa*. Because we observed no negative values for these trials, we found that *L. grandis* was always at least as affected by the crab predator as *L. nassa*.

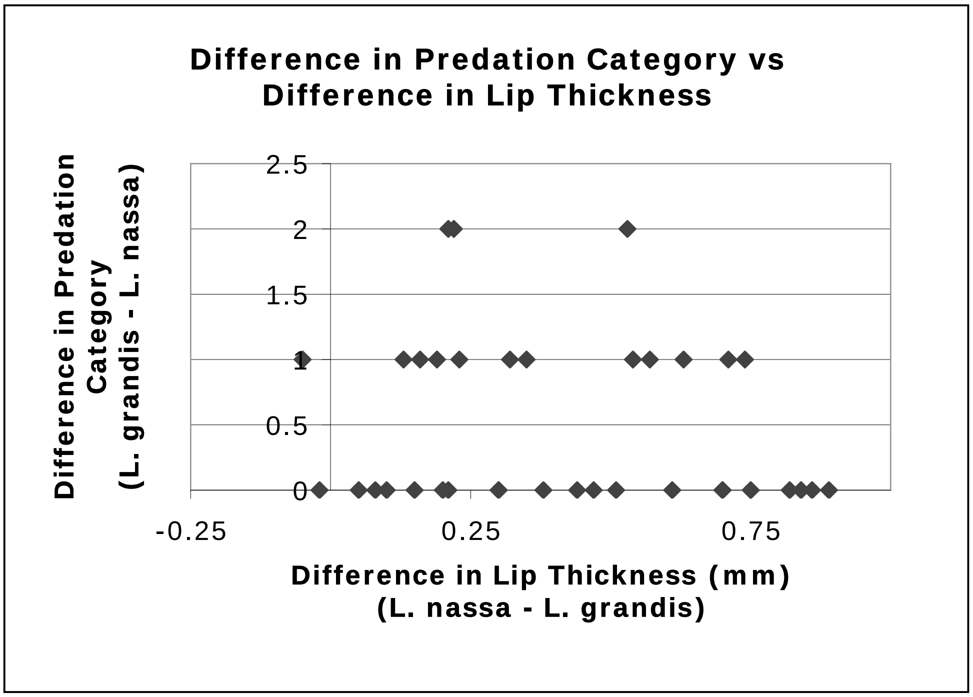

Figure 5 Effect of difference in lip thickness between paired snails on predation category. Lip thickness measurements of *L. nassa* were subtracted from those for *L. grandis* for each paired trial and graphed against the difference in predation category as described in Figure 4. If crabs were choosing *L. grandis* based on its thinner lip, we would expect to see points on this graph clustered in the lower left and upper right corners. We do not see this trend and therefore conclude that something more than just lip thickness is important is going into the decision.