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DIET IN MANGROVE SNAILS: PRELIMINARY DATA ON GUT CONTENTS AND STABLE ISOTOPE ANALYSIS

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ABSTRACT Microscopic analysis of gut contents performed on three *Littoraria* species from mangrove forests in Thailand revealed differences in diet among species. Analysis of carbon and nitrogen stable isotopes was used as an alternative way of tracing food sources. *Rhizophora* leaves, scrapings from both leaf and prop-root surfaces, and local particulate organic matter (POM) were well separated on the basis of their $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values. In contrast, the three *Littoraria* species exhibit considerable overlap and scatter in both carbon and nitrogen isotope ratio values, suggesting that the snails are opportunistic feeders sharing similar food resources. The wide range of $\delta^{13}\text{C}$ values of *Littoraria* (-17.2‰ to -26.3‰) is consistent with carbon assimilation from multiple sources (epiphytes from leaves and prop roots, suspended POM, and *Rhizophora* detritus). *Littoraria intermedia* and *L. pallescens*, the smallest species, had similar $\delta^{13}\text{C}$ values, whereas *L. scabra* was significantly more ^{13}C depleted. A diet of microalgae and cork cells from prop roots could explain this pattern, with *L. scabra* being larger, consuming relatively more cork cells. However, only a few of the *L. scabra* and *L. intermedia* individuals had $\delta^{15}\text{N}$ values consistent with such a diet, and the remaining *L. scabra* and *L. intermedia* and all *L. pallescens* individuals were too depleted, indicating that these individuals must derive a significant amount of their food from a strongly ^{15}N -depleted source. Such a source is present on *Rhizophora* leaf surfaces ($\delta^{15}\text{N} = 0.30 \pm 0.05$; $n = 2$). Some very low values of *Littoraria* $\delta^{15}\text{N}$, down to -7‰, indicate that some individuals have assimilated a yet unknown, highly ^{15}N -depleted food source or that other unknown fractionation processes are involved.

KEY WORDS: *Littoraria*, diet, mangroves, stable isotopes, $\delta^{13}\text{C}$, $\delta^{15}\text{N}$

INTRODUCTION

Mangrove snails of the littorinid genus *Littoraria* are found throughout the tropics with an especially large number of species in the Indo-Pacific region (Reid 1986, Reid 1989). Indo-Pacific species all have planktonic larvae and spend their adult lives on stems, prop roots, and leaves of the trees. Like most littorinids they feed on biofilms, and the mangroves presumably act merely as substrates.

Littoraria scabra (L., 1758), *L. intermedia* (Philippi, 1846), and *L. pallescens* (Philippi, 1846) have been studied in Thailand, where their stomachs have been found to contain fungal hyphae and spores, among other items (Christensen 1998). *L. scabra* and *L. intermedia* live on stems and prop roots of mangroves with *L. scabra* found lower and usually on the seaward edge of the forests. *L. pallescens* is found mostly on the leaves of mangroves where *L. intermedia* is also occasionally seen. *L. pallescens* feeds on the leaf surface without damaging the leaf epidermis, although crescent-shaped necrotic marks may be seen on leaves where the snails rest during the day. However, Ohgaki (1990) reported radular marks on leaves of *Rhizophora stylosa*. The snails move up and down with the tides and come in direct contact with the water only when releasing offspring. The zonation of the three species may be seen as degrees of terrestrialization of snails with marine origins, and it is therefore interesting to compare their diets.

Direct microscopic analysis of gut contents may reveal the range of food items ingested by animals, but the method has certain drawbacks. First of all, the method will depend strongly on the skills of the observer with respect to identifying fragmented objects. Indeed, most of the material in the gut contents of animals may not be visually identifiable. Hard structural tissues may be more easily identified than soft tissues, resulting in a bias in the

relative importance of items, and this bias may be further enhanced by differences in degradability. Finally, gut contents analysis does not reveal the extent to which food items are actually assimilated.

Stable isotope analysis of animal tissues can provide alternative information on sources of food. The stable carbon isotope profile of animal tissues resembles that of the food taking into account a fractionation of about 1‰ per trophic level. Thus, for food sources differing in carbon isotope profile, the isotopic composition of the tissue may ideally indicate the relative contribution of each source to the diet. With respect to nitrogen, animals are usually enriched in ^{15}N (about 3‰) relative to the diet (Michener & Schell 1994).

The purpose of this study was to provide some preliminary information on diet characteristics of these species and to serve as a possible starting point for more detailed analyses.

MATERIALS AND METHODS

The samples analyzed were collected on different occasions; however, all samples were taken during the south-west monsoon (July-September) at the island of Phuket, Thailand. Gut contents analysis was performed on a sample (Sample 1) of snails collected on *R. apiculata* mangroves at Chalong Bay and dropped into 70% ethanol (five individuals of each species were analyzed). Another sample (Sample 2) was collected on *Avicennia marina* (*L. scabra* and *L. intermedia*) at Tang Khen Bay and *Rhizophora* (*L. pallescens*) at Chalong Bay and fixed in 70% ethanol after cracking the shell (10 individuals of each species). The contents of the stomachs were removed under a dissecting microscope, smeared onto microscope slides, and imbedded in glycerol gelatin. From each snail three smears were prepared, and from each smear three fields were scored by ocular grid (81 intersections per field). Objects at each intersection were classified (i.e., 729 scores per individual).

Snails were collected for carbon and nitrogen stable isotope analysis from *R. apiculata* mangroves at Chalong Bay and kept cool for 3 days in plastic containers during transport to Denmark

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before being frozen. For analysis the soft tissue was separated from the shell and operculum and lyophilized prior to being ground with a mortar and pestle. Prior to analysis the tissue was acidified (10% HCl) to ensure the removal of any carbonate debris, rinsed with distilled water, and then freeze-dried again. Analysis of carbon and nitrogen isotopes was also performed on stomach contents removed from snails that had been fixed in 70% ethanol (Sample 1), but only nitrogen results are presented.

Potential food sources were collected from *R. apiculata* mangroves at Chalong Bay. *Rhizophora* leaves were picked from trees, dried at 60°C, and ground for stable isotope analysis. Leaf surfaces were also carefully scraped with a surgical blade without damaging the epidermis. Scrapings from about 50 leaves were pooled in each sample and dried at 60°C before analysis. Wetted surfaces of prop roots and stems were lightly scraped with surgical blades. These scrapings were taken from random spots distributed within the range occupied by the snails, and the obtained material was shaken in a bottle with distilled water and filtered through 250- μ m and 63- μ m filters and finally onto pre-combusted Whatman GF/C-filters (Whatman Intl. Ltd., Maidstone, UK). The three fractions were dried at 60°C along with nonfractionated scrapings. Particulate organic matter (POM) was filtered from the waters of the bay onto precombusted Whatman GF/C filters and dried at 60°C.

Samples were prepared and analyzed as reported by Handley et al. (1991, 1993). The mass spectrometric analyses were done on a Europa 20-20 IRMS with an ANCA-SL sample converter (PIDZ Europa, Cheshire, UK). A routine precision of approximately 0.1‰ for both C and N for invertebrate samples have been obtained. Stable isotope ratios are reported in standard δ notation as $\delta I = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1,000$, in units of per mil, where I is the element in question, and R is the ratio of the heavy to the light isotope. Standards were a CO₂-C standard previously calibrated against the universal Pee Dee Belemnite standard and atmospheric nitrogen.

RESULTS

The three *Littoraria* species exhibited clear differences in composition of the stomach contents with respect to identifiable components (Fig. 1). However, the major part of the stomach contents could not be identified microscopically. Cork cells from mangroves contributed significantly to the stomach contents of *L. scabra* and was also the dominant identifiable item in the stomachs of *L. intermedia*. In *L. pallescens*, fungal hyphae and spores were the most prominent identifiable objects, but these items were also present in the other two species. Diatoms, other algae, and cyanobacteria were present in all species, but only in *L. scabra* and *L. intermedia* could algae in any significant amounts be identified. Kruskal-Wallis tests revealed significant differences among species for all food items except diatoms (Sample 2): hyphae: $K = 15.8$, $P < 0.001$; spores: $K = 21.3$, $P < 0.001$; other algae: $K = 10.9$, $P = 0.004$; cork cells: $K = 19.1$, $P < 0.001$ (d.f. = 2 in all cases).

Stomach contents from snails that had the shell cracked had a higher diversity of identifiable items than snails that had been dropped directly into 70% ethanol, but also in the latter (Sample 1) were there clear differences between species, with cork cells being most prominent in *L. scabra* and fungal hyphae most prominent in *L. pallescens*.

Isotopic signatures of the soft tissue of the three *Littoraria* species exhibited considerable individual variation (Fig. 2). In *L.*

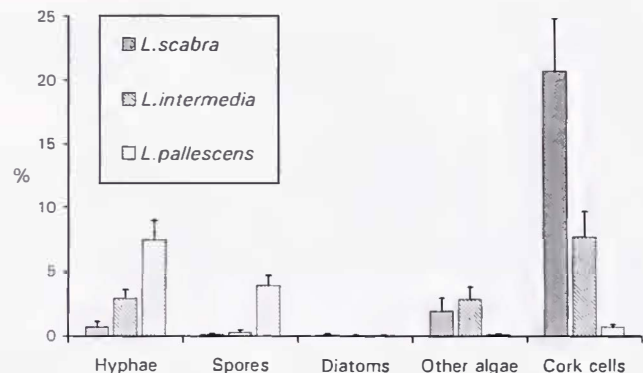


Figure 1. The occurrence of identifiable items in stomachs of three *Littoraria* species expressed as percentages (mean + standard error) of the total number of objects observed under an ocular grid in microscopic smears. Items include fungal hyphae and spores, diatoms, other algae (including cyanobacteria), and cork cells from mangroves. Animals fixed in 70% ethanol after cracking of the shell (Sample 2). *Littoraria pallescens* collected on *Rhizophora apiculata*; *L. scabra* and *L. intermedia* collected on *Avicennia marina*.

scabra the mean $\delta^{13}\text{C}$ value was -24.22‰ , and the range was -26.34 to -22.67‰ ($n = 16$). *L. intermedia* had a mean $\delta^{13}\text{C}$ of -22.51‰ and a range of -24.82 to -20.21‰ ($n = 16$), and *L. pallescens* had a mean $\delta^{13}\text{C}$ of -22.45‰ and a range of -24.86 to -17.27‰ ($n = 15$). A Kruskal-Wallis test revealed significant differences among species ($K = 9.21$; $P = 0.01$; d.f. = 2), with *L. scabra* being significantly more ^{13}C depleted than the other two species. Also, nitrogen isotopic signatures of the three snail species were highly variable. *L. scabra* had a mean $\delta^{15}\text{N}$ of 1.89‰ and a range of -4.64 to 6.11‰ ($n = 16$). *L. intermedia* had a mean $\delta^{15}\text{N}$ value 1.43‰ and a range of -6.97 to 6.80‰ ($n = 16$), and *L. pallescens* had a mean $\delta^{15}\text{N}$ of -1.80‰ and a range of -6.12 to 2.00‰ ($n = 15$). Differences among species were significant ($K = 9.42$; $P < 0.01$; d.f. = 2), and *L. pallescens* was significantly more ^{15}N depleted than *L. scabra* and *L. intermedia*; the latter showed the largest individual variation.

Isotope analyses performed on stomach contents of snails fixed in 70% ethanol resulted in a mean $\delta^{15}\text{N}$ of 1.33‰ (0.22 to 3.52‰ ; $n = 7$) in *L. scabra*, a mean $\delta^{15}\text{N}$ of 2.38‰ (-1.91 to 7.31‰ ; $n = 9$) in *L. intermedia*, and a mean $\delta^{15}\text{N}$ of -1.24‰ (-2.91 to -0.19‰ ; $n = 9$) in *L. pallescens*. Again, *L. pallescens* was the most ^{15}N depleted, and *L. intermedia* was the most variable of the species.

Rhizophora leaves, scrapings from both leaf and prop-root surfaces, and local POM were well separated on the basis of their $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures (Fig. 2). POM was the least ^{13}C depleted of the sources, and *Rhizophora* leaves, leaf scrapings, and the $>250\text{-}\mu\text{m}$ fraction of the prop-root scrapings were the most depleted. Leaf scrapings were highly depleted in ^{15}N compared with the other sources. A few of the *L. scabra* and *L. intermedia* individuals had $\delta^{15}\text{N}$ values consistent with a diet of mixed mangrove and POM, whereas the remaining *L. scabra* and *L. intermedia* and all the *L. pallescens* were too ^{15}N depleted.

DISCUSSION

Although only small amounts of the stomach contents could be identified, the differences among species with respect to the identifiable fraction of the diet appear to reflect true differences in diet. Whether the differences are solely a result of differences in com-

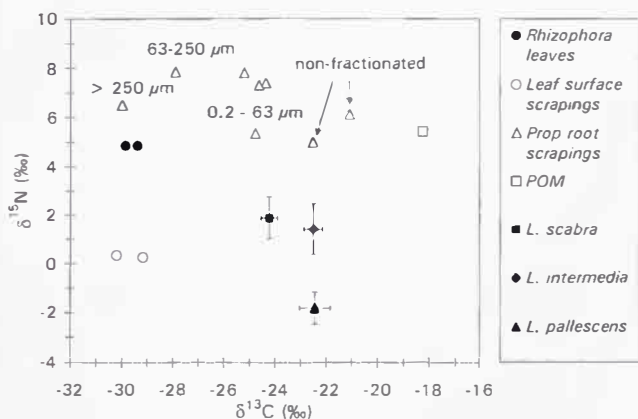


Figure 2. Stable isotopic carbon and nitrogen signatures of the soft tissue of three species of *Littoraria* collected on *Rhizophora apiculata* (means \pm standard error of means) and of some potential sources of food for these snails presented as individual sample measurements.

position of the substrates upon which the snails feed (i.e., a result of zonation) or of the snails' ability to actively select among available items cannot be discerned on the basis of available data. Neither can the extent to which different items contribute to assimilated matter in the three species. Cork cells, fungal hyphae, and spores are major identifiable structural components of the diet, but whether they contribute significantly to the snails' energy budget is unknown. Fungal material in the diet was also reported by Kohlmeyer and Bebout (1986) in *L. angulifera* and by Newell and Bärlocher (1993) in *L. irrorata*.

The difference between snails that had their shells cracked prior to fixing and those fixed whole with respect to diversity of items is probably a result of continued breakdown of easily degradable food items in those snails dropped whole into ethanol. This underlines the importance of rapid and effective fixing of material for stomach analysis. The diet analyses demonstrate that there are differences between species as far as structural components in the gut contents are concerned, but these differences should not be overinterpreted because only a fraction of the gut contents can be identified.

The considerable overlap and scatter in isotope ratio values suggest that the snails are opportunistic feeders and that they to some extent share food resources. The wide range of *Littoraria* $\delta^{13}\text{C}$ values (-26.3 to 17.3‰) suggest carbon assimilation from multiple sources (epiphytes from leaves and prop roots, deposited POM, and *Rhizophora* detritus). *L. intermedia* and *L. pallescens*, the smallest species, had identical mean $\delta^{13}\text{C}$ values, whereas *L. scabra* was significantly more ^{13}C depleted. A diet of microalgae and cork cells from prop roots could explain this pattern with *L. scabra*, which is larger and consumes relatively more cork cells. However, all three species were on average more ^{15}N depleted than these food sources. *L. pallescens* had a significantly lower mean $\delta^{15}\text{N}$ value than the other two species, and it is clear that it does not derive its food directly from the mangrove leaves upon which it lives. It must derive a significant amount of its food from a strongly ^{15}N -depleted source. Such a source was present in scrapings from leaf surfaces ($\delta^{15}\text{N} = 0.3 \pm 0.05\text{‰}$; $n = 2$), but it is as yet unknown what it represents and how it is related to the diet of the snail.

Rodelli et al. (1984) reported stable carbon isotope ratios in plants and animals from Malaysian mangrove forests. They found

a $\delta^{13}\text{C}$ of -27.2‰ in *R. apiculata*, which is acceptably close to our value of -29.6‰ ($n = 2$). In *L. melanostoma* they found a $\delta^{13}\text{C}$ of -24.6‰ (mean of 3) comparable to that of *L. scabra* in our study, and a value of -21.5 (mean of 10) in *L. undulata* (a rock-dwelling species). They did not report nitrogen isotope ratios.

L. irrorata in a North Carolina salt marsh had $\delta^{13}\text{C}$ values of -16.6 to -15.1‰ and $\delta^{15}\text{N}$ values of 2.2 to 3.7‰ (diet 0.1 to 3.8‰) (Currin et al. 1995), and in a Kenya mangrove forest the herbivorous snail *Terebralia palustris* had a $\delta^{13}\text{C}$ value of -24.23‰ , similar to that of its presumed mangrove leaf diet ($\delta^{13}\text{C} = -24.28\text{‰}$), and a $\delta^{15}\text{N}$ signal consistent with the normal pattern of enrichment relative to the diet (Marguillier et al. 1997). If we assume that the on average very depleted ^{15}N signatures of the studied *Littoraria* tissues are not in conflict with the generally observed 3‰ enrichment per trophic level in animals (Owens 1987, Michener & Schell 1994), we must conclude that all of the three *Littoraria* species have assimilated a yet unknown and very ^{15}N -depleted food source, but other unknown fractionation processes may be involved.

The fact that the snails were kept alive for 3 days before being frozen could be invoked as a source of ^{15}N depletion. However, it is difficult to identify a mechanism leading to this result. First of all, such a mechanism should affect individuals differently because not all individuals were highly depleted. Furthermore, starvation is known to lead to ^{15}N enrichment of the tissues (e.g., Hobson et al. 1993), and, finally, stomach contents had equally ^{15}N -depleted signatures, again with *L. pallescens* being on average the most depleted. The ^{15}N signatures of stomach contents were obtained from snails that were killed without delay by dropping them into 70% ethanol, a procedure that is expected not to affect nitrogen isotope ratios. Mangrove snails may stay inactive for days during dry periods, so being deprived of food for 3 days is not entirely unnatural for them.

A purely hypothetical scenario explaining the ^{15}N depletion is that nitrogen excreted by the snails (uric acid) is recycled (e.g., through fungi), involving fractionation (depletion), and that the fungi subsequently are ingested by the snails. Supporting such a hypothesis is the fact that excretion products (ammonia, urea, and uric acid) are depleted compared with the dietary source and the tissues of the animals excreting them (Gannes et al. 1997) and that fungi are most prominent in the diet of the most ^{15}N -depleted species (*L. pallescens*). Fungi are among the microorganisms known to degrade uric acid (Kieslich 1976). Microorganisms like the cyanobacterium *Anabaena* grown on nitrate or ammonia show large fractionations and ^{15}N depletions (Macko et al. 1987).

The results underline the usefulness of multiple isotope analyses. A more limited conclusion would have been reached had the analysis been based on gut contents and stable carbon isotopes alone. The wide range of isotope ratios within species further stresses the importance of large samples in food chain studies. Considerable bias or loss of information may result if one attempts to deduce trophic relationships based on pooled samples of three or four individuals. During the last three decades, most isotopic studies have been based on small samples under the assumption that there is insignificant variation between individuals within species. Our data demonstrate that this is not always the case and that variation can be large, possibly due to small-scale heterogeneity in the occurrence and accessibility of food items. Detailed studies are needed to explain the highly ^{15}N -depleted tissues and the range of variation in N isotope ratios in these snails.

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