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MORPHOMETRICS AND MINERAL COMPOSITION OF SHELL WHORLS IN THREE SPECIES OF GIANT AFRICAN SNAILS FROM ABEOKUTA, NIGERIA

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Abstract: *Archachatina marginata* (Swainson), *Achatina achatina* (Linnaeus) and *Achatina fulica* Bowdich were examined for their shell whorl morphometrics (20 snails per species) and the calcium, iron and phosphorus content in the shell whorls (10 snails per species). The species differed in the degree of shell mineralization (the highest in the medium-sized *A. achatina*, intermediate in the smallest-sized *A. fulica* and the smallest in the largest-sized *A. marginata*) which was not age- or environment-dependent, since all the experimental snails were 12 months old adults of 6 whorls, originating from the same snail farm. In all three species the degree of shell mineralization decreased from the apical (oldest) to the body (youngest) whorl, thus depending entirely on the whorl's age. The inter-specific differences in calcium content should be considered when using shells for medical or agricultural purposes.

Key words: land snails, shell mineralization, inter-specific differences, age-dependent differences

INTRODUCTION

When the snail grows, its shell (and body) expands only at the aperture margin while the shell thickness and the degree of mineralization increase all the time on all the shell internal surface (Solem 1974, Barker 2001). Apart from the effect of such factors as calcium supply, food quality, rainfall (and thus duration of activity periods: feeding), or temperature (metabolic rate) (Gärdenfors et al. 1993, Barker 2001, Ademolu et al. 2007, Mierzwa 2008), the shell thickness and its saturation with calcium should vary among conspecific individuals of different age. Since whorls of each shell differ in age, they should also differ in their mineral content, especially in tall-shelled, long-lived species; the literature information on the mineral composition pertains to whole shells but not to individual whorls. Inter-specific differences should also exist. For example, two species of *Achatina* differ in their haemolymph composition (Idowu et al. 2008) which has a direct bearing on the shell composition.

The problem has also practical aspects. In many countries snails are eaten: the flesh is removed, and the shells are either discarded or used for various purposes. Using them as, for example, ornaments or containers is not affected by their mineral composition, but in some regions of Africa they are used in traditional medicine, as addition to herbal remedies for measles, cough and gonorrhoea, or to treat wounds, since calcium improves blood clotting, to store medicinal concoctions, as material to produce tooth powder, or as a component of feed for farm animals, particularly layer hens (Agbelusi & Ejidike 1992, Houndonougbo et al. 2012, Amubode & Fafunwa 2014, Ademolu et al. 2015).

This study was aimed at ascertaining to what extent the mineral content in the shell depended on the age of the whorl, and at checking if and to what extent it was species-specific in a group of closely related species.

MATERIAL AND METHODS

Three species of giant snails are common in Nigeria, and these were used in the analyses: *Archachatina marginata* (Swainson, 1821), *Achatina achatina* (Linnaeus, 1758) and *Achatina fulica* Bowdich, 1822. Ninety adult (6 whorls) snails (30 snails per species), aged 12 months, were purchased from the snail farm of the Forestry and Wildlife Department, Federal University of Agriculture, Abeokuta, Nigeria. The snails were maintained on pawpaw (*Carica papaya*) leaves while on the farm. Twenty snails per species for the shell measurements (diameter and height of whorls) were randomly selected from the stock purchased from the farm. The height was measured using measuring tape while the diameter was measured with Vernier calliper. The remaining ten snails were used for the shell mineral analysis. The snails were killed by submerging in deoxygenated water. The bodies were removed, the shells were air-dried and cut into separate whorls using carpenter's hack saw. Each whorl sample was oven-dried at 50°C for two days, ground into powder and subject to spectrophotometric analysis (KJ23A Spectrophotometer, KINGJOE, NIG LTD). Calcium, iron and phosphorus content in each sample were determined following A.O.A.C. (1980) methods and the analyses were run in triplicates. The results were analysed using IBM SPSS (version 21.0). The data were subject to two-way analysis of variance (ANOVA) and the Tukey test was used to determine the statistical significance of the differences. The Spearman correlation was used to test relationships between dependent variables.

RESULTS

The height and diameter measurements of the whorls are shown in Tables 1 and 2.

The relation between the dependent variables (whorl height and diameter) and the species and whorl age is shown is Table 3. Both the species and the whorl age had a significant effect on the whorl height and diameter.

The content of the three elements in the studied shells is shown in Tables 4–6.

The calcium content in all three species is the highest in the apical whorls and decreases with the age of the whorls. Overall, it is the highest in the shells of *A. achatina*, followed by *A. fulica*, and the smallest in *A. marginata*. It is thus the highest in the medium-sized species, intermediate in the smallest species and the smallest in the largest species (see also Tables 2, 3).

The distribution of values of iron and phosphorus content among the species is the same as that of calcium content: the greatest in *A. achatina* (medium-sized species), followed by *A. fulica* (smallest species) and then *A. marginata* (largest species).

Like calcium, the content of iron and phosphorus is the greatest in the upper (older) whorls of all three

Table 1. Whorl height [cm] in the three examined species. Mean values marked with different superscript in the same column are significantly different ($p<0.05$)

Whorl/Species	*A. achatina*	*A. marginata*	*A. fulica*
6th	7.20±0.01[a]	9.44±0.12[a]	6.26±.021[a]
5th	2.12±0.11[b]	3.42±0.01[b]	2.16±0.23[b]
4th	1.48±0.32[c]	2.38±0.23[c]	0.62±0.15[c]
3rd	0.60±0.52[d]	1.76±0.11[d]	0.34±0.43[c]
2nd	0.34±0.14[d]	1.38±0.01[d]	0.24±0.23[c]
1st	0.24±0.43[d]	0.42±0.10[d]	0.21±0.11[c]

Table 2. Whorl diameter [cm] in the three examined species. Mean values marked with different superscript in the same column are significantly different ($p<0.05$)

Whorl/Species	*A. achatina*	*A. marginata*	*A. fulica*
6th	15.60±0.10[a]	20.16±0.21[a]	15.50±0.42[a]
5th	11.38±0.11[b]	14.90±0.23[b]	7.12±0.32[b]
4th	5.52±0.21[c]	8.10±0.14[c]	6.26±0.11[b]
3rd	4.12±0.34[c]	5.50±0.20[d]	4.20±0.27[c]
2nd	2.06±0.25[d]	3.56±0.17[d]	2.14±0.10[d]
1st	1.24±0.32[d]	2.60±0.02[d]	1.58±0.01[d]

Table 3. Relations between dependent variables and other factors

Dependent variable	Factor	df	MS	F	P
Whorl height	Species (S)	2	35.327	103.583	0.00
	Whorl age (W)	5	224.600	658.555	0.00
	S × W	10	2.217	6.501	0.000
	Error	162	0.341		
Whorl diameter	Species (S)	2	159.364	230.805	0.000
	Whorl age (W)	6	1043.353	1511.079	0.000
	S × W	10	20.802	30.128	0.000
	Error	162	0.690		

Table 4. Calcium content [mg/g] in whorls of the three examined species. Mean values marked with different superscript in the same column are significantly different ($p<0.05$)

Whorl/Species	*A. achatina*	*A. marginata*	*A. fulica*
6th	110.71 ± 0.02^c	31.34 ± 0.10^b	47.61 ± 0.40^b
5th	110.93 ± 0.01^c	31.68 ± 0.11^b	47.91 ± 0.17^b
4th	112.29 ± 0.00^b	31.83 ± 0.13^b	48.11 ± 0.11^a
3rd	112.68 ± 0.12^b	32.11 ± 0.11^a	48.37 ± 0.21^a
2nd	116.13 ± 0.21^a	32.13 ± 0.10^a	48.82 ± 0.10^a
1st	116.35 ± 0.01^a	32.45 ± 0.01^a	48.92 ± 0.03^a

species, indicating that overall the degree of mineralization is higher in the upper whorls. The Spearman correlation analysis revealed a significant relationship between whorl height and calcium (0.001), iron (0.000) and phosphorus (0.02) on the one hand and between whorl diameter and calcium (0.005), iron (0.001) and phosphorus (0.006) on the other.

Table 5. Iron content [mg/g] in whorls of the three examined species. Mean values marked with different superscript in the same column are significantly different ($p<0.05$)

Whorl/Species	*A. achatina*	*A. marginata*	*A. fulica*
6th	51.48 ± 0.02^c	12.10 ± 0.01^b	21.50 ± 0.21^b
5th	53.13 ± 0.30^b	12.42 ± 0.21^b	22.16 ± 0.02^b
4th	53.21 ± 0.01^b	14.34 ± 0.10^a	26.45 ± 0.10^a
3rd	56.02 ± 0.23^a	15.11 ± 0.04^a	27.16 ± 0.01^a
2nd	56.12 ± 0.11^a	15.42 ± 0.32^a	27.72 ± 0.07^a
1st	56.74 ± 0.05^a	15.46 ± 0.12^a	27.89 ± 0.16^a

Table 6. Phosphorus content [mg/g] in whorls of the three examined species. Mean values marked with different superscript in the same column are significantly different ($p<0.05$)

Whorl/Species	*A. achatina*	*A. marginata*	*A. fulica*
6th	62.01 ± 0.22^c	10.03 ± 0.01^b	22.02 ± 0.01^b
5th	62.12 ± 0.04^c	10.19 ± 0.02^b	22.12 ± 0.12^b
4th	62.01 ± 0.26^c	10.19 ± 0.01^b	22.12 ± 0.40^b
3rd	64.31 ± 0.65^b	12.01 ± 0.22^a	24.13 ± 0.20^a
2nd	68.61 ± 0.32^a	12.15 ± 0.30^a	24.50 ± 0.09^a
1st	68.68 ± 0.20^a	12.39 ± 0.08^a	24.52 ± 0.46^a

DISCUSSION

A. marginata has the largest shell (height and diameter) while that of *A. fulica* is the smallest. There is a significant correlation between the whorl's age and its size: the younger the whorl, the larger its size (ADEMOLU et al. 2008, IDOWU et al. 2008, ALUKO et al. 2014). The large size of *A. marginata* may be responsible for its common name, "Big Black Snail", in West Africa (COBBINAH et al. 2008).

In the three examined species the apical whorl has the greatest and the body whorl the smallest mineral content. The apex is the oldest whorl in the shell and was formed during the embryonic stage (BAUR 1994, YOLOYE 1994). As the snail ages, its shell becomes gradually thicker and stronger, hence it is not surprising that the apex contains more minerals than the younger whorls; it has been accumulating them over a longer period of time. The difference should be even more pronounced in long-lived species. The small mineralization of the last part of the body whorl, at the aperture, is responsible for its brittleness (AMUSAN & OMIDIJI 1998). This seems to indicate that the degree of mineralization depends directly on the age of the whorl. On the other hand, it is advantageous to have the shell apex with the next few whorls mechanically resistant, since they are as exposed to mechanical damage as the body whorl, but with a smaller repair ability (HELLER 1990).

As could be expected, in all three species calcium was the most abundant element in the shell, while the content of Fe^{2+} was the smallest. Snail shells are primarily built of calcium carbonate which contributes to the shell's mechanical resistance (JORDAENS et al. 2006, BEEBY & RICHMOND 2007, ADEMOLU et al. 2015). The high calcium content is the reason for adding snail shells to the fodder of farm animals, particularly layer hens (HOUNDONOUGBO et al. 2012). Likewise, their use in wound treatment in traditional medicine is associated with their high Ca^{2+} content, since calcium is a prerequisite to blood clotting in animals. However, the role of phosphorus and iron in the shell structure is unknown.

The content of the three minerals is radically different among the three species: the shell of *A. achatina* contains more minerals than those of the other two species. Though rather closely related, they differ in the degree of shell mineralization and the differences are not size-related. Considering the same breeding and raising conditions (the snails were purchased from the same snail farm and fed the same diet) and the very small individual variation in the content of calcium, iron and phosphorus, the degree of shell mineralization seems to be indeed species-specific. One reason for the difference may be that, unlike the other two species, *A. achatina* has a pattern of black wavy streaks on a yellowish background (COBBINAH et al. 2008). These streaks consist of pigments, such as melanin, which are produced by glandular cells of the mantle edge (HELLER 2015). The snail melanin pigments are known to bind or accumulate various metal ions due to their cation exchange activity with free carboxyl groups in the melanin polymer as the main binding sites (Larsson & Tjälve 1978).

The practical conclusion of the study is rather obvious: shells with higher calcium content provide better material to produce calcium-rich medicines or feed, so it is not unimportant which species is used for the production. Similarly, the high concentration of minerals (Ca, Fe and P) in the shell of *A. achatina* probably makes the shell rigid and more resistant to breakage, predators and dehydration. Likewise, it probably aids dispersal, since thick-shelled snails can survive in the bird digestive as reported for medium-sized forest snails, for example *Alinda biplicata* (Montagu, 1803), *Cochlodina laminata* (Montagu, 1803), or *Discus rotundatus* (O. F. Müller, 1774) (Simonova et al. 2016).

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