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The concentration of calcium carbonate in shells of freshwater snails

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Abstract: The range of concentration of calcium carbonate in the shells of various freshwater gastropods was determined using ion chromatography. Individuals of *Helisoma trivolvis* (wild) were collected from three ponds in Pennsylvania and New Jersey; individuals of *Physa* sp. were collected from one pond in New Jersey; individuals of *H. trivolvis* (Colorado strain) and *Biomphalaria glabrata* (NMRI strain) were raised in the laboratory; and individuals of *Pomacea bridgesii* were purchased commercially. The concentrations of calcium carbonate (mean % by dry weight) of the shells were as follows: *H. trivolvis* (wild), 97.0; *Physa* sp., 97.8; *H. trivolvis* (CO), 97.6; *B. glabrata*, 98.8; *P. bridgesii*, 98.2. Our data support and validate the previous claim that snail shells are comprised of 95-99.9% calcium carbonate.

Key words: Gastropoda, ion chromatography, *Biomphalaria glabrata*, *Helisoma trivolvis*, *Physa* sp., *Pomacea bridgesii*

According to Hare and Abelson (1965) and Marxen *et al.* (2003), molluscan shells consist of 95-99.9% calcium carbonate and 0.1-5% organic material by weight. However, detailed analyses of the concentration of calcium carbonate in the shells of individual species of pulmonate and prosobranch snails are for the most part not available. Some values of calcium carbonate in the shells of several pulmonates were given incidental to a study that examined the effects of parasitism by larval trematodes on the proportion of calcium carbonate in the shells of selected gastropods (White *et al.* 2005). To provide further confirmation of the 95-99.9% range of calcium carbonate reported for molluscan shells, we examined the calcium carbonate concentration of individuals from several species of field collected (wild) snails, *Helisoma trivolvis* (Say, 1816) and *Physa* sp., two species of laboratory-reared snails, *H. trivolvis* and *Biomphalaria glabrata* (Say, 1816), and one snail species purchased from a commercial supplier, *Pomacea bridgesii* (Reeve, 1856).

METHODS

Collection and maintenance of snails

Individuals of *Helisoma trivolvis* (Pennsylvania and New Jersey strain) were collected from Amwell Lake, Wildlife Management Area, East Amwell Township, Hunterdon County, New Jersey, USA (40°26′N, 74°49′W) on 15 July 2004 and from Hoch Pond, Northampton County, Pennsylvania, USA (40°47′20″N, 75°27′15″W) on 1 July 2004. Individuals of *H. trivolvis* and *Physa* sp. were collected from Delaware Pond, Knowlton Township, Columbia, New Jersey, USA (40°55′19.1″N, 75°03′49.5″W) on 1 July 2004. All wild snails were analyzed within 1-2 days of collection. A sample of water from each pond was also collected and analyzed for calcium carbonate as well.

One of us (B. Fried) has maintained a continuous culture of *Helisoma trivolvis* (Colorado strain) for more than 15 years (Fried *et al.* 1987). Stock cultures of *Biomphalaria glabrata* (NMRI strain) were obtained from Dr. Fred Lewis, Schistosomiasis Laboratory, Biomedical Research Institute, Rockville, Maryland, USA. Individuals of *Pomacea bridgesii* were purchased from Carolina Biological Supply Company (Burlington, North Carolina, USA). The specific identity of these snails was confirmed by Dr. Robert H. Cowie, University of Hawaii, Center for Conservation Research and Training, Honolulu, Hawaii, USA. Twenty individuals of *H. trivolvis* (Colorado strain), 20 of *B. glabrata*, or 2 to 3 of *P. bridgesii* were maintained at 23 ± 1°C in aerated glass jars containing 800 mL artificial spring water (ASW) as prepared by Ulmer (1970) under diffuse fluorescent light for a photoperiod of 12 h per day. The cultures were fed *ad libitum* on boiled romaine lettuce leaves, and the water was changed three times a week. Individuals of *H. trivolvis* (CO) and *B. glabrata* were maintained in the laboratory for about 6 weeks prior to use. At the time of analysis, they were sexually mature and exceeded 10 mm in shell diameter. Individuals of *P. bridgesii* were used within about 1 week of receipt from the supplier. All of the snails were negative for larval trematodes at necropsy.

Calcium carbonate determination

Ten snails were randomly selected from each population for analysis. At necropsy, snails were measured with a vernier caliper to the nearest 0.1 mm for *Helisoma trivolvis, Biomphalaria glabrata,* and *Physa* sp. and to the nearest 1 mm for *Pomacea bridgesii*. Measurements were taken of the maximum length of individuals of *Physa* sp. and *P. bridgesii* and of the maximum diameter of *H. trivolvis* and *B. glabrata*. The shell was dissected from the body and the body was

discarded. The operculum of *P. bridgesii* was also discarded. Each sample consisted of one snail shell, which was prepared and analyzed as described in White *et al.* (2005) using a Dionex DX-120 Ion Chromatograph (Dionex, Sunnyvale, California, USA) with a Dionex AS40 automated sampler, an IonPac CG12A guard column (4 × 50 mm), an IonPac CS12A cation exchange analytical column (4 × 250 mm), and a conductivity detector. The column was eluted isocratically with 20 mM methanesulfonic acid at a flow rate of 1.0 mL/min. A Dionex cation self-regenerating suppressor ultra (100 mA) was utilized to suppress background conductivity. Standard solutions of the calcium ion were prepared at 1.00 mg/L, 5.00 mg/L, 10.0 mg/L, 25.0 mg/L, 50.0 mg/L, 100 mg/L, and 200 mg/L and used for calibration. Each sample was analyzed in triplicate with an injection volume of 25 µL and the mean concentration of calcium ion (% by dry weight) was calculated. The retention time for the calcium ion was 8.15 ± 0.50 min. Values of concentration of calcium carbonate of the test solutions were determined by PeakNet version 5.1 software as described in White *et al.* (2005). For each population, a blank was prepared in the same manner and was taken into account in calculating the final percentage of calcium carbonate of each snail shell. A single water sample from each collection site and ASW were analyzed by ion chromatography following the same procedure.

Statistical analysis

For all experiments in which multiple sample means were compared, a single factor analysis of variance (ANOVA) was used to determine whether there was a significant difference between the concentrations of calcium carbonate of the shells of various populations of snails. If a significant difference ($P < 0.05$) was found, the data were subjected to the Bonferroni method to determine among which populations the difference occurred. When two means were compared, Student's *t*-test was used to determine whether there was a significant difference ($P < 0.05$) in the calcium carbonate concentration in the shells of the two populations. SPSS version 12.0 software was used for all data analyses.

RESULTS

The percentage of calcium carbonate of the shells, the sizes of the snails used, and the concentrations of calcium carbonate of the water in which the snails were maintained are presented in Table 1. The samples from *Helisoma trivolvis* from Hoch Pond showed a significantly lower concentration of calcium carbonate than any other snail population studied (ANOVA, $P < 0.05$). Shell size could not be analyzed as a factor when looking at the concentration of calcium carbonate among all populations due to the natural size differences between species. There was no significant difference in the concentrations of calcium carbonate between the two species of laboratory-reared snails and the commercially purchased snails (ANOVA, $P > 0.05$). The shells of wild *H. trivolvis* showed no significant difference in the concentration of calcium carbonate from the laboratory-reared individuals of *H. trivolvis* (Student's *t*-test, $P > 0.05$); however, the shells of the wild population of *H. trivolvis* as a group were significantly smaller in diameter than the shells of the laboratory-reared *H. trivolvis* (Student's *t*-test, $P < 0.05$).

Table 1. Percentage of calcium carbonate by dry weight of snail shell, shell size, and calcium carbonate content (mg/L) of water.

	Species	Pond or ASW[1]	$CaCO_3$ content in shells (%) (mean ± SE)	Size of snail (mm) (mean ± SE)	$CaCO_3$ content in water (mg/L)[2]
Wild snails	*Helisoma trivolvis*[3]	Amwell Lake	97.6 ± 0.2[4]	15.0 ± 0.5[7]	141.1
	H. trivolvis[5]	Hoch Pond	95.2 ± 0.4[4]	10.4 ± 0.2[7]	39.9
	H. trivolis	Delaware Pond	98.1 ± 0.6	10.6 ± 0.3[7]	190.4
	Physa sp.[3]	Delaware Pond	97.8 ± 0.5	8.4 ± 0.1[4,6]	190.4
Laboratory-reared snails	*H. trivolvis* (CO)	ASW	97.6 ± 0.4[4]	13.2 ± 0.3[7]	32.0
	Biomphalaria glabrata[3]	ASW	98.8 ± 0.2	11.1 ± 0.3[7]	32.0
Commercially purchased snails	*Pomacea bridgesii*	ASW	98.2 ± 0.4	36 ± 2[6]	32.0

[1] ASW = artificial spring water.

[2] Water from the collection sites for wild snails and ASW for all others.

[3] From White *et al.* (2005).

[4] One sample was determined by the Q-test (90% confidence interval) to be an outlier and was excluded from the results and all statistical analyses, giving $n = 9$.

[5] This sample had a mean concentration of calcium carbonate that was significantly lower than the other samples (ANOVA, $P < 0.05$).

[6] Maximum length.

[7] Maximum diameter.

When considering only shells from the wild populations of *H. trivolvis*, the group collected from Hoch Pond had a significantly lower concentration of calcium carbonate in their shells than did the groups collected from Amwell Lake and Delaware Pond.

A correlation between the hardness of the water and the concentration of calcium carbonate in the shells was found only for the wild snails. Hoch Pond had the softest water (although still above the 20 mg/L $CaCO_3$ minimum found to limit the number of species of snails that can survive; Boycott 1936, Macan 1950). Shells of snails obtained from that pond had the lowest concentrations of calcium carbonate. Delaware Pond had the hardest water and the shells of snails collected from it had the highest concentrations of calcium carbonate. Amwell Lake had a calcium content between that of Hoch Pond and Delaware Pond. Shells of snails from that lake had concentrations of calcium carbonate between those of the snails from Hoch Pond and the snails from Delaware Pond.

The ASW had the lowest concentration of calcium carbonate, yet the shells of the laboratory-reared and commercially-purchased snails that were maintained in it had concentrations of calcium carbonate that were significantly higher than those of the wild snails (Student's *t*-test, $P<0.05$). According to the standard classification for water hardness of the U.S. Geological Survey (2006), the water from Hoch Pond and the ASW were soft, the water from Amwell Lake was hard, and the water from Delaware Pond was very hard.

DISCUSSION

In spite of considerable variation in the calcium content (mg/L) of the water in which the snails were maintained, all of the snails showed concentrations of calcium carbonate in their shells in the range of 95.2-98.8 % by weight, similar to the range reported by Hare and Abelson (1965). Thus, under conditions of variable concentrations of calcium carbonate in the water, freshwater snails are able to maintain a high concentration of calcium carbonate in their shells. No clear trend between the concentrations of calcium carbonate of the external media and the concentrations of the snail shells was found when both wild and laboratory-reared populations were considered. Freshwater snails obtain their calcium from the surrounding water and their food source (van der Borght and van Puymbroeck 1966, Young 1975), first localizing the calcium in the mantle before depositing it in the shell (Bevelander 1952). It is surprising that the laboratory-reared snails, which were raised in the softest water, had relatively high concentrations of calcium carbonate in their shells. One important difference between the laboratory-reared snails and the wild snails was that the laboratory-reared snails were fed *ad libitum*; we do not know how adequate the food supply was for the snails obtained from the wild. Individuals of *Lymnaea peregra* (Müller, 1774) and *Planorbarius corneus* (Linnaeus, 1758) reared in calcium-rich water, obtain calcium more from the water than from the food. Individuals reared in calcium-poor water, however, obtain two to four times more calcium from the food than from the water (Young 1975). This relationship between water hardness and the source of calcium may be responsible in part for the results of the present study; however, the relative role of food versus water as a source of calcium for the snails was undetermined in our study.

The data showed no direct correlation between size of the shell and concentration of calcium carbonate. Within the wild populations of *Helisoma trivolvis*, the snails from Amwell Lake were the largest; however, this did not correspond to a lower or higher concentration of calcium carbonate in the shell. The shells from the wild population of *H. trivolvis* showed no difference in concentration of calcium carbonate from those in the laboratory-reared strain, but the wild population was significantly smaller in diameter.

The data supported the claim that shells of freshwater snails are comprised of 95-99.9 % calcium carbonate by weight.

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LITERATURE CITED

Bevelander, G. 1952. Calcification in mollusks. III. Intake and deposition of Ca^{45} and P^{32} in relation to shell formation. *Biological Bulletin* **102**: 9-15.

Borght, O. van der and S. van Puymbroek. 1966. Calcium metabolism in a freshwater mollusk. Quantitative importance of water and food as supply for calcium during growth. *Nature* **210**: 791-793.

Boycott, A. E. 1936. The habitats of fresh-water Mollusca in Britain. *Journal of Animal Ecology* **5**: 116-186.

Fried, B., S. Scheuermann, and J. Moore. 1987. Infectivity of *Echinostoma revolutum* miracidia for laboratory raised pulmonate snails. *Journal of Parasitology* **73**: 1047-1048.

Hare, P. E. and P. H. Abelson. 1965. Amino acid composition of some calcified proteins. *Carnegie Institution of Washington Yearbook* **64**: 223-232.

Macan, T. T. 1950. Ecology of freshwater Mollusca in the English Lake District. *Journal of Animal Ecology* **19**: 124-146.

Marxen, J. C., W. Becker, D. Finke, B. Hasse, and M. Epple. 2003. Early mineralization in *Biomphalaria glabrata*: Microscopic and structural results. *Journal of Molluscan Studies* **69**: 113-121.

Ulmer, M. J. 1970. Notes on rearing of snails in the laboratory. *In:* A. J. MacInnis and M. Voge, eds., *Experiments and Techniques in Parasitology*. W. H. Freeman, San Francisco. Pp.143-144.

U.S. Geological Survey. 2006. Explanation of hardness. Available at: http://water.usgs.gov/owq/Explanation.html 7 November 2006.

White, M.M., M. Chejlava, B. Fried, and J. Sherma. 2005. Effects of various larval digeneans on the calcium carbonate content of the shells of *Helisoma trivolvis, Biomphalaria glabrata,* and *Physa* sp. *Parasitology Research* **95**: 252-255.

Young, J.O. 1975. A laboratory study, using ^{45}Ca tracer, on the source of calcium during growth in two freshwater species of Gastropoda. *Proceedings of the Malacological Society of London* **41:** 439-445.

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