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# Similar Shell Features between Rear and Wild *Cornu aspersum* Snails

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**Abstract** Body weight, shell's diameter, shell's' thickness, shell's mechanical strength and chemical composition of the hypostracum of reared and wild snails (*Cornu aspersum*) were analyzed. Wild adult individuals were collected from Crete (Greece) and compared with adult individual snails reared under laboratory conditions. A standard layer diet supplemented with 20% calcium was used as snails' diet. An EDS (Energy Dispersive Spectrometry) method was used for the analysis of the hypostracum. The EDS analysis was made by the use of a scanning electron microscope. Calcium, oxygen, carbon and silicon were the chemical elements that they were detected at the *C. aspersum* hypostracum. No statistical difference was recorded between the chemical elements of the hypostracum of reared snails and of the wild ones. Both groups had similar body weight, shell mechanical strength and shell thickness.

***Keywords:*** *Cornu aspersum, hypostracum, EDS analysis, shell fracture*

## 1. Introduction

The snails constitute trophic species, which is consumed by millions of people in the world. Intensive stock farming is possible and economically profitable for the species *Cornu aspersum*, which presents high commercial interest in Greece and Europe. Especially in Europe the snail consumption is significantly high. European snails' imports from 1995 to 2010 had a 49% increment [1]. The snail's meat has low calorie and fat content and high amounts of minerals, essential amino-acids and polyunsaturated fatty acids [2,3,4]. Recent research ranked snail meat as one of the positive factors of Mediterranean diet [5]. Various aspects of its biology, ecology and ecogenetics have been studied, as this species is the principal subject for snail farming (heliciculture) in Greece [6,7]. The dietary calcium in snail farming is still a point of disagreement among many researchers [8-17].

A snail, like most other mollusks, uses its mantle to develop its shell by releasing from glands a liquid made up of shell materials. Gradually the liquid hardens and forms the shell. The shell protects the snail's internal organs, prevents water loss, provides shelter from the cold, as well as protection from predators. It consists of three layers: the hypostracum - the innermost layer which is closest to the snail's body, the ostracum – the middle shell layer and the periostracum - the outermost layer. The hypostracum which grows layer by layer, is thicker in some areas and thinner in some others. The inner shell is almost exclusively made of crystals of calcium carbonate called aragonite (95-99% by weight), packed together by conchiolin [18]. The remaining 1-5% is made of organic macromolecules which play an important role in the crystal morphology and mechanical properties [19]. The calcium carbonate crystals of the hypostracum are oriented parallel to the shell surface. The ostracum consists of prism-shaped calcium carbonate crystals and organic molecules. The ostracum and the periostracum are secreted by a marginal band of cells, so that the shell grows at its outer edge. The oxygen, the carbon and probably the calcium, which are used from snails to build their shell, derive from the water that the snails either absorb through their skin or drink from the upper layers of the soil [20,21,22,23,24]. The mechanical strength of the shells is related to their chemical composition and the level of their structural maturity. The mechanical strength is crucial for the survival of the individuals.. Shells containing a higher percentage of calcium were characterized by lower mechanical strength than those containing a lower amount [25].

The snails' shells are used widely in food industry by refilling the empty shells with cooked or salted snails' meat [26]. A recent study suggests the use of the snails' shells for the treatment of waste water of the food industry [27]. A high level of success can be achieved in reducing dissolved solids, nitrates, sulphate and of removal phosphate completely from the waste water.

In this study body weight shell thickness, shell diameter, shell weight and required shell fracture force of wild and reared brown garden snails (*C. aspersum* Müller, 1774) were determined. Also the chemical elements of which the hypostracum is made of were detected and quantified by using a scanning electronic microscope and microanalysis with X-rays (Energy dispersive X-ray spectroscopy). To our knowledge this is the first study of *C. aspersum* hypostracum chemical composition and the required

fracture force of the shell, as well as one of the few in molluscs in general.

## 2. Materials and Methods

*C. aspersum* were borned, bred and raised in the laboratory under standard conditions of temperature (20°C), humidity (90%), photoperiod (12L: 12D). A standard layer diet supplemented with 20% calcium, provided by Pappas Ltd, was used as the snails' daily diet. The approximate composition of this diet is given in Table 1. After reaching their adult stage in a 90-day growing period, five snails were randomly chosen as experimental animals. Their shell diameter was measured (Table 2).

**Table 1. Approximate composition of the diet fed to the laboratory-raised snails**

| Proximate composition | | Vitamin and mineral composition (per Kg of diet) | | | |
|---|---|---|---|---|---|
| Total proteins | 20% | Vitamin A | 12000 IU | Folic acid | 1mg |
| Total celluloses | 3.5% | Vitamin $D_3$ | 3000 IU | Nicotinic acid | 35mg |
| Total lipid | 3.8% | Vitamin E | 60mg | Choline | 300mg |
| Moisture | 12% | Vitamin $K_3$ | 6mg | Iron | 50mg |
| Ash | 5.2% | Vitamin $B_1$ | 2mg | Manganese | 100mg |
| Calcium | 20.9% | Vitamin $B_2$ | 7mg | Iodine | 1mg |
| Total phosphorus | 0.6% | Vitamin $B_6$ | 6mg | Cobalt | 2mg |
| Sodium | 0.15% | Vitamin $B_{12}$ | 0.3mg | Zinc | 55mg |
| Lysin | 1% | Biotin | 0.2mg | Selenium | 0.1mg |
| Sulfur amino acids | 0.6% | Pantothenic acid | 10mg | Coccidostat | 500mg |

**Table 2. Measurements of the body weight, shell weight, shell diameter, shell thickness and shell fracture force of rear and wild snails (Mean±SE)**

| Rear snails | | Wild snails | |
|---|---|---|---|
| Body weight (g) | 8.85±1.37 | Body weight (g) | 8.06 ±1.97 |
| Shell weight (g) | 1.46±0.12 | Shell weight (g) | 2.28±0.29 |
| Shell diameter (mm) | 29.98±1.95 | Shell diameter (mm) | 32.94±1.90 |
| Shell thickness (mm) | 0.38±0.06 | Shell thickness (mm) | 0.44±0.06 |
| Shell fracture force (N) | 13.0±2.5 | Shell fracture force (N) | 15.1±2.2 |

Wild adult *C. aspersum* of similar marketable size to the reared ones were collected from Krousonas (35°13′50″N 24°58′58″E), Crete (Greece) in April 2010. The area is at 700m altitude, a typical Mediterranean phrygana ecosystem and is characterized by calcareous rocky soils. Five snails were used as control animals. Their shell diameter was measured and is shown at Table 2.

All snails were fully anaesthetized when sacrificed during the shell fracture procedure. For anaesthesia, we used clove tree oil and water. 20 drops of *Eugenia caryophyllus* oil were diluted in 50ml water [28]. Snails were kept in this emulsion for 2h at room temperature in order to relax and extend their body.

The required fractured force of the shells was determined by the use of an ADMET eXpert 5600 testing machine, equipped with a SM-250 force sensor connected with a ram of 1.2cm diameter. Each snail was put with the upper side of its shell under the ram. The ram began to move downwards with low velocity and stopped only when the shell was fractured. Then it returned automatically to its original position.

After fracture the body weight of each snail was measured and all the parts of the shell were collected in order to determine the shell weight and the shell thickness (Table 2). At least three parts of the fractured shell of each snail were used for an EDS (Energy Dispersive Spectrometry) analysis of the hypostracum. The EDS analysis was made by the use of a JEOL JSM-5600 scanning electron microscope equipped with an OXFORD Link ISIS 300 system. Each measurement was last 360 sec and was made in a 0.25$mm^2$ area.

Measurements of the body weight, shell weight, shell diameter, shell thickness and shell fracture force of rear and wild snails were checked for normality using the Shapiro-Wilk test. Statistical comparisons between the groups were made using a two sample t- test. All values are presented as means ± standard error and differences presented at 5% level are considered significant.

## 3. Results

All data were tested for normality and found to be drawn from a normally distribution population. The mean values of the body weight were 8.06 ± 1.97g and 8.85± 1.37g for wild and rear snails, respectively. A two sample t-test (p=0.48) showed that the mean values of both groups were similar. A same t-test showed that the shells of the reared snails (1.46± 0.12g) appeared to be significant lighter (p= 0.03) than the shells of the wild snails (2.28± 0.29g). The shell diameter of the rear snails (29.98±1.95mm) was also statistically different (p=0.04) from the shell diameter of the wild snails (32.94± 1.90mm). As regarding the shell thickness, the mean values were 0.44±0.06mm and 0.38±0.06mm for wild and rear snails, respectively. Statistically these values were similar (p=0.36). The shells of the wild snails needed a slightly bigger force for being fractured than the shells of the reared snails (15.1±2.2 N and 13.0±2.5 N respectively). This shell fracture force difference was statistically insignificant (p=0.55). All measurements are shown in Table 2.

An EDS analysis showed that the hypostracum of the rear snails contained carbon (C), calcium (Ca), oxygen (O) and silicon (Si) in a conciseness of 31.165%, 12.9%, 55.86% and 0.075%, respectively (Figure 1). Same results turned up for the wild snails. Their hypostracum conciseness at carbon, calcium, oxygen and silicon was 30.64%, 11.144%, 58.13% and 0.086%, respectively (Table 3). A Shapiro-Wilk normality test was applied to the hypostracum EDS analysis data. For all the chemical elements of the hypostracum for both groups of rear and wild snails, the data were found to be drawn from a

normally distribution population. For each chemical element of rear and wild snails' hypostracum a two-sample t-test was applied. At the 0.05 level no statistical difference was found between the chemical elements of the hypostracum of reared snails and of the wild ones (p=0.40, p=0.89, p=0.32, p=0.16 for oxygen, carbon, calcium and silicon, respectively).

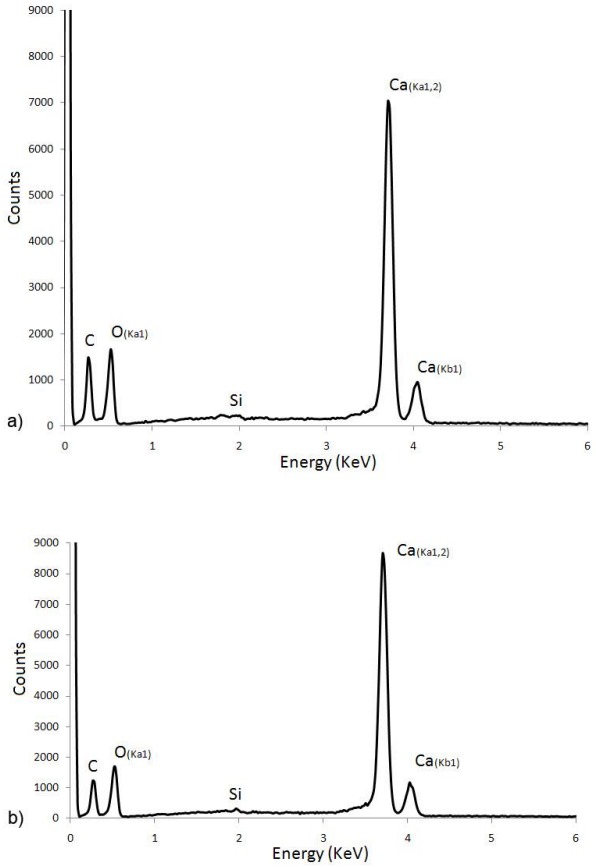


**Figure 1.** a) X ray spectrum from the EDS analysis of wild *Cornu aspersum* hypostracum, b) X ray spectrum from the EDS analysis of rear *Cornu aspersum* hypostracum

**Table 3. Rear and wild snails' hypostracum composition (%)**

| Chemical Elements | Reared snails' hypostracum composition (%) | Wild snails' hypostracum composition (%) |
|---|---|---|
| O | 55.86 | 58.13 |
| C | 31.165 | 30.64 |
| Ca | 12.9 | 11.144 |
| Si | 0.075 | 0.086 |

## 4. Discussion

The objective of this study was to determine if there are any differences in the shell formation of the wild and reared *C. aspersum*, to provide information about the constitution of the their hypostracum and to determine their shell fracture force. The results showed a non - significant difference concerning shell fracture force and shell thickness of rear and wild snails. Also a non - significant difference was recorded for the chemical constitution of the shell's hypostracum. Empty snails' shells have a remarkable commercial value because they are promoted to the industries and are refilled with salt snails' meat or snails' flesh treatment with butter, parsley, garlic and other spices [26]. For that reason the production of high quality snails' shells is one of the objectives of the snail farming industry. In order for snail farming to fulfill its role in providing a high quality product it has to adopt practices which will ensure that the end product is comparable to that from wild populations.

Helicids and similar snails have been traditionally regarded as generalist herbivores feeding on a wide variety of living or dead plant material. Their diet usually includes leaves, stems, soft barks, fruit and vegetables [29,30]. However, in some places there are localized species that are carnivores or omnivores. In our study the reared snails were fed daily on a formulated diet enriched with 20% calcium, vitamins and minerals (Table 1). Shells containing a higher percentage of calcium are characterized by lower mechanical strength than those containing a lower amount [25]. The hypostracum calcium conciseness is important for the formation of the whole shell. Prolonged starvation leads to shell thinning as a result of the release of mineral salts from the ostracum and hypostracum [31,32]. These salts are transported via the hemolymph to the calcic cells. The periostracum, which is further removed from the mantle, is little affected by these processes of accumulation and release.

The standard layer diet (supplemented with 20% calcium) that was used in our study for rearing the snails and the standard lab conditions of temperature (20 ℃), humidity (90%) and photoperiod (12L:12D) were suitable for the production of marketable size snails in a 90 days period. In nature the same size is reached within 2 years. Snail farmers often ask about the rate of calcium inclusion in snail diets. Thompson and Cheney [17] reported that 40% limestone flour promoted good growth in *C. aspersum* (former *Helix aspersa*). Daouda [33] used 15% oyster shell for growing *Achatina achatina* while Amubode and Ogogo [34] used 20% bone meal and 30% oyster shell in diets for *Archachatina marginata*. Our study showed that snails (*C. aspersum*) fed a standard layer diet supplemented with 20% calcium can produce a shell with similar thickness and mechanical resistance of the wild ones, despite the fact that their shells were smaller and lighter than the wilds' ones. Their hypostracum's chemical composition is also similar with the wild ones. Thus, the reared snails were able to absorb all the necessary mineral salts from the calcium enriched standard layer diet and to produce a "healthy" shell.

In conclusion, the qualitative and the quantitative EDS analysis revealed that the hypostracum's of the rear and wild *C. aspersum* snails do not differ. The body weight, shell fracture force and shell thickness were also similar. The above lead to the conclusion that the used calcium enriched standard layer diet was suitable for a rapid (90 days) growth and its mineral salts were absorbable from the rear snails in order to produce a similar to the wilds shell.

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