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Source: American Malacological Bulletin, 38(2) : 23-33

Published By: American Malacological Society

URL: <https://doi.org/10.4003/006.038.0204>

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Size related differences in organic and mineral components of molluscan shell

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Abstract: The organic component of the molluscan shell allows for orderly biomineralization and ensures structural integrity that is crucial to survival. This organic contribution to the shell typically composes 2-5% of the total adult shell by weight. Because macro- and microstructure of the shell is known to vary with ontogeny and across taxa, we examined if the organic to mineral ratio components in shell also varied with growth across taxa. To assess intraspecific differences in the organic to mineral ratio of the shell during growth, we examined ratios in three marine [*Crepidula fornicata* (Linnaeus, 1758), *Littorina littorea* (Linnaeus, 1758), and *Littorina saxatilis* (Olivi, 1792)] and two freshwater [*Corbicula fluminea* (Müller, 1774) and *Bellamyia chinensis* (Gray, 1834)] mollusks across size ranges. In the marine gastropods, the average organic component by weight of the small size class was significantly larger than the average organic proportions of the medium and large size classes. The smallest size class of *L. saxatilis* had an average shell organic proportion of 11.12%, while the smallest size classes of *C. fornicata* (3.53%) and *L. littorea* (2.60%) had percentages below 5%. The smallest size class of *C. fluminea* had a greater average shell organic proportion than the largest size class (6.19% vs 2.68% organics). Adult specimens of *B. chinensis* had an average shell organic proportion of 3.93%, while *in utero* shelled juveniles had an average of 10.05%. In both freshwater and marine species, the smallest size class had a greater organic proportion. As the organic matrix is energetically more expensive than the calcified shell portion, we hypothesize that energy expended in these smaller (usually pre-reproductive maturity) stages of growth allows for a more rapid production of shell and that this “expense” is a valuable trade-off for the protection the shell offers young mollusks.

Key words: organic matrix, calcification, mollusc shell, Gastropoda, Bivalvia

The molluscan shell is typically composed of 2-5 calcified layers plus an external organic periostracum (Marin *et al.* 2012). The calcified layers, which naturally occur as aragonite, calcite, and (rarely) vaterite (Nebel and Epple 2008), have an organic infrastructure, or organic matrix that comprises a small percentage of the shell and is of fundamental importance to the orderly biomineralization of the shell.

This organic matrix, typically making up 2-5% of the adult mollusk shell, has been suggested to act as a calcification inhibitor and a regulator for shell growth, shell structure (macro and micro), crystal nucleation, and crystal orientation (Suzuki *et al.* 2017). The organic matrix is composed of lipids, proteins, and polysaccharides such as chitin (Suzuki *et al.* 2017), and forms the periostracum, intracrystalline matrix, and intercrystalline matrix (Marin *et al.* 2012). For example, organic components both surround nacre tablets as the intercrystalline matrix and are found within tablets as the intracrystalline matrix (Marin *et al.* 2013). The typically thin periostracum helps prevent corrosion of the calcified shell, but also serves to inhibit the invasion of parasitic/boring organisms, provides a foundation for shell growth, may assist in camouflaging the animal, and aids in sealing the extrapallial space, which is instrumental in mineral deposition that furthers shell growth (Watabe 1988, Marin *et al.* 2012, Clark *et al.* 2020).

In ocean habitats, where carbonate and calcium ions are abundant, physiological regulation is required to use those components to form structurally strong and organized shells. Orderly biomineralization and microstructure are imperative within the molluscan shell and here the organic matrix is, in large part, responsible (Wheeler and Sikes 1984). With that, we question if the proportion of organic to mineral components in the mollusk shell changes with shell size?

Nearly 50% of metabolic energy expended in producing the shell is associated with the organic matrix (Palmer 1992). Contributing half the total energy allocated for shell production to creating such a small component (< 5%) could seem out of proportion, but not when considering the essential roles of the organic matrix and the fact that metabolic rates are typically higher in younger animals than older (Schöne 2008, Butler *et al.* 2011, Glazier *et al.* 2015, Suzuki *et al.* 2017, Ruiz *et al.* 2018).

Most of the relevant literature suggests that the proportion of shell organics in mollusks hovers around 5% or less by weight (Weiner and Traub 1984, Glover and Kidwell 1993, Marin *et al.* 2007, De Paula and Silveira 2009, Marin *et al.* 2012, Osuna-Mascaró *et al.* 2014, Clark *et al.* 2020). Using chemical extraction with 0.1 M trichloroacetic acid, Goulletquer and Wolowicz (1989) determined that shell organic material comprised 5.16% and 9.60% of the total organism organics (body + shell) for *Cardium glaucum* (Bruguère,

1789) and *Cardium edule* (Linnaeus, 1758), respectively. Price *et al.* (1976) found organic percentages in shells ranged from 1.4% in *Argopecten irradians* (Lamarck, 1819), a species with a very thin periostracum, to 21.4% in *Solemya velum* (Say, 1822), a species with an extensive periostracum. The periostracum alone in *S. velum* likely accounts for the large organic content. This would not necessarily reflect a change in intra- or intercrystalline organic matrix with growth but a change in the proportion associated with the periostracal cover. However, some suggest that younger mollusks have a larger organic proportion within their shells (Price *et al.* 1976, Gouletquer and Wolowicz 1989, Prezant *et al.* 2006, Thomsen *et al.* 2013). The shell organic material in younger individuals of *Mytilus edulis* (Linnaeus, 1758) was on average greater than 10% by weight of the shell, while adult individuals of the same species had shell organic content ranging from 1 to 6% (Thomsen *et al.* 2013).

Preliminary work by the current authors found that the organic proportion in the shells of the freshwater Asian clam *Corbicula fluminea* (Müller, 1774) decreased with age. This suggests a proportional decrease in organic shell component with growth. In this study, we expand on this work to determine if the relative proportion of organic to mineral component in mollusks decreases as they grow/age using various common species from freshwater and marine coastal mid-Atlantic sites [marine gastropods *Crepidula fornicata* (Linnaeus, 1758), *Littorina littorea* (Linnaeus, 1758), and *Littorina saxatilis* (Olivier, 1792), along with freshwater gastropod *Bellamya chinensis* (Gray, 1834)]. We hypothesized that the proportion of organic to mineral components associated with the shell decreases with age and ontogenetic size.

MATERIALS AND METHODS

Specimens of both freshwater and marine mollusks from southern Connecticut, USA, were collected and processed to determine relative proportions of organic to mineral components in shells through growth. Individual methodologies for these groups are comparable but are detailed below.

Corbicula fluminea

Specimens of the freshwater bivalve *Corbicula fluminea* were collected in shallow, slow moving waters of the Wepe-waug River in Eisenhower Park, Milford, Connecticut, USA

(41°15'0"N, 73°3'25"W), on 8 September 2018. All organisms used in this study were collected under Connecticut Department of Energy and Environmental Protection permit no. 1821006 and all were from large populations. Clams were found burrowed beneath or just at the surface of sandy sediments. Clam shell length ranged from 7.8 – 8.1 mm in the small size class, 10.4 – 11.4 mm in the medium size class, and 15.1 – 16.1 mm in the large size class (Table 1). Clams were maintained in small aquaria using river water at ambient room temperature through 4 December 2018 and acted as a control group for an unpublished study performed by the authors. They were preserved in 70% ethanol on 4 December 2018. Wet weight (nearest 0.0001 g), length (nearest 0.01 mm), height (nearest 0.01 mm), and breadth (nearest 0.01 mm) measurements were recorded (after preservation) with an analytical balance and caliper respectively (Kosnik *et al.* 2006).

Soft tissues were carefully extracted from shells using forceps. Shells surfaces were dried with soft, lintless tissue, wet-weighted and heat dried at 100°C for 24 hours (Ricciardi and Bourget 1998). The shells were removed from the oven and reweighed to determine dry weight. All shells were stored in a desiccator after drying.

Prior to combustion to determine shell organic content, each shell was crushed with a mortar and pestle and placed individually in a pre-weighed crucible. The overall mass of the crucible and the shell was recorded. The crucible was then placed in a combustion oven at 550°C for 60 minutes (Prezant *et al.* 2006). Crucibles were removed from the oven, and briefly cooled to room temperature to allow weighing to determine post-combustion weight. Percent shell organic content was determined with the following formula: ((mass of dried shell – mass of combusted shell)/mass of dried shell) (Prezant *et al.* 2006).

Bellamya chinensis

Specimens of the viviparid gastropod *Bellamya chinensis* were collected from shallow waters of Mondo Ponds, Milford, CT (41°13'6"N, 73°5'20"W) on 8 July 2020. Nine females and 6 males were collected. The substratum from the collection site was smooth rock surfaces covered with short epiphytes and nearby muddy, soft sediments. Snails were brought to the laboratory and shells were cleaned of surficial epibionts and then manually cracked before placing specimens in 70% ethanol to ensure internal penetration of the preservative. Animal

Table 1. Size (length in mm) ranges for specimens of *Crepidula fornicata*, *Littorina littorea*, *Littorina saxatilis*, *Corbicula fluminea*, and *Bellamya chinensis*.

| Size Class | <i>C. fornicata</i> | <i>L. littorea</i> | <i>L. saxatilis</i> | <i>C. fluminea</i> | <i>B. chinensis</i> |
|------------|---------------------|--------------------|---------------------|--------------------|---------------------|
| Small | 5.9 – 10.5 | 9.4 – 11.55 | 5.0 – 8.0 | 7.8 – 8.1 | 3.7 – 9.0 |
| Medium | 17.45 – 22.7 | 14.0 – 15.85 | 10.0 – 14.0 | 10.4 – 11.4 | |
| Large | 35.65 – 41.7 | 16.9 – 20.0 | 15.0 – 17.55 | 15.1 – 16.1 | 41.7 – 52.7 |

wet weight (nearest 0.0001 g) along with shell length (nearest 0.01 mm) and width (nearest 0.01 mm) were recorded with an analytical balance and caliper respectively after animals were preserved in 70% ethanol (Kosnik *et al.* 2006). After preservation, specimens were sorted based on sex and any shelled juvenile gastropods found *in utero* were removed and placed in separate vial of 70% ethanol. Developing juveniles were found in eight of the nine adult female gastropods collected. All *in utero* specimens had shell length, width, and wet weight recorded using an ocular micrometer and analytical balance, respectively. *In utero* gastropods were separated into three mixed size groups with an even distribution of different shell lengths ranging from 3.7 mm to 9.0 mm in shell length. Adult and *in utero* groups were treated the same as the specimens previously described to determine shell organic proportion. Average shell organic proportions of *B. chinensis* were compared based on sex as well as size class.

Marine gastropods

Specimens of various sized coastal gastropods *Crepidula fornicata*, *Littorina littorea*, and *Littorina saxatilis* were collected from or near the intertidal zone at Silver Sands Beach, Milford, Connecticut, USA (41°11'49.5"N 73°04'11.9"W). Specimens of *C. fornicata* and *L. saxatilis* were collected on 25 May 2019. Additional specimens of *L. saxatilis* plus specimens of *L. littorea* were collected on 6 October 2019. Specimens were preserved in 70% ethanol in the field upon collection either on 25 May 2019 or 6 October 2019.

Thirty specimens were chosen randomly and placed in categories of "small," "medium," and "large," with 10 individuals in each size class. The determination of gastropod sizes used was based on the relative size range within populations sampled (Table 1).

The presence of barnacles and other epibionts on littorines were noted and photographed before and after ultrasonic bath treatment. Ultrasonic bath treatment was completed to help remove any epibionts left after manual removal. Any epibionts remaining after sonication were carefully removed prior to weighing the gastropods. Wet weight (nearest 0.0001 g), length (nearest 0.01 mm), and width (nearest 0.01 mm) were recorded with an analytical balance and caliper respectively after specimens were preserved (Kosnik *et al.* 2006). Organic proportion was determined using the same methodology as previously described.

Statistical analysis

Length (mm) vs. percent organics and wet weight (g) vs. percent organics were plotted and a linear regression was produced for the marine gastropods and *Corbicula fluminea*, resulting in R^2 , slope, and p-values for each graph. A one-way ANOVA was performed using Microsoft Excel (Edis *et al.* 2018, Huang and Shih 2020) to assess if there were intraspecific differences present in the percent organic for the marine gastropods and *C. fluminea*. Tukey's honest significance post-hoc test was performed to assess pair-wise differences between size classes within each species.

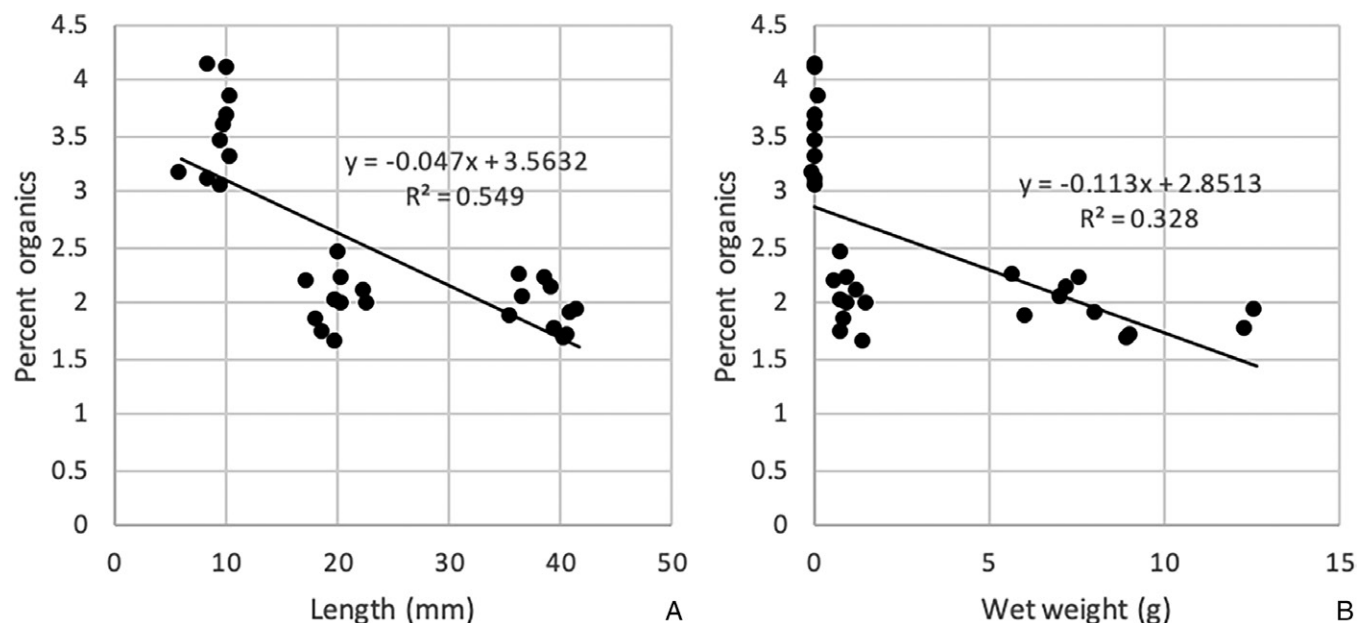


Figure 1. (A) Length (mm) vs. percent organics and (B) wet weight (g) vs. percent organics averages measured in the shell of *Crepidula fornicata*.

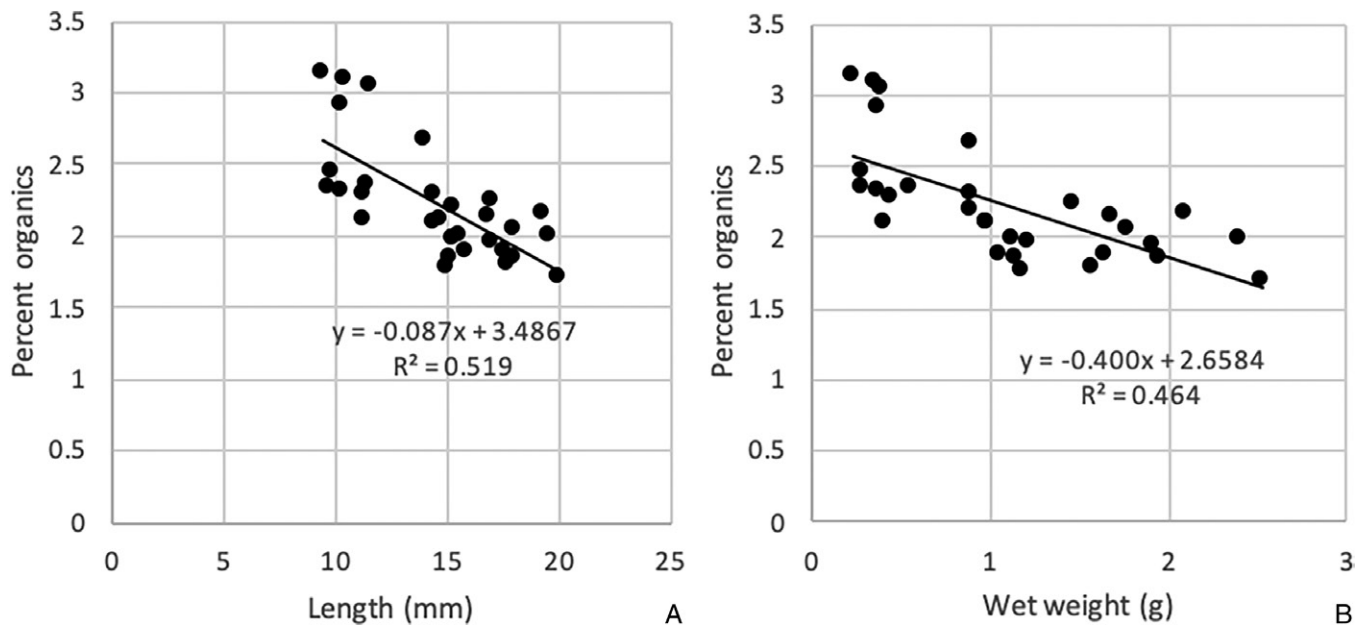


Figure 2. (A) Length (mm) vs. percent organics and (B) wet weight (g) vs. percent organics averages measured in the shell of *Littorina littorea*.

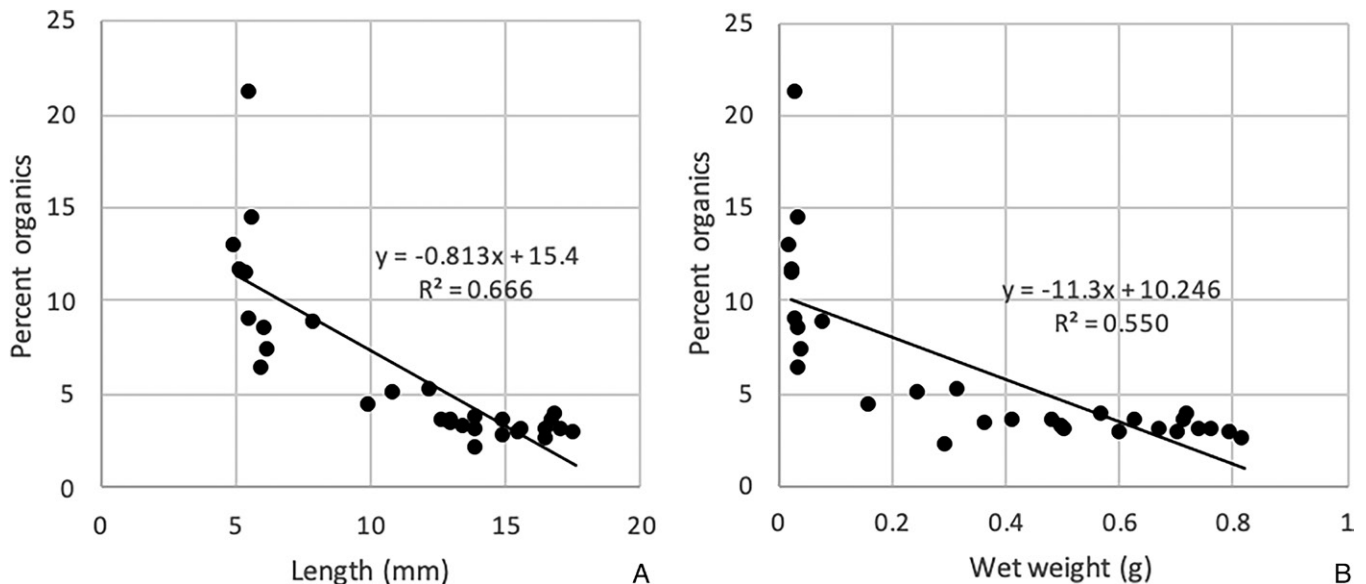


Figure 3. (A) Length (mm) vs. percent organics and (B) wet weight (g) vs. percent organics averages measured in the shell of *Littorina saxatilis*.

A one-way ANOVA test was performed on Microsoft Excel (Edis *et al.* 2018, Huang and Shih 2020) in order to test if there was a significant difference between the shell organic proportions measured in male and female *Bellamya chinensis* individuals.

RESULTS

As wet weight and length increased, shell organic proportion decreased for *Crepidula fornicata* (length, $R^2 = 0.549$, $m = -0.047x$, $p < 0.001$; wet weight, $R^2 = 0.328$, $m = -0.113x$,

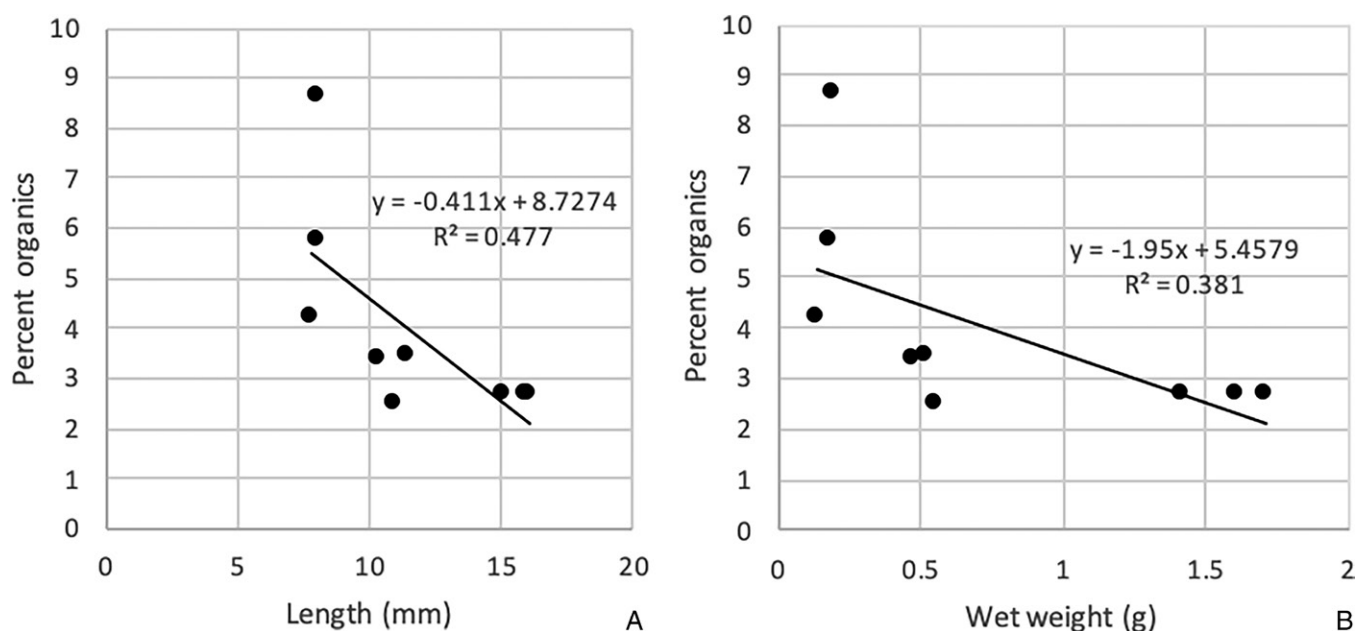


Figure 4. (A) Length (mm) vs. percent organics and (B) wet weight (g) vs. percent organics averages measured in the shell of *Corbicula fluminea*.

$p < 0.001$), *Littorina littorea* (length, $R^2 = 0.519$, $m = -0.087$, $p < 0.001$; wet weight, $R^2 = 0.464$, $m = -0.400x$, $p < 0.001$), *Littorina saxatilis* (length, $R^2 = 0.666$, $m = -0.813x$, $p < 0.001$; wet weight, $R^2 = 0.550$, $m = -11.3x$, $p < 0.001$), and *Corbicula fluminea* (length, $R^2 = 0.477$, $m = -0.411x$, $p = 0.039$; wet weight, $R^2 = 0.381$, $m = -1.95x$, $p = 0.077$; Figs. 1–4). *Crepidula fornicata* specimens had an average of 3.525 ± 0.401 (st dev) % in the smallest size class, $2.007 \pm 0.240\%$ in the medium size class, and $1.934 \pm 0.208\%$ in the largest size class. Individuals of *L. littorea* showed an average of $2.60 \pm 0.395\%$ in the smallest size class, then $2.08 \pm 0.259\%$ in the medium size class, and $1.97 \pm 0.174\%$ in the largest size class. *Littorina saxatilis* specimens had an average of $11.12 \pm 4.31\%$ in the shell organics of the smallest size class, $3.66 \pm 0.920\%$ in the medium size class, and $3.06 \pm 0.413\%$ in the large size class. Significant differences in shell percent organics were detected within species when comparing the three size classes of *Crepidula fornicata* (One-Way ANOVA, $F_{2,27} = 92.55$, $p < 0.001$), *Littorina littorea* (One-Way ANOVA, $F_{2,27} = 13.41$, $p < 0.001$), and *Littorina saxatilis* (One-Way ANOVA, $F_{2,27} = 30.73$, $p < 0.001$).

The smaller specimens of *C. fluminea* had an average percent organic of $6.19 \pm 2.25\%$ with a maximum of 12.07% . Medium and large sized clams had average organic proportions of $3.10 \pm 0.545\%$ and $2.68 \pm 0.021\%$, respectively. Significant differences in shell percent organics were detected within species when comparing the three size classes of *Corbicula fluminea* (One-Way ANOVA, $F_{2,6} = 6.187$, $p < 0.05$).

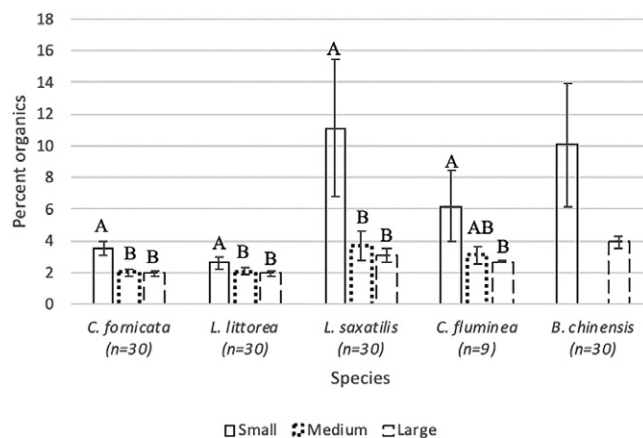


Figure 5. Mean percent shell organics in each size class of species analyzed (note: the “small” size class for *B. chinensis* refers to shelled *in utero* juveniles, while the “large” size class refers to fully grown adults). Groups marked with different letters were significantly different from one another as indicated by Tukey’s honest significance post-hoc test. Specific size classes are denoted in Table 1.

Adult specimens of *Bellamya chinensis* had an average shell organic proportion of $3.93 \pm 0.385\%$. *In utero* shelled juveniles had shell organic proportions averaging $10.05 \pm 3.86\%$. Sex did not influence adult shell organic proportion (One-Way ANOVA, $F_{1,10} = 1.078$, $p > 0.05$).

Shell organics decrease as the mollusks reach larger sizes (Fig. 5, Table 2). This trend was consistent in each of the

Table 2. Average shell organic percent for each size class of *Crepidula fornicata*, *Littorina littorea*, *Littorina saxatilis*, *Corbicula fluminea*, and *Bellamya chinensis*. s = standard deviation

| Size Class | <i>C. fornicata</i> | <i>L. littorea</i> | <i>L. saxatilis</i> | <i>C. fluminea</i> | <i>B. chinensis</i> |
|------------|---------------------|--------------------|---------------------|--------------------|---------------------|
| Small | 3.525% s = 0.401 | 2.60% s = 0.395 | 11.12% s = 4.31 | 6.19% s = 2.25 | 10.05% s = 3.86 |
| Medium | 2.007% s = 0.240 | 2.08% s = 0.259 | 3.66% s = 0.920 | 3.10% s = 0.545 | |
| Large | 1.934% s = 0.208 | 1.97% s = 0.174 | 3.06% s = 0.413 | 2.68% s = 0.021 | 3.93% s = 0.385 |

Table 3. Tukey’s honest significance test (HSD) values. These values test for significant differences in mean shell organic proportions between size classes (within species).

| Species | HSD Value |
|----------------------------|-----------|
| <i>Crepidula fornicata</i> | 0.327 |
| <i>Littorina littorea</i> | 0.314 |
| <i>Littorina saxatilis</i> | 2.84 |
| <i>Corbicula fluminea</i> | 3.34 |

five species analyzed. There were no significant differences between the average organic proportion in medium vs large shells in any marine gastropod species (Table 3). There were no significant differences between specimens of *L. saxatilis* collected in May or October of 2019.

DISCUSSION

The physiological and structural importance of the organic component of molluscan shell has long been recognized (Wheeler and Sikes 1984, Dauphin 2001, Nishida *et al.* 2011, Suzuki *et al.* 2017, Jain *et al.* 2018, Schoeppler *et al.* 2018, Bruggmann *et al.* 2019). Similarly, the functional microstructure and ontogenetic changes of shell structure and microstructure are also well known (Kemp and Bertness 1984, Bandel 1991, West and Cohen 1994, Román-González *et al.* 2017, Checa 2018, Wan *et al.* 2019). Absent from the literature are details of ontogenetic changes that could take place in the shell’s organic matrix with growth and any functional significance of these possible changes.

The current study demonstrates that smaller shells of three species of marine (*Crepidula fornicata*, *Littorina littorea*, *Littorina saxatilis*) and two species of freshwater (*Corbicula fluminea*, and *Bellamya chinensis*) mollusks, when compared to larger specimens, have a greater shell organic to mineral ratio. While the shell is the exoskeletal protection of most mollusks through most of their lives, and there is a recognized correlation between shell thickness and shell strength

(Zuschin and Stanton 2001, Zuschin *et al.* 2003, Vasconcelos *et al.* 2011), we suggest that the proportionally greater percent of organic matrix in juvenile shells lends additional protection to the thinner shells of younger mollusks. The actual source of the higher organic component we believe is primarily from a greater content within the shell’s organic matrix and not a proportional change (decrease) in mineral content nor increase in relative periostracal thickness. In specimens used here, the periostracum was typically intact and very thin in littorinid specimens, exceptionally thin to absent in specimens of *Crepidula*, thin in *Corbicula* with some umbonal erosion typical, and well developed but also thin in *Bellamya* with erosion often found in the older parts of the shell. While the thickness, and thus overall weight contribution of the periostracum, could be a variable when considering shell organics and could change over time and age (but only at the growing edge), it is unlikely that this would change the trends in organics seen here since the surface of the forms a uniform “mold” for this outer and proportionally uniform cover in species examined here.

In one of the few reports that examined proportionalities of shell organics, Vinogradov (1953) stated that bivalve shells had a greater organic to mineral content than gastropods, and estuarine and marine mollusks a greater organic content than freshwater mollusks. In addition, periostracum in freshwater mollusks tends to be thicker than those that live in typically warmer ocean waters (Watabe 1988). The results here (albeit limited in species examined) found that specimens of the freshwater bivalve *C. fluminea* had average organic proportions less than that of the marine gastropod *Littorina saxatilis* in each size class. However, average organic proportions measured in each of the size classes of *C. fornicata* and *L. littorea* were not consistently greater than those of *C. fluminea*. Neither Vinogradov (1953) nor Watabe’s (1988) suggestions regarding the relative proportion of organic shell in marine/estuarine or freshwater environments is supported by the limited data in this study.

Predation and shell organics

A greater proportion of organic matrix in juvenile shells is one possible strategy to increase chances of survival for

younger (smaller) mollusks (Prezant *et al.* 2006) that are threatened by a compromised shell. The organic matrix adds flexibility, adds paths for microfractures (that prevent major shell failure) along inter- and intracrystalline matrices, and increases overall resistance to shell fracture (Li *et al.* 2017). It is not unusual for juvenile gastropods to be targets of shell cracking or crushing predators, such as the blue crab *Callinectes sapidus* (Rathbun, 1896), which prefers small specimens of *Littorina irrorata* (Vaughn and Fisher 1988). With a higher organic proportion in the shells of younger mollusks, a failed and sublethal attack would better support shell repair and survival. In many cases, younger, thinner shelled mollusks can be at higher risk of predation than larger, thicker shelled adults, the latter creating an “ontogenetic refuge” (Harding 2003, Grey *et al.* 2005). Here, a possible trade-off in distribution of energy towards shell production could better secure early life stages.

Adult *C. fornicata* are sedentary and are preyed upon by various crabs (Pechenik *et al.* 2010). The thicker adult shell offers some level of protection not found in juveniles. Vasconcelos *et al.* (2011) suggest that shell thickness is a strong correlate with shell strength (as demonstrated in species of Mytilidae, Veneridae, and Arcidae). Zuschin and Stanton (2001), however, note that shell size is measured in height, width, and length and thus not necessarily always a direct correlate to shell thickness. As such, care must be taken in drawing too strong a link between overall shell size, rather than shell thickness, as a defense of soft tissue. The association between predation and prey size, in fact, is not universal. Pechenik *et al.* (2010), for instance, demonstrated that the crab *Hemigrapsus sanguineus* (De Haan, 1853) preyed heavily upon juveniles of *C. fornicata* in the lab but the rate of predation increased as juveniles grew. This could be related to consumer energy dynamics and the balance between predation effort and nutritive value of a larger accessible food resource – an example of energy maximization (Griffiths 1975). Similarly, the freshwater crab, *Zilchiopsis collastinensis* (Pretzmann, 1968) in the Paraná River, Argentina, selectively consumed larger invasive golden mussels, *Limnoperna fortunea* (Dunker, 1857) despite the extended time needed to gain access to the soft tissue (Torres *et al.* 2012). It is possible that this is a result of the larger visual cue the larger mussels offer making them more readily available, the difficulty the crabs have handling smaller shells, or a result of optimal foraging (all reviewed by Torres *et al.* 2012). We have little information relating energy balance, shell development, shell organics, shell size, and predator/prey interaction.

Size and shell thickness does matter for *C. fluminea*. Predatory crayfish more readily feed upon smaller specimens of the Asian clam (Covich *et al.* 1981, Pereira *et al.* 2016). The larger and thicker shells of adult clams offer increased structural resistance for crayfish claws. Pereira *et al.* (2016) suggest

that the preference for smaller clams by the crayfish *Procambarus clarkii* (Girard, 1852) was related to the greater effort, and thus energy expended, in attempting to open a larger and thicker shelled clam. The cyprinid fish *Luciobarbus bocagei* (Steindachner, 1864) is only limited in the ingestion of *C. fluminea* by the diameter of its own oral cavity as these barbels ingest clams in their entirety (Pereira *et al.* 2016). While not relevant to a predator that consumes the entire animal through a suctorial mouth, for the smaller clams that are preferred by crayfish an interrupted attack that leaves only a partially damaged shell could mean a greater chance for healing and survival.

Development, reproduction and shell organics

Crepidula fornicata can have shells that grow up to 59 mm long and 26 mm wide (Emerson and Jacobson 1976, Prezant *et al.* 2002). Males that belong to this species usually reach sexual maturity within 2 months of life, measuring around 4 mm in length (Henry *et al.* 2010). The smallest size class representing this species in this study ranged from 5.9 mm to 10.5 mm; all were presumed to be sexually mature and male. The smallest size class, although sexually mature, still had the greatest shell organic proportion on average in comparison to the other larger size classes. The largest size class of *C. fornicata* used in this experiment ranged from 35.65 mm to 41.7 mm and were female. The energy allocation shift from growth to reproduction is well documented (for example, see Ishida 2004). For this species, the energy involved in supporting sexual maturity in the smaller males is also much lower than that of the larger females who develop and support large, nutritive ova (Broquet *et al.* 2015). The larger females also likely distribute energy away from shell growth and towards reproduction as demonstrated by Chaparro and Flores (2002) for *Crepidula fedunda* (Gallardo, 1979). In the latter, considerable energy is focused on generation of gametes during brooding. Similarly, in *Crepidula dilatata* (Lamarck, 1822), energy expended on broods increases as the brooding female gets larger (Chaparro *et al.* 1999). Similar energy trends are seen in *Conus pennaceus* (Born, 1778) (Perron and Corpuz 1982). The rapid growth of juveniles is essential to produce thicker shells that are more resistant to predation but there is an energy cost. We suggest that with age, maturity and concomitant growth, there is a reallocation of energy from producing proportionally more shell organics to reproduction and gamete and young development.

Predators of *C. fornicata* might gain entry by cracking the shell proper or dislodging their “footing” from their hard substratum perch, possibly cracking the shell margin in the process. A greater proportion of organic material in the thinner shell of smaller specimens could lend the shell flexibility and

resistance to fracture and may be a strategic way to increase survival.

Littorina littorea are oviparous with shells morphology influenced by habitat (Kemp and Bertness 1984). Larvae are planktotrophic with planktonic existence lasting several weeks (Grahame 1973), thus environmental conditions at ultimate settling site can vary. Offspring from the same parent, although closely related genetically, may look different and might have different shell organic proportions reflecting their environmental homes after dispersal. Individuals of *L. littorea* can grow up to 42 mm in height (Emerson and Jacobson 1976). In this study, the largest specimen used was 20.0 mm. Thus, the mineral to organic ratio throughout the lifespan and full-size range is not documented. *Littorina littorea* typically reach maturity at about 13 mm in length (Saier 2000) and specimens used here did span each side of that marker with the smallest specimens ranging from 9.4 mm to 11.55 mm in length. The average shell organic proportion found in each size class was within the lower range of the usual 2-5% range, however, the smallest group (assumed sexually immature) had the greatest average shell organic proportion at 2.60% out of the three size classes. This again supports the inverse relationship between relative organic shell content and size and the likelihood that sexually immature individuals allocate a greater proportion of energy towards production of the organic shell component.

Littorina saxatilis is ovoviviparous and also shows developmental plasticity with habitat. Specimens of *L. saxatilis* that reside in the upper shore of Galician beaches have ridged, banded, and overall larger shells, while those in the lower shore have smooth, non-banded, and smaller shells (Carballo *et al.* 2005, Conde-Padín *et al.* 2008). The live-born juveniles must endure the intertidal region of a wave impacted rocky intertidal zone. Clearly here a shell that has structural integrity is crucial. In the upper shore, gastropods are most threatened by predation and heat desiccation, while in the lower shore, wave action is the greatest environmental threat (Conde-Padín *et al.* 2008). The support, flexibility, and fracture resistance the juvenile organic matrix lends to shells is likely important in this challenging environment.

Adult specimens of *Littorina saxatilis* can measure up to 18 mm (Emerson and Jacobson 1976). Sexually mature individuals are usually greater than 6 mm in length (Daka and Hawkins 2002). The smallest size class of *L. saxatilis* analyzed in this work ranged from 5.0 mm to 8.0 mm in length and had organics composing, on average, 11% of the shell. Medium and larger size classes had shell organic proportions within the 2-5% range. There was a significant difference in shell organic proportion between the sexually mature and immature gastropods of this species.

Individuals of the invasive and hermaphroditic freshwater bivalve *Corbicula fluminea* have been reported to grow to 60 mm in length (Hornbach 1992). Sexual maturity occurs within the first 4 to 6 months of life in clams as small as 6 mm in length (Prezant and Chalermwat 1984). All specimens of *C. fluminea* analyzed here were sufficiently large to be considered sexually mature. Therefore, no conclusions can be drawn regarding relevant shell organic differences and sexually mature vs immature individuals. The shell lengths in the largest size class of Asian clams examined did not extend up to the maximum recorded shell length. Still, the smallest size class had a greater shell organic proportion than the medium and large size classes.

The viviparous gastropod *Bellamya chinensis* has been reported to grow to a shell height of 70 mm (Olden *et al.* 2013). Shells of this species are thick and resistant to desiccation (Olden *et al.* 2013). Brooding females contain each stage of embryo through juvenile shelled stages within the uterine sac (Prezant *et al.* 2006). Periostracal hairs measuring up to 0.4 mm are distributed on the surface of shells *in utero* and in recently released juveniles; these hairs add to the measured organic component that composes the shell (Soes *et al.* 2011). To our knowledge, other than experimental work done by Prezant *et al.* (2006) who found newly born juvenile shells to have 13.6% organic content, no other organic measurements of developing, *in utero* shells representing this species have been reported. Similarly, here we found *in utero* shells to contain just over 10% organics compared to just under 4% in adult shell. Prezant *et al.* (2006) suggested that the higher organic shell content in newly released juveniles would be advantageous in mitigating possible predator-induced shell fractures.

CONCLUSIONS

The inverse relationship between total percent organic material vs. mineral component composing the shell of some freshwater and marine mollusks seems robust. This difference is unlikely to be a result of presence of different periostracal thicknesses, erosional loss of periostracum with growth, differential production of periostracum, a decrease in inter- or intracrystalline organic shell material, or a proportional increase in CaCO_3 in the shell with growth. None of the species examined here had a thick enough periostracum to account for the trends seen. In addition, changes from proportional erosion and proportional periostracal growth/thickness would not account for the minor influence this outer shell covering might have. With no obvious rationale for a disproportionate increase in mineral content or disproportionate or allometric changes in shell thickness, the change in organic content is most likely due to a greater proportion in organic production compared to mineral material within the smaller,

juvenile shell. Ongoing research will focus on extending taxa examined plus determine any correlates between organic/mineral ratios and shell strength and fracture resistance.

ACKNOWLEDGMENTS

Part of this work is derived from an undergraduate honors college research thesis (SR) at Southern Connecticut State University. We would like to thank the thesis committee, Drs. Vince Breslin, Emma Cross and Sean Grace and acknowledge the important statistics support received from Dr. Raymond Mugno. Lastly, thank you to Dr. Gary Dickinson and anonymous reviewers for their important suggested edits. The editorial work of Dr. Wallace M. Meyer made this a substantially improved and more cohesive manuscript. Funding for this work came from Southern Connecticut State University.

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Submitted: 20 September 2020; **accepted:** 24 May 2021; **final revisions received:** 15 June 2021; **Published:** 16 December 2021