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## Contribution of organic shell matter to biomass estimates of unionid bivalves

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The amount of acid extractable organic matter in the shell is significantly different in *Anodonta cataracta* ( $3.75 \pm 0.25\%$  w/w), *Elliptio complanata* ( $2.79 \pm 0.04\%$ ) and *Lampsilis ochracea* ( $2.26 \pm 0.15\%$ ). Within a species values do not change with size or sex. The percentage ash and caloric content do not differ among the species and pooled data give values of  $5.13 \pm 0.27\%$  and  $4567.2 \pm 283.5$  cal/g dry weight respectively. The organic content of the shell accounted for between 4.6 and about 30% of the total energy content, depending on species and size.

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La quantité de matière organique extraite à l'acide, dans la coquille, diffère chez *Anodonta cataracta* ( $3.75 \pm 0.25\%$  p/p), *Elliptio complanata* ( $2.79 \pm 0.04\%$ ) et *Lampsilis ochracea* ( $2.26 \pm 0.15\%$ ). Cependant, chez une même espèce, ces valeurs restent indépendantes de la taille ou du sexe. Le pourcentage des cendres et le contenu calorifique ne diffèrent pas d'une espèce à l'autre et leurs valeurs respectives sont de  $5.13 \pm 0.27\%$  et de  $4567.2 \pm 283.5$  cal/g poids sec, telles que calculées d'après les données regroupées. Le contenu organique de la coquille représente, selon l'espèce et la taille, de 4.6 à 30% du contenu énergétique total.

[Traduit par le journal]

### Introduction

Bivalve molluscs frequently contribute a substantial proportion of the standing stock of many freshwater and marine benthic communities. In spite of an abundant literature, it is difficult to compare the contribution of bivalves to such communities because of the wide variation in methods of expressing standing stock. The standing stock is commonly expressed as one or more of total wet weight (including water in the mantle cavity), total dry weight, wet or dry tissue weight, wet or dry shell weight, tissue calories or, most frequently, as numerical abundance. A number of authors have used standing stock estimates as a basis for measuring net production and (or) total energy flow through bivalve populations (Dame 1972, 1976; Dare 1976; Hughes 1970; Kuenzler 1961; Lewandowski and Stanczykowska 1975; Magnin and Stanczykowska 1971; Münch-Petersen 1973; Negus 1966; Wolff *et al.* 1975), although difficulties again arise in attempting to compare the various studies because of the variability of the units selected for expression of production.

In addition to the difficulties that arise because of the selection of units for expression of results, there is the possibility that many studies have the inherent error that they have ignored, or assumed to be negligible, the organic compounds, collectively termed conchiolins (Beedham 1958), contained

within the bivalve shell. Daniel (1921) determined the organic content of the shell of *Mytilus edulis* to average 3.4% based on loss of weight on ignition. Dare (1976) in a study of production of two populations of this species used Daniel's value and assumed a caloric content of 5.65 kcal/g (1 cal = 4.1868 J). His results (Dare 1976) indicate that organic shell matter was equal to between 32 and 34% of the total ash-free tissue weight over 3 years in one population and 30 and 71% during the 2 years in the other. Dame (1972) determined that in *Crasostrea virginica* the organic content of the shell amounted to  $0.896 \pm 0.096\%$  by weight when the organic material was extracted by solution of the shell in 0.1N HCl + 10% trichloroacetic acid (TCA). This average value is substantially lower than the 3.04% recorded by Price *et al.* (1976), determined by loss on ignition at 475°C. Price *et al.* (1976) showed that the organic content of the shell in 14 species of marine bivalves ranged from 1.4 to 21.4%, revealing specific differences. Hughes (1970) included shell organic material in calculation of an energy budget for *Scrobicularia plana*, but did not present data on the amount of organic material per unit shell weight. Based on a small number of samples Hughes (1970) determined energy content of the shell organic material as 5.037 kcal per ash-free gram. In studies on *Modiolus demissus* Kuenzler (1961) included the contribution of con-

TABLE 1. The relationship of dry tissue weight (grams) and dry shell weight (grams) to shell length (centimetres) in four populations of freshwater bivalves. All data fitted to  $\log_{10} W = a + b \log_{10} L$ , where  $W$  = weight and  $L$  = length

	Dry tissue weight			Dry shell weight		
	<i>n</i>	<i>a</i>	<i>b</i>	<i>n</i>	<i>a</i>	<i>b</i>
<i>Elliptio complanata</i>	281	-2.403 ± 0.071 *	2.770 ± 0.089	320	-1.936 ± 0.085	3.530 ± 0.100
<i>Anodonta cataraacta</i>						
Morice Lake	154	-2.597 ± 0.076	2.915 ± 0.094	321	-2.021 ± 0.052	3.121 ± 0.065
Layton's Lake	249	-2.121 ± 0.047	2.729 ± 0.059	400	-1.956 ± 0.033	3.156 ± 0.038
<i>Lampsilis ochracea</i>	186	-2.197 ± 0.107	2.931 ± 0.148	191	-1.784 ± 0.048	3.530 ± 0.106

\* ± one standard error.

chiolin to the annual net production and energy flow within the population. The conchiolin abundance was calculated as 10.6% of shell weight, based on loss of ignition measurements. However, for purposes of calculating the overall conchiolin contribution, it was assumed that the ratio of shell weight to tissue weight remained constant throughout the size range of the species. He determined that almost 66% of the dry organic standing stock of *M. demissus* is in the form of shell organic matter. The value of 10.6% determined by Kuenzler (1961) is substantially higher than the values (4.63–6.16%) determined by Price *et al.* (1976) for five populations of this species.

As part of an ongoing study of energy flows through freshwater bivalve populations and communities in southeastern New Brunswick, the current study was undertaken to determine the contribution of organic shell material (OSM) to standing stock biomass of the species and to determine the extent of interspecific variation.

### Methods

*Elliptio complanata* (Solander), *Anodonta cataraacta* (Say), and *Lampsilis ochracea* (Say) were collected by dragging in Morice Lake, a 1.5 km<sup>2</sup> polymictic, mesotrophic reservoir, located 1.5 km north of Sackville, N.B. *Anodonta cataraacta* was also collected by hand from Layton's Lake, N.S., a eutrophic meromictic lake, 14.5 km southwest of Sackville, N.B.

Samples of approximately 25 of each species were collected at about 3-week intervals throughout the ice-free period. Each individual was gently scrubbed to remove shell concretions and then the maximum anterior–posterior length determined to the nearest 0.5 mm. The bivalve was opened by severing the adductor muscles and the sex was determined by observing eggs and (or) glochidia in the marsupia, or by examining wet mounts of gonadal material. All tissue was transferred to a preweighed aluminum drying dish and both shell and tissue dried at 60°C for 48 h, after which the weight of tissue and shell was determined.

Dried shells were then selected to represent small, medium, and large individuals of both sexes for all populations. Both valves were crushed and then passed through a soil grinder with a 60 mesh screen. Between 0.25 and 0.50 g of the ground shell was dissolved in excess 0.1 N HCl. The HCl solution was then filtered through preweighed 0.45 µm Millipore filters, which

were dried at 60°C for 48 h and reweighed. For some samples the remaining ground shell material was also dissolved in acid and then the acid was decanted off the settled organic material. This was washed twice in distilled water and dried at 60°C for 48 h. Part of this material was used to determine the caloric content in a Phillipson microbomb calorimeter, while the remainder was used to determine the loss on ignition at 575°C for 6 h. The Phillipson microbomb calorimeter was also used to determine energy content of selected samples of bivalve tissue.

Other samples of about 0.25 g of ground shell for each of the three Morice Lake populations were transferred to weighed crucibles that were then reweighed. After ignition at 575°C for 2 h the crucibles were cooled in a desiccator and weighed.

### Results

Regression equations for the relationship between dry tissue weight and shell length and between dry shell weight and shell length for the pooled data of each of the four populations are given in Table 1. Individual regression lines were initially constructed for both sexes for each sampling date. While some minor variations with sex and season could be detected, these have been ignored for the purpose of the present study. In all cases the slope of dry tissue weight against length is less than the slope of shell weight against length. Consequently, as an individual of any of the four populations increases in length there is an increase in the ratio between shell weight and dry tissue weight. The two populations of *Anodonta cataraacta* show significant differences in their growth relationships. For any given length an individual from Morice Lake has a slightly lighter shell weight and a much lighter dry tissue weight than an individual of the same length from Layton's Lake.

The energy content of the dry tissue of the four populations is given in Table 2. There is no significant difference between the caloric content of male and female individuals with the exception of *A. cataraacta* from Layton's Lake where males have a significantly higher energy content than females. No change in dry tissue caloric content with increasing bivalve size was detected.

TABLE 2. Caloric content of dry tissue of four populations of freshwater bivalves

	Number	Calories per gram, $\bar{x} \pm 1 \text{ SE}$
<i>Elliptio complanata</i> *	91	4454.6 $\pm$ 92.3
<i>Anodonta cataracta</i>		
Morice Lake*	86	4611.3 $\pm$ 140.5
Layton's Lake		
Males	44	5162.8 $\pm$ 91.1
Females	52	4731.6 $\pm$ 90.1
<i>Lampsilis ochracea</i> *	9	4757.1 $\pm$ 130.0

\*Males and females not significantly different.

The percentage (w/w) of acid-extractable organic shell material (OSM), percentage ash of this material, and its caloric content for the four populations are presented in Table 3. Shells were initially divided on the basis of bivalve sex and further divided into categories of either small, medium, or large size. No significant differences in any of the components could be detected that related to either sex or shell size. The percentage OSM in shells of the two populations of *A. cataracta* does not differ significantly and a pooling of these data gives an average OSM content of  $3.75 \pm 0.25$  ( $\pm$  SE)% of total shell weight. However, the concentration of OSM in *A. cataracta* shells is significantly greater than in *Elliptio complanata* ( $2.79 \pm 0.04\%$ ), which is in turn significantly greater than the concentration in *Lampsilis ochracea* ( $2.26 \pm 0.15\%$ ). In spite of the differences in abundance of OSM in the three species, no significant differences are found in the percentage ash in OSM (pooled average  $5.13 \pm 0.27\%$ ) or in the caloric value (pooled average  $4567.2 + 283.5$  cal/g dry weight).

Determination of percentage OSM by loss on ignition at  $575^\circ\text{C}$  gave values of  $7.25 \pm 0.60\%$  (*Elliptio complanata*,  $n = 14$ ),  $6.75 \pm 0.42\%$  (*Anodonta cataracta*,  $n = 15$ ) and  $6.75 \pm 0.53\%$  (*Lampsilis ochracea*,  $n = 11$ ). In all cases the values are significantly greater than those determined by acid extraction.

The acid extraction results can be used to determine the contribution of OSM to the total energy content of an individual of any size for the four populations (Fig. 1). The contribution of shell organic material increases with increased bivalve length because of the increasing ratio between shell weight and tissue weight with growth. As shown in Fig. 1, the contribution of OSM can range from as low as 4.6% in small male individuals from Layton's Lake to a high of approximately 30% in large *E. complanata* from Morice Lake. It is interesting to note that, while the shape of the curves for *A. cataracta* from Layton's Lake and Morice

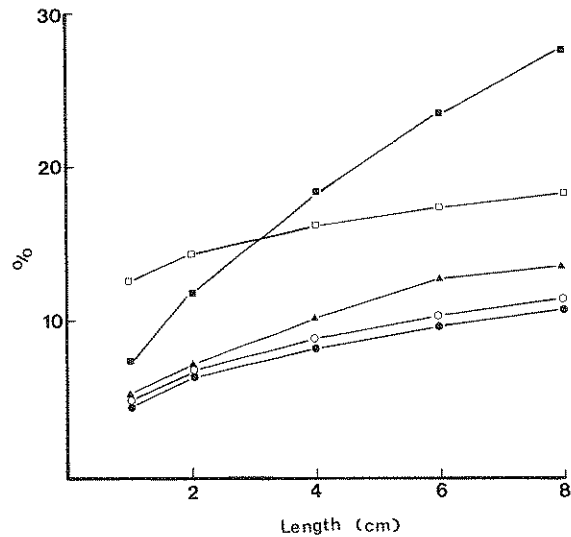


FIG. 1. The contribution of energy content of organic shell material to the total energy content of four populations of unionid bivalves. ■, *Elliptio complanata*, Morice Lake; □, *Anodonta cataracta*, Morice Lake; ▲, *Lampsilis ochracea*, Morice Lake; ○, female *A. cataracta*, Layton's Lake; ●, male *A. cataracta*, Layton's Lake.

Lake are similar, there is approximately an 8% difference in contributions of OSM to total energy content. This results from the differential increase in tissue weight and shell weight with length in the two populations.

### Discussion

The present study indicates that there is no difference in the concentration of OSM in two distinctive populations of the same species. Further studies may indicate that the concentration of organic material within the shell of a species remains constant, although our results for *E. complanata* and *L. ochracea* suggest that within the Unionidae there are substantial interspecific differences. However, it appears that the ash fraction of the OSM and the caloric value do not differ with species. The percentages of the shell composed of OSM in species included in this study are relatively similar to the value of 3.4% found in *Mytilus edulis* by Dare (1976), but are substantially lower than the 10.6% suggested by Kuenzler (1961) for *Modiolus demissus*. The high values given by Kuenzler (1961) may reflect extensive intergeneric differences or may be a product of the determination of OSM by loss on ignition, as heating of  $\text{CaCO}_3$ -rich samples to ashing temperatures might drive off substantial amounts of  $\text{CO}_2$  and thus overestimate the OSM concentration. The second possibility appears the most likely explanation as estimation of OSM concentration by loss on ignition at  $575^\circ\text{C}$  for

TABLE 3. The percentage of acid extractable organic shell material (OSM) and its ash and caloric content for four populations of freshwater bivalves. Number of replicates in parentheses

	OSM, %	Ash, %	Caloric content, cal/g dry weight
<i>Elliptio complanata</i>	2.79 ± 0.04* (25)	5.06 ± 0.22 (18)	4290.7 ± 267.3 (33)
<i>Anodonta cataracta</i>			
Morice Lake	3.82 ± 0.10 (54)	5.16 ± 0.30 (38)	4735.9 ± 235.6 (48)
Layton's Lake	3.66 ± 0.21 (39)	5.03 ± 0.25 (51)	4630.4 ± 103.5 (54)
<i>Lampsilis ochracea</i>	2.26 ± 0.15 (61)	5.62 ± 0.36 (11)	4471.5 ± 212.8 (25)

\*Mean ± one standard error.

the three Morice Lake species produced values that ranged from 1.77- to 2.97-fold greater than estimated by acid extraction.

The results of the present study indicate that measures of standing stock biomass, production, or energy flow in bivalve populations can be extensively underestimated unless the energy content of the organic material contained within the shells is included in the estimates. When a bivalve is lost from a population by predation or by some other mortality factor, the tissue energy will be rapidly incorporated into the food web of the community. The organic matter contained in shells undoubtedly will cycle at a much slower rate. However, if over a period of time the population can be considered to approximate a steady-state condition, then the amount of energy released through breakdown of shells would equal the energy content of the total shell material made available for breakdown by bivalve mortality during the same time period. Consequently, to accurately determine the standing stock biomass in a bivalve population it may be necessary to include not only the tissue energy and OSM energy of living bivalves, but the OSM contained in empty shells and shell fragments. Nixon *et al.* (1971) in a study of standing stocks in a *Mytilus edulis* bed collected empty shells and shell fragments in addition to the living organisms. Their results indicate that the dry tissue weight of *M. edulis* amounted to 12.8% of the shell weight of living bivalves. When the total weight of shell material, including empty shells and shell fragments, is included in the calculation, dry tissue weight is only 7.14% of the total shell weight. However, in order to include empty shells and shell fragments in such calculations it would be necessary to determine if there is a differential in the rate of loss of OSM from the shell relative to the rate of dissolution of the shell inorganic material.

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