THE SHELL OF *CARDIUM EDULE, CARDIUM GLAUCUM* AND *RUDITAPES PHILIPPINARUM*: ORGANIC CONTENT, COMPOSITION AND ENERGY VALUE, AS DETERMINED BY DIFFERENT METHODS

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(Figures 1-3)

Assessments of the quantity of organic matter in shells were made both by ignition at 475°C and by chemical extraction in 0·1M trichloroacetic acid.

The quantity of organic matter obtained by ignition at 475°C was over-estimated by 2 to 4·8 times, depending on the species studied (*Ruditapes philippinarum, Cardium edule, Cardium glaucum*). A technique of extraction by 0·1M trichloroacetic acid, filtration on Whatman GF/C filter and weighing of remaining ashes after ignition (450°C) is proposed. Energy values of the shell organic matter varied from 17·00 (±0·60) J mg⁻¹ to 24·0 (±2·87) J mg⁻¹ depending on the species and the geographic location. In the energy budget, these methods have made it possible to obtain an estimate of the energy mobilised in the production of shell organic matter. Variability in the energy values was the result of variation in the relative proportion of the different biochemical components. The proportion of proteins (Kjeldahl method) varied from 66·7% to 89·7% according to the species and geographical locations. The lipid content varied from 0·84% to 2·88% and carbohydrates from 0·15% to 0·29%.

INTRODUCTION

The organic content of the shells of molluscs can represent a significant fraction of the total organic content (Bernard, 1974) but it is often neglected in calculations of energy budgets in these animals. This may be in part due to uncertainty about the true values, since published estimates of the organic content of shells show quite wide variation. The species examined and also the provenance of the selected samples contribute to this variation as does also the method of measurement. The methods principally used have been by ignition at various temperatures from 400 to 550°C for various durations between 2 and 36 h (see Shumway & Newell, 1984; Jørgensen, 1976; Mohlenberg & Kiørboe, 1981; Vahl, 1981; Shafee, 1979; Price et al., 1976) or by acid extraction using different extraction proceedures (see Ivell, 1979; Dame, 1972; Horn, 1986; Griffiths & King, 1979). To calculate the energy content of the organic component some investigators have used the Hughes (1970) coefficient of 5·037 cal mg⁻¹, while others have used Paine's (1971) protein coefficient of 2·39 J g⁻¹. Wilbur & Saleuddin (1983) have called attention to the need for more study of these analyses. We present here the results of a study of the shell organic content of three species of molluscs, using two methods for the measurement and giving data on biochemical composition and energy value.

MATERIALS AND METHODS

Study area and sampling

Cardium edule (Linné) and *Ruditapes philippinarum* (Adams and Reeve) were collected from the intertidal populations located in the northern and southern parts of the Bay of Marennes-Oleron from regions of different salinity and sediment type, the characteristics of which are defined in Table 1. Because of its wide distribution, *Cardium glaucum* (Poiret) was collected around the European coast as shown in Figure 1.

In most cases, about a hundred individuals of each species were analysed separately. Each individual was measured and weighed. The exterior of the shells was brushed smooth and cleaned. The flesh was removed from the shells and was lyophilized for 48 h. The shells were rinsed with double-distilled water and dried at 60°C (12 h) to constant weight.

Table 1. *Characteristics of the sampling sites; nature of sediment and water variabilities*

| Site		Species	Nature of the sediment	Water column over one year			
				Temperature mean (± SD)		Salinity mean (± SD)	
Atlantic coast							
Marennes-Oléron Bay	(1)	*Ruditapes philippinarum*	Mud	13.5	(4.8)	31.1	(1.7)
			Sand	13.4	(4.6)	31.9	(1.6)
	(2)	*Cardium edule*	Sand	14.6	(4.6)	31.8	(2.1)
Moeze	(3)	*Cardium glaucum*	Mud	14.0	(6.58)	18.6	(4.9)
Arcachon Bay	(4)	*Cardium glaucum*	Mud	16.3	(6.67)	19.5	(11.2)
Mediterranean Sea							
Embiez island	(5)	*Cardium glaucum*	Sandy mud	15.7	(5.6)	39.5	(0.6)
Baltic Sea							
Sopot	(6)	*Cardium glaucum*	Sand	8.8	(6.5)	7.4	(0.3)
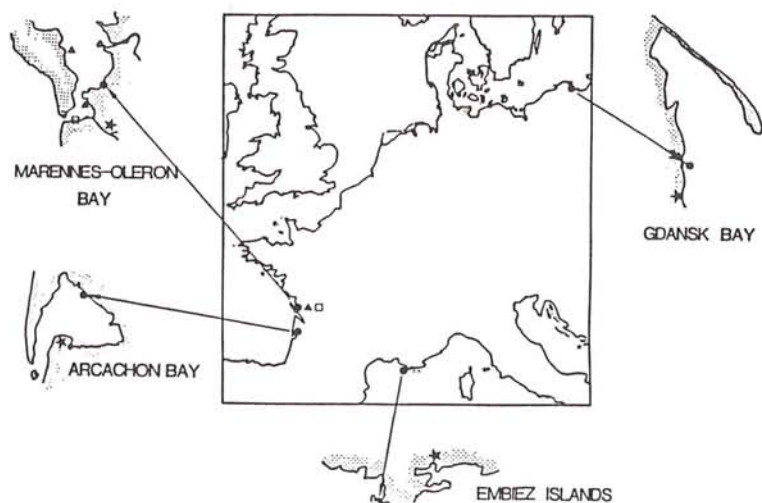
Figure 1. Geographical locations of the populations of *Ruditapes philippinarum* (▲), *Cardium glaucum* (●) and *Cardium edule* (□).

Organic content

The amount of shell organic matter was estimated by two methods. For the first, a random sample of 30 to 50 shells was placed in individual alumina boxes and heated in a muffle furnace at 475°C±5°C for 36 h to remove any organic material (Price *et al.*, 1976). The organic content of shells was estimated from the loss of weight. According to Paine (1964), this temperature is sufficient to oxidize organic carbon to carbon dioxide but not to break down inorganic carbonate compounds.

The other method consisted in an extraction in 0·1M trichloroacetic acid (TCA). Forty shells of each species were taken at random and were individually decalcified in 30 ml TCA solution at 18°C. The organic content was estimated by filtering the solution on to Whatman GF/C filter paper. Retained material was rinsed with double-distilled water and dried to constant weight at 60°C for 48 h (Dame, 1972).

A subsample of ten filters per species, after ignition in a muffle furnace at 450°C for 48 h, gave an estimate of the percentage of ash remaining in the organic fraction (Rodhouse *et al.*, 1984) and thus the ash-free dry weight of organic matter (AFDW).

Biochemical composition

Carbohydrates in aliquots were analysed as described in Dubois *et al.* (1956) with additional extraction in 0·15M TCA for 1 h at 6°C.

Lipids were extracted at room temperature in chloroform-methanol (1 V/2 V) (Bligh & Dyer, 1959) and analysed by the procedure published by Marsh & Weinstein (1966).

Proteins were assessed according to Lowry *et al.* (1951) after extraction in 1M sodium hydroxyde for 12 h.

The total amount of nitrogen was estimated on a sample of 15 whole shells per species by the Kjeldahl method. A coefficient of 6·25 (Giese, 1967) was used to calculate the proteins. In order to compare the total amount of proteins, the same procedure was used on a sample of shell organic content from previous extraction in 0·1M TCA.

The energy value of the organic matter was measured with a microbomb calorimeter (Phillipson, 1964), which was calibrated with benzoic acid. Samples, consisting of individual extracts of six to eight shells, were pooled and homogenized. Measurements of aliquots were always carried out in triplicate.

To compare the different regressions under various conditions, we have used an ANCOVA as described in Snedecor & Cochran (1967). The *t*-test was used to ascertain the significance of differences between means.

RESULTS

Estimation of the shell organic content

The relative proportions of the total organic matter present in the soft tissues and the shell are shown in Table 2. The fraction of the total represented by the shell organic matter varies by a factor of from 2 to 4 depending on the species and the method used for the organic part in the shell. The quantity of shell organic matter in the different species of

Table 2. *Characteristics of bivalve samples*

The shell organic part varies noticeably depending on the method used, ignition (2) or chemical extraction (1).

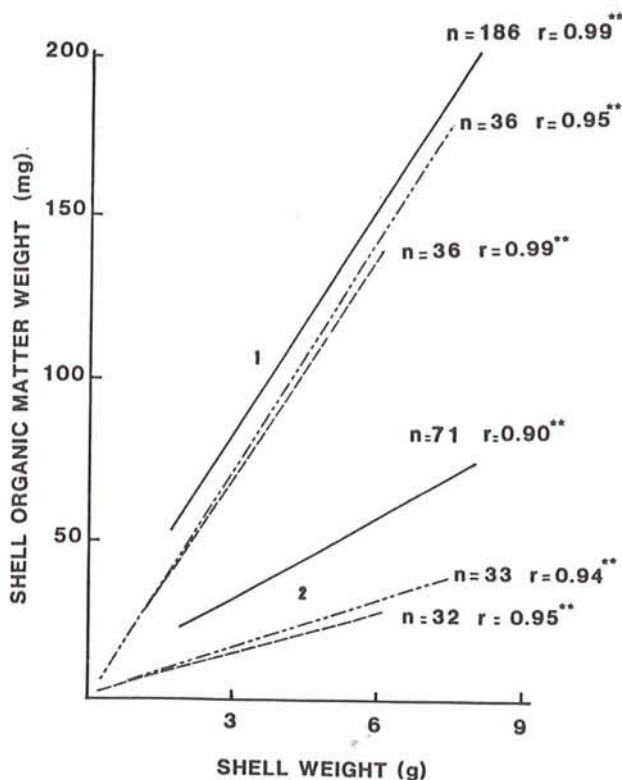|	n	Length (mm) (±SD)	shell weight (g) (± SD)	(1) % tissue	(1) % shell	(2) % tissue	(2) % shell
R. philippinarum	295	34.67 (5.45)	5.77 (2.48)	85.58	14.42	67.61	32.39
C. glaucum	145	25.47 (3.22)	2.80 (1.05)	94.84	5.16	79.16	20.84
C. edule	100	22.91 (6.26)	2.47 (1.70)	90.40	9.60	67.87	32.13

Figure 2. Regression lines of organic matter in the shells of *Ruditapes philippinarum* (solid line), *Cardium edule* (long and short broken line) and *Cardium glaucum* (dashed line) estimated by;(1), ignition and (2), chemical extraction (0·1M TCA).

bivalves is shown in Figure 2. The quantities obtained by the ignition method were noticeably higher than those resulting from the 0·1M TCA extraction. The average percentage of shell organic matter, as calculated by ignition, varied from 2·38±0·12% for *Cardium edule* and 2·34±0·13% for *Cardium glaucum* to 2·80±0·05% for *Ruditapes philippinarum*. On the same species, the TCA extraction resulted in an average percentage of 0·9±0·09% to 0·48±0·06%. When comparing the slope coefficient by using the F test from the ANCOVA, significant differences were found between *R. philippinarum* and the Cardiidae (Table 3). The significant difference at the 5% level between *C. edule* and *C. glaucum* when using the TCA extraction method, may be related to the difference in the

sizes analysed. According to Price *et al.* (1976) young specimens show a higher percentage of organic matter.

Table 3. *Comparison between the slope, using analyses of covariance (ANCOVA) and F test (see Snedecor & Cochran, 1967, p. 433), for quantity of organic matter versus shell weight by both methods, ignition or chemical extraction*

Significance level: NS, not significantly different; * significantly different $P<0.05$; ** significantly different $P<0.01$; *** significantly different $P<0.001$.

Method		Ignition		Extraction (0.1M TCA)	
Comparison		F for slope	degree free	F for slope	degree free
Ruditapesphillipinarum vs *Cardium edule*		78.51**	1,220	84.24**	1,102
Ruditapes philippinarum vs *Cardium glaucum*		42.65**	1,220	96.61**	1,101
Cardium edule vs *Cardium glaucum*		0.63 NS	1,70	4.20*	1,63

Table 4. *Estimation of the organic part and approximate biochemical composition in the shell*

Columns: A=per g shell biomass, B=per % organic matter. *t*-test was used to provide the significances of differences between means (NS, not significantly different; * significantly different, $P<0.05$; ** significantly different, $P<0.01$; *** significantly different, $P<0.001$). One significant difference was observed between sites 2 and 4 in carbohydrates (A, per g shell biomass; B, per % organic matter).

Extraction TCA	n	R. philippinarum 35	36	C. edule 33	32	C. glaucum pool	pool	pool
% organic matter mean (±SD)		1.12 (0.01)	0.82 (0.01)	0.53 (0.1)	0.48 (0.01)	0.53 (-)	0.37 (-)	0.45 (-)

Site	1		2		3		4		5		6		7	
	A	B	A	B	A	B	A	B	A	B	A	B	A	B
Proteins (mg) (n=3) Lowry														
	5.70 (0.27)	51.32 (1.91)	4.95 (0.28)	60.38 (3.46)	2.586 (0.14)	48.81 (2.65)	3.461 (0.06)	72.11 (1.39)	3.328 (0.116)	62.82 (2.15)	3.33 (0.01)	90.20 (0.29)	2.897 (0.09)	64.38 (2.02)
Proteins (mg) (n=3) Kjeldahl														
	7.47 (0.02)	66.73 (0.19)	6.493 (0.07)	79.18 (0.92)	4.337 (0.02)	81.83 (0.47)	3.848 (0.02)	80.16 (0.38)	4.303 (0.04)	81.18 (0.78)	- -	- -	4.035 (0.06)	89.67 (1.32)
Carbohydrates (µg) (n=3)														
	16.93 (3.55)	0.151 (0.03)	18.51 (2.52)	0.226 (0.03)	19.14 (6.51)	0.293 (0.05)	11.36 (0.34)	0.237 (0.01)	-	-	-	-	-	-
Lipids (µg) (n=3)														
	94.08 (15.68)	0.84 (0.14)	71.344 (14.76)	0.87 (0.18)	52.96 (2.26)	1.00 (0.04)	42.28 (5.18)	0.95 (0.09)	84.64 (5.88)	1.60 (0.11)	106.46 (1.59)	2.88 (0.04)	88.27 (3.51)	1.96 (0.08)

cont...

Table 4 (continued)

Proteins (Kjeldahl)

Site		1	2	3	4	5	6
2	A	***					
	B	***					
3	A	***	***				
	B	***	*				
4	A	***	***	**			
	B	***	NS	NS			
5	A	***	***	NS	**		
	B	NS	NS	NS	NS		
6	A	***	***	**	**	*	
	B	**	**	*	*	*	
7	A	***	***	**	*	*	**
	B	***	***	**	**	**	**

Proteins (Lowry)

Site		1	2	3	4	5	6
2	A	*					
	B	*					
3	A	***	***				
	B	NS	**				
4	A	**	**	**			
	B	***	*	**			
5	A	***	***	**	NS		
	B	**	NS	**	*		
6	A	**	**	**	NS	NS	
	B	***	**	***	**	***	
7	A	***	***	*	**	**	**
	B	**	NS	**	*	NS	***

Lipids

Site		1	2	3	4	5	6
2	A	NS					
	B	NS					
3	A	*	NS				
	B	NS	NS				
4	A	**	*	*			
	B	NS	NS	NS			
5	A	NS	NS	***	***		
	B	**	**	**	**		
6	A	NS	*	***	***	**	
	B	***	***	***	***	***	
7	A	NS	NS	***	***	NS	**
	B	***	**	***	***	**	***

Biochemical composition of the extracts

The results of biochemical composition analyses, obtained in different ways, are summarized in Table 4. According to these data it appears that proteins constitute the major component, up to 90% of the shell organic matter in some cases. Carbohydrates represent only a small proportion from 0·15% to 0·29% according to sites and species, while the proportion of lipids varies from 0·8% to 2·9%. It appears that the quantity and quality of shell organic matter differed from one geographical location to another, especially for *Ruditapes philippinarum* ($F_{1,69}=67·34$, $P<0·001$).

Table 5. *Energetic value of the shell and the organic part of the shell*

n = number of samples ; 23·65 J mg^{-1} is the energetic protein coefficient (Brody, 1945). *t*-test was used to provide the significances of differences between means. NS, not significantly different ; * significantly different, P<0·05; ** significantly different, P<0·01; *** significantly different, P<0·001

Site	1		2		3		4		5		6		7	
	J g^{-1} shell mass	J mg^{-1} organic matter	J g^{-1} shell mass	J mg^{-1} organic matter	J g^{-1} shell mass	J mg^{-1} organic matter	J g^{-1} shell mass	J mg^{-1} organic matter	J g^{-1} shell mass	J mg^{-1} organic matter	J g^{-1} shell mass	J mg^{-1} organic matter	J g^{-1} shell mass	J mg^{-1} organic matter
Energy value (1) (n = 3)	190.35	17.00	157.12	19.16	99.2	18.72	101.42	21.13	124.24	23.44	88.92	24.03	106.80	23.73
	(6.76)	(0.60)	(1.77)	(0.21)	(2.6)	(0.49)	(6.67)	(1.39)	(5.97)	(1.13)	(10.65)	(2.87)	(5.71)	(1.27)
ratio: (Energetic value (1) /23·65)x 100	71.86		81.02		79.14		89.33		99.12		101.62		100.36	
	(2.55)		(0.90)		(2.07)		(5.88)		(4.76)		(12.17)		(5.37)	

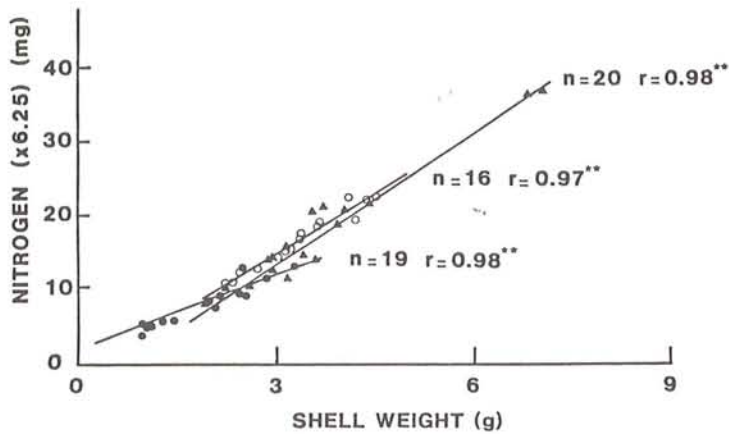|	R.p. 1		R.p. 2		C.e. 3		C.g. 4		C.g. 5		C.g. 6	
2	**	**										
3	*	***	NS	***								
4	**	***	NS	***	*	NS						
5	***	**	**	**	**	**	NS	*				
6	*	***	*	**	*	NS	NS	NS	NS	NS		
7	**	***	**	***	**	NS	NS	NS	NS	*	NS	NS

Figure 3. Protein content (N x 6·25) in the whole shell of *Ruditapes philippinarum* (▲), *Cardium glaucum* (○) and *Cardium edule* (●).

Comparison between species of the total nitrogen in the whole shell

Analyses of the total nitrogen content in the whole shell (Figure 3) did not show significant difference in the regression slope between *R. philippinarum* and *C. glaucum* ($F_{1,34}$=1·0, NS). However, differences were significant between *C. edule* and *R. philippinarum* ($F_{1,37}$=20·03, P<0·01) and between *C. edule* and *C. glaucum* ($F_{1,33}$=76·67, P<0·01). Some differences may result from the different size ranges studied. Alternatively, the protein contents may be compared with those obtained after 0·1M TCA extraction and analysed by the Kjeldahl method: *Ruditapes philippinarum* (6·49±0·07; 4·86±0·75 mg g^{-1} shell), *Cardium glaucum* (4·07±0·26; 4·96±0·24 mg g^{-1} shell), *Cardium edule* (4·33±0·02; 4·27±0·42 mg g^{-1} shell). The results are similar if we consider the different size ranges studied and sample preparations (pool or individual analyses).

Energy value

Differences between the species in the energy value of the organic matrix were noted. The energy varied from a minimum value for *R. philippinarum* (17·00±0·6 J mg^{-1}) to a maximum for *C. glaucum* (24·03±2·87 J mg^{-1}) (Table 5). This value for *C. glaucum*. is very close to the energy coefficient of proteins (23·65 J mg^{-1}) given by Brody (1945). This must be related to the high protein content in this species (ratio from 89% to 100%).

DISCUSSION

The estimate of shell organic content varied by 2·5 to 4·8 times, depending on the species and the method, ignition or chemical (0·1M TCA) extraction. The results obtained by ignition (2·38% for *C. edule* and 2·8% for *R. philippinarum*) may be compared with those obtained by Hibbert (1976) using the same method (2·04% for *C. edule* and 2·55% for *Venerupis aurea*). According to Beukema (1981) the organic content is underestimated

when using the extraction method, due to some tightly bound water of crystallization. An ignition temperature of 460°C (Jørgensen, 1976), overestimates the loss of organic matter because of loss of CO_2 from calcium carbonate. Rodhouse et al. (1984) also overestimate the loss of organic matter by a factor of 2·5 at 540°C. Such a loss of CO_2 could account for the varying estimates given by the different methods. The chemical extraction method gives results which correspond to those obtained by the Kjeldahl method on the whole shells either before or after chemical extraction. Thus, ignition overestimates the organic content, especially at and above 475°C. The overestimation factor is ranked between 2 and 5. TCA (0·1M) extraction appeared to be suitable for chemical extraction because of the existence of a fraction of organic matter which is soluble in hydrochloric acid but insoluble in TCA (Crenshaw, 1972). Percentages of shell organic matter vary from 5·2% to 14·4% of the total organic content . These results show that it is necessary to consider this production in the energy budget as mentioned by Jørgensen (1976), Rodhouse et al. (1984) and Hawkins & Bayne (1985) on Mytilus edulis.

Reviews on the biochemical composition of the organic content of shell have been presented by Wilbur & Simkiss (1968), Kennedy et al. (1969), Gregoire (1972). The shell matrix is composed mainly of proteins and glycoproteins with soluble and insoluble parts. These authors show that their proportion varies according to species and environmental conditions. It seems prudent to use the Kjeldahl method for proteins because it estimates both structural proteins and Lowry positive substances (Table 4). But the estimation of proteins is not alone sufficient to estimate the quantity of organic matter because of the variability of shell proteins between species. Our results show individual and interspecific variations in the proportion of organic matter and in its biochemical composition. Ivell (1979) has found that the shell of C. glaucum contained 1·43% of organic matter, 56% of which were proteins when using the Lowry method, as compared to 0·5% (74·1% protein) obtained in this study. Such biochemical data provide energy conversion coefficients required to estimate the energy mobilised in production of shell organic matter. For example, the energy mobilised in the production of shell organic matter, may be assessed by using different energy conversion coefficients, such as those given by Hughes (1970) on Scrobicularia plana (21·08 J mg^{-1}) and by Griffiths (1981) on Chromomytilus meridionalis (28 J mg^{-1}) or a proteins conversion coefficient of 23·95 J mg^{-1} (Paine, 1971).

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