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Comparisons of European populations of the *Cerastoderma glaucum/C. lamarcki* complex based on reproductive physiology and biochemistry

Reproduction
Biochemistry
Physiology
Cerastoderma
Ecotypes

Reproduction
Biochimie
Physiologie
Cerastoderma
Écotypes

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ABSTRACT

Five European populations of the *Cerastoderma glaucum/C. lamarcki* complex were studied to elucidate differences between Mediterranean, Western European and Baltic populations: one from the French Mediterranean; two from the west coast of France; one from the Limfjord, Denmark; and one from the metal contaminated Gdansk Bay, Poland. The biochemical tissue composition related to five reproductive stages was analysed, and the results for the five populations were compared. The comparison showed that at similar maturity stages the biochemical composition of the cockle tissue was different for populations from each of the three regions, Northern Europe (region I), the French West coast (region II), and the Western Mediterranean (region III). These differences may reflect individual acclimation to different habitat conditions, as well as genetically-based adaptation at population or subspecies level.

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RÉSUMÉ

Comparaisons de populations allopatriques de *Cerastoderma larmarcki* et *C. glaucum* à travers leur cycle de reproduction et leur composition biochimique

Cinq populations allopatriques de *Cerastoderma glaucum/C. lamarcki* ont été étudiées afin de clarifier certaines différences écophysiologiques entre les différentes populations européennes : l'une d'origine méditerranéenne, deux de l'Atlantique à l'ouest de la France, l'une du Limfjord, au Danemark, et l'une de la Mer Baltique en Pologne

Ces populations ont été comparées à partir d'analyses biochimiques tissulaires réalisées à cinq stades de développement des gonades. Les résultats montrent que, pour un même stade de développement, la composition biochimique tissulaire diffère selon la situation géographique. Trois régions ont ainsi été distinguées : l'Europe du Nord (région I), la côte ouest de la France (région II) et la Méditerranée occidentale (région III). Ces différences entre les populations peuvent traduire une acclimatation aux conditions ambiantes de l'habitat ou une adaptation établie génétiquement au niveau des populations ou des sous-espèces.

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INTRODUCTION

The complex of *Cerastoderma glaucum* (Bruguière) and *C. lamarcki* (Reeve) occurs in the Baltic Sea, the Mediterranean Sea, the Black Sea, the Red Sea and in sheltered areas along the other European coasts (*e. g.* Russel, 1971; Brock, 1979 and 1980; Wolowicz, 1984). Existing results show no consistent morphological differences to separate the northern and southern populations (Brock, 1991); however, differences in reproductive patterns have been documented (Riva, 1976; Ivell, 1979 *a* and *b*; Zaouali, 1980; Wolowicz, 1991). As morphological criteria failed to distinguish between *e. g.* the Baltic and Mediterranean types of the complex, they have for the last two decades been considered conspecific (Bowden and Heppel, 1968; Rygg, 1970; Boyden and Russell, 1972; Petersen and Russell, 1973; Barnes, 1973; Brock, 1979; Labourg and Lasserre, 1980; Wolowicz, 1987 *a*). The discovery of immunological differences between populations of *Cerastoderma* from the Baltic Sea and the Limfjord, on the one hand, and from the Mediterranean, Black, and Red Seas, on the other (Brock, 1987), together with the demonstration of substantial chromosomal DNA differences between Danish and Mediterranean forms (Brock and Christiansen, 1989) give rise to a new perspective. These biotechnological studies show genetic distances of 0.84 between *Cerastoderma edule* and *C. lamarcki* from the Baltic and the Limfjord, 0.69 between *C. edule* from the Baltic and the Limfjord and C. glaucum from the Mediterranean, and 0.52 between *C. lamarcki* and *C. glaucum.*

While the status of *C. edule* as a distinct species is clear in relation to the *C. glaucum*/*C. lamarcki* complex (Brock, 1978), it is uncertain for the types *C. lamarcki* and *C. glaucum*. The demonstrated genetic differences between these two forms could reflect the results of selection by distinct environmental factors. *Cerastoderma glaucum* easily adapts to different habitat conditions and has been considered a pioneer species (Zaouali, 1980); indeed, its penetration into the Red Sea (Brock, 1987) is strong evidence of this.

Initial studies by one of us (M.W.) showed the biochemical composition of *C. glaucum* from Ile des Embiez to be very different from that of the Gdansk population. In the present study we compare the eco-physiology as well as the eco-biochemistry of reproduction for five populations of the *Cerastoderma glaucum*/*C. lamarcki* complex.

The comparison considers the types referred to as *C. lamarcki* (from the Limfjord and the Bay of Gdansk), *C. glaucum* [from Ile des Embiez (Brock, 1987)], and two populations from the west coast of France (Marennes-Oléron basin and Arcachon basin) that may be either *C. lamarcki*, *C. glaucum*, a mixture of the two types, or hybrids between them. The variables: sampling time, size, sex, age and reproductive stage were considered when comparing populations by their content of protein, lipid, glycogen, and carbohydrates other than glycogen.

MATERIALS AND METHODS

Records of reproductive patterns in Gdansk Bay were based on thirty individuals sampled at monthly intervals from March to October in the years 1976-1978 and 1984-1985.

Thirty individuals, sampled in Gdansk Bay in March, April and May 1984 and 1985 were used to compare inter-annual variations with respect to biochemical content for the two sexes.

Approximately thirty live cockles were sampled at monthly intervals during the reproductive period from each of the five localities shown in Figure 1. The locality at Gdynia in Gdansk Bay is in open connection with the Baltic (salinity: 7); sampling period: March to October 1984 and 1985. The Limfjord locality is part of the open Nibe Bredning (26 %), sampling period, April to October 1987. The locality in the Marennes-Oléron area is a sheltered ditch at Brouage (19 %), and the Arcachon locality is a saline pool ("claire") at Certes (21 %); sampling period for both localities: March to August 1986. The Mediterranean material was sampled in a saline pool at Ile des Embiez (46 %) in the period March to June 1986; no haloclines were observed. For temperature records, *see* Figure 2. The cockles were kept unfed in seawater for 24 hours, and stored in a deepfreeze prior to analysis. Their age was determined by the number of carefully identified annual rings (Orton, 1926; Bourget and Brock, 1990).

Metric values were measured with a caliper to the nearest 0.1 mm: width as maximum width, height as the distance from the umbo to the shell edge parallel to the ribs, and length as the distance from the anterior to the posterior edge perpendicular to the height measurement (not necessarily maximal length distance). Weight in

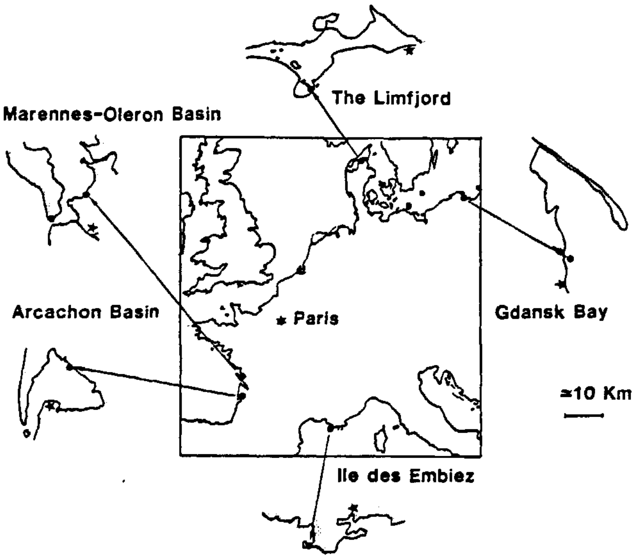

Figure 1

Map of Europe showing the five sampling localities for the Cerastoderma glaucum/C. lamarcki complex*: Gdynia in Gdansk Bay, asterisk: Gdansk. Nibe Bredning in the Limfjord, asterisk: Ålborg. Brouage in the Marennes-Oléron basin, asterisk: Marennes. Certes in the Arcachon Bay, asterisk: Arcachon. Ile des Embiez, asterisk: Toulon.*

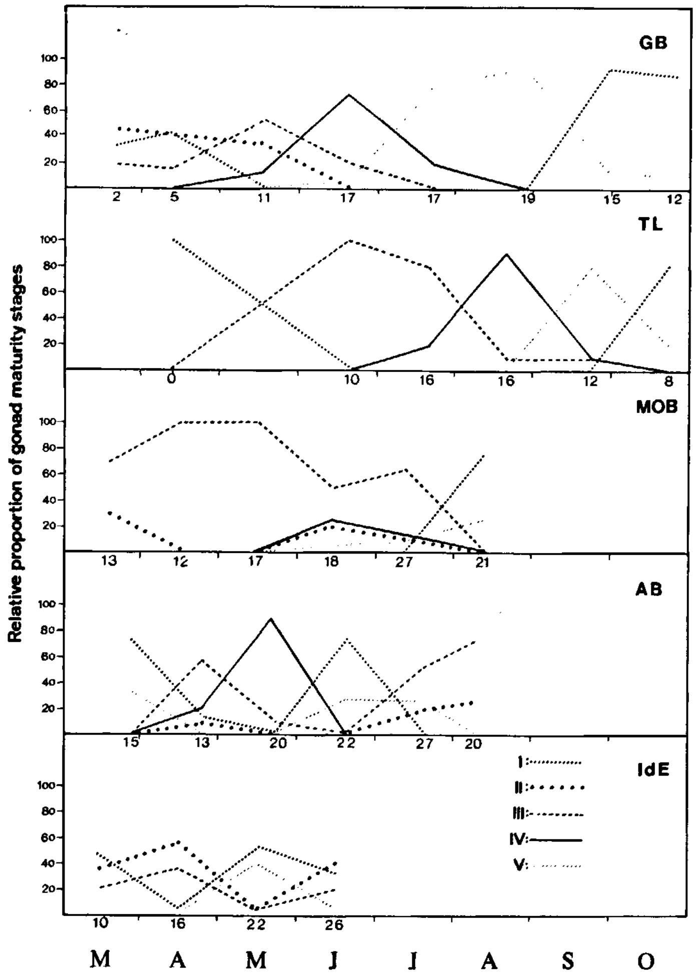

Figure 2

Reproductive condition indicated by gonad maturity stages I-V at different sampling dates for the Cerastoderma glaucum/C. lamarcki *complex sampled at the five localities shown in Figure 1. GB: Gdansk Bay; TL: the Limfjord; MOB: Marennes-Oléron basin; AB: Arcachon basin; IdE: Ile desEmbiez. The abscissa shows the sampling months, while the numbers at the axis shows the temperature in degrees centigrade at the sampling time. Spawning may occur whenever maturity stage IV has been achieved; maturity stage V is the post-spawning stage. Note that spawning occurs later and over a more prolonged period in the Northern (upper) than in the Southern (lower) localities.*

milligrams was obtained for the intact cockle, the shells, the wet and dry tissue, and for the fractions of tissue homogenate used for analysis of ash content and for biochemical components. Very small individuals were not analysed for biochemical content. The physiological index, tissue dry weight divided by shell weight (Lucas and Beninger, 1985) was chosen for comparison of the cockle condition for the two sexes at five different maturity stages at the five actual localities.

The content of dissolved protein (not including structural proteins) was analysed according to Lowry *et al.* (1951); lipid content was extracted according to Bligh and Dyer (1959) and analysed in accordance with Marsh and Weinstein (1966). Glycogen and total sugar were analysed according to the method of Dubois *et al.* (1956) using hot (80°C) rather than boiling water. Individual relative content of a biochemical component was calculated as the amount of the component in g x 100 divided by the ash free dry weight in grams.

The determinations of sex and maturity of gonads (stages I-V) were made according to Wolowicz (1987 *b*). Comparisons of index and biochemical values (continuous, normal distributed variables) for regions and maturity stages (non-significant interaction, category variables) were based on analysis of variance (ANOVA) on individual data.

RESULTS

Table 1 demonstrates a repeated strictly monocyclic pattern of the gonad maturation cycle in Gdansk Bay during the five years for which we possess records.

When maturity stage IV is achieved, spawning may take place; maturity stage V is the post-spawning stage. As seen from Figure 2, there is a clear relation between the latitude and the spawning period; the onset of spawning occurs earlier and takes place over a shorter period in the Southern localities than in the Northern. At Ile des Embiez, Arcachon, and possibly Marennes-Oléron, the rapid return to stage III after spawning shows that a second spawning takes place in late summer (*see* Discussion).

Index values were independent of sex (ANOVA, $p > 0.05$) and the results for the two sexes were pooled for comparisons between populations. As Table 2 shows, the index values seemed generally high for the Limfjord and Gdansk populations, intermediate for the two populations from the west coast of France and low for the Mediterranean population. For all populations except that from Marennes-Oléron, maturity stage and condition index were interrelated, albeit differently. For the Limfjord and the Ile des Embiez populations, index values were highest at maturity stage III, whereas this value was highest at stage II for the populations from Gdansk andArcachon.

For the following comparisons, all individuals were "normalized" according to their maturity stages; thus, comparisons of *e. g.* biochemical composition of populations were carried out for individuals at similar maturity stages and not for individuals sampled in the same period.

Figure 3 shows the biochemical composition of the cockle tissue at gonad maturity I-V for the five different populations. By combining the amount of dry weight accounted for by the biochemical analyses with the ash weight, 70 % of total dry weight could be accounted for in the Limfjord population, 68 % in the Marennes-Oléron population, 65 % in the Gdansk Bay population, 63 % in the Arcachon basin population, and 72 % in the Ile des Embiez population. While 2-3 % may have been caused by residual water (Beukema and de Bruin, 1977), the residual dry weight is mainly constituted by structural proteins and nucleic acids.

The analysis of variance showed no pronounced relations ($P > 0.99$) between maturity stages of all individuals and the composition of either protein, lipid, glycogen or

Table 1

Monocyclic reproductive pattern of maturity stages I-V of Cerastoderma *sampled in Gdansk Bay during the period March to October, 1976, 1977, 1978, 1984 and 1985. Left-hand figure: females, right-hand: males; number = percentage of the actual maturity stage. Number of individuals per sample: 30.*

		I		II		III		IV		V	
	1976	-	-	-	-	-	-	-	-	-	-
	1977	17.2	76.6	64.1	23.4	18.7	-	-	-	-	-
M	1978	35.9	87.5	64.1	12.5	-	-	-	-	-	-
	1984	10.7	71.1	57.1	28.9	32.2	-	-	-	-	-
	1985	41.7	82.8	58.3	17.2	-	-	-	-	-	-
	1976	-	-	-	-	-	-	-	-	-	-
	1977		76.6	90.6	23.4	9.4	-	-	-	-	-
A	1978	12.5	79.7	55.2	20.3	32.5	-	-	-	-	-
	1984	-	80.0	68.0	20.0	32.0	-	-	-	-	-
	1985	17.7	61.4	48.1	38.6	34.2	-	-	-	-	-
	1976	-	-	-	-	-	-	-	-	-	-
	1977	-	-	-	-	-	-	-	-	-	-
M	1978	6.2	-	84.4	65.6	9.4	34.4	-	-	-	-
	1984	13.8	-	62.1	55.6	24.1	44.4	-	-	-	-
	1985	3.1	-	89.1	43.4	7.8	53.3	-	3.3	-	-
	1976	-	-	-	-	-	-	-	-	-	-
	1977	-	-	-	-	42.2	28.2	57.8	71.8	-	-
J	1978	-	-	-	-	9.4	15.6	75.0	75.0	15.6	9.4
	1984	-	-	-	-	22.2	18.2	66.7	81.8	11.1	-
	1985	-	-	-	-	7.7	6.1	78.5	93.9	13.8	-
	1976	-	-	-	-	-	-	-	-	-	-
	1977	-	-	-	-	-	-	60.9	76.6	39.1	23.4
J	1978	-	-	-	-	-	-	15.6	14.1	84.4	85.9
	1984	-	-	-	-	-	-	22.2	15.4	77.8	84.6
	1985	-	-	-	-	-	-	4.7	8.2	95.3	91.8
	1976	14.1	3.1	-	-	-	-	-	-	85.9	96.9
	1977	-	-	-	-	-	-	23.4	-	76.6	100.0
A	1978	-	-	-	-	-	-	-	-	-	-
	1984	-	-	-	-	-	-	12.0	-	88.0	100.0
	1985	-	-	-	-	-	-	-	-	-	-
	1976	51.6	57.8	-	-	-	-	-	-	48.4	42.2
	1977	57.8	90.6	-	-	-	-		-	42.2	9.4
S	1978	84.4	93.7	-	-	-	-	-	-	15.6	6.3
	1984	78.3	91.3	-	-	-	-	-	-	21.7	8.7
	1985	-	-	-	-	-	-	-	-	-	-
	1976	84.4	40.6	-	-	-	-	-	-	15.6	59.4
	1977	65.6	7.8	-	-	-	-	-	-	34.4	92.2
O	1978	100.0	62.5	-	-	-	-	-	-	-	37.5
	1984	89.7	86.4	-	-	-	-	-	-	10.3	13.6
	1985	-	-	-	-	-	-	-	-	-	-

carbohydrates other than glycogen. The absolute contents of dissolved protein were high and comparable for the five populations; however, the relative protein (fraction of total tissue dry weight constituted by dissolved protein) differed statistically ($p < 0.001$) from region I to region III.

According to the ANOVA, the relative lipid content of cockles from Gdansk Bay ($\bar{X}$ = 10.57, sd = 2.39, N = 433) equalled that of the cockles from the Limfjord ($\bar{X}$ = 10.47, sd = 1.99, N =123). Their lipid content was significantly ($p < 0.001$) higher than that found in the Marennes-Oleron population ($\bar{X}$ = 6.15, sd = 1.50, N = 139), the Arcachon population ($\bar{X}$ = 7.69, sd = 1.51, N = 92) and the Ile des Embiez population ($\bar{X}$ = 6.37, sd =1.09, N = 118). Hence, the two populations originating in region I are separated from the three populations from regions II and III.

Relative glycogen content in the population from Gdansk Bay and the Limfjord population was ($\bar{X}$ = 1.25, sd = 0.77,

Table 2

Cerastoderma condition index ($\bar{X}$ sd), meat dry weight x 100 divided by shell weight related to maturity stage for pooled males and females from the five populations. Sample number in paranthesis.

	I	II	III	IV	V
Limfjord	9.2 ± 1.6 (21)	8.1 ± 0.9 (29)	13.6 ± 1.8 (50)	6.3 ± 1.9 (31)	6.9 ± 1.3 (21)
Gdansk	5.7 ± 1.3 (141)	12.7 ± 1.5 (85)	10.7 ± 1.2 (66)	8.8 ± 1.2 (54)	6.8 ± 0.9 (87)
Brouage	6.7 ± 1.0 (37)	5.2 ± 0.8 (19)	6.6 ± 1.3 (63)	7.4 ± 0.9 (6)	5.7 ± 1.0 (14)
Arcachon	8.3 ± 2.2 (19)	10.6 ± 1.6 (17)	6.6 ± 1.9 (34)	4.4 ± 1.3 (12)	5.0 ± 1.0 (10)
Embiez	4.9 ± 1.1 (41)	5.3 ± 1.5 (38)	6.3 ± 0.8 (27)	5.2 ± 0.9 (13)	2.8 ± 0.7 (12)

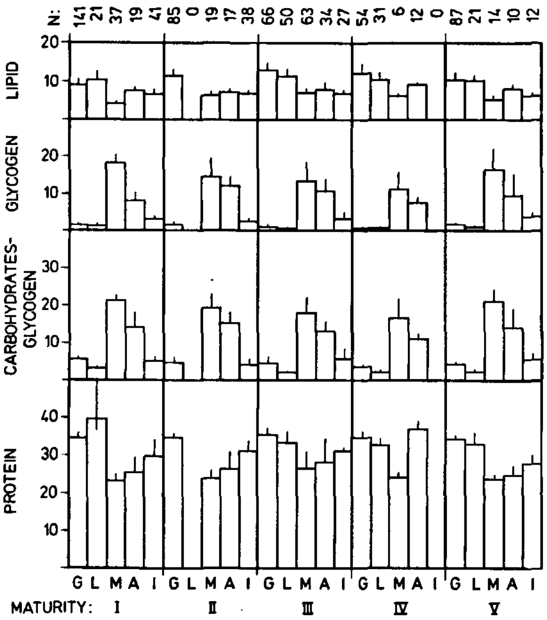

Figure 3

Relative content of dissolved protein, lipid, glycogen, and carbohydrates other than glycogen in dry tissue for the Cerastoderma glaucum/C. lamarck *complex at maturity stages I-V. Localities, G: Gdansk Bay, L: the Limfjord, M: Marennes-Oléron basin, A: Arcachon, I: Ile des Embiez. Columns indicate mean of relative content, and bars show standard deviation. The number of individuals is shown at the top of the figure.*

N = 433, and $\bar{X} = 0.56$, sd = 0.45, N = 123, respectively). Both results were significantly ($p < 0.001$) lower than those of the three populations from Marennes-Oléron, Arcachon, and Ile des Embiez ($\bar{X} = 14.01$, sd = 4.89, N = 139; $\bar{X} = 9.64$, sd = 3.36, N = 92; $\bar{X} = 3.02$, sd = 1.22, N = 118). ANOVA also separates region I from regions II and III. Further, the population from Ile des Embiez contained significantly ($p < 0.001$) less relative glycogen than the two French west coast populations, thus separating regions II and III.

The relative content of carbohydrates other than glycogen was significantly ($p < 0.001$) lower in the populations from Gdansk Bay and the Limfjord ($\bar{X} = 4.56$, sd = 1.41, N = 433 ; $\bar{X} = 2.31$, sd = 0.56, N = 123) than in the Marennes-Oléron and Arcachon populations ($\bar{X} = 18.59$, sd = 4,39, N = 139; $\bar{X} = 13.35$, sd = 3.51, N = 92), and also different ($p < 0.001$) from the Ile des Embiez population ($\bar{X} = 5.19$, sd = 1.80, N = 118). Thus, ANOVA of carbohydrates other than glycogen differentiates the three regions from each other.

DISCUSSION

Critical temperatures, and especially temperature changes, are known to initiate spawning in cockles (Gimazane, 1971; Kingston, 1974; Lucain and Martin, 1974; Brock, 1982), and it is not surprising, therefore, that the spawning period of populations of the *Cerastoderma glaucum/C. lamarcki* complex is to some extent correlated with latitude. Due to excessively lengthy sampling intervals, our data do not directly show a second spawning period in the Mediterranean population; but a bicyclic reproductive pattern including a spawning in late summer has been clearly demonstrated (Ivell, 1979 *a*; Wolowicz, 1991). Inter-annual condition variation is recorded for *C. edule* (Hancock and Franklin, 1972; Guillou *et al.*, 1990), and such variation was found for *Cerastoderma* from Gdansk Bay in different months, even when comparing animals at the same maturity stage (Tab. 3). Annual variation was also found when comparing individuals of each of the other populations at the same maturity stage at different dates [*see* for example the variation in glycogen content for the Arcachon population (Fig. 2 and 3)]. By normalizing the individuals according to their maturity stages and thereafter comparing their biochemical composition using the same methods, we were able to identify possible differences between the populations.

Thus, as demonstrated by standard deviations (Fig. 3), the above-mentioned differences were minor when compared to the overall pattern of differences between populations at the various maturity stages.

Table 3

Cerastoderma *from Gdansk Bay at maturity stage II in March, April and May in 1984 (upper results) and 1985 (lower results), sampling number:30. The relative meat dry weight content of dissolved protein, lipid, glycogen, carbohydrates other than glycogen, and ash.*

MONTH	March		April		May	
Sex	Females	Males	Females	Males	Females	Males
Prot.	33.87 ± 0.89 34.27 ± 1.12	36.73 ± 2.30 36.14 ± 0.87	33.83 ± 1.11 36.82 ± 2.10	33.84 ± 1.30 34.83 ± 1.83	36.48 ± 1.36 37.15 ± 1.88	36.49 ± 1.24 35.43 ± 1.28
Lip.	9.02 ± 0.99 10.31 ± 0.78	9.86 ± 0.61 9.86 ± 0.23	10.93 ± 0.89 12.08 ± 0.82	11.92 ± 0.64 12.41 ± 0.96	11.22 ± 0.78 10.61 ± 0.63	15.00 ± 0.30 11.82 ± 0.71
Gly.	2.43 ± 0.63 0.47 ± 0.12	1.76 ± 0.51 0.23 ± 0.11	2.13 ± 0.31 - -	2.39 ± 0.38 - -	- - - -	- - - -
Carb.+ Gly.	5.61 ± 0.51 3.00 ± 0.48	5.78 ± 0.74 2.15 ± 0.56	6.33 ± 0.73 1.12 ± 0.72	6.11 ± 0.47 0.91 ± 0.80	2.00 ± 0.31 1.39 ± 0.63	2.04 ± 0.21 1.97 ± 0.97
Ash	9.14 ± 1.21 13.29 ± 2.17	10.03 ± 2.09 16.38 ± 2.47	9.89 ± 1.17 13.87 ± 2.41	9.29 ± 0.74 17.90 ± 2.96	11.44 ± 1.14 14.13 ± 2.30	11.62 ± 1.33 15.66 ± 2.45

Other studies of cockles have shown seasonal variation in reproductive condition as well as in tissue dry weight, chemical content, and calorific value using monthly sampling periods without demonstrating obvious spawning related changes in the condition of the animals (Ivell, 1979 *a* and *b*; Newell and Bayne, 1980; Wolowicz, 1987 *b*). By analysing the dry weight and biochemical composition of individuals and relating the findings to their actual maturity stage, only significant ($p < 0.01$) changes due to spawning were found for the Mediterranean population. It is possible that temporary deficiency in biochemical components disappears after a rather short recovery period; therefore, the use of shorter sampling intervals may help to establish whether more dramatic changes in relation to spawning actually occur.

The high index values at maturity stage III found for the Limfjord and the Embiez populations reflect expected conditions for bivalves before the onset of spawning, while the results from the other populations, especially from Gdansk and Brouage, raises the question of whether their physiology has been stressed to some degree. Initial screening for toxic metals such as mercury, lead and cadmium has revealed relatively low amounts in the material from Nibe and Embiez (Hg less than 100 ppb dry weight, Pb less than 400 ppb, and Cd less than 50 ppb), and high cadmium content in *C. lamarcki* from the Gdansk Bay (*ca.* 3 000 ppb). No data are available for the other populations. Mercury tissue content of 2 000-3 000 ppb has been shown not to affect the growth of the species in environmental samples, and laboratory-exposed bivalves exhibited no growth reduction while accumulating mercury to the extremely high tissue content, 792 ppm (Brock, 1992). Other metals and combinations of other metals may, however, affect growth differently, thus we do not exclude that differences between *e. g.* the Gdansk and the Limfjord localities reflect antropogenic impact.

The biochemical composition was found to be size-independent ($p > 0.995$) for the following properties: relative protein, relative lipid, relative glycogen, and relative carbohydrate minus glycogen. Therefore, these properties were useful for comparisons of populations with non-matching size classes. Our finding that biochemical differences between the two sexes were small was probably influenced by the long sampling intervals.

The relative lipid content was higher in the population from region I than in those from regions II and III, which contrasts with the results obtained by Ivell (1979 *a* and *b*); in his Northern population (England), the lipid contents constituted a smaller fraction (3.56 to 6.10 %) than in his Southern population (Italy, 7.59 to 8.8 %). The comparison is, however, weakened by the fact that the two studies use different methods to quantify lipid content.

Comparisons of glycogen contents indicate that the population from region III (3.02 %) resembles the region I populations from the Baltic (1.25 and 0.56 %) more than the region II populations (14.01 and 9.64 %). However, Riva (1976) found a glycogen content of 15.8 to 20.0 % in *Cerastoderma* from the same region III locality and of 8.6 % in a neighbouring locality, and Ivell (1979 *a*) found glycogen contents of 9.5-32,8 % in his Mediterranean population, results that resemble our results from region II populations. Finally, the analysis of carbohydrates other than glycogen indicates a difference between the populations from region II and those from the two other regions.

An obvious weakness in all population comparisons based on phenotypes is that demonstrated differences between populations may simply reflect the effect of differential habitat conditions, such as food availability, predation, and physical and chemical factors. By comparing populations at similar maturity stages we have attempted to "normalize" the populations and thereby reduce some of the above-mentioned weakness. The differences between the populations from the three regions may reflect acclimation responses to different habitat properties as well as genetically-based adaptations. The populations from Marennes-Oléron and from Arcachon show some differences in comparison with the Mediterranean as well as the Northern European populations; since we have no information about the genetic distances between these region II populations and the populations from the other regions, any interpretation of the demonstrated similarities and differences can only be speculative.

The demonstrated difference between the populations from regions I and III can be related to, and accords with the genetic differences between these populations. The differences between the populations from each of the three regions do not prohibit first-generation hybrid formation. Hybridization experiments between Baltic and Mediterranean *C. lamarcki* and *C. glaucum* were carried out in 1981 (Y. Martin and V. Brock, not published). They gave viable larvae which did not metamorphose. Similar experiments combining individuals from the Baltic and Atlantic, the Baltic and Mediterranean, and the Atlantic and the Mediterranean were carried out in 1988 (M. Wolowicz, not published). These experiments resulted in metamorphosed hybrids for all three combinations.

Given the wide geographical distribution pattern of the complex, it is not surprising that distant populations exhibit different physiological properties. We find it probable that the demonstrated differences between the populations from the three regions reflect acclimation as well as adaptation to different habitat properties, such as food availability, temperature, salinity, and toxic substances. We have identified three different population groups in three ecologically different regions. Future studies of the population genetics of the complex may reveal whether the *Cerastoderma glaucum*/*C. lamarcki* forms a continous ecocline or whether it is composed of semi-isolated sub-populations.

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