

Chemical composition of *Leptocythere psammophila* (Crustacea: Ostracoda) as influenced by winter metabolism and summer supplies

A. M. Boderгат¹, G. Carbonnel¹, M. Rio¹, D. Keyser²

¹ Centre de Paléontologie stratigraphique et Paléocéologie associé au CNRS, URA 11, Université Claude Bernard Lyon 1, 27-43 bd du 11 novembre, F-69622 Villeurbanne Cedex, France

² Zoologisches Institut und Zoologisches Museum, Martin-Luther-King-Platz 3 D, W-2000 Hamburg, Germany

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Abstract. *Leptocythere psammophila* is well known for the ecophenotypic variations of the ornamentation of its carapace. To test the respective influences of the environmental parameters on its ornamentation, 41 living specimens were collected in the Baltic Sea, the North Sea and in the English Channel during spring, summer and winter (April 1987, September and December 1988). The carbonate carapaces of these specimens were analyzed by means of electron microprobe. Thirteen elements were detected. Statistical comparisons of means and variances were performed using classical *F* and *t*-tests. Data were submitted to Normalized Principal Component Analysis and Discriminant Analysis. There are no significant differences between chemical composition of the carapaces from the different stations. However, the variability of Baltic Sea specimens is lower than that of other stations. Nonetheless, strong and significant differences appear when samples are gathered by season. The chemical composition of summer individuals is controlled by variations in water salinity and in fine grain terrigenous sediment supply. The composition of winter samples is related to the incorporation of Mg. In the case of spring specimens, both these factors have to be considered. Discriminant analysis between winter and non-winter samples sets correctly 84% of the individuals. The wrongly classified specimens are interpreted as ostracods that did not molt during the season of sampling. By attributing them to their possible season of molting, the result of the Normalized Principal Component Analysis improves. Differences in ornamentation are slight. They occur between the samples from the Baltic Sea and those from the other stations. The former have larger and less numerous punctations and lack the smooth surface in the antero-ventral part of the carapace. The punctation diameter is more variable during summer than during spring. In the range of environments investigated, ornamentation of the carapace of *L. psammophila* seems unaffected by the seasonal environmental variations whereas its composition exhibits strong differences.

Introduction

Three cases can be recognized concerning the variability of the ornamentation of individuals from species that have a shell or a carapace: (1) There are no obvious variations. (2) The variability cannot be associated with environmental modifications; this phenomenon is called polymorphism (Reyment 1985, 1988) and a genetic control is assumed. (3) The variability is associated with modifications in the composition or characteristics of the environment (Boderгат 1983, Via and Lande 1985, Reyment and Kennedy 1991); this case corresponds to so-called ecophenotypic variations (Clark 1976, Benson 1981, Reyment 1985, 1988). If the environmental modifications are too weak, they will not have any consequence on the ornamentation. Thus, testing their influence requires the development of a sampling procedure which takes the following into account: the choice, for the study, of an organism that records instantaneously the characteristics of the surroundings; the observation of this organism in a stable and essentially closed environment; the study of the same organism in an environment where fluctuations are known or can be recorded; the evaluation of the environmental modifications on the shape, the ornamentation or any other morphological character of this organism; and the repetition of these observations at different periods in the year to determine if the fluctuations are seasonal.

Ostracods are small bivalve crustaceans that shed their carapace several times in order to grow. As adults, they do not molt again. The new cuticle is formed beneath the old one, separated from it only by a thin liquid-filled space, the so-called molting space (Keyser 1983, 1990). The liquid contains powerful enzymes which dissolve the innermost noncalcified layer of the former carapace and make its substances available again to the ostracod. An epicuticle is secreted and is calcified after molting. The speed of calcification is fairly rapid in the first few hours and decreases with time. During this process, several trace elements are incorporated together with the calcium in the carapace. Therefore the carapace can record some

characteristics of the environment during calcification. Tupen and Angel (1971) have shown that the whole amount of Ca used by the organism to build its carapace is taken from the ambient water just at the time of molting. Mg and Sr may be incorporated into the carbonate lattice at that time. However, other elements cannot generally be incorporated into these minerals. They are situated elsewhere in the carapace, either linked to organic compounds or as mineral inclusions.

Many authors have pointed out the relationships between chemical composition of carapaces and one or more characteristics of the ambient water: different environmental parameters (Sohn 1958) temperature (Cadot and Kaesler 1977, Bodergat 1983, Chivas et al. 1985, 1986a and b, Engström and Nelson 1991), seasonal cycles (Durazzi 1975), salinity (Chivas et al. 1985, Anadon and Julia 1990, Engström and Nelson 1991), sedimentation rate and amount of dissolved oxygen (Bodergat 1983, 1985, Bodergat et al. 1991). For similar surroundings, Cadot et al. (1975), Durazzi (1975), Bodergat (1983) and Chivas et al. (1983, 1985) have shown that the chemical composition of the carapace is related to the species. Finally, in some specimens, it has been shown, after a chemical study of the carapace and of ambient water, that ecophenotypic variations can be interpreted as an answer to change in some environmental parameters (Bodergat 1983, Tölderer-Farmer 1985, Bodergat et al. 1991).

Leptocythere psammophila is common on sandy substrates, near estuaries, from the Baltic Sea to the French Atlantic coast (Guillaume 1988). The well known variation in the ornamentation of its carapace could be related to environmental variations (Guillaume 1976, Hartmann and Kuhl 1978, Kuhl 1980).

Materials and methods

Ostracods

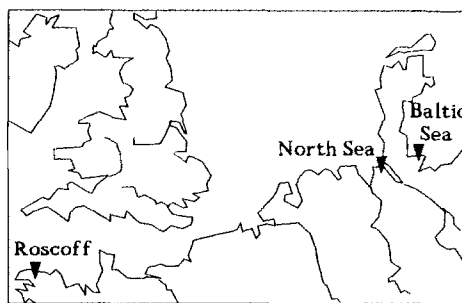
The specimens selected for this work were specifically determined by studying carapaces and soft parts. Only living adult females were chosen. It seems that *Leptocythere psammophila* has two generations a year (Kuhl 1980). The results provided by discriminant analysis on the chemical elements of the carapace confirm that opinion (see "Discussion").

Sampling sites (Fig. 1)

Sampling was initiated at low tide, in small puddles, on the shelf of the Baltic Sea, of the North Sea and of the English Channel, at the beginning of spring (April 1987), the end of summer (September 1988) and during winter (December 1988) in each case, within a few days.

Stn 1 is located at the mouth of the Kieler Förde, on the southern coast of the Baltic Sea. It is a brackish water marsh area. In this part of the Baltic Sea, the tidal range is 0.5 m and salinity does not exceed 16‰. Therefore, this is the maximum value for the salinity of the water in which *Leptocythere psammophila* lives except during very hot summer days. The ostracods are gathered near small puddles, remaining at low tide on slightly muddy sand. They are probably feeding on diatoms. During high tide they shelter in the pores between sand grains.

The sampling site is surrounded by the industrial city of Kiel and the large sailing harbour of Wentorf Marina. Pollution related to



▼ Sampling areas

Fig. 1. Location map of the sampling areas

activities in these areas probably has an influence on the composition of the water but it will not be investigated in this work.

Stn 2 lies on the North Sea near Sahlenburg, next to Cuxhaven at the mouth of the River Elbe. The sampling site is the Wadden Sea area, where at low tide, the shore extends over 10 miles. The salinity varies much more than in the previous station from 27‰ in April to 22‰ in September whereas the usual salinity of North Sea water is 33 to 35‰. The temperature range is fairly wide, as the substrate (light grey, fine silty mud) is covered with ice during winter and is completely dry during summer. The proximity of the Elbe and the Weser has to be pointed out because they are among the most polluted rivers in the North Sea.

Stn 3 is situated west of the small town of Roscoff at the southern end of the English Channel. During sampling, the salinity was that of normal seawater but, during summer, in some pools, salinity may reach 50‰. The substrate is a coarse well-sorted sand. The effects of a large oilspill which happened a few years ago are no longer evident, and there is no pollution at present. From one season to another, the ecological conditions are rather different. From spring to autumn, the development of phytoplankton lowers the amount of nutrients in the seawater (Rosenfeld 1979). During winter, and at the beginning of spring, primary production is low and the amount of available nutrients and level of dissolved O₂ rises.

Scanning electron microscopy

During molting, just before the ostracod sheds its carapace, the epidermal cells secrete a new procuticle (Okada 1982) which possesses the ornamental elements of the future carapace. When the former carapace is shed, this structure is inflated with water (Benson 1981) and not fully calcified. Nevertheless, there is not always a direct relationship between the degree of calcification and the importance of ornamentation. Indeed, for some species, the result of calcification is a smoothing of the carapace; this is called celation (Sylvester-Bradley and Benson 1971).

The surface of the carapace of *Leptocythere psammophila* has more or less rough and rounded punctations. Their diameter varies greatly, indicating large differences in calcification between individuals. These punctations can be completely obliterated as a result of a very high calcification of the carapace (Kuhl 1980). This process could be responsible for the presence of a smooth surface in the antero-ventral part of the carapace (Fig. 2).

The right valve of each analyzed individual has been observed by means of scanning electron microscopy. It appears that there are large differences in the number and size of the punctations. They have been counted on photographs, at the same magnification, on the same area, located between the dorso-median sulcus and the posterior side of the carapace (Fig. 2a). Most of the individuals have a smooth area whose surface is highly variable; it spreads between the post-ocular sulcus and the dorso-median sulcus (Fig. 2f, g, h and i). The micrographs of the right valve of each analyzed individual have been digitalized and the surface of the

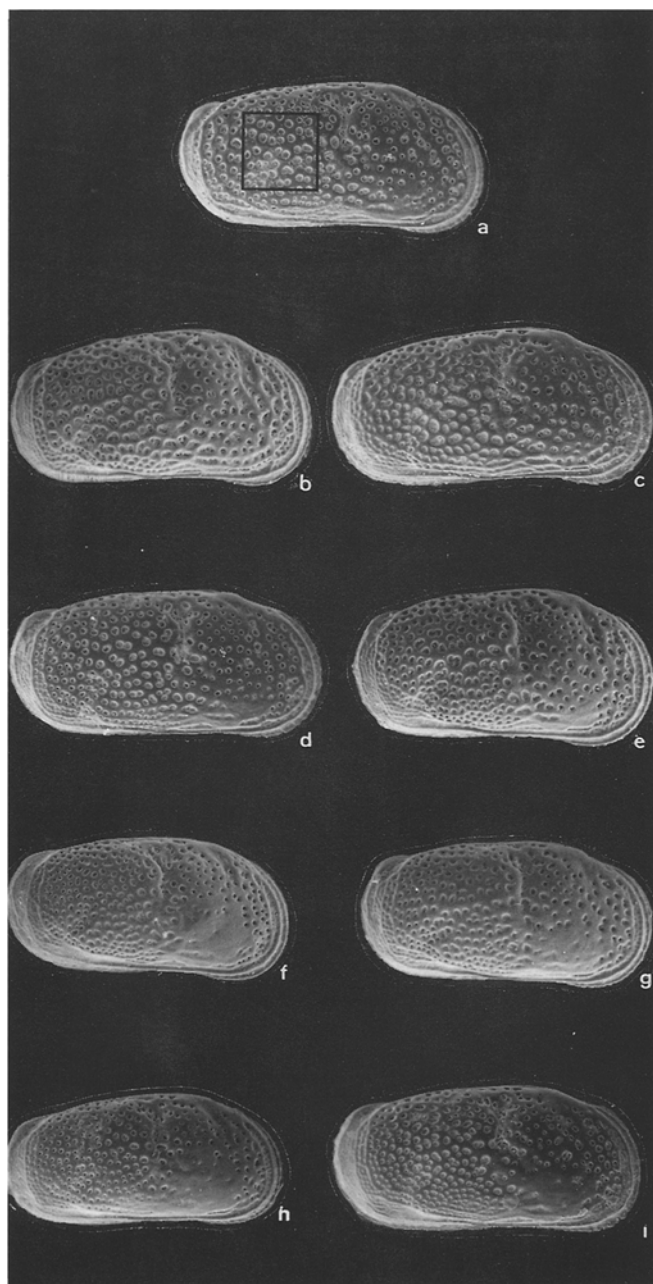


Fig. 2. *Leptocythere psammophila*. Right valve of the studied live female ostracod *L. psammophila* ($\times 70$). (a) Summer specimen from the North Sea (square indicates area on which punctations were counted); (b) winter specimen from the Baltic Sea; (c) spring specimen from the Baltic Sea; (d) spring specimen from Roscoff; (e) summer specimen from the Baltic Sea; (f) winter specimen from the North Sea; (g) summer specimen from Roscoff; (h) spring specimen from the North Sea; (i) winter specimen from Roscoff. Note that smooth anterior area is absent in specimens from the Baltic Sea [(b), (c) and (e)]

smooth area calculated. On the surface used for counting the punctations, the maximum length of the pores cut by four equidistant lines was measured on the same digitalized micrographs.

Electron microprobe

The left valve of each specimen was enclosed in an EPOXY resin, cut and polished parallel to a plane containing the hinge. These

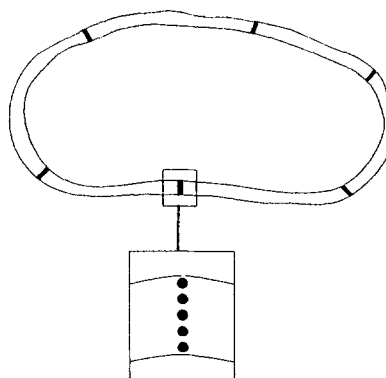


Fig. 3. *Leptocythere psammophila*. Location of points analyzed by means of electron microprobe on a section of a valve

surfaces were carbon-coated, film. We had the opportunity to perform the analyses with an electron microprobe CAMECA CAMEBAX (Centre des Sciences de la Terre, Université Blaise Pascal, Clermont-Ferrand, France). For each sample, the analysis was made at 30 points situated on five wreath and six sections (Fig. 3). We assume that the mean value of these 30 measurements is representative of the composition of the whole carapace. Thirteen elements were determined: Ca, Ba, Cl, S, Sr, Fe, Mn, Na, Mg, Al, Si, P and O. The results are expressed as element atomic percent (Table 1).

Statistical analysis

Before performing any computation, we calculated means and standard deviations for the whole elements in each sample (i.e., for the 30 analyzed points of each carapace). A detailed examination of the analytical results revealed that some points of analysis gave completely abnormal results with respect to analytical bias or to mineral inclusions. These results were discarded when they exceeded an interval of three standard deviations. We had to discard 213 values, i.e., 2.3% of the total results. Means for each sample were then recalculated. Means and variances of each elements for the samples set by stations and by seasons were also calculated (Tables 2 and 3).

Variances and means were compared by using *F* and *t*-tests. The table was also submitted to a Normalized Principal Component Analysis (NPCA). We computed the same analysis for the samples gathered by stations and by seasons. In the tables submitted to NPCA and Discriminant Analysis (DA), we removed O and Ca whose variances are too low and concentrations too high, as were Sr and Ba due to their high variances in the DA.

Results

Ornamentation and punctation

Analysis of the number of punctations shows no differences between individuals either from the different stations or from different seasons (Table 4). The only point to be mentioned is the low variability of the number of punctations from Baltic Sea specimens. The analysis of the sizes of the punctations (Table 4) shows that individuals living in the Baltic Sea have significantly larger punctations than those from the other stations. The same comparison with respect to seasons does not reveal any differences except that variability is greater in those individuals sampled during summer than for those sampled

Table 1. *Leptocythere psammophila*. Results of chemical analysis of the right valve of the carapace of *L. psammophila*. Each data is the mean of the results obtained on 30 points of analysis except when

this result exceeded an interval of 3 SD around the mean. P: spring; S: summer; W: winter

Stn Season	Element											
	Ca	Ba	Cl	S	Sr	Fe	Mn	Na	Mg	Al	Si	P
Roscoff												
03 P	46.577	0.011	0.338	0.244	0.127	0.029	0.034	0.796	0.765	0.029	0.232	0.698
48 P	47.573	0.012	0.070	0.160	0.110	0.012	0.013	0.348	0.887	0.006	0.005	0.545
49 P	47.359	0.011	0.051	0.192	0.099	0.012	0.018	0.249	1.169	0.011	0.008	0.555
50 P	46.941	0.015	0.176	0.220	0.116	0.051	0.013	0.362	1.183	0.005	0.009	0.672
63 P	47.242	0.017	0.046	0.214	0.120	0.017	0.013	0.269	1.217	0.004	0.007	0.578
07 S	45.766	0.022	0.480	0.214	0.104	0.044	0.019	0.943	1.477	0.033	0.088	0.628
08 S	47.126	0.016	0.198	0.146	0.118	0.049	0.015	0.707	0.803	0.018	0.053	0.605
43 S	47.152	0.011	0.190	0.143	0.107	0.014	0.014	0.508	1.027	0.015	0.008	0.586
62 S	47.527	0.011	0.110	0.119	0.107	0.010	0.011	0.392	0.847	0.004	0.013	0.632
34 W	47.383	0.012	0.087	0.135	0.099	0.017	0.007	0.202	1.134	0.005	0.005	0.604
35 W	47.764	0.015	0.123	0.124	0.098	0.014	0.012	0.208	0.795	0.005	0.009	0.541
36 W	47.686	0.017	0.165	0.144	0.138	0.027	0.017	0.188	0.800	0.019	0.020	0.563
64 W	46.876	0.016	0.090	0.208	0.123	0.020	0.057	0.401	1.113	0.010	0.020	0.713
67 W	47.545	0.012	0.077	0.206	0.105	0.028	0.014	0.264	0.858	0.012	0.019	0.576
North Sea												
05 P	47.350	0.017	0.043	0.101	0.142	0.021	0.032	0.549	0.972	0.005	0.012	0.535
57 P	47.324	0.014	0.079	0.271	0.087	0.022	0.055	0.215	0.953	0.009	0.015	0.715
58 P	46.361	0.014	0.118	0.250	0.100	0.024	0.043	0.607	1.455	0.048	0.015	0.651
59 P	47.112	0.019	0.117	0.211	0.130	0.020	0.039	0.401	1.133	0.005	0.008	0.571
11 S	46.606	0.017	0.114	0.180	0.117	0.033	0.034	0.529	1.245	0.022	0.075	0.662
12 S	44.928	0.013	0.499	0.317	0.122	0.348	0.033	1.367	1.232	0.153	0.265	0.537
51 S	47.594	0.016	0.057	0.141	0.133	0.022	0.039	0.366	0.907	0.008	0.008	0.501
113 S	46.685	0.015	0.170	0.151	0.124	0.060	0.008	0.618	1.149	0.054	0.103	0.600
19 W	47.686	0.012	0.074	0.119	0.111	0.016	0.023	0.202	0.954	0.007	0.005	0.507
21 W	47.857	0.014	0.054	0.146	0.094	0.019	0.024	0.209	0.737	0.010	0.007	0.551
54 W	47.136	0.012	0.107	0.102	0.115	0.106	0.038	0.353	1.124	0.013	0.011	0.595
55 W	47.136	0.012	0.107	0.102	0.115	0.106	0.038	0.353	1.124	0.013	0.011	0.595
56 W	47.492	0.016	0.061	0.107	0.120	0.012	0.025	0.312	0.965	0.023	0.010	0.542
Baltic Sea												
44 P	47.226	0.011	0.051	0.170	0.156	0.023	0.029	0.214	1.139	0.009	0.020	0.505
45 P	47.532	0.017	0.071	0.135	0.146	0.018	0.013	0.389	1.026	0.009	0.010	0.434
46 P	47.298	0.013	0.037	0.168	0.139	0.021	0.025	0.412	1.068	0.006	0.025	0.540
47 P	47.323	0.014	0.071	0.185	0.135	0.014	0.016	0.376	1.079	0.007	0.010	0.535
13 S	46.257	0.016	0.394	0.125	0.130	0.069	0.017	0.921	1.266	0.025	0.098	0.539
14 S	46.478	0.012	0.107	0.209	0.126	0.075	0.025	0.353	1.643	0.018	0.085	0.579
38 S	47.690	0.012	0.112	0.083	0.132	0.019	0.014	0.366	0.961	0.003	0.007	0.448
39 S	47.527	0.013	0.084	0.114	0.141	0.015	0.021	0.266	1.083	0.007	0.013	0.482
40 S	47.175	0.014	0.159	0.149	0.135	0.020	0.016	0.395	1.262	0.013	0.013	0.487
23 W	47.623	0.013	0.129	0.094	0.111	0.020	0.010	0.234	0.978	0.012	0.006	0.512
24 W	47.332	0.011	0.157	0.115	0.123	0.017	0.016	0.278	1.074	0.007	0.006	0.573
25 W	47.665	0.016	0.046	0.103	0.149	0.017	0.017	0.227	0.908	0.009	0.006	0.518
60 W	47.196	0.012	0.081	0.139	0.131	0.013	0.017	0.188	0.943	0.006	0.017	0.719
61 W	47.242	0.017	0.059	0.103	0.121	0.006	0.017	0.208	1.163	0.008	0.014	0.625

Table 2. *Leptocythere psammophila*. Chemical composition of the carapace of *L. psammophila*. Means of each element calculated for each season and station

Stn	Element											
	Ca	Ba	Cl	S	Sr	Fe	Mn	Na	Mg	Al	Si	P
Roscoff	47.180	0.014	0.157	0.176	0.112	0.025	0.018	0.417	1.005	0.012	0.035	0.607
North Sea	47.021	0.015	0.123	0.169	0.116	0.062	0.033	0.468	1.073	0.028	0.042	0.582
Baltic Sea	47.254	0.014	0.111	0.135	0.135	0.025	0.018	0.345	1.113	0.010	0.024	0.535
Season												
Spring	47.171	0.014	0.098	0.194	0.124	0.022	0.026	0.399	1.080	0.012	0.029	0.580
Summer	46.808	0.015	0.206	0.161	0.123	0.060	0.020	0.595	1.146	0.029	0.064	0.561
Winter	47.463	0.014	0.090	0.130	0.115	0.023	0.020	0.248	0.969	0.011	0.014	0.584

Table 3. *Leptocythere psammophila*. Chemical composition of the carapace of *L. psammophila*. Variances of each element calculated for each season and station

Element	Element										
	Ca	Ba	Cl	S	Sr	Fe	Mn	Na	Mg	Al	P
Stn											
Roscoff	2.570 × 10 ⁻¹	1.039 × 10 ⁻⁵	1.363 × 10 ⁻²	1.607 × 10 ⁻³	1.317 × 10 ⁻⁴	1.842 × 10 ⁻⁴	1.473 × 10 ⁻⁴	5.265 × 10 ⁻²	4.289 × 10 ⁻²	7.960 × 10 ⁻⁵	3.484 × 10 ⁻³
North Sea	5.406 × 10 ⁻¹	4.625 × 10 ⁻⁶	1.292 × 10 ⁻²	4.785 × 10 ⁻³	2.203 × 10 ⁻⁴	7.760 × 10 ⁻⁴	1.220 × 10 ⁻³	8.670 × 10 ⁻²	3.121 × 10 ⁻²	1.528 × 10 ⁻³	3.737 × 10 ⁻³
Baltic Sea	1.619 × 10 ⁻¹	4.145 × 10 ⁻⁶	7.555 × 10 ⁻³	1.272 × 10 ⁻³	1.296 × 10 ⁻⁴	3.905 × 10 ⁻⁴	2.393 × 10 ⁻⁴	3.139 × 10 ⁻²	3.272 × 10 ⁻²	3.002 × 10 ⁻⁵	4.993 × 10 ⁻³
Season											
Spring	1.149 × 10 ⁻¹	6.486 × 10 ⁻⁶	6.223 × 10 ⁻³	2.113 × 10 ⁻³	3.919 × 10 ⁻⁴	9.317 × 10 ⁻⁴	1.712 × 10 ⁻⁴	2.580 × 10 ⁻²	2.704 × 10 ⁻²	1.453 × 10 ⁻⁴	6.164 × 10 ⁻³
Summer	6.056 × 10 ⁻¹	9.179 × 10 ⁻⁶	2.107 × 10 ⁻²	3.257 × 10 ⁻³	1.267 × 10 ⁻⁴	7.353 × 10 ⁻⁴	8.584 × 10 ⁻⁵	9.236 × 10 ⁻²	5.523 × 10 ⁻²	1.468 × 10 ⁻³	4.090 × 10 ⁻³
Winter	6.761 × 10 ⁻²	3.834 × 10 ⁻⁶	1.399 × 10 ⁻³	1.172 × 10 ⁻³	2.464 × 10 ⁻⁴	5.161 × 10 ⁻⁴	1.476 × 10 ⁻⁴	3.761 × 10 ⁻³	1.674 × 10 ⁻²	4.124 × 10 ⁻²	3.968 × 10 ⁻³

Table 4. *Leptocythere psammophila*. Diameter and number of pores of the carapace of *L. psammophila* for the different stations and seasons. SD: standard deviation

	Pore diameter		Number of pores	
	Mean	SD	Mean	SD
Stn				
Baltic Sea	38.69	5.66	101.1	18.21
North Sea	28.99	5.96	131.2	27.44
Roscoff	28.37	7.37	131.2	37.14
Season				
Spring	31.00	5.82	124.8	37.59
Summer	34.14	9.97	126.0	23.49
Winter	33.69	7.87	112.5	31.95

Table 5. *Leptocythere psammophila*. Chemical composition of the carapace of *L. psammophila*. Comparison of variances and means of chemical elements between seasons. Variances significantly different at the 95% confidence level labeled with the letter corresponding to the season whose variance is the greatest. P: spring; S: summer; W: winter

Element	Summer–Winter		Spring–Winter		Summer–Spring	
	F	t	F	t	F	t
Al	S 25.28	0.79	P 2.53	0.12	S 10.00	0.70
Fe	S 14.16	0.60	W 5.49	0.05	S 77.78	0.61
Na	S 23.26	1.58	P 6.57	1.22	S 3.53	0.78
Cl	S 21.23	1.01	P 6.07	0.13	S 3.50	0.89
Si	S 50.56	0.99	P 30.33	0.38		1.67 0.53
S	S 3.03	0.68		2.02 1.61		1.50 0.63
Sr		1.98 0.62		2.02 0.51	P 4.00	0.06
Mn		1.10 0.00		2.02 0.48		2.22 0.48
Mg	S 3.27	0.92		1.61 0.73		2.04 0.31
P		1.01 0.35		1.52 0.05		1.50 0.26
Ba		2.28 0.38		1.52 0.00		1.50 0.35
Ca	S 9.01	1.12		1.71 0.93	S 5.27	2.62

Table 6. *Leptocythere psammophila*. Chemical composition of the carapace of *L. psammophila*. Comparison of variances and means of chemical elements between stations. Variances significantly different at the 95% confidence level labeled with the letter corresponding to the station whose variance is the greatest. N: North Sea; R: Roscoff

Element	North Sea Baltic Sea		Roscoff North Sea		Roscoff Baltic Sea	
	F	t	F	t	F	t
Al	N 67.06	0.55	N 25.15	0.46	R 2.67	0.39
Fe	N 20.12	0.56	N 40.24	0.57		2.00 0.00
Na	N 2.82	0.49		1.65 0.07		1.71 0.34
Cl		1.63 0.11		1.07 0.28		1.75 0.42
Si	N 6.29	0.33		1.68 0.11	R 3.75	0.24
S	N 5.03	0.60		2.51 0.13		2.00 1.04
Sr		2.01 1.42		2.01 0.32		1.00 2.12
Mn	N 5.03	1.89		1.00 1.44	R 5.00	0.00
Mg		1.06 0.22		1.07 0.01		1.30 0.53
P		1.24 0.66		1.34 0.41		1.67 1.08
Ba		1.26 0.45		1.99 0.35		2.50 0.00
Ca	N 3.36	0.38		1.59 0.43	R 3.34	0.27

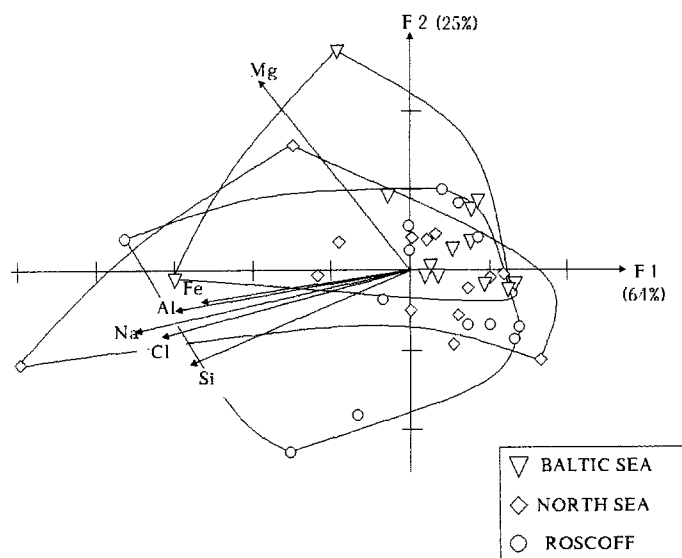


Fig. 4. *Leptocythere psammophila*. Normalized Principal Component Analysis (NPCA). Plane of the first two factors of a NPCA performed with 11 elements analyzed in 41 left valves of *L. psammophila*. Only elements correlated to the factors have been pointed. Samples gathered according to stations. Dispersion of the sets is rather equivalent for every station. F₁: first factor; F₂: second factor. Number in parentheses: % of inertia of the corresponding factor

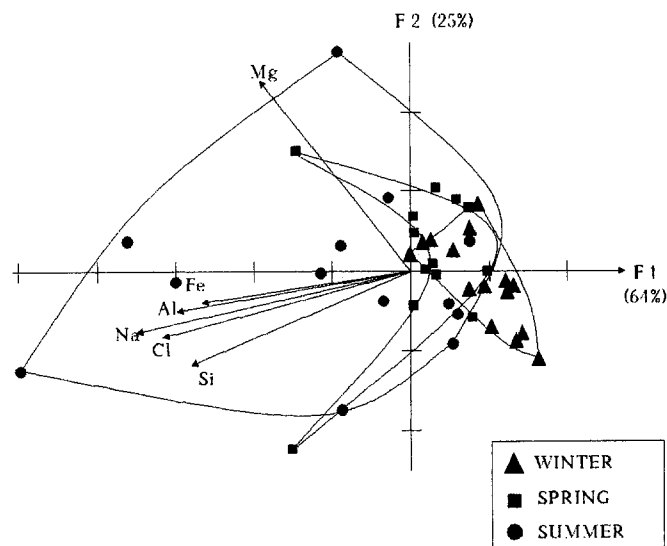


Fig. 5. *Leptocythere psammophila*. Normalized Principal Component Analysis (NPCA). Plane of the first two factors of a NPCA performed with 11 elements analyzed in 41 left valves of *L. psammophila*. Only elements correlated to the factors have been pointed. Samples gathered according to seasons. There are strong differences between sets. F₁: first factor; F₂: second factor. Number in parentheses: % of inertia of the corresponding factor

during spring. The average surface of the smooth area is the same everywhere and at all seasons, except for the specimens from the Baltic Sea which do not have such an area (Fig. 2b, c–e). It is slightly more variable during summer than during winter.

Therefore, it appears that the mean characteristics of ornamentation do not vary significantly in time or space, with the exception of the individuals sampled in the Baltic Sea whose carapaces have larger and less numerous punctations and which do not possess an anterior smooth area (Fig. 2b, c–e).

Chemical composition

We have compared the bulk results by means of classical *F* and *t*-tests. It is noteworthy that variances are often different but that means are not (Tables 5 and 6). The differences are important between seasons for Al, Fe, Na, Cl and Si. They are weak or nil for S, Sr, Mn, Mg, P, Ba and Ca. They are less marked between stations since only Al, Fe and Si and at a lower level Na, S and Mn present differences between variances. It is noteworthy that the variability of the data from the Baltic Sea is always the lowest. Thus, we must keep in mind that the chemical composition of the carapaces is mainly controlled by the time variability of the different components and not by their available amount in one place at one time.

The results of NPCA provide a more complete illustration of these remarks. They were computed on the whole samples and the chemical elements, Ca and O excepted. As usual, we can study variables and samples grouping. We will only comment on the F₁ (first factor)–

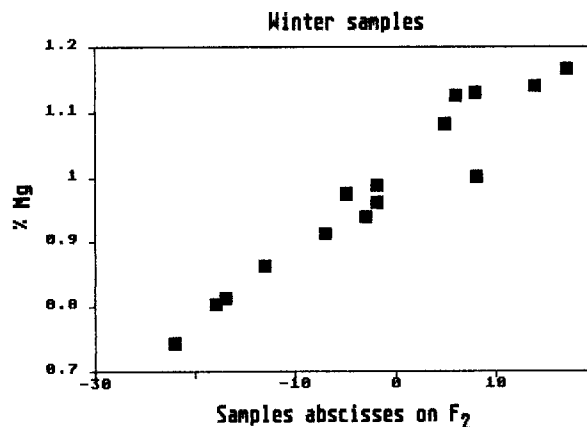


Fig. 6. *Leptocythere psammophila*. Ordering of winter samples according to their abscisses on F₂ (second factor). Also ordered according to Mg content

F₂ (second factor) plan for it contains 89% of the total inertia.

Five elements, Na, Cl, Si, Al and Fe, are linked to F₁; Mg is associated with F₂. These are the elements, previously mentioned, whose variances are significantly different from one season to another.

The samples can be gathered in two ways, by stations and by seasons. In the first case (Fig. 4), the sets are similarly dispersed and completely superimposed. As for the variances, the Baltic Sea sample set is a little more grouped than the other ones. In contrast, the shape of the sets of seasons are completely different (Fig. 5). Winter samples are well gathered in a triangle lengthened in the direction of the Mg vector. These samples can be ordered

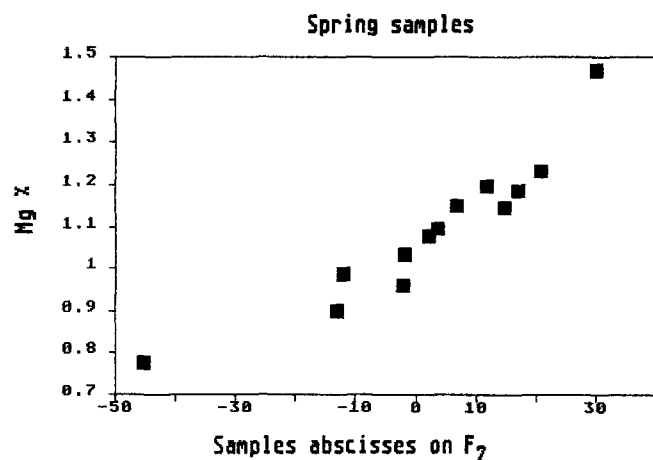


Fig. 7. *Leptocythere psammophila*. Ordering of spring samples according to their abscisses on F_2 (second factor). Also ordered according to Mg content

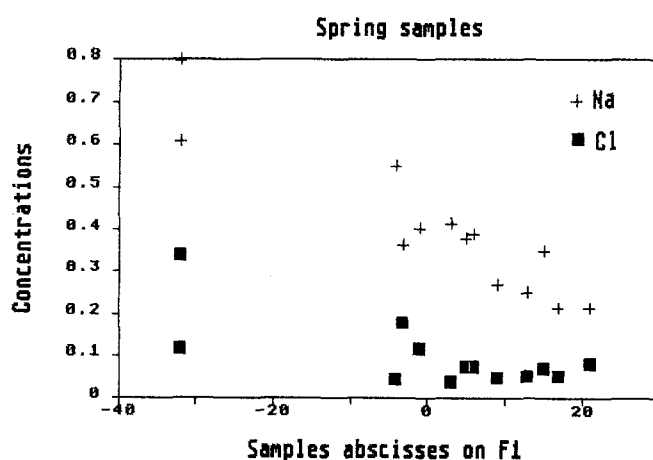


Fig. 8. *Leptocythere psammophila*. Ordering of spring samples according to their abscisses on F_1 (first factor). Na and Cl content roughly ordered

according to their abscissa on F_2 ; their Mg contents are then also well ordered (Fig. 6). Spring sample sets form a crescent whose focus is approximately situated near the barycentre. They can be ordered in the same way as the winter samples, and, in point of fact, according to their Mg contents (Fig. 7). But they can also be ordered according to their abscissa on F_1 , and their Na, Cl, Si, Al and Fe contents is then also correctly ordered (Fig. 8). The summer sample cluster is the most extended; it seems lengthened in the direction of the bundle of Na, Cl, Si, Al and Fe vectors. The classification of these samples, according to their F_1 abscissa, gives a good ordering of the Na and Cl contents (Fig. 9a). The Si, Al and Fe contents are arranged only for the samples whose abscissa is negative (Fig. 9b).

The surface of the fields occupied by the samples of each season corresponds to the variability of their composition revealed by comparisons of the variances. Thus, the chemical composition of the carapaces is mainly controlled by variations of salinity (Na and Cl), and fine grained detrital sediment supply (Si, Al and Fe) during summer, by Mg during winter and by all the elements during spring. These conclusions can be confirmed by NPCA performed for each season and each station. The results (Fig. 10) are similar to those obtained on the whole samples except for the winter and the Baltic Sea. In every case, salinity and terrigenous supply are the main sources of inertia, Mg being the next most important. For the Baltic Sea samples, there is a separation between salinity and detrital supply, the former being dominant over the latter which has the same role as Mg. For winter, the dominant elements are completely different. Salinity and terrigenous supply do not appear, but other elements, especially P and S, associated with F_3 in the first analysis, have an important inertia. These observations tend to separate the winter samples from the others. We have used discriminant analysis (DA) to see whether or not these samples are different from the others. Three groups were made: (1) winter samples from every station; (2) spring and summer samples from the Baltic Sea and

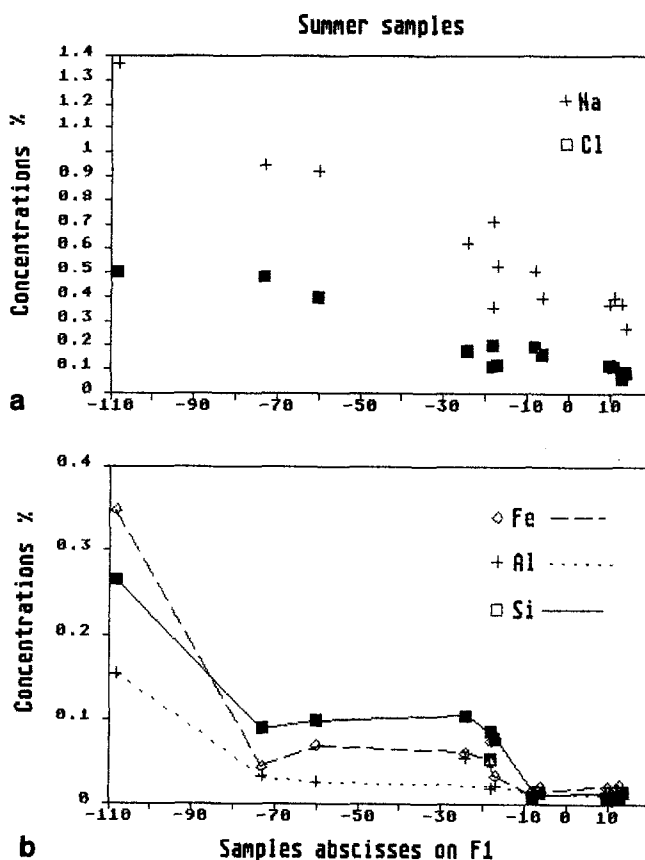


Fig. 9. *Leptocythere psammophila*. Ordering of summer samples according to their abscisses on F_1 (first factor). (a) Na and Cl content ordered; (b) Si, Al and Fe content ordered only for samples whose abscissa is negative

from the North Sea; these two groups were used to calculate the best discriminant function; (3) the spring and summer samples from Roscoff were used as a test group to validate the function. The discriminant function is:

$$Za = 0.5 \text{ Na} + 0.55 \text{ S} + 0.15 \text{ Mg} - 0.55 \text{ Cl} - 1.12 \text{ Fe.}$$

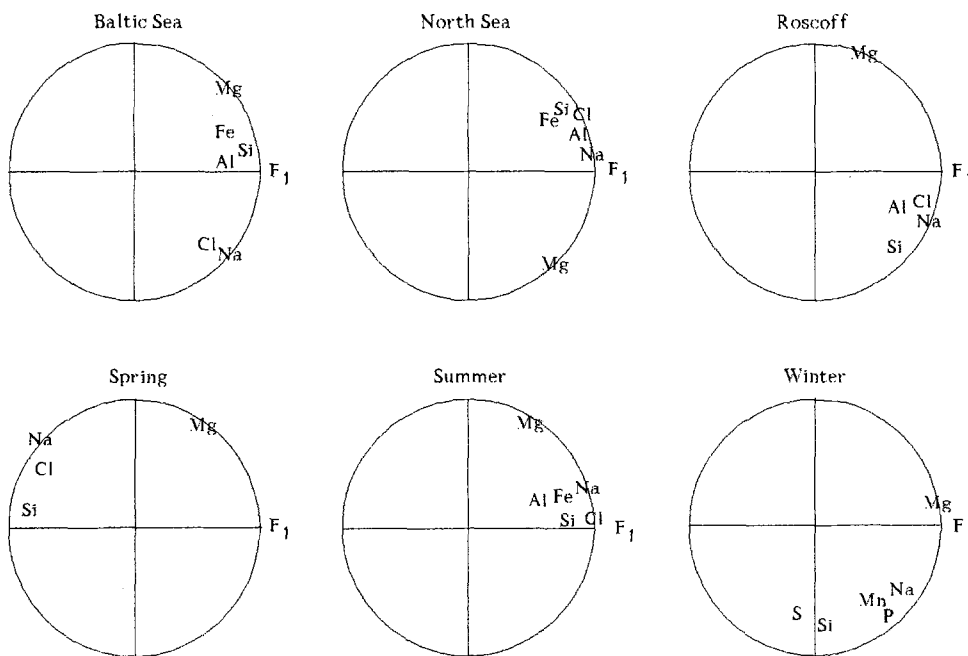


Fig. 10. *Leptocythere psammophila*. Correlations circles after Normalized Principal Component Analysis (NPCA) of samples from different stations and different seasons. Note that only the relative and not the absolute position of the variables must be considered. Results equivalent except in the case of winter and Baltic Sea. F_1 : first factor; F_2 : second factor

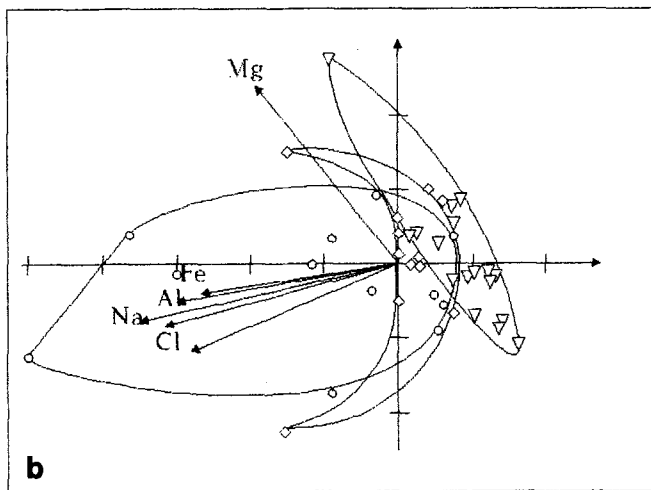
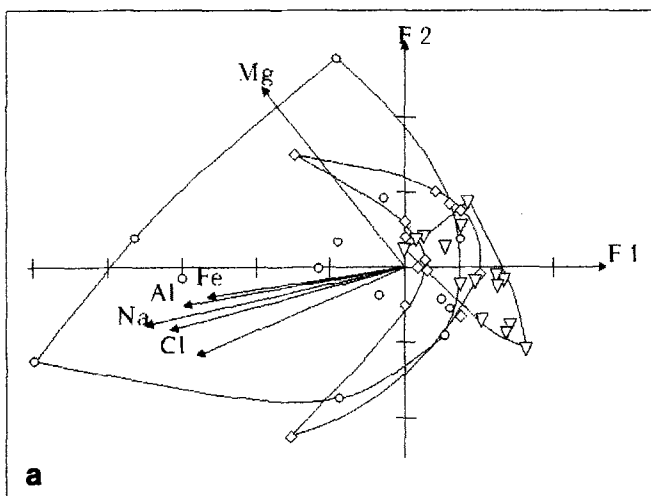


Fig. 11. *Leptocythere psammophila*. Normalized Principal Component Analysis (NPCA). Plane of first two factors of a NPCA performed with 11 elements analyzed in 41 left valves of *L. psammophila*. (a) Same as Fig. 5, samples gathered according to seasons; (b) samples gathered according to discriminant analysis. Discrimination between sample sets is improved

It is noteworthy that the elements associated with this function are the same as those revealed by NPCA. The distance between the barycenters of the two sets reaches 4.29; this value is highly significant ($F=26.6$ for 5,26 df). The discriminant function sets correctly 84% of samples: 14 over 15 in the winter group, 13 over 17 in the non-winter group. In the test group, 8 over 9 samples (90%) are correctly classified. It is noteworthy that changing the seasonal attribution of the samples in Fig. 11a gives a better discrimination between the three sets (Fig. 11b). The winter group is clearly elongated parallel to the Mg vector; the shape of the spring crescent does not change; the summer set is smaller and obviously oriented by the Na, Cl, Si, Al and Fe vector bundle. We can then infer that DA could give us a more accurate definition of the time at which the individuals molted. We will discuss the consequences of this idea later.

Discussion

The chemical composition of the carapace is controlled by the variations of salinity (Na and Cl), terrigenous supply (Si, Al, Fe) and by metabolism controlling Mg incorporation. The latter is discernible only during winter, when the effect of the former are very weak. Microprobe measurements have revealed pinpoint abnormal concentrations in Si (quartz or siliceous skeleton remains), Si, Al and Fe (clays) or Na and Cl (halite). They represent inclusions of minute particles in the carapace of the ostracod.

Thus, when the variation range of the environmental conditions is wide enough (during summer and partly during spring), the ostracod cannot control the composition of its carapace: it incorporates some of the available minute grains. Only when an environment is more stable (during winter) does the influence of metabolic control appear: most of the time, *Leptocythere psammophila*

seems passive towards the environmental composition. We could guess that when the temperature is the highest, the molt process occurs most quickly and does not allow an important incorporation of detrital elements. Some other studies on *Cyprideis torosa* show some relation between these elements and periods of erosion in the environment (author's unpublished data, work in progress).

The previous discussion concerning differences in the chemical compositions is based on seasonal variations. On the other hand, the only differences in the ornamentation of the carapaces were observed between the samples from the Baltic Sea and those from the other two stations. Chemical composition seems to be time dependent, ornamentation space dependent. This is a clear indication that, in the case of *Leptocythere psammophila*, there is no relationship between ornamentation and the chemical composition of the carapace. This conclusion is in contrast to the results obtained for *Cyprideis torosa* (Bodergat 1983, Bodergat et al. 1991). But it must be pointed out that these results have been obtained with two different genera, and numerous studies have shown that the composition of carapaces or skeletons are related to the species (Durazzi 1975, Cadot and Kaesler 1977, Bodergat 1983, Chivas et al. 1985). Further *Cyprideis torosa* was studied in environments whose compositions, and salinities, were highly variable (from 1 to 120 g l⁻¹; Bodergat 1983), whereas, in the case of *Leptocythere psammophila* they are much lower.

Therefore, these conclusions should be tested with others species living in the same or other environments.

Another consequence of our calculations concerns the life cycle of this species. According to DA, four of the 17 specimens (ca. 25%) sampled during spring or summer have a chemical composition similar to that of winter individuals. Three of them come from the Baltic Sea and one from the North Sea. These exceptions might be considered to be individuals that molted during winter and that lived up to the time of sampling. On the other hand, only one individual from 15 (7%) sampled during winter has a composition characteristic of spring or summer. The survival ratio is greater in the first case than in the second one, and, moreover, this ratio might be better for ostracods from the Baltic Sea than for the others. The survival ratio is higher in the northern part of the study area than in the southern part. Individuals which molted during winter can survive until summer whereas the opposite case is uncommon. Thus, the ostracods unduly attributed to summer (because they were sampled during this season) and really belonging to winter (because of their chemical composition) are evidence of longevity and physiological resistance.

Further geochemical studies of carapaces of the genus *Leptocythere* should be limited to the only important elements extracted by data analyses presently used (NPCA and DA), Na, Cl, Al, Si, Fe and Mg.

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