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Comparative ultrastructure of the cuticle of some pelagic, nektobenthic and benthic malacostracan crustaceans

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Abstract. The ultrastructure of malacostracan integument was examined and compared in 11 species collected primarily from the western Baltic Sea in 1989, of which eight species were studied for the first time (indicated below by an asterisk). We attempted to relate cuticle structure and thickness to swimming aptitude. The pelagic euphausiid *Meganyctiphanes norvegia* and the mysids *Praunus flexuosus** and *Neomysis integer** displayed a thin, little-mineralized, and thus light-weight cuticle. Laminae of the endocuticle were very thin (0.1 μm) relative to those of the exocuticle (1 μm). In contrast, laminae in the procuticles of the benthic amphipods *Gammarus locusta, Caprella linearis**, *Corophium volutator**, *Orchestia gammarellus**, and the isopod *Idotea baltica* were evenly distributed, comparatively thick (1 to 2 μm), and more heavily mineralized. The nektobenthic amphipod *Hyperia galba**, the cumacean *Diastylis rathkei** and the decapod *Crangon crangon** migrate between pelagic and benthic regions. Only near the hypodermis did these organisms exhibit the characteristically pelagic fine-layered endocuticle. A membranous layer was lacking in all species investigated. In contrast to the less-mineralized cuticles of the species analyzed here, a membranous layer appears to be restricted to crustaceans with heavily calcified shells. Ultrastructural results were substantiated by morphometric calculations, which indicated differences in thickness of the total cuticle relative to body volume. In the pelagic malacostracans, thickness of the cuticle did not increase with body volume over the size range investigated.

Introduction

Basic knowledge on structure and chemical composition of the crustacean shell was obtained by Drach (1939). Subsequent histological examination elucidated the fine structure of the cuticle and led to generalized models of crustacean integument (Dennell 1947, Richards 1951, Hackmann 1971, Stevenson 1985; cf. Fig. 8). Generally, the integument is bordered by a relatively thin outermost epicuticle. The underlying procuticle consists of a massive multilayered exo- and endocuticle, typically followed by a thinner membranous layer. Underneath, a single layer of cells, the epidermis, secretes the cuticle before and after ecdysis. Buchholz and Buchholz (1989) investigated the ultrastructure of the cuticle of Antarctic krill *Euphausia superba*, and found remarkable deviations from this basic scheme. In particular, the membranous layer was missing, and the number of cuticular laminae was very high. These features were discussed with respect to possible adaptations in the pelagic life style of the krill.

Interrelations between environmental requirements and chemical composition were also noted. In insects and spiders (Hadley 1986), choice of habitat is directly reflected in contents of chitin, protein, and lipids, and in the degree of sclerotization of the various cuticle layers. In decapod crustaceans, the proportion of shell made up of cuticle-sublayers is closely related to the shell's physical properties (Voss-Foucart and Jeuniaux 1978). Such differences in chemical and physical features should also be reflected in the structure of the arthropod integument.

Crustaceans inhabit all regions of the aquatic environment in great abundance, from the pelagic to the benthic and littoral zone. Physical conditions of the particular regions are equally diverse, resulting in different requirements to the exoskeleton. A pelagic crustacean, e.g., must have a light-weight cuticle to facilitate swimming. In contrast, benthic crustaceans need protection from hard bottom structures. The need and ability to swim are greatly reduced in these latter forms. Accordingly, adaptive differences in ultrastructural properties of cuticles were to be expected.

Our aim was first to describe and compare the ultrastructure of cuticles in a series of malcostracan species, which we were able to obtain from Kiel Bight, and in a euphausiid from the Kattegat. To date, a wide variety of preparatory methods for ultrastructural study of crustacean cuticle have been used, which makes interspecific comparison difficult. We used standardized methods and transmission electron microscopy (TEM) to compare the

fine structural properties of the cuticle of 11 malacostracan species. Eight of these species were studied for the first time.

Secondly, we examined the cuticular structures with respect to interspecific swimming aptitudes, in order to determine possible adaptive features. Morphometric analyses of cuticular dimensions and of body volume were conducted to facilitate a description of ecotypes. However, this was only possible in a broad sense. Overall our study must be considered an initial attempt to relate some behavioural and environmental constraints to cuticle structure.

The 11 species investigated were classified into three broad categories; pelagic, nektobenthic and benthic, according to the species' known habits (see references below). Pelagic species, i.e., those that swim virtually continuously, were represented by the euphausiid *Meganyctiphanes norvegica* (Buchholz and Boysen-Ennen 1988) and the mysids *Praunus flexuosus* and *Neomysis integer* (Green 1968). Species frequently migrating between pelagic and benthic regions were characterized as "nektobenthic" (Pérès 1982); among these were the amphipod *Hyperia galba* (Dittrich 1988), the cumacean *Diastylis rathkei* (Habermehl et al. 1990) and the decapod *Crangon crangon* (Hagerman 1970). Predominantly benthic crustaceans include species that rarely swim or are entirely sessile. This group was represented by the isopod *Idotea baltica* (Gessner 1957) and by the amphipods *Gammarus locusta*, *Caprella linearis* (Dahl 1977), *Corophium volutator* (Icely and Nott 1985), and *Orchestia gammarellus* (Vogel 1985).

Materials and methods

With the exception of *Meganyctiphanes norvegica*, which was caught using a ring-trawl in the Scandinavian Kattegat near the island of Laesoe, all specimens were dredged during 1989 in the Kiel Bight ("Kieler Bucht") in the western Baltic Sea.

To ensure standardized comparison, only the dorsal region of segments carrying appendages was analyzed (see Table 1). A total of ca. 400 specimens was investigated. Moult stages were first assessed by testing the cuticle hardness through compressing the lateral body parts with fine forceps. An exact determination was possible by analyzing semithin sections and electron micrographs. Only individuals in moulting stages C to D_1 (Drach 1939, Buchholz 1982) were evaluated.

Ultrastructure

All specimens were dissected immediately after being caught. Segments carrying appendages were detached with fresh razor blades and immersed in Karnovsky's (1965) fixative in 0.1 *M* phosphate buffer at 4 °C for light microscopy and TEM. Subsequently, the tissues were rinsed in cold 0.1 *M* phosphate-buffered saline with 7.5% sucrose (pH 7.7), post-fixed for 5 h in 2% 0.1 *M* phosphate-buffered OsO_4, dehydrated in a graded series of ethanol, passed through propylene oxide and embedded in araldite. Hackman (1984) pointed out that decalcification dissolves the water-soluble proteins of the cuticle. Therefore, to preserve the natural structure, samples were not decalcified. This meant that, due to the ensuing difficulties in cutting, some sections could not be optimally presented. Semithin sections were obtained with a glass knife, stained in Methylene Blue according to Richardson et al. (1960) and viewed on a Zeiss Photomicroscope III. Diamond-cut thin sections were mounted on formvar-coated copper grids and double-stained with a saturated solution of uranyl acetate and lead citrate according to Reynolds (1963). The grids were viewed and photographed on a Zeiss electron microscope EM 9 S-2.

Morphometry

To reduce the different shapes of the crustaceans to a common scheme, the volume of each specimen was calculated as a cylinder. Semithin sections (237) were photographed on a Zeiss Photomicroscope III, and the cross-sectional area of each segment was determined on an Interactive Image Analysis System IBAS 1. This cross-sectional area was multiplied by individual body length, measured to 0.5 mm accuracy, to give an estimated body volume. Cuticular thickness was measured from the electron micrographs. Regressions of crustacean volumes against cuticular thicknesses were calculated and compared.

Results

General features

The integument of all species was divided into an outer, narrow epicuticle, followed by a laminated procuticle and an underlying hypodermis. The procuticle exhibited the typical helicoidal arrangement of fibrils and was vertically pervaded by pore canals. With the exception of *Caprella linearis*, the procuticle was always divided into two sublayers, which were identified as an outer exocuticle and an inner endocuticle. In all species, the endocuticle represented the thickest cuticular layer. The relative proportions of layer thickness in the species investigated were distinctly different. A membranous layer was lacking in all specimens analyzed.

Pelagic species

Cuticles of the three pelagic species displayed pronounced differences in laminar thickness of the exocuticle and endocuticle. The exoskeleton of *Megancytiphanes norvegica* had an electron-dense exocuticle with laminar thickness of 1 μm, and an electron-translucent endocuticle with laminae of only 0.1 μm (Fig. 1 a). The epicuticle was always artificially detached from the underlying exocuticle, and the resulting gap was filled with electron-dense material of unknown origin. A similar pattern was found in the cuticle of *Praunus flexuosus*, except that the endocuticle was the electron-dense layer (Fig. 1 b). A transition zone between epicuticle and exocuticle was often visible. Compared to that of the other species, the epicuticle in *P. flexuosus* was relatively thick and showed cylindrical electron-dense structures in the distal region. The mysid *Neomysis integer* had an almost homogeneous electron-dense procuticle (Fig. 1 c). Laminae in the exocuticle were up to 0.3 μm thick, whereas those of the endocuticle were thinner than 0.1 μm. Cylindrical structures in the epicuticle were far less common than in *P. flexuosus*.

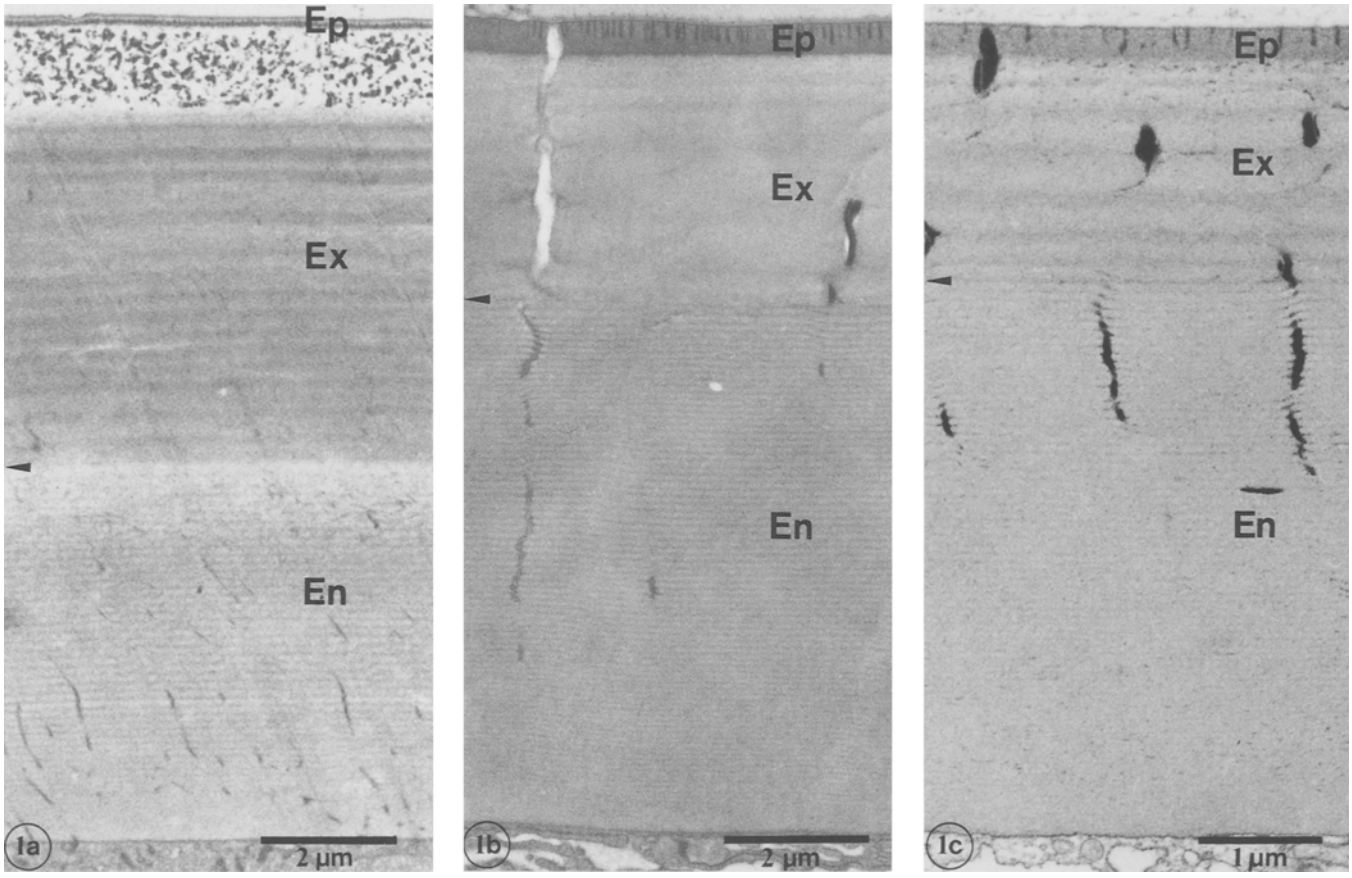

Fig. 1. Electron micrographs of cuticles of pelagic malacostracans. (a) *Meganyctiphanes norvegica*, ×8 900; (b) *Praunus flexuosus*, ×9 500; (c) *Neomysis integer*, ×16 200. Ep: epicuticle; Ex: exocuticle; En: endocuticle; arrows indicate border of procuticular sublayers

Nektobenthic species

Exoskeletons of the nektobenthic species were generally distinguished by relatively thick endocuticular laminae followed by thin ones close to the hypodermis. Both exo- and endocuticle of *Crangon crangon* increased gradually in electron density from distal to proximal regions (Fig. 2a). In some samples, a small and less electron-dense transition zone between epicuticle and exocuticle was visible, and/or the endocuticle was divided into two layers of different electron density. The laminae of the exocuticle and the distal region of the endocuticle were equally thick (max. 2 µm). However, in the proximal part of the endocuticle, the laminae became increasingly narrow (min. 0.3 µm). Laminar borders of the exocuticle of *Diastylis rathkei* were not well defined, in contrast to those of the endocuticle (Fig. 2b). Laminae of the endocuticle in the distal region were almost 1 µm thicker than those of the exocuticle (0.6 µm). In the inner part of the endocuticle the laminae became thinner (min. 0.3 µm). The epicuticle was very thin (0.2 µm). *Hyperia galba* and *D. rathkei* displayed similar cuticles. The procuticle of *H. galba* consisted of an electron-translucent exocuticle with laminae of 0.5 µm thickness, and an electron-dense endocuticle with laminae thicknesses ranging from 1 µm near the exocuticle to 0.3 µm close to the hypodermis (Fig. 2c).

Benthic species

Laminae in the cuticles of the benthic crustaceans were considerably thicker (2 µm) than those of the nektobenthic species. The procuticle of the amphipod *Gammarus locusta* consisted of an electron-dense exocuticle and an electron-translucent endocuticle (Fig. 3a). Lamina thickness was between 2 and 3 µm in the distal region and became thinner in the inner zone of the endocuticle (0.5 µm). Between epicuticle and exocuticle a small transition zone was always visible. The epicuticle of the isopod *Idotea baltica* was very thick (max. 3.3 µm) and contained elongated and vertically oriented structures (Fig. 3b). Between epicuticle and exocuticle a less electron-dense transition zone was visible. The laminae in the middle of the exocuticle were very thick (max. 7 µm), while those of the periphery became thinner. The electron-translucent endocuticle consisted of laminae which in the outer part were of similar size to those of the exocuticle but which were much thinner in the inner part at the level of the hypodermis (min. 1 µm). The integu-

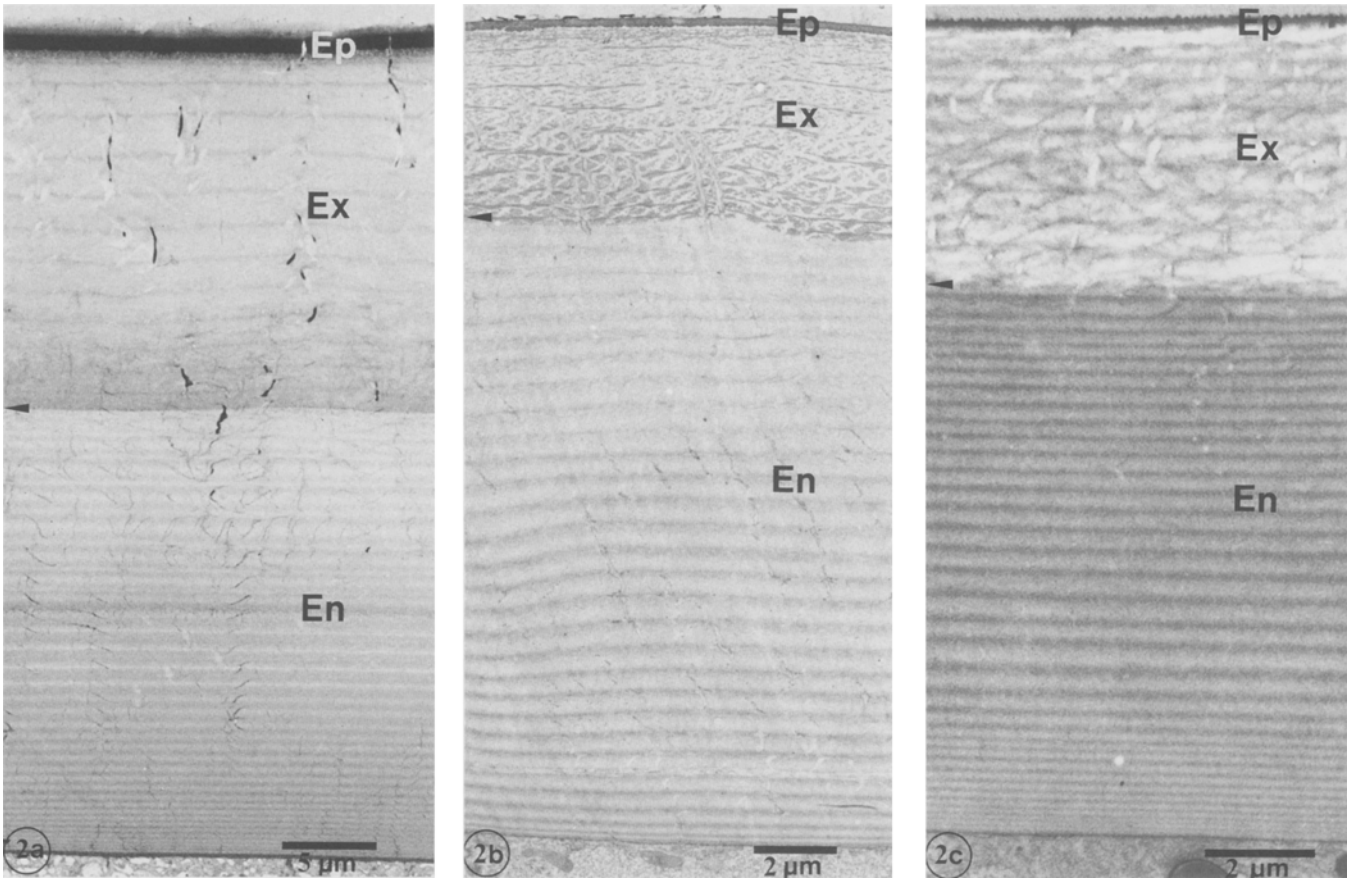

Fig. 2. Electron micrographs of cuticles of nektobenthic malacostracans. (a) *Crangon crangon*, ×2 500; (b) *Diastylis rathkei*, ×5 400; (c) *Hyperia galba*, ×7 200. Abbreviations and arrows as in Fig. 1

ment of *Caprella linearis* (Fig. 3c) was clearly distinguishable from that of all other species investigated in that the procuticle was divided into three, instead of two, sublayers. The outermost layer of the procuticle was an electron-dense exocuticle. The laminae in the central part of this sublayer were ca. 1.5 µm thick but became thinner in the peripheral zone. The central sublayer, here called the "mesocuticle", contained laminae 1 µm thick. This sublayer was clearly distinguishable from other sublayers by its lesser electron-density and a symmetrical arrangement of laminae. The inner sublayer, the endocuticle, had laminae 0.6 µm thick, which became thinner in the proximal zone.

The epicuticle of *Corophium volutator* was relatively thick and contained recesses which were open to the outside and filled with electron-dense material. In some instances a connection between these cavities and the pore canals was recognizable. There was a narrow transition zone between epicuticle and exocuticle. Laminar thickness in the procuticle was about 0.6 µm (Fig. 4a). The epicuticle of *Orchestia gammarellus* exhibited the same features as that of *C. volutator* except that recesses were void. Exocuticle and endocuticle were separated by an electron-dense transition zone. Laminar thickness was 1 µm (Fig. 4b). The cuticles of *C. volutator* and *O. gammarellus* were the most difficult to cut, indicating a high grade of mineralization.

Schematic representation and comparison of cuticles

To enhance interspecific cuticle comparison, cuticles were adjusted to equal thicknesses. This approach is based on the assumption that the relative proportion of each integumental sublayer is constant and does not vary considerably with body size in the different species (Fig. 5). Additional dimensional cuticle data are summarized in Table 1.

Morphometric calculations

We attempted to examine whether swimming aptitude influenced not only cuticular structure but also the relationship between cuticle thickness and body volume (Table 2). To accomplish this, average cuticle thickness was regressed against the estimated body volume for each species (Fig. 6). Considerable differences in regression slopes were apparent. The pelagic species had a negative or very slightly increasing (and therefore statistically insignificant) slope, as follows: *Meganyctiphanes norvegica*, 0.004; *Neomysis integer*, 0.001; *Praunus flexuosus*, −0.02. A slight but significant increase (correlation coefficient R: $p \leq 0.05$) in cuticle thickness was apparent in the nektobenthic *Crangon crangon* (slope = 0.15) and benthic *Gammarus locusta* (0.13). Species with a comparatively

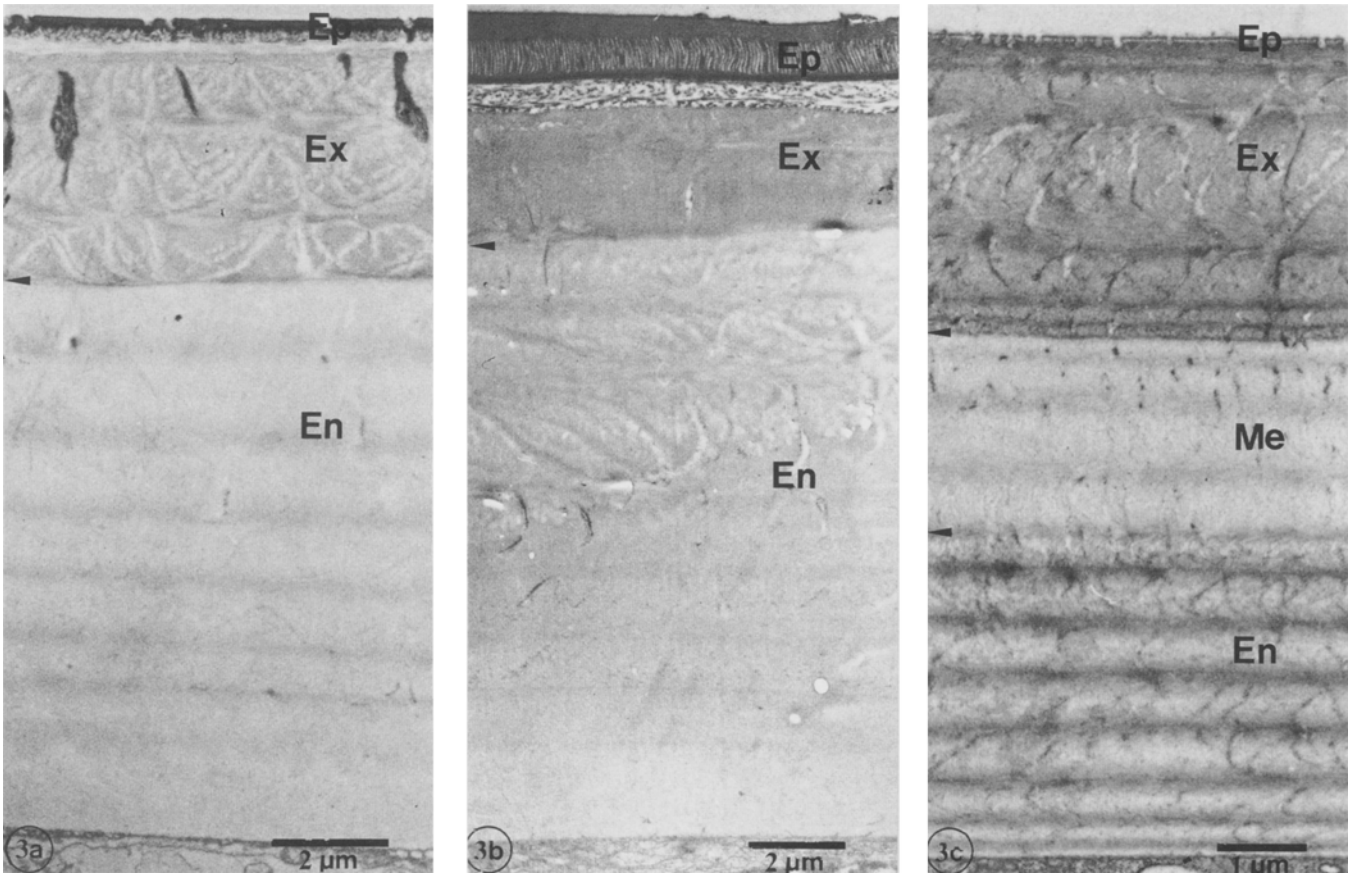

Fig. 3. Electron micrographs of cuticles of benthic malacostracans. (a) *Gammarus locusta*, ×7 600. (b) *Idotea baltica*, ×6 200. (c) *Caprella linearis*, ×11 800. Me: mesocuticle; arrows and other abbreviations as in Fig. 1

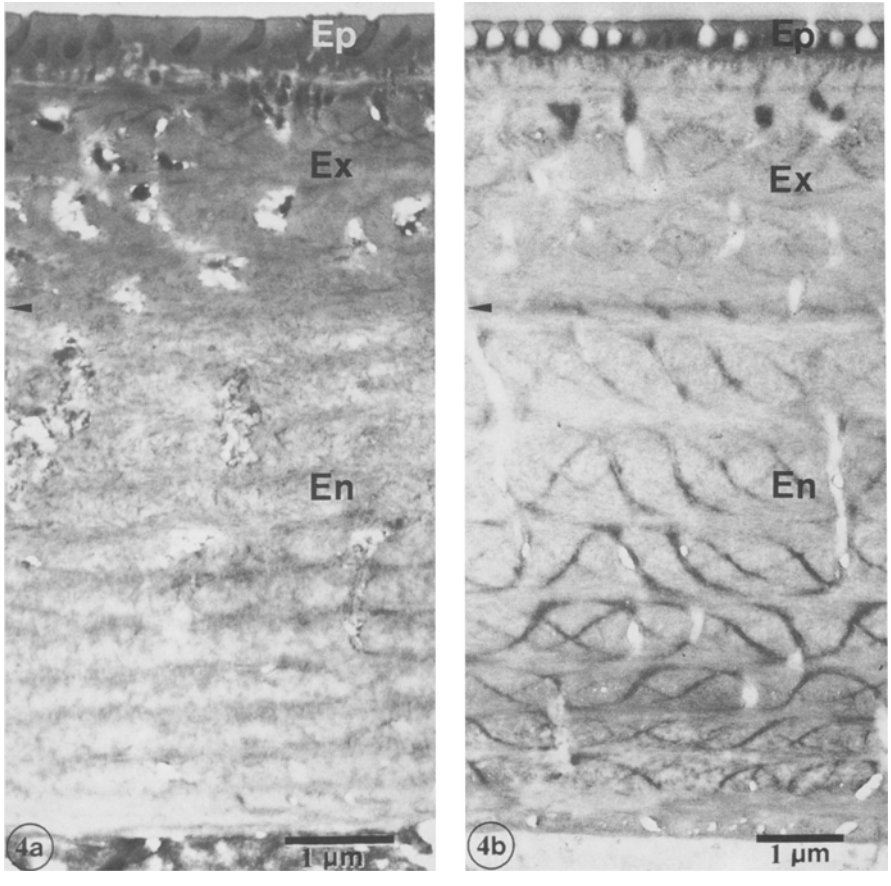

Fig. 4. Electron micrographs of cuticles of benthic malacostracans. (a) *Corophium volutator*, ×14 200. (b) *Orchestia gammarellus*, ×11 200. Abbreviations and arrows as in Fig. 1

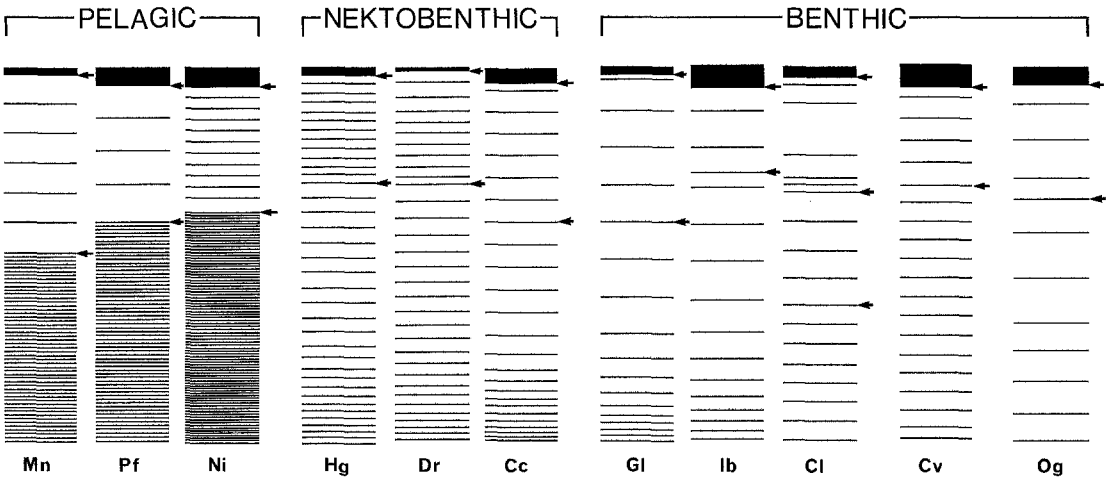

Fig. 5. Schematic representation and comparison of malacostracan cuticle. Cuticles are presented in order of pelagic, nektobenthic and benthic species. Diagrams are in sequential order of structural similarity. Cuticles were adjusted to equal thicknesses. Solid black portion represents epicuticle; arrows mark border between exo- and endocuticle. Mn: *Meganyctiphanes norvegica*; Pf: *Praunus flexuosus*; Ni: *Neomysis integer*; Hg: *Hyperia galba*; Dr: *Diastylis rathkei*; Cc: *Crangon crangon*; Gl: *Gammarus locusta*; Ib: *Idotea baltica*; Cl: *Caprella linearis*; Cv: *Corophium volutator*; Og: *Orchestia gammarellus*. For *Caprella linearis*, lower arrow marks lower border of "mesocuticle"

Table 1. Cuticles of various malacostracan species. Minimal and maximal thickness of cuticle sublayers, and their average relative proportions (%), of the segments analyzed. pl.: pleon; pe.: pereion

Species (*n*; segment)	Epicuticle			Exocuticle			Endocuticle		
	μm		%	μm		%	μm		%
	Min.	Max.		Min.	Max.		Min.	Max.	
Pelagic									
Meganyctiphanes norvegica (11; 5th pl.)	0.2	0.3	2	3.5	7.4	48	2.8	7.5	50
Praunus flexuosus (16; 5th pl.)	0.5	0.8	5	3.3	6.9	37	6.8	9.2	58
Neomysis integer (12; 5th pl.)	0.3	0.5	5	1.7	3.0	34	3.1	4.8	61
Nektobenthic									
Hyperia galba (22; 6th pl.)	0.1	0.2	2	0.8	6.1	29	1.6	24.6	69
Diastylis rathkei (16; 4th pl.)	0.1	0.2	1	1.9	9.7	31	4.2	23.9	68
Crangon crangon (15; 5th pl.)	0.2	1.8	4	4.8	20.5	38	7.6	26.4	58
Benthic									
Gammarus locusta (16; 6th pe.)	0.2	0.8	2	5.1	21.0	40	9.4	26.1	58
Idotea baltica (12; 6th pe.)	0.5	3.3	6	2.5	16.5	23	11.2	38.3	71
Caprella linearis[a] (19; 4th pe.)	0.2	0.5	3	1.9	5.8	32	4.8	11.9	65
Corophium volutator (10; 6th pe.)	0.3	0.7	6	1.6	2.8	27	3.4	6.3	67
Orchestia gammarellus (11; 6th pe.)	0.5	0.9	5	3.3	5.0	31	6.5	11.5	64

[a] The endocuticle comprises the intermediate layer, or "mesocuticle"

high increase in cuticle thickness with respect to body volume were the nektobenthic *Hyperia galba* (0.53) and the benthic *Idotea baltica* (0.58) and *Orchestia gammarellus* (0.37). The greatest increases were found in the nektobenthic *Diastylis rathkei* (1.98) and the benthic *Corophium volutator* (1.10) and *Caprella linearis* (1.09). Correlation coefficients of all the latter species were significant.

The significance of differences between slopes of regressions was tested using analysis of covariance (ANCOVA; Sokal and Rohlf 1969) in conjunction with Duncan's (1970) multiple range test. Firstly, the slopes of amphipods were compared. Those of *Hyperia galba*, *Gammarus locusta* and *Caprella linearis* were significantly different ($p \leq 0.05$). The slopes of *Orchestia gammarellus* and *Corophium volutator* were statistically indistinguishable from each other and from those of the other amphipods. Secondly, representatives of different orders were compared. In this case, differences between *Meganyctiphanes norvegica* (pelagic), *Crangon crangon* (nektobenthic) and *Idotea baltica* (benthic) were significant, whereas *Corophium volutator* (benthic) and *O. gammarellus* (benthic) were indistinguishable.

Table 2. Morphometric data for cuticles of various species of malacostracan crustaceans. For full genus names see Table 1. *R*: correlation coefficient; *p*: probability of *R* [calculated according to Clauß and Ebener (1972)]

Species	*n*	Cuticle thickness (μm)		Length (mm)		Volume (mm^3)		Cuticle thickness vs volume		Standard error of slope	*R*	*p*
		Min.	Max.	Min.	Max.	Min.	Max.	Intercept	Slope			
Pelagic												
M. norvegica	27	6.6	13.7	29	43	25	158	14.80	0.004	0.02	0.04	0.827
P. flexuosus	17	10.9	16.6	15	34	7	58	13.11	−0.02	0.04	−0.13	0.615
N. integer	11	5.9	7.6	15	18	10	38	5.61	0.001	0.06	0.01	0.984
Nektobenthic												
H. galba	20	2.8	30.1	5	11	3	58	−1.47	0.53	0.05	0.94	0.000
D. rathkei	20	6.4	31.5	4	17	0.2	11	10.67	1.98	0.03	0.85	0.000
C. crangon	23	12.6	47.5	16	53	13	240	14.30	0.15	0.02	0.83	0.000
Benthic												
G. locusta	26	16.5	47.6	10	26	6	141	16.49	0.13	0.04	0.57	0.002
I. baltica	39	14.2	57.8	4	17	0.8	59	17.49	0.58	0.06	0.84	0.000
C. linearis	28	7.5	17.5	8	27	0.6	15	11.12	1.09	0.21	0.71	0.000
C. volutator	13	5.7	8.9	4	7	1	7	5.70	1.01	0.33	0.68	0.011
O. gammarellus	13	10.5	17.0	8	12	6	23	7.87	0.37	0.09	0.79	0.001

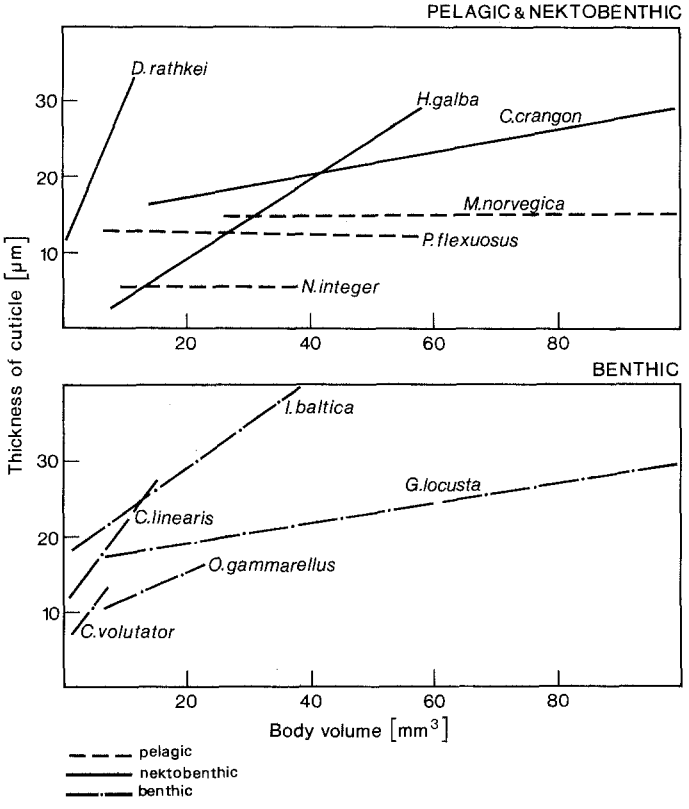

Fig. 6. Regression of cuticle thickness vs body volume of malacostracan crustaceans. For full genus names see Fig. 5

Discussion

With the exception of *Caprella linearis*, general cuticular organisation of the examined crustaceans corresponded to the classical scheme with respect to epicuticle, exocuticle and endocuticle, but not with respect to the membranous layer. Overall, there were clear distinctions in relative thickness and construction of the different layers in the crustaceans investigated, which could be related to taxonomic order and environmental requirements.

Membranous layer

A membranous layer is generally accepted as a necessary component of the crustacean integument (Drach 1939, Dennell 1947, Richards 1951, Hackman 1971, Stevenson 1985). This layer is deposited during moult stage C_3 and is located between the endocuticle and the hypodermis. The membranous layer is not mineralized or sclerotized and has poorly organized laminae (Drach 1939, Skinner 1962, Travis 1965, Bouligand 1971, Green and Neff 1972, Gharagozlou-van Ginneken and Bouligand 1975, Voss-Foucart and Jeuniaux 1978). Generally, this layer has been reported in the carapace of decapod crustaceans. (Skinner 1962, Keller and Adelung 1970, Green and Neff 1972, Welinder 1975, Hegdahl et al. 1977, Voss-Foucart and Jeuniaux 1978, Sarda 1981, Goffinet and Compere 1986). Moreover, the membranous layer has also been found in some copepods and one ostracod (Okada 1982), but is reported to be missing in other ostracods (Bate and East 1972). In several reports this layer is neither mentioned nor visible in cuticle sections (Halcrow 1976, 1978, Schultz and Kennedy 1977, Vallabahn 1982, Arsenault et al. 1984, Powell and Halcrow 1984, Cameron 1985, Cuzin-Roudy and Tchernigovtzeff 1985). Buchholz and Buchholz (1989) investigated the cuticle of Antarctic krill *Euphausia superba* and found no membranous layer. A comparison of the cuticle of krill with that of other crustaceans (Buchholz et al. 1989) indicated that the membranous layer might not be an integral feature of the crustacean integument. Voss-Foucart and Jeuniaux (1978) compared the relative thickness of the membranous layer in the carapace of several decapod species and found interrelations between the grade of mineralization and the thickness of this layer. Our results confirm the suggestion of Buchholz et al. (1989): in none of the mala-

costracans analyzed, not even in the relatively large decapod *Crangon crangon,* was a membranous layer visible. In accordance with the results of Voss-Foucart and Jeuniaux (1978), we suggest the membranous layer to be an additional layer which only occurs in very large decapods possessing a heavily calcified shell. Further investigations should demonstrate whether the membranous layer is characteristic in decapods and whether it is restricted to certain parts of the body, in particular to the carapace.

Cuticle and swimming aptitude

General trends in cuticular construction relative to swimming aptitude among pelagic, nektobenthic and benthic species are presented in Table 3. In pelagic crustaceans body density should be as close as possible to that of seawater to minimize the energy demand for swimming. One obvious way to reduce specific weight is to form a thin and little-mineralized cuticle.

Accordingly, our morphometric calculations showed that cuticle thickness of the pelagic species increased only to a certain minimum during growth, then remained constant. This is surprising in view of Drach's (1939) hypothesis, whereby relative dimensions of cuticular sublayers are reported to be constant with respect to body size, at least in benthic crustaceans. Furthermore, unpublished data from chemical analyses by Ballschmieter and Buchholz (Fig. 7) reveal a decreasing degree of cuticular mineralization in benthic vs pelagic crustaceans.

In addition, mineralization following ecdysis is a relatively slow process. In pelagic species, the time of actual moulting must be short – otherwise the organisms would quickly sink into oxygen-depleted depths during moult. The process of sclerotization takes place early during the formation of the new cuticle and ensures sufficient rigidity already shortly after moulting (Buchholz 1991). This is important, since muscle insertions are anchored to the cuticle.

Cuticle hardness may be further increased, without increasing specific weight, by development of thin laminae. The more closely packed the laminae are in any one layer, the stronger that layer becomes, even without mineralization. Accordingly, in pelagic forms such as krill and mysids, the procuticle is characterized by multiple thin endocuticular laminae. These are likely to add substantially to the rigidity of this layer. This is necessary because muscles are anchored here via tonofibrils that branch in the endocuticle (Buchholz and Buchholz 1989 and unpublished results). The same features are visible in cuticular sections of the mysid *Siriella armata* (Cuzin-Roudy and Tchernigovtzeff 1985). It is reassuring that the same structural features are found in pelagic peracarid as well as eucarid malacostracans.

Nektobenthic species frequently change their habitat, migrating between the benthic and pelagic zones or, as in the case of *Hyperia galba*, partly parasitising medusae. For swimming in the open water the cuticle should be as light as possible, whereas for dwelling on the sea bottom the cuticle should be robust. Does the cuticular structure represent a compromise between these two requirements in nektobenthic species?

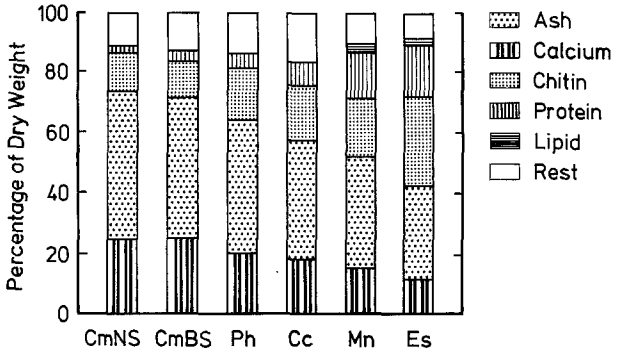

Fig. 7. Chemical composition of various malacostracan cuticles (Ballschmieter and Buchholz unpublished). CmNS: *Carcinus maenas,* North Sea; CmBS: *Carcinus maenas*, Baltic Sea; Ph: *Portunus holsatus*; Cc: *Crangon crangon*; Mn: *Meganyctiphanes norvegica*; Es: *Euphausia superba*

Table 3. General trends in cuticle contruction relative to swimming aptitude among pelagic, nektobentic and benthic malacostracan species

Parameter	Habitat		
	Pelagic	Nekto-benthic	Benthic
Specific weight	——→ increasing ——→		
Mineralization	——→ increasing ——→		
Cuticle thickness vs body volume	——→ increasing ——→		
Lamina number	←—— increasing ←——		
Lamina thickness	——→ increasing ——→		

In contrast to those in the pelagic species, laminar thicknesses in nektobenthic species were not as clearly divided between exo- and endocuticle (Fig. 5). Nevertheless, within the proximal part of the endocuticle, laminar thickness almost attained values typical of the pelagic species. Compared to those in the benthic species, the laminae are still relatively thin, particularly in *Hyperia galba* and *Diastylis rathkei.* In view of the need for cuticular strength without too much weight gain, this can be interpreted as an adaptation to the pelagic component of life in nektobenthic species.

Mineralization as well as sclerotization of the cuticle becomes increasingly important as activity becomes more benthic. Calcium salts are stored in the interstices of the macrofibrils (Neville 1975) and are arranged parallel to the fibrils (Hegdahl et al. 1977). Presumably, the additional storage of salts requires sufficient space between the fibrils. In heavily calcified decapods, for example, laminar thicknesses reach 20 μm (Hegdahl et al. 1977). This explains the greater distance between laminae in the nektobenthic species relative to those in the pelagic species.

The nektobenthic decapod *Crangon crangon* is similar to pelagic species in that the cuticle does not increase substantially in thickness relative to body volume, probably due to the necessity of frequent excursions into the pelagic zone. However, the other two nektobenthic species considered exhibited steeper regression slopes: in *Hyperia galba* this could reflect an adaptation to its partly

parasitic life style, since the thicker cuticle would protect the crustacean against the digestive enzymes of the host *Aurelia aurita*. The greatest relative increase in cuticle thickness, found in the cumacean *Diastylis rathkei*, is presumably due to the organism's essentially infaunal life style. Protection against the surrounding medium is of greater importance than energy loss during the relatively short pelagic excursions (Habermehl et al. 1990).

In benthic crustaceans, specific weight is less critical than in pelagic and nektobenthic species, because swimming occurs infrequently. However, in order to hide under bottom structures when in danger or while preparing to moult, these organisms require a robust cuticle. Consequently, laminae in the procuticles of *Gammarus locusta* and *Idotea baltica* are, as in the nektobenthic species, more or less evenly thick. Laminae are, however, substantially thicker in the benthic than in the nektobenthic species. In addition, the border zone with thin laminae close to the hypodermis is smaller or completely absent. Cuticles of the benthic species *Corophium volutator* and the supralittoral species *Orchestia gammarellus*, with their evenly thick laminae across the whole procuticle, apparently gain further strength by extensive mineralization. The occurrence in *Caprella linearis* of an additional sublayer in the procuticle, which contains altogether three uniformly thick sublayers, is perhaps a consequence of the organism's "sessile" life style.

An interesting feature among the benthic species is the epicuticular structure of the isopod *Idotea baltica* and the amphipods *Orchestia gamarellus* and *Corophium volutator*, in which elongated and vertically oriented cavities are visible (Figs. 3b and 4). This feature is explained by Powell and Halcrow (1985) for the isopod as an adaptation to its specific habitat. The structures are believed to strengthen the epicuticle so as to protect underlying parts from damage.

The morphometric parameters of the benthic species are typical of benthic crustaceans. In the amphipod *Gammarus locusta* the cuticle increases only slightly in thickness relative to body volume. Significantly, *G. locusta* is often found swimming freely close to the sea bottom (Dahl 1977 and authors' personal observations). The regression slopes of cuticle thickness vs body volume for *Idotea baltica*, *Caprella linearis* and *Corophium volutator* are substantially steeper. Here, the increasingly thick cuticle is presumably a direct consequence of necessary protection against environmental structures. The particularly steep regression slope in *C. volutator* may be linked to the infaunal ecotype. The lower slope in *Orchestia gammarellus* is presumably due to the terrestrial environment, and particularly to its typical saltatory locomotion.

Statistical evaluation of morphometric measurements supported the conclusion that apart from taxonomic order, behavioural and environmental constraints influence the structure of crustacean cuticle. Significant differences were seen not only among different orders of crustaceans but also within orders, in nektobenthic, as well as benthic amphipods. However, this could only be accomplished by reducing the organisms' very different shapes to a common simplified scheme. Further structural research is therefore necessary in order to differentiate more clearly between phylogenetic characteristics and patterns determined by the life style of the malacostracans.

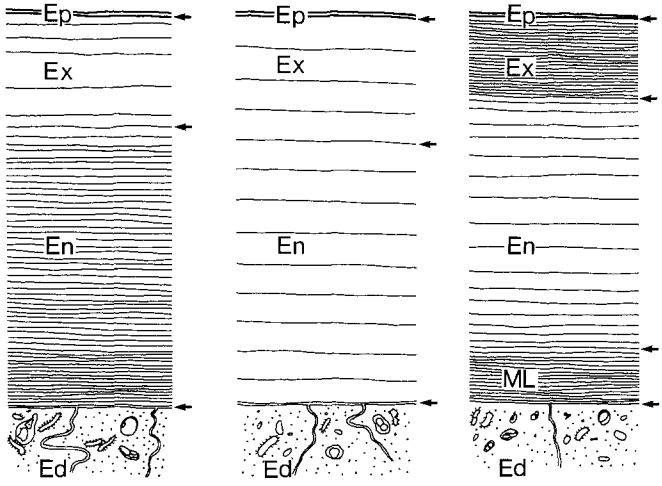

Fig. 8. Schematic drawing of basic models of malacostracan cuticle. From left to right: krill, benthic amphipod and crab. Ed: epidermis; ML: membranous layer. Supplemented, with permission, from Buchholz et al. (1989)

Conclusions

The integumental structure of malacostracan crustaceans is not as uniform as previously supposed. Apart from distinct features related to taxonomic order of the organisms, cuticular structure appears to be influenced by the necessity of and aptitude for swimming in the respective environment. The degree of mineralization and sclerotization and the laminar dimensions are subject to variation, whereby mineralization and laminar thickness seem to be interdependent. In pelagic species the cuticle is little mineralized, resulting in low specific weight. Cuticle thickness does not increase with increasing body volume over the size range investigated. Sufficient hardness is achieved by sclerotization and the formation of many thin and tightly packed laminae in the endocuticle. In contrast, the cuticle of the benthic species appears to be hardened mainly by mineralization. Here, laminae are evenly distributed and thick relative to body volume. The cuticular structure of the nektobenthic species can be viewed as a compromise between these properties. The membranous layer appears to be an additional layer which is found only in larger decapods. Accordingly, the schematic comparison in Buchholz et al. (1989) of the laminated procuticular structure of decapods and euphausiids can be expanded (Fig. 8). Nevertheless, these schemes represent only basic models, and it should be remembered that structural transitions occur according to the crustaceans' specific life style.

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