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Fluoride in Antarctic marine crustaceans

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Abstract The concentration of fluoride in the body parts of a range of Antarctic crustaceans from a variety of habits was examined with the aim of determining whether fluoride concentration is related to lifestyle or phylogenetic grouping. Euphausiids had the highest overall fluoride concentrations of a range of Antarctic marine crustaceans examined; levels of up to 5477 μg g^{-1} were found in the exoskeleton of *Euphausia crystallorophias*. Copepods had the lowest fluoride levels (0.87 μg g^{-1} whole-body); some amphipods and mysids also exhibited relatively high fluoride levels. There was no apparent relationship between the lifestyle of the crustaceans and their fluoride level; benthic and pelagic species exhibited both high and low fluoride levels. Fluoride was concentrated in the exoskeleton, but not evenly distributed through it; the exoskeleton of the head, carapace and abdomen contained the highest concentrations of fluoride, followed by the feeding basket and pleopods, and the eyes. The mouthparts of *E. superba* contained almost 13 000 μg F g^{-1} dry wt. Antarctic krill tail muscle had low levels of fluoride. After long-term (1 to 5 yr) storage in formalin, fluoride was almost completely lost from whole euphausiids.

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Introduction

In animals, naturally occurring fluoride is usually localised in hard tissues such as bone or exoskeleton. Terrestrial vertebrates contain, on average, 100 to 150 μg F g^{-1} dry wt in the bones (Hodge and Smith 1965). Soevik and Braekkan (1979) found high levels of fluoride (1500 μg g^{-1} whole-body dry wt) localised in the exoskeleton of the Antarctic krill *Euphausia superba* and North Atlantic krill *Meganyctiphanes norvegica*. Such high levels had previously been reported only in animals exposed to abnormally high fluoride levels. Vertebrates such as penguins and seals, which rely on krill as a major part of their food source, have bone fluoride levels as high as 10 000 μg g^{-1} dry wt, with no apparent adverse effects (Schneppenheim 1980; Culik 1987). In comparison, animals which do not include krill in their normal diet suffer injurious effects at lower fluoride concentrations in their food when fed artificial high-fluoride diets (Hodge and Smith 1965). This has limited the use of krill as a food for domestic animals (Budzinski et al. 1985). Soft-tissue levels, even in animals fed artificial high-fluoride diets, rarely increase far above the normal, low levels (up to 100 μg g^{-1} dry wt) (Harvey 1952, 1953; Suttie et al. 1958). In animals which normally include krill in their diet, muscle fluoride levels are low and within the range of soft tissue fluoride levels reported for terrestrial vertebrates. High quantities of fluoride have occasionally been reported in the soft tissues of crustaceans, e.g. Moore (1971), but as with vertebrates this is usually as a result of exposure to artificially high fluoride levels caused by industrial pollution, or applied under laboratory conditions.

The high levels of fluoride in the exoskeleton of some crustacean species is unexplained. Initially it was suggested that deposition of fluoride in the exoskeleton of krill was a means of storing a toxic element until it could be discarded along with the moult (in krill this occurs regularly every 16 to 26 d; Murano et al. 1979). There is evidence for this sort of disposal mechanism in *Daphnia*

magna (Dave 1984); however, the concentration of fluoride attained in *D. magna*, even under experimentally enhanced fluoride conditions, is considerably lower than the concentrations found naturally in krill.

Our study determined the fluoride content and its distribution in a number of Southern Ocean crustacean species, to reveal any relationships between taxonomic groups, habit, and fluoride concentration. The stability of fluoride in the exoskeleton of samples preserved by different methods was also examined.

Materials and methods

Effect of preservative

Euphausia superba have been maintained live in the laboratory of the Australian Antarctic Division (AAD) headquarters at Kingston, Southern Tasmania, since 1981; experimental individuals were drawn from this stock. The AAD also stores bulk krill at a temperature of ca. −20 °C; smaller batches are also preserved in formalin. In order to determine the effect of these storage methods on the concentration and distribution of fluoride within the krill, samples of frozen (−20 °C) and formalin-preserved krill were taken for ion-selective electrode (ISE) fluoride-analysis. For comparison, fresh samples were analysed using the same procedure.

Inter-species comparisons

Several species of Antarctic crustaceans were collected from Prydz Bay during January and February 1993, using trawls from the RSV "Aurora Australis". These were: a mysid, *Antarctomysis maxima*; the amphipods *Cyllopus lucasii* and *Hyperia macrocephala*; three species of gammarids (*Cyphocaris micronyx* and two unidentified species: sp. 1, benthic; sp. 2, pelagic); the copepods *Calanus propinquus* and *Euchaeta antarctica*; three euphausiids, *Euphausia crystallorophias*, *E. superba* and *Thysanoessa macrura*; and the decapod *Notocrangon antarcticus*. Some specimens were frozen immediately in liquid nitrogen for later analysis in Hobart; others were kept alive in individual 250, 500 or 1000 ml bottles in filtered sea water pumped directly from the ocean and temperature-regulated to 0 °C. The water in these bottles was changed weekly. Bottles were kept in the dark and checked daily for moults or deaths. Moults and dead krill were frozen in liquid nitrogen for later analysis. In cases where moults were found, either the krill and moult were both frozen for later analysis, or the moult alone was frozen and the krill was kept alive to collect further moults.

Sample preparation

Frozen krill, stored at –20 °C for 2 yr, were separated into exoskeleton and muscle. Formalin-preserved samples, stored for 1 to 5 yr, were divided into exoskeleton, muscle and head.

As well as providing a direct comparison with frozen and preserved krill, fresh krill were used for fluoride-localisation studies. They were separated into thoracic exoskeleton (carapace), thoracic muscle, feeding basket, eyes, head, abdominal exoskeleton, pleopods, and pleon muscle with micro-scissors and fine forceps. All the above samples were then prepared for ISE fluoride-analysis.

Where possible, krill collected from Prydz Bay were dissected into separate exoskeletal and muscle tissues. Exceptions were *Calanus propinquus* and *Euchaeta antarctica*, which were too small to make dissections practical. Only whole-body analyses were carried out on these two species.

Since chlorine in sea water can interfere with fluoride analyses, all samples were rinsed thoroughly in distilled water to remove sea water. Washed samples were then freeze-dried at −60 °C for at least 48 h.

Fluoride analyses

Fluoride analyses were carried out at the Australian Government Analytical Laboratory using the ion-selective electrode method of fluoride analysis outlined by Lewis et al. (1987) with the following minor modifications.

Rinsed and freeze-dried samples were weighed to within 10 μg in individual petri dishes. Fluoride was liberated from the sample using 70% perchloric acid saturated with Ag_2SO_4 to prevent chloride diffusion. Digestion was carried out at 50 °C for 16 h. Liberated fluoride was diffused into an alkaline medium (1% mV NaOH in 97% EtOH) which coated the petri-dish lids. After diffusion, the alkaline mixture was dissolved in a 1:1 solution of distilled water and TISAB II buffer (58 g NaCl and 4 g cyclohexylenedinitrilo tetraacetic acid in 57 ml glacial acetic acid and 500 ml water, and pH adjusted to 5–5.5 with 20% NaOH). Fluoride concentration was measured with an Orion Model 96-09 combination fluoride electrode and a Radiometer Ion 85 pH/mV meter (resolution 0.1 mV). For calibration, the ISE potential was plotted against the log of concentration using known standard fluoride solutions (0.1 to 200 μg F g^{-1} dry wt). Measurements were made at a constant temperature in a 25 °C water bath. Because of the relative sizes of the electrode and the sample vessels, constant mixing was not required. Unless otherwise stated all fluoride levels are in μg F g^{-1} dry wt.

Statistics

For statistical analyses, a homoscedastic, two-sample, two-tailed, Student's *t*-test (confidence level 0.05) was used.

Results

All exoskeletal regions of fresh *Euphausia superba* contained fluoride concentrations of ~2000 μg g^{-1}, except for the feeding basket and pleopods, which displayed significantly lower levels (Table 1). Muscle had lower fluoride levels than the exoskeleton, averaging 74 μg g^{-1}. This fluoride concentration in the muscle may have been artificially high because of contamination with shell particles during dissection (see "Discussion-Fluoride in marine crustaceans"). A large range of concentrations (average 299 μg F g^{-1}) was recorded for the eye fluoride measurements. During frozen storage (2 yr at −20 °C), considerable fluoride contamination of muscle tissues occurred, although this did not seem to affect the exoskeletal fluoride levels. During storage in formalin, almost complete loss of fluoride from all body parts was noted.

Results of the fluoride determinations on fresh and deep-frozen samples of 12 species of Southern Ocean crustaceans are shown in Table 2. Whole-body concentrations ranged widely, from 0.878 μg F g^{-1} in *Euchaeta antarctica*, to 1466 μg F g^{-1} in the benthic gammarid sp. 1. Exoskeletal results were also wide, with values ranging from 148 μg F g^{-1} in *Notocrangon antarcticus* to 5977 μg F g^{-1} in *Euphausia crystallorophias*. Muscle fluoride values varied little. In all cases fluoride appeared to be localised in the exoskeleton.

Table 1 *Euphausia superba*. Fluoride in body parts of Antarctic krill (μg F g^{-1} dry wt ± average of absolute deviations of data points from their mean). Frozen specimens were stored at −20 °C for 2 yr (*nd* no data)

Body part	Fresh (n = 5)	Frozen (n = 5)	Formalin-preserved (n = 8)
Cephalothorax			
Exoskeleton	1899 ± 470	nd	nd
Muscle	96 ± 42	nd	nd
Feeding basket	667 ± 112	nd	nd
Eyes	299 ± 168	nd	nd
Mouth parts	12 876[a]	nd	nd
Remainder (no muscle)	2096 ± 551	nd	15 ± 4.2
Abdomen			
Exoskeleton	2035 ± 724	3914 ± 909[b]	0.86 ± 0.28[c]
Pleopods	762 ± 108	nd	nd
Tail muscle	52 ± 35	1119 ± 287	10.6 ± 0.19

[a] Single analysis on batched mouthparts from several specimens
[b] Includes exoskeleton of thorax, feeding basket, pleopods and head
[c] Includes thoracic exoskeleton, feeding basket and pleopods

Discussion

Effects of sample storage on fluoride content and distribution in *Euphausia superba*

Frozen storage

The obvious state of partial decomposition (evidenced by dark coloration) and the very high fluoride levels (1119 ± 328 μg F g^{-1}) in the muscle tissues of krill which had been stored frozen for 2 yr at −20 °C clearly demonstrate the migration of fluoride into muscle tissues, even at low storage temperatures. Adelung et al. (1987) suggested post mortem migration of fluoride during storage as an explanation for some of the high muscle-fluoride levels that had been reported in earlier studies. In a definitive study, Christians and Leinemann (1983) demonstrated that even at −20 °C a 10-fold enrichment of muscle fluoride occurs within 1 yr. A storage temperature of −10 °C resulted in specimen decomposition evidenced by black coloration, as observed in the present study. Only at −30 °C or lower, was the fluoride leaching significantly reduced. The present study supports Christians and Leinemann's in finding that for long-term storage of krill, temperatures below −20 °C are necessary to prevent fluoride contamination of muscle tissues.

Preservation in formalin

Fluoride leaches out of all sections of krill during storage in formalin. Previous investigations have shown fluoride migration from the exoskeleton into muscle tissues in dead animals, even in cold storage (Christians and Leinemann 1983; present study), and from cast exoskeletons into the surrounding water (Nicol and Stolp 1989). The present study is the first to report fluoride leaching during storage in formalin, and demonstrates that although formalin preserves the tissues, almost all the fluoride is lost during 1 to 5 yr storage. After 1 to 5 yr, there was no significant difference between exoskeleton and muscle fluoride levels (Student's t-test, $t = 0.26$), but the heads contained a significantly higher ($t = 1 \times 10^{-5}$), although still very low, level of fluoride (Table 1).

Fluoride leaching in formalin is perhaps not surprising given its rapid mobility on contact with aqueous

Table 2 Fluoride concentration (μg g^{-1} dry wt ± average of absolute deviations of data points from their mean) in Southern Ocean crustaceans (sample size in parentheses) (*nd* no data)

Species	Exoskeleton	Whole-body	Muscle
Antarctomysis maxima	1254.31 ± 200 (6)[a]	510.35 ± 65 (4)	23.52 ± 8 (6)
Calanus propinquus	nd	1.44 ± 0.5 (8)	nd
Cyllopus lucasii[c]	1373.87 ± 563 (19)[b]	111.38 ± 104 (22)	nd
Euchaeta antarctica	nd	0.87 ± 0.4 (4)	nd
Euphausia crystallorophias	3492–5977 (4)[b]	nd	nd
Euphausia superba	2232.28[a]	nd	74.44
Cyphocaris micronyx	1041–4602 (3)[b]	117.61 ± 47 (18)	nd
Hyperia macrocephala	361.60 ± 88 (6)[b]	2.92 ± 2 (18)	nd
Notocrangon antarcticus	148–271 (2)[a]	nd	0.45–0.78 (2)
Thysanoessa macrura	5103.72 ± 1308 (7)[a]	nd	257.18 ± 233 (7)
Gammarid sp. 1 (benthic)	nd	578–1466	nd
Gammarid sp. 2 (pelagic)	nd	935–1409	nd

[a] Dissected exoskeleton
[b] Moulted exoskeleton
[c] Whole sample of *C. lucasii*, separated into individuals that had just moulted (1409.78 μg F g^{-1} in exoskeletons and 33.1 μg F g^{-1} in whole-body), and those at unknown stage of the moult cycle (1351.11 μg F g^{-1} in exoskeletons and 170.94 μg F g^{-1} in whole-body)

media (Nicol and Stolp 1989). However, formalin is a well known tissue-fixative, and the penetration of formalin is rapid and uniform (Gabe 1976), so the loss of fluoride is difficult to account for. Formalin is a good fixative for lipids, but does not fix carbohydrates (Humason 1972). One possibility may be that fluoride is linked with a carbohydrate and is released during storage in formalin as the carbohydrate dissolves. It is also possible that formalin does not halt enzymatic degradation of the krill tissue. Formaldehyde reacts with amino groups of proteins, and preservation of enzyme activity is inversely proportional to the number and speed at which cross-links are introduced (Robinson 1982).

A procedure for producing low-fluoride krill-protein concentrates using either organic acid washings or simple water washings (Tenuta-Filho 1993) results in fluoride concentrations of <21 μg g^{-1} compared to untreated protein concentrates with values of $\sim$250 μg g^{-1}. This further emphasises the high solubility of the fluoride in krill exoskeletons.

Fluoride in body segments of *Euphausia superba*

Krill mouthparts contained the highest level of fluoride (12 876 μg F g^{-1}; Table 1) – some six times higher than the whole exoskeleton. This result was from a single analysis of batched mouthparts, and there are no other reports in the literature for comparison. However, Boone and Manthey (1983) reported that the head, although its fluoride concentration is similar to that of other exoskeletal segments, contains 29.8% of the krill's total fluoride compared to the abdominal exoskeleton, which contained the highest concentration of fluoride, but only 17.2% of the total body chloride (Table 3). This finding suggests that some part of the head has a higher than average level of fluoride which is being diluted by other tissues in the head; the present study suggests that the mouthparts would contain such high concentration of fluoride.

All exoskeletal sections in the present study contained roughly equal fluoride concentrations, except for the feeding basket and pleopods which had significantly lower concentrations. This could result from dilution of fluoride by the muscles, which it was impractical to remove from these appendages. The only other report of fluoride content in appendages comes from Boone and Manthey (1983), who found 1777 μg F g^{-1} in the pleopods. This is more than twice the pleopod fluoride level found in the present study. From their description, Boone and Manthey used a similar dissection method to that used in the present study, and their other results were comparable to the present results. However, they made no mention of the number or size of the specimens they analysed, and did not include any errors or confidence intervals with their data. The impracticality of separating the muscle from these body parts means that the size of the krill will have an important effect on the fluoride level measured in the appendages because of surface area:volume differences. Boone and Manthey's specimens may have been smaller than those used in the present study.

Fluoride in Antarctic crustaceans

The euphausiids *Euphausia crystallorophias* and *Thysanoessa macrura* contained the highest fluoride levels in the range of species examined (Table 2); i.e. up to 5977 μg g^{-1} in moulted exoskeletons of *E. crystallorophias*. Schneppenheim (1980) reported whole-body fluoride levels of 1370 μg F g^{-1} for *E. crystallorophias*; this is consistent with the exoskeletal levels recorded in our study. The levels reported here for the exoskeletons of *E. crystallorophias* and *T. macrura* are slightly higher than previously reported levels for other euphausiids: *E. superba* up to 4028 μg F g^{-1} (Zhang et al. 1993a); *Meganyctiphanes norvegica* up to 3300 μg F g^{-1} (Adelung et al. 1987).

Other species with high fluoride levels included the mysid, *Antarctomysis maxima*, the amphipod *Cyllopus lucasii* and the two gammarid species; there are no previous reports of fluoride levels in these groups. The fluoride concentrations we detected in mysids and gammarids were high compared to values reported for crustaceans other than euphausiids.

The amphipod *Hyperia macrocephala* contained low whole-body fluoride levels but higher levels were present in its moults. The two copepods *Calanus propinquus* and *Euchaeta antarctica* contained very low whole-body fluoride levels; their small size made it impractical to dissect exoskeletons for separate analyses, and no moults were collected. For comparison, the northern copepod *C. finmarchicus* was reported to have 13 to 15 μg F g^{-1} wet wt in its whole-body (Soevik and Braekkan 1979). The only species reported to have fluoride concentrations as low as the copepods in the present study, are the crab, shrimp and prawn studied by Wright and Davison (1975) which were found to contain $\sim$11 μg F g^{-1} dry wt in their exoskeletons. Other low values reported include 2.6 μg F g^{-1} wet wt in whole bodies of the brown shrimp *Crangon crangon* (Spaargaren 1988) and 18 to 91 μg g^{-1} dry wt in the deep-sea prawn *Pandalus borealis* (Soevik and Braekkan 1979).

The fluoride levels in *Notocrangon antarcticus* were lower than those reported by Schneppenheim (1980) for the same species, but some of these differences could be explained by variations accompanying the moult cycle, since one recently moulted specimen in the present study had still lower levels. Such moult cycle-related changes in fluoride levels have been reported for Antarctic krill (Adelung et al. 1987; Nicol and Stolp 1989). Moult-cycle-related variation was also apparent in *Cyllopus lucasii*, whereby whole-body fluoride levels in freshly moulted (within the last 24 h) individuals was significantly lower than in individuals at unknown stages of

Table 3 *Euphausia superba*. Comparison of fluoride content (μg F g^{-1} dry wt ± average of absolute deviations of data points from their mean) in body sections of Antarctic krill. Sample size in parentheses (*ISE* ion-selective electrode analysis; *nd* no data)

Analysis, Methodology, Body segment	Present study (fresh specimens)	Boone and Manthey (1983)[a]	Szewielow (1981)	Adelung et al. (1987)	Soevik and Braekkan (1979)[b]	Zhang et al. (1993a)
Analysis:	Acid digest/ISE	Acid digest/ISE	Hydrolysis/ISE	(1) Reverse extraction/ colorimetry (2) acid digest/ISE	Acid digest/ISE	ISE
Sample preparation	Fresh specimens	Dissected then deep-frozen (−30°C)	Fresh specimens	Dissected then frozen (−20°C)	Deep-frozen	Frozen then dissected
Cephalothorax						
Muscle	96.56 ± 42.24 (5)	nd	nd	nd	nd	nd
Feeding basket	667.42 ± 111.65 (5)	nd	nd	nd	nd	nd
Eyes	298.73 ± 168.53 (5)	334	nd	nd	nd	nd
Carapace	1898.67 ± 470.28 (5)	1077	1290 ± 360 (4)		4.260	4028 (range: 3828–4278)
Stomach	nd	1202	nd	nd	nd	nd
Hepatopancreas	nd	7.6	nd	nd	nd	nd
Remainder	2076.25 ± 550.77[c] (5)	1162	2000 (2)	nd	nd	2724 (range: 2338–3028)
Abdomen						
Exoskeleton	2035.47 ± 724.26 (5)	1958	nd	2594 ± 661 (82)	nd	2828[d] (range: 2338–3028)
Pleopods	761.88 ± 108.08 (5)	1777	nd	nd	nd	nd
Tail muscle	52.33 ± 35.04 (5)	70	60 ± 20 (4)	2.94 ± 0.8 (14) 4.5 ± 2.4 (42)	570	226 (range: 178–285)
Haemolymph	nd	nd	nd	4.4 ± 2.4 (26)	nd	nd
Whole krill	nd	1009	780 ± 140 (5)	1058 ± 108 (17)	2400	1232 (range: 1102–1432)
Exoskeleton	2232.28	nd	nd	nd	3300	nd

[a] No significant difference from 10 determinations on batched whole krill using two separate methods. All other data were determined once using each method and averaged
[b] No indication of sample size or variance
[c] Excluding feeding basket
[d] Including pleopods

the moult cycle (33.1 vs 170.94 μg F g^{-1} dry wt; Student's $t = 0.005$).

Fluoride in marine crustaceans

A range of fluoride levels has been reported for marine crustaceans (Table 4). Fluoride levels of the nine species examined in the present study cover the full range of previously reported levels. As the species examined in the present study are all from the same geographical region and in water of similar temperature and composition, these variables cannot explain the differences in fluoride levels we recorded. Nor can the fluoride concentrations in the various species be related to their way of life (both benthic and pelagic species exhibit high fluoride levels) or degree of calcification (many of the species in the present study would be regarded as "lowly-calcified species"; Roer and Dillman 1984); nevertheless, their fluoride levels differed considerably. Oehlenschlager and Manthey (1982) reported a range of fluoride concentrations in benthic invertebrates. Low fluoride levels were found in a pycnogonid, a polychaete and an octopod (7.7, 7.9 and 17 μg F g^{-1} wet wt respectively) and high levels were found in two echinoderms (1400 and 1500 μg F g^{-1} dry wt). Of three benthic crustaceans examined, two species of *Paracerodocus* contained 2200 and 2500 μg F g^{-1} dry wt, and an isopod, *Serolis cornuta*, contained 900 μg F g^{-1} dry wt. On the other hand, the pelagic copepods from the present study contained only 0.87 to 1.44 μg F g^{-1} dry wt. Finally, the fluoride levels of freshwater species can also be high; Ma (1994) reports 433 μg F g^{-1} in the exoskeleton of the freshwater yabby *Cherax destructor*. This is higher than the exoskeletal levels reported for many marine crustaceans.

Some of the variation in the fluoride levels of the various tissue types can be explained by the contamination of the (usually) low-fluoride muscle with the (often) high-fluoride exoskeleton. When separating muscle from exoskeletons, muscle tissues can become contaminated by fragments of exoskeleton. Individuals with higher exoskeletal levels of fluoride generally also had more F in the muscle, consistent with contamination of muscle samples by fragments of exoskeleton. For all species examined in our study, the generally low muscle-fluoride levels, compared to the high exoskeletal levels, indicates that the fluoride is concentrated primarily in the exoskeleton, as with all other species examined to date. Where no muscle fluoride levels were analysed, whole-body levels were always considerably lower than exoskeletal levels.

Work on *Euphausia superba* and *Meganyctiphanes norvegica* has suggested that fluoride is associated with the structural proteins of the integument and plays a role in the hardening of the exoskeleton (Zhang et al. 1993a). The major inorganic constituents of the krill exoskeleton, Ca and P, will combine readily with fluoride to give $Ca_5(PO_4)_3F$, a known hardener (Zhang et al. 1993a).

Spaargaren (1988) suggested that fluoride plays a role in the release of metabolic energy in the brown shrimp

Table 4 Reported fluoride levels in marine crustaceans (μg F g^{-1} dry wt)

Species	Exoskeleton	Whole-body	Muscle	Source
Antarctomysis maxima	1254	510	24	Present study
Calanus propinquus	–	1.44	–	
Cyllopus lucasii	1351	171	–	
Euchaeta antarctica	–	0.87	–	
Euphausia crystallorophias	3492–5977	1370	–	Present study; Schneppenheim (1980)
Euphausia superba	1290–4028	980–2400	< 6–570	Present study, Soevik and Braekkan (1979); Szewielow (1981); Adelung et al. (1987); Zhang et al. (1993a)
Cyphocaris micronyx	1041–4602	117.6	–	Present study
Hyperia macrocephala	362	2.92	–	
Notocrangon antarcticus	148–271	–	0.45–0.78	
Thysanoessa macrura	5104	–	257	
Calanus finmarchicus	–	13–15	–	Soevik and Braekkan (1979)
Callinectes sapidus	298	–	10	Moore (1971)
Crangon crangon (wet wt)	–	2.6	1.8	Spaargaren (1988)
Crangon vulgaris (wet wt)	6–17	2.3–7.1	0.9–2.7	Wright and Davison (1975)
Euphausia frigida	–	2440	–	Schneppenheim (1980)
Heterocarples silogal	434	170	17	Ma (1994)
Leander serratus (wet wt)	4.3–18.1	2.9–6.7	0.9–3.3	Wright and Davison (1975)
Meganyctiphanes norvegica	3300	2300	–	Soevik and Braekkan (1979); Adelung et al. (1987)
Metanephros neptunus	1350	–	6	Ma (1994)
Nyctiphanes australis	–	277–3507	–	Virtue et al. (1995)
Ovalipes australiensis	200	30	9.1	Ma (1994)
Pandalus borealis	36–169	18–91	0.5–2.2	Soevik and Braekkan (1979)
Paracerodocus sp.	–	2200–2500	–	Oehlenschlager and Manthey (1982)
Penaeus semisculcatus	73	26–53	2.7	Ma (1994)
Portunus depurator (wet wt)	6–17	3.1–3.9	1–2.2	Wright and Davison (1975)
Serolis cornuta	–	900	–	Oehlenschlager and Manthey (1982)

Crangon crangon. During extremes of temperature and salinity, internal fluoride concentrations increased, leading Spaargaren to suggest that the difference in fluoride levels observed between various species may be related to temperature. Fluoride concentrations in brown shrimp were, however, very low ($\sim$2.6 $\mu g\ g^{-1}$ wet wt) in the whole-body; Spaargaren suggested that at very low temperatures, such as in the Antarctic, fluoride concentration may increase to much higher levels.

The chemical form of fluoride and its function in the exoskeleton in certain crustaceans remains uncertain. If fluoride acts as a hardener (Zhang et al. 1993a, b), it would most probably be associated with the more mineralised tissues of the exo- and endocuticles. Since the new exoskeleton contains considerably less fluoride than the old, and the fluoride level rises steeply after moulting, it could be argued that fluoride is associated with the endocuticle since this is the only layer remaining to be deposited after the moult (Skinner 1962; Green and Neff 1972; Buchholz and Buchholz 1989). On the other hand, although the epi- and exocuticles are deposited pre-moult, neither is calcified until after the moult (Roer and Dillman 1984). The deposition of calcium is but one of several processes involved in postmoult hardening. Hardening also involves chitin synthesis in the exo- and endocuticles (Travis 1965), but Zhang et al. (1993a) concluded that fluoride is not associated with chitin in krill: however, Zhang et al. isolated chitin by treatment of the exoskeleton with acid and alkali, a process which is also likely to remove any fluoride. A third factor in the process of postmoult hardening is the synthesis and deposition of a water-soluble protein, arthropodin and a water-insoluble protein, sclerotonin (Fraenkel and Rudall 1947). The rapid dissolution of fluoride from the exoskeleton suggests that water-soluble proteins may be the relevant location of fluoride involvement in postmoult hardening. Significantly, Zhang et al. (1993b) reported that there is more fluoride on the inner surface than the outer surface of the exoskeleton. The higher concentration of fluoride in the mouthparts of Antarctic krill in the present study adds weight to the suggestion that its role is that of a hardener, since these body parts are, by necessity, the hardest parts of the exoskeleton.

Fluoride is apparently actively taken up by certain crustaceans and is deposited in the hardening exoskeleton. This can lead to high overall levels of fluoride, and this has implications for products from species that are the targets of commercial fisheries (Tenuta-Filho 1993). Our study has shown that a wider range of crustacean species exhibit high concentrations of exoskeletal fluoride than has been reported previously. Fluoride in euphausiids is in a water-soluble form. It is not immobilised by formaldehyde preservation, but is immobilised by boiling (Budzinski et al. 1985); however, its chemical form and function in the exoskeleton remain the subject for further study.

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