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Effects of season and temperature on long-term *in situ* loss rates of Pu, Am, Np, Eu, Ce, Ag, Tc, Zn, Co and Mn in a Baltic *Mytilus edulis* population

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ABSTRACT: Loss of 10 radionuclides by *Mytilus edulis* was studied near its salinity minimum in the Bothnian Sea (northern Baltic Sea) by labelling mussels in the laboratory and then allowing them to depurate during a 10 mo period in the field at 2 locations: one with normal temperatures, the other with temperatures 8 to 10 C° above normal. During winter, the clearest effect of heating was accelerated loss of silver. Also zinc was apparently lost more rapidly in warm water, whereas none of the remaining nuclides showed loss rates significantly different from zero at either temperature. At normal temperatures during spring and summer all analysed elements were lost faster than in the heated winter experiment despite similar average temperature conditions. It is concluded that temperature differences play only a minor role in the observed seasonal effect on long-term loss rates. Food availability appears to be a key factor. Loss rates are apparently faster than in full-salinity waters. Possible reasons for this are discussed. Plutonium to americium ratios decreased during depuration. The plutonium fraction lost after 300 d was estimated to be twice the corresponding fraction of americium. Europium to americium ratios remained unchanged during all seasons and temperatures, whereas cerium to americium ratios decreased to half during the initial loss phase, after which they remained unaltered. It is concluded that europium behaved as an ideal analogue to americium in this experiment.

INTRODUCTION

The common edible mussel *Mytilus edulis* is widely used as a 'bioindicator' for several different pollutants in coastal waters (e.g. Phillips 1977, 1978, Goldberg et. al. 1978, 1983, Harris et al. 1979, Davies & Pirie 1980, Popham et al. 1980, Satsmadjis & Voutsinou-Taliadouri 1983, Palmieri et al. 1984). This implies that differences in concentrations of metals and radionuclides in mussel soft parts, sampled at different locations and at different times, indicate differences and changes in the general environmental level of the pollutants measured. Knowledge of the metabolism of these elements by mussels, as influenced by different environmental parameters, is essential for a correct interpretation. Several investigations have elucidated parts of this complex problem.

With *Mytilus* only a few loss-rate studies have been conducted over a sufficient time span to elucidate biological half lives of the slowest pools. Among these, the results of Young & Folsom (1967) on ^{65}Zn, Clifton et al. (1983) on americium, plutonium, caesium, cerium and ruthenium, and Guary & Fowler (1977) on neptunium, applied specifically to *Mytilus* soft parts, whereas several others (Table 5) were obtained simply by repeated whole-body countings.

The Forsmark biotest facility (Fig. 1) at the Bothnian Sea (northern Baltic Sea) was chosen as experimental site because of its logistic possibilities and stable low salinity. The idea was to obtain long-term loss rates under northern Baltic Sea conditions, i.e. close to the mussels minimum salinity requirement and during a long, cold winter. The final goal was to distinguish between a pure temperature effect and a general seasonal effect including temperature changes.

MATERIALS AND METHODS

Location. The experimental site, the Forsmark Biotest facility (Fig. 1), is run by the National Swedish Environmental Protection Board. The main Biotest

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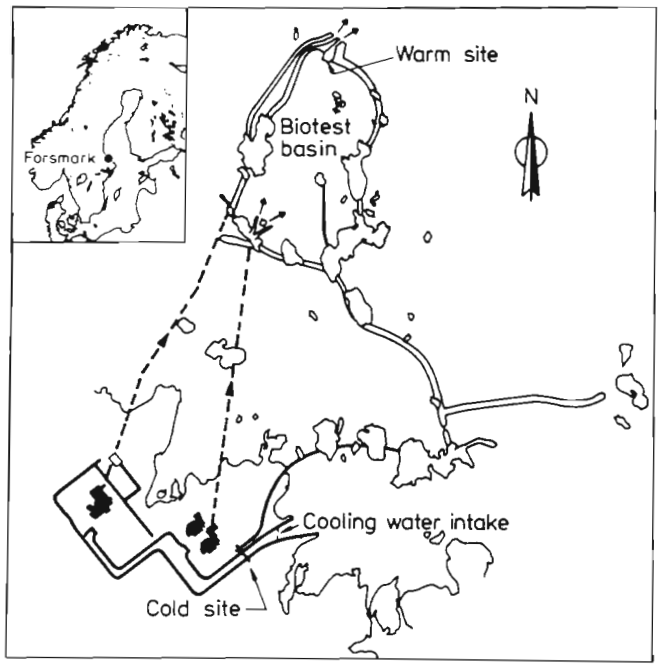

Fig. 1. Experimental localities at Forsmark Biotest facility. Cold and warm sites are indicated. Insert: Position of Forsmark on Swedish coast of Bothnian Sea

basin is a 1 km^2 embanked area of the coastal archipelago flushed and heated by cooling water from the 2 first units of the Forsmark Nuclear Power Plant. In the present study the open channel that leads cooling water to the reactors was used as the site at normal temperature ('cold'), whereas the 'warm' site was at the outlet of the Biotest basin. Temperatures measured at the 2 locations by the Swedish National Meteorological and Hydrological Institute (SMHI) are shown in Fig. 2. The warm site is heated 8 to 10 C° above normal throughout winter.

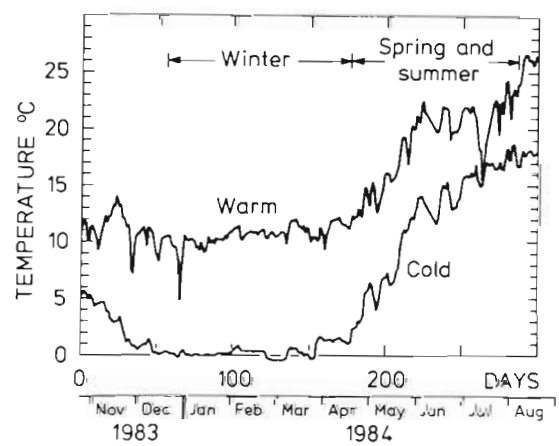

Fig. 2. Water temperature (°C) at cold and warm sites as recorded by the Swedish Meteorological and Hydrological Institute

Salinity at the experimental sites is 5 to 6 ‰ throughout the year, except for some lower transients during ice-melting in spring.

Mytilus edulis approaches its ultimate lower salinity regime in the Northern Baltic Sea (Kautsky & Wallentinus 1980, Kautsky 1981). From another Baltic environment Kautsky (1982) reports decreasing growth rates above 20 mm – corresponding to ca 10 yr of age – and an asymptotic value of 32 mm.

Accumulation. Mytilus edulis were collected 2 km NE of the Biotest basin and kept in outdoor basins flushed by Biotest seawater (~ 10 °C) for 3 d before 200 of them were brought to a controlled isotope laboratory for 'contamination'. Mean shell length was 27.7 ± 3.5 (SD) mm. Accumulation was performed in a modified version of the turbidostatic mussel-feeding set-up described earlier (Bøtter-Jensen & Dahlgaard 1981). In the set-up 60 l of local seawater was circulated at 10 °C. After the mussels had cleared off the natural suspended matter, the set-up automatically kept a stable phytoplankton concentration by injecting *Phaeodactylum tricornutum* cells from a chemostatic culture, at the same rate as the mussels cleared them from the water. Phytoplankton cell density was measured by a Coulter Counter during the period of accumulation and found to be 2100 ± 780 (SD) cells ml^{-1} (ca 25 µg organic dry cell material l^{-1}). During 3 periods of the accumulation phase, clearance rate of phytoplankton cells was 12.1 ± 1.3 (SD) ml min^{-1} $mussel^{-1}$. This implies that each mussel ingested on the average 435 µg organic dry cell material or ca 0.5 % of its soft part dry weight per day. The 10 radionuclides (Table 1) were added to the experimental sea water 8 h prior to the mussels, and the pH, which declined due to acid added with the isotopes, was adjusted back to the original value, 7.9. During accumulation, the pH declined to 7.6.

Depuration. After 136 h accumulation was stopped by changing to clean seawater. For 21 h, the mussels were allowed to depurate. To facilitate gut clearance, phytoplankton cell density was elevated to ~10 000 cells ml^{-1} in this phase. Activity remaining in soft parts after this initial loss is listed in Table 1 as per cent of the activity when the accumulation was stopped. The mussels were then divided into 2 groups and returned to field conditions, suspended in small enclosures, at the 2 locations indicated by 'cold' and 'warm' in Fig. 1. The suspended cages had plastic netting on all 6 sides allowing a free flow of water and due to the huge amounts of cooling water (up to 86 m^3 s^{-1}), water exchange at the 2 sites was very high. During the next 10 mo 5 to 6 mussels were sampled from each site on 11 occasions.

Analysis. Only soft parts were analysed. For shells, length and weight were recorded. All measurements were performed on individual mussels. Before ship-

Table 1. Radionuclides in experimental water during accumulation (time-weighted averages) and concentration ratios in *Mytilus edulis* (Bq g^{-1} dry soft parts/time-weighted average Bq ml^{-1}) obtained when accumulation was stopped after 136 h. i.e. at time zero on loss curves. Activity remaining in *M. edulis* soft parts after the initial 21 h of depuration is given as %. Nuclide concentrations (water and particles) were measured after 15, 24, 36, 70, 94, 118 and 136 h

Isotope and chemical form	Average nuclide concentration kBq l^{-1}	Element added (carrier + isotope) $\mu g\ l^{-1}$	Average particulate fraction (0.4 µm Nuclepore)	Concentration ratio after accumulation ± SD (n = 5)	Remaining activity after initial depuration %
$^{54}MnCl_2$	14	0.008	0.0010	350 ± 56	74
$^{60}CoCl_2$	3.6	0.008	0.0022	900 ± 560	82
$^{65}ZnCl_2$	5.1	0.13	(0.0005)	950 ± 250	84
$^{95\,m}TcO_4$	21	25×10^{-6}	(0.00002)	12 ± 2	36
$^{110\,m}AgNO_3$	0.64	0.006	0.046	6500 ± 2000	69
$^{144}CeCl_3$	8.8	75×10^{-6}	0.051	2000 ± 620	72
$^{155}EuCl_3$	15	0.8×10^{-3}	0.013	350 ± 105	63
$^{237}NpO_2NO_3$	0.33	12	0.0007	10 ± 2	68
$^{239}Pu(NO_3)_4$	0.012	0.005	0.020	400 ± 170	86
$^{241}Am(NO_3)_3$	0.012	0.10×10^{-3}	0.023	400 ± 130	66

ment to the laboratory, soft parts were dried in numbered and preweighed borosilicate glass tubes. The tubes were calibrated allowing γ-counting to be performed directly without changing the container. Gamma counting was performed on a high-resolution semiconductor (Ge[Li]) detector connected to a 2K multichannel analyser. The samples were ashed at 550 °C in the glass tubes, dissolved in HNO_3 and spiked with ^{236}Pu and ^{243}Am for yield determination; the actinides then were separated from the bulk material by ion-exchange (Holm & Persson 1979) and finally electroplated on polished stainless steel discs (Hallstadius 1984). The samples were then counted in an α-spectrometer with a high-resolution surface-barrier Si-detector. The 5 different α-emitters (3 from the sample and 2 spikes) were easily separated. Yields were generally high (50 to 90 %) although some samples were far less successful. Since a suitable yield determinant for Np was unavailable, ^{237}Np-results were calculated utilizing the plutonium spike. Previous tests had shown that plutonium followed neptunium more closely through the separation and electroplating than did americium, but this did of course add an extra uncertainty to the neptunium results. Counting errors were generally low: <5 % for the great majority of samples. However, for low levels of ^{95m}Tc and ^{110m}Ag values with counting errors up to 30 % have been included. All radionuclide results were decay-corrected to the beginning of the loss experiment.

RESULTS

Effects of season and temperature on losses of manganese, cobalt, zinc, technetium, silver, cerium, europium, neptunium, plutonium and americium in *Mytilus edulis* soft parts are shown in Fig. 3. The results document actual loss from mussels without interference from concentration changes due to growth or weight loss. This also applies to the biological half-lives and retention percentages listed in Tables 2 to 4. Biological half-lives were calculated as $T_{½b} = \log 2/-\lambda$, where λ = slope of linear regression line fitted by least-squares method to log (results) versus time.

Regression lines have been calculated and evaluated using a computerized version of the methods described by Sokal & Rohlf (1981). A comparison of the regression line slopes for the cold and warm experiment during the winter period shows a significant difference (P > 95 %) only for ^{110m}Ag whereas during spring and summer both ^{65}Zn and ^{155}Eu clearly show significantly different slopes at the 2 temperatures (>99 %); the same is true for ^{110m}Ag, ^{144}Ce, ^{239}Pu and ^{241}Am at a little less significant level (P > 95 %). If both periods (i.e. all 8 mo not considered initial) are included in the calculation there are no significant differences between cold and warm experiments.

Comparison of regression line slopes for ^{239}Pu and ^{241}Am reveals no significant differences. However, by calculating plutonium-to-americium ratios in individual mussels, and by computing linear regressions of log (ratio) versus time, it is seen that plutonium is lost significantly faster than americium (P > 95 %) at both temperatures (Fig. 4 & 5). As the ratio of one exponential function to another is also exponential, it is reasonable to calculate the 'biological half-life' of the plutonium to americium ratio. This gives 318 and 267 d for the cold and warm experiments, respectively; in other words, after ~300 d, the fraction lost of plutonium is ca twice that of americium. Clifton et al. (1983) observed a slower loss of $^{239, 240}Pu$ (but not ^{238}Pu) than of ^{241}Am. These differences could be an effect of different oxidation states.

Corresponding ratios of ^{237}Np, ^{155}Eu and ^{144}Ce to

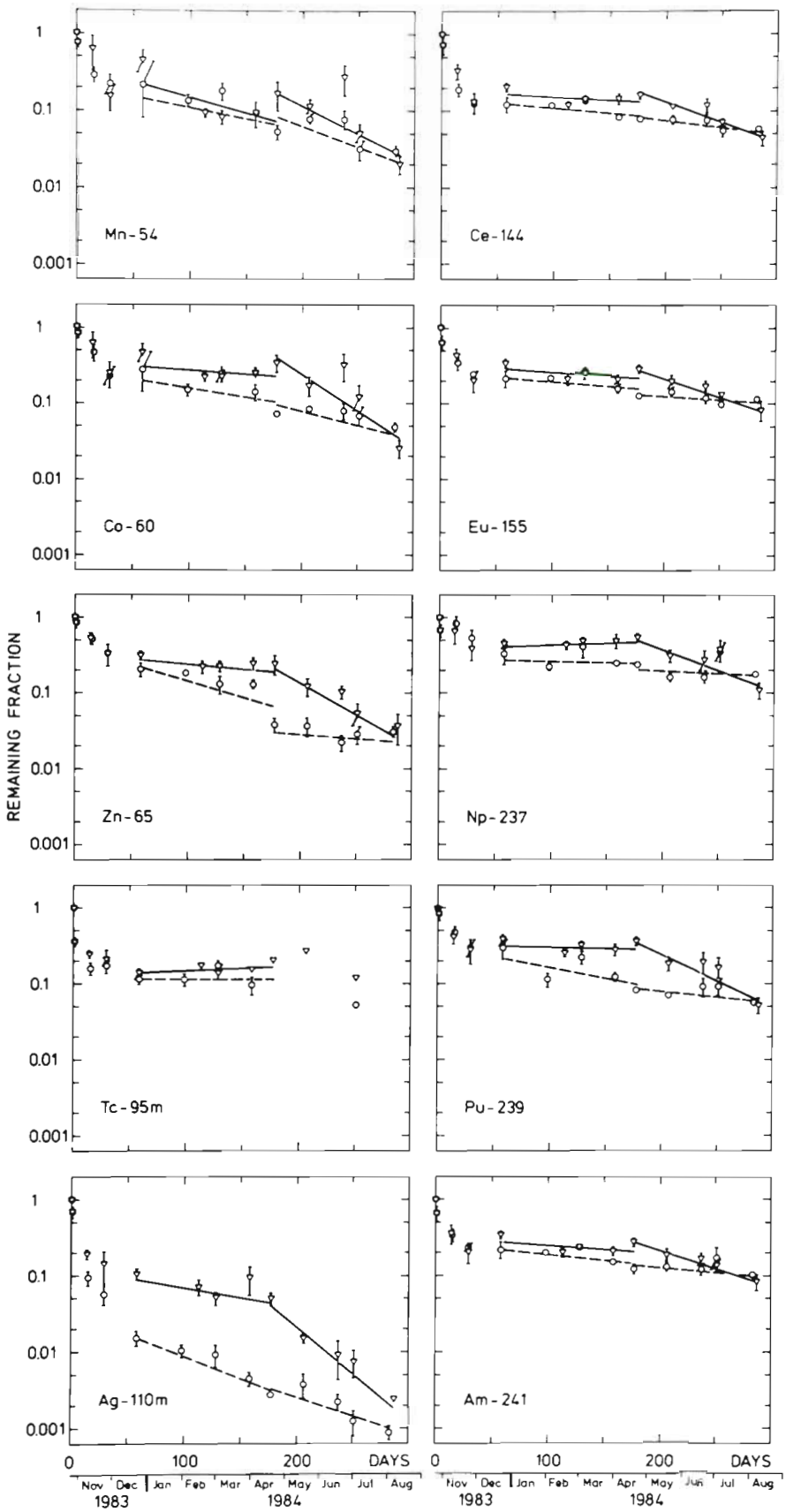

Fig. 3. *Mytilus edulis* soft parts. Loss curves for the 10 nuclides shown as total activity in soft parts normalized to time zero. Values are decay corrected. Error bars: ± SE (n = 5 to 6). (▽) cold; (o) warm. Regression lines calculated for winter and spring + summer periods as indicated in Fig. 2; solid lines: cold; broken lines: warm

Table 2. *Mytilus edulis* soft parts. Biological half-lives in days. Calculations based on linear regressions of log (activity per mussel) versus time. Cold is the normal temperature. Confidence intervals are given in Table 3

Nuclide	Winter		Spring and summer		Both periods	
	Cold	Warm	Cold	Warm	Cold	Warm
^{54}Mn	74^{+}	107^{+}	**42**	**56**	**90**	**81**
^{60}Co	295^{+++}	131^{++}	**33**	**77**	**79**	**88**
^{65}Zn	234^{+}	**62**	**36**	283^{+++}	**67**	**67**
^{95m}Tc	–	–	BDL	BDL	–	–
^{110m}Ag	**123**	**53**	**25**	**61**	**45**	**58**
^{144}Ce	335^{+++}	247^{++}	**59**	**175**	**134**	**176**
^{155}Eu	299^{+++}	243^{+++}	**61**	**302**	**137**	**194**
^{237}Np	–	–	**53**	–	**133**	**298**
^{239}Pu	–	102^{++}	**43**	185^{+}	**98**	**119**
^{241}Am	296^{+++}	197^{++}	**65**	255^{++}	**141**	**195**

Values in bold: perfect regressions ($P > 95\,\%$). For other values the confidence level of slopes, being different from zero (i.e. $T_{½b}$ is different from ∞), are: $^{+}$, $P > 90\,\%$; $^{++}$, $P > 80\,\%$; $^{+++}$, $P > 50\,\%$; –, $P < 50\,\%$ (values not given). BDL: below detection limit

Table 3. Confidence intervals on 95 % level (after Sokal & Rohlf 1981) for biological half-lives (d) in Table 2

Nuclide	Winter		Spring and summer		Both periods	
	Cold	Warm	Cold	Warm	Cold	Warm
^{54}Mn	34–∞	49–∞	23–274	32– 215	52–310	60– 125
^{60}Co	84–∞	55–∞	20– 90	52– 148	50–194	70– 117
^{65}Zn	127–1500	35–2400	27– 53	108–∞	49–102	51– 96
^{95m}Tc	234–∞	84–∞	BDL	BDL	313–∞	84–∞
^{110m}Ag	71–442	45–65	20– 32	36– 180	34– 65	51– 68
^{144}Ce	102–∞	96–∞	43– 92	117– 342	90–267	136– 248
^{155}Eu	94–∞	79–∞	47– 87	154–6700	94–252	138– 326
^{237}Np	600–∞	130–∞	34–126	84–∞	81–367	160–2300
^{239}Pu	135–∞	46–∞	31– 72	92–∞	66–191	87– 188
^{241}Am	94–∞	82–∞	51– 91	112–∞	98–251	142– 311

Table 4. *Mytilus edulis* soft parts. Long-term retention expressed as size of slow winter-compartment in per cent of activity present at start of loss experiment. Values calculated as intercepts with the Y-axis of winter-regression lines in Fig. 3

	^{54}Mn	^{60}Co	^{65}Zn	^{95m}Tc	^{110m}Ag	^{144}Ce	^{155}Eu	^{237}Np	^{239}Pu	^{241}Am
Cold (normal)	37	35	32	12	12	19	32	39	34	32
Warm	21	27	40	11	3	14	26	29	33	27

^{241}Am showed no significant effect of temperature or time (after initial loss) (Fig. 4 & 5). The values of ^{155}Eu remain at unity during the entire release period, indicating that the mussels did not distinguish between these 2 nuclides. Thus europium behaved as an ideal analogue to americium in this release experiment. Had the data been normalized to the value after 1 to 2 mo of loss the same would have been the case for cerium; in other words: after an accelerated initial release period, cerium is lost at the same rate as europium and americium. The mussels remaining for the last 4 to 5 samplings became successively smaller (Fig. 6). Regression lines and biological half-lives were, therefore, also calculated on the basis of Bq g^{-1} soft part and Bq in total soft parts per g shell as suggested by Fischer (1983). However, a statistical comparison of the slopes showed no significant differences. Results document that a loss of soft part weight (Fig. 7) during winter is not accompanied by a similar loss of metals (Fig. 3). Thus, the elements in question are in fact concentrated

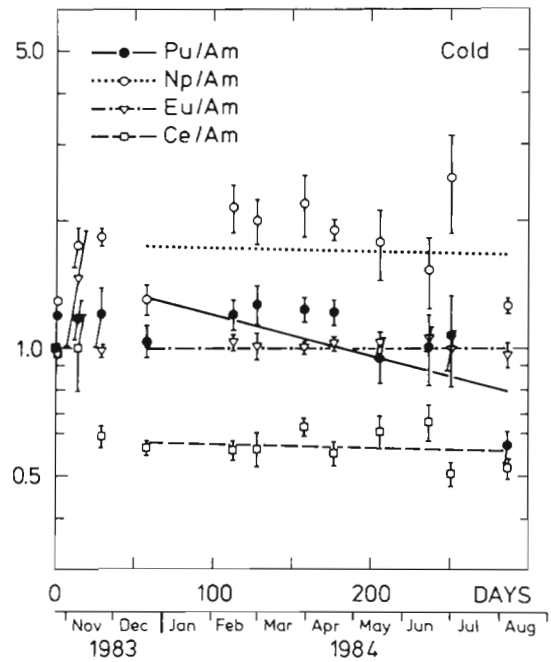

Fig. 4. *Mytilus edulis* soft parts. Cold site: amounts of plutonium, neptunium, europium and cerium relative to americium calculated for individual soft parts and normalized to time zero. Regresion lines indicate period for which they were calculated. Error bars: ± SE (n = 5 to 6)

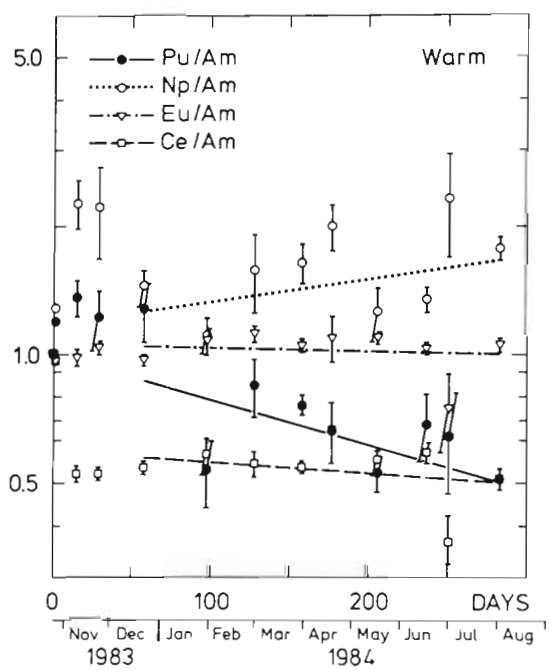

Fig. 5. *Mytilus edulis* soft parts. Warm site: amounts of plutonium, neptunium, europium and cerium relative to americium (see Fig. 4)

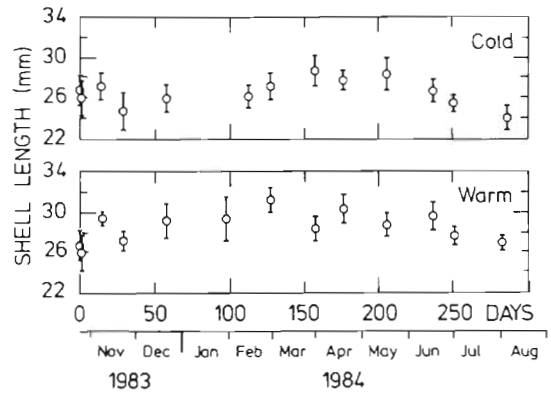

Fig. 6. *Mytilus edulis*. Average shell lengths for different samplings. Error bar: ± SE (n = 5 to 6)

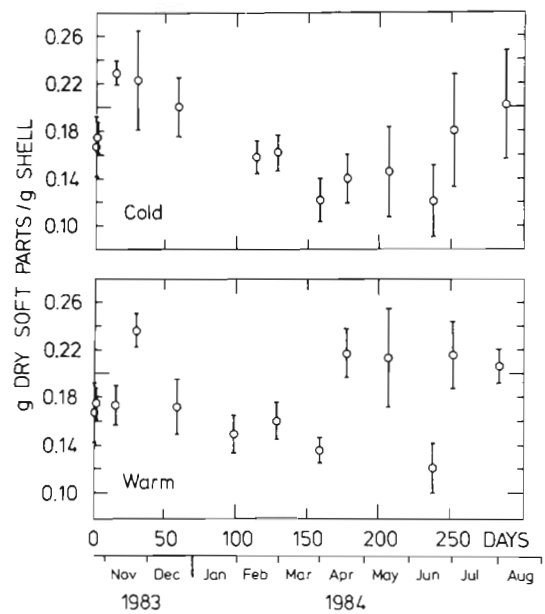

Fig. 7. *Mytilus edulis*. Condition indicated as means of g dry soft parts/g shell calculated for individual mussels. Error bars: ± SE (n = 5 to 6)

in the mussel soft parts during the winter weight-loss period. The same effect has been suggested by Phillips (1976).

DISCUSSION

Baltic Sea environment

It is well known that 'biological variation' often makes comparisons between different experiments questionable. This problem was solved in the present experiment by using multi-element techniques for making comparisons of loss rates of different nuclides. However, as the present work consists of only a single

overall experiment, the variation in loss rates between different locations or different mussel populations, and the reproducibility between different years at the same location is unknown. Effects of Baltic Sea environmental conditions – including the stable, low salinity and the distinct seasonal variation in phytoplankton abundancy (Eriksson et al. 1977) – can be estimated by comparing the present results with loss rates obtained under other conditions. This has been attempted in Table 5, which should be compared with values from the present study in Table 2. The 3 first references are the only ones directly comparable to the present work, as they report loss from soft parts in long-term field studies. The remainder are from whole-body countings, where the shell may be dominant. Furthermore, most of the results in the whole-body group were calculated over a period considered as the initial release phase in the present work. The general impression is that where comparisons are possible the all-seasons loss rates in the Baltic Sea (Table 2) are similar or faster than loss rates reported at full salinity. As presumably none of the results in Table 5 were obtained from mussels experiencing a long, cold winter with food deficiencies, the results obtained at natural (cold) temperatures during spring and summer (Table 2) could be expected to be the most comparable. In this case, the higher loss rates from Baltic mussels, relative to values in Table 5, are conspicuous. Probably not only salinity but also size (Boyden 1977) and energy metabolism influence these differences.

Temperature versus food

Several authors have indicated that temperatures above 20 °C are unfavorable for *Mytilus edulis* (Widdows 1978, Incze et al. 1980, Almada-Villela et al. 1982). This could explain the slower loss rate for warm mussels during spring and summer. Comparison of cold winter values and warm winter values (Table 2), reveals that below 20 °C elevated temperature actually accelerates the losses of silver and zinc, whereas no significant effect is seen for the other elements.

Cold summer and spring experiments and the warm winter experiments cover more or less the same temperature range. The present results document a distinctly higher loss rate during cold summer than during warm winter; this cannot be due to a temperature effect as such. It has previously been observed that the elimination of neptunium, arsenic, mercury and zinc from bivalves is faster *in situ* than in the laboratory without feeding (Romeril 1971, Guary & Fowler 1977, Fowler et al. 1978, Ünlü & Fowler 1979). Borchardt (1983) also reported an increasing loss of cadmium from mussels with increasing food availability in the laboratory. Furthermore, Wallace (1980) found that mussels feeding on fish-farm debris maintained a high growth rate throughout the North-Norwegian winter, whereas comparable mussels that had no access to this food source ceased growing and formed distinct winter-rings. Therefore, the seasonal loss pattern observed in the present work is probably due to a lack

Table 5. *Mytilus edulis*. Biological half-lives (d) for the 10 elements from other works. 1: Young & Folsom (1967), 2: Clifton et al. (1983), 3: Guary & Fowler (1977), 4: Beasley et al. (1982), 5: Guary & Fowler (1981), 6: Dahlgaard (1981), 7: Fowler et al. (1981), 8: Van Weers (1973), 9: Fowler et al. (1975), 10: Bjerregaard et al. (1985)

	Source										
	1	2	3	4	5	5	6	7	8	9	10
Field/lab	Field	Field	Field	Field	Field	Field	Field	Field & lab	Lab	Lab	Lab
Tissue	Soft	Soft	Soft	Whole	Whole	Whole	Whole	Whole	Whole	Whole	Whole
Duration	1 yr	1 yr	7 mo	2 mo	1 yr	4 mo	1 yr	4 mo	2 mo	6 mo	2 mo
Mn							642				
Co							580		64•		
Zn	82						159		55•		
Tc				130				121•			
Ag											
Ce		319									
Eu											
Np			331								
Pu		240, 708••				192				776	80•
Am		303			480						88•

• Authors' mean values •• Two plutonium isotopes

of suspended food particles throughout winter, resulting in a much lower metabolic rate than that induced by the low temperature alone. This implies that decreased metabolic rates include metabolism of essential as well as non-essential trace elements, and further that the seasonal variation often observed in trace element concentrations in mussels and other bivalves (Bryan 1973, Phillips 1976, Orren et al. 1980, Latouche & Mix 1981, Popham & D'Auria 1982, Palmieri et al. 1984), can, at least to some extent, be explained by variations in the available food. Bryan (1973) earlier suggested such a relationship. It could probably also explain why California mussels do not show the same seasonal variation in trace element levels as those from Rhode Island in the US mussel watch (Goldberg et al. 1983, Palmieri et al. 1984). Furthermore, reduced energy metabolism and slow growth (Seed 1980) might explain the higher concentrations of plutonium and cadmium observed in mussels sampled highest up in the tidal zone (Clifton et al. 1983, Goldberg et al. 1983, Palmieri et al. 1984).

CONCLUSIONS

The most significant finding of the present work is that the observed seasonal effect on trace element loss rates should probably be explained by seasonal variations in food availability rather than by direct temperature effects. This emphasizes the difficulties in drawing conclusions on cause and effect relations in environmental studies. It furthermore stresses the unique possibilities of the Forsmark Biotest facility, which gives access to a continuous flow of the same water mass at different temperatures.

Europium was treated by the mussels exactly as was americium, whereas plutonium was lost at a faster rate. The concordance between Eu measured by gamma-spectrometry and Am measured by alpha-spectrometry can be taken as a proof of the reproducibility of the 2 methods. The results show the advantage of multi-element experiments, as a variation between single experiments would have made these conclusions questionable. They also show the compatibility of the simplified multi-alpha technique (Holm & Persson 1979) as compared e.g. to using the short-lived and extremely expensive plutonium-237.

For all the 10 trace elements the initial loss phase appeared to last from 1 to 2 mo. Any conclusions based on relatively short loss curves should thus be drawn with much more care than is seen in part of the published literature.

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