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Uptake and depuration of ^{241}Am, $^{239+240}$Pu, ^{238}Pu, ^{137}Cs and ^{106}Ru by *Mytilus edulis* under natural stress

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ABSTRACT: Rates of uptake of ^{241}Am, ^{137}Cs and ^{106}Ru by both the soft tissue and the shell of transplanted *Mytilus edulis* L. were determined; soft tissue data are compared to theoretically derived values based on the biological half-life and the steady state concentration of these isotopes. The rates of loss of ^{241}Am, $^{239+240}$Pu, ^{238}Pu, ^{137}Cs and ^{106}Ru by *M. edulis* under conditions of stress, manifest as extended periods of shell closure as a consequence of high ambient concentrations of copper and zinc and extended periods of aerial exposure, were examined. Both types of stress did not significantly alter the rate of loss of the Pu isotopes, ^{137}Cs or ^{106}Ru from the flesh of the mussel but the ^{241}Am concentration increased significantly over the depuration period of February to June – a probable consequence of the remobilisation of ^{241}Am associated with the inner nacreous layer of the shell.

INTRODUCTION

In the marine environment, for a variety of pollutants and contaminants including radionuclides (Goldberg et al. 1978, The International Mussel Watch 1983), the mussel *Mytilus edulis* L. has been identified as a useful sentinel organism (Bayne et al. 1981). At a specific site, uptake of a particular contaminant may be affected by a variety of different factors: for example, age, sex, size, tidal position, concentration of other contaminants, physiological state and various environmental variables such as temperature, salinity, particulate load and pH (Bayne & Widdows 1978, Goldberg et al. 1978, Bayne et al. 1979, Simpson 1979, Strömgren 1982). Previously, Clifton et al. (1983) described the effects of mussel size, tidal position and seasonal variability of the condition index (C. I.) of the mussel in relation to the concentration and depuration of ^{106}Ru, ^{137}Cs, ^{238}Pu, $^{239+240}$Pu and ^{241}Am for mussels exposed to low-level radioactive waste, released into the northeast Irish Sea from the British Nuclear Fuel plc (BNF) spent fuel reprocessing plant at Sellafield, Cumbria, UK.

Here we determine the rates of uptake of ^{241}Am, ^{137}Cs and ^{106}Ru by both the soft tissue and the shell of *Mytilus edulis*, transplanted from the Lynher estuary in Cornwall, UK, to Lowsy Point in Cumbria (see Fig. 1). We also consider the influence of stress on transplanted mussels in relation to the rate of loss of these radionuclides. For mussels transplanted from the Esk to the Restronguet Creek, the consequences of 2 types of stress are considered:

(1) The presence of high levels of copper and zinc in ambient water which result in an enhanced production of metallothioneins and granules and subsequent sequestration of the elements in a variety of mussel tissues and organs, especially the digestive gland, pericardial gland and kidney (George et al. 1979, Morse et al. 1985). This study was implemented to determine whether or not certain radionuclides which enter the mussels body fluids are associated with metallothioneins or granules in a manner which might influence depuration rates.

(2) Extended periods of shell closure, especially those induced by prolonged aerial exposure, and the subsequent fate of any shell-bound radionuclides following the partial dissolution of the inner nacreous layer (aragonite) of the shell by the acidic products of anaerobiosis (Wada 1972, Bayne et al. 1979).

SAMPLING AND TRANSPLANT AREAS

Mussels were transplanted from the Esk to Millbay in September 1980 (Clifton et al. 1983), from the Esk to

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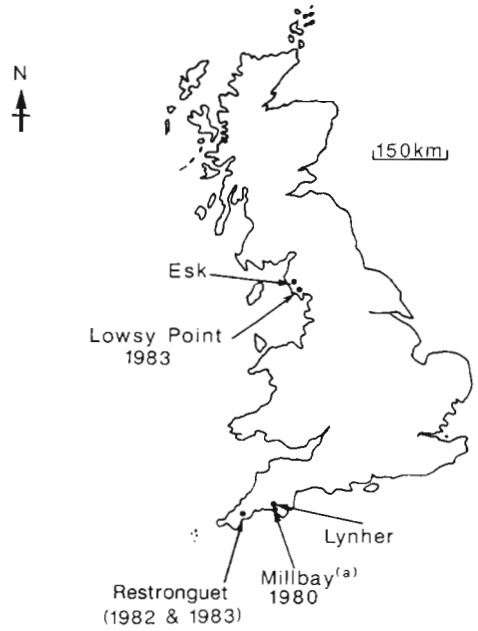

Fig. 1. Sampling sites, UK, showing transplants with dates. [a]Clifton et al. 1983

Restronguet in December 1982 and September 1983 and from the Lynher to Lowsy Point in September 1983. The locations of the study areas are illustrated in Fig. 1.

Millbay site, Plymouth Sound, Devon. This site, described by Clifton et al. (1983), is situated at the mouth of the River Tamar; native mussels are rare, but the submerged cages of Esk transplants became covered with a large number of juvenile mussels indicating that they are potentially viable in this area; the absence of an established mussel bed is probably due to the lack of a suitable substrate.

Esk Estuary, Cumbria. An area supporting well established mussel beds, but also one which receives a variety of radionuclides originating from BNF; see Hamilton & Clarke (1984) for details of the region.

Lynher River, Cornwall. This river enters the Tamar estuary, Devon, 6 km north of Plymouth Sound, and supports an established mussel population which has been described by Worrall & Widdows (1984).

Lowsy Point, Cumbria. Adjacent to the north end of Walney Island and subject to inputs of radioisotopes originating from BNF which is about 20 km to the north. The total beta radioactivity of the fine sediments of this area is 2 to 5 times lower than that measured in the sediments of the Esk estuary (Table 1). At this site there is a small local population of mussels which can be observed at low water. Mussels from the Lynher site, free of BNF radionuclides, were transplanted to Lowsy Point rather than to the Esk estuary in order to comply with regulations imposed to control the spread of parasitic infestations of shellfish. The mortality of the transplanted mussels was less than 5 % over the transplant period.

Table 1. Concentration (Bq kg^{-1}) of ^{241}Am, $^{239+240}Pu$, ^{137}Cs and ^{106}Ru in fine silts adjacent to the Esk mussel beds (ES), Restronguet transplant site (RC), Walney island (WI). Note very low ambient concentration of these radionuclides at Restronguet Creek relative to the Esk estuary

Site	Concentration			
	^{241}Am	$^{239+240}Pu$	^{137}Cs	^{106}Ru
ES 1983[a]	2700	3000	6000	6900
RC 1983	<0.47	<0.1	5.1	<2.13
WI 1983	450	NA	1600	3600

[a] From Hunt (1985)

Restronguet Creek, Fal Estuary, Cornwall. High concentrations of dissolved metals (e.g. Fe, As, Cu, Zn) are present, originating from past metalliferous mining activities within the region (Dines 1969, Bryan & Gibbs 1983). There is no native population of mussels at this site, but Boyden (1977), Bryan & Gibbs (1983) and Klumpp & Petersen (1979) have successfully transplanted *Mytilus edulis* to this area. Mortality of the transplanted mussels was ca 25 %. It was not possible to carry out experiments at this site during July and August due to disturbances associated with the tourist season.

MATERIALS AND METHODS

Determination of copper and zinc. Copper and zinc were determined in the Esk mussels transplanted to Restronguet Creek: groups of 10 to 20 mussels, pretreated in the same way as that described by Clifton et al. (1983) for radiochemical analyses, were freeze-dried and then homogenised in an agate ball-mill (Glen Creston). Duplicate samples weighing about 0.5 g were then wet ashed in concentrated HNO_3 and H_2O_2 (30 %) to obtain a white residue which was then dissolved in 10 ml 1*N* HCl. Copper and zinc were determined using a Pye Unicam SP9 flame atomic absorption spectrophotometer with a background correction facility. Instrumental errors were ca 1 % relative standard deviation (RSD) and errors on duplicate samples were < 10 % RSD. These determinations were based on standards obtained from British Drug Houses Ltd.

Radionuclide measurements. Gamma-emitting radionuclides, ^{106}Ru, ^{137}Cs, ^{241}Am, were determined by high resolution gamma spectrometry using an intrinsic germanium detector and ^{238}Pu and $^{239+240}Pu$ by surface barrier alpha spectrometry as previously described by Clifton et al. (1983). All radioisotopes were determined using reference standards obtained from Amersham International plc.

Determination of Condition Index (C. I.). Although there are a variety of indicators of the physiological condition of the mussel (Bayne et al. 1976, Cossa et al. 1979, Widdows et al. 1980, Zandee et al. 1980, Bayne et al. 1981, Martin et al. 1984) we continue to use the C. I. for 2 reasons: (a) to maintain continuity with our previous work (Clifton et al. 1983); and (b) because we are still of the opinion that the C. I. is probably the most meaningful measurement in this type or work; it provides a quantitative estimate of the seasonal variation in soft tissue weights which are essential for the interpretation of radionuclide concentrations in tissues and the estimation of total body burdens. The methods employed to determine C. I. are described by Clifton et al. (1983).

Physical measurements. Once every 6 wk, during periods when the mussels were submerged, 10 to 12 l aliquots of seawater were taken every 30 min from above the mussels using a 12 V submersible pump (Jabsco products, ITT); salinity was determined using a MC5 salinometer (Electronic Instruments Ltd); particulate loading of the water was determined gravimetrically by filtering (0.45 µm Millipore) known aliquots of water. The pH was determined with a battery-operated pH meter (Orion Research model 201). The period of aerial exposure of the mussels was also noted at all 3 sites during both spring and neap tides.

Uptake and depuration of radionuclides. ***Lynher to Lowsy Point transplants.*** From the River Lynher 150 mussels were transplanted to Lowsy Point; sub-samples were collected, together with samples of the resident population, 2 and 57 d after the transplant. The samples were returned to the laboratory where they were maintained in seawater, obtained from Plymouth Sound, for 48 h to remove gut contents before determining the concentration of radionuclides in the total soft tissue and the shell.

The relative radioisotope levels in the inner and outer shell surfaces were determined by subdividing the shells of the transplant mussels into 2 batches: one was analysed, unprocessed, in order to determine the activity of the nuclides associated with the total shell and the other was analysed after the removal of the periostracum and about 0.1 mm of the outer shell surface with a steel brush and abrasive paper.

Esk to Restronguet transplants. Two batches of mussels were transplanted from the Esk to Restronguet Creek – one in December 1982 (R1) and the other in September 1983 (R2).

The radionuclides ^{106}Ru, ^{137}Cs, ^{238}Pu, $^{239+240}$Pu and ^{241}Am were determined in sub-samples (n = 40) taken at intervals of 4 to 6 wk in order to calculate depuration time profiles which could then be compared with those obtained at the Millbay site in 1981–82 (Clifton et al. 1983).

Esk to Millbay transplants. These experiments have been described previously by Clifton et al. 1983.

RESULTS

The uptake (expressed as a percentage of the concentration in the native population) of ^{241}Am, $^{239+240}$Pu, ^{238}Pu, ^{137}Cs and ^{106}Ru by the soft tissue (after 57 d) and shell (after 2 and 57 d) for mussels transplanted from the Lynher (Cornwall) to Lowsy Point (Cumbria), are given in Tables 2a and 2b respectively. Percentage uptake values and an estimate of the time required for these isotopes to attain ca 80 % of the steady state values in the mussel are also listed in Tables 2a and 2b, together with the relative distribution of ^{241}Am, ^{137}Cs and ^{106}Ru on the inner and outer surfaces of the shell.

The uptake of Cu and Zn by the Esk mussels transplanted to Restronguet and Millbay is illustrated in Fig. 2a, b.

The depuration of ^{241}Am, $^{239+240}$Pu, ^{238}Pu, ^{137}Cs and ^{106}Ru from mussels transplanted to Restronguet and Millbay is illustrated in Fig. 3a to e. The concentrations of the isotopes are expressed, on a natural log scale, as a percentage of the original concentration determined in the Esk mussels prior to transplant, but after depuration of the digestive gland contents for 48 h. All data has been corrected to account for changes in the soft tissue weight as determined by the C. I. (Clifton et al. 1983). The ranges (representing the maxima and minima recorded over the transplant period) of temperature, pH, particulate load, salinity and aerial exposure time experienced by the native Esk and the Millbay and Restronguet transplant mussel populations are listed in Table 3.

Seasonal variation in C. I. of the 3 transplant populations is plotted in Fig. 4.

DISCUSSION

Radionuclide uptake by Lynher mussels transplanted to Lowsy Point

If the uptake of a contaminant by the soft tissue of the mussel is solely dependent on a constant environmental level and a constant tissue weight, then it can be expressed by the equation:

$$C_t = C_{ss}(1-e^{-kt}) \quad (1a)$$

where C_t = concentration of nuclide at time t; C_{ss} = asymptotic or steady state concentration; k = 0.693/T, where T = the biological half-life of the nuclide; t = exposure time.

The absolute concentration of a contaminant in the

Table 2. *Mytilus edulis*. Transplant data Lynher to Lowsey Point.
(a) Soft tissue. Comparison of percentage steady-state values determined from the Lowsy Point transplant data and those calculated using the biological half-lives determined from the Millbay transplant data. An estimate of the time to attain 80 % steady-state is also included

Isotope	Biological half-life[a] (d)	% Steady-state at 57 d		Time to attain 80 % steady state
		Calculated	Found[b]	
^{241}Am	303 ± 34.1	12.3 ± 1.3	15.4	704
$^{239+240}$Pu	708 ± 228	5.4 ± 2.5	15.2	1644
^{238}Pu	240 ± 35.1	15.2 ± 2.4	22.2	557
^{137}Cs	38.7 ± 0.9	64.0 ± 0.8	65.9	90
^{106}Ru	261 ± 56.8	14.0 ± 3.6	22.3	606

[a] Calculated from Millbay data (Clifton et al. 1983)
[b] Determined from Lowsy Point transplant data

(b) Shell-uptake and distribution. Percentage of steady-state values attained 2 and 57 d after transplant. Distribution of the isotopes on the outer and inner surfaces of the shell are compared

Isotope	% Steady-state		Distribution of activity (%)	
	2 d	57 d	Outer shell	Inner shell
^{241}Am	35.5	55.4	'100'	'0'
^{137}Cs	17.2	67.4	66.3	34.1
^{106}Ru	10.4	58.0	52.1	48.2

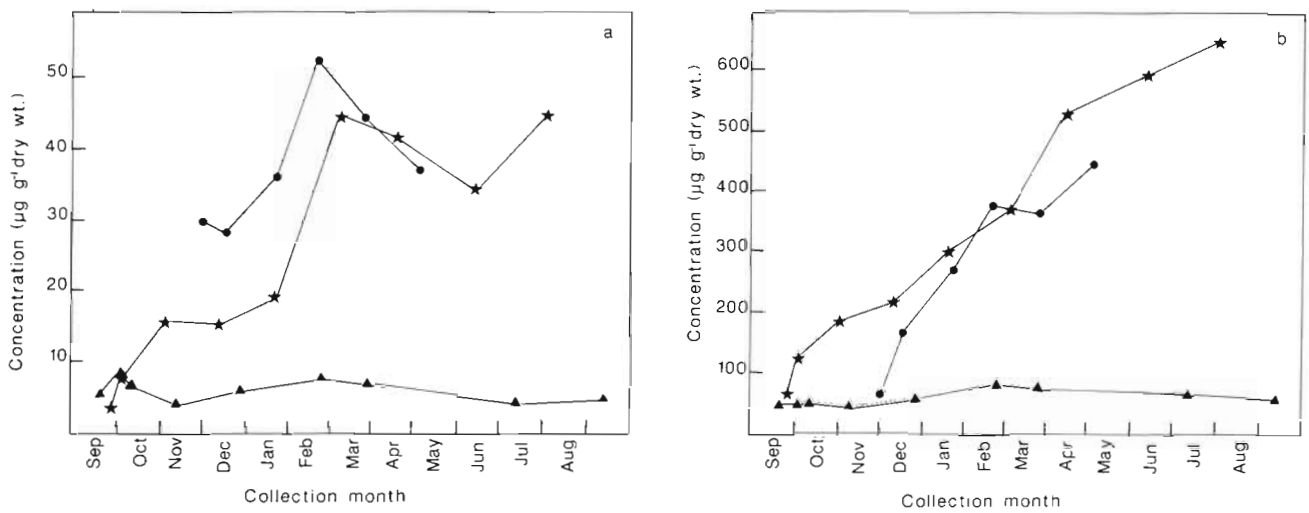

Fig. 2. *Mytilus edulis*. Time-related uptake of (a) Cu and (b) Zn by Esk mussels transplanted to: (▲) Millbay, (●) Restronguet 1, (★) Restronguet 2

soft tissue of the mussel will depend on a variety of factors, for example: (1) period of exposure to the contaminant; (2) food availability and the degree to which the contaminant is associated with both food and water; (3) the concentration factor for contaminants in the mussel tissues relative to those in food and water; (4) the biological half-life of the contaminant in the different components of the soft tissue and their relative proportions.

Previous work (Clifton et al. 1983), together with the data presented here, shows that, over the period 2 to 360 d, the depuration profiles of the radionuclides studied cannot be resolved into more than one component. Over the transplant period, it is reasonable to assume that conditions (2) to (4) are the same for both the native mussel population and those transplanted to the same area. Therefore, a single compartment model should describe the uptake of a particular isotope for a

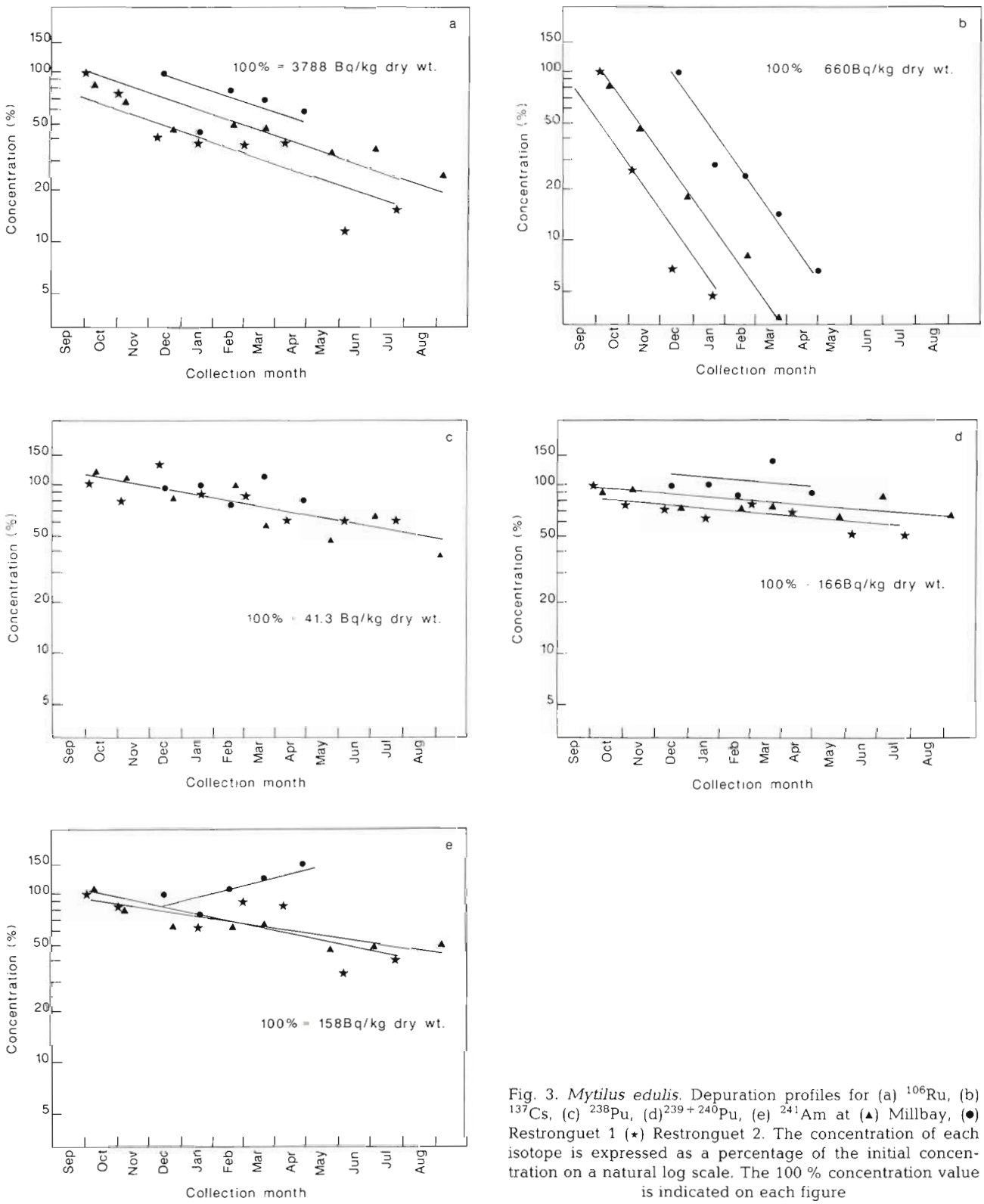

Fig. 3. *Mytilus edulis*. Depuration profiles for (a) ^{106}Ru, (b) ^{137}Cs, (c) ^{238}Pu, (d) $^{239+240}$Pu, (e) ^{241}Am at (▲) Millbay, (●) Restronguet 1 (⋆) Restronguet 2. The concentration of each isotope is expressed as a percentage of the initial concentration on a natural log scale. The 100 % concentration value is indicated on each figure

Table 3. Physical and chemical characteristics of the Esk, Millbay Restronguet and Lowsy Point sites

Parameter	Site			
	Esk	Millbay	Restronguet	Lowsy Pt
Temperature	4.3–16.4	6.0–17.8[a]	5.5–18.1	14.1–15.9
pH	7.8–8.3	7.8–8.4[a]	8.0–8.3	8.0–8.3
Particulate load (mg l^{-1})	3.6–33.2	1–15[a]	4.2–53.2	2.3–16.2
Salinity (‰)	28.9–34.5	30[a]	26.7–33.0	31.2–32.6
Aerial exposure time (%) (at sampling site)	30–46	0–0.04	51–68	29–38

[a] From Morris et al. (1982)

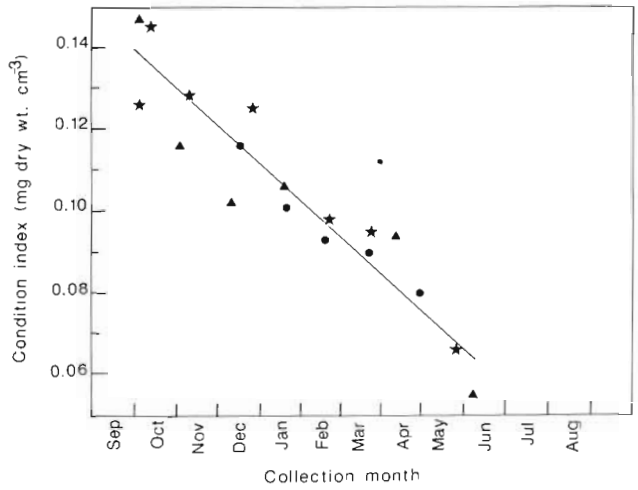

Fig. 4. *Mytilus edulis*. Condition index of mussels transplanted to (▲) Millbay, (●) Restronguet 1, (★) Restronguet 2. Condition index is expressed as mg dry tissue weight per cm^3 of shell cavity volume

given exposure period if it is expressed as a fraction of the concentration in the native mussels which are assumed to have attained a 'quasi steady state'; i.e.

$$C_t/C_{ss} = 1-e^{-kt} \quad (1b)$$

It is assumed radionuclides in the Lowsy Point native mussels approach steady state concentrations and hence the calculated uptake values may be compared, using the biological half-life of the nuclide determined from the Millbay transplants (Clifton et al. 1983), to those measured at Lowsy Point after 57 d. With the exception of $^{239+240}$Pu, which has a large error associated with the determination of its biological half-life (Clifton et al. 1983), the calculated and observed uptake values are in reasonable agreement (Table 2a). These findings serve to validate the biological half-lives determined by Clifton et al. (1983) for the depuration of ^{106}Ru, ^{137}Cs, ^{238}Pu and ^{241}Am from the total soft tissue of the mussel. Using these values for biological half-life we have also calculated that the time required for an isotope to reach ca 80% of its steady state concentration in the soft tissue of the mussel would be about 90 d for ^{137}Cs with a biological half-life of 39 d and 550 to 700 d for ^{241}Am, ^{238}Pu and ^{106}Ru with biological half-lives in the region of 250 to 300 d (Table 2a). Hence there is a need for caution when comparing data from mussels acutely exposed to radionuclides in the laboratory to those obtained from mussels chronically exposed in the field.

The shell approaches steady state values more rapidly than the soft tissue (Table 2b) and the relative affinities for ^{241}Am, ^{137}Cs and ^{106}Ru for the outer and inner surfaces of the shell are quite different (Table 2b): ^{241}Am is taken up almost entirely by the outer shell surface (i.e. periostracum); ^{137}Cs is distributed in the ratio 2:1 between the outer and inner surfaces while ^{106}Ru is distributed evenly between the two. However, this situation is almost certainly atypical as the mussels transplanted to Lowsy Point were mature and previously uncontaminated by these isotopes; their radionuclide distribution will be very different to those found in mussels native to the area as shell growth processes will result in a more even distribution of these radioisotopes.

Depuration of radionuclides from Esk mussels transplanted to Restronguet

A multi-regression analysis of the 3 depuration profiles for ^{106}Ru, ^{137}Cs, and $^{239+240}$Pu for total soft tissue, obtained at Millbay, Restronguet 1 (R1) and Restronguet 2 (R2), indicates that although the intercepts are different, the 3 curves are parallel at the 95% significance level (Fig. 3a, b, d). However, the ^{241}Am depuration profiles (Fig. 3e) are different, especially at R1 which shows a significant increase in the ^{241}Am concentration over the depuration period February to June. A multi-regression analysis of the 3 profiles confirms that, at the 95% level, they are different (slope and intercept). The relatively low concentrations of ^{238}Pu result in depuration profiles which have higher errors associated with each data point (30 to 40% for

^{238}Pu vs 10 to 15% for other isotopes) and are not significantly different. With the exception of ^{137}Cs, the concentration of all the isotopes in the R1 transplants appear to increase during the period February to June. However, unlike the ^{241}Am increase, these increases are not statistically significant (at the 95% confidence level).

Analyses of Restronguet sediments indicate that environmental levels of these isotopes are negligible relative to those found in the silts adjacent to the Esk mussel beds (Table 1). Transfer of these radioisotopes from the periostracum, byssus or faeces to the soft tissue via ventilation and food uptake is highly improbable. The ^{241}Am increase cannot be attributed to changes in the total soft tissue weight as all these data have been normalised to the mussels' C.I.; data not corrected in this manner would indicate an even greater increase in the concentration of ^{241}Am in total soft tissue over the same period. One possible explanation for this anomaly is that the environmental conditions which prevail at the Restronguet site impose a stress (Bayne et al. 1985) on the mussel which is greater than that experienced by mussels transplanted to the other sites. The temperature, pH, suspended particulate load and salinity ranges of the water, measured at all 3 sites, were similar (Table 3). However, there were 2 significant differences between the environmental conditions experienced by the Restronguet and the Millbay transplant populations: the Restronguet mussels were subjected to longer periods of aerial exposure (Table 3); and they were exposed to much higher fluxes of copper and zinc which is reflected (confirming the findings of Bryan & Gibbs 1983) by the fact that the concentration of these elements in the soft tissue of the transplanted mussels (R1 and R2) increased by approximately one order of magnitude over the 290 d transplant period (Fig. 2a, b).

Hamilton & Sims (unpubl.) have demonstrated that mussels from regions which contain relatively low concentrations of trace elements, when transplanted to Restronguet Creek, rapidly produce metallothioneins and granules in the digestive gland, the pericardial gland and the kidney. However, the prime function of metallothionein and granule production is sequestration of elements; any effects they have on the retention of radionuclides would be manifest as changes in their depuration rates. The authors are not aware of any biochemical process (including readsorption from shell) involving metallothionein or granule production which could result in the observed increase of ^{241}Am in the R1 transplants.

The stress imposed by exposure to high levels of dissolved metals will be enhanced by the extended period of aerial exposure which in turn will result in prolonged periods of shell closure and anaerobiosis.

Concentrations of radionuclides approach steady state values more rapidly in the shell than in the total soft tissues of the mussel (Table 2b). Shanbhay & Morse (1982) have described the uptake of americium onto synthetic calcite and aragonite and shown that aragonite absorbs at a rate 40 times that of calcite. However, for the macrobiogenic calcite of the echinoderm *Clypeaster* sp. and aragonite of the coral *Fungus* sp. americium adsorption is similar for both materials, but uptake is influenced by the microporous nature of both materials and the degree of surface recrystallisation of the carbonate matrix. Nevertheless, in the instance of the inner nacreous layer of the mussel shell, dissolution of the surface aragonite occurs during periods of shell closure and anaerobic metabolism, in order to maintain pH of the pallial fluid.

Normal erosion of the inner shell surface is counterbalanced by the deposition of calcium carbonate (aragonite) with an overall bias towards deposition (Lutz & Rhodes 1977, Brand et al. 1987). However, during extended periods of shell closure there will be a bias towards dissolution which in turn could result in the release of shell-bound radionuclides from the inner nacreous layer and the observed changes in the slope of the ^{241}Am depuration curve over the period February to June. Visual examination of the nacreous layer shows that there is a change from the 'pearl-like' appearance of the native Esk mussels to a more 'chalky' appearance several months after transplant which, according to Wada (1972), is indicative of shell erosion.

The total plutonium and americium content of shells is 3.5 to 4.4 times greater than that of soft tissues. Koide et al. (1982) have described the advantages of using shell and byssal thread of bivalves as sentinels of metal and radionuclide pollution monitoring in marine waters. Studies are in progress to determine the distribution and concentration of americium in mussel shell, particularly the partitioning between the inorganic and organic phases.

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