PRECIS

A la suite de l'identification des zones locales où les populations naturelles de moules ont été contaminées par le mercure, un procédé technique de biocontrôle sur place a été mis au point qui reflète avec précision la teneur en mercure totale moyenne de l'eau de mer avoisinante. La limite de détection de cette technique est estimée à 5-20 ng Hg l^{-1}, et par conséquent, cette méthode permet de détecter des accroissements relativement faibles des concentrations de mercure habituelles de l'eau de mer et de l'eau des estuaires.

International Council for the Exploration of the Sea

C.M.1977/E:36
Fisheries Improvement Committee

The use of the mussel, Mytilus edulis as a bio-assay organism for mercury in sea water

by

I.M. Davies and J.M. Pirie
Marine Laboratory
Victoria Road
Aberdeen, Scotland, UK

Abstract

Following the identification of local areas of mercury contamination in natural mussel populations, a field bio-assay technique was developed which accurately reflects the mean total mercury concentration in the surrounding sea water. The detection limit of the technique is estimated at 5-20 ng Hg l^{-1}, and consequently the method can detect comparatively small enhancements over background mercury concentrations in estuarine and sea water.

Introduction

The accumulation of heavy metals in marine organisms arising from exposure to polluted environments or, in some species, from natural processes in relatively unpolluted areas, is well known. Some results for important food organisms are contained in recent reports, for example HMSO (1976). Metal accumulation can be of importance from the public health point of view, and can also be used in the assessment of environmental quality (eg Eganhouse and Young, 1976). The mussel, Mytilus edulis, is a sedentary, filter-feeding mollusc of wide distribution in coastal waters and comparative ease of collection and would therefore appear to have certain favourable characteristics for use as an indicator in studies of environmental quality. Consequently, considerable effort has been expended in the analysis of metals in mussels from areas thought to be at risk from pollution (eg Jones et al., 1972; Eganhouse and Young, 1976); in international projects (Holden, 1973), suggestions have been made for world-wide monitoring of the coastal marine environment (Goldberg, 1975) using the mussel as a biotic heavy metal integrator.

To be of general use as an indicator species for comparing environmental conditions, it is necessary to establish that mussels are reliable and consistent in their response to the stresses imposed. The collection of comparable samples is of prime importance when studying natural populations (Philips, 1976). Growth rate and size range of mussels vary markedly with locality and position on the beach with respect to tidal range (Seed, 1976), presenting immediate practical sampling problems. Philips (1976) has recently described the variation of trace metal content in mussels from fixed localities with season and position in the water column, and has recommended sampling procedures.

Unpublished results of measurements of the heavy metal content of fish and shellfish from Scottish waters by this laboratory have shown enhanced mercury concentrations in some mussel samples from the Firth of Forth. A previous paper

(Davies, 1976) presented the results of a further more detailed survey of the mercury content of intertidal mussels from the Forth to establish the extent of the enhancement. The variation of mercury concentration with size of animal was also assessed. In this paper the analyses of mercury in native mussels are compared with the measured distribution of total mercury in the water of the estuary. A bio-assay technique, using introduced mussels of the same age and similar in size exposed in moored cages is also described, the results from which can be more readily related to the total mercury concentration in the water.

Materials

The mussels used in the bio-assay experiments were cultivated, sub-tidal mussels from the Dornoch Firth (N. Scotland). They were of a single year class, similar in size, and proved to have a low mercury content. Batches of 70 mussels were placed in plastic-coated wire mesh (2 mm gauge, 2.5 cm mesh) cages (60 x 45 x 10 cm), suspended 2 m below a surface buoy anchored by two weights (see Fig. 1). The mooring locations were such that the cages would not touch the sea-bed at low tide.

Four samples of mussels were removed from the cages at intervals and kept for 24 hours in clean seawater to flush out particulate residues. They were then individually shucked, the flesh homogenised by Ultra-Turrax homogeniser, and stored frozen until analysis.

Two litre water samples from 2 m depth were collected on eight occasions at various states of the tide over a two day period in August 1976 using a PVC Van Dorn sampling bottle.

Chemical analysis

a) Mussels

Samples of wet homogenate (0.5-1.0 g) were ashed in a stream of oxygen for 10 mins at 1000°C in a silica lined furnace. The liberated mercury was trapped in a mixture of 10 cm^3 50% H_2SO_4 plus 10 cm^3 2% $KMnO_4$ (Topping et al., 1975).

b) Water

The total mercury content of the unfiltered water samples was determined as described by Topping and Pirie (1972), modified to include an oxidative pre-treatment with 20 cm^3 2% $KMnO_4$ plus 20 cm^3 50% H_2SO_4.

In both analyses the final determination was made by cold vapour atomic absorption on a Techtron 120 spectrophotometer (Topping and Pirie, 1972). The coefficient of variation of the techniques were 5% and 10% for mussels and water respectively at the concentrations encountered.

Results

a) Water analyses

The mean mercury concentrations in the water at the twelve stations sampled in the inner Firth of Forth are shown in Figure 2. The highest results (80 - 119 ng Hg l^{-1}) were obtained upstream off Grangemouth and progressively lower concentrations eastwards to Rosyth (16 - 22 ng l^{-1}). Our previous unpublished data showed concentrations of <10 ng l^{-1} east of the Queensferry Bridges. There was wide variation of total mercury concentration at each station over the tidal cycle, e.g. 22 - 111 ng Hg l^{-1} at station 2, and 5 - 20 ng Hg l^{-1} station 11.

The survey of native mussels (Davies, 1976) had found uniformly high tissue mercury concentrations in the inner Firth of Forth (Queensferry to Grangemouth), and elevated concentrations for some distance east of Queensferry. This may be contrasted with the observed distribution of mercury in the seawater.

b) Bio-assays using selected cultured mussels

Experiments with caged cultured mussels were carried out at five positions (A - E) shown in Figure 2. The results for unfiltered water indicate that the mean total mercury concentrations in the water at these stations could range from approximately twice background level (~ 10 ng l^{-1}) at position E to five times background at position A.

The results of the analyses of mussels after various periods of exposure are given in Table 1. They show a rapid accumulation of mercury at all positions with the largest increase at position A. At all positions the increase in concentration continued throughout the period of the experiment. After approximately 150 days the exposed mussels reached mercury concentrations similar to those observed in natural populations of mussels in the upper reaches of the Firth of Forth.

A plot of total mercury per mussel against the average concentration found in the water at the position of exposure (Fig. 3) shows that on the first sampling occasion a linear relationship existed between mercury content and water mercury concentration. Similar trends were maintained on subsequent sampling occasions, although the correlation tended to become less good.

Discussion

Our studies using resident mussel populations to indicate mercury distribution within a small estuary have highlighted the problems of using natural populations of mussels. The levels of total mercury in water showed enhancement within the inner estuary falling to typical background levels in the outer estuary. The natural populations of mussels did not reflect this gradient, but indicated a wide area of mercury contamination. Further detailed studies of a population of local mussels showed that a considerable part of the variation of mercury concentration in tissue could arise from differences in water content and size of mussel. Some possible explanations for this variation of concentration with size have been discussed by Davies (1976).

The experiment to minimise these problems by exposing standardised cultivated mussels in moored cages has clearly many fewer degrees of variation. This simple, relatively cheap technique, provides a quick and accurate bio-assay which reflects the total mercury concentration in the water. After only 20 days exposure at mercury levels of approximately 20 ng l^{-1} a significant increase in the mercury concentration of the mussels was detectable. Figure 3 shows that the mercury content of the mussels was closely related to the mercury concentration in the water after 20 days exposure. Longer exposure times resulted in increased uptake of mercury, but the relationship between uptake and water concentration deteriorated. The spread of concentrations obtained from the group of individuals drawn from each cage also increased with time. Schulz-Baldes (1974) found a linear uptake of lead with time by mussels in experimental systems, and also noted that the standard deviation of the mean concentration of lead in tissues increased with exposure time.

An important criterion for the usefulness of an assay organism is that it should show measurable response to the environmental concentrations likely to occur in polluted waters. Schulz-Baldes (1974) deduced concentrations of lead in water of 0.06-0.18 µg l^{-1} from analyses of mussel tissue from the Weser estuary, although his experimental conditions embraced the range 5-5000 µg l^{-1}. Stebbing (1976) has reviewed the sensitivity of bio-assay techniques for mercuric chloride and concluded that only the most sensitive measurable response was appropriate for the determination of even the greatest reported field concentrations of mercury. The bio-assay technique described in this paper has been calibrated using exposures under natural conditions and has been shown to respond markedly to the prevailing mercury concentrations encountered which are not excessively high. The standard deviation of the mercury content of the caged mussels sample after 20 days exposure at position A is 0.18 µg Hg/mussel; B 0.16 µg Hg; C 0.41 µg Hg; D 0.19 µg Hg; E 0.10 µg Hg. If the 'detection limit' of the method is taken as that water concentration which will induce a measurable response (2σ), then the detection limit is in the range 5 - 20 ng Hg l^{-1}. This is at least 2 orders of magnitude lower than the most sensitive method listed by Stebbing (1976) and is comparable to the concentrations reported in open ocean water, consequently the method should be adequate for light - moderately polluted inshore and coastal waters.

Bearing in mind the difficulties (Davies, 1976) of the use of natural populations of mussels as indicators of environmental conditions, it is recommended that where surveys of natural populations of mussels have indicated possible enhanced mercury concentrations in the water, these areas should be further investigated using the bio-assay technique described above to obtain more reliable information on the magnitude and extent of the mercury contamination. The bio-assay technique described should be readily adaptable for use with other organisms and pollutants, and may well be applicable in relation to water quality criteria directives.

Acknowledgements

We wish to thank the Forth River Purification Board for considerable assistance with field work, Imperial Chemical Industries Ltd. for helpful discussion, and making available accommodation and facilities for analytical work. Mr C.G. Moscati, Meikle Ferry, Tain, kindly provided mussels for the bio-assay experiments, and various members of the Marine Laboratory have assisted with sampling, analysis, and many other things.

References

Davies, I.M. 1976 Some observations on mercury in mussels (Mytilus edulis) from the Firth of Forth. ICES C.M. 1976/E:43.

Eganhouse, R.P. and Young, D.R. 1976 Mercury in tissues of mussels of Southern California. Mar. Pollut. Bull. 7, 145-7.

Goldberg, E.D. 1975 The mussel watch - a first step in global marine monitoring. Mar. Pollut. Bull. 6, 111.

H.M.S.O. 1976 Environmental mercury and man. Pollution Paper No 10, Dept. of the Environment, Central Unit on Environmental Pollution, 92 pp.

Holden, A.V. 1973 International co-operative study of organochlorine and mercury residues in wildlife, 1968-71. Pest. Monitor. Bull. 7, 37-52.

Jones, A.M., Jones, Y. and Stewart, W.D.P. 1972 Mercury in marine organisms of the Tay region. Nature, 238, 164-5.

Philips, D.J.H. 1976 The common mussel Mytilus edulis as an indicator of pollution of zinc, cadmium, lead and copper. I Effects of environmental variables on uptake of metals. Mar. Biol. 38, 59-70.

Schulz-Baldes, M. 1974 Lead uptake from sea water and food, and lead loss in the common mussel Mytilus edulis. Mar. Biol. 25, 177-193.

Seed, R. 1976 Ecology, In: Marine mussels: their ecology and physiology, pp 13-66 B.L. Bayne (Ed) Cambridge Univ. Press, 506 pp.

Stebbing, A.R.D. 1976 The sublethal effects of metals on clonal hydroids. J. Mar. Biol. Assoc. UK 56, 977-994.

Topping, G and Pirie, J.M. 1972 Determination of inorganic mercury in natural waters. Anal. Chim. Acta., 62, 200-203.

Topping, G., Pirie, J.M., Graham, W.C. and Shepherd, R.J. 1975 An examination of the heavy metal levels in muscle, kidney and liver of saithe in relation to year class, area of sampling, and season. ICES C.M.1975/E:37.

Table 1

Concentrations of mercury in cage mussels after various exposure times

POSITION	EXPOSURE PERIOD (DAYS)				
	20	55	106	153	
A	0.19	0.14	0.26	0.41	µg Hg/g wet wt
	2.00	2.21	1.95	3.43	µg Hg/mussel
B	0.13	0.12	*	*	µg Hg/g wet wt
	1.34	1.62	-	-	µg Hg mussel
C	0.10	0.08	0.13	0.15	µg/g wet wt
	1.04	1.19	1.21	1.74	µg/mussel
D	0.09	0.08	0.16	0.18	µg/g wet wt
	1.08	1.15	1.58	1.96	µg/mussel
E	0.06	N.D.	0.09	0.19	µg/g wet wt
	0.79	N.D.	0.98	2.16	µg/mussel

Control mussels (prior to exposure) 0.03 µg Hg/g wet wt

0.30 µg Hg/mussel

* The cage at position B was lost after two months

N.D. Not determined

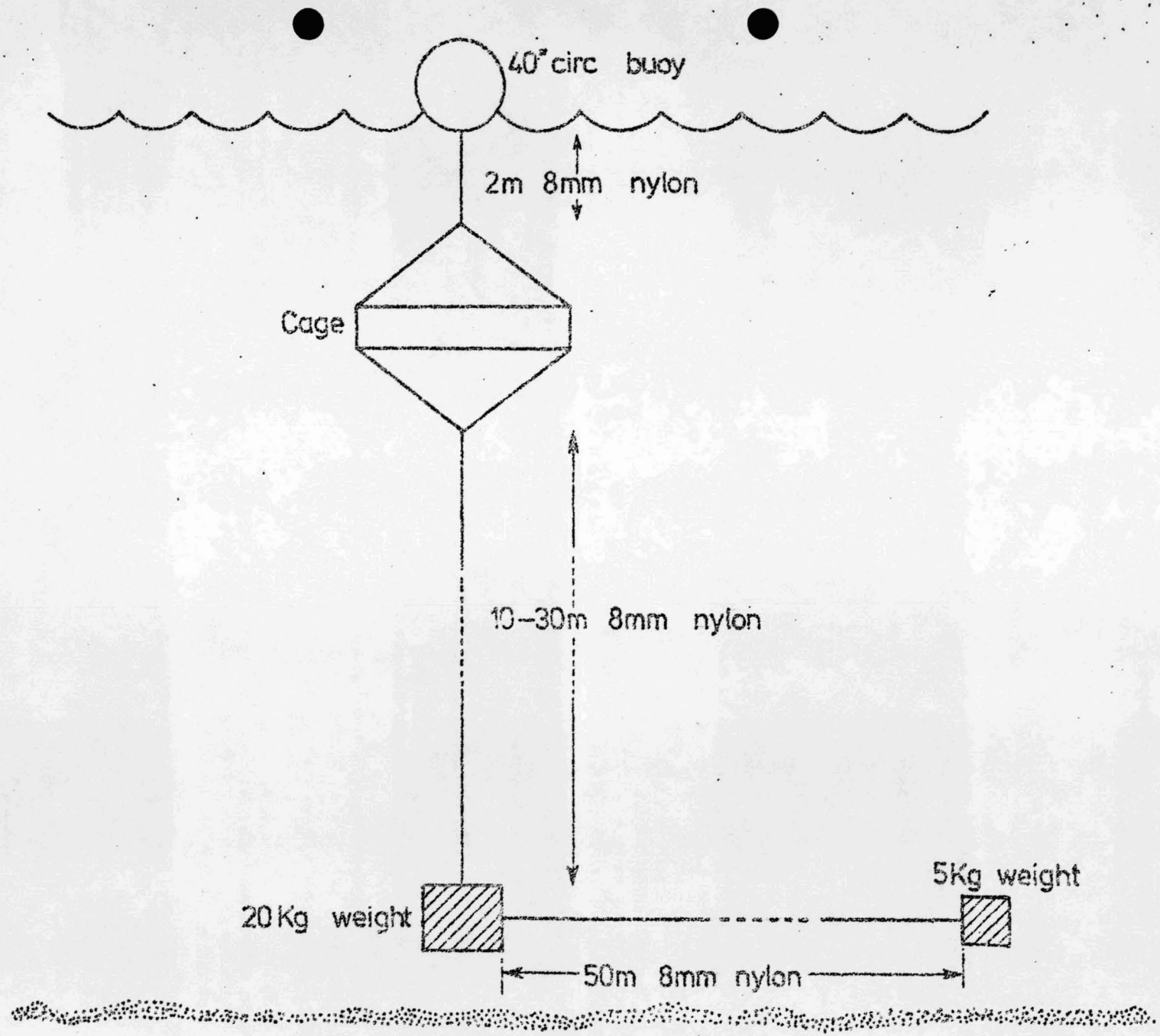

Figure 1. Mussel cage mooring system.

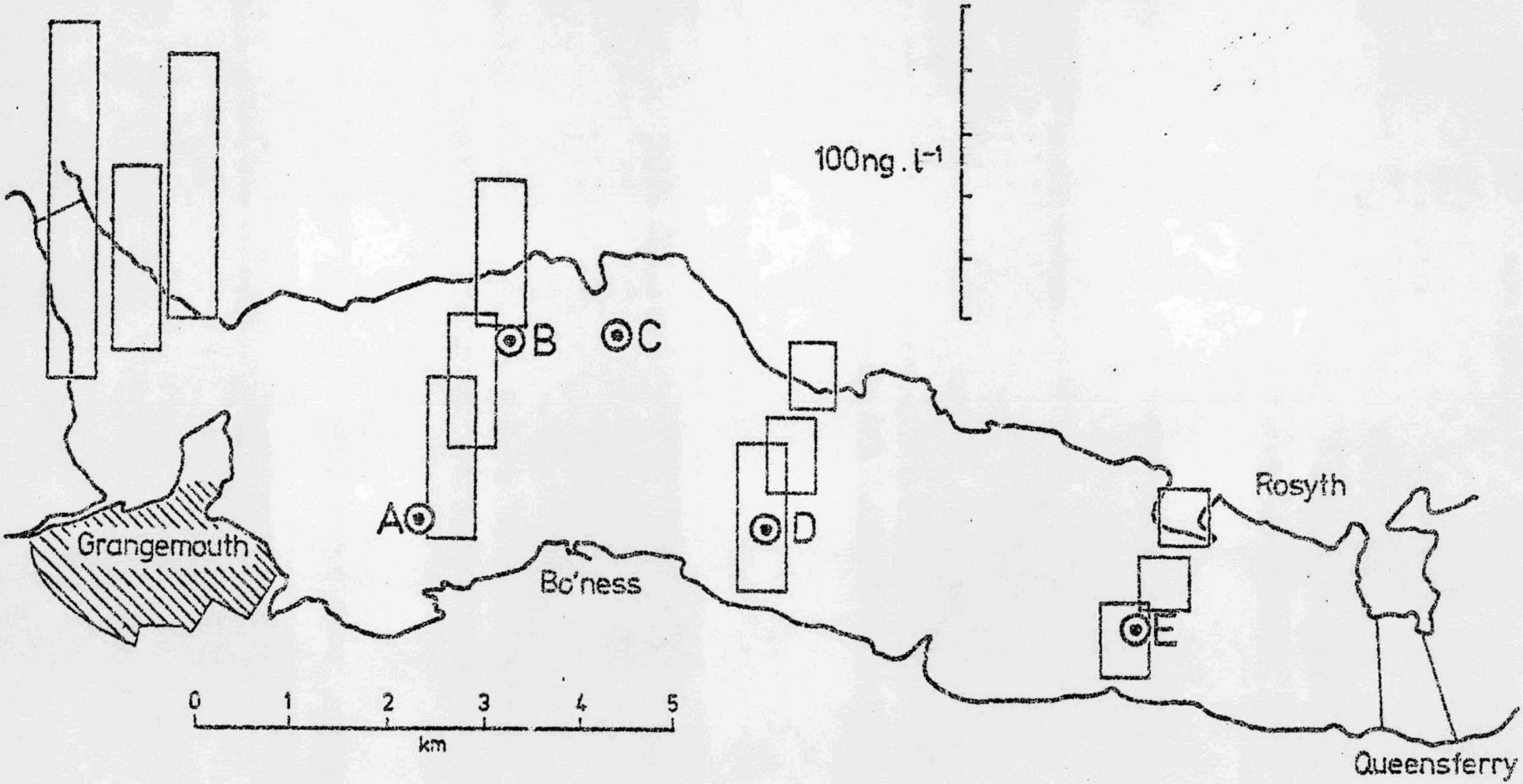

Figure 2. The mean concentration of mercury in the water of the Firth of Forth at twelve sampling points. Mussel cage mooring positions (A-E) are also shown.

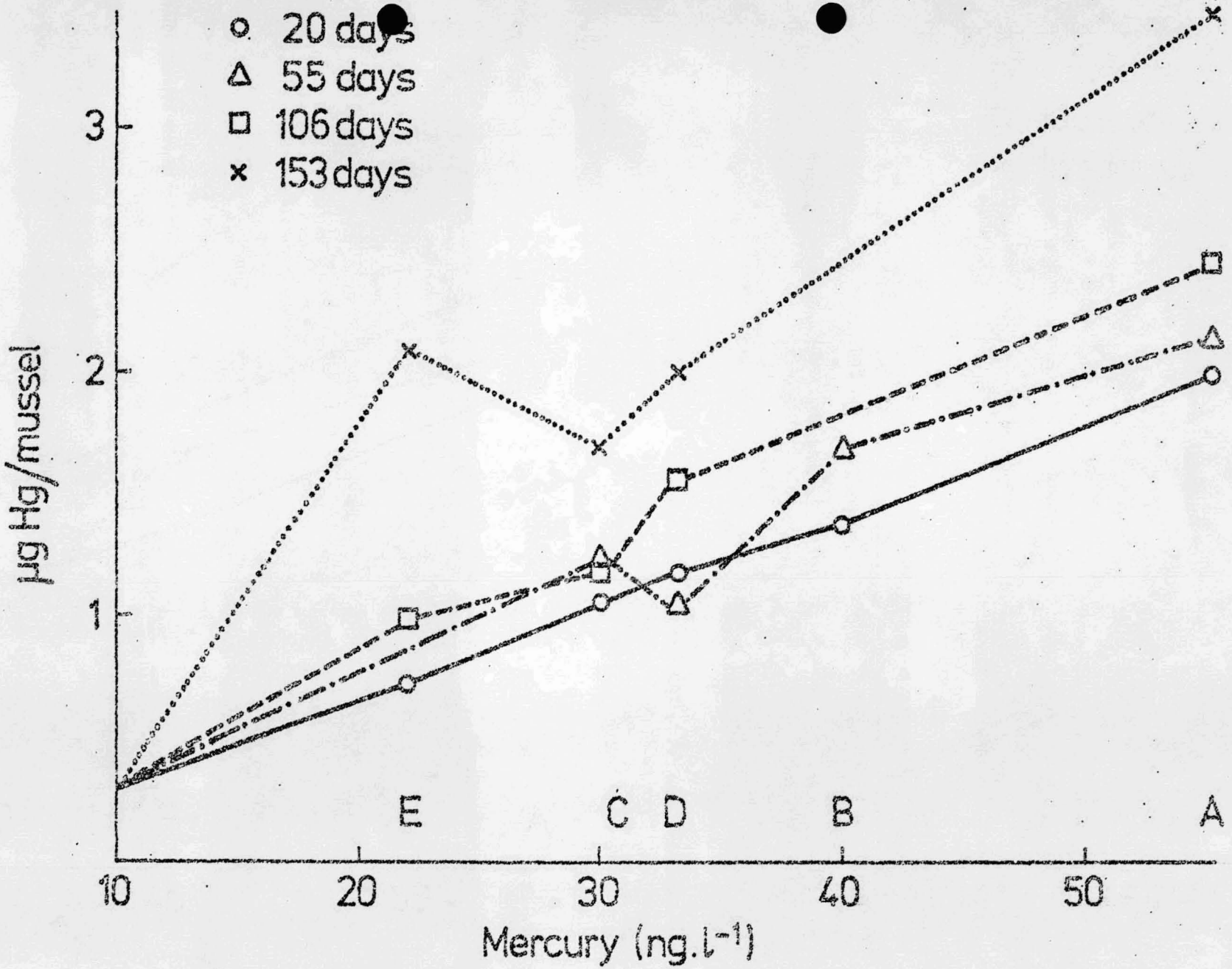

Figure 3. The mercury content of caged mussels after various exposure times. The cage positions (A-E) are plotted at their estimated mean water mercury concentration.