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May 1981

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BIOINDICATORS FOR MONITORING RADIOACTIVE POLLUTION OF THE MARINE ENVIRONMENT

Experiments on the feasibility of Mytilus as a bioindicator in estuarine environments - with some comparisons to Fucus

Henning Dahlgaard

Abstract. Mussels (Mytilus edulis) are globally used as bioindicators for pollution of coastal and estuarine environments by metals and radionuclides. The aim of this work has been to improve the use of Mytilus edulis as a bioindicator by gaining knowledge on its accumulation and loss of certain radionuclides (^{65}Zn, ^{57}Co, ^{54}Mn, ^{51}Cr, ^{59}Fe and ^{134}Cs) under different field-comparable environmental conditions.

A laboratory set-up in which natural concentrations of suspended phytoplankton are kept constant for weeks was evolved for the accumulation experiments with mussels. It is argued that continuous feeding at very low (natural) levels is necessary if field-comparable experiments are to be performed with suspension feeding bivalves.

Accumulation via food intake was studied by comparing experiments with different concentrations of contaminated phytoplankton (Phaeodactylum tricornutum). This comparison showed no effect of varying the phytoplankton concentration.

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Decreasing the salinity and increasing the temperature elevated the influx (initial rate of accumulation) of the radionuclides.

During one year excretion experiments were performed by weekly wholebody countings of laboratory contaminated mussels which had been re-introduced in their natural environment. A seasonal effect on the biological half life was detected for ^{65}Zn.

It is concluded that mussels are useful bioindicators provided the variability due to environmental factors, e.g. season and salinity, is taken into consideration.

Brown algae, expecially Fucus vesiculosus, were used to trace the controlled liquid discharges (mainly ^{60}Co, ^{58}Co, ^{65}Zn, ^{54}Mn and ^{110m}Ag) from two Swedish nuclear power plants (Barsebäck and Ringhals). Fucus showed higher accumulation than Mytilus.

Transfer factors between discharge and sample from a specified location are presented. It is argued that these transfer factors may be useful in estimating the magnitude of an uncontrolled accidental release of activity and its transport to man.

INIS descriptors: AQUATIC ECOSYSTEMS; BIOLOGICAL HALF-LIFE; BIOLOGICAL INDICATORS; CESIUM 134; CHROMIUM 51; COBALT 57; CONTAMINATION; ESTUARIES; FUCUS; IRON 59; MANGANESE 54; MOLLUCS; RADIOECOLOGICAL CONCENTRATION; RADIONUCLEDE KINETICS; ZINC 65.

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ABBREVIATIONS AND UNITS

Bq: becquerel: the unit of radioactivity in the SI-system = 1 disintegration s^{-1} (= 27 pCi)

Ci: curie: the "old" unit of radioactivity used in this report = 3.7×10^{10} Bq (= 2.22×10^{12} dpm)

m: milli: 10^{-3}; mCi = 37×10^{6} Bq
μ: mikro: 10^{-6}; μCi = 37×10^{3} Bq
n: nano: 10^{-9}; nCi = 37 Bq
p: pico: 10^{-12}; pCi = 0.037 Bq

CR: concentration ratio: $\frac{\text{pCi g}^{-1}\text{ of organism}}{\text{pCi ml}^{-1}\text{ of water}}$ after a specified period of accumulation

CF: concentration factor: CR at steady-state

SD.: standard deviation: $\sqrt{\frac{\Sigma(\bar{x}-x_i)^2}{(n-1)}}$

SE.: standard error: $\sqrt{\frac{\Sigma(\bar{x}-x_i)^2}{n(n-1)}}$

In the significance test the following symbols were used:

* : probably significant (P > 95%)
** : significant (P > 99%)
***: highly significant (P > 99.9%)

In Appendix III the following symbols were used for counting errors:

A: relative standard deviation 20-33%
B: relative standard deviation >33%, such results are not considered significantly different from zero activity

1. GENERAL INTRODUCTION

The use of bioindicators in monitoring several pollutants in aquatic environments is often described (see e.g. Philips 1977a; Goldberg et al. 1978). In this context "bioindicators" are organisms whose radionuclide or stable metal contents are used to indicate the level of radioactive or trace metal pollution in the sampling area. Several reasons for using bioindicators in stead of water samples can be mentioned: 1) An integration of fluctuating levels is made over a period of time, 2) due to accumulation, the limit of detection is lowered, 3) an estimation can be made of transfer to humans via edible organisms and 4) there is the highest sensitivity for the most biologically available physicochemical species, i.e., if a radionuclide exists on a certain form, which is not accumulated in the bioindicator, it is - ideally - neither accumulated in species used for human food, and is thus less important than if it had existed on a highly available form.

However, the period of integration and the rate of accumulation and loss may vary, e.g. with season and location. Such variations have often been stressed (cf. the discussion), but much work is to be done before the mechanisms are so well understood that they can be accounted for in bioindicator programmes. Furthermore, if the bioindicator is used to map the distribution of total concentrations in water, irrespective of chemical and physical form and potential transport to man, the above mentioned point 4 is to be considered as a possible source of error.

Irrespective of the relatively limited knowledge on different bioindicators' ability to concentrate different pollutants and on the variation of their accumulation with e.g. season, location and salinity, or, in other words, irrespective of lacking knowledge of the radioecological sensitivity and variability (cf. Aarkrog 1979), bioindicators are already widely used to identify polluted areas. The American "Mussel Watch" (Goldberg et al. 1978) is perhaps the

most widely known example, but all over the world several other projects are utilizing the common mussel, Mytilus edulis, as a bioindicator. Jørgensen (1975a) stressed the importance of field-comparability in laboratory experiments on suspension feeding and regretted that this point has often been neglected. The lack of field comparability is even more obvious in experiments on metal accumulation by suspension feeding mussels, conducted without feeding and without control of filtering activity (cf. Chapter 2.4.1.).

The idea of the present work has been primarily to establish a laboratory set-up in which food conditions are made relatively field-comparable and in which mussels show natural filtering activity; and secondly then to improve the available knowledge of accumulation and loss of certain radionuclides by Mytilus edulis in experiments which are as field-comparable as possible with respect to food, filtering activity and metal concentrations. Furthermore it was intended to evaluate the quality of the laboratory work and to gain experience in the practical use of bioindicators by running a field programme near two Swedish nuclear power plants Barsebäck and Ringhals.

2. EXPERIMENTS ON ACCUMULATION AND LOSS OF ^{65}Zn, ^{57}Co, ^{54}Mn, ^{51}Cr, ^{59}Fe AND ^{134}Cs BY THE MUSSEL MYTILUS EDULIS

2.1. Introduction

The common blue mussel, Mytilus edulis, is a sedentary suspension feeder. It's food consists of particles above ~1 µm, e.g. phytoplankton cells, which it filters out of huge volumes of water (cf. Chapter 2.4.1). The water to be filtered is transported through the large and highly specialized gills due to ciliary movements, and the gills are also responsible for the particle retainment and the transport to the mouth (Jørgensen 1966).

The sedentary habit and the filtering activity are important aspects when selecting Mytilus as a bioindicator organism. The tendency to accumulate trace elements is also important although other bivalves show higher concentrations (Brooks and Rumsby 1965, Goldberg et al. 1978, Harris et al. 1979). However, the reasons why Mytilus has been chosen as an international bioindicator in stead of other bivalves is, that it is nearly globally distributed except in tropical areas, it is commonly found in very dense populations in coastal waters and it is found far into estuarine areas and even in metropolitan harbours.

Another point sometimes mentioned is that Mytilus is a suitable laboratory animal with relatively limited demands. This is, however, only partly true. As will be further elucidated in the discussion, nearly all previously published radionuclide and metal accumulation experiments with mussels have been conducted without intended feeding, and if the mussels are fed, it is not done in a field-comparable way. The ease with which mussels are brought to survive in long-term laboratory experiments has led to numerous results. However, as a response to poor laboratory conditions mussels may stop the water transport and even close the valves for periods of time leading to results without interest for natural field conditions.

In the evolved set-up, described in Chapter 2.2.4 and Appendix I, the mussels are continuously fed low levels of phytoplankton, and

their natural filtering activity is demonstrated by measuring the rate of clearance. This set-up has been used to elucidate the effect of different levels of food, temperature and salinity on the initial rate of accumulation of 6 radionuclides by Mytilus edulis.

Loss studies should be made over very long periods of time, at least several months, in order to separate the very slow compartments, which are indeed the most interesting from a bioindicator point of view. As mentioned above, it is not easy to maintain mussels under natural conditions in long term laboratory studies, and most experiments on loss of radionuclides from mussels are probably not comparable to field conditions. In the study described in this report, whole body loss measurements during one year were made on mussels contaminated in the laboratory and reintroduced to their natural environment (Appendix II and Chapter 2.2.6).

2.2. Materials and Methods

2.2.1. Sea Water

Sea water for the experiments was collected below the halocline in the Kattegat near Hesselø (56°10'N, 11°47'E). Salinity was 28-34°/oo as measured by titration with $AgNO_3$ or densitometric. The water was stored in 50 l polyethylene containers with open lids at approximately 5°C. Initially pH decreased to about 6 and increased again to 7-8 before use. Immediately before use the sea water was diluted to the desired salinity with demineralized (ion-exchanged) or glass-distilled water and if the pH was below 7.7, it was adjusted to 7.8-8.0 with NaOH.

Throughout the experiments the total radionuclide level was measured by evaporating 5.00 ml samples of experimental sea water on aluminium trays for γ-spectrometric analysis (Chapter 2.2.7).

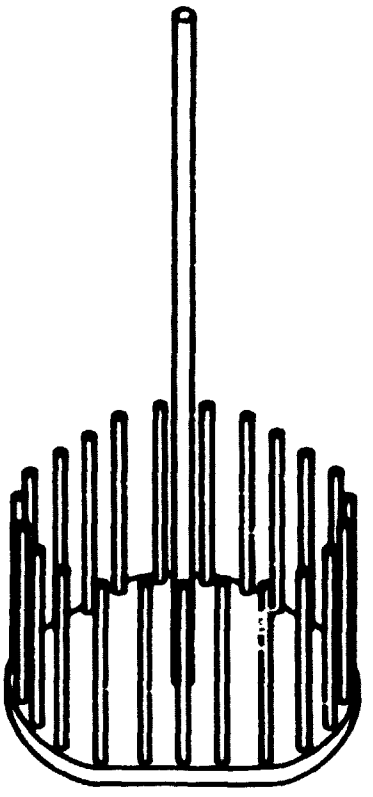

Figure 2.2.2.1. Perspex basket on which mussels were attached by byssus threads during the acclimation and experiments. Three baskets fitted into the mussel aquarium (Figure 1 in Appendix I).

2.2.2. Mussels

Common blue mussels (Mytilus edulis) with shell lengths of 3-4 cm were collected in Roskilde Fjord from the Risø-pier, at salinities of 14-15°/oo (cf. Table 2.2.8.2). It was the intention to conduct all the experiments with these animals, but this appeared to be impossible as they stopped filtering at the low salinity (8°/oo). Experiments at 8°/oo are thus conducted with animals from the southern part of Øresund collected near Rødvig at salinities of 9-10°/oo.

The water temperature at sampling varied between 3 and 20°C. Epifauna and byssus threads were removed from the shells, and the mussels were transported to the laboratory in sea water from the

sampling location. Prior to the experiment, the animals were "acclimated" to experimental temperature and salinity for 3-4 days. During the acclimation, 6-10 animals on a Perspex basket (Figure 2.2.2.1) were immersed in 1 l of aerated experimental sea water, which was changed 1-2 times pr. day. The animals had attached themselves to the Perspex baskets with byssus threads before experiments started, and was not removed from it until the time of sacrificing. Three baskets fitted into the mussel aquarium of the set-up (see Figure 1 of Appendix I). Before they were sacrificed, the mussels were washed in running demineralized water and opened by cutting the posterior adductor muscle. The open animals were quickly washed and soft tissues were removed from the shells. The soft parts were dried at 105°C in glass vials for γ-counting. Fresh and dry weight of soft parts and dry weight and length of shells were recorded (cf. Table 2.2.8.2).

Stable metals were not measured during the experiments, but levels of Zn, Mn and Fe in animals from the two locations were not atypical compared to the rest of Denmark (location 41 and 35 in Philips 1977c and 1978).

2.2.3. Supply of phytoplankton cells: Chemostatic Culture

The supply of algal cells was provided from a chemostatic culture of Phaeodactylum tricornutum. Stock cultures were kindly delivered by Dr. Grethe Møller Christensen, Marine Biological Laboratory, Helsingør.

The culture was reared in 10 l glass flasks (Figure 2.2.3.1) and kept at constant temperature (16-17°C) in a cooling bath. The algae were continuously illuminated by 7 fluorescent light tubes (Philips TL 20W/33). The spectral composition of this light source is equivalent to common coastal water types at a depth of 6 m except for some additional radiation in the interval 400-500 nm (Steemann-Nielsen and Willemoës 1971). The light intensity was approximately 7 k lux at the surface of the culture flask as measured by a "H & B Beleuchtungmesser EBLX 3". The chemostat was aerated and stirred by a constant flow of sterile (0.22 µm Millipore filtered) compressed air bubbling slowly through the culture.

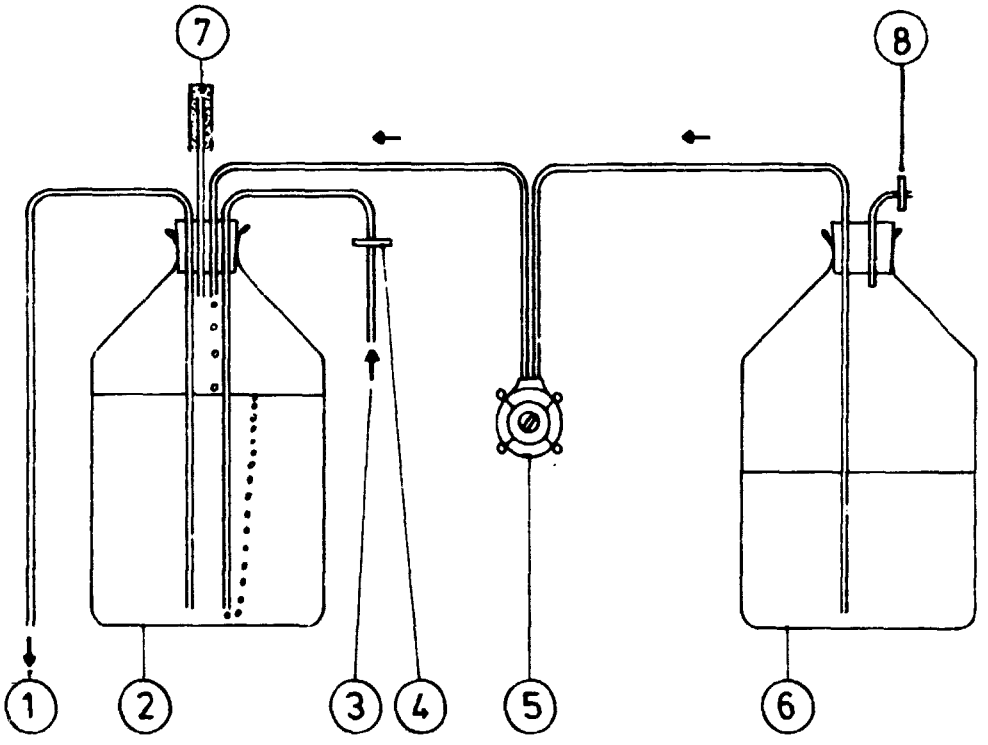

Figure 2.2.3.1. Chemostatic set-up for phytoplankton culturing. Medium (6) is continuously pumped into the culture (2) by a peristaltic pump (5). The culture is aerated by 0.22 µm Millipore-filtered compressed air (3,4). A sterile air vent (7) and air inlet in the medium flask through a 0.22 µm Millipore filter (8) prevents airborn infections. The culture flask is directly connected to the mussel feeding apparatus by sterile tubing (1) through a peristaltic pump.

The algal medium used was sterile N- and P-enriched stored natural sea water. This simple medium, only providing relatively slow growth but obviously supporting the basic requirements of Phaeodactylum tricornutum, was used in order to minimize additions of trace metals and complexing agents to the mussel experiments. Sea water for the medium was collected and stored as described in Chapter 2.2.1. After dilution with glass-distilled water to the desired salinity (20°/oo), the sea water was filled into 10-ℓ glass medium flasks. If the pH was below 7.7 it was adjusted to 7.8 - 8.0 with NaOH. The medium was sterilized by two heatings to approximately 75°C in a water bath with an interval of one day. After cooling, 1 g $NaNO_3$ and 0.2 g Na_2HPO_4 were added as sterile solutions. The medium was continuously pumped into the chemostat by a peristaltic pump (Cole Parmer Masterflex).

Only glass and silicone rubber was used for the chemostatic set up. These materials have been found non-toxic to marine phytoplankton cultures, whereas several other materials used in laboratory equipment are more of less toxic (Bernhard and Zattera 1970). All equipment used was sterilized by autoclaving, and care was taken not to contaminate the chemostat when siphoning off culture. During mussel experiments with the turbidostatic set-up, the peristaltic pump for algal injection was connected directly to the culture through sterile silicone tubing. This connection did not lead to contamination as the peristaltic pump (Cole Parmer Masterflex) tightly closed the tubing. New sterile tubing and clean sterile culture flasks were provided when wall-growth or precipitation of algae occurred.

The growht rate of the culture was relatively slow. The mean culture generation time during steady state growth:

$$\tau = \frac{1}{\omega}$$

where ω is rate of dilution (d^{-1}) was calculated during one year of culturing to 15.4±1.3 (SE, n=14) days. The corresponding mean cell generation time calculated as

$$T = \tau \ln 2$$

was thus 10.7 days. The culture generation time and cell generation time were calculated as indicated by Kubitschek (1970).

The culture and medium were contaminated by the same concentrations of radionuclides as the sea water in the mussel experiments (Table 2.2.7.1). At some occasions it was observed that the addition of radionuclides including carrier trace metals to a slowly growing culture accelerated the growht rate for some days, implying that one or more trace metals have been present in minimal amounts. However, experiments conducted by D'Elia et al. (1979) do not demonstrate a vitamin or trace metal requirement in excess of that present in N- and P-enriched natural sea water. Unlike other cultured diatoms, not even a silicium requirement could be demonstrated. This can be explained by the cell wall of

Phaeodactylum tricornutum which is almost entirely organic (Borowitzka and Volcani, 1978).

Some authors considers Phaeodactylum tricornutum a poor bivalve food and make an effort to avoid it in marine farming (D'Elia et al. 1979), while Wilson (1978) claims it to be a desirable food species in bivalve hatcheries. This is further supported by Riisgård and Randløv (1981) who showed Phaeodactylum tricornutum was able to support growth of Mytilus edulis successfully in the laboratory.

2.2.4. The mussel feeding apparatus - a turbidostatic set-up

The evolution of an extremely sensitive turbidostatic set-up designed for maintaining low and relatively constant levels of phytoplankton in laboratory experiments with mussels was one of the primary aims of this work. The idea of making this set-up was inspired by discussions with Riisgård, who constructed an improvement of Winter's (1973) apparatus (Riisgård and Møhlenberg 1979). The benefits of using this type of set-up in pollutant accumulation studies will be dealt with in Chapter 2.4.1 and Appendix I. It should only be underlined that the set-up facilitates continuous feeding at low levels which assures an optimal digestion of the contaminated food particles, and that clearance rates are measured continuously, thus obtaining an index of animal condition.

The design and performance of this set-up has been published (Appendix I), and will, therefore, be only briefly described here.

The principle of the apparatus is a turbidostatic regulation of the algal concentration. The experimental sea water is continuously pumped through a 60-cm "cuvette" where the turbidity is monitored by a photocell system. The turbidity is kept constant by automaticly injecting phytoplankton cells from a chemostatic culture (Chapter 2.2.3). By placing mussels in a small volume of water which is exchanged several times per minute, recirculation of already filtered water is effectively prevented (see Appendix

I). Thus, in this set-up, the measured rate of clearance is identical to the mussels' pumping rate. Clearance rates are calculated from particle countings (Chapter 2.2.5) of the experimental water and the phytoplankton culture throughout the experiments and from the recorded amount of phytoplankton culture injected during the time in calculation (see Appendix I).

Figure 2.2.4.1 shows selected parts of a recorder track from one of the experiments. Initially (Section A), the turbidity decreases sharply due to the mussels' filtering of suspended matter in the sea water. The apparatus cannot operate properly unless "background" turbidity is constant. A decrease in the background turbidity results in an increase in the phytoplankton concentration, as the apparatus maintains a constant total turbidity by regulating the injection of phytoplankton culture. At zero time, the automatic algal injection is started after the desired concentration is made up. During the rest of the experiment the actual phytoplankton concentration is repeatedly measured by a Coulter Counter (Chapter 2.2.5), and when deviations from the desired level is noted, the turbidity controller is adjusted. Often a decrease in algal concentration is observed indicating an increase in the background turbidity. This may be explained by resuspension of faeces or by coloring from mussel or phytoplankton excretion products. In an ultimate 3-week experiment (see Appendix I), this was equilibrated by continuously renewing the water at a rate of 4.6 liters of the 30 liters per day. The function of the apparatus will probably be further improved by applying a more efficient settling aquarium for trapping the faecal particles. The differences of Sections B and C illustrates the flexible function of the apparatus. At the high total clearance rate (25 animals), the injections are much more numerous than at a lower clearance rate (4 animals), and the algal concentration is thereby kept constant. After removal of the last animals, the turbidity was slowly increasing (Figure 2.2.4.1 - D) partly due to resuspension or cell division of the plankton algae. Based on the Coulter Counter measurements, a total negative "clearance" of -4 ml/minute was measured. As compared to the 123 ml/min measured with 4 animals in the set-up, this is without importance. Settling or resuspension should however be considered if the apparatus is used with extremely low total clearance rates.

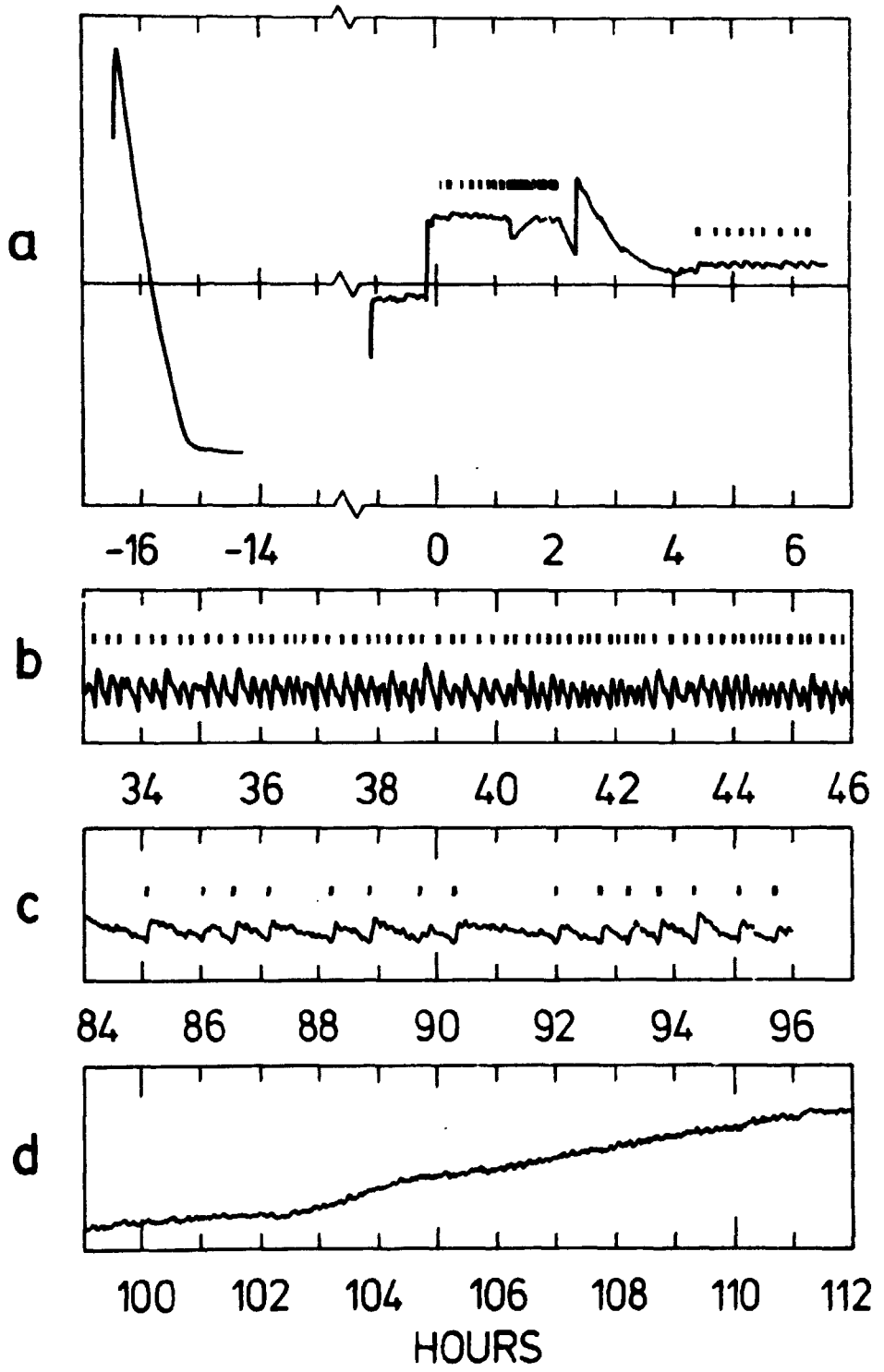

Figure 2.2.4.1. Mussel feeding. Selected parts of a pen-recorder track showing turbidity variations in the water throughout an experiment. Increasing turbidity upwards (relative units). The sensitivity of the recorder is 5 times higher in b-d than in a. Rectangels above the track show periods of phytoplankton injection. a: 30 mussels are added to the experimental water at -16.5 hours. At zero hours, algal culture and radionuclides are added. 1-4 hours: disturbances followed by adjusting the turbidity level. b: 25 animals, high total rate of clearance: 580 ml/min. c: 4 animals, low total rate of clearance: 123 ml/min. d: no animals, after termination of the experiment turbidity slowly increased; a negative "clearance" of -4 ml/min. was calculated from particle countings.

The sensitivity of the apparatus is very high. In one experiment the sensitivity, expressed as the difference in algal concentration before the injector pump started and after it stopped, was 106 ± 19 (SE, n=7) cells per milliliter corresponding to 1.3 x 10^{-9} g organic dry substance per milliliter (Appendix I). In this experiment the limit of performance was thus not determined by the electronic units but rather by changes in background turbidity. As suggested above, this might be improved. However, the current set-up can operate at sufficiently low levels to maintain the mussels at or even below the maintenance ration (Chapter 2.4.1). The apparatus and set-up are therefore significantly improved compared to similar ones described by Riisgård and Møhlenberg (1979) and Winter (1973).

2.2.5. Particle counting and filtration

The Coulter Counter

The concentration of phytoplankton cells in the chemostatic culture and in the turdibostatic set-up during the mussel experiments was determined by an electronic particle counter, a "Coulter Counter" model Z_B. A counting tube with an orifice of 50 µm was found convenient for the size of Phaeodactylum tricornutum - approximately 25 $(\mu m)^3$.

During the countings, a lower and higher discriminator level was used to minimize the number of non-algal particles counted. Countings of the phytoplankton culture were performed on a 1:25 dilution with 0.45-µm Millipore-filtered sea water of the same salinity. The diluent was used for background countings, which was very low: usually 2-10 particles per milliliter. The phytoplankton concentration in the mussel experiments was determined directly by measuring 0.5 ml of the suspension. It is impossible to correct for non-algal particles, e.g. detritus, in the size-range covered. However, as countings of experimental water with mussels and without algal injection showed considerably lower values than the lowest algal concentration maintained, and as the size interval measured is effectively filtered by the mussels, this has probably not led to important faults. Furthermore, the faults would result in an overestimation of the low algal con-

centrations especially, that is, the difference in algal concentration between the high-concentration and low-concentration experiments would be underestimated. As the experiments showed no effect of the algal concentration used (Chapter 2.2.3), this is without significance.

Millipore-filtration

The fraction of particulate activity in the experimental sea water was determined by filtration on 0.45-µm Millipore filters (type HA). One liter of water was filtered through 2 Millipore membranes by suction. The filters were washed with 25 ml of sea water and the difference in radionuclide concentration between the two filters was used as a measure of the particulate activity. This doubble filter technique has been used succesfully in radiocaesium experiments to distinguish between activity associated with phytoplankton cells and activity adsorbed on the filters (Dahlgaard 1977). As the other nuclides included in this work has a greater tendency to be associated with other particles (cf. Chapter 2.4.2) this method is probably not feasible for the measurement of these nuclides in phytoplankton. However, it has been assumed that the technique is sufficient to distinguish between activity associated with total particulate matter retained by the filter and activity adsorbed on the filter.

2.2.6. Semi-field Experiment on Loss of the 6 Radionucledes by Mytilus

As the method and results from this experiment have been published elsewhere (see Appendix II), only a brief outline will be given here.

Some mussels from 3 accumulation experiments were placed singly in small perforated Perspex tubes (Figure 2.2.6.1 and Appendix II) and wholebody-counted. The rate of loss was followed during 3 months for 12 animals, and during a whole year for 4 of them. During the loss experiment the mussels persisted in their cages which were placed in the natural estuarine environment from which the animals were collected prior to contamination, i.e. the Risø pier in Roskilde Fjord.

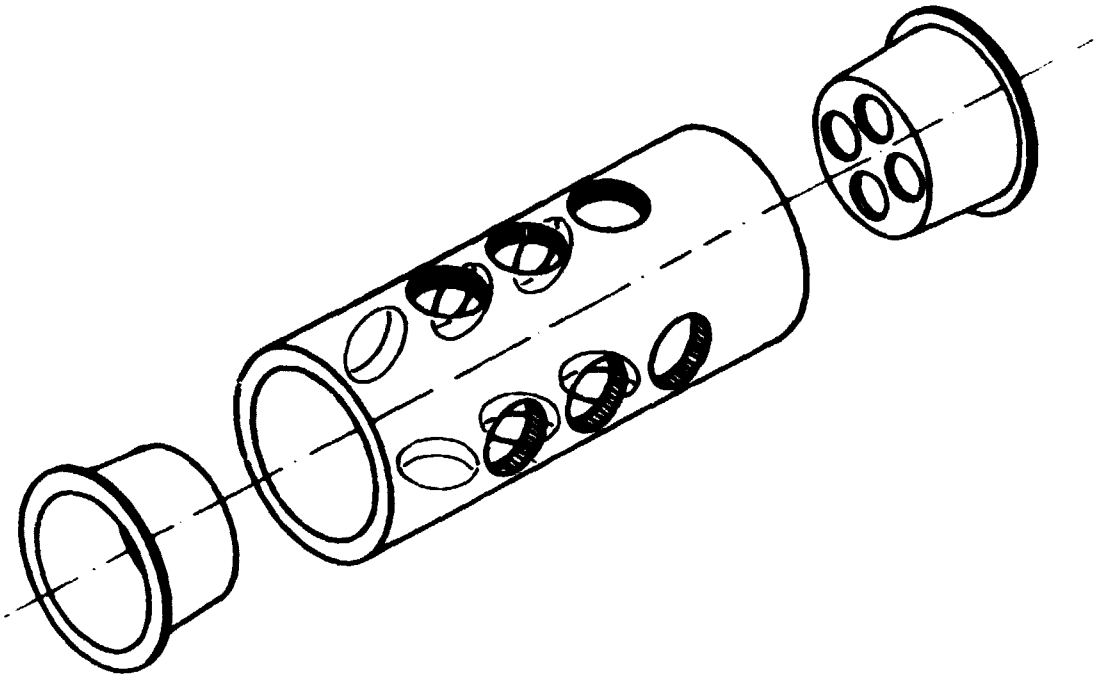

Figure 2.2.6.1. Perforated Perspex tube in which one mussel was placed during the one-year loss experiment in the field. Overall length is 75 mm and inner diameter 24 mm. Mussels were not removed from the tube during whole-body countings.

During the wholebody countings the Perspex tubes with the animals were placed in a holder inside a γ-counting beaker and it was assured that the mussel always had the same position relative to the detector. Furthermore, the detector was not very sensitive for small changes in position of the mussel. During countings the animals were out of the water and the valves were closed. Salinity was very constant (14-15°/oo), but the temperature changed during the year between ~20°C and 0°C (cf. Chapter 2.3.4 and Appendix II).

2.2.7. Isotopes and γ-counting

In all experiments, radioisotopes of zinc, cobalt, manganese, chronium, iron, and caesium, as listed in Table 2.2.7.1, were added to the experimental sea water. Low levels of carrier were chosen in order not to elevate natural metal levels in the sea water significantly.

All 6 radionucledes were measured simultaneously by γ-spectrometry using a Ge(Li) detector and a 1024-channel analyser (Figure 2.2.7.1). All activity levels were decay corrected to the start of the experiments. The calculation of the activity in the samples was computerized (Lippert, 1978). Three geometries were used for the countings, each calibrated with solutions of all 6 nuclides. The one geometry used for the mussel soft parts and crushed shells was a 15-mm diameter glass γ-counting tube. The soft parts were dried in the tube. The other geometry was a flat 36-mm disc used for water and filter countings. For water countings, 5.00 ml of the water was pipetted into a thin aluminium tray and evaporated. A piece of lens paper prevented non-uniform evaporation patterns. The effective filter diameter of the 47-mm Millipore membrane filters was approximately 35 mm, and the calibration for the water trays was adequate for the filters too. The third geometry used for wholebody countings of

Table 2.2.7.1. Mytilus edulis laboratory experiments. Radionuclides and amount of carrier added to the experimental sea water. 0.1 ml of stock-solution in 0.01 - 0.1 M HCl was added per liter of sea water and per isotope.

Isotope	Chemical form	Physical half life, days	Specific activity, Ci/g of element (Approx.)	Experimental sea water: Nuclide concentration, pCi/l (Approx.)	Experimental sea water: Carrier metal added µg/l (Approx.)	Element in sea water.*) µg/l
^{65}Zn	$ZnCl_2$	243.8	2.6	1	0.4	4.9
^{57}Co	$CoCl_2$	270	10^4	2	0.0002	0.05
^{54}Mn	$MnCl_2$	312.5	28	1	0.04	0.2
^{51}Cr	$CrCl_3$	27.8	360	10	0.03	0.3
^{59}Fe	$FeCl_3$	44.6	5	2	0.4	2
^{134}Cs	CsCl	747.3	8	1	0.1	0.4 **)

*) *Reference:* Brewer, 1975.

**) Sea water with a salinity of 34 °/oo contains 3.8×10^5 µg/l of the chemically and physiologically similar K.

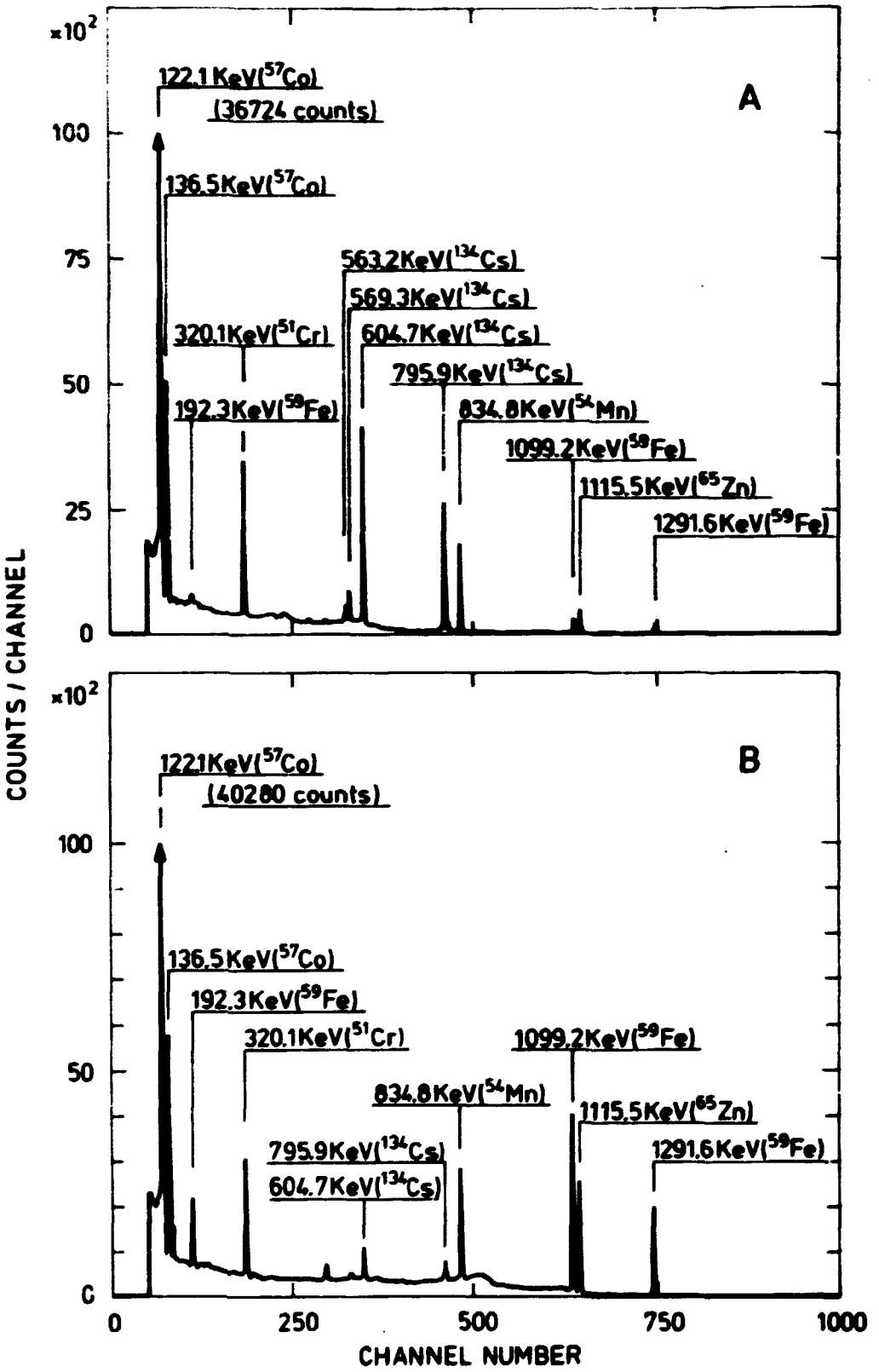

Figure 2.2.7.1. Two Ge(Li)-gamma spectra showing photon energies of the 6 radionuclides. A: water (5 ml) counted 129 minutes and B: mussel soft parts (128 mg dry) counted 66 minutes. Both are from experiment no. 790713.

live mussels is described in Chapter 2.2.6. Generally, counting times were chosen to assure counting errors (± 1 SD) of less than 5%. However, in a few countings errors for certain isotopes (mostly ^{134}Cs and ^{51}Cr) were up to ~20%.

2.2.8. Experimental Design and Statistical Analysis

The radionuclide accumulation experiments were designed as a 2^3 factorial experiment, that is 8 combinations of two levels of food, salinity and temperature. Each single experiment was planned as a determination of a 72-hour accumulation curve (see Chapter 2.3.3). Three to five mussels were sampled 2, 8, 24, 48, and 72 hours after radionuclides and contaminated phytoplankton was added. In addition, 6-10 mussels on one Perspex basket were placed in the experimental set-up 24 hours after time zero in order to evaluate effects of changes in the physicochemical state of the radionuclides during the first 24 hours in sea water. These mussels were sampled after 24, 48, and 72 hours of contamination, and as no differences were observed, the results were included in the main uptake curve (Chapter 2.3.3).

Before experiments started, the experimental plan depicted in Table 2.2.8.1 was designed. The "high" level of algal concentration is, in fact, a rather low concentration as compared to similar experiments (Winter 1973, Riisgård and Møhlenberg 1979).

Table 2.2.8.1. Planned experimental design. The 2^3 factorial experiment was subdivided in 1): a, b, c, abc and 2): 1, ab, ac, bc thus confounding the three-factor interaction against seasonal variation between the two blocks. (After Davies, 1963).

C: Temperature		15°C		5°C	
B: Salinity		20 °/oo	8 °/oo	20 °/oo	8 °/oo
A: Algal concentration	low	1	b	c	bc
	high	a	ab	ac	abc

The 8000 Phaeodactylum tricornutum cells per milliliter corresponds to approximately 10^{-7} g organic dry weight per milliliter, and this relatively low level was chosen in order not to exceed the concentrations where the mussels start producing pseudofaeces (Riisgård and Møhlenberg, 1979). The production of pseudofaeces would presumeably lower the uptake of activity from the phytoplankton and other particulate matter, and thereby make a comparison of the two phytoplankton concentration levels impossible. The low level was chosen as the lowest readily attainable, and is approximately 2000 cells per milliliter, i.e. one fourth of the "high" concentration. As discussed in Chapter 2.4.1 this low concentration is apparantly very near the maintenance ration for the mussels.

Salinity was chosen to be representative of the inner Danish waters which can be considered as part of a vast estuary between the Baltic (~8‰) and the North Sea (~34‰). A high salinity of 35‰, i.e. an atlantic salinity, would be relevant, but was considered too difficult with relation to water supply and collection of animals. Temperatures were chosen as a compromise between natural levels in the area, which oscillates between 0°C and approximately 20°C (Figure 2.2.8.1), and available laboratory equipment. A low temperature of approximately 0°C would be interesting and relevant, but was considered too difficult to attain. The data obtained were analyzed by three and two sided analysis of variance as described in Chapter 2.3.3.

The 8 combinations of two levels of food, salinity and temperature (Table 2.2.8.1) were planned to be run at least twice in order also to elucidate seasonal variations. As the performance of 8 experiments is time consuming, they were planned to be performed in two blocks with 4 experiments in each (Table 2.2.8.1), so only the three-factor interaction was "confounded" against the seasonal variation between the two sub-blocks, i.e. the single experiments were performed in a certain order assuring that a seasonal variation between the two sub-blocks would not affect the primary parameters investigated (food level, salinity, temperature) or two-factor interactions among them (Davies 1963).

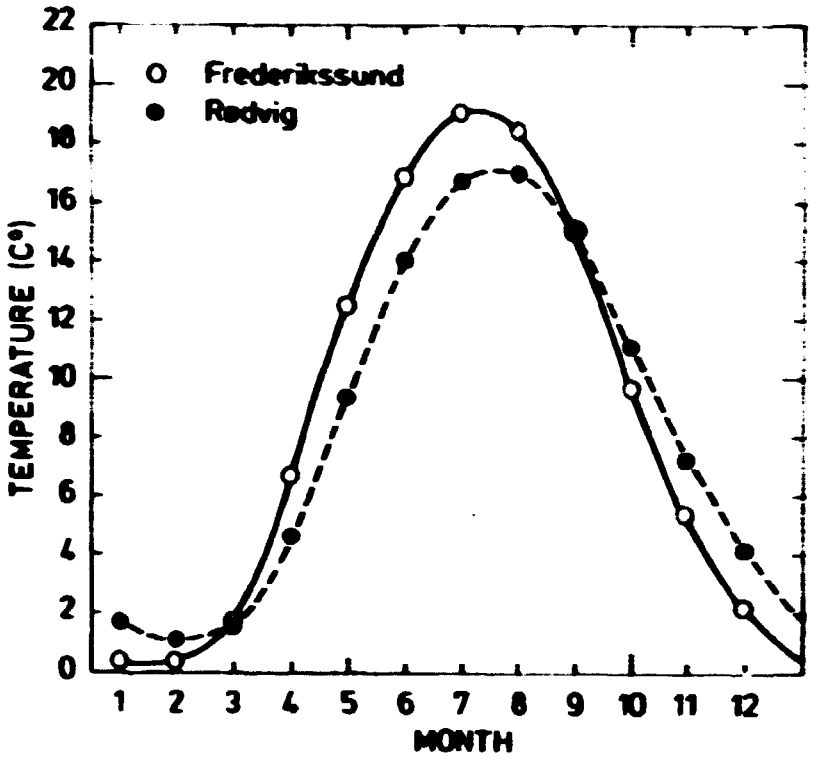

Figure 2.2.0.1. Surface water temperatures in Roskilde Fjord (Frederikssund) and at Rødvig. Grand monthly means for the period 1931-1960 (Det Danske Meteorologiske Institut 1976).

Table 2.2.0.2. Mytilus experiments, actual experimental design and basic data on the animals. The experimental number refer to the day of sampling. The 20 °/oo animals were collected in Roskilde Fjord at the Risø pier (16-15 °/oo) while the 8 °/oo animals are from Rødvig (9-10 °/oo). Error term: ± SE

Temperature		15°C			5°C		
Salinity		20 °/oo	20 °/oo	8 °/oo	20 °/oo	20 °/oo	8 °/oo
Experiment no:		790630	790805	781214	-	781008	790827
Low Algal concentration	Soft part dry weight, mg	195±7	203±8	29.3±2.2		119±6	75.1±4.1
	Soft part water contents, %	83.5±0.3	84.0±0.2	91.7±0.2		85.6±0.2	88.7±0.3
	Shell length, mm	35±0.5	38±0.6	25±0.5		33±0.3	30±0.5
	Shell dry weight, mg	779±29	1078±88	319±17		603±24	429±22
	Number of animals (n)	32	40	30		24	26
Experiment no:		-	781110	781201	790713	790708	790820
High algal concentration	Soft part dry weight, mg		124±5	18.8±1.6	171±7	218±8	59.6±4.5
	Soft part water contents, %		85.6±0.2	90.2±0.2	86.6±0.3	82.7±0.3	89.5±0.3
	Shell length, mm		33±0.6	19±0.6	35±0.6	36±0.5	28±0.6
	Shell dry weight, mg		669±32	165±9	755±38	758±29	427±36
	Number of animals (n)		24	35	28	64	29

However, the experiments turned out to be not that simple! All experiments with the low salinity (8°/oo) were lost because the animals sampled in Roskilde Fjord stopped filtering, and several other experiments failed, e.g. due to spawning. The problems with the low salinity were solved by sampling mussels in a low-salinity area (see Chapter 2.2.2), but this introduced a new "confounding", namely salinity effect against sampling location, i.e. a significant salinity effect could be explained alternatively as an effect of sampling location. The animals from the two locations differed in e.g. size and water content. This is shown in Table 2.2.8.2 which also depicts the actual experimental design.

In addition to these experiments, a single three-week experiment (No. 790718) was conducted at 10°C and 14°/oo salinity (Chapters 2.3.1 and 2.3.3). Animals for this experiment were sampled in Roskilde Fjord and had a soft parts dry weight of 170 ± 6 mg (SE, n=49) and a shell length of 35 ± 0.3 mm (SE, n = 49).

2.3. Results

Laboratory Experiments

2.3.1. Activity in Water and Particles

Figure 2.3.1.1A shows the variation in the total activity concentration of the 6 radionuclides in the experimental sea water during one of the ten accumulation experiments with Mytilus edulis. It is seen that, except for ^{134}Cs, the concentration is declining. The largest loss of activity from the water column is found for ^{51}Cr and ^{59}Fe, and the decline is most pronounced during the first day of the experiment. This is seen for all 10 experiments. A similar situation is observed in a single 3-week accumulation experiment (Figure 2.3.1.2A), where the 30 ℓ of experimental sea water was renewed continuously at a rate of 4.6 ℓ/day.

Due to the declining water activities, the concentration ratios for Mytilus during the accumulation experiments (2.3.3) are cal-

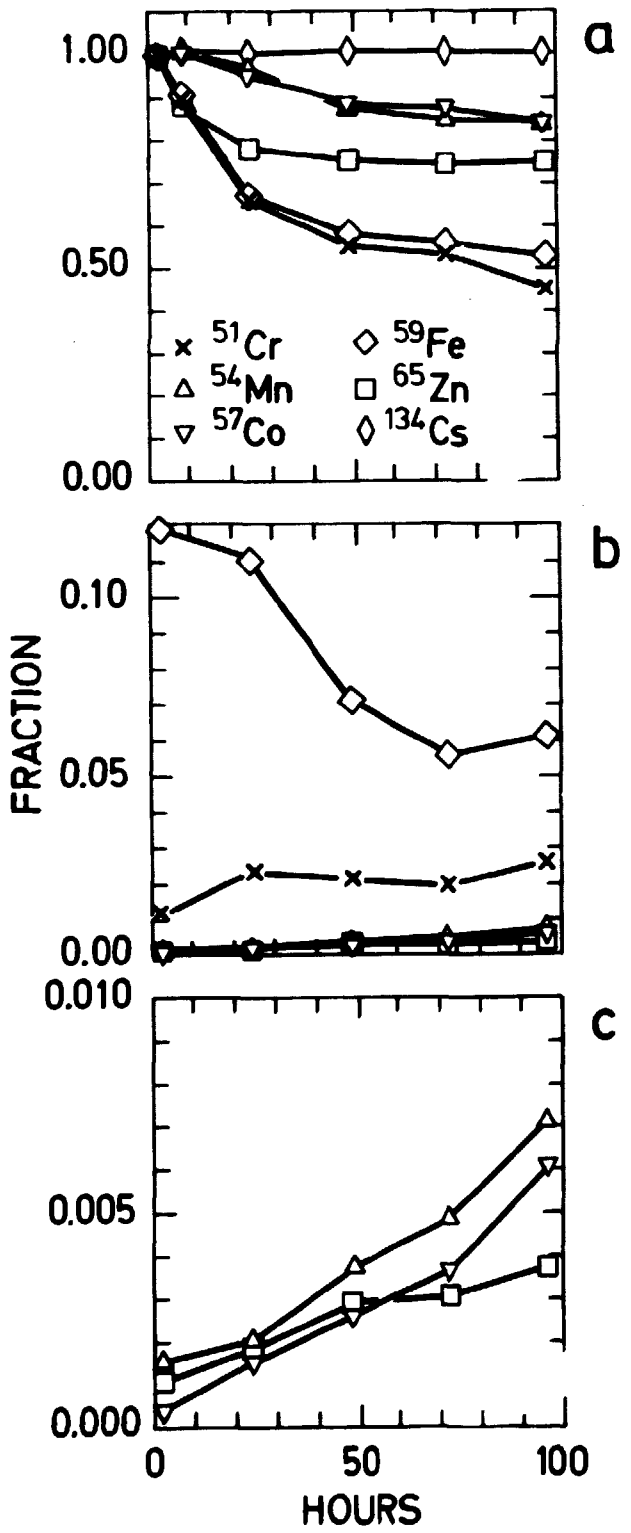

Figure 2.3.1.1. Mytilus edulis experiment 781201 (one of ten experiments). A: Total activity in water as fraction of initial activity. B and C: Particulate (0.45-µm) activity fraction. Compare with Figure 2.3.3.1.

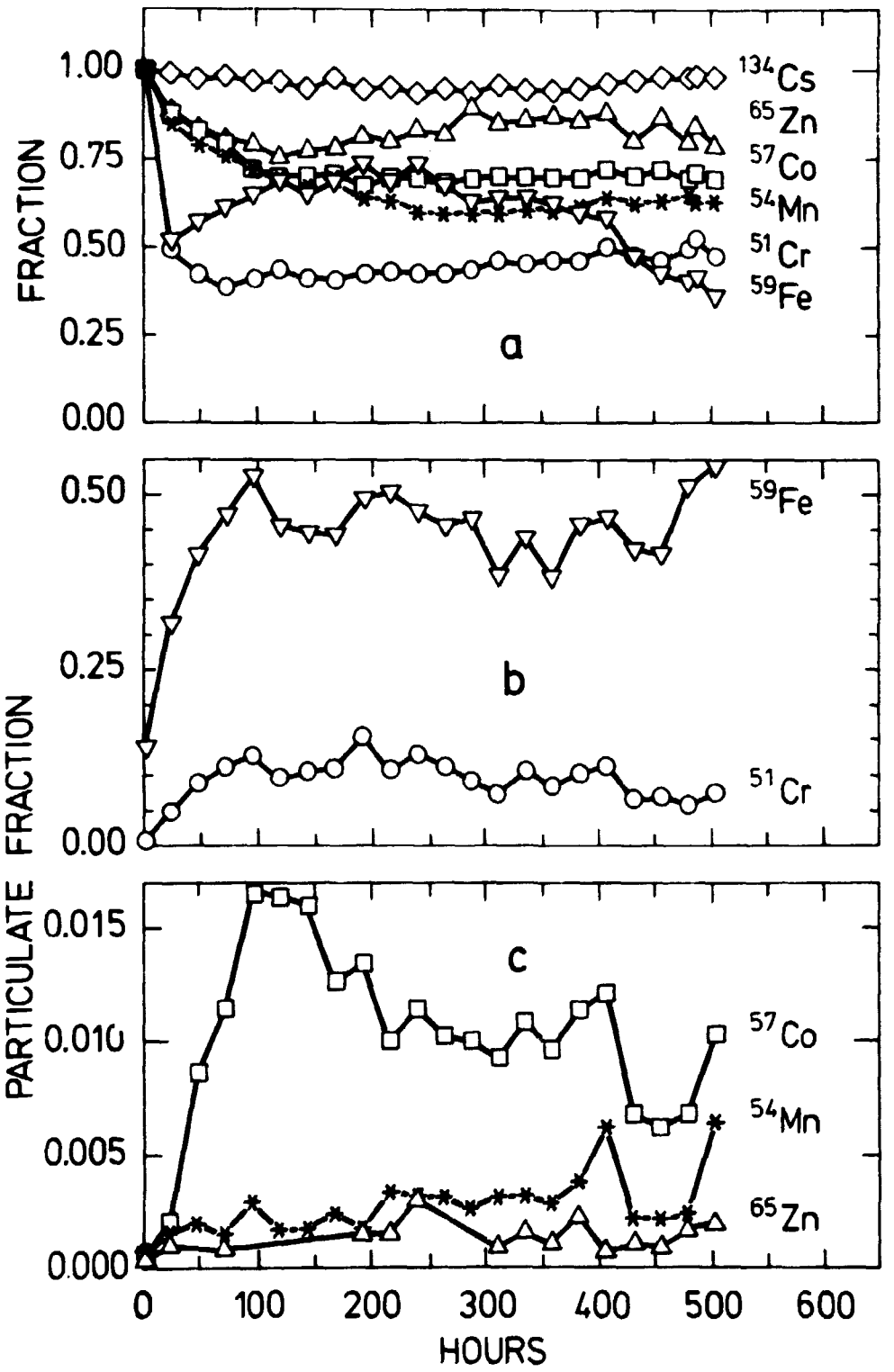

Figure 2.3.1.2. Mytilus edulis, single 3-week experiment (790718). A: Total activity in water as fraction of initial activity. B and C: Particulate (0.45-um) activity fraction. Compare with Figure 2.3.3.2.

culated on the basis of a time-weighted mean water activity. For t^* hours of contamination starting at t = 0 this is calculated as:

$$V_{t^*} = \frac{\sum_{t=0}^{t^*} \frac{1}{2}(V_i+V_j)\ |t_i-t_j|}{t^*} \qquad (pCi\ ml^{-1}) ,$$

where V_i and V_j is the activity in the water t_i and t_j hours after incubation, respectively, i.e. as the total number of "pCi x hours" during contamination divided by length of contamination in hours.

The particulate activity fraction collected by filtration through a 0.45-µm Millipore membrane filter was generally increasing during the experiments (Figure 2.3.1.1., B and C) except for ^{59}Fe, the behaviour of which was somewhat variable. In the 3-week experiment (Figure 2.3.1.2, B and C) the increase stops after approximately 4 days. Time-weighted means of the particulate (0.45-µm) activity fractions measured during the ten Mytilus experiments are shown in Table 2.3.1.1. The values are calculated as:

Table 2.3.1.1. Particulate activity (0.45 µm) as % of total activity during the Mytilus edulis experiments. Time-weighted means of values measured 2, 8, 24, 48, 72, and 96 hours after addition of nuclides, for each of ten experiments, and the means of these values for high and low algal concentration. (Cf. text and figure 2.3.1.1, B and C).

Temperature		15°C			5°C			
Salinity		20 °/oo	20 °/oo	8 °/oo	20 °/oo	20 °/oo	8 °/oo	
Experiment no:		790630	780605	781214	-	781008	790427	Mean ± SE(n=5)
Low algal concentration	^{51}Cr	1.10	4.18	7.26		1.55	1.60	3.14 ± 1.16
	^{54}Mn	0.523	0.243	0.239		0.137	0.630	0.35 ± 0.09
	^{57}Co	1.49	0.687	0.301		0.132	0.321	0.59 ± 0.24
	^{59}Fe	12.4	18.5	17.8		6.34	8.51	12.7 ± 2.4
	^{65}Zn	0.310	0.368	0.238		0.093	0.153	0.23 ± 0.05
	^{134}Cs	-	-	-		-	-	-
Experiment no:		-	781110	781201	790713	780708	790420	Mean ± SE(n=5)
High algal concentration	^{51}Cr		3.58	2.04	1.75	0.936	2.39	2.14 ± 0.43
	^{54}Mn		0.566	0.346	0.49	0.226	0.295	0.38 ± 0.06
	^{57}Co		0.348	0.248	1.86	0.391	0.217	0.61 ± 0.031
	^{59}Fe		18.5	8.31	16.0	13.4	7.51	12.7 ± 2.1
	^{65}Zn		0.459	0.248	0.163	0.328	0.189	0.27 ± 0.06
	^{134}Cs		-	-	-		-	-

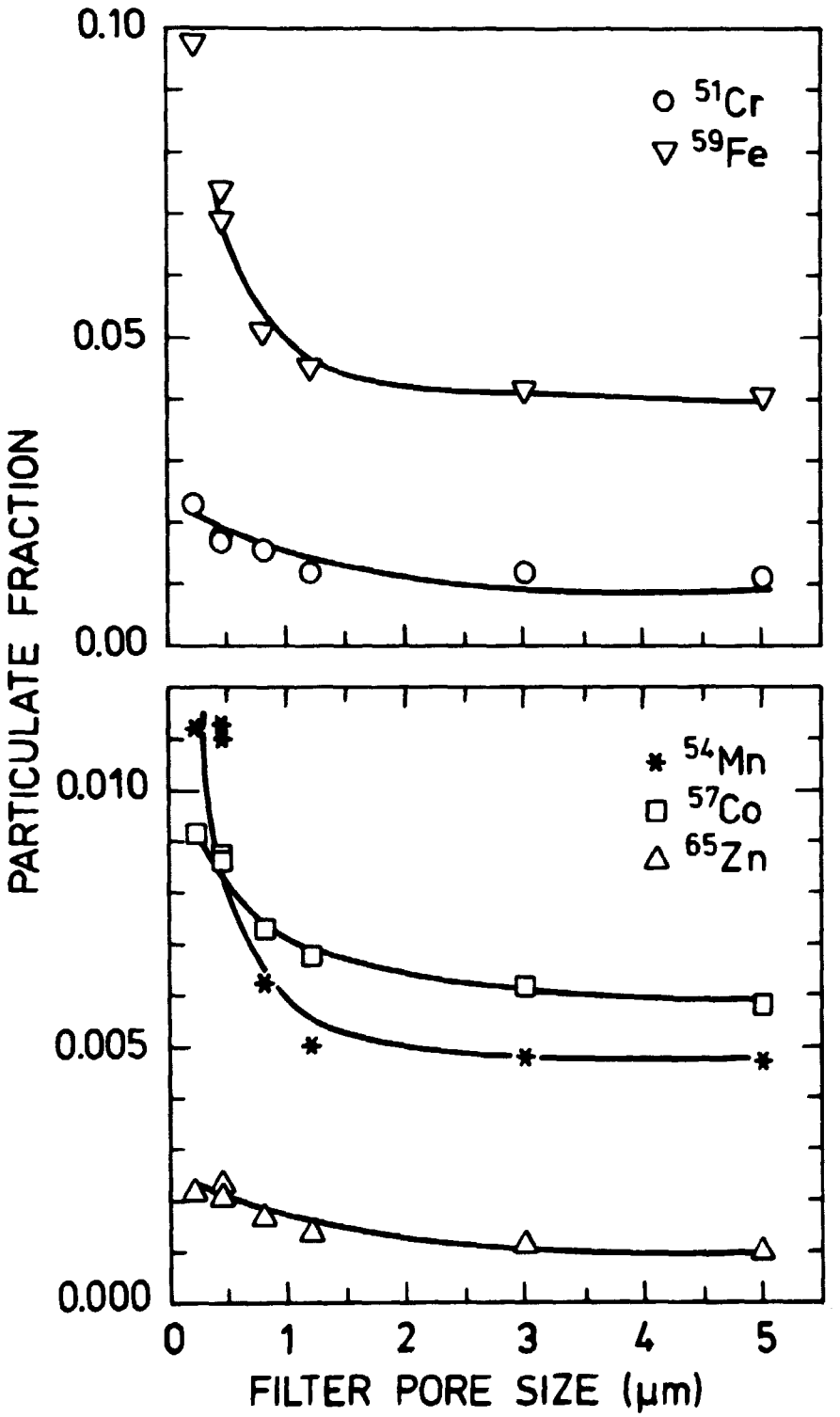

Figure 2.3.1.3. Particulate activity fraction in experimental sea water as measured by Millipore membrane filters of different pore size after experiment 790427. See text for further explanation. Curves are fitted by eye.

$$\bar{A} = \frac{\sum_{t=0}^{96} \frac{1}{2}(A_i + A_j) \, |t_i - t_j|}{96}$$

where A_i and A_j are particulate activity fractions measured t_i and t_j hours after incubation, respectively. From the mean values it is seen that the particulate fraction is declining in the order $^{59}Fe > ^{51}Cr > ^{57}Co > ^{54}Mn > ^{65}Zn$. Furthermore, it is noted that no difference is measured between the experiments with high and low algal concentration, although the two groups of experiments differ by a factor of 3.1 in their concentration of contaminated phytoplankton. The causes and consequences of this lack of difference is discussed in Chapter 2.4.6.

The mussel gill is a coarser filter than the 0.45 µm Millipore membrane which is often used to define "particulate matter". The retention efficiency of the mussel gill is more comparable to a 5.0 µm membrane filter (see Chapter 2.4.1). The effect of filter pore size on the measured particulate activity was therefore investigated (Figure 2.3.1.3). It is seen that a smaller pore size (0.22 µm) increases the measured particulate activity, while the decrease in particulate activity with increasing pore sizes (0.8 µm, 1.2 µm, 3.0 µm, and 5.0 µm) is modest. The ratio between particulate activity measured by the standard 0.45 µm filter and a 5.0 µm filter, was determined for ^{59}Fe, ^{51}Cr, ^{57}Co, ^{54}Mn, and ^{65}Zn after two experiments (790427 and the 3-week experiment: 790718) as 1.7 ± 0.3 (SD).

2.3.2. Filtration Rate and Food Intake

As mentioned earlier (Chapter 2.2.4), one of the benefits of using the automatic mussel-feeding apparatus is that the rate of clearance is continuously monitored, thus applying an index of animal condition to the experiments. In these experiments, the rate of clearance is a measure of the filtration rate, as the retention efficiency of the phytoplankton cells used, Phaeodactylum tricornutum, is 100% in one passage of the mussel gill (Møhlenberg and Riisgård 1978), and as water already filtered by the mussels is not refiltered before its algal concentration has been brought back to the desired level (Chapter 2.2.4 and Appendix I).

Table 2.3.2.1. Filtration rate (clearance) and food intake in the 10 mussel experiments calculated over the entire experiment (96 hours) (cf. text), and mean values during selected periods, i.e. optimum values. The mean algal concentration during these periods is shown. All weights refer to total soft parts. (Cf. Figure 2.3.2.1 and Table 2.2.8.2). Error term: ± SE.

Temperature		15°C			5°C		
Salinity		[illegible] ‰	20 ‰	8 ‰	20 ‰	20 ‰	8 ‰
Experiment no.:		791030	780605	781216	-	781008	790427
Low Algal concentration	Filtration rate (total) ml min^{-1} g^{-1} dry	101	115	435		103	160
	Food intake algae min^{-1} g^{-1} dry	30.9×10^4	33.6×10^4	89.6×10^4		26.9×10^4	37.9×10^4
	Filtration rate (optimal) ml min^{-1} g^{-1} dry	117±15(n=8)	120±9(n=5)	456±28(n=8)		120±23(n=6)	213±19(n=6)
	Algal concentration, ml^{-1}	2805±185(n=8)	2912±162(n=7)	2048±138(n=8)		2606±165(n=6)	2371±236(n=6)
Experiment no.:		-	781110	781201	790713	780708	790420
High Algal concentration	Filtration rate (total) ml min^{-1} g^{-1} dry		292	501	40	48	112
	Food intake algae min^{-1} g^{-1} dry		218×10^4	396×10^4	31.3×10^4	38.6×10^4	100×10^4
	Filtration rate (optimal) ml min^{-1} g^{-1} dry		257±13(n=9)	517±33(n=10)	63±5(n=5)	68±5(n= 6)	143±8(n=12)
	Algal concentration, ml^{-1}		7462±175(n=9)	7916±218(n=10)	7822±629(n=5)	8042±290(n=6)	8160±238(n=12)

Table 2.3.2.1 shows two different values for the filtration rate calculated during the mussel experiments and the rate of food intake. As the number of animals in the set up changes due to sampling, the mean rate of food intake during an experiment is calculated as

$$E = \frac{C}{\sum(n\ t)\ W} \qquad \text{(cells } min^{-1}\ g^{-1} \text{ dry)}$$

where C is the total number of algal cells removed from suspension by the mussel during the experiment (96 hours), $\sum n\ t$ is the total number of "animal-minutes", and W is the mean dry weight (g) of soft parts per mussel. From this rate of food intake, or "eating rate", a total rate of filtration can be calculated as

$$F_{total} = \frac{E}{A} \qquad \text{(ml } min^{-1}\ g^{-1} \text{ dry)}$$

where A is the mean algal concentration (cells/ml) during the experiment. This total rate of filtration can be considered as a time-weighted mean value including periods in which the mussels are disturbed, e.g. during sampling.

The optimal rate of filtration measured during periods of steady, non-disturbed function, i.e., during periods with constant number

of animals in the aquarium, has been calculated as

$$F_{optimum} = \frac{a}{t\ n\ W}\left(\frac{C_s}{C_e} - 1\right) \qquad (ml\ min^{-1}\ g^{-1}\ dry)$$

where a is ml algal culture injected during t minutes, C_s is algae/ml culture, C_e is algae/ml experimental sea water, n is number of aninals, and W is mean dry weight of soft parts per mussel.

The formula, which is adopted from Riisgård and Møhlenberg (1979), allows for "dilution" of experimental sea water with water from the algal culture. Furthermore, in the 3-week experiment, allowance was taken for dilution due to the continuous water exchange (Appendix I).

Mean values of this "optimal" filtration rate (Table 2.3.2.1) is expected to be higher than the "total" value averaging the whole experimental period if disturbances, e.g. during sampling, are serious. A t-test shows the difference between the two filtration rates to be probably significant (P > 95%) in experiments 790427, 781110, and 780708; of these 781110 happens to show the highest value for the total filtration rate. The two values of the filtration rate is equal in all other experiments implying that disturbances has not been of major importance.

In Figure 2.3.2.1, the "total" filtration rates of Table 2.3.2.1 are plotted against the mean soft parts weight per animal (Table 2.2.8.2) showing a decrease in filtration rate per mg soft parts with increasing weight. The rate of filtration is 2.5 times higher at 15°C than at 5°C, based on values transformed to the same dry weight (100 mg) by a ln-ln extrapolation with an exponent of -0.28. This function, found in a systematic study of the filtration rate versus animal size under optimal laboratory conditions at 15°C (Riisgård and Møhlenberg 1979), is included in Figure 2.3.2.1. Obviously, most of the values measured at 15°C are in good agreement with Riisgård and Møhlenberg's data although the heavier animals (experiments 79063C, 780605, 790713, and 780708) show a somewhat lower rate of filtration than expected.

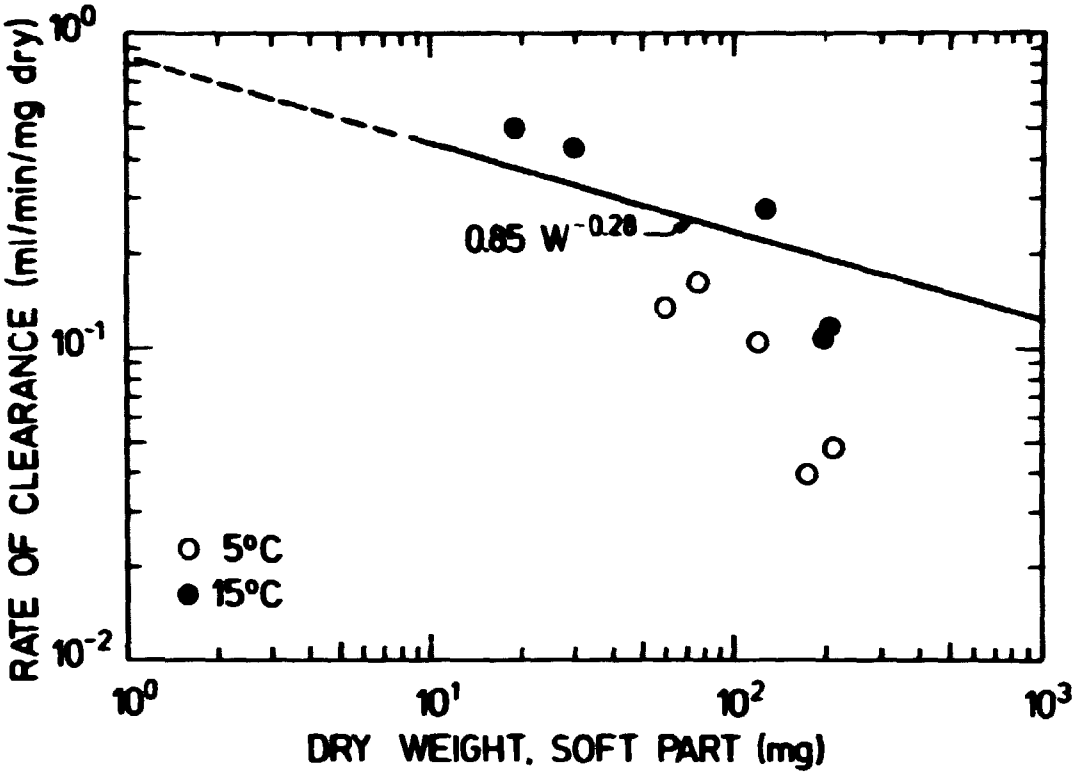

Figure 2.3.2.1. Mytilus edulis experiments. Filtration rate (clearance) per mg dry soft parts over the entire experiment (96 hours) (cf. text and Table 2.3.2.1) as a function of soft parts dry weight. The line $F = 0.85\ W^{-0.28}$ is from Riisgård and Mohlenberg's work (1979) at 15°C.

No differences in filtration rate are seen between the high and low algal concentration. For concentrations slightly lower (less than 1500 cells/ml), Riisgård and Randløv (1981) observed a reduced rate of filtration.

2.3.3. Time-dependent accumulation of radionuclides in the mussels

Figure 2.3.3.1 shows the accumulation of the 6 radionuclides in one of the 10 72-hour experiments given as a "concentration ratio" versus time. The concentration ratio for an animal sampled after t hours of contamination is calculated as

$$CR_t = \frac{A_t}{V_t} \quad ,$$

where A_t is the radionuclide concentration (pCi/g fresh weight) in the soft parts and V_t is the time-weighted mean water con-

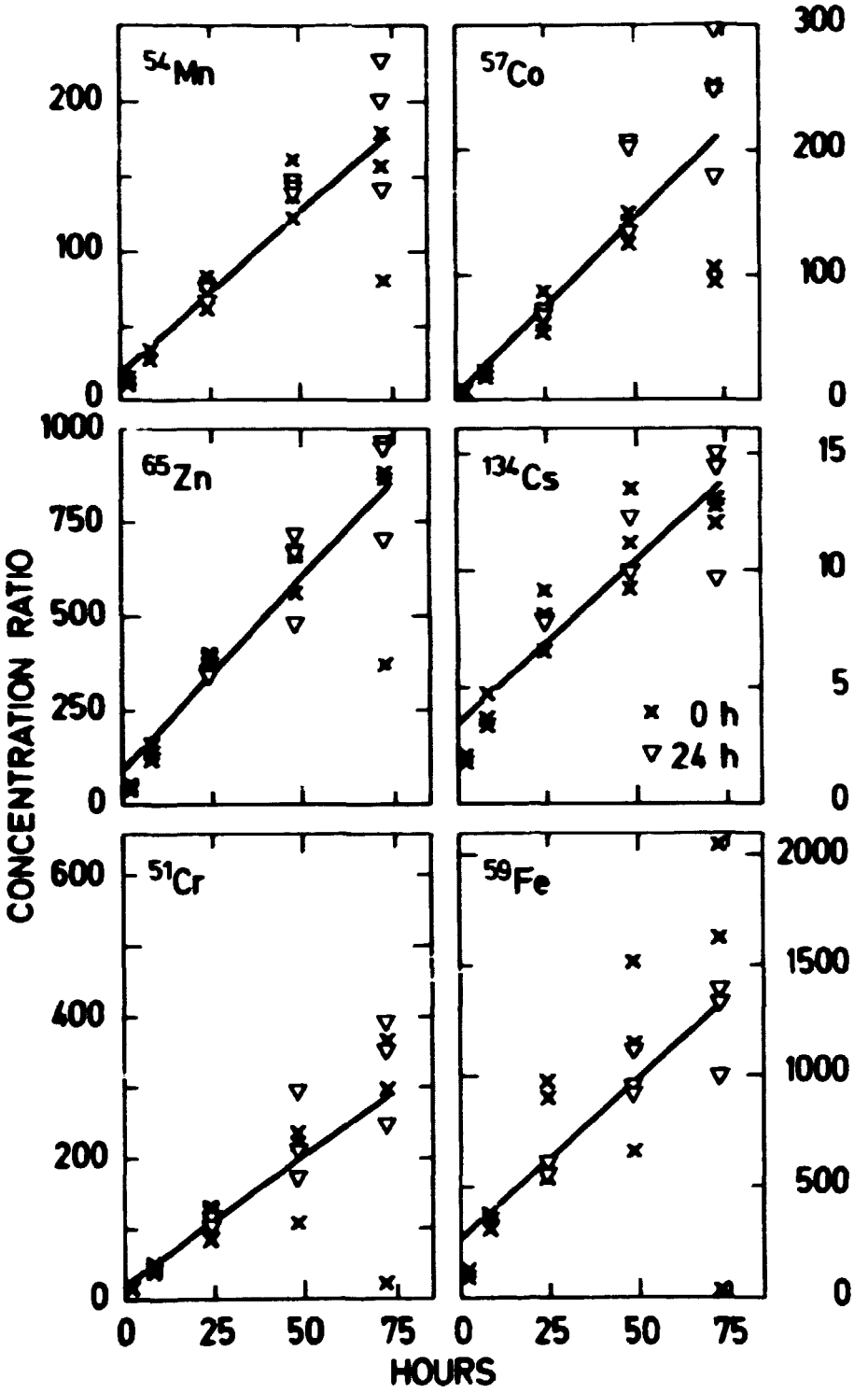

Figure 2.2.2.1. Mytilus edulis experiment 781201 (one of ten experiments). Rate of accumulation. The concentration ratio (ml^{-1} parts, fresh weight) is based on a time-weighted mean water activity (cf. text). The rates of accumulation are calculated as the slope of the regression lines indicated. Triangles denotes animals contaminated 24 hours after experimental start.

centration (pCi/ml) during the contamination (see Chapter 2.3.1). This value is often termed "concentration factor" or "CF" (see e.g., Pentreath 1973, Fowler et al. 1978, Anon. 1975). However, by "concentration factor" several authors understand the concentration ratio attained in steady state field conditions e.g., with stable metals (see Lowman et al. 1971). The term "concentration ratio" is thus an attempt to avoid common confusion.

It should be noted that the time axis of the accumulation curves (Figure 2.3.3.1) is 72 hours, whereas it is 96 hours for the water samples (Figure 2.3.1.1). This difference is caused by the 24-hour delay in contamination of some of the animals (see Chapter 2.2.8). Comparisons of the rates of accumulation calculated for animals contaminated 24 hours after experimental start and those present in the set-up at the time of radionuclide addition showed no significant differences. Therefore, the two sets of data are collected in one.

Table 2.3.3.1. Mytilus edulis experiments. Rate of radionuclide accumulation expressed as "radionuclide clearance": ml h^{-1} g^{-1} dry soft parts. Cf. text.

Temperature		15°C			5°C		
Salinity		20 °/oo	20 °/oo	8 °/oo	20 °/oo	20 °/oo	8 °/oo
Experiment no.:		790630	780605	781214	-	781008	790427
Low Algal concentration	^{51}Cr	3.67	3.48	79.7		4.95	11.9
	^{54}Mn	6.12	0.850	16.4		2.28	12.1
	^{57}Co	9.51	5.39	18.6		3.76	11.1
	^{59}Fe	40.9	14.4	204		33.4	72.8
	^{65}Zn	27.5	21.9	78.9		11.4	33.4
	^{134}Cs	0.43	0.475	1.08		0.322	1.14
Experiment no.:		-	781110	781201	790713	780708	790420
High Algal concentration	^{51}Cr		13.4	38.0	4.93	1.18	8.29
	^{54}Mn		7.26	22.0	3.11	1.32	5.18
	^{57}Co		9.59	28.9	5.52	2.27	6.66
	^{59}Fe		98.0	148	56.0	30.1	42.5
	^{65}Zn		33.6	104	13.3	11.6	21.3
	^{134}Cs		0.541	1.45	0.327	0.277	1.12

The rate of accumulation shown in Table 2.3.3.1 is calculated as the slope of the line of regression (Figure 2.3.3.1). This method is used because the accumulation curves are too short to show equilibration tendencies. The unit of the slope and thereby of the rate of radionuclide accumulation can be expressed as ΔCR/h, i.e., the hourly concentration ratio increament. As CR can alternatively be expressed as milliliters of water containing the same amount of the radionuclide as one gramme of mussel, ΔCR/h is equivalent to a "radionuclide clearance": ml h^{-1} g^{-1}, i.e., milliliter of water cleared for activity per hour and gramme of mussel. The rate of accumulation is thus directly comparable to the phytoplankton clearance (see below). It should be noted, however, that the values in Table 2.3.3.1 are calculated on a dry weight basis due to differences in soft parts water contents (Table 2.2.8.2).

In some of the experiments, e.g. the one used as an example in Figure 2.3.3.1, the ^{134}Cs curve apparently deviates from a straight line; this can result in an underestimate of the initial rate of accumulation and an overestimate of the intercept.

Some of the differences observed in the rate of accumulation (Table 2.3.3.1) might be explained by differences in animal size (Table 2.2.8.2) as several physiological processes, e.g. metabolic rate, are not directly proportional to animal weight. Zeuthen (1953) describes the oxygen consumption as a function of animal weight over several orders of magnitude with a power function, $Y = aX^b$, with b-values of ~ 0.7 - 0.9; these values are equivalent to -0.3 - -0.1 for the oxygen consumption per gramme of animal as a function of animal weight. Table 2.3.3.2 thus shows the rates of accumulation per g dry weight extrapolated along the line $Y = k\ X^{-0.28}$ found for the rate of filtration per gramme soft parts in Mytilus edulis (Riisgård and Møhlenberg 1979, cf. Chapter 2.3.2).

A three-way analysis of variance (temperature x salinity x algal concentration) on the results from Tables 2.3.3.1 and 2.3.3.2 showed no effects of algal concentration on the rate of accumulation (cf. Table 2.3.3.5). Results from the two algal concentrations and the two replicates are thus averaged in Tables 2.3.3.3 and 2.3.3.4 to show the mean rates of accumulation found

Table 2.3.3.2. Mytilus edulis experiments. Rate of radionuclide accumulation extrapolated to animals of 100 mg dry weight (soft parts). "Radionuclide clearance": ml h^{-1} g^{-1} dry. Values from Table 2.3.3.1 normalized to 100 mg by a ln x ln extrapolation on the line $Y = k\ x^{-0.28}$ (cf. text, Figure 2.3.2.1 and Table 2.2.8.2).

Temperature		15°C			5°C		
Salinity		20 °/oo	20 °/oo	8 °/oo	20 °/oo	20 °/oo	8 °/oo
Experiment no.:		790630	780605	781214	-	781008	790427
Low Algal concentration	^{51}Cr	4.42	4.41	56.4		5.20	11.0
	^{54}Mn	7.38	1.08	11.6		2.40	11.2
	^{57}Co	11.5	6.83	13.1		3.95	10.2
	^{59}Fe	49.3	18.3	144		35.0	67.2
	^{65}Zn	33.2	27.8	55.9		12.0	30.8
	^{134}Cs	0.519	0.602	0.768		0.338	1.05
Experiment no.:		-	781110	781201	790713	780708	790420
High Algal concentration	^{51}Cr		14.3	23.8	5.73	1.46	7.16
	^{54}Mn		7.71	13.8	3.61	1.62	4.47
	^{57}Co		10.2	18.1	6.41	2.80	5.76
	^{59}Fe		104	92.6	65.1	37.1	36.7
	^{65}Zn		35.7	65.1	15.4	14.2	18.4
	^{134}Cs		0.575	0.91	0.380	0.342	0.971

for the four combinations of two temperatures and two salinities. The mean temperature and salinity effects shown in Table 2.3.3.5 are calculated from mean values in Tables 2.3.3.3 and 2.3.3.4, whereas the two-way analysis of variance (temperature x salinity) has been performed on values from Tables 2.3.3.1 and 2.3.3.2 ignoring algal concentration. Generally, a higher rate of accumulation is observed for higher temperature and for lower salinity. However, the effects are lower for values extrapolated to 100 mg dry. The interaction (temperature x salinity) observed for ^{134}Cs-values extrapolated to 100 mg dry is explained by an "inverse" temperature effect at 8°/oo (Tables 2.3.3.2 and 2.3.3.4).

The regression analysis often showed a significantly positive intercept with the Y-axis, especially for the ^{134}Cs accumulation. Table 2.3.3.6 shows mean values from the 10 experiments. A t-test showed ^{134}Cs-values to be significantly different from zero ($P > 99\%$) and ^{65}Zn values to be probably significantly different ($P > 95\%$). The interpretation of a positive intercept will be discussed in Chapter 2.4.4 together with a discussion of the relation between rate of accumulation and influx.

Table 2.3.3.3. Mytilus experiments. Mean rates of accumulation ignoring food levels. "Radionuclide clearance": ml h^{-1} g^{-1} dry. Values from Table 2.3.3.1. Error term: ±SE.

Temperature	15°C		5°C	
Salinity	20 °/oo	8 °/oo	20 °/oo	8 °/oo
	(n = 3)	(n = 2)	(n = 3)	(n = 2)
^{51}Cr	6.9±3.3	59±21	3.7±1.2	10±2
^{54}Mn	4.7±2.0	19±3	2.2±0.5	9±3
^{57}Co	8.2±1.4	24±5	3.9±0.9	9±2
^{59}Fe	51±25	176±28	40±8	50±15
^{65}Zn	28±3	91±13	12±1	27±6
^{134}Cs	0.48±0.03	1.27±0.18	0.31±0.02	1.13±0.01

Table 2.3.3.4. Mytilus experiments. Mean rates of accumulation ignoring food levels. "Radionuclide clearance": ml h^{-1} g^{-1} dry extrapolated to 100 mg dry weight animals (cf.text). Values from Table 2.3.3.2. Error term: ±SE.

Temperature	15°C		5°C	
Salinity	20 °/oo	8 °/oo	20 °/oo	8 °/oo
	(n = 3)	(n = 2)	(n = 3)	(n = 2)
^{51}Cr	7.7±3.3	40±16	4.1±1.3	9±2
^{54}Mn	5.4±2.2	13±1	2.5±0.6	8±3
^{57}Co	9.5±1.4	16±3	4.4±1.1	8±2
^{59}Fe	57±25	118±26	46±10	52±15
^{65}Zn	32±2	61±5	14±1	25±6
^{134}Cs	0.57±0.02	0.84±0.07	0.35±0.01	1.01±0.04

Table 2.3.3.5. Mytilus edulis experiments. Effect of environmental factors expressed as ratios between rates of accumulation from Tables 2.3.3.1-2.3.3.4.

Factor:		Temperature $\frac{15^oC}{5^oC}$	Salinity $\frac{8\ ‰}{20\ ‰}$	Algal concentration high/low
Measured values	^{51}Cr	3.9	5.6**	0.64
	^{54}Mn	2.1	4.1*	1.0
	^{57}Co	2.4*	2.6**	1.1
	^{59}Fe	2.2	2.5	1.0
	^{65}Zn	2.9***	2.8***	1.1
	^{134}Cs	1.3**	3.1***	1.1
Values extrapolated to 100 mg dry (cf.text)	^{51}Cr	3.2	3.7*	0.64
	^{54}Mn	1.9	2.8	0.93
	^{57}Co	2.1*	1.8*	0.95
	^{59}Fe	1.8	1.6	1.1
	^{65}Zn	2.4**	1.8**	0.93
	^{134}Cs	1.2 +++	2.2	0.95

Analysis of variance significance tests:
*: P > 95%, **: P > 99%, ***: P > 99.9%
+++: highly significant (P > 99.9%) interaction

Accumulation curves from the single 3-week experiment is shown in Figure 2.3.3.2. For ^{57}Co, ^{54}Mn, ^{59}Fe and ^{51}Cr the curves were not deviating from straight lines, whereas ^{134}Cs- and ^{65}Zn-curves could be fitted to exponential models (cf. discussion, Chapter 2.4.5).

As mentioned above, the rate of accumulation (Table 2.3.3.1) and the rate of filtration (Table 2.3.2.1) is expressed in comparable units, namely as amount of water "cleared" for radionuclides and algal cells, respectively, per unit time and per gramme dry soft parts. As the retention efficiency for the phytoplankton cells used is 100% (Møhlenberg and Riisgård 1978), this enables us to calculate a retention efficiency for particulate activity (R)

Table 2.3.3.6. Mytilus experiments. Mean values of the intercept with the Y-axis from analysis of regression expressed as "radionuclide clearance": ml g^{-1} dry soft parts. Cf. text and Figure 2.3.3.1.

Isotope	Mean ± SE(n = 10)
^{51}Cr	65 ± 29
^{54}Mn	43 ± 20
^{57}Co	- 9 ± 13
^{59}Fe	661 ± 322
^{65}Zn	246 ± 99*
^{134}Cs	12.2 ± 3.6**

Difference from zero:
*: probably significant (P > 95%)
**: significant (P > 99%)

under the assumption, that radionuclides are accumulated exclusively from particles:

$$R = \frac{A \times 100}{p \times F} \quad (\%),$$

where A is the rate of radionuclide accumulation (ml/h/g) from Table 2.3.3.1, p is the time-weighted mean particulate activity from Table 2.3.1.1, recalculated to a fraction, and F is the filtration rate from Table 2.3.2.1, recalculated to ml/h/g.

To assume an accumulation takes place from particles only is of course erroneous, but it can be used to clarify some of the data. If one of the nuclides was actually accumulated exclusively via particles, and if, furthermore, the particulate fraction sampled by filtration on a 0.45 µm membrane filter was exactly and completely retained by the mussel - neither more nor less - then a retention efficiency of 100% would be calculated for that nuclide. If, in addition to the accumulation via particles, the animal accumulated a similar quantity of activity from the "dissolved"

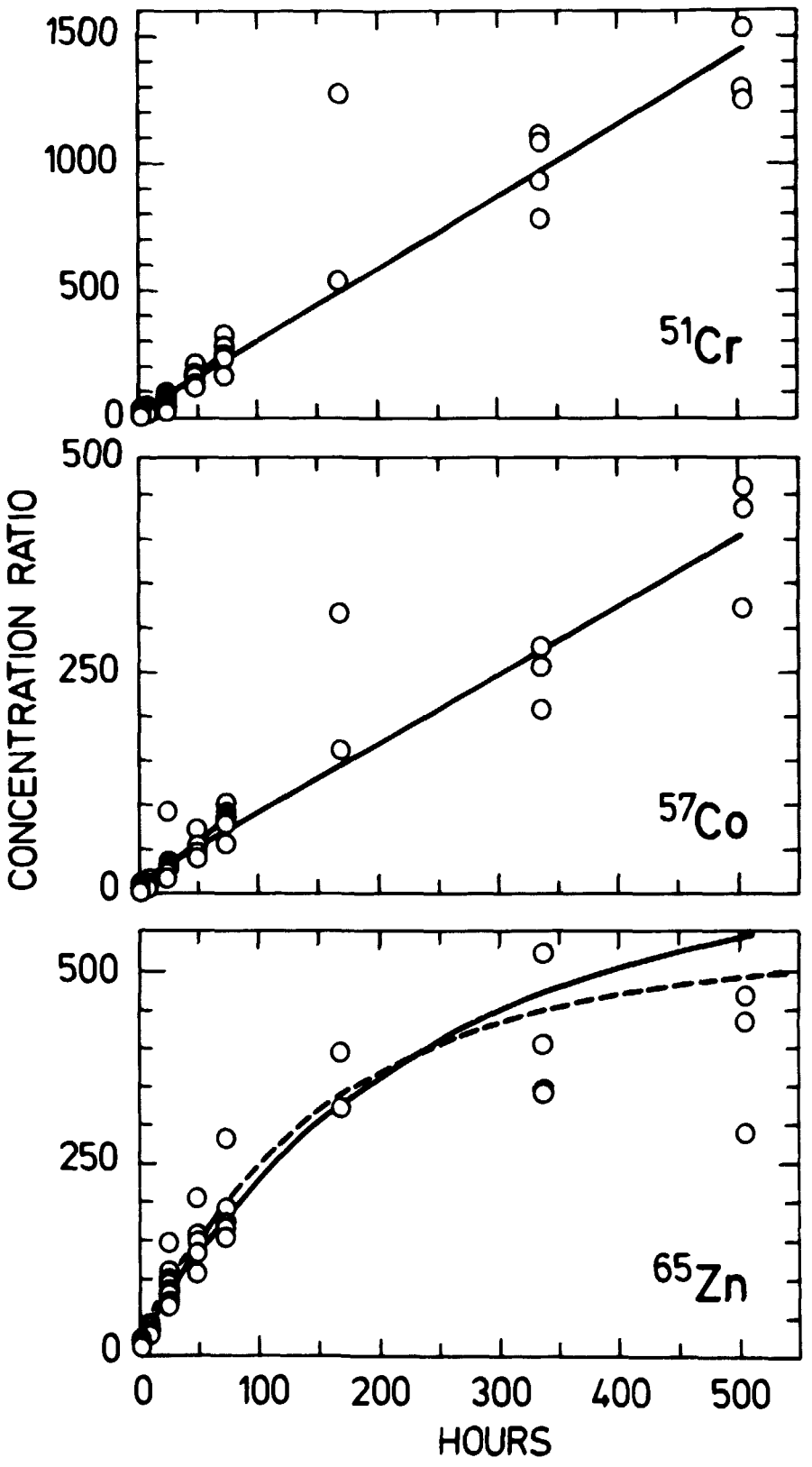

Figure 2.3.3.2. Mytilus edulis experiment 790718. Rate of accumulation in 3-week experiment (14‰ salinity, 10°C). The concentration ratio (soft parts, fresh weight) is based on a time-weighted mean water activity (cf. text). Regression lines are indicated for 0-72 hours and (^{57}Co, ^{54}Mn, ^{59}Fe, ^{51}Cr) for all results The monoexponential accumulation curves (broken lines) fitted to the experimental results for ^{65}Zn and ^{134}Cs and the ^{65}Zn accumulation curve (solid line) based on a two-compartment model are explained and discussed in Chapter 2.4.5.

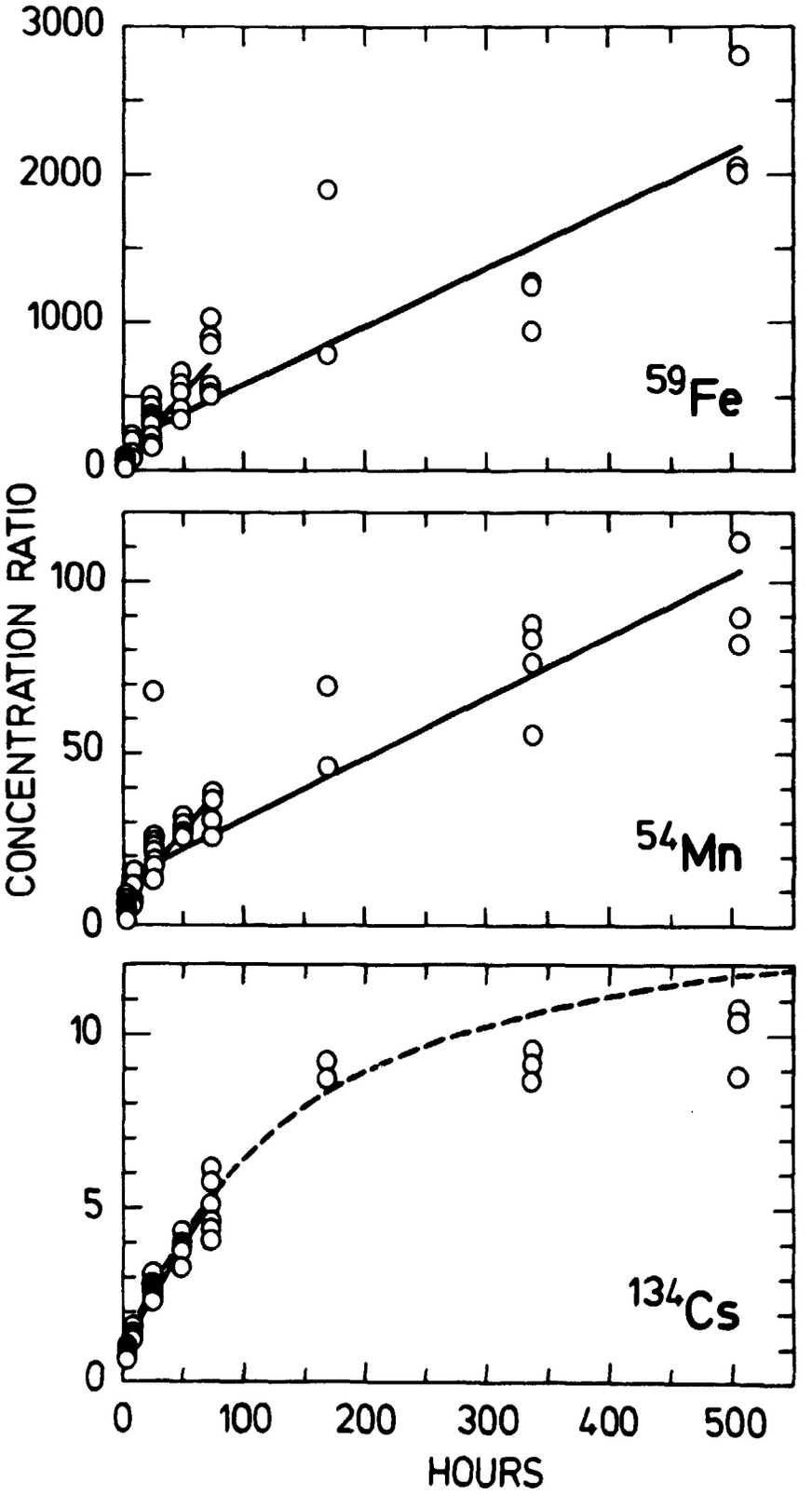

Figure 2.3.3.2. continued

Table 2.3.3.7. Mytilus edulis experiments. Efficiency of retention for particulate (0.45 µm) activity (Table 2.3.1.1.) pretending radionuclide uptake from particles only, i.e., an overestimate (cf. text). Unit: % of algal retention.

Isotope	Mean ± SE(n=10)
^{51}Cr	5.2 ± 0.9
^{54}Mn	20 ± 2
^{57}Co	25 ± 4
^{59}Fe	6.7 ± 1.2
^{65}Zn	159 ± 25

pool, then the method of calculation used would give a "retention efficiency" of 200%, and if the accumulation of "dissolved" activity was 9 times the accumulation from particles, then a "retention efficiency" of 1000% would be calculated.

The lowest values of Table 2.3.3.7 are, however, far below 100% indicating that the efficiency of retention of particulate activity was much lower for the mussel than for the 0.45 µm membrane filter. This will be interpreted further in Chapter 2.4.2.

Field Experiments

2.3.4. Loss of Radionuclides from Mussels in the Field

The whole-body loss of ^{51}Cr, ^{54}Mn, ^{57}Co, ^{59}Fe, ^{65}Zn, and ^{134}Cs was measured during 3 months on 12 individuals contaminated during experiments 790630, 790713, and the three-week experiment, 790718. Four of the animals contaminated for 3 weeks were monitored during one year. During the loss measurements the animals were placed in small perforated Perspex cages (Chapter 2.2.6) in the natural environment from which they were sampled prior to contamination. The results from this experiment have been published (Appendix II) and will therefore be only briefly summarized here.

The loss curves for the first 3 months, i.e., autumn 1979 with temperatures decreasing from ~ 19°C to ~ 5°C, were resolved in

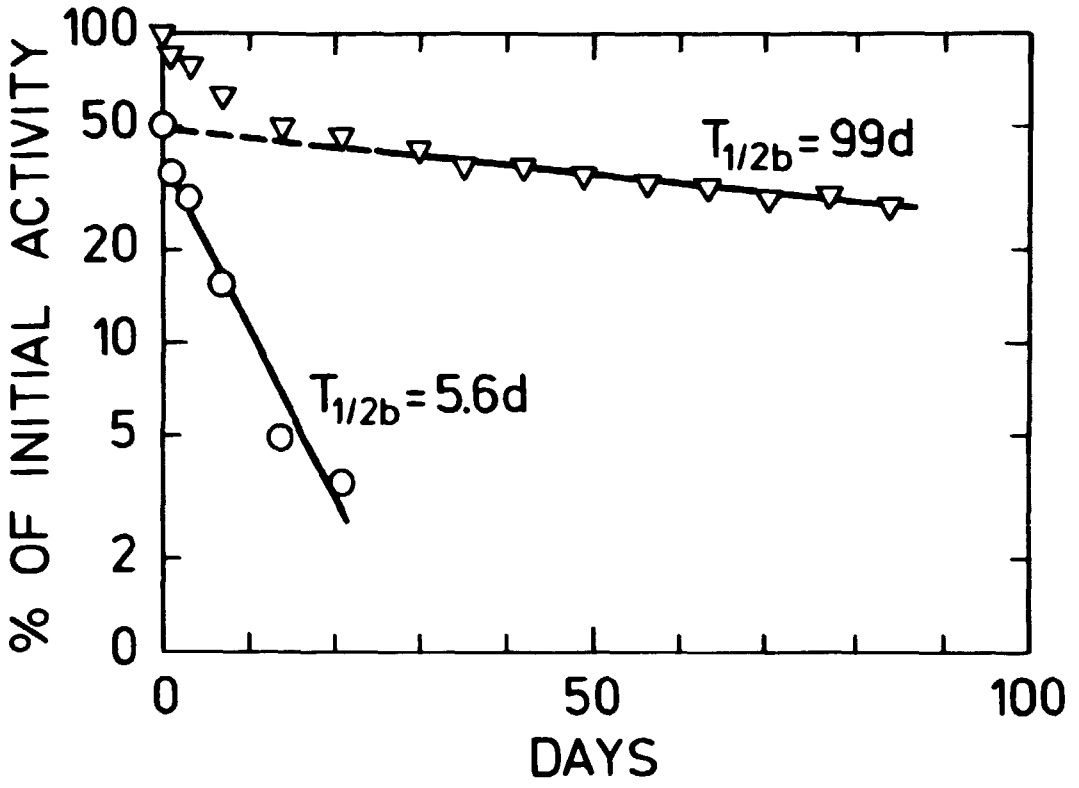

Figure 2.3.4.1. Mytilus edulis. Whole body loss of ^{65}Zn in the field (one of 12 animals). The loss curve shown by triangles is resolved in two exponential compartments by analysis of regression (cf. text).

two exponential components as exemplified in Figure 2.3.4.1. A line of regression (ln activity versus time) on the straight part of the curve is subtracted from the results to form a new curve, on which another analysis of regression is performed. Thus the loss curves are satisfactorily described by the sum of two exponential functions:

$$A_t = b\, e^{-\lambda_1 t} + c\, e^{-\lambda_2 t}$$

where A_t is the percentage of initial activity after t days of loss, the rate constants λ_1 and λ_2 (d^{-1}) are found as the slope of the two lines of regression, and b and c (%) are their intercepts with the Y-axis and express the relative magnitude of the two exponential components. The magnitude of a very fast component, a, is found by subtraction:

$$a = 100 - (b + c) \qquad (\%).$$

As all activity concentrations are decay corrected to the start of the experiment, the biological half life of the two exponential components are calculated as

$$T_{\frac{1}{2}b} = \frac{\ln 2}{\lambda} \quad \text{(days)}.$$

Mean values of the biological half-lives and the magnitude of the different components are shown as Table II in Appendix II.

The whole-body loss of ^{65}Zn and ^{57}Co during one year is depicted in Figure 2.3.4.2 along with a temperature curve. As indicated, the year has been divided into 4 sections: in Section I the fast components are not yet washed out, whereas the loss of activity in Sections II, III, and IV is considered as from one slow exponential compartment. For these periods the curves are thus not resolved but the loss rates are calculated for each period separately by means of regression analysis (ln activity versus time). Period II is autumn 1979 above 5°C, III is winter 1979-1980 with temperatures below 5°C, and IV is spring and summer 1980.

From Figure 2.3.4.2 it is evident that the half-lives increase during the winter, whereas they decrease again for ^{65}Zn but not for ^{57}Co during spring and summer 1980. The corresponding biological half-lives are shown in Table III of Appendix II. It is seen that ^{54}Mn behaves as ^{57}Co.

A possible explanation of the difference observed between ^{65}Zn, which shows a seasonal variation in its biological half-life, and ^{57}Co and ^{54}Mn, which has shown an extremely slow component after several months of loss, is shown in Table IV of Appendix II: the major part of the ^{57}Co and ^{54}Mn activity remaining in the animals after one year of loss is found in the shell, whereas most of the ^{65}Zn activity is still situated in the soft parts.

It should be mentioned here that the description of the loss curve or sections of the loss curve by exponential functions, and the resolution of the loss curves in two exponential components, and thereby the calculation of the biological half-lives, is based merely on the straight lines found in the semi-logarithmic

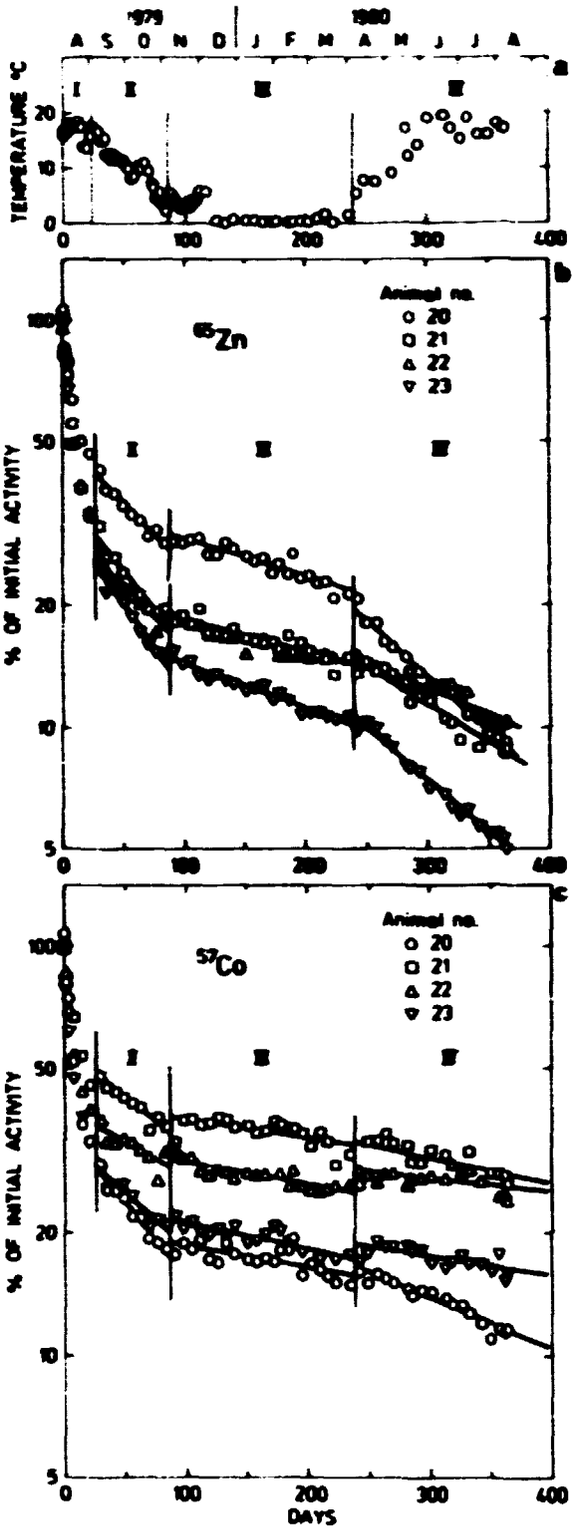

Figure 2.3.6.2. Mytilus edulis. Whole body loss of ^{65}Zn (B) and ^{57}Co (C) from 4 individual animals in the field after laboratory contamination, and the water temperature in Roskilde Fjord during the experiment (A). Regression lines are indicated for three periods (cf. text). All values are decay corrected to the start of the experiment.

plots of the loss curves (Figures 2.3.4.1 and 2.3.4.2), indicating that the loss is satisfactorily described by one or two exponential functions. The exponential "components" or "compartments" are thus defined entirely mathematically and have not been correlated to anatomically or functional compartments.

2.4. Discussion

2.4.1. Feeding in Mussel Experiments

The natural way of life for a mussel is to continuously filter huge amounts of water with low concentrations of phytoplankton. As demonstrated, e.g., in Figure 2.3.2.1, a relatively small mussel, say 100 mg dry soft parts or approximately 3 cm long, can filter ~ 23 ml of water per minute or more than 30 l a day. Thus, on a fresh weight basis, this small mussel filters an amount of water corresponding to 45,000 times its own soft parts weight daily. From this vast amount of water, the mussel completely retains particles as small as approximately 4 µm, whereas the retention efficiency of 1 µm particles has been measured at approximately 50% (Møhlenberg and Riisgård, 1978).

One of the reasons for using Mytilus as a bioindicator, namely the relatively high "concentration factors" reported for several pollutants (see, e.g. Phillips 1977a), is probably correlated to this fast and efficient filtration. However, in spite of this, the rate of filtration, to my knowledge, has never been mentioned in the large number of radionuclide accumulation studies published till now. The amount of water actually "cleared" for particles in laboratory experiments on particle retention may be considerably lower due to an inconvenient set-up resulting in recirculation of already-filtered water (Riisgård 1977) or due simply to bad water quality. The filtration rate, or at least some indication of the animal function, e.g. state of valve opening (cf. Jørgensen 1975b) is thus badly needed when evaluating laboratory experiments on pollutant accumulation by Mytilus.

The most natural way of performing filtration rate measurements is to feed the mussels phytoplankton cells and then monitor the

particle clearance. However, in nearly all radionuclide accumulation experiments performed till now, mussels were not fed. The accumulation is thus often referred to as "accumulation from water" contrasting "accumulation via food" (Pentreath 1973, Shimizu 1975). This strict differentiation is difficult to draw, however, as normally filtering mussels, although not fed by intention, ingest natural particles in the water, e.g. bacteria and detritus, and thereby pollutants associated with these particles.

When mussels are not fed the rate of filtration declines (Riisgård and Randløv 1981) and the mussels may even close their valves for shorter or longer periods of time. On the other hand, if mussels are fed concentrations of phytoplankton that are too high they react by creating "pseudofaeces", i.e., part of the filtered material is discharged before entering the digestive tract. Furthermore, part of the food ingested during periods when particle concentrations are too high may pass the intestine without proper digestion (Riisgård and Møhlenberg 1979).

From two growth experiments performed by Riisgård and Randløv (1981) during spring (March) and winter (January), approximately 800 and 2200 Phaeodactylum tricornutum cells per ml, respectively, can be considered to sustain "zero growth", i.e., these concentrations can give rise to a "maintenance ration". As heavy pseudofeaces production has been observed at a concentration of 41,000 cells/ml (Riisgård and Møhlenberg 1979), field-comparable laboratory experiments on metal and radionuclide accumulation via food should preferably be conducted well below this concentration. A reasonable concentration range for such experiments seems thus to be 1500-30,000 Phaeodactylum tricornutum cells per ml, or approximately 18-360 µg organic dry substance per liter. As, furthermore, the food assimilation seems to decrease with increasing concentration inside this range (Riisgård and Randløv 1981, Bayne et al. 1976), food concentrations should preferably be as stable as possible in order to decrease variability.

In addition to a decreased rate of filtration, starvation will, of course, imply loss of weight. Phillips (1976a) and Simpson (1979) suggest that the seasonal variation observed for metal

concentration in mussels could be explained by a seasonal variation in weight combined with a relatively stable total metal load. From this we would infer, that weight loss in laboratory experiments without feeding is associated with increasing metal concentrations. Nevertheless, in some tank experiments the loss in weight was associated with decreasing metal concentrations (Pentreath 1973, Simpson 1979). If the metal load of the rearing water was comparable with that of the sampling area, this might indicate anomalous metal metabolism during starvation.

The reduced rate of filtration and possible anomalous metal metabolism makes the field comparability of laboratory experiments with starved mussels questionable. In this light, the automatic maintenance of constant phytoplankton concentrations in experiments on accumulation of metals and radionuclides by mussels seems indispensable. Furthermore, by using the set-up described here (Chapter 2.2.4 and Appendix I) the continuous monitoring of the filtration rate applies an index of animal condition or "function" to the experiment.

2.4.2. Compartmentalization and chemical speciation of the radionuclides

Chemical and physical speciation of trace metals in sea water

The metal contents of sea water can be subdivided into several "compartments" depending on chemical and physical species. This subject is very complex and not fully understood. The following summary of physical and chemical species of the trace metals Zn, Co, Mn, Cr, Fe and Cs in sea water is based mainly on Florence and Batley's (1980) review.

Physically, two main species can be distinguished, namely the "dissolved" and the particulate fraction. These two fractions are usually separated by filtration through a 0.45 µm membrane filter (Millipore or equivalent) (See e.g. Riley 1975). The "dissolved" fraction defined in this way contains most of the smaller sizes of particulates, e.g. colloidal solids (Riley 1975, Parks 1975, Bowen 1979), but, on the other hand, the 0.45 µm Millipore filter retains part of these microparticulate

and colloidal species expected to pass the filter (Davis et al. 1974). Thus, the division into particulate and dissolved metal by membrane filtering is not clearcut.

Florence and Batley (1980) divide possible chemical forms of metals in natural waters into several groups and indicate the size ranges in each group (Table 2.4.2.1). A similar scheme is presented by Stumm and Brauner (1975). Of the forms indicated only ions and complexes are considered in true solution while even colloids are mainly in solution according to membrane filter separations. Considering colloids to the particulate fraction, the probable main dissolved species in sea water calculated for the 6 nuclides added are listed in Table 2.4.2.2 according to reviews by Florence and Batley (1980) and Stumm and Brauner (1975). However, for Zn in particular some disagreements exist in the recent literature (Florence 1980).

The 6 radionuclides are all added in the ionic forms as chlorides (Table 2.2.7.1), but only caesium is likely to remain in this form throughout the experiment. The divalent trace metals manganese, cobalt and zinc can probably exist in significant proportions in sea water as free metal ions, whereas the proportion of free ions of the trivalent metals iron and chromium existing in natural sea water is very small.

Table 2.4.2.1. Possible chemical forms (speciation) of metals in natural waters. (From Florence and Batley 1980).

Chemical form	Possible examples	Approximate diameter (nm)
Particulate	Retained by 0.45 µm filter	>450
Simple hydrated metal ion	$Zn(H_2O)_6^{2+}$	0.8
Simple inorganic complexes	$Zn(H_2O)_5Cl^+$	1
Simple organic complexes	Cu-glycinate	1-2
Stable inorganic complexes	PbS, $ZnCO_3$	1-2
Stable organic complexes	Cu-fulvate	2-4
Adsorbed on inorganic colloids	Cu^{2+}-Fe_2O_3, Cd^{2+}-MnO_2	10-500
Adsorbed on organic colloids	Pb^{2+}-humic acid, Zn^{2+}-organic detrius	10-500

Table 2.4.2.2. Probable main dissolved species of the added metals in sea water, according to Florence and Batley (1980) and Stumm and Brauner (1975). (Calculated results).

Element	probable main species
Zn	Zn^{2+}, $ZnCl^{+}$, $ZnCO_3$
Co	Co^{2+}, $CoCO_3$
Mn(II)	$MnCl^{+}$, Mn^{2+}
Fe(III)	$Fe(OH)_3$
Cr(III)	$Cr(OH)_3$
Cs	Cs^{+}

In estuaries the concentration of dissolved iron decreases as the salinity increases resulting in flocculation and precipitation of iron originating from fresh-water run-off. Other elements, e.g. manganese, are coprecipitated and part of this complex precipitate remains probably in colloidal suspension (Florence and Batley 1980). The particulate activity measured during the present experiments showed, however, no significant differences with salinity (Chapter 2.3.1). This is not surprising as only insignificant quantities of carrier metals were added to the experimental sea water (Table 2.2.7.1), which was furthermore diluted to the desired salinity with demineralized water.

In natural, oxygenated water the trivalent state of Fe is the dominant over Fe(II), whereas information on the distribution between Cr(III) and Cr(VI) in natural waters is contradictory (Florence and Batley 1980). However, the trivalent form added is probably relevant for chromium too. The divalent manganese added is unstable in oxygenated waters where it is oxidized to Mn(IV) which precipitates as MnO_2. The precipitation of MnO_2 and the anoxic solubilization in sediments is probably important for phosphorus and trace metal balances. The added amount of carrier manganese is so low (cf. Table 2.2.7.1) that it does not elevate the total concentration above normal levels of soluble manganese in oxygenated waters. This is in agreement with the

low particulate ^{54}Mn fractions found in these experiments (Table 2.3.1.1). However, the actual ratio between Mn^{2+}, colloidally bound Mn(II) and colloidal MnO_2 in the "soluble" fraction is not known neither in these experiments nor in the oceans (Florence and Batley 1980).

As all the radionuclides are in the ionic forms when added to the sea water, the proportion of radioactive to stable isotopes, i.e. the specific activity, will probably be highest in the ionic fractions and successively lower in other chemical and physical species depending on rate constants. Piro et al. (1973) found, that $^{65}Zn^{2+}$ added to sea water exchanged with ionic and particulate zinc, but was not in equilibrium with complexed zinc even one year after addition. This may be of significant importance for the rate of uptake and will thus limit the comparability to stable field conditions. Similarly, Jennings (1978) reports ^{55}Fe from nuclear test explosions to be accumulated approximately 2 orders of magnitude higher than stable iron by zooplankton in the Pacific Ocean presumeably due to differences in physical or chemical speciation. This might also occur when using bioindicators to monitor discharges from nuclear industries. To calculate steady state conditions from stable metal data, i.e. to use a specific activity approach in radionuclide accumulation studies, is thus not necessarily feasible when studying bioindicators.

Adsorption and formation of particulate activity during radionuclide experiments and its significance for accumulation by Mytilus

According to Table 2.4.2.1, the metal fraction not being in true solution can be grouped in metals associated with conventional particles retained by e.g. a 0.45 µm membrane filter and in metals associated to different forms of colloids. Colloids of the metal species itself, e.g. new formation of pure collidal ironhydroxy complexes, are thought to be not of major importance for the radionuclide distribution in the present experiments, as maximally a few nano-moles of carrier metal are added per liter (Table 2.2.7.1).

Generally, the total concentration of the radionuclides decreases and the particulate fraction (0.45 µm) increases during the laboratory experiments except for ^{134}Cs (Chapter 2.3.1). Apart from the significant quantities removed by the animals these observations can probably be explained mainly by sorption to aquarium walls and other surfaces of the equipment and to existing suspended particulate surfaces in the experimental sea water. The similarity of the "size-spectrum" (Figure 2.3.1.3) for the 5 radionuclides detected in the particulate matter supports the hypothesis that the formation of particulate activity should be explained by adsorption to existing particles, and that flocculation of the individual metal species themselves are negligible, at least in the filterable size ranges. However, these "size spectra" are apparently modified by the filtering activity of the mussels, as the "plateau" observed for membrane filters above 0.45 µm pore size could be explained by the spectrum of particle retention observed for mussels (cf. Chapter 2.4.1).

A decreasing activity concentration in the water is a severe problem in laboratory experiments (see, e.g., Shimizu 1975). However, by using the time-weighted mean water level (Chapter 2.3.1) when calculating concentration ratios, the rates of accumulation are probably not seriously biased due to this. The increasing particulate activity fraction might be another source of error when compared to natural conditions. Ideally, accumulation studies should be performed under more stable conditions concerning total activity concentrations and particulate activity in order to increase field comparatility. This might be accomplished by using the procedure of continuous water exchange from the 3-week experiment (Figure 2.3.1.2 and Appendix I) and discarding animals present in the set-up during equilibration. As no significant differences were found between animals contaminated 24 hours after experimental start and animals present in the set-up at contamination (Chapter 2.3.3), the errors due to these sources are probably not of major importance. As equilibrium is not reached at 24 hours, and as the number of animals was low and the variation high, an effect cannot be totally ruled out, however.

Although the phytoplankton concentration was unimportant in the present experiments (Chapters 2.3.3 and 2.4.6), accumulation via particles is probably of major importance. The "retention efficiency" of particulate (0.45 µm) activity by the mussels, as calculated under the assumption of the exact and complete retention of the fraction sampled on 0.45 µm Millipore filters and negligible uptake from other sources (Table 2.3.3.7), show the lowest values (5-7%) for ^{51}Cr and ^{59}Fe and the highest (160%) for ^{65}Zn. This could indicat , that the accumulation via particles is relatively important for ^{51}Cr and ^{59}Fe, whereas the accumulation of ^{65}Zn from other sources, e.g. from $^{65}Zn^{2+}$, might be predominant. Several suggestions on an explanation of the apparently low retention efficiencies of particulate activity (Table 2.3.3.7) can be made: 1) The difference between the standard 0.45 µm Millipore filter and a 5.0 µm filter, which should more closely resemble the mussel gill (cf. Chapter 2.4.1), is not enough, as the ratio between 0.45 µm and 5.0 µm filters was only 1.7±0.3 (SD) (Chapter 2.3.1). 2) Assuming a transport time for the ingested particles through the intestinal tract of a few hours, an assimilation efficiency of the radionuclides below 5-10% could, in addition to point 1, explain the results. However, Nakahara and Cross (1978) reported ^{60}Co retention efficiencies of up to 50% in bivalves from 3 phytoplankton species, and even higher values have been reported (Amiard and Amiard-Triquet 1975). 3) Finally the results could indicate a retention of microparticulates or colloids not retained by the mussel gill even in the 5.0 µm Millipore filter. An adsorption of colloids to the constituent materials of the Millipore filter with a relatively low efficiency would be corrected for in the double-filter procedure used (Chapter 2.2.5); however, if the retention of some species is high this correction is insufficient. Davis et al. (1974) found a high retention efficiency of sub-micron radiocolloids (^{198}Au) by 5.0 µm Millipore filters, thus supporting this hypothesis. Davis et al. suggest the use of Nucleopore polycarbonate membranes, which acts as a sieve, instead of Millipore or similar "membranes", which are actually depth filters.

Probably all the above-mentioned points should be considered in explaining the values of particulate activity retention. However, it can be stated that the standard use of a 0.45 µm

Millipore membrane when defining particulate matter is questionable. In future work more light should be shed on this problem as a better description of the physical as well as chemical speciation is necessary when interpreting laboratory experiments and when evaluating field comparability.

2.4.3. On stable metal concentrations

An "ideal" bioindicator accumulates metals and radionuclides in direct proportion to the concentrations in water, i.e., the rate of accumulation expressed as the concentration factor increament per time unit and the concentration factor at steady state should ideally be unaffected by metal concentrations. Organisms which efficiently regulate metals at a fixed level irrespective of environmental concentrations, as is seen for zinc in crabs (Carsinus maenas) over more than an order of magnitude (Bryan 1971), cannot of course be used as indicators for stable metal pollution. If they are used to indicate a radionuclide, e.g. ^{65}Zn, it might, however, prove useful provided the concentration of the stable metal is relatively constant in the monitored environment.

In the present studies stable metal concentrations were not measured due to lack of time and equipment. However, in order to maintain metal concentrations at relatively constant natural background levels, water for the experiments was sampled below the halocline in the Kattegat (Chapter 2.2.1); and minimal amounts of carrier metals were added with the radionuclides (Table 2.2.7.1). Furthermore, Phillips' (1977c & 1978) data indicate that metal concentrations in mussels from the two sampling areas are equal and at a natural level.

Bivalves are used to indicate point sources of metal pollution in the environment, i.e., e.g. mussels, oysters, and Scrobicularia from metal-polluted environments show higher levels than animals from relatively clean areas (Goldberg et al. 1978, Phillips 1976b & 1977a, Davies and Pirie 1980, Bryan and Hummerstone 1978). This shows that bivalves do not completely regulate metal concentrations. A lack of regulation has been shown for some metals in laboratory studies, too, although a tendency to non-linearity is observed especially at high concentrations (Harrison 1973,

Schultz-Baldes 1974, Ünlü and Fowler 1979, George and Pirie 1980).

It is therefore assumed that the results reported here are unaffected by metal levels. However, the possibility of metal regulation has not been fully studied at or below background levels. A change in the stable caesium level is probably without effect as it is metabolised as potassium (Bryan 1963). However, the other 5 elements studied should be considered as trace elements most of which are known to be essential (Bowen 1979) and an increased accumulation at very low environmental levels cannot be ruled out. In the future this point should be elucidated further as it might lead to misinterpretations.

2.4.4. On the estimate of the radionuclide influxes

In Chapter 2.3.3 the accumulation of radionuclides in the mussel soft parts was expressed by the inclination of a line of regression calculated for a 3-day accumulation curve (see Figure 2.3.3.1). This inclination was termed "rate of accumulation" and should be considered as a net uptake, i.e., the difference between the total uptake of a radionuclide and the simultaneous loss. In, e.g., compartment-model calculations (cf. Chapter 2.4.5) the total rate of uptake or the "influx" is an important parameter. As the 3-day curves of accumulation (Chapter 2.3.3) did not deviate significantly from a straight line, the measured rate of accumulation is probably only slightly smaller than the actual influx.

In this estimate of the influx, the possible existence of a component with a fast rate constant of the magnitude of 1 d^{-1} or higher, is ignored.

Such a component was indicated in the loss studies for all 6 radionuclides investigated (Table II of Appendix II). In the accumulation studies, a fast component will result in a positive Y-intercept. Of the 6 radionuclides only ^{65}Zn and ^{134}Cs showed a significantly positive intercept (Table 2.3.3.6). In bioindicator contexts a relatively small and very fast compartment is not relevant as such but it may influence the results of short-term laboratory experiments substantially, especially if the rate of

accumulation is estimated assuming linearity. This is also the case if an intercept should be explained by water adhering to the animals when sampled. In this case the effect of water adhesion is, however, insignificant as the intercepts, expressed as ml water/g animal, are different for different nuclides and as the amount of adhering water should be greater than the total sample in order to explain the intercept.

A positive intercept could, however, also be explained by a significant loss even in the initial accumulation phase giving rise to a non-linear 3-day accumulation curve. As the intercept was significantly positive for ^{134}Cs and ^{65}Zn only (Table 2.3.3.6) and as the 3-week accumulation curves for these two nuclides were non-linear (Figure 2.3.3.2) this is probably the explanation for at least part of the intercept for ^{134}Cs and ^{65}Zn. This will result in an underestimate of the influx of ^{134}Cs and ^{65}Zn. The magnitude of this error is not known but as the 3-day accumulation curves did not deviate from a straight line as a rule, it must be limited. As the same procedure has been used in all experiments, the evaluation of environmental variable effects (salinity, temperature and food level) is probably not influenced. Thus, ignoring the probable existence of a relatively small and very fast compartment, the best estimate of the radionuclide influx from these studies is the rate of accumulation (Chapter 2.3.3).

2.4.5. Compartment models describing the accumulation of radionuclides

The exchange of radionuclides between water and aquatic organisms is often conveniently described by exponential compartment models. In this chapter a simple compartment model, which sufficiently describes the accumulation of radionuclides in Mytilus soft parts, is presented.

In chapter 2.4.4 it was argued, that an "intercept-compartment", i.e. a very fast compartment appearing as a Y-intercept on the accumulation curves, exists in these experiments. Since its rate constants are not known it will be included in the model as a constant only. This is possible because the intercept-compartment

is not included in the influx estimates (cf. Chapter 2.4.4). The intercept compartment is indicated in Figures 2.4.5.1 - 2.4.5.3 as compartment No. 1.

Figure 2.4.5.1 depicts the simplest exponential model describing radionuclide uptake and loss in an aquatic organism. Although three compartments are included, only one of these is exponential, and the model is here termed "one-compartment model" or "mono-exponential model", although it might alternatively be

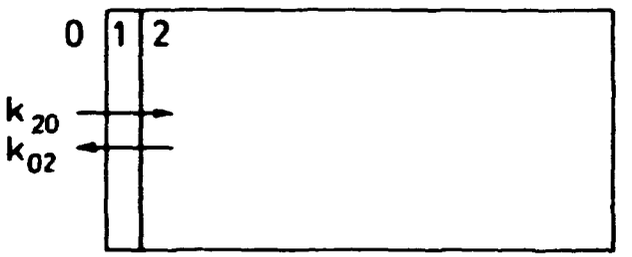

Figure 2.4.5.1. Compartment model with one exponential compartment (mono-exponential model). Although three compartments are included it is here termed "one-compartment model". Accumulation of a radionuclide in accordance with this model can be expressed as equation (3). Cf. Table 2.4.5.1 for symbols.

termed a 3-compartment model (Atkins 1969, Jaquez 1972). Compartment 0 represents the radionuclide amount q_0 in the surrounding experimental water. In these model calculations q_0 is assumed "infinite", i.e. the radionuclide pool in the water is so big that it is not significantly influenzed by accumulation in the animals. This has not been quite true in these experiments, especially not for ^{59}Fe. Compartment 1 which represents the radionuclide amount q_1 in the intercept-compartment is, as mentioned above, considered constant. The single exponential compartment in this model, compartment 2, contains the radionuclide amount q_2. Its exchange with q_0 is described by the rate constants k_{20} and k_{02} (d^{-1}). In Figure 2.4.5.1 these exchanges are shown as passing through the intercept-compartment. Whether this is actually happening is not known. In accordance with this mono-exponential model the exchange of activity between the animal and the

water, i.e. between compartments 2 and 0 of Figure 2.4.5.1 can be described by the differential equation

$$\frac{d(q_2)}{dt} = k_{20}q_0 - k_{02}q_2 \qquad (1)$$

where symbols are explained above and in Table 2.4.5.1. By integration (cf. e.g. Atkins 1969), the total amount of radionuclide in the animal during accumulation, i.e. in compartments 1 and 2, is thus described by

$$q_{1+2} \cdot q_1 + \frac{k_{20}q_0}{k_{02}}(1 - e^{-k_{02}\,t}) \qquad (2)$$

(cf. Table 2.4.5.1 for symbols). Dividing through by animal weight (g fresh) and radionuclide concentration in the water (pCi/ml) gives the concentration ratio CR (cf. Chapter 2.3.3). As

$$q_2 \to \frac{k_{20}q_0}{k_{02}} \quad \text{for } t \to \infty$$

the accumulation in accordance with the mono-exponential model

Table 2.4.5.1. Symbols and abbreviations used in the compartment models.

0, 1, 2, 3	: Compartment number. 0 is surrounding experimental water, 1 is "intercept-compartment" and 2 and 3 denotes exponential compartments.
q_0, q_1, q_2, q_3	: Amount of activity in different compartments (pCi).
k_{20}, k_{02},...k_{ij}	: rate constant (e.g. d^{-1}) for transport to compartment i from compartment j.
t	: time (e.g. days) since start of contamination.
CR_i	: concentration ratio contribution from compartment no. i, i.e. pCi in compartment i per gramme of animal divided by pCi per milliliter of water.
$CR_{tot.}$	: concentration ratio of total animal
CR^t, CR^{ss}	: concentration ratios after t days of contamination and at steady state, respectively.
$\Delta CR_i d^{-1}$	: influx to compartment i (equivalent to ml cleared $d^{-1}g^{-1}$) (cf. Chapter 2.3.3).

(Figure 2.4.5.1) is thus described by

$$CR^t_{tot} = CR_1 + CR^t_2 = CR_1 + CR^{ss}_2(1 - e^{-k_{02}t}) \qquad (3)$$

(cf. Table 2.4.5.1 for symbols).

This mono-exponential model or a similar one without the intercept compartment is often used when describing radionuclide accumulation (see e.g. Pentreath 1973). Equation (3) can be rearranged to

$$\ln\left(1 - \frac{CR^t_{tot.} - CR_1}{CR^{ss}_2}\right) = - k_{02}t \qquad (4)$$

(cf. Table 2.4.5.1 for symbols). If the model is correct, the rate constant of the outflux, k_{02}, can thus be calculated from an accumulation experiment by an analysis of regression on the left side of the last equation against t, and the biological half life of the exponential compartment can be calculated from

$$T_{1/2b} = \frac{\ln 2}{k_{02}} . \qquad (5)$$

This mono-exponential curve-fitting procedure has been applied to the ^{65}Zn and ^{134}Cs data of the 3-week experiment (Figure 2.3.3.2). Intercept values (CR_1) of 0.86 and 16 were used for ^{134}Cs and ^{65}Zn, respectively. These values were calculated by regression (cf. Chapter 2.3.3). Guessing total steady-state concentration ratios (or concentration factors) of 12 for ^{134}Cs and 500 for ^{65}Zn, rate constants corresponding to biological half lives of 4.4 days and 4.3 days, respectively, were calculated fitting experimental data up to 72 hours. The corresponding accumulation curves are included in Figure 2.3.3.2 (broken lines).

In the loss experiments (Chapter 2.3.4 and Appendix II) a ^{65}Zn compartment of comparable biological half life (6 days) was identified. However, an additional slow compartment ($T_{1/2b}$ ~ 87 days) was also found indicating that the mono-exponential model

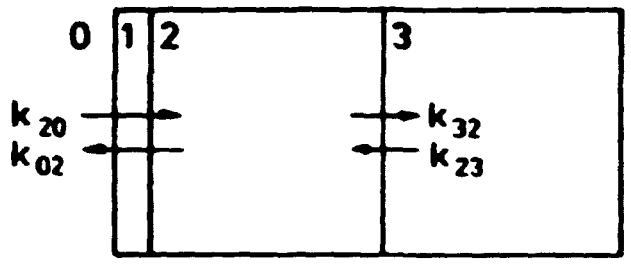

Figure 2.4.5.2. Compartment model with two exponential compartments. This model is not solved in the text. Cf. Table 2.4.5.1 for symbols.

is not sufficient in describing long term experiments or in extrapolations to steady-state. The necessary introduction of a second exponential compartment in the model can be done in several ways. Figure 2.4.5.2 shows an extra exponential compartment (3) as exhanging only with compartment 2. As in the above-mentioned model, differential equations describing the change of activity in the two compartments are easily arranged. The i..tegrated solution of these are, however, more complicated and cannot be tested on the experiments available. Furthermore, an excretion directly from a slow compartment has been proposed (George and Pirie 1980).

The model in Figure 2.4.5.3, containing two independent exponential compartments exchanging with the surrounding medium, is therefore proposed. The changes in activity contents in these compartments are simply the sum of two differential equations similar to equation (2) above and the integrated solution can subsequently be expressed as

$$CR_{tot}^{t} = CR_1 + CR_2^{ss} (1 - e^{-k_{02}t}) + CR_3^{ss} (1 - e^{-k_{03}t}) \qquad (6)$$

(cf. Table 2.4.5.1 for symbols). As for the one-compartment model (cf. above) the amount of radionuclide at steady-state can, for each of the two exponential compartments, be expressed as

$$q_2^{ss} = \frac{k_{20}q_0}{k_{02}} \quad \text{and} \quad q_3^{ss} = \frac{k_{30}q_0}{k_{03}}$$

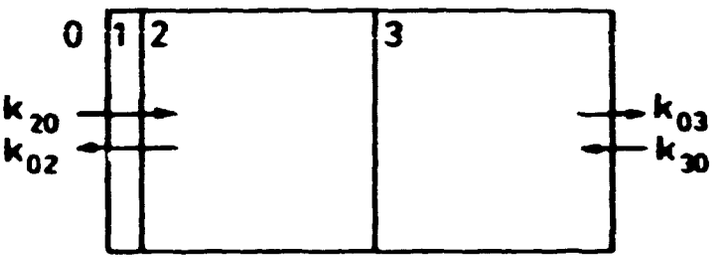

Figure 2.4.5.3. The "two-compartment model" exemplified in the text (double-exponential model). Accumulation of a radionuclide in accordance with this model can be expressed as equation (6). Cf. Table 2.4.5.1 for symbols.

i.e. as the ratio between influx (pCi/d) and the rate constant of outflux (d^{-1}) for the respective compartments. From the present experiments a rough estimate of the steady state concentration ratio can now be made, assuming that the model (figure 2.4.5.3 and equation (6)) is sufficient. The two equations above can be re-calculated to

$$CR_2^{ss} = \frac{\Delta CR_2 d^{-1}}{k_{02}(d^{-1})} \quad \text{and} \quad CR_3^{ss} = \frac{\Delta CR_3 d^{-1}}{k_{03}(d^{-1})} \qquad (7)$$

(cf. Table 2.4.5.1 for symbols). The total influx of ^{65}Zn (ΔCR_2 + ΔCR_3) in the 3-week experiment (Figure 2.3.3.2) was measured to 62.4 ml cleared per day and gramme of fresh soft parts, and from the loss-study (Table II of Appendix II) the relative size of the two exponential compartments ("medium"/"slow" ~ CR_2/CR_3) was estimated to 1.71 and the rate constants of the two exponential compartments (k_{02} and k_{03}) were measured to 0.1136 d^{-1} and 7.97 x 10^{-3} d^{-1}, respectively. The two equations (7) can now be solved giving steady state concentration ratios of 528 and 308, respectively, for compartments 2 and 3 which, including the intercept value of 16, gives a total steady-state concentration ratio of 852. In Figure 2.3.3.2 an accumulation curve in accordance with these results and the double-exponential model (Figure 2.4.5.3 and equation (6)) is included for ^{65}Zn (solid line). Apparantly the fitting to the experimental values is not as good as for the mono-exponential model (broken line), but it should be remembered that rate constants and relative sizes of the two compartments

are from a different experiment. It therefore demonstrates that the fitting of a mono-exponential model to an accumulation curve does not exclude the existence of a slow compartment. Pentreath (1973) fitted a mono-exponential model to his accumulation curves for Mytilus and found lower steady-state concentration ratios than expected from comparison with stable metal levels. He concluded that the higher concentration factors in the field were due to accumulation via food. In accordance with the above mentioned findings and the insignificant accumulation via food in these experiments (Chapter 2.3.3 and 2.4.6) the conclusion could alternatively be, that a slow compartment was not accounted for.

In Table 2.4.5.2 the above mentioned method has been used to calculate steady-state concentration ratios (or concentration factors) utilizing mean values from the loss study (Appendix II) and 10 accumulation experiments. The results should only be taken as a calculation example. A comparison with natural stable metal concentration factors is not possible as no comparable measurements were made on the experimental animal population and the corresponding sea water. It should furthermore be noted that values are calculated on the basis of influx results obtained with two different animal populations, two salinities and two temperatures (see e.g. Table 2.2.8.2) and that rate constants of outflux and relative size of compartments are from a semi-field experiment at 15 °/oo and changing temperature (Appendix II).

Table 2.4.5.2. Mytilus edulis, soft parts. Example of calculated steady-state concentration ratios (CR^{ss}, fresh weight) assuming two independent exponential compartments (2 and 3) (cf. Figure 2.4.5.3). Rate constants (k) and relative sizes of compartment 2 and 3 are from the loss study (Appendix II) and intercept and influx are mean values of 10 experiments (Tables 2.3.3.6 and 2.3.3.3) recalculated to fresh weight. Cf. text and Table 2.4.5.1 for further details.

	k_{02} (d^{-1})	k_{03} (d^{-1})	CR_2/CR_3	Influx ml $d^{-1}g^{-1}$ or a CR d^{-1}	CR_1 (Intercept)	CR_2^{ss}	CR_3^{ss}	$CR_{tot.}^{ss}$
^{65}Zn	0.1136	7.97×10^{-3}	1.71	725	30	1057	618	1705
^{57}Co	0.1333	3.63×10^{-3}	1.26	35.9	- 1	264	209	472
^{54}Mn	0.1005	1.24×10^{-3}	0.68	27.3	5	255	530	790
^{51}Cr	0.1359	4.59×10^{-3}	2.16	61.2	8	443	205	659
^{59}Fe	0.1650	4.05×10^{-3}	3.13	260	81	1561	699	2141
^{134}Cs	0.0912	-	-	2.53	1.5	27.7	-	29

Even if the model was assumed completely correct, the results will never be better than the basic data put into it. In the future more work should therefore be aimed at improving the different rate constants under relevant environmental conditions and at making the results more field comparable.

2.4.6. Effect of food, salinity, and temperature on uptake

The relative role of food and "water" in radionuclide accumulation by marine organisms has been a very controversial subject for years (see e.g. Polikarpov 1966, Lowman et al. 1971). One of the main aims when designing the experimental plan was therefore to make a reasonable approach to this problem for the accumulation of ^{51}Cr, ^{54}Mn, ^{57}Co, ^{59}Fe, ^{65}Zn, and ^{134}Cs in mussels.

As discussed in Chapter 2.4.1 it is not convenient to separate the routes of uptake in filter-feeding mussels between food and water. The amount of water passing the gills is decreased, if particle concentrations are too low, e.g. when the mussels are not fed. Thus, when the mussels are starved, accumulation via non-food particles and probably also from "solution" may be decreased. It was, therefore, decided to evaluate the role of food in radionuclide accumulation by comparing experiments with two relatively low food levels instead of with and without food.

As indicated in Chapter 2.3.3, the rates of accumulation of all 6 radionuclides were unaffected by food level. This finding is not thought to be affected by the changed experimental plan (cf. Chapter 2.2.8), as the two food levels for identical temperature and salinity were run almost without simultaneous changes in sampling locality and season. This indicates that accumulation of the 6 radionuclides via Phaeodactylum tricornutum is of minor importance as a route of uptake in mussels. Further studies should, however, be conducted to establish the actual contribution, as differences in rate of filtration made the differences in eating rate smaller and more variable than intended (Table 2.3.2.1). Furthermore, uptake via other algal species should be considered, as Nakahara and Cross (1978) reported differences in the retention of ^{60}Co by Mercenaria from 3 species of contaminated phytoplanktor. From the present experiments it can, however, be concluded

that accumulation via other sources than food, i.e. non-food particles and radionuclides in "solution", is likely to be the main route of accumulation of the 6 radionuclides in Mytilus. This conclusion disagrees with several authors (Pentreath 1973, Preston 1971, Phillips 1977a, Lowman et al. 1971) who assumed uptake from food to be the main route of metal uptake in mussels as laboratory experiments with starved animals resulted in too low a concentration factor as compared with stable metal field conditions. This lack of comparability has been observed by several other authors, but might alternatively be explained by, e.g., effects of starvation (cf. Chapter 2.4.1), bad laboratory conditions, and, perhaps, poor modelling (cf. Chapter 2.4.5). It should, however, be mentioned that no differences were observed in particulate (0.45 µm) activity between high and low algal concentrations (Chapter 2.3.1), i.e., the particulate activity was mainly associated with particles other than food. It is not known whether this situation is field comparable. If not, the above-mentioned conclusion might be erroneous.

For all 6 nuclides the rate of accumulation at 8 °/oo was higher than at 20 °/oo, although the salinity effect for ^{59}Fe was not significant (Table 2.3.3.5). However, due to the changed experimental plan (cf. Chapter 2.2.8) the effect of salinity cannot be separated from a possible effect of sampling location and other parameters that were different at the two sampling locations (Table 2.2.8.2). Of these, the different sizes of animals is thought to be the most important, as an increased rate of accumulation and a higher concentration factor of several metals have been reported for smaller sizes (Lucu and Jelisavcic 1970, Schulz-Baldes 1974, Boyden 1974 & 1977, Phillips 1977a, Fowler et al. 1978, Cossa et al. 1979, Harris et al. 1979, Ünlü and Fowler 1979). However, in stable field conditions the opposite relationship with weight have occasionally been observed for some metals (Bryan and Hummerstone 1978, Harris et al. 1979). An increased rate of accumulation for small animals may be explained by the higher metabolic rate (Zeuthen 1953) and the higher rate of filtration (Riisgård and Møhlenberg 1979) observed per gramme of small animals (cf. Chapters 2.3.2 and 2.3.3). As the two parameters show a comparable relationship with weight the measured rates of accumulation (Tables 2.3.3.1

and 2.3.3.3) have been weight-normalized (Tables 2.3.3.2 and 2.3.3.4) by the function found by Riisgård and Mohlenberg (1979) for the rate of filtration (Figure 2.3.2.1). From the normalized data a lower salinity effect appears indicating that size differences might have biased the results. Still, however, a significant effect is found for ^{51}Cr, ^{57}Co, and ^{65}Zn (Table 2.3.3.5). The normalized data for ^{134}Cs show a highly significant interaction between the effects of temperature and salinity due to an "inverse" temperature effect. However, for 15°C and 5°C separately, t-tests show a significant salinity effect on the accumulation of ^{134}Cs.

The effect of salinity on the rate of accumulation and the concentration factor of metals and radionuclides in marine invertertebrates is poorly investigated except for caesium, where an increased concentration factor with decreased salinity seems well documented (Bryan 1963, Wolfe and Coburn 1970, Lucu and Jelisavcic 1970, Amiard-Triquet 1974). In contrast to these studies, Phillips (1976a, 1977a, 1977b) was unable to see a salinity effect in laboratory experiments on the accumulation of zinc in Mytilus perhaps because of poor laboratory conditions. Phillips (1977c and 1979) measured higher zinc concentrations in mussels from the Baltic at low salinity than from the Swedish west coast at high salinity in spite of similar levels in the water, whereas the brown alga Fucus vesiculosus showed no differences. The conclusion was that Baltic phytoplankton concentrates metals more than phytoplankton from higher salinities, as Phillips assumed no true salinity effect on zinc accumulation in mussels and that phytoplankton was the major route of zinc uptake. As mentioned above none of these assumptions are consistent with this investigation. However, in the field the salinity effect cannot be separated from other factors varying with salinity. Although Mytilus edulis tolerates very low salinities, it has a lower growth rate and a smaller maximum size than at higher salinities (Seed 1976). Furthermore, animals with the same shell size often have lower soft parts weight and higher water contents at low salinities (see e.g., Table 2.2.8.2). As metal and radionuclide concentrations often vary inversely with animal size (see above), the high metal concentration in Baltic mussels should thus be explained not only by a salinity effect but also

by differences in the sampled animals, e.g. size and condition. As a salinity effect is very important for bioindicator studies in estuarine areas, a more determined effort should be aimed at the effects of salinity and of physico-chemical state of the metals which varies with salinity (see e.g., Mantoura et al. 1978 and Chapter 2.4.2), and therefore probably explains part of the observed effects of salinity on the accumulation of certain metals.

For all 6 nuclides, the accumulation rates were higher at 15°C than at 5°C, although only differences for ^{65}Zn, ^{134}Cs, and ^{57}Co were significant. This result is not surprising as metabolic rates are temperature dependent (see e.g. Bayne et al. 1976), and as the rate of filtration at 15°C was found to be 2.5 times higher than at 5°C (Chapter 2.3.2). A positive temperature effect on metal accumulation by marine bivalves has previously been reported for caesium, cobalt, iron, arsenic, and cadmium (Wolfe and Coburn 1970, Nakahara et al. 1977, Frazier and Ancellin 1975, Ünlü and Fowler 1979, Phillips 1976a), whereas Phillips (1976a and 1977b) found no temperature effect at all on zinc accumulation by Mytilus. However, as suggested for the salinity effect, Phillips' results might have been biased by laboratory conditions.

The "inverse" temperature effect observed for weight-normalized ^{134}Cs results at 8 °/oo (Table 2.3.3.4) and thereby the significant temperature-salinity interaction (Table 2.3.3.5) might be explained by an artifact caused by the changed experimental plan (cf. Chapter 2.2.8), as the two 15°C experiments at 8 °/oo were performed with smaller animals than the experiments at 5°C, and as the 15°C experiments were performed in December, whereas the 5°C experiments were performed in April. A seasonal effect or an inaccuracy in the weight normalisation function might thus be the explanation. The conclusion to be drawn from this woven explanation is that it is very important to make a feasible experimental plan.

2.4.7. Radionuclide exchange in the field

In this study, measurements of the loss of radionuclides from mussels were performed by repeated whole-body countings on

animals reared in the field after contamination in the laboratory (Chapters 2.2.6 and 2.3.4 and Appendix II). The biological half-lives were calculated for whole animals. Thus, the decrease in concentration due to growth did not influence the results as it would if the half-lives were calculated on the basis of activity per gramme of animal. As mentioned in Appendix II, the biological half-life of the long-lived component of ^{65}Zn during autumn 1979 (87 days) is identical with Young and Folsom's (1967) results without growth dilution (82 days) measured in the field whereas it is more difficult to compare with laboratory results (Van Weers 1973, Baudin 1973). Most results obtained in the laboratory are not directly field comparable (Patel 1975). Thus, the biological half-life excluding growth "dilution" for cadmium, mercury, and arsenic in mussels was found to be longer in laboratory experiments than in the field (Fowler and Benayoun 1974, Fowler et al. 1978, Ünlü and Fowler, 1979), presumably because of the higher metabolic rate assiciated with the higher growth rate in the field. This is further evidence for the need to establish natural food conditions in laboratory experiments (cf. Chapter 2.4.1).

The growth rate of mussels during one year in this study (Table I of Appendix II) is much smaller than the maximum growth rates measured during summer months (Kiørboe et al. 1981), but is in good agreement with comparable measurements over one year in several Danish Fjords (Theisen 1975). The results depicted in Chapter 2.3.4 and Appendix II are thus thought to represent real field conditions.

A seasonal variation of stable metal concentrations in bivalves is often recorded and several explanations have been proposed (Bryan 1973, Phillips 1976a and 1977a). Of these, differences in soft parts weight and growth rates are probably important (cf. Chapter 2.4.6). For zinc the rate of accumulatior declines with declining temperature (Chapter 2.3.3) and the loss rate is lowest during winter (Chapter 2.3.4). The combined seasonal effect of this, if any, is unclear. Although the apparent correlation with the temperature curve (Figure 2.3.4.2), could indicate a temperature effect, the long winter half-life for ^{65}Zn might as well be caused, at least partly, by lack of food during the dark winter resulting in low metabolic activity. This would be consistent

with the above-mentioned explanation that the different half-lives in field and laboratory experiments found by Fowler and co-workers could be explained by higher metabolic rate due to normal food conditions in the field. Furthermore, it is supported by the finding that the growth stop during winter resulting in year-marks on Mytilus shells is not caused by low temperatures but rather by lack of food as mussels feeding on suspended food particles in tne vicinity of marine fish farms in northern Norway grow all year irrespective of low winter temperatures (Wallace 1980).

Odum and Golley (1963) supposed the loss rate of ^{65}Zn to be so tightly correlated with metabolic rates that it could be used as a long-term "activity meter" in free-living organisms. This might be supported by the discussion of seasonal variation of the ^{65}Zn loss rate above. However, Hoss et al. (1978) concluded that the loss rate of ^{65}Zn is not a practical method for estimating field metabolism of fish as no correlation with, e.g., oxygen metabolism could be established. As several parameters other than temperature change with season, e.g. light, food amount and quality, growth rate, biochemical composition, and sexual activity (Jørgensen 1966, Zandee et al. 1980), the explanation of seasonal variation in metal metabolism and concentration in bivalves is probably complex.

In the future, an important task must be to elucidate the effects of environmental and physiological variations on metal and radionuclide accumulation, loss, and concentration in mussels and other bioindicators e.g. Fucus.

Contradictory conclusions should be elucidated by further data as the problems are important for the interpretation of bioindicator data.

3. BIOINDICATOR RESULTS ON DISTRIBUTION OF NUCLEAR POWER PLANT RELEASES IN TWO ESTUARINE ENVIRONMENTS, BARSEBÄCK AND RINGHALS, SWEDEN

3.1. Introduction

Since 1974-75 Sweden has operated nuclear power plants at Ringhals and Barsebäck on the Swedish west coast, and in 1976 neutron activated corrosion products were detected in bioindicators sampled in the estuarine environments near both sites (Aarkrog and Lippert 1977). From 1977 and onwards the controlled liquid discharges from Barsebäck and Ringhals have been utilized to investigate the feasibility of especially Fucus vesiculosus and Mytilus edulis as bioindicators of nuclear discharges. This ongoing project is performed in cooperation with the Department of Radiation Physics at the University of Lund and the National Swedish Environment Protection Board, Drottningholm, Sweden. The project is supported in part by grants from the Nordic Liaison Committee for Atomic Energy (Nordisk Kontaktorgan for Atomenergispørgsmål).

The main aim of these field investigations is to perform a practical test of the use of bioindicators and thereby to collect knowledge of their advantages and limits. It should be realized that accumulation- and loss-rates in the field are the "real values" with which laboratory results should be compared. Mainly due to excessively low discharges such comparisons have not yet been satisfactory.

Primary results from the Danish part of this work are published yearly (Dahlgaard 1978, 1979, 1980), and the results to be discussed here are furthermore included in Appendix III.

3.2. Materials and methods

Brown algae, benthic invertebrates, sediments and fish are sampled regularely at both sites. Samples were kept frozen until preparation, which consisted in drying at 105°C and grinding. Most fish samples were furthermore partly ashed (24 hours at 400°C). The grinded or ashed samples were packed in containers of up to 1700 milliliter carefully calibrated for gamma-spectrometric analysis. The countings were performed, usually overnight, with solid state detectors (Ge(Li)) connected to multichannel analyzers (1024 or 2048 channels). The detectors were placed in 10 cm lead shields. The activity concentration of the γ-active nuclides in the samples were calculated by computer (Lippert 1978).

The power plant companies report controlled radioactive discharges to the Swedish authorities monthly (Sydkraft, Vattenfall). The liuqid discharge data were calculated from a pooled sample of aliquots of all controlled discharges during the month. These monthly discharge data were utilized to calculate "transfer factors" to selected bioindicator samples. "Transfer factor" is here defined as a ratio between the activity concentration in a sample from a specified location and the discharge rate from the power plant averaged as indicated below. In the present work two different transfer factors have been used: the normal transfer factor (TF) and the decay-corrected transfer factor (DTF).

The normal transfer factor is calculated as

$$TF = \frac{A_i}{\frac{1}{m}\sum_{}^{m} D_j} \qquad \text{(pCi month kg}^{-1}\text{ mCi}^{-1}\text{)}$$

and the decay-corrected transfer factor as

$$DTF_m = \frac{A_i}{\sum_{}^{m} D_j e^{-\lambda(i-j)}} \qquad \text{(pCi (m months) kg}^{-1}\text{ mCi}^{-1}\text{),}$$

where A_i is the activity of a sample collected in month i (pCi kg^{-1} fresh weight), D_j is the discharge during month j (mCi $month^{-1}$), m is the number of months in the calculation and λ is the radioactive decay constant ($month^{-1}$). For the TF-calculations m is 12 months, whereas in the DTF-calculation m is chosen as the number of months before sampling which must be included in the calculation in order to make DTF-values for ^{60}Co and ^{58}Co equal. The "integration-time" is defined as this last m-value. If more months than the "integration time" are included in the calculation, DTF for ^{60}Co($T_{\frac{1}{2}}$ ~ 1922 d) will be smaller than DTF for the relatively short-lived ^{58}Co ($T_{\frac{1}{2}}$ ~ 71.3 d), as also ^{60}Co releases not included in the sample, are included in the calculation, whereas the corresponding ^{58}Co discharges have already mainly decayed. Calculation of the "integration time" by DTF-values are thus made on the assumption that the algae cannot distinguish between the two cobalt-isotopes, i.e. they are assumed to be in the same physicochemical state. Figure 3.2.1 exemplifies the estimation of the ingration time (m months).

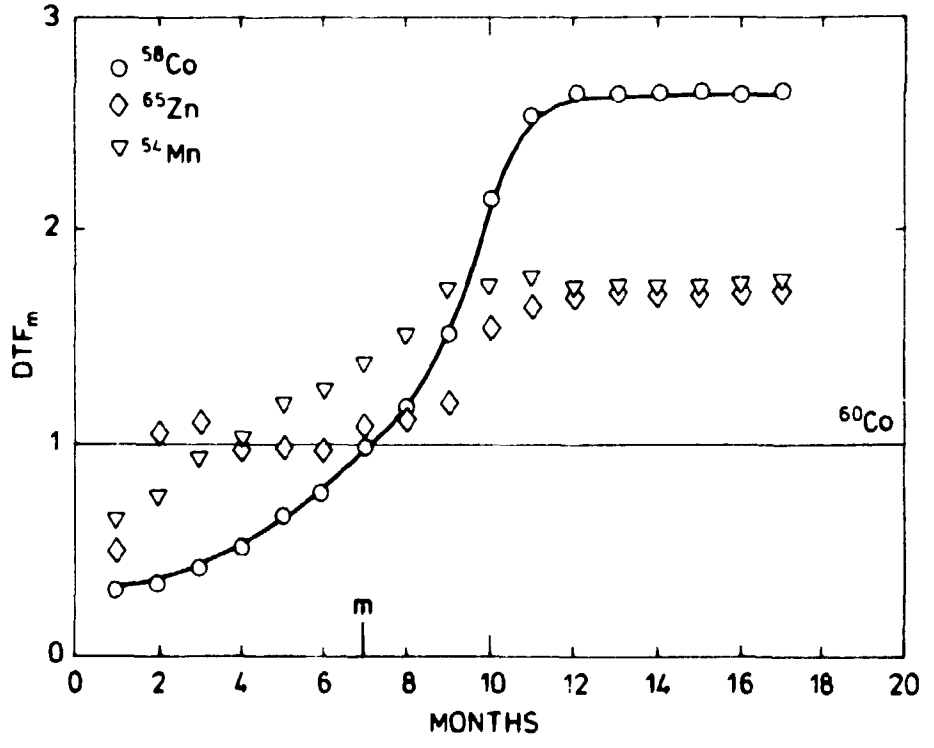

Figure 3.2.1. Calculation example showing the estimation of the integration time (m months) from calculations of the decay-corrected transfer factor (DTF) (cf. text). Values are normalized to DTF for ^{60}Co.

Data on these transfer factors have been published previously (Dahlgaard 1978, 1979, 1980) and the results will be summarized and discussed below.

3.3. Results and discussion

Activity decrease with distance

The variation with distance is pronounced for the nuclides discharged from the power plants. As an example Figure 3.3.1 shows the concentration of γ-active corrosion and fission products in Fucus vesiculosus collected near Barsebäck as a function of distance from the point of discharge. The plot illustrates not only the dilution of activity with distance but also the differ-

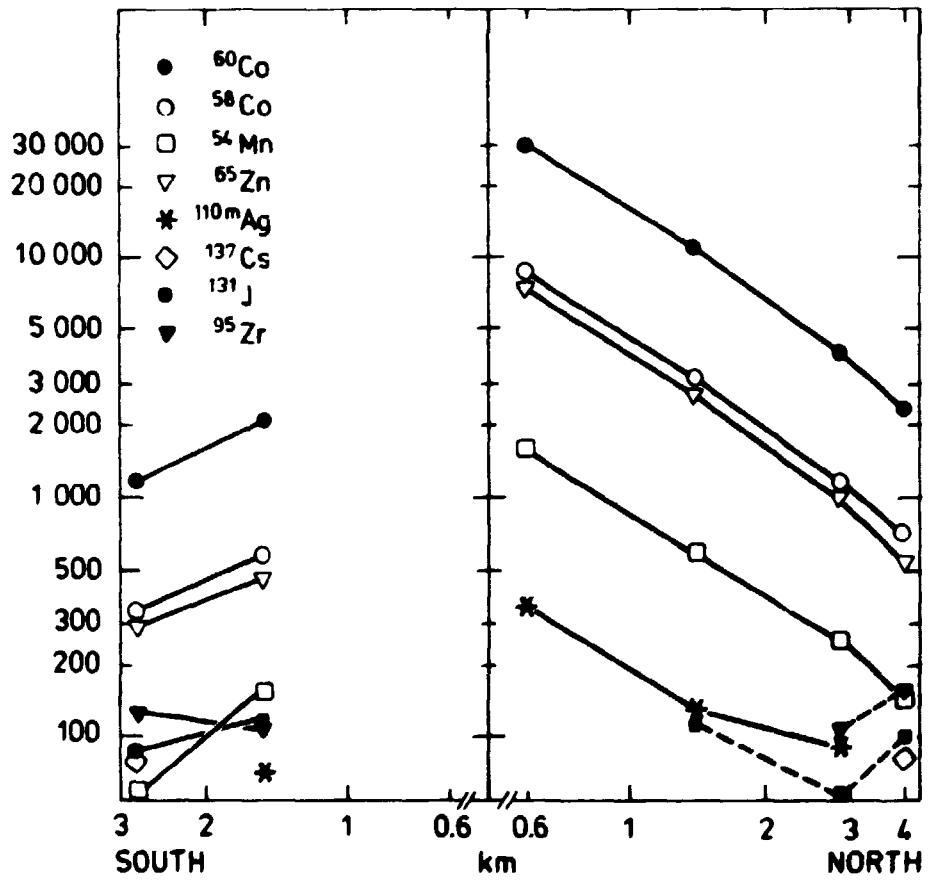

Figure 3.3.1. Effect of distance from point of discharge on radionuclide concentrations in Fucus vesiculosus (pCi/kg fresh) collected near Barsebäck September 1977. Cf. text and Appendix III.

ence between transport southwards and northwards, and it helps in the identification of sources.

The decreasing activity concentration northwards can, for ^{60}Co, ^{58}Co, ^{54}Mn and ^{65}Zn be described by a power function

$$A = k\ X^b$$

where X is distance from point of discharge in kilometers, k is a constant and b is found as the slope of a line of regression (ln activity concentration versus ln distance). From the results depicted in Figure 3.3.1 a mean b-value of - 1.33 was found. The power function has been used to describe the decrease in activity concentration 125 kilometers northwards from Barsebäck along the Swedish west coast at several instances since 1977 with a mean b-value of - 1.4 ± 0.1(SD) (Mattsson et al. 1980). If the decrease in activity with distance from Ringhals is described by the same power function, results from 1977 - 1979 (Appendix III) give b-values of -0.79 ± 0.09(SD) till 6.3 km northwards and -0.93 ± 0.10(SD) southwards till 4.1 km.

Assuming that the changes in environmental parameters along the Swedish west coast, e.g. the increase in salinity from ~10 °/oo to ~20 °/oo, are without significant importance for the accumulation of the nuclides and assuming that changes in physical and chemical form of the nuclides between 0.6 km and 125 km from Barsebäck are also without importance, the above mentioned function shows the approximate decrease in time-integrated radionuclide concentration in the water along the Swedish coast.

Preliminary unpublished experiments indicate that the accumulation of Co, Zn, Mn, Ag and Cs in Fucus vesiculosus might increase with decreasing salinity as was the case for Mytilus (cf. Chapters 2.3.3 and 2.4.6). This indicates that the decrease in water concentration might be overestimated by the Fucus measurements as salinity increases northwards from Barsebäck. An increased accumulation in brown algae with distance due to a time-dependent change in chemical speciation, as is demonstrated for ^{125}Sb-discharges from Cap de la Hague (Ancellin and Bovard 1979) is unlikely for cobalt, zinc and manganese as they

show similar patterns whereas ^{110m}Ag behaves different (see below). Provided the above mentioned effects are either insignificant or well described, the Fucus measurements give us a method of tracing water currents and pollutant dispersion. Figure 3.3.1 shows e.g. that most of the activity is transported northwards from Barsebäck. This is in agreement with hydrological measurements (Anon. 1979).

A detailed knowledge of the decreasing activity concentration in Fucus with distance can be used when calculating potential transport to man of the nuclides discharged, as the distribution function is probably similar for edible organisms. A direct measurement on fish samples from different locations along the coast would be impossible due to the low concentration in fish. An important problem in such a use of the Fucus measurements is that commercial fish and Fucus might be representing different water masses especially near the point of discharge.

The pronounced decrease in concentration of ^{58}Co, ^{60}Co, ^{54}Mn, ^{65}Zn and ^{110m}Ag (Figure 3.3.1) with increasing distance from the point of discharge identifies the source of these nuclides. For ^{137}Cs the source is identified as global fallout and Windscale discharges whereas the origin of ^{131}I and ^{95}Zr is uncertain. Although these nuclides could originate from the power plant, Figure 3.3.1 shows this is not the case. For ^{95}Zr the source was probably recent fallout from Chinese weapon tests, whereas ^{131}I was probably from hospital or laboratory discharges.

The transfer factor, TF

Table 3.3.1 shows mean values of the normal transfer factor (TF) from 1977 to 1979, i.e. ratios between activity concentration in Fucus (pCi/kg fresh) and mean discharge rate averaged over the preceding 12 months (mCi/month). More detailed data can be found in Dahlgaard 1978, 1979 and 1980. In order to facilitate a comparison, the values are recalculated to the same distance north of Barsebäck and Ringhals, respectively, by the power functions mentioned above.

Table 3.3.1. Transfer factors (TF) from discharge (mCi/month) to Fucus (pCi/kg fresh). Mean values 1977-1979 recalculated to 2,9 km north of Barsebäck and Ringhals, respectively (cf. text). Data from Dahlgaard 1978, 1979, 1980. Unit: pCi month kg^{-1} mCi^{-1}. Error term: ± SE.

	Barsebäck	Ringhals	ratio Barsebäck/ Ringhals
^{60}Co	217±38 (n=10)	8.5±1.7 (n=5)	26
^{58}Co	83±21 (n=10)	3.7±0.8 (n=5)	23
^{54}Mn	138±18 (n=10)	9.7±3.1 (n=5)	14
^{65}Zn	204±38 (n=10)	26 ±8 (n=5)	8
^{110m}Ag	23± 4 (n=7)	46 ±28 (n=4)	0.5

The discharge from the nuclear power plants oscillates throughout the year showing distinct peaks during and after the yearly refuelling and servicing which normally takes place during late summer at Barsebäck and in spring and late summer at the two Ringhals reactors. Apparently the transfer factors show a similar oscillation.This is not surprising as discharges in the start of the period in calculation (12 months) are expected to give a lower TF-value than discharges just before sampling, since the TF-calculation does not correct for radioactive decay, dilution with growth, and loss of activity from the Fucus plants. Similar seasonal variations have been observed for stable metals in Fucus (Van Weers 1972, Fuge and James 1973). It is thus not simple to distinguish between the effects of seasonal variation on metal or radionuclide accumulation, growth rate and other eco-physiological parameters on one side and effects of the yearly discharge patterns on the other.

However, from the present data (see e.g. table 3.3.1), the order of magnitude of activity expected to be found in one kg of Fucus after a certain release is known for Barsebäck and Ringhals. In case of an accidental release of unknown magnitude these data, together with the analysis of some Fucus samples, can be used to

estimate the order of magnitude of the release and furthermore, after comparison with existing data on activity ratios between Fucus and edible organisms, quickly indicate if fishing restrictions are required on radiological reasons. Due to the above mentioned variation in the calculated transfer factors, giving highest values within the first weeks after a discharge, an estimate of an accidental release on the basis of mean values (e.g. from Table 3.3.1) will be too high, whereas peak-values are expected to give a more correct answer.

As mentioned above the decrease with distance from the point of discharge is different for different sites, depending on local hydrological conditions. Furthermore, Table 3.3.1 shows a pronounced difference in transfer factors recalculated to the same distance from Barsebäck and Ringhals, respectively. This could perhaps be explained by differences in local hydrological conditions, whereas the salinity effect (~10 °/oo at Barsebäck and ~20 °/oo at Ringhals) is probably too small to contribute significantly. The difference between Barsebäck and Ringhals is, however, not the same for different nuclides. This can only be explained by differences in physical or chemical form of the nuclides discharged from the two plants or perhaps partly by different "speciation" patterns due to different water qualities. This indicates clearly, that results obtained from one site should not be used to describe another site, without supplementary investigations.

One might get the impression from Table 3.3.1 that ^{110m}Ag behaves similarly at the two sites and that the other nuclides show greater differences. If, however, hydrological conditions result in a greater dilution at Ringhals than at Barsebäck, ^{110m}Ag is in fact accumulated to a higher level at Ringhals than at Barsebäck probably due to differences in chemical form. A hypothesis could be that ^{110m}Ag is released from Barsebäck in a less available form than from Ringhals, and that the Barsebäck-silver during its transport northwards along the Swedisn coast slowly "dissolves" to a more available form, as was clearly demonstrated for ^{125}Sb released from Cap de la Hague (Ancellin and Bovard 1979). This would be consistent with the slower decrease of activity

in Fucus with distance from Barsebäck observed for ^{110m}Ag as compared to ^{58}Co, ^{60}Co, ^{65}Zn and ^{54}Mn, all of which show a similar dependence on distance, as mentioned above (Nilsson et al. 1981).

The decay-corrected transfer factor, DTF

As mentioned above some of the variation in the normal transfer factor (TF) is due to physical decay of nuclides released some months before sampling. As an example Table 3.3.1 shows that TF-values for ^{58}Co ($T_{\frac{1}{2}}$ = 71 days) are lower than values for ^{60}Co ($T_{\frac{1}{2}}$ = 1922 days). Assuming similar behaviour of the two isotopes and therefore similar TF-values immediately after a single release of activity, the explanation would be that the ^{58}Co accumulated e.g. 5 months before sampling has decayed to approximately 25% (approximately 2 half-lives), whereas 95% of the ^{60}Co accumulated at the same time is still present. By calculating the decaycorrected transfer factor, DTF (cf. Chapter 3.2), this difference is utilized to make an evaluation of the period during which the Fucus plants have actually "integrated" the discharges (m months). Values of the decay corrected transfer factor, DTF_m (pCi (m months) kg^{-1} mCi^{-1}) represent the radioactive concentration (pCi kg^{-1} fresh weight) found in Fucus after a decay corrected discharge accumulated over m months to one mCi. Thus, DTF_m values will ideally show the transfer of the metals independent of the decay constant of the specific isotope.

Table 3.3.2 shows values of the decay-corrected transfer factor (DTF) and the corresponding integration time (m months) calculated at several instances during 1977-1980 for one location near Barsebäck. It is seen that the integration time, m, varies from 4 to 16 months and it is also seen that the DTF-values show a seasonal variation which, as for the TF-values, might be explained partly by oscillating discharge patterns.

Preliminary, unpublished laboratory experiments indicate that Co and Zn might be accumulated partly "irreversible" by Fucus vesiculosus, whereas the loss of Mn, Cr and Ag is measureble. Irreversible accumulation of Zn in brown algae has been suggested earlier (Young 1975, Skipnes et al. 1975, Gutknecht 1963, Bryan

Table 3.3.2. Decay-corrected transfer factors, DTF. Fucus vesiculosus collected at Barsebäck, location 26, 1.4 km north of the outlet. (Unit: pCi (a months) kg^{-1} wCi^{-1})

Date of sampling	770615	771022	780204	780417	780615	780908*	781210	790406	790619	790907
m month	7.5	10.7	11.2	8	8	6	10	13	16	16
^{60}Co	20.8	20.6	19.8	3.33	8.13	22.5	29.9	20.6	7.1	9.2
^{58}Co	20.1	21.1	19.9	3.30	7.67	22.5	30.3	20.6	7.1	8.7
^{54}Mn	30.8	17.3	18.1	3.22	9.01	15.3	18.6	12.3	7.8	7.9
^{65}Zn	30.8	21.3	22.8	5.16	9.36	22.8	46.8	30.9	19.7	19.2
^{110m}Ag		1.90		0.66			3.74	2.53	1.45	1.88
^{51}Cr				8.77				15.6		

* Mean of 2 samples.

1969). Field experiments with Ascophyllum nodosum indicates however, that Zn is actually lost, at least from this species, but with a very long half life (Eide et al. 1980). Wheather this is also the case for Fucus remains to be documented, as none of the above mentioned experiments were run for so long periods of time that biological half-lives above a few months could have been detected. The biological half-lives of Zn and Co are, however, in any case so long, that a dilution of the activity concentration with new biomass due to growth is probably more important than a loss of activity from the plants, at least during the spring and summer. Thus Brinkhuis (1977) measured up to a 5-fold increase in biomass of young Fucus vesiculosus plants during two spring months and estimated the biomass to "turn over" twice per year on average. If biological half-lives are measured on an activity per unit weight basis in a growing population, this growth dilution is included in the estimate. In the above mentioned loss-experiments growth dilution was not included.

The long biological half-lives indicate that the estimated periods of integration (m in Table 3.3.2) might be correct. Much more data should, however, be gathered and analyzed before a proper evaluation of the DTP-method is possible. If the cross-point determining the integration time (cf. Figure 3.2.1) should actually fall in a period with extremely low discharges of radiocobalt, the estimate will be un-precise as the two lines will run nearly parallel. Another problem has been that in several samples

from Ringhals the $^{58}Co/^{60}Co$ ratio has been too high compared to discharge reports (cf. Dahlgaard 1980). This might indicate differences in speciation of the two cobalt isotopes from this plant or simply inaccuracy in the reported discharges. For this purpose discharge reports should be much more precise than required from a radiological protection viewpoint.

Mussel data

When this project started it was the idea to compare field measurements with laboratory results for mussels (cf. Section 2) in order to explain observed variations in the field and in order to evaluate the field comparability of data obtained in the laboratory. It was, however, difficult to get sufficiently large mussel samples near Barsebäck, and discharges were too small for distant measurements. Mussel data from Ringhals are summarized in Table 3.3.3 as a comparison with Fucus. It is seen that even ^{65}Zn, which is quickly accumulated to high levels in mussels, is found in higher concentrations in Fucus. When furthermore samples up to 5-6 kg (fresh) have been measured, it is obvious that Fucus is much easier to sample and process in sufficient amounts for this purpose than Mytilus.

Table 3.3.3. Activity ratios on fresh weight basis, Mytilus edulis soft parts to brown algae (Fucus vesiculosus and Fucus serratus) collected at Ringhals 1977-1979.

Isotope	Mean ± SE
^{58}Co	0.19 ± 0.05 (n=7)
^{60}Co	0.17 ± 0.03 (n=8)
^{54}Mn	0.08 ± 0.02 (n=2)
^{65}Zn	0.32 ± 0.04 (n=6)
^{110m}Ag	0.24 ± 0.14 (n=3)
^{137}Cs	0.38 ± 0.08 (n=5)

In order to improve the measurements on Mytilus, several thousands of young individuals were filled in nettings developed for commercial mussel farming (Bøhle 1974) and transplanted to buoys near the discharge pipe at Barsebäck. The idea was to measure on a population which had been present in the area during a well known period of time and afterwards to measure excretion of the nuclides after transplanting the mussels to a non-contaminated area. This project failed as the mussels disappeared from the nettings during periods with rough weather. In the future these experiments should be reconsidered near Ringhals where conditions for mussels are much better (higher salinity). Such studies will facilitate an important evaluation of the excretion measurements on laboratory-contaminated mussels (cf. Chapters 2.3.4 and 2.4.7 and Appendix II).

4. CONCLUSIONS

The experiments presented here have shown, that it is possible to make accumulation experiments with Mytilus edulis in the laboratory under reasonably natural feeding conditions, i.e. at a constant, low concentration of phytoplankton. As the filtration (phytoplankton clearance) may stop or be decreased when laboratory conditions do not fit the animals, e.g. if water quality is bad or if no particles are present in the water, natural food levels are indispensable in metal or radionuclide accumulation studies on suspension feeding bivalves. Furthermore, by maintaining a constant food level the clearance rate can be calculated and used as a measure of natural filtering activity.

It was found that the accumulation of the 6 radionuclides via contaminated food (Phaeodactylum tricornutum) by Mytilus was insignificant as compared to an accumulation directly from water and via non-food particles.

In laboratory experiments with Mytilus decreasing salinity and increasing temperature elevated the influx (initial rate of accumulation) of the nuclides. In the field, the biological half life of ^{65}Zn was longer during the winter than in spring and autumn. The combined effect of these findings is to be studied further. Bioindicator programmes with Mytilus can give useful information. It can however be concluded, that seasonal effects and local conditions, such as salinity, have to be taken into account.

Field results have shown, that for low levels of radioactive releases Fucus may be a more sensitive bioindicator than Mytilus.

Transfer factors from controlled discharges to Fucus can be used to estimate the magnitude of an uncontrolled discharge, especially if a long time-series of data obtained with controlled discharges can elucidate variation between seasons and years. Differences between Barsebäck and Ringhals shows clearly, that data

from one site should not be used for another site without supplementary investigations.

Still much work remains to be done before the total effect of environmental parameters on the performance of Mytilus and Fucus as bioindicators can be satisfactorily described. The continuation of this bioindicator research will aim at describing the effect of environmental parameters such as season and salinity-conditions on the performance and limits of Mytilus and Fucus as bioindicators for several radionuclides and metals. Much of this future work has to be done in the laboratory, but in order to elucidate and improve field-comparability, as much data as possible will be gained from bioindicator programmes utilizing "tracers" originating from nuclear power plants, fuel reprocessing plants and testing of nuclear weapons.

It can be concluded, that in spite of some lacking data on the bioindicator organisms, monitoring programmes can give much valuable information not only on the pollution to be monitored but also on the organisms used.

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APPENDIX I

OCEAN SCIENCE AND ENGINEERING, 6(1), 59-69 (1981)

A SENSITIVE TURBIDITY CONTROLLER FOR PHYTOPLANKTON SUSPENSIONS APPLIED TO BIOACCUMULATION STUDIES OF MUSSELS

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KEY WORDS: Filter-feeders, Mussels, Mytilus, Phytoplankton, Suspension, Turbidity, Bioaccumulation

ABSTRACT

An apparatus designed to maintain phytoplankton cell concentrations at a constant and very low level in long-term bioaccumulation studies with suspension-feeding mussels is described. Phytoplankton concentrations of 2000 Phaeodactylum tricornutum cells/ml (approximately 24 x 10^{-9} g organic dry weight/ml) can be maintained for weeks. The set up facilitates continuous control of animal condition by measuring the clearance rate. Circuit diagrammes are given and results are presented to demonstrate the performance.

INTRODUCTION

Marine and estuarine bivalves, especially the common mussel Mytilus edulis, are widely used as "bioindicators" in monitoring pollution of estuaries and coastal waters with radionuclides, metals,

petroleum hydrocarbons and halogenated hydrocarbons (Goldberg et al., 1978, Phillips, 1977). The use of mussels as bioindicators demands field-comparable experiments on the effects of environmental variables on accumulation and loss of pollutants by the mussels.

The natural way of life for a mussel is to filter huge volumes of water continuously with low concentrations of phytoplankton. If the concentration of particles becomes too high, the mussels react by creating "pseudofaeces", i.e. a certain part of the filtered material is discharged before it enters the digestive tract. For phytoplankton concentrations of 0.5 mg organic dry weight/l, heavy pseudofaeces production has been observed (Riisgård and Møhlenberg, 1979). It is therefore necessary to maintain concentrations well below 0.5 mg/l, and the apparatus described here was developed to maintain concentrations as low as 2000 Phaeodactylum tricornutum cells/ml, i.e. approximately 0.024 mg dry weight per liter.

Winter (1973) described an apparatus for maintaining constant concentrations of phytoplankton cells in filtration experiments with Mytilus, and an improved set-up was described by Riisgård and Møhlenberg (1979). The present apparatus was designed to operate at even lower concentrations and for longer periods of time. In addition to continuous filtration rate measurements, the apparatus was designed for radionuclide accumulation experiments (Dahlgaard, 1979). Thus it is possible to remove some animals from the set-up to measure the radionuclide concentration without seriously disturbing the running experiment.

A description of the instrument design is given and results are presented to demonstrate its performance.

EXPERIMENTAL SET UP

The laboratory set-up used for radionuclide uptake experiments with Mytilus edulis is depicted schematically in Figure 1. Mussels, which are attached to perspex baskets by their byssus threads, are placed in a small mussel-aquarium containing approx. 0.8 l seawater. The experimental water is circulated by a siphoning system from a photoaquarium, via the mussel-aquarium to a settling aquarium at a flow rate of 6.5 l/min. The water from the settling aquarium is pumped back to the photoaquarium via a glass heat exchanger that

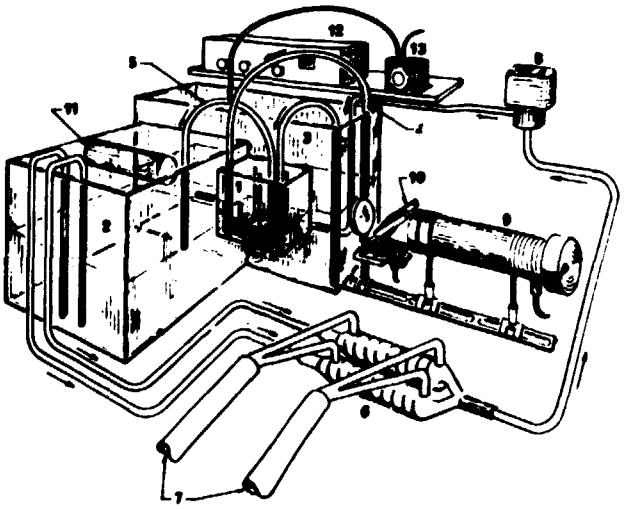

FIG. 1. The laboratory set-up, schematically. (1) Mussel-aquarium, (2) settling aquarium, (3) phoaquarium, (4+5) siphoning system, (6) heat exchanger, (7) antifreeze-liquid, (8) pump, (9) quartz lamp light source, (10) beam splitter, (11) measuring photodiode, (12) level control amplifier, (13) peristaltic pump.

keeps the water at a constant preset temperature (± 0.1° C). Increasing background turbidity and bad water quality is compensated by a continuous injection of 4.6 l of fresh seawater per day. The total amount of experimental water is kept constant at 30 l by an overflow arrangement. The water is aerated by bubbling with compressed air.

The relatively large volume of the settling aquarium (approx. 17 l) provides for settling of faeces. The photoaquarium is equipped with a photometric measuring system that controls the turbidity. An electronic turbidity control amplifier provides compensation for declining turbidity caused by the mussels' filtering activity. It controls a peristaltic dosing pump that injects phytoplankton suspension from a chemostatic culture to the experimental water. An algal concentration at a preset level is thus maintained. A coulter counter, model Z_B, is used for calibration of the apparatus to selectively maintain the desired concentration of algal cells and to further control this concentration throughout the experiment.

Materials in contact with the seawater are glass, silicone-rubber and perspex which have been found to be non-toxic to marine organisms (Bernhard and Zattera, 1970). In addition an Eheim aquarium pump and nylon fittings are used.

The electronic turbidity controller system basically consists of a photometric measuring system in connection with a servo amplifier. The photometric system which is attached to the photoaquarium (see figure 2) consists of a halogen quartz lamp in a cylindrical housing, a beam splitter, focusing lenses, a reference photodiode

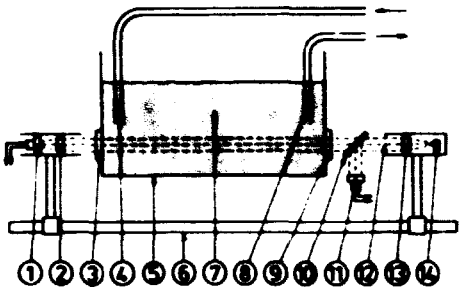

FIG. 2. The photoaquarium, schematically. (1) Measuring photodiode, (2) focusing lens, (3) petri dish, (4) suspension inlet, (5) photoaquarium, (6) optical bench, (7) experimental algal suspension, (8) suspension outlet, (9) petri dish, (10) beam splitter, (11) reference photodiode, (12) diaphragm, (13) optics, (14) quartz lamp.

and a measuring photodiode; all are mounted on an optical bench physically separated from the aquarium. The beam splitter placed in the light-path directs approx. half of the light to the reference photodiode; the remaining fraction passes the photoaquarium before it is detected by the measuring photodiode. The difference signal between the two detectors is a measure of the turbidity and is thus independent of variations of the light source.

In order to minimize problems with dew, two perspex Petri dishes containing a few dry silica-gel crystals were glued onto the aquarium walls in the light path. The light passing through the aquarium is focused by optical lenses to optimize the light quantity and to avoid interference with other light sources. The set-up is shielded against direct sunlight.

The signals from the two detectors are fed to the level-control amplifier shown schematically in Figure 3. The two photodiode signals are amplified by identical input amplifiers with equal am-

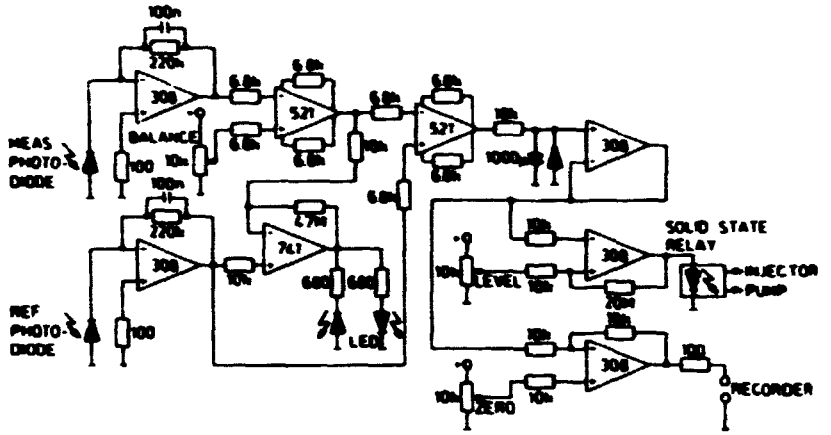

FIG. 3. Circuit diagram of the level-control amplifier.

plifications thus correcting for errors caused by temperature drift. The outputs are fed to a comparator that allows for an initial zero-set by balancing two light-emitting diodes. An injection of algal suspension produces a difference signal which is proportional to the preset algal concentration. The difference signal is further amplified and fed to a final comparator which activates the algal dosing pump via a solid-state relay when a selectively preset concentration-level is exceeded. To obtain a graph of the turbidity variations a separate amplifier provides an output signal suitable for a recorder.

An opto-coupler on the injector pump counts the revolutions which are registered on an electromagnetic counter; this indicates the injection rate.

The rate of phytoplankton clearance (ml/min/animal) was calculated from the formula

$$F = \frac{a}{t\ n}\left(\frac{C_s}{C_e} - 1\right) - \frac{r}{n}$$

where a is ml algal culture injected during t minutes, C_s = algae/ml culture, C_e = algae/ml experimental seawater, n = number of animals and r = rate of water exchange (ml/min) (Riisgård and Mohlenberg, 1979). The formula allows for dilution of experimental water with algal medium and fresh seawater and for removal of algal cells by overflow and water sampling.

PERFORMANCE AND RESULTS

The function and performance of the set-up is exemplified by briefly describing a radionuclide uptake experiment lasting for 3 weeks.

Mussels (Mytilus edulis) were sampled at 17° C and were acclimated to the experimental temperature (10° C) for 2 days only. Salinity was 14 o/oo in the experiment and in the sampling area as well. The mean dry weight of soft parts was 170 mg. 24 mussels on perspex baskets were placed in the aquarium the day before algal injection. The initial decrease in experimental seawater turbidity caused by the mussels filtering activity is of the same order of magnitude or even larger than the increase in turbidity caused by the added phytoplankton cells. Consequently a constant phytoplankton concentration is maintained only after this drop in seawater turbidity has equilibrated. Radionuclides were added at time zero and phytoplankton concentration was brought up to the desired level by adding algal culture. After an initial zero setting of the ampli-

fier, the algal injector was turned on. During the rest of the experiment, the apparatus maintained a constant turbidity.

Clearance rates calculated during the 3-week experiment are shown in Figure 4. A linear analysis of regression shows that clearances do not change with time. The rate of clearance is thus not affected by the number of animals or by the duration of the experiment.

Phytoplankton cells were taken from a chemostatic culture of Pheaodactylum tricornutum. According to Riisgård and Mahlenberg (1979), the organic dry weight of cells in the same stock culture was 12.0×10^{-12} g/cell.

The sensitivity of the turbidostatic equipment to changes in turbidity and its accuracy in maintaining a constant algal concentration when "background turbidity" is constant, can be expressed as the difference in algal concentration before the start and after the stop (Δ start/stop) of the peristaltic pump injecting algal culture. In the actual 3-week experiment Δ start/stop was

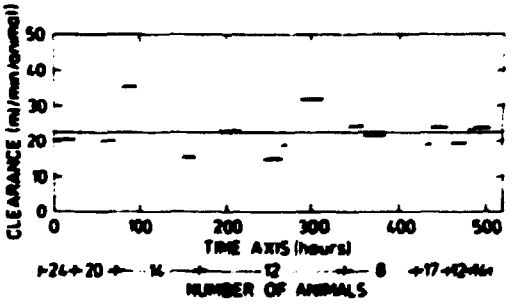

FIG. 4. Clearance rates for Mytilus edulis measured during a 3-week experiment. The line of regression is indicated.

431 ± 39 (SE, n=12), algal cells/ml, which is 18% of the mean concentration maintained during the experiment (2391 cells/ml). The sensitivity appeared to be dependent on an accurate calibration of the lamp and the optical unit. An earlier experiment showed a Δ start/stop of 106 ± 19 (SE, n=7) cells/ml, which is 4% of the controlled mean concentration (2371 cells/ml). This sensitivity is very high as 106 Phaeodactylum tricornutum cells/ml correspond to 1.3×10^{-9} g organic dry matter/ml.

The exact measurement of the filtration rate is based on the assumption that the mussels do not recirculate water which has already been filtered by other mussels. Results depicted in Figure 5 demonstrate that the total rate of clearance, measured with the actual set-up, is independent of the pumping rate. Recirculation is thus effectively prevented by pumping at a rate of 6.5 l/min through a small mussel aquarium (0.8 l).

DISCUSSION

The benefits of using the apparatus described above in long-term filtration experiments with suspension feeding bivalves are obvious. The apparatus is also excellent for the study of pollutant uptake via particles as the filtering activity of the mussels is continuously recorded.

High sensitivity and stability are obtained by the establishment of the relatively long light path (60 cm) and the use of an optical bench system combined with the specially designed noiseless servo amplifier.

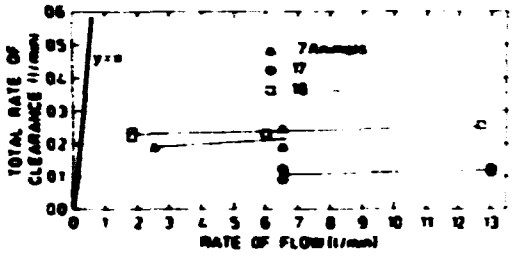

FIG. 5. Total rate of clearance as a function of flow rate through the mussel aquarium.

As the background turbidity is kept constant by the settling arrangement and the continuous reneval of the experimental water, the set-up will maintain an ultra low concentration of algal cells even in long-term experiments. Furthermore, this arrangement meets the need for an acceptable and constant water quality. The prevention of recirculation, see figure 5, is very important. If mussels recirculate already-filtered water, the clearance rate will consequently be lower. This may give seriously misleading results in filtration experiments (see e.g. Riisgård, 1977). In pollutant accumulation experiments recirculation not only decrease the uptake via particles but also increase the individual variation.

We have considered a more efficient settling tank formed as a cone with continuous removal of the precipitate from the pointed bottom. Furthermore, the sensitivity and stability of the turbidostatic equipment can be raised by 1) reflecting the light beam by a mirror whereby the light path is doubled and 2) by installing the measuring photo-diode in the same metal block as the reference photo-diode to avoid operating the two photo-diodes at different ambient temperatures.

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APPENDIX II

INTERNATIONAL ATOMIC ENERGY AGENCY
OECD NUCLEAR ENERGY AGENCY

INTERNATIONAL SYMPOSIUM ON THE IMPACTS
OF RADIONUCLIDE RELEASES INTO THE MARINE ENVIRONMENT

Vienna, 6–10 October 1980

IAEA-SM-248/ 106

LOSS OF ^{51}Cr, ^{54}Mn, ^{57}Co, ^{59}Fe, ^{65}Zn and ^{134}Cs BY THE MUSSEL Mytilus edulis.

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IAEA-SM-248/106

LOSS OF ^{51}Cr, ^{54}Mn, ^{57}Co, ^{59}Fe, ^{65}Zn and ^{134}Cs BY THE MUSSEL Mytilus edulis.

ABSTRACT

The loss of ^{51}Cr, ^{54}Mn, ^{57}Co, ^{59}Fe, ^{65}Zn and ^{134}Cs from naturally growing mussels (Mytilus edulis) was followed in a temperate estuarine environment - a Danish fjord - by individual whole-body countings on a Ge(Li)-detector. The mussels accumulated the radionuclides in the laboratory from food and water and were brought back to their natural environment in small plastic cages.

The loss curves for 12 animals from July-August 1979 till November 1979 (20-5°C) were resolved in a slow compartment with 140-215 days biological half-life for ^{57}Co, ^{54}Mn, ^{51}Cr and ^{59}Fe, and 87 days for ^{65}Zn, and a medium compartment with a biological half-life of 4 to 7 days for all nuclides.

The long-lived compartments of ^{65}Zn, ^{57}Co and ^{54}Mn were followed in 4 individual animals from August 1979 to August 1980. For ^{65}Zn a seasonal effect was clearly demonstrated as the biological half-life was prolonged from 87 days during autumn 1979 to 347 days in the cold period (0 - 5°C), whereas it decreased again during the summer of 1980. For ^{57}Co and ^{54}Mn the long-term excretion study revealed an extra-slow compartment, as the long half-life in the cold period (~ 600 days) persisted during the summer of 1980. This is explained by association with the shell.

1. INTRODUCTION

The common blue mussel, Mytilus edulis, is widely used as a bioindicator when monitoring several pollutants in coastal marine and estuarine environments [1,2]. Measurements of pollutant levels in mussels or other bioindicators are often preferred for water analysis as fluctuating levels are integrated in the bioindicator tissues over a period of time and as levels are higher due to accumulation. Furthermore, for radioactive pollutants dose commitment to humans can be calculated, as Mytilus is edible.

Uptake and excretion of pollutants may, however, vary under differing environmental conditions, e.g. season. Knowledge of such variations from field or field-comparable experiments are very important when bioindicator data are to be interpreted. This paper reports a study of loss of several radionuclides from mussels contaminated in the laboratory and transferred to the natural environment, where the excretion from individual animals was followed for one year.

2. MATERIALS AND METHODS

2.1. Accumulation of radionuclides

The excretion experiments described here were conducted as the termination of a series of accumulation experiments in the laboratory. Mytilus edulis averaging 3.5 cm in length were collected in Roskilde Fjord at a salinity of 14-15 o/oo and kept in the laboratory for 3 days at the experimental temperature and salinity before ^{51}Cr, ^{54}Mn, ^{57}Co, ^{59}Fe, ^{65}Zn and ^{134}Cs were added in the chloride form. Two animals were contaminated 2 hours at 5°C, 20 o/oo, 6 animals were allowed to accumulate the radionuclides for 3 days at 15°C, 20 o/oo, and 4 animals accumulated for 21 days in 10°C, 14 o/oo. The animals accumulated the radionuclides from both water and food. A constant and low level of contaminated phytoplankton (Phaeodactylum tricornutum) was maintained during the accumulation by means of a turbidostatic set-up described elsewhere [3]. Thus, the radionuclides were accumulated in a more field-comparable way than in experiments without feeding, and the condition of the animals was continuously controlled by calculating the filtering activity (phytoplankton clearance). Results from the experiments on accumulation of the 6 radionuclides as a function of salinity, temperature and food level will be published elsewhere.

2.2. Excretion and whole-body counting

After accumulation, the animals were washed in running water, prior to the start of the first whole-body counting. All activity concentrations have been decay corrected to the beginning of the experiment and are calculated as a percentage of the initial counting. The animals were recounted after 1-2 hours, 1 day, 3 days, 1 week, and after that weekly. After accumulation the animals were whole-body counted and reared in small cages with sufficient holes to ensure free passage of water. The cages were constructed of 75-mm long Perspex tubings, inner diameter 24 mm, with 12 10-mm holes. The ends were secured with a grid of nylon threads or plugged with a holed polypropylene cap. The caged animals were placed one meter below mean water level in Roskilde Fjord at Risø National Laboratory. The animals were out of the water only during the weekly counting for 1-2 hours. The whole-body countings were performed on a Ge(Li)-detector connected to a 1024-channel analyzer ensuring resolution of the different radionuclides.

The 4 animals contaminated for 3 weeks had accumulated so much of the radionuclides that the excretion could be followed for one year. Growth data for these animals are reported in Table I where they are compared with 15 animals originally from the same experiment and originally also with the same shell size. Assuming exponential growth the growth-constant for the soft parts during one year was 0.0034 d^{-1} based on fresh weights from Table I. However, as the excretion was measured by a total whole-body counting, the growth dilution does not contribute to the biological half life. The remaining 8 animals in the experiment were killed in the autumn of

1979, as activity levels were too low for continuous counting.

During the loss measurements from July/August 1979 to August 1980 salinity in the Fjord was 14-15 o/oo. A temperature curve is shown in Figure 1. From the middle of December to the end of February the Fjord was mainly ice-covered and the water over the mussel-cages were prevented from freezing by a flow of compressed air.

3. RESULTS

Biological half-lives based on weekly whole-body countings of 12 individual mussels from July/August till November 1979, (20 - 5°C) are reported in Table II.

Caesium was accumulated to "concentration factors" of approximately 10 and was lost so rapidly that the excretion could be followed for a few weeks only. Half of the activity was lost with a biological half-life of 7.6 days, whereas the rest was lost very quickly.

The other nuclides showed "concentration-factors" (soft parts, fresh weight basis) after 3 weeks of uptake of up to 100 for ^{54}Mn, 450 for ^{65}Zn and ^{57}Co, 1500 for ^{51}Cr, and 2500 for ^{59}Fe. As indicated in Table II, 15 - 40% of this initial activity was lost very quickly with half-lives below 1 day. The remaining loss curve was resolved in two compartments with medium and slow loss rates. The medium compartment comprising 20 - 55% of the initial activity shows a biological half-life for all nuclides of 4 - 7 days, whereas the slow compartment including 15 - 40% of the initial activity shows biological half-lives of 87 days for ^{65}Zn, and 143 - 214 days for ^{57}Co, ^{54}Mn, ^{51}Cr and ^{59}Fe.

The duration of the period of accumulation, from 2 hours to 21 days, had no effect on the biological half-lives of the different compartments, but a longer period of contamination increased the relative magnitude of the long-lived compartment of ^{65}Zn, ^{57}Co, ^{54}Mn and ^{134}Cs approximately by a factor of 2. For ^{51}Cr and ^{59}Fe no effect was observed. The material is, however, too small for a proper elucidation of this problem.

Figures 2, 3 and 4 show whole-body loss-curves for 4 animals during one year from August 1979 to August 1980. The biological half-lives for the slow compartment have been calculated for 3 periods as indicated on the excretion curves and the temperature curve (Fig. 1). Period II, autumn 1979, is the same period as for the slow compartment in Table II, i.e. for temperatures above 5°C. Period III, the winter of 1979-80, shows temperatures below 5°C, and the last period, spring and summer of 1980, has water temperatures of 5 - 20°C.

The biological half-lives calculated from the regression lines indicated in Figures 2, 3 and 4 are reported in Table III. For ^{65}Zn the biological half-life is seen to increase during the winter and decrease again during spring and summer 1980, i.e., a seasonal effect on the loss rate of ^{65}Zn is clearly demonstrated. For ^{57}Co and ^{54}Mn the biological half-life is also increased during the winter, but it does not decrease again during the warm period. This indicates that the remaining activity is bound in a compartment with longer half-life than the slow compartment calculated during the autumn

1979. This is further elucidated by Table IV showing the distribution of the remaining activity between soft parts and shells after 364 days excretion. It is seen that for ^{57}Co and ^{54}Mn the major activity is associated with the shell, whereas most of the ^{65}Zn activity is found in the soft parts.

4. DISCUSSION

Loss of the radionuclides from Mytilus was adequately described by exponential functions:

$$A_t = A_o e^{-kt},$$

where A_t is the activity at time t, A_o at zero time, and k is the elimination constant. As the results are decay corrected, the half-life calculated from the k-value by

$$T_{\frac{1}{2}} = \ln 2/k$$

is denoted the biological half-life. As mentioned earlier (Table II) elimination constants and biological half-lives for two compartments could be calculated for most isotopes. The radioactive nuclides decay after the same equation, and the combined loss in activity due to physical decay of the nuclides and the loss of the radioactive metals from the animals, i.e. the effective half life, $T_{\frac{1}{2},eff.}$, can be calculated from

$$\frac{1}{T_{\frac{1}{2},eff.}} = \frac{1}{T_{\frac{1}{2},biol.}} + \frac{1}{T_{\frac{1}{2},phys.}} \quad .$$

The dilution of the activity concentration due to growth of the animal tissues is not included in the biological half-lives described here, as results from the whole-body countings are calculated as total activity in the animals. However, dilution of the activity due to growth (cf. Table I) can be included in the calculation of the biological half-lives if necessary. In a bioindicator and radio-protection programme, the relevant parameter is the effective half-life including growth dilution.

The biological half-life for ^{65}Zn is the one most readily compared with published results. Young and Folsom [4] translocated 100 kg of Mytilus californianus from the vicinity of the contaminated Columbia River to California, where the loss of ^{65}Zn was followed for one year, during which the mussels lived in a natural environment. From this study a biological half-life excluding growth dilution of 82 days was found for the soft parts and probably somewhat higher for the shells. However, as the activity in the soft parts makes up for the major part of the whole-body activity this figure is comparable with the biological half-life for ^{65}Zn reported in Tables II and III. Except for the cold period the similarity is remarkable. In laboratory experiments with Mytilus, van Weers found a somewhat lower biological half-life for ^{65}Zn: 48-60 days [5], while Baudin [6], found a biological half-life for the slow compartment of 214 days. Also for ^{60}Co, van

Weers [5] reported a lower biological half-life, 57-72 days, than found in this study. It is, however, difficult to compare the long-term laboratory studies with field conditions, as, for instance, feeding in laboratory studies is usually not field comparable. However, several other parameters may explain the differences, e.g. size, salinity, temperature and water quality.

For Mya arenaria and Crassostrea gigas long-lived compartments of radiocesium have been demonstrated [7,8]. The lack of a similar compartment in these investigations might be explained by the relatively low ^{134}Cs contamination resulting in loss curves which are too short.

For the loss of ^{65}Zn a seasonal effect was clearly demonstrated (Table III and Figure 2). Seasonal oscillations in the contents of metals in marine organisms are often observed (see e.g. [9] and may be explained by growth dilution. However, as the effect of growth dilution is eliminated in this study, the actual rate of loss from whole animals was lower - perhaps because of diminished physiological activity due to low temperatures and low food supply during the winter. The long biological half-life of ^{57}Co and ^{54}Mn during the winter persists during the summer of 1980. This indicates that the remaining activity is associated to a very slow compartment, which is apparently situated in the shell as the major part of the activity of ^{57}Co and ^{54}Mn after one year of excretion was found here (Table IV).

Due to the uncertainties in separating the seasonal effect observed for ^{65}Zn from the long-term effect of a very slow compartment observed for ^{57}Co and ^{54}Mn, the very slow compartment has not been resolved from the rest of the loss curve. This may result in biological half-lives of the "slow compartment" in Table II that are too long.

The high contribution of the shell to the total activity of ^{57}Co and ^{54}Mn is a drawback in this study as the soft parts of the animals are the most interesting from a bioindicator and radio-protection viewpoint. However, as the only absolutely safe way of making field-comparable experiments is to conduct them in the field, the results from this and similar studies can at least be seen as a demonstration of the possibilities of the methods described here. These studies should therefore be followed up by similar field studies where groups of animals are dissected during the period of loss.

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Table I. *Mytilus edulis*. Growth data for 4 animals (no 20–23) in the 364 days excretion experiment. Mean values. Error term : ± 1 SE.

	soft parts		shell length
	fresh weight g.	water cont. %	mm
August 1979 (n = 15)	1.10±0.07	84.9±0.5	35±1
August 1980 (n = 4)	3.80±0.40	81.3±1.3	46±1

Table II. Mytilus edulis. Whole-body biological half-lives (days) July/August till November 1979. Whole-body countings and resolution of loss-curves was performed on 12 individual animals reared in Roskilde Fjord after contamination in the laboratory. Error term : ± 1 SE.

Isotope	Slow compartment		Medium compartment		Fast compartment
	$T_{\frac{1}{2},b}$, d.	%	$T_{\frac{1}{2},b}$, d.	%	%
^{65}Zn	87±5	28±3	6.1±0.5	48±2	24
^{57}Co	202±35	38±3	5.2±0.7	48±3	14
^{54}Mn	214±31	42±5	6.9±0.9	20±2	38
^{51}Cr	151±15	25±2	5.1±0.6	54±5	21
^{59}Fe	143±9	15±1	4.2±0.5	47±5	38
^{134}Cs	-	-	7.6±1.4	49±5	51

Table III. Mytilus edulis. Whole-body biological half-lives (days) in "slow compartment" for three periods during one year. Whole-body countings on 4 individual animals reared in Roskilde Fjord after contamination in the laboratory. Refer to Fig. 1 - 4. Error term : ± 1 SE.

Period	Animal no.	^{65}Zn	^{57}Co	^{54}Mn
II	20	99	83	84
Autumn	21	82	132	135
1979	22	101	185	260
> 5°C	23	72	117	136
	Mean	89±7	129±21	154±38
III	20	322	610	645
Winter	21	377	696	741
79/80	22	413	553	617
< 5°C	23	276	472	551
	Mean	347±30	583±47	638±40
IV	20	112	243	281
Spring-	21	166	486	577
summer 1980	22	238	930	891
> 5°C	23	118	656	818
	Mean	159±29	579±145	642±138

Table IV. Mytilus edulis. Activity in soft parts as a percentage of whole-body activity after termination of the 364 days excretion experiment. (cf. Table III).

Animal No	^{65}Zn	^{57}Co	^{54}Mn
20	79	19	0.0
21	53	4.5	0.3
22	53	9.4	0.3
23	64	7.9	0.2

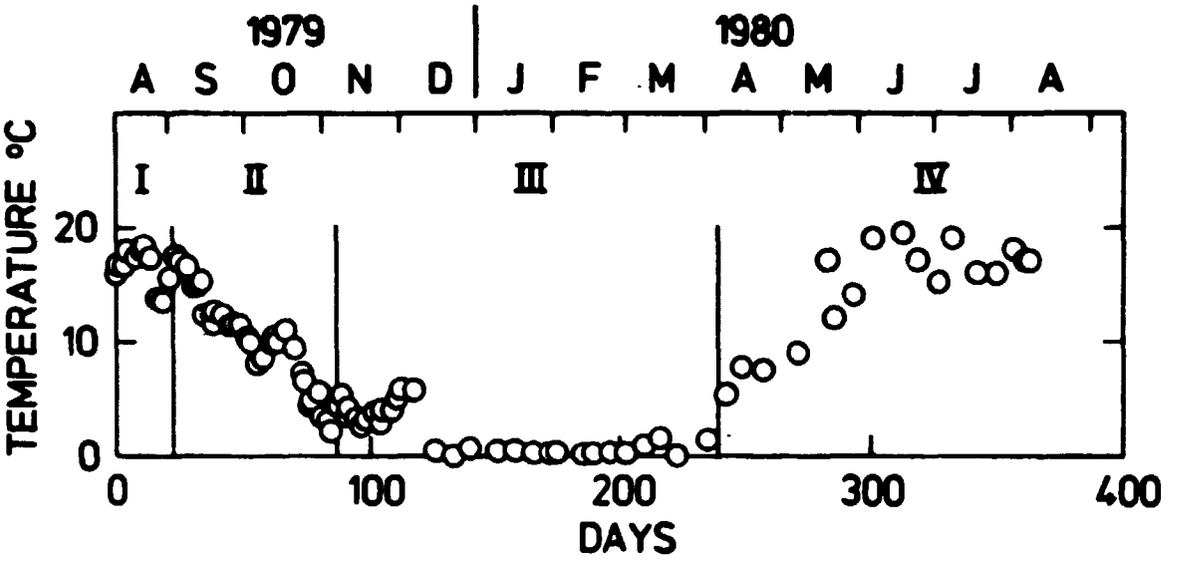

Figure 1. Water temperature in Roskilde Fjord during the long-term excretion study.

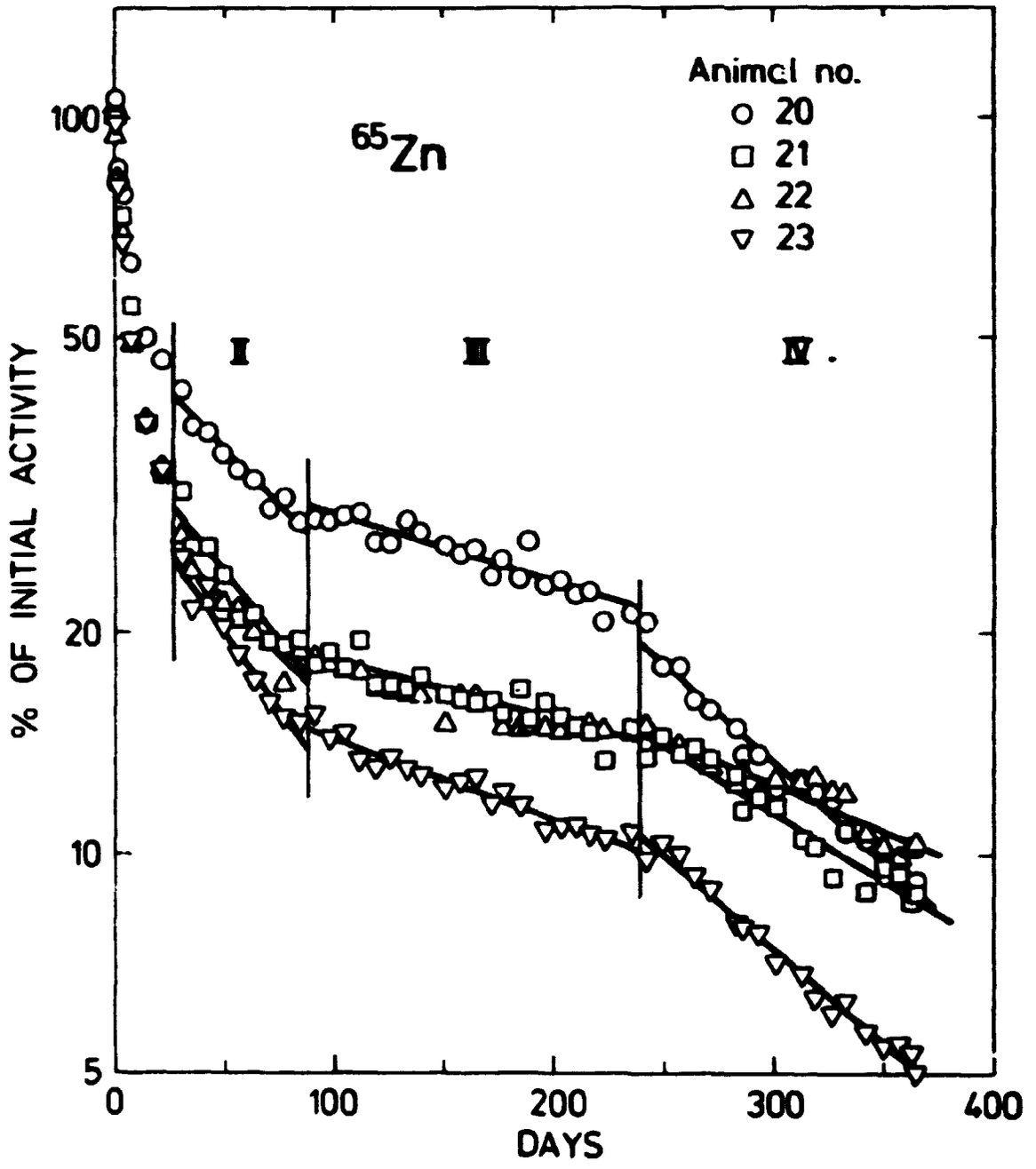

Figure 2. Mytilus edulis. ^{65}Zn whole-body loss curves for 4 individual animals. Regression lines for 3 periods indicated (cf. Table [illegible]T).

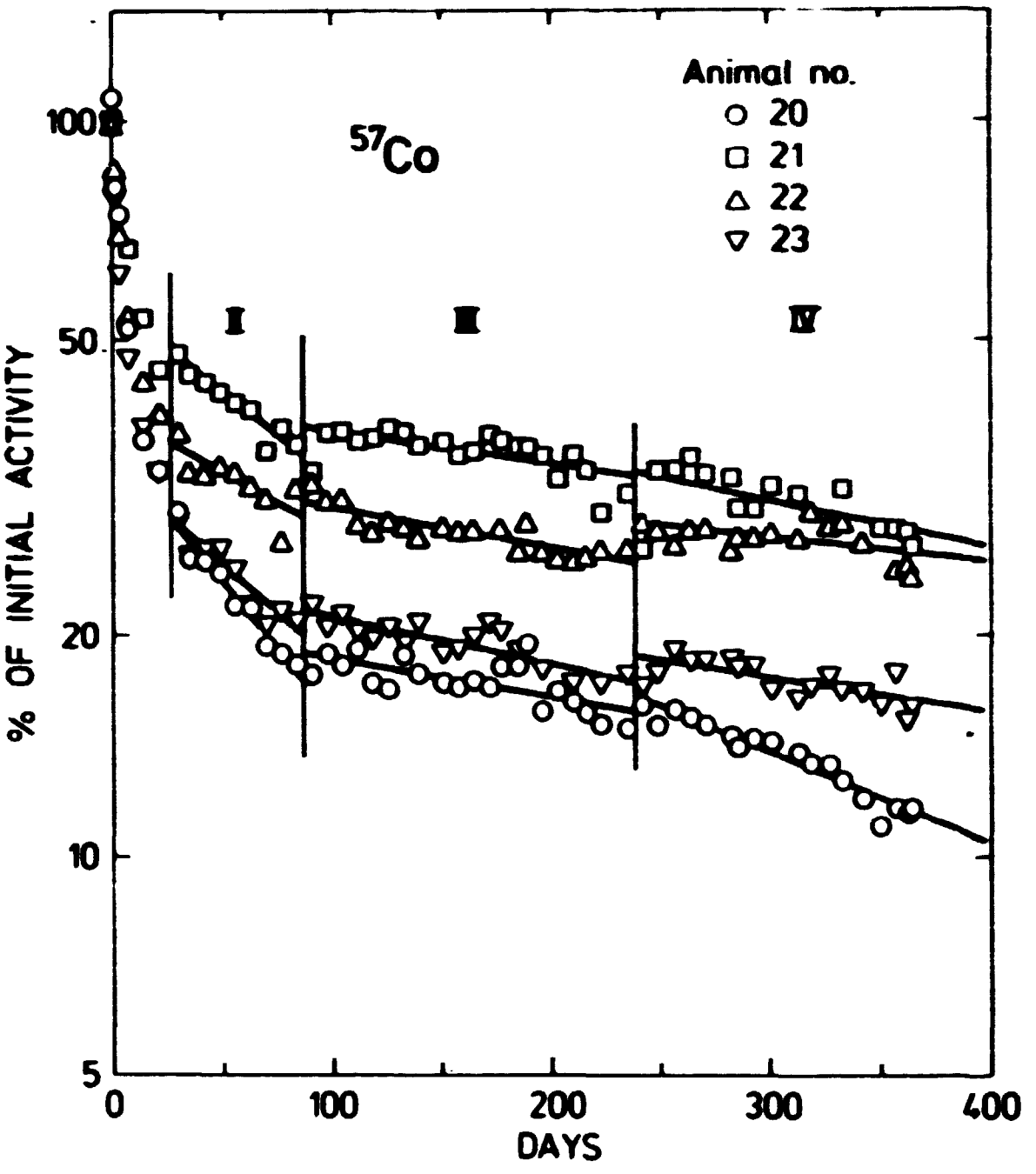

Figure 3. Mytilus edulis. ^{57}Co whole-body loss curves for 4 individual animals. Regression lines for 3 periods indicated (cf. Table III).

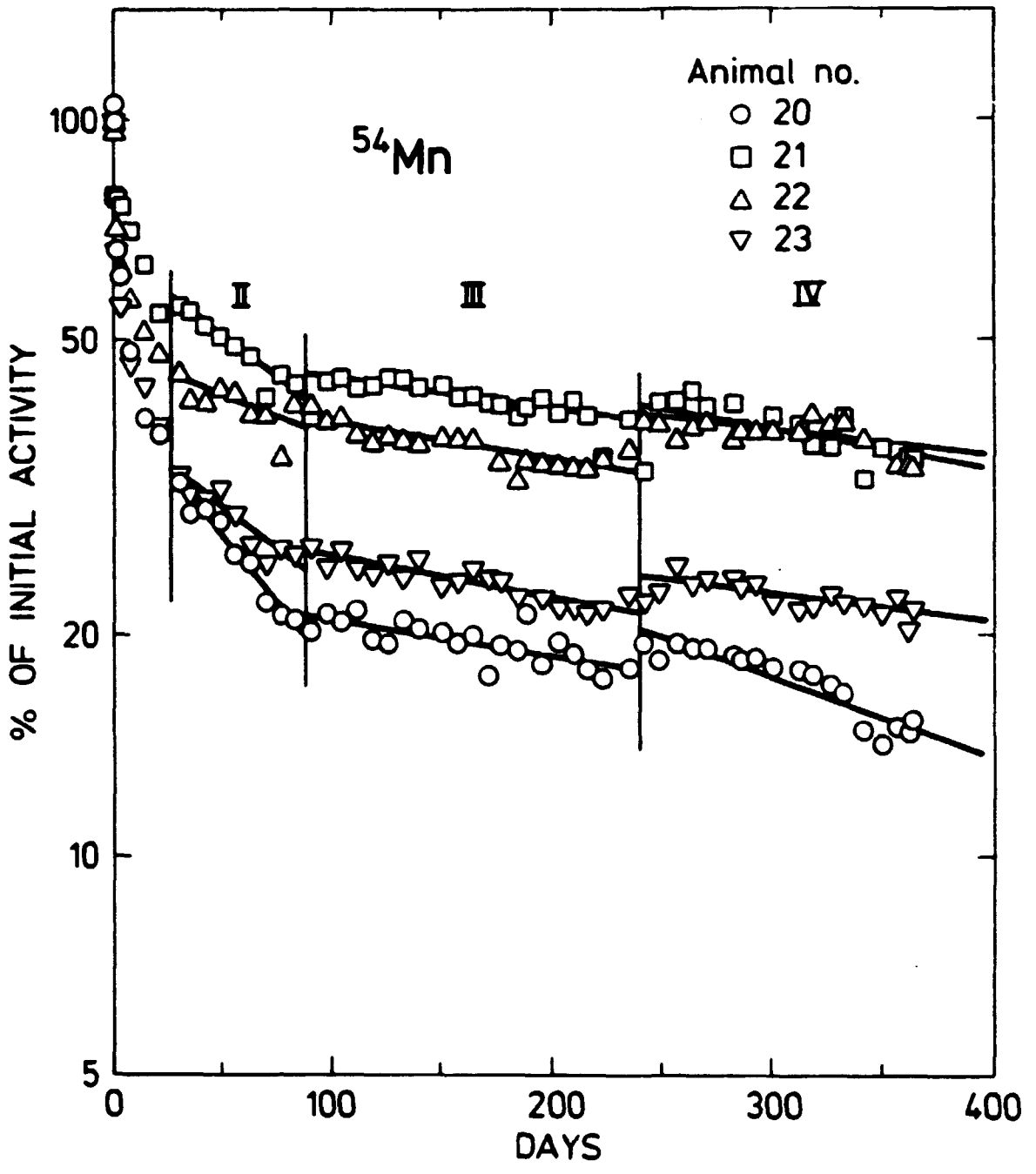

Figure 4. Mytilus edulis. ^{54}Mn whole body loss curves for 4 individual animals. Regression lines for 3 periods indicated (cf. Table III).

APPENDIX III

Selected bioindicator data from Barsebäck and Ringhals 1977-1979. Data from Dahlgaard 1978, 1979, 1980 (cf. Chapter 3).

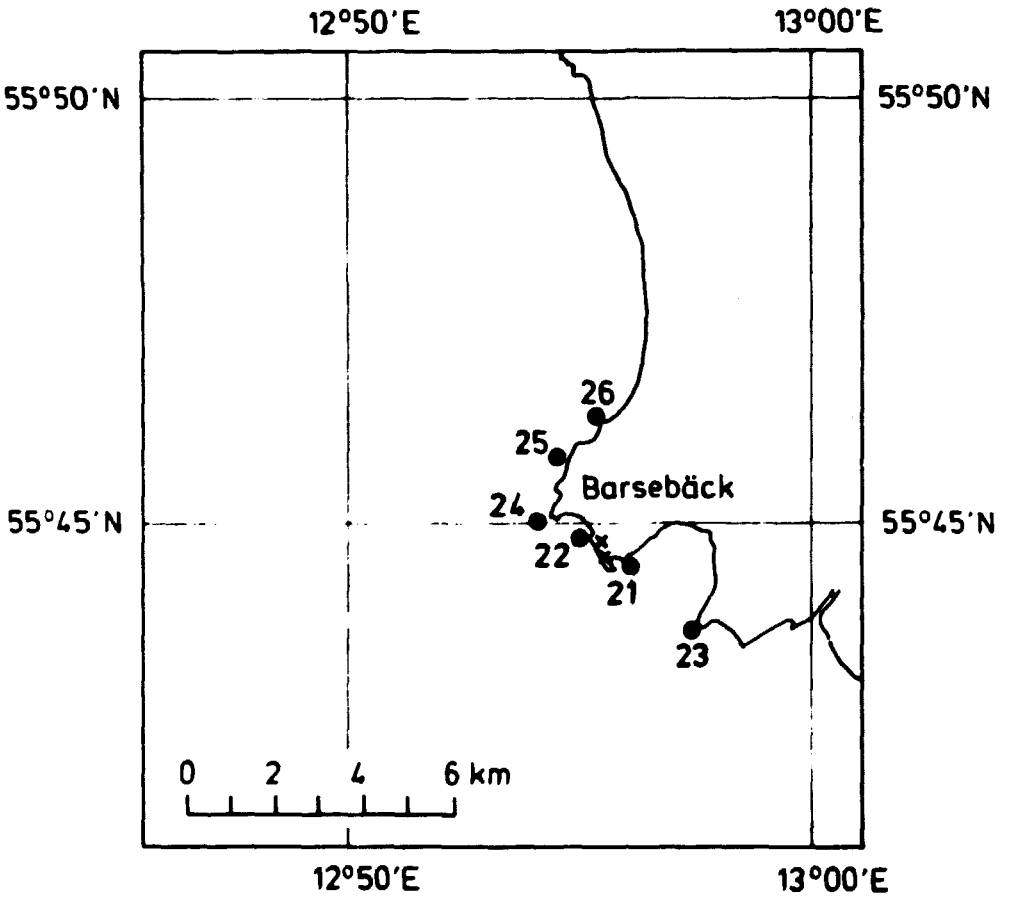

Figure III.1. Sampling locations at Barsebäck. The discharge pipe is located between the two reactors (crosses).

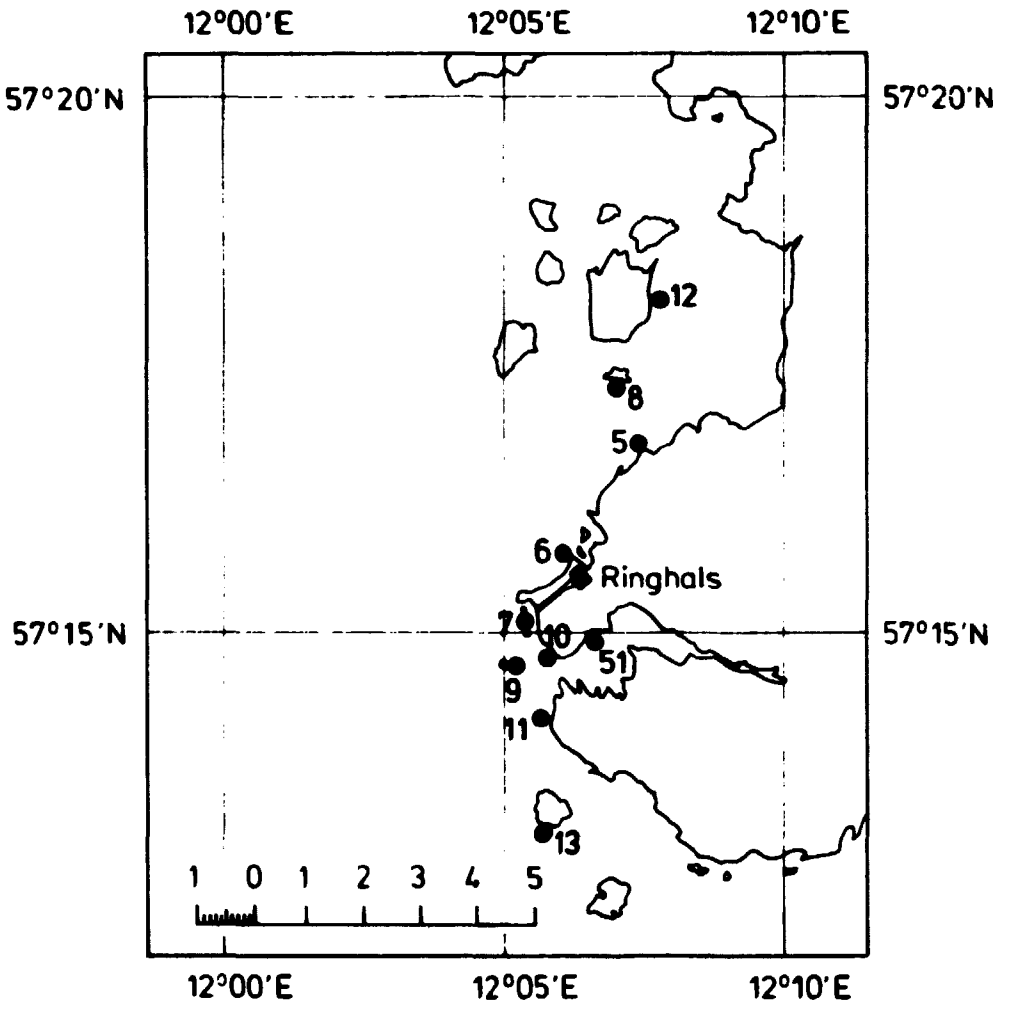

Figure III.2. Sampling locations at Ringhals. The discharge pipes are shown opposite location 7.

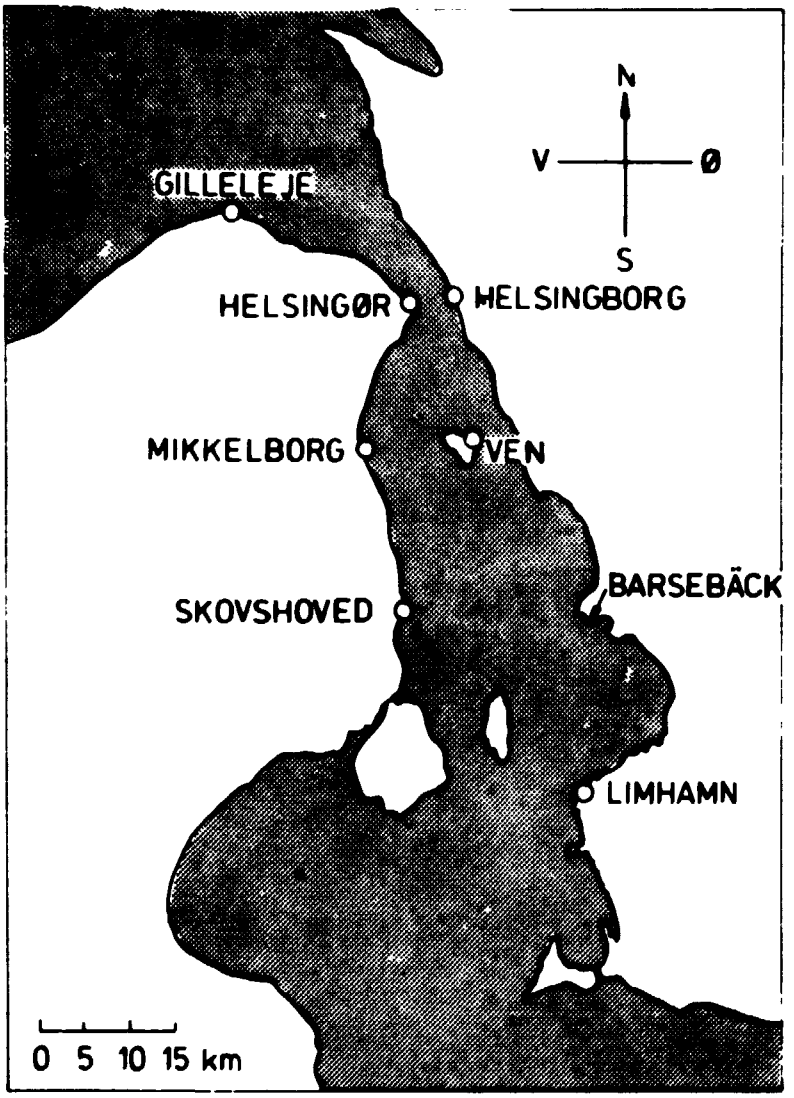

Figure III.3. Sampling locations in the Sound.

Table III.1. Gamma-emitting radionuclides in Fucus vesiculosus collected at Barsebäck 1977 (Unit: pCi kg^{-1} fresh weight)

Date of sampling	15 June						22 October						6 December	
Station No.	22	24	25	26	21*	23*	22	24	25	26	21*	23*	22	24
Distance from outlet in km	0.6	1.4	2.9	4.0	1.5	2.8	0.6	1.4	2.9	4.0	1.5	2.8	0.6	1.4
^{60}Co	3,790	2,540	609	285	182	164	29,600	11,200	4,070	2,320	2,130	1,190	30,500	13,400
^{58}Co	2,490	1,450	394	196	33	32	8,710	3,150	1,130	694	576	340	5,710	2,560
^{54}Mn	465	387	108	60	39	32	1,660	585	248	136	157	61	1,840	757
^{65}Zn	851	557	164	82	54	82	7,480	2,690	989	530	476	299	7,840	3,270
^{110m}Ag	67 A	34 B	123	-	-	-	361	129	91	-	75	-	302	-
^{51}Cr	144 B	92 B	-	-	-	-	-	-	-	-	-	-	-	-
^{137}Cs				57	47	91				82		80		
^{131}I	51 A	33 A	-	-	-	-	-	112	57 A	98	118	87	-	-
^{95}Zr	234	132	115	372	211	173	-	-	107	150	115	126	-	-

*South of the outlet.

Table III.2. Gamma-emitting radionuclides in Fucus vesiculosus collected at Barsebäck in 1978
(Unit: pCi kg^{-1} fresh weight)

Date of sampling	17 April						15-16 June					8 September						10 December
Station No.	22	24	25	26	21*	23*	22	24	25	26	23*	22	24	25	26	21*	23*	24
Distance from outlet in km	0.6	1.4	2.9	4.0	1.5	2.8	0.6	1.4	2.9	4.0	2.8	0.6	1.4	2.9	4.0	1.5	2.8	1.4
^{60}Co	5,090	2,720	1,318	856	553	231	8,350	5,470	1,600	780	406	24,600	14,700±1,200	3,320	1,490	1,710	667	36,800
^{58}Co	482	220	98	60	35	11.5	2,320	1,070	358	134		14,540	9,040±740	1,870	861	1,060	408	8,380
^{54}Mn	225	134	61	38	25	10.4	576	382	108	61	25 A	1,400	775± 47	172	103	153	53	1,300
^{65}Zn	1,120	713	277	186	96	58	1,500	879	267	144		4,760	2,640±220	655	219	212	144	7,920
^{110m}Ag		26 B	19 A									393						225
^{51}Cr	238	79 A					98					1,080						
^{137}Cs	93			71	53	44	130	113	82	77	92		82± 33	94	66	60	63	
^{131}I	67	34		28 A	16 B	20	46	58						21				
^{95}Zr		31 A	43	49	48	28												

* Locations south of the outlet; the other locations were situated north of the outlet.

Table III.3. Gamma-emitting radionuclides in Fucus vesiculosus collected at Barsebäck in 1979 (Unit: pCi kg^{-1} fresh weight)

Date of sampling	6 April			19 June					7 January 1980	
Station No.	24	21*	23*	22	24	25	26	21*	23*	24	23*
Weight fresh/dry	4.40	3.26	4.08	5.60	5.52	4.68	5.13	4.85	5.39	7.14	4.52
Distance from outlet in km	1.4	1.5	2.8	0.6	1.4	2.9	4.0	1.5	2.8	1.4	2.8
^{60}Co	25,100	2,530	1,470	9,140	9,270	2,080	1,520	1,730	505	8,530	431
^{58}Co	2,050	184	118	685	464	111	75	78	55	705	31
^{54}Mn	716	103	55 A	708	386	86	54	65	31	373	25
^{65}Zn	4,270	490	282	1,380	1,410	327	230	240	78	1,680	84
^{110m}Ag	122			88	62 A					28	
^{51}Cr	1,080			188 B							
^{137}Cs		71	90			60	60	70	81	49	71

* Locations south of the outlet; the other locations were situated north of the outlet.

Table III.4. Gamma-emitting radionuclides in Fucus vesiculosus (Fu.ve.) and Fucus serratus (Fu.se.) collected in the Sound in 1979. (Units: pCi kg^{-1} fresh weight)

Date of sampling	9 April	7 April	19 April			19 June	7 January 1980		
Location	Skovshoved havn	Ven	Mikkelborg	Helsingør	Gilleleje	Skovshoved havn	Helsingborg	Helsingør	Limhamn
Weight fresh/dry	4.74	3.82	6.34	5.83	7.49	5.50	6.12	6.33	4.49
Distance from outlet in km	20.0	22.4	30.2	39.6	60.2	20.0	39.0	39.6	17.8
Species	Fu.se.	Fu.ve.	Fu.ve.	Fu.se.	Fu.ve.	Fu.ve.	Fu.ve.	Fu.se.	Fu.ve.
^{60}Co	79	82	15.9	31.4	4.2		29.7	16.5	21.8
^{54}Mn	9.0 A							4.0 A	
^{137}Cs	58	40	59	40	35	60	55	51	69
^{131}I	71 A								
^{58}Co		11.0 B							
^{65}Zn								10.2 A	

Table III.5. Gamma-emitting radionuclides in Fucus vesiculosus (Fu.ve.), Fucus serratus (Fu.se.) and Ascophyllum nodosum (As.no.) collected at Ringhals in 1977. (Unit: pCi kg^{-1} fresh weight).

Date of sampling	7-9 July									10 November					
Station No.	7	6	5	8	12	10*	9*	11*	13*	7	6	5	8	12	9*
Species	As.no.	Fu.ve.	Fu.se.	Fu.ve.	Fu.ve.	Fu.se.	Fu.se.	Fu.se.	Fu.se.	As.no.	Fu.se.	Fu.se.	Fu.ve.	Fu.ve.	Fu.se.
Distance from outlet in km	0.2	1.9	4.1	4.8	6.3	0.9	1.1	1.9	4.1	0.2	1.9	4.1	4.8	6.3	1.1
^{60}Co	4,320	892	492	400	237	2,440	1,150	1,080	152	4,940	1,460	852	257	246	1,880
^{58}Co	9,480	1,270	1,160	908	592	3,260	2,380	2,390	213	1,890	645	470	147	139	1,020
^{54}Mn	1,670	525	152	151	102	505	319	318	48	272	193	113	61	58	243
^{65}Zn	576	115	88	56	-	503	289	204	45 A	6,260	2,510	1,030	439	264	2,750
^{110m}Ag	-	-	-	-	-	-	-	-	-	422	-	50 A	-	-	94
^{51}Cr	-	-	-	-	-	-	-	-	-	1,040	-	-	-	-	-
^{137}Cs	85	53	44	58	74	43 A	38	44	49	-	103		64	72	
^{134}Cs	132	24 B	28 A	19	-	58	42	36	-	-	-	-	-	-	-
^{131}I	-	-	-	-	-	-	-	-	-	-	-	-	18 A	-	-
^{95}Zr	294	859	488	522	920	244	420	236	726	118	128	101	120	178	111

*Locations south of the outlet; the other locations were situated north of the outlet.

Table III.6. Gamma-emitting radionuclides in Fucus vesiculosus (Fu.ve.) and Fucus serratus (Fu.se.) collected at Ringhals May 20, 1978.
(Unit: pCi kg^{-1} fresh weight)

Station No.	7	6	5	8	12	10*	9*	11*	13*
Species	Fu.se.	Fu.ve.	Fu.ve.	Fu.ve.	Fu.ve.	Fu.se.	Fu.se.	Fu.se.	Fu.se.
Distance from outlet in km	0.2	1.9	4.1	4.8	6.3	0.9	1.1	1.9	4.1
^{60}Co	2270	373	267	118	67	887	530	896	145
^{58}Co	951	155	120	52	26	192	309	167	38 A
^{54}Mn	233	52	49	21	16	61	56	88	17 A
^{65}Zn	2810	418	242	113	76	1300	517	1034	185
^{110m}Ag	241	25 A				102		51 A	
^{137}Cs			58	53	46		105		51
^{131}I				23 A					
^{95}Zr		22 A		8 A	37		46 A	65 A	59 A

* Locations south of the outlet; the other locations were situated north of the outlet.

Table III.7. Gamma-emitting radionuclides in Fucus vesiculosus (Fu.ve.), Fucus serratus (Fu.se.), Fucus spiralis (Fu.sp.) and Ascophyllum nodosum (As.no.) collected at Ringhals September 3, 1978. (Unit: pCi kg^{-1} fresh weight)

Station No.	7 East	7 East	7 South	7 South	7 South	6	5	8	12	9*	11*	51*	13*	37*
Species	Fu.ve.	As.no.	Fu.ve.	Fu.sp.	Fu.se.	Fu.ve.	Fu.ve.	Fu.ve.	Fu.ve.	Fu.ve.	Fu.ve.	Fu.ve.	Fu.ve.	Fu.ve.
Distance from outlet in km	0.2	0.2	0.2	0.2	0.2	1.9	4.1	4.8	6.3	1.1	1.9	2.0	4.1	19.4
^{60}Co	2,070	3,830	1,320	1,950	2,170	274	105	95	80	273	422	491	40	12.0 A
^{58}Co	1,010	591	538	901	551	55	37	30	34	111	127	187	10.3	9.8 A
^{54}Mn	267	115	170	367	203	38	22 A	19 A	25 A	34	76	54	8.5	
^{65}Zn	14,560	11,830	6,290	10,400	6,650	389	481	252	229	1,320	1,190	1,560	134	
^{110m}Ag	1,580	1,190	610	660	530	52	69	42 A	37 A	52	81 A	72 A	15 A	
^{51}Cr			210 A		110 B	66 A								
^{137}Cs														54
^{131}I	292	359	69	230 B	69	12 B								
^{95}Zr	93 B		78		73	57							13 A	13 A

* Locations south of the outlet; the other locations were situated north of the outlet.

Table III.8. Gamma-emitting radionuclides in Fucus vesiculosus (Fu.ve.), Ascophyllum nodosum (As.no.) and Fucus serratus (Fu.se.) collected at Ringhals may 12 and 13, 1979. (Unit: pCi kg^{-1} fresh weight)

Station No.	7	7	6	5	8	12	9*	11*	13*	13*	37*
Weight fresh/dry	4.40	5.63	3.64	3.91	3.32	4.65	4.13	4.25	3.85	4.64	3.71
Species	Fu.ve.	As.no.	Fu.se.	Fu.se.	Fu.se.	Fu.ve.	Fu.se.	Fu.se.	Fu.se.	Fu.ve.	Fu.se.
Distance from outlet in km	0.2	0.2	1.9	4.1	4.8	6.3	1.1	1.9	4.1	4.1	19.4
^{60}Co	712	846	398	200	175	53	404	320	70	34	19.8
^{58}Co	603	99	135	88	61	24 A	246	68	21		
^{54}Mn	112	18 A	38	17 A	20 A	8.8 B	35	20 A	11 A		7 B
^{65}Zn	1409	1039	682	407	268	75	805	916	187	71 A	
^{137}Cs						66			74	58	62
^{110m}Ag	98	100	48	28 A	18 A		52	43			

* Locations south of the outlet; the other locations were situated north of the outlet.

Table III.9. Gamma-emitting radionuclides in Fucus vesiculosus (Fu.ve.), Fucus serratus (Fu.se.) and Ascophyllum nodosum (As.no.) collected at Ringhals November 15, 1979. (Unit: pCi kg^{-1} fresh weight)

Station No.	7	7	8	8	8	12	9*	13*
Weight fresh/dry	4.57	4.16	4.42	4.23	3.64	3.94	4.56	4.34
Species	Fu.ve.	Fu.se.	Fu.ve.	Fu.se.	As.no.	Fu.ve.	Fu.ve.	Fu.se.
Distance from outlet in km	0.2	0.2	4.8	4.8	4.8	6.3	1.1	4.1
^{60}Co	2,260	2,560	140	157	66	102	202	171
^{58}Co	690	820	45	48	15.0		70	66
^{54}Mn	520	380	33	27		20 B	44	21 A
^{65}Zn	26,700	36,100	2050	1230	764	793	3280	1470
^{137}Cs	135	101				101		84
^{110m}Ag	270	320	22 A	13 B	24		27	
^{134}Cs	30 A	27 A						

* Locations south of the outlet; the other locations were situated north of the outlet.

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