Chapter 6

DESIGN OF LABORATORY RADIOTRACER STUDIES IN MARINE RADIOECOLOGY

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Abstract

A condensed description of methods used in laboratory radiotracer studies in marine radioecology is presented showing also the difficulties which may be encountered in order to obtain realistic and comparable information on the general behaviour of radionuclides in marine organisms. Practical guidance on the choice of the biological material and how to set-up laboratory experiments and to control properly important experimental conditions are given. Key parameters like concentration factors and biological half-lives are defined and the theoretical estimation and practical determination of input, uptake, accumulation and loss of radionuclides in marine biota are formulated by the aid of mathematical equations. Examples of uptake and loss curves obtained in the laboratory are shown. The importance of some environmental factors (temperature, food, growth) on uptake and loss of radionuclides are demonstrated. Comparison of experimental and field data of concentration factors is reported to show the difficulty in extrapolating from laboratory experiments to nature.

6.1 INTRODUCTION

During a survey of certain nuclear sites or, in general, when considering marine radioecological studies in the field, one is faced with problems to accurately measure and determine concentration factors (CFs) and biological half-lives or half-times in biota. This fact results in nature from very low concentrations of radioactivity in the water and in the organisms so that concentration may be beneath the detection limits of the equipment used for measurements. Moreover, it is very rarely possible to follow the kinetics of radioisotope behaviour (accumulation and loss) in marine organisms in the field, especially in those species which are commercially important and/or of economic value. Such difficulties and problems may be surmounted only by undertaking well defined laboratory radiotracer studies to determine these parameters.

6.1.1. Scope

Laboratory studies with living organisms should, therefore, serve for obtaining such information under defined laboratory conditions that is difficult or impossible to achieve in the field. The results which are most important should enable us to extrapolate the information obtained in the laboratory to field conditions. The knowledge of the behaviour and physiology of radionuclides in fish is of general importance because fish represent the higher trophic levels in the marine ecosystem, and thus constitute one of the most direct transfer routes of radioactivity back to man.

6.2. EQUIPMENT

6.2.1. Aquaria

Fish are normally the most difficult marine organisms to maintain in a healthy state in the laboratory (aquaria), since they have precise requirements for temperature, salinity, light, food, diet, and adequate volumes of water. For the purpose of experimentation, it is advisable to start with a stock population of fish in an open-circulation system in order to furnish an adequate supply of fresh water to maintain the fish in a healthy state. Moreover, fish should remain for a sufficiently long time (weeks) in the stock aquaria for acclimatisation to the artificial environment and to ensure good health.

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The experiments with radioisotopes should be carried out in a system with closed circulation of water in order to avoid loss of radioactivity. Thus, two distinct aquaria sets are necessary, the stock population of fish, acclimated to the artificial, unnatural conditions in the laboratory, and the smaller experimental group of fish generated from the stock population to be used in radioecological studies. After a certain time of acclimatisation only healthy organisms will be chosen for the experiments in order to extrapolate the data obtained to the natural environment.

6.2.2. Radioisotopes

It is preferable to use gamma-emitting radionuclides with suitable and appropriate physical half-lives (days, weeks, months) according to the duration of the experiments. The commercial market offers a variety of radionuclides (beta-, and gamma-emitters) with half-lives which easily match the requirements of the laboratory experiments. The gamma-emitters also have the advantage to enable a non-destructive measurement of the experimental organisms, since the living organisms can be measured for accumulated radioactivity and analysed again in the same experiment. This possibility will decrease experimental variability between organisms and the quantity of radioactivity to be used. Furthermore, the same experimental animals can be utilised for the total length of the experiments which reduces the numbers of animals necessary and, hence, the size of the aquaria and the quantity of water which is contaminated.

6.2.3. Counting facilities

Unfortunately, most of the commercial counting facilities are unsuitable for radiobiological or radioecological purposes because they are constructed for medical and/or clinical use and, therefore, normally have relatively small crystals and, hence, also very small volumes for counting vials (which may be used for small mussels or crustaceans but are unsuitable for fish). Thus, often it will be necessary to construct counting facilities with bigger crystals and a counting chamber which may respond to the requirements of the measurements.

6.3. MULTI-COMPARTMENTAL EXPERIMENTS (Mesocosm)

In laboratory experiments on uptake and accumulation of radioactivity, usually only two compartments are considered: for example, water and fish, or food and fish, in order to enable following the kinetics of uptake in the biota. In more complicated systems, involving several compartments like water, sediments, prey organism, predator, and second stage predator, often the results and/or observations are difficult to explain. Therefore, it may be advisable to make a step by step approach to such a complicated system and/or food web by considering transfers of radioactivity in food chains using separate experiments with to the different trophic levels.

6.4. LABORATORY EXPERIMENTS IN RADIOECOLOGY

6.4.1. Uptake pathways

Uptake pathways are quite variable according to species and their habitats. A fish in nature normally encounters different uptake routes for radionuclides present in the environment. Radioactivity will enter the organism via contaminated food or will be accumulated directly from the surrounding water body through drinking, by absorption across the gills, and to a minor extent by absorption through total surface of the fish. Fish living on bottom sediments often will feed on bottom dwelling organisms and, hence, will eat sediment particles together with the food.

6.4.2. Uptake from water

Various factors may influence the uptake of radionuclides from water by fish. These are the initial concentration of the radioisotope in the water, the physico-chemical state of the selected radioisotope because it may be soluble, colloid, or in a particulate form, all of which may influence the final or effective uptake. The stability of the tracer in the water is also important since it may form a compound or absorb to surfaces, container walls or other parts in the system. The loss of the initial radioactivity in solution has to be corrected in order to maintain a relatively constant concentration in the experimental system. A varying concentration of radioactivity will not result in a reliable value of the concentration factor (CF).

The radioisotopes used may or may not be regulated by the organism. If a radioisotope of an element is used which is metabolically regulated by the fish, the behaviour of the radioisotope in the organism changes with respect to an isotope which is not regulated and the resulting concentrations factors may vary considerably. This holds for chemical analogues for example Ca (Sr) and K (Cs) which are regulated and treated by the organism as physiologically essential elements.

The quantity of the stable isotope of the same element present in the system will create a so-called isotopic dilution of the radioisotope used since the fish cannot distinguish between the stable and the radioactive isotope and will take up equal portions of that element. The specific activity (isotopic dilution) will not affect the bioavailability of the radioisotope, which may vary according to the physico-chemical state. The stable element may also exert a certain toxicity on the organism so that synergistic effects may occur.

6.4.3. Concentration factor (Measure of uptake from water)

In the literature different denominations for the term "concentration factor" exist; these are "concentration coefficient", "concentration ratio", "bioaccumulation factor", etc.. All these terms mean basically the same thing and they refer to the ratio between the activity in the organism (fresh weight, assuming a relative density of 1), divided by the activity found in the same quantity (or volume in this case, g/ml) of water. Thus, the definition of the concentration factor is a ratio of activities in equal units of the organism and water.

$$CF = \frac{activity\ (cpm, Ci, Bq) / g\ organism\ (FW)}{activity\ (cpm, Ci, Bq) / ml\ water} \qquad (1)$$

where

CF	is the concentration factor
cpm	is counts per minute
FW	is fresh weight

Furthermore, CFs refer to equilibrium conditions between the organism and the surrounding water and can by definition be calculated only at steady-state. This means that an equilibrium exists where intake of radioactivity by the organism equals the excretion rate, so that the concentration of radioactivity in the body remains constant.

The scope of the calculation of concentrations factors in the environment is to relate all concentrations of radionuclides in environmental samples to a common value, and this value is the corresponding activity in the water. The disadvantage of the use of concentration factors is that the CF is just a ratio and not a numeric or an absolute value. Concentrations factors originally refer to uptake from water and not from other sources like food in which case the term "transfer factor" should be normally used.

6.4.4. Potential parameters influencing the calculation of concentration factors

The calculation of a concentration factor is strongly influenced by environmental as well as by inherent factors of the organisms. The relative concentration of radioactivity in water may vary by a factor of 100 whether filtered or unfiltered water is considered (e.g. total water: particulate matter, phyto-, zooplankton). The use of the type of filters is crucial, of course, because the definition of the "soluble phase" of filtered water depends on the mesh size (e.g. 0.45 or 0.25 μm). Therefore, the question arises where the limit between particulate and soluble can be set because some "soluble" components will not pass through a filter of 0.22 μm.

With respect to organisms the parameters which influence the CF are season, temperature and salinity of the water, as well as the general physiology, sex and size of the experimental organisms.

Considering uptake from food other parameters may influence the CF. This depends mainly on whether natural food or artificial food is used in the experiments and how the radioactivity was accumulated in the food. Whether the radioactivity was introduced artificially or if it was accumulated by the organism physiologically (i.e. assimilated) the CFs obtained may change considerably. In the latter case the type of compound in the food may be readily bioavailable for the organism of the next trophic level. This is also true for artificial food; however, the situation may be somewhat less clear because all characteristics and constituents of the food have to be known (stable element content, physico-chemical state of the radioisotope, the different compounds of the radionuclide in the artificial food) in order to assess the bioavailability of the radioactivity in that specific food. Some examples in the literature serve to illustrate the influence of size of organisms on the CF (Fig.1).

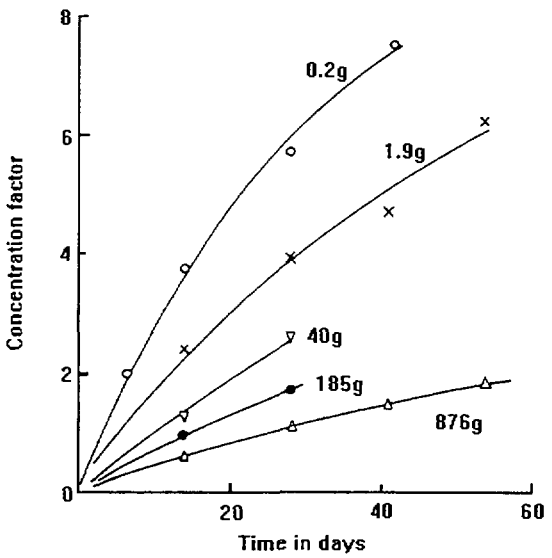

Fig.1. Concentration of ^{134}Cs by plaice of different weights [1]

The uptake and/or concentration factor of Cs-134 by plaice of different weights is indirectly related to size (weight). Small fish take up Cs faster and to a greater extent than bigger fish. The figure shows the initial uptake phase but at equilibrium (steady state) the differences between the different CFs will be more pronounced.

In Figure 2 a clear relationship is shown between weight and uptake of Cs-134 (total body-burden) by plaice and eels. This demonstrates that beside temperature and salinity one of the most important factors affecting the rate of uptake and accumulation of an isotope is body size.

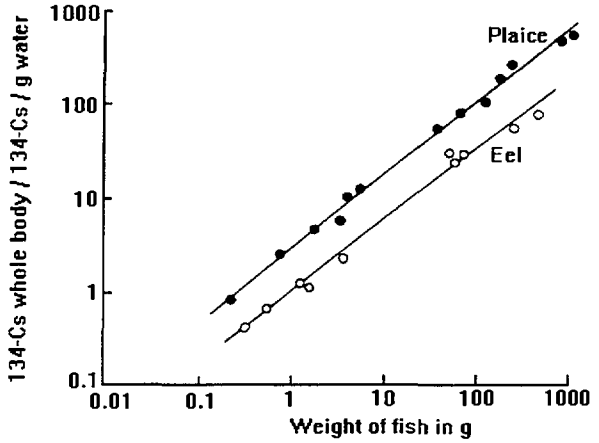

Fig.2. Relation between weight and uptake of ^{134}Cs by plaice and eel [1]

6.4.5. Accumulation from water

Accumulation from water can be expressed by a simple model, i.e. accumulation is intake minus excretion; therefore, at equilibrium intake equals excretion. This can be derived from the following formula where the change in concentration with time is equal to intake minus the concentration at any time multiplied by the coefficient **k**, which is the absorption or fixation coefficient and also the excretion coefficient.

$$\frac{dC_t}{dt} = I - kC_t; \quad I = k\,C_{ss} \text{ (steady state; asymtotic value: } C_{ss} = \frac{I}{k}\text{)}$$

$$\frac{dC_t}{dt} = k\,C_{ss} - k\,C_t; \quad C_t = C_{ss}(1-e^{-kt}); \quad k = \frac{\ln 2}{T_{1/2(b)}} = \frac{0.693}{T_{1/2(b)}} \qquad (2)$$

where

C_t	is the concentration of a radioisotope at time *t*
I	is the intake/unit weight/unit time
C_{ss}	is the concentration at equilibrium (steady state)
k	is a constant, i.e. the coefficient of adsorption or fixation (excretion)
$T_{1/2(b)}$	is the biological half-life

At equilibrium between the organism and the environment (steady state) intake balances excretion, that means the maximum value for the CF will reach the asymptotic value of the accumulation curve.

6.4.6. Accumulation from water: Multi-compartmental equation

Considering a normal radionuclide uptake curve, the initial linear increase is represented by uptake only and no interaction between uptake and excretion occurs. After a certain time excretion will take place until an asymptotic value at equilibrium is reached. This occurs if the organism as a whole is considered, but in laboratory experiments a different response may occur. Organisms are composed of different compartments, different organs which may behave differently with respect to the radioisotope introduced into the organism. In this case a multi-compartmental equation is considered.

$$C_t = A_1 \times C_{ss}\left[1 - e^{-(k_1 + l)t}\right] + A_2 \times C_{ss}\left[1 - e^{-(k_2 + l)t}\right] + A_n \times C_{ss}\left[1 - e^{-(k_n + l)t}\right] \quad (3)$$

(not corrected for physical decay: $T_{1/2(p)}$)

where

$C_t = CF_t$ is the concentration factor at time t
$C_{ss} = CF_{ss}$ is the concentration factor at equilibrium
A_1; A_2; A_n are fractions of the concentration at equilibrium of compartment 1, 2, and n
k_1; k_2; k_n are constants of the biological fixation rates of compartment 1, 2, and n
t is the time in days

$C_{ss} = \frac{I}{k}$; (I and k are functions of temperature, weight, growth, age of organism)

$l = \frac{0.693}{T_{1/2(p)}}$ is the physical decay constant

$k = \frac{\ln 2}{T_{1/2(b)}} = \frac{0.693}{T_{1/2(b)}}$

This is effectively the same equation as before but enlarged by coefficients A_1, A_2, A_n, which are fractions of the concentration at equilibrium in compartments 1, 2, 3, and so on. The fractions are normally expressed in percentages of the whole radionuclide concentration or the total body burden of the radioisotope in the organism. These values must be corrected for physical decay by introducing the physical decay constant. In order to satisfy the multi-compartmental equation all factors and coefficients have to be calculated and/or determined. Later on methods will be demonstrated how to determine the concentrations of the radionuclide which are lacking in the different compartments of the experimental organism.

Some examples of laboratory experiments on uptake of radionuclides by crustaceans and fish considering both the whole body and specific organs will illustrate the general accumulation behaviour and the possibilities to describe the uptake curves by simple first order kinetics or by multi-compartmental equations.

Fig. 3 clearly shows the effect of different temperatures on Tc uptake. There is effectively a different uptake velocity expressed by different k-values which is the coefficient of

fixation, and also the excretion rate. In both curves a directly temperature-dependent process with different velocities was observed; however, after a sufficiently long period (35-40 days), the asymptotic value, expressed as a CF is reached. The uptake process can be expressed either by a first order equation, i.e. the organism is considered as a single compartment **or** by a multi-compartmental equation.

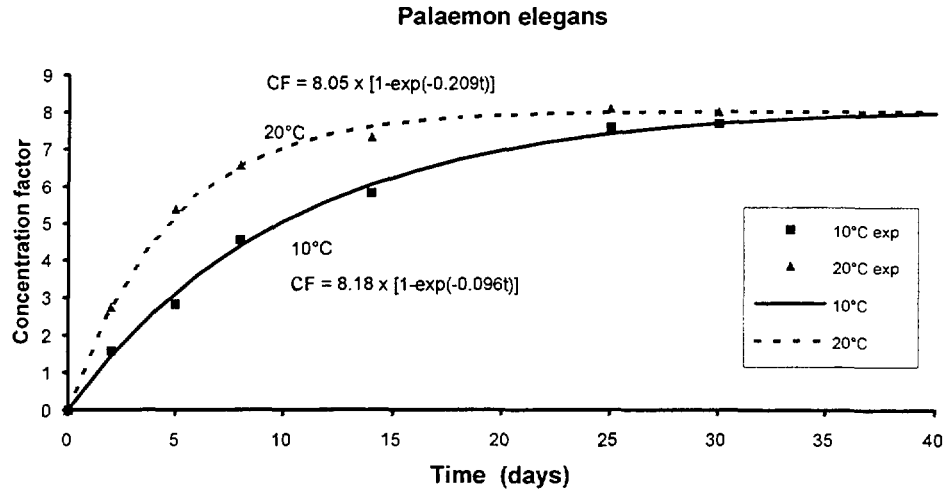

Fig.3. Influence of temperature on the concentration factor of ^{95m}Tc in the shrimp Palaemon elegans; after Schulte et al. [2].

If in the case of the shrimp *Palaemon elegans* where more than one compartment will be considered, e.g. the whole body and the hepato-pancreas (Fig.4), the uptake process can be expressed as a second order equation where 90% and 10% of the radioactivity is located in the hepato-pancreas and rest of the body, respectively.

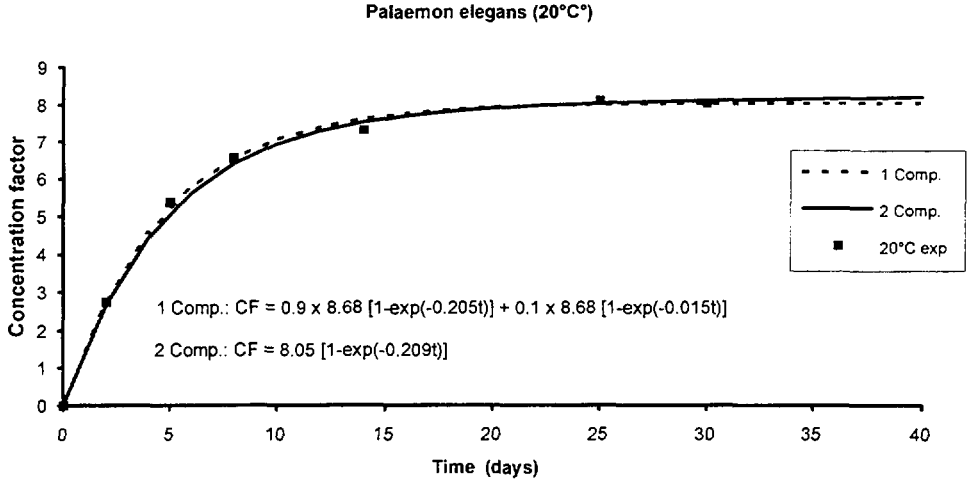

Fig.4. Uptake and accumulation curves (CF) of ^{95m}Tc expressed by simple first order kinetics and by a two-compartmental equation in shrimp using same experimental data; after Schulte et al. [2]

If concentrations of the radioisotope in organs are measured, different uptake velocities will be noted, and, hence, different final CFs measured according to the different

physiological features of the organs. Consequently, two different k-values will be found which represent the respective uptake velocities in the two compartments. As mentioned above, the different organs represent the different compartments (fractions) in the formula.

In another laboratory experiment with a the crab *Pachygrapsus marmoratus* (Fig.5) an uptake and accumulation curve of Tc-95m could be described by a more complicated equation which comprises several body compartments and the respective fractions of radioactivity found in those compartments (53%, 20%, and 27%). This approach may appear confusing, but it will become clearer after the execution of loss and elimination experiments through which all percentages in the corresponding body compartment or organ can be determined.

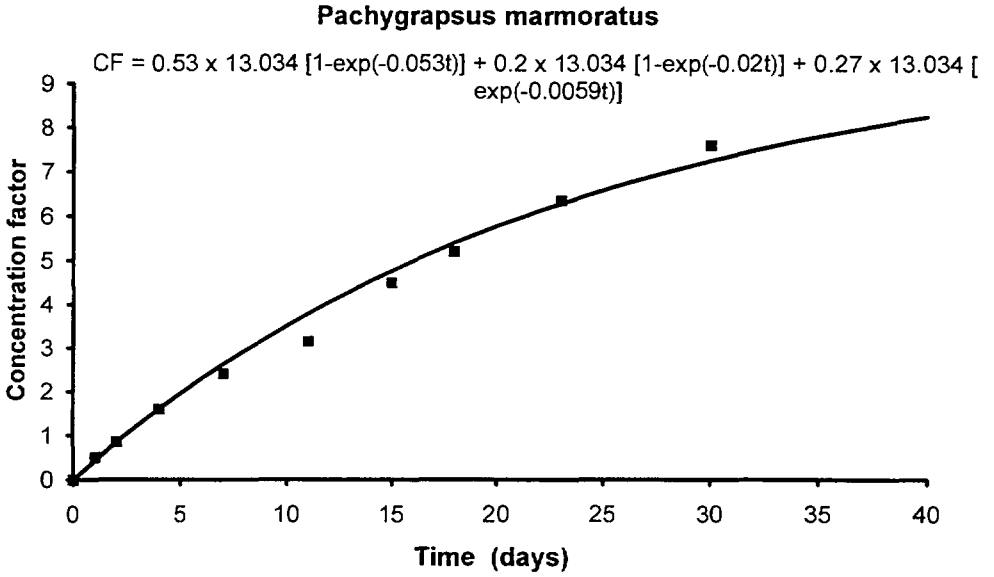

Fig.5. Uptake and accumulation (CF) curve of ^{95m}Tc in the crab Pachygrapsus marmoratus described by a multi-compartmental equation; after Schulte et al. [4].

6.4.7. Accumulation from water and food

On some occasions one has to consider the combined uptake of radionuclides from water **and** from food. Coming back to the initial formula, the simple model describes uptake or accumulation as intake minus excretion. The formula remains practically the same as before except that two factors, which correspond to the intake from food plus the intake from water must be introduced. Furthermore, one has to consider various parameters and factors mentioned previously which potentially influence the uptake. All those factors enter into the formula, i.e. concentration of the radioisotope in the water and food, body size (weight) of the organism, and the temperature (uptake velocity is dependent on temperature and weight of the organism at a certain time).

$$\frac{dC_t}{dt} = \dot{I} - kC_t; \quad \text{(Accumulation = Intake - Excretion)}$$

$$\frac{dW_tC_t}{dt} = (I_{ft} + I_{wt})W_t - k_TW_tC_t \qquad (4)$$

where

C_t	is the concentration of the radionuclide (Bq/g) at time *t*
W_t	is weight of organism (g) at time *t*
I_{ft}	is intake from food
I_{wt}	is intake from water
k_t	is the fixation rate (elimination) in function of temperature and weight (W_t)

The former formula does not consider growth since it is sufficiently difficult to control all the afore-mentioned parameters of the experiment. Nevertheless, under normal experimental conditions one should maintain growth. However, sometimes it may be impossible to control growth, thus, during the time of the experiment exponential growth of the organisms, especially when using small organisms, may occur. That situation will complicate the formula because organisms, depending on the initial weight at time zero, will exponentially change weight according to their growth constant, i.e. it will increase.

$W_t = W_0 e^{\lambda_g \cdot t}$; λ_g = growth constant/day

$$C_t = \frac{I_{wt} + I_{ft}}{k_T + \lambda_g}\left[1 - e^{-(k_T+\lambda_g)\,t}\right] \qquad (5)$$

A rise in temperature T (°C) increases the value C_t, but not the asymptotic value at equilibrium. Uptake curves obtained at different temperatures will reach the same final constant CFs, regardless of the temperature used during the experiment. Equilibrium (steady state) will only be reached faster (note the two curves at 10° and 20°C; Fig. 3). The increase in weight, however, will normally decrease the value of C_t, due to variations in physiology and "dilution" of tissues in the organism. Also the asymptotic value (CF) will decrease.

If intake from water and food are considered separately, then the following formulas can be used. Once again it holds that uptake or accumulation is intake minus excretion. In both cases the same parameters as before have to be considered, i.e. the weight of the organism, the temperature at time t, and the increase in weight per day.

$$C_t = \frac{I_{wt}}{k_T + \lambda_g}\left[1 - e^{-(k_T+\lambda_g)\,t}\right]$$

$$I_{wt} = \frac{C_t(k_T + \lambda_g)}{\left[1 - e^{-(k_T+\lambda_g)\,t}\right]}; \quad \text{at equilibrium (steady state): } C_t = C_{ss}(1 - e^{-(k+\lambda)t})$$

$$I_{wt} = C_{ss}(k_T + \lambda_g) \qquad (6)$$

The value C_t may be corrected for the weight increase (λ_g). After integration the intake at equilibrium (steady state concentration) is $\mathbf{1\text{-}e^{-k}}$, which is the excretion factor, plus λ, which is the physical decay of the radioisotope. By introducing that expression into the former

formula, the intake will depend directly on the excretion rate, which itself depends on the temperature and the daily growth increase.

Food is considered to be used for two reasons, maintenance of the fish and for growth. During growth a certain coefficient of maintenance must be considered, i.e. not all of the food will be assimilated by the organism. The coefficient of maintenance from food depends on body weight of the fish and will affect the absorption, which will be directly proportional to the assimilation factor. In this case the main parameters are body weight at time (t), efficiency of food utilisation (assimilation efficiency), the coefficient of maintenance, and the concentration of the radionuclide in the food at time (t).

$$I_{ft} = C_{ft}\ \xi\ W_t^j + C_{ft}\ \frac{1}{\varepsilon} \cdot \frac{dW_t}{dt} \qquad (7)$$

where

I_{ft}	is the intake from food
C_{ft}	is the concentration in food at time *t*
ξ	is the maintenance food coefficient, related to weight (W) by the exponent j
W_t	is the body weight at time *t*
ε	is the efficiency of food utilisation for growth (assimilation coefficient)

Some examples of laboratory experiments (Fig. 6) may demonstrate the influence of food regimes and changes in weight of the experimental organisms on the concentration factor.

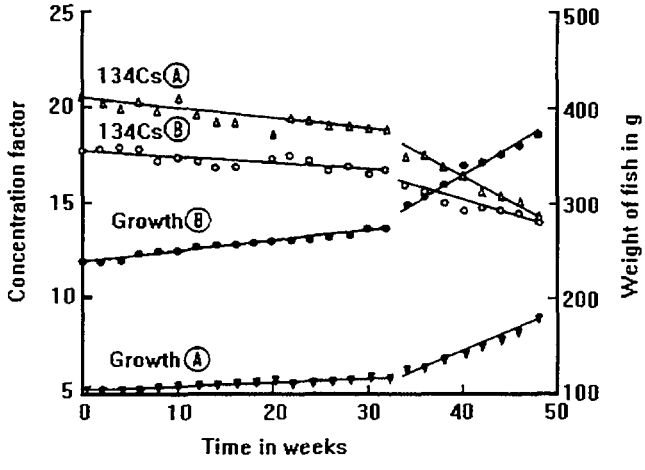

Fig.6. Effect of change in weight on the concentration factor of ^{134}Cs in plaice [1]

In two distinct experiments **(situation A and B)**, plaice were maintained at a steady growth and feeding regime. While growing, the concentration factor in the fish decreased slightly. When the feeding regime was drastically changed, the growth increased and the CF dropped considerably. One has to bear this in mind in order to obtain reliable CFs in the laboratory, because if the conditions during the experiment change, CFs will not be comparable; therefore, all experimental conditions should be maintained constant for the duration of the experiments.

6.4.8. Elimination

Once a certain value of CF is reached or a certain quantity of the radioisotope has accumulated in the organism, which may be close to the steady state condition or the asymptotic value, it may be of interest to examine the loss, or loss velocity or elimination rate of the radioisotope by the organism. For this reason an elimination or loss experiment has to be performed in uncontaminated aquaria. Generally, the contaminated organism which had accumulated a certain amount of radioactivity is placed into a non-contaminated environment. This can be done in two ways, either in a flow-through sea water system or in a aquarium with a re-circulation system for uncontaminated water. Normally, aquaria with a sufficiently large volume are satisfactory in order not to change the water frequently.

The quantity of radioactivity in the organism at the time of start of the elimination experiment (C_0) is measured at 100%, regardless of the absolute quantity in the organism. The elimination or loss followed over time can be described by the following formula.

$$C_t = C_0\, e^{-k_{eff}\cdot t}; \quad k_{eff} = \frac{0.693}{T_{1/2eff}} \quad \text{(constant of effective elimination)} \qquad (8)$$

where

C_t is the concentration at time t.
C_0 is the concentration at time t_0 (body burden) which can reach C_{ss} (100%).

This correlation shows that the concentration at time t is dependent on the concentration of the radionuclide in the organism at time zero. That means 100% in this case, i.e. $C_0 e^{-k_{eff}\, t}$. This constant of effective (*eff.*) elimination consists of two rates or two factors of elimination: a real physiological elimination of the quantity of radioactivity in the organism, and the elimination by physical decay. This may be of varying importance according to the radioisotope used. The coefficient of effective elimination can be derived from the formula for effective half-life ($k_{eff} = 0.693/T_{1/2\ eff}$).

The calculation of the **effective half-life** or half-time of a radioisotope in an organism is a relation between physical half-life or decay and the biological half-life which still has to be determined. The equation can be solved for the biological half-life and elimination rate or turnover time in the organism.

$$T_{1/2eff} = \frac{T_{1/2(p)} \times T_{1/2(b)}}{T_{1/2(p)} + T_{1/2(b)}}; \qquad T_{1/2(b)} = \frac{T_{1/2(p)} \times T_{1/2eff}}{T_{1/2(p)} - T_{1/2eff}}; \qquad (9)$$

p = physical
b = biological

6.4.9. Elimination (Multi-compartmental equation)

The elimination equation resembles very much the uptake equation (3). In both expressions the same fractions of the radioisotope present in the different compartments (A_1,

A_2, A_n) of the experimental organisms are considered with their respective elimination constants (k_1, k_2, k_n).

$$C_t = A_1 \cdot C_{ss}\, e^{-k_1 \cdot t} + A_2 \cdot C_{ss}\, e^{-k_2 \cdot t} + A_n \cdot C_{ss}\, e^{-k_n \cdot t} \qquad (10)$$

where

C_t	is the concentration at time *t*.
A_1; A_2	are the fractions in compartment 1 and 2 of the value C_{ss}.
k_1; k_2	are the elimination constants (rates) in compartment 1 and 2.
t	is the time in days

All parameters of the equation are subjected to the same factors as during accumulation, i.e. body size, growth rate, temperature, etc.. In order to solve the elimination equation, all unknown items have to be determined. This can only be achieved by knowing the organ distribution of the radioactivity in the organism by means of dissection.

Therefore, when planning a laboratory experiment in radioecology one has to bear in mind from the very beginning how many organisms will be needed for the elimination experience in order to have a sufficient number of organisms left for dissection. Because without dissecting the organisms, none of the components representing the different elimination velocities and different behaviour of the radioisotope in the organism can be determined.

The general interest of radioecological laboratory studies is the knowledge of the behaviour of a certain radionuclides in body compartments or organs of the organism under study which may be of important from the standpoint of health protection. Thus, it will be most interesting to know CFs and biological half-lives in edible parts like the muscle, or where most of the radioactivity is accumulated (critical organ). For this purpose it is essential to know exactly from the very beginning how many organisms are needed effectively in order to get reliable values when dissecting the organisms at the end of the experiment.

Examples of laboratory studies on elimination of Tc (Fig. 7) by three marine organisms have revealed quite large differences in loss velocities and general behaviour of elimination. In all three cases the organisms as a whole were considered.

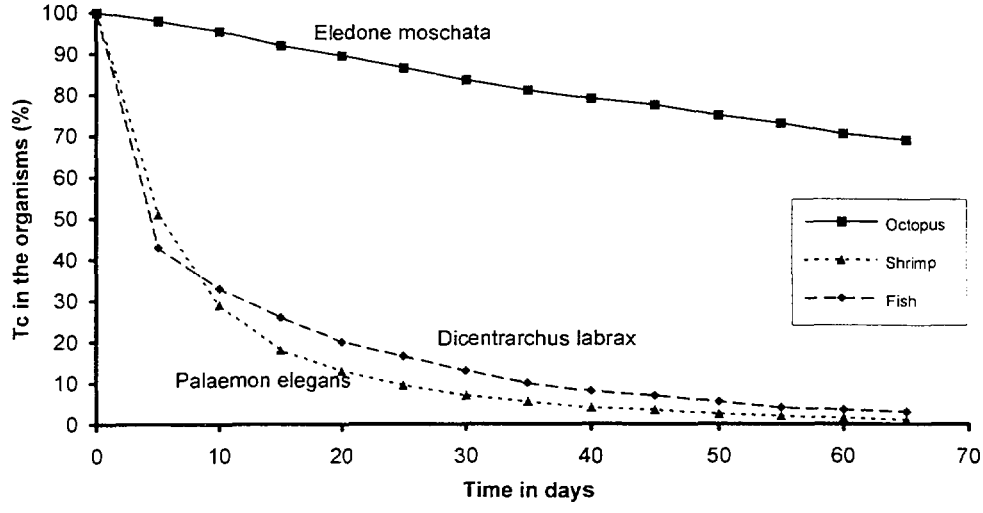

Fig.7. Elimination curves of ^{95m}Tc by three marine organisms (octopus, shrimp, and fish); after Schulte et al. [5].

Both fish (*Dicentrarchus labrax*) and crustacean (*Palaemon elegans*) showed a fast initial elimination rate slowing down after 15 to 20 days. The cephalopod (*Eledone moschata*) lost the accumulated radioisotope very slowly and at a constant rate. The fast loss in fish and crustaceans can be explained by the fact that most of the radioactivity was located in the liver and hepato-pancreas which both have a high metabolic activity and, hence, a fast excretion and elimination while the radioactivity in the cephalopod was fixed in body parts with characteristically low metabolic activity.

General speaking, the cephalopod seemed to behave as one compartment while fish and the shrimp showed at least two different velocities of elimination; therefore, more than two compartments may be attributed to those loss curves.

As shown for the accumulation of Tc in shrimp (*Palaemon elegans*) temperature has also a striking effect on the elimination velocity (Fig.8). The loss curves are described by equations which consider two compartments in the organism, i.e. the hepato-pancreas and the whole body (75% and 25%; 70% and 30% respectively).

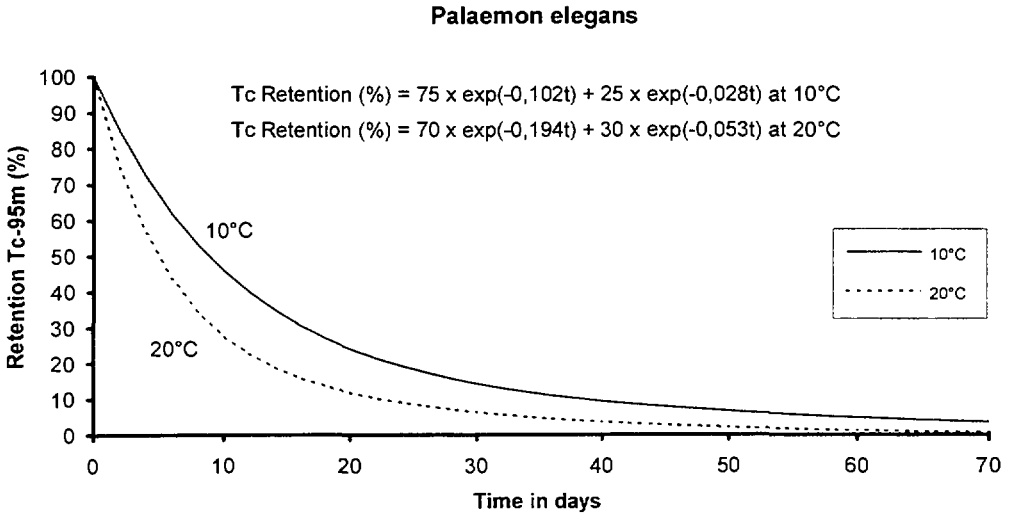

Fig.8. Effect of temperature on the elimination velocity of ^{95m}Tc in the shrimp Palaemon elegans; *after Schulte et al. [2].*

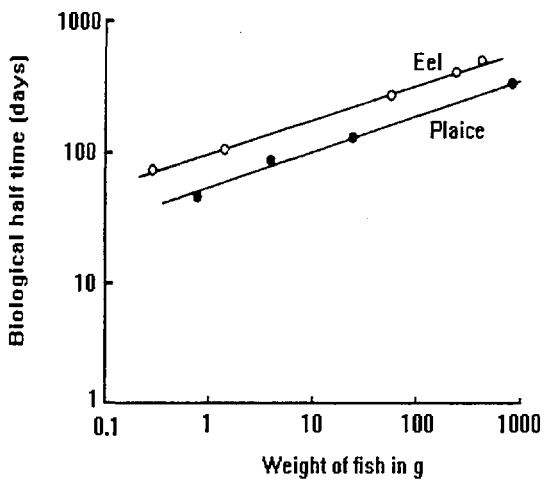

Fig.9. Relation between biological half-time and weight for plaice and eels [1]

Among the various physiological factors, growth i.e. size and the respective physiological activity influences strongly the elimination rate of incorporated radioactivity in fish. Younger (smaller) fish show faster elimination rates (shorter biological half-times) than older ones as clearly depicted in Fig 9. where the biological half-times increase exponentially with the weight of the specimens.

6.4.10. Graphical determination of parameters

The parameters of for the elimination equation can be determine graphically and this is often used to determine the elimination coefficient and the percentage of radioactivity in the different compartments. The loss curve (points) drawn on semi-logarithmic paper will be represented by one or more straight lines. The number of lines to be fitted to the experimental points should correspond to the number of compartments found in the organisms by dissection.

If the graphic presentation of the experimental results for radionuclide loss indicates a single straight line, then the organism can be considered as only one compartment. In this case the quantity of radionuclide **transferred** (accumulated) per unit of time **from water to the organism** is proportional to the concentration of the radionuclide in the water (C_W) and a constant ($\mathbf{k}_{W.org.}$), the coefficient of adsorption, while the quantity **eliminated** from the organism (**lost from the organism to water**) per unit of time is proportional to the radionuclide concentration in the organism itself and the constant ($\mathbf{k}_{org.w.}$), the coefficient of elimination or excretion. Under equilibrium conditions, the two rates, $\mathbf{k}_{W\ org.}$ and $\mathbf{k}_{org.w}$, are equal, i.e. the velocity of transfer of radioactivity from the water to the organism (accumulation, intake), depending directly on the concentration of the radioisotope in the water, balances the velocity of loss from the organism to the water. This indicates steady state where intake equals loss or release.

At equilibrium (steady state):

$$k_{w\,org.} \cdot C_w = k_{org.\,w} \cdot C_{org.}; \qquad \frac{C_{org.}}{C_w} = CF_\infty = C_{ss}\,(steady\ state) \tag{11}$$

$$k_{w\,org.} = k_{org.\,w} \cdot C_{ss}$$

(intake) (loss)

where

$k_{W\ org.}$ is the rate constant water → organism
$k_{org\ w}$ is the rate constant organism → water

A practical demonstration of the procedure of the graphical method is given using the experimental data for a crab (*Pachygrapsus marmoratus*) obtained in a laboratory study (Fig.10).

The straight lines arbitrarily drawn correspond to three different velocities and compartments in the organism. During the observation phase of 120 days, no more than three compartments could be identified. A longer observation time would perhaps have revealed additional compartments with very slow elimination velocities like bone.

Starting with the slowest compartment one can directly read the percentage of radioactivity which was present at time zero for this part of the curve, in this case 27%. For the faster compartments the differences between each other and 100% are 20% for the intermediate and 53% for the fastest compartment. By dissecting the organisms afterwards one may find the corresponding compartments which show similar contents (percentages) of the total radioactive body burden. In this particular case the three percentages could be attributed to hepato-pancreas (53%), digestive system (stomach and gut, 20%), and gills and other organs (27%), the latter which had the slowest exchange or turnover rate.

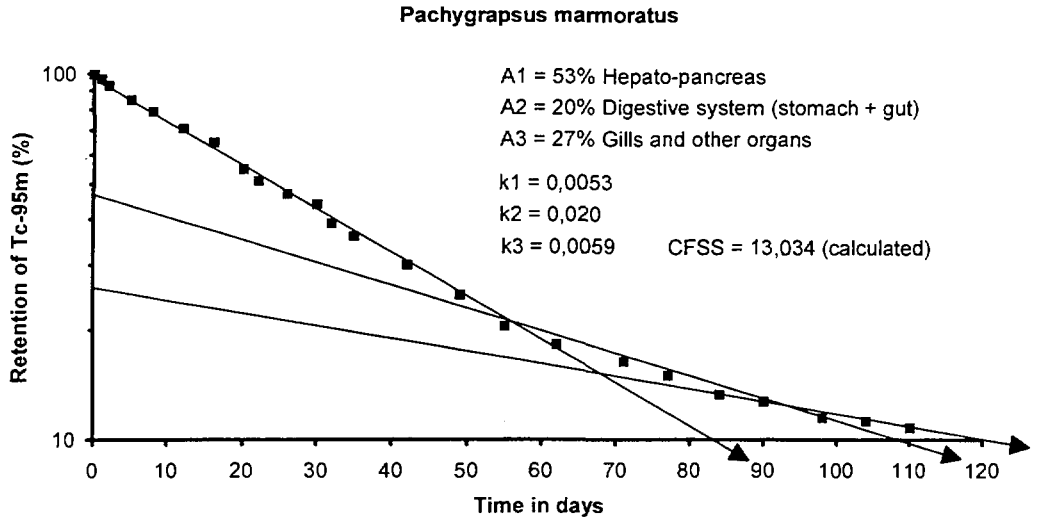

Fig.10.Graphical determination of the percentages of radioactivity present in the different compartments in the crab Pachygrapsus marmoratus *using a semi-logarithmic plot of experimental data. Intersections of the lines with the ordinate indicate percentages of radioactivity in the different compartments; after Schulte et al.[4].*

The remaining elimination coefficient k necessary to describe completely the elimination process can be calculated by the formula ***k = 0.693/biological half-life(days)***. The biological half-life (time necessary to reduce the initial activity 50%) of a certain radioisotope e.g. Tc in the different organs can be read directly from the graph by subtracting the slow loss components from the faster ones since the value of 100% is the sum of all components. The completed loss curve for Tc and its formula for the crab is shown in Fig. 11.

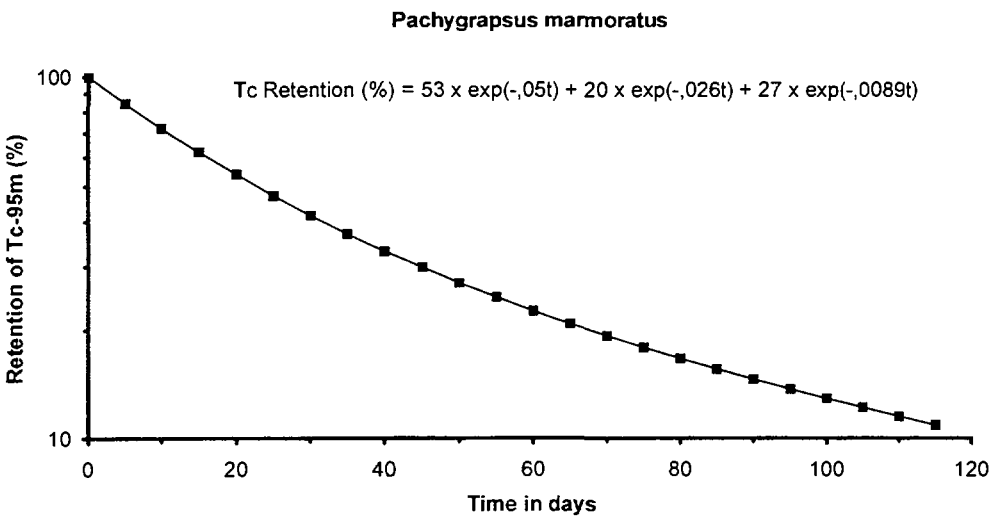

Fig.11.Loss of Tc-95m in the crab Pachygrapsus marmoratus *and its corresponding three-compartmental equation; after Schulte et al.[4].*

The practical execution of the graphical determination of biological half-lives and the percentages present in the different compartments is demonstrated in the Fig. 12.

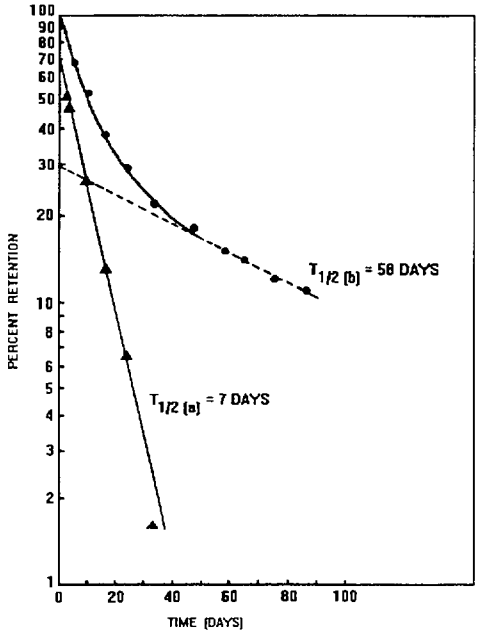

Fig.12. Retention of Tc-95m by Haliotis rufescens *following direct uptake from seawater. •, whole animal retention curve; Δ, rapid loss component obtained by subtracting slow loss component (----) from total retention curve [6].*

In this two component loss curve the low velocity component, which represents 30% of the total body burden, has to be subtracted from 100%. The resulting fast velocity component which represents 70% has to be back-extrapolated to the initial original part of the loss curve. Then starting from 70%, the biological half-life can be found by reading the value on the abscissa vertically below the point of intersection between 35% and the constructed line (7 days). The same holds for the low velocity component.

6.5. COMPARISON OF CFs OBTAINED UNDER LABORATORY AND FIELD CONDITIONS

Concentration factors obtained under laboratory conditions may differ considerably from those measured in the field (Tab. I).

TABLE I. Comparison of concentration factors of marine biota measured in the field and under laboratory conditions

BIOTA	LABORATORY	Ref.	FIELD	Ref.	AREA
ALGA					
Phaeophyceae					
Fucus sp.	250 - 2500	7 - 11	85000	14	Scottish coast
"	-		75000	14; 15	Norwegian coast
"	-		25000	16	Baltic Sea
"	-		21000 - 89000	17	Atlantic (Channel)
Rhodophyceae	8	12	400	13	Atlantic (Channel)
MOLLUSCA					
Gastropoda					
Patella sp.	10	8	1000 - 7000	17	Atlantic (Channel)
Haliotis tuberc.	30	13	4300	13	Atlantic (Channel)

The question arises whether the field or the laboratory data have to be considered as artefacts. The difficulty to measure and determine CFs in the natural environment is caused by the high uncertainty of correct measurements of radioactivity concentrations in the natural water body. Often very low concentrations of a certain radionuclide are found which are difficult to detect (large water volumes to be handled) or vary considerable between locations and within short time periods, so that it is difficult to decide on a realistic value. On the other hand, under laboratory conditions certain marine species may behave atypically which results in concentration factors that may not reflect reality.

These discrepancies between laboratory results and values found in nature can be easily encountered during radioecological studies as shown by actual results from the radioecology literature (Fig.13). For example, brown algae (*Fucus sp.*) were sampled at different times for ten years to define continuous uptake as CF (Bq/kg dry). The algae accumulated Tc-99 continuously from water showing a constant rate of increase in radioactivity in their tissues. This fact is a result mainly of the physiology of the plants which grew continuously as well as low Tc concentrations found in the water. Generally speaking, the growing algae did not reach an equilibrium with the surrounding water and therefore did not display a constant concentration factor.

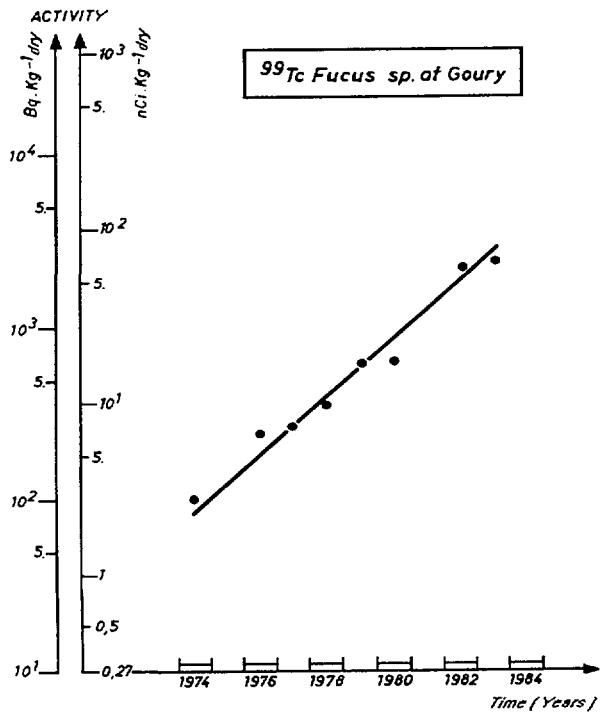

Fig.13.Evolution of the activity of ^{99}Tc with time at Goury between 1974 and 1983 [17]

Frequently biota in nature are subjected to fluctuating concentrations of radionuclides (Fig.14). Variation of concentrations of Tc-99 in the effluent of a reprocessing plant were reflected with a certain time lag in *Fucus sp.* and the mollusc *Patella sp.*.

Both species showed also synchronous behaviour with respect to increase and decrease of radioactivity in the surrounding water body. Thus, during the time of observation both species could not reach a constant concentration factor and therefore, did not reach equilibrium. The reasons may be found either in the fluctuating concentration of the radioisotope, or in the physiology of the organisms, i.e. their growth, or both .

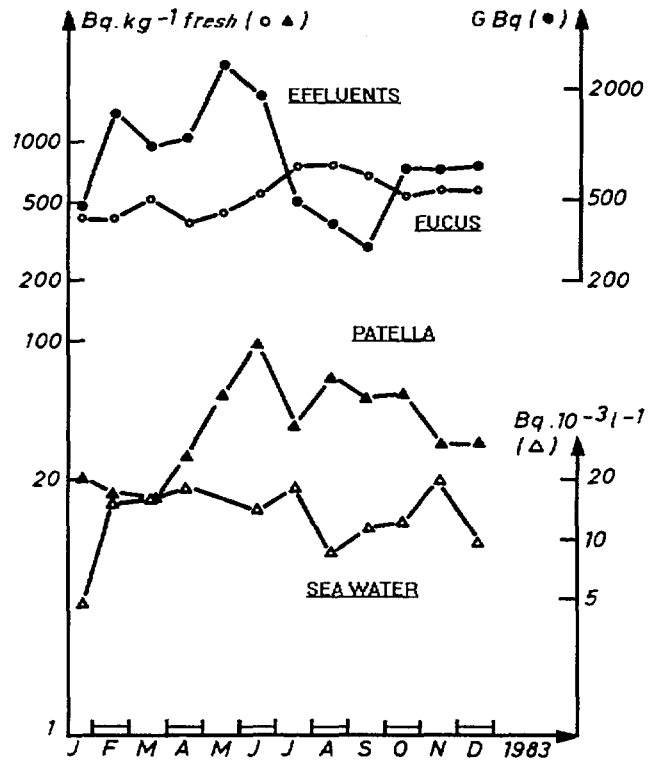

Fig.14.Annual variation of ^{99}Tc activity in discharge waters, sea water, Fucus sp. *and* Patella sp. *(sea water, fucus and patella were sampled at Goury at the same time) [17]*

6.6. CONCLUSION

Although the experimental approach to measurements and determinations of radioecological parameters in marine biota (concentration factors, assimilation rates, biological half-lives etc.) seemed to be quite simplistic, experience on the general behaviour of radionuclides in marine species gained over several decades has demonstrated that much useful information can be obtained from laboratory simulations providing proper controls on the experiments and their conditions were set and maintained. However, data obtained under laboratory conditions with species deprived of their natural environment can only be but indicative since realistic values can only be found and/or measured in the field. For several reasons direct measurements of concentration factors and still more seldom biological half-lives or residence-times are often very time consuming and difficult to obtain in the field because of fluctuating concentrations of the radionuclide or high infra-species variability. Nevertheless, data from laboratory experiments have often given "values" that could have been otherwise obtained much more difficultly or not at all with field measurements. In conclusion, one should consider laboratory experiments, even if performed as close as possible to natural conditions, as a means, technical tool and/or simulation of what may happen under natural conditions. Therefore, data and results obtained in the laboratory should always be taken with care and, if possible, be validated against measurements in the field.

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