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Applied radiotracer techniques for studying pollutant bioaccumulation in selected marine organisms (jellyfish, crabs and sea stars)

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Abstract Obtaining specific information on contaminant biokinetics in marine biota is often necessary for properly interpreting monitoring data on trace contaminant levels in bioindicator species living under varying environmental conditions. Radiotracers have been employed in laboratory experiments to assess the uptake, distribution and retention of selected heavy metals and PCB congeners in three potential marine bioindicators occupying different ecological niches in the coastal zone. Pelagic and benthic jellyfish readily accumulated Co, Ag, Zn, Cd, ^{137}Cs and ^{241}Am from both water and food and retained them with biological half-lives ($T_{b1/2}$) ranging from a few days to several weeks. Zinc and silver were accumulated to the greatest degree (CF $\sim 4 \times 10^2$), with benthic jellyfish having a greater affinity for metals than the pelagic species. Results from light–dark experiments indicate that the enhanced metal uptake in the benthic jellyfish is due to the presence of endosymbiotic photosynthetic zooxanthellae situated in the arms of organisms. Shore crabs ingesting Ag, a sewage-related contaminant, readily accumulated the metal with male crabs assimilating some 71% and female crabs 51% of the Ag from their food. Moreover, the assimilated fraction of Ag remained virtually immobile in their tissues as evidenced by an extremely long $T_{b1/2}$ for depuration of 7.3 years. Sea stars exposed to ^{14}C-labelled PCB congener #153 in sea water accumulated the congener mainly in the body wall and podia reaching lipid weight CFs that ranged between approximately 2×10^5 to 4×10^5. In contrast, following exposure in radiolabelled sediments, the corresponding PCB transfer factors in the same tissues were much lower, *viz*., 3×10^2 to 5×10^2. Nevertheless, regardless of the exposure mode, CFs of PCB in the other tissues (digestive system, gonads, pyloric and rectal caeca) were consistently one to two orders of magnitude lower, an observation which suggests that sea star body wall and podia could serve as target tissues in biomonitoring studies assessing these toxic compounds.

Key words radiotracers • bioaccumulation • metals • radionuclides • PCB • marine invertebrates

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Introduction

Inorganic and organic contaminants entering coastal waters may be concentrated by edible marine organisms to varying degrees from either water, their food or sediments [3]. Understanding the transfer of contaminants through the food web is critical to predict the exposure of humans to contaminants (either through subsistence or commercial consumption of seafood) and the possible health consequences of such exposure. In addition, such information is crucial in making accurate risk assessment for seafood safety purposes, a topic which is attracting much national and international attention.

Nuclear techniques can be used to improve our understanding of the processes involved in the transfer of radionuclides and conventional contaminants through coastal marine food chains. In particular, the ability to radioanalyse live organisms and the increased sensitivity of radiotracer detection allows: 1) reducing considerably biological variation in experiments; 2) measuring contaminant biokinetics over the long term in a limited number of individuals; 3) studying marine organisms and contaminant transfer mechanisms that cannot be easily investigated using standard analytical techniques. Furthermore, it allows working experimentally at contaminant concentrations that closely reflect those present in the environment. This overview

highlights some of the advantages of radiotracer techniques used to assess contaminant flow through marine biota by illustrating some examples with different taxonomic species from three case studies carried out in the experimental radioecology facilities at IAEA-MEL.

Case studies

Heavy metal bioaccumulation in gelatinous plankton

Jellyfish are prey for numerous invertebrate and vertebrate species and as such play a central role in the trophic organization of many marine food chains. Furthermore, they are known to prey abundantly and selectively upon certain zooplankton species including fish larvae [6], and thereby may exert a major impact on the structure and dynamics of mesozooplankton communities as well as fish stocks. Their impact on the environment is particularly important during summer blooms, when jellyfish may occur in very dense aggregations containing millions of individuals. Despite the well-known ecological importance of jellyfish, data are extremely sparse on the accumulation of metals and other contaminants in coelenterates, although their abundance, trophic position, and planktonic behaviour suggest they can affect the fluxes and fate of these contaminants in marine waters. Therefore, our objective in this study was to investigate the biokinetics of heavy metal and radionuclide transfer in jellyfish in order to assess their role in the marine fluxes of these contaminants. Jellyfish are a primary example of organisms whose study involves several practical problems due to their delicate gelatinous nature and high water content. Hence, the use of radiotracer approaches has allowed us, using live organisms, to delineate the biokinetics of selected heavy metals and radionuclides accumulated both from sea water and food.

Two species of jellyfish, the benthic *Cassiopea andromeda* and the pelagic *Aurelia aurita,* were acclimated to laboratory conditions (open circuit aquaria; water renewal 10% per hour; salinity 38‰, T = (18 ± 1)°C; fed daily with *Artemia salina* brine shrimp nauplii) for approximately 8 weeks prior to experimentation. Both species were then exposed to radiotracers of four heavy metals (^{57}Co, ^{65}Zn, ^{110m}Ag, ^{109}Cd) and to long-lived artificial radionuclides (^{134}Cs, ^{241}Am) directly from water or through their food (*viz.* live *A. salina* nauplii previously exposed to the tracers for 48 h). Jellyfish as well as brine shrimp were exposed to the following activities: 0.2 Bq ml^{-1} ^{241}Am, 0.5 Bq ml^{-1} ^{57}Co, ^{65}Zn and ^{110m}Ag, and 1 Bq ml^{-1} ^{109}Cd and ^{134}Cs. Uptake and excretion of the radioisotopes were followed in the whole live animals for 1–3 months to determine concentration factors (CF), assimilation efficiencies (AE), and retention ($T_{b½}$) of the contaminants [4]. Tissue distribution of the isotopes was also determined by dissecting a few individuals at different times during the experiment. In addition, the possible influence of the symbiotic zooxanthellae on contaminant bioaccumulation in *C. andromeda* was examined by performing uptake experiments under light and dark conditions. All radiotracer analyses were carried out using a high-resolution γ-spectrometry system consisting of coaxial Ge (N- or P-type) detectors (EGNC 33-195-R, Intertechnique) connected to a multichannel analyser and a computer with spectra analysis software (Interwinner, Intertechnique). The detectors were calibrated with appropriate standards for each of the counting geometries used, and measurements were corrected for background and physical decay of the radiotracers. Counting times were adapted to obtain relative propagated errors less than 5%.

With either exposure mode (sea water or food), ^{110m}Ag and ^{65}Zn were the metals accumulated to the greatest degree by both jellyfish species (Table 1). Furthermore, in all cases both radionuclides, ^{134}Cs and ^{241}Am, were always taken up much less efficiently and lost more rapidly than the heavy metals. Except in the case of zinc, which was taken up with similar efficiency by the two species, *C. andromeda* accumulated all other isotopes much more efficiently than *A. aurita* (Table 1).

Dissection of *C. andromeda* showed that the vesicles, situated along the arms and containing the endosymbiotic zooxanthellae, always displayed the highest CF for the metals tested. The CFs in vesicles ranged from 110 ± 8 (^{57}Co) to 1080 ± 230 (^{110m}Ag), and were 2 to 17 times higher that those calculated in the other body compartments (*viz.* umbrella, tentacles, gut and mesoglea). This suggests that autotrophic metabolism of the photosynthetic zooxanthellae is actively involved in metal uptake by the jellyfish. Indeed, all the metals were more readily concentrated by the benthic species under light conditions (Table 1). In contrast, no significant difference was observed in uptake of the radionuclides under light and dark conditions.

Elimination of metals and radionuclides previously accumulated via sea water was also species dependent. Retention capacity for metals in *A. aurita* was quite weak in that all the accumulated isotopes were rapidly excreted with biological half-lives ($T_{b1/2}$) ranging from 3 to 6 days only, whereas *C. andromeda* retained metals much more efficiently with $T_{b1/2}$ ranging from 25 to 60 days. Accordingly, zooxanthellae may also be involved in the processes of metal release.

The feeding experiments demonstrated that, except for ^{134}Cs and ^{241}Am, both jellyfish species readily accumulated and assimilated these heavy metals from their prey. Furthermore, heavy metal assimilation efficiency (AE) and resultant retention time ($T_{b1/2}$) were always higher in *C. andromeda* (AE, 65 to 94%; $T_{b1/2}$, 28 to 65 days) than in *A. aurita* (AE, 37 to 57%; $T_{b1/2}$, 20 to 29 days).

Such radiotracer studies have shown that jellyfish take up heavy metals and retain them in their tissues quite efficiently, in particular Zn and Ag. Both sea water and food are important pathways for metal accumulation in their tissues. Metal assimilation from food was particularly

Table 1. Whole-body concentration factors (mean ± sd; n = 9) calculated in jellyfish exposed to radioisotopes for 14 days in sea water.

Isotope	*A. aurita*	*C. andromeda* (in light)	*C. andromeda* (in dark)
^{57}Co	6.0 ± 0.5	82 ± 3.9	64 ± 2.2
^{65}Zn	317 ± 37	412 ± 39	281 ± 23
^{110m}Ag	28 ± 3.2	455 ± 25	305 ± 10
^{109}Cd	20 ± 3.3	224 ± 16	148 ± 11
^{134}Cs	1.6 ± 0.2	3.6 ± 0.4	4.1 ± 0.5
^{241}Am	1.2 ± 0.2	12 ± 1.3	10 ± 0.6

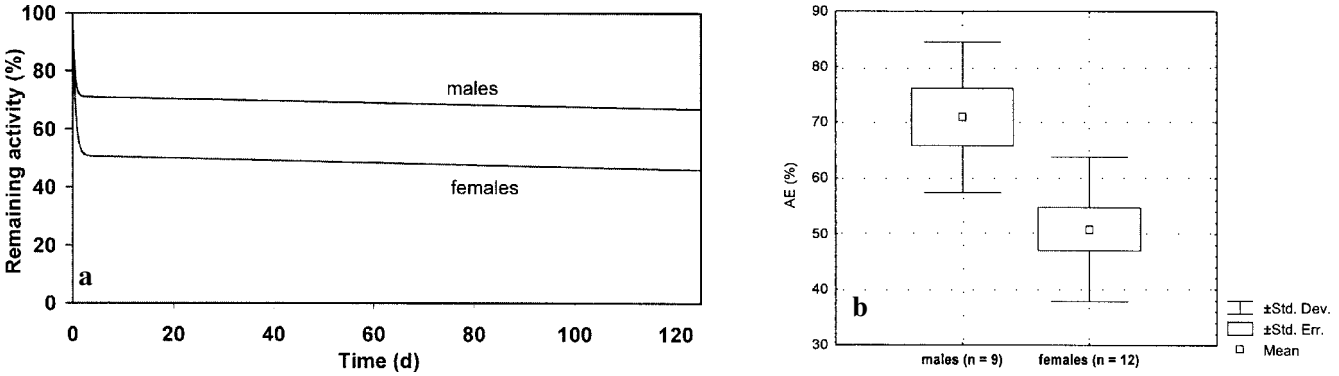

Fig. 1. Loss kinetics of ^{110m}Ag from male and female crabs *Pachygrapsus marmoratus* after a single feeding on radiolabelled shrimp (a) and difference in assimilation efficiency (AE) of ^{110m}Ag ingested with food in males and females (b).

elevated in *C. andromeda*, a benthic species. High metal assimilation from ingested prey coupled with a strong retention in tissues indicates that over the long term, dietary intake might be the predominant source of metal contamination in this benthic medusa. Jellyfish, which are key representatives of the gelatinous plankton community, constitute an important biomass in the oceans. Given they are also efficient metal bioaccumulators, these ubiquitous gelatinous plankton likely play an important role in biological transfer and recycling of heavy metal contaminants in the marine environment.

Trophic transfer and retention of silver in crabs

Another example of a major constraint of using classical analytical techniques for experimental studies is evaluating contaminant biokinetics in a given organism over the long term. Benthic crabs, like many other crustaceans, are known to accumulate many heavy metals and radionuclides [1], and are an important commercial food source for many coastal countries. The heavy metal silver is a common marine contaminant often associated with untreated urban sewage [5]. Using γ spectrometric techniques (i.e. Ge detectors), we were able to follow Ag behaviour in a small number of crabs that were allowed to ingest during a short period of time (pulse-chase feeding technique) shrimp previously labelled alive with the ^{110m}Ag radiotracer.

Crabs (*Pachygrapsus marmoratus*; size from 0.7 to 11.4 g wet wt; $n = 30$) were allowed to feed for 9 h on small shrimp (*Palaemonetes varians*) previously exposed to 0.5 Bq ml^{-1} ^{110m}Ag for 15 d. Loss kinetics of ingested ^{110m}Ag were then followed individually in each crab for 4 months, using a high-resolution γ-spectrometry system as the one described above. Calibration and correction for background and physical decay of the radiotracers were realised as previously described and counting times were adapted to obtain relative propagated errors less than 5%. In parallel, the distribution of ^{110m}Ag in their tissues was investigated using whole-body autoradiography (WBARG) with crabs N_2-frozen at different times.

Whole-body loss kinetics of ingested ^{110m}Ag were best fitted by a two-component exponential model. The short-lived compartment described loss of ^{110m}Ag along with the faeces ($T_{b1/2}$ = 8 h) and the long-lived compartment described turnover of ^{110m}Ag actually assimilated by the organisms. The retention capacity of this metal was extremely strong ($T_{b1/2}$ = 7.3 y), underscoring the usefulness of *P. marmoratus* as an excellent long-term recorder of silver contamination in coastal environments.

Not surprisingly, the amount of food ingested increased with crab size, particularly in males (correlation coefficient $r = 0.77$); however, crab size (and, hence, amount of ingested food) had little or no effect on the assimilation efficiency (AE) of ^{110m}Ag from food. In contrast, $T_{b1/2}$ of the short-lived compartment increased with increasing crab size (r = 0.71 in females and 0.87 in males) indicating that the smaller the crab, the faster the gut transit time for the metal.

Interestingly, the results also revealed that AE of ^{110m}Ag was related to the sex of the crab ($p_{T\,\text{test}} = 0.006$); *viz.* AE was (71 ± 13)% in male crabs *vs.* (51 ± 13)% in females (Fig. 1). Nevertheless, all other biokinetic parameters of the radioisotope were not significantly different between females and males.

Based on 15 independent observations during the experiment, molting did not affect loss kinetics of ingested ^{110m}Ag indicating that the strong retention of this metal did not involve silver sequestration in the organism's exoskeleton. Furthermore, autoradiography (WBARG) indicated that incorporated ^{110m}Ag was exclusively localized in the hepatopancreas of the crabs. Therefore, mechanism(s) responsible for the very efficient sequestration of Ag in this crustacean are most probably related to hepatic metabolism.

It should be noted that crabs were growing during the experiment and gained about 50% of their initial weight after 4 months. Therefore, the long-term retention of Ag could be determined only because of the use of a radiotracer that could be counted as whole-body activity against a background virtually equal to zero. Indeed, any observation using classical metal measurements (AAS, ICPS) would have resulted in Ag concentrations decreasing with time due to growth dilution.

PCB bioaccumulation in echinoderms

The high degree of sensitivity of radiodetection methods are also useful for investigating bioaccumulation of organic contaminants which occur at very low concentration, such as individual PCB congeners. Echinoderms are increasingly being used as coastal biomonitors of organic contaminants, since they are known to accumulate toxic compounds such as PCBs to relatively high levels [2]. Therefore, the uptake

Table 2. Concentration and transfer factors (CF and TF; maximum, minimum and mean values) of ^{14}C-PCB#153 in the body compartments of the sea star *Asterias rubens* after 34 days of exposure via sea water or sediments.

	Body wall	Digestive system	Gonads	Rectal caeca	Pyloric caeca	Podia
			Sea water exposure			
Max. CF*	3.91×10^5	9.16×10^4	6.01×10^4	7.90×10^3	4.75×10^4	2.43×10^5
Min. CF	3.52×10^5	5.44×10^4	2.96×10^4	4.58×10^3	1.05×10^4	1.72×10^5
Mean CF	3.74×10^5	7.50×10^4	4.62×10^4	6.76×10^3	2.31×10^4	2.17×10^5
			Sediment exposure			
Max. TF*	417	109	150	3.43	70	863
Min. TF	286	81	111	2.91	55	258
Mean TF	343	94	137	3.10	61	479

* CF and TF are calculated as the ratio between PCB#153 concentration in the sea star body compartments ($ng \cdot g^{-1}$ total lipids) and its concentration ($ng \cdot g^{-1}$) in sea water (CF) or sediments (TF).

of ^{14}C-labelled PCB congener #153 (*viz.* the most abundant congener found in biota) was investigated in a key echinoderm species, the common NE Atlantic sea star *Asterias rubens.* After 4 weeks of acclimation, individuals were experimentally exposed for 5 weeks to labelled PCB congener at realistic PCB concentrations via sea water (31.4 $ng \cdot l^{-1}$) or sediments (9.5 $ng \cdot g^{-1}$ dry wt). Water, sediment and sea star tissue samples were prepared as described in Danis *et al.* [2] and ^{14}C-radioactivity was measured using a 1600 TR Liquid Scintillation Analyzer (Packard), compared to standards of known activities, and corrected for quenching, background and physical decay of the radiotracer. Counting times were adjusted to obtain counting rates with relative propagated errors less than 5%. ^{14}C-activities were eventually expressed as PCB concentrations on a total lipid content basis. Owing to the high sensitivity of the detection technique, ^{14}C-PCB biokinetics could be measured in individual organs, most of which are too small to be analyzed by standard PCB measurement techniques, such as GC-ECD or GC-MS.

Results indicate that PCB bioaccumulation by *A. rubens* varies with both the body compartment and the exposure mode (Table 2). When exposed to PCB in ambient sea water, uptake was always the most efficient, i.e. concentration factors were up to 3 orders of magnitude higher compared to organisms exposed to spiked sediments. Concentrations of congener #153 attained in the body compartments at the end of the experiments were in the range of values reported from several field studies. This indicates that the experimental procedure adequately simulated exposure conditions in the field. These radiotracer experiments also allowed determining the main sea star organs which bioaccumulate PCB, i.e. body wall and podia. Therefore, these specific organs and tissues should be considered for purposes of PCB biomonitoring in coastal benthic environments.

Conclusions

We conclude that radioisotopic techniques are a very unique tool for studying under carefully controlled conditions transfer and transport processes of inorganic and organic contaminants in a variety of marine species. Such nuclear techniques are extremely sensitive and allow working with live organisms at realistic pollutant concentrations as well as examining biokinetics of certain organic contaminants in target organs which are often too small for quantifying these compounds by classical detection methods. Furthermore, the usefulness of radiotracers to obtain rapid information on contaminant bioaccumulation and retention potential in organisms selected for biomonitoring has widespread application to monitoring programmes and coastal zone management strategies in general.

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