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An experimental study on the bioaccumulation and turnover of polonium-210 and lead-210 in marine shrimp

Fernando P. Carvalho, Scott W. Fowler

International Atomic Energy Agency, Marine Environment Laboratory, PO Box 800, MC-98012 Monaco Cedex

ABSTRACT: The experimental accumulation of ^{210}Po and ^{210}Pb from seawater alone and from seawater and food was studied in the laboratory with the benthic shrimp *Lysmata seticaudata*. Shrimp accumulated both radionuclides from water for 21 d, reaching whole body concentration factors [CF = (Bq g^{-1} of shrimp wet wt)/(Bq ml^{-1} filtered water)] of 139 ± 28 for ^{210}Po and 682 ± 149 for ^{210}Pb. When uptake was from water only, tissue analyses revealed that radionuclide accumulation was mainly due to external adsorption. Accumulation in internal tissues also occured through the intake of seawater for osmotic regulation. Exposure of shrimp to radionuclides dissolved in water and labelled food produced a noticeable accumulation of ^{210}Po in internal tissues which was not accompanied by an identical accumulation of ^{210}Pb. It is concluded that in shrimp ^{210}Po was mainly accumulated from food, whereas ^{210}Pb was largely taken up from the fraction dissolved in seawater. A much higher digestive assimilation efficiency for ^{210}Po, about 5-fold that of ^{210}Pb, was observed in *L. seticaudata*. This difference can account for the enhancement of ^{210}Po:^{210}Pb ratios observed in the lower trophic levels of marine food chains. Turnover of ^{210}Po in shrimp tissues is much slower than for ^{210}Pb, a fact which also contributes to the enhanced ^{210}Po:^{210}Pb concentration ratios observed in marine crustaceans.

KEY WORDS: Bioaccumulation · Crustaceans · Lead · Natural radioactivity · Polonium

INTRODUCTION

The relative importance of food and water in the bioaccumulation of trace elements including polonium-210 (^{210}Po) and lead-210 (^{210}Pb) in marine species has been debated by researchers for many years; however, surprisingly, very few attempts have been made to differentiate these routes of uptake (Fowler 1982). In an early study on the euphausiid *Meganyctiphanes norvegica*, Heyraud & Cherry (1979) deduced that the ingestion of food should play a major role in the accumulation of ^{210}Po. Later, Cherry et al. (1989) reported the results of an experimental study on ^{210}Po ingestion by anchovy and pilchard which demonstrated that the ^{210}Po content of fish flesh reflects to some degree the concentrations of ^{210}Po in the food. The potential use of these radionuclides as natural tracers of the diet of marine organisms has thus been suggested (Carvalho 1988a, Heyraud et al. 1988, Cherry et al. 1989, Miquel et al. 1993). However, as generally acknowledged by these authors, the use of ^{210}Po or the ^{210}Po-^{210}Pb pair as natural tracers of the diet of marine organisms is based on the assumption that all the radionuclide accumulation occurs via the food chain and that there is no direct accumulation from seawater.

In the marine environment ^{210}Pb and ^{210}Po originate from the radioactive decay of radium-226 dissolved in seawater and from the atmospheric deposition of radon-222 daughters. The concentrations of ^{210}Pb and ^{210}Po in seawater, suspended matter and marine organisms have been well documented (Hoffman et al. 1974, Heyraud & Cherry 1979, Cherry & Heyraud 1981, McDonald et al. 1986, Carvalho 1988a, b, Heyraud et al. 1988, Skwarzec & Bojanowski 1988).

In contrast to terrestrial organisms, many marine species are highly enriched in these radionuclides. For example, ^{210}Po concentrations of 40 Bq kg^{-1} (dry wt) in

phytoplankton (Skwarzec & Bojanowski 1988), 15 to 1700 Bq kg^{-1} dry wt in different shrimp species (Cherry & Heyraud 1981), and from 1 to 7000 Bq kg^{-1} dry wt for a wide variety of marine fish (Carvalho 1990) have been reported. While some tissues, such as muscle, consistently display low ^{210}Po concentrations, certain organs have been found to contain unusually high ^{210}Po levels. This is the case for crustacean hepatopancreas (100 to 27 000 Bq kg^{-1} dry wt, Heyraud et al. 1988), and liver (2000 Bq kg^{-1}), gonad (1000 Bq kg^{-1}) and the intestinal wall (1×10^5 Bq kg^{-1}) of common clupeoid fish such as sardines (Carvalho 1988a, 1990). ^{210}Pb is also always present in the tissues of marine organisms but, in general, at concentrations lower than ^{210}Po. For example, reported ^{210}Po:^{210}Pb activity concentration ratios typically vary between 10 and 10^3 (Heyraud & Cherry 1979, Carvalho 1988a); however, the reason for elevated ^{210}Po:^{210}Pb ratios in many marine organisms remains unclear.

For several other reasons, interest in the bioaccumulation of ^{210}Po and ^{210}Pb by marine organisms and their transfer through food chains has broadened in recent years. First, the high concentration of alpha-emitting ^{210}Po in certain tissues in marine biota results in some of the highest known natural radiation doses (Cherry & Heyraud 1982, Carvalho 1988a). Second, the relatively high concentration of ^{210}Po in marine organisms commonly used in the human diet can increase the radiation dose to man by a significant factor (Watson 1985, Carvalho 1990). Furthermore, the enhancement of natural levels of ^{210}Po and ^{210}Pb in estuaries and coastal zones, from various phosphate-rock processing industries (elemental phosphorus, phosphoric acid and phosphate fertilizer manufacturing plants), poses the added potential risk of their transfer to humans through consumption of sea food (Koster et al. 1992).

To investigate the pathways involved in ^{210}Po and ^{210}Pb accumulation, and to identify the relative importance of the sources of these radionuclides to marine organisms, a series of controlled laboratory radiotracer experiments was conducted with marine shrimp. Here, results are presented on the accumulation of ^{210}Po and ^{210}Pb from seawater and from food, and the relative distribution and turnover of these radionuclides in the different shrimp tissues.

MATERIAL AND METHODS

Radiotracers and radioanalytical techniques. A standard solution of ^{210}Pb ($t_{1/2}$ = 22.2 ± 0.2 yr) as lead nitrate in 3 M HNO_3 was purchased from ORIS, France. The concentration of the ^{210}Pb was 0.737 MBq g^{-1} solution and was in radioactive equilibrium with its progeny ^{210}Bi ($t_{1/2}$ = 5.01 d) and ^{210}Po ($t_{1/2}$ = 138.4 d). From the ^{210}Pb stock, dilute working solutions were prepared as well as radioactive sources with adequate activities and geometries for calibrating the gamma spectrometry system. The 46.5 keV gamma emission (4.05% intensity) of ^{210}Pb was measured using a large volume NaI well-type detector connected to a multichannel analyser. The absolute detection efficiency of ^{210}Pb was 3.4 ± 0.2% under the conditions selected.

Measurements of ^{210}Po were made through its 5.30 MeV alpha particle emission, using ^{209}Po (4.88 MeV alpha emission, $t_{1/2}$ = 102 yr) as the internal tracer. After a standard addition of ^{209}Po (purchased from Oak Ridge, TN, USA), each sample was completely dissolved with mineral acids and polonium spontaneously plated onto a silver disc in 0.5 N HCl in the presence of ascorbic acid (Flynn 1968). Recovery was generally between 80 and 100%. Measurements were made on silicon surface-barrier detectors connected to a multichannel analyser. The detectors were calibrated with an electroplated ^{239}Pu reference source. Concentrations of both ^{210}Po and ^{210}Pb in samples were much above detection limits. Counting times of samples were adjusted to obtain relative standard errors of approximately 5%, and resultant concentrations of ^{210}Po and ^{210}Pb are reported per unit of fresh weight of tissue. As ^{210}Po analyses were made immediately, no correction was necessary for either the radioactive decay of ^{210}Po or the ^{210}Po ingrowth from ^{210}Pb.

Organisms and experimental procedures. The common Mediterranean shrimp *Lysmata seticaudata* (Risso) (Hyppolytidae), a detritivore, was selected for this study. Shrimp were collected in baited traps located near the port of Monaco and were subsequently acclimated in laboratory aquaria for about 1 wk prior to use.

A solution of ^{210}Pb-^{210}Po in radioactive equilibrium was used to elevate the concentrations of naturally occurring ^{210}Po and ^{210}Pb (ca 1 mBq l^{-1}) to 260 Bq l^{-1} in the experimental seawater. All aquaria containing 5 l of seawater were maintained at 14 °C under a controlled 12 h light : 12 h dark cycle. To prevent the buildup of shrimp exometabolites in the aquaria, the labelled seawater was replaced every 2 to 3 d by fresh seawater (filtered through a ca 10 µm sand filter) spiked with ^{210}Po-^{210}Pb. The frequent renewal of radioactive water minimized the fluctuation of radionuclide levels throughout the uptake experiment.

Two separate groups of shrimp were used to study the kinetics of ^{210}Po and ^{210}Pb accumulation from water and from water plus food. Shrimp in Group I (mean individual wet wt 0.72 ± 0.24 g, n = 12) were never fed in the experimental aquaria during the 21 d exposure period. Instead, they were regularly fed brine shrimp *Artemia* sp. (from a frozen stock) for short periods

(< 0.5 h) in non-radioactive seawater inside the counting vials during the whole-body gamma measurements.

Shrimp in Group II (0.54 ± 0.06 g, n = 12) were always fed ad libitum in the aquaria containing the radioactive seawater during the 14 d exposure period. *Artemia* sp. carcasses supplied as food were allowed to reside for long periods in the aquaria. In this way shrimp were exposed both to ^{210}Po-^{210}Pb dissolved in water as well as to the radionuclides adsorbed on the food particles. A separate experiment with brine shrimp provided information that adsorption of ^{210}Pb from water by *Artemia* sp. occurs rapidly. Measurements of *Artemia* sp. collected from experimental aquaria at the end of the feeding periods indicated that identical ^{210}Po and ^{210}Pb activities were sorbed on food.

Throughout both the experiments, concentrations of radionuclides in seawater and on suspended matter (filterable on 0.45 µm pore size Millipore filters) were monitored. In addition, radioactivity in Group I shrimp over time was measured on a whole-body basis. The ^{210}Pb measurements were made *in vivo* and most shrimp were returned to the aquaria for further uptake, whereas the ^{210}Po measurements required the periodic sacrifice of individual shrimp during the experiment.

In the case of Group II shrimp, uptake measurements were only made for ^{210}Pb. For purposes of comparison, at the end of the exposure period detailed ^{210}Pb and ^{210}Po analyses were made in tissues of shrimp from both Groups I and II. Thereafter all the remaining shrimp in Group II were transferred into aquaria receiving flowing seawater (unlabelled) to follow the elimination of the radionuclides from whole shrimp and their tissues over time.

RESULTS

The uptake of dissolved ^{210}Pb and ^{210}Po from seawater by shrimp (Group I) is shown in Fig. 1. At the end of a 3 wk exposure to elevated concentrations of these radionuclides in water, the increasing whole-body concentration factors (CFs) indicated that equilibrium concentration of nuclides between shrimp and water had not been reached. By the end of the experiment, average CFs were 139 ± 28 (n = 3) for ^{210}Po and 682 ± 149 (n = 3) for ^{210}Pb. Comparison of these 2 mean values using *t*-test statistics indicates that the CFs are significantly different at $p < 0.01$ level.

Periodic analyses of the water resulted in an average concentration of 262 ± 35 Bq l^{-1} for ^{210}Pb and 230 ± 23 Bq l^{-1} for ^{210}Po (n = 6) in the dissolved phase. Before renewal, the radionuclides in particulate phase

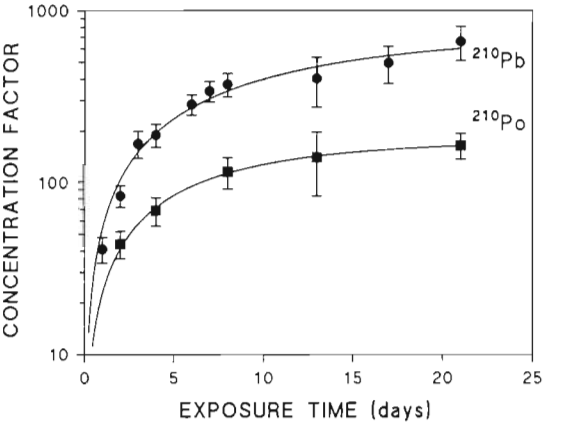

Fig. 1. *Lysmata seticaudata*. Uptake of ^{210}Pb and ^{210}Po from seawater by shrimp. Each point is the mean ± 1 SD of 3 to 10 individual measurements for ^{210}Pb and 2 to 3 measurements for ^{210}Po. Concentration Factor (CF) = (Bq g^{-1} of shrimp wet wt)/(Bq ml^{-1} of water, dissolved)

(> 0.45 µm) were 10 to 15% of the total radioactivity in the water. Therefore, 85% or more of the total ^{210}Pb and ^{210}Po activities remained in the dissolved phase throughout the exposure period. A control aquarium with seawater and no shrimp showed that adsorption of ^{210}Pb and of ^{210}Po on aquaria walls was negligible, and that the ^{210}Po-^{210}Pb radioactive equilibrium was maintained in solution (^{210}Po:^{210}Pb = 0.97 ± 0.09, n = 4).

Group I shrimp were never fed in the experimental aquaria. Frozen *Artemia* sp., containing a low concentration of natural ^{210}Po (0.030 ± 0.014 Bq g^{-1} dry wt), were fed to shrimp in unlabelled seawater. Gamma measurements of this water after the feedings indicated that a small amount of ^{210}Pb, less than 10% of the shrimp whole-body activity, had been released from shrimp. Nevertheless, control adsorption experiments utilizing *Artemia* sp. demonstrated that very little of the ^{210}Pb would have been recycled and accumulated by the shrimp during the short time they fed on dead *Artemia* sp. under these conditions.

During the accumulation phase, Group II shrimp were always fed in the radioactive aquaria in order to include the accumulation of both nuclides via ingested food. Whole-body gamma measurements of ^{210}Pb in these shrimp (not shown) gave results identical to those in Fig. 1 for ^{210}Pb accumulation from radioactive water only. However, analyses of water from the Group II aquarium indicated that the fraction of activity associated with particles was higher than in the seawater from Group I, i.e. it ranged from 25 to 40% of the total activity before renewal. This was most likely due to the higher concentration of residual particles in the form of *Artemia* sp. debris in Group II water. Average concentrations of radionuclides in the soluble phase were 250 ± 33 Bq l^{-1} for ^{210}Pb and 207 ± 35 Bq l^{-1} for ^{210}Po (n = 9).

Table 1. *Lysmata seticaudata*. Specific activity (Bq g^{-1} wet wt) and concentration factor [CF = (Bq g^{-1} tissue)/(Bq ml^{-1} water, dissolved)] of ^{210}Po and ^{210}Pb in experimental and control shrimp. CFs for control group were computed using ambient concentrations in seawater: 1 mBq l^{-1} for ^{210}Po and 1.5 mBq l^{-1} for ^{210}Pb. Three individuals from each group were pooled for analysis to obtain a more representative sample. ^{210}Pb in control shrimp was measured by a second plating of ^{210}Po approximately 6 mo later. Relative standard errors of activity concentrations are generally 5% for ^{210}Po and between 5 and 10% for ^{210}Pb. Whole shrimp dry wt : wet wt ratio = 0.26 ± 0.01 (n = 3)

Tissue	% Wet wt	^{210}Po (Bq g^{-1})	CF	^{210}Pb (Bq g^{-1})	CF	^{210}Po:^{210}Pb
Group I: 21 d uptake from water						
Hepatopancreas	2.8	73	316	77	297	0.95
Muscle	39.5	5.5	24	12	47	0.46
Exoskeleton	18.1	63	272	452	1738	0.14
Remainder	39.6	65	281	350	1348	0.18
Whole body	100	32	139	177	682	0.18
Group II: 14 d uptake from water and food						
Hepatopancreas	7	958	4628	232	928	4.13
Muscle	40	11	54	8	32	1.38
Gut	2.1	65	314	53	212	1.21
Exoskeleton	12.8	303	1465	454	1813	0.66
Remainder	41.1	227	1097	302	1210	0.75
Whole body	100	168	810	166	663	1.01
Control						
Hepatopancreas	3.6	1.66	1600000	0.024	16000	69
Muscle	35.7	0.008	8000	0.0001	67	80
Exoskeleton	15.2	0.029	29000	0.0021	1400	14
Remainder	45.5	0.015	15000	0.0004	267	37
Whole body	100	0.075	75000	0.0016	1067	47
Group II: after 20 d of depuration						
Hepatopancreas	3.3	109		8		14
Muscle	26.8	3.5		0.03		117
Gut	3.2	40		17		2
Exoskeleton	20.3	12		0.8		15
Remainder	46.4	19		1.3		15
Whole body	100	16		1.5		11

After a 14 d uptake period, shrimp from Group II were dissected and compared with the tissue results obtained from Group I shrimp (Table 1). A comparison of the radionuclide contribution of each tissue to the whole-body burden of ^{210}Po and ^{210}Pb in *Lysmata seticaudata* from these 2 groups, as well as in control shrimp containing natural concentrations of these nuclides, is given in Fig. 2. In the control group the concentration of ^{210}Po was higher in hepatopancreas than in other tissues, accounting for about 80% of the total ^{210}Po in *L. seticaudata* despite the small weight of this organ (Fig. 2). Exoskeleton, which represents a much larger fraction of the shrimp weight, contained only ca 9% of the total ^{210}Po. A similar distribution was found for ^{210}Pb; however, concentrations were 10 to 100 times lower than those of ^{210}Po. In our experiment *L. seticaudata*, exposed to ^{210}Po and ^{210}Pb dissolved in water (Group I), displayed the highest concentrations of both radionuclides in the external surfaces (exoskeleton and remainder) which contained most of the total activity in shrimp (body burden) for both accumulated radionuclides (Table 1, Fig. 2). The internal tissues (hepatopancreas + muscle) contributed only 10% of the total ^{210}Po and 5% of the total ^{210}Pb; therefore, CFs computed for these experimental conditions were generally higher in the external organs (Table 1). Furthermore the Po:Pb activity ratios in exoskeleton and in the remainder fraction account for the low Po:Pb concentration ratio measured on a whole-body basis (Table 1). Concentration of both radionuclides in internal tissues of these shrimp were higher than concentrations measured in control shrimp. Since no labelled food was supplied in this experiment (see above), this observation implies that some direct absorption from seawater and internal transfer took place.

The results of Group II shrimp show a different distribution of the radionuclides (Table 1, Fig. 2). While the exoskeleton and remainder fraction still contribute most to the ^{210}Pb and ^{210}Po whole-body burden, the relative contribution of internal tissues is much higher

than in Group I. The internal tissues (hepatopancreas + muscle) account for 35% of total ^{210}Po but only 9.5% of total ^{210}Pb (Fig. 2). The enhanced accumulation of food-associated radionuclides in hepatopancreas is expected considering the absorptive and storage functions of this organ (Waterman 1960, Mantel & Farmer 1983). Moreover, comparison of the ^{210}Pb concentrations (Bq g^{-1}) and CFs in Table 1 shows that the uptake of ^{210}Pb in the exoskeleton of these Group II shrimp is identical to the ^{210}Pb uptake by exoskeleton of Group I shrimp. This result indicates that ^{210}Pb has essentially been directly adsorbed from water in both cases.

Further examination of ^{210}Po and ^{210}Pb concentrations in tissues is instructive (Table 1). For example, the hepatopancreas and muscle in Group II shrimp display much higher concentrations of ^{210}Po than in the first group (significantly different at $p < 0.05$). In the case of ^{210}Pb only the hepatopancreas concentration increased following uptake from food and water. Such a difference would be expected due to ingestion of radioactive food in the experimental aquaria, nevertheless the increase in ^{210}Pb concentrations (hepatopancreas) was markedly less than those observed for ^{210}Po. Furthermore, the concentrations of ^{210}Po in exoskeleton and remainder of Group II shrimp were also 4 to 5 times higher than those in Group I, whereas ^{210}Pb concentrations were identical in both groups within the experimental error. This suggests that ^{210}Po assimilated from food was transferred through internal organs to the exoskeleton more rapidly than ^{210}Pb, resulting in an increase of the Po:Pb ratio in exoskeleton from approximately 0.1 in Group I to 0.7 in Group II. In addition Group II shrimp displayed a much enhanced whole-body Po:Pb concentration ratio of 1.0 (Table 1). In general, comparison of concentrations in the tissues of the 2 groups indicates that the ingestion of food has produced a higher accumulation of the radionuclides, particularly ^{210}Po, in the internal organs.

Following the uptake phase, radiolabelled shrimp from Group II were transferred into aquaria with running seawater to follow the kinetics of ^{210}Po and ^{210}Pb elimination (Fig. 3). On a whole-body basis, as well as in individual tissues, the elimination of ^{210}Pb proceeded faster than that of ^{210}Po. Biological half-lives for radionuclide turnover in whole shrimp and their tissues ranged from 7 to 28 d for ^{210}Po and 2 to 11 d for ^{210}Pb (Table 2). Molting of shrimp which occurred on Day 9 resulted in the observed sudden decrease in radionuclide concentrations. The cast molts removed 80 to 90% and 30 to 40% of the whole-body concentration of ^{210}Pb and ^{210}Po respectively. The high percentages of radioactivity associated with these molts confirm that surface adsorption of these nuclides onto shrimp external parts occurred during the uptake phase. The molts displayed Po:Pb ratios which were

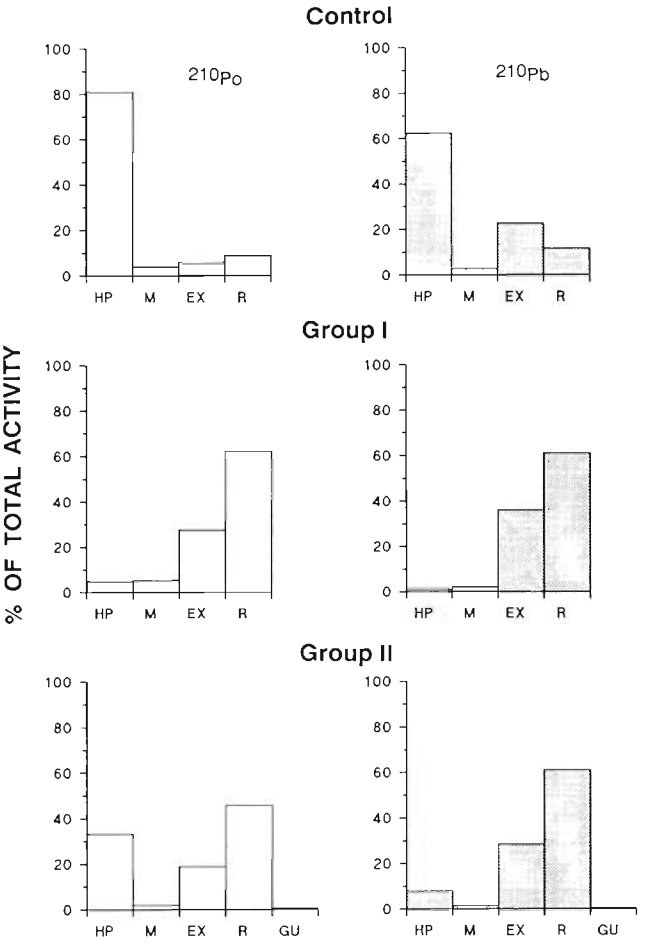

Fig. 2. *Lysmata seticaudata*. Percent contribution of tissues to the whole-body burdens of ^{210}Pb and ^{210}Po in shrimp. Control: naturally occurring radionuclides; Group I: after 21 d of uptake from water; Group II: after 14 d of uptake from water and from labelled food; HP: hepatopancreas; M: muscle; EX: exoskeleton; GU: gut; R: remainder

greater than unity due to the more rapid desorption of ^{210}Pb from the shrimp. Furthermore, the new cuticle produced by shrimp also displayed Po:Pb ratios much higher than unity (Fig. 4).

DISCUSSION

It has been experimentally verified that ^{210}Po and ^{210}Pb dissolved in seawater are accumulated to a certain degree by shrimp. However, when water is the only source, the accumulation of these radionuclides by shrimp does not result in CFs similar to those found in natural waters. Instead, the experimental CFs are approximately 1 to 2 orders of magnitude lower (Table 1). Furthermore, detailed analyses of shrimp tissues indicate that resultant radionuclide distributions

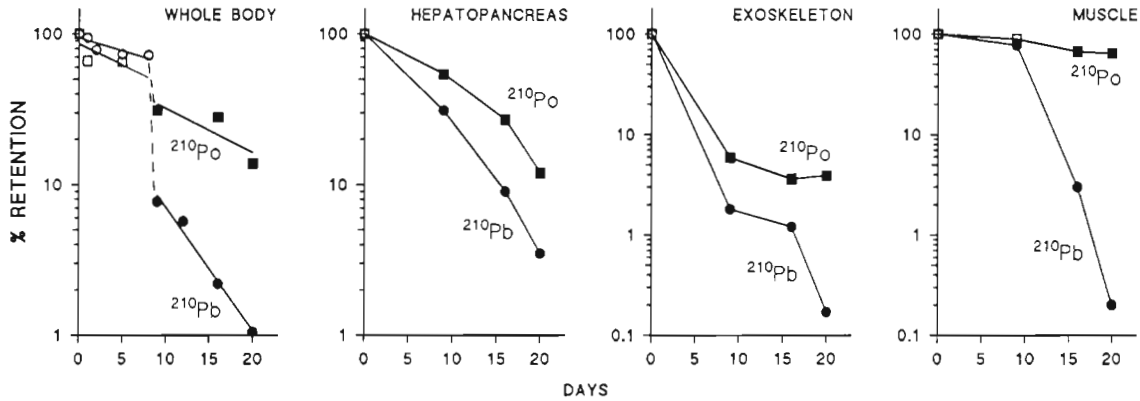

Fig. 3. *Lysmata seticaudata*. Retention of ^{210}Pb and ^{210}Po in whole shrimp and in shrimp tissues. Each point corresponds to the concentration (Bq g^{-1} wet wt) in 2 or 3 individuals pooled in a composite sample as a percent of the concentration at time zero. Dashed lines indicate molting of shrimp. Open symbols and solid symbols indicate measurements in shrimp and their tissues before and after molting, respectively

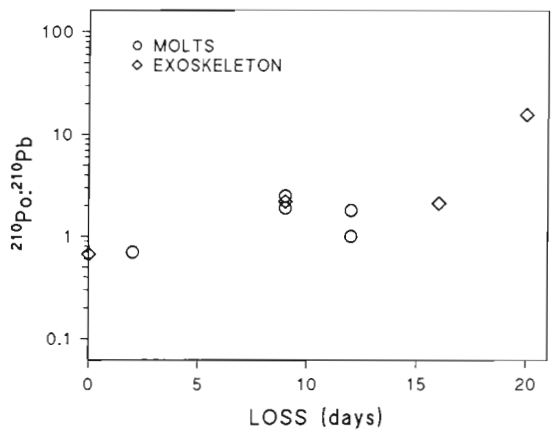

Fig. 4. *Lysmata seticaudata*. Evolution of ^{210}Po:^{210}Pb concentration ratio in the exoskeleton of shrimp as a function of depuration time. Molts: exuviae naturally released by shrimp; exoskeleton: samples obtained by dissection

do not agree with the distribution of naturally occurring ^{210}Po and ^{210}Pb in this crustacean (Fig. 2). In fact, the analyses of tissues demonstrated that the experimental uptake of ^{210}Po and ^{210}Pb from water was mainly due to adsorption onto shrimp exoskeleton (>90% of total body burden), whereas absorption into shrimp internal organs, although noticeable, was comparatively low. In contrast to uptake from water, the additional ingestion of labelled food produced a notable increase in ^{210}Po concentration in shrimp tissues which was not mirrored by a similar increase in ^{210}Pb. This resulted in elevated ratios (>1) of Po:Pb concentrations in shrimp, which tended toward those (>10) usually found in nature (Table 1). Therefore, ^{210}Po dissolved in seawater is not the primary source of this radionuclide to crustaceans. Moreover, passive adsorption from seawater is not the main mechanism for the accumulation of the high polonium concentrations

Table 2. *Lysmata seticaudata*. Biological half-lives ($t_{1/2,\,b}$) of ^{210}Po and ^{210}Pb in shrimp tissues. Percent retention data fitted to a single exponential model, $R(t) = R(0)e^{-kt}$ · $t_{1/2,\,b} = (\ln 2)\,k^{-1}$; R = correlation coefficient of the log-linear least square fit

Tissue	^{210}Po					^{210}Pb				
	Interval of fit	*y*-intercept	$t_{1/2,\,b}$ (d)	n	R	Interval of fit	*y*-intercept	$t_{1/2,\,b}$ (d)	n	R
Whole body										
Before molting	Days 0–5	85	11	3	−0.678	Days 0–5	97	11	4	−0.911
After molting	Days 9–20	62	10	3	−0.845	Days 9–20	49	4	4	−0.992
Hepatopancreas	Days 0–20	114	7	4	−0.974	Days 0–20	113	4	4	−0.992
Muscle	Days 0–20	103	28	4	−0.967	Days 0–20	256	2	4	−0.908
Exoskeleton										
After molting	Days 9–20	134	18	3	−0.868	Days 9–20	754	3.5	3	−0.881

measured in crustaceans. If this were the case, ^{210}Pb concentrations should be higher than those of ^{210}Po due to the sorptive nature of ^{210}Pb (Fig. 1). It is noteworthy that even after the additional input of ^{210}Po through food for up to 14 d (Group II), the final distribution of ^{210}Po in the experimental shrimp did not closely agree with that in the control group (Fig. 2). This is likely due to the differences in ^{210}Po concentration factors of natural food and *Artemia* sp. labelled for only short periods of time, and the time factor necessary for experimental shrimp to achieve isotopic equilibrium with their surrounding environment.

Polonium and lead, which are stable in seawater as divalent species Po^{2+} and Pb^{2+} (Brookins 1988), are adsorbed onto shrimp exoskeleton most likely through chelation by functional groups on organic surfaces, as is the case for other divalent metals (Stumm 1992). However, the degree of adsorption will vary with surface properties; e.g. results from similar experiments with fish showed that external adsorption of polonium is negligible (Carvalho & Fowler in press).

Accumulation of both these radionuclides from seawater into the internal tissues of *Lysmata seticaudata* most likely is closely linked to the intake of water for osmotic regulation. It has been suggested that net water absorption in intermolt crustaceans takes place through the hepatopancreas and the gut wall, and, especially before ecdysis, through the integument (Mantel & Farmer 1983). Interestingly, as in water, the concentrations of ^{210}Pb and ^{210}Po in the hepatopancreas of Group I *L. seticaudata* are nearly identical, suggesting that absorption of water with the radionuclides takes place through this organ. The body fluids (blood) of *L. seticaudata* are hypoosmotic relative to seawater (Spaargaren 1972). To compensate for water loss by osmosis, shrimp drink seawater and this is followed by active excretion of salts and retention of water in order to maintain a balance of water and salt in tissues. In this way, as for other electrolytes dissolved in seawater, ^{210}Po and ^{210}Pb can enter internal tissues. For example, based on measurements of the extra-renal NaCl efflux from *L. seticaudata* (Spaargaren 1972), we estimate the drinking rate to be 2.4 $ml^{-1}\ g^{-1}\ d^{-1}$ in a 38‰ seawater salinity (2.2 to 2.6 $ml^{-1}\ g^{-1}\ d^{-1}$ for a range from 4 to 22 °C water temperature). This rate corresponds to an average influx of dissolved ^{210}Po into shrimp of 0.55 Bq $g^{-1}\ d^{-1}$ (0.50 to 0.60 Bq $g^{-1}\ d^{-1}$). Using this influx rate, and taking into account the biological half-life of ^{210}Po in muscle tissue (Table 2) as well as ^{210}Po radioactive decay constant (0.005 d^{-1}), the activity accumulated in this tissue after 21 d of exposure should be approximately 5.7 (5.2 to 6.2) Bq g^{-1}. This is in close agreement with the 5.5 Bq g^{-1} measured in muscle of the experiment Group I shrimp (Table 1). Using similar calculations for ^{210}Pb, the intake of seawater can explain at least 60% of the ^{210}Pb concentration measured in muscle (12 Bq g^{-1}). Therefore, the direct intake of seawater alone can account approximately for the increased concentrations of ^{210}Po and ^{210}Pb measured in the muscle of shrimp exposed to the radionuclides in seawater .

Results of the Group II experiment suggest a higher digestive absorption of ^{210}Po than ^{210}Pb, which results in an increase in the Po:Pb ratio from about 1 in water and food particles to much greater than unity in shrimp hepatopancreas and muscle. In fact with the exception of the hepatopancreas, the ingestion of food did not significantly increase the concentration of ^{210}Pb in shrimp tissues, an observation which suggests that most of the accumulated ^{210}Pb originates from the water. The accumulation of ^{210}Po was, however, significantly increased by food. The Group II data in Table 1 demonstrate that 80% of the whole-body ^{210}Po in shrimp originated from the food. Under natural conditions with low environmental concentrations of ^{210}Po in seawater and relatively high levels typical in food organisms (Heyraud & Cherry 1979), the percent contribution from food would be much higher.

Comparison of Po:Pb ratios in whole shrimp and tissues in Groups I and II (Table 1) also leads to the conclusion that the ratios were increased by a factor of about 5 due to the ingestion of labelled food. This implies that absorption of ^{210}Po from food is approximately 5 times more efficient than the absorption of ^{210}Pb. Interestingly, a significant difference in gut assimilation efficiencies of Po and Pb has not been observed in mammals (Moroz & Parfenov 1972, Holtzman 1978) and no comparable data are available for marine organisms. Nevertheless, the observed difference in the assimilation efficiencies of these radionuclides enables interpreting ^{210}Po:^{210}Pb ratios commonly found in marine crustaceans. For example, *L. seticaudata* feeds upon algae and detritus in which ^{210}Po:^{210}Pb ratios vary from 2 to 10 (Carvalho 1990, author's unpubl. data). Using the assimilation efficiency ratio of 5 given above, consumption of this food should lead to environmental ^{210}Po:^{210}Pb ratios in shrimp between 10 and 50 which agrees with the measurements here (cf. Table 1, control group). A wide range in ^{210}Po concentrations and ^{210}Po:^{210}Pb concentration ratios between 10 and 1000 have been reported for marine crustaceans and were tentatively explained as being due to variations in the composition of the diet of different species (Heyraud et al. 1988). The observation of the overriding importance of food in the accumulation of ^{210}Po in shrimp supports this interpretation.

The elimination experiment provides the first estimates of biological half-lives for ^{210}Po and ^{210}Pb turnover in shrimp. The biological half-lives for ^{210}Po in whole shrimp (10 d) and hepatopancreas (7 d) are quite

similar to those obtained for whole euphausiid (5.6 d) and euphausiid hepatopancreas (6.5 d) (Heyraud et al. 1976, Heyraud & Cherry 1979). There appears to be no other turnover data for ^{210}Po in marine species. Turnover data determined for *Lysmata seticaudata* also indicate that the elimination of ^{210}Pb from hepatopancreas, muscle, and exoskeleton is generally much faster than that of ^{210}Po (Table 2). Apparently, no turnover data exist in the literature for ^{210}Pb in marine organisms.

The difference in turnover rates of Po and Pb has the net effect of producing an increase in the enhanced Po:Pb concentration ratios in tissues over time, a process possible in nature. Combined with the higher assimilation efficiency of ^{210}Po, the slower turnover of polonium in tissues provides a mechanism for the increase in Po:Pb ratios observed throughout marine food chains. These Po:Pb ratios increase from a range of 1 to 4 in phytoplankton and suspended particles to a range of 50 to 1000 in mysids, copepods, euphausiids and certain pelagic shrimp (Cherry & Heyraud 1981, Heyraud et al. 1988, Carvalho 1990).

The sulphur-analog properties of polonium (Moroz & Parfenov 1972, Heyraud et al. 1987) may explain its longer turnover time in marine organisms, through binding and cycling with amino acids and sulphur-containing proteins. Lead seems less efficiently absorbed and definitely is more rapidly eliminated by crustaceans through detoxification mechanisms, viz. elimination with cast molts or sequestration by metallothioneins and calcium phosphate granules formed in the hepatopancreas, that also operate on other metals and transuranium elements (Rainbow 1988, Fisher & Reinfelder 1991, Engel & Brouwer 1993).

Since the food chain clearly is the main transfer pathway for polonium in marine crustaceans, ^{210}Po concentration in organisms should reflect the ^{210}Po content of their prey to a degree dependent upon food assimilation efficiency. Therefore, the use of ^{210}Po as a natural tracer in diet studies of marine organisms, as proposed by Heyraud et al. (1988) and Cherry et al. (1989), would appear to be well justified. However, the rapid turnover of ^{210}Po displayed by internal organs of crustaceans will result in large variations in ^{210}Po content among individuals of the same species. Thus, the length of time between feeding and capture of the organism for analyses will be a crucial factor in the ^{210}Po levels observed. ^{210}Po concentrations in crustacean hepatopancreas will be particularly sensitive, while muscle should contain ^{210}Po levels less affected by daily fluctuations in feeding and, therefore, will better reflect ambient (typical) ^{210}Po concentrations.

^{210}Pb accumulated in shrimp displays a completely different behaviour. Since the assimilation efficiency of ^{210}Pb from food by shrimp is very low and external adsorption from seawater relatively high, the use of ^{210}Pb as a tracer of the diet seems very limited. Furthermore, organisms with different types of external surfaces will display ^{210}Pb concentrations with variable contributions from surface adsorption.

In view of these experimental results, which are particularly relevant to studies related to the enhancement of environmental ^{210}Po and ^{210}Pb levels from non-nuclear industries, it can be predicted that ^{210}Po dissolved in water will not be greatly accumulated in the tissues of shrimp (this study) and fish (Carvalho & Fowler in press). Instead, to be effectively accumulated, ^{210}Po would require previous binding to organic matter at lower trophic levels in order to enter the food chain and build up higher concentrations in the immediate prey of these organisms.

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