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Bioaccumulation of polonium-210 in marine copepods

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Abstract

^{210}Po, a naturally occurring radioisotope that is ubiquitous in seawater, is especially enriched in proteinaceous tissues of marine organisms and may therefore be useful as a tracer of organic carbon flux in marine systems. Due in part to its biomagnification in marine food chains, ^{210}Po provides the largest radiation dose to any organism under natural conditions. To better understand the extent to which zooplankton can influence the fluxes of ^{210}Po and serve as a conduit between phytoplankton, which concentrate it greatly from ambient water and higher trophic levels, we conducted a series of laboratory experiments with the calanoid copepod *Acartia tonsa. A. tonsa* was presented with either dissolved ^{210}Po or with one of eight different phytoplankton species and sterile glass beads, all labeled with ^{210}Po. Assimilation efficiencies (AEs) of ingested ^{210}Po in copepods ranged from 19% to 55% among the phytoplankton diets, and correlated directly with ^{210}Po's cytoplasmic distributions in the algal cells. The AE of ^{210}Po from ingested glass beads was 0%. The high AE and low efflux rates (mean of 3% d^{-1}) of ^{210}Po in copepods can explain its biomagnification in marine food chains. Uptake and loss parameters of ^{210}Po in copepods measured in these experiments were used in a model to quantify the relative sources of ^{210}Po for copepods. Under all realistic scenarios, $>$90% of ^{210}Po in copepods appears to be taken up through diet. Model-predicted ^{210}Po concentrations in copepods in different ocean regions closely matched independent measurements, suggesting that we understand the processes governing this element's enrichment in zooplankton and thus can make quantitative predictions of its bioconcentration on a site-specific basis.

Zooplankton have long been recognized to influence the cycling and fluxes of elements in the ocean. By producing relatively large, dense fecal pellets that transport material out of surface waters during their descent, zooplankton can reduce the residence time of metals in the mixed layer (Cherry et al. 1978; Fowler and Knauer 1986). In contrast, by concentrating other elements through assimilation in their tissue, zooplankton can increase the time these elements spend in surface waters and in the marine food web (Fisher and Reinfelder 1995). Furthermore, zooplankton are important intermediates between phytoplankton, which can greatly concentrate metals from seawater and higher trophic levels, which are consumed as seafood.

The trophic transfer and cycling of many elements by zooplankton have been well studied (Fisher and Reinfelder 1995), but one element that has received relatively little attention in this regard is polonium. ^{210}Po is the final radioactive product in the ^{238}U decay series and is of interest as a potential tracer of organic carbon flux in the ocean (Friedrich and Rutgers van der Loeff 2002). The alpha decay of ^{210}Po accounts for most of the radioactive dose to marine organisms (Cherry 1964), and human consumers of seafood receive their largest dose of natural radiation from the polonium in their food (Cherry 1964; Bulman et al. 1995). Thus, in addition to ^{210}Po's potential as a geochemical tracer, its interactions with marine biota are of interest to risk assessment modelers in the context of understanding the implications of nuclear waste discharge in the marine environment beyond the natural radiation background.

Measurements of ^{210}Po concentrations in biota (Shannon et al. 1970; Folsom and Beasley 1973; Germain et al. 1995) can be used to infer the trophic transfer of ^{210}Po in marine food chains, but few studies have assessed the rates and mechanisms involved in the uptake of ^{210}Po from food (Carvalho and Fowler 1994). Understanding these processes may explain observations that ^{210}Po is highly concentrated in the tissue of commercially important vertebrates and invertebrates (Dahlgaard 1996; Stepnowski and Skwarzec 2000).

It has been recently shown that ^{210}Po concentrates to varying degrees in marine phytoplankton in a way that is dependent on the size and protein content of the cells (Stewart and Fisher 2003). Other laboratory studies have also investigated the accumulation of ^{210}Po in bacteria and phytoplankton and related its cytological distribution to that of protein (Fisher et al. 1983; Cherrier et al. 1995), consistent with observations that ^{210}Po is associated with protein in animal tissues (Heyraud et al. 1976; Carvalho and Fowler 1994; Durand et al. 1999). Phytoplankton may serve as a highly enriched source of ^{210}Po for grazers, as they do for other metals (Fisher and Reinfelder 1995), but dissolved ^{210}Po may also contribute appreciably to the body burden of this element in marine animals (Jeffree et al. 1997; Wang and Fisher 1999).

We have therefore conducted a series of experiments to quantify the rates and routes of bioaccumulation of ^{210}Po in marine copepods and have used a simple biokinetic model to evaluate the relative importance of food and water as source terms of this element for zooplankton. Because sinking fecal matter from zooplankton can greatly influence the fluxes of organic and inorganic matter in the sea, it might also be possible that ^{210}Po can be used, along with the traditional particle-reactive radiotracers ^{234}Th and ^{210}Pb, as a tool to follow these fluxes. Because of differences between

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Table 1. Algal species and clonal designations of cells used as food in feeding experiments. For more information on cell characteristics, see Stewart and Fisher (2003).

Species	Clone	Phytoplankton type
Chlorella autotrophica	CCMP 243	Chlorophyte
Dunaliella tertiolecta	DUN	Chlorophyte
Emiliania huxleyi	CCMP 2112	Coccolithophore
Heterocapsa triquetra	OB 21019305	Dinoflagellate
Isochrysis galbana	ISO	Prymnesiophyte
Rhodomonas salina	CCMP 1319	Cryptophyte
Tetraselmis levis	PLATY 1	Prasinophyte
Thalassiosira pseudonana	3H	Diatom

Table 2. The mean and standard deviations of ^{210}Po concentrations (kBq L^{-1}) in feeding suspensions, phytoplankton cells, or glass beads, and the dissolved phase after 2-h copepod feedings.

	kBq L^{-1} total	kBq L^{-1} particles	kBq L^{-1} dissolved
Glass beads	1.9±0.2	1.4±0.1	0.50±0.17
C. autotrophica	2.3±0.3	1.7±0.3	0.58±0.30
D. tertiolecta	3.8±0.6	3.0±0.3	0.77±0.70
E. huxleyi	1.5±0.2	1.4±0.1	0.18±0.28
H. triquetra	2.2±0.2	1.7±0.1	0.50±0.18
I. galbana	3.0±0.4	2.3±0.2	0.87±0.15
R. salina	2.7±0.2	2.4±0.2	0.27±0.15
T. levis	3.0±0.2	2.6±0.1	0.40±0.25
T. pseudonana	2.0±0.2	1.8±0.1	0.36±0.20

uptake and cytological distributions among these three radioisotopes, a quantitative understanding of ^{210}Po trophic transfer may be useful in developing conceptual models for marine transport of organic matter. For example, the ratio of polonium to thorium or polonium to lead in suspended or sinking material may reveal what fraction of particulate matter is organic or proteinaceous (Nozaki et al. 1997; Friedrich and Rutgers van der Loeff 2002).

Materials and methods

The basic protocol for evaluating the assimilation of radioactive metals in copepods (Fisher et al. 1991; Reinfelder and Fisher 1991; Wang and Fisher 1998) was modified only to enable analysis of ^{210}Po by liquid scintillation counting. Eight species of phytoplankton representing seven algal divisions (Table 1) were cultured in sterile-filtered (0.2-μm sterilized polycarbonate membrane) surface seawater enriched with f/2 nutrients (Guillard and Ryther 1962). These species were selected because they represented a range of cell characteristics and possible prey items for copepods in the ocean. Late log-phase cells were harvested by filtering or centrifuging and resuspended into filtered seawater containing f/2 nutrients and 60–90 kBq L^{-1} (7.7–11.6 nmol L^{-1}) dissolved ^{210}Po as described in Stewart and Fisher (2003). Cells were grown for at least 48 h to allow three to four cell divisions to uniformly radiolabel the cells. Characteristics of the cells were measured including C : N ratios, surface area, volume, and protein content (Stewart and Fisher 2003). Aliquots of cells were fractionated using osmotic disruption and differential centrifugation (Sheeler 1981) and the ^{210}Po associated with the cell membranes and cell walls was distinguished from the cytoplasmic ^{210}Po (Fisher et al. 1983). Nonporous sterile glass beads (5.35 ± 1.37 μm) were also labeled with ^{210}Po in the same manner as the phytoplankton. All phytoplankton cells and beads were filtered out of labeling suspensions before being presented to the copepods so that the animals were presented with labeled particles suspended in 0.2-μm filtered, unlabeled water. The DOC content of this water, measured with a Shimadzu TOC 5000 analyzer, was 1 mg L^{-1}.

Adults of the calanoid copepod *Acartia tonsa* were collected with a plankton net (200-μm nylon mesh) from Stony Brook Harbor, New York during the spring and summer. Immediately after collection, the copepods were visually identified and separated from other plankton using a dissecting microscope. The copepods were counted and placed in 0.2-μm filtered surface seawater for 12–24 h to evacuate their guts of any previously ingested material. Copepods were then immediately introduced into either filtered seawater containing 0.8 kBq L^{-1} (0.1 nmol L^{-1}) of ^{210}Po to measure uptake from the dissolved phase, or into unialgal suspensions of ^{210}Po-labeled phytoplankton or glass beads resuspended in nonradioactive seawater for feeding experiments.

For each algal species, 60 copepods were placed in 100 ml of a cell suspension contained in a 250-ml polystyrene screw-top culture flask, held at 15°C. Three replicate bottles were used for each time point during depuration, plus three for the initial time point at the end of feeding. The algal cell densities ranged from 3 to 4 × 10^4 cells ml^{-1}, corresponding to 100–500 μg C L^{-1}, depending on the species. The cells were labeled with 1.4–3.0 kBq ^{210}Po L^{-1} (Table 2). The copepods were allowed to feed for 2 h. This short feeding period precluded extensive excretion and recycling of the ^{210}Po. The cell densities in the feeding suspensions were counted before and after the feeding using a Coulter Counter Multisizer II and AcuComp software, and a 1-ml sample of the feeding suspension was measured for total radioactivity at the beginning and end of the feeding period. The time of feeding, the concentration of cells, and the number of animals were selected so that there would be sufficient activity in the animals for liquid scintillation counting, but neither the algal cell density nor the radioactivity of each cell would change by more than 5% during grazing. Control experiments without phytoplankton and with inorganic particles are described later.

After feeding, the copepods were separated from the radiolabeled phytoplankton using a 200-μm mesh. Sixty copepods from each of three replicate flasks were then immediately dissolved using Packard Solvable to determine the average amount of ^{210}Po ingested by the copepods. These samples were prepared by adding 2 ml Solvable to filtered samples in glass scintillation vials, heating at 60°C for 2–3 h, and then cooling before counting. The remaining copepods were resuspended into flasks containing 5–10-fold more dilute suspensions of nonlabeled phytoplankton cells, the same cell type as they fed on during the radioactive feeding period. The copepods from each of the triplicate flasks were then periodically harvested during this depura-

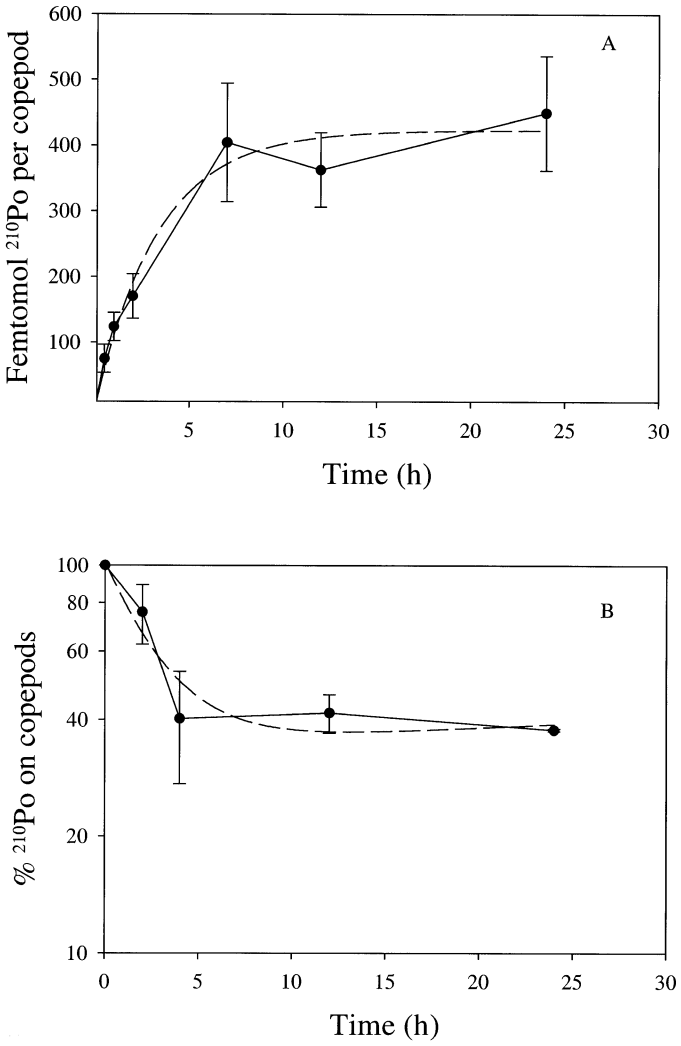

Fig. 1. (A) Femtomoles of ^{210}Po per copepod over 24 h while exposed to 0.1 nmol L^{-1} of ^{210}Po in the dissolved phase. The data were fit with a nonlinear regression curve (dashed line) with the equation $F(x) = 422.67(1 - e^{-0.307x})$, $r^2 = 0.97$. This model was used to calculate the initial (0–2 h) uptake rate of dissolved ^{210}Po. (B) The loss of ^{210}Po from copepods during depuration after exposure to dissolved ^{210}Po. Time zero represents the time when the radiolabeled copepods had 100% of their adsorbed ^{210}Po and were first resuspended into nonradioactive filtered seawater. Values represent the means from three replicate flasks; error bars denote 1 SD. The data were fit with a nonlinear regression curve (dashed line) with the equation $F(x) = 34.0 + 68.0e^{-0.37x} + 0.20x$, $r^2 = 0.97$.

tion period, with more frequent sampling at the beginning of depuration. Copepods were filtered onto 200-μm mesh and dissolved in scintillation vials using Solvable as above. Phytoplankton cells from each feeding suspension were filtered onto 1-μm membranes that were analyzed by liquid scintillation counting as was 5 ml of filtrate. The ^{210}Po activity in all samples was counted in 20-ml glass vials with a Packard TRI-Carb 2100TR liquid scintillation counter using 10 ml Packard Ultima Gold XR as scintillation fluid (Stewart and Fisher 2003).

A similar experiment was conducted with the ^{210}Po-labeled glass beads, but the feeding occurred in 150-ml polycarbonate screw top centrifuge tubes attached to a slowly rotating (1 rpm) plankton wheel to prevent the beads from sinking out of suspension. Beads were counted before and after the feeding period to ensure ingestion. The original concentration of beads was approximately 3×10^4 beads ml^{-1}. After the copepods were sieved out of the bead feeding suspension, they were resuspended in a dilute culture of unlabeled *T. pseudonana* to prevent starvation and promote gut evacuation of unassimilated material. Depuration was followed as for the phytoplankton experiments.

Because copepods can accumulate ^{210}Po from both the dissolved phase and from ingested food, the contribution of radioisotope sorbed by the copepods during the feeding period was evaluated. Copepods were exposed to dissolved ^{210}Po (0.8 kBq L^{-1}, or 0.1 nmol L^{-1}) that had been equilibrated with the seawater for 24 h; this concentration was based on earlier measurements (Stewart and Fisher 2003) of desorbed ^{210}Po from algal cells into the dissolved phase over a 2-h period. The range of concentrations of dissolved ^{210}Po in the feeding suspensions at the end of the 2-h feeding exposures encompassed 0.8 kBq L^{-1} (Table 2), the concentration used to evaluate ^{210}Po uptake solely from the dissolved phase. The ^{210}Po content of copepods obtained from the dissolved phase during the 2-h feeding experiments (always $<4\%$ of the total body burden) was subtracted from the copepod ^{210}Po body burdens in the feeding suspensions to determine the contribution of food alone as a source of ^{210}Po for the copepods. Uptake of dissolved ^{210}Po was also measured over a 24-h exposure period to more fully understand the capacity of the zooplankton to accumulate ^{210}Po from the dissolved phase and to measure ^{210}Po release rates from the copepods following solute exposure, using the same approach as that following feeding experiments.

Assimilation efficiencies (AEs) and excretion rates of ^{210}Po in copepods were determined from the depuration curves following feeding experiments and dissolved exposures (Wang and Fisher 1999). We used three different methods to estimate assimilation efficiency. First, we fit a double exponential loss model to the depuration data. This model assumes two compartments of metal depuration, with the first compartment representing ^{210}Po lost due to defecation followed by a slow loss that reflects the metabolic turnover (excretion) of assimilated ^{210}Po in the animal (Wang and Fisher 1999). The model equation was

$$F(t) = U_0 e^{(-dt)} + A_0 e^{(-rt)} \quad (1)$$

where $F(t)$ is the radioactivity retained in the copepods at time t (Bq $copepod^{-1}$), U_0 is the initial amount of ^{210}Po in the rapidly exchanging pool (unassimilated ^{210}Po, Bq $copepod^{-1}$), d is the depuration rate constant of this first rapidly exchanging pool (h^{-1}), t is time in hours, A_0 is the initial amount of ^{210}Po in the slowly exchanging pool (assimilated ^{210}Po, Bq $copepod^{-1}$), and r is the depuration rate constant of this second pool (h^{-1}). The initial ^{210}Po in both pools was constrained to sum to 100%. All parameters were estimated using log-normally distributed errors, and 95% confidence intervals were calculated using likelihood ratios (Hilborn and Mangel 1997).

A second approach to determine AE involved fitting a

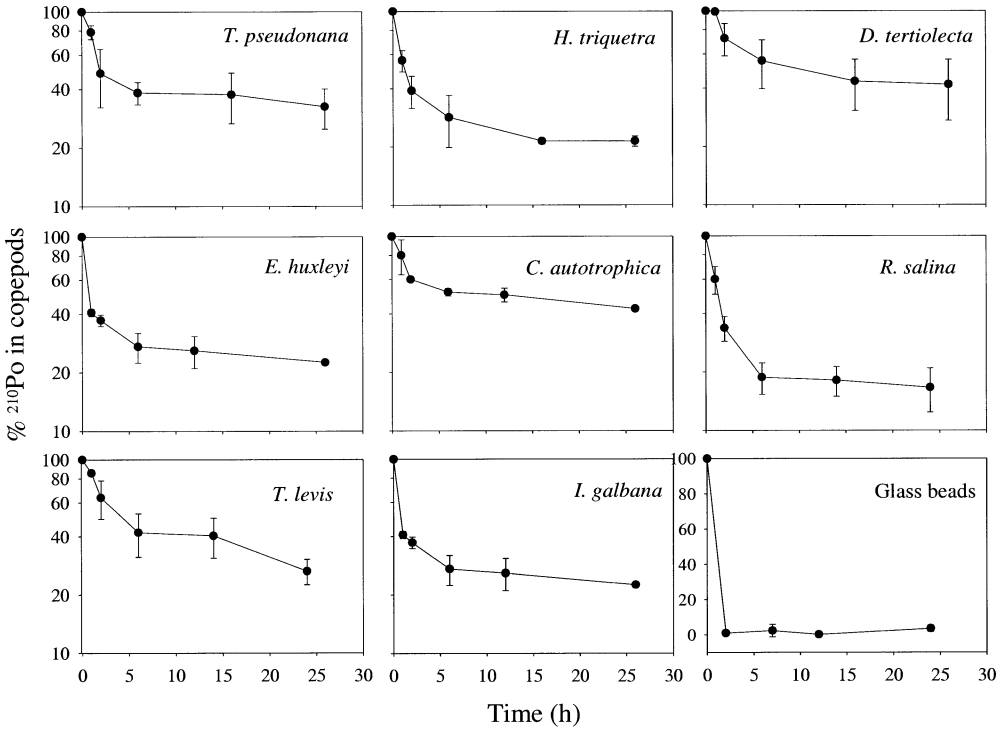

Fig. 2. Depuration curves from all the feeding experiments. Time zero represents resuspension of the copepods out of radioactive feeding suspensions and into nonlabeled feeding chambers. In all cases, the ^{210}Po adsorbed onto the animals from the dissolved phase was subtracted. Values represent the means from three replicate flasks; error bars denote 1 SD.

linear model to the slow phase of each depuration curve. All data points after the inflection points of the curves, starting with the concentration in the copepods at 6 h in all cases except the glass beads, were included in a simple first-order equation,

$$F(t) = rt + A_0 \quad (2)$$

In this case, r is the slope and reflects the depuration rate constant (h^{-1}) and A_0, the y-intercept, represents the amount of ^{210}Po assimilated by the copepod immediately after feeding (Bq copepod^{-1}) (Wang and Fisher 1999). Finally, we estimated AE by recognizing that the efflux rate of ^{210}Po from the copepods was very small (on the order of 1–7% d^{-1}) and that the first measurement of ^{210}Po in the copepods after the rapid egestion (again assessed at 6 h for most feeding suspensions) divided by the total ^{210}Po ingested would generate a conservative estimate of AE. For all three AE estimates, AE was calculated as A_o divided by ^{210}Po ingested.

Results

Copepods accumulated ^{210}Po from the dissolved phase during the 24-h exposure; the initial uptake of ^{210}Po during the first 2 h of exposure was rapid, but after 8 h, the net uptake had essentially stopped, with each copepod having an equilibrium value of approximately 400 fmol (Fig. 1A).

Table 3. Mean percentage of ^{210}Po in the cytoplasm of phytoplankton cells and three estimates of assimilation efficiencies for each food type with standard deviations or 95% confidence intervals.

	% ^{210}Po in cytoplasm (±SD)	Mean AE 6-h time point (±SD)	Mean AE linear model (95% CI)	Mean AE double-exponent model (95% CI)
Glass beads	0(±0)	2.4(±3.6)	0.9(−1.5, 3.8)	1.14(−0.45, 1.78)
C. autotrophica	58(±2.1)	51.8(±2.3)	55.1(48.7, 57.3)	54.6(47.2, 61.8)
D. tertiolecta	52(±0.6)	55.2(±15.6)	51.3(32, 74.2)	51.3(30.2, 70.3)
E. huxleyi	28(±1.7)	27.2(±4.8)	28.7(22.1, 32.1)	31.5(26.1, 36.8)
H. triquetra	35(±2.2)	28.4(±8.5)	27.9(18.5, 34.8)	32.6(21.6, 37.3)
I. galbana	43(±2.1)	27.2(±4.8)	32.7(22.7, 36.0)	35.3(26.6, 45.1)
R. salina	31(±1.5)	18.7(±3.5)	19.6(13.7, 24.5)	19.8(15.8, 32.6)
T. levis	45(±1.3)	42.0(±10.7)	49.3(32.0, 60.1)	50.1(31.2, 67.9)
T. pseudonana	39(±0.8)	38.4(±5.1)	40.8(29.8, 48.1)	40.0(30.2, 52.3)

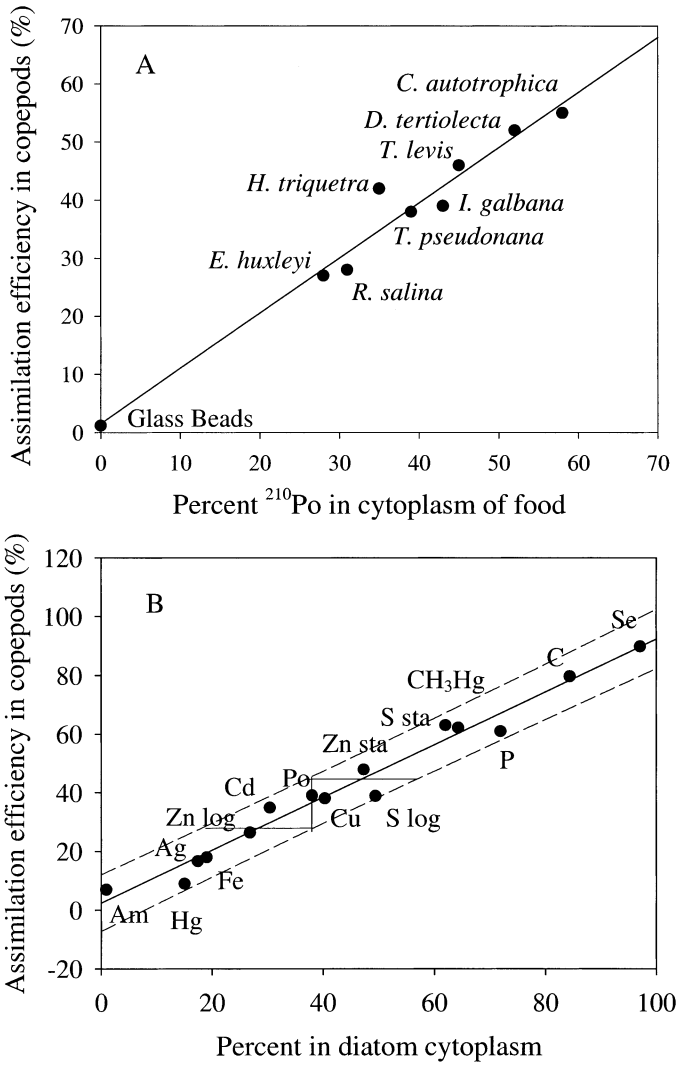

Fig. 3. (A) Relationship of assimilation efficiency of ^{210}Po in copepods with the distribution of ^{210}Po in cytoplasm of the algal cells that served as food. In this figure, the AE estimates were based on the double-exponential model describing ^{210}Po retention in copepods. The line drawn represents a least-squares regression through the points with the equation $F(x) = -1.86(\pm 1.96) + 0.93(\pm 0.05)x$, ($r^2 = 0.98$). When we compare the linear model estimate of AE with ^{210}Po cytoplasmic distribution, the regression equation is $F(x) = -1.80(\pm 5.03) + 0.91(\pm 0.14)x$, ($r^2 = 0.90$). Finally, using the AEs determined by ^{210}Po measurements at 6 h, the regression equation is $F(x) = -1.08(\pm 5.70) + 0.91(\pm 0.14)x$, ($r^2 = 0.86$). None of these regression lines is statistically distinct from a 1:1 line passing through the origin ($p > 0.11$ in all cases). (B) Comparison between the cytoplasmic distribution and copepod AE of ^{210}Po from diatom (*Thalassiosira* spp.) prey and those of other elements. Data from Reinfelder and Fisher (1991), Hutchins et al. (1995), Mason et al. (1996), Chang and Reinfelder (2000), and this study. The solid line is the linear regression ($F(x) = 2.21(\pm 2.20) + 0.90(\pm 0.04)x$, ($r^2 = 0.97$)) and the dashed lines represent the 95% prediction interval based on the strength of this linear regression. Rectangulation from the mean ^{210}Po number shows the predicted range of possible values for the assimilation of this metal. Other metals with AEs within this range are not statistically different, but those with AEs beyond this range are statistically distinct at the 95% confidence level. The growth stage of the phytoplankton cells when they were consumed by copepods is designated as "sta" for stationary phase and "log" for exponential growth phase for certain elements where effects of growth phase were specifically measured.

When resuspended into unlabeled seawater, the copepods quickly lost approximately 60% of their ^{210}Po (Fig. 1B), after which the rate of loss from the copepods was negligible.

The copepods ingested the radiolabeled cells and glass beads during all feeding exposures. Figure 2 shows the depuration curves of the ^{210}Po from the copepods after being removed from the radiolabeled feeding suspensions; all values depicted in the figure are corrected for the contribution (ranging from 2 to 4%) of the ^{210}Po body burden accumulated from the dissolved phase in the feeding suspensions. Note that the radioactivity in the copepods was related to the radioactivity measured at time zero of the depuration period (i.e., immediately after feeding), taken to be 100%. After 2 h of depuration, the rate of ^{210}Po loss decreased for all phytoplankton food types, and after 5 h, the slow rate generally stabilized at 1–7% d^{-1} (mean = 3% d^{-1}) until the end of the experiment. For animals that fed on glass beads, >98% of the ^{210}Po was lost in the first 2 h.

Table 3 presents the results for the three AE calculations for each species. The assimilation efficiencies of ^{210}Po in the copepods differed significantly for 6 out of 8 algal diets. Overall, among the algal species examined, AEs were lowest for *R. salina* (about 20%) and highest for *C. autotrophica* and *D. tertiolecta* (51–55%). The AE of ^{210}Po obtained from glass beads was not significantly different from zero using all three estimates of AE ($P > 0.05$).

Based on earlier studies that found very strong correlations between copepod AEs of different metals and the distribution of these metals within *T. pseudonana* cells (Reinfelder and Fisher 1991), we plotted in Fig. 3A the AEs for ^{210}Po against the percentage of ^{210}Po found inside the cytoplasmic fraction of the food cells (Table 3). For this figure, we used the estimated AEs based on the double exponential model of ^{210}Po retention in the copepods. Clearly, there was a close fit ($r^2 = 0.98$) between copepod AE and cytoplasmic distribution of ^{210}Po, with a slope not significantly different from 1 and the *y*-intercept not significantly different from 0.

Discussion

^{210}Po was assimilated in copepods from algal food to a varying degree, with AE values ranging from 19% to 55%, depending on the algal diet. The different AE values among algae and glass beads can be explained by examining the cytological distribution of ^{210}Po in the food particles. As shown for other trace elements in copepods and bivalve larvae (Reinfelder and Fisher 1991, 1994), there was a strong correlation between assimilation efficiencies and the cytoplasmic distribution of ^{210}Po in the algal cells. This relationship between cytological distribution and AE values was also evident in the study by Hutchins et al. (1995), in which the cytological distribution of one single metal, iron, in a diatom was manipulated and the AE values in copepods showed corresponding changes in a one-to-one fashion. In the present study, we have shown for the first time that the AE in copepods of a single element (^{210}Po) from different algal species also follows the one-to-one pattern with cytoplasmic distribution. Therefore, cells into which ^{210}Po penetrates more readily have the potential to introduce more

Table 4. The ^{210}Po concentrations used to generate the model predictions shown in Fig. 4. Standard deviations are shown where available. All values represent mBq g^{-1} dry weight, using a factor of 10 conversion between wet and dry weight.

Measured seawater concentration (mBq L^{-1})*	Measured phytoplankton concentration (mBq g^{-1})	Measured zooplankton concentration (mBq g^{-1})	Location	Reference
3.7†	188±69‡	249±115‡	Cape of Good Hope	Cherry (1964)
No data	104	174	Cape of Good Hope	Shannon and Cherry (1967)
0.73±0.3	40±23	148±59	Cape of Good Hope	Shannon et al. (1970)
2.4±3.8	126±26§	167±84§	Misaki Coast, Japan	Tateda and Yamamoto (1997)

* Total seawater concentration, including particulate matter.
† Calculated from concentration factors.
‡ Values from ''Section III'' for phytoplankton and ''Section II'' for zooplankton, same location.
§ Values for phytoplankton (100–300 μm) and for zooplankton (300–1,000 μm).

^{210}Po to herbivores, especially those with short gut-transit times. The species composition of phytoplankton communities, or selective feeding by grazers, may explain varying ^{210}Po concentrations in top predators and shellfish from one ecosystem to another.

The copepods did not assimilate any ^{210}Po associated with the glass beads despite the fact that 6,500 beads were ingested by each copepod during the 2-h feeding (Fig. 2). Most of the ^{210}Po associated with the copepods after feeding was adsorbed to the copepods from the dissolved phase, a reflection of the rapid loss of ^{210}Po from the beads into the dissolved phase of the feeding suspension (Stewart and Fisher 2003). All of the ingested ^{210}Po bound to the beads was defecated. This result is also consistent with the assimilation findings of Reinfelder and Fisher (1991), in which cytoplasmic metals penetrate the gut linings of marine copepods whereas surface-bound metals get packaged with unassimilated waste in fecal pellets. Because less than 1% of the total ^{210}Po associated with the beads remained in the copepods after depuration, it appears that ^{210}Po desorbed from the beads into the gut fluid does not cross the gut linings of copepods. Perhaps cytoplasmically distributed metals, including ^{210}Po, are bound to molecules such as amino acids or polypeptides that the gut linings of zooplankton have evolved to select for, and the associated metal gets transported across the gut lining.

The relationship of ^{210}Po's AE in marine copepods to cytoplasmic distribution in algal food (diatoms of the genus *Thalassiosira*) is comparable with that of other essential and nonessential elements (Fig. 3B). The assimilation of ^{210}Po exceeds that of some other metals, including Fe, and is comparable with that of Cu and Cd. Given polonium's known association with protein (Fisher et al. 1983; Cherrier et al. 1995; Stewart and Fisher 2003), the similarity between assimilation efficiencies for ^{210}Po and S are consistent with the uptake mechanism hypothesized above.

Another distinguishing feature of ^{210}Po dynamics in copepods is its extremely slow rate of loss from the animals. The loss rates found for ^{210}Po—about 1–3% d^{-1} following uptake from the dissolved phase, 1–7% d^{-1} following dietary uptake—are significantly lower than those of Ag, Cd, Co, Se, and Zn from copepods, where loss rates of 11–17% d^{-1} following uptake from the dissolved phase and 8–30% d^{-1} following dietary uptake from algal food were reported (Wang and Fisher 1998). The relatively high AE and the slow loss rate of assimilated ^{210}Po suggest that ^{210}Po may biomagnify in planktonic food chains. The trophic transfer potential (TTP) for any metal can be estimated from the ratio of its assimilation efficiency to that of its efflux rate (Reinfelder et al. 1998). For copepods obtaining metals from phytoplankton food, AE values like those displayed by ^{210}Po would result in biomagnification (e.g., ^{210}Po concentration in copepod/^{210}Po concentration in phytoplankton > 1) in copepods under all feeding conditions when efflux rates are $<5\%$ d^{-1} (Wang 2002). Thus, our laboratory results indicate that ^{210}Po would likely biomagnify in marine plankton communities, and this may explain the higher ^{210}Po concentrations found in field-collected zooplankton than phytoplankton (Table 4). Methyl mercury, zinc, and selenium also have TTPs >1 (Reinfelder et al. 1998; Wang 2002).

Consistent with the idea that ^{210}Po can biomagnify up the food chain and that it is accumulated through dietary exposure, shrimp and cephalopods that eat larger prey and have faster ingestion rates have been shown to have higher ^{210}Po concentrations than those that eat smaller prey more slowly (Heyraud and Cherry 1979). Cherry and Heyraud (1981) also hypothesized that seasonal changes in ^{210}Po concentrations in marine shrimp reflected changes in the ^{210}Po content of their diet. Further, the relatively high AEs and low efflux rates for ^{210}Po in copepods may explain the lower ^{210}Po/^{210}Pb ratios in zooplankton fecal pellets than in the phytoplankton food from which they were derived (Heyraud and Cherry 1979). Pb shows little assimilation in marine zooplankton from phytoplankton food (Fisher et al. 1987, 1995). Consequently, the ^{210}Po/^{210}Pb ratios in phytoplankton, zooplankton, and fecal pellets reflect the relative trophic transfer of these two isotopes in marine plankton assemblages. Because ^{210}Po and ^{210}Pb distribute differently in plankton cells, with ^{210}Po much more closely associated with protein inside algal cells and ^{210}Pb more associated with cell walls and other structural components (Fisher et al. 1983), it may be possible to examine their ratios in settling planktonic debris to differentiate between labile and refractory cell components (Friedrich and Rutgers van der Loeff 2002).

In addition to uptake from food, zooplankton can accumulate trace elements directly from the dissolved phase (Wang and Fisher 1998), as shown here with ^{210}Po. To quantify the relative contribution of food and water as sources of

^{210}Po for copepods, we used a simple kinetic model, described in detail elsewhere (Wang and Fisher 1999). Metal bioavailability may depend on many parameters, including food quantity and quality, cellular partitioning, digestive physiology, the behavior and speciation of the dissolved chemical, and the associations with different fractions of the food (Wang and Fisher 1999). This simple model, which has been successfully field tested for copepod metal accumulation in the Mediterranean (Fisher et al. 2000), represents the parameters involved in metal uptake from food and from water and efflux following both

$$C_{ss} = \frac{C_w \times k_u}{k_{ew} + g} + \frac{C_f \times IR \times \text{AE}}{k_{ef} + g} \quad (3)$$

where C_{ss} is the concentration of the ^{210}Po in an animal at steady state (Bq g^{-1}), C_w and C_f are the ^{210}Po concentrations in the water (Bq L^{-1}) and food (Bq g^{-1}), respectively, k_u is the uptake rate constant from water (d^{-1}), k_{ew} and k_{ef} are the efflux rates (d^{-1}) after uptake from water and food, g is the growth rate constant (d^{-1}), and IR is the ingestion rate (d^{-1}).

This model is useful in evaluating rates of metal uptake in organisms and distinguishing the relative contribution of dietary and solute uptake pathways. In our modeling, we used mean AE, k_{ef}, k_{ew}, and k_u values for ^{210}Po determined in the laboratory, and literature values for *A. tonsa* growth (0.03 d^{-1}) and ingestion (0.33 d^{-1}) rates (Mauchline 1998). Mean k_{ew} and k_{ef} were 0.03 d^{-1} and 0.05 d^{-1}, respectively, k_u was 1.13 ml g^{-1} d^{-1} (calculated using a first-order regression describing the copepod uptake of ^{210}Po from the dissolved phase, using the data in Fig. 1A), and AE was 39.4% (calculated as the mean of double-exponential model determinations). While we recognize that, as with other metals, no single AE value for Po can accurately reflect the assimilation of this element from diverse algal diets in copepods, we used 39.4% to represent assimilation from ''generic phytoplankton'' for modeling purposes. Even with the lowest AE value measured for phytoplankton (18.7%, in the case of *R. salina*), no less than 92% of the ^{210}Po measured in copepods would be obtained from their food. This high proportion deriving from food is comparable with that of Se in copepods, and significantly higher than that of Zn, Ag, Cd, and Co (Wang and Fisher 1998).

Relative to dietary sources, the low uptake of ^{210}Po by copepods from the dissolved phase found here may help explain results from a study by Jeffree et al. (1997) in which the ^{210}Po concentrations in zooplankton in oligotrophic waters of French Polynesia were found to relate inversely with zooplankton biomass. Jeffree et al. assumed copepod uptake of ^{210}Po came principally from the dissolved phase and argued that the dissolved concentration was inversely related to zooplankton biomass because copepod fecal pellets removed ^{210}Po from surface waters, thereby lowering the ambient dissolved ^{210}Po concentration. An alternative explanation for their observed inverse relationship between zooplankton biomass and ^{210}Po tissue concentrations, given that <8% of the ^{210}Po in copepods comes directly from the dissolved phase, may be that only a small biomass of phytoplankton was available for grazing by the copepods in these oligotrophic waters. Thus, in waters in which there were dense patches of zooplankton, each animal was able to graze less phytoplankton food than in waters with lower copepod biomass, limiting the ^{210}Po intake per animal. Indeed, it may be possible that ^{210}Po concentrations in copepods can be used as a chemical tracer of food limitation in these animals or possibly shifts in phytoplankton community structure.

In addition to using the model to distinguish the relative importance of dietary and solute sources of ^{210}Po for copepods, we used the model to predict ranges of ^{210}Po concentrations in copepods from different regions and compared these predictions to independent field measurements. For the model calculations, we again used the means of measured field concentrations of ^{210}Po in water and phytoplankton at each site and the laboratory-derived parameter values noted above. We used values on the low end of the reported ranges for g (0.03 d^{-1}) and IR (0.33 g g^{-1} d^{-1}) in *A. tonsa* (Mauchline 1998) because we assumed most of the zooplankton in these studies lived in oligotrophic waters. Of course, there is no way to accurately derive the ingestion rate or the phytoplankton species eaten by copepods in the field without further data collection. Likewise, the growth rate and the laboratory-derived parameters of ^{210}Po uptake/loss in copepods are only estimates. For comparing model predictions of ^{210}Po concentrations in copepods with measured values, we only used the dietary uptake term to make predictions about natural samples because the dissolved exposure term would be smaller than the possible range around the food-derived term. Table 4 shows the ^{210}Po concentrations in water, phytoplankton, and zooplankton from four different studies for which field data are available, and Fig. 4 compares

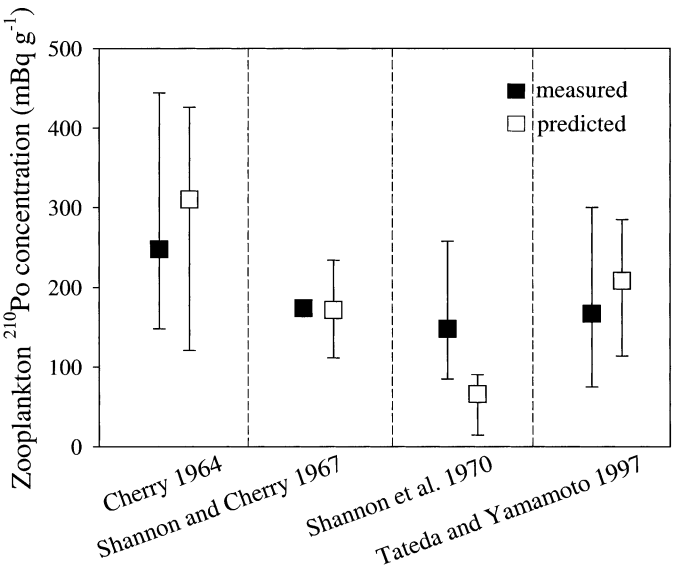

Fig. 4. Comparison between measured ^{210}Po concentrations (mBq g^{-1}) in field-collected zooplankton from different regions and the ranges of concentrations predicted from the kinetic model using laboratory-derived kinetic parameters. Values shown are means and ranges of measured and model-predicted ^{210}Po concentrations in zooplankton. Note there was only one measurement given in Shannon and Cherry (1967). The means of the modeled values were 326, 171, 66, and 208 mBq g^{-1} in chronological order.

our site-specific model-predicted ranges for zooplankton concentrations of ^{210}Po with independent measurements.

The mean measured zooplankton ^{210}Po concentrations were close to the mean model-predicted values, and were certainly well within the range of predicted concentrations, in three of the four cases (Fig. 4). This similarity between predicted and measured ^{210}Po concentrations in zooplankton was found even though some of the referenced studies only measured the ^{210}Po concentration in bulk zooplankton and our model variables refer specifically to the copepod *A. tonsa*. Earlier work noted similar behaviors for other metals in different calanoid copepod species (Reinfelder and Fisher 1991); thus, our data for *A. tonsa* may well reflect the response of other copepods to ^{210}Po at the various sites considered. In addition, it is clear that some of the ''phytoplankton'' values used in the model encompass the ^{210}Po concentration in phytoplankton, some microzooplankton, and protozoa as well as any other particles within a certain size range and that the ''zooplankton'' values include the ^{210}Po values in both herbivores and predators that may, in fact, eat other zooplankton. Nevertheless, the match between predicted and measured ^{210}Po concentrations in zooplankton suggests that we can account for the major processes governing ^{210}Po accumulation in these animals and that the laboratory-derived kinetic parameters are applicable to natural conditions.

It therefore appears that it is possible to predict the concentration of ^{210}Po in copepods on a site-specific basis given the concentration of ^{210}Po in local phytoplankton. Recently, it was shown that the concentration of ^{210}Po in phytoplankton cells is predictable with knowledge of the size and protein content of the cells (Stewart and Fisher 2003). Thus, with few measured parameters, the concentration of ^{210}Po in marine phytoplankton and zooplankton can be calculated. Given their relatively high assimilation of ^{210}Po and slow loss rates, zooplankton may indeed be effective conduits for the transfer of this radioisotope to higher levels of marine food webs.

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