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Tagging crustacean larvae: Assimilation and retention of trace elements

Abstract—The uptake, assimilation, and retention of Se, Co, Ag, Ce, Eu, and Gd by crustacean larvae were measured to assess the suitability of these elements as tags for determining larval dispersal patterns and mortality rates. Gamma-emitting radioisotopes were used to determine the uptake and retention of each element by larvae of three crabs, *Dyspanopeus sayi*, *Uca pugnax*, and *Sesarma reticulatum*, and one barnacle, *Semibalanus balanoides*. Selenium was readily taken up by larvae from their food, was assimilated at efficiencies of 61–93%, was retained at concentrations detectable above background levels for weeks, and did not consistently affect larval survival or development in the laboratory. The other elements were not absorbed sufficiently from food or were not retained long enough to serve as appropriate tags for monitoring larval dispersal. Thus, only Se seemed to be transferred efficiently between trophic levels and is suitable as a larval tag.

Marine benthic invertebrates typically produce thousands of poorly swimming microscopic larvae that develop in the plankton for weeks before settling to the substrate (Young and Chia 1987; Morgan 1995). The vast majority of larvae are thought to disperse randomly and die, which has profound implications for population dynamics, community structure, and life histories in the sea (Gaines and Lafferty 1995; Morgan 1995). However, direct tests of this hypothesis are rare because it is difficult to track minute widely dispersing larvae. Although larval mortality and dispersal have been measured reliably by following large ascidian larvae after their release (Olson and McPherson 1987; Stoner 1992), measurements for other taxa usually are inferred from passively drifting drogues and dyes, tracking cohorts of larvae while ignoring emigration from or immigration to the patch, contrasting the number of larvae produced with the number that settle, or contrasting abundances of larvae in consecutive stages of development (reviewed by Levin 1990; Rumrill 1990).

The best method of measuring larval mortality and dispersal for most marine organisms would be to release and recapture marked individuals, but the development of a larval tag that is easily applied and detected, persistent, non-toxic, inexpensive, and invisible to predators has been elusive. Investigators have tried tagging larvae with stains, calcium carbonate, genetic markers, radioactive labels, and trace elements but most of these techniques are of limited utility (Levin 1990). Rare-earth elements are promising because they occur at extremely low levels in wild zooplankters, are easily applied, and are environmentally safe. However, lutetium (Lu), samarium (Sm), and europium (Eu) tags persisted only briefly and larval survival was reduced by the high doses applied (Levin et al. 1993).

To develop a larval tag, we have adapted the techniques of entomologists who have been successful in marking insects with rubidium (Rb), cesium (Cs), strontium (Sr), and dysprosium (Dy) to study dispersal, population size, mortal-

ity, feeding, and mate competition (Hayes 1991). Increased concentrations of elements in insect tissues were detectable above trace background levels for weeks. These elements are inexpensive, safely applied on a broad scale, and can be detected using atomic absorption spectrometry (AAS) or inductively coupled plasma–mass spectrometry (ICP-MS), which are widely available, relatively inexpensive, and sensitive at concentrations of $\mu\text{g kg}^{-1}$ of dry sample weight.

Most of the elements that have been used to tag insects are not concentrated appreciably by organisms living in seawater (Bewers et al. 1985). Therefore, we selected other elements that are concentrated appreciably by some marine organisms (Bewers et al. 1985), including selenium (Se), cobalt (Co), silver (Ag), europium (Eu), gadolinium (Gd), and cerium (Ce). Cobalt and Ag were chosen based on their low background levels and the likelihood of being concentrated sufficiently by animals out of seawater (Bewers et al. 1985) to serve as tracers, which reflects accumulation and retention in the animals. We also chose Se for the latter reason. Cerium, Eu, and Gd were chosen because other rare-earth elements showed some promise as larval tags (Levin et al. 1993). Uptake and depuration of these elements by larvae from food and water were studied nondestructively by using γ -emitting radiotracers, which enabled us to work with low, environmentally realistic concentrations of elements. Survival and development also were assayed to ensure that tagging did not harm larvae.

Elements are concentrated by phytoplankton from seawater and are transferred through planktonic communities, which affects the vertical flux and residence times in the water column (Fisher and Reinfelder 1995). Therefore, the uptake, assimilation, and retention of elements studied here are useful in understanding digestion and absorption of nutrients by zooplankters and in evaluating processes that regulate the biogeochemical cycling of these elements.

We studied omnivorous crab larvae and herbivorous barnacle nauplii to compare trophic differences in the uptake and retention of elements. Crustaceans are ideal organisms for this study because they have dispersing larvae and are related to insects. Moreover, larval distributions and dispersal mechanisms have been relatively well studied for these animals, thereby preparing the way for subsequent field trials.

Ovigerous female crabs of three species, *Dyspanopeus sayi*, *Uca pugnax*, and *Sesarma reticulatum*, were collected from Flax Pond and West Meadow Creek (Long Island, New York) in June 1994. Crabs were maintained in the laboratory in seawater with a salinity of 30 and a temperature of 25°C in a 14:10 L/D cycle until larvae were released. Newly hatched larvae were transferred to filtered, ultraviolet-sterilized seawater until experiments began 1–4 h later. Adult barnacles, *Semibalanus balanoides*, were collected from rocks near Stony Brook, Long Island. Barnacles were maintained in seawater with a salinity of 35 and a temperature

Table 1. Experimental conditions during labeling experiments. Regression equations of the physiological loss curves and their r^2 values, assimilation efficiencies (AEs), and biological half-lives ($tb_{1/2}$) of each element are also shown. In the regression equations, $y = \ln(\% \text{ initial activity})$ and $x = \text{time (d)}$. The efflux rate constants are the slopes of these regression lines and the AEs are the y-intercepts. The AEs of the REEs were taken as the minimum percentage of each element retained by larvae during the first day of depuration.

Element	Food type	Elemental concn in food (amol per cell)	Food concn (10^5 cells ml^{-1})	Larval species	Final concn of element (nmol g^{-1})	Regression equation		r^2	AE (%)	$tb_{1/2}$ (d)
						Slope	Intercept			
^{75}Se	<i>Thalassiosira pseudonana</i>	6.1	5	<i>Uca pugnax</i>	809	-0.05	4.2	0.94	61	13
^{75}Se	<i>Artemia</i> nauplii	310*		<i>Sesarma reticulatum</i>	43	-0.091	4.6	0.96	94	7.6
				<i>Dyspanopeus sayi</i>	23	-0.085	4.5	0.69	84	8.2
^{75}Se	<i>T. pseudonana</i>	3.3	5	<i>S. reticulatum</i>	240	-0.082	4.2	0.96	63	8.4
				<i>D. sayi</i>	241	-0.097	4.5	0.97	78	7.1
^{141}Ce	<i>T. pseudonana</i>	0.63	5	<i>D. sayi</i>	4.8				0	
			5	<i>U. pugnax</i>	11					
^{152}Eu	<i>T. pseudonana</i>	0.36	5	<i>D. sayi</i>	28				20	
			5	<i>U. pugnax</i>	47					
^{153}Gd	<i>T. pseudonana</i>	0.46	5	<i>D. sayi</i>	0.9				33	
			5	<i>U. pugnax</i>	0.9					
^{75}Se	<i>T. pseudonana</i>	9,300	10	<i>Semibalanus balanoides</i>	47	-0.034	4.5	0.92	93	20
^{110}Ag	<i>T. pseudonana</i>	152,000	10	<i>S. balanoides</i>	185	-0.011	1.9	0.005	10	63
^{57}Co	<i>T. pseudonana</i>	0.03	10	<i>S. balanoides</i>	0.06	-0.12	3.8	0.95	40	5.7

* Per nauplii, not per cell.

of 4°C in a 10:14 L/D cycle. Adult barnacles were cracked open to release larvae as needed for experiments. The diatom *Thalassiosira pseudonana* (clone 3H) was used in labeling experiments. Cultures of *T. pseudonana* were maintained in sterile glass-fiber-filtered (GF/C) seawater that was enriched with f/2 nutrients (Guillard and Ryther 1962).

The experimental protocol for radiolabeling crab larvae through their food was adapted from Fisher et al. (1991) and Reinfelder and Fisher (1994), and the experimental conditions are summarized in Table 1. Experimental inocula for uptake of elements by phytoplankton were obtained by resuspending cells from a 1- μm Nuclepore polycarbonate membrane in sterile-filtered seawater to obtain a concentration of 5×10^4 cells ml^{-1} (equal to 1.1 mg dry wt liter^{-1}). This medium was enriched with f/2 nutrients without copper, zinc, or EDTA. Algal cells were exposed separately to 55.5 kBq liter^{-1} (1.8×10^{-8} M) ^{75}Se , 74 kBq liter^{-1} (1.2×10^{-8} M) ^{141}Ce , 74 kBq liter^{-1} (1.2×10^{-9} M) ^{152}Eu , or 74 kBq liter^{-1} (8.5×10^{-10} M) ^{153}Gd (1 Bq = 1 dps). The ^{75}Se (obtained from Amersham) was added from a stock solution of sodium selenite in distilled water, and the ^{152}Eu (obtained from Amersham), ^{141}Ce (obtained from New England Nuclear), and ^{153}Gd (obtained from New England Nuclear) were added from stocks dissolved in 0.1 N HCl, 0.5 N HCl, and 0.5 N HCl, respectively. The pH was adjusted to 8.0 with NaOH, as necessary. Control flasks contained the same concentrations of radioisotopes but did not contain diatoms. Uptake of each element by diatoms was monitored for 4–5 d until an equilibrium in isotope partitioning between particles and the dissolved phase ($<0.2 \mu\text{m}$) was attained, as described elsewhere (Fisher et al. 1983a). The radioactivity of all samples was determined in an LKB Compugamma gamma counter that was equipped with a large well NaI (TI) detector. Counting times were adjusted to give propagated counting errors $<5\%$. Gamma emissions were detected at

264 keV for ^{75}Se , 145 keV for ^{141}Ce , 344 keV for ^{152}Eu , and 97 keV for ^{153}Gd .

Radiolabeled diatoms in experimental feeding flasks were resuspended from 1- μm Nuclepore filters into 250 ml or 500 ml unlabeled, unenriched GF/C filtered seawater to attain phytoplankton concentrations of 11 mg dry wt liter^{-1} . Larvae of each crab species were added separately to these cell suspensions to attain densities of about 1 larva per 2 ml of seawater. The experiment was not replicated because similar experiments have revealed very little intraspecific variability in uptake rates and assimilation efficiencies (AEs) (Fisher and Reinfelder 1991; Wang and Fisher 1996). Larvae fed on radiolabeled diatoms for ~3–6 d and were allowed to purge their guts of undigested radioactive food by transferring them to flasks containing unlabeled *T. pseudonana* (11 mg dry wt liter^{-1}). Uptake and depuration of elements by larvae were monitored after 2, 4, 8, and 24 h during the first day and once per day for the remainder of the experiment. At each sampling, we determined the fractionation of radioisotopes between cells and the dissolved phase. Ten larvae per treatment were collected on a 118- μm mesh sieve, were washed with 10 ml of filtered seawater, and were rinsed into a counting tube for measuring radioactivity. Individual larvae were not analyzed because counting errors would have been too high at these low elemental concentrations.

Attempts were made to label crab larvae with Se through the dissolved phase (by adding ^{75}Se , as selenite, directly to seawater in which larvae were placed) and through feeding on labeled *Artemia* nauplii (which were labeled by feeding on labeled phytoplankton as previously described for crab larvae). Neither of these attempts was successful.

Based on the results of labeling experiments with crab larvae, barnacle nauplii were labeled with ^{75}Se -, ^{110}Ag -, and ^{57}Co -labeled phytoplankton. *T. pseudonana* was labeled with 111 kBq liter^{-1} (3.6×10^{-8} M) ^{75}Se as previously de-

scribed. In a separate culture, algal cells simultaneously were exposed to $185 \text{ kBq liter}^{-1}$ ($1.9 \times 10^{-7} \text{ M}$) ^{110}Ag and $111 \text{ kBq liter}^{-1}$ ($6.6 \times 10^{-12} \text{ M}$) ^{57}Co . The ^{75}Se was added from a stock solution of selenite in distilled water, ^{110}Ag was added from a stock in 0.1 N HNO_3 , and ^{57}Co was added from a stock in 0.1 N HCl . Gamma emissions of ^{110}Ag were detected at 658 keV and ^{57}Co emissions were detected at 122 keV . Double labeling the same larvae with these radioisotopes eliminated variability between elements in the uptake and depuration rates due to differences among individual larva.

Two replicate feeding flasks were prepared as described above. Phytoplankton concentrations were $20 \text{ mg dry wt liter}^{-1}$ and nauplii were held at densities of $1 \text{ nauplius ml}^{-1}$ in all treatments. Barnacle nauplii were fed ^{75}Se -labeled cells for 10 d, and they were fed ^{110}Ag - and ^{57}Co -labeled cells for 4 d. Nauplii then were transferred to filtered seawater containing unlabeled phytoplankton and their guts were cleared. Sampling during the uptake and depuration periods was as described for crab larvae.

Physiological turnover curves describe the release of elements over time after the unassimilated tracers have been egested. Because zooplankters typically evacuate their guts within 1 h (Fisher and Reinfelder 1991), we safely assumed that larvae had evacuated their guts within 4 h. Samples taken before 4 h were omitted from the least-squares regression of the log-linear depuration curve (plot of fraction of initial activity vs. time). The log-linear physiological turnover curves of radiotracers then were extrapolated to the origin and the resulting y-intercepts revealed the AEs.

The toxicity of Se to crab larvae was tested using larvae of *U. pugnax* and *S. reticulatum*. Each species was separately labeled with three concentrations of Se. Low, medium, and high concentrations of Se were obtained by adding $55.5 \text{ kBq liter}^{-1}$ ($1.8 \times 10^{-8} \text{ M}$) of ^{75}Se and either $7.75 \times 10^{-7} \text{ M}$, $7.75 \times 10^{-6} \text{ M}$, or $7.75 \times 10^{-5} \text{ M}$ of stable Se (as selenite) to phytoplankton cultures and growing the cells for 5 d. The labeled treatment and unlabeled control cultures were prepared as previously described, and $\sim 2 \text{ Artemia nauplii ml}^{-1}$ were added to each culture. The experiments were replicated three times for *U. pugnax* and twice for *S. reticulatum* by using larvae that were hatched from different females. After 2 d, water in the feeding cultures was changed, and new *Artemia* and labeled diatoms were added at the initial concentrations. After feeding on labeled phytoplankton for 4 d, the radioactivity of larvae was determined as previously described. Mean concentrations ($\pm 1 \text{ SE}$) of Se in high-, medium-, and low-Se treatments at the end of the labeling period were $3.4 (\pm 0.4)$, $0.2 (\pm 0.008)$, and $0.03 (\pm 0.003) \mu\text{mol g}^{-1}$ for *U. pugnax* and $1.7 (\pm 0)$, $0.2 (\pm 0)$, and $0.03 (\pm 0.004) \mu\text{mol g}^{-1}$ for *S. reticulatum*.

Fifty larvae from each replicate treatment and control were transferred to small culture dishes that contained filtered seawater and $2 \text{ Artemia nauplii ml}^{-1}$. Survivors were counted daily and transferred to new culture dishes with clean filtered seawater and freshly hatched *Artemia* nauplii. Larvae were reared until they metamorphosed to megalopae to determine the effects of Se on survival and development time.

The toxicity of Se to barnacle nauplii was tested using

labeled ($0.05 \mu\text{mol g}^{-1}$) and unlabeled *S. balanoides* nauplii. Fifty nauplii labeled with ^{75}Se were placed in each of three replicate culture dishes that contained 50 ml of filtered seawater. Fifty unlabeled larvae that had hatched on the same day as the labeled larvae also were reared in three replicate dishes. All larvae were fed unlabeled phytoplankton ($20 \text{ mg dry wt liter}^{-1}$). Survivors were counted daily and transferred to clean culture dishes that contained filtered seawater and phytoplankton.

Because Se held the most promise for future tagging studies, only the background concentration of this element was determined. Background concentrations of Se were determined for three samples of $1,300\text{--}7,400$ larvae that were hatched by two *U. pugnax* females and one *D. sayi* female, and they were determined for three samples of $7,000\text{--}8,700$ nauplii that were hatched from three barnacles. Crab larvae were fed *Artemia* nauplii and phytoplankton, while barnacle nauplii were fed only phytoplankton. All larvae were fed in unfiltered seawater for 2 d to allow them to accumulate elements. Crab and barnacle larvae were washed on $118\text{-}\mu\text{m}$ sieves with deionized water and rinsed into a beaker with deionized water. Larvae were lyophilized for 24 h, weighed and digested using the following $\text{HNO}_3\text{--HClO}_4$ acid digestion technique. Ten milliliters of trace metal-grade HNO_3 was added to the dried larval tissue. The mixture was allowed to stand for 2 h, was refluxed at 70°C for 4 h, and was cooled at room temperature. Five milliliters of $1:1$ nitric acid:distilled deionized water was added to each of the samples, which again were refluxed for 2 h at 70°C and allowed to cool. HClO_4 (1 ml) was added and the samples were refluxed for 1 h at 70°C . The samples were allowed to cool and the final step was repeated. The samples were evaporated at 70°C to a volume of 1 ml and were centrifuged to remove remaining tissue. Se analyses of the digest solutions were performed using a Perkin-Elmer Zeeman 5000 AAS that was equipped with a HGA-500 graphite atomizer and an AS-40 autosampler. Se was analyzed using a nickel matrix modifier in a flameless analysis.

The expected background concentration of Se in zooplankton also was calculated by multiplying the concentration of Se in surface seawater (Bruland 1983) by its concentration factor for zooplankton (1×10^4 ; Bewers et al. 1985). Mean individual dry weights ($\pm 1 \text{ SE}$) of larvae, measured by weighing thousands of larvae that had been dried at 60°C for 24 h, were $2.63 (\pm 1.2) \mu\text{g}$ ($n = 3$), $1.18 (\pm 0.15) \mu\text{g}$ ($n = 3$), $2.73 \mu\text{g}$ ($n = 1$), and $2.89 \mu\text{g}$ ($n = 1$) for *S. balanoides*, *U. pugnax*, *D. sayi*, and *S. reticulatum* larvae, respectively.

The concentration of Se in crab larvae that were exposed to dissolved ^{75}Se for 4 d was only $4\text{--}9 \text{ nmol g}^{-1}$ for *D. sayi* and $8\text{--}11 \text{ nmol g}^{-1}$ for *S. reticulatum*. Because crab larvae did not take up substantial amounts of Se from the dissolved phase, they were labeled with elements through their food.

^{75}Se was taken up readily by *T. pseudonana*. After 5 d, 78% of Se in the flask was associated with diatom cells. The volume/volume concentration factor (VCF) is a unitless measure of the extent to which an element is concentrated from seawater by an organism and it was measured as described in Fisher et al. (1983a). The VCF for Se is $4.0 \times$

Fig. 1. Uptake and depuration of selenium by larvae of three crabs, *Uca pugnax*, *Sesarma reticulatum*, and *Dyspanopeus sayi*, and larvae of one barnacle, *Semibalanus balanoides*. A. The uptake of ^{75}Se by crab and barnacle larvae feeding on labeled phytoplankton, *Thalassiosira pseudonana*. B. Linear regressions of the loss of ^{75}Se from the same larvae. Depuration of Se is the percentage of the initial concentration remaining per larva. The first molts to megalopae, seen in each of several replicates, occurred between days 10 and 14 for *U. pugnax*, between days 11 and 15 for *D. sayi*, and between days 7 and 11 for *S. reticulatum*. The calculated background levels of Se (see text) are expressed as the percentage of Se per larva at the beginning of the depuration period and are 2.2% for *U. pugnax*, 3.0% for *S. reticulatum*, 3.1% for *D. sayi*, and 38% for *S. balanoides*.

10^4 and the final concentration of ^{75}Se in the cells was $0.3 \mu\text{mol g}^{-1}$.

^{75}Se was readily accumulated by larvae from their food (Fig. 1A). Assimilation efficiencies (the percent of ingested element retained by the organism after gut evacuation) of ^{75}Se were 61, 78, and 63% for *U. pugnax*, *D. sayi*, and *S. reticulatum* larvae (Table 1). More than 40% of initial Se was retained by all larvae after 1 wk of depuration. Forty-seven percent, 21%, and 28% of assimilated Se was retained by *U. pugnax*, *D. sayi*, and *S. reticulatum* megalopae following 5, 4, and 3 molts, respectively (Fig. 1B). The efflux rate constants were 0.050 d^{-1} for *U. pugnax*, 0.097 d^{-1} for *D. sayi*, and 0.082 d^{-1} for *S. reticulatum* (Table 1). The biological half-life [$t_{1/2} = (\ln 2)/\text{efflux rate constant}$] of Se was 13, 7.1, and 8.4 d in *U. pugnax*, *D. sayi*, and *S. reticulatum*.

After 4 d, diatom VCFs were 1.6×10^6 for ^{141}Ce , $1.2 \times$

10^6 for ^{152}Eu and 3.9×10^5 for ^{153}Gd . Assimilation efficiencies of these elements were low in *U. pugnax* and *D. sayi* larvae, and depuration was rapid (Table 1).

Barnacle nauplii also concentrated ^{75}Se from labeled phytoplankton cells (Fig. 1A). The ^{75}Se concentration was $0.04 \mu\text{mol g}^{-1}$ at 3 d and it reached a maximum of $0.05 \mu\text{mol g}^{-1}$ at 9 d. The AE of ^{75}Se in barnacle nauplii was 93% and depuration of the element was very slow, with an efflux rate constant of 0.034 d^{-1} and a $t_{1/2}$ of 20 d (Table 1).

Uptake of ^{110}Ag by barnacle nauplii was rapid initially, reaching $0.2 \mu\text{mol g}^{-1}$ within 8 h, but the concentration of ^{110}Ag fluctuated greatly thereafter. Although the physiological turnover of ^{110}Ag was slow, the AE was only 10% (Table 1), and most of the element already had been lost during the initial depuration period. Barnacle nauplii also concentrated ^{57}Co from their food. However, the uptake and AE were low and depuration was rapid (Table 1). Less than 20% of both ^{110}Ag and ^{57}Co was in the dissolved phase, and therefore uptake from this source was negligible.

Artemia nauplii concentrated ^{75}Se from labeled phytoplankton cells, but the final concentration of ^{75}Se was only 17 nmol g^{-1} (mean dry wt of nauplius $\pm 1 \text{ SE} = 1.83 \pm 0.07 \mu\text{g}$). Consequently, the final concentration of ^{75}Se in crab larvae that were fed radiolabeled nauplii was lower ($23\text{--}43 \text{ nmol g}^{-1}$) than for those that were fed radiolabeled phytoplankton (240 nmol g^{-1}) (Table 1).

Mean percent survival ($\pm 1 \text{ SE}$) of *U. pugnax* larvae to megalopae at control, low, medium, and high levels of Se was $34 (\pm 3)$, $13 (\pm 2)$, $21 (\pm 4)$, and $12 (\pm 4)$, respectively (Fig. 2). Although survival of unlabeled controls was higher than survival of labeled larvae for this species, we wanted to determine if there was a consistent trend where increased Se concentration led to reduced survival. Polynomial regression was performed on the data and better fits were not obtained by using higher order regressions. Linear regression therefore was used to determine the effects of elemental concentration on percent survival and development time of larvae that metamorphosed to megalopae. Larval survival on day 19 for *U. pugnax* and day 10 for *S. reticulatum*, when the first larva metamorphosed to megalopa, was regressed on Se concentration. The concentrations of Se in *U. pugnax* ($r^2 = 0.15$) and *S. reticulatum* ($r^2 = 0.07$) did not affect larval survival. Postlarval survival of both species declined at similar rates in all treatments. Because only one concentration of Se was used in the barnacle toxicity study, a Student's *t*-test was performed for each day to determine if survival of controls differed from survival of unlabeled nauplii. Survival between the two groups was not significantly different (for example, at 1 week, $t = 0.14$, $\text{df} = 4$) until the last 3 d of the experiment (Fig. 3). At this point, survival was in general very low and nauplii may have been looking for a cue to settle that was not present.

Exposure to Se also did not affect larval development (Fig. 2). The percentage of individuals that metamorphosed to megalopae (on the first day that all larvae metamorphosed in one of the culture dishes, which was day 25 for *U. pugnax* and day 13 for *S. reticulatum*) was not related to the concentration of Se in either *U. pugnax* ($r^2 = 0.19$) or *S. reticulatum* ($r^2 = 0.14$) larvae. The effect of Se on development

Fig. 2. Survival and development of *Uca pugnax* and *Sesarma reticulatum* larvae labeled with three concentrations of selenium (mean $\pm$ SE: 0.03 ± 0.003 , 0.2 ± 0.008 , and $3.4 \pm .04 \mu\text{mol g}^{-1}$ for *U. pugnax* and 0.03 ± 0.004 , 0.2 ± 0 , and $1.7 \pm \mu\text{mol g}^{-1}$ for *S. reticulatum*) and unlabeled controls. Upper graphs show survival of both larvae and megalopae, and lower graphs show the cumulative percentage of larvae that metamorphosed to megalopae. Arrows indicate when labeling was stopped and monitoring of survival and development began. Error bars represent ± 1 SE.

Fig. 3. Survival of barnacle, *Semibalanus balanoides*, larvae labeled with ^{75}Se ($0.05 \mu\text{mol g}^{-1}$) vs. unlabeled controls. Error bars represent ± 1 SE.

of barnacle nauplii was not assessed, because nauplii did not metamorphose to cyprids.

The background concentrations of Se were $29 (\pm 4) \text{ nmol g}^{-1}$ in crab larvae and $6 (\pm 1) \text{ nmol g}^{-1}$ in barnacle larvae. The mean Se concentration in the crustacean larvae used in this study was 18 nmol g^{-1} , which is identical to the concentration in zooplankton that was calculated from the literature (Bruland 1983; Bewers et al. 1985).

Among the six elements tested in this study, Se holds the most promise as a larval tag. It is readily accumulated in phytoplankton at high levels, which makes it easy to tag large numbers of larvae by labeling their food. Larvae assimilated Se with efficiencies of 61–93% and lost it slowly. Enhanced concentrations of Se are retained at detectable levels for several weeks, making it suitable to study dispersal and mortality during the entire planktonic phase of development. Larval survival and development were not consistently affected by the concentrations used in this study. Finally, Se can be easily and inexpensively detected using AAS or ICP-MS, which are both widely available.

Radiolabeled REEs were not assimilated by crab larvae probably because they are associated with cell walls and plasmalemmae of diatoms that are not easily digested by zooplankters (Reinfelder and Fisher 1991, 1994). Microbial

grazers also assimilated Gd poorly (Twiss and Campbell 1995), and AEs of ^{110}Ag and ^{57}Co in our study were similar to those for bivalve larvae—16–33% for Ag and 19–27% for Co (Reinfelder and Fisher 1994).

The AEs of zooplankters affect the biogeochemical cycling and oceanic residence times of elements (Fisher and Reinfelder 1995). Elements that are not assimilated will be concentrated in fecal pellets of zooplankton and will rapidly sink to the benthos (Fowler and Knauer 1986). Therefore, REEs and, to a lesser extent, Ag and Co will have shorter residence times in surface waters than Se and other highly assimilated elements that will be recycled with organic matter.

Crab larvae did not accumulate considerable amounts of ^{75}Se from the dissolved phase. Larvae were not labeled with REEs through the dissolved phase because these highly reactive elements likely would be associated with larval surfaces (Fisher et al. 1983b). Therefore, concentrations of these elements in larvae should decrease rapidly as observed by Levin et al. (1993).

The rapid loss of each element from larvae within the first few hours of depuration was due to gut evacuation, and the long slow loss resulted from physiological turnover. In calculating depuration rates and AEs, we assumed a constant loss of each element during physiological turnover. This may not be accurate for organisms that molt if elements are associated with exoskeletons. However, pulsed loss associated with molting was not evident in our study, and linear regressions likely provide good estimates of elemental depurations. Essentially, all radiolabeled REEs were lost during the first few hours of the experiment, suggesting that they were not assimilated. Approximately 90% of the ^{110}Ag and half of the ^{57}Co were lost through gut evacuation. Silver concentrations in barnacles probably remain above background levels for much longer than 18 d but the sporadic uptake, low assimilation (10%), and rapid initial depuration of Ag make its suitability as a larval tag questionable. Barnacle nauplii retained Co above the calculated background concentration for only a few hours and this element could not be used to mark larvae.

In contrast, ^{75}Se was lost slowly; only 6–39% of it was lost during gut evacuation, suggesting that most of it was assimilated into tissues. Larval concentrations of Se remained above background for 35 d in *D. sayi*, 37 d in *S. reticulatum*, >60 d in *U. pugnax*, and >18 d in *S. balanoides*. Thus, Se was retained at levels detectable above background for the duration of the larval phase for all four species and is the most suitable element tested for tagging crustacean larvae.

Previous studies have shown that cellular concentrations of Se as high as $1.3 \mu\text{mol g}^{-1}$ are not toxic to phytoplankton (Wheeler et al. 1982), and the concentrations (0.24 – $0.81 \mu\text{mol g}^{-1}$) used in this study also did not appear to be toxic to crustacean larvae. Although early survival of *U. pugnax* larvae that were labeled with Se appeared to be lower than unlabeled controls, larvae that were labeled with intermediate concentrations of Se ($0.2 \mu\text{mol g}^{-1}$) survived better than those that were labeled either with low ($0.03 \mu\text{mol g}^{-1}$) or high (1.7 to $3.4 \mu\text{mol g}^{-1}$) Se concentrations. Because the variability in larval survival was not consistently related to larval concentrations of Se, doses used in this study probably did not lower survival of *U. pugnax* larvae. Furthermore, of the three species of crustacean larvae studied, only *U. pug-*

nax appeared to be affected at all by the Se concentrations used. Unlabeled larvae survived worse and had longer development times than has been reported previously (Costlow and Bookhout 1962; Christy 1989), but this may be improved by providing more food or suitable substrates for settlement (Crisp 1976; Tegtmeyer and Rittschof 1989).

Studying dispersal and mortality with tagged larvae still presents problems that must be overcome. If larval mortality and advection are extensive in the field, then hundreds of thousands or even millions of tagged larvae must be released to recover any of them. These problems may be overcome if Se is passed from females to larvae, because entire broods of thousands or millions of offspring may be marked by dusting a proscribed area with Se as has been done with insects (Hayes 1991). If intergenerational studies and mass marking of field populations are not possible, then a variety of other approaches may be used to facilitate the recovery of tagged larvae. For example, tagged larvae may be released in confined areas or in oceanic features that concentrate larvae (e.g. fronts, eddies). Alternatively, larvae may be released in conjunction with dye tracers, drogues, or computerized larval mimics to increase sampling efficiency. Despite these obstacles, we suggest that tracking Se-labeled larvae will dramatically enhance our understanding of the factors that affect larval dispersal and mortality.

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Contrasts between marine and freshwater bacterial community composition: Analyses of communities in Lake George and six other Adirondack lakes

Abstract—The bacterial communities of seven freshwater lakes in the Adirondack Mountains of New York state were examined using culture-independent methods. *β-Proteobacteria* 16S rRNA sequences were recovered from all seven lakes and their presence was confirmed by direct DNA hybridization. The results are consistent with phylogenetic and in situ hybridization-based studies in other freshwater environments, but are significantly different than the results of marine oceanic studies, where *β-Proteobacteria* are noticeably absent. This relationship between evolutionary history and environmental distribution is striking, since these phylogenetic clades have not been correlated with consistent physiological features or biochemical capabilities, and there is no a priori reason to expect differences in phylogenetic composition between the

environments. In contrast, freshwater relatives to marine phylogenetic clusters, in particular the SAR 11 cluster of the *α-Proteobacteria*, were identified. The data imply an underlying physiological distinction between the *β*- and other *Proteobacteria* groups and potentially an important difference between the composition of bacterial communities in marine and freshwater environments.

The differences between marine and freshwater bacterial populations have never been well defined (Hobbie 1988). Classical microbiology studies initially indicated differences in relative abundance of rods and cocci, as well as Gram-