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Flexible digestion strategies and trace metal assimilation in marine bivalves

Abstract—Pulse-chase experiments show that two marine bivalves take optimal advantage of different types of particulate food by varying food retention time in a flexible two-phase digestive system. For example, carbon is efficiently assimilated from bacteria by subjecting nearly all the ingested bacteria to prolonged digestion. Prolonging digestion also enhances assimilation of metals, many of which are toxic in minute quantities if they are biologically available. Detritus-feeding aquatic organisms have always lived in environments naturally rich in particle-reactive metals. We suggest that avoiding excess assimilation of metals could be a factor in the evolution of digestion strategies. We tested that suggestion by studying digestion of particles containing different Cr concentrations. We show that bivalves are capable of modifying the digestive processing of food to reduce exposure to high, biologically available, Cr concentrations. The evolution of a mechanism in some species to avoid high concentrations of metals in food could influence how effects of modern metal pollution are manifested in marine ecosystems.

Most trace elements are particle reactive and it is increasingly evident that particle ingestion is a pathway whereby elements can enter benthic food webs (Decho and Luoma 1991; Luoma et al. 1992; Reinfelder and Fisher 1991). Bivalves are an important component of benthic food webs. Digestive system morphology, gut residence time, and digestive processing are unique and complicated in bivalves (Owen 1974). Food is first processed in the intestine where digestion is rapid and primarily extracellular (Reid and Rauchert 1972; Bayne et al. 1989; Widdows et al. 1979). A selected portion of food is held for a longer period in the digestive gland where intracellular digestion occurs and processing is most intensive (Fig. 1). One cost of prolonged food retention during digestion is a reduction in the mass of food processed per unit time. Prolonged food retention also could result in greater uptake of potentially toxic, food-associated metals, imposing an additional cost.

Chromium(III) is a common anthropogenic pollutant and a potent toxin when it is biologically available (Nriagu and Neibor 1988). Cr(III) has been used as an inert tracer in carbon assimilation studies because earlier studies suggested it was not biologically available when ingested by certain animals (Mertz 1969; Lopez et al. 1989). Recent studies of bivalves have shown that Cr(III) is biologically available from some food particles, depending on how the particles are digested (Decho and Luoma 1991, 1994).

We investigated the digestive processing and utilization of C and Cr(III) associated with different types of food particles in the deposit-feeding bivalve *Macoma balthica* and the suspension-feeding clam *Potamocorbula amurensis*. We further examined whether these bivalves are capable of modifying the digestive processing of food particles to reduce exposure to high, biologically available Cr concentrations during prolonged digestion. Both bivalves are adaptable, opportunistic, and abundant in San Francisco Bay (Nichols et al. 1986). Previous studies showed significant differences in the way these bivalves processed bacterial food particles through the two digestive pathways (Decho and Luoma 1991). We expected that these bivalves also might exhibit differences in the digestive processing of other types of food particles.

All bivalves we used were collected from San Francisco Bay. Between 5 and 15 replicate bivalves were used for each experimental treatment because initial levels of label uptake by individuals was often variable. All experiments were conducted at 20‰ salinity and 20°C.

A first set of experiments was conducted to compare the digestive processing and assimilation of C and Cr(III) among several natural food sources of the animals: diatoms, bacterial cells, a bacterial exudate (exopolymer or EPS) in suspension or adsorbed to sediments, and flocculent surficial sediment particles from a mudflat. Diatoms were from cultures of the marine plankton *Thalassiosira pseudonana*. Cells were grown in f/2 media (Guilliard and Ryther 1962), uniformly labeled with $NaH^{14}CO_3$ (37 kBq per 100 ml of culture), and harvested during the early stationary phase of growth. Bacteria and exopolymer were from cultures of the marine bacterium *Alteromonas atlantica*. Cultures were grown in media consisting of 0.5% (wt/vol) D-glucose and 0.2% peptone in the presence of [U-D-^{14}C]glucose (37 kBq per 50 ml of culture). Exopolymers were extracted according to methods already described (Decho and Lopez 1993). ^{51}Cr(III) was actively incorporated into living diatom and bacterial cells and adsorbed to purified exopolymer and natural sediment particles. ^{14}C was actively incorporated into living cells and exopolymers; C assimilation from natural sediments was not determined. The isotope ^{51}Cr (half-life, 27.8 d) was detected by gamma counting. All samples containing both ^{14}C and ^{51}Cr were initially gamma counted, fixed (5% Formalin) for analysis of ^{14}C, and analyzed by liquid scintillation counting (LSC). Quench was corrected with the external standards ratio method. The ^{51}Cr beta signal in LSC was corrected with internal standards.

Feeding experiments, using the pulse-chase approach, were conducted with radioisotopes to assess differences in assimilation efficiencies (Decho and Luoma 1991; Luoma et al. 1992; Reinfelder and Fisher 1991; Fisher et al. 1991). Bivalves were placed in microcosms and fed a short pulse (20 min) of labeled food [^{14}C- and ^{51}Cr(III)-labeled food particles]. Egestion and retention of label was followed as animals fed on unlabeled flocculent sediment from San Francisco Bay.

In previous experiments, animals were fed beads impregnated with unavailable ^{51}Cr(III) to determine total gut residence time and to define the phases of digestion (Decho and Luoma 1991). The output of ^{51}Cr(III)-labeled feces was the same during the first few hours of digestion for impregnated beads as for other types of food particles. These results showed that ^{51}Cr(III) was not assimilated from any type of food during intestinal digestion. Therefore, the proportion of ^{51}Cr(III) egested during the early phase of digestion (3 h in *P. amurensis* and 20 h in *M. balthica*) was used to estimate the proportion of food subjected only to intestinal digestion in the present pulse-chase experiments. The percentage of food processed by the digestive gland was determined by difference. The ^{14}C:^{51}Cr(III) ratio in feces (Lopez and Cheng 1983) in the early phase of digestion indicated ^{14}C assimilation in the intestine. Overall assimilation efficiency and corrections for $^{14}CO_2$ loss during respiration were determined as described earlier (Decho and Luoma 1991; Luoma et al. 1992).

Mathematically, the proportion of food processed by the intestinal pathway was determined as the (total ^{51}Cr egested during intestinal phase) / (total ^{51}Cr ingested). The percent absorption efficiency (%AE) of carbon in the intestine pathway = (^{14}C:^{51}Cr intest. feces)/(^{14}C:^{51}Cr in food). The percent of total carbon assimilated by intestinal digestion = (%AE in intest.) × (% food processed only by the intestinal pathway). The percent total C assimilated by the digestive gland (DG) = (overall % AE) − (% of total C assimilated by intest.). The %AE in DG = (% of total C assimilated by DG)/(% food processed by DG).

The pathway of digestion strongly affected carbon assimilation. Half of the carbon in diatoms was assimilated during the early phase of digestion (Table 1), probably because extracellular intestinal digestion frees labile carbon from the cytosol of diatom cells (Reinfelder and Fisher 1991). Longer retention in the digestive gland was necessary for assimilation of the other half of the carbon. In contrast, less carbon was assimilated from bacteria than

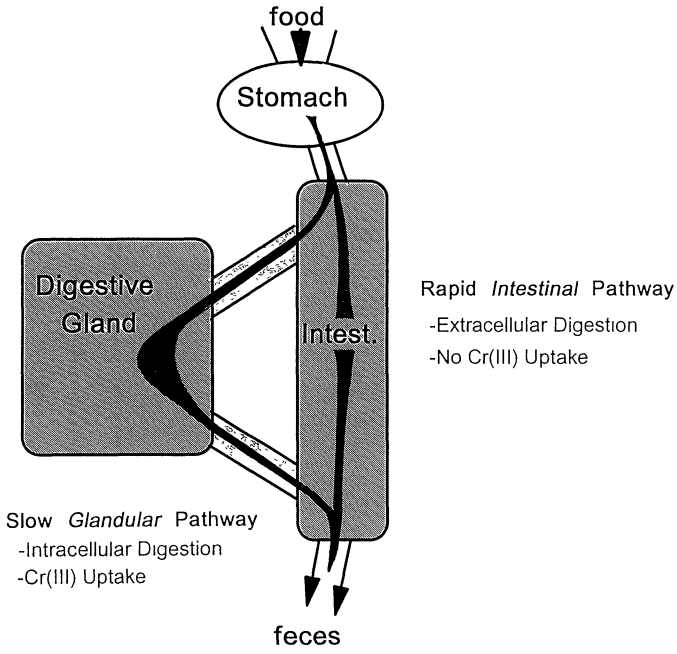

Fig. 1. Two major digestive pathways in bivalves. Bivalves possess a rapid intestinal pathway that utilizes primarily extracellular digestion. This pathway is generally less efficient in assimilating many types of carbon. A selected portion of food material is processed by a second, much slower, glandular pathway, within the digestive gland, which primarily utilizes intracellular digestion.

Table 1. Food processing and assimilation in the bivalves *Macoma balthica* and *Potamocorbula amurensis* fed different types of food particles. Values in the table are explained in text. All values were multiplied by 100 to achieve percent units. Assimilation efficiency—AE; digestive gland—DG; intestine—gut. Overall assimilation efficiency values having the same superscript are not significantly different from each other ($P < 0.05$) by two-way factorial ANOVA (Sokal and Rohlf 1981). (Not determined: nd; not detectable: —.)

Particle	Food processed by DG	^{14}C:^{51}Cr		AE in gut C	C from gut	AE in DG		Overall AE	
		Food	Feces			C	Cr	C	Cr
				M. balthica					
Diatoms	47±26	79	4.1	95±3	50	91	6	92[a]	3[c]
Bacteria (No. 1)	93±3	169	48	73±7	5	97	92	96[a]	86[a]
Bacteria (No. 2)	41±5	0.9	0.6	43±13	24	99	66	69[a]	27[b]
EPS solution	62±19	6.2	0.05	97±1	38	78	73	85[a]	47[b]
EPS sediment	44±5	5.2	0.9	80±10	46	20	78	53[a]	35[b]
Natural sediment	6±7	nd	nd	nd	—	nd	14	nd	1[c]
				P. amurensis					
Diatoms	53±2	79	4.7	94±3	44	91	4	92[a]	2[c]
Bacteria (No. 1)	98±1	169	299	—	0	98	97	96[a]	95[a]
EPS solution	75±13	6.2	0.8	88±6	22	39	54	49[b]	42[b]
EPS sediment	82±14	5.2	2.0	61±20	11	22	36	33[b]	26[b]
Natural sediment	26±19	nd	nd	nd	—	nd	12	nd	3[c]

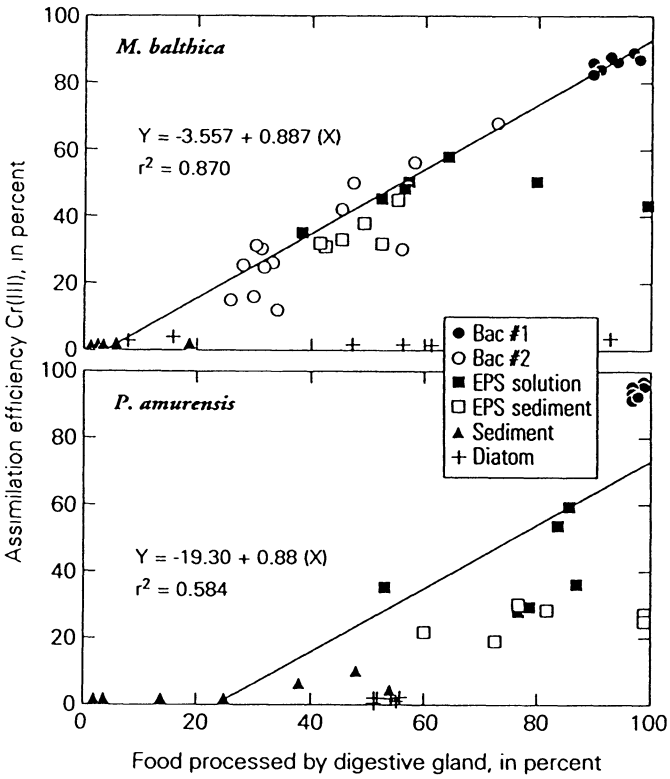

Fig. 2. The relationship of Cr(III) assimilation and percent food processed via the prolonged digestion pathway in individual bivalves (*Potamocorbula amurensis* and *Macoma balthica*). Each data point represents an individual bivalve. Linear regressions for each used arc-sin-transformed data; $P \leq 0.001$ for both bivalves across all particles types, except diatoms.

from diatoms during the early phases of digestion. Only when a high proportion of bacteria was subjected to prolonged digestion was carbon efficiently assimilated. Prolonging digestion was essential for both bivalves to take fullest advantage of the more recalcitrant carbon in bacteria (Table 1).

Assimilation of ^{51}Cr(III) from diatoms and natural sediments was inefficient (Table 1). However, bivalves were highly efficient in assimilating ^{51}Cr(III) from bacteria (Table 1). Both clams also assimilated ^{51}Cr(III) from suspended EPS and EPS-coated sediments.

Assimilation of ^{14}C and ^{51}Cr(III) from sediment, bacteria, and exopolymer was related to the proportion of the particles subjected to prolonged digestion (Table 1, Fig. 2) in both bivalves. Almost all the bacteria ingested by *P. amurensis* were retained in the digestive tract for more than 3 h, and almost all the ^{14}C and ^{51}Cr(III) was assimilated from these bacteria (Table 1). *M. balthica* retained >90% of bacteria through glandular digestion in one experiment and assimilated 96% of ^{14}C and 86% of ^{51}Cr(III). During a second experiment, when *M. balthica* retained only 41% of bacteria in the digestive gland, only 69% of carbon and 27% of Cr(III) was assimilated (Fig. 2, Table 1). Reasons for the observed pliancy in digestive processing in *M. balthica* between the two experiments are not understood. Preliminary studies showed that processing of bacteria by *P. amurensis* was consistently high. Linear regression analysis showed a highly significant relationship between digestive partitioning and the degree of assimilation of Cr(III) from bacteria, exopolymer, and perhaps sediments, in both bivalves (Fig. 2).

Geochemical form was also important in assimilation (Fig. 2). Even when subjected to prolonged digestion, ^{51}Cr(III) was not assimilated from diatoms. This food source, therefore, was excluded from the regression equation. Earlier studies showed low assimilation of Cr(III) associated with Si surfaces typical of diatoms (Decho and Luoma 1994). The same studies showed little assimilation of Cr(III) from iron oxides and humic substances; thus, the low assimilation from mudflat sediments also may have been influenced by geochemical factors.

A second set of feeding experiments was conducted to determine whether bivalves are capable of modifying the digestive processing of food to reduce exposure to high, biologically available Cr concentrations. These experiments examined specifically the bivalve *P. amurensis*; bacterial cells were used as a food source for biologically available Cr. These choices were based on the above results, which showed that *P. amurensis* processed most (>95%) ingested bacterial cells with the prolonged digestion pathway, and this resulted in a highly efficient uptake of Cr(III) from bacteria (Table 1).

Bivalves were initially acclimated to different concentrations of bacterial Cr as food. A 6-d pre-exposure period was used in case adaptation was necessary for any food processing response. This pre-exposure consisted of feeding the bivalves 3 times d^{-} (for 6 d) with one of three treatments consisting of natural food sources and either bacteria spiked with high concentrations of Cr(III) (nominal concentration = 200–500 μg^{-1}), bacteria spiked with moderate concentrations of Cr(III) (nominal concentration = 2–5 μg^{-1}), or bacteria with no added Cr(III). We used 27 individuals in each treatment. Each treatment was separated into two identical exposure chambers containing 13 or 14 individuals. No statistical differences were observed between replicate exposure chambers within each treatment. Thus, differences between treatments were ascribed to Cr(III) pre-exposure effects.

At the conclusion of the 6-d pre-exposure, pulse-chase feeding experiments were conducted to access differences in assimilation efficiencies resulting from the metal pre-exposures. Experiments were conducted by methods similar to those described above, except that bivalves were placed in microcosms containing no added Cr(III), and then fed a short pulse (20 min) of labeled food [^{14}C- and ^{51}Cr(III)-labeled bacteria at identical concentrations and activities]. Egestion and retention of labeled material was followed while animals were fed unlabeled flocculent sediment from San Francisco Bay.

One way in which *P. amurensis* avoids Cr(III) is by reducing ingestion rates. The mean ingestion rate of bacterial particles was significantly ($P < 0.01$) reduced for bacterial particles with both the moderate and highest Cr(III) concentrations and the variability of ingestion rate increased (Fig. 3A). The C.V. for mean ingestion rate was

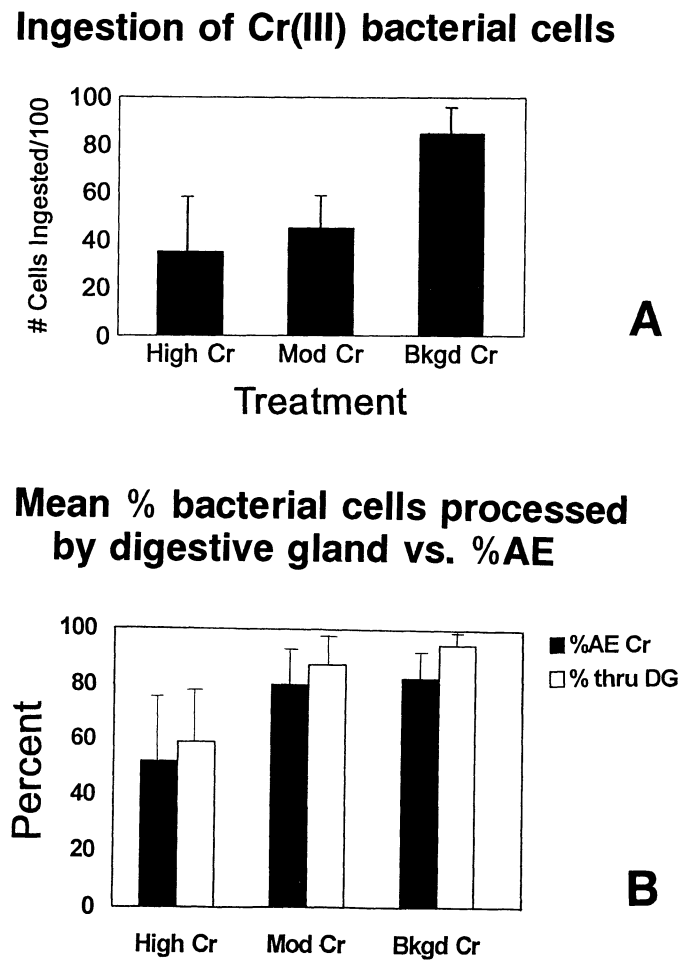

Fig. 3. Mean ingestion (A) and digestive processing (B) of Cr-III bacterial cells by *Potamocorbula amurensis*. Ingestion rates represent mean number of cells ingested per 100 cells present as food. Percent of ingested bacterial cells processed by the digestive gland—% thru DG; mean percent absorption efficiencies of Cr(III) from bacterial cells for each treatment—%AE Cr. Vertical bars—standard error of mean.

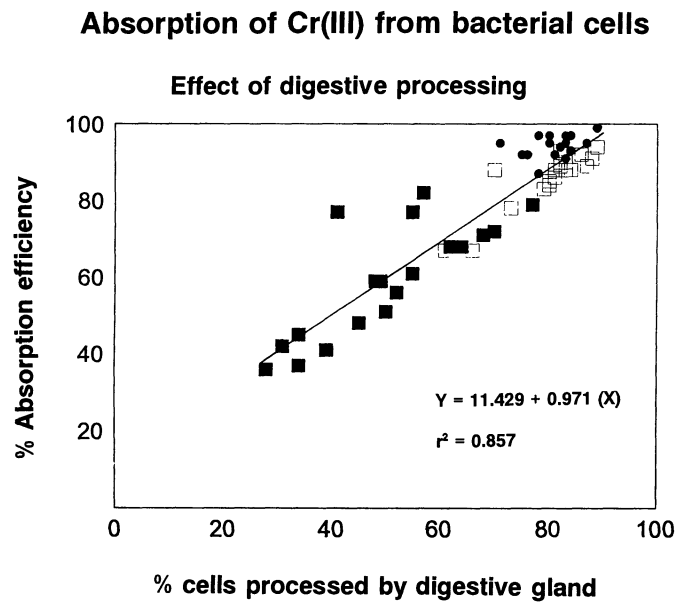

Fig. 4. Relationship of digestive partitioning and assimilation efficiency in *Potamocorbula amurensis* during pulse-chase experiments. Bivalves were fed natural food sources plus bacteria amended with three different concentrations of Cr(III) for 6 d immediately before pulse-chase experiments. Percent absorption efficiencies (%AE) of individual bivalves are plotted against the amount of bacterial food processed by glandular digestion. Bacterial cells were loaded with three concentrations of Cr(III): ■—high Cr loading; □—moderate Cr loading; ●—no-added Cr. Linear regression was significant ($P \leq 0.001$) and used arc-sin-transformed data.

31% for no Cr(III), 29% for moderate Cr(III), and increased to 62% for high Cr(III). Reducing the ingestion rates of contaminated particles may represent a first response in reducing the uptake of potentially toxic compounds, since this response occurred at moderate Cr(III) concentrations.

A second way to reduce Cr(III) exposure is in the digestive processing of food particles. The concentration of Cr(III) in bacteria (a bioavailable form) affected retention time of bacteria in the digestive system. There was a significant decrease in the mean proportion of bacteria processed by glandular digestion at the highest Cr(III) concentration (Fig. 3B). A corresponding change in the overall assimilation of Cr(III) occurred, declining from 81±4 to 51±14%. Assimilation efficiency of bacterial Cr(III) within the digestive gland itself remained the same whatever the contamination (86, 93, and 86% in the different treatments); thus, the decrease in overall assimilation at the highest Cr(III) concentrations resulted from a change in digestive processing of Cr(III) bacteria by the animal. Linear regression of individual bivalves showed a significant ($P < 0.001$) dependence of %AE of Cr(III) on digestive processing (Fig. 4).

Digestive processing is a remarkably pliant process in *M. balthica* and *P. amurensis* and that pliancy affects assimilation of metals and carbon. Both bivalves can maximize nutrient gain from recalcitrant particles (like bacteria) by prolonging digestion. The nutritional benefits of prolonged digestion are balanced by costs that include increased assimilation of at least some types of potential toxins. *P. amurensis* reduces its reliance on prolonged digestion as a direct response to high concentrations of a toxin, bioavailable Cr(III), in food. Such a mechanism would seem to have evolved only if exposure to natural toxins was a factor exerting selective pressure during the evolution of this digestive strategy.

The flexible digestive strategy demonstrated by these bivalves could influence how the effects of modern metal pollution are manifested. The flexibility represents a cost-benefit that the animal can regulate in response to its environment. Differing capabilities of organisms to avoid contaminated food could determine species survival in a polluted environment. Indirect nutritional deficits are a possible secondary effect of ingesting contaminated food. If contamination affects selection of food, the community

structure of species that are food sources could be affected. Such subtle, but ecologically important, responses to modern pollutants could be the result of mechanisms developed in the evolution of feeding strategies in detritus-feeding organisms.

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