

Differential uptake of dissolved and particulate organic carbon by the marine mussel *Perna viridis*

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Abstract

We used radiotracers in laboratory experiments to determine the organic carbon uptake by the marine mussel *Perna viridis* from different diets (phytoplankton and detritus) and from the dissolved phase (colloidal and low molecular weight organic carbon). Standard compounds (glucose, arginine, and leucine, and the carbohydrate macromolecular dextran with sizes ranging from 3–2,000 kDa) were used to study organic matter flux into the green mussels. Carbon absorption was significantly affected by the food type and quality. The highest absorption efficiency (AE, 80%) was from the diatom *Thalassiosira pseudonana*, while the AE from *Chlorella autotrophica* was comparable with those from three types of algal detritus (20–30%). Mussels were able to directly ingest macromolecular dextran colloidal materials. Significant accumulation of these compounds was found both in the gills and the digestive glands of the mussels within the 12–17-h exposure period. Similarly, a significant accumulation of biogenic colloidal organic carbon (COC) and low molecular weight ultrafiltered organic carbon (UOC) was found. Uptake of UOC and COC resulting from the decomposition of different algal sources was comparable, with an uptake rate constant of $6.08 \times 10^{-4} \text{ L g}^{-1} \text{ dry wt h}^{-1}$ and an absorption efficiency of 0.0053%. A kinetic model was subsequently applied to quantitatively evaluate the carbon contribution from dissolved and particulate sources to general organic carbon uptake by the mussels. Mussels accumulated organic carbon predominantly from the particulate phase, with very little (<0.2%) coming from the DOC. However, the apparent ingestion and accumulation of colloids suggest that they can actively participate in the food chain dynamics in marine systems.

For bivalve mollusks, both dissolved organic and particulate organic sources can contribute to their carbon acquisition. Accumulation of particulate organic constituents from such sources as living particles (phytoplankton, bacteria, and heterotrophic protists) and nonliving particles (detritus and seston) occurs by filtration, ingestion, and absorption, and is controlled by the absorption efficiency (AE), the carbon content of the ingested particles, and the ingestion rate of the bivalves (Dame 1993, 1996; Wang and Fisher 1996). Absorption from the food source is affected by food characteristics, such as food quality and quantity, and represents a first-order physiological process to be contrasted among different food types (Wang and Fisher 1996).

Dissolved organic matter (DOC), defined as the fraction passing a filter of 0.22- μm pore size, is the largest organic carbon reservoir in seawater, playing an important role in the marine carbon cycle and other biogeochemical processes in the ocean. The bulk DOC pool is heterogeneous in size, or molecular weight, and may originate from phytoplankton and zooplankton processes (Münster 1993). The labile organic carbon pool represents 10–30% of the bulk DOC in the marine environment and mainly consists of amino acids and carbohydrates (Münster 1993). The role of DOC as an essential nutrient source for heterotrophic bacteria and protists has been well recognized (Hill and Wheeler 2002), but

its importance to marine bivalves or other suspension-feeding invertebrates is less well documented. In contrast with the carbon absorption efficiency from their diets, there is essentially no reported value for the carbon absorption efficiency from the DOC pool for these animals. Roditi et al. (2000) found that the DOC in freshwaters contributed up to 50% of the carbon demand of the zebra mussel, *Dreissena polymorpha*.

Marine bivalves, especially the suspension-feeding mussels, pump a large amount of water through their mantle cavity due to their large gill surface areas. They can play an important role in carbon cycling because of their dense assemblages in many coastal ecosystems (Dame 1993, 1996). Their suspension-feeding behavior exposes these animals to large volumes of seawater containing DOC, which can be released as photosynthate or by autolysis of senescent algae (Sundh 1992), by the grazing activity of zooplankton (Nagata and Kirchman 1991), or by the solubilization of organic particles and virus-induced lysis of algae and bacteria (Fuhrman 1987). Earlier studies have demonstrated that dissolved free amino acids can serve as an important nutritional supplement for the marine bivalves *Mytilus edulis* and *Modiolus modiolus*, and the net accumulation by carrier-mediated transport occurred at a relatively low concentration (1 $\mu\text{mol L}^{-1}$) (Wright et al. 1975; Wright and Manahan 1989).

Moreover, the bivalves may potentially ingest components of the colloidal fraction of DOC (Tack and Polk 1995). Sub-micron colloidal organic carbon (COC, 1 kDa - 0.22 μm) is abundant in natural waters and acts as the intermediary between low molecular weight ultrafiltered organic carbon (UOC) and particulate organic carbon (POC) (Guo and Santschi 1997a; Pettine et al. 2001). Tranvik et al. (1993)

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demonstrated the importance of colloidal particles (55–2,000 kDa) to marine heterotrophic flagellates through the active ingestion of fluorescein isothiocyanate-labeled carbohydrates (dextran) and proteins at substrate concentrations of 1–10 mg L⁻¹. Although very little is known of COC as a nutrient source for marine bivalves, a few recent studies have shown that colloidal metals were bioavailable to these species (Wang and Guo 2000; Pan and Wang 2002, 2004).

The green mussel, *P. viridis*, is widely distributed in tropical and subtropical coastal systems. This study employed a radiotracer technique to quantify their carbon absorption from different food types (phytoplankton and phytodetritus) and the influx of different organic compounds from the dissolved phase into the mussels. Arginine, which is a hydrophobic neutral acid and can contribute up to 9% of the total dissolved amino acid pool in seawater, together with leucine and glucose, which may present a potential supplemental source of nutrition to the mussels (Wright et al. 1975; Wright and Manahan 1989), were chosen as the tested substrates. Furthermore, a variety of high molecular weight (HMW) carbohydrate macromolecules (dextran conjugates, 3–2,000 kDa) were used to explore the direct ingestion of colloidal particles by this bivalve. Dextran, a group of hydrophilic polysaccharides synthesized by bacteria, are characterized by their high molecular weight, good water solubility, low toxicity, and relative inertness (e.g., less digestible). In marine environments, high molecular weight polysaccharides can form an important fraction of DOC and COC (Pakulski and Benner 1994; Santschi et al. 1998). Recent studies have demonstrated that polysaccharides can aggregate and represent an important pathway for the formation of transparent exopolymeric particles (Engel et al. 2004). These organic aggregates may then be a potential source of nutrition for suspension-feeding bivalves in nearshore regions rich in natural seston (Alber and Valiela 1996). Thus, a food web pathway may exist from the primary producers to DOC to organic aggregates to metazoan consumers (Alber and Valiela 1994). Finally, following these experimental determinations, we then attempted to predict the relative importance of DOC and POC in the overall carbon uptake by the mussels. The results will have implications to our understanding of the vital role of COC and DOC in the nutrition of marine invertebrates, as well as their potential roles in the carbon cycles in coastal ecosystems.

Materials and methods

Organisms and chemicals—Green mussels, *Perna viridis*, with a shell length of 3.0–3.5 cm, were collected from Wu Kai Sha, Tolo Harbor, Hong Kong. Following the removal of epibionts on the shells, mussels were acclimated in the laboratory for 1–2 weeks in seawater with a salinity of 30 prior to the experiments. During the acclimation period, they were continuously fed with the diatom *Thalassiosira pseudonana* (clone 3H) with a ration of 2% of their tissue dry weight per day. Before experimental exposure, individual mussels were starved overnight in ultrafiltered seawater (<1 kDa, collected by cross-flow ultrafiltration as described in Pan and Wang 2002), to avoid any production of feces during the exposure period.

Radioisotope-labeled glucose (D-[¹⁴C(U)]-), leucine (L-[¹⁴C(U)]-), arginine (L-[¹⁴C(U)]-), and amino acid mixtures (L-[¹⁴C(U)]-) (Perkin Elmer Life Sciences) were used to examine the flux of these standard compounds into the mussels. Fluorescein-labeled high molecular weight carbohydrate macromolecules (dextran), with a range of molecular weights from 3–2,000 kDa (Molecular Probes), were used to investigate colloidal particle ingestion by the mussels. Radioisotope ¹⁴C (NaH¹⁴CO₃) was used to radiolabel the algae for measurements of carbon absorption efficiency from the food particles and carbon absorption efficiency from the DOC.

Direct ingestion of modeled dextran compounds by the mussels—This experiment specifically tested whether the mussels were able to ingest compounds of different sizes. The mussels were exposed to dextrans with the molecular weights or sizes of 3, 10, 40, 500, or 2,000 kDa for 2 h in the ultrafiltered seawater (1 liter) at concentrations ranging from 0.22–2.4 mg L⁻¹. For each treatment, we had four replicate mussels. In the control treatment, ultrafiltered seawater containing the corresponding size and concentration of dextrans, but without the presence of mussels, was monitored for any changes in fluorescence during the exposure period. After 2 h of exposure, the mussels were removed and placed in clean filtered seawater for 5 min to remove any absorbed compounds. The gills and digestive glands were then dissected with a stainless-steel knife and were weighed, treated with 1 mol L⁻¹ Suprapur NaOH and placed in a shaker overnight. The solubilized soft tissues were centrifuged for 10 min at 3,000 × g. The fluorescence of the supernatants was determined by a CytoFluor™ II microplate fluorescence reader (Biosearch) at absorption/emission maxima of 490/520 nm. In addition, five mussels without fluorescent particle exposure were dissected, and the fluorescence values in the blanked gills and digestive glands were similarly quantified.

In another experiment, green mussels were exposed to a medium containing 1 mg L⁻¹ of dextran particles of different sizes for 12–17 h. At different time intervals, six individuals were removed and their fluorescence readings were measured as described above. The exposure medium was also sampled for fluorescence measurement.

Preparation of radiolabeled algae, detritus, DOC, and COC—All the seawater used for the labeling and decomposition experiments was autoclaved. Phytoplankton, *T. pseudonana* (CCMP 1335), *Prorocentrum minimum* (CCMP 696), and *Chlorella autotrophica* (CCMP 243), in their early stationary phases were gently filtered onto a 1- or 3- μ m polymembrane, rinsed with 0.22 μ m filtered seawater, then separately resuspended into 0.22 μ m filtered seawater enriched with N, P, Si, vitamins and trace metals at f/2 levels (Guillard and Ryther 1962) and spiked with ¹⁴C at 925 kBq L⁻¹ (as NaH¹⁴CO₃). The macroalga *Ulva lactuca*, collected from a fish farm in Sai Kung, Hong Kong, was carefully cleaned and cut into pieces (dry weight 30–50 mg), then suspended in 0.22 μ m filtered seawater containing the f/2 levels of macronutrients and ¹⁴C at 925 kBq L⁻¹ (as NaH¹⁴CO₃). After 6 days of radiolabeling (i.e., at least four divisions for all algal species and thus uniform radiolabel-

ing), the microalgae were collected by filtering onto a 1- or 3- μm polycarbonate membrane and rinsed with nonradioactive seawater. Whether the *Ulva* was uniformly radiolabeled was not known from this study, although significant growth was evident over the 6 days of the radiolabeling period. A portion of the resuspended radiolabeled algae was used for the absorption experiments (described following), and the remaining radiolabeled algae (except *C. autotrophica*) were resuspended into 0.22 μm seawater to decompose for 1 month under dark conditions to produce radiolabeled phytodetritus and DOC or COC. The radiolabeled macroalga *U. lactuca* was ground and then placed in 0.22 μm seawater to decompose for 1 month to produce the radiolabeled detritus. Whether the detrital particles contained any bacteria was not quantified. The pH of the detritus-producing medium was monitored during the decomposition period, and was adjusted to 8.20 using 0.5 N Suprapur NaOH when necessary. After the decomposition, the detritus was filtered onto a nylon mesh and the size fraction between 0.22 μm and 40 μm was used for the absorption experiments. The filtrates were then ultrafiltered to yield the UOC or COC (as follows).

Absorption from living phytoplankton and nonliving detritus—Carbon AE was measured by the mass balance method using ^{14}C as a radiotracer (Wang and Fisher 1996). The radiolabeled living (*C. autotrophica* and *T. pseudonana*) and decomposed microalgal cells (*T. pseudonana* and *P. minimum*) and macroalga *U. lactuca* were pulse fed to the green mussels at 0.4 mg dry wt L^{-1} biomass for 30 min. Food was added at 10-min intervals to maintain a constant food biomass in the feeding suspension. The mussels were subsequently depurated of their ingested radiolabeled food in 0.22- μm filtered seawater for 25 h, during which the water was changed every 6 h, and the mussels were fed with the unradiolabeled diatom *T. pseudonana* at a comparable cell biomass (0.4 mg L^{-1}). There were eight replicate individuals for each treatment. Fecal pellets were collected immediately after the feeding and at 1–4-h intervals during the depuration period. After 25 h of depuration, the mussels were dissected and homogenized with 1 ml Solvable after wet-weight measurements. Following the tissue solubilization, aliquots of dissolved soft tissue were mixed with cocktail. The ^{14}C radioactivity was analyzed after 12 h (described below). The carbon AE was estimated using the mass balance method by the following equation:

$$\text{AE (\%)} = ({}^{14}\text{C}_{\text{food}} - {}^{14}\text{C}_{\text{feces}}) / {}^{14}\text{C}_{\text{food}} \times 100 \quad (1)$$

where ${}^{14}\text{C}_{\text{food}}$ is the total radioactivity ingested by the mussels, calculated as the sum of radioactivity in the cumulative feces as well as the radioactivity in the mussel's tissues after 25 h of depuration, and ${}^{14}\text{C}_{\text{feces}}$ is the radioactivity in the cumulative feces.

Another experiment also compared the carbon AEs determined by the mass balance method and the $^{14}\text{C} : {}^{51}\text{Cr}$ dual tracer ratio method (Wang and Fisher 1996). The diatom *T. pseudonana* was dual-radiolabeled with ^{14}C at 925 kBq L^{-1} (as $\text{NaH}^{14}\text{CO}_3$) and ${}^{51}\text{Cr}$ at 92.5 kBq L^{-1} (in 0.1 mol L^{-1} HCl, corresponding to 0.55 nmol L^{-1}) and fed to the mussels at a biomass of 0.4 mg L^{-1} . The same pulse-chase feeding

was employed for 30 min, and the mussels were depurated in 0.22 μm seawater for 25 h. The absorption was estimated using both the ratio method and the mass balance method. In the ratio method, the carbon AE was calculated by the following equation (Calow and Fletcher 1972):

$$\text{AE (\%)} = [1 - ({}^{14}\text{C}/{}^{51}\text{Cr})_{\text{feces}} / ({}^{14}\text{C}/{}^{51}\text{Cr})_{\text{food}}] \times 100 \quad (2)$$

where $({}^{14}\text{C}/{}^{51}\text{Cr})_{\text{feces}}$ is the ratio of the radioactivity of ^{14}C to ${}^{51}\text{Cr}$ in the cumulative feces and $({}^{14}\text{C}/{}^{51}\text{Cr})_{\text{food}}$ is the ratio of the radioactivity of ^{14}C to ${}^{51}\text{Cr}$ in the food.

The clearance rate of mussels feeding on the diatom *T. pseudonana* were measured using the methods described by Wang and Dei (1999). Briefly, individual mussels were placed in 1.5 liters of filtered seawater containing 20,000 cells ml^{-1} of diatoms. Any decrease of cell concentration due to mussel's feeding was quantified by a Coulter Counter over a period of 40 min.

Uptake of dissolved organic matter by the mussels—The time series of organic compound uptake by the mussels was first determined. Glucose ($\text{D}-[{}^{14}\text{C}(\text{U})]$) was used as one model compound in these carbohydrate uptake experiments. The radioisotope was spiked into 800 ml of 0.22- μm filtered seawater (in a 1-liter beaker) at 0.04 $\mu\text{mol L}^{-1}$, and the green mussels were individually exposed to this solution to examine whether uptake proceeded linearly. The total duration of exposure was 1 h. At each 15-min interval, four individual mussels were removed and their soft tissues were dissected. The ^{14}C radioactivity in the soft tissues was quantified. Uptake of glucose was expressed as the concentration factor (L kg^{-1} wet wt), calculated as the radioactivity per tissue wet weight to the radioactivity in the exposure medium.

In a second experiment, the uptake of radiolabeled glucose and amino acids (arginine and leucine) by the green mussels were examined at 6–7 different ambient concentrations ranging from 0.4 to 100–200 $\mu\text{mol L}^{-1}$. The total concentration included both the radioisotopes and the non-radiolabeled chemicals. The spiked radioactivity of the radiolabeled chemicals for all treatments was 22.2 kBq L^{-1} . For the amino acid mixture, only the radiolabeled chemicals were used in the experiment at a corresponding concentration of 0.4 $\mu\text{mol L}^{-1}$ as a reference control. Mussels (four replicates) were individually placed in 800 ml of filtered seawater (in 1-liter beaker). At the end of the 1-h exposure, the mussel soft tissues were dissected and weighed, then homogenized and analyzed for ^{14}C radioactivity.

Uptake of organic matter (arginine, leucine, and glucose) by the mussels was analyzed using the Michaelis–Menten kinetic equation:

$$J = J_{\text{max}}[S] / (K_m + [S]) \quad (3)$$

where J is the flux or rate of transport of solute from a concentration $[S]$, J_{max} is the maximal rate of transport, and K_m is the Michaelis constant, defined as the solute concentration at which transport is half maximal.

In a third experiment, green mussel uptake of UOC (<1 kDa) and COC (≤ 1 kDa–0.22 μm) that resulted from decomposing *T. pseudonana*, *U. lactuca*, and *P. minimum* was quantified. The 1-month decomposed medium was ultrafiltered using an Amicon stirred cell with a 1-kDa ultrafiltra-

Table 1. Biogenic organic carbon concentrations ($\mu\text{mol L}^{-1}$) obtained by 1-month decomposition of the algae *Thalassiosira pseudonana*, *Prorocentrum minimum*, and *Ulva lactuca*. UOC: ultrafiltered organic carbon (<1 kDa). COC: colloidal organic carbon (1 kDa–0.22 μm).

Organic carbon ($\mu\text{mol L}^{-1}$)	<i>T. pseudonana</i>	<i>U. lactuca</i>	<i>P. minimum</i>
UOC	184	510	409
COC	122	1120	395

tion membrane at a N_2 pressure of 30 Pascals, using the concentration factor of 20, i.e., 100 ml of retentate (the colloidal fraction of the decomposed medium, which had excluded any bacterial contamination) was collected from 2 liters of the decomposition medium. The permeate from the ultrafiltration was the UOC fraction (<1 kDa). During the uptake experiments, the radioactive colloids and UOC were suspended into nonradioactive UOC seawater at a dilution factor of 15, and the mussels were subsequently exposed to the medium for 1 h. The experimental DOC concentrations used in the experiment for each treatment are shown in Table 1. At the end of exposure, the mussels were rinsed and dissected for ^{14}C radioactivity measurements as described above.

Modeling carbon acquisition by the mussels from different fractions—The overall influx (I_c) of organic carbon into the mussels can be described by the following equation, assuming that influx from each source is additive:

$$I_c = I_{\text{DOC}} + I_{\text{POC}} \quad (4)$$

where, the influx of carbon from the particulate source (I_{POC}) is a function of carbon AE, carbon concentration in this fraction (C_{POC} , $\mu\text{mol L}^{-1}$), and the clearance rate of the mussels (CR, $\text{L g}^{-1} \text{h}^{-1}$) (Wang and Fisher 1996). The carbon influx from the dissolved phase (I_{DOC}) can be calculated as the uptake rate constant (k_u , $\text{L g}^{-1} \text{h}^{-1}$, calculated from the slope of the linear regression of the influx rate against the DOC concentration; see below) times the DOC concentration (C_{DOC} , $\mu\text{mol L}^{-1}$). Thus,

$$I_{\text{DOC}} = k_u \times C_{\text{DOC}} = k_u \times C_{\text{TOC}} \times \% \text{DOC} \quad (5)$$

$$\begin{aligned} I_{\text{POC}} &= \text{CR} \times \text{AE} \times C_{\text{POC}} \\ &= \text{CR} \times \text{AE} \times C_{\text{TOC}} \times \% \text{POC} \end{aligned} \quad (6)$$

where, %DOC is the fraction of total organic carbon (TOC) as DOC, and %POC is the fraction of TOC as POC. Thus, the fraction of DOC uptake (f_{DOC}) can be calculated with the following equation:

$$f_{\text{DOC}} = \frac{I_{\text{DOC}}}{I_c} = \frac{k_u \times \% \text{DOC}}{k_u \times \% \text{DOC} + \text{CR} \times \text{AE} \times \% \text{POC}} \quad (7)$$

Analytical and radioactivity measurements—The DOC concentration was quantified using a Shimadzu TOC-5000A total organic carbon analyzer using the high temperature catalytic combustion (HTC) method (Guo et al. 1994). To mea-

sure the ^{14}C radioactivity, the mussels were dissected and the soft tissues were homogenized with 1 ml Solvable™ (Packard). After 12 h, 0.5-ml aliquots of the solubilized tissues were added to 5 ml of cocktail (Fisher Chemicals). After another overnight equilibration, the radioactivity of ^{14}C was measured using a Beckman LS6500 multipurpose scintillation counter by the external standard ratio method. The counting times were adjusted to yield a propagated counting error of less than 5%. The ^{51}Cr radioactivity of the samples was measured using a gamma counter at 230 keV. In this specific experiment, where both ^{14}C and ^{51}Cr had been simultaneously spiked, the spillover of ^{51}Cr onto ^{14}C counting was calibrated by running a standard curve for ^{51}Cr in the scintillation counter.

Results

Ingestion of fluorescein dextran nanoparticles—In the control treatment (containing the corresponding size and concentration of dextrans but without the presence of mussels), no change in fluorescence during the exposure period was observed, suggesting that adsorption of fluorescent dextrans onto the beakers was negligible. The carbohydrate fluorescent dextrans (3–2,000 kDa) were all effectively accumulated by the mussels over the 12–17-h exposure period (Fig. 1). Significantly higher fluorescence readings from dextran nanoparticles were found in both the mussel's gills and digestive glands than those in the control mussels without dextran exposure, implying that the mussels were able to accumulate the compounds. Generally, the accumulation of dextrans in the gills and digestive glands increased significantly over the exposure time. In a few cases, the relationships were not statistically significant. The regressed slopes for the digestive glands were higher than those for the gills, suggesting that the dextrans were accumulated in the digestive glands at a faster rate than in the gills. However, there was no consistent pattern of the calculated slopes among different sizes of dextrans.

In experiments with different dextran concentrations (0.2–2 mg L^{-1} or 2.4 mg L^{-1} for 500 kDa), dextrans (3–2,000 kDa) showed positive linear patterns of accumulation in the mussel's gills and digestive glands (Fig. 2). In this experiment, the slopes of the linear regression between the fluorescence accumulation and the dextran concentration were comparable between the digestive glands and the gills, and there was no consistent pattern among the different sizes of dextran.

Carbon absorption in green mussels—The clearance rate of the mussels ranged from 8.8–14.1 $\text{L g}^{-1} \text{dry wt h}^{-1}$, with a mean of $11.5 \pm 1.5 \text{ L g}^{-1} \text{h}^{-1}$. After the ingestion of radiolabeled living algae or phytodetritus, the highest carbon egestion rate was recorded after 2 h of depuration, and >70% of the unassimilated diets was egested within 6 h (Fig. 3). There was very little loss of ^{14}C by the mussels after 12 h of depuration. The proportion of ingested ^{14}C and ^{51}Cr retained in the mussels following ingestion of different types of diets is shown in Fig. 4. After 25 h of depuration, only 3.4% of the Cr was retained by the mussels, indicating that it was essentially inert to the animals. The carbon AE

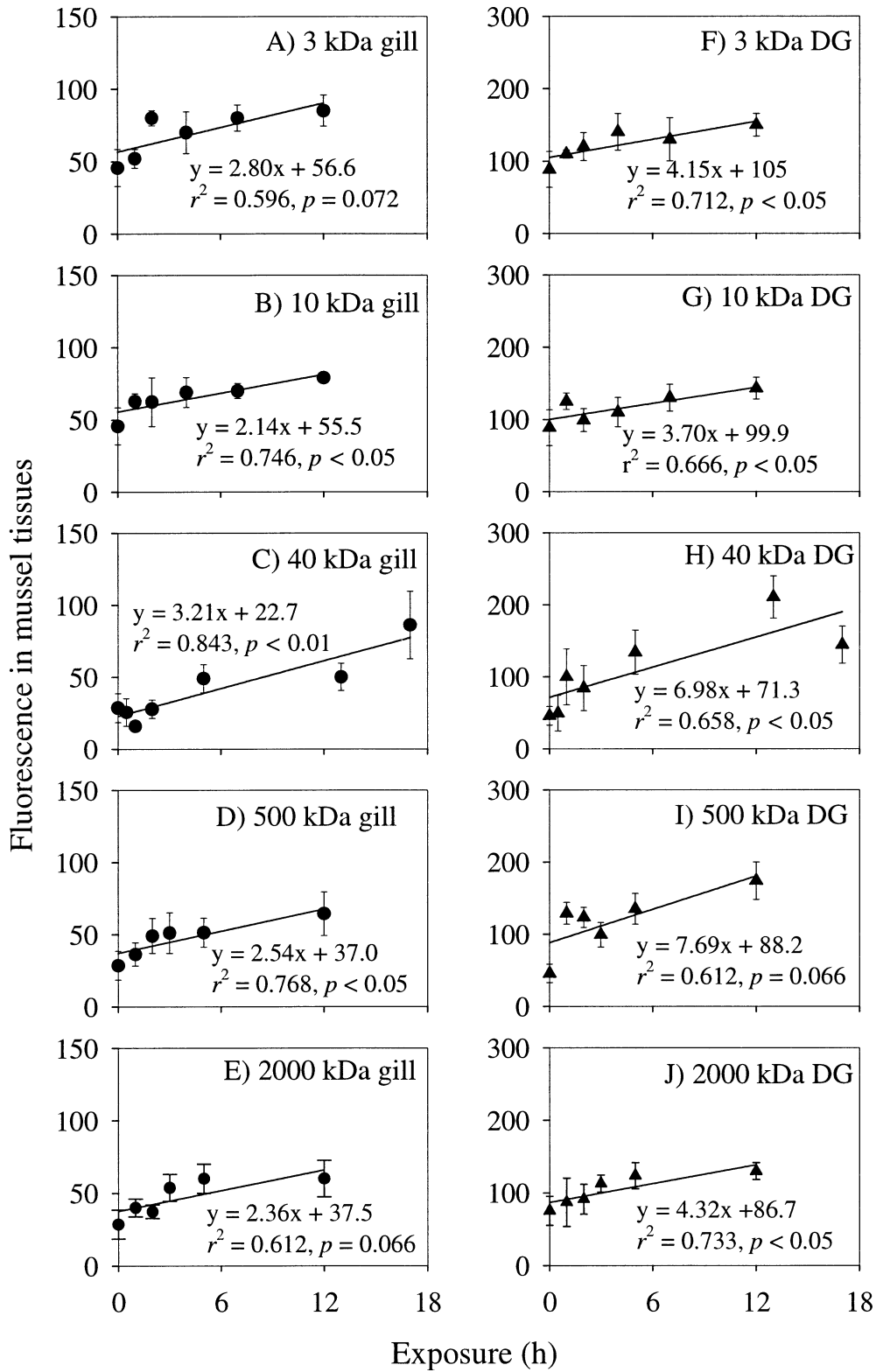


Fig. 1. Uptake of different sized fluorescent dextran compounds by the green mussel *Perna viridis* over time. Mean \pm SD ($n = 6$). The accumulation in (A–E) the gills and (F–G) in the digestive gland (DG) were quantified.

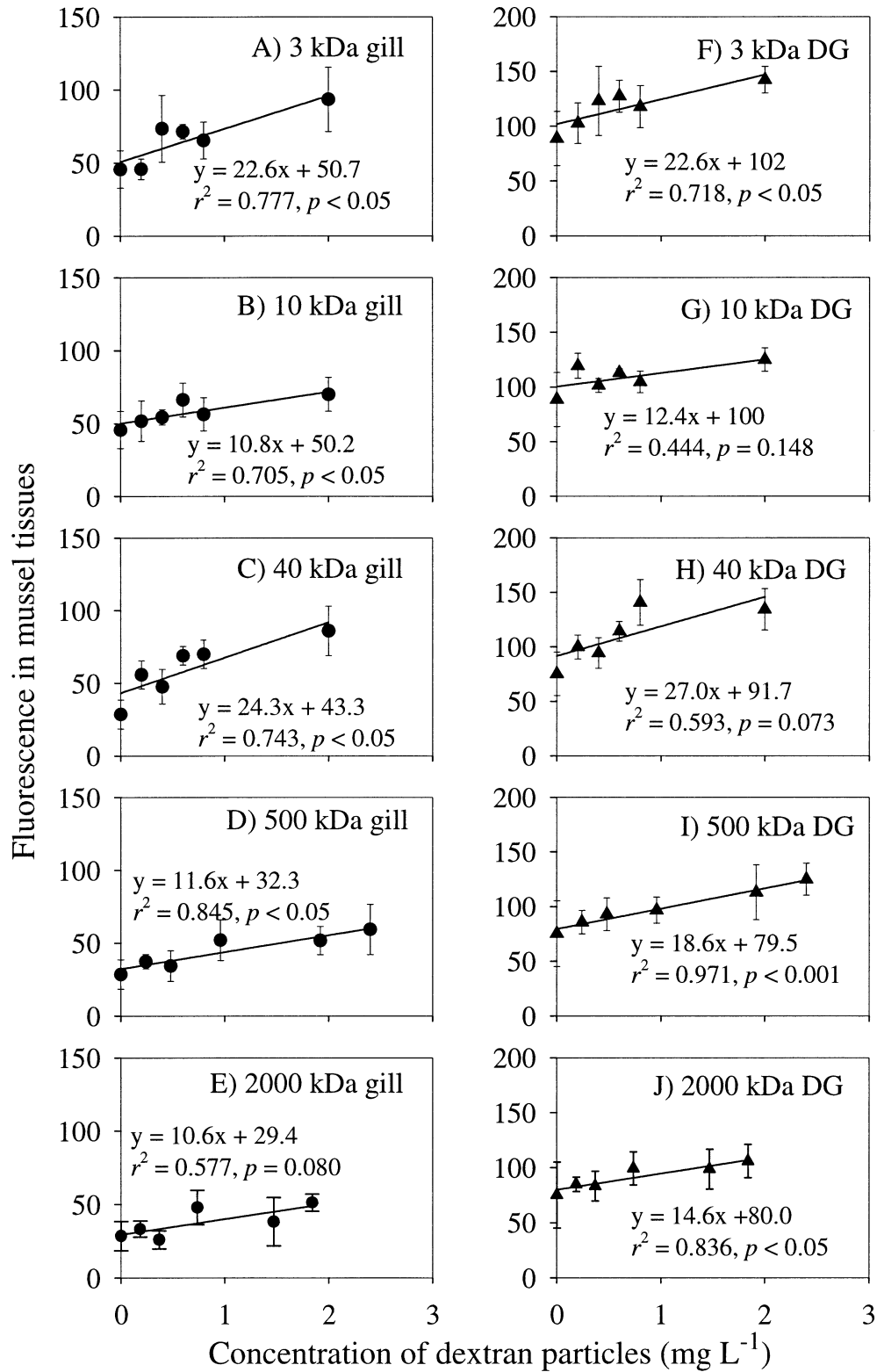


Fig. 2. Uptake of different sized fluorescent dextran compounds by the green mussel *Perna viridis* at different concentrations. Mean \pm SD ($n = 4$). The accumulation in (A–E) the gills and (F–G) in the digestive gland (DG) were quantified.

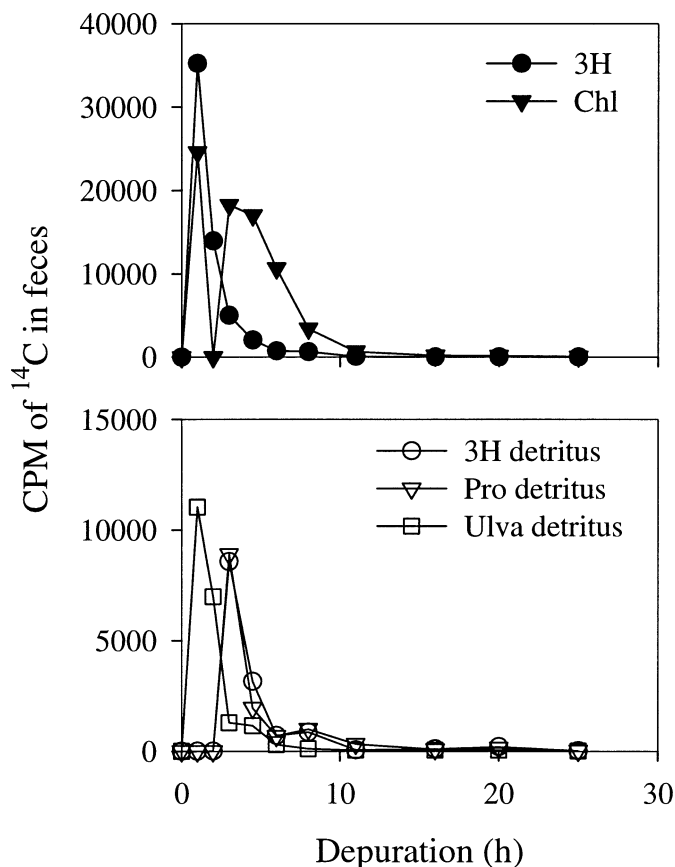


Fig. 3. Instantaneous egestion of ^{14}C from the green mussel *Perna viridis* following a pulse ingestion of radiolabeled food particles. Mean \pm SD ($n = 8$). 3H: *Thalassiosira pseudonana*, Chl: *Chlorella autotrophica*, 3H detritus: dead cells of *Thalassiosira pseudonana*, Pro detritus: dead cells of *Prorocentrum minimum*, *Ulva* detritus: detritus produced by decomposition of *Ulva lactuca*.

was calculated by the mass balance method and defined as the fraction retained in the mussel's soft tissues after 25 h of depuration. Mussels had the highest carbon AE for the living diatom *T. pseudonana* ($68.8\% \pm 3.7\%$ SD) among the five types of food tested. The AEs for the green algae *C. autotrophica* ($20.7\% \pm 1.5\%$), detrital diatoms ($19.5\% \pm 7.8\%$), detrital dinoflagellates ($30.4\% \pm 8.2\%$), and detrital *Ulva* ($25.3\% \pm 7.5\%$) were comparable. Furthermore, the carbon AEs estimated using the ratio method ($73.0\% \pm 6.3\%$) were comparable with that estimated using the mass balance method ($68.8\% \pm 3.7\%$).

Amino acid and organic carbon uptake by the mussels—In the first experiment, glucose uptake by the green mussels, calculated as the concentration factor, increased linearly during the 1-h exposure period (Fig. 5). The uptake rate constant derived by regressing the concentration factor against the exposure time was $4.46 \times 10^{-4} \text{ L g}^{-1} \text{ wet wt min}^{-1}$. The uptake rate of glucose at this concentration ($0.04 \mu\text{mol L}^{-1}$) was $1.070 \text{ nmol g}^{-1} \text{ wet wt h}^{-1}$.

Transport of amino acids (arginine and leucine) and glucose into the mussels can be modeled using the Michaelis-Menten saturation kinetic equation (Fig. 6). At the same con-

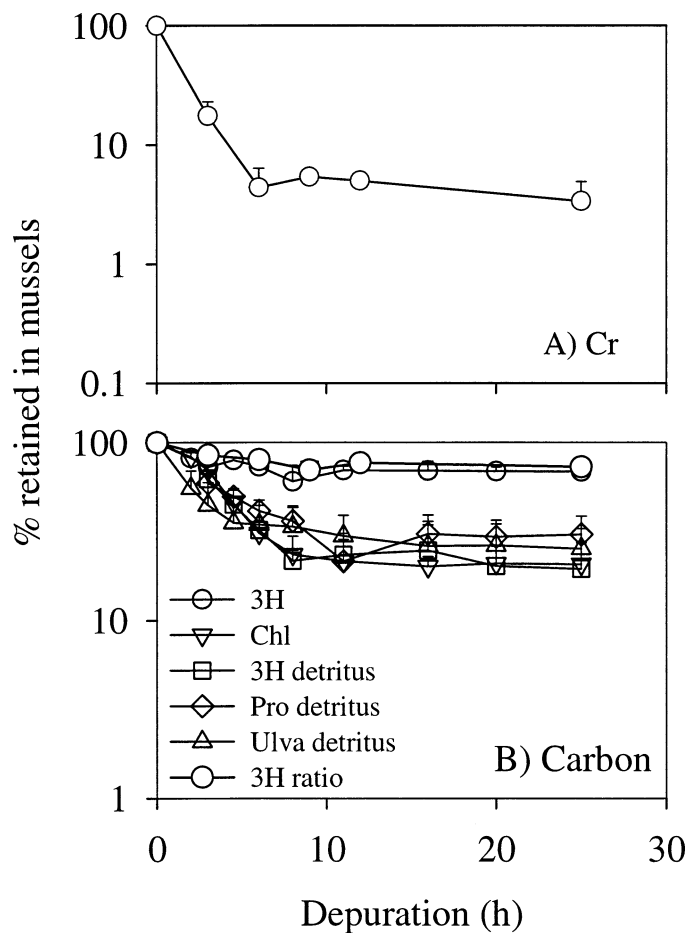


Fig. 4. Retention of (A) ^{51}Cr and (B) ^{14}C in the green mussel *Perna viridis* following a pulse ingestion of radiolabeled food. Mean \pm SD ($n = 8$). 3H: *Thalassiosira pseudonana*, Chl: *Chlorella autotrophica*, 3H detritus: dead cells of *Thalassiosira pseudonana*, Pro detritus: dead cells of *Prorocentrum minimum*, *Ulva* detritus: detritus produced by decomposition of *Ulva lactuca*, 3H ratio: *Thalassiosira pseudonana* absorption estimated using the ratio method.

centration of $0.4 \mu\text{mol L}^{-1}$, the uptake rates of arginine, leucine, and the amino acid mixture were 30.5 , 24.5 , and $16.5 \text{ nmol g}^{-1} \text{ wet wt h}^{-1}$, respectively. For glucose at the same concentration ($0.4 \mu\text{mol L}^{-1}$), its uptake rate ($18.9 \text{ nmol g}^{-1} \text{ wet wt h}^{-1}$) was similar to that of the amino acid mixture. The calculated J_{max} of glucose was, however, $13.1\times$ and $1.2\times$ higher than that of arginine and leucine, respectively. The K_m of glucose was also the highest among the three compounds examined.

In contrast with the uptake of amino acids and glucose, the influx of the ultrafiltered UOC ($<1 \text{ kDa}$) and the COC was proportional to the organic carbon concentration when all the organic matter in different fractions (UOC or COC) and from different sources (diatoms, dinoflagellates, and *Ulva*) was considered together (Fig. 7). The uptake rate constant, the slope of the linear regression of the influx rate against the DOC concentration, was $1.07 \times 10^{-4} \text{ L g}^{-1} \text{ wet wt h}^{-1}$ (or $6.08 \times 10^{-4} \text{ L g}^{-1} \text{ dry wt h}^{-1}$, with a dry weight and wet weight ratio of 1:5.68). The absorption efficiency

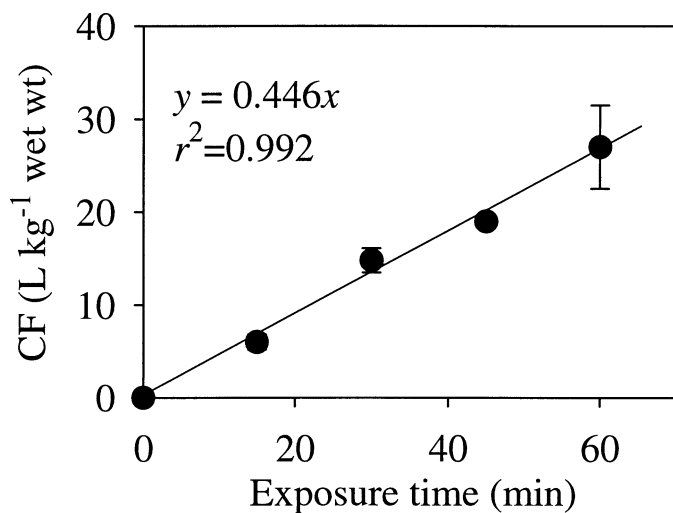


Fig. 5. Glucose uptake by the green mussel *Perna viridis* over time at a concentration of $0.04 \mu\text{mol L}^{-1}$, as quantified by the concentration factor (CF). Mean \pm SD ($n = 4$).

of DOC was calculated as the uptake rate constant divided by the clearance rate of the mussels, and was 0.0053%.

Modeling carbon uptake from different fractions—The parameters used for modeling the relative contributions of DOC and POC to the overall organic carbon uptake by the mussels included k_u , %DOC as TOC, CR, AE, and %POC as TOC. Because the uptake rate constant k_u was the same for both the UOC and COC ($6.08 \times 10^{-4} \text{ L g}^{-1} \text{ h}^{-1}$), we only considered the DOC (including both the UOC and COC) in the calculation. However, the k_u was determined based on the uptake of biogenic DOC, whereas it is recognized that the bulk DOC pool in natural seawater represents a continuum of biological lability, ranging from refractory materials that are biologically resistant (thus cannot be utilized by marine organisms) to labile materials that are biologically reactive (Keil and Kirchman 1991). The refractory component represents approximately 70% of surface bulk DOC (Druffel et al. 1992; Münster 1993). In our calculation, we assumed that about 50% of the total coastal DOC pool is refractory and another 50% is biologically active and may be potentially bioavailable to the mussels. For the POC, we also assumed a single pool (including both the living and nonliving particles) and a range of 20–70% for carbon AE. The mean CR as measured in this study was $11.5 \text{ L g}^{-1} \text{ h}^{-1}$. Various authors have reported that the average DOC concentration as TOC from different regions ranges between 70% and 90% (Guo and Santschi 1997b; Hama 2000; Hill and Wheeler 2002), so a range of 60–90% was used in the calculations.

Our calculated results suggest that the majority of organic carbon accumulation by marine mussels comes from the ingestion of POC and a negligible fraction originates from DOC uptake (<0.22%) under all conditions. Varying the AE (between 20% and 70%) and the fraction of TOC as DOC (60–90%) does not result in any change in the dominance of POC as the carbon source for the mussels. The dominance

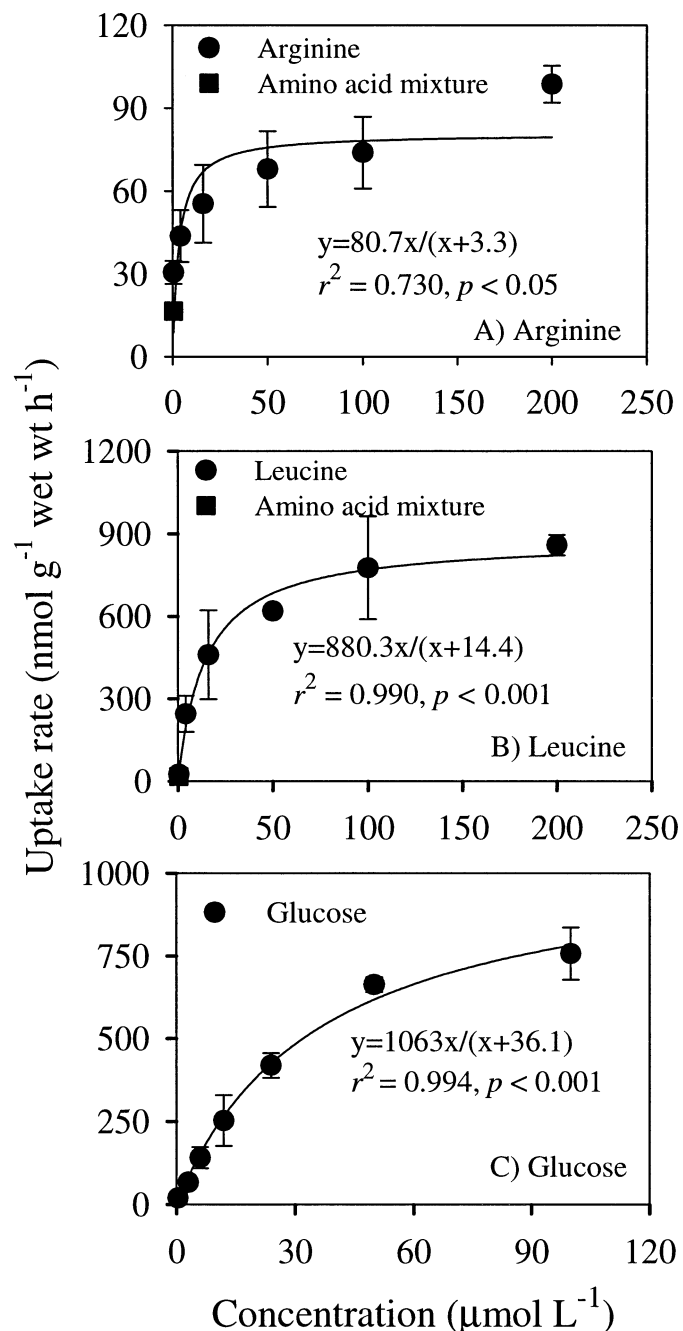


Fig. 6. Uptake of (A) arginine, (B) leucine, and (C) and glucose at different substrate concentrations by the green mussel *Perna viridis*. The influx of an amino acid mixture at $0.4 \mu\text{mol L}^{-1}$ (the lowest concentration used for arginine and leucine) was also quantified for reference. Mean \pm SD ($n = 4$).

of POC uptake was primarily a result of the exceedingly low k_u for DOC uptake by the mussels.

Discussion

Colloidal ingestion by mussels—Our results obtained with the dextran compounds demonstrate for the first time that mussels are able to ingest colloidal nanoparticles of different

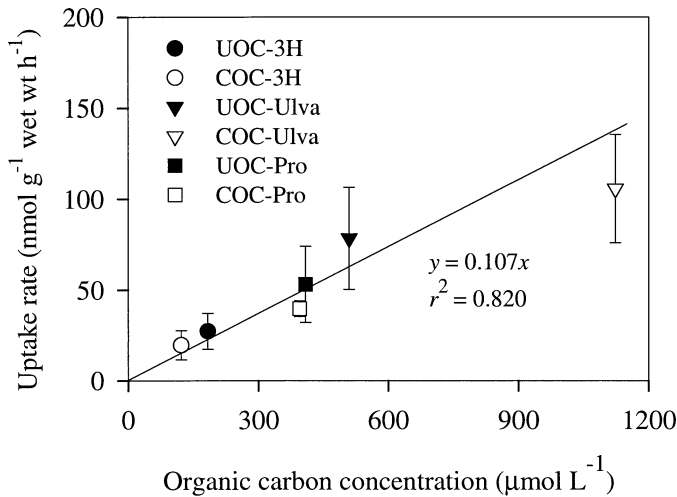


Fig. 7. Uptake of ultrafiltrated organic carbon (UOC, <1 kDa) and colloidal organic carbon (COC, 1 kDa to 0.22 μm) produced by algal decomposition by the green mussel *Perna viridis*. Mean + SD ($n = 4$). 3H: *Thalassiosira pseudonana*, Ulva: *Ulva lactuca*, Pro: *Prorocentrum minimum*.

sizes. Although previous studies have emphasized the significance of colloids in understanding the biogeochemical cycling of organic C and trace elements (Guo and Santschi 1997a), direct evidence for the uptake of colloidal matters by marine bivalves has been rare. In our study, in addition to the significant accumulation of dextrans by the mussels' gills, we also found that the digestive glands contained significant amounts of dextran following the exposure. The uptake of the fluorescein-labeled colloidal carbohydrate dextrans provided direct experimental evidence for the colloid ingestion by the mussels. Tack and Polk (1995) used the colloidal melanin from the ink sac of the cuttlefish, *Sepia* sp., which was prefiltered through a Whatman glass-fiber filter with 1.2-μm nominal pore size, to measure the uptake of colloidal DOC (0.2–1.2 μm) by 13 species of bivalves. The gut contained the blackened compounds, suggesting that the colloidal ink particles were in fact ingested by the bivalves.

In previous studies, bivalves have generally been considered to graze primarily on phytoplankton and detritus, and any DOC released into the ambient water was subsequently taken up by the heterotrophic organisms (Siebers 1982). The large gill surface areas and the great amounts of water pumped through their mantle cavity suggested that the bivalves may potentially obtain dissolved organic matter. In addition to the removal of particulate organic foods from the water column, the mussels may also utilize DOC (including COC and UOC) and play a role in DOC cycling in the water column or at the benthic–water interface. However, the colloid pool as a whole is still poorly understood and largely uncharacterized. To better understand carbon and other element cycling in the marine environment, more studies on the chemical and biological characterization of COC in marine systems are indispensable.

We used the fluorescein-tagged dextran conjugates to investigate whether the mussels were able to directly ingest

the nanocolloidal particles. Dextrans are highly branched carbohydrates and are not easily digested by the animals and thus have very low nutritional value. In the marine environments, a significant fraction of natural colloidal particles is of a polysaccharide nature (Santschi et al. 1998), and thus the employment of dextrans may be partially representative of natural colloidal particles. It is therefore likely that natural colloidal particles may directly enter the marine food chain as a result of ingestion by suspension feeders. In addition, recent evidence indicated that the polysaccharides may aggregate to form transparent exopolymer particles (TEP) (Engel et al. 2004), which may also enter marine food chain and constitute potential food sources for marine bivalves. Several previous studies have demonstrated that organic aggregates produced from dissolved organic materials (released from macrophytes and phytoplankton) were potentially incorporated by bay scallops (*Argopecten irradians*) and mussels (*Geukensia demissa* and *Mytilus edulis*) (Alber and Valiela 1994, 1995, 1996).

Carbon absorption by the mussels—The food particle sources of marine suspension-feeding bivalves include living particles such as phytoplankton, bacteria, and protists, as well as the nonliving detrital particles (Langdon and Newell 1990; Wong et al. 2003). Absorption efficiency (AE) is generally dependent on the digestibility of each food type and can be influenced by the food quantity and cell characteristics, such as cell-wall structure and biochemical composition (Wang and Fisher 1996). In our study, the green alga *C. autotrophica*, which have highly refractory cell walls, was much less digestible than the living diatom *T. pseudonana*, similar to the results of a previous study (Wang and Fisher 1996). Its quantified AE was comparable with those of the three types of detritus decomposed from phytoplankton and macroalgae (20–30%). Particle selection may also be important in carbon acquisition by the marine bivalves (Bayne 1993; Ke and Wang 2002). For example, the suspension-feeding oysters *Crassostrea virginica* and *Crassostrea gigas* were able to select living particles from nonliving detritus on the gills based on particle surface properties (Ward et al. 1997).

The importance of phytoplankton as food items for suspension-feeding bivalves has been confirmed in earlier studies (Newell et al. 1989), and phytoplankton stocks can be depleted by bivalve feeding in coastal and estuarine ecosystems (Nichols 1985; Dame 1993, 1996). A great deal of algal production in marine ecosystems ends up as detritus that can be exposed to suspension feeders (Mann 1988; Charles et al. 1992). In the ribbed mussel *Aulacomya ater*, the absorption of kelp detritus was about 50% (Stuart et al. 1982). Other studies using similar radiotracer pulse-chase feeding techniques also showed that cellulose detritus can be efficiently assimilated by marine bivalves (Kreeger and Newell 2001; Huang et al. 2003). In our study, detritus was utilized with a reasonably high AE (20–30%), consistent with other previous studies (Kreeger and Newell 2001). Organic detritus often has attached bacteria that might be assimilated by suspension-feeding bivalves (Crosby et al. 1990). However, the actual contribution of attached bacteria has been estimated at <5% of the metabolic carbon requirement for intertidal

mussels (Langdon and Newell 1990). In our algal culturing and decomposing experiments, we used axenic medium, thus the influence of bacteria on detritus absorption may be relatively small—a conclusion that will require experimental testing.

DOC uptake by the mussels—DOC has long been hypothesized to constitute a potential source of nutrition to marine organisms (Stephens 1968). Despite this, some studies have suggested that the uptake of DOC is not nutritionally significant for marine invertebrates because bacteria respond more quickly to DOC and reduce its concentration to a low level (Siebers 1982; Ambariyanto and Hoegh-Guldberg 1999). The DOC, mostly released by biogenic activity (Biddanda and Benner 1997; Wang and Guo 2001), consists of all types of biochemical products, including carbohydrates (Biersmith and Benner 1998). We quantified the bioavailability of the well-characterized organic compounds (glucose, amino acids) in the DOC pool as well as the biogenic DOC derived from decomposing diatoms to the mussels. The marine environment contains diverse types of organic material, and the quantified uptake of amino acids and glucose yields only an indirect and incomplete estimate of the importance of DOC to energy metabolism. Quantitative assessment of the biogenic DOC can help in evaluating the remaining elusive contribution of the naturally occurring DOC pool to the total nutritional needs of mussels.

Arginine and leucine account for 2–9% of the total dissolved amino acid pool (Hubberten et al. 1994). Siebers and Winkler (1984) reported that mussels took up 30–50% of the dissolved amino acids and obtained nutritional benefits from the uptake equivalent to 5–38% of their metabolic requirements. Rice and Stephens (1987) documented that 10 amino acids were taken up by the mussel *Mytilus edulis* and the oyster *Crassostrea gigas*, and then distributed rapidly into the internal tissues. In these previous studies, the isolated gills were exclusively used as an experimental system, with the advantage of easy manipulation and without variable pumping activity in vivo. However, the kinetics of in vitro transport in the gills can be different from those detected using the intact animals. We used intact mussels, similar to a few earlier studies (Péquignat 1973; Wright and Stephens 1978; Manahan et al. 1982). The transport of amino acids was analyzed using the Michaelis–Menten equation describing the kinetics of a saturable process.

Uptake of carbon from the dissolved phase may occur primarily across the gills of organisms (Péquignat 1973; Wright and Manahan 1989). Among the organic compounds tested, glucose was transported at the highest rate (with a maximum transport rate of $1.063 \mu\text{mol g}^{-1} \text{ wet wt h}^{-1}$), followed by leucine ($0.880 \mu\text{mol g}^{-1} \text{ wet wt h}^{-1}$), and arginine ($0.081 \mu\text{mol g}^{-1} \text{ wet wt h}^{-1}$). Ambariyanto and Hoegh-Guldberg (1999) also reported a faster uptake of neutral amino acids (e.g., leucine) than basic amino acids (arginine) by the coral *Galaxea fascicularis*. A faster uptake of glucose may be due to the different transport mechanisms in the bivalves. For example, the transport of glucose in the gills, which requires coupling between the movements of glucose and sodium (cation specific), was different from the transport of amino acids in the mussels, which exhibited weak cation

selectivity (Pajor et al. 1989). In the green mussels, the K_m of arginine ($3.3 \mu\text{mol L}^{-1}$) was the lowest, followed by leucine ($14.4 \mu\text{mol L}^{-1}$) and then glucose ($36.1 \mu\text{mol L}^{-1}$), indicating the higher binding affinity and the effective transport of arginine. Preston and Stevens (1982) proposed two different groups of transport systems, one with $K_m < 10 \mu\text{mol L}^{-1}$, where DOC concentrations are very low, and another with $K_m > 10 \mu\text{mol L}^{-1}$, where higher DOC concentrations can be found.

Our experimental data strongly suggest that biogenic DOC can be accumulated by mussels as a potential nutrient. In contrast with the uptake of amino acids and glucose, the uptake of DOC was proportional to the dissolved DOC concentrations, with no evidence of reaching saturation, even at a very high DOC concentration ($1120 \mu\text{mol L}^{-1}$). Thus, the transport of biogenic DOC had lower affinity for the transporters in the mussels. Organic matter with different chemical properties may have different potentials in being accumulated by the mussels.

Although the transport of different organic compounds was markedly different, the k_u of UOC and COC with different origins (diatoms, green algae, and dinoflagellates) was rather comparable, suggesting that the compositions of biogenic UOC and COC from these sources were mostly similar. In a study of the diverse organic matter excreted by marine phytoplankton, Biddanda and Benner (1997) reported distinct HMW DOC ($>1,000 \text{ Da}$, C:N ratio ~ 21) and UOC DOC ($<1,000 \text{ Da}$, C:N ratio ~ 6) produced by different groups of phytoplankton species (*Synechococcus bacillaris*, *Phaeocystis* sp., *Emiliania huxleyi*, *Skeletonema costatum*). However, the organic matter synthesized by phytoplankton was mainly of polysaccharide origins (70–94%) (Biddanda and Benner 1997), with similar contributions from several aldoses (galactose, glucose, mannose, xylose, and arabinose) (Biersmith and Benner 1998). Such similarity may partially account for the same k_u observed in our study.

Using a simple kinetic equation, our results demonstrated that the majority of carbon accumulation by marine mussels originated from the ingestion of POC, with very little contribution from DOC. In Hong Kong coastal water, the average DOC concentration is typically $100 \mu\text{mol L}^{-1}$ (Pan and Wang 2004). Assuming that 50% of the DOC is potentially labile and bioavailable (Middelboe and Sondergaard 1995), the calculated influx rate would be $0.0304 \mu\text{mol C g}^{-1} \text{ dry wt h}^{-1}$, with a k_u of $6.08 \times 10^{-4} \text{ L g}^{-1} \text{ dry wt h}^{-1}$. For the POC, assuming an average AE of 30% (the mean value for living particles and detritus), a CR of $11.5 \text{ L g}^{-1} \text{ dry wt h}^{-1}$ and a POC concentration of $20 \mu\text{mol C L}^{-1}$, the influx rate from POC is $69 \mu\text{mol C g}^{-1} \text{ h}^{-1}$. Consequently, DOC uptake would contribute only 0.8% to the mussels' TOC acquisition. This prediction is generally consistent with several previous studies showing that marine bivalves are unable to take any nutritionally significant DOC from seawater (Siebers 1982; Ambariyanto and Hoegh-Guldberg 1999). The negligible contribution to overall carbon uptake from the bulk DOC pool is due to the exceedingly low carbon absorption efficiency from the dissolved phase (0.0053%), which is >3 orders of magnitude lower than the carbon AE from food particles.

Nevertheless, given the importance of colloidal organic

materials as scavenging sites for particle-reactive contaminants, the pumping and ingestion of these COCs by the marine bivalves may provide an important pathway for the transport of contaminants to the animals. A few recent studies have in fact demonstrated that marine bivalves are able to accumulate metals bound with colloidal materials, possibly through the ingestion of these colloidal carriers (Wang and Guo 2000; Pan and Wang 2002, 2004). Clearly, studies of contaminant transport in marine organisms need to consider the potential ingestion of these colloidal nanoparticles. Furthermore, the direct ingestion of the colloidal materials suggests that these macromolecular particles may actively participate in the transport of materials in marine food chains. Given the recent finding that marine protists may constitute a source of carbon acquisition for bivalves (Kreeger and Newell 1996; Wong et al. 2003), it is also likely that marine dissolved organic carbon may play an additional role in the nutritional ecology of marine bivalves through its indirect influence on microbial food chains.

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