FS/BIO-IATR//91-29

# *Potamocorbula amurensis:* Comparison of Clearance Rates and Assimilation Efficiencies for Phytoplankton and Bacterioplankton

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Technical Report 29
October 1991

## Interagency Ecological Studies Program for the Sacramento-San Joaquin Estuary

A cooperative program by the:

California Department of Water Resources
State Water Resources Control Board
U.S. Bureau of Reclamation
U.S. Army Corps of Engineers
California Department of Fish and Game
U.S. Fish and Wildlife Service
U.S. Geological Survey

# ABSTRACT

This study investigated clearance rates and assimilation efficiencies of natural bacterioplankton and cultured phytoplankton by the introduced Asian bivalve *Potamocorbula amurensis*. Clearance rates were measured by following decreases in particle (bacteria or phytoplankton cells) concentrations in containers over time using a microscope. Ingestion, excretion, and respiration rates, determined using bacterioplankton radiolabeled with a $^{14}C$-amino acid mixture and phytoplankton radiolabeled with $NaH^{14}CO_3$, were used to calculate gross and net assimilation over periods of up to 69 hours.

The study showed that *P. amurensis* is able to retain natural bacterioplankton ($< 1.2\,\mu m$) more efficiently than other bivalve species investigated so far. The average clearance rate for bacteria was 45 mL/h/clam, which represented a retention efficiency of 29%-13% for clams of 1-2 cm shell length compared to retention of the marine flagellate *I. galbana* (about 5 $\mu$m). Whereas the clearance rates on *I. galbana* were highly correlated with animal size, there was no correlation between shell length and bacterioplankton clearance rates. Bacteria were readily assimilated by *P. amurensis*. Gross assimilation was 73% after 49 hours compared to 90% for *I. galbana*. Net assimilation was 45% when feeding on bacteria and 53% when feeding on the flagellate. Bacterial carbon appeared to be respired faster than the algal carbon. As seen in other bivalves, feces production increased at higher food concentration, and assimilation efficiencies decreased accordingly.

Natural bacterioplankton constitute an additional, albeit minor under most circumstances, food source for *P. amurensis*. Its significance is dependent on the relative concentrations of bacteria and other food sources (*i.e.* detritus, phagotrophic or heterotrophic flagellates, microzooplankton, etc., which were not evaluated), as well as the size of the clam. Given the mean bacterioplankton and phytoplankton standing stocks in Suisun Bay during the summer of 1988 and the clearance rates we measured, calculations indicate that bacterioplankton would have supplied about 8% and 10% of the sum of bacteria and phytoplankton C and N, respectively, consumed by a 1-centimeter *P. amurensis*. The potential range is much larger: 2% to 84% of C and 3% to 88% of N. From the net assimilation efficiencies measured, we calculate that a 1-centimeter clam could have doubled its biomass C in 139 and 16. 4 days by feeding on bacterioplankton and phytoplankton, respectively, during this period. Clearly, phytoplankton dominate the nutrition of *P. amurensis* populations in Suisun Bay, and even the low standing stocks found there presently (about 1-2 $\mu$g chlorophyll/L) appear to be adequate to ensure continued rapid growth of *P. amurensis* populations.

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# EXECUTIVE SUMMARY

The study discussed in this report was mounted to provide information on the diet of the Asiatic clam *Potamocorbula amurensis*. This clam was introduced to San Francisco Bay in 1986 and has had a devastating effect on the food chain in portions of the bay where it has achieved its highest densities. One of these areas is Suisun Bay, where phytoplankton plant biomass dropped 10- to 50-fold (from 35-50 to 0.5 to 4 $\mu$g chlorophyll/L) following establishment of clam beds with densities in excess of 2,000 clams/m$^2$. The production of new phytoplankton biomass has decreased 5-fold, and zooplankton communities have experienced similar declines.

Growth of the clam population in Suisun Bay has remained high in spite of the precipitous decline in phytoplankton biomass and production. This prompted us to hypothesize that *P. amurensis* might be able to use food sources other than phytoplankton. The ability to use other food sources might be one of the keys to the success of this species as an invader and could have significant implications for the transfer of material (including pollutants) and energy through the food web of San Francisco Bay.

At present, the biomass of planktonic bacteria in Suisun Bay is roughly half that of phytoplankton, but the production of new biomass is roughly 5 times that of the phytoplankton. Their growth appears to depend on organic matter brought into Suisun Bay with fresh water, rather than on phytoplankton. These facts, and observations that other clams consume bacteria, suggested that planktonic bacteria might be important as an alternative food source to *P. amurensis* in Suisun Bay. We tested this by comparing *P. amurensis*' ability to consume and digest phytoplankton and planktonic bacteria.

We found that *P. amurensis* is able to harvest free-living (as opposed to particle-bound) bacterial cells from the water, albeit with lower efficiency than for phytoplankton cells, which are much larger (0.5 to 1 $\mu$m versus 5-20 $\mu$m). Clearance rates (equivalent to the volume of water swept free of particles per unit time) for bacteria were 44.7 mL/h/clam and were independent of clam size. Clearance rates for phytoplankton varied depending on the size of the clam. A regression equation relating clearance rate and size is given in the text. A clam 1 centimeter long cleared the phytoplankton from 154 mL of water in an hour; for a 2-cm clam the rate was 337 mL/hr. Relative to phytoplankton, bacteria are a more important food source for small than for larger clams.

Given these rates and the densities of clams in Suisun Bay, we calculate that the clam population could filter the water over shallow water beds (1 meter deep) 12.8 times in a day. Water over deeper beds (10 meters deep) could be filtered 1.3 times per day. These calculations ignore the possible restriction of exchange between water within a few centimeters of the bottom and overlying water due to bottom-induced physical processes.

Both phytoplankton and bacteria were good food for *P. amurensis*. Of the phytoplankton biomass consumed, 53% had been incorporated into tissue, 42% had been respired and 10% had been excreted as feces within 49 hours of consumption. For bacteria, these ratios are: 45% incorporated, 39% respired, and 24% excreted. Bacteria appear to be digested more rapidly than phytoplankton.

Based on the concentrations of free-living bacteria and phytoplankton presently found in Suisun Bay and ignoring other possible food sources, we calculate that bacteria provide 12.2% of the C and 15.5% of the N consumed by a 1-cm clam and 6.0% of C and 7.7% of N, for a 2-cm clam. Furthermore, a 1-cm clam would double its biomass in 139 days from the bacteria and 16.4 days from the phytoplankton in Suisun Bay water. For a 2-cm clam, these values are 976 days for bacteria and 52.6 days for phytoplankton. Including particle-bound bacteria in these calculations could roughly double the importance of bacteria (20% of the population harvested with 100% efficiency for particle-bound versus 80% of the population harvested with 20% efficiency for free-living bacteria).

We conclude that direct consumption of free-living bacteria is a minor source of food for *P. amurensis*. Second, the phytoplankton populations presently found in Suisun Bay appear adequate to support rapid growth of small clams. Larger clams may supplement their diet with other food sources such as detritus, dissolved organic matter, or protozoa such as flagellates and ciliates that feed on bacteria. Simple calculations indicate that indirect consumption of bacteria mediated by protozoa can provide 2.5 times the food that is available from phytoplankton growth.

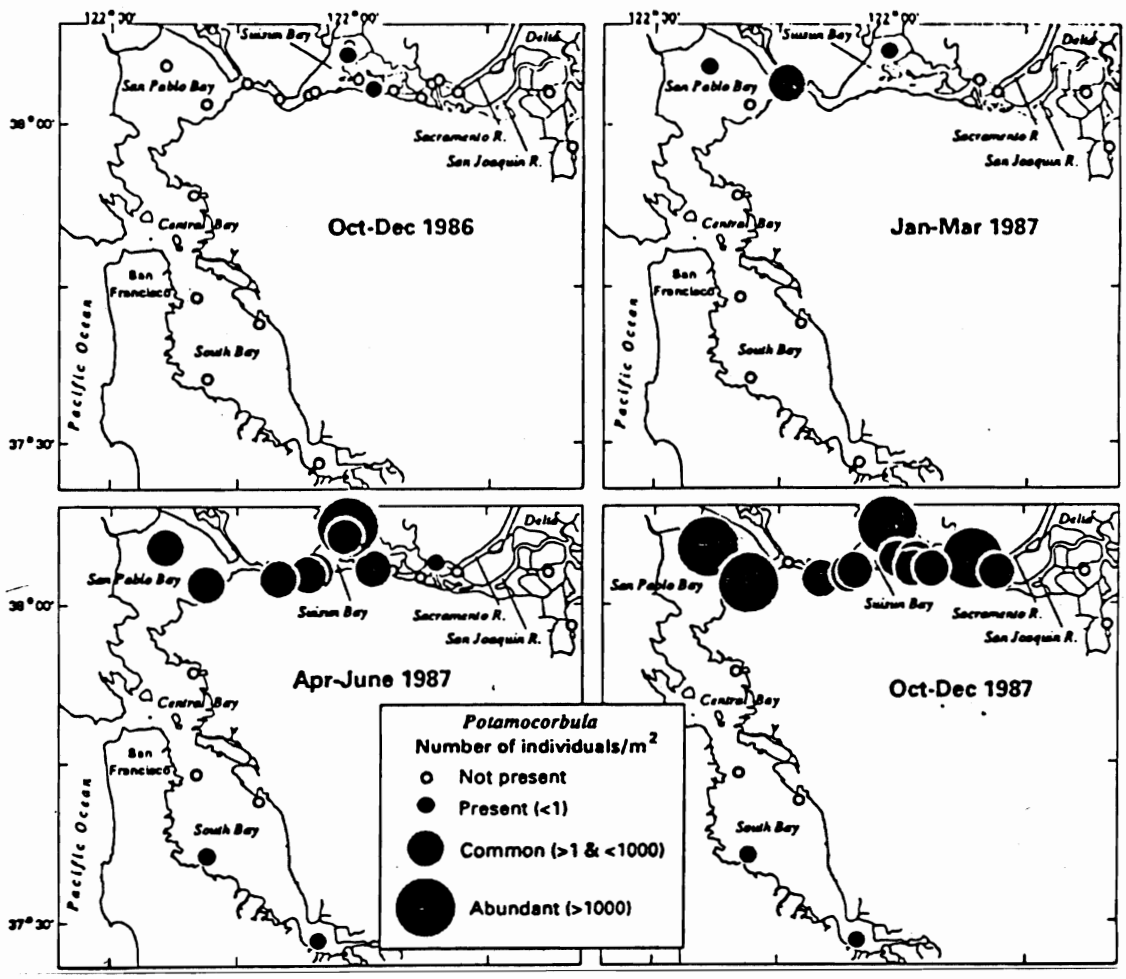
# INTRODUCTION

San Francisco Bay has been the site of numerous invasions by exotic species (Carlton 1979). Among the factors thought to be responsible for the abnormally successful colonization by exotics is the geologically young age of the estuary, about 5,000 years (Nichols and Pamatmat 1988; Carlton *et al* 1990). In recent years, transport and release of seawater ballast from cargo vessels has become a major source of introduced species (Carlton 1985). Improved relations with the People's Republic of China and the opening up of new Chinese ports in the mid-1980s coincides with the appearance of *Potamocorbula amurensis* (Fam: Corbulidae), which was first discovered in Grizzly Bay in October 1986.

The spread of *P. amurensis* through San Francisco Bay has been monitored by a joint program of the State Water Quality Control Board and the United States Geological Survey (Carlton *et al* 1990). From one reported specimen in late 1986, the *P. amurensis* population has increased to peak densities of >12,000 animals/m$^2$ (Figure 1; Carlton *et al* 1990). It is now the dominant species in northern San Francisco Bay benthos and has become established throughout the Bay (Nichols *et al* 1990; Figure 2).

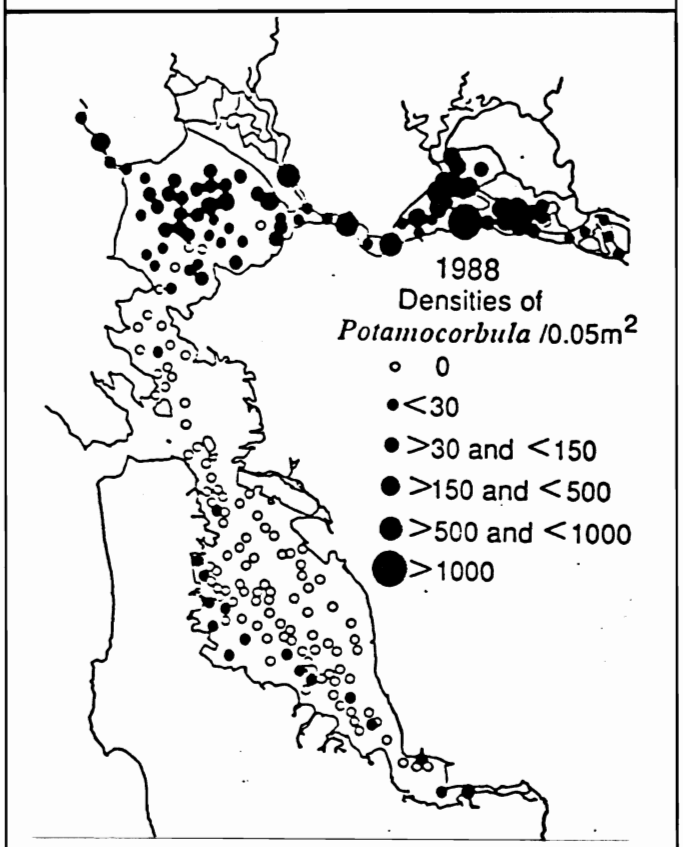
Coincident with the increase of *P. amurensis* populations in Suisun Bay, a significant drop in phytoplankton standing crop was observed by scientists of the U.S. Geological Survey and the Department of Water Resources. The decline in phytoplankton standing crop has been attributed to increased benthic grazing (Alpine and Cloern 1991). The Suisun Bay phytoplankton standing crop decreased from a summer average of 35-50 mg chla/m$^3$ in 1980 (Cloern *et al* 1985) to <4 mg chla/m$^3$ in 1988 (Alpine and Cloern 1991). Phytoplankton production in Suisun Bay declined fivefold, from 100 g C/m$^2$/yr in 1980 (Cloern *et al* 1985) to 20 g C/m$^2$ in 1988 (Alpine and Cloern 1991).

Figure 1
DISTRIBUTION OF *P. AMURENSIS* IN SAN FRANCISCO BAY, 1986-1987




*From Carlton et al 1990.*

Figure 2
DISTRIBUTION OF *P. AMURENSIS* IN SAN FRANCISCO BAY, 1988




*From Carlton et al 1990.*

Despite the considerable reduction in phytoplankton standing crop and productivity, the *Potamocorbula* population continued to increase at a rapid rate. This suggested *P. amurensis* might use sources of organic carbon other than phytoplankton for growth.

Bacteria are abundant in estuaries and bacterial production is high (Ducklow 1983; Coffin and Sharp 1987). Bacteria are important agents of organic matter decomposition and nutrient regeneration as well as food for higher organisms (Wright 1978). Depending upon the efficiency with which its biomass can be utilized, bacterioplankton could represent an important food source in estuarine ecosystems. Recent work (Wright *et al* 1982; Langdon and Newell 1990) demonstrates bacteria can be an important food source for bivalves, depending on the availability of phytoplankton and detritus.

Consumption of bacteria has been reported for a number of invertebrates, generally smaller than bivalves (Gophen *et al* 1973; Fenchel *et al* 1975; Peterson and Hobbie 1978; King *et al* 1980; Hollibaugh *et al* 1980). The textbook statement that "bivalves are exclusively herbivorous" has been challenged for over 50 years. Both detritus (Jorgensen 1966; Langdon and Newell 1990) and bacteria have been investigated as potential food sources for bivalves (Zobell and Feltham 1938; Tunnicliffe and Risk 1977; Prieur 1981; Wright *et al* 1982; Harvey and Luoma 1984; Amouroux *et al* 1989; Langdon and Newell 1990). However, little is known of the ability of bivalves to retain particles as small as natural bacterioplankton, whose size ranges from 0.3-2.0 $\mu$m, with about 90% of the cells being smaller than 1 $\mu$m (Azam and Hodson 1977; Wright *et al* 1978).

Zobell and Feltham (1938) obtained growth of *Mytilus californianus* on a diet of bacteria, using very high concentrations of cultured bacteria (500-5000 x $10^6$ cells/mL). Although Davis (1953) was not able to demonstrate bactivory oyster larvae, a number of other studies found that bacteria are important sources of food for marine bivalves. Tunnicliffe and Risk (1977) have shown that densities of *Macoma balthica* are positively correlated with densities of bacteria in the sediment, whereas correlations with tidal elevation and organic carbon content of the sediment were not significant. They conclude that bacteria fulfill dietary requirements for *Macoma* and point out the importance of bacteria as a nitrogen source. Harvey and Luoma (1984) found clearance rates of 0.168-0.172 liter/day/animal (20 mm length) for *M. balthica* grazing on suspended cultured bacteria. Langdon and Newell (1990) reported that free-living bacteria ($<1\,\mu$m) were retained by *Geukensia demissa* with 15. 8% of the efficiency of removal of 3.9 $\mu$m diameter microspheres, whereas oysters *(Crassostrea virginica)* filtered unattached bacteria with an efficiency of only 5.0% that for 3.9-$\mu$m microspheres. Wright *et al* (1982) found that the retention efficiency of natural bacteria was 18.3% for *G. demissa*, 0% for *M. edulis*, and 4.7% for *Mya arenaria* compared to graphite particles 1-2.3 $\mu$m in diameter.

The importance of bacteria to bivalve nutrition is not limited to the calories they supply. Enzymatic activities associated with ingested bacteria are reported to increase the efficiency with which other food sources are utilized (Langdon and Newell 1990). Bacteria also form the base of the "microbial loop" (Azam *et al* 1983). Nanozooplankton have been shown to feed primarily on bacteria (Fenchel 1982; Sherr *et al* 1987). If size is the major factor for retention in bivalve gills, these ciliates and flagellates (2-20 $\mu$m in diameter) can be retained more efficiently by suspension feeding bivalves and may be an important link in the transfer of nutrients from bacteria to bivalves.

These considerations suggest the ability to directly exploit an abundant food source like bacteria might constitute a competitive advantage in certain environments and/or during certain times of the year. This question is especially interesting with regard to "invasive" species like *P. amurensis*, because this ability might contribute to their success in dominating benthic communities.

Bacterial production now exceeds phytoplankton production in Suisun Bay and the western Delta (Hollibaugh, unpublished data). This suggested that bacterioplankton might be important to *P. amurensis*' nutrition, raising the following questions:

- Is *P. amurensis* able to retain natural bacterioplankton in its filtering apparatus?
- What are *P. amurensis*' assimilation efficiencies for bacteria and phytoplankton?
- What are the relative importances of bacterioplankton and phytoplankton to *P. amurensis*' nutrition?

These questions were addressed in a series of laboratory experiments using *P. amurensis* collected in San Francisco Bay, natural bacterioplankton populations from San Francisco Bay, and cultures of phytoplankton. In the first part of this study, size and weight specific clearance rates for *Isochrysis galbana* and natural bacterioplankton were measured and compared. We found that *P. amurensis* is a very efficient filter feeder and is capable of retaining natural bacterioplankton, albeit at a lower efficiency than phytoplankton (13-29%, depending on the size of the clam). The second part of the study examines the ability of *P. amurensis* to assimilate phytoplankton and bacterial C. We found that both bacterioplankton and phytoplankton C are assimilated with high efficiency (>80% in 3-hour experiments, >70% in 2-day experiments). In addition, *P. amurensis* appears to have limited capabilities for direct utilization of dissolved organic matter.

Chapter 2

# CLEARANCE RATE MEASUREMENTS

The commercial value of bivalves, especially of oysters and mussels, evoked early interest in their feeding mechanisms (e.g. Lotsy, 1895). The most commonly used measurement of filtering activity was and still is the filtration rate (clearance rate). It is defined as the volume of water completely cleared of particles per unit time. Pumping rate (ventilation rate), which is frequently confused with the filtration rate, is defined as the volume of water actually flowing through the gills per unit time. Filtration rate is equal to pumping rate only if particles are retained with 100% efficiency.

Filtration rate is most commonly measured indirectly, by following the removal of particles from the water; the filtration rate is calculated from the exponential decrease in particle concentration. The resulting parameter is often called the clearance rate. Clearance rate measurements permit the animal's impact on food organisms to be estimated.

The methods and conditions under which clearance rate measurements are made vary widely, which often makes it difficult to compare results (see reviews by Owen 1966, 1974; Jorgensen 1966; Winter 1978; Mohlenberg and Riisgard 1979). Moreover, there is no agreement on the units to use in expressing clearance and pumping rates. More recently, mL/gram dry weight/unit time or mL/animal/unit time seem to be preferred.

The simplest way to determine clearance rates is in a static system, where the bivalve is placed in a volume of water containing a suspension of food particles. The decrease in particle concentration is monitored either photometrically (Jorgensen 1966; Walne 1972), by measuring the removal of radiolabeled food from the water or its accumulation in grazers (Rice and Smith 1958; Mohlenberg and Riisgard 1978; Hollibaugh *et al* 1980; King *et al* 1980; Harvey and Luoma 1984; Amouroux *et al* 1989) or by direct counting methods (Utermöhl, Coulter counter; Mohlenberg and Riisgard 1978). Experimental conditions such as duration, vessel geometry, the volume of water in relation to the size of the animal, etc. , have to be chosen carefully. Also, the amount of particulate matter available to the bivalve is continually decreasing, which introduces an additional variable. However, values recorded in static systems by indirect methods are comparable with measurements subsequently made in flow systems (Bayne *et al* 1976). In any case, extrapolation of rates determined under artificial laboratory conditions to field rates must be treated with caution. For example, in the field most clams live buried in sediment, but experimental protocols often exclude sediment.

Clearance rates have been shown to be influenced by a number of factors:

- The size of the animal,
- The size of the food particles,
- The particle concentration,
- The temperature, and
- The flow velocity of the surrounding water.

Seasonal and/or tidal cycles, salinity, and pollutants may also affect feeding.

## Methods

Clams were collected from various sites in northern San Francisco Bay, mainly Carquinez Strait, where the salinity is normally lower than near the Tiburon Center (5-15 *vs* 25-34 ppt, respectively). They were held in tanks at the Tiburon Center for 2 months to acclimate before being used in experiments. Water flowing through the tanks was pumped from about 15 meters offshore and a depth of about 3. 5 meters (bottom). The water was not filtered and no food was added. Chlorophyll *a* levels off Tiburon Center are similar to Suisun Bay. The flow through the tanks was about 18 liters/hour (residence time about 0.5 hour). The clams were allowed to bury themselves in mud that settled out of the inflowing bay water. They were kept in flat glass dishes to facilitate removing them for experiments. Clams increased in size under these conditions, indicating they were happy.

One to two days before the experiments, the clams were measured, weighed, and moved to smaller, empty glass vessels in the tanks. This was done to acclimate them to not being able to bury themselves in mud. We found that animals treated this way did not move as much during the experiments and began feeding sooner than animals removed from sediment immediately before commencing the experiment. The clams were not starved before the experiments.

### Food Suspensions

Unialgal, axenic cultures of *Skeletonema costatum* (Grev.) Cleve, *Phaeodactylum tricornutum* Bohlin, *Platymonas subcordiformis* (Wille) Hazen, and *Isochrysis galbana* Parke were obtained from the UTEX Culture Collection of Algae, Austin, Texas, and maintained under unialgal but not axenic conditions in f/2 medium (Guillard and Ryther 1962). Initial experiments were performed with each of these species. Of the species we examined, *I. galbana*, a marine flagellate (Fam.: Prymnesiophyceae), was found to be the best suited phytoplankton species for this study. It is a flagellated, spherical cell about 5 $\mu$m in diameter that grows slowly and does not form chains.

Phytoplankton were washed on 3 $\mu$m Nuclepore filters with particle-free bay water (filtered through a 0.45-$\mu$m Millipore filter) to remove most of the bacteria growing in the culture (which are larger than natural bacterioplankton), then resuspended in a small volume of particle-free water. Microscopic examination of washed cultures revealed no lysed phytoplankton cells. The volume filtered depended on the concentration of phytoplankton in the culture. The washed phytoplankton suspension was then added to glass-fiber-filtered (GF/C) bay water to yield final concentrations

of 5,000 to 12,000 cells/mL. For *Phaeodactylum* the final concentration was 25,000 cells/mL. These concentrations are believed to be lower than the saturation threshold beyond which clearance rates decrease. They are also similar to concentrations currently found in San Francisco Bay.

Water and bacterioplankton assemblages used in experiments were taken from the water supply system feeding the tanks. For each experiment, 500 mL of water was filtered through Whatman GF/C filters (nominal pore size: 1.2 $\mu$m) to remove detritus, larger mud particles, zooplankton, and phytoplankton. About 75-90% of the natural bacterioplankton (Wright *et al* 1982; Lee and Fuhrman 1986; Hollibaugh, unpublished data) pass through GF/C filters.

## Experimental Protocol

Grazing experiments were conducted in 1000 mL Pyrex beakers containing 500 mL of the food suspension. Magnetic stir bars, rotating at the slowest possible speed (< 120 rpm), kept the suspension well mixed. Stirring bars were enclosed in perforated inverted Petri dishes attached to the bottom of the beaker to dampen water flow and prevent formation of vortices that might disturb the animals (Figure 3).

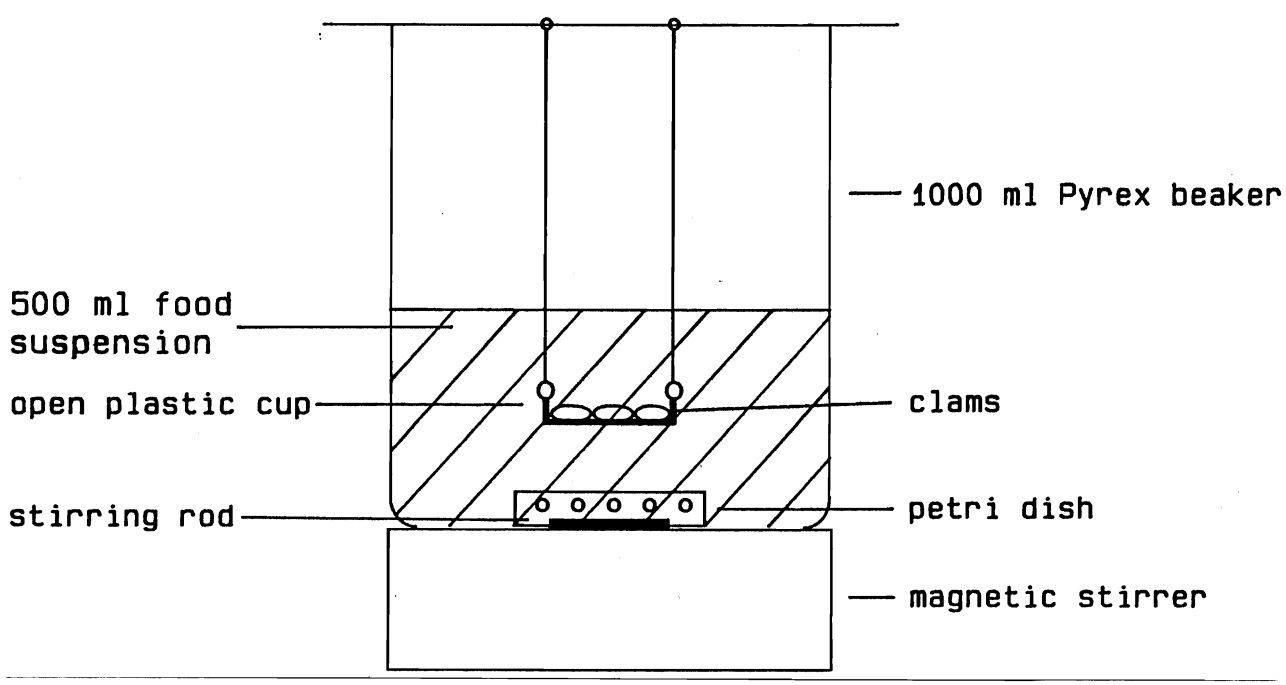
The clams were scrubbed clean of attached material, then three animals of similar shell length were put in perforated plastic cups and suspended in the beakers (Figure 3). Care was taken not to contaminate the water with mud or bacteria other than the bay water bacterioplankton when placing the clams into the beakers.

Experiments were run at *in situ* water temperatures (September 1989 to January 1990; 12-25°C) so animals were adapted to the experimental temperatures. Although bacteria and phytoplankton experiments were run at different times, we tried to run both sets of experiments at comparable temperatures. The temperature change during an experiment was never more than 2°C. Salinity ranged from 25-34‰, also according to the conditions in the bay and in the tanks where the clams were kept.

Each experiment consisted of five complete treatments (clams plus suspension) and one control (no clams). The control was used to assess changes in bacterial and algal concentration due to growth, contamination, lysis, or attachment of the cells to the glass or plastic. The duration of the experiments was 75 to 105 minutes. The doubling time of bacteria in San Francisco Bay is 2 to 5 days (Hollibaugh, unpublished data). The short duration of the experiments relative to bacterial doubling times suggested that bacterial growth during the experiments would be small. The experiments were run in the light, and phytoplankton growth could have occurred. However, no significant changes in either bacterial or phytoplankton cell concentrations were observed in the control beakers.

Clams were allowed to acclimate to the beakers for 15 minutes before initiating sampling. This was usually enough time for the majority of clams to begin filtering following the disturbance of handling. Clams that did not open their valves at all were considered non-feeding and excluded from the calculations. The active, *i.e.* feeding, clams opened and closed their valves at varying frequencies. This could not be accounted for and is probably responsible for some of the variation in the clearance rate data.

Figure 3
APPARATUS FOR MEASURING CLEARANCE RATES



## Sample Enumeration

Water samples (4 mL) were removed from the beakers every 15 minutes for 75 to 105 minutes. They were placed in plastic vials and preserved with 0.2 mL borate-buffered formaldehyde (bacteria) or 0.4 mL Lugol's solution (algae). Bacterial concentrations were determined by enumerating samples stained with DAPI (4',6'-diamidino-2-phenylindole) using an epi-illuminated fluorescence microscope (Porter and Feig 1980). Initial concentrations ranged from $0.5 \times 10^6$ to $1.5 \times 10^6$ cells/mL. The average standard error of the counts was 17±11% (n = 412). Phytoplankton concentrations were determined by counting the cells with an inverted microscope and Utermöhl chambers (Utermöhl 1958). The average standard error of the counts was 15±9% (n = 122).

## Calculations

Clearance rates were obtained by applying the following equations:

(1) $C_t = C_o * e^{-ft}$

(2) $\ln C_t = \ln C_o - f*t$

(3) $f = (\ln C_o - \ln C_t)/t$

where:

$f$ = clearance rate in volume/time

$t$ = incubation time in minutes

$C_o$ and $C_t$ = concentrations of particles at the start of the measurement and after $t$ minutes.

The slope $f$ of equation (2) was calculated from model 1 least squares linear regressions of $\ln C_t$ against $t$. To obtain $f$ in mL/h/clam or mL/h/gram wet weight, $f$ was multiplied by the volume of water in the experimental container (500 mL) and divided by the number of feeding clams or by the sum of their weights. Each clearance rate estimate, therefore, represents the mean clearance rate of the active clams in one beaker. Where $f$ in controls was significantly different from zero ($p < 0.05$) the slope was subtracted from the slope for beakers containing clams.

To facilitate comparison between studies, we measured the shell lengths and widths and dry weight of a number of clams. These data were used to develop regressions for converting $f$ into units of mL/g dry weight/unit time. Conversion to rates on an ash-free dry weight was accomplished using a regression of ash-free dry weight ($W$, mg) against shell length ($L$, mm) developed by J. Thompson and F. Nichols (pers. comm. ):

$$\ln (W) = -4.81 + 2.81*\ln(L)$$

We also ran controls to determine whether or not clams released bacteria into the experiments in their feces and/or pseudofeces, or by simply opening their valves. Three clams, treated exactly as described above, were placed in particle-free bay water and left there for 65 minutes. This experiment was run in duplicate and feces production was observed in both replicates. Bacteria concentration increased in one of the treatments by about $10^4$ cells/mL, but decreased in the other one. An increase of this magnitude represents only 1% of the average concentrations used in all other experiments and is negligible.

Apparent clearance rate might also be affected by attachment of bacteria to the clams, since many bacterioplankton are both chemotactic and sticky. To assess this problem, three clams were killed by cutting them open and placing them in $NaN_3$. They were rinsed in particle-free bay water before being used for the experiments, which were carried out according to the protocol described above. No change in bacterioplankton concentration was observed, indicating that attachment of bacterioplankton to clams was not a significant experimental artifact.

The relationship between clearance rates and animal size was determined by linear regression analysis. A total of 14 experiments were performed, 7 with bacteria only and 7 with both bacteria and phytoplankton.

# Results

The lack of consistency in units used for reporting clearance rates presents a problem when trying to compare results obtained by different investigators. We have chosen to present our results in units of mL/h/clam and mL/h/gram wet weight. To facilitate comparison of our results with those of other studies where other units (*e.g.* mL/h/g dry weight or mL/h/mg dry tissue weight) were used, graphs were prepared of the relationship between:

- Shell width and shell length (Figure 4)
- Shell weight and shell length (Figure 5)
- Wet weight and shell length (Figure 6)
- Dry tissue weight and shell length (Figure 7)

Shell width shows a clearly linear relationship with shell length, whereas shell weight and wet weight (weight of wet tissue plus shell) increase exponentially with shell length. Figure 7 shows the dry tissue weights of the clams plotted against shell length. The open squares represent clams collected in fall 1989, whereas the solid squares are clams collected in spring 1990.

The measurements of dry tissue weight were made in spring 1990, using both the clams kept in tanks at Tiburon Center over the winter and the clams collected in March and May from sand and mudflats in Carquinez Strait and San Pablo Bay. Clams collected in spring, especially the larger ones, had lower dry-tissue-weight to shell-length ratios, which could be due to spawning. Clams held in flowing sea water at the Tiburon Center did not show this effect. Both water temperature and salinity can be important environmental cues of gametogenesis and spawning in bivalves. The Tiburon Center is located on central San Francisco Bay; hence the water pumped through the tanks is colder, saltier, and less subject to temporal variation in temperature and salinity than water over sand and mudflats in San Pablo and Suisun Bays.

Figure 4
RELATIONSHIP BETWEEN SHELL WIDTH AND SHELL LENGTH

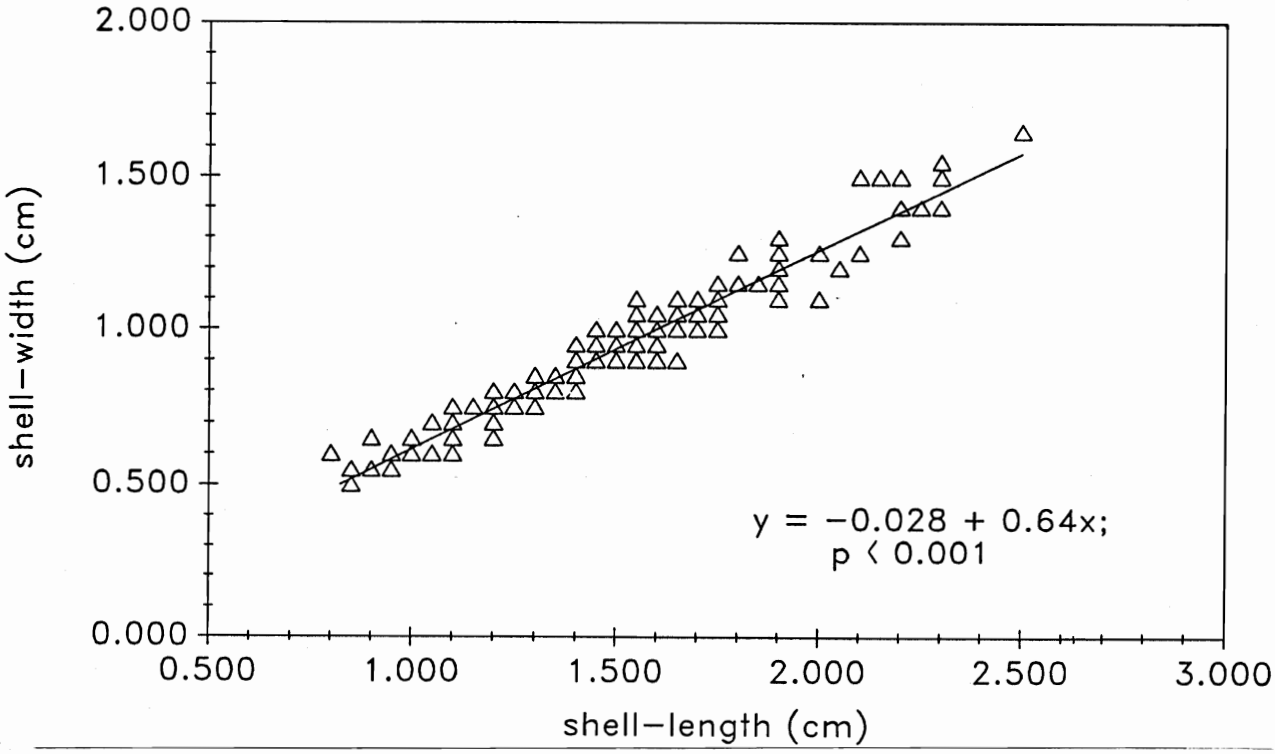



Figure 5
RELATIONSHIP BETWEEN SHELL WEIGHT AND SHELL LENGTH

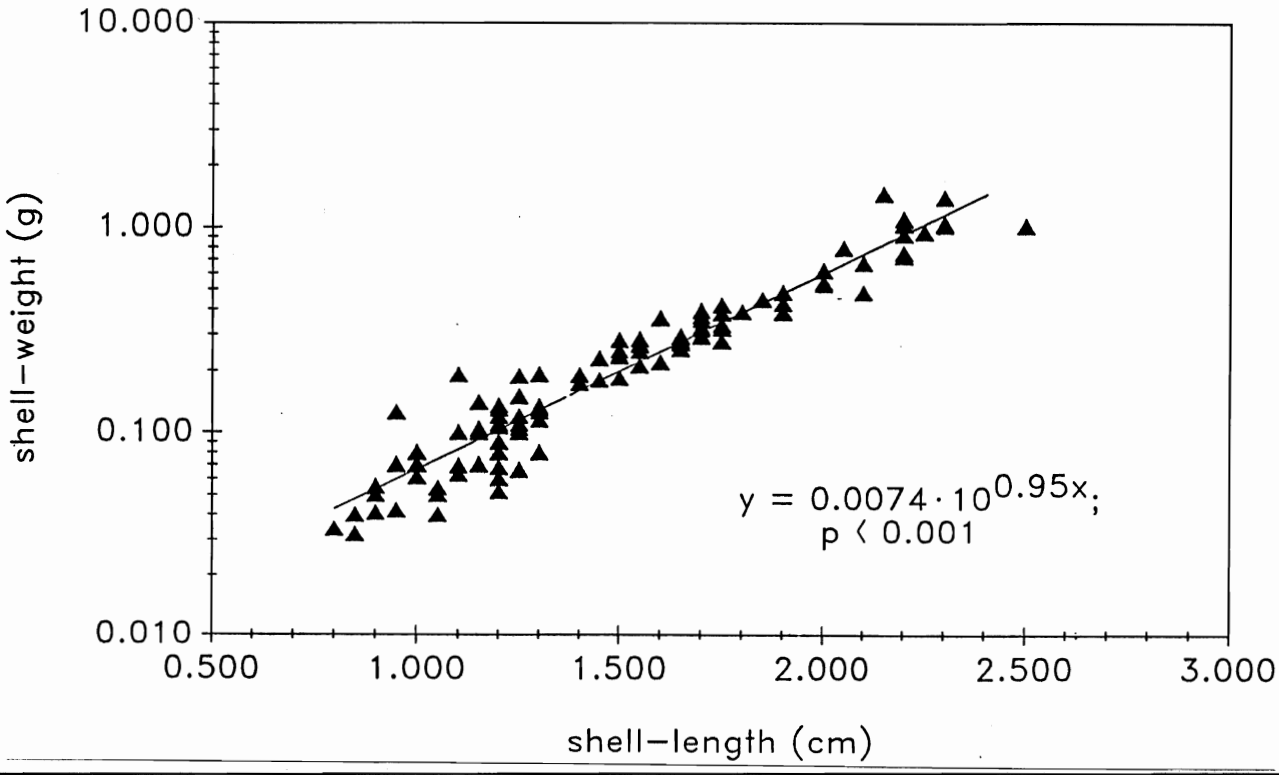


Figure 6
RELATIONSHIP BETWEEN WET WEIGHT (including shell) AND SHELL LENGTH

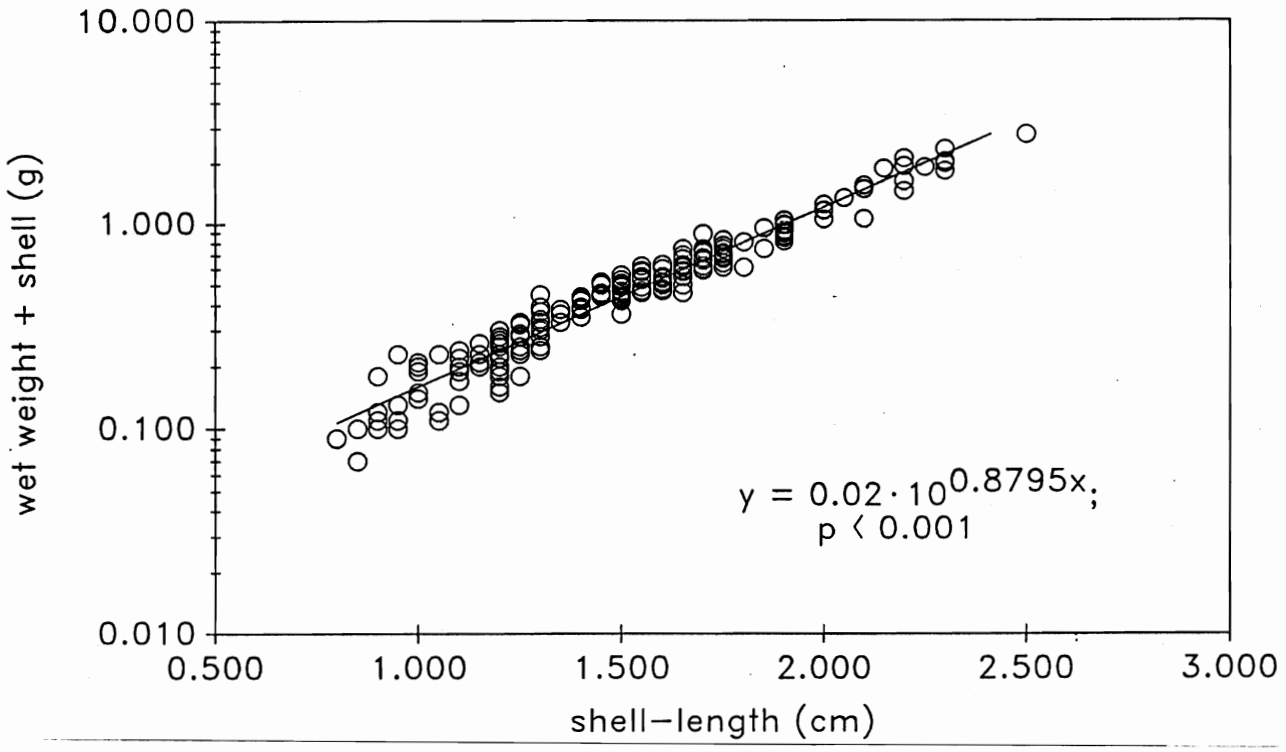
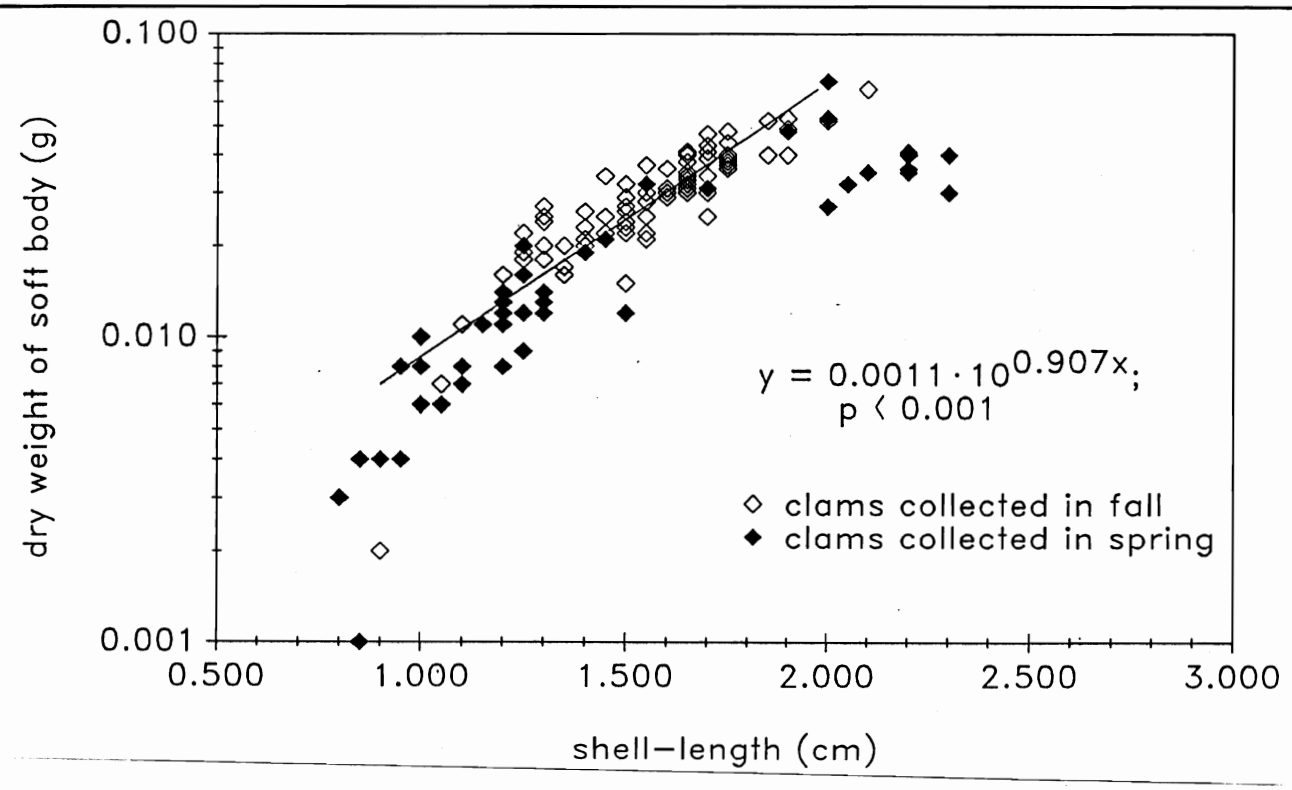



Figure 7
RELATIONSHIP BETWEEN DRY TISSUE WEIGHT AND SHELL LENGTH



## Clearance Rates on Phytoplankton

Clearance rates on phytoplankton were highly correlated with the size (in cm shell length) and weight (in gram wet weight + shell) of the clams. They ranged from 74 mL/h/clam for clams of 1.2-cm shell length to 492 mL/h/clam for clams of 2.3-cm shell length. The relationship between clearance rate $f$ and shell length is:

$$f = 179.2*L - 20.5; \; p < 0.01$$

Using this equation, the clearance rates of standard 1- and 2-cm clams are 154 and 337 mL/h/clam, respectively. These rates will be used for modeling purposes elsewhere in the text.

Figure 8 and Table 1 present results of the experiments carried out with *I. galbana* and of initial experiments with *Phaeodactylum tricornutum* and *Skeletonema costatum*, which were not used in regression calculations. Clearance rates on *P. tricornutum* and *S. costatum* agreed well with rates determined for *I galbana*. This suggests that *I. galbana* (5 $\mu$m) is filtered with the same efficiency as S. costatum (15 $\mu$m) and *P. tricornutum* (10 $\mu$m). Experiments where regression slopes were not significantly different from 0 ($p > 0.1$, 2 experiments) are not included.

Some of the variation in clearance rates was probably due to slight differences in conditions between experiments. In the experiments with *I. galbana* temperatures ranged from 15 to 20°C. These temperatures are well within the range experienced by natural populations of *P. amurensis* and were the same temperatures to which the animals were adapted prior to the experiments. Temperatures did not vary by more than 2°C during a single experiment, so the temperature effect during an experiment is likely to be small. The experiments were run in daylight, which might have disturbed the clams to some degree. Some moved a lot and obviously tried to bury themselves, which was not possible.

Clearance rates were converted to wet and dry weight equivalents using Figures 5, 6, and 7. A comparison of clearance rates for clams of 1 and 2 cm shell length is presented in Table 2. It is apparent that smaller clams exert greater grazing pressure per unit biomass than larger clams.

## Clearance Rates on Bacteria

Clearance rates on natural bacterioplankton were measured in two series of experiments:

- With only free-living bacteria as a food source.
- With both free-living bacteria and phytoplankton.

The purpose of this distinction was to find out whether more bacteria were retained when phytoplankton were present. This could be achieved by clogging the gills with phytoplankton cells, resulting effectively in a finer mesh size that would retain small particles more efficiently. Alternatively, the presence of phytoplankton might have stimulated mucus production on the gill surface, resulting in more efficient trapping of bacteria. The results of these experiments are presented in Table 3 and Figure 9. The presence or absence of phytoplankton cells made no difference in the ability of *P. amurensis* to retain bacteria.

Figure 8
PHYTOPLANKTON CLEARANCE RATES PER INDIVIDUAL AS A FUNCTION OF SHELL LENGTH

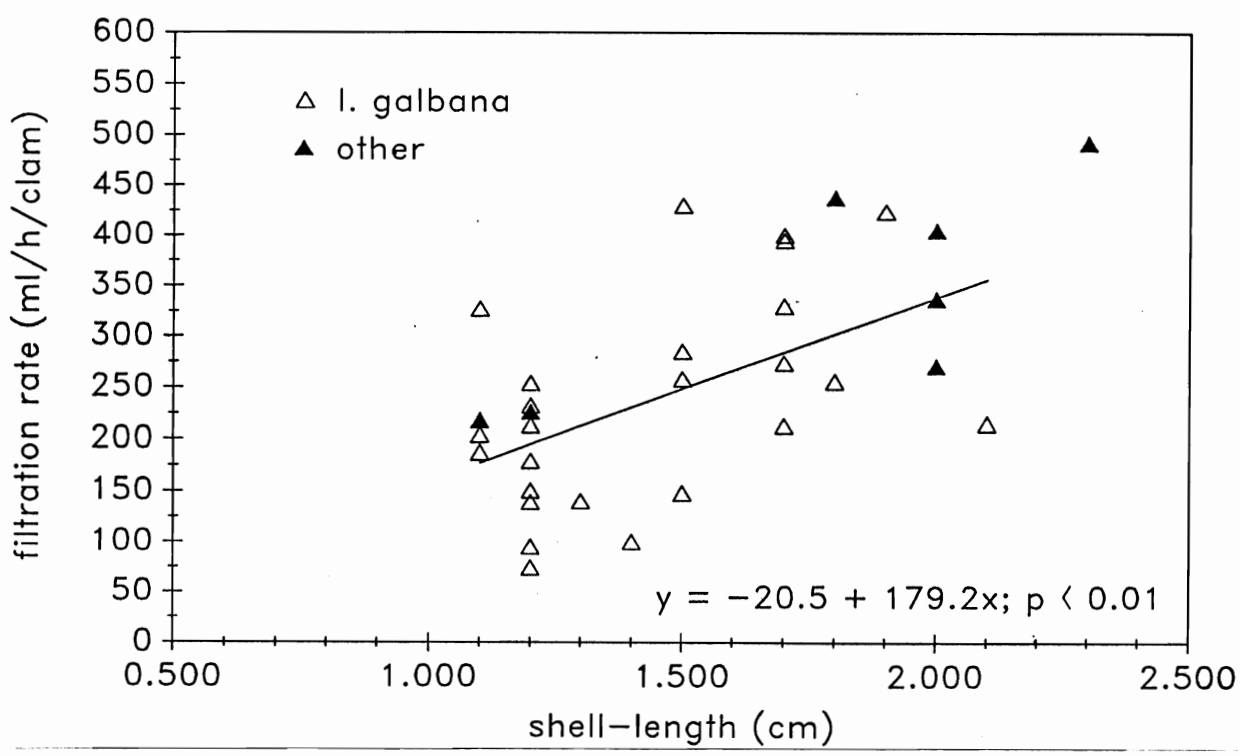


Table 1
CLEARANCE RATES OF PHYTOPLANKTON BY *P. AMURENSIS*

| Date | Concentration (cells/mL) | Alga | Temperature (°C) | Length (cm) | Average *f* (mL/h/clam) | |
|---|---|---|---|---|---|---|
| 09/89 | 25,000 | *Phaeodactylum tricornutum* | 20-20.5 | 1.2 | 226 | ($p<0.1$) |
| | | | | 1.1 | 218 | ($p<0.05$) |
| 09/89 | 8,000 | *Skeletonema costatum* | 23-24.5 | 2.0 | 337 | ($p<0.05$) |
| | | | | 1.8 | 438 | ($p<0.01$) |
| | | | | 2.1 | 271 | ($p<0.01$) |
| | | | | 2.3 | 492 | ($p<0.1$) |
| | | | | 2.0 | 405 | ($p<0.1$) |
| 10/89 | 7,000 | *Isochrysis galbana* | 16-17 | 1.2 | 95 | ($p<0.05$) |
| | | | | 1.3 | 140 | ($p<0.05$) |
| | | | | 1.2 | 74 | ($p<0.05$) |
| | | | | 1.5 | 148 | ($p<0.01$) |
| | | | | 1.7 | 213 | ($p<0.05$) |
| 10/89 | 9,000 | *I. galbana* | 19-20 | 1.2 | 213 | ($p<0.001$) |
| | | | | 1.1 | 327 | ($p<0.1$) |
| | | | | 1.2 | 232 | ($p<0.1$) |
| | | | | 1.5 | 285 | ($p<0.05$) |
| 11/89 | 4,000 | *I. galbana* | 15 | 1 2 | 139 | ($p<0.05$) |
| | | | | 1.1 | 204 | ($p<0.01$) |
| | | | | 1.2 | 150 | ($p<0.001$) |
| | | | | 1.5 | 258 | ($p<0.01$) |
| | | | | 1.7 | 400 | ($p<0.001$) |
| 11/89 | 7,000 | *I. galbana* | 17.5-16 | 1.2 | 254 | ($p<0.01$) |
| | | | | 1.1 | 187 | ($p<0.01$) |
| | | | | 1.2 | 179 | ($p<0.01$) |
| | | | | 1.7 | 330 | $p<0.01$) |
| | | | | 1.8 | 256 | ($p<0.01$) |
| 12/89 | 12,000 | *I. galbana* | 18 | 1.9 | 424 | ($p<0.01$) |
| | | | | 1.4 | 100 | ($p<0.05$) |
| | | | | 2.1 | 215 | ($p<0.01$) |
| | | | | 1.7 | 395 | ($p<0.01$) |
| | | | | 1.5 | 430 | ($p<0.001$) |

Date = Date the experiment was run.
Concentration = Initial phytoplankton concentration.
Alga = Species of phytoplankton used in the experiment.
Temperature = Temperature at which experiment was run.
Length = Average shell length in each replicate.
f = Average clearance rate per beaker and significance of log-linear regression used to calculate clearance rate.
Apparent clearance rates in controls containing no clams were not significantly different from 0.

Table 2
CLEARANCE RATES OF *P. AMURENSIS* ON PHYTOPLANKTON *(I. galbana)*

| Shell Length (cm) | mL/h/clam | Wet Weight Including Shell mL/h/g | Dry Weight Including Shell mL/h/mg | Dry Tissue Weight mL/h/mg | Ash-Free Dry Weight mL/h/mg |
|---|---|---|---|---|---|
| 1.0 | 154 | 854 | 2.7 | 22 | 29.1 |
| 2.0 | 337 | 248 | 0.51 | 4.13 | 9.1 |

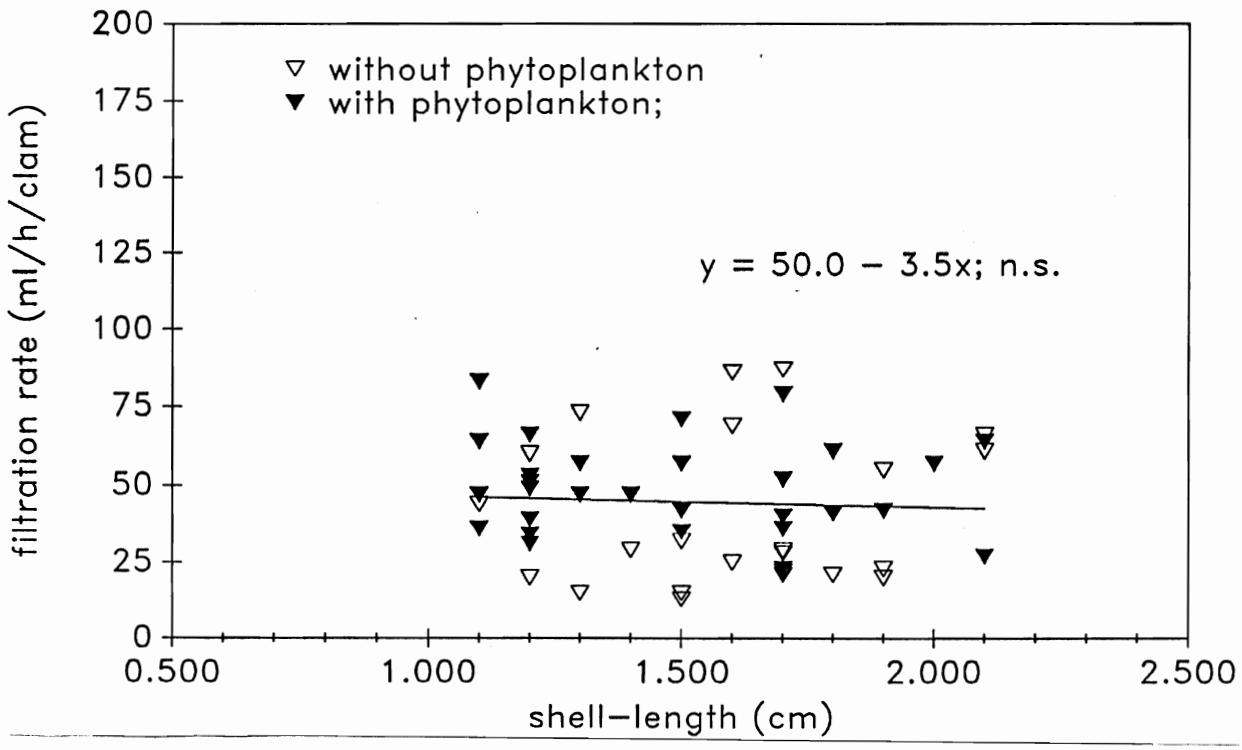
Results are given in various units to facilitate comparison with results of other studies.

Table 3
CLEARANCE RATES OF NATURAL BACTERIOPLANKTON BY *P. AMURENSIS*

| Date | Conc Bact (cells/mL) | Alga (cells/mL) | Temperature (°C) | Length (cm) | Average f (mL/h/clam) | Control f (mL/h) |
|---|---|---|---|---|---|---|
| 09/89 | $0.7 \times 10^6$ | No Algae Present | 20-21 | 1.7 | 87 (p <0.1) | NS |
| | | | | 1.6 | 86 (p <0.01) | |
| | | | | 1.6 | 69 (p) <0.05 | |
| | | | | 1.2 | 60 (p) <0.01 | |
| 09/89 | $0.5 \times 10^6$ | *P. tricornutum* (25,000) | 20-20.5 | 1.7 | 79 (p <0.01) | NS |
| | | | | 1.2 | 66 (p <0.01) | |
| | | | | 1.1 | 47 (p <0.05) | |
| | | | | 1.3 | 57 (p <0.001) | |
| 09/89 | $1.3 \times 10^6$ | No Algae Present | 24-25 | 2.1 | 61 (p <0.05) | +14 |
| | | | | 1.3 | 73 (p <0.05) | |
| 09/89 | $1.4 \times 10^6$ | *S. costatum* (8,000) | 23-24.5 | 1.8 | 61 (p <0.1) | NS |
| | | | | 2.1 | 64 (p <0.1) | |
| | | | | 2.0 | 57 (p <0.01) | |
| 10/89 | $0.6 \times 10^6$ | *I. galbana* (7,000) | 16-17 | 1.2 | 39 (p <0.001) | −10 |
| | | | | 1.3 | 47 (p <0.001) | |
| | | | | 1.2 | 34 (p <0.05) | |
| | | | | 1.5 | 42 (p <0.01) | |
| | | | | 1.7 | 40 (p <0.1) | |
| 10/89 | $1.4 \times 10^6$ | *I. galbana* (9,000) | 19-20 | 1.1 | 36 (p <0.05) | NS |
| | | | | 1.2 | 31 (p <0.1) | |
| | | | | 1.5 | 57 (p <0.05) | |
| 11/89 | $0.7 \times 10^6$ | *I. galbana* (4,000) | 15 | 1.2 | 49 (p <0.01) | NS |
| | | | | 1.1 | 64 (p <0.01) | |
| | | | | 1.5 | 71 (p <0.05) | |
| | | | | 1.7 | 23 (p <0.1) | |
| 11/89 | $1.0 \times 10^6$ | No Algae Present | 16-17 | 1.1 | 44 (p <0.05) | NS |
| | | | | 1.2 | 20 (p <0.05) | |
| | | | | 1.9 | 20 (p <0.1) | |
| 11/89 | $1.5 \times 10^6$ | *I. galbana* (7,000) | 17.5-16 | 1.2 | 53 (p <0.001) | NS |
| | | | | 1.1 | 83 (p <0.001) | |
| | | | | 1.2 | 51 (p <0.01) | |
| | | | | 1.7 | 52 (p <0.01) | |
| | | | | 1.8 | 41 (p <0.01) | |
| 11/89 | $0.7 \times 10^6$ | No Algae Present | 14-12 | 1.5 | 15 (p <0.05) | NS |
| | | | | 1.7 | 29 (p <0.05) | |
| | | | | 1.9 | 23 (p <0.1) | |
| 12/89 | $0.6 \times 10^6$ | No Algae Present | 14-15 | 1.5 | 13 (p <0.1) | −8 |
| | | | | 2.1 | 66 (p <0.01) | |
| | | | | 1.7 | 22 (p <0.1) | |
| 12/89 | $0.6 \times 10^6$ | *I. galbana* (12,000) | 18 | 1.9 | 42 (p <0.01) | NS |
| | | | | 1.4 | 47 (p <0.01) | |
| | | | | 2.1 | 27 (p <0.05) | |
| | | | | 1.7 | 36 (p <0.1) | |
| | | | | 1.5 | 35 (p <0.05) | |
| 02/90 | $1.1 \times 10^6$ | No Algae Present | 16.5-17 | 1.9 | 55 (p <0.01) | NS |
| | | | | 1.4 | 29 (p <0.01) | |
| | | | | 1.5 | 32 (p <0.01) | |
| | | | | 1.7 | 28 (p <0.1) | |
| 03/90 | $1.0 \times 10^6$ | No Algae Present | 17-19 | 1.3 | 15 (p <0.01) | NS |
| | | | | 1.7 | 21 (p <0.01) | |
| | | | | 1.6 | 25 (p <0.01) | |
| | | | | 1.7 | 23 (p <0.01) | |
| | | | | 1.8 | 21 (p <0.01) | |

Experiments were performed as described in text.
Conc Bact = initial concentration of bacterioplankton
Alga = species of alga present and concentration
Length = Average length of clams in a replicate
Average f = Average clearance rate in the replicate and statistical significance of log-linear regression used to calculate f, corrected for control f
Control f - apparent clearance rate in a control containing no clams.
NS = Change in bacteria abundance in control not statistically significant ($p > 0.05$).

Figure 9
BACTERIOPLANKTON CLEARANCE RATES PER INDIVIDUAL AS A FUNCTION OF SHELL LENGTH




Comparison of clearance rates at upper and lower ends of the temperature range used in these experiments (12-25°C) suggested that the influence of temperature was important. Regressions of clearance rate against animal size (shell length) were not statistically significant (Figure 9). The mean clearance rates were:

- All experiments 44.7 mL/hr/clam ($n = 53$, S.E. = 2.74)
- 12-19°C 39.8 mL/hr/clam ($n = 32$, S.E. = 2.96)
- 19-25°C 52.2 mL/hr/clam ($n = 21$, S.E. = 4.89)

Clearance rates in the high and low temperature groups were significantly different from each other ($p < 0.05$, 1-tailed t-test), but not significantly different from the overall mean rate. The average size of clams in the two temperature groups was not significantly different ($p > 0.1$).

Bacterioplankton clearance rates were converted to dry weight equivalents using Figures 5, 6, and 7. A comparison of clearance rates for clams of 1- and 2-cm shell length is presented in Table 4. It is again apparent that smaller clams exert greater grazing pressure per unit biomass than larger clams. Compared to *I. galbana*, bacteria were retained with only 29% and 13% efficiency (1- and 2-cm clams, respectively; compare Tables 2 and 4).

Table 4
CLEARANCE RATES OF NATURAL BACTERIOPLANKTON BY *P. AMURENSIS*

| Shell Length (cm) | mL/h/clam | Wet Weight Including Shell mL/h/g | Dry Weight Including Shell mL/h/mg | Dry Tissue Weight mL/h/mg | Ash-Free Dry Weight mL/h/mg |
|---|---|---|---|---|---|
| 1.0 | 45 | 250 | 0.60 | 5.06 | 8.50 |
| 2.0 | 45 | 39 | 0.07 | 0.63 | 1.22 |

Results are given in various units to facilitate comparison with results of other studies.

## Discussion

To assess the competitive advantage that *P. amurensis* might enjoy as a result of its ability to retain bacterioplankton, the clearance rates found in this study have to be compared with those of other bivalves found in San Francisco Bay. Some of the problems in comparing results from different studies have been discussed above. Moreover, there is still a dearth of information on clearance rates of natural bacterioplankton by bivalves. Table 5 presents clearance rates reported in the literature for the dominant bivalve species found in San Francisco Bay.

It can be seen that the clearance rates we measured for *P. amurensis* are not exceptionally high. *Tapes japonica* had much higher rates with respect to dry tissue weight. Whether these rates are increased or decreased by the high concentrations of suspended inorganic particles found in northern San Francisco Bay remains to be determined. Kiorboe *et al* (1981) report that *M. edulis* depends on resuspended bottom material to exploit fully its clearance potential.

The grazing impact of *P. amurensis* can be substantial. At the densities at which it is now found in northern San Francisco Bay (>2,000 animals/m$^2$) and using a clearance rate of 267 mL/h/clam, the entire water column can be filtered 1.28 times per day in deeper areas (10 meters) and 12.8 times in shallow areas (1 meter). Since the water column is generally well mixed to the bottom in San Francisco Bay, all the food is potentially available to the benthos (Wolff 1977). How food availability is affected by stratification patterns, water movement, and food depletion within the benthic boundary layer (Frechette and Bourget 1985a, b; Frechette *et al* 1989) is unknown. Cloern (1982) attributed the control of phytoplankton biomass in South San Francisco Bay to benthic grazing. Another example of phytoplankton control by benthic feeding has been reported by Cohen *et al* (1984). They attributed a minimum in phytoplankton concentrations in one stretch of the Potomac River, Maryland, to high densities of the Asiatic clam *Corbicula fluminea*, which was introduced in 1977.

Of the bivalve species listed in Table 5, only *Mya arenaria* can be compared to *P. amurensis* with regard to retention of natural bacterioplankton. Harvey and Luoma (1984) used cultured bacteria, which are 2 to 10 times larger than natural bacteria, in their studies of *Macoma balthica* clearance rates. Since particle retention is size-specific, their results cannot be compared directly to the results of this study. Weight-specific bacterioplankton clearance rates are much higher in small clams than in larger clams, which is also true for the clearance rates on *I. galbana*. They are up to two orders of magnitude higher than weight-specific rates reported for *Mya arenaria* (Wright *et al* 1982). The animals used by Wright *et al.* (1982) were probably much larger than most of the clams used in this study, because adult *M. arenaria* are larger than *P. amurensis*. Bacterioplankton clearance rates per animal are in the same range, but are higher for *P. amurensis*.

The retention efficiency of *P. amurensis* for bacteria of < 1.2 $\mu$m diameter was 29% for clams of 1 cm shell length, decreasing to 13% for 2.0 cm animals compared to *I. galbana* cells of about 5 $\mu$m diameter. The retention efficiency for *I. galbana* is assumed to be 100%. Langdon and Newell (1990) report retention efficiencies of mussels (Geukensia demissa) for unattached natural bacteria to be 15.8% of the efficiency of removal for 3.9 $\mu$m diameter microspheres. Wright *et al* (1982) found comparable values of 18.4% retention efficiency for natural bacteria compared to graphite particles of 1-2.5 $\mu$m diameter. It is not clear whether the relatively small graphite particles and microspheres used in those experiments were retained with 100% efficiency. Higher clearance rates on graphite particles in these experiments were attributed to higher concentrations in the "food" suspension.

Table 5
CLEARANCE RATES (*f*) FOR THE DOMINANT BIVALVES OF SAN FRANCISCO BAY

| Species | *f* on Phytoplankton | *f* on Bacteria | Reference |
|---|---|---|---|
| *Macoma balthica* (20 mm) | 20 mL/h/animal | 7 mL/h/animal<br>0.167-0.171 mL/h/mg dwt | Harvey & Luoma (1984) |
| *Mya arenaria* (57-82 mm) | 220 mL/h/g dtwt<br>600-1300 mL/h/animal | 20 mL/h/g dtwt | Wright et al (1982)<br>Allen (1962) |
| (10 mm) | 120 mL/h/animal | | Kiorboe & Mohlenberg (1981) |
| *Gemma gemma* (0.2-0.4 mg dwt) | 20-37.5 mL/h/animal | | Cloern (1982) |
| *Tapes japonica* (27.5-39.5 mg dwt) | 629-804 mL/h/animal | | Cloern (1982) |
| *Musculus senhousia* (12-22 mg dwt) | 362-542 mL/h/animal | | Cloern (1982) |
| *Potamocorbula amurensis* (10-20 mm) | 154-337 mL/h/animal | 45 mL/h/animal<br>5.1-0.6 mL/h/mg dtwt | This Study |

dwt = Dry weight including shell.
dtwt = Dry tissue weight.

We compared the relative contribution of bacterioplankton and phytoplankton C and N to the daily ration of *P. amurensis* in our experiments as follows (Table 6). The average concentration of unattached bacteria in the water used in this study is about $1 \times 10^6$ cells/mL. Assuming a mean volume of $0.2\,\mu^3$ per cell (Wright *et al* 1982) and a conversion factor of $1.21 \times 10^{-13}$ g C/$\mu^3$ (Watson *et al* 1977), the organic carbon present in the form of bacteria is 24 $\mu$g C/L. Using a C:N:P ratio by atoms of 45:9:1 (Goldman *et al* 1987), bacterioplankton biomass is 5.7 $\mu$g N/L. The average concentration of *I. galbana* used in this study was 10,000 cells/mL. This is the equivalent of 130 $\mu$g C/L and 22.9 $\mu$g N/L, using Strathmann's (1967) relationship between biovolume and biomass and the Redfield ratio for phytoplankton composition of C:N:P = 106:16:1 by atoms. It can be seen (Figure 10) that bacterioplankton in the suspensions would have supplied a relatively small portion of the daily ration of C and N. Bacterioplankton are more important to smaller clams than to larger ones and might supply a significant portion of the diet of very small, newly settled clams, assuming the size/clearance rate relationship we found can be extrapolated reliably to clams outside the size range we used in our experiments.

Similar calculations indicate that bacterioplankton may represent a more significant source of nutrition for *P. amurensis* populations in Suisun Bay due to differences in the relative abundance of bacterioplankton and phytoplankton. Between July 1988 and July 1990, bacterioplankton biomass in Suisun Bay averaged $1.62 \times 10^9$ cells/L (range 0.47 to 3.77). Using the conversion factors given above, the average bacterioplankton biomass is 39.2 $\mu$g C/L and 9.1 $\mu$g N/L (ranges 11.4 to 91.2 $\mu$g C/L and 2.7 to 21.3 ug N/L). During the summer of 1988, Suisun Bay phytoplankton biomass averaged about 1.5 $\mu$g chl/L (range 0.1 to 3.0; Alpine and Cloern 1991). Assuming a C:chl ratio of 55:1 (Wienke and Cloern 1987), mean phytoplankton C and N biomass is 82.5 $\mu$g C/L and 14.5 $\mu$g N/L (ranges 5.5 to 165 $\mu$g C/L and 1.0 to 29.1 $\mu$g N/L). Using these concentrations in the calculations above, it can be seen (Table 6) that bacterioplankton are, on the average, contributing about 12% of the C and 15% of the N consumed by a 1-cm clam.

Data collected during the summer of 1988 were used on a sample-by-sample basis to calculate the contribution of bacterioplankton to the total daily ration of a 1-cm *P. amurensis* in Suisun Bay. Assuming *P. amurensis* feeds only on bacterioplankton and phytoplankton, the contribution of bacterioplankton averaged 7.8% for C and 10.4% for N (ranges: 2.6 to 17.1% for C and 3.5 to 22.7% for N).

When the ranges of measured phytoplankton and bacterioplankton biomass are considered independently (bacterioplankton: 12.2 to 98.02 $\mu$g C/L and 3.0 to 24.2 $\mu$g N/L; phytoplankton: 20.32 to 610 $\mu$g C/L and 3.6 to 107 $\mu$g N/L), the potential range of the relative contribution of bacteria to the nutrition of this hypothetical 1-cm clam in Suisun Bay is large: 2.0 to 83.8% of C and 2.7 to 87.7% of N when bacteria are low and phytoplankton are high and *vice versa*. Thus, bacterioplankton can be a significant portion of the total daily ration of *P. amurensis* when phytoplankton biomass is low, although that total daily ration is likely to be greatly reduced. Bacterioplankton biomass is somewhat less variable than, and not well correlated with, phytoplankton biomass in Suisun Bay. The importance of bacterioplankton to *P. amurensis* nutrition is likely to vary with seasonal and annual variations in phytoplankton biomass.

Unattached bacteria account for the majority of bacteria in San Francisco Bay and other estuaries. In Suisun Bay they represent 75-80% of the total bacterial biomass; in South San Francisco Bay they represent 88-98% (Harvey, personal communication). The higher percentage of attached bacteria in Suisun Bay may increase the availability of bacteria as a food source for suspension feeders since retention is facilitated by the larger size of the particles to which they are attached. Attached bacteria are also often larger than their free-living counterparts. Kiorboe *et al* (1981) report that suspended bottom material serves as an additional food source to *M. edulis*. Grant *et al* (1990) obtained similar results with *Ostrea edulis*. Most of the suspended sediment in San Francisco Bay is resuspended and, thus, possibly enriched with benthic microbes. Although we did not investigate the role of attached bacteria in the nutrition of *P. amurensis*, a simple calculation suggests that including

Table 6

COMPARISON OF THE *C* AND *N* RATION SUPPLIED BY FILTERING BACTERIOPLANKTON AND PHYTOPLANKTON IN EXPERIMENTS AND IN SUISUN BAY

| Length (cm) | Food Type | *f* (L/d) | Standing Crops $\mu$g C/L | Standing Crops $\mu$g N/L | Daily Ration $\mu$g C/D | Daily Ration $\mu$g N/d | % of Total *C* | % of Total *N* |
|---|---|---|---|---|---|---|---|---|
| Lab Experiments | | | | | | | | |
| 1.0 | Bacteria | 1.1 | 24.0 | 5.7 | 25.9 | 6.2 | 5.1 | 6.8 |
| | Phytoplankton | 3.7 | 130.0 | 22.9 | 480 | 84.6 | | |
| 2.0 | Bacteria | 1.1 | 24.0 | 5.7 | 25.9 | 6.2 | 2.4 | 3.2 |
| | Phytoplankton | 8.1 | 130.0 | 22.9 | 1051 | 185 | | |
| Suisun Bay | | | | | | | | |
| 1.0 | Bacteria | 1.1 | 39.2 | 9.1 | 42.3 | 9.8 | 12.2 | 15.5 |
| | Phytoplankton | 3.7 | 82.5 | 14.5 | 305 | 53.6 | | |
| 2.0 | Bacteria | 1.1 | 39.2 | 9.1 | 42.3 | 9.8 | 6.0 | 7.7 |
| | Phytoplankton | 8.1 | 82.5 | 14.5 | 667 | 117.3 | | |

Standing crops calculated as indicated in text.

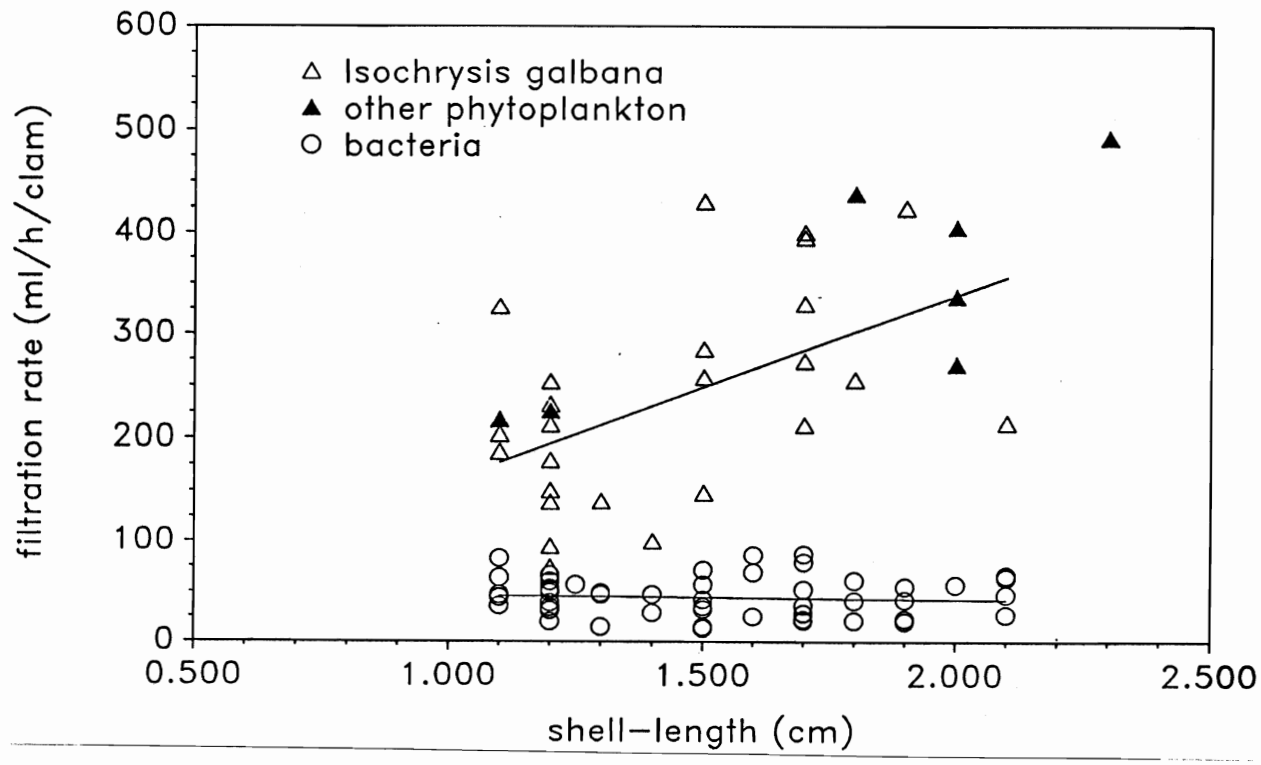
% of Total = Contribution of bacterioplankton to total daily ration (bacterioplankton + phytoplankton, other sources such as detritus not included), expressed as a percentage.

attached bacteria could double the significance of bacteria as a food source for *P. amurensis* (20% of the bacterioplankton population grazed with 100% efficiency for attached bacteria *versus* 80% of the population grazed with 20% efficiency for free-living bacteria).

Natural bacterioplankton may be an important additional food source for filter-feeding bivalves, depending on the availability of phytoplankton, detritus, and other potential food sources and bacteria concentrations in the water column. It is unlikely that *P. amurensis* could depend solely on bacteria as food, but during periods of low phytoplankton abundance, the ability to exploit bacterioplankton directly as a food source may help *P. amurensis* maintain its population more successfully than other bivalves.

The extent to which phytoplankton and bacteria are actually utilized by *P. amurensis* depends on the clams' ability to assimilate these food items. To examine this question, we conducted experiments to determine assimilation efficiencies of phytoplankton and bacterial C by *P. amurensis*. These experiments are described in Chapter 3.

Figure 10
COMPARISON OF CLEARANCE RATES PER INDIVIDUAL FOR PHYTOPLANKTON AND BACTERIOPLANKTON



Chapter 3

# ASSIMILATION EFFICIENCIES

Early studies on assimilation efficiencies in bivalves were based mainly on growth rates observed in long-term experiments (Zobell and Feltham 1938; Walne 1963). Conover (1966a,b) proposed a method by which assimilation efficiency may be determined from the ash-free dry weight to dry weight ratios of food and fecal samples. The method is based on the assumption that only the organic component of the food is significantly affected by digestion and allows an estimation of net assimilation as defined below. The use of $^{14}C$ for the estimation of primary production (Steemann-Nielsen 1952) led the application of $^{14}C$ to studies of trophic relations in aquatic environments (Sorokin 1966), making it possible to trace labeled food in the organism and in its excretory and respiratory products.

The amount of food assimilated or taken up by an organism, expressed as a percentage of the amount of food ingested, is defined as the assimilation efficiency. In this study, a distinction is made between net and gross assimilation: gross assimilation (= ingestion – excretion) is a measure of the amount of energy available to an organism. Bayne and Newell (1983) call it "absorption efficiency" or "absorbed ration". Net assimilation *(= ingestion – excretion – respiration = gross assimilation – respiration)* is the part of ingested material actually built into the animal's body, *i.e.* used for growth and reproduction. This "assimilated ration" (Bayne and Newell 1983) is important for estimating secondary production in an ecosystem.

Because unassimilated food may be released in soluble form, assimilation efficiency calculated from ingestion, respiration, and excretion overestimates the true assimilation efficiency by some amount. This error is difficult to quantify, because it requires measuring dissolved organic carbon *(DOC)* and distinguishing between material released from the gut or feces (*i.e.* unassimilated) and that originating from other surfaces (*i.e.* assimilated, metabolized, and released) (Bayne *et al* 1976).

Studies on assimilation of algae are numerous (Walne 1963; Winter 1970,1978; Foster-Smith 1975a, b; Bayne *et al* 1976; Griffiths 1980; Kiorboe *et al* 1980, Epifanio 1982; Webb and Chu 1982; Amouroux 1986; Amouroux *et al* 1989). When comparing assimilation efficiencies reported by various authors (see Winter 1978), it became apparent that the duration of the experiments and the particle concentration were important influencing factors. Because of differences in experimental design, results vary widely and are difficult to compare.

Growth of bivalves fed exclusively on bacterial cultures provides direct evidence for the nutritional value of bacteria. Zobell and Feltham (1938) documented growth of bivalves fed on bacteria but found differences in digestibility of gram negative and gram positive bacteria. Prieur (1981) reported growth on cultured bacteria, but was unable to detect any difference in growth on and digestion of gram negative and gram positive bacteria.

The use of radioisotopes as tracers in feeding experiments permits more detailed analysis of partitioning in ingested material, but information on the assimilation efficiencies of bivalves fed with bacteria is still scarce. Amouroux (1986) reports assimilation efficiencies of 40-50% for *Venus verrucosa* fed with *Lactobacillus* sp. Langdon and Newell (1990) use an assimilation rate of 52% for their calculations on the food value of bacteria for oysters. They, like Tunnicliffe and Risk (1977), found the contribution of bacterial nitrogen to be more important than bacterial carbon in meeting the respective metabolic requirements of oysters. By comparing different organisms, Tietjen (1980) found that bacteriovores *(Rhabditis marina, Monhystera disjuncta)* assimilate significantly more of the ingested food than herbivores *(Chromadorina germanica)*.

## Influence on Assimilation of Food Quality and Quantity

Assimilation efficiency in bivalves and other organisms depends primarily on the quality (Walne 1963; Conover 1966a,b; Winter 1978; Langdon and Newell 1990) and secondarily on the quantity (Bayne *et al* 1976) of the food ingested. For a given food, assimilation efficiency in various bivalve species is reported to decrease with increasing ingestion rate (Allen 1962; Winter 1969; Foster-Smith 1975b; Bayne *et al* 1976; Kiorboe *et al* 1980; Morton 1983).

The amount of food the digestive gland can handle is believed to be limited. If this limit is exceeded, an increasing proportion of the ingested material does not enter the digestive gland but is channeled directly into the intestine and excreted (Bayne *et al* 1976). Thus the percentage of material excreted rises with ingestion rate (Morton 1983; Allen 1962; Foster-Smith 1975a). Winter (1969) reported that efficiency of protein assimilation by *Modiolus modiolus* was high (80-90%) and was independent of the food concentration. Because most studies use bulk organic matter (*i.e.* whole phytoplankton cells, *etc.*), it is difficult to compare his observations on assimilation with those of others.

## Influence on Assimilation of Body Size and Temperature

Whereas Winter (1978) reports that assimilation efficiency for organic matter is independent of body size and temperature, Bayne *et al* (1976) found that both body size and temperature affected assimilation of suspended matter in *M. edulis*. Since respiration increases with increasing temperature, net assimilation efficiency might be expected to be inversely correlated with temperature. Also, temperature is likely to have a positive effect on the activity of the digestive enzymes. However, this effect appears to be species-specific. Newell *et al* (1977) report that energetic costs in *Ostrea edulis* rise sharply with environmental temperature

temperature increases, whereas they remain essentially constant in *Crepidula fornicata*. They relate this effect to the temperature at which maximum clearance rates are reached. Increased respiration in larger animals is compensated by an increased ingestion rate.

## Factors Affecting Excretion

There is evidence that bivalves have various types of feces. Allen (1962) describes two types of fecal material in *M. edulis*:

- Material that is rejected by the digestive diverticulum and excreted through the intestine which represents the bulk of feces; and
- Thin ribbons of material ejected from the digestive diverticulum.

Dinamani (1969) reported the first type to be excreted rapidly, sometimes within 20 minutes of ingestion, while the second type was expelled 12-20 hours after ingestion by *M. edulis*. It is important to distinguish between these two types of excretory products in studies of assimilation efficiencies. One would expect:

- The first type to be dependent on food concentration (because at high food concentrations the capacity of the digestive diverticulum is exceeded).
- The second type to be dependent on food quality (high quality food will be more completely assimilated in the digestive diverticulum).

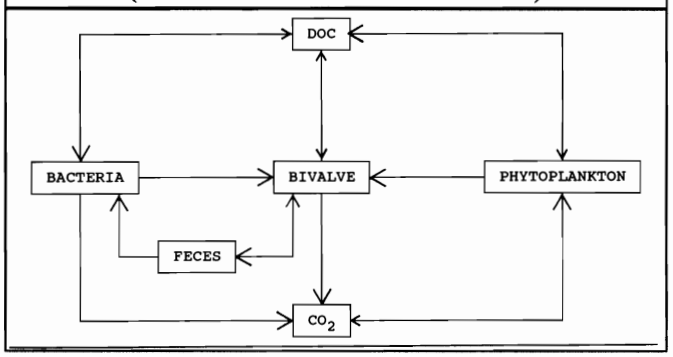
## Role of Suspended Inorganic Particles

In turbid water such as that of San Francisco Bay, the concentration of suspended inorganic matter could influence ingestion, assimilation, energetics, and growth in suspension feeding bivalves. Kiorboe *et al* (1981) found that clearance rates in *M. edulis* increased by 32-43% by the addition of 5 mg silt/L as compared to clearance in a pure algal suspension (0-20,000 cells/mL). Growth rates were increased 30-70%. They concluded that resuspended sediment not only serves as an additional food source by providing attached and bound organic material, but is also necessary for *M. edulis* to fully exploit its clearance potential. Moreover, it was hypothesized that ingested algae are more efficiently digested in the presence of silt in the stomach, thus increasing assimilation efficiency (Kiorboe *et al* 1980; 1981). Griffiths (1980) attributes this to dilution of food by silt particles, whereas Kiorboe *et al* (1981) suggest that silt aids mechanical disruption of food particles in the stomach.

## Using $^{14}C$ to Determine Assimilation Efficiencies

A study of assimilation efficiencies involving the use of $^{14}C$ in a "phytoplankton-bacteria-bivalve" system has to consider the factors described above and the complexity of interactions between the different radiolabeled compartments (Conover and Francis 1973; Amouroux *et al* 1989; Figure 11). Only by knowing the quantitative importance of the various possible metabolic pathways for $^{14}C$ ($DO^{14}C$ – dissolved organic carbon, $^{14}CO_2$, and radiolabel in bivalve feces) can results obtained for the main compartments (bivalves, bacteria, phytoplankton) of such a system be correctly interpreted.

Figure 11
INTERACTIONS IN A
PHYTOPLANKTON-BACTERIA-BIVALVE SYSTEM
(Modified from Amoroux *et al* 1989.)




## $DO^{14}C$

Release of DOC by phytoplankton and its subsequent uptake by bacteria is one of the interactions taking place in the above model. Phytoplankton are known to excrete organic substrates essential for bacterial metabolism (Hellebust 1965; Bell and Sakshaug 1980; and many others). Such products may serve as low molecular weight substrates for unattached bacteria. The amount of organic carbon entering microbial food chains via algal extracellular products has been the subject of numerous studies. However, Sharp (1977) and Coffin and Sharp (1987) report it to be relatively small, especially when the phytoplankton are in the log phase of growth. Bell and Sakshaug (1980) estimated that about 1.9 $\mu$g C/L/day was released during a bloom of *Skeletonema costatum* based on a rate of release of extracellular products by *S. costatum* of 5% of net primary production.

Bacteria are also known to release metabolic products, *e.g.* exopolymers, into their environment (Harvey and Luoma 1985). Depending on the nature of these bacterial exopolymers, they might be utilized by bivalves, other bacteria, and/or algae.

Uptake of dissolved organic compounds such as amino acids and sugars by bivalves has been demonstrated by a number of authors(Swift *et al* 1975; Rice and Stephens 1988; Manahan 1989). In their studies of oysters *(Crassostrea gigas)* Swift *et al* (1975) found glucose uptake rates of 10-15 ng/h/g wet weight at an initial concentration of 50 mg/L. Rice and Stephens (1988) found influx rates in *M. edulis* (wet mass of soft tissue: about 6 g) of 2.03, 1.66 and 1.72 $\mu$mol/h for the amino acids alanine, cycloleucine and taurine, respectively, at initial concentrations of 5 $\mu$M. Normal environmental levels of individual free amino acids in oceanic environments range from $< 0.01$ to $0.2\,\mu$M. The question of whether dissolved organic matter uptake contributes significantly to bivalve nutrition or is simply a mechanism for osmoregulation is still open.

Amouroux *et al* (1989) reported that DOC was released by the bivalve *Abra alba* in their feeding experiments, which ran for 50 hours. These organic compounds are likely to be utilized by heterotrophic bacteria. However, the DOC released is probably of small quantitative importance, and its main role might be osmoregulation (Rice and Stephens 1988).

## $^{14}CO_2$

Bacteria and bivalves release respiratory $CO_2$ into the surrounding water. If experiments are run in light, photosynthetic incorporation of respired $^{14}CO_2$ can take place. Even if experiments are run in the dark, limited uptake of $^{14}CO_2$ by algal cells cannot be excluded. However, isotope dilution by the seawater $CO_2$ pool is probably enough to reduce $^{14}CO_2$ uptake by algae to negligible levels.

## Bivalve Feces

It is possible that $^{14}C$ is released into the water via feces as particulate material (bacteria, undigested organic material). Undigested $^{14}C$ containing organic matter could then be taken up or recycled by bacteria in the system. In his study of assimilation, Allen (1962) carried out control experiments in which feces from feeding experiments using $^{32}P$-labeled algal cells were placed in glass-filtered sea water. No release of $^{32}P$ into the water was observed for more than 48 hours. However, in feeding experiments using radiolabeled bacteria, the possibility of labeled bacteria being excreted and released from the feces into the surrounding water cannot be excluded.

The purpose of the second part of the present study was to determine assimilation efficiencies of *Potamocorbula amurensis* for natural bacterioplankton and for the marine flagellate *I. galbana* by using $^{14}C$-labeled food. This was done to complement the feeding rate study and compare ingestion and assimilation of the two food organisms, making it possible to assess the nutritional significance of bacteria to *P. amurensis*.

# Methods

For the determination of assimilation efficiencies, natural bacterioplankton from San Francisco Bay and *I. galbana* cells were labeled with $^{14}C$. Experiments were carried out with labeled bacteria in the absence and presence of unlabeled *I. galbana* cells, and with labeled *I. galbana* plus unlabeled bacteria. The apparatus was the same as that used for clearance rate measurements. Most experiments were run for 2.5 hours (labeled phytoplankton) and 3 hours (labeled bacteria); however, two prolonged experiments were carried out, one with labeled phytoplankton cells (49 hours) and the other with labeled bacteria (69.5 hours).

## Preparing $^{14}C$-Labeled *I. galbana*

Algae were cultured in f/2 medium, and 8 $\mu$Ci $NaH^{14}CO_3$ were added to 200 mL of culture 3 to 4 days before the experiments (Welschmeyer and Lorenzen 1984). Prior to the experiment, the culture was filtered through 3 $\mu$m Nuclepore filters, washed twice with a few mL of particle-free bay water and resuspended in GF/C-filtered bay water. Care was taken to prevent the filter from going dry to avoid lysis or adhesion of cells to the filter.

## Preparing $^{14}C$-Labeled Bacteria

The protocol for preparing radiolabeled bacteria is essentially that of Hollibaugh *et al* (1980) except that $^{14}C$ amino acids were used instead of methyl-$^{3}H$-thymidine. An amino acid mixture was used rather than a specific compound to provide the highest probability of achieving uniform $^{14}C$ labeling of the cells. Twenty-five $\mu$Ci of algal protein hydrolysate (ICN, specific activity: 50 mCi/mg-atom C) was added to 1. 5 L of GF/C-filtered bay water. The flask was covered and incubated for about 18 hours. Previous work (Hollibaugh, unpublished data) indicated that the bacterioplankton population would double at least once and increase in size during this period, assuring that cells would become labeled with $^{14}C$. Changes in number or size of cells during this period were irrelevant, because this part of the study dealt only with assimilation and not with grazing efficiency. However, clearance rates were calculated from radioisotope uptake data were compared to rates calculated from the decrease in food particle concentration determined by microscopy. Clearance rates calculated from radioisotope uptake were comparable to rates measured in the first part of this study.

Before beginning the grazing experiment, $^{14}C$-labeled bacteria were washed and concentrated to a small volume by filtration through 0.2 $\mu$m Nuclepore filters. It is important that the filter is not sucked dry during the concentration or rinse steps, as the bacteria adhere to the filter under these conditions, resulting in poor recoveries of labeled cells. The suspension of concentrated bacteria was then filtered through a 1-$\mu$m-pore-size Nuclepore filter to remove any clumps of bacteria that may have formed during the concentration and washing steps. About 15% of the $^{14}C$ remained on the Nuclepore filters during the concentration and screening steps. The filtrate contained a mean of 64±15% of the total $^{14}C$ added. The concentrated and screened labeled bacteria were then suspended in 3150 mL (later 3050 mL) of GF/C-filtered bay water. These suspensions were used in the experiments.

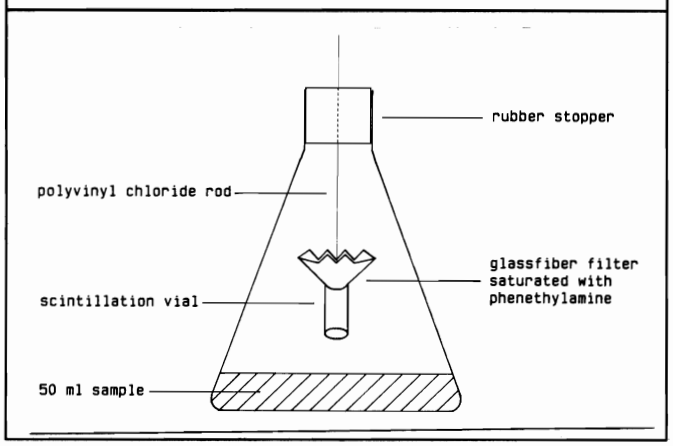
## Determining Particulate $^{14}C$, $^{14}CO_2$, and $DO^{14}C$

Particulate $^{14}C$ was determined by filtering a known volume of sample through 0.45 $\mu$m Millipore filters at < 5-inch Hg vacuum. The filter was rinsed briefly with particle-free bay water, then placed in a scintillation vial with Aquasol 2.

Respired $^{14}CO_2$ and $DO^{14}C$ were determined by the method described by Hobbie and Crawford (1969) as modified by Hollibaugh (1979). The apparatus is shown diagrammatically in Figure 12. Fifty mL samples were filtered through 0.45-$\mu$m-pore-size Millipore filters to remove bacteria and placed in 250-mL conical flasks, which were closed

with rubber stoppers. A piece of polyvinyl chloride rod that was bent into a circular clip at one end was inserted into a hole in the stopper. The clip held a scintillation vial vertically inside the flask a few millimeters above the surface of the filtrate. Half of a 45-mm-diameter glass fiber filter was placed in the mouth of a scintillation vial and saturated with phenethylamine (about 0.2 mL, Packard). The vial was clipped to the stopper, then about 1 mL of concentrated sulfuric acid was carefully pipetted into the bottom of the flask. The flask was immediately stoppered, then swirled to mix the acid and the filtrate. After a minimum of 12 hours, the scintillation vial was removed from the flask. The filter was pushed to the bottom of the vial then radioassayed. Two milliliters of the acidified filtrate was pipetted into another scintillation vial for measurement of $DO^{14}C$.

Figure 12
APPARATUS FOR TRAPPING $^{14}CO_2$




## Measuring $^{14}C$ in Clam Tissue

After rinsing the clams as described above, they were cut open, soft tissue was removed, lyophilized and ground using a mortar and pestle (large clams only), then placed in small centrifuge tubes. The weight of the lyophilized tissue was determined to 1 $\mu$g on a Mettler type H6 scale. Next, 1. 5 mL of hypotonic solution of NaCl (0.08 mol NaCl, 0.01 mol EDTA-$Na_2$, pH 8) was added to lyse cells. After 1 hour, the mixture was centrifuged (5 min, 5900 rpm). The supernatant was removed and placed in a scintillation vial. The pellet was digested with protease (Pronase E; Sigma Chemical Co.) for 2 days at 38.5°C. One hundred $\mu$L of 10 mM $NaN_3$ was added to inhibit bacterial growth. Digested tissue and rinse water were combined with the hypotonic solution and radioassayed.

## Radioassay Methods

All samples were counted using Aquasol 2 (New England Nuclear) as the scintillation cocktail, which was added to samples immediately after collection. Filters were counted in 7 mL glass scintillation vials with 4 mL of scintillation cocktail. Liquids, including digested clams, were pipetted into 20 mL plastic or glass scintillation vials with 8 mL of scintillation cocktail. All samples were counted in a Beckman LS 3801 liquid scintillation spectrometer after having been stored in the dark overnight to allow the filters to clear and chemiluminescence to decay. Corrections for counting efficiency and quenching were made using the Compton edge shift (H#) external standard method and appropriately quenched standards. Radioassay data were converted to disintegrations per minute (DPM) for use in calculations.

## Experimental Protocol for *I. galbana*

Three sets of experiments containing 5 replicates plus 1 control were conducted on the assimilation of $^{14}C$-labeled *I. galbana* by *P. amurensis*. These experiments used a suspension of $^{14}C$-labeled *I. galbana* and unlabeled bacteria. The algal concentration was 180,000 cells/mL in one set and about 47,000 cells/mL in all others. Temperatures ranged from 19-22°C. Based on results of clearance rate experiments, the experiments were run for one hour and used 2 large or 3 smaller clams per beaker containing 500 mL food suspension. This prevented depletion of labeled food particles before the end of the experiments. Each measurement is, therefore, the mean assimilation efficiency of 2 or 3 clams, if all animals were feeding.

To detect bacterial uptake of $DO^{14}C$ released by the phytoplankton cells, 2 mL samples were filtered through 3-$\mu$m and 0.45-$\mu$m filters and radioassayed as described above. In all cases, $DO^{14}C$ uptake by bacteria was below the limit of detection.

## Experimental Protocol for Bacteria

Before each experiment, three 50 mL samples (later one) of the final suspension were used to determine particulate $^{14}C$, $^{14}CO_2$ and $DO^{14}C$ as described above. Experiments with labeled bacterioplankton were run in the absence (4) and presence (4) of unlabeled phytoplankton.

In the first part of the experiment (feeding), labeled bacteria were fed to the animals for 1.5 hours. The subsequent rinse period also lasted 1.5 hours in most experiments. Each experiment used 3 or 4 clams of similar size. The clams were placed in beakers, each containing 500 mL of $^{14}C$-labeled bacterioplankton or $^{14}C$-labeled bacterioplankton plus *I. galbana* (20,000 cells/mL, see below) suspension. The experiments were run as described for clearance rate measurements. Two sets of samples (2 mL) were initially taken every 15 minutes for 1.5 hours. In later experiments this sampling was limited to the beginning and end of the experiments. One set of samples was fixed in buffered formaldehyde (2% final concentration) for subsequent enumeration as described in the clearance rate section.

The second set of 2-mL samples was used to determine particulate $^{14}C$ as described above. The filtrate was retained, and a few drops of concentrated sulfuric acid were added to volatilize $^{14}CO_2$ so that only $DO^{14}C$ remained in the filtrate. Both the filter and the filtrate were radioassayed. One control without clams was run for every set of experiments (5 beakers) to account for changes in $^{14}C$ distribution due to uptake of $DO^{14}C$ by the bacteria and to release of $DO^{14}C$ or respired $^{14}CO_2$ by the bacteria.

The unlabeled *I. galbana* culture was washed and resuspended as described in the clearance rate section. The resuspended algae were then added to the suspension of 14C-labeled bacteria. To account for uptake of radioactive $CO_2$ by *I. galbana*, 2-mL samples were filtered through 3-$\mu$m-pore-size Nuclepore filters. Bacteria adhering to phytoplankton or to the filter were assumed to be negligible. The filtrate was collected in test tubes and subsequently filtered through 0.45-$\mu$m Millipore filters. The $^{14}C$ content of both filters and the filtrate was determined.

## Attachment of Bacteria to Clams

Control experiments were conducted to determine the significance of attachment of labeled bacteria to the clams, which would have caused ingestion rates and assimilation efficiencies to be overestimated. Clams were killed using $NaN_3$, then rinsed with filtered bay water. Four clams were placed in a suspension of labeled bacteria, 3 were placed in 0.2-$\mu$m filtered water from an experiment where radiolabel was present as $DO^{14}C$. The killed clams were incubated for 1.5 hours, then placed in rinse water. After 1.5 hours in the rinse water, radioactivity remaining in clam tissue was measured.

## $DO^{14}C$ Uptake by Bivalves

Bacterioplankton were labeled using algal protein hydrolysate. Despite washing the labeled bacteria on 0.2-$\mu$m Nuclepore filters, some dissolved organic label was always present in the final suspension used in the experiments. Furthermore, we observed that $DO^{14}C$ concentrations in samples taken at the end of the experiments were often slightly lower in the beakers containing clams than in the controls. This suggested uptake of $DO^{14}C$ by bivalves, which has been reported by others (Swift *et al* 1975; Rice and Stephens; 1988; Manahan 1989). Significant uptake of $DO^{14}C$ would cause ingestion rates and assimilation efficiencies to be overestimated.

Accordingly, a set of experiments was conducted to determine the extent of $^{14}C$ organic matter uptake by the clams. Filtrate from the bacteria washing process was diluted with 0.2-$\mu$m Nuclepore-filtered bay water. The filtrate contained $^{14}C$-labeled amino acid mixture that had not yet been metabolized by the bacteria, as well as respired $^{14}CO_2$- and $^{14}C$-labeled metabolites released by the bacteria. Activity of the $DO^{14}C$ in the control experiments was adjusted to about 450 dpm/mL. This is an average of the activities present in the feeding experiments (290-645 dpm/mL). Three or four clams were placed in each beaker, and experiments were run as described above. The control contained 3 clams that had been killed with $NaN_3$ and placed in $DO^{14}C$ with their valves open.

## Determining Excretion and Respiration

After 1.5 hours (labeled bacteria) or 1 hour (labeled phytoplankton), the animals were transferred into unlabeled suspensions of bacteria or algae and bacteria (250 mL) and left there for 1.5 hours to excrete $^{14}C$- containing feces. The gut passage time of *P. amurensis* is reported to be 37.6±16 minutes (Alan Deco, U.S. Geological Survey, personal communication ). While the clams were rinsing, 50-mL samples were taken from each beaker containing the radiolabeled food suspension and filtered through 0.45-$\mu$m Millipore filters. Particulate $^{14}C$, $^{14}CO_2$ and $DO^{14}C$ were determined as described above. Feces were collected on a 3-$\mu$m Nuclepore filter using a Pasteur pipette, then sucked dry.

The $^{14}CO_2$ content of rinse water at the end of the rinse period, determined as described above, was used to calculate the respiration rate of *P. amurensis*. The $DO^{14}C$ content of rinse water was very small, and $DO^{14}C$ uptake by unlabeled bacteria and the resulting production of $^{14}CO_2$ by bacterial respiration was considered negligible. Feces produced in the rinse beaker were collected, as described above, and measured separately.

## Prolonged Experiments

Two experiments were carried out to investigate excretion and respiration of labeled food over a period of days. In one experiment, labeled *I. galbana* and unlabeled bacteria were fed to the animals (50,000 cells/mL, fed 1 hour, rinsed 48 hours, 20-22°C). In the other one, food consisted of labeled bacteria and unlabeled *I. galbana* (30,000 cells/mL, fed 1.5 hour, rinsed 68 hours, 20-22°C).

The experiments were conducted as described above except that after being fed radiolabeled food, all clams were transferred into one vessel containing 1 liter of unlabeled food suspension. Unlabeled bacterioplankton and *I. galbana* cells were continuously supplied over 48 and 68 hours. Care was taken to maintain relatively constant food concentrations throughout the experiments by transferring the animals into fresh unlabeled feeding suspension (GF/C-filtered bay water with added algal cells) every time samples were taken. Particulate $^{14}C$, $^{14}CO_2$, $DO^{14}C$, and $^{14}C$ in animals and in feces were determined as described above.

## Calculations

Total ingestion was calculated by adding the radiolabel found in the feces and the $^{14}CO_2$ respired during the experiment to the radiolabel found in the clam tissue according to the following equation:

$$I = A + R + E$$

where:

$I$ = Ingestion
$A$ = Assimilation
$R$ = Respiration
$E$ = Excretion

Clams that did not open their valves during the initial feeding period and that contained <500 dpm were excluded from the calculations (see below). Gross and net assimilation, respiration, and excretion were determined as percentages of ingestion or of gross assimilation according to the definitions given above. These parameters are time-dependent as long as radiolabel is excreted or respired by the bivalves. The arithmetic means and standard deviations of

the results obtained in the different experimental groups (labeled bacteria with and without unlabeled phytoplankton, labeled phytoplankton) were tested for statistically significant differences with one-tailed t-tests.

## Results

### Assimilation of *I. galbana*

Neither net nor gross assimilation of *I. galbana* or bacteria was correlated with animal size. Most animals were feeding during the experiments, which were usually conducted in the dark. An estimated 90% of the feces excreted by the clams within 2.5 or 3 hours was collected, which means ingestion values are slightly underestimated. Production of pseudofeces was not observed.

Four sets of experiments were carried out on assimilation efficiencies of *P. amurensis* fed *I. galbana*. Table 7 presents results of these experiments. Results of one experiment (7 May 1990) were not used in calculations because the feeding period was 1.5 hour instead of 1 hour, as in all other short-term experiments, and because the concentration of *I. galbana* cells was not determined. At a concentration of 47,000 cells/mL of *I. galbana*, net assimilation 2.5 hours after beginning to feed on $^{14}C$-labeled algal cells was 82.9±3.4% (n = 10) of ingestion. Gross assimilation was 94.2±3.1% of ingestion. Respiration and feces production were 12.1±1.4% and 5.8±3.1% of the total ingestion, respectively.

Table 8 compares the results of experiments with high and low cell concentrations (180,000 and 47,000 cells/mL, respectively). Gross and net assimilation were significantly lower and feces production and the amount of radioactivity detected in the feces was significantly higher in experiments with high cell concentrations. Respiration as a percentage of gross assimilation was also lower at high *I. galbana* concentrations.

### *Respired $^{14}CO_2$*

Respiration rates were obtained by extrapolating the rate of respiratory $^{14}CO_2$ production during the rinse period to the feeding period. Because there is probably a lag before labeled $CO_2$ is released by the clams, this assumption might lead to slight overestimations of true rates of ingestion, gross assimilation, and respiration and underestimation of net assimilation. $^{14}CO_2$ was also measured at the end of the incubation with radiolabeled *I. galbana*. This $^{14}CO_2$ was attributed to clam respiration, since changes in $^{14}CO_2$ concentrations in controls without clams were small and inconsistent (Table 9).

$^{14}CO_2$ release by bacteria in experiments with $^{14}C$-labeled *I. galbana* was probably negligible, since $DO^{14}C$ was present in only small amounts (excretion of $DO^{14}C$ by the algal cells was below the limit of detection). Particulate $^{14}C$ in control beakers showed no consistent change during either the feeding or rinse periods of these experiments. Release of $^{14}CO_2$ by phytoplankton respiration was believed to be negligible because they were rapidly consumed by the bivalves and $^{14}CO_2$ release in controls was negligible.

Table 7
ASSIMILATION OF RADIOLABELED *ISOCHRYSIS GALBANA* BY *P. AMURENSIS*

| Date | Temp (°C) | Concentration (cells/mL) | Duration (hours) | Length (cm) | Gross Assimilation | Net Assimilation | Respiration %I | Respiration %A | Excretion % |
|---|---|---|---|---|---|---|---|---|---|
| 05/07/90 | 20-22 | Unknown | 3 | 1.9 | 86.6 | 70.4 | 16.2 | 19 | 13.4 |
| | | | | 1.25 | 95.8 | 72.1 | 21 | 22.5 | 7.1 |
| | | | | 1.65 | 90.6 | 71.4 | 19.2 | 21 | 9.4 |
| | | | | 1.05 | 95.4 | 78.5 | 17 | 18 | 4.6 |
| | | | | 1.2 | 95.8 | 76.8 | 19 | 20 | 4.2 |
| 05/24/90 | 19-20 | 180,000 | 2.5 | 1.3 | 88 | 79.4 | 8.6 | 9.7 | 12 |
| | | | | 1.2 | 84.3 | 75.4 | 8.9 | 10.5 | 15.7 |
| | | | | 0.9 | 76.6 | 67.1 | 9.4 | 12.3 | 23.4 |
| | | | | 1.5 | 83.3 | 73.5 | 9.8 | 11.8 | 16.7 |
| | | | | 2.0 | 87.6 | 79.5 | 8.5 | 9.7 | 12.0 |
| 06/01/90 | 19-20 | 46,400 | 2.5 | 1.2 | 96 | 85.2 | 10.8 | 11.2 | 4.0 |
| | | | | 1.3 | 97.4 | 85.4 | 12.0 | 12.3 | 2.6 |
| | | | | 1.2 | 96.6 | 84 | 12.6 | 13.1 | 3.4 |
| | | | | 1.0 | 95 | 82.5 | 12.5 | 13.1 | 5.0 |
| | | | | 1.2 | 86.5 | 74.9 | 11.6 | 13.4 | 13.5 |
| 06/10/90 | 21-22 | 47,000 | 2.5 | 1.15 | 95.2 | 86.6 | 8.6 | 9.1 | 4.8 |
| | | | | 1.0 | 92.8 | 81.1 | 11.7 | 12.6 | 7.2 |
| | | | | 1.45 | 92.2 | 80.9 | 11.3 | 12.2 | 7.8 |
| | | | | 1.3 | 95.9 | 85.8 | 10.1 | 10.6 | 4.1 |
| | | | | 1.1 | 94.5 | 82.1 | 12.4 | 13.1 | 5.5 |

Temp = Incubation temperature.
Duration = Duration of experiment, sum of feeding + rinse periods.
Length = Average shell length of clams in a replicate.
Gross and Net Assimilation = Assimilation of $^{14}C$ expressed as a percentage of ingestion (see text).
Respiration = Respiration as percentage of ingestion (I) and gross assimilation (A).
Excretion = Excretion (feces only) as percentage of ingestion.

Changes in $^{14}CO_2$ concentrations measured during experiments with labeled *I. galbana* are presented in Table 9. Control values have been subtracted from the respective values for replicates containing clams. The rates of $^{14}CO_2$ production were greater in the feeding period than the rinse period in all experiments except one, where the control value was unusually high. $^{14}CO_2$ must, therefore, be released very shortly after *P. amurensis* starts feeding on labeled algal cells. $^{14}CO_2$ release also appears to decrease rapidly once the clams are no longer consuming radiolabeled food.

### *Prolonged Experiment with Labeled I. galbana*

Results of this experiment are presented in Figures 13 and 14. After 49 hours, the radiolabel collected as feces was 10% of ingestion and gross assimilation was 90.0%. Respired $^{14}CO_2$ was 41.6% of gross assimilation, and net assimilation was 52.6% of ingestion. This is in contrast to net assimilation values of 82.9% (47,000 cells/mL) and 75% (180,000 cells/mL) seen in the 2.5-hour experiments.

Fifty percent of $^{14}C$-labeled feces were excreted during the first 2.5 hours. A peak in labeled feces production was observed around 24 hours after feeding the clams radiolabeled *I. galbana*. Following this peak, only small quantities of radiolabel were found in the feces, but radiolabel continued to be excreted. Of the total quantity of labeled feces produced during 49 hours, 86% had been collected 30 hours after transfer to nonlabeled water (Figure 13).

Release of labeled dissolved organic matter appeared to coincide with excretion of labeled feces, but the amount of $DO^{14}C$ produced was at the limit of detection. Particulate radiolabel collected during the rinse period was negligible (data not shown).

$^{14}CO_2$ is released very soon after the animals start feeding on the labeled cells. During the first 2.5 hours, 22.3% of the $^{14}CO_2$ produced by the clams during the experiment was released: 42.5% during the first 7 hours, and 83.4% after 31 hours (Figure 14).

To compare long- to short-duration experiments, rates in the long-duration experiment were calculated for a 2.5-hour period (Table 10). After 2.5 hours, gross assimilation was 95.0% and net assimilation was 85.2%; feces represented 5.0% of ingested labeled cells. Respired $^{14}CO_2$ amounted to 8.8% of gross assimilation. These results compare well to values for the short-duration experiments except that the calculated percentage of food respired as $^{14}CO_2$ after 2.5 hours in the long-duration experiment is lower than the value determined directly in the short-duration experiment.

Table 8
ASSIMILATION EFFICIENCIES OF *P. AMURENSIS* FED *I. GALBANA* DETERMINED 2.5 HOURS AFTER INITIATING FEEDING

| Concentration (cells/mL) | Gross Assimilation | Net Assimilation | Respiration % A | Excretion % I | Number of Replicates |
|---|---|---|---|---|---|
| 180,000 | 84.0±4.6 | 75.0±5.1 | 10.8±1.2 | 16.0±4.7 | 5 |
| 47,000 | 94.2±3.1 | 82.9±3.4 | 12.1±1.4 | 5.8±3.1 | 10 |
| t-test | $p<0.028$ | $p<0.002$ | $p<0.007$ | $p<0.0001$ | |

Gross and Net Assimilation = Mean and standard deviation of assimilation efficiencies as percentages of ingestion.
Respiration = Respiration as a percentage of gross assimilation.
Excretion = Excretion (feces only) as a percentage of ingestion.
t-test = Statistical significance of the difference between parameters.

Table 9
$^{14}CO_2$ RELEASE IN EXPERIMENTS WITH LABELED *I. GALBANA*

| Control | Replicate Number I | II | III | IV | V |
|---|---|---|---|---|---|
| + 630 | (1880)<br>650 | (1790)<br>870 | (2550)<br>760 | (1080)<br>880 | (1000)<br>940 |
| +1810 | (–)<br>555 | (100)<br>530 | (–)<br>315 | (1100)<br>425 | (510)<br>430 |
| – 440 | (1450)<br>735 | (1850)<br>965 | (1350)<br>810 | (1530)<br>535 | (1460)<br>400 |
| + 210 | (980)<br>350 | (360)<br>270 | (700)<br>350 | (750)<br>430 | (840)<br>365 |

Values are given in dpm/mL.
Numbers in parentheses represent the labeled feeding period; values without parentheses were measured in the rinse water (1.5 hour).

Figure 13
PRODUCTION OF $^{14}C$-LABELED FECES DURING A PROLONGED EXPERIMENT WITH *I. GALBANA*

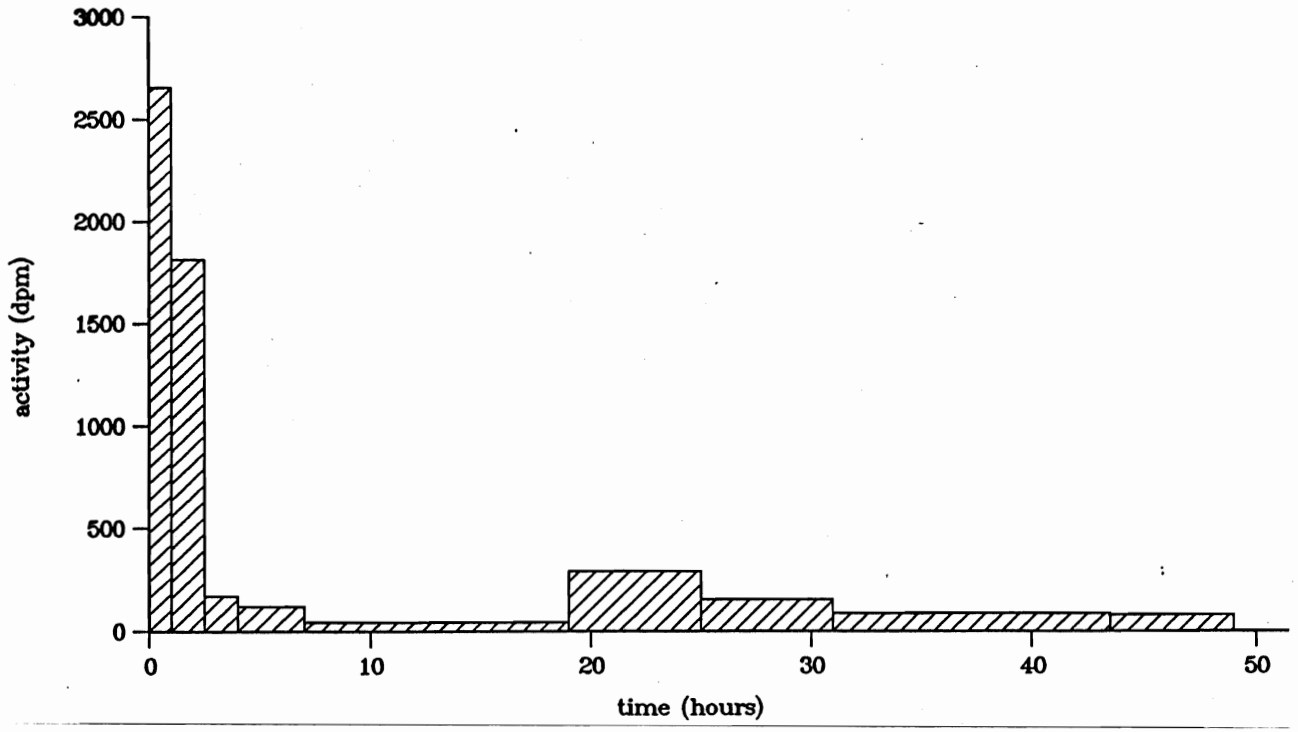



Figure 14
PRODUCTION OF $^{14}CO_2$ DURING A PROLONGED EXPERIMENT WITH *I. GALBANA*

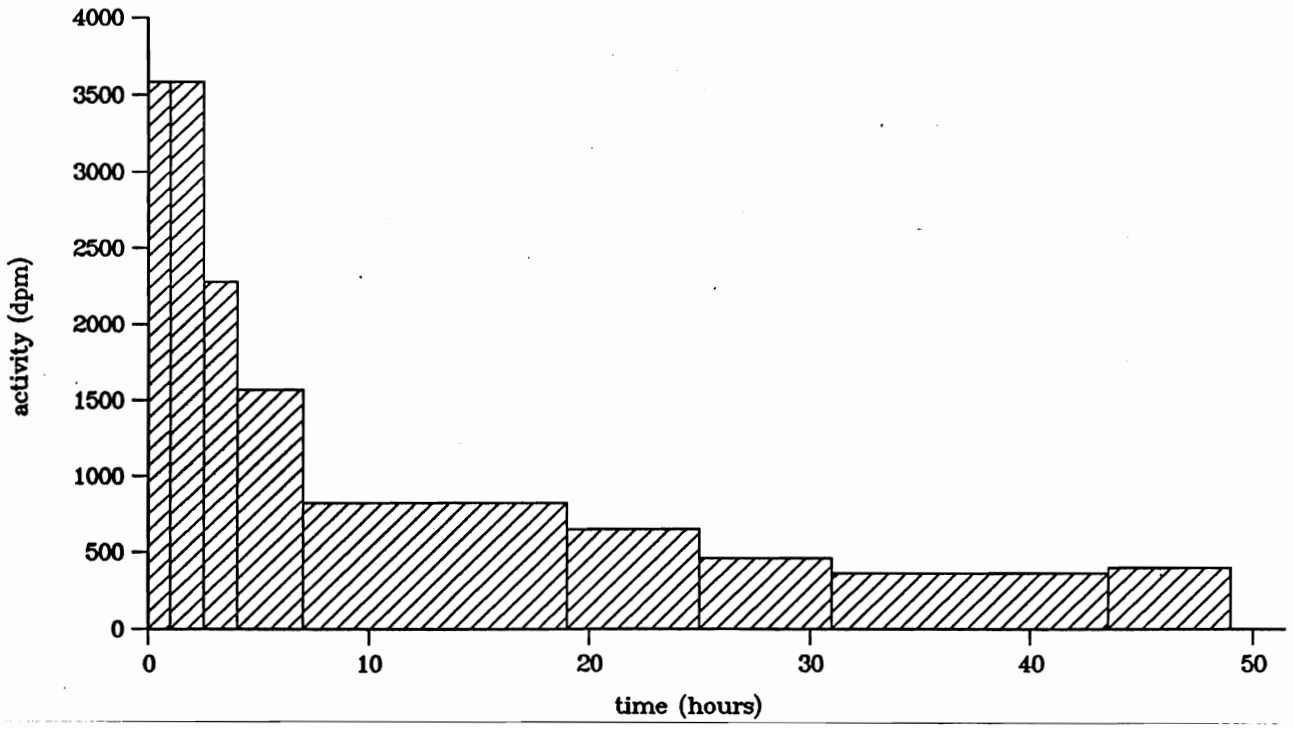


Table 10
CHANGE IN ASSIMILATION EFFICIENCIES OF *I. GALBANA* AFTER A PROLONGED RINSE PERIOD

| Duration (hours) | Gross Assimilation | Net Assimilation | Respiration % | Excretion % |
|---|---|---|---|---|
| 2.5 | 95% | 85.2% | 8.8 | 5.0 |
| 49 | 90% | 52.6% | 41.6 | 10.0 |

Clams used were 1.4, 1.5, 1.35, 1.2, 1.45, 1.45, 1.25, and 1.3 cm in shell length.
Duration = Time after beginning the feeding period.
Gross and Net Assimilation = Gross and net assimilation as a percentage of ingestion
Respiration = Respired $^{14}CO_2$ as a percentage of gross assimilation.
Excretion = Excretion (feces only) as a percentage of ingestion.

A possible explanation for this difference is simply error associated with detecting near background $^{14}C$ activities. In the short-term experiments, radiolabeled $CO_2$ was measured in the rinse water separately for each treatment involving 3 or 4 clams. Activities measured were low, and background counts were a larger proportion of total counts, decreasing the precision, and possibly the accuracy, of rates calculated in short-duration experiments. In extended experiments, all clams were placed in one container to rinse, and only one sample was taken. Higher activities were obtained. Therefore, we assume the prolonged experiment provided better estimates for the true respiration rate and assimilation efficiencies.

## Assimilation of Natural Bacterioplankton

Results of these experiments are presented in Table 11. Table 12 compares assimilation efficiencies in the presence and absence of *I. galbana*. Gross assimilation of bacteria was 90.7±5.0% (n=8) of ingestion after 3 hours when only bacteria were available as a food source, and 86.6±8.5% (n=16) when unlabeled *I. galbana* was added to the suspension. Experiments with 1.5-hour rinse periods were excluded from the calculations in Table 12. Respired $^{14}CO_2$ for both food suspensions was 25.1±5.6% of gross assimilation at the end of the experiments. After 3 hours, net assimilation was 68.8±5.0% and 65.2±9.1% of ingestion, respectively. The quantity of $^{14}C$ excreted in the feces increased with increasing food concentration from 9.3±5.0% (n=8) to 13.4± 8.6% (n=16) in the presence of algae. Although higher food concentration (+ *I. galbana* treatments) decreased bacterioplankton assimilation and increased excretion, these differences were not statistically significantly (Table 12). Bacteria concentrations were about 1-2 x $10^6$ cells/mL throughout the experiments.

### *Respired $^{14}CO_2$*

The mean respiration rate calculated for experiments with *I. galbana* present was 21.4±4.3% of total ingestion and 25.1±5.6% of gross assimilation (= ingestion – feces). These rates were then used to calculate ingestion and assimilation values for the rest of the experiments. Based on the lack of a significant difference in the clam's respiration rate when the concentration of labeled phytoplankton cells varied, it was assumed that the rate of respiration of bacterial cell *C* would be constant regardless of the presence or absence of phytoplankton cells.

Respiration data from the feeding period were inconclusive and were not included in the calculations. The trend during experiments with only bacteria was that rates were lower than those found during the rinse period. Variation was high, and it was difficult to determine the source of the $^{14}CO_2$ (bacteria or clams). An increase in the lag phase for $^{14}CO_2$ production when bacteria were the radiolabeled food or inhibition of bacterial respiration by the feeding activity of the clams (Amouroux 1986) are among the factors that might be responsible for the lower values. A more likely explanation is that $^{14}CO_2$ production might have been overestimated by assuming a linear increase during the feeding period.

Data obtained by measuring $^{14}CO_2$ in the 3-$\mu$m filtrate of control beakers at the beginning and end of the experiments were inconclusive, and uptake of $^{14}CO_2$ by *I. galbana* was not consistently detected (Table 13). The experiments were kept in darkness except when sampling, and the algal cells were consumed rapidly by *P. amurensis*. We conclude that uptake by unlabeled phytoplankton of $^{14}CO_2$ respired by bacteria did not affect results of these experiments.

There was a general decrease of in the amount of label in bacteria during the course of control experiments with one exception (Table 14). Loss values ranged from –7.9% to –28.2% of the label initially present, with a mean of –13.8± 8.6% (calculated from Table 14). Some of this decrease can be attributed to respired $^{14}CO_2$, since $^{14}CO_2$ concentrations in the control beakers increased throughout the experiments. These results do not affect the calculation of assimilation efficiencies, since respiration was calculated from respiration during the rinse period, when no radiolabeled bacteria were present.

### *Attachment of Labeled Bacteria to the Clams*

Table 15 presents the results of experiments to determine whether attachment of labeled bacteria to clams resulted in a significant error in apparent ingestion rates. The results indicate that attachment was a negligible source of error. Based on these results, clams were declared non-feeding when radioactivity in clam tissue was lower than 500 dpm.

Table 11
ASSIMILATION OF $^{14}C$-LABELED BACTERIOPLANKTON BY *P. AMURENSIS* IN THE PRESENCE (*) OR ABSENCE OF 20,000 CELLS/mL UNLABELED *I. GALBANA*

| Date | Temperature (°C) | Duration (hours) | Length (cm) | Gross Assimilation | Net Assimilation | Respiration %I | Respiration %A | Excretion % |
|---|---|---|---|---|---|---|---|---|
| 03/01/90 | 16.5-17 | 2.25 | 1.9 | 93.3 | 75.9 | 17.4 | | 6.7 |
| | | | 1.4 | 94.3 | 76.9 | " | | 5.7 |
| | | | 1.5 | 94.9 | 77.5 | " | | 5.1 |
| | | | 1.75 | 95.8 | 78.4 | " | | 4.2 |
| | | | 2.0 | 74.6 | 57.2 | " | | 25.4 |
| 03/03/90 | 18 | 2.5 | 1.2 | 94.9 | 76.0 | 18.9 | | 5.1 |
| | | | 1.3 | 97.0 | 78.1 | " | | 3.0 |
| | | | 1.6 | 98.5 | 79.6 | " | | 1.5 |
| | | | 1.9 | 96.6 | 77.7 | " | | 3.4 |
| 03/06/90 | 17-19 | 3.0 | 1.3 | 92.9 | 71.0 | 21.9 | | 7.0 |
| | | | 1.6 | 94.7 | 72.8 | " | | 5.3 |
| | | | 1.55 | 94.2 | 72.3 | " | | 5.8 |
| | | | 1.7 | 89.0 | 67.1 | " | | 11.0 |
| | | | 1.8 | 92.9 | 71.0 | " | | 7.1 |
| 03/17/90 | 18-20 | 3.0 | 1.85 | 88.2 | 66.3 | 21.9 | | 11.8 |
| | | | 1.55 | 93.9 | 72.0 | " | | 6.1 |
| | | | 1.6 | 79.8 | 57.9 | " | | 20.2 |
| 03/27/90* | 19-20 | 3.0 | 1.4 | 94.2 | 72.3 | 21.9 | | 5.8 |
| | | | 1.2 | 90.2 | 70.5 | 19.7 | | 9.8 |
| 04/02/90* | 18-20 | 3.0 | 1.9 | 87.1 | 51.8 | 35.3 | 40.5 | 12.8 |
| | | | 1.7 | 85.2 | 63.3 | 21.9 | 25.7 | 14.8 |
| | | | 1.6 | 96.7 | 70.9 | 25.8 | 26.6 | 3.3 |
| | | | 1.3 | 97.5 | 78.2 | 19.3 | 19.8 | 2.5 |
| | | | 1.5 | 96.4 | 74.5 | 21.9 | 22.7 | 3.6 |
| 04/10/90* | 20-22 | 3.0 | 1.7 | 92.9 | 71.3 | 21.6 | 23.3 | 7.1 |
| | | | 1.0 | 82.3 | 65.4 | 16.9 | 20.6 | 17.7 |
| | | | 2.1 | 79.5 | 61.1 | 18.4 | 23.2 | 20.5 |
| | | | 2.2 | 73.8 | 51.7 | 22.1 | 30.0 | 26.3 |
| | | | 2.15 | 86.6 | 67.1 | 19.5 | 22.5 | 13.4 |
| 04/17/90* | 19-21 | 3.0 | 1.3 | 83.4 | 65.2 | 18.2 | 21.9 | 16.6 |
| | | | 1.0 | 67.6 | 45.2 | 22.4 | 33.1 | 32.5 |
| | | | 2.2 | 80.6 | 62.9 | 17.7 | 22.0 | 19.4 |
| | | | 1.9 | 91.5 | 72.3 | 19.2 | 20.9 | 8.5 |

Date = Date the experiment was performed.
Duration = Length of experiment, sum of feeding period + rinse period.
Length = Average shell length of clams in a replicate.
Gross and Net Assimilation = $^{14}C$ assimilation efficiency as a percentage of ingestion.
Respiration = Respiration as percentage of ingestion (I) and gross assimilation (A).
Excretion = Excretion (feces only) as percentage of ingestion.

Table 12
COMPARISON OF ASSIMILATION EFFICIENCIES FOR BACTERIOPLANKTON BY *P. AMURENSIS* IN THE PRESENCE AND ABSENCE OF UNLABELED *I. GALBANA*

| Concentration of *I. galbana* (cells/mL) | Gross Assimilation | Net Assimilation | Respiration % A | Excretion % I | Number of Replicates |
|---|---|---|---|---|---|
| 0 | 90.7±5.0 | 68.8±5.0 | 25.1 | 9.3±5.0 | 8 |
| 20,000 | 86.6±8.5 | 65.2±9.1 | 25.1±5.6 | 13.4±8.6 | 16 |
| t-test | $p>0.1$ | $p>0.1$ | $>0.1$ | $p>0.1$ | |

Raw data are from Table 11, definitions of parameters are as given in that table.
t-test = Significance of a one-tailed t-test of the difference between the means of the parameters.

Table 13
$^{14}CO_2$ UPTAKE BY *I. GALBANA* DURING EXPERIMENTS WITH RADIOLABELED BACTERIA

| Date | $T_o$ (dpm/mL) | $T_E$ (dpm/mL) | %Change |
|---|---|---|---|
| 03/27/90 | 122 | 269±29 | +120 |
| 04/02/90 | 751±116 | 750±56 | – |
| 04/10/90 | 108±51 | 184±112 | – |
| 04/17/90 | 306±61 | 197±15 | – 64 |

Mean and S.E. of $^{14}CO_2$ activity in the 3-$\mu$m fraction was measured in the control beakers at the beginning ($T_o$) and the end ($T_E$) of the feeding period.

Table 14
LOSS OF $^{14}C$ FROM BACTERIA AND INCREASE OF DISSOLVED $^{14}CO_2$ IN CONTROL BEAKERS

| | Bacteria | | | $^{14}CO_2$ | | |
|---|---|---|---|---|---|---|
| Date | $T_o$ | $T_E$ | Change | $T_o$ | $T_E$ | Change |
| 03/03/90 | 1494 | 1184 | – 310 | 4265 | 5677 | +28 |
| 03/06/90 | 4060 | 2915 | – 1145 | 5871 | 5412 | – 9 |
| 03/17/90 | 3610 | 4045 | + 435 | 6209 | 6721 | +10 |
| 03/27/90 | 1071 | 973 | – 98 | 7962 | 8871 | +18 |
| 04/02/90 | 3578 | 3276 | – 302 | 7723 | 9781 | +41 |
| 04/10/90 | 4683 | 4290 | – 393 | 7793 | 9275 | +30 |
| 04/17/90 | 4270 | 3935 | – 335 | 11701 | 12899 | +24 |

$T_o$ = Beginning of feeding period.
$T_E$ = End of feeding period (1.5 hour).
Change = $T_o$ minus $T_E$.
Units are DPM/mL in all cases.

Table 15
ATTACHMENT OF RADIOLABELED BACTERIA TO CLAMS KILLED WITH $NaN_3$

| Length (cm) | Temperature (°C) | Bacteria (DPM/mL) | $DO^{14}C$ (DPM/mL) | Tissue (DPM/Clam) |
|---|---|---|---|---|
| 1.6 | 18-20 | 3,600 | 600 | 388 |
| 1.6 | " | " | " | 429 |
| 1.6 | " | " | " | 231 |
| 1.5 | " | " | " | 250 |
| 1.6 | 19-20 | 0 | 400 | 59 |
| 1.5 | " | " | " | 271 |
| 1.65 | " | " | " | 95 |

$^{14}C$ content of clam tissue was determined after 1.5 hours exposure to $^{14}C$-labeled bacteria and/or $DO^{14}C$ and 1.5 hours of rinse.
Length = Shell length.
Temperature = Temperature at which experiment was run.

## *$DO^{14}C$ Uptake by Bivalves*

The amount of radiolabel recovered in clam tissue after exposure to $DO^{14}C$ is given in Table 16. The result of a preliminary experiment with a much higher $DO^{14}C$ concentration is also shown. *P. amurensis* appears to be able to take up $DO^{14}C$ directly from the water. The average uptake of $DO^{14}C$ from $^{14}C$-labeled algal protein hydrolysate was calculated (1716 DPM/clam, 450 DPM/mL, 50 $\mu$Ci/$\mu$mole C, 5 C atoms/amino acid, 125 $\mu$g/$\mu$mole average molecular weight for amino acids in algal protein hydrolysate) to be 3.1 x $10^{-3}$ nmole amino acid/clam in 1.5 hours (about 0.39 ng amino acid/clam) at a radiolabeled amino acid concentration of 0.8 nM (100 ng/L). In the experiment with high $DO^{14}C$ concentrations (46 nM, 5.8 $\mu$g/L) the greatest uptake of labeled amino acid in 1.5 hours was 0.76 nmol/clam (95 ng/clam). Both radiolabeled feces and $CO_2$ were detected in some of these experiments.

The nutritional significance of this mechanism is unclear. The amounts of amino acids taken up are small, yet calculations were performed assuming the specific activity of the amino acid mixture reported by the manufacturer. If, as is highly probable, the specific activities were lower, perhaps due to unlabeled amino acids present in the bay water used for the experiments or released by clams, the amounts of amino acids taken up would be larger.

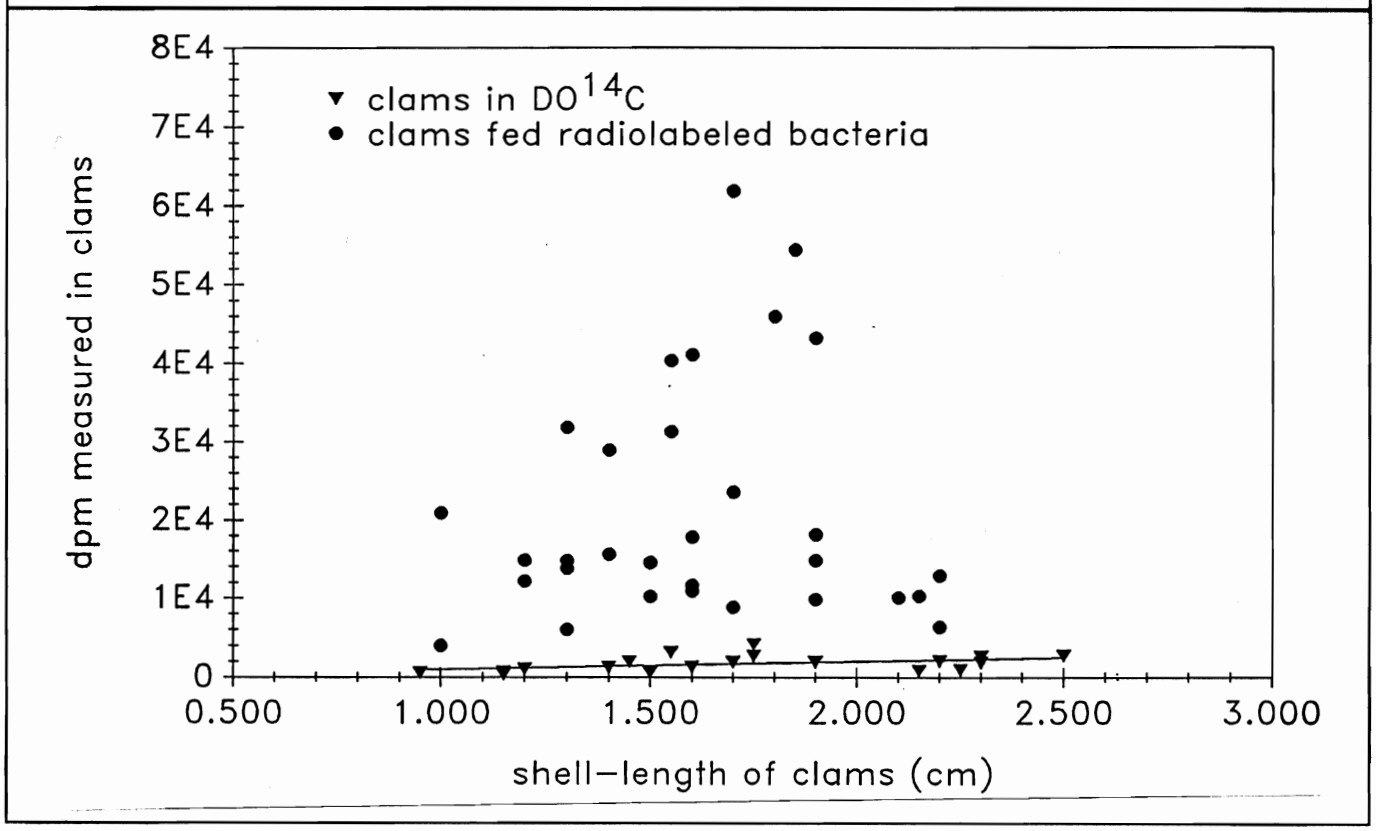
The extent to which $DO^{14}C$ uptake might influence results of assimilation experiments is illustrated in Figure 15, which compares $^{14}C$ content of clams fed labeled bacteria to clams exposed only to $DO^{14}C$. $DO^{14}C$ concentrations were similar in all experiments (about 450 dpm/mL). The activity of the labeled bacteria in the feeding experiments varied. It can be seen that $DO^{14}C$ uptake by the bivalves is negligible in most cases, resulting in, at most, slight overestimation of bacteria *C* assimilation.

Table 16
UPTAKE OF $DO^{14}C$ BY *P. AMURENSIS*

| Length (cm) | Temperature (°C) | $DO^{14}C$ (dpm/mL) | Clams (dpm) | Feces (dpm) | Rinse Water $TD^{14}C$(dpm/mL) | Rinse Water $^{14}CO_2$(dpm/50mL) |
|---|---|---|---|---|---|---|
| 1.7 | 18-19 | 25,638 | 168,680 | 8,186 | 1,600 | – |
| 1.7 | | | 269,738 | | | |
| 1.7 | | | – 2,108 | | | |
| 1.7 | | | 423,947 | | | |
| 1.65 | 17-19 | 280 | 2,353 | 913 | – | – |
| 1.65 | | | 1,152 | | | |
| 1.55 | | | 2,637 | | | |
| 1.55 | | | 4,366 | | | |
| 1.45 | 18-20 | 450 | 1,953 | – | – | 211 |
| 1.15 | | | 365 | | | (23% of Uptake) |
| 0.95 | | | 550 | | | |
| 1.15 | | | 672 | | | |
| 1.4 | | | 1,247 | – | – | Not Measured |
| 1.5 | | | 628 | | | |
| 1.2 | | | 1,064 | | | |
| 1.55 | | | 3,131 | | | |
| 1.75 | | | 4,117 | – | – | 517 |
| 1.7 | | | 1,920 | | | (19.6% of Uptake) |
| 1.75 | | | 2,663 | | | |
| 1.9 | | | 1,927 | | | |
| 2.2 | | | 2,053 | – | – | 417 |
| 2.25 | | | 945 | | | (23.1% of Uptake) |
| 2.3 | | | 2,607 | | | |
| 1.6 | | | 1,318 | | | |
| 2.5 | | | 2,727 | – | – | Not Measured |
| 2.15 | | | 851 | | | |
| 2.3 | | | 1,867 | | | |
| Dead Clams | | | | | | |
| 1.6 | 17-19 | 280 | 59 | – | – | |
| 1.5 | | | 271 | | | |
| 1.65 | | | 95 | | | |

These experiments were performed in April 1990.
Length = Shell length of clams.
$TD^{14}C$ = Total dissolved $^{14}C$.

Figure 15
COMPARISON OF $DO^{14}C$ UPTAKE TO INGESTION OF $^{14}C$-LABELED BACTERIOPLANKTON IN FEEDING EXPERIMENTS




*Prolonged Experiment With Labeled Bacterioplankton*

Table 17 and Figures 16, 17, and 18 present results of an experiment to examine partitioning of bacterial $^{14}C$ by *P. amurensis* over a period of days. To facilitate comparison with the prolonged experiment using labeled phytoplankton and with short-duration experiments, rates were calculated for 3, 49.5, and 69.5 hours both excluding (A) and including (B) particulate $^{14}C$ found in rinse water, which was high in this experiment (see below).

Labeled feces collected within the first 3 hours represented 53% of the total labeled feces collected over the duration of the experiment (Figure 16). After 30 hours of rinse, 90% of the total labeled feces had been excreted. We saw the same excretion peak around 24 hours that was observed with labeled phytoplankton (Figure 13).

Release of $DO^{14}C$ and production of particulate $^{14}C$ coincided with peaks in feces excretion. $DO^{14}C$ production was negligible with respect to the $^{14}C$ mass balance; therefore, it was not included in further calculations. The origin of the $DO^{14}C$ is unclear. It is possible that soluble organic compounds are excreted by the bivalves at the same time as feces are released. The extent to which excreted labeled compounds have been altered by the clams' metabolism is not known.

Some of the particulate $^{14}C$ may have resulted from uptake of $DO^{14}C$ by bacteria in the rinse water (Figure 17). This material could also be due simply to fragmentation of feces into particles too small to be detected with the naked eye and, therefore, were not collected, or to labeled bacteria that passed through the clams without being digested or bound into fecal pellets or strings. The particulate fraction of rinse water in the corresponding experiment with labeled phytoplankton contained negligible $^{14}C$. Whatever mechanism, the only possible source of the label is the clams. We conclude that this material was derived from feces and have included it as excreted $^{14}C$ in calculations of assimilation efficiencies. We assumed that the particulate $^{14}C$ was undigested bacteria released from feces and calculated the amount of particulate radiolabel that should have been produced during the feeding period by extrapolation from the first 1.5 hours of rinse. This was added to the inventory of excreted $^{14}C$.

Of the total amount of $^{14}CO_2$ released during the experiment (69.5 hours), 36% was collected within the first 3 hours of the experiment, 58% after 7.5 hours, and 87% after 31.5 hours (30 hours rinse) (Figure 18). After 49.5 hours, 94% had been released. $^{14}CO_2$ continued to be released after 68 hours, albeit at a very low rate. Compared to the short-term experiments with labeled bacterioplankton, production of respiratory $^{14}CO_2$ and feces are low, similar to the amounts produced in experiments with labeled *I. galbana*. In both experiments that included extended rinses, respiration rates are about two-thirds of the rates determined in short-duration experiments, probably due to counting errors associated with the small amounts of $^{14}CO_2$ released in the short-duration experiments, as discussed above.

Table 17
RESULTS OF THE LONG-TERM EXPERIMENT WITH LABELED BACTERIA PERFORMED IN JULY 1990

| | Duration (hours) | Gross Assim % | Net Assim % | Respiration % | Excretion (%) Feces | Excretion (%) Particles |
|---|---|---|---|---|---|---|
| (A) | 3 | 97.4% | 82.7% | 15.1 | 2.9 | |
| | 49.5 | 95.3% | 56.6% | 40.6 | 4.7 | |
| | 69.5 | 95.1% | 54.0% | 41.2 | 4.9 | |
| (B) | 3 | 90.1% | 79.6% | 11.7 | 1.8 | 8.1 |
| | 49.5 | 72.9% | 44.7% | 38.6 | 3.4 | 23.8 |
| | 69.5 | 69.1% | 39.2% | 43.3 | 3.5 | 27.4 |

Clams used had shell lengths of 1.25, 1.25, 1.1, 1.15, 2.0, 1.15 and 1.0 cm.
Respiration = Respiratory $^{14}CO_2$ as a percentage of gross assimilation.
Excretion = Excretion as a percentage of ingestion.
Fecal material was readily visible to the unaided eye, particles were collected on 0.45 m pore size membrane filters.
In (A) particles were not included in the feces inventory. In (B) they were.

Figure 16
PRODUCTION OF $^{14}C$-LABELED FECES DURING A PROLONGED EXPERIMENT WITH BACTERIOPLANKTON

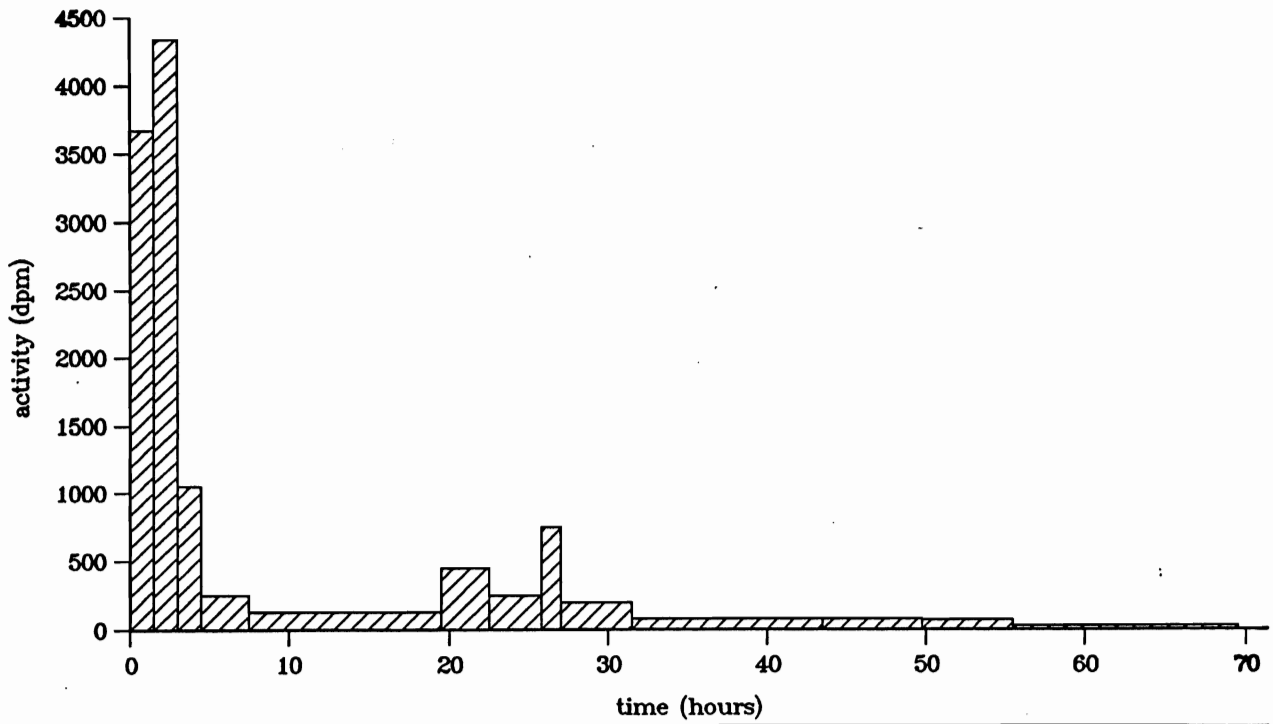


Figure 17
PRODUCTION OF $^{14}C$-LABELED PARTICULATE MATTER IN RINSE WATER DURING A PROLONGED EXPERIMENT WITH BACTERIOPLANKTON

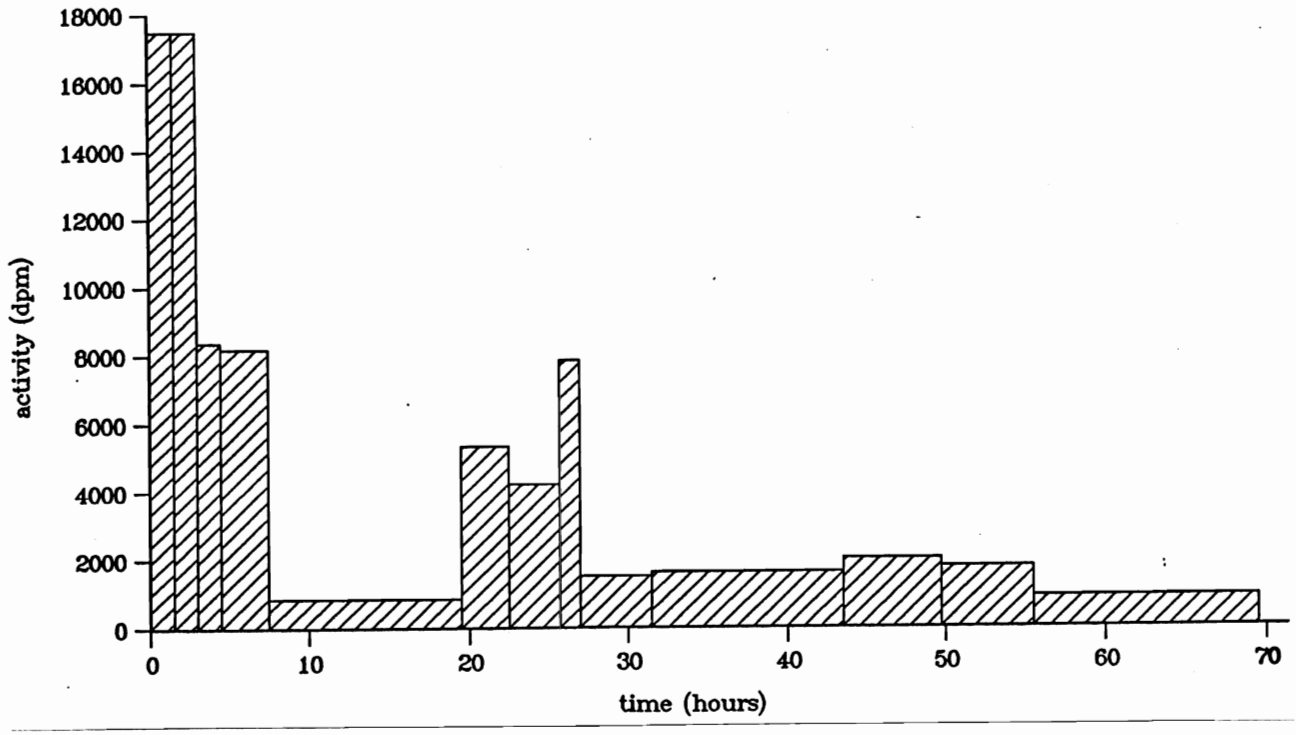
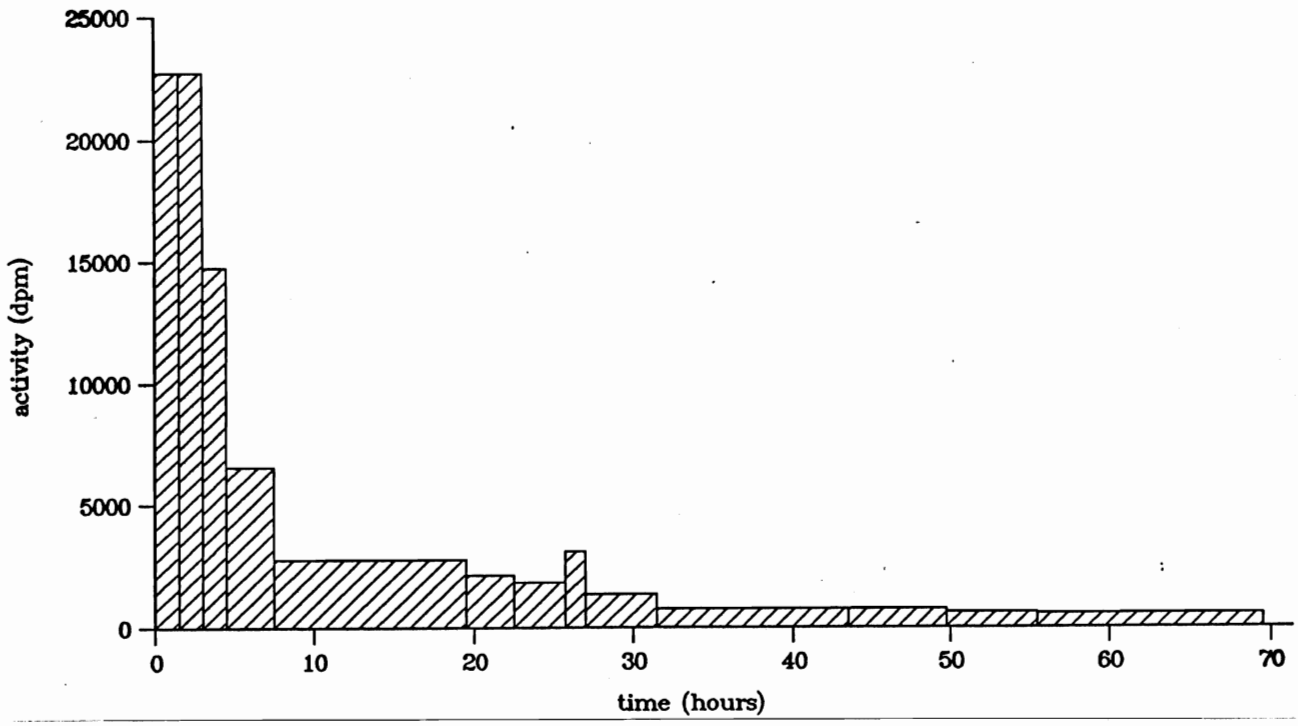



Figure 18
PRODUCTION OF $^{14}CO_2$ DURING A PROLONGED EXPERIMENT WITH BACTERIOPLANKTON



## Discussion

The terms "gross assimilation" and "net assimilation" have to be applied with caution to experiments of short duration because of the limited time bivalves have had to process their food. Most of the assimilation efficiencies reported above represent processing periods of only 2.5 or 3 hours after the bivalves started feeding on radiolabeled food particles. They can be used to compare utilization of *I. galbana* and bacterioplankton, but probably overestimate long-term assimilation rates. We intentionally conducted experiments with prolonged rinse periods (48 and 68 hours) to obtain better estimates of the true assimilation efficiencies for bacterioplankton and *I. galbana*.

The experiments described above show that bacteria are readily assimilated by *P. amurensis* and suggest bacterial $^{14}C$ is respired more quickly than that of *I. galbana*. Net assimilation efficiencies are similar for the two food organisms used.

*I. galbana* is reported in the literature to be a good food source for bivalves (Davis 1953; Walne 1963). Net and gross assimilation efficiencies of *P. amurensis* for *I. galbana* found in this study are comparable to rates observed in other studies with different bivalves and food organisms.

Although only one set of experiments used high concentrations of *I. galbana* (180,000 cells/mL), it was obvious that assimilation efficiency decreased and excretion increased with increasing food concentration. Similar observations were made in the experiments with bacteria in the presence and absence of phytoplankton, although the difference could not be demonstrated statistically. Both $^{14}C$-labeled feces production and $DO^{14}C$ excretion increased with higher food concentration. Pseudofeces production was not observed in any experiments we performed. *Crassostrea virginica* begins to produce pseudofeces at a *Chlorella* (5 $\mu$m, about the same size as *I. galbana*) cell density of 450,000 cells/mL (Winter 1978).

Gross assimilation is the amount of food that is assimilated from the gut or that enters the digestive diverticulum of the animal. It includes some compounds that are rapidly utilized and respired by the animal. Most of the fecal matter excreted within the first 1.5 hours after the animals were fed is material that passed directly through the stomach and gut and did not undergo thorough digestion. The bulk of feces consists of this rejected material (Allen 1962), and the production of this portion of the feces is most influenced by food concentration. The degree to which nutrients in this fraction are taken up while passing through the intestine is not known.

Indigestible material that enters the digestive diverticulum generally appears as feces within a few days (Allen 1962; Dinamani 1969). The amount excreted is dependent on the food value of the ingested material; *i.e.* the greater the content of indigestible material, the greater the amount excreted. The prolonged experiments carried out in this study, both with labeled algae and labeled bacteria, confirmed Dinamani's (1969) observations for other bivalves that this "secondary excretion" occurs after about 24 hours.

Gross assimilation efficiencies in short experiments were similar for both bacteria and *I. galbana* (Tables 8 and 12) and lower in experiments with high concentrations of food organisms. This suggests more efficient utilization of food when it is scarce. Gross assimilation did not change appreciably with time, whereas net assimilation decreased due to respiration. During the first few hours of the experiments, more $^{14}CO_2$ was respired when $^{14}C$-labeled bacteria were fed than in experiments with radiolabeled *I. galbana*, suggesting bacterial *C* is metabolized more readily. Higher respiration rates due to increased pumping activity are unlikely, since respiration of bacterial and *I. galbana* $^{14}C$ was measured in experiments that contained about the same amount of bacteria and *I. galbana*. This may have been due to differences in the labeling patterns resulting from use of $^{14}C$ amino acids versus $NaH^{14}CO_3$ or due to differences in the composition and digestibility of truly uniformly labeled bacterioplankton and algae.

These data suggest that food ingested by *P. amurensis* is rapidly metabolized. According to Prieur (1981), distribution of bacteria through the digestive tract is very rapid in *M. edulis*. In his experiments, cultured bacteria were found intact in the stomach 15-30 minutes after feeding commenced. In both extended experiments, the fractions of bacterial and algal $^{14}C$ respired after 49 hours and 69.5 hours were similar. Further losses of $^{14}C$ from the clams' tissue are likely to be small. If particulate $^{14}C$ in the rinse water is considered as excreted matter, gross assimilation efficiency for bacteria is lower than for *I. galbana*. Net assimilation found in the extended experiments probably comes close to true net assimilation rates, since loss of $^{14}C$ by both respiration and excretion was small after about 26 hours.

Assimilation efficiencies for natural bacterioplankton are rarely reported in the literature. Langdon and Newell (1990) cite Crosby (1987, PhD thesis, Univ. of Maryland), who found a net assimilation efficiency of 52% for cultured cellulolytic bacteria in *Crassostrea virginica*. Amouroux *et al* (1989) report a net assimilation efficiency of 40-50% in *V. verrucosa* fed with cultured *Lactobacillus* sp. Saunders (1969) reported assimilation efficiencies between 52% and 14% for *Daphnia* fed cultured bacteria. The net assimilation efficiency for natural bacterioplankton that we found in the extended experiment is comparable to that reported by Amouroux *et al* (1989).

The effect of suspended inorganic particles on assimilation rates of *P. amurensis* has not been examined. San Francisco Bay, especially Suisun Bay, is very turbid (photic zone depths of tens of centimeters are common [Cloern *et al* 1985]) due to suspended inorganic particulate material. It has been suggested that diluting food with inorganic particles increases assimilation (Griffiths 1980). However, this mechanism does not seem applicable at the low phytoplankton concentrations found in northern San Francisco Bay.

It is not surprising that bacteria are readily assimilated once they are ingested by the bivalve. As early as 1938, Zobell and Feltham (1938) observed bacteria in different stages of lysis in stomach contents of mussels *(M. edulis)* fed with dense bacteria suspensions (500-5000 x $10^6$ cells/mL). They isolated digestive enzymes that lyse bacteria from the mussels' viscera and reported resistance to digestion for acid-fast Mycobacteria, Actinomyces-like rods and spores of *Bacillus* sp. Prieur (1981) found that gram-negative *Vibrio*-type

bacteria were less sensitive to enzymatic digestion in *M. edulis*, but others were digested extracellularly in the stomach. Intracellular digestion of bacteria is described by Cheng and Rudo (1976). Its importance, according to the authors, lies in its function as a defense mechanism, but they also point out its contribution to the nutrition of *Crassostrea virginica*. Glycogen was synthesized from bacterial constituents and first detected in the body tissues 16 hours after injection of the bacteria.

We can compare the contribution of bacterioplankton and phytoplankton *C* to *P. amurensis* biomass production as follows.

Clearance rates calculated in Table 6 are assumed to be the same as ingestion rates (*i.e.* no pseudofeces production).

The net assimilation found in this study (from Tables 10 and 17 after 49 hours of rinse, with particulate $^{14}C$ in rinse water assumed to be of fecal origin) and the ingestion rate are then used to calculate the daily net assimilation of *C*.

Clam biomass, in mg *C*, is calculated from Nichols and Thompson's (personal communication) regression of afdwt against shell length by assuming that 50% of afdwt is *C*.

Finally, the length of time, in days, required for a clam to double its biomass can be approximated by dividing biomass by daily net assimilation.

Results of these calculations are given in Table 18. It can be seen that clams can attain reasonable growth rates on phytoplankton, but that growth on bacterioplankton alone would be slow or negligible. This study was not designed to investigate assimilation of nitrogen, and the question of the importance of bacterial *N* for *P. amurensis*' growth remains open.

Table 18
COMPARISON OF THE CONTRIBUTION OF BACTERIOPLANKTON AND PHYTOPLANKTON TO *P. AMURENSIS* GROWTH IN LABORATORY EXPERIMENTS AND IN SUISUN BAY
(As described in text.)

| Shell Length (cm) | Food Type | Ingested (μg C/d) | Net Assimilation Efficiency (%) | Net *C* Assimilation (μg C/d) | Biomass (mg C/clam) | Biomass Turnover (d) |
|---|---|---|---|---|---|---|
| Laboratory Experiments | | | | | | |
| 1.0 | Bacterioplankton | 25.9 | 44.7 | 11.6 | 2.63 | 227 |
| | Phytoplankton | 480 | 52.6 | 252 | 2.63 | 10.4 |
| 2.0 | Bacterioplankton | 25.9 | 44.7 | 11.6 | 18.45 | 1590 |
| | Phytoplankton | 1051 | 52.6 | 553 | 18.45 | 33.4 |
| Suisun Bay | | | | | | |
| 1.0 | Bacterioplankton | 42.3 | 44.7 | 18.9 | 2.63 | 139 |
| | Phytoplankton | 305 | 52.6 | 160 | 2.63 | 16.4 |
| 2.0 | Bacterioplankton | 42.3 | 44.7 | 18.9 | 18.45 | 976 |
| | Phytoplankton | 667 | 52.6 | 351 | 18.45 | 52.6 |

Chapter 4

# CONCLUSIONS

Results of the present study show that the Asian bivalve *Potamocorbula amurensis* is able to retain natural bacterioplankton (< 1.2 $\mu$m) more efficiently than other bivalve species investigated so far. The average clearance rate for bacteria found was 45 mL/h/clam, which represented a retention efficiency of 29%-13% for clams of 1-2 cm shell length, compared to retention of the marine flagellate *I. galbana* (about 5 $\mu$m). Whereas the clearance rates on *I. galbana* were highly correlated with animal size, no correlation with shell length could be detected for clearance rates on bacterioplankton. Bacteria were readily assimilated by *P. amurensis*. Gross assimilation was 73% after 49 hours, compared to 90% for *I. galbana*. Net assimilation was 45% when feeding on bacteria and 53% when feeding on the flagellate. Bacterial carbon appeared to be respired faster than the algal carbon. As seen in other bivalves, feces production increased at higher food concentration, and assimilation efficiencies decreased accordingly.

It can be concluded that natural bacterioplankton constitute an additional, albeit minor under most circumstances, food source for *P. amurensis*. Its significance is dependent on bacteria concentrations and the abundance of other food sources. During times of low phytoplankton biomass, as found in recent years in northern San Francisco Bay, the ability to derive nutrition from bacterioplankton could give *P. amurensis* a competitive advantage over other bivalves. However, the small ration that bacterioplankton appear to supply suggests production of *P. amurensis* in Suisun Bay continues to be fueled by the limited phytoplankton production in that embayment, or possibly by detritus, dissolved organic material, or indirectly by bacterioplankton production processed through the microbial loop. If it is assumed that nanoflagellates can be grazed by *P. amurensis* with the same efficiency as *I. galbana*, and that nanoflagellates assimilate bacterioplankton biomass with a net efficiency of 50%, about half of the bacterioplankton production in Suisun Bay (0.5 * 100 g C/m$^2$/yr or 50 gC/m$^2$/yr) is available to *P. amurensis*. This is 2.5 times the present phytoplankton production in Suisun Bay (Alpine and Cloern 1991).

# ACKNOWLEDGMENTS

The authors would like to thank the Department of Water Resources for providing support for this study through contract B-57360 to J.T. Hollibaugh. Ingeborg Werner would like to thank Studienstiftung des Deutschen Volkes for their support of her studies. We would both like to thank Ms. Jan Thompson, Mrs. Pat Wong, and Dr. S. Obrebski for their help and advice and J. Orsi and Z. Hymanson for their review of an earlier draft of the report.

This study formed the basis of an M.S. thesis submitted to the Fakultät für Biologie an der Albert-Ludwigs-Universität, Freiburg im Breisgau by Ingeborg Werner.

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