Vol. 197: 181–192, 2000 | MARINE ECOLOGY PROGRESS SERIES
Mar Ecol Prog Ser | Published May 12

# Digestive kinematics of suspension-feeding bivalves: modeling and measuring particle-processing in the gut of *Potamocorbula amurensis*

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ABSTRACT: Particle digestion in lamellibranch bivalves is partitioned between 2 paths, an 'intestinal' path through the stomach and intestine and a 'glandular' path through the stomach, digestive gland and intestine. In the Asian clam *Potamocorbula amurensis* (Schrenck, 1867), the relative importance of the intestinal path increases compared to the glandular path as food availability and ingestion rate increases. The effects of changes in food availability and ingestion rate on digestive partitioning are at least as important as the effect of changes in diet observed by other investigators. Analyses of residence-time distributions of inert 9 and 44 µm particle tracers show that the gut of *P. amurensis* can be modeled as an ideal mixing reactor (stomach and digestive gland) and an ideal plug-flow reactor (intestine) in series. This model appears to be valid for the processing of particles ≤9 µm in size. For particles of ≥15 µm, the ideal mixing component of the model must be modified to account for channeling of particles through the stomach to the intestine. Larger particles can enter the digestive gland, but are probably not phagocytized for intracellular digestion. Instead they may clog the ducts and tubules, limiting phagocytosis of smaller particles and potentially reducing the extent of digestion and absorption. Mixing, and the resultant intragut particle-sorting thus appear to be necessary components of a digestive strategy that incorporates intracellular digestion.

KEY WORDS: Bivalve · Digestion · Gut residence time · Gut model · Internal particle sorting · Suspension feeding

## INTRODUCTION

Much researfch on suspension-feeding bivalves has focused on quantifying diets and aspects of ingestion (e.g. Widdows et al. 1979, Kiørboe et al. 1980, Cole et al. 1992, Werner & Hollibaugh 1993, Ward et al. 1998). The existence of detailed summaries of gut anatomy (e.g. Purchon 1977) and schematic compartmental models for bivalve guts (e.g. Widdows et al. 1979, Decho & Luoma 1996) suggests there is a similar amount of quantitive information about digestion and particle-processing in bivalve guts. However, much of what is known or assumed about the kinematics of digestion in lamellibranch bivalves is derived from qualitative studies that are primarily anatomical or histological, and often static (e.g. Yonge 1926, Owen 1955, Van Weel 1961, Mathers 1972, Owen 1974). There are relatively few observations and measurements related to aspects of gut kinematics (e.g. Bayne et al. 1987, 1989, Hawkins et al. 1990, Decho & Luoma 1991).

Information about patterns and rates of particle-processing within bivalve guts is difficult to obtain, in part because the guts of lamellibranch bivalves are structurally complex. A bivalve's gut has 4 main components, a tubular esophagus, an expanded sac-like stomach (including a crystalline style and style sac), digestive gland, and a tubular intestine. Digestion is both extracellular and intracellular (Purchon 1977). Ingested particles bound in a mucus string pass down the esophagus and into the bivalve stomach, where the mucus string is disaggregated and particles can be broken down by physical and chemical processes.

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From the stomach there are 2 paths of material-processing, an 'intestinal' path and a 'glandular' path (Yonge 1926, Owen 1955, Van Weel 1961, Purchon 1977). Particles processed via the intestinal path pass through the stomach to the intestine and are subjected only to extracellular digestion during gut passage. Particles processed via the glandular path pass from the stomach to the digestive gland and are subjected to both extracellular digestion (in the stomach) and intracellular digestion (in the digestive gland). Particulate wastes from the digestive gland are shunted from the digestive gland ducts to the intestine where they may again be subjected to extracellular digestion before being egested. Rates and patterns of particle movement within a bivalve's gut and the partitioning of material between the intestinal and glandular paths affect extents of digestion and absorption (Van Weel 1961, Bayne & Newell 1983, Decho & Luoma 1996), but these processes and factors that affect them are usually not quantified directly.

I use tracer experiments to describe digestive kinematics of the Asian clam *Potamocorbula amurensis* (Schrenck, 1867), a eulamellibranch bivalve. *P. amurenesis* is the dominant benthic suspension feeder in regions of San Francisco Bay (Carlton et al. 1990, Alpine & Cloern 1992), and some limited gut tracer data exist for it (Decho & Luoma 1991). In addition, it has been demonstrated or inferred to exploit a variety of food resources ranging in size from bacteria to copepod larvae (Alpine & Cloern 1992, Werner & Hollibaugh 1993, Kimmerer et al. 1994). I quantify how particle size and ingestion rate affect patterns and rates of particle movement within the gut of *P. amurenesis* and how they affect partitioning of material between the intestinal and glandular paths. Reactor theory and gut reactor models (Penry & Jumars 1987) provide the context for this study of gut kinematics. The theory is used in interpretation of tracer residence time distributions to derive a gut reactor model that is applicable to lamellibranch bivalves with gut morphologies similar to *P. amurenesis*.

## MATERIALS AND METHODS

*Potamocorbula amurensis* (Schrenck, 1867) were collected from San Francisco Bay in October 1997. Shell lengths ranged from 10 to 14 mm. Clams were held at 16°C and 26 psu and were fed a maintenance diet of laboratory-cultured algae, *Isochrysis galbana* (Prymnesiophyceae) (CCMP 462, Provasoli-Guillard National Center for Culture of Phytoplankton, West Boothbay Harbor, Maine) and *Rhodomonas salina* (Cryptophyceae) (CCMP 1319), and a slurry of field-collected surface sediment until used in experiments.

Clams were transferred to individual 400 ml beakers filled with a suspension of *Rhodomonas salina*, and were acclimated to 20°C for 24 h before use in experiments. My previous work with *Potamocorbula amurensis* showed that it acclimates easily and quickly to this temperature change. Only clams that showed evidence of overnight feeding (presence of fecal material) and had siphons extended were selected for use in experiments.

Dual-tracer experiments were designed to examine the effects of particle size and ingestion rate on particle residence-time and patterns of particle-processing in the gut of *Potamocorbula amurensis*. Two sizes of fluorescent polystyrene beads were used as ingested tracers:

A small bead tracer, 9 µm blue fluorescent beads (Polysciences, Warrington, Pennsylvania; 8 to 12 µm equivalent spherical diameter), was used as a tracer of the movement of particles of the size of *Rhodomonas salina*. It was selected to be as close as possible in size to *R. salina* (5 to 8 µm equivalent spherical diameter) and yet still be distinguishable from *R. salina* when bead and algal mixtures were enumerated using an electronic particle-size analyzer (Coulter Multisizer II).

A large bead tracer, 44 µm yellow-green fluorescent beads (Polysciences, Warrington), was selected as a test particle for use in comparisons with the 9 µm beads. The choice of 44 µm polystyrene beads was based on several considerations. Beads of this size fall in the size range of large phytoplankton and microzooplankton that *Potamocorbula amurensis* can encounter in San Francisco Bay (Cloern 1996). Pretests had shown that clams would readily ingest 44 µm beads and, if bead concentrations were kept low, they would not reject them as pseudofeces. I wanted to estimate the residence-time distribution of particles in the stomach unaffected by subsequent passage through the digestive gland. Although large, heavy particles (e.g. sand grains) generally seem to be moved quickly to the ciliary rejection gutter in the stomach and shunted out of the stomach to the intestine (Yonge 1926, Owen 1955, Van Weel 1961, Purchon 1977), I expected that the 44 µm polystyrene beads, while large, might be light enough to be retained and mixed with bulk stomach contents instead of being shunted to the ciliary gutter. I also expected that 44 µm particles, by virtue of their large size, would be unlikely to enter the digestive gland.

Clam ingestion rates were manipulated by varying food concentrations. Three concentrations of *Rhodomonas salina* were used: 3000 cells $ml^{-1}$ (low-food), 6000 cells $ml^{-1}$ (mid-food) and 12 000 cells $ml^{-1}$ (high-food). *R. salina* is a common component of spring bloom communities in San Francisco Bay (Cloern 1996), and the high-food concentration is typical of

phytoplankton concentrations observed during blooms in San Francisco Bay (Cole et al. 1992). Pretests had shown that these food concentrations did not result in significant production of pseudofeces. Pseudofeces were loose, amorphous, mucus-bound clumps of material and were easily distinguished from the well-formed cylindrical strings of fecal material.

Seven experimental runs were performed during January and February 1998. Individual clams were used only once. Actively feeding clams were randomly assigned to 400 ml beakers containing 300 ml of low-, mid- or high-food concentrations and were allowed to feed for 30 min before being transferred to experimental beakers containing 300 ml of the same food concentration with beads added. At each food level there were 2 bead treatments: algae + 9 µm beads or algae + 9 µm and 44 µm beads together. In 1 run there was a third bead treatment, algae + 44 µm beads. Bead concentrations were generally 5 to 10% of algal concentrations.

A pulse-chase tracer protocol was used. After feeding for 30 min on low-, mid- or high-food suspensions with beads, each clam was quickly rinsed with a gentle stream of seawater to remove any beads adhering to its shell and then transferred to a beaker containing the same food concentration without beads. Clams were transferred to beakers with fresh food suspensions at 30 min intervals for the next 8 h. At the end of 8 h clams were monitored at 2 to 3 h intervals for the next 6 to 8 h and then at 8 to 12 h intervals for 48 h or longer, until no or very few beads were collected in the feces.

An electronic particle-size analyzer (Coulter Multisizer II) was used to measure particle concentrations in experimental beakers before clams were added and after clams were removed, and in control beakers with food suspensions without clams. Ingestion rates were calculated for each clam for each 30 min interval using the equations of Marin et al. (1986) and then averaged over the first 8 h of the experiment. Pseudofeces production can affect both feeding-rate estimates and descriptions of tracer residence-time distributions, but very little pseudofeces production was noted during the experiments. When pseudofeces were observed, they were removed and discarded so they would not affect tracer residence-time distributions.

After particle concentrations had been measured, suspensions were sonicated to disaggregate fecal strings and then filtered onto grey polycarbonate filters (25 mm diameter, 0.2 µm pore size; Poretics, Livermore, California). The filters were stored in the cold and dark for later enumeration by epifluorescence microscopy. Examination of the filters showed that sonication was effective in disaggregating fecal strings and that beads were usually evenly distributed on the filters.

## RESULTS

### Relationships among particle concentration, ingestion rate, and median gut-residence time

Ingestion rate of *Potamocorbula amurensis* increased with increasing particle concentration (Fig. 1). The median algal ingestion rate was significantly greater for clams in the high-food treatment ($8.8 \times 10^5$ cells clam$^{-1}$ h$^{-1}$; n = 33) than for clams in the low ($3.1 \times 10^5$ cells clam$^{-1}$ h$^{-1}$; n = 21) and mid ($3.6 \times 10^5$ cells clam$^{-1}$ h$^{-1}$; n = 26) food treatments (Kruskal Wallis, $p < 0.001$; followed by Conover's method for multiple comparisons [Conover 1980]). Since there was no significant difference between median ingestion rates for clams in the low- and mid-food treatments, these groups of clams were combined in subsequent analyses.

Gut throughput time is the time required for an individual to process 1 gut volume of ingested food (Penry & Jumars 1987). I use median gut-residence time, the time for egestion of 50% of ingested beads, as a measure of gut throughput time. I chose median gut-residence time instead of mean gut-residence time because egestion and fecal sampling were both discontinuous, and egested bead counts are thus best assigned to a time interval. The length of collection intervals also varied, and feces were aggregated over longer time intervals after the first 8 h of the experiment. The residence time distributions thus are described with less accuracy at longer residence times than shorter ones, and use of median residence times is a way to minimize the potential effects of any associated biases.

The rate at which material moves through an organism's gut is directly related to its ingestion rate when ingestion is steady, even when the volume material in the gut decreases as a result of digestion and absorp-

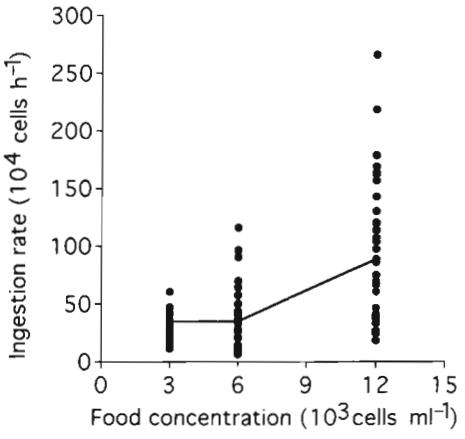


Fig. 1. *Potamocorbula amurensis*. Individual ingestion rate as a function of concentration of *Rhodomonas salina*. Line connects median data points

tion. Gut throughput time, measured as median gut-residence time, should be inversely related to ingestion rate. I predicted that median gut-residence time of 9 µm beads (tracers of bulk gut contents) would decrease with increasing ingestion rate.

The correlation between median gut-residence time and ingestion rate is negative but not significant when data for all clams are combined (n = 76) (Kendall's tau, p = 0.45). However, the relationship observed between ingestion rate and food concentration suggests that determinants of ingestion rate may be different for clams in the low- and mid-food treatments and clams in the high-food treatment, so I re-examined this prediction for clams in the low- and mid-food treatments as a group (Fig. 2A), and for clams in the high-food treatment as a group (Fig. 2B). There was no correlation between median gut residence time of 9 µm beads and ingestion rate among clams in the low- and mid-food treatments (n = 47) (Kendall's tau, 1-tailed test, p = 0.35), but there was, as predicted, a significant negative correlation between median gut-residence time of 9 µm beads and ingestion rate for clams in the high food treatment (n = 33) (Kendall's tau, 1-tailed test, p = 0.011).

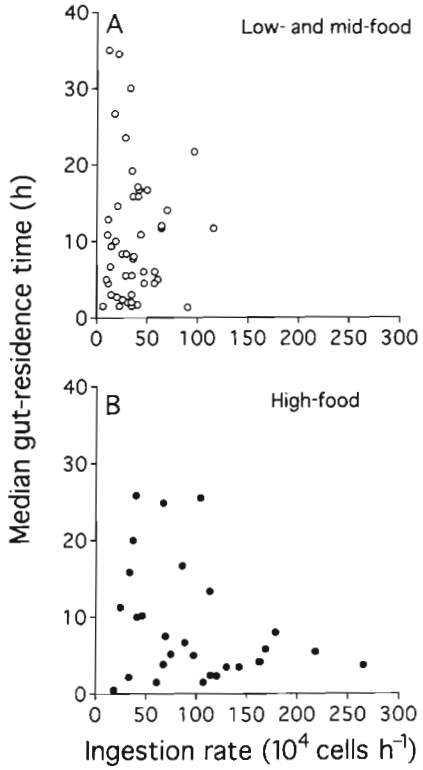


Fig. 2. *Potamocorbula amurensis*. Individual median gut-residence time vs individual ingestion rate for (A) clams in low- and mid-food treatments and (B) clams in high-food treatment. Median gut-residence time: time for egestion of 50% of ingested 9 µm beads

### Effects of particle size on patterns and rates of particle-processing within gut

When clams ingested both 9 and 44 µm beads, I expected that 44 µm beads would pass through the gut more quickly than 9 µm beads because I expected 9 µm beads to enter the digestive gland and 44 µm beads to bypass it. I used cross-correlation analysis (SYSTAT 1992) to look for relationships between time series of 9 and 44 µm beads egested by individual clams. I tested the null hypothesis that egestion of 9 and 44 µm beads was contemporaneous. The alternative hypothesis was that egestion of 44 µm beads either led or lagged egestion of 9 µm beads over time. These analyses were restricted to data from the first 8 h of the experiments, because resolution of bead egestion over time was greatest during that period and sampling intervals were equal. The longer sampling intervals used later in the experiments would tend to force contemporaneous correlations.

Egestion time-series from 39 clams were analyzed (12, 14 and 13 clams in the low-, mid- and high-food treatments respectively). In 25 of these 39 clams, cross-correlations of the time-series of egestion of 44 µm beads with egestion of 9 µm beads were significant (Fig. 3). Of the significant cross-correlations, 50% indicate that egestion of 44 µm beads was contemporaneous (Interval 0) with egestion of 9 µm beads. Of the remaining significant cross-correlations, 12.5% indicate that egestion of 44 µm beads led egestion of 9 µm beads by 30 min (Interval −1), and 12.5% indicate that egestion of 44 µm beads lagged egestion of 9 µm beads by 30 min (Interval +1). Given the discontinuous nature of both egestion and sampling of egested material, a lead or a lag of 30 min (the sampling interval)

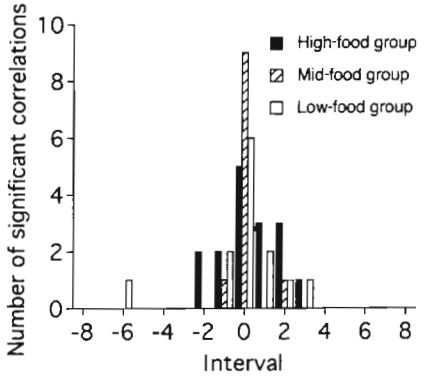


Fig. 3. *Potamocorbula amurensis*. Results of time-series correlations between egestion of 9 and 44 µm beads for individual clams. Each interval unit = 30 min. Correlations at negative intervals indicate that egestion of 44 µm beads led egestion of 9 µm beads, correlations at positive intervals that egestion of 44 µm beads lagged egestion of 9 µm beads, and correlations at zero that egestion of 44 µm beads was contemporaneous with that of 9 µm beads

cannot be interpreted as significant, and I accept the null hypothesis that egestion of 44 µm beads was generally contemporaneous with egestion of 9 µm beads.

Median gut residence time of 9 µm beads was affected by the presence of 44 µm beads. In the low- and mid-food treatments the median gut residence time of 9 µm beads was significantly shorter in clams fed both 9 and 44 µm beads (n = 22) than in clams fed 9 µm beads alone (n = 25) (Wilcoxon 2-sample test, 2-tailed test, $0.001 < p < 0.01$). Results for the high food treatment suggest a similiar pattern in median gut residence time of 9 µm beads between clams fed both beads (n = 13) and clams fed only the small beads (n = 15) (Wilcoxon 2-sample test, 2-tailed test, $p \sim 0.1$).

### Characteristics of tracer residence-time distributions and comparisons with ideal reactor models

Observed tracer residence time distributions were characterized by 3 features (Fig. 4): an early relatively short pulse of bead egestion, and a later more prolonged period of bead egestion, separated by a period of low or no bead egestion. This pattern was observed for about two-thirds (45 of 70) of actively feeding clams, and was generally most clear for clams in the high food treatment (Fig. 4C,D). The early pulse and later prolonged period of bead egestion were evident in residence-time distributions for clams in the low- and mid-food treatments, but the lag period was often less clearly defined than for clams in the high-food treatment (e.g. Fig. 4A).

The duration of the first pulse of bead egestion ranged from ~1 to 4 h, and the median percentage of beads egested in the first pulse was 71% (range: 10 to 97%). Clams in the high-food group egested a greater percentage of 9 µm beads in the first pulse of tracer (n = 7; median = 78%; range = 50 to 97%) than clams in the low- and mid-food groups (n = 16; median = 67.5%; range = 31 to 78%) (Wilcoxon 2-sample test, 2-tailed test, $0.02 < p < 0.05$). Median time for egestion of 90% of 9 µm beads was 25 h for clams in the low- and mid-food treatments (range = 2.5 to 97 h; 1st and 3rd quartiles = 22 and 31 h) and 22.5 h for clams in the high-food treatment (range = 3 to 62 h; 1st and 3rd quartiles = 8 and 25 h).

Interpretation of tracer data in the context of reactor theory is based on 2 ideal reactors, the ideal plug-flow reactor (PFR) and the ideal mixing reactor (continuous-flow, stirred-tank reactor, CSTR) (Penry & Jumars 1987). Deviations of the behavior of real reactors, e.g. clam guts, from these ideal models are estimated by determining actual residence-time distributions and comparing them with the ideal residence-time distributions (Levenspiel 1972, Carberry 1976, Smith 1981). For each clam, I plotted my observations as the cumulative fraction of tracer egested versus time, and I have illustrated 4 typical examples in Figs. 5 to 8. I then calculated the cumulative fractions of tracer egested with time that would be predicted by the ideal plug-flow and mixing models. Comparisons of observed and predicted cumulative-tracer curves for each clam and among clams are easier if time is expressed as nondimensional time $T^*$. $T^*$ is the time of each observation divided by some reference time, in this case, median gut-residence time.

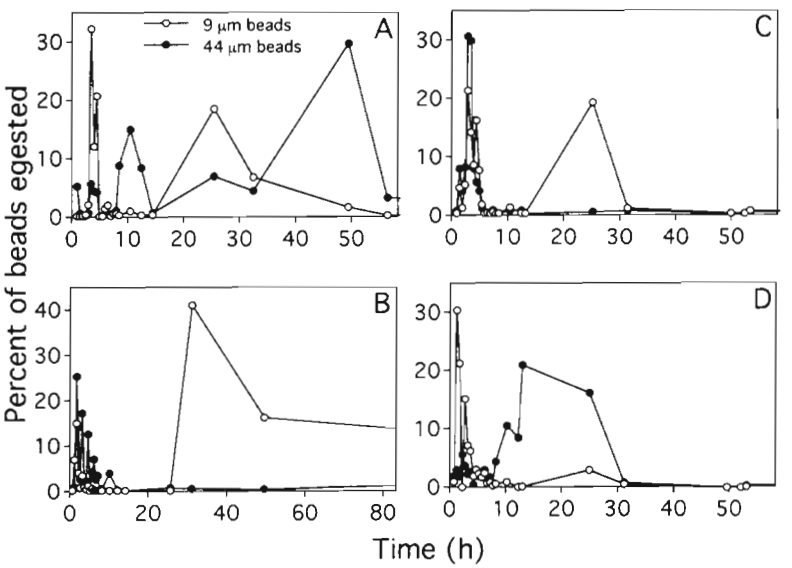


Fig. 4. *Potamocorbula amurensis*. Individual residence-time distributions for (A) Clam #2/25-8 in low-food treatment, (B) Clam #2/18-8 in mid-food treatment, and (C, D) Clams #2/4-10 and #2/4-11 in high-food treatment. Each clam illustrated here was fed 9 and 44 µm beads together. These 4 examples are typical of individual residence-time distributions observed for about two-thirds of actively-feeding clams (including clams fed 9 µm beads alone and clams fed 9 and 44 µm beads together)

The characteristics of the ideal plug-flow reactor require that tracer residence-time is constant (Penry 1989). Pulsed tracer input to an ideal plug-flow reactor at time $t = 0$ results in pulsed tracer output 1 gut throughput time later. Since I use median gut-residence time as an estimate of throughput time, the cumulative fraction of tracer egested steps from 0 to 1 for the ideal plug-flow model when $T^* = 1$ (i.e. when $t$ = median gut-residence time) (Figs. 5 to 8).

The ideal mixing reactor is characterized by a large spread of particle residence times, and tracer output in response to pulsed tracer input is proportional to

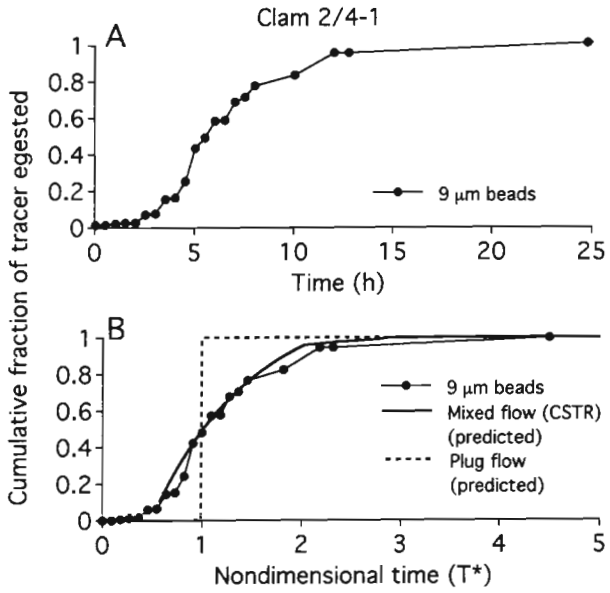


Fig. 5. *Potamocorbula amurensis*. Cumulative egestion of 9 µm beads vs nondimensional time (i.e. time of each observation divided by median gut-residence time) for Clam #2/4-1. CSTR: continuous-flow stirred-tank reactor

$e^{(-T^*)}$ (Smith 1981). The curve describing the cumulative fraction of tracer egested from an ideal mixing reactor thus increases gradually over time and is easily distinguished from the cumulative curve for the ideal plug-flow reactor (Figs. 5 to 8). If a clam's gut were to operate in its entirety as an ideal mixing reactor, then the largest single fraction of tracer egested would be observed at time $t = 0$. In other words, in a gut that operates entirely as an ideal mixing reactor, an input pulse of tracer at time $t = 0$ would be instantaneously mixed through the entire gut, and the largest single fraction of tracer egested would appear immediately in feces. The initial, predicted value for the cumulative fraction of tracer egested is thus non-zero.

The clam's tubular esophagus and intestine are unlikely to act as mixing components (Penry & Jumars 1987). Instead of instantaneous mixing and egestion of tracer, some time delay related to the time required for tracer to pass through the esophagus and intestine would be expected before tracer would begin to appear in the feces. The predicted cumulative tracer-egestion curves for the mixing model thus would not be expected to start at time $t = 0$. They would be expected to be offset from zero by the amount of time required for the tracer to pass through the esophagus and intestine, but there is no independent *a priori* way to know what that time is. I have therefore started the plot of each predicted cumulative-tracer egestion

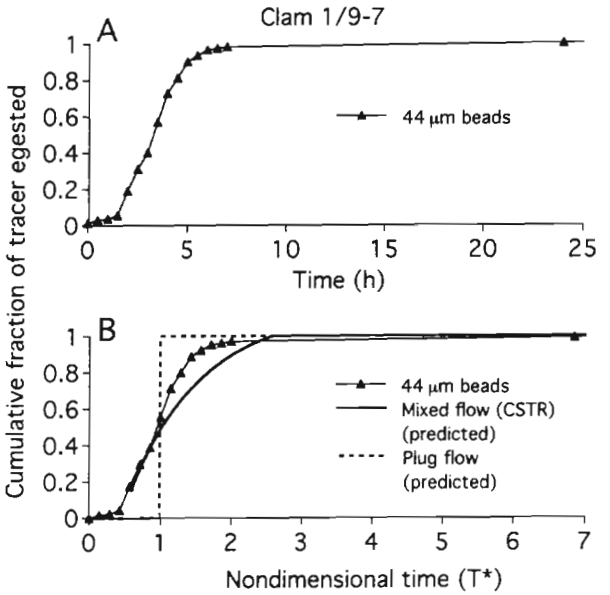


Fig. 6. *Potamocorbula amurensis*. Cumulative egestion of 44 µm beads vs nondimensional time for Clam #1/9-7

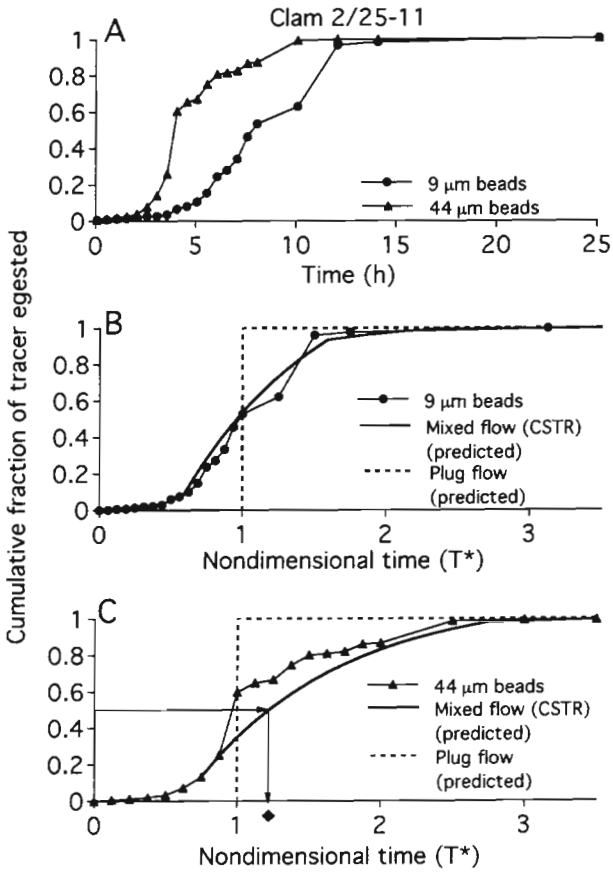


Fig. 7. *Potamocorbula amurensis*. Cumulative egestion of 9 and 44 µm beads vs nondimensional time for Clam #2/25-11. Arrows and diamond on *x*-axis = median residence-time for 44 µm beads predicted by ideal mixing model is longer than that observed

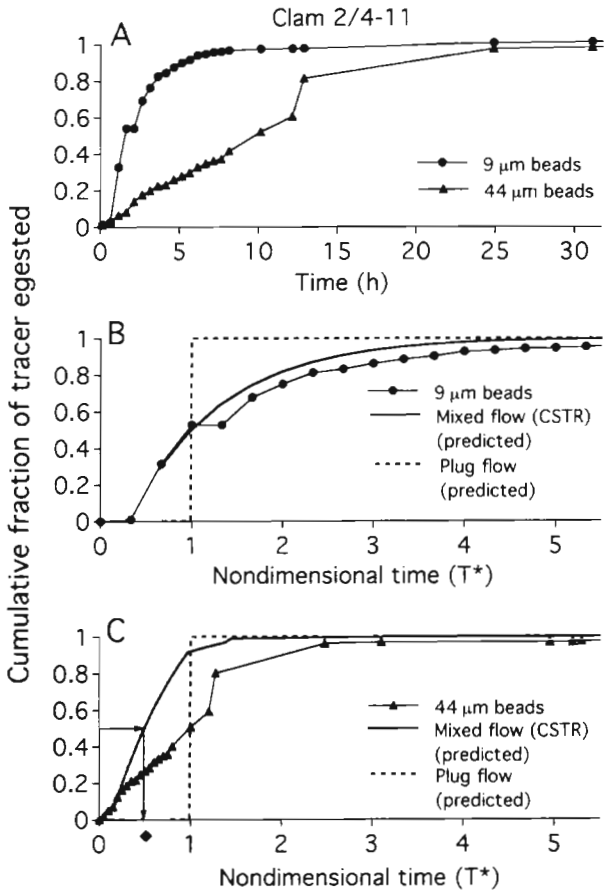


Fig. 8. *Potamocorbula amurensis*. Cumulative egestion of 9 and 44 µm beads vs nondimensional time for Clam #2/4-11. Arrows and diamond on *x*-axis = median residence-time for 44 µm beads predicted by ideal mixing model is shorter than that observed. (See Fig. 4D for corresponding tracer residence-time distributions for this clam)

curve for the mixing model at the point in time where the initial, predicted value for the cumulative fraction of tracer egested equals the observed value.

The cumulative egestion of 9 µm beads predicted by the ideal mixing model agrees quite well with observed egestion (Figs. 5B, 7B & 8B). The model seems to hold true for the passage of 9 µm beads alone (Fig. 5B) and also in the presence of 44 µm beads (Figs. 7B & 8B). The fit of the ideal mixing model to observed egestion of 9 µm beads suggests that the gut of *Potamocorbula amurensis* can be modeled in part as an ideal mixing reactor.

Neither the ideal plug-flow model nor the ideal mixing model describes egestion of 44 µm beads (Figs. 6B, 7C & 8C). There is no correspondence between observed cumulative-tracer egestion curves for 44 µm beads and curves predicted by the ideal plug-flow model, except at long times (i.e. at $T^* > 2$) when the plug-flow model, the mixing model and the observations all approach 1. There is limited correspondence between the observed cumulative-tracer egestion curves for 44 µm beads and the curves predicted by the ideal mixing model for times shorter than the median residence time (i.e. at $T^* < 1$), but the mixing model overestimates or underestimates observed median-gut residence times for 44 µm beads (e.g. Figs 6B, 7C & 8C).

In 11 of 39 clams that ingested both tracers, the median residence time of 44 µm beads was significantly longer than the median residence time of 9 µm beads, and a greater fraction of 44 µm beads than 9 µm beads was egested during the later, more prolonged period of bead egestion (cf. Fig. 4A,D). These results suggest that, contrary to my expectations, a large number of 44 µm beads probably did enter the digestive gland of these clams. Subsequent qualitative observations of the digestive glands of other individuals fed suspensions of *Rhodomonas salina* and 9 and 44 µm beads showed that 44 µm beads can indeed enter and sometimes pack the tubules of the digestive gland.

## DISCUSSION

### Ingestion rate and gut-residence time

Bivalve ingestion rates generally increase with increasing food concentration until some maximal, saturated ingestion rate is attained (e.g. Widdows et al. 1979, Bayne et al. 1989). This study was not designed to investigate the effects of food concentration on ingestion rates in *Potamocorbula amurensis*, but the data are suggestive of patterns observed for other suspension-feeding bivalves. The highest ingestion rates for *P. amurensis* were observed at the highest food concentration (12 000 cells $ml^{-1}$), and since no significant pseudofeces production occurred, the highest ingestion rates observed were probably less than maximal. The ingestion-saturating food concentration is probably greater than 12 000 cells $ml^{-1}$. There appears to be some threshold food concentration for *P. amurensis* around 6000 cells $ml^{-1}$ below which ingestion rates seem to have been constant in these experiments.

Ingestion rate was 1 important determinant of median gut-residence time (a measure of gut throughtput time), but median residence-times were quite variable, especially at the lowest ingestion rates, indicating that factors other than ingestion rate affected median residence-time. The cluster of short median residence-times at low ingestion rates (Fig. 2) suggests that those clams had relatively empty guts. The effects of gut fullness, the volume of material in an organism's gut, on digestive processing are not usually considered, and gut fullness, if considered, is assumed to be constant (e.g. Penry & Jumars 1987). These and other experi-

mental results (e.g. Bayne et al. 1984, 1987, 1989, Penry 1989, Penry & Frost 1990), however, show that this assumption is unlikely to be true. Gut fullness can be an important variable determining gut throughput time and patterns of particle-processing within an organism's gut.

Gut residence-time was also affected by the size composition of particles in the gut. The median gut-residence time of 9 µm beads was shorter in clams that also ingested 44 µm beads. It is not surprising that material was processed differently in clams that ingested large beads because, although about 2 to 10 times fewer 44 µm beads than 9 µm beads were ingested, the total volume of 44 µm beads ingested was about 2 to 10 times the total volume of 9 µm beads ingested. Ingestion of large particles such as diatoms, protozoans or invertebrate larvae thus might have a greater effect on the mechanics of particle-processing and bulk movement of material in a bivalve's gut than the numbers in which they are ingested might otherwise suggest.

### Relative importance of intestinal and glandular processing paths

Features of the tracer residence-time distributions for *Potamocorbula amurensis* reveal important elements of particle-processing in the stomach and digestive gland and confirm previous descriptions of stomach and digestive gland operations in lamellibranch bivalves (Yonge 1926, Owen 1955, Van Weel 1961, Purchon 1977, Decho & Luoma 1991). The fact that there are generally 2 'peaks' of tracer egestion indicates that there are 2 particle-flow paths through the stomach/digestive gland compartment, each generating a portion of the overall residence-time distribution (Levenspiel 1972). The early, relatively short pulse of tracer egestion is most likely associated with particles processed via the intestinal path, while the later more prolonged period of tracer egestion is most likely associated with material processed via the glandular path (Decho & Luoma 1991). The passage of particles processed via the intestinal path would be expected to be quicker than the passage of particles processed via the glandular path, because particles in the glandular path pass through an additional compartment, the digestive gland, and an additional process, intracellular digestion. Intracellular digestion in the digestive gland may be slower than extracellular digestion in the stomach and intestine and may contribute to the longer retention of particles in the glandular path.

In species in which intestinal and glandular fractions can be distinguished visually in feces, the qualitative appearance of feces changes with food availability. The intestinal fraction of feces appears to increase with food availability and appears to comprise the larger fraction of total fecal material when food availability is high (Van Weel 1961). Intestinal feces also appear less well digested than feces resulting from the glandular path (Van Weel 1961). Observations that absorption efficiency decreases with increasing food availability suggest that glandular digestion may saturate as food availability increases, and thus larger fractions of material may bypass the digestive gland to be processed via the intestinal path (Thompson & Bayne 1972).

Overall, *Potamocorbula amurensis* processed about 2.4 times more material via the intestinal path than the glandular path, and the relative importance of the intestinal path increased compared to the glandular path as food availability increased. The proportion of material processed via the intestinal path (i.e. the fraction of 9 µm beads egested in the early pulse) increased by 16% (from 67.5 to 78%) when concentrations of *Rhodomonas salina* increased from 3000–6000 cells $ml^{-1}$ to 12 000 cells $ml^{-1}$.

The effect of this change in food availability on digestive partitioning between the intestinal and glandular paths is at least as important as effects of changes in diet. The proportion of material processed via the intestinal path by *Potamocorbula amurensis* has been reported to be 2% for a diet of cultured *Alteromona atlantica* (bacterium) (Decho & Luoma 1996), 18% for a diet of bacterial exudates adsorbed to sediments (Decho & Luoma 1996), 25% for bacterial exudates in suspension (Decho & Luoma 1996), 47% for cultured *Thalassiosira pseudonana* (diatom) (Decho & Luoma 1996), 71% for cultured *Rhodomonas salina* (cryptophyte), and 8 and 74% for 2 diets both characterized as natural surficial sediment-flocs (Decho & Luoma 1991, 1996). Food concentrations and ingestion rates, however, were not quantified for any of the diets except *R. salina*, and variation in these parameters could potentially be responsible for at least some of the observed differences among diets (Thompson & Bayne 1972, Widdows 1978, Bayne et al. 1987).

### Modeling particle-processing in gut of a lamellibranch bivalve

The bivalve esophagus and intestine are long and tubular and can be modeled as plug-flow compartments (Penry & Jumars 1987). Since tracer residence-time distributions for plug-flow compartments simply reflect patterns of tracer input, I can assume that an ingested tracer pulse is unaltered by passage through the esophagus, and I can assume that tracer output from the stomach and digestive gland is unaltered by intestinal passage. Interpretation of tracer residence-

time distributions for bivalve guts thus focuses on particle-processing in the stomach and digestive gland.

Features of the tracer residence-time distributions and cumulative tracer residence-time curves for 9 µm beads show that the stomach and digestive gland can be modeled together as an ideal mixing reactor. Overall, the gut of a lamellibranch bivalve can be modeled as an ideal mixing reactor (the stomach and digestive gland) and an ideal plug-flow reactor (the intestine) in series. Even given the complexities of the structure and function of the stomach and digestive gland, my results suggest that no initial modifications of the ideal mixing model are needed. Future modifications may involve development of separate models for the stomach and digestive gland, but modeling the stomach and digestive gland separately will require the development of methods (perhaps using the endoscopic techniques of Ward et al. [1998]) that allow collection of tracer residence-time data for the stomach and digestive gland independently.

While there are no initial modifications of the ideal mixing model for the stomach and digestive gland, there are some qualifications. About 70% of the 9 µm beads passed through the stomach to the intestine (i.e. the intestinal path), and the remaining 30% passed through the stomach and digestive gland to the intestine (i.e. the glandular path). Passage through the stomach was thus the strongest determinant of the residence-time distributions. It is possible that a shift in partitioning between the intestinal and glandular paths may change the model, but other evidence argues against it. The mixing model can describe processing of bacterial cells of ≈1 µm in size by *Potamocorbula amurensis* and by *Macoma balthica*, another lamellibranch bivalve, (Fig. 3 in Decho & Luoma 1991), and bacteria appear to be processed predominantly (98%) via the glandular pathway (Decho & Luoma 1996). The mixing model thus appears to be valid for the stomach and digestive gland regardless of relative partitioning between the intestinal and glandular paths.

The bivalve stomach is sac-like, and physical mixing of particles is known to occur (see following subsection). The digestive gland is a mass of blind tubules connected to the stomach by larger ciliated ducts (Yonge 1926). There may be some physical mixing associated with particle movement in the ducts, but it is unlikely that the degree of mixing is comparable to that in the stomach. The digestive gland may appear to operate as a mixing compartment simply because tracer input from the stomach (a mixing compartment) to the digestive gland would yield a residence-time distribution for the digestive gland that is characteristic of a mixing compartment whether or not physical mixing actually occurs. However, I do not think that this explanation is complete. The process of intracellular digestion in the digestive gland is likely to result in differential particle retention, and thus a mixing model may fit particle residence-time distributions for the digestive gland even though physical mixing may not occur.

The ideal mixing model for the stomach and digestive gland holds for processing of relatively small particles ≤9 µm in size, that generally comprise the bulk of the diet of most suspension-feeding bivalves (Widdows et al. 1979, Bayne & Newell 1983). Suspension-feeding bivalves, however, can ingest particles >9 µm, and larger particles may seasonally constitute the greatest fraction of particles available for ingestion (Bayne et al. 1989). The ideal mixing model does not necessarily hold true for larger particles.

In some clams, median residence-times for 44 µm beads were shorter than predicted by the ideal mixing model, indicating that 'channeling' of flow most likely occurred (Levenspiel 1972). Particle-sorting is known to occur in the stomach of lamellibranch bivalves (Yonge 1926, Owen 1955, Purchon 1960), and can result in the channeling of larger particles through the stomach. A laminar-flow model (Smith 1981) might offer a useful approximation of the behavior of larger particles in this case, but this potential model modification requires further investigation of how larger particles are processed.

In some clams, median residence times for 44 µm beads were longer than predicted by the ideal mixing model, indicating particles were held up in some way and retained for longer times (Levenspiel 1972). It is most probable that 44 µm beads entered the ducts, and possibly the tubules of the digestive gland, and were retained there. Evidence from clams that ingested both 9 and 44 µm beads suggests that 44 µm beads can clog the ducts of the digestive gland and block the entrance of 9 µm beads. In fact, particles as small as 15 µm may clog the digestive gland in *Potamocorbula amurensis*. A cumulative residence time curve for 15 µm beads observed by Decho & Luoma (1991; their Fig. 3A) showed that 15 µm beads were retained longer than would be predicted by the mixing model, suggesting difficulty in eliminating them from the digestive gland. These artifacts associated with feeding clams big plastic beads do not need to be incorporated into models, but they do provide additional, indirect evidence that the later, prolonged phase of egestion does, in fact, represent the glandular digestive path.

### Advantages and disadvantages for bivalves of a mixing gut

An *a priori* model for a the gut of a lamellibranch bivalve based solely on consideration of digestive-

reaction kinetics would not have a mixing component at all. For reactions with rates that decrease monotonically as reactant concentration decreases (i.e. most enzyme-mediated reactions), a gut that operates as an ideal plug-flow reactor will always yield greater extents of digestion in less time and gut volume than a gut that operates, even in part, as an ideal mixing reactor (Penry & Jumars 1987). Thus, for lamellibranch bivalves, advantages associated with mixing must outweigh disadvantages associated with reductions in reaction rate and extents of digestion.

Mixing is associated with particle-sorting in guts (Penry 1989), and intragut particle-sorting appears to be an advantageous, and perhaps even necessary, component of a digestive strategy that incorporates intracellular digestion. Clams naturally encounter and can ingest very large particles such as sand grains 80 to 320 µm in size (Owen 1955), ciliates ~50 µm in size (Dupuy et al. 1999) and copepod nauplii ~100 µm in size (Kimmerer et al. 1994), but the largest particles reported to be phagocytized in the digestive gland are whole algal cells ≤9 µm in size (Mathers 1972). When larger particles enter the digestive gland, they are most likely not phagocytized but may clog the ducts and tubules, limiting phagocytosis of smaller particles and potentially reducing extents of digestion and absorption. The potential nutritional losses associated with reductions in intracellular digestion and absorption when particles that cannot be phagocytized enter the digestive gland are mostly probably greater than nutritional losses associated with the reductions in rates and extents of extracellular digestion that result from mixing. In other words, potential gains from intragut sorting outweigh losses associated with mixing. Mixing can also help to break down larger particles into smaller ones that can be more easily digested extracellularly or more easily phagocytized in the digestive gland.

## Implications for modeling and measuring energy and nutrient acquisition by suspension-feeding bivalves

Performance equations associated with a reactor model for a bivalve's gut can be incorporated into analytical models of energy and nutrient acquisition and absorption (e.g. Dade et al. 1990, Levinton et al. 1996). Similar direct incorporation of reactor-performance equations is not always possible with empirical models of energy and nutrient acquisition and absorption (e.g. Willows 1992, Hawkins et al. 1998), but there are some modifications that are possible for and relevant to empirical models as well.

Analytical and empirical parameterizations of digestion by suspension-feeding bivalves must include consideration of intragut particle-sorting and the effects of particle size on digestive processing, with particular attention to processing of large particles. My results define endpoints of a continuum of fates of large particles. Large plastic beads can either be channeled rapidly through the stomach to the intestine, or can end up clogging digestive gland ducts. The former path is likely to be important for bivalves feeding on natural particle suspensions, while the latter path is most probably an artifact of processing large light particles that cannot be broken down into smaller ones. Some large organic particles may be processed in the stomach in ways similar to large plastic beads and channeled relatively rapidly through the stomach. Unlike large plastic beads, however, many large organic particles can be broken down by mixing and extracellular digestion in the stomach, yielding smaller particles and dissolved materials that can then enter the digestive gland for further digestion and absorption. The effects of particle size on digestive processing will thus be related to the size distribution of ingested particles and the size distribution of particles resulting from degradation in the stomach.

Both analytical and empirical parameterizations of digestion by suspension-feeding bivalves must also include partitioning between the intestinal and glandular paths and how it is affected by ingestion rate. Diet can also affect partitioning (Decho & Luoma 1996), but diet effects probably represent an aggregate of interactions among particle concentration, size, and quality and ingestion rate. Incorporation of partitioning in empirical models would require at least 2 equations for digestion, 1 for the intestinal path and 1 for the glandular path. Incorporation of partitioning in analytical models may require a more complex reactor model, with the stomach and digestive gland modeled as separate mixing reactors in series (with bypass streams: see Penry [1989]).

Digestive gains from intestinal and glandular digestion need to be parametrized separately. In existing models, extent of digestion is directly related to gut throughput time and inversely related to ingestion rate (e.g. Dade et al. 1990, Willows 1992). An increase in ingestion rate results in a decrease in gut throughput time and a decrease in extent of digestion. This parametrization is likely to hold true for intestinal digestion, but not for glandular digestion. Ingestion rate will affect rates of particle supply to the digestive gland, but rates of phagocytosis and intracellular digestion are more likely to affect particle residence-time and gains from digestion in the digestive gland.

It is clear that more information is required about digestion in the digestive gland. Detailed qualitative descriptions of the digestive gland (e.g. Owen 1955, Van Weel 1961, Mathers 1972, Purchon 1977) exist, but

the information necessary for model development (e.g. kinematics of phagocytosis, kinetics of vacuole formation and disappearance, kinetics of digestion within vacuoles, rates of regeneration of cells in which intracellular digestion occurs) is still generally lacking (but see Hawkins et al. 1983).

Existing models of energy and nutrient acquisition by suspension-feeding bivalves (e.g. Willows 1992, Levinton et al. 1996, Hawkins et al. 1998) lack explicit consideration of the kinematics of particle-processing in the gut. The first-generation gut-reactor model proposed here provides a framework for explicit treatment of gut kinematics, and is readily modified to account for observed complexities of digestion in lamellibranch bivalves. Experimental investigations of feeding and digestion in suspension-feeding bivalves need to quantify patterns and rates of particle processing in addition to parameters such as ingestion rate and digestion and absorption efficiencies in order to understand how variations in diet affect gains of energy and nutrients and scope for growth.

*Acknowledgements.* I thank Mary Helen Garcia, Jan Thompson and the crew of the RV 'Polaris' for help collecting clams, Lara Gulmann for help with the experiments and for patiently counting fluorescent beads, and Anne Slaughter for help with data entry and manipulation. I also thank Don Weston and 4 anonymous reviewers for comments that helped to improve earlier versions of the manuscript. This work was supported by the Alan T. Waterman Award (OCE 93-20572) from the National Science Foundation to D.L.P.

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*Editorial responsibility: Otto Kinne (Editor), Oldendorf/Luhe, Germany*

*Submitted: June 18, 1999; Accepted: October 18, 1999*
*Proofs received from author(s): April 25, 2000*