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ABSTRACT

Suspension-feeding in various larval stages of the bivalve *Mercenaria mercenaria* (Linne) was observed and characterized using high speed video microscopy (60–240 images·s^{-1}). Larvae were tethered in a micro flow-through chamber and exposed to varying prey type (*Isochrysis* aff. *galbana,* TISO and *Synechococcus* spp., SYN) and concentration (10^2 to 10^6 cells·ml^{-1}). Feeding appeared to consist of a series of distinct, independent steps: (1) capture of a cell by the pre- and post-oral cirri of the velum, (2) transport to the mouth by the food groove, (3) concentration of cells into a bolus at the mouth, (4) selection or rejection of cells for entry into the esophagus and, (5) activation of a ciliated sphincter either to allow a cell to enter the stomach or to reject it from the esophagus. Probabilities of steps one and two appeared to be a function of the encounter rate with cells in the medium as both prey types were captured and transported to the mouth with equal efficiency. However, steps three, four and five were related to particle characteristics and the degree of gut satiation. Ingestion rate of *Isochrysis* cells was proportional to the encounter rate between concentrations of 10^2 and 10^3 cells·ml^{-1}, but declined to an incipient level specific to larval size at cell concentrations above 10^4 cells·ml^{-1}. The rate of cell rejection increased with increasing concentration and was inversely proportional to ingestion rate above 10^3 cells·ml.$^{-1}$ Both the water flow through the velum per unit velar edge and clearance rate per unit edge increased by a factor of two between 2 and 10 days of development. Ten-day-old larvae with empty guts were exposed to a constant concentration of *Isochrysis* cells. Ingestion was high for the first 6 min (93 cells·min^{-1}) and proportional to the rate of capture and transport to the mouth, but then fell to 4.3 cells·min^{-1} within 9 min of initial exposure. A total of 635 cells were ingested before rejection exceeded ingestion rate after 9 min of feeding.

Despite a wealth of information regarding optimal conditions for laboratory culture of marine bivalve larvae (Walne, 1974), the mechanisms responsible for their feeding behavior have largely been ignored. Quite the opposite is true for many groups of invertebrate larvae (e.g., Gastropods, Werner, 1955; Thompson, 1959; Fretter and Montgomery, 1968; rotifer, pluteus and trochophore larvae, Strathmann et al., 1972) and holoplankton, particularly copepods (Cannon, 1928; Frost, 1972; Longhurst, 1976; Paffenhöfer, 1976; Strickler, 1985). Behavioral responses to food species, concentration, and their trophic interactions, have been studied for many years in an attempt to elucidate fundamental patterns structuring plankton communities.

In coastal waters, adult bivalve molluscs are important members of the benthic community, while their larvae, as ciliated suspension feeders in the plankton, can numerically dominate the zooplankton at certain times of the year and exert considerable grazing pressure on nanoplankton populations (Jørgensen, 1981). Despite this fact, only the recent study of Crisp et al. (1985) has attempted to describe feeding behavior of bivalve larvae in context of existing theoretical models of predator-prey relationships. However, this approach will not be successful until basic mechanisms regulating food capture and processing have been determined. Although the technology has been available for such detailed studies of feeding behavior in small zooplankton since the introduction of high-speed microcinematography by Storch (1929), only recently has it been applied in comparative

studies of foraging on a microscale (Strathmann et al., 1972; Alcaraz et al., 1980, Paffenhöfer et al. (1982) and Strickler (1982a).

Also using microcinematographic techniques, Strathmann and Leise (1979) showed that particle capture by larvae of three molluscs was a highly efficient process, dependent on specific morphological and physiological aspects of the organisms such as length and velocity of the pre-oral cilia and length of the velar edge. However, a quantitative description of the processes controlling particle handling after capture remained wanting. This is particularly so in light of the observation made by Fretter and Montgomery (1968) that there appears to be some mechanism to allow for decoupling of particle capture from ingestion to avoid overloading the digestive system at high food concentrations.

Observations presented here address three basic questions concerning the feeding behavior of bivalve larvae: (1) How does the rate of particle encounter by the pre-oral cilia of the velum influence feeding activity over a wide range of particle concentrations? (2) How efficiently are particles captured and transported to the mouth after being encountered in the medium? (3) What mechanism(s) are available for decoupling particle encounter, capture and ingestion at satiating food levels?

The normal and high-speed video microscopic techniques used in this study proved to be a valuable tool for characterizing feeding in bivalve larvae. Using frame-by-frame analysis, the fate of individual particles was traced through the steps of capture, transport to the mouth by the food groove, accumulation at the mouth, and ingestion and rejection from the esophagus.

Methods and Materials

Larval Treatment.—Larvae of the venerid mollusc *Mercenaria mercenaria* (Linne) were reared in the laboratory following the procedures outlined in Gallager and Mann (1986) and were fed monocultures of *Isochrysis* aff. *galbana* (TISO) at a concentration of 10^4 cells·ml^{-1}. At 2 and 10 days post-fertilization, subsamples of veligers were removed from the culture and placed in a shallow dish to allow attachment of a micro-suction pipet similar in design to that used by Yule and Crisp (1983). Alternatively, water was wicked away from the larval shells and a glass pipet, drawn out to approximately 10 μm in diameter, was attached to the dorso-lateral aspect of the umbo using isocyanoacrylate cement. On the day of an experiment, a minimum of 20 larvae were tethered and immediately replaced in seawater containing *Isochrysis* at a concentration of 10^4 cells·ml^{-1} to await use in a feeding experiment. Tethered larvae that did not extend their velum immediately upon returning to seawater were not used in experiments.

Larvae were prepared for scanning electron microscopy (SEM) using the procedures outlined in Gallager et al. (1988). After narcotization with $MgCl_2$, cilia were pre-fixed for one minute by rapid addition of 2% osmium in seawater, followed by fixation for 10 min in glutaraldehyde (2% in 23 mM sodium cacodylate in seawater, pH 8.1), dehydration in a graded series of acetone, and critical point drying with liquid CO_2. Dried larvae were mounted on aluminum stubs, coated with gold-palladium (approximately 400 Å) and viewed with a JEOL JSM-840 SEM at 15 KeV.

Video and Optical Equipment.—Depending on the type of observations to be made, one of three video microscope systems were employed. For low magnification (50× maximum) recordings of the flow field generated by the velar cilia, single tethered larvae were positioned in a 20·20·10-cm-deep plexiglass chamber that rested on the dark-field illuminated stage of a Zeiss dissecting microscope. Ten-μm polystyrene particles (Duke Scientific) were added to the chamber at a final concentration of about 10^5 particles·ml^{-1} and mixed by hand to achieve a uniform distribution. Larvae usually resumed "swimming" within a few minutes and approximately 10 min of video recordings were made at normal speed (60 fields·s^{-1}) using a Panasonic high resolution B/W camera and a Sony (VO 2600) 0.75-inch cassette tape recorder. Flow fields established by various stages of larvae were analyzed using a Sony 5800H recorder and Mini-Com (United Media) tape editor by plotting in-focus particle positions every 10 video frames (20 fields) from prints made with a Mitsubishi video printer. The use of the printer avoided corrections that would be necessary to account for distortion of the video images on a monitor. Recordings were made at room temperature (22 ± 1°C).

Observations of particle capture, transport to the mouth and ingestion/rejection necessitated higher

magnification. These were made on tethered larvae mounted in micromanipulators and placed in a flow-through chamber. The 5-mm deep chamber received 0.22-μm filtered seawater from an aspirator jar to which algae were added at a concentration depending on the experimental protocol (see below). The cell concentration in the aspirator bottles was verified by quadruplicate counts with a Coulter Counter (Model IIA) and, where necessary, adjusted to the correct value ($\pm$5%) before initiating an experiment. The velocity through the chamber was adjusted to between 0.3 and 1.0 mm$\cdot$s^{-1}, calculated from particle positions on the monitor between successive frames, to supply larvae with a constant source of food and maintain their temperature at 22 $\pm$ 2°C (normal vertical swimming speed of *M. mercenaria* larvae ranges from 0.2 to 8 mm$\cdot$s^{-1} depending on the developmental stage, Gallager, 1985; in prep.). The chamber rested on the stage of a Zeiss compound microscope and recordings were made using either a 32 or 40× LD Plan objective (working distance ~2.8 and 1.9 mm, respectively), a 10 or 15× ocular and the camera-recorder combination described above. The output from a GE Strobotac was modified for use as the microscope light source and synchronized with the video camera to provide a single flash during each video field scan.

Although the strobe was able to stop movements of particles and cilia, allowing individual cells to be counted as they were captured, transported by the food groove and ingested or rejected, the temporal resolution at 60 fields$\cdot$s^{-1} (i.e., ~16.6 ms between fields) did not allow observation of cilia velocities in relation to uncaptured or captured particles. This was accomplished using a Dage video camera modified by Xybion, Inc. to provide high-speed imaging. Briefly, the system works by increasing the scan rate of the camera from a normal 60 fields$\cdot$s^{-1} to user-selected speeds of 120, 180 or 240. A maximum of four complete images are transmitted to the monitor during each video field resulting in a temporal resolution of 8.3, 5.6 or 4.2 ms between images, respectively. Since the duration of the effective stroke of the frontal cirri operating at a beat frequency of 20 Hz is about 15 ms (pers. obs.), the 180 or 240 images$\cdot$s^{-1} setting on the camera was used to calculate cilia and particle velocities. Unfortunately, there is a loss of resolution concomitant with increasing the imaging rate, making particles less than about 1 μm in diameter difficult to resolve.

Particle Concentration.—The effect of particle concentration on feeding behavior of *Mercenaria* larvae was characterized over a wide range of particle concentrations, from 10^2 to 10^6 cells$\cdot$ml^{-1} at 10-fold increments. A tethered larva was mounted in the flow-through chamber and allowed to acclimate for about 30 min to a concentration of 10^2 *Isochrysis* cells$\cdot$ml^{-1} followed by five-10 min periods of recording at 60 fields$\cdot$s^{-1} (experiments described below suggested that the actual time for acclimation was probably not more than 10 min). The concentration was then increased to 10^3 cells$\cdot$ml^{-1}; the larva acclimated for another 30-min period and was again recorded for a similar length of time. This process was repeated for each concentration up to 10^6. The feeding response of 10 larvae was characterized this way on day 2 and day 10 of planktonic development. Between 5 and 8 min of recordings for each larva were analyzed field by field for the following parameters: number of cells appearing at the mouth, cells ingested, cells rejected from the mouth and esophagus and cells accumulated into a bolus in front of the mouth.

Particle Mixtures.—Observations of larval feeding behavior in the presence of a mixture of two particle types were made using equal numbers ($3\cdot10^4$ cells$\cdot$ml^{-1}) of both *Isochrysis* cells (4.5 μm-diameter, ~47.7 μm^3) and cells of the cyanobacterium *Synechococcus* spp. (SYN, WH8109) (1$\cdot$0.5 μm, ~0.19 μm^3). On day 2 and day 10 following fertilization of another larval culture, 10 tethered larvae were acclimated to the particle mixture for 2 h in a dish, then mounted sequentially in the flow-through chamber and their feeding activity recorded for 15 min.

Gut Satiation.—Another experiment was designed to evaluate the effect of gut satiation on feeding activity. Ten-day-old veligers were allowed to empty their guts in 0.22-μm filtered seawater for 2 h prior to tethering and observation in the flow-through chamber. Contents of the stomach and crystaline style sac were observed to verify their condition. Larvae were recorded in flowing, filtered seawater (velocity = 0.5 mm$\cdot$s^{-1}) for 2 to 3 min before cells of *Isochrysis* were added to the aspirator jar at a final concentration of 10^4 cells$\cdot$ml^{-1}. Feeding activity was recorded for 30 min following addition of cells and the video tapes were analyzed as above.

Encounter Rate.—The rate at which particles were encountered from the flow field and their potential for capture by the frontal cirri was considered to be a function of the area of the velum in which particles are actually captured (capture zone), particle velocity through the capture zone and particle concentration. Calculation of the encounter rate required estimates of the following parameters: cilia length, area within the ciliar sublayer covered by the return stroke, and particle paths, trajectories and velocities. Measurements of these parameters were made on prints of video images. Cirral beat frequency was estimated by synchronizing the strobe with the velar metachronal wave while a calibrated digital frequency counter gave a direct reading of strobe rate in Hz. Measurements of velar length (antero-posteriorly) and width (left to right) were made from ventral views of tethered organisms and

used to calculate circumference of the velum assuming the shape of the velum to approximate an ellipse. Shell lengths were measured to the nearest micron.

RESULTS

Flow Field Visualization.—Low magnification video recordings of larvae in the static chamber in the presence of 10-μm latex spheres revealed the type of particle paths illustrated in Figure 1. Particles directly in front of the velum began to accelerate rapidly when they were about 600 μm away from the organism. At a distance of about 200 μm, particles veered in the direction of the edge of the velum and followed streamlines through the velar cirri. Two almost symmetrical streamlines, apparently parabolic in shape in two dimensions, were formed, one on either side of the organism. When particles that had followed streamlines through the velar cirri were traced back to their origin, the maximum distance from which a particle originated orthogonal to the veliger's centerline was about 300 μm at a distance of 600 μm from the velum. Thus, veligers appeared to be encountering a much larger area of water than just that directly in front of the organism.

Capture Zone.—Figure 2 illustrates morphological relationships between velar cirri, food groove and mouth in a 2-day-old larva. Each ribbon-like compound cirrus is composed of about 10 simple cilia at the base and tapers towards the tip. Two cirri are arranged in each orthoplectic row (parallel to fluid flow) and the cirral spacing within diaplectic rows (normal to fluid flow) is about 0.5 μm. Cirri beat in diaplectic metachrony (i.e., groups of orthoplectic rows in sequential stages of their effective stroke) and the wave is propagated in a laeoplectic pattern (i.e., clockwise when viewed ventrally). Figure 3 illustrates a cross-sectional view through the velum of a 2-day-old veliger tethered in the flow-through chamber (ambient velocity = 0.5 mm$\cdot$s^{-1}) in the presence of 10^5 cells$\cdot$ml^{-1} of *Isochrysis*. The diagram represents a composite of many high-speed video images, such as those shown in Figure 4, where particles were either captured and transported to the mouth or lost into the flow field. The two-phase cycle of the velar cirri is shown in Figure 3 at intervals of 16.7 ms, indicating a maximum tip velocity of about 2.6 mm$\cdot$s^{-1} during the effective stroke and a much slower return stroke. Cells passing about 10 μm outside of the ciliar sublayer (i.e., the layer of water between the base and tip of the cirri, Blake and Sleigh, 1975) were accelerated to a velocity of 1.3 mm$\cdot$s^{-1}, but their trajectory took them away from the velum. Algal cells passing just within the sublayer accelerated to about 80% of the maximum cirral tip velocity, passed through the cirri, and were not captured. Cells of *Isochrysis* were captured only when they entered the sublayer no more distal to the region covered by the return stroke than about 15 μm (n = 85). Although not all cells entering this area, defined as the capture zone, were ultimately captured, a consistent feature of captured cells was their clockwise rotation within the sublayer. The speed of rotation apparently corresponded to the velocity of the cirri in their return stroke. The approximate dimensions of the capture zone and the proportion of cells captured remained remarkably consistent throughout larval development (see below).

Particle velocities within and adjacent to the sublayer of 10-day-old veligers are shown in Figure 5. Velocities increased rapidly between the area covered by the recovery stroke and cirral tip and then declined to a level equal with the ambient velocity through the flow-through chamber at a distance of about 90 μm from the base of the cirri. The particle capture zone is depicted as an area of about 15 μm within the viscous sublayer.

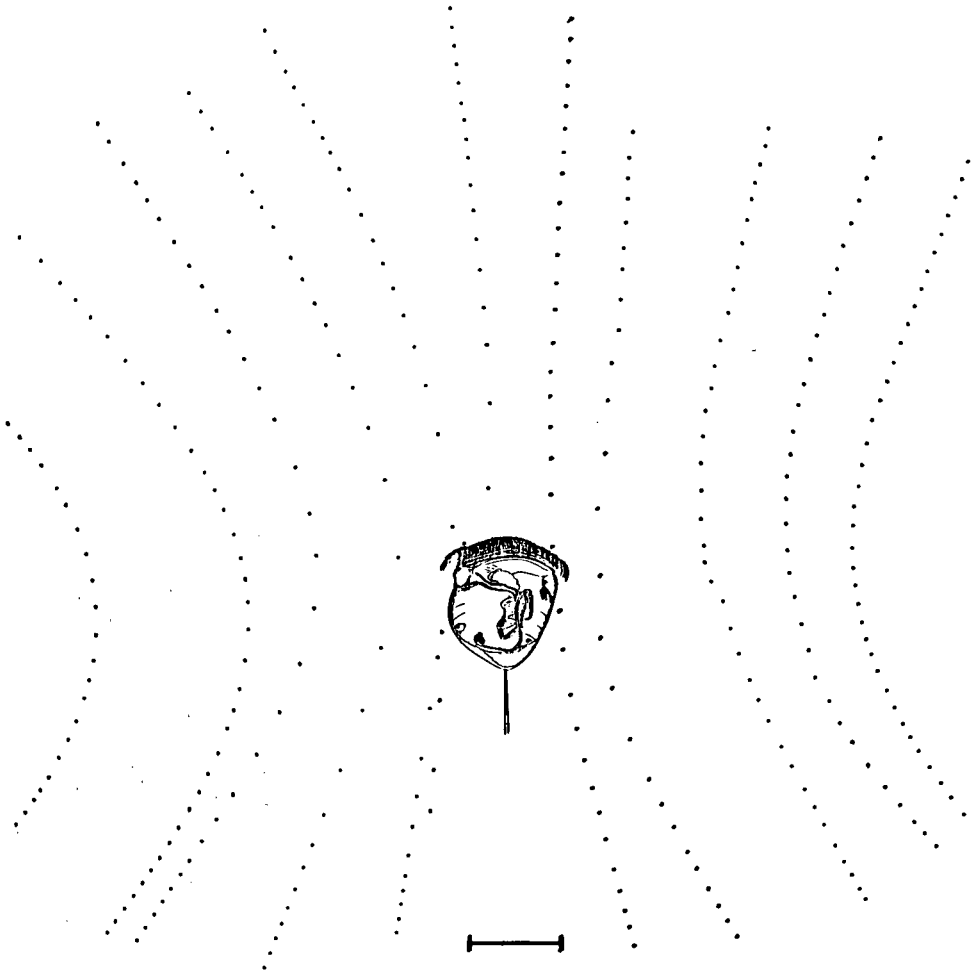

Figure 1. Two dimensional view of the flow field generated by a tethered larva of *Mercenaria mercenaria*. Larva was suspended in a 20·20·10 cm chamber with 10-μm latex spheres and observed with dark-field illumination at a magnification of 50×. Particle streamlines were plotted at 83-ms intervals. Bar = 200 μm.

Velar Flow Rate.—Data for various parameters of the frontal cirri of two stages of *Mercenaria* veligers (Table 1) were used to calculate total flow through the velum and the proportion of that flow which would pass through the capture zone. The rationale for these calculations followed that of Strathmann and Leise (1979). The total pie-shaped area (At) swept by a single cirri through its effective stroke is $(R^2a)\cdot 2^{-1}$, where R is cirral length (radius of a sector) and a is the angle subtended by the cirral shaft in radians ($a = S\cdot R^{-1}$ where S is the sector length described by the cirral tip). The area of the capture zone (Ac) becomes:

$$((r_1 + r_2)^2a)\cdot 2^{-1} - Ar$$

where r_1 is the radius of cirri within the recovery stroke, r_2 is the cirral length associated with the capture zone (i.e., 15 μm) and Ar is the area encompassed by the recovery stroke (i.e., $(r_1^2a)\cdot 2^{-1}$). Total flow through the velum should be the

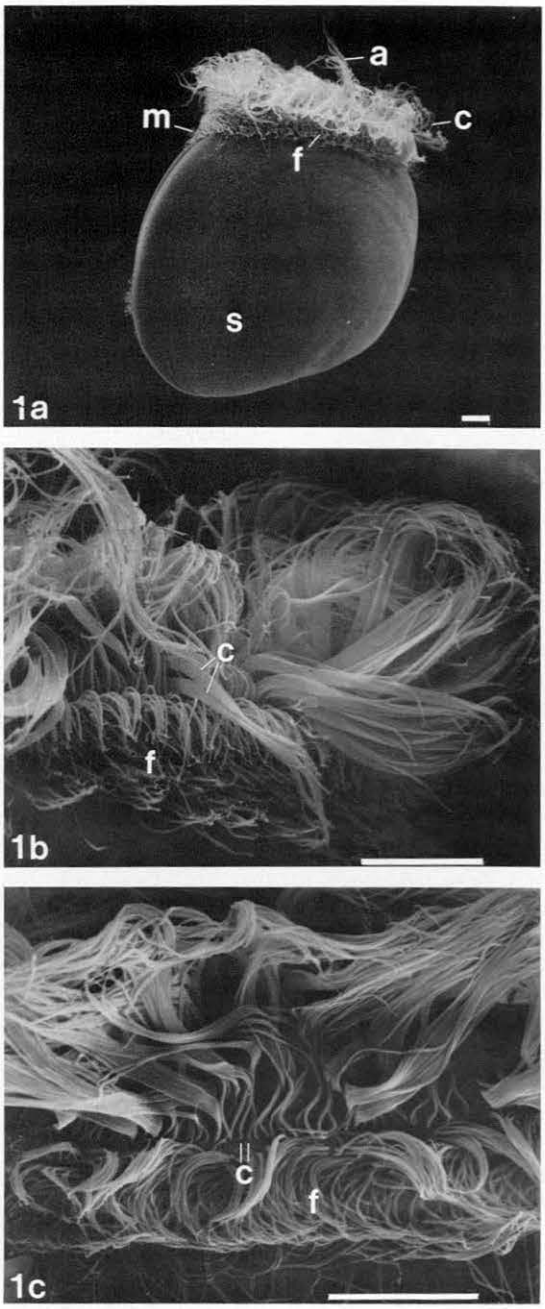

Figure 2. Scanning electron micrographs of 2-day-old *Mercenaria* larvae. (a) whole organism; (b) magnified area of velum; pre-oral cirri, C, are at end of effective stroke. Note diaplectic metachrony of the beat cycle. (c) Spacing between orthoplectic rows is about 0.5 μm. f, food groove; M, mouth; apical tuft of cilia; S, shell. Bars = 10 μm.

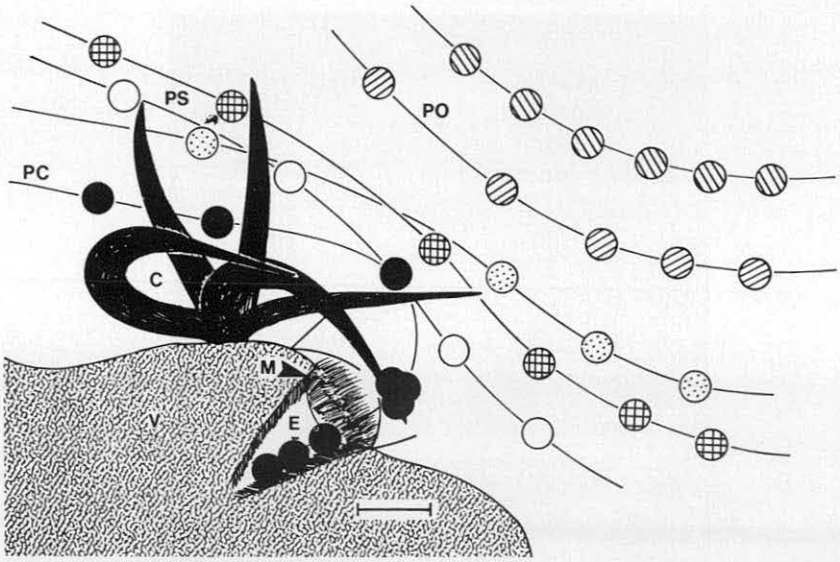

Figure 3. Cell trajectories and velocities through the pre-oral cirri of *Mercenaria* larvae. The position of a single cirrus and six cells of *Isochrysis galbana* are plotted at 17-ms intervals. Spatial configuration of cells and cirri have no relationship. Only cells following paths depicted by the solid circle ± 5 μm were captured. PO: cell paths outside the cilia sublayer; PS: cell paths within the sublayer, but not captured; PC, paths of cells that were captured; C, pre-oral cirri; M, mouth; E, esophagus; V, velum. Bar = 10 μm.

total area swept by the cirri minus the area blocked by the return stroke multiplied by the angular velocity (w) and the circumference of the velum (B). But cilia do not move water with 100% efficiency (Sleigh and Aiello, 1972). The mean ratio of cirral to particle velocity found in the capture zone was 1.3 (range = 1.01–2.1, n = 24), suggesting a 77% efficiency in movement of water (assuming that suspended particles reflect water velocity). The result obtained above for total flow must, then, be divided by the cirral to particle velocity ratio to obtain an accurate estimate of water flow through the velum (Ft). Thus:

$$Ft = ((At - Ar)wB) \cdot 1.3^{-1}$$

where w (radians/s) = Vt/R and Vt is the cirral tip velocity. Flow through the capture zone (Fc) may be calculated in a similar manner by the following:

$$Fc = (Ac\ w\ B) \cdot 1.3^{-1}$$

There was no significant difference in cirral beat frequency, angular velocity or tip velocity for organisms of the same age exposed to particle concentrations ranging between 10^2 to 10^6 *Isochrysis* cells·ml^{-1} (two-way ANOVA: $P > 0.01$). The data in Table 1 for these parameters, therefore, are means ± 1 SEM of pooled data from all cell concentrations and were used to make the calculations above.

Total flow through the velum of 10-day-old veligers was eight fold greater than in 2-day-old organisms. This was due to the combination of a greater velar circumference available for water propulsion, longer cirri and the higher angular velocity in the larger larvae. Interestingly, the increase in flow through the capture zone in large larvae was not directly proportional to larval size since the percentage of total flow through the capture zone decreased. This result reflects the relatively constant dimensions of the capture zone observed throughout development.

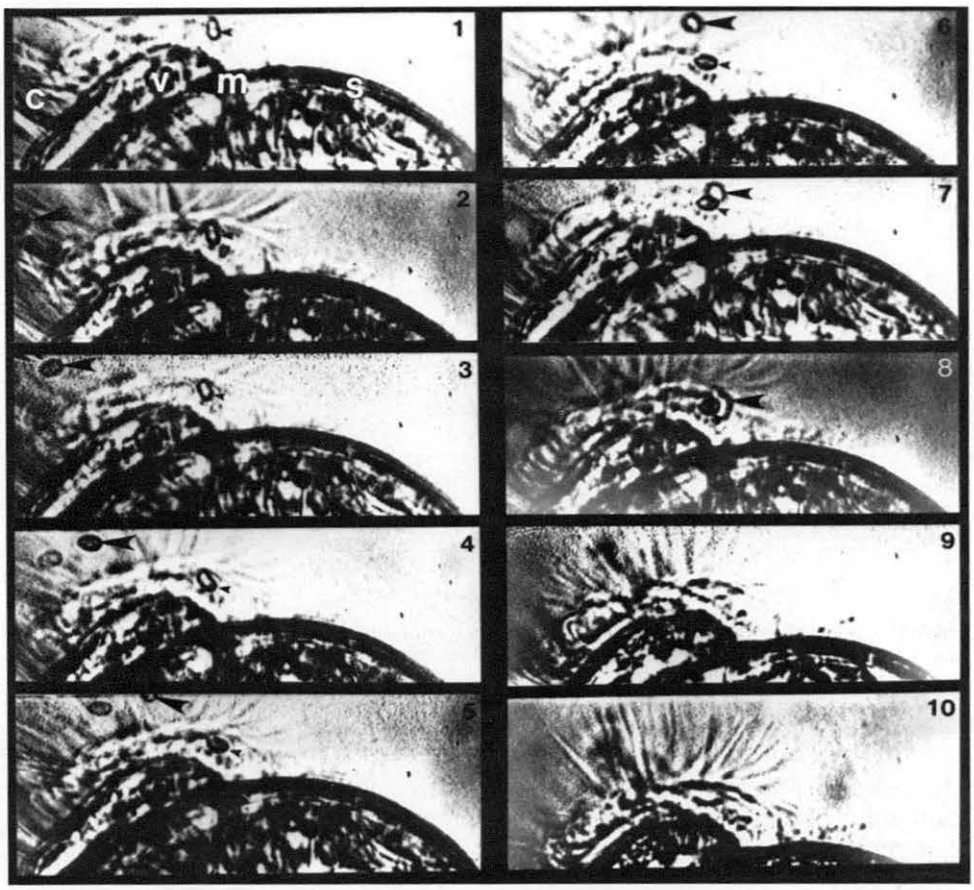

Figure 4. Optical cross-section through the velum of a tethered larva. High-speed video images were photographed from the monitor and show two particles being independently manipulated prior to their ingestion. Large arrow follows an *Isochrysis* cell as it enters the ciliar sublayer (2–3) passes through the capture zone just distal to cilia in their return stroke (4–6, particle ghost due to light-lag of video tube), forced into the food groove (7–9) and ingested (10). Small arrow shows a cell counter-rotating above the mouth relative to the direction of flow generated by the velum (1–8) and ingested (10). Images are 8.3 ms apart, strobe duration about 10 μsec. C, pre-oral cirri; V, velum; M, mouth; S, shell.

Encounter Rate.—Calculations of encounter rate (ER) of particles by the velar cirri were made using the following equation: (ER) = FcC, where C is the particle concentration in the ambient medium. Although previous studies have shown that ER may be a function of particle properties such as shape, radius and volume for various zooplankton (Gerritsen and Strickler, 1977; Gigeure et al., 1982), the present calculations assumed no dependence of ER on properties of particles other than their abundance. This is important for a first assessment of the mechanisms of particle capture and transport in bivalve veligers.

Particle Concentration.—The ER of *Isochrysis* cells within the capture zone of 2- and 10-day-old veligers increased linearly with increasing cell concentration from 10^2 to 10^6 cells·ml^{-1} (Fig. 6). The rate at which cells were captured and transported to the mouth (defined as the rate of cells appearing at the mouth in

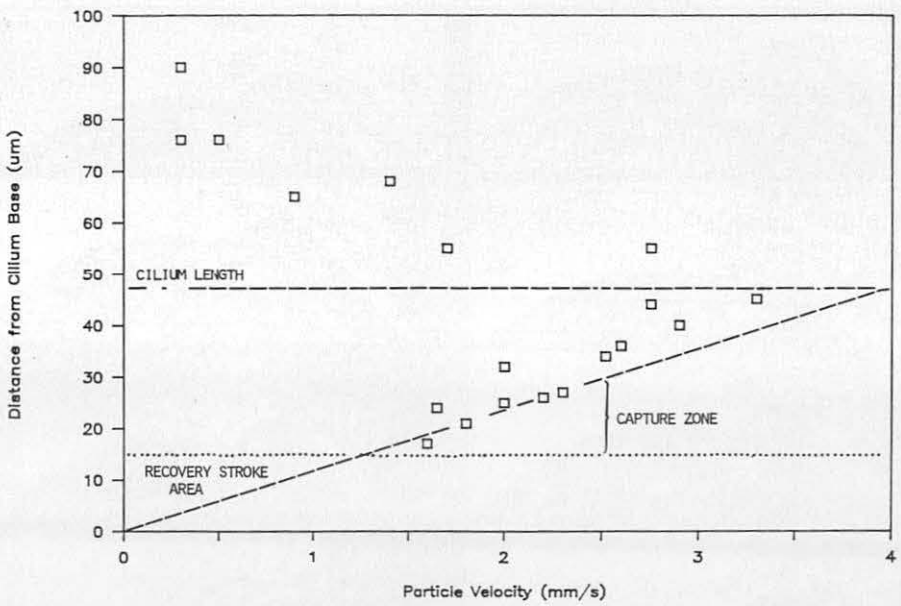

Figure 5. Particle velocity profile through cilia sublayer of 10-day-old larvae of *Mercenaria*. Dashed line represents cilium velocity along its length, dotted line is the area within the viscous sublayer associated with the recovery stroke. Particle capture zone extends approximately 15 μm distal to the top of recovery stroke. Figure includes data from eight larvae.

the video recordings) was proportional to the ER up to a concentration of 10^5 cells·ml^{-1}, beyond which a slight decrease was noted in both age groups of larvae. The proportion of cells transported to the mouth to those encountered ranged between 0.49 and 0.80 with a mean of 0.60 up to a concentration of 10^5 cells·ml^{-1}. This decreased to 0.13 at the highest concentration.

Between 10^2 and 10^3 cells·ml^{-1}, ingestion rates were virtually identical to the rate at which cells were captured and transported to the mouth (a mean of only 12 cells·h^{-1} were lost). Ingestion decreased at concentrations above 10^4 cells·ml^{-1} to an apparent satiation rate of 86 and 388 cells·h^{-1} in 2- and 10-day-old veligers, respectively.

Table 1. Various parameters of the frontal cirri of *Mercenaria* veligers over development

	2	10
Age (days)		
Shell length (μm)	100 ± 2	234 ± 4
Velar circumference at top of recovery stroke (μm)	259 ± 9	574 ± 10
Length of frontal cirri (μm)	32 ± 1	45 ± 2
Cirral shaft length covered by recovery stroke (μm)	8 ± 1	11 ± 1
Cirral beat frequency (Hz)†	12.8 ± 0.9	22.8 ± 0.9
Cirral arc length (μm) (S)	82 ± 2	115 ± 5
Angular velocity (rad·S^{-1})† (W)	94 ± 7	168 ± 8
Cirral tip velocity (mm·S^{-1})† (V_t)	2.9 ± 0.2	7.5 ± 0.4
Flow rate through whole velum (μl·h^{-1})	107 ± 8	876 ± 87
Flow rate through capture zone (μl·h^{-1})	30 ± 2	150 ± 10
Percentage of flow through capture zone (%)	29 ± 2	17 ± 1

All values are $\bar{x}$ + 1 SEM, n = 10 except noted † where n = 50.

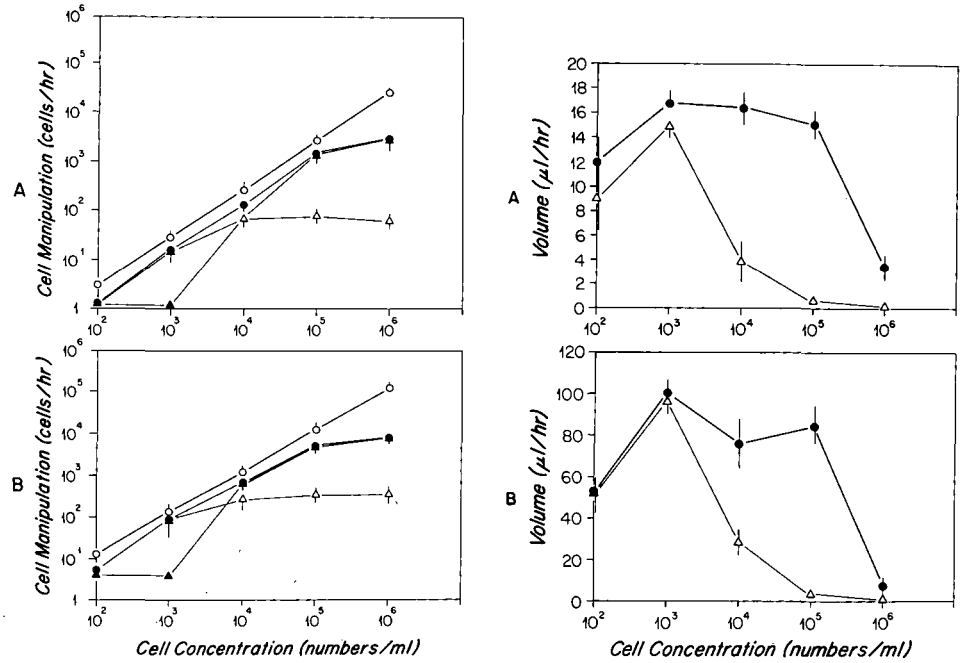

Figure 6. (left) Rates of encounter (○), capture and transport to the mouth (●), ingestion (△), and rejection (▲) in (A) 2- and (B) 10-day-old tethered larvae over a wide range of cell concentrations (n = 10 ± 1 SEM).

Figure 7. (right) Filtration (●) and clearance (△) rates for (A) 2- and (B) 10-day-old tethered larvae over a wide range of cell concentrations (n = 10 ± 1 SEM). Filtration rate is based on cells captured and transported to the mouth, clearance rate is based on cells ingested.

Cell rejection from the area of the mouth and esophagus appeared as the inverse of ingestion. Rejection remained low (i.e., 0.6 to 1.2 cells·h^{-1} in 2-day-old larvae and 5 to 10 cells·h^{-1} in 10-day-old larvae) up to an ambient concentration of 10^3, but then increased to a level almost equivalent to the rate of capture and transport at 10^4 cells·ml^{-1}. At ambient concentrations in which rejection of cells became a dominant feature of the feeding behavior, a bolus of 2 to 15 cells formed in front of the mouth and counter-rotated with respect to the dorsally-directed flow generated by the velar cirri. As new cells were transported to the mouth by the food groove and joined the bolus, others were rejected from the bolus at an equivalent rate. Cells were periodically removed from the bolus and directed into the mouth and esophagus at a rate of about 1.4 and 6.5 cells·min^{-1} for 2- and 10-day-old veligers, respectively. Most cells that entered the esophagus passed by a ciliated sphincter at the junction to the stomach and were ingested, but a small number (about 1 out of 15) were ejected from the esophagus by muscular contractions and, apparently, by reversal of the sphincter cilia. This behavior was particularly evident when on a number of occasions four to six cells were lined up within the esophagus, ejected as a unit and lost into the flow of the velar cirri.

Filtration and Clearance Rates.—In most studies of feeding behavior in zooplankton, filtration and clearance rates have been equated to the volume of water swept clear of particles in unit time (Cushing, 1968; Frost, 1972; Crisp et al., 1985). Rates are usually based on the number of particles removed from suspension by

grazing organisms in declining food concentrations, and are directly related to ingestion. Particles that are temporarily removed from suspension, only to be returned upon their rejection, are usually not differentiated from those that are ingested and permanently removed from the medium when clearance rates are calculated. In the present study, the filtration rate is defined as the volume of water from which particles are removed by the velar cirri and transported to the mouth. The clearance rate, on the other hand, is defined in conventional terms as the volume of water swept clear of particles that are ingested.

The filtration rate in both stages of larvae remained relatively high between the concentrations of 10^3 and 10^5, but decreased sharply below or above this food level (Fig. 7). Interference and clogging of the velar cirri or food groove probably occurred at the highest food concentrations since the proportion of cells captured and transported to the mouth to those encountered fell to 0.13. The reason for the decrease at low food levels is not as apparent since no statistical difference was found between cell concentrations for cirral beat frequency and angular velocity, two parameters directly influencing flow through the capture zone. At very low cell concentrations, however, even minor variations in these parameters may have induced a decrease in filtration rate.

Clearance rates decreased, apparently hyperbolically (Crisp et al., 1985), above a concentration of 10^3, and were also depressed at 10^2 cells·ml^{-1}. Maximum clearance rates of 10-day-old larvae were more than six fold greater than in 2-day-old larvae.

Gut Satiation.—Ten-day-old larvae were exposed to a concentration of 10^4 cells·ml^{-1} after evacuating their guts in 0.22-μm filtered seawater for 2 h. As an example of the feeding response, data from a single individual showed that cells were captured and transported to the mouth at nearly a constant and mean rate of 99 cells·min^{-1} (range: 85–133) over a 19-min-period (Fig. 8). The ingestion rate was initially high (93 cells·min^{-1}) but gradually fell to an incipient level of 4.3 cells·min^{-1} 9 min after first being exposed to food. A bolus first appeared in front of the mouth after 5 min of exposure while, concomitantly, cells began to be rejected from the mouth region. As the gut became more satiated, the number of cells in the bolus increased to a maximum of 16 before it was lost into the dorsally-directed flow of the velar cirri. A new bolus was then formed within a few seconds. Post-satiation ingestion rates observed in this experiment (4.3 cells·min^{-1}) were similar to acclimated rates of ingestion at 10^4 cells·ml^{-1} (4.9 $\pm$ 1.0 cells·min^{-1}).

Particle Selection.—Although cells of *Isochrysis* were easily enumerated as they appeared at the mouth and were either ingested or rejected, the small size and low refractive index of *Synechococcus* made it much more difficult to observe their fate in the feeding process. It was necessary to make triplicate counts of cells over a specific sequence of video fields to reduce the coefficient of variation (SD/mean) to 8 to 14%. Nevertheless, errors introduced into the data presented below probably resulted in underestimates of capture and ingestion of *Synechococcus* since: 1) only particles appearing at the mouth that could be positively identified were included in the results and 2) the optical resolution of the recording system (about 1 μm) may not have been sufficient to differentiate adjacent cells.

Larvae feeding on a mixture of equal numbers of *Isochrysis* and *Synechococcus* ($3 \cdot 10^4$ cells·ml^{-1} of each species) captured and transported cells of both species to the mouth in statistically equivalent proportions ranging from 0.42 to 0.64 (Table 2). The proportion of cells ingested to those transported to the mouth was significantly different between *Isochrysis* and *Synechococcus* ($P < 0.01$). The ratio of cells ingested (SYN/TISO) ranged between 17 and 162:1 with a mean of 48:1

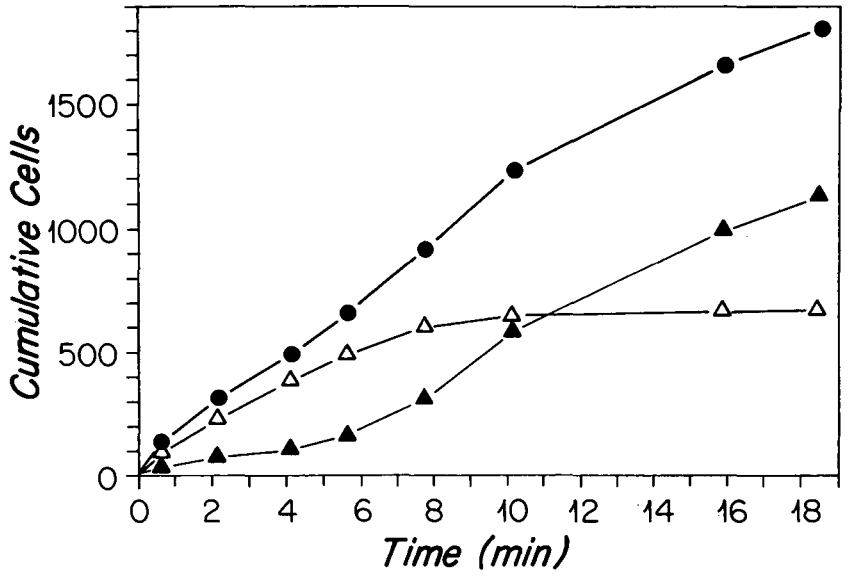

Figure 8. Short-term feeding activity of a single tethered larva exposed to 10^4 cells·ml^{-1} *Isochrysis* after 2 h of gut evacuation in 0.22-μm filtered seawater. Rate of capture (●), ingestion (△) and rejection (▲).

in 2-day-old larvae and between 2 and 22:1 with a mean of 3:1 in 10-day-old veligers. However, when cell numbers were converted to volumes using 47.7 and 0.19 μm^3·cell^{-1} for *Isochrysis* and *Synechococcus,* respectively, four times as much *Isochrysis* biomass was ingested compared with *Synechococcus* in 2-day-old larvae and 33 fold more algal biomass was ingested by 10-day-old larvae.

Synechococcus cells, which autofluoresce orange to phycoerytherin (Waterbury et al., 1980; Caron, 1983), were observed in the gut of *Mercenaria* veligers after the feeding experiments.

Discussion

Werner (1959) described the feeding behavior of *Crepidula fornicata* (L.) veligers as being a four step process: 1) producing a feeding current, 2) clearing particles from it, 3) transporting particles to the mouth, and 4) ingesting them. Fretter and Montgomery (1968) also noted that feeding in prosobranch veligers consisted of at least four distinct phases and observed inorganic particles being transported through the gut while algal cells were retained. Strathmann and Leise (1979) documented particle capture by mollusc veligers using high-speed microcinephotography, but no attempt was made to described the feeding process as a whole (this was not the objective of their research). The normal- and high-speed video techniques employed in the present study extends these qualitative observations while providing a quantitative link between the functional morphology of veligers and their feeding behavior.

Crisp et al. (1985) showed that the functional response of *Ostrea edulis* larvae over a range of cell concentrations ($1.2 \cdot 10^4$ to $2.5 \cdot 10^5$ cells·ml^{-1} of *Pavlovia lutheri*) fit a hyperbolic relationship identical to Holling's (1959) disk equation as modified by Cushing (1968) and Fenchel (1980): $I = FC/(1 + FCth)$ where I is the ingestion rate, F is maximum filtration rate, C is the particle concentration

Table 2. Comparison of various parameters associated with larvae feeding on *Isochrysis galbana* (TISO) and *Synechococcus* sp. (WH8109) (SYN) (both species were added at a concentration of $3 \cdot 10^4$ cells·ml^{-1})

	Age		
	2 days	10 days	Level of significant difference between larval stages
Encounter rate (ER) of both TISO and SYN† (cells·h^{-1})	891 ± 70	1,469 ± 81	**
Cells transported (T) to mouth (Cells·h^{-1})			
TISO	381 ± 17 *	937 ± 48 *	**
SYN	532 ± 53	695 ± 64	*
Cells ingested (I) (cells·h^{-1})			
TISO	11 ± 2 **	205 ± 41 **	**
SYN	523 ± 51	672 ± 65	*
Ratio cells ingested (SYN/TISO)	48 ± 14	3 ± 2	
Ratio volume ingested SYN/TISO	0.24 ± 0.05 **	0.03 ± 0.001 **	*
Cells rejected (R) (cells·h^{-1})			
TISO	369 ± 33 **	732 ± 72 **	**
SYN	10 ± 3	23 ± 19	
Ratio T/ER			
TISO	0.42 ± 0.06	0.64 ± 0.04	
SYN	0.60 ± 0.07	0.47 ± 0.04	
Ratio I/T			
TISO	0.03 ± 0.01 **	0.22 ± 0.02 **	**
SYN	0.98 ± 0.08	0.97 ± 0.09	
Filtration Rate (μl·h^{-1})			
TISO	12.6 ± 1.0 *	93.6 ± 4.8 **	**
SYN	18.0 ± 1.7	69.6 ± 6.4	**
Clearance Rate (μl·h^{-1})			
TISO	0.38 ± 0.1 **	20.4 ± 4.1 **	**
SYN	17.4 ± 5.6	67.2 ± 6.4	**

(Wilcoxon Test and Student *t*-test; Ho: group means same, reject if $P < 0.01$; $P < 0.05$, *; $P < 0.01$, **; $\bar{x} \pm 1$ SEM, n = 10; †, assumes dimensions of capture zone to be equivalent for both cell species.)

and th is the time to handle one cell. The general view of veliger feeding has historically been that it is a relatively continuous process with particle capture, ingestion and digestion all occurring simultaneously, but under independent control. Both Holling's and Cushing's models assume that the time available for food capture, handling, and digestion are independent processes. Crisp et al. (1985), then, hypothesized that there might be a mechanism for decoupling the processes of particle capture and ingestion in bivalve larvae to allow for continued clearance of particles from suspension independently of ingestion once digestion had been saturated. Indeed, this behavior is precisely what Werner (1955, 1959) and Fretter

and Montgomery (1968) had described for gastropod veligers and Strathmann et al. (1972) described for a trochophore larva.

The present study substantiates the observations made by previous authors by demonstrating that the capture rate of particles is proportional to their encounter rate in the medium up to an incipient level (i.e., 10^5 cells·ml^{-1}), beyond which physical interference probably limits capture efficiency. Ingestion rate was proportional to the rate of capture and transport to the mouth at low food concentrations, but declined to an age or size-specific level at high concentrations. Double reciprocal plots of ingestion rate data gave handling times (th) of 42 and 9.3 s·cell^{-1} for 2- and 10-day old larvae, respectively. Handling times for older larvae was similar to that calculated by Crisp et al. (1985) for larvae of *Mytilus edulis* from Jesperson and Olsen's (1982) data (i.e., 8.2 s·cell^{-1}), but 42 s·cell^{-1} for the young larvae is much higher than any published value. This is probably due to the size of the gut at this stage and the fact that most other investigations have used only late stage veligers in their studies.

There was no visual evidence that the increase in fluid viscosity associated with cell concentration by the velar cirri, food groove or mouth interfered with ingestion, as postulated by Crisp et al. (1985) to be a potential mechanism for limiting food intake. Even at the maximum cell concentration tested (10^6), where the concentration factor was calculated to be well over 10^5, cells were periodically removed from the bolus in front of the mouth and ingested with no apparent decrease in efficiency.

Cell concentrations of mixed flagellates in coastal waters typically supporting populations of bivalve larvae range between 10^2 and $3.7 \cdot 10^5$·ml^{-1} (Caron, 1983; Sherr and Sherr, 1984) and can rarely exceed 10^6 cells·ml^{-1} under extreme bloom conditions. Its interesting to note that maximum ingestion rates occurred within this range and were associated with minimum energy expended for rejection of superfluous cells. Poor growth of larvae at very high food concentrations has been observed in the laboratory by a number of investigators (Loosanoff et al., 1953; Walne, 1974; pers. obs.). Perhaps the energy required for rejection of cells becomes limiting under such conditions as described for *Daphina* sp. by Porter et al. (1982).

The decrease in clearance rate (based on cells ingested) above a cell concentration of 10^3 in this study is typical of a variety of planktonic grazers (e.g., echinoderm larvae, Strathmann, 1971; copepods, Mullin et al., 1975; cladocera, Porter et al., 1982; bivalve larvae, Sprung, 1984). Depressed clearance rate at low food levels, although described for copepods by a number of authors (Frost, 1972; Price and Paffenhöfer, 1984), is not as typical for bivalve veligers and may be due to one or more of the following: (1) experimental artifact induced by variability in cell counts at low concentrations (the Coulter Counter gave a coefficient of variation of 6% between quadruplicate cell counts at 10^2), (2) a real reduction of feeding response of larvae, although no statistical difference was found in cirral beat frequency or angular velocities at low cell levels or, (3) a change in some parameter of the capture mechanism not taken into account in the present calculations. I have no good explanation for the result except that there may have been a reduction in a number of parameters that, when compounded together, caused the observed decrease in clearance. This seems likely since the filtration rate of particles (based on the proportion of particles captured and transported to the mouth) was depressed as well. Theoretical energy optimization models (Lam and Frost, 1976; Lehman, 1976) predict reduced feeding efforts at food levels lower than that necessary to support growth. Since bivalve larvae probably rarely encounter cell concentrations much below 10^3 cells·ml^{-1}, one would not expect larvae to have adopted such a strategy. Nevertheless, in laboratory cultures, larvae routinely

form dense patches or rafts in which local cell levels may be depleted by grazing. Because of this, motor activity might be depressed along with clearance rates, forcing larvae to sink out of the center of the patch and begin swimming and feeding in a new area of the water column, thereby rejuvenating their food supply. Whether this behavior occurs under turbulent conditions in the field is purely speculative, but warrants further consideration. Studies on the relationship between food concentration and swimming path geometry are in progress in an attempt to explain these results.

Clearance rates per unit length of the velum ranged between $4.8 \cdot 10^4$ and $9.7 \cdot 10^2$ $\mu m^3 \cdot s^{-1} \cdot \mu m^{-1}$ in 10-day-old larvae and between $1.6 \cdot 10^4$ and 10 $\mu m^3 \cdot s^{-1} \cdot \mu m^{-1}$ in 2-day-old larvae depending on concentration (lowest clearances were at highest cell concentrations). Strathmann and Leise (1979) gave a value of 3,600 $\mu m^3 \cdot s^{-1} \cdot \mu m^{-1}$ for larvae of *Crassostrea gigas* (unspecified shell length) and quoted Bayne (1965) as calculating clearances of 10,000 to 15,000 $\mu m^3 \cdot s^{-1} \cdot \mu m^{-1}$ of velar edge in 250-μm long free swimming veligers of *Mytilus edulis*. Because the food concentration was not given in either of these studies, it is not possible to compare their results with those obtained here other than to note that maximum clearances per length of band appear to fall within similar orders of magnitude for different species of bivalve veligers.

Strathmann et al. (1972) discussed how the flexibility of a feeding mechanism may be enhanced by compounding cilia into discrete functional units that possess a greater bending couple than simple cilia. They argued that, with compound cirri, it may be possible to increase clearance rate per unit length of ciliated band by increasing beat frequency and cirral length. Present data show that velar circumferance, cirral length, beat frequency and cirral tip velocity all increased by almost a factor of two over development of *Mercenaria* veligers. This led to a four-fold increase in total flows per unit length of the velum and a three- to 97-fold increase in clearance rate per unit length, depending on cell concentration, between 2 and 10 days of development. This substantiates the postulate made by Strathmann et al. (1972) and suggests that compound cirri have enabled veligers to achieve a degree of developmental plasticity not otherwise possible. When one considers the wide range of reproductive strategies within the mollusca alone, it is not hard to imagine how important this one developmental trait has been to the evolution of suspension-feeding organisms.

The importance of pretreatment of larvae before a feeding experiment is apparent from the present study. Feeding rates of larvae with empty guts were 25 times greater than those of larvae with satiated guts. Sprung (1985) noted that starved larvae of *Mytilus edulis* had ingestion rates several times higher than well fed larvae. Besides introducing experimental variability, the effect of gut satiation may have important consequences for larvae in the field where plankton distributions may not be homogenously distributed. Effects of feeding experience in the copepod *Eucalanus pileatus* were described by Price and Paffenhöfer (1984) to include behavioral shifts in the particle size spectra of cells captured and ingested while the organisms became more experienced in handling specific cells over time. The data cannot be evaluated in terms of cell size since only one algal species was used in the gut satiation studies presented here, however, it is certainly clear that every attempt should be made to acclimate larvae at the desired cell concentration prior to conducting a feeding experiment when it is desired to measure steady-state feeding rates.

The results of the feeding trials using mixtures of *Isochrysis* and *Synechococcus* are in conflict with data in the literature on size-related retention of particles by bivalve larvae. Both Riisgard et al. (1980) and Sprung (1985) showed data for the

clearance efficiency of *Mytilus edulis* larvae feeding on natural particles from seawater. They concluded that between the size range of 1 and 9 μm, retention efficiency was 10–100% when normalized to the maximum clearance of three micron particles. There was no clearance of particles below 1 μm. Size distributions of particles in static experimental chambers were measured using a Coulter Counter before and after the experiments in both of these studies. Data from our laboratory (Gallager, Stoecker and Waterbury, unpubl.) shows that bivalve larvae produce fecal aggregates in the size range of 0.2 to 10 μm in diameter as measured with a Coulter Counter. Short bursts of particle production over 5 to 10 min can result in negative clearance rates greater than 80 $\mu l \cdot larva^{-1} \cdot h^{-1}$ in the 0.2- to 3-μm size fraction. Since particle production at these rates could lead to gross underestimation of clearance of small particles, results from grazing experiments employing closed vessels and electronic particle counters should be interpreted with caution. It is likely that bivalve larvae clear and feed on very small cells under natural conditions; indeed, Crisp et al. (1985) calculated that the concentration of flagellates in coastal waters was not great enough to support normal growth and development of *Ostrea edulis* larvae, and postulated that bacteria, as well as dissolved nutrients, may play a major role in larval nutrition.

Although cells of *Synechococcus* and *Isochrysis* appear to be captured and transported by *Mercenaria* larvae in statistically similar proportions, the differential ingestion and clearance rates suggest some form of selection at the mouth, be it passive or active. More experiments at various concentrations and particle sizes are needed to completely evaluate this effect, but the most probable reason is based on the difference in volume between *Isochrysis* and *Synechococcus.* This is particularly evident in the younger larvae where the diameter of the esophagus and volume of the gut may limit the size and number of particles. It is clear that although the numerical ratio of SYN/TISO ingested is relatively high, the volumetric ratio indicates that the contribution of *Synechococcus* biomass, at the concentration and proportion of cells used in our experiments, is considerably less than the biomass contributed by *Isochrysis.*

Observations of feeding behavior in larvae of *Mercenaria* described in this study demonstrate independent control over particle capture and transport to the mouth and ingestion and rejection from the esophagus. From these observations, a relatively simple scenario may be developed as a basis for further discussion and testing of hypotheses (Fig. 9). In the water column, bivalve larvae cycle through alternate phases of swimming and sinking which are proportional to size, age and relative density of the organism (Gallager, 1985). To initiate the swimming phase, the velum is extended and the long, compound frontal cirri begin to beat. The beating cirri are immediately coordinated into a metachronal wave through viscous coupling (Blake and Sleigh, 1975), with a wavelength and beat frequency proportional to the seawater viscosity (Machemer, 1972). Water movement past the velum generates a flow field more or less symmetrical in shape with dimensions being a function of the velocity of water past the velum and the forward velocity of the organism. As in the description of the flow field for the copepod *Eucalanus crassus* by Strickler (1982b; 1985), the shape of the flow field generated by a veliger should consist of a cylindrical motion core extending parallel to the body axis in front of the animal and entraining particles that will always be encountered by the velum regardless of forward velocity of the organism. A parabolically shaped viscous core of sheared water is also generated with dimensions increasing as restraint is applied to the larva by some external force, be it gravity when freely swimming in the water column or a tether in an experimental vessel (Strickler, 1982b; Emlet and Strathmann, 1985). If the cirral beat frequency and angular

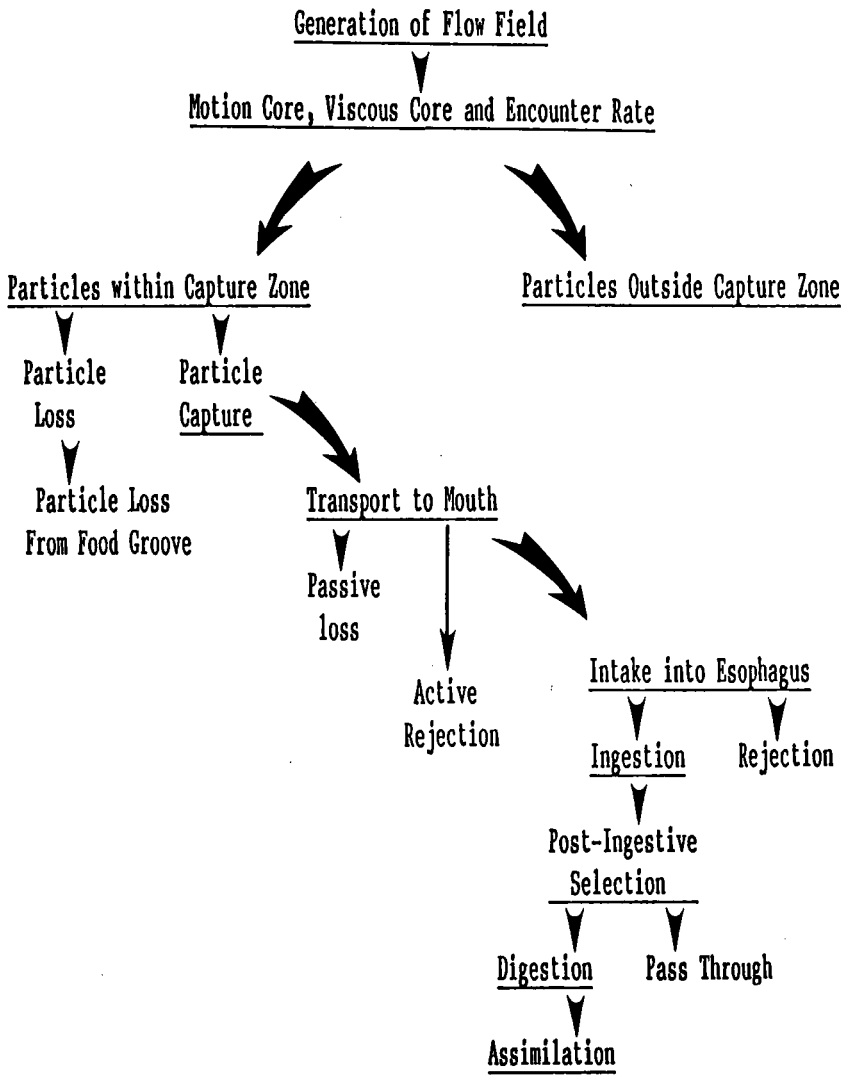

Figure 9. Proposed sequence of events in the feeding behavior of bivalve larvae. See text for details.

velocity remain constant, and thus the volume of water passing through the velar cirri, under restrained and unrestrained conditions (an assumption yet to be fully verified) then the rate at which particles are encountered will be a function of their ambient concentration and the flow rate through the velum. Homogenously distributed particles, therefore, should have an equal chance of being captured by both restrained and unrestrained organisms. Particles lateral to the organism, however, will more likely be encountered when an animal is either hovering in the water column or tethered in an experimental chamber. This would probably not have much effect on results obtained in laboratory vessels with a well mixed food supply, but horizontal variability in particle concentration in the field (i.e., patchiness) may be more effectively sampled when negatively buoyant organisms are moving very slowly or hovering in place. This may be particularly important

for bivalve larvae since buoyancy and locomotion are held in tenuous balance with the hydrostatic effects of lipid accumulated during planktonic life (Gallager, 1985; unpubl.).

Particles entrained within the flow field may or may not pass within the capture zone (defined in the present study as an area about 15 μm in width juxtaposed to the most distal boundary of the return stroke where particles were observed to be captured). Those that do appear to have a 42–64% chance of being captured and transported by the food groove to the mouth. The probability of capture may be related to the phase of the beat cycle when particles enter the sublayer and, if so, the length of the metachronal wave may be important in determining the proportion of particles passing through the capture zone that are directed into the food groove. The observation that cells in the process of being captured rotate in association with the shearing boundary layer around cirri that are in their return stroke, points to the importance of the the beat cycle in particle capture. Future studies should include recordings of feeding larvae from a dorsal perspective to observe displacement of particles relative to the metachronal wave.

Using high-speed microcinephotography, Strathmann and Leise (1979) reported that cells of *Dunaliella tertiolecta* and *Monochrysis lutheri* (diameters ranging from 5 to 10 μm) were captured by veligers of a bivalve and two gastropod species within the viscous sublayer of the velar cirri. Greatest capture efficiency was noted when particles encountered cirri within the lower half of their length where fluid velocity was lower than at the cirral tip. In the present study, fluid velocity appeared lowest in the capture zone adjacent to cirri in the return phase of their stroke. Blake and Sleigh (1975) observed that a beating cilium brings water from zero velocity close to its base (the no-slip condition) to maximum velocity at its tip. Fluid velocity decays toward the base of a cilium as the inverse square of the distance from the tip resulting in a three-dimensional cone of shearing fluid increasing in diameter distal to the cilium base. Although the present data appear to corroborate the observations of Blake and Sleigh (1975), compound cirri of the velum of *Mercenaria* larvae are much greater in length and width than simple cilia, and are arranged and beat in two orthoplectic rows. Extrapolations from the hydrodynamics of simple cilia are, therefore, tenuous at best and should be treated with caution. Future studies concerning the mechanism of particle capture should focus on the spatial and temporal relationship of particles, cilia and fluid velocity within the viscous sublayer.

Once captured, particles are entrained within the flow generated by the cilia of the food groove cilia (Strathmann and Leise, 1979) and transported to the mouth. No attempt was made in this study to estimate the proportion of cells lost from the food groove, but this should be possible since the transport process can be readily observed with the optical configuration described. Cells transported to the mouth may be lost, apparently by passive processes, accumulated into a bolus, ingested or actively rejected from the esophagus. The rates at which each of these processes occur are most likely a function of cell concentration in the medium, degree of gut satiation and particle digestibility.

Selection at the mouth and the esophagus may not be necessarily based on similar particle properties. Rejection from the the mouth may be a function of particle size (volume) and shape, while biochemical sensory mechanisms may be invoked within the esophagus where particles are in close association with the ciliated epithelia. Thus, rejection or selection by the sphincter cilia could be based on chemical information obtained at this level. The importance of chemosensory mechanisms in particle selection by copepods has been reviewed by Strickler (1985), who showed that the diffusional shell of algal metabolites can be detected

several particle diameters away from a feeding appendage. Furthermore, copepods appear to detect biochemicals directly in seawater (Strickler, 1985; Van Alstyne, 1986). Future studies on the location of mechano- and chemosensory structures in bivalve larvae may be revealing.

I have observed 2-μm latex spheres passing through the gut of veligers as quickly as they were ingested, while cells of *Isochrysis* were retained in the crystaline style sac and digested. Similar observations of post-ingestive selection were reported by Fretter and Montgomery (1968) for inorganic particles traveling through the digestive system of prosobranch veligers and by Robinson (1981) for fluorescent microspheres in the gut of *Mercenaria* larvae. This may be an important process for eliminating dense, non-nutritious material from the gut during periods of rapid ingestion when the gut is relatively empty.

Normal and high-speed video microscopy has been shown to be a useful tool in studying feeding behaviors of zooplankton. Using this technology, future experiments should concentrate on three areas of research: (1) the extent of the flow field generated by both free-swimming and tethered organisms to the test the hypothesis that a retarding force increases the encounter probability of particles lateral to the velum, (2) the size of the capture zone relative to particle characteristics such as size, shape, specific gravity, surface electrostatic charge and the functional morphology of the velum over development (i.e., cirral length, beat frequency and metachronal wavelength) and, (3) an analysis of the physical and biochemical characteristics of particles that mediate their selection and rejection in relation to the nutritional value of the potential food item. Controlled laboratory experiments coupled with studies of gut contents of larvae collected from the field should increase our understanding of the the trophic relationships responsible for regulating population dynamics of these invertebrate larvae.

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APPENDIX: DISCUSSION AFTER GALLAGER

C. Boyd: How does the feeding mechanism in your ciliated invertebrate larvae compare with that in single-celled organisms which must also use cilia for both locomotion and feeding?

S. Gallager: From the work of Fenchel and others, many ciliated protozoans appear to use fused cirri or membranelles as a sieve for capturing particles. They usually show a specific efficiency of particle capture based on particle size and can capture very small particles down to about 0.2 μm, such as bacteria. Bivalve larvae, on the other hand, appear not to use their pre-oral cirri as a sieve since both large (4.5 μm) and small (0.5 μm) particles are captured with equal efficiency. Moreover, the diaplectic metachrony found in bivalve larvae suggests that there must be temporal as well as spatial components to particle capture—particles appear to be captured when they enter the cilia sublayer at a point of the metachronal wave where the cirri are at the end of their effective stroke, or during the return stroke. Patterns of fluid flow and shear gradients within the sublayer probably govern particle capture rather than direct interception by pre-oral cirri. The functional response of bivalve larvae is very similar to ciliated protozoans, but clearance rates, particularly on very small particles like *Synechococcus,* are in the tens to hundreds of $\mu l \cdot h^{-1} \cdot larva^{-1}$ and are much higher than observed in most protozoans feeding on similar sized particles.

P. Verity: You've shown that bivalve larvae can capture and ingest cells of *Synechococcus,* but what do they do with them after ingestion? Are they assimilated or just passed through the gut intact? Apparently there are very few

microzooplankters that can actually utilize *Synechococcus* as a nutritional source.

S. Gallager: I have taken a number of approaches to answer that question but the results are somewhat ambiguous. Bivalve larvae will retain autofluorescence of phycoerytherin in the gut and digestive gland for many days after a pulse feeding of *Synechococcus.* Phycoerytherin is also present in larvae collected from the field. When ^{14}C-labeled cells are ingested, about 50% of the cell carbon is retained by the larvae after a chase period of two days. Moreover, there is a rapid production of $^{14}CO_2$ by larvae grazing on labeled cells, suggesting efficient digestion and metabolism of cellular carbon. Interestingly, larvae do not grow and develop at normal rates on a diet of *Synechococcus* alone, however, when offered in combination with cells of good nutritional value, *Synechococcus* apparently provides some benefit (perhaps an essential amino or fatty acid) since growth is enhanced over mono-specific diets.

Unknown: Then there is no reason why larvae should want to specifically select *Synechococcus* over other bacterioplankton—there is no feed-back message for selecting cells of good nutritional value.

S. Gallager: Probably not. The most probable reason why larvae ingested many more cells of *Synechococcus* than *Isochrysis* in this study is because of particle size. As long as the gut is not satiated, small particles may be ingested indiscriminately while large particles may be actively sorted and selected. A similar size-related mechanism appears to occur in some copepods, as shown in the studies of Price and Paffenhöfer.

D. Checkley: You and several others have used tethered animals in your studies of feeding behavior. How might tethering be interfering with this process?

S. Gallager: Ciliated organisms may be different from copepods when the effects of tethering are considered. From the studies I have done by exposing tethered larvae to various flow regimes, it appears that the shape of the flow field around the organisms changes with simulated forward velocity. When water is passed over an organism at a velocity equivalent to their normal swimming speed, parallel streamlines in front of the larva diverge around the body and then reconverge in back (Reynolds number is about 0.5 to 1.5 in this situation). However, when water flowing over the organism is stopped, simulating hovering by the larva, the flow field becomes parabolic in shape and water appears to be pulled in lateral to the larva. Feeding rates are comparable between conditions of simulated swimming and hovering. Although there may be a slight increase in water flow through the velum when larvae are hovering (Childress et al., 1987; J. Fluid Mech. 177: 407–436), it appears that the main affect tethering has on bivalve larvae is to increase the rate of sampling of particles lateral to the organism, rather than what is just in front of the organism. Hovering may be important in the field where particles may be patchy on a horizontal scale, but there should be little effect in laboratory experiments when they are homogeneously distributed.

H. Price: I would like to point out that there has been progress towards conducting these studies on free-swimming animals. Rudi Strickler's new system is set up for 3-D tracking of free-swimming copepods and their prey as small as 10 μm. Also, long-distance microscopes have recently been developed which claim a resolution of 3 μm at a distance of 1 m away. Studies of tethered animals have been a useful first step while we are moving towards making high-resolution observations on free-swimming individuals.

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