

# Effect of flow and sediment transport on feeding rate of the surface-deposit feeder *Saccoglossus kowalevskii*

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**ABSTRACT:** We studied the feeding behavior of the surface-deposit feeder *Saccoglossus kowalevskii* under oscillatory flows in the laboratory. Low oscillatory flow (peak speed =  $10 \text{ cm s}^{-1}$ ) without associated sediment transport had no effect on egestion rates in comparison to no-flow conditions. Moderate flows (peak speed = 15 and  $17.5 \text{ cm s}^{-1}$ ) with incipient sediment transport caused an increase in egestion rates of 35 to 100% relative to low-flow rates. High flow (peak speed =  $25 \text{ cm s}^{-1}$ ) with bulk sediment transport caused a decrease in egestion rates to one-third the low-flow rates. When compared to a functional response curve previously determined for *S. kowalevskii*, our data show that under high flow *S. kowalevskii* does not feed at rates predicted by the available food resources (as measured by chlorophyll *a* concentration). Under moderate flow, *S. kowalevskii* is stimulated to feed at higher rates than predicted by the available food resources. And under low flow, *S. kowalevskii* appears to be unaffected by flow and continues feeding at rates predicted by the available food resources. Flow and the associated sediment flux thus lead to an envelope of egestion rates about the no-flow functional response, indicating that the feeding behavior of *S. kowalevskii* is a function both of available food resources and flow regime. Laboratory still-water functional responses and simple feeding models are thus inadequate. Accurate extrapolation and prediction require that flow and sediment flux parameters be incorporated in feeding models.

**KEY WORDS:** *Saccoglossus kowalevskii* · Deposit feeder · Oscillatory flow

## INTRODUCTION

The feeding behavior of deposit feeders has been the subject of much study (see Lopez & Levinton 1987 for a review), but not until recently have the effects of flow and sediment flux on feeding behavior been examined (Taghon et al. 1980, Dauer et al. 1981, Jumars & Self 1986, Miller & Jumars 1986, Nowell et al. 1989, Levinton 1991, Turner & Miller 1991, Miller et al. 1992, Taghon & Greene 1992). Often, deposit feeders respond with an immediate change in feeding behavior from no-flow to flow conditions, while behavior under increasingly stronger flows appears to be a modulation of the initial response (Miller et al. 1992). As such, feeding behavior changes are most definitive at low flow rates; as flow rates increase, feeding behavior

changes are more or less a continued progression from the initial behavioral change, and in many cases lead to an eventual ceasing of feeding behavior (Levinton 1991, Miller et al. 1992).

Flow may effect the feeding behavior of deposit feeders in both direct and indirect ways. In a direct way, increasing flow rates may cause a switch from deposit to suspension feeding by some species (Taghon et al. 1980, Dauer et al. 1981, Levinton 1991, Turner & Miller 1991) or a reduction in the area of the feeding pits of obligate deposit feeders (Miller et al. 1992). Flow regime may indirectly affect feeding behavior by causing sediment transport and, as a result, changes in available food resources (Taghon & Greene 1992, Bock & Miller 1995). Changes in food resource quality may cause deposit feeders to alter their rates of ingestion or egestion (Cammen 1989, Taghon & Greene 1990, Karrh & Miller 1994). Sedi-

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ment flux may have positive (through input of new materials) or negative (by removal or dilution of high quality materials within feeding pits) effects on the food resource availability, and likewise on feeding rates, depending on the degree of sediment flux and conditions upstream of the feeding pit (Miller et al. 1984). Removal of fecal material may also be an indirect effect of flow regime on feeding rates (Miller & Jumars 1986).

To examine the effects of flow on deposit feeder behavior, we performed 2 experiments with the surface-deposit feeder *Saccoglossus kowalevskii*. *S. kowalevskii* is a good test organism for this task. First, it is an obligate deposit feeder, which prevents it from switching to suspension feeding in the presence of flow. Second, *S. kowalevskii* is a non-selective feeder with respect to particle size (Knight-Jones 1953, Brandon 1991), which reduces the potential for behavioral response to flow due to resuspension and/or winnowing away of smaller particles. Third, we previously quantified its feeding behavior under no-flow conditions (Karrh & Miller 1994), which provided necessary background information on which to base a predicted response to food resource availability. And finally, previous observations of this organism under oscillatory flows (Miller et al. 1992) indicated that the feeding area is reduced as flow rate increases and feeding at the surface ceases at the highest flows. For these reasons, *S. kowalevskii* represents a simple case for quantifying feeding response to flow.

Based on the observations of Miller et al. (1992), our hypothesis for these experiments was that *Saccoglossus kowalevskii* feeding rates would decrease as flow rate increased due to a reduction in the amount of space covered and time spent feeding at the surface. We expected that any response to food quality would be removed from the analysis by comparison to the no-flow functional response (Karrh & Miller 1994).

## MATERIALS AND METHODS

**Organism.** We used the obligate surface-deposit feeder *Saccoglossus kowalevskii* (phylum Hemichordata: class Enteropneusta), commonly called an acorn worm. *S. kowalevskii* feeds on the top 1 mm layer of loose sediment (Carey & Mayer 1990). Fecal material is packaged into mucous encased coils (Miller 1992) and deposited in characteristic piles on the sediment surface. Fecal coils of darkened, reduced sand are rarely seen in the laboratory, indicating that *S. kowalevskii* feeds primarily on surface sediment. *S. kowalevskii* has distinct areas for feeding and fecal piles, and the presence of fecal material is not likely a deterrent to feeding behavior in this organism (Miller 1992).

The functional response of *Saccoglossus kowalevskii* in the absence of flow (Karrh & Miller 1994) involves increasing egestion rates to a maximum as food quality (as measured by chlorophyll *a* concentration) increases to moderate levels, followed by a slight decline in egestion rates as food quality further increases. Miller et al. (1992) observed that, under oscillatory flows, *S. kowalevskii* feeds at the sediment surface until stronger flows (peak speeds  $\geq 18 \text{ cm s}^{-1}$ ) begin. The proboscis is retracted into the burrow under these stronger flows, and *S. kowalevskii* may either ingest particles swept into its burrow by passing water, feed below the surface, or cease to feed altogether until the flow returns to lower intensities. *S. kowalevskii* returns to surface feeding soon after the flow is returned to lower intensities.

**Experiments.** We performed 2 experiments in the Lofquist oscillatory water tunnel (Turner & Miller 1991). The water tunnel allowed us to better simulate flow conditions found on the intertidal sandflat at Cape Henlopen, Delaware, USA, than is possible in a more traditional, 1-directional flume. In the water tunnel, we were able to regulate both amplitude and period of the simulated waves. However, for practical reasons, we only modified the period of oscillation in defining treatment flows. Flow treatments were characterized by peak near-bottom orbital speed,  $U_{max}$ , in  $\text{cm s}^{-1}$ . A more extensive description of the water tunnel can be found in Turner & Miller (1991).

Sand was collected from the field site, sieved through a 1 mm mesh to remove macrofauna, and placed in the bottom 20 to 25 cm of the water tunnel's acrylic working section. Individual plastic containers (10 × 10 × 13 cm), each containing 1 previously collected and acclimated worm, were placed within the bed such that the tops of the containers were below the sediment surface and did not interfere with the flow. Removal of 3 water-tight hatches in the top of the water tunnel's working section allowed for access to the bed and the organisms within it.

Chlorophyll *a* and protein concentrations in the bed sediment were measured to determine food resource availability during the experiment. Sediment samples for these analyses were taken from randomly assigned locations within the bed surface. Five replicate sediment samples for each analysis were taken using a modified 20 ml plastic syringe to core a 2.84  $\text{cm}^2$  area within the assigned location. The top 1 cm of each core was retained for analysis.

For chlorophyll *a* analysis, we used the acetone extraction method of Parsons et al. (1984), modified for sediment samples (Ray 1989, Brandon 1991). Samples were frozen at  $-20^\circ\text{C}$  upon collection until analysis, which was generally within a few days. For sediment protein analysis, we used a modification of the method

of Mayer et al. (1986). This involved replacement of the standard additions with a casein standard curve for determining protein content of extracts (Bock & Miller 1994, Karrh & Miller 1994). Samples were frozen at  $-20^{\circ}\text{C}$  upon collection and generally analyzed within 1 mo.

Feeding behavior was monitored by quantifying egestion rates. Due to the high percentage of the inorganic portion of the sediment, the mass of egested material is essentially equal to the mass of ingested material, and egestion rates are often used instead of ingestion rates (Miller & Jumars 1986, Taghon & Greene 1990, Miller 1992). Fecal samples were taken at hourly or 2 h intervals by gently pipetting intact coils from the sediment surface and placing them into individual, weighed petri dishes. Excess seawater was gently suctioned off, and the fecal samples were dried overnight at approximately  $60^{\circ}\text{C}$ . Samples were not rinsed to remove salt because previous work indicated this was not a large source of error (Miller 1992). Egestion rate was determined by calculating grams sediment (dry weight) egested per unit time (in hours). Typically, fecal samples weighed hundreds of milligrams for 1 h feeding.

The design of both experiments was complicated by the limitation of having only 1 water tunnel in which to conduct the experiments. For this reason, we could only administer 1 flow treatment at a time. Therefore, flow treatments were administered on consecutive days. The whole group of 15 worms was subjected to each day of the flow treatments, and the same group of worms was used in both experiments. As a result, we had repeated measurements of egestion rates for each worm for each day of each treatment. The data were therefore analyzed using a repeated-measures ANOVA (see 'Data analysis'). This experimental design and analysis allowed us to remove the variability due to differences between individual worms while statistically allowing for possible correlations among individuals' responses, both of which are inherent in this experimental design.

**Expt 1.** *Saccoglossus kowalevskii* were collected from the sandflat at Cape Henlopen ( $38^{\circ} 47' \text{N}$ ,  $75^{\circ} 06' \text{W}$ ) in September 1992 and kept in plastic containers in a flow-through seawater table until used for Expt 1, which was conducted in October 1992. Fifteen worms of various sizes were used for this experiment and worm size was not controlled. The worms, each in a separate plastic container, were placed in the water tunnel bed and allowed to acclimate for 2 d prior to the experiment.

We used 3 flow treatments for this experiment. Each treatment was characterized by the flow speed and the amount of particle movement associated with it. The low-flow treatment ( $U_{\text{max}}$  of  $10 \text{ cm s}^{-1}$ ) consisted of

water movement alone with no sediment movement except for the flocculent material on the surface of the bed. The moderate-flow treatment ( $U_{\text{max}}$  of  $17.5 \text{ cm s}^{-1}$ ) consisted of flow sufficient to begin movement of the sediment. The high-flow treatment ( $U_{\text{max}}$  of  $25 \text{ cm s}^{-1}$ ) consisted of flow sufficient to cause bulk sediment movement and ripple formation. Each flow treatment was administered on 3 consecutive days in a randomly assigned sequence: high (Days 1 to 3), low (Days 4 to 6), moderate (Days 7 to 9).

Each day, the flow was set at the appropriate treatment level and *Saccoglossus kowalevskii* were allowed to feed undisturbed for 12 h. After this period, the flow was turned off and fecal samples were collected for the  $\text{ER}_{\text{FLOW}}$  sample. The  $\text{ER}_{\text{FLOW}}$  samples represent egestion rates (ER) under flow. Fecal samples were subsequently taken at hourly intervals for 3 h (samples  $\text{ER}_{+1\text{h}}$ ,  $\text{ER}_{+2\text{h}}$  and  $\text{ER}_{+3\text{h}}$ ) each day. Although these later samples represent feeding rates under no flow, they were taken to assess feeding rates under higher flows when fecal material is eroded away and uncollectable. Our intent was to compare  $\text{ER}_{\text{FLOW}}$  to the ER during the immediately following no-flow period ( $\text{ER}_{+1\text{h}}$ ,  $\text{ER}_{+2\text{h}}$  and  $\text{ER}_{+3\text{h}}$ ) to determine if a lag period in egestion rates occurred as has been previously documented (Miller 1992). Such a relationship would allow us to monitor feeding rates under higher flows which otherwise are unmeasurable by direct sampling.

In the design of this experiment, we had expected to be able to collect  $\text{ER}_{\text{FLOW}}$  fecal samples under the low- and moderate-flow treatments. Unfortunately, the moderate-flow treatment was high enough to erode fecal material, so we were unable to collect reliable  $\text{ER}_{\text{FLOW}}$  fecal samples under this treatment. This left only the low-flow treatment data to establish a relationship between the  $\text{ER}_{\text{FLOW}}$  and the other samples ( $\text{ER}_{+1\text{h}}$ ,  $\text{ER}_{+2\text{h}}$  and  $\text{ER}_{+3\text{h}}$  samples). Since no difference was found subsequently (see 'Results — Expt 1'), we compared the flow treatments using the  $\text{ER}_{+1\text{h}}$ ,  $\text{ER}_{+2\text{h}}$  and  $\text{ER}_{+3\text{h}}$  samples collected following each treatment, and our inferences are contingent upon this ER lag-period assumption.

Sediment samples were taken after collection of the  $\text{ER}_{+3\text{h}}$  fecal samples, and the worms were left under still water for approximately 6 h. We then collected all accumulated fecal material for each worm and resealed the tunnel for the next flow period. The tunnel was turned on each evening and left under the assigned treatment flow for the next 12 h.

**Expt 2.** This experiment was conducted in January 1993 and consisted of 2 flow treatments, low flow ( $U_{\text{max}}$  of  $10 \text{ cm s}^{-1}$ ) and moderate flow ( $U_{\text{max}}$  of  $15 \text{ cm s}^{-1}$ ). This slower moderate-flow speed was chosen in an attempt to reduce erosion of fecal material observed under the moderate-flow treatment in Expt 1. The

order of treatments was specifically chosen to include all possible sequences of the 2 flow speeds to test whether the previous day's flow treatment affected the feeding response to the next day's flow treatment. Treatment order was: low, moderate, moderate, low, low, moderate. The worms used in Expt 2 were the same 15 used in Expt 1. Each worm, in its container, was randomly assigned and buried in a new position relative to the others in the tunnel bed.

Each day, the water tunnel was set to the appropriate flow speed and the worms were left to feed under flow for 21 h. At the end of this period, the tunnel was turned off and fecal samples were taken for an  $ER_{FLOW}$  sample. The worms were then allowed to feed undisturbed for 2 h, and fecal samples were again taken ( $ER_{+2h}$  samples). Following collection of the  $ER_{+2h}$  fecal samples, sediment samples were taken as described above. We then resealed the tunnel and set the flow to the assigned flow treatment level. *Saccoglossus kowalevskii* were allowed to feed undisturbed under this flow for the next 21 h period.

As in Expt 1, total erosion of fecal material occurred under the moderate-flow treatment and partial erosion of some fecal piles occurred under the low-flow treatment. This left very few  $ER_{FLOW}$  samples that could be collected. Instead, we used the  $ER_{+2h}$  data for comparisons between the treatments and days.

Partial erosion of fecal piles under the low-flow treatment was likely the result of 2 factors. First, many of the fecal mounds were at the crests of ripples in the bed where they were under more erosional stress. Second, the extended period of flow (21 h as opposed to 12 h in the first experiment) allowed for further degradation of the mucus encasing the fecal material and may have expedited erosion.

**Behavioral observations.** Qualitative observations of feeding behavior were made during flow periods. Observations under high flow were made on the first day of Expt 1. Five of the worms had burrowed against the acrylic side of the working section, and these worms were visible within the uppermost portion of their burrows. We watched and recorded the behavior of these 5 worms during the first 2 h after the flow had been turned on. No randomized, systematic method was used; instead, notations were made of the activity of these worms at the times they were observed.

Behavior under the moderate- and low-flow treatments was observed during Expt 2. All 15 worms were observed within 3 separate groups. Groups were based on the position of individual worms within the water tunnel bed. Each group was watched for 35 min, with a switch from one side of the water tunnel working section to the other mid-way through. By switching sides we were in many cases able to see worms feeding that had not been visible from the other side. Notation

was made of the time an activity was observed along with the worm's identity. Behavior under each flow was observed on the second consecutive day of each treatment (i.e. experiment Day 3 for moderate flow and Day 5 for low flow) so that possible effects of the previous day's flow would not be a factor.

**Data analysis.** Statistical analysis of egestion rate data for both experiments was completed using univariate repeated-measures ANOVAs (Kirk 1982), with repeated measures on day in both experiments and also on hour in Expt 1. Statistical analysis of the chlorophyll *a* and protein concentration data was completed using 1-way nested ANOVAs with day nested within treatment. All tests were evaluated at an  $\alpha$ -level of 0.05 for statistical significance.

Tests of the assumptions on which repeated-measures ANOVAs and nested ANOVAs are based were also performed. Homogeneity of variances was tested according to Bartlett's testing, normality of the data was tested according to a Kolmogorov-Smirnov's comparison to a Lilliefors normal distribution, and symmetry was tested within the univariate repeated-measures ANOVA algorithm (SYSTAT software; Wilkinson 1990). Tukey's honestly significant difference multiple comparison test (Zar 1984) was used to determine where significant differences occurred between means. The within-factor error mean square from the repeated-measures ANOVA was used in the Tukey test as the measure of variance. Honestly significant difference values were used to depict error bars in graphing the egestion rate data for each experiment. Non-overlapping error bars on the ordinate axis indicate significant difference between means (Andrews et al. 1980) at an overall family-wise  $\alpha$ -level of 0.05. This allows for quick visual interpretation of the data in these graphs. In graphs where data from more than 1 experiment are presented (and hence not analyzed using a single ANOVA), error bars represent 1 standard error of the mean for comparisons.

## RESULTS

### Expt 1

Chlorophyll *a* concentrations and protein concentrations were not significantly different throughout Expt 1 ( $p \geq 0.1$ ). Due to erosion of fecal mounds under the moderate and high flows, the treatments were compared using the  $ER_{+1h}$ ,  $ER_{+2h}$  and  $ER_{+3h}$  data. One worm was dropped from the repeated-measures ANOVA due to missing data for 1 d, leaving a sample size of 14. Violation of the assumptions upon which univariate repeated-measures ANOVA testing is based were indicated, and standard transformations

(logarithmic and inverse) of the data did not yield substantial improvement. Using the raw data, the Greenhouse-Geisser and Huynh-Feldt corrected probabilities, each a conservative adjustment of the univariate  $F$ -ratio probability (Wilkinson 1990, Zolmon 1993), both gave significance results consistent with the univariate  $F$ -ratio probability, so no transformation of the data was used for final analysis and unadjusted univariate  $p$ -values are reported below.

Treatment overall mean egestion rates (all samples together) were significantly different ( $p < 0.001$ ), but there were no significant daily ( $p = 0.2$ ) or hourly ( $p = 0.1$ ) differences, and no significant interactions (all  $p \geq 0.05$ ). The moderate-flow treatment overall mean egestion rate was significantly higher than those of the other 2 flow treatments, which were not significantly different from one another (Fig. 1). Treatment overall mean egestion rate increased in the same order as treatments were administered, suggesting the possibility of an artifact in the data due to treatment order. This possible problem was further addressed in Expt 2.

The low-flow treatment  $ER_{\text{FLOW}}$  samples are the only data available for egestion rates under flow. We used a repeated-measures ANOVA (with repeated measures on day and hour) to compare the  $ER_{\text{FLOW}}$  data with the data from  $ER_{+1h}$ ,  $ER_{+2h}$  and  $ER_{+3h}$  samples. There was no significant difference between daily ( $p = 0.3$ ) or hourly ( $p = 0.1$ ) mean egestion rates. The non-significant difference between hours suggests that a lag in egestion rate response of at least a few hours occurred, or that egestion rates under the low-flow treatment were not significantly different from egestion rates under no flow (Fig. 2). In either case, it appears valid to use the  $ER_{+1h}$ ,  $ER_{+2h}$  and  $ER_{+3h}$  data in comparisons with the other 2 treatments to represent egestion rates under the low-flow treatment.

We were unable to test directly for a lag effect under the moderate and high flows so we tentatively assumed that the  $ER_{+1h}$ ,  $ER_{+2h}$  and  $ER_{+3h}$  data collected following flow periods adequately represent egestion rates under the previous flow treatments (an unmeasurable  $ER_{\text{FLOW}}$ ). This assumption is consistent with our finding (above) of no time effects (day or hour) or interactions except those attributable to treatments. This assumption is further supported by analysis of the data by separate repeated-measures ANOVAs (Table 1). The results reveal the same pattern in the treatment effect on egestion rates over the 3 h period following the flow treatments (Fig. 3), even though these samples were taken following still-water periods. The consistent, highly significant pattern over the several hours following the flow treatments, and the hours-long lag period in egestion rates response found by Miller (1992), support the assumption that egestion rates following the flow treatments are representative

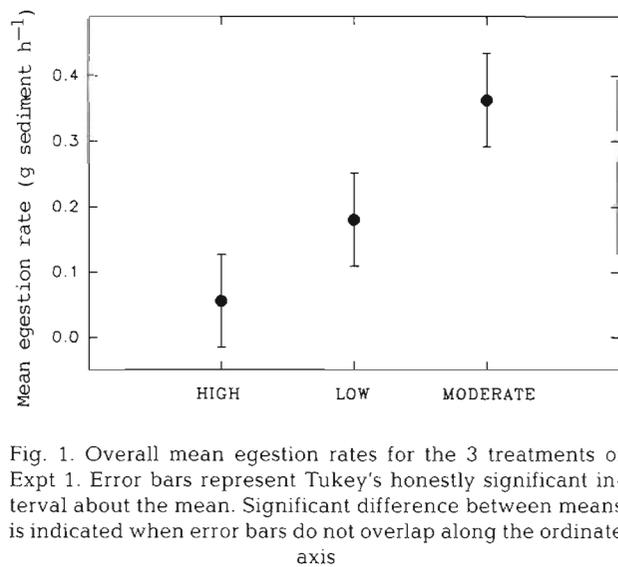


Fig. 1. Overall mean egestion rates for the 3 treatments of Expt 1. Error bars represent Tukey's honestly significant interval about the mean. Significant difference between means is indicated when error bars do not overlap along the ordinate axis

of egestion rates during the flow treatment and that our comparisons represent valid treatment differences. Expt 2 was designed to verify further these results.

## Expt 2

As in Expt 1, chlorophyll  $a$  and protein concentrations throughout Expt 2 were not significantly different ( $p \geq 0.3$ ). Comparisons between the treatments and days was done using the  $ER_{+2h}$  data. No violation of the assumptions on which univariate repeated-measures ANOVAs are based was found in these egestion rate data, so the data were used without transformation and unadjusted univariate  $p$ -values are reported below.

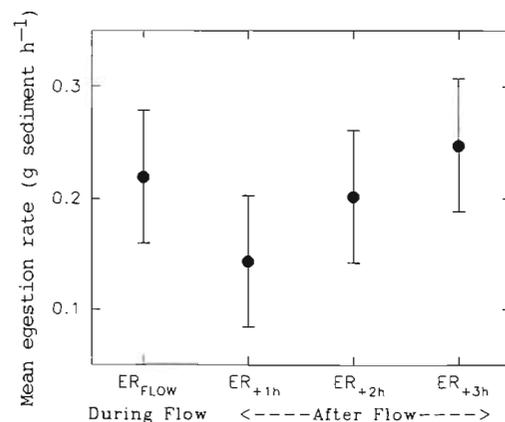


Fig. 2. Hourly mean egestion rates for the low-flow treatment from Expt 1. Error bars represent Tukey's honestly significant interval about the mean. The overlap of error bars indicates no significant difference among these means

Table 1. Results of separate analysis of the ER<sub>+1h</sub>, ER<sub>+2h</sub> and ER<sub>+3h</sub> data from Expt 1. Analysis was done by a univariate repeated-measures ANOVA (with repeated measures on day)

| Sample                        | Source of variation | df | F-ratio | p      |
|-------------------------------|---------------------|----|---------|--------|
| ER <sub>+1h</sub><br>(n = 14) | Treat               | 2  | 11.2    | <0.001 |
|                               | Error               | 26 |         |        |
|                               | Day                 | 2  | 2.05    | 0.2    |
|                               | Error               | 26 |         |        |
|                               | Treat × Day         | 4  | 2.28    | 0.07   |
|                               | Error               | 52 |         |        |
| ER <sub>+2h</sub><br>(n = 15) | Treat               | 2  | 6.56    | 0.005  |
|                               | Error               | 28 |         |        |
|                               | Day                 | 2  | 1.02    | 0.4    |
|                               | Error               | 28 |         |        |
|                               | Treat × Day         | 4  | 1.32    | 0.3    |
|                               | Error               | 56 |         |        |
| ER <sub>+3h</sub><br>(n = 15) | Treat               | 2  | 27.1    | <0.001 |
|                               | Error               | 28 |         |        |
|                               | Day                 | 2  | 2.60    | 0.09   |
|                               | Error               | 28 |         |        |
|                               | Treat × Day         | 4  | 2.56    | 0.05   |
|                               | Error               | 56 |         |        |

Again, treatment overall mean egestion rates were significantly different ( $p = 0.03$ ). As before, daily egestion rates were not significantly different ( $p = 0.5$ ) within the treatments, and there was no interaction ( $p = 0.08$ ). The moderate-flow overall mean egestion rate was again higher than the low-flow overall mean egestion rate (Fig. 4A). On a daily basis, however, there was some variability in effect magnitude (Fig. 4B), though the only significant differences are attributable to flow treatments. This may indicate a small latent effect of the previous day's treatment on the daily egestion rates or some other unmeasured factor influencing the egestion rates. However, on the days which were the second consecutive day of the same treatment (Day 3 for the moderate treatment and Day 5 for the low treatment), the daily means were significantly different between treatments. Also, the overall mean for each treatment (Fig. 4A) indicates a significant difference in egestion rates, suggesting that any influence of treatment order (particularly on Days 2 and 4 of the experiment) is smaller than the effect of the treatment flow speed on feeding behavior.

For those worms whose fecal piles remained intact under the low-flow treatment ( $n = 4$ ), we compared the ER<sub>LOW</sub> data, representing feeding under flow, to the

ER<sub>+2h</sub> data, representing feeding rates following the flow treatment. There was no significant difference between daily ( $p = 0.08$ ) or hourly ( $p = 0.1$ ) egestion rates, and no interaction ( $p = 0.9$ ). The results were the same in this experiment as those in Expt 1, again suggesting that the egestion rates following the flow treatments are representative of the egestion rates during the flow treatments and that the statistical comparisons made accurately reflect flow treatment differences.

### Behavioral observations

Qualitatively, *Saccoglossus kowalevskii* fed differently under the 3 treatment flows. Under the low-flow treatment of Expt 2, *S. kowalevskii* fed by extending the proboscis approximately 1 cm from the burrow opening, as opposed to approximately 2 to 3 cm under no flow. The proboscis was generally visible for 2 to 5 s per feeding bout, and most worms were seen feeding during the observation periods. At least 1 worm per group was observed to defecate during the observation period. Defecation events may not have been accurately noted, however, due to the remaining fecal

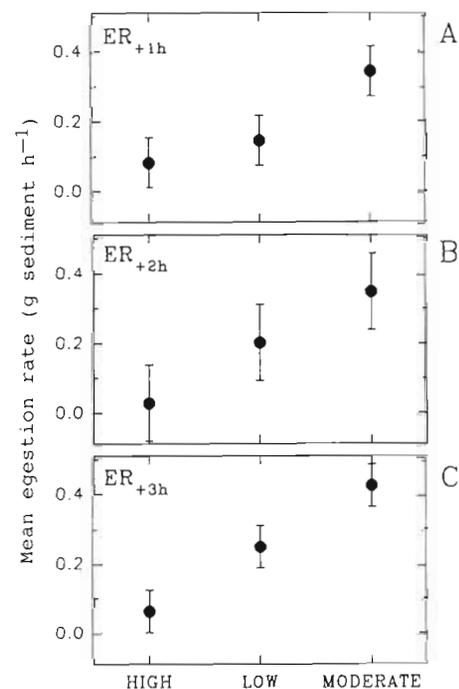


Fig. 3. Hourly mean egestion rates for all 3 treatments of Expt 1 (A) Results when only the ER<sub>+1h</sub> data are analyzed; (B, C) similar results when only the ER<sub>+2h</sub> and ER<sub>+3h</sub> data, respectively, are analyzed. Error bars represent Tukey's honestly significant interval about the mean. Significant difference between means is indicated when error bars do not overlap along the ordinate axis

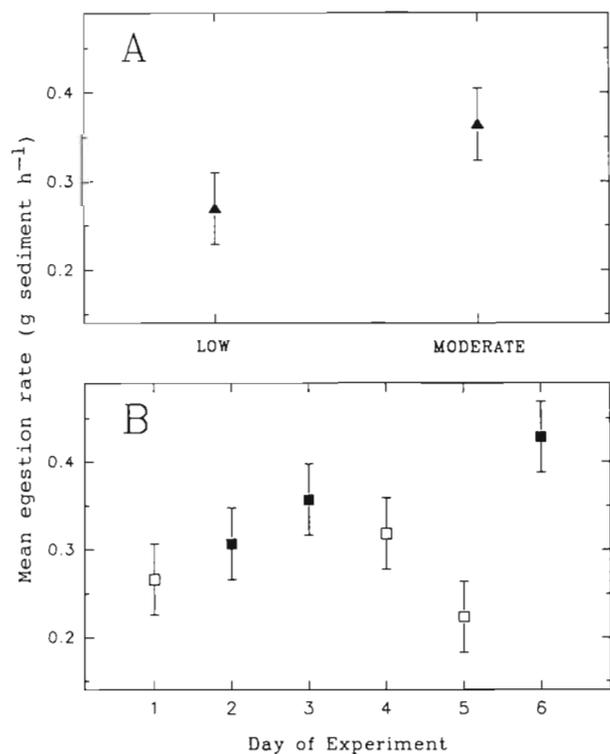


Fig. 4. Expt 2. (A) Overall mean egestion rates for treatments. (B) Daily mean egestion rates in administered order. (□) Low-flow data; (■) moderate-flow data. In both graphs, error bars represent Tukey's honestly significant interval about the mean. Significant difference between means is indicated when error bars do not overlap along the ordinate axis

material left in piles on the surface. Subsurface material, indicated by darker feces suggesting anoxic material, occurred in many of the fecal piles, but was generally not a large portion (visually estimated to be less than 25%) of any one fecal pile. Subsurface material in the feces could have resulted from subsurface feeding, daily maintenance of their burrows, or other unobserved activities.

Under the moderate-flow treatment of Expt 2, we observed at least 2 worms in each group actively feeding and most of the worms defecated during the observation periods. Defecation was easier to note under this treatment due to erosion of fresh fecal coils within minutes of egestion. Time spent feeding was generally longer under this treatment (2 to 10 s) while feeding areas under this flow were smaller (radius of approximately 0.5 to 1 cm from the burrow opening). At other times, only the proboscis tip (2 to 3 mm) was extended for 1 to 2 s. Subsurface material was contained within a few of the fecal piles but was not as prevalent as under the low-flow treatment and in general was a small portion (estimated to be less than 10%) of the total fecal matter in any one pile.

Under the high-flow treatment of Expt 1, worms approached the tops of their burrows, momentarily extended the very tip of their proboscis out into the flow, then immediately retracted them into the burrow and descended. The worms were visible within their burrows and were often seen moving up and down within the upper portions of their burrows, perhaps feeding subsurface or maintaining their burrows. Due to the flow rate and sediment flux, it was difficult to observe defecation. However, the momentary presence of coils still 'attached' to the sediment as they were presumably defecated suggested that the worms were indeed feeding under the high-flow treatment. Within 30 min of turning off the flow, fecal piles appeared on the sediment surface, again suggesting that the worms had been feeding under the high-flow treatment. In a few cases, subsurface material was noted in fecal samples taken after the flow was turned off, in approximately the same amounts as under the moderate-flow treatment (estimated to be less than 10% of any one pile).

## DISCUSSION

This study represents a first attempt to quantify the feeding rates of *Saccoglossus kowalevskii* under oscillatory flows similar to those found at Cape Henlopen (Ray 1989, Bock & Miller 1994). The presence of a lag effect in egestion rate response is a key assumption in these experiments. The consistency of the results when the data from the first experiment were examined in several different ways suggests that a lag period did occur. Without a lag period in behavioral response, we would expect that feeding rates (measured by  $ER_{+1h}$ ,  $ER_{+2h}$  and  $ER_{+3h}$ ) would quickly become similar to one another and to no-flow rates. This did not occur (Fig. 1). Because there was no difference in chlorophyll *a* or protein concentrations between treatments, these persistent differences in egestion rates cannot be attributed to food availability. Based on this, we feel there is strong evidence that a lag period in response did occur in our experiments. This is reinforced by the fact that we found the same overall results in the second experiment as in the first. Therefore, we believe that the egestion rate data collected following the flow periods in both experiments reliably estimate the actual egestion rates under flow.

In recent literature, the term 'functional response' has been used with regard to deposit feeders to describe a change in feeding rate with changes in sediment food quality (Taghon & Greene 1990). Such a response curve was previously described for *Saccoglossus kowalevskii* feeding in the absence of flow (Karrh & Miller 1994). This response curve can be com-

pared to the measured changes in feeding rates from these experiments, making it possible to separate the indirect effect of changes in food quality due to flow from the direct effect of flow on feeding rates. Because it is reasonable to assume that food quality and particle transport are strongly coupled in the field, and should covary, it makes little sense to discuss flow effects independently.

Though there were no treatment differences, we used overall mean chlorophyll *a* concentrations for each treatment from each experiment as the abscissa value to plot the overall treatment mean egestion rates atop the functional response curve (Fig. 5). The graph clearly shows a decrease in feeding rates under the high-flow treatment of Expt 1 relative to no-flow conditions, indicating that the worms were in some way directly inhibited by the flow and were not utilizing the available food source as would be predicted by the functional response curve. Moderate-flow egestion rates in both experiments were substantially higher than those under no-flow conditions, showing a response to the flow regime in addition to the response to food quality. And low-flow egestion rates in both experiments were close to those predicted by the functional response curve, suggesting that *Saccoglossus kowalevskii* feeding rates are not affected by low-flow rates.

A relationship between egestion rates and protein concentration (as measured here and previously) was not found in our previous experiments (Karrh & Miller 1994). However, our data indicate that protein concen-

tration did not significantly change between days in either experiment. This suggests that any influence of protein concentration on food quality and feeding behavior would not be a factor in the observed changes in feeding behavior in these experiments.

Given our past (Miller et al. 1992) and present observations, it is not surprising that high flow had a negative effect on feeding rates in Expt 1. Studies of other deposit-feeding species have found similar results (Levinton 1991, Miller et al. 1992). The behavioral observations made of *Saccoglossus kowalevskii*, both here and elsewhere (Miller et al. 1992), suggest that *S. kowalevskii* does not feed at the sediment surface under the higher flows, which could account for the decline in feeding rates. We observed the worms to travel to the tops of their burrows under high flow, but to retreat immediately once they reached the burrow opening. Our observations also indicate that the worms were still feeding under this treatment, as evidenced by defecation by many worms upon cessation of flow. It is not possible to determine from the results presented here whether the worms continued to feed subsurface or on particles that were swept into their burrows by the flow. More detailed observation of feeding under high flows, possible with the use of videographic imaging over longer periods than we used in our observations, is a potential next step to address this question.

Under low flow and in the absence of sediment flux, *Saccoglossus kowalevskii* appears to be unaffected and continues to feed at approximately the same rates as under no flow. Our visual observations indicate that the worms did feed in more limited areas under low flow than under no flow, which may indicate some influence of flow on their feeding behavior. However, any change in egestion rates, if it occurred, was smaller than detectable by our methods. Further study of *S. kowalevskii* under the conditions represented by this treatment would be required to address this question, if some method could be designed to enable more sensitive measurement of egestion rate.

Feeding rates were significantly higher under the moderate-flow treatments than under the other treatments in both experiments. Importantly, feeding rates were also higher than predicted based on the available food quality from still-water experiments. It would be advantageous to feed rapidly under conditions of sediment transport if the rate of input of new food materials met or exceeded the removal rates due to ingestion (Miller et al. 1984).

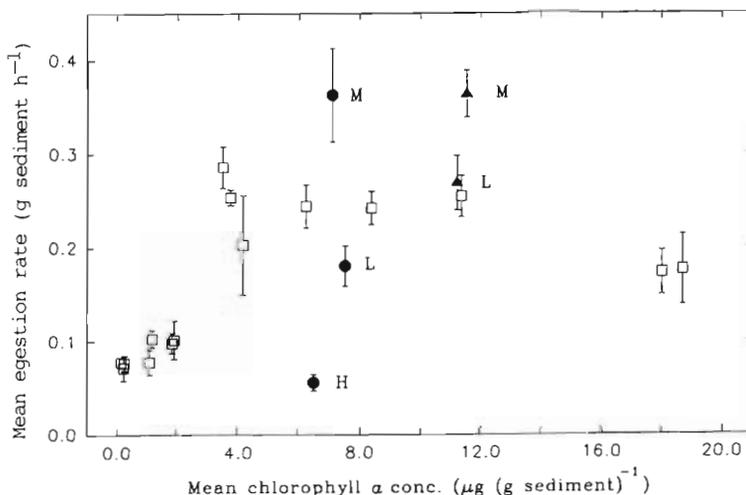


Fig. 5. *Saccoglossus kowalevskii*. Comparison of the results of the water tunnel experiments to the functional response curve. Data points represent overall treatment means, and error bars represent 1 SEM, i.e. mean values would have to differ by more than 2 SE to be judged significantly different. (□) No-flow functional response. (●) Expt 1 results, labeled according to treatment (L: low; M: moderate; H: high). (▲) Expt 2 results, similarly labeled

The worms were observed to spend more time at the sediment surface, although they fed within smaller areas than under the low-flow treatment. This may indicate that, when resupplied often, a smaller feeding pit offered the same amount of food material as a larger pit under no flow without sediment transport. However, our results indicate that food quality alone cannot fully account for the increased feeding rates. We believe that flow rate and sediment transport have a clear effect on the feeding rates of *Saccoglossus kowalevskii*.

In hindsight, it is appreciated that the actual feeding pit resources under the treatments were probably not well characterized by our sampling methods. Possible bias in the chlorophyll *a* and protein data may have resulted for 2 reasons. First, we sampled from random locations within the water tunnel bed rather than actual feeding pits. In the absence of sediment transport, food resources in feeding pits may have been depleted relative to the overall bed conditions, as was demonstrated for *Spiochaetopterus oculatus* and *Marenzelleria viridis* in the field at Cape Henlopen (Bock & Miller 1995). If so, our measurements may have overestimated the available food quality, but only under the low-flow treatment; under the moderate- and high-flow treatments sediment transport was sufficient to resupply food materials within feeding pits and prevent depletion (as evidenced by the erosion of fecal mounds). Second, we sampled the top 1 cm of sediment, while *Saccoglossus kowalevskii* feeds on only the top 1 mm. By sampling to a depth of 1 cm, we may have diluted the food resources at the sediment surface, where algal and microbial growth would lead to higher concentrations, with deeper sediment of lower food quality. This would lead to an underestimate of the available food quality for *S. kowalevskii*, but this was not likely under high flow, which was sufficient to homogenize the sediment to a depth of greater than 1 cm.

If food quality under the low-flow treatment was overestimated, the egestion rates should be shifted to the left relative to the functional response curve; if it was underestimated, they should be shifted to the right (Fig. 5). In both cases, the egestion rates following low flow would still fall within the range predicted to be due to food quality alone. If food quality under the moderate flow was underestimated, the egestion rates should be shifted to the right relative to the functional response curve, but it is still clear that *Saccoglossus kowalevskii* was feeding at rates higher than predicted based on the functional response curve.

Regardless of the potential biases in food quality determination, the overall results remain unchanged. Moderate flows consistently and significantly increased feeding rates relative to low- and high-flow treatments, and the response cannot be attributed to the available

food quality alone. With so few studies of deposit feeders under flow, little is known about the possible cues or causes that lead to the changes in feeding rates. High flows capable of bedload transport often result in a cessation of surface feeding (Levinton 1991, Miller et al. 1992). For some organisms this may result from an inability to control the feeding appendage in these high flows due to drag forces (Levinton 1991, Miller et al. 1992). *Saccoglossus kowalevskii* has a large, muscular proboscis which likely is able to withstand the drag effects of flow. Under moderate flows, the proboscis was still extended out of the burrow opening without an observed loss of control, suggesting that drag forces do not prevent *S. kowalevskii* from feeding at the surface.

An unstudied but potential cause could be the abrasiveness of the transported sediment. A 'sand-blasting' effect under high flows (Nowell et al. 1989) could potentially damage the soft tissue of the proboscis, making feeding at the surface under high flow detrimental. This risk of injury might then cause *Saccoglossus kowalevskii* to remain in the burrow and to retreat rapidly, as we observed, on the occasions it does venture to the top of the burrow. Still other explanations for the cessation of surface feeding are feasible and should be explored.

Overall, our results indicate that the feeding rate response of *Saccoglossus kowalevskii* is not simply predicted by food quality and that feeding rates are closely tied to flow regime. Our laboratory findings provide testable hypotheses for further study of these organisms under true field conditions. Flow regime in the field often transports enough sediment to replenish feeding pits of deposit feeders at rates faster than feeding rates (Miller & Sternberg 1988, Bock & Miller 1995). Therefore, we believe that the moderate-flow feeding rates will be most characteristic of feeding rates in the field. The high-flow treatment represents the effects of storm conditions on the intertidal sandflat at Cape Henlopen (Bock & Miller 1995), indicating that under such conditions *S. kowalevskii* ceases to feed at the surface until the flow rates subside. And finally, the low-flow treatments approximate the conditions within tide pools or at low tide.

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