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**GUT-MARKER AND GUT-FULLNESS METHODS FOR ESTIMATING  
FIELD AND LABORATORY EFFECTS OF SEDIMENT TRANSPORT ON  
INGESTION RATES OF DEPOSIT-FEEDERS<sup>1</sup>**

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**Abstract:** By developing a method for quantifying feeding rate in the presence of sediment transport, we determined whether sediment transport or one of its correlates influenced feeding rate of deposit-feeders in the field. The method entailed application and subsequent removal of magnet-susceptible marker particles, so that no marker was present in the ambient sediments during the period over which feeding rate actually was measured. Error variance was reduced by a paired statistical design in which the technique was used twice in the same individuals, to assess feeding rate immediately prior to the sediment transport event as well as during it.

Low levels of sediment transport significantly affected feeding rates of *Pseudopolydora kempj japonica* Imajima & Hartman (a spionid polychaete) both in a small, imprecise, laboratory flume and in an intertidal environment characterized from late spring through early fall by a short pulse of sediment transport coincident with the incoming tide. Deposit-feeding rates immediately after the latter pulse were enhanced by a (geometric) mean of 50% in field trials from May through October. Both in the laboratory and in the field, however, the magnitude of the (albeit statistically significant) sediment transport effect was overshadowed by variation due to nutritional history of individuals and to other unidentified factors.

The form of the field data for *P. kempj japonica* (feeding rates estimated for two successive intervals in the same individuals) allowed us, in addition, to analyze the response of gut fullness to mean feeding rate (averaged over the two feeding bouts), previous feeding rate (first bout), most recent feeding rate (second bout) or recent change in feeding rate (difference between first and second bouts). The strongest correlation was found with the last of these variables, suggesting that gut fullness may provide a tracer-free method of gaining some kinetic information on continuously feeding animals. Contrary to prevailing impressions, gut fullness in such continuous feeders may provide more information on recent change in feeding rate than on feeding rate itself.

We gathered additional data on *P. kempj japonica* that differed in emersion history (in tide-pools versus outside them prior to the pulse of sediment transport with the incoming tide) but were not exposed to markers. Animals both inside and outside tide-pools showed fuller guts immediately after the sediment transport pulse, consistent with an interpretation of accelerated feeding caused by some aspect of the sediment transport event. Incidentally collected individuals of *Hobsonia florida* (Hartman) (an intertidal ampharetid polychaete), on the other hand, showed no obvious response to the same conditions that triggered accelerated feeding and fuller guts in *Pseudopolydora kempj japonica*.

**Key words:** deposit-feeding; gut fullness; *Hobsonia*; polychaetes; *Pseudopolydora*; sediment transport

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## INTRODUCTION

Recent, empirically focused laboratory observations (Taghon *et al.*, 1980; Dauer *et al.*, 1981; Dauer, 1983; Nowell *et al.*, 1984) have revealed the importance of even low levels of sediment transport in determining the feeding modes and rates of those functionally deposit-feeding, benthic microphages that feed on the sediment surface. The scaling arguments of Miller *et al.* (1984) further suggest that sediment transport can act to increase food availability to a deposit-feeder when or where local production (within the foraging area of a sedentary deposit-feeder) is limited. If food quality is increased by sediment transport, foraging theory and the results of its tests suggest that feeding rates can be expected to increase as well (Sibly, 1981, his Fig. 5.2, p. 113; Taghon, 1981; Taghon & Jumars, 1984), or at least to change (Whitlatch & Weinberg, 1982). If food quantity is limited, transport again can be expected to enhance feeding rates. When imported material of low food value acts as a diluent, on the other hand, sediment transport may decrease food availability (Miller *et al.*, 1984). At some high rate (even if the quality of transported food is high) sediment movement is likely to decrease feeding rate simply because erosion or burial of the animal itself occurs.

Despite such strong arguments and corroborative laboratory observations, to date there have been no demonstrations that feeding rate of facultative deposit-feeders changes in the field in response to, or at least correlated with, sediment transport. Our primary goal was to determine whether indeed there is such a response in the field. We took such a determination as prerequisite to the effort of developing more precise laboratory (Miller & Jumars, 1986) and field quantification both of the stimulus that alters feeding rate and of the ensuing feeding response.

The methods needed to document changes in feeding rate with changes in sediment transport did not prove straightforward. High or even modest sediment transport rates presented problems in attempted applications of the usual methodologies for estimating feeding rates. When fecal pellets begin to transport, it becomes difficult to use the simple method (e.g., Rhoads, 1963; Mangum, 1964; Nichols, 1974; Dauer *et al.*, 1981; Taghon & Jumars, 1984) of measuring fecal weight or volume produced per unit time as an estimate of ingestion rate. In muddy sediments, transport makes viewing all but impossible. Hence, we attempted to use the tracer methodology of Cammen (1980) for estimating effects of sediment transport rate. Our attempts (unpubl. data available from P.A.J. on request) failed because the tentacle-feeders we examined were able to concentrate even seemingly low-dosage applications of tracers applied to the sediment surface. Thus, the material did not enter the gut in tracer quantities, and the ingested amounts were sufficient to change feeding rates, clean 44–62- $\mu\text{m}$  glass beads depressing feeding rates because of their low food value (Taghon & Jumars, 1984) and DayGlo™ paint pigments of <9  $\mu\text{m}$  diameter (Cammen, 1980) accelerating it, apparently because of the mechanical ease of picking up the latter particles (Jumars *et al.*, 1982, their Fig. 7e). More important for our purposes, we could not expect any artifacts from these or other tracers to remain constant as the tracer was diluted by geophysical sediment transport, so we discontinued this approach.

We developed instead a gut-marker methodology that does allow comparison of feeding rates among laboratory and field environments varying in sediment transport characteristics. (We use the term “marker” to suggest that it is not necessarily present in trace amounts.) Our method entailed the use of magnet-susceptible marker particles and their removal from the system before feeding rates on the underlying or transporting sediments were measured. With the benefit of this methodology, we present laboratory flume results with a species that is functionally capable of deposit-feeding, and we describe the first field measurements evaluating effects of sediment transport on deposit-feeding rates. Observations throughout the development and application of this marker methodology suggested that, given adequate knowledge of the kinetics of gut filling and emptying, some short-term kinetics of ingestion rate may be resolved by monitoring gut fullness – without recourse to tracers except as a means of calibration.

## MATERIALS AND METHODS

### MARKERS

To decrease the potential for artifacts (i.e., feeding rates altered by the presence or ingestion of the marker), we decided to remove the markers used to time feeding bouts before the nominal period during which feeding rate was measured. We elected to try magnet-susceptible particles. (Removal even of easily eroded tracers by hydrodynamic means confounds transport effects with artifacts of the removal process; our qualitative observations showed definite responses to “puffs” of sediment generated in such attempts with DayGlo pigments.) Microtaggants (3M Company, St. Paul, Minnesota; color-coded, plastic particles with an embedded layer of magnetite, specific gravity  $\approx 1.8$ ) were used for most of the marker experiments that entailed removal before the feeding bouts of concern. They have the advantage of chemical inertness, but they are difficult to fracture into particles much smaller than  $150\ \mu\text{m}$  without loss of the embedded metallic layer. Iron powder (particles  $< 45\ \mu\text{m}$  in diameter, specific gravity  $\approx 7.9$ ; Pyron Corporation, Niagara Falls, New York) was used in some experiments as a check that the results with Microtaggants were not seriously affected by the large size of individual Microtaggant particles. Iron powder has the advantage of small particle size, but the disadvantage of chemical reactivity. We were unable to find commercially available particles that were susceptible to magnets, small, and inert.

Particles were applied with a pepper shaker having appropriately sized openings. They were removed with a small, permanent, horseshoe magnet. In pre-trials we found it important to hold the magnet just close enough to the sediment-water interface to lift the particles. Holding it closer caused more rapid upward acceleration of the markers and sometimes entrained underlying sediments. All removals (after practice) were accomplished in  $< 10\ \text{s}$ .

## LABORATORY EXPERIMENTS

We ran laboratory experiments with the particles in order to gain familiarity with them in an easily observed and controlled system. We would not have gone to the field without these laboratory tests. They were conducted at the Friday Harbor Laboratories, San Juan Island, Washington, with adult *Pseudopolydora kempji japonica* Imajima & Hartman dug from False Bay. Animals were kept in their natural sediments in sea-water tables at the Friday Harbor Laboratories until experiments were set up (periods of 1 to 6 days) in the small, water- and sediment-recirculating flume described by Miller (Fig. 1 in Miller, 1984), using 300  $\mu\text{m}$ -sieved sediments from False Bay (median grain size  $\approx 125 \mu\text{m}$ ). The flume has a working section 1 m long and 15 cm wide and fluid volume of  $\approx 25$  l. To prevent changes in temperature (from heat produced by the pump) during experiments, the flume was modified to allow water from the laboratory sea-water system to exchange with water in the flume. A slow ( $1 \text{ litre} \cdot \text{min}^{-1}$ ) flow under pressure into the middle of the tail box was balanced by an overflow at the top of the tail box (out of the path of settling silt and sand). This exchange was sufficient to maintain the flume's sea-water temperature at the seasonal level ( $8\text{--}11^\circ\text{C}$  in spring, and  $12\text{--}14^\circ\text{C}$  in fall of 1983) but was too slow to remove a substantial quantity of transported sediment ( $< 1\%$ ) from the system during experiments.

This flume is too small for precise quantification either of boundary-layer flow or of sediment transport (cf. Nowell & Jumars, 1984), but is fully adequate for work at the ordinal level of transport parameters and for qualitative behavioral observations. We set the pump to conditions of no mean flow (pump turned off, water recirculating through the tail-box exchange mechanism to maintain oxygenation), flow at a level insufficient to move the sediments (subcritical) and/or flow competent to move sediments at a substantial rate (supercritical). It should be noted that the absolute value of the shear stress that defines the transition between critical and subcritical flows ( $u_{*cr}$ ) will vary with the sediment type. We use the terms in the sense that they are employed by geophysicists, namely based on whether the sediment grains move. Especially in a small flume with extensive side-wall effects, this characterization of flow forces is more reliable than an arbitrarily determined flow velocity (Nowell & Jumars, 1984). Operationally, however, flocculent material (not usually considered by sediment-transport workers) moved as bedload at both the subcritical and supercritical levels and also went into suspension in the more energetic (supercritical) flows.

Vials containing *P. kempji japonica* individuals were implanted in the working section, and water velocity and water depth were set to the pretreatment levels for 20 h (spring 1983) or 1 h (fall 1983). After the pretreatment, the flow was slowed to zero, and a magnet-susceptible tracer was applied. To minimize artifacts due to the marker's particle size, the smallest Microtaggant particles we could separate ( $150\text{--}350 \mu\text{m}$ ) without loss of magnetic susceptibility were used in one set of experimental runs (spring 1983), and iron powder  $< 45 \mu\text{m}$  in grain diameter was used in another (fall 1983). ( $250 \mu\text{m}$  is near the upper limit for frequent sediment particle ingestion by *P. kempji*

*japonica*, and particles of this size conceivably could affect the mechanics of gut passage, although we have observed this animal to ingest whole tanaids (*Tanais* sp.) 1 cm long.) The marker was left for either 20 min (Microtaggants™) or 5 min (iron powder); these periods were the shortest that allowed reliable marker horizons to form in the gut in pre-tests. The flow conditions were then set to treatment levels for 30 min (spring 1983) or 25 min (fall 1983). After the feeding period, the flume was drained and the animals retrieved, fixed in 20% formalin for  $\approx 24$  h, and subsequently preserved in 80% ethanol.

Weight of sediment in the worm guts ( $\mu\text{g}$ ) anterior to the tracer horizon was determined, along with an individual's total volume ( $\text{mm}^3$ ) to give size-specific ingestion rate ( $\mu\text{g}$  sediment ingested  $\cdot \text{mm}^{-3}$  worm volume  $\cdot \text{min}^{-1}$ ). Specimens were immersed in 100% glycerol in a depression (welled) slide and viewed under a dissecting microscope for: (1) measurement of prostomial length (anteriormost point to midpoint of second setiger; providing a good estimate of preserved *Pseudopolydora kempji japonica* volume via an established regression (cf. Self & Jumars, 1978; Taghon, 1982); (2) determination of maturity (presence or absence of gametes in swollen parapodial pockets of the middle setigers); and (3) counts of anteriormost setigers in which sediments and tracer occurred. Animals were then returned to 80% ethanol and all sediment attached to the epidermis, removed. Worms were severed at the sediment-tracer interface, and the anterior, sediment-containing portions were dried at 60 °C overnight. After drying, sediment and flesh were weighed with a Cahn 25 Electrobalance. Sediment and flesh were rehydrated in tap water, and (under a dissecting microscope) the sediment was teased from the gut. Only the flesh was again dried overnight at 60 °C and reweighed. Weight of sediment ingested was estimated by the difference between flesh and sediment plus flesh.

#### FIELD EXPERIMENTS AND ANCILLARY OBSERVATIONS

The sandflat (125  $\mu\text{m}$  median grain size) between the north and south forks of the Skagit River, northern Puget Sound, Washington, was selected on the basis of several characteristics: accessibility (intertidal), the presence of an easily identifiable pulse of sediment transport (coincident with the incoming tide), and the existence of natural tide-pools to allow resolution of the effects of immersion from those of sediment transport. At low tide, the flat is dotted with small pools, a few centimeters to a few meters in diameter and 1–5 cm deep. The leading edge of the incoming tide is distinct as a "tidal bore,"  $\approx 2$  cm in height, and is associated with a visually obvious pulse of sediment movement ( $\approx 10$  s long at any one location) that ceases abruptly (is followed by clear water) after passage of the bore. The rate of approach of the bore to the experimental site between the +5 and +6 tidal level (1.5–1.8 m above mean low water) is  $\approx 6$ –9  $\text{m} \cdot \text{min}^{-1}$ , but with a component of unpredictable variability due to winds.

In the field experiments, an area  $\approx 100 \text{ cm}^2$  within a tide-pool was covered completely with 250–500- $\mu\text{m}$  (sieved) Microtaggants for a period of 5–10 min that pre-tests

suggested would allow *P. kempji japonica* reliably to ingest them. That marker was removed, and the spionids were allowed to feed on natural sediments for 5–10 min, when a second batch of Microtaggants, differing only in color code, was sprinkled over the same area as the first. This second marker was removed just as the tidal bore immersed the tide-pool (Treatments) or after a predetermined time in the absence of sediment transport (Controls). *P. kempji japonica* were again allowed to feed on natural sediments for the same period as after the first marker before a sediment core was taken and immediately fixed in 20–40% formalin for sieving, animal sorting, and gut analysis in the laboratory. The timing used for these procedures (Table I) was determined from pre-tests to both retain the first marker in the gut and allow measurable volumes of sediment to accumulate between markers and after (anatomically anterior to) the last marker.

To evaluate potential biases and artifacts of the procedure, additional variations and controls were implemented. A “time control” was instituted because the time of arrival of the bore could not be predicted with perfect accuracy; the second tracer of the pair in the pre-bore control was left in place twice as long as the first to see whether this asymmetry (i.e., short-term starvation on a diet of large particles of no food value) might account for the burst of feeding after the tidal bore. “Jar controls” were instituted to eliminate the pulse of sediment transport, providing further evidence of its effects. They were of two kinds to ensure that the observations were not artifactual. Two adjacent areas in a tide-pool were isolated by pressing the cut-off upper portions of plastic gallon jars into the sediment. The first tracer was dispersed and retrieved and the second tracer dispersed as noted previously. Just prior to immersion by the tidal bore, the second tracer was retrieved from both areas, one jar was covered with its lid, and the other jar was removed, exposing the latter group of animals to the pulse of sediment transport. To isolate artifacts potentially caused by the large size of Microtaggant particles, in some treatments they were replaced with iron powder.

Preserved *P. kempji japonica* individuals containing both colors of Microtaggants or both plugs of iron powder were immersed in 100% glycerol in a wetted slide and viewed under a dissecting microscope equipped with a camera lucida and dark-field lighting. Body volume again was estimated from prostomial length. Tracings were made of the two sediment plugs (respectively between and anterior to the tracers), and their projected areas were estimated by planimeter. Volume of sediment in each plug was estimated from area and length of segments of these plugs by assuming simple geometric objects of revolution (spheres, ellipsoids, cones, or cylinders). Tracer volumes were not included in any of the feeding-rate calculations. We used this volumetric approach rather than the gravimetric one used in the laboratory experiments because we found the latter to be ungainly in handling the two, necessarily smaller, sediment plugs arising in a double-marking procedure. In the animals from cores both with and without marker application, the first sediment-filled setiger was noted. Operationally, it was defined as the anteriormost setiger containing more than a single sediment grain.

The experiments were supplemented by a set of ambient cores, with the animals fixed,

extracted, and preserved similarly, but taken in the absence of any marker application. Their purpose was to estimate natural variation in gut fullness without any effect of the marker and to examine its dependence on one natural variable, i.e., emersion or lack thereof. Cores were taken  $\approx 3$  min before the tidal bore as well as 10 min after it. They were collected both from tide-pools and from areas that were emersed before the bore.

#### STATISTICAL ANALYSES

Standard nonparametric one- and two-way layouts (Hollander & Wolfe, 1973) were used throughout. The experimental design of the laboratory flume experiments with *P. kempji japonica* was sufficiently complex that a nonparametric procedure capable of resolving statistical interactions (Wilson, 1956) was employed as well. The complexity of this data set and of the field data with paired tracers led us eventually to resort to parametric ANOVA procedures (Hull & Nie, 1981) as well.

Care was taken to randomize individuals by size across treatments in all laboratory experiments. In both field and laboratory experiments, results were tested against size (estimated body volume) of individuals as an additional or alternate treatment variable. No significant correlation was found in any case; for brevity, these negative results will be omitted.

### RESULTS

#### LABORATORY

In the laboratory (spring animals ranging from 0.12 to 5.24 mm<sup>3</sup> in estimated body volume, and fall animals 0.16 to 1.32 mm<sup>3</sup>) *P. kempji japonica* deposit-fed when not exposed to flow, suspension-fed at the subcritical flow level, and used a combination of the two methods at the supercritical level, deposit-feeding primarily when in the lee of a migrating ripple. At the supercritical level, sand particles in characteristic ballistic trajectories during bedload transport frequently could be seen to impinge directly on a tentacle and to be captured. Also at the supercritical level, *P. kempji japonica* spent substantial time in tube maintenance, building its tube up as a ripple crest approached and tearing it down toward the new sediment surface after the crest went by. Whenever flow conditions were changed, animals spent time adjusting tube height. Like the sabellid polychaete *Eudistylia vancouveri* (Merz, 1984), *Pseudopolydora kempji japonica* builds its tube higher above the substratum at lower shear velocities (Taghon, in prep.). Pretreatment conditions were such strong determinants of feeding rates in the spring flume runs that pretreatment exposure was cut to 1 h in the second set of laboratory experiments. Even so, pretreatment conditions accounted for a major portion of the observed variation in feeding rates with sediment transport rates.

The general pattern observed was for the highest feeding rates in those individuals preconditioned under no flow and for the lowest feeding rates in those individuals preconditioned at the supercritical level. For individuals preconditioned under no flow

TABLE I

ANOVA results for normalized,  $\log_{10}$ -transformed rates of feeding ( $\mu\text{g ingested} \cdot \text{mm}^{-3}$  of worm  $\cdot \text{min}^{-1}$ ) by *P. kempji japonica* exposed to varying sediment transport regimes in a laboratory flume: under the implicit assumption that they behave independently within each season's treatment, individuals are treated as replicates in the analysis.

Source of variation	Sum of squares	d.f.	Mean square	F	P
Covariates	0.406	2	0.203	1.501	0.225
Days since collection	0.017	1	0.017	0.125	0.724
Reproductive state	0.386	1	0.386	2.856	0.092
Main effects	5.260	5	1.052	7.778	0.001
Season	0.015	1	0.015	0.114	0.736
Pretreatment	3.166	2	1.583	11.704	0.001
Treatment	1.209	2	0.604	4.470	0.013
Two-way interactions	1.712	8	0.214	1.583	0.131
Season-Pretreatment	0.264	2	0.132	0.975	0.379
Season-Treatment	0.110	2	0.055	0.405	0.667
Pretreatment-Treatment	1.108	4	0.277	2.048	0.089
Three-way interactions	0.445	4	0.111	0.823	0.512
Explained	7.823	19	0.412	3.044	0.001
Residual	29.754	220	0.135		
Total	37.577	239	0.157		

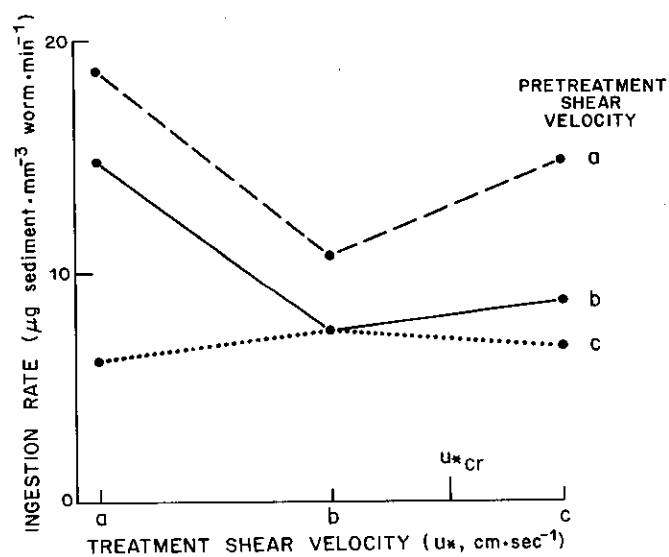


Fig. 1. Back-transformed cell means from the ANOVA of Table I, showing feeding rates of *Pseudopolydora kempji japonica* as functions of treatment and pretreatment sediment transport conditions in a laboratory flume, where  $u_{*cr}$  is the threshold shear velocity ( $u_{*}$ ,  $\text{cm} \cdot \text{s}^{-1}$ ) at which sediments begin to move; means sharing pretreatments are connected by lines.

or subcritical flow, lowest feeding rates were observed at the subcritical treatment level. This trend was lacking or even slightly reversed in the animals preconditioned under supercritical flow, accounting for the observed statistical interaction. We present the parametric ANOVA results (Table I, Fig. 1) for efficiency. Despite problems with heteroscedasticity – small but detectable via Cochran's *C* and the Bartlett-Box *F* tests even after logarithmic transformation – the parametric ANOVA yields probability levels nearly identical to those obtained by the much more cumbersome approach of running Wilson's (1956) non-parametric equivalent separately on the two seasons and using Fisher's (1970) method to combine probabilities for the spring and fall runs. The parametric design also can easily remove effects due to unintended covariates, i.e., reproductive state and time since isolation from the field, whose potential influence became a concern due to observed trends (slower feeding with greater isolation time and with gonadal maturity) after the first experiment had been run. Although neither effect reached statistical significance at the 0.05 alpha level, we conservatively present the ANOVA with these covariates analyzed and removed (Table I), especially because the gonadal condition variate verged on statistical significance. Lastly, the residual sum of squares of the parametric approach gives the reader an approximation for the proportion of the total variance that goes unexplained even in controlled laboratory experiments.

#### FIELD

On the Skagit sand-flats, *P. kempji japonica* (estimated body volumes ranging from 0.88 to 9.39 mm<sup>3</sup>) showed  $\approx 50\%$  increase in mean feeding rate in the time interval immediately following passage of the tidal bore (bore versus non-bore difference of Fig. 2 as a percentage of the non-bore, geometric mean feeding rate). Individuals were observed to deposit, rather than suspension, feed during both measurement intervals. It was difficult to obtain many specimens on any one date, due both to limitation (by expense and the necessity for complete and simultaneous removal) in areal coverage by Microtaggants and to the necessity of using only individuals that had fed on both tracers and were anatomically entire in the preserved samples. To remedy the problem of too few individuals, we had to repeat the experiments several times over the field season (Fig. 2). Given the importance of prior conditioning in determining feeding rate (Fig. 1, Table I), this pattern of replication resulted in a great deal of variability in results among dates (Table II). Logarithmic transformation again was the most successful of the common approaches in producing homoscedasticity for parametric analysis, but was not entirely so. Parametric ANOVA, however, again proved both to be the most efficient means of summary and to be in close accord with nonparametric analyses of all the significant effects. The parametric ANOVA readily allows display of the effect of varying exposure time to the tracer; while the effect is small, its removal prior to nonparametric analysis is problematic. Parametric ANOVA also readily reveals how large a fraction (77%) of the total sum of squares remains unexplained after the analysis. We further

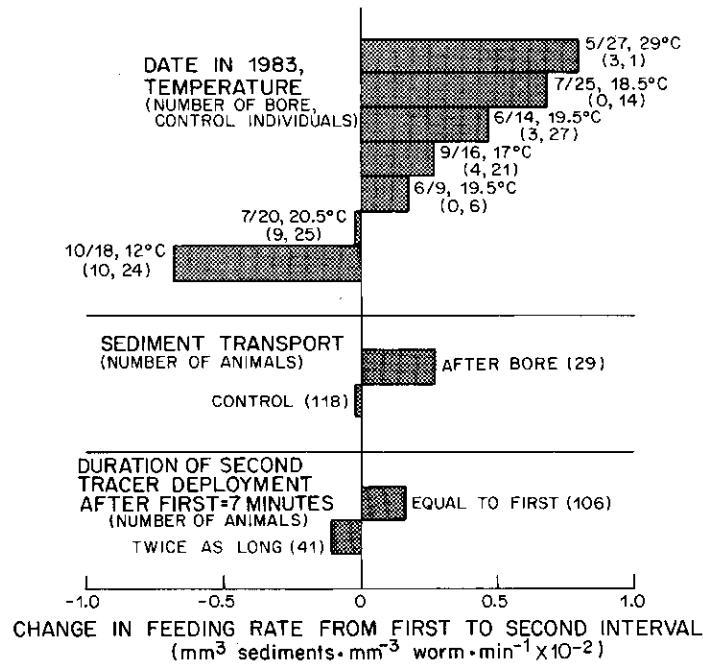


Fig. 2. Back-transformed cell means from the main treatment effects of the ANOVA of Table II, showing the relative magnitude of the sediment transport (bore) effect on *Pseudopolydora kempji japonica* in the field: these changes over successive feeding intervals (7 min long and spaced either 7 min or 14 min apart) compare with a geometric mean feeding rate (both intervals of all non-bore or control worms)  $0.574 \text{ mm}^3 \text{ sediments} \cdot \text{mm}^{-3} \text{ worm} \cdot \text{min}^{-1}$ .

TABLE II

ANOVA results for normalized ( $\text{mm}^3$  of sediment ingested  $\cdot \text{mm}^{-3}$  of worm  $\cdot \text{min}^{-1}$ ), and transformed, tracer-derived differences in feeding rate over successive 7-min intervals within 147 individual *P. kempji japonica* variously exposed to sediment transport (bore effect) over 7 widely separated days in 1983 and using both equal and unequal exposure times to the paired tracers: for ease of manipulation and to minimize problems with heteroscedasticity, data have been transformed as follows, where  $v_1$  and  $v_2$  refer, respectively, to feeding rates during the first and second intervals:  $\log_{10} \{ [v_2 - v_1] \times 10^4 \} + 400$ ; probability levels again utilize individuals as replicates (assume they behave independently within days).

Source of variation	Sum of squares	d.f.	Mean square	F	P
Main effects	0.486	8	0.061	3.716	0.001
Bore	0.077	1	0.077	4.726	0.031
Days	0.439	6	0.073	4.474	0.001
Duration of tracer deployment	0.044	1	0.044	2.686	0.104
Two-way interaction	0.180	4	0.045	2.757	0.030
of Bore with Days*					
Explained	0.666	12	0.056	3.396	0.001
Residual	2.190	134	0.016		
Total	2.856	146	0.020		

\* Other two-way interactions irretrievable in this unbalanced design.

used parametric ANOVA to analyze for significant covariate effects of animal size and gonadal condition. Finding none, we have omitted those results.

We anticipated problems from temperature changes as the incoming tide advanced. The main body of Puget Sound normally stays in the range from 7–12 °C, so we expected water temperatures to fall after passage of the tidal bore, making conservative our estimates of the magnitude of any feeding rate-enhancing, sediment transport effect. The tidal flats are so extensive and uniform, however, that no temperature changes in excess of 0.5 °C were observed during the experiments in readings taken at 5 cm depth in the sediments, in the water of tidal pools and behind the bores at the termination of experiments. Temperature undoubtedly did affect feeding rate, but it affected both treatments and controls equally.

The “jar control” (run on 16 Sept. and 18 Oct.) showed no obvious differences from other treatments, except a small suggestion of a disturbance artifact (slightly but not significantly enhanced feeding rates in jars, corresponding with our qualitative impression of slightly greater feeding activity immediately following jar emplacement) irrespective of whether a bore effect was included in or excluded from the jar. Hence jar controls were lumped with the rest of the treatments in the analysis of Table II and Fig. 2. Similarly, no significant difference was observed in Microtaggant versus iron powder treatments run on 18 Oct., and they were combined.

The other check on the methodology could not be lumped with these treatments, but it did (Fig. 3) corroborate the conclusion that feeding rate increased in *P. kempji japonica* immediately following the bore-induced pulse of sediment transport. The inference we drew is that a fuller gut corresponded with recently enhanced levels of feeding. Interestingly, gut fullness in a simultaneously collected species of ampharetid, *Hobsonia florida* (Hartman), did not (Fig. 3) follow the same pattern. It would appear that *H. florida* continued to feed at a relatively constant rate so long as it was immersed, while *Pseudopolydora kempji japonica* was stimulated to feed faster after the transport event. It is unfortunate that *Hobsonia florida* mixed its gut contents, precluding use of the twin tracer technique on the same scales as chosen for *Pseudopolydora kempji japonica* to corroborate this impression. As a consequence of this mixing, the second tracer frequently appeared first in the anatomical sequence of gut contents retained by *Hobsonia florida*.

As a check on our interpretation of gut fullness, we used the marker data with *Pseudopolydora kempji japonica* ( $n = 147$ ). We had measures of feeding rate during each of the two feeding bouts and created two derivative variables from them. One was simply the mean feeding rate over both intervals. The other was the difference in feeding rate between the two intervals. In a stepwise, multiple linear regression with the number of the first full setiger as a measure of (inverse) gut fullness, only the difference in feeding rate proved a significant predictor of gut fullness ( $P = 0.017$ ). For reasons we will explain in the discussion, we plotted (Fig. 4) these data after exchanging the dependent and independent variables and recalculating the regression accordingly. The correlation coefficients of first full setiger (number) with difference in feeding rate (second- minus

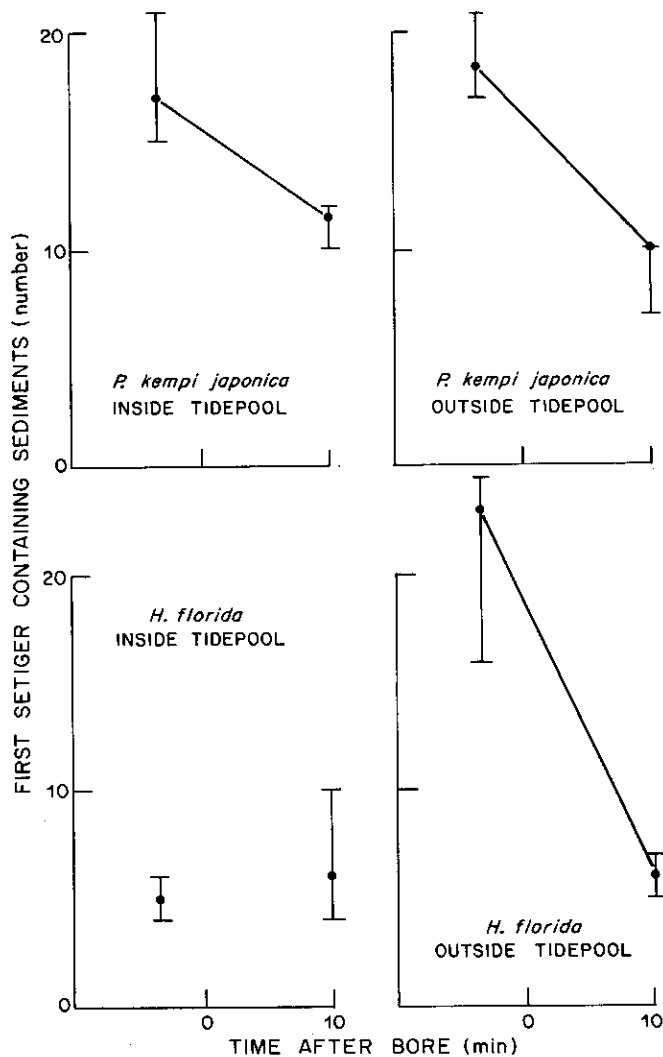


Fig. 3. Gut fullness, measured as the first setiger bearing sediments, in field-collected specimens of *Hobsonia florida* and *Pseudopolydora kempj japonica* under varying conditions of immersion and sediment transport (24 June 1983 at 23 °C): while both species respond to immersion, only *P. kempj japonica* appears to accelerate its feeding after a pulse of sediment transport; indicated are medians and their 95% confidence limits based on 20 individuals in each category (except that only nine post-bore *Hobsonia florida* were collected); data gathered with the help of Mr. S. Brumsickle.

first-period rate), most recent rate, prior rate, and mean rate were, respectively,  $-0.175$ ,  $-0.128$ ,  $0.082$ , and  $-0.043$ .

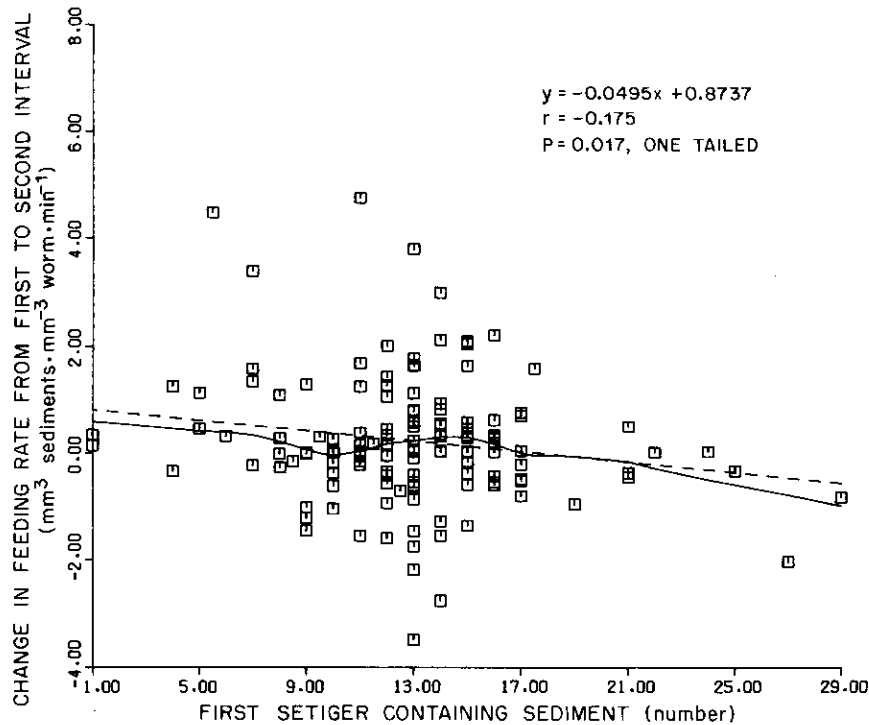


Fig. 4. Observed differences (period 2 - period 1) in feeding rate during successive 7-min periods in individuals of *Pseudopolydora kempji japonica* versus an index of their gut fullness (most anterior setiger in which sediments occur): the broken line is the best-fit least-squares regression (for which parameters are indicated, treating individuals as replicates), while the solid line is an unbiased LOWESS (Cleveland, 1979) smoothing, insensitive to outliers, of the scatterplot.

#### DISCUSSION AND CONCLUSIONS

We should point out before we begin discussing our own results that our experience with potential, non-magnet-susceptible tracers should not be used to cast immediate doubt on the results of Cammen (1980). He worked with a nereid polychaete that does not have an obvious mechanical means (such as the tentacles of *P. kempji japonica*) of concentrating the tracer in its gut from a large feeding area. Other spionids with greater proclivity for rejection after collection (Dauer, 1985) may also be less sensitive than *P. kempji japonica* to tracer-induced (or marker-induced) artifacts, but they may also be more difficult to mark.

Conversely, we by no means assert that removing the marker with a magnet removes all the potential for artifacts, namely for changes in feeding rate due to either food-quality or mechanical differences between the marker and the particles the animal would have eaten in the marker's absence. Removal does, however, preclude an interaction of those

artifacts (due for example to gradual marker dilution) with sediment transport treatments applied after removal. Furthermore, there is no grossly obvious effect of the marker on the natural range of gut fullness (Fig. 3 compared with Fig. 4). One still should question absolute feeding rates determined via the magnetic-marker methodology until a dose-response curve (the equivalent of a tracer-dilution curve) is generated with it. Rather than attempting to generate absolute values, however, we sought to compare the relative magnitudes of feeding rates under varying sediment transport treatments. For these purposes and with the controls applied, removal of the marker enhanced reliability of the results. We hasten, however, to point out the sensitivity of *P. kempji japonica* to any disturbance that suspends sediments (Taghon *et al.*, 1980); the magnet must be held far enough from the sediments that the initial acceleration of the marker particles does not entrain underlying sediments. Following this caution, we did not observe any obviously artifactual behavior, even when particles were lifted from their transport paths on the animals' palps.

We anticipated treatment differences in the laboratory flume experiments on *P. kempji japonica*, based on the qualitative observations of differing feeding modes and differing times allocated to tube maintenance. A low ingestion rate, in  $\mu\text{g}$  of gut contents  $\cdot \text{mm}^{-3}$  of worm  $\cdot \text{min}^{-1}$  was anticipated under conditions of subcritical flow, because only material of low density could come into contact with the coiled, elevated feeding palps (Taghon *et al.*, 1980). Under conditions of no net flow, animals actively picked up sediment grains from the bed, while under supercritical conditions, sand grains were transported onto their palps. The treatment differences in feeding rate do not, then, allow one to draw the conclusion that less food in any terms other than bulk weight is obtained under subcritical or supercritical flow than under no flow, since the quality of the food changes over this range of conditions. These results do underscore the importance of basic natural history information in interpreting laboratory or field gut fullness data. Without direct observations and the results of Taghon *et al.* (1980), it would be very easy to misinterpret the observed changes in gravimetric feeding rate. In general, any change in feeding mode that changes the kind of food ingested (mechanical properties or quality) can be expected to change the kinetics of gut passage.

Completely unanticipated was the strength of the pretreatment effect. The pretreatment period was shortened in the fall 1983 run series to investigate the sensitivity of the results to its duration. Surprisingly, the pretreatment effect remained strong (not significantly different from the 20-h results and therefore lumped with them). We do not yet know, either empirically (e.g., through a signal-processing, input-output analysis of the gut by methods such as those of Goreau, 1977) or mechanistically, the reasons for this effect. There are many possible choices. We have already mentioned the fact that animals spent some time adjusting tube height, and consequently less time feeding, when flow conditions changed. Varying food quality also changes gut kinetics (Taghon & Jumars, 1984). Our experiment was not intended to isolate such cues, but rather was a pre-test of methodology for field application. More recent experiments have been designed for clearer isolation of potential stimuli (Miller & Jumars, 1986) and show that

removal of the fecal pellets of *P. kempji japonica* from its feeding area enhances feeding rate whether or not food is provided in unlimited supply. Thus, the dynamics of pellet removal probably were also involved in the results of Fig. 1. For present purposes, the laboratory results were most important in pointing out short-term (1 h or less) sensitivity to prior conditions and in lacking blatant artifacts of the marker technology (e.g., morbidity or aberrant feeding behavior with either Microtaggants or iron powder). They made us optimistic that the field approach would work, and they indicated differing feeding histories as a likely source of natural variability among individuals.

The field experiments with magnet-susceptible markers differed from the laboratory approaches in important ways. Notably, when transport conditions were changed in the laboratory flume during an experiment, the new conditions were maintained for the duration of the post-marker measurement. In the field setting chosen, sediment transport occurred as a short pulse. Because the gut in the field experiments retained two marker plugs rather than one, there was more abundant opportunity for marker artifacts, necessitating controls lacking only the tidal bore. Our need to measure two small sediment plugs also made volumetric estimates of sediment consumption easier than gravimetric ones in the field design.

Both field and laboratory approaches, then, concurred in suggesting that sediment transport does significantly influence rate of feeding in *P. kempji japonica*. *Hobsonia florida* (unfortunately not amenable to marker analysis), on the other hand, showed no apparent increase in feeding rate (Fig. 3) in response to sediment transport at low rate or frequency, suggesting that large differences may be found in the sensitivity of surface deposit-feeders to the stimuli of sediment transport. Animals that do respond to the tidal bore in the field may be taking advantage of the front of organic-rich particulate material that moves along with the foam line (Wissmar & Simenstad, 1984) and under it (Anderson & Mayer, 1984). Alternatively, enhanced feeding rate may be due to the stimulus of fecal removal (Miller & Jumars, 1986). In any case, as yet we neither know how long the elevated feeding rate is maintained in *Pseudopolydora kempji japonica* nor whether the observed enhancement provides a significant entry in the individuals' energy budgets.

The large residual error in controlled laboratory experiments (Table I), in paired-design field trials (Table II), as well as in paired-design laboratory experiments (Taghon & Jumars, 1984) aimed at deposit-feeding rate measurements, adds to the difficulty of the problem of precisely quantifying the biological responses. One family of reasons for the variability in response has become clearer, however. Namely, nutritional "history" over several time scales seems to influence strongly an animal's response to food of a given quantity and quality either in transport or on the bed. Pretreatment conditions over periods of 1 h to a day dominated the results of the laboratory experiments with *P. kempji japonica*. Between-day variability (Days effect of Table II, including spring-neap as well as seasonal variability) dominated response to the tidal bore; even if variation in feeding rate due to temperature was taken out by second-order, polynomial regression, the days effect and the interaction between days and the bore in an ANOVA

remained stronger than the bore effect itself in determining feeding rate. There is even reason to suspect (at the  $P = 0.1$  level) that a few minutes of additional starvation on a food-poor marker (Times effect of Table II) altered the results.

The emerging picture is of strongly time-varying and imperfectly synchronized nutritional status among individuals. Nutritional condition of inhabitants of local topographic highs (Fig. 3) certainly varied with the stage of the tide. On even shorter time scales, our results provide some insight into the kinematics of gut passage in *P. kempji japonica* and perhaps in other species that feed more or less continuously. These results suggest that (Fig. 4) acceleration in feeding causes the gut to become fuller. We are careful to distinguish this suggestion from the suspect idea, recently reviewed and criticized by Murtaugh (1984, 1985), that guts are fuller at faster feeding rates. At steady feeding rates – fast or slow – guts may reach the same equilibrium level of fullness, but temporary dis-equilibrium between ingestion and egestion may still occur when feeding rates change. As well as being consistent with the results presented here, such dis-equilibrium could explain the results Taghon & Jumars (1984) observed in *P. kempji japonica* and *Hobsonia florida*, whose feeding rates increased more on the short term than on the long term when transferred to food of higher quality.

The results in Fig. 4 raise the exciting possibility that some gut kinetic information can be gained without the repeated need for tracers. The coefficient of determination for the regression is low, and we would hardly recommend taking this equation to the field to estimate recent changes in feeding rate. For a number of reasons, we were surprised to see any significant relation at all. The data are from varied seasons and a range of temperatures from 12 to 29 °C. There are very few individuals whose feeding rates decelerated (lower right quadrant of the graph) because there was either little stimulus to change (controls) or a stimulus to accelerate (tidal bore). Setiger number of the anteriormost segment containing sediments is not a very precise measure of gut fullness. Time intervals for the feeding measurements were not adjusted to maximize the sensitivity of this method. The species was not chosen with this application in mind.

We therefore believe that it is worthwhile to pursue the methodology of tracer-free estimation of gut kinematics further with models and measurements that expressly are designed to improve the chances of success. The models we are exploring (Penry & Jumars, 1986) are based on the principles of chemical reactor analysis and design (Froment & Bischoff, 1979); they can be used to predict how gut fullness should change with food quantity and quality. We believe that the present results demonstrate good potential for the magnetic-marker technology either as a means of calibration and verification of the tracer-free approach if the latter becomes practical or as an alternative if the tracer-free method cannot be made sufficiently accurate and precise. We are at present experimenting with making small and inert marker particles by polymerizing iron particles in acrylic spheres, following the methodology of Frish & Webb (1981). A spherical shape is desirable also because it minimizes sediment entrainment when retrieving the marker. The motivation to develop tracer- or marker-free methods for estimating gut kinetic parameters from unmanipulated field samples is substantial. They

would greatly facilitate evaluation of the importance of natural sediment transport events to deposit-feeders in a representative array of species and environments, including the subtidal (Grant *et al.*, 1984) and deep sea (Hollister *et al.*, 1984).

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