



Responses of deposit-feeding spionid polychaetes to dissolved chemical cues

Matthew C. Ferner, Peter A. Jumars*

School of Oceanography, University of Washington, Box 357940, Seattle, WA 98195-7940, USA

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Abstract

Few studies have investigated capabilities or ecological implications of chemical sensing in deposit feeders. We tested three species of spionid polychaetes (*Boccardia proboscidea* Hartman, *Polydora cornuta* Bosc, and *Pseudopolydora kempii japonica* Imajima and Hartman) for responses to a variety of compounds: amino acids, carbohydrates, organic acids, nucleotides, and plant and animal extracts that might have been used as general indicators of food quality or of the presence of specific foods. Five individuals of each species were exposed to 44 different dissolved chemical cues introduced in slow, laminar flow to control location and dose (1 mM for most cues) of delivery. Cues that altered feeding behaviors of one or more species were then tested against ten additional individuals of each species, in part to overcome dangers of multiple testing in the original screening. We observed differences among the three species both in their background behavioral patterns and in their responsiveness to dissolved cues applied at palp level just above the sediment–water interface, although significant changes in feeding behavior occurred only in *Boccardia* and *Pseudopolydora*. Taurine, threonine and valine acted as strong phagodepressants, whereas proline was significantly phagostimulatory to *Boccardia*. No hexoses elicited significant responses. Natural extracts were more stimulatory than the low-molecular-weight monomers tested. © 1999 Elsevier Science B.V. All rights reserved.

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1. Introduction

Marine environments contain a spatially and temporally dynamic array of dissolved chemical compounds; from a biological perspective, “the sea is indeed a rich broth of potential chemical stimuli” (Atema, 1985). Marine organisms ranging from bacteria to

*Corresponding author. Tel.: +1-206-543-7615; fax: 1-206-543-6073; e-mail: jumars@ocean.washington.edu

vertebrates use chemical signals to mediate a variety of processes (reviewed in Mackie and Grant, 1974; Carr, 1988), including recruitment and metamorphosis (Pawlik, 1992), predator avoidance (Mackie, 1970), reproduction (Miller, 1989), and feeding (Carr and Derby, 1986). Varied effects of dissolved chemical cues on feeding behavior have been described for a wide range of taxa, including protozoans (Verity, 1988), cnidarians (Lenhoff and Lindstedt, 1974), copepods (Gill and Poulet, 1988), decapod crustaceans (Zimmer-Faust, 1989), molluscs (Sakata, 1989), and teleosts (Hara, 1994). Many specific compounds (e.g., glycine, ATP), classes of compounds (e.g., amino acids, nucleotides) and complex organic mixtures (e.g., extracts of plant and animal tissues) are known to affect directly the initiation, intensity, or duration of feeding activity.

Although there have been numerous studies of chemoreception in herbivores and carnivores, information on sensory-regulated ingestion in deposit feeders is limited. The importance of deposit feeding is acknowledged in stratigraphy (Wheatcroft et al., 1990), sediment transport (Eckman et al., 1981), and the flow of both natural substances (Widbom and Frithsen, 1995) and pollutants (Mayer et al., 1996) in benthic food webs. Despite this importance, the food materials utilized by most deposit feeders remain obscure (Lopez et al., 1989). One recent approach to this question has been to measure absorption rates of specific compounds *in vitro*, under the assumption that animals will show active uptake of growth-rate-limiting substances (Self et al., 1995). Another possibility is to obtain clues of utilized diets by identifying phagostimulants and phagodepressants, compounds that directly affect rates of ingestion.

Among deposit feeders, chemical mediation of feeding behavior has been investigated in several species of sand fiddler crabs (Robertson et al., 1981; Rittschof and Buswell, 1989; Weissburg and Zimmer-Faust, 1991), with a variety of sugars, amino acids and peptides being implicated as phagostimulants. Sediment chlorophyll *a* concentrations are strongly correlated with feeding rate in a deposit-feeding enteropneust (Karrh and Miller, 1994), and organic content of sediments is an important determinant of ingestion rates in a variety of deposit feeders (Levinton, 1989). Although dissolved chemical cues stimulate responses in a facultatively deposit-feeding ghost crab (Trott and Robertson, 1984), the various ocy podid (fiddler and ghost) crab studies that surveyed effects of specific compounds focused on chemosensory capabilities during emersion when the crabs are active, and hence on relatively high dissolved concentrations in surficial pore waters and on adsorbed cues.

Diets of deposit feeders are best known in intertidal species, and best known among them, in turn, are taxa that clearly specialize on diatoms, in particular ocy podid crabs and hydrobiid snails (reviewed by Jumars, 1993). Consonant with this dietary specialization, fiddler crabs show strongest responses to simple sugars (Robertson et al., 1981) that in general dissolve well and adsorb poorly. Juvenile deposit feeders of several polychaete species in the intertidal zone rely extensively on diatoms for their nutrition, but the adults broaden their resources to include detritus of lower quality (Hentschel and Jumars, 1994; Hentschel, 1998). We hypothesized, therefore, that specific sugars or amino acids that are abundant in diatoms (e.g., serine) might be generally useful cues for the presence of fresh diatom food of high quality and hence might elicit enhanced feeding activity in adult polychaete deposit feeders. Deposit feeders in general process a milieu rich in carbon and comparatively poor in available nitrogen; a second prediction

is that amino acids, which are perhaps the most available nitrogen species because they require no hydrolysis before absorption, should be in general phagostimulatory.

It is also striking that the intertidal deposit feeders whose diets are best known are mobile, and that their food preferences have been learned in large part from studying patch use (Jumars, 1993). By contrast, methods to deliver cues to immersed, sedentary deposit feeders and to classify their feeding responses are still rudimentary. A large part of our experimental effort, therefore, was devoted to developing a system for delivering cues reliably and evaluating changes in behavior. It is not immediately obvious that deposit feeders would benefit from detecting dissolved cues instead of 'tasting' adsorbed ones. In order to stimulate deposit feeders to ingest particles without food value (e.g., clean glass beads) for studies of mechanics of selection (e.g., Jumars et al., 1982), however, we routinely add dissolved cues from either commercial aquarium fish foods. Occasionally, we have used only filtrates. We therefore know that dissolved extracts from complex food mixtures are effective phagostimulants, at least temporarily. One reason to suspect rapid response to dissolved cues is that the sedentary animals in question cannot pursue advected food and are known to switch from feeding on deposits to feeding on suspended material when the suspended flux is sufficient (e.g., Taghon et al., 1980).

Herein we were especially interested in testing the general hypothesis that the stimulatory effect could be elicited by simple, well-defined monomers, in each case against the null hypothesis of no significant change in behavior. Having an identified chemical instead of a complex mixture with phagostimulatory activity would greatly ease the task of developing a dose–response relationship. Dade et al. (1990) and Jumars (in review) have pursued the hypothesis that deposit feeders ingest material at the rate that maximizes rate of absorption of growth-rate-limiting compounds from the gut. This hypothesis is supported by the feeding-rate and growth-rate data of Taghon and Greene (1990). If it is correct, then application of phagostimulants or phagodepressants, without changing the diet available for ingestion, would provide a test of the hypothesis because both should decrease absorption rate by displacing ingestion rate from its optimum. Because low-molecular-weight compounds are particularly common feeding cues (Carr, 1988), we first selected amino acids and simple sugars as potential feeding stimulants and then expanded our investigation to include more complex carbohydrates, organic acids, nucleotides and natural extracts.

We tested this variety of dissolved compounds against an array of closely related, sympatric species of spionid polychaetes. Spionids are representative surface deposit or 'interface' feeders, living and feeding at the sediment–water interface in most soft-bottom marine environments (Fauchald and Jumars, 1979). Adults typically live in vertically oriented tubes constructed of mucus and sediment grains and collect particles from the sediment surface with two ciliated feeding tentacles or palps. Orientations and movements of the feeding palps are often highly directed, and different taxa of spionid polychaetes can display quite different behaviors (Dauer et al., 1981). Spionids spend a high percentage of their time in plain view, actively probing into and feeding on surficial sediments, and they are relatively easy to maintain in the laboratory. Specific aspects of spionid feeding have been examined in some detail, including effects of temperature (Yokoyama, 1988), particle size (Lopez and Levinton, 1987), particle specific gravity

(Mayer et al., 1993), palp diameter (Hentschel, 1996), flow velocity (Taghon and Greene, 1992), sediment transport (Jumars and Self, 1986), and protein content of sediments (Taghon and Greene, 1990).

Although spionids are known to increase feeding in response to dissolved chemicals, particularly those emanating from high-nutrient substances such as commercial baby food (Hentschel, 1996) or a recently injured amphipod (E. Gallagher, pers. comm.), effects of specific dissolved cues on spionid feeding have not been previously documented. The three species tested showed very different behavioral patterns and responsiveness to cues. In general, natural extracts were the most effective stimulants and single amino acids had a depressant effect if any at all.

2. Materials and methods

2.1. Collection and maintenance of animals

The three species of spionid polychaetes (*Boccardia proboscidea* (Hartman), *Polydora cornuta* (Bosc), and *Pseudopolydora kempji japonica* (Imajima and Hartman)) were collected with a 1-mm sieve from False Bay (San Juan Island, WA, USA) in late September 1995. Individuals that showed no signs of gametogenesis, loss of segments, or visible bodily damage and measured 10–15 mm long were introduced to sediment-filled culture tubes (6 ml Falcon). The lower half of each culture tube was filled with foundry sand (125 μm median grain diameter, Salmon Bay Gravel) to slow the onset of anoxia by excluding oxidizable organic matter, whereas the upper half was filled with False Bay sediment passed through a 125- μm sieve and thrice frozen and thawed to restrict living meiofauna and bacteria. (All natural sediments used in this study were treated similarly.) Spionids that constructed sediment tubes and exhibited typical behaviors within 1 h were selected for observation. Five culture tubes containing selected individuals of the same species (one individual per tube) were positioned in a plastic tray (35 \times 25 \times 10 cm) filled with foundry sand and topped with a 2-cm layer of False Bay sediment. To prevent interference between adjacent individuals and to avoid boundary effects of the tray edges on the flow, animals were spaced 4 cm apart (across the flow, so that none would be downstream from another) and 5 cm from the leading edge of the sediment tray. Trays were then placed under a laminar flow of non-recirculating, unfiltered seawater (mean velocity = 0.5 cm s^{-1} , measured by timing dye spots 1 cm above the bottom). Fecal pellets were removed every 8 h, and since the accumulation of fecal material may inhibit feeding rates in spionids (Miller and Jumars, 1986; but see Taghon, 1992), pellets were removed whenever more than 4 or 5 (or equivalent unconsolidated material) accumulated during the course of experimental trials. Sediment trays were topped with False Bay sand (approximately 1-mm layer) every 24 h to assure liberal food supply and a smooth sediment surface.

2.2. Behavioral classification

Animals were illuminated with cool (fiber-optic) light and their behaviors closely

observed under a dissecting microscope mounted on a movable arm above the sea table. Although it was possible to see several mm down into an individual's tube and therefore to observe the ingestion of particles in detail, movements of the tentacular feeding palps were of particular interest. Palps initiate feeding through particle collection from the sediment surface, and while extended are in continuous contact with overlying water. Conversely, the head and mouth typically are held within the tube below the sediment surface, only occasionally extending into the water column. Palps are thus the primary interface between spionids and their feeding environment, and are the structures most likely to encounter dissolved chemical cues.

Palp behaviors were classified as inactive, active but non-feeding, or actively feeding. Visible behaviors were grouped further into seven basic states (Fig. 1), ranging from completely withdrawn into tubes to vigorously deposit feeding. To document the background frequencies of these behaviors, eight individuals per species were each observed in twenty separate, 5 s intervals in the absence of introduced chemical cues. Time between observations was random, but always longer than the average time spent in any one behavior. Observations were recorded only if there was no change of behavior within the 5 s interval. As an added measure of background activity, randomly selected animals ($n = 5$) from each species were observed continuously through a series of 15 behavioral transitions. The time and nature of each transition was recorded and served as an independent measure of the frequency of time spent in various behaviors. In addition to these seven basic states, other changes in behavior occurred, such as partial extension or retraction of the feeding palps, changes in the rate of palp movements, rapid twisting and knotting of the palps (distinct from the palp coiling associated with suspension feeding; Taghon et al., 1980), and active removal of fecal pellets or egestion of unconsolidated fecal material. Because of the ambiguity regarding their classification as short-term feeding responses, these additional behaviors were recorded but not analyzed in the present study.

In deposit feeding, the fraction of the palp surface occupied by moving sediment grains ranged from very low (one or two particles in total) to very high (effectively 100% of the palps covered by particles). Differences in palp coverage by sediment grains were divided into three levels (Fig. 1). Combined with observations of particle transport speed along the palps (designated as fast or slow), changes in palp coverage were used to indicate apparent changes in ingestion rate. A simple set of criteria was developed to classify behavioral responses to cues and controls. A response was scored as positive if the worm either began feeding or increased ingestion rate through either an increase in palp coverage or an increase in particle transport speed (or both). A response was scored as negative if the worm ceased feeding, decreased ingestion rate through a decrease in palp coverage (and) or particle transport speed, or completely retracted into its tube from a non-feeding but active state.

2.3. Cue-delivery apparatus

Preliminary observations indicated that at least 50% of all individuals showed marked responses when artificial seawater (ASW) was introduced gently with a pipette 1–2 cm upstream. In addition to creating velocity fluctuations around the palps, the hand

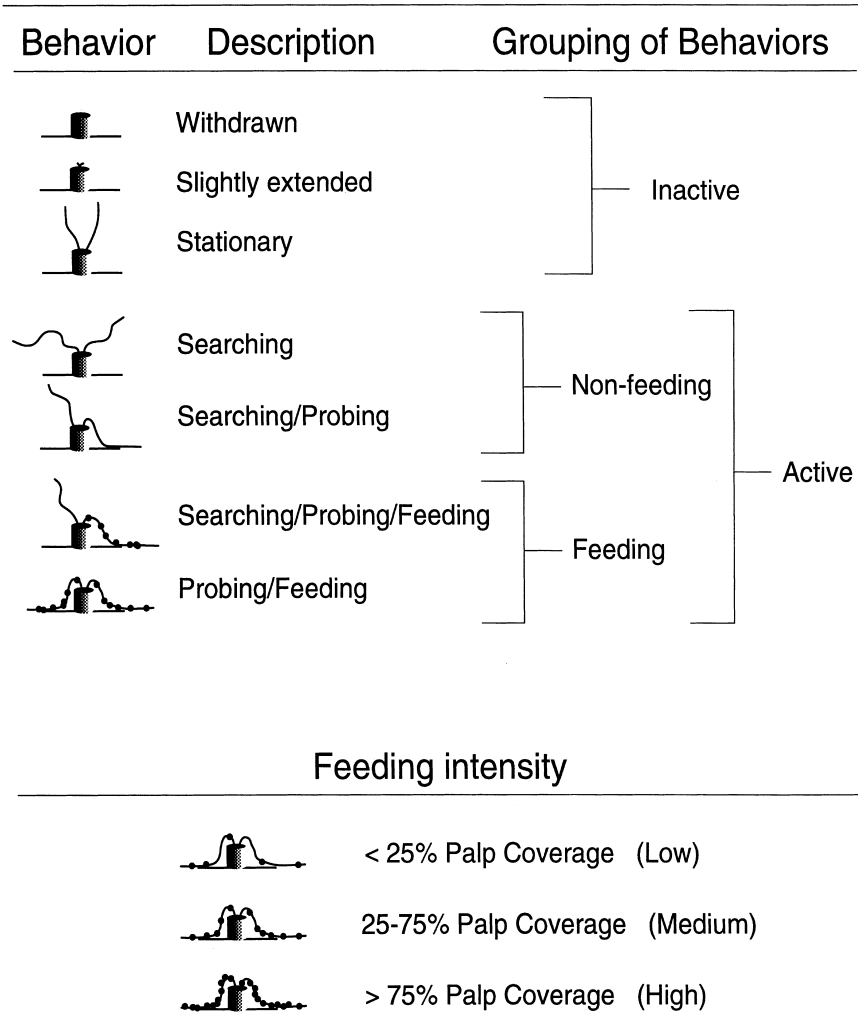


Fig. 1. Classification of seven basic behavioral states, applicable to all three species in the present study. Schematic side views of spionid feeding palps and exposed tube. Tubes are typically 1–2 mm in diameter, and range from flush with the sediment surface to built up several mm (as shown). It should be noted that suspension-feeding behaviors were not observed at the low current velocities imposed (0.5 cm s^{-1} at 1 cm above bottom). Also shown are three levels of palp coverage by sediment grains used in assessing short-term changes in apparent ingestion rate.

injections may have flushed out pore water from surficial sediments, further complicating interpretation of observed behavioral changes. Although some cues still elicited detectable responses over seawater controls when introduced manually, the mechanical stimulus associated with such an injection underscored the need for steadier cue delivery to avoid confusing hydromechanical with chemical cues. A reservoir of filtered seawater fed five separate lines, each extending down to an outlet (15-gauge blunt needle) 1 cm

Cue-Delivery Apparatus

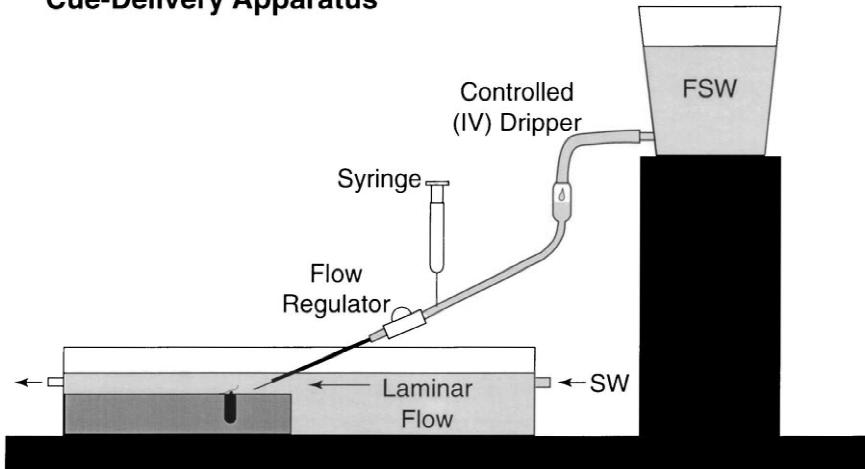


Fig. 2. Side view of the experimental apparatus used to introduce cues and seawater controls in steady, laminar flow over individual polychaetes. See text for description.

directly upstream from an individual polychaete (Fig. 2). Filtered seawater was added continuously to the reservoir, and the water level was maintained with an overflow valve to ensure constant pressure in the lines. Water in each line passed through an airtight dropper (from an intravenous [IV] administration system), enabling flow rate to be estimated by monitoring drip speed. Cues and controls were injected into the line with a sterile needle and syringe. Flow rate was controlled precisely by altering resistance in the line with a thumbscrew positioned just below the site of injection, yielding a predictable (from dye injection) delay between the time of stimulus injection and the time of contact with a spionid tube. Dye experiments confirmed laminar flow and absence of velocity fluctuations in the vicinity of the worms, particularly at the moment of stimulus injection. All lines were flushed with hydrogen peroxide at the end of each day of experimental trials.

2.4. Experimental design

Of the 24 animals on which background behavioral observations were made, five individuals of each species were selected at random and screened for their responsiveness to singly presented cues and controls. A suite of 44 different cues (Table 1) and 25 ASW control replicates was tested against each individual. Treatments were done in batches. For each batch, randomly chosen subsets of five cues (plus one ASW control) were prepared and presented in random order to all 5 five individuals from one species. Each cue was presented once to each individual, and only when that individual was active (feeding or non-feeding). Each trial consisted of a 70-s period of observation during which behaviors were recorded continuously. The first 30 s were used to indicate

Table 1
Dissolved chemical cues tested singly against individuals ($n = 5$) of each species

Category	Chemical cue
Amino acids ^a	Alanine, arginine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, histidine, isoleucine, leucine, methionine, phenylalanine, proline, serine, taurine, threonine, tryptophan, tyrosine, valine, betaine ^l
Carbohydrates ^a	Galactose, glucose, maltose, mannitol, sucrose, trehalose, casein ^b , cornstarch ^{b,e}
Organic acids ^a	Alginate ^c , ascorbic acid ^f , citric acid, succinic acid, oxalic acid ^g , glycolic acid ^h
Nucleotides ^a	Adenosine monophosphate (AMP), adenosine diphosphate (ADP), adenosine triphosphate (ATP)
Nitrogenous wastes ^a	Ammonium ⁱ , urea
Natural extracts ^d	Crustacean (mixed and <i>Corophium</i> spp.), macroalga (<i>Ulva</i> sp.)
Nutrient-enriched mixtures ^c	Baby food ^j , fish food ^k

^a 1 mM concentration in ASW; ^b 0.2 g in 100 ml ASW; ^c 0.6 g in 100 ml ASW, 0.2- μ m filtered; ^d blended in ASW, 0.2- μ m filtered; ^e Argo®; ^f MCB Reagents; ^g Mallinckrodt; ^h Aldrich Chemical; ⁱ J.T. Baker; ^j Gerber® mixed cereal; ^k Atlantis® goldfish flakes; ^l a common amine.

immediate feeding history. If individuals produced a fecal pellet or disappeared completely during this time interval, the trial was aborted. After a 30- μ l stimulus (cue or control) was introduced, the polychaete was monitored closely for 10 s. The score for each trial of positive, negative, indeterminate, or no response was determined from responses (or lack thereof) solely during this 10-s period. Observations for each trial were extended an additional 30 s to collect information on longer-term responses, although these results are not presented here.

Largely due to the effectiveness of the cue-delivery apparatus (in limiting physical disturbances), all species showed a low number of responses to ASW. A Chi-squared heterogeneity test revealed no significant differences among individuals within a species ($p > 0.75$). The number of responses to ASW controls was used as an indication of the frequency of response absent a cue (i.e., spontaneous response). Probabilities of spontaneous positive and negative responses were used to set significance thresholds for the number of responding individuals in the experimental trials. Using simple binomial expansion, a significant number of responses was determined as that which had < 0.1% chance of occurring spontaneously. With the exception of negative responses in *Polydora* (where 100% of individuals responding indicated a significant effect), a threshold of 60% (i.e., three of five individuals responding) was necessary for a cue to be considered to have significant effect on feeding behavior. Among-species differences in thresholds were due to among-species differences in frequency of spontaneous responses.

Because of the large number of screening trials and small number of individuals per species, problems associated with multiple testing were expected. To overcome this potential complication, cues that elicited the most significant responses were tested further against ten new individuals per species, collected from the same area in False Bay in February 1996. Specimens were more scarce at that time. Although several hundred worms were sorted, most were not in ideal physical condition and about one-third (*Boccardia*, $n = 5$; *Polydora*, $n = 3$; and *Pseudopolydora*, $n = 2$) of the

individuals chosen for observation either were damaged or showed signs of gametogenesis. Animals with developing gametes have been avoided in other studies of spionid feeding (e.g., Taghon, 1992), as the active formation of reproductive tissue may alter nutritional requirements or preferences. We were forced to use some gametogenic individuals. Despite these differences in physiological state, individual responses to ASW controls confirmed probabilities of spontaneous response that were not significantly different from those obtained in the original screening. Therefore, the same significance thresholds were used to evaluate the responses to presented cues.

2.5. Preparation of stimuli

Chemical cues were mixed in ASW, and salinity, pH and temperature were adjusted to ambient levels before each set of trials (salinity = 31.0–32.5‰; pH = 7.75–7.85; $T = 9.0$ – 11.0°C). Chemicals were purchased from Sigma Chemical, with exceptions noted in Table 1. All amino acids were 1 mM in concentration, and other compounds were also mixed to this concentration when possible (see Table 1). This level is several orders of magnitude higher than typical mean levels of dissolved free amino acids in interstitial and near-bottom waters (Jørgensen, 1979; Henrichs and Farrington, 1979; Burdige and Martens, 1990), and was assumed to be perceived by the spionids as a spike above normal background. Artificial-seawater suspensions of commercial baby food and fish food (both filtered through a 0.2- μm cellulose–acetate filter) represented nutrient-enriched mixtures. For the original screening, natural extracts were obtained from a macroalga (*Ulva* sp.) and from a mixed sample of crustaceans (copepods, amphipods, and barnacle nauplii). These samples were rinsed and blended in ASW, vacuum filtered through a 0.2- μm cellulose–acetate filter, and adjusted to ambient pH. Crustacean extract was intended to be from benthic amphipods (*Corophium* spp.) commonly occurring in False Bay, but specimens could not be found at the time of the original screening. For the experiments conducted in February, *Corophium* was used to prepare the crustacean extract. The macroalgal extract used in this second set of experiments was prepared from freeze-dried *Ulva* because no fresh specimens could be located. All cues were used for behavioral assays within 2 h of preparation.

3. Results

3.1. Background behavioral patterns

We observed strong differences in background behavioral patterns among species (Fig. 3). A Chi-squared heterogeneity test verified that individuals within a species were not significantly different from one another in the proportion of time spent in classified behavioral states ($p > 0.75$), although individuals were internally consistent and often unique in the finer detail of their behaviors. *Polydora cornuta* exhibited patterns of behavior distinct from and more stereotyped than the other two species in that palps were usually either withdrawn into the tube or intensely deposit feeding with little or no extension into the water column. *Pseudopolydora kempii japonica* showed the broadest

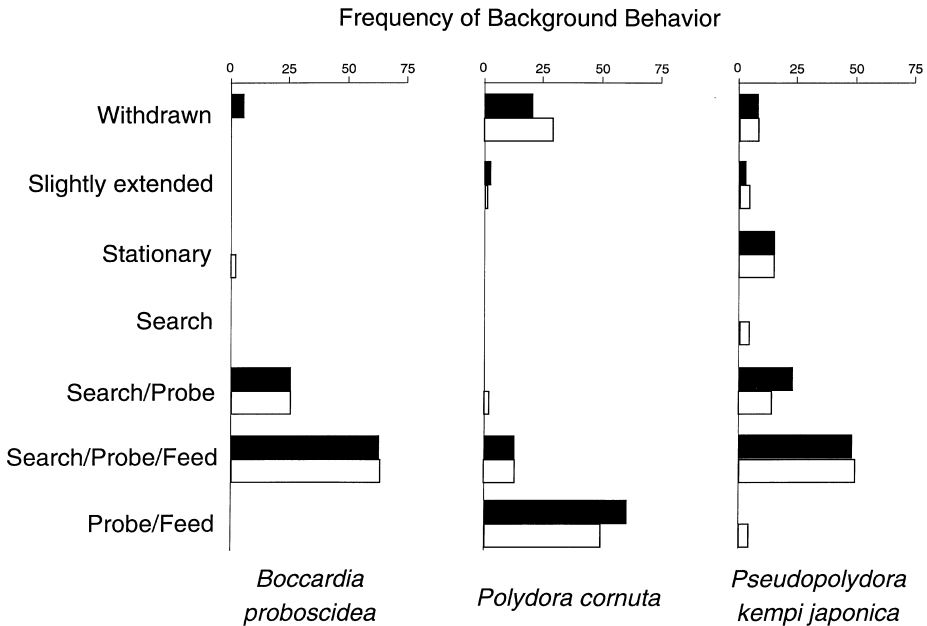


Fig. 3. Background behavioral patterns in the absence of introduced stimuli. Black bars represent median frequencies of behaviors for each species (twenty independent observations per individual; $n = 8$ individuals per species), and white bars represent the frequency of time spent in each behavior when observed over a continuous series of fifteen behavioral transitions ($n = 5$ per species).

range of behaviors and was similar to *Boccardia proboscidea* in that palps often were extended into the water column. Besides exhibiting a wider variety of behaviors, the latter two species also changed from one state to another more quickly than did *Polydora*.

General observations also supported several notable differences among species. *Boccardia* almost always fed with the head partly exposed or flush with the tube opening, sometimes extending 3–5 setigers into the water column. Palps searched the water column in broad, sweeping motions, and often performed a curious ‘pore-water sniffing’ behavior. It involved raising the palps to an erect position and then lowering them very slowly, seemingly passively, to the sediment surface. After contacting the sediments, the palps quickly returned to an upright position and proceeded to repeat the passive sinking. These motions continued in a systematic treatment of the circular area surrounding an individual’s tube. When 30 μl of a nutrient-enriched mixture (e.g., fish-food extract colored with fluorescein dye) was injected gently into the pore water of sediments surrounding an individual, a touchdown and withdrawal of the palps in this area caused the dyed mixture to be whisked out of the sediments and to come into clear contact with the palps. Vigorous deposit feeding ensued in the treated area. In contrast, *Polydora* seemed purposefully to avoid extending palps into the overlying water. Feeding proceeded almost exclusively with palps flat against the sediment surface, although many times the tip of the palp was bent into the water, and particles were

collected at the most distal point of contact by the palps with the sediment surface. *Polydora* ingested a larger volume of sediment per unit of time than did the other two species, and its palps often were packed densely with particles travelling towards the mouth. Its palps were highly sensitive to mechanical disturbances of the surrounding water and seemed to collect only easily detached particles. An individual's tube usually was flush with the sediment surface, and the head was almost never exposed. *Pseudopolydora* often built up its tube above the sediment–water interface. Its individuals ingested a volume of sediments per unit of time intermediate between the other two species studied. Palp behaviors shifted more rapidly than in either of the other species, and the head was rarely exposed except during fecal-pellet release.

Characteristics of egestion also differed among species. *Boccardia* withdrew into the tube for 5–10 s and made distinct rods or pellets, pushing them out of the tube with the head. Pellets therefore accumulated close around the tube opening and at times seemed physically to hinder deposit feeding. Under turbulent flow, pellets released in this way likely would be removed from the vicinity of the animal. *Pseudopolydora* withdrew for 10–30 s before releasing a fecal pellet, and held the pellet between the palps with the ciliary tracts reversed so as to deposit it at some distance from the tube. The head rarely had any visible contact with a pellet. *Polydora* established a U-shaped tube and expelled mounds of unconsolidated fecal material, usually from the opening not actively being used for feeding. Unlike both other species, expulsion of fecal material appeared to be nearly continuous. As with *Boccardia* this material likely would be washed away under more turbulent flow.

3.2. Responses to cues

Of the 44 cues tested in the original screening, only 12 elicited significant responses in at least one species (Fig. 4). *Boccardia* showed definite negative responses when exposed to the amino acids taurine, threonine and valine. All three terminated any feeding activity and led to withdrawal of the palps, with the withdrawal response to taurine being most rapid. Although not as dramatic, *Pseudopolydora* showed similar withdrawal responses to taurine, and most individuals quickly ceased feeding and withdrew palps when exposed to glycine. Exposure to urea resulted in some twisting and partial withdrawal of palps, along with variable decreases in palp coverage. Significant responses to ammonium were characterized by the termination of feeding and complete withdrawal of palps.

Although ATP was not stimulatory to any of the species tested, introduction of ADP to *Boccardia* resulted in an increase in palp coverage and particle transport speed, as well as twisting and extension of the palps. Exposure to the macroalgal extract also stimulated sizable increases in apparent ingestion rate, evidenced by increases in palp coverage, particle transport speed, and rates of palp movement. Responses by *Boccardia* to the crustacean and fish-food extracts were characterized by twisting and extension of palps along with increases in palp coverage. Although the response was less pronounced, proline initiated feeding and increased palp coverage, and the baby-food extract increased rate of palp movements and palp extension, along with some increases in palp coverage. Significant responses by *Pseudopolydora* to the crustacean and fish-food

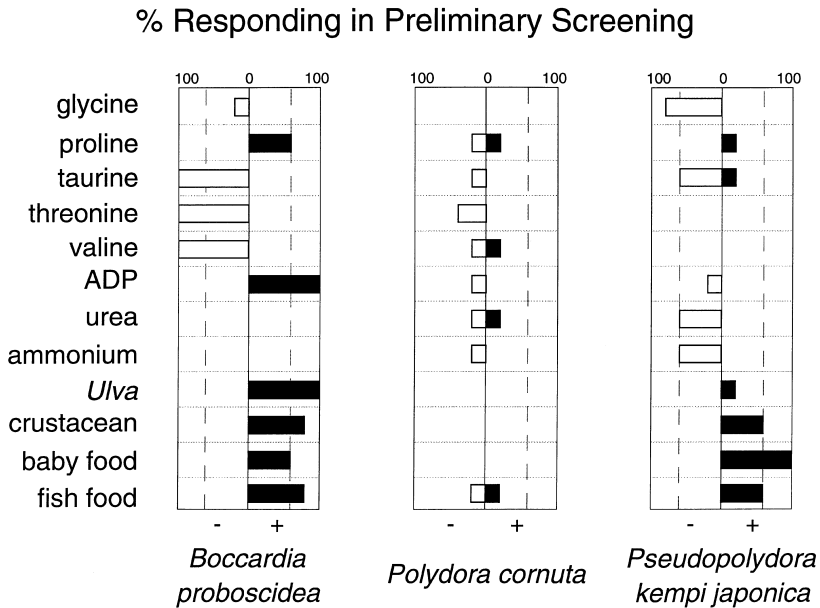


Fig. 4. Statistically significant responses shown in the preliminary screening. All compounds listed in Table 1 were tested against each individual, but only results for cues that elicited significant responses in at least one species are shown as varying numbers of positive (black bars) and negative (white bars) responses. Significance thresholds are indicated by vertical dashed lines, with the negative threshold for *Polydora* falling at 100% (see text).

extracts were characterized by increases in palp coverage and rate of palp movements. Baby-food extract was the most stimulatory cue presented, causing rapid twisting and extension of the palps and large increases in both palp coverage and particle transport speed. Unlike the other two species, *Polydora* showed no significant responses to any of the cues tested, including the nutrient-enriched mixtures and natural extracts.

Testing this subset of cues against ten additional individuals per species resulted in similar responses (Fig. 5), with a few notable differences. Exposing *Boccardia* to ADP elicited fewer and less dramatic responses than in the original screening, and positive responses to baby food fell below the significance threshold. Although the *Ulva* extract used in these confirming tests was not a fresh preparation as in the original screening, positive responses by *Boccardia* were still statistically significant. In addition, the number of positive responses to the *Corophium* extract increased over responses to the mixed crustacean extract presented in the screening. *Pseudopolydora* showed a similarly strong increase in positive responses to the *Corophium* extract. Negative responses to ammonium, however, fell below the significance threshold.

4. Discussion

We conducted these experiments to provide a first survey of the effects of dissolved

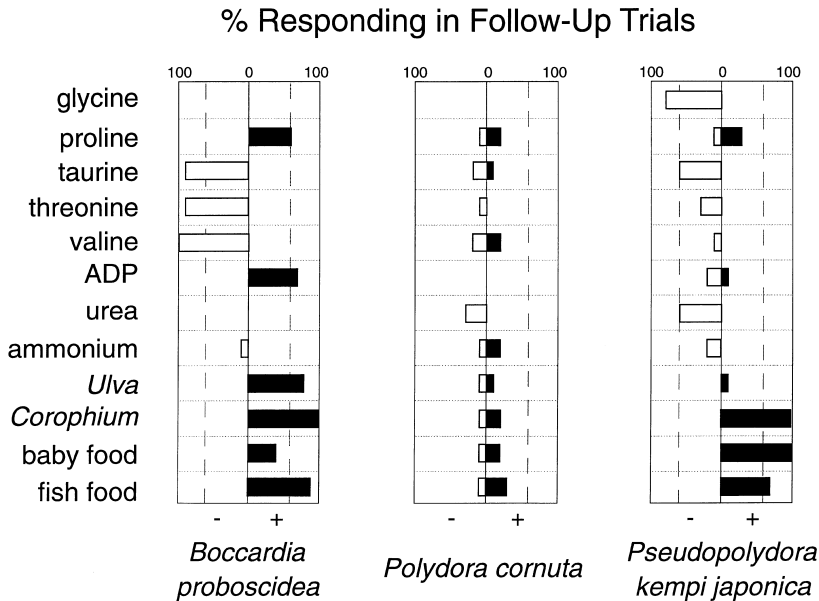


Fig. 5. Follow-up test responses to cues that were significant phagostimulants (black bars) or phagodepressants (white bars) in the original screening (see Fig. 4). Significance thresholds are indicated by vertical dashed lines, with the negative threshold for *Polydora* again at 100%. Results generally support those from the preliminary screening, with notable differences discussed in the text.

cues on the feeding behaviors of one group of surface deposit feeders. Based on prior results with ocypodid crabs during emersion, we expected phagostimulation by plant sugars. Based on low supplies in detrital environments, we expected perhaps a general phagostimulation response to amino acids. Among the three species of spionid polychaetes, there were strong interspecific differences. At the concentrations tested, however, amino acids tended to elicit negative responses if any, and the other monomers presented were not significantly stimulatory, contrary to our initial expectations. We found complex mixtures to be the most stimulatory cues tested, in accordance with many studies on other taxa (e.g., Carr and Derby, 1986).

The environment provided in the laboratory carefully restricted conditions to steady, laminar flow and was selected intentionally to be a small subset of the natural one. The purpose of this restriction was to preclude suspension feeding (as hydrosols and other cues of rapid flow were lacking) and to create episodes of known and high exposure to the stimulus plume. Although our results therefore probably do not represent times spent in these various behaviors in the field, except perhaps at slack water, they do suffice to show strong interspecific differences that help, along with past work, better to understand foraging differences among superficially similar species.

Results from the present study clearly demonstrate that spionid polychaetes are capable of detecting and responding to dissolved chemical compounds. Although the species tested showed variable responses, their background behavioral patterns reinforce these differences in sensitivity. Both *Boccardia* and *Pseudopolydora* spend a large

fraction of time 'monitoring' the water column, and likewise show rapid responses to several dissolved cues. The repeated observations of 'pore-water sniffing' by *Boccardia* also suggest that this species exhibits stimulus-acquisition behavior to direct profitable feeding activity from selected regions within the reach of its palps. *Polydora* on the other hand, rarely extends its palps into the water column and shows no significant responses to dissolved cues. Although the lack of responses may simply reflect a higher sensitivity threshold (in terms of concentration of cue or duration of exposure), *Polydora* typically emerged only long enough to deposit feed vigorously before retreating back into its tube. This pattern suggests that individuals of that species may not rely heavily on water-borne cues to indicate favorable conditions, and may instead feed at near-maximal ingestion rates unless a sufficiently negative stimulus is encountered. The preference for loose sediment grains observed for *Polydora* in this study, and for *Pseudopolydora* by Jumars and Self (1986), would help ensure a high ingestion rate if unconsolidated material is present, and lack of consolidation may be a physical indicator of food quality as a surrogate of freshness (recent deposition).

Methods of egestion may also correlate with sensitivity to dissolved stimuli. Allowing fecal material to accumulate in close proximity to the tube opening would elevate local concentrations of metabolic wastes, at least over short periods or in still water. Both *Boccardia* and *Polydora* followed this basic strategy for egestion, and both were remarkably insensitive to either urea or ammonium at the concentrations presented. It should be stressed that the worms were not immersed in this concentration but rather had their palps exposed to a plume of it. We were not testing toxicity but rather chemosensory response. In contrast to the other two species, *Pseudopolydora* purposefully released fecal pellets at some distance from the tube opening, and individuals reacted negatively to both urea and ammonium in the original screening. The decreased responsiveness to ammonium in the second set of experiments (conducted in February) could reflect seasonal changes in sensitivity. Building their tubes well above the sediment surface also could aid in separating egested material from the active feeding area, although this behavior also is correlated with lack of a strong current (Nowell et al., 1989). Even low-velocity flow may be sufficient to remove the majority of dissolved waste products (Taghon, 1992), but our results suggest a possible link between sensitivity to such substances and patterns of egestion. Previous studies with other taxa also implicate metabolic waste products as effective phagodepressants (e.g., Zimmer-Faust, 1987), although further testing is necessary to confirm this pattern in spionids. Dauer et al. (1981) present a more detailed description of egestion in several species of spionid polychaetes, but correlative data on chemical sensitivities to waste products are not yet available.

In addition to metabolic wastes, other phagodepressants seem to play an important role in regulating spionid feeding behavior. Surface deposit feeders in general are highly susceptible to predation by pelagic and epibenthic predators (Holland et al., 1980), and spionid feeding palps are typical targets of browsing predators (DeVlas, 1979; Woodin, 1982). Although sensitivity to vibrations may be a primary means of predator avoidance and is a common cause of withdrawal responses in spionids (M. Ferner, pers. obs.), the detection of chemical signals released from a nearby predator or damaged prey could greatly facilitate these survival mechanisms (Carr, 1988). Two of the compounds that

elicited negative responses in this study may serve as an early warning of such a threat. Both taurine and glycine often are associated with vertebrate bile salts (Matthews and van Holde, 1990), and are two of the most common organic osmolytes in marine invertebrates (Carr et al., 1996). The observed negative responses to threonine and valine are less revealing, but may be driven by similar factors related to predator avoidance. Uhazy et al. (1978) found glycine, threonine and valine to act as repellents to an aquatic mollusc but did not speculate as to possible correspondence with natural events. We would expect spionids to respond negatively to both predator exudates and extracts of conspecific tissues, although tests of these substances have not yet been performed.

Stimulatory cues should provide an indication of the types of organic matter used by deposit feeders. Self et al. (1995) suggested that, based on the high uptake ratio of sugars to amino acids, a deposit-feeding holothuroid (*Parastichopus californicus*) is a functional herbivore. Hentschel and Jumars (1994) and Hentschel (1998) document the importance of diatoms to juvenile deposit feeders, including the *Pseudopolydora* and *Polydora* at False Bay. If such a preference exists (i.e., selection for plant-derived organic matter), one might expect to see a feeding response to plant extracts. Although *Boccardia* and *Pseudopolydora* both showed positive responses to several cues, the striking difference between these two species in their responsiveness to the macroalgal extract may reflect a fundamental difference in nutritional sources. Uhazy et al. (1978) demonstrated that romaine-lettuce homogenates attracted an aquatic mollusc and identified proline as a primary attractant in the mixtures presented. The positive responses shown by *Boccardia* to proline may follow a similar mechanism for detecting nutritionally valuable substances. These responses to proline also could relate to the abundance of that amino acid in crustacean tissues (Carr et al., 1996), since both *Boccardia* and *Pseudopolydora* showed strong responses to the animal extracts. Because of its ubiquitous presence in animal tissue, one might expect ATP to be an informative and stimulatory molecule, as has been shown for a carnivorous crustacean (Zimmer-Faust, 1987, 1993). Surprisingly, ATP was not stimulatory to any of the species that we tested. Although *Boccardia* did show strong positive responses to ADP, we cannot speculate with confidence as to the adaptive advantage of this response. Behavioral data coupled with data on hydrolysis and absorption of digesta could help identify potential diet of deposit-feeding animals. If preferences for plant- or animal-derived organic matter can be shown for opportunistic deposit feeders, an informative restructuring of perceived trophic pathways may result.

We deliberately presented dissolved cues. Whereas adsorbed cues and organically enriched sediments may drive long-term feeding responses, dissolved cues may be the first indicators of rapidly changing conditions. It may be that a portion of some of the compounds tested began adsorbing to sediments immediately after introduction, but the direct observations of positive responses indicated that the animals were detecting a stimulus in the water column, not only on particles being ingested. Repeated and swift reaction of both *Boccardia* and *Pseudopolydora* to phagodepressants also suggested that signals were detected in dissolved state. For most of our cues, partition coefficients are too low and porosities are too high for adsorption to be significant (Henrichs, 1992). Given the system that we employed it would be unreasonable to expect adsorption levels

to be more than 2–3%, and it is therefore very likely that all stimuli were detected in dissolved state. A more complicated experimental design would be needed, however, to resolve effects of adsorbed versus dissolved cues on sponiid feeding behavior.

Our cues, by design, fell largely in two extremes; monomers and complex mixtures. Complex mixtures showed generally more frequent and more intense responses, but the molecules accounting for the responses remain unidentified. Monomers give the problem of limited information content. All predators and prey, for example, contain many if not most amino acids in common. Two complementary means are available to obtain more information about the source of a chemical signal. One is to sense oligomers that are more specific to a particular source. Although it was in a very different context, Tamburri et al. (1996) recently demonstrated the power of a dissolved trimer of amino acids to elicit settlement in oysters. Another strategy is to respond to specific mixtures of monomers. Our experiments do not allow us to say whether the enhanced effect of mixtures is due to primarily one of these means of garnering more information, to both or to responses to larger molecules (not mixtures). The obvious problem in exploring these possibilities is the staggering number of possible permutations and combinations (e.g., of 21 amino acids as trimers or in mixtures of monomers). At the time that we conducted these experiments, it was not possible for us to choose particular amino-acid oligomers for bioassay. By relating chemical structure with stimulus strength, Browne et al. (1998) very recently have provided a rational means to narrow the choices. Chemosensory cues thus have not yet given an answer to the long-standing question of nutritional sources used by deposit feeders (Lopez et al., 1989), but they continue to provide one of many promising avenues for clues to narrow the spectrum of possibilities further.

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