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Winsor H. Watson III

University of New Hampshire, Durham, win.watson@unh.edu

Charles M. Chester

University of Washington

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The Influence of Olfactory and Tactile
Stimuli on the Feeding Behavior of
Melibe leonina (Gould, 1852)
(Opisthobranchia: Dendronotacea)

by

WINSOR H. WATSON III¹

AND

CHARLES M. CHESTER

Zoology Department, Coastal Marine Laboratory, Center for Marine Biology,
University of New Hampshire, Durham, New Hampshire 03824, USA

Abstract. The nudibranch *Melibe leonina* feeds using the rhythmic movements of its large oral hood to capture small crustaceans that are present in the water column. The frequency of these feeding movements, or hood closures, is proportional to the concentration of available prey. The purpose of this study was to determine what qualities of prey cause the rate of these feeding movements to change. Animals were observed during exposure to the following treatments: (1) filtered seawater; (2) *Artemia*-conditioned seawater (smell); (3) small particles in seawater; (4) particles soaked in *Artemia*-conditioned seawater; (5) frozen *Artemia* and; (6) live *Artemia*. Both conditioned water and particles caused appetitive behavior (orientation of the oral hood) and a significant increase in the frequency of hood closures. This increase in rate had a rapid onset and was maintained throughout the duration of the 20-min test period. The major difference between the effects of the two stimuli was that smell alone led to incomplete feeding cycles while particle treatments yielded normal feeding behavior. When applied together these stimuli produced a larger response than either one did alone. However, no combination of stimuli was as effective as live prey. We conclude that both tactile and chemical cues are sufficient to elicit an increase in the feeding movements of *Melibe leonina*, but some additional stimulus provided by live prey, such as vibrations, may play an important role as well. The information provided by these stimuli helps initiate appetitive and early aspects of the consummatory phases of feeding, and also influences full expression of the rhythmic feeding motor program.

INTRODUCTION

In most mollusks, both chemical and tactile stimuli have a strong influence on feeding behavior (KOHN, 1983; AUDESIRK & AUDESIRK, 1985). The presence of chemoreceptors in the oral region of many mollusks has been well documented, as has the ability of food extracts to elicit both the appetitive and consummatory phases of feeding

(KOHN, 1961; CROLL, 1983). Mechanoreceptors, which often have centrally located somata, are found both around the mouth and in various regions of the esophagus and gut, and they appear to be capable of either exciting or inhibiting feeding and swallowing behaviors. However, the relative influence of each type of stimulus on feeding behavior has only been examined closely in a few species.

In *Aplysia*, as in many mollusks, there is a clear appetitive response to the presence of chemical stimuli (PRESTON & LEE, 1973; KUPFERMANN, 1974). Animals wave their head, and lift the anterior two-thirds of their body off the

¹ To whom requests for reprints should be sent at Zoology Department, U.N.H., Durham, New Hampshire 03824.

substrate. If head waving does not bring them in contact with food, they alternate locomotion and head waving until food is localized. Chemical stimuli alone (seaweed extract), when applied to the lips, or mouth region, will elicit a biting response, while tactile stimuli alone (a glass rod) will not (ROSEN *et al.*, 1982). Nevertheless, tactile stimuli will enhance the response to chemical input, resulting in regular biting. *Tritonia diomedea* (Bergh, 1894) also bites repeatedly in response to chemical input (sea whip extract), and as with *Aplysia*, tactile stimuli in the mouth or esophagus modulates this behavior (AUDESIRK & AUDESIRK, 1979). Some interesting mechanoreceptor cells, which also receive excitatory chemical input from the oral veil and mouth region, appear to be at least one site where the two modalities might undergo peripheral integration (AUDESIRK & AUDESIRK, 1980a, b).

The dendronotacean opisthobranch *Melibe leonina* (Gould, 1852) is an unusual gastropod lacking jaws, a radula, and a well-defined buccal mass (GOSLINER, 1987). It feeds by removing small planktonic animals from the water column using a specialized oral hood (AGERSBORG, 1921; HURST, 1968; AJESKA & NYBAKKEN, 1976). This structure is equipped with sensory, muscular, and vascular elements that allow for the efficient capture of free-swimming prey (HURST, 1968). The oral hood surrounds prey that are in the water column, closes to force water out through the tentacles on the edge of the veil, and then contracts further to bring the captured animals into the mouth (WATSON & TRIMARCHI, 1992). If sufficient prey are available, the behavior is rhythmic, with a frequency ranging from 0.5 to 3 cycles/min, depending on the concentration of food in the water (WATSON & TRIMARCHI, 1992).

At the present time little is known about the motor programs underlying expression of rhythmic feeding in *Melibe*, or the sensory inputs that control and influence their expression. The fact that the feeding rhythm is stereotyped (WATSON & TRIMARCHI, 1992), and occurs with a slow rhythm in the absence of prey (AJESKA & NYBAKKEN, 1976; THOMPSON & CRAMPTON, 1984) suggests that a central pattern generator may be involved. *Melibe* is sensitive to tactile stimulation (BICKELL & KEMPF, 1983) and there is some evidence that the feeding cycles are triggered by contact of prey with the oral hood (HURST, 1968). However, no information is available about the possible role of chemoreceptors. The hypothesis put forth by WATSON & TRIMARCHI (1992) is that the feeding rhythm is under the control of a central pattern generator (CPG), and both chemical and tactile stimuli modulate this CPG. The goal of this study was to determine the relative influence of chemical and mechanical stimuli on *Melibe* feeding behavior.

MATERIALS AND METHODS

All animals were collected, using SCUBA, from an eelgrass bed located along the border of the San Juan Channel, near an area of Shaw Island called Neck Point. Shaw

Island is part of an archipelago of 172 islands in the upper Puget Sound, Washington, known collectively as the San Juan Islands. Animals were shipped to New Hampshire and maintained in recirculating aquaria at 10–15°C, in the Zoology Department, U.N.H., Durham, New Hampshire. Animals were starved at least 7 days prior to testing. Feeding experiments were performed in a 15-L aquarium, at 12°C. Three to four animals were placed in the aquarium and allowed to acclimate for 30 min. The feeding activity of each animal (number of hood closures/min) was monitored for 20 min before and throughout each 20-min treatment. In addition, we determined whether each feeding act was complete, according to the criteria described by WATSON & TRIMARCHI (1992). This allowed us to calculate the percent of feeding cycles that were prematurely terminated for each treatment.

Animals were exposed to the following substances: (1) filtered seawater (control); (2) water conditioned with *Artemia* (smell); (3) small (350 μ m) Sephadex beads in filtered seawater (particles); (4) Sephadex beads soaked overnight in *Artemia*-conditioned seawater (smell and particles); (5) frozen *Artemia*; and (6) live *Artemia*. Stimuli were added as concentrated 50-mL aliquots so that when they were diluted in the 15-L aquarium, a final concentration of 1500 particles, or *Artemia*/L, or the odor equivalent to 1500 *Artemia*/L, was obtained. Although *Artemia* is not a normal component of the diet of *Melibe* it was used as a food source because it provides a well-defined and quantifiable diet, and our subjects ate them as voraciously as natural prey. Several preliminary studies with natural prey yielded comparable results.

Statistical analyses were performed using the program SYSTAT (SYSTAT Inc., Evanston, Il.). Ten *Melibe* were randomly chosen for each treatment. The effects of each treatment on complete and on incomplete feeding cycles were analyzed using a one-way analysis of variance (ANOVA) model (SOKAL & ROHLF, 1981). In some cases data were $\ln(x + 1)$ transformed to uncouple the variance from the mean and to give a positive value (KREBS, 1989). A Student-Newman-Keuls multiple comparison was used to detect differences between treatments. To compare the control with individual stimuli, *t*-tests were utilized.

In some experiments animals were sequentially exposed to different treatments to more accurately compare the relative responsiveness of individuals to different stimuli. For example, after 20 min exposure to control conditions, animals were subjected to a 20 min period during which only particles were present, followed by 20 min exposure to smell and particles. These treatments were not independent and were not used for statistical analysis. Rather, they provided information about the additive effects of stimuli and the time course of their influences.

RESULTS AND DISCUSSION

In the absence of any stimuli, in control seawater, *Melibe* maintained a hood closure rate of 0.20 cycles/min ($n = 51$, SEM = 0.017). None of the controls for the various

treatments was significantly different from each other (ANOVA $F = 1.854$, $df = 5, 54$ $P = 0.122$). This allowed comparisons between experimental treatments to be made. The addition of *Artemia*-conditioned water (smell) caused a significant, and rapid, increase in rate ($t = 2.79$ $df = 18$, $P = 0.0016$) (Figures 1, 2, 3). This elevation in rate was maintained for approximately 15 min, before beginning to decline. All the *Melibe* tested ($n = 10$) became more active and oriented their oral hood toward the source of the stimulus. The majority (70%) of the animals tested also began to swim shortly after application of the stimulus. However, this swimming activity was transient, lasting 2–7 min.

The addition of a tactile stimulus (Sephadex beads, particles) also caused a significant increase in hood closure rate ($t = 3.318$, $df = 18$, $P = 0.006$) (Figures 1, 2, 3). As observed with the olfactory stimulus, animals oriented with their oral veil facing the source of the particles, and in 30% of the cases they swam for periods of time ranging from 1 to 9 min. In contrast to their response to the olfactory stimulus, a high hood closure rate was maintained throughout the observational period.

There was no significant difference between the effectiveness of odor and particles as feeding stimulants, although the rate obtained in the tactile treatment was slightly higher (Figures 1, 2). Both treatments resulted in increases in hood closure rate that were approximately one-third as great as those obtained when live food was present (Figure 2). In order to try and mimic the stimuli present when real prey is available, we exposed animals to smell and particles together, particles soaked in *Artemia*-conditioned water, or frozen *Artemia*. These treatments did elicit greater responses than either stimulus applied alone, but there was no significant difference between the soaked particle, frozen *Artemia*, or inert particle treatment (Figure 2). Moreover, none of these treatments was nearly as effective as live prey. Thus, both tactile and chemical cues are sufficient to elicit an increase in the rate of feeding movements of *Melibe leonina*, but some additional stimulus provided by live prey, such as vibrations, probably plays an important role as well.

In order to determine if olfactory and tactile stimuli had additive influences on feeding movements we examined the effects of adding them sequentially. Both treatments, by themselves, resulted in a rapid increase in hood closure rate (Figure 3). However, the addition of a second, different stimulus, 20 min after the initial stimulus, did not result in any further increase in the hood closure rate of the animals tested. In contrast, addition of live prey to the observation chamber, produced a significant increase in rate. The findings of this experiment reinforce the hypothesis that live prey provide an additional feeding stimulus which excites *Melibe* feeding activity more than any combination of smell or inert particles.

WATSON & TRIMARCHI (1992) proposed that the feeding motor program of *Melibe* consists of a central pattern generator which is modulated and regulated throughout the feeding cycle by sensory input. They noted that animals

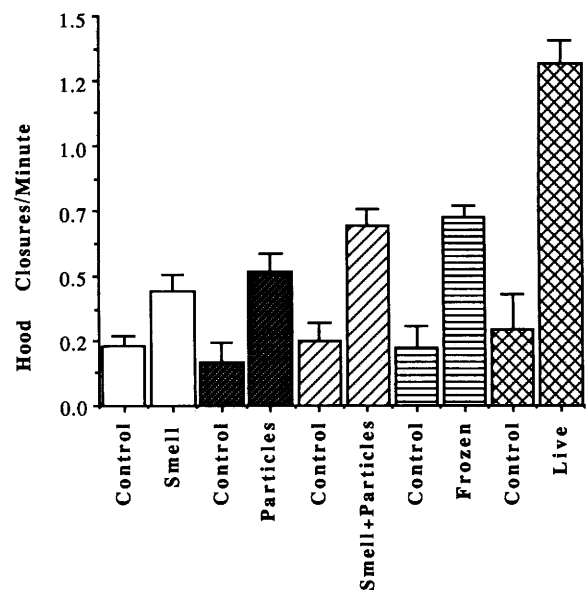


Figure 1

The influence of olfactory and tactile stimuli on the feeding frequency of *Melibe*. Five separate experiments are depicted in this figure, each with separate controls. In the first experiment animals were exposed to *Artemia*-conditioned water (smell), in the second Sephadex beads (particles), the third particles soaked in *Artemia*-conditioned water, the fourth frozen *Artemia*, and in the final experiment animals were exposed to live *Artemia*. In all cases we observed a significant increase in their rate of hood closures following the addition of one of the stimulants. Bars represent standard error of measurement.

often prematurely terminate feeding cycles if food is not present. We also observed this phenomenon in our experiments. When animals were stimulated to feed with an olfactory stimulus they increased their hood closure rate, but they rarely completed a feeding cycle; more than 70% of the feeding cycles they initiated ended before reaching the final consumption phase (Figure 4). This proportion of incomplete episodes was comparable to that observed in controls; however, the feeding movements of control animals are much less frequent and regular. In contrast, all the treatments that provided something to consume, whether inert or otherwise, resulted in a high proportion of complete feeding cycles, and consumption of the objects. As in our other experiments, live prey were the most effective stimuli. Therefore, it appears as if the type of stimuli present influence both the rate of food capture and the sequential expression of movements associated with food acquisition and consumption.

Adult *Melibe* are normally found in eelgrass beds or kelp forests, where they feed on epifaunal crustaceans, or planktonic crustaceans such as copepods and nauplii. Like most gastropods their vision is limited and therefore they must rely heavily on olfaction and mechanoreception to locate food and discriminate appropriate prey from other objects. Our laboratory studies indicate that *Melibe* is sim-

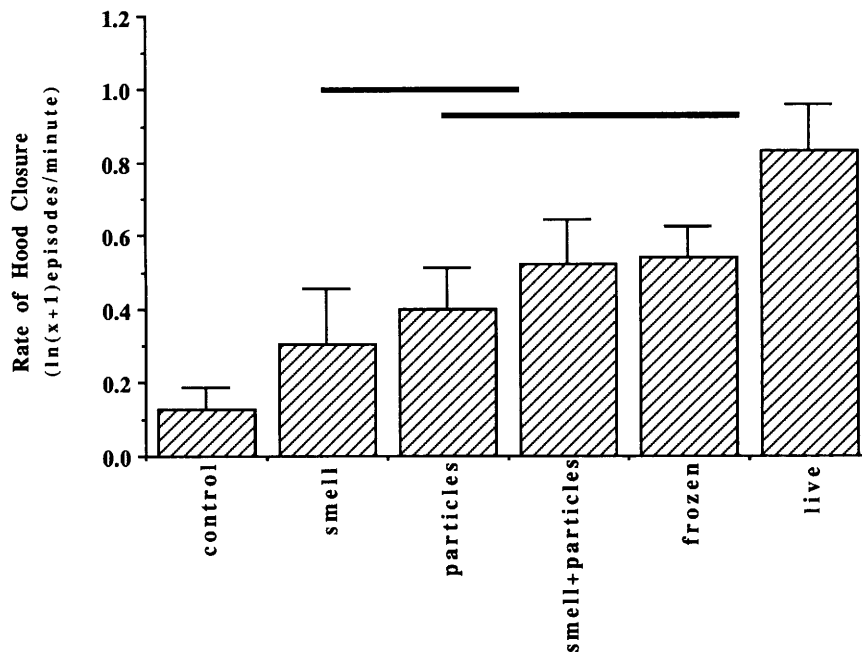


Figure 2

The relative potency of various feeding stimulants. Overall, the treatments were significantly different from each other (ANOVA $F = 44.133$, $df: 5, 54$, $P > 0.0001$). Horizontal lines indicate treatments that are not statistically significant from each other, using the Student-Newman-Keul's multiple comparison test. Both olfactory and tactile stimuli enhance feeding frequency to a limited extent, but neither stimulus alone, or when combined with each other (smell and particles, frozen *Artemia*), are as effective as live animals. The data were transformed using the natural log of $(x + 1)$. Bars represent standard error of measurement.

ilar to other gastropods in their use of chemoreceptors to initiate appetitive aspects of feeding such as changes in locomotion and orientation toward the source of food (KUPFERMANN, 1974; CROLL, 1983; AUDESIRK & AUDESIRK, 1985; TEYKE *et al.*, 1992). It has been suggested that chemical stimuli serve primarily to evoke a food-induced state of arousal in *Aplysia* (KUPFERMANN *et al.*, 1991), and it may serve a similar role in *Melibe* as well. Chemical stimuli cause *Melibe* to change their rate of locomotion, orient toward the source of the stimuli, and increase their rate of feeding movements. However, it appears as if they are merely sampling the water, not feeding, because they do not carry out complete feeding cycles. This increase in the frequency of hood movements may also serve to enhance the ability of putative chemoreceptors on the oral veil to detect prey; comparable to antennule flicking in many crustaceans. Then, once preylike objects make contact with the oral hood, they are captured and brought in contact with the mouth, and normal feeding behavior is initiated.

Most opisthobranchs are well endowed with chemo- and mechanoreceptors (CROLL, 1983) and it has been postulated that these two groups of receptors converge on neurons which regulate different aspects of feeding (ROSEN *et al.*, 1982). Evidence from *Tritonia* also indicates that some mechanoreceptors receive direct input from chemorecep-

tors which modulates their responsiveness (AUDESIRK & AUDESIRK, 1980a), as well as input from some aspect of the swim circuit (AUDESIRK & AUDESIRK, 1980b). Thus a certain amount of integration and discrimination appears to take place very early in the circuit which links sensory input to the feeding circuit, and as a result the presence of certain odors can have an important impact on the responsiveness of the animal to tactile stimulation. This appears to be the case in *Aplysia* (ROSEN *et al.*, 1982) and certain other gastropods, where the biting response to mechanical stimuli is limited unless a chemical cue is also present. In some cnidarians chemical cues actually alter the tuning properties of mechanoreceptors involved with prey capture. WATSON & HESSINGER (1989) found that the receptors controlling the discharge of sea anemone nematocysts are activated by 30–75 Hz vibrations and the chemical cues associated with prey modulate these receptors so they shift their sensitivity to a range of 5–40 Hz, which precisely matches the swimming movements of their prey (they also used *Artemia* in their study). In our experiments it was clear that live *Artemia* stimulated feeding much more effectively than any combination of odor and touch. We are presently searching for receptors in *Melibe* that are most sensitive to the vibrations produced by swimming prey. The possibility that these receptors are also

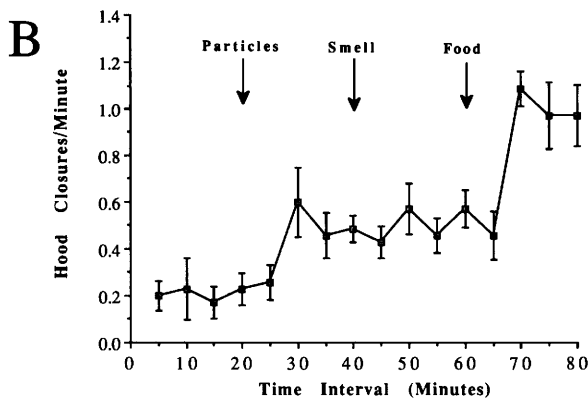
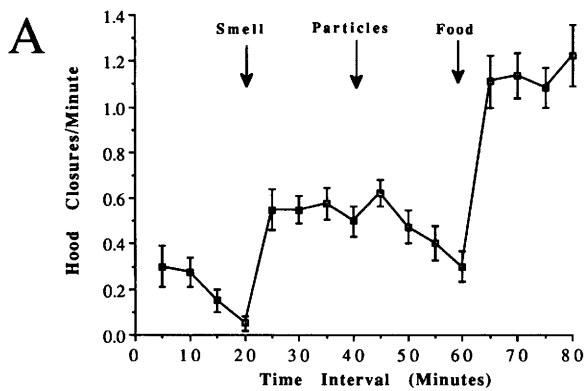


Figure 3

The response of *Melibe* to sequential addition of olfactory and tactile stimulants. A. After 20 min in filtered seawater, conditioned water (smell) was added to the observation tank, resulting in a rapid increase in feeding frequency, which was maintained for 20 min. Addition of particles did not cause any further increase in feeding rate during the next 20 min. However, live prey (food) had a much greater effect on feeding than the combination of particles and food odor. B. This experiment was similar to the one described in A, except particles were added first, followed by smell, and then live prey (food). As in A, addition of a second feeding stimulant did not cause any additional increase in feeding rate, while live prey did. Bars represent standard error of measurement.

modulated by the odor of prey, or the behavioral state of the animals, is also a subject worthy of further investigation.

The stereotyped, rhythmic movements involved in *Melibe* feeding behavior have characteristics typical of fixed action patterns that are under the control of a central pattern generator or motor program (AJESKA & NYBAKKEN, 1976; WATSON & TRIMARCHI, 1992). This motor program is expressed at a very low frequency (0.2 cycles/min) even in the absence of prey, and when it senses prey, through a combination of the cues discussed in this paper, the

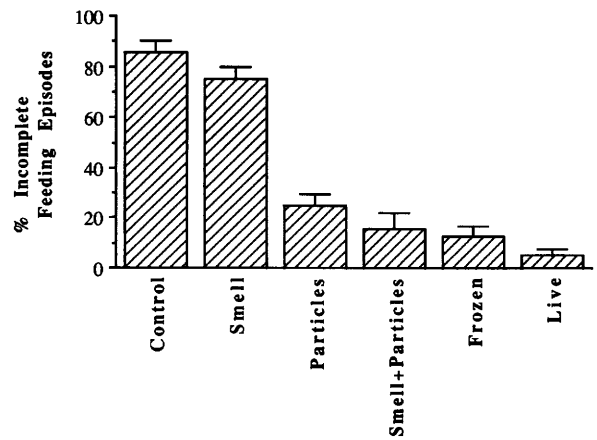


Figure 4

The influence of feeding stimulants on the sequential expression of *Melibe* feeding movements. The typical *Melibe* feeding cycle consists of a series of movements designed to capture prey and bring them in close proximity to the mouth for consumption. If prey are sparse or absent, animals often terminate a feeding cycle before the tilt and squeeze phase of the cycle, which brings food to the mouth. This figure shows the proportion of such prematurely terminated feeding cycles during different treatments. It is clear that while the odor of prey stimulates feeding activity (Figures 1, 2), the type of feeding movements displayed are seldom complete. In contrast, when any type of particle is present, animals usually attempt to engulf the objects they capture, resulting in complete feeding cycles.

cycling rate of the motor program increases. In addition, the quality and quantity of sensory input appear to influence the full expression or completeness of the feeding cycle. If only the odor of food is present, animals become aroused and sample the water column for food, often terminating their feeding cycle prior to making the movements that normally bring prey in close proximity to the mouth. However, if particles are present, or live prey, most feeding cycles are complete. We hypothesize that *Melibe* feeding behavior consists of a series of flexible motor programs that are centrally programmed, triggered by sensory input, and modulated by sensory feedback throughout the feeding cycle. This hypothesis is derived, in part, from an emerging view of central pattern generators as broader more flexible *motor pattern networks*, which combine elements of traditional motor programs with a high level of sensory modulation (HARRIS-WARRICK & JOHNSON, 1989). Our present studies are designed to test this hypothesis and examine this relatively new view of "stereotyped" behavior.

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