

NOTE

Effects of phytoplankton taste and smell on feeding behavior of the copepod *Centropages hamatus**

Kathryn L. Van Alstyne**

Woods Hole Oceanographic Institution, Woods Hole, Massachusetts 02543, USA

ABSTRACT: Adult copepods *Centropages hamatus* were induced to feed on 10 to 40 μm Sephadex beads by adding *Thalassiosira weissflogii* or *Scrippsiella trochoidea* whole-cell extract, or filtrate from a *T. weissflogii* culture, to a bead suspension. Beads were neither ingested in the absence of a chemical stimulus, nor in the presence of filtrates from cultures of *S. trochoidea* and *Olisthodiscus luteus*, or *O. luteus* extracts. Extracts from both *S. trochoidea* and *O. luteus*, and filtrate from an *O. luteus* culture, appear to exert an inhibitory effect when applied in combination with *T. weissflogii* extract. Copepods acclimated to *T. weissflogii* for 24 h did not significantly increase their feeding rate on *T. weissflogii*-flavored beads and did not ingest *S. trochoidea*-flavored beads.

Food choice by herbivorous copepods can be influenced by the size, shape and chemical composition of potential prey. Earlier studies of food choice by copepods focused on the effects of phytoplankton size because copepods were thought to be passive filter feeders and limited in their ability to actively select food items (e.g. Boyd 1976, Nival & Nival 1976, Frost 1977). However, since Alcaez et al. (1980) and Koehl & Strickler (1981) have shown that copepods actively select their prey, more studies have focused on the role of chemical stimuli produced by the prey in determining food choice by copepods (e.g. Donaghay & Small 1979, Huntley et al. 1983). Huntley et al. (1986) have shown that *Calanus pacificus* and *Paracalanus parvus* reject particular dinoflagellate species and that in one case the rejection is based upon the presence of exudates released by the alga.

Here, I show that feeding behavior in the herbivorous copepod *Centropages hamatus* can be stimulated by *Thalassiosira weissflogii* whole cell homogenates or

by filtrates taken from cultures of these cells. I further document that the presence of homogenates or filtrates of 2 other phytoplankton species, *Scrippsiella trochoidea* and *Olisthodiscus luteus*, can inhibit feeding in these copepods.

Material and Methods. *Thalassiosira weissflogii* (Bacillariophyceae), *Scrippsiella trochoidea* (Dinophyceae) and *Olisthodiscus luteus* (Xanthophyceae) were grown in unialgal cultures in f/2 culture medium (based on Guillard's F₁ medium from Guillard & Ryther 1962). Whole-cell extracts were made by centrifuging 13 mm³ of cells taken from cultures exhibiting exponential growth. They were put into 10 ml of filtered seawater (henceforth meaning seawater that was filtered through a 0.8 μm filter) and sonicated for three 30 s intervals, cooling the cells for intervals of 3 to 5 min after sonicating. The extract was then centrifuged at 5000 rpm for 15 min. The supernatant was poured off, filtered through a 0.8 μm filter and brought up to a volume of 25 ml with filtered seawater. Exudates from phytoplankton cultures were collected by vacuum filtering cells from the medium in which they were growing using a 0.8 μm filter at pressure differentials of no more than 5 mm Hg.

Adult *Centropages hamatus* were collected from the Woods Hole Oceanographic Institution dock in Woods Hole, Massachusetts (USA) in July and August of 1983. Unless otherwise specified, the collected copepods were starved in filtered seawater for 6 to 8 h prior to the start of each experiment. The term 'acclimated copepods' refers to copepods fed *Thalassiosira weissflogii* for at least 24 h prior to the experiment.

Each experiment consisted of 3 replicate bottles. Each bottle contained 400 ml of filtered seawater, 20 copepods, and an initial bead concentration of about 500 Sephadex G-50 Superfine (10 to 40 μm) beads ml⁻¹. The beads had been rehydrated in filtered sea-

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** Present address: Department of Zoology NJ-15, University of Washington, Seattle, Washington 98195, USA

water for 1 h prior to the start of the experiment. To each set of bottles a total of 2 ml of phytoplankton extract or filtrate from a phytoplankton culture was added singly or in combinations of 1 ml of each to determine the effects of these substances on copepod feeding behavior. Additions of 2 ml of filtered seawater or *f/2* culture medium were used as controls. Initial bead concentrations were determined by averaging Coulter counter measurements from 12 to 15 replicate samples from each bottle. Copepods were allowed to feed for 1 h while the bottles were rotated at 1 to 2 rev min^{-1} to keep the beads suspended. Limiting the feeding experiments to 1 h ensured that there was insufficient time for the beads to pass through the guts of the copepods and then become resuspended in the seawater. Copepods were subsequently removed from the bottles and final bead concentrations were measured in 15 replicate bead samples from each bottle.

Differences in initial and final concentrations of beads were compared using a 2-way ANOVA without replication in which differences in initial bead concentrations were considered random effects and changes over time were examined. Single-classification ANOVA's were used to compare differences in ingestion rates. Multiple comparisons of mean ingestion rates were made using the T-method for unplanned comparisons (Sokal & Rohlf 1981).

Results. There was no significant change over time in initial and final bead concentrations (based on the time component of a 2-way ANOVA; $p < 0.05$) when filtered seawater alone was added to the bottles (Fig. 1), indicating that *Centropages hamatus* would not ingest Sephadex beads in the absence of a chemical feeding stimulus. Likewise, addition of *f/2* culture medium did not stimulate feeding. When *Thalassiosira weissflogii* and *Scrippsiella trochoidea* extracts or *T. weissflogii* medium were added there was a significant decrease in bead concentration ($p < 0.025$). Ingestion of beads flavored with *T. weissflogii* extract was significantly higher ($p < 0.05$; T-method) than with *S. trochoidea*-flavored beads. There was no difference ($p > 0.75$) between ingestion rates when *T. weissflogii* extract and *T. weissflogii* medium were added. Ingestion rates were also not significantly different in the presence of 1 rather than 2 ml of *T. weissflogii* extract. And, there was no significant ($p > 0.05$) change in bead concentrations when either *S. trochoidea* medium or *Olisthodiscus luteus* extract were added. Copepods acclimated to live *T. weissflogii* for 1 d prior to the experiments did not significantly increase ($p > 0.05$) their consumption of beads flavored with *T. weissflogii* extract relative to copepods that had not been acclimated to *T. weissflogii* (Fig. 2). However, acclimated copepods did not ingest beads flavored with *S. trochoidea* extract.

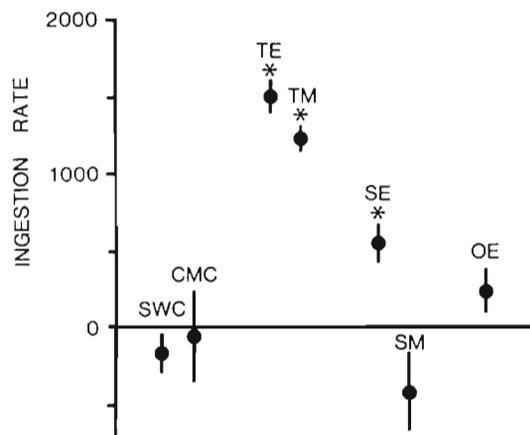


Fig. 1. *Centropages hamatus*. Mean ingestion rates of Sephadex beads (beads copepod⁻¹ h⁻¹) when phytoplankton extracts and exudates are added to the bead suspension. Vertical lines: ± 1 SE; asterisks: additives causing significant ($p < 0.05$) changes in bead concentration. SWC: seawater control; CMC: *f/2* culture medium control; TE: *Thalassiosira weissflogii* extract; TM: *T. weissflogii* medium; SE: *Scrippsiella trochoidea* extract; SM: *S. trochoidea* medium; OE: *Olisthodiscus luteus* extract

In order to determine whether any of these phytoplankton extracts and exudates might have an inhibitory effect on feeding behavior, *Scrippsiella trochoidea* and *Olisthodiscus luteus* extracts and media were added in combination with *Thalassiosira weissflogii* extract. Addition of *S. trochoidea* extract with *T. weissflogii* extract resulted in significantly lower ingestion rates ($p < 0.05$ using the T-method) than when either substance was added separately (Table 1). Addition of *O. luteus* extract in combination with *T. weissflogii* extract resulted in ingestion rates intermediate be-

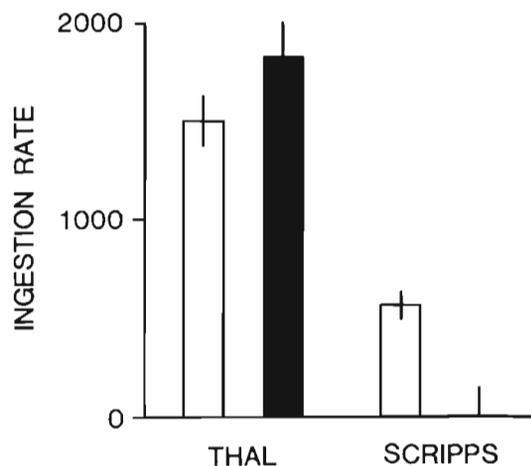


Fig. 2. *Centropages hamatus*. Mean ingestion rates (beads copepod⁻¹ h⁻¹) of Sephadex beads flavored by *Thalassiosira weissflogii* and *Scrippsiella trochoidea*. Solid bars: acclimated copepods; open bars: non-acclimated copepods; vertical lines: ± 1 SE

tween the ingestion rates obtained when either was added separately. Mean ingestion rates in the presence of *O. luteus* medium and *T. weissflogii* extract were 43 % lower than those obtained when only *T. weissflogii* extract was added; however, due to high variance in ingestion rates these numbers were not significantly different.

Discussion. The responses by *Centropages hamatus* to the exudates and extracts of the 3 phytoplankton species correspond well with preferences shown by other copepod species. Digestive enzyme activity in *Calanus pacificus* drops when fed *Scrippsiella trochoidea* over a 24 d period (Hassett 1986). When fed *Thalassiosira weissflogii*, cellobiose activity drops but laminarase and maltase activity remain relatively constant. Hassett (1986) also showed that *C. pacificus* had a much lower survival rate when fed *S. trochoidea* than when fed *T. weissflogii*. And, *Olisthodiscus luteus* is known to be a less preferred food item for *Acartia* spp. (Tomas & Deason 1981). Thus, *S. trochoidea* and *O. luteus* tend to be less preferred food items because of their chemical composition rather than because of their size or morphological features.

The negative responses of *Centropages hamatus* to *Scrippsiella trochoidea* and *Olisthodiscus luteus* are similar to the responses obtained by Huntley et al. (1986). They found that exudates but not homogenates of *Protoceratium reticulatum* inhibit feeding by *Calanus pacificus* on *Gyrodinium resplendens*. Many phytoplankters are known to produce toxins (Carmichael & Gorham 1977, Schmidt & Loeblich 1979, White 1980) or structures such as trichocysts (Ukeles & Sweeney 1969) which could deter feeding by herbivorous zooplankton. Many other substances, which might serve as feeding cues, are released by marine algae. These include both simple and complex polysaccharides, sugar alcohols, amino acids, small peptides and larger proteins (Hellebust 1965, 1967). Chemo-receptor-like structures have been found on the heads and mouths of various copepod species (e.g. Elofsson 1971, Friedman & Strickler 1975). However, it is not

known at this time what kind of stimuli these receptors will respond to. Similar structures in decapod crustaceans serve as receptors for amino acids and simple sugars (Hartman & Hartman 1977).

Responses to filtrates are probably more ecologically relevant than responses to extracts because copepods in nature are more likely to encounter exudates from whole cells rather than products coming from broken cells. These allelopathic substances that are released by phytoplankton are probably produced as feeding deterrents, however they might also be waste products of cell metabolism (Lewis 1986). Andrews (1983) has modelled the distribution of exudates being released from a single phytoplankton cell in the presence of a feeding current based on empirically derived values obtained from films of feeding copepods. High concentrations of phytoplankton exudates may be found within dense localized blooms of a particular phytoplankton species. For a single-celled organism such as a phytoplankter, being bad-smelling would be more advantageous than being bad-tasting. A predator would have to kill a cell in order to learn that it is bad-tasting, whereas it could recognize and avoid a bad-smelling prey without harming it. However, producing a scent is energetically more expensive because the warning substances must be continuously produced and released. Being bad-tasting could be selectively advantageous if genetically identical clones would benefit from a predator's exposure to such cells. Such could be the case for asexually reproducing phytoplankters that are not rapidly dispersed. However, it is probably more likely that any bad-tasting compounds in these cells are simply being stored and then later released to dissuade potential predators.

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Table 1. *Centropages hamatus*. Response to phytoplankton extracts and exudates. Initial and final concentrations of 10 to 40 μm Sephadex beads (beads ml^{-1}) and ingestion rates (beads copepod $^{-1}$ h $^{-1}$) are given as means \pm the standard error of the mean

Substances added	Initial concentration	Final concentration	Ingestion rate
<i>Thalassiosira weissflogii</i> extract (1 ml of extract)	495 \pm 23	438 \pm 21	1150 \pm 77*
<i>Scrippsiella trochoidea</i> extract & <i>T. weissflogii</i> extract	560 \pm 33	557 \pm 9	67 \pm 64
<i>S. trochoidea</i> extract	634 \pm 18	605 \pm 16	567 \pm 82*
<i>Olisthodiscus luteus</i> extract & <i>T. weissflogii</i> extract	511 \pm 21	474 \pm 18	740 \pm 110*
<i>O. luteus</i> extract	593 \pm 16	585 \pm 19	253 \pm 85
<i>O. luteus</i> medium & <i>T. weissflogii</i> extract	577 \pm 7	541 \pm 23	720 \pm 359

* Ingestion rates significantly ($p < 0.05$) greater than zero using a t-test

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