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Evaluation of the copepod *Tigriopus californicus* as a bioassay organism for the detection of chemical feeding deterrents produced by marine phytoplankton

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Abstract Marine phytoplankton have been shown to use chemical feeding deterrents to reduce or inhibit zooplankton grazing. In order to screen phytoplankton species for feeding deterrent production and to isolate and identify feeding deterrent compounds, a new, rapid, and reliable laboratory bioassay was developed. This bioassay used the laboratory-reared harpacticoid copepod *Tigriopus californicus* and measured inhibition of feeding by measuring the fecal pellet production rate. The bioassay was capable of detecting deterrent compounds: (1) adsorbed onto ground fish food (a normally palatable food); (2) dissolved in a mixture of seawater and live *Thalassiosira pseudonana* cells (a species of diatom which had no feeding deterrent activity); and (3) present in live cell cultures. Method (2) was recommended for use in bioassay-guided fractionation (isolation of chemical compounds), as it was reliable, rapid, accurate, and easy to perform with large numbers of samples. The total bioassay time was < 48 h, and data collection required only a microscope. Methanolic cell extracts of several phytoplankton species were screened for feeding deterrent activity. Extracts from the diatom *Phaeodactylum tricornerutum* and the dinoflagellate *Gonyaulax grindleyi* gave feeding deterrent responses, while extracts from the diatom *Thalassiosira pseudonana* gave no feeding deterrent responses. Live *P. tricornerutum* cells deterred feeding at densities of 6×10^5 cells ml⁻¹. This bioassay should provide a valuable tool in screening phytoplankton for feeding deterrent compounds and determining the chemical nature of these compounds.

Introduction

Previous work has shown that marine phytoplankton produce compounds which reduce or inhibit the grazing of

various herbivorous zooplankton. These investigations range from field observations to laboratory feeding studies and have focused on the behavior of copepods feeding on a number of phytoplankton species. In the field, the calanoid copepods *Calanus pacificus* collected from a dinoflagellate bloom of *Gymnodinium flavum* had emptier guts than those caught outside the bloom (Huntley 1982). In laboratory experiments, *C. pacificus* preferred a smaller diatom, *Thalassiosira weissflogii* ($\approx 17 \mu\text{m}$), over *G. flavum* ($\approx 35 \mu\text{m}$). To determine if copepod feeding rates varied with dinoflagellate species, a number of laboratory experiments were conducted using *C. pacificus* (Huntley et al. 1986). The feeding rates of *C. pacificus* on various dinoflagellates were compared to the feeding rates on plastic beads of similar size. The dinoflagellates *Alexandrium tamarense*, *Gonyaulax grindleyi*, *Gymnodinium breve* and *Scrippsiella trochoidea* were consumed at rates lower than plastic beads of the same size. Furthermore, filtrate from exponentially growing *G. grindleyi* caused a decrease in feeding upon the normally palatable *Gyrodinium resplendens*, while the cell homogenate of *G. grindleyi* did not alter the filtration rate. This suggested that *G. grindleyi* was producing and secreting a compound which inhibited feeding. Filtrates of the dinoflagellate *Gymnodinium nagasakiense* and the raphidophyte *Heterosigma carterae* contained deterrent compounds which decreased the feeding rates of the inshore marine copepods *Pseudodiaptomus marinus* and *Acartia omorii* (Uye and Takamatsu 1990). Based on these observations, Huntley et al. (1986) proposed that the production of feeding deterrents allowed slow growing species (e.g. dinoflagellates) to attain bloom concentrations.

The feeding deterrents produced by some dinoflagellates caused marked behavioral changes in *Calanus pacificus*. In order to determine the mechanisms by which feeding was inhibited, a video system was used to observe and record the behavior of restrained copepods when presented with dinoflagellates producing feeding deterrents (Sykes and Huntley 1987). Copepods presented with *Gonyaulax grindleyi* failed to maintain full guts and often regurgitated after 45 to 120 min. In the presence of *Gymnodinium breve*,

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copepods exhibited rapid heart rate and loss of motor control. Copepods fed *Scrippsiella trochoidea* occasionally displayed mouth part twitching or failure to maintain gut fullness. Thus, these feeding deterrents appeared to work by reducing the efficiency with which the copepod could filter and absorb food particles.

Work has also been done on the effects of feeding deterrents on other zooplankton species. Tintinnids (Verity and Stoecker 1982), marine rotifers (Chotiyaputta and Hirayama 1978; Egloff 1986), and bivalves (Ali 1970; Ward and Targett 1989) have all been shown to have decreased feeding rates in the presence of certain phytoplankton.

Feeding deterrents have both commercial and ecological significance. A number of phytoplankton species are used in mariculture. Production of feeding deterrents by such species may decrease growth rates or increase mortality in mariculture species (e.g. oysters). Identification of deterrent producing species will improve mariculture practices. In the natural environment, production of feeding deterrents may control grazing and determine which species will bloom and how long the bloom will persist.

Although certain phytoplankton produce feeding deterrents, very little research has been done on the isolation and structural characterization of these compounds. Bioassay-guided chemical fractionation of extracts from deterrent producing phytoplankton provides a means to isolate deterrent compounds. Therefore, there is a need for a simple, rapid, cost-efficient, reliable, and accurate bioassay, as many samples must be bioassayed in order to isolate a single compound.

The present paper describes a new bioassay for feeding deterrents which fulfills the above criteria. The bioassay used *Tigriopus californicus*, an easily cultured, extremely robust harpacticoid copepod (Burton et al. 1979; Dethier 1980). Feeding rate, measured by fecal pellet production, was used to assess sub-lethal responses to feeding deterrents. The bioassay was evaluated using a known producer of a feeding deterrent, *Gonyaulax grindleyi* (Huntley et al. 1986), and a suspected producer of a feeding deterrent, *Phaeodactylum tricornerutum* (Epifanio et al. 1981).

Materials and methods

Phytoplankton cultures

Cultures of the diatoms *Thalassiosira pseudonana* and *Phaeodactylum tricornerutum*, and the dinoflagellate *Gonyaulax grindleyi* were grown and harvested to yield cellular extracts suitable for testing in the feeding deterrent bioassay. Unialgal cultures of *P. tricornerutum* Bohlin (NEPCC #640), *T. pseudonana* (Hustedt) Hasle and Heimdal clone 3H (NEPCC #58), and *G. grindleyi* (NEPCC #535) were obtained from the Northeast Pacific Culture Collection (NEPCC), Department of Oceanography, University of British Columbia. Natural seawater (salinity $\approx 28\%$) was collected from West Vancouver, British Columbia at a site 100 m from shore and 15 m depth. Seawater used for culturing was filtered through activated charcoal to remove organics (Craigie and McLachlan 1964) and then filtered through a 0.8- μm Millipore filter to remove particulates. The seawater was sterilized by autoclaving. All cultures were grown using full enriched natural seawater (ES) (Harrison et al. 1980) at 19 °C under continuous irradiance ($\approx 70 \mu\text{mol m}^{-2} \text{s}^{-1}$) with stirring at ≈ 60 rpm.

Culture growth was measured by in vitro fluorescence. Cell densities of *Thalassiosira pseudonana*, *Phaeodactylum tricornerutum*, and *Gonyaulax grindleyi* were determined using either a Coulter Counter (model TAIL) or an inverted compound microscope (400 \times power).

Cultures were harvested in late log or early senescence phase. Phytoplankton cells were collected by a series of gentle filtration (< 50 mm Hg) techniques. Initially, the cultures were filtered through a 30- μm (nominal size) Nitex screen to remove large cell clumps, followed by filtration through a 5- μm (nominal size) Nitex screen. Cells were rinsed from both screens with methanol and collected in a grinding tube. The filtrate which passed through the screens was then filtered through a GF/F glass-fiber filter, and this filter was added to the grinding tube. Cellular extracts of the phytoplankton cells were obtained by grinding the harvested cells in methanol and rinsing the cell debris repeatedly in methanol until all pigments were removed. The methanol was then filtered through a GF/F glass-fiber filter to remove remaining cell debris. The methanolic extract underwent rotary evaporation to yield a dry solid which was used in the bioassay procedures.

Copepod cultures

A culture of the harpacticoid copepod *Tigriopus californicus* was maintained to provide a continuous supply of organisms for the bioassay. *T. californicus* (Fig. 1) was originally isolated from splash pools on the West Coast of Vancouver Island by Dr. A. G. Lewis, University of British Columbia, in 1966 (Sullivan and Bisalputra 1980). Copepods used for the bioassay were maintained in filtered natural seawater in 1-litre Pyrex flasks at 18 °C at an irradiance of $\approx 100 \mu\text{mol m}^{-2} \text{s}^{-1}$ and a 18 h light:6 h dark cycle. The copepods were fed either a diet of ground fish food (Wardley's Basic Fish Food for Tropical Fish) or a diet of the diatom *Thalassiosira pseudonana*. To reduce variability in the bioassay due to differences in the sex or life stage of the copepods, only the adult (C6) male copepods were used.

Copepods with reduced bacterial contaminants were prepared by soaking the copepods in a solution of 800 mg penicillin-G, 180 mg dihydrostreptomycin, 8 mg chloramphenicol, and 20 mg dextrose in 100 ml of filtered, autoclaved natural seawater for 24 h (adapted from Wootton 1989). Before being used in a bioassay, antibiotic-treated copepods were placed in autoclaved seawater without food for 24 h, while untreated copepods were placed in autoclaved seawater without food for 48 h.

Differences in grazing rates were determined by measuring fecal pellet production. The water in which the copepods were incubated was quantitatively transferred to a 10-ml cylindrical settling chamber, the contents were settled for 30 min, and fecal pellets were counted at low power (100 \times) using an inverted compound microscope.

Egestion rate experiments

Using egestion as an index of feeding, an experiment was designed to determine the feeding response of *Tigriopus californicus* to *Thalassiosira pseudonana* cells and to determine the concentration of *T. pseudonana* cells required to saturate the egestion rate of *T. californicus*. Preliminary experiments demonstrated that fecal pellet production was constant over a period of 100 h. Microscopic examination of the fecal pellets showed no signs of coprophagy or fecal pellet degradation over that period. Feeding experiments using heat-killed, fluorescently stained *T. pseudonana* cells (Sherr et al. 1991) indicated that the first stained fecal pellet was produced within 30 min of the addition of the food source and that fecal pellet production had reached a constant rate within 4 h. Thus a convenient incubation period of 24 h was selected, as this time was long enough to ensure a constant rate of pellet production.

Various concentrations of *Thalassiosira pseudonana* cells were placed in the wells (volume ≈ 15 ml) of a tissue culture plate and two male C6 copepods were added to each well. The copepods were incubated for 24 h at 18 °C at an irradiance of $\approx 100 \mu\text{mol m}^{-2} \text{s}^{-1}$ and

a 18 h light:6 h dark cycle. The fecal pellet production rate of the copepods was then measured. To determine if treatment of copepods with antibiotics had any effects, this experiment was repeated twice, once with untreated copepods and once with antibiotic-treated copepods. The change in fecal pellet production rate with food concentration was fit to the equation:

$$F = (F_{\max} \times C) / (K_c + C), \quad (1)$$

where F = the fecal pellet production rate (fecal pellets h^{-1} copepod $^{-1}$), F_{\max} = the maximum fecal pellet production rate, C = the concentration of *T. pseudonana* cells, and K_c = the concentration of *T. pseudonana* cells required to reach the half-saturation fecal pellet production rate.

Bioassay method using feeding deterrents adsorbed onto ground fish food

Extracts from phytoplankton cells were adsorbed onto ground fish food (Wardley's Basic Fish Food for Tropical Fish), and this treated fish food was presented as a food source to copepods to determine if the presence of a feeding deterrent on the surface of a normally palatable food particle would produce a decrease in feeding. Fish food particles are very lipophilic and were expected to behave in a manner similar to the reverse phase microparticles used by Ward and Targett (1989) to adsorb dissolved ectocrines. Four experimental treatments ($n=6$) were done: untreated copepods fed fish food with no adsorbed cell extract, antibiotic-treated copepods fed fish food with no adsorbed cell extract; untreated copepods fed fish food with adsorbed cell extract; and antibiotic-treated copepods fed fish food with adsorbed cell extract.

A weighed sample of the dry methanolic cell extract was dissolved in methanol and added to autoclaved ground fish food. The fish food was dried under vacuum to remove the methanol, then re-ground. The treated fish food contained 20 to 28% cellular extract by weight. 2 mg of this treated fish food was suspended in 10 ml of autoclaved seawater, the mixture was placed in a single well (volume ≈ 15 ml) of a tissue culture plate with one male C6 copepod and incubated for ≈ 65 to 75 h at 18°C at an irradiance of $\approx 100 \mu\text{mol m}^{-2} \text{s}^{-1}$ and a 18 h light:6 h dark cycle. Fecal pellets were settled and counted at the end of the bioassay.

Bioassay method using dissolved feeding deterrents

Extracts from phytoplankton cells were dissolved in seawater and mixed with live *Thalassiosira pseudonana* cells to determine if the presence of a dissolved feeding deterrent would produce a decrease in feeding by copepods on a normally palatable phytoplankton species. Two experimental treatments ($n=6$) were done: antibiotic-treated copepods fed *T. pseudonana* cells in the presence of dissolved cell extract from *Phaeodactylum tricornutum*; and antibiotic-treated copepods fed *T. pseudonana* cells in the presence of dissolved cell extract from *T. pseudonana*.

2 mg of the dried methanolic cell extract to be bioassayed was dissolved in 5 ml of autoclaved seawater and sonicated (1 min in bath sonicator at 60 Hz) to mix. If the extract to be tested was not soluble in seawater, it was dispersed using four drops of dimethylsulfoxide (DMSO). When DMSO was used to dissolve the extract, it was also added to the control.

5 ml of an exponentially growing culture of *Thalassiosira pseudonana* was added to the dissolved cell extract. If the cell cultures were clumped, cell clumps were broken up using sonification (1 min in bath sonicator at 60 Hz) and filtration (forced through a 200- μm screen at a rate of $\approx 50 \text{ ml min}^{-1}$ using a syringe; repeated four times). Microscopic observations indicated that these procedures did not lyse the *T. pseudonana* cells. The final concentration of *T. pseudonana* cells was kept at or above the level required for saturation of the egestion rate of *Tigriopus californicus* ("Results"), so decreases in fecal pellet production would not be due to decreased food levels.

The mixture of dissolved cell extract and live *Thalassiosira pseudonana* cells was placed in one well (volume ≈ 15 ml) of a tissue cul-

ture plate and two antibiotic-treated male C6 copepods were added. The assay was incubated for ≈ 20 h at 18°C at an irradiance of $\approx 100 \mu\text{mol m}^{-2} \text{s}^{-1}$ and a 18 h light:6 h dark cycle. Fecal pellets were settled and counted at the end of the bioassay.

Bioassay method using feeding deterrents produced by live cells

To determine if live cells produced feeding deterrents in concentrations sufficient to inhibit feeding, copepods were presented with live cell cultures at a density of 6×10^5 cells ml^{-1} . Two experimental treatments ($n=5$) were done: antibiotic-treated copepods fed live *Phaeodactylum tricornutum* cells; and antibiotic-treated copepods fed live *Thalassiosira pseudonana* cells.

Cell clumps in 10 ml of exponentially growing *Phaeodactylum tricornutum* or *Thalassiosira pseudonana* cell culture were broken up using sonification (1 min in bath sonicator at 60 Hz) and filtration (forced through a 200- μm screen at a rate of $\approx 50 \text{ ml min}^{-1}$ using a syringe; repeated four times). The cell culture was placed in one well (volume ≈ 15 ml) of a tissue culture plate and two antibiotic-treated male C6 copepods were added. The assay was incubated for ≈ 20 h at 18°C at an irradiance of $\approx 100 \mu\text{mol m}^{-2} \text{s}^{-1}$ and a 18 h light:6 h dark cycle. Fecal pellets were settled and counted at the end of the bioassay.

Results

Egestion rate experiments

The egestion rate response of *Tigriopus californicus* to varied concentrations of *Thalassiosira pseudonana* cells is shown in Fig. 1. The data were analyzed using the Wilkinson method for estimating the parameters of a Michaelis-Menten type equation (Wilkinson 1961). Untreated copepods had a maximum fecal pellet production rate of 1.6 fecal pellets h^{-1} (SE=0.1) and a half-saturation constant of 2×10^4 cells ml^{-1} (SE= 1×10^4). Antibiotic treated copepods had a maximum fecal pellet production rate of 1.9 fecal pellets h^{-1} (SE=0.2) and a half-saturation constant of 2×10^4 cells ml^{-1} (SE= 1×10^4). Using a two-tailed *t*-test, the maximum fecal pellet production rates ($n=18$, $t=1.40$, $\alpha=0.09$) and the half-saturation constants ($n=18$, $t=0.21$, $\alpha=0.25$) of treated and untreated copepods were not significantly different. For both treatments, a cell density of greater than 1×10^5 cells ml^{-1} produced a saturated egestion rate response in *T. californicus*. Therefore, all bioassays using *T. pseudonana* were run with cell densities at or above this concentration.

Bioassay method using feeding deterrents adsorbed onto ground fish food

A cellular extract from *Gonyaulax grindleyi* was tested for feeding deterrents by adsorbing this extract onto ground fish food. The data from this experiment were arranged into four blocks (untreated copepods fed fish food with no adsorbed cell extract, antibiotic-treated copepods fed fish food with no adsorbed cell extract, untreated copepods fed fish food with adsorbed *G. grindleyi* cell extract, and antibiotic treated copepods fed fish food with adsorbed *G. grindleyi* cell extract) and analyzed using two-factor analysis

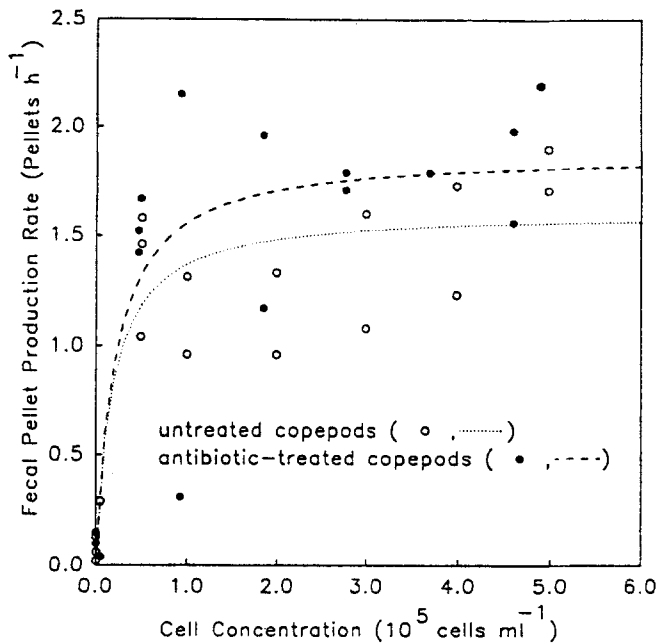


Fig. 1 *Tigriopus californicus*. Feeding response, as measured by egestion rate, for untreated and antibiotic-treated *T. californicus* feeding on *Thalassiosira pseudonana*

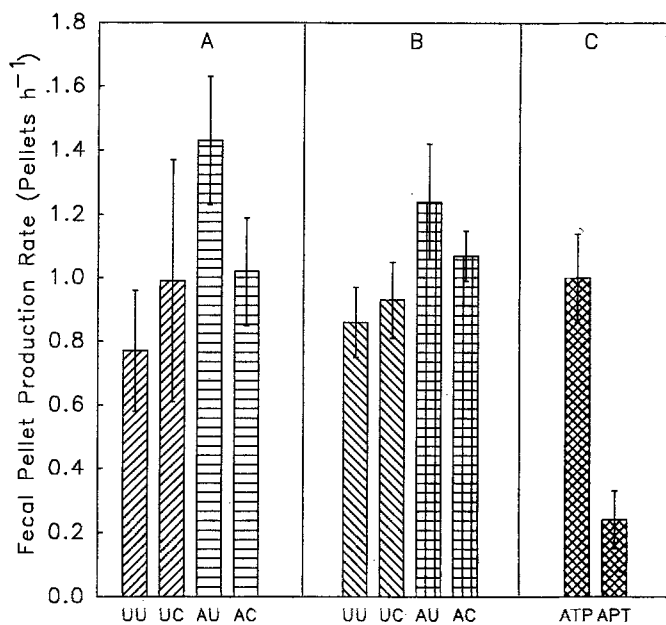


Fig. 2 *Tigriopus californicus*. Feeding response, as measured by egestion rate, of *T. californicus* to treatment of copepods with antibiotics and treatment of ground fish food with cellular extracts from three phytoplankton species. **A** (UU untreated copepods fed uncoated fish food; UC untreated copepods fed fish food coated with *Gonyaulax grindleyi* cellular extract; AU antibiotic-treated copepods fed uncoated fish food; AC antibiotic-treated copepods fed fish food coated with *G. grindleyi* cellular extract.) **B** (UU untreated copepods fed uncoated fish food; UC untreated copepods fed fish food coated with *Thalassiosira pseudonana* cellular extract; AU antibiotic-treated copepods fed uncoated fish food; AC antibiotic-treated copepods fed fish food coated with *T. pseudonana* cellular extract.) **C** (ATP antibiotic-treated copepods fed fish food coated with *T. pseudonana* cellular extract; APT antibiotic-treated copepods fed fish food coated with *Phaeodactylum tricoratum* cellular extract.) Error bars are $\pm 1 \sigma$ ($n=6$)

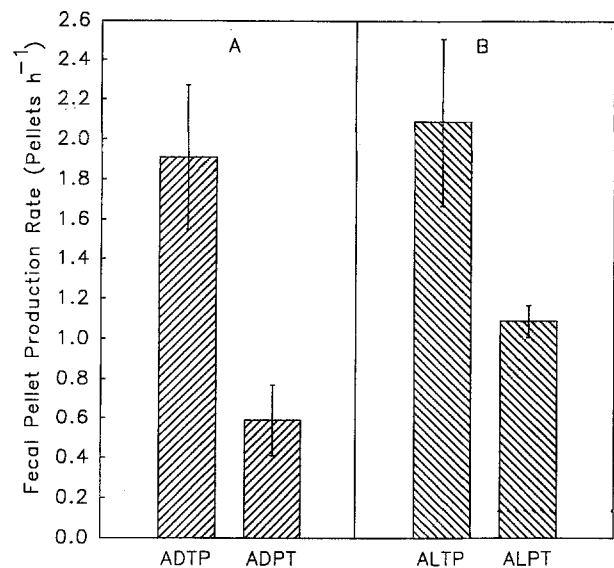


Fig. 3 *Tigriopus californicus*. Feeding response, as measured by egestion rate, of *T. californicus* when presented with a diet of live phytoplankton cells. **A** (ADTP antibiotic-treated copepods fed live *Thalassiosira pseudonana* cells suspended in dissolved cellular extract from *T. pseudonana*; ADPT antibiotic-treated copepods fed live *T. pseudonana* cells suspended in dissolved cellular extract from *Phaeodactylum tricoratum*). **B** (ALTP antibiotic-treated copepods fed live *T. pseudonana* cells; ALPT antibiotic-treated copepods fed live *P. tricoratum* cells.) Error bars are $\pm 1 \sigma$ ($n=6$)

of variance (Fig. 2A). There was significant interaction between the two treatments (treatment of copepods with antibiotics; treatment of fish food with cell extracts) ($n=24$, $F=9.81$, $\alpha=0.005$). Protected two-tailed *t*-tests showed that there were significant differences between antibiotic-treated copepods and untreated copepods and between fish food with no adsorbed cell extract and fish food with adsorbed cell extract. One-factor ANOVA showed that the antibiotic-treated copepods produced significantly fewer fecal pellets on a diet of fish food with adsorbed cell extract than on a diet of fish food with no adsorbed cell extract ($n=6$, $F=15.4$, $\alpha=0.004$). This indicates that *G. grindleyi* does produce an extractable cellular feeding deterrent. These results correlated with work done by Huntley et al. (1986), who also showed that *G. grindleyi* inhibited feeding. However, untreated copepods did not show a significantly different response between the two diets ($n=6$, $F=1.72$, $\alpha=0.23$), suggesting that the bioassay is affected by bacteria on the copepods or in the medium.

A cellular extract from *Thalassiosira pseudonana* was bioassayed for feeding deterrents. The data were arranged into four blocks, as in the previous experiments, and analyzed using two-factor analysis of variance (Fig. 2B). There was no significant interaction between the two treatments (treatment of copepods with antibiotics; treatment of fish food with cell extracts) ($n=24$, $F=3.75$, $\alpha=0.07$). Data from both groups fed fish food with no adsorbed cell extract and both groups fed fish food with adsorbed *T. pseudonana* cell extract were combined and analyzed by one-factor ANOVA. There was no significantly different re-

sponse between a diet of fish food treated with *T. pseudonana* cell extract and a diet of untreated fish food ($n=12$, $F=1.07$, $\alpha=0.31$). It appears that *T. pseudonana* does not produce any cellular feeding deterrents that affect this copepod. ANOVA also showed that antibiotic-treated copepods produced more fecal pellets than untreated copepods ($n=12$, $F=19.9$, $\alpha<0.001$), indicating that bacterial contaminants have a significant affect on the copepods.

In both of the above experiments, antibiotic-treated copepods produced significantly more fecal pellets than untreated copepods. As bacteria affect the feeding of *Tigriopus californicus*, subsequent bioassays were performed with antibiotic-treated copepods to decrease this effect.

A cellular extract from *Phaeodactylum tricornerutum* was bioassayed for feeding deterrents (Fig. 2C). A cellular extract from *Thalassiosira pseudonana* was used as the control. ANOVA showed that copepods on a diet of *P. tricornerutum* treated fish food produced significantly fewer fecal pellets than copepods on a diet of *T. pseudonana* treated fish food ($n=6$, $F=128$, $\alpha<0.001$). Therefore, *P. tricornerutum* produced a cellular substance which is a feeding deterrent.

By adsorbing cell extracts onto ground fish food, the bioassay simulated the situation where an algal cell has various compounds attached to or closely associated with the cell surface [similar to the "phycosphere" concept proposed by Bell and Mitchell (1972)]. The copepod was then able to handle the food particle and accept or reject it on the basis of its surface properties. This was a more realistic model than simply dissolving the cell extracts in the medium, and it provided some information on the mechanisms involved (e.g. the copepods detected the feeding deterrent when handling the food particles). Using cell extracts eliminated feeding preferences based on size, shape, and texture of the algal cell. However, many of the fish food particles were of a similar size and shape as the fecal pellets. This made counting fecal pellets slow and subjective, since fecal pellets were difficult to distinguish from fish food particles. Therefore, adsorbing cell extracts onto fish food was not a suitable method for bioassay-guided chemical fractionation.

Bioassay method using dissolved feeding deterrents

A cellular extract from *Phaeodactylum tricornerutum* was tested for feeding deterrents by presenting the copepods with the extract dissolved in seawater and mixed with live *Thalassiosira pseudonana* cells as the food source (Fig. 3A). Copepods produced significantly fewer fecal pellets when presented with live *T. pseudonana* cells mixed with dissolved cellular extract from *P. tricornerutum* cells than when presented with live *T. pseudonana* cells mixed with dissolved cellular extract from *T. pseudonana* cells ($n=6$, $F=61$, $\alpha<0.001$). The feeding deterrent produced by *P. tricornerutum* affects *Tigriopus californicus* when dissolved in seawater.

The fecal pellets were more easily and quickly counted when the feeding deterrent was presented to the copepods

as a dissolved material in the presence of live phytoplankton cells than when the copepods were provided with ground fish food onto which the feeding deterrent was adsorbed. There was no confusion in distinguishing fecal pellets from phytoplankton cells. This removed the subjective bias found in the ground fish food method resulting from the difficulty in correctly identifying food particles from fecal pellets. The speed and ease of performing this method made it suitable for bioassay-guided chemical fractionation. As in the previous method, using cell extracts eliminated feeding preferences based on size, shape, and texture of the algal cell. However, presenting the feeding deterrent in a dissolved form may be unrealistic, especially if the feeding deterrent is actually intracellular or closely associated with the cell surface. In these cases, the feeding deterrent response in the natural environment may be different.

Bioassay method using feeding deterrents produced by live cells

To determine if feeding deterrents produced by *Phaeodactylum tricornerutum* could be detected in live cultures, *Tigriopus californicus* was exposed to both *Thalassiosira pseudonana* and *P. tricornerutum* live cells (Fig. 3B). Copepods fed live *P. tricornerutum* cells produced significantly fewer fecal pellets than copepods fed live *T. pseudonana* cells ($n=5$, $F=32$, $\alpha=0.0003$). Thus, a feeding deterrent can be detected from live *P. tricornerutum* cells.

Presenting live phytoplankton cells to the copepods allowed the bioassay to simulate the natural environment. The copepod was able to detect the feeding deterrent at concentrations produced by live cells. Whether the feeding deterrent was present as a component of the cell's "phycosphere", as a dissolved exudate, or intracellularly was not determined from this method. In addition, this method did not control for changes in feeding due to differences in the size, shape, and texture of the phytoplankton cells, or preconditioning of the copepod to feeding on a certain cell type.

Discussion

Many species of marine phytoplankton reduce or completely inhibit feeding in various zooplankton (Ali 1970; Chotiyaputta and Hirayama 1978; Huntley 1982; Verity and Stoecker 1982; Egloff 1986; Huntley et al. 1986; Sykes and Huntley 1987; Ward and Targett 1989; Uye and Takamatsu 1990). No feeding deterrent compound has been isolated and structurally characterized from marine phytoplankton, although toxins from freshwater cyanobacteria (microcystin-LR, nodularin) have been found to be feeding deterrents (DeMott et al. 1991). Isolation of an active compound from a crude extract requires bioassay-guided chemical fractionation of the crude extract to be performed.

Therefore, a fast and reliable bioassay is needed, since many samples must be bioassayed to isolate one active compound. Most feeding deterrent bioassays presently in use are tedious, require expensive and specialized equipment and supplies, or demand high levels of skill in culturing or collecting delicate bioassay organisms (Huntley et al. 1986; Sykes and Huntley 1987).

In previous work, feeding deterrents have been detected by measuring ingestion rate (Huntley et al. 1986). This approach is often tedious and time consuming (e.g. cell/bead counts). Fecal pellet production rate (egestion) has been correlated with ingestion rate for copepods (Huntley et al. 1983; Ayukai and Nishizawa 1986; Tsuda and Nemoto 1990). Ayukai (1987) used fecal pellet volumes and production rates of the copepod *Acartia clausi* to estimate ingestion rates, and thus assess discriminate feeding between polystyrene beads and various species of phytoplankton. Therefore, fecal pellet production rate was used as a measure of ingestion rate in the bioassay described by the present paper. Counting fecal pellets is rapid, easy, and requires little skill. However, as egestion rate is an indirect measure of feeding, it is not always tightly coupled to ingestion rate, and a bioassay using egestion rate may have decreased accuracy. The bioassay presented in our work has two main advantages over previous bioassays: (1) the bioassay organism, *Tigriopus californicus*, is readily cultured in the lab using commercially available fish food, thus freeing the researcher from the seasonality and difficulties involved in collecting copepods from the natural environment; (2) since the copepods are reared under controlled conditions, they have known life histories and have been exposed to the same conditions.

Bacteria associated with *Tigriopus californicus* or the culture medium reduced the feeding of the copepod on ground fish food. When *T. californicus* was treated with antibiotics, the egestion rate increased and the copepods were more responsive to the presence of a feeding deterrent. Bacteria may be harmful to the copepods (decreasing egestion rate), may be producing their own feeding deterrent compounds (decreasing egestion rate), may be breaking down the feeding deterrent being bioassayed (decreasing sensitivity of the bioassay), or may be providing an additional food source for the copepods (decreasing sensitivity of the bioassay). Antibiotic treatment is recommended for copepods with heavy bacterial contamination.

The type of food source used in the bioassay is important. Food sources with large particle sizes, such as ground fish food, make fecal pellet counts difficult, while food sources with small particle sizes, such as diatom cells, produce a background against which fecal pellets are easily counted. For greatest accuracy, small phytoplankton (< 20 μm) should be used.

Using *Tigriopus californicus* as the indicator organism, a feeding deterrent was detected in the cell extracts from both the diatom *Phaeodactylum tricornerutum* and the dinoflagellate *Gonyaulax grindleyi*. Therefore, it appears that the production of feeding deterrents is not limited to dinoflagellates (marine) or cyanobacteria (freshwater), and it is possible that many classes of phytoplankton produce

these compounds. Further screening of other phytoplankton is required.

Tigriopus californicus is an extremely tolerant organism (Burton et al. 1979; Dethier 1980), capable of surviving long periods of starvation. It is possible that compounds which would deter the feeding of a less tolerant copepod will have little or no effect on *T. californicus*. However, if a compound elicits a deterrent response from *T. californicus*, that compound may completely inhibit the feeding of a less tolerant species. Thus, feeding deterrents detected by *T. californicus* would certainly be of ecological significance.

This work showed that *Phaeodactylum tricornerutum* at a density of 6×10^5 cells ml^{-1} was capable of producing feeding inhibition in *Tigriopus californicus*. Although this density is too high for a normal ecological system, further studies of feeding inhibition by *P. tricornerutum* at lower cell densities is merited. If phytoplankton which produce feeding deterrents are found in bloom concentrations in nature, these blooms could deter predators. Thus, feeding deterrents may control which phytoplankton species are palatable and assimilated by zooplankton. This, in turn, may initiate or extend blooms of non-palatable species. Ultimately, feeding deterrents may control transfer of energy along some paths in the food web.

A new, rapid, and reliable bioassay for feeding deterrents has been developed. This bioassay is capable of detecting deterrent compounds from both live cells and cell extracts, and should provide a valuable tool in screening other phytoplankton for the production of feeding deterrent compounds and determining the chemical nature of these compounds.

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