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Copepod grazing selection and particle discrimination on the basis of PSP toxin content

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ABSTRACT: Omnivorous copepods are capable of discriminatory feeding using mechano- and chemosensory mechanisms. The presence of phycotoxins in phytoplankton often results in reduced consumption of such potential prey by copepods, though it has not been clear if this is the result of discriminatory feeding by either tactile (mechanosensory) or chemosensory recognition of toxic prey, or perhaps a physiological response to ingested neurotoxic compounds. In this study, experiments were performed to determine whether 3 species of marine copepods (*Acartia tonsa*, *Centropages hamatus*, and *Eurytemora herdmani*) that commonly co-occur with toxic *Alexandrium* spp. dinoflagellates were capable of discriminating between cultured *Alexandrium* spp. strains on the basis of paralytic shellfish poisoning (PSP) toxin content, i.e. by chemosensory means, using live fluorescently labeled cells. Additional experiments investigated whether toxic cells in mixtures with non-toxic alternate species of dinoflagellates affected either prey selection or total carbon consumption rates of copepods, and whether daily carbon rations could be maintained on both toxic and non-toxic *Alexandrium* spp. monoculture diets. Results indicated that all 3 copepod species could discriminate between toxic and non-toxic *Alexandrium* spp. cells by chemosensory means, suggesting that selective behavior, rather than physiological effects, governs the grazing response of copepods exposed to toxic prey. Prey selection in mixtures of several dinoflagellate species depended on whether the *Alexandrium* spp. cells present were toxic or non-toxic. *C. hamatus* and *E. herdmani* (but not *A. tonsa*) maintained daily carbon rations despite the presence of toxic *Alexandrium* spp., chiefly through increased consumption of alternate prey. For *A. tonsa* and *C. hamatus*, carbon rations were not equivalent between toxic and non-toxic *Alexandrium* spp. monoculture diets, indicating strong aversions to PSP toxins, and the potential for physiological effects when no other food is available. In all experiments feeding behavior varied among copepod species, suggesting that grazing pressure on toxic *Alexandrium* spp. is not uniform throughout the zooplankton community. The grazer-deterrent effects observed have implications for the function of PSP toxins.

KEY WORDS: *Alexandrium* · Copepod · Grazing behavior · PSP toxins

INTRODUCTION

Dinoflagellates of the genus *Alexandrium* often produce neurotoxic compounds commonly known as paralytic shellfish poisoning (PSP) toxins. *A. tamarense* (Lebour) Balech and *A. fundyense* Balech occur and may proliferate in the coastal waters of northeastern North America. Toxins produced by these species can contaminate filter-feeding shellfish, resulting in economic losses and a threat to public health (Shumway et al. 1988). Toxigenic *Alexandrium* spp. can also adversely affect other components of the marine food web, including finfish and marine mammals, probably via a zooplankton vector (White 1979, 1980, 1981, Geraci et al. 1989). Grazing by zooplankton and filter-feeding shellfish may also serve to moderate *Alexandrium* spp. bloom formation (Turner & Anderson 1983, Watras et al. 1985). In general, grazing (or the lack thereof) may be a principal biological mechanism by which harmful algal blooms are either terminated or allowed to persist (Smayda 1992). Furthermore a large

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proportion of the total toxins produced in a bloom may pass through grazers, with the potential to spread throughout the food web. It is therefore important to understand the mechanisms governing the response of grazers to harmful algal blooms, so that we can understand both the reasons for bloom formation and the potential impact on food webs.

The effect of toxigenic phytoplankton on the feeding behavior of omnivorous zooplankton can vary greatly among zooplankton species (see Turner & Tester 1997 and references therein). In experiments with *Alexandrium* spp., some studies have reported a reduced grazing response of zooplankton to toxic prey cell types compared with non-toxic alternatives (Ives 1985, 1987, Turriff et al. 1995), although reasonably high ingestion rates of toxic *Alexandrium* spp. have been observed with no apparent physiological effects (Teegarden & Cembella 1996). The mechanism of potentially reduced zooplankton grazing rates on toxic *Alexandrium* spp. has been the subject of some investigation (Ives 1987, Turriff et al. 1995, Teegarden & Cembella 1996). Earlier evidence supported a physiological basis for reduction of copepod grazing rates (Ives 1987; for other species see Huntley et al. 1986, Uye & Takamatsu 1990), whereas later studies have concluded that discriminatory feeding selection is important in the grazing response of omnivorous copepods (Turriff et al. 1995, Teegarden & Cembella 1996). Nevertheless it has been unclear whether the postulated selective grazing can be attributed to the actual toxin content of the cells, or to other factors affecting palatability, or indeed whether grazers may have to sample toxic cells and learn to recognize them by tactile means to avoid subsequent ingestion (Sykes 1991).

This study was designed to determine whether PSP toxins have a role in the selective grazing response of 3 coastal copepods that commonly occur along with *Alexandrium* spp. in the estuaries and embayments of the Gulf of Maine. *Acartia tonsa* Dana, *Centropages hamatus* Lilljeborg, and *Eurytemora herdmani* Thompson & Scott were tested for the ability to distinguish between toxic and non-toxic clonal cultures of *Alexandrium* spp. that are virtually identical in other respects, in mixtures containing equal numbers of each clone. Additional experiments investigated the feeding response of these copepods to mixtures of several species of dinoflagellates including either toxic or non-toxic *Alexandrium* spp., to determine if both prey cell selection and overall carbon consumption rates differed between treatments as a result of the presence of toxic prey. Finally each copepod species was tested in grazing trials with monocultures of toxic or non-toxic *Alexandrium* spp., to determine whether carbon consumption rates could be maintained when only 1 prey type was available.

MATERIALS AND METHODS

Phytoplankton culture. Three clones of *Alexandrium* spp. were used in various experiments. *A. tamarense* (Lebour) Balech clone CCMP 115 (= PLY 173) was obtained from the Guillard/Provasoli Center for the Culture of Marine Phytoplankton, Boothbay Harbor, Maine. *A. tamarense* clone GTCN 16 and *A. fundyense* clone GTCA 28 were provided by the laboratory of Dr Donald M. Anderson, Woods Hole Oceanographic Institution. The CCMP 115 clone was identified as non-toxic, while GTCN 16 has been reported as a low-toxicity clone and GTCA 28 as a moderate- to high-toxicity clone (D. M. Anderson pers. comm.). Samples from experiments were analyzed by high performance liquid chromatography with fluorescence detection (HPLC-FD; see Teegarden & Cembella 1996 for details) under the direction of Dr Allan Cembella at the Institute for Marine Biosciences, NRC, Halifax, Nova Scotia. HPLC-FD analysis confirmed CCMP 115 samples to be below detection limits for PSP toxins. GTCN 16 samples also tested below detection limits, such that the clonal cultures used in these experiments may be considered functionally non-toxic. GTCA 28 samples tested moderately toxic as expected (Table 1). There was some variation in toxicity of the *A. fundyense* GTCA 28 culture over time, but toxicity remained within the normal range for this clone. Consistent experimental results (presented below) suggest that such toxin variation had no effect on experimental outcomes.

Additional species of dinoflagellates used in mixed species experiments were *Lingulodinium polyedrum* (Stein) Dodge clone CCMP 407 (Guillard/Provasoli CCMP), and *Gonyaulax* cf. *cochlea* Meunier clone B199J (= CCMP 1592) and *Prorocentrum micans* Ehrenberg clone C 26 from the laboratory of Dr P. E. Hargraves, Graduate School of Oceanography, University of Rhode Island. None of these dinoflagellate clones produce known toxins.

All of the aforementioned phytoplankton clones were cultured in an environmental room at 18°C, with a 14 h : 10 h light:dark cycle using cool white fluorescent lamps. Culture medium was prepared from autoclaved 0.45 µm filtered Narragansett Bay water, approx. 28 to 30 PSU, enriched with f/2 - Si (Guillard 1975). Stock cultures were used to inoculate 8 l bottles every 2 wk to provide quantities of exponentially growing phytoplankton for experiments; only exponential phase cultures were used.

Zooplankton collection. Zooplankton were collected from the Damariscotta River estuary in Walpole, Maine, an area that has experienced blooms of toxic and non-toxic *Alexandrium* spp. in recent years (Yentsch et al. 1978, Maine Dept Marine Resources

Table 1. Toxicity of *Alexandrium* spp. clones used in grazing experiments with the 3 species of copepods. The first column indicates copepod species, followed by the type of experiment performed, the *Alexandrium* spp. clone tested, and the toxicity measured for that clone, in pg saxitoxin equivalents (STXeq) per cell

Copepod sp. Experiment	*Alexandrium* clone	Toxicity (pgSTXeq cell^{-1})
Acartia tonsa		
Selection	GTCA 28	19.8
	CCMP 115	0.0
	GTCN 16	0.0
Mixed species	GTCA 28	20.7
	CCMP 115	0.0
Carbon ingestion	GTCA 28	25.0
	CCMP 115	0.0
Eurytemora herdmani		
Selection	GTCA 28	31.0
	CCMP 115	0.0
	GTCN 16	0.0
Mixed species	GTCA 28	20.7
	CCMP 115	0.0
Carbon ingestion	GTCA 28	15.4
	CCMP 115	0.0
Centropages hamatus		
Selection	GTCA 28	31.0
	CCMP 115	0.0
Mixed species	GTCA 28	17.5
	CCMP 115	0.0
Carbon ingestion	GTCA 28	12.7
	CCMP 115	0.0

PSP monitoring reports; L. Bean pers. comm.). A 150 µm mesh net was suspended in the tidal flow, allowing gentle collection of larger zooplankton. Adult females of the copepod species *Acartia tonsa*, *Centropages hamatus*, and *Eurytemora herdmani* were sorted out and used to establish cultures in laboratory facilities at the University of Rhode Island. Copepods were kept in multiple 22 l containers with aeration and regular water changes, and fed a diet of mixed dinoflagellate species (*Heterocapsa triquetra* clone HT984 and *Gymnodinium sanguineum* clone B4). *A. tonsa* was maintained at 19°C, and *C. hamatus* and *E. herdmani* were maintained at 17°C, with 14 h : 10 h light:dark cycles. All 3 species exhibited vigorous growth and reproduction in culture, and, although cultures could be maintained indefinitely, they were occasionally augmented with freshly collected zooplankton.

Alexandrium* spp. selection experiments. *Staining of dinoflagellates: The *Alexandrium* spp. clones used in experiments reported here are virtually identical in size and shape, carbon and nitrogen per cell, etc. *A. tamarense* and *A. fundyense* are separated on the basis of a pore on the 1' apical plate of *A. tamarense* which *A. fundyense* lacks (Balech 1990). The 2 species can be crossed in sexual reproduction, and are considered by many to be conspecific (A. Cembella pers. comm.). Mixtures of toxic *A. fundyense* and non-toxic *A. tamarense* were used to test copepods for the ability to discriminate between particles on the basis of PSP toxin content. In order to be able to distinguish between the 2 virtually identical cell types in a mixture, one of the cell types was labeled with a live fluorescent vital stain, Cell Tracker® Blue CMAC (7-amino 4-chloromethylcoumarin, Molecular Probes Inc.), which fluoresces blue when excited by UV light at 354 nm. Chambers constructed for the labeling procedures consisted of polycarbonate screw top bottles fitted with a 5 µm mesh bottom and a slow drip flat funnel supporting the mesh, with controllable outflow. Exponentially growing cells were stained with the label at a final concentration of 5 µM in filtered seawater, and incubated at 18°C for 4 h. The cells were then gently rinsed free of excess label by transferring the solution to the chamber, reducing the solution volume and replacing with filtered seawater, repeating several times. Cells were allowed to recover from the treatment overnight at 18°C, and checked for label efficacy with epifluorescence microscopy and for cell viability. Only cultures that had 100% of sampled cells labeled and viable were used in experiments. As a precautionary control measure, unlabeled cells were subjected to the same handling procedure without CMAC. Preliminary experiments, prior to grazing trials, tested cells for label retention through cell divisions, and samples maintained visible label in 100% of the cells through 3 d (division rates approx. 0.25 d^{-1}). The label does not appear to leak from cells, and any released through disruption (e.g. grazing) should not affect unlabeled cells, due to the relatively high concentrations needed to effectually label *Alexandrium* spp. dinoflagellates. Additional preliminary experiments confirmed that the presence of label did not affect palatability of cells to the copepods tested.

Experimental design: The 3 copepod species were tested for the ability to discriminate between toxic *Alexandrium fundyense* GTCA 28 and non-toxic *A. tamarense* CCMP 115 in mixtures by labeling one clone and mixing with equal numbers of the other (subjected to equal treatment without label). As an added control, 2 treatments were run simultaneously for each grazing trial; one treatment with labeled toxic *A. fundyense* GTCA 28 mixed with unlabeled non-toxic *A. tamarense* CCMP 115, and another treatment with unlabeled GTCA 28 mixed with labeled CCMP 115. Although preliminary experiments showed no effect of label on palatability, such treatments should guard against any possible discrepancies. I also tested for differences in palatability between non-toxic clones

of *A. tamarense* on 2 occasions, by feeding *Acartia tonsa* and *Eurytemora herdmani* mixtures of *A. tamarense* GTCN 16 (functionally non-toxic) and *A. tamarense* CCMP 115 (non-toxic).

Grazing trials were performed the day after cultures of *Alexandrium* spp. were labeled. Cell concentrations were determined with a Coulter® Counter model ZM, and cultures were mixed to give experimental solutions at a concentration of 200 cells ml^{-1} of each type (400 cells ml^{-1} total). These concentrations allow acceptable confidence in post-experimental visual cell counts, and also ensure that the potential for selection is not affected by possible food limitation which might constrain grazers to consume less palatable prey. Adult females of the copepod species being tested were sorted from cultures and, without delay, triplicate experimental containers were prepared for each treatment containing 10 copepods in 280 ml of experimental *Alexandrium* spp. cell mixtures. Replicate control containers and initial containers without animals were prepared at the same time. Control and experimental containers were placed on a grazing wheel rotating at 1 rpm in a temperature controlled water bath, at 19°C for *Acartia tonsa*, or 17°C for *Centropages hamatus* and *Eurytemora herdmani*. Experiments were run for 24 h on a 14 h:10 h light:dark cycle. Initial container samples were processed immediately at the start of an experiment. At the end of an experiment, copepods were removed and examined for physiological condition. Replicate 2 ml subsamples of control and experimental cell mixtures were gently filtered down onto gridded membranes (Millipore RA), which were cleared with a drop of immersion oil, and cell counts were made using an Olympus microscope with epifluorescence illumination. The counting procedure took generally less than 6 h, but additional subsamples were preserved with 1.25% formaldehyde as a precaution, and counted if necessary (formaldehyde does not affect the fluorescent label). Algal growth rates in control containers and copepod grazing rates in experimental containers were calculated from the equations of Frost (1972) and analyzed with factorial ANOVA.

Mixed dinoflagellate species grazing experiments. To determine whether the presence or absence of toxin in *Alexandrium* spp. affected the grazing response of the 3 copepod species (selective preferences and total carbon consumption rates) when multiple dinoflagellate species were available, additional grazing trials were conducted using mixtures of *Alexandrium* spp. and 3 alternate non-toxic dinoflagellate species. Mixtures contained *Lingulodinium polyedrum* clone CCMP 407 (~29 µm diameter), *Gonyaulax cochlea* clone B199J (~24 µm diameter), *Prorocentrum micans* clone C 26 (~27 µm diameter), and either *A. tamarense* CCMP 115 (non-toxic) or *A. fundyense* GTCA 28 (toxic; both ~27 µm diameter). Cell mixtures were prepared at approx. 300 µg C l^{-1} of each type (1200 µg C l^{-1} total). As before, adult females of the copepod species being tested were sorted out from cultures and immediately placed into 280 ml experimental containers, 20 copepods each in triplicate containers for each of the 2 treatments (mixtures containing toxic *A. fundyense* GTCA 28, and mixtures containing non-toxic *A. tamarense* CCMP 115). Control containers with no grazers were prepared at the same time, and then incubated with experimental containers on a grazing wheel in a temperature controlled water bath for 24 h. Initial samples were preserved immediately with Lugol's iodine solution. At the end of the incubation, copepods were removed and examined for physiological condition, and dinoflagellate mixtures were preserved with Lugol's solution. Replicate subsamples were then counted for all dinoflagellate species using a Sedgwick-Rafter chamber with phase contrast microscopy. Algal growth rates and copepod grazing rates were calculated with the Frost (1972) equations, and results analyzed by ANOVA with LSD post-hoc testing using SAS® (Cody & Smith 1991).

Monoculture grazing experiments. To determine whether equivalent carbon consumption rates could be maintained with either toxic or non-toxic cells as the sole food source, grazing trials were conducted with the 3 species of copepods using monocultures of either toxic *Alexandrium fundyense* GTCA 28 or non-toxic *A. tamarense* CCMP 115. *Alexandrium* spp. cultures were harvested and counted with a Coulter® Counter Multisizer, and diluted in filtered seawater to 500 cells ml^{-1} (approx. 1000 µg C l^{-1}). Triplicate experimental containers were prepared for each treatment, with 20 adult females in 280 ml in each container, along with control and initial containers. Triplicate initial samples of 20 adult female copepods were also taken and prepared for CHN analysis. Experimental and control containers were incubated as before on the grazing wheel in a temperature controlled water bath for 24 h; initial containers were counted immediately with the Multisizer. At the end of an experiment copepods were removed and examined for physiological condition, then placed into tin boats and dried for CHN analysis. Cell concentrations in experimental and control containers were determined with the Multisizer (using volume % mode to minimize artifacts), and grazing rates were calculated by the Frost (1972) equations and analyzed with 2-way ANOVA. Subsamples of algal cultures used were collected on precombusted glass fiber filters (10^5 cells $filter^{-1}$), and copepod and algal samples were analyzed with a Carlo Erba CHN analyzer.

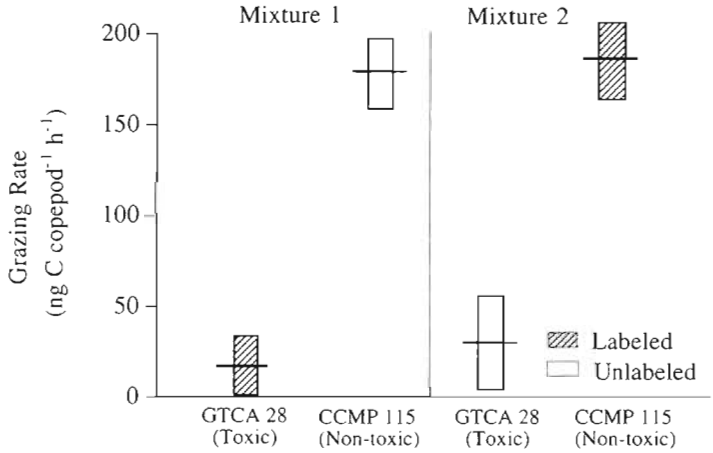

Fig. 1. *Acartia tonsa* grazing rates on *Alexandrium* spp. dinoflagellates in mixtures of toxic and non-toxic cells. Central bars denote mean rates, and boxes indicate 1 standard deviation. Mixture 1 contained fluorescently labeled toxic *A. fundyense* GTCA 28 and unlabeled non-toxic *A. tamarense* CCMP 115 in equal numbers; in mixture 2 the label sequence was reversed. In either mixture, non-toxic *Alexandrium* spp. cells were consumed at significantly higher rates ($p < 0.001$) than toxic cells, which were avoided despite their physical similarity

RESULTS

Alexandrium spp. selection experiments

Acartia tonsa and *Centropages hamatus* revealed a striking ability to distinguish between toxic and non-toxic *Alexandrium* spp. cultures in mixtures, whereas *Eurytemora herdmani* was capable of distinguishing between cell types, but was less likely to do so. When *Acartia tonsa* females grazed in mixtures of toxic GTCA 28 (*A. fundyense*) and non-toxic CCMP 115 (*A. tamarense*), regardless of the presence or absence of fluorescent label, the non-toxic *Alexandrium* clone was always consumed at a much higher rate (Fig. 1). *C. hamatus* also grazed non-toxic *Alexandrium* clone CCMP 115 in preference to toxic GTCA 28 (Fig. 2), but consumed the toxic clone at a relatively higher rate than *A. tonsa*. The difference in *C. hamatus* grazing rates between the 2 clones of *Alexandrium* spp. in mixtures was highly significant ($p < 0.001$), but only 3- to 4-fold, whereas *Acartia tonsa* exhibited an 8- to 10-fold difference between grazing rates on the 2 *Alexandrium* spp. clones ($p < 0.001$).

When first tested, *Eurytemora herdmani* did not exhibit any significant differences between grazing rates on either the toxic or non-toxic *Alexandrium* spp. clones, in either of the 2 treatment mixtures (Fig. 3a). Slight (non-significant, $p = 0.07$) preferences for non-toxic *A. tamarense* CCMP 115 encouraged a replication of the experiment. When the same experiment was performed 1 wk later, *E. herdmani* displayed a significant preference ($p < 0.001$) in both treatments for the non-toxic CCMP 115 *A. tamarense* clone over the toxic GTCA 28 *A. fundyense* clone (Fig. 3b), although the average difference in grazing rates was not as great as those exhibited by *Acartia tonsa* or *Centropages hamatus*. In experiments with all 3 copepod species, some toxic cells were ingested, though the extent to which toxic cells contributed to the diet varied among species of copepod. In all the experiments, copepods examined after grazing trials were in good condition, did not show signs of physiological impairment, and had food in the guts.

Acartia tonsa and *Eurytemora herdmani* were also tested with *Alexandrium tamarense* clones that were both functionally non-toxic, CCMP 115 and GTCN 16 (Fig. 4). Grazing trials with both copepod species revealed that in no cases were grazing rates significantly different between the *A. tamarense* clones in mixtures, indicating a lack of selective preference.

Mixed dinoflagellate species grazing experiments

All 3 copepod species exhibited significantly different grazing responses in mixtures of dinoflagellate species depending on the presence or absence of toxin

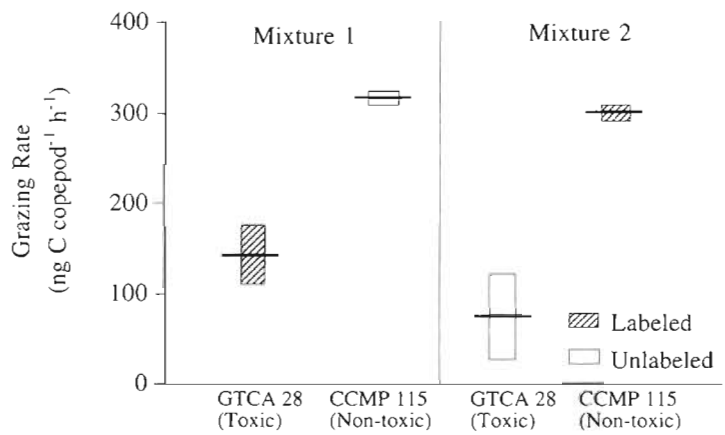

Fig. 2. *Centropages hamatus* grazing rates on *Alexandrium* spp. dinoflagellates in mixtures of toxic and non-toxic cells. Central bars denote mean rates, and boxes indicate 1 standard deviation. Non-toxic *A. tamarense* cells were consumed at significantly higher rates than toxic *A. fundyense* cells ($p < 0.001$). Nevertheless toxic cells constituted up to a third of the diet

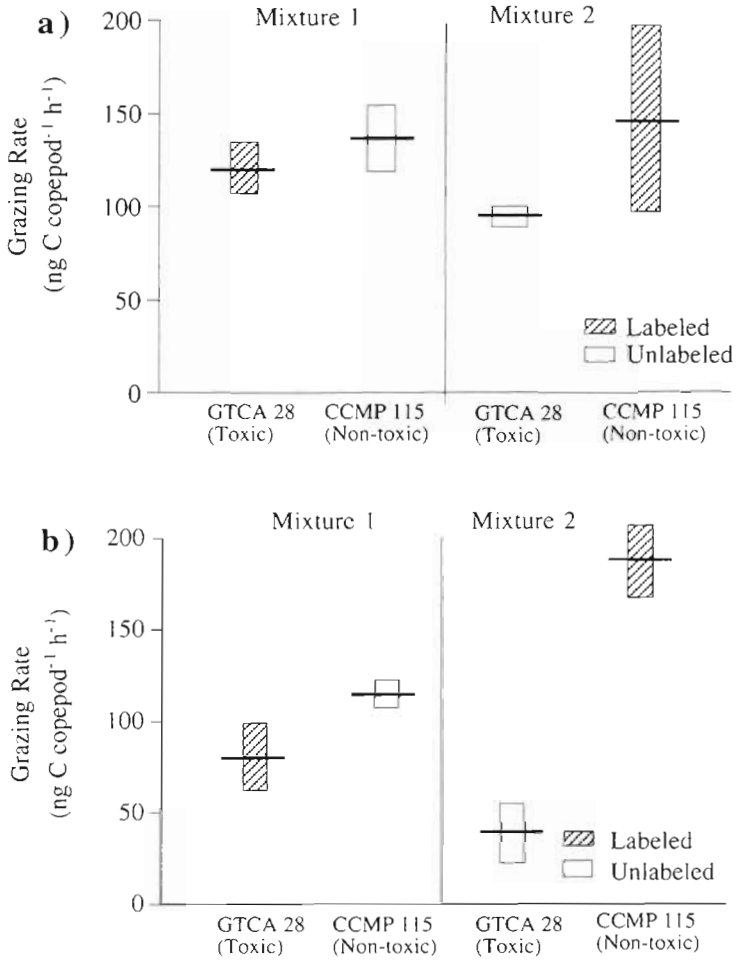

Fig. 3. *Eurytemora herdmani* grazing rates on *Alexandrium* spp. dinoflagellates in mixtures of toxic and non-toxic cells. Central bars denote mean rates, and boxes indicate 1 standard deviation. (a) The first trial resulted in no significant differences between grazing rates on toxic and non-toxic cells in a mixture ($p = 0.073$). (b) In the second trial, *E. herdmani* demonstrated significantly higher grazing rates on non-toxic *A. tamarense* CCMP 115 over toxic *A. fundyense* GTCA 28, though variability was high and the 2 mixture treatments were not identical (see 'Discussion')

in the *Alexandrium* spp. clone included in the mixture. Only 1 species (*Acartia tonsa*) had a significantly lower total carbon consumption rate as a result. In mixtures containing non-toxic *A. tamarense* CCMP 115, *A. tonsa* consumed this dinoflagellate clone at a significantly higher rate (100 ng C copepod^{-1} h^{-1}, $p < 0.01$) than any other dinoflagellate species (Fig. 5a). *Prorocentrum micans* was consumed at significantly lower ($p < 0.01$) rates than either *Gonyaulax cochlea* or *Lingulodinium polyedrum*, which were consumed at moderate rates of 60 to 70 ng C copepod^{-1} h^{-1}. When presented with a mixture containing toxic *A. fundyense* GTCA 28 (Fig. 5b), *A. tonsa* grazed this clone at 16 ng C copepod^{-1} h^{-1}, similar to the rate on *P. micans*, and more than 6-fold lower than the rate on non-toxic *A. tamarense* CCMP 115 from the previous treatment. *L. polyedrum* and *G. cochlea* were grazed at significantly higher rates than the 2 other dinoflagellate species, *L. polyedrum* being grazed at the highest rate ($p < 0.01$). *A. tamarense* CCMP 115 constituted a large proportion (39%) of the total carbon intake of *A. tonsa* in the first treatment, and the substitution of toxic *A. fundyense* GTCA 28 in the second treatment induced a shift in dependence for carbon ration to *L. polyedrum* (54%). Nevertheless, *A. tonsa* had a lower total carbon intake rate in the second treatment compared with the first treatment (4.15 and 6.23 µg copepod^{-1} d^{-1}, respectively, $p = 0.02$).

Centropages hamatus also demonstrated a significant ($p < 0.01$) preference for non-toxic *Alexandrium tamarense* CCMP 115 over all other dinoflagellate species in mixed species experiments (Fig. 6a), obtaining half of the measured daily carbon ration from that clone (220 ng C copepod^{-1} h^{-1}); *Lingulodinium polyedrum* and *Gonyaulax cochlea* were grazed at similar lower rates (~100 ng C copepod^{-1} h^{-1}), and *Prorocentrum micans* was grazed at the lowest rate (~30 ng C copepod^{-1} h^{-1}). Substitution of toxic *A. fundyense* GTCA 28 in the second treatment resulted in a great reduction in the carbon consumption of *Alexandrium* spp. by *C. hamatus* (Fig. 6b). Grazing rates on *G. cochlea* and *L. polyedrum* increased relative to the first treatment, and were equivalent at about 140 ng C copepod^{-1} h^{-1}, whereas toxic *A. fundyense* GTCA 28 was grazed at a significantly lower rate, and *P. micans* at the lowest rate ($p < 0.01$). Increased consumption of *L. polyedrum* and *G. cochlea* in the second treatment relative to the first treatment allowed *C. hamatus* to maintain equivalent daily carbon rations in both treatments (~10.5 µg copepod^{-1} d^{-1}), despite the difference in consumption of *Alexandrium* spp.

As with the other copepod species, *Eurytemora herdmani* fed upon non-toxic *Alexandrium tamarense* CCMP 115 at a significantly higher ($p < 0.01$) rate, 50 ng C copepod^{-1} h^{-1}, than any other species of dinoflagellate in the first treatment, and consumed *Prorocentrum micans* at a significantly lower rate ($p < 0.01$, Fig. 7a). *Gonyaulax cochlea* and *Lingulodinium polyedrum* were consumed at similar, intermediate rates. In the second treatment containing toxic *A. fundyense* GTCA 28, the grazing rate on that *Alexandrium* clone was reduced 3-fold compared to *A.*

tamarense in the first treatment (Fig. 7b). The grazing rate on *L. polyedrum* nearly doubled in the second treatment, and was significantly higher ($p < 0.01$) than grazing rates on any other dinoflagellate species; grazing rates on *G. cochlea*, *A. fundyense* and *P. micans* were not significantly different. The means of total daily carbon intake of *E. herdmani* in the non-toxic and toxic treatments (6.5 µg copepod^{-1} d^{-1} and 5.4 µg copepod^{-1} d^{-1}, respectively) were not significantly different ($p = 0.19$), suggesting that *E. herdmani* can obtain equivalent carbon rations in the presence or absence of toxic prey.

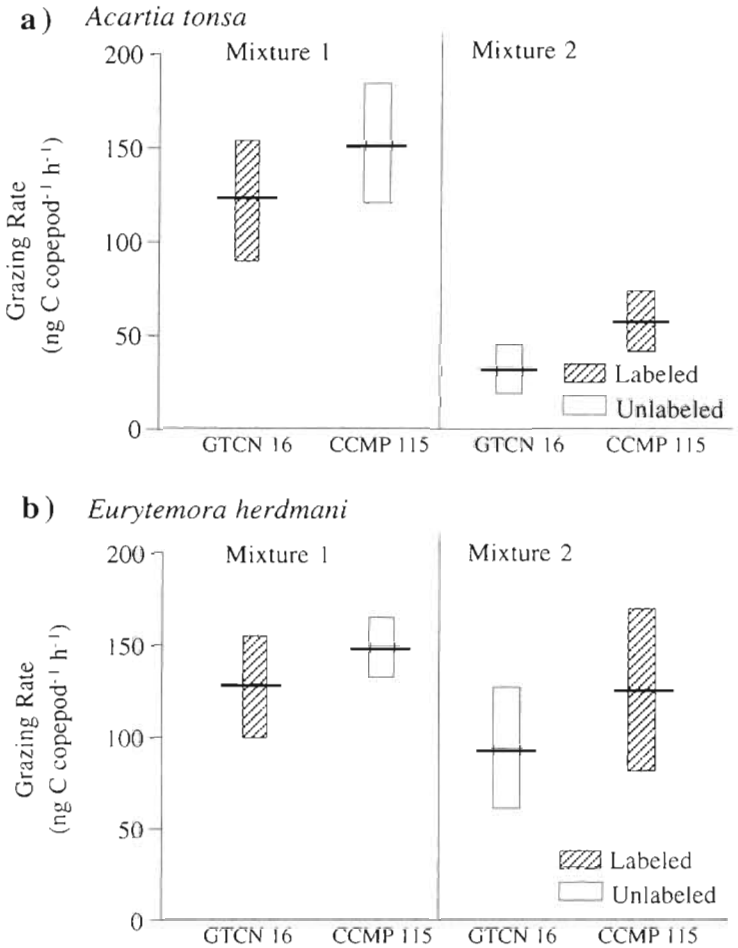

Fig. 4. (a) *Acartia tonsa* and (b) *Eurytemora herdmani* grazing rates in mixtures of 2 functionally non-toxic clones of *Alexandrium tamarense*, CCMP 115 and GTCN 16. Central bars denote mean rates, and boxes indicate 1 standard deviation. Although individual grazing rates and total carbon consumed were variable, there were no significant differences between grazing rates in any mixture treatment, for either copepod species

Monoculture grazing experiments

Acartia tonsa and *Centropages hamatus* consumed significantly different total carbon rations when grazing monocultures of non-toxic *Alexandrium tamarense* CCMP 115 or toxic *A. fundyense* GTCA 28, but *Eurytemora herdmani* did not exhibit significantly different grazing rates or total carbon rations between the 2 *Alexandrium* spp. clone monoculture diets ($p = 0.18$, Fig. 8). *A. tonsa* consumed an average of 196% of their mean body carbon d^{-1} when grazing a monoclonal culture of non-toxic *A. tamarense* CCMP 115, and showed a significant increase in carbon biomass ($p < 0.01$) over initial copepod samples of 18% (Table 2). When grazing toxic *A. fundyense* GTCA 28, *A. tonsa* only consumed an average of 61% of their body carbon d^{-1}, and lost 28% ($p < 0.01$) of the body carbon contained in initial copepod samples (Table 2), indicating starvation that should result in mortality if maintained over longer periods. Copepods from this treatment examined after experiments were clearly impaired, and were incapable of directed swimming or pipette avoidance, but did appear to have some food in the guts. Copepods from the non-toxic monoculture treatment were healthy, active, and had food in the guts.

Centropages hamatus consumed an average of 47% of their mean body carbon d^{-1} when grazing non-toxic *Alexandrium tamarense* CCMP 115, and showed a significant ($p < 0.05$) increase in body carbon of 24% over initial copepod samples (Table 2). *C. hamatus* consumed an average of 32% mean body carbon d^{-1} when grazing toxic *A. fundyense* GTCA 28, with no significant difference in body carbon between initial and experimental copepods on that diet (Table 2). Copepods examined after experiments were apparently unaffected in either treatment, being equally active and with food in the guts in both cases. Despite the lack of observable impairment, consumption of the toxic GTCA 28 clone was significantly lower than the non-toxic CCMP 115 clone (Fig. 8), indicating a lack of either ability or willingness to consume a normal daily ration when toxic *Alexandrium* spp. cells are the only available food.

Unlike the other copepod species, *Eurytemora herdmani* did not exhibit significantly different grazing rates on monoculture diets of non-toxic *Alexandrium tamarense* CCMP 115 or toxic *A. fundyense* GTCA 28 (Fig. 8). The average percent mean body carbon consumed per day was 76% for a diet of *A. tamarense* CCMP 115, with a significant ($p < 0.05$) carbon gain of 14% over initial samples (Table 2). On a diet of toxic *A.*

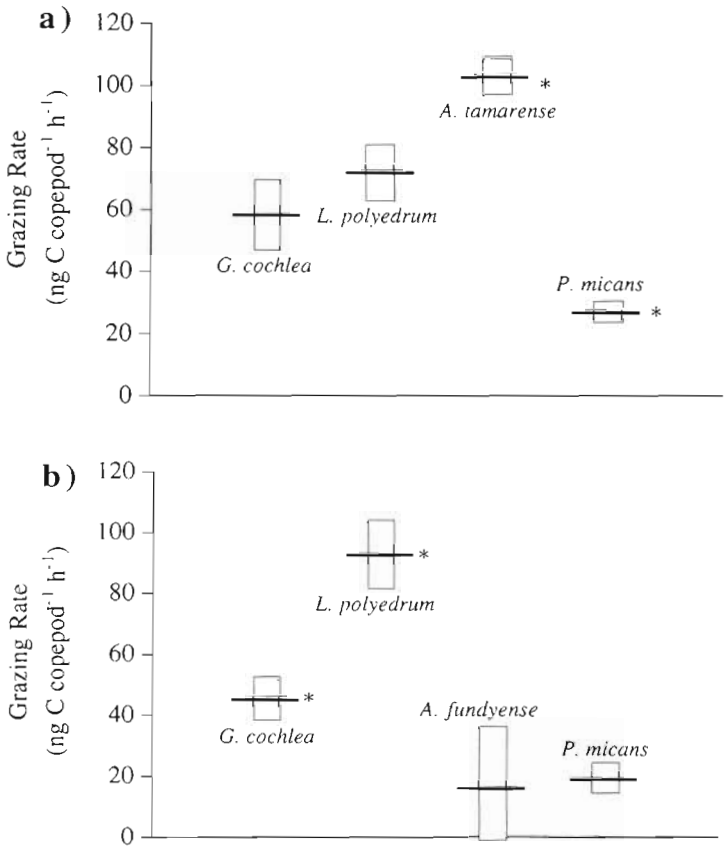

Fig. 5. *Acartia tonsa* grazing rates on mixtures of 4 species of dinoflagellates. Central bars denote mean rates, and boxes indicate 1 standard deviation. (a) In mixtures containing non-toxic *Alexandrium tamarense* CCMP 115, the CCMP 115 clone was consumed in preference to all other particles. (b) In the second treatment containing toxic *A. fundyense* GTCA 28, the *Alexandrium* clone was consumed at much lower rates, and *Lingulodinium polyedrum* and *Gonyaulax cochlea* were consumed at significantly higher rates than other species. *Significant difference $p < 0.05$ in grazing rates from other dinoflagellate species

fundyense GTCA 28, 63% of total body carbon was consumed, and there was no significant difference in body carbon between initial and experimental copepods (Table 2). Mean grazing rates were not significantly different between the 2 treatments. Furthermore, in both treatments, copepods observed after the experiment showed no signs of impairment, and were equally active and healthy, with food in the guts and production of eggs observed in both treatments.

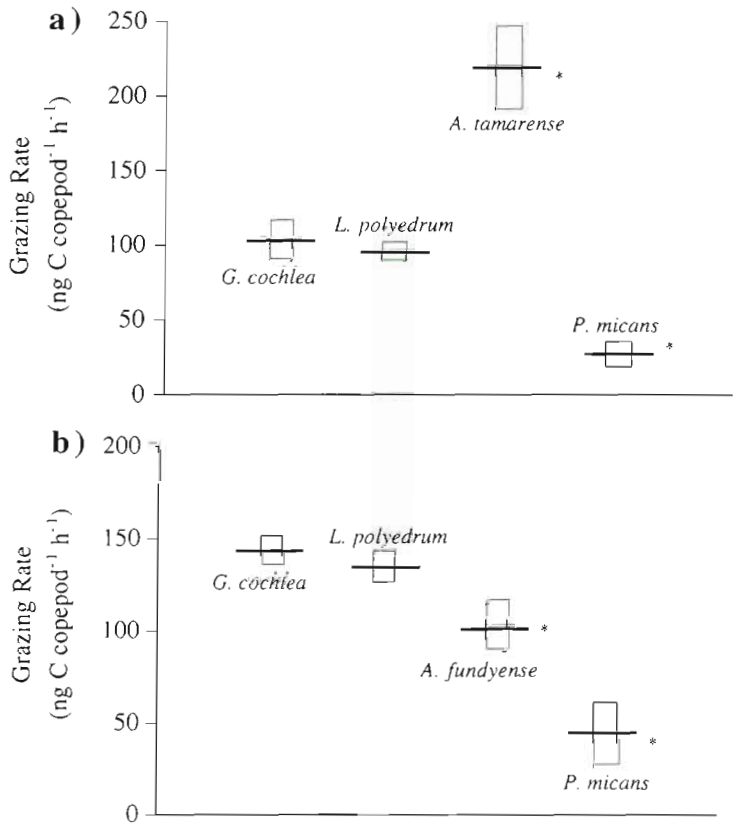

Fig. 6. *Centropages hamatus* grazing rates on mixtures of 4 species of dinoflagellates. Central bars denote mean rates, and boxes indicate 1 standard deviation. (a) As with *Acartia tonsa*, *Alexandrium tamarense* CCMP 115 was consumed in preference to all other species. (b) In the treatment containing toxic *A. fundyense* GTCA 28, grazing rates on *Alexandrium* were half of what they were in the first treatment, and rates on *Gonyaulax cochlea* and *Lingulodinium polyedrum* increased; grazing rates on *A. fundyense* and *Prorocentrum micans* were significantly lower than on the former species. *Significant difference $p < 0.05$ in grazing rates from other dinoflagellate species

DISCUSSION

Selection among *Alexandrium* spp. on the basis of PSP toxin content

The results of this study strongly suggest that selective grazing by omnivorous copepods, on the basis of PSP toxin content, is not only possible, but it is also likely to govern grazing response before physiological effects would cause impairment of grazing processes. This conclusion contrasts with a number of pioneering studies which suggested a physio-

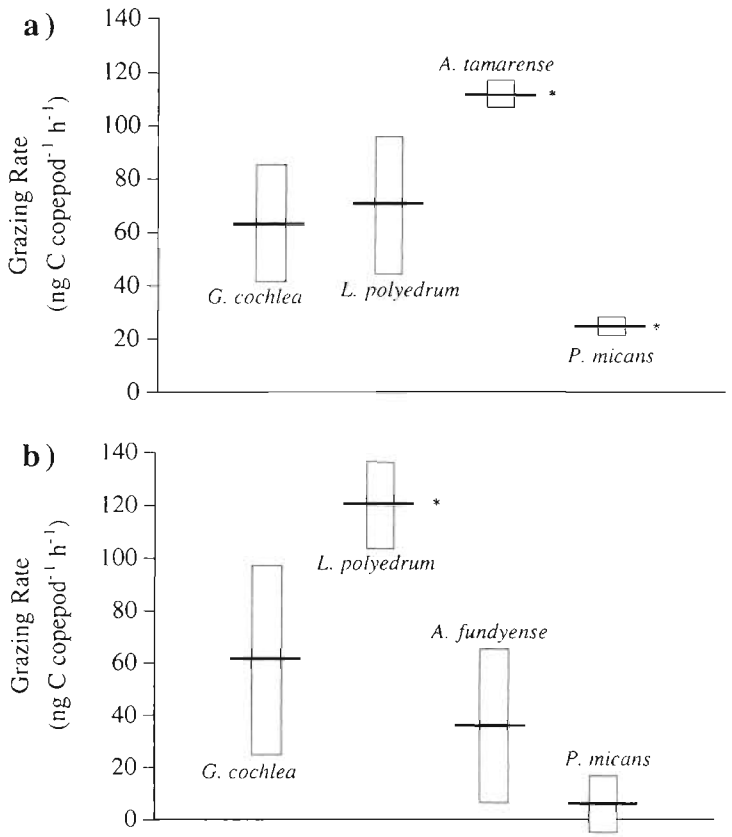

Fig. 7. *Eurytemora herdmani* grazing rates on mixtures of 4 species of dinoflagellates. Central bars denote mean rates, and boxes indicate 1 standard deviation. (a) *Alexandrium tamarense* was grazed at significantly higher rates than other species, while *Prorocentrum micans* was grazed at lower rates than all other species. (b) Grazing rates on *A. fundyense* were less than half of those on *A. tamarense* CCMP 115, while rates on *Lingulodinium polyedrum* increased and were significantly higher than rates on any other species. *Significant difference $p < 0.05$ in grazing rates from other dinoflagellate species

logical basis for observed reduced consumption or rejection of such phytoplankton. Among the first to address the question of the mechanism of zooplankton grazing response were Ives (1985, 1987) and Huntley et al. (1986), who concluded that ingestion and subsequent physiological impairment caused rejection or avoidance of certain dinoflagellates, including *Alexandrium* spp. Uye & Takamatsu (1990) tested several species of 'red tide' flagellates (not *Alexandrium* spp.) as food for copepods, and determined that intracellular chemicals caused feeding inhibition in some instances. These studies concluded that particle rejection did not take place prior to ingestion, and thus was not a result of chemosensory recognition of potential feeding deterrents present in certain phytoplankton. Huntley et al. (1986) and Ives (1987) concluded that toxins or feeding deterrents, including those produced by *Alexandrium* spp., may produce effects through the actual disabling of coordinated feeding appendage movement in copepods, rendering the grazer incapable of feeding. Uye & Takamatsu (1990) speculated that trial-and-error consumption of harmful phytoplankton may allow grazers to learn which species should be avoided, after suffering impairment or recognizing unpalatable intracellular substances. Sykes (1991) lent support to this hypothesis. He found that, although *Calanus pacificus* could not recognize noxious *Gonyaulax grindleyi* (= *Protoceratium reticulatum*) prior to ingestion (and thus selectively avoid initial consumption), *C. pacificus* could

Table 2. Results from copepod carbon consumption experiments. The first column indicates copepod species, followed by the sample indicating which clone was grazed by copepods (or initial sample). Adjacent columns indicate the mean carbon copepod^{-1} measured by CHN analysis, carbon ingested copepod^{-1} calculated from grazing rates, the percentage of initial body carbon ingested d^{-1}, and the total change in body carbon over 24 h, expressed in µg and as a percentage of initial body carbon (*significant differences)

Copepod sp.	Sample/clone grazed	Carbon (µg copepod^{-1})	Carbon ingested (µg C copepod^{-1} h^{-1})	% body carbon ingested d^{-1}	Change in body carbon (µg, % initial body carbon)
Acartia tonsa	Initial	4.18 ± 0.12	–	–	–
	CCMP 115	4.93 ± 0.04	0.37 ± 0.03	196 ± 15	+0.75, +18%*
	GTCA 28	3.02 ± 0.29	0.09 ± 0.01	61 ± 6	−1.16, −28%*
Centropages hamatus	Initial	9.91 ± 0.76	–	–	–
	CCMP 115	12.25 ± 0.72	0.22 ± 0.03	47 ± 7	+2.34, +24%*
	GTCA 28	10.48 ± 1.39	0.14 ± 0.02	32 ± 4	+0.57, +6%
Eurytemora herdmani	Initial	7.47 ± 0.42	–	–	–
	CCMP 115	8.55 ± 0.47	0.25 ± 0.06	76 ± 18	+1.08, +14%*
	GTCA 28	7.16 ± 0.29	0.19 ± 0.02	63 ± 7	−0.31, −4%

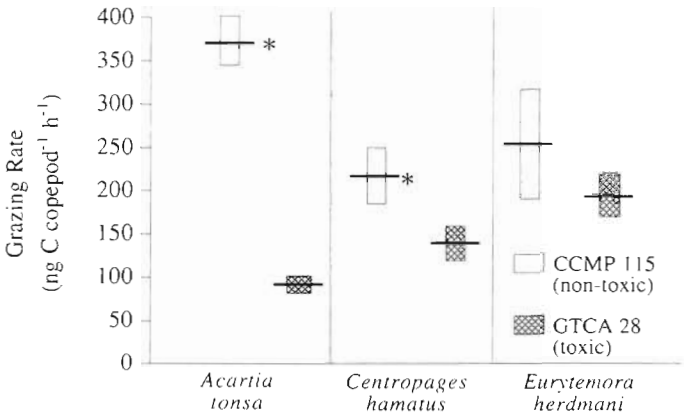

Fig. 8. *Acartia tonsa*, *Centropages hamatus*, and *Eurytemora herdmani* grazing rates on monocultures of either *Alexandrium tamarense* CCMP 115 or *A. fundyense* GTCA 28. Central bars are mean values, boxes indicate 1 standard deviation. *Significant differences. Both *A. tonsa* and *C. hamatus* demonstrated significantly higher grazing rates on monocultures of *A. tamarense* CCMP 115 than on *A. fundyense* GTCA 28 ($p < 0.001$, $p = 0.02$, respectively). *E. herdmani* grazed either monoculture at roughly equal rates (no significant difference, $p = 0.2$)

'remember' *G. grindleyi* from initial grazing trials and avoid subsequent ingestion upon re-exposure, but that this memory lasted only about 12 h.

Recent experimental work has suggested that copepods do not need to consume harmful phytoplankton to the point of impairment to recognize and avoid potentially toxic cells, or distinguish such cells from palatable alternatives in mixtures. DeMott & Moxter (1991) showed that a freshwater copepod (*Diaptomus birgei*) ingested the non-toxic cyanobacteria *Oscillatoria tenuis* (non-planktonic), while rejecting toxic (planktonic) *O. rubescens* or *O. agardhii* (which produce presumably toxic polypeptides) in mixtures of the species. Working with *Alexandrium* spp., Turriff et al. (1995) found that *Calanus finmarchicus* preferred the non-toxic, smaller diatom *Thalassiosira weisflogii* in mixtures with toxic *A. excavatum*, but non-toxic *A. tamarense* clone PLY 173 was grazed in preference to *T. weisflogii* in mixtures. In these experiments the prey available could easily be distinguished by size, cell abundance, and capture method, but the results certainly suggest that toxicity could be recognized and used by the copepods as a basis of rejection. The results do not however conclusively demonstrate that copepods could 'taste' PSP toxins, as it was possible (and the authors suggested) that trial-and-error consumption, with subsequent inimical effects, allowed the copepods to learn to recognize the unpalatable toxic species and avoid further grazing. Teegarden & Cembella (1996) found selection by grazing copepods in mixtures of toxic *Alexandrium* spp. and the non-toxic dinoflagellate *Lingulodinium polyedrum*, present in equal numbers. Although the prey used in that study were of similar size and equal concentration, the 2 cell types could probably be distinguished by the copepods via mechano- and chemoreceptors. In any case toxin content did not appear to be the principal factor governing selection, as a low toxicity *A. tamarense* clone was grazed in preference to *L. polyedrum* by one of the copepod species tested.

The results presented here strongly suggest that the copepods tested could recognize and reject toxic cells prior to ingestion, by chemosensory means. The 2 *Alexandrium* spp. clones used, considered by many to be conspecific, are virtually identical in all respects save toxin content, and could not be distinguished by mechanosensory abilities of the copepods (i.e. the copepods could not 'learn' to distinguish one cell type from another based on mechanical cues). In a mixture of such identical cell types, the only plausible explanation for the observed preference for non-toxic cells over toxic cells is that grazers could capture cells, use chemosensors to assess palatability, and reject less palatable cells, in this case cells producing PSP toxins. Since all dinoflagellates used were in exponential growth, culture age and physiological state should not have affected palatability. Furthermore the clear preference for non-toxic over toxic *Alexandrium* spp. demonstrated on several different experimental dates, and across 3 species of calanoid copepods, reinforces the conclusion that PSP toxin content is the factor affecting palatability. In experiments with 2 functionally non-toxic *Alexandrium* spp. dinoflagellates (Fig. 4) there was no significant difference in grazing rates, i.e. no grazing preference. All of these results lead to the following conclusions: (1) the copepods examined here can recognize *Alexandrium* spp. cells containing PSP toxins by chemosensory means, (2) if all else is equal PSP toxins render cells less palatable, (3) when such copepods are grazing in suspensions of more than 1 cell type, as would be the case in most natural environments, grazing of *Alexandrium* spp. dinoflagellates is governed by behavioral selection of prey, not physiological impairment due to ingested toxins.

One aspect of the results seems troubling; if toxic *Alexandrium* spp. cells are less palatable, why were they consumed in any quantity in all of the selection experiments? Could this be explained as a trade-off between the increased handling time involved in selection, and the apparent susceptibility to PSP toxins of each of the copepod species examined? *Acartia tonsa* is intolerant of PSP toxins (as seen in the strong impairment suffered in the monoculture grazing experiment),

Centropages hamatus is more tolerant, but still will not consume monocultures of toxic cells at the same rate as non-toxic cells, and *Eurytemora herdmani* is moderately tolerant, showing no significant difference in grazing rates on toxic or non-toxic monoculture diets. In the selection experiments reported here, *A. tonsa* demonstrated very strong avoidance of toxic *Alexandrium* spp., *C. hamatus* avoided toxic cells to a lesser degree, and *E. herdmani*, while capable of avoiding toxic cells (Fig. 3b), was not constrained to do so (Fig. 3a). Physiological impairment was not observed in any of the selection experiments, and thus it is unlikely that ingestion of toxic cells by each of the 3 copepod species was sufficient to affect grazing processes. Since each *Alexandrium* spp. clone was present in equal, moderately high concentrations (200 cells ml^{-1} each), it is likely that copepods consumed toxic cells in proportion to their ability to tolerate PSP toxins.

Lehman (1976) presented one of the first models of optimal foraging for selective 'filter feeders' such as copepods. In this and subsequent models, handling time was assumed to be negligible, and gut residence or evacuation was considered to be the principal time constraint. Such models further predict that selective feeding would be most noticeable when food concentrations are high, but that grazers would be less selective at lower food concentrations. If a copepod species such as *Acartia tonsa* (which is intolerant of PSP toxins) must practice selective grazing, but has ample alternate food, then this food should be consumed at rates limited only by digestive capacity. Yet individual *A. tonsa* consumed on average 4.7 µg (estimated 105% body carbon) d^{-1} in selection experiments (Fig. 1), while in a monoculture of non-toxic *Alexandrium tamarense* CCMP 115 each *A. tonsa* consumed an average of 8.88 µg or 196% body carbon d^{-1} (Table 2). Clearly the copepods from the selection experiments were not maximizing their daily intake. The very low number of toxic cells ingested argues against a physiological basis for this result; at the same time, it is difficult to imagine that the grazing rates, which correspond to less than 100 cells $copepod^{-1}$ h^{-1}, were limited by handling time. At this time there is not sufficient evidence to rule out the cost of handling as a factor that may affect grazing rates in mixtures containing high concentrations of toxic cells.

On the other hand, *Eurytemora herdmani* consumed equivalent daily rations in virtually all of the experiments in this study (60 to 80% body carbon d^{-1}), whether it practiced selection or not, even in monocultures of toxic *Alexandrium* spp. Addressing selective grazing mechanisms of freshwater and marine zooplankton in the context of optimal foraging models, DeMott (1990) remarked that 'calanoid copepods show strong, invariant selection against toxic algae,' arguing that abstinence is still preferable to consumption of inimical food items. The fact that *E. herdmani* would consume significant quantities of toxic cells when non-toxic cells were abundant and food was not limiting, and the species is apparently capable of toxin detection and selection by chemosensory means, raises interesting questions. It is likely that this species has developed a tolerance to PSP toxins, similar to that postulated for certain cladocera and cyanobacterial toxins (DeMott & Moxter 1991). Are both *Acartia tonsa* and *E. herdmani* practicing optimal foraging? Not all copepod species must reject toxic cells, nor do species capable of selection necessarily obtain a maximal rate of energy gain when alternate food is abundant. It is possible by using ad hoc arguments to conclude that these results may be recognized as optimal foraging within the physiological constraints of the individual species, but the utility of optimal foraging models to *predict* copepod behavior in the presence of toxic cells is limited. One must conclude that grazer response to toxic prey will vary among species, and that grazing impact on blooms of *Alexandrium* spp. will depend on the composition of the zooplankton community.

Mixed dinoflagellate species grazing experiments

The experiments with several species of dinoflagellate prey performed in this study provide perhaps the best illustration of the difference in palatability between toxic and non-toxic *Alexandrium* spp. One of the most striking results from these experiments is the preference of all 3 copepod species for non-toxic *A. tamarense* CCMP 115 over other non-toxic and apparently palatable and nutritious dinoflagellate species (Figs. 5a, 6a & 7a). Such consistent results strongly suggest that non-toxic *Alexandrium* spp. are perceived as high-quality food, and may be selectively consumed by zooplankton relative to other available prey. Dinoflagellates typically have high nitrogen content and large cytoplasmic volume relative to many other phytoplankton types (Hitchcock 1982), and zooplankton preference for dinoflagellates over other phytoplankton has been suggested from both laboratory and field work (e.g. Morey-Gaines 1980, Kleppel et al. 1991). Turriff et al. (1995) found selective grazing by *Calanus finmarchicus* on non-toxic *A. tamarense* over the diatom *Thalassiosira weisflogii*, and Teegarden & Cembella (1996) observed preference for *A. tamarense* over *Lingulodinium polyedrum* by the copepod *Eurytemora herdmani*, despite the presence of a low level of toxin in the *A. tamarense* clone. The results of the present study and those mentioned above suggest that non-toxic or low-toxicity *Alexandrium* spp. are highly susceptible to grazing losses.

Substitution of toxic *Alexandrium fundyense* GTCA 28 in the second mixed-species treatments resulted in significant reductions in grazing rates on that clone compared to non-toxic *A. tamarense* CCMP 115 for all 3 copepod species, underscoring the fact that the presence of PSP toxins renders *Alexandrium* spp. less palatable to grazers. Nevertheless the toxic clone was not universally rejected, nor was it necessarily consumed at much lower rates than alternative prey species. *Acartia tonsa* grazed toxic *A. fundyense* GTCA 28 at significantly lower rates than most other dinoflagellate species (except *Prorocentrum micans*, Fig. 5b), consistent with the apparent inability of *A. tonsa* to tolerate PSP toxin ingestion (see monoculture experiment discussion). *Centropages hamatus* grazed toxic *A. fundyense* GTCA 28 at a significantly higher rate than *P. micans*, and *Eurytemora herdmani* grazed *A. fundyense* GTCA 28 at rates comparable to *Gonyaulax cochlea* and *P. micans* (Figs. 6b & 7b). Thus for these 2 copepod species, consumption of the toxic clone was not prevented or even strongly inhibited, but merely reduced to a level comparable to those on alternate dinoflagellate species. One may conclude from these results that the presence of PSP toxins in the *A. fundyense* GTCA 28 clone confers the definite advantage of reducing that dinoflagellate's palatability or desirability compared to *Alexandrium* spp. without PSP toxins, but does not cause them to be avoided or selected against by all grazer species.

Reduction of grazing pressure on *Alexandrium* spp. due to the presence of toxin often resulted in increased grazing pressure on alternate species of dinoflagellates in mixtures. If selective grazing is practiced in natural environments, copepods may exert a greater grazing pressure on alternate phytoplankton species, essentially competitors of *Alexandrium* spp., in order to maintain daily rations. *Centropages hamatus* and *Eurytemora herdmani* maintained equivalent rations in both treatments. These 2 species of copepods were therefore flexible enough to switch particle preference in the presence of toxic prey to ensure consumption of a sufficient ration. Only *Acartia tonsa* failed to maintain equivalent carbon rations in both treatments. In the first (non-toxic) treatment, 39% of *A. tonsa* total carbon ration came from non-toxic *A. tamarense* CCMP 115, significantly more than any other dinoflagellate species. Although *A. tonsa* switched grazing preference to *Lingulodinium polyedrum* (54% of the total ration) in the second (toxic) treatment, consumption of all species was not sufficient to equal total carbon consumed in the first treatment. The reason for this discrepancy is not clear at this time; the copepods were healthy and active at the end of experiments, so it is unlikely that physiological impairment inhibited grazing activity. It is possible that the increased costs of handling and selective ingestion in the second treatment were sufficient to depress overall carbon consumption, but without actual measurements of energetic expenditures it is impossible to suggest this with confidence. It is also possible that the *Gonyaulax cochlea* and *Prorocentrum micans* were not sufficiently palatable to *A. tonsa*, and without the preferred non-toxic *A. tamarense*, *A. tonsa* was not induced to increase consumption of those alternate species to make up for reduced consumption of *Alexandrium* spp.

Monoculture grazing experiments

When *Acartia tonsa*, *Centropages hamatus* and *Eurytemora herdmani* were offered monocultures of *Alexandrium* spp. as an exclusive diet, the differential susceptibility of the copepod species to the effects of PSP toxins was apparent. *A. tonsa* and *C. hamatus* did not maintain equivalent carbon consumption rates between the 2 treatments. The greatest discrepancy occurred in *A. tonsa* grazing trials. *A. tonsa* fed non-toxic *A. tamarense* CCMP 115 grazed at a very high rate, >196% body C d^{-1} (Table 2), and copepods were healthy and active, and had produced eggs. Copepods from the concurrent treatment fed a monoculture diet of toxic *A. fundyense* GTCA 28 consumed only 61% body C d^{-1}, and were clearly impaired; copepods were motionless at the bottom of the experimental containers, and displayed no response upon examination, although most were still living (mortality <10%). *A. tonsa* is a species that relies on regular consumption of food, and does not tolerate starvation (Dagg 1977, Durbin et al. 1983). This requirement may have overridden a behavioral aversion to consumption of toxic *Alexandrium* spp., and induced sufficient grazing to effect physiological incapacitation when no other food was available. The fact that severe impairment was apparent at a moderate rate of consumption of toxic *A. fundyense* GTCA 28 indicates that this species has a low tolerance for ingestion of PSP toxins, and if selective grazing is not allowed, as in the very unusual case of a virtually monospecific bloom of *Alexandrium* sp., grazing (and thus growth and production) of *A. tonsa* may be inhibited by physiological impairment.

Centropages hamatus also displayed significantly different grazing rates between monoculture *Alexandrium* spp. diets. Rates of consumption were 47 and 33% of body carbon d^{-1} for non-toxic *A. tamarense* CCMP 115 and toxic *A. fundyense* GTCA 28 respectively (Table 2). Consumption of toxic cells was moderately high, yet did not result in any observable impairment of copepods, and food was observed in the guts of

copepods from that treatment. This species apparently can tolerate moderate consumption of toxic cells, and is not likely to ever suffer impairment in natural situations. The reduced consumption by *C. hamatus* of toxic compared to non-toxic *Alexandrium* spp. cells in monocultures appears to reflect a behavioral bias against toxic prey. Although maximum daily rations were not attained on toxic *Alexandrium* spp. cells alone, severe starvation is unlikely, since in this experiment *C. hamatus* maintained average body carbon over 24 h (Table 2).

Eurytemora herdmani did not exhibit significant differences in grazing rates on the 2 *Alexandrium* spp. monoculture diets. This is consistent with the conclusion that *E. herdmani* is generally tolerant of PSP toxin consumption, augmented by the fact that impairment of this species was never observed in any of the experiments conducted in this study. Such tolerance probably allows *E. herdmani* to obtain its daily ration when no other food is available. Significant growth did occur in the non-toxic treatment (Table 2), but there was no significant difference between initial copepod samples and copepods which had fed on toxic *A. fundyense* GTCA 28. It is not known at this time whether ingestion of toxin has any effects on the copepod's ability to assimilate food, or why there should be a difference in growth between the 2 diets when no difference in grazing rates was discerned. Possible effects of long term exposure to PSP toxins on physiology and production are also not known at this time.

On the function of PSP toxins

The idea that PSP toxin production in *Alexandrium* spp. is an adaptation for grazer defense, and that defense is the function (*sensu* Williams 1966) of PSP toxins, has not been universally accepted, often because of the failure of PSP toxins to provide perfect defense, e.g. as demonstrated here with the copepod *Eurytemora herdmani*. Another problem stems from earlier arguments that PSP toxins could not be detected prior to ingestion, and that effects were physiological (Ives 1985, 1987, Huntley et al. 1986). If defense cannot be effected prior to ingestion, then the only benefit of the chemical deterrent would be for the species, when some cells in a population are saved by the sacrifice of other individuals. Such a case suggests natural selection at the group level, which most biologists are unwilling to support as powerful enough for development of an adaptation (Williams 1966). Numerous 'effects' on grazers have been attributed to PSP toxins (see Turner & Tester 1997 and references therein), including reduced growth and inhibited production of future generations, but unless a defense can be discerned which benefits individual *Alexandrium* spp. cells, it is difficult to argue that the 'function' of PSP toxins in *Alexandrium* spp. is to provide defense against grazers.

The results of the experiments reported here strongly suggest that cells containing PSP toxins can be discerned by grazers prior to ingestion. The results of the mixed dinoflagellate species experiments in particular demonstrate that non-toxic *Alexandrium* spp. cells are highly susceptible to grazing losses, while the presence of toxin in *Alexandrium* spp. renders such cells much less palatable; even to grazers such as *Eurytemora herdmani* that are apparently tolerant of PSP toxins. The selection experiments further demonstrate that cells which 'taste bad' can be rejected without mortal damage. Shaw et al. (1997), in a study combining experimental and modeling analyses of copepod response to purified toxins (including PSP toxins), argued convincingly that PSP toxins act as deterrents, which can be recognized by chemosensors, rather than as toxins which induce physiological impairment or mortality. Haney et al. (1995) concluded that the cladoceran *Daphnia carinata* responded to purified saxitoxin in a manner consistent with chemosensory stimulation, not physiological inhibition. Yamamori et al. (1988) have postulated the existence of chemosensors in fish which can 'taste' PSP toxins. One may conclude from all of these results that PSP toxins are distasteful, and that a good number of marine species are probably capable of sensing and avoiding prey containing PSP toxins.

This defense is not infallible, since toxic *Alexandrium* spp. cells may be consumed at moderately high rates, as seen in this study with *Eurytemora herdmani* and (to some extent) *Centropages hamatus*. But a perfect defense is not required for the evolution of a chemical deterrent. The possibility that some zooplankton species (e.g. *E. herdmani*) may have developed tolerance to toxin ingestion does not alter the fact that non-toxic *Alexandrium* spp. cells were consistently preferred in the diet relative to toxic *Alexandrium* spp. cells. Across all experiments and all copepod species examined here, toxic *Alexandrium* spp. cells enjoyed a distinct advantage over non-toxic *Alexandrium* spp. cells in the presence of selective grazers. If a toxic *Alexandrium* spp. cell is more likely to avoid being consumed than a non-toxic *Alexandrium* spp. cell, and it can be rejected without suffering mortal damage, it therefore has a greater probability of surviving long enough to reproduce and proliferate. Thus natural selection at the individual level should favor the production of PSP toxins by *Alexandrium* spp. in many environments, and it is reasonable to consider PSP toxin production an evolved adaptation for grazer deterrence.

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