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Copepod feeding selectivity on microplankton, including the toxigenic diatoms *Pseudo-nitzschia* spp., in the coastal Pacific Northwest

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ABSTRACT: As part of the Pacific Northwest ECOHAB project, we measured clearance rates and feeding selectivity of calanoid copepods off the coast of Washington State, USA, during fall of 2003. We tested the hypothesis that copepods discriminate amongst prey, particularly against the toxic diatoms *Pseudo-nitzschia* spp. in natural assemblages from this highly productive, upwelling environment. Seven grazing experiments were conducted across and along the shelf using the copepods *Calanus pacificus*, *Metridia pacifica*, *Acartia longiremis* and a small community assemblage dominated by *Acartia* spp., with minor contributions from *Pseudocalanus* spp., *Paracalanus* spp. and *Oithona* spp. Three general patterns emerged from our experiments. First, all copepods, except *A. longiremis* in 1 experiment, showed neutral preference or discriminated against *Pseudo-nitzschia*, but preference did not appear related to cellular domoic acid concentrations. Second, the dominant prey biomass contributors in each experiment were cleared at low rates relative to other prey types. In most cases the dominants were the diatom *Thalassiosira* spp. or the autotrophic dinoflagellates *Ceratium* spp. and *Prorocentrum* spp. The third pattern was high preference for microzooplankton. High clearance on microzooplankton can result in trophic cascades, which were evident in our size-fractionated chlorophyll data. These patterns indicate that copepods could have both direct and indirect effects on the plankton community composition on the Washington coast. However, our estimates of total potential grazing suggest that copepod grazing impact on *Pseudo-nitzschia* populations is negligible.

KEY WORDS: Copepod grazing · Prey selectivity · *Pseudo-nitzschia* · Microzooplankton

INTRODUCTION

Toxigenic diatoms in the genus *Pseudo-nitzschia* are widespread off the Washington coast where unique oceanographic characteristics (MacFadyen et al. 2005) appear to promote their growth, bloom formation, retention and toxicity (Horner et al. 2000, Trainer et al. 2002). At least 7 species of *Pseudo-nitzschia* have been found to occur on the Washington coast (Stehr et al 2002). Not all *Pseudo-nitzschia* spp. produce the marine neurotoxin domoic acid (DA), and for those that do, production of DA varies spatially and temporally (Trainer et al. 2002). The stimulus that induces DA production in the field remains ambiguous; there is evidence for increased DA production under phosphate and silicate limitation (e.g. Fehling et al. 2004), the presence of bacteria (Bates et al. 1995), elevated pH (Lundholm et al. 2004) and trace metal concentration (Maldonado et al. 2002).

Pseudo-nitzschia spp. are often the sole diatoms remaining after the spring and summer upwelling-induced diatom bloom events, and are found co-occurring and proliferating with taxa identified as members typical of 'recycled nutrient' communities (e.g. autotrophic dinoflagellates). Their wide spatial distribution, and more specifically, their long retention in the surface layer in diverse environmental conditions, implies that either reduced mortality rates, unique nutrient acquisition strategies (Wells et al. 2005), or both, play a significant role in the population ecology of these diatoms.

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As with any alga, the formation of a *Pseudo-nitzschia* bloom depends on imbalance that favors phytoplankton growth over mortality rate (Banse 1994). Once a bloom forms and exponential growth ceases, the retention and maintenance of the bloom in the water column becomes a near exclusive function of mortality rate. *Pseudo-nitzschia* is one of the few phytoplankton genera that are consistent and prevalent members of the global plankton community (Rhodes 1998, Cusack et al. 2002, Kaczmarska et al. 2005); this suggests that the balance between growth and mortality is consistently biased in favor of the former. In order to understand the population dynamics of *Pseudo-nitzschia* it is therefore important to study not only the factors that influence rates of cell division, but also the rates and relevant agents of mortality. Arguably, the most significant source of external mortality to phytoplankton is zooplankton grazing (Calbet & Landry 2004). Consequently, any exploration of *Pseudo-nitzschia* mortality should start there.

Potential grazers of *Pseudo-nitzschia* spp. include the calanoid copepods. Unfortunately, our confidence in the knowledge that copepods will unreservedly graze on *Pseudo-nitzschia* is poor; to date no field experiments have explored this relationship in detail. Some copepods are selective feeders, discriminating prey based on a multitude of factors. Laboratory studies have established some of these factors to be size (Frost 1972), food quality (e.g. Cowles et al. 1988), chemical cues (Huntley et al. 1986) and toxin content of the prey (e.g. Teegarden 1999). These findings would suggest that, when offered a diverse prey suite, discriminate feeding by copepods may shape both microplankton community size structure and species composition. However, the degree to which selective grazing behavior shapes natural communities remains contradictory (Huntley 1982, Turner & Tester 1989, Meyer-Harms et al. 1999, Irigoien et al. 2000), and may vary under different environmental conditions, grazer communities and prey suites — including those that harbor toxic phytoplankton (Teegarden et al. 2001, Colin & Dam 2002, Kozlowsky-Suzuki et al. 2005).

The fastidiousness with which copepods feed, coupled with the ubiquity of *Pseudo-nitzschia* spp., both on the Washington coast and worldwide, led us to pose the hypothesis that this genus escapes intense copepod grazing. Among the candidate mechanisms that would reduce grazing mortality, an inviting hypothesis is to assume a functional role of the *Pseudo-nitzschia*-produced toxin, domoic acid (DA). Compared to the suite of dinoflagellate-produced saxitoxins (e.g. Colin & Dam 2002 and references therein) little is known about the role of DA in grazer interactions (Lincoln et al. 2001, Bargu et al. 2003, Maneiro et al. 2005). Regardless of the cause, a refuge from grazing mortality will positively affect *Pseudo-nitzschia* population ecology by 3 routes: (1) directly through reduced grazing mortality, (2) by reducing competition through copepod consumption of other phytoplankton, and (3) through grazing on microzooplankton — another potential *Pseudo-nitzschia* grazer. To test this hypothesis, we conducted grazing experiments with the locally dominant copepod species in natural plankton assemblages along the coast of Washington State, USA. This study site is an ideal location to test this hypothesis, as physical and chemical properties, prey biomass and community composition vary greatly on short time and space scales. Clear patterns of reduced grazing on *Pseudo-nitzschia* under these wide-ranging prey conditions would indicate habitual grazing behaviors and, ultimately, suggest that copepod grazing influences *Pseudo-nitzschia* population ecology.

MATERIALS AND METHODS

Copepod grazing experiments were conducted on the Washington coast on board the RV 'Wecoma' from September 1 to 16, 2003, as part of the Pacific Northwest ECOHAB (ECOlogy of Harmful Algal Blooms) project. The sole criterion for selecting experimental stations was the presence of ample *Pseudo-nitzschia* cells and, because of this single constraint, experimental stations (Fig. 1) were widespread spatially, representing both onshore and offshore locations, and resulted in diverse chemical/physical conditions (Table 1) and prey communities and concentrations (see Table 2). The cross-shelf spacing of our experimental locations provided the opportunity to work with the regional dominant copepod genera *Calanus*, *Metridia* and *Acartia*, which represented a large size spectrum and contrasting feeding behaviors. The numerically dominant species of copepod at each location was used for experiments. Initial experimental conditions, copepod species tested and treatment replicates are shown in Table 1.

Grazing experiments. We conducted 7 copepod grazing experiments. Copepods were collected with vertical net tows using a 1 m diameter ring net with 200 µm mesh. Vertical tows were done to a maximum depth of 200 m, but at shallow stations to ~5 m off the bottom. Once on deck, healthy copepods were immediately diluted into 2 l polyethylene containers holding raw seawater where they were allowed to 'recover' (for ~0.5 h) from the net tows. Individual adult female copepods were selected under a stereomicroscope with an inverted Pasteur pipette and placed in 30 ml beakers containing filtered seawater until the start of the experiment (~1 h).

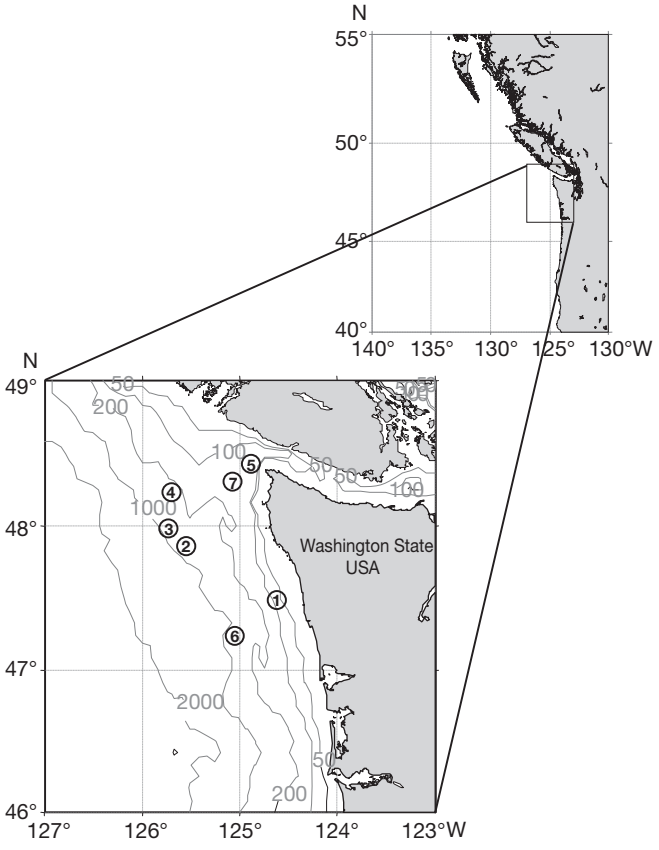

Fig. 1. Locations of Stns 1 to 7 off the coast of Washington State, USA, used for the grazing experiments. Depth contours (m) are shown

Seawater for grazing experiments (GEW) was collected from the depth of ~50% surface PAR using Niskin bottles attached to a CTD rosette equipped with Sea-Bird environmental sensors. Once on deck, the Niskin bottles were gently drained through acid-cleaned silicone tubing (8 mm inner diameter) into acid-cleaned 50 l polyethylene carboys. Undesired mesozooplankton were removed from the GEW with 200 µm mesh netting attached to the silicone tubing. The plankton in the GEW were kept homogenized by slowly raising and lowering a polyethylene plunger through the water. At the same time, the well-mixed GEW was gently siphoned into 2.5 l acid-cleaned polycarbonate bottles for the control and grazing experiment treatments (see Table 1 for replicate numbers). To ensure that phytoplankton growth rates were unaffected by nutrient depletion or copepod grazing-induced nutrient remineralization, we added a nutrient mixture to all bottles: 10 µM NO_3^- added as $NaNO_3$, 10 µM $Si(OH)_4$ added as $Na_2O_3Si \cdot 9H_2O$, 0.63 µM PO_4^{3-} added as $NaH_2PO_4 \cdot H_2O$, 3 nM Fe dissolved in 2% HCl and 0.3 nM Mn added as $MnCl_2 \cdot 4H_2O$. While filling grazing and control bottles, samples for initial size fractionated (>5 µm and <5 µm) chlorophyll *a* (chl *a*) and microplankton counts were randomly taken in between filling experimental bottles. Separate samples for microplankton were immediately fixed in 5% Lugol's acid or fixed within ~20 min of collection in 0.5% glutaraldehyde.

Once the grazing bottles had been filled to near capacity, the copepods were added. The number added to the experimental bottles varied with species and location (Table 1). Control and grazing bottles were placed in clear tubes covered with mylar screening to approximate *in situ* light levels. The tubes were secured to a revolving wheel (1 rpm) which was submerged in a Plexiglas on-deck incubator. The temperature inside the incubator was maintained by continuous flow of surface seawater.

Incubations lasted 24 h, after which the bottles were removed from the incubator and the copepods' condition immediately assessed. Copepods appeared healthy and mortality was negligible; therefore corrections for copepod mortality were not made to final rate measurements. Samples were taken for size fractionated chl *a* analysis and microplankton abundances for all control and treatment bottles as per experimental set up. Net growth rates of chlorophyll size fractions and microplankton taxa, as well as copepod clearance were all calculated using the equations of Frost (1972).

Sample processing. ***Microplankton enumeration and biomass estimation:*** Aliquots from the Lugol's pre-

Table 1. Initial conditions for copepod selection experiments in 2003. Temperature, salinity and nutrient salts measured in samples from water depth that corresponded to 50% surface irradiance. Number(s) following individuals per bottle are numbers of treatment replicates

Expt. no.	Date (m/dd)	Location °N Lat	Location °W Long	Copepod species	Ind. bottle^{-1}	Salinity	*T* (°C)	Nutrients (µM) NO_3^-	PO_4^{3-}	SiO_4^{2-}
1	9/01	47 31.67	124 38.49	*Acartia longiremis*	25 (3)	32.8	10.1	18.3	1.31	36.6
2	9/03	47 52.99	125 32.02	*Metridia pacifica*	20 (2)	32.0	13.4	1.1	0.36	12.1
3	9/06	47 59.03	125 43.39	*Metridia pacifica*	50 (3)	31.2	13.9	4.1	0.50	26.2
4	9/09	48 15.70	125 40.04	*Acartia longiremis*	75 (2)	31.6	13.4	9.3	0.87	29.6
5	9/12	48 26.25	124 51.36	*Calanus pacificus*	20 (2)	32.5	11.5	6.2	0.58	25.5
6	9/14	47 15.62	125 03.70	*Metridia pacifica*	40 (2)	31.9	15.0	0.7	0.37	6.0
7	9/16	48 19.36	125 04.11	Small assemblage	150 (4)	32.2	11.8	14.1	1.28	32.0

served GEW were allowed to settle for 24 h in either Utermöhl chambers or graduated cylinders, depending on the volume needed to accurately quantify the sample. For abundant prey taxa, several millilitres were settled directly in chambers; for the more infrequent prey taxa, larger volumes were settled in graduated cylinders for 24 h. The following day, the top filtrate was decanted off, while the bottom 10 ml containing the prey was resuspended and allowed to settle in a counting chamber for 24 h prior to counting. Slides of glutaraldehyde-fixed cells were prepared for epifluorescent microscopy according to Lessard & Murrell (1996). Prey cells in Utermöhl chambers and glutaraldehyde-fixed slides were counted and measured with a digitizing pad, and linear dimensions were converted to biovolume according to Roff & Hopcroft (1986). Prey biovolume was converted to cellular C using the equations of Menden-Deuer & Lessard (2000). Between 30 and 500 cells were counted for each prey type.

Pseudo-nitzschia spp. were separated into 2 size categories, small and large, based upon the width of the cell valve. We considered cells with a transapical axis narrower than 3 µm to be small cells and part of the *P. pseudodelicatisima/delicatissima/cuspidata* species complex. Cells with a transapical axis wider than 3 µm were considered large cells and part of the *P. australis/fraudulenta/heimii* and *P. pungens/multiseries* species complexes (Horner 2002).

Chlorophyll: Change in size-fractionated chl *a* over the course of the experiments was used as a proxy to determine phytoplankton community growth and grazing rates. Chlorophyll concentrations were calculated according to the acidification method of Parsons et al. (1984).

Domoic acid: Particulate DA, at or near the depth of the experimental water, was measured using the receptor binding assay (Van Dolah 1997, Trainer et al. 2002). Total particulate DA was normalized to *Pseudo-nitzschia* spp. cell biomass to give an approximate index of cellular toxicity.

Electivity indices. In order to determine the magnitude of copepod prey preference, we calculated electivity indices (Ivlev 1961). We used the selection coefficient and electivity index of Vanderploeg & Scavia (1979a,b):

$$W_i = F_i / \sum F_i$$

where W_i is the selection coefficient for each food type i, and F_i and ΣF_i are the clearance rate for food type i and the sum of clearance rates for all food types, respectively. From this, the electivity index (E_i^*) for all major food types within an experiment is calculated by

$$E_i^* = [W_i - (1/n)]/[W_i + (1/n)]$$

where n is the total number of prey types within the given experiment. This method is recommended by Lechowicz (1982) when prey abundances are unequal, and was used in a study analogous to ours by Teegarden et al. (2001). This method is convenient in that the index ranges between –1 and 1. Neutral preference for a prey type (i.e. prey is ingested in direct proportion to its availability in the environment) is indicated by 0, whereas positive values indicate preference and negative values indicate avoidance. The degree to which these values deviate from zero indicates the magnitude of preference.

RESULTS

Initial prey biomass and composition

Prey biomass across experiments varied by nearly 2 orders of magnitude, ranging from 34 to 1258 µg C l^{-1} (Table 1). The highest biomass occurred at the mouth of the Juan de Fuca Strait and the lowest biomass was found off the shelf. Total phytoplankton carbon ranged from 18 to 1212 µg C l^{-1}. The diatoms *Thalassiosira* spp., and the autotrophic dinoflagellates *Ceratium*

Table 2. Initial chlorophyll (µg chlorophyll l^{-1}), prey carbon concentration (µg C l^{-1}) and domoic acid concentrations in copepod selection experiments, September 2003. Ciliates include aloricate choreotrichs, holotrichs and tintinnids. H.dino: heterotrophic dinoflagellates; *Pseud*: *Pseudo-nitzschia* spp. nd: no data

Expt no.	Chlorophyll (µg l^{-1})			Biomass (µg C l^{-1})						Domoic acid	
				Autotrophic			Heterotrophic			ng l^{-1}	ng $µg^{-1}$
	>5 µm	<5 µm	Total	Diatom	Other	Total	Ciliates	H.dino	Total		*Pseud* C
1	10.12	0.60	10.72	187.7	8.3	196.0	5.5	14.0	19.5	0	0
2	8.89	0.93	9.82	47.0	22.6	69.6	15.3	10.2	25.5	40	11.5
3	13.44	2.82	16.27	24.3	94.6	118.9	28.9	23.4	52.3	378	180.0
4	3.26	1.77	5.03	5.6	45.1	50.7	16.6	18.1	34.7	93	116.3
5	21.28	0.58	21.86	848.1	364.0	1212.1	17.8	28.7	46.5	56	13.0
6	0.56	0.44	1.00	1.8	21.4	23.2	4.7	6.4	11.1	2014	1549.0
7	1.46	1.05	2.52	9.6	8.8	18.4	11.0	7.9	18.9	nd	nd

Table 3. Mean (± SE) initial prey carbon and abundance estimates in experiments conducted with the natural plankton assemblage at locations off the coast of Washington State in 2003. Dates given in form m/dd/yy. Number(s) following date of experiment are experimental locations (Fig. 1). Auto: autotrophic; Gymnodinoid: gymnodinoid heterotrophic dinoflagellates. –: prey negligible

Prey	*Acartia longiremis*		*Metridia pacifica*			*Calanus pacificus*	Small assembl.
	8/31/03 (1)	9/9/03 (4)	9/3/03 (2)	9/6/03 (3)	9/14/03 (6)	9/11/03 (5)	9/16/03 (7)
Small *Pseudo-nitzschia* spp.							
Cells ml^{-1}	199.0 ± 43.2	31.4 ± 3.7	109.2 ± 17.5	68.5 ± 6.3	4.2 ± 0.9	156.4 ± 5.3	19.7 ± 4.3
µg C l^{-1}	2.4 ± 0.3	0.3 ± 0.0	1.2 ± 0.1	0.6 ± 0.1	0.1 ± 0.0	1.7 ± 0.1	0.2 ± 0.1
Large *Pseudo-nitzschia* spp.							
Cells ml^{-1}	19.4 ± 0.6	6.6 ± 2.6	19.2 ± 0.3	15.6 ± 0.7	11.3 ± 2.4	32.0 ± 1.4	5.2 ± 1.9
µg C l^{-1}	1.8 ± 0.1	0.5 ± 0.3	2.3 ± 0.1	1.5 ± 0.2	1.2 ± 0.3	2.6 ± 0.2	0.3 ± 0.1
Thalassiosira spp.							
Cells ml^{-1}	785.9 ± 24.6	5.3 ± 0.1	46.0 ± 1.2	30.6 ± 3.1	–	3708.8 ± 70.0	0.5 ± 0.1
µg C l^{-1}	178.0 ± 4.2	1.5 ± 0.1	35.3 ± 0.7	19.4 ± 4.2	–	843.8 ± 14.3	0.2 ± 0.0
Chaetoceros spp							
Cells ml^{-1}	24.2 ± 5.3	–	6.2 ± 0.4	–	–	–	–
µg C l^{-1}	2.2 ± 0.3	–	0.7 ± 0.1	–	–	–	–
Corethron spp.							
Cells ml^{-1}	–	–	–	–	–	–	0.5 ± 0.1
µg C l^{-1}	–	–	–	–	–	–	0.4 ± 0.0
Coscinodiscus spp.							
Cells ml^{-1}	–	0.7 ± 0.2	–	–	–	–	0.8 ± 0.1
µg C l^{-1}	–	3.2 ± 0.1	–	–	–	–	8.4 ± 0.8
Guinardia spp.							
Cells ml^{-1}	12.7 ± 2.0	0.6 ± 0.1	1.4 ± 0.8	8.8 ± 1.7	–	–	–
µg C l^{-1}	2.5 ± 0.6	0.2 ± 0.1	0.7 ± 0.4	2.8 ± 0.5	–	–	–
Leptocylindrus spp.							
Cells ml^{-1}	27.9 ± 4.4	–	–	–	–	–	–
µg C l^{-1}	0.9 ± 0.2	–	–	–	–	–	–
Dictyocha spp.							
Cells ml^{-1}	–	0.2 ± 0.0	–	–	–	–	0.6 ± 0.1
µg C l^{-1}	–	0.0 ± 0.0	–	–	–	–	0.2 ± 0.0
Ceratium spp.							
Cells ml^{-1}	0.2 ± 0.0	2.8 ± 0.0	2.5 ± 0.1	7.9 ± 1.7	5.2 ± 0.6	26.4 ± 2.5	0.3 ± 0.1
µg C l^{-1}	1.6 ± 0.3	17.3 ± 0.9	17.5 ± 0.4	52.2 ± 12.2	19.3 ± 0.6	330.0 ± 37.1	1.4 ± 0.5
Prorocentrum spp.							
Cells ml^{-1}	1.7 ± 0.2	40.6 ± 0.6	3.5 ± 0.1	60.2 ± 5.7	0.4 ± 0.0	39.4 ± 1.1	7.6 ± 0.0
µg C l^{-1}	1.5 ± 0.3	19.3 ± 0.2	2.7 ± 0.1	29.8 ± 5.0	0.3 ± 0.0	24.7 ± 1.1	4.4 ± 0.6
Auto. dinoflagellate < 20 µm							
Cells ml^{-1}	24.4 ± 1.9	80.6 ± 22.0	19.1 ± 1.9	110.0 ± 12.5	22.5 ± 2.0	90.0 ± 6.4	31.2 ± 1.8
µg C l^{-1}	3.5 ± 0.0	6.7 ± 0.0	1.57 ± 0.6	9.5 ± 3.0	1.6 ± 0.2	7.8 ± 1.8	2.7 ± 0.1
Cryptophytes							
Cells ml^{-1}	33.7 ± 2.5	55.3 ± 23.4	15.0 ± 1.5	77.5 ± 17.5	7.2 ± 0.2	44.0 ± 1.1	12.9 ± 0.3
µg C l^{-1}	1.7 ± 0.0	1.8 ± 1.0	0.8 ± 0.3	3.1 ± 0.4	0.2 ± 0.0	1.5 ± 0.1	0.3 ± 0.1
Tintinnids							
Cells ml^{-1}	0.1 ± 0.0	1.1 ± 0.1	1.1 ± 0.2	1.7 ± 0.0	–	4.7 ± 0.3	1.0 ± 0.1
µg C l^{-1}	0.1 ± 0.0	0.6 ± 0.3	0.4 ± 0.1	0.8 ± 0.3	–	1.4 ± 0.2	0.2 ± 0.0
Ciliates > 40 µm							
Cells ml^{-1}	0.6 ± 0.0	1.7 ± 0.1	4.2 ± 0.1	4.2 ± 0.2	0.4 ± 0.6	2.5 ± 0.3	1.3 ± 0.0
µg C l^{-1}	2.5 ± 0.1	8.4 ± 0.6	12.4 ± 0.1	18.7 ± 1.5	1.3 ± 0.2	12.0 ± 2.4	3.1 ± 0.3
Ciliates 20–40 µm							
Cells ml^{-1}	1.7 ± 0.0	1.4 ± 0.1	1.5 ± 0.5	2.4 ± 0.1	0.7 ± 0.2	2.4 ± 0.1	2.9 ± 0.7
µg C l^{-1}	2.2 ± 0.1	2.6 ± 0.1	1.6 ± 0.5	4.3 ± 1.1	0.8 ± 0.2	3.6 ± 0.4	3.9 ± 1.9
Ciliates < 20 µm							
Cells ml^{-1}	3.1 ± 0.5	19.4 ± 2.8	2.7 ± 0.3	19.4 ± 0.2	10.0 ± 3.8	1.9 ± 0.4	16.6 ± 2.8
µg C l^{-1}	0.7 ± 0.1	5.0 ± 0.4	0.9 ± 0.1	5.1 ± 0.5	2.6 ± 0.9	0.8 ± 0.2	3.8 ± 0.5
Protoperidinium spp.							
Cells ml^{-1}	0.3 ± 0.0	0.5 ± 0.1	0.4 ± 0.0	0.6 ± 0.1	0.3 ± 0.0	1.0 ± 0.2	0.3 ± 0.0
µg C l^{-1}	1.7 ± 0.5	3.1 ± 1.8	2.0 ± 0.5	3.7 ± 0.4	2.9 ± 0.5	5.5 ± 1.6	0.9 ± 0.1
Gymnodinoid >40 µm							
Cells ml^{-1}	0.3 ± 0.0	1.9 ± 0.1	0.6 ± 0.0	1.8 ± 0.6	0.9 ± 0.0	3.3 ± 0.3	0.8 ± 0.2
µg C l^{-1}	0.4 ± 0.0	3.9 ± 0.0	1.4 ± 0.0	3.6 ± 0.7	0.9 ± 0.0	8.5 ± 1.1	1.0 ± 0.3
Gymnodinoid <40 µm							
Cells ml^{-1}	92.5 ± 5.0	99.3 ± 23.4	46.1 ± 0.4	113.7 ± 1.3	25.2 ± 0.8	105.0 ± 6.4	43.4 ± 1.5
µg C l^{-1}	11.9 ± 1.2	11.1 ± 2.0	6.8 ± 0.1	16.1 ± 7.0	2.6 ± 0.2	14.7 ± 0.4	6.0 ± 0.2

spp., *Prorocentrum* spp. contributed most to total biomass (Table 3). *Pseudo-nitzschia* spp. never contributed greatly to total biomass, but still comprised an appreciable portion of the prey suite, as represented by their cellular abundance (Table 3). Cyanobacteria and other picoeukaryotes were abundant at all sites, but because they are inefficiently grazed by calanoid copepods (Nival & Nival 1976), they were not included in the available prey suite. Total heterotrophic biomass ranged from 11 to 52 µg C l^{-1}. Heterotrophic dinoflagellates contributed most to microzooplankton biomass in 4 of the 7 experiments and contributed most to cell numbers in all experiments. Biomass differences between heterotrophic dinoflagellates and ciliates were not statistically different (paired *t*-test, $p >> 0.05$); however, because of the low sample size, statistical power was weak. The most dominant microzooplankton in both biomass and cell concentration were naked gymnodinoid dinoflagellates <40 µm in length of the longest axis. The larger heterotrophic dinoflagellates *Gyrodinium* cf. *spirale* and *Protoperidinium* spp. were present in all cases with appreciable biomass, especially during Expts 3, 4 and 5 (Table 3). Aloricate choreotrich ciliate cell concentration and biomass was substantial. Ciliate cell numbers were dominated by cells <20 µm in length and, in general, biomass varied evenly amongst experiments; 2 exceptions were Expts 3 and 5, in which biomass of ciliates >40 µm in length were 18.7 and 12.0 µg C l^{-1}, respectively. Holotrich ciliates were extremely rare and, with 1 exception (Expt 5), tintinnid ciliates were sparse.

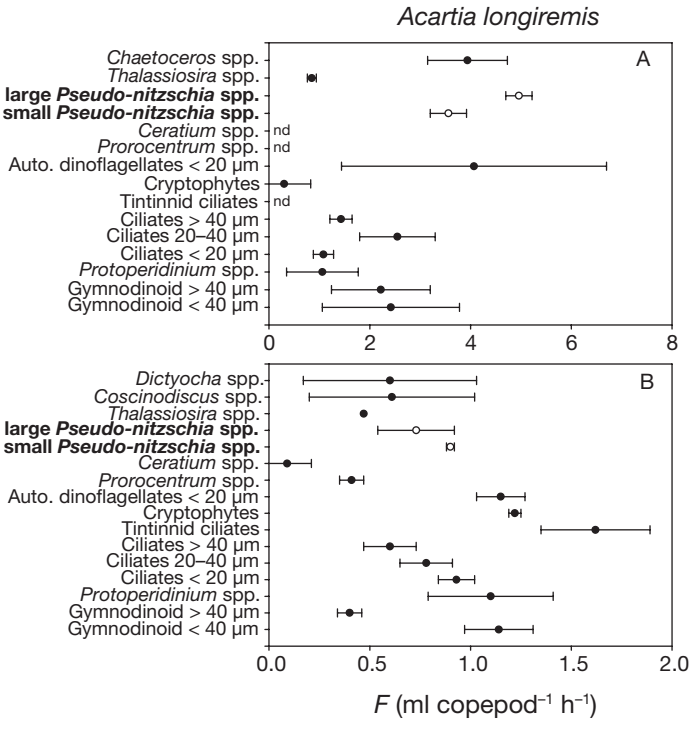

Fig. 2. *Acartia longiremis*. Mean ± SE clearance (i.e. filtering, *F*) rates on natural prey assemblages in (A) Expt 1 and (B) Expt 4. *Pseudo-nitzschia* spp. denoted by open symbols for clarity. nd: negative rate calculated; Gymnodinoid: gyrodinium and gymnodinium-like dinoflagellates; Auto.: autotrophic. Note different scales of abscissas

Copepod clearance rates and selectivity on prey taxa

No consistent pattern of copepod clearance was seen for any given prey species, including *Pseudo-nitzschia*, across all experiments (Figs. 2 to 5). *Acartia longiremis* cleared small and large *Pseudo-nitzschia* spp. cells at high rates in Expt 1, both in terms of total clearance and relative to other prey types. In Expt 4, *A. longiremis* total clearance was low for the entire prey suite, with *Pseudo-nitzschia* being cleared at rates equivalent to those for other prey (Fig. 2B). The highest clearance rates by *A. longiremis* in Expt 1 were on autotrophic prey, but were low for the biomass dominant *Thalassiosira* spp., which represented 83% of available prey. In Expt 4, *A. longiremis* cleared ciliates, specifically tinitinnid ciliates, and heterotrophic dinoflagellates at high rates. As in Expt 1, the biomass dominants *Ceratium divaricatum* and *Prorocentrum* spp. were cleared at the lowest rates.

Metridia pacifica cleared *Pseudo-nitzschia* spp. at low absolute and comparative rates in 2 of the 3 experiments illustrated (Fig. 3B,C). The highest rates of *Pseudo-nitzschia* clearance by *M. pacifica* was during Expt 2, which was coincident with the highest *Pseudo-nitzschia* biomass of the 3 experiments. *M. pacifica* cleared microzooplankton, especially large dinoflagellates and ciliates (>40 µm), at high rates in all experiments. As with *Acartia longiremis*, *M. pacifica* clearance rates of the biomass dominants was low. The exception to this was Expt 6, in which *M. pacifica* cleared *Ceratium furca* and *C. lineatum* (representing 57% of total prey biomass) at high rates (Fig. 3C).

Calanus pacificus cleared both small and large *Pseudo-nitzschia* spp. at ~2 ml copepod^{-1} h^{-1}, but these rates were low compared to those for the rest of the prey suite (Fig. 4). With the exception of autotrophic dinoflagellates <20 µm in diameter, only microzooplankton were cleared at rates higher than 3 ml copepod^{-1} h^{-1}. The highest clearance rates were on gymnodinoid dinoflagellates, including *Gyrodinium* cf. *spirale* (max. >8 ml copepod^{-1} h^{-1}). The high clearance rates on microzooplankton are surprising given that of 1258 µg C l^{-1}, only 48 µg C l^{-1} were represented by these heterotrophic prey.

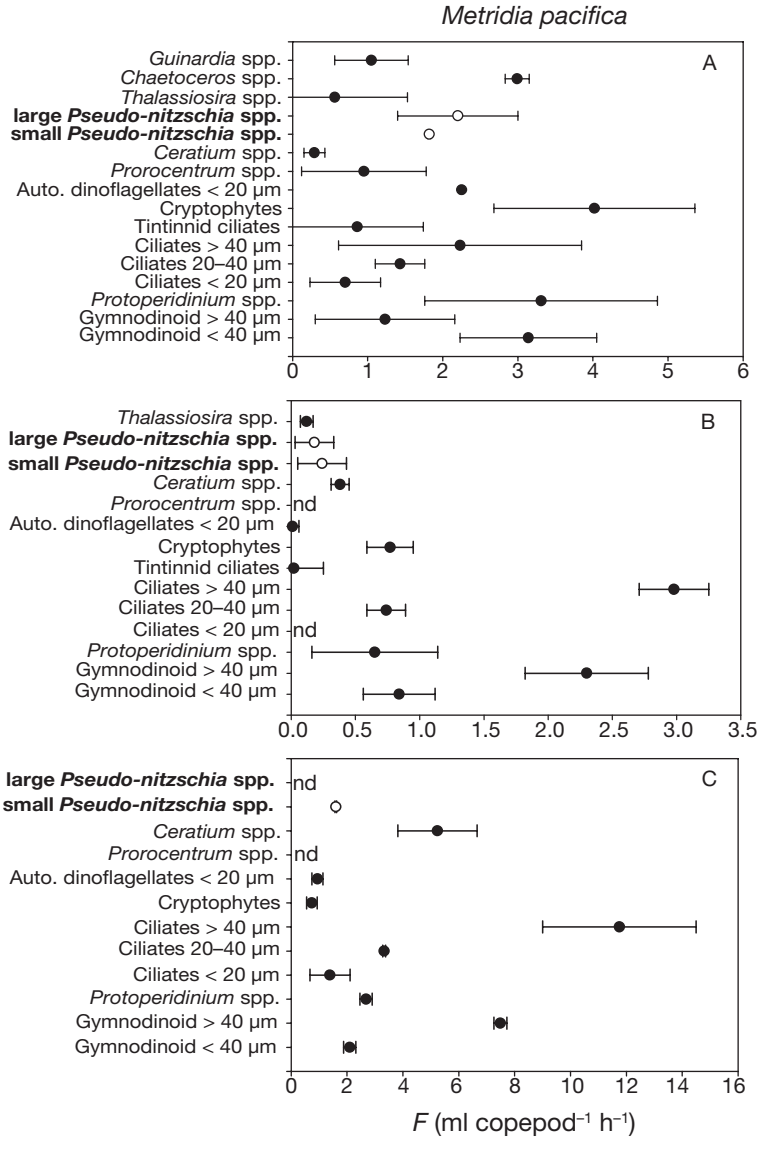

Fig. 3. *Metridia pacifica*. Mean ± SE clearance rates in (A) Expt 2, (B) Expt 3 and (C) Expt 6. Further details as in Fig. 2. Note different scales of abscissas

The small copepod assemblage cleared all prey types at extremely low rates, but especially for *Pseudo-nitzschia* spp., *Ceratium* spp. and *Dictyocha* spp. (Fig. 5). The highest clearance rates for this mixed copepod assemblage was on *Thalassiosira* spp., heterotrophic gymnodinoid dinoflagellates and ciliates <40 µm, and cryptophytes.

The electivity indices confirm the trends seen in the clearance rates. For *Acartia longiremis*, the E_i^* for both size classes of *Pseudo-nitzschia* spp. in Expt 1 were positively selected for, whereas in Expt 4, E_i^* was essentially zero, indicating no preference for *Pseudo-nitzschia* (Fig. 6). In both *A. longiremis* experiments prey biomass dominants were selected against. This was most evident in Expt 4 on the prey type *Ceratium* spp.

Of the 3 species of calanoid copepods, *Metridia pacifica* appears to be the most selective feeder. The mean absolute E_i^* value for the 3 *M. pacifica* experiments was 0.36, whereas for *Acartia longiremis*, *Calanus pacificus* and the small community, E_i^* values were 0.27, 0.28 and 0.31, respectively. *M. pacifica* showed a particularly high affinity for heterotrophic gymnodinoid dinoflagellates and ciliates >40 µm, with E_i^* values ~0.5 for both prey types in 2 of the 3 experiments illustrated (Fig. 7).

Calanus pacificus showed strong selection against the 2 large autotrophic dinoflagellates, *Ceratium* spp. and *Prorocentrum* spp., and for all diatoms present in the prey suite, including *Pseudo-nitzschia* spp. (Fig. 8). Positive selection was seen only for dinoflagellates and ciliates.

The small copepod community assemblage showed substantial selectivity (Fig. 9). The highest level of negative selection was seen on the large autotrophic dinoflagellates *Ceratium* spp. and on the silicoflagellates *Dictyocha* spp. Both large and small *Pseudo-nitzschia* spp. were also strongly selected against. Highest positive selection was seen on *Thalassiosira* spp., ciliates of 20 to 40 µm and heterotrophic dinoflagellates <40 µm.

DISCUSSION

Copepods generally selected against or had no preference for *Pseudo-nitzschia* spp. The sole exception was *Acartia longiremis*, which showed positive selection for *Pseudo-nitzschia* (perhaps slightly more for the larger cells) during Expt 1 (Fig. 6A). *Acartia* spp. are known to switch feeding modes between suspension feeding and ambush feeding (Kiørboe et al. 1996). The switch is an apparent attempt by the copepod to maximize energy intake rates, and may depend on the motile behavior of the most abundant prey types. For example, low abundance of non-motile prey (e.g. diatoms) and high abundance of motile prey (e.g. dinoflagellates) would induce a change from filter feeding to ambush feeding. In Expt 1, diatoms dominated prey biomass (85%), with *Thalassiosira* spp. representing 83% of the total biomass. It is likely that in this experiment *A. longiremis* was feeding by filtration and, consequently, the non-motile *Pseudo-nitzschia* were ingested at rates higher than those for motile prey. In contrast, motile prey, most specifically dinofla-

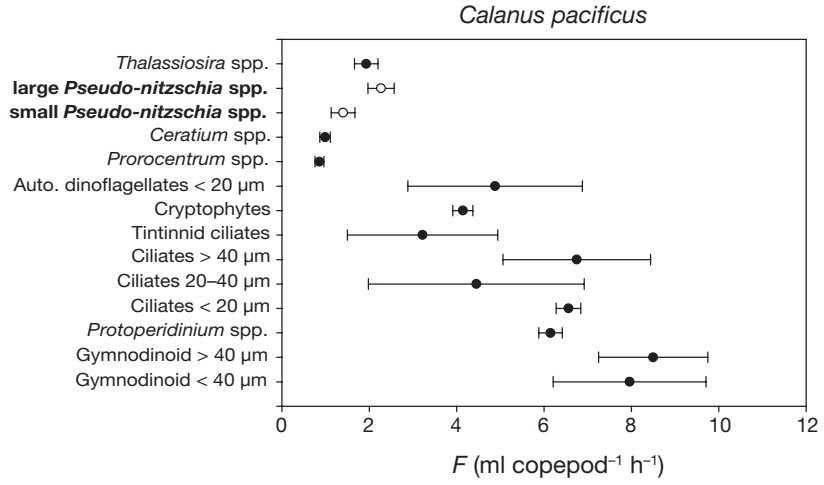

Fig. 4. *Calanus pacificus.* Mean ± SE clearance rates in Expt 5. Further details as in Fig. 2

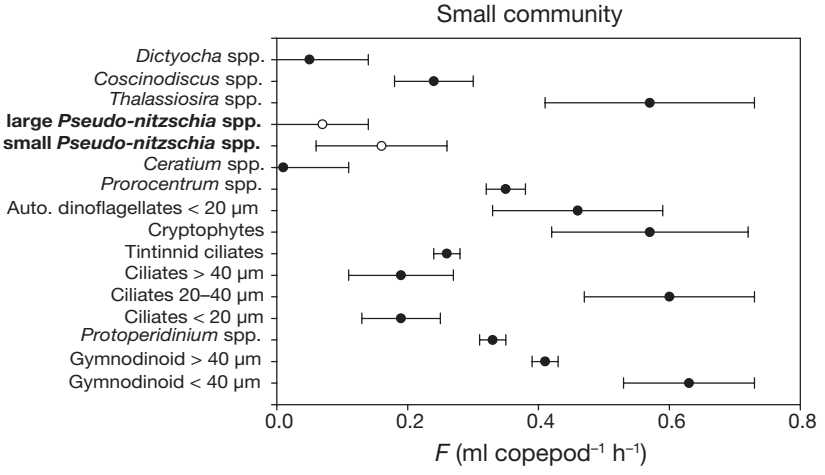

Fig. 5. Small copepod community assemblage. Mean ± SE clearance rates in Expt 7. Further details as in Fig. 2

gellates and ciliates, were the dominant biomass in Expt 4. In this experiment clearance rates were low; *Pseudo-nitzschia* appeared to be neutrally selected while other diatoms were *negatively* selected (Fig. 6B).

The fact that *Pseudo-nitzschia* spp. were generally not a preferred prey type is likely to be a consequence of the rich diversity of the prey assemblages in our study, rather than the toxicity of *Pseudo-nitzschia* cells. Several lines of reasoning support this conclusion. First, although strong positive selection for *Pseudo-nitzschia* by *Acartia longiremis* was observed in the single experiment (Expt 7) in which DA was undetectable (Table 2), the data do not show a consistent trend of stronger negative selection towards the most toxic cells. Moreover, most negative preference indices towards *Pseudo-nitzschia* were within (and usually much less than) the range for non-toxic prey types. Second, other studies (Lincoln et al. 2001, Maneiro et al. 2005) have found calanoid copepod feeding rates and selectivity to be the same between toxic and non-toxic *Pseudo-nitzschia.* Third, it is likely that in order for the copepods to achieve the required balanced diet, selection towards non-diatom prey is essential when diatoms dominate the prey suite (Jones & Flynn 2005). In our experiments, diatom biomass was consistently substantial and, in 2 experiments, quite high. Therefore, *Pseudo-nitzschia* spp. may have been avoided because they are diatoms.

Perhaps another candidate mechanism that prevents *Pseudo-nitzschia* spp. from being selected is their needle-like shape and propensity to form chains. Due to the higher abundance of small *Pseudo-nitzschia* cells, on a per cell basis a copepod is more likely to encounter a small rather than a large *Pseudo-nitzschia* cell. Based upon our average per cell biomass estimates (data not shown), the ingestion of ~19 small *Pseudo-nitzschia* cells would be required to equal the ingested carbon from one *Thalassiosra* spp. cell. Assuming a copepod were to encounter a large *Pseudo-nitzschia* cell, then ~2.6 *Pseudo-nitzschia* spp. cells would be required to achieve the equivalent carbon content of one *Thalassiosra* spp. cell. From an energetic standpoint it makes little sense for a copepod to select for *Pseudo-nitzschia*, which are frequently in long chains and would require significant handling time, over other equally abundant prey types.

The overall low clearance rates on *Pseudo-nitzschia* spp. imply that the adult copepod community has little direct effect on their population ecology. To estimate this, we used the equation

$$PPC = (IR_c \cdot N)/(GM \cdot \mu)$$

where PPC is the percent of *Pseudo-nitzschia* spp. production consumed, IR_c is the ingestion rate copepod^{-1} in a given experiment, N is the estimated copepod population size, GM is the geometric mean population size of *Pseudo-nitzschia*, and µ is the intrinsic rate of *Pseudo-nitzschia* growth estimated from concurrent dilution experiments (Olson et al. unpubl.). Using published estimates of copepod abundance on the Washington and Oregon coasts (Postel et al. 1980, 1982, Morgan et al. 2003) and our clearance rate measurements, we calculated the potential impact by the adult

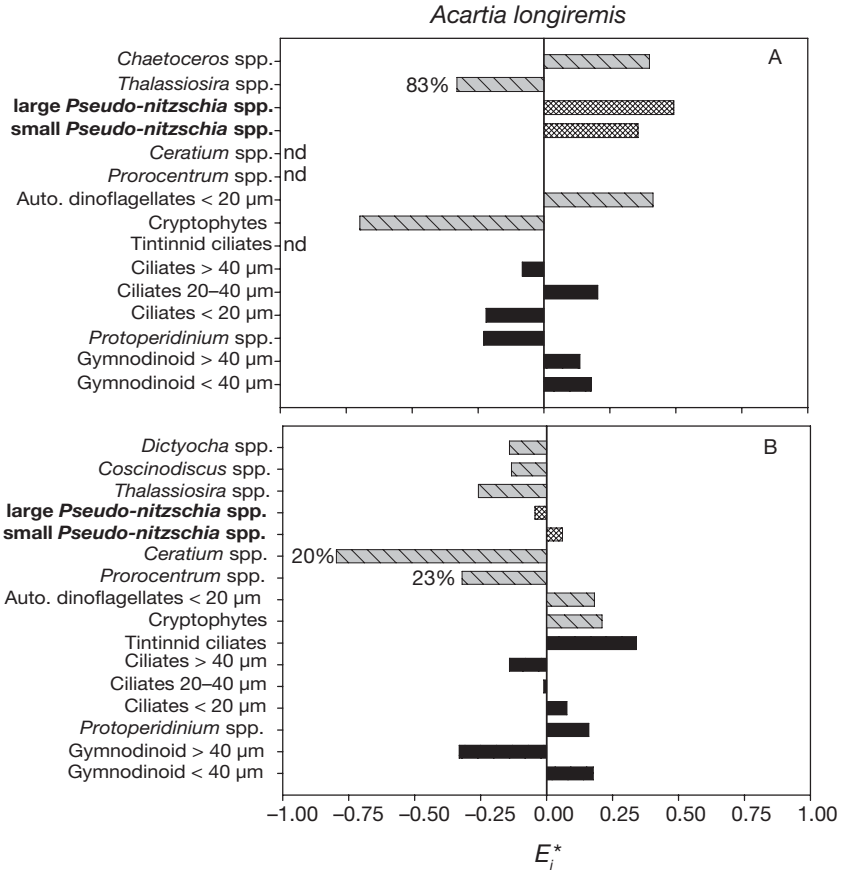

Fig. 6. *Acartia longiremis*. Electivity indices (E_i^*) for copepods feeding on natural prey assemblages in (A) Expt 1 and (B) Expt 4. Cross-hatched bars: *Pseudo-nitzschia* spp.; hatched bars: diatoms and all other autrophs; black bars: heterotrophs. Percent contribution to total biomass of biomass dominants in each experiment is shown. nd: prey types whose E_i^* were incalculable due to negative clearance estimates; Gymnodinoid: gyrodinium and gymnodinium-like dinoflagellates; Auto.: autotrophic

copepod community on *Pseudo-nitzschia* production. Using mean copepod abundance values, the grazing impact on *Pseudo-nitzschia* production varied amongst the copepod species but was always minimal (Table 4). Due to their higher abundance, *Acartia longiremis* and the small copepod community have the greatest potential to impact *Pseudo-nitzschia* populations. However, at mean typical copepod concentrations, we calculated a consumption of only 7 and 10% *Pseudo-nitzschia* production by the *A. longiremis* and small copepod community. If, however, the maximum values of copepod abundance were to co-occur with a *Pseudo-nitzschia* population growing at rates equal to those seen in this study, the potential impact on *Pseudo-nitzschia* production would be significant, but, again, only for *A. longiremis* and the small copepod community.

Perhaps the most discernible and interesting trend we found was the negative selection towards the prey types whose biomass contributed most to total biomass in each experiment. In nearly all of our experiments, the biomass dominant prey types were the diatoms *Thalassiosira* spp. and the autotrophic dinoflagellates *Ceratium* spp. and *Prorocentrum* spp. The only exception was Expt 7, in which the diatoms *Coscinodiscus* spp. and heterotrophic gymnodinoid dinoflagellates <40 µm dominated prey biomass. With regard to the avoidance of *Thalassiosira* spp., among the many possible explanations are poor nutritional quality (Jones & Flynn 2005), morphological defenses (Hamm et al. 2003) and (perhaps most likely) toxic aldehyde production (e.g. Miralto et al. 1999). In an enclosed region of the Puget Sound geographically very near to our study site, Leising et al. (2005) also found that the dominant diatoms (usually *Thalassiosira* spp.) were avoided. Their study reported lower copepod naupliar survival when the prey field was limited to *Thalassiosira* spp. diatoms, which were subsequently isolated and shown to produce high levels of aldehydes (Wichard et al. 2005). Leising et al. (2005) further found that when other prey were abundant in later weeks, these same *Thalassiosira* spp. diatoms were avoided. Although we did not measure diatom aldehyde production and naupliar survival, these same *Thalassiosira* spp. were present in our experiments; by extension, it is plausible that the copepods in our experiments were avoiding *Thalassiosira* spp. for similar reasons.

It is less obvious why the copepods tested in our study avoided the autotrophic dinoflagellates *Ceratium* spp. (mostly *C. divaricatum*) and *Prorocentrum* spp., especially since the latter has been used as staple food for copepods (Rey-Rassat et al. 2002). One explanation could be size selection; *Ceratium* spp. are generally considered too large to be effectively grazed by many copepod species (Nielsen 1991, Granéli et al. 1993). In Expts 2, 3 and 6, *Ceratium* spp. dominated the prey biomass with contributions of 20, 30 and 57%, respectively. In Expts 2 and 3, *C. divaricatum*, with an average biovolume of 3.43×10^4 µm^{-3}, had negative E_i^* values of −0.72 and −0.38, respectively. During Expt 6, *C. divaricatum* was replaced in dominance by *C. lineatum*, with an average biovolume one-third (1.27×10^4 µm^{-3}) that of *C. divaricatum*. In this experiment, *C. lineatum* contributed 57% to total prey biomass and

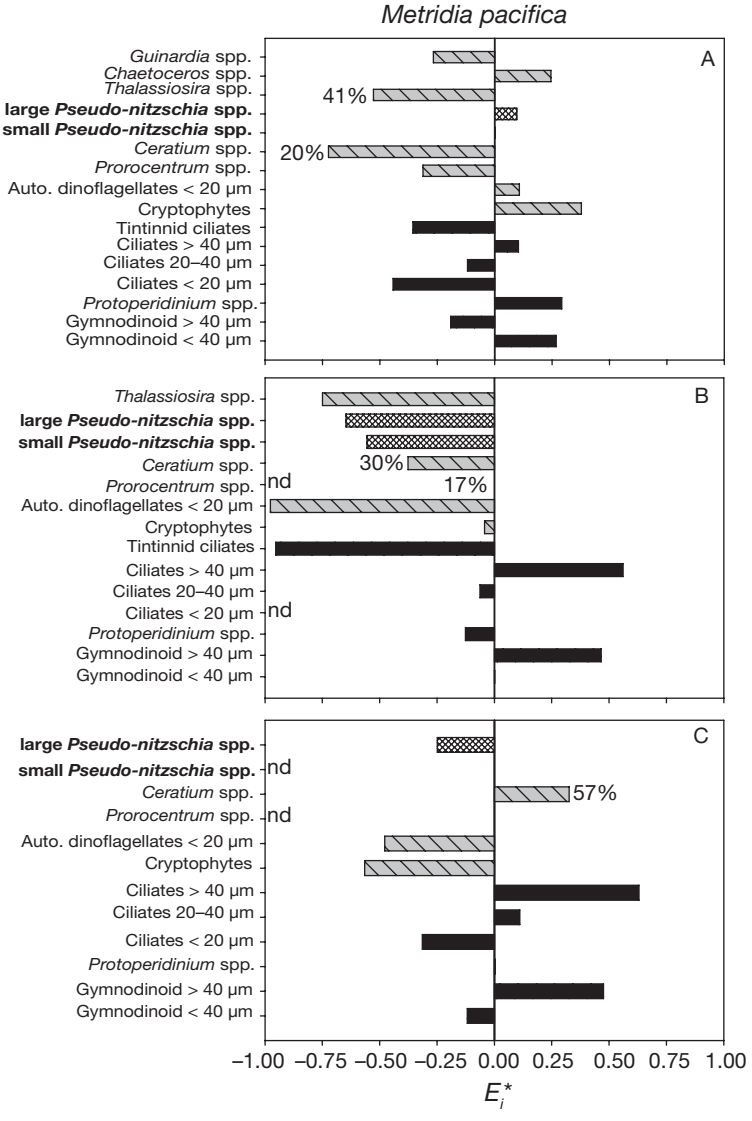

Fig. 7. *Metridia pacifica*. Electivity indices (E_i^*) for copepods feeding on natural prey assemblages in (A) Expt 2, (B) Expt 3 and (C) Expt 6. Further details as in Fig. 6

had an E_i^* of 0.33, indicating considerable preference. In 6 of the 7 experiments *Ceratium* spp. and *Prorocentrum* spp. dinoflagellates were either not ingested or highly selected against. These large autotrophic dinoflagellates grow relatively slowly; consequently, their accumulation in the plankton must be partly the result of lower predation.

The results of our study agree with those of many other studies showing that heterotrophic protists are an important (e.g. Fessenden & Cowles 1994, Calbet & Saiz 2005), if not preferred (Wiadnyana & Rassoulzadegan 1989, Leising et al. 2005), component of a copepod's diet. In every experiment, we found that microzooplankton were cleared at high rates and were highly preferred based on E_i^* values, even at locations where autotrophic biomass was much greater than heterotrophic biomass. The most striking example was Expt 5 with *Calanus pacificus*. In this experiment, microzooplankton contributed only 4 % out of a possible 1259 µg C l^{-1} of prey carbon, yet were cleared at rates 8× higher than those of the most abundant phytoplankters, *Thalassiosira* spp. and *Ceratium divaricatum*. These results agree with those of Leising et al. (2005) who found that *C. pacificus* cleared microzooplankton preferentially and at high rates, even when diatom biomass far exceeded that of microzooplankton.

In addition to aldehydes being a reasonable hypothesis as to why copepods would select for non-diatom prey, the need for copepods to achieve a balanced diet may also play a role (Jones & Flynn 2005). Klein Breteler et al. (1999) showed that 2 copepod species, *Temora longicornis* and *Pseudocalanus elongatus*, were unable to survive on a diet of the phytoplankton *Dunaliella* sp. However, when these same copepods grazed on the heterotrophic dinoflagellate *Oxyrrhis marina*, which had been grown on *Dunaliella* sp., they rapidly matured from nauplii to adult. These authors hypothesized that, by way of their high unsaturated fatty acid and sterol content, heterotrophic protists not only transfer energy and cellular constituents from lower trophic levels, but also 'upgrade' the food the copepod receives with their own cellular additions. The nutritional benefit achieved by a copepod grazing on microzooplankton, however, may be species- or essential fatty acid-specific (Broglio et al. 2003); in monospecific experiments, Ederington et al. (1995) and Klein Breteler et al. (2004) found no nutritional benefit to copepods grazing on 2 ciliate species compared to algal diets. Nonetheless, by increasing the prey fatty acid spectrum, selective grazing on microzooplankton may be nutritionally advantageous for copepods. This being so, reasonable questions are: 'How does this intense grazing pressure on microzooplankton affect productive, coastal ecosystems?' and, 'Can the population ecology of *Pseudo-nitzschia* spp. and the other dominant phytoplankton encountered in this study be *positively* impacted by copepod predation on microzooplankton?'

In our experiments, selective grazing behavior of copepods on microzooplankton clearly impacted the <5 µm chlorophyll size fraction (Fig. 10), as evidenced by the significant increase in <5 µm chl *a* net growth rate when copepods were present. This is evidence of a 'trophic cascade,' whereby the grazing mortality of ciliates and smaller (≤30 µm) heterotrophic dinoflagellates releases

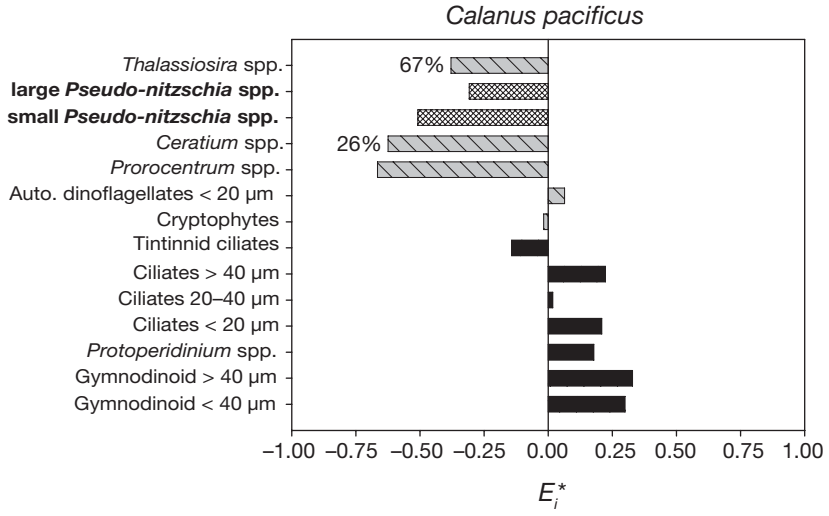

Fig. 8. *Calanus pacificus*. Electivity indices (E_i^*) for copepods feeding on natural prey assemblages in Expt 5. Further details as in Fig. 6

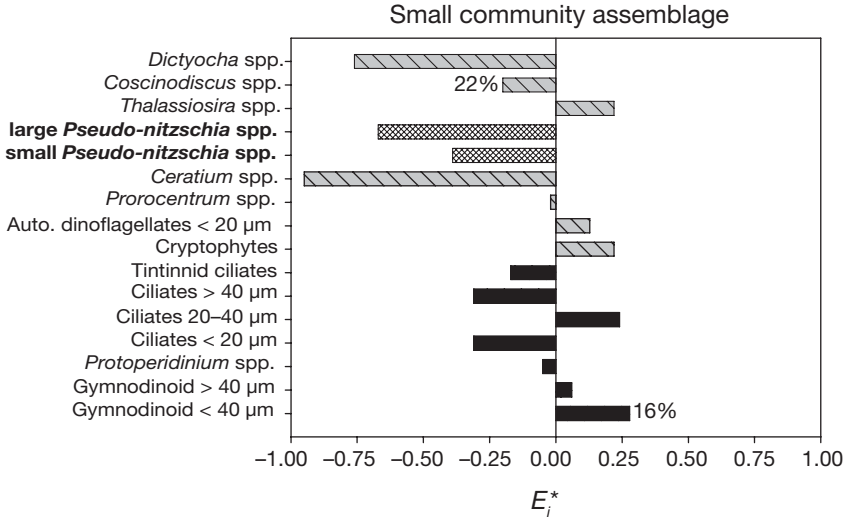

Fig. 9. Small copepod community assemblage. Electivity indices (E_i^*) for copepods feeding on natural prey assemblages in Expt 7. Further details as in Fig. 6

<5 µm phytoplankton from grazing. Trophic cascades were not evident on the phytoplankton community >5 µm. Additionally, direct estimates of *Pseudo-nitzschia* spp. growth did not show evidence of trophic cascades positively affecting their net growth. Likely microzooplankton grazers of the large and dominant phytoplankton *Thalassiosira* spp., *Ceratium* spp. and *Prorocentrum* spp. are the naked heterotrophic dinoflagellates *Gyrodinium* spp. and the thecate dinoflagellates *Protoperidinium* spp. Although these 2 heterotrophic dinoflagellate genera were cleared at high rates by all copepods (thus possibly initiating a trophic cascade), their abundance in the plankton was usually low. Additionally, grazing rates on large phytoplankton and, most significantly, on *Pseudo-nitzschia* spp., by microzooplankton during this study was low compared to their growth rates (Olson et al. unpubl.). Because of this, it is not surprising that trophic cascades were not apparent in the large phytoplankton size class.

The lack of a trophic cascade in the large phytoplankton, specifically the biomass dominants, indicates: (1) that microzooplankton grazing pressure on these large cells is low, and (2) that any structuring mechanisms the copepod population exerts on the large phytoplankton must result from direct grazing. Because we found very little clearance and negative E_i^* values on the biomass dominants, the ability of these plankton to bloom and dominate the community composition must, in part,

Table 4. Published copepod abundance estimates for the Washington or Oregon coasts, *Pseudo-nitzschia* spp. production and estimated *Pseudo-nitzschia* spp. production grazed for each experiment (present study: see 'Materials and methods' for calculations). Numbers in parentheses following mean copepod abundance are sample sizes. Sources were: 1, Postel et al. (1980); 2, Postel et al. (1982); 3, Morgan et al. (2003)

Expt No.	Copepod species	Copepod abundance (m^{-3}) Min.	Max.	Mean	Source	*Pseudo-nitzschia* production (mg C m^{-3} d^{-1})	*Pseudo-nitzschia* production grazed (%) Min.	Max.	Mean
1	*Acartia longiremis*	4	8033	1266 (22)	1,2	11.04	0.02	44.0	7.0
2	*Metridia pacifica*	4	72	25 (8)	1,2,3	1.44	0.09	1.6	0.6
3	*Metridia pacifica*	4	72	25 (8)	1,2,3	0.29	0.007	0.01	0.004
4	*Acartia longiremis*	4	8033	1266 (22)	1,2	0.58	0.01	22.3	3.51
5	*Calanus pacificus*/*C. marshallae*	18	291	131(8)	1,2,3	5.3	0.08	1.3	0.6
6	*Metridia pacifica*	4	72	25 (8)	1,2,3	0.44	0.02	0.41	0.14
7	Small community	461	22290	7045	1,2	0.09	0.7	31	9.9

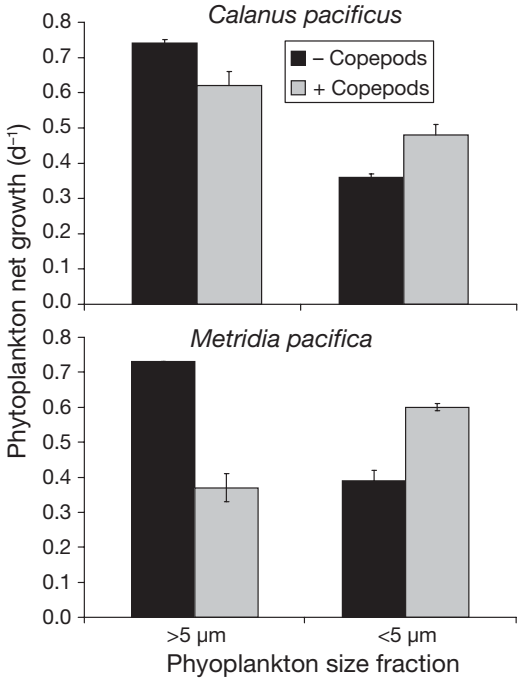

Fig. 10. Mean (± SE) net phytoplankton growth rates for 2 chlorophyll size fractions (>5 µm and <5 µm) incubated with or without copepods *Calanus pacificus* and *Metridia pacifica*

be due to reduced mortality from copepod grazing. This must especially be the case for *Ceratium* spp. and *Prorocentrum* spp., whose intrinsic growth rates are much lower than that of diatoms (Chan 1978).

Finding a consistent pattern of selective grazing by copepods in natural prey assemblages is, by itself, extremely challenging due to the variability associated with grazing experiments. Intrinsic variability can be expected due to the dynamic nature of the Washington coast, where prey populations vary in species composition and biomass concentration on short time and space scales. A grazer's feeding behavior may be shaped by the current and previous prey suite (Uye 1996). Additionally, the grazing behavior of a copepod on a specific prey can be shaped by the copepod's evolutionary history (Colin & Dam 2002, 2004). Colin & Dam (2004) show that a population of *Acartia hudsonica* unaccustomed (i.e. 'naïve') to a diet of the toxic dinoflagellate *Alexandrium fundyense* exhibited lower somatic growth, size at maturity, egg production and survival compared to populations of *A. hudsonica* historically exposed to these toxic dinoflagellates. Additionally, they show that when reared on toxic *A. fundyense*, succeeding generations of the naïve copepod populations can increase egg production and ingestion rates, supporting their argument that toxic phytoplankton, specifically *A. fundyense*, can be a selective agent creating genetically distinct populations. However, it is unlikely that the evolutionary history of the copepods we used explains the observed difference in selection between prey types. First, the prey species we examined, including toxic *Pseudo-nitzschia* spp., are at times ubiquitous across our study region. Second, unlike the geographically isolated bays from which the copepods were collected in the Colin & Dam (2004) study, the geography and hydrography of our study region probably precludes continued geographic isolation. Consequently, it is highly unlikely any single selective force (including prey types) would be geographically distinct in our open-coast study site, especially on evolutionary timescales.

Nonetheless, we have clearly shown that, within any given copepod species and experiment, selective feeding occurred, especially on microzooplankton. Our data show that selective feeding behavior, both positive and negative, affect the plankton community composition on the Washington coast. This is exemplified directly by our evidence of trophic cascades reducing mortality in the <5 µm chl *a* community, and indirectly by indicating that the biomass dominants achieve dominance by being largely omitted from the copepod diet, thus reducing their mortality rates compared to competing phytoplankton.

Pseudo-nitzschia, which is often a numerical dominant, should also be considered as a phytoplankter that benefits from low copepod grazing mortality. Our data highlight how important a reduction in mortality is to a phytoplankter's population ecology. Therefore, increased efforts should be made to study plankton mortality if we are to truly understand the population ecology of influential plankton.

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