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Grazing by the calanoid copepod *Neocalanus cristatus* on the microbial food web in the coastal Gulf of Alaska

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Neocalanus cristatus feeding on phytoplankton and microzooplankton was measured in the coastal Gulf of Alaska during spring and early summer of 2001 and 2003. Neocalanus cristatus CV fed primarily on particles >20 μm. Particles in the 5- to 20-μm size range were ingested in some experiments under nonbloom conditions but not under bloom conditions. Particles <5 μm were not ingested but increased during incubations because N. cristatus consumed their microzooplanktonic predators. Neocalanus cristatus are sufficiently abundant in nature to induce such a cascade effect in situ. Microzooplankton provided >70% of the carbon ingested by N. cristatus under nonbloom conditions but only ~30% under bloom conditions. Neocalanus cristatus ingested about two times more carbon under bloom conditions (average 21.4 μg C copepod^{-1} day^{-1}) than under nonbloom conditions (average 10.0 μg C copepod^{-1} day^{-1}), but these rates were inadequate to meet nutritional demands for growth and metabolism, estimated to be between 40 and 140 μg C copepod^{-1} day^{-1}. We believe our ingestion rates are underestimates of in situ rates because (i) we are underestimating consumption rates of large particles, (ii) we may not be including some very large particles that should be considered as diet items and (iii) we are not properly accounting for the ingestion of aggregates. The feeding behavior of N. cristatus, one of the most abundant copepods in the North Pacific Ocean, remains incompletely understood.

INTRODUCTION

Three *Neocalanus* species dominate mesozooplankton biomass throughout the entire subarctic Pacific Ocean and its marginal seas in the spring and early summer. *Neocalanus cristatus* is the least abundant of the three but it is the largest, with copepodid stage V having a prosome length between ~6.0–7.5 mm (Kobari *et al.*, 2003) and a body weight typically >1 mg C. *Neocalanus cristatus* has an annual life cycle and completes the majority of its growth and development over several months in the spring and summer, during a similar time span to the other two *Neocalanus* species in this region, *Neocalanus plumchrus* and *Neocalanus flemingeri*. Because the absolute growth rate of *N. cristatus* is greater than that of *N. plumchrus* (Vidal and Smith, 1986), its ingestion rate is also higher. However, *N. cristatus* does not appear to have a raptorial feeding mode (Greene and Landry, 1988) and instead is a true suspension feeder that relies on the establishment of a feeding current to collect food particles. In oceanic regions, measured rates of ingestion of phytoplankton and microzooplankton are inadequate to support nutritional demands for growth and metabolism (Dagg, 1993a; Gifford, 1993; Tsuda and Sugisaki, 1994), and sinking aggregates appear to be a major dietary component (Dagg, 1993b). In marginal seas, phytoplankton concentration is often higher than in the High Nutrient Low Chlorophyll (HNLC) oceanic regions, and ingestion rates of *N. cristatus* can be much higher (Dagg and Wyman, 1983).

In the coastal Gulf of Alaska, onshore transport of surface water is strongest in the late winter and early spring. This results in the seeding of shelf waters with the oceanic zooplankton community, including *N. cristatus*, prior to the development of the spring phytoplankton bloom in April. Consequently, *N. cristatus* typically contributes significantly to the zooplankton biomass on the

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Alaska continental shelf during the spring and early summer. Here, *N. cristatus* encounters a wide range of food environments, and this situation has provided us an opportunity to further examine its feeding behavior and its contributions to structuring the food web. In this article, we report the results from feeding experiments done in the coastal Gulf of Alaska with *N. cristatus* CIVs (four experiments) and CVs (25 experiments) on shipboard using natural seawater as food medium during spring and early summer of 2001 and 2003.

METHOD

Neocalanus cristatus were collected by vertically hauling a plankton net with mesh size of 202 μm and an Aquarium cod end from 50 m to the surface. Cod-end contents were carefully poured into an insulated container. In the laboratory, healthy *N. cristatus* were sorted using ladles and pipettes and transferred into small jars containing surface seawater. These animals were kept at the temperature of collection for ~30–90 min before being transferred to experimental bottles.

For food medium, seawater was collected from the depth of 50% light level with clean Niskin bottles mounted to a CTD rosette, except for experiments 16 and 17 which used water from deeper layers. Seawater from several Niskin bottles was gently drained into a 20-L polycarbonate carboy and then through a 202-μm mesh during the filling of 2.3-L polycarbonate bottles. All carboys, bottles, tubing and other labware used in collecting seawater were acid cleaned and rinsed with Milli-Q water before use.

Staged *N. cristatus* were placed in the experimental bottles, and bottles were placed in an incubator rack on deck for 24 h. Bottles were not agitated or stirred, but limited mixing was provided by ship motion. In each 2001 experiment, a series of different concentrations of copepods was incubated with 2–4 replicate bottles for each treatment. In 2003 experiments, only one concentration of copepods was incubated but with greater replication. In all experiments, a series of bottles was sampled at the beginning of the incubation to provide the initial conditions, and 2–3 bottles with no copepods were incubated as controls. All experimental bottles were tightly capped after filling, and one layer of neutral screen was applied to each bottle to decrease light by 50%. During incubation, temperature was controlled by running surface seawater. At the end of the 24-h incubation, we took samples from all bottles for measurement of chlorophyll *a* (Chl *a*), picoplankton, heterotrophic nanoplankton and microplankton and allocation of plankton into size classes.

Chl *a* concentration was determined in three size classes: <5 μm, 5–20 μm and >20 μm, assigned to picoplankton, nanoplankton and microplankton, respectively. Each water sample was filtered with a cascade filtration system fitted with a 20-μm polycarbonate filter, a 5-μm polycarbonate filter and a GF/F glass-fiber filter. Filters were placed in 90% acetone for 24 h at –20°C, and the extract was measured with a Turner Designs fluorometer to determine Chl *a* concentrations (Strickland and Parsons, 1972).

For picoplankton, 1 mL of seawater was taken from each experimental bottle before and after incubation, preserved with paraformaldehyde (0.2% final concentration), quick frozen and stored in liquid nitrogen for flow cytometric analysis. A BD LSR flow cytometer equipped with 20-mW blue (488 nm) and 8-mW UV (325 nm) lasers was used to enumerate the picoplankton. Forward and right-angle light scattering (FSC and SSC) and green (515–545 nm), orange (564–606 nm) and red (>650 nm) fluorescence were collected, saved and analysed with CYTOWIN software written by Daniel Vaulot (downloaded from http://www.sb-roscoff.fr). All signals were normalized to that of the 1-μm Fluoresbrite YG beads (Polysciences, Warrington, PA) that were added to each sample. *Synechococcus* spp. were distinguished from picoeukaryotes primarily by the strong orange fluorescence from phycoerythrin.

For heterotrophic nanoplankton, 30–60 mL samples were preserved in 10% gluteraldehyde (final concentration), stained with 4-6-diamidino-2-phenylindole (DAPI) and filtered onto 1-μm pore-size black polycarbonate membrane filters. The filters were mounted in immersion oil (type B) on slides and kept frozen until analysis with an epifluorescence microscope (Sherr *et al.*, 1993). Usually >300 individuals were enumerated in two size fractions, <5 μm and >5 μm in the longest dimension.

For microplankton analysis by FlowCAM (Sieracki *et al.*, 1998), live samples (~250 mL) stored at incubation temperature in the dark after collection from incubation bottles were analysed within 2 h of collection. Gentle stirring was used to keep particles uniformly suspended during FlowCAM analysis; 500 particles per sample were counted and imaged. The FlowCAM was standardized by comparison with manual counts of cultured phytoplankton, including species with a range of cell sizes. Counted particles were binned into 10-μm size classes according to their equivalent spherical diameter (ESD).

For microplankton enumeration by microscopy, 100 mL of seawater was preserved with 10% acid Lugol's solution. In the laboratory, subsamples of 10–50 mL were placed in sedimentation chambers to settle for >24 h (Utermöhl, 1958). Microplankton cells including diatoms, dinoflagellates and ciliates (including aloricate oligotrichs, loricate tintinids and autotrophic *Mesodinium*) were identified and counted under an inverted

microscope (Nikon-TMD 300) at 200× or 400× magnification. For ciliates, >30 cells of each species were measured, cell volumes were calculated assuming standard geometric forms (Ota and Taniguchi, 2003), and carbon biomass estimated using a volume to carbon conversion factor of 0.19 pg C μm^{-3} (Putt and Stoecker, 1989). For dinoflagellates, since we are not able to separate heterotrophs from autotrophs in the samples fixed by Lugol's solution, we identified, counted and measured the cell size of heterotrophic and autotrophic dinoflagellates on the slides mentioned above. Biomass of heterotrophic dinoflagellates was calculated using an equation derived from Menden-Deuer and Lessard (Menden-Deuer and Lessard, 2000):

$$\text{pgC cell}^{-1} = 0.760 \times \text{volume}(\mu m^3)^{0.819} \quad (1)$$

Phytoplankton carbon was estimated by applying a C : Chl ratio of 25 in the experiments conducted under phytoplankton bloom conditions and 75 for the experiments conducted under nonbloom conditions. These ratios are based on direct measurements made in the same region during the same period by Lessard *et al.* (Lessard *et al.*, 2003), who reported C : Chl ratios ranging from 20 to 85 from near shore (high Chl) to the shelf break (low Chl) in April and May 2001.

Neocalanus cristatus clearance rates (F) on Chl *a* and microplankton were calculated following the formulae of Frost (Frost, 1972).

$$F(\text{L cop}^{-1}\text{d}^{-1}) = \frac{V(k_c - k_t)}{z} \quad (2)$$

where V is the volume of the incubation bottle (in L) and z is number of *N. cristatus* added to the incubation bottle, k_c and k_t are the net or apparent prey growth rates in the controls and treatments, respectively, which are calculated by

$$k(\text{d}^{-1}) = \text{LN}\frac{C_0}{C_e} \quad (3)$$

for 24-h incubations, where C_0 is the initial concentration of prey, and C_e is the concentration of prey in the control and treatment bottles at the end of the incubation.

Ingestion rate is calculated by

$$I(\mu\text{g C copepod}^{-1}\text{ day}^{-1}) = C \times F \quad (4)$$

where C is the mean concentration of prey (L^{-1}) throughout the 24-h incubation period which is calculated by

$$C = C_0\frac{e^{k_t} - 1}{k_t} \quad (5)$$

RESULTS

In all, we did 25 feeding experiments with *N. cristatus* CVs and 4 experiments with CIVs collected from the coastal Gulf of Alaska (Fig. 1). These experiments spanned a wide range of conditions as indicated by the initial Chl *a* concentration, which ranged from 0.29 to 11.44 $\mu g\ L^{-1}$ (Table I). The food environment was divided into three types based on Chl.

One type of water represented what we refer to as nonbloom conditions. In experiments done using this water as food medium, between 62 and 83% of the total Chl *a* was in the <5-μm size category (Table I). This type of water usually had a low total Chl *a* concentration (<0.50 $\mu g\ L^{-1}$), but a few stations dominated by small cells had elevated concentrations of total Chl *a* (Table I). All nonbloom stations had very low concentrations of large cells, <0.07 μg Chl *a* L^{-1} in the >20-μm size category.

Type-2 water had a more even distribution of Chl *a* across all three size categories (Table I), although large cells were the largest component (40–50% of total Chl). Total Chl *a* concentration was low (0.46–0.72 $\mu g\ L^{-1}$), but the concentration of particles >20 μm was higher than in nonbloom water, between 0.18 and 0.38 $\mu g\ L^{-1}$. Only three experiments were done with this type of water, which was only observed in Prince William Sound (PWS).

The third type of water was dominated by the large cell category, with >20 μm Chl *a* containing particles

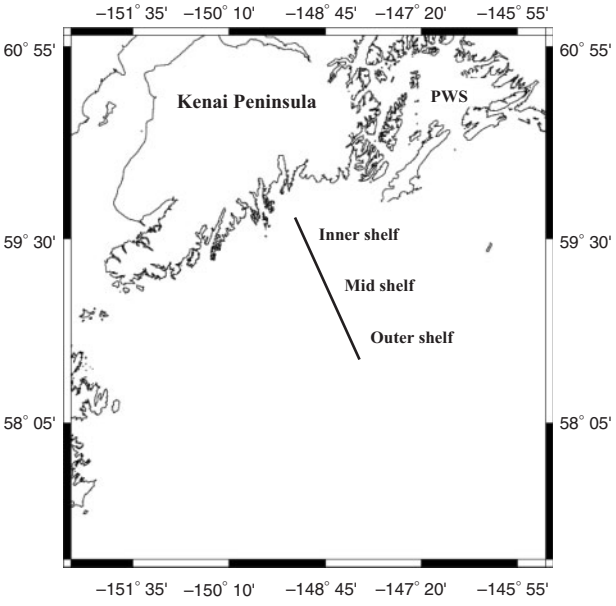

Fig. 1. Study area and approximate station locations along the Global Ocean Ecosystem Dynamics (GLOBEC) Seward line for all feeding experiments with *Neocalanus cristatus*.

Table I: Initial chlorophyll a (Chl a) concentrations and the percentage of Chl in each size category for all grazing experiments with Neocalanus cristatus

Experiment number	Water	Stage	Date	Location	Initial Chl (μg L^{-1})				% of Chl		
					<5 μm	5–20 μm	>20 μm	Total Chl	<5 μm	5–20 μm	>20 μm
1	Nonbloom	V	17 April 2001	Outer	0.236	0.061	0.047	0.342	69.0	17.8	13.7
2	Nonbloom	V	18 April 2001	Outer	0.191	0.059	0.036	0.286	66.8	20.6	12.6
3	Nonbloom	IV	19 April 2001	Outer	0.192	0.071	0.047	0.310	61.9	22.9	15.2
4	Nonbloom	IV	20 April 2001	Mid	0.280	0.060	0.030	0.931	83.0	14.7	2.3
5	Nonbloom	V	17 May 2001	Outer	0.773	0.137	0.021	0.369	75.9	16.3	8.1
6	Nonbloom	V	19 May 2001	Outer	0.698	0.145	0.053	0.897	77.8	16.2	5.9
7	Nonbloom	V	24 May 2001	Mid	0.272	0.048	0.054	0.374	72.7	12.8	14.4
8	Nonbloom	V	26 May 2001	Mid	0.392	0.060	0.066	0.518	75.7	11.6	12.7
9	Nonbloom	V	16 July 2001	Mid	0.813	0.227	0.072	1.112	73.1	20.4	6.5
10	Nonbloom	IV	19 July 2001	Outer	0.350	0.105	0.032	0.487	71.9	21.6	6.6
11	Nonbloom	V	19 July 2001	Outer	0.350	0.105	0.032	0.487	71.9	21.6	6.6
12	Nonbloom	V	28 April 2003	Outer	0.199	0.075	0.050	0.324	61.4	23.1	15.4
13	Nonbloom	V	13 May 2003	Outer	0.999	0.244	0.058	1.301	76.8	18.8	4.5
14	Type 2	V	21 May 2001	Prince William Sound	0.240	0.171	0.308	0.719	33.4	23.8	42.8
15	Type 2	V	23 May 2001	Prince William Sound	0.170	0.102	0.183	0.455	37.4	22.4	40.2
16	Type 2	V	23 July 2001	Prince William Sound	0.158	0.105	0.264	0.526	30.0	20.0	50.2
17	Bloom	V	26 April 2001	Prince William Sound	0.104	0.266	1.343	1.713	6.1	15.5	78.4
18	Bloom	V	29 April 2001	Prince William Sound	0.163	0.184	0.732	1.069	15.2	17.2	68.5
19	Bloom	V	25 May 2001	Mid	0.402	0.161	2.840	3.403	11.8	4.7	83.5
20	Bloom	V	29 May 2001	Inner	0.228	0.199	4.766	5.192	4.4	3.8	91.8
21	Bloom	V	30 May 2001	Inner	0.240	0.168	1.648	2.056	11.7	8.2	80.2
22	Bloom	V	13 July 2001	Inner	0.743	0.541	1.343	2.628	28.3	20.6	51.1
23	Bloom	V	14 July 2001	Inner	0.196	0.213	0.756	1.164	16.8	18.3	64.9
24	Bloom	IV	25 July 2001	Inner	0.278	0.231	4.254	4.763	5.8	4.8	89.3
25	Bloom	V	30 April 2003	Prince William Sound	0.166	0.201	1.317	1.684	9.9	11.9	78.2
26	Bloom	V	2 May 2003	Prince William Sound	0.291	0.147	0.610	1.048	27.8	14.0	58.2
27	Bloom	V	3 May 2003	Prince William Sound	0.041	0.081	1.151	1.273	3.2	6.4	90.4
28	Bloom	V	6 May 2003	Inner	0.281	0.458	2.050	2.789	10.1	16.4	73.5
29	Bloom	V	12 May 2003	Inner	0.456	0.457	10.523	11.438	4.0	4.0	92.0

accounting for 51–92% of total Chl *a* (Table I). We refer to this category of conditions as bloom conditions. Total Chl *a* concentration was typically high, ranging between 1.07 and 11.44 μg L^{-1}.

With the exception of PWS stations, the water in our experiments appeared to be either the nano- and pico-plankton-dominated system typical of the HNLC oceanic region or the diatom-dominated conditions of phytoplankton blooms that commonly occur on the shelf. Although water types were not completely separated in an onshore-to-offshore direction, almost all bloom stations were in the inner shelf or in PWS, and all nonbloom stations were in the middle or outer shelf (Table I). Water types were not completely separated by season, and each type was observed on all of the cruises.

A more complete description of the planktonic community composition is provided in Table II for three nonbloom stations (experiments 1, 6 and 8) and three bloom stations (experiments 17, 18 and 19). At these nonbloom stations, an average of 74% of the Chl *a* was found in <5-μm fraction, whereas at the bloom stations 77% of the Chl *a* was in the >20-μm fraction (Table II). This pattern was reflected in the diatom abundance which was 40× greater at the bloom stations than at the nonbloom stations. At nonbloom stations, diatom concentrations were low, 1.68–4.48 × 10^3 cells L^{-1}, and the dominant diatoms were *Nitzschia* spp. At bloom

Table II: Chlorophyll a (Chl a) concentration and abundance of major components of the planktonic food web at each experimental site

Experiment	Type	Location	Date	Chl *a* concentration				Abundance of major components of planktonic food web							
				Total	<5 μm	5–20 μm	>20 μm	Ciliates	*Mesodinium*	Dinoflagellates	Diatoms	<5 HNF	>5 HNF	*Synechococcus*	Picoeukaryotes
1	Nonbloom	Outer	17 April 2001	0.363	0.238 (65.5)	0.075 (20.7)	0.050 (13.8)	3.68	1.22	3.44	3.30	425	84	32.1	26.6
6	Nonbloom	Outer	19 May 2001	0.901	0.698 (77.5)	0.152 (16.8)	0.051 (5.7)	6.95	0.09	7.93	4.48	326	87	107.0	37.0
8	Nonbloom	Mid	26 May 2001	0.518	0.392 (75.7)	0.060 (11.5)	0.066 (12.8)	8.48	0.69	9.80	1.68	197	34	136.0	43.0
17	Bloom	Prince William Sound	26 April 2001	1.713	0.104 (6.1)	0.200 (15.5)	1.343 (78.4)	4.15	1.53	14.3	84.0	131	29	0.7	3.8
18	Bloom	Prince William Sound	29 April 2001	1.069	0.163 (15.2)	0.184 (17.2)	0.723 (67.6)	9.18	5.53	15.4	16.7	628	74	—	—
19	Bloom	Mid	25 May 2001	3.403	0.402 (11.8)	0.161 (4.7)	2.840 (83.5)	7.59	0.79	17.4	282	261	156	50.6	22.4

The units are μg L^{-1} for Chl *a*, 10^3 cells L^{-1} for microplankton and heterotrophic nanoflagellates (HNF) and 10^3 cells mL^{-1} for picoplankton.

stations, the dominant diatom species varied, and concentrations were higher, between 16.7 and 282.0 × 10^3 cells L^{-1}. *Coscinodiscus* sp. and *Thalassiosira gravida* dominated in experiment 17, while *Coscinodiscus* sp. and *Chaetoceros* spp. dominated in experiment 18. Experiment 19, in the mid-shelf, occurred during a large diatom bloom with *Chaetoceros radicans*, *Nitzschia* spp. and *T. gravida* being the dominant species.

At nonbloom stations, concentrations of ciliates and dinoflagellates were approximately equal, whereas at bloom stations, dinoflagellates were approximately twice as abundant as ciliates (Table II). Gymnodinoid forms dominated the dinoflagellates at all stations, but prorocentroid and gonyaulacoid dinoflagellates were also abundant, especially at bloom stations. There was no apparent difference in the abundance or composition of ciliates between bloom and nonbloom conditions (Table II). Dominant ciliate species were *Lohmanniella oviformis* and various species of *Strombidium*. At both bloom and nonbloom locations, *Mesodinium* were abundant in April (>1000 cells L^{-1}) and became rare in May (Table II). Tintinnids were always rare, usually <100 cells L^{-1}.

The abundance of heterotrophic nanoflagellates (HNF) was highly variable and without a clear difference between bloom and nonbloom stations (Table II). HNF in the <5-μm size fraction varied from 131 to 628 cells mL^{-1}, exceeding concentrations of the >5-μm HNF on average by a factor of 4.3. This ratio appears to be higher in the three experiments conducted in April (4.6–8.5, mean 6.0) than in the May experiments (1.7–5.7, mean 3.7). Picoplankton abundance was lower in bloom conditions than in nonbloom conditions (Table II).

Results from grazing experiment 6, done under nonbloom conditions, and experiment 18, done under bloom conditions, are presented graphically in Fig. 2. Direct consumption of the >20-μm phytoplankton is indicated by the decrease of net growth rate with increasing numbers of *N. cristatus* in the incubation bottles and is apparent in both experiments (Fig. 2). In the 5- to 20-μm size fraction, the effects of *N. cristatus* were insignificant in these experiments. In the smallest size phytoplankton category, <5 μm, both experiments showed a positive slope (Fig. 2) indicating a significant enhancement of net growth (particle production) with *N. cristatus* inclusion in the incubation bottles. The data from these experiments are used to calculate feeding rates.

In many of our experiments, we noticed large declines in clearance rates at concentrations of three or more *N. cristatus* per bottle. This was more evident in experiments done under nonbloom conditions, where clearance rates were highest and indicated three *N. cristatus* could filter the entire bottle of water (~2.3 L) in 1 day.

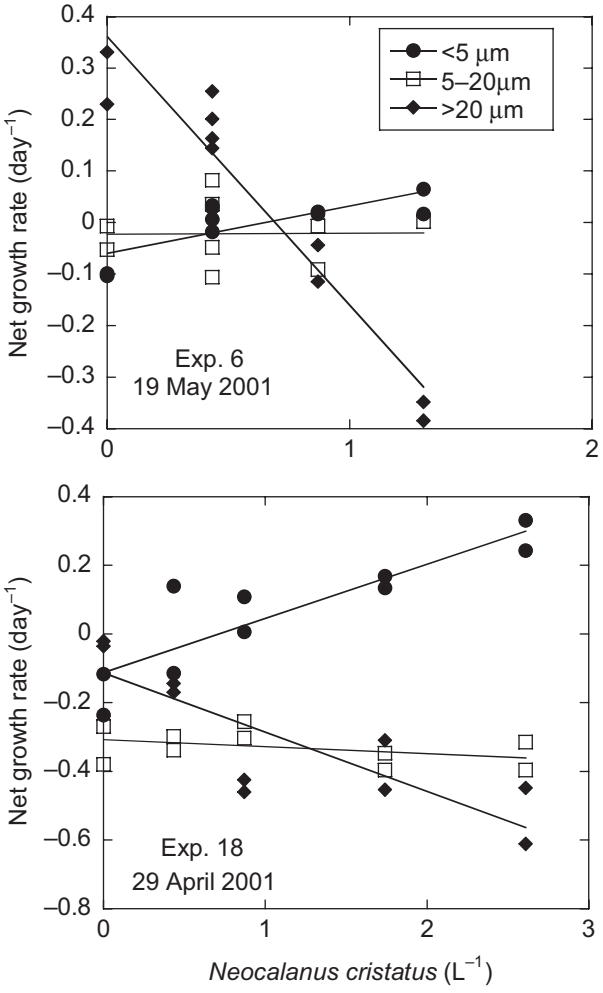

Fig. 2. Sample grazing experiments under nonbloom (experiment 6) and bloom (experiment 18) conditions (see text for explanation). The net growth rate of >20-μm chlorophyll *a* (Chl *a*) in these two experiments was highly correlated to the number of *Neocalanus cristatus* grazers in the incubation bottles (r^2 = 0.92 and 0.71 for experiments 6 and 18, respectively), and the slope of each regression line is significantly different from 0 (P < 0.001 and 0.01, respectively). The negative correlations between <5-μm Chl *a* growth rate and *N. cristatus* grazing were also significant (r^2 = 0.60 and 0.77, P < 0.01 and 0.001, respectively). Slopes for the intermediate-sized cells were not significant.

Consequently, we only used data from experimental bottles with one or two *N. cristatus* L^{-1}.

In experiments done under nonbloom conditions, experiments 1–13 in Tables I, *N. cristatus* clearance rates on cells <5 μm ranged from slightly positive (experiment 1) to strongly negative (experiment 8) (Table III). In general, negative clearance rates were observed in this cell-size category, indicating particle production occurred during the incubation (Table III). Clearance rates on intermediate-sized cells were negative in 6 of 13 experiments and positive in 7 experiments. Clearance rates on particles >20 μm were strongly positive in all but one of the experiments

Table III: Calculated clearance rates and ingestion rates

Experiment number	Water	Stage	Date	Location	*n*	Mean clearance rate (L copepod^{-1} day^{-1})			Mean ingestion rate (ng Chl copepod^{-1} day^{-1})		
						<5 μm	5–20 μm	>20 μm	<5 μm	5–20 μm	>20 μm
1	Nonbloom	V	17 April 2001	Outer	4	0.042	0.707	0.656	9.7	47.7	33.8
2	Nonbloom	V	18 April 2001	Outer	4	0.008	0.133	0.153	1.6	6.3	5.9
3	Nonbloom	IV	19 April 2001	Outer	2	0.012	0.469	0.198	2.1	27.0	8.7
4	Nonbloom	IV	20 April 2001	Mid	3	–0.031	–0.059	0.195	–9.6	1.2	5.5
5	Nonbloom	V	17 May 2001	Outer	6	–0.087	0.337	0.539	–65.2	45.1	11.2
6	Nonbloom	V	19 May 2001	Outer	8	–0.200	–0.025	0.343	–141.2	–3.2	16.9
7	Nonbloom	V	24 May 2001	Mid	3	–0.102	0.174	0.464	–36.7	7.7	22.8
8	Nonbloom	V	26 May 2001	Mid	4	–0.393	0.166	0.805	–180.8	9.4	45.0
9	Nonbloom	V	16 July 2001	Mid	4	–0.124	–0.272	–0.089	–119.7	–87.8	–9.4
10	Nonbloom	IV	19 July 2001	Outer	4	–0.144	–0.036	0.136	–40.3	–9.1	0.5
11	Nonbloom	V	19 July 2001	Outer	2	–0.191	–0.157	0.101	–70.3	–5.1	5.8
12	Nonbloom	V	28 April 2003	Outer	4	–0.189	–0.015	0.471	–48.1	–1.4	19.3
13	Nonbloom	V	13 May 2003	Outer	3	–0.017	0.004	0.517	–22.3	0.4	219.7
14	Type 2	V	21 May 2001	Prince William Sound	4	–0.408	–0.292	0.103	–116.1	–51.5	26.3
15	Type 2	V	23 May 2001	Prince William Sound	3	–0.365	–0.275	0.197	–68.2	–22.8	23.9
16	Type 2	V	23 July 2001	Prince William Sound	2	–0.191	–0.157	0.101	–42.6	–20.8	43.5
17	Bloom	V	26 April 2001	Prince William Sound	4	–0.238	–0.031	0.524	–27.3	–8.8	703.9
18	Bloom	V	29 April 2001	Prince William Sound	4	–0.366	–0.034	0.401	–60.9	–5.4	247.4
19	Bloom	V	25 May 2001	Mid	6	–0.154	–0.107	0.211	–63.0	–16.3	639.0
20	Bloom	V	29 May 2001	Inner	1	0.063	–0.387	–0.341	9.8	–55.9	–1490.0
21	Bloom	V	30 May 2001	Inner	4	–0.099	–0.290	–0.252	–23.8	–48.8	–414.8
22	Bloom	V	13 July 2001	Inner	4	–0.200	–0.270	0.101	–103.8	–134.1	178.1
23	Bloom	V	14 July 2001	Inner	4	–0.163	–0.271	0.256	–50.2	–67.3	335.5
24	Bloom	IV	25 July 2001	Inner	6	–0.131	–0.178	–0.200	–40.2	–43.5	–1143.5
25	Bloom	V	30 April 2003	Prince William Sound	4	–0.230	–0.330	0.255	–50.4	–71.5	363.3
26	Bloom	V	2 May 2003	Prince William Sound	4	–0.224	–0.454	0.379	–44.5	–49.2	170.5
27	Bloom	V	3 May 2003	Prince William Sound	3	–0.072	–0.191	–0.008	–6.1	–31.6	–16.4
28	Bloom	V	6 May 2003	Inner	4	–0.180	–0.378	0.045	–44.9	–143.2	113.1
29	Bloom	V	12 May 2003	Inner	4	–0.162	–0.694	–0.033	–70.7	–440.4	–426.7

(Table III). Although large cells were rare in all nonbloom experiments (Chl *a* concentration in the >20-μm size category was only 0.02–0.07 μg L^{-1}), clearance rates on these particles were high. Using only positive clearance rates on all three cell sizes to calculate ingestion rate indicates that between 20 and 100% (mean = 72%) of total ingestion of Chl *a* was derived from the large cell category, in spite of the very low concentration of cells in this category. Under nonbloom conditions, there did not appear to be any relationship between the concentration of cells in a given category and the clearance rate of cells in that category (plots not shown).

Although total Chl *a* concentrations in the three experiments done in category-2 water were similar to those in nonbloom conditions, the size composition of Chl-containing particles was intermediate between nonbloom stations which were heavily dominated by cells <5 μm and bloom stations which were heavily dominated by cells >20 μm. *Neocalanus cristatus* clearance rates on small particles were strongly negative in all three experiments, and this was also the pattern in the intermediate-sized cells (Table III). Only in the >20-μm size category was there a net positive clearance (removal) of cells. All measurable ingestion of Chl-containing particles in these experiments was derived from cells in the >20-μm size category.

In experiments done under bloom conditions, *N. cristatus* clearance rates on both the small- and

intermediate-sized cells were negative, with the exception of experiment 20 which consisted of only a single bottle (Table III). Even in the >20-μm size category, net clearance rates in several experiments were negative, although most were positive. The highest ingestion rates observed in all our experiments were under bloom conditions where clearance rates on large particles were high and concentrations of large particles were high.

For all experiments combined, clearance rate was negative or not significantly different from 0 on particles <5 μm, and there was no relationship between clearance rate and concentration of cells <5 μm (Tables I and III, Fig. 3a). For cells in the 5- to 20-μm category, there was a tendency for clearance rates to be positive at the lower concentrations and decline to negative rates as concentration increased (Tables I and III, Fig. 3b). In about half of the nonbloom experiments, clearance rates on cells in the intermediate-size category were strongly positive, whereas all were negative in Category-2 water and under bloom conditions. In the large cell category, clearance rates also tended to decline with increasing cell concentration, although rates were almost all positive except at the highest concentrations (Tables I and III, Fig. 3c).

Three experiments were analysed with a FlowCAM. Data from this instrument were used to calculate clearance rates for 10-μm bin sizes for particles >11 μm (Fig. 4). This additional data provides further evidence for high *N. cristatus* clearance rates on large particles. In experiment 8 (Fig. 4a, nonbloom conditions), clearance rates on particles >40-μm ESD were ~2.0 L day^{-1}. This size category was dominated by a chloroplast-retaining ciliate, *Laboea* sp. Particles between 11 and 40 μm were cleared at substantially lower rates, between ~0.5 and 1.0 L day^{-1}, about the same rate as we found for Chl-containing particles >20 μm (Table III). This experiment indicates that *N. cristatus* can remove larger particles (>40 μm) at substantially higher rates than indicated by our measurements of size-fractionated Chl. These FlowCAM-derived rates are consistent with the high clearance rates we measured by microscopic counts of ciliates and dinoflagellates in this experiment (Table IV), which ranged between 1.7 and 3.2 L day^{-1}. In experiment 17 (Fig. 4b) conducted under bloom conditions, the FlowCAM analysis indicated a different and more complex pattern. Clearance rates on particles >50 μm were ~0.5 L day^{-1}, similar to those we observed for Chl >20 μm (Table III). Clearance rates on particles between 31 and 50 μm, however, were negative indicating these particles increased in abundance in the presence of *N. cristatus*. Last, clearance rates on particles between 11 and 30 μm were positive and very high. This pattern, discussed below, is suggestive of complex trophic interactions within the microplankton food web. Experiment 18 was also analysed with the FlowCAM (Fig. 4c). Clearance rates were generally high, 0.4–0.5 L day^{-1} for particles between 21 and 60 μm. These rates are similar to those we measured by size-fractionated Chl for the >20-μm sized particles (Table III). The largest particles (>60 μm) were rare, and their scarcity

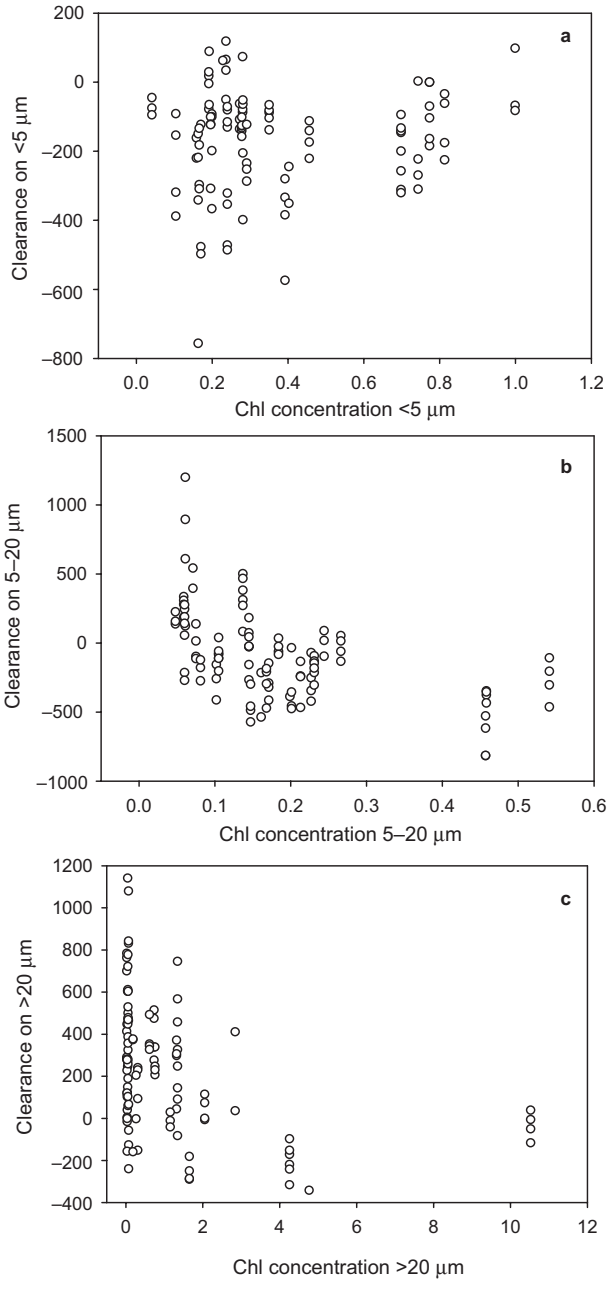

Fig. 3. Calculated clearance rates (L copepod^{-1} day^{-1}) of *Neocalanus cristatus* on chlorophyll containing particles (**a**) <5 μm, (**b**) between 5 and 20 μm and (**c**) >20 μm, as a function of the concentration of chlorophyll *a* (Chl *a*) in each size category.

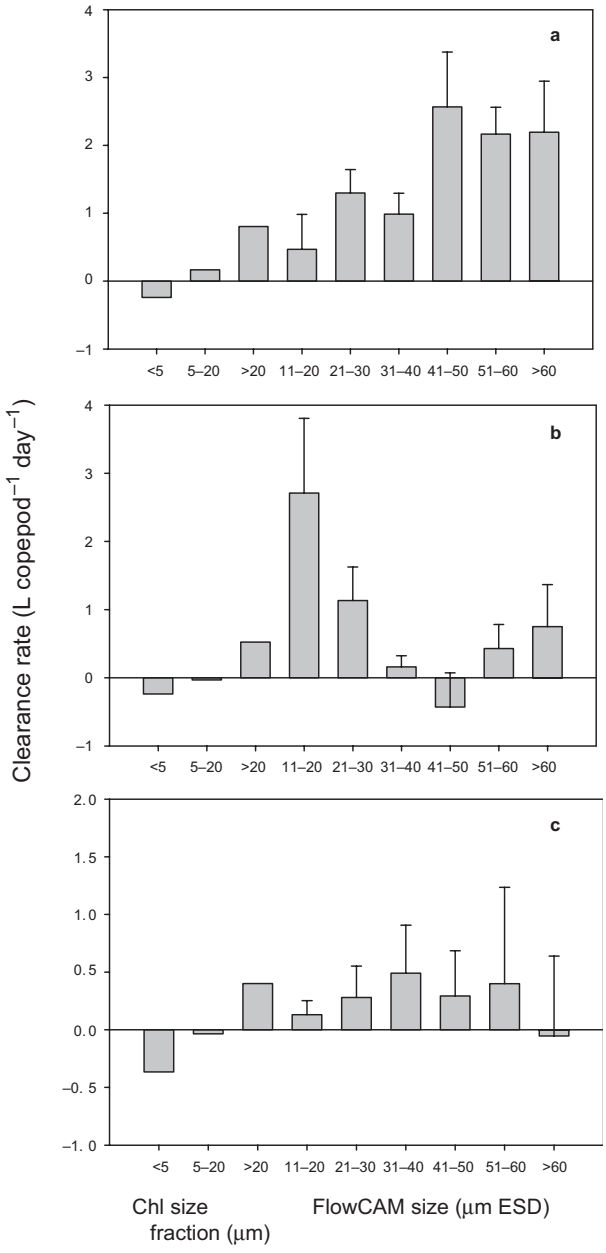

Fig. 4. FlowCAM-determined clearance rates (L copepod^{-1} day^{-1}) on large particles in (**a**) experiment 8, (**b**) experiment 17 and (**c**) experiment 18. Rates derived from size-fractionated chlorophyll analyses (Table III) are represented by the leftmost three bars in each panel.

probably contributes to the wide range observed in these clearance rates.

Size-fractionated Chl data were used to calculate ingestion rates of phytoplankton by *N. cristatus*. For initial calculations of ingestion rate, negative ingestion rates are set to 0. With the exception of experiment 13, high ingestion rates were only observed under bloom conditions (Table III). Ingestion was weakly related to total Chl *a* concentrations and only slightly better related to Chl *a* concentration in the >20-μm category (Fig. 5). The large amount of variability in Fig. 5 indicates factors controlling ingestion, for *N. cristatus* are much more complex than this simple expression of food concentration.

Six experiments were selected to provide more detailed analysis of particle composition in grazing experiments under nonbloom conditions (experiments 1, 6 and 8) and bloom conditions (experiments 17, 18 and 19). Microscopic counts of microplankton, HNF and picoplankton in these six experiments were used to determine clearance rates on each of these prey categories (Table IV). Patterns are consistent with those seen in bulk Chl *a*, which is also shown in Table IV for these specific experiments. Clearance rates were highest on large particles and higher under nonbloom conditions than bloom conditions. *Neocalanus cristatus* grazed heavily on oligotrich ciliates and *Mesodinium* ciliates in all experiments and grazed equally heavily on diatoms, except on May 25 when a diatom bloom of very high concentrations occurred (total = 2.82×10^5 cells L^{-1}, *C. radicans* = 1.56×10^5 cells L^{-1}). For large ciliates and diatoms, clearance rates averaged between 1 and 2 L copepod^{-1} day^{-1} under nonbloom conditions. The highest clearance rate observed was 3.2 L copepod^{-1} d^{-1} in experiment 6 under nonbloom conditions (Table IV). We observed comparatively low grazing on dinoflagellates. Overall, clearance rates of *N. cristatus* on phytoplankton and microzooplankton >20 μm were much higher in nonbloom, picoplankton-dominated conditions than in bloom conditions dominated by large phytoplankton. In contrast, clearance rates were typically negative on the small particles including HNF <5 μm, *Synechococcus* and eucaryotic ultraplankton. The presence of *N. cristatus* led to an increase in apparent growth rate of these small cells. These patterns are easily seen by comparing the averages of all data from these six experiments (Fig. 6).

Based on carbon biomass, ciliates were the most important prey for *N. cristatus* in nonbloom waters, whereas phytoplankton contributed the majority of food for *N. cristatus* under bloom conditions (Fig. 7). In nonbloom conditions, *N. cristatus* ingested 5.36 μg C copepod^{-1} day^{-1} from ciliates, 3.64 μg C copepod^{-1} day^{-1} from phytoplankton and 0.97 μg C copepod^{-1} day^{-1} from heterotrophic dinoflagellates. On the other hand, under bloom conditions, *N. cristatus* ingested 13.39 μg C copepod^{-1} day^{-1} from phytoplankton, much more than from heterotrophic dinoflagellates (6.06 μg C copepod^{-1} day^{-1}) and ciliates (1.92 μg C copepod^{-1} day^{-1}). Overall, *N. cristatus* ingested two times more carbon under bloom conditions than under nonbloom

Table IV: Clearance rates (L cristatus^{-1} day^{-1}) of Neocalanus cristatus on prey items of different sizes during 6 experiments in 2001

Water type	Nonbloom				Bloom			
Date	17 April	19 May	26 May		26 April	29 April	25 May	
Experiment number	1	6	8	Mean	17	18	19	Mean
>20 μm Chl *a*	0.656 (0.360)	0.286 (0.146)	0.805 (0.254)	0.540 (0.329)	0.524 (0.177)	0.401 (0.112)	0.190 (0.203)	0.372 (0.210)
Ciliates	0.574 (0.223)	1.396 (0.544)	1.704 (0.544)	1.057 (0.785)	0.228 (0.405)	0.175 (0.241)	0.458 (0.379)	0.271 (0.332)
Mesodinium	1.961 (1.234)	1.229 (0.076)	3.183 (2.227)	2.148 (1.572)	1.011 (0.437)	0.475 (0.369)	0.924 (1.395)	0.803 (0.825)
Dinoflagellates	–0.310 (0.382)	0.301 (0.886)	–0.028 (0.302)	–0.044 (0.619)	0.332 (0.186)	0.264 (0.157)	0.092 (0.381)	0.242 (0.239)
Diatoms	1.104 (0.777)	1.786 (1.022)	2.403 (1.814)	1.546 (1.132)	0.297 (0.118)	0.063 (0.108)	–0.789 (0.560)	–0.084 (0.535)
5–20 μm Chl *a*	0.707 (0.355)	–0.025 (0.163)	0.166 (0.092)	0.239 (0.378)	–0.031 (0.082)	–0.034 (0.052)	–0.167 (0.274)	–0.077 (0.166)
5–20 μm heterotrophic nanoflagellates	0.267 (0.841)	–1.074 (0.968)	–0.752 (0.482)	–0.498 (0.962)	0.061 (0.128)	–0.002 (0.205)	0.086 (0.254)	0.055 (0.175)
<5 μm Chl *a*	0.042 (0.071)	–0.229 (0.079)	–0.240 (0.134)	–0.198 (0.193)	–0.238 (0.139)	–0.366 (0.272)	–0.149 (0.075)	–0.266 (0.190)
<5 μm heterotrophic nanoflagellates	0.008 (0.168)	–0.865 (0.464)	–0.267 (0.253)	–0.385 (0.494)	–0.497 (0.087)	–0.177 (0.055)	–0.421 (0.308)	–0.388 (0.219)
Picoeukaryotes	–0.040 (0.067)	–0.109 (0.031)	–0.289 (0.148)	–0.142 (0.143)	0.053 (0.151)		–0.209 (0.193)	–0.056 (0.210)
Synechococcus	–0.016 (0.021)	0.006 (0.014)	–0.110 (0.091)	–0.028 (0.058)	0.096 (0.106)		–0.035 (0.059)	0.048 (0.110)

Numbers in parentheses are standard deviations.

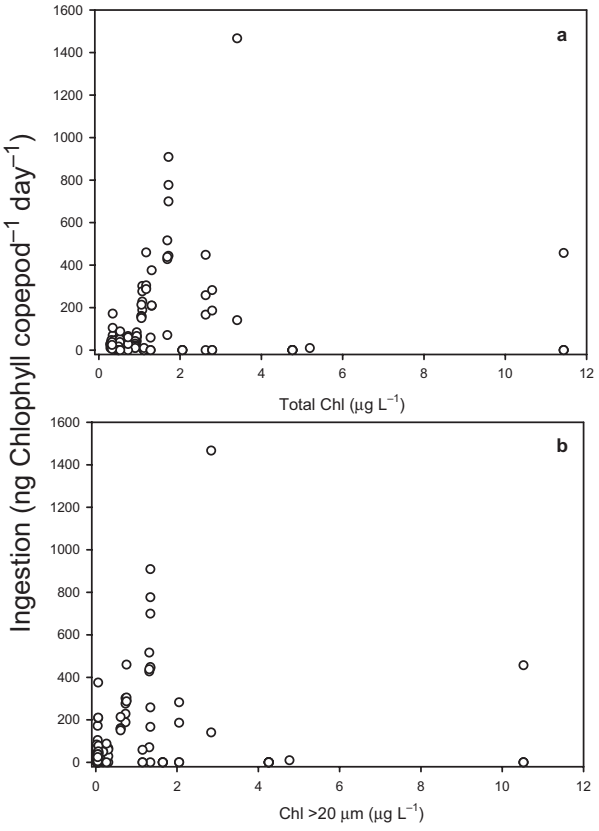

Fig. 5. Ingestion of chlorophyll *a* (Chl *a*) by *Neocalanus cristatus* as a function of (**a**) total Chl *a* concentration and (**b**) concentration of Chl *a*-containing particles >20 μm.

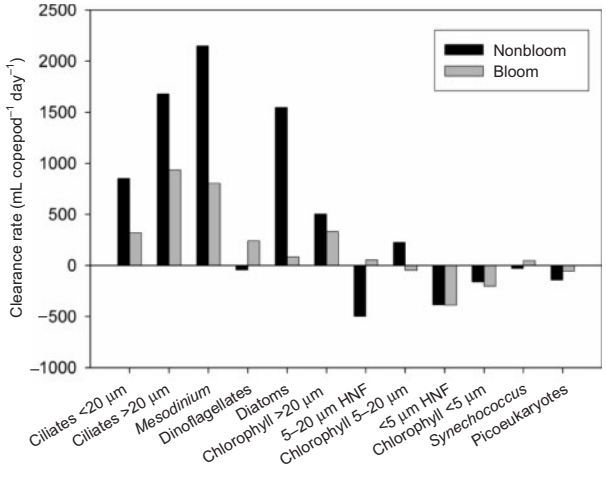

Fig. 6. Clearance rates on each food category under nonbloom and bloom conditions.

conditions (21.27 versus 9.97 μg C copepod^{-1} day^{-1}, respectively). During nonbloom conditions, 73% of ingested C was from heterotrophs and 27% from autotrophs. In contrast, heterotrophs were less important than autotrophs in the diet of *N. cristatus* under bloom conditions, 37 and 63% respectively.

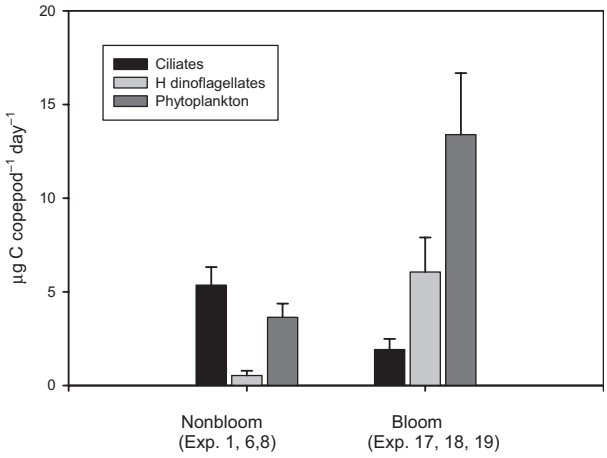

Fig. 7. The contribution of phytoplankton and heterotrophs to the diet of *Neocalanus cristatus* under nonbloom and bloom conditions.

DISCUSSION

All our bottle incubations indicate that large particles are the primary food source for late copepodid stages of *N. cristatus*. Changes in Chl *a* concentration during incubation indicated small particles (<5 μm) were not consumed, intermediate-sized particles (5–20 μm) were only consumed in a few experiments, and almost all consumption was from the large particle category (>20 μm). This pattern was verified by detailed microscopic analysis of particles in six selected experiments and FlowCAM data from three experiments. Clearance rates were highest on large ciliates and other large microplankton, and there was no net removal of HNF <5 μm, picoeukaryotes or *Synechococcus*.

Clearance rates on the largest particles are probably underestimates, particularly in the experiments done under nonbloom conditions. Calculated clearance rates of 1–3 L copepod^{-1} day^{-1} indicate that, even with only one copepod in a 2.3-L bottle, the abundance of large particles would have greatly decreased before the 24-h incubation was complete. Incubation bottles typically contained between one and three copepods L^{-1}, although we presented only data from bottles containing one or two copepods L^{-1}. We believe that *N. cristatus* in its natural environment is able to clear large particles at considerably higher rates than our experiments indicate. Furthermore, it is possible that larger, rarer particles we did not account for in our experiments may also be important to the *N. cristatus* diet. FlowCAM analysis of the experiment done under nonbloom conditions (Fig. 4a) indicated clearance rates on particles >60 μm were as high as those

observed on the 41- to 50-μm and 51- to 60-μm categories. *Neocalanus cristatus* however is not a raptorial feeder and is unable to capture large mobile prey the way a strong carnivore-like *Euchaeta* spp. does. Laboratory work (Greene and Landry, 1988) indicates that 'carnivory' in *N. cristatus* results from capture of animals via feeding currents. Because of its large size, *N. cristatus* can probably generate a strong feeding current, sufficient to capture large protists and some small metazoans that might escape the feeding currents of smaller copepods. Additional experiments are required to identify the upper limits of food size and clearance rate of *N. cristatus*.

In our study, total consumption under nonbloom conditions averaged 10.0 μg C copepod^{-1} day^{-1} and under bloom conditions averaged 21.4 μg C copepod^{-1} day^{-1}. *Neocalanus cristatus* body size in May 2004 averaged 511 μg C individual^{-1} (n = 12) in the inner shelf, 670 μg C individual^{-1} (n = 26) in the middle shelf and 892 μg C individual^{-1} (n = 31) in the outer shelf. Applying a relationship between body size and respiration derived for Bering Sea plankton (Dagg *et al.*, 1982) indicates metabolic demands would be 25.0, 31.8 and 41.0 μg C copepod^{-1} day^{-1} (i.e. ~0.047 day^{-1}) in the inner-, middle- and outer-shelf regions. These are probably slight underestimates because Bering Sea temperatures were a few degrees cooler. Additional demands for growth would be considerable because this large copepod has an annual life cycle and completes most of its growth in a few months. Growth rates in Bering Sea CV *N. cristatus* were between 0.03 and 0.07 day^{-1} (Vidal and Smith, 1986), equivalent to between 21 and 100 μg C copepod^{-1} day^{-1}. Thus, the 10–21 μg C copepod^{-1} day^{-1} (~0.03 day^{-1}) ingested in our experiments is inadequate to meet respiratory and growth demands of ~40–140 μg C copepod^{-1} day^{-1} for this copepod.

We may have underestimated the ingestion of intermediate-sized particles (5–20 μm) in our experiments. Particle increases in small- and intermediate-size categories were observed in most of our bottle incubations. This could be due to several factors, including (i) a growth stimulus from nutrients excreted by the large copepods and an inability of *N. cristatus* to feed on small- and intermediate-sized particles and (ii) the ingestion of microzooplankton consumers of small- and intermediate-size particles by *N. cristatus*, with subsequent increase in the net population growth rates of these small particles.

We do not believe that nutrient excretion contributed to this pattern. Ambient nutrient concentrations were high in most of the experiments in April 2001 and May 2001 (Table V) and nutrients were added to all bottles in July 2001 and in all 2003 experiments. Experimental water was almost always collected from the 50% light depth (between ~3–10 m depending on the location), and so initial nutrient concentrations in the incubation bottles would be somewhere between the surface and 10-m concentrations reported in Table V. Only experiments 14 and 15 had low nutrient levels at the onset of incubation. We concluded that the enhanced growth of small- and intermediate-sized particles in our incubations was not due to the release of excretory nutrients by the grazers.

Table V: Nutrient concentrations in ambient water used for grazing experiments

Experiment	Nutrients not added—ambient levels			
	Depth (m)	PO_4 (μM)	$Si(OH)_4$ (μM)	NO_3 (μM)
1 and 2	0	1.36	22.2	14.4
	10	1.33	21.7	14.2
3	0	1.27	21.7	13.8
	10	1.29	21.5	14.0
4	0	1.34	26.3	15.3
	10	1.36	26.0	15.5
5	0	1.64	28.2	19.8
	10	1.48	24.0	16.8
6	0	1.13	21.2	10.7
	10	1.11	21.3	10.8
7	0	1.08	21.4	9.9
	20	1.09	20.9	10.3
8	0	1.00	20.7	9.6
	10	1.01	21.4	9.6
14	0	0.16	0.0	0.2
	5	0.17	0.0	0.1
	10	0.16	0.1	0.1
15	0	0.25	0.2	0.4
	5	0.36	0.4	0.8
	10	0.53	1.9	2.3
17	0	0.35	1.2	1.7
	5	0.39	1.7	2.1
	10	0.46	2.4	3.0
18	0	0.30	1.1	0.9
	8	0.38	2.0	1.8
19	0	0.59	6.5	3.2
	10	0.77	9.9	5.1
20	0	0.14	0.4	0.3
	5	0.28	3.2	1.5
	10	0.94	15.9	8.9
21	0	0.47	3.3	2.3
	5	0.82	9.2	6.4
	10	0.94	12.6	8.2

Experiments not listed had nutrients added to all incubation bottles to attain a final concentration of 5-μM NO_3, 1-μM NH_4 and 0.3-μM PO_4.

Strong coupling of nonadjacent trophic levels via indirect cascade effects is a well-established phenomenon in freshwater systems (e.g. Carpenter *et al.*, 1985; Pace *et al.*, 1998) and has also been observed in marine systems (Landry *et al.*, 1993; Calbet and Landry, 1999; Vadstein *et al.*, 2004). In our experiments, removal of microzooplankton by *N. cristatus*, combined with the inability of *N. cristatus* to feed directly on individual particles <5 μm, resulted in a decrease in the grazing mortality of small particles, allowing them an enhanced population growth rate. This was evident in both the Chl and the microscopic analyses. It is this cascade effect that resulted in the increased apparent growth rate of particles <5 μm in all our experiments. A cascade effect was also always observed in intermediate-sized cells (5–20 μm) under bloom conditions and often under nonbloom conditions, suggesting *N. cristatus* is capable of ingesting cells in the 5- to 20-μm size range but does not do so under bloom conditions. Ingestion of cells in this size range was also observed by Frost *et al.* (Frost *et al.*, 1983). Possibly *N. cristatus* directly consumed intermediate-sized cells only under nonbloom conditions and shifted its diet away from intermediate sizes under bloom conditions, allowing the observed enhanced growth rate.

There may be trophic interactions and cascade effects more complex than those indicated by our size-fractionated Chl data. In the FlowCAM analysis of experiment 17 (Fig. 4b), it appeared that the consumption of the >50 μm particles by *N. cristatus* resulted in a release on the 31– to 50-μm particles. Both particle groups were comprised of dinoflagellates and ciliates, but the species were different. A mix of chain-forming diatom genera was present throughout both groups. Particles in the 11- to 30-μm size range were removed at very high rates during the incubations. It seems unlikely that *N. cristatus* would remove particles 11–30 μm and particles >50 μm but not particles between those sizes (31–50 μm), so it is not clear what the trophic interactions are in this experiment, except that they are complex. In the control bottles of this experiment (Fig. 8), with no *N. cristatus*, the particle-size spectrum shifted dramatically toward the smaller particles, suggesting intense activity within the microzooplankton that is somehow kept in check by the presence of *N. cristatus*. The details of the trophic interactions initiated by *N. cristatus* are not fully elucidated by these analyses, but the effect is a strong one and suggests that *N. cristatus* feeding behavior is complex and flexible.

A clear cascade effect was observed in our experimental bottles and such an effect would also occur *in situ*. In our study area, the highest abundance of *N. cristatus* CV in May 2001 was between 0.2 and 0.3 copepod L^{-1} (J. Napp, National Marine Fisheries, Seattle, personal communication). Clearance rates of 3 L copepod^{-1} day^{-1} on large

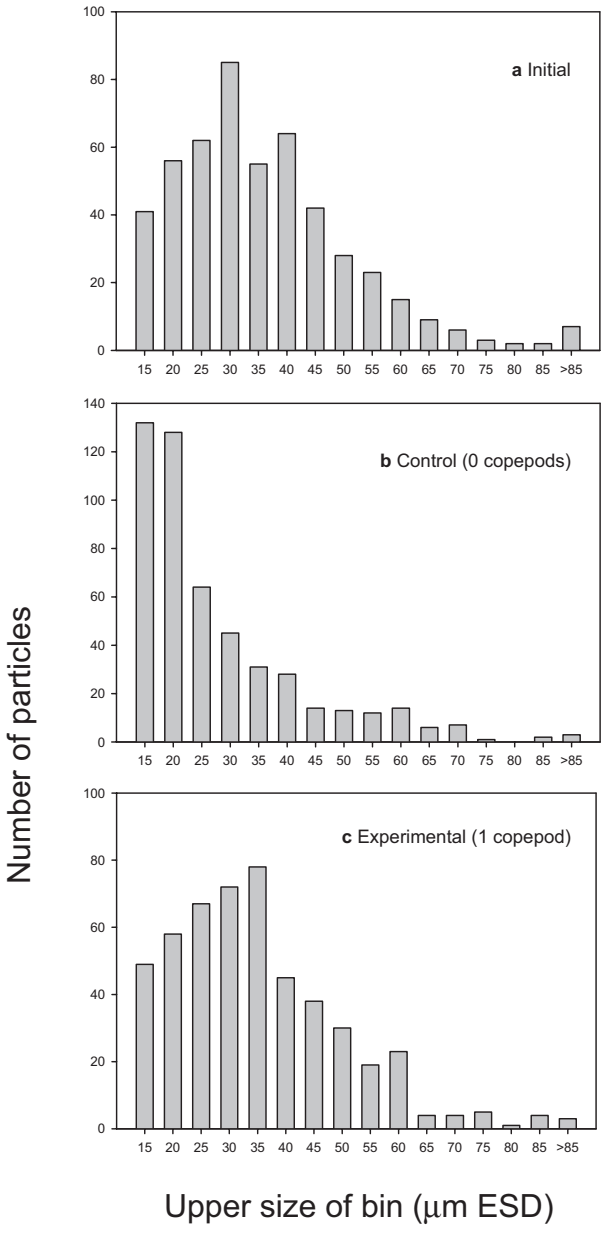

Fig. 8. FlowCAM counts from (**a**) the initial water, (**b**) a control bottle after 24 h and (**c**) experimental bottle after 24 h, in experiment 17. Five hundred particles were counted in each sample.

particles such as ciliates could easily induce a cascade effect *in situ* and modify the structure and dynamics of the microplankton food web.

In 2001, more ciliates would have been consumed under nonbloom conditions, because ciliate concentrations were approximately the same under bloom and nonbloom conditions and *N. cristatus* clearance rates were higher under nonbloom conditions. Consequently, we might expect to see a stronger cascade effect under nonbloom conditions. This did not appear to be the

case, however, either in bulk Chl *a* <5 μm or in the various categories of small cells, where no significant differences were observed between bloom and nonbloom conditions. This is probably due to compensatory mechanisms within food webs that dampen or eliminate cascades (Polis and Strong, 1996). If our system was a simple food chain such that each component represented one trophic role, then copepods would feed on ciliates which fed on HNF which fed on cyanobacteria. Under these conditions, we would expect to see a decrease in ciliates, an increase in HNF and a decrease in cyanobacteria as a result of trophic cascading induced by *N. cristatus* grazing. However, the microbial food web is complex and most components have multiple trophic roles and feed across more than one trophic level. For example, both ciliates and HNF feed on picoplankton (Christoffersen, 1994; Christaki *et al.*, 1998; Callieri *et al.*, 2002), and heterotrophic dinoflagellates can feed on a wide range of organisms from bacteria to metazoans, including chains of diatoms (Lessard, 1991). This complexity dampens and suppresses trophic cascades. In our experiments, *N. cristatus* was feeding on large particles, both autotrophs and heterotrophs, and most of the heterotrophs consumed were also omnivores. Therefore, it is very difficult to predict the detailed outcome of food web changes that would be caused by *N. cristatus* grazing except to say that some enhancement in growth rate of populations of <5-μm–sized particles will occur.

Direct ingestion of particles in the 5- to 20-μm size range and enhanced growth due to a cascade effect may be occurring simultaneously. Under these conditions, the net effect of copepod grazing and cascade-induced enhancement may still result in net particle production in this size category though some ingestion is occurring. This ingestion would not be included in our calculations. The degree to which we are underestimating ingestion due to this process is not clear, but an estimate can be made. If we assume *N. cristatus* is unable to ingest individual cells <5 μm, then the difference between the cascade-induced enhancement of small cells and intermediate-sized cells can be attributed to ingestion of intermediate-size cells. In every nonbloom experiment, the net growth in the small-cell category was greater than in the intermediate-cell category, suggesting the ingestion of intermediate-sized cells. The average difference was 56 ng Chl copepod^{-1} day^{-1}, equivalent to 4.2 μg C copepod^{-1} day^{-1}. This simplistic calculation assumes that growth rates of small- and intermediate-sized cells are the same, all the ingestion is attributable to *N. cristatus*, and there is no cascade effect of large particles on intermediate-sized particles. Nevertheless, it provides an initial determination of our underestimate of ingestion of intermediate-sized cells under nonbloom conditions. In bloom experiments, the differences were sometimes positive (small cell > intermediate cell, $n = 3$) and sometimes negative (intermediate cell > small cell, $n = 9$), but the overall average was negative (–44 ng Chl copepod^{-1} day^{-1}), although not significantly different from 0, indicating no ingestion of intermediate-sized cells. We conclude that the maximum amount we are underestimating the ingestion of intermediate-sized cells is 4.2 μg C copepod^{-1} day^{-1}, and this applies only to nonbloom conditions.

Conditions at our nonbloom stations were similar to those encountered across the entire HNLC region of the subarctic Pacific and earlier incubation experiments in the open subarctic Pacific, at Station 'P', gave comparable results. For *N. cristatus* CV, ingestion of protists averaged 4.1 μg C copepod^{-1} day^{-1} (Gifford, 1993) and ingestion of phytoplankton averaged 6.3 μg C copepod^{-1} day^{-1} (Dagg, 1993a) for a total of 10.4 μg C copepod^{-1} day^{-1}. Gifford suggested small mesozooplankon may make an additional contribution of 2.1–4.3 μg C copepod^{-1} day^{-1} yielding a total between 12.5 and 14.7 μg C copepod^{-1} day^{-1}. In another study, done in the western subarctic Pacific, ingestion rate of phytoplankton by CV *N. cristatus* was also very low, only 3.3 μg C copepod^{-1} day^{-1}, much less than the metabolic requirement of 18.1 μg C copepod^{-1} day^{-1} (Tsuda and Sugisaki, 1994). More recently, Kobari *et al.* (Kobari *et al.*, 2003) measured total ingestion rates of only a few μg C copepod^{-1} day^{-1} in the same region under nonbloom conditions. In the southeast Bering Sea under nonbloom conditions, ingestion rates of phytoplankton by *N. cristatus* CV were considerably higher, between 10 and 64 μg C copepod^{-1} day^{-1} (Dagg and Wyman, 1983). Microzooplankton in the diet were not measured in the latter two studies (Dagg and Wyman, 1983; Tsuda and Sugisaki, 1994).

Under bloom conditions in our study, average ingestion rate was approximately double that observed under nonbloom conditions, 21.4 μg C copepod^{-1} day^{-1} versus 10.0 μg C copepod^{-1} day^{-1}. In an earlier study done in the southeast Bering Sea under bloom conditions, ingestion rates of phytoplankton by *N. cristatus* CV were much higher, between 67 and 438 μg C copepod^{-1} day^{-1} (Dagg and Wyman, 1983). Under bloom conditions in the western subarctic Pacific, ingestion rates were also higher than ours, >100 μg C copepod^{-1} day^{-1} during the April bloom and ~30 μg C copepod^{-1} day^{-1} during the immediate post-bloom period when phytoplankton was moderately high (Kobari *et al.*, 2003).

These different studies indicate that *N. cristatus* CV ingestion rates are highly variable even within apparently similar situations of bloom or nonbloom conditions. Some of this variability may be due to active selectivity by *N. cristatus*. In our experiments, we

observed *N. cristatus* feeding on 5–20 μm of phytoplankton under nonbloom conditions but not under bloom conditions. On the other hand, *Neocalanus* spp. respond to small increases in phytoplankton concentration by large increases in their feeding rate (Frost *et al.*, 1983), so subtle differences in the food that we would not detect in our coarse size fractions might have important effects on feeding rate. In addition, some of this variability among different studies may be attributed to methodological differences. For example, based on gut contents and other evidence, it was determined that, in addition to individual particles, *N. cristatus* in the open north Pacific consumes sinking aggregates comprised of small particles, detritus and their associated microzooplankton communities (Dagg, 1993b). High ingestion rates of phytoplankton measured under bloom conditions using gut-content methods (Dagg and Wyman, 1983) may also result from the ingestion of aggregates, not simply from clearing individual phytoplankton. These conditions would not be mimicked in incubation bottles, resulting in an underestimation of ingestion in bottle incubations. We suggest that even under bloom conditions, bottle experiments do not provide a representative food environment for *N. cristatus*.

All existing information on *N. cristatus* indicates a flexible feeding behavior, although it remains clear that large particles are the most important component of the diet. Kobari *et al.* (Kobari *et al.*, 2003) observed that *N. cristatus* in the Oyashio region obtained >80% of their ingested carbon from diatoms during April diatom blooms, but 78% of their ingested carbon from ciliates during July when the water column was oligotrophic. Total carbon ingested in July was low, ~5 μg C copepod^{-1} day^{-1}, but >100 μg C copepod^{-1} day^{-1} in April (Kobari *et al.*, 2003). Our bottle incubations are consistent with this pattern in that ciliates and heterotrophic dinoflagellates constituted >70% of the *N. cristatus* diet when Chl *a* concentration was low and phytoplankton was dominated by <5-μm cells. Microzooplankton in our experiments accounted for only ~30% of *N. cristatus* carbon ingestion when large diatoms were abundant. In general, the contribution of microzooplankton to copepod diet varies widely (Halvorsen *et al.*, 2001) and appears to be dependant on the biological conditions of the study sites, particularly the biomass and size composition of phytoplankton and microzooplankton. Where the initial concentrations of microzooplankton are high and Chl *a* concentrations are low and dominated by small phytoplankton cells, the contribution of microzooplankton to the copepod diet is higher (Fessenden and Cowles, 1994; Ohman and Runge, 1994).

The feeding behavior of *N. cristatus*, one of the most abundant copepods in the entire North Pacific Ocean, remains incompletely understood. We believe our ingestion rates are underestimates of *in situ* rates because (i) we are underestimating removal rates of large particles, (ii) we may not be including some very large particles that should be considered as diet items and (iii) we are not properly accounting for the ingestion of aggregates. Significant amounts of carbon flow directly through *Neocalanus* spp. in the North Pacific Ocean (e.g. Kobari *et al.*, 2003) and refinement of carbon budgets will require better information on *N. cristatus* feeding. More significantly, *N. cristatus* does not indiscriminantly ingest all sizes of particles but instead primarily impacts the large particles and thereby modifies the size composition and structure of the microplankton community, indirectly altering the carbon flow through the pelagic system.

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