

# Grazing by mesozooplankton from Kiel Bight, Baltic Sea, on different sized algae and natural seston size fractions

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**ABSTRACT:** Grazing experiments were conducted with natural mesozooplankton from Kiel Bight, Germany, using radioactive labelled phytoplankton cultures and seston size fractions. The results of experiments using phytoplankton cultures indicated that bivalve veligers performed highest clearance of particles within a size range of 4.7 to 6.3  $\mu\text{m}$ , whereas optimum particle size for copepods was 15  $\mu\text{m}$ . The results of experiments using labelled natural seston size fractions identified bivalve veligers and appendicularians as those responsible for the removal of particles within the smallest size class (<2  $\mu\text{m}$ ). Seston size fractions larger than 5  $\mu\text{m}$  were mainly cleared by copepods and nauplii. As particle size increased, the contribution of copepod clearance to total zooplankton clearance within size classes increased from 57% (<5  $\mu\text{m}$  size class) to more than 81% (30 to 100  $\mu\text{m}$  size class). When the nauplii clearance rates were included, the total copepod clearance accounted for 90 to 97.6% of the total volume cleared of particles bigger than 10  $\mu\text{m}$ . Despite low abundances of bivalve veligers and appendicularians in Kiel Bight at the time of the experiment, we calculated that approximately 10 and 8.5%, respectively, of the carbon ingested by total mesozooplankton was due to veliger and appendicularian grazing. The importance of bivalve veligers might be seen in their grazing on seston particles that escape predation by copepods and on the amount of energy that is therefore directed from the water column to the benthos when larvae settle.

**KEY WORDS:** Bivalve veligers · Copepods · Size-selectivity · Community grazing

## INTRODUCTION

In most marine pelagic ecosystems calanoid copepods are the most abundant mesozooplankton guild and provide the bulk of food for commercially important planktivorous fish (Cushing 1975). Therefore, in terms of energy, the main carbon flow from phytoplankton towards fish stocks is expected to be mediated via copepods. Gelatinous zooplankton and meroplanktonic larvae can be regarded as planktonic groups that temporarily direct energy out of this food

chain to the benthos (settling meroplanktonic larvae) or into trophic guilds (e.g. coelenterates), which are not heavily grazed upon by commercial fish stocks (Cushing 1975).

Copepods have been found to feed on a wide range of particles of auto- and heterotrophic seston organisms (for review Kleppel 1993) which can be selectively captured (Alcaez et al. 1980), tested for their nutritional quality (DeMott 1988, 1989) and perceived by specialized mechanoreceptors located on the first antennae (Bundy et al. 1998). Although the freshwater copepod *Diaptomus sicilis* has been found to ingest particles as small as  $\leq 4 \mu\text{m}$  due to a passive feeding mode (Vanderploeg & Pfaffenhöfer 1985), optimum particle sizes for

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copepods reported in the literature usually lie beyond 10  $\mu\text{m}$  (Harris 1982, Vanderploeg & Pfaffenhöfer 1985, Berggreen et al. 1988, Nakamura & Turner 1997). In contrast, meroplanktonic larvae, such as bivalve veligers, have been reported to ingest particles as small as heterotrophic bacteria (Prieur 1983, Douillet 1993) or the cyanobacterium *Synechococcus* (Gallager et al. 1994). Whereas some authors have found selective grazing by bivalve veligers on particles > 10  $\mu\text{m}$  (Fritz et al. 1984) or even > 20  $\mu\text{m}$  (Baldwin & Newell 1991), a preference of particles sized  $\leq 4$   $\mu\text{m}$  has been reported by others (Riisgård et al 1980, Baldwin 1995).

We performed grazing experiments with the natural mesozooplankton composition present at Kiel Bight, Germany (54° 19' 27" N, 10° 08' 01" E). Major marine groups such as echinoderm larvae and salps are missing in the plankton of the Baltic Sea, reflecting the general characteristic of the Baltic as an area of uniquely low species biodiversity. Large mussel banks down to 30 m depth are formed by *Mytilus edulis* dominating animal biomass (Kautsky 1981), which may be one explanation why bivalve veliger larvae compose up to 28% of the mesozooplankton community in Kiel Bight (Fig. 1).

Using radioactive labelled cultures of marine phytoplankton and seston size fractions as tracers, we (1) evaluated clearance rates for copepods and bivalve veligers in order to determine optimum particle sizes, (2) calculated the contribution of the main mesozooplankton guilds to total zooplankton grazing within seston size fractions and (3) quantified the amount of carbon ingested by zooplankton guilds.

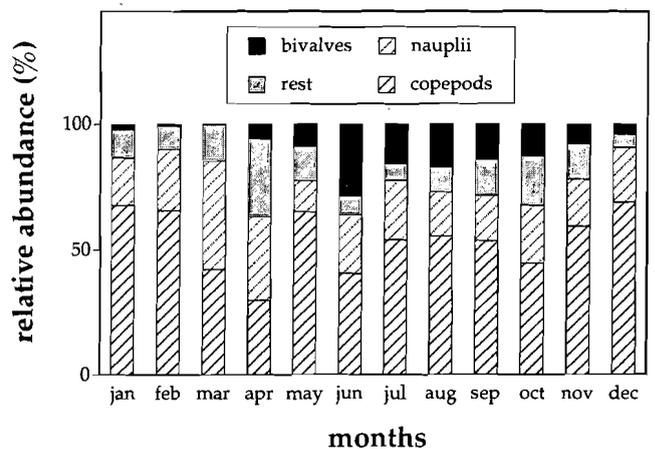


Fig. 1. Relative monthly mean abundances of zooplankton (1985 to 1992) at Boknis Eck, Kiel Bight. Data used to calculate stacks were taken from Behrends (1996)

## MATERIAL AND METHODS

**Cultures.** Various cultures of marine phytoplankton were obtained from the Department of Marine Botany, Institut für Meereskunde (Table 1). The algae were classified to the species level except for 2 that are referred to as pennate diatoms 1 and 2. All phytoplankton cultures were grown in 0.2  $\mu\text{m}$  membrane-filtered Baltic seawater medium to which nutrients and trace metals were added (Von Stosch & Drebes 1964; modified). Surface seawater was filtered through 0.8  $\mu\text{m}$  membrane filters in order to remove

Table 1. Phytoplankton cultures serving as tracers in grazing experiments: values represent means of cell dimensions ( $n = 50$ ) and of clearance rates ( $n = 3$ ). Values in brackets are 1 SD; \* $p < 0.05$ ; \*\* $p < 0.005$

Tracer	Cell dimensions		Clearance rate ( $\mu\text{l ind.}^{-1} \text{h}^{-1}$ )
	Length or diameter ( $\mu\text{m}$ )	Width ( $\mu\text{m}$ )	
<b>Bivalve veligers</b>			
Heterotrophic bacteria	<0.8	<0.8	5.2 (1.44)
<i>Chlorella sp.</i>	2.7 (0.4)	–	3.4 (1.35)
<i>Emiliana huxleyi</i>	4.7 (0.6)	–	17.3 (4.08)**
Pennate diatom 1	6.3 (0.3)	4.0 (0.5)	21.9 (6.18)**
<i>Prymnesium parvum</i>	8.7 (0.9)	–	5.3 (2.91)
<i>Chrysochromulina polylepis</i>	9.7 (0.4)	9.0 (0.7)	2.7 (0.90) <sup>a</sup>
<i>Thalassiosira weissflogii</i>	10.0 (0.4)	5.2 (0.7)	2.1 (0.94)
Pennate diatom 2	14.3 (2.5)	7.0 (1.3)	0.97 (0.45) <sup>a</sup>
<i>Heterocapsa triquetra</i>	21.0 (1.8)	15.3 (1.2)	1.5 (0.88)
<b>Copepods</b>			
Pennate diatom 1	5.6 (0.7)	5.0 (0.4)	21.9 (3.16)
<i>Emiliana huxleyi</i>	6.3 (1.1)	–	13.2 (0.59)
<i>Chrysochromulina polylepis</i>	9.8 (1.9)	8.3 (0.7)	21.2 (5.46)
<i>Thalassiosira weissflogii</i>	15.0 (2.0)	8.8 (0.9)	58.1 (17.22)*
<i>Nitzschia closterium</i>	49.5 (3.3)	3.5 (0.3)	26.7 (7.14)
<i>Nitzschia longissima</i>	73.0 (4.1)	7.2 (0.6)	25.1 (12.85)

<sup>a</sup> $n = 2$

all cells except suspended heterotrophic bacteria. The 0.8  $\mu\text{m}$  filtrate as well as algal cultures were stored at  $18 \pm 0.5^\circ\text{C}$  and  $80$  to  $100 \mu\text{E m}^{-2} \text{s}^{-1}$  (PAR 400 to 800 nm; LiCor Li 189). Algae were selected for grazing experiments in accordance with the reported range of particle sizes ingested by copepods and bivalve veligers, respectively. On the day prior to grazing experiments, algal cell dimensions of at least 50 individuals were determined to the closest 0.5  $\mu\text{m}$  at  $630\times$  magnification under a Zeiss Axioplan microscope (Table 1).

**Seston size fractions.** Baltic surface seawater was serially fractionated in order to establish the (1) 30 to 100  $\mu\text{m}$ , (2) 10 to 30  $\mu\text{m}$ , (3) 5 to 10  $\mu\text{m}$  and (4)  $<5 \mu\text{m}$  seston size classes. Part of the 5  $\mu\text{m}$  fraction was filtered through a 2.0  $\mu\text{m}$  membrane filter creating the (5)  $<2 \mu\text{m}$  size class. Medium (as above) was added to 80–100 ml of each fraction and stored in 600 ml TC (tissue culture) flasks at  $11 \pm 0.5^\circ\text{C}$  and at  $80$  to  $100 \mu\text{E m}^{-2} \text{s}^{-1}$  (12 h light:12 h dark cycle). Steps of fractionation and radioactive labelling are given in Fig. 2.

The taxonomic composition of the seston size fractions, as determined in 10 ml Utermöhl chambers (Drebes 1974), remained fairly constant over the growth period. Because of cell growth and the formation of colonies, the above-described sieving and filtration procedure was repeated on the day of radioactive labelling in order to attain the original size cut-off of seston size classes. After repeating the fractionation procedure, species composition of all size fractions was determined in 10 ml Utermöhl chambers and additionally, by means of epifluorescence microscopy (Leica Type DMRB) for both the  $<2$  and  $<5 \mu\text{m}$  size fractions (Table 2). Samples for epifluorescence microscopy were prepared by filtering 5 ml of each seston size fraction onto black membrane filters (0.2  $\mu\text{m}$  pore size) and staining them with 400  $\mu\text{l}$  of DAPI (4', 6-diamino-2-phenylindole) solution ( $10 \mu\text{g ml}^{-1}$ ) for 10 min.

**Radioactive labelling.** Twenty-four hours prior to the grazing experiments, 40 ml of each seston size fraction or phytoplankton culture was labelled with 1 ml of  $^{14}\text{C}$  bicarbonate ( $\text{NaH}^{14}\text{CO}_3$ ; Hartmann Analytic, Braunschweig; specific activity =  $25 \mu\text{Ci ml}^{-1}$ ; final concentration =  $0.6 \mu\text{Ci ml}^{-1}$ ). Heterotrophic bacteria were inoculated with 5 ml of radioactive glucose (Hartmann Analytic, Braunschweig; specific activity =  $5 \mu\text{Ci ml}^{-1}$ ; final concentration =  $0.6 \mu\text{Ci ml}^{-1}$ ). Due to the presence of cyanobacteria in the  $<2 \mu\text{m}$  fraction (Table 2), this

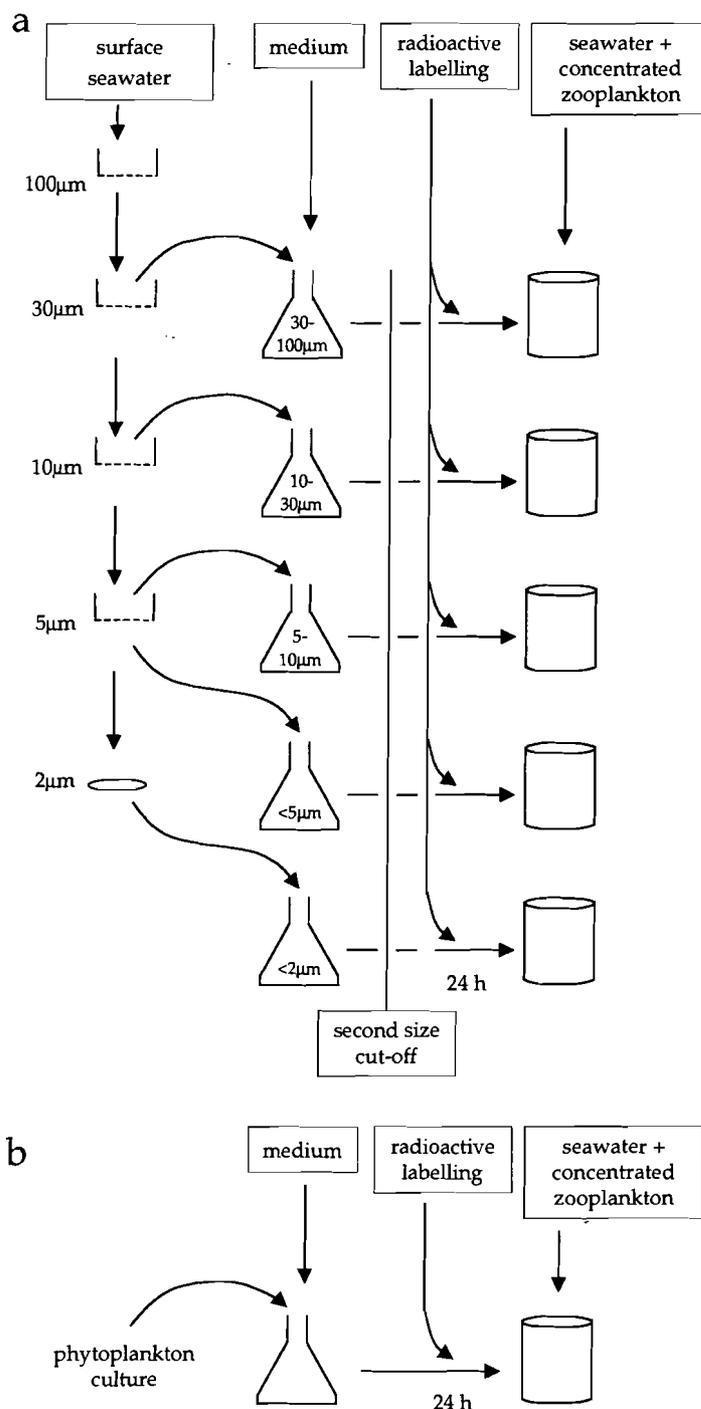


Fig. 2. Scheme illustrating the procedures of plankton fractionation and radioactive labelling in experiments using (a) seston size fractions and (b) phytoplankton cultures. For details see text

size class was labelled with both  $^{14}\text{C}$  bicarbonate and radioactive glucose at a final concentration of  $1.1 \mu\text{Ci ml}^{-1}$ . The bottles were stored under the same conditions as during growth of the seston size fractions until experiments commenced.

Table 2. Taxonomic composition of the seston size fractions determined in Utermöhl chambers prior to radioactive labelling

Taxon	Seston size fraction				
	30 to 100 µm	10 to 30 µm	5 to 10 µm	<5 µm	<2 µm
Centric diatoms	<i>Thalassiosira weissflogii</i>	<i>T. weissflogii</i>	<i>T. weissflogii</i>		
	<i>Thalassiosira nitzschoides</i>				
	<i>Coscinodiscus</i> sp.				
	<i>Chaetoceros</i> sp.	<i>Chaetoceros</i> sp.			
	<i>Chaetoceros debilis</i>				
Pennate diatoms	<i>Chaetoceros curvisetus</i>				
	<i>Skeletonema costatum</i>	<i>S. costatum</i>	<i>S. costatum</i>	<i>S. costatum</i>	Cyanobacteria <sup>a</sup> ( <i>Pseudanabaena</i> ?)
	<i>Thalassionema nitzschioides</i>				
	<i>Ditylum brightwellii</i>				
	<i>Nitzschia longissima</i>	<i>N. longissima</i>			Heterotrophic bacteria <sup>a</sup>
Chrysophyceae	<i>Rhizosolenia pungens</i>	<i>R. pungens</i>			
	<i>Dictyocha speculum</i>	<i>D. speculum</i>			Auto- and heterotrophic flagellates <sup>a</sup>
Dinoflagellates	<i>Dinophysis norvegica</i>				
	<i>Peridinium pellucidum</i>	<i>Peridinium</i> sp.			
	<i>Ceratium tripos</i>	<i>Prorocentrum</i> sp.			
	<i>Ceratium lineatum</i>	<i>Leptocylindris danicus</i>			
	<i>Ceratium fusus</i>				
Ciliates	<i>Strobilium</i> sp.	<i>Strobilium</i> sp.			
Copepods	Nauplii				

<sup>a</sup>Determined by means of epifluorescence microscopy

**Manipulation of zooplankton.** Zooplankton were collected at the institute's pier in Kiel Bight by means of several vertical plankton hauls (mesh size 200 µm). The contents of all plankton hauls were collected in a cool-box that had been previously filled with unfiltered surface water, hence concentrating mesozooplankton >200 µm. Concentration was necessary in order to increase the amount of individuals of otherwise numerically poorly represented guilds in the plankton. In the laboratory, glass jars (800 ml) were filled with this seawater and stored under the same conditions as during growth of the seston size fractions. Zooplankton was thereby allowed to adapt to experimental conditions for 2 h before the beginning of the grazing experiments.

In order to calculate ingestion rates, triplicate samples of surface seawater (500 ml) were first screened through a 100 µm sieve (thereby removing zooplankton) and then filtered onto precombusted Whatman GF/C filters. Filters were dried for several days at 45°C and later analyzed for their carbon content with a Fisons NA 1500N elemental analyzer.

**Experimental procedures.** Triplicate grazing trials were run with each phytoplankton culture or seston size class, respectively, serving as tracers for size-specific clearance. Radioactive labelled tracer (10 ml) was added to the glass jars containing concentrated zooplankton (800 ml). After a grazing period of 30 min, zooplankton were rapidly anaesthetized with carbonized water. Then zooplankton were collected on a 100 µm screen and thoroughly rinsed with 0.2 µm filtered seawater in order to remove adhering labelled

cells. Finally, zooplankton were transferred into bottles with 0.2 µm filtered seawater and preserved with formalin (4 % final concentration). Within 2 d, zooplankton were separated into major guilds and counted under a Leica WILD M3Z dissecting microscope before pipetting them into scintillation vials (20 ml). Tissue solubilizer (0.5 ml of Soluene-350) was added and digestion accelerated by exposing the vials to 40°C for several hours. After allowing the scintillation vials to cool off, 10 ml of scintillation cocktail (Ultima Gold, Packard) was added.

Passive adsorption of radioactive tracer by zooplankton was assessed using formalin-killed zooplankton. As for treatments using live zooplankton, 10 ml of each tracer was added to the glass jars containing formalin-killed zooplankton. After 30 min, the zooplankton were removed and further manipulated as mentioned above. Radioactive values assessed for passive adsorption of radioactive tracer were used to correct for values obtained in grazing trials.

In order to determine the amount of radioactivity added to the glass jars in which grazing occurred, 1 ml samples of each labelled tracer were filtered onto Whatman GF/C filters (0.2 µm membrane filters were used for <0.8 µm heterotrophic bacteria and the <2 µm size fraction). Filters were exposed to vapours of concentrated HCl in order to remove non-incorporated, residual radioactive bicarbonate as <sup>14</sup>CO<sub>2</sub>. After 2 h, the filters were placed into scintillation vials (6 ml) together with 5 ml of scintillation cocktail (Lumagel SB, Baker).

**Assessment of clearance and ingestion rates.** Radioactivity was measured by means of a liquid scintillation analyzer (TRI-CARB 2100 TR) with external standards for determining efficiencies. Values of radioactivity expressed in dpm were corrected by subtracting background  $^{14}\text{C}$  activity and mean passive adsorption of radioactive tracer by formalin-killed zooplankton. Individual clearance rates were computed using the equation provided by Haney (1971):

$$F = (\text{dpm ind.}^{-1}/\text{dpm ml}^{-1} \text{ grazing suspension}) \times (60/t)(1)$$

where  $F$  = clearance rate ( $\text{ml ind.}^{-1} \text{ h}^{-1}$ ), grazing suspension = volume in which grazing occurred and to which radioactive labelled tracer was added (ml), and  $t$  = grazing time (min).

In experiments using seston size fractions, we further calculated group clearance rates of major taxonomic guilds by multiplying individual clearance rates with the appropriate mean abundances calculated for  $1 \text{ m}^3$  seawater. Group clearance rates of major functional guilds were graphically presented as the percentage of total group clearance of mesozooplankton within each seston size fraction. Ingestion rates of mesozooplankton guilds were calculated by multiplying the sum of group clearance rates of all size fractions with the carbon content assessed on the day of the experiment. When calculating the sum of group clearance rates,

clearance for the 2 to 5  $\mu\text{m}$  size class was computed by subtracting values obtained in the  $<2$  and  $<5 \mu\text{m}$  size fractions.

**Data analysis.** Differences among treatments were tested for significance using 1-way ANOVA. Original data were log-transformed in cases where basic assumptions of ANOVA (Cochran's test, Kolmogorov-Smirnov) were not fulfilled. Where significant differences were indicated, Tukey's HSD or Tukey's HSD for unequal sample sizes were used at a significance level  $p = 0.05$ . For all statistical analyses STATISTICA 4.0, 1993, software was used.

## RESULTS

### Bacteria and phytoplankton cultures

Mesozooplankton composition and abundance were determined for the experiment in which copepod grazing was tested (Table 3a). Zooplankton was highly concentrated (27-fold) within the experimental jars, with copepods representing more than 90% of total zooplankton abundance. Among copepods, 3 species were dominant: the cyclopoid *Oithona similis* and the calanoids *Paracalanus parvus* and *Pseudocalanus elongatus*.

Table 3. Mesozooplankton composition in experiments (a) using phytoplankton cultures testing copepods and (b) using seston size fractions. Absolute and relative (%) mean number of individuals in experimental jars (values in brackets are 1 SD) and in  $1 \text{ m}^3$  seawater as well as copepod species contribution to total copepod abundance

Taxonomic group	(a) Cultures (copepods)			(b) Seston size fractions		
	Mean in jars	%	% of total copepods	Mean in jars	Mean in $1 \text{ m}^3$ seawater	% of total copepods <sup>a</sup>
<b>Holoplankton</b>						
Copepods (total)	150 (21.3)	90.1		91 (10.2)	5197	61.5
<i>Paracalanus parvus</i>			49			30
<i>Pseudocalanus elongatus</i>			3			11
<i>Centropages hamatus</i>			2			1
<i>Temora longicornis</i>			41			55
<i>Oithona similis</i>			–			3
<i>Acartia</i> sp.			–			1
Harpacticoida			3			–
Unidentified copepods			2			–
Copepod nauplii						
Nauplii	–	–		17 (4.5)	977	11.6
Appendicularians	–	–		8 (2.7)	458	5.4
Chaetognaths	–	–		1 (0.6)	85	1.0
Ostracods	–	–		2 (1.0)	123	1.5
Ctenophores	0.3 (0.1)	0.2		–	–	–
<b>Meroplankton</b>						
Nectochaeta	11.7 (4.9)	7.0		3 (1.3)	181	2.1
Gastropod veligers	2.2 (1.3)	1.3		2 (1.2)	152	1.8
Bivalve veligers	1.9 (0.3)	1.1		22 (6.5)	1265	15.0
Cyphonautes	0.3 (0.2)	0.2		–	–	–

<sup>a</sup>Calculated from Behrends (1996)

The size spectrum of food types presented to bivalve veligers and copepods is given in Table 1. The highest clearance rates of bivalve veligers were found for particles sized 6.3 and 4.7  $\mu\text{m}$ , being insignificantly different from each other. Clearance values of all other particle sizes were significantly lower ( $p < 0.005$ ) from veliger maximum clearance rates, but again differences between these treatments were insignificant. Copepods performed maximum clearance of particles sized 15  $\mu\text{m}$ . All other particle sizes were cleared at significantly lower rates ( $p < 0.05$ ). The ranges of optimum particle sizes best cleared by bivalve veligers and copepods in our experiments were clearly separated (Fig. 3).

**Seston size fractions.** Mesozooplankton  $>200 \mu\text{m}$  were dominated by nauplii and copepods, which together accounted for more than 73% of zooplankton composition (Table 3b). Bivalve veligers and holoplanktonic appendicularians represented 15 and 5% of the mesozooplankton, respectively. All other guilds including gastropod veligers, nectochaetae, chaetognaths and ostracods accounted for less than 7%, being numerically too low to allow reliable assessment of clearance rates. Hence, clearance rates of seston size fractions were only determined for copepods, nauplii, bivalve veligers and appendicularians, which were the 4 most abundant taxonomic guilds.

Mean clearance rates of all 4 guilds ranged from 0 to 138  $\mu\text{l ind.}^{-1} \text{h}^{-1}$  (Fig. 4). Copepods and nauplii cleared no particles of the  $<2 \mu\text{m}$  seston size class. Whereas copepod clearance rates of all size classes were not significantly different, nauplii clearance of particles of the  $<5 \mu\text{m}$  size class was significantly higher ( $p < 0.05$ ) than of the 10 to 30 and 30 to 100  $\mu\text{m}$  size classes. In contrast to copepods and nauplii, both bivalve veligers and appendicularians cleared particles of the  $<2 \mu\text{m}$

size class. Whereas no significant differences could be found between clearance rates for bivalve veligers, all appendicularian clearance rates were significantly different ( $p < 0.05$ ), except for the 5 to 10 and 30 to 100  $\mu\text{m}$  size classes in which no clearance was performed.

The contribution of the 4 main taxonomic guilds to total zooplankton clearance within seston size fractions is shown in Fig. 5. Particles of the smallest size fraction ( $<2 \mu\text{m}$ ) were exclusively cleared by appendicularians and bivalve veligers. As particle size increased, the portion cleared by copepods increased from 57% ( $<5 \mu\text{m}$  size class) to more than 81% (30 to 100  $\mu\text{m}$  size class). When the relative clearance of adult copepods and nauplii was summed, the total copepod clearance accounted for 90 to 97.6% of the total volume cleared from particles bigger than 10  $\mu\text{m}$ . The contribution of bivalve veligers dropped from 19% in the  $<5 \mu\text{m}$  size class to less than 3% in the 30 to 100  $\mu\text{m}$  seston size fraction. Appendicularians, in contrast, mainly cleared particles of the  $<2 \mu\text{m}$  size class, accounting for more than 62% of the total clearance in this size fraction.

Mean carbon ingestion rates of seston particles  $<100 \mu\text{m}$  calculated for mean abundances of all 4 guilds in 1  $\text{m}^3$  ranged from 0.024 to 0.186  $\text{mg C h}^{-1}$ . The highest ingestion rate was found for copepods (0.186  $\text{mg C h}^{-1}$ ); all other guilds ingested particles at rates lower than 0.050  $\text{mg C h}^{-1}$ . Nauplii and copepod ingestion rates thereby accounted for 66.1 and 15.4% of total carbon ingestion; bivalve veligers and appendicularians contributed 10.0 and 8.5%, respectively.

## DISCUSSION

### Methodological aspects

In 2 out of 4 cases the concentration of animals in the experimental jars significantly affected radioactive uptake by zooplankton (Fig. 6). In both cases, higher abundances of zooplankton in the experimental jars led to a reduced uptake of radioactive tracer per individual, which proved to be highly significant ( $p < 0.005$ ) for copepods in experiments using seston size fractions, and significant ( $p < 0.05$ ) for bivalve veligers in experiments using phytoplankton cultures. Tracer cell concentration, however, did not seem to affect radioactive uptake by copepods (Fig. 7) (no data are available for bivalve veligers). Copepods, which are known to behave as optimal foragers, change food selectivity with particle type abundance (DeMott 1989). The addition of tracer cells at high concentrations might have induced copepods to switch to these as an alternative food source as the encounter rate with tracer-sized particles was enhanced. In our experiments the addition of tracer cells apparently did not affect copepod feeding strategy.

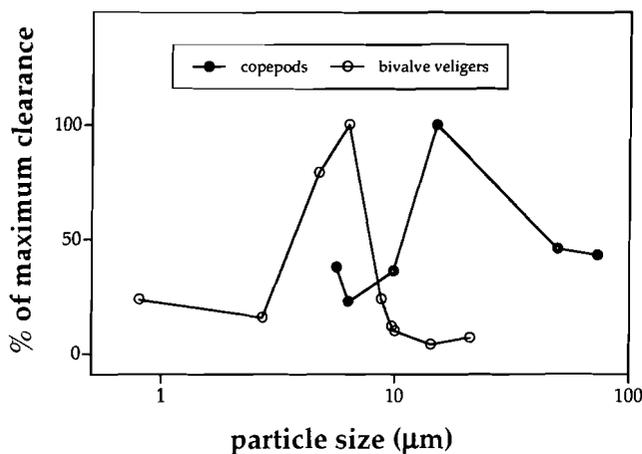


Fig. 3. Clearance curves of mesozooplankton guilds as evaluated in experiments using phytoplankton cultures. Note logarithmic scale of particle size axis

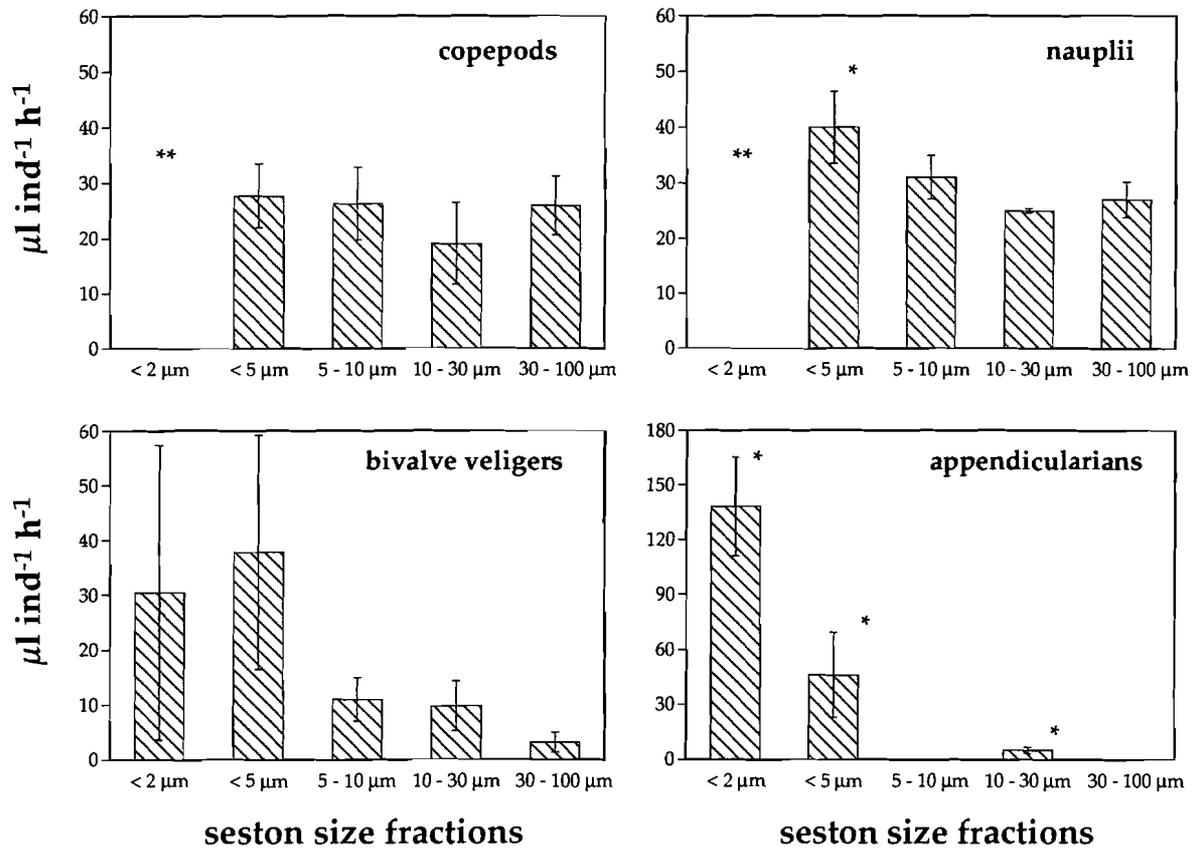


Fig. 4. Clearance rates ( $\mu\text{l ind}^{-1} \text{h}^{-1}$ ) of the main mesozooplankton guilds, evaluated using radioactive labelled seston size fractions. Bars are means of triplicates; error bars represent 1 SD; \* $p < 0.05$ , \*\* $p < 0.005$  (for details see text)

The clearance rates of appendicularians ( $<165 \mu\text{l ind}^{-1} \text{h}^{-1}$ ) and copepods ( $<75 \mu\text{l ind}^{-1} \text{h}^{-1}$ ) in our experiments are lower than values reported in literature (e.g. Harris 1982, Berggreen et al. 1988, Bedo et al. 1993, Nakamura & Turner 1997). Low clearance might have been a result of high particle concentrations in the seston or may be attributed, at least partly, to loss of radioactive label from the gut due to fixation in 4% formalin (Haney & Hall 1973, Holtby & Knoechel 1981). Other sources of error in using the isotope grazing technique are discussed in Roman & Rublee (1980), White & Roman (1992) or Roman & Gauzens (1997).

#### Size-specific clearance of bacteria and phytoplankton cultures

Using a radioactive labelling technique, we determined the highest clearance of bivalve veligers and copepods for particles sized 4.7 and 6.3  $\mu\text{m}$  and for particles sized 15  $\mu\text{m}$ , respectively. The highest veliger clearance of particles  $<10 \mu\text{m}$  is in accordance with previous results of some authors using phytoplankton cultures and natural seston (Table 4). A preference for

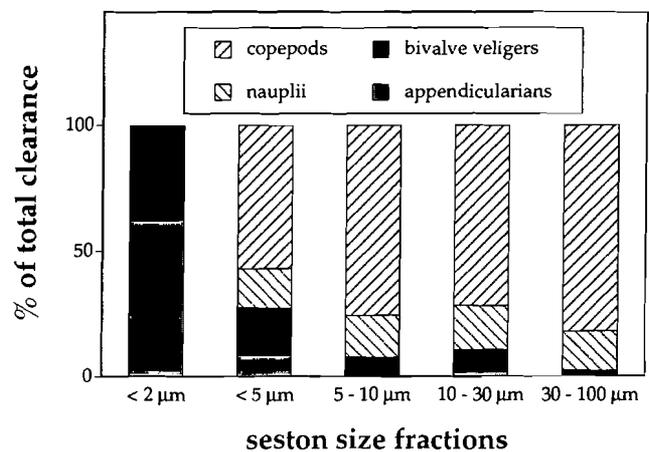


Fig. 5. Contribution of the main mesozooplankton guilds to total clearance within each seston size fraction. Clearance was calculated for mesozooplankton densities in  $1 \text{ m}^3$  seawater (see 'Material and methods')

particles sized 2.5 to 3.5  $\mu\text{m}$  (Riisgård et al. 1980) and 2 to 4  $\mu\text{m}$  (Baldwin 1995) has been reported for bivalve veligers, and of 4 to 6  $\mu\text{m}$  particles for a gastropod veliger (Hansen 1991). Furthermore, a study on the

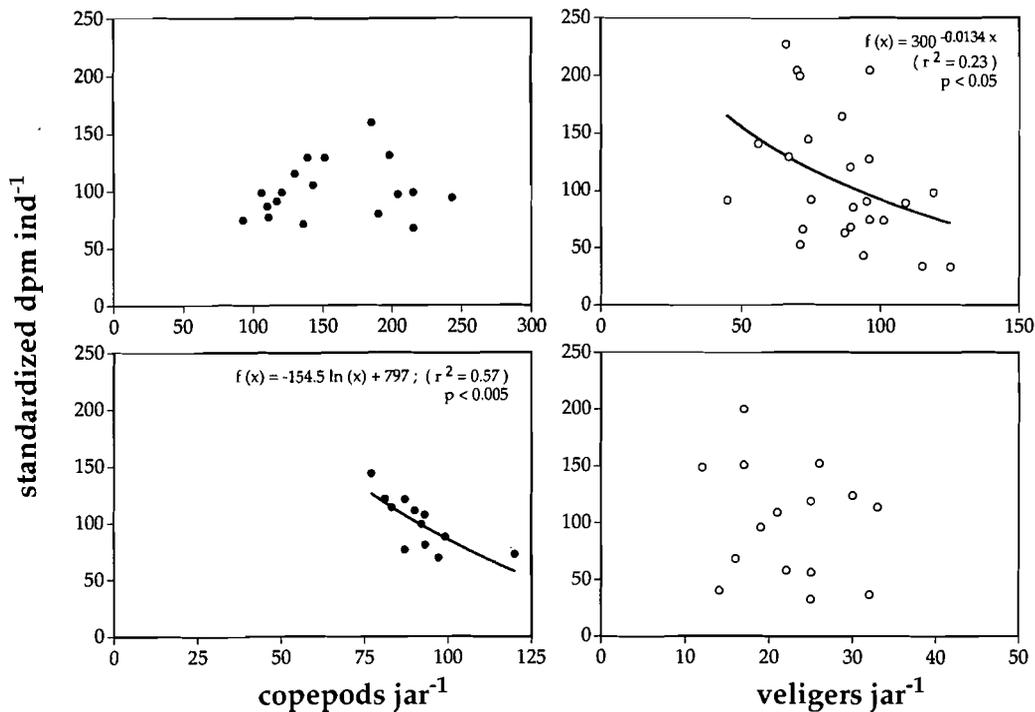


Fig. 6. Uptake of radioactive tracer by copepods (left panels) and bivalve veligers (right panels) as a function of grazer density in experimental jars. Each data point represents one value of experiments using labelled phytoplankton cultures (top panels) or seston size fractions (bottom panels). Dpm values were standardized by dividing replicate values by sample means and subsequent multiplication with 100. Note different axis scaling.

stomach contents of bivalve veliger larvae that had grazed upon the natural seston community revealed that all veligers contained cyanobacteria  $< 2 \mu\text{m}$  and autotrophic flagellates  $< 5 \mu\text{m}$ , and only 55% of the larvae examined contained particles sized 5 to 15  $\mu\text{m}$ . (Raby et al. 1997), supporting our results, which have shown main clearance of particles sized 4.7 and 6.3  $\mu\text{m}$ .

Size-specific clearance by copepods can be related to 3 species that almost exclusively composed the copepod community: *Pseudocalanus elongatus*, *Paracalanus parvus* and *Oithona similis*, which together

accounted for more than 90% of all copepods (Table 3). A study on the morphologies of the mouth parts of these 3 species classified *P. elongatus* and *P. parvus* as morphologically similar herbivores, capable of ingesting small particles, whereas *O. similis* has morphological traits typical of carnivores feeding on larger particles (Schnack 1982). Although a preference of bigger-sized cells ( $>10$  to  $>20 \mu\text{m}$ ) has been found for the herbivorous *Paracalanus* sp. (Pfaffenhöfer 1984) and *Pseudocalanus minutus* (Harris 1982), particles as small as 4.5  $\mu\text{m}$  can be cleared (Pfaffenhöfer 1984). Thereby *Paracalanus* sp. probably employs a passive feeding mode in capturing the small algae (4 to 6  $\mu\text{m}$ ), whereas bigger—in fact preferred—particles are actively grazed upon (Vanderploeg & Pfaffenhöfer 1985). Ingestion of generally large particles ( $>10$  to 250  $\mu\text{m}$ ) has also been found for other marine copepods, considered herbivores, including *Calanus pacificus* (Frost 1972), *Acartia tonsa* (Berggreen et al. 1988) and *Eurytemora affinis* (Merrell & Stoecker 1998). Removal experiments using the carnivore copepod *Oithona similis* showed no significant clearance of particles  $<10 \mu\text{m}$  and the highest clearance of particles  $>20 \mu\text{m}$  were mainly by ciliates and heterotrophic flagellates (Nakamura & Turner 1997).

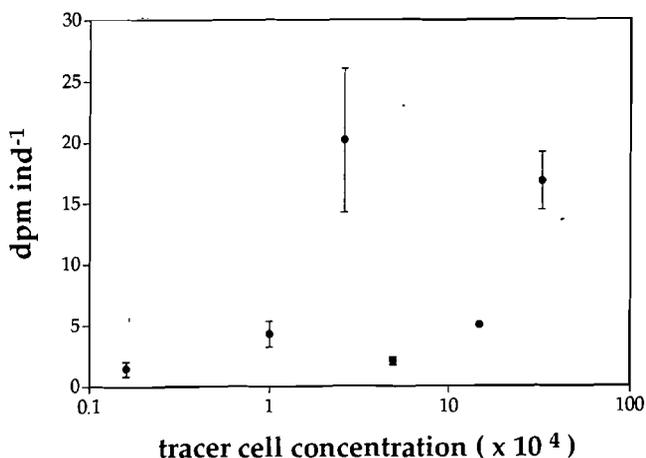


Fig. 7. Uptake of radioactive tracer by copepods as a function of tracer cell concentration in experiments using labelled phytoplankton cultures. Data points are means ( $n = 3$ ); error bars represent 1 SD. Note logarithmic scale of particle size axis

#### Seston size fractions

The results obtained in grazing experiments using seston size fractions showed that the smallest seston

Table 4. Clearance rates and size preference of different veliger larvae

Species	Size/age	Food	Clearance rate ( $\mu\text{l ind.}^{-1} \text{h}^{-1}$ )	Particle size preference ( $\mu\text{m}$ )	Method	Source
<i>Mytilus edulis</i>	No data	<i>Isochrysis galbana</i>	11.4	2.5–3.5	Removal (Coulter)	Riisgård et al. (1980)
<i>Mercenaria mercenaria</i>	2–10 d	<i>Isochrysis galbana</i>	0.38–20.4		Video image analysis	Gallager (1988)
	2–10 d	<i>Synechococcus</i>	17.4–67.2			
<i>Crassostrea gigas</i>	95–283 $\mu\text{m}$	Bacterial strains	1.0–3.6		Radioactive labelling	Douillet (1993)
	98 $\mu\text{m}$	<i>Isochrysis galbana</i>	1.9			
<i>Mercenaria mercenaria</i>	6 d	<i>Synechococcus</i>	2–23		Epifluorescence microscopy	Gallager et al. (1994)
<i>Crassostrea virginica</i>	179 $\mu\text{m}$	0.2–30 $\mu\text{m}$ phototrophs	82.5	20–30	Radioactive labelling	Baldwin & Newell (1991)
	179 $\mu\text{m}$	0.2–30 $\mu\text{m}$ heterotrophs	1.7			
	237 $\mu\text{m}$	<i>Isonema papillatum</i>	64.0			
	273 $\mu\text{m}$	Ciliate Smcil	109.3			
<i>Phine operta</i> (gastropod larva!)	149–392 $\mu\text{m}$	1.3–18 $\mu\text{m}$ algae	–	4–6	Removal (Coulter)	Hansen (1991)
<i>Crassostrea virginica</i>	63–157 $\mu\text{m}$	<44 $\mu\text{m}$ seston	–	>10	Removal	Fritz et al. (1984)
<i>Crassostrea virginica</i>	106–290 $\mu\text{m}$	0.2–30 $\mu\text{m}$ seston	–	2–4	Removal (Coulter)	Baldwin (1995)
Natural community <sup>a</sup>	185–405 $\mu\text{m}$	Seston	–	<5	Epifluorescence microscopy	Raby et al. (1997)
Natural community	>200 $\mu\text{m}$	<0.8–21 $\mu\text{m}$ tracer	0.97–21.9	4.7–6.3	Radioactive labelling	This study
	>200 $\mu\text{m}$	<100 $\mu\text{m}$ tracer	3.1–37.8			

<sup>a</sup>A natural veliger community composed of *Placopecten magellicanus*, *Mytilus edulis* and *Mya arenaria*

particles (<2  $\mu\text{m}$ ) were exclusively ingested by appendicularians and, less pronounced, by bivalve veligers, whereas particles >5  $\mu\text{m}$  were grazed upon by copepods, nauplii and veligers. As clearance within the <5  $\mu\text{m}$  seston size fraction can still be attributable to the removal of particles visible in Utermöhl chambers (e.g. *Skeletonema costatum*), clearance within the <2  $\mu\text{m}$  seston size fraction would imply the ingestion of particles detectable only under epifluorescent light (Table 2), thus suggesting that heterotrophic bacteria, cyanobacteria and flagellates ( $\leq 2 \mu\text{m}$ ) escape predation by copepods.

Baldwin & Newell (1991) employed a similar method of radioactive labelled size fractionation and found that veligers of *Crassostrea virginica* cleared 0.2 to 30  $\mu\text{m}$  <sup>14</sup>C- and <sup>3</sup>H-labelled seston particles at a (summed) rate of 84.2  $\mu\text{l larva}^{-1} \text{h}^{-1}$  (Table 4). In our experiments, veligers cleared particles <2 to 30  $\mu\text{m}$  at a similar (summed) rate of 119  $\mu\text{l larva}^{-1} \text{h}^{-1}$ . The particle size preference found by Baldwin & Newell (1991) was for the 20 to 30  $\mu\text{m}$  fraction (Table 4), which is in contrast to later experiments by Baldwin (1995), which indicated a preference for smaller-sized particles (2 to 4  $\mu\text{m}$ ). The results of our experiments using seston size fractions, however, did not show any significant differences between veliger clearance rates over a broad size spectrum of food particles (<2 to 100  $\mu\text{m}$ ) and failed to confirm the significant particle size preference (4.7 and 6.3  $\mu\text{m}$ ) found in phytoplankton culture experiments. As stated for the larvae of *C. virginica*, veliger larvae apparently extend their maximum ingested particle size in the presence of blooms (Baldwin & Newell 1995a) and seem to select particles based

more on their volume and nutritional quality than on particle size or abundance (Baldwin & Newell 1995b), a fact that has been well documented for copepods (e.g. Houde & Roman 1987, Cowles et al. 1988, DeMott 1988, 1989).

Copepods and nauplii, which represented more than 61 and 11% of the mesozooplankton in Kiel Bight (Table 3), accounted for 66.1 and 15.4% of the total ingested carbon of seston particles <100  $\mu\text{m}$ , respectively. Bivalve veligers and appendicularians contributed 10.0 and 8.5%, respectively, to the total carbon ingestion rate. White & Roman (1992) performed grazing measurements using radioactive labelling in a mesohaline bay that, similar to Kiel Bight, lacked planktonic groups such as echinoderm larvae and salps. They found, except for a bloom situation of polychaete larvae, similarly high values of contribution to total carbon ingestion for copepods (46.5 to 77.5%), higher values for nauplii (18.9 to 52.3%) and much lower ones for bivalve veligers (0 to 2.5%). The contribution of copepods and nauplii to total carbon ingestion in this bay proved to be even higher (87.6 to 98.8%) than in our experiments with zooplankton from Kiel Bight (81.5%).

## CONCLUSIONS

### Food size spectrum

The results of our experiments with phytoplankton cultures are in accordance with earlier findings and suggest different optimum food particle sizes for bi-

valve veligers and copepods (Fig. 3). Such a clear separation of optimum particle size, however, seems subject to flexible variation as the composition and nutritional quality of seston particles (e.g. blooms) varies (Fig. 4, Baldwin & Newell 1995a).

### Ecological implications in Kiel Bight

Due to high abundances throughout the year (Fig. 1) and the capabilities of clearing particles within a broad size range (Fig. 5), copepods proved to be the functionally dominant mesozooplankton guild in Kiel Bight. Nauplii and adult copepods together accounted for 81.5% of the total particulate carbon removed per hour. However, main grazers on particles in the range of the smallest members of the plankton were found to be bivalve veligers and, even more pronounced, appendicularians (Fig. 5).

Grazing by bivalve veligers can be seen as an important trophic link in the water column (other than the main carbon flow phytoplankton-copepod-fish) which utilizes carbon sources, such as heterotrophic bacteria and small flagellates ( $\leq 2 \mu\text{m}$ ), which escape predation by copepods. In terms of energetics, the ingestion of these small particles directs carbon towards the benthos when veliger larvae settle.

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