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Grazing during early spring in the Gulf of Aqaba and the northern Red Sea

Ulrich Sommer[1,*], Ulrike G. Berninger[1,2], Ruth Böttger-Schnack[3], Astrid Cornils[2], Wilhelm Hagen[3], Thomas Hansen[1], Tariq Al-Najjar[4], Anton F. Post[5], Sigrid B. Schnack-Schiel[2], Herwig Stibor[6], Dorothea Stübing[3], Stephen Wickham[5,7]

[1]Institut für Meereskunde an der Universität Kiel, Düsternbrooker Weg 20, 24105 Kiel, Germany
[2]Alfred-Wegener-Institut für Polar- und Meeresforschung, 27515 Bremerhaven, Germany
[3]Marine Zoologie, Universität Bremen (NW2), Postfach 33040, 28334 Bremen, Germany
[4]Marine Science Station, PO Box 195, Aqaba, Jordan
[5]Interuniversity Institute, PO Box 469, 88103 Eilat, Israel
[6]Zoologisches Institut, Ludwig-Maximilians-Universität, Karlsstraße 23-25, 80333 München, Germany
[7]Zoologisches Institut, Universität Köln, Weyertal 119, 50923 Köln, Germany

ABSTRACT: Zooplankton grazing on bacterio- and phytoplankton was studied in the Gulf of Aqaba and the Northern Red Sea during Meteor Cruise Me 44-2 in February-March 1999. Protozoan grazing on bacterioplankton and autotrophic ultraplankton was studied by the Landry dilution method. Microzooplankton grazing on phytoplankton >6 µm was studied by incubation experiments in the presence and absence of microzooplankton. Mesozooplankton grazing was studied by measuring per capita clearance rates of individual zooplankton with radioactively labelled food organisms and estimating *in situ* rates from abundance values. Protozoan grazing rates on heterotrophic bacteria and on algae <6 µm were high (bacteria: 0.7 to 1.1 d^{-1}, ultraphytoplankton: 0.7 to 1.3 d^{-1}), while grazing rates on *Synechococcus* spp. were surprisingly low and undetectable in some experiments. Mesozooplankton grazing was weak, cumulative grazing rates being ca. 2 orders of magnitude smaller than the grazing rates by protozoans. Among mesozooplankton, appendicularians specialised on smaller food items and calanoid copepods on larger ones.

KEY WORDS: Phytoplankton · Protozoa · Bacteria · Zooplankton · Grazing · Red Sea · Gulf of Aqaba

INTRODUCTION

Phytoplankton in the oligotrophic northern Red Sea and in the Gulf of Aqaba (Klinker et al. 1978, Reiss & Hottinger 1984) are characterised by a low biomass (<0.8 µg chlorophyll l^{-1}) dominated (>95%) by phytoplankton <8 µm (Lindell & Post 1995, Li et al. 1998, Yahel et al. 1998). Except for the early summer and fall summer bloom of the cyanobacterium *Trichodesmium* spp., algae measuring 8 to several 100 µm are scarce and contribute <10% of chlorophyll *a* (Yahel et al. 1998). Although not totally absent, they are usually countable in samples from plankton nets or by sedimentation of several 100 ml of water (Kimor & Golandsky 1977). Similar to phytoplankton, both protozoan and metazoan plankton are characterised by low biomass and low abundance. Nevertheless, all major functional and taxonomic groups of marine zooplankton are represented. However, neither total grazing pressure on phytoplankton nor the relative importance of different functional categories of zooplankton as grazers of different phytoplankton size-classes have been studied so far. The different size classes of phytoplankton and of zooplankton require different methods

*E-mail: usommer@ifm.uni-kiel.de

of grazing measurements. In this article, we present the synthesis of several simultaneously run, but independent, grazing studies performed during Meteor cruise Me 44-2 in the Gulf of Aqaba and the northern open Red Sea (15 February to 9 March 1999) together with data on zooplankton abundance and copepod lipid and gut contents as long-term (lipids) and short-term (gut contents) indicators of previous diet. This choice of season allowed comparison of plankton from a deeply mixed water column (Gulf of Aqaba, mixing depth >300 m) with plankton from a stratified water column (Red Sea, mixing depth <50 m at most stations). This contrast in the mixing regime is typical for this period, whereas a few weeks later summer stratification also begins in the Gulf of Aqaba (Wolf-Vecht et al. 1992, Genin et al. 1995).

MATERIALS AND METHODS

Phytoplankton size spectrum. Water samples were obtained from 10 l Go-Flo bottles on an CTD-rosette and passed through 100 µm mesh. Duplicate 1.5 ml samples were preserved in 1.8 ml cryotubes (Nunc) with 75 µl of 2.0% paraformaldehyde solution, quickly frozen, and stored in liquid nitrogen until flow cytometry was performed. Picoplankton were analysed on a FACScan flow cytometer (Becton Dickinson) modified to permit quantification of all groups including the dimly fluorescent surface populations of *Prochlorococcus* spp. (Li et al. 1993, Dusenberry & Frankel 1994); 10 µl of a 0.474 µm Fluoresbrite microsphere (Polysciences, Inc.) suspension were added as internal standard. Flow cytometry histograms were analysed with Cytowin software (Vaulot 1989).

Table 1. List of stations and positions of grazing experiments and mesozooplankton counts mentioned in this study. F: flow cytometry samples for picoplankton; M: microzooplankton grazing on nano- and microzooplankton according to Sommer (2000); P: grazing on picoplankton according to Landry et al. (1995); Z: mesozooplankton net hauls

Stn; Expt	Site	Date (dd.mm.yy)	North	East
118; F, M	Gulf of Aqaba	21.02.99	28.582′	34.651′
122; F	Gulf of Aqaba	22.02.99	29.492′	34.950′
123; P	Gulf of Aqaba	22.02.99	29.284′	34.817′
124; F	Gulf of Aqaba	22.02.99	29.083′	34.766′
126; F	Gulf of Aqaba	23.02.99	28.585′	34.650′
127; F	Gulf of Aqaba	23.02.99	28.334′	34.550′
130; F	Red Sea	24.02.99	27.418′	34.668′
132; F, M, P	Red Sea	24.02.99	27.298′	34.368′
134; F	Gulf of Aqaba	25.02.99	28.334′	34.550′
136; F	Gulf of Aqaba	25.02.99	28.834′	34.733′
138; F	Gulf of Aqaba	26.02.99	29.284′	34.817′
139; F	Gulf of Aqaba	26.02.99	29.492′	34.950′
145; F, M	Red Sea	27.02.99	27.654′	34.668′
147; F, P	Red Sea	28.02.99	27.183′	34.666′
148; F	Red Sea	28.02.99	27.397′	34.368′
152; M, Z	Gulf of Aqaba	02.03.99	28.334′	34.551′
156; Z	Red Sea	04.03.99	27.416′	34.083′
157; P	Gulf of Aqaba	05.03.99	29.491′	34.951

Zooplankton abundance. As standard devices for the quantitative collection of zooplankton, 2 multiple opening-closing nets were used: a smaller one (mouth opening 0.25 m^2) consisting of 5 nets of 55 µm mesh and a larger one (mouth opening 0.5 m^2) comprising 9 nets of 150 µm. Stratified vertical hauls covered the entire water column between the surface and the sea bottom. The filtered volume of the larger net was measured by a flowmeter, while the filtered volume of the smaller net was calculated on the basis of the vertical distance covered by the net's mouth area assuming 100% efficiency. All samples were fixed in a 4% formaldehyde seawater solution. For the present study, abundance data from 2 stations and the uppermost depth strata (0 to 50 and 50 to 100 m) were used. Samples from the 150 µm net were used for the counts of adults and late copepodids of calanoids and other large zooplankton, while samples from the 55 µm net were used for juveniles, small-bodied copepod taxa, and appendicularians.

Nano- and microzooplankton grazing on picoplankton. Microzooplankton grazing dominated by protozoa on autotrophic and heterotrophic picoplankton was studied by Landry et al.'s (1995) modification of the dilution method of Landry & Hasset (1982). This method is based on a dilution series of natural plankton suspension with filtered seawater. Net growth rates are calculated from cell counts at the beginning and at the end of the incubation period (in our case ca. 36 h). In more dilute samples, the encounter rates of predators with their prey is reduced, resulting in a higher net growth rate of the prey in comparison to less dilute treatments.

Water samples were collected at 5 different locations (Stns 123, 132, 147, 152, 157: Table 1). Particle-free water was prepared by filtration through a 0.2 µm cartridge filter. This water was mixed with unfiltered water to obtain dilution grades of 35, 50, 60, 80, 90, and 100% unfiltered water. Experiments were conducted in 2.5 l transparent polycarbonate bottles, incubated for 36 h in on-deck incubators cooled by a flow-through of surface water and exposed to ca. 30% of incident light. All experiments were run in duplicate bottles. Nutrients (12 µM N, 6 µM Si, 0.7 µM P) and vitamins were added to avoid nutrient limitation in the bottles. In 3 of the experiments, unenriched bottles were also incubated to estimate the extent of nutrient limitation. Samples were taken at the beginning and end of incubation for later microscopic counts. Samples for the enumeration of picoplankton and nanoplankton

were preserved in 1.25% glutaraldehyde and stored at 4°C; 15 ml of the fixed sample were filtered onto 0.2 µm Nuclepore filters and stained with the fluorochrome DAPI (1.0 µg l^{-1}). Heterotrophic pico- and nanoplankton and autotrophic ultraplankton (<8 µm) were counted using a blue filter set, whereby autotrophs were differentiated from heterotrophs on the basis of their red or orange autofluorescence. Samples of phytoplankton >8 µm were fixed in Lugol's iodine and counted under an inverted microscope.

Growth rates were calculated individually for each bottle. A linear regression of growth rate on the fraction of undiluted water yielded an estimate of the gross growth rate (μ; equivalent to the *y*-axis intercept) and of the grazing rate (γ; equivalent to the negative slope of the regression). Addition of nutrients in a parallel dilution series prevents nutrient limitation at lower dilutions. It yields an estimate of the maximal growth rate (μ_{max}) and, by comparison with the gross growth rate (μ) of the unenriched bottle, an assessment of the extent of nutrient limitation.

Microzooplankton grazing on nano- and microphytoplankton. Grazing rates on phytoplankton taxa >6 µm were obtained from shipboard experiments designed for the comparison of the relative importance of nutrient versus grazing control on medium-sized phytoplankton (Sommer 2000). Nutrient manipulations ranged from controls (no addition), over silicate enrichment (4 µM Si), to a full enrichment (4 µM Si, 4.5 µM N, 0.3 µM P) that ensured all phytoplankton were free of nutrient stress. Grazer manipulations consisted of sieving the sample through net-screens with mesh sizes of 100 µm (removal of adult metazoa, but not of protozoa and the smallest nauplii), 20 µm (removal of larger protozoa) and 10 µM (removal of medium-sized protozoa). Protozoa <10 µm were not removed because they usually feed on pico- but not on nanoplankton and their removal would also have removed all the target algae of this study.

The water samples for the experiments were taken from 10 m depth at 2 stations in the Gulf of Aqaba (Expt 1: Stn 118; Expt 4: Stn 152) and 2 stations in the open Red Sea (Expt 2: Stn 132; Expt 3: Stn 145). The manipulated water samples were incubated in 2 l polycarbonate bottles floating in a deck incubator. Each treatment was duplicated in separate bottles. The deck incubator was cooled by a flow-through of surface water (21 to 23°C) and shielded against direct sunlight by a Plexiglas cover which absorbed ca. 70% of incident radiation.

Subsamples (250 ml) from the incubation bottles were taken at the start of the experiments and after 2 and 5 d, and were preserved with Lugol's iodine for identification at the genus level and cell counts of the nano- and microplankton species. The scarcity of nano- and microphytoplankton (0.05 to 10 cells ml^{-1} in the initial samples) required the sedimentation of 200 ml samples prior to microscopic counting in an inverted microscope. If cell numbers were sufficient, 100 individuals were counted per taxon, thus giving 95% confidence limits of ca. ±20% (Lund et al. 1958). However, in many cases counting of the entire counting chamber resulted in ≪100 cells. The response of individual taxa to the experimental treatments was assessed by calculating net growth rates from the cell density data on Days 0 and 2: $r = \ln(N_2/N_0)/2$. Grazing rates (γ; d^{-1}) were calculated as the difference between net growth rates of the fully enriched treatments without microzooplankton removal and the fully enriched treatments sieved through 10 µm mesh (or 20 µm mesh in the case of phytoplankton taxa which did not pass the 10 µm screen). Data from the non-enriched bottles were not used, because nutrient excretion by zooplankton might have enhanced cell division rates relative to grazer-free treatments. It is assumed that microzooplankton grazing rates in the bottles do not respond to nutrient enrichment within 2 d and conform to the *in situ* rates (Sommer 2000). For the purpose of synthesis, the dependence of grazing rates on phytoplankton particle size was analysed by regression analysis. Particle size was defined as the greatest axial linear dimension (GALD). In the case of colonial or filamentous algae, colony or filament length were used instead of cell length.

Size-specific grazing rates of mesozooplankton. Grazing was measured by using radioactively labelled algae and bacteria of different sizes as tracers and freshly collected zooplankton individuals as grazers. The phytoplankton tracers were obtained from stock cultures. Sizes of the tracer algae (see Table 5) are characterised by the greatest axial linear dimension and the equivalent spherical diameter (ESD). All phytoplankton cultures were grown in 0.2 µm membrane-filtered Red Sea water with nutrients and trace metals added (von Stosch & Drebes 1964). Suspended heterotrophic bacteria were isolated by filtering seawater through 0.8 µm membrane filters. All cultures were kept at 22°C and an irradiance of about 100 µE cm^2 s^{-1}; 24 h prior to a grazing experiment, phytoplankton cultures were labelled with ^{14}C bicarbonate (250 µCi); bacteria were inoculated with radioactive glucose (25 µCi).

Zooplankton were collected by vertical plankton hauls (mesh size 100 µm). To keep gelatinous plankton intact, we replaced the original net beaker at the end of the net by a plastic bag. Zooplankton were separated into functional groups immediately after collection. For each taxonomic group used in a grazing experiment, 10 to 30 animals of the same size class were transferred to 1 l jars filled with <100 µm filtered

seawater to allow acclimation to experimental conditions. After approximately 4 h the animals were checked for viability and mobility to ensure that only intact individuals were introduced into our experimental set-up. Aliquots of equally-sized individuals were dried and analysed for carbon in a Fisons 1500 CN analyser.

Triplicate grazing trials were run with each size class of food organisms. Experiments were repeated 2 or 3 times. Radioactively labelled food organisms were added to 30 ml glass jars filled with Red Sea water filtered through a 100 µm mesh. Each glass jar contained between 1 and 3 experimental animals. Independent of the number of animals in the jar, a jar was always treated as 1 replicate. After a grazing period of 15 min, zooplankton were anaesthetised with carbonated water and collected on a 100 µm screen. Animals were thoroughly rinsed with GF/F-filtered seawater to remove adhering labelled cells. The animals were then pipetted into a scintillation vial. Tissue solubiliser was added (0.5 ml of Soluene 350), and digestion was accelerated by exposing the vial to 60°C for several hours. Additionally, Carbosorb was added to the vials to prevent loss of radioactive carbon. Passive adsorption of radioactive tracer on zooplankton was assessed using formalin-killed zooplankton which were introduced into the grazing experiments as controls. In order to determine the amount of particulate radioactivity in the grazing jars, 1 ml from each jar was filtered onto GF/F filters (algae) or 0.2 µm membrane filters (bacteria). Filters were exposed to vapours of concentrated HCl to remove non-incorporated radioactive bicarbonate.

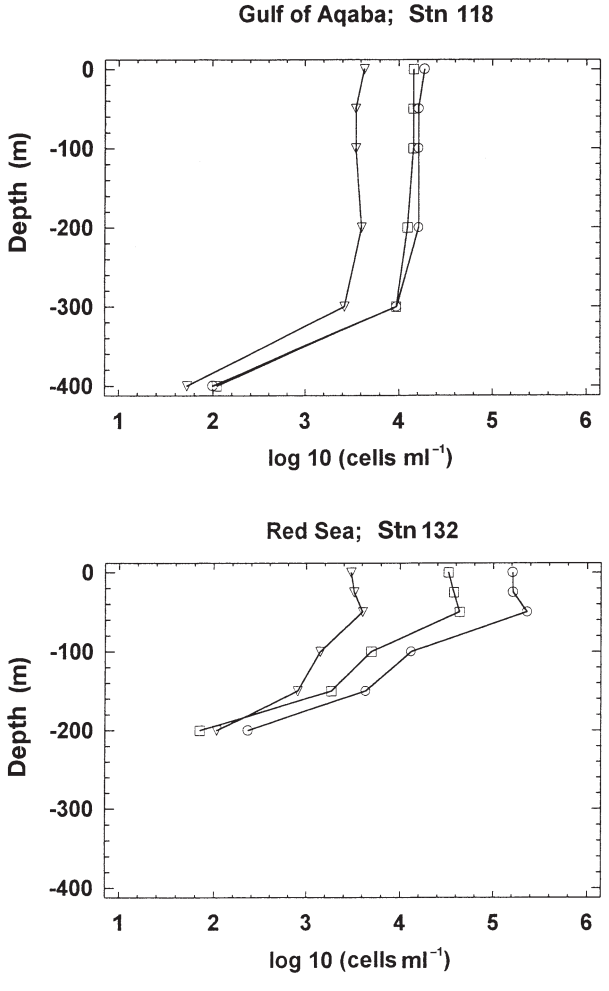

Fig. 1. Depth profiles of the 3 major picophytoplankton groups, *Prochlorococcus* spp. (○), *Synechococcus* spp. (□) and eukaryotes (▽) at Stns 118 (Gulf of Aqaba) and Stn 132 (northern Red Sea)

Radioactivity was measured by a liquid scintillation analyser (TRI CARB 2100 TR); Ultima Gold was used as a scintillation cocktail. Values of radioactivity (expressed in dpm) were corrected by subtracting passive adsorption of radioactive tracer by formalin-killed zooplankton. Individual clearance rates (ml ind.$^{-1}$ d^{-1}) for each tracer size class were computed using the equation provided by Haney (1971):

$$F = (\text{dpm ind.}^{-1}/\text{dpm ml}^{-1}\text{ grazing suspension}) \times (1440/t)$$

where F = clearance rate (ml ind.$^{-1}$ d^{-1}); grazing suspension = volume in which grazing occurred and to which specific tracer was added; and t = grazing time in minutes.

Lipid content and composition of copepods. Copepods, especially *Rhincalanus nasutus* CV and females, were collected at various stations in the Gulf of Aqaba and the northern Red Sea. The specimens were sampled by vertical multinet hauls from depths between 600 m to the surface. They were immediately transferred to a container with filtered seawater and sorted according to species and stages. The individuals were pooled for each sample (usually 40 to 50 specimens) and directly deep-frozen at –80°C or stored frozen in dichloromethane/methanol (2:1 by vol.) until analysis.

Lipids were extracted with dichloromethane/methanol (2:1 by vol.) after Folch et al. (1957) and Hagen (2000), and lipid classes were determined according to Fraser et al. (1985) by thin-layer chromatography/flame-ionisation detection (TLC-FID) with a Iatroscan MARK II. After transesterification of the lipids with 3% concentrated sulphuric acid in methanol, fatty acid methyl esters and free alcohols were simultaneously analysed in a gas chromatograph equipped with a DB-FFAP column. The gas chromatographic oven was temperature-programmed. The fatty acids and alcohols were identified with known standards. For analytical details refer to Kattner & Fricke (1986), Kattner et al. (1994), and Hagen (2000).

Table 2. Abundance (cells ml^{-1}; mean and minimum to maximum range) and biomass (biovolume based on mean cell size and mean abundances; μm^3 ml^{-1}) of small-sized phytoplankton groups in the Gulf of Aqaba and the Red Sea. Samples for fluorescence microscopy were the initial samples of the dilution experiments taken from Stns 123, 152, and 157 for the Gulf of Aqaba and from Stns 132 and 147 for the Red Sea. Sampling depth was 10 m, except for Stn 123 (50 m). Samples for flow-cytometry were taken from 10 m depth at Stns 118, 122, 124, 126, 127, 134, 136, 138, 139 (Gulf of Aqaba) and Stns 130, 132, 145, 147, 148 (Red Sea)

Phytoplankton group	Cell size (µm)	Vol. (μm^3)	Gulf of Aqaba		Red Sea	
			Abundance	Biomass	Abundance	Biomass
Estimated by flow cytometry						
Prochlorococcoccus spp.	0.6–0.8	0.18	18 100 5 100–48 000	3 240	204 000 150 000–260 000	36 720
Synechococcus spp.	0.9–1.2	0.70	16 500 8 200–30 800	11 550	29 100 15 400–35 000	20 370
Pico-eukaryotes	1.0–2.0	1.77	4 200 1 970–5 860	7 434	2 660 1 640–3 420	4 708
Estimated by fluorescence microscopy						
Synechococcus spp.	0.9–2.0	1.6	17 800 4 500–43 500	28 480	17 500 7 500–27 500	28 000
Phytoplankton <8 µm (without *Synechococcus* spp.)	0.6-4.0	6	3 990 580–7 180	23 940	1 530 860–2 200	9 180
Larger phytoplankton	>8	400[a]	2.42 1.26–3.80	968	2.47 2.13–2.81	988

[a]Based on thin, spindle-shaped *Rhizosolenia* spp., *Nitzschia* spp. and *Pseudonitzschia* spp., the dominant phytoplankton taxa >8 µm

RESULTS

Phytoplankton and bacterioplankton size-spectrum

Water-column conditions had a distinct effect on the abundance and composition of phytoplankton communities. Fig. 1 shows typical depth profiles for picoplankton abundance in the deeply mixed Gulf of Aqaba (Stn 118) and in the stratified northern Red Sea (Stn 132). Cells were distributed in a uniform way over 300 m at Stn 118, and *Synechococcus* spp. and *Prochlorococcus* spp. were present in equal abundance. However, at Stn 132 picoplankton depth distributions showed a distinct stratification, with highest cell numbers in the upper 60 m (Fig. 1). While picoeukaryote abundance at this site was about half that at Stn 118, *Synechococcus* spp. and *Prochlorococcus* spp. were more abundant by factors of 2 and 10 times, respectively.

A comparison of flow-cytometry analysis of samples at all sites taken at 10 m depth revealed considerable differences in the abundance of phototrophic picoplankton, with the smallest group (*Prochlorococcus* spp.) being 10 times more abundant in the stratified Red Sea, and picoplankton eukaryotes being 2 times more abundant in the deeply mixed waters of the Gulf of Aqaba (Table 2). Thus, the composition of phototrophic picophytoplankton in the Red Sea was similar to the composition in the Gulf of Aqaba during the period of summer stratification (Lindell & Post 1995). The ranges for the flow cytometer counts and for the microscopic counts indicate a pronounced spatial variability in both systems. Epifluorescence counts and inverted microscope counts were performed at fewer stations and, although they are probably less precise, they do permit an estimate of larger phytoplankton also. Phytoplankton >8 µm contributed ca. 0.01% to total cell number and ca. 2% to total biomass, as estimated from cell volumes.

The abundances of heterotrophic bacteria were not affected by the physical structure of the water masses. Numbers of heterotrophic bacteria were in the range to be expected for oligotrophic environments (5 to 5.5×10^5 ml^{-1} in the Red Sea and 3 to 5.3×10^5 ml^{-1} in the Gulf of Aqaba).

Zooplankton abundance

Copepods (including nauplii) were the most numerous group among the mesozooplankton in the Gulf of Aqaba and the northern Red Sea. Appendicularians ranked next in abundance in the upper strata, followed by chaetognaths, ostracods and gastropods, as usually found in the Red Sea (Böttger-Schnack 1995). Among the copepods, calanoids were most abundant, contributing about 40 to 50% of the total numbers (Fig. 2). Cyclopoids and poecilostomatoids ranked next, whereas harpacticoids were found in small numbers

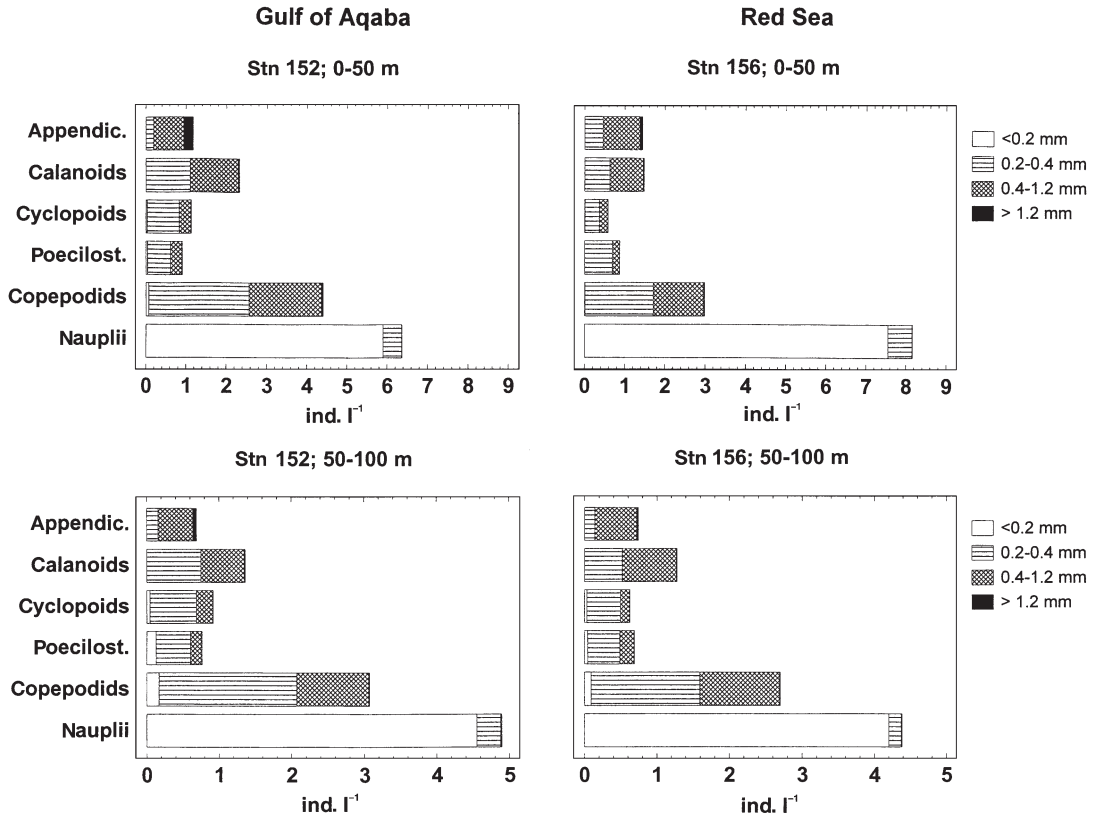

Fig. 2. Abundance of major mesozooplankton groups by size classes at Stn 152 (Gulf of Aqaba) and Stn 156 (northern Red Sea) Copepodids and nauplii were not sorted taxonomically. Appendic.: appendicularians; Poecilost.: poecilostomatoids

only. Most calanoid species belonged to the family Clausocalanidae (e.g. *Ctenocalanus vanus*, *Clausocalanus* spp.) and were of small size (about 1 to 2 mm). Larger calanoids such as *Rhincalanus nasutus* and *Pleuromamma indica* accounted for only a small portion of the calanoid abundance in the upper 100 m of the water column.

Table 3. Minimum to maximum ranges of greatest axial linear dimension of individual cells or chains/colonies (*) (GALD; µm) and microzooplankton grazing rates (γ; d^{-1}) on phyto plankton genera

Genus	GALD	γ
Small dinoflagellates	6.5–11	0.95–1.25
Pyramimonas	8–10	0.98–1.27
Rhodomonas	9.5–12.5	0.85–1.25
Emiliana	10–14	0.51–0.82
Navicula	12–18	0.78–1.10
Nitzschia	24–36	0.50–0.84
Thalassiosira	22–39	0.46–0.86
Gymnodinium	40–56	0.16–0.34
Rhizosolenia	50–102	0.30–0.64
Leptocylindrus	300–450*	0.05–0.45
Pseudonitzschia	330–470*	0.08–0.32

Nano- and microzooplankton grazing on picoplankton

Cell division rates (μ) of heterotrophic bacteria were moderately high (0.61 to 0.72 d^{-1} in the Red Sea and 0.86 to 1.30 d^{-1} in the Gulf of Aqaba) and were roughly balanced by grazing (γ = 0.73 to 0.89 d^{-1} in the Red Sea and 0.75 to 1.06 d^{-1} in the Gulf of Aqaba). There was no effect of nutrient addition on bacterial growth or grazing. The growth rates of bacteria in the dilution experiments were higher than those (<0.15 d^{-1}) obtained by *in situ* incorporation of ^{14}C-labelled leucine (Grossart & Simon 2002). This difference between the dilution method and tracer techniques has also been observed elsewhere, and has not yet been explained satisfactorily.

Neither *Synechococcus* spp. nor ultraphytoplankton showed a response to nutrient addition in either system. For *Synechococcus* spp., moderate growth and grazing rates could only be determined for 1 station in the Red Sea (Stn 147, μ = 0.64 d^{-1}; γ = 0.19 d^{-1}), while data scatter permitted no calculation for the other stations. Ultraphytoplankton responded to grazing pressure in both stations of the Red Sea (γ = 0.72 to 1.3 d^{-1}), but not in the Gulf of Aqaba.

Microzooplankton grazing on nano- and microphytoplankton

The grazer-removal experiments provided microzooplankton grazing rates on phytoplankton species ranging from ca. 6.5 to 225 µm particle length (GALD) and from 77 to 15 000 µm^3 cell volume. Grazing rates declined with increasing algal size, but were high throughout the entire range of phytoplankton sizes (Table 3; for further data see Sommer 2000). The best fit was obtained with linear regressions of the grazing rate (γ; d^{-1}) on log GALD (Table 4). Other size parameters (cell volume, ESD) provided poorer fits, but the trends were always similar. Grazing rates decreased with increasing size of algae. Differences between the experiments and the sites (Red Sea, Gulf of Aqaba) were analysed by ANOVA with log GALD as covariate. Both ANOVAs showed marginally insignificant effects of site (F-ratio = 3.136; p = 0.0868) and experiment (F-ratio = 2.448; p = 0.0845), but highly significant effects of log GALD (p < 0.0001). The small differences between the different experiments are plausible because of a very restricted range of microzooplankton abundances in the grazed treatments: nauplii 10 to 18 ind. l^{-1}; tintinnids 20 to 42 ind. l^{-1}; *Strombilidium* spp. 13 to 55 ind. l^{-1}; small unidentified ciliates 100 to 260 ind. l^{-1} (mean values for the grazer treatment; for further details see Sommer 2000).

Table 4. Regression of grazing rates by microzooplankton on greatest axial linear dimension (GALD) of phytoplankton (g = a.GALD^b). n: number of cases

Region; date	Stn	a	b	r^2	n	p
Southern Gulf; 21 Feb	118	1.99	−0.30	0.50	8	0.0325
Red Sea; 24 Feb	132	2.06	−0.30	0.78	8	0.0039
Red Sea; 27 Feb	145	3.88	−0.48	0.96	8	0.0001
Southern Gulf; 2 Mar	152	2.11	−0.40	0.76	8	0.0050
Pooled data		2.73	−0.41	0.69	32	<0.00001

Mesozooplankton grazing

Grazing by mesozooplankton fell into 2 categories of size preferences. Microphageous zooplankton have had the highest per capita clearance rates on bacteria (Appendicularia, Ostracoda) or on 3 µm algae (*Doliolum* spp.). Macrophageous zooplankton included all copepods studied and showed the highest per capita clearance rates for the largest (*Rhincalanus nasutus*) or second-largest (*Calanus* sp. and small calanoids) algal size category (Table 5). Animal sizes in the experiments were ca. 3.5 to 4 mm for *R. nasutus*, 3.5 mm for the appendicularians, 2.5 mm for *Doliolum* spp., 2.3 mm for *Calanus* sp., 1.5 mm for the ostracods, and 1.1 mm for the small calanoid copepods. Copepod lengths were measured from the front end of the head to the telson without taking the appendages into account; appendicularian lengths are trunk lengths without tail. The average carbon content of the different zooplankton groups was: small calanoids 4.36 ± 1.02 (SD) µg C ind.$^{-1}$; *Calanus* sp. 35.32 ± 3.78 µg C ind.$^{-1}$; Appendicularia 11.3 ± 2.3 µg C ind.$^{-1}$; *Rhincalanus* 73.3 ± 10.8 µg C ind.$^{-1}$; *Doliolum* 3.38 ± 0.94 µg C ind.$^{-1}$; Ostracods 25.87 ± 3.8 µg C ind.$^{-1}$.

Calculation of total community clearance rates (1 m^{-3} d^{-1}) from species-specific individual rates and species abundances could only be performed imperfectly, because of some mismatch between the animal taxa used in the grazing experiments and the most abundant groups (Fig. 2). Moreover, the majority of *in situ* zooplankton individuals were much smaller than the experimental individuals. Thus, assumptions about taxonomic/functional analogy and a size correction were needed. It was assumed that both the category 'calanoids' and the category 'copepodids' in Fig. 2 were best represented by the category 'small calanoids' in the grazing experiments. Phytoplankton grazing by adult cyclopoids and poecilostomatoids was considered negligible, because many of them are considered carnivorous, at least when small phytoplankton cells are dominant (Satour et al. 2000). Naupliar grazing was neglected as well in this calculation, because it was already included in microzooplankton grazing (see foregoing subsection). This means that appendicularians and small calanoids

Table 5. Clearance rate (ml ind.$^{-1}$ d^{-1}) of mesozooplankton on different-sized (GALD/ESD in µm) categories of food

Zooplankton	Bacteria (1/0.8 µm)	*Chlorella* spp. (3/2.7 µm)	Small pennate diatoms (15/8.259 µm)	*Thalassiosira* spp. (40/22 µm)	*Nitzschia* spp. (100/18 µm)
Appendicularia	126	113	107	15	3.8
Doliolium spp.	71	86	36	29	17
Ostracoda	19	7.6	7.6	3.8	0.95
Rhincalanus nasutus	0	5.5	10	21	91
Calanus sp.	0	1.2	7.8	39	29
Small calanoids	0	2	9	22	16.5

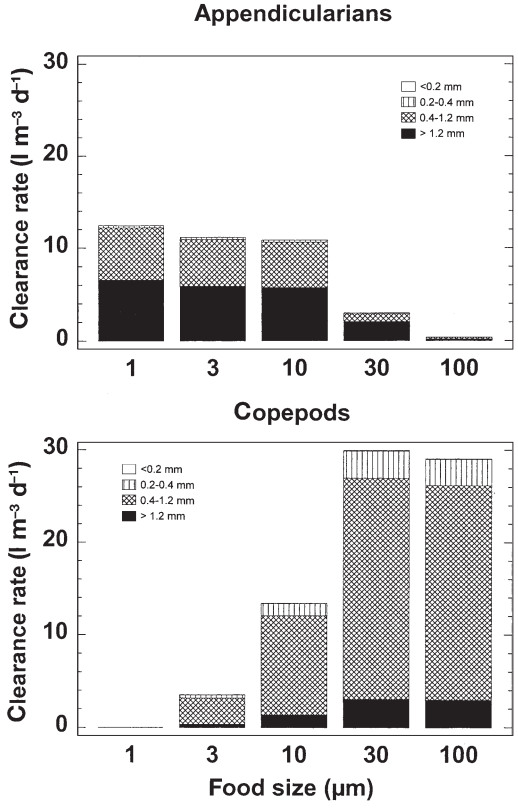

Fig. 3. Clearance rates of appendicularians and calanoid copepods on food particles of different size; cumulative plot of zooplankton size classes (open: <0.2 mm; vertically hatched: 0.2 to 0.4 mm, cross hatched: 0.4 to 1.2 mm, black: >1.2 mm

were considered the only significant mesozooplankton grazers.

Size correction was performed assuming that clearance rates scale with the square of linear body measurements because of the 2-dimensional nature of feeding structures, e.g. appendicularian filters. For the size class <0.2 mm, the calculated per capita clearance rate of 0.2 mm animals was used. For the size class 0.2 to 0.4 mm, the mean of the mean clearance rates of 0.2 and 0.4 mm animals was used. For the size class 0.4 to 1.2 mm, the mean of the clearance rates for 0.4 and 1.2 mm animals was used. For the >1.2 mm size class of small calanoids, the clearance rate of 1.2 mm animals was used, while for appendicularians >1.2 mm, the value for 2.65 mm animals was used (mean length of appendicularians >1.2 mm in the 100 µm net samples).

Based on the mean abundances of the stations and depth intervals in Fig. 2, appendicularian clearance rates of ca. 10 l m^{-3} d^{-1} were found for the smallest food particles and copepod clearance rates of ca. 30 l m^{-3} d^{-1} for large food particles (Fig. 3). This corresponds to instantaneous grazing rates (γ) of 0.01 and 0.03 d^{-1}, respectively.

Copepod lipid composition

The analyses of late copepodid stages (CV) and female *Rhincalanus nasutus* showed that the lipid contents of females and CV stages during the investigation period ranged around 30% of dry mass. For females (18 samples), the lipid content averaged 30.8 ± 4.9%; for the CV stages (5 samples) the mean was 30.3 ± 9.6%. Lipids were dominated by wax esters, which made up 87.3% of total lipids (10 samples). Other lipid classes comprised triacyglycerols (mean 3.2%), phospholipids (mean 9.1%) and cholesterol (0.3%). The principal fatty acids in the total lipid extract (8 samples) were 18:1(n-9) with 30.7%, 16:1(n-7) with 23.7%, 16:2 with 8.5% and 20:5(n-3) with 5.5% for females. These percentages were very similar to those of the CV stages (2 samples). The fatty alcohols consisted mainly of 2 shorter-chain components, 16:0 with 78.5% and 14:0 with 21% in the females, and 16:0 with 81.1% and 14:0 with 18.3% in the CV stages, respectively.

DISCUSSION

The summarised grazing data of zooplankton functional groups on phytoplankton functional groups are rough estimates only. Among other sources of error, experimentally obtained grazing-rate data from a few species of mesozooplankton only were extrapolated to functional groups, ignoring the small size classes. Moreover, stations from which the material for the different shipboard experiments was obtained and stations for the abundance samples did not always match because of logistic constraints. Nevertheless, the trends are sufficiently obvious to permit some clear semi-quantitative conclusions about overall grazing pressure and the relative importance of different zooplankton functional groups of zooplankton (Fig. 4).

A further methodological problem lies in the incubation time of the Landry et al. (1995)-type dilution experiments (36 h) that was needed to achieve a statistically significant response of the larger components of the microbial food web. A shorter incubation time (e.g. 6 h) would have reduced the bottle artifacts (e.g. wall growth), influencing bacterial growth rates, but incubation times <6 h would have missed any diel effects. Bacterial growth rates measured by the dilution technique were higher and clearly better in balance with grazing rates than the extremely low estimates obtained from leucine-uptake measurements (Grossart & Simon 2002). Calculating growth rates from the uptake of 1 substrate only involves some risky assumptions, which might not always apply.

The calculated grazing rates on bacteria and medium-sized phytoplankton were rather high, but

low or even undetectable for the picoplanktonic cyanobacteria *Synechococcus* spp. For heterotrophic bacteria and medium-sized algae, the system appears highly dynamic in spite of low nutrient levels and low phytoplankton biomass. In fact, calculated bacterial growth rates were in the middle of the range estimated for the same season in the slightly more eutrophic central Red Sea using metabolic inhibitors (0.34 to 2.3 d^{-1}: Weisse 1989). In order to balance grazing losses, a high production:biomass ratio of phytoplankton is required. This agrees with the high assimilation numbers measured during our cruise (C. Häse & M. Tilzer pers. comm.). High growth rates balanced by high loss rates at a low standing stock is the core of Goldman's (1984) 'spinning wheel' concept for the oligotrophic ocean. He even hypothesised maximal growth rates despite low nutrient concentrations (Goldman et al. 1979). Our dilution experiments have shown that phytoplankton dominated by pico- and small nanoalgae are indeed not limited by nutrients, while some nutrient limitation was found for stations with a higher contribution of large algae. This agrees too with the experiments by Sommer (2000), who found moderate but significant nutrient limitation for phytoplankton species >6 µm; nevertheless, even algae of this size are grazed at high rates. However, the period of deep mixing is the period with the weakest nutrient stress in the Gulf of Aqaba; stronger nutrient stress would be expected in the stratified period (Lindell & Post 1995).

The low growth and grazing rates of *Synechococcus* spp. do not fit into Goldman's (1984) concept and can only tentatively be explained from the available data. Low grazing rates are easiest explained by assuming that *Synechococcus* spp. are either avoided actively or is too large (0.9 to 1.2 µm) for ingestion by heterotrophic nanoflagellates and too small for ciliates. Indeed, heterotrophic flagellates were unusually small in the study area, and a preference for smaller food items (heterotrophic bacteria) might therefore be expected. A control of picoplanktonic cyanobacteria by heterotrophic nanopflagellates does not seem to be a universal rule in the oligotrophic ocean, but seems to vary between sites and seasons. Low grazing rates of flagellates on *Synechococcus* spp. were found by Dolan & Simek (1999) in the western Mediterranean Sea, while high rates were reported by (e.g.) Burkill et al. (1993) for the oligotrophic part of the Indian Ocean and by Sakka et al. (2000) for French Polynesia. But how can the low growth rates despite nutrient sufficiency be explained? Viral lysis is possible, but no proof is available, and it would probably have operated in the experimental bottles as well as *in situ*. Experimental artefacts such as artificial light inhibition are also possible, because algae were arrested at 30% surface irradiance instead of being circulated in the mixed water column. However, any explanation by artefacts would require an additional limiting factor *in situ*, since unrestricted growth and resultant blooms of autotrophic picoplankton were never observed.

Grazing by mesozooplankton is a negligible loss factor for any of their prey, be it bacteria, algae or protozoa. Mesozooplankton grazing rates are ca. 2 orders of magnitude lower than grazing rates by microzooplankton, and much smaller than the scatter of protozoan grazing rates or phytoplankton growth rates estimated. Admittedly, thus far, these estimates rest on many assumptions and abundance data for only 2 stations (data for more stations will become available in the future: R. Böttger-Schnack, S. B. Schnack-Schiel). However, no alternative way of calculating clearance rates and no conceivable level of mesozooplankton spatial variability would lead to estimates indicating that biomass loss due to mesozooplankton grazing is of equal importance to that due to protozoan grazing. It remains an open question to what extent a correction would be necessary it poecilostomatoid and cyclopoid copepods feed significantly on phytoplankton, contrary to our assumption.

Our results add to the well-established evidence that planktonic food webs of oligotrophic seas are dominated by the 'microbial loop' (picoplankton + bacteria + heterotrophic flagellates→ciliates→copepods) instead of the classic food chain (phytoplankton→copepods→

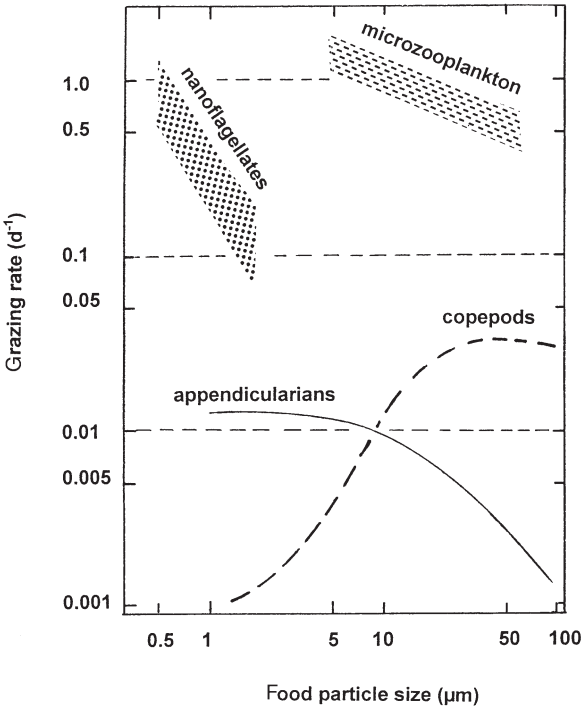

Fig. 4. Double logarithmic plot of the grazing rates of major functional groups of zooplankton on food particle size

fishes). The microbial loop has been proposed as a second major pathway of energy and carbon flow in the pelagic food web (Pomeroy 1974, Sieburth & Davis 1982, Azam et al. 1983). Because of the predominance of picophytoplankton in the oligotrophic ocean (Raven 1986, Stockner & Antia 1986) and the inability of copepods to feed on picoplankton, it is assumed that the microbial loop dominates over the classic food chain in oligotrophic systems (Sanders et al. 1992, Caron et al. 1995, 1999, Reckermann & Veldhuis 1997).

In addition, we have found that pelagic tunicates are more important grazers of pico- and smaller nanoplankton than are copepods within the size range of dominant phytoplankton in the Red Sea. Because of their high efficiency in capturing extremely small food particles, their high clearance rates, and their high intrinsic growth rates (Heron 1972, Deibel 1982a,b, Crocker et al. 1991, Bochdansky & Deibel 1997, Bone et al. 1997), it might be expected that tunicates could play an important role at least locally under suitable conditions. Therefore, we suggest that picoplankton feeding by tunicates should be considered a third major pathway in the pelagic food web.

The inability of copepods to feed on picoplankton and small (<5 µm) nanoplankton forces them to rely on protozoans as a major food source or on episodic blooms of larger algae. There was evidence of diatom-feeding by the strong signal of the fatty acid 16:1(n-7) (e.g. Graeve et al. 1994). This contrasts with a seasonal study of copepod gut contents at a station off Aqaba (Al-Najjar 2000), which did not find the diatom marker fucoxanthin, except in 1 sample of cyclopoid copepods on 7 March 1999 (7.26 ng ind.$^{-1}$). However, the 4 wk sampling regime of Al-Najjar's study was insufficient to detect episodic feeding events; lipid analysis would seem to provide more reliable information. Lipid composition is the result of dietary inputs over several weeks to months, depending on the life cycle of the species concerned.

In conclusion, phytoplankton grazing in the northern Red Sea and in the Gulf of Aqaba during the spring period is clearly dominated by microzooplankton as a result of the small size of the phytoplankton available. Appendicularians also feed on these algae, but are of less importance than microzooplankton. Copepods are the dominant mesozooplankton by number and biomass, but they occupy the third rather than the second trophic level.

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LITERATURE CITED

Al-Najjar T (2000) The seasonal dynamics and grazing control of phyto- and mesozooplankton in the northern Gulf of Aqaba. PhD thesis, Universität Bremen, Germany

Azam F, Fenchel T, Field JG, Meier-Reil LA, Thingstad F (1983) The ecological role of water column microbes in the sea. Mar Ecol Prog Ser 10:257–263

Bochdansky AB, Deibel D (1997) Functional feeding response and behavioral ecology of *Oikopleura vanhoeffeni* (Appendicularia, Tunicata). J Exp Mar Biol Ecol 233: 181–211

Bone Q, Braconnot JC, Carre C, Ryan KP (1997) On the filter-feeding of *Doliolum* (Tunicata: Thaliacea). J Exp Mar Biol Ecol 179:179–193

Böttger-Schnack R (1995) Summer distribution of micro- and small mesozooplankton in the Red Sea and Gulf of Aden, with special reference to non-calanoid copepods. Mar Ecol Prog Ser 118:81–102

Burkill PH, Leakey RJG, Owens NJP, Mantoura RFC (1993) *Synechococcus* and its importance to the microbial food-web in the northwestern Indian Ocean. Deep-Sea Res Part II 40:773–782

Caron DA, Cam HG, Kremer P, Lessard EJ and 6 others (1995) The contribution of microorganisms to particulate carbon and nitrogen in surface waters of the Sargasso Sea near Bermuda. Deep-Sea Res 42:943–972

Caron DA, Peele ER, Lim EL, Dennett MR (1999) Picoplankton and nanoplankton and their trophic coupling in surface waters of the Sargasso Sea south of Bermuda. Limnol Oceanogr 44:259–272

Crocker KM, Alldredge AL, Steinberg DK (1991) Feeding rates of the doliolid, *Dolioletta gegenbauri*, on diatoms and bacteria. J Plankton Res 13:77–82

Deibel D (1982a) Laboratory determined mortality, fecundity and growth rates of *Thalia democratica* Forskal and *Dolioletta gegenbauri* Uljanin (Tunicata, Thaliacea). J Plankton Res 4:143–153

Deibel D (1982b) Laboratory-measured grazing and ingestion rates of the salp, *Thalia democratica* Forksal and *Dolioletta gegenbauri* Uljanin (Tunicata, Thaliacea). J Plankton Res 4:189–201

Dolan JR, Simek K (1999) Diel periodicity in *Synechococcus* populations and grazing by heterotrophic nanoflagellates: analysis of food vacuole contents. Limnol Oceanogr 44: 1565–1570

Dusenberry JA, Frankel SL (1994) Increasing the sensitivity of FACScan flow cytometer to study oceanic picoplankton. Limnol Oceanogr 39:206–209

Folch J, Lees M, Sloane-Stanley GH (1957) A simple method for the isolation and purification of total lipids from animal tissue. J Biol Chem 226:497–509

Fraser AJ, Tocher DR, Sargent JR (1985) Thin-layer chromatography—flame ionization detection and the quantification of marine neutral lipids and phospholipids. J Exp Mar Biol Ecol 88:91–100

Genin A, Lazar B, Brenner S (1995) Vertical mixing and coral death in the Red Sea following the eruption of Mount Pinatubo. Nature 377:507–510

Goldman JC (1984) Oceanic nutrient cycles. In: Fasham MJ (ed) Flows of energy and materials in marine ecosystems: theory and practice. Plenum Press, London, p 137–170

Goldman JC, McCarthy JJ, Peavey DG (1979) Growth rate influence on the chemical composition of phytoplankton in oceanic waters. Nature 279:210–215

Graeve M, Kattner G, Hagen W (1994) Diet-induced changes in the fatty acid composition of Arctic herbivorous cope-

pods: experimental evidence of trophic markers. J Exp Mar Biol Ecol 182:97–110
Grossart HP, Simon M (2002) Bakterioplankton dynamics in the Gulf of Aqaba and in the northern Red Sea in early spring. Mar Ecol Prog Ser 239:263–276
Hagen W (2000) Lipids. In: Harris R, Wiebe P, Lenz J, Skjoldal HR, Huntley M (eds) ICES zooplankton methodology manual, Academic Press, San Diego, p 113–119
Haney JF (1971) An *in situ* method for the measurement of zooplankton grazing rates. Limnol Oceanogr 16:970–977
Heron AC (1972) Population ecology of a colonizing species: the pelagic tunicate *Thalia democratica*. Oecologia 10: 269–293
Kattner G, Fricke HSG (1986) Simple gas-liquid chromatographic method for the simultaneous determination of fatty acids and alcohols in wax esters of marine organisms. J Chromatogr 361:263–268
Kattner G, Graeve M, Hagen W (1994) Ontogenetic and seasonal changes in liquid and fatty acid/alcohol compositions of the dominant Antarctic copepods *Calanoides propinquus*, *Calanus acutus* and *Rhincalanus gigas*. Mar Biol 118:637–644
Kimor B, Golandsky B (1977) Microplankton of the Gulf of Elat: aspects of seasonal and bathymetric distribution. Mar Biol 42:55–67
Klinker J, Reiss Z, Kropach C, Levanon J, Harpaz H, Shapiro Y (1978) Nutrients and biomass distribution in the Gulf of Aqaba (Elat), Red Sea. Mar Biol 45:53–64
Landry MR, Hassett RP (1982) Estimating the grazing impact of marine micro-zooplankton. Mar Biol 67:283–288
Landry MR, Kirshtein J, Constantinou J (1995) A refined dilution technique for measuring the community grazing impact of microzooplankton, with experimental tests in the central equatorial Pacific. Mar Ecol Prog Ser 120: 53–63
Li H, Veldhuis MJW, Post AF (1998) Alkaline phosphatase activities among planktonic communities in the northern Red Sea. Mar Ecol Prog Ser 173:107–115
Li WKW, Zohary T, Jacobi YZ, Wood AM (1993) Ultraphytoplankton in the eastern Mediterranean Sea: towards deriving phytoplankton from biomass flow cytometric measurements of abundance, fluorescence and light scatter. Mar Ecol Prog Ser 102:79–87
Lindell D, Post AF (1995) Ultraphytoplankton succession is triggered by deep winter mixing in the Gulf of Aqaba (Eilat), Red Sea. Limnol Oceanogr 40:1130–1141
Lund JWG, Kipling C, LeCren ED (1958) The inverted microscope method of estimating algal numbers and the statistical basis of estimations by counting. Hydrobiologia 11: 143–170
Mackey MD, Mackey DJ, Higgins HW, Wright SW (1996) CHEMTAX—a program for estimating class abundance from chemical markers: application to HPLC measurements of phytoplankton. Mar Ecol Prog Ser 144: 265–283
Pomeroy LR (1974) The ocean foodweb, a changing paradigm. BioScience 24:499–504
Raven JA (1986) Physiological consequences of extremely small size for autotrophic organisms in the sea. Can Bull Fish Aquat Sci 214:1–70
Reckermann M, Veldhuis MJW (1997) Trophic interactions between picophytoplankton and micro- and nanozooplankton in the western Arabian Sea during the NE monsoon 1993. Aquat Microb Ecol 12:263–273
Reiss Z, Hottinger L (1984) The Gulf of Aqaba: ecological micropaleontology. Springer-Verlag, Berlin
Sakka A, Legendre L, Gosselin M, Delesalle B (2000) Structure of the oligotrophic planktonic food web under low grazing of heterotrophic bacteria: Takapoto Atoll, French Polynesia. Mar Ecol Prog Ser 197:1–17
Sanders RW, Caron DA, Berninger UG (1992) Relationship between bacteria and heterotrophic nanoplankton in marine and fresh waters: an inter-ecosystem comparison. Mar Ecol Prog Ser 86:1–14
Sautour B, Artigas LF, Delmas D, Herbland A, Laborde P (2000) Grazing impact of micro- and mesozooplankton during a spring situation in coastal waters off the Gironde estuary. J Plankton Res 22:531–552
Sieburth JM, Davis PG (1982) The role of heterotrophic nanoplankton in the grazing and nurturing of planktonic bacteria in the Sargasso and Caribbean Sea. Annu Inst Océanogr 58:285–296
Sommer U (1996) Plankton ecology: the past two decades of progress. Naturwissenschaften 83:293–301
Sommer U (2000) Scarcity of medium-sized phytoplankton in the Red Sea explained by strong bottom-up and weak top-down control. Mar Ecol Prog Ser 197:19–25
Stockner JG, Antia NJ (1986) Algal picoplankton from marine and freshwater ecosystems: a multidisciplinary perspective. Can J Fish Aquat Sci 43:2472–2503
Vaulot D (1989) CYTOPC: processing software for flow cytometric data. Signal Noise 2:8
Weisse T (1989) The microbial loop in the Red Sea: dynamics of pelagic bacteria and heterotrophic nanoflagellates. Mar Ecol Prog Ser 55:241–250
von Stosch HA, Drebes G (1964) Entwicklungsgeschichtliche Untersuchungen an zentrischen Diatomeen. IV. Helgol Wiss Meeresunters 11:209–257
Wolf-Vecht A, Paldor N, Brenner S (1992) Hydrographic indications of advection/convection effects in the Gulf of Eilat. Deep-Sea Res 39:1393–1401
Wright SW, Thomas DP, Marchant HJ, Higgins HW, Mackey MD, Mackey DJ (1996) Analysis of phytoplankton of the Australian sector of the Southern Ocean: comparison of microscopy and size fraction data with interpretations of pigment HPLC data using 'CHEMTAX' matrix factorisation program. Mar Ecol Prog Ser 144:285–298
Yahel G, Post AF, Fabricius K, Marie KD, Vaulot D, Genin A (1998) Phytoplankton distribution and grazing near coral reefs. Limnol Oceanogr 43:551–563

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