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NOTE

Gut content analysis of neritic copepods *Acartia omorii* and *Oithona davisae* by a new method

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ABSTRACT: Two neritic copepods, *Acartia omorii* and *Oithona davisae*, were collected from an area rich in diatoms. Their gut contents, treated with concentrated nitric acid and hydrogen peroxide, were qualitatively analysed with a scanning electron microscope coupled with an energy dispersive X-ray spectrometer. Results indicated *A. omorii* as a diatom feeder, but *O. davisae* as a non-diatom feeder

Knowledge of the kind of food ingested by dominant species of neritic copepods is essential for studying their feeding impact and dependence upon primary producers and their relation to consumers at higher trophic levels. Marine cyclopoid copepods of the genus *Oithona* are often predominant in neritic environments. Their feeding habit, however, is not well understood in spite of a considerable number of studies. They have generally been known as diatom feeders (cf. Lampitt & Gamble 1982, Turner 1986) though Marshall & Orr (1962) suggested non-diatom feeding in *O. similis*. Uchima & Hirano (1986a) demonstrated non-diatom feeding in *O. davisae* from laboratory experiments and observations, but did not examine gut contents.

Oithona davisae Ferrari & Orsi often attains a population density of more than 1000 l^{-1} and over 90 % of the species composition of copepods in the inner part of Tokyo Bay, Japan, during the diatom and flagellate bloom period from June to August (Uchima unpubl.). In February and March when diatoms bloom at lower concentrations, *O. davisae* comprises ca 50 % of total copepods though the population density remains below 100 l^{-1} If they ingest diatoms in nature, their population growth will depend on the blooming of diatoms; but on the other hand, if they do not, they should contribute to promoting, not depressing, the diatom bloom through excretion.

The present study was carried out to investigate whether or not *Oithona davisae* ingests diatoms under natural conditions during a diatom bloom in the inner part of Tokyo Bay. Food of a co-existing species *Acartia omorii* Bradford, formerly identified as *A. clausi* in Japanese waters (Ueda 1986), was also examined to gain understanding of interactions between the 2 species. Gut contents were analysed by a new method instead of the usual SEM analytic method for fecal pellets.

In January 1988, planktonic diatoms occurred abundantly and sustained high concentrations, and formed a red tide in early February (Table 1). *Acartia omorii*

Table 1 Concentrations of phytoplankton collected with a 2 l bottle sampler between surface and 0.5 m depth in the inner part of Tokyo Bay at 21:50 h on 8 Feb 1988. Flagellates were divided into 2 categories, small and large. Dinoflagellates were categorized into the latter

Species	Concentration (cells ml^{-1})	Totals	
Flagellates			
Small (≦ 10 µm)	32480	37760	
Large (> 10 µm)[a]	5280		
Diatoms			
Chaetoceros affinis	82	1262	2801
C. brevis	142		
C. compressus	628		
C. debilis	221		
C. didymus	94		
C. radicans	38		
C. sp.	57		
Coscinodiscus sp.	6		
Eucampia zoodiacus	2		
Nitzschia closterium	4		
Rhizosolenia setigera	9		
Skeletonema costatum	1043		
Thalassionema nitzschioides	30		
Thalassiosira anguste-lineata	288	445	
T rotula	157		

[a] *Euglena* sp. comprised ca 70 % of large flagellates

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Table 2. Specimens used for gut content analysis. *Acartia omorii* and *Oithona davisae* were collected in horizontal hauls at 0.2 m depth in the inner part of Tokyo Bay at 23:00 h on 8 Feb 1988. CIV and CV: Copepodite IV and V, respectively

Species	Stage and sex	No. of specimens	Total
With full gut			
A. omorii	Adult male	10	20
	Adult female	10	
O. davisae	Adult male	50	300
	Adult female	100	
	CV male	50	
	CV female	50	
	CIV[a]	50	
With empty gut: starved for 30 h (control)			
A. omorii	Adult male	10	20
	Adult female	10	
O. davisae	Adult male	50	100
	Adult female	50	

[a] CIV is sexually indistinguishable (Uchima 1979)

and *Oithona davisae* comprised more than 90 % of copepods. At 23:00 h on 8 February, a 59 µm mesh net was towed horizontally over a distance of 5 m at 0.2 m depth at a speed of ca 1 m s^{-1}; net-tows were repeated 20 times over 30 min. Copepods collected by the first 10 net-tows were immediately preserved in 5 % buffered formalin/seawater solutions and then, in the laboratory, 20 *Acartia* and 300 *Oithona* specimens with full guts were sorted under a stereomicroscope (Table 2). Live copepods taken by the second 10 net-tows were sorted within 30 min after collection and rinsed 5 times with in situ seawater (27 ‰ S) passed through a 0.45 µm Millipore filter. Twenty *Acartia* and 100 *Oithona* adults were individually transferred to 1 l plastic dishes filled with the filtered seawater. They were kept at in situ water temperature of 12.5 °C in darkness, starved for 30 h and then preserved for use as control specimens; their empty guts were all observed.

Preserved specimens were each rinsed 5 times with distilled water to remove seawater salts and/or diatoms possibly entangled in feeding appendages and swimming legs. They were dried at 60 °C and digested at 100 °C for 2 h with concentrated nitric acid and hydrogen peroxide to remove organic matter; silica skeletons are not digested by this treatment. The residue was washed by centrifugation 3 times with distilled water and then collected on a small section of 1.0 µm Nuclepore filter. These samples were carbon-coated to about 30 nm thickness and analysed qualitatively with a scanning electron microscope (SEM) coupled with an energy-dispersive X-ray spectrometer (EDS) that detects elements heavier than neon. Residual matter was regarded as diatom skeletons when only silicon was detected with EDS, as clay minerals when other metal elements were detected, and as organic matter when no prominent elements were detected (Fig. 1).

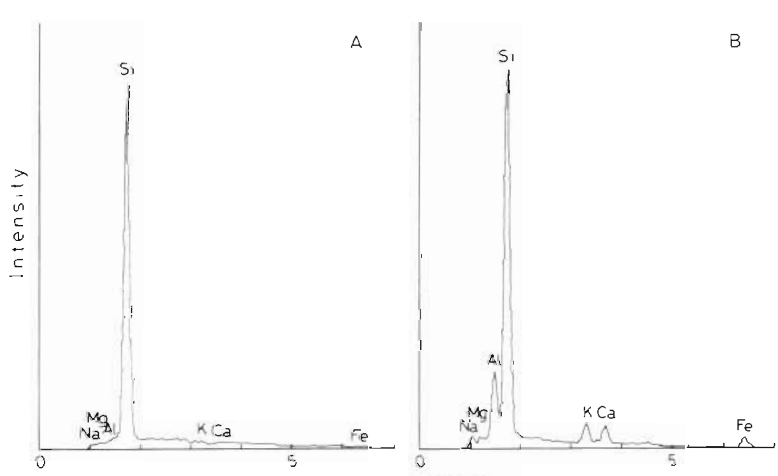

Fig. 1. Elemental composition of (A) diatom skeletons and (B) clay minerals analysed with an energy-dispersive X-ray spectrometer

Gut content analyses indicated that adult *Acartia omorii* fed on diatoms, *Chaetoceros, Skeletonema* and *Thalassiosira* species in abundance in situ (Fig. 2; Table 1). The many fine slender particles observed can be regarded as fragments of setae of *Chaetoceros* spp., since they are distinguishable in shape and composition from clay minerals and organic matter undigested with nitric acid (Figs. 1 and 2).

In the gut contents of adults and copepodites of *Oithona davisae,* silica skeletons, even fragments, were not observed at all (Fig. 3 A, B). The skeleton-search with SEM produced the same result as for a control sample (Fig. 3 C). There is little possibility of all fragments of diatom skeletons passing through a Nuclepore filter, since the digestive ability of copepods to break diatom skeletons into pieces (< 1 μm) is unlikely. Possible intake of only the contents of diatoms after breakage of frustules has not been observed in *Oithona davisae* (Uchima & Hirano 1986a).

Marshall & Orr (1962) suggested *Oithona similis* as a non-diatom feeder by ^{32}P measurements, although later they (1966) stated that it ingested *Ditylum* sp. Other workers reported diatom feeding in *O. nana* (Murphy 1923, Haq 1965, Lampitt 1979, Lampitt & Gamble 1982), *O. similis* (Lebour 1922, Eaton 1971), *O. plumifera* and *O. simplex* (Turner 1986). There are, however, no studies showing that oithonid copepods grow or produce eggs in Millipore-filtered seawater containing only diatoms as food (cf. Eaton 1971, Uchima & Hirano 1986a); notice that 'seawater' enriched with *Phaeodactylum tricornutum* was used by Haq (1965) in his successful experiments on the growth of *O. nana,* and that this diatom was not available for *O. davisae* (Uchima & Hirano 1986a). On the other hand, flagellates and/or dinoflagellates led to success (Eaton 1971, Uchima 1979, 1985, Lonsdale 1981, Uchima & Hirano 1986a). Whether oithonid copepods ingested diatoms as a nutrient or did so accidentally may need to be re-examined.

Oithona davisae never feeds on diatoms in laboratory experiments (Uchima & Hirano 1986a). The present study indicates that *O. davisae* does not ingest diatoms

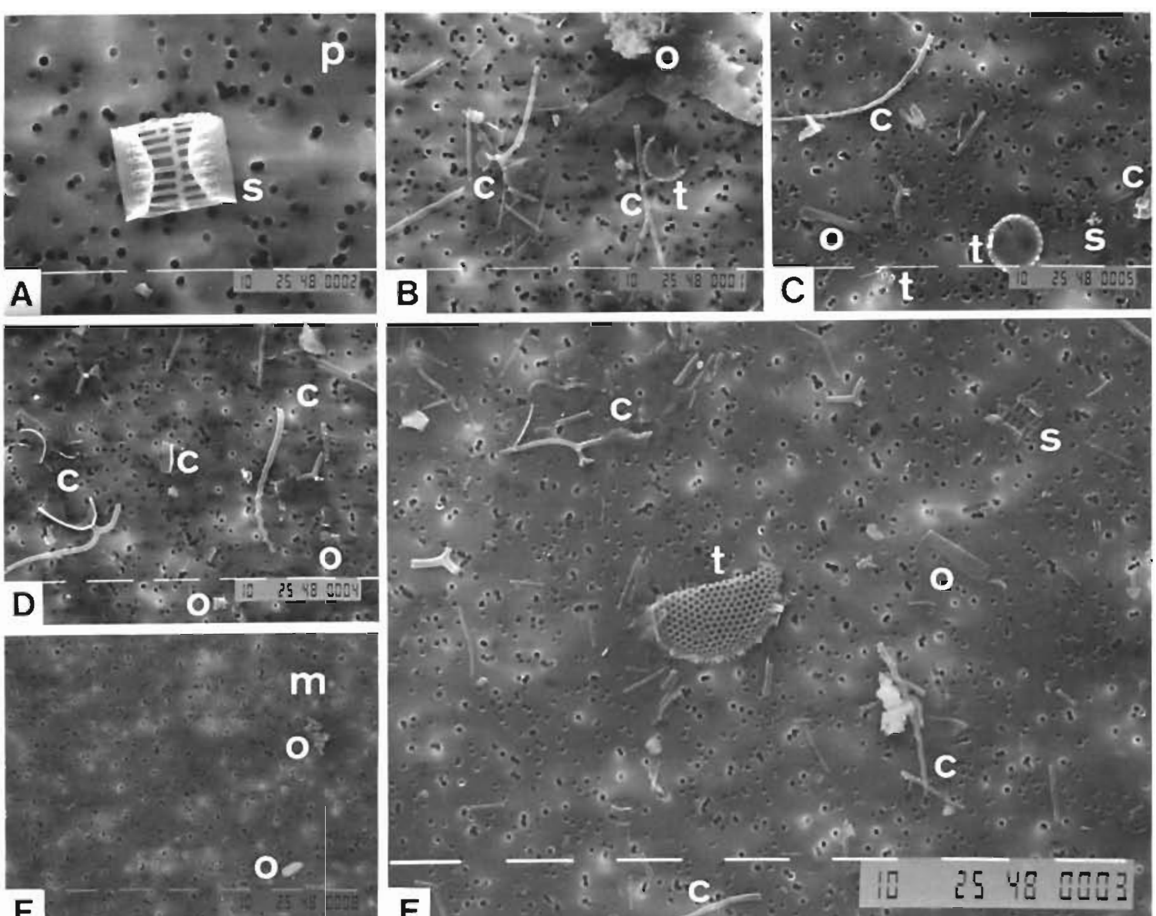

Fig. 2. *Acartia omorii.* Gut contents. (A to E) From specimens with full guts. (F) From control specimens with empty guts. c: *Chaetoceros* spp.; m: clay minerals; o: organic matter; p: pores of Nuclepore filter; s: *Skeletonema costatum*; t: *Thalassiosira* spp. Magnifications: (A) 3500×; (B, C) 2000×; (D) 1500×; (E, F) 1000×. Each scale bar = 10 μm

even in the sea rich in them. This finding suggests feeding segregation between *O. davisae* and *Acartia omorii*, i.e. between co-occurring species, although *A. omorii* also ingests flagellates and dinoflagellates (Iwasaki et al. 1977, Tsuda & Nemoto 1984). In the study area, the population density of *A. omorii* gradually increased between February and April and reached a peak of over 100 l^{-1}, while that of *O. davisae* generally remains less than 100 l^{-1} (Uchima unpubl.). This feeding segregation may moderate possible interactions between the 2 species (Uchima & Hirano 1986b), especially during the diatom bloom period.

The present method was first applied to gut content analysis of zooplankters. Although the method provides qualitative information only on food organisms with silica skeletons, it prevents suspended diatoms and/or detrital particles from contaminating matter ingested as food, and allows the examination of diatom feeding in a number of specimens in a short period. Using a common SEM analytic method, Turner (1986) examined fecal pellets defecated by *O. simplex*, which is smaller than *O. davisae*. My attempts to use his method to analyse fecal pellets of *O. davisae* ended in failure, with difficulty in distinguishing fecal pellets from detrital particles in preliminary experiments. Since the question whether or not copepods ingest diatoms is an important matter in marine ecology, diatom feeding in small copepods should be examined by this new method if their fecal pellets are difficult to collect.

Further studies on the feeding behaviour of zooplankters will clarify whether or not a planktonic community forming a marine food web can be divided into 2 categories, diatom-basis and flagellate-basis links.

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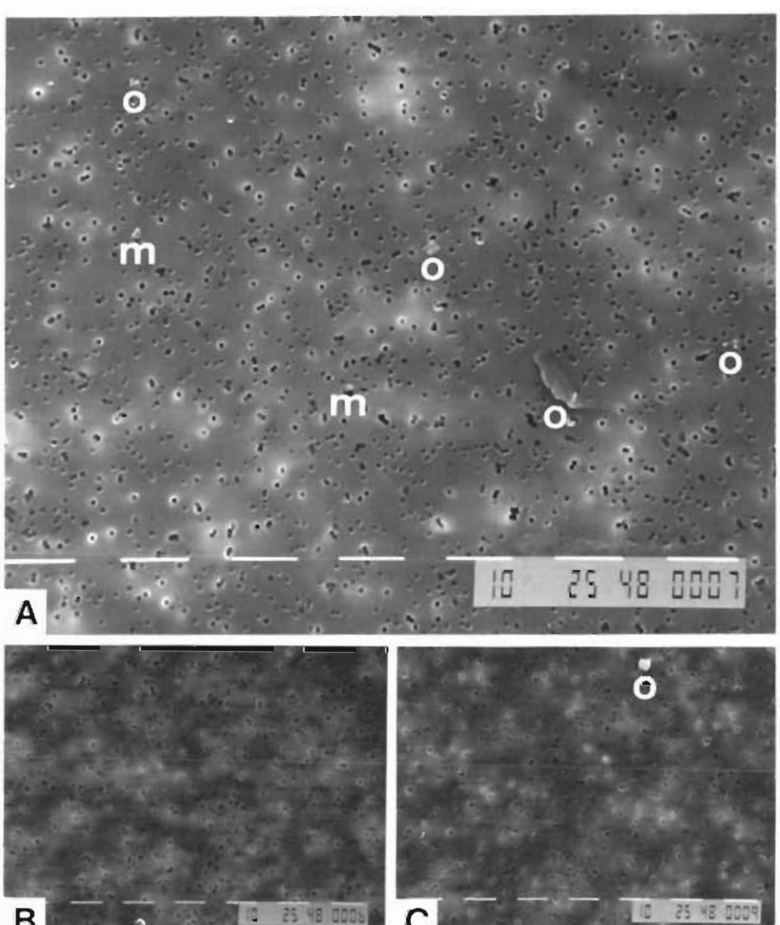

Fig. 3. *Oithona davisae*. Gut contents. (A, B) From specimens with full guts. (C) From control specimens with empty guts. m: clay minerals; o: organic matter. Magnification: 1000×. Each scale bar = 10 μm

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