

# Temporally sustained dietary niche partitioning in two mesopelagic copepod species and their mouthpart morphology

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**ABSTRACT:** We compared seasonal feeding habit patterns, mouthpart structures, body sizes and vertical distributions of the aetideid copepods *Undeuchaeta major* and *Chirundina streetsii* in Sagami Bay, Japan. We focused on niche partitioning as one of the mechanisms sustaining co-existence of these mesopelagic copepods, by integrative application of stable-isotope analysis, microscopic observation and elemental analysis of the gut contents. While the body sizes and vertical distribution of both species were similar, they showed marked differences in their feeding habits throughout the year, i.e. carnivory in *U. major* and omnivory in *C. streetsii*, suggesting that food niche partitioning was a mechanism enabling their co-existence. Of particular interest was the consistently lower  $\delta^{15}\text{N}$  values in *U. major*, attributable to its selective feeding on oncaeid copepods. Previous reports of low  $\delta^{15}\text{N}$  in oncaeids, whose major food source has been assumed to be nanoplankton attached to appendicularian houses, suggest that the size of the primary producer is important in  $\delta^{15}\text{N}$  analyses of pelagic food webs. Comparison of mouthparts of the 2 species indicated slight differences in the maxilliped morphology. The relationship between feeding habit and maxilliped morphology was examined for the species above, other omnivorous aetideids and the Euchaetidae, a carnivorous sister family of the Aetideidae. The results suggest that carnivores have a broad basis and long unguiform setae, while omnivores have a narrower basis and shorter, more slender setae. The feeding habits of Aetideidae, which have so far been considered as exclusively omnivorous, comprise omnivory and carnivory, and can be distinguished by their maxilliped morphology.

**KEY WORDS:** Aetideidae · Vertical distribution · Food chain · Marine snow · Feeding · Stable isotope · Elemental analysis

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## INTRODUCTION

Among the most striking features of the biodiversity in oceanic pelagic communities is the high species diversity at a local scale, in contrast to the low species diversity on a global scale, which is attributable largely to the high species diversity in the mesopelagic zone (Angel 1993, Mauchline 1998). Among others, the highest copepod species diversity was observed in the mesopelagic zones of many areas of the world's oceans (Roe 1972, Yamaguchi et al. 2002,

Kuriyama & Nishida 2006). However, the mechanisms generating and maintaining such a diversity pattern are still not fully understood. While recent studies have suggested vertical habitat partitioning and food niche partitioning as important mechanisms for the co-existence of copepods in the mesopelagic zone (Kuriyama & Nishida 2006, Laakmann et al. 2009, Matsuura et al. 2010, Sano et al. 2013), little is known about the temporal changes in the food-web structure and the relationships among species in the mesopelagic zone. Exceptions are a few studies

that investigated the feeding habits of mesopelagic copepods throughout the year, which highlighted a seasonal change of food composition of the targeted species (Gowing & Wishner 1998, Schnetzer & Steinberg 2002).

To investigate food niche partitioning, size-independent feeding selectivity of taxonomically related and/or morphologically similar species (Kuriyama & Nishida 2006) should be taken into consideration. Sano et al. (2013) examined food source differences in 2 such pairs of species during a spring-bloom season. They compared the scolecitrichids *Scottocalanus helenae* and *Scottocalanus securifrons*, which had a similar vertical distribution and body size, and found significant differences in nitrogen stable-isotope ratios, but no differences in other feeding-related features, such as gut contents, as indicated by microscopic and elemental analyses and by morphology of the feeding appendages. On the other hand, another species pair belonging to the Aetideidae, *Chirundina streetsii* (Fig. 1a) and *Undeuchaeta major* (Fig. 1b), which were also similar in body size and general body shape, showed marked differences, not only in the nitrogen stable-isotope ratios, but also in other

feeding-related features, suggesting carnivory for *U. major* in contrast to the marine-snow feeding of *C. streetsii* (Sano et al. 2013). This finding urged reconsideration of previously held beliefs that Aetideidae species, one of the largest families of the Calanoida and encompassing ~180 species distributed in mainly the meso- and bathypelagic zones, are essentially mixed feeders without any apparent food selectivity (Boxshall & Halsey 2004). For this reason, we compared the feeding habits and distributions of these 2 species throughout the year.

Until recently, most deep-sea copepods have been considered to be either omnivorous or carnivorous because of limited data in the feeding analysis of deep-sea copepods (Sano et al. 2013). To investigate the detailed feeding habits of deep-sea copepods, a multidisciplinary, integrative approach is recommended (Kosobokova et al. 2002, Laakmann et al. 2009, Sano et al. 2013). However, it would be impossible to analyze all species comprising the targeted copepod community. An alternative option is an analysis of the mouthpart morphology, which would provide a useful body of information on their feeding habits, accompanied by other supporting evidence (e.g. Itoh 1970, Ohtsuka & Onbé 1991, Nishida & Ohtsuka 1996). An example of this approach was carried out by von Vaupel Klein (1998) for the Aetideidae, in which he compared lengths of the maxilliped parts of some *Euchirella* species and suggested size differences of food items. The function of the Aetideidae maxillipeds is to hold and/or seize prey (von Vaupel Klein 1982, von Vaupel Klein & Koomen 1994 and references therein). On the basis of these observations, we investigated the relationship between feeding habits and mouthpart morphology, with special reference to the maxillipeds of *C. streetsii* and *U. major* and other aetideid species reported to be omnivorous (Mauchline 1998). For a comparison, we also examined the mouthpart morphology of Euchaetidae species, a sister family of the Aetideidae (Park 1994), which are essentially carnivorous (Boxshall & Halsey 2004) and use the maxillipeds for capturing prey (Wickstead 1962, Yen 1983).

This study aims at examining temporal change in the feeding habits of the 2 related mesopelagic copepods, focusing on their possible niche partitioning throughout the year, and investigating appropriate morphological indices to identify feeding habits of mesopelagic copepods. We applied integrative analysis of gut contents (microscopic and elemental analysis), stable-isotope ratios, mouthpart morphology and vertical distributions for these purposes.

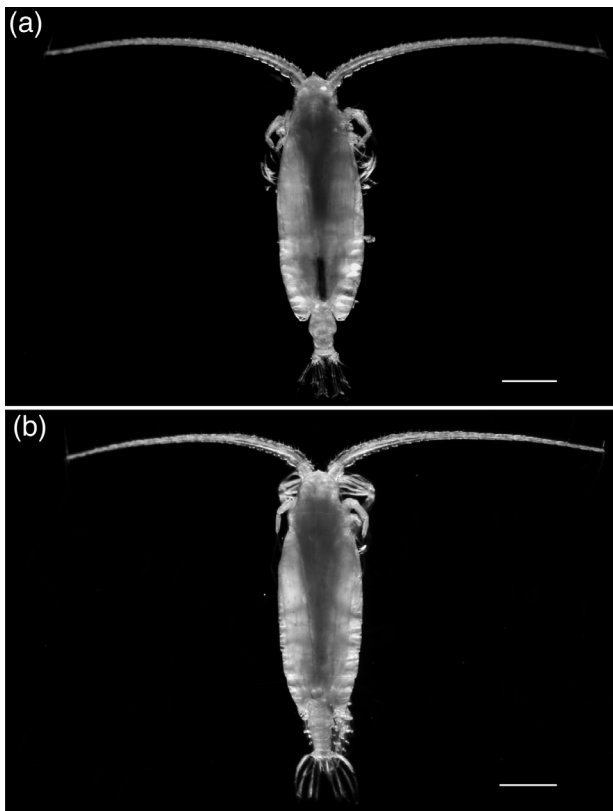


Fig. 1. Dorsal view of 2 aetideid copepods: (a) *Chirundina streetsii* and (b) *Undeuchaeta major*. Scale bars = 1 mm

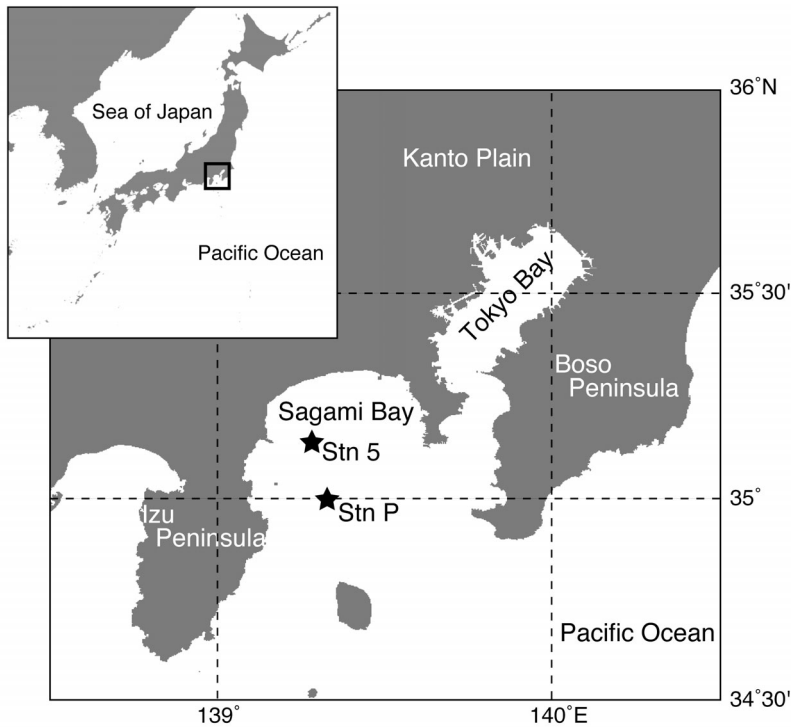


Fig. 2. Sampling stations (Stn P: 1450 m water depth; Stn 5: 1200 m water depth) in Sagami Bay, Japan

The term 'carnivory' in the present study applies to feeding mainly on metazoans (excluding direct feeding on heterotrophic microbes), while 'omnivory' refers to feeding mainly on macroscopic aggregates (including microbes) with or without metazoans, although 'omnivory' in the referenced literature may include broader definitions.

## MATERIALS AND METHODS

### Sampling

Plankton samples were collected from 2 stations (Stn P, 35°00'N, 139°20'E, depth ~1450 m; Stn 5, 35°10'N, 139°17'E, depth ~1200 m; Fig. 2) in Sagami Bay in April, June, September, and November 2009 and March 2010 (Table 1).

In April, November and March, plankton samples were collected using Motoda horizontal closing nets (MTD nets: 56 or 80 cm mouth diameter, 0.33 mm mesh aperture; Motoda 1971) during voyages of the RV 'Tansei Maru'. The MTD nets were towed horizontally at 8, 11, or 15 varying depths at Stn P (Table 1). Attachment positions of the MTD nets to the wires were preset based on the intended sampling depth and the wire angle, which was maintained at 45° dur-

ing the hauls, assuming a straight wire. The depth of the deepest nets was monitored using a depth meter (Compact-TD, JFE Advantech Co. Ltd.), which indicated that the real depths of tows fluctuated within  $\pm 10\%$  of the preset depths. Hence, the preset depths were applied for presentation of the vertical patterns. The samples were fixed and preserved in 5% borax-buffered formalin-seawater or immediately frozen and preserved at  $-80^{\circ}\text{C}$ . The volume of water filtered was estimated from readings taken by a flow meter (Rigoshia Co. Ltd.) attached to the mouth of each net. In March, plankton samples were also collected at Stn 5 using an Ocean Research Institute (ORI) net (160 cm mouth diameter, 0.33 mm mesh aperture; Omori 1965) towed obliquely from epi- and mesopelagic depths ( $>864$  m). The collected samples were treated in the same way as in the MTD-net samples. In June and September, plankton samples were collected using an Intelligent Operative Net Sampling System (IONESS, effective mouth area 1 m<sup>2</sup>, 0.33 mm mesh aperture), an opening/closing net system modified from the MOCNESS

(Wiebe et al. 1985), during voyages of the TS 'Seiyo Maru'. The IONESS was towed obliquely in 8 discrete depth layers in June and September. The samples were divided with a box-type splitter (Motoda 1959) into aliquots, of which one was fixed and preserved in 5% borax-buffered formalin-seawater, and the others were immediately frozen and preserved at  $-20^{\circ}\text{C}$  on board, then preserved at  $-80^{\circ}\text{C}$  in the laboratory until analysis.

Marine snow samples were collected using Knauer-type cylindrical sediment traps (Knauer et al. 1979) deployed at 4 depths (100, 200, 300 and 400 m) in April and 5 depths (25, 100, 200, 300 and 400 m) in March. The trap system at each depth consisted of 8 acrylic tubes with a mouth area of 39 cm<sup>2</sup>. A collecting cup attached to the bottom of each tube was filled with 0.2- $\mu\text{m}$ -filtered seawater to which we added NaCl (5 g l<sup>-1</sup>) and borax-buffered formalin (1% final concentration) to the April sample, and NaCl (5 g l<sup>-1</sup>) and HgCl<sub>2</sub> (50 mg l<sup>-1</sup>) to the March sample to minimize microbial activities and decomposition of the marine snow. The traps were left to drift for ~1 d (22 April 2009, 08:51 h to 23 April 2009, 08:30 h; 11 March 2010, 17:42 h to 12 March 2010, 15:57 h) during which time they shifted ~6 km (34°59'55"N, 139°14'59"E to 34°56'59"N, 139°14'23"E in April; 34°59'55"N, 139°20'01"E to 34°59'55"N, 139°20'16"E

Table 1. Sampling records from Motoda (MTD) nets, the Intelligent Operative Net Sampling System (IONESS) and the Ocean Research Institute (ORI) net in Sagami Bay

Cruise	Date	Station	Net type	Time (h)		Sampling depth (m)	Preservation
				Net in	Net out		
KT-09-4	22 Apr 2009	Stn P	MTD	19:05	20:50	95, 100, 195, 200, 295, 300, 395, 400	Frozen
	23 Apr 2009	Stn P	MTD	19:58	22:10	300, 400, 500, 600, 800, 1000	5% formalin
	23 Apr 2009	Stn P	MTD	22:21	23:05	0, 50, 100, 150, 200	5% formalin
	24 Apr 2009	Stn P	MTD	04:23	06:42	100, 200, 300, 400, 600, 800, 1000	Frozen
	24 Apr 2009	Stn P	MTD	10:52	12:53	300, 400, 500, 600, 800, 1000	5% formalin
	24 Apr 2009	Stn P	MTD	12:56	13:45	0, 50, 100, 150, 200	5% formalin
'Seiyo maru' June Cruise	3 Jun 2009	Stn P	IONESS	15:28	18:33	1400–1000, 1000–800, 800–600, 600–500, 500–400, 400–300, 300–200, 200–0	1/4 aliquot: 5% formalin 3/4 aliquot: frozen
'Seiyo maru' September Cruise	10 Sep 2009	Stn P	IONESS	11:59	14:27	1000–800, 800–600, 600–500, 500–400, 400–300, 300–200, 200–100, 100–0	1/2 aliquot: 5% formalin 1/2 aliquot: frozen
KT-09-25	30 Nov 2009	Stn P	MTD	10:15	12:20	1000, 800, 600, 500, 400, 300, 200, 100	5% formalin
	30 Nov 2009	Stn P	MTD	19:45	22:17	1000, 800, 600, 500, 400, 300, 200, 100	Frozen
KT-10-2	11 Mar 2010	Stn P	MTD	23:59	02:05	1000, 800, 600, 500, 400, 300	5% formalin
	12 Mar 2010	Stn P	MTD	02:12	03:00	200, 150, 100, 50, 0	5% formalin
	12 Mar 2010	Stn P	MTD	09:01	10:55	1000, 800, 600, 500, 400, 300	5% formalin
	12 Mar 2010	Stn P	MTD	11:00	11:41	200, 150, 100, 50, 0	5% formalin
	13 Mar 2010	Stn 5	ORI	14:53	16:12	864–0	5% formalin or frozen

in March). After recovery, some samples from each depth were filtered through a 1 mm mesh to remove swimmers and then immediately filtered through pre-combusted (450°C for 3 h) Whatman GF/F filters and preserved at –80°C until analysis.

Water samples were collected at Stn P from discrete depth layers using a CTD system (ICTD, Fal-mouth Scientific Inc.) equipped with 8 or 12 l Niskin bottles (General Oceanics). Water samples to determine chlorophyll *a* concentration were filtered through Whatman GF/F filters (25 mm diameter).

### Chlorophyll *a* concentration

The chlorophyll *a* concentrations of the April, June and September water samples were analyzed using a Turner fluorometer (10-AU) following the methods of Holm-Hansen et al. (1965) and Suzuki & Ishimaru (1990), and, for the November and March samples, those of Suzuki & Ishimaru (1990) and Welschmeyer (1994).

To examine the seasonal change of primary producers, the chlorophyll *a* concentration in the upper 50 m (0 to 38 m in June) were vertically integrated, applying the trapezoidal rule.

### Stable-isotope analysis

Nitrogen and carbon stable-isotope ratios were analyzed for the 2 targeted species of aetideid copepods and their main potential food, the oncaeid copepods and marine snow (Sano et al. 2013). The frozen plankton samples were thawed and sorted on an ice-cooled tray to prevent degradation, and were then identified under a stereo-microscope. The aetideid copepods were analyzed individually, while the oncaeids were analyzed as pooled samples of up to ~50 copepods, according to their body mass. All of the sorted copepods were adult females. The sorted samples were dried at 60°C for 24 h and packed in tin capsules. For the isotopic analysis, 0.4 to 1.2 mg (dry weight) of tissue was used from each sample. The plankton samples were not defatted because biomass values were generally low, and other studies have shown a bias in  $\delta^{15}\text{N}$  after lipid extraction (Bodin et al. 2007, Mintenbeck et al. 2008). Based on the atomic C/N ratios, which were measured simultaneously with the analysis of the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values, we corrected the  $\delta^{13}\text{C}$  of the plankton samples for lipids using the mass balance model for zooplankton recommended by Smyntek et al. (2007: Eq. 5).

The GF/F filtered marine snow samples were treated with HCl fumes to remove inorganic carbon, dried, folded, wrapped in tin capsules, and molded into small tablets. We used formalin to inhibit biological activity in the sediment-trap samples from April. Altabet (2001) reported that formalin did not appear to add sufficient organic C to sediment trap samples to alter the  $\delta^{13}\text{C}$ , based on comparison with  $\text{NaN}_3$  and  $\text{HgCl}_2$  preserved samples. Altabet (2001) used 3% formalin and deployed the sediment traps for 1 yr. The concentration of formalin in the present study was 1%, and the traps were deployed for only 1 d. Therefore, we considered the effect of formalin on  $\delta^{13}\text{C}$  to be negligible in this analysis.

The  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of plankton and filter samples were determined by an elemental analyzer connected to an isotope-ratio mass spectrometer (Flash EA-ConFloIII-Delta<sup>plus</sup>XP, Thermo-electron). The  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values were obtained in parts per thousand (‰) relative to the standard, according to the following formula:

$$\delta^{13}\text{C} \text{ or } \delta^{15}\text{N} = [(R_{\text{sample}} / R_{\text{standard}}) - 1] \times 10^3 \quad (1)$$

For  $\delta^{13}\text{C}$ ,  $R_{\text{sample}}$  and  $R_{\text{standard}}$  were  $^{13}\text{C}/^{12}\text{C}$  of the sample and the standard (Vienna Pee Dee Belemnite), respectively. For  $\delta^{15}\text{N}$ ,  $R_{\text{sample}}$  and  $R_{\text{standard}}$  were  $^{15}\text{N}/^{14}\text{N}$  of the sample and the standard (atmospheric  $\text{N}_2$ ), respectively. To obtain atomic C/N ratios of the samples, glycine was used to calibrate the quantification of C and N.

### Microscopic gut contents analysis

Morphological microscopic and elemental analyses of the gut contents to ascertain seasonal differences were carried out using the formalin-preserved samples collected during the daytime, as they contained larger numbers of specimens with sufficient gut contents (ascertained from microscopic inspection of intact specimens) than those collected at night in April. Adult females of the targeted copepods containing gut contents were sorted. Images of the sorted copepods were taken by a digital camera (E5000, Nikon) attached to a stereo-microscope, and the body lengths of the copepods were measured from the images using digital imaging software (Photoshop CS4 extended, Adobe Systems). The sorted copepods were then rinsed with 0.2- $\mu\text{m}$ -filtered 1% formalin-seawater and dissected in the same medium with insect pins under a stereo-microscope. Gut contents were detached from the digestive tracts and

examined under a compound microscope. The gut contents were then suspended in 100 to 200  $\mu\text{l}$  of the same medium to loosen them, and a 1 to 1/4 aliquot of each suspended gut content sample was filtered via vacuum through 0.2  $\mu\text{m}$  pore-size Nucleopore filters (Whatman). The filters were rinsed with Milli-Q water to remove salt before being dried in a desiccator. The dried filters were affixed to brass stubs with electric conductive tape and coated with carbon using a high-vacuum evaporator (JEE-4B or JEE-420, JEOL). The carbon-coated filters were examined in a scanning electron microscope (SEM: S-4500 or S-4800; Hitachi) at an accelerating voltage of 5 kV.

### Elemental analysis of gut contents

The gut contents samples for elemental analysis were chosen after microscopic analysis. Samples with very small particle numbers were not used in the analysis. The filtered gut contents samples were analyzed using an electron probe microanalyzer (EPMA: JXA-8900; JEOL) coupled with an automated particle-recognition program (APRP: XM-87562; JEOL). An energy dispersive X-ray spectrometer (EDS) was used for the elemental analysis because it was believed to be more suitable for analyzing the filters than a wavelength-dispersive X-ray spectrometer, owing to the rough surfaces of the particles. After the gut contents were identified on the backscattered electron images, ~160 to 330 particles with diameters of 1 to 20  $\mu\text{m}$  from each specimen were analyzed for elemental contents. This size range was applied, based on the range deemed suitable for identifying the particles by APRP. The conditions for the analyses were as follows: accelerating voltage, 15 kV; beam current,  $1.5 \times 10^{-8}$  A; collection time, 20 s particle<sup>-1</sup>. The methods generated semi-quantitative data for Mg, Al, Si, P, S, K, Ca, Ti, Mn, Fe and Ba. We followed Iwamoto et al. (2009) for the experimental conditions.

### Vertical distributions

From the formalin-preserved samples collected by the MTD nets or IONESS, we sorted and counted adult females of the targeted aetideids. To describe the vertical distribution, copepod density (individuals  $\text{m}^{-3}$ ) was calculated from the volume of water filtered with the nets and the numbers of each species.



### Morphological analysis of mouthparts

The mandibles, first maxillae, second maxillae and maxillipeds of adult females of *Chirundina streetsii* and *Undeuchaeta major* were dissected from the formalin-preserved specimens under a stereo-microscope, and their morphology was examined under a compound microscope. Edge indices of mandibles were calculated according to Itoh (1970). For the maxilliped morphological analysis, adult females from 13 aetideid species reported as omnivorous, 1 aetideid species with unknown feeding habits and 10 euchaetid species were sorted from the formalin-preserved samples. Maxillipeds of the sorted specimens were dissected under a stereo-microscope and photographed under a compound microscope using a digital camera (DMC-G1, Panasonic). We referred to illustrations of maxillipeds of 3 aetideid species of unknown feeding habits: *Chiridiella kuniae* from Grice & Hulsemann (1965), and *Lutamator elegans* and *Pseudeuchaeta arctica* from Markhaseva (1996), which were chosen as suitably illustrated for the present purposes. On the basis of these photographs and illustrations, the length of the basis along the median line (LB), the maximum width of the basis (WB) and the length of the longest seta on the endopod (LS) (Fig. 3) were measured using image-processing software ImageJ (Ver. 1.33u, National Institute of Health). We calculated WB/LB and LS/LB ratios as measures of robustness and prey-capture abilities of the maxillipeds, respectively, based on the following observations: the second basipodal segment of the maxilliped of the *Euchaeta* is used to hold prey animals (Yen 1983), the maxilliped setae of the *Paraeuchaeta* are used to form a kind of rake underneath the body (see Fig. 2 on p. 553 of Wickstead 1962) and the setae of predatory copepods are very large and unguiform (Arashkevich 1969). Measure-

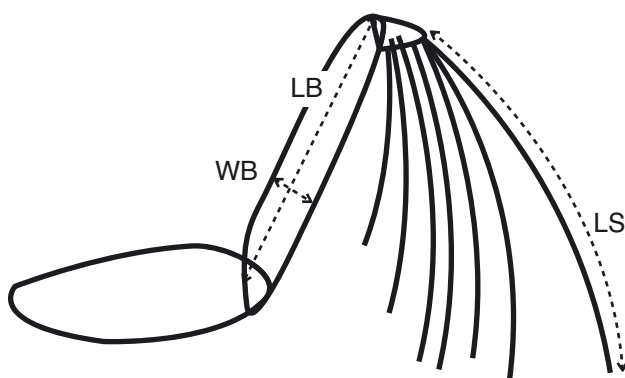


Fig. 3. Maxilliped measurements. LB: length of the basis along the median line; WB: maximum width of the basis; LS: length of the longest seta

ments of the maxillipeds were carried out for 3 individuals per species, except when the illustrations were used instead.

### Data analysis

In the stable-isotope analyses, the fractionation values per trophic level of  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  are often assumed to be 3.4‰ (Minagawa & Wada 1984, Post 2002) and 0 to 1‰ (Peterson & Fry 1987), respectively. While our knowledge is limited on the fractionation of stable isotopes in deep-sea species, lower  $\delta^{15}\text{N}$  enrichment in marine ammonotelic crustaceans has been reported by a meta-analysis of consumer-diet  $\delta^{15}\text{N}$  enrichment (Vanderklift & Ponsard 2003). Additionally, Fanelli et al. (2009) reported that a fractionation value of  $\delta^{15}\text{N}$  of 2.54‰ (Vanderklift & Ponsard 2003) is consistent with the dietary information available for some of the species analyzed in their study of mesopelagic–suprabenthic food webs. On this basis, we assumed the fractionations of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  to be 0 to 1‰ and 2.54 to 3.4‰, respectively, so as not to miss potential copepod food sources.

For the elemental analysis, particles were placed in 4 groups, Al-Si, Si-rich, Ca-rich and others, based on the relative weight percentages (wt%) of the elements (Table 2) following Iwamoto et al. (2009) and Sano et al. (2013), for the classification of particles. The ratio of each particle type was calculated based on the total area of particles on backscattered electron images. The areas were measured by the automated particle-recognition program. We compared only the ratios of Si-rich and Al-Si particles, which are assumed to be appropriate proxies of diatoms and mineral particles, respectively (Iwamoto et al. 2009), justifying application of these particles as proxies of the food items in combination with microscopic analysis. On the other hand, Ca-rich and other particles are unsuitable as such proxies, since they represent materials of highly diverse origins: the former including e.g. coccoliths (Iwamoto et al. 2009), foraminifers and pteropod shells; and the latter including mineral particles and other organic materials (Sano et al. 2013).

Table 2. Classification scheme of particle analysis

Class name	Criteria
Si-rich	Si >60 wt%
Al-Si	Al: 10–40 wt%, Si >20 wt%
Ca-rich	Ca >60 wt%
Others	Those not meeting the above criteria

The differences in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ , areas of Si-rich and Al-Si particles, and body lengths between *C. streetsii* and *U. major* in each season were tested using JMP 9 (SAS Institute). Normal distribution (Shapiro-Wilk test) and homogeneity of variances (*F*-test) of the data were first examined, followed by a Wilcoxon rank sum test for the data showing non-normal distribution. For those showing normal distribution, Welch's *t*-test was applied if variances were unequal; otherwise, Student's *t*-test was performed.

Cluster analysis, based on normalized Euclidean distances of the average WB/LB and LS/LB ratios of maxillipeds, was performed using the R statistical software (Ver. 2.10.0), to describe similarities in the morphological features of the maxillipeds of the aetideid and euchaetid species.

## RESULTS

### Chlorophyll *a* concentration

Vertically integrated chlorophyll *a* concentrations changed seasonally (Fig. 4), with higher values in the spring-bloom seasons; i.e. April 2009 ( $94.1 \text{ mg m}^{-2}$ ) and March 2010 ( $79.4 \text{ mg m}^{-2}$ ), than in June ( $34.0 \text{ mg m}^{-2}$ ), September ( $23.8 \text{ mg m}^{-2}$ ) and November ( $34.8 \text{ mg m}^{-2}$ ).

### Stable-isotope ratios

Although the  $\delta^{15}\text{N}$  values of the 2 species changed seasonally (Fig. 5), but with a fluctuation of  $<4\%$ , the values of *Chirundina streetsii* were significantly higher than those of *Undeuchaeta major* throughout the year (Student's *t*-test,  $p < 0.05$ ), except for September, when the difference could not be tested due to the low number of specimens (Table S1 in the Supplement at [www.int-res.com/articles/suppl/m518p051\\_supp.pdf](http://www.int-res.com/articles/suppl/m518p051_supp.pdf)). The  $\delta^{15}\text{N}$  values of the marine snow and oncaeid copepods also changed on a seasonal basis. The  $\delta^{15}\text{N}$  values of marine snow, a potential food source for *C.*

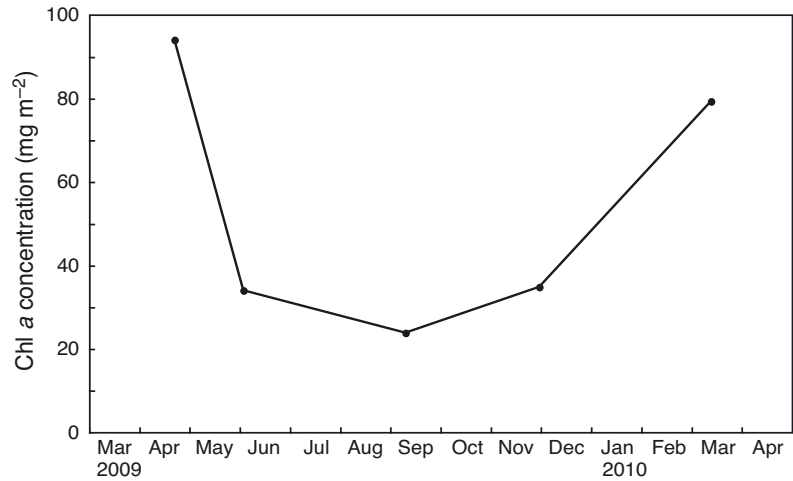


Fig. 4. Changes in the integrated chlorophyll *a* concentration at Stn P from April 2009 to March 2010. Chlorophyll *a* values were integrated above 50 m depth (above 38 m depth in June) according to the trapezoidal rule

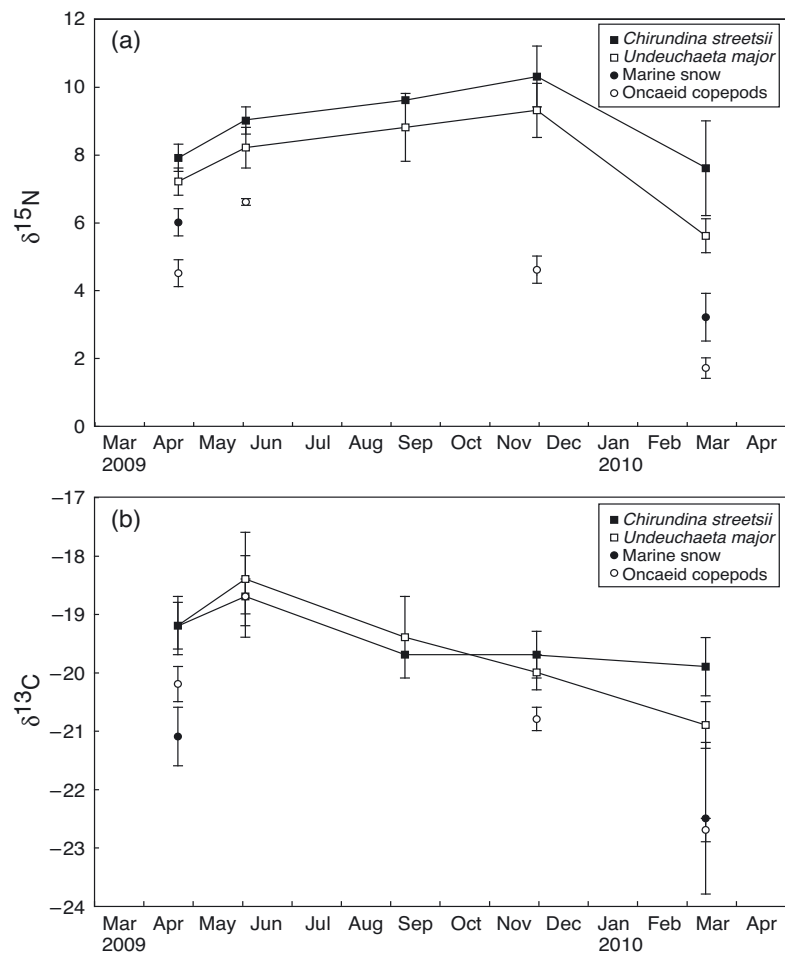


Fig. 5. Temporal changes of mean ( $\pm$ SD) (a) nitrogen and (b) carbon stable-isotope ratios of *Chirundina streetsii*, *Undeuchaeta major*, marine snow and oncaeid copepods. The lack of values for oncaeid copepods in September is due to an instrumental failure. Original data are in Table S1 in the Supplement at [www.int-res.com/articles/suppl/m518p051\\_supp.pdf](http://www.int-res.com/articles/suppl/m518p051_supp.pdf)

*streetsii*, were lower than those of *C. streetsii* by 1.9‰ in April and 4.4‰ in March. The  $\delta^{15}\text{N}$  values of the oncaeid copepods, the potential food of *U. major*, were lower than those of *U. major* throughout the year, with differences of 2.7‰ in April, 1.6‰ in June, 4.7‰ in November and 3.9‰ in March.

The  $\delta^{13}\text{C}$  values of the 2 species also changed seasonally (1.2 and 2.5‰, respectively), but there was no significant difference between the values of these species (Student's *t*-test,  $p > 0.05$ ) for each month, except for September and March (Student's *t*-test,  $p < 0.05$ ). The  $\delta^{13}\text{C}$  values of marine snow were lower than those of *C. streetsii* by 1.9‰ in April and 2.6‰ in March. The  $\delta^{13}\text{C}$  values of the oncaeids were lower than those of *U. major* throughout the year, with differences of 1‰ in April, 0.3‰ in June, 0.8‰ in November and 1.8‰ in March.

### Gut contents analysis

Table 3 lists the gut contents of *C. streetsii* and *U. major*, with values of food items shown as percentages of copepods containing each food to all examined copepods (note that in September, the sample size was only 3 for both species, due to the low number of individuals with gut contents). There was no marked change in the occurrence of each item in *C. streetsii*, except in September. Diatom fragments, coccoliths and silicoflagellates were found in nearly all individuals of *C. streetsii*. Among the items of zooplankton origin, crustacean fragments were found in >50% of *C. streetsii*. Nematocysts, foraminifers, radi-

olarian fragments and tintinnids were also ingested by *C. streetsii*.

In *U. major*, diatoms were found in >70% of the specimens with gut contents in April and November, but in <60% in June, September and March. The other phytoplankton-origin items were found in only a few individuals of *U. major* throughout the year. Among the items of zooplankton origin, crustacean fragments were ingested by all but 8% of *U. major* individuals in November. Oncaeid copepod fragments were found in all seasons, and corycaeid copepod fragments were found in April and March.

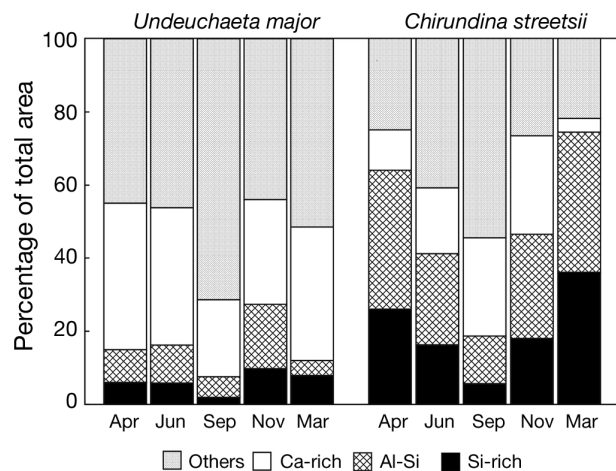


Fig. 6. Temporal pattern of mean percentages of total area of each particle type in the gut contents of *Chirundina streetsii* and *Undeuchaeta major*. Original data are in Table S2 in the Supplement at [www.int-res.com/articles/suppl/m518p051\\_supp.pdf](http://www.int-res.com/articles/suppl/m518p051_supp.pdf)

Table 3. Gut contents and body lengths of *Chirundina streetsii* and *Undeuchaeta major* in each season. Body length values denote means  $\pm$  standard deviation. Values of food items denote percentages of specimens containing each food. n: number of specimens; DF: diatom fragments; CO: coccoliths; SF: silicoflagellate fragments; DI: dinoflagellates; CF: crustacean fragments; CR: corycaeid fragments; OF: oncaeid fragments; NE: nematocysts; FO: foraminifers; RF: radiolarian fragments; TI: tintinnids. Corycaeid fragments and oncaeid fragments were counted as not only 'corycaeid fragments' or 'oncaeid fragments' but also 'crustacean fragments'

Season	n	Body length (mm)	Phytoplankton origin				Zooplankton origin						
			DF	CO	SF	DI	CF	CR	OF	NE	FO	RF	TI
<b><i>Chirundina streetsii</i></b>													
Apr	10	5.0 $\pm$ 0.2	100	70	80	0	100	0	0	20	10	60	30
Jun	13	4.9 $\pm$ 0.2	92	46	8	0	69	0	0	8	0	8	23
Sep	3	4.9 $\pm$ 0.2	67	0	0	0	100	0	0	0	0	0	0
Nov	12	5.2 $\pm$ 0.2	100	83	67	8	92	0	0	25	0	8	8
Mar	12	5.0 $\pm$ 0.2	100	33	17	0	67	0	0	33	0	17	0
<b><i>Undeuchaeta major</i></b>													
Apr	21	5.0 $\pm$ 0.2	71	38	10	0	100	24	48	0	0	0	0
Jun	12	4.9 $\pm$ 0.1	58	8	8	0	100	0	17	8	0	0	8
Sep	3	4.9 $\pm$ 0.2	0	0	0	0	100	0	33	0	0	0	0
Nov	12	5.0 $\pm$ 0.2	100	8	25	8	92	0	33	0	0	0	0
Mar	11	4.9 $\pm$ 0.1	45	0	0	0	100	27	18	9	0	0	0



### Elemental analysis

The ratios of Si-rich particles in the gut contents of *C. streetsii* were significantly higher than those in *U. major* (April and June, Student's *t*-test,  $p < 0.05$ ; November and March, Wilcoxon rank sum test,  $p < 0.05$ ), except in September (Student's *t*-test,  $p > 0.05$ ), when the difference was not significant (Fig. 6), but the mean value remained higher in *C. streetsii* than in *U. major*. Although the ratio of Si-rich particles in *U. major* did not change significantly throughout the year, it decreased in *C. streetsii* from April to September, and increased from September to March. The ratios of Al-Si particles in *C. streetsii* were significantly higher than those in *U. major* in April, June and March (April and June, Wilcoxon rank sum test,  $p < 0.05$ ; September, Wilcoxon rank sum test,  $p > 0.05$ ; November, Welch's *t*-test,  $p > 0.05$ ; March, Student's *t*-test,  $p < 0.05$ ).

### Vertical distribution

In the daytime, the 2 species were distributed mostly within the mesopelagic zone from ~200 to 1000 m, with the exception of an occurrence at epipelagic depths in April (Fig. 7). The distribution peaked at comparable depths, and it changed slightly according to the season: 400 m in April and March, 300 to 400 m in June, 500 to 800 m in September and 500 m in November. The nighttime sampling conducted in April and March indicated an up-shift of the main populations of both species to shallower depths, including the epipelagic zone (>200 m).

### Morphological analysis

The body lengths of *C. streetsii* and *U. major* were ~5 mm, with no marked changes throughout the year (Table 3), and no significant differences between the species, except in November (April and September, Wilcoxon rank sum test,  $p > 0.05$ ; June and March, Student's *t*-test,  $p > 0.05$ ; November, Welch's *t*-test,  $p < 0.05$ ).

The shapes of the mandible teeth of both species were similar, with edge indices of 560 in *C. streetsii* and 507 in *U. major* (Fig. 8a,e). The shapes of the other mouthpart appendages were also largely similar in both species, but showed the following differences: the setae on the epipodite of the first maxilla of *C. streetsii* were slightly longer than those of *U. major* (Fig. 8b,f), the basis of the maxilliped of

*U. major* was thicker, and the setae on the endpods were unguiform and longer than those of *C. streetsii* (Fig. 8d,h).

The WB/LB and LS/LB ratios of all species examined showed different trends according to their feeding habits (Fig. 9). The euchaetid copepods (E1 to E10) showed higher WB/LB (0.28 to 0.36) and LS/LB ratios (1.37 to 1.61) than the omnivorous aetideid copepods (A5 to A13; WB/LB, 0.16 to 0.27; LS/LB, 1.03 to 1.29), except the *Gaetanus* spp. (A1 to A3), which showed high LS/LB (1.51 to 1.62) and low WB/LB ratios (0.18 to 0.21). *U. major* (A4), an aetideid species considered as carnivorous, showed high LS/LB ratios (1.50) and relatively high WB/LB ratios (0.25), which are close to the ratios of the euchaetid copepods. *C. streetsii* (A7), aetideid copepods considered as omnivorous, showed low LS/LB (1.20) and WB/LB ratios (0.20) similar to those of the omnivorous aetideid copepods. The WB/LB and LS/LB ratios of the aetideid species of unknown feeding habits (a1 to a4) deviated into 2 groups, one similar to the euchaetid copepods (*Undeuchaeta plumosa*, *Lutamator elegans*, *Pseudeuchaeta arctica*) and the other similar to the omnivorous aetideid copepod *Chiridiella kuniae*. A cluster analysis based on the mean values of the above ratios identified 3 groups: Group 1 consisted mainly of omnivorous copepods; Group 2 consisted of *Gaetanus* species; and Group 3 consisted mainly of euchaetid copepods (Fig. 10). *C. streetsii* and *C. kuniae* were included in Group 1, while *U. major*, *U. plumosa*, *L. elegans* and *P. arctica* were in Group 3.

## DISCUSSION

### Feeding habits of *Chirundina streetsii* and *Undeuchaeta major*

Our analysis demonstrated the carnivorous feeding habits of *Undeuchaeta major*, which feed especially on oncaeids throughout the year, from the following observations: (1) the occurrence of crustacean fragments, including those of oncaeids throughout all months; (2) the  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values of the oncaeids being close to the values of the potential food sources, except for November and March, respectively; and (3) the lower abundance of Si-rich particles compared to *Chirundina streetsii*. The mismatch between gut contents and stable isotope values on both sampling occasions is possibly attributable to feeding on crustaceans at higher trophic levels, which could not be identified on the basis of the frag-

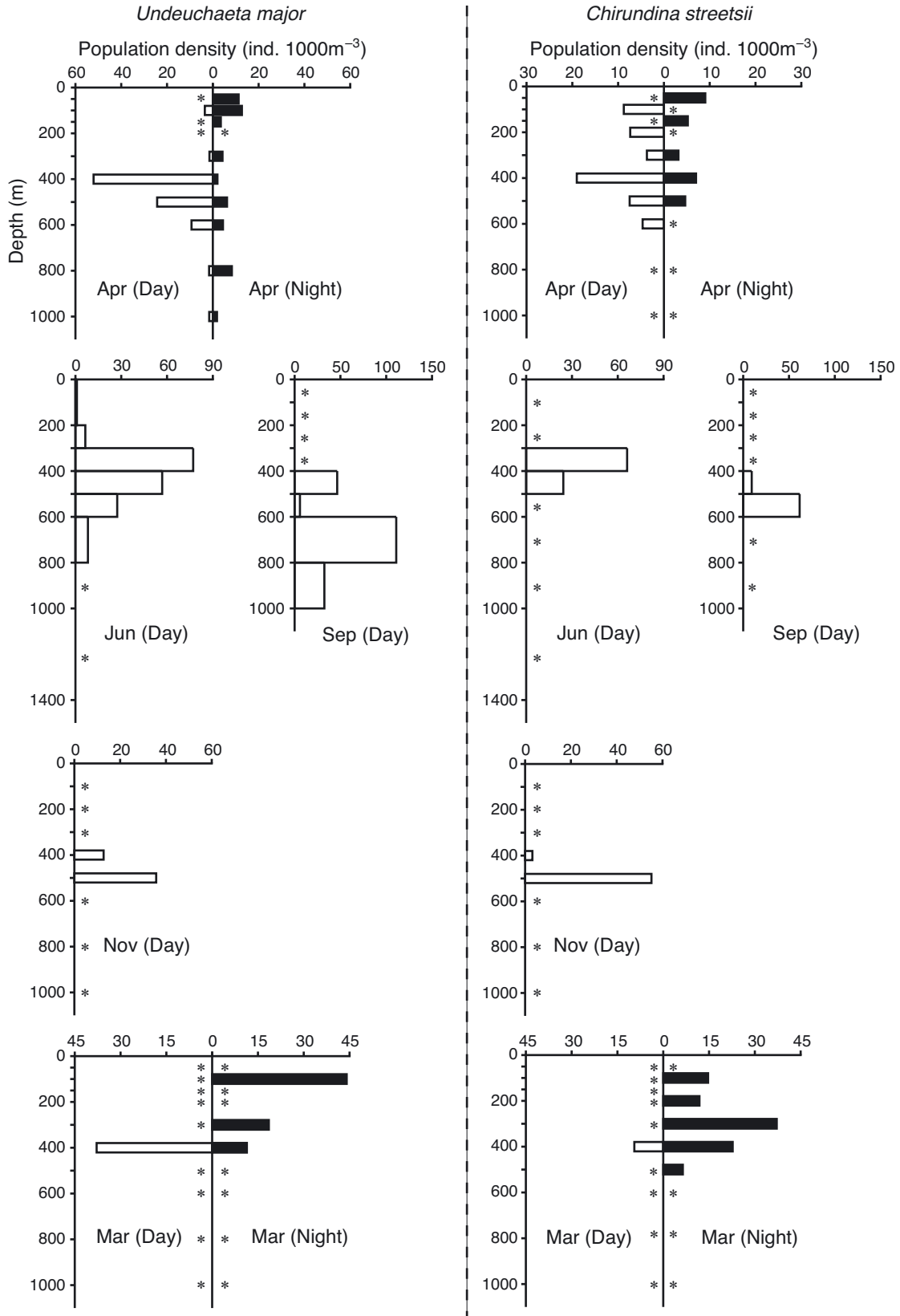


Fig. 7. Vertical distributions of *Chirundina streetsii* and *Undeuchaeta major*. The asterisks indicate collected depths with no specimens

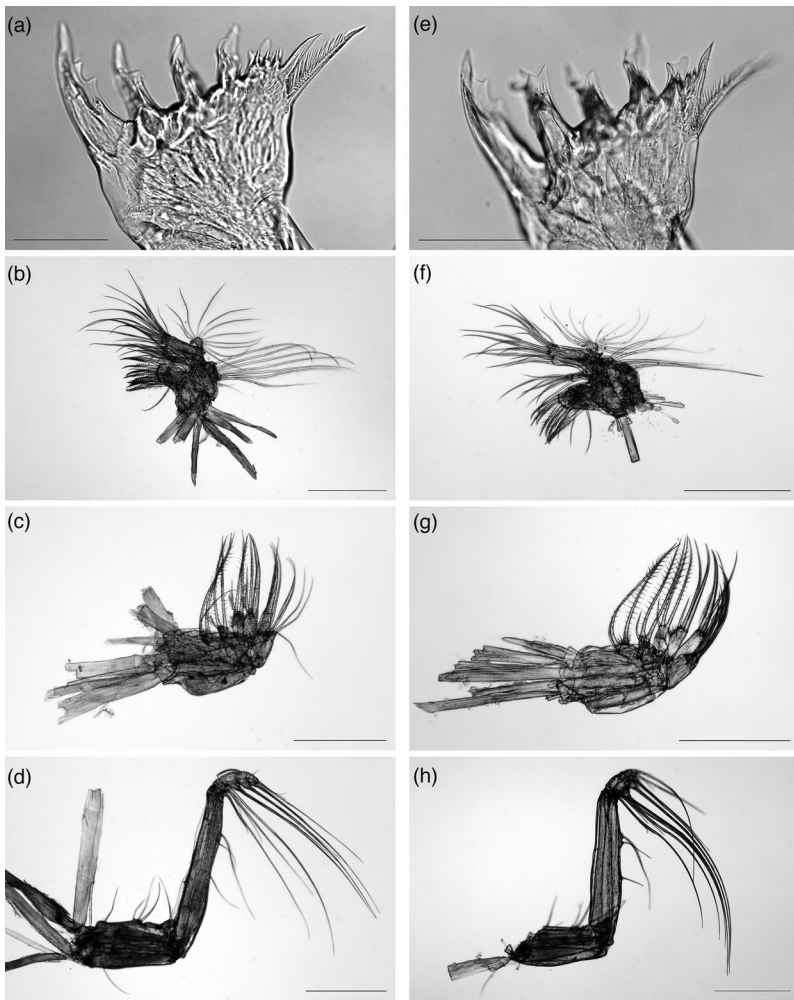


Fig. 8. Feeding appendages of (a–d) *Chirundina streetsii* and (e–h) *Undeuchaeta major*: (a,e) mandible, (b,f) first maxilla, (c,g) second maxilla and (d,h) maxilliped. Scale bars = (a,e) 100  $\mu\text{m}$ ; (b–d, f–h) 500  $\mu\text{m}$

ments alone. As Si-rich particles originate mainly from diatoms (Iwamoto et al. 2009), the contribution throughout the year of diatoms to the diet of *U. major* is minor, and this agrees with the results obtained from the microscopic analysis. The small amount of diatoms found in *U. major* may be derived from the gut contents of ingested zooplankton, such as oncaeid copepods (Turner 1986).

The composition of gut contents of *C. streetsii* changed seasonally. Although the gut contents included various items which were similar all year-round, the ratios of Si-rich particles in *C. streetsii* decreased from April to September and increased from September to March, which agrees with the seasonal change of vertically integrated chlorophyll *a* concentration. Because Al-Si particles were considered to be of geological origin (Iwamoto et al.

2009) and represent mineral particles, they might not have been actively eaten by copepods, and therefore the ingestion of Al-Si particles indicates non-selective feeding by copepods. Ratios of Al-Si particles in *C. streetsii* were significantly higher than those in *U. major* in April, June and September, but not in November and March, which suggests that *C. streetsii* ingested food containing Al-Si particles, such as marine snow, on a more non-selective basis than *U. major*. The differences in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values between *C. streetsii* and marine snow deviated slightly from the fractionation values per trophic level, suggesting that *C. streetsii* ingested not only marine snow but also other food. These results indicate that *C. streetsii* is a highly versatile omnivore that feeds mainly on marine snow throughout the year.

The present observations on the differences in gut contents, stable-isotope ratios and maxilliped morphology (see the penultimate subsection of the 'Discussion') between the 2 species suggest that *U. major* ingested active/live crustaceans, including *Oncaea* and *Corycaeus*, while *C. streetsii* ingested immotile crustacean fragments that are contained in marine snow. The contribution of digestible items, such as ciliates and phytoplankton without hard exoskeletons or with

indigestible cell walls, are difficult to evaluate by the methods applied in the present study. However, the targeted copepod species do not have fine setules, similar to those in epipelagic particle feeders, in their mouthparts (Fig. 8 and Sano et al. 2013). Accordingly, we assumed that these species cannot directly ingest such small, digestible particles. The other possibility may be indirect ingestion of these small, digestible particles as constituents of marine snow, which cannot be detected by the present analysis either. However, this should not be a cause of bias in the present analysis, since our focus was on the feeding modes of the copepods (carnivory or marine-snow feeding) regardless of the presence of digestible phytoplankton in the ingested particles, and marine-snow feeding can be sufficiently evaluated by the occurrence of other indigestible items.

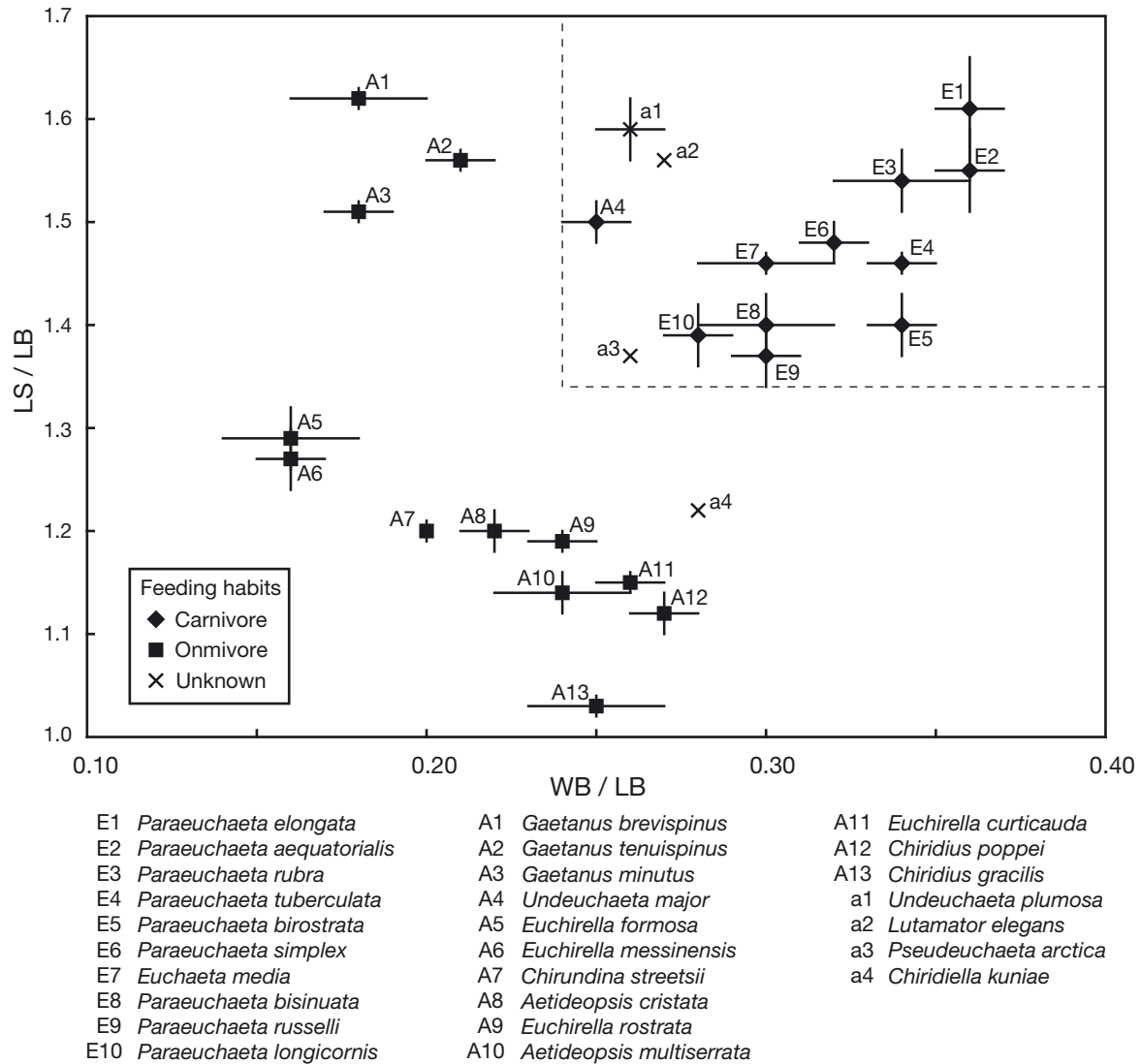


Fig. 9. WB/LB and LS/LB ratios of maxillipeds in aetideid and euchaetid copepods. Error bars indicate standard deviation. E1–10: euchaetid copepods; A1–13: aetideid copepods; a1–4: aetideid copepods whose feeding habits were unidentified. The feeding habits of the copepods were based on Mauchline (1998). Dotted lines indicate LS/LB ratios of 1.34 and WB/LB ratios of 0.24. LB: length of the basis along the median line; WB: maximum width of the basis; LS: length of the longest seta

### $\delta^{15}\text{N}$ and copepod food

In this study, the  $\delta^{15}\text{N}$  of the carnivorous *U. major* was lower than that of the omnivorous *C. streetsii* throughout the year, which seems at odds with the general rule that  $\delta^{15}\text{N}$  is higher at higher trophic levels. This may be due to the  $\delta^{15}\text{N}$  of their specific food: oncaeid copepods and marine snow, the former being lower in  $\delta^{15}\text{N}$  than the latter. The  $\delta^{15}\text{N}$  values of oncaeid copepods were close to or lower than those of herbivorous zooplankton all year-round (Aberle et al. 2010), and were almost identical to those of suspended particles (Sano et al. 2013). These paradoxical observations may be reasonably interpreted by

focusing on the particle size; oncaeid copepods have been reported to be associated with appendicularian houses (Alldredge 1972, Ohtsuka et al. 1993, Steinberg et al. 1994) and feed on the houses (Ohtsuka et al. 1996). A high abundance of autotrophic nanoplankton attached on the discarded larvacean houses have been reported (Davoll & Silver 1986), including effective utilization of this nanoplankton by oncaeid copepods (Alldredge 1972, Ohtsuka et al. 1996). Feeding on small particles (e.g. Turner 1986, Metz 1998) and large diatoms (Turner 1986) other than those with larvacean houses has also been reported. Altabet (1988) reported  $\delta^{15}\text{N}$  of particulate nitrogen in  $<3\ \mu\text{m}$  fractions was 2‰ lower than that in  $>3\ \mu\text{m}$

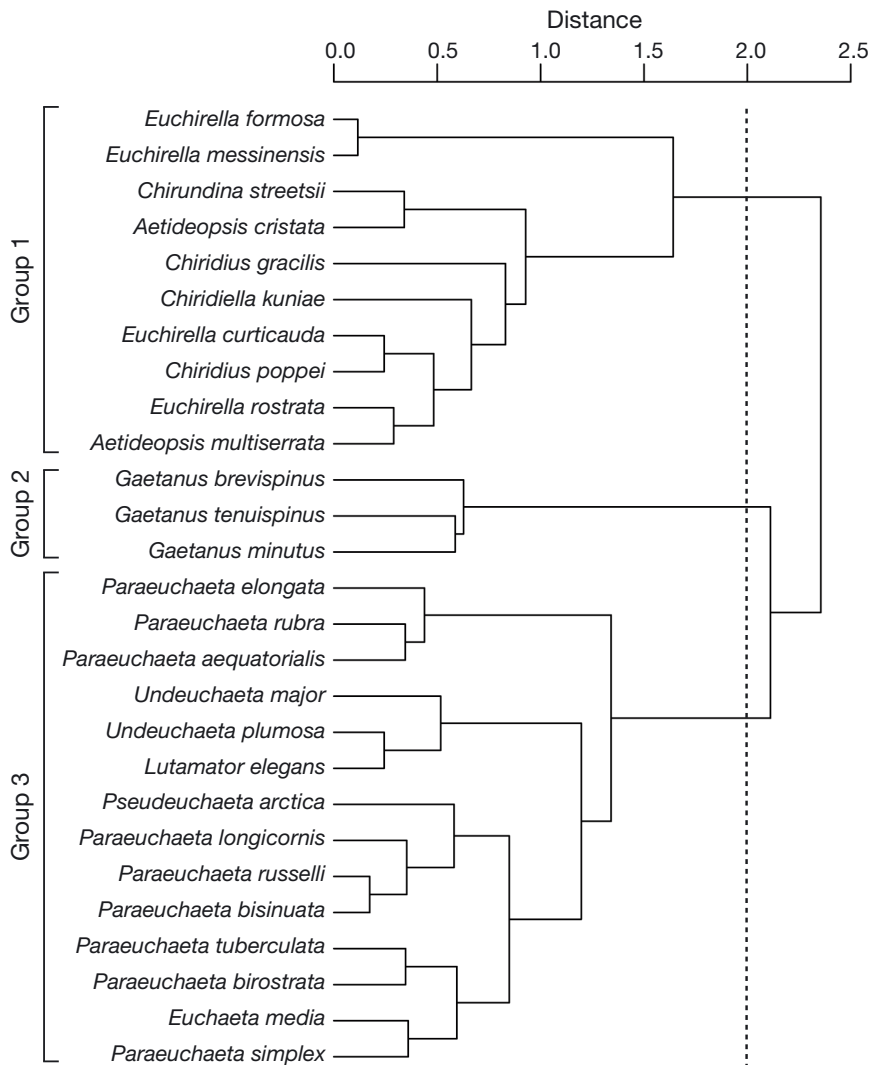


Fig. 10. Cluster analysis (normalized Euclidean distance and group average method) using mean values of WB/LB and LS/LB ratios of maxillipeds

factions in the Sargasso Sea. Rau et al. (1990) reported that the  $\delta^{15}\text{N}$  of particulate organic matter (POM) of  $<8\ \mu\text{m}$  was lower than that of  $>8\ \mu\text{m}$  POM in the Mediterranean Sea. Karsh et al. (2003) reported that the  $\delta^{15}\text{N}$  of small particles (1 to 5  $\mu\text{m}$ ) was  $\sim 3\%$  lower than that of large particles ( $>200\ \mu\text{m}$ ). Kang et al. (2009) reported that the  $\delta^{15}\text{N}$  of fine POM ( $<20\ \mu\text{m}$ ) was  $2.1 \pm 0.9\%$  (mean  $\pm$  standard deviation) lower than that of coarse POM (20 to 180  $\mu\text{m}$ ) throughout the year in the coastal waters of Korea. Karsh et al. (2003, and references therein) suggested that the cause of the lower values of the smaller POM could be differences in phytoplankton biomass among size fractions, species-specific  $^{15}\text{N}$  fractionation accompanying nitrate uptake, and differences in assimilation of different nitrogen forms, such as

ammonium and urea, between nano-plankton and microplankton. If oncaeid copepods ingested large diatoms, the  $\delta^{15}\text{N}$  of oncaeid copepods would have been larger than that of POM. Thus, oncaeid copepods are considered not to have ingested large particles, at least in April, in Sagami Bay. Meanwhile, the  $\delta^{15}\text{N}$  of the appendicularian houses themselves may not greatly affect the  $\delta^{15}\text{N}$  of house consumers because of the relatively low nitrogen content of newly secreted houses compared with discarded houses (see Table 2 in Alldredge 1976). Thus, the most plausible explanation for the low  $\delta^{15}\text{N}$  values of oncaeid copepods would be the consumption of nanoplankton with low  $\delta^{15}\text{N}$  values as a result of feeding on appendicularian houses or direct feeding on small particles. This suggests that the food web in epi- and mesopelagic zones contains at least 2 major pathways of organic matter: one based on large POM (e.g. diatoms) and the other on small POM (e.g. nanoplankton). Therefore, an evaluation of the role of oncaeid copepods in the food web may be important for understanding the carbon flux, and sizes of primary producers should be taken into consideration in estimating pelagic food webs by  $\delta^{15}\text{N}$ . However, data on  $\delta^{15}\text{N}$  values of nano- and picoplankton are still very limited and, as far as we

know, nothing has been reported on the  $\delta^{15}\text{N}$  of natural appendicularian houses. Further studies on  $\delta^{15}\text{N}$  of size-fractionated POM, appendicularian houses and oncaeid copepods are needed.

### Niche partitioning

The present study demonstrated that the difference in feeding habits between the 2 aetideid species, which had similar body shapes, sizes and vertical distributions, was sustained throughout the year, indicating that food-niche partitioning is a major mechanism for their co-existence. We assume that the 2 targeted species are among the most competitive aetideids in Sagami Bay for the following rea-



sons: (1) *C. streetsii* is the only species of the genus *Chirundina* reported to date in Sagami Bay. (2) *Undeuchaeta plumosa*, a suspected congeneric competitor of *U. major*, which is more abundant but distributed at similar depths to *U. major* throughout the year (M. Sano unpubl. data), is much smaller than *U. major* (ratio of total length: *U. major/U. plumosa* is ~1.3; M. Sano unpubl. data). A ratio of 1.3 is the minimum size ratio for co-existing congeners through food-niche partitioning, as suggested by Hutchinson (1959). (3) *U. major* and *C. streetsii* are similar in body size and general body shape. Tanaka (1957) suggested the genus *Undeuchaeta* is closely allied to the genus *Chirundina*. These genera are distinguished by the number of outer margin spines on the exopod of leg 1 (*Chirundina*: 3, *Undeuchaeta*: 2) and symmetrical (*Chirundina*) or asymmetrical (*Undeuchaeta*) genital segments with small projections or spines on the right-hand side. Food-niche partitioning of other aetideid species has been previously reported by Laakmann et al. (2009), who proposed the differences in food composition on the basis of fatty-acid analysis between the genera *Gaetanus*, *Chiridius* and *Aetideopsis*, which were distributed at the same depth. Our results show that these differences in feeding habits between the sympatric aetideid species were sustained in Sagami Bay throughout the year. To date, limited year-round feeding analyses of deep-sea copepods have been carried out. Schnetzer & Steinberg (2002) reported that the gut contents of *Pleuromamma xiphias* and *Euchirella messinensis* changed seasonally, but their feeding selectivity did not change. Our results suggest that the differences in feeding habits between more taxonomically related and morphologically similar species are also sustained throughout the year.

#### Feeding habits and mouthpart morphology of the Aetideidae and Euchaetidae

There was no clear difference in the morphological features of the mouthparts between *C. streetsii* and *U. major*, except for the maxillipeds. The maxillipeds of *U. major* were similar to those of other carnivorous copepods, with unguiform setae (Arashkevich 1969). The reason the morphological differences appear only in the maxillipeds may be because the aetideid copepods use them for seizing and holding prey (e.g. von Vaupel Klein 1982, von Vaupel Klein & Koomen 1994 and references therein) and the carnivorous euchaetid species, a sister family of the Aetideidae, use the second basipodal segment of the maxilliped

to hold prey animals (Yen 1983). Sufficient muscles and long, unguiform setae are needed to capture prey animals. The long setae of the maxillipeds of the euchaetid species and *U. major* enable them to form a net underneath the body, as shown by Wickstead (1962) (see Fig. 2 in Wickstead 1962). Therefore, carnivorous euchaetid species have long setae and a thick basis for prey capture, while the omnivorous aetideid species do not (Fig. 11a,b). The 3 *Gaetanus* species have long setae, as shown by the high LS/LB ratios, but the setae are straight and thin (Fig. 11c). The basis of the maxillipeds of these species were slim, as indicated by low WB/LB ratios, suggesting that they do not have as much muscle as the euchaetids. Therefore, the long setae of the *Gaetanus* spp. may not be used for carnivorous feeding,

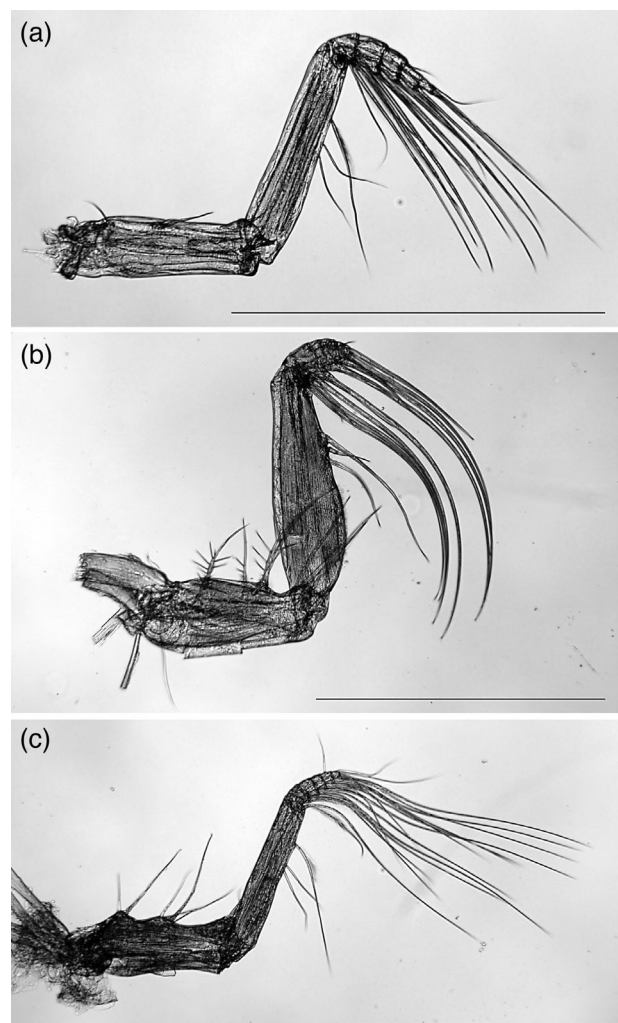


Fig. 11. Maxillipeds of (a,c) omnivorous aetideid copepods and (b) carnivorous euchaetid copepod: (a) *Aetideopsis cristata*, (b) *Euchaeta media*, (c) *Gaetanus brevispinus*. Scale bar = 1 mm

rather for feeding on other, less active/inactive items such as marine snow. *Gaetanus* spp. are reported as being omnivorous or particle feeders (e.g. Yamaguchi et al. 2007, Laakmann et al. 2009). Thus, species with LS/LB ratios above 1.34 and WB/LB ratios above 0.24 are considered carnivorous (Fig. 9). On the basis of this criterion, *U. plumosa*, *L. elegans* and *P. arctica* are considered carnivores and *C. kuniae* is considered an omnivore. Those assumptions correspond with the results of cluster analysis (Fig. 10).

Aetideid copepods have been considered omnivores based on gut contents, as well as morphological and chemical analyses (e.g. Arashkevich 1969, Hopkins 1985, Laakmann et al. 2009). This study suggested that *U. major*, and some other aetideid copepods, are carnivorous, on the basis of the morphological features of the maxillipeds. Euchaetidae and Aetideidae are considered sister families on the basis of their morphological characteristics (Park 1994) and molecular genetic analyses (Blanco-Bercial et al. 2011, Laakmann et al. 2012). Boxshall & Halsey (2004) suggested that Euchaetidae may be a derived lineage within the Aetideidae. The presence of carnivorous aetideid species and the morphological features of the maxillipeds of the Aetideidae and Euchaetidae suggest that the Euchaetidae possibly derived from the Aetideidae through a shift in feeding habits. Ohtsuka et al. (1997) showed an outburst of species diversity in the Heterorhabdidae that was associated with the acquisition of novel feeding tactics. An integrative analysis applying the molecular phylogeny of the families and the molecular genetic identification of gut contents, along with the analytical techniques applied here, would provide a better understanding of the evolutionary changes in feeding habits of the Aetideidae and Euchaetidae.

### Summarizing conclusions

The present study demonstrated that differences in feeding habits between the taxonomically related and morphologically similar species, which had similar sizes and vertical distributions, were sustained throughout the year, suggesting that food-niche partitioning is a major mechanism for their co-existence. The  $\delta^{15}\text{N}$  analysis also indicated a close link between food-particle sizes and food pathways, suggesting that the mesopelagic food web, rather than being simply dependent on marine snow, may be more complex than previously thought. These observations invite further studies on the seasonal patterns of feeding habits and food-niche partitioning among

other mesopelagic copepods and estimations of the structure of the mesopelagic food web.

The present study also provides a morphological index for estimating the feeding habits of aetideid copepods, which were considered to consist not only of omnivores but also carnivores. Aetideidae is one of the major families in the mesopelagic zone; hence, these results may provide new information about consumers in the mesopelagic zone, which should facilitate a more accurate estimation of sinking-flux consumption by copepods. Further research on the phylogeny of Aetideidae and Euchaetidae, a sister family of Aetideidae, will provide a better understanding of the evolution and feeding strategy of copepods in the mesopelagic zone.

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