Vol. 333: 205–212, 2007 | MARINE ECOLOGY PROGRESS SERIES
Mar Ecol Prog Ser | Published March 12

Association of the copepod *Macrosetella gracilis* with the cyanobacterium *Trichodesmium* spp. in the North Pacific Gyre

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ABSTRACT: The harpacticoid copepod *Macrosetella gracilis* is found in pelagic habitats in tropical and subtropical oceans associated with colonies of the N_2 fixing cyanobacterium *Trichodesmium* spp. In the central North Pacific near Hawaii, *M. gracilis* was abundant (1.8 ± 1.4 [SD] *M. gracilis* adults m^{-3} and 4.7 ± 3.9 *M. gracilis* copepodites m^{-3}) and constituted an average of 10.8% of the total copepod population. However, we observed no statistically discernable correlation between *M. gracilis* and *Trichodesmium* spp. abundances, suggesting that availability of *Trichodesmium* spp. did not limit the abundance of *M. gracilis* during our study. In previous laboratory studies *M. gracilis* had been shown to have the ability to ingest *Trichodesmium* spp. trichomes and appeared immune to cyanobacterial toxins harmful to other species of copepods. Natural abundance of stable isotopes ($\delta^{15}N$, $\delta^{13}C$) in copepod tissue from field samples suggested that the diet of *M. gracilis* was not predominately composed of *Trichodesmium* spp. as proposed by previous research. Natural abundance of $\delta^{15}N$ was similar for *M. gracilis* (3.06 ± 2.29), *Miracia efferata* (1.83 ± 0.88), and calanoid copepods (2.7 ± 1.95). No *Trichodesmium* spp. were observed in *M. gracilis* gut contents. *Trichodesmium* spp. was not a predominant food in the diet of this copepod, but colonies of the toxic cyanobacterium could provide shelter from predation and be used as a floating substrate for adult and juveniles of *M. gracilis*.

KEY WORDS: *Macrosetella gracilis* · *Trichodesmium* · Copepod feeding · Stable isotopes · Floating substrate · Zooplankton · Oligotrophic oceans · Life history

INTRODUCTION

The scarcity of buoyant substrates in the open ocean prevents many species with poor swimming ability from living in pelagic habitats unless they can become part of a multi-species association that serves as a float and habitat. The nitrogen-fixing cyanobacterium *Trichodesmium* spp. is an important species that can provide a floating habitat in the open ocean (Sheridan et al. 2002). *Trichodesmium* spp. is one of the most abundant primary producers in oligotrophic tropical and subtropical oceans and can provide an important nitrogen source for higher trophic levels (Letelier & Karl 1996, Capone et al. 1997, Carpenter et al. 1999, Post et al. 2002). *Macrosetella gracilis* is a harpacticoid copepod that occurs globally in tropical and subtropical oceans, and is found in association with blooms of *Trichodesmium* spp. (Huys & Böttger-Schnack 1994). Harpacticoid copepods are predominately benthic, but *M. gracilis* and other copepods in the family Miraciidae are some of the few species that have evolved a pelagic life style (Huys & Böttger-Schnack 1994). *M. gracilis* is a poor swimmer (Hwang & Turner 1995) and uses *Trichodesmium* spp. colonies as a float, as a nursery ground for its nauplii, and as a potential food source (Huys & Böttger-Schnack 1994, O'Neil 1998).

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While an ecological link between *Macrosetella gracilis* and *Trichodesmium* spp. is apparent, few data exist concerning the abundance, life-history, and feeding habits of *M. gracilis* in its natural habitat. The relative abundance of *M. gracilis* among tropical copepods is poorly known, although Böttger-Schnack & Schnack (1989) estimated that *M. gracilis* comprises 1 to 3% of adult tropical copepods in the Red Sea. Calef & Grice (1966) showed that *M. gracilis* abundance in the western tropical Atlantic closely tracked *Trichodesmium* spp. abundance, and they found no *M. gracilis* in the absence of *Trichodesmium* spp. A positive correlation between *Trichodesmium* spp. and *M. gracilis* abundances was also found in the Red Sea (Calef & Grice 1966, Böttger-Schnack & Schnack 1989, Böttger-Schnack 1991). However, no abundance data appears to be available for the North Pacific Gyre. This study used quantitative stratified sampling to determine the abundance of *M. gracilis* near Hawaii and to determine how this copepod species is distributed in relation to *Trichodesmium* abundance.

The diazotroph *Trichodesmium* spp. provides a nitrogen source that is not readily accessible to generalized grazers in oligotrophic waters due to the production of neurotoxins that may lead to grazer avoidance (Hawser et al. 1991, Cox et al. 2005). Hawser et al. (1992) demonstrated that *Trichodesmium thiebautii* was toxic to all copepods tested except *Macrosetella gracilis* and *Miracia efferata.* It has been shown in laboratory experiments that *M. gracilis* can feed on *Trichodesmium* spp. (Roman 1978, O'Neil & Roman 1994, O'Neil et al 1996). The mechanism *M. gracilis* employs to protect itself from the effects of the cyanobacterial toxin is not known, nor is the extent to which *M. gracilis* feeds on *Trichodesmium* spp. in the wild. It is unclear, whether *M. gracilis* feeds on other species of pelagic phytoplankton, but results from laboratory studies by O'Neil et al. (1996) suggest that it does not.

The present study investigated the natural diet of *Macrosetella gracilis* by using 2 methods: gut content analysis and natural abundance of stable isotopes in copepod tissue. Autofluorescence of photosynthetic pigments can be used for identification of gut contents with epifluorescence microscopy. Studies of naturally occurring nitrogen and carbon stable isotopes have been successfully used in ecological studies to investigate trophic relationships in aquatic environments (DeNirot & Epstein 1981, Lajtha & Michener 1994, Michener & Schell 1994, Cabana & Rasmussen 1996, Jennings & Warr 2003). Organisms that can fix molecular nitrogen will have characteristically lower $\delta^{15}N$ ratios than those that must assimilate other forms of inorganic nitrogen such as ammonium or nitrate (DeNirot & Epstein 1981). It has been shown that the diazotroph *Trichodesmium* spp. has the lowest $\delta^{15}N$ and highest $\delta^{13}C$ values of any phytoplankter reported to date (Minagawa & Wada 1986, Carpenter et al. 1997). Grazing animals show an average enrichment per trophic level of around 3.5‰ for nitrogen and 1‰ for carbon (Peterson & Fry 1987, Michener & Schell 1994). Thus, one would expect *M. gracilis* and *Miracia efferata*, as grazers on *Trichodesmium* spp., to have distinctly lower $\delta^{15}N$ and higher $\delta^{13}C$ in their tissues than calanoid copepods that do not feed on *Trichodesmium* spp.

The goals of this study were to: (1) obtain quantitative data on the distribution of *Macrosetella gracilis* and other zooplankton in the North Pacific Gyre and discern how they correlate with *Trichodesmium* spp. distribution; and (2) determine the natural diet of *M. gracilis* through gut content analysis and natural abundance of stable isotopes in copepod tissue.

MATERIALS AND METHODS

Sample collection and enumeration. Zooplankton samples were collected near the Hawaiian Islands (18° 30.01′ N to 21° 00.78′ N and 154° 49.41′ W to 162° 13.06′ W) with a 0.5 m mouth diameter multiple

Table 1. Sampling design for *Macrosetella gracilis* and *Trichodesmium* spp. during Cruise MPO9, with station number, sampling dates, station coordinates, sea-surface temperature (SST), salinity (Sal), MOCNESS sampling for zooplankton collection and Niskin bottle sampling for *Trichodesmium* spp. abundance (MOC), natural isotope data of copepods (I) and number of *M. gracilis* sampled for gut content analysis (G)

Stn	Date (2003)	Latitude (N)	Longitude (W)	SST (°C)	Sal	MOC	I	G
14	8 Aug	18° 30.01′	157° 00.00′	26.85	34.77	Yes	No	–
16	10 Aug	19° 29.59′	157° 00.01′	26.50	34.91	Yes	Yes	6
17	12 Aug	19° 32.14′	158° 58.75′	27.36	35.05	Yes	Yes	3
18	14 Aug	20° 32.11′	160° 59.87′	27.38	35.02	Yes	Yes	5
19	15 Aug	21° 00.78′	158° 59.70′	27.07	34.91	Yes	Yes	–
21	18 Aug	20° 15.18′	159° 11.32′	27.39	35.11	No	Yes	–
22	20 Aug	19° 06.26′	162° 13.62′	27.35	35.02	No	Yes	4
23	21 Aug	20° 30.04′	161° 30.02′	27.58	34.97	No	Yes	–

opening/closing net and environmental sensing system (MOCNESS), fitted with 7 nets (64 µm mesh), and towed at 1 knot for 10 to 20 min (Table 1, Fig. 1). Samples were taken between 0 and 150 m at approximately 20 m depth intervals. Samples were preserved in 4% buffered formaldehyde, and the total sample volume was counted under a dissecting microscope.

Water samples for *Trichodesmium* spp. abundance were collected using 10 l Niskin bottles mounted on a CTD rosette system. Samples were collected at the same stations and depths within a maximum of 3 h of the MOCNESS tow. Concentrations of free trichomes and trichomes in colonies of *Trichodesmium* spp. for each sample were determined following gravity filtration methods described by Carpenter et al. (2003). This method allowed minimal disturbance of colonies and permitted counting of trichomes in both colonial and free states. Trichomes were enumerated on board within 24 h of collection at 400× magnification using a Zeiss Axioskop microscope with epifluorescence and green excitation.

Abundance data were examined for departure from homogeneity of variances using Levene's test and for normality using Andersen-Darling tests. One-way ANOVA (Minitab 13) was used to test whether there were statistical differences in *Macrosetella gracilis* adult and *M. gracilis* copepodite abundances among either different depths or different stations (Sokal & Rohlf 1995). A Pearson's product-moment correlation coefficient (ρ) was calculated (Minitab 13) to determine the relationships between the abundance of *M. gracilis* adults and *Trichodesmium* spp., and *M. gracilis* copepodites and *Trichodesmium* spp.

Gut content analysis. *Macrosetella gracilis* were collected from surface plankton tows (0 to 20 m) at 4 different stations (Table 1), rinsed 3 times in 0.2 µm filtered seawater (FSW) to remove phytoplankton attached to their carapaces, and frozen in liquid nitrogen with 50 to 100 µl of FSW within an hour after collection and kept frozen until dissection. Fullness level of *M. gracilis* guts was rated as full, approximately half full, or empty by visual inspection prior to dissection. Copepods were dissected, and gut contents were observed under epifluorescence green and blue light excitation using 40× and 100× magnification. Only cells with sufficient shape and size distinctiveness that allowed identification of the cell origin were counted and photographed.

Natural abundance of stable isotopes. Copepods collected at 7 different stations (Table 1, Fig. 1) were removed from plankton tows (0 to 20 m) and sorted. Individual *Macrosetella gracilis*, *Miracia efferata*, and calanoid copepods from the same station were rinsed 3 times in FSW. Twenty to thirty copepods of either *M. gracilis*, *M. efferata*, or calanoid copepods were separated by species or group for calanoid copepods, and each category was filtered through a Swinnex filter onto pre-combusted (550°C, 24 h) Whatman GF/F filters. The filters were frozen at –20°C until analysis. In the laboratory, the filters with the copepods were dried for 3 d at 50°C. Dried filter samples were pelletized in tin foil and analyzed for isotopic and elemental composition using an Isoprime mass spectrometer configured with continuous flow to an elemental analyzer. Atmospheric air was used as the standard for nitrogen, and Pee Dee Belemite, as the standard for carbon. Results of natural abundance of stable isotopes are reported as delta values ($\delta^{13}C$ and $\delta^{15}N$), and all units are per mille (‰). Delta values are not absolute abundances, but differences between sample readings and a standard, such that:

$$\delta\,[‰] = [(R_{sample}/R_{standard}) - 1] \times 1000 \quad (1)$$

where R is expressed as the ratio of the heavy to the light isotope.

One-way ANOVA (or Kruskal-Wallis tests when the data did not fit the assumption of normality) (Minitab 13) were used to examine differences in the natural abundance of stable carbon and nitrogen isotope ratios among the different groups of copepods (Sokal & Rohlf 1995).

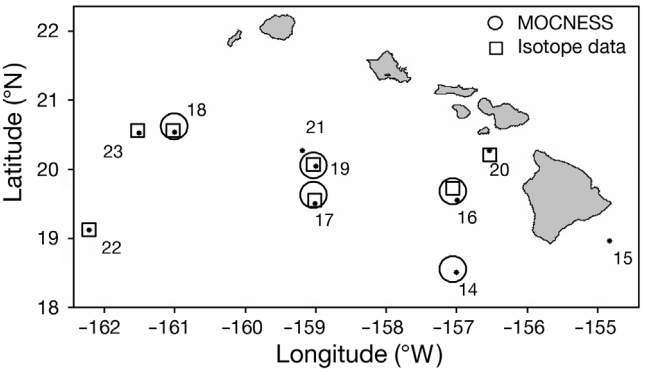

Fig. 1. *Macrosetella gracilis* and *Trichodesmium* spp. sampling stations during Cruise MPO9 in the Pacific near the Hawaiian islands

RESULTS

Zooplankton and *Trichodesmium* spp. abundances

Hydrographic conditions during August 2003 were typical for the season, with sea-surface temperatures ranging from 26.5 to 27.6°C and surface salinity ranging from 34.8 to 35.1 (Table 1).

Counts of the total sample volume averaged 523 ± 231 (SD) individual zooplankters. Individuals of *Macrosetella gracilis*, including both adults and copepodites,

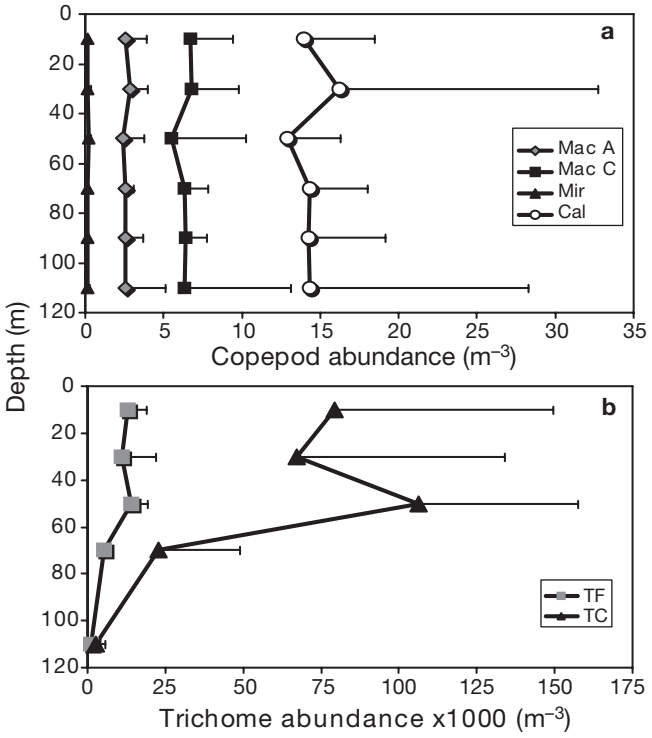

Fig. 2. Average copepod and *Trichodesmium* spp. abundances (+SD) at different depths: (a) *Macrosetella gracilis* adults (Mac A) and copepodites (Mac C), *Miracia efferata* (Mir), and calanoid copepods (Cal) and (b) free trichomes (TF) and trichomes in colonies (TC) (×1000 m^{-3})

were present in all our samples, with abundances ranging from 0.4 to 4.3 adults m^{-3} and from 0.5 to 19.1 copepodites m^{-3} (Fig. 2a). We found on average 1.8 ± 1.4 *M. gracilis* adults m^{-3} and 4.7 ± 3.9 *M. gracilis* copepodites m^{-3}. The number of *M. gracilis* copepodites was higher than the number of *M. gracilis* adults for all samples. *Miracia efferata*, also in the family Miraciidae, had consistently lower overall abundances than *M. gracilis*, with abundances ranging from 0 to 0.6 ind. m^{-3}. Total copepod abundance averaged 18.4 ± 16.2 adult copepods m^{-3}, with *M. gracilis* comprising, on average, 10.8 ± 5.1% of the total number of copepods, but never <3.5%, whereas calanoid copepods comprised 61.9 ± 7.7% of all copepods (Fig. 2a).

Trichomes of *Trichodesmium* spp. were present in all samples, with highest abundances in the water column above 60 m (Fig. 2b), and declined when the light level decreased below 5 to 10% of surface irradiance. We did not find a similar decline in *Macrosetella gracilis* densities at lower depths. Total trichome densities averaged 77.0 ± 88.9 × 10^3 trichomes m^{-3} of seawater (Fig. 2b). On average, 86.4% of trichomes occurred in colonies; the rest existed as free trichomes. Overall, we found no statistically discernable correlation between *Trichodesmium* spp. abundance and the abundance of either *M. gracilis* adults (ρ = 0.088, p = 0.697) or copepodites (ρ = 0.052, p = 0.818) when abundances from all stations and depths were pooled (Fig. 3). However, there seems to be a clear trend in the copepodite to *Trichodesmiun* correlation, slope = 0, indicating only a small abundance of *Trichodesmium* is necessary for copepodite survival.

Gut content analysis

Gut contents of a total of 18 copepods from 4 stations were examined (Table 1). Visual inspection of gut fullness before dissection revealed that 35% of the copepods had completely full guts, 24% partially full guts, and 41% empty guts. No *Trichodesmium* spp. filaments were noted during dissection, but single coccoid cyanobacterial cells were observed in 6 of 18 individuals (33.3%). When unicellular cyanobacteria were observed, they were found in copepods with either full (n = 4) or partially full guts (n = 2). One individual contained a fragment of the diatom *Rhizosolenia* sp. with 2 individuals of the symbiotic cyanobacterium *Richelia intracellularis*. Most of the gut contents, however, could not be identified.

Natural abundance of stable isotopes in copepod tissue

Natural abundances of $\delta^{15}N$ were similar for *Macrosetella gracilis* (3.06 ± 2.29), *Miracia efferata* (1.83 ±

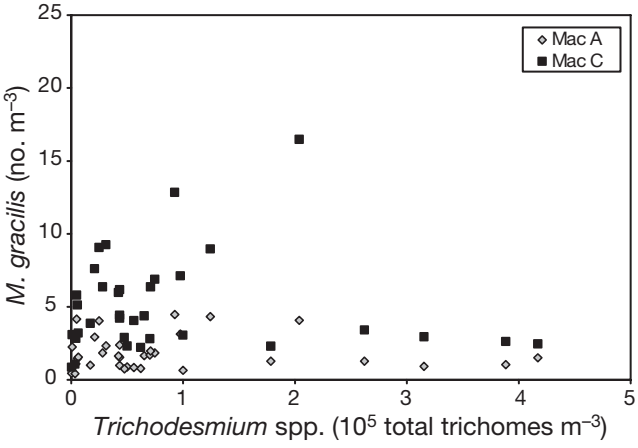

Fig. 3. *Macrosetella gracilis* and *Trichodesmium* spp. Correlation plot of *M. gracilis* adults (Mac A) and *M. gracilis* copepodites (Mac C) with *Trichodesmium* spp. (total trichomes) abundance shows no significant correlation between the abundance of *M. gracilis* adults and *Trichodesmium* spp. (Mac A: ρ = 0.088, p = 0.697) or between *M. gracilis* copepodites and *Trichodesmium* spp. (Mac C: ρ = 0.052, p = 0.818)

Table 2. Natural abundance of stable isotopes ($\delta^{15}N$, $\delta^{13}C$) in copepod tissue for different taxa. n: number of samples

Taxon	n	$\delta^{15}N$		$\delta^{13}C$	
		Mean	SD	Mean	SD
Macrosetella gracilis	13	3.06	2.29	–22.28	0.67
Miracia efferata	7	1.83	0.88	–20.91	0.45
Calanoid copepods	3	2.70	1.95	–19.42	0.45

0.88), and calanoid copepods (2.7 ± 1.95) (Kruskal-Wallis, $H = 0.764$, $p = 0.682$; Table 2). However, there were statistically discernible and ecologically significant differences in $\delta^{13}C$ values among the different copepod groups (1-way ANOVA, df = 2, $F = 32.175$, $p < 0.005$; Table 2). There do appear to be 2 clusters within the *Macrosetella* distributions, with little overlap with *Miracia* and no overlap with the calanoids. These clusters were not statistically discernible in the present study. A dual stable isotope plot of $\delta^{15}N$ and $\delta^{13}C$ data was used to compare the 3 different copepod groups analyzed in this study with published natural isotope values of *Trichodesmium* spp. (Fig. 4). Natural abundance of stable isotopes for all 3 groups of copepods differed from the published values for *Trichodesmium* spp. by amounts other than the expected 3.5 and 1‰ enrichment per trophic level for nitrogen and carbon, respectively (Fig. 4).

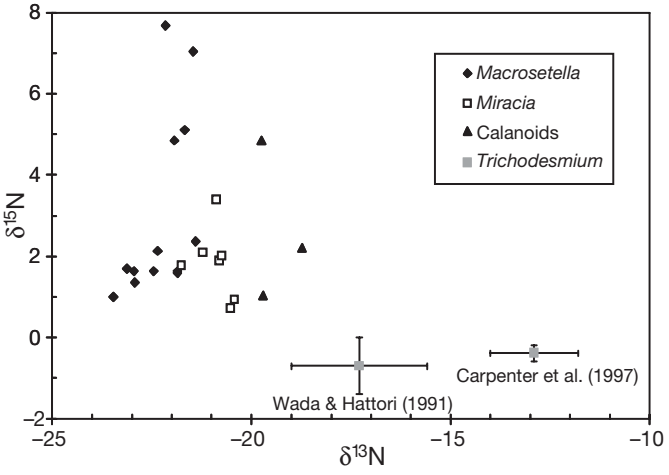

Fig. 4. *Macrosetella gracilis*, *Miracia efferata* and calanoid copepods. Dual isotope plot of natural abundance of stable isotopes $\delta^{15}N$ and $\delta^{13}C$ of copepod tissue (published *Trichodesmium* spp. values are shown for comparison)

DISCUSSION

Macrosetella gracilis and *Trichodesmium* spp. abundance

Macrosetella gracilis abundances from this study in the Pacific were lower than peak abundance estimates from the Red Sea (Böttger-Schnack & Schnack 1989, Böttger-Schnack 1991) and the Atlantic (Calef & Grice 1966). *Trichodesmium* spp. were present in all samples analyzed in this study, yet no significant positive correlation between *M. gracilis* and *Trichodesmium* spp. abundance was detected, suggesting that factors other than substrate availability may limit the abundance of the copepod. Despite its poor swimming ability and the specialized use of *Trichodesmium* spp. as a floating substrate for nauplii and copepodites, *M. gracilis* adults may not be highly dependant on *Trichodesmium* spp. colonies during all life-history stages. *M. gracilis* was found at depths >100 m, where *Trichodesmium* spp. no longer occurred due to light limitation. Furthermore, *M. gracilis* has been encountered on rare occasions in waters too cold to sustain *Trichodesmium* spp. (Huys & Böttger-Schnack 1994). *M. gracilis* was also found in a deep population below the photic zone in the Red Sea, where no *Trichodesmium* spp. occur (Böttger-Schnack & Schnack 1989). Presence of *Trichodesmium* spp. colonies may only be necessary to confer reproductive success (Björnberg 1965, but may have less influence on abundance of adults than previously assumed (Calef & Grice 1966). It should be acknowledged, however, that sampling in this study occurred only in areas where *Trichodesmium* was present, ranging between 23×10^3 and 136×10^3 trichomes m^{-3}, and *Trichodesmium* spp. may therefore have always been present in quantities that were high enough not to limit the abundance of the copepod. Additionally, the short duration of the sampling regime did not allow tracking of copepod abundances over multiple lifecycles. Future studies should include abundance data for earlier life-history stages of *M. gracilis* that could not be quantitatively sampled in this study.

While it is possible that *Macrosetella gracilis* uses other types of aggregates as a floating substrate, we have no data to confirm this. Other copepod species—for example the morphologically similar, but not closely related harpacticoid genus *Microsetella*—are often found associated with marine snow (Alldredge 1972, Ohkutsa et al. 1993, Steinberg 1994), but we have found no record that *M. gracilis* is associated with marine snow. Since *Trichodesmium* spp. is toxic, the association with the cyanobacterium could provide a shelter from predation. This shelter hypothesis needs to be tested in future studies.

Our study did not specifically address the question of selective predation on *Macrosetella gracilis.* The hydroid *Pelagiana trichodesmiae* and chaetognaths are known to feed on *M. gracilis* adults and juveniles

(Borstad & Brinckmann-Voss 1978, Post et al. 2002) and could have a significant impact on copepod abundance. The hydroid *P. trichodesmiae*, reported to date only from the Atlantic Ocean, preys extensively on nauplii and copepodites of *M. gracilis* (Borstad & Brinckmann-Voss 1978), but did not occur in our samples, so this source of predation likely did not play a role in limiting the abundance of the *M. gracilis* populations sampled. Chaetognaths were present in all samples (range 0.5 to 11.9 ind. m^{-3}), and predation by chaetognaths could be important in reducing *M. gracilis* abundance. Based on analysis of omega fatty acids of zooplankton samples from the Red Sea, Post et al. (2002) concluded that chaetognaths fed on *M. gracilis*.

The consistently higher abundances of *Macrosetella gracilis* copepodites compared to adults and the high number of gravid females (41.3% of all adults; n = 298) suggest that *Trichodesmium* spp. abundance encountered in our samples—with an average of 68.5 ± 33.4% of all trichomes in colonies—is highly favorable to *M. gracilis* reproduction. Previous studies encountered nauplii, copepodites, and gravid females of *M. gracilis* only in areas and at depths of high *Trichodesmium* abundance (Böttger-Schnack & Schnack 1989, Böttger-Schnack 1991). Females of *M. gracilis* carry between 5 and 12 eggs (an average of 6 eggs) in each of the 2 egg sacs, and nauplii hatch successively (Huys & Böttger-Schnack 1994). Females have been observed to actively attach eggs to *Trichodesmium* spp. colonies or swim with hatched nauplii—still holding on to the females' caudal rami with modified appendages—to colonies in order to transfer nauplii to the nursery habitat of the colonies (O'Neil 1998). Females were also observed to wait at a colony until all nauplii hatched successively (Björnberg 1965, Huys & Böttger-Schnack 1994). As an extreme example of parental care, female *M. gracilis* dropped their caudal rami, and nauplii used them as 'rafts' until they encountered a *Trichodesmium* spp. colony (R. Eberl pers. obs.), though this observed behavior may be an artifact of culturing conditions in the laboratory, and we do not know if it occurs in the wild. Nauplii and copepodites of *M. gracilis* cling to *Trichodesmium* spp. filaments with hook-like appendages (Björnberg 1965, Tokioka & Bieri 1966, Huys & Böttger-Schnack 1994), but there is no clear consensus as to whether or not nauplii are feeding on *Trichodesmium*, or using it primarily as a floating substrate to prevent sinking, conserve energy, or find protection from predation (Krishnaswamy 1949, Huys & Böttger-Schnack 1994).

Since physical substrate in the open ocean is rare, the association of *Macrosetella gracilis* nauplii and copepodites with *Trichodesmium* spp. is important for their pelagic existence and evolutionary significant for reproductive success. Björnberg (1965) documented significantly higher survival and developmental rates of *M. gracilis* nauplii when *Trichodesmium* spp. colonies were added to the culture. Personal observations of sinking and perishing *M. gracilis* nauplii during culturing experiments as part of a different study affirmed the reliance of *M. gracilis* on *Trichodesmium* spp. colonies as a means to remain pelagic. This obligate association of *M. gracilis* nauplii with *Trichodesmium* spp. was further corroborated by Sheridan et al. (2002) who found *M. gracilis* nauplii and copepodites only on *Trichodesmium* spp. colonies and not swimming freely in the water column.

Diet of *Macrosetella gracilis*

Gut content and natural abundance of stable isotopes in this study suggested that the natural diet of *Macrosetella gracilis* is not predominately composed of *Trichodesmium* spp. as previously proposed (O'Neil et al. 1996, O'Neil 1998, 1999). While the above-mentioned studies by O'Neil and colleagues demonstrated in a laboratory setting that *M. gracilis* could ingest *Trichodesmium* spp. and was not harmed by *Trichodesmium* toxins, our findings indicated that *M. gracilis* did not always feed on *Trichodesmium* spp. in the field when alternate food sources were present.

While *Trichodesmium* spp. filaments were not observed among the gut contents of *Macrosetella gracilis* in this study, this finding does not disprove per se that *M. gracilis* feeds on *Trichodesmium* spp. Gut content analysis is a descriptive rather than quantitative estimate of the food that *M. gracilis* has consumed recently. The copepod's feeding behavior was not observed in the present study, and it is possible that *Trichodesmium* cellular contents, and not the cell itself, were ingested by the copepods; however, our isotope data do not support this possibility. Several factors may affect whether or not *Trichodesmium* spp. (or any other food particles) are found in the gut. Gut residence time of food is difficult to discern, since digestion rates for different phytoplankters may vary. While copepod samples were processed immediately after collection, it is possible that some of the copepods voided their guts during processing (Barmstedt et al. 2000), which could explain the high number (44%) of copepods with empty guts. Alternatively, it is possible that not all the copepods were feeding at the time of collection.

However, stable isotope analyses of copepod tissue also suggested that *Macrosetella gracilis* did not feed predominately on *Trichodesmium* spp. in the field, and may feed on a mixed diet similar to other copepods examined in this study. Our results for $\delta^{15}N$ showed no significant difference between the supposed *Trichodesmium* spp. feeders (*M. gracilis* and *Miracia effer-*

ata) and other copepods that have been shown to be negatively affected by *Trichodesmium* toxin and were expected not to feed on *Trichodesmium* spp. The average $\delta^{13}C$ values for *M. gracilis* (–22.3 ± 0.7‰) were statistically lower than those of calanoid copepods (–19.4 ± 0.6‰) and closer to $\delta^{13}C$ values of non-N_2-fixing phytoplankton than of *Trichodesmium* spp. Assuming a 3.5‰ enrichment per trophic level for nitrogen and a 1‰ enrichment for carbon (Lajtha & Michener 1994, Michener & Schell 1994, Carpenter et al. 1997), none of the copepod samples in this study fell within the expected range for $\delta^{15}N$ and $\delta^{13}C$ values when compared to published *Trichodesmium* spp. data (Minagawa & Wada 1986, Wada & Hattori 1991, Carpenter et al. 1997). Lack of significantly different ^{15}N values among all the groups of copepods investigated suggested that all copepods utilized similar nitrogen sources. The natural abundances of stable isotopes in this study did not support the hypothesis that *M. gracilis* or *M. efferata* fed to a large degree on whole *Trichodesmium* spp. or on their cellular contents. Natural abundance of stable isotope measurements for *Trichodesmium* spp. collected at the same temporal and geographic location as the copepods used for diet analysis is needed to better understand the degree to which *M. gracilis* consumes and assimilates *Trichodesmium* spp. Montoya et al. (2002) showed that the low $\delta^{15}N$ of suspended particles and zooplankton in the North Atlantic during a bloom of *Trichodesmium* was consistent with input of new nitrogen by nitrogen fixation. Their study was conducted on size-fractioned zooplankton samples and showed increasing $\delta^{15}N$ values with increasing size class. No information, however, was provided on species composition of the samples. Therefore, it is not known whether *M. gracilis* was present in their samples, or what the relative abundance of *M. gracilis* was.

One possible explanation for our findings that *Macrosetella gracilis* did not feed on *Trichodesmium* spp. is the recent identification of the neurotoxins β-N-methylamino-L-alanine (BMAA) produced by *Trichodesmium* spp. collected near Hawaii (Cox et al. 2005). The amounts of BMAA measured from seawater samples collected in Hawaii during the time period of this study were 0.0079 and 0.0071 µg g^{-1} wet weight. More data are necessary to determine temporal and spatial variation of toxin concentrations during *Trichodesmium* blooms. While it has been shown that *M. gracilis* is not affected by the toxins that can harm other zooplankters (O'Neil et al. 1996), *M. gracilis* may choose other dietary sources if they are available. Future studies on the diet of *M. gracilis* should include analyses of the amount of toxicity present in the micro-layers of water surrounding *Trichodesmium* spp. colonies, as variable toxin content of *Trichodesmium* spp. could elicit different feeding responses. Future work should also investigate a possible correlation between toxin production by *Trichodesmium* spp. and grazing pressure.

Our data suggest that *Macrosetella gracilis* does not use *Trichodesmium* spp. directly for nutrition, but we posit that it may use *Trichodesmium* colonies as floating habitat for reproduction and possibly as shelter from predation. Whether *M. gracilis* gets nourishment from mucilage and microorganisms associated with colonies as suggested by Borstadt & Borstadt (1977) requires further studies. *M. gracilis* is a species with low reproductive output per individual, and the association with *Trichodesmium* spp. may have been a key step in the global distribution of this harpacticoid copepod that would not have been possible with continuation of a benthic lifestyle of adults and nauplii. It appears likely that *M. gracilis* may prefer *Trichodesmium* species with lower toxin content, as suggested by O'Neil & Roman (1994), if it feeds at all on the cyanobacterium, but this requires further investigation. In the absence of other food sources, *M. gracilis* may feed on *Trichodesmium* spp. as was suggested by previous research (O'Neil & Roman 1994, O'Neil et al. 1996), but the findings from this study suggest that it feeds on a more varied diet that does not always include *Trichodesmium* spp. The proportionally higher abundance of copepodites, as well as reproductive females in samples from this study, combined with the lack of evidence that *M. gracilis* feeds predominately on *Trichodesmium* spp., suggests that the association of *M. gracilis* with *Trichodesmium* spp. may have developed mainly from the need for a floating habitat rather than for nutritional dependence. We hypothesize that *Trichodesmium* spp. colonies could act as both nursery ground and/or shelter for predation.

Acknowledgements. We thank D. Capone (University of Southern California) and the participants of Research Cruise MPO9 on RV 'Roger Revelle' for assistance with this research. We thank A. Subramaniam (Columbia University) and T. Gunderson (University of Southern California) for help with MOCNESS sampling, and T. Gunderson for running the stable isotope analyses. We thank the following individuals for valuable discussions of research ideas and review of this manuscript: L. Falcon, R. Cohen, J. Durand, A. Slaughter, and R. Welch. Funding for this project was provided by NSF Grant OCE-0196186 awarded to E.J.C.

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Editorial responsibility: Howard Browman (Associate Editor-in-Chief), Storebø, Norway

Submitted: May 5, 2006; Accepted: August 10, 2006
Proofs received from author(s): February 22, 2007