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Quality and Quantity of Particulate Organic Carbon in a Coral Reef at Tioman Island, Malaysia

(Kualiti dan Kuantiti Karbon Partikulat Organan di Terumbu Karang Pulau Tioman, Malaysia)

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ABSTRACT

The quality and quantity of particulate organic carbon (POC) were investigated in a fringing coral reef of Tioman Island, Malaysia to better understand the food sources for reef meso-zooplankton. Phytoplankton biomass in the water column was on average 0.22 (± 0.07) mg Chl-a m^{-3}, of which picophytoplankton was the most important (size <3 µm, 50-70% of the total Chl-a). The proportion of C biomass by phytoplankton and other plankton to particulate organic carbon (POC) was low (6% and 5%, respectively) and the major portion of POC was occupied by detritus (89%), suggesting that the diet of particle-feeding or suspension feeding meso-zooplankton would chiefly consist of detritus.

Keywords: Detritus; food; phytoplankton; POC; zooplankton

ABSTRAK

Kualiti dan kuantiti karbon partikulat organan POC di sekitar terumbu pinggir Pulau Tioman Malaysia telah dikaji untuk memahami dengan lebih mendalam sumber makanan untuk mesozooplankton terumbu. Biojisim fitoplankton di dalam turus air puratanya adalah 0.22 (± 0.07) mg Chl-a m-3, di mana pikofitoplankton merupakan yang terpenting (size <3 µm, 50-70% daripada jumlah Chl-a). Nisbah biojisim karbon oleh fitoplankton dan plankton lain dengan karbon partikulat organan didapati rendah (6% and 5%, masing-masing) dan jumlah utama bahagian POC terdiri daripada detritus (89%), menunjukkan bahawa diet pemakan partikulat atau pemakan enapan mesozooplankton terutamanya terdiri daripada detritus.

Kata kunci: Detritus; fitplankton; makanan; POC; zooplankton

INTRODUCTION

In order to better understand the meso-zooplankton ecology, it is important to investigate the stock and composition of particulate organic matter (POM) as their food source. In this instance, the POM consists of living and non-living organic particles smaller than meso-zooplankton. The living organic particles include pico-, nano-, and microplankton such as phytoplankton, protozoans, and bacteria, while the non-living organic particles basically consist of detritus. Although picoplankton cells (<3 µm) are believed not to be readily utilized by some net-zooplankton such as copepods and other crustaceans because they are too small to be captured (Nival & Nival 1976), the picoplankton production may be converted to a size range that is available to meso-zooplankton through the microbial loop by heterotrophic flagellates and ciliates (Azam et al. 1983). Detritus is also considered as one of the important food sources for meso-zooplankton.

Coral reefs are characterized by low input of new nutrients and active nutrient recycling (Hatcher & Frith 1985), thus resulting in low primary production of phytoplankton. In these oligotrophic environments, it is considered that some zooplankton utilize detritus to compensate for the low available phytoplankton stocks (Roman et al. 1990). In fact, gut contents of meso-zooplankton (copepod and larvaceans) are reported to consist of >80% detritus in an atoll lagoon of Marshall Islands (Gerber & Marshall 1974). At present, relatively little is known about the stock (mg m^{-3}) of detritus over coral reefs (Charpy-Roubaud 1990; Charpy & Sakka et al. 2002; Gerber & Marshall 1982; Roman et al. 1990) though the biomass of the pico-, nano-, microplankton has been quantified in several reefs (Ferrier-Pagès & Furla 2001). The objective of this study is to measure the quality and quantity of POM including pico-, nano- and microplankton and detritus in a coral reef of Malaysia to better understand coral reef zooplankton ecology.

MATERIALS AND METHODS

SAMPLE COLLECTION

This study was carried out in a fringing coral reef at Tioman Island, off the east coast of Peninsular Malaysia. Sampling was conducted at a jetty in the Marine Park Centre at Tioman Island, off the east coast of Peninsular

Malaysia (2°50´00˝; 104°10´00˝) (Figure 1). The study site forms a typical fringing coral reef, with a dominance of *Acropora* corals. The water depth varied from 7.5 to 10.2 m depending on the tide.

Sampling was conducted every 3 hours for 2 consecutive days (48 hours) during 22-24 August and 1-3 October in 2004, and 25-27 February and 2-4 June in 2005, which amounted to 16 replicate sampling times for each study period. Seawater was collected with a 10 L Niskin bottle at 1 m below the surface and 1 m above the sea bottom for measurements of particulate organic carbon (POC), chlorophyll-*a* (Chl-*a*), inorganic nutrient concentrations, and community composition of pico-, nano-, and microplankton. The water from the two depths were pre-filtered through a 100 μm mesh screen to remove net-zooplankton and later combined. The combined water sample (ca. > 20 L) was brought back to the laboratory of the Marine Park Centre within 10 min.

Duplicate subsamples (10 mL) from the pre-filtered seawater (<100 μm) were filtered through a 0.45 μm syringe filter (Millipore) and stored at -20ºC for inorganic nutrients analysis ($SiOH_4$, PO_4, NO_2 and NO_3). A subsample (2000 mL) of the pre-filtered seawater was used for POC analysis and filtered onto a GF/F filter (Whatman), which was pre-combusted (500ºC, 4 h) and pre-weighted. The nominal pore size of GF/F filter is defined as 0.7-0.8 μm (Maske & Garcia-Mendoza 1994; Hashimoto & Shiomoto 2000); we expediently defined the pore size at 0.8 μm in the present study. The GF/F filter was rinsed with distilled water and placed over fuming HCl in a closed glass container for 24 h to remove carbonates (Strickland & Parsons 1972). The filters were then dried for 24 h at 60°C and stored in a desiccator until POC analysis.

To measure the size structure of plankton, 18 L of the pre-filtered seawater (<100 μm) was gently filtered through a 35 μm mesh screen to obtain two size-fractions, i.e. <35 μm and 35-100 μm. Analysis of the filtrate which contains particles of <35 μm and the particles retained on the 35 μm mesh screen (35-100 μm) were handled as follows. Chl-*a* concentrations were determined on a subsample (2000 mL) of the filtrate (<35 μm) that were successively filtered through a 3 μm pore-size membrane filter (Millipore) and a GF/F filter to measure the Chl-*a* contents in the fractions 0.8-3 μm and 3-35 μm. The filters were placed in *N,N*-dimethylformamide (DMF) and stored at -20ºC until analysis (Suzuki & Ishimaru 1990). A 100 mL subsample of the filtrate (<35 μm) was fixed in 1% glutaraldehyde seawater and stored in darkness at -20ºC until microscopic analysis. The particles retained on the 35 μm mesh screen (35-100 μm) were divided into two aliquots with a Folsom plankton splitter (Omori & Ikeda 1984). One aliquot was used for Chl-*a* measurement and the other for microscopic analysis. The aliquot for Chl-*a* measurement was filtered onto a GF/F filter, which was placed in DMF for pigment extraction and stored at -20ºC until analysis as described above. The aliquot for microscopic analysis was fixed with buffered formalin to a final concentration of 2% and stored at 5ºC until observation duration at time before observation.

SAMPLE ANALYSIS

The inorganic nutrient concentrations were analyzed according to Parsons et al. (1984) using Bran+Lubbe AASC II Autoanalyzer. The POC concentration was measured following Nagao et al. (2001), using a CN analyzer (Fisons

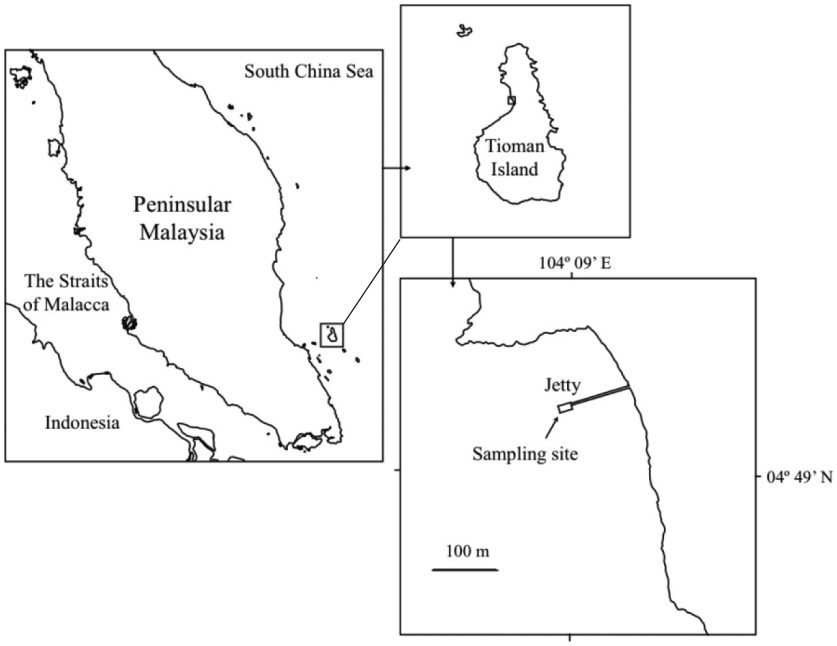

FIGURE 1. Map of the sampling site at Tioman Island off the east-coast of Peninsular Malaysia (2°50´00˝ N, 104°10´00˝ E)

EA 1108 CHNS/O). Particulate organic N (PON) was not detected due to the low amount of subsamples that were put into the CN analyzer. Chl-*a* concentrations were determined using a fluorometer (Turner Designs 10-AU) according to Holm-Hansen et al. (1965). The carbon biomass of phytoplankton was estimated using a C:Chl-*a* ratio of 50 (Charpy-Roubaud et al. 1989).

MICROSCOPIC OBSERVATION

To enumerate heterotrophic bacteria (hereafter bacteria), cyanobacteria and heterotrophic flagellates, the glutaraldehyde fixed sample was divided into two aliquots and each filtered onto a black polycarbonate filter (0.8 μm, Millipore). For bacteria and cyanobacteria, the filter was stained with SYBR Gold (Molecular Probes) according to Shibata et al. (2006). For heterotrophic flagellates, the filter was stained with primulin (Sigma) following Sherr et al. (1993). Cyanobacteria, bacteria, heterotrophic flagellates were counted with an epifluorescence microscope (Zeiss Axioskop 2 plus) using blue and green excitation at 1000 times magnification. For bacteria and cyanobacteria, at least 400 cells were counted per slide. For heterotrophic flagellates, at least 100 microscope fields per slide were scanned. Cell volumes of bacteria and heterotrophic flagellates were calculated from the length and width measured by an image analysis software (Zeiss AxioVision) and a digital camera (Zeiss AxioCam MRc5) mounted on the microscope. The cell volume of bacteria and heterotrophic flagellates were converted to carbon units using a conversion factor of 0.209 pg C μm^{-3} (Kogure & Koike 1987) and 183 fg C μm^{-3} (Caron et al. 1995), respectively. The cell volumes of heterotrophic flagellates were multiplied by a factor of 2.13 to correct for shrinkage in glutaraldehyde fixation (Choi & Stoecker 1989). Cyanobacterial cell numbers were converted to carbon units using a conversion factor of 200 fg C $cell^{-1}$ (Caron et al. 1995). The microscopic sample of the fraction 35-100 μm (microzooplankton) was categorized into ciliates such as tintinnids and naked ciliates and metazoans (e.g. copepods, polychaetes and larvaceans), and counted under a stereomicroscope. The body length of ciliates was measured to determine lorica volume of tintinnids and cell volume of naked ciliates using the image analysis software and the digital camera mounted on the microscope. The lorica volume of tintinnids was converted to carbon weight (*CW*) using the regression equation: *CW* (pg) = 444.5 + 0.053 LV (Verity & Langdon 1984), and the cell volume of naked ciliate was converted to carbon unit using a factor of 0.14 pg C μm^{-3} (Putt & Stoecker 1989). These equations do not take into account cell shrinkage due to formalin preservation and thus a factor of 1.15 for tintinnids and 1.25 for naked ciliates was multiplied for correction (Choi & Stoecker 1989). The body lengths of metazoans were also measured and their carbon weight was calculated using previous regression equations (Fisheries Agency 1987; e.g. Hirota 1986; Uye & Ichino 1995) taking into account body shrinkage by formalin preservation (Scheinberg et al. 2005; Szyper 1976; Wang et al. 1995).

DETRITUS ESTIMATION

The detritus mass (mg C m^{-3}) was estimated by subtracting the value of living organic C (i.e. sum of the carbon biomass of bacteria, heterotrophic flagellates, phytoplankton including cyanobacteria and microzooplankton) from that of POC (Andersson & Rudehäll 1993).

RESULTS

INORGANIC NUTRIENTS AND POC CONCENTRATIONS

The silicate ($SiOH_4$) concentration was relatively high among the nutrients and ranged from 2.76 (± 0.51) to 7.52 (± 0.98) μM (Table 1). The phosphate (PO_4) concentration was below the detectable level in August and October but was 0.07 (± 0.004) μM in June. The nitrite (NO_2) concentration ranged from 0.02 (± 0.01) to 0.32 (± 0.15) μM. The nitrate (NO_3) concentration varied from 0.14 (± 0.04) to 0.39 (± 0.11) μM. POC concentration in the water column ranged from 166.9 (± 36.6) to 275.6 (± 43.3) mg C m^{-3}, with an overall mean of 188.9 (± 65.7) mg C m^{-3} (Figure 2).

TABLE 1. Average concentrations of inorganic nutrients at Tioman Island. ud means undetectable. Concentration of inorganic nutrients in February 2005 were not measured. Mean values expressed with standard error. n means number of replicates

Sampling date (d/m/y)	Silicate (uM)	Phosphate (uM)	Nitrate (uM)	Nitrite (uM)
22-24/08/2004	7.52 ± 0.98 (n = 16)	ud (n = 16)	0.17 ± 0.03 (n = 10)	0.07 ± 0.004 (n = 16)
01-03/10/2004	6.94 ± 0.60 (n = 16)	ud (n = 16)	0.14 ± 0.04 (n = 10)	0.02 ± 0.01 (n = 7)
25-27/02/2005	–	–	–	–
02-04/06/2005	2.76 ± 0.51 (n = 16)	0.07 ± 0.02 (n = 7)	0.39 ± 0.11 (n = 11)	0.32 ± 0.15 (n = 6)

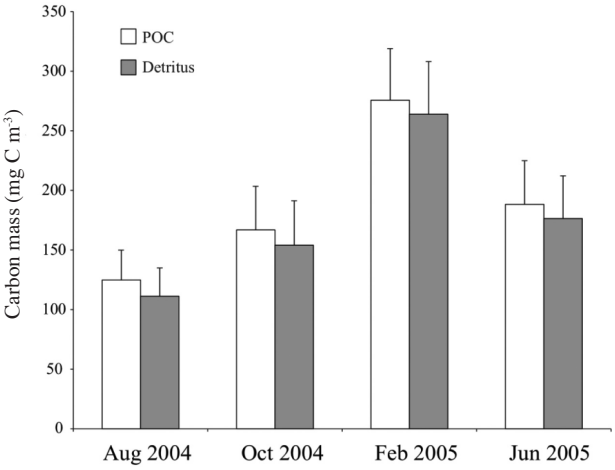

FIGURE 2. Particulate organic carbon (POC) and detritus at Tioman Island, Malaysia. Error bars represent standard deviation of the POC and detritus for 16 replicate measurements

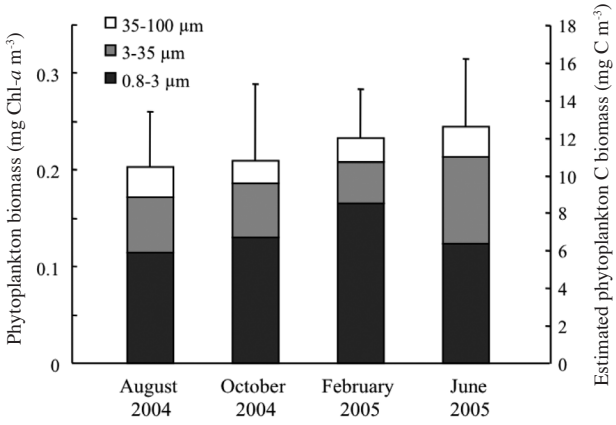

FIGURE 3. Size based distribution of phytoplankton biomass expressed with Chl-*a* concentration and estimated carbon (C) at Tioman Island, Malaysia. Phytoplankton C biomass was estimated using a C:Chl-*a* ratio of 50 (Charphy-Roubaud et al. 1989). Error bars represent standard deviation of the total phytoplankton biomass for 16 replicate measurements

PLANKTON COMMUNITIES AND DETRITUS

The total phytoplankton biomass was low and varied from 0.20 (± 0.05) to 0.24 (± 0.07) mg Chl-*a* m^{-3}, with an overall average of 0.22 (± 0.07) mg Chl-*a* m^{-3} (Figure 3). Phytoplankton assemblage was dominated by pico- and nanoplankton (size <35 µm, 85.0-89.4% of the total Chl-*a*), especially by picophytoplankton cells (size <3 µm, 50.7-70.6%). The total phytoplankton C biomass ranged from 10.1 (± 2.9) to 12.2 (± 3.5) mg C m^{-3} (average: 11.1 ± 3.3 mg C m^{-3}) and contributed only 3.8% to 9.8% of the POC concentrations (average: 6.5%).

Bacterial abundance (>0.8 µm) ranged from 1.07 (± 0.55) to 1.90 (± 0.67) × 10^4 cells L^{-1}, while the biomass ranged from 0.29 (± 0.15) to 0.51 (± 0.18) mg C m^{-3} (Figure 4a). The abundance of cyanobacteria varied from 1.0 (± 0.5) to 1.8 (± 1.1) × 10^7cells L^{-1}, whereas the biomass ranged from 1.97 (± 0.98) to 3.68 (± 2.19) mg C m^{-3} (Figure 4b). The abundance of heterotrophic flagellates ranged from 3.4 (± 2.2) to 7.7 (± 6.2) × 10^3 cells L^{-1}, while the biomass ranged from 35.5 (± 29.3) to 93.3 (± 55.2) × 10^{-3} mg C m^{-3} (Figure 4c). In microzooplankton (35-100 µm), protozoans (ciliates) were poorly represented

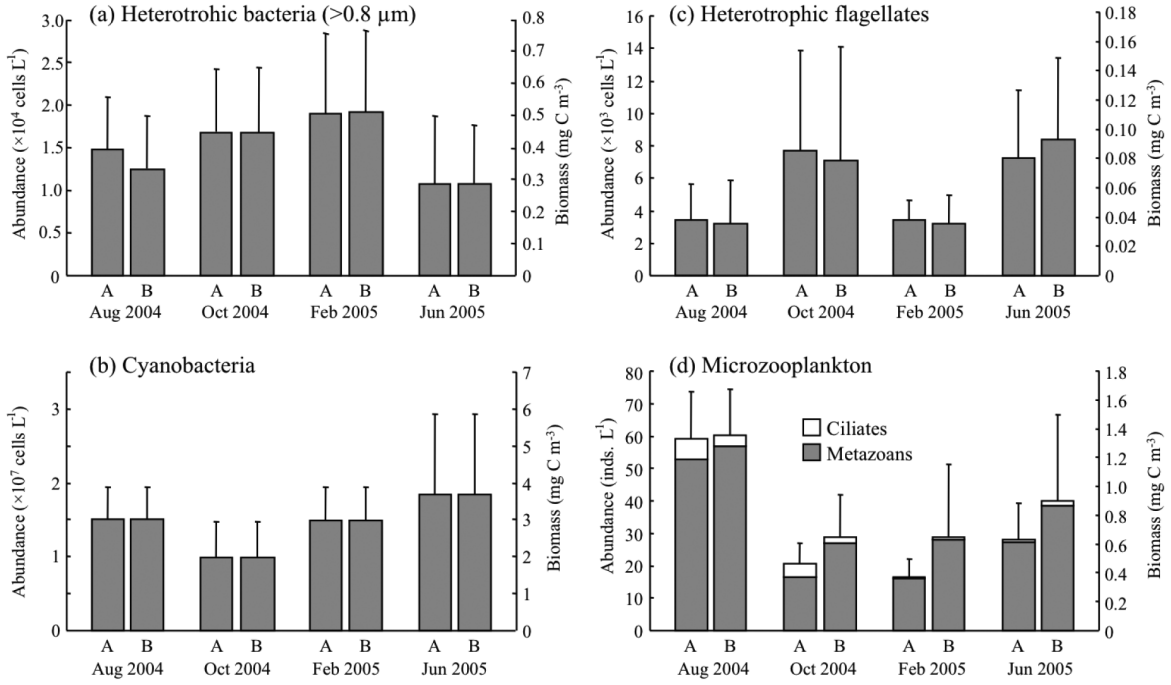

FIGURE 4. Abundance (A) and biomass (B) of heterotrophic bacteria (a), cyanobacteria (b), heterotrophic flagellates (c) and microzooplankton (d) at Tioman Island, Malaysia. Error bars indicate standard deviation for 16 replicate measurements

and metazoans constituted 79.1% to 97.5% of the total number and 93.3% to 97.5% of the total biomass (Figure 4d). The abundance of metazoans ranged from 16.34 (± 4.16) to 52.61 (± 13.36) inds. L^{-1}, while the biomass ranged from 0.48 (± 0.44) to 6.55 (± 3.43) mg C m^{-3}. Total living C (0.8-100 μm, sum of the carbon biomass of bacteria, phytoplankton including cyanobacteria, heterotrophic flagellates and microzooplankton) varied from 11.6 (± 4.1) to 13.5 (± 3.8) mg C m^{-3}. The size distributions of each plankton organism are shown in Figure 5.

The detritus mass ranged from 106.7 (± 23.9) to 252.4 (± 44.0) mg C m^{-3} with an overall average of 168.5 (± 63.8) mg C m^{-3}. The detrital contribution to the POC varied from 85.5% to 91.6% (average: 89.2%) (Figure 2).

Discussion

This paper describes the quality and quantity of micro organic particles (0.8-100 μm) in a coral reef of Tioman Island, Malaysia, to better understand the food sources for reef meso-zooplankton. The proportion of C biomass by phytoplankton and other plankton to POC was low (5.9% and 4.9%, respectively) and the major portion of POC was occupied by detritus.

Phytoplankton assemblage was mostly dominated by picophytoplankton (<3 μm, 51-71% of the total phytoplankton biomass), which may reflect the nutrient-poor environment ($PO_4 \leq 0.1$ μM, $NO_2 \leq 0.4$ μM, $NO_3 \leq 0.4$ μM), though the concentration of silicate was relatively high ($SiOH_4 \leq 8$ μM). Picophytoplankton (i.e. cyanobacteria) is known to be dominant in such oligotrophic waters because its small size and large surface area to volume ratio are an advantage over larger cells for nutrient uptake (Chisholm 1992; Dufour et al. 1999). Although picoplankton cells are believed to not be readily utilized by some particular species of meso-zooplankton such as copepods (e.g. Nival & Nival 1976), pelagic tunicates (e.g. larvaceans), one of the important components of net-zooplankton in tropical waters (Hopcroft & Roff 1998), have the ability to feed on a wide spectrum of particle size including <1 μM (e.g. Flood et al. 1992) and they are in favor of ingesting picoplankton biomass. The picoplankton (cyanobacteria) production may also be converted to a size range that is available to meso-zooplankton through the microbial loop by heterotrophic flagellates and ciliates (Azam et al. 1983).

Flagellates and ciliates are known to be widely and abundantly represented in coral reefs (reviewed by Ferrier-Pagès & Furla 2001). However, the mean numbers of heterotrophic flagellates and ciliates at the study site (5,400 cells L^{-1} and 3 cells L^{-1}, respectively) are one of the lowest reported in literature (Table 2). The maximum numbers are two orders lower than those in other reefs. Fixation procedures may have caused some underestimation in their numerical abundance (Bloem et al. 1986; Stoecker et al. 1994). In this study, 1% glutaraldehyde and 2% formalin were used for heterotrophic flagellates and ciliates fixation, respectively. Although flagellate is reported to be well preserved with 1% glutaraldehyde (Bloem et al. 1986) and Tsuji & Yanagita (1981) noted a loss of only 10% cells after 6 months storage at -20ºC, a relatively prolonged storage for nearly one year in the present study may have caused further decreases in cell numbers. For ciliate cells, formalin fixation is reported to cause loss of cells up to 20% compared to live counts (Dale & Burkill 1982). However even if we take into account 20% loss of cell numbers of both flagellates and ciliates in the present study, their numerical abundance is still lower than those in other reef systems. As previously demonstrated in various oceanic areas, heterotrophic flagellates are the major picoplankton grazers, whereas ciliates are the major grazers on flagellates (Strom 2000). Considering that they are a very important component in the microbial loop to transfer picoplankton production to higher trophic levels, i.e. meso-zooplankton, the contribution of the typical microbial loop (picoplankton-flagellates-ciliates)

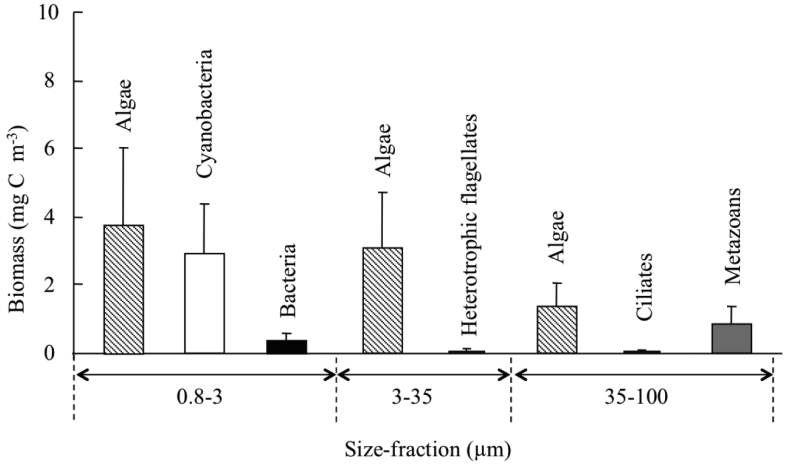

FIGURE 5. Size based distribution of plankton biomass (<0.8 μm, 0.8-3 μm, 3-35 μm, 35-100 μm) at Tioman Island, Malaysia. Error bars indicate standard deviation for 16 replicate measurements

TABLE 2. Summary of abundance of cyanobacteria, flagellates and ciliates measured in coral reef waters from other reports

Study site	Cyanobacteria ($\times 10^7$ cells l^{-1})	Heterotrophic flagellates ($\times 10^4$ cells l^{-1})	Ciliates ($\times 10^3$ cells l^{-1})	Source
Tikehau Atoll (French Polynesia)	15.13	7.1	6.68	Blanchot et al. (1989)
Sinton Atoll (South China Sea)		1.0-130.0		Sorokin (1991)
Tuamotu Atoll (French Polynesia)	5.10-19.2			Charpy et al. (1992)
Seychelles Island (Aldabra)		20.0-310.0		Sorokin (1995)
GBR (Australia)	0.37-2.51	1.2-4.9	0.018-0.071	Ayukai (1995)
Takapoto Atoll (French Polynesia)	1.60-3.50			Charpy & Blanchot (1996)
Tuamotu Atoll (French Polynesia)	6.80-14.1			Blanchot et al. (1997)
Tuamotu Atoll (French Polynesia)	11.0-40.0			Charpy & Blanchot (1998)
Tikehau Atoll (French Polynesia)		30.0-150.0		Gonzáles et al. 91998)
Mayotte Atoll (Comoros Islands)		2.0-10.0		Vacelet et al. (1999)
Great Astrolabe Reef (Fiji)		530		Charpy & Blanchot (1999)
Tioman Islands (Malysia)	1.5 (± 0.7)	0.54 (±0.23)	0.003 (± 0.001)	This study

GBR: Great Barrier Reef

to meso-zooplankton energetics may probably be small in the study site. Yet, the number of cyanobacteria was not uncommon compared with other coral reefs (Table 2). The picoplankton production may be removed by other organisms such as larvaceans (Flood et al. 1992), copepod nauplii (Roff et al. 1995), bivalve larvae (Douillet 1993), corals (Sorokin 1973) and sponges (Ribes et al. 2003).

The high detrital contribution to POC in our study site (86-92%, average 89%) confirmed previous measurements performed in other coral reefs. For instance, detrital carbon represented 77% of POC in Davies reef, GBR (Roman et al. 1990), 84% in Tikehau atoll, French Polynesia (Charpy & Charpy-Roubaud 1990), and 59% in Takapoto atoll, French Polynesia (Sakka et al. 2002). The values above appear lower than the values in the present study (89%), but the present value is lower than the 93% detritus of POC in Enewetak atoll in winter (Gerber & Marshall 1982). The amount of detritus was obtained by subtracting the value of living C from that of POC in the present study, but the detritus generally undergo biological decomposition and many microbes would be attached to the detritus. The biomass of the attached microbes was not distinguished from the detritus in the present study and thus the estimated amount of detritus may be somewhat overestimated. Detritus is considered as one of the important food sources for meso-zooplankton in oligotrophic environments including coral reefs. For instance, the planktonic harpacticoid copepod *Microsetella norvegica* is a typical herbivore in phytoplankton-rich eutrophic waters but this species is regarded as a detritivore in phytoplankton-scarce, oligotrophic waters (Ohtsuka & Nishida 1997; Ohtsuka et al. 1993). Gerber and Marshall (1974) analyzed the gut contents of the calanoid copepod *Undinula vulgaris* and the larvacean *Oikopleura longicaudata* at an atoll lagoon of Marshall Islands and reported that their gut materials consisted of >80% detritus. Since detritus was the major component of POC in the present study, most of the diet of particle-feeding or suspension feeding net-zooplankton at the study site would consist of detritus. Further investigation of detritus production and the consequent utilization by meso-zooplankton would be important for better understanding of reef pelagic ecosystem.

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REFERENCES

Andersson, A. & Rudehäll, Å. 1993. Proportion of plankton biomass in particulate organic carbon in the northern Baltic Sea. *Marine Ecology Progress Series* 95: 133-139.

Ayukai, T. 1995. Retention of phytoplankton and planktonic microbes on coral reefs within the Great Barrier Reef, Australia. *Coral Reefs* 14: 141-147.

Azam, F., Fenchel, T., Field, J.G., Gray, J.S., Meyer-Reil, L.A. & Thingstad, F. 1983. The ecological role of water column microbes in the sea. *Marine Ecology Progress Series* 10: 257-263.

Blanchot, J., André, J.M., Navarette, C. & Neveux, J. 1997. Picophytoplankton dynamics in the equatorial Pacific: diel cycling from flow-cytometer observations. *Comptes Rendus de l'Académie des Sciences* 320: 925-931.

Blanchot, J., Charpy, L. & Le Borgne, R. 1989. Size composition of particulate organic matter in the lagoon of Tikehau atoll (Tuamotu archipelago). *Marine Biology* 102: 329-339.

Bloem, J., Bär-Gilissen, M.B. & Cappenberg, T.E. 1986. Fixation, counting, and manipulation of heterotrophic manoflagellates. *Applied and Environmental Microbiology* 52: 1266-1272.

Caron, D.A., Dam, H.G., Kremer, P., Lessard, E.J., Madin, L.P., Malone, T.C., Napp, Q.J.M., Peele, E.R., Roman, M.R. & Youngbluth, M.J. 1995. The contribution of microorganisms to particulate carbon and nitrogen in surface waters of the Sargasso Sea near Bermuda. *Deep-Sea Research Part I* 42: 943-972.

Charpy, L. & Blanchot, J. 1996. Prochlorococcus contribution to phytoplankton biomass and production in Takapoto atoll (Tuamotu Archipelago). *Comptes Rendus de l'Académie des Sciences* 319: 131-137.

Charpy, L. & Blanchot, J. 1998. Photosynthetic picoplankton in French Polynesian atoll lagoons: estimation of taxa contribution to biomass and production by flow cytometry. *Marine Ecology Progress Series* 162: 57-70.

Charpy, L. & Blanchot, J. 1999. Picophytoplankton biomass, community structure and productivity in the Great Astrolabe Lagoon, Fiji. *Coral Reefs* 18: 255-262.

Charpy, L., Blanchot, J. & Lo, L. 1992. Cyanobacteria *Synechochoccus* spp. contribution to primary production in a closed atoll lagoon (Takapoto, Tuamotu, French Polynesia). *Comptes Rendus de l'Académie des Sciences* 314: 395-404.

Charpy, L. & Charpy-Roubaud, C.J. 1990. Trophic structure and productivity of the lagoonal communities of Tikehau atoll (Tuamotu Archipelago, French Polynesia). *Hydrobiologia* 207: 43-52.

Charpy-Roubaud, C.J., Charpy, L. & Lemasson, L. 1989. Benthic and planktonic primary production of an open atoll lagoon (Tikehau, French Polynesia). In *Proceedings of the 6th International Coral Reef Symposium Vol. 2*., edited by J.H. Choat, D. Barnes, M.A. Borowitzka, Coll, J.C., Davies, P.J., Flood, P., Hatcher, B.G., Hopley, D., Hutchings, P.A., Kinsey, D., Orme, G.R., Pichon, M., Sale, P.F., Sammarco, P., Wallace, C.C., Wilkinson, C. Wolanski, E., Bellwood, O. p. 551-556 Townsville, Australia.

Chisholm, S.W. 1992. Phytoplankton size. In *Primary Production in the Sea*, edited by P.G. Falkowski & A.D. Woodhead. p. 213-237. New York: Plenum Press.

Choi, J.W. & Stoecker, D.K. 1989. Effects of fixation on cell volume of marine planktonic protozoa. *Applied and Environmental Microbiology* 55: 1761-1765.

Dale, T. & Burkill, P.H. 1982. "Living counting": a quick and simple technique for enumerating pelagic ciliates. *Annales de L'Inst Océanograpique* 58: 267-276.

Douillet, P.A. 1993. Carbon contribution through bacterivory in larvae of the Pacific oyster *Crassostrea gigas*. *Marine Ecology Progress Series* 102: 303-314.

Dufour, P., Charpy, L., Bonnet, S. & Garcia, N. 1999. Phytoplankton nutrient control in the oligotrophic south pacific subtropical gyre (Tuamotu Archipelago). *Marine Ecology Progress Series* 179: 258-290.

Ferrier-Pagès, C. & Furla, P. 2001. Pico- and nanoplankton biomass and production in the two largest atoll lagoons of French Polynesia. *Marine Ecology Progress Series* 211: 63-76.

Fisheries Agency. 1987. *Zooplankton research manual*. Tokyo: Fisheries Agency Research Division. (in Japanese)

Flood, P.R., Deibel, D. & Morris, C.C. 1992. Filtration of colloidal melanin from sea water by planktonic tunicates. *Nature* 355: 630-632.

Gerber, R.P. & Marshall, N. 1974. Ingestion of detritus by the lagoon pelagic community at Enewetok Atoll. *Limnology and Oceanography* 19: 815-824.

Gerber, R. P. & Marshall, N. 1982. Characterization of the suspended particulate organic matter and feeding by the lagoon zooplankton at Enewetok Atoll. *Bulletin of Marine Science* 32: 290-300.

Gonzáles, J.M., Torréton, J.P., Dufour, P. & Charpy, L. 1998. Temporal and spatial dynamics of the pelagic microbial food web in an atoll lagoon. *Aquatic Microbial Ecology* 16: 53-64.

Hashimoto, S. & Shiomoto, A. 2000. Comparison of GF/F filters and 0.2 and 0.6 μm nuclepore filters on the chlorophyll a retention. *Bulletin / National Research Institute of Far Seas Fisheries* 37: 45-48.

Hatcher, A.I. & Frith, C.A. 1985. The control of nitrate and ammonium concentrations in coral reef lagoon. *Coral Reefs* 4: 101-110.

Hirota, R. 1986. Zooplankton. In: *Coastal Environmental Research Manual*, edited by The Oceanographic Society of Japan. p. 177-191 Kouseishakouseikaku, Tokyo. (in Japanese)

Holm-Hansen, O., Lorenzen, C.J., Holmes, R.W. & Strickland, J.D.H. 1965. Fluorometric determination of chlorophyll. *ICES Journal of Marine Scinece* 30: 3-15.

Hopcroft, R.R. & Roff J.C. 1998. Production of tropical larvaceans in Kingston Harbour, Jamaica: are we ignoring an important secondary producer? *Journal of Plankton Research* 20: 557-569.

Kogure, K. & Koike, I. 1987. Particle counter determination of bacterial biomass in sea water. *Applied and Environmental Microbiology* 53: 274-277.

Maske, H. & Garcia-Mendoza, E. 1994. Adsorption of dissolved organic matter to the inorganic filter substrate and its implications for 14C uptake measurements. *Applied and Environmental Microbiology* 60: 3887-3889.

Nagao, N., Toda, T., Takahashi, K., Hamasaki, K., Kikuchi, T. & Taguchi, S. 2001. High ash content in net-plankton samples from shallow coastal water: possible source of error in dry weight measurement of zooplankton biomass. *Journal of Oceanography* 57: 105-107.

Nival, P. & Nival, S. 1976. Particle retention efficiencies of an herbivorous copepod, *Acartia clausi* (adult and copepodite stages): effects on grazing. *Limnology and Oceanography* 21: 24-38.

Ohtsuka, S, Kubo, N., Okada, M. & Gushima, K. 1993. Attachment and feeding of pelagic copepods on larvacean houses. *Journal of Oceanography* 49: 115-120.

Ohtsuka, S. & Nishida, S. 1997. Reconsideration on feeding habits of marine pelagic copepods (Crustacea). *Oceanography in Japan* 6: 299-320.

Omori, M. & Ikeda, T. 1984. *Methods in Marine Zooplankton Ecology*. New York: John Wiley and Sons.

Parsons, T.R., Maita, Y. & Lalli, C.M. 1984. *A Manual of Chemical and Biological Methods for Seawater Analysis*. Oxford: Permagon Press.

Putt, M. & Stoecker, D.K. 1989. An experimentally determined carbon, volume ratio for marine "oligotrichous" ciliates from estuarine and coastal waters. *Limnology and Oceanography* 34: 1097-1103.

Ribes, M., Coma, R., Atkinson, M.J. & Kinzie III, R.A. 2003. Particle removal by coral reef communities: picoplankton is a major source of nitrogen. *Marine Ecology Progress Series* 257: 13-23.

Roff, J.C., Turner, J.T., Webber, M.K. & Hopcroft, R.R. 1995. Bacterivory by tropical copepod nauplii: extent and possible significance. *Aquatic Microbial Ecology* 9: 165-175.

Roman, M.R., Furnas, M.J. & Mullin, M.M. 1990. Zooplankton abundance and grazing at Davies Reef, Great Barrier Reef, Australia. *Marine Biology* 105: 73-82.

Sakka, A., Legendre, L., Gosselin, M., Niquil, N. & Delesalle, B. 2002. Carbon budget of the planktonic food web in an atoll lagoon (Takapoto, French Polynesia). *Journal of Plankton Research* 24: 301-320.

Scheinberg, R.D., Landry, M.R. & Calbet, A. 2005. Grazing of two common appendicularians on the natural prey assemblage of a tropical coastal ecosystem. *Marine Ecology Progress Series* 294: 201-212.

Sherr, E.B., Caron, D.A. & Sherr, B.F. 1993. Staining of heterotrophic protists for visualization via epifluorescence microscopy. In *Handbook of Methods in Aquatic Microbial Ecology*, edited by P.F. Kemp, B.F. Sherr, E.B. Sherr & J.J. Cole. Boca Raton, FL.: Lewis Publishers, CRC Press.

Shibata, A., Goto, Y., Saito, H., Kikuchi, T., Toda, T. & Taguchi, S. 2006. Comparison of SYBR Green I and SYBR Gold stains for enumerating bacteria and viruses by epifluorescence microscopy. *Applied and Environmental Microbiology* 43: 221-231.

Sorokin, Y.I. 1973. On the feeding of some scleractinian corals with bacteria and dissolved organic matter. *Limnology and Oceanography* 18: 380-385.

Sorokin, Y.I. 1991. Parameters of productivity and metabolism of coral reef ecosystems off central Vietnam. *Estuarine, Coastal and Shelf Science* 33: 259-280.

Sorokin, Y.I. 1995. *Coral Reef Ecology*. Berlin Hiedelberg New York: Springer-Verlag.

Stoecker, D.K., Gifford, D.J. & Putt, M. 1994. Preservation of marine planktonic ciliates: losses and cell shrinkage during fixation. *Marine Ecology Progress Series* 110: 293-299.

Strickland, J.D.H. & Parsons, T.R. 1972. *A Practical Handbook of Seawater Analysis*. 2nd Ed. Canada: Fisheries research board of Canada.

Strom, S.L. 2000. Bacterivory: Interactions between bacteria and their grazers. In *Microbial Eecology of the Oceans*, edited by D. L. Kirchman. New York: Wiley-Liss.

Suzuki, R. & Ishimaru, T. 1990. An improved method for determination of phytoplankton chlorophyll using *N,N*-dimethylformamide. *Journal of Oceanography* 46: 190-194.

Szyper, J.P. 1976. The Role of Sagitta Enflata in the Southern Kaneohe Bay Ecosystem. Ph.D. thesis, Hawaii: University of Hawaii (unpublished).

Tsuji, T. & Yanagita, T. 1981. Improved fluorescent microscopy for measuring the standing stock of phytoplankton including fragile components. *Marine Biology* 64: 207-211.

Uye, S. & Ichino, S. 1995. Seasonal variations in abundance, size composition, biomass and production rate of *Oikopleura dioica* (Fol) (Tunicate: Appendicularia) in a temperate eutrophic inlet. *Journal of Experimental Marine Biology and Ecology* 189: 1-11.

Vacelet, E., Arnoux, A., Thomassin, B.A. & Travers, M. 1999. Influence of freshwater and terrigenous material on nutrients, bacteria and phytoplankton in a high island lagoon: Mayotte, Comoro Archipelago, Indian ocean. *Hydrobiologia* 380: 165-178.

Verity, P.G. & Langdon, C. 1984. Relationships between lorica volume, carbon, nitrogen, and ATP content of tintinnids in Narragansett Bay. *Journal of Plankton Research* 6: 859-868.

Wang, Z., Thiébaut, E. & Dauvin, J.C. 1995. Spring abundance and distribution of the ctenophore *Pleurobrachia pileus* in the Seine estuary: advective transport and diel vertical migration. *Marine Biology* 124: 313-324.

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