

Coupling of thraustochytrids and POM, and of bacterio- and phytoplankton in a semi-enclosed coastal area: implication for different substrate preference by the planktonic decomposers

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ABSTRACT: Abundances of planktonic thraustochytrids and bacterioplankton were investigated in the Seto Inland Sea, Japan, with reference to concentrations of potential growth substrates such as dissolved organic carbon (DOC) and particulate organic carbon (POC) concentration and of phytoplankton (chlorophyll *a* concentration). Thraustochytrid and bacterioplankton cells were stained with acriflavine and acridine orange, respectively, and directly counted by epifluorescence microscopy. Thraustochytrids occurred in the water column at a density of 0.9×10^2 to 2.1×10^4 cells l^{-1} , with an overall average (\pm SD) of $3.1 \pm 4.0 \times 10^3$ cells l^{-1} . Bacterioplankton were found in the water column at a density of 1.1×10^9 to 6.6×10^{10} cells l^{-1} , with an overall average of $4.8 \pm 7.0 \times 10^9$ cell l^{-1} . Thraustochytrid abundance was significantly correlated primarily to POC concentration ($r = 0.547$, $p < 0.001$) and secondarily to chlorophyll *a* concentration ($r = 0.318$, $p < 0.001$). By contrast, bacterioplankton concentration was significantly correlated primarily to chlorophyll *a* concentration ($r = 0.498$, $p < 0.001$) and secondarily to DOC concentration ($r = 0.236$, $p = 0.002$) but negatively to POC concentration ($r = 0.143$, $p = 0.068$). Phytoplankton-derived POC accounted for a certain fraction (only ambiguously estimated at 20 to 60 %) of total POC, and about 20 % of POC was ascribable to bacterioplankton biomass. The remaining POC was assumed to be non-phytoplanktonic, non-bacterioplanktonic sources such as phytodetritus and terrigenous matter. Therefore, it is suggested that bacterioplankton abundance may depend primarily on phytoplankton-derived DOC whereas thraustochytrid abundance is more influenced by non-phytoplankton, non-bacterioplankton POC. That is, planktonic degraders, thraustochytrids and bacterioplankton, decompose substrates of different origins and sizes. The potential ecological importance of the thraustochytrids as active degraders of POC and as an alternative food source is discussed.

KEY WORDS: Thraustochytrid · Fungoid protist · Bacterioplankton · Abundance · Particulate organic carbon · Dissolved organic carbon · Chlorophyll *a*

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INTRODUCTION

Thraustochytrids are obligately marine- and estuarine-dwelling protists (Moss 1986), and were formerly classified as the lower fungi, heterotrophic eukaryotic microorganisms. This group is characterized by the presence of an ectoplasmic net and a cell wall composed of non-cellulosic, sulfurylated scales (Darley et

al. 1973). Thraustochytrids are now classified in the phylum Heterokonta within the kingdom Stramenopila (Patterson 1999). The phylum Heterokonta also includes the species of diatoms and brown algae as well as the oomycetes (Cavalier-Smith et al. 1994).

Previous studies have determined the abundance of thraustochytrids over a wide range of habitats, including the water column, algae, particulate detritus and invertebrates (Raghukumar et al. 1990, Riemann & Schaumann 1993, Frank et al. 1994, Naganuma et al. 1998, Kimura et al. 1999). A rapid direct detection tech-

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nique using the fluorogenic dye acriflavine was developed and used for the enumeration of thraustochytrids (Raghukumar & Schaumann 1993). The acriflavine fluorescence technique relies on the fact that the wall and nucleus of thraustochytrid cell fluoresce differently in red and blue-green, respectively, under blue-light excitation. This dual fluorescence distinguishes thraustochytrids from other protists and detritus. In recent studies, 18S rDNA analysis identified thraustochytrids in the pico- and nanoplanktonic assemblages from the equatorial Pacific Ocean and the Antarctic polar front (López-García et al. 2001, Moon-van der Staay et al. 2001). Note that the enumeration based on acriflavin-staining may include protozoan cysts (leading to overestimation) and exclude thraustochytrid zoospores (leading to underestimation). Contribution of this over- and underestimation should be evaluated in future studies by using a more specific technique such as immunofluorescence microscopy.

The fungoid protists, thraustochytrids, are sized largely between 3.5 and 20 µm in diameter (Raghukumar & Schaumann 1993, Naganuma et al. 1998), and are larger than bacterioplankton (<1 µm, Naganuma & Miura 1997). Thus, thraustochytrids are thought to serve as important food sources for picoplankton feeders and contribute to the increase in the efficiency of pelagic secondary production (Naganuma et al. 1998, Kimura et al. 1999). Thraustochytrids uniquely produce polyunsaturated fatty acids such as docosapentaenoic acid and docosahexaenoic acid (Nakahara et al. 1996), which may be important nutritionally for the growth of fish larvae (Raghukumar 1986, Raghukumar & Balasubramian 1991).

Another ecological role of thraustochytrids is decomposition of refractory organic substrates in marine ecosystems (Perkins 1973). Thraustochytrids have been reported to occur in association with decaying fecal pellets of zooplankton such as salps (Raghu-

kumar & Raghukumar 1999) and plant material such as algal tissue (Miller & Jones 1983, Sathe-Pathak et al. 1993, Sharma et al. 1994), cellulosic material (Fell & Master 1973, Findlay et al. 1986) and mangrove leaf (Ulken 1984, Raghukumar 1988, Raghukumar et al. 1994, Bremer 1995). These observations suggest that thraustochytrids may play a definite role in the dynamics of particulate organic matter (POM) in the marine ecosystem. On the other hand, it is known that dissolved organic matter (DOM), such as exudate DOM from phytoplankton (Larsson & Hagström 1979) and terrigenous DOM in river water (Zweifel et al. 1995, Kuparinen et al. 1996, Rolff & Elmgren 2000), is used as a growth substrate for bacterioplankton (Zweifel et al. 1993, Moran & Hodson 1994, Hullar et al. 1996). However, no work has been published on the correlation between the abundances of planktonic thraustochytrids and bacterioplankton and the concentrations of dissolved organic carbon (DOC), particulate organic carbon (POC) and chlorophyll *a* (chl *a*).

We report the distribution of planktonic thraustochytrids and bacterioplankton abundances in the Seto Inland Sea and their correlation with DOC, POC and chl *a* concentrations.

MATERIALS AND METHODS

Sample collection and site description. In total, 165 water samples were collected for determining the abundances of thraustochytrids and bacterioplankton and the concentrations of DOC, POC and chl *a*. Samples were collected with Van Dorn samplers from 42 sites at 3 depths (1, 5 and 15 m) during cruises by RV 'Toyoshio-maru' in July and September 2000. The samples were collected in the Seto Inland Sea and the Hyuga Nada area, Japan (Table 1, Fig. 1). The Van Dorn samplers were pre-washed and pre-rinsed with distilled water, and re-washed with seawater of the same site and depth immediately before sample collection.

Vertical profiles of water temperature, salinity and dissolved oxygen were monitored at each sampling site with a Sea Bird conductivity, temperature, depth profiler (CTD). Water temperature ranged from 15.1°C (Site 6, 15 m depth) to 29.0°C (Site 20, 1 m depth) in July, and from 23.1°C (Site 11, 15 m depth) to 27.6°C (Site 1, 1 m depth) in September. Salinity ranged from 27.2 psu (Site 34, 1 m depth) to 33.9 psu (Site 33, 15 m depth) in July, and from 30.7 psu (Site 17, 1 m

Table 1. Sites and months of sample collection, corresponding area names, and range of surface water temperature observed during each cruise (July and September 2000). –: samples were not collected; +: samples were collected

Site no.	Area name		Jul 2000 15.1–29.0°C	Sep 2000 23.1–27.6°C
1 to 3	Hyuga Nada	Kuroshio-affected ^a	–	+
4	Bungo Channel	Outer Seto Inland Sea	–	+
5 to 14	Iyo Nada	Seto Inland Sea	+	+
15 to 17	Hiroshima Bay	Seto Inland Sea	+	+
18 to 22	Hiuchi Nada	Seto Inland Sea	+	–
23	Bisan Seto	Seto Inland Sea	+	–
24 to 29	Harima Nada	Seto Inland Sea	+	–
30 to 37	Osaka Bay	Seto Inland Sea	+	–
38 to 42	Kii Channel	Seto Inland Sea	+	–

^aKuroshio Current, NW Pacific

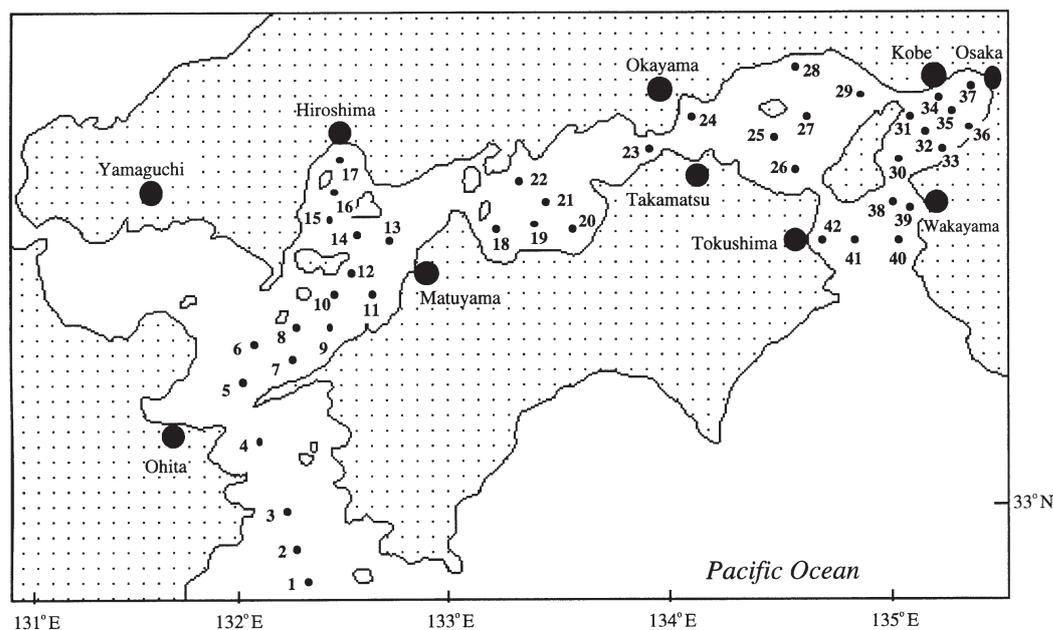


Fig. 1. Site locations of sample collection in and near the Seto Inland Sea, Japan

depth) to 34.2 psu (Site 1, 15 m depth) in September. Dissolved oxygen concentration was within the range from 2.64 mg O₂ l⁻¹ (Site 37, 15 m depth) to 5.23 mg O₂ l⁻¹ (Site 17, 1 m depth) in July, and from 2.81 mg O₂ l⁻¹ (Site 17, 15 m depth) to 4.90 mg O₂ l⁻¹ (Site 17, 1 m depth) in September.

Thraustochytrid and bacterial cell staining and counting. Planktonic thraustochytrid and bacterial cells were collected on pre-blackened polycarbonate filters (Isopore, Millipore; pore size, 0.2 μm; diameter, 25 mm) immediately after water collection. Each 10 to 20 ml of sample water was filtered for thraustochytrid cell counts, and each 0.5 to 2 ml was filtered for bacterial cell counts. The remaining water samples were fixed with 0.2 μm-filtered formalin at a final concentration of 5% v/v and kept refrigerated for later observation.

Thraustochytrid cells on filters were immediately stained with 4 ml of 0.2 μm-filtered 0.05% acriflavine (Sigma) in 0.1 M citrate buffer at pH 3.0 (prepared from a 0.5% stock solution of acriflavine in distilled water) for 4 min, and rinsed with 0.2 μm-filtered 75% isopropanol for 1 min. (Raghukumar & Schaumann 1993). On the other hand, bacterial cells were stained with 0.01% acridine orange in 10 mM phosphate buffer at pH 8.0 for 5 min (Hobbie et al. 1977, Zimmermann et al. 1978). Thraustochytrid and bacterial cells were counted over 20 to 30 microscopic fields per filter, and 2 filters for each sample were counted by epifluorescence microscopy. Acriflavine stained both the nuclear DNA (Gahan 1984) and the cell wall sulfurylated polysaccharides (Pearse 1968), fluorescing blue-green and red, respectively.

The limitation of the current staining technique is that zoospores of most thraustochytrid species lack cell walls (Moss 1986). Also, very small cells (<5 μm) were not easily distinguishable because the cell wall-associated red fluorescence was weaker than the nucleus-associated green fluorescence. Thus, very small cells were not counted, and the counts reported in this study probably are underestimations.

Determination of DOC, POC and chl *a* concentrations. Immediately after sampling, 200 ml of water samples was filtered through pre-combusted glass fiber filters (450°C, 3 h; 25 mm, Whatman GF/F), and both filtrate and filters were kept frozen at -20°C until determination. DOC concentration in each filtrate was measured with a total organic carbon analyzer (Shimadzu Co. Ltd, Kyoto, Japan). POC on each filter was measured with a CHN analyzer (Model MT-5, Yanaco CO. Ltd, Kyoto, Japan). Chl *a* concentration was calculated from the fluorescence intensity, whose vertical profile was monitored at each sampling site with a Sea Bird CTD. The fluorescence intensity was calibrated with known amounts of chl *a* (Sigma) dissolved in dimethyl formamide at -20°C (Parsons et al. 1984).

RESULTS AND DISCUSSION

DOC, POC and chl *a* concentrations

General distributions of DOC, POC and chl *a* concentrations were as follows: DOC concentration varied from 0.3 (Site 4, 5 m depth, September) to 4.3 mg l⁻¹ (Site 9, 1 m depth, September). POC concentration

ranged from 0.11 (Site 2, 5 m depth, September) to 3.3 mg l⁻¹ (Site 17, 15 m depth, July). Calculated chl *a* concentration was within the range of 0.172 (Site 1, 5 m depth, September) to 30.0 µg l⁻¹ (Site 34, 1 m depth, July).

Abundance of planktonic thraustochytrids and bacterioplankton

The abundance of planktonic thraustochytrids in the Seto Inland Sea (Table 2) varied from 0.86 × 10² (Site 7, Iyo Nada, 1 m depth in September) to 2.14 × 10⁴ cells l⁻¹ (Site 17, Hiroshima Bay, 1m depth in July), with an overall average (±SD) of 3.11 ± 4.00 × 10³ cells l⁻¹ (n = 165). The population density of bacterioplankton varied within a relatively narrow range from 1.10 × 10⁹ (Site 3, Hyuga Nada, 1 m depth in September) to 6.56 × 10¹⁰ cells l⁻¹ (Site 33, Osaka Bay, 1m depth in July), with an overall average of 4.79 ± 7.02 × 10⁹ cells l⁻¹ (n = 165). Previous studies reported an average thraustochytrid abundance of 10³ to

10⁴ cells l⁻¹ (Naganuma et al. 1998, Kimura et al. 1999) and an average bacterioplankton abundance of 10⁹ cells l⁻¹ (Naganuma & Miura 1997) in the coastal Seto Inland Sea and adjacent open waters. The abundance determined in this study was in good agreement with the previous reports. The depth-to-depth variation in the abundances of thraustochytrids and bacterioplankton is shown in Table 3. Abundances of thraustochytrids and bacterioplankton were generally high in the top 1 m of water and decreased with depth. However, the overall correlation (n = 55) of abundance and depth was not significant for thraustochytrids (r = 0.107, p = 0.170) and bacterioplankton (r = 0.125, p = 0.109).

The highest abundance of thraustochytrids, both site-/depth-specific and site-/depth-averaged, was found in Hiroshima Bay (Sites 15 to 17), where all the determined parameters were above the averages (Table 2). The highest POC concentration was also seen in Hiroshima Bay, and this coincidence suggested that thraustochytrid abundance and POC concentration are connected.

Table 2. Area-/depth-averaged abundances of thraustochytrids (Thra) and bacterioplankton (Bact), and concentrations of dissolved organic carbon (DOC), particulate organic carbon (POC) and chlorophyll *a* (Chl *a*) in the Seto Inland Sea, Japan

Site	Sea area name	Thra (×10 ³ cells l ⁻¹)	Bact (×10 ⁹ cells l ⁻¹)	DOC (mg l ⁻¹)	POC (mg l ⁻¹)	Chl <i>a</i> (µg l ⁻¹)
July						
5 to 14	Iyo Nada	4.46 ± 4.51	2.47 ± 0.42	2.13 ± 0.29	1.11 ± 0.38	2.00 ± 1.26
15 to 17	Hiroshima Bay	8.62 ± 7.93	6.41 ± 1.15	2.30 ± 0.43	1.96 ± 1.02	7.03 ± 6.20
18 to 22	Hiuchi Nada	1.64 ± 2.11	3.12 ± 1.04	1.43 ± 0.57	0.88 ± 0.13	1.44 ± 1.22
23	Bisan Seto	1.87 ± 0.81	3.39 ± 0.29	1.52 ± 0.49	1.41 ± 0.51	4.21 ± 0.14
24 to 29	Harima Nada	3.68 ± 3.64	3.70 ± 0.39	2.64 ± 0.34	0.78 ± 0.54	4.11 ± 2.12
30 to 37	Osaka Bay	4.00 ± 4.50	15.7 ± 13.8	2.23 ± 0.39	0.71 ± 0.48	7.81 ± 7.68
38 to 42	Kii Channel	4.14 ± 2.43	4.16 ± 1.66	2.39 ± 0.27	0.52 ± 0.10	4.04 ± 2.15
5 to 42	Average	4.09 ± 4.44	5.88 ± 8.14	2.11 ± 0.48	0.94 ± 0.59	4.21 ± 4.72
September						
1 to 3	Hyuga Nada	1.43 ± 1.21	1.34 ± 0.14	1.60 ± 0.69	0.13 ± 0.02	0.28 ± 0.08
4	Bungo Channel	1.16 ± 0.12	1.87 ± 0.04	1.09 ± 0.76	0.21 ± 0.01	2.90 ± 0.53
5 to 14	Iyo Nada	0.48 ± 0.27	1.91 ± 0.47	1.69 ± 0.59	0.27 ± 0.08	4.92 ± 2.21
15 to 17	Hiroshima Bay	1.83 ± 1.33	5.19 ± 3.11	2.05 ± 0.61	0.46 ± 0.15	7.16 ± 4.29
1 to 17	Average	0.93 ± 0.93	2.38 ± 1.86	1.70 ± 0.64	0.27 ± 0.13	4.38 ± 3.24
1 to 42	Total Average	3.11 ± 4.00	4.79 ± 7.02	1.99 ± 0.56	0.73 ± 0.58	4.26 ± 4.31

Table 3. Average abundance of thraustochytrids (Thra) and bacterioplankton (Bact), phytoplankton (Chl *a*), and eutrophical parameters: dissolved organic carbon (DOC), particulate organic carbon (POC) concentration and salinity (S)

	Thra (×10 ³ cells l ⁻¹)	Bact (×10 ⁹ cells l ⁻¹)	Chl <i>a</i> (µg chl <i>a</i> l ⁻¹)	DOC (mg l ⁻¹)	POC (mg l ⁻¹)	S (psu)
Overall (n = 165)	3.11 ± 4.00	4.79 ± 7.00	4.26 ± 4.31	1.99 ± 0.56	0.73 ± 0.58	32.76 ± 0.88
1 m (n = 55)	3.75 ± 4.50	5.94 ± 9.85	4.44 ± 4.91	2.13 ± 0.63	0.87 ± 0.69	32.57 ± 1.19
5 m (n = 55)	3.00 ± 4.08	4.71 ± 6.08	4.64 ± 5.22	1.92 ± 0.58	0.66 ± 0.43	32.75 ± 0.80
15 m (n = 55)	2.61 ± 3.32	3.71 ± 3.51	3.70 ± 2.15	1.91 ± 0.45	0.67 ± 0.58	32.96 ± 1.19

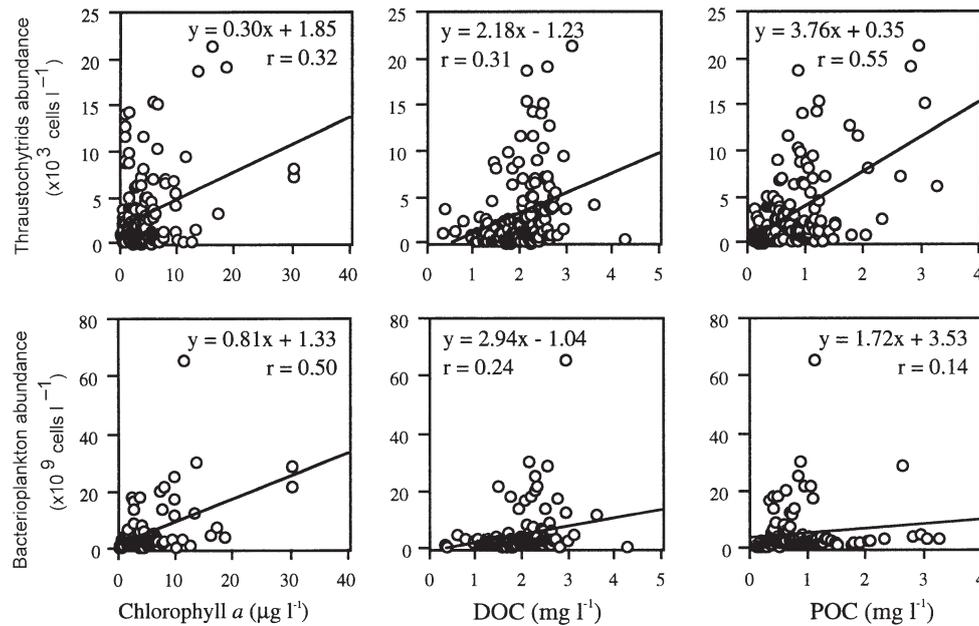


Fig. 2. Correlation between the decomposers (abundances of thraustochytrids and bacterioplankton) and environmental parameters of phytoplankton (chlorophyll *a*, $\mu g\ l^{-1}$), dissolved organic carbon (DOC, $mg\ C\ l^{-1}$) and particulate organic carbon (POC, $mg\ C\ l^{-1}$) concentrations. $n = 165$

In contrast, the highest bacterioplankton abundance, both site-/depth-specific and site-/depth-averaged, was found in Osaka Bay (Sites 30 to 37). The highest chl *a* concentration was also observed there, and thus a connection between bacterioplankton abundance and chl *a* concentration is implied in Osaka Bay.

Both Hiroshima Bay and Osaka Bay are highly eutrophic areas of the Seto Inland Sea (Okaichi & Yanagi 1997). However, these bays are contrasted in the postulation that Hiroshima Bay has a close thraustochytrid-POC connection whereas Osaka Bay is rather dominated by a bacterioplankton-chl *a* connection. This postulation suggests that different ecological processes occur in these similarly eutrophic bays.

Correlation between decomposer abundances and environmental parameters

The measured parameters thraustochytrid abundance ($\times 10^3$ cells l^{-1}), bacterioplankton abundance ($\times 10^9$ cells l^{-1}), chl *a* concentration ($\mu g\ l^{-1}$), DOC concentration ($mg\ C\ l^{-1}$) and POC concentration ($mg\ C\ l^{-1}$) were correlated (Fig. 2). The correlation coefficients are shown in Table 4.

The abundance of planktonic thraustochytrids was positively corre-

lated to that of bacterioplankton and phytoplankton (chl *a* concentration) and to DOC and POC concentrations by linear regression ($y = ax + b$). In particular, thraustochytrid abundance was most significantly correlated to POC concentration ($r = 0.547$). The contribution of thraustochytrid-C to POC was calculated to be $0.076 \pm 0.079\%$ of POC, based on $1.65 \times 10^{-4}\ \mu g\ C\ cell^{-1}$ (Kimura et al. 1999). Therefore, the involvement of thraustochytrid-C in POC is negligible. In a previous study (Raghukumar et al. 1990), thraustochytrid abundance and POC concentration were not significantly correlated in the pelagic Arabian Sea water. The study area, Seto Inland Sea, receives a river discharge of, for example, $62\ km^3\ yr^{-1}$ in 1985 (Yanagi 1996). This accounts for 7.6% of the volume of Seto Inland Sea ($816\ km^3$), and the residence time of river water was

Table 4. Correlation coefficients among thraustochytrids (Thra), bacterioplankton (Bact), phytoplankton (Chl *a*), dissolved organic carbon (DOC), particulate organic carbon (POC) and salinity (S) by linear regression equations ($n = 165$). * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

	Thra	Bact	Chl <i>a</i>	DOC	POC	S
Thra	1					
Bact	0.250**	1				
Chl <i>a</i>	0.318***	0.498***	1			
DOC	0.307***	0.236**	0.210**	1		
POC	0.547***	0.143	0.277***	0.291***	1	
S	-0.261***	-0.544***	-0.709***	-0.330***	-0.345***	1

estimated to be 1.2 yr (Okaichi & Yanagi 1997). The riverine influence with the input of terrigenous organic matter may favor the habitation of thraustochytrids. Although the riverine influence is rather small and locally limited compared with other major estuaries receiving greater river discharges (e.g., Biggs & Howell 1984), the high abundance of thraustochytrids was connected to river discharge (Naganuma et al. 1998, Kimura & Naganuma 2001). In this study, the correlation of thraustochytrid abundance with salinity was significant but correlation with POC, DOC and chl *a* concentrations was more significant (Tables 4 & 5).

It is thus suggested that microbial processes such as secondary production and degradation may be influenced by riverine input of terrigenous DOC and POC. An average of 40% of POC in this area is estimated to be from phytoplankton and bacterioplankton biomass (discussed below), and a certain fraction of the remaining 60% would be of terrestrial origin. Terrigenous organic matter often contains refractory substrates such as cellulosic and phenolic compounds (Ittekkot 1988). Thraustochytrids can degrade these refractory substrates (Heald & Odum 1970). Therefore, it might be hypothesized that the abundance of planktonic thraustochytrids is influenced by riverine input of terrigenous POC. This hypothesis is yet to be tested; however, circumstantial but supportive evidence has been reported (Kimura & Naganuma 2001).

On the other hand, bacterioplankton abundance was most significantly correlated to chl *a* concentration ($r = 0.498$) and secondarily to DOC ($r = 0.236$) concentration, as shown by the simple linear regression equation (Fig. 2), as previously shown in the Seto Inland Sea (Naganuma & Miura 1997). The correlation between bacterio- and phytoplankton is well known for a wide variety of waters over large temporal and spatial scales (e.g., Fuhrman et al. 1980, Bird & Kalff 1984, Cole et al. 1988, Cho & Azam 1990, Bird & Karl 1999). The bacterio- and phytoplankton coupling is viewed as bacterial dependence on the exudate DOC from phytoplankton (e.g. Cole et al. 1982, Chrost & Faust 1983).

The abundances of planktonic thraustochytrids ([thra], $\times 10^3$ cells l^{-1}) and bacterioplankton ([bact], \times

10^9 cells l^{-1}) were further correlated to the POC, DOC and chl *a* concentrations and salinity ([S], psu) by the multiple regression analysis as follows:

$$[\text{thra}] = 3.287 [\text{POC}] + 1.138 [\text{DOC}] + 0.227 [\text{chl } a] + 0.595 [S] - 22.024 \quad (r = 0.596, n = 165)$$

$$[\text{bact}] = 0.381 [\text{chl } a] + 1.059 [\text{DOC}] - 0.930 [\text{POC}] - 2.994 [S] + 99.846 \quad (r = 0.517, n = 165)$$

Previous studies reported that the fungoid protists, thraustochytrids, are among the major marine decomposers of: plant-derived, often refractory, organic matter in riverine input (Bremer 1995, Bremer & Talbot 1995, Valiela 1995); mangrove leaf litter (Ulken 1984, Raghukumar 1988, Raghukumar et al. 1994, Bremer 1995); phanerogamic material (Fell & Master 1973, Findlay et al. 1986); brown algal tissue (Miller & Jones 1983, Sathe-Pathak et al. 1993, Sharma et al. 1994); and fecal pellets of tunicate salp (Raghukumar & Raghukumar 1999). It is presumed that relatively large-sized (3.5 to 20 μm) thraustochytrids degrade and utilize relatively large organic materials (micrometer-sized POM).

Estimation of POC composition: implication of different preferences for decomposing substrates

A certain fraction of POC is composed of phytoplankton biomass that is represented by chl *a* concentration. The ratio of phytoplankton-C to chl *a* concentration is generally regarded as 30 to 90 g C per g [chl *a*] (Banse 1977). The ratio is calculated as the slope of the linear regression of [POC]-[chl *a*] correlation. The slope obtained in this study was 37.4 (Fig. 3), which is regarded as the minimum model ($y = ax + b$). The y-intercept ($0.57 \text{ mg C } l^{-1}$), 78.1% of total POC, is

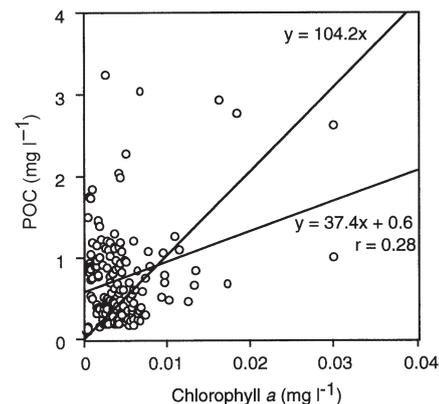


Fig. 3. Correlation between phytoplankton (chlorophyll *a*, $\text{mg } l^{-1}$) and particulate organic carbon (POC, $\text{mg C } l^{-1}$) concentrations. $n = 165$

Table 5. Standard partial regression coefficients, or beta weight (b), for the multiple regression between the planktonic decomposers (thraustochytrids [thra] or bacterioplankton [bact]) and the environmental parameters [chl *a*], [DOC], [POC] and [S] ($n = 165$). * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

	[chl <i>a</i>]	[DOC]	[POC]	[S]
[thra]	0.245**	0.160*	0.478***	0.131
[bact]	0.235*	0.085	-0.077	-0.376***

regarded as non-phytoplanktonic POC by this model. On the other hand, forcing the y -intercept to zero (i.e., assuming that all POC is phytoplanktonic), the slope would be 104.2 (Fig. 3). This is regarded as the maximum model ($y = a'x$). Then the overall average concentration of chl a ($4.26 \mu\text{g l}^{-1}$) yields a phytoplankton biomass of 0.16 and 0.44 mg C l^{-1} , accounting for 21.7 and 60.3%, respectively, of the overall average POC concentration (0.73 mg C l^{-1}).

Similarly, a certain fraction of POC is composed of bacterioplankton biomass. This accounts for 19.7% of the overall average POC concentration. However, it should be noted that some fraction of bacterioplankton cells might be missed from the POC collection by GF/F filtration and thus from the POC determination. This may have caused overestimation of the bacterioplankton-C contribution to the total POC pool.

These estimates suggest that a certain fraction (only ambiguously estimated as 20 to 60%) of total POC is composed of phytoplankton-C, and about 20% of POC was ascribable to bacterioplankton biomass. The remaining POC was assumed to be non-phytoplanktonic, non-bacterioplanktonic sources such as phytodetritus and terrigenous matter. The contributions of microzooplankton-C and net-zooplankton-C were negligible, accounting for only 0.1 to 3.9% and 1.8 to 4.8%, respectively, of total POC in the Seto Inland Sea (Uye et al. 1996, Uye & Shimazu 1997). The statistical observation that thraustochytrid abundance was most significantly correlated to POC concentration leads to the idea that thraustochytrid and POC are closely coupled, particularly the POC of non-phytoplankton and non-bacterioplankton-C. In contrast, bacterioplankton abundance was more highly correlated to chl a concentration and insignificantly correlated to POC concentration. This suggests that bacterioplankton abundance responds mainly to chl a concentration but not to the non-phytoplankton-dominated POC pool. The apparent coupling of thraustochytrid abundance and POC concentration implies certain functional activity of thraustochytrids, such as exoenzymatic degradation, on POM. Further study using concentrated DOM and POM on thraustochytrid growth is underway to evaluate the role of thraustochytrids in the production and cycling of aquatic organic materials.

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