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The pelagic microbial food web structure in Sanggou Bay, Yellow Sea: Spatial variation over four successive seasons

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Abstract. Sanggou Bay (Yellow Sea, China) is a small semi-closed bay in the eastern part of the Shandong Peninsula. In order to characterise the Sanggou Bay microbial food web (MFW) structure, we first documented, over four successive seasons, the distributions of environmental variables and abundances and biomasses of heterotrophic prokaryotes (HP), *Synechococcus* (SYN), picoeukaryrotes (PEUK), heterotrophic and pigmented nanoflagellates (HNF & PNF) and ciliates. The four season distributions in the Sanggou Bay of environmental variables and MFW components were submitted to cluster analysis, leading to distinguish Inner Bay and Outer Bay clusters at each season. In addition, Outer Bay MFW was found identical to the Inner Bay one but with a delay of one season, thus limiting to 4 the number of MFW characterising Sanggou Bay in that survey. We confirmed the existence of a strong relationship between HNF and HP, and extended this empirical relationship to the other MFW components: SYN, PEUK, PNF and ciliates. We also established upper and lower empirical linear boundaries for all the MFW component relationships with HP. The existence of these boundaries in the complex system made by the MFW stresses the need for systemic studies like the ones conducted for multi-enzyme systems and metabolic pathways that lead to the metabolic control theory. To better determine the MFW structure, we normalised for each sample, the biomass of the MFW components by that of HP. The normalised biomasses of SYN, PEUK, PNF and HNF had obvious seasonal variations with high values in summer or autumn, while ciliate normalised biomasses were low in summer and exhibited high values in winter. The main MFW-structure difference between Inner and Outer Bay clusters came from biomass differences for SYN, PEUK and PNF, whereas other component biomass-values were similar between Inner and Outer Bay clusters. Our study showed that the normalisation method could be used in other marine area to study the microbial food web structure. Indeed, the efficiency of this approach to determine MFW structure was demonstrated by successfully applying it to a similar data set from the literature and related to the Arabian Sea.

1 Introduction

The marine planktonic microbial food web (MFW) encompasses viruses, heterotrophic (HP) and autotrophic (*Synechococcus* and *Prochlorococcus*) prokaryotes, eukaryotic phytoplankton, nanoflagellates and ciliates (Pomeroy, 1974; Azam et al., 1983; Sherr and Sherr, 1988; Kirchman,

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2010). One way to describe the MFW structure was to establish numerical relationships between its different components with respect to their abundances or biomasses. The relationship between HP and flagellate abundances was studied by Sanders et al. (1992) and Gasol (1994) by compiling available data collected from many ecosystems. Similarly, Fenchel (2008) stated that bacteria and flagellate abundances in the water column were around 10^6 and 10^3 cells cm^{-3}, respectively. Miki and Jacquet (2008) clearly stated that typical "relative abundance" for viruses, bacteria and heterotrophic nanoflagellates (HNF) was 10^{-1} to 10^{-3}. To our knowledge, relationships between other MFW members were not previously reported.

The coast geomorphology increases the habitat diversity (Pierrot-Bults and Angel, 2012). In many places around the world ocean, the shoreline curvature forms bays, some of them trapping seawater inside and thus generating environmental conditions different from outside the bay. How to decide whether MFWs in different sea areas or periods are different, is another less studied question. Garrison et al. (2000) were the first to apply cluster analysis to the biomass of the MFW components in order to assess whether one MFW could be different from others. However, the cluster analysis could not provide information on what the difference is. Because of the insufficient taxonomic information for most of the components (except tintinnids), taxonomic differences could not be used to compare different MFW structures.

The present study aimed at (i) determining distribution patterns of environmental variables and MFW-component abundances and biomasses, (ii) establishing relationships between HP and MFW components other than HNF and HP as they were not yet documented and (iii) characterising the MFW structure in a given environment. To conduct this investigation, we monitored abundances and biomasses of MFW components over four successive seasons in Sanggou Bay. Cluster analysis distinguished between Inner Bay and Outer Bay. By normalising MFW-component biomasses by that of the related HP biomass, we defined a way to characterise the MFW structure. We could demonstrate that MFW structure inside and outside Sanggou Bay could be distinguished due to environmental differences and by taking into account additional tintinnid taxonomic data. We also successfully applied this approach to a similar data set from the literature (Garrison et al.; 2000) to determine the MFW structure in the Arabian Sea.

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2 Materials and Methods

2. 1 Study site and sampling strategy

Sanggou Bay is a small (144 km^2) semi-enclosed (mouth of 11.5 km) bay in the east part of Shandong Peninsula in the Yellow Sea (Fig. 1) where the seasonal temperature variation is the largest (> 12 °C) in the world ocean (Mackas et al., 2012). The maximum and average depths are 21 and 7.5 m, respectively.

The Sanggou Bay MFW was assessed through 19 study sites in an area delimited by 37.02-37.15 ℃N and 122.45-122.65 ℃E (Fig.1). Four cruises were conducted on April 23-25 (spring), August 2-4 (summer), October 26-27 (autumn), 2011 and January 5-6 (winter), 2012, on board the R/V *Lurongyuyang-65577* and the same 19 stations displayed in Fig. 1 were occupied and sampled at each cruise.

At each station, surface seawater samples were collected by bucket. Surface temperature and salinity were determined by using a portable water quality analyzer YSI (Professional Plus made in USA) by dropping the probe into the bucket seawater. Different subsamples were collected for determining chlorophyll *a* (Chl *a*) and nutrient concentrations, for flow cytometry analysis, and for determining flagellate and ciliate abundances. In the case of flagellates, a few spring samples were lost.

2. 2 Chlorophyll *a* and nutrients

The chlorophyll *a* concentration was determined by an ACLW-RS chlorophyll turbidity temperature sensor (ALEC Electronics Co., Ltd., Japan) with a precision of ± 0.1 μg dm^{-3}.

Water samples (1 dm^3) from every station were filtered through an acid pre-cleaned 0.45 μm pore-size acetate cellulose filter (Development Center of Water Treatment Technology, Hangzhou, China), and the filtrates were poisoned by addition of saturated $HgCl_2$ (ca. 1.5×10^{-3} v/v), preserved in low-density polyethylene bottles at room temperature and then analysed in the laboratory. Nutrients including NO_3^-, NO_2^- were determined spectrometrically using an autoanalyzer (Model: SKALAR SAN plus), while NH_4^+ and PO_4^{3-} were determined according to a manual method (Maita et al., 1984). The concentration of dissolved inorganic nitrogen (DIN) is the sum of NO_3^-, NO_2^- and NH_4^+ concentrations.

2. 3 Flow cytometry

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For flow cytometry analysis, subsamples (5 cm^3) were fixed onboard with paraformaldehyde (final concentration 1%), kept at room temperature for 10 to 15 minutes, and then freeze-trapped and stored in liquid nitrogen on the boat (Thyssen et al., 2005). Samples were stored at -80 ℃ once in the laboratory where they were processed within 3 months. Before analysis, the seawater samples were thawed at room temperature (about 20 min). Picoplankton, including *Synechococcus* (SYN), phototrophic picoeukaryotes (PEUK), and heterotrophic prokaryotes (HP) were analysed with a FACSVantage SE flow cytometer (Becton Dickinson) equipped with a Coherent water-cooled Argon laser (488 nm, 1 W). When analysing autotrophic picoplankton, subsamples (1 cm^3) were initially supplemented with 1 mm^3 bead (2 μm, Polysciences) suspension to be used as internal standard, and red fluorescence was set as the trigger signal to discard signals from inorganic particles and heterotrophic prokaryotes. SYN and PEUK were resolved on the basis of their side scatter and red fluorescence signals. For HP analysis, seawater subsamples (50 mm^3) were diluted 5 fold with TE buffer (Tris-EDTA, 100 mM Tris-Cl, 10 mM EDTA, pH=8.0, Sigma, USA), then stained with the nucleic acid dye SYBR Green I (Molecular Probes, USA) (final dilution 10^{-4}, v/v) and let incubate 20 min in the dark before analysis. HP were resolved on the basis of their green fluorescence from staining and scatter properties. Data was collected and analysed with CellQuest software (Version 3.3, Becton Dickinson). Biomass values of SYN, PEUK and HP were calculated by using the following conversion factors: 200 fg C $cell^{-1}$ (Mackey et al., 2002), 1393 fg C $cell^{-1}$ (Verity et al., 1992), 20 fg C $cell^{-1}$ (Lee and Fuhrman, 1987) respectively.

2. 4 Nanoflagellates

Samples for the enumeration of nanoflagellate (NF) cells were pre-filtered by gravity through a nylon mesh of 20 μm pore size, then fixed with cold glutaraldehyde (final concentration 0.5 %, v/v). Subsamples (20 cm^3) were filtered onto 0.2 μm pore size black polycarbonate membrane filters at <100 mm Hg pressure. When 1 cm^3 of the sample remained in the funnel, the vacuum pump was turned off and the sample was stained with DAPI (final concentration 10 μg cm^{-3}) for 5 min, then the pump was turned on again, to let the residual liquid completely pass through the membrane filter. The filter was then mounted on a microscope slide, a few drips of paraffin were dropped on the filter center and a coverslip was placed on the top. Finally, the sample slide was immediately stored in the dark at -20°C.

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Nanoflagellate cells were counted by epifluorescence microscopy (Leica DM4500B) at 1000× magnification. Pigmented NFs (PNF) were distinguished from heterotrophic NFs (HNF) by the presence of red-autofluorescence in the former with a blue excitation filter set (Tsai et al., 2005). At least 30 fields of view were examined. The abundance of flagellates was calculated from the average of cell counts made on duplicate samples. The mean cell volumes were estimated and converted to carbon biomass using a conversion factor of 0.22 pg C μm^{-3} (Børsheim and Bratbak, 1987).

2. 5 Ciliates

Seawater samples (1 dm^3) for ciliate counts were fixed with Lugol (final concentration 1%). Each ciliate sample was concentrated to about 100 cm^3 by gently siphoning out the supernatant after 48 h settling. A subsample of 20 or 25 cm^3 of the concentrated sample was settled in an Utermöhl counting chamber for 24 h and examined using an Olympus IX 71 inverted microscope at 100× or 200× magnification. Aloricate ciliates and tintinnids were counted and their abundances calculated respectively. Species of tintinnids were identified based on their morphological characteristics according to references (Kofoid and Campbell, 1929; 1939; Zhang et al., 2012). Ciliate dimensions including body length, oral diameter, etc., were measured and average bio-volume of each taxon was estimated from appropriate geometric shapes. Biomass values of aloricate ciliates were calculated from their bio-volume multiplied by a conversion factor (0.19 pg C μm^{-3}) (Putt and Stoecker, 1989). The tintinnid biomass was assumed to occupy 30% of the lorica volume (Gilron and Lynn, 1989).

2. 6 Statistical analysis

Univariate correlation analyses based on Spearman rank correlation coefficient were carried out using the statistical program SPSS V16.0. Cluster analysis was performed using Primer 5.0 based on (i) environment variables (temperature, salinity, Chl *a* and nutrient concentrations), (ii) biomasses of six microbial groups, and (iii) abundances of different tintinnid species. Group-average linkage based on Bray-Curtis similarity matrix of fourth root transformed from original data was used.

3 Results

3. 1 Environmental variables and Chl *a* concentration surface distribution

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Temperature varied within a large range (Fig. 2; see also Table S in supplementary material). The lowest temperature (1.90°C) was observed in winter and the highest (24.20°C) in summer. In spring and summer, the inside bay exhibited higher temperature than the outside bay (Fig. 2A, B). In autumn and winter, temperature out of the bay was higher than inside (Fig. 2C, D).

Salinity fell in the range 26.17-31.57 (Fig. 2; see also Table S1 in supplementary material). In summer, autumn and winter, salinity was higher in the outside bay than in the inside bay (Fig. 2B, C, D). It was the opposite in spring, except for St. 14 which had the lowest salinity (30.21) (Fig. 2A).

Over the one year survey, Chl *a* concentration varied from 0.42 to 38.74 μg dm^{-3} (Fig. 2; see also Table S1 in supplementary material). In spring, high Chl *a* concentration was equally distributed in both outside and inside bay with a narrow band of low values in between (Fig. 2A). In summer and autumn, Chl *a* concentration was very high in the inside bay (Fig. 2B, C) and sharply decreased towards the outside bay. In winter, it was higher in the outside bay than in the inside bay (Fig. 2D).

3. 2 Microbial-component surface distribution

Annually, HP abundance fell in the range of 2-68 $\times 10^5$ cells cm^{-3}, and subsequently, HP biomass varied in the range of 3.77-135.77 μg C dm^{-3} (see Table S1 in supplementary material). Surface HP biomass showed higher values in the inside than outside bay over all seasons (Fig. 2). SYN was present all year round with abundance in the range 0.01-264$\times 10^3$ cells cm^{-3} and with biomass in the range 0.00-52.84 μg C dm^{-3} (see Table S1 in supplementary material). Higher SYN biomass was observed in the outside bay in spring and winter (Fig. 2A, D), and in the inside bay during summer and autumn (Fig. 2B, C). PEUK and PNF abundances varied in ranges 0.40-245$\times 10^3$ cells cm^{-3} and 144-136442 ind. cm^{-3}; biomasses varied in ranges 0.55-340.85 μg C dm^{-3} and 0.38-512.52 μg C dm^{-3}, respectively (see Table S1 in supplementary material), and always exhibited higher values in the inside bay (Fig. 2). HNF abundance fell in the range 171-35183 ind. cm^{-3} and HNF biomass fell in the range 0.38-116.19 μg C dm^{-3} over the study (see Table S1 in supplementary material). In summer, autumn and winter, HNF biomass was higher in the inside bay than in the outside bay (Fig. 2B, C, D). In spring, HNF biomass reached higher values in both inside and outside bays with a narrow low-value band in between (Fig. 2A). The annual range of ciliate abundance was 500-61667 ind. dm^{-3} and their biomass range was 0.44-33.09 μg C dm^{-3} (see also Table S1 in supplementary material). The surface ciliate biomass

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exhibited higher values in the inside bay over the four covered seasons (Fig. 2).

3. 3 Tintinnid surface distribution

The surface abundance distribution of tintinnids varied with seasons. In spring, the tintinnid abundance reached a maximum value at St.19, in the inside bay, and decreased from the inside to the outside bay (Fig. 3A). In summer and autumn, the tintinnid abundance was higher in the central part of the bay than around it (Fig. 3B, C). In winter, tintinnid abundance increased from the inside to the outside bay and followed an opposite distribution pattern in spring (Fig. 3D).

Twenty six tintinnid species belonging to seven genera were identified during the whole survey. Different species had different surface-abundance-distribution patterns. In spring, among the five observed species, four belonged to *Tintinnopsis*. *T. beroidea* was mainly present south of the bay. *T. rapa* was identified at almost every station. *T. acuminate* was mainly present in the outside bay, and *T. brasiliensis* in the inside bay (Fig. 3A).

In summer, the species richness was 15. Four species were found at more than 6 stations. *T. beroidea* was present at almost every station. *T. acuminate* mainly occupied north of the bay. *Tintinnidium primitivum* was mainly found in the outside bay, while *T. kofoidi* was mainly found in the inside bay (Fig. 3B).

In autumn, the species richness was 13. Five species were found in more than 6 stations. *T. beroidea* was present in almost every station. *T. tubulosoides* occupied the center of the bay. *T. nana* and *T. primitivum* were mainly present in the outside bay, while *Eutintinnus tubulosus* was mainly distributed in the inside bay (Fig. 3C).

In winter, 10 species were identified, but only *T. nana* occurred in more than 6 stations, being mainly present northeast of the bay. *T. beroidea* and *T. acuminate* were the dominant species over the other seasons. *T. beroidea* was mainly observed in the outside bay, and *T. acuminate* was only found at St. 5 in the southeast of the bay (Fig. 3D).

3. 4 Cluster analysis

Three distinct cluster analyses were run on three data sets, each encompassing 4 seasons and covering (i) environmental conditions (including temperature, salinity, Chl *a* and nutrient

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concentrations, figure not shown), (ii) biomasses of the MFW components (Fig. 4) and (iii) abundances of the tintinnid community (Fig. 5). For each season, all cluster analyses could divide the stations into similar Inner Bay stations and Outer Bay cluster thus discriminating distributions between inside and outside bay (Fig. 6). Though the shape and position of the division lines between clusters were different from one season to another, they were similar to the nearest isothermal generally.

When applied to the biomasses of all microbial groups over the four seasons, the cluster analysis showed that the Outer Bay MFW was one season ahead the Inner Bay MFW. For example the Outer Bay MFW in summer was similar to that of the Inner Bay in autumn (Fig. 4, Fig. 6). We artificially defined the MFW as Spring MFW, Winter MFW, Autumn MFW and Summer MFW as in Fig 4 and consequently, if we refer to seasons, in summer, the Summer MFW occurs in the Outer Bay whereas the Spring MFW occurs in the Inner Bay. Similarly, the tintinnid community could be defined as Spring Tintinnid Community, Winter Tintinnid Community, Autumn Tintinnid Community and Summer Tintinnid Community, too (Fig. 5). Thus, in spite of the existence of two distinct sub-domains (Inner and Outer Bay MFW), only four MFW types could be distinguished over seasons and they were shifted by one season between Outer and Inner Bay.

3. 5 Relationships

3. 5. 1 Relationships between abundances of HP and the other microbial groups

Considering the empirical relationship between the logarithm of HP and HNF abundances reported by Gasol and Vaqué (1993) and further explored by Gasol (1994), we found that our data were satisfying such a relationship (Table 1; Fig. 7). We found significant positive correlations between (log) abundances of HP and other five microbial groups when taking into account all the survey data (Table 1; Fig. 7). The strongest correlation was between HNF and HP.

Gasol (1994) defined a boundary upper limit to HNF abundance for a given HP abundance called the maximum attainable abundance line:

$$\text{Log HNF}_{max} = -2.47 + 1.07 \text{ Log HP} \quad (1)$$

After verifying that this empirical boundary upper limit was also valid for our HNF-HP data set (Fig. 7), we formulated empirical construction as detailed in supplementary material (S1) and defined similarly a boundary lower limit for HNF abundance (Fig. 7). We further applied this empirical

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construction to define upper and lower boundaries for the abundance relationships of the other MFW components with HP (Fig. 7). All these boundaries are defined by the same slope and different intercepts linked to the level of the related component abundance values. PNF had the same upper boundary (Eq. (1)) as HNF, and the boundary upper limits of SYN, PEUK and ciliate abundances were separately defined by:

$$\text{Log SYN}_{max} = -1.74 + 1.07 \text{ Log HP} \quad (2)$$

$$\text{Log PEUK}_{max} = -1.45 + 1.07 \text{ Log HP} \quad (3)$$

$$\text{Log Ciliates}_{max} = -4.83 + 1.07 \text{ Log HP} \quad (4)$$

The empirical lower limit boundaries (minimum attainable abundance line) for SYN, PEUK, HNF, PNF and ciliate abundances were defined by the following equations as explained in the supplementary material:

$$\text{Log SYN}_{min} = -6.21 + 1.07 \text{ Log HP} \quad (5)$$

$$\text{Log PEUK}_{min} = -4.42 + 1.07 \text{ Log HP} \quad (6)$$

$$\text{Log PNF}_{min} = -4.82 + 1.07 \text{ Log HP} \quad (7)$$

$$\text{Log HNF}_{min} = -4.36 + 1.07 \text{ Log HP} \quad (8)$$

$$\text{Log Ciliates}_{min} = -7.44 + 1.07 \text{ Log HP} \quad (9)$$

3. 5. 2 Relationships between HP and other microbial group biomasses

HP biomass had significant positive correlations with the biomass of the other five microbial groups (Table 2) when taking into account all the survey data (Fig. 8). The strongest correlation was between PNF and HP.

In winter, the biomass of all microbial groups was low, and the variation range was narrow. It was thus difficult to find out a relationship between HP biomass and the biomasses of the other microbial groups. In contrast, a linear relationship could be established in spring, summer and autumn. SYN biomass remained a low value in spring and summer, and increased with HP biomass in autumn. PEUK biomass kept increasing with HP biomass at all seasons but winter. PNF biomass remained low in autumn, and increased with HP biomass in spring and summer. HNF remained relatively low and constant in spring and autumn, and increased with HP biomass in summer. Ciliate biomass remained relatively low and constant in spring, whereas in summer and autumn, it first increased and then

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decreased dramatically (Fig. 8).

Despite the different consecutive seasons, every MFW component biomass varied within a limited range as shown on Fig. 8. The biomass of HP was larger than that of the other MFW components in spring and winter. In summer and autumn, SYN, HNF and ciliates still had biomasses lower than that of HP, but PEUK and PNF biomasses could surpass that of HP biomasses (Fig. 8).

3. 5. 3 Abundance relationships between predators (HNF, ciliates) and their preys

When taking into account all the survey data, we found significant positive correlations between (log) abundances of HNF and that of HP, SYN and PEUK (Table 3; Fig. 9A). In contrast, the correlation between (log) abundances of ciliates and NF (nanoflagellates) was quite weak but still significant (Table 3, Fig. 9B).

When considering seasons separately, the corresponding relationships between biomasses of predators (HNF, ciliates) and their preys varied with seasons. In spring and winter, biomasses of both predators and their preys were very low. In summer, HNF biomass increased with that of HP, SYN and PEUK, whereas in autumn it kept relatively stable in spite of the biomass increase of HP, SYN and PEUK (Fig. 10A). In summer and autumn, the ciliate biomass increased significantly while the range of NF biomass was narrow. However, the ciliate biomass dropped drastically when the NF biomass exceeded 400 μg C dm^{-3} (Fig. 10B).

3. 6 MFW structure based on biomass standardisation

To better assess the structure of the MFW, the biomass values of the MFW components were normalised by that of HP belonging to the same sample (Table S2). The annual averaged structure of MFW was thus HP: SYN: PEUK: PNF: HNF: ciliates=1: (0.10 ± 0.14): (0.96 ± 0.87): (0.95 ± 0.85): (0.36 ± 0.29): (0.17 ± 0.14) (Fig. 11). Among them, PNF normalised biomass had the largest variation range from 0.07 (St.7 in winter) to 4.42 (St. 18 in summer). SYN normalised biomass had the narrowest variation range from 0.00 (St.3 in spring) to 0.51 (St.11 in autumn) (Fig.11). The relative biomasses of the MFW components exhibited large differences with respect to seasons, except that of ciliates that was rather constant. The lowest averaged relative biomasses of SYN, PEUK were observed in spring and that of PNF and HNF in winter. The largest averaged relative biomasses of PEUK, PNF

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and HNF occurred in summer and that of SYN in autumn. For ciliates, the lowest averaged relative biomass value was observed in summer and the largest in winter (Fig. 11).

The discrimination between Inner Bay and Outer Bay was brought by the cluster analysis of abiotic and biotic features. It was also supported by the existence of different MFW structures (Fig. 12). Differences between Inner Bay and Outer Bay were mainly caused by PEUK and PNF in spring and summer where the PNF biomass was higher in Inner than Outer Bay, while the PEUK biomass was higher in Inner than Outer Bay in spring, and the reverse in summer. In autumn, the difference between Inner and Outer Bay was mainly due to SYN and PEUK whose biomass was higher in Inner Bay than in Outer Bay. In winter, the difference between Inner and Outer Bay was mainly caused by PNF, which also had higher biomass in Inner Bay than in Outer Bay (Fig. 12).

4 Discussion

4.1 Distribution of the environmental variables and MFW-component abundances

The investigation of the Sanggou Bay environmental features over 4 successive seasons confirmed the report by Mackas et al. (2012) stating that its seasonal temperature variation was the largest in the world ocean. Indeed, in this study, the surface water temperature varied from 1.90°C to 24.20°C. The Sanggou Bay exhibited different trophic regimes, from oligotrophy to eutrophication with Chl *a* concentration varying from 0.42 to 38.74 μg dm^{-3}. In warm summer and autumn, there were large temperature and trophic gradients from inner to outer part of the bay. These large gradients corresponded to a large range of HP abundance, (0.2-6.3) $\times 10^6$ cells cm^{-3}, similar to the HP abundance ranges (0.04-15.85) $\times 10^6$ cells cm^{-3} and (0.16-15.85) $\times 10^6$ cells cm^{-3} reported by Sanders et al. (1992) and Gasol (1994) respectively. Tintinnids were the only MFW components that could be identified at the species level which was instrumental in distinguishing Inner Bay from Outer Bay.

4.2 Cluster analysis

In our study, the data from all stations sampled over the 4 successive seasons were submitted to cluster analysis with respect to environmental condition parameters, MFW parameters and tintinnid communities. They distinguished two parts in the bay that we reported as Inner and Outer Bay cluster which were characterised by distinct environmental features (Fig. 6) and hosted two different MFWs.

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This was demonstrated by comparing the relative biomasses of the MFW components in the Inner and Outer bay respectively (Fig. 12). Consequently, at this stage, it would have been reasonable to foresee the existence of 8 distinct MFW over the 4 investigated seasons. However, in addition to generating different MFWs in the Inner and Outer Bay, the topography of the bay also influenced the seasonal succession of the MFW components. Indeed, the cluster analysis applied to the whole set of biomass data only distinguished 4 MFWs labeled Spring MFW, Summer MFW, Autumn MFW and Winter MFW that occurred with one season phase shift in Inner and Outer Bay, the Outer Bay MFW being one season ahead the Inner Bay MFW. To our knowledge, this study is the first one to show that a small bay could host a MFW different from the one in neighboring coastal water.

4. 3 Abundance relationships between HP and the other MFW components

Gasol (1994) established an empirical relationship between HP and HNF abundances. We first showed that this relationship was also satisfied by HP and HNF abundances in Sanggou Bay. Thus we demonstrated that a similar empirical relationship could be defined between HP and the other MFW component abundances. We also found that the empirical ceiling limit of HNF abundance for any HP abundance value defined by Gasol (1994) was also valid for the Sanggou bay data. Similarly, we empirically determined (see supplementary material) a minimum attainable abundance in the log/log representation of HNF abundance versus HP abundance (Fig. 7). Finally, in addition to the extension of the empirical relationship of Gasol (1994) to the other MFW components we could extend the existence of upper and lower limits to the other MFW components as well (Fig. 7). The lower limit boundaries (minimum attainable abundance line) of PEUK, HNF, PNF were similar. That of SYN was lower because of its very low abundance in spring. Abundances of ciliates were much lower than those of the other MFW components, and occasionally were < 1 ind. cm^{-3} which made the ciliate lower limit boundary the lowest in the MFW.

When considering biomass relationships, we found that SYN, HNF and ciliates biomasses could not exceed that of HP during four seasons. However, PNF and PEUK biomass could sometimes exceed that of HP in summer where their mean values were the highest, and autumn for PEUK only. Usually, HP abundance (biomass) increases after the onset of the phytoplankton spring bloom when more dissolved organic matter is made available. During spring bloom the development of large

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phytoplankton cells is favoured at the expense of the small ones (PEUK). This trend is reversed by depletion of nutrients, on the way to oligotrophy and this is why PEUK and PNF mean abundance (biomass) values were the highest in summer (Table S1, supplementary material). When HP biomass was over 100 μg C dm^{-3}, ciliate biomass decreased which could result from a more effective predation on ciliates.

To our knowledge, this is the first report on the extension of the relationship between HP and HNF abundances to the other MFW components (SYN, PEUK, PNF, and ciliates). More work should be done to check whether these relationships remain valid in other marine environments. The MFW being a multi-component system, the reported empirical boundaries that could not apply to monospecific cultures, highly suggest the need for a systemic approach like the one developed for multienzyme systems and metabolic pathways that lead to the metabolic control theory (Cornish-Bowden, 1995).

4. 4 Predators (HNF, ciliates) and their preys in MFW

HNF can ingest HP, SYN and PEUK as predators in MFW, and be ingested by ciliates (Azam et al., 1983). There were significant positive correlations between (log) abundances of HNF and that of HP, SYN and PEUK, which indicated strong bottom-up control of HNF abundance by HP, SYN and PEUK in Sanggou Bay. The significant but quite weak relationship between (log) abundances of ciliates and NF (nanoflagellates: HNF & PNF) would reflect a top-down control of ciliates (likely by copepods) also playing an important role in Sanggou Bay.

The biomasses of HP, SYN and PEUK were too low to support the growth of HNF in winter and spring. In summer, SYN biomass and to a lesser extent PEUK biomass were highly limited by HNF. The biomass of HNF increasing while that of SYN and PEUK remained relatively constant can be interpreted as SYN and PEUK being consumed as soon as produced (strong top-down control). In autumn, HNF biomass appeared limited despite high biomasses of HP, SYN and PEUK. This could reflect predation on HNF. Field observations generally support that in summer, the increasing NF-biomass favors the ciliate-biomass increase whereas in autumn, ciliates are limiting NF-biomass. In Sanggou Bay, we observed that the ciliate biomass decreased dramatically when NF biomass exceeded 400 μg C dm^{-3} (Fig. 10B) which could reflect a more effective predation on ciliates.

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4. 5 MFW structure

The finding of the relationships between MFW component abundances/biomasses singled out the role of HP. Because of the spreading of individual abundances over 6 orders of magnitude, we chose to normalise the component biomasses by that of HP to address the structure of the MFW in a relevant way. This approach enabled to identify distinct MFW structures at each season (Fig. 11).

Even though the idea of using relative abundance and relative biomass to depict MFW structure was brought up by several authors (Garrison et al., 2000; Fenchel, 2008; Miki and Jacquet, 2008), our study is the first attempt to describe the MFW structure by normalising the different component biomasses with respect to HP biomass. We found only one data set in the literature, from Garrison et al. (2000), to which we could apply this new approach and demonstrate its usefulness to characterise MFW structure in different areas and distinct periods. The study reported by Garrison et al. (2000) was the first and only one evaluating the seasonal and spatial changes of MFW structure using cluster analysis of different MFW components and large phytoplankton biomass. The sampling dates in Garrison et al. (2000) were March 14th to April 10th, August 17th to September 15th, November 28th to December 27th, and January 8th to February 4th, and are similar to the sampling dates in Sanggou Bay, so that applying to the Arabian Sea the MFW structure determination developed for the Sanggou Bay was fully relevant.

The data from Table 5 in Garrison et al. (2000) were normalised with respect to HP biomass, yielding the following normalised biomasses: HP: SYN: PEUK: PNF: HNF: Ciliate = 1: (0.45 ±0.35): (0.07 ±0.05): (0.48 ±0.32): (0.38 ±0.19): (0.10 ±0.07) (Fig. 13). On that basis, the Arabian Sea MFW structure was determined for each season and the overall survey as illustrated by Fig 13. The results displayed in Fig. 13 show that the Arabian Sea MFW structure varied with seasons, in agreement with the finding of Garrison et al. (2000).

The resulting MFW structures showed that, compared with that of Sanggou Bay (Fig. 11), the Arabian Sea MFW structure exhibited a very low PEUK biomass and higher SYN biomass (Fig. 13) which can reasonably be assigned to differences between trophic regimes, eutrophy in coastal area and oligotrophy in oceanic area.

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5 Conclusions

The four season distributions in the Sanggou Bay of environmental variables and microbial food web (MFW) components were submitted to cluster analysis leading to distinguish Inner and Outer Bay cluster at each season. In addition, Outer Bay MFW was found one season ahead Inner Bay MFW, limiting to 4 the number of MFW characterising Sanggou Bay in that survey.

We confirmed the existence of a strong relationship between HNF and heterotrophic prokaryotes (HP), and extended the empirical relationship of Gasol (1994) to the other MFW components: *Synechococcus* (SYN), picoeukaryrotes (PEUK), pigmented nanoflagellates (PNF) and ciliates. We also established upper and lower empirical linear boundaries for all the MFW component relationships with heterotrophic prokaryotes (HP). Systemic approaches should be developed to investigate MFW as were multi-enzyme systems and metabolic pathways, leading to the metabolic control theory. There was strong bottom-up control of HNF abundance by HP, SYN and PEUK, and the top-down (likely by copepods) control of ciliates also played an important role in Sanggou Bay. There were also strong relations ships between predators (HNF and ciliates) and their preys.

The present study demonstrated for the first time that the microbial food web structure in a given environment and at a given season can be established by considering the biomasses of its components and normalising them by HP biomass. In the case of Sanggou Bay, this approach established distinct microbial food web structures with seasons and could account for environmental differences by resolving distinct MFWs between inside and outside bay.

The usefulness and efficiency of this approach was demonstrated by applying it to the only similar data set available in the literature and determining the microbial food web structure in the Arabian Sea at four successive seasons.

Author contributions. X. Chen did all the statistical analysis. W. Zhang and T. Xiao supervised the projects. M. Denis helped to organize the manuscript. Y. Zhao provided the data of picoplankton including heterotrophic prokaryotes, *Synechococcus*, picoeukaryrotes. L. Huang provided the nanoflagellates data. Z. Jiang provided the environmental variable and nutrient data. X. Chen prepared the manuscript with contributions from all authors.

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6

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Table 1. Characteristics of the relationships between HP and the other microbial group abundances

Microbial group	N	r^2	Constant	Slope	P
SYN	76	0.285	-5.90	1.51	< 0.01
PEUK	76	0.743	-5.48	1.56	< 0.01
PNF	72	0.792	-5.57	1.46	< 0.01
HNF	72	0.834	-3.41	1.09	< 0.01
Ciliates	76	0.321	-2.91	0.58	< 0.01

Note that the relationships were logarithmic. SYN: *Synechococcus*; PEUK: picoeukaryotes; HNF: heterotrophic nanoflagellates; PNF: pigmented nanoflagellates; HP: heterotrophic prokaryotes.

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Table 2. Characteristics of the relationships between biomasses of HP and the other microbial groups

Microbial group	N	r^2	Constant	Slope	P
SYN	76	0.285	-1.56	0.26	< 0.01
PEUK	76	0.743	-5.36	0.94	< 0.01
PNF	72	0.823	-28.08	2.12	< 0.01
HNF	72	0.794	-4.34	0.57	< 0.01
Ciliates	76	0.581	1.28	0.10	< 0.01

SYN: *Synechococcus*; PEUK: picoeukaryotes; HNF: heterotrophic nanoflagellates; PNF: pigmented nanoflagellates; HP: heterotrophic prokaryotes.

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Table 3. Characteristics of the abundance relationships between predators (HNF, ciliates) and their preys

Predator	Prey	N	r^2	Constant	Slope	P
HNF	HP	72	0.834	-3.41	1.09	< 0.01
HNF	SYN	72	0.349	2.34	0.26	< 0.01
HNF	PEUK	72	0.679	1.02	0.55	< 0.01
Ciliates	NF	72	0.211	-0.44	0.30	< 0.01

Note that the relationships were logarithmic. SYN: *Synechococcus*; PEUK: picoeukaryotes; NF: HNF + PNF; HNF: heterotrophic nanoflagellates; PNF: pigmented nanoflagellates; HP: heterotrophic prokaryotes.

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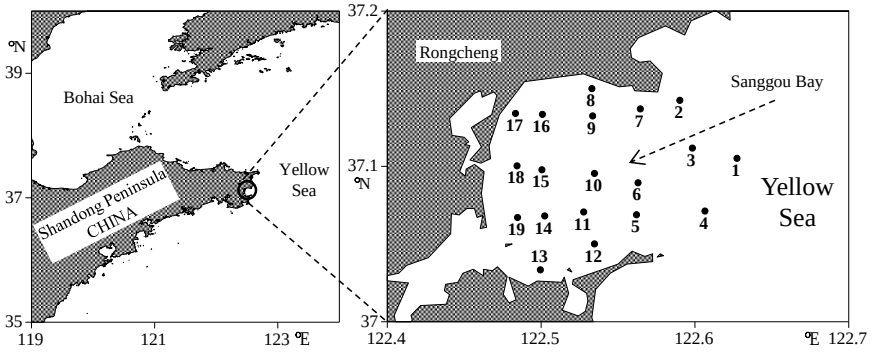

Figure 1. Sampling area and location of the sampling stations

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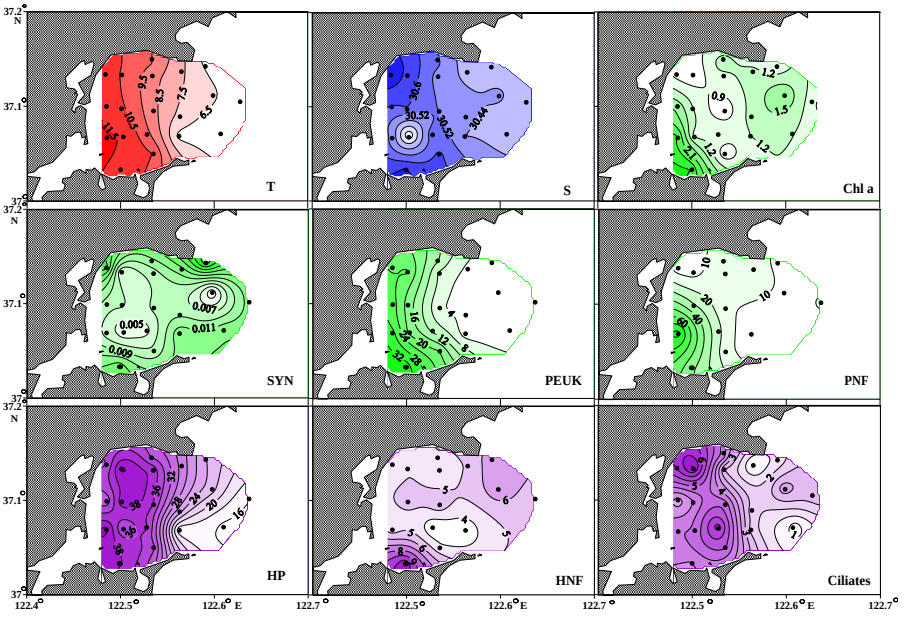
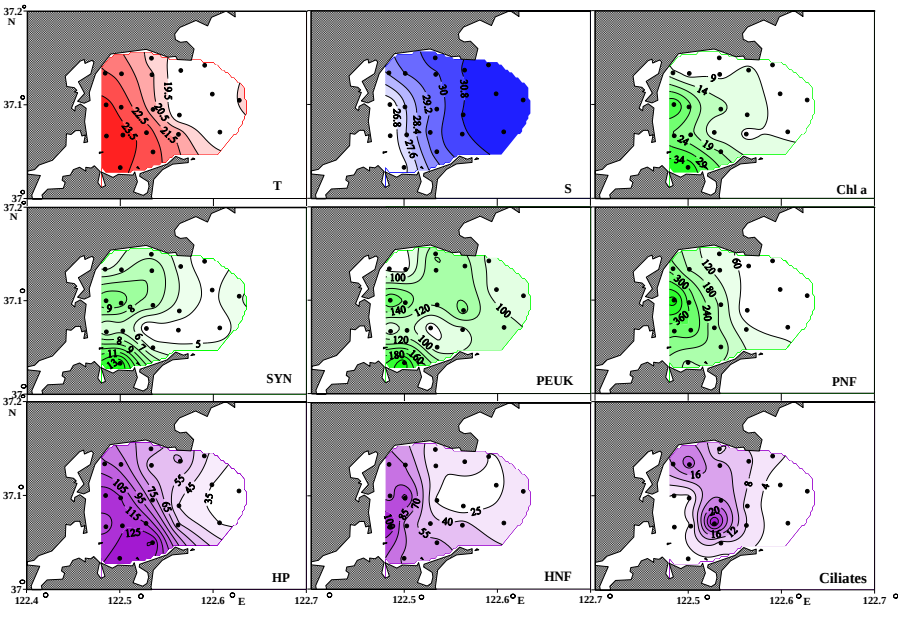
A - spring



B - summer



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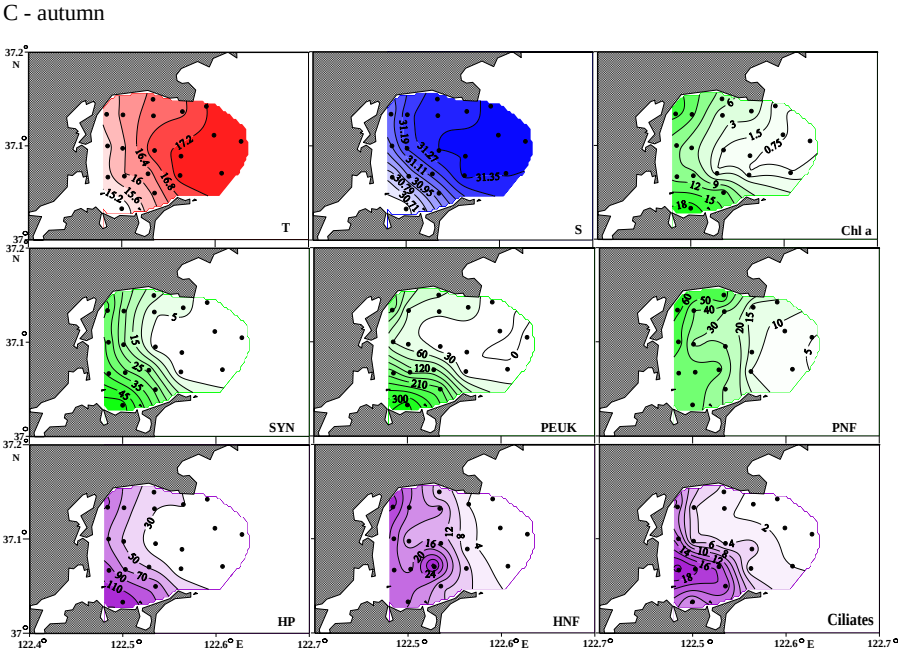

Figure 2. Surface distribution of temperature (T, °C), salinity (S), Chl *a* concentration (µg dm^{-3}), and MFW-component biomasses (µg C dm^{-3}) over the four investigated seasons.
SYN: *Synechococcus*; PEUK: picoeukaryotes; PNF: pigmented nanoflagellates; HP: heterotrophic prokaryotes; HNF: heterotrophic nanoflagellates.

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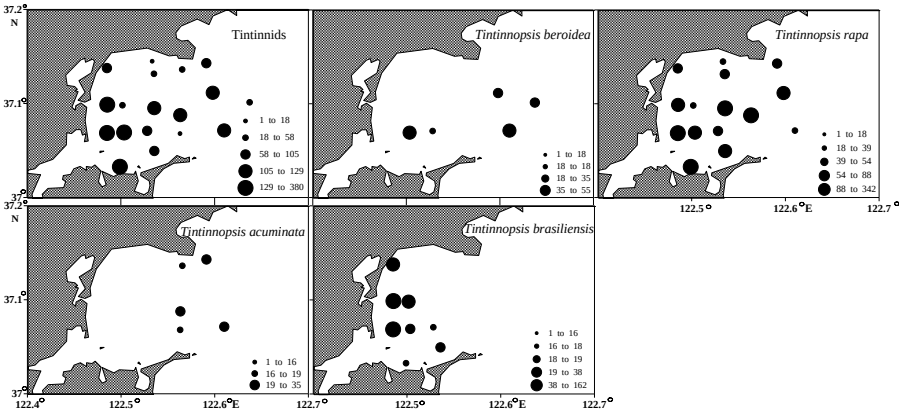
A - spring



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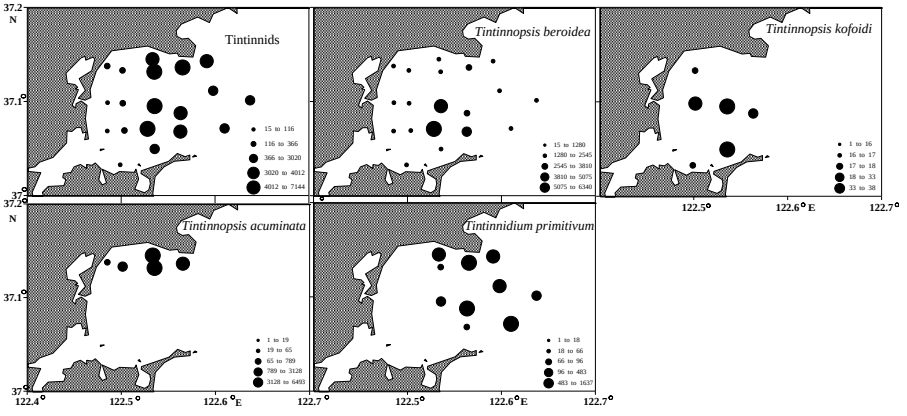

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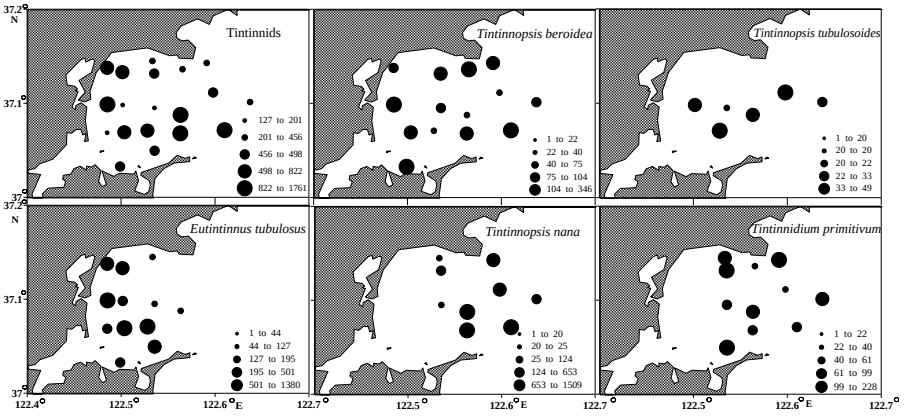
C- autumn



D -winter

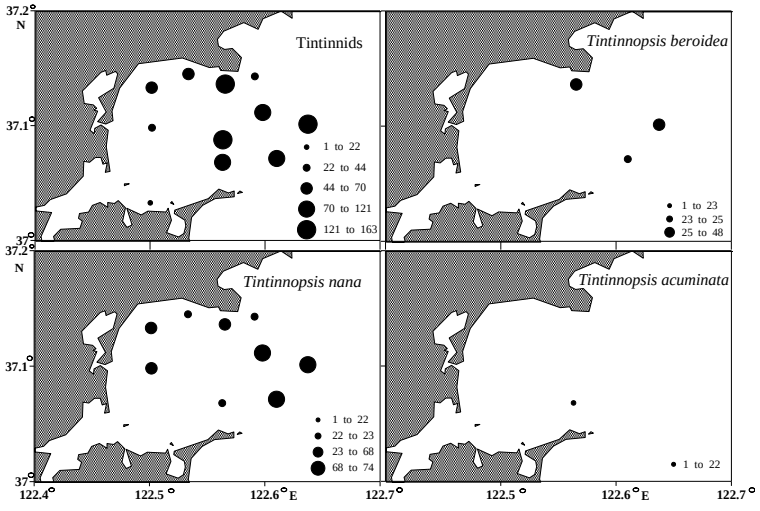


Figure 3. Tintinnids and main species abundance (ind. dm^{-3}) surface distribution over the four investigated seasons.

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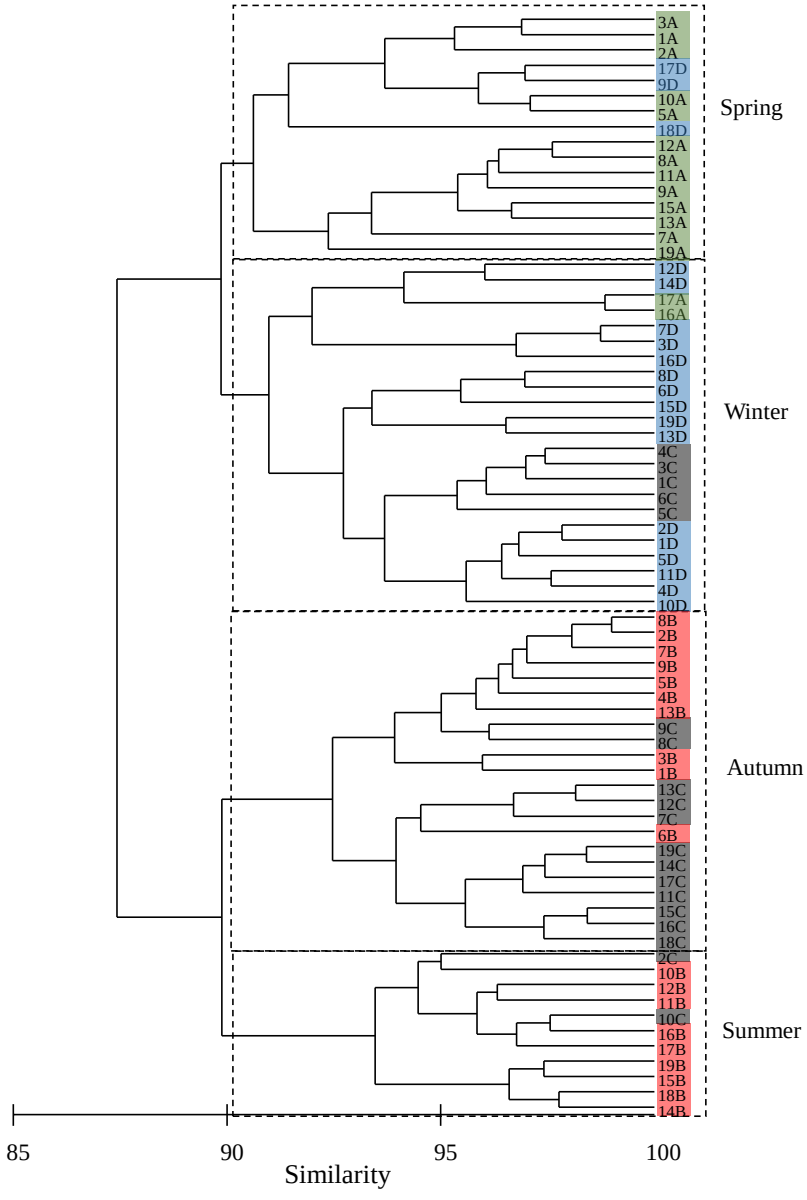

Figure 4. Output of the cluster analysis based on MFW-component biomasses (µg C dm^{-3}) from the whole survey. Note that the April stations with missing data were removed.

A&■: spring data collected from 15 stations;

B&■: summer data collected from 19 stations;

C&■: autumn data collected from 19 stations;

D&■: winter data collected from 19 stations.

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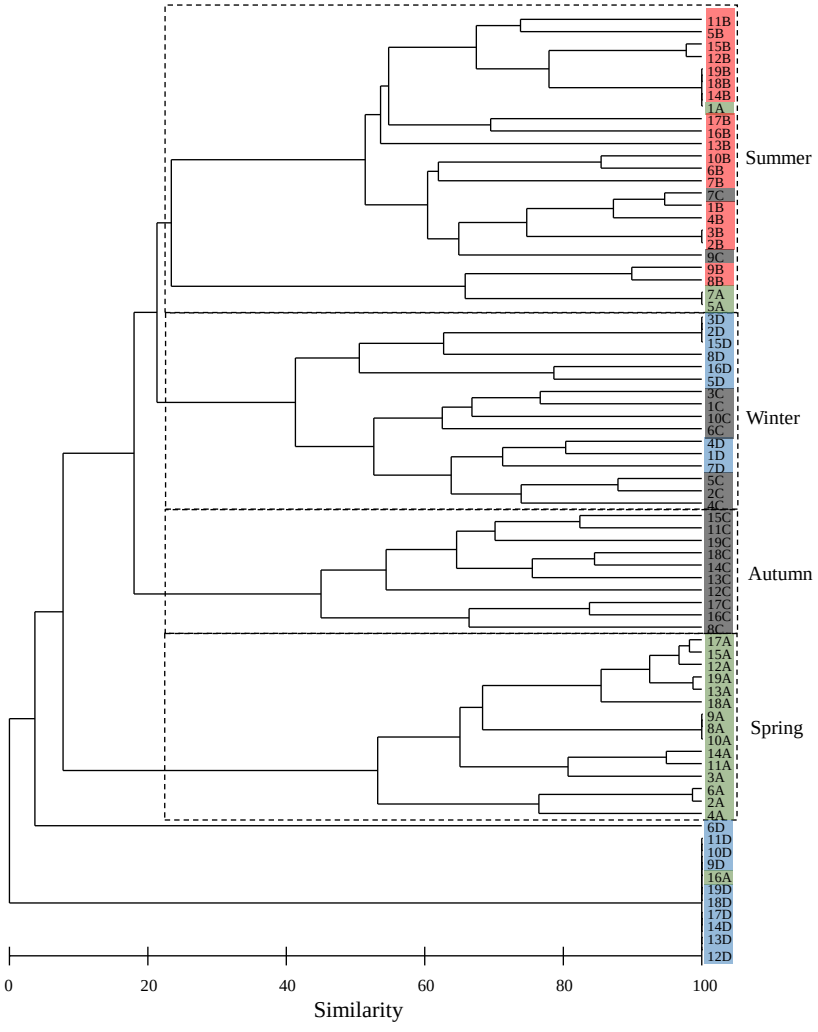

Figure 5. Output of the cluster analysis based on tintinnid abundances (ind. dm^{-3}) determined during the four seasons.

A&■: spring data collected from 19 stations;

B&■: summer data collected from 19 stations;

C&■: autumn data collected from 19 stations;

D&■: winter data collected from 19 stations.

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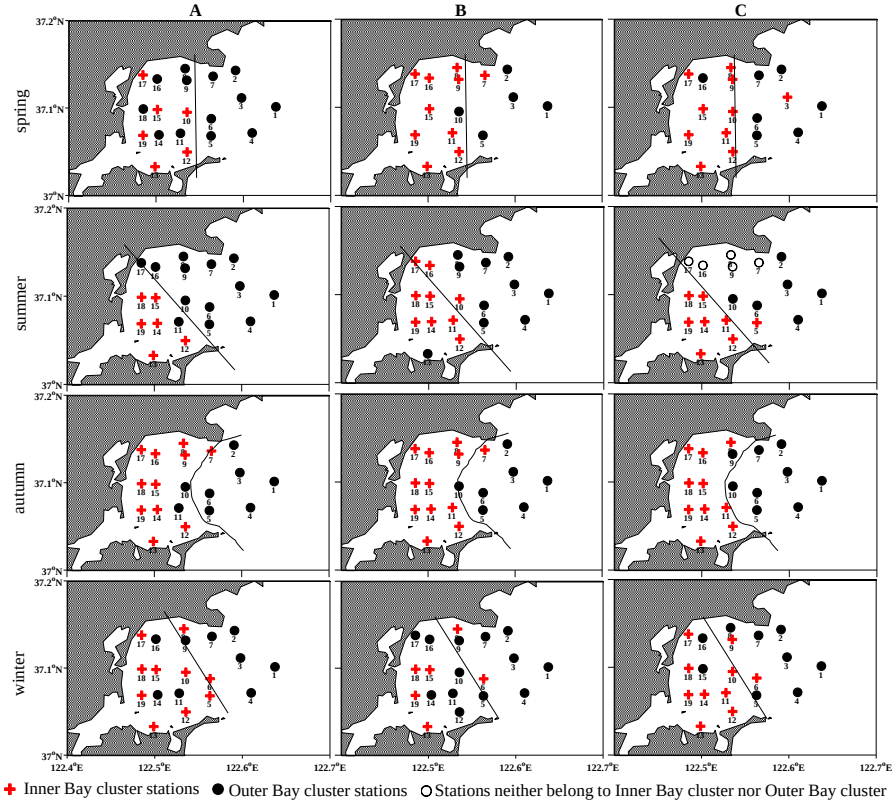

Figure 6. Sampling stations showing cluster results (A: based on environmental conditions; B: based on biomass; C: based on tintinnid abundances). The solid lines showed the approximate dividing position between Inner Bay stations and Outer Bay stations.

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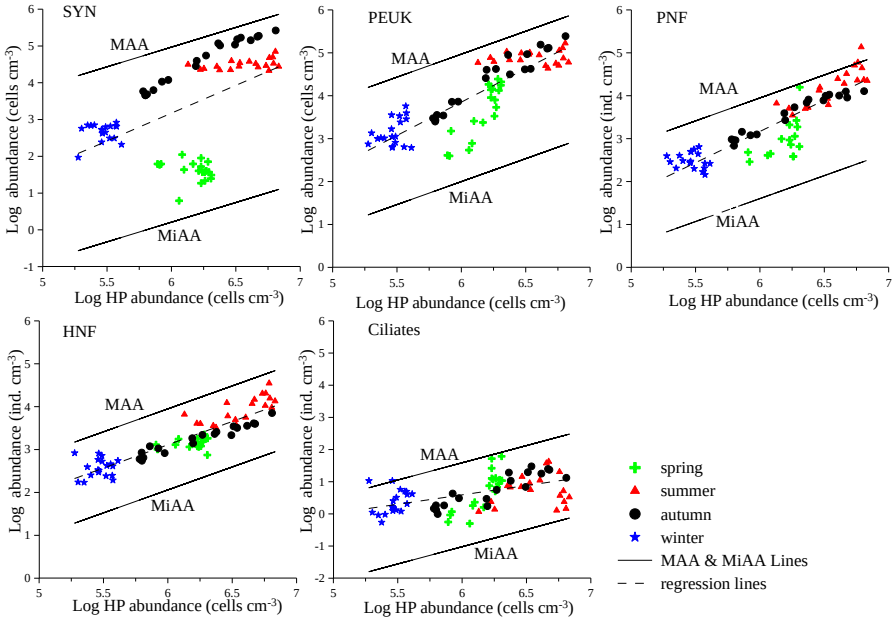

Figure 7. Scatter graphs between Log HP abundance (cells cm^{-3}) and Log abundance (cells cm^{-3} or ind. cm^{-3}) of the other five biological groups by taking into account data from the whole survey. Note that the scale is logarithmic.
SYN: *Synechococcus*; PEUK: picoeukaryotes; PNF: pigmented nanoflagellates; HP: heterotrophic prokaryotes; HNF: heterotrophic nanoflagellates. MAA: maximum attainable abundance line; MiAA: minimum attainable abundance line.

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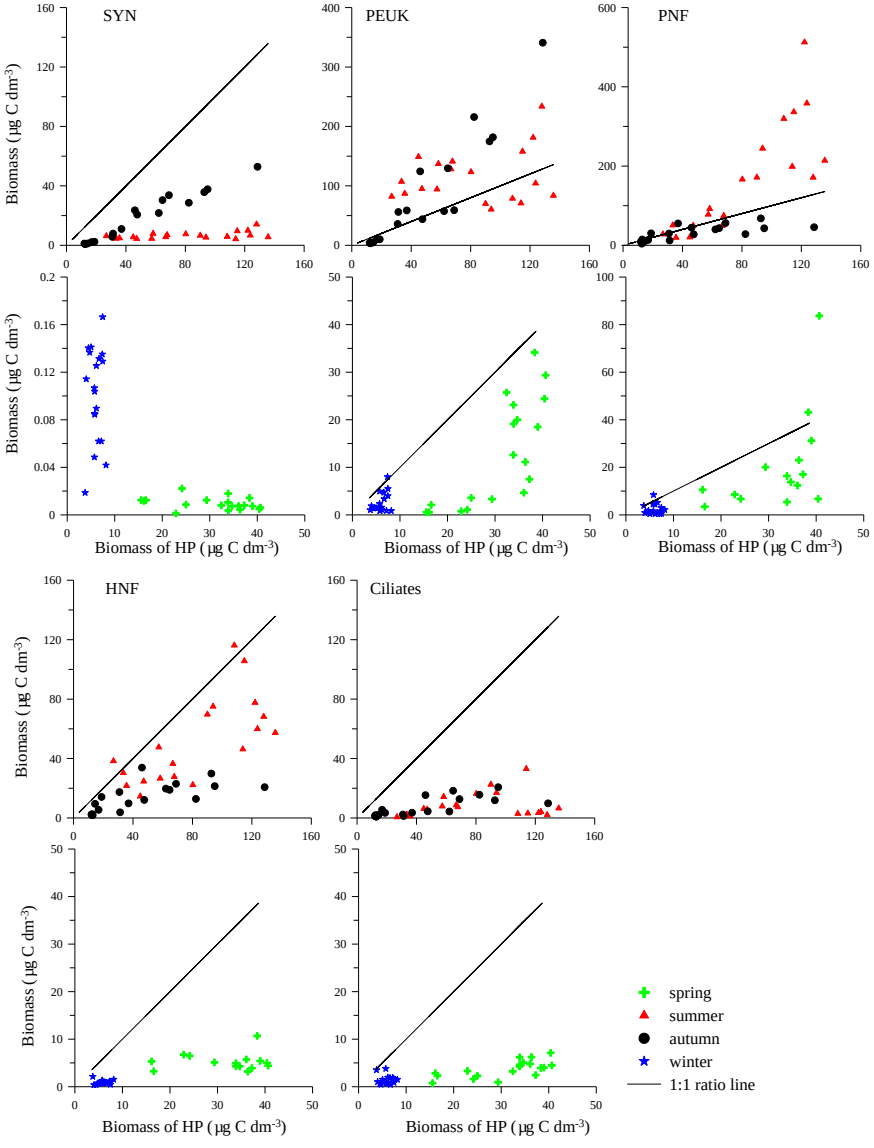

Figure 8. Relationship of MFW component (SYN, PEUK, PNF, HNF and ciliates) biomasses (µg C dm^{-3}) with respect to HP biomass (µg C dm^{-3}) by taking into account data from the whole survey.

SYN: *Synechococcus*; PEUK: picoeukaryotes; HNF: heterotrophic nanoflagellates; PNF: pigmented nanoflagellates; HP: heterotrophic prokaryotes.

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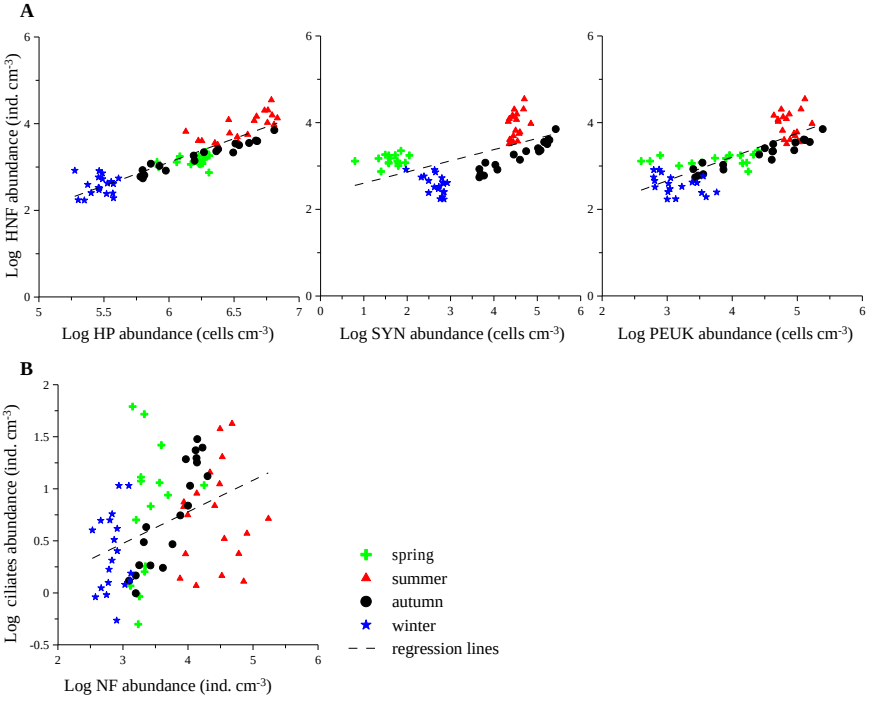

Figure 9. Abundance relationships between predators (A. HNF, B. ciliates) and their preys. HP: heterotrophic prokaryotes; SYN: *Synechococcus*; PEUK: picoeukaryotes; NF: nanoflagellates (HNF+PNF); HNF: heterotrophic nanoflagellates; PNF: pigmented nanoflagellates.

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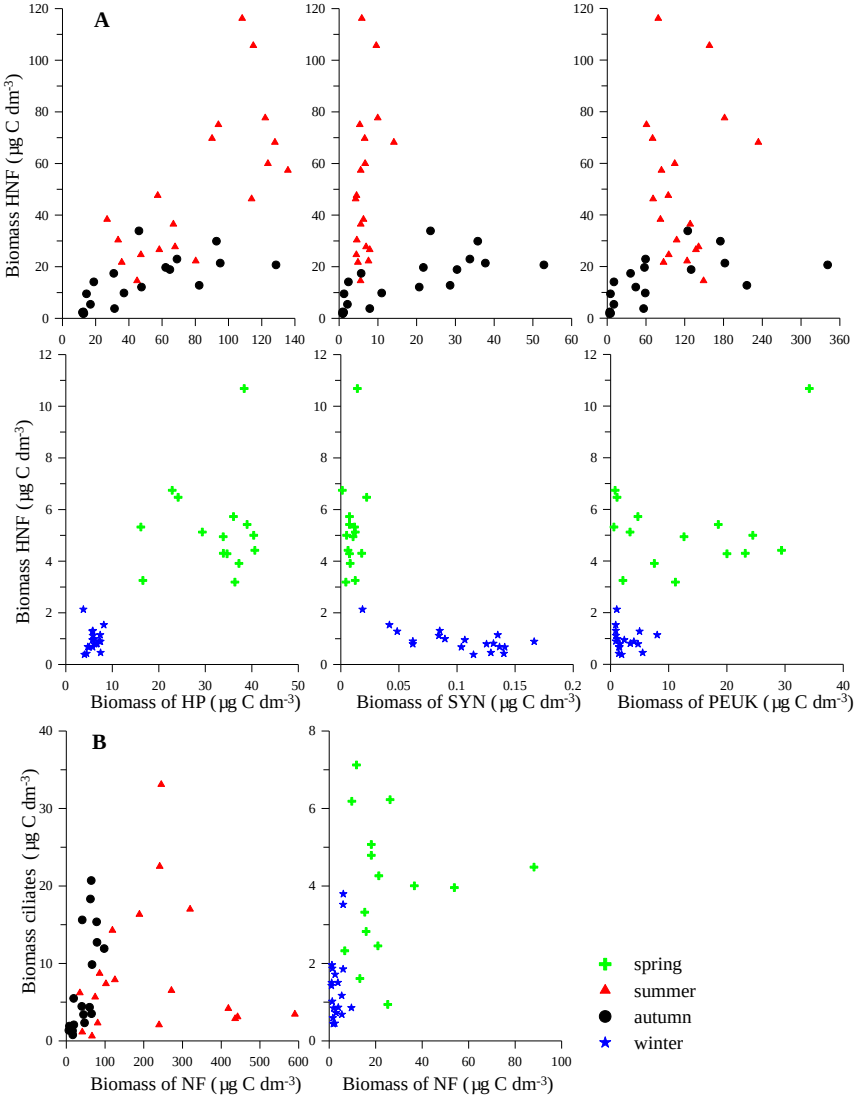

Figure 10. Biomass relationships between predators (A. HNF, B. ciliates) and their preys. HP: heterotrophic prokaryotes; SYN: *Synechococcus*; PEUK: picoeukaryotes; NF: nanoflagellates (HNF+PNF); HNF: heterotrophic nanoflagellates; PNF: pigmented nanoflagellates.

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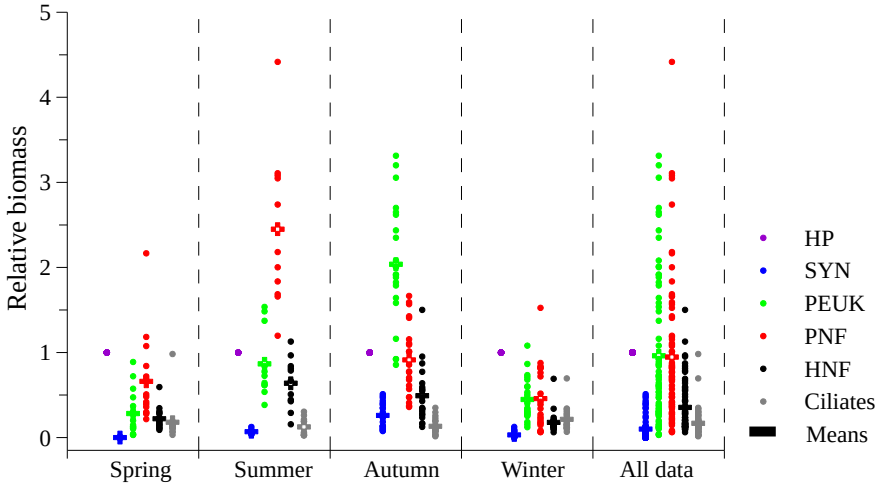

Figure 11. Microbial food web structure in Sanggou Bay at four successive seasons. The relative biomasses of the MFW components were normalised by the HP biomass belonging to the same sample.

HP: heterotrophic prokaryotes; SYN: *Synechococcus*; PEUK: picoeukaryotes; PNF: pigmented nanoflagellates; HNF: heterotrophic nanoflagellates.

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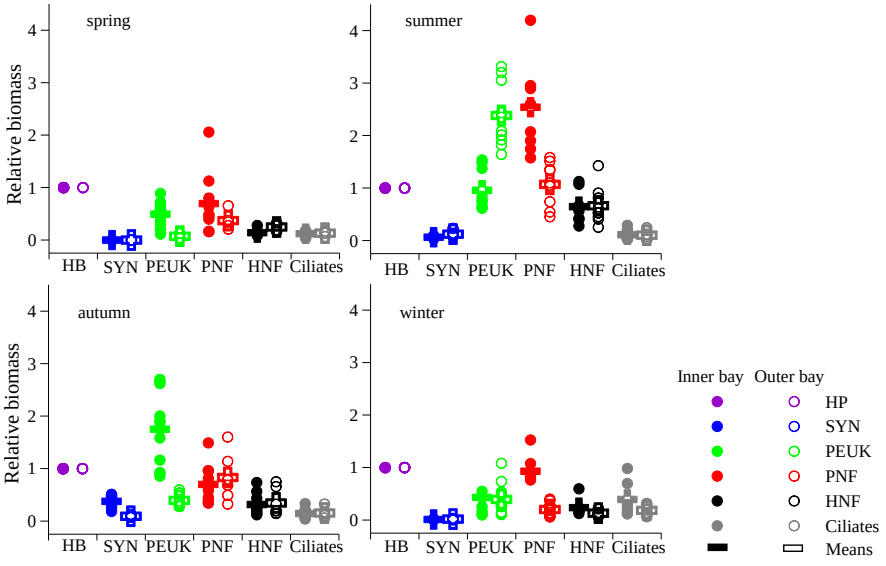

Figure 12. Microbial food web structure in inner and outer Sanggou Bay at four successive seasons. Relative biomasses of the MFW components were normalised by the HP biomass belonging to the same sample.

HP: heterotrophic prokaryotes; SYN: *Synechococcus*; PEUK: picoeukaryotes; PNF: pigmented nanoflagellates; HNF: heterotrophic nanoflagellates.

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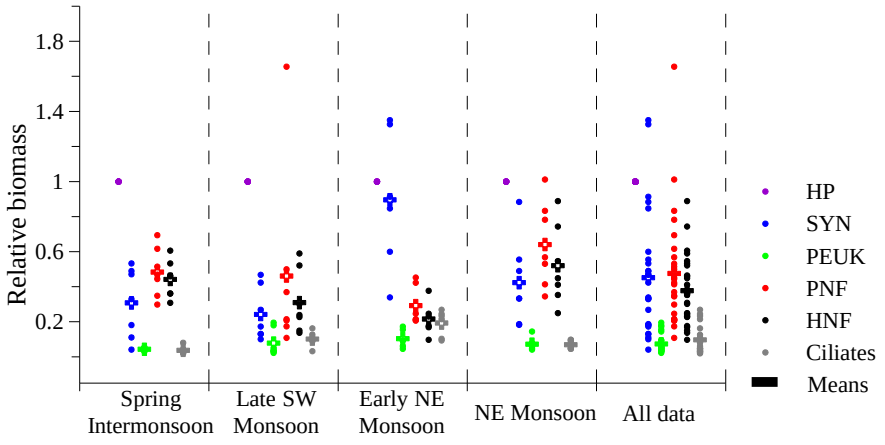

Figure 13. Microbial food web structure in the Arabian Sea. The data were extracted from Garrison et al. (2000). Biomasses of the MFW components were normalised by the HP biomass belonging to the same sample, as reported in the present study.
HP: heterotrophic prokaryotes; SYN: *Synechococcus*; PEUK: picoeukaryotes; PNF: pigmented nanoflagellates; HNF: heterotrophic nanoflagellates.