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In situ diet of the copepod *Calanus sinicus* in coastal waters of the South Yellow Sea and the Bohai Sea

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Abstract

Copepods are a key trophic link between primary producers and predatory animals at higher trophic levels in the marine ecosystem. Knowledge of the *in situ* composition of the copepod diet is critical for the accurate evaluation of trophic relationships and energy transfer in marine food webs. In this study, we applied a PCR-based cloning technique developed previously to investigate the *in situ* diet of *Calanus sinicus*, an ecologically important large-sized calanoid copepod that dominates in the shelf waters around China, Japan and Korea. Analyses of the 18S rDNA sequences obtained from the copepod diet revealed the diverse food composition of *C. sinicus* from two stations (Y19 in the South Yellow Sea and B49 in the Bohai Sea). A total of 43 operational taxonomic units (OTUs) were detected, which belonged to 13 diverse lineages: Bacillariophyta, Dinoflagellata, Dictyochophyceae, Chrysophyta, Katablepharidophyta, Pelagophyceae, Apusozoa, Hydrozoa, Ctenophora, Echinodermata, Tunicata, Chaetognatha and marine fungi. The results indicate that during an algae bloom, *C. sinicus* can graze on the bloom causative species. When the abundance of phytoplankton in ambient water is relatively low, *C. sinicus* can choose eggs, larvae, or organic particles/detritus of various metazoans, especially hydrozoans and ctenophores, as alternative food sources. Our result suggests that *C. sinicus* is an omnivorous species, and its prey choice may depend on the food availability in the ambient waters.

Key words: copepod, *in situ* diet, molecular analysis, 18S rDNA, ciliate blocking primer

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1 Introduction

Copepods, especially the planktonic copepods such as those in the order Calanoida, are considered as the key trophic linkage between phytoplankton and higher trophic levels. The trophic relationship between copepods and other organisms has had a great impact on biogeochemical cycling and energy transfer in marine food webs (Ban et al., 1997; Calbet, 2008; Zöllner et al., 2009). To better understand the complexity of the copepod-based food chains, an accurate estimation of the diet composition is very important, because it provides information on how copepods obtain their required nutrients as well as on the nutrient recycling and energy flow of marine food webs (Turner, 2004). Information on copepod diets has largely stemmed from methods for analyzing gut pigments (Irigoien et al., 2004), dissection of grazers' guts followed by microscopic analyses of the gut content (Buffan-Dubau et al., 1996) and use of stable isotope tracers (Verschoor et al., 2005). However, pigment-based methods are very sensitive to differential pigment breakdown, and pigments are not often specific to particular species (Irigoien et al., 2004). Besides, pigment-based methods do not yield data for heterotrophic prey (Nejstgaard et al., 2008). In the case of gut dissection, food content may not be easily identified, especially when they are partially digested and morphologically altered (Sautour et al., 2000). The usage of stable isotope tracers also could not exactly reflect the *in situ* prey composition of the copepod in the natural environment (Schmidt et al., 2003). Therefore, the lack of *in situ* diet information largely due to unavailability of a proper methodology, has hindered our understanding of the trophic relationships of plankton in the pelagic ecosystem.

The PCR-based molecular technique has an obvious advantage over the traditional methods of studying *in situ* copepod diets in its sensitivity, specificity and efficiency (Nejstgaard et al., 2003; Motwani and Gorokhova, 2013; Hu et al., 2014; Huang et al., 2014). Numerous studies aiming to explore the trophic relationships of marine organisms have been carried out (King et al., 2008; Pompanon et al., 2012 and refs therein). Eukaryotic small subunit ribosomal RNA gene (SSU rDNA, or 18S rDNA) is the most extensively used DNA marker for taxonomic classification of organisms due to its high copy number in the genome and overall good phylogenetic resolution (López-García et al., 2001; Moon-van der Staay et al., 2001; Bråte et al., 2010). In the previous study, we developed several non-copepod 18S rDNA primer

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sets to successfully depress the 18S rDNA amplification of the copepods, especially for the calanoida (Guo et al., 2012). The non-copepod18SF2&R2 primer set proved to be useful in amplifying this gene from most of eukaryotic lineages while excluding that of the copepod in the *in situ* diet study of the copepod (Hu et al., 2014; Huang et al., 2014). However, the presence of symbiotic apostome ciliate DNA compromises the efficacy of this method to detect the genuine diet species (Guo et al., 2012). To overcome this shortcoming, we, as well as the other researchers, have developed blocking primers which bind to the regions unique to the 18S rDNA of the apostome ciliates, and the results show that the co-amplification of the 18s rDNA of the apostome ciliates could be minimized largely by the addition of the block primers (Hu et al., 2014; Yi et al., 2014).

Calanus sinicus is an ecologically important large-sized calanoid copepod which is distributed widely in the shelf waters of China, Japan and Korea, and dominates the mesozooplankton in the coastal waters including the South Yellow Sea and the Bohai Sea (Chen, 1964; Hulsemann, 1994; Uye, 2000). Many studies have been carried out on diet composition of *C. sinicus*. Initially, herbivorous diatom, e.g., species in Genera *Coscinodiscus*, *Melosira* and *Cyclotella*, were considered as the main food source of this copepod (Li, 1964; Yang, 1997). More recent studies indicated that *C. sinicus* could ingest ciliates, bacteria, and even copepods besides phytoplankton (Huo et al., 2008; Chen et al., 2010; Liu et al., 2011). However, methodological limitations of previous studies (as mentioned above) could not accurately estimate the prey organisms of *C. sinicus* in natural waters, and the relative importance of different food sources within the natural diet of *C. sinicus* remains uncertain.

In this study, we explored the diet composition and the feeding strategies of the *C. sinicus* population in the coastal waters of the South Yellow Sea and the Bohai Sea of China using a PCR-based protocol developed previously. Our data suggests that *C. sinicus* feeds on a variety of prey including those previously known, as well as those that were unsuspected. *C. sinicus* in the South Yellow Sea are likely to prey on the eggs, larvae or organic particles/detritus of metazoans, especially hydrozoans and ctenophores, as supplementary food sources when phytoplankton abundance is low, while *C. sinicus* in the Bohai Sea can graze on the brown tide alga *Aureococcus anophagefferens* when the bloom occurs.

2 Materials and methods

2.1 *Ambient water sample collection and microscopic analyses*

To explore the plankton composition that might be potential food of *C. sinicus*, ambient water samples were collected in the South Yellow Sea and the Bohai Sea on June 15 and 25, 2011 at Stas Y19 and B49, respectively (Fig. 1 and Table 1). Water samples of three depths (3 m, 10 m and 20 m; 750 mL each) were collected simultaneously using Niskin Water Sampler assembled on a CTD profiler (Seabird 911 plus, USA). Water samples were fixed on site immediately as mentioned above. Plankton samples were concentrated through settling down by gravity for three days in the dark for further microscopic analyses as reported (Lin et al., 2009).

2.2 *Copepod sampling and DNA isolation*

Copepods were collected at the same sites. Net tow was performed from the bottom to the surface of the sea at a speed of 0.5 m/s using a plankton net (mouth diameter 31.6 cm, 160-μm mesh size) with a filtering cod end. To minimize stimulation of the copepods which might cause defecation, the net contents for genomic DNA isolation were gathered without washing the net. Samples were fixed on site immediately using 2% Utermöhl's solution (Utermöhl, 1958) and stored at 4°C for a short period of time until copepod sorting and DNA extraction (within 1 month), a storage condition without detectable DNA degradation in the samples (Zhang and Lin, 2002).

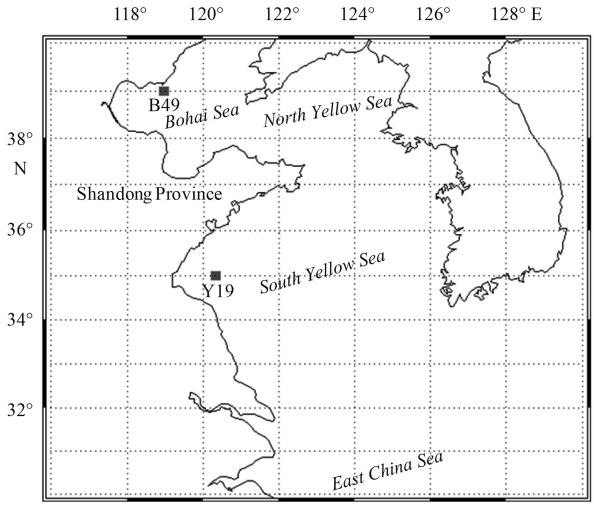

Fig. 1. Sampling locations in the South Yellow Sea and the Bohai Sea.

Table 1. Information on sampling sites in the South Yellow Sea and the Bohai Sea

	Sta. Y19	Sta. B49
Coordinates	34°59.823′N, 120°20.600′E	39°00.010′N, 118°58.264′E
Sampling date	Jun. 15, 2011	Jun. 25, 2011
Temperature/°C	15.8	17.5
Dissolved oxygen/μmol·kg^{-1}	193	260
Salinity	31.9	31.3
Chlorophyll *a*/μg·L^{-1}	0.578	14.8

C. sinicus adults were sorted under a stereomicroscope (Leica S8APO, Leica Corporation) in the laboratory. The appendages of the copepods were carefully removed and the remaining body parts were washed five times individually in 0.45 μm-filtered and autoclaved seawater (salinity 31 to eliminate the detritus and/or associated phytoplankton cells loosely attached to the body. The surface of each copepod was examined under stereomicroscope to confirm no visible microorganisms, especially the apostome ciliates, attached (Guo et al., 2012). To study the population as a whole, we pooled 30 copepods obtained into a single sample for each of the station because the diet profiles differed greatly among the copepod individuals (Guo et al., 2012; Huang et al., 2014). Then, 30 copepod individuals from the same station were homogenized with a pestle in a 1.5 mL micro-tube with 0.5 mL DNA buffer (1% SDS, 0.1 mol/L EDTA, 200 μg/mL proteinase K), and incubated at 55°C for 2 d and the genomic DNAs were extracted following a CTAB protocol (Zhang and Lin, 2005). DNA concentration and quality were measured using a NanoDrop 2000C spectrophotometer (Thermo Scientific, USA).

2.3 *PCR amplification, cloning and sequencing for copepod samples*

Since in *C. sinicus* DNA samples isolated from both Stas B49

and Y19, apostome ciliates was detected, we combined apostome ciliate blocking primer (Ciliate18Sblk1, Table 2) with non-copepod18S primers (10:1) and the PCR was performed with 1 cycle of initial denaturing for 3 min at 94°C, 5 cycles of denaturing 10 s at 95°C, 60 s at 68°C, followed by 30 cycles of 95°C for 10 s, 54.2°C for 30 s, 72°C for 40 s, and finally 10 min at 72°C (Yi et al., 2014). The PCR products were purified and cloned into T-vector, and 60 random clones of each sample were sequenced after plasmid isolation.

The 18S rDNAs of the copepod samples of the two stations were also PCR- amplified with the universal primer set 18ScomF1 and 18ScomR1 (Table 2) using the genomic DNAs isolated from the *in situ* fixed copepods as templates. PCR was performed with 1 cycle of initial denaturing for 3 min at 94°C, 35 cycles of 95°C for 30 s, 56°C for 30 s, 72°C for 40 s, and finally 10 min at 72°C, and the amplicons were purified and directly sequenced as reported (Zhang et al., 2005) to confirm the identity of the copepods.

2.4 *Sequence and phylogenetic analyses*

All original sequences were manually inspected to remove the vector and primer sequences and to correct reading errors. The sequences were searched against the NCBI nt database using the Basic Local Alignment Search Tool (BLAST) (Altschul et al., 1997). Homologous sequence information was determined based on top hits from BLAST. The best hits were aligned with our cloned sequences using Clustal X v2.1 (Larkin et al., 2007). Maximum Likelihood (ML) tree was inferred with 1000 resampling bootstrap analyses using MEGA 5.0 (Tamura et al., 2011). Rarefaction analyses and diversity indices were estimated by Mothur (Schloss et al., 2009).

3 Results

3.1 *Phytoplankton community and A. anophagefferens bloom*

Water samples from different depth were analyzed microscopically. Results revealed 26 different species for each station respectively, which belonged to diatoms, dinoflagellates, cryptophytes and pelagophytes (Tables A1–A4). For the phytoplankton community in Sta. Y19, the total cell abundance was 3.8×10^4 cells/L. Diatoms dominated the species number (50%) and accounted for 37% of the phytoplankton abundance, followed by cryptophytes, dinoflagellates, and pelagophytes in cell abundance, accounting for 33%, 22% and 8%, respectively. For Sta. B49 in the Bohai Sea, a brown tide bloom of *A. anophagefferens* was breaking out during sampling period with cell concentration reaching 1.4×10^7 cells/L. This species accounted for >99% cell abundance of the whole phytoplankton community. Besides *A. anophagefferens*, 15 species of diatoms, 9 species of dinoflagellates and 1 species of pelagophyte were also detected (Table A2).

Microscopic analyses clearly showed that the phytoplankton abundance at Sta. B49 (1.4×10^7 cells/L) was significantly higher than that at Sta. Y19 (3.8×10^4 cells/L), even when the bloom causative species *A. anophagefferens* was excluded, cell abundance at Y19 in the South Yellow Sea was still lower, about one half of that at B49 in the Bohai Sea. The cell abundance of phytoplankton matched the biomass measured by chlorophyll *a* concentration of two stations. Chlorophyll *a* of the algal bloom at Sta. B49 was 14.8 μg/L, 5 times higher than that of Sta. Y19 (Table 1).

3.2 *Molecular confirmation of C. sinicus and identification of apostome ciliates*

The sequence of 1.75-kb 18S rDNA amplified from the copepod samples showed 99% identity to the reported *C. sinicus* sequence in GenBank (GU969174). This result molecularly confirmed the identity of *C. sinicus* collected in the two stations in addition to morphological identification.

The PCR using the apostome ciliate-specific 18S rDNA primer set (Vampe18SF and Vampe18SR) gave positive results for the copepod samples from both Y19 and B49, indicating *C. sinicus* from both stations hosted the parasitic ciliates.

3.3 *Analyses of in situ diet of C. sinicus*

18S rDNA libraries were constructed for *in situ* diet analyses of *C. sinicus*, and 60 clones were randomly picked and sequenced for each sample respectively. The partial 18S rDNA sequences obtained in this study had been deposited in the GenBank under accession numbers KT825583-KT825682. Fifty-one (Sta. Y19) and 49 (Sta. B49) high quality sequences of ~0.75 kb were obtained, which were clustered into 26 and 23 unique OTUs using 99.5% cut-off, respectively (Tables 3 and 4). The OTUs corresponded to species from 13 diverse lineages including Bacillariophyta (diatoms), Dinoflagellata (dinoflagellates), Dictyochophyceae (dictyochales), Chrysophyta (chrysophytes), Katablepharidophyta (katablepharidophytes), Pelagophyceae (pelagophytes), Apusozoa, Hydrozoa (hydrozoans), Ctenophora (ctenophores), Echinodermata (echinoderms), Tunicata (tunicates), Chaetognatha (arrow worms) and marine fungi (Fig. 2). No apostome ciliates were detected. The rarefaction curve (Fig. 3) showed that the current sequencing depth has not exhausted the diet diversity. Chao and the ACE parameter estimator indicated that the maximal number of OTUs to be 61 and 109.5 respectively (Table 5). The Shannon and Simpson index indicated that the species distribution of Sta. Y19 was more uniform.

Sequence analyses revealed a wide range of organisms including phytoplankton, metazoans and marine fungi. The number of phytoplankton (marine algae) sequences accounted for 18% and 73% respectively in Stas Y19 and B49, while the proportion of metazoans were 59% and 17%, and the marine fungi accounted for 23% and 10%, respectively (Fig. 4).

Sequences of diatoms, dinoflagellates, chrysophytes and katablepharidophytes were represented in both diet clone libraries, while sequences of pelagophytes (*A. anophagefferens*, 99% identity) and dictyochales (*Florenciellales* sp., 95% identity) were unique in Sta. B49. In Sta. Y19 diet library, dinoflagellates were

Table 2. Primers used in the study

Primer/oligo name	Sequence (5′→3′)	Application	Reference
Ciliate18Sblk1	CGCGTAAATTACCCAATCCTGATC CACAATGAGA	apostome ciliate 18S rDNA blocking	Yi et al. (2014)
18ScomF1	GCTTGTCTCAAAGATTAAGCCATGC	DNA quality test	Zhang et al. (2005)
18ScomR1	CACCTACGGAAACCTTGTTACGAC	DNA quality test	Zhang et al. (2005)
Vampe18SF	CGCGTAAATTACCCAATCCTGATC	apostome ciliate	this study
Vampe18SR	ACTTTAATTTCTCATACAGTGCTGACC	apostome ciliate	this study
Non-copepod18SF2	AGCAGGCGCGHAAATTRCCCAATCY	PCR gene of prey	Guo et al. (2012)
Non-copepod18SR2	CCGTGTTGAGTCAAATTAAGCCG	PCR gene of prey	Guo et al. (2012)

Table 3. Taxonomic distribution of diets retrieved from *Calanus sinicus* at Sta. Y19 in the South Yellow Sea

OTUs[1)]	Best hit Accession	Best hit species	E-value	Identity/%	Category	Count/%
Y-01	FR865512	*Phaeodactylum tricornutum*	0	99	Bacillariophyta	2
Y-02	DQ351882	*Aidanosagitta neglecta*	0	96	Chaetognatha	4
Y-03	DQ351882	*Aidanosagitta neglecta*	0	96	Chaetognatha	2
Y-04	DQ351890	*Sagitta bipunctata*	0	95	Chaetognatha	2
Y-05	HQ877920	*Isochrysis* sp.	0	99	Chrysophyta	2
Y-06	DQ068051	*Clytia gracilis*	0	99	Hydrozoa	18
Y-07	DQ068051	*Clytia gracilis*	0	99	Hydrozoa	2
Y-08	FJ550583	*Eudendrium glomeratum*	0	99	Hydrozoa	2
Y-09	EU305496	*Hydrichthys boycei*	0	99	Hydrozoa	2
Y-10	HE647719	*Euplokamis* sp.	0	99	Ctenophora	10
Y-11	JF790988	*Karlodinium micrum*	0	95	Dinoflagellata	8
Y-12	EF492490	*Karlodinium micrum*	0	99	Dinoflagellata	2
Y-13	JF790988	*Karlodinium micrum*	0	92	Dinoflagellata	2
Y-14	DQ073779	*Archaeopneustes hystrix*	0	99	Echinodermata	6
Y-15	DQ073779	*Archaeopneustes hystrix*	0	99	Echinodermata	4
Y-16	DQ073779	*Archaeopneustes hystrix*	0	99	Echinodermata	2
Y-17	DQ073779	*Archaeopneustes hystrix*	0	99	Echinodermata	2
Y-18	JN934894	*Katablepharis* sp.	0	98	Katablepharidophyta	2
Y-19	KP872533	*Aspergillus* sp.	0	100	marine fungi	2
Y-20	JX273057	*Cladosporium* sp.	0	99	marine fungi	8
Y-21	HM116764	*Cadophora* sp.	0	99	marine fungi	2
Y-22	KM096178	*Cladosporium* sp.	0	100	marine fungi	2
Y-23	EU192367	*Malassezia restricta*	0	99	marine fungi	4
Y-24	JQ008922	*Asterotremella musci*	0	99	marine fungi	2
Y-25	DQ862056	*Valsa ambiens*	0	100	marine fungi	4
Y-26	AB013015	*Oikopleura* sp.	0	99	Tunicata	4

Note: [1)] OTUs are shown as station followed by numbers. Y represents the South Yellow Sea.

Table 4. Taxonomic distribution of diet organisms retrieved from *Calanus sinicus* at Sta. B49 in the Bohai Sea

OTUs[1)]	Best hit Accession	Best hit species	E-value	Similaritiy/%	Category	Count/%
B-01	AB430590	*Stephanopyxis turris*	0	91	Bacillariophyta	2
B-02	DQ351882	*Aidanosagitta neglecta*	0	96	Chaetognatha	4
B-03	AJ246273	*Chrysochromulina campanulifera*	0	100	Chrysophyta	2
B-04	HQ877901	*Emiliania huxleyi*	0	99	Chrysophyta	6
B-05	EU876559	*Ectopleura crocea*	0	100	Hydrozoa	8
B-06	HE647719	*Euplokamis* sp.	0	99	Ctenophora	2
B-07	U41087	*Gymnodinium beii*	0	99	Dinoflagellata	2
B-08	JF790988	*Karlodinium micrum*	0	96	Dinoflagellata	6
B-09	JN986577	*Karlodinium veneficum*	0	99	Dinoflagellata	2
B-10	JF715165	*Prorocentrum minimum*	0	100	Dinoflagellata	2
B-11	JF715165	*Prorocentrum minimum*	0	96	Dinoflagellata	2
B-12	FR690459	*Woloszynskia cincta*	0	100	Dinoflagellata	4
B-13	JN934894	*Katablepharis* sp.	0	98	Katablepharidophyta	2
B-14	HM116765	*Cadophora luteo-olivacea*	0	99	marine fungi	2
B-15	AF385443	*Dioszegia zsoltii*	0	99	marine fungi	2
B-16	EU192367	*Malassezia restricta*	0	99	marine fungi	2
B-17	AB263120	*Rhodotorula lamellibrachiae*	0	99	marine fungi	2
B-18	JQ008922	*Asterotremella musci*	0	99	marine fungi	2
B-19	JQ420081	*Aureococcus anophagefferens*	0	99	Pelagophyceae	37
B-20	JQ420081	*Aureococcus anophagefferens*	0	99	Pelagophyceae	2
B-21	JQ420084	*Aureococcus anophagefferens*	0	99	Pelagophyceae	2
B-22	JQ340338	*Ancyromonas atlantica*	0	98	Apusozoa	2
B-23	AB518483	*Florenciellales* sp.	0	95	Dictyochophyceae	2

Note: [1)] OTUs are shown as station followed by numbers. B represents the Bohai Sea.

relatively abundant (12%). The library of Sta. B49 samples was dominated by *A. anophagefferens*, accounting for 41% of the all sequences obtained. In the two clone libraries, chrysophytes were represented by the three species, *Isochrysis* sp., *Emiliania huxleyi* and *Chrysochromulina campanulifera*. Diatoms included *Phaeodactylum tricornutum* and *Stephanopyxis turris*, while kat-

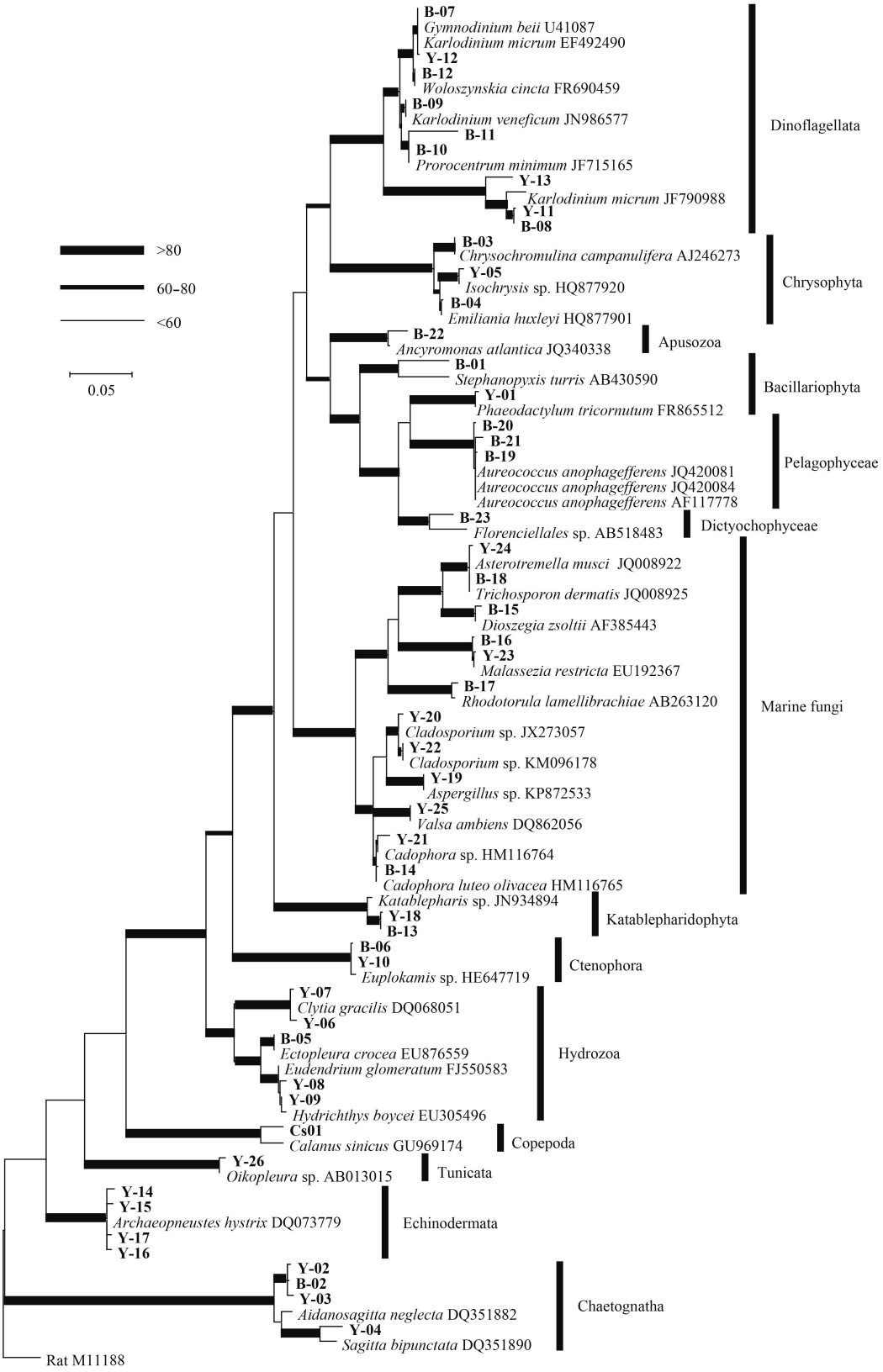

Fig. 2. Maximum Likelihood tree for 18S rDNA of *Calanus sinicus* diet organisms at Stas Y19 and B49.ML tree was inferred with 1 000 bootstrap resampling. Branch support estimated from Approximate Likelihood Ration Test was indicated with the branch thickness. Representative sequences (OTUs) of the diet organisms obtained in this study are shown in bold, Y represents the South Yellow Sea and B the Bohai Sea (corresponding to data in Tables 3 and 4). Cs01 stands for 18S rDNA sequences obtained from *C. sinicus*. 18S rDNA sequences of the representative species from each major lineages shown as species name followed by GenBank accession number. Rat 18S rDNA was used to root the tree.

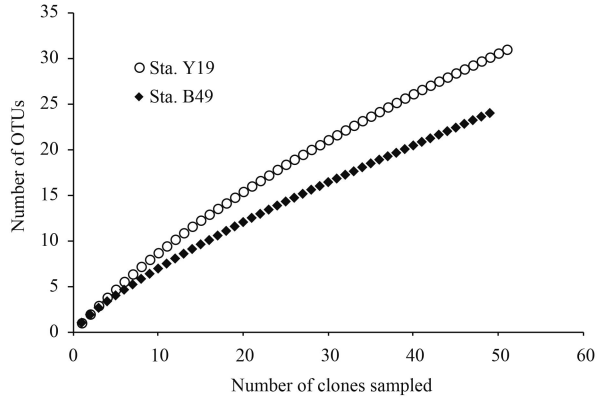

Fig. 3. Rarefaction curves of *Calanus sinicus* diet 18S rDNA clone libraries.

Table 5. Diversity indices of diets retrieved from *Calanus sinicus* at Stas Y19 and B49

Stations	Clones	OTUs	Chao	ACE	Simpson	Shannon
Y19	51	26	61	113.86	0.96	0.94
B49	49	23	109.5	137.39	0.30	0.80

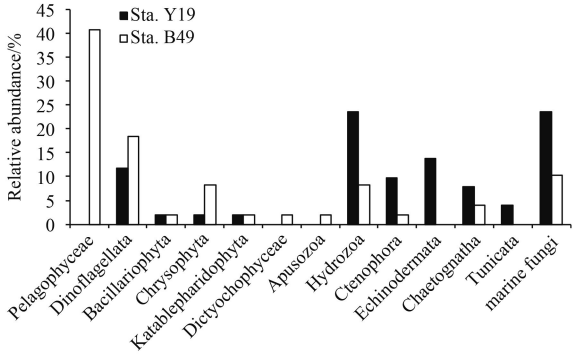

Fig. 4. Relative abundance of the potential *Calanus sinicus* diet organisms at Stas Y19 and B49.

ablepharidophytes only included *Katablepharis* sp.. Metazoans were the substantial components of the diet. Sequences of gelatinous jellyfish, including hydrozoans and ctenophores, and arrow worms, were detected in both station samples, while sea urchin (*Archaeopneustes*) and tunicate (*Oikopleura* sp.) were only detected at Sta. Y19. Jellyfish sequences were the most abundant in the diet library of Sta. Y19 sample, and *Clytia gracilis* (100% identity) accounted for the majority of all the jellyfish sequences. The detected jellyfish species including *Clytia gracilis*, *Hydrichthys boycei*, *Ectopleura crocea*, *Eudendrium glomeratum*, *Euplokamis* sp., and the species of Chaetognatha were *Aidanosagitta* sp. and *Sagitta* sp.. Also notable in both diet libraries were the marine fungi. *Asterotremella music* (99% identity), *Cadophora*-like marine fungi (99% identity) and *Malassezia restricta* (99% identity) were detected in both stations. *Aspergillus* sp. (100% identity), *Cladosporium* sp. (99% identity) and *Valsa ambiens* (100% identity) were only found in Sta. Y19, while *Dioszegia zsoltii* (99% identity) and *Rhodotorula lamellibrachiae* (99% identity) were unique to Sta. B49.

4 Discussion

It is evident that PCR-based molecular technique has advantages over the traditional morphology-based methods on unveiling the *in situ* diet information of copepods and other zooplankton in the natural environment (Nejstgaard et al., 2003; Boling et al., 2012; Maloy et al., 2013; Hu et al., 2014; Huang et al., 2014; Yi et al., 2014; Hu et al., 2015), largely because oftentimes the prey organisms in the gut of the predators are already partly digested and morphologically indistinguishable, yet they can still be detected as long as the DNA of the targeted gene (18S rDNA for example) is intact.

Our results expanded the knowledge of the copepod *in situ* diet composition using PCR-based molecular technique in this study. *C. sinicus* likely feeds on various organisms which were unknown previously, ranging from phytoplankton to metazoans. Various marine microalgae including diatoms, dinoflagellates, chrysophytes, pelagophyceae, dictyochales, and katablepharidophytes were detected in the *C. sinicus* diet. Ctenophores and hydrozoans make up a substantial part of the *C. sinicus* diet in the South Yellow Sea, as well as echinoderms, an important metazoan in the study area. It has been found that jellyfish (ctenophores and hydrozoans) sequences dominated the 18S rDNA diet libraries of *C. sinicus* in the Huanghe (Yellow River) Estuary, along with various algae and mollusks (Huang et al., 2014). Tunicates, cladocerans, ophiuroids (brittle stars) and various algae have been detected in the diet of the calanoid copepod *Canthocalanus pauper* from the South China Sea (Hu et al., 2015). It is worthwhile to note that we also observed the diet compostion of *C. sinicus* during a brown tide bloom.

4.1 *Prey diversity*

The dietary analyses of *C. sinicus* in this study generated 43 unique OTUs, among which 26 and 23 were found at Stas Y19 and B49, respectively. Dinoflagellates and diatoms were present in both ambient water samples and copepod diet samples. However, although sequences of chrysophytes, katablepharidophytes, and dictyochales were detected in the diet samples, they were not found in the ambient water, likely due to the lower resolution power of the morphology-based methods. The sequences of the brown tide species *A. anophagefferens* dominated B49 diet samples. *A. anophagefferens* was also the dominant species found in the B49 ambient water sample through microscopic analyses (Table A2), so it was not surprising to find many *A. anophagefferens* sequences in the library. Diatoms have been considered the main prey of *C. sinicus* (Li, 1964; Yang, 1997). Our data showed that in both stations, compared to diatoms represented by *P. tricornutum* and *Skeletonema* sp., dinoflagellates were more abundant in the copepod diet (Fig. 4). In a study on the diet of copepod *Acartia tonsa*, it was found that diatoms were of lower nutritional value than dinoflagellates (Jones and Flynn, 2005), also the development of the copepod *C. helgolandicus* was found to be arrested when feeding exclusively on the diatom *Skeletonema costatum* (Ianora et al., 2004). Whether or not *C. sinicus* selectively feed on dinoflagellates requires in deep sequencing of the diet organisms using technique such as Illumina high throughput sequencing.

Metazoans, such as hydrozoans, ctenophores, arrow worms, echinoderms and tunicates, are rarely considered as prey for copepods. In this study, however, sequences of these metazoans were detected in relatively high frequency, especially in Sta. Y19 (Fig. 4 and Table 3). In the previous study, using PCR-based methods, we detected many sequences of ctenophores and hydrozoans (Huang et al., 2014). Other researchers have also found that tunicate *Oikopleura* sp., echinoderm *Ophionereis reticulate*, arrow worms and other metazoans could constitute the potential diet of the calanoid copepod *C. pauper* (Hu et al., 2014, 2015).

Considering body size of adult *C. sinicus* collected in this study, 2.6–3.5 mm, and the diameter of its mouthpart, it is unlikely that *C. sinicus* could eat the adults of the metazoans such as hydrozoans, ctenophores, echinoderms, arrow worms and tunicates. Instead, *C. sinicus* could eat eggs, larvae, organic particles or detritus of these metazoans. A study using gut dissection and microscopic examination method also suggested that copepods could prey on hydrozoans and arrow worms (Schnetzer and Steinberg 2002). Using the gut pigments analyses method, Zhang et al. (2006) found that non-phytoplankton food accounted for 6%–60% of the diet composition of *C. sinicus* in June. Our result is consistent with these studies.

Microscopic analyses indicated that in June 2011, the abundance of phytoplankton was low in the South Yellow Sea, but zooplankton was abundant (average abundance 12 288 ind./m^3). Among zooplankton, species proportion of planktonic larva, cnidarians, tunicates, chaetognath accounted for 28%, 13%, 3%, 3% respectively. Abundance of tunicate (*Oikopleura dioica*) were high especially in the western part of the South Yellow Sea including our sampling site (700 ind./m^3), and the larvae of sea urchins (echinoderms) were also detected microscopically in this region. On the other hand, the metazoan excluding crustaceans were few (84 ind./m^3) in the Bohai Sea. The larvae or eggs of these metazoans could be the dominant food supply for the *C. sinicus* population at Sta. Y19 when the phytoplankton abundance was low (Table 1 and Tables A1–A4).

The marine fungi sequences were found in both Stas Y19 and B49. As an important component of microorganisms in the marine ecosystem, most marine fungi are either parasitic or symbiotic to the marine organisms including copepods, or attach to nutrient-rich sediments. Only a minority are free-living (Richards et al., 2012). So far, there is little research on the relationship between marine fungi and copepods. It is possible that the fungi detected in our samples were either parasites or constituted the diet of *C. sinicus*. Marine fungi were also found in the diet of European eel larvae in the Sargasso Sea (Riemann et al., 2010) and in the diet of calanoid copepods *C. pauper*, *Termora turbinate* and *Subeucalanus subcrassus* in the Sanya Bay, China (Hu et al., 2015) through molecular approach. More studies combining the molecular approach with other methodologies are needed to confirm the trophic relationship between marine fungi and copepods such as *C. sinicus*.

Free-living ciliates are an important food group of calanoid copepods (Saiz and Calbet, 2011), but we did not detect any of their sequence in this study. Altough the blocking primer was desgined based on the apostome ciliate 18S rDNA sequence, it might also inhibit the amplification of free-living ciliates 18S rDNA. The addition of blocking primer is necessary in the cloning based Sanger sequncing method (Guo et al., 2012; Hu et al., 2014). In the furture study, to overcome this shortage, instead of using blocking primers to block the amplification of apostome ciliate 18S rDNA, development of new primers that are sutable for high throughput sequenicg method is needed.

4.2 *Eggs, larvae or particles/detritus of ctenophores and hydrozoans as food of C. sinicus*

Gelatinous jellyfish including hydrozoans and ctenophore are substantial component detected in diet composition, especially *Clytia* and *Euplokamis*. The abundance of both hydrozoan and ctenophore species are high in the South Yellow Sea and the Bohai Sea in summer, and a common ctenophore in both waters is *Pleurobrachia globose* (Du et al., 2013). Intriguingly, instead of *P. globose*, *Euplokamis* sp. was the only ctenophore species detected in the diet libraries of *C. sinicus*. Our previous study (Huang et al., 2014) also showed that the *Euplokamis* sequences dominated (40%) the diet library of *C. sinicus* in the adjacent waters of the Huanghe Estuary in the Bohai Sea. The hydrozoans *Clytia gracilis*, *Eudendrium glomeratum* and *Hydrichthys boycei* were found in Sta. Y19, and *Ectopleura crocea* was detected in B49 diet library (Tables 3 and 4), while *Rathkea octopunctata* and *Eirene menoni* were detected in *C. sinicus* near the Huanghe Estuary (Huang et al., 2014). All these hydrozoan species were not detected microscopically. These results suggest that *C. sinicus* may have a low digestion rate toward the ingested ctenophore and hydrozoan eggs or larvae, that these jellyfish have higher gene copy numbers of 18S rDNA in the genome, or that *C. sinicus* selectively prey on this rather less abundant jellyfish in the form of eggs or larvae to support their reproduction and development.

Besides in the form of eggs or larvae, jellyfish could also be ingested in the form of organic particles/detritus. Although plankton (both phyto- and zooplankton) are the dominant food composition of copepod like *C. sinicus*, there are some researches showed that other food sources such as marine snow could also be part of the diet (Chen et al., 2010). Marine snow is always combinations of fecal pellets from zooplankton, fish, and organic aggregates (such as discarded appendicularian houses) from some metazoans (Turner, 2015). Particles or detritus of jellyfish could be part of marine snow and be eaten by copepod like *C. sinicus*.

4.3 *Potential control of C. sinicus to brown tide*

During our sampling at Sta. B49, a brown tide bloom broke out. Both microscopic and molecular analyses confirmed the bloom species was *A. anophagefferens*. *A. anophagefferens* brown tides were first recorded in several bays on the northeastern coast of USA (Sieburth et al., 1988). China is the third country in the world where *A. anophagefferens* brown tide bloom has been reported (Zhang et al., 2012). The temperature, salinity and chlorophyll *a* concentration data obtained in our sampling (Table 1) were consistent with those reported in Zhang et al. (2012). It is clear that both studies detected the same brown tide bloom in the coastal waters of the Bohai Sea.

Our diet data of *C. sinicus* sampled in the *A. anophagefferens* bloom clearly showed that this brown tide causative species was the dominant food of the copepod. Zooplankton (such as copepods) grazing over phytoplankton cells is an important regulatory factor of algal blooms (Calbet et al., 2003; Barofsky et al., 2010). In our study when brown tide broke out, the phytoplankton in the ambient water was overwhelmingly dominated by *A. anophagefferens* (more than 99% of total cells counted), *C. sinicus* mainly grazed on this tide causative species. The grazing of *C. sinicus* on *A. anophagefferens* cells provided more evidences that this copepod may play an important ecological role in the marine ecosystem by regulating the dynamics of certain harmful algal blooms.

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References

Altschul S F, Madden T L, Schäffer A A, et al. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Research, 25(17): 3389–3402

Ban S, Burns C, Castel J, et al. 1997. The paradox of diatom-copepod interactions. Marine Ecology Progress Series, 157: 287–293

Barofsky A, Simonelli P, Vidoudez C, et al. 2010. Growth phase of the diatom *Skeletonema marinoi* influences the metabolic profile of the cells and the selective feeding of the copepod *Calanus* spp. Journal of Plankton Research, 32(3): 263–272

Boling W B, Sinclair G A, Wawrik B. 2012. Identification of calanoid copepod prey species via molecular detection of carbon fixation genes. Marine Biology, 159(5): 1165–1171

Bråte J, Logares R, Berney C, et al. 2010. Freshwater Perkinsea and marine-freshwater colonizations revealed by pyrosequencing and phylogeny of environmental rDNA. The ISME Journal, 4(9): 1144–1153

Buffan-Dubau E, de Wit R, Castel J. 1996. Feeding selectivity of the harpacticoid copepod *Canuella perplexa* in benthic muddy environments demonstrated by HPLC analyses of chlorin and carotenoid pigments. Marine Ecology Progress Series, 137(1–3): 71–82

Calbet A. 2008. The trophic roles of microzooplankton in marine systems. ICES Journal of Marine Science, 65(3): 325–331

Calbet A, Vaqué D, Felipe J, et al. 2003. Relative grazing impact of microzooplankton and mesozooplankton on a bloom of the toxic dinoflagellate *Alexandrium minutum*. Marine Ecology Progress Series, 259: 303–309

Chen Qingchao. 1964. A study of the breeding periods, variation in sex ratio and in size of *Calanus sinicus* Brodsky. Oceanologia et Limnologia Sinica (in Chinese), 6(3): 272–288

Chen M R, Kâ S, Hwang J S. 2010. Diet of the copepod *Calanus sinicus* Brodsky, 1962 (Copepoda, Calanoida, Calanidae) in northern coastal waters of Taiwan during the northeast monsoon period. Crustaceana, 83(7): 851–864

Du Mingmin, Liu Zhensheng, Wang Chunsheng, et al. 2013. The seasonal variation and community structure of zooplankton in China sea. Acta Ecologica Sinica (in Chinese), 33(17): 5407–5418

Guo Zhiling, Liu Sheng, Hu Simin, et al. 2012. Prevalent ciliate symbiosis on copepods: high genetic diversity and wide distribution detected using small subunit ribosomal RNA gene. PLoS One, 7(9): e44847

Hu Simin, Guo Zhiling, Li Tao, et al. 2014. Detecting *in situ* copepod diet diversity using molecular technique: development of a copepod/symbiotic ciliate-excluding eukaryote-inclusive PCR protocol. PLoS One, 9(7): e103528

Hu Simin, Guo Zhiling, Li Tao, et al. 2015. Molecular analysis of *in situ* diets of coral reef copepods: evidence of terrestrial plant detritus as a food source in Sanya Bay, China. Journal of Plankton Research, 37(2): 363–371

Huang Yousong, Xu Donghui, Chen Hongju, et al. 2014. PCR-based *in situ* dietary analysis of *Calanus sinicus* Brodsky in Yellow River estuary and adjacent waters. Periodical of Ocean University of China (in Chinese), 44(3): 83–91

Hulsemann K. 1994. *Calanus sinicus* Brodsky and *C. jashmovi*, nom. nov. (Copepoda: Calanoida) of the North-western Pacific Ocean: a comparison, with notes on the integumental pore pattern in *Calanus* s. str. Invertebrate Taxonomy, 8(6): 1461–1482

Huo Yuanzi, Wang Shiwei, Sun Song, et al. 2008. Feeding and egg production of the planktonic copepod *Calanus sinicus* in spring and autumn in the Yellow Sea, China. Journal of Plankton Research, 30(6): 723–734

Ianora A, Miralto A, Poulet S A, et al. 2004. Aldehyde suppression of copepod recruitment in blooms of a ubiquitous planktonic diatom. Nature, 429(6990): 403–407

Irigoien X, Meyer B, Harris R, et al. 2004. Derek Harbour Using HPLC pigment analysis to investigate phytoplankton taxonomy: the importance of knowing your species. Helgoland Marine Research, 58(2): 77–82

Jones R H, Flynn K J. 2005. Nutritional status and diet composition affect the value of diatoms as copepod prey. Science, 307(5714): 1457–1459

King R A, Read D S, Traugott M, et al. 2008. Molecular analysis of predation: a review of best practice for DNA-based approaches. Molecular Ecology, 17(4): 947–963

Larkin M A, Blackshields G, Brown N P, et al. 2007. Clustal W and Clustal X version 2.0. Bioinformatics, 23(21): 2947–2948

Li Shaojing. 1964. Preliminary studies on the food and feeding habits of some marine planktonic copepods in Amoy waters. Journal of Xiamen University (Natural Science) (in Chinese), 11(3): 93–109

Lin Senjie, Zhang Huan, Hou Yubo, et al. 2009. High-level diversity of dinoflagellates in the natural environment, revealed by assessment of mitochondrial *cox1* and cob genes for dinoflagellate DNA barcoding. Applied and Environmental Microbiology, 75(5): 1279–1290

Liu Mengtan, Li Chaolun, Sun Song. 2011. Seasonal variation in fatty acid composition of seston and the copepod *Calanus sinicus* (Brodsky, 1962) in Jiaozhou Bay and its trophic implications. Chinese Journal of Oceanology and Limnology, 29(6): 1164–1173

López-García P, Rodríguez-Valera F, Pedrós-Alió C, et al. 2001. Unexpected diversity of small eukaryotes in deep-sea Antarctic plankton. Nature, 409(6820): 603–607

Maloy A P, Culloty S C, Slater J W. 2013. Dietary analysis of small planktonic consumers: a case study with marine bivalve larvae. Journal of Plankton Research, 35(4): 866–876

Moon-van der Staay S Y, De Wachter R, Vaulot D. 2001. Oceanic 18S rDNA sequences from picoplankton reveal unsuspected eukaryotic diversity. Nature, 409(6820): 607–610

Motwani N H, Gorokhova E. 2013. Mesozooplankton grazing on picocyanobacteria in the Baltic Sea as inferred from molecular diet analysis. PLoS One, 8(11): e79230

Nejstgaard J C, Frischer M E, Raule C L, et al. 2003. Molecular detection of algal prey in copepod guts and fecal pellets. Limnology and Oceanography: Methods, 1(1): 29–38

Nejstgaard J C, Frischer M E, Simonelli P, et al. 2008. Quantitative PCR to estimate copepod feeding. Marine Biology, 153(4): 565–577

Pompanon F, Deagle B E, Symondson W O C, et al. 2012. Who is eating what: diet assessment using next generation sequencing. Molecular Ecology, 21(8): 1931–1950

Richards T A, Jones M D M, Leonard G, et al. 2012. Marine fungi: their ecology and molecular diversity. Annual Review of Marine Science, 4(1): 495–522

Riemann L, Alfredsson H, Hansen M M, et al. 2010. Qualitative assessment of the diet of European eel larvae in the Sargasso Sea resolved by DNA barcoding. Biology Letters, 6(6): 819–822

Saiz E, Calbet A. 2011. Copepod feeding in the ocean: scaling patterns, composition of their diet and the bias of estimates due to microzooplankton grazing during incubations. Hydrobiologia, 666(1): 181–196

Sautour B, Artigas L F, Delmas D, et al. 2000. Grazing impact of micro-and mesozooplankton during a spring situation in coastal waters off the Gironde estuary. Journal of Plankton Research, 22(3): 531–552.

Schloss P D, Westcott S L, Ryabin T, et al. 2009. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Applied and Environmental Microbiology, 75(23): 7537–7541

Schmidt K, Atkinson A, Stübing D, et al. 2003. Trophic relationships among Southern Ocean copepods and krill: Some uses and limitations of a stable isotope approach. Limnology and Oceanography, 48(1): 277–289

Schnetzer A, Steinberg D. 2002. Natural diets of vertically migrating zooplankton in the Sargasso Sea. Marine Biology, 141(1): 89–99

Sieburth J M, Johnson P W, Hargraves P E. 1988. Ultrastructure and ecology of *Aureococcus anophageferens* gen. et sp. nov. (Chrysophyceae): the dominant picoplankter during a bloom in Narragansett Bay, Rhode Island, summer 1985. Journal of Phycology, 24(3): 416–425

Tamura K, Peterson D, Peterson N, et al. 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Molecular Biology and Evolution, 28(10): 2731–2739

Turner J T. 2004. The importance of small planktonic copepods and their roles in pelagic marine food webs. Zoological Studies, 43(2): 255–266

Turner J T. 2015. Zooplankton fecal pellets, marine snow, phytodetritus and the ocean's biological pump. Progress in Oceanography, 130: 205–248

Utermöhl H. 1958. Zur vervollkommnung der quantitativen phytoplankton-methodik. Mitteilingen der Internationalen Vereinigung fur Theoretische und Angewandte Limnologie, 9: 1–38

Uye S. 2000. Why does *Calanus sinicus* prosper in the shelf ecosystem of the Northwest Pacific Ocean?. ICES Journal of Marine Science, 57(6): 1850–1855

Verschoor A M, Boonstra H, Meijer T. 2005. Application of stable isotope tracers to studies of zooplankton feeding, using the rotifer *Brachionus calyciflorus* as an example. In: Herzig A, Gulati R, Jersabek C, et al., eds. Rotifera X. Netherlands: Springer, 535–549

Yang Jiming. 1997. Primary study on the feeding of the Bohai Sea *Calanus sinicus*. Oceanologia et Limnologia Sinica (in Chinese), 28(4): 376–382

Yi Xiaoyan, Zhang Huan, Liu Guangxing. 2014. Primers to block the amplification of symbiotic apostome ciliate 18S rRNA gene in a PCR-based copepod diet study. Chinese Journal of Oceanology and Limnology, 32(3): 515–521

Zhang Huan, Bhattacharya D, Lin Senjie. 2005. Phylogeny of dinoflagellates based on mitochondrial cytochrome b and nuclear small subunit rDNA sequence comparisons. Journal of Phycology, 41(2): 411–420

Zhang Guangtao, Li Chaolun, Sun Song, et al. 2006. Feeding habits of *Calanus sinicus* (Crustacea: Copepoda) during spring and autumn in the Bohai Sea studied with the herbivore index. Scientia Marina, 70(3): 381–388

Zhang Huan, Lin Senjie. 2002. Detection and quantification of *Pfiesteria piscicida* by using the mitochondrial cytochrome *b* gene. Applied and Environmental Microbiology, 68(2): 989–994

Zhang Huan, Lin Senjie. 2005. Development of a cob-18S rRNA gene real-time PCR assay for quantifying *Pfiesteria shumwayae* in the natural environment. Applied and Environmental Microbiology, 71(11): 7053–7063

Zhang Qingchun, Qiu Limei, Yu Rencheng, et al. 2012. Emergence of brown tides caused by *Aureococcus anophagefferens* Hargraves et Sieburth in China. Harmful Algae, 19: 117–124

Zöllner E, Hoppe H G, Sommer U, et al. 2009. Effect of zooplankton-mediated trophic cascades on marine microbial food web components (bacteria, nanoflagellates, ciliates). Limnology and Oceanography, 54(1): 262–275

Appendix:

Table A1. Microscopic analysis of phytoplankton in ambient water samples at different depths of Sta. Y19

Depth	Genus	Cell density/cells·L^{-1}	Species number
Y19-3 m			
	Diatoms		
	Thalassiosira	12 600	2
	Nitzschia	5 600	2
	Skeletonema	3 255	1
	Navicula	1 155	1
	Diploneis	1 400	1
	Dinoflagellates		
	Prorocentrum	12 600	2
	Peridinium	1 050	1
	Gymnodinium	140	1
	Gyrodinium	105	1
	Ornithocerus	105	1
	Cryptophyta		
	Cryptomonas	29 400	1
	Pelagophyceae		
	Aureococcus	8 120	1
Y19-12 m			
	Diatoms		
	Thalassiosira	3 285	2
	Nitzschia	2 985	2
	Diploneis	1 349	1
	Phacodactylum	745	1
	Pennatae	570	1
	Skeletonema	550	1
	Coscinodiscus	30	2
	Dinoflagellates		
	Prorocentrum	3 008	2
	Protoperidinuim	180	1
	Gymnodinium	120	1
	Karenia	90	1
	Pyrophacus	90	1
	Cryptophyta		
	Cryptomonas	1 035	1
	Pelagophyceae		
	Aureococcus	120	1
Y19-26 m			
	Diatoms		
	Thalassiosira	4 080	2
	Nitzschia	1 360	2
	Pennatae	1 020	1
	Coscinodiscus	765	2
	Phacodactylum	680	1
	Skeletonema	680	1
	Diploneis	170	1
	Pleurosigma	129	1
	Dinoflagellates		
	Prorocentrum	7 480	2
	Gymnodinium	790	1
	Cryptophyta		
	Cryptomonas	7 140	1
	Pelagophyceae		
	Aureococcus	1 360	1

Table A2. Microscopic analysis of average cell abundance of phytoplankton in ambient water sample at Sta. Y19

Genus	Cell destiny/cells·L^{-1}	Percentage of abundance/%	Species number	Species percentage/%
Diatoms				
Thalassiosira	6 655	17.31	2	7.69
Nitzschia	3 315	8.62	3	11.54
Skeletonema	1 495	3.89	1	3.85
Diploneis	973	2.53	1	3.85
Pennatae	530	1.38	1	3.85
Phacodactylum	475	1.24	1	3.85
Navicula	385	1.00	1	3.85
Coscinodiscus	265	0.69	2	7.69
Pleurosigma	43	0.11	1	3.85
Dinoflagellates				
Prorocentrum	7 696	20.02	4	15.38
Gymnodinium	350	0.91	1	3.85
Peridinium	350	0.91	1	3.85
Protoperidinuim	60	0.16	1	3.85
Ornithocerus	35	0.09	1	3.85
Gyrodinium	35	0.09	1	3.85
Karenia	30	0.08	1	3.85
Pyrophacus	30	0.08	1	3.85
Cryptophyta				0.00
Cryptomonas	12 525	32.58	1	3.85
Pelagophyceae				
Aureococcus	3 200	8.32	1	3.85
Total	38 447	100	26	100.00

Table A3. Microscopic analysis of phytoplankton in ambient water samples from different depths of Sta. B49

Depth	Genus	Cell density/cells·L^{-1}	Species number
B49-3 m			
	Diatoms		
	Melosira	5 920	1
	Phacodactylum	4 500	1
	Thalassiosira	3 060	2
	Nitzschia	2 700	1
	Diploneis	1 800	1
	Skeletonema	1 800	1
	Pennatae	540	1
	Coscinodiscus	360	1
	Planktoniella	180	1
	Pleurosigma	180	1
	Trachyneis	180	1
	Dinoflagellates		
	Gymnodinium	14 400	1
	Prorocentrum	14 400	2
	Scripciella	2 700	1
	Cryptophyta		
	Cryptomonas	12 600	1
	Pelagophyceae		
	Aureococcus	28 800 000	1
B49-10 m			
	Diatoms		
	Thalassiosira	19 200	2
	Nitzschia	9 600	1
	Meuniera	1 920	1
	Diploneis	960	1
	Pennatae	960	1
	Dinoflagellates		
	Prorocentrum	19 200	2

to be continued

Continued from Table A3

Depth	Genus	Cell density/cells·L^{-1}	Species number
	Gymnodinium	14 400	1
	Scripciella	960	1
	Cryptophyta		
	Cryptomonas	28 800	1
	Pelagophyceae		
	Aureococcus	7 200 000	1
B49-20 m			
	Diatoms		
	Thalassiosira	32 400	2
	Melosira	5 720	1
	Phacodactylum	720	1
	Nitzschia	1 620	1
	Asterolampra	360	1
	Diploneis	360	1
	Gyrodinium	360	1
	Navicula	360	1
	Coscinodiscus	180	1
	Pennatae	180	1
	Pleurosigma	180	1
	Dinoflagellates		
	Prorocentrum	3 600	2
	Gymnodinium	3 240	1
	Protoperidinuim	540	1
	Scripciella	180	1
	Cryptophyta		
	Cryptomonas	10 800	1
	Pelagophyceae		
	Aureococcus	6 480 000	1

Table A4. Microscopic analysis of average cell abundance of ambient water sample at Sta. B49

Genus	Cell destiny/cells·L^{-1}	Percentage of abundance/%	Species number	Species percentage/%
Diatoms				
Thalassiosira	18 220	0.13	2	7.69
Nitzschia	4 640	0.03	1	3.85
Melosira	3 880	0.03	1	3.85
Phacodactylum	1 740	0.01	1	3.85
Diploneis	1 040	0.01	1	3.85
Meuniera	640	4.49×10^{-5}	1	3.85
Skeletonema	600	4.22×10^{-5}	1	3.85
Pennatae	560	3.93×10^{-5}	1	3.85
Coscinodiscus	180	1.26×10^{-5}	1	3.85
Asterolampra	120	8.43×10^{-6}	1	3.85
Navicula	120	8.43×10^{-6}	1	3.85
Pleurosigma	120	8.43×10^{-6}	1	3.85
Planktoniella	60	4.22×10^{-6}	1	3.85
Trachyneis	60	4.22×10^{-6}	1	3.85
Dinoflagellates				
Prorocentrum	11 867	0.08	5	19.23
Gymnodinium	10 680	0.08	1	3.85
Scripciella	1 280	0.01	1	3.85
Protoperidinuim	180	1.26×10^{-5}	1	3.85
Gyrodinium	120	8.43×10^{-6}	1	3.85
Cryptophyta				
Cryptomonas	17 400	0.12	1	3.85
Pelagophyceae				
Aureococcus	14 160 000	99.48	1	3.85
Total	14 233 507	100	26	100