

## Dietary separation between co-occurring copepods in a food-limited tropical coral reef of the Sanya Bay

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### Abstract

Food differentiation among coexistent species in the field is important strategy for copepods to acquire materials and maintain population stabilization. *In situ* diet analysis of co-occurring six copepod species in coral waters of the Sanya Bay was conducted using a PCR protocol based on 18S ribosomal gene. Various prey organisms were uncovered, including dinoflagellate, diatom, green algae and plant, protozoa and metazoan. All these spatially co-existing six species showed different dietary diversity, with the food niche breadth ( $B$ ) ranging from 1.00 (*Temora turbinata* in morning) to 10.68 (*Calanopia elliptica* in night). While food overlap between all these copepods were low, with the average value of the diet niche overlap index being approximately 0.09. Even temporally co-existing species sampled from the same time point fed on different groups of prey items with the food overlap index of 0.04 to 0.07 in midday and night but 0 in morning. As the most important dominant copepod in the Sanya Bay, *Subeucalanus subcrassus* seems to be capable to regulate its feeding, by exhibiting a rhythm of herbivorous feeding in midday and carnivorous feeding in morning and night, to better coordinate with other competitors for utilization of food resources. For most copepods, none of the prey items belonged to the dominant phytoplankton in the ambient water, indicating that copepod can better their survival by widening the choice of potential food resources in food limited environment. The dietary separation observed here might be important strategy for copepod to maintain population stabilization and thriving in the Sanya coastal waters.

**Key words:** copepod, food partitioning, Sanya Bay, coral reef ecosystem

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### 1 Introduction

The question of resource partitioning often arises when considering the ecology of plankton in marine ecosystem, which was first discussed as a paradoxical situation of plankton by Hutchinson (1961). Namely, it is very important question how it is possible for plankton to coexist in the same environment competing for the same materials. For maintaining coexistence, these species might exhibit different strategies of resource utilization and thus occupy different niche. Differences in the niche of different species could be important functional traits to express the ecosystem functioning (Aranguren-Riaño et al., 2018). Among all these functional traits, feeding is the most vital process which determines energy supply and thus sustains the stabilization of the cooccurrence of different species (Lee et al., 2012). While it was difficult to perform field observation of food use for different species, especially for small-sized zooplankton.

Copepods are the most abundant zooplankton in marine ecosystem and play an important role in energy transfer to higher trophic levels and many copepod species are often found in the

same area with very high abundance (Kleppel, 1993). As most of copepod species showed a similar way using food resources, various feeding strategies had been reported for copepod to deal with the food competition. Laakmann et al. (2009) found that co-occurring large-sized copepod species exhibited different ecological niche by vertical partitioning and different food preferences among water layers in pelagic deep-sea ecosystems (Laakmann et al., 2009; Sato et al., 2011). Unlike the almost homogeneous environment in deep sea, coastal waters were characterized by variable hydrodynamic factors and uneven food environment, and thus copepod in coastal waters was potentially vulnerable to food limitations and they might be especially dependent on sufficient food supply within close range (David et al., 2006). Previous studies using amino acid composition or marker pigment as indicator to distinguish distinct food sources had found that co-occurring copepod showed asymmetric reaction in incubation experiment even there is no apparent resources competition among them in the field (Arroyo et al., 2007).

Although previous studies had shown that dominant zo-

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oplankton taxa exhibited different trophic level and feeding selectivity in variable coastal environment (Carrasco and Perissinotto, 2011), copepod was found to apply an “opportunistic feeding” strategy in food-limited conditions and had to utilize insufficient food resources according to their availability, which would exacerbate feeding competition among different species (Lee et al., 2012; Lombard et al., 2013; Hu et al., 2014). In this circumstance, it is vital for these co-existence copepods to allocate limited resources properly and efficiently in order to maintain their population stabilization. As it was difficult to perform field observation of food use of copepod, rare data had obtained about exact diets composition of different species in the same community though previous studies using amino acids or pigments analysis had revealed trophic niche of different populations (Guisande et al., 2002). The benefit from development of molecular methods for feeding studies makes it possible now to obtain exact diets information of copepods in natural sea (Nejstgaard et al., 2003; Hu et al., 2014, 2015).

The Sanya Bay located in the southernmost coast of Hainan Island in the South China Sea. With an area of about 120 km<sup>2</sup> and mean depth of 16 m, the bay features several coastal coral reefs situated at Luhuitou, Dongmao Island and Ximao Island (Huang et al., 2003). While in the coastal coral waters, the primary production was low (with an average chlorophyll *a* concentration of about 0.95 mg/m<sup>3</sup>) but high biomass of copepod was always observed, with *Subeucalanus*, *Temora*, *Calanopia*, *Paracalanus* and *Acartia* being the most abundant groups (Ke et al., 2011). In our previous study of copepod *in situ* diets conducted in summer 2010, three dominant copepod species, *Temora turbinata*, *S. subcrassus* and *Canthocalanus pauper*, consumed large amount of terrigenous detritus as supplementary food resources in phytoplankton-limited condition (Hu et al., 2015). Considering high concentration of terrigenous materials brought by the Sanya River or coastal land plants, copepod seemed to apply an “opportunistic feeding” strategy in such food-limited environment. As a tropic open bay, seasonal succession of dominant species was

not obvious in the Sanya Bay, thus the mechanism supporting coexistence of these copepod groups is of great importance for understanding the production of zooplankton but remains unclear. The aim of this study was to determine *in situ* diet compositions of co-occurring dominant copepod populations by a PCR protocol, which was proved to be effective in detecting copepod preys in field (Hu et al., 2014), in order to demonstrate the food use strategy of copepod in the Sanya coastal waters.

## 2 Materials and methods

### 2.1 Sample collection

Copepods and ambient water samples were collected at Sta. SY-C (18°13.199'N, 109°28.799'E) near Luhuitou coral waters in the Sanya Bay on May 21, 2011 at three time points (6:00, 12:00, 18:00) (Fig. 1). Copepods were collected using a plankton net (505 μm) and were fixed immediately in neutral Lugol's solution at 2% final concentration. Ambient water samples were also collected using a Niskin bottle and 500 mL was fixed immediately. The samples were covered with black bags and taken to the laboratory for next analysis.

### 2.2 Microscopic analysis of water sample

Water samples were gently mixed by inversion and 50 mL subsample was taken into a 50 mL centrifuge tube. Then the tube was kept for >24 h and concentrated to 1 mL according to the Utermöhl Settling method. From the concentrated samples plankton were identified and enumerated in a Sedgwick-Rafter counting chamber under an Olympus BX51 microscope.

### 2.3 *In situ* diet analysis of copepod samples

Dominant copepod species from different time were identified under stereomicroscope and sorted. The sorted copepod samples were serially rinsed thoroughly with autoclaved 0.45 μm filtered seawater for >3 times and then with sterilized water for several times, examined under stereomicroscope to ensure no at-

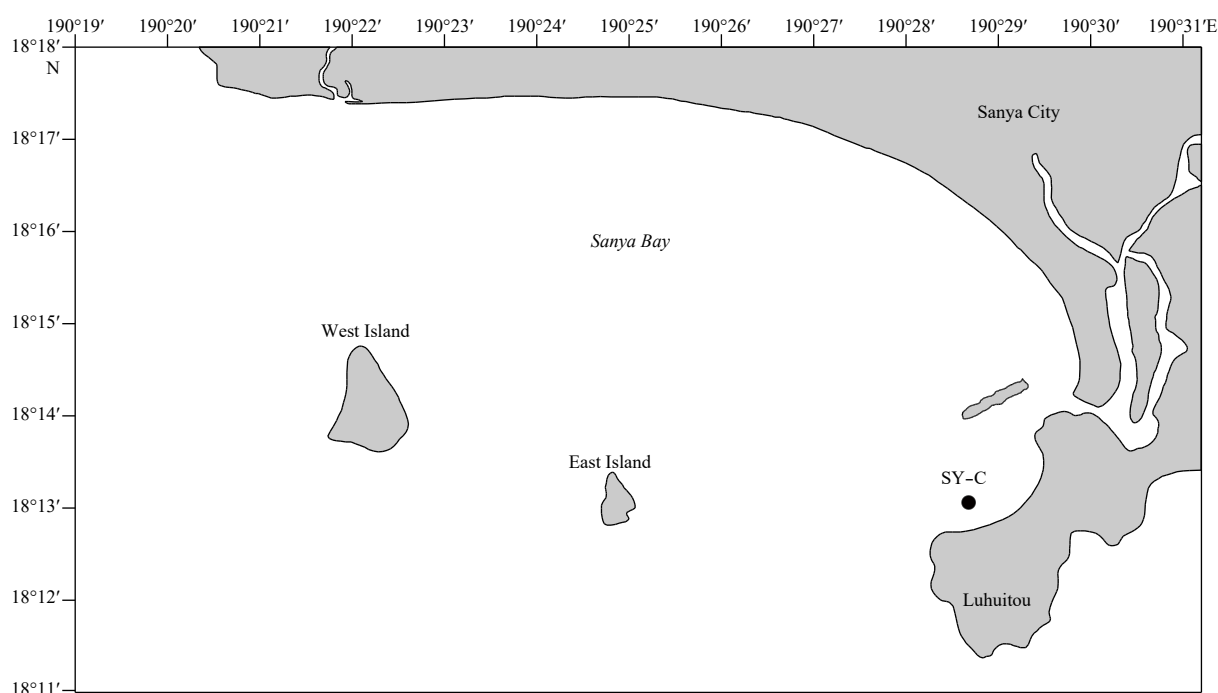


Fig. 1. Sampling location (SY-C) in coral waters near Luhuitou of the Sanya Bay.

tachment of other visible organisms on the surface body, and homogenized in a microfuge tube using a disposable micro-pestle. The homogenates were re-suspended in 500  $\mu\text{L}$  DNA buffer (1% SDS, 100 mmol/L EDTA, and 200  $\mu\text{g}/\text{mL}$  proteinase K) and incubated for 3 d at 55°C for thorough cell lysis and DNA from all samples were extracted. 18S rDNA of copepod samples were PCR amplified using the copepod-excluding eukaryote-inclusive primer set (CEEC) and the PCR products were purified, cloned and 30–50 clones for each sample were picked randomly and sequenced (Hu et al., 2014).

#### 2.4 Phylogenetic analyses

Sequences obtained were searched against the GenBank database using the Basic Local Alignment Search Tool. The best hits were aligned with new sequences obtained in this study using CLUSTAL W (1.8) after the primer sequences were trimmed. Then the alignment results were exported to MEGA 4.0 and a Neighbor Joining (NJ) tree was inferred from the aligned dataset.

#### 2.5 Food niche overlap analysis

A percentage of each sequence from the whole clone library of each copepod sample was used to estimate the relative diet proportion. The percentage was then recorded to calculate diet diversity, which was described by food niche breadth ( $B$ ), a reciprocal of Simpson's diversity index, following Levins (1968):

$$B = \frac{1}{\sum_{i=1}^n P_i^2}, \quad (1)$$

where  $P_i$  is the relative abundance of prey  $i$  and  $n$  is the total taxa of all the preys in the diet of copepod.

Diet niche overlap ( $O$ ) was also calculated by the percentage of overlapped diet species between different copepod species (Pianka, 1973):

$$O_{xy} = 1 - \frac{1}{2} \times \sum |P_{ix} - P_{iy}|, \quad (2)$$

where  $x$  and  $y$  denote different species of copepod.

Bipartite network was also used based on percentage of each diet on species-level, to intuitively reflect main dietary specialization and overlaps of different copepods, and visualization was performed with the R package "bipartite".

### 3 Results

#### 3.1 Phytoplankton and copepod community

Microscopic analysis of ambient water identified 35 phytoplankton species totally, including diatom, dinoflagellates, chrysophyta and cyanobacteria (Table 1). Diatom dominated the community both in species numbers and cell density with 68.4% and 74.2%, 85% and 95.9%, 85% and 52.6% in morning, midday and night, respectively. And the cell density presented an increase trend in time series morning ( $9.7 \times 10^3$  cells/L), midday ( $25.4 \times 10^3$  cells/L) and night ( $73.3 \times 10^3$  cells/L). *Skeletonema* sp. was dominant group both in morning and midday, while *Trichodesmium erythraeum* occupied almost half of the total abundance (46.5%) in night. *Rhizosolenia* sp. was also abundant group in midday and night.

Different composition patterns were also observed for copepod community in different time, 13, 10 and 19 species were

**Table 1.** Phytoplankton community of ambient water from different times

	Species number	Percentage /%	Density/ $10^3$ cells·L <sup>-1</sup>	Percentage /%
Morning				
Diatom	13	68.4	7.2	74.2
Dinoflagellate	5	26.3	2.2	22.6
Others	1	5.3	0.3	3.2
Sum	19	100	9.7	100
Midday				
Diatom	17	85	24.3	95.9
Dinoflagellate	3	15	1.0	4.1
Others				
Sum	20	100	25.4	100
Night				
Diatom	17	85	38.6	52.6
Dinoflagellate	2	10	0.6	0.9
Others	1	5	34.1	46.5
Sum	20	100	73.3	100

Note: Others include Cyanobacteria (mostly) and Chrysophyta.

identified in morning, midday and night, respectively. The highest density of copepods ( $38.1 \text{ ind./m}^3$ ) presented in night, being much higher than that in morning ( $8.26 \text{ ind./m}^3$ ) and midday ( $7.93 \text{ ind./m}^3$ ), respectively. As for the species community, small copepods (<2 mm), such as *Paracalanus*, *Acrocalanus*, *Temora*, *Centropages* and *Acartia*, showed similar but lower densities in morning, midday and night, but occupied almost 95% of total community in midday. Large copepods in genus of *Labidocera*, *Pontella*, *Calanopia* and *Undinula* appeared only in night and dominated the copepod community. *Subeucalanus subcrassus* was the dominant species in all three times, and other species with different sizes, such as *Neocalanus tenuicornis*, *Acrocalanus gibber*, *Temora turbinata*, *Acrocalanus gibber*, *Acartia negligens* and *Calanopia elliptica*, which were sorted out for diets analysis, were also abundant in different community.

#### 3.2 Food diversity

The 18S rDNA clone libraries were constructed for diet analyses of all these six species of copepod from different time points. Different taxa (1–10) of preys were revealed by randomly sequenced clones for each library. Chao1 estimates indicated that the actual numbers of taxa were similar with sequenced taxa, showing adequate coverage of diversity (Table 2). Diverse diets were detected for all the copepods as shown by the wide phylogenetic range and taxonomic distribution of these prey species

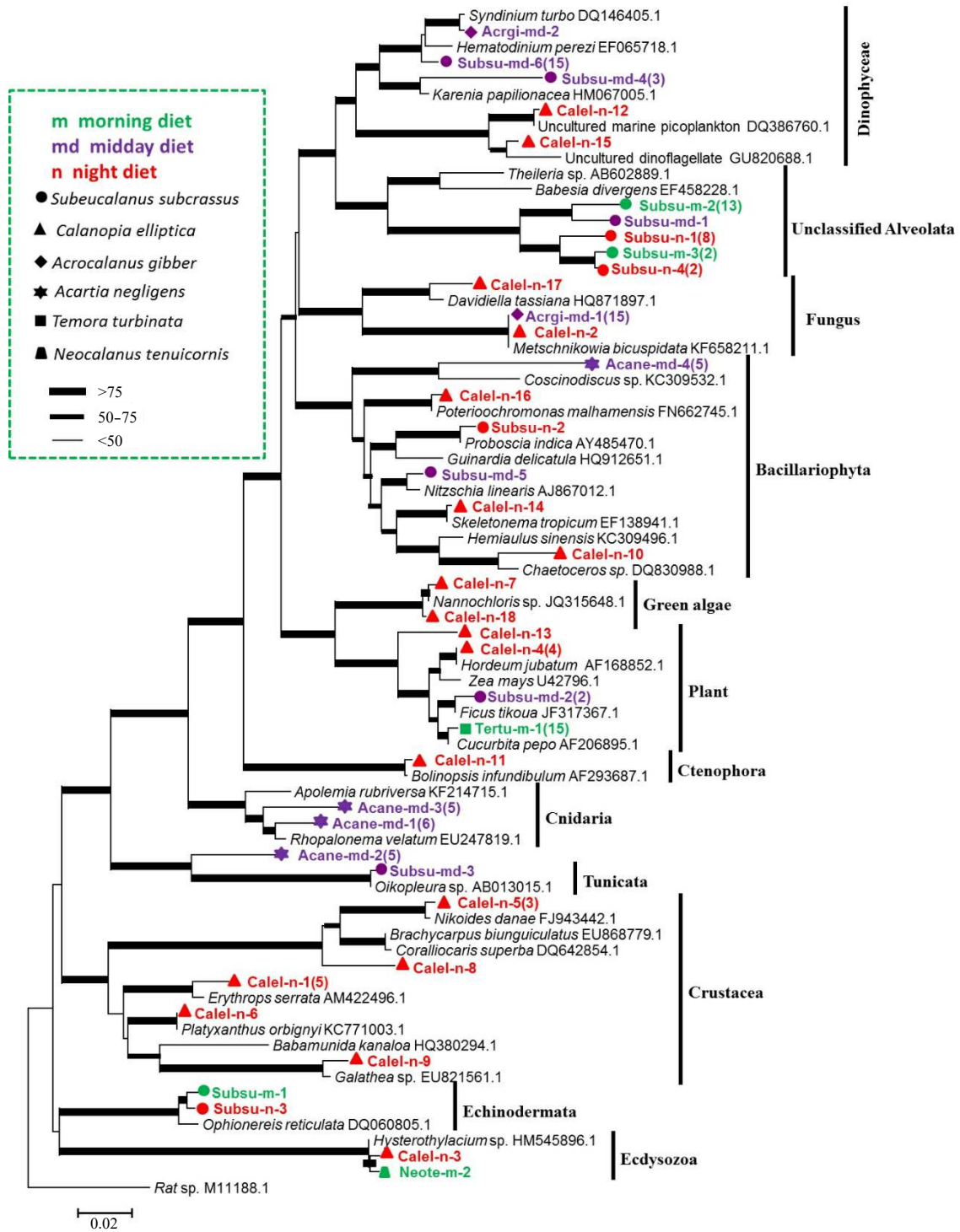
**Table 2.** Diversity indices of prey organisms in the eight copepod samples analyzed

Sample ID	Taxa_S	Individuals	Simpson_1-D	Shannon_H	Chao-1
Subsu-m	2	15	0.124 4	0.244 9	2
Subsu-md	6	23	0.544 4	1.166	7.5
Subsu-n	3	12	0.291 7	0.566 1	4
Temtu-m	1	15	0	0	1
Neote-m	2	22	0.0867 8	0.184 9	2
Acrne-md	3	20	0.625	1.04	3
Acrge-md	3	17	0.214 5	0.443 8	4
Calel-n	10	27	0.776 4	1.867	12.5

Note: Subsu represents *Subeucalanus subcrassus*, Temtu *Temora turbinata*, Neote *Neocalanus tenuicornis*, Acrne *Acartia negligens*, Acrge *Acrocalanus gibber*, Calel *Calanopia elliptica*, m morning, md midday, and n night.

(Fig. 2), including phytoplankton (e.g., diatom, dinoflagellate, green algae), plant, protozoa and different groups of metazoan (e.g., cnidarian, tunicate, crustacean). Metazoan was among the most abundant and diverse groups of prey items, with a variety of crustacean preys, such as *Erythrotrips* (Mysis), *Processidae*, and

*Galathea* (coral shrimp). Other metazoan included ctenophora, cnidaria (jellyfish), tunicate (appendicularia), and Echinodermata (brittle star). Diatoms were also among the most diverse groups of prey items, including *Coscinodiscus*, *Chaetoceros*, *Skeletonema*, *Proboscica* and *Nitzschia*, which were all microscopic



**Fig. 2.** 18S rDNA phylogram for different copepod species from different sampling time in the Sanya Bay. Neighbour-Joining (NJ) phylogenetic tree was shown here and only representative clones from each major lineage were included in the tree. Different colors denote different sampling time and different symbols denote different copepods. Canle-n represents *Calanopia elliptica* from night, Temtu-m *Temora turbinata* from morning, Neote-m *Neocalanus tenuicornis* from morning, Acane-md *Acartia negligens* from midday, Acargi-md *Acrocalanus gibber* from midday, Subsu-m *Subeucalanus subcrassus* from morning, Subsu-md *S. subcrassus* from midday, and Subsu-n *S. subcrassus* from night.

identified in the ambient water. Relatively, dinoflagellates were less abundant prey items only for some copepod species (e.g., *S. subcrassus*), with *Karenia*, *Syndinium* and two unclassified species being detected. Land plant was also abundant prey items for some copepod species (e.g., *C. elliptica*, *T. turbinata*, *S. subcrassus*), with *Hordeum*, *Ficus* and *Cucurbita* being detected. A species of green algae (*Nannochloris* sp.) was also detected in *C. elliptica*. Some other organisms, such as Fungus and unclassified Alveolata, were also detected in some copepod species.

### 3.3 Diet composition of different species

The diets composition of spatially co-occurring six species were different from each other as shown by the wide taxonomic distribution of these prey species and diverse phylogenetic affiliations that received strong bootstrap supports (Fig. 2). Large copepod *S. subcrassus* fed mostly on protozoa and dinoflagellates, and *N. tenuicornis* diet consisted of a large fraction of metazoan and fungus. While another large copepod *C. elliptica*, which was the most abundant species in night, exhibited a much wider prey spectrum, including diatom, dinoflagellates, green algae and plant, ctenophore and a large amount of crustacean. Relatively, small copepods showed much lower diet diversity. *Acartia negligens* consumed a large fraction of metazoan (e.g. cnidarian, appendicularia) and a little diatom (*Coscinodiscus* sp.), while large amount of fungus and plant were detected in *A. gibber* and *T. turbinata*. Generally, metazoan, plant and phytoplankton were common prey items for these copepods, but with different preference in different populations. For instance, metazoan dominated the diet of *A. negligens* with two species of cnidarian, and *C. elliptica*'s with a large fraction of crustacean and a species of ctenophore, respectively. *Proboscia indica* and *Skeletonema tropicum*, which were abundant in ambient water, were detected in the diet of *S. subcrassus* and *C. elliptica*, respectively.

Food niche breadth (*B*) of six copepod species differed greatly from each other. *Calanopia elliptica* showed the highest *B* index of 10.48, followed by *A. negligens* with a value of 4. The *B* index for other species varied from 1.0 to 2.2.

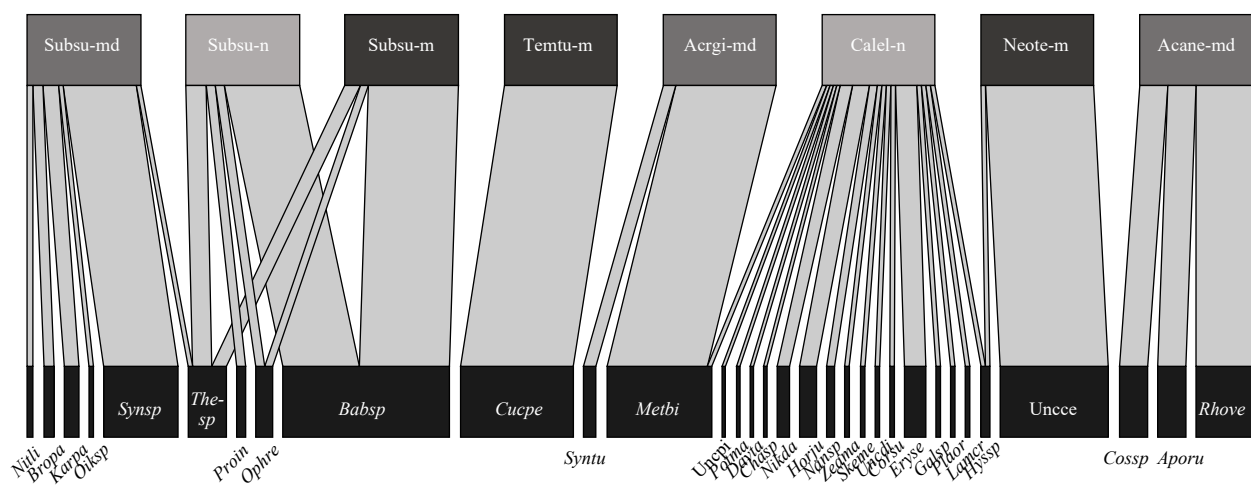
On the other hand, although temporally co-existing copepod populations sharing the same restricted food resources, diet differentiation was also observed. Thirty-eight feeding events were observed totally by the bipartite network analysis (Fig. 3). All these copepod species showed very limited food overlap as demonstrated by low (<0.2) diet niche overlap (*O*) index (Table 3). The three species from morning showed no overlap with each other in diet composition, with an *O* index of 0. Copepods from midday and night showed very limited diet overlap as the *O* index ranging from 0.04 to 0.07.

## 4 Discussion

Competition for food is generally thought to exert a strong evolutionary pressure, driving trophic niche separation, either by specialization and/or by widening the choice of potential food resources, and determining the final assemblage (Guisande et al., 2003). To keep population stabilization for coexistence copepods in a competitive food environment was important question to help understanding of the ecosystem function (Kimmel, 2011). Previous studies had revealed several potential mechanisms for this question in coastal waters, such as variations of vertical distribution among different populations, different feeding rhythm and selective feeding of different species (Ishii, 1990; Pierson et al., 2013). All these results were obtained indirectly by trophic biomarker analysis which can provide dietary signals over longer time periods of days to several weeks (Teuber et al., 2014). In our study, exact *in situ* food species of copepods was uncovered directly by the molecular detection. The real time diets of different species provided intuitive evidence of food niche separation among coexisting copepods, which could support food partitioning and thus decrease competition for food resources between species. This feeding strategy for copepod populations was of great importance for their survival and production in food limited coastal waters like the Sanya Bay.

### 4.1 Widened food spectrum

Diverse diet items were detected from the clone libraries of



**Fig. 3.** Bipartite networks depicting the main dietary specialization and overlaps of different copepod samples. Bars on the top line represent different copepod sampled from different time (Canle-n represents *Calanopia elliptica* from night, Temtu-m *Temora turbinata* from morning, Neote-m *Neocalanus tenuicornis* from morning, Acane-md *Acartia negligens* from midday, Acrgi-md *Acrocalanus gibber* from midday, Subsu-m *Subeucalanus subcrassus* from morning, Subsu-md *S. subcrassus* from midday, and Subsu-n *S. subcrassus* from night). Bars on the bottom line represent different food items on species level (shortening the names with the first three letters of the genus name and first two letters of the species names), and the width of bars denote the relative percentage of each food items. A line connecting the bars between copepod and food species represents an intaking event.

**Table 3.** Diet niche overlap indexes of all the copepods samples

	Subsu-m	Temtu-m	Neote-m	Subsu-md	Acrigi-md	Acane-md	Subsu-n	Calel-n
Subsu-m	1	0 <sup>1)</sup>	0 <sup>1)</sup>	0.04	0	0	0.9	0
Temtu-m	0	1	0 <sup>1)</sup>	0.09	0	0	0	0.19
Neote-m	0	0	1	0	0.06	0	0	0.04
Subsu-md	0.04	0.09	0	1	0.06 <sup>1)</sup>	0.04 <sup>1)</sup>	0.09	0.17
Acrigi-md	0	0	0.06	0.06	1	0 <sup>1)</sup>	0	0.11
Acane-md	0	0	0	0.04	0	1	0.09	0.18
Subsu-n	0.9	0	0	0.09	0	0.09	1	0.07 <sup>1)</sup>
Calel-n	0	0.19	0.04	0.17	0.11	0.18	0.07	1

Note: <sup>1)</sup>The diet niche overlap indexes of copepods from the same sampling time (morning, midday and night).

six copepods with 35 taxa totally, including not only common food reported by previous studies, such as diatoms, dinoflagellates, chlorophyta and protozoan, but also diet species uncovered recently by new methods, such as metazoan and land plants. Metazoan and land plants were the most common prey items among six copepod species analyzed here, followed by diatom, and this was consistent to some extent with previous study conducted in the same area in the Sanya Bay (Hu et al., 2015). Diatom was abundance group in prey items but with relatively low percentage of clone libraries. *Skeletonema tropicum*, *Chaetoceros* sp. and *Proboscia indica* were also identified in ambient water. However, among all the prey items detected, metazoan exhibited highest species diversity and relative abundance, with different phyla (e.g., Ctenophora, Ophionereididae, Crustacea). It had been confirmed that copepod could feed on metazoan by both gut content analysis (Schnitzer and Steinberg, 2002) and molecular detection (Hu et al., 2014, 2015). Most of the crustacea detected here were from Malacostraca, such as *Erythrope*, *Nikoides*, *Coralliocaris*, *Galathea* and *Platyanthus*, which might be the eggs or larva. In the Sanya Bay of spring (April to June), almost all the aquatic organisms were in spawning or breeding stage, and planktonic larvae could reach a percentage of 25% in zooplankton community with diverse species (e.g., Mysidacea larva, Luciferinae larva and Zoea larva) (Yin et al., 2004). Ophiuroids were also common benthic echinoderms in coral reef ecosystem (Lewis and Bray, 1983) and had only a spawning period during spring to summer (Yokoyama et al., 2008). Another group of metazoan worth noting was Hydrozoa (jellyfish), which was considered to be predators of copepod (Ishii and Tanaka, 2001), indicating complex trophic relationships among zooplankton in coastal waters. Land-plant detritus were another abundant food resource for copepods, and this was consistent with our previous results which found that copepods consumed large amounts of land plant detritus as a supplementary food source when faced with food limited situation (Hu et al., 2015). Widened food spectrum of these copepods could help to reduce food competition to some extent, and this might be important strategy for copepod populations to acquire food for their community development in complex natural environment (Leising et al., 2005; Kjørboe et al., 2010).

#### 4.2 Dietary separation of different copepod species

Copepods differed in both diet diversity and food preference in our results. The highest diversity was observed in larger copepod populations in night, with 18 and 4 taxa in *C. elliptica* and *S. subcrassus* respectively. This was consistent with previous studies that copepods would increase feeding activity from dusk, which could decrease the danger of predation (Saito and Taguchi, 1996; Calliari and Antezana, 2001). While there were also some species which showed enhanced feeding in daytime, such

as *S. subcrassus* in this study with 6 taxa detected in midday (Wong et al., 1991). *Subeucalanus subcrassus* fed mostly on protozoa and dinoflagellates, while *C. elliptica* and *A. negligens* consumed a large fraction of metazoan. Plant dominated the diet of *T. turbinata* and protozoa (Rhizaria) dominated the diet of *N. tenuicornis*.

The spatially co-existing species in our study could be divided into different ecological groups according to their ecological habits and distribution characters. *Calanopia elliptica* and *A. negligens* were subtropical coastal epipelagic species, and *S. subcrassus* and *T. turbinata* were warm water nearshore groups, while *N. tenuicornis* was widely-distributed warm water pelagic group (Yin et al., 2004). It is obvious that coastal population groups, such as *C. elliptica*, *T. turbinata* and *A. negligens* consumed large amount of metazoan and plant, which might originate from organic detritus, indicating a detritivorous feeding of these omnivorous copepods. These copepods were considered to be omnivores and they might apply a filter feeding during the day time, as the time-averaged fluid signal and the consequent predation risk is much less for copepods >1 mm using this feeding pattern (Kjørboe et al., 2010). We also found that the population structure varied by copepod size-fractions. The abundance of small copepods (<2 mm), such as *Paracalanus*, *Acrocalanus*, *Temora* and *Acartia*, showed little variations from morning, midday and night, but they occupied a large fraction (>50%) of the total biomass in midday, while large copepods (>2 mm) were more abundant and diverse in night. Dagg (1977) measured starvation time of different copepods, and showed that small coastal species, such as *Acartia* spp., were more vulnerable to starvation than larger ones (such as *Pseudocalanus minutus* and *Calanus finmarchicus*), so they might reside within specific environment to reduce energy consumption.

As the sampling site was reported to have high suspended matter and terrigenous input accounted for 44.5% of total sediments, primary production was much lower and might be insufficient to support zooplankton biomass (Zhao et al., 2013). Lombard et al. (2013) found that copepod can detect chemical trails originating from sinking marine snow particles, indicating that organic detritus in the water column might be potential sources of food supplements for coastal copepods like the coastal groups detected in this study. While as the most important dominant copepod in the Sanya Bay, *S. subcrassus* exhibited a more flexible feeding pattern due to its high capacity of migration and ambush feeding, indicating that organic detritus might be important supplementary food sources for omnivorous coastal populations (Lee et al., 2012). As the only pelagic group, *N. tenuicornis* consumed mostly unknown rhizaria, which was not detected in other copepods, indicating they might occupy different ecological niche through food partitioning even if spatially collocated in the same environment.

Moreover, copepods sampled from the same time also showed different food preference. Sequences of metazoan and protozoa were abundant in diets of *S. subcrassus* and *N. tenuicornis* in the morning, while *T. turbinate* consumed large amount of plant. Dominant small copepod, *A. negligens*, in midday community also consumed a large amount of organic detritus from metazoan (e.g., tunicate, cnidaria), while the larger population of *S. subcrassus* mainly fed on dinoflagellate. Even though the night community was dominated by two large species with similar size (*S. subcrassus* and *C. elliptica*), they showed apparent differences in diets diversity. The food partitioning here might be important strategy for the stabilization and survival of copepod assemblage (Guisande et al., 2003). In the morning, all the three copepods showed lowest diet diversity and none of the dominant phytoplankton in ambient water were detected for prey. This was consistent with previous conclusions that copepod would decrease feeding activity since dawn, especially pigment prey which would make them more visible to predators (Ishii, 1990). While in night, the diversity of all dominant populations was high, as the copepod biomass was highest in night and the strengthened feeding competition make the copepod more selective to acquire nutritional needs.

#### 4.3 Potential significance of dietary separation for copepod in coastal ecosystem

Variations of the phytoplankton community in our results suggested a changing food environment in the study area during the sampling time in the Sanya coastal waters. Copepod was reported to be capable of switching feeding pattern to better utilize the food resources in such conditions. *Subeucalanus subcrassus* copepods here could switch their feeding behavior to cooperate with co-occurring copepods in food allocation, with a more herbivorous feeding at midday and relatively carnivorous feeding in morning and night. *Subeucalanus subcrassus* was dominant species during the sampling time and they had strong ability to migrate throughout the whole water column in the Sanya Bay (Yin et al., 2004). When faced with harsh competition and relatively lower predation risk in night, they consumed mainly protozoa by ambush feeding, while in midday with high predation risk they fed on more diverse preys by filter feeding. Different feeding mode will generate different fluid signal and hence exposes the grazers differently to predators. For such large copepods as *S. subcrassus*, fluid signal and the consequent predation risk is much less by filter feeding than ambush feeding (Kjørboe et al., 2010). It was clear from the results that *S. subcrassus* consumed a large amount of dinoflagellates in midday, even though diatom dominated the phytoplankton. The discrepancy between preys detected and ambient phytoplankton community might indicate selective feeding of *S. subcrassus*, consisting with that Carrasco and Perissinotto (2011) demonstrated copepod was more selective in complex coastal ecosystem and hence support food partitioning between species (Pagano et al., 2003). These feeding switching and selectivity might be vital mechanism to ensure *S. subcrassus* being the most important ecological groups throughout the year in the Sanya Bay (Mackas et al., 1993, Yin et al., 2004).

The variations of dominant species like *S. subcrassus* in community will bring different extent of feeding competition for co-occurring populations, as copepods exhibit a wide array of foraging behaviors across many spatial scales, depending on copepod species and ambient food environment (Leising et al., 2005). The selective differences amongst copepods could well avoid inter-specific competition within assemblage temporally ap-

peared in the same water column (Arroyo et al., 2007; Laakmann et al., 2009). Such varied food niche differentiation pattern in field might be an important adaptive mechanism for copepod to survive in complex environment like the Sanya coastal water here.

#### 5 Conclusions

Coastal coral ecosystem in the Sanya Bay was characterized by low primary production (phytoplankton biomass). The Sanya Bay was also an open bay with diverse ecological groups of copepod collocated. As reported previously, diverse prey items detected here for different copepod species suggested they may potentially be able to feed opportunistically and opportunistic feeding may intensify feeding competition (Hu et al., 2015). In this study, we found that spatially co-existing six copepod species exhibit an obvious dietary separation as almost none of them have food overlap. Furthermore, the dietary difference was not caused by ambient food environment as temporally co-existing species also consumed different preys even they were caught in the same site. Dietary separation observed here indicates that different copepod populations could occupy non-overlapping trophic niche to meet their energy and material requirement in such complex food environment. This *in situ* evidence indicated that trophic-niche differentiation might play a key role in stabilization and survival of copepod assemblage in coastal ecosystems.

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