Possible aplanochytrid (Labyrinthulea) prey detected using 18S metagenetic diet analysis in the key copepod species *Calanus sinicus* in the coastal waters of the subtropical western North Pacific

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**Abstract:** Metagenetic diet analyses of the 18S V9 region were conducted in 40 adult female *Calanus sinicus* during winter in Tosa Bay (Japan). The majority of prey items were small crustaceans (of Copepoda and Cirripedia) and diatoms, taxa that are dominant in the environment and have been previously reported as important prey items of *Calanus*. The abundance of sequences attributable to Dinophyta and Chlorophyta was significantly lower in *C. sinicus* gut contents than in environmental plankton communities, suggesting that *C. sinicus* avoids prey from these groups. Hydrozoans were also observed, and aplanochytrids (Labyrinthulea) were detected for the first time as a major prey of *C. sinicus*. Additionally, high proportions of unclassified eukaryote material were observed, suggesting undetected predator–prey relationships in key copepod species in marine ecosystems. The dietary importance of aplanochytrids, heterotrophic protists that accumulate unsaturated fatty acids such as docosahexaenoic acid, has been overlooked in previous research. *Calanus sinicus* is a key copepod species in the subtropical coastal regions of the western North Pacific, and a major food source for the larvae of commercially important fish; therefore, further investigation into novel prey items such as aplanochytrids is recommended to understand the complex food web structures in marine ecosystems.

**Key words:** aplanochytrid, *Calanus sinicus*, diet, Labyrinthulea, metagenetics

**Introduction**

*Calanus sinicus* (Brodsky, 1962) is a large calanoid copepod (approximately 2.1–3.3 mm in total length) common to the coastal waters of the subtropical western North Pacific (Hulsemann 1994, Chihara & Murano 1997). *Calanus sinicus* is a key species in the food webs adjacent to the Kuroshio Current, off Japan, where it makes up most of the zooplankton biomass, and where its immature stages are the primary prey for larvae of commercially important fish (Nakata & Hidaka 2003, Hirai et al. 2017). Although phytoplankton is considered its primary food source, *C. sinicus* is omnivorous, and also feeds on various microzooplankton (Uye & Murase 1997, Yang et al. 2009). It has also been reported that feeding habits, such as the proportion of phytoplankton contributing to its diet, vary temporally and spatially, according to food availabilities in ambient waters (Zhang et al. 2006). In addition to water temperature, food availability is one of the primary factors controlling development and egg production in *C. sinicus* (Uye & Murase 1997), suggesting that detailed investigation of predator–prey relationships involving *C. sinicus* is important to understand planktonic food webs in the western North Pacific.

Various approaches have been used to analyse the diets of copepods, including morphological observations, stable isotope analyses, and pigment measurements; however, it
is difficult to obtain detailed taxonomic information from damaged gut contents using such methods. For example, observations using scanning electron microscopy (SEM) frequently detect fragments of diatoms in the gut contents of C. sinicus; however, the majority of gut contents remain unidentifiable (Chen et al. 2010). In contrast, a molecular-based method can detect detailed taxonomic information in damaged gut contents. The metagenetic method, which uses high-throughput sequencing, is a particularly powerful approach to investigating predator–prey relationships at high taxonomic resolutions in marine taxa such as copepods (Pompanon et al. 2012, Cleary et al. 2016). Diets of C. sinicus have been investigated in the South Yellow Sea and the Bohai Sea using the cloning technique (Yi et al. 2017), and in the coastal waters around Taiwan and Hong Kong using metagenetic analysis (Ho et al. 2017). These studies confirm that C. sinicus is omnivorous and that feeding habits vary spatially and temporally, and furthermore identified novel prey items in the gut contents belonging to gelatinous taxa classified as Hydrozoa and Ctenophora.

In the present study, we collected C. sinicus during winter in Tosa Bay (Japan). 18S metagenetic analyses of gut contents were carried out in adult female C. sinicus to characterise the diet and to detect novel prey items previously undetectable using conventional means. Considering its strong ecological role in linking higher and lower trophic levels, investigating in detail the prey items taken by C. sinicus is an important step in understanding marine ecosystems in the coastal waters of the subtropical western North Pacific.

### Materials and Methods

#### Samplings

Samples were collected during the cruise SY-15-02 aboard the research vessel (R/V) Soyo-maru (National Research Institute of Fisheries Science, Japan Fisheries Research and Education Agency), at two stations, L3250 (32°49.8’N; 133°10.2’E) and N3320 (33°20.1’N; 133°29.9’E), in the coastal area of Tosa Bay, off the southern coast of Japan (Fig. 1). Samples were collected on 15 and 17 February 2015, respectively, to correspond with the spawning seasons of the Japanese sardine Sardinops melanostictus and the Pacific round herring Etrumeus teres (Hirai et al. 2017). Bulk zooplankton samples were collected using ORI or NORPAC nets with a mesh size of 335 μm at depths of 0–50 m, once during the day and once at night at each station, and were preserved at −20°C. Water samples were used to characterise the environmental plankton communities at the sampling sites, according to the methods detailed by Hirai et al. (2017). We collected a water sample at three depths (0, 10, and 30 m) at each station. Immediately after collection, 1 L of each sample was filtered using sterile polycarbonate filters with pores 1 μm wide (Nuclepore membrane; GE Healthcare).

![Fig. 1. Sampling locations in the present study. Black dots show sampling stations. Isobaths are indicated by grey lines.](image-url)

**High-throughput sequencing**

Frozen bulk zooplankton samples were thawed in the laboratory, and we identified 10 adult female C. sinicus individuals, morphologically, from each sample. In total, 40 individuals (10 at each station, day and night) were dissected, and the whole gut of each specimen was collected. Genomic DNA was extracted from the guts of each C. sinicus individual using 30 μL 5% Chelex buffer (Bio-Rad Laboratories) according to the methods proposed by Nagai et al. (2012). Protocols of library preparations for high-throughput sequencing were performed for gut contents of C. sinicus, according to the method of Hirai et al. (2017). The 18S V9 region (approximately 130 bp) was amplified by the polymerase chain reaction (PCR) using eukaryotic universal primers 1389F (5′-TTG TAC ACA CCG CCC-3′) and 1510R (5′-CCT TCGCA GGT TCA CCT AC-3′; Amaral-Zettler et al. 2009). Adaptors for Illumina MiSeq and index sequences for discriminating samples were attached by second and third PCRs (Hirai et al. 2017). DNA concentrations of PCR products were measured using a Qubit 3.0 Fluorometer (Life Technologies). An equal amount of DNA was combined from each of the PCR products of gut contents, before being purified using the QIAquick PCR Purification Kit (QIAGEN). Finally, the PCR products of gut contents were transferred to Bioengineering Lab. Co. Ltd. (Atsugi, Japan), where a single sequencing procedure was carried out using MiSeq Reagent Kit v2 on an Illumina MiSeq platform to obtain 2×250 bp paired-end sequence reads (DDBJ Sequence Read Archive accession number DRA005779). The sequence data for environmental seawater samples were already available from a previous study (Hirai et al. 2017); therefore, sequencing data were not newly obtained for environmental plankton communities in the present study.

#### Data analyses

The raw sequence data for C. sinicus gut contents were
combined with those of the environmental plankton communities reported by Hirai et al. (2017). According to the methods described by Hirai et al. (2017), sequences with low quality and short fragment length were removed using Trimmomatic (Bolger et al. 2014). The remaining paired-end sequences were merged and further quality-filtered in MOTHUR (Schloss et al. 2009). These high-quality sequences were aligned using Silva 119 databases (Quast et al. 2013) in MOTHUR. After single-linkage pre-clustering (Huse et al. 2010), we removed singleton reads according to Unno (2015), in order to remove redundant and erroneous molecular operational taxonomic units (MOTUs). Chimeric sequences were removed using UCHIME (Edgar et al. 2011). We added four sequences from the GenBank database (Paracalanus parvus, GenBank accession numbers KX364939.1 and JF326205.1; Labidocera japonica, AB200178.1; and Calanus sinicus, KR048707.1) to the V9 PR2 reference database of the Tara Oceans project (de Vargas et al. 2015) to carry out taxonomic classification of sequence reads using a naïve Bayesian classifier (Wang et al. 2007). Following selections of Eukaryota, sequence reads belonging to the family Calanidae, unclassified Copepoda, or unclassified calanoid copepods were removed accordingly from the subsequent analysis, because those sequences might be derived from the hosts of the present study. The sequences were also removed if they were classifiable into parasitic or mammalian sources. The taxonomic groups of parasites were based on those detected in 18S metagenetic analysis of environmental seawaters in Cleary & Durbin (2016), who reviewed the literature to determine parasitic taxa. The proportions of sequence reads in the gut contents were calculated for all eukaryotic supergroups as well as for major taxonomic groups after excluding the supergroup Opisthokonta. The proportions of sequence reads attributable to each taxonomic group in the gut contents were averaged across the day and night samples, respectively, at each station. Moreover differences in the proportional contribution of taxonomic groups between all gut contents and environmental samples were tested using Mann-Whitney U tests. In addition to averaged proportions of sequence reads, detection frequencies for each major taxonomic group were investigated in all 40 C. sinicus individuals. In this frequency analysis, we assigned each individual to a category based on the proportion of sequence reads in the gut contents: low (0–1%), medium (1–10%), or high (>10%). The number of individuals assigned to each category for each taxonomic group was counted both for Eukaryota as a whole and for Eukaryota after excluding Opisthokonta. All eukaryotic sequence reads were clustered into MOTUs at a similarity threshold of 99%. For obtaining detailed taxonomic information of major prey, the Basic Local Alignment Search Tool (BLAST) in the National Center for Biotechnology Information (NCBI) database was used for the 10 most abundant MOTUs in the gut contents. The putative taxon of each MOTU was investigated based on the results of the BLAST analysis.

### Results

#### Massive sequence data

After quality filtering all sequence data, a total of 2,919,386 reads (average 72,985 reads per C. sinicus individual) were obtained in the gut content analysis. Among the gut content data, large proportions (>94%) of sequence reads were attributed to the host (Table 1). Sequence reads classified as parasites or mammals were comparatively rare (<1%; Table 1). The remainder were attributed to prey species. The number of sequence reads in each of six environmental plankton community samples ranged from 19,778 to 42,720. In total, 287,165 sequence reads from 40 gut content samples and six environmental community samples were used for the subsequent analysis.

#### Opisthokonta taxa

High proportions of Opisthokonta were detected among the sequence reads of Eukaryota (Fig. 2A). In Opisthokonta, sequences belonging to Copepoda were particularly prominent, accounting on average for 28.9–57.1% of sequences in the gut content and 28.0–45.4% in the environmental samples (Fig. 2A). Cirripedia were abundant in the gut contents during the day at station L3250, accounting for 24.2% of the sequences; however, the abundance of Cirripedia sequences in the gut contents was lower in other samples, accounting for up to 4.8% of the sequence reads. Hydrozoa were relatively abundant at station L3250 (day:

Table 1. Numbers and percentages of sequence reads detected in the gut contents of Calanus sinicus taken in Tosa Bay, off Japan, at two stations (L3250 and N3320) during the day and at night. Sequence read numbers and percentages represent averages calculated from 10 individuals collected during each sampling. Sequence reads were classified into host, parasite or mammal, or prey materials.

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>All</th>
<th>Host</th>
<th>Parasite or Mammal</th>
<th>Prey</th>
</tr>
</thead>
<tbody>
<tr>
<td>L3250 Day</td>
<td>10</td>
<td>74,351</td>
<td>70,405 (94.7%)</td>
<td>449 (0.63%)</td>
<td>3,497 (4.65%)</td>
</tr>
<tr>
<td>L3250 Night</td>
<td>10</td>
<td>74,094</td>
<td>72,234 (97.6%)</td>
<td>39 (0.05%)</td>
<td>1,821 (2.33%)</td>
</tr>
<tr>
<td>N3320 Day</td>
<td>10</td>
<td>68,016</td>
<td>64,883 (95.3%)</td>
<td>126 (0.19%)</td>
<td>3,007 (4.49%)</td>
</tr>
<tr>
<td>N3320 Night</td>
<td>10</td>
<td>75,478</td>
<td>73,051 (96.9%)</td>
<td>39 (0.05%)</td>
<td>2,388 (3.09%)</td>
</tr>
<tr>
<td>All</td>
<td>40</td>
<td>72,985</td>
<td>70,143 (96.1%)</td>
<td>163 (0.23%)</td>
<td>2,678 (3.64%)</td>
</tr>
</tbody>
</table>
6.2%, night: 5.6%); however, smaller proportions were observed at station N3320 (day: 1.3%; night: 1.5%). Other Opisthokonta material was relatively common, accounting for 10.3–24.3% of sequence reads in the gut contents. When all sequence data were combined, Cirripedia were proportionally more abundant in gut contents than in environmental plankton communities, although the difference was not significant (Fig. 3). The abundances of copepods and hydrozoans were approximately equal in gut contents and environmental plankton communities. Ninety percent of *C. sinicus* individuals had high abundances of sequence reads (>10%) in the gut contents attributable to Copepoda (Table 2). Low proportions (<1%) were observed in one individual. Cirripedia and Hydrozoa frequently contributed moderate proportions (1–10%); however, 25% and 42.5% of *C. sinicus* individuals had low proportions of Cirripedia and Hydrozoa, respectively.

**Stramenopile taxa**

Sequences classified as Stramenopiles were also abundant among all Eukaryota, comprising 8.6–38.1% and 7.7–17.1% in gut contents and in environmental plankton, respectively (Fig. 2A). After removing Opisthokonta, the proportion of Bacillariophyta (Stramenopiles) material was particularly high both in gut contents (33.1–51.0%) and in environmental plankton (13.6–23.8%; Fig. 2B). Labyrinthulea (Stramenopiles) was also abundant. After removing Opisthokonta, the highest observed proportion, 32.7%, was measured in the night sample from station L3250; a

![Fig. 2.](image)

Average sequence compositions of major taxonomic groups detected in the gut contents of female *Calanus sinicus* sampled at two stations (L3250 and N3320) in Tosa Bay, off Japan, sorted by sampling time (day: N=10 per station; night: N=10 per station). Average environmental plankton compositions (Environment) at both sites are also included (seawater sample N=3 for each station). (A) Supergroups within Eukaryota. Opisthokont materials are further classified into the major taxonomic groups Cirripedia, Copepoda, and Hydrozoa. (B) Major taxa after removing Opisthokonta.

![Fig. 3.](image)

Comparisons of relative abundances of major taxonomic groups between all *Calanus sinicus* gut contents and environmental seawater. The abundance of sequence reads are presented as log\(_{10}+1\) transformed averages of all available samples both during day and night at two sampling sites (gut contents: N=40; seawater samples: N=6). Scale bars indicate standard deviation. Asterisks (*) indicate a significant difference (p<0.01) detected by the Mann–Whitney U test.

**Table 2.** Numbers of *Calanus sinicus* individuals with low (0–1%), medium (1–10%), or high (>10%) proportions of sequence reads attributable to major taxonomic groups in the gut contents. For each major taxonomic group, all specimens of female *Calanus sinicus* (n=40) were categorised into one of the three frequency groups based on the proportions of sequence reads of a given taxonomic group in the gut contents. This frequency analysis was carried out for taxonomic groups identified in all taxonomic groups of Eukaryota (left), and for Eukaryota excluding Opisthokonta (right).

<table>
<thead>
<tr>
<th>All-Eukaryota</th>
<th>Non-Opisthokonta</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;1%</td>
</tr>
<tr>
<td>Bacillariophyta</td>
<td>8</td>
</tr>
<tr>
<td>Chlorophyta</td>
<td>34</td>
</tr>
<tr>
<td>Cirripedia</td>
<td>10</td>
</tr>
<tr>
<td>Copepoda</td>
<td>1</td>
</tr>
<tr>
<td>Dinophyta</td>
<td>27</td>
</tr>
<tr>
<td>Hydrozoa</td>
<td>17</td>
</tr>
<tr>
<td>Labyrinthulea</td>
<td>22</td>
</tr>
<tr>
<td>Rhizaria</td>
<td>36</td>
</tr>
</tbody>
</table>
proportion of 6.3% was measured in the day sample from station L3250, and proportions of 9.3% and 6.9% were measured in the day and night samples, respectively, from station N3320. In a comparison of all samples, the proportion of Bacillariophyta was lower in gut contents than in environmental plankton communities, whereas the proportion of Labyrinthulea was higher; however, these differences were not significant (Fig. 3). Other Stramenopiles, including unclassified taxa, comprised 3.2–24.9% of sequences in gut contents, after removing Opisthokonta. Fifty-five percent of C. sinicus individuals had medium proportions of Bacillariophyta sequence reads; however, high proportions were observed in 75.0% of individuals after removing Opisthokonta from the analysis (Table 2). Almost half of C. sinicus individuals had low proportions of Labyrinthulea both in the all-Eukaryota (22/40 individuals) and non-Opisthokonta analyses (19/40 individuals). Large proportions of Labyrinthulea sequence reads were found in 12.5% of C. sinicus in the all-Eukaryota analysis, and in 40.0% after removing Opisthokonta.

**Other taxa**

Alveolata and Archaeplastida were relatively abundant in environmental samples, comprising 15.5–24.5% and 12.2–13.1% sequences, respectively, of all sequences (Fig. 2A). These supergroups were primarily represented by Dinophyta and Chlorophyta, comprising 25.1–47.0% and 20.8–22.4%, respectively, of environmental plankton communities, after removing Opisthokonta (Fig. 2B). However, Dinophyta and Chlorophyta comprised 3.5–13.9% and 1.0–8.0% sequences in the gut contents, respectively, even after removing Opisthokonta. Rhizaria comprised 0.1–1.7% of all gut contents, or 0.4–6.0% after removing Opisthokonta. The relative abundances of Rhizaria, Dinophyta, and Chlorophyta were significantly lower in gut contents than in environmental samples (p<0.01; Fig. 3). These taxonomic groups were also rare in the frequency analysis, which showed that 85% of C. sinicus individuals had <1% Chlorophyta material in the gut (70% after removing Opisthokonta), 67.5% had <1% Dinophyta material (47.5% after removing Opisthokonta), and 90% had <1% Rhizaria in the gut (87.5% after removing Opisthokonta; Table 2). Unclassifiable Eukaryota material comprised 0.3–9.9% of all gut contents, or 9.3–25.3% after removing Opisthokonta. Other taxa, including Apusozoa, Excavata, Hacrobia, and Picozoa, were comparatively uncommon across all sequence reads.

**Taxonomy of abundant MOTUs**

BLAST results indicated that several of the 10 most abundant MOTUs in the gut contents were copepods (Table 3). The most abundant MOTU belonged to the copepod family Clausocalanidae. The families Oithonidae (rank 2) and Paracalanidae (rank 5), as well as unclassified Calanoid copepods (rank 10) and another crustacean, attributable to Cirripedia (rank 4), also appeared among the 10 most abundant MOTUs. The third most abundant Bacillariophyta MOTU was attributed to Thalassiosirales. The remaining MOTUs comprised Labyrinthulea material with a high similarity to Aplanochytrium (rank 7), two unclassifiable eukaryotes (ranks 6 and 8), and one hydrozoan (rank 9).

**Discussion**

The major finding in the present study was the detection of aplanochytrids (Labyrinthulea) previously unrecognised as playing a role in C. sinicus diets; however, the results of the present study also supports those of previous reports that diatoms (Bacillariophyta) and small crustaceans are major prey groups in C. sinicus. Although Eukaryota-wide use of the metagenetic method is possibly prone to bias (e.g. copy numbers of rRNA genes, primer mismatches), metagenetic analysis concerning 18S rRNA has been suggested as a viable, semi-quantitative method for providing snapshots of eukaryotic community compositions useful for accurately characterising predator–prey relationships.

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**Table 3.** BLAST results for the 10 most abundant MOTUs identified in the gut contents of Calanus sinicus. The average percentage of sequence reads was calculated from data obtained from 40 female C. sinicus collected from Tosa Bay, off Japan. Query coverage and sequence identity are provided as percentages. Asterisks (*) denote cases where multiple best-hit species were detected with the same score; representative species are listed in such cases.

<table>
<thead>
<tr>
<th>Rank</th>
<th>Average</th>
<th>Best hit species</th>
<th>Coverage</th>
<th>Identity</th>
<th>Accession</th>
<th>Putative taxon</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10.09%</td>
<td>Pseudocalanus sp.</td>
<td>95%</td>
<td>98%</td>
<td>GU594644.1</td>
<td>Copepoda (Clausocalanidae)</td>
</tr>
<tr>
<td>2</td>
<td>8.39%</td>
<td>Oithona similis</td>
<td>100%</td>
<td>99%</td>
<td>KF153700.1</td>
<td>Copepoda (Oithonidae)</td>
</tr>
<tr>
<td>3</td>
<td>7.67%</td>
<td>Thalassiosira mala</td>
<td>100%</td>
<td>100%</td>
<td>HM991693.1</td>
<td>Bacillariophyta (Thalassiosirales)</td>
</tr>
<tr>
<td>4</td>
<td>7.64%</td>
<td>Galkinus sp.*</td>
<td>100%</td>
<td>100%</td>
<td>KM217491.1</td>
<td>Cirripedia</td>
</tr>
<tr>
<td>5</td>
<td>5.62%</td>
<td>Paracalanus sp.</td>
<td>100%</td>
<td>99%</td>
<td>KX364940.1</td>
<td>Copepoda (Paracalanidae)</td>
</tr>
<tr>
<td>6</td>
<td>3.96%</td>
<td>Aegyria foissneri</td>
<td>30%</td>
<td>100%</td>
<td>KX364493.1</td>
<td>Unclassifiable (Eukaryota)</td>
</tr>
<tr>
<td>7</td>
<td>3.49%</td>
<td>Aplanochytrium kerguelense</td>
<td>100%</td>
<td>98%</td>
<td>AB022103.1</td>
<td>Labyrinthula (Aplanochytrium)</td>
</tr>
<tr>
<td>8</td>
<td>3.27%</td>
<td>Acartia hongi</td>
<td>27%</td>
<td>100%</td>
<td>GU969195.1</td>
<td>Unclassifiable (Eukaryota)</td>
</tr>
<tr>
<td>9</td>
<td>2.85%</td>
<td>Muggiaea sp.*</td>
<td>100%</td>
<td>100%</td>
<td>AF358073.1</td>
<td>Hydrozoa</td>
</tr>
<tr>
<td>10</td>
<td>2.80%</td>
<td>Sinocalanus tenellus</td>
<td>100%</td>
<td>95%</td>
<td>GU969144.1</td>
<td>Calanoid copepod</td>
</tr>
</tbody>
</table>
C. sinicus samples were generally abundant; therefore, the contributions of crustaceans might not be appropriately reflected in these previous studies. Although overall proportions were lower in the gut contents than in the environmental samples, possibly because of their toxic effects on copepods (Ianora & Miralto 2010), diatoms, particularly Thalassiosirae, nevertheless contributed substantially to gut contents in this study. Although cannibalism could not be evaluated using the metagenetic methods deployed in the present study, crustacean material attributable to Copepoda and Cirripedia comprised substantial proportions of the diet in almost all individuals of C. sinicus analysed. Predation on crustaceans by C. sinicus has been reported only rarely (Uye & Murase 1997); however, such behaviour is frequently observed in other Calanus species (Landry 1981). The BLAST results reported in the present study indicate that small copepods functioned as important prey items for adult female C. sinicus, suggesting that the role played by small copepods in marine ecosystems has been underestimated (Turner 2004). Previous molecular-based diet studies used methods to prevent amplifying host sequences during PCR (Yi et al. 2017, Ho et al. 2017), which may prevent amplification of crustacean DNA other than the host species; therefore, the metagenetic data provide no information on the distribution of different aplanochytrids forms in the environment, the community structures of such taxa change seasonally, occasionally reaching high abundances in coastal waters (Ueda et al. 2015), suggesting that they may play an important ecological role. The biomass of Labyrinthulea sometimes exceeded that of bacterioplankton, and Aplanochytrids have commonly been observed in the oceans, including coastal waters of southern China (Liu et al. 2017) and oceanic Hawaiian waters (Li et al. 2013). Aplanochytrid material was also detected in the guts and faecal pellets of copepods in the tropical Indian Ocean (Damare & Raghukumar 2006, 2010), supporting our findings that aplanochytrids are an important food source for C. sinicus. Given the higher proportions of aplanochytrids in gut contents than in the environmental samples in the present study, we suggest that C. sinicus positively selects aplanochytrids as prey. Because metagenetic data provide no information on the distribution of different aplanochytrids forms in the environment, it is unclear precisely how C. sinicus consumed aplanochytrids. Labyrinthulea have been frequently observed to attach to aggregations in the environment (Li et al. 2013; Bochdansky et al. 2017); therefore, these aggregations may be consumed by zooplankton. Calanus sinicus uses DHA in lipid metabolism (Wang et al. 2017), suggesting that aplanochytrids may act as important sources of DHA for C. sinicus. Finally, we recommend investigating the role of aplanochytrid prey in larvae of commercially important fish, including Japanese sardine and Pacific round herring.

The results we obtained using the 18S metagenetic approach in this study support those of previous studies indicating that diatoms, small crustaceans, and hydrozoans play important roles in the diet of C. sinicus, and further
suggest a new predator–prey interaction involving aplanochytrids. However, the scope of the present study was confined to a single copepod species during the winter in Tosa Bay, off Japan; therefore, further studies are recommended to investigate the ecological roles of the prey organisms newly detected in this study. Additionally, we frequently encountered large proportions of eukaryote, opisthokont, and stramenopile material that could not be identified to major taxa, indicating that further DNA barcoding efforts are necessary for eukaryotic marine plankton taxa, and suggesting the possibility that several predator–prey relationships remain undetected in marine planktonic ecosystems. The number of metagenetic analyses of plankton community composition and zooplankton gut contents published continues to increase. We recommend that studies continue to be conducted in this area at different spatial and temporal scales, as such data contribute to our understanding of the complex food web structures in marine ecosystems.

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