# Large-scale metagenetic community analysis of epipelagic and mesopelagic copepods in the Pacific

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Planktonic copepods are among the most important zooplankton in marine food webs and biogeochemical cycles, and their community structures have a pronounced effect on global marine ecosystems. Marine planktonic copepods are ubiquitous and diverse with more than 2,500 described species, and there are many undescribed or cryptic species in the ocean. Because morphological identifications of copepods are time-consuming and require sophisticated expertise, few studies have focused on large-scale community structures of copepods especially in the Pacific Ocean. We investigated the large-scale community structures of epipelagic and mesopelagic copepods in the Pacific using metagenetic analysis of nuclear large subunit ribosomal DNA (28S). This method is rapid and taxonomically comprehensive, revealing community structure of copepods based on massive sequence data without depending on morphological classification. VMPS net was used to collect zooplankton samples at both epipelagic (0-200 m) and mesopelagic (200-500 and 500-1000 m) layers in each sampling site. Total >100 community samples covering 40°S-68°N and 138°E-95°W were collected during 2011-2015. After DNA extraction and PCR amplification of 28S D2 region, massive sequence data were obtained using high-throughput sequencers Illumina MiSeq. All sequence reads were quality-filtered, and approximately 18,000 sequence reads in each sample were clustered into Molecular Operational Taxonomic Units (MOTUs) for community analysis. Community structures of copepod]s were distinct between cold and warm waters both at epipelagic and mesopelagic layers, and community boundary existed between subtropical gyre and transition zones. In the epipelagic layer, copepod communities were almost corresponded with water masses including arctic, subarctic, transition, subtropical and equatorial zones. Within warm water regions in the low latitude, chlorophyll a concentration highly correlated with copepod communities, and communities in the Kuroshio region were more similar to those in the equatorial regions than to those in the subtropical regions. We also observed several groups of copepod communities even within the subtropical gyres. In addition to epipelagic layer, mesopelagic layer in the low latitude also showed different communities of copepods in the tropical, subtropical, and Kuroshio regions both at 200-500 and 500-1000 m depth. Therefore, small-scale community structure existed even within warm water regions in the epipelagic regions, which might affect community structures in the mesopelagic layer within relatively stable water environments. In the analysis of copepod diversity, latitudinal gradients were evident in the epipelagic layer. High diversity was observed in the tropical and subtropical areas, with a peak in the North Pacific subtropical gyre. This pattern was also observed in the mesopelagic layer, suggesting the influence of epipelagic ecosystems on mesopelagic communities. Although diversity was higher in the mesopelagic layer than in the epipelagic layer, the epipelagic community was composed of various taxonomic groups with high genetic diversity. Specific taxonomic groups were diverse in the mesopelagic layer, indicating that different mechanisms maintain diversity within each layer. Distribution patterns were clearly different between low-latitude and high-latitude groups. Phylogenetic analysis revealed recent divergence of the high-latitude group, suggesting that a relatively short evolutional history is a factor underlying the low diversity at high latitudes. These results indicate that both environmental and evolutionary factors have determined the current diversity and biogeography of planktonic copepods.

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### Molecular cross-talk between a chemosymbiotic *Calyptogena* clam and its endosymbiont shown through meta-transcriptome sequencing

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Hydrothermal vents and methane seeps are deep-sea habitats rich in reduced chemicals, such as sulfide and methane, and support dense communities of megafauna. Many endemic species harbor chemosynthetic symbionts for nutrition. Vesicomyid clams in the genus Calyptogena is one such case, and especially notable as they obtain endosymbionts through vertical transmission. The present study aims to understand the host-symbiont relationship in an undescribed Calyptogena species recently discovered from a cold seep in the South China Sea. The gill, mantle, adductor muscle, and foot tissue were dissected from one individual and subjected to RNA-Seq in an Illumina HighSeq platform. De novo assembly of the reads resulted in 246,150 contigs, among them 25,530 were translated into protein sequences. Comparing the expression level of the host proteins in the four different tissues revealed that Ras protein family, especially the Rab proteins that play an important role in the fusion of endosome and lysosome during endocytosis, are highly expressed in the gill. This indicate that rate of lysis is increased in the gill, where the endosymbionts are housed. Meanwhile, mapping the sequencing reads from the gill to the closest sequenced endosymbiont genome that is from Calyptogena okutanii, revealed high expression of genes involved in sulfur oxidizing and ATP synthesis in the symbiont. These results show that the new Calyptogena species also rely on sulfur-oxidizing symbionts for energy, and the high expression level of Rab proteins may help the host control the symbiont population.

Keywords: Vertical transmission, Host-symbiont relationship, Cold seep, Calyptogena clam

# Carbon fixation by endosymbiotic algae within protistan microzooplankton

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Endosymbiosis with algae (photosymbiosis) is one of the styles of acquired phototrophy seen in marine protistan microozooplankton such as radiolarians and foraminifers. Although their biomass in the ocean is not so large, the number of cells of their symbiotic algae within one host organism (< ca. 500  $\mu$ m) is well over a few thousands or even said to reach several tens of thousands. It means that the amount of photosynthesis mediated by their symbiotic systems might be "hot spots" of primary production especially in oligotrophic oceans. Of those photosymbiotic organisms, planktic foraminifers that precipitate calcite tests contribute to both inorganic carbon production by calcification, and organic carbon production via photosynthesis of their symbiotic system found in coral reefs, the basic knowledge of photosymbiosis and the photosynthesis itself in pelagic microzooplankton are quite limited and have been overlooked.

Here, we performed <sup>14</sup>C-tracer experiment to estimate photosynthetic carbon-fixation rates (CFR) of the symbionts within planktic foraminifers, together with photophysiological measurement using an active fluorometry (fast repetition rate fluorometry, FRRF). The main purpose of this study is (1) to estimate the CFR of the symbionts under different light conditions, (2) to evaluate the photophysiology of the symbionts, and as a preliminary investigation, (3) to extrapolate the CFR information to field data to estimate the contribution of the symbiosis-related photosynthesis in oligotrophic oceans. The samples for the laboratory experiments were collected from Sagami Bay by surface plankton net towing. The target species was *Globigerinoides sacculifer* which has dinoflagellate symbionts. In the laboratory experiment, FRRF measurement was firstly performed on each individual under certain actinic light level (either of the three irradiance levels, 220, 150, 70  $\mu$  mol photons m<sup>-2</sup> s<sup>-1</sup>). Right after the measurement, each specimen was transferred to a jar with NaH<sup>14</sup>CO<sub>3</sub> and incubated for 1h, then radioactivity of the fixed organic carbon was measured with a liquid scintillation counter. The CFR was estimated accordingly. The field observation for the purpose (3) was conducted by R/V Shinsei-Maru in the subtropical Northwestern Pacific (KS-16-9). Vertically stratified samples were collected by VMPS towing. The standing stock, the species composition, and the size structure of the samples were analyzed.

The photophysiological parameters of the symbionts photosynthesis all demonstrated the healthy state of their photosynthetic system, indicating fine relationships between the host and the symbionts. The chlorophyll-based CFRs (median values) were 16.5, 18.2, and 9.1 mmol C (mol Chl)<sup>-1</sup> s<sup>-1</sup> for 220, 150, 70  $\mu$  mol photons m<sup>-2</sup> s<sup>-1</sup>, respectively. Using a size-chlorophyll relationship of the nominal species, the above results enable us to make an assumption of the CFR from the shell size of foraminiferal specimens as long as the information of the light environment of their habitat is available. In the presentation, we also show the rough estimation of the vertical structure of the symbiosis-related primary production in the studied site, their contribution, and insight of their importance in the oligotrophic ocean ecosystem.

キーワード:炭素固定、光共生、マイクロ動物プランクトン、浮遊性有孔虫

Keywords: carbon fixation, photosymbiosis, microzooplankton, planktic foraminifers

## ミズクラゲポリプの増殖:機能的応答とエネルギー収支 Reproduction of *Aurelia aurita* s.l. (Cnidaria: Scyphozoa) polyps: Functional response and energy budget

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#### 1. 緒言

鉢クラゲ類メデューサの出現量は一般に顕著な年々変動を示し、時に大発生する。その原因究明のカギはポ リプにある。ポリプは特異的な無性生殖方法により個体群の増大を図ることができ、さらに季節的にストロビ ラに変態して、多くのエフィラを水中に放出するからである。そうなると、クラゲ大発生の原因究明において 最も肝要なことは、ポリプの再生産や個体群動態に影響する要因を抽出し、それらの影響を定量的に評価する ことである。そこで、本研究ではミズクラゲのポリプ期におけるエネルギー収支を明らかにし、ポリプの再生 産速度に及ぼす水温と餌密度の影響を評価した。

2. 材料と方法

実験に使用したミズクラゲのポリプは、2011年に博多湾で採取した成熟メスに由来し、以後継続飼育した ものである。ポリプの呼吸速度に及ぼす水温と塩分の影響、捕食速度に及ぼす餌(カイアシ類、ベントス幼 生)の質的違い、餌密度、水温の影響を調査した。それらの結果に基づいて炭素収支モデルを構築した。その モデルを福山湾のポリプ個体群に当てはめ、純生産(体重増加と再生産)速度の季節変動を算出した。

結果と考察

3-1. 呼吸速度

呼吸速度に及ぼす水温の影響は顕著で、単位炭素重量当たりの呼吸速度は8℃から28℃への水温上昇に伴い、指数関数的に増大した。一方、塩分は影響せず、15~33の範囲では呼吸速度はほぼ一定であった。

3-2. 捕食速度

ポリプは与えたいずれの餌でも捕食可能で、しかもその捕食速度は既報の微小動物プランクトンを餌とした 場合よりもはるかに高かった。ポリプは中型動物プランクトンを主要餌生物にしていると考えられる。複数の 分類群で構成される中型動物プランクトン群に対してポリプは次のような機能的応答を示した。即ち、捕食速 度は餌密度の上昇に伴い直線的に増大し、実験した餌条件下では飽和することはなかった。ただし、捕食速度 は餌のサイズや遊泳速度による影響を受けた。また、濾水速度は餌密度に関係なく一定で、水温と正相関を示 した。

3-3. 炭素収支モデルの構築とその野外ポリプ個体群への適用

上記実験結果に基づいて炭素収支モデルを構築し、水温と餌密度を変数としてポリプの純生産速度を定式化 することができた。その式を福山港(水温範囲: 9.7~27.2°C、カイアシ類現存量範囲: 9.7~82.7 mg C m<sup>-3</sup> )のポリプ個体群に当てはめた。富栄養化したこの湾内でポリプは常に純生産を行い、その速度は 0.0039~0.34  $\mu$ g C  $\mu$ g C<sup>-1</sup> d<sup>-1</sup>の範囲で変動した。モデル式から、ポリプの呼吸速度を賄えない餌濃度

 $(4.6 \sim 8.6 \text{ mg C m}^{-3})$ 以下の貧栄養海域ではポリプは生息できないことも明らかになった。

4. まとめ

ミズクラゲのポリプの再生産速度には餌供給量が顕著な影響を及ぼすことから、富栄養化はクラゲ大発生の 主要な原因であることが確かめられた。広義のミズクラゲは世界中の沿岸域に分布するが、ここで使用したミ ズクラゲと同様の生活史パターンや生理生態的特性を有する種類には、本モデル式が適用可能である。

キーワード:クラゲ大発生、ポリプ、機能的応答、エネルギー収支

Keywords: jellyfish bloom, polyp, functional response, bioenergetics

## 音響を用いた動物プランクトン生態研究 Ecological studies on zooplankton by using acoustic technic

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Since acoustic backscatter data has higher temporal and vertical resolution with a longer sampling duration, the backscatter data obtained from several types of acoustic devices (e.g. ADCP, AZFP, echo sounder, TAPS) have been used to describe the temporal and spatial variability of zooplankton biomass and migration over decade. Mooring observation of the acoustic device allows the sampling of interannual and seasonal variability as well as episodic events such as eddies and storms. Usefulness of the acoustic mooring in barely accessible area such as seasonal ice zones is also well known. Acoustic backscattering strength from organisms is known to be dependent on target size, anatomical characteristics and orientation, and the frequency of the incident sound. These dependences can be used to make inferences about classification of target organisms and their size distribution. Recent studies have used the differences in backscatter measurements at multiple frequencies to attempt to identify the scatterers. In this presentation, we will introduce our acoustic studies on zooplankton ecology; vertical migration and its seasonal variability of zooplankton in the subtropical western North Pacific by using moored ADCP, seasonal dynamics of zooplankton and identification of the dominant scatterers in the southern Chukchi Sea, Pacific Arctic, by using moored AZFP (Acoustic Zooplankton Fish Profiler), and high-resolution profiling of zooplankton vertical distribution by vertical haul of AZFP in the subarctic western North Pacific. Additionally, we will also discuss problems found out through the studies and future direction for the acoustical zooplankton studies.

キーワード:音響手法、動物プランクトン生態、ADCP、AZFP Keywords: acoustic technic, zooplankton ecology, ADCP, AZFP

## A challenge to evaluate effect of climate change on Japanese anchovy (*Engraulis japonicus*) in the East China Sea III

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We have evaluated climate change (global warming) effects on Japanese anchovy (*Engraulis japonicus*) in the East China Sea by integrating a fish-migration and growth model using environmental conditions derived from simulations of a coupled ocean circulation and ecosystem model with contemporary and future climate forcing. For the ocean circulation model, a high resolution (1/16 deg.) CHOPE (Max-Planck-Institute Ocean Model) was used. For the marine ecosystem model, eNEMURO, an extended version of NEMURO (North Pacific Ecosystem Model for Understanding Regional Oceanography) was used. For future climate forcing, the output of MIROC-3.2(high) with the SRES A1B scenario was used. The initial spawning grounds were assumed in the area which depth is less than 1000 m and the sea surface temperature (SST) is between 14.1 and 20.1 degC and 27.2 and 27.8 degC based on the field data analyses. We estimated the spawning grounds in March, April and May. The fish growth and migration model was integrated for one year since the spawning.

Under the contemporary condition, the number of anchovy larvae advected to the northwestern side of Kyushu (NWK) showed the maximum in April, while it showed the maximum in March in the southwestern side of Kyushu (SWK). However, under the future climate during 2050-60, it showed the maximum in March both in the NWK and SWK. Therefore, the peak timing was advanced in the NWK under the future condition. Regarding the body size, larvae advected to the NWK showed the maximum mode body length in May, while those advected to the SWK showed it in April under the contemporary condition. Under the future condition during 2050-60, the timing was advanced by one month (April in the NWK and March in the SWK). Under the future condition during 2050-60, the number of larvae advected to the SWK in April and May and those advected to the NWK in May were drastically decreased. This result heavily depends on the assumption that the spawning ground is not formed in the region which SST is between 20.2 and 27.1 degC. Under the future condition during 2050-60. However, the number of anchovy larvae advected to SWK was much more reduced. The results highly depend on the initial spawning distribution. As a future work, the estimation of spawning area should be improved.

キーワード:海洋生態系モデル、魚類成長-回遊モデル、カタクチイワシ、地球温暖化 Keywords: ecosystem model, fish growth-migration model, Japanese anchovy, climate change