

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 evolutionary 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.

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. Vesicomid 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 microzooplankton 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 μm) 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 symbionts, which should be an important key player in the carbon cycle. However, compared to the famous photosymbiotic 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 ^{14}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 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$). Right after the measurement, each specimen was transferred to a jar with $\text{NaH}^{14}\text{CO}_3$ 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 $\text{mmol C (mol Chl)}^{-1} \text{s}^{-1}$ for 220, 150, 70 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, 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.

Keywords: carbon fixation, photosymbiosis, microzooplankton, planktic foraminifers

Reproduction of *Aurelia aurita* s.l. (Cnidaria: Scyphozoa) polyps: Functional response and energy budget

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1. Introduction

It is important to clarify why scyphozoan medusa populations show such remarkable annual fluctuations, sometimes developing massive blooms, sometimes not. The benthic polyps play a key role to control the medusa population size, because they reproduce asexually by multiple modes and release numerous planktonic ephyrae, which develop into medusae. Hence, it is ultimately necessary to clarify which factors are responsible for the reproduction and population dynamics of benthic polyps. In this study, we constructed a bioenergetic model in the scyphozoan polyps for the first time, and used it to determine the net growth (somata and offspring) rates of the polyp stage of *Aurelia aurita* sensu lato, a cosmopolitan jellyfish species.

2. Materials and Methods

Aurelia aurita polyps used in this study were derived from the stock-cultures, which originated from medusae caught in Hakata Bay, northern Kyushu, in 2011. We examined the effects of temperature and salinity on carbon-weight-specific respiration rate, and the effects of temperature, prey type and prey density on carbon-weight-specific ingestion rate on various natural zooplankton taxa (e.g. copepods and benthos larvae) in the laboratory. A carbon budget model constructed from these experiments was applied to the natural polyp population in Fukuyama Harbor, the Inland Sea of Japan.

3. Results and Discussion

3-1. Respiration rate

The carbon-weight-specific respiration rate of *A. aurita* polyps increased exponentially with increasing temperature from 8 to 28°C. However, salinity did not affect the respiration rate, which was constant at salinities ranging from 15 to 33.

3-2. Ingestion rate

Polyps could capture and ingest various natural mesozooplankton from the Inland Sea of Japan at much higher rates than those feeding on microzooplankton (i.e. ciliates), indicating that the major food for polyps is mesozooplankton. The general functional response of polyps feeding on various prey taxa was expressed by a linear increase in ingestion rate as a function of prey density, although it was affected by the size and/or swimming ability of prey organisms. Accordingly, the clearance rate was constant irrespective of prey density, and increased linearly with temperature from 8 to 26°C.

3-3. Carbon budget model and its application to natural polyp population

Integrating above-mentioned results, we constructed a carbon budget model of *A. aurita* polyps as a function of temperature and mesozooplankton prey density. An application of this model to polyps in Fukuyama Harbor (monthly average temperature: 9.7–27.2°C, monthly average copepod biomass: 9.7–82.7 mg C m⁻³) suggested that they consistently attain a positive growth rate, ranging from 0.0039 to 0.34 μg C μg C⁻¹ d⁻¹. Our model also predicts that oligotrophic areas where mesozooplankton biomass is always less than the critical level, not meeting the minimum requirement for metabolism, i.e., 4.6–8.6 mg C m⁻³, may not constitute polyp habitat.

4. Conclusion

As the growth rate of polyps elevates with the increase of mesozooplankton biomass, eutrophication is considered a major driving force for medusa blooms. The moon jellyfish formerly known as *A. aurita* is now a species complex, which consists of at least 10 cryptic species by genomics in addition to *A. aurita* s.s. If

the seasonal life cycle and physio-ecological characteristics of these species are similar to our *A. aurita*, being tentatively designated as *Aurelia* sp. 1, our bioenergetic model may be applicable to the polyp populations in any temperate coastal waters.

Keywords: jellyfish bloom, polyp, functional response, bioenergetics

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.

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 2090-2100, the timing of the maximum advected larval number and growth was the same as those 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.

Keywords: ecosystem model, fish growth-migration model, Japanese anchovy, climate change