

Morphological changes in kleptochloroplasts after ingestion in the unarmored dinoflagellates

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Dinoflagellates are ubiquitous unicellular protists and the evolutionary scenario of their chloroplast evolution is quite complex one. About half of the dinoflagellates are photosynthetic, while rests are heterotrophic species, and the latter are thought to have evolved from the photosynthetic ancestors by losing their chloroplasts. Additionally, some dinoflagellates have replaced their original chloroplasts with those of haptophyte, diatom or green alga. In addition, some dinoflagellates possess 'kleptochloroplast', which is the temporary chloroplast 'stolen' from other photosynthetic algae.

The unarmored dinoflagellate *Amphidinium poecilochroum* (marine) and *Gymnodinium aeruginosum* (freshwater) are closely related to each other, and both possess kleptochloroplasts derived from cryptomonads. These dinoflagellates ingest cryptomonad cell and retain its chloroplast temporarily, but eventually lose the chloroplast due to cell division or digestion. Previous studies revealed that several differences exist between marine and freshwater representatives with regard to the cryptomonad-dinoflagellate specificity and the dynamics of kleptochloroplast processing. *A. poecilochroum* is capable of ingesting any species of cryptomonads, and synchronised division of kleptochloroplast with the host cell has never been observed. By contrast, *G. aeruginosum* can accept only members of the genus *Chroomonas* as prey and the kleptochloroplast is simultaneously divided with the host cell and being inherited by each daughter cell. Thus, the kleptochloroplastity in *G. aeruginosum* seems to represent much more advanced stage toward acquisition of 'true chloroplast' within the lineage. Therefore, unraveling the differences between the two species in detail might give us clue to understand evolutionary significance and contribution of kleptochloroplast during the quest for true chloroplasts. Although the general ultrastructure of these dinoflagellates has been studied, the morphological changes from ingestion of cryptomonad to disappearance of kleptochloroplast have never been focused and remain unclear. In this study, we observed the morphological changes of kleptochloroplast in *A. poecilochroum* and *G. aeruginosum* using light and transmission electron microscopes, and compared the differences between the two species.

The both species ingested cryptomonad chloroplast, nucleus, nucleomorph, mitochondria and ejectosomes with surrounding cytoplasm directly into the dinoflagellate cytoplasm. In *A. poecilochroum*, cryptomonad mitochondria and ejectosomes were removed together with cytoplasm, by transferring them into the food vacuole within 1 h after ingestion. The kleptochloroplast was enlarged gradually, and the cryptomonad nucleus was digested after 3 h. In *G. aeruginosum*, the cryptomonad cytoplasm, containing cryptomonad nucleus and mitochondria, was retained around the chloroplast. The chloroplast was enlarged drastically after 6 h, and eventually occupied most of the host cytoplasm by the 3rd day, forming a cup shape with several pyrenoids. The cryptomonad nucleus was positioned inside the cup-shaped chloroplast. By the day 5, the nucleomorph has undergone multiplication at the vicinity of the cryptomonad nucleus. This study revealed that *G. aeruginosum* can expand its kleptochloroplast more extensively and is capable of retaining the cryptomonad nucleus for a longer period than *A. poecilochroum*. Previous study on the kleptochloroplastidic ciliate *Mesodinium rubrum* showed that the endosymbiont nucleus plays an important role to maintain its kleptochloroplast. The diatom-harboring dinoflagellates possess diatom nucleus, and can divide the latter nucleus simultaneously with the host cell division. The differences between *G. aeruginosum* and *A. poecilochroum* indicated in this study also support that retention of endosymbiont nucleus is advantageous to maintain its chloroplast stably.

Keywords: dinoflagellate, kleptochloroplast, ultrastructure

Algivore or Phototroph? *Plakobranthus ocellatus* (Gastropoda) Continuously Acquires Kleptoplasts and Nutrition

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The sea slug *Plakobranthus ocellatus* (Sacoglossa, Gastropoda) retains photosynthetically active chloroplasts from ingested algae (functional kleptoplasts) in the epithelial cells of its digestive gland for up to 10 months. While its feeding behavior has not been observed in natural habitats, two hypotheses have been proposed: 1) adult *P. ocellatus* uses kleptoplasts to obtain photosynthates and nutritionally behaves as a photoautotroph without replenishing the kleptoplasts; or 2) it behaves as a mixotroph (photoautotroph and herbivorous consumer) and replenishes kleptoplasts continually or periodically. To address the question of which hypothesis is more likely, we examined the source algae for kleptoplasts and temporal changes in kleptoplast composition and nutritional contribution. By characterizing the temporal diversity of *P. ocellatus* kleptoplasts using *rbcl* sequences, we found that *P. ocellatus* harvests kleptoplasts from at least 8 different siphonous green algal species, that kleptoplasts from more than one species are present in each individual sea slug, and that the kleptoplast composition differs temporally. These results suggest that wild *P. ocellatus* often feed on multiple species of siphonous algae from which they continually obtain fresh chloroplasts. By estimating the trophic position of wild and starved *P. ocellatus* using the stable nitrogen isotopic composition of amino acids, we showed that despite the abundance of kleptoplasts, their photosynthates do not contribute greatly to the nutrition of wild *P. ocellatus*, but that kleptoplast photosynthates form a significant source of nutrition for starved sea slugs. The herbivorous nature of wild *P. ocellatus* is consistent with insights from molecular analyses indicating that kleptoplasts are frequently replenished from ingested algae, leading to the conclusion that natural populations of *P. ocellatus* do not rely on photosynthesis but mainly on the digestion of ingested algae.

Keywords: kleptoplasty, sacoglossan, ulvophyceae, symbiosis

Evolution of symchlosomes driven by endosymbiosis of zoochlorellae in freshwater protozoa and metazoa

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Freshwater micro-predators bearing zoochlorellae (intracellular symbiotic green algae) have been reported in various protozoan and metazoan groups. In order to extract common features among endosymbiosis of zoochlorellae in various host organisms, four 'green' species, *Mayorella viridis* (amoeboid protozoan), *Paramecium bursaria* (ciliated protozoan), *Stentor polymorphus* (ciliated protozoan), and *Hydra viridissima* (Cnidaria) were observed with a transmission electron microscope by freeze-substitution technique. Their endosymbiotic zoochlorellae formed very regulative membrane-bound photosynthetic organelles, which we named **symchlorosomes**. Symchlorosomes can be found in many freshwater micro-predatory species with a very wide genetic variety, which are ecologically important as they can provide a new niche for such mixotrophic organisms in freshwater micro-environment. We are going to introduce a possibility of ecological and evolutionary researches on symchlorosomes through our resent ultrastructural study.

Keywords: protozoa, algae, endosymbiosis, zoochlorella, symchlorosome

Fine-structure and molecular analyses of symbiotic algae in Radiolaria

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Marine, holoplanktonic protist radiolarians retain the algal symbionts within the cytoplasmic bodies. The majority of modern symbionts-bearing radiolarians appear to depend on their symbionts to provide photosynthetically fixed carbon and to maintain the radiolarians in low nutrient environments (e.g., Anderson 1978). Therefore, acquisitions of the photo-symbionts may have had their survival under low nutrient condition in the geologic time. During symbiotic state, algal symbiont within radiolarians generally appear as yellow-brown minute spheres, several micrometers in diameter. Cyanobacteria, dinoflagellates, prasinophytes, and haptophytes have all been identified as symbionts of radiolarians (e.g. Anderson 1983; Foster et al. 2006; Yuasa et al. 2012). However, the accurate taxonomic affiliation of these symbionts has not been clarified by the lack of diagnostic morphological features, such as theca or flagella, during the symbiotic state. Among them, I was able to establish cultures of the symbiotic dinoflagellate and compared the motile cell morphology and the molecular phylogeny of the SSU rDNA sequences with those of related species. The features of the thecal plate pattern and the molecular phylogenetic analysis indicate that the symbiotic dinoflagellate belongs to the peridinioid genus and species. In addition, based on the ultrastructural features by scanning electron and transmission electron microscopy and the molecular phylogenetic analyses of non-motile cells of other symbiotic algae, I found that radiolarian species contained some other partners; *Synechococcus* sp. (Cyanobacteria), *Chrysochromulina* sp. (Haptophyte) and Chlorophyta gen. sp. This symbiont diversity is in contrast to many corals, which host only dinoflagellates (*Symbiodinium* spp. and others). On the other hand, the symbionts have never co-occurred in a single host radiolarians, so the notion of only one kind of symbiotic algae per individual host has been maintained. A hypothesis would be that radiolarian symbionts originated from some free-living algae. This hypothesis is in agreement with the concept that radiolarians can easily acquire cyanobacteria symbionts *Synechococcus* sp. and *Prochlorococcus* sp. from environmental pools (Foster et al. 2006; Yuasa et al. 2012). Very little is known, however, about the distribution of free-living dinoflagellate, and, as far as we know, there is no evidence for the presence of radiolarian specific dinoflagellate symbionts in the natural environment.

Keywords: Radiolaria, Symbiosis, algae, ultrastructure, molecular phylogeny

Symbiotic relationship between *Braarudosphaera bigelowii* and cyanobacteria

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Braarudosphaera bigelowii (Haptophyta, Prymnesiophyceae) is a single-celled coastal coccolithophores, which is characterized by regular dodecahedral exotheca consists of regular pentagonal calcareous scales called pentoliths. Fossil records of the Family Braarudosphaeraceae and *B. bigelowii* extend back to the early and late Cretaceous, respectively. Living and fossil *B. bigelowii* have significant variation in size of pentoliths. Molecular phylogenetic study of living *B. bigelowii* revealed that morphotypes of living *B. bigelowii*, which was classified based on the size of pentoliths, can be related to the 18S rDNA genotypes. Therefore, it is thought that living *B. bigelowii* is a species complex consists of at least four discrete species which can be differentiated from each other based on size of pentoliths and of 18S rDNA sequences (Hagino et al. 2009). A recent study revealed close phylogenetic relationships among *B. bigelowii* sensu stricto (morphotype Intermediate form B, 18S rDNA Genotype III), *Chrysochломulina parkeae* (Prymnesiophyceae) and a prymnesiophyte cell that has symbiotic association with a nitrogen-fixing cyanobacterium UNYN-A. The prymnesiophyte host cell receives nitrogen from the cyanobacterium in exchange for transferring fixed carbon (Thompson et al., 2012). It was an unexpected relationship since *B. bigelowii* dissimilar to *C. parkeae* in general morphology, and *B. bigelowii* differs from UCYN-A in geographic distribution; living *B. bigelowii* is a notable coastal-neritic dweller, while the UCYN-A were abundantly reported from oligotrophic open ocean. In order to examine their relationships, we have conducted transmission electron microscopic and molecular phylogenetic studies of *B. bigelowii* and *C. parkeae*. In this talk, we will present an overview of geological history of the Family Braarudosphaeraceae, and morphological and genetic diversity in living *B. bigelowii*. We will also discuss about relationships among *B. bigelowii*, *C. parkeae* and the prymnesiophyte host of the UCYN-A based on the results from our morphological and molecular phylogenetic studies.

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Keywords: coccolithophores, cyanobacteria, symbiosis

Putative functions of kleptoplast in *Planoglabratella opercularis* (foraminifera)

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A rocky-shore benthic foraminifera, *Planoglabratella opercularis*, constructs specific host-symbiont relationships that has chloroplast as kleptoplast. Host organisms may have some benefit from kleptoplast, such as organic matters, or amino acids. To understand the functions of kleptoplast, we conducted culture experiment, ultrastructural observations, oxygen micro-sensor observations and nitrogen stable isotope of amino acid analyses. The trophic position of individuals with or without kleptoplast, we measured stable isotopic composition of amino acid to understand whether their nutritional requirements come from kleptoplast or not. As a result, trophic position (TP) of the individual with kleptoplast shows 1.2. In contrast, TP of cultured individual specimens that digested kleptoplast shows 2.0. It is possible that *P. opercularis* behave as a primary producer, phyto-benthos, in nature.

Keywords: Kleptoplast, benthic foraminifera, nitrogen isotope of amino acid, oxygen micro-sensor, transmission electron microscope

The effect of temperature on the composition of lipid biomarkers produced by *Chrysolita lamellosa*

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Long chain alkenones are synthesized by several species of Haptophyte, and used for quantitative paleo-sea surface temperature reconstructions. Alkenones have also been found in many lakes around the world, although their origin is not clear. Recent phylogenetic study suggested that typical lake alkenones with high content of tetra-unsaturated compounds are possibly produced by *Chrysolita lamellosa*, *Isochrysis galbana* or their intimately-associated species. However, only two investigations hitherto reported the lipid composition for *C. lamellosa* as a function of culture temperature. Intraspecific variation in the physiological response are noted by culture experiments of *Emiliana huxleyi* strains (Conte et al., 1998), which is less understood in coastal/limnic species including *C. lamellosa*. Here, we report $U^{K'}_{37}$ and U^K_{37} values for a *C. lamellosa* strain which no alkenone composition ever studied.

Keywords: Alkenone, Alkene, Haptophyte, *Chrysolita lamellosa*, UK'37, UK37

The ecological role of green sulfur bacteria in the chemocline of Lake Suigetsu

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Phototrophic sulfur bacteria are characterized by their oxidation of reduced sulfur compounds, which serve as electron donors during carbon fixation and anoxygenic photosynthetic growth in aquatic environments when the anoxic layers containing reduced sulfur compounds are exposed to light. Phototrophic sulfur bacteria often form dense blooms in the oxic-anoxic interfaces of stratified lakes. Furthermore, a high level of carbon fixation was detected at the oxic-anoxic interface in some meromictic lakes, indicating that phototrophic sulfur bacteria contribute significantly to primary production during the anaerobic carbon cycle. However, there is no evidence about in situ CO₂ fixation by phototrophic sulfur bacteria and the importance of CO₂ fixation by phototrophic sulfur bacteria in environment is still speculative. Lake Suigetsu is a meromictic lake, which is characterized by a permanent chemocline at a depth of 3-8 m that separates the oxic low salinity mixolimnion from the anoxic saline sulfidogenic monimolimnion. Green sulfur bacteria dominated at the chemocline of the Lake Suigetsu through the year. In this study, we evaluate the contribution of phototrophic sulfur bacteria to carbon fixation in the Lake Suigetsu.

The identity of active CO₂-fixing bacteria in the chemocline was assessed by DNA-stable isotope probing. The water at the chemocline was incubated with ¹³C-labelled sodium bicarbonate and under light or dark condition. The community composition of active CO₂-fixing bacteria was revealed by analysis of ¹³C-labelled DNA fractions. The diversity of 16S rRNA gene was analyzed using clone libraries. And productivity was measured in light or dark conditions by ¹⁴C method.

Chemotrophic carbon fixation accounted for about 80% of the carbon fixation rate in the chemocline. This indicates the contribution of chemotrophic bacteria to carbon fixation was larger than phototrophic bacteria in the chemocline. Clone sequences related to sulfide-oxidizing *Thiomicrospira* and sulfur-reducing *Thioreductor* were frequently recovered from ¹³C-DNA fraction library under dark condition, suggesting that these bacteria assimilate CO₂ using sulfur compounds in the water in the dark. Most of 16S rDNA sequences amplified from ¹³C-DNA under light condition were related to the genera *Chlorobium*. This indicated green sulfur bacteria assimilate CO₂ in the light. And sulfur-disproportionating *Desulfocapsa* also recovered from ¹³C-DNA fraction library under light condition. Although *Desulfocapsa* grow chemolithotrophically, clones related to *Desulfocapsa* did not detect from in dark incubation. In light condition, green sulfur bacteria also main bacteria and they accumulate elemental sulfur on its cell surface coupled with photosynthesis. We speculated *Desulfocapsa* use sulfur deposited on green sulfur bacteria as energy source for CO₂ fixation.

This study indicated that green sulfur bacteria fix carbon in the chemocline. And chemolithotrophic bacteria also play a significant role in the anaerobic CO₂ fixation in the chemocline of Lake Suigetsu. Our results suggest new ecological role of green sulfur bacteria serving energy for chemotrophic bacterial CO₂ fixation.

Keywords: meromictic lake, CO₂ fixation, green sulfur bacteria, stable isotoping method

Steroid analysis in culture samples of Parmales: Search for Parmales biomarker

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Palmales is picoplankton that has siliceous tests, and may be closely related to diatom, which is a main important primary producer in the Cenozoic ocean. There have been no reports for siliceous fossil of Parmales. It is known to well preserve siliceous diatom fossil in ancient sediment, and however, such fossil is frequently lost through its dissolution by diagenesis during post-deposition. Therefore, very small siliceous tests of Parmales must be easily dissolved by diagenesis, and it cannot evaluate the timing of first appearance and reconstruct productivity of Parmales by using its siliceous fossil. Thus, we clarified the Parmales biomarkers and their compositions, and these biomarkers are used as molecular fossils for giving understanding evolution processes and historical variations of productivity of this alga. In the present study, we try to search lipid biomarkers, especially steroid, of the Parmales, and to give understanding for taxonomic variability for steroid composition and concentration.

We use culture strains of *Triparma laevis*, *Triparma laevis f. longispina* and *Triparma strigata* for analysis of lipid biomarker. Wet culture samples were extracted with methanol/ dichloromethane, and the extracts were fractionated by silica gel chromatography. Polar fraction was silylated by BSTFA before analyses using GC/MS (Sawada and Shiraiwa, 2004, *Phytochem.* 65, 1299).

We can identify C_{21:6} n-alkene, C_{20:5} and C_{22:6} n-alkenoic acids as well as C₂₇-C₂₉ sterols as Parmales biomarkers. These lipids have been detected from diatom cultures as reported previously (e.g. Rampen et al., 2010, *Limnol. Oceanogr.* 55, 91). In particular, *T. laevis* strain is found to be characterized by overwhelmingly abundance of C₂₉ beta-sitosterol. However, C₂₈ sterol as ostreasterol is more abundant rather than C₂₉ sterols in *T. strigata*. These results indicate that there is possibly interspecies variability in sterol composition within *Triparma* genus. In addition, we can detect a number of unknown polar compounds with higher molecular weight. These unknown compounds may have potential as specific *Triparma* biomarkers.

Keywords: Parmales, biomarker, culture, steroid, evolution of diatom, chemotaxonomy

Chlorophyll detoxification catabolism associated with protistan phycophagy and evolution of phototrophic symbiosis

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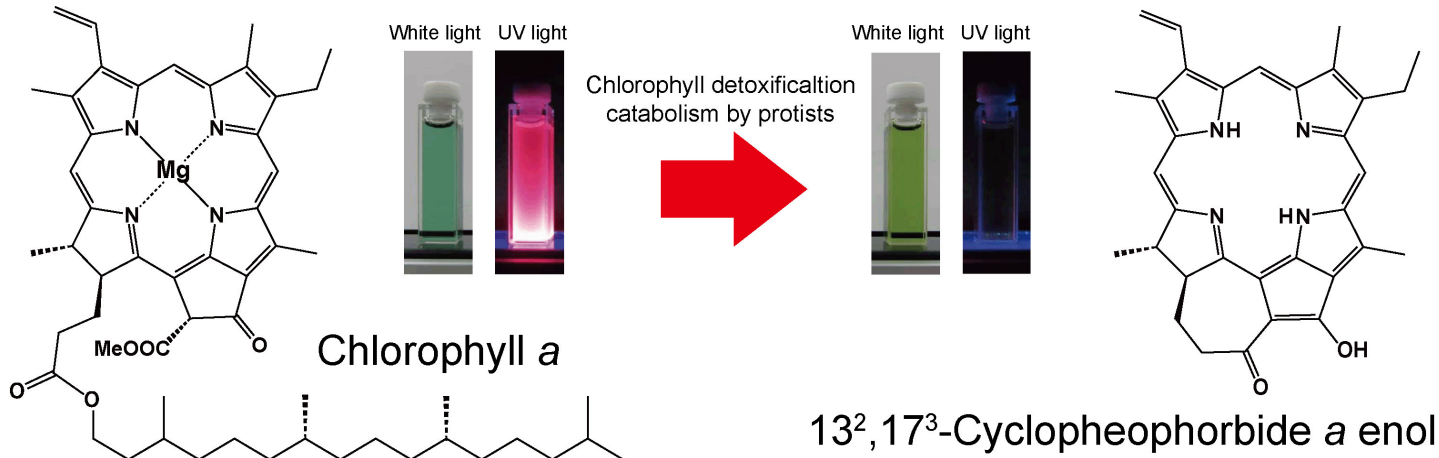
Chlorophylls are highly phototoxic and thus potentially problematic during their biosynthesis, organization, and degradative processes [1]. We have recently reported that metabolic conversion of chlorophyll to 13²,17³-cyclopheophorbide enol ("cyclo-enol") is a major detoxification mechanism for phycophagic protists (i.e., unicellular eukaryotes feeding on algae).[2] Significantly, a cyclo-enol is completely non-fluorescent and proven to be non-photosensitive in spite of their intact cyclic tetrapyrrole structure exhibiting green color in a solution. We cultured a series of phycophagic protists feeding on uniclonal algae and identified cyclo-enols as a sole major chlorophyll derivative presenting in extracts of the cultures. In addition, we demonstrated in microscopic observations of phycotrophic protists a quick disappearance of the autofluorescence of chlorophylls in the chloroplasts of ingested algae in an early stage of their digestion in phagocytosis, suggesting very rapid and nonradiative quenching of the presumable chlorophyll degradative product therein. We also infer that the cyclo-enol catabolism would be significant for the evolutions of algae that possess chloroplasts originating in secondary symbionts.

References

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Keywords: phototoxicity of chlorophyll, protists, phototrophic symbiosis, evolution of secondary algae, cyclopheophorbide enol



Spatio-temporal relationship between chlorophyll derivatives and eukaryotic microorganisms in a coastal water.

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Chlorophylls(Chls) are essential components of photosynthetic organism (algae), which include Chls-*a*, *b*, *c*, *d*, and *f*. The composition of the photosynthetic pigments including Chls as well as carotenoids and phycobiliproteins can be used as the taxonomic character or the biomarker to distinguish the dominant species in the aquatic ecosystems. While various Chl metabolites are known, their sources in the nature are not clear. Recently, ubiquitous occurrence of cyclophorbide *a* enol (cPPB-*a*E) is reported, and its producers, herbivorous protists, were elucidated. Therefore, we inspected that cPPB-*a*E can be able to be the biomarker to detect the feeding activity of protists. To understand the spacial-temporal relationships between the Chl derivatives and microorganisms, pigment analysis by HPLC, calculation of the cells and quantitative analysis using the environmental sequencing were performed.

The results demonstrated that quantity of the Chls and microorganisms were co-related. Chl-*a* was an extremely abundant pigment and much detected in shallow water. A quantitative trend of the cPPB-*a*E was similar to Chl-*a*, but the quantity in deep water in mid summer to early winter was much larger than shallow water. Even though a considerable amount of Chl-*a* was detected, cPPB-*a*E in winter was less abundant than in summer. Those trend shown in cPPB-*a*E was consistent with the abundances of the heterotrophic protists indicated by the environmental sequences.

Keywords: Chlorophyll derivatives, Cyclophorbide *a* enol, Protist, Algae

Distribution of chlorophyll *f* within hot spring microbial mat

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Chlorophyll (Chl) *f* is a recently discovered photosynthetic pigment, which absorbs far-red (FR) light (700-750 nm) in vivo. The distribution and role of Chl *f* in natural environments were still unclear. We have isolated Chl *f*-containing cyanobacteria from various habitats by cultivation using FR-LED as their sole light sources. These cyanobacteria produced Chl *f* only when the cells were grown under FR-LED. Therefore, we hypothesized that Chl *f* was distributed only in certain environments where FR light mainly existed, and it contributed to the oxygenic photosynthesis at those habitats. We thought that the inner layer of microbial mat was one of such environments, because photosynthetically active radiation (PAR 400-700 nm) was absorbed by phototrophs in surface layer. In this study, we aimed to reveal the vertical distribution of Chl *f* and the light environment within hot spring microbial mats.

We collected 20 microbial mat samples at 6 hot springs in Nagano and Gifu prefectures in Japan. Chl *f* was detected from 5 samples of them. Vertical profiles of Chl *f* and downward spectral irradiance within microbial mats were measured by using HPLC and fiber optic spectrophotometer, respectively. Community structure analysis in mats was also performed by PCR-DGGE to reveal the vertical distribution of Chl *f*-producing cyanobacteria. In this poster, we discuss the adaptive significance of Chl *f* in microbial mats.

Keywords: chlorophyll *f*, cyanobacteria, microbial mat

Spatio-temporal dynamics of chlorophylls and chlorophyll-derived catabolites in Lake Biwa

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Chlorophylls in aquatic samples have been regarded as important biomarker for phototrophic microbes such as cyanobacteria and algae. Chlorophyll *a* (Chl-*a*) in particular has been treated as a proxy for photosynthetic production in oceans and lakes. Recently, Kashiya, Yokoyama et al. (2012) [1] reported that ¹³C²,¹⁷O³-cyclophorbide *a* enol (cPPB-*a*E), a pigment derived from Chl-*a*, occurs ubiquitously from most of aquatic environments. cPPB-*a*E comprises 7-16% of total Chl-*a* derivatives in euphotic water column and 51% in the surface sediment at the center of Lake Biwa. We herein report monthly changes in pigment concentrations of vertical water column profile to discuss on year-around variations in activities of phototrophic and phycophagic microbes in Lake Biwa.

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Keywords: Lake Biwa, Protists, cycloenls, algae, microbial loop