

# **The 13<sup>th</sup> Symposium on Polar Science**

15 – 18 November 2022

National Institute of Polar Research  
Research Organization of Information and Systems

## **Session OB**

### **Polar Biology**

#### **Program and Abstracts**

Conveners

Nobuo Kokubun, and Ryosuke Makabe (NIPR)

# 【OB】 Polar Biology

## Scopes

This session covers the following research topics.

Polar marine ecosystem -biogeochemistry, environmental DNA, and ecology of phytoplankton to apex predators

Polar terrestrial ecosystem -environment, biodiversity, and biological responses

## Real-time Oral presentations (10:00 – 11:35, 14:25 – 16:00)

Date: Wed. 16 November

*Note: [I] represents an invited talk.*

Chair: Tohru Hirawake (NIPR)			
10:00 - 10:05		Opening remark	
OBo1	10:05 - 10:35	[I] The thermophilization of Arctic marine ecosystems from climate change	*Jorge García Molinos (Hokkaido Univ.)
Chair: Sakae Kudoh (NIPR)			
OBo2	10:35 - 10:55	Discrepancies of fungi and plants in the pattern of beta-diversity with environmental gradient imply a comprehensive community assembly rule	*Shota Masumoto (Univ. of Tsukuba), Ryo Kitagawa (Kansai Research Center, Forestry and Forest Products Research Institute), Ryo Kaneko (National Institute of Polar Research), Keita Nishizawa (The Univ. of Tokyo), Shunsuke Matsuoka (Kyoto Univ.), Masaki Uchida (NIPR & SOKENDAI), Akira Mori (The Univ. of Tokyo)
OBo3	10:55 - 11:15	Recovery of Soil Fungal Community: Monoculture Plantation versus Natural Regeneration	*Minagi Naka (Yokohama National Univ.), Keita Nishizawa (Univ. of Tokyo), Shota Masumoto (Univ. of Tsukuba), Yuta Kobayashi (Univ. of Tokyo), Kureha Suzuki (Yokohama National Univ.), Tomoya Kawakami (Hokkaido Univ. ), Noboru Katayama (Otaru Univ. of Commerce), Makoto Kobayashi (Hokkaido Univ. ), Keichi Okada (Tokyo Univ. of Agriculture ), Shinichi Tatsumi (Forestry and Forest Products Research Institute), Shunsuke Matsuoka (Yokohama National Univ.), Uchida Masaki (NIPR & SOKENDAI), Takagi Kentaro (Hokkaido Univ.), Akira S Mori (Univ. of Tokyo)
OBo4	11:15 - 11:35	Fungi in the Arctic and Antarctic's ice cores, and new attempts to use fungi in polar regions	*Masaharu Tsuji (National Institute of Technology, Asahikawa College)
Lunch			
Chair: Nobuo Kokubun and Ryosuke Makabe (NIPR)			
12:45 - 13:05		1-minute poster appeal (10 short talks of OBp1 – OBp10)	
13:05 - 13:40		Poster session core time_1	
13:40 - 14:15		Poster session core time_2	
14:15 - 14:25		Break	
Chair: Akinori Takahashi (NIPR)			
OBo5	14:25 - 14:55	[I] Inter-specific overlap in foraging habitat use and diet but segregation in utilization of winds in Antarctic fulmarine petrels	*Nina Dehnhard (Norwegian Institute for Nature Research)

OB06	14:55 - 15:15	Eat-in or take-away? Quantification of Antarctic resident and non-resident seabirds in the pelagic ecosystem in the eastern Indian sector of the Southern Ocean	*Nobuo Kokubun (NIPR & SOKENDAI), Kohei Hamabe (Japan Fisheries Research and Education Agency (FRA) & TUMSAT), Nodoka Yamada (Hokkaido Univ.), Hiroko Sasaki (FRA), Bungo Nishizawa (Hokkaido Univ. & FRA), Yutaka Watanuki (Hokkaido Univ.), Hiroto Murase (TUMSAT & FRA)
OB07	15:15 - 15:35	The choice of prey for parents own and for chicks in Rhinoceros Auklet	*Jumpei Okado (Hokkaido Univ.), Motohiro Ito (Toyo Univ.), Yutaka Watanuki (Hokkaido Univ.)
OB08	15:35 - 15:55	Biologging reveals behavioral coordination of Adélie penguins traveling in groups	*Toshitaka Imaki (SOKENDAI), Nobuo Kokubun (NIPR & SOKENDAI), Kozue Shiomi (Tohoku Univ.), Akinori Takahashi (NIPR & SOKENDAI)
15:55 - 16:00		Closing remark	
Chair: Nobuo Kokubun and Ryosuke Makabe (NIPR)			
16:00 - 16:20		1-minute poster appeal (12 short talks of OBp11 – OBp22)	
16:20 - 16:55		Poster session core time_3	
16:55 - 17:30		Poster session core time_4	

## Real-time Poster presentations (12:45 – 14:15, 16:00 – 17:30)

Date: Wed. 16 November

	Core time	Chair: Nobuo Kokubun and Ryosuke Makabe (NIPR)	
<b>OBp1</b>	<b>13:05 - 13:40</b>	DEHP degrading bacteria isolated from Antarctica	*Yudai Inagaki (Shibaura Institute of Technology), Siti Aqlima Ahmad (Universiti Putra Malaysia), Azham Zulkharnain (Shibaura Institute of Technology)
<b>OBp2</b>	<b>13:05 - 13:40</b>	Bacterial microbiota associated with <i>Umbilicaria</i> spp. lichens in continental and maritime Antarctic, Arctic and Alpine regions	*Zichen He (Hiroshima Univ.), Takeshi Naganuma (Hiroshima Univ.), Ryosuke Nakai (AIST), Megumu Tsujimoto (Keio Univ.), Hiroshi Kanda (NIPR), Satoshi Imura (NIPR), Jun Uetake (Hokkaido Univ.), Martin W. Hahn (Univ. of Innsbruck), Peter Convey (BAS)
<b>OBp3</b>	<b>13:05 - 13:40</b>	Meteorological features of Syowa station and ice-free areas for bryophyte ecosystem.	*Shunichi Arai (SOKENDAI), Satoshi Imura (NIPR & SOKENDAI), Koyomi Nakazawa (Toyama Prefectural Univ.), Tomotake Wada (NIPR & SOKENDAI), Sakae Kudoh (NIPR & SOKENDAI & ROIS)
<b>OBp4</b>	<b>13:05 - 13:40</b>	Characterization of Polylactic Acid Degrading Bacterium strain N-3 from Antarctic Soil	*Kenta Sato (Shibaura institute of technology), S. A. Ahmad (Universiti Putra Malaysia), A Zulkharnain (Shibaura institute of technology)
<b>OBp5</b>	<b>13:05 - 13:40</b>	Applying ATR-FTIR spectroscopy to Characterize and Discriminate Tundra Plant Leaves	*Takashi Osono (Doshisha Univ.), Weitong Lin (Doshisha Univ.), Motohiro Hasegawa (Doshisha Univ.), Masaki Uchida (NIPR & SOKENDAI)
<b>OBp6</b>	<b>13:40 - 14:15</b>	Microbial diversity around Syowa Station in 2021	*Sakae Kudoh (ROIS & NIPR & SOKENDAI), Shu Kuan Wong (AERC & NIPR), Kenichi Watanabe (NIPR), Mizuho Mori (NIPR), Koyomi Nakazawa (Toyama P. Univ.)
<b>OBp7</b>	<b>13:40 - 14:15</b>	Outbreaks of bdelloid rotifers in and at adjacent wetland of a marine relict lake, Mago Ike on Soya Coast, East Antarctica: climate and limnological conditions	*Sakae Kudoh (ROIS & NIPR & SOKENDAI), Kunio Takahashi (NIPR & SOKENDAI), Tomotake Wada (SOKENDAI)

<b>OBp8</b>	<b>13:40 - 14:15</b>	Bacterial community structures of environmental sample and enrichment cultures of the hyper-saline lake Zakuro in the Langhovde, East Antarctica.	*Kaori Iiyama (Soka Univ.), Hanae Seki (Soka Univ.), Akinori Kawamata (EPSM), Satoshi Imura (NIPR & SOKENDAI), Hiroyuki Sakai (Soka Univ.), Norio Kurosawa (Soka Univ.)
<b>OBp9</b>	<b>13:40 - 14:15</b>	Seasonal changes of chytrid infection of glacier algae in Alaska	*Kino Kobayashi (Chiba Univ.), Nozomu Takeuchi (Chiba Univ.), Maiko Kagami (Yokohama National Univ.)
<b>OBp10</b>	<b>13:40 - 14:15</b>	Influences of ground moss cover and summer precipitation on the occurrence of tar spot disease of polar willow in Ny-Ålesund, Spitsbergen Is., Norway	*Motoaki Tojo (Osaka Metropolitan Univ.), Masahiro Suzuki (Osaka Metropolitan Univ.), Shota Masumoto (Univ. of Tsukuba), Masaki Uchida (NIPR & SOKENDAI)
<b>OBp11</b>	<b>16:20 - 16:55</b>	Tooth morphologies of <i>Pusa</i> and <i>Phoca</i> seals and its relation to their diet	*Uno Ishihara (SOKENDAI), Yuuki Watanabe (NIPR & SOKENDAI)
<b>OBp12</b>	<b>16:20 - 16:55</b>	Thermal sensitivity of metabolic rate mirrors different biogeographies between teleosts and elasmobranchs	*Yuuki Watanabe (NIPR & SOKENDAI), Nicholas Payne (Trinity College Dublin)
<b>OBp13</b>	<b>16:20 - 16:55</b>	Animal-borne video camera provides new insights into predator-prey interactions between the Adélie penguin <i>Pygoscelis adeliae</i> and the prey fish <i>Pagothenia borchgrevinki</i>	*Soma Tokunaga (SOKENDAI), Yuuki Kawabata (Nagasaki Univ.), Akinori Takahashi (NIPR & SOKENDAI)
<b>OBp14</b>	<b>16:20 - 16:55</b>	Prokaryotic and eukaryotic community structures in East Antarctic coastal sediments	*Hideaki Ochi (Soka Univ.), Ryo Matsuda (Soka Univ.), Ryosuke Makabe (NIPR & SOKENDAI & TUMSAT), Masayoshi Sano (NIPR & AORI, The Univ. of Tokyo), Shintaro Takao (NIES), Masato Ito (NIPR), Shin Sugiyama (ILTS, Hokkaido Univ.), Masato Moteki (NIPR & TUMSAT), Norio Kurosawa (Soka Univ.)
<b>OBp15</b>	<b>16:20 - 16:55</b>	Empirical equations and image analyses for estimating zooplankton biomass in the Southern Ocean	*Shunji Yukawa (TUMSAT), Ryosuke Makabe (NIPR & SOKENDAI & TUMSAT), Masayoshi Sano (AORI, Univ. of Tokyo & NIPR), Masato Moteki (TUMSAT & NIPR)
<b>OBp16</b>	<b>16:20 - 16:55</b>	Particulate organic matters in sea ice floe in the Indian sector of the Southern Ocean	*Ryosuke Makabe (NIPR & SOKENDAI & TUMSAT), Masayoshi Sano (AORI, Univ. of Tokyo & NIPR), Keigo Takahashi (SOKENDAI), Shintaro Takao (NIES), Ryo Matsuda (Soka Univ.), Masato Ito (NIPR), Michiyo Yamamoto-Kawai (TUMSAT), Daiki Nomura (Hokkaido Univ.), Norio Kurosawa (Soka Univ.), Masato Moteki (TUMSAT & NIPR)
<b>OBp17</b>	<b>16:55 - 17:30</b>	Transport of live Antarctic fishes from Syowa Station to Port of Nagoya Public Aquarium	*Tsuyoshi Matsuda (Port of Nagoya Public Aquarium), Akinori Takahashi (NIPR), Kotaro Ichikawa (Kyoto Univ.), Hideaki Nishizawa (Kyoto Univ.), Saki Asai (TUMSAT), Yoshinori Miyamoto (TUMSAT), Kiyonori Nakajima (Port of Nagoya Public Aquarium), Masanori Kurita (Port of Nagoya Public Aquarium)
<b>OBp18</b>	<b>16:55 - 17:30</b>	Development of animal-borne dissolved oxygen loggers to examine the foraging behavior of northern elephant seals in the oxygen-limited mesopelagic zone	*Atsuya Ogata (SOKENDAI), Arina Favilla (UCSC), Rachel Holser (UCSC), Theresa Keates (UCSC), Daniel Costa (UCSC), Akinori Takahashi (NIPR & SOKENDAI)
<b>OBp19</b>	<b>16:55 - 17:30</b>	King penguins adjust their fine-scale traveling and foraging behavior to spatial and diel changes in feeding opportunities	*Hina Watanabe (SOKENDAI), Kozue Shiomi (Tohoku Univ.), Katsufumi Sato (AORI, The Univ. of Tokyo), Akinori Takahashi (NIPR), Yves Handrich (Université de Strasbourg, CNRS), Bost Charles-André (CEBC, UMR 7372 CNRS-Université de la Rochelle)

<b>OBp20</b>	<b>16:55 - 17:30</b>	Impacts of climate change and variability on penguins: a review	*Akinori Takahashi (NIPR & SOKENDAI)
<b>OBp21</b>	<b>16:55 - 17:30</b>	Temporal changes in protist fluxes during sea ice melt off Wilkes land, East Antarctica	*Chiho Tsuchiya (TUMSAT), Keigo Takahashi (SOKENDAI), Masayoshi Sano (AORI, Univ. of Tokyo & NIPR), Ryosuke Makabe (NIPR & SOKENDAI & TUMSAT), Shintaro Takao (NIES), Masato Moteki (TUMSAT & NIPR)
<b>OBp22</b>	<b>16:55 - 17:30</b>	The occurrence of a dinoflagellate species <i>Gyrodinium rubrum</i> in sea ice in the Indian sector of the Southern Ocean	*Ryo Matsuda (Soka Univ.), Masayoshi Sano (AORI, The Univ. of Tokyo & NIPR), Keigo D. Takahashi (SOKENDAI), Shintaro Takao (NIES), Ryosuke Makabe (NIPR & SOKENDAI & TUMSAT), Michiyo Yamamoto-Kawai (TUMSAT), Masato Moteki (TUMSAT & NIPR), Norio Kurosawa (Soka Univ.)

# The thermophilization of Arctic marine ecosystems from climate change

Jorge García Molinos<sup>1</sup>

<sup>1</sup>*Arctic Research Center, Hokkaido University, Japan*

One of the most pervasive and widespread signatures of anthropogenic climate change is the global redistribution of biodiversity with an overarching pattern of species range shifts towards cooler environments found at higher latitudes (Poloczanska et al. 2016). As a result, temperate and high-latitude ecosystems are experiencing an influx of warm-affinity, climate migrants that are gradually dominating and replacing the local, cold-affinity flora and fauna. This global thermophilization is well documented in marine ecosystems (e.g., Burrows et al. 2019), characterized by less fragmented habitats and hosting species having typically lower thermal safety margins than terrestrial ecosystems, particularly at biogeographical transition zones where species live at or close to their distributional range limits (Ferro and Morrone 2014). Among them, marginal Arctic seas are particularly exposed and sensitive to the effects of a rapidly warming climate. Here, I review recent evidence on the ongoing thermophilization of marginal Arctic seas, and the resulting consequences ranging from documented shifts in species ranges, to changes in community composition, functional diversity, food webs and, ultimately, ecosystem functioning (e.g., Alabia et al. 2018; Fossheim et al. 2015; Kortsch et al. 2015; Emblemståg et al. 2022). As gateways of the Arctic, connecting the Pacific and Atlantic Oceans to the Arctic Ocean, these ongoing changes provide a glimpse into the transformations that are likely to spread into other Arctic marine ecosystems in the future.

## References

- Alabia, I.D., J. García Molinos, S.I. Saitoh, T. Hirawake, T. Hirata, F.J. Mueter. Distribution shifts of marine taxa in the Pacific Arctic under contemporary climate changes. *Diversity and Distributions*, 24, 1583-1597, 2018.
- Burrows, M.T., A.E. Bates, M.J. Costello, M. Edwards, G.J. Edgar, C.J. Fox, B.S. Halpern, J.G. Hiddink, M.L. Pinsky, R.D. Batt, J. García Molinos, B.L. Payne, D.S. Schoeman, R.D. Stuart-Smith and E.S. Poloczanska. Ocean community warming responses explained by thermal affinities and temperature gradients. *Nature Climate Change*, 9, 959–963, 2019.
- Emblemståg, M. L. Pecuchet, L.G. Velle, A. Nogueira and R. Primicerio. Recent warming causes functional borealization and diversity loss in deep fish communities east of Greenland. *Diversity and Distributions*, 28, 2071-2083, 2022.
- Ferro, I. and J.J. Morrone. Biogeographical transition zones: a search for conceptual synthesis. *Biological Journal of the Linnean Society*, 113, 1–12, 2014.
- Fossheim, M., R. Primicerio, E. Johannesen, R.B. Ingvaldsen, M.M. Aschan and A.V. Dolgov. Recent warming leads to a rapid borealization of fish communities in the Arctic. *Nature Climate Change*, 5, 673–677, 2015.
- Kortsch, S., R. Primicerio, M. Fossheim, A.V. Dolgov and M. Aschan. Climate change alters the structure of arctic marine food webs due to poleward shifts of boreal generalists. *Proceedings of the Royal Society B*, 282, 20151546, 2015.
- Poloczanska, E. S., M.T. Burrows, C.J. Brown, J. García Molinos, B.S. Halpern, O. Hoegh-Guldberg, C.V. Kappel, P.J. Moore, A.J. Richardson, D.S. Schoeman and W.J. Sydeman. Responses of marine organisms to climate change across oceans. *Frontiers in Marine Science*, 3, ea62, 2016.

# Discrepancies of fungi and plants in the pattern of beta-diversity with environmental gradient imply a comprehensive community assembly rule

Shota Masumoto<sup>1\*</sup>, Ryo Kitagawa<sup>2</sup>, Ryo Kaneko<sup>3</sup>, Keita Nishizawa<sup>4</sup>, Shunsuke Matsuoka<sup>5</sup>, Masaki Uchida<sup>3,6</sup>, Akira S. Mori<sup>4</sup>

<sup>1</sup> Faculty of Life and Environmental Sciences, University of Tsukuba

<sup>2</sup> Kansai Research Center, Forestry and Forest Products Research Institute

<sup>3</sup> National Institute of Polar Research

<sup>4</sup> Research Center for Advanced Science and Technology, the University of Tokyo

<sup>5</sup> Field Science Education and Research Center, Kyoto University

<sup>6</sup> The Graduate University for Advanced Studies, School of Multidisciplinary Sciences

Beta-diversity partitioning has been showed that the nestedness component develop with environmental stress in a variety of taxa (Ulrich et al 2012). However, soil fungal community may maintain its turnover components despite the development of plants' nestedness component, and the potential causes remain unclear.

To investigate the process of species turnover of soil fungi along stress gradient in the Arctic, we divided species turnover component into sub-components;  $\beta_{sim\_hete}$  and  $\beta_{sim\_homo}$  representing species turnover with or without a change in the guilds.

As the result, fungal community maintain its turnover component unlike plant community, but its  $\beta_{sim\_hete}$  increased under stressful (Fig. 1). Additionally, GDM analysis resulted in that  $\beta_{sim\_hete}$  were mainly explained by stress gradient and plant nestedness (Fig. 2), suggesting that functionality of soil fungi was ecologically filtered by environmental stress and plant community structure.

The discordant trend of beta-diversities between plant and fungi (i.e. development of plant nestedness and maintaining of fungal turnover) would be not caused by different assembly rules working parallelly on the two taxa, but according to an ecological rule that reflects plant-fungal interaction.

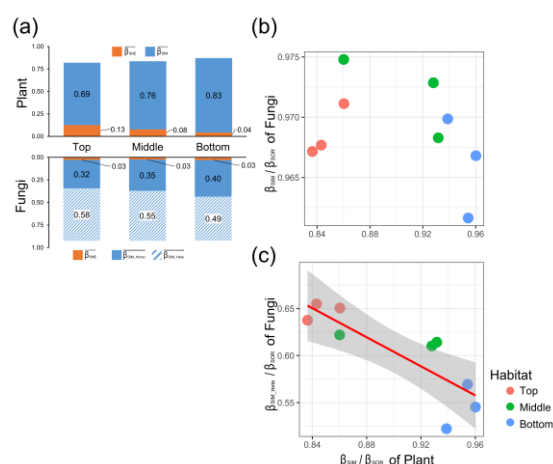


Figure 1. (a) Visualization of multi-site dissimilarity of plant and fungal communities.  $\beta_{SOR}$  is the Sørensen dissimilarity and  $\beta_{SIM}$  and  $\beta_{NES}$  are the turnover and nestedness components of dissimilarity, respectively.  $\beta_{SIM\_Hete}$  and  $\beta_{SIM\_Homo}$  are the turnover components with and without guild exchange, respectively. The correlations of the  $\beta_{SIM}$  to  $\beta_{SOR}$  ratios of soil fungi and plants (b) and (c)  $\beta_{SIM\_Hete}$  to  $\beta_{SIM}$  ratio of soil fungi with the  $\beta_{SIM}$  to  $\beta_{SOR}$  ratio of plants. Red lines represent statistically significant ( $P < 0.05$ ) linear model fits; the shaded area represents the corresponding 95% confidence intervals.

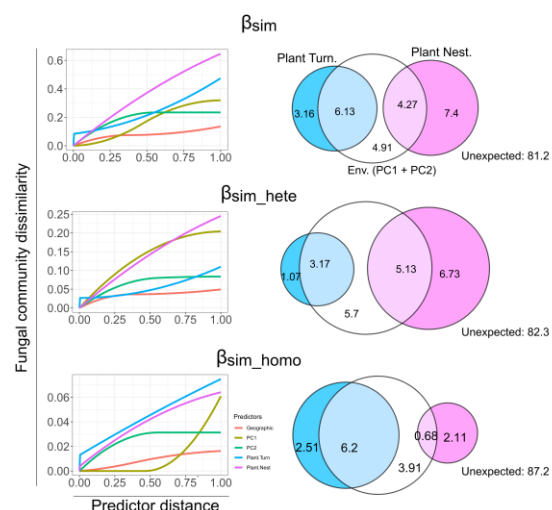


Figure 2. Predictors for soil fungal turnover components:  $\beta_{sim}$ ,  $\beta_{sim\_hete}$ ,  $\beta_{sim\_homo}$ . The left graphs show the effect of each predictor from the generalized dissimilarity modeling (GDM) analysis and Venn diagrams on the right show the relative importance of each predictor to the variation partitioning based on the GDM results.

## References

Ulrich W, Almeida-Neto M. On the meanings of nestedness: Back to the basics. *Ecography (Cop)* 35, 865–71, 2012.

# Recovery of Soil Fungal Community: Monoculture Plantation versus Natural Regeneration

Minagi Naka<sup>1</sup>, Keita Nishizawa<sup>2</sup>, Shota Masumoto<sup>3</sup>, Yuta Kobayashi<sup>2</sup>, Kureha Suzuki<sup>1</sup>, Tomoya Kawakami<sup>4</sup>, Noboru Katayama<sup>5</sup>, Makoto Kobayashi<sup>4</sup>, Keichi Okada<sup>6</sup>, Shinichi Tatsumi<sup>7</sup>, Shunsuke Matsuoka<sup>8</sup>, Masaki Uchida<sup>9</sup>, Kentaro Takagi<sup>4</sup> and Akira S Mori<sup>2</sup>

<sup>1</sup>*Yokohama National University*

<sup>2</sup>*University of Tokyo*

<sup>3</sup>*University of Tsukuba*

<sup>4</sup>*Hokkaido University*

<sup>5</sup>*Otaru University of Commerce*

<sup>6</sup>*Tokyo University of Agriculture*

<sup>7</sup>*Forestry and Forest Products Research Institute*

<sup>8</sup>*Kyoto University*

<sup>9</sup>*National Institute of Polar Research*

The recovery of ecosystem functioning to the natural forests is one of the main challenges of forest restoration worldwide these days. While monoculture plantations have been the most common practice of forest restoration, a number of studies have demonstrated that these forests have lower levels of biodiversity and ecosystem functioning, especially in terms of carbon storage, than natural forests. Forests in the northern area identified as a critical carbon sink, monoculture plantations have been expanding rapidly on the deforested land but there is a growing interest in natural regeneration (also as known as passive forest restoration). Some studies have compared vegetation recovery with and without human intervention. However, little is known about the succession of soil fungi during forest development even though they play a vital role in regulating multiple ecosystem functions such as nutrient cycles, plant growth and carbon budget. Here we aimed to compare the successional development and the recovery of soil fungal communities among different forest types.

The study sites were the Teshio experimental forest of Hokkaido University located in the northern part of Hokkaido, Japan (Fig. 1). We established 33 study sites in three forest types: natural forest, *Picea-glehnii* plantation and secondary forest. To assess the fungal succession trajectory in each forest restoration type, these sites were designed to have different stages of forest development (chronosequence approach from 0 to 50 years). Soil samples were collected in three replicates at each plot and sequenced using DNA metabarcoding. The procedures used for bioinformatics analyses followed those described by Matsuoka et al., 2019. The Jaccard dissimilarity index between all samples was calculated and visualized using principal coordinate analysis (PcoA). We showed the change in OTU richness and the dissimilarity index along the forest age, and then fitted a linear model on the soil fungal community data.

The Ascomycota was the most abundant bacterial phylum, followed by the Basidiomycota, Mucoromycota, Chytridiomycota, Zoopagomycota, Cryptomycot and Blastocladiomycota, as well as 5% of the OTUs could not be identified to phylum. OTU richness significantly decreased with time in plantations but not significantly in natural regeneration forests (Fig. 2). In the plantation, there was no relationship between the Jaccard dissimilarity index with natural forest and forest age while the index significantly decreased in the regeneration forest (Fig. 3). In addition, the fungal communities of the secondary forest became similar to those in the natural forest as the stage developed (Fig. 4).

We found that there were different successional patterns of fungal communities across the forest restoration types. Previous studies confirmed that the plant community plays an important role in the soil microbial community structure via litter chemical properties and root exudation. Differences in tree species composition could have affected the soil fungal community structure. Moreover, our findings imply that natural regeneration could be a successful approach for restoring fungal communities.



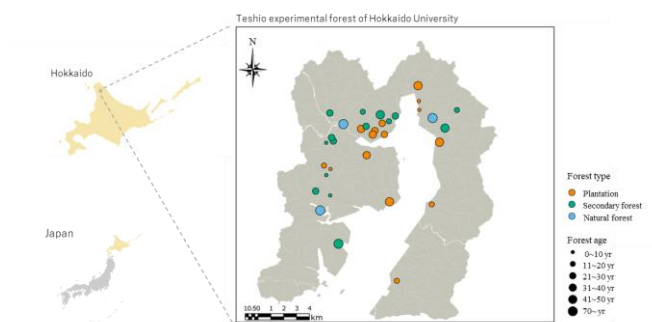


Fig. 1: The study site of the Teshio experimental forest of Hokkaido University. Different colors of points refer to three different forest types. Orange, plantation; Green, secondary forest; Blue, natural forest.

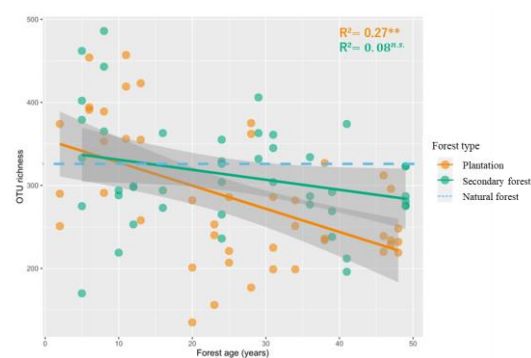


Fig. 2: Operational taxonomic unit (OTU) richness of fungi during forest succession. Different colors of points refer to three different forest types. A dotted line indicates the average number of OTUs in all plots of natural forest. \*\*  $P < 0.01$ . n.s.  $P > 0.05$ .

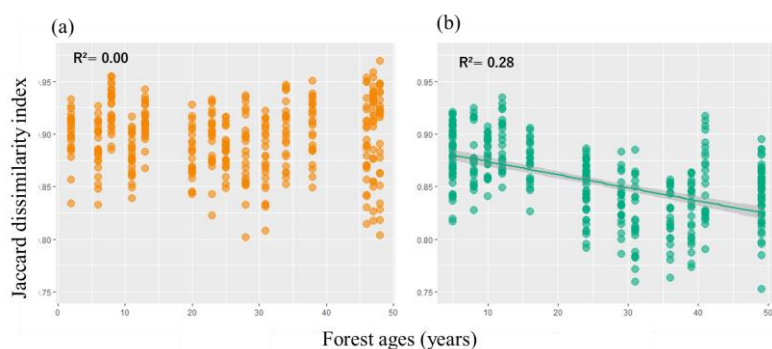


Fig. 3: Relationships between forest age and similarity of fungal communities. Jaccard similarity coefficient with natural forest calculated (0: similar, 1: dissimilar) for all plots of plantation (a) and secondary forest (b). If linear regression with forest age appears significant for  $p < 0.05$ , the trend line and 95% credible intervals are given.

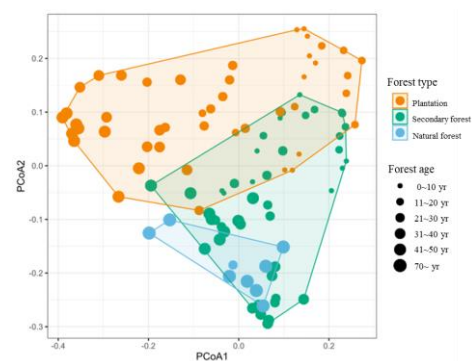


Fig. 4: Principal coordinate analysis on the Jaccard similarity index matrix of all plots highlighting the three different forest-type.

## References

Matsuoka, S., Sugiyama, Y., Sato, H., Katano, I., Harada, K., and Doi, H, Spatial structure of fungal DNA assemblages revealed with eDNA metabarcoding in a forest river network in western Japan, *Metabarcoding and Metagenomics*, 3, 37–47, 2019.

# **Fungi in the Arctic and Antarctic's ice cores, and new attempts to use fungi in polar regions**

Masaharu Tsuji<sup>1</sup>

<sup>1</sup> *National Institute of Technology, Asahikawa College*

Ice sheet deposited in Arctic and Antarctica entraps viable and nonviable fungi, as well as biomolecules and air. They become temporal atmospheric records. Despite exposure to conditions adversely affecting their survival, such as subzero temperatures and low nutrient and water availability, some of fungi that entrapped in ice sheet can survive.

Fungi in the Antarctic and Arctic are known to have a high cold and freezing tolerance, as they can survive in extreme environments below -40°C. Because the polar environment is oligotrophic, the fungi that inhabit it play an important role in nutrient cycling, and even changes in the fungal diversity due to climate changes can have a significant impact on material production in the polar regions. Therefore, isolation of fungi in ice cores and investigation of their genomic and physiological characteristics are important not only for understanding the evolution of fungi in polar regions, but also for elucidating polar ecosystems.

In this presentation, I would like to talk about my attempts to isolate ancient fungi from ice cores in the Arctic and Antarctica.

According to the IPCC Special Report, if global warming continues at its current pace, the annual average temperature is expected to increase by about 3.2°C by 2100, which corresponds to a temperature difference of about 360 km (equivalent to the distance between Shinjuku Ku, Tokyo and Sendai City, Miyagi) in terms of Japanese latitude. Since most of the fungi in Antarctica and the High Arctic are specialized for cold environments, this rise in temperature could have a serious impact on the survival of fungi around Syowa Station, Antarctica, in the Canadian High Arctic etc.

Fungi in Antarctica and the High Arctic are threatened with habitat shrinkage and extinction due to climate change because they are specialized for growth at low temperatures. These fungi have the unique characteristic of growing even at sub-zero temperatures and have recently begun to attract attention as a microbial resource.

In addition, fungi in polar regions, which inhabit one of the world's harshest environments, are attracting attention as a new genetic resource. However, the genome sequences of Antarctic and High Arctic fungi are not available without advanced bioinformatics technology, since only about 20 whole genome data are stored in DDBJ, NCBI, and EBI, which are international nucleotide sequence databases.

In the second topic, I would like to talk about the attempt to make the National Institute of Polar Research a research hub on fungi in Polar regions.

# Inter-specific overlap in foraging habitat use and diet but segregation in utilization of winds in Antarctic fulmarine petrels

Nina Dehnhard<sup>1</sup>

<sup>1</sup> Norwegian Institute for Nature Research (NINA), Trondheim, Norway

Antarctic fulmarine petrels, as all procellariiform seabirds, rely heavily on wind for their energy-efficient, gust-soaring flight. According to physics, flight speeds and thus commuting costs are highly dependent on wind speeds and wind direction, and differences in wing loading (mass per surface area) between species may further affect flight behaviour. With global climate change, wind patterns in the Southern Hemisphere are predicted to change, as is the amount and distribution of sea ice, which is an important foraging habitat for these species. Identifying the currently utilized foraging habitats and diets and how wind conditions affect the travel to and from foraging sites is essential to understand the species' biology and help make predictions about how future changes will affect the species. I will here present data that have been previously published (see Dehnhard et al. 2020, 2021) and discuss the results in the context of ecological niche theory and climate change.

In 2015/16 the foraging behaviour and diet of southern fulmars (*Fulmarus glacialis*), Antarctic petrels (*Thalassoica antarctica*) and cape petrels (*Daption capense*) breeding sympatrically on Hop Island (68.82°S, 77.68°E) in the Prydz Bay region (East Antarctica) was investigated. The three species show a gradient in mass and wing loading, with southern fulmars being approximately twice as heavy as cape petrels, but Antarctic petrels having the highest wing loading. Using lightweight GPS loggers, a total of 270 foraging trips were recorded, covering the entire 2015/16 breeding season from incubation to late chick-rearing in all three species, including multiple foraging trips made by several individuals. Blood, feathers and egg membranes were collected from the same species.

GPS data were intersected with environmental data from remote sensing (e.g. bathymetry, sea ice concentration, wind speed and wind direction). Foraging locations were identified using wet/dry data recorded by the GPS loggers and Expectation-Maximization binary Clustering (EMbC). Generalized Additive Models (GAMs) were used to identify habitat characteristics associated with foraging locations, and kernel analyses to assess inter-specific overlap in spatial habitat use. Stable isotopes were used to explore the dietary overlap between the three seabird species using isotopic niche parameterisation and estimates of resource use through mixing models. The commuting sections (outbound and inbound legs) of foraging trips during the chick-rearing period were used to investigate the relationship between wind speed and wind direction and the birds' flight speed over ground as well as trip distances.

All three species showed a high overlap in utilized foraging areas and isotopic niches as well as timing of foraging. There was also no indication for individual specialisation in foraging behaviour or habitat characteristics. Wind direction relative to flight direction (and thus whether birds were encountering head- or tailwinds) did not affect how far away from the colony birds were feeding. However, wind speeds and wind direction had distinct effects on flight speeds of the three species, with Antarctic petrels reaching higher flight speeds than the other two species. All three species reached higher flight speeds under tailwinds, especially on return trips from foraging, when wing loading was increased since birds carried food for their chicks.

The high overlap in space use and diet among the three study species is likely a result of high food availability in the sea ice zone during the Antarctic summer and may further be driven by high spatial mobility of prey swarms. This allowed the three sympatrically breeding species to coexist despite feeding on the same resources within the same spatial area. The only inter-specific differences in foraging behaviour were linked to flight speeds and utilizing winds, likely caused by the morphological differences among the three species. These results highlight the importance of winds for the long commutes to their foraging locations. Changes in sea ice and resulting prey availability as well as changes in wind patterns that affect the ability to fly at low cost may have significant implications for this species group in the future.

## References

Dehnhard, N., Achurch, H., Clarke, J., Michel, L., Southwell, C., Eens, M. and L. Emmerson, High inter- and intraspecific niche overlap among three sympatrically breeding, closely-related seabird species: Generalist foraging as an adaptation to a highly variable environment? *Journal of Animal Ecology* 89: 104-119, 2020.

Dehnhard, N., Klekociuk, A.R. and L. Emmerson, Interactive effects of body mass changes and species-specific morphology on flight behavior of chick-rearing Antarctic fulmarine petrels under diurnal wind patterns, *Ecology and Evolution* 11: 4972–4991, 2021.

# **Eat-in or take-away? Quantification of Antarctic resident and non-resident seabirds in the pelagic ecosystem in the eastern Indian sector of the Southern Ocean**

Nobuo Kokubun<sup>1,2</sup>, Kohei Hamabe<sup>3,4</sup>, Nodoka Yamada<sup>5</sup>, Hiroko Sasaki<sup>4</sup>, Bungo Nishizawa<sup>4,5</sup>, Yutaka Watanuki<sup>5</sup>, and Hiroto Murase<sup>3,4</sup>

<sup>1</sup> *National Institute of Polar Research, Japan*

<sup>2</sup> *Graduate University of Advanced Studies (SOKENDAI)*

<sup>3</sup> *Tokyo University of Marine Science and Technology*

<sup>4</sup> *Japan Fisheries Research and Education Agency*

<sup>5</sup> *Hokkaido University*

Seabirds are among the top predators in the Southern Ocean marine ecosystem because of their high biomass and metabolic rates. While the Antarctic neritic areas, including on-shelf, slope, and adjacent sea ice zones, are the main habitat of abundant central place foraging Antarctic residents such as Adélie penguins (*Pygoscelis adeliae*) and fulmarine petrels during summer, seabird abundance in the off-shelf pelagic areas between the northern edge of the sea ice and 60°S is not well documented. In off-shelf pelagic areas, non-residents flying from sub-Antarctic breeding sites are expected to be more abundant than Antarctic residents. Given the wide range of foraging and the high mobility of non-residents, quantification of seabird abundance in pelagic areas may provide insights into ecological functions to characterize the area. This study aimed to quantify seabird abundance in pelagic areas in the data-limited eastern Indian sector of the Southern Ocean based on an at-sea observational study conducted during the 2018/19 austral summer season. We estimated food consumption by seabirds based on their biomass, conceivable field metabolic rates, number of days spent in the areas, and diet composition. Among the five functional seabird groups (penguins, albatrosses/giant petrels, shearwaters, petrels/ Charadriiformes, prions/storm-petrels), shearwaters, non-Antarctic resident, were the most dominant taxa both by abundance (15,650,000 birds) and biomass (9,300 tons) in the study area during the summer. Most of the prey consumed by all seabirds in the area was presumed to be Antarctic krill (51,500 tons) and pelagic fishes (91,100 tons), such as myctophids. Although the total food consumption by the seabirds during the summer (204,000 tons) was lower than that reported in the Antarctic neritic areas (e.g., 753,000 tons in the neighboring Prydz Bay region), the higher proportion of non-resident shearwaters in biomass and estimated food consumption (88%) were the characteristic of the study area. Less coverage of krill-rich Antarctic neritic areas in the present study could be the reason for the lower contribution of Antarctic resident species and total food consumption by seabirds found in this study. Our results suggest the characteristics of the ecological function of the study area in which seabirds consumed Antarctic krill and pelagic fishes in the upper 60 m layer of the water column, and its nutrients are transported by subcutaneous fat or stomach oil to the outside of the areas mainly towards the northeast, even to the northern Pacific.

# The choice of prey for parents own and for chicks in Rhinoceros Auklet

Jumpei Okado<sup>1\*</sup>, Motohiro Ito<sup>2</sup> and Yutaka Watanuki<sup>1</sup>

<sup>1</sup> Graduate school of Fisheries Sciences, Hokkaido University

<sup>2</sup> Department of Applied Biosciences, Toyo University

\* Present address: Salmon Research Department, Fisheries Resources Institute, Japan Fisheries Research and Education Agency

Central-place foraging theory predicts that seabird parents feed on prey species that maximize foraging efficiency (kJ/foraging effort unit) for self-feeding but on prey species that maximize energy delivery (total energy in meal-loads) for chicks. The difference of prey for parents and chicks have been in some species but not in some others. To understand the underlying factors for those prey choice in seabirds, we collected prey for parents (stomach contents) and for chicks (meal-load) from Rhinoceros Auklet *Cerorhinca monocerata* at Teuri Island in 2004–2009, 2014–2015 and Daikoku Island in 2014–2015. We categorized the extent of the difference in prey species between meal-loads and stomach contents as “same”, “partly different”, and “different” ( $n = 4–61$  per year colony). The birds at Teuri Island fed on age $\geq 1+$  anchovy both for chicks and themselves in 2004–2009 but fed on age-0 greenling and age-0 sand lance for chicks and also krills and squids adding to the two species for themselves in 2014–2015. Proportion of individuals categorized as the “same” in Teuri Island was high (67–100%) in 2004–2009 but small (8–47%) in 2014–2015. The birds in Daikoku Island fed on age-0 salmon for chicks but age-0salmon and squid for themselves in 2014–2015 so the proportion of individuals categorized as the “same” was small (0–16%). Of the 61 birds that were categorized as “partly different” or “different” (combining all year and colony), 56% brought the prey species giving higher meal-load energy to chicks. As age $\geq 1+$  anchovy maximize the meal-load energy and also increase foraging efficiency (kJ/diving time) by eight times than age-0 greenling and age-0 sand lance, the parents fed on anchovy for chicks and for themselves as well in 2004–2009 when anchovy was abundant. While in 2014–2015 when anchovy was not abundant, the parents fed on various prey species opportunistically for themselves but fed on prey species that relatively increase meal-load energy such as greenling0+ or salmon0+ for chicks. This study suggests that Rhinoceros Auklet change the strategy of prey choice for themselves and chicks depending on the availability of the most profitable prey species.

# **Biologging reveals behavioral coordination of Adélie penguins traveling in groups**

Toshitaka Imaki<sup>1</sup>, Nobuo Kokubun<sup>1,2</sup>, Kozue Shiomi<sup>3</sup>, and Akinori Takahashi<sup>1,2</sup>

<sup>1</sup> *Department of Polar Science, The Graduate University for Advanced Studies, SOKENDAI, Japan.*

<sup>2</sup> *National Institute of Polar Research, Japan.*

<sup>3</sup>*Frontier Research Institute for Interdisciplinary Sciences, Tohoku University, Japan*

Collective behavior is prevalent in wild animals and brings various benefits to members of the group. However, collective behavior can also be costly, as each individual in a group needs to coordinate their behaviors to maintain group cohesion. Documenting when and how wild animals coordinate their behavior in a group is important to understand the cost and benefit of collective behavior, but there have been limited studies on free-ranging animals due to the difficulties in the observation. Penguins are known to breed colonially, travel, and forage at sea in groups, though the dynamics and behavioral coordination of group members remain unknown.

Here, we used biologging to examine the behavioral coordination of Adélie penguins traveling in groups from the breeding colony to the land-fast sea-ice edge for foraging near the Syowa Station, East Antarctica. GPS tracking showed that three groups of seven birds (2, 2, and 3, out of 14 tracked birds) departed the colony, traveled 39.7-41.3 km for 14.8-16.7 hours, and reached the ice edge together. In all three groups, individuals adjusted their traveling speed to each other, maintaining group cohesion until reaching the ice edge. We also collected records of the 3-axis acceleration for one group of three birds, which allowed us to classify behaviors (resting, walking, or tobogganing) and examine the coordination of behaviors among group members in detail. The results showed two possibilities of mechanisms of synchronous traveling. First, penguins matched the timing of rest between individuals, suggesting that the rest of one individual initiated the rest of the others. Second, the time spent for walking/tobogganing differed among individuals, reflecting differences in locomotion speed. The individual of slower travel speed moved a relatively longer distance by tobogganing, a fast way of moving, to catch up with the group. Our results suggest that Adélie penguins continuously maintain group cohesion while traveling to the foraging site by coordinating behaviors depending on the social context.

# DEHP degrading bacteria isolated from Antarctica

Yudai Inagaki<sup>1</sup>, S. A. Ahmad<sup>2</sup> and A. Zulkharnain<sup>1</sup>

<sup>1</sup>*Department of Bioscience and Engineering, College of Systems Engineering and Science, Shibaura Institute of Technology, 307 Fukasaku, Minuma-ku, Saitama, 337-8570, Japan*

<sup>2</sup>*Department of Biochemistry, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia*

Plasticizers are widely used in the processing of plastics. Plasticizers are persistent and they are known to have an adverse effect on ecosystems when eluted from plastics into the environment. Di(2-ethylhexyl)(DEHP) is the most used plasticizer, and bacteria had been reported to degrade it recently. It was reported *Bacillus mojavensis* completely degrades DEHP in 2018<sup>1)</sup>. The purpose of this study is to isolate DEHP degrading bacteria from Antarctic soil and to investigate its chemical properties, DEHP degrading ability, and protein concentration in the medium.

DEHP was added to the mineral salt medium to a final concentration of 0.1% and incubated by shaking. As a result of the culture, isolated bacteria showed Gram-positive rod-shaped. This isolate was designated as strain HP5. HP5 showed positive response in catalase test. Furthermore, it showed negative response in oxidase test, indole test and motility test. To evaluate the biodegradation rate of DEHP, it was incubated for 168h. The number of viable bacteria was measured by the CFU counting method and the concentration of DEHP after 48h was analyzed by gas chromatography. The DEHP completely degraded after 120h. Furthermore, the number of bacteria peaked at 72h and started to decrease gradually after 120h. Protein concentrations of uninoculated medium were measured at 0.01551µg/mL, and that of HP5 inoculated medium were measured at 0.2171µg/mL. These results confirmed that the strain HP5 has the ability to degrade DEHP.

## References

- 1) Jingfan Zhang, Chengnan Zhang, Yunping Zhu, Jinlong Li, Xiuting Li, (2018), Biodegradation of seven phthalate esters by *Bacillus mojavensis* B1811, International Biodeterioration & Biodegradation 132, 200-207, doi:10.1016/j.ibiod.2018.04.006



# Bacterial microbiota associated with *Umbilicaria* spp. lichens in continental and maritime Antarctic, Arctic and Alpine regions

Zichen He<sup>1</sup>, Takeshi Naganuma<sup>1</sup>, Ryosuke Nakai<sup>2</sup>, Megumu Tsujimoto<sup>3</sup>, Hiroshi Kanda<sup>4</sup>, Satoshi Imura<sup>4</sup>, Jun Uetake<sup>5</sup>, Martin W. Hahn<sup>6</sup>, and Peter Convey<sup>7</sup>

<sup>1</sup> Graduate School of Integrated Sciences for Life, Hiroshima University, Higashi-hiroshima, 739-8528, Japan

<sup>2</sup> Bioproduction Research Institute, National Institute of Advanced Industrial Science and Technology, Sapporo, Japan

<sup>3</sup> Faculty of Environment and Information Studies, Keio University, Fujisawa, 252-0882, Japan

<sup>4</sup> Bioscience Group, National Institute of Polar Research, Tachikawa, 190-8518, Japan

<sup>5</sup> Field Science Center for Northern Biosphere, Hokkaido University, Sapporo, 060-0811, Japan

<sup>6</sup> Research Department for Limnology, University of Innsbruck, Mondsee, A-5310, Austria

<sup>7</sup> British Antarctic Survey, High Cross, Madingley Road, Cambridge, CB3 0ET, UK

Lichens are common and widely distributed symbiotic organisms. Increased research attention is being given to the bacterial diversity associated with lichens. This study aimed to analyze the bacterial diversity and their physiological or ecological roles in the lichen symbiosis using culture-independent phylogenetic analyses.

A total of 44 lichen samples were obtained from continental and maritime Antarctic, Arctic and Alpine regions. In the two Antarctic regions, 18 samples were obtained from the continental Antarctic (Syowa station) and four samples from the maritime Antarctic (Signy Island); in the Arctic region, two samples were obtained from Kugluktuk (Canada), two from Gamvik (Norway), and five from Enotekio and Levi (Finland); in the Alpine region, five samples were obtained from the Eastern Alps (Austria) and 11 from the Rwenzori Mountains (Uganda). All the sampled lichen-forming fungi were confirmed to be affiliated with the ascomycete genus *Umbilicaria* through Sanger sequencing.

Lichen-associated bacterial diversity was assessed using Illumina MiSeq targeting the V3-V4 region of the bacterial 16S rRNA gene. A total of 2,038,816 reads were obtained for analysis. Phylotypes (OTUs) were classified using a 97% similarity cutoff. 1,028,426 reads were obtained from Antarctic samples, 439,303 from Arctic samples and 571,087 from Alpine samples. In Antarctic samples, the average number of reads per sample was 46,747 representing an average of 478 OTUs; in Arctic samples, these values were 48,811 and 254, and in Alpine samples 35,693 and 426. Bacterial phylum composition identified from samples from Syowa Station was distinctly different from the other sampling regions. The dominant phylum in the 18 Syowa samples was *Bacteroidota* (66.46%), while in the 26 samples from other regions (including Antarctic Signy Island) it was *Pseudomonadota* (51.65%). This difference was confirmed using Principal Component Analysis. The assigned OTUs included four cosmopolitan taxa at species rank. These four OTUs affiliated with *Gluconacetobacter* or new genera of the *Acetobacteraceae* family, which is known to include nitrogen-fixing members.

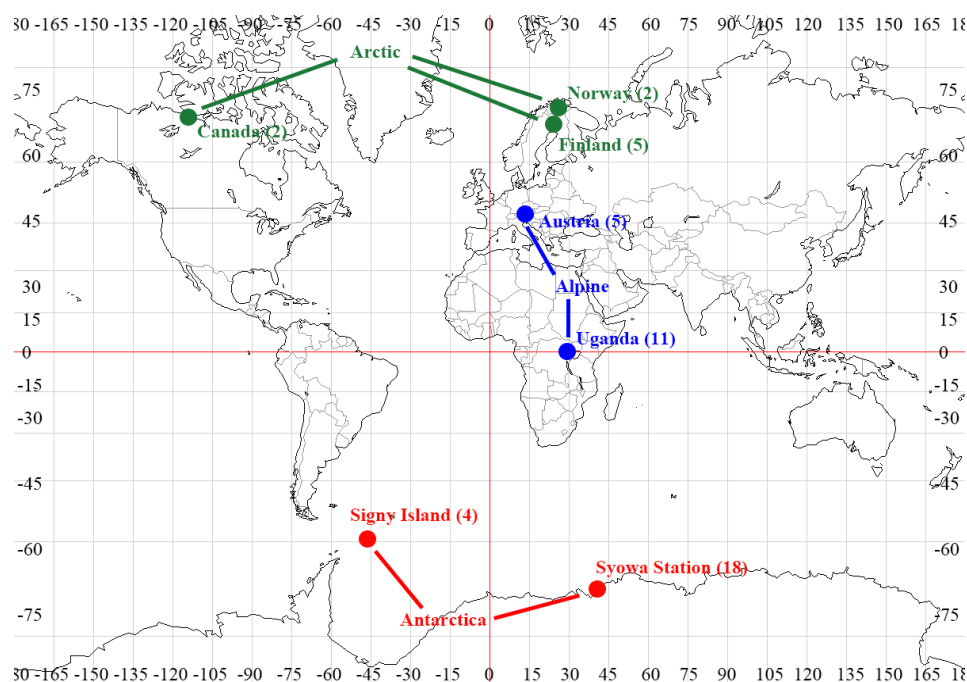


Figure 1. Lichen sampling locations in Arctic (green), Alpine (blue) and Antarctic (red) regions, indicating the number of samples obtained at each location.

## **Meteorological features of Syowa station and ice-free areas for bryophyte ecosystem.**

Shunichi Arai<sup>1</sup>, Satoshi Imura<sup>1,2</sup>, Koyomi Nakazawa<sup>3</sup>, Tomotake Wada<sup>1</sup>, Sakae Kudoh<sup>1,2,4</sup>

1) *Department of Polar Science, SOKENDAI (The Graduate University for Advanced Studies)*

2) *National Institute of Polar Research, Research Organization of Information and Systems*

3) *Department of Environmental and Civil Engineering, Toyama Prefectural University*

4) *Office of Strategic Planning, Research Organization of Information and Systems*

Ice-free areas around Syowa station, Langhovde, Skarvsnes, and Skallen harbor prosperous bryophyte colonies. Bryophyte colonies in Langhovde are known as Antarctic Specially Protected Area (ASPA) 147. Meteorological conditions strongly affect the growth and reproduction of bryophyte. Hence, National Institute of Polar Research (NIPR) installed Automatic Weather Station (AWS) in each ice-free area and published the recorded meteorological data until 2018. 63rd Japanese Antarctic Research Expedition (JARE) member retrieved the AWSs' data recorded from August 2019 until January 2022. We analyzed the data recorded by each AWS and the data at Syowa station reported by Japan Meteorological Agency. We will discuss the relationship between meteorological conditions and bryophyte communities in the session.

# Characterization of Polylactic Acid Degrading Bacterium strain N-3 from Antarctic Soil

Kenta Sato<sup>1</sup>, S. A. Ahmad<sup>2</sup> and A. Zulkharnain<sup>1</sup>

<sup>1</sup>Department of Bioscience and Engineering, College of Systems Engineering and Science, Shibaura Institute of Technology,  
307 Fukasaku, Minuma-ku, Saitama, 337-8570, Japan

<sup>2</sup>Department of Biochemistry, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400 UPM  
Serdang, Selangor, Malaysia

Plastic is widely used in our daily life. However, it is difficult to be degraded in the natural environment and remains persistent for an extended period of time. In this environmental problem, ecologically friendly bioplastics have been attracting a lot of attention as these categories of plastics can be degraded by microorganisms. To date, several strains of bacteria including psychrophilic have been reported to possess the ability to degrade different types of plastics<sup>1</sup>). Polylactic acid (PLA) is a type of bioplastic that is synthesized from biological resources and is biodegradable. It is used for prosthetic devices and food packaging due to its biologically safe characteristic. In addition, its strength and functionality are being studied to improve its usability as a plastic. Previously, we reported on *Massilia* (Naxibacter) sp. N-3 strain isolated from Antarctic soil sample, collected from Signy Island, with confirmed ability to degrade PLA. Strain N-3 degraded PLA granules for four weeks. The objective of this study was to investigate the biochemical, microbiological, and genetic properties of *Massilia* sp. N-3 strain, in order to characterize the strain and elucidate the mechanism of PLA degradation.

Comparison of growth at different incubation temperatures revealed that strain N-3 was not a psychrophilic bacterium but a psychrotolerant. The genomic DNA of this bacterium was extracted and whole genome sequencing was performed. Sequencing was performed on Illumina Hiseq and PacBio RS II platforms, and assembly results revealed the sequence to have a length of 5.58 Mbp, an average coverage of 136.05, and a GC content of 65.44%. The gene annotation results that it had 5187 genes (including RNA genes) and identified eight genes, including PHB depolymerase and hydrolytic enzymes, as PLA-degrading genes. In addition, three sequences were annotated as unknown genes belonging to alfa/beta hydrolases. Three and seven genes were identified as esterases and proteases, respectively, which are known enzymes that degrade PLA. Besides, genes for enzymes that assimilate lactic acid, a metabolite of PLA, were identified. Strain N-3 was found to possess dehydrogenase genes that metabolize L- and D-body lactic acid, respectively. These results indicated that strain N-3 has complete genes to metabolizes PLA.

In the future, we will use RT-PCR to confirm whether this enzymatic expression during PLA degradation, and conduct functional analyses of these genes using *E. coli* as host.

## References

### References

1)Atanasova, N.; Stoitsova, S.; Paunova-Krasteva, T.; Kambourova, M. Plastic Degradation by Extremophilic Bacteria. *Int. J. Mol. Sci.* **2021**, *22*, 5610. DOI: 10.3390/ijms22115610

# Applying ATR-FTIR spectroscopy to Characterize and Discriminate Tundra Plant Leaves

Takashi Osono<sup>1</sup>, Weitong Lin<sup>1</sup>, Motohiro Hasegawa<sup>1</sup>, Masaki Uchida<sup>2</sup>

<sup>1</sup>*Faculty of Science and Engineering, Doshisha University, Kyotanabe, Kyoto 610-0394, Japan*

<sup>2</sup>*National Institute of Polar Research, Tachikawa, Tokyo 190-8518, Japan*

The chemical property of leaves is an important aspect of functional trait of tundra plants as it represents the biochemical features explaining not only their response to environmental change but also their effects on ecosystem function. Understanding chemical properties of leaves is thus crucial for the understanding of arctic terrestrial ecosystems, where plant growth is strongly limited by such environmental factors as low temperature, low moisture, low nutrient availability, and a short growing season. The attenuated total reflection Fourier transform infrared (ATR-FTIR) spectroscopy is a powerful tool of investigating functional traits of plants, but its applicability to tundra plant leaves was yet to be addressed.

The purpose of the present study was to apply the ATR-FTIR measurement to characterize biochemical fingerprint of tundra plant leaves and to discriminate it between plant species. We used a total of 50 samples each for live and dead leaves from 14 plant species of shrubs, forbs, graminoids, and mosses collected in the proglacial field of the southern front of Arklio Glacier in the Kreiger Mountains near Oobloyah Bay, Ellesmere Island, Nunavut, Canada (Osono et al., 2006). The ATR-FTIR measurement allows complex assemblages of organic constituents to be displayed as distinctive spectral features in the mid infrared range (4000-400 cm<sup>-1</sup>). Fundamental vibration modes of specific functional groups yield characteristic spectral absorption features that provide comprehensive biochemical fingerprint of various organic compounds in leaf samples (Silverstein et al., 2012).

The ATR-FTIR spectra in the fingerprint region of live and dead leaves from 14 tundra plant species of shrubs, forbs, graminoids, and mosses showed a variability in overall appearance between plant species and a degree of similarity between live and dead leaves of the same plant species. At least 16 peaks were obvious in the spectra of live and dead leaves of 14 plant species, 5-9 peaks per plant species. Of these, four highest peaks were found between 1575 and 1637 cm<sup>-1</sup>, 1406 and 1452 cm<sup>-1</sup>, 1313 and 1325 cm<sup>-1</sup>, and 1022 and 1058 cm<sup>-1</sup> and are attributed to chemical features of lignin, cellulose, and/or oxalate. The overall spectra in the fingerprint region were significantly different between plant species both for live and dead leaves [one-way permutational multivariate analysis of variance (PERMANOVA), d.f. =13, P<0.001].

Cluster and principal component analyses showed that leaves of *Oxyria digyna* and other forbs had distinctive spectral characteristics attributable to the content of oxalate and other putative compounds. The spectra of shrubs had greater values of relative height at 1575 and 1637 cm<sup>-1</sup> and 1406 and 1452 cm<sup>-1</sup> than those of graminoids and mosses. This difference is due to the fact that contents of lignin relative to cellulose were generally greater in shrubs than in graminoids and mosses.

In conclusion, the ATR-FTIR spectroscopy can detect a suite of organic components that characterize live and dead leaves of tundra plant species. Such spectral measurements can be used as a tool to describe functional traits of plants (Mori et al., 2017). The ATR-FTIR measurement will also be potentially useful in documenting the process of microbial decomposition and soil formation in arctic tundra. Future studies using the ATR-FTIR spectroscopy are needed for tundra plants in polar regions to characterize the process of decomposition of plant leaves and depolymerization of organic constituents.

## References

- Osono, T., Mori, A., Uchida, M., Kanda, H., 2006. Chemical property of live and dead leaves of tundra plant species in Oobloyah Valley, Ellesmere Island, high arctic Canada. *Mem. Natl. Inst. Polar Res. Spec. Issue* 59, 144-155.
- Silverstein, R.M., Webster, F.X., Kiemle, D.J., Bryce, D.L., 2012. *The Spectrometric Identification of Organic Compounds*, 8th edition. Wiley, New York.
- Mori, A.S., Osono, T., Cornelissen, H., Craine, J., Uchida, M., 2017. Biodiversity-ecosystem function relationships change through primary succession. *Oikos* 126, 1637-1649.

# Microbial diversity around Syowa Station in 2021

Sakae Kudoh<sup>1,2,3</sup>, Shu Kuan Wong<sup>1</sup>, Kenichi Watanabe<sup>1</sup>, Mizuho Mori<sup>1</sup> and Koyomi Nakazawa<sup>4</sup>

<sup>1</sup>National Institute of Polar Research

<sup>2</sup>SOKENDAI

<sup>3</sup>Office of Strategic Planning, ROIS

<sup>3</sup>Toyama Prefectural University

Bulk DNA extracted from the soil samples collected around Syowa Station as a soil monitoring study in the 63rd Japanese Research Expedition (Fig. 1) was used to amplify the V3-V4 region of the 16S rRNA gene, and they were MiSeq-sequenced. The V3-V4 sequences were grouped into ASVs (amplicon sequence variants), and bacterial flora and their diversity against the distance and direction from the center of the Syowa Station were compared.

## Important Results

**Variation Partitioning analysis:** Direction accounts for almost 15.7% of the variations in the bacterial community structure; while distance of sampling explained 12.7% of the variation in bacterial community structure. 74.5 % of the variation in the bacterial community structure were still unexplained (affected by other factors not included in this study).

**Principal Component Analysis (PCA) Plot based on Bray-Curtis Distance** indicates samples collected 100 m from the center of the Station and 1400 m or more far from the Station were plotted together in different positions each other, means soil bacterial diversity near the station and far from the Station differ (Figure 2).

**Alpha diversity (summarized by direction and by distance):** Indexes of alpha diversity summarized to direction of samples indicated lower diversity for samples collected from ESE, N, NE & NNW where activity of expeditioners are relatively high (Machine maintenance building, oil tanks, Power plant building, etc.), and diversity summarized to distance followed a Bell curve pattern, increasing from 100m until it peaks at 800m and dropping thereof as the distance increases until 1800m, also supported bacterial diversity seemed to poor in active areas near the center of the Station, but it also took poor values at southern edge areas where almost no human activity in the island.

Based on ASVs, phylum, class, order, and family level of bacterial dominance will be listed in the poster.

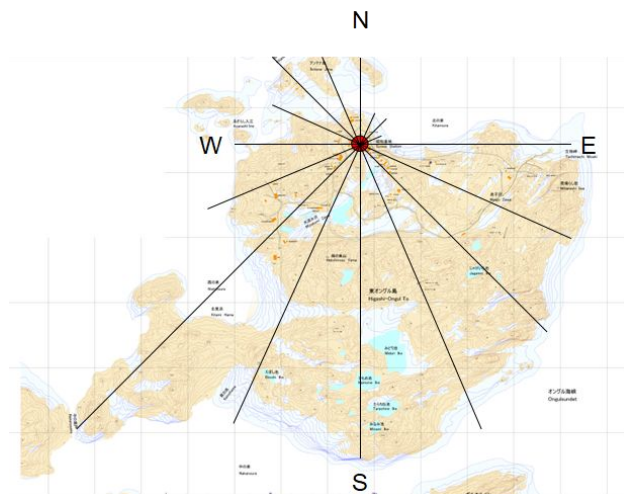


Figure 1. Map of East Ongul island and axis of soil sampling points.

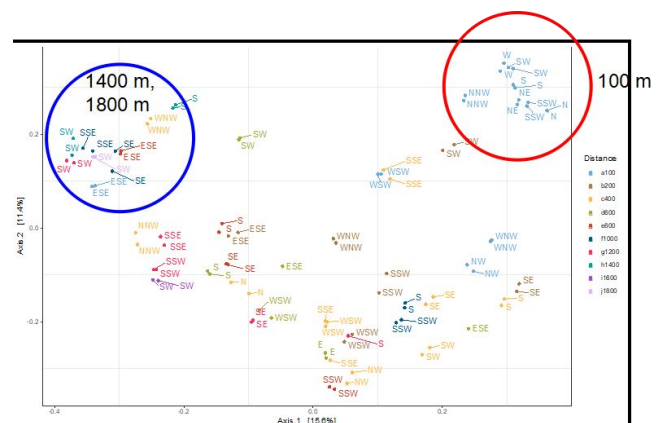


Figure 2. PCA Plot based on Bray-Curtis Distance

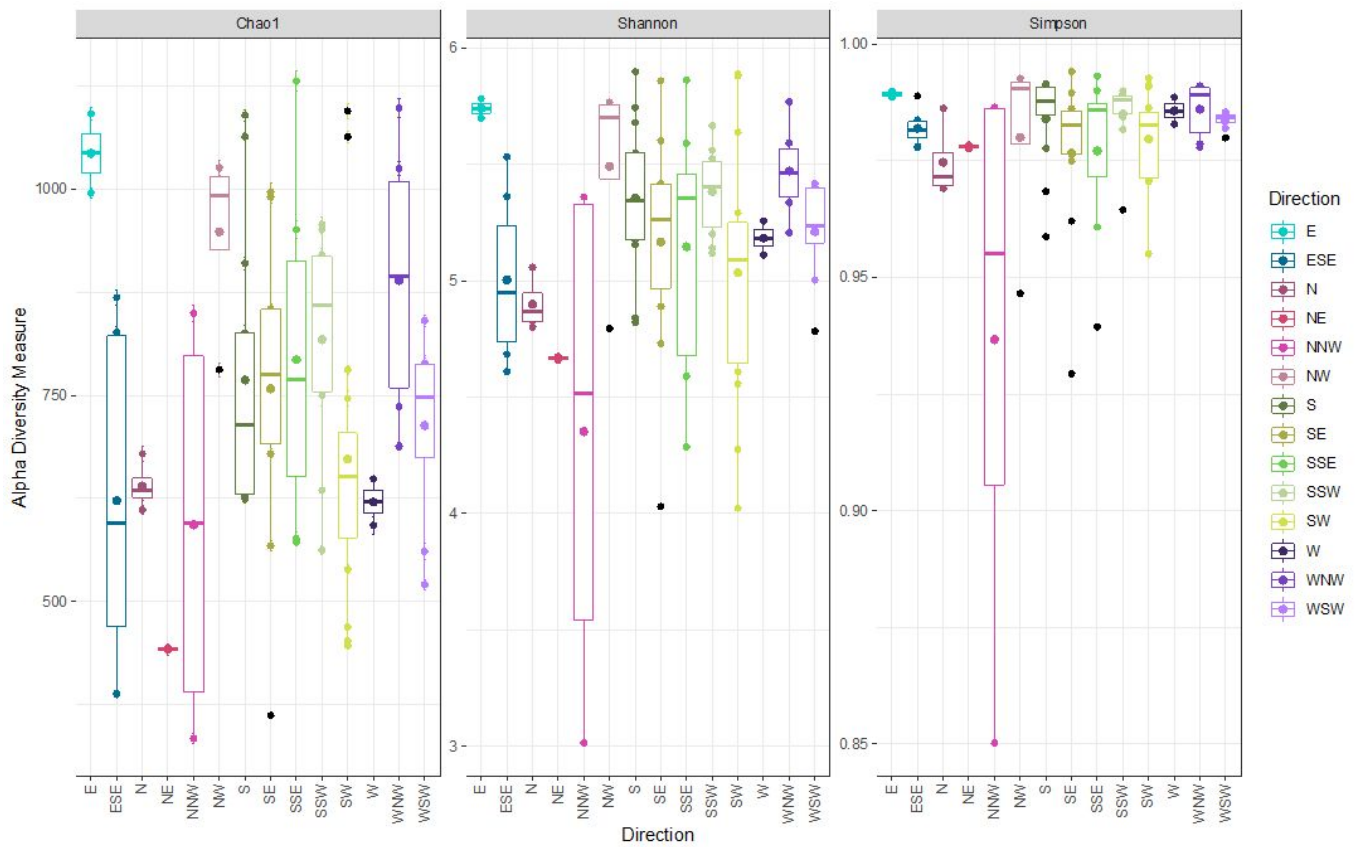


Figure 3, Alpha diversity summarized by sampling direction

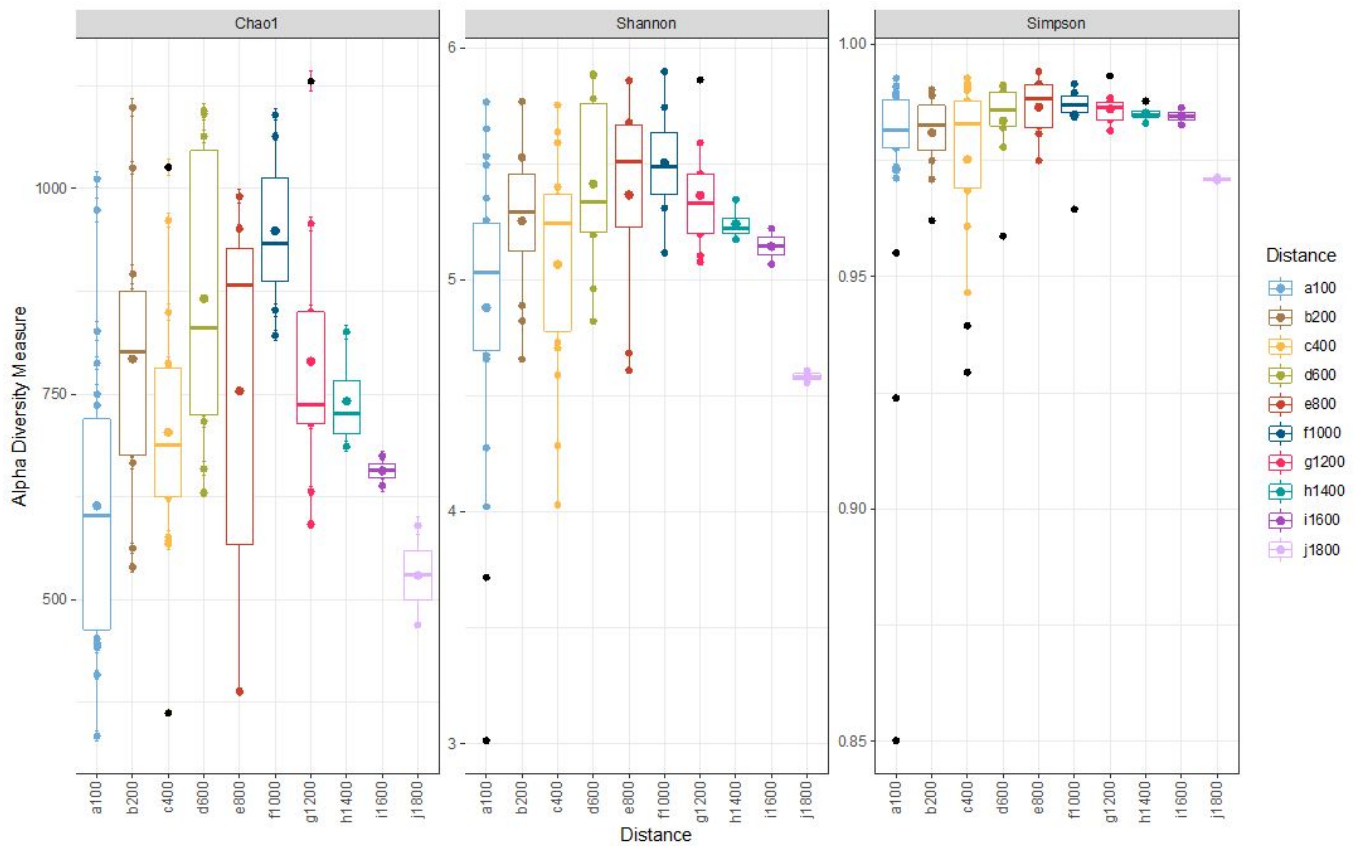


Figure 4. Alpha diversity summarized by sampling distance

# Outbreaks of bdelloid rotifers in and at adjacent wetland of a marine relict lake, Mago Ike on Soya Coast, East Antarctica: climate and limnological conditions.

Sakae Kudoh<sup>1,2,3</sup>, Kunio T. Takahashi<sup>2,3</sup> and Tomotake Wada<sup>3</sup>

<sup>1</sup>*Office of Strategic Planning, Research Organization of Information and Systems*

<sup>2</sup>*National Institute of Polar Research*

<sup>3</sup>*SOKENDAI*

Outbreaks of bdelloid rotifers have been sometimes reported by Antarctic researchers, but almost all cases lack scientific description of environmental condition. We found spotty congregation of red-colored rotifers in a wetland adjacent to a lake, Mago Ike in December 2013, and started limnological surveys to 27 January 2014. The congregated rotifers were recognized visually during our study period at the wetland, and another congregated outbreak was noticed on the biofilms at rocky littoral zone of the west coast of the lake on 27 January. In December, the wetland was already fed by seepage of snow melt and a small stream, and rusty colored biofilms and microbial mat had been developed on the floors of the wetland and stream (Plate 1). However, the lake was covered by ice during in December, the ice disappeared during twice windy days in late December. Water temperature rose gradually from *ca.* 1°C up to 12°C in mid-Jan, and decreased to 6°C in late Jan. Water of the stream and the wetland contained relatively higher inorganic nutrients such as nitrate and silicate than the lake water, while the lake water contained higher chlorophyll *a* and organic matters. Disturbance by the ice cover which scraped shallow bottom of the lake occurred when it develops, breaks and moves, may affect and delay the development of microbes such as bacteria and autotrophs which can be expressed chlorophyll *a* standing stock on shallow lake floors; it was *ca* 5 times smaller at lake littoral zone than in the stream. By late Jan. biofilm developed and it covered on shallow rocky littoral zone, and the second outbreak of rotifers was noticed on the biofilm surface at western shore of the lake (Plate 1). In this stage, we cannot directly evaluate why the rotifer could proliferate on the wetland and the lake in this summer, however, we expect further studies on growth and life cycle property of rotiferes against on the present environmental features might give answers.

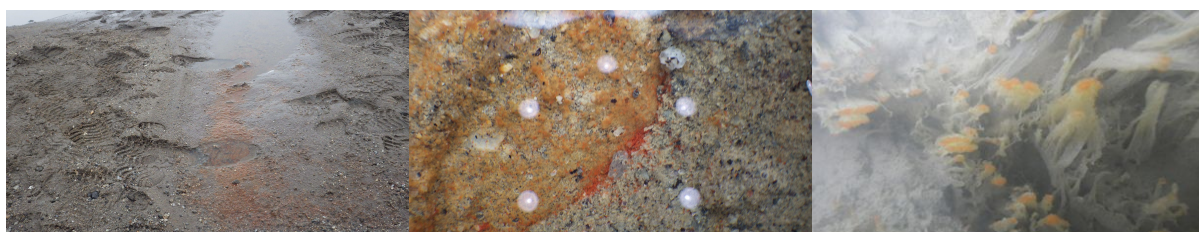


Plate 1 Biofilms developed in wetland (left), congregation of red-colored rotifers in a shallow paddle (middle), and on epilithic biofilm (right)

Table 1 Weather features of several summer seasons at Kizahashi Hama, Skarvsnes, Soya Coast.

The first day appeared above 0°C in each summer, number of days above 0°C, and the integrated temperature above 0°C

	2010/2011	2011/2012	2012/2013	2013/2014	2014/2015
JARE* term	52nd	53rd	54th	55th (present study)	56th
The first day >0°C	2010/11/6	2011/12/12	2012/11/10	2013/11/10	2014/12/3
Days >0°C	49	34	68	58	38
Integrated temperature >0°C	110.8	60.1	158.9	92.5	61.7

## References

Wada et al., Abundance and biomass of bdelloid rotifers in the microbial mats from East Antarctica; the ecological relations between microscopic phototrophs and invertebrates Ecological Research, *in press* , 2022.



# **Bacterial community structures of environmental sample and enrichment cultures of the hyper-saline lake Zakuro in the Langhovde, East Antarctica.**

Kaori Iiyama<sup>1</sup>, Hanae Seki<sup>2</sup>, Akinori Kawamata<sup>3</sup>, Satoshi Imura<sup>4, 5</sup>, Hiroyuki D. Sakai<sup>2</sup> and Norio Kurosawa<sup>1, 2</sup>

<sup>1</sup>*Graduate School of Science and Engineering, Soka University*

<sup>2</sup>*Faculty of Science and Engineering, Soka University*

<sup>3</sup>*Ehime Prefectural Science Museum*

<sup>4</sup>*National Institute of Polar Research*

<sup>5</sup>*The Graduate University for Advanced Studies (SOKENDAI)*

Antarctica has the greatest diversity of lake types on Earth (Laybourn-Parry & Pearce, 2016; Pickard et al., 1986). Indeed, many lakes, consisting of various salinity, area, and depth, are located in Antarctica. These lakes have been considered low primary production because of the low temperature and significant periods of ice formation. Therefore, (micro)organisms in these lakes were generally considered oligotrophic with low biodiversity. However, decades of research have revealed that considerably diverse prokaryotes exist in those lakes (Vincent et al., 2008; Kurosawa et al., 2010; Laybourn-Parry & Pearce, 2016; Chaya et al., 2019). Lake Zakuro is a marine relic lake located in the Langhovde, East Antarctica, which possesses unique physicochemical parameters (i.e., hyper-saline, oligotrophic, and low temperature) that are not observed in temperature zones. Therefore, Bacteria in Lake Zakuro probably have unique physiology and ecology to adapt to this harsh environment. This study examined the bacterial community structures of environmental sample and enrichment cultures derived from Lake Zakuro by 16S rRNA gene amplicon sequence analyses.

Lake water sample, including the bottom mud, was collected on January 22, 2013. At that time, the water temperature and pH were 9.9°C and 8.0, respectively. To reveal the bacterial community structure in Lake Zakuro, DNA was directly extracted from the sample and used for the 16S rRNA amplicon sequence analysis. The representative sequences were then applied to the Basic Local Alignment Search Tool (BLAST) of the National Center for Biotechnology Information for species identification. To find out what species of bacteria detected in the lake sample could be cultured, enrichment cultivations were conducted. The lake water sample was inoculated into the enrichment medium (pH 8.0) consisting of artificial seawater (salinity 80‰), 1xMBS (Kurosawa et al., 1998), and yeast extract (0.1%). Incubation temperatures were set at 5, 10, 15, 20, and 25°C. After reaching the stationary phase, the cultures were centrifuged, and the enriched microbial DNAs were extracted, followed by the 16S rRNA amplicon sequence analyses.

The bacterial Community in Lake Zakuro consisted of 19 phyla and was further classified into 179 genera. The most frequently detected phylum was Proteobacteria (39% of total sequences), in which  $\alpha$ -Proteobacteria was mostly dominant (66% in the phylum), followed by  $\gamma$ -Proteobacteria (33%). Oligoflexia,  $\beta$ - and  $\delta$ -Proteobacteria were also detected as minor components (<1%). This proteobacterial composition has the characteristics of marine bacteria. Other than Proteobacteria, phyla Bacteroidetes (19%), Verrucomicrobia (14%), Cyanobacteria (14%), Planctomycetes (2.0%), Actinobacteria (1.7%), and Campylobacterota (1.2%) were also detected. In addition, four phyla belonging to the Candidate Phyla Radiation (CPR) were detected at 1.4%. The CPR is a supergroup of uncultured enigmatic species that desired to be cultured and characterized in detail. As a result of enrichment cultivation, the growth of microorganisms was confirmed in the cultures at all temperatures. Based on the BLAST analysis, bacterial sequences in all the cultures were classified into four phyla, 30 genera, and 57 species. Thirty of the 57 species showed less than 98.2% homology to the described species, suggesting that these species are novel bacteria. However, their detection frequencies were at a maximum of 1.8%. It may be difficult to separate them from the enrichment cultures by the extinction dilution method. As mentioned above, 179 genera were detected in the environmental sample, but only 30 were detected in the enrichment cultures. In this enrichment, we used a single culture medium under aerobic conditions and changed only the temperature. To enrich the novel bacteria detected in Lake Zakuro to a much higher percentage, it is necessary to perform enrichment cultivation under more conditions with various media types.



## References

- Chaya, A., Kurosawa, N., Kawamata, A., Kosugi, M. and Imura, S., Community structures of bacteria, archaea, and eukaryotic microbes in the freshwater glacier lake Yukidori-Ike in Langhovde, East Antarctica. *Diversity*, 11(7), 105, 2019.
- Kurosawa, N., Itoh, Y. H., Iwai, T., Sugai, A., Uda, I., Kimura, N., Horiuchi, T. and Itoh, T. *Sulfurisphaera ohwakuensis* gen. nov., sp. nov., a novel extremely thermophilic acidophile of the order Sulfolobales. *Int. J. Syst. Bacteriol.*, 48(2), 451, 1998.
- Kurosawa, N., Sato, S., Kawarabayasi, Y., Imura, S. and Naganuma, T., Archaeal and bacterial community structures in the anoxic sediment of Antarctic meromictic lake Nurume-Ike. *Polar Science*, 4(2), 421-429, 2010.
- Laybourn-Parry, J. and Pearce, D., Heterotrophic bacteria in Antarctic lacustrine and glacial environments. *Polar Biology*, 39(12), 2207-2225, 2016.
- Pickard, J., Adamson, D. A. and Heath, C. W., The evolution of Watts Lake, Vestfold Hills, East Antarctica, from marine inlet to freshwater lake. *Palaeogeography, Palaeoclimatology, Palaeoecology*, 53(2-4), 271-288, 1986.
- Vincent, W.F. and Laybourn-Parry (Eds.), J., *Polar lakes and rivers: limnology of Arctic and Antarctic aquatic ecosystems*. Oxford university press, 79, 2008.

# Seasonal changes of chytrid infection of glacier algae in Alaska

Kino Kobayashi <sup>1</sup>, Nozomu Takeuchi <sup>2</sup> and Maiko Kagami <sup>3</sup>

<sup>1</sup> Graduate school of Science and Engineering, Chiba University

<sup>2</sup> Graduate school of Science, Chiba University

<sup>3</sup> Faculty of Environment and Information Sciences, Yokohama National University

Recent darkening of the glacier surface has accelerated the melting rate of the ice. One of the reasons for the darkening is blooming of glacier algae, which have dark-colored pigments in their cells. The blooming of glacier algae is likely controlled not only by environmental conditions of the glacier surface, but also by chytrid fungus infections. Chytrids are a group of fungi which produce zoospores having flagellum. Parasitic chytrids are known to have a great impact on aquatic ecosystems, controlling population dynamics of host species and sometimes causing host extinction (Kagami et al., 2006). Yet, the impacts of parasitic chytrids on glacier algae and ecosystems are still unclear. We have revealed that the prevalence of chytrid infection on glacier algae, *Ancydonema nordenskioldii* (Fig.1), was higher in cryoconite holes than on ice surface. However, it is unknown how the prevalence of infection changes throughout the melting period. The purpose of this study is to describe the seasonal changes of chytrid infection of glacier alga in summer (June, August, and September) in cryoconite holes and on ice surface on the bare ice surface of Gulkana Glacier in Alaska.

Microscopic observation revealed there were chytrids infection glacier algae throughout three months (Fig.2). In June, chytrids infection started as soon as the algae appeared on the ice surface. In August, the prevalence of infection was 21.8% in cryoconite holes, and continued to be high in September. On the other hand, the prevalence of infection on the ice surface significantly increased from August (9.1%) to September (26.7%). This is probably due to increasing of melting water on the ice surface in which chytrid zoospores actively move to find the host algal cells. Increases of melting water and prevalence of infection may have been caused by the collapses of cryoconite holes and/or the changes of physico-chemical conditions on the ice surface due to frequent covering of new snow. Furthermore, algal conditions may have influenced the prevalence of infection. The filament length of *A. nordenskioldii* significantly differed between cryoconite holes and the ice surface. In cryoconite holes, most of the algal filament consisted of a single cell, which had higher infection rate in August and September. On the other hand, on the ice surface, the filament length was longer, and increased from June to August and decreased from August to September. The prevalence of infection was higher for short filament on the ice surface as well. These results indicate that chytrid infection occurred throughout three months of the melting period on the ice surface but accelerated in the late of the season and caused the shortening of algal filament.



Figure.1 Glacier algae *Ancydonema nordenskioldii*.

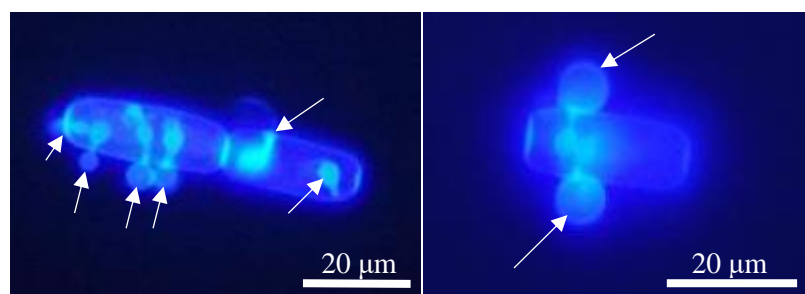


Figure.2 Chytrids infecting glacier algal cells under a fluorescence microscope.

All arrows indicate chytrid fungi.

## References

Maiko Kagami, Tek Bahadur Gurung, Takehito Yoshida and Jotaro Urabe, To sink or to be lysed: Contrasting fate of two large phytoplankton species in Lake Biwa, Limnology and Oceanography 51, 2775-2786, 2006.

# Influences of ground moss cover and summer precipitation on the occurrence of tar spot disease of polar willow in Ny-Ålesund, Spitsbergen Is., Norway

Motoaki Tojo<sup>1</sup>, Masahiro Suzuki<sup>1</sup>, Shota Masumoto<sup>2</sup>, Masaki Uchida<sup>3</sup>

<sup>1</sup>Graduate School of Agriculture, Osaka Metropolitan University, Japan

<sup>2</sup>University of Tsukuba, Faculty of Life and Environmental Sciences, Japan

<sup>3</sup>National Institute of Polar Research, Japan

## Introduction and Objectives

Plant pathogens commonly occur on mosses and vascular plant species in the polar regions (Tojo and Newsham 2012). Tar spot disease caused by *Rhytisma polare* is commonly found on leaves of polar willow (*Salix polaris*) in Ny-Ålesund, Spitsbergen Is., Norway (Masumoto et al. 2014). *R. polare* has been known to have a large impact on the host carbon balance under the natural polar ecosystem (Masumoto et al. 2018a). Since *R. polare* requires the wet conditions of the host plant leaves for the dispersion of the ascospores (Masumoto et al. 2018b), keeping the wet leaves is important for the occurrence and development of the disease. During the long-term field surveys of plant pathogens in Ny-Ålesund (Tojo and Nishitani 2005; Tojo et al. 2021), we found that tar spot disease highly occurred when the host plant was grown on the ground covered with mosses which can keep the wet condition required for the disease occurrence. The objective of this study was to clarify the influences of ground moss cover on the occurrence of tar spot disease of polar willow. Summer precipitation which provides wetting of the host plant leaves was also evaluated on the influence of the disease occurrence.

## Materials and Methods

The experiment was conducted at the north side cliff in Ny-Ålesund, Spitsbergen Is. from July to August of 2008, 2010, 2012, 2013, 2016, 2018, and 2022. Fifteen plots, each consisting of a 15 x 15 cm square containing up to 223 shoots of polar willow, were examined for the number of plant shoots that had diseased leaves. The ground covering mosses were examined for each plot on the percentage of ground area covered by moss colonies consisting mainly of *Sanionia uncinata* and *Orthothecium* sp.

## Results

Positive correlations were observed between tar spot occurrence and the moss-covered area in all three observation years with coefficients of 0.85, 0.59, 0.68, and 0.54 for 2008, 2010, 2012, and 2018 respectively. The disease occurrence increased with increasing the moss-covered area in the other years investigated, although the positive correlations were unclear in these years. The disease showed high occurrence when the summer precipitation was increased.

## Conclusion

The results demonstrated that the ground moss cover and summer precipitation influenced the occurrence of tar spot disease of polar willow in Ny-Ålesund, Spitsbergen Is. The ground moss cover was thought to keep the wet condition required for the disease occurrence. Since the distribution of moss communities and precipitation patterns are changing year by year in Ny-Ålesund (Ren et al. 2021), more long-term monitoring is needed to have a better understanding of the occurrence factors of tar spot disease.

## References

- Masumoto S, Tojo M, Uchida M, Imura S (2014) *Rhytisma polaris*: Morphological and molecular characterization of a new species from Spitsbergen Island, Norway. *Mycological Progress* 13:181–188.
- Masumoto S, Uchida M, Tojo M, Herrero ML, Mori AS, Imura S (2018a). The effect of tar spot pathogen on host plant carbon balance and its possible consequences on a tundra ecosystem. *Oecologia* 186:843–853.
- Masumoto S, Tojo M, Imura S, Herrero ML, Uchida M (2018b) Occurrence pattern of the parasitic fungus *Rhytisma polare* (Ascomycota) on the polar willow (*Salix polaris*) under limited water conditions in a high-Arctic semi-desert. *Polar Biology* 41:1105–1110.
- Tojo M, Nishitani S (2005) The effects of the smut fungus *Microbotryum bistortarum* on survival and growth of *Polygonum viviparum* in Svalbard, Norway. *Canadian Journal of Botany* 83:1513–1517.
- Tojo M, Newsham KK (2012) Snow moulds in polar environments. *Fungal Ecology* 5:395–402
- Tojo M, Fujii N, Yagi H, Yamashita Y, Tokura K, Kida K, Hakoda A, Herrero ML, Hoshino T, Uchida M (2021) Identification and isolation pattern of *Globisporangium* spp. from a *Sanionia* moss colony in Ny-Ålesund, Spitsbergen Is., Norway from 2006 to 2018. *Microorganisms* 9:1912.
- Ren G, Wang J, Lu Y, Wu P, Lu X, Chen C, Ma Y (2021) Monitoring changes to Arctic vegetation and glaciers at Ny-Ålesund, Svalbard, based on time series remote sensing. *Remote Sensing* 13:3845.

# Tooth morphologies of *Pusa* and *Phoca* seals and its relation to their diet

Uno Ishihara<sup>1</sup>, Yuuki Watanabe<sup>1,2</sup>

<sup>1</sup>Department of Polar Science, The Graduate University for Advanced Studies, SOKENDAI, Tokyo, Japan

<sup>2</sup>National Institute of Polar Research, Tokyo, Japan

Tooth shape is often related to its diets in many animals. The relationships between tooth morphology and diets are well studied for Antarctic seals but are poorly known for seals that inhabits in the northern hemisphere. In this study, we address this gap by examining the tooth morphology of *Phoca* and *Pusa* spp., which have different feeding ecology. We hypothesized that tooth jaggedness would be higher in the species that consume greater amount of zooplankton than those who consume exclusively on fish. To examine this hypothesis, maxilla and mandible were photographed using museum skull samples. In three species of genus *Pusa*, tooth bluntness increased while tooth jaggedness decreased with age. Of the three species of genus *Pusa*, two species showed an increase in tooth gap with age, while the rest depicted no significant relationship between tooth gap and its age. As such, a trend of decreasing jaggedness with age was observed in the three *Pusa* species, but regardless of age, the jaggedness of baikal seal (*Pusa sibirica*) was higher than that of the other two species. Zooplankton consumer, genus *Pusa* had relatively high tooth jaggedness, whereas genus *Phoca*, fish consumers, had relatively small tooth gap size. Despite this propensity, baikal seal showed explicitly higher jaggedness than other *Pusa* group, and had slightly greater tooth gap as *Phoca* group had. Overall, our result highlights a unique tooth morphology of baikal seal compared to its relative species.

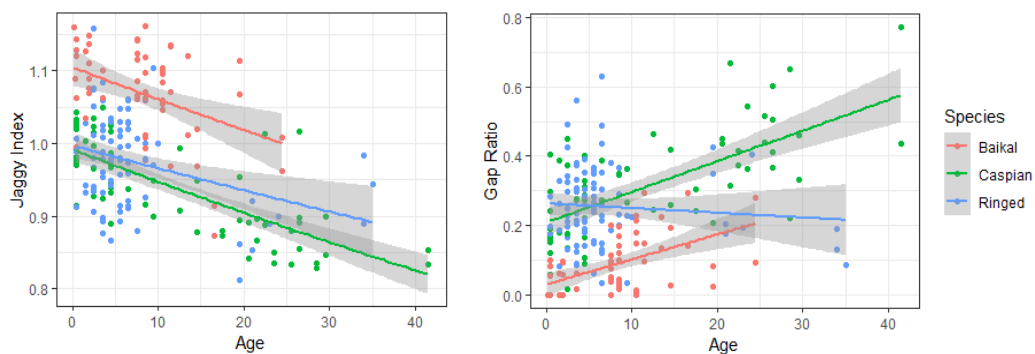


Figure1. (a) Jaggy Index: measurement of tooth jaggedness, plotted against age estimation data. (b) Gap ratio: proportion of tooth gap to its teeth width, plotted against age estimation data. Lines for each data were drawn by generalized linear model using package ggplot2 of R. Grey bands represent 95% credible intervals.

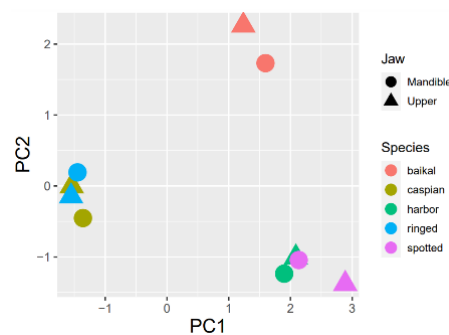


Figure2. Principal component analysis based on tooth morphologies of *Pusa* and *Phoca* species. Variables used for PCA were: Jaggy Index, Gap Ratio, Cusp bluntness: using Area of the teeth 2 mm from the cusp tip measurement directly, Tip bluntness: computed by calculating radius of the cusp tip from circumference of the cusp tip, and tooth height.

# **Thermal sensitivity of metabolic rate mirrors different biogeographies between teleosts and elasmobranchs**

Yuuki Watanabe<sup>1</sup> and Nicholas Payne<sup>2</sup>

<sup>1</sup>*NIPR & SOKENDAI*

<sup>2</sup>*Trinity College Dublin*

Environmental temperature affects physiological functions, representing a barrier for the range expansions of ectothermic species. To understand the link between thermal physiology and biogeography, a key question is whether among-species thermal sensitivity of metabolic rates is mechanistically constrained or buffered through physiological remodeling over evolutionary time. The former conception, the Universal Temperature Dependence (UTD) hypothesis, predicts similar among- and within-species thermal sensitivity. The latter conception, the Metabolic Cold Adaptation (MCA) hypothesis, predicts lower among-species thermal sensitivity than within-species sensitivity. Previous studies that tested these hypotheses for fishes overwhelmingly investigated teleosts with elasmobranchs understudied. In this study, we show that among-species thermal sensitivity of resting metabolic rates is lower than within-species sensitivity in teleosts but not in elasmobranchs. Moreover, species richness declines with latitude more rapidly in elasmobranchs than in teleosts. MCA exhibited by teleosts might underpin their high diversity at high latitudes, whereas the inflexible thermal sensitivity approximated by UTD of elasmobranchs explains their low diversity at high latitudes.

# **Animal-borne video camera provides new insights into predator-prey interactions between the Adélie penguin *Pygoscelis adeliae* and the prey fish *Pagothenia borchgrevinki***

Soma Tokunaga<sup>1</sup>, Yuuki Kawabata<sup>2</sup>, Akinori Takahashi<sup>1,3</sup>

<sup>1</sup>*Department of Polar Science, The Graduate University for Advanced Studies, SOKENDAI, Tachikawa, Tokyo 190-8518, Japan*

<sup>2</sup>*Graduate School of Fisheries and Environmental Sciences, Nagasaki University, Bunkyo, Nagasaki 852-8521, Japan*

<sup>3</sup>*National Institute of Polar Research, Tachikawa, Tokyo 190-8518, Japan*

Predation acts as a strong selective pressure on the behaviors of both predators and prey. However, the opportunity to observe predator-prey interactions in the wild is limited, especially for aquatic animals. Animal-borne video cameras (i.e., video loggers) would be a powerful tool to solve this problem. Here, we examined the animal-borne videos obtained from the Adélie penguin (*Pygoscelis adeliae*) that showed interactions with the prey fish (*Pagothenia borchgrevinki*) under Antarctic sea-ice. A video logger and an accelerometer were attached to eight penguins at Hukuro Cove colony, Antarctica, during December 2012 to January 2013. The videos showed that the penguins foraged on krill and small fishes, consistent with a previous study. While, one out of eight penguins targeted two large *P. borchgrevinki*. During these two predation events, the penguin pursued the prey five times. After correcting camera rotation and attach angle, it is suggested that the penguin did not swim toward the prey (i.e., tracking strategy) but rather toward the prey's future position or swimming path (i.e., interception strategy). We also found that *P. borchgrevinki* curls the body into a circle or a "C" shape in six out of nine captures. Based on these findings, we raise the following two hypotheses: 1) Adélie penguins can intercept the maneuvering prey, and 2) the prey fish *P. borchgrevinki* takes a curling posture to increase the likelihood of escape. Our observations reinforce the effectiveness of animal-borne videos in understanding predator-prey interactions in natural environments.

# Prokaryotic and eukaryotic community structures in East Antarctic coastal sediments

Hideaki Ochi<sup>1</sup>, Ryo Matsuda<sup>1</sup>, Ryosuke Makabe<sup>2,3,4</sup>, Masayoshi Sano<sup>2,5</sup>, Shintaro Takao<sup>6</sup>, Masato Ito<sup>2</sup>, Shin Sugiyama<sup>7</sup>, Masato Moteki<sup>2,4</sup>, Norio Kurosawa<sup>1</sup>

<sup>1</sup>Graduate School of Science and Engineering, Soka University

<sup>2</sup>National Institute of Polar Research

<sup>3</sup>The Graduate University for Advanced Studies (SOKENDAI)

<sup>4</sup>Tokyo University of Marine Science and Technology

<sup>5</sup>Atmosphere and Ocean Research Institute, The University of Tokyo

<sup>6</sup>National Institute for Environmental Studies

<sup>7</sup>Institute of Low Temperature Science, Hokkaido University

The Southern Ocean plays a crucial role in the uptake and storage of carbon. Carbon uptake of the region is estimated as more than 10% of the anthropogenic carbon dioxide. The study on the Southern Ocean sea ice is essential to understand the variation of carbon uptake and biogeochemical cycle. Sea ice incorporates high levels of iron during its formation triggering phytoplankton blooms at the sea-ice edge during the melt season (Duprat et al., 2020). Recent observations reported that frazil ice could incorporate resuspended sediment through suspension freezing in a coastal polynya (Ito et al., 2019). Turbulent conditions formed a mixture of frazil/grease ice and open water in the active-frazil area (Nakata et al., 2021). These studies implied that sea ice incorporates resuspended sediment in the coastal area and releases it offshore via sea ice advection and melts. Therefore, studying the seafloor sediment is necessary to understand the Antarctic ecosystem and biogeochemical cycle. However, there are few reports on comprehensively investigating biological communities of Antarctic coastal sediments. This study aims to reveal the prokaryotic and eukaryotic communities of the East Antarctic coastal sediment that may be transferred offshore via sea ice advection.

The seafloor surface sediment samples were collected from Cape Darnley Polynya, Lützow-Holm Bay, and Langhovde Glacier by the 59th Japan Antarctic Research Expedition (Table 1). The sediment DNAs were extracted, and the 16S rRNA gene V3-V4 region and 18S rRNA gene V9 region were amplified by PCR. The PCR products were sequenced with an Illumina MiSeq sequencing system, and the sequence data were analyzed using mothur software. Sequences were classified using trainset18 for 16S rRNA genes and PR2-database for 18S rRNA genes, and those with 97% or more sequence homology were designated as one OTU (Operational Taxonomic Unit). In addition, representative sequences of OTU were identified at the species level by BLAST (Basic Local Alignment Search Tool).

**Table 1. Sampling sites**

Sampling sites	Abbreviations	Latitude, Longitude	Depth (m)
Cape Darnley Polynya	CDP	-67.4063, 68.5537	205
Lützow-Holm Bay	LHB	-68.5652, 39.4058	149
Langhovde Glacier 1	LG1	-69.2025, 39.8219	381
Langhovde Glacier 2	LG2	-69.1966, 39.8083	464
Langhovde Glacier 3	LG3	-69.1922, 39.8144	504
Langhovde Glacier 4	LG4	-69.1886, 39.7925	479

In prokaryotic community structures, phylum *Proteobacteria* was most frequently detected at all sites, ranging from 57 to 96%. Among this phylum,  $\alpha$ -*Proteobacteria* and  $\gamma$ -*Proteobacteria* were dominant. In addition to *Proteobacteria*, phyla *Actinobacteria*, *Bacteroidetes*, *Cyanobacteria*, and *Planctomycetes* were detected relatively frequently. On the other hand, site-to-site differences were observed when comparing at lower taxonomic levels. For example, the order *Chromatiales* accounted for most of the  $\gamma$ -*Proteobacteria* detected in CDP and LHB, whereas it was less abundant under the ice shelf. Conversely, the order *Alteromonadales* of  $\gamma$ -*Proteobacteria*, detected in a high proportion under the ice shelf, especially in LG2, were rarely detected in CDP and LHB. In eukaryotic community structures, phylum *Ochrophyta* was detected at a relatively high frequency at all sites. Phylum *Dinoflagellata* and *Rhizaria* were also detected at a relatively high frequency at most sites but not in LG1 and LG2 under the ice shelf close to land. As with the prokaryotic community, the differences between the sites

become clear when comparing at lower taxonomic levels. For example, orders *Thalassiosirales*, *Dictyochales*, and *Pedinellales* were found only in CDP, whereas the genus *Fragilariopsis* was found only in LHB.

As a result of this study, it was found that there are many similarities between the community structures in the sediments in Cape Darnley Polynya and Lützow-Holm Bay. In contrast, the community structure under the Langhovde Glacier differs from other sites. These findings provide useful information for a deeper understanding of biological activities and the ecosystem dynamics affected by them in the Southern Ocean. In addition, the DNA of organisms specifically detected in sediments in specific areas may be used as tracers of sea ice formed there. However, this study uses sediments collected from only one point in each area, so it is not easy to generalize at this time. By increasing the number of sampling sites, it is thought that the characteristics of the three areas can be captured more accurately.

## References

- Duprat, L., Corkill, M., Genovese, C., Townsend, A. T., Moreau, S., Meiners, K. M., Lannuzel, D. (2020). Nutrient distribution in east Antarctic Summer Sea Ice: A potential iron contribution from glacial basal melt. *Journal of Geophysical Research: Oceans*, 125(12).
- Ito, M., Ohshima, K. I., Fukamachi, Y., Hirano, D., Mahoney, A. R., Jones, J., Takatsuka, T., Eicken, H. (2019). Favorable conditions for suspension freezing in an Arctic Coastal polynya. *Journal of Geophysical Research: Oceans*, 124(12), 8701–8719.
- Nakata, K., Ohshima, K. I., Nihashi, S. (2021). Mapping of active frazil for Antarctic Coastal Polynyas, with an estimation of sea-ice production. *Geophysical Research Letters*, 48(6).



# Empirical equations and image analyses for estimating zooplankton biomass in the Southern Ocean

Shunji Yukawa<sup>1</sup>, Ryusuke Makabe<sup>1,2,3</sup>, Masayoshi Sano<sup>2,4</sup> and Moteki Masato<sup>1,2</sup>

<sup>1</sup>Tokyo University of Marine Science and Technology

<sup>2</sup>National Institute of Polar Research

<sup>3</sup>The Graduate University for Advanced Studies (SOKENDAI)

<sup>4</sup>Atmosphere and Ocean Research Institute, The University of Tokyo

Zooplankton plays important roles as secondary producers of marine food webs and drivers of biological carbon pump. The Antarctic krill (*Euphausia superba*), known as a key player in such ecological roles in the Southern Ocean, have been centered in various ecological studies. However, recent studies have shown the heterogeneous distribution of this species, and the importance of the food web structure centered on other zooplankton species is increasing. This food web structure has not been studied as much as the krill-dependent food web. Therefore, the players that take the place of krill in this food web structure have rarely been quantitatively evaluated. These players include diverse taxonomic groups such as copepods, amphipods, ostracods, molluscs, pteropods, and salps. Although these groups are diverse in size, feeding, and body structure, they have often been evaluated as a single group. This prevent us to quantitative understand of the ecosystem structures and the energy flow in the Southern Ocean. One of the major reasons why zooplankton community structure with biomass of each taxon have not yet been accumulated is that analysis requires a great deal of time and labor. This is because manual analysis has been the main method used in the past, either by directly measuring weight or by measuring body length and converting it to weight. To solve this problem, a method has been developed to automatically measure surface area from image data of a sample and obtain quantitative data from the relationship between surface area and weight, although there are only two studies conducted around the Antarctic Peninsula.

Zooplankton samples were collected off Vincennes Bay in the Southern Ocean by using ORI net, MOHT net during TV *Umitaka maru* cruise in January 2019. The samples were frozen by liquid nitrogen immediately after collection and stored at  $<-60^{\circ}\text{C}$  until analyses. The frozen samples were thawed, photographed, dried, and weighted on land laboratory. Body length and width data were measured using the image processing software ImageJ (National Institute of Health), and surface area was calculated by assuming an ellipse with these values as its diameter. After then, each zooplankton specimen was dried at  $60^{\circ}\text{C}$  for 24 hours for measuring dry weight using a microbalance. Totally, 408 specimens (covers 26 taxonomic groups) were analyzed. For many taxa, relationship between surface area and dry mass showed better correlations than those of length-dry mass relationship. Crustaceans showed similar regression equations for the relationship between surface area and weight regardless of taxonomic group, while the equations of two copepods, *Calanoides acutus* and *Rhincalanus gigas*, which known as dominant suspension feeder, were different from those of other crustaceans. The regression equations of the other taxa were completely different from those of crustaceans. In addition, we examined the best method for the area detection using the ImageJ. The binarization methods "RenyiEntropy" and "Default" recorded high detection rates for many taxa. However, both methods have their strengths and weaknesses, and some individuals were not recognized at all. We will continue to discuss which method to use and how to use it in the binarization process.



Figure 1. Lengths measured for area calculation. Length and height.

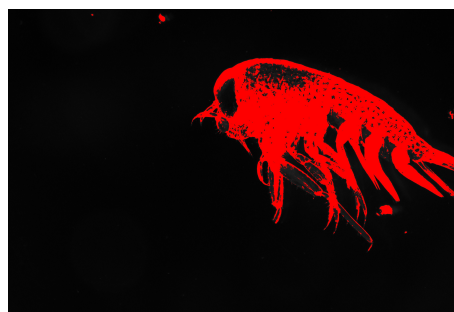


Figure 2. Image after binarization using the "Default" binarization method

# Particulate organic matters in sea ice floe in the Indian sector of the Southern Ocean

Ryosuke Makabe<sup>1,2,3</sup>, Masayoshi Sano<sup>1,4</sup>, Keigo D. Takahashi<sup>3</sup>, Shintaro Takao<sup>5</sup>, Ryo Matsuda<sup>6</sup>, Masato Ito<sup>1</sup>, Michiyo Yamamoto-Kawai<sup>3</sup>, Daiki Nomura<sup>7</sup>, Norio Kurosawa<sup>6</sup>, Masato Moteki<sup>1,3</sup>

<sup>1</sup> National Institute of Polar Research

<sup>2</sup> The Graduate University for Advanced Studies (SOKENDAI)

<sup>3</sup> Tokyo University of Marine Science and Technology

<sup>4</sup> Atmosphere and Ocean Research Institute, The University of Tokyo

<sup>5</sup> National Institute for Environmental Studies

<sup>6</sup> Soka University

<sup>7</sup> Hokkaido University

The Seasonal Ice Zone (SIZ) covers broad area in the Southern Ocean. Various organisms live in this area have a life cycle strategies closely related with sea ice dynamics. For example, sea ice is the important habitat for protozoans and small metazoans, and ice algal production accounting ca. 10% of total primary production in this area. Ice edge blooms are also important event not only for primary producer but also various consumers whose growth and/or reproduction largely depend on this event. On the other hand, occurrence of the ice edge bloom is quite heterogeneous compared to that of filter feeding consumers mainly depend on primary production. We hypothesize, therefore, alternative food sources other than ice edge production, e.g. particulate organic matters (POM) in the sea ice, could explain the gap. POM contents in sea ice have been investigated by a number of previous studies, but most of them conducted using ice cores collected from large ice floes or fast ice mainly in coastal area.

Field samplings were conducted in Indian sector of the Southern Ocean during the Antarctic cruises of the training vessel (TV) *Umitaka-maru* of the Tokyo University of Marine Science and Technology (2015/16, 2016/17, 2017/18, 2018/19 and 2019/20), the cruise of the icebreaker *Shirase* (2015/16, 2017/18, and 2019/20), and the cruises of *RV Hakuho-maru* (2018/19 and 2019/20). Sea ice floes in the marginal ice zone were collected by using a stainless steel basket (50 × 60 × 20 cm) or a large ring net frame (1.6 m diameter) with an attached canvas. In most case, surrounding water was also collected for measuring chlorophyll *a*, macro-nutrients and particulate organic carbon and nitrogen using the pump-underway ship intake system (intakes were located 5-9 m depth). Sea ice samples were kept in freezer, and then melted at 4°C in dark. For measuring chlorophyll *a*, sea ice was melted with addition of filtered sea water (sea ice:filtered sea water = 1:9, w/w).

Particulate organic carbon (POC), particulate organic nitrogen (PON) and Chl. *a* in sea ice was  $1368 \pm 1008 \text{ mg C m}^{-3}$  ( $n = 155$ ),  $121 \pm 125 \text{ mg N m}^{-3}$  and  $17 \pm 41 \text{ mg m}^{-3}$ , respectively (Fig. 1). The carbon:chlorophyll ratio usually quite high compared to the traditional of phytoplankton (Fig. 2). In addition, C/N ratio was also high ( $14.1 \pm 5.8$ ). These results suggest that high carbon contents in the sea ice floes were mainly caused by detrital materials rather than living organisms, such as ice algae and the other microorganisms. The high POC contents with low variability compared to Chl. *a* implies that such detrital POC input from sea ice to upper water column could contribute as stable food source for zooplankton community in MIZ.

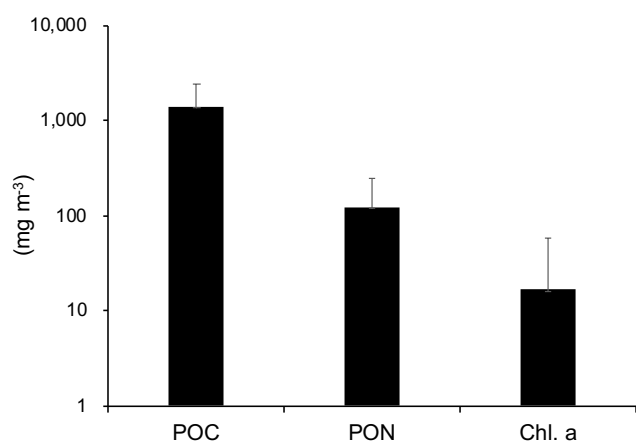


Fig. 1. Mean particulate organic carbon (POC), nitrogen (PON), and Chlorophyll *a* (Chl. *a*) in collected sea ice floes. error bars show standard deviation.

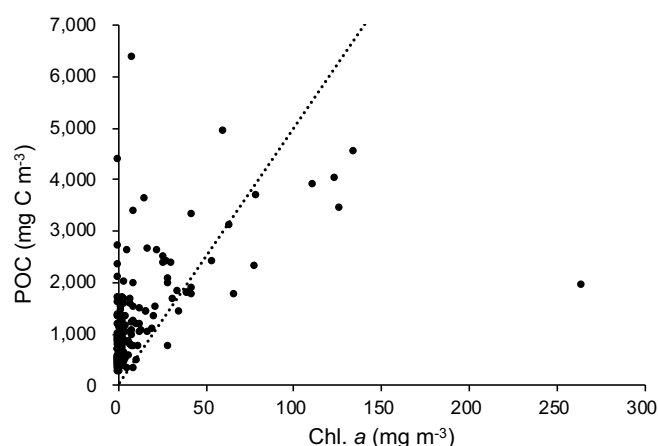


Fig. 2. Chlorophyll *a* (Chl. *a*) and particulate organic carbon (POC) in sea ice floes. A dashed line shows carbon:chlorophyll ratio = 50.

# Transport of live Antarctic fishes from Syowa Station to Port of Nagoya Public Aquarium

Tsuyoshi Matsuda<sup>1</sup>, Akinori Takahashi<sup>2</sup>, Kotaro Ichikawa<sup>3</sup>, Hideaki Nishizawa<sup>4</sup>, Saki Asai<sup>5</sup>, Yoshinori Miyamoto<sup>6</sup>,  
Kiyonori Nakajima<sup>1</sup>, Masanori Kurita<sup>1</sup>

<sup>1</sup>Port of Nagoya Public Aquarium

<sup>2</sup>National Institute of Polar Research

<sup>3</sup>Field Science Education and Research Center, Kyoto University

<sup>4</sup>Graduate School of Informatics, Kyoto University

<sup>5</sup>Graduate School of Marine Science and Technology, Tokyo University of Marine Science and Technology

<sup>6</sup>Tokyo University of Marine Science and Technology

Four individuals of two species of Notothenioidei fishes (one ploughfish *Gymnodraco acuticeps* and three emerald rockcod *Trematomus bernacchii*) were collected near Syowa Station in January 2019 and transported alive to Port of Nagoya Public Aquarium (Nagoya, Japan). Here we provide some detailed records on how the transport was successfully conducted.

The fishes were collected by fishing in Kita-no-ura cove (Lützow-Holm Bay, East Antarctica) (Nishizawa et al. 2019). One ploughfish was collected on 18 Jan., and five emerald rockcod were collected between 10 and 17 Jan. After the collection, the fishes were divided into two sealed buckets (Toslon SN-20) filled with seawater and aerated by dry cell air pump and held under the Environmental Science Building of Syowa Station. The fishes were transported by helicopter to the icebreaker "SHIRASE" on 19 Jan. On board "SHIRASE", the fishes were housed in the sealed buckets with 20 litres of seawater, one or two individuals in one bucket, and aerated with a dry-cell air pump. The sealed buckets were housed in C-packs (CES-4) and kept cool with freshwater ice around them. The C-packs were placed in the refrigerator (room temperature 2-5°C) in Observation Room 2 of "SHIRASE". During the transport in "SHIRASE", water was changed every 3-10 days at a rate of 7-8 litres per bucket. Ice for cold storage and air pump batteries were replaced every three days. After "SHIRASE" arrived at the Port of Sydney, Australia, on 18 March, the fishes were unloaded from "SHIRASE" and packed for air transport after quarantine. Each fish was sealed in a plastic bag with seawater and oxygen and placed in a sealed bucket. Sealed buckets were placed in C-packs and kept cold around them with freshwater ice and refrigerants. The fishes left Sydney Airport on 19 March and arrived at Chubu Centrair international Airport the same night. Then they were transported to Port of Nagoya Public Aquarium and housed in aquaria. After the collection, the fishes were not fed for approximately two months, including during transport, until they were housed in aquaria at Port of Nagoya Public Aquarium. The water temperature during transport temporarily rose to 4.0°C during loading onto "SHIRASE" and temporarily rose to 2.7°C during transshipment in Sydney, but otherwise remained between -1.0°C and 1.3°C. During transport, one emerald rockcod died on 2 Feb and another emerald rockcod on 16 Feb.

The transport from Sydney to Nagoya could not be treated as an export because none of our staff at the scene resided in Australia. This case, we adopted the procedure of a transshipment of fishes in Sydney. However, a problem was what an appropriate treatment could be to enter them into Japan. After discussions with Nagoya Customs, we came to the conclusion that it would be treated as domestic cargo from Syowa Station to Nagoya. The fishes brought into Port of Nagoya Public Aquarium are still kept in good health after three and a half years.

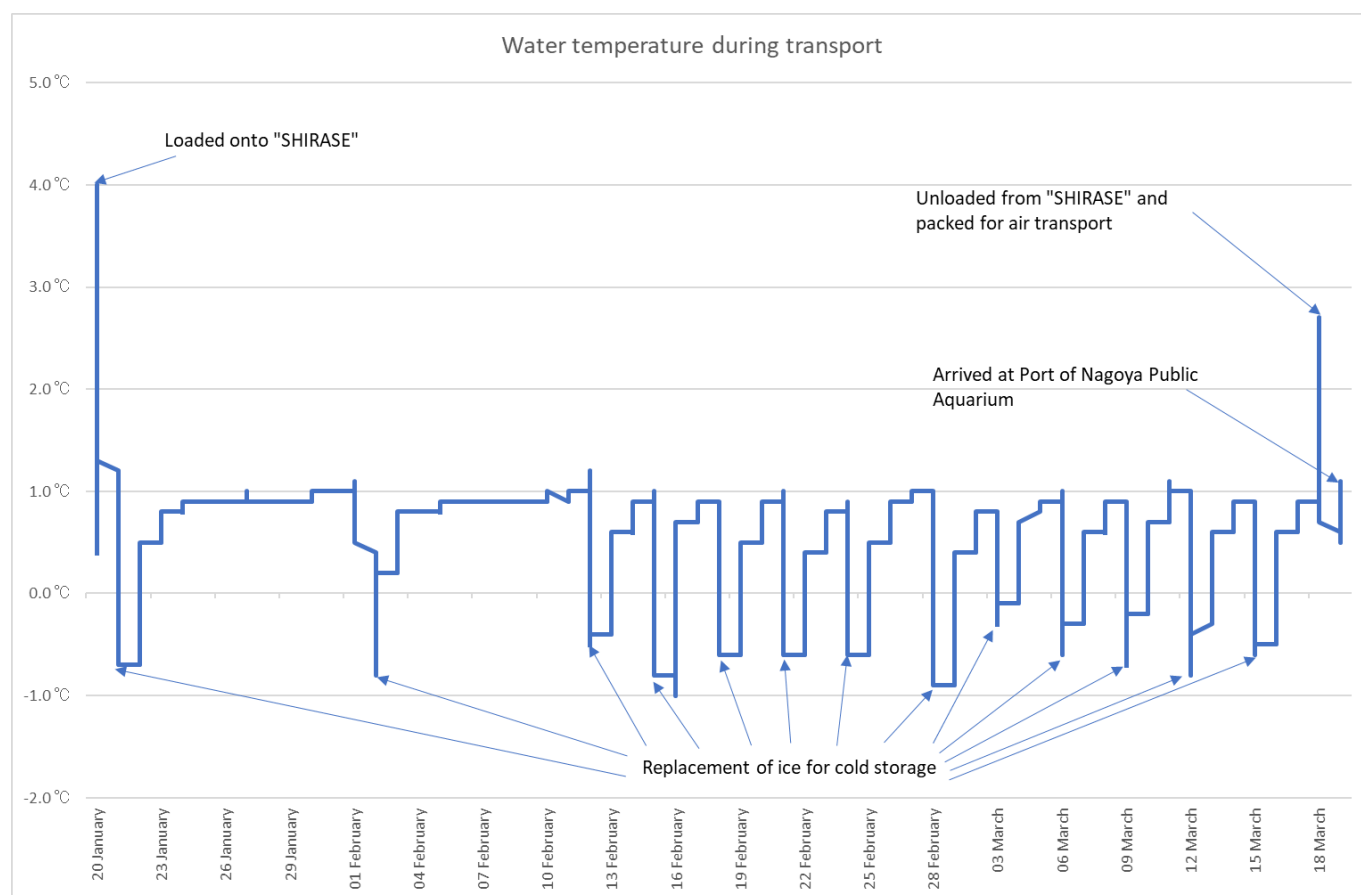


Figure 1. Water temperature during transport

## References

Nishizawa, H., K. Ichikawa, S. Asai, N. Arai, H. Mitamura, Y. Miyamoto and T. Iwami, Record of fishes sampled in Kita-no-ura cove (Lützow-Holm Bay, East Antarctica) in January 2019, *Antarctic Record*, 63, 27-55, 2019.

# **Development of animal-borne dissolved oxygen loggers to examine the foraging behavior of northern elephant seals in the oxygen-limited mesopelagic zone.**

Atsuya Ogata<sup>1</sup>, Arina Favilla<sup>2</sup>, Rachel Holser<sup>2</sup>, Theresa Keates<sup>2</sup>, Daniel Costa<sup>2</sup>, Akinori Takahashi<sup>1,3</sup>

<sup>1</sup>*Department of Polar Science, The Graduate University for Advanced Studies, SOKENDAI, Japan*

<sup>2</sup>*University of California Santa Cruz, USA*

<sup>3</sup>*National Institute of Polar Research, Japan*

The ocean's mesopelagic zone (200-1000 m) holds large fish biomass and is a critical foraging habitat for deep-diving marine mammals. The mesopelagic zone is characterized by decreasing dissolved oxygen (DO) concentration with depth, but mid- and deep-water DO levels often vary spatially. Understanding the foraging behavior of deep-diving marine mammals relative to variability in midwater DO levels will provide insight into the ecological effects of climate change on the mesopelagic zone. Deep-diving northern elephant seals are hypothesized to feed on sluggish fish in mesopelagic oxygen minimum zones (Naito et al., 2017 Ecol Evol). However, it has been challenging to obtain in situ midwater DO levels where marine mammals forage due to a lack of suitable animal-borne devices. Here, we report preliminary results on the relationship between midwater DO levels and feeding behavior of northern elephant seals, by using newly developed animal-borne DO loggers together with other behavioral loggers. We obtained data on midwater DO levels from female northern elephant seals ( $n = 5$ ) during a portion (1-7 days) of their post-breeding trips from back-mounted DO loggers (PRE1300-ODT, manufactured by Little Leonardo Ltd.). We also investigated concurrent measurements of feeding events from jaw-mounted accelerometers and movement tracks from head-mounted satellite transmitters. Our results showed that seals fed at different depths and therefore experienced different DO levels between daytime and nighttime dives. They fed at mean depths of 557-609 m and 337-440 m (mean DO: 0.55-1.51 mg/L and 1.74-3.54 mg/L) during daytime and nighttime, respectively. DO levels at mean feeding depths were lower than upper DO thresholds of oxygen-limited zone (OLZ: 2 mg/L) and oxygen minimum zone (OMZ: 0.7 mg/L) for 93.5% and 14.0% of all daytime feeding dives ( $n = 399$  dives), respectively. In comparison, DO levels at mean feeding depths were lower than upper DO thresholds of OLZ and OMZ only for 23.1% and 1.6% of all nighttime feeding dives ( $n = 707$  dives), respectively. Nighttime dives had slightly more feeding events per dive (12.6- 20.9 events / dive) and more feeding events per unit dive time (0.010- 0.021 / s) than daytime dives (8.2- 14.0 events / dive, and 0.007- 0.010 / s). Further investigations are required if seals target on prey of larger size or different types to compensate for apparently fewer feeding events in deep daytime dives in OLZ.

# King penguins adjust their fine-scale traveling and foraging behavior to spatial and diel changes in feeding opportunities

Hina Watanabe<sup>1,\*</sup>, Kozue Shiomi<sup>2</sup>, Katsufumi Sato<sup>3</sup>, Akinori Takahashi<sup>1,4</sup>

Yves Handrich<sup>5</sup>, Charles-André Bost<sup>6</sup>

<sup>1</sup>*Department of Polar Science, The Graduate University for Advanced Studies, SOKENDAI*

<sup>2</sup>*Frontier Research Institute for Interdisciplinary Sciences, Tohoku University*

<sup>3</sup>*Atmosphere and Ocean Research Institute, University of Tokyo*

<sup>4</sup>*National Institute of Polar Research*

<sup>5</sup>*Université de Strasbourg, CNRS*

<sup>6</sup>*Centre d'Etudes Biologiques de Chizé, UMR 7372 CNRS-Université de la Rochelle*

Central place foragers such as pelagic seabirds often travel large distances to reach profitable foraging areas. King penguins (*Aptenodytes patagonicus*) are well known for their large-scale foraging movements to the productive Antarctic Polar Front (APF), though their fine-scale traveling and foraging characteristics remain unclear. Here, we investigated the horizontal movements and foraging patterns of king penguins to understand their fine-scale movement decisions during distant foraging trips. We attached multi-channel data loggers that can record depth, speed, tri-axis acceleration, tri-axis magnetism, and environmental temperature to the penguins and obtained data (n=8 birds) on their horizontal movement rates from reconstructed dive paths and their feeding attempts estimated from rapid changes in swim speed. During transit toward main foraging areas, penguins increased the time spent on shallow traveling dives (< 50 m) at night and around mid-day, and increased the time spent on deep foraging dives (≥ 50 m) during crepuscular hours. Once penguins reached to the main foraging areas near or in the APF, they increased the time spent on deep foraging dives (≥ 50 m) throughout the daytime. The horizontal movement rates during deep dives were negatively correlated with maximum dive depths, suggesting that foraging at greater depths is associated with a decreased horizontal traveling speed. Penguins concentrated their foraging efforts (more deep dives and higher rates of feeding attempts) at twilight during transit, when prey may be more accessible due to diel vertical migration, while they traveled rapidly at night and mid-day when prey may be difficult to detect and access. Such behavioral adjustments correspond to a movement strategy adopted by avian deep divers to travel long distances while feeding on prey exhibiting diel vertical migration.

# **Impacts of climate change and variability on penguins: a review**

Akinori Takahashi

*National Institute of Polar Research, Japan*

Climate change and variability has been shown to affect a broad range of wildlife worldwide. Seabirds are likely to be affected by changing climate while breeding on-land and foraging at-sea, and their population status and ecological changes are monitored as indicators of ecosystem change. This paper reviewed the ecological processes and population consequences of climate impacts on penguins. The review showed that climate change and variability is an important factor driving the changes in the population size and breeding success of penguin species (up to 13 of 18 extant species) via changes in the breeding and foraging environments. However, climate change and variability affected penguins in a complicated way: for example, the effects of warming climate can vary from negative to positive in different regions of a single species or different life-history parameters in a single population (e.g., breeding success vs. adult survival rates). Some simulation studies produced future population projections in Antarctic and subantarctic penguins based on multiple climate change scenarios, emphasizing the importance of climate mitigations. These simulation results should still be interpreted with caution by appreciating uncertainties associated with climate projections and penguin responses to future climate. More research on the foraging ecology is needed, especially during data-poor non-breeding or juvenile periods, to elucidate the processes of the climate impacts on penguins fully. Finally, mitigating the existing human impacts would be essential to safeguard the penguin species, which should help penguin populations to be more resilient to the existing and future climate impacts.

# Temporal changes in protist fluxes during sea ice melt off Wilkes land, East Antarctica

Chiho Tsuchiya<sup>1</sup>, Keigo D. Takahashi<sup>2</sup>, Masayoshi Sano<sup>3,4</sup>, Ryosuke Makabe<sup>1,2,3</sup>, Shintaro Takao<sup>5</sup>, Masato Moteki<sup>1,3</sup>

<sup>1</sup>Tokyo University of Marine Science and Technology

<sup>2</sup>The Graduate University for Advanced Studies. SOKENDAI

<sup>3</sup>National Institute of Polar Research

<sup>4</sup>Atmosphere and Ocean Research Institute, The University of Tokyo

<sup>5</sup>National Institute for Environmental Studies

Seasonal Ice Zone (SIZ) is one of the most important area of the Southern Ocean ecosystem. Sea ice, which regulate environmental conditions (e.g. light penetration and iron supply) in SIZ, strongly affect primary production (Smith and Nelson 1985; Lancelot et al. 2009) and material cycling through the food web. The relationships between surface environments and the biological pump has usually been investigated by using sediment traps attached to a mooring. However, in the Southern Ocean, mooring observation could not cover the surface environment due to the existence of icebergs. In this study, we conducted a drifter observation using a newly developed ice-resistant GPS buoy to understand the relationship between surface environment and the protist composition in sinking particles during sea ice melt.

Deployment (December 9, 2019) and retrieval (February 16, 2020) of the drifter were conducted from the icebreaker *Shirase* under the 61st Japanese Antarctic Expedition. Sinking particles were collected at depths of 60 m and 150 m by time-series sediment traps. Water temperature, salinity, Photosynthetically Active Radiation (PAR), and chlorophyll fluorescence were measured using sensors placed at multiple depths (0, 10, 20, 30, 40 m). CTD observations were made when the drifter was deployed and retrieved, and seawater samples at each depth were collected by a bucket and Niskin bottles. Sea ice around the vessel was collected when the drifter was deployed. A similar observation was also conducted near the path of the drifter on January 22, 2020 during the *Umitaka-maru* cruise (Takahashi et al. 2022). Samples of seawater, sea ice, and sinking particles were fixed using neutral Lugol-iodine solution. Composition and flux of protist were analyzed using an inverted microscope.

Sea ice concentration was almost 100% when the buoy was deployed, then rapidly decreased from December 27 to January 5, finally reaching 0% on January 12. PAR increased with decreasing sea ice concentration. During the period, a peak of chlorophyll fluorescence was found at 20 m, and then, shifted to deeper depths. The chlorophyll fluorescence peaks were always found in pycnocline, which existed below the euphotic layer, suggesting that they were caused by phytoplankton accumulation rather than their production at the pycnocline. Nanoflagellates (mainly *Phaeocystis antarctica*) and *Fragilariopsis cylindrus* were dominant protists in sinking particles. Cluster analysis using the protist composition of sinking particles, seawater, and sea ice showed that the composition in sinking particles was divided into two clusters, one similar to seawater and the other similar to sea ice. Cluster A is a cluster whose protist composition is similar to sea ice, and Cluster B is a cluster whose protist composition is similar to seawater. Of the clusters in Cluster B, those that resemble seawater were classified as Cluster B1. The composition in sinking particles was similar to that of seawater before the sea ice melted and became similar to that of sea ice during the period of rapid sea ice melting. Therefore, organisms released from sea ice settle faster and are expected to contribute to the flux during the period of sea ice melting.

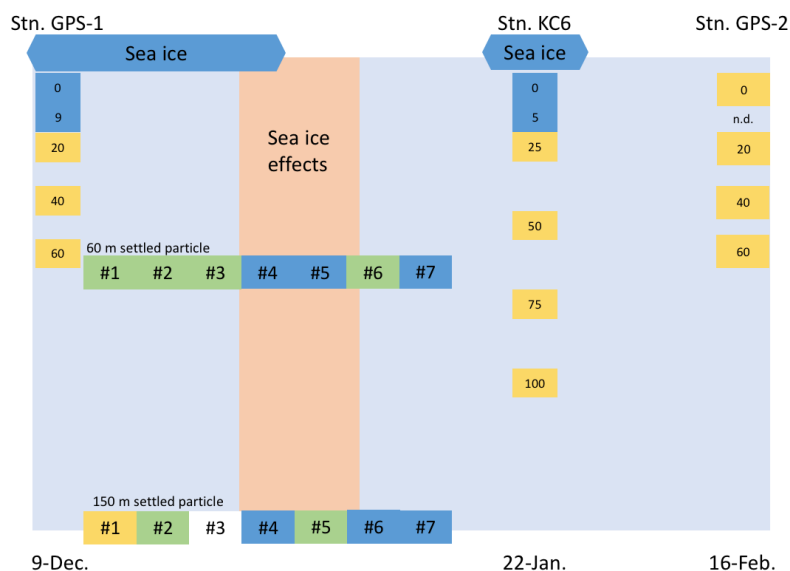


Fig.1. Schematic diagram of results of cluster analysis.

Cluster A: Clusters similar to the protist composition in sea ice

Cluster B: Clusters similar to the protist composition in sea water

B1: Groups with composition similar to seawater, especially

B2: Groups of similar composition to seawater that are common in settled particles

Stn. GPS-1: GPS buoy deployment point

Stn. GPS-2: GPS buoy retrieval point

Stn. KC6: Observation point by *umitaka\_maru*



# The occurrence of a dinoflagellate species *Gyrodinium rubrum* in sea ice in the Indian sector of the Southern Ocean

Ryo Matsuda<sup>1</sup>, Masayoshi Sano<sup>2,3</sup>, Keigo D. Takahashi<sup>4</sup>, Shintaro Takao<sup>5</sup>, Ryosuke Makabe<sup>2,4,6</sup>,  
Michiyo Yamamoto-Kawai<sup>6</sup>, Masato Moteki<sup>2,6</sup>, Norio Kurosawa<sup>1</sup>

<sup>1</sup>Graduate School of Science and Engineering, Soka University

<sup>2</sup>National Institute of Polar Research

<sup>3</sup>(Present address) Atmosphere and Ocean Research Institute, The University of Tokyo

<sup>4</sup>The Graduate University for Advanced Studies, SOKENDAI

<sup>5</sup>National Institute for Environmental Studies

<sup>6</sup>Tokyo University of Marine Science and Technology

## Introduction

The biological carbon pump is a crucial for carbon sequestration in the deep sea. A better understanding of the complex processes of the biological carbon pump is essential for understanding the global carbon cycle. Previously, we identified faecal pellet-like dinoflagellates (FLD) in the Southern Ocean as *Gyrodinium rubrum* and *G. heterogrammus* (Matsuda et al., in prep). In the Weddell Sea, a phagotrophic athecate dinoflagellate that grows larger (50  $\mu\text{m} \times 50 \mu\text{m}$ ) was observed in both the floating sea ice and the adjacent water column in the coastal area (Buck et al., 1990). By contrast, FLD of a size similar to ellipsoidal faecal pellet found in sinking particles has never been reported in offshore waters. Based on their appearance, these might have been classified as faecal pellets, not living dinoflagellates. As a result, their role in the food web and carbon cycle may have been significantly overlooked. FLD cells excrete faecal pellets with a sinking rate of  $225 \pm 139 \text{ m d}^{-1}$ , which is comparable with that of faecal pellets generated by metazoans, such as Antarctic krill and copepods. These FLD contributes up to 20% of total particulate organic carbon (POC) flux. These results suggest that FLD have an important role in carbon export.

Recently, Li et al. (2021) demonstrated that the association between sea ice and plankton assemblage is critical for carbon cycles (Li et al., 2021). Using the high-throughput sequencing technic, *G. rubrum* has been frequently detected in the Ryder Bay after sea ice melts (Piquet et al., 2011). These results imply that *G. rubrum* cells are released from sea ice. If *G. rubrum* inhabits in sea ice and spreads geographically via sea ice advection, it potentially has a role in carbon export in the Southern Ocean. In this study, we investigated the occurrence and abundance of *G. rubrum* in sea ice from the Indian sector of the Southern Ocean.

## Material and Methods

Field observations were conducted in the Indian sector of the Southern Ocean, off Cape Darnley, off Lützow-Holm Bay, off Totten Glacier from December 2019 to March 2020. During cruises on the icebreaker *Shirase* as part of the 61<sup>st</sup> Japanese Antarctic Research Expedition, the training vessel *Umitaka-maru* of the Tokyo University of Marine Science and Technology, and the cruise KH20-1 on R/V *Hakuho-maru*, brash ice and newly formed ice (total of 96 samples from 21 stations) were collected using a plankton net with a cover, small fishing nets with a stick, or a stainless steel cage. All sea ice samples were stored at  $-20^{\circ}\text{C}$  in the dark until laboratory analysis. The cell abundance of *G. rubrum* was examined by quantitative polymerase chain reaction (qPCR). A 200 g sea ice sample from each station was melted in 800 mL of 3% NaCl artificial seawater. The sea-ice meltwater was filtered and DNA extracted from the filters using the DNeasy PowerWater Sterivex Kit. To develop species-specific primers and a TaqMan probe set, the internal transcribed spacer 1 and 2 regions of *G. rubrum* cells sampled in the Southern Ocean were determined. qPCR was performed using the species-specific primers and a TaqMan probe set on the QuantStudio 1 real-time PCR system (Thermo Scientific) under the following thermal cycling conditions:  $95^{\circ}\text{C}$  for 20 s, followed by 40 cycles of  $95^{\circ}\text{C}$  for 1 s and  $60^{\circ}\text{C}$  for 20 s. Genomic DNA was extracted from the 30 cells of *G. rubrum* cells, and a mixture of the genomic DNA was used as a positive and standard control. The sea-ice meltwater was filtered through a Whatman GF/F filter for analyses of chlorophyll a (Chl. a), POC, and particulate organic nitrogen (PON). The Chl. a concentration was measured using a pre-calibrated fluorometer (10-AU, Turner Designs, USA) after filtration and pigment extraction. POC and PON concentrations were measured using an elemental analyser connected to an isotope-ratio mass spectrometer (Thermo Scientific), after filtration and drying.

## Results and Discussion

Using qPCR, *G. rubrum* was found at 13 stations (36 samples), including 4, 1, and 31 sea ice samples from the waters off Cape Darnley, Lützow-Holm Bay, and the Totten Glacier, respectively. *G. rubrum* was observed in all regions, with cell

abundances ranging from 0.0 to 3.4 cells/L ( $< 1$  cells/L in most of the sea ice samples). The cell abundance in samples off the Totten Glacier was  $0.25 \pm 0.69$  cells/L, compared with  $0.13 \pm 0.085$  cells/L in other regions. At station “GPS”, where a drifter with a sediment trap was deployed in our previous study, the cell abundance ranged from 0.0 to 0.2 cells/L. The cell abundance at several stations off Cape Darnley and the Totten Glacier was similar to that at station “GPS”. No significant correlations between cell abundance and environmental parameters (Chl. a concentration, POC, or PON) were observed. In our previous study, the FLD flux at 60 m depth, which was identified as *G. rubrum*, increased as the sea ice concentration decreased from December 2019 to January 2020, although the cell abundance in most of the sea ice samples was lower than 1 cell/L. The increased *G. rubrum* flux could be supported by its abundance not only in the sea ice but also in the surface water. Its abundance in the surface water needs to be evaluated to reveal the relationship between sea ice melt and *G. rubrum* flux.

In this study, we detected *G. rubrum* in sea ice from off Cape Darnley, Lützow-Holm Bay, and the Totten Glacier by qPCR analysis. However, no significant correlation between the abundance of *G. rubrum* and environmental parameters was observed. Previous studies detected *G. rubrum* in the water column in Ryder Bay, Kerguelen Plateau, Antarctic Peninsula, and in the Scotia Sea in the Southern Ocean using high-throughput sequencing of seawater DNA (Piquet et al., 2011; Georges et al., 2014; Duret et al., 2019; Liu et al., 2022). This is the first observation of *Gyrodinium* cells in the Indian sector of the Southern Ocean.

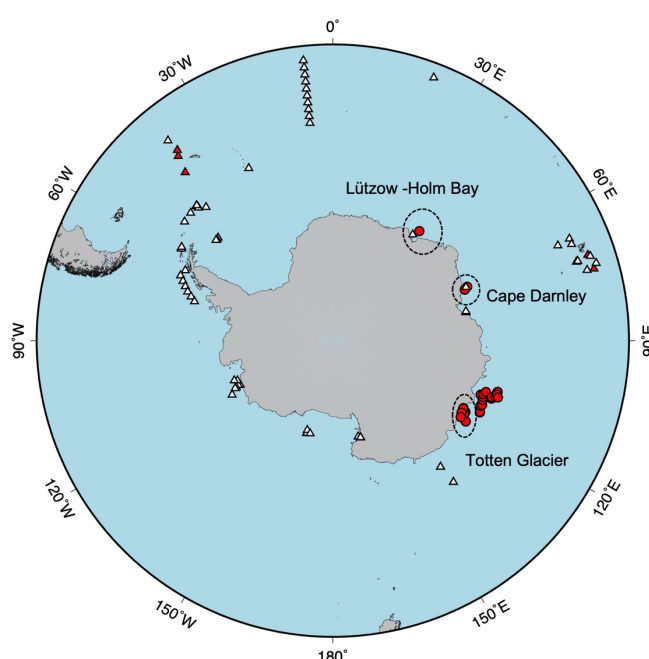


Fig. Occurrence of *Gyrodinium* species in the Southern Ocean (circle, this study; triangle, studies published from 1989 to 2022, red, *Gyrodinium rubrum*; white *Gyrodinium* species.)

## References

- Buck KP, Bolt PA, Garrison DL, Phagotrophy and fecal pellet production by an athecate dinoflagellate in Antarctic Sea ice. *Mar Ecol Prog Ser*, 60, 75–84, 1990.
- Duret MT, Lampitt RS, Lam P, Prokaryotic niche partitioning between suspended and sinking marine particles, *Environ Microbiol Rep*, 11, 386–400, 2019.
- Georges C, Monchy S, Genitsaris S, Christaki U, Protist community composition during early phytoplankton blooms in the naturally iron-fertilized Kerguelen area (Southern Ocean), *Biogeosci*, 11, 5847–5863, 2014.
- Lin Y, Moreno C, Marchetti A, Ducklow H, Schofield O, Delage E, Meredith M, Li Z, Eveillard D, Chaffron S, & Cassar N. Decline in plankton diversity and carbon flux with reduced sea ice extent along the Western Antarctic Peninsula. *Nat Com*, 12, 1–9, 2021.
- Liu C, Zhang X, Wang X, DNA metabarcoding data reveals harmful algal-bloom species undescribed previously at the northern Antarctic Peninsula region. *Polar Biol.*, 2022.
- Piquet AMT, Bolhuis H, Meredith MP, Buma AGJ, Shifts in coastal Antarctic marine microbial communities during and after melt water-related surface stratification, *FEMS Microbiol Ecol*, 76, 413–427, 2011.