

Room:201B

Time:May 26 08:30-08:45

Introduction to the Joint session

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Principal goal of the session is to discuss proper approaches to reconstruct the Earth climate system. Many of marine calcified organisms are used as biological proxies for climate change researches at present and past. For progressing the field of this science, we should precisely understand the biocalcification mechanisms and the incorporation of proxy signals. Thus, we have invited contributions related to the biocalcification, calibration and validation of marine proxies.

Keywords: biogeosciences, proxy development, culture, biology, chemical characters, biomineralization



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## Vesicle Dynamics In Foraminifera

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The calcium carbonate produced by foraminifera contains a wealth of information (so called "proxies") for paleoceanographical studies. The environmental conditions, under which they calcified, such as temperature, salinity, productivity and the ocean carbonate chemistry, are recorded in the geochemical composition of their shells (isotopic signature, trace metals concentrations). However, foraminifera maintain their own distinct (trace) metal homeostasis, which results in characteristic and species specific elemental ratios as well as in particular isotope fractionation ("vital effect"), which is offset from inorganic-thermodynamic equilibrium. In order to improve the predictive capability of the proxies it is important to identify the physiological processes controlling the chemical and isotopic composition of foraminiferal calcite.

Foraminifera precipitate calcite from modified seawater vacuoles, which are incorporated via the process of endocytosis (Erez 2003). We studied this vacuolisation process on juvenile foraminifera using fluorescent dyes (FITC-Dextran and Calcein) and confocal imagery. Specimens of the shallow, benthic foraminifer Ammonia tepida were incubated for various periods (ranging from 2-10 hrs) in seawater labelled with fluorescent dyes. After incubation the Petri dishes containing the foraminifera were carefully washed with seawater to remove the dye. Once the dishes were filled with fresh, unlabelled seawater the dynamics of the formed endocytosis vesicles were followed by means of confocal microscopy until chamber formation occurred. Depending on the length of the incubation period and the physiological status of the cell (prior/ after chamber formation) different patterns of vesicle cycling could be observed.

Keywords: foraminifera, cytology, biomineralization



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## Characterization of the shell matrix proteins of calcareous foraminifera

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Foraminifera are unicellular marine organism. Many of them have calcitic shells and are among the major calcium carbonate producers in the oceans. Their calcareous shells are widely used for stratigraphic and paleoenvironmental analyses. Like the case of skeletons of many other organisms, foraminiferan shell formation is thought to be controlled to a large extent by organic macromolecules such as proteins. But none of them have been identified so far.

Baculogypsina sphaerulata and Calcarina gaudichaud are common calcareous foraminiferan species, and were collected from Okinawa, Japan.

Both of the soluble and insoluble organic shell materials of B. sphaerulata and C. gaudichaudii were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). For the soluble shell materials of the two foraminifera, no signal was detected when stained with Coomassie Brilliant Blue (CBB), but strong smear bands were seen when stained with silver. CBB is known to stain most proteins, but it does not usually stain very acidic proteins. Thus we also stained the soluble shell materials with Stains-all which stains cation-binding proteins blue and the other proteins pink. When stained with Stains-all, the soluble shell material of B. sphaerulata appeared as a blue smear, and that of C. gaudichaudii showed a blue protein band of 66kDa as well as a blue smear along the lane. These results suggest that the shells of both B. sphaerulata and C. gaudichaudii contain soluble acidic proteins.

For the insoluble shell materials of the two foraminifera, no signal was detected when stained with CBB. But blue smears were seen for both species when stained with Stains-all. The signals did not appear so blue as that of soluble materials. Thus, insoluble materials are unlikely to be so very acidic, and perhaps they play a different role from that of the soluble materials.

In order to clarify the process and the underlying mechanism of foraminiferan shell formation, it is necessary to understand the function of shell matrix proteins. The results of this study provide a basis for those experiments in future.



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# Foraminiferal cellular pH control under low pH environment in the laboratory

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Foraminifera have been considered one of the major carbonate producers in marine environments. Their calcareous tests are broadly utilized as paleo-environmental indicators in various studies of earth science because their tests act as geochemical archives. Although foraminifers are unicellular organisms, they precipitate a highly complex and fine-decorated test. Their calcification mechanisms must thereby be strongly controlled by biological activity and knowledge about the cytological control on carbonate precipitation is rapidly accumulating. In particular, mechanisms of calcium and carbonate ion uptake into foraminiferal cells from ambient seawater are of great interest. Our previous studies showed the potential to understanding the biomineralization of foraminifera by the application of fluorescent indicators. Development of fluorescent indicators allow us to visualize the spacial distributions of cytological chemical environment (e.g. pH and calcium concentrations) and organelles in living cell. Observed results show that foraminifera operate their biomineralization by controlling their intracellular environments. Acidified oceanic condition by antigenic high pCO2 becomes global environmental problem in the near future. Therefore, we have extended our approach in order to observe foraminiferal cellular environment under a range of environmental pH's through laboratory culture experiments.

Keywords: foraminfiera, calcium carbonate, ocean acidification



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### Improvement of culturing experiment of planktic foraminifera using the fluorescent indicator calcein

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To reconstruct the paleoenvironmental variations using trace elements and stable isotopes of planktic foraminiferal calcite, it is necessary to determine the coefficient by vital effect based on culturing experiment. In the conventional culturing method, however, it was necessary to conduct the culturing experiment every one individual to distinguish the newly formed chambers. In that case, a lot of time was spent to obtain enough number of cultured specimens. Therefore, we attempted to construct a new culturing method of planktic foraminifera using the fluorescent indicator calcein.

Living planktic foraminifera were collected using a plankton net from the Tosa Bay, south of Kochi in Japan. Specimens of planktic foraminifera were cultured in calcein solution, which was adjusted at a concentration of 10 mg/L. We cultured individuals in calcein solution under two conditions for 48 hours and 72 hours temporarily to determine time necessary for the dye. Cultured individuals were moved to natural seawater condition after the culturing experiment in calcein solution.

We observed the calcein labeled chambers using a fluorescence microscope. As a result, fluorescence was observed in not only the newly formed final chamber but also the chamber that had been added before. Therefore, it is suggested that the surface of chambers, which were added before started culture, were also calcified in the experiment. On the other hand, it has been observed that a final chamber, which was added in natural seawater after culturing in calcein solution for 48 hours did not fluorescence. Based on our culturing experiment, the fluorescent indicator calcein is a good tool to distinguish the newly formed chamber. In addition, a temporarily culturing in the calcein solution is effective to culture more individuals of planktic foraminifera. Time necessary for temporary culturing in calcein solution is enough in 48 hours.

Keywords: planktic foraminifera, culture, fluorescent indicator calcein



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# Effects of thermal and salinity stresses on coral calcification: approach by aposymbiotic and symbiotic primary polyps

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In order to better understand the effects of high thermal and low salinity stresses on coral calcification from the aspect of coralalgal symbiosis, aposymbiotic (lacking symbionts) and symbiotic coral primary polyps were experimentally exposed to several seawater temperatures  $(27 \ 33 \ 0C)$  and salinities  $(26 \ 34 \ psu)$ . Symbiotic polyps showed non-linear calcification responses to thermal stresses whereas aposymbiotic demonstrated linear increase of calcification responses according to the increase of temperature. Both aposymbiotic and symbiotic polyps showed the linear decreases of calcification rates according to the decrease of salinity. Our results suggest that future global warming might have positive and negative impact on coral calcification, and low salinity stress, which would be caused by increase of the frequency of local floods related to future climate change, would certainly decrease coral calcification despite the existence of symbiotic algae.

Keywords: coral polyp, growth rate, environmental stress, alagal symbiosis



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# Interindividual differences in stable isotopes of benthic foraminifera: the profile of isotopic disequilibrium

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For more than 50 years, variations in stable carbon and oxygen isotopic compositions of calcium carbonate, especially foraminiferal shells, have been used for estimating paleoenvironments, such as global sea-level changes, paleotemperature, global deep-sea circulation, and huge methane release events from the seafloor. In particular, the stable isotopic compositions of calcium carbonate careous benthic foraminiferal shells have been used as tracers to determine the paleoenvironment at the seafloor.

Several factors determine the isotopic values of benthic foraminiferal shells, including the isotopic composition and temperature of the bottom water, the organic carbon flux, and "vital effects". Major isotopic variations in some species of benthic foraminifera are already being utilized as paleoindicators of bottom water conditions, but recently researchers have begun to quantify in detail the relationship between the isotopic composition of benthic foraminiferal shells and ecological characteristics such as species' microhabitat and the organic carbon flux. Such precise calibration and validation of isotopic indicators in benthic foraminifera will broaden the range of their application as paleoenvironmental tracers. However, until recently, it has not been possible to analyze the stable isotopic compositions of small carbonate samples of less than 20 micrograms and obtain results with an acceptable error range, thus, each sample used for analysis included multiple individuals, resulting in isotopic values averaged across individuals. This limitation prevented precise association of shell compositions with microenvironments.

In this study, by using a custom-made analytical system to determine stable carbon and oxygen isotopes in specimens with submicrograms calcium carbonate; the quantity required by the system is less than 1/100 of that required by conventional analytical methods, we determined the stable isotopic values of small individual shells of deep sea benthic foraminifera from core-top samples from three sites in marginal seas of the northwestern Pacific to characterize the magnitude of interindividual variation in their stable isotope ratios. We expect the results to be useful for exploring which species are most appropriate to use as pale-oindicators in paleoenvironmental studies.

Keywords: stable isotope, foraminifera, vital effect, proxy, microscale analysis, calcification

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## Effects of carbonate leaching on foraminifer stable isotopes ratios

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Stable carbon and oxygen isotope ratios were measured on 125 individual epifaunal and infaunal benthic foraminifers from two discrete intervals in a shallow-water sediment core ( $\sim$  450 m) from the Timor Sea. Methane seeps are common in the area, likely resulting in significant precipitation of secondary calcite, the effects of which were assessed by subjecting foraminifers to varying degrees of pretreatment. All foraminifers received standard cleaning with ethanol and brief sonication. A subset were further cleaned and sonicated in a dilute HCl solution ( $\sim$  0.003 M). Foraminifer tests were photographed using both reflected light and scanning electron microscopes during the course of treatment to monitor the changing degree of secondary calcite contamination as increasingly aggressive cleaning methods were employed. While foraminifers subjected to treatment with HCl exhibit a lower relative standard deviation, the variance remains high relative to expected results. Therefore, a similar experiment is being conducted on living individuals (stained with Rose Bengal) taken from a nearby multicore with complementary isotope ratios of overlying seawater, porewater, and DIC.

Keywords: stable isotopes ratios, foraminifera, carbonate leaching



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### A Holocene diatom oxygen isotopes record from the Indian Sector of the Southern Ocean

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The oxygen isotope ratio (d<sup>18</sup>O) of diatom frustules are promising as a quantitative proxy of past seawater temperature and seawater d<sup>18</sup>O, analogous to foraminifer d<sup>18</sup>O. Therefore, diatom d<sup>18</sup>O is expected to be used for paleoceanographic studies in polar regions where foraminifer shells are hard to be preserved in sediments. However, conventional methods for measurements on diatom  $d^{18}O$  have various limitations, including difficulty of processing and necessity for large amount of samples. Here we present diatom d<sup>18</sup>O records from the Southern Ocean in order to reconstruct paleoceanographic change in the Southern Ocean and the Antarctic Ice Sheet fluctuations during the Holocene. Our analytical system is composed of inductive high temperature carbon reduction and continuous-flow isotope mass spectrometry. In this system, silica is reduced by carbon at 1600 degree C to produce carbon monoxide for isotope analysis. The carbon monoxide gas is directly introduced into mass spectrometry by helium gas. Our system is capable to measure ~100 microgram of diatom d<sup>18</sup>O safely without any cumbersome procedures. A piston core COR-1PC (54016.04'S, 39046.00'E; 2,864 m water depth; 408 cm core length) was collected from the Conrad Rise, Indian sector of Southern Ocean in January 2008 by R/V Hakuho-Maru. Sediments were mostly composed of diatom ooze. The age model was established using AMS 14C dating on planktonic foraminifera (Neogloboquadrina pachyderma, sinistral) with core top and bottom ages estimated as 813 cal BP and 10,192 cal BP, respectively. We measured diatom d<sup>18</sup>O from 43 samples. We found that; i) diatom d<sup>18</sup>O in the Conrad Rise fluctuates between 38 per mil and 43 per mil through the Holocene, ii) diatom d<sup>18</sup>O show periodic variation during early to middle Holocene, iii) fluctuations of diatom d<sup>18</sup>O are different from those of foraminifer  $d^{18}O$ . We interpret fluctuations in diatom  $d^{18}O$  to be dominated by a sea water  $d^{18}O$  signal because the diatom  $d^{18}O$  fluctuations exhibit more volatility than is expected for sea surface temperature. The periodic variation of early to middle Holocene seen in the Conrad Rise is also seen in other parts of the Southern Ocean, which coincides with the Holocene Thermal Maximum. This suggests that the Antarctic Ice Sheet underwent periodic melting associated with warming since the end of the last ice age.

Keywords: diatom d18O, foraminifer d18O, Southern Ocean, Holocene, Holocene Thermal Maximum



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# Temperature dependence of Mg isotope fractionation in deep-sea coral: paleoceanographic implications as a new proxy

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This study presents magnesium isotopic composition and its temperature dependence of high Mg biogenic calcium carbonates to evaluate their potential proxy of paleo seawater temperature. Degrees of Mg isotope fractionation compared to present seawater were measured in deep-sea coral. The mean d26Mg value of deep-sea corals was -2.5 permil. Moreover, Mg isotope fractionation in deep-sea coral showed a clear temperature dependence from 2.5 to 19.5 degree. The observed temperature dependence of Mg isotope fractionation in deep-sea coral skeletons implies that a combination of proxy developments and further high-precision isotope analysis allows potential application of Mg isotopes of high-Mg calcite to an environmental proxy for water temperature. The mean Mg isotope value of large benthic foraminifera which are also composed of high-Mg calcite was -2.34 permil. Even though the precipitation rates of deep-sea coral, benthic foraminifera were several order of magnitude different, they both plot on the same regression line within uncertainty. This result suggests that kinetic isotope fractionation may not be a major controlling factor, and indicate a possible further application of Mg isotope values as temperature proxy. Deep-sea corals and benthic foraminifera also showed similar Mg isotope fractionation factor to inorganically precipitated calcite, and the slope of temperature dependence in Mg isotope fractionation is similar to that for an inorganically precipitated calcite speleothem. Moreover, Mg concentrations and the relationship between Mg/Ca and temperature were also similar between deep-sea corals and inorganically precipitated calcite. Taking into account elemental partitioning and the calcification rate of biogenic  $CaCO_3$ , the similarity among inorganic minerals, deep-sea corals and benthic foraminiferas may indicate a strong mineralogical control on Mg isotope fractionation for high-Mg calcite.

Keywords: magnesium isotope, precious coral, MC-ICP-MS, temperature dependence, proxy



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### Assessment of foraminiferal richness from deep-sea benthos using Illumina massive sequencing technology

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New massive sequencing technologies are especially relevant to assess the diversity of environmental samples from the Deep Sea since they allow retrieving extensive genetic information from limited amount of material uneasy to collect at such remote places.

We collected unsieved sediment samples from the Deep Sea of five distinct geographic regions and sequenced a very short (36 nt) fragment of the foraminiferal SSU rDNA hypervariable region.

Among the numerous phylotypes resulting from our analyses, some of them have been identified to the genus or species level and most of identified OTUs were assigned to monothalamous (single chambered) taxa. Our results, in which multi-chambered orders account for only a minority part of the richness, contrast with the classical view of foraminiferal diversity based on micropaleontologically oriented study of fossilized species.

This study emphasizes the usefulness of such technology for environmental biomonitoring perspectives regarding climate changing and human activities impact on deep-sea environment.

Keywords: benthic foraminifera, deep sea, massive sequencing

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BPO003-12

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## Genetic diversity of planktic foraminifera and the bipolarity of genotypes in the Pacific

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Molecular phylogenetic analyses have revealed high genetic diversity within planktic foraminifer morphospecies. Molecular studies of planktic foraminifera suggest these genotypes exhibit distinct ecological preferences. Moreover, their potential differences of their ecology and habitats could affect their chemical and isotopic composition of the test. However, the phylogeography of planktic foraminifera in the South Pacific is yet to be revealed. This study shows the phylogeography of *Globigerina bulloides* in the South Pacific. Living planktic foraminifera specimencs were collected during R/V Mirai cruise (MR08-06). Molecular phylogeny and Identification of genotypes were based on partial small subunit ribosomal RNA gene (rDNA). We confirmed that one bipolar genotype in the Atlantic (type IIa) also exhibits bipolar distribution in the Pacific. Our results also suggest that trans-equatorial dispersal occurred in the East Pacific genotype.

Keywords: Planktic foraminifera, Genetic diversity



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### Chlorophyll derivatives as proxies of the marine photosynthetic production and succeeding biogeochemical processes

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Chlorophylls produced in the oceanic environment undergo potentially both abiotic and biotic decompositions. A majority of chlorophylls is to be destroyed through so-called Type II process that cleaves tetrapyrrole macrocycle oxidatively. A portion of chlorophylls, however, experiences the Type I process in which tetrapyrrole macrocycles are preserved intact with various degree of defunctionalization, hence surviving into sediments. These survived chlorophyll derivatives could be further altered chemically to be fossil porphyrins, red pigments extracted from sedimentary rock as old as the Proterozoic.

Although chlorophyll derivatives in marine sediments have been studied for almost a quarter century, our new HPLC method that excludes analytical artifacts revealed that major chlorophyll derivatives are pyropheophytins, cyclopheophorbide enols, chlorin carotenyl esters, and chlorin sterile esters. The chemical structures of these derivatives strongly suggest their formations through metabolic processes, not a simple abiotic product. In addition, derivatives of chlorophyll b, chlorophyll d, and bacteriochlorophyll a that should be derived from specific taxa are also commonly identified in marine surface sediments. These chlorophyll derivatives are thus potential proxies of not only photosynthetic productions but also metabolic, biochemical processes in the water column.

We pursue uses of these chlorophyll derivatives as proxies of water column biogeochemical processes by understanding taphonomy of chlorophylls. Here, we particularly focused in cyclopheophorbide enols which has turned out to be quantitatively most important after our new analytical method. I is thus important to elucidate the environment of it's formation and the organisms/processes concerned. We considered various analytical processes for the best method quantifying cyclopheophorbide enols from both water and sedimentary samples. We also determined physicochemical properties on hemi-synthesized cyclopheophorbide enols.

Keywords: chlorophyll derivatives, chlorophyll d, fossil porphyrin, cyclopheophorbide enols, marine photosynthetic production, biogeochemical processes



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# Distributions of archaeal membrane lipid and DNA within the modern coastal shallow marine water column

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TEX86 thermometry, based on the number of cyclopentane moieties in the glycerol dialkyl glycerol tetraether (GDGT) lipids of membranes of Crenarchaeota, has been utilized as a paleotemperature proxy for the last ten years (Schouten et al., 2002 and others). This proxy was derived from an empirical relationship between annual mean sea surface temperatures and TEX86 values of core top sediments (Kim et al., 2008). This empirical relationship has been subsequently supported by the results of incubation experiments of marine Crenarchaeota (Wuchter et al., 2004; Schouten et al, 2007). Based on analyses of particulate organic matter from the modern open ocean, the membrane lipids preserved within core top sediments were biosynthesized at about 100 m deep within a water column (Wuchter et al., 2005). The distributions of isoprenoid chain produced within a water column are finally preserved within deep sea sediments (Takano et al, 2010). However, because this proxy has been developed and designed for open ocean settings, applicability of this unique proxy to the shallow coastal ocean, which can be easily affected by the anthropogenic climate change, is still uncertain.

Here we present abundances and distributions of archaeal membrane lipids within the water column at Beppu bay in the Seto Inland Sea. Beppu bay is an archetypal silled basin of 70 m depth, with bottom water that are decoupled from the surface water in summer, producing anoxic conditions in the bottom water. The abundance of GDGTs in the anoxic water mass was considerably higher than those of the oxygen rich water mass. Nonetheless, low particulate organic carbon content in the anoxic water mass indicates that the excess concentration of GDGTs implies in situ biosynthesis rather than accumulation of organic matter settled from the overlying oxygen rich water mass. Calculated TEX86 values from these lipids show a significant positive correlation with the in situ water temperatures observed, confirming the appropriateness of this proxy for shallow coastal ocean paleothermometry. On the other hand, at least two different genotypes were identified from archaeal DNA distributions within the water column. One genotype identified in samples recovered from water depth greater than 30 m was not found in samples recovered from the very shallow water, implying GDGTs in deeper water are synthesized by the different genotype than those found in the surface water.

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Keywords: Archaea, TEX86, DNA, coastal shallow marine



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## Effect of surface ocean stratification on the distribution of planktic foraminifers

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Production and abundance of planktic foraminifer species are correlated to a varying degree to surface ocean temperature (SST), salinity, primary production (PP), i.e., nutrient utilization, availability of prey, and the turbidity of surface waters. The regional and seasonal variability of surface ocean stratification does encompass different environmental parameters. In turn, planktic foraminifers facilitate the reconstruction of past surface ocean stratification on a seasonal and longer term, for example, the modern and Pleistocene surface ocean of the northern Arabian Sea. Changes in stratification are confirmed by the ecological significance of planktic foraminifer species.

Keywords: planktonic foraminifera, stratification



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# Stable isotopic composition of polar planktonic foraminifera: Results from sediment trap study in the North Pacific

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Planktonic foraminifera provide a record of the upper ocean environment through their species assemblage and individual tests. Oceanographic condition at high-latitude plays an important role in the changes in global ocean environment, thus it is significant to assess the detailed past ocean situation in this region. Neogloboquadrina pachyderma (Ehrenberg) mainly distribute and dominate in sub-polar and polar region, thus it is an important species for the reconstruction of paleo-oceanography in the high latitude. In the study, we investigated the changes in oxygen isotope of N. pachyderma (sin.) using a 3.5-year sediment trap sample (about two-weeks resolution), and inferred 1) vital offset value in the area, 2) size effect, and 3) apparent calcification depth. In this study area, offset values were approximately 1 permil throughout the sampling period for both size except for 2000 (around 0.8 permil offset). Oxygen isotope values of N. pachyderma (sin.) exhibit definite seasonal variation throughout the sampling period, 1998 -2001. d<sup>18</sup>O values of both small (125-180 um) and large (180-250 um) shells decreased in autumn (September-October) with a minimum around September-October, and increased in spring with a maximum value around April-May. They ranged from 0.58 to 2.53 permil for smaller shells and 0.52 to 2.27 permil for larger shell throughout the study period. The differences in d<sup>18</sup>O between small and large shells generally degreased during winter, and increased summer. During winter, water column is well mixed, and differences is small (0.14-0.21 permil), while water column become stratified during summer, and differences became big (0.30-0.51 permil). During stratified water column period, larger shell mostly represented lighter  $d^{18}O$  values, up to 0.54-0.79 permil lighter values. The large seasonal change in difference of  $d^{18}O$  suggests that the different d<sup>18</sup>O between shell size would be mainly affected by water column situation rather than individual kinetic/metabolic effect. During stratification period, larger shell and smaller ones would mainly calcify at 24-35m and ~45m water depth, respectively. On the other hand, both size mainly reflect the water environment at 45-55m to their shells during water column mixed.

Keywords: planktonic foraminifer, Neogloboquadrina pachyderma, oxygen isotope ratio, sediment trap, northwestern North Pacific