

Culture experiments to better understand biomineralization under varying geochemical conditions

--Zombie factory in Japan: the first trial on Scandinavian foraminifera--

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Ocean acidification is a consequence of ongoing global climate change, and it may have severe impacts on calcifying organisms. This process may be amplified in coastal regions where erosion, land run-off and eutrophication contribute in lowering the pH.

The aim of our study is to define how bottom-dwelling foraminifera will respond to pH changes in the Skagerrak/Baltic Sea region in addition to other environmental stressors such as temperature and salinity changes.

First results demonstrate variations in shell preservation of the living foraminiferal fauna. The more marine foraminifera have pristine shells, while the low salinity Baltic ones are more or less dissolved and only inner organic linings are still visible. However, these "zombie" foraminifera are still alive, as determined from the CellTracker Green labelling. The dissolution can be linked to the lower pH in the Baltic.

In order to investigate the zombie foraminifera further, we set up culture experiments on healthy foraminifera under controlled geochemical parameters. We discovered that lowering the pH is not sufficient to create zombie foraminifera and that abrasion between foraminifera and sediment is probably involved in the shell loss. Those results highlight the multiple factors affecting the balance of benthic ecosystems subjected to environmental stressors.

Keywords: benthic foraminifera, ocean acidification, laboratory experiment

浮遊性有孔虫に対する分子系統・形態計測・地球化学の複合分析

Approach to comprehensive analyses of molecular phylogeny, morphometrics, and geochemistry of planktonic foraminifera

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Planktonic foraminifera have been widely used for the studies of paleontology and paleoceanography. Stable oxygen and carbon isotope ratios of planktonic foraminiferal shells are the major proxies for paleo-environment, as these isotopes are affected by inhabiting water temperature and the chemical components. Most of previous studies have been conducted with the traditional species concept based on the morphological differences of their calcareous shells. However, molecular phylogenetic studies have unveiled the presences of multiple cryptic species in a single morphospecies of planktonic foraminifera. Such a high diversity of planktonic foraminifera suggests that the current paleoceanographic proxies are underestimated due to the mixed information of multiple biological species. Therefore, it is the urgent task to re-assess the ecological and geochemical characters at each biological species. For this purpose, we need to work out the implementation method, which combines the multiple analyses: DNA, morphological, and geochemical analyses, for a single individual. The DNA extraction method by using the buffer based on the guanidium isothiocyanate now enables us to preserve the calcareous shells after the extraction. By using this method, we can detect the morphological and geochemical characters on a same individual, which is identified by the molecular technique. However, the thermal and chemical reactions of this DNA extraction method to the calcareous shells are still unknown. In this study, we test whether or not the method of the molecular experiment physically and chemically damage the calcareous shells. We collected the living specimens of planktonic foraminifera and divided them into three experiment sets. In the first set, the specimens were applied to the DNA extraction with incubation process at 70 °C for 40 minutes as usual. In the second set, the time for incubation was three times longer than the first one. Through the comparison between these two patterns, the effect of the incubation time to the calcareous shells can be detected. We also prepared the specimens just collected from the sea-water but without the process for the DNA extraction, as a control. The densities of the calcareous shells were measured by the micro-focus CT scanning, and then their stable oxygen and carbon isotopes were analyzed one by one. The comparisons of physical and geochemical characters of the planktonic foraminiferal shells showed that the heat and chemical treatments concerning the DNA extraction never changed the shell component. Thus, we succeed to establish the experimental methods, which investigate the morphological and geochemical features for each biological species from single individual.

キーワード：浮遊性有孔虫、分子系統、CTスキャン、安定同位体

Keywords: planktonic foraminifera, Molecular phylogeny, micro-focus CT scan, Stable isotopes

放散虫アカンタリア目骨格中のシリカ (SiO₂) 含有についての検討

A study on chemical composition of living acantharian (Radiolaria) shell

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放散虫とは、オパール(SiO₂+nH₂O)やSrSO₄からなる鉱物質の内殻を持つ海洋性動物プランクトンのことである。現生の放散虫類はオパールの殻をもつコロダリア目、ナセラリア目、スプメラリア目、SrSO₄から成る骨格をもつアカンタリア目、そしてタクソポディア目の5目に分類されている。本研究では、放散虫の内骨格形成過程の観察のため、黒潮表層海域の放散虫を採取し室内における飼育実験を実施した。殻の成長観察については、蛍光試薬を用いオパールの付加成長部位を発光させる手法(Ogane et al., 2010)を用いた。その結果、従来SiO₂成分が含まれていないと考えられてきたアカンタリア目の生体骨格にも Siが含有される可能性が示されたのでそれについて発表する。

検討に用いた現生放散虫は、黒潮海域であり放散虫が多種生息する高知県柏島近海にて採取した。プランクトン採集は2015年の7月12日、11月30日、2016年1月11日の全3回行い、採取した放散虫を目別に数個体ずつピックアップし、専用の飼育装置に入れて飼育を行った。飼育の際、水温を常に27℃に保ち、12時間LEDライトの照射、消灯を相互に行なった。飼育装置にて24時間静置した後、HCK-123蛍光試薬を投薬し、24時間~30時間経ったものをスライドに封入した。蛍光試薬を入れてから生体内に試薬が取り込まれその部分を蛍光発光させる事で、SiO₂を含む骨格の付加成長が起きた部分と生体内でのSi元素分布を知ることができる。作成したスライドは愛媛大学理学部設置の共焦点レーザー顕微鏡(Carl Zeiss LSM510)を用いて観察した。スライドには波長488nmのArレーザー光を照射し、HCK-123蛍光試薬投薬後の蛍光発光を観察した。また、Siの含有についての真偽を検討するため、さらにアカンタリア骨格のFE-SEMおよびWDS分析を行った。

採集した放散虫類の内、今回は4個体のアカンタリア目の結果について報告する。蛍光試薬投薬後

Acanthometra muelleri (個体番号: 20150712A-1)、*Amphilonche complanata* (個体番号: 20151130A-1)は24時間飼育、*Acanthostaurus conacanthus* (個体番号: 20160111A-1)、*Acanthometron pellucidum* (個体番号: 20160111A-2)は30時間飼育し、スライドに封入した。各個体を共焦点レーザー顕微鏡にて観察したところ、*Acanthometra muelleri*は表層の骨針と中央部、*Amphilonche complanata*は骨針の根元と中央の一部、*Acanthostaurus conacanthus*は骨針の根元の表層部、*Acanthometron pellucidum*は折れた骨針と新しく成長中の骨針の一部にSiO₂の付加が確認できた。更に、アカンタリア目*Amphilonche complanata*の異なる個体でFE-SEMおよびWDS分析を行った。アカンタリア目の主成分はSrSO₄なので、今回はSr、S、Oと、今回注目しているSiについて元素マッピング分析を行った。主成分であるSr、S、Oは骨格全体に広く含まれていたが、その中に部分的にSiの分布も確認できた。殻周辺の付着物以外でSiの反応が確認できた箇所を拡大して分析したところ、骨針の先端表層部分に集中してSiが含まれていることが確認できた。

以上の蛍光試薬を用いた飼育実験結果および生体骨格の元素マッピング分析により、従来骨格にSiO₂を含まないと考えられてきたアカンタリア目は、生きている状態においては部分的にSiO₂を含有する事が示された。またそれは、殻の成長活発な部位において顕著で、アカンタリア目の骨格形成においてSiO₂成分が重要な役割を果たす事を示唆している。

キーワード: 放散虫、アカンタリア、骨格組成

Keywords: Radiolaria, Acantharia, skeletal composition

アワビ類2種の殻・軟体部成長、貝殻の安定同位体組成への海洋酸性化影響

Effects of ocean acidification on shell and somatic growth, and stable isotopes of shell carbonate of two species of abalones

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Ocean acidification is now one of the important issue to appreciate the impact on marine calcifiers (IPCC, 2013), and potentially affects their survival, calcification, growth, physiology and development. To evaluate the effects of elevated CO₂ levels on shell and somatic growth, and stable isotope compositions of two species of abalones (*Haliotis discus discus*, *Haliotis gigantea*), we conducted culture experiments at three treatment levels of seawater pCO₂ (400, 750, and 1200 μatm), at approximately 23 °C.

The effects of seawater pH on calcification (shell width, shell weight) was non-significant in both species. On the other hand, the positive relationships between pH and wet weight of soft tissue of two species were observed. Their adjusted wet weight of soft tissue at 1200 μatm was significantly greater than that at 400 μatm. These results suggest that elevated pCO₂ affected their metabolism (e.g. higher metabolic rates to maintain homeostasis).

Stable oxygen isotope compositions of outer (calcite) and inner (aragonite) shell layers of two species showed non-significant relationships with pH. The negative correlations between carbon isotope compositions and pH of both layers appeared in both species, and the slopes of these relationships of shells were lower than that of dissolved inorganic carbon (DIC) of seawater. We estimated the equilibrium values of carbon isotope compositions at each pCO₂ treatment, and the difference between the carbon isotope compositions of shell and equilibrium values showed gradual increases in shell carbon isotope compositions with decreasing pH. Thus, the pCO₂-induced change in metabolism of abalones might appear in carbon isotope compositions of shells as the metabolic effect.

キーワード：海洋酸性化、飼育実験、安定同位体、アワビ類、代謝、バイオミネラリゼーション

Keywords: ocean acidification, culture experiment, stable isotopes, abalone, metabolism, biomineralization