

## Analysis of benthic community food web at gas hydrate deposits of Joetsu Basin

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The ocean floor ecosystem depends largely on photosynthetically-derived organic matter and the degraded products. Also, we can find some ecosystems supported by chemosynthetically-derived carbon in deep seafloor. Chemosynthetic organisms use inorganic material, such as sulfur and methane as source of energy. In some methane seeps, benthic chemosynthetic communities are dominated by macrobenthos, such as gastropod, bivalve, crustacean and pogonophoran. It is known that many red snow crabs often congregate around cold seep in the Sea of Japan and that *Provanna* known as a chemosynthetic gastropod species in hydrothermal vent and cold seep also inhabits. However, their relationship between their ecology and chemical energy derived methane seep remains unknown.

To better understand the ecology of benthic community at gas hydrate deposits of Joetsu Basin, we analyzed their food web using the stable isotopic ratios of carbon and nitrogen ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) for the surface sediment and benthic fauna, such as red snow crab, eelpout, whelk and polychaete. Carbon and nitrogen signature reflects carbon source and trophic level, respectively.

Sampling of benthic fauna at seeps using a slurp gun and a strainer was conducted during 24-30 September 2013 at Umitaka Spur and Torigakubi Spur. We obtained surface sediment sample using MBARI ROV coring system at depths of 0-2.5 and 2.5-5 cm below seafloor. Sampling at reference site was conducted at Joetsu Knoll on 23 September 2013. Macrofaunal sample was dissected on board and frozen at -20 °C. Meiobenthos were removed by sieving of sediment samples and frozen. In laboratory, faunal sample was powdered after freeze drying. We removed carbonate from the sample by HCl steam treatment. Sample was refilled after neutralization by NaOH steam and drying. We measured stable isotope signature using IRMS (Flash 2000, Thermo Scientific Inc.). Similarly, sediment sample was removed carbonate by HCl solution, dried on a hotplate and measured.

Our result shows that no distinct difference between the isotopic signature of red snow crab and one species of eelpout, *Bothrocara hollandi* collected both at seep site and reference site. Other macro carnivores such as a squid and some individuals of whelk also have similar isotope signature. Biplot of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values suggests that the red snow crab mainly eat small crustaceans including amphipods and shrimps although we know the crab eats squids and cannibalizes by ROV observation at sea floor. Our results indicate that food habitat of red snow crab depends on photochemically-produced organic matter even in an individual inhabiting around methane seep. That means the red snow crab is a component of phototrophic ecosystem.

In this investigation, isotopic signature of *Provanna* collected at Joetsu Basin shows similar value with that of polychaete. We collected another species of eelpout, *Petroschmidia toyamensis*, which have isotopic value showing they eat *Provanna* and polychaete differently from *B. hollandi* as a component of photosynthesis ecosystem. Our direct observation of gut supports the predator-prey relationship between the former eelpout and *Provanna*. Small  $\delta^{13}\text{C}$  of *Provanna*, polychaete and *P. toyamensis* indicates that they depend on methane-derived carbon microorganism produced. That suggests that they are components of chemosynthetic ecosystem at Joetsu Basin.

Meanwhile, the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of a whelk, *Buccinum tenuissimum* varied at site in spite of same species. We considered that the food habit of the whelk was affected by site-specific factors rather than the maturity.

Our research showed that we could analyze the benthic food web at seep site more precisely rather than direct observation and elucidate the dependence of benthic community on methane-derived carbon using stable isotope analysis of widespread species.

This work was conducted as a part of METI's methane hydrate exploration project.

Keywords: methane hydrate, benthic fauna, food web, stable isotope analysis