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# RAPID CREATION OF A REDUCED ENVIRONMENT AND AN EARLY STAGE OF A CHEMOSYNTHETIC COMMUNITY ON CATTLE BONES AT THE DEEP-SEA BOTTOM IN SAGAMI BAY, CENTRAL JAPAN

*An experimental approach to test the stepping-stone hypothesis  
for chemosynthetic community dispersion*

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CATTLE-BONE COMMUNITY  
CHEMOSYNTHETIC COMMUNITY  
DEEP SEA  
REDUCED ENVIRONMENT  
STEPPING-STONE HYPOTHESIS  
BIOGEOGRAPHY

CARCASSES DE VERTÉBRÉS  
ET COMMUNAUTÉ INDUITE  
COMMUNAUTÉ CHIMIO SYNTHÉTIQUE  
MER PROFONDE  
ENVIRONNEMENT RÉDUIT  
MICROBIOTOPES RELAIS ?  
BIOGÉOGRAPHIE

**ABSTRACT.** –The ability to create a locally reduced environment that harbors a deep-sea chemosynthetic community in the deep sea was tested using thigh bones of cattle as an analogue of a dead fall of a large marine vertebrate. The experiment was carried out in December 1993 by placing the bones on the bathyal seafloor in Sagami Bay, Japan using the manned submersible Shinkai 2000. By one year later, a reduced environment had developed in the sediment around the bone. On the bone, a white microbial mat grew thickly and a galatheid crab and lithoid crab gathered. Our results confirmed that bones of a large animal can result in a locally reduced environment and become stepping stones that may aid long-distance dispersion of the larvae of animals of chemosynthetic communities.

**RÉSUMÉ.** – La possibilité de l'apparition d'un environnement localement réduit avec une communauté chimiosynthétique profonde a été testée dans les grands fonds en utilisant des fémurs de bétail simulant l'arrivée sur le fond de carcasses de grands vertébrés marins. L'expérience, réalisée en décembre 1993, a consisté à déposer les fémurs sur le fond de la zone benthique de la Baie de Sagami, au Japon, à l'aide du submersible Shinkai 2000. Une année plus tard, un environnement réduit s'était développé dans les sédiments voisins. Un épais tapis microbien blanc s'étendait sur les fémurs et des Crustacés galathéides et lithoïdes étaient présents. Ces résultats confirment que les os de grands vertébrés marins peuvent être à l'origine d'un environnement local réduit et peuvent constituer des microbiotopes relais participant à la dispersion à grande échelle des larves d'organismes en provenance de communautés chimiosynthétiques.

## 1. INTRODUCTION

Deep-sea environments are generally oxic because they are nutrient-poor, particularly in comparison to many coastal margins (Thiel, 1975). In special geological or oceanographic settings such as ocean floor spreading centers, subduction zones, petroleum seeps, oceanic upwelling areas and anoxic basin floors, however, anoxic or euxinic conditions exist (Ohta, 1990). Chemosynthetic communities supported by the autotrophic activity of sulfide- or methane-oxidizing bacteria and characterized by large benthic animals such as vesicomyids (Vrijenhoek *et al.*, 1994), limpets (McLean, 1992) and vestimentiferan tube worms,

develop in such areas. These chemosynthetic communities are distributed patchily in the deep sea, because of the patchy distribution of the special settings. The question is how the members of these communities colonize distant sites across stretches of oxic ocean floor.

Recently, communities with similar species compositions to cold seep fauna were found on whale bones lying on the sea floor of the Santa Catalina Basin, California (Smith *et al.*, 1989; Smith, 1992; Bennett *et al.*, 1994) and on the Torishima Seamount, Japan (Fujioka *et al.*, 1993). The establishment of such whale-bone communities has been explained as follows. The organic matter contained in the bone marrow comes out slowly like the interstitial water of a cold-seep,

creating a reduced environment around the bone, and the hydrogen sulfide produced by sulfide-reducing bacteria growing in the anoxic conditions is used by sulfide oxidizing bacteria as the substrate of chemosynthesis (Smith, 1992; 1994). Smith *et al.* (1989) proposed the hypothesis that whale bones are stepping stones that harbor the larvae of hydrothermal vent or cold seep fauna and help the dispersion of these animals to other vent or seep sites. This hypothesis may be supported by the results of a molecular biological study of the mitochondrial DNA of *Calyptogena* species around Japan (Kojima *et al.*, 1994). The study revealed that RFLP pattern of *Calyptogena soyoae* of Sagami Bay and an undescribed *Calyptogena* species from Iheya Ridge of Okinawa Trough are very close. The similarity suggests the occurrence of recent gene flow between populations that are isolated by great distances. To test the stepping-stone hypothesis in a direct way, we performed an experiment at a permanent deep-sea station in Sagami Bay, central Japan. Instead of whale bones, thigh bones of cattle were placed on the sea floor. The same site was visited one year later, and the change of environment around the bones and colonization of organisms on and around the bones were determined.

## 2. MATERIALS AND METHODS

Cattle bones were placed on the deep-sea floor adjacent to a platform built for geophysical studies at a permanent deep-sea observing station (St. OBB) in Sagami Bay, central Japan (35°00.86'N, 139°21.59'E, depth 1445m; Fig. 1). The Japan Marine Science and Technology Center (JAMSTEC) operates shuttle dives of the deep-sea submersible "Shinkai 2000" to this site every year. Fifteen kilometers both west and east from the site, there are cold seeps associated with active fault systems, and chemosynthetic communities develop at these seeps (Ohta, 1990).

Cattle bones were prepared in two different ways. In the first method, two thigh bones, 50 cm long and 10 cm in diameter, were bound with rope. At an end of the rope, a floating ball was tied to create a site marker. The bones had practically no flesh except for a small amount of fatty materials (Fig. 2(A)). These bones were placed directly on the sea floor close to the platform at St. OBB. For the second method, cattle ribs were cut into pieces 8 cm long and put inside a glass bottle. The bottles were placed on an in situ culture tray (Kitazato, 1995), and the tray was placed near the platform. These bones were stationed on December 4, 1993.

The submersible visited St. OBB again on December 2 of the next year. Organisms on and around the thigh bones were observed in detail, and sediment cores 36 mm in inner diameter and 30 cm in length were collected beside and about 50 cm away from the bones. The culture tray that held the vials that contained the cattle ribs was also recovered on deck.

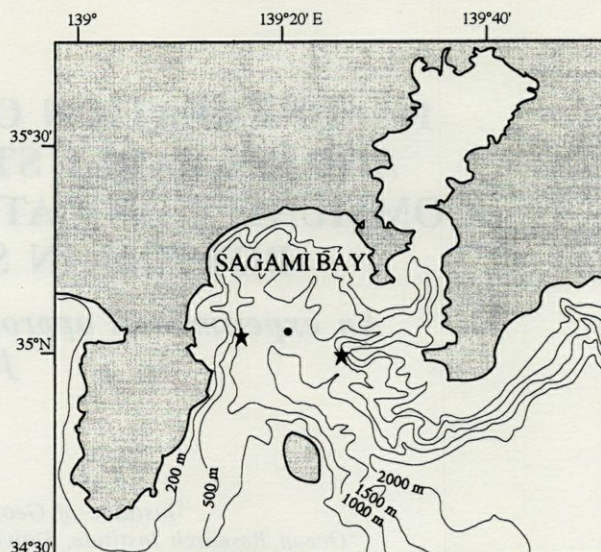


Fig. 1. - Map showing the study area. Closed circle shows the permanent deep-sea station OBB. Asterisks indicate the cold seep sites.

## 3. RESULTS

After one year, the thigh bones were patchily covered with yellowish-white filamentous organisms (Fig. 2 (B)). The morphological characters of the organisms is similar to those of sulphide oxidizing bacteria, though we have not yet identified the organisms unequivocally. Some parts of the bones had changed from ivory-white to black in color. The bones were still hard to break after one year.

When we collected sediment core samples beside the bones, we noticed that the subsurface sediments around the bones had changed from greenish brown to black (Fig. 2 (B)). Observation of the core on board revealed that black sediments developed between 1 and 4 cm deep. We smelled hydrogen sulfide, suggesting that this black color is derived from reduction of iron by sulfur in an anoxic environment.

A reducing environment was also created in the bottles where ribs had been placed, so that both ribs and newly deposited sediments in the bottles were black. A weak smell of hydrogen sulphide came from these bottles as well. No evidence of settlement of macrobenthic animals was found in the bottles.

One individual of galatheid crab was associated with the bones. Using its claws, the crab frequently fed on bacterial mats growing on the bones. One individual of an anomuran stone crab (*Paralomis multipina*) burrowed itself in the hollow that existed under the thigh bones.

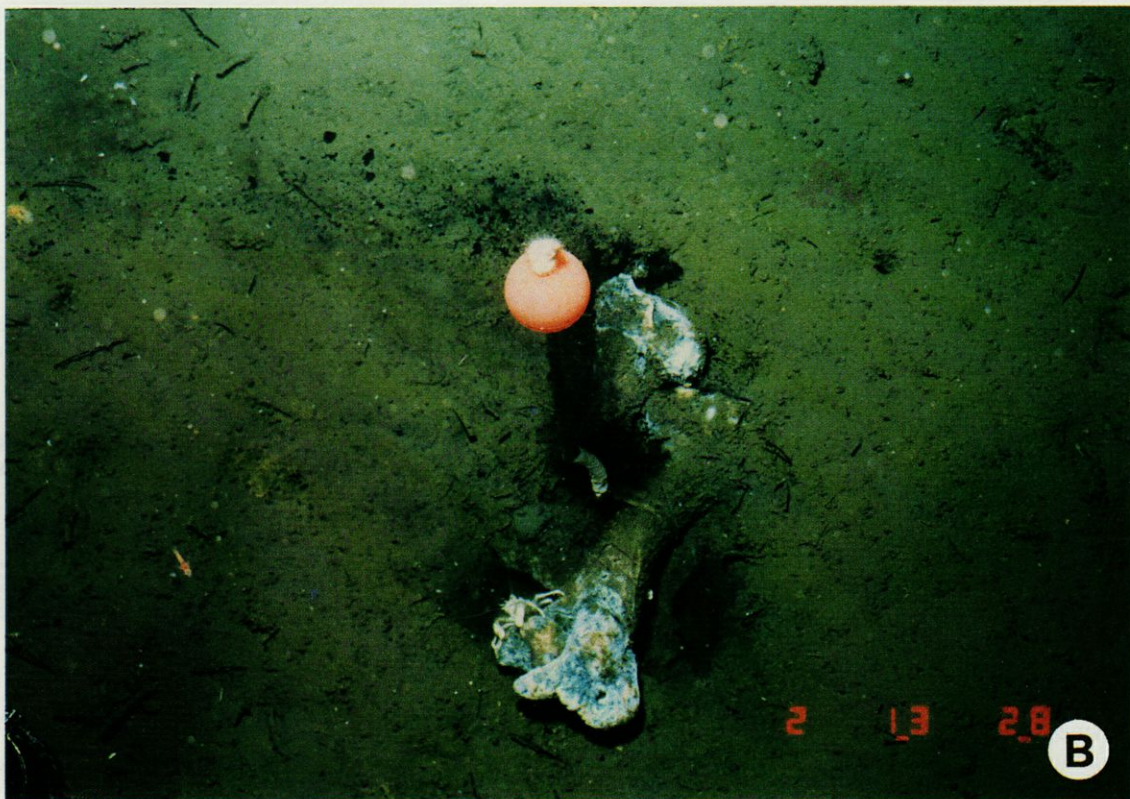
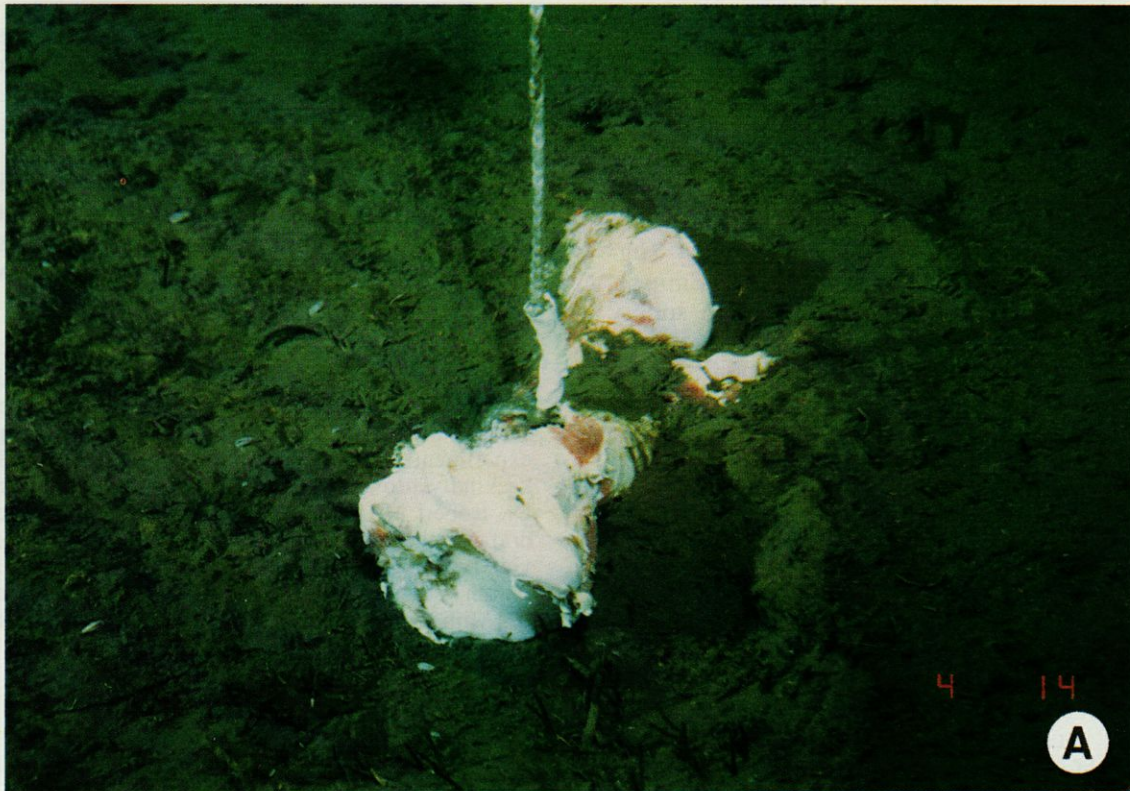


Fig. 2. - Thigh bones of cattle placed on the sea floor. (A) Immediately after the placement on December 4, 1993. (B) The same bone after one year. The surface was covered patchily with a mat of an organism resembling the bacterium *Beggiatoa*. A galatheid crab was found on the lower-left corner of the bones. A spiny stone crab (*Palalomis multispina*; not shown in this photograph) inhabited a hollow that was present under the bones. Note the black sediment distributed to the upper-left of the bones. This sediment dropped off when a core sample was taken adjacent to the bones. The black sediment suggests that a reduced environment was created in the subsurface layer of the sediment around the bones.

#### 4. DISCUSSION

We observed distinct environmental changes of the experimental site around the bones one year after we placed the cattle bones on the sea floor. The occurrence of black sediment strongly suggests development of a reduced environment, which is thought to be created by a rich supply of organic matter through seepage of the bone marrow. The flesh attached to the bone is not likely to be a source of the organic matter, because we observed that immediately after we placed the bones on the sea floor, such materials were intensively fed upon by deep-sea fishes and amphipods. Our result suggests that there is enough organic material in the bone marrow to create and maintain a reduced environment on and around it for at least one year.

It is probable that feces of amphipods that gathered immediately after we placed the bones are a source of the organic matter. So far as we observed at the sediment surface, however, amounts of fecal pellets at the sediment surface were not much different between the bone site and the normal sea floor.

The reduced environment seems to produce hydrogen sulfide, the basic material supporting chemosynthetic communities. If yellowish white microbial mats on the bones are a species of sulfide oxidizing bacteria, they may be supported by hydrogen sulfide produced by the sulfide-reducing bacteria growing in the anaerobic conditions around the cattle bones.

The galatheid crab is a typical member of cold seep and whale bone communities. The existence of the crab on our experimental bones suggests that the environment around the bone is similar to that of other chemosynthetic communities. Our result also suggests that the crab can find and colonize newly-established reduced environments at least within one year.

Other large animals characteristic of chemosynthetic communities such as vesicomyids, limpets and vestimentiferan tube-worms were not observed on or around the bones. Adults of these species are not likely to migrate for a long distance. The larvae of these species might settle around the experimental site. The larvae that settle in cold seeps or around bones, however, would not grow as rapid as those in hydrothermal vents (Lutz *et al.*, 1994), because the ambient temperature is lower and the supply of hydrogen sulfide is less than at hydrothermal vent settings. Even if the larvae of vesicomyids settled around the experimental bones, it is not likely that they would grow large enough to be found within one year by observation from the window of the submersible.

Results of the present experiment suggest that deadfall of large marine vertebrates such as whales can create an environment suitable to the establishment of chemosynthetic communities in the deep sea. To prove the stepping stone hypothesis, however, it is necessary to carry out longer observations of the experimental bone until we confirm that the reduced environment lasts long enough to allow settled individuals to grow and reproduce another generation.

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