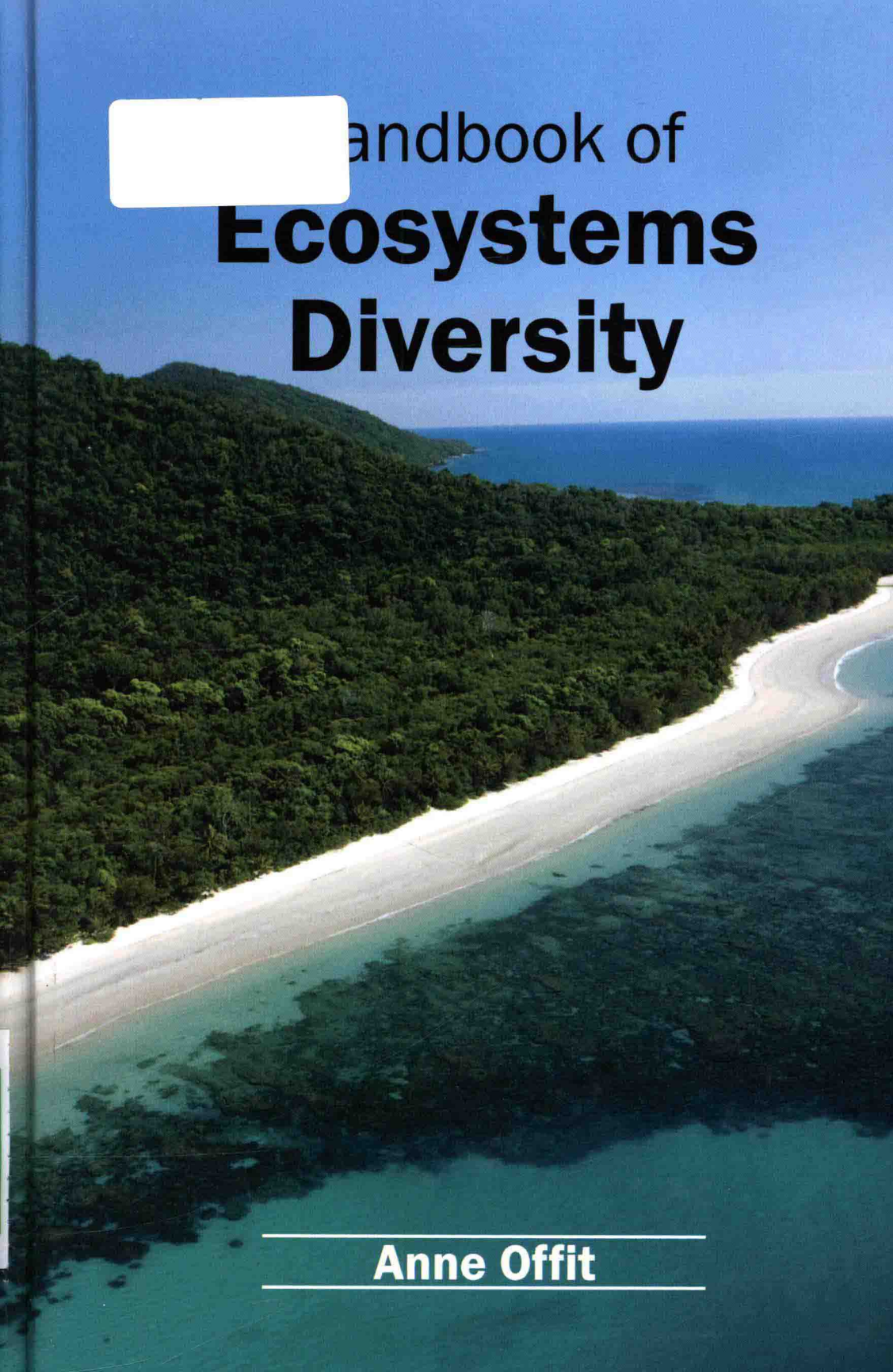
andbook of
Ecosystems
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Anne Offit

Handbook of Ecosystems Diversity

Edited by **Anne Offit**

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Handbook of Ecosystems Diversity

Preface

In my initial years as a student, I used to run to the library at every possible instance to grab a book and learn something new. Books were my primary source of knowledge and I would not have come such a long way without all that I learnt from them. Thus, when I was approached to edit this book; I became understandably nostalgic. It was an absolute honor to be considered worthy of guiding the current generation as well as those to come. I put all my knowledge and hard work into making this book most beneficial for its readers.

Ecosystems offer huge diversity around the globe and facilitate several advantages with different regions. Certainly ecosystems provide diverse services to humankind through their composition and structure but the bearable levels are unidentified. In this new backdrop of frivolity and climatic variations, these ecosystems suffer notable modifications enlarged by domestic uses of which it was subjected to. The conservation of these eco-systemic services needs a fair perception of their complexity. The function of research is not only to describe these ecosystems but also to undoubtedly describe the allowable usage levels. Their description proves to be significant not only for the local citizens that use them but also for the protection of biodiversity. So the measurement, management and protection of ecosystems need unique and varied techniques. For all these reasons, the purpose of this book is to introduce a universal view on functioning of ecosystems, a species ecological modeling - homotopy analysis and extreme climatic events as drivers of ecosystem change.

I wish to thank my publisher for supporting me at every step. I would also like to thank all the authors who have contributed their researches in this book. I hope this book will be a valuable contribution to the progress of the field.

Editor

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How to Keep Deep-Sea Animals

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1. Introduction

The ocean covers 71% of the surface of the Earth. The deepest trench is the Mariana Trench, with a depth of 11000 m. The average elevation of terrestrial areas is about 840 m high, while the average depth of the oceans is about 3800 m. This means that the average elevation above the rock substrate for the Earth is -2440 m – i.e. within the seas. Moreover the volume of the ocean is 300 times the volume of all terrestrial areas combined. The coastal area of the ocean is less than 10% of the total area of the oceans. The deep sea lies under the epipelagic zone, which is the layer from the surface to 200 m depth. The volume of the deep sea is over 95% of the total volume of the oceans. The deep-sea realm is the largest biosphere on the Earth. There are three categories of habitat in the deep-sea realm. One is the deep-sea floor, the second is the bentho-pelagic layer, which is the layer from the deep-sea floor up to an altitude above the bottom of 100m, and the third is the mid-water zone, which is between the epipelagic layer and the bentho-pelagic layer. The mid-water is an extremely important realm, and holds the key to elucidate the cycles of matter in the ocean, carbon transportation from the surface layer to the deep sea, and the interaction between the behavior of oceanic circulation with global warming and the lives of deep-sea animals. However, information about mid-water biology is extremely limited because of the difficulty of sampling swimming (nekton) and floating (plankton) animals in the mid-water. The mid-water community is one of the most mysterious of all deep-sea communities.

The deep sea is a mysterious kind of Inner Space for us, even though the ocean is closer than Outer Space. High water pressure in the deep sea keeps us from easily exploring this realm. Studies of deep-sea animals have long been carried out through net sampling using tools such as dredges, trawl nets, plankton nets and line fishing. However, the development of deep-sea crewed submersibles and remotely-operated vehicles (ROVs) has drastically changed the way we study deep-sea biology. These deep-sea survey tools allow us to visit places where fishing nets cannot trawl, e.g. deep-sea valleys, outcrops of base rocks, cliffs, gaps, hydrothermal vent areas and cold seep areas. Moreover, they allow us to observe deep-sea animals and their behavior *in-situ*. This is particularly true of gelatinous zooplankton, which are vastly understudied because their fragile bodies are easily damaged and destroyed by fishing nets. Crewed submersibles and ROVs have enabled rapid progress

in the study of gelatinous zooplankton because they allow us to observe their behavior and collect them in pristine condition (Miyake et al., 2001; Robison, 2004).

The most history-changing moment for deep-sea biology is undoubtedly the discovery of a deep-sea chemosynthetic ecosystem off the Galapagos Islands in 1977 (Corliss & Ballard, 1977; Corliss et al., 1979). This discovery is widely considered to be one of the greatest discoveries of the 20th century. Hydrothermal vents can spew hot water at temperatures above 300 °C at 2600 m depth. Many animals live around hydrothermal vents and most of the animals do not depend on solar energy like we do, but on heat and chemical energy from inside the Earth. Hydrothermal vent fluids can include hydrogen sulfide and methane from deep within the Earth. Bacteria use these chemicals for chemosynthesis - the making of organic molecules using chemical energy. Primary production in deep-sea chemosynthetic ecosystems relies on chemosynthetic bacteria rather than the photosynthesis of plants. Seven years after the discovery of the first hydrothermal vent, a cold seep was discovered off the coast of Florida in the Gulf of Mexico (Paull et al., 1984). Cold seeps are often located on the seafloor close to faults or the margins of oceanic plates. Chemosynthesis-based associations also occur at cold seeps. After the discovery of hydrothermal vents and cold seeps, the first whale fall community was discovered in the Santa Catalina Basin in 1987 (Smith et al., 1989). Many animals that were related to animals from hydrothermal vents and cold seeps were discovered there. Dead whales sink to the deep-sea floor and are eaten by animals such as sharks, hagfish, crabs, and so on. Remnants of the whale carcass then remain on the deep-sea floor, still containing abundant organic matter such as blubber and bone marrow. Rotten fat promotes the formation of hydrogen sulfide and methane. Therefore many animals that are related to animals from hydrothermal vents and cold seeps are able to inhabit such whale carcasses.

The study of deep-sea biology has progressed rapidly since deep-sea submersibles began to be used for science (Gage & Tyler, 1991; Van Dover, 2000; Herring, 2002; Fujikura et al., 2008). However, many aspects of deep-sea biology still need to be investigated. Surveys using deep-sea submersibles can obtain data on behavior, systematics, evolution, symbioses, and biodiversity, using video images, samples preserved using chemicals, and frozen samples. It is now possible to observe deep-sea animals *in situ* with the naked eye or with HDTV cameras. Many questions have arisen from such real time observations of deep-sea animals. However, it is difficult to observe deep-sea animals over the long term *in situ*. Our observations of deep-sea animals using these tools are limited to only a few points during the space-time of their lives. One method to connect these points over time is the rearing and observation of deep-sea animals. One of the next important and necessary steps in deep-sea biology is to study live deep-sea animals in land-based aquaria using rearing and observation methods. The keeping of deep-sea animals has been tried in many institutes and public aquaria around the world because the rearing of deep-sea animals is considered by many to be an essential development for the future. In this chapter, we would like to introduce methods for the collection and maintenance of deep-sea animals, especially midwater animals and chemosynthetic ecosystem-associated animals.

2. Environment of the deep sea

The deep sea is a realm of darkness, cold, and high water pressure. Water temperature and salinity of the surface water layer vary from one locality to another according to season, latitude, or ocean currents. However, the differences in water temperature and salinity are

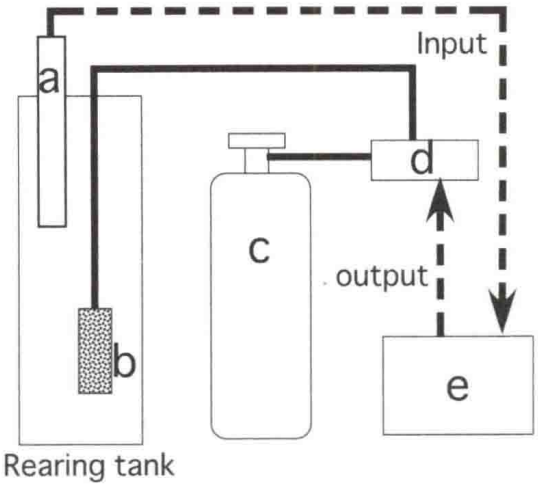

Fig. 3. Dissolved Oxygen Concentration Control System (DOCCS)
a: DO sensor, b: Air stone, c: Nitrogen tank, d: Electromagnetic valve, e: Control unit DO electric value which is measured at DO sensor (a) is processed by control unit (e). When DO value is higher than preset DO value, the control unit send a signal to electromagnetic valve (d) to open the valve, and nitrogen gas added to the aquarium. When DO reaches to the preset value, the electromagnetic valve is closed and nitrogen input stops.

Sometime an ultraviolet lamp is installed at the outlet of the filtration tank, because the mid-water generally has low abundances of bacteria. As for lighting, no statistically-proven, quantitative data exists, but experience suggests that lighting affects the survival of deep-sea animals in aquaria. Strong white light can make deep-sea animals blind (Herring et al., 1999). Some pigments of deep-sea animals such as porphyrin-derivatives can turn toxic when exposed to light (Herring, 1972). At public aquaria, artificial lighting such as by white fluorescent lamps or halogen lamps, as well as exposure to daylight over long periods, can lead to early death of deep-sea animals. We use red LED lamps to decrease mortality. An added advantage is that deep-sea animals show natural behaviors under red LED light.

4.1 Gelatinous zooplankton

Observations and experiments on live jellyfishes are necessary to understand their life history strategies in the deep sea. Therefore establishment of how to collect and keep jellyfishes that inhabit the mid-water and benthopelagic zones is necessary. However, it is difficult to collect many individuals of one species of jellyfish during a deep-sea dive. Jellyfishes have complex life cycles, including alternation of generations between a planktonic medusa stage (sexual generation), and a benthic polyp stage (asexual generation). This polyp stage has a great ability to regenerate. If we can collect a deep-sea polyp that also has a medusa generation, we can raise medusae from the polyp. The collection of polyps from the deep-sea in order to raise and keep deep-sea jellyfish in the aquarium is a very useful method.

We collect hard-bodied benthos like snails, as well as other substrates such as sunken wood, rocks and deep-sea litter, in order to find polyps. We also deploy and recover pot-plant pots

with marker buoys on the deep-sea floor using manned submersibles and ROVs. Some polyps of hydrozoans and scyphozoans were successfully collected on these substrates and were subsequently kept in aquaria with temperatures regulated to the same temperature as their in situ habitats (4 ~ 12 °C) and at atmospheric pressure. These polyps were kept in small aquaria (30 cm – 20cm -25cm H) with a sponge filter and fed *Artemia* nauplii twice a week. Every species of polyp formed a colony on the substrates through asexual reproduction. Some polyps in the colony were collected using a needle under a stereoscopic microscope and transferred into a petri-dish (φ 80mm - 4cm H). After the polyps attached to the bottom of the petri-dish, *Artemia* nauplii were fed to them twice a week. About four hours after feeding, each time, all the rearing water was changed. Using this method, we have been able to observe the growth, degrowth, regeneration, colony formation, medusa bud formation, and strobilation of polyps in the laboratory.

The hydroid from a *Ptilocodium* sp. was collected on a pot-plant pot with marker buoy deployed at a depth of 1170m off Hatsushima Island, Sagami Bay (Fig. 4). This species attached to the pot-plant pot substrate itself rather than to the rope or marker. There were two types of polyp in the colony – gastrozooids and dactylozooids. Dactylozooids had four tentacles and lacked mouths. Gastrozooids ate food that the dactylozooids caught with their tentacles. One to four medusa buds were formed on the basal part of gastrozooids. Medusae just after liberation had four short tentacles. This species was kept successfully at 4 °C and was unable to be kept at temperatures above 10°C.

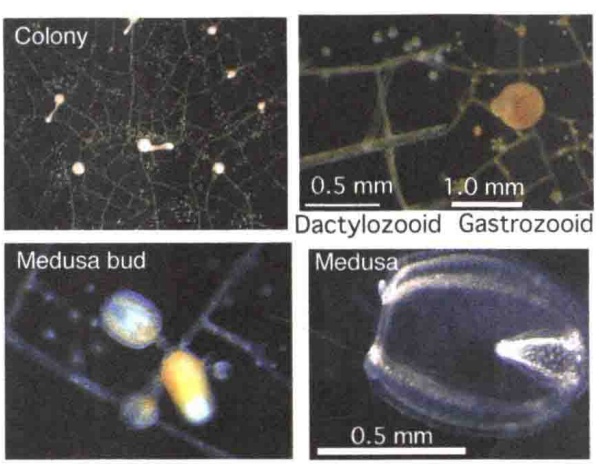

Fig. 4. *Ptilocodium* sp.

A large species of hydrozoa, *Corymorpha* sp., was collected using a suction sampler at a depth of 530m off Mera, Suruga Bay (Fig. 5a). This species inhabits sandy substrates. Just after sampling, this specimen was 15cm in height and was composed of a long hydrocaulus and a terminal hydranth. Five polyps were found at the base of the hydrocaulus. The number of aboral tentacles was about 60 and the number of oral tentacles was about 50. Blastostyles were located just above the aboral tentacles of the hydranth and had many medusa buds (Fig. 5b). Some medusae were liberated from the blastostyles (Fig. 5c). After sampling, all tentacles on the hydranth become atrophied and the hydranth dropped off from the hydrocaulus. The remaining hydrocaulus degenerated into a tissue mass. The

tissue mass regenerated into a polyp after one month and started asexual reproduction(Fig. 5d). The liberated hydranth also regenerated tentacles and caught *Artemia* nauplii. One polyp degenerated into a tissue mass and was divided into two separate tissue masses. Each of these tissue masses regenerated into a new individual. Lighting had a detrimental effect on this species during rearing, causing atrophication of tentacles and degeneration into a tissue mass.

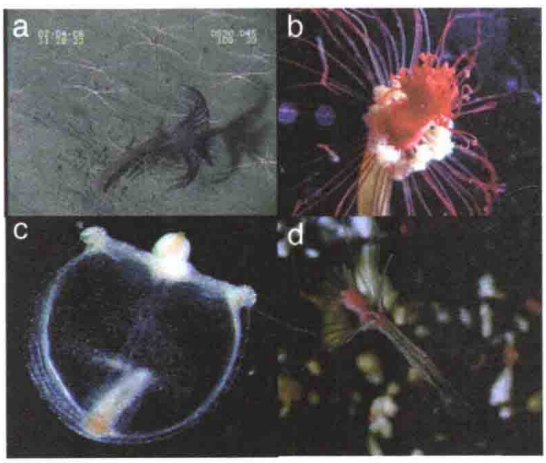

Fig. 5. *Corymorpha* sp.

It is necessary to rear the medusae that are released from such polyps until they are fully grown in order to clarify the life history of the species. Recently, some studies have been published about the life cycles and new species descriptions of deep-sea jellies from Monterey Bay (Widmer, 2007; Widmer et al., 2010; Widmer, 2011). Keeping deep-sea jellyfish in the laboratory makes it much easier to obtain detailed data about them that we would be unable to obtain in situ.

4.2 Mid-water shrimp, *Bentheogennema borealis*

Bentheogennema borealis is a mid-water shrimp referred to as Shinkai Ebi in Japanese (Shinkai means deep-sea and Ebi means shrimp in Japanese). This species is colored deep-red (Fig. 6). Sampling was done using a multiple opening/closing net - the IONESS (Intelligent Operative Net Sampling System). Many *B. borealis* were collected from 600m to 1500m depths in Sagami Bay, Japan. However, more than half of the shrimps were dead when the IONESS was retrieved onto the deck. To increase the survival rate when rearing the shrimp, it is essential to sample them in the winter season when surface water temperature is low. Lively shrimps were selected from the collected animals and transferred into buckets filled with chilled water (4°C). Selected shrimps were kept in the aquaria that were developed for jellyfish rearing (outlined above) at 4°C. Most of the shrimps had damaged antennae and could not swim normally but instead spiraled as they swam. Weakened individuals did not show any escape response. Such individuals must be eliminated from the rearing tank before the water becomes clouded and water quality worsens due to the fats and oils exuded from weakened individuals. When water became

clouded, it was exchanged with fresh sea water. White light was also considered not to be good for them so red LED lights were used for observation. They could not catch food by themselves because of the damage incurred during sampling. Therefore, we fed them a piece of krill or mysid meat directly to the mouth of each individual. The survival rate was about 11% for the first month, and about 6% over three months. Maximum duration of survival was more than 575 days. Some individuals molted and regenerated antennae and/or legs.

Fig. 6. *Bentheogennema borealis*

5. Chemosynthesis-based ecosystem animals

Deep-sea biologists can observe and collect live animals from deep-sea chemosynthetic ecosystems using ROVs or manned submersibles. However, it has been difficult to keep them alive over the long-term in order to perform a large variety of biological studies. Keeping deep-sea animals from chemosynthetic ecosystems in captivity enables many useful studies of these animals, as researchers can conduct experiments at any time, without needing to participate in deep-sea diving cruises. Keeping these animals is also very useful for public aquaria or science museums as part of a deep-sea biology outreach program to the public.

The rearing of animals from deep-sea chemosynthesis-based ecosystems has been difficult due to problems in maintaining high pressure, low pH, optimizing H_2S and high CO_2 concentrations, low dissolved oxygen, and keeping low light and low temperature conditions. These conditions are far removed from the normal rearing conditions for most fishes in aquaria. To overcome these problems, artificial hydrothermal vent tank and cold seep system tanks have been developed (Miyake et al., 2006; Miyake et al., 2007) (Fig. 7).

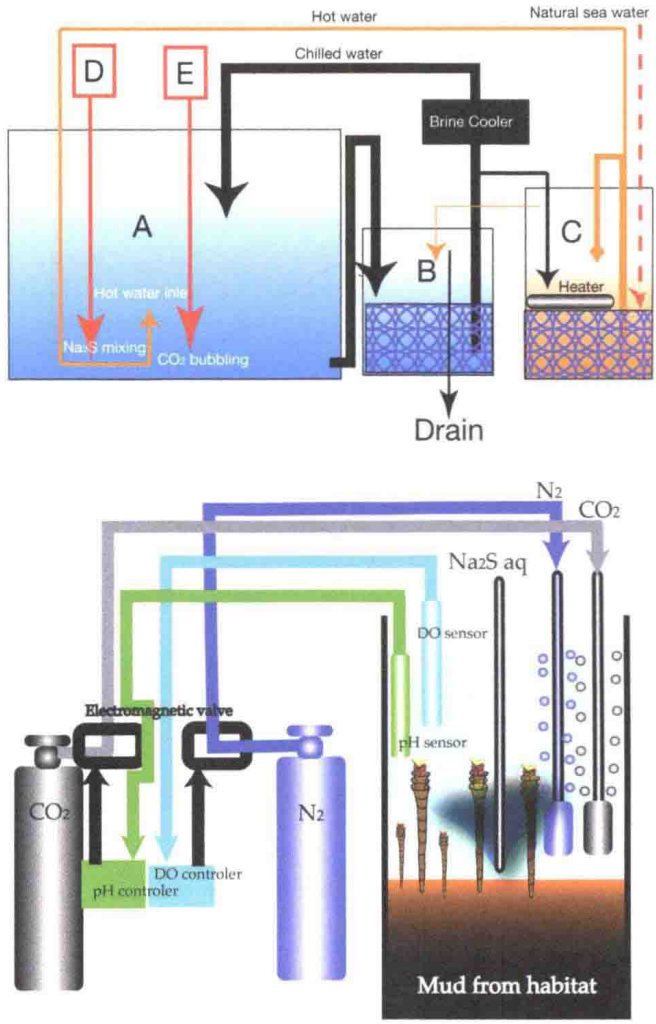

Fig. 7. Artificial hydrothermal vent and cold seep systems
Upper: Artificial hydrothermal vent system. A: Main rearing tank. Temperatures were 12 °C and 4 °C. B: Filtration tank. Filtration material was coral sand. C: Heating tank. Heating tank was also for filtration. D: Sodium sulphide tank. E: CO_2 cylinder.
Lower: Artificial cold seep systems

In 2006, the Deep-sea Chemosynthetic Ecosystem Tank (Fig. 8), with an artificial hydrothermal vent and cold seep system, was opened in the Deep-sea Corner at Enoshima Aquarium. This tank (3000WX1000-1500HX1000D (mm)) consists of three parts, a hydrothermal vent area, a cold seep area and a whale fall area. In the whale fall area, bones of a whale are displayed. In the cold seep area, the reduction zone is made using 30cm of mud mixed with decaying organic matter. The system of the tank is composed of a heating tank, a hot water outlet with added Na_2S as a source of H_2S, and added CO_2 for chemosynthetic bacteria and pH regulation. When the need arises, a DO control unit (DOCCS) is attached. There are some artificial chimneys, which act as hot water vents. The

maximum temperature of hot water is 60°C. Ambient water is 2 to 6°C, with an average temperature of 4°C.

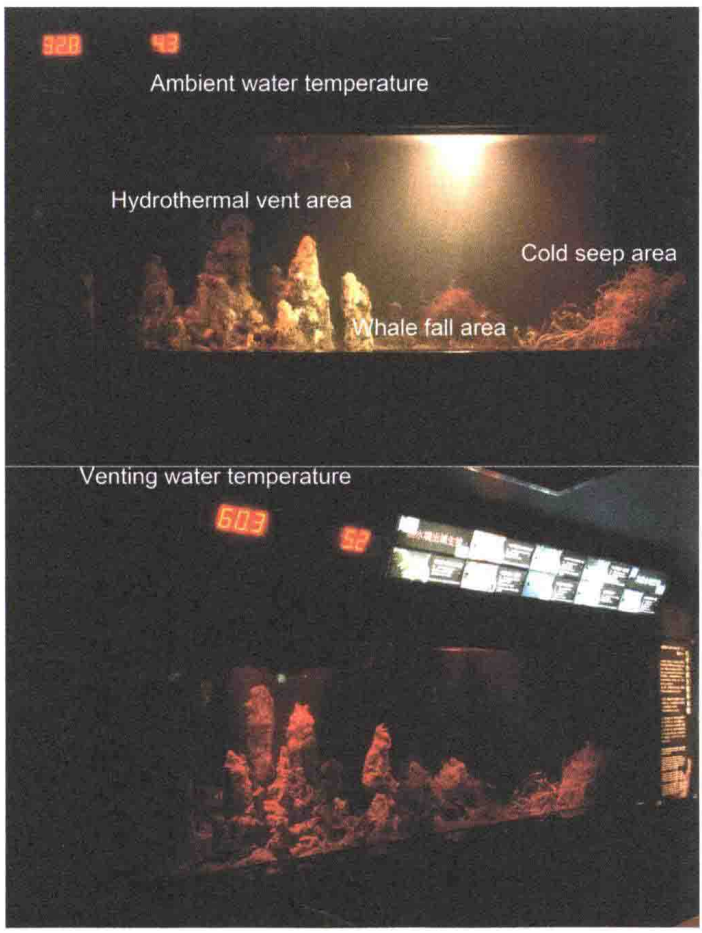

Fig. 8. The Deep-sea Chemosynthetic Ecosystem Tank, the main tank in the Deep-sea Corner in Enoshima Aquarium. Upper: Front view of the tank. Hydrothermal vent area venting hot water from imitation chimneys is located on the left side of the tank. Cold seep area is located at the right side of the tank. Whale fall area includes whale bone collected from Sagami Bay (924m). Two temperature signs (red) are displayed, the left sign is the hot water temperature, and the right sign is ambient water temperature. Below: Lighting is always red light. Hot water from imitation chimney is a maximun of 60°C.

5.1 Hydrothermal vent crabs and shrimps (Fig. 9)

Hydrothermal vent crabs, *Gandalfus yunohana, Austinograea alayseae, A. rodriguezensis* and the vent shimps, *Alvinocaris longirostris, Opaepele loihi* and *Opaepele* spp. have been kept in the hydrothermal vent system tank. *Alvinocaris longirostris* did not show any tendency to keep close to hot water vents, while the others have a strong tendency to keep close to hot water vents and gather around the outlet for hot water. Survival rates of these animals, except for

A. longirostris, were higher in the tank with hot water vents than in a tank without any hot water vents. Experience shows that white lighting was not good for the animals, with white light sometimes killing them, but red LEDs were a good source of illumination. They are fed krill, mysid or fish meat twice a week.

The vent crab *G. yunohana* exhibits behavior where females are guarded by the males before they molt. After molting, copulation has been observed. On the other hand, males just after molting are often cannibalized by other individuals in the aquarium. Some females spawned in the rearing tank. Gravid crabs maintained eggs on their abdomen in the hot water vent. Some eggs collected from females were kept in petri dishes with controlled temperatures at 12°C and 20°C. 12°C is the ambient water temperature in the rearing tank. Larvae hatched at 20°C, but did not hatch at 12°C. Adult crabs have no eyes, however hatched larvae have eyes. Unfortunately, rearing of the larvae has not yet been successful.

The vent shrimp graze on filaments and mats of chemosynthetic bacteria attached around the inlets for hot water, as well as eating krill, mysid or fish meat. *O. loihi* was brooding eggs when collected from the field and continued to brood them in the tank. Adult shrimps have no eyes, but larvae have eyes and were observed to swim upward in an upside-down posture. However, rearing of these larvae has not yet been successful.

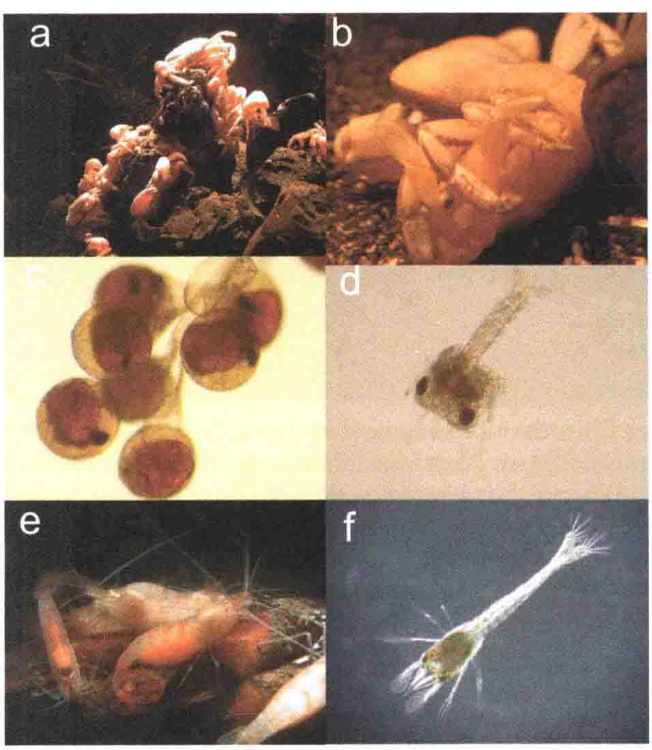

Fig. 9. Behaviors and breeding of vent crab and shrimp in the aquarium.
a: *Gandalfus yunohana* gathered around artificial hydrothermal vent, b: mating behavior of *G. yunohana*, c: eyed eggs of *G. yunohana*, d: hatched larva of *G. yunohana*, e: gravid female of *Opaepele loihi*, f: hatched larvae of *O. loihi*

5.2 Hydrothermal vent squat lobsters

The hydrothermal vent-associated squat lobster *Shinkaia crosnieri* was collected at a hydrothermal vent area at depths of 1000 ~ 1600m in the Okinawa Trough (Fig. 10a). *S. crosnieri* has a unique feeding ecology. This species has bushy white hairs, upon which dense chemosynthetic bacterial filaments are attached, on its ventral side (Fig. 10b). This species does not eat normal foods such as krill, mysid or fish meat, but instead grazes on the bacterial filaments on its bushy ventral hairs. This species lives near hydrothermal vents and farms chemosynthetic bacteria on its bushy ventral hairs. *S. crosnieri* has been kept in the vent system tank, but it showed no tendency to remain close to the hot water vent inlets like vent crabs do. Suitable rearing temperatures were 4~7 °C, and temperatures less than 3°C or more than 10°C were not suitable for long-term rearing.

S. crosnieri had bushy white hair with dense bacterial filaments attached at the time of sampling. However, a few days after sampling these dense bacterial filaments had disappeared from animals kept in a normal non-vent environment-simulating tank. In hydrothermal vent system tanks, bacterial filaments on the white hairs on the ventral side of *S. crosnieri* increased in bushiness within a few days. It has been observed that *S. crosnieri* graze on these bacteria using their mouthparts. A combination of optimum hydrogen sulfide concentrations and jetting water stream speed may be a key to emulating their *in situ* environment. A single molting was observed. The individual had no bacterial filaments on its ventral hairs after molting and died about one month later.

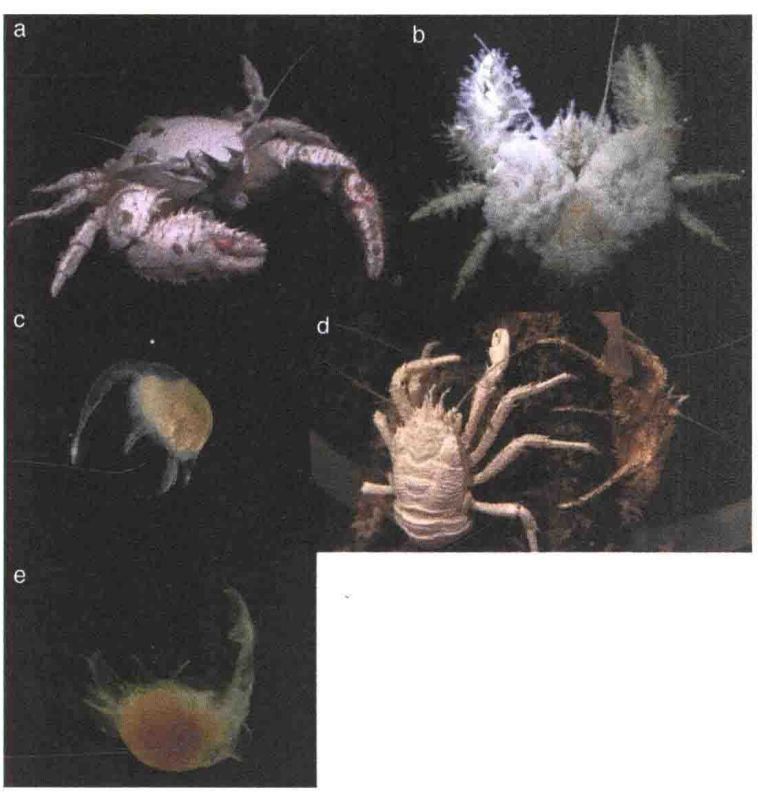

Fig. 10. *Shinkaia crosnieri* (a~c) and *Munidopsis myojinensis* (d~e)