Prus, T., 1970. Caloric value of animals as an element of bioenergetical investigations. Polskie Archiwum Hydrobiolii 17, 183–199.
Raymont, J.E.G., Srinivasagam, R.T., Raymont, J.K.B., 1969. Biochemical studies on marine zooplankton. VIII. Observations on ertains deep sea zooplankton. Internationale Revue der Gesanten Hydrobiologie 54, 357–365.
Reyss, D., 1971. Les canyons sous-marins de la mer Catalane: le rech du Cap et le rech Lacaze-Duthiers. III. Les peuplements de macrofaune benthique. Vie et milieu 22, 529–613.
Sardà, F., Cartes, J.E., Company, J.B., Albiol, T. (in press). A modified commercial trawl used to sample deep-sea megabentos. Fisheries Science.
Schmidt-Nielsen, K., 1990. Animal Physiology. Adaptation and Environment. Cambridge University Press, Londres.
Slobodkin, L.B., Richman, S., 1961. Calories/gm in species of animals. Nature 191, 299.
Smith, K.L. Jr., 1978. Benthic community respiration in the N. W. Atlantic Ocean: in situ measurements from 40 to 5200 m. Marine Biology 47, 337–347.
Teal, J., 1971. Pressure effects on the respiration of vertically migrating decapod crustacea. American Zoologist 11, 571–576.
Teal, J., Carey, F.G., 1967. Effects of pressure and temperature on the respiration of euphausiids. Deep-Sea Research 14, 725–733.
Thayer, G.W., Schaaf, W.E., Angelovic, J.W., LaCroix, M.W., 1973. Caloric measurements of some estuarine organisms. Fishery Bulletin 71 (1), 289–296.
Torres, J.J., Belman, B.W., Childress, J.J., 1979. Oxygen consumption rates of midwater fishes as a function of depth of occurrence. Deep-Sea Research 26, 185–197.
Torres, J.J., Somero, G.N., 1988. Metabolism, enzymic activities and cold adaptation in Antarctic mesopelagic fishes. Marine Biology 98, 169–180.
Wacasey, J.W., Atkinson, E.G., 1987. Energy values of marine benthic invertebrates from the Canadian Arctic. Marine Ecology – Progress Series 39, 243–250.

PERGAMON Deep-Sea Research I 45 (1998) 1881–1889

DEEP-SEA RESEARCH PART I

In situ spawning of a deep-sea vesicomyid clam: Evidence for an environmental cue

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Received 27 January 1997; received in revised form 30 June 1997; accepted 29 October 1997

Abstract

Spawning of the deep-sea vesicomyid clam *Calyptogena soyoae* was shown to be induced by short-term changes in water temperature. It was induced *in situ* by artificially increasing the ambient water temperature using the submersible *Shinkai 2000* off Hatsushima Island in Sagami Bay, Japan. Furthermore, we recorded *in situ* spawning events associated with natural changes in temperature at a deep-sea observatory on 11 occasions over a period of 1.5 years at the same site. The potential benefit of thermal regulation of reproduction in an otherwise aperiodic, dark, and unstable environment suggests that this strategy may be common for reproduction, particularly near chemosynthetic communities.

1. Introduction

Fertilization is critical for successful reproduction (Giese and Kanatani, 1987; Himmelman, 1981), and many marine organisms have evolved strategies to maximize this important step. For most broadcast spawners, fertilization occurs in the water column after gametes are released by both sexes, and the rate of fertilization (percentage fertilized) is enhanced by the synchronization of spawning (Ghiselin, 1987). Environmental factors, including bright sunlight, lunar period, phytoplankton, rain storms, sunrise, sunset, temperature change, and wave action, are known to be environmental cues for spawning of shallow-water animals (Galtsoff, 1932; Ghiselin, 1987; Giese and Kanatani, 1987; Loosanoff, 1937; Loosanoff and Davis, 1963; Young

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0967-0637/98/$–see front matter
PII: S0967-0637(98)00033-8

and Eckelbarger, 1994). In deep-sea habitats, environmental cues are less available, and little information on spawning or the related effects of environmental cues exists (Young and Eckelbarger, 1994). Moreover, very few deep-sea animals have been observed to spawn *in situ*. There are notable exceptions, such as the observation of spawning behavior in the hydrothermal vent tubeworm, *Riftia pachyptila*, on the East Pacific Rise (Van Dover, 1994).

Calyptogena soyoae is a conspicuous species at cold seeps in Sagami Bay, Japan, forming aggregations of dozens to thousands of individuals (Hashimoto *et al.*, 1989; Okutani and Egawa, 1985). Like other vesicomyid clams, *C. soyoae* is thought to rely on endosymbiotic chemoautotrophic bacteria for nutrition (Cavanaugh, 1983; Endow and Ohta, 1990; Vetter, 1985). Such symbiosis likely decreases biological linkages to seasonal sinking fluxes of organic debris, thereby decreasing further the number of potential cues to reproduce. *C. soyoae* is a diecious, broadcast spawner with planktonic larval stages.

The spawning behavior of *C. soyoae* was observed *in situ* on 11 days between December 1993 and June 1995 (Momma *et al.*, 1995). Among all factors measured at the benthic observatory, spawning activity was significantly correlated with water temperature only. Spawning events were not related to seasonal or lunar periods, as has been reported for other marine invertebrates (Young and Eckelbarger, 1994). The initiation of each spawning event followed an increase in water temperature of greater than 0.1°C in the preceding hour. Spawning did not, however, occur after each temperature increase. Gametes (sperm and eggs) were easily distinguished on video images from the observatory. Males spawned first during each event, and females often spawned 10 min later.

In order to test the hypothesis that temperature increase would stimulate spawning, an *in situ* heating experiment was performed on a vesicomyid clam bed.

2. Materials and methods

2.1. Animal collection

All the clams used for electron microscopic observations in this study were collected in 1991 and 1996 during dives of the submersible *Shinkai 2000* from seep sites off Hatsushima Island in Sagami Bay, Japan. Upon recovery, the clams were transferred immediately to fresh, chilled (about 4°C) seawater.

2.2. Environmental data set on natural spawning

During natural spawning, observations, including video recordings of clam aggregations, pressure (i.e. tidal height), current speed and direction, water temperature and salinity, underground thermal profiles and heat flux, and seismographic information, were made using a deep-sea observatory at a depth of 1,174 m off Hatsushima Island in Sagami Bay between December 1993 and June 1995 (Momma *et al.*, 1994 and 1995). Records on natural spawning and water temperature are shown in Table 1 and Fig. 1.

Table 1
Record of natural spawning of *Calyptogena soyoae* between December 1993 and June 1995 using a long-term observatory off Hatsushima Island in Sagami Bay (34°59.97'N, 139°13.69'E, 1174 m depth). "Time" marks the beginning of spawning. "Male" and "Female" indicate the sex of spawning. "Lunar phase" represents the lunar day (e.g. 0.0 days = new moon) at noon in Japan

			Sex		Lunar phase
	Date	Time	Male	Female	
1993	Dec. 21	17:06	+		7.7
		17:14		+	
1994	Jan. 6	13:46	+		23.7
	Feb. 9	12:50	+		28.2
	Feb. 16	22:03	+		5.5
		22:10		+	
	Mar. 11	10:34	+		28.5
		16:28	+		
		16:39		+	
	May. 26	20:41	+		15.4
		20:50		+	
	Jul. 22	15:26	+		13.2
	Oct. 27	17:50	+		22.0
1995	Jan. 26	9:31	+		24.7
		9:37	+		
		9:50	+		
		10:00		+	
	Mar. 2	15:36	+		0.6
		15:45		+	
	Jun. 8	18:23	+		9.7
		18:34	+		
		18:41		+	

2.3. Heating experiment

An *in situ* heating experiment was performed on a vesicomyid clam bed during the *Shinkai 2000* dive #831 (25 November 1995) at 34°59.99'N, 139°13.69'E, 1175 m depth off Hatsushima Island in Sagami Bay. A heating chamber (23 cm diameter polycarbonate dome with a halogen light [DEEPSEA POWER & LIGHT, SeaLite-120/250]) was placed over an aggregation of clams (less than 10 individuals) (Fig. 2). The distance between each specimen was negligible. After waiting for an hour for clams to recover from any mechanical disturbance, the light was turned on to heat the water within the dome. Temperature was measured by a thermometer (RIGOSHA RMT, temperature range: 0–100°C) within the heating dome. Seawater within the dome was collected using a hand-pump operated by the *Shinkai's* manipulator in a polyethylene collection bag.

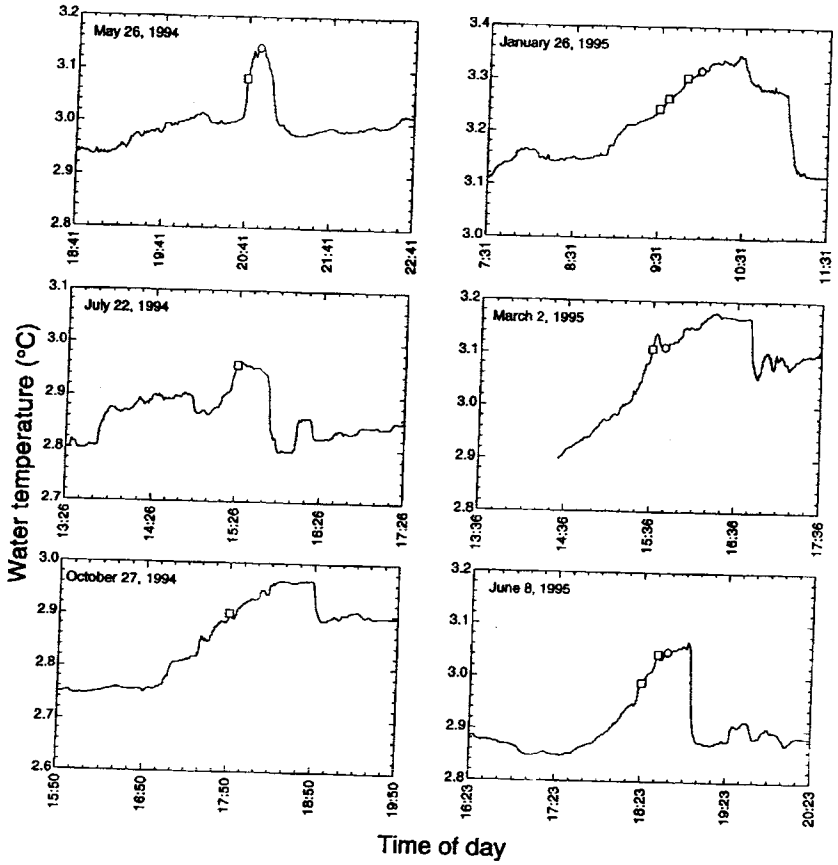

Fig. 1. Water temperature changes during natural spawning off Hatsushima Island in Sagami Bay. These measurements were made at a long-term observatory off Hatsushima Island. Open squares and circles represent male and female spawning, respectively.

2.4. Treatment for SEM observations

The particles present in the water collected from the heating device were prefixed in 2% formalin for 1 month at room temperature. After rinsing twice with the seawater, post-fixation was carried out for 30 min with 1% OsO_4 in 0.1 M phosphate buffer (pH 7.4) and 0.5 M NaCl at 0°C. After centrifugation, the precipitates were dehydrated, freeze-dried in 2-methyl–2-propanol, coated with platinum–palladium and observed using a HITACHI S-4100 scanning electron microscope.

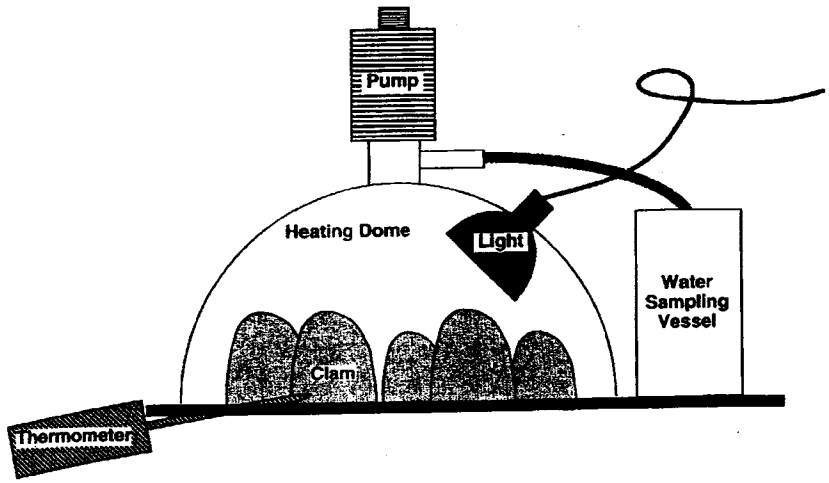

Fig. 2. Schematic diagram of the heating chamber for the *in situ* heating experiment on *Calyptogena soyoae*. The heating chamber was composed of a polycarbonate dome that was 23 cm in diameter, an underwater halogen light (DEEPSEA POWER & LIGHT, SL-120/250) for heating, a hand-pump that was manipulated by the mechanical arm of the submersible to collect heated seawater, a polyethylene bag for collection of seawater and a thermometer (RIGOSHA RMT, temperature range: 0–100°C).

2.5. Treatment for TEM observations

Small pieces of testes were prefixed with 2.5% glutaraldehyde in the buffered saline (1% $K_2Cr_2O_7$–KOH buffer (pH 7.4) containing 0.5M NaCl) for 3 h at room temperature. After rinsing three times with the buffered saline, post fixation was carried out for 1 h with 1% OsO_4 in the buffered saline at 0°C. Tissues were then dehydrated and embedded in Spurr resin. Ultra-thin sections of the specimens were stained with uranyl acetate and lead citrate, and were observed using a HITACHI H-600 transmission electron microscope.

3. Results

Spawning commenced 5 min after heat was applied (Fig. 3) and temperature had risen from 2.8 to 5°C. At least 5 individuals spawned (some repeatedly), with a total of 19 spawning events occurring during 70 min of observation. Spawning by an individual continued for about 1 min forming a cloud of apparently neutrally buoyant gametes. As in the natural spawning events, female spawning followed male spawning. In addition to spawning within the dome, spawning down stream outside the dome was also observed (within 50 cm of the device), within the flow of heated water and gametes.

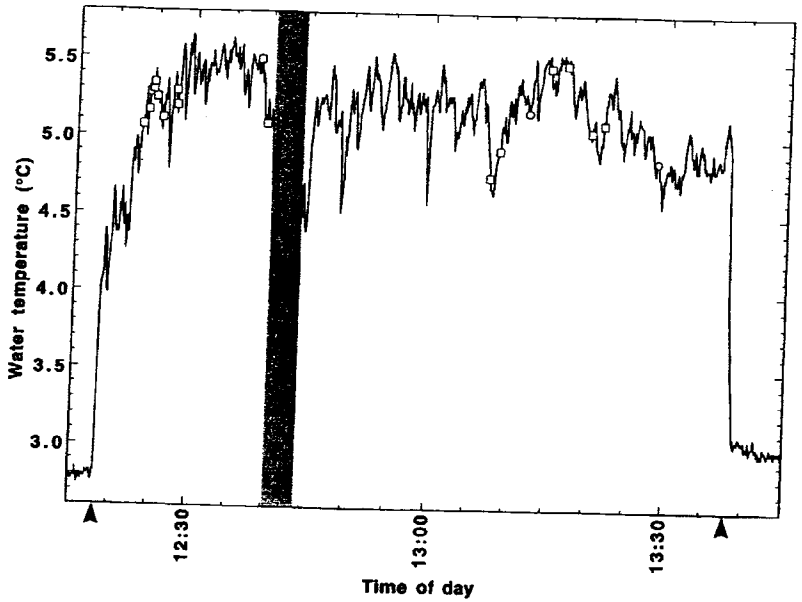

Fig. 3. Experimental water temperature change using the heating device deployed by the submersible *Shinkai 2000* dive #831 (November 25, 1995) off Hatsushima Island in Sagami Bay (34°59.99'N, 139°13.69'E, 1175 m depth). Open squares and circles represent male and female spawning, respectively. The period between arrowheads indicates the heating time. The shaded area denotes the time of seawater collection.

Seawater from the dome was collected to verify the presence of gametes using a hand-pump operated by the *Shinkai's* mechanical arm and a polyethylene collection bag after spawning of several males. Many spermatozoa were observed in the precipitates from the water using SEM (Fig. 4a). They were approximately of the same size and shape as the spermatozoa in the testes of *C. soyoae* seen under SEM and transmission electron microscopy (TEM) observations (Fig. 4b, c). No ova were observed in the precipitates.

Non-heated seawater was also collected as a control using the same device without heating on the *Shinkai* dive #834 (30 November 1995) and treated as above. Collection was made within a bed of clams close to the site where the heating experiment was performed. No gametes were observed under SEM in the control water.

4. Discussion

Synchrony of spawning behavior is a critical process to the reproductive success of many underwater organisms. Most of the cues used by shallow-water animals for

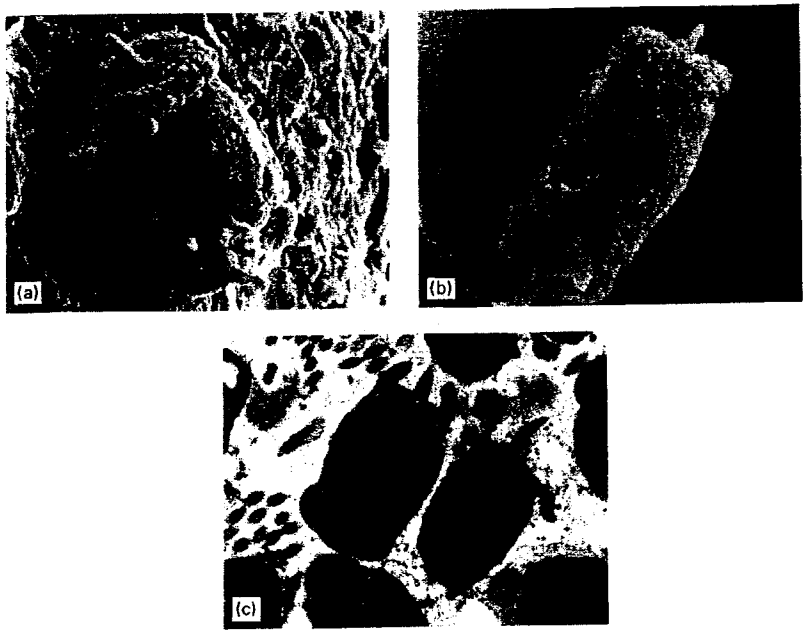

Fig. 4. Spermatozoa of *C. soyoae*. (A), (B) Scanning electron micrographs: (A) Spermatozoan collected from heated seawater; (B) Spermatozoan collected from clam testis. (C) Transmission electron micrograph showing the spermatozoa in the testis of a clam. Scale bar, 1.5 μm.

synchronizing spawning are not available to organisms living in continuously dark and relatively stable habitats of the deep sea (Young and Eckelbarger, 1994). Temperature change, which stimulated spawning in this clam, was one of the few possibilities for an environmental cue in the deep sea, and is also a known cue of shallow-water bivalves (Galtsoff, 1932; Loosanoff, 1937). The cause of natural temperature increase associated with spawning is not clear. Heat flow related to fluid seepage was excluded, because the underground temperature did not change during natural spawning events.

The presence of gametes in the water column may act as a secondary cue for spawning. Females released eggs only after males had begun spawning, which may increase their reproductive success by maximizing release of energetically expensive eggs during periods when fertilization is more likely. Male spawning may also be induced by the presence of gametes. A similar behavior has been recorded in some species of shallow-water bivalves (Loosanoff and Davis, 1963). There is a possibility that both sexes respond to temperature increase and that the male reaction is faster than that of the female.

The influence of light on spawning cannot be excluded completely, because all observations were made under lighted conditions. However, the possibility that light

is the immediate spawning cue can be excluded, because spawning was observed only downstream from the chamber and not in well-lit locations upstream. Furthermore, spawning never occurred during unheated observatory experiments. Finally, since sunlight does not penetrate to 1200 m depth, light cannot be an effective natural spawning cue.

The possibility that the spawning cue is chemical cannot be excluded completely. However, rates of chemical flow in seeps are thought to affect the underground temperature (Nakanishi *et al.*, 1995), and there was no change of the underground temperature around the natural spawning events.

Continuous spawning (i.e. no seasonal peak) by *C. soyoae* was consistent with the TEM observations of their mature gonads. Gonad maturity was similar between specimens collected in August and November 1991, and July and September 1996 (data not shown), which was also similar to that of other vesicomyid clam species inhabiting the Japanese subduction zones and in East Pacific (Berg, 1985; Fiala-Medioni and Le Pennec, 1989). The clams require much energy to keep mature gametes throughout the year. Continuous spawning might be a reproductive strategy of invertebrates from deep-sea chemosynthetic communities, because the activities of the vent and seep systems are thought to be unstable and relatively short-lived (Fiala-Medioni and Le Pennec, 1989; Tunnicliffe, 1991).

The apparent neutral buoyancy of gametes suggests that progeny are planktonic and disperse far downstream, depending on current patterns. This may explain why *C. soyoae* lives in seep areas separated by distances of tens of kilometers in Sagami Bay, in spite of the nutritional limitation of their habitat.

Chemosynthetic environments are rare and thought to be ephemeral habitats owing to dramatic changes in the energy available to support a resident fauna. In such environments, the combination of aggregate and repeated spawning events triggered by temperature changes could serve to maximize the likelihood of both fertilization success and larval dispersal between chemosynthetic habitats. Since this is the first discovery of an environmental cue used to synchronize spawning for a chemosynthetic species, it is not known to what extent other species rely on this cue to synchronize spawning.

Acknowledgements

We would like to thank Hiroyasu Momma, Kyohiko Mitsuzawa, Yuka Kaiho and Ryoichi Iwase for the development of the deep-sea observatory, observations, and their collection of non-heated sea water. We are grateful to James C. Hunt and James P. Barry for their useful suggestions and critical reading of the manuscript. We also thank the operation team of the submersible *Shinkai 2000* and the captain and crew of the *R/V Natsushima*.

References

Berg, C.J. Jr., 1985. Reproductive strategies of mollusks from abyssal hydrothermal vent communities. Bulletin of the Biological Society of Washington 6, 185–197.

Cavanaugh, C.M., 1983. Symbiotic chemoautotrophic bacteria in marine invertebrates from sulphide-rich habitats. Nature 302, 58–61.

Endow, K., Ohta, S., 1990. Occurrence of bacteria in the primary oocytes of vesicomyid clam *Calyptogena soyoae*. Marine Ecology Progress Series 64, 309–311.

Fiala-Medioni, A., Le Pennec, M., 1989. Adaptative features of the bivalve molluscs associated with fluid venting in the subduction zones off Japan. Palaeogeography, Palaeoclimatology, Palaeoecology 71, 179–192.

Galtsoff, P.S., 1932. Spawning reactions of three species of oysters. Journal of Washington Academy of Sciences 22, 65–69.

Ghiselin, M.T., 1987. Evolutionary aspects of marine invertebrate reproduction. In: Giese, A.C., Pearse, J.S., Pearse, V.B. (Eds.), Reproduction of Marine Invertebrates. Vol. 9, Blackwell Scientific Publications and Boxwood Press, California, pp. 609–665.

Giese, A.C., Kanatani, H., 1987. Maturation and spawning. In: Giese, A.C., Pearse, J.S., Pearse, V.B. (Eds.), Reproduction of Marine Invertebrates. Vol. 9, Blackwell Scientific Publications and Boxwood Press, California, pp. 251–329.

Hashimoto, J., Ohta, S., Tanaka, T., Hotta, H., Matsuzawa, S., Sakai, H., 1989. Deep-sea communities dominated by the giant clam, *Calyptogena soyoae*, along the slope foot of Hatsushima Island, Sagami Bay, central Japan. Palaeogeography, Palaeoclimatology, Palaeoecology 71, 179–192.

Himmelman, J.H., 1981. Synchronization of spawning in marine invertebrates by phytoplankton. In: Clark, W.H.Jr., Adams, T.S. (Eds), Advances in Invertebrate Reproduction, Elsevier, North-Holland, New York, pp. 3–19.

Loosanoff, V.L., 1937. Seasonal gonadal changes of adult clams, *Venus mercenaria* (*L.*). Biological Bulletin 72, 406–416.

Loosanoff, V.L., Davis, H.C., 1963. Rearing of bivalve mollusks. Advances in Marine Biology 1, 1–136.

Momma, H., Mitsuzawa, K., Kaiho, Y., Hotta, H., 1994. Long term and real time observation on the deep sea floor off Hatsushima Island in Sagami Bay. JAMSTEC Journal of Deep-Sea Research 10, 363–371.

Momma, H., Mitsuzawa, K., Kaiho, Y., Iwase, R., Fujiwara, Y., 1995. Long-term deep sea floor observation off Hatsushima Island in Sagami Bay—one year in the *Calyptogena soyoae* clam colony. JAMSTEC Journal of Deep Sea Research 11, 249–268.

Nakanishi, M., Fujimoto, H., Murakami, H., 1995. Temperature Variations in the Calyptogena community off Hatsushima Island, Sagami Bay—Correlation with earthquakes swarms. Zisin 48, 71–79.

Okutani, T., Egawa, K., 1985. The first underwater observation on living habit and thanatocoenoses of *Calyptogena soyoae* in bathyal depth of Sagami Bay. Venus 44, 285–289.

Tunnicliffe, V., 1991. The biology of hydrothermal vents: ecology and evolution. Oceanographic Marine Biology Annual Review 29, 319–407.

Van Dover, C.L., 1994. *In situ* spawning of hydrothermal vent tubeworms (*Riftia pachyptila*). Biological Bulletin 186, 134–135.

Vetter, R.D., 1985. Elemental sulfur in the gills of three species of clams containing chemoautotrophic symbiotic bacteria: a possible inorganic energy storage compound. Marine Biology 88, 33–42.

Young, C.M., Eckelbarger, K.J., 1994. Reproduction, Larval Biology, and Recruitment of the Deep-Sea Benthos, Columbia University Press, New York, 336 pp.