Short Communications

MARINE BIOTECHNOLOGY

Survival of Deep-Sea Shrimp (*Alvinocaris* sp.) During Decompression and Larval Hatching at Atmospheric Pressure

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Abstract

We report successful larval hatching of deep-sea shrimp after decompression to atmospheric pressure. Three specimens of deep-sea shrimp were collected from an ocean depth of 1157 m at cold-seep sites off Hatsushima Island in Sagami Bay, Japan, using a pressure-stat aquarium system. Phylogenetic analysis of *Alvinocaris* sp. based on cytochrome *c* oxidase subunit gene sequences confirmed that these species were a member of the genus *Alvinocaris*. All 3 specimens survived to reach atmospheric pressure conditions after stepwise 63-day decompression. Two of the specimens contained eggs, which hatched after 10 and 16 days, respectively, of full decompression. Although no molting of the shrimp larvae was observed during 74 days of rearing under atmospheric pressure, the larvae developed conventional dark-adapted eyes after 15 days.

Key words: pressure-stat aquarium — deep-sea crustacean — hatching — low-pressure tolerance

Introduction

In comparison with extensive reports of studies dealing with shallow water-dwelling organisms, fewer studies have investigated the reproduction of deep-sea multicellular organisms. Environmental cues for the reproduction of shallow-water animals include a wide variety of factors such as bright sunlight, lunar period, phytoplankton, rainstorms, sunrise, sunset, temperature change, and wave action (reviewed in Giese and Kanatani, 1987). There are fewer environmental cues in deep-sea habitats (reviewed in Van Dover, 2000), and most deep-sea multicellular organisms investigated to date appear to undergo asynchronous gametogenesis. Valuable observations have been made of spawning behavior in the hydrothermal vent tubeworm *Riftia pachyptila* on the East Pacific Rise (Van Dover, 1994) and of the vesicomyid clam *Calyptogena soyoae* at the cold seep in Sagami Bay, Japan (Momma et al., 1995; Fujiwara et al., 1998). In *C. soyoae*, spawning was synchronized with natural temperature (Momma et al., 1995) and induced by an artificial temperature increment of in situ water created using the submersible *Shinkai 2000* carrying a polycarbonate dome with a halogen light (Fujiwara et al., 1998).

To investigate whether gametogenesis is induced in abyssal organisms by alterations in external environmental factors such as pressure, temperature, and artificial light exposure, we have developed a novel piezostat aquarium system (Koyama et al., 2002). The pressure-stat aquarium system is composed of pressure-retaining suction capture and pressure-stat rearing devices. The system, designed for operation by submersibles, captures deep-sea organisms with a suction servomotor. The system can also control the pressure range up to 20 MPa (1 atm = 0.101325 MPa), with fluctuation of ±0.1 to 0.2 MPa, by exchanging the seawater contained within. After slow decompression using this system, the deep-sea eel *Simenchelys parasiticus* collected at

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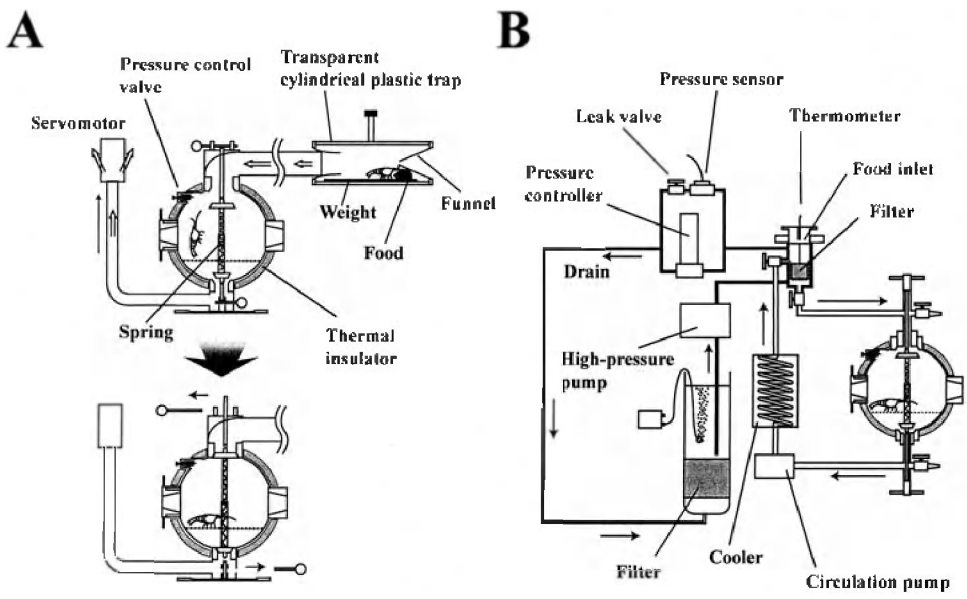

Fig. 1. Schematic of the pressure-stat aquarium system.**A**: Suction sampling device to be carried by a submersible. Length of the trap is 560 mm; inside diameter, 109 mm; outside diameter, 115 mm. **B**: Pressure-stat rearing device.

1162 m was kept alive under atmospheric pressure conditions for 2 to 5 days, and pectoral fin cells were successfully cultivated (Koyama et al., 2003).

In the present study, we succeeded in maintaining 3 live deep-sea *Alvinocaris* sp. specimens under atmospheric pressure conditions, withstep wise decompression over a 63-day rearing period. Furthermore, the shrimp larvae hatched after 10 and 16 days of full decompression, while the 2 original specimens continued to swim.

Materials and Methods

Collection of Deep-sea Shrimp. Transparent cylindrical plastic traps (Figure 1, A) were placed on the deep-sea bottom in Sagami Bay, Japan, for a few days. A slice of fresh mackerel purchased from a fishmonger was placed in each trap. Three specimens of the deep-sea shrimp *Alvinocaris* sp. were collected from the traps with our pressure-stat aquarium system (Figure 1; Koyama et al., 2002) at a depth of 1157 m (*Shinkai 2000* dive 1396, October 15, 2002; 35°00.07(N, 139°13.45(E) and maintained at 4.5°C with fluctuation of ±0.3°C at hydrostatic pressure of 11.5 Mpa (corresponding to a depth of 1130 m). The body length of the 2 deep-sea shrimps containing eggs was 5 cm, while that of the third was 4 cm. We placed 10 L of artificial seawater containing 400 g of marine salt (Tetra) and 20 L of the collected deep-sea water with the shrimp specimens in the pressure-stat aquarium. The seawater was exchanged by injecting 10 L of fresh artificial seawater into the pressure-stat aquarium 5 or 6 times weekly. Tuna purchased from a fishmonger and lyophilized krill (Tetra Krill-E, Warner-Lambert Inc., Tetra Division) were fed to the deep-sea shrimp (Figure 2). We supplied 3 to 4 g of tuna or 2 to 3 krill to the 3 deep-sea shrimp under pressure at each feeding. Any uneaten food fell through a stainless steel net with the action of seawater circulation and was removed by the filter in the food inlet box (Figure 1, B; Koyama et al., 2002).

Alteration of Pressure, Temperature, and Illumination Intensity. Pressure change was controlled using the feedback circuit of the pressure controller and pressure sensor, both of which adjust the amount of seawater drainage and maintain the pressure in the aquarium (Figure 1). Compressed seawater was supplied at a rate of 36 ml/min. The 3 shrimp were exposed to light through the side-view window of the pressure-stat aquarium using a 50-W halogen tungsten lamp (Model TGH, Olympus) for 30 minutes per day (5 or 6 days per week). The illumination intensity was 60 lux as measured using a Handy Digital Lux Meter (LX-1330, Atex) placed at the window on the opposite side of the aquarium filled with artificial seawater (Tetra). We shut down the cooling unit for 1.5 hours to perform a temperature increment experiment on day 22 at 8 MPa (Figure 2). The seawater temperature measured with a thermometer (Figure 1, B) was increased slowly up to 9°C over 1.5 hours. After 1.5 hours cooling resumed, and the water was chilled to 4.5°C over a 30-minute period. The stability of temperature control was ±0.3°C in the aquarium system.

Measurement of Dissolved Oxygen and Ammonium Ion Concentrations. Dissolved oxygen was monitored in the injection and drainage seawater

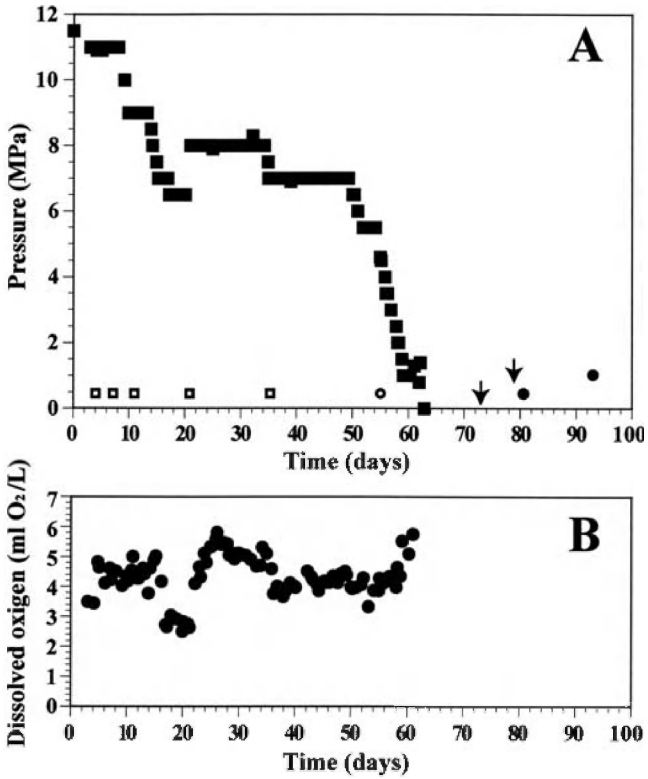

Fig. 2. Pressure and dissolved oxygen concentration in the aquarium system. **A**: Rate of pressure reduction from 11.5 MPa to atmospheric pressure. The arrows and black circles indicate hatching and molting of deep-sea mother shrimp, respectively. The white squares and the white circle indicate tuna and krill supply, respectively. **B**: Variation in dissolved oxygen concentration. Air-saturated seawater (6.5 ml O_2/L) was supplied at a rate of 36 ml/min.

with a portable dissolved oxygen sensor (DO-21P, Toa Electronics). Air-saturated seawater (6.5 ml O_2/L) was supplied into the aquarium system using a high-pressure pump (Figure 1, B, NP-KX-500-40, Nihon Seimitsu Kagaku) at a rate of 36 ml/min. Ammonium ion concentrations were measured using an ammonium-ion-selective pack test (WAK-NH_4, Kyoritsu Chemical-check Lab.). The detectable limit of the ammonium ion concentration was 0.2 mg/L. The ammonium ion concentration in the system was 0.5 mg/L between day 0 and day 39 of rearing. It began to decrease after day 39 and was undetectable by day 52.

Maintenance of Deep-Sea Shrimp Larvae. The hatched *Alvinocaris* sp. larvae were transferred to a 50-L tank and cultured in 40 g/L of artificial seawater (Tetra Marine Salt, Tetra) under atmospheric pressure conditions at 4.5°C in a cold room. The major and minor axes of the elliptical-spherical eggs were approximately 1100 μm and 830 μm, respectively. Seawater was pumped out with an Eheim 2213 filter

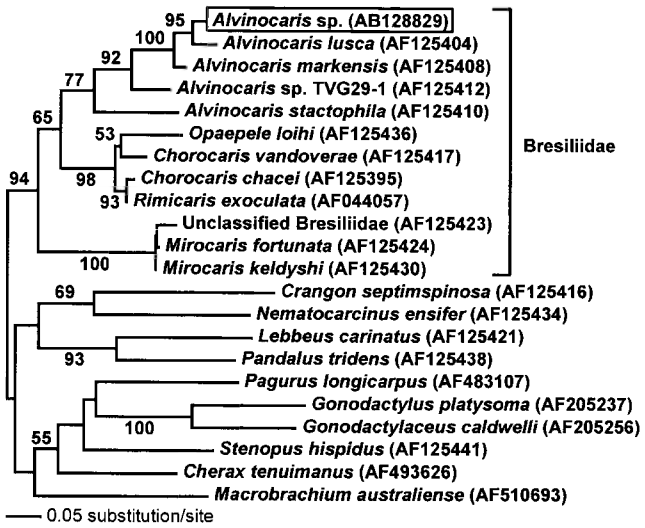

Fig. 3. Phylogenetic position of *Alvinocaris* sp. inferred from nucleotide sequences of the *COI* gene. The ML tree was constructed as described in the text. Numbers are bootstrap values for nodes supported by more than 50% (100 replicates). Bar indicates 0.05 substitution per site.

pump (Warner-Lambert Inc., Tetra Division) placed on the bottom of the tank with the suction site on the surface layer of the seawater to suspend the larvae in the tank. The larvae had only weak ability to swim and adhered to the sponge on the suction site. Half of the water in the tank was exchanged 3 to 6 times weekly. Liquid food containing 2.9% fat, 1.3% protein, 1.3% fiber, 5.7% minerals, 4.8% carbohydrates, and 930 mg/kg of vitamins (Marine Deluxe, Redsea-Japan; available at http://www.reefcreation.uk/marine_deluxe.htm) was fed to the deep-sea larvae 3 to 6 hours before the water was exchanged. Deep-sea larvae are not cannibalistic by our observation and therefore were cultured communally.

Molecular Phylogenetic Analysis. Molecular phylogenetic analysis of *Alvinocaris* sp. (Figure 3) was conducted for the cytochrome *c* oxidase subunit I (*COI*) nucleotide sequence using PAUP* 4.0b10 (Swofford, 1998). Phylogenic analysis was performed with one of the 3 deep-sea shrimps. The *COI* nucleotide sequence was registered in the DNA Data Bank of Japan (DDBJ; accession number AB128829). DNA extraction for Polymerase chain reaction (PCR) was performed using the QIAamp DNeasy Tissue Kit (QIAGEN). The primers used for amplifying and sequencing the mitochondrial *COI* were those described by Folmer et al. (1994). The PCR product was purified using ExoSAP-IT (USB) and sequenced using a DNA sequencer (model 4000L, Li-Cor). Maximum likelihood (ML) analyses (Felsenstein, 1981) were

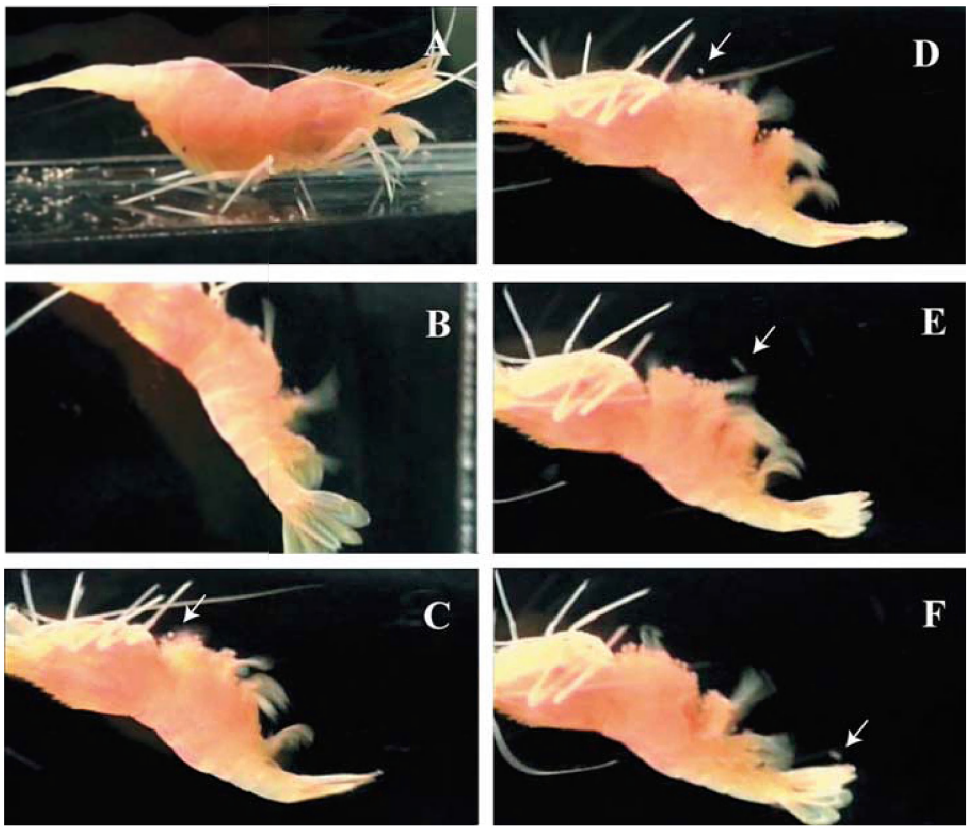

Fig. 4. Hatching of deep-sea *Alvinocaris* sp. specimens after full decompression. Deep-sea shrimp hatched while swimming in the water after 10 days of full decompression. The body length of the shrimp is 5 cm. Arrows indicate the time immediately after hatching.

performed using heuristic searches with random stepwise addition of 100 replicates and tree bisection-reconnection (TBR) rearrangements. An optimal model of nucleotide evolution for ML analyses was determined using hierarchical likelihood ratio tests as implemented in MODELTEST 3.06 (Posada and Crandall, 1998). The model selected as the best fit was GTR+I+G. For bootstrap analyses (Felsenstein, 1985), 100 replicates were generated with 5 random additions and TBR rearrangements.

Results

The shrimps were tentatively identified as *Alvinocaris* sp. by morphologic observation. One of the shrimps was later confirmed by phylogenetic analysis of the *COI* gene sequence (Figure 3). Two of the 3 *Alvinocaris* sp. specimens contained eggs at capture, and we investigated the physical parameters that triggered spawning and/or hatching of the shrimp eggs. To trigger spawning or hatching of the deep-sea shrimp, we altered such environmental factors as pressure, temperature, and illumination in the pressure-stat aquarium system. However, no effect of temperature increases up to 9°C (gradual increase to 9°C over 1.5 hours and then chilled to 4.5°C over a 30-minute period), light exposure (60 lux for 30 minutes), or lunar phase on the *Alvinocaris* sp. in inducing spawning or hatching was found.

Neither hatching nor spawning was observed during 50 days of rearing, even when the aquarium pressure was varied between 11.5 and 6.5 MPa, corresponding to a depth of 1130 m and 640 m, respectively (Figure 2). After 10 and 16 days of full decompression (Figure 2), corresponding to 73 and 79 days of rearing, respectively, the shrimp larvae hatched while the 2 original specimens continued to swim (Figure 4). The 2 shrimp had 417 and 326 larvae, respectively. No spawning events were observed in either of the deep-sea shrimp, however. The complete hatching of the brood required 7 and 14 days, respectively, after which the mother shrimp exuviated to remove the empty egg cases and any remaining undeveloped eggs.

Next, we investigated whether the larvae of *Alvinocaris* sp. could grow under atmospheric pressure conditions. *Alvinocaris* sp. larvae metamorphosed after 15 days of rearing, and a pair of compound eyes developed in each (Figure 5). The larvae were fed commercially purchased plankton, and more than 80% remained alive after 50 days at

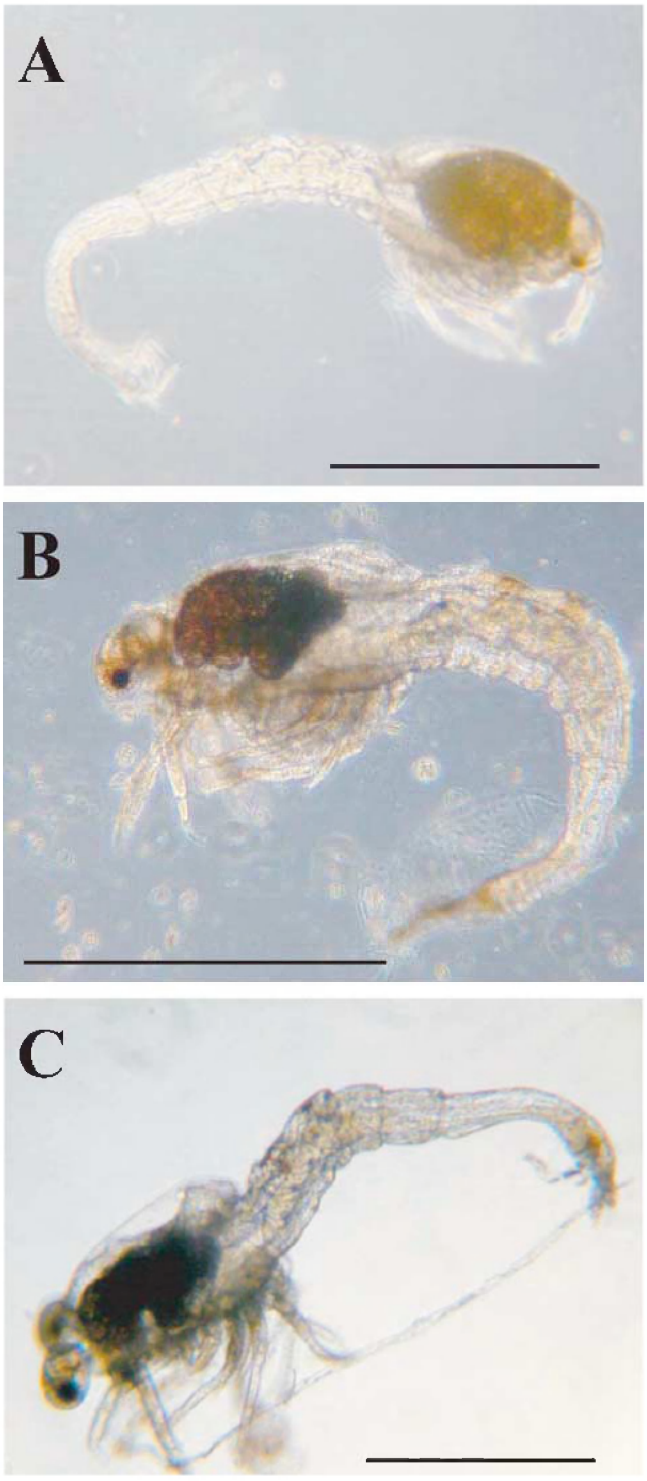

Fig. 5. Deep-sea shrimp larva at day 0 (**A**), day 15, (**B**), and day 50 (**C**) of the larval stage. Scale bars indicate 1 mm.

atmospheric pressure. After 74 days of rearing, all of the larvae died owing to an outbreak of mold. No molting was observed during 74 days of rearing.

Discussion

We found that all 3 shrimp survived at atmospheric conditions after gradual 63-day decompression, and that the shrimp larvae hatched while the 2 original specimens continued to swim after 10 and 16 days of full decompression (Figures 2 and 4). Our results raise the question of where the reproduction of vent/seep/deep-sea crustaceans takes place, and where the larvae exist in the water column. Several researchers reported that invertebrate larvae and adults in general are able to tolerate pressure change (Yayanos, 1981; Tsuchida et al., 1998; Tyler and Young, 1998; Koyama and Aizawa, 2000). For example, using a windowed pressure-retaining trap, Yayanos (1978, 1981) showed that the captured deep-sea amphipod *Paralicella capresca* remained alive during decompression from 601 to 300 atm without loss of locomotor activity. When *P. capresca* was left at atmospheric pressure for 4 minutes, locomotor activity was immediately regained following recompression (Yayanos, 1981). Tolerance in the early embryos of sea urchin from shallow water is limited to depths of less than 1000 m, whereas the larvae were reported to be capable of tolerating pressures down to 2000 m (Tyler and Young, 1998). Although *Alvinocaris* sp. larvae hatched at atmospheric pressure in the present study (Figure 4), all died after 74 days of rearing. This suggests that larval growth requires hydrostatic pressure. When the pressure changed from 2.5 to 2 MPa, corresponding to depths of 250 and 200 m, respectively (58 days in Figure 2), the 3 adult shrimps began to move to the bottom of the stainless steel net in the aquarium. Therefore it is physiologically possible for *Alvinocaris* sp. to ascend to the upper limit of 200 to 250 m in depth under natural conditions for reproduction and larval growth.

Williams and Chace (1982) established the genus *Alvinocaris* of the family Bresiliidae for the single species *Alvinocaris lusca* (Figure 3) collected from the thermally influenced fields on the Galapagos Rift. Since then a few species have also been described from deep-sea hydrothermal fields in Japan (*Alvinocaris longirostris*, Kikuchi and Ohta, 1995; *Alvinocaris brevitelsonis* and *Alvinocaris leurokolos*, Kikuchi and Hashimoto, 2000). Our 3 captured deep-sea shrimp species were determined to be members of the genus *Alvinocaris* on the basis of morphologic observation and phylogenetic analysis using the *COI* gene sequences (Figure 3). The topology within the family Bresiliidae in this tree was consistent with that in a previous study (Shank et al., 1999). Evolutionary distances between *Alvinocaris* sp. and *A. lusca* as the closest relative seemed

to correspond to the divergence of different species. Some known *Alvinocaris* species have not been examined for *COI* sequences, however. In the morphologic characteristics, each specimen collected had a remarkably long rostrum; therefore, they were expected to be closely related to *A. longirostris*. Specimens of *A. longirostris* have been found in hydrothermal vents in the Okinawa Trough, whereas our *Alvinocaris* sp. specimens were collected at cold seeps in Sagami Bay. Because it was uncertain whether they were identical species, we regarded this species as *Alvinocaris* sp.

A pair of compound eyes appeared in each *Alvinocaris* sp. larva after 15 days of rearing under atmospheric conditions (Figure 5), although adult *Alvinocaris* sp. do not have conventional eyes (Figure 4). The adult vent shrimp *Alvinocaris markensis* (Figure 3) is truly blind and lacks photoreceptor cells (Wharton et al., 1997). Gaten et al. (1998) reported that the vent shrimp *Rimicaris exoculata* (Figure 3) possesses conventional eyes in the larval and early postlarval stages. The vent crabs *Bythograea thermydron* possess eyes in which spectral absorbance shifts toward longer wavelengths from the larval to postlarval to adult stage (Jinks et al., 2002). Larvae of 7 deep-sea taxa (*Benthonella tenella, Laeviphitus verduini, Oocorys-type, Amphissa acutecostata, Drilliola loprestiana, Benthomangelia macra*, and *Famelica-type*) have been recognized in surface waters (Bouchet and Warén, 1994). Therefore, the deep-sea shrimp larvae that possess conventional eyes (Figure 5) could exploit phytoplankton as a food resource in the photic zone.

Prawn larvae are known to go through the nauplius, zoea, mysis, and postlarval developmental stages, accompanied by molting (Hudinaga, 1942). Prawns in the nauplius stage were reported to enter the zoea stage after 6 meltings within 36 to 37 hours after hatching (Hudinaga, 1942). The yolk of nauplius prawn larvae, which fills the body upon hatching, is almost completely absorbed before entering the zoea stage (Hudinaga, 1942). The *Alvinocaris* sp. larvae differed from the prawn larvae in that they did not molt during 74 days of rearing. In addition to hydrostatic pressure, deep-sea larvae may also require more time and/or additional nutrients to reach the next developmental growth stage.

We used a one-variable-at-a-time program to investigate the triggering effects of pressure, temperature, and illumination on shrimp spawning and hatching, because it was extremely difficult to collect numerous gravid deep-sea shrimp with eggs. Future research will elucidate the details of these effects if it is possible to capture more gravid deep-sea shrimp with eggs.

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