J. Mar. Biol. Ass. U.K. (2004), **84**, 743–745
Printed in the United Kingdom

Larval development and intermoult period of the hydrothermal vent barnacle *Neoverruca* sp.

H. Watanabe*[∫], R. Kado[†], S. Tsuchida[‡], H. Miyake[‡], M. Kyo[‡] and S. Kojima*

*University of Tokyo, Ocean Research Institute, Minamidai, Nakano, Tokyo 164-8639, Japan. [†]School of Fisheries Sciences, Kitasato University, Okkirai, Sanriku-cho, Ofunato, Iwate 022-0101, Japan. [‡]Japan Marine Science and Technology Center, Natsushima-cho, Yokosuka, Kanagawa 237-0061, Japan. [∫]Corresponding author, e-mail: hwatanabe@ori.u-tokyo.ac.jp

Larvae of the hydrothermal vent barnacle *Neoverruca* sp. were reared under laboratory conditions and larval development was observed. Under these conditions, the larvae were released from adults as first-stage nauplii, although the larvae of other deep-sea barnacles have generally been considered to be released at a later larval stage such as the cyprid stage. The larvae of *Neoverruca* sp. were lecithotrophic through six naupliar stages and the subsequent cyprid stage. The larval period of *Neoverruca* sp. was more than 96 days under the present rearing conditions, which is the longest yet reported for barnacles. Most cyprid larvae, however, exhibited abnormal morphology and no larvae settled successfully on the substrate. These observations suggest that such a long larval period might enable neoverrucid barnacles to disperse between vent fields.

INTRODUCTION

Biological communities associated with hydrothermal vent fields contain many endemic species (Tunnicliffe & Fowler, 1996). The vent fields are patchily distributed on the vast deep-sea floor and individual vents are thought to be ephemeral. Therefore, how vent-endemic animals disperse and establish new populations is an interesting issue. For vent-endemic sessile organisms, their planktonic periods (larvae or gamete) must be the most plausible opportunity to migrate from one vent field to another.

In the western Pacific, vent barnacles belonging to the family Neoverrucidae (Cirripedia: Thoracica) are widely distributed and can be quite abundant in some hydrothermal vent fields (Newman, 1989; Newman & Hessler, 1989; Fujikura et al., 2001). Larvae can easily be obtained from adults as all barnacles brood their embryos inside the mantle cavity until hatching. Thus vent barnacles provide a good opportunity to study the larval dispersal of hydrothermal vent-endemic animals. This study investigated the larval period of *Neoverruca* sp. reared under laboratory conditions.

MATERIALS AND METHODS

Thirty-one living adult neoverrucids (JAMSTEC registration no. 054404-054434) were collected from the hydrothermal vent field on the Dai-yon (No. 4) Yonaguni Knoll (24°50.9′N 122°42.03′E, 1340 m depth), located in the Okinawa Trough, by the remotely operated vehicle (ROV) 'HyperDolphin', belonging to the Japan Marine Science and Technology Center (JAMSTEC) during dive 124 on 29 November 2002 (Kyo et al., 2003). These neoverrucids were assignable to the genus *Neoverruca* but exhibited some differences in shell arrangement from the sole described species in this genus, *Neoverruca brachylepadoformis* Newman & Hessler, 1989. After the cruise, adults were reared in artificial seawater (Rote Marine, Rei-Sea Co., Tokyo) at 5°C in a 100-l aquarium volume, including filtration system and holding tank, adding 142 mg of sodium sulphide per 10 min and keeping the salinity at 34.5–35 psu. They were covered with plankton netting of 100-μm mesh size. Thirty-six larvae were collected on 25 and 26 December and another six larvae were collected on 31 December 2002 inside the netting. These larvae were reared individually in 6-well plates (Microplate 3810-006, IWAKI Co., Tokyo) at 4°C with 5 ml of filtered seawater (pore size: 0.22 μm) which did not contain any food or additional chemicals such as hydrogen sulphide. Seawater was replaced daily and, at the same time, the larval stages were recorded.

RESULTS AND DISCUSSION

The present study revealed that the larvae of *Neoverruca* sp. are lecithotrophic throughout the naupliar stages (N1, N2, N3, N4, N5 and N6) and the subsequent cyprid stage. Neither naupliar nor cyprid eyes were visible under the microscope (Figure 1). The larvae of *Neoverruca* sp. were released from the parent at the first naupliar stage under the present rearing conditions, whereas non-vent deep-sea barnacles are generally considered to reach the cyprid stage inside the mantle cavity of their parents (Anderson, 1994). In the first larval stage (N1), they could not swim well and mainly floated at the surface due to their positive lipid-induced buoyancy. Within a few days, they moulted and started swimming, no longer floating at the surface. The duration and survival rate of each naupliar stage are shown in Table 1. In the final larval (cyprid) stage, most of the larvae developed with unusual morphology (Figure 1A,B). Their cyprid carapace remained open and failed to fold their bodies, and they were unable to swim using their thoracopods as ordinary cyprids do. None of the larvae were able to settle and all died without

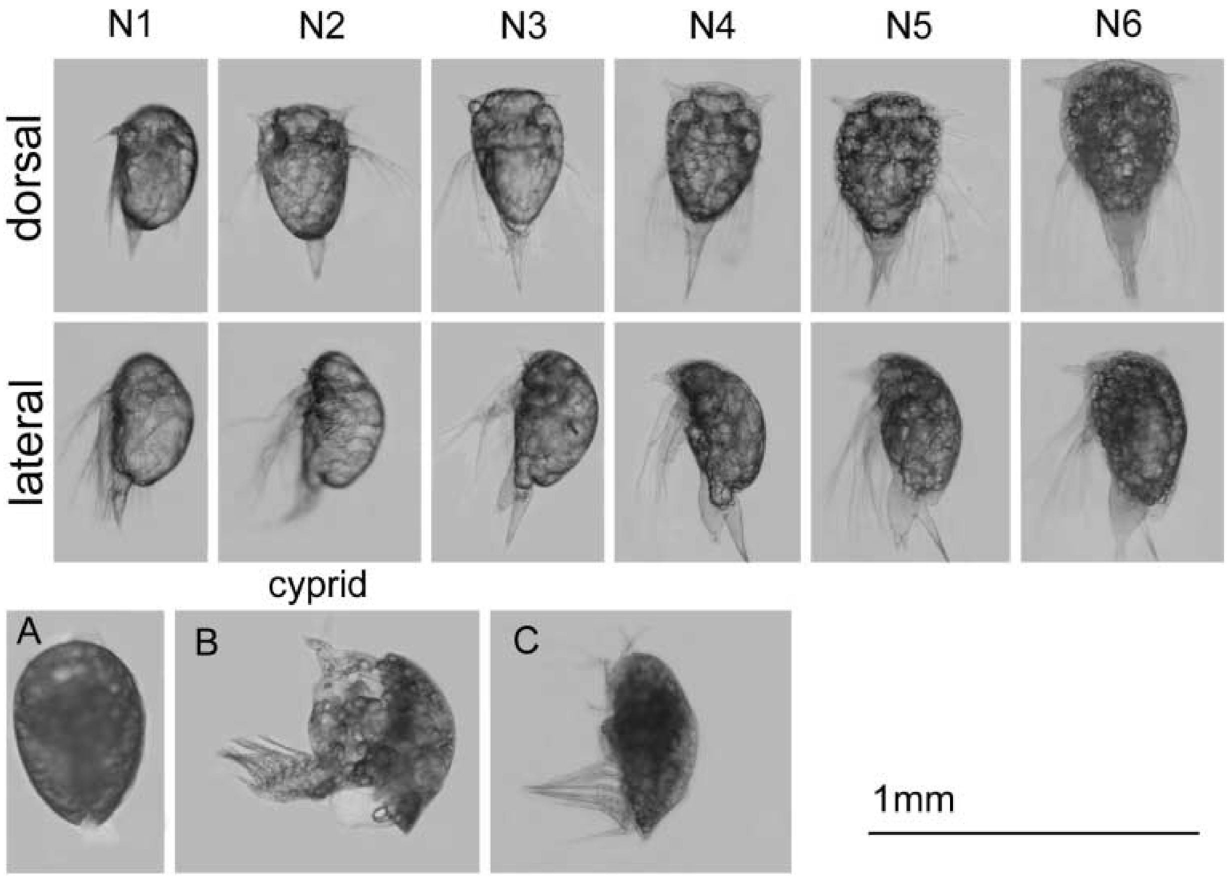

Figure 1. Successive larval development of *Neoverruca* sp. Dorsal and lateral features are shown. Neither naupliar nor compound eyes were visible in any larval stage. Endopods of antennae and mandibles were poorly developed through the six naupliar stages. The N1 nauplius lacks the front-lateral horns. Abnormal (A, dorsal view of unfolded carapace; B, lateral view of extruded thorax); and normal (C) cyprids are shown. All photographs are of the same magnification.

Table 1. *Intermoult period of naupliar larvae of* Neoverruca *sp. The exact period of the first larval stage (N1) was unable to be determined as the larvae hatched inside the parent's mantle cavities. Two individuals in each larval stage were fixed for morphological observations, and therefore the number of individuals decreased for each larval stage regardless of the survival rate.*

Stage	N	Mean	±SE	Maximum	Minimum	Survival rate
N1	42	Within three days				100
N2	40	9.8	0.49	16	5	100
N3	37	9.7	0.25	14	5	97
N4	36	10.7	0.26	16	8	97
N5	24	15.1	0.48	27	12	97
N6	19	62.2	1.64	85	53	56
N2–N6	19	105	1.99	127	96	–

N, number of individuals; SE, standard error.

metamorphosing into juveniles within 183 days after their release.

The number of stages and general morphology of *Neoverruca* sp. nauplii was the same as those of other planktotrophic and lecithotrophic nauplii of barnacles, except for the lack of front-lateral horns in the N1 stage. N1 larvae moulted within three days. N2, N3 and N4 larvae had similar intermoult periods and N5 was 1.5 times longer, although the duration of the N6 stage was extraordinarily long (four times longer than N5). Increases in the duration of intermoult periods have been observed previously in shallow-water species (Kado, 1982).

In the present study, nauplii were reared under atmospheric pressure, rather than 13.5 MPa (134 atm). However, Kon-ya & Miki (1994) reported that the larvae of the intertidal barnacle *Balanus amphitrite* survived and settled under a hydrostatic pressure of 20 MPa, suggesting that the larvae of vent barnacles might not be affected greatly by changes in hydrostatic pressure. *Neoverruca* sp. larvae were also successfully reared in filtered artificial seawater without food, addition of vent-associated chemicals or vibration, which suggests that they might not be affected greatly by such environmental cues during this period. This ability should enable *Neoverruca* sp. to migrate to new habitats through regular non-vent oceanic environments. As the larvae have positive buoyancy, and assuming they can withstand elevated temperatures, they could be transported to upper oceanic layers and drift with faster currents enabling greater dispersal.

In contrast to the N1–N5 stages, the N6 stage had an extraordinarily long average intermoult period, and most cyprids exhibited abnormal morphology, probably due to the present rearing conditions, such as lack of hydrostatic pressure or sulphide although these cues may not be important for the development of N1–N5 stages. Warén & Bouchet (1989) suggested that vent gastropods might

https://doi.org/10.1017/S0025315404009841h Published online by Cambridge University Press

delay settlement for several months until they reach a suitable environment. In barnacles, as the cyprid stage is the stage at which settlement occurs *Neoverruca* sp. might be able to extend the duration of their planktonic period by controlling the length of the N6 stage. The present rearing conditions did not emulate any factors usually characterizing hydrothermal vent fields. Consequently, the N6 stage nauplii might regard this environment as inappropriate for a lifelong habitat and therefore did not metamorphose and settle. For successful settlement and metamorphosis, cyprids of shallow-water barnacles need some extra cues such as light, water temperature, vibration, organic and inorganic chemicals (including settlement-inducing pheromones derived from conspecific adults) and bacterial films (Rittschof et al., 1998; Clare & Matsumura, 2000). By detecting these cues, the barnacle larvae can recognize a suitable habitat for settlement. Settlement failure in *Neoverruca* sp. might be caused not only by abnormal morphology but also by a dissatisfaction with various environmental cues. These cues, the absence of which under the present rearing conditions may have led to the long intermoult period of N6 and abnormal development of cyprid larvae, might be relevant to the vent endemism of *Neoverruca* sp.

We are grateful to the on-board scientists of cruise KY02-11, the officers and crew of the Research Vessel 'Kaiyo', and the operation team of the ROV 'HyperDolphin' under Commander Kazuya Mitsufuji. We also thank Dr Dhugal J. Lindsay for useful discussions. Part of this study was supported by a grant from the Ministry of Education, Culture, Sports, Science and Technology of Japan (no. 12NP0201).

REFERENCES

Anderson, D.T., 1994. *Barnacle structure, function, development and evolution*. London: Chapman & Hall.

Clare, A.S. & Matsumura, K., 2000. Nature and perception of barnacle settlement pheromones. *Biofouling*, **15**, 57–71.

Fujikura, K. et al., 2001. Report on investigation of hydrothermal vent ecosystems by the crewed submersible 'Shinkai 2000' on the Dai-yon (No. 4) Yonaguni Knoll and the Hatoma Knoll, the Okinawa Trough. *JAMSTEC Deep Sea Research*, **19**, 141–154. [In Japanese text with English abstract, tables and figures.]

Kado, R., 1982. *Ecological and taxonomical studies on the free-living nauplius of barnacles (Crustacea, Cirripedia)*. PhD thesis, University of Tokyo, Tokyo, Japan. [Japanese text, English tables and figures.]

Kon-ya, K. & Miki, W., 1994. Effect of environmental factors on larval settlement of the barnacle *Balanus amphitrite* reared in the laboratory. *Fisheries Science*, **60**, 563–565.

Kyo, M., Shitashima, K., Maeda, Y., Koike, Y., Fujii, T. & Sairaiji, M., 2003. Trial of hydrothermal plume sensing using newly developed ISFET pH sensor. *Proceedings of Oceans 2003*, 1732–1737.

Newman, W.A., 1989. Juvenile ontogeny and metamorphosis in the most primitive living sessile barnacle, *Neoverruca*, from an abyssal hydrothermal spring. *Bulletin of Marine Science*, **45**, 467–477.

Newman, W.A. & Hessler, R.R., 1989. A new abyssal hydrothermal verrucomorphan (Cirripedia; Sessilia): the most primitive living sessile barnacle. *San Diego Society of Natural History Transactions*, **21**, 256–273.

Rittschof, D. et al., 1998. Cues and context: larval response to physical and chemical cues. *Biofouling*, **12**, 31–44.

Tunnicliffe, V. & Fowler, C.M.R., 1996. Influence of sea-floor spreading on the global hydrothermal vent fauna. *Nature, London*, **379**, 531–533.

Warén, A. & Bouchet, P., 1989. New gastropods from East Pacific hydrothermal vents. *Zoologica Scripta*, **18**, 67–102.

Submitted 22 August 2003. Accepted 18 March 2004.