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Effects of temperature on the development and survival of *Nanomia bijuga* (Hydrozoa, Siphonophora)

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Abstract. The effects of temperature on the development of marine invertebrates have been studied primarily in benthic species. For this study, gametes were collected from *Nanomia bijuga,* a mesopelagic siphonophore, and were self-crossed. Fertilized eggs kept at 8 and 12°C developed more slowly at the lower temperature. Developing stages were preserved after 2, 4, 6, and 9 days of development for comparative analysis with SEM. Concurrent experiments compared survival. Eggs collected from two additional colonies were placed in four temperature treatments: 4, 8, 12, and 18°C. The young developed normally in all treatments, but survived longer at lower temperatures. Young of *N. bijuga* will develop to siphonulae possessing tentacles, nematocysts, and a functional gastrozooid without being fed. Nonetheless, it is likely that food constraints, rather than temperature, limited survival in this study.

Additional key words: mesopelagic, midwater, physonect, planulae, siphonulae

The physonect siphonophore *Nanomia bijuga* (CHIAJE 1841) occurs in a broad geographic range from the subtropics (Alvariño 1971) to the Mediterranean (Totton 1965) and polar regions (Alvariño et al. 1990; Pages & Gili 1992). In Monterey Bay, California, these siphonophores are common members of the mesopelagic community. Although they are most common between 200 and 400 m, colonies of *N. bijuga* occupy depths ranging from just below the surface to 800 m (Robison et al. 1998). Within this broad depth range, temperatures can vary from about 4.5 to 16°C.

We often collect colonies of *N. bijuga* with gametes. From May 1995 to September of 1999, we found adult colonies with ripe male gonophores and eggs in all calendar months except February and July (unpubl. data). Larval tolerances generally fall within the environmental extremes encountered over a given species' range and reproductive seasons (Thorson 1950; Morgan 1995a). If mature colonies reproduce throughout the year, as the presence of gametes indicates, then larvae should be able to survive and develop normally at a wide range of temperatures as well.

Few studies have addressed the development of midwater animals such as siphonophores. Embryogenesis and development of *N. bijuga* (Carré 1969; Mackie et al. 1987) and development of *N. cara* (Freeman 1983) have been investigated. The taxonomic distinctness of *N. cara* in the Pacific has been questioned and it is likely that Freeman, in fact, worked with *N. bijuga* (P.R. Pugh, C.E. Mills, pers. comm.). Carré (1969) established that development was indeterminate at all stages preceding organogenesis (young planulae) and that developing embryos progressed from blastulae to periblastulae to gastrulae, becoming planulae after 36 h at 14°C. She found that cleavage was superficial and partial, and that gastrulation occurred by primary delamination. After 60 h, the rudiments of a pneumatophore developed. Week-old stages possessed a float, tentacle(s), and a proto-gastrozooid. Freeman's (1983) results differed notably only in that regulative development ceased before the 8-cell stage (4–6 h at 11–12°C).

Whether or not young of *N. bijuga* are larval is an equivocal issue. The definition is not critical to this paper, but we point out that discrepancies exist in the use of the term. Both Carré (1969) and Freeman (1983) have described the development of these siphonophores as direct, while at the same time referring to planulae and siphonulae as 'larval.' Direct development usually refers to species that progress from embryo to adult without a larval stage (McEdward & Janies 1997). For cnidarians, the typical larval stage is a planula (Fautin et al. 1989; Levin & Bridges 1995). Development is indirect, as the planktonic planula eventually settles and develops into a benthic polyp. *N. bijuga* is a holoplanktonic species and planulae de-

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velop into siphonulae without a polyp stage. In this paper, we chose to follow the precedent set forth by Carré (1969), Freeman (1983), Kirkpatrick & Pugh (1984), and Mackie et al. (1987) in referring to developing stages of *N. bijuga* as 'larval' despite their lack of a radical metamorphosis.

Planktonic larvae inhabit a more dynamic environment than do benthic larvae. Generally, planktonic larvae are thought to have little or no control over their fate (Morgan 1995a), particularly if they are not strong swimmers. Siphonophores in the genus *Nanomia* posses an apical pore in their pneumatophore through which carbon monoxide can be released (Pickwell 1966). Thus, at least some physonect siphonophores are capable of regulating float volume during vertical migrations by controlling the amount of gas in their pneumatophore (Mackie et al. 1987).

During development, *N. bijuga* forms a pneumatophore in about 3–5 days (unpubl. obs.). Thus, it is possible that siphonulae have some buoyancy control early in their development and can maintain themselves within a preferred depth and temperature range, optimizing development. Alternatively, siphonulae could be positively buoyant and relegated to developing at or near the surface, where they would remain within a narrower and warmer temperature range than that experienced by adult colonies. In either case, larval temperature exposure would be more limited than that of adults. The present study was conducted to investigate the effects of temperature on the development and survival of larvae of *N. bijuga.*

Methods

Mature specimens of *Nanomia bijuga* were collected with the ROV *Ventana* (Robison 1993) at depths between 200 m and 400 m in Monterey Bay. All specimens were kept in a darkened, 8°C cold room at the Monterey Bay Aquarium Research Institute (MBARI). Colonies were inspected daily and gametes were obtained by one of two methods once the male gonophores developed. After being kept in the dark for a minimum of 12 h, siphonophores were light-shocked to induce spawning (Miller 1979; Freeman 1983). When light shocking failed to initiate spawning, gametes were gently stripped free with dissecting needles.

In various trials, we attempted to feed the developing larvae using several prey types—rotifers, nauplii, copepods, and ground krill—but had little success. Consequently, larvae were not fed during these studies. Antibiotics are harmful to some larvae (M. Strathmann 1987) and thus were not added to these cultures. Filtered (0.2 μm), autoclaved seawater was changed daily.

Development and growth

Ripe gametes were manually stripped from a single colony (collected 6 May 1997) and then self-crossed. After 15 min, the fertilized eggs were divided between 2 temperature treatments: 8 and 12°C (N = 75–100 per treatment). These are within the temperature range at depths where adult siphonophores are most abundant (Robison et al. 1998). Development was monitored hourly for the first 12 h, and daily thereafter. For the first 6 days of development, larvae were video-taped every 24 h for subsequent image analysis. Siphonulae were video-taped under the dissecting microscope and a size index was calculated for each siphonophore. Between the 2 temperatures, size was compared with a 2-way ANOVA (N = 48 per treatment, $\alpha = 0.05$). Viable larvae were preserved in 2% glutaraldehyde with cacodylate buffer and seawater after 2, 4, 6, and 9 days of development. Osmium tetroxide was used as a secondary fixative, and samples were rinsed with reverse osmosis (RO) water before being put through a dehydration series in ethanol. Finally, planulae and siphonulae were critical point dried and sputter coated in preparation for scanning electron microscopy.

Survival

A colony collected on 8 May 1997 spawned spontaneously after more than 15 h in the light, at 8°C. Cleaving eggs were separated and allowed to develop at 8°C until they became planula larvae. After 40 h of development at 8°C, planulae were divided among 4 temperature treatments (N = 60 per treatment): 4, 8, 12, and 18°C. The number of surviving larvae was recorded daily by counting each larva as it was transferred via pipette to a dish of clean, filtered seawater. Dead larvae were removed and preserved in 2% glutaraldehyde and seawater.

Another colony, collected on 30 April 1999, had eggs and ripening male gonophores. This colony was exposed to light after being kept for 3 days in the dark. After ~1 h in the light, the colony released eggs. The male gonophores were dissected with needles, and the sperm were used to fertilize the eggs. After ~4 h at 8°C, when the developing embryos were between first and second cleavage, they were divided among 4 temperature treatments (N = 28 per treatment): 4, 8, 12, and 18°C. Previous observations indicated that developing embryos are sensitive to osmotic changes, so after fertilization, eggs were allowed to develop briefly in the water in which they had been spawned. Once they became planulae (1–2 days), they were transferred via pipette to a dish of clean, filtered seawater. Surviving larvae were counted every 2–3 days for 22

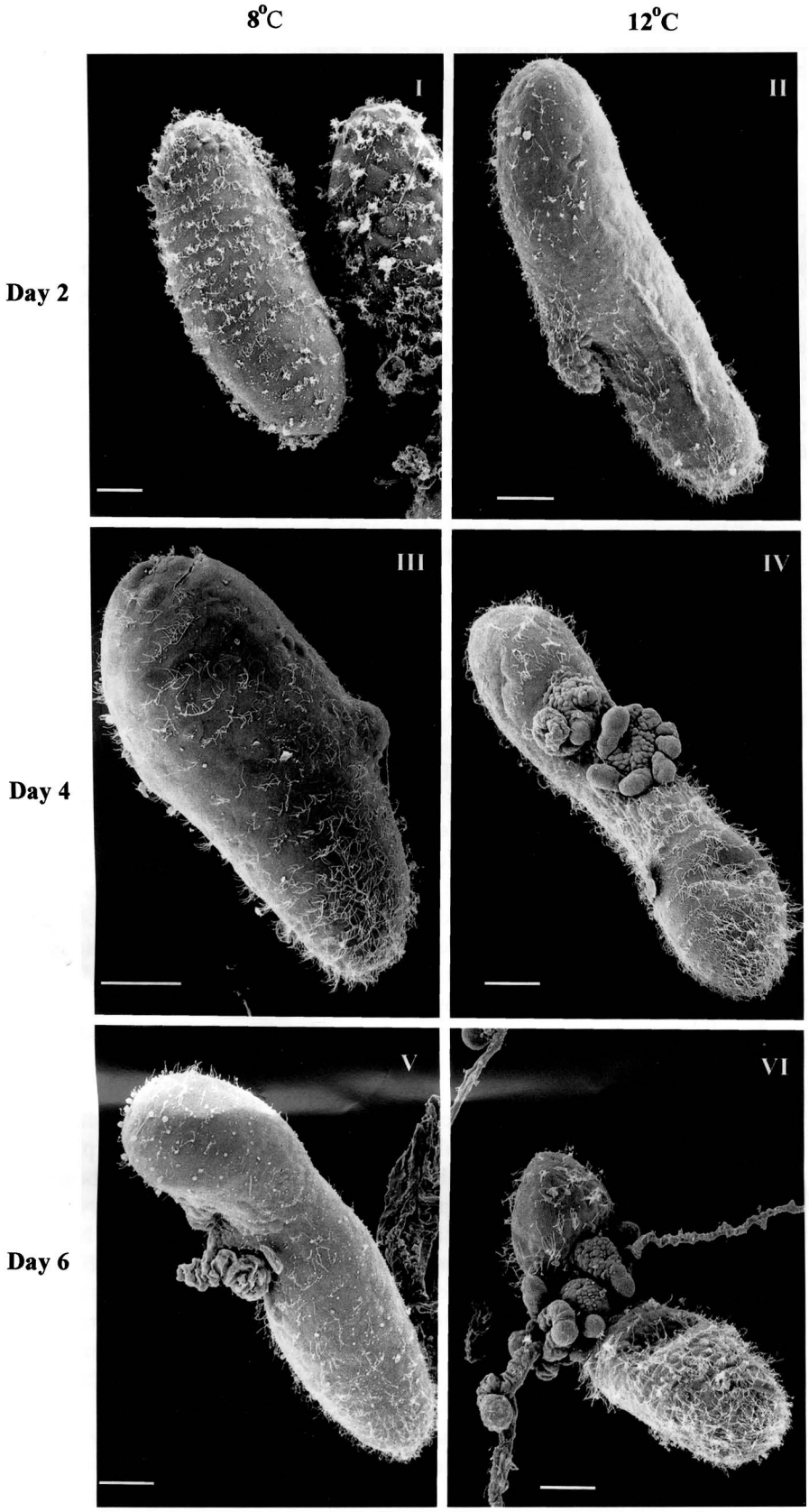

Fig. 1. Time series of *Nanomia bijuga* exposed to two temperatures: 8°C (left) and 12°C (right).

At 2 days:

I. A ciliated planula.

II. A late-stage planula/early siphonula with tentacle rudiment.

At 4 days:

III. Late-stage planula with tentacle rudiment. At this stage, no gas was apparent in the pneumatophore.

IV. Siphonula with gas-filled float rudiment (upper left), proto-gastrozooid (lower right), and coiled tentacle. Nematocyst batteries hang from the tentacle but cnidobands and terminal filaments have not yet formed.

At 6 days:

V. Siphonula without nematocyst batteries.

VI. Well-developed siphonula with two tentacles and developing nectophores (swimming bells). Scale bars, 50 μm.

Table 1. Development at 8 and 12°C. Stages given are the majority seen through the dissecting microscope and do not represent all stages present. The experiment was terminated after 9 days.

Time	8°C	12°C
6 h	2- to 4-cell stage.	8-cell stage.
12 h	16-cell stage. Some embryos motile.	64-cell stage and gastrulae. All embryos actively motile.
24 h	Gastrulae-early planulae.	Planulae.
2 d	Planulae.	Planulae with tentacle rudiment.
4 d	Late planulae, few pigmented. No float rudiment. No tentacles.	Early siphonulae pigmented and with float rudiment. All with a tentacle.
6 d	Early siphonulae with some pigment. Float with some gas. No tentilla on tentacles.	Siphonulae with pigmented gastrozooid and float. Well-developed pneumatophore. Feeding tentacle(s) with tentilla.
9 d	Similar to siphonula at 12°C and 6 d, but tentacles less well developed.	As above, but with 2 tentacles, both with tentilla. Body size shrinking.

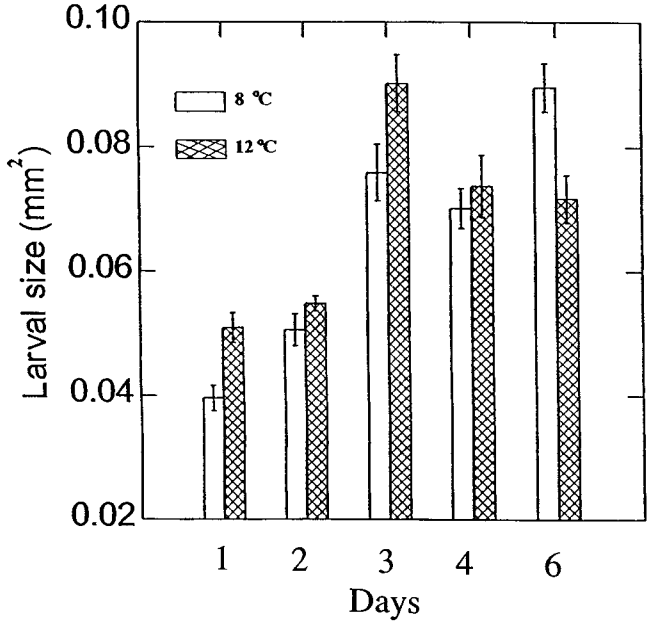

Fig. 2. Larval size over 6 days of development. Larvae kept at 8°C increased in size throughout the experiment. Larvae kept at 12°C increased in size up to 74 h, then decreased. Bars represent standard error. N = 48.

days. Dead larvae were removed and preserved in 2% glutaraldehyde and seawater with a cacodylate buffer.

Results

Adult specimens of *Nanomia bijuga* from Monterey Bay generally possessed eggs and often had male gonophores. If male gonophores were not present at the time of collection, they generally formed 2–4 days thereafter. Gametes, if absent at the time of collection, usually developed in colonies that were kept in the dark. Light was only occasionally successful as a spawning cue. On 5 occasions, temperature shock was tried without success. Mechanically stripping eggs from the siphosome with a needle was effective. However, stripping yielded fewer embryos than did the spontaneous spawning events, probably because mechanical damage may have rendered some eggs unviable, or gametes may have differed in developmental state. Eggs from 2 adult specimens averaged 282 μm in diameter (range 271–299 μm, SD = 6 μm, N = 18).

Development and growth

The eggs of *N. bijuga* were ciliated and slightly motile, and cilia became more numerous throughout development (Fig. 1). Within 20 h at 12°C and 40 h at 8°C, 90% of the embryos had progressed from gastrulae to more elongate planulae. After becoming planulae, larvae formed a tentacle bud, then a pneumatophore/float. The proto-gastrozooid continued to elongate, and a gastric cavity began to form. Larvae were considered siphonulae when they possessed a tentacle, float rudiment, and proto-gastrozooid. Healthy siphonulae were most often observed in the middle or upper half of the culture dish, exhibiting neutral or slightly positive buoyancy.

Larvae developed normally at both temperatures, but larvae kept at 12°C developed more rapidly (Fig. 1). At 8°C, all larvae had developed to early planulae after 2 days, but they did not possess tentacle buds and were not pigmented. In contrast, at 12°C, larvae had grown tentacle buds and were pigmented on both the float and proto-gastrozooid (Fig. 1, Table 1). Even after 3 days, few larvae at 8°C had developed tentacle buds or a pneumatophore, and none had tentacles or pigmentation on the proto-gastrozooid. The pneumatophore, unlike that of larvae at 12°C, did not obviously contain gas.

Differences in developmental and growth rates between treatments were most obvious after the larvae became planulae (Fig. 2). Larval size differed significantly over time ($p<.01$) and between the 2 temperatures ($p = .049$). The ANOVA showed a significant

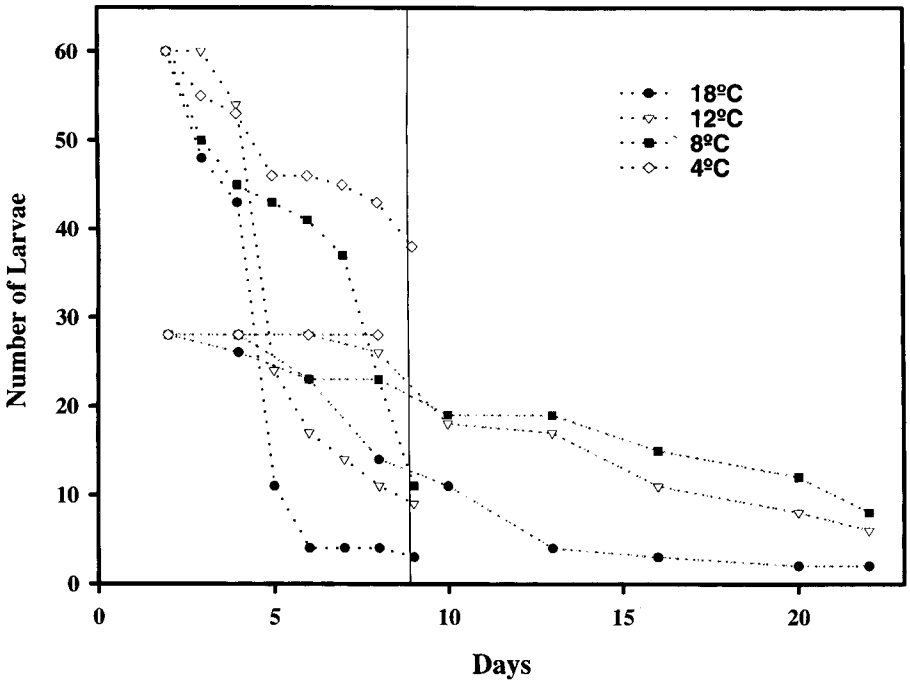

Fig. 3. Survival of larvae at 4 temperatures. The solid line indicates the termination of the shorter experiment at 9 days (N = 60 per treatment). The second experiment ended after 22 days (N = 28 per treatment).

interaction between temperature and time ($p<.01$). Larvae kept at 8°C were still growing larger at 6 days, whereas larvae from the 12°C treatment began to decrease in body size after 3 days (Fig. 2).

Survival

Two siphonophores released gametes after exposure to light. One released eggs and sperm, whereas the other released only eggs and its ripe male gonophores required manual stripping. The first set of embryos were 40 h old and had developed to planulae at 8°C before being placed in the 4 temperature treatments. The second set of embryos were only 4 h old and were between the first and second cleavage when they were placed in the 4 temperature treatments.

When the first experiment was terminated, 9 days after fertilization, survival was inversely proportional to temperature, although the animals developed normally at all temperatures. Only 5% of the larvae in the 18°C treatment remained alive, in contrast to 63% of the larvae at 4°C (Fig. 3). Although not quantified during this experiment, growth rate was visibly affected after planulae had spent one day in each temperature treatment.

The second set of larvae showed the same trend—the larvae survived longest at lower temperatures—although they survived longer over all temperatures than did the first set (Fig. 3). After 22 days, when the experiment was terminated, 7% of the larvae remained alive at 18°C compared to 28% of the larvae at 8°C. The 4°C chiller failed after 8 days, at which time all larvae in that treatment had developed to planulae.

Discussion

In this study, development progressed in a manner comparable to that described by Carré (1969) and by Freeman (1983). Many factors (e.g., egg size, nutrition) can influence the rate of development, but temperature is arguably the most important parameter (Hoegh-Guldberg & Pearse 1995). Not surprisingly, lower temperatures generally result in slower developmental rates. At colder temperatures, development took longer for siphonophores from Monterey Bay than was observed by Carré at higher temperatures (1969). The growth rate of *Nanomia bijuga* varied directly with temperature, while survival varied inversely with temperature (Figs. 2, 3). The different slopes seen for the two survival experiments (Fig. 3) may result from differences in egg quality and/or crowding, since the same-sized culture dishes were used for both experiments. For the development experiment, siphonophores developing at 12°C initially grew more quickly than those at 8°C. Subsequently, 12°C siphonulae began to decrease in size while the siphonulae at 8°C were still growing. We suspect that the significant interaction between temperature and time was the result of different timing of growth and de-growth.

Nutrition is obviously critical for developing organisms (Clarke 1982, 1990, 1992; R. Strathmann 1987; Jaeckle 1995). A siphonula can feed after the proto-

gastrozooid develops a terminal opening, but we have been unable to feed developing young consistently. Because development progressed normally over the range of experimental temperatures, the inverse relationship between survival and temperature is probably an artifact of starvation. Starvation probably affected the rate of development as well as survival in the present study. Nonetheless, it is notable that young stages of *N. bijuga* can develop without external nutrition. They almost surely feed during embryogenesis but can develop without food until well after they are capable of feeding.

The ability of these siphonophores to develop normally over widely varying temperatures seems consistent with the broad geographic and vertical distributions over which adult colonies of this species are found. Yet, larval behaviors (e.g., swimming, phototaxis, barokinesis) can be quite complex. Some larvae control their depth, thereby enhancing or limiting dispersal, and larvae have been shown to react predictably to various physical and chemical aspects of their environment (Young & Chia 1987; Morgan 1995b; Young 1995). Developing young of *N. bijuga* have a visible gas bubble in their pneumatophore by the time they reach an early siphonula stage (~3 days to 1 week). Healthy siphonulae are either positively or neutrally buoyant. Because they possess a pneumatophore, it seems likely that siphonulae have some ability to control their depth and thus the temperature at which they develop. Unfortunately, we lack any data to indicate where developing stages of *N. bijuga* are most commonly found. Such data might help us to understand why there is a sharp seasonal peak in the abundance of adult colonies, when reproductive colonies occur throughout the year (Robison et al. 1998).

Life cycles evolve through changes in developmental modes: indirect to direct development or planktotrophy to lecithotrophy (McEdward & Janies 1997). Without a radical metamorphosis from planula to siphonula, our use of "larva" may seem inaccurate. Yet, a simple larval structure may be the result of selection to eliminate structures that are not necessary (Wray 1995) because siphonulae and adult siphonophores live in a broadly similar environment and encounter broadly similar selective pressures. Defining life histories and changes in developmental modes is seldom simple, but it is even harder when the environmental and selective pressures to which larvae are subjected differ radically from those affecting adults. For this reason, studies on midwater organisms such as the siphonophore *N. bijuga* can help us to further our understanding of life histories and embryogenesis. However, we must understand the basics regarding the development of more midwater species before we can draw comparisons to the abundant literature describing development in benthic organisms.

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