

A novel mutualistic relationship between a doliolid and a cnidarian, *Bythotiara doliioeques* sp. nov.

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A description of the first known mutualistic interaction between a cnidarian and a pelagic tunicate is presented. Calyccopsid anthomedusan polyps, belonging in the genus *Bythotiara*, were discovered associated with individual zooids of a new doliopsid (Thaliacea: Doliolida: Doliopsidae) collected from the mesopelagic waters of Monterey Bay. One hundred and seventy five polyp-bearing doliopsid colonies have been observed and 43 were collected from 1995 to 2004. Colonies were observed *in situ* and then brought back to the laboratory and maintained in aquaria. More than 250 individual anthomedusan polyps were removed from positions at the buccal siphon of their doliopsid host zooids and maintained in the laboratory until they released medusae. The morphology and taxonomy of the polyps and medusae are described. A unique tentacle-based asexual reproduction strategy is discussed. The relationship between the doliopsid and the polyp appears to be mutualistic, with the doliolid obtaining both nutrition and protection from the anthomedusan polyps, while the polyps gain access to a mobile, food-rich substrate.

INTRODUCTION

Most medusae require a benthic habitat to complete their life cycle. The archetypal pattern involves free-swimming medusae which give rise to gametes that form a zygote that settles onto the benthos before metamorphosis. This metamorphosis results in a feeding polyp, or hydroid, which often has the capability of asexual propagation of additional polyps. This benthic polyp produces the medusae asexually, which then start the cycle anew. However, benthic substrate suitable for settling is often in limited supply, and deep benthic substrates may be physiologically challenging because of depth related factors. Cnidarian polyps must compete with many other organisms for these limited resources. Algae, sponges, other cnidarians, polychaetes, bryozoans, ascidians, and others all compete with polyps for space in benthic habitats (Dales, 1957; Osman, 1977; Gili & Hughes, 1995). Many hydroids have specialized in colonizing atypical substrates, such as other organisms, thereby reducing competition (Dales, 1957, 1966; Gili & Hughes, 1995). Among these are hydroids which have adapted to an associated lifestyle, utilizing the exterior of a potential competitor as the substrate. In many species, these interspecific associations go beyond epibiosis to include symbiosis, and these symbioses can range

from parasitism to mutualism (see Dales, 1957; Henry, 1966; Rees, 1967 for terminology).

The hydroids *Hydrichthys* spp. and *Podocorella minoi* are known parasites of fish (Dales, 1966; Boero et al., 1991). The hydroid *Halocoryne epizoica* feeds on the lophophoral tentacles of a bryozoan (Piraino et al., 1992). Narcomedusae are known to parasitize other medusae. Many species of the genus *Cunina* parasitize *Geryonia* spp. and *Turritopsis nutricula* (Mayer, 1910; Dales, 1966), as well as *Solmissus incisa*, *Ptycogena lactea*, and *Foersteria purpurea* (K.A. Raskoff, unpublished data; Osborn, 2000). In *Cunina*, the parasitic polyps not only adsorb the reproductive tissues of the host but also ingest food captured by the host (K.A. Raskoff, unpublished data). *Cunina* spp. are effectively reproductive castrators, as the host's reproductive capability is destroyed by the budding *Cunina* polyps.

Many other examples of symbiotic interaction between hydroids and other taxa have been reported. The best studied are the cases of *Hydractinia* spp. living on gastropod shells occupied by decapods. These interactions are frequently termed mutualistic, with the decapod gaining increased protection from the polyp's stinging tentacles, as well as camouflage. The benefits to the polyps include a home in a substrate-limited habitat, transport, and access to food items from the crabs' feeding (Brooks & Mariscal, 1985;

Yund & Parker, 1989; Gili & Hughes, 1995). Hydroids have also been found on pelagic heteropods and pteropods, but the relationship between these gastropods and cnidarians is unclear (Lalli & Gilmer, 1989). Other examples of hydroid symbiotic partners include other cnidarians (Hughes, 1975), sabellid worms (Hand & Hendrickson, 1950; Hand, 1954), polychaetes (Dales, 1966), bryozoans (Osman & Haugsness, 1981; Boero & Hewitt, 1992; Piraino et al., 1992), bivalves (Hand, 1957; Dales, 1966; Rees, 1967; Vance, 1978; Kubota, 1992; Piraino et al., 1994), octopods (Rees, 1967), crustaceans (Dales, 1966; Moore, 1985; Cerrano et al., 1998), holothuroids (K.A. Raskoff, unpublished data), and tunicates (Brinckmann-Voss, 1979; Rees, 1979; Schuchert, 1996). All these interactions, from epibiotic to symbiotic, have the commonality of a cnidarian utilizing another organism as substrate, and often obtaining some nutritive benefit. The majority of these interactions have been found in benthic systems; there are few examples of pelagic interactions, except the associations with, *Cunina* spp., and pelagic gastropods noted above. These few pelagic examples are signifi-

cant because the cnidarians reside on organisms which have tremendous dispersive ability in comparison to those with benthic hosts. In addition, since the polyps are off the bottom and in the open water, a completely different array of food is made available to them.

Presented here is the first known report of a hydroid associated with a pelagic tunicate. The hydroid has been found exclusively on a newly described genus and species of doliolid (Thaliacea: Doliolida: Doliopsidae; see Robison et al., this issue). The hydroid is a new species of *Bythotiar*a. All known *Bythotiar*a spp. hydroids have been found living in the incurrent siphons of benthic tunicates (Fraser, 1937; Brinckmann-Voss, 1979; Rees, 1979; Schuchert, 1996), but never on pelagic species. More than 250 individual anthomedusan polyps were observed attached in positions at the buccal siphons of their doliopsid host zooids. A detailed description of both the hydroid and medusa stages is presented, along with a description of a unique, tentacle-based, asexual reproductive strategy. The relationship is apparently mutualistic, with both the hydroid and the doliolid benefiting from the interaction.

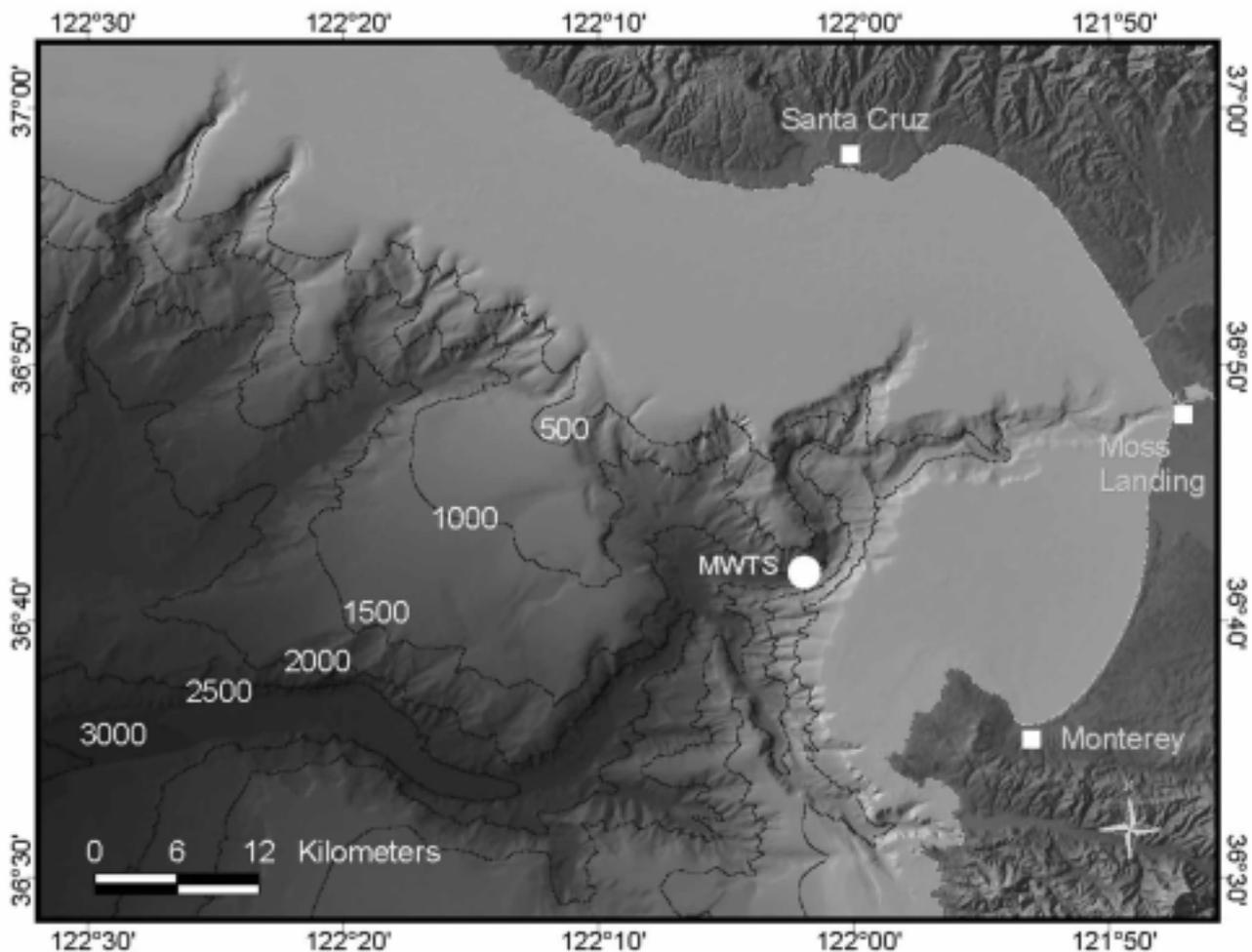


Figure 1. Map of Monterey Bay showing the MBARI midwater time-series site (MWTS), in 1600 m of water over the axis of the canyon.

MATERIALS AND METHODS

One hundred and seventy five polyp-bearing doliopsid colonies have been observed and 43 were collected from 1995 to 2004. Live doliopsid colonies and their associated hydroids were observed and collected using remotely operated vehicles (ROVs) at the Monterey Bay Aquarium Research Institute's (MBARI) midwater time-series site at 36°42.00'N 122°02.00'W, over the axis of the Monterey Canyon in 1600 m of water (Figure 1). The ROVs Ventana and Tiburon are specialized tools with a variety of sensors and modifications for midwater research (see Robison, 1993, 2000). Doliopsid colonies with their attached hydroids were captured in 7.5 l detritus samplers (Youngbluth, 1984; Robison, 1993).

Colonies were observed *in situ* then collected and brought back to the laboratory ashore where they were maintained in dark, temperature-controlled chambers. Intact doliopsid colonies were observed and the behaviours of the hydroids were noted. More than 250 individual polyps were removed from the doliopsid colonies and separated into 100 ml glass bowls or 10 ml culture chambers with 0.2 µm double filtered seawater, and were maintained at *in situ* temperatures (6–8°C).

Polyps and medusae were hand fed on 1–2 day old *Artemia* sp. nauplii, macerated bivalve digestive gland (*Mytilus* sp.), and nutrient infused agar (Raskoff et al., 2003). Morphology and development was followed and recorded using a video-equipped Nikon SMZ-U dissection microscope with 35 mm and digital cameras. Cnidae were photographed with a Zeiss Axioplan 2 phase contrast compound microscope with a Spot SP100 CCD digital camera.

RESULTS

The Doliolid

The Thaliacean suborder *Doliopsidina* is a little-known taxon in the order Doliolida (Godeaux, 1998, 2003; Godeaux & Harbison, 2003). The suborder shares the general morphological patterns of other doliolids, with a few major differences. The body is more spherical than the barrel-shaped bodies typical of other doliolids (Figure 2). The characteristic eight muscle bands of the suborder Doliolida are reduced to paired bands at the buccal and atrial siphons, and single lateral bands on each side of the animal's mid-body. In Monterey Bay, colonies were found between 78 and 2843 m depth. The zooids of this previously undescribed doliopsid occurred in colonies of 6–232 elements, connected by a contractile stolon. The taxonomic status of the new doliopsid and studies of its

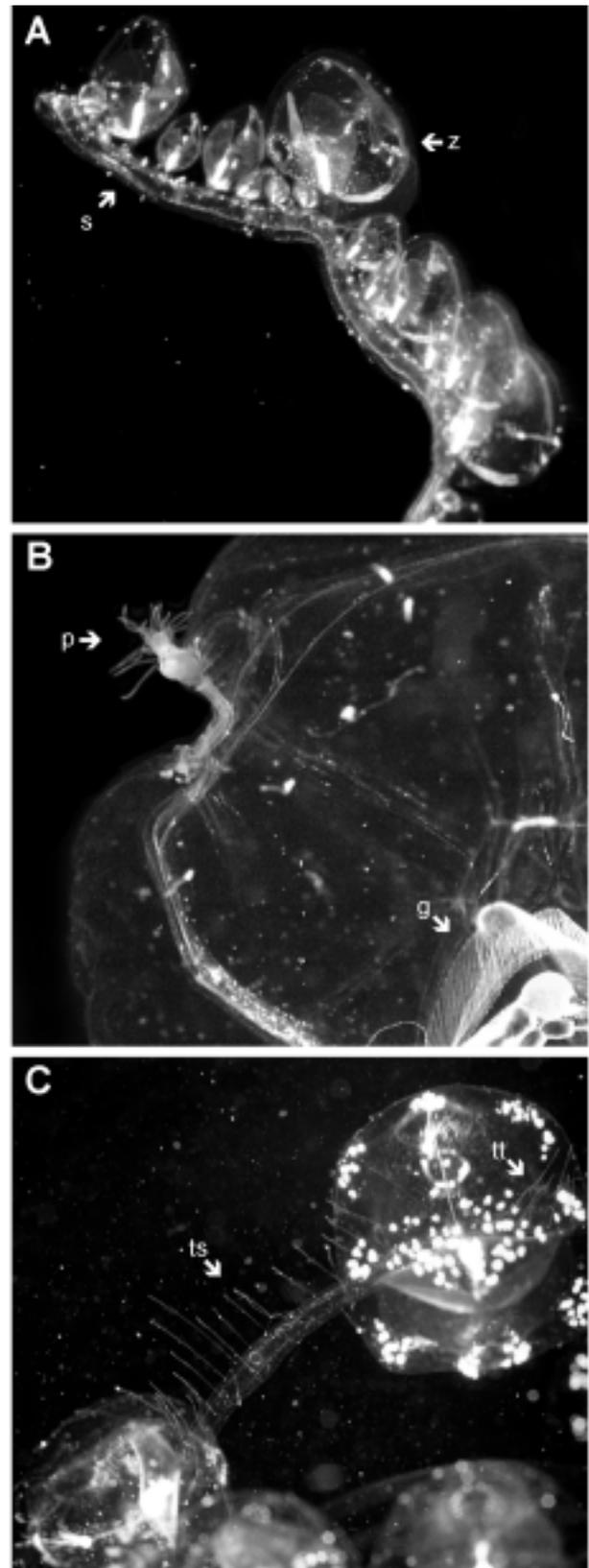


Figure 2. Doliopsid colony with *Bythotiara dolioeques* polyps and autonomous tentacles. (A) Small chain of Doliopsids, with contractile stolon and zooids; (B) close up of one zooid showing polyp and gill apparatus; (C) autonomous tentacles on the stolon and tunic of a doliopsid colony. s, stolon; z, zooid; p, polyp; g, gill apparatus; ts, autonomous tentacles on stolon; tt, autonomous tentacle on tunic.

ecology and distribution are described in Robison et al., (this issue). According to Harbison (1998) and Dales (1957, table 1), no hydroid polyps are known to inhabit any pelagic Thaliacean, therefore this appears to be the first description of a hydroid-thaliacean interaction. This association is similar to the described interaction between *Bythotiara huntsmani* and solitary benthic ascidians in the north-east Pacific (Brinckmann-Voss, 1979; Rees, 1979).

The Cnidarian

SYSTEMATICS

Class HYDROZOA

Order ANTHOMEDUSAE

Family CALYCOPSIDAE

Genus *Bythotiara*

Bythotiara dolioeques sp. nov.

Type material

One holotype and three paratypes of both the polyp and medusa are deposited in the California Academy of Sciences, Department of Invertebrate Zoology & Geology collection, CASIZ nos. 162750-162753. Specimens were collected at the MBARI midwater time-series site, Monterey Bay, California (36°42.00'N 122°02.00'W), collected at 477 m depth on 31 October 1997.

Comparative material examined

Holotype and paratypes of *Bythotiara stilbosa* (Mills & Rees, 1979) were examined from the California Academy of Sciences, Department of Invertebrate Zoology & Geology collection. Specimens were collected from Bodega Bay, CA.

Diagnosis of hydroid

Hydroid found attached to the buccal siphon of *Doliolula equus* (Thaliacea: Doliolida: Doliopsidae) (Robison et al., this issue). Polyps are unbranched, monomorphic, and singular, arising from root-like hydrorhiza (Figure 3B). The hydroid is not covered with perisarc. Polyps are 1.0 to 12.0 mm in height and have a highly contractile column. Hydrocalus is simple, with 3–5 irregular whorls of filliform, oral tentacles. Tentacles are hollow and highly elastic; their length can range between 0.5 mm to more than 15.0 mm. The hypostome is conical to clavate, with a circular mouth (can be flat or pulled back over the tentacles when everting stomach contents). The cnidome of the polyp consists of desmonemes, microbasic mastigophores, and microbasic euryteles (Figure 4A–C). Gonophores are borne on the side of the polyp, just below the tentacles. One to three medusae buds can

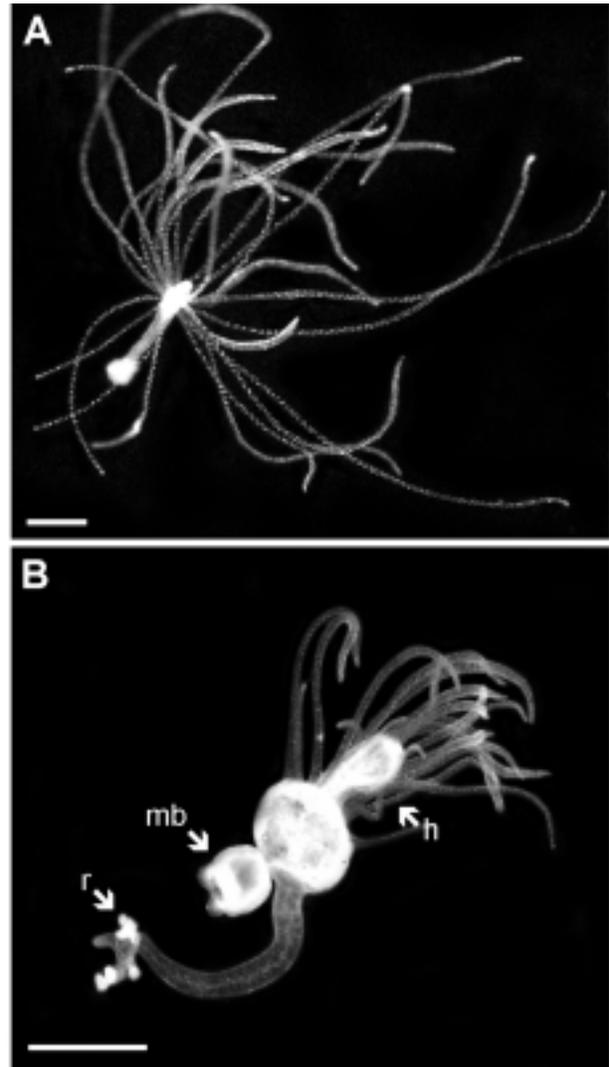


Figure 3. Polyp of *Bythotiara dolioeques*. (A) Polyp with extended tentacles; (B) polyp with root-like attachments, a medusa bud, full gastric cavity, and extended clavate hypostome. r, root-like attachments; mb, medusa bud; h, hypostome. Scale bars: 1.0 mm.

be found, with one always in an advanced stage of development compared to the others. Medusae develop to approximately 2.0 mm in diameter before they are released. Colour of hydroid and developing medusae is opaque white, with occasional green to orange internal gut coloration which is heavily influenced by prey coloration.

More than 500 doliopsid zooid-polyp associations have been observed, and over 250 polyps have been maintained in the laboratory, many of which gave rise to medusae. With very rare exceptions, only a single polyp was found on each zooid of the doliopsid colonies. Virtually all doliopsid zooids, excluding the very smallest, immature individuals, had an associated polyp. The polyps were located on the tunic next to the flaps of the buccal siphon (incurrent area) (Figure

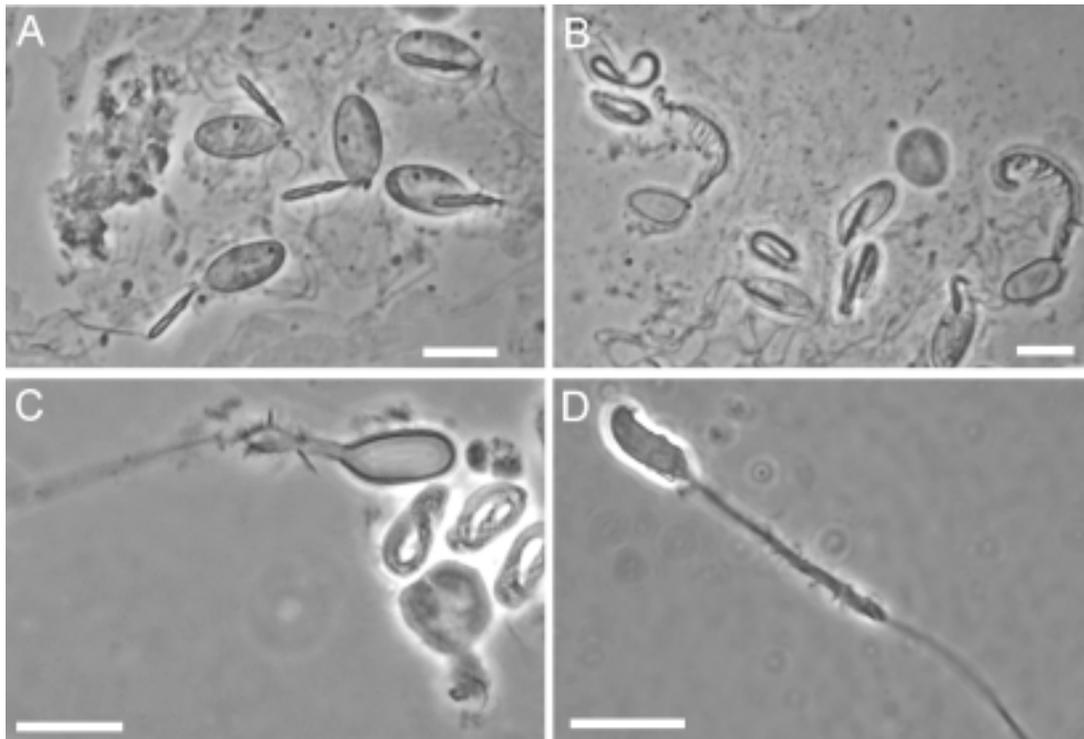


Figure 4. Cnidom of *Bythotia doliioeques*. A-C are from polyp, D is from medusa. (A) Microbasic mastigophores; (B) desmonemes and microbasic mastigophores; (C) microbasic eurytele with undischarged desmonemes; (D) macrobasic eurytele from tentacle tip of medusa. Scale bars: 10 μm .

2B). The attachment was by way of short, root-like protrusions off the base of the polyp's stalk (Figure 3B). The roots were sunk into the tunic of the doliopsid, but no damage or reaction was ever observed. On one occasion, a doliopsid colony of this genus and species was found which had no associated polyps. Several other doliolid species occur in Monterey Bay but none of these have been observed to bear polyps.

When in an undisturbed state, a polyp would bend into the buccal chamber of the doliopsid and extend its tentacles so they filled the buccal chamber. Any food brought into the chamber by the pumping and ciliary actions of the doliopsid would be filtered through the tentacles of the polyp. *Artemia* nauplii placed next to the buccal siphon were carried into the chamber on the feeding currents of the doliopsid and were captured by the polyp's tentacles. On one occasion in the laboratory, a copepod, which was captured along with the doliopsid as by-catch in the sampler, was observed to enter the buccal cavity of the doliopsid and then was captured and ingested by the polyp.

Polyps were also routinely observed everting their gastric cavities to clear their gut contents. The polyp would pull its mouth backward over its tentacles, effectively turning itself inside out. This behaviour has been noted previously in this genus by Fraser (1937). When this undigested material was free from the polyp, it was caught in the doliopsid feeding filter and consumed by the zooid.

Diagnosis of medusa

Medusae are released when ~ 2.0 mm in diameter and reach a maximum diameter of 4.6 mm. Newly released medusae are globular, slightly wider than high. Mesoglea is thick at apex and thins at the margin. Young medusae have an umbilical canal for 1–4 days after release, at which point the tissue fills in from the stomach to the exumbrella. The exumbrellar and subumbrellar surfaces are covered with small cnidocyst clusters (microbasic mastigophores) that are retained throughout development. The manubrium begins quadratic, then develops into a cruciform shape by the first week of life. Manubrium length is one half to two thirds of the subumbrellar height. The mouth is simple and cruciform, with nematocyst batteries. Four simple, unbranched, radial canals connect to the ring canal. There are eight adradial gonads, with no noticeable folding (specimens may not have been fully reproductive). The velum is wide, spanning one third of the radius, and highly contractile. The base of each of the four tentacles is slightly swollen and pigmented. The four perradial tentacles terminate in swellings that become heavily armed with nematocysts (macrobasic euryteles) in the first week of development. Tentacles are highly elastic, extensible to over three times the diameter of the bell (>12 mm), and contractile to less than one eighth the diameter.

Fifty-eight medusae were budded from polyps and raised in the laboratory for a maximum of 94 days.

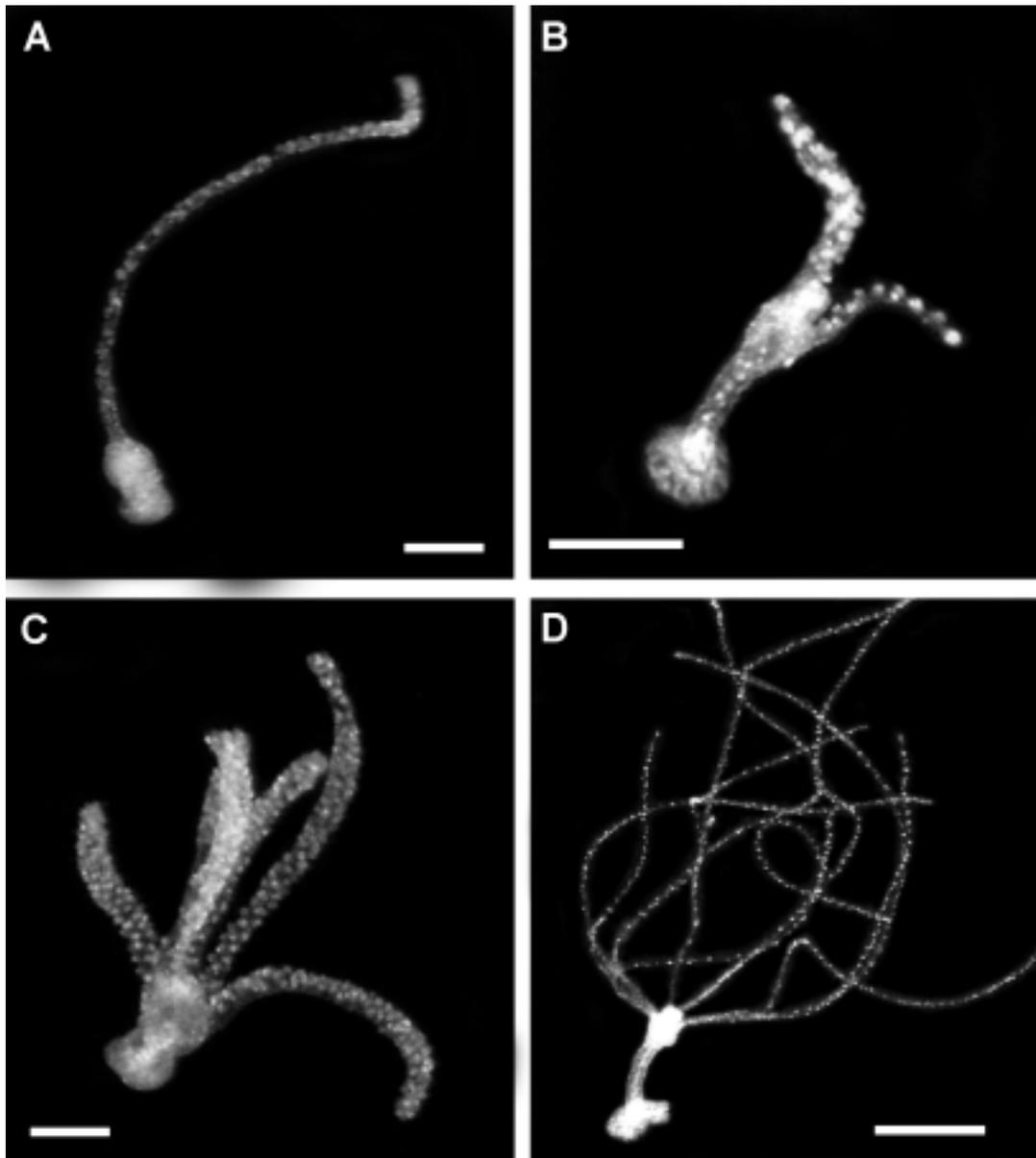


Figure 5. Autonomous tentacle development. (A) Autonomous tentacle at time of removal from doliopsid tunic; (B) tentacle five days after removal. The mouth parts are formed and it can feed; (C) tentacle 12 days after removal; (D) tentacle at 20 days after removal. Morphology is indistinguishable from that of a polyp. Scale bars: A–C, 200 μm ; D, 1.0 mm.

Figure 6 shows the general morphology of a 28-d-old medusa. Figure 7 shows the development of a single medusa from polyp to 45 days old. The velum of the medusa was quite thin at rest, but when swimming or eating *Artemia*, the velum would cinch almost completely shut, effectively trapping prey items inside the bell.

Etymology

The specific name *dolioeques* (Latin: *dolio* = small barrel; *eques* = horseman, or rider) refers to the novel habitat in which this species is found, attached to the buccal siphon of a doliolid.

Autonomous tentacles

Entire doliopsid colonies were frequently found covered with hundreds of autonomous tentacles (Figure

2C). These tentacles lacked a mouth or any outward signs of hydroid morphology, except for a small swelling at the base of attachment. The tentacles still retained their ability to contract and had active nematocysts. These tentacles were found attached on all surfaces of the outer tunic of the doliopsid colony. They survived and were reactive to touch for several months when removed and kept in filtered water in the laboratory, with no care except for occasional water changes.

On three occasions in the laboratory, the tentacles of a *Bythotia dolioeques* polyp were observed to extend to maximal length and attach their distal tip to the stalk or tunic of a nearby doliopsid zooid. These tentacles then separated from the polyp at their proximal

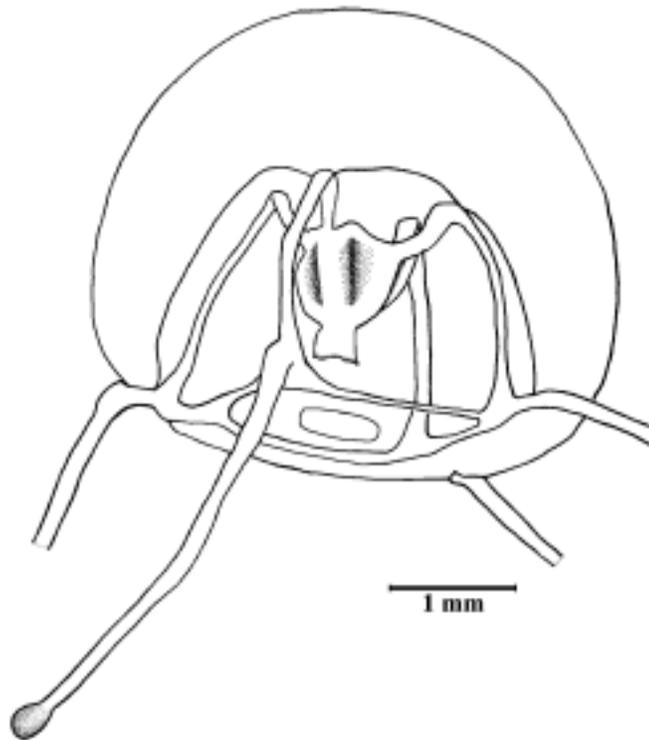


Figure 6. *Bythotiarra dolioeques* medusa at 25 days after release from polyp.

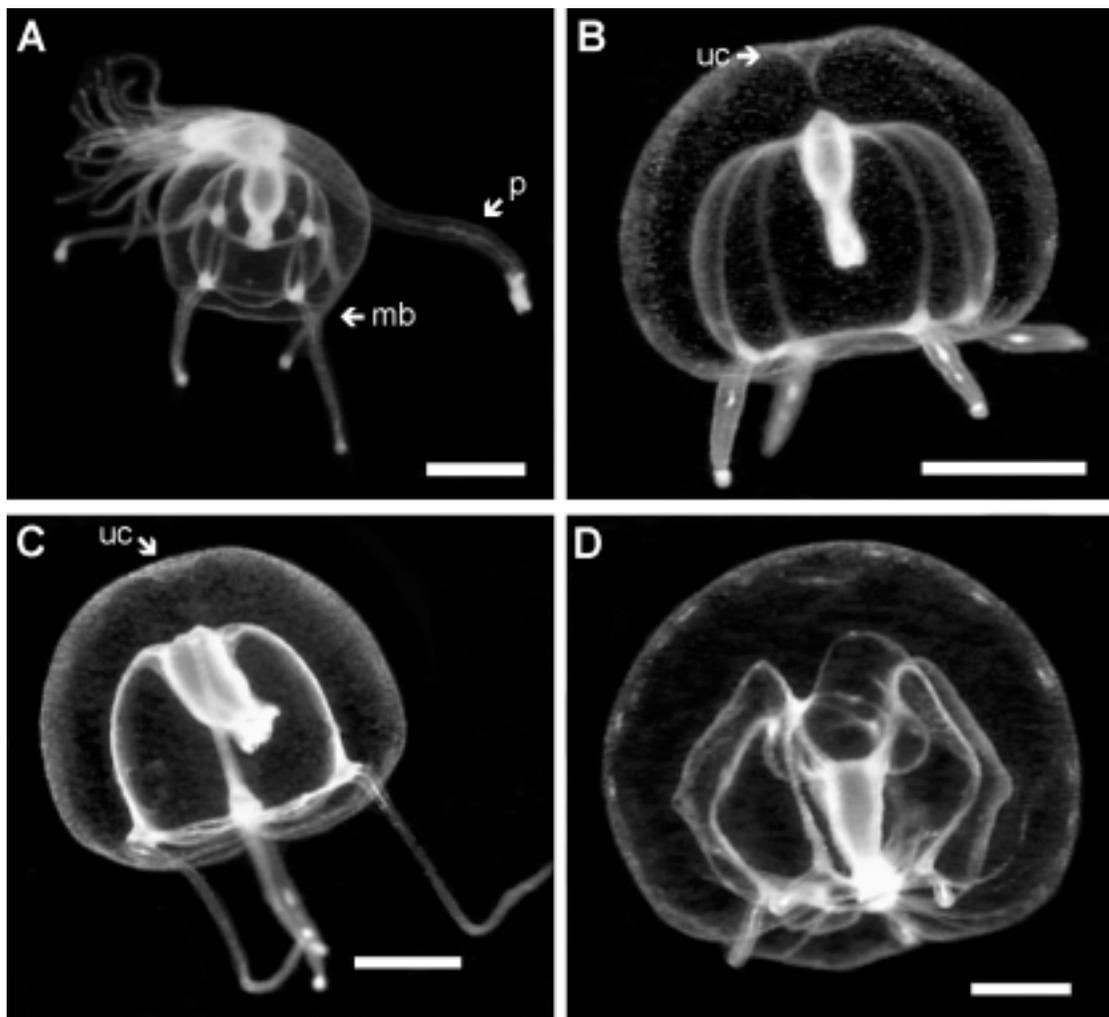


Figure 7. Development of *Bythotiarra dolioeques* medusa. (A) Medusa bud attached to polyp three days before release; (B) young medusa two days after release with umbilical canal beginning to fill; (C) medusa at 14 days after release; (D) medusa at 45 days after release. p, polyp; mb, medusa bud; uc, umbilical canal. Scale bars: 1.0 mm.

end. Over the course of several days, these autonomous tentacles would swell at the distal attachment point. At this stage, the tentacles appeared to be identical to those found covering the doliopsid colonies. In the laboratory, removed from the doliopsid tunic, autonomous tentacles would occasionally (<5%) develop into new polyps (Figure 5). This development was not preceded by any manipulation. The top of the swelling at the former base of the tentacle would then begin to differentiate into a circular mouth, and 1–3 days later an additional tentacle would form. Single tentacles were added over the course of days to weeks. Autonomous tentacles developed into fully formed polyps in 2–3 weeks.

DISCUSSION

Taxonomic status of Bythotiarra dolioeques

The polyps of only two species of *Bythotiarra* have been found, *Bythotiarra huntsmani* (Fraser) (Fraser, 1911, 1912, 1937 [as *Endocrypta huntsmani*]; Brinckmann-Voss, 1979; Rees, 1979 [as *Endocrypta huntsmani*]) and *Bythotiarra parasitica* (Kirk) (Schuchert, 1996), both found on benthic tunicates. The polyps of *B. dolioeques* resemble those of both *B. huntsmani* and *B. parasitica*, with a few important differences. Unlike the other species mentioned, no polyp of *B. dolioeques* has ever been found with a hydrorhiza connecting it to another polyp. The polyps are singular, rarely sharing their space on the doliolid with another polyp. Nor did they ever form a colony while being raised in the laboratory. Polyps of both *B. huntsmani* and *B. parasitica* occur in colonies. Polyps of *Bythotiarra dolioeques* can grow to 12.0 mm in length, while those of *B. huntsmani* reach 2–8 mm and *B. parasitica*, can be 3 mm in length. The tentacles of *Bythotiarra dolioeques* grow to 13.0 mm in length, while those of *B. huntsmani* are 1–1.5 mm (estimated from figures) and those of *B. parasitica* may be to 1.0 mm in length. Lastly, 1–3 medusae buds are found in *B. dolioeques*, while *B. huntsmani* has 2–8 medusae buds and *B. parasitica* has 1–4 medusae buds per polyp.

Four species in the genus *Bythotiarra* have medusae similar to *B. dolioeques*: *B. parasitica*, *B. dryglaskii*, *B. huntsmani*, and *B. stilbosa* (Kramp, 1968; Mills & Rees, 1979; Brinckmann-Voss, 1979; Rees, 1979; Pages et al., 1991; Schuchert, 1996). These medusae all have four tentacles and four unbranched radial canals. However, there are differences in size, cnidome, and habitat between these species and *B. dolioeques*. *Bythotiarra dryglaskii* is substantially larger than *B. dolioeques* (11.0 mm as opposed to 3.8 mm in height), and *B. parasitica* is taller than wide (2.5 mm wide by 3.8 mm tall), while *B. dolioeques* is wider than tall (4.6 mm wide by 3.8 mm tall). *Bythotiarra huntsmani* and *B. stilbosa* are differentiated by general shape (*B. huntsmani* is taller than wide, *B. stilbosa* is wider than

tall), differences in mesoglea thickness, and differences in tentacle tip morphology (Brinckmann-Voss, 1979). *Bythotiarra dolioeques* is very similar to *B. stilbosa* in all three of these characters. However, there are important differences between these species in the location and number of the umbrellar nematocysts, and the sizes of the microbasic mastigophores.

Bythotiarra stilbosa has nematocysts primarily on the exumbrellar surface of the medusae, with sparse nematocysts on the subumbrella (Mills & Rees, 1979). *Bythotiarra dolioeques* also has nematocysts on both of these surfaces, but they are much more dense. The exumbrellar nematocyst density in *B. dolioeques* (~512 mm²) is roughly an order of magnitude greater than in *B. stilbosa* (~65 mm²; calculated from figure 3 in Mills & Rees, 1979). The subumbrellar nematocysts are equally dense in *B. dolioeques*, but very sparse in *B. stilbosa*. In addition, the microbasic mastigophores found in *B. dolioeques* are much smaller than those found in *B. stilbosa*. They are ~7.0×12.0 µm in *B. dolioeques*, but ~17.0×20.0 µm in *B. stilbosa*. Other nematocyst types found were similar in size between the two species.

Little is known about the life history of *B. stilbosa*, as it has been collected on only three occasions in the same location, and its polyp is not known. However, young *B. stilbosa* were found in a marina, 2 km from the open ocean in Bodega Bay, California (Mills & Rees, 1979). Given the fact that the umbilical canals fill in within a few days of liberation, these medusae most probably were not more than two days old. This information lends support to the hypothesis that these *B. stilbosa* medusae were produced locally within the harbour, perhaps from tunicate-bound polyps, as is the case with *B. huntsmani*. In contrast, the polyps of *B. dolioeques* are known only from the mesopelagic open ocean. The medusae of *B. dolioeques* have not yet been collected *in situ*, and there is no information on where they might reside. However, it seems unlikely that they could be transported from the deep midwaters offshore into harbours 2 km from shore over the course of 1–2 days. Given the differences in umbrellar nematocyst density, the size of microbasic mastigophores, and the habitats in which they are found, *B. stilbosa* and *B. dolioeques* are considered distinct species.

Natural history

Medusae of *Bythotiarra* spp. examined in previous studies reached a maximum age of 27 days (Mills & Rees, 1979), while those of *B. dolioeques* were raised for a maximum of 94 days. Development of the medusae closely followed the sequence described by Mills & Rees (1979) and Brinckmann-Voss (1979), aside from

gonad formation and longevity. The bases of the tentacles were swollen and had a red/orange pigmentation which stretched into the associated radial canal. This pigmentation appeared to be primarily from their laboratory diet of *Artemia*. The highly contractile velum of the medusa was quite thin at rest, but would rapidly cinch shut, effectively trapping prey items inside the bell. Previous reports of *Bythotia* spp. have described the formation of the gonads at 9 to 14 days (Brinckmann-Voss, 1979; Mills & Rees, 1979; Rees, 1979). In contrast, gonads did not reach full development in any of the *B. dolioeques* raised in the current study, even after 94 days.

The lack of gonadal development may be attributable to a number of factors. The first is that the life span of *B. dolioeques* may be much longer than in other species of the genus, and the medusae have yet to be observed at their reproductive peak. However, given that numerous medusae were kept in the laboratory for more than two months, this seems unlikely. Another reason may be that the medusae received insufficient nutrition. Although the medusae ate *Artemia*, they did not appear to be well nourished. Other prey items were presented, including several types of copepods, rotifers, agar-based food mixes, and mussel digestive gland (see Raskoff et al., 2003), but none were readily ingested. An additional factor could be an unnaturally low culture temperature. Medusae were raised at the temperature in which the polyps were caught (6–8°C), however, it is possible that the medusae migrate to a shallower or deeper depth, and thus higher or lower temperature, after being released from the polyps. Therefore, the medusae may have been subjected to a delay of growth due to unnaturally low or high temperatures. A short trial at raising medusae at 12°C was not successful, as the specimens' health declined rapidly.

Previous studies have suggested that the medusae of other species of *Bythotia* migrate from shallow levels down to greater depths as they mature (Brinckmann-Voss, 1979). *Bythotia dolioeques* may show the opposite pattern, with the polyps residing at depth and the medusae migrating towards the surface. Future studies will need to address this question in greater detail.

As was the case in other studies, *Bythotia* medusae are difficult to raise in captivity (Edwards, 1967; Brinckmann-Voss, 1979; Rees, 1979; Schuchert, 1996). Medusae had to be hand-fed by bringing prey into direct contact with the mouth, at which time the medusae would cinch their velum closed, trapping the prey inside the subumbrellar cavity, as has been observed in other studies (Brinckmann-Voss, 1979; Schuchert, 1996). The medusae often swam weakly, with their tentacles contracted and held slightly upwards. When at rest, the tentacles would elongate

and radiate out from the bell, perpendicular to the swimming axis. No prey were ever observed being caught by the tentacles.

Settlement and asexual reproduction

Many facets of the Doliopsid–*Bythotia* association are unclear. It is not presently known how the hydroid initially colonizes the doliopsid. Possible mechanisms include the planula settling directly onto the tunic, or being swept into the buccal chamber by the doliopsid feeding currents, and then evading capture by the feeding filter and migrating out onto the tunic for further development. It is not known if all the polyps found on a doliopsid chain are from the same genetic stock (i.e. all being derived from an initial founding polyp), or if they are the result of multiple settlement events.

Our data support the premise that all polyps arise from one initial settlement event. On a number of occasions, polyp tentacles were observed to re-attach themselves to another location on the doliolid colony. These autonomous tentacles often densely covered the stolon and tunic of freshly caught colonies (Figure 2C). This movement of polyp tissue from one area of the colony to another may be an asexual reproductive mechanism for founding polyps. Observations in the laboratory have shown that these autonomous tentacles have the capacity to form fully functioning polyps. Previous studies have found that isolated tentacles from the polyps of the scyphozoan *Aurelia aurita* have the capacity to reform complete polyps (Lesh-Laurie et al., 1991). The process took 6 to 12 weeks and was only observed in experimentally isolated tentacles removed from the polyp. It has not been observed in the wild nor has it been observed to occur spontaneously, as is the case with this study.

We hypothesize that the polyp from the initial settlement of a *B. dolioeques* planula colonizes the rest of the doliopsid chain by way of these autonomous tentacles. When these tentacles happen to attach in a region by the buccal siphon of a feeding zooid, they form polyps. If they attach in another region of the doliopsid, they will remain as autonomous tentacles. In this way, the tentacles are the primary asexual reproductive device of the polyp. The mechanisms of this developmental control are unknown.

This hypothesis is supported by the fact that only the doliopsid zooids which are mature enough to feed have associated polyps. Very young, non-feeding zooids were never found with a polyp, but did occasionally have autonomous tentacles attached to their tunics. Additionally, when a doliopsid colony is encountered, if a polyp is found on one zooid, they are found on virtually all mature zooids. However, one doliopsid colony

was collected which had no associated polyps. Presumably this was an example of a doliopsid which had not yet been settled by a founding planula/polyp.

Mutualistic relationship

Previous studies have explored the possibility of mutualistic relationships between hydroids and their 'hosts' (see Vance, 1978; Piraino, 1994). The symbiotic relationship between the *Bythotiara doliioeques* polyps and the doliopsid colony is hypothesized to be mutualistic. There are many benefits for the polyps. Substrate is a limited resource in the mesopelagic habitat. The ability of *B. doliioeques* to utilize pelagic animals as its substrate permits exploitation of the pelagic realm, and the hydroid is able to utilize pelagic food resources unavailable to benthic organisms. *Bythotiara doliioeques* is able to live in the productive upper levels of the oceanic water column, where the nearest benthic substrate is below, in water deep enough to be physiologically hostile because of pressure, temperature, and the lack of food. Additionally, its position at the incurrent siphon of the doliopsid allows the continuous flow of water generated by the doliolid to bring food items past the waiting tentacles of the polyp. There is also no competition for space with other species, which is so pronounced in benthic systems. Finally, there may be a substantial reduction in the potential predators of the polyps in the pelagic environment.

There are two primary advantages for the doliopsid in this system. The first is protective. The autonomous tentacles of *B. doliioeques* were found covering the tunic and stolon of the doliopsid colonies. These tentacles were reactive to the touch and contained active nematocysts which would immobilize *Artemia* in the laboratory. They were shown to persist for months in the laboratory. These autonomous tentacles, along with the whorl of tentacles on the polyp itself, would provide an effective deterrent to potential predators of the doliopsid. The second advantage to the doliopsid is nutritive. The polyp has the capability to capture large prey which the doliopsid may be unable to capture and ingest, and the polyps can act as a pre-filter for the delicate mucous feeding webs of the doliopsid. The polyp's undigested faecal matter is later captured by the feeding apparatus of the doliolid and ingested. This provides direct nutrient flow from the polyp to the doliopsid. This unidirectional flow of nutrients is unusual in other epibiotic relationships (Wahl, 1989) and lends strong support for a mutualistic, rather than strictly epibiotic, relationship.

We wish to thank Kim Reisenbichler, Rob Sherlock, and Bill Hamner for assistance with this research. Claudia Mills, John T. Rees, and Francesc Pagès all provided very helpful comments regarding the biology and taxonomy of *Bythotiara*,

as well as encouragement toward its study. Kimberly Shirley, Bill Hamner, and three anonymous referees provided helpful comments on drafts of this manuscript. Special thanks go to the captains and crews of the RV 'Pt. Lobos' and RV 'Western Flyer' and to the talented pilots of the ROVs Ventana and Tiburon. All funding for this work was provided by the David and Lucile Packard Foundation through the MBARI Midwater Ecology Group.

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Submitted 2 September 2004. Accepted 12 February 2005.