Hydrobiologia **216/217**: 171–179, 1991.
R. B. Williams, P. F. S. Cornelius, R. G. Hughes & E. A. Robson (eds),
Coelenterate Biology: Recent Research on Cnidaria and Ctenophora.

Observations on the anatomy, behaviour, reproduction and life cycle of the cubozoan *Carybdea sivickisi*

R.F. Hartwick
Department of Marine Biology, James Cook University of North Queensland, Townsville, Q. 4811, Australia

Key words: *Carybdea sivickisi*, life cycle, reproduction, behaviour, anatomy, adhesion

Abstract

Substantial numbers of the very small carybdeid jellyfish, *Carybdea sivickisi*, have been observed feeding actively by divers in the vicinity of fringing coral reefs. During the day specimens were seen in close association with the substrate and with sessile macroalgae. They appeared able to adhere to substrates by contact with an area on the aboral surface of the bell. Individuals maintained in the laboratory showed the same adhering ability. The adhesive organs correspond to 4 areas of raised secretory epithelium on the aboral surface.

Following capture, one isolated specimen extruded a long gelatinous strand containing several thousand developing embryos and numerous functional nematocysts. The embryos developed into characteristic cubozoan planulae, which were able to attach themselves temporarily to substrates by means of nematocysts. After 4 days of free swimming, planulae began to attach permanently and to develop into sessile polyps with 3 or 4 tentacles, each armed with a single stenotele.

The species displays sexual dimorphism and evidence for spermatophore formation and transfer which parallel that reported for *Tripedalia cystophora*. The presence of sperm-storage sacs in both sexes, the production of an embryo mass, the adhering behaviour and an apparent association with algal substrates are among features apparently unique to the species.

Introduction

Carybdea sivickisi Stiasny is a very small ($\leqslant 14$ mm diam.) carybdeid cubomedusa reported from coastal water at a few locations in the tropical and temperate Indo-Pacific (Thailand and Philippines, Stiasny (1922, 1926); Japan, Uchida (1970); New Zealand, Hoverd (1985); and Vietnam, specimens held in Zoological Museum, Copenhagen). The species appears infrequently in plankton samples, and only a few observations on its behaviour in captivity exist (Hoverd, 1985). Nothing has been reported on its ecology, reproduction or early life stages, though these aspects are critical to the unique status of the cubozoans. Internal fertilization appears to be typical of carybdeid reproduction (Arneson, 1976) and, in the case of another small species, *Tripedalia cystophora* Conant, includes such hitherto unparalleled features as sexual dimorphism, pair formation, spermatophore transfer and larviparity (Werner, 1973, 1975). Evidence for simpler reproductive behaviour in the chirodropid cubozoans (Yamaguchi & Hartwick, 1980) points to a need for broader knowledge of the reproductive biology of cubozoan species, with the aim of clarifying their taxonomic and phylogenetic status. A carybdeid which resembles

T. cystophora in certain features and reveals some apparently unique variations is reported here.

During late October and early November of 1982, SCUBA divers on fringing reefs of Magnetic Island, Townsville, Australia, noted substantial numbers of the distinctively coloured medusae swimming in the water column (depth = 1.5 to 3.0 m) above the reef during night dives. The jellyfish swam among broadcast coral gametes and large numbers of polychaete heteronereids, gammarid amphipods and cumaceans which were swarming at the time. The swarming species were being stung and ingested by the medusae, which also caused frequent painful stings to the exposed skin of the divers. Erythema and blistering persisted for hours after the sting, though no systemic effects were reported, despite multiple stings. The medusae appeared to be attracted to the divers' lights.

During later daytime dives in the same area other individuals were observed swimming in close association with the substrate and with benthic macroalgae (*Sargassum* and *Colpomenia* spp.). The jellyfish appeared to be able to adhere to the surface of the algae (and also to items of the diver's equipment) by contact with the exumbrellar surface of the bell. On 2 occasions a specimen was captured by hand in a glass jar and returned to the laboratory alive.

Methods

The first specimen (a female, perradial diam. 6.3 mm) was captured at about 13.00 h on 22 October and transferred the next morning to a modified planktonkreisel previously used for the rearing of young medusae of *Chironex fleckeri* (see Yamaguchi & Hartwick, 1980). The second specimen (Fig. 1) (a male, perradial diam. 5.0 mm) was caught 10 d later and transferred to the same kreisel. In October 1989, 3 sexually mature specimens (2 female, diam 6.9 and 8.0 mm, and a male, 7.8 mm) were also collected by the author under a night light.

Planula larvae produced by 2 females (one in 1982, another in 1989), were transferred to a series of 100 ml glass beakers of filtered coastal seawater, in some cases with a range of artificial and natural substrates (glass, ceramic tile, pebbles, dead coral, live *Colpomenia* and *Sargassum*) with varied degrees of bacterial/algal film covering them. Aeration and circulation were provided in a few cases; in others the water was changed at 12–24 h intervals (water temperature was 24.8° ± 0.5 °C). Specimens were examined and photographed with a Leitz Diavert microscope and a Tessovar photomacroscope.

Observations on anatomy are supplemented from inspection of an additional male (diam. 3.7 mm) captured and preserved in the same area in October 1984, and 2 females (diam. 7.2 mm and 6.5 mm) captured in October 1986. Also, 14 preserved specimens (including type material) held in the Royal Museum of Natural History, Leiden, and the Zoological Museum, Copenhagen, have been examined.

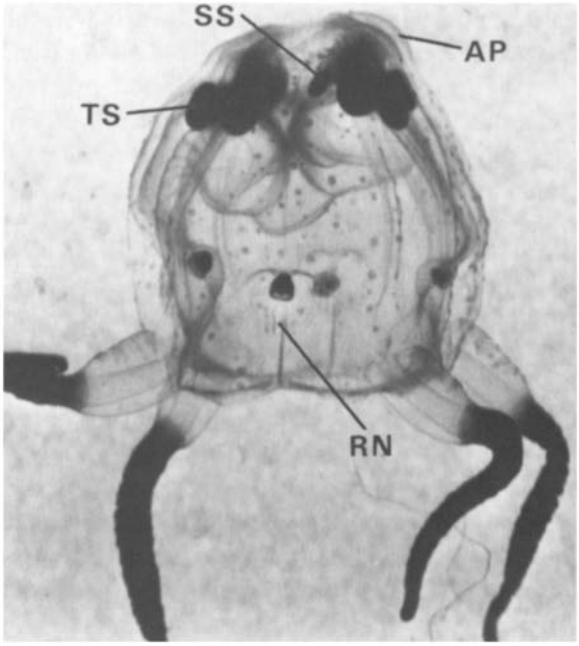

Fig. 1. Mature male *Carybdea sivickisi* photographed alive, with bell apex contracted (perradial bell diam. 5.0 mm). AP = Adhesive pad; TS = Testis; SS = Subgastral sac; RN = Rhopalar niche opening.

Results

Medusa anatomy and behaviour

The captured specimens, having been minimally disturbed, were in excellent condition for anatomical and behavioural study. Due to their absence from our local plankton samples, I could not immediately identify them, but later found them to agree in all particulars with Stiasny's *Carybdea sivickisi* type material from Puerto Galera, Philippines. Their distinctive features include:

1) Rhopalar niches lack flap-like covering 'scales', leaving an opening rather higher than wide, with an oval to keyhole-shaped outline.
2) Dense mammilation patterns of nematocysts on the body include 7 or 8 parallel bars in a column down the abaxial margin of each pedalium (often abraded in net-haul specimens).
3) Four subequal, broadly palmate velar canals per quadrant. (Hoverd (1985) states 4 per octant). In some specimens, one or two small (up to 0.18 mm) darkly pigmented club-shaped structures occur at termini of the short digitations of each canal. Filled with a fine granular material, their function is unknown but they appear only in females and may, therefore, serve in reproduction.
4) Fresh specimens contain substantial orange pigment (faded in preserved material), associated variously with the gonads, gastric region and tentacles; in the last case, in broad bands alternating with white. The females have 8 pigmented, roughly hemispherical (mean diam. 0.34 mm), sac-like protuberances from the subumbrellar surface, each located beneath an outer corner of one of the gastric phacellae and opening into a gastric ostium. After specimen preservation, a micropipette was inserted into 2 of the sacs and the contents proved to be dense clusters of tailed sperm (Fig. 2) and numerous cnidocytes containing microbasic euryteles (mean diam. 11.0 μm). The males have 2 sets of 8 red-orange bodies arranged around the body axis. One, in the form of sacs on the gastric subumbrella, is essentially identical to that of the females. The second set, comprising larger flattened ovoid thickenings of the hemigonads, are arranged in pairs in the gastric pockets, on either side of the interradial septa (the typical location for the gonads in the carybdeids). In some specimens the structures are relatively short (Fig. 1); in others they extend further toward the bell margin, until up to 4 times longer than wide. Squash preparations showed them to be composed primarily of mature sperm and nematocysts like those found in the subgastral sacs of both sexes. The female gonads, in contrast, are the pale, thin sheets typical of other carybdeids, and contains euryteles about twice as large as those in the testis.
5) The exumbrella is prominantly sculptured, including 4 rounded triangular to quadrate raised areas on the apical surface. Though unique to the species, no function has been proposed for these areas (Hoverd (1985) labels them 'phacellae', which actually lie below them in the gastric cavity). Their surface has a 'raw' (Stiasny, 1926) or satiny appearance and a somewhat granular underlying tissue, suggesting a specialized function, such as secretion. Stiasny (1926) described a pattern of parallel

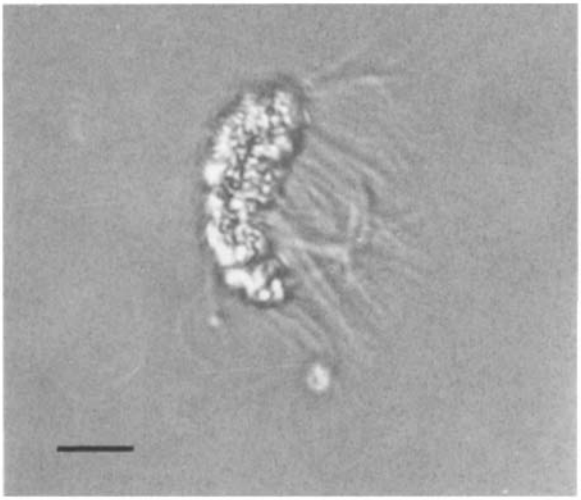

Fig. 2. Sperm bundle ('spermozeugma') from the subgastral spermatheca of the spawned female *C. sivickisi*, following preservation (Nomarski differential interference contrast). (Scale bar = 10 μm).

> ridges on their surface in preserved material, but this was not present in my living specimens or in other preserved material.

Observation of living specimens of *C. sivickisi* soon made it clear that the apical thickenings serve as adhesive pads. Repeatedly, animals swam up to a substrate surface, made contact with one or more of the pads, and stuck fast. Swimming at once ceased, and the tentacles relaxed. The animal would hang motionless for varied periods of time, from a few minutes to more than an hour, punctuated by occasional single bell pulsations. Eventually it would detach, either spontaneously or in response to some disturbance, such as turning off the aquarium light, by initiating a rapid series of spasmodic bell contractions. Swimming then recommenced.

A variety of substrates served for attachment, including ceramic tile, coral rocks, live *Colpomenia* and *Sargassum*, and a length of plastic twine used to lower the alga into the aquarium (Fig. 3). No specimen was ever seen to attach to the transparent Perspex walls of the kreisel, although they frequently swam into them inadvertently.

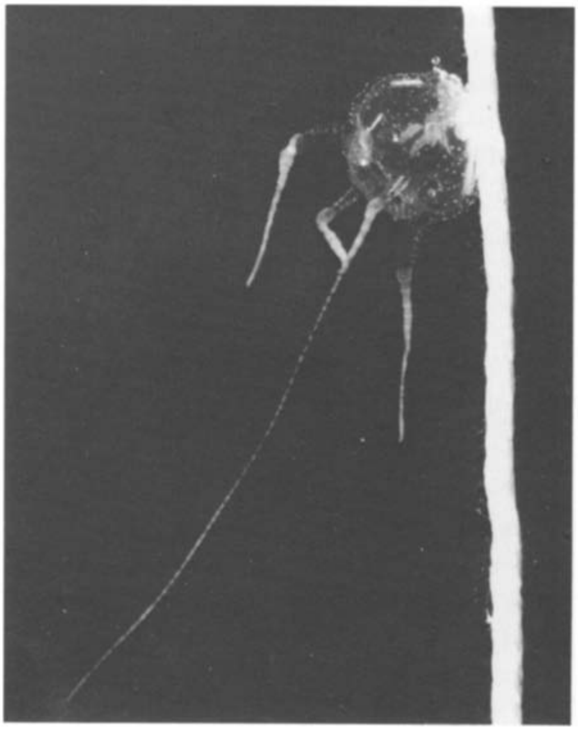

Fig. 3. Mature female *C. sivickisi* photographed alive, 10 d after spawning, while adhering to near-vertical plastic twine in aquarium (perradial bell diam. 6.3 mm). Note redeveloping ovaries and detritus adhering to edge of adhesive pads.

The adhesive surface most probably resulted from a sticky secretion rather than agglutinant nematocysts. Although the complex pad epithelium contained a scattering of isolated spherical isorhizas like those forming the mammilation clusters on the rest of the body, the predominant cell type was a 20 μm tall columnar cell packed with spherical 1.5 μm acidophilic granules. Interspersed with them were irregularly elongate pale sacs filled with an amorphous substance. The spherical granules appear very similar to those reported in the basal adhesive disc of *Hydra* by Philpott *et al.* (1966). Varied detritus (e.g., *Artemia* eggs) soon began to adhere to the pads, making them more prominent, though probably reducing their efficiency of operation.

Reproductive biology and life cycle

When first captured on 22 October 1982, the female specimen had a great deal of dense whitish material nearly filling the gastric spaces. At 24 h after capture the animal was swimming steadily at the top of the kreisel. Four hours later it was found to have extruded from its manubrium a whitish-yellow gelatinous strand about 3 times the length of the bell. The medusa, with its dangling strand, was transferred to a small beaker for examination with the photomacroscope, revealing thousands of developing embryos, at about the 8-cell stage, scattered through the gelatinous matrix. A small quantity of material containing eggs or embryos was still in each gastric pocket, and was seen to be flowing through the gastric ostia into the stomach to be joined with the developing strand. (These observations also revealed the active muscular control of the rhopalia with their prominent eyes, permitting vision both adaxially and abaxially – see Discussion).

Within an hour after return to the kreisel, the medusa released the strand, which sank to the bottom, leaving the animal nearly empty of white material and with no sign of paired interradial gonads. The embryo strand (Fig. 4), *ca* 40 mm long and 2.2 mm wide) was transferred to a glass dish and reexamined at intervals with the inverted microscope. Partial counts indicated that it contained 4000–5000 subspherical embryos (mean diam. 127 μm). Interspersed with them were many cnidoblasts containing functional microbasic euryteles of 2 size classes: the more common being essentially identical in size and shape to those in the ovary; the rarer type – half as large – matched those found in the subgastral sacs and in the testes of males. The strand was quite adherent to the bottom of the dish. (A nearly identical spawning event was observed in the 6.9 mm female captured in 1989; the following description of development synthesizes the 2 sets of observations.)

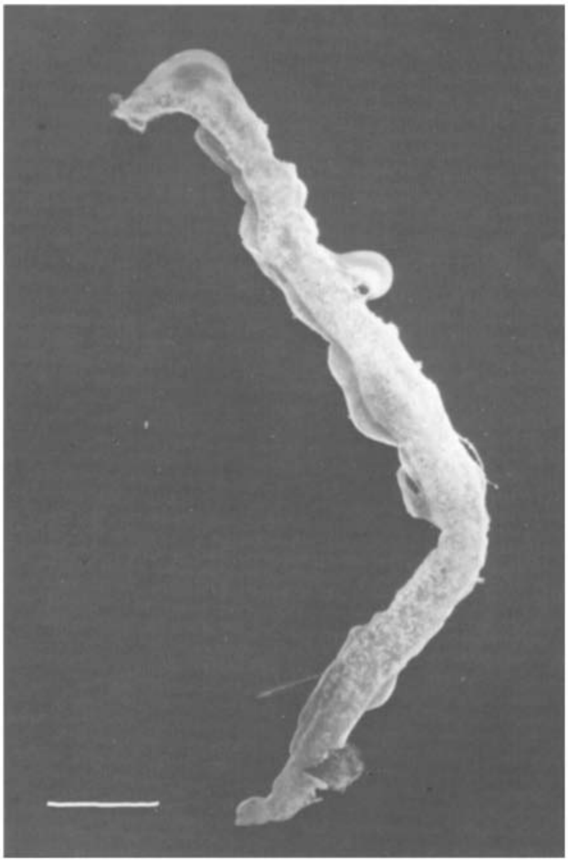

Fig. 4. Gelatinous embryo strand of *C. sivickisi* 4 d after release by female (release of planulae commenced). (Scale bar = 5 mm).

Embryonic development proceeded until, 72 h after production of the strand, the appearance of a belt of about 30 dark red-brown pigment bodies around the mid region heralded a typical cuboplanula. By this time the gelatinous matrix of the strand showed signs of decomposition, and this led, by the following day, to the commencement of the release of pear-shaped planulae (Fig. 5A) up to 160 μm long. These swam about for at least 4 d before settlement began.

During this dispersal period, planulae were able to attach temporarily to a clean glass surface (e.g., a cover slip) such that moderate agitation would not dislodge them. After a few minutes in place they would begin an accelerating rotation which broke them free. At the attachment spot were usually found 1 to 4 small (6.2 μm diam.) oval microbasic euryteles, their discharged tubules firmly adhering to the glass with a surrounding agglutinant material. The nematocysts were visible in significant numbers in the ectoderm of the planula.

Planula settlement and metamorphosis commenced 8 d after production of the embryos. There was no convincing pattern of site preference, although the culture conditions may have been inappropriate for determining this. Settlement did occur on the clean glass bottoms of the culture beakers. Metamorphosis to a sessile primary polyp required about 24 h, during which time the pigment bodies gradually faded and 3 to 4 tentacles developed (Fig. 5B). The latter contained at the tip a single stenotele (Fig. 5C). In some cases polyps transformed into a motile 'creeping' form similar to that seen in other cubopolyps (Werner, 1975; Yamaguchi & Hartwick, 1980).

The polyps lived for 2–6 weeks after settlement before dying. The cause of this barrier to further development, a common occurrence in cubozoan rearing efforts (see Hartwick, 1991), is uncertain, but may be the result of unsuitable food. Although *Artemia* nauplii were the principal food provided in 1982, a much wider range of food types and sizes were offered in 1989. These included crustacean nauplii, harpacticoid copepods, dinoflagellates, ciliates, rotifers and polychaete trocho-

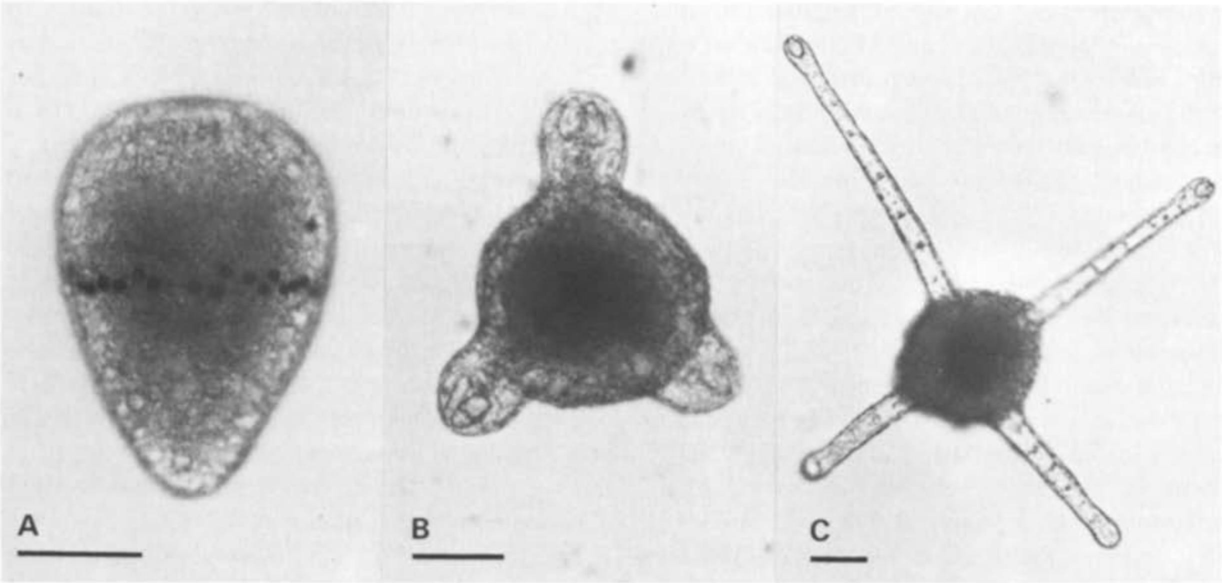

Fig. 5. Early developmental stages (photographed alive) of *C. sivickisi* after release from embryo strand. A) Free-swimming planula; B) Recently-settled primary polyp; C) Primary polyp 6 d after settlement (Scale bars = 50 μm).

phores. In no case was there evidence of successful feeding.

Addition of the second 1982 medusa (a male) to the kreisel containing the spent female did not lead to a mating interaction like that reported for *Tripedalia* (Werner, 1973, 1976). Given the recent spawning of the female, such behaviour was perhaps improbable, although her ovaries appeared to be rapidly redeveloping. The animal died 16 d after spawning. About 48 h after their capture in 1989, the 8.0 mm female was put together in an aquarium with the male. On the following morning the female was found swimming with an irregularly ovoid, red-orange mass (mean diam. 1.0 mm) attached to the midpoint of one tentacle by a short strand. A similar mass could be seen in one side of the stomach cavity; neither had been present before.

The exposed mass was removed with a pipette and examined microscopically to reveal thousands of aggregates of active sperm, very similar in appearance, though smaller (mean dimensions: 15.3 × 10.8 μm), than the spermozeugmata of *Tripedalia* (Werner, 1973). The spermatophore-carrying female spawned a mass of developing embryos during the next night.

Discussion

Carybdea sivickisi is the smallest representative of its genus (and possibly the smallest cubozoan). Its reproductive biology parallels that of another small species, *Tripedalia cystophora* (see Werner, 1973, 1976) in several aspects, including: the sexual dimorphism of the gonads, their shape and pigmentation, the formation of sperm bundles (spermozeugmata), their collection in invaginations of the stomach floor adjacent to the gastric ostia and their assembly into spermatophore-like masses for transfer. *C. sivickisi* is distinct from *Tripedalia* in that these 'subgastral sacs' (effectively, seminal vesicles) are 8 in number, rather than 4, and that similar structures, with similar contents (effectively, spermathecae) are found in the female as well. Although the act of spermatophore transfer has not been observed in *C. sivickisi*, there is little reason to doubt that a process similar to that in *T. cystophora* occurs. An additional sex-linked characteristic found only in *C. sivickisi* is the small pigmented bodies associated with the velar canals of the female.

Details of the fertilization process in *C. sivickisi* are incomplete, but it appears likely that sperm can be stored in the spermathecae of the female for extended periods, and fertilization delayed until formation of the embryo strand commences with transfer of the eggs from the gastric pockets through the gastric ostia, over the openings of the spermathecae and into the stomach. When first examined, about 28 h after capture of the 1982 female and, therefore, at least that long after mating, the embryos in the strand had undergone only about 3 cleavage divisions. The rate of subsequent cleavage as well as reported development rates for cubozoans (*Chironex fleckeri* (see Yamaguchi & Hartwick, 1980); *Carybdea alata* (see Arneson, 1976); *C. marsupialis* (see Studebaker, 1972)) and other coelenterates (e.g., Donaldson, 1974) make it improbable that fertilization occurred more than 2–3 h before. This would approximately correspond to the onset of strand formation, since no strand was apparent when the female was last observed, 4 h previously. It is reasonable to hypothesize that fertilization occurs not in the gastric pockets, as reported in other carybdeids, but in the stomach, as the eggs pass the spermathecae.

Production of the embryo strand is unprecedented among cubozoans, contrasting with the individual release of blastulae, as in other *Carybdea* species (Arneson, 1976), planulae, as in *Tripedalia* (see Conant, 1898) or, apparently, unfertilized eggs, as in *Chironex* (see Yamaguchi & Hartwick, 1980). Although simply dropped by the female in captivity, the possibility remains that the strand, in nature, is more selectively deposited in a suitable location, e.g., in a cryptic niche or attached to macroalgae. The strand was quite adherent, even to clean glass. The 2 size classes of euryteles could serve for adhesion and/or predator determent. Carré & Carré (1980) have suggested the latter role for the euryteles in the gelatinous egg coat of *Nausithoe punctata* Kölliker.

The early life stages of *Carybdea sivickisi* closely resemble those of other cubozoans, and further reinforce the coherent picture of ontogeny within the class. The planulae, as in the case of *Chironex fleckeri* (see Hartwick, 1991) and possibly of most other cnidarian planulae, can make temporary attachment to substrates by means of agglutinant nematocysts. There is no evidence, in the conditions of culture, of the extreme specificity of attachment reported by Donaldson (1974) for *Proboscidactyla flavicirrata* Brandt, but a lack of the appropriate substrate cues in culture may lead to a lowering of settlement preference thresholds. Nothing is known of the location of the polyps of *C. sivickisi* in nature, although the rarity of medusae in open-water plankton samples implies that their shallow coastal habitat is not far removed from that of the polyps. It is notable that *C. sivickisi* is the only cubozoan in which all stages of the life cycle, from medusa through developing embryo, planula and polyp, have the ability to adhere to substrates. The species is thus well adapted to minimize dispersal, and may reveal an unusually confined life cycle, perhaps in association with macroalgae.

The apical adhesive pads of the medusa of *C. sivickisi* have no parallel in the Class Cubozoa, nor any direct equivalent in other medusae. Their operation appears intentional and controlled: animals never adhered 'accidentally' when colliding with the kreisel walls, and rarely appeared to 'struggle' for an extended period to free themselves. They predictably detached within a few seconds of a significant disturbance such as switching off the aquarium light. The pads would provide them with the ability to remain immobile for extended periods while in a cryptic location, such as a clump of macroalgae. This behaviour would afford both energy savings and predator avoidance, and may be typical of their distribution during daylight. They have only been observed swimming freely in the water column at night. Such activity may provide the bulk of their feeding opportunities, since gut contents invariably reveal night-swarming benthic organisms: heteronereids, cumaceans, gammarid amphipods and (in two Vietnamese specimens) isopods.

There are indications that vision may play a role in the approach to attachment, in that all chosen substrates were opaque, in contrast to the transparent Perspex walls. Furthermore, the narrow strand of plastic twine suspended in the kreisel was approached slowly, in a direct and controlled path on several occasions, not as an apparent random contact. Although anatomists have long remarked on the sophisticated 'image-forming' structure of the rhopalial eyes of cubomedusae (Hyman, 1940), little is known of the importance of visual responses in their behaviour. There is a tendency to discount this with the observation that the principal eyes appear to be directed toward the interior of the bell (e.g., Studebaker, 1972), implying a major preoccupation with food items, and a questionable disinterest in the surrounding environment. It needs to be emphasized that, although the orientation of the major rhopalial eyes in *preserved* cubozoans is usually (though not always) adaxial, when alive the rhopalia are under active control of the musculature associated with the stalk, and can be rapidly directed both adaxially and abaxially. This is most readily observed in small, easily handleable species such as *C. sivickisi* or in the early reared medusae of larger species such as *Chironex fleckeri*. Thus the animal has an unobstructed view of most of its surroundings, with some possible limitation directly forward along the body axis. The transparency of the bell permits each eye considerable multidirectional vision, but the shape of the opening of the rhopalar niche, hitherto solely of taxonomic significance, should be reexamined as an adaptation to provide a field of clear vision in some particular region of the visual field. In most cubozoans this appears directed laterally and behind the animal, but the distinctive 'keyhole' openings in *C. sivickisi* would seem to emphasize forward vision. This may well relate to a search for and approach to habitat substrates, but visual responses may also be pertinent to reproductive behaviour. The sexually dimorphic pigmentation patterns and the distinctive banding of the tentacles are striking, even to preoccupied human SCUBA divers, and may well be at least as meaningful to *C. sivickisi*. Their role in species and mate recognition awaits critical test.

The apparently unique characters of *Carybdea sivickisi* mark it as one of the most highly adapted of the cubozoans, a group already recognized as a 'highpoint' in the development and evolution of coelenterates (Werner, 1976). Its apical adhesive pads alone would set it apart from other cubozoans to at least generic level, and its reproductive system, though showing similarities to *Tripedalia cystophora*, is likewise distinctive. The latter species shows an interesting ecological parallel in its reported association with mangrove roots (Conant, 1898), but it may be that this plant-animal association has evolved to its logical extreme in *Carybdea sivickisi*.

Acknowledgements

Thanks are due to Matthew Cockburn, who noted this curious cubozoan in the field, and returned it for study; and also to J. Collins, R. Babcock and P. Harrison, who also supplied specimens and field reports. C. Alexander and L. Winsor assisted with equipment and techniques. A. Sharp prepared the manuscript.

References

Arneson, A. C., 1976. Life history of *Carybdea alata* Reynaud, 1830 (Cubomedusae). M.Sc. thesis, Univ. Puerto Rico.

Carré, D. & C. Carré, 1980. On triggering and control of cnidocyst discharge. Mar. Behav. Physiol. 7: 109–117.

Conant, F. S., 1898. The Cubomedusae. Mem. biol. Lab. Johns Hopkins Univ. 4(1): 1–61.

Donaldson, S., 1974. Larval settlement of a symbiotic hydroid: specificity and nematocyst responses in planulae of *Proboscidactyla flavicirrata*. Biol. Bull. 147: 573–585.

Hartwick, R. F., 1991. Distributional ecology and behaviour of the early life stages of the box-jellyfish *Chironex fleckeri*. Hydrobiologia 216/217: 181–188.

Hoverd, W. A., 1985. Occurrence of the order Cubomedusae (Cnidaria: Scyphozoa) in New Zealand: collection and laboratory observations of *Carybdea sivickisi*. N.Z. J. Zool. 12: 107–110.

Hyman, L. H., 1940. The Invertebrates: Protozoa through Ctenophora. McGraw-Hill, N.Y., 726 pp.

Philpott, D. E., A. B. Chaet & A. L. Burnett, 1966. A study of the secretory granules of the basal disc of *Hydra*. J. Ultrastruct. Res. 14: 74–84.

Stiasny, G., 1922. Die Scyphomedusen-Sammlung von Dr. Th. Mortensen nebst anderen Medusen aus dem zoologischen Museum der Universität in Kopenhagen. Vidensk. Meddr. dansk naturh. Foren. 73: 33–558.

Stiasny, G., 1926. Über einige Scyphomedusen von Puerto

Galera, Mindoro (Philippinen). Zool. Meded. Leiden 9: 239–248.

Studebaker, J. P., 1972. Development of the cubomedusa, *Carybdea marsupialis*. M.Sc. thesis, Univ. Puerto Rico.

Uchida, T., 1970. Revision of Japanese Cubomedusae. Publs Seto mar. biol. Lab. 17: 289–297.

Werner, B., 1973. Spermatozeugmen und Paarungsverhalten bei *Tripedalia cystophora* (Cubomedusae). Mar. Biol. 18: 212–217.

Werner, B., 1975. Bau und Lebensgeschichte des Polypen von *Tripedalia cystophora* (Cubozoa, class nov., Carybdeidae) und seine Bedeutung für die Evolution der Cnidaria. Helgoländer wiss. Meeresunters. 27: 461–504.

Werner, B., 1976. Killermedusen und ihr Liebesspiel. Umschau 76(3): 80–81.

Yamaguchi, M. & R. Hartwick, 1980. Early life history of the sea wasp, *Chironex fleckeri* (Class Cubozoa). In P. Tardent & R. Tardent (eds), Development and Cellular Biology of Coelenterates, Elsevier/North Holland Biomedical Press, Amsterdam: 11–16.