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Strobilation, Budding and Initiation of Scyphistoma Morphogenesis in the Rhizostome *Cassiopea andromeda* (Cnidaria: Scyphozoa)

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Abstract

Scyphistomae of *Cassiopea andromeda* Forskål, 1775 containing symbiontic zooxanthellae did not develop medusae at a constant temperature of ~20°C, but monodisc strobilation was initiated after transfer of the polyps to ~24°C. After release of the ephyrae and regeneration of the hypostome and tentacular region, the recovered polyps either produced vegetative buds or entered a new strobilation phase. Formation of motile, planula-like buds was not found to be indicative of unfavourable environmental conditions. Intensity of budding was positively correlated with available food and with increase of temperature. Budding was negatively correlated with the number of polyps maintained per dish and with the conditioning of the sea water. Under optimal feeding and temperature conditions, polyps could simultaneously produce chains of buds at 2 to 4 budding regions. Settlement and development of buds into scyphistomae was suppressed in pasteurized sea water and in pasteurized sea water containing antibiotics, but polyps developed from buds in the presence of algal material taken from the aquarium, debris or egg shells of *Artemia salina*, or on glass slides which had been incubated in used *A. salina* culture medium. Several species of marine bacteria were detected after staining these slides. One, a Gram-negative coccoid rod, which was identified as a nonpathogenic *Vibrio* species, was isolated, cultivated as a pure strain, and was proved to induce the development of *C. andromeda* buds into polyps. Millipore filter-plates coated with *Vibrio* sp. cells grown in suspension culture were ineffectual, but diluted filtrate initiated polyp morphogenesis. The inducing factor is obviously not a constituent of the bacterial cell surface, but is a product of growing *Vibrio* sp. cells released into the medium. This product was found to be relatively heat-stable and dialyzable. As to the basic mechanism involved in the induction of polyp formation, it is suggested that the inducing factor(s) acts bimodally by inducing pedal disc development and by eliminating a head inhibitor originating from the basal end of the bud. The life history, and various aspects of medusa-formation and of vegetative reproduction in scyphozoans are reviewed and discussed with particular reference to rhizostome species. Special attention has been paid to some reports of larval metamorphosis controlled by marine bacteria.

Introduction

The anatomy and some aspects of development in the rhizostome species *Cassiopea xamachana* have already been dealt with in the classic paper of Bigelow (1900). Gohar and Eisawy (1960a, b) largely confirmed and extended Bigelow's findings in their studies on the biology and the development of *C. andromeda* from the Red Sea, so far as medusa formation by monodiscous strobilation of scyphistomae is concerned. These authors succeeded in describing the entire life cycle of this gonochoric species, and were able to compare the development of the scyphopolyp from planulae reared from artificially fertilized eggs, with that from vegetative buds produced by fully developed polyps. It is interesting to note that *C. andromeda* revealed developmental features which are considerably similar to those found in two other rhizostome species from the Pacific Ocean: *Mastigias papua* (Uchida, 1926; Sugiura, 1964, 1965) and *Cephea cephea* (Sugiura, 1966).

In contrast to the semaestome species *Aurelia aurita* and *Chrysaora quinquecirrha*, in which factors involved in the induc-

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tion of strobilation have been analysed in recent years (Spangenberg, 1971; Loeb, 1972, 1974a, b), rather little experimental work has been performed on this aspect in *Cassiopea* species. Ludwig (1969) found that the presence of zooxanthellae (for detailed description see Freudenthal, 1962) was prerequisite for the onset of medusa formation in *Cassiopea andromeda*. Sugiura (1964, 1965) had mentioned earlier that in *Mastigias papua* not only the presence of symbiontic algae, commonly named zooxanthellae, but also a change in temperature controlled strobilation. We demonstrate in this paper that the latter environmental factor (which has proved to be important in many other coelenterate species) also effects medusa formation in *C. andromeda*.

Another aim of our work was to examine the development of *Cassiopea andromeda* polyps from vegetative buds. As early as 1900, Bigelow, for *C. xamachana*, wrote: "it is an important, if not the chief factor in the perpetuation of the species", whereas Gohar and Eisawy (1960b), for *C. andromeda*, suggested such development was exceptional and that it indicated unfavourable conditions for this species. Curtis and Cowden (1971) stated that the buds of *C. xamachana* did not settle and give rise to polyps on clean glass-surfaces, but only on a suitable substrate such as the algal layer in the aquaria. In our study, observations on budding in *C. andromeda* under various external conditions are reported, and experimental results are presented which indicate that the development of scyphopolyps from vegetative buds is initiated by a factor of microbial origin.

Evidence that a head inhibitor originating from the presumptive foot end of the buds is involved in the control of scyphistoma morphogenesis in *Cassiopea andromeda* has been presented elsewhere by one of the present authors (Neumann, 1977).

Materials and Methods

All specimens of *Cassiopea andromeda* (Forskål, 1775) used for this study were reared from a batch kindly supplied by the Löbbecke Museum & Aquarium, Düsseldorf, FRG. The species was determined according to the criteria indicated by Gohar and Eisawy (1960a) for ephyrae and young medusae. The polyps of our stock were maintained in aerated glass aquaria containing 4 l of natural sea water at 19.5°C ± 0.5 C°. Artificial light was administered for 16 h per day. The scyphopolyps were fed to repletion twice a week with recently hatched *Artemia salina* nauplia; the sea water of the aquaria was renewed some hours thereafter.

In the experimental series concerned with strobilation and propagation of vegetative buds, either single full-grown polyps or groups ranging in number from 10 to 30 were isolated from the substrate, cleaned of adhering algal material, and transferred to Boveri dishes containing about 150 ml pasteurized, natural sea water. They were subjected to various environmental and feeding conditions and kept in constant-temperature rooms or in incubators, as indicated in the respective sections.

Sea water containing antibiotics was prepared by adding 129 mg streptomycin-sulfate and 100 mg penicillin-K (SERVA) to 1000 ml of pasteurized sea water. Sterile glassware or sterile disposable dishes were used throughout the studies on polyp formation from vegetative buds. Marine bacteria present on the substrate of the aquaria and in medium of our brine shrimp culture which was several days old were either grown at 22° to 25°C on 1.5% sea water-agar containing nutrients in various combinations, or as suspension cultures in sea water to which nutrients had been added. Pure strains were isolated according to routine techniques. The most effective strain (as judged from its ability to initiate polyp formation from vegetative buds) was maintained in vials with agar slant containing 0.5% peptone (Difco). Before a test, agar plates of the same type were inoculated with the bacteria and incubated for 12 to 18 h. Thereafter, 500 ml of sterile culture broth containing 0.2 mg peptone ml sea water^{-1}, was inoculated with the rapidly growing bacteria from the plates, aerated, and incubated for various periods of time. As a first approach, overall bacterial growth was densitometrically measured with a Bausch & Lomb photometer. For the bioassay at 22° to 25°C, bacteria were separated from the culture medium by Millipore filtration (pore size = 0.22 µm) with the aid of filter syringes; the filtrate and the filter coated with bacteria could then be assayed separately. Living bacteria were examined using phase-contrast optics and by Nomarsky-contrast techniques. After heat-fixation of smears and Gram-staining the slides were checked using conventional optics. Photomicrographs were taken either with a camera mounted on a Leitz dissecting microscope or with a Zeiss photomicroscope.

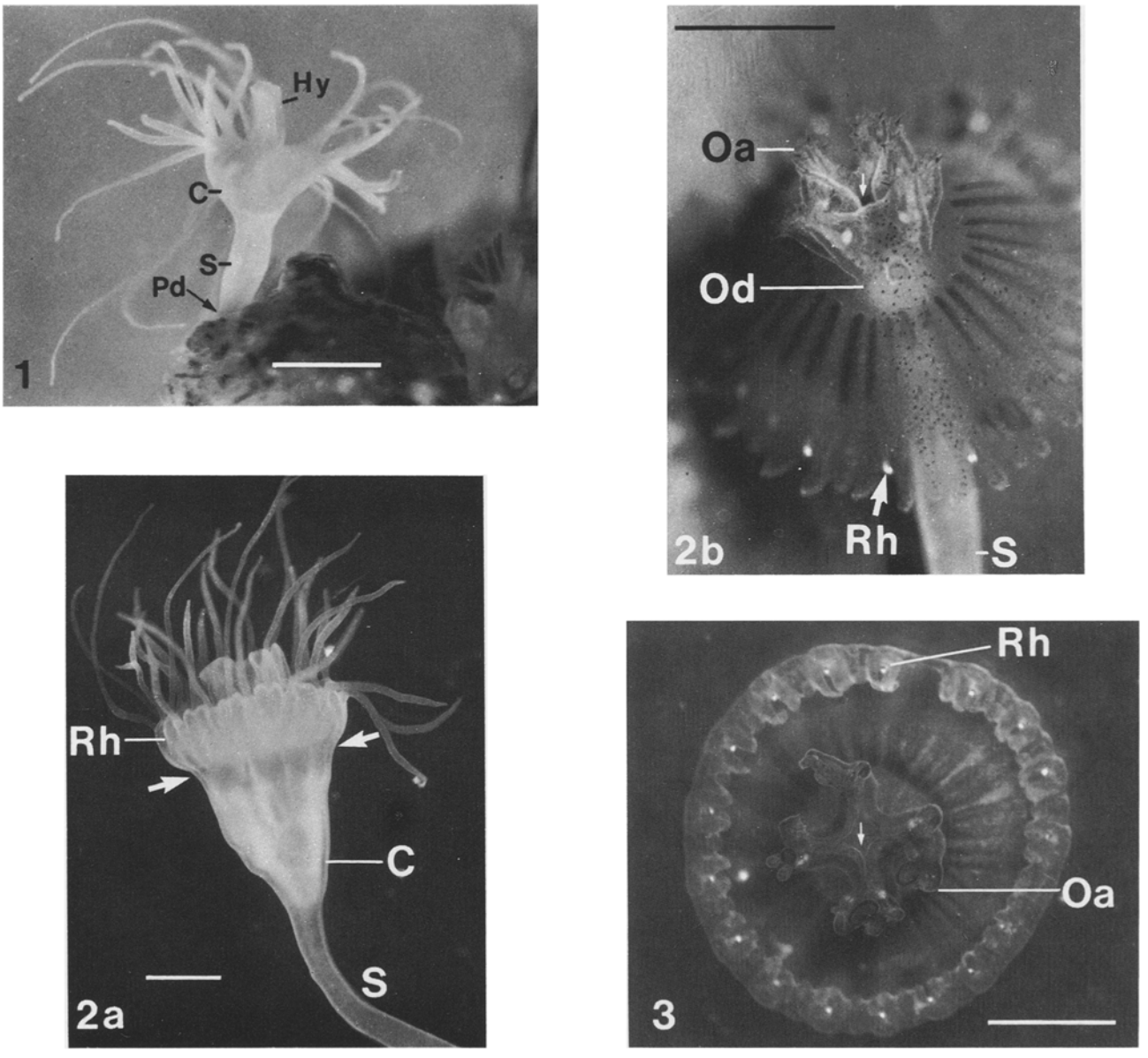

Fig. 1. *Cassiopea andromeda*. *1*: Syphistoma; C, calyx with tentacles ("head"); Hy, hypostome with mouth opening; Pd, pedal disc (covered by algal material); S, stalk (slightly contracted). *2*: Scyphistomae at (a) early stage of strobilation, note rhopalial lobes developing at margin of the calyx and beginning of the constriction (arrows); (b) advanced stage of strobilation, ephyra is about to separate from scyphistoma; arrow, original mouth opening; C, calyx; Oa, Od, oral disc with oral arms developing from the perradial parts of the hypostome; Rh, rhopalial lobes with rhopalia; S, stalk. *3*: Ephyra some days after separation from polyp, seen from oral side; note reduced central mouth opening (arrow); Oa, oral arms; Rh, rhopalial lobes with rhopalia. (All scale bars = 1 mm)

Results

Formation of the Medusae

The development of the medusae from scyphistomae in the genus *Cassiopea* was studied by Bigelow (1900), and has more recently been investigated by Gohar and Eisawy (1960b) and by Ludwig (1969). Therefore, only some main developmental events are briefly recapitulated here.

In contrast to *Aurelia aurita*, which simultaneously produces a considerable number of medusae-anlagen, *Cassiopea andromeda* forms only one anlage at a time in the distal region of the scyphistoma (Fig. 1: *1*, *2*). This process, termed monodiscous strobilation, includes the

reduction of tentacles, the formation of marginal lobes and rhopalia, and the gradual formation of a circular constriction below the tentacle region, by means of which one strongly pulsating discoid ephyra finally becomes separated from the basal polyp (Fig. 1: *3*). The ephyra subsequently acquires the adult structures of the rather specialized rhizostome medusa, which settles on the bottom, exumbrella down. The basal polyp undergoes regeneration of the apical organs lost during strobilation, i.e., the hypostome and the 32 marginal tentacles. We can confirm the earlier observation of Bigelow (1900) that regeneration begins even prior to separation of the ephyra from the basal polyp. Formation of an ephyra occurs within a period of about 1 week.

The above-described medusae formation was never observed in our stock cultures, which we maintained at 19.5°C ± 0.5 C° for a long day (16 h light:8 h dark) and fed twice a week. Only vegetative propagation took place. This was unexpected, since the scyphistomae were derived from a strain which in fact did produce medusae and which contained the indispensable zooxanthellae (Ludwig, 1969). As some influence by frequency of feeding and/or by temperature was presumed, both factors were successively altered:

In the first experiment, 80 full-grown scyphistomae lacking buds were isolated from the stock culture and subdivided into 4 groups of 20 polyps. They were kept in Boveri dishes at 19.5°C ± 0.5 C° and were now fed 6 times a week. During a period of 7 weeks none of the polyps showed strobilation, only numerous vegetative buds were formed.

In the second experiment, 4 groups of 20 polyps were isolated from the stock culture, and fed 6 times a week, but they were kept now at 24°C ± 1 C°. The observations during this series are summarized in Fig. 2. After 24 days the first polyps were about to strobilate, and after another 14 days each of the 80 scyphistomae had developed an ephyra. Subsequently, the basal polyps regenerated their apical parts. Some did not show any further development, but the majority (54 polyps) successively started budding. Twenty four scyphistomae resumed strobilation without an intervening budding-phase, while 30 polyps first formed buds and then strobilated. After 69 days, a total of 55 polyps had formed a second ephyra. At this time, a few scyphistomae had already begun to produce buds. It should be noted that during this experiment all basal polyps survived and that budding sometimes started before strobilation was achieved in the respective polyps. Furthermore, the presence of a bud apparently did not inhibit the onset of medusa formation.

One group of 20 scyphistomae which was likewise kept at 24°C ± 1 C°, but was fed only twice a week, displayed a development similar to that of the above-mentioned individuals. After 26 days the first polyp began to strobilate, and after 10 more days all of the 20 polyps had formed an ephyra. Thereafter, budding started but proceeded at a rather low rate. Similar experiments have repeatedly confirmed that strobilation can be induced by raising the temperature from ca. 20°C to ca. 24°C or even to 28°C. It appears, therefore, that under laboratory conditions temperature is the

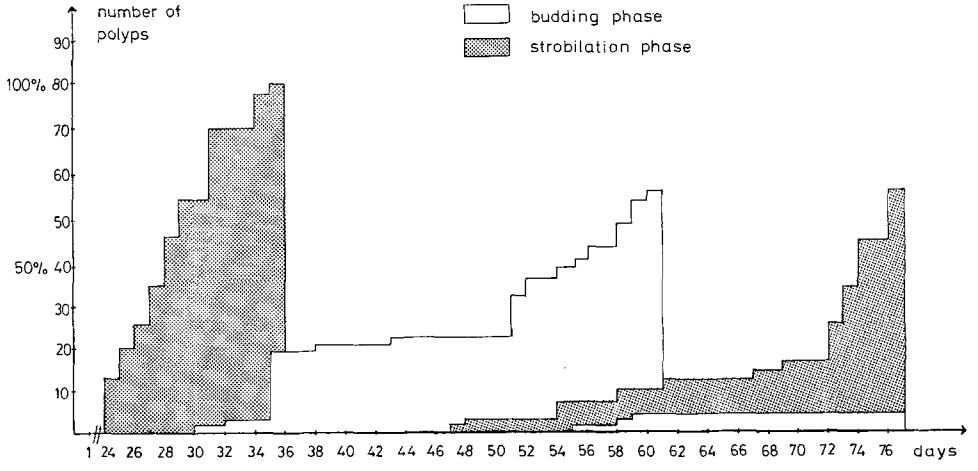

Fig. 2. *Cassiopea andromeda*. Cumulative frequency curve (%) of strobilation in a total of 80 scyphistomae after transfer from 19.5°C ± 0.5 C° to 24°C ± 1 C°. Food was supplied 6 times a week. Ordinate: cumulative frequency of polyps having undergone strobilation or budding; abscissa: days after transfer

main environmental factor controlling medusae formation in *Cassiopea andromeda*.

Formation of Vegetative Buds

Bigelow (1892, 1900), in scyphistomae of *Cassiopea xamachana*, noticed that in certain, perradial areas in the inferior region of the calyx a particular type of vegetative bud is formed. First, a roundish protrusion of ectoderm, mesogloea and endoderm appears; this elongates, and later becomes spindle-shaped; it finally remains attached to the polyp only by a thin ectodermal tube. This connection is then disrupted by movements of the scyphistoma and by the ciliary motion of the bud. The latter swims away, rotating around its longitudinal axis, with the original distal knoblike pole in front. Propagation of another bud can proceed before the first one is fully developed and before it is separated from the polyp (Fig. 3: *1*, *2*). Under certain conditions, even chains with 3 or 4 buds at different stages may occur. The buds are released one by one. The detached, spindle-shaped bud contains some zooxanthellae and, as stated by Curtis and Cowden (1971), it is definitely morphologically and physiologically polarized. The knoblike structure directed forward invariably constitutes the future site of attachment to the substrate and represents the presumptive foot region of the scyphistoma which eventually develops from the bud.

We found that in well-fed polyps not only 1, but 2, 3 or even 4 budding regions can be present which simultaneously produce chains of buds. Budding regions are usually located perradially, but may occasionally be situated interradially. If several budding regions occur in a single scyphistoma, either all are located perradially, or only some are found perradially with the others situated interradially. Bud formation is not restricted to polyps fixed on the substrate by their perisarc-covered basal disc region: buds were readily formed by scyphistomae which had carefully been isolated from the substrate; they were even produced by calyces cut off from the stalk.

Intensity of Budding under Different Experimental Conditions

Budding was observed in two experimental series, each consisting of 4 batches of

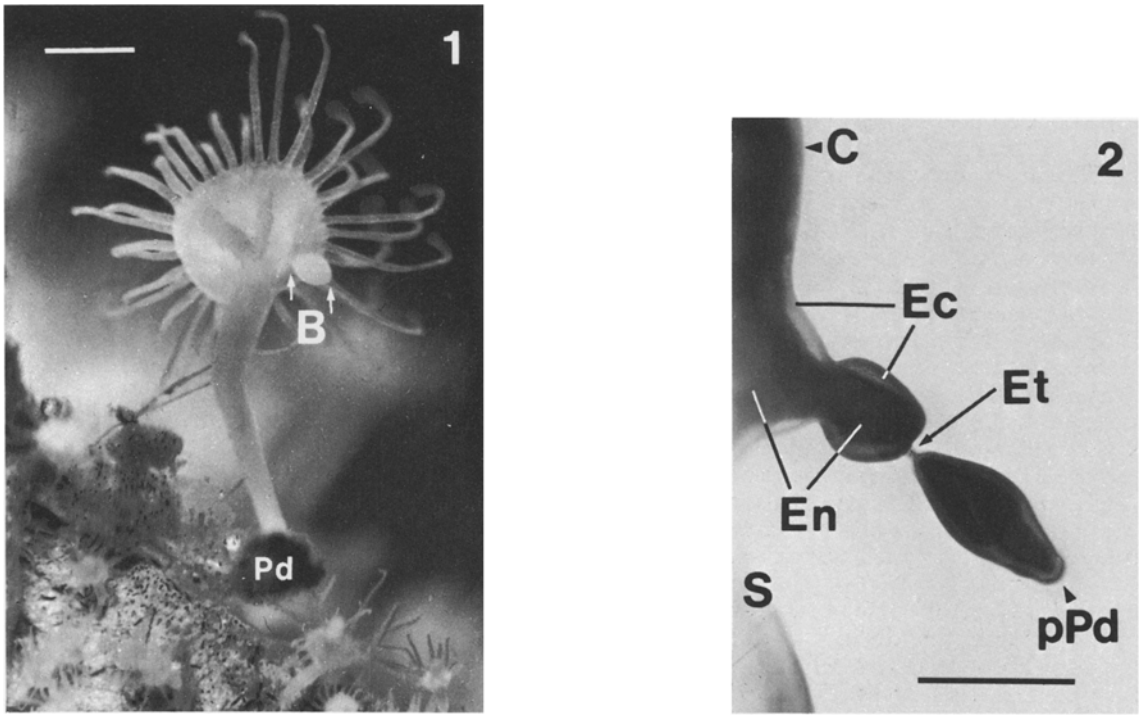

Fig. 3. *Cassiopea andromeda*. *1*: Budding polyp with 1 bud which will shortly be constricted off; B, bud; Pd, pedal disc (covered by algal material) (scale bar = 1 mm). *2*: Budding region of scyphistoma with 2 buds at different stages of development; endoderm of proximal bud is continuous with endoderm of polyp, distal bud is connected only by an ectodermal tube; C, calyx; Ec, ectoderm; En, endoderm; Et, ectodermal tube; pPd, presumptive pedal disc region; S, stalk (scale bar = 0.5 mm)

20 full-grown non-budding scyphistomae selected from the stock culture (19.5°C ± 0.5 C°, fed twice a week).

The individuals of the first series were continously kept at 19.5°C ± 0.5 C°, but were now fed 6 times a week. The number of polyps forming buds was counted before food was administered. In the 4 batches, the first polyps started to produce buds after 4, 5 and 10 days, respectively. After 2 weeks, bud formation was in progress in 30 of the 80 scyphistomae, and after 30 days 60 polyps displayed buds.

In the second series of 4 batches of 20 individuals, the polyps were likewise fed 6 times a week, but were maintained at 24°C ± 1 C°. With the raising of temperature and the increase of feeding frequency, budding occurred earlier and in a higher proportion of individuals than in the preceding series. The first budding polyps were noticed in the batches after 2, 4 and 8 days, respectively; after 2 weeks 68 individuals had begun to form buds, and after 24 days all polyps had produced at least 1 bud. At this time, the first polyps of this series began to strobilate.

Several experimental series were run to test whether the number of buds produced in a certain period of time could be influenced by varying external conditions: frequency of feeding, temperature of the medium, quality of the sea water and illumination cycle. Furthermore, budding in single polyps and in groups of 10 polyps was compared.

The results of 6 sets of experiments are compiled in Table 1, they are ranked according to the mean number of buds formed during the observation period. The total number of buds liberated from the scyphistomae was determined from counting the buds, which were collected from the dishes usually once a day. The data were obtained from series in which strobilation did not occur within the first 37 days of the experiments.

The observations indicate that the intensity of budding in fact depends on external conditions and can be modified by combinations of different factors. The results reflect a rather systematic tendency which can be characterized as follows:

In the first series (cultivated at 24°C) single polyps produced considerably more buds than polyps which were kept in groups of 10. This "crowding effect" was likewise apparent in the other series. Furthermore, in recycled sea water, single and/or grouped polyps were less efficient in budding than those maintained in fresh sea water. In the series cultivated at 28°C ± 1 C°, the number of buds increased when the food supply was raised. If polyps (kept at 28°C) were fed 5 times a week and grown in recycled sea water, those with 16 h light administered per day formed more buds than those maintained in a dark room and only exposed to light during bud collection, feeding and subsequent renewal of sea water. The data suggest a positive correlation of budding with feeding frequency, temperature and, perhaps, with duration of the diurnal photophase. Bud formation appears to be negatively correlated with the number of polyps per dish and with pollution of the medium by soluble, heat-stable waste products.

Our results prove that the production of vegetative buds in *Cassiopea andromeda* is numerically rather important. In regard to the dynamics of budding under various experimental conditions, we cannot agree with the assumption of Gohar and Eisawy (1960b) that budding is indicative of unfavourable environmental conditions. On the contrary, our findings substantiate the suggestion of Bigelow (1892, 1900) and Curtis and Cowden (1971) that the formation of buds and their subsequent development into scyphopolyps is a most important pathway for the perpetuation of this species.

Polyp Formation from Buds in Cassiopea andromeda

General Observations

Once liberated from the scyphistoma, the ciliated planula-like bud swims about with its knoblike, distal pole forwards. Eventually the swimming bud comes into contact with a substrate, settles, and fixes provisionally, probably by means of secretions of gland cells in the region of the knob. The latter then flattens and develops into a basal disc, which accomplishes permanent attachment to the substrate. Within 24 h at most, the attached, flask-like bud forms a hypostome at its upper end, with mouth opening and surrounding tentacle-forming region. Finally, a prominent head or calyx with numerous tentacle-anlagen is segregated from the lower portion of the bud, which itself differentiates into a slender, contractile stalk. Our observations, except for very minor variations, fit well with the detailed description of the process in *Cassiopea xamachana* given by Curtis and Cowden (1971). The division into 6 stages of this developmental sequence proposed by these authors proved to be convenient, and was used throughout this study (see Fig. 4a). Formation of a polyp from a bud, however,

Table 1. *Cassiopea andromeda*. Bud production under various environmental conditions

Temperature[a] and no. of polyps per Boveri dish	Illumination cycle (light:dark)	Feeding frequency	Culture medium	No. of buds produced within 37 days	
				Total no.	Mean no. per polyp
24°C 10	16h:8h	Twice a week	Pasteurized, recycled sea water[b]	28	2.8
10				29	
1				18	15.2
1				15	
1				14	
1				12	
1				22	
1				19	
1				17	
1				17	
1				6	
1				12	
24°C 10	16h:8h	Twice a week	Pasteurized sea water	95	11.3
10				135	
10				117	
8				82	
28°C 10	16h:8h	3 times a week	Pasteurized sea water	171	20.7
10				242	
1				49	52.6
1				52	
1				62	
1				44	
1				79	
1				53	
1				50	
1				47	
1				43	
1				47	
28°C 10	Continuous darkness	5 times a week	Pasteurized, recycled sea water[b]	352	33.3
10				314	
1				64	62.0
1				59	
1				58	
1				52	
1				67	
1				60	
1				47	
1				53	
1				57	
1				103	
28°C 10	16h:8h	5 times a week	Pasteurized, recycled sea water[b]	581	58.1
1				89	84.8
1				78	
1				93	
1				77	
1				87	
28°C 10	16h:8h	5 times a week	Pasteurized sea water	830	83.0
1				107	99.6
1				72	
1				92	
1				109	
1				118	

[a]All temperatures ± 1 C°.
[b]Sea water in which cultures of various hydrozoan species were maintained for 3 to 4 days, then collected, pasteurized, stored and repasteurized before use.

occurs only in the presence of "suitable substrate" (mainly represented by the algal covering of the aquarium), as stated by Curtis and Cowden (1971). In the absence of such substrate, the swimming buds do not settle but nevertheless undergo a limited morphogenesis: 2 to 6 days after detachment the mouth opening appears at the slightly conical, future oral end, and a hypostomal area becomes delineated, with 3 to 5 tentacle rudiments developing below the stomodeal region. The tentacles remain stout, and there is no further growth in the continuously swimming bud, which may survive up to more than 5 weeks. If sub-

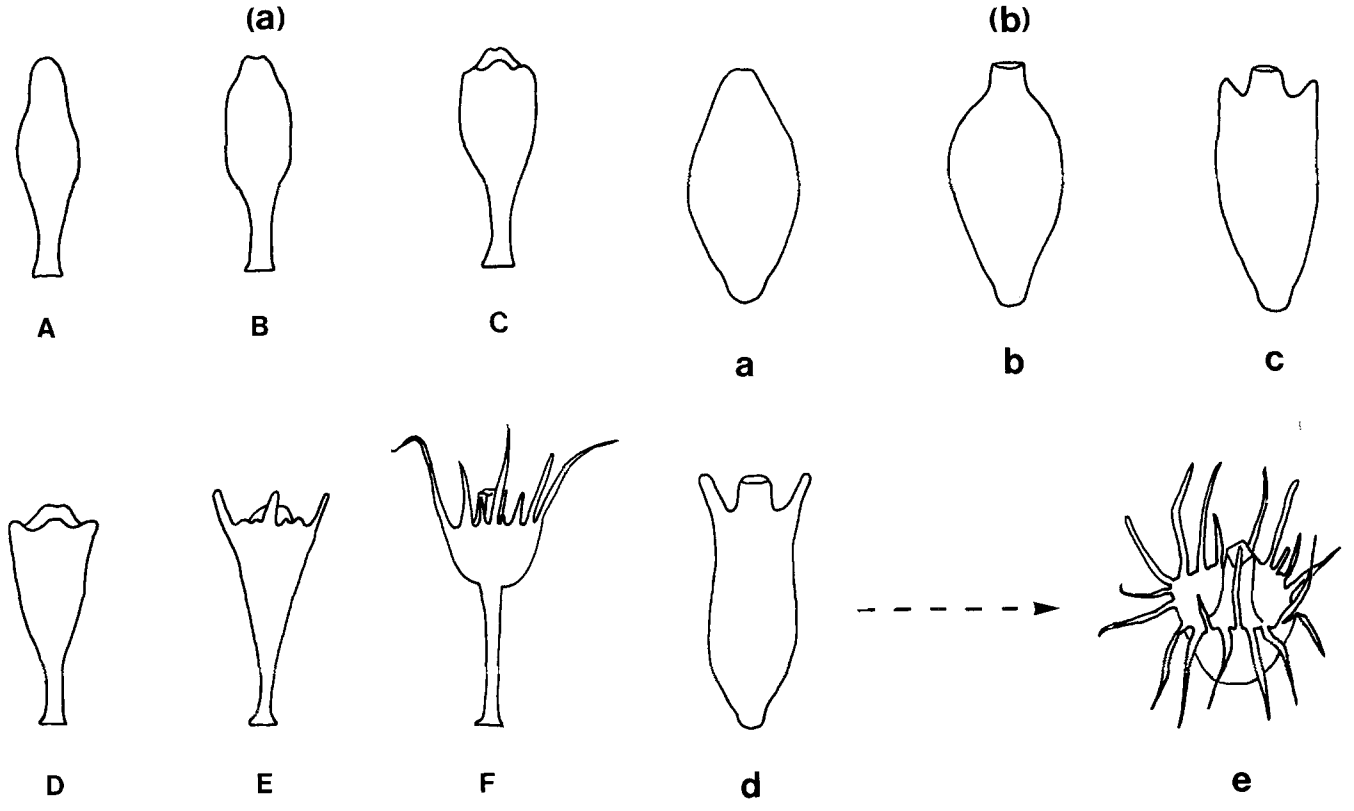

Fig. 4. *Cassiopea andromeda*. (a) Development of buds into polyps in presence of suitable substrate or bacterial inducer; (b) development of buds maintained in sterile medium in absence of suitable substrate or bacterial inducer. Stages redrawn, with minor modifications, from Curtis and Cowden (1971). For details see text

strate is later added, these partly transformed buds are still able to settle and to develop into normal polyps. This course of development has been divided into 4 arbitrary stages by Curtis and Cowden (1971) (see Fig. 4b).

We found in some, but not in all our cultures, that a small number of buds kept without suitable substrate displayed not only hypostome and tentacle-bud formation, but underwent complete transformation into a solitary polyp head without any rudiment of a stalk or a pedal disc. Such polyp heads developed, at the earliest, 10 days after the buds had been liberated from the parent scyphistoma; they formed long tentacles and were able to capture *Artemia salina* nauplii and to produce vegetative buds. This infrequent, nontypical morphogenesis has hitherto been observed only in oral fragments of transected buds of *Cassiopea* (Curtis and Cowden, 1971; Neumann, 1977).

Control of Polyp Morphogenesis: Preliminary Observations

Provided that the swimming buds were maintained in sterilized dishes in pasteurized sea water, and that dishes and medium were daily changed, no bud settled and developed into a scyphistoma. All 90 buds (6 batches of 15 buds maintained for 4 to 5 weeks either at ~20 or ~24°C) showed only the partial development described above. The same applies to buds which were kept in pasteurized sea water containing penicillin and streptomycin: polyp formation was completely inhibited in all 120 buds (8 batches of 15 buds maintained for 4 to 8 weeks at ~20° or ~24°C). However, if no antibiotics were added to the medium and if the dishes and the sea water were not changed during the observation period, the buds began to settle even on the glass surfaces, and all 75 buds (5 batches of 15 buds kept at ~20° or ~24°C) developed into normal scyphistomae within 1 to 27 days. However, it is worth noting that in 4 batches of 15 buds which were supplied with pieces of algal substrate from the aquarium, all 60 buds settled and developed into scyphopolyps within 6 days.

Two other sets of experiments demonstrate that the capacity to induce bud attachment and subsequent development is

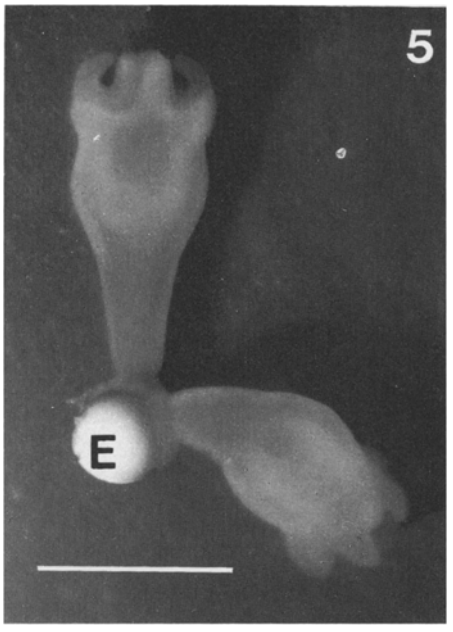

Fig. 5. *Cassiopea andromeda*. Two polyps developing from buds which settled on an *Artemia salina* egg shell (E). (Scale bar = 0.5 mm)

not restricted to the very complex algal covering of the aquarium:

In the first series, buds were transferred to dishes with pasteurized sea water to which a small quantity of egg shells and other debris from a brine-shrimp culture was added. Eleven of 12 buds had settled within 24 h and all had transformed into polyps after 3 days. It is of interest that 3 polyps were attached to the bottom of the dish, 1 was hanging from the surface pellicle, and 7 had settled on brine-shrimp egg shells or debris (Fig. 5). Similar results were obtained when brine-shrimps killed by heating were introduced into the dishes.

In the second series, sterilized glass-slides were incubated for at least 24 h with the culture medium of a several-day-old brine-shrimp culture. Thereafter, the slides were transferred into Boveri-dishes and buds stored in sterile medium were added. In 4 sets of this experiment, with a total number of 240 individuals, many buds attached to the glass slides or to the dishes and already showed tentacle-anlagen after 16 h. After 4 days, 82 to 95% of the buds had developed into polyps, which were frequently found in clusters. As in many other experiments, and as already reported by Curtis and Cowden (1971), some stragglers were present which, for unknown reasons, did not develop into polyps within the observation period.

In an attempt to determine the reasons for the settling of buds on certain parts of the glass surfaces, the incubated slides with the attached buds, and also some slides which had proved to be inefficient in the assay, were fixed and stained according to bacteriological routine methods. The preparations revealed, as expected, a layer of a variety of bacteria. At least 5 morphologically different types could be distinguished: short, stout rods; long chains of small rods; threads or clusters of coccoid rods of different size; and long, filamentous bacteria. The incidence of certain Gram-negative coccoid rods on the slides with attached buds was conspicuous, and it was of particular interest to note that ineffective slides lacked this type of bacteria.

Since planula-metamorphosis in the hydroid *Hydractinia echinata* (Müller, 1969, 1973) and metamorphosis of the larva of the tentaculate *Actinotrocha branchiata* (= *Phoronis muelleri*) (Herrmann, 1975, 1976) are known to be initiated by certain marine bacteria, it appeared consistent with our results to investigate whether factors of microbial origin guide settlement and induce attachment of the buds and their subsequent morphogenesis in *Cassiopea andromeda*.

Control of Polyp Morphogenesis by Bacteria

Based on the aforementioned findings, numerous efforts were made to grow bacteria present in the brine-shrimp culture and the algal substrate either in suspension cultures, or on agar plates or agar slant, and to test whether or not buds settled, formed a basal disc and developed into polyps upon exposure to the bacteria. Several pure strains could be isolated and tested separately, and one of them proved to be efficient. Gram-stained smear preparations revealed that the strain consisted of Gram-negative, coccoid rods which were probably identical with those detected on the incubated glass slides. Phase-contrast and Nomarsky-contrast observation of living bacteria, followed by differential determination tests permitted us to identify these bacteria as a nonpathogenic species of the genus *Vibrio*. This particular strain of *Vibrio* was used throughout the experiments reported below.

Toxic effects very soon interfered with the development when buds were transferred to aliquots (for example 3 ml) of the undiluted suspension culture. But settlement and/or pedal disc

formation, and polypoid development were to some extent observed in buds if the bacterial culture was diluted with pasteurized sea water in the range 1:2 to 1:10. However, typical polyps with long tentacles did not develop. This might be due to accumulation of metabolic products, since bacterial growth continued in all these assays as long as nutrients were available. In subsequent series, we therefore transferred the buds after various times of treatment in diluted bacterial cultures into sea water containing antibiotics. Buds which had developed a pedal disc and which eventually displayed a head segregated from a stalk (this was frequently observed after about 16 h) generally achieved the differentiation of complete scyphistomae. Buds beyond the pedal disc stage at the time of transfer usually resumed the shape of swimming buds or occasionally became abnormal.

As centrifugation proved insufficient to separate bacteria of suspension cultures from a noncontaminated supernatant, separation was performed by Millipore filtration with the aid of filter-syringes. Samples were taken after about 2, 4, 6, 8 and 24 h of incubation of the suspension cultures. The data presented here are based on several independent repetitions, usually 10 buds were employed per assay.

If brought into contact with the densely packed bacterial layer on the Millipore filter, which was immersed in pasteurized sea water with or without antibiotics, buds neither settled nor exhibited any sign of polypoid development; they essentially retained the features of swimming buds. Buds displayed the same behavior if they were tested in pasteurized sea water with the pellet of bacteria separated by centrifugation from submerse cultures, or with bacteria colonies scratched off an agar plate. It is important to state that the filtered or centrifugated bacteria were still alive, and could resume growth when resuspended in the appropriate medium.

Millipore filtrate of *Vibrio* sp. cultures incubated for less than 2 h caused polypoid development only in a few batches, but filtrate of cultures about 2 to 4 h old proved very effective. In cultures incubated for 6 to 24 h, however, toxic effects prevailed. For the filtrate of 2 to 4 h-old cultures (diluted or non-diluted), transfer of the buds into pasteurized sea water or pasteurized sea water containing antibiotics after about 16 h of treatment always led to the formation of normal polyps. It is worth noting that all buds from the same batch always synchronously reacted to the different treatments.

Heating to about 80°C for 5 min did not reduce the efficiency of the 2 to 4 h filtrate, as proved by the transfer test. Autoclaved filtrate (60 min at 110°C at 0.5 bar), however, only exceptionally induced polypoid development, and mainly provoked abnormities in the buds.

For a further characterization of the rather heat-stable bacteria factor(s), Millipore filtrates were submitted to dialysis. Aliquots (5 ml) of filtrate were filled in sterilized Visking tubes (exclusion limit: 10.000 to 20.000 Daltons), which then were immersed in 2.5 ml of sea water with antibiotics. If dialysis of filtrates of suspension cultures incubated for 2 to 4 h was performed in the presence of the buds, the buds predominantly attached to the membrane, formed particularly large pedal discs (see Fig 6), and developed remarkably synchronously into normal polyps. The rest of the buds fixed on the wall or the bottom of the dishes. In control assays with pieces of sterilized Visking tube immersed in sea water containing antibiotics, buds neither settled nor showed any further development.

If the buds were added when the tube was withdrawn after 24 h of dialysis, they quantitatively developed into scyphistomae which, as in the aforementioned series, displayed enlarged pedal discs. The latter observation establishes that a morphogenetic agent in fact passes through the membrane and was present in the surrounding medium.

Buds tested in autoclaved dialysates did not react unequivocally: polypoid development was rare, but lethal, atypical or nontransformed buds prevailed.

As observed in both diluted and undiluted Millipore filtrate from *Vibrio* sp. cultures incubated for 6 to 8 and 24 h, buds tested in the respective dialysates reacted atypically: in many cases pedal disc formation occurred without preceding settlement of the buds and without subsequent differentiation of a typical head; in one batch, all buds died within 24 h.

From the aforementioned observations it follows that the typical morphogenesis of buds of *Cassiopea andromeda*, e.g. settlement on a substrate, formation of the pedal disc, and development into scyphistomae with a slender stalk and a head with a final number of 32 long tentacles, can be induced by bacteria of a strain of the genus *Vibrio*. The bacterial factor(s) is apparently not a constituent of the bacterial cell surface, but is a soluble, dialysable and relatively

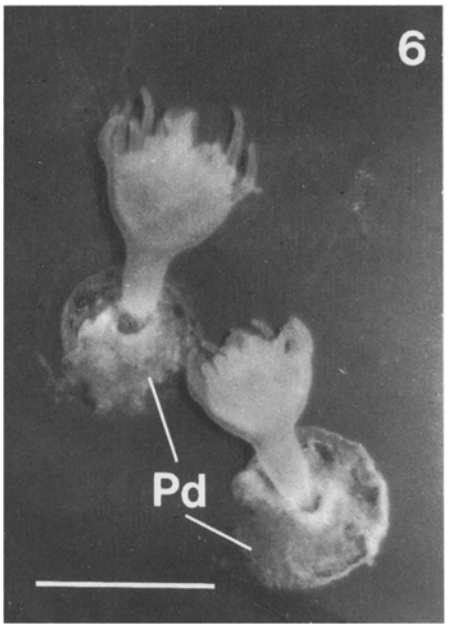

Fig. 6. *Cassiopea andromeda*. Two polyps which developed on a dialysis tube containing Millipore filtrate of a *Vibrio* sp. suspension culture. Note considerably enlarged pedal discs (Pd). (Scale bar = 0.5 mm)

heat-stable product of a molecular weight inferior to 10.000 to 20.000 Daltons which is released into the culture medium by growing bacteria. The findings do not permit us to decide whether abnormal development, in some cases tentatively interpreted as toxic effects, are merely dose effects of the morphogenetic factor(s), or are due to other exogenous metabolic products.

Discussion

Life-History

Investigations on the developmental biology of rhizostome species are available for *Cotylorhiza tuberculata* (Claus, 1881, 1892; Hein, 1902, 1903; Goette, 1887, 1893), *Rhizostoma cuvieri* (Claus, 1881), *R. pulmo* (Paspaleff, 1938), *Cassiopea xamachana* (Bigelow, 1892, 1900), and *Mastigias papua* (Uchida, 1926). Most of the classical work has been reviewed by Berrill (1949). The life history, essentially established with the aid of laboratory-culture techniques, is now known for *M. papua* (Uchida, 1926; Sugiura, 1963), *Cassiopea andromeda* (Gohar and Eisawy, 1960a, b), *Cephea cephea* (Sugiura, 1966), and *Rhopilema verrilli* (Calder, 1973). These investigators usually started from embryonic or planula stages collected from mature medusae. Except for *Cassiopea xamachana* (Bigelow, 1892, 1900) and *R. verrilli* (Cargo, 1971), the scyphistomae of other rhizostomae have not yet been observed in the natural habitat, but are only known from laboratory work.

Strobilation

The development of the strobila, separation of the ephyra, and the subsequent development of the young medusa, has been described in detail for *Cotylorhiza tuberculata*, *Cassiopea xamachana*, *Cassiopea andromeda*, *Mastigias papua*, *Cephea cephea* and *Rhopilema verrilli* (correct name *R. verrilii*). Monodiscous strobilation is obviously typical for the rhizostomae, but Calder (1973) found strobilae with two ephyrae formed at the same time to be common in *R. verrilli*. Polydiscous strobilae with 12 to 18 ephyra-anlagen were obtained from *Rhizostoma pulmo* upon experimental stimulation by Paspaleff (1938). However, this case appears atypical, since the liberated "ephyrae" did not develop into medusae but gave rise to young polyps which settled after a short period of vagile life.

In polyps of *Mastigias papua* (Sugiura, 1965), strobilation could be induced by raising the temperature above 20°C. In *Cephea cephea* (Sugiura, 1966) and also in *Rhopilema verrilli* (Calder, 1973) an increase of temperature likewise led to strobilation. In our experiments, polyps of *Cassiopea andromeda* did not strobilate at 20°C, neither when food was administered and sea water was renewed twice a week, nor when polyps were fed and were supplied with fresh sea water 6 times a week. Raising the temperature to about 24°C (or above) however, caused medusa formation. As in *M. papua* (Sugiura, 1963), strobilation in *C. andromeda* is further dependent upon an intrinsic factor: the symbiontic zooxanthellae in the entoderm and the mesogloea (Ludwig, 1969). These organisms have been described by Freudenthal (1962) as *Symbiodinium microadriaticum* nov. gen. nov. sp. The distribution of zooxanthellae in the scyphistoma is axially graded, with a maximal density in the subtentacular region and with minima in the proboscis and in the pedal region. Rapid multiplication of these cells was found to be concomitant with (perhaps even causative factor in) the onset of strobilation, and leads to a particular colour pattern of the strobila. Most of the symbionts are then located in the subtentacular region and the proboscis and become, therefore, incorporated into the ephyra. Scyphistomae

which were experimentally deprived of their symbionts proved unable to develop ephyrae. They resumed strobilation in a rather low proportion following reinfection with zooxanthellae according to the methods of Sugiura (1964). The findings of Ludwig (1969) show further that an experimentally reduced stock of symbionts results in a low rate of strobilation. Causal analysis of strobilation control in *C. andromeda* requires elucidation of the role of the symbionts in the metabolism of the coelenterates which is still obscure at present (see Ludwig, 1969 and Kremer, 1976 for more detailed discussions).

Experimental and biochemical investigations provided information on control of strobilation in two semaeostome species. In *Aurelia aurita* (Spangenberg, 1971), polydiscous strobilation can be induced by warming the sea water to 27°C, provided that the scyphistomae have previously been chilled for 6 to 8 weeks at 19°C. In addition, iodine must be present in the medium. Strobilating *A. aurita* polyps were found to produce three protein-bound iodocompounds and to secrete one of them into the medium. This latter compound, tentatively identified as thyroxine, proved to induce strobilation in prechilled polyps maintained in iodine-free sea water.

Earlier attempts by Paspaleff (1938) to induce strobilation by temporal or chronic treatment with rather large amounts of KJ-solutions added to the medium were reported to have succeeded in *Aurelia aurita* and in *Rhizostoma pulmo*. In the latter species, however, the "ephyrae" resulting from the polydiscous strobilae developed into polyps but not into medusae.

In *Chrysaora quinquecirrha* (Loeb, 1972), strobilation can be induced by warming polyps to 26°C, after previous chilling at 20°C for more than 7 weeks. Two hours after warming of the polyps a factor can be detected in the surrounding medium which is only produced for two further hours (Loeb, 1974a). This factor is able to stimulate the polyps to elongate the lips of the proboscis and to form the circular indentation (neck) below the tentacle region which is the first visible symptom of strobilation. This neck-inducing factor (NIF) can be dissociated into polypeptide subunits which recombine under appropriate conditions (Loeb, 1974b). This author states that the NIF is probably involved only in the initial steps, since normal, complete strobilation in NIF-treated individuals was exceptional.

Budding

Several types of vegetative propagation of the polyp-form occur among scyphozoans. In the Semaeostomae, polyps may form by fission budding, from *Hydra*-type lateral buds, from stolonial buds, from pedal stolons and from dormant podocysts (see Berrill, 1949 for review). An entirely different type of budding has been described for most of the rhizostome species. Goette (1887, p. 25) first described and depicted buds arising from the inferior region of the calyx in polyps of *Cotylorhiza tuberculata* and Claus (1892, his Plate I and Figs. 1-3) presented a somewhat different illustration of the ciliated "planula-like" buds in the same species. In the same year, Bigelow (1892) described the development of a scyphistoma from such a swimming bud after it had been separated from the parent polyp in *Cassiopea xamachana*. Budding is apparently similiar in *Mastigias papua* (Sugiura, 1963). In *Cephea cephea* (Sugiura, 1966), some of the buds were of the aforementioned planula-like type, but others developed tentacle-anlagen and a short stalk before separation from the adult individual. The latter very much resembled those buds of *Cotylorhiza tuberculata* depicted by Goette (1887). It is common to all these species that the polarity of their buds is inverse to bud-polarity in species of *Hydra*: in the latter, the distal end of the bud represents the future oral region of the new *Hydra*, whereas the distal, knoblike end of the rhizostome buds invariably develops into the aboral (= pedal) region of the young scyphistoma.

According to Calder (1973), the rhizostome *Rhopilema verrilli* notably differs from the species considered above. Scyphistomae, being rather stout, were never observed to produce ciliated, motile buds, but were found to form stolons and to develop podocysts, either at the pedal region of the polyp or at the tip of the stolon. From the viewpoint of external morphology and mode of vegetative reproduction, *R. verrilli* represents rather the semaeostome-type than the rhizostome type. However, strict classification according to the mode of asexual reproduction is not possible, since *Rhizostoma pulmo*, as indicated by the observations of Paspaleff (1938), seems to exhibit almost every known type of vegetative polyp formation. New scyphistomae are reported to arise from motile, planula-like buds, from *Hydra*-type buds and stolonial buds, as well as from two different types of podocysts. The experimentally induced polyp formation from strobilae (which is not very likely

to reflect a normal way of development) has already been mentioned above.

Most authors agree with the view that production of ciliated, lateral buds is not a response to unfavourable conditions. In *Mastigias papua*, the frequency of budding was found to be closely related to the quantity of food available (Sugiura, 1963). The same author stated (again without giving quantitative data) that food quantity and water temperature are two major factors influencing the budding rate in *Cephea cephea* (Sugiura, 1966). Our numerical results prove the importance of both factors for budding in *Cassiopea andromeda*.

Unlike strobilation, which requires the presence of symbiontic algae, budding proceeds in scyphistomae of both *Mastigias papua* and *Cassiopea andromeda* without zooxanthellae (Sugiura, 1964; Ludwig, 1969), however, no quantitative data are available to compare the budding rate of polyps with and without symbionts.

Throughout our experiments, the intensity of budding was observed to be negatively correlated with the number of *Cassiopea andromeda* polyps maintained per dish. A single, similar result was mentioned by Ludwig (1969), who found that the budding rate in a batch of 50 polyps was lower than that in a batch of 15, whereas medusa formation did not appear to be affected by population density. To our knowledge, only the paper of Coyne (1973) deals with the rate of vegetative reproduction in relation to initial population density in another scyphozoan, *Aurelia aurita*. Although employing different methods (i.e., the newly produced buds and polyps were not collected but were left together with their parental polyps in the same dishes), Coyne found that in all replicates the reproduction rate was inversely correlated to the initial population size. He further observed that the use of highly conditioned medium from *A. aurita* stock cultures instead of fresh artificial sea water strongly reduced the growth rate. This tendency was likewise found in *C. andromeda* kept in recycled, repasteurized sea water taken from hydrozoan cultures (see Table 1 of this paper). The question remains open as to whether unspecific, soluble waste products are accumulated in the respective mediums and suppress budding in scyphistomae of both species, or whether more specific substances (such as nematocyst toxins in the case of regeneration inhibition in hydroid species, Davis, 1967) or even quite different factors are responsible. Furthermore, it is questionable as to whether such a mechanism to reduce population growth, which appears useful in the limited space of laboratory dishes, is of any significance in the natural habitat where a variety of limiting factors may interfere with maintenance and growth of populations.

Initiation of Polyp Morphogenesis

Few papers have attributed more than casual interest to conditions of settlement and causative extrinsic or intrinsic factors in subsequent polyp morphogenesis of both sexually produced larvae and vegetative buds in coelenterates. In his study on the development of *Haliclystus octoradiatus* (Lucernariidae), Wietrzykowski (1912) found larval stages and young polyps up to the 4-tentacle stage only attached to filamentous red algae, particularly *Ceramium* sp., whereas later stages were specifically found on *Zostera* thalli (not identified to species). Weill (1934) questioned such a strict, stage-dependent substrate specifity, and reported that *H. octoradiatus* settled on virtually every kind of algae.

A preference by the hydroid *Coryne uchidai* to develop colonies on thalli of the alga *Sargassum tortile* was shown to be due to algal constituents which stimulate planula larvae to settle and to form polyps. δ-tocotrienol and its two epoxides, determined by analysis and synthesis, were identified as active components by Kato *et al.* (1975).

Curtis and Cowden (1971) considered the algal covering of the aquarium as a "suitable substrate" for the development of the planula-like buds of *Cassiopea xamachana*; however, our results rather indicate that the algal layer is only indirectly involved in the control of polyp morphogenesis from buds of *C. andromeda*, serving as carrier or substrate for microorganisms. Conclusive evidence for our hypothesis that microbial factors might induce buds to develop into scyphistomae was obtained after an efficient strain of Gram-negative coccoid rods, identified as a nonpathogenic *Vibrio* species, was isolated and used for assays. Probably there are still other bacterial species with inductive capacities.

Induction of planula metamorphosis in the hydroid *Hydractinia echinata* (Müller, 1969, 1973) is at present the most thoroughly investigated example of bacterial involvement in developmental control by marine invertebrates. Cameron and Hinegardner (1974) reported that metamorphosis of pluteus larvae of *Arbacia punctulata* and *Lytechinus pictus* was triggered by bacteria. Furthermore, Herrmann (1976) recently presented a de-

tailed study which proved that metamorphosis of larvae of the tentaculate *Phoronis muelleri* is induced by a variety of species of marine bacteria. Bacteria may also be concerned with substrate specifity and settlement of larvae in some sedentary polychaetes (see Herrmann, 1976 for references).

Müller (1969, 1973) and Herrmann (1976) stated that induction activity was detectable in bacteria harvested on Millipore filters from suspension cultures but not in the cell-free filtrate. Contrary to these findings, *Vibrio* sp. cells which were separated by Millipore filtration or centrifugation and hindered from growth by transferring them into sterile sea water, proved to have no effect on buds of *Cassiopea andromeda*. Since Millipore filtrate of growing suspension cultures induced polyp development, we concluded that the factor(s) which induce buds to develop into polyps obviously are not integral surface-components of *Vibrio* sp. cells, but are metabolic products released into the medium by growing bacteria, only. The product was found to be dialysable and relatively heat-stable.

In the presence of this product, bud settlement, attachment and formation of scyphistomae can proceed on sterile glass surfaces and dialysis tubes. Polyps even develop without recognizable involvement of solid substrate at the air-water interface or floating in the medium. In a short comment by Eiben *et al.* (1976), which refers also to the mechanisms of transformation of buds in *Cassiopea* sp., it is said that the stimulus to settle originates from living bacteria which must be present in a sessile state. These conditions are certainly realized on the incubated glass slides to which buds of *C. andromeda* attached and developed. Indeed, it has been reported above that the inductive stimulus is exerted by bacteria in growing suspension cultures. It must be admitted that the conditions encountered by *C. andromeda* buds in the natural habitat or in the biocenose of an aquarium considerably differ from the situation in log-phase bacterial suspension cultures. The latter imply complications such as toxic effects, which have to be overcome by dilution series and transfer experiments.

It is not known whether the buds are "trapped" by chance by the bacterial agents when they, in a random trial-and-error-manner touch various substrates and obstacles, or are directed by short- or long-range chemotaxis to settle on the appropriate substrate. In either case, the bacterial morphogenetic influence must persist until stable determination of the organism is achieved, i.e., until a bud has formed its pedal disc. As to the ecological and microenvironmental aspects of the problem, it is unproved whether or not the presence of effective bacteria indicates suitable conditions of life for *Cassiopea andromeda*.

A remarkable concept of the primary target in the induction of metamorphosis in *Hydractinia echinata* planulae has been proposed by Müller (1973). The isolated inducing agent (a bacterial leakage-product which is probably an instable, nondialysable phospholipid), when administered to the larvae as a pulse, led to dose-response curves with Michaelis-Menten-like saturation kinetics. Furthermore, the inducer could be antagonized by the alkaloid ouabain. It was also shown that pulse application of monovalent cations (Cs^+, Rb^+, Li^+) provoked planula metamorphosis which could also be blocked by ouabain (Müller and Buchal, 1973). These facts lead to the conclusion that stimulation of an enzyme or carrier system in larval cell membranes which is responsible for active cation transport (i.e., Na^+/K^+-ATPase), can be interpreted as a primary effect in planula metamorphosis initiated by both bacterial and ionic induction. The ouabain-insensitive K^+-induction, however, is said to be a passive reaction directed by the Gibbs-Donnan principle.

Herrmann (1976) favours the view that an alteration of the electrokinetic potential, brought about by immediate contact with the inductive bacteria (or by Cs^+-ions) rather than by a distinct bacterial substance, is causative for the successive metamorphic events in *Phoronis muelleri* larvae. However, direct evidence for this theory is not yet available.

Actually, statements on the initial effect leading to polyp morphogenesis in buds of *Cassiopea andromeda* cannot be made in precise biophysical or biochemical terms. However, results of dissection experiments using *C. andromeda* buds (Neumann, 1977) permit us to speculate on the basic mechanism. When buds, selected at various times following detachment from the parent polyps, were transected into fragments of different sizes, then, depending on the original position in the organism, on their size and on the age of the operated buds, the fragments either regenerated or developed into a solitary polyp's head without stalk and peduncle. Whereas basal fragments tended to regenerate complete buds, young apical parts mostly differentiated polyp heads. Apical and middle parts of progressively older buds regenerated buds with increasing frequency. To explain the par-

ticular alteration in the developmental capacities, a head inhibitor was postulated which originates from the basal region of the buds and which expands apically with increasing age of the buds. Apart from the question of the structural and chemical properties of this head inhibitor, we propose as a working hypothesis that the bacterial inducer which stimulates a bud to develop into a complete scyphistoma acts bimodally: direct action on the knoblike end of the bud is assumed to induce formation of the pedal disc, indirect action by abolishment or elimination of the head inhibitor is thought to permit segregation and differentiation of the polyp's head.

As mentioned above, in some cases solitary polyp heads without stalk and peduncle developed from aged, entire buds in the absence of bacterial inducer. This indicates that head formation, which appeared to occur only after previous pedal disc development, can be realized autonomously. These observations give further support to our view that control of head and pedal disc formation is exerted by different pathways, head development being under indirect control of the bacterial inducer.

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