Marine Biology 23, 47—57 (1973)

Studies on Reef Corals. I. Skeleton Formation by Newly Settled Planula Larva of *Pocillopora damicornis**

J. H. Vandermeulen[1] and N. Watabe[2]

[1] Department of Biology, University of California; Los Angeles, California, USA
and
Department of Zoology, Duke University; Durham, North Carolina, USA
and
[2] Electron Microscope Laboratory, University of South Carolina; Columbia, South Carolina, USA

Abstract

Initiation of coral-skeleton formation was studied in the reef-coral *Pocillopora damicornis* Lamarck. Observations were made on sequential skeletal growth stages of newly settled planula larvae during the first 22 days following settling onto glass microscope slides. Techniques used include phase light microscopy, scanning and transmission electron microscopy, and powder X-ray and selected area electron micro-diffraction. Formation of the skeleton is initiated immediately on settling of the larva. The primary calcareous elements are of two types — flattened spherulitic platelets, and smaller rod-like granules. Rudimentary primary septa are clearly defined within 6 h after settling. Fusion of the primary calcareous elements results in the formation of the larval basal disc within 48 to 72 h. With transmission electron microscopy, this basal disc is found to differ from subsequent adult calcification in (1) considerably lesser degree of mineralization, (2) smaller crystal size, (3) more random orientation of the crystals, and (4) the presence of trace amounts of calcite in addition to aragonite. The basal disc with its septal rudiments constitutes a true larval skeleton, differing in morphology, micro-architecture, and crystal type from the fibrous growth characterizing the adult skeleton.

Introduction

The characteristically sculpted, species-specific skeleton of the reef-building corals is an outstanding example of cellular control over its extracellular environment: "the spatial extension of cellular activities" (Picken, 1960). The coral skeleton is an extracellular, apparently non-varying, composite of an organic matrix, in intimate relationship with the calcareous (aragonite) component. The skeleton is made up of closely apposed crystalline fibers, each in turn composed of slender aragonite crystals with their c-axis oriented parallel to the long axis of the fiber (Wainwright, 1964). All crystal fibers[1] during their formation lie normal to the surface of the skeleton, and thus to the exposed surface of the overlying calicoblast (=skeletogenic) epithelium. Both the gross morphology and the microarchitecture of the coral skeleton is species specific (viz. Vaughan and Wells, 1943), and apparently dictated by the overlying coral tissues.

The mechanism of calcification is not known. In fact, the site of skeleton formation, i.e., whether intracellular (von Heider, 1882; Ogilvie, 1897; Kawaguti and Sato, 1968), or extracellular (von Koch, 1882; Duerden, 1904; Goreau, 1959; Vahl, 1966), is still a matter of debate. The sum of the evidence suggests an extracellular formation (viz. most recently, Vandermeulen and Muscatine, 1973; also Vandermeulen, 1973b). In this scheme, the coral's calicoblast epithelium secretes an organic matrix which becomes included in some manner, whether actively or passively is not known, in the aragonitic skeletal elements.

The larva of the stony corals, however, leads a non-calcifying planktonic existence following its release from the adult colony. It is only on settling onto a suitable hard substrate that the previously non-calcifying larva abruptly changes its life mode, and begins to lay down the extensive extracellular calcareous elements which will lead to the specifically organized adult skeletal structure. The settling stage of the larva then presents a unique system in which to study initiation of skeleton formation. Further, this stage also provides opportunity to follow the extension of cellular control over extracellular organization of structure by following skeletal development during the initial stages of settling.

* Contribution 419, Hawaii Institute of Marine Biology, University of Hawaii.

[1] The authors agree with Sorauf (1970) on the acceptance of the term "crystal fiber" (Wainwright, 1964) in preference to "fasciculus" (Wise, 1969), to describe the polycrystalline aggregate of aragonite crystal units comprising the crystal bundle of the adult skeleton. Whereas "fasciculus" satisfactorily describes the cluster or bundle of crystal units, the term "crystal fiber" more correctly describes its crystalline structure and optical properties and is, therefore, more acceptable to biologists, geologists, and crystallographers.

Knowledge of larval settling has caught scientific attention sporadically, and has centered largely on spawning rates and times, growth rates, and gross tissue changes (e.g. Stephenson, 1931; Abe, 1937; Atoda, 1947; Harrigan, 1972). Studies on skeleton formation have concentrated on gross changes of skeletal structure, primarily as these relate to taxonomic relatonships. Nothing is known of the mechanism of skeleton initiation. The little detail available is set out in only two, widely separated, studies, those of von Koch (1882), and of Wainwright (1963). Indeed, details have been figured in the literature only in camera lucida drawings (viz. von Koch, 1882).

Larval calcification is known to include formation of spherulitic crystal clusters or "spherulites" (von Koch, 1882; Wainwright, 1963), and a "larval skeleton" is described as precursor to the appearance of the adult skeleton. Fusion of the rapidly formed and numerous spherulites is then supposed to result in the larval basal disc, a flat two-dimensional calcareous disc on which all subsequent structures arise. The parallel crystal growth characterizing the adult skeleton is viewed as resulting from loss of the spherulitic radiating character of the larval deposit, the individual crystal being forced into parallel growth through space competition (for initial analysis see Bryan and Hill, 1941; Barnes, 1970).

Wainwright (1963) reported calcite in the interseptal regions of the first-formed basal disc. This is in contrast to the crystal form of the adult skeleton, which is entirely composed of aragonite. Recently this observation has been re-interpreted as possibly being due to bacterial calcitic contamination, formed during settling in the larval organic exudate (Wainwright, *in* Siegel, 1973).

The sum of these few and scattered observations suggest that the coral larval skeleton, both in structure and crystal type, differs from that of the adult. The present study presents detailed observations on skeleton development during the first 22 days following settling of the planula larva of the reef-coral *Pocillopora damicornis*. Sequential growth stages are examined by scanning and transmission electron microscopy, and the crystal structure of the various stages is monitored by powder X-ray diffraction and selected area electron micro-diffraction. Results are discussed in terms of biological control over the extracellular formation of calcareous structure. The tissue ultrastructural changes accompanying settling and initiation of skeleton formation are the subject of two papers published elsewhere (Vandermeulen, 1973a, b).

Materials and Methods

Larval Settling and Culture (see Reed, 1971)

Living colonies of *Pocillopora damicornis* Lamarck were collected from reefs in Kaneohe Bay, Oahu, Hawaii, and were maintained in plastic aquaria at the University of Hawaii's Marine Laboratory on Coconut Island, Oahu.

The planula larvae, which are released nightly, were collected daily from the aquaria with a Pasteur pipette and transferred to 4″ × 4″ (10 cm × 10 cm) polyethylene containers (settling dishes) lined with glass microscope slides, approximately 200 planulae per dish. Each morning the settling dishes were inspected, and slides with attached planulae were transferred to large glass bowls containing filtered ("Aquapure", 5 μ pore size) seawater, and were maintained under a 12 h light 12 h dark light regime (15,000 to 18,000 lux at the water surface from fluorescent lights). Seawater was changed daily, and slides were cleaned routinely with cotton-tipped applicator sticks to remove algal growth.

Preparation of Skeletal Material

Bleached skeletons of various settled stages of coral polyps were obtained by placing slides with attached specimens in a 1:20 dilution of commercial sodium hypochlorite (Chlorox or Purex) in distilled water to remove the soft tissues.

Light Microscopy

Rinsed and dried skeletons were examined directly on the glass slides.

Scanning Electron Microscopy

Fragments of glass microscope slides with attached skeletons were cemented directly onto brass stubs with Duco cement, and vacuum-coated with 40% palladium-60% gold before examination in a Jeolco JSM-2 scanning electron microscope at 15 kv. Fragments of adult coral branches were treated similarly.

Transmission Electron Microscopy (and Selected Area Electron-Microdiffraction)

Both attached and detached skeletons were embedded in plastic (either Vestopal W or Spurr), and sectioned with a diamond knife (DuPont) in a Porter-Blum ultramicrotome. Gold sections were examined in a Siemens Elmiskop I at 80 kv.

Polished-Etched Material

Adult coral branches were embedded in Epon 812, and polished on a rotating lap proceeding through aluminum oxide, and two grades of diamond paste. Etching of polished surfaces was performed either with 2% Na-EDTA (ethylene diamine tetra-acetic acid) for 2½ h, or with 1% formic acid for 1 min.

Powder X-ray Diffraction

Five 18-day juvenile skeletons were ground in a ceramic mortar, and the resulting powder examined with a Phillips Norelco X-ray diffractometer (CuK_α radiation, 40 kv, 30 ma, lithium fluoride monochromator). Spectrum scans were obtained from 25° to 30° 2θ, so as to include the major aragonite peaks at 26.18° and 27.24° (corresponding to crystal lattice planes 111 and 021) and the major calcite peak at 29.36° (crystal plane 104) (National Bureau of Standards, 1950).

Observations

Considerable variation is found between individual *Pocillopora damicornis* polyps both in their calcification rate and budding rate, and in time of formation of the various skeletal features such as septa, coenosteal spines, basal plate diameter, etc.

Skeleton Development

6 h to 36 h

The first calcareous elements which can be recognized after settling of the larva are circular platelets (25 to 50 μ in diameter) interspersed with numerous smaller rod-shaped granules, the latter up to 10 μ in length. These platelets and granules lie directly on the glass substrate. By 6 h after settling, some polyps show calcareous aggregates: the rudiments of the primary septa (Fig. 1:*1*). These rudiments are clearly defined at 12 h. Except for the radially arranged septal rudiments, the platelets and granules lie distributed without apparent organization over the glass substrate beneath the base of the polyp. Within 24 h, almost the entire surface covered by the flattened and rapidly spreading polyp has become calcified, forming the basal plate of the young polyp skeleton (Fig. 1:*2*).

The building blocks of the basal plate are the platelets and granules. The platelets are seen mainly in the central columella region which is the slowest to calcify; (Fig. 1:*3*), underlying the primary and secondary septa (Fig. 1:*4*), and constitute most of the interseptal areas as a thin pavement. Externally, these platelets have the shape of flattened circular structures (Fig. 2:*6*). When viewed with the phase microscope, these appear to be composed of radiating acicular elements (Fig. 2:*5*). Granules fill in the interstices of the basal plate, and masses of granules constitute the septal growth points (Fig. 2:*7*). The periphery of the basal disc also is composed mainly of granules (Fig. 2:*8*).

48 h Skeleton

By 48 h, most of the basal plate appears to be completely unified into a solid calcareous mat. There is no evidence of the surface nodules typical of the adult fibrous growth, neither on the surface of the basal plate, on septal growth points (Fig. 2:*9*), nor on more fully formed septal rudiments (Fig. 2:*10*).

72 h Skeleton

At this stage, the surface character of the central region has undergone change, with the appearance of the large nodules characteristic of adult fibrous growth (Fig. 3:*12*). Meanwhile the margin of the basal plate still consists of the featureless conglomeration of granules typical of earlier stages (Fig. 3:*11*). The hexagonal configuration of the scleractinian skeleton is now clearly defined, and the skeleton contains a full complement of 1°, 2°, and 3° septa, and a fully calcified columella.

14-Day Skeleton

At 2 weeks, the skeleton consists of a three-dimensional palisade of crystal fibers (Fig. 3:*15, 16*), oriented more or less normal to the skeleton surface. These fibers are seen both in the basal plate, and in the vertical structures (Fig. 3:*13, 14*). At this stage, the juvenile skeleton (i.e., still possessing the septa which are lacking in the adult skeleton) is structurally identical to the adult skeleton in its fibrous growth (Fig. 4:*17, 18*).

Transmission Electron Microscopy, 22-Day Skeleton

In external appearance, the 22-day skeleton is characteristically adult in surface features and in structure. Many specimens show secondary and tertiary polyps budding off from the main polyp. In section, the skeleton is massively calcified, consisting of closely packed crystal fibers, each composed of lath-like crystal units. Individual crystal units are elongate, varying in width from 1200 to 1500 Å, and approximately 500 Å in thickness (Fig. 4:*19*). Their full length was not determined, but it is in excess of 15 μ. The crystals appear to be homogeneous, lacking substructure.

In contrast to the massive skeleton of adult growth, the thin basal layer representing the basal disc of the 12 h polyp is only lightly mineralized (Fig. 4:*20*), and is composed of smaller, randomly oriented crystals (Fig. 4:*20, 21*). One also frequently finds non-mineralized "pockets" (Fig. 4:*21*). These "pockets" are found both in the basal plate layer and in the rudimentary septa formed immediately after settling.

Crystallography

X-ray diffraction of the powder of entire 18-day skeletons revealed only aragonite peaks (26.18° and 27.24° 2θ) (Fig. 5).

Whereas X-ray diffraction yields significant results, it remains possible to miss detection of trace amounts of calcite in a predominantly aragonitic deposit. Therefore, selected area electron-microdiffraction was utilized to examine specific regions of thin sections of larval skeletons.

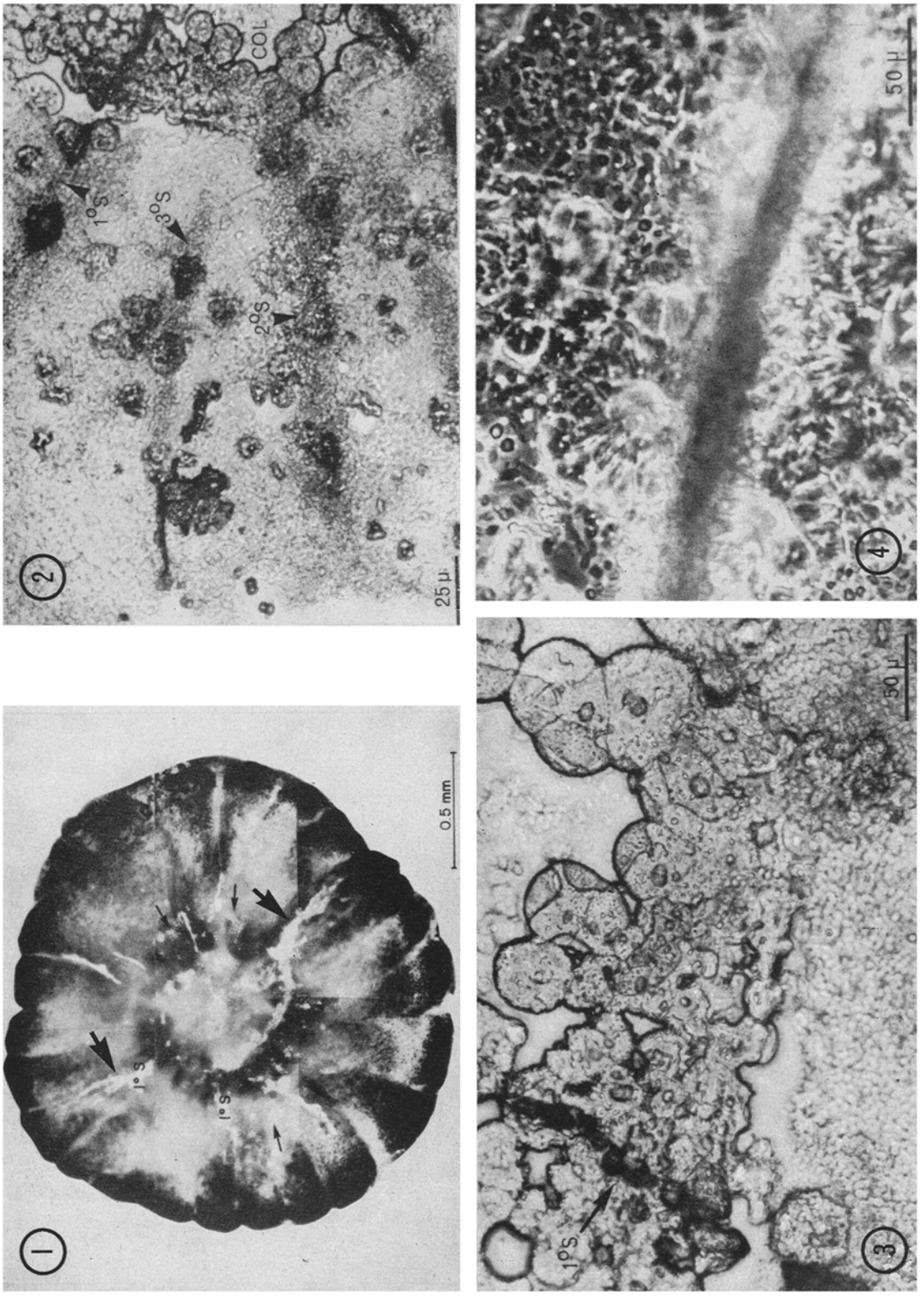

Fig. 1. *Pocillopora damicornis*. *1* Top view of 6 h settled planula larva. Specimen was fixed in glutaraldehyde and osmium tetroxide before examination with incident light (Leitz Ultraphot). Note underlying newly formed rudiments of primary septa (1° S, large arrows) and circular platelets (small arrows) visible through larval tissues. Primary septa are radially arrayed in hexa-coral pattern. *2* Sector of basal plate of 24 h skeleton, with central region on right, and basal plate periphery on left. Note circular platelets in columella region (COL). 1°, 2°, 3° S: primary, secondary, and tertiary septa. *3* Part of columella region (36 h skeleton), showing pavement of platelets, and adjacent accumulation of granules in interseptal areas. 1° S: primary septum. *4* Phase photomicrograph of primary septum (36 h stage) and interseptal areas composed of spherulitic platelets and smaller granules

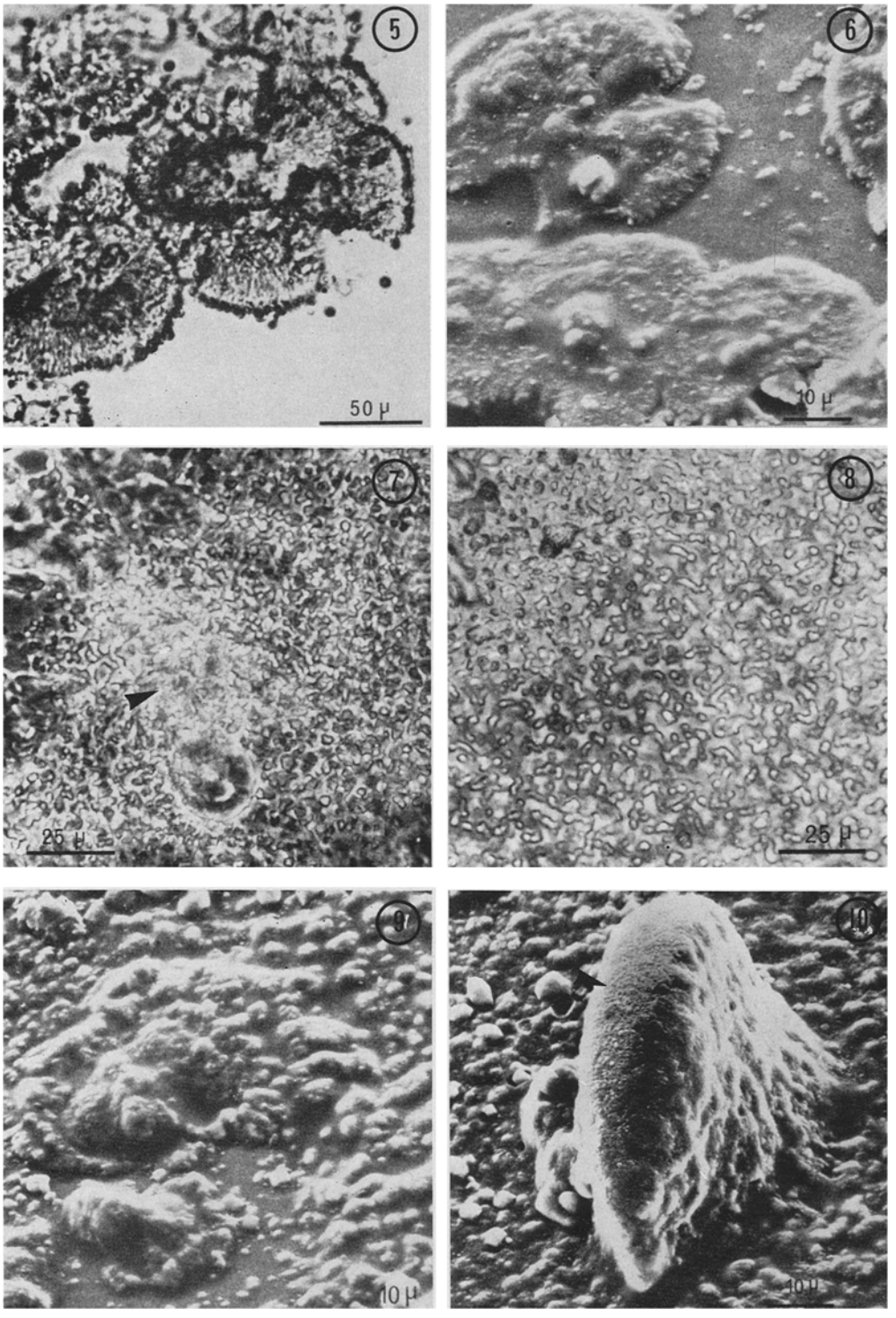

Fig. 2. *Pocillopora damicornis*. *5* Phase photomicrograph of platelets, showing spherulitic acicular substructure. *6* Scanning electron micrograph of circular platelet; note flattened appearance. *7* Growth point of a secondary septum (arrow), apparently consisting of local accumulation of small crystals or granules on larval basal disc (36 h stage). *8* Edge of 36 h basal plate, composed entirely of small granules. *9* Scanning electron micrograph of growth point of secondary septum in 48 h skeleton. *10* Primary septum rudiment in 48 h skeleton; note finely granular surface texture of septum

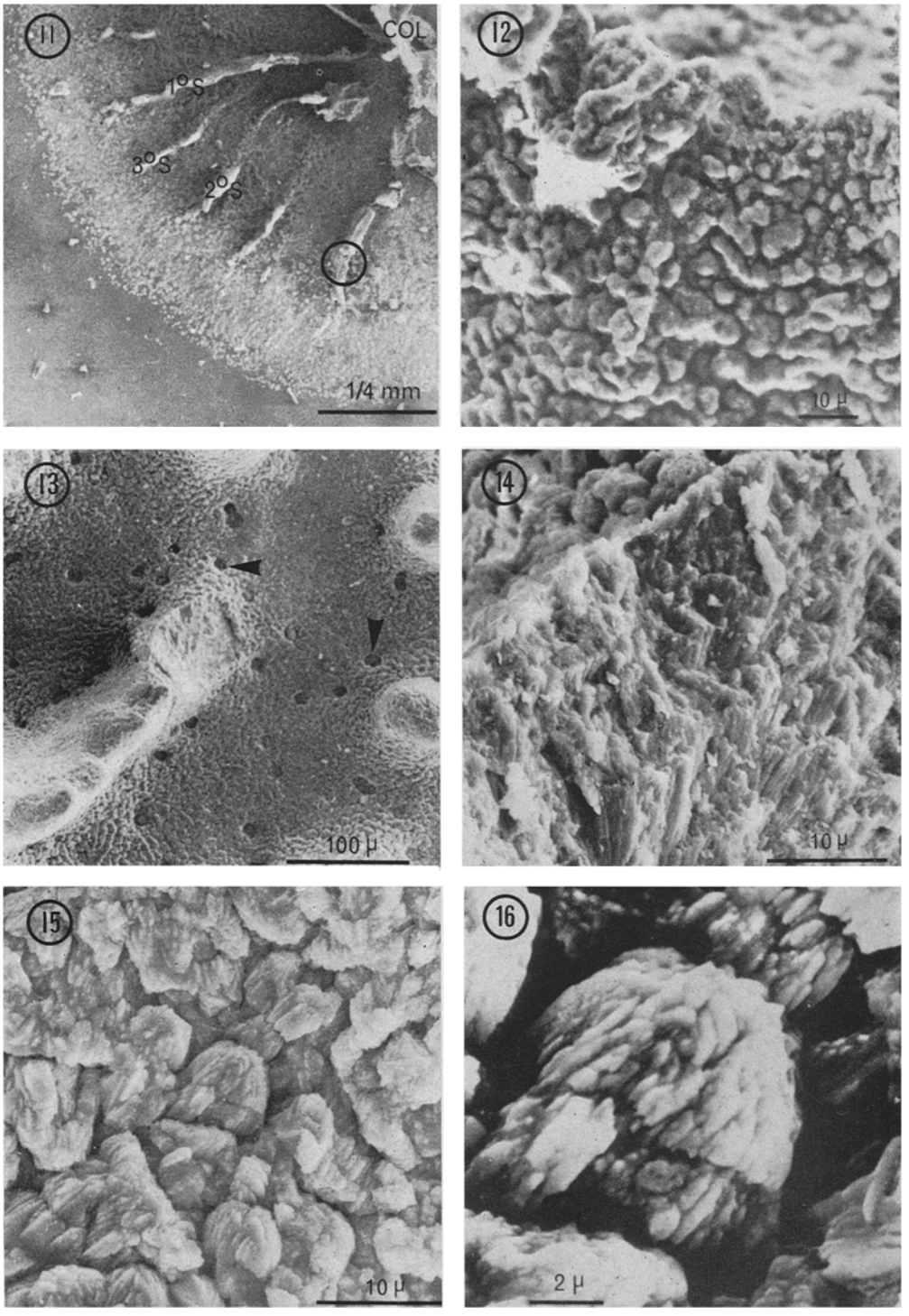

Fig. 3. *Pocillopora damicornis*. *11* Quadrant of 72 h skeleton. At this stage, columella in center of basal disc is fully formed. 1° S, 2° S, 3° S: septa; COL: columella. *12* Detail of primary septum circled in *11*. Note nodular surface texture, indicating adult-type fibrous skeletal growth. *13* Surface view of portion of 22-day skeleton, showing part of a septum and adjacent coenosteal spines. Surface pits (arrowed) probably represent skeletal attachment sites for specialized polyp attachment processes, the "desmoidal processes" (Wise, 1970; Vandermeulen, 1973b). *14* Detail of fractured septal tip in *13*, showing fibrous composition of skeleton (22-day stage). *15* Higher magnification of nodular surface of 22-day coral skeleton. *16* High magnification view of detail from *15*, showing polycrystalline composition of nodule

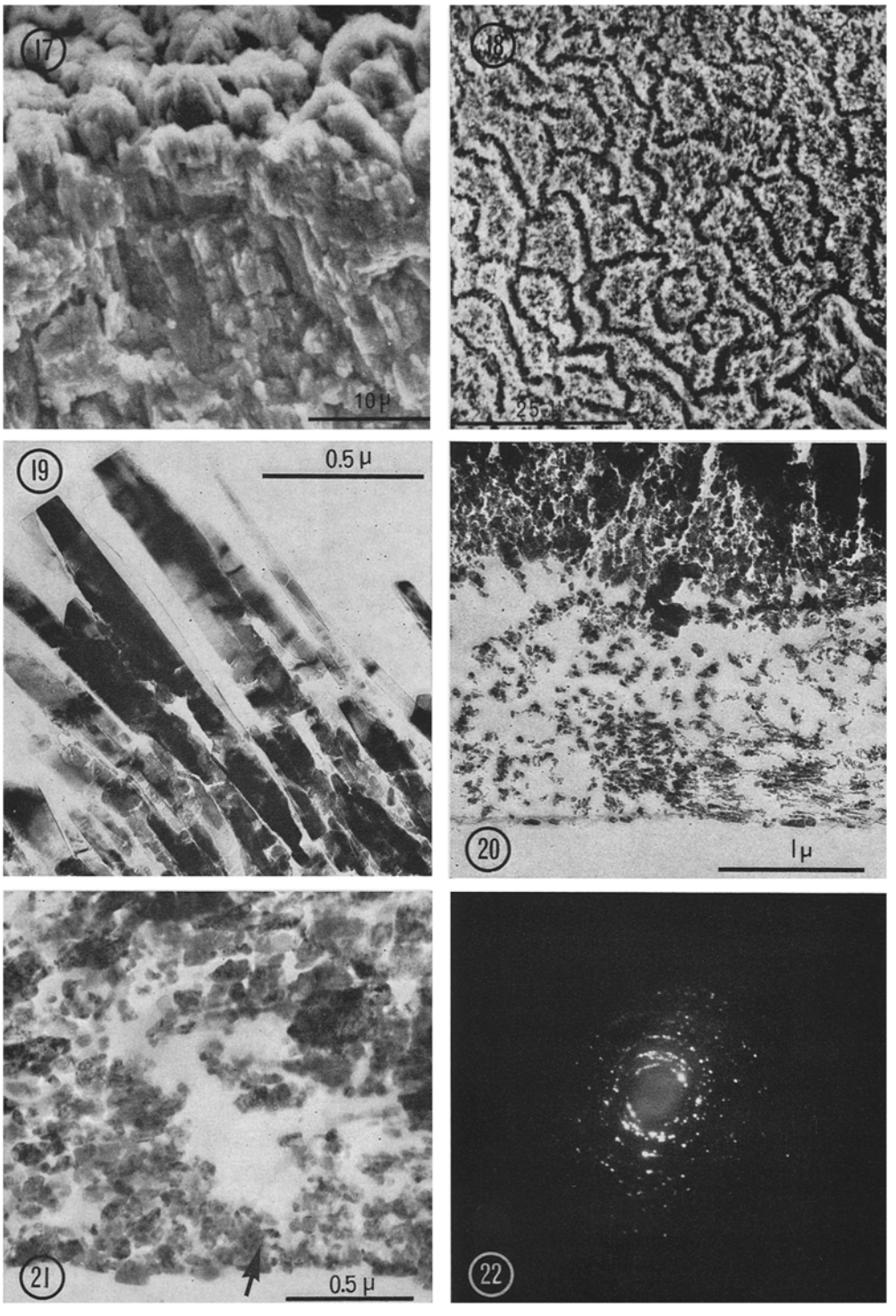

Fig. 4. *Pocillopora damicornis*. *17* Fracture through branch of adult, showing nodular surface features and underlying fibrous structure. *18* Etched transverse section through field of crystal fibers of common orientation; note variation in cross-sectional shapes of fibers (polished and formic acid-etched adult coral skeleton). *19* Transmission electron micrograph of adult coral crystal units. Crystals are oriented normal to surface of skeleton. Section was cut in direction transverse to long axis of crystals (22-day skeleton, Spurr embedded). *20* Section through basal region of 22-day skeleton. Lower, lightly calcified zone corresponds to larval calcification during initial 72 h following settling. The upper massively calcified material is subsequent adult (fibrous) growth. Sectioning direction from left to right. *21* Detail of basal, lightly calcified layer, showing non-calcified "pocket". Arrow points to crystals examined with electron diffraction. *22* Electron diffraction pattern obtained from crystals indicated in *21*. See Table 1 for D-spacing analysis

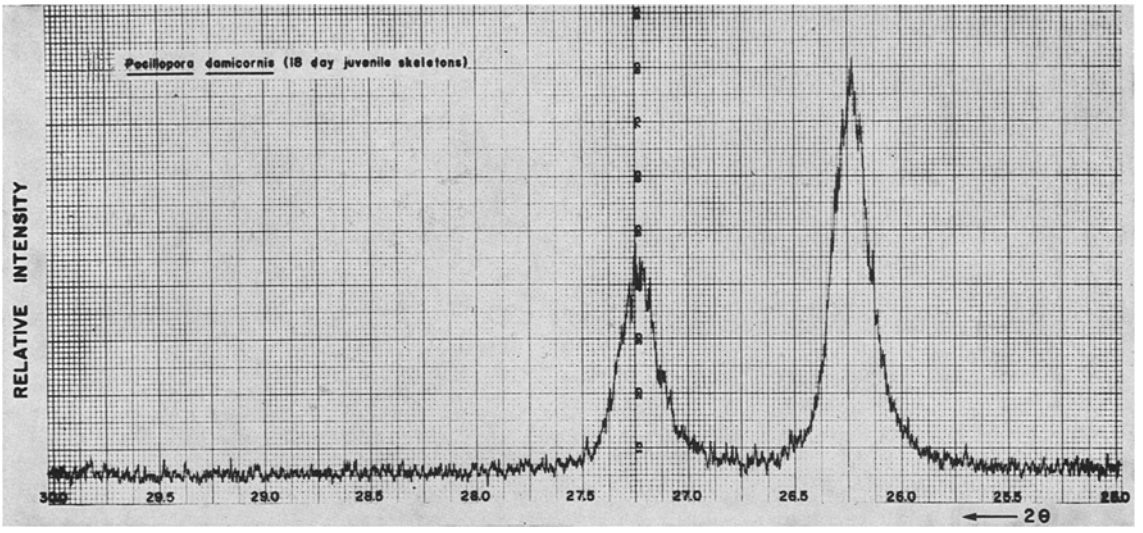

Fig. 5. *Pocillopora damicornis.* Powder X-ray diffraction pattern obtained from entire, powdered 18-day skeletons. Pattern includes only major peaks at 26.18° and 27.24° 2θ, characteristic of aragonite, and does not show the major peak for calcite at 29.36° 2θ

Table 1. *Pocillopora damicornis. D-spacings from selected area electron micro-diffraction pattern obtained from larval basal disc*

Basal disc	Aragonite[a]	Calcite[a]
4.25 A	4.21 A	3.86 A
3.38	3.40	
3.27	3.27	
3.00		3.04
2.87	2.87	2.85
2.74	2.73	
	2.70	
2.50	2.48	2.50
	2.34	
2.29	2.33	2.29
2.18	2.19	
2.09	2.11	2.10
1.98	1.98	
		1.92
1.90	1.88	1.88
1.81	1.81	
	1.76	
	1.74	
	1.73	
1.68	1.70	
1.63		1.63

[a] From ASTM powder diffraction file.

Patterns obtained in this manner from the heavily mineralized, i.e. adult, growth are consistent with those for aragonite. However, patterns obtained from the basal, lightly mineralized region show traces of calcite in addition to aragonite as the main form of calcium carbonate in the larval deposit. Such patterns, indicating a mixture of calcite and aragonite, were obtained from the central columellar region, the base of larval septa, and from interseptal regions (Fig. 4:*22*, Table 1), and were obtained both from the most basal crystals, and from crystals some distance from the settling surface.

Interpretation and Discussion

The two noteworthy points arising out of these observations are: (1) the new *Pocillopora damicornis* polyp, on settling, does not form a junior version of the adult skeleton (i.e., fibrous parallel growth, massive calcification), but proceeds via a true larval skeleton, differing in smaller crystal size, with random orientation, not grouped into bundles or fibres. In addition, it is lightly mineralized, and contains calcite as well as aragonite; (2) the larval skeletal septa are formed in precise order and position immediately following settling, suggesting pre-programming of skeleton information in the planktonic larval stage prior to settling.

The mechanism of nucleation during initial calcification is not known. Mineralization occurs in the micro-environment at the interface between the larval calicoblast epidermis and the underlying substrate. Nucleation, i.e., formation of calcium carbonate crystals of sufficient size to lead to further continued crys-

tal growth, can occur either on nucleation points in the surface of the substrate, or by biological i.e., larval means. If one eliminates the substrate as a potential source of nucleation sites, and there is in fact no evidence for doing so, then the alternative choice is that the settling larva can mediate nucleation in two ways: by providing calcareous seed nuclei or, alternatively, through secretion of some catalytic matrix. There is to date no firm evidence for the formation of calcareous particles, either intracellular or extracellular, which may act as seed nuclei (Vahl, 1966; Vandermeulen, 1973b). However, there are considerable data indicating intimate participation of an organic component in coral calcification (Wainwright, 1963; Muscatine and Cernichiari, 1969; Young, 1971, 1973; Young *et al.*, 1971), which is probably present also at the time of settling of the larva (Vandermeulen, 1973b).

The primary calcareous elements formed at settling and constituting the building blocks of the larval skeleton, and the flattened spherulitic platelets and rod-like granules, probably represent two distinct populations. No intermediate stages have been observed which might have suggested derivation of the larger platelets from the smaller granules. Fusion of these primary elements results in formation of the larval basal plate and its vertical structures, the septa and the columella.

No evidence has been found for the formation and consolidation of the larval skeletal basal plate through a succession of "concentric rings" (Barnes, 1971, p. 130), although this may be a feature unique to certain hexacoral genera. In *Pocillopora damicornis*, in fact, the most central part of the basal plate, the columella (a larval feature missing in the adult calyx) is the last of all basal structures to calcify completely. Although there is considerable variation in skeletal growth rates between specimens, in *P. damicornis* the normal sequence is that of initial deposition of septal rudiments, with subsequent filling in of interseptal regions by platelets and granules, their coalescence, and finally formation of the central columella.

That the basal disc differs from later growth in both degree of mineralization and in crystallography is not entirely unexpected. In bivalve shell formation, the crystal structure of the larval shell is frequently different from that of the adult shell (Kennedy *et al.*, 1969). Also, preliminary studies of larval shell formation in the oyster *Crassostrea virginica* show that the larval shell not only is less heavily mineralized than the adult shell, but differs also in crystallography and in mineral composition (Watabe, unpublished observations). This pattern of larval mineralization may well be more widespread than hitherto suspected.

With respect to the presence of trace amounts of calcite in the basal layer, the possibility of bacterial calcitic contamination can not be eliminated on the basis of these observations. However, the fact that calcite patterns were obtained from regions throughout the basal disc suggests that calcite is indeed a permanent feature of larval calcification, possibly attributable to non-organic (i.e., non-biologically mediated) physico-chemical precipitation in the super-saturated micro-environment created between the larval calicoblast epidermis and the substrate at time of settling.

Previous workers viewed the crystal fibers, characteristic of the adult skeleton, as vertical extensions of larval spherulites (Bryan and Hill, 1941; Vaughan and Wells, 1943; Barnes, 1970), formed either by vertical stacking of spherulites, or by the continuous elongation of the spherulite crystals in the vertical direction only. One should expect, therefore, some degree of correspondence between larval spherulite cross-sectional shape and diameter and those of the adult skeleton's crystal fiber. However, the size and shape of the spherulites, up to 50 μ in diameter, and approximately circular, argues against either scheme, the adult fibers being of much smaller diameter and of more varying cross-section. Rather, the formation of the adult crystal fiber is probably that outlined by Wainwright (1964), i.e., a random grouping of parallely oriented and closely apposed crystal units.

Skeletal growth in the adult probably proceeds simply by overgrowth of crystals on the skeletal surface. The alternative modes of skeletal growth are (1) episodic deposition[2] of pre-formed calcareous particles or structures, or (2) formation of a superficial lightly mineralized zone with subsequent "maturation" into the massive final product. To date, there is no evidence to support either of these alternatives. Rather, it appears likely that crystal growth occurs external to the tissues, possibly some distance from the calicoblast cells (ca. 5 to 10 μ, Vandermeulen, 1973b), on the face of the skeleton. It is probably a combination of simple physico-chemical precipitation, either onto existing crystal surfaces or onto an organic matrix and of cellular control of crystal growth and skeletal modelling through modulating production of the organic matrix and supply of calcium ions.

Summary

1. Formation of the skeleton of the stony reef-coral *Pocillopora damicornis* Lamarck is initiated immediately on settling of the planula larva.

2. The primary calcareous elements formed on settling are of two types — flattened spherulitic platelets, and smaller rod-like granules. The platelets form a flat pavement in the central columella region of the developing skeleton, and underlie the septal rudiments.

[2] This episodic crystal growth is not to be confused with the "episodic" formation of the coral tabula, the calcareous floor of the coral polyp which is fully formed at irregular intervals (Vaughan and Wells, 1943).

The smaller granules coalesce with the platelets to form a calcareous circular mat, the basal disc. These are also found in large numbers at the growing edge of the basal disc, and constitute the growth points of the secondary and tertiary septa.

3. The fibrous growth characterizing adult skeletal growth is not evident until approximately 72 h after settling.

4. The thin basal disc differs from later adult growth form in the following respects — it is only lightly mineralized, it is composed of smaller crystals which are more or less randomly oriented, and the deposit includes calcite in addition to aragonite. For these reasons, the basal disc formed during the first 48 to 72 h after settling constitutes a distinct larval skeleton.

5. The crystal units comprising the adult skeleton appear to be homogeneous, single crystals, lacking substructure, when viewed with the transmission electron microscope.

Acknowledgements. Much of this work represents part of a study by the senior author for the Ph. D. degree in biology at the University of California, Los Angeles. We acknowledge the generous aid of Dr. P. Helfrich and staff of the Hawaii Institute of Marine Biology; the invaluable assistance of Dr. J. W. Schopf and of Mrs. C. Lewis and L. Flesché, Department of Geology, UCLA; and the expert technical assistance of Mr. D. Dunkelberger, Electron Microscope Laboratory, University of South Carolina. Drs. H. Lowenstam, G. Chapman, and C. D. Curtis commented valuably during the course of this study. Special thanks to Dr. S. A. Wainwright, Duke University, for critical reading of the manuscript. Sustained, contributive advice and unfailing support of Dr. L. Muscatine, UCLA, is deeply appreciated. Research was supported by the following sources: NSF grant 11940 (L. Muscatine), NASA grant 9-9941 (J. W. Schopf), NIH grant 1R01-DE03424-01 (N. W.), and an Edwin Pauley Research Grant (J. H. V.). Manuscript preparation was supported by a Cocos Foundation post-doctoral fellowship to J. H. V.

Literature Cited

Abe, N.: Post-larval development of the coral *Fungia actiniformis* var. *palawensis* Doderlein. Palao trop. biol. Stn Stud. **1**, 73—93 (1937).

Atoda, K.: The larva and postlarval development of some reef-building corals. I. *Pocillopora damicornis cespitosa* (Dana). Scient. Rep. Tôhoku Univ. (4th Ser. Biol. **18**, 24—47 (1947).

Barnes, D. J.: Coral skeletons: an explanation of their growth and structure. Science, N.Y. **170**, 1305—1308 (1970).

— A study of growth, structure and form in modern coral skeletons. Ph. D. dissertation, University of Newcastle Upon Tyne 1971.

Bryan, W. H. and D. Hill: Spherulitic crystallization as a mechanism of skeletal growth in the hexacorals. Proc. R. Soc. Qd **52**, 78—91 (1941).

Duerden, J. E.: Recent results on the morphology and development of coral polyps. Smithson. misc. Collns **47**, 93—111 (1904).

Goreau, T. F.: The physiology of skeleton formation in corals. I. A method for measuring the rate of calcium deposition by corals under different conditions. Biol. Bull. mar. biol. Lab., Woods Hole **116**, 59—75 (1959).

Harrigan, J. F.: Behavior of the planula larva of the scleractinian coral *Pocillopora damicornis* (L.). Am. Zool. **12**, p. 723 (1972).

Kawaguti, S. and K. Sato: Electron microscopy on the polyp of staghorn corals with special reference to its skeleton formation. Biol. J. Okayama Univ. **14**, 87—98 (1968).

Kennedy, W. J., J. D. Taylor and A. Hall: Environmental and biological controls on bivalve shell mineralogy. Biol. Rev. **44**, 499—530 (1969).

Muscatine, L. and E. Cernichiari: Assimilation of photosynthetic products of zooxanthellae by a reef coral. Biol. Bull. mar. biol. Lab., Woods Hole **137**, 506—523 (1969).

National Bureau of Standards: Tables for conversion of X-ray diffraction angles to interplanar spacings. Appl. Math. Ser. **10** (1950).

Ogilvie, M. M.: Microscopic and systematic study of madreporarian types of corals. Phil. Trans. R. Soc. (Ser. B) **187**, 83—345 (1897).

Picken, L.: The organization of cells, 629 pp. Oxford: University Press 1960.

Reed, S. A.: Techniques for raising the planula larvae and newly settled polyps of *Pocillopora damicornis*. *In:* Experimental coelenterate biology, pp 66—72. Ed. by H. M. Lenhoff, L. Muscatine and L. V. Davis. Honolulu: University of Hawaii Press 1971.

Siegel, F. R.: Scleractinian corals: strontium and aragonite. Why? *In:* Caribb. geol. Confer. (V: Margarita, Venezuela, 1971). (In press). (1973).

Sorauf, J. E.: Microstructure an formation of dissepiments in the skeleton of the recent Scleractinia (hexacorals). ForschBer. Biomineral. Akad. Wiss. Lit., Mainz **2**, 1—22 (1970).

Stephenson, T. A.: Development and the formation of colonies in *Pocillopora* and *Porites*. Part I. Scient. Rep. Gt Barrier Reef Exped. **3**, 113—134 (1931).

Vahl, J.: Sublichtmikroskopische Untersuchungen der kristallinen Grundbauelemente und der matrix Beziehung zwischen Weichkörper und Skelett an *Caryophyllia* Lamarck 1801. Z. Morph. Ökol. Tiere **56**, 21—38 (1966).

Vandermeulen, J. H.: Studies on reef corals: II — fine structure of planktonic planula larva epidermis. Tissue Cell (1973a).

— Studies on reef corals: III — Fine structural changes in larva epidermis during settling and calcification. Tissue Cell (1973b). (In preparation).

— and L. Muscatine: Influence of symbiotic algae on calcification in reef coral: critique and progress report. *In:* Symbiosis in the sea, Vol. II. Ed. by W. B. Vernberg and F. J. Vernberg. University of South Carolina Press. (1973). (In press).

Vaughan, T. W. and J. W. Wells: Revision of the suborders, families, and genera of the Scleractinia. Spec. Pap. geol. Soc. Am. **44**, 1—363 (1943).

Von Heider, A.: Die Gattung *Cladocora* Ehrenberg. Sber. Akad. Wiss. Wien (1) **84**, 634—667 (1882).

Von Koch, G.: Über die Entwicklung des Kalkskelettes von *Asteroides calycularis* und dessen morphologische Bedeutung. Mitt. zool. Stn Neapel **3**, 284—292 (1882).

Wainwright, S. A.: Skeletal organization in the coral, *Pocillopora damicornis*. Q. Jl microsc. Sci. **104**, 169—183 (1963).

— Studies on the mineral phase of coral skeleton. Expl Cell Res. **34**, 213—230 (1964).

Wise, S. W., Jr.: Organization and formation of fasciculi in the skeletons of scleractinian corals. Bull. geol. Soc. Am. (Abstr.) **7**, p. 241 (1969).
— Scleractinian coral exoskeletons: surface microarchitecture and attachment scar patterns. Science, N.Y. **169**, 978—980 (1970).
Young, S. D.: Organic material from scleractinian coral skeletons. I. Variation in composition between several species. Comp. Biochem. Physiol. **40** B, 113—120 (1971).
— Calcification and synthesis of skeletal organic material in the coral, *Pocillopora damicornis* (L.). Comp. Biochem. Physiol. **44A**, 669—672 (1973).
—, J. D. O'Connor and L. Muscatine: Organic material from scleractinian coral skeletons. II. Incorporation of ^{14}C into protein, chitin and lipid. Comp. Biochem. Physiol. **40B**, 945—958 (1971).

First author's address: Dr. J. H. Vandermeulen
Fisheries Research Board of Canada
Marine Ecology Laboratory
Bedford Institute
Dartmouth, Nova Scotia
Canada

Date of final manuscript acceptance: July 13, 1973. Communicated by J. Bunt, Miami