nal SAMs. A large number of different organic functionalities should be compatible with this procedure. The last entry for Table 1 illustrates, however, one important current limitation of the technique. Although either hydrophobic or hydrophilic SAMs can be formed on gold, only hydrophobic SAMs can presently be formed on alumina: molecules $X(CH_2)_nY$ in which both X and Y are hydrophilic appear to adsorb with both polar functionalities bound at the alumina surface, and thus appear to form looped structures that are hydrophobic. This phenomenon occurs not only with alumina but seems to be common to metal oxides.

The technique of simultaneous formation of two different SAMs on a common, microlithographically prepared substrate exposing patterns of two different materials provides a new method for controlling and modifying the characteristics of surfaces. Since SAMs afford a high degree of control at the 2 Å scale perpendicular to the plane of the monolayer, and since lithography provides the ability to form lateral features having dimensions as small as 50 Å (*13*), the combination of the two offers a highly versatile protocol for the control of surface structure and properties.

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9. The gold substrate was prepared by evaporation of 100 Å of 99.99% chromium onto a polished Si(111) wafer followed by 2000 Å of 99.999% gold with the use of a cryogenically pumped E-beam evaporator operating at 10^{-6} torr.
10. For adsorbates only partially soluble in isooctane, substitution of anhydrous ethanol for isooctane yielded similar XPS and Auger results.
11. XPS spectra were obtained on a Surface Science Instruments spectrometer (Model SSX-100) operated at $<3 \times 10^{-9}$ torr with a 1-mm spot size.
12. Auger analysis was performed using a PHI 660 scanning Auger spectrometer operating at $<2 \times 10^{-9}$ torr. Mapping of SAMs proved to be extremely difficult at normal beam parameters. Although beam damage was minimized by using very low beam energies (0.5 to 3 nA, 8 to 15 kV), damage to some SAMs was so significant that element maps could only be obtained on the first pass.
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Evidence from Sediments of Long-Term *Acanthaster planci* Predation on Corals of the Great Barrier Reef

PETER D. WALBRAN, ROBERT A. HENDERSON, A. J. TIMOTHY JULL, M. JOHN HEAD

Since 1962 the crown-of-thorns starfish, *Acanthaster planci*, has caused the devastation of living coral in large tracts of the Great Barrier Reef, Australia. Some authorities view this as a modern phenomenon, resulting from ecological disturbance caused by man. Evidence from skeletal remains in sediment suggests that large *A. planci* populations have been part of the Great Barrier Reef ecosystem for at least 8000 years. Coral predation by *A. planci* is likely to have influenced the morphological fabric of the Great Barrier Reef in its post-glacial development and may also have influenced species richness of the reef biota.

THE CROWN-OF-THORNS STARFISH (*Acanthaster planci* L.) is a major predator of hermatypic scleractinian corals, particularly in the western Pacific region (*1, 2*). Its population dynamics are considered to be cyclic (*3*) with low population densities alternating with dramatic population outbreaks that result in massive damage to the living coral cover of individual reefs and reef tracts (*4, 5*). The Great Barrier Reef has experienced two documented contemporary cycles, with outbreaks affecting reefs between Princess Charlotte Bay and the Swain Reefs Complex in the periods 1962 to 1977 and 1979 to 1986 (*6, 7*). It is thought that outbreaks occur first in the Cairns section of the Great Barrier Reef with reefs further south becoming progressively infested (*6*). Extensive regional surveys undertaken during 1985–86 indicated that, of 228 reefs surveyed in the Great Barrier Reef Province, approximately 27% had been affected by *A. planci* in the outbreaks of the present cycle (*7*).

The temporal context of the present crown-of-thorns predation cycles is a contentious issue. Some investigators argue that the cycles are a contemporary phenomenon directly attributable to interference by man in reefal ecosystems (*8*) thereby raising a major conservation issue in Australia and elsewhere. A converse view is that crown-of-thorn outbreaks represent an enduring ecological pattern that escaped notice until recently (*9*). The antiquity of *A. planci* outbreaks in the Great Barrier Reef is an important question to be answered.

Acanthaster planci contains a complex skeleton of small, arm-supporting ossicles and protective spines made of calcite. After death, these skeletal elements accumulate with reef sand as a recoverable fossil record. They are distinctive in color, shape, and microtexture and can be distinguished with certainty from other carbonate grains (*10*). Although the potential of this record in assessing the temporal distribution of *A.*

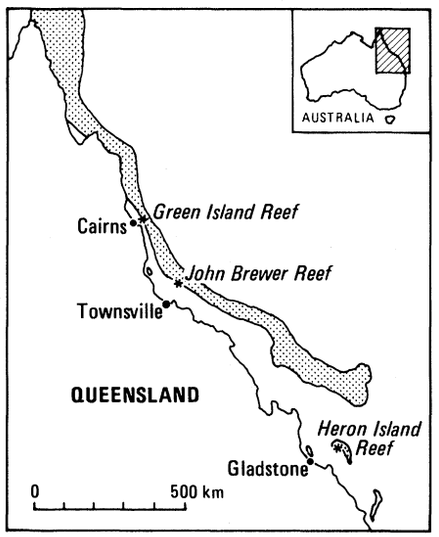

Fig. 1. Location of Great Barrier Reef sites examined for *A. planci* skeletal elements in the sediment record.

P. D. Walbran and R. A. Henderson, Geology Department, James Cook University of North Queensland, Townsville, Queensland, 4811, Australia.
A. J. T. Jull, NSF Accelerator Facility for Radioisotope Analysis, University of Arizona, Tucson, Arizona 85721.
M. J. Head, Radiocarbon Dating Research Laboratory, Australian National University, G.P.O. Box 4, Canberra, A.C.T., 2601, Australia.

Table 1. Recovery of *A. planci* skeletal elements in sediment samples from John Brewer, Green Island, and Heron Island reefs. Note that surface samples (1 kg) were four times as large as subsurface samples (250 g).

Reef	Number of samples	Samples without elements	Average elements per sample
John Brewer			
Surface	59	17	11.2 ± 21.5
Subsurface	663	288	1.3 ± 1.6
Green Island			
Surface	46	2	21.7 ± 23.5
Subsurface	592	84	3.3 ± 2.7
Heron Island			
Surface	55	53	0.04

planci has been recognized for some time (*11*) the results of earlier work have been regarded as inconclusive (*12*).

Our initial approach has been to count *A. planci* skeletal elements in surface sediment samples from reefs known to have experienced contemporary outbreaks. These results were compared with samples from a control reef for which population densities of the starfish are known to have been consistently low for several decades. Green Island and John Brewer reefs (Fig. 1) have both experienced two major episodes of crown-of-thorns predation since 1962 (*3*). Heron Island Reef (Fig. 1) has maintained a low-density population for at least 35 years, ever since routine ecological surveys first began (*13*).

Summary results of *A. planci* skeletal element contents in the ≥0.5-mm size fraction of 1-kg surface sediment samples from widely scattered localities on these three reefs are given in Table 1. They show that outbreaks are marked by the contribution of a significant number of skeletal elements to the surface sediment on Green Island and John Brewer reefs. In contrast, only low numbers of skeletal elements were found in surface sediment from Heron Island Reef, where our sample suite was representative of shallow-water environments across all sectors of the reef. That the skeletal elements in these surface sediments generally represent the remains of contemporary *A. planci* populations has been confirmed by radiocarbon dating of groups of elements drawn from individual surface sediment samples (*14*).

Having established a relation between outbreak events and the contribution of skeletal elements to surface sediment, we next examined subsurface sediment from Green Island and John Brewer reefs. Subsurface sediment samples were obtained by taking cores of reefal sediment bodies in water depths ranging from 1 to 39 m. Paired replicate cores, 76 mm in diameter and ranging in length from 1.5 to 4 m, were obtained from six sites on Green Island Reef and from seven sites on John Brewer Reef. Each core was split longitudinally and one-half divided into 250-g samples, each representing 8 to 10 cm of core length. The ≥0.5-mm size fraction of each sample was then counted for *A. planci* skeletal elements. Replicate cores from individual shallow-water sites yielded closely comparable patterns, such that element counts from the replicate pairs may be combined to give a generalized distribution of elements with respect to depth in the sediment pile at individual sites (Fig. 2). Summary results for samples obtained from all cores are presented in Table 1.

The number of *A. planci* skeletal elements in ancient, subsurface sediment obtained from Green Island and John Brewer reefs is comparable with that recovered from surface sediment at these localities. We conclude that substantial populations of *A. planci* have had a long history on Green Island and John Brewer reefs and that past patterns are likely to have been similar to those presently observed.

Detailed stratigraphic interpretation of the *A. planci* record within the cores is complicated by biogenic sediment recycling processes. In particular, callianassid shrimps are ubiquitous in shallow-water sand-grade sediment of the Great Barrier Reef. They are known to burrow deeply and to recycle substantial quantities of sediment, commonly resulting in a closely spaced pattern of seafloor mounds up to 30 cm in height (*15*). The detailed stratigraphic integrity of shallow water reef sediment is almost certainly impaired because of such biogenic activity.

Radiogenic carbon dating of bulk sediment samples from all cored sediment bodies shows an ordered age structure (*14*). We consider the age structure to be depositional on the basis that postdepositional biogenic movement of individual grains is generally random rather than vectored. Accelerator mass spectrometer ages for individual *A. planci* skeletal elements show little relation to ages obtained by bulk-dating associated sed-

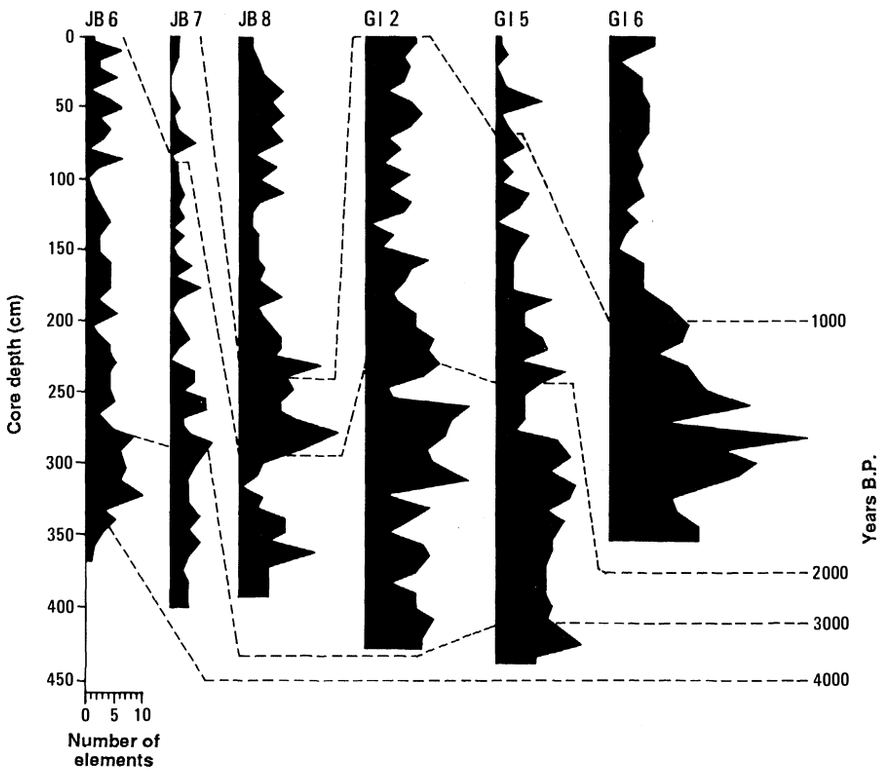

Fig. 2. Down-core distribution of *A. planci* skeletal elements at sites on John Brewer and Green Island reefs. Each graph represents pooled data from replicate core pairs. Age calibration is based on five to eight individual dates obtained from bulk sediment samples spaced down one core from each site.

iment, or to within-core stratigraphic position (Table 2). These data conform with conclusions drawn from biological reworking of the sediment pile. The down-core distribution of *A. planci* cannot be interpreted in detail to individual, short-period outbreak events that currently are less than 5 years in duration, or to large-scale cycles of outbreak events if such have occurred. We do note, however, that core intervals with indicated ages exceeding some 1000 to 2000 years (Fig. 2) are significantly richer in *A. planci* skeletal elements than core intervals of younger age. This coarse pattern suggests enhanced *A. planci* abundance on both Green Island and John Brewer reefs at a much earlier period in their history in comparison with that at present.

The activity of callianassid shrimp is largely limited to water depths of less than 20 m (*15*). Accordingly, the stratigraphic record of sediment bodies deposited in water depths exceeding that offers a much higher degree of reliability. We have obtained cores from the well-developed back-reef sediment apron in the lee of John Brewer Reef in water depths of more than 30 m. The best of these, in terms of *A. planci* element recovery, shows consistent down-core distribution of elements below 80 cm, and the age structure, determined by dating of bulk sediment horizons spaced down the core, suggests a high level of stratigraphic integrity (Fig. 3). We interpret this core to indicate the presence of *A. planci* on John Brewer Reef, viewed in terms of time units of approximately 250 years, for an enduring period extending back to 8000 years before present (B.P.). Given that Holocene reef growth first commenced in the Great Barrier Reef some 9000 years ago (*17*), *A. planci* predation has probably been an aspect of Holocene reef ecology from its inception.

Data on coral recovery following major predation events for some reefs in the Great Barrier Reef indicate a period of 10 years before coral cover is substantially restored (*5*). Given the high rates of degradation known to apply to exposed carbonate skeletons in coral reef environments generally (*18*), it might be thought that long-term *A. planci* predation should have accelerated the production of detrital sediment relative to reef framework and thereby changed the constructional mode of reefs so affected.

Data for the Great Barrier Reef indicate that most reef flats had equilibrated to present sea level by 4000 years B.P. (*17*). Subsequently, framework construction has been almost exactly balanced by degradation to detrital sediment. Lateral accretion of framework has been constrained by lagoonal sediment on the inner side of reef flats and by steep slopes on the outside, descending to water depths where framework construction cannot prosper. Although an agent of framework degradation, *A. planci* in the present regime does not induce a process that would otherwise not occur.

Tracts of submerged reef, fossil in the sense that their framework is no longer actively accreting, are widespread in the Great Barrier Reef and are thought to date from the early Holocene, prior to sea level reaching its present level 6500 years ago (*19*). *Acanthaster planci* predation may have been a factor in suppressing coral growth on such tracts during the period 9000 and 7000 years ago when sea level is thought to have been rising about 9 mm per year (*20*) and the capacity of framework growth to keep pace was under stress. Episodic growth in reef framework during this period has been noted for a number of elements within the Great Barrier Reef (*17, 21*), to which *A. planci* predation may have contributed.

Short-term catastrophic disruption of tracts of the Great Barrier Reef due to *A. planci* outbreaks is well recognized (*8*). Viewed in the context of a longer time frame as suggested here, *A. planci* predation is likely to have exerted a major influence on the ecological setting of the Great Barrier Reef for most of the Holocene and perhaps longer.

It has been argued that species richness may result from both stable ecosystems and those subject to repeated disturbance with quite separate mechanisms maintaining diversity in each case (*22*). From the observed pattern of *A. planci* predation in the Great Barrier Reef, it appears that some individual reef tracts experience severe cyclical preda-

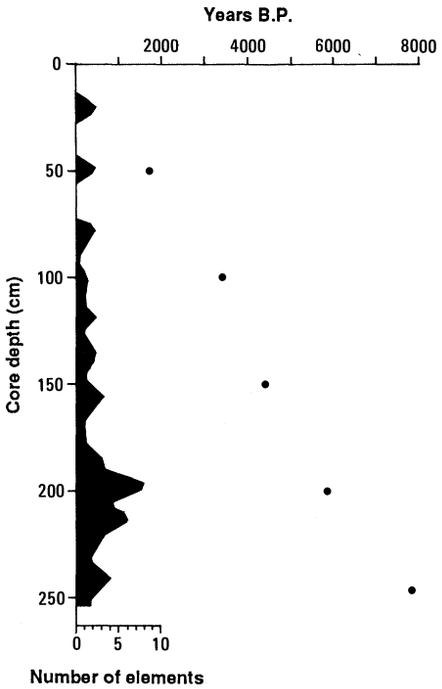

Fig. 3. Distribution of *A. planci* skeletal elements obtained from a single core taken at John Brewer leeward slope site 13, in a water depth of 39 m. Also shown are radiocarbon dates determined from bulk sediment samples spaced down the core.

Table 2. Accelerator mass spectrometer dates (prefix AA to sample number) for individual *A. planci* skeletal elements obtained from subsurface sediment and subjacent bulk sediment dates (prefix ANU to sample number). Real ages represent conventional ages adjusted for minor peculiarities of ^{14}C systematics that are specific to nearshore Australian marine environments (*16*). Error limits reflect the analytical precision in the measurement of isotope ratios for individual samples.

Core number	Core interval (cm)	Carbon-14 sample	Conventional age	Real age
JB6c	149.0–153.0	ANU-5865	2790 ± 80	2340 ± 90
	169.0–177.0	AA2918	3155 ± 140	2705 ± 145
	169.0–177.0	AA2919	4050 ± 160	3600 ± 165
	169.0–177.0	AA2920	4290 ± 160	3840 ± 165
	221.0–223.0	ANU-5866	3070 ± 80	2620 ± 90
JB8b	236.0–242.5	ANU-5655	1420 ± 70	970 ± 80
	243.0–251.5	AA2921	1710 ± 145	1260 ± 150
	243.0–251.5	AA2922	2170 ± 145	1720 ± 150
	243.0–251.5	AA2923	3075 ± 190	2625 ± 195
	251.5–259.0	ANU-5474	1780 ± 60	1330 ± 70
GI6a	198.0–200.0	AA2927	2035 ± 105	1585 ± 110
	198.0–200.0	AA2933	1735 ± 145	1285 ± 150
	200.0–202.0	AA2928	1515 ± 120	1065 ± 125
	200.0–202.0	AA2929	1955 ± 105	1505 ± 110
	208.0–212.0	ANU-6561	1620 ± 70	1170 ± 80
	216.0–218.0	AA2930	1965 ± 120	1515 ± 125
	218.0–220.0	AA2931	1815 ± 130	1365 ± 130
	218.0–220.0	AA2932	1485 ± 105	1035 ± 110
GI6b	213.0–215.0	ANU-5478	1380 ± 70	930 ± 80
	215.0–217.0	AA2924	980 ± 530	530 ± 530*
	215.0–217.0	AA2925	2055 ± 120	1605 ± 125
	217.0–224.5	AA2926	1535 ± 130	1085 ± 135

*Unreliable date.

tion and massive ecological disturbance whereas others remain untouched. Long-term existence of reefal enclaves with stable community structure intermingled with others subject to disequilibrium and repeating patterns of ecological succession should have important evolutionary consequences. Induction of a biotic province with an enhanced capacity for speciation and the generation of unusually high diversity levels should result.

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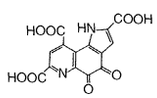
Nutritional Importance of Pyrroloquinoline Quinone

JOHN KILLGORE, CARSTEN SMIDT, LISA DUICH, NADIA ROMERO-CHAPMAN, DONALD TINKER, KAREN REISER, MARIA MELKO, DALLAS HYDE, ROBERT B. RUCKER*

Mice fed a chemically defined diet devoid of pyrroloquinoline quinone (PQQ) grew poorly, failed to reproduce, and became osteolathyritic. Moreover, severely affected mice had friable skin, skin collagen that was readily extractable into neutral salt solutions, and decreased lysyl oxidase. The identification of functional defects in connective tissue and the growth retardation associated with PQQ deprivation suggest that PQQ plays a fundamental role as a growth factor or vitamin.

THE QUINOPROTEINS WERE RECOGnized in the late 1970s as a novel class of bacterial oxidoreductases that utilize PQQ (or Methoxatin) as a cofactor (*1*). PQQ also functions as a cofactor for important plant and animal enzymes (*2*). It is present in a number of common foods and food components (*3*), and it is a product of fermentations (*4*). Such observations raise questions regarding its metabolism and possible role as an essential dietary factor or vitamin.



The inhibition of lysyl oxidase, which in mammals requires PQQ, leads to osteolathyrism characterized by decreased cross-linking of collagen and elastin (*5*). We show that PQQ deprivation causes decreased levels of lysyl oxidase in mice, which results in mice that are lathyritic, grow poorly, and fail to reproduce. Although severe measures are required to render animals deficient in PQQ, deprivation can be achieved with an experimental protocol (*6*) similar to that used to produce deficiencies of essential ultratrace minerals or vitamins.

When PQQ was omitted from a chemically defined diet containing antibiotics (*6*), growth impairment was observed (Fig. 1). A normal growth response was observed when PQQ was added to the deficient diet, or whenever PQQ was detectable in the fecal samples or as a diet or water contaminant. The latter often occurred when resistance was developed to the succinyl sulfathiozole that was added to inhibit growth of intestinal microflora.

About 20 to 30% of mice, for which no PQQ was detectable in food or excreta, had clear external signs from the PQQ deprivation. These signs included friable skin, mild alopecia, and a hunched posture. The signs were occasionally quite severe (Fig. 2). Of a total of 40 mice assigned to PQQ-deficient diets (maternal dams plus the offspring in four separate litters), eight died by week 8 of deprivation. Three of these deaths were due to an aortic aneurysm or abdominal hemorrhages. Diverticuli were also common in the deficient animals. In contrast, only one of the mice in PQQ-supplemented groups (33 total) died during the course of the experiments.

A striking response was the friability of skin in those mice that appeared most affected by dietary PQQ deprivation. The response suggested that decreased maturation or deposition of skin collagen was a component of the lesion. Indeed, collagen extractability (*7*), a measure of collagen maturation and cross-linking, was increased abnormally in skin from mice fed deficient diets. The total amount of collagen extracted from skin of PQQ-deprived mice was about twice that of supplemented mice (*7*). Although it was not possible to confirm unequivocally that the increase in collagen extractability was due entirely to reduced amounts of cross-linking amino acids, a significant reduction occurred in the tissue levels of lysyl oxidase (*8*). PQQ deprivation caused a net decrease in lysyl oxidase to 10 to 30% of the normal values for skin, as estimated by an enzyme-linked immunosorption assay (*8*).

In addition, attempts to breed young female mice, which were fed the PQQ-deficient diet for 8 to 9 weeks, resulted in either no litters or in pups that were immediately cannibalized at birth. Such responses or behaviors were not observed for female mice fed the supplemented diet; greater than

J. Killgore, C. Smidt, L. Duich, N. Romero-Chapman, D. Tinker, M. Melko, Department of Nutrition, School of Agricultural and Environmental Sciences (AES), University of California, Davis, CA 95616.
K. Reiser, Division of Pulmonary Medicine, School of Medicine, University of California, Davis, CA 95616.
D. Hyde, Department of Anatomy, School of Veterinary Medicine, University of California, Davis, CA 95616.
R. B. Rucker, Departments of Nutrition (AES) and Biological Chemistry (School of Medicine), University of California, Davis, CA 95616.

*To whom correspondence should be addressed.