Table I

Antimicrobial activity of accessory nidamental gland (ANG), egg cases, and bacterial isolates from Loligo pealei

	ANG	Egg cases	Str. Sq2 #1E, #2E	Str. Ro1 #3E, #4E	Str. 303 #5E, #6E, #8E, #9E	#7E
Escherichia coli	−	−	−	+	−	±
Staphylococcus aureus	−	−	±	−	−	++
Streptomyces griseus	++	−	−	++	−	+++
Vibrio anguillarum	+	−	+	+	−	+++
Aeromonas salmonicida	+	−	+	+	−	++
Laegenidium myophilum	nd	nd	+	+++	−	nd

− = negative; ± = slight activity (<0.5 mm); + = weak activity (<2.5 mm); ++ = good activity (2.5–5 mm); +++ = strong activity (5–15 mm); nd = not determined.

ture supernatants after 4 days of fermentation at room temperature, and concentrated by evaporation under nitrogen flux. Butanol-extracted homogenate from both fresh ANG and egg case jelly membranes was also tested in all assays.

Phenotypical analysis showed nine types of colonies (#1E to #9E). RFLP grouped these colonies as follows: *Shewanella*-like, #1E and #2E; *Alteromonas*-like, #3E and #4E; *Roseobacter*-like, #5E and #6E. The other three colonies showed a different RFLP pattern. Results obtained from the antimicrobial activity assay are presented in the Table I.

Considering that one or more antimicrobial metabolites could be induced under nutritional stress, we also tried a butanol extraction of nutrient-limited growth media, following methods described elsewhere (6). Only Ro1, grown on marine broth diluted (1:9) plus 0.1% ANG, inhibited growth of the marine fungus *L. myophilum* (up to 5 mm).

The antifungal activity from *Alteromonas* Str. Ro1 suggests that an activity similar to that previously demonstrated in the eggs of shrimp (7) could also occur in the squid. Further studies are planned using more concentrated extracts and larger numbers of potential pathogens to better understand mechanisms of how the bacterial symbionts of the squid ANG could affect their microhabitat, competitors, and predators.

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Coral Bleaching on Johnston Atoll, Central Pacific Ocean

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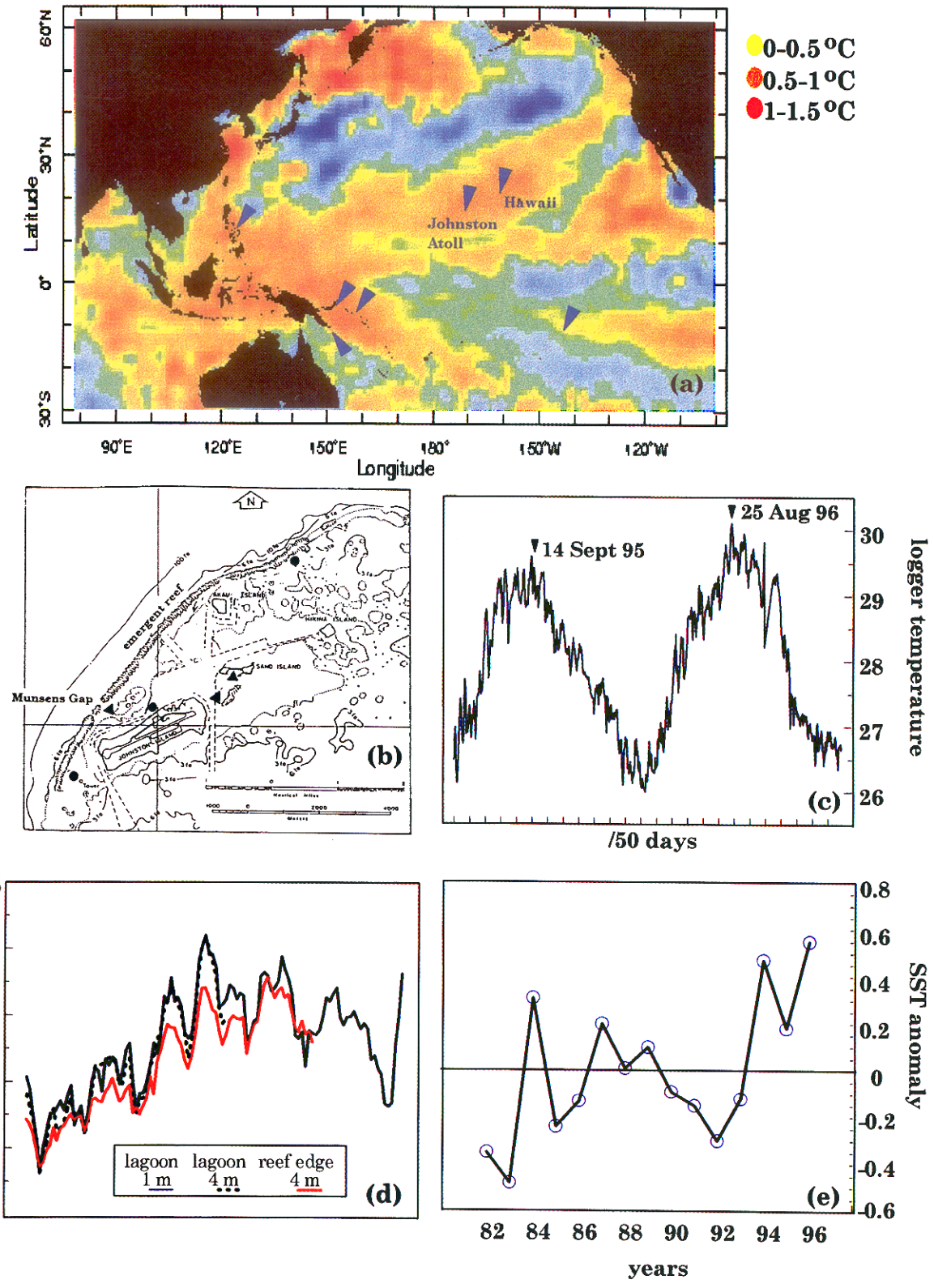
On 10 September 1996, extensive coral bleaching was noted on Johnston Atoll (JA), an isolated coral reef ecosystem in the central Pacific Ocean (16°N, 169°W). Between September 1996 and March 1997 we monitored the nature and extent of the bleaching, as well as the anomalous conditions of ocean temperature.

Coral "bleaching," or the loss of zooxanthellae and their photosynthetic pigments, is one of the first visible signs of thermal stress (1). The association between mass reef bleaching, and subsequent coral mortality, with elevated ocean temperatures is of concern in light of predicted global temperature increases over the next century (2). Mass bleaching is most often associated with anomalous ocean temperatures during the warmest month of the year. A temperature increase of 1°–2°C above the historical mean summer maximum is considered necessary to induce coral bleaching in tropical and subtropical environments (1).

The 1996 JA bleaching event did not occur in isolation but appears to have been part of a global-scale bleaching episode that began in the western Caribbean and Gulf of Mexico in the summer of 1995 and was observed at several sites in the western and central Pacific the following year (3) (Fig. 1a). Satellite images indicate a basin-wide sea surface temperature anomaly (SSTA) of between 0.5°C and 1.5°C in September 1996 (4),


0-0.5 °C
0.5-1 °C
1-1.5 °C
Hawaii
Johnston Atoll
(a)
Latitude
Longitude
60°N
30°N
0°
30°S
90°E
120°E
150°E
180°
150°W
120°W
Munsens Gap
emergent reef
(b)
25 Aug 96
14 Sept 95
(c)
logger temperature
/50 days
lagoon 1 m
lagoon 4 m
reef edge 4 m
(d)
(e)
SST anomaly
years

coincident with reports of coral bleaching on the Hawaiian Islands and on JA (Fig. 1a).

On JA, we examined six lagoonal and reef-edge sites to depths of 5 m (Fig. 1b) during three field excursions: 2–4 October, 21 October–5 November 1996, and 4–16 March 1997. Affected corals were tagged and photographed at one lagoonal site to monitor recovery rates. We distinguished between colonies that were fully bleached and those that showed partial bleaching, *i.e.,* bleaching confined to localized regions on a single colony. Our observations were as follows:

a. Bleaching was confined to corals in lagoonal sites (Fig. 1b). No bleaching occurred along the emergent reef with the exception of one bleached colony (*Pocillopora meandrina*) noted on the inside of the eastern reef edge.

b. Bleaching was species-specific. All *Montipora* spp. and *Pocillopora* spp. were affected, although the degree of bleaching of individual colonies varied from completely unaffected to partially bleached to complete loss of skeletal pigment. Bleaching was not observed in *Acropora cytherea,* the dominant coral species on JA.

c. Tissue loss from affected colonies did not occur during the first 3 weeks after the bleaching was noted, *i.e.,* 10 September–4 October 1996. Tissue loss was noted for several bleached *Pocillopora* colonies in late October. On the contrary, bleached *Montipora* colonies maintained living polyps for the duration of the bleaching event.

d. We estimated the areal extent of the bleaching in lagoonal sites to be from 15% to 20% between 0 to 5 m depth. Bleached corals were observed to a depth of 5 m.

e. By March 1997, 50% of the affected, tagged colonies had made a full recovery and regained pigment. Recovery was unrelated to the degree of bleaching experienced by individual colonies but was to some degree species-specific in that *Pocillopora* colonies without tissue were eventually overgrown by algae.

Daily temperatures at two lagoonal sites and one reef-edge site were recorded using temperature loggers (Brancker Instruments) (Fig. 1b, c, d). The reef-edge site is a 6-m-deep channel (referred to as Munsens Gap; Fig. 1b) in the emergent reef structure. We regard temperatures recorded at this site to be representative of open-ocean mixed layer temperatures. *In-situ* temperature records in combination with IGOSS NMC satellite-derived SSTs (4) (Fig. 1e) allow us to make the following observations:

a. Temperature loggers recorded a maximum summer lagoon temperature of 31.1°C on 25 August 1996, compared with a maximum summer temperature of 29.7°C in the previous year (14 September 1995) (Fig. 1c).

b. Average daily temperatures at the reef edge were 0.2°C lower than those recorded in the lagoon between 3 July and 21 October 1996. The maximum recorded SST at Munsens Gap was 29.8°C, and the maximum recorded difference between reef-edge and lagoonal sites was 0.4°C in late August 1996 (Fig. 1d).

c. Satellite-derived summer (JAS) SSTAs for a 1° × 1°C grid square centered on 16.5°N, 169.5°W indicate an anomaly of 0.6°C in 1996 (compared with the historical mean since 1982) (Fig. 1e).

In timing, nature, and extent—including the species affected, tissue loss, and rate of recovery—the bleaching episode on JA was very similar to the one that was observed on the Hawaiian Islands in the same year (3; Paul Jokiel, University of Hawaii, pers. comm.) and that was predicted on the basis of laboratory manipulations of temperature (5, 6). However, whereas temperatures between 28°C and 29°C are sufficient to induce bleaching of *Montipora* spp. and *Pocillopora* spp. on Hawaii (1), congenerics on JA appear tolerant of temperatures up to 29.8°C. Although the exact date when bleaching first occurred was not documented, the distribution of bleaching across the atoll, the timing of SST maxima in the lagoon, and the timing of the first observation of extensive bleaching lead us to conclude that the most sensitive coral species on JA have an upper thermal limit of about 30°C. This apparent difference in coral thermal tolerances between Hawaii and JA corresponds to differences in the maximum summer temperatures between these two sites.

Although *in situ* temperature data enable us to estimate the upper thermal limit of the affected JA coral species, the time series are too short to determine how high this limit is above normal summer SST maxima. The longer satellite-derived temperature record shows an anomaly of 0.6°C during the summer of 1996; according to field and laboratory observations, this anomaly is not high enough to induce coral bleaching by temperature alone (1, 5, 6). However, in assessing whether temperature was the sole cause of the JA coral bleaching event or whether other factors were involved, it is important to recognize the spatial resolution over which satellite-derived SSTs are averaged (1° × 1°). Satellite temperatures are therefore representative of relatively large-scale open-ocean conditions and do not distinguish fine-scale temperature variability across the atoll. Our logger data indicate that small but important differences in SST occurred between sites on JA. SSTs in the lagoon, where bleaching occurred, were up to 0.4°C higher than those at the reef edge, where bleaching did not occur. Thus, we deduce that lagoonal temperatures were at least 1°C higher than the long-term ambient summer SST, which is in good agreement with that predicted to induce coral bleaching (1, 5, 6).

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Figure 1. *Sea surface temperatures (SSTs) for the Pacific basin and for Johnston Atoll during the 1996 bleaching event. (a) Satellite-derived SST anomalies across the Pacific basin during September 1996 (Lamont-Doherty Earth Observatory Climate Data Catalog—ref 4). Arrows indicate sites where coral bleaching was reported to NOAA's Coral Health and Monitoring Program bleaching website during 1996 (3). (b) Map of Johnston Atoll showing monitored reef and lagoonal sites. Triangles denote sites where temperature loggers were deployed. (c) Daily SSTs at 1 m depth in Johnston Atoll lagoon: May 1995–February 1997. (d) Daily temperatures between 1 m and 5 m depth recorded at lagoonal and reef-edge sites on Johnston Atoll: 3 July 1996–31 October 1996. (e) Average summer (JAS) satellite-derived SSTs for a 1° × 1° grid, centered at 169.5°W 16.5°N: 1982–1996 (4).*

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PCB Contamination Relative to Age for a Pacific Damselfish, *Abudefduf sordidus* (Pomacentridae)

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Coral reef fishes grow rapidly during the first and second years of their life, attaining from 50% to 87% of their adult size (1, 2). During this period, growth is determined by various density-dependent and environmental factors (3, 4). Once a fish reaches maturity, somatic growth slows dramatically as energy is allocated for reproduction. The combination of these factors often produces fishes of different ages for a given size (5). Concentrations of polychlorinated biphenyls (PCBs) were highly variable in tissues of adult damselfish of about the same size collected from the same area at Johnston Atoll. These fish were aged to determine if there was a correlation with contaminant concentration. The hypothesis is that age equals exposure time, and therefore older fish are predicted to contain higher contaminant levels.

Adult damselfish, *Abudefduf sordidus* (Forsskal 1775) were collected to determine if PCBs sorbed to sediments were accumulating in their tissues. *A. sordidus* is a territorial spawner and a benthic omnivore, making it ideal for monitoring anthropogenic effects on coral reefs. *A. sordidus* gut contents include algae, benthic invertebrates, and sediments. Fish were collected from four areas in the lagoon of Johnston Atoll: (a) West Sand Island, with high (up to 389.0 μg/kg, or ppb) sediment PCB concentrations; (b) Buoy 14, with low (up to 0.5 ppb) PCB concentrations; (c) the former Herbicide Orange site, also with low (up to 7.2 ppb) PCB concentrations; and (d) East Sand Island, where additional fish were collected for aging only. The west end of Sand Island was the primary study site, where localized PCB contamination had been detected at concentrations exceeding ecological screening levels. The Buoy 14 and Herbicide Orange sites were used as background sites for PCB levels since no source, or substantial levels, of PCBs had been measured in the sediments there. However, other contaminants, including dioxins and metals, also occur in sediments at the Herbicide Orange site. PCBs in sediments had a patchy distribution within and between sites (6). Sediments were composed of carbonate sand mixed with diatoms, dinoflagellates, and microinvertebrate infauna.

Fish (n = 18) were measured (standard length), weighed, and sexed; the otoliths were removed and weighed. Fish at each site were collected at the same time and were reproductively mature. Individual differences in recent reproductive output were not determined. Age was determined from thin, transverse sections of the sagittal otolith, mounted on a microscope slide and etched for 45 min with 2% hydrochloric acid. Otolith sections were viewed with transmitted light at 40× magnification. We found that discriminating opaque and translucent zones for aging was possible but difficult because the otoliths lacked a clear internal structure. Results from examination of 29 species of coral reef fishes, including three damselfishes, indicate that the alternating translucent/opaque patterns in the otoliths of coral reef fishes represent annual growth patterns (7). PCB and lipid content of the fish (without viscera) was analyzed by the Toxic Contaminant Research Laboratory, Wright State University, Ohio. Viscera were removed for other analyses. PCBs were analyzed using LRMS/HRGC and HRMS/HRGC (Modified EPA Method 8082 and Draft EPA Method 1668). Individual congeners of di- to deca-chlorinated PCBs were analyzed and summed to give total PCB concentration. Linear regression analysis was used to determine relationships between length, age, weight, and lipid content for all fish. Linear regression within sites was used to determine relationships between lipid and PCB concentration, and age and PCB concentration within sites because of the differing exposures at the sites.

There were significant relationships between length and weight ($r^2 = 0.913$, $P < 0.001$) and length and age ($r^2 = 0.767$, $P < 0.001$) for all fish. Ages varied from 5 to 9 years among fish at all sites, but individuals within a site were very close

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