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Gross and microscopic pathology of lesions in *Pocillopora* spp. from the subtropical eastern Pacific

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ABSTRACT

Coral reefs are threatened by a variety of factors including diseases that have caused significant damage in some regions such as in the Caribbean. At present, no data are available on coral diseases in the Mexican Pacific where *Pocillopora* spp. is a dominant component of coral communities. Here, we describe gross and microscopic morphology of lesions found in pocilloporids at four sites in the Mexican Pacific. Corals were identified and their lesions photographed and quantified in the field. Tissue samples were collected from healthy and affected colonies for histopathology. We recorded seven species of pocilloporids at the study sites with Isla Isabel being the location with the highest coral diversity ($H' = 1.27$). Lesions were present in 42% of the colonies and included discoloration (32%), predation-induced tissue loss (30%), unexplained tissue loss (3%) and overgrowth by sponges or algae (35%). The most affected species, *P. damicornis* (50%), was also one of the most common in the region. No species was more prone to a particular lesion, but there was a significant association between location and the presence of lesions. Northern Islas Marietas (61%) and Isla Isabel (41%) had the highest prevalence of lesions, followed by Manzanillo (37%) and Bahías de Huatulco (23%). Histological changes included atrophy of the surface body wall with depletion of zooxanthellae (91%) in corals with discoloration (bleaching). Ablation of tissue from mesoglea (18%) was also observed. Colonies with unexplained tissue loss showed atrophy and thinning of the epidermis (89%), characterized by cuboidal instead of pseudocolumnar cells normally found in healthy pseudocolumnar ciliated epithelium. Bacterial aggregates between the mesoglea and gastrodermis (11%) were very conspicuous in healthy and diseased corals. Lesions produced by fish bites and gastropods were associated with tissue atrophy (40%) and, in some cases, algal overgrowth near the lesion (20%). No infectious agents associated with cell pathology were detected microscopically. Bleaching and overgrowth by algae and sponges, as well as unexplained tissue loss, are common in *Pocillopora*. These lesions and anatomical changes warrant further study since their incidence is potentially indicative of reef degradation.

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1. Introduction

Coral reefs are subject to widespread degradation globally due to many factors, including environmental changes and human activities (Bruno et al., 2007; Hoegh-Guldberg et al., 2007; Ruiz-Moreno et al., 2012). In addition, the increased incidence of coral diseases has caused major declines in coral populations in the Caribbean (Aronson and Precht, 2001; Hughes, 1994) and is gaining importance in the Pacific (Weil et al., 2006, Williams et al., 2010).

A large number of diseases has been described in different scleractinian corals, but only a few have been characterized at the microscopic level (Weil et al., 2006; Williams et al., 2010; Work and Aeby, 2006). This has led to ambiguous descriptions because few attempts are generally made to characterize at the cellular

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level what is killing the corals (Work and Aeby, 2006; Work and Rameyer, 2005; Work et al., 2008; Work and Meteyer, 2014). To avoid these problems, systematic descriptions of lesions at the gross and cellular level must be considered as the initial step to disentangle the dynamic interactions between host, pathogen, and environment (Auffret, 1988; Work et al., 2008). This allows for the detection of potential causative agents of disease and the host response to those agents, thereby guiding additional analytical procedures (e.g., molecular, laboratory experimentation) intended to confirm the identity of suspect causative agents and allowing for a more complete understanding of disease pathogenesis.

In the eastern Pacific, including Western Mexico, *Pocillopora* is the dominant genus of scleractinian corals (López-Pérez et al., 2012; Reyes-Bonilla and Barraza, 2003). The low diversity of reefs highly dominated by *Pocillopora* spp., is related to their high growth rate, their capacity of propagating by asexual reproduction, and by aggressive interspecific interactions (Glynn et al., 1982). Even though coral communities in the Mexican Pacific are constantly disturbed by natural events, such as El Niño Southern Oscillation (ENSO), it appears that these ecosystems are resilient and can recover rapidly even in areas with high coral mortalities (Reyes-Bonilla et al., 2002). However, anthropogenic impacts such as tourism and change in land use are important risk factors leading to eutrophication, algal overgrowth, and the decline of live coral cover (López-Pérez et al., 2012).

Several diseases have been reported for *Pocillopora* spp. in the Indo-Pacific, including skeleton eroding band, Black band, *Vibrio coralliilyticus* induced bleaching, white plague type I, and skeletal growth anomalies (Sutherland et al., 2004). *Pocillopora* is also susceptible to bleaching events (Flot and Tillier, 2004; Galloway et al., 2007). However, in-depth studies describing diseases of pocilloporids are scarce, particularly in reefs where they dominate and have a central ecological role as ecosystem engineers. Furthermore, there is no information about the health status of coral species in the Mexican Pacific. Hence, our objective was to assess the incidence and distribution (taxonomic and geographic) of lesions naturally occurring in *Pocillopora* spp. at the gross and microscopic level to improve our understanding of coral health in the region.

2. Materials and methods

2.1. Sampling and fieldwork

Four sites in the Mexican Pacific were surveyed between November 2010 and September 2011 including Isla Isabel (Nayarit), Islas Marietas (Nayarit), Manzanillo (Colima), and Bahías de Huatulco (Oaxaca) (Fig. 1; Table 1).

Isla Isabel is an oceanic island located 62 km off coast of the Nayarit state declared as a national park and used as a maritime refuge with temporary settlement of fishers (CONANP, 2005). It is in a transition zone with a complex oceanic structure and dynamics affected by the California Current, the North Equatorial Current, and the Gulf of California's water mass (Badan, 1997). Seawater around the island is mostly oligotrophic, with temperatures between 26 and 29 °C, and salinity between 33‰ and 35‰ (CONANP, 2005). Ten scleractinean species are found around the island (Carriquiry and Reyes Bonilla, 1997), with *Pocillopora* being the dominant genus. Isla Isabel is the most remote site from the coast of those included in this survey and probably has the lowest anthropogenic influence on coral communities.

Islas Marietas is a national park formed by two islands (Isla Larga and Isla Redonda) located off the coast of the Nayarit state in Bahía de Banderas. Marietas was recently declared a Biosphere Reserve by the United Nations Educational, Scientific and Cultural Organization, UNESCO (CONANP, 2008). Reefs on this site are dominated by *Pocillopora* and *Pavona* mainly distributed off the eastern shore of the islands where they are protected from currents and winds and in some coves in the west coast of Isla Redonda (CONANP, 2008). The islands are uninhabited but are a popular tourist attraction and therefore have some human impacts.

The seaport of Manzanillo comprised two locations (Carrizales and La Boquita). The main human activities in Manzanillo are commercial and sport fisheries, tourism and maritime transportation (Gobierno de la Ciudad de Manzanillo, 2009). Reefs in this region are dominated by *Pocillopora verrucosa*, *Porites panamensis* and *Pocillopora capitata*, with 13 species of scleractinian corals documented in the region (Reyes-Bonilla et al., 2013).

Finally, Bahías de Huatulco was the southernmost locality and constitutes a national park comprising nine bays with depths up to 200 m. The coral community in Huatulco comprises 14 scleractinian species dominated by *Pocillopora* spp. (CONANP, 2003). Huatulco National Park is also a tourist destination, with activities such a scuba diving, fishing, and other water sports (CONANP, 2003).

At each site, three to five consecutive 25 × 2-m belt transects separated by ca. five m were deployed end-to-end parallel to the coastline, following depth contours as much as possible (Table 1). Colonies along transects were identified to species following Veron (2000) and counted. Gross lesions were photographed with a Sea Life DC 1200 camera and described according to their distribution, location on the colony, margins, shape, and color following Work and Aeby (2006). Lesions were classified as discoloration, tissue loss, and overgrowth of live tissue by algae or sponges. Discoloration was further subdivided into bleaching (white or pale brown) or purple; tissue loss was subdivided into (a) fish bites (multifocal tissue loss associated with bare eroded skeleton), (b) snail predation (multifocal to diffuse tissue loss revealing intact bare skeleton with presence of snails nearby), (c) crab predation (same as snail predation but with crabs nearby) and (d) unexplained tissue loss consisting of multifocal to diffuse acute to subacute tissue loss revealing intact bare white skeleton with no evidence of predators. Overgrowth of live tissue was categorized according to the overgrowing organism: filamentous or fleshy macroalgae or sponges. Categories of lesions were not mutually exclusive. Therefore, a single colony could have more than one lesion, and consequently the total number of lesions could exceed the number of colonies.

Sea surface temperature during the study was obtained from Giovanni – Interactive Visualization and Analysis (NASA, 2013).

2.2. Gross and microscopic description of lesions

Coral fragments were taken directly from lesions and healthy regions of affected colonies as well as from non-affected colonies. They were fixed for a minimum of 48 h in zinc-formaldehyde solution (Z-fix, Anatech) diluted 1:5 in seawater, and then submerged in Zenker Solution (for 1 L of fresh seawater: 25 g potassium dichromate, 45 g mercury chloride, 10 g sodium sulfate, and 50 ml acetic acid) for approximately 18 h to improve staining of the mesoglea with the Mallorýs trichrome procedure. Subsequently, samples were rinsed in fresh water and preserved in 70% ethanol. Samples were decalcified in HCl (10%) buffered solution (0.7 g EDTA, 0.14 g sodium tartrate, 0.008 potassium sodium tetrahydrate), embedded in paraffin, and sectioned (5 μm thick). Tissues were stained with Harris's hematoxylin and eosin procedure, with Mallory's trichrome procedure to highlight connective tissue (Humason, 1967), and with Gram's stain for bacteria (all in Kiernan, 1999).

Microscopic morphological changes were described relative to normal tissues from healthy and diseased colonies. Observations

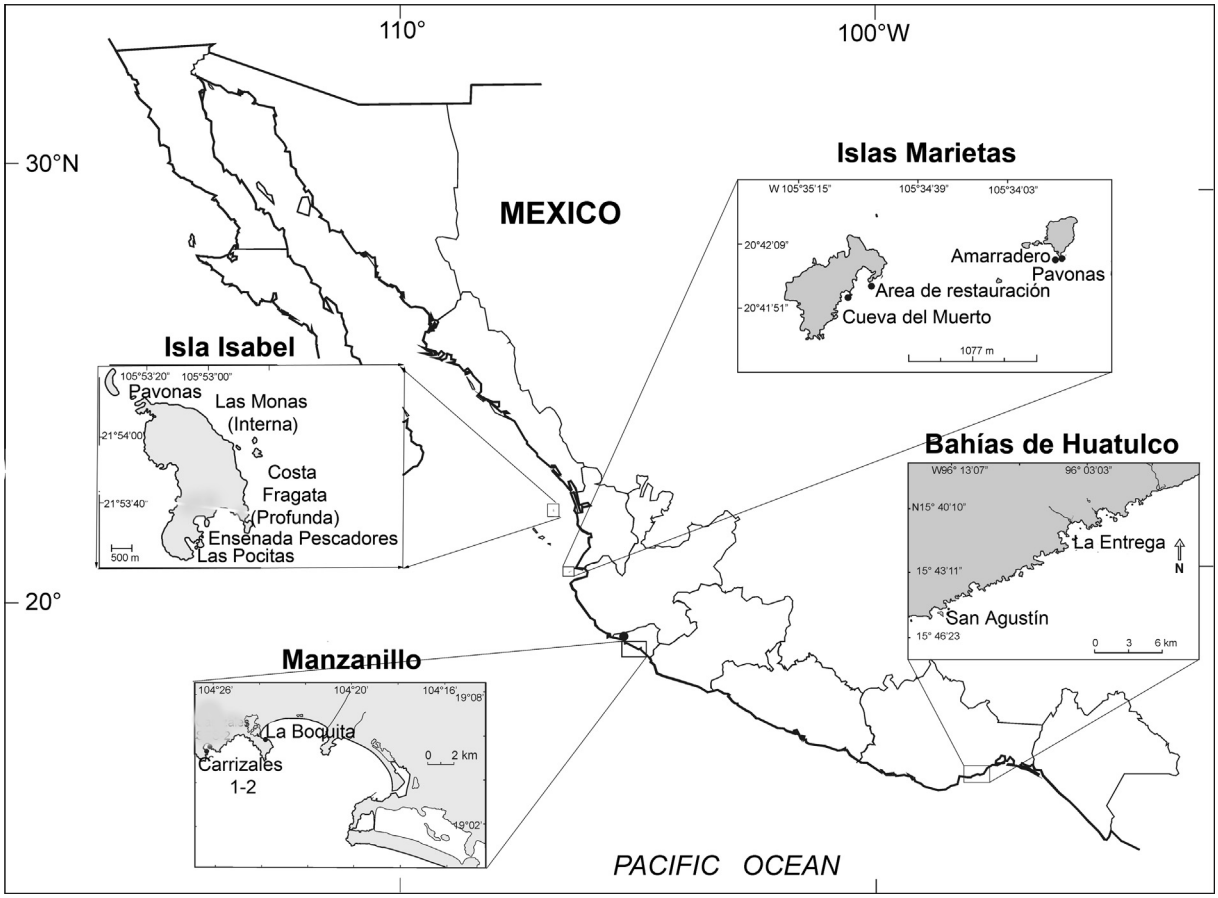

Fig. 1. Location of coral communities studied in the Mexican Pacific.

Table 1
Names, GPS location, depth and area surveyed for coral lesions in individual sites for four locations (Isabel, Marietas, Manzanillo, Huatulco) in the Mexican Pacific.

Sites surveyed	N	W	Depth range (m)	Area surveyed (m^2)
Isla Isabel				
Pavonas	21.853	−105.8907	7–10	150
Ensenada Pescadores	21.8429	−105.8843	2–5	250
Costa Fragata Profunda	21.8453	−105.8805	6–10	250
Las monas (interna)	21.8489	−105.8852	3–6	250
Las Pocitas	21.8412	−105.8844	7–11	250
Islas Marietas				
Amarradero	20.7009	−105.5649	6–9	150
Pavonas profunda	20.7014	−105.5649	10–11	250
Cueva del Muerto	20.6973	−105.5827	4–5	250
Area de restauración	20.6994	−105.5809	2–6	250
Manzanillo				
Carrizales 1	19.458	−104.6422	4–7	250
Carrizales 2	19.458	−104.6422	4–8	250
La Boquita	19.19	−104.6703	1–3	250
Bahías de Huatulco				
San Agustín	15.6877	−96.2250	3–7	250
La Entrega	15.7416	−96.1222	3–7	100

(qualitative data) per colony were recorded on histopathology datasheets that included the history of the sample and a full histopathological description according to Galloway et al. (2007). Changes such as atrophy, sloughing of epithelia from mesoglea, calicodermal hypertrophy, zooxanthellae depletion, presence of bacterial aggregates and suspect endolithic algae or fungi were also recorded on the datasheets (quantitative data) to relate gross and microscopic pathology findings.

2.3. Data analyses

The diversity of *Pocillopora* communities was quantified with the Shannon–Wiener index (H'), N_i and the effective number of species as follows:

$$H' = -\sum_{i=1}^{s}(p_i)\ln(p_i) \quad (1)$$

where H' is the information content of sample = index of diversity, s the number of species, p_i the proportion of total sample belonging to ith species, N_i the number of equally common species which would produce the same diversity as H' and the effective number of species =exp(N_i), sometimes called the true diversity of a community (Jost, 2006).

Prevalence of lesions in each *Pocillopora* species was defined as the percent of *Pocillopora* colonies surveyed that had a particular lesion type. Overall prevalence was the percent of colonies surveyed that had a particular lesion type with all surveys combined. A chi-square was performed to test geographical homogeneity in the number of lesions. A logistic regression was used to test for relationships between the prevalence of lesions with coral abundance and diversity. To perform the logistic regression, the dependent variable was coded as lesion or non-lesion and natural logarithm transformed, as implemented in Statistica ver 7.1 (StatSoft, Inc., 2005).

3. Results

3.1. Pocillopora community in the Mexican Pacific

Seven species of *Pocillopora* (*P. verrucosa*, *P. damicornis*, *P. capitata*, *P. eydouxi*, *P. meandrina*, *P. inflata*, and *P. effusus*) were recorded comprising a total of 1006 colonies with the highest diversity seen in Isla Isabel (H' = 1.27) followed by Manzanillo, Islas Marietas, and Bahías de Huatulco (Table 2). The most and least common species were *P. verrucosa*, representing 54% (n = 545) of the colonies and *P. effusus* seen only in Manzanillo, respectively (Table 2).

3.2. Lesion occurrence and gross description

Fifty-eight percent (n = 584) of the colonies did not show any gross lesions and presented a vivid and uniform color that varied from brown to green depending on the species; some displayed an altered morphology secondary to the presence of symbiotic crabs (Fig. 2A). Discoloration (32%), tissue loss (33%) and overgrowth (35%) were equally frequent overall in the Mexican Pacific. Bleaching (Fig. 2B and C) comprised a majority (98%) of discoloration with purple discoloration (Fig. 2D) being rarely seen. For tissue loss, fish bites (88%) (Fig. 2E) dominated followed by unexplained tissue loss (Fig. 2F) with snails (Fig 2G) or crabs being uncommon. Algae were the predominant overgrowing organism (91%) (Fig. 2H). Corals manifesting discoloration predominated in Isla Marietas (85%), tissue loss predominated (55%) in Manzanillo, and overgrowth of live tissues was equally dominant (ca. 33% each) in Manzanillo and Huatulco. In contrast, discoloration was rare (<5%) in Manzanillo and Huatulco, and tissue loss was absent in Marietas (Table 3).

We found significant geographic heterogeneity in the presence of lesions among sites (Chi square = 8.9, p = 0.04), with Islas Marietas having the highest prevalence of lesions (61%), followed by Isla Isabel (42%), Manzanillo (37%) and Bahías de Huatulco (23%) (Table 4).

For the three dominant species of corals, *P. damicornis*, *P. verrucosa*, and *P. capitata*, there was no significant difference in prevalence of dominant lesions (bleaching, fish bites or algal overgrowth) (P > 0.05). However, when partitioned by location, there was considerable heterogeneity among sites. For example, *P. damicornis* possessed the highest frequency of fish bites in Isabel; whereas in Marietas *P. capitata* was the commonly affected species. Likewise, *P. damicornis* was most susceptible to bleaching in Marietas. *P. verrucosa* appeared most susceptible to algal overgrowth at all sites (Table 5). Chi-square test revealed a significant association between type of gross lesion and coral species affected (P < 0.01). We saw no relationships between coral abundance or diversity and the prevalence of lesions (p > 0.05).

3.3. Histopathology

Sixty-six tissue samples from healthy (15), lesioned (31) and apparently healthy regions from lesioned (22) colonies were examined by microscopy. In healthy tissues, surface body wall comprised pseudostratified columnar epithelium separated from gastrodermis by mesoglea. Gastrodermis consisted of simple cuboidal cell layer with zooxanthellae that were uniformly round with distinct nuclei and pyrenoids. Mesenteries had a core of mesoglea covered by pseudostratified columnar epithelium (Fig. 3A and B). Bleached corals manifested diffuse atrophy of the epidermis and gastrodermis (91%, n = 10) with absence or reduced numbers of zooxanthellae (91%, n = 10) (Fig. 3C). In some colonies with patches of pale brown discoloration, atrophy was not always evident, however, there was reduced number of zooxanthellae (Fig. 3D), and zooxanthellae in some polyps appeared degraded manifesting enlargement to almost twice the normal size with cytoplasmic vacuolation present and pale staining.

Only one tissue with purple discoloration was examined histologically, and it displayed infiltrations of macrophytic tissues and hypertrophy of coenenchyme characterized by enlargement and thickening of the epithelial cells and overall tissue fragmentation (Fig. 3E). Zooxanthellae adjacent to algae were depleted but their abundance increased with distance from the lesion (Fig. 3F).

Tissue loss: The remnant tissue in samples from colonies with tissue loss generally had uniform atrophy of epidermis and gastrodermis (40%) with the epidermis most often affected (Fig. 3G). Tissues from healthy colonies, normal, and lesion tissues from *P. verrucosa*, *P. damicornis*, *P. capitata*, and *P. inflata*, all had basophilic

Table 2
Abundance (n = number of colonies surveyed) and diversity of *Pocillopora* species recorded at each location.

Species	Isla Isabel	Islas Marietas	Manzanillo	Bahías de Huatulco	All surveys combined
P. capitata	4	41	75	22	142
P. damicornis	60	54	17	72	203
P. verrucosa	103	157	213	72	545
P. eydouxi	45	13	12	0	70
P. efusus	0	0	1	0	1
P. meandrina	11	0	8	0	19
P. inflata	1	0	24	1	26
Total	224	265	350	167	1006
Diversity indices					
H'	1.3	1.1	1.2	1.0	
Shanon index	3.6	2.9	3.3	2.8	
Effective species	36.0	18.5	26.1	16.1	

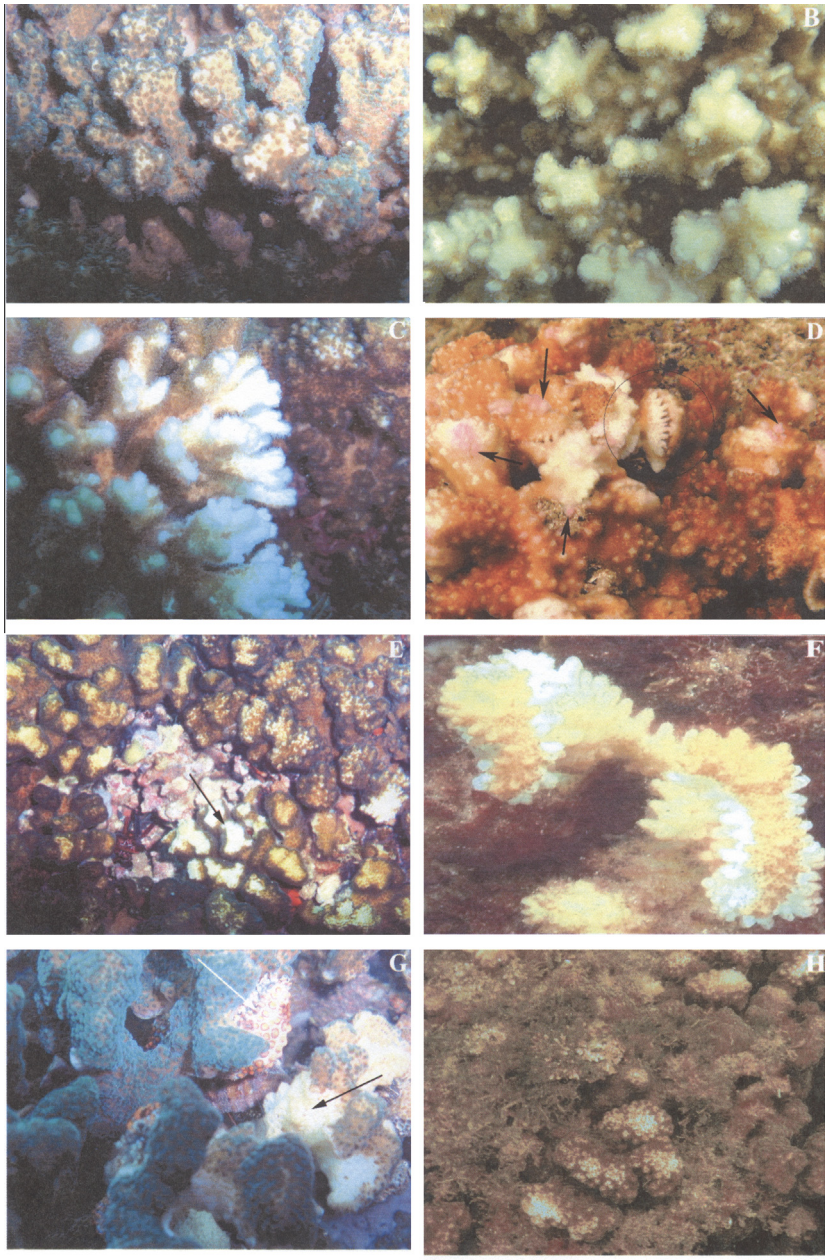

Fig. 2. Gross lesions observed in colonies of *Pocillopora* spp in comparison with a healthy colony (A). (B and C) Bleached colonies; note white skeleton visible through polyps. (D) Purple discoloration on branch tips. Arrows indicate abnormal growth of the skeleton (E) Predation by fishes. (F) Predation by *Jenneria pustulata* and hermit crabs. (G and H) Overgrowth of live tissue by sponge (arrow) (G) and algae (H) over the branches of *Pocillopora* spp.

aggregates of bacteria located between the epidermis and the mesoglea (38%) (Fig. 3H) that stained Gram negative and averaged 31.73 ± 3.88 μm ($n = 9$ aggregates) in Bahías de Huatulco and 24.68 ± 12.11 ($n = 4$ aggregates) in Manzanillo. These aggregates were not seen in Isla Isabel or Islas Marietas.

4. Discussion

Lesions are a normal component of tropical coral reef building communities as documented by Vargas-Ángel, 2009 in remote islands of the US Pacific, where even under low levels of anthropogenic influence, lesions in corals were always present. However, there are many drivers that could increase their prevalence and incidence and so compromise the stability of the entire ecosystem. Thus, understanding the geographic distribution of lesions and their potential causes can prove informative to the assessment of reef health and may provide guidance for management.

Tissue loss was one of the most common lesions reported in the Mexican Pacific, dominated by fish predation. Fish bites by species from the families Scaridae, Tetraodontidae and Monachantidae are recurrent morphological lesions that are present in corals all over the world (Rotjan and Lewis, 2008). In *Pocillopora* species, some studies have identified the predator species by the characteristics of the scars, because observing fish actively feeding on corals can be challenging (Jayewardene et al., 2009). For example, in Hawaii, *Cantherhines dumerilii* (Monacanthidae) was the species responsible for the lesions found on branch tips of *P. meandrina* (Jayewardene et al., 2009). *Arothron meleagris* (Tetraodontidae) have been identified as a general predator that could feed on branched corals as well as on algae, mollusks and other species (Glynn et al., 1972; Guzmán and López, 1991). In the Gulf of

Table 3
Number of *Pocillopora* colonies from the Mexican Pacific with a particular gross lesion partitioned by location and lesion category (discoloration, tissue loss, overgrowth) along with percentage of particular lesion type by category or overall (total).

Lesion	Isabel	Marietas	Manzanillo	Bahías de Huatulco	*n*	% Category	% Total
Discoloration							
Bleaching	18	124	1	6	149	98	31
Purple tips	0	3	0	0	3	2	1
Total	18	130	1	6	152	100	32
Tissue loss							
Fish bites	47	0	67	22	136	88	29
Unexplained	0	0	13	0	13	8	3
Snail	0	0	5	0	5	3	1
Crab	0	0	1	0	1	1	0
Total	47	0	86	22	155	100	33
Overgrowth of live tissues							
Algae	32	53	57	13	155	91	32
Sponges	0	3	12	0	15	9	3
Total	32	56	57	13	170	100	35

Table 4
Percent (*n*) of a given species at a particular location with lesions (all types) and percent (*n*) of all lesions for all species for a given location (prevalence per locality). Total number of colonies enumerated for all locations was 1006.

	Isla Isabel	Islas Marietas	Manzanillo	Bahías de Huatulco	All surveys combined
P. capitata	50(4)	42(41)	45(75)	23(22)	41(142)
P. damicornis	63(60)	83(54)	24(17)	21(72)	50(203)
P. verrucosa	41(103)	61(157)	35(213)	26(72)	42(545)
P. eydouxi	20(45)	31(13)	42(12)	0(0)	26(70)
P. efusus	0(0)	0(0)	0(1)	0(0)	0(1)
P. meandrina	27(11)	0(0)	12.5(8)	0(0)	21(19)
P. inflata	0 (1)	0(0)	42(24)	0(1)	38(26)
Prevalence per locality	41 (224)	61(265)	37(350)	23(167)	42(1006)

Table 5
Prevalence of dominant lesions (bleaching, fish bites, algal overgrowth) for dominant species partitioned by location.

Species	Isabel	Marietas	Manzanillo	Huatulco	All
Algal overgrowth					
P. capitata	25	9.8	6.7	4.5	11.5
P. damicornis	8.3	27.8	5.9	1.4	10.8
P. verrucosa	16.5	20.4	22.5	15.3	18.7
Bleaching					
P. capitata	25	31.7	1.3	4.5	15.6
P. damicornis	10	81.5	0	2.8	23.6
P. verrucosa	7.8	42.7	0	4.2	13.7
Fish bite					
P. capitata	0	0	34.7	18.2	13.2
P. damicornis	43.3	0	11.8	18.1	18.3
P. verrucosa	19.4	0	13.6	6.9	10

California, *A. meleagris* has been reported as a predator of *Pocillopora* species (Moreno et al., 2009; Reyes-Bonilla and Calderon-Aguilera, 1999.

Lesions caused by predation, (e.g., fishes), should be included in studies of lesions in coral reefs in order to gain a better understanding of the dynamics of coral pathologies (Aeby and Santavy, 2006; Rotjan and Lewis, 2008) and reef health. For instance, the presence of a large number of corals with fish bites could imply a healthier ecosystem with larger fish populations and more intact food webs (Sandin et al., 2008). Alternatively, excessive fish bites could increase in marine environments perturbed by overfishing. For example, depletion of larger fish feeding on corallivores could release the latter from predation control leading to population growth and increased predation on corals (Raymundo et al., 2009). Understanding these processes would require focused studies on fish community structure and observational studies of time-activity budgets of reef fish communities and their interactions with corals. Microscopically, more than 50% of fish bite cases had no evident microscopic changes. Tissue regeneration could explain the lack of microscopic lesions. A couple of weeks after artificially induced mechanical damage, *Montipora capitata* tissues regenerated completely, making the regenerated tissue microscopically undistinguishable from the original (Work and Aeby, 2010), yet scarred lesions remained macroscopically visible.

Discoloration of tissues was frequently observed and dominated by complete (white) or partial (brown) bleaching. Coral bleaching secondary to low water temperatures is a known phenomenon in the Mexican Pacific and Gulf of California. Thermal stress induced by a decrease in water temperature 7 °C below the average (26 °C) has been observed during the spring (Cupul Magaña and Calderon-Aguilera, 2008; LaJeunesse et al., 2010; Paz-García et al., 2012) leading to massive mortalities of *Pocillopora* (Wilson, 1990). Bleaching was most prevalent in Islas Marietas during December 2010 to March 2011 when water temperature in the area dropped from 28 °C in November 2010 to 21 °C in February 2011. A similar bleaching event at that time was reported in the Gulf of California where water temperature dropped from 21 °C to 18 °C during February 2011 resulting in bleaching of 84% of colonies sampled in April (Paz-García et al., 2012). *P. damicornis* in Islas Marietas was the most susceptible species (82% prevalence). Although *Pocillopora* has a wide range of responses to elevated temperatures depending on which clades of endosymbiotic algae it harbors, degree of exposure to the stressor, and spatial distribution (D'Croz and Maté, 2004), *P. damicornis* has a particular low tolerance to thermal stress in the eastern Pacific (Glynn et al., 2001). *P. damicornis* also was the most affected species in the bleaching event in the Gulf of California, where 36% of colonies presented bleached tissues. Recent physiological assays on colonies from Islas Marietas exposed to low and high water temperature, found that

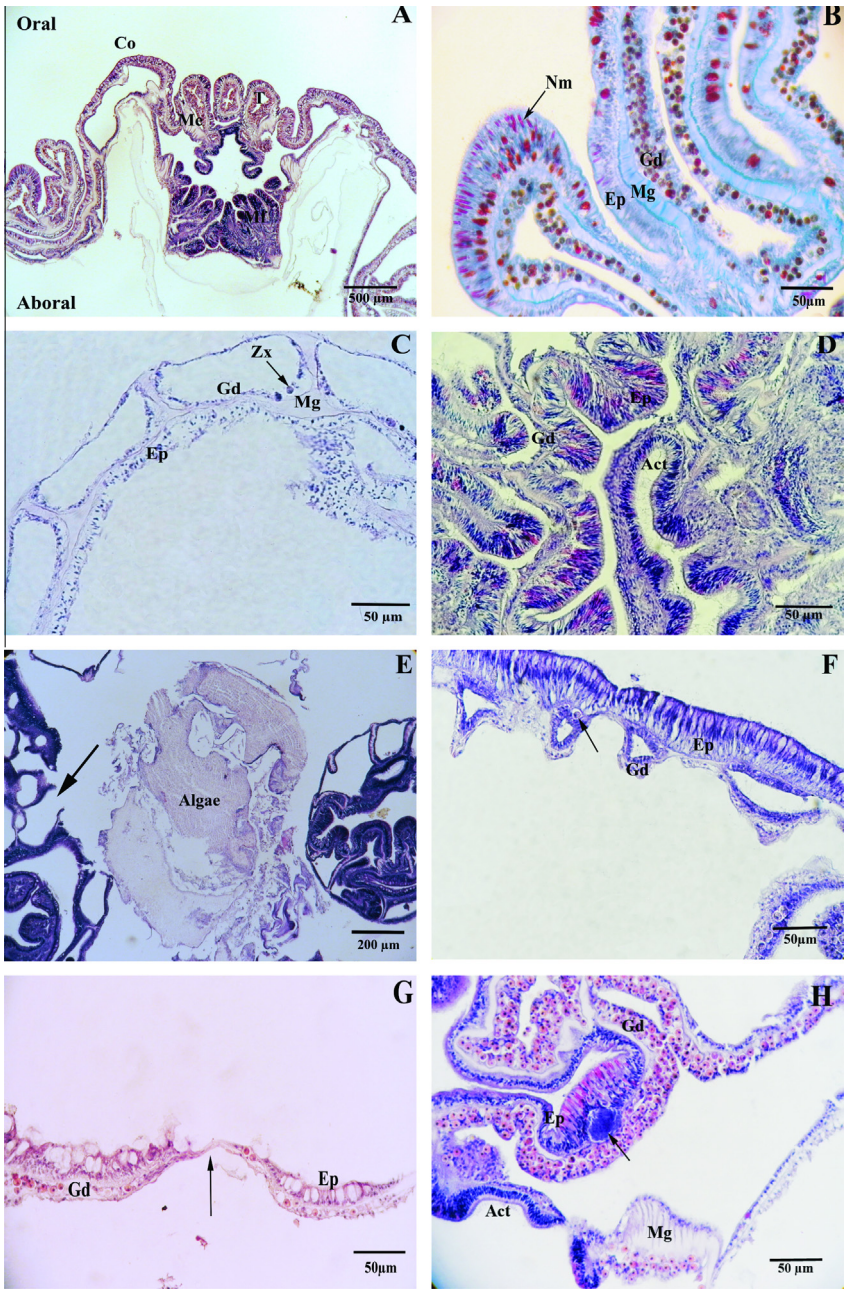

Fig. 3. (A and B) Normal *Pocillopora* spp.; note the characteristics of the epidermis (Ep), gastrodermis (Gd) and mesoglea (Mg), abundance of zooxanthellae inside the gastrodermal cells, and nematocytes (Nm) in the epidermis. (C) Bleached colony; note atrophy of the tissues with thinning epithelia (Ep) and gastrodermis (Gd) and absence of zooxanthellae (Zx). (D) Partially bleached colony; note epithelia, (Ep) and actinopharynx (Act) with normal characteristics but complete absence of zooxanthellae in gastrodermis (Gd). (E) Endolithic algae between two adjacent polyps of *Pocillopora* spp; note (Arrow) the atrophy and fragmentation of the calicodermis. (F) Tissue loss. Note coenenchymal tissue showing few zooxanthellae (arrow) within gastrodermis and normal epidermis (Ep) and mesoglea (Mg). (G) Tissue loss; note thinning in the gastrodermis and epidermis, reduced number of zooxanthellae. (H) Normal tissue with bacterial aggregate between the mesoglea and gastrodermis (arrow) with normal epidermis (Ep), nematocytes (Nm), gastrodermis (Gd) and actinopharynx (Act). All photos hematoxylin and eosin except B, which is Mallory Trichrome.

bleaching is the common response of colonies to thermal stress (Rodriguez-Troncoso et al., 2013a,b).

Although bleaching events observed here were likely caused by low water temperature, the microscopic morphology of the lesion was similar to that seen in bleached corals exposed to elevated water temperature. Specifically, atrophy of tissues associated with depletion of zooxanthellae has been well documented in bleached corals in the Pacific (Brown et al., 1995; Gates et al., 1992; Glynn et al., 1985) and the Atlantic (Hayes and Bush, 1990; Vargas-Ángel et al., 2007). It is likely that atrophy was the result of loss of nutrients from depleted algal symbionts. Mechanisms associated with this process are not fully understood but probably are linked to programmed cell death (Weis, 2008). Similar changes in the general tissue condition have been observed in colonies living naturally under thermal stress in the field and under special conditions in the laboratory (Rodríguez-Troncoso et al., 2010). The apparent lack of alteration of tissues from some samples of bleached corals has been explained by Brown et al. (1995). They proposed a bleaching mechanism by which endosymbiotic cells are degraded *in situ*, with either the subsequent reabsorption of waste material in the gastrodermal epithelia or their expulsion through the gastric cavity. This mechanism does not necessarily

involve a destruction or breakdown of coral tissues as seen in our samples. Another possible explanation for the high prevalence of discoloration in this genus is senescence. *Pocillopora* is one of the few scleractinian genera possessing determinate growth, and perhaps bleaching is a sign of old age (Kinzie Iii and Sarmiento, 1986). This hypothesis merits further investigation.

Corals with purple discoloration showed microscopic evidence of algae associated with tissue fragmentation and depletion of zooxanthellae in the gastrodermis. Competition between corals and algae is well known but the mechanisms of the interaction are not completely understood (Barott et al., 2009; McCook et al., 2001). Peters (1984) found that corals that were overgrown with algae (*Colpophyllia natans*, *Diploria strigosa*, *Favia fragum*, *Montastraea annularis*, *Porites astreoides*, *Siderastrea radians*, *Siderastrea siderea*, *Stephanocoenia michelinii*, *Acropora cervicornis*, and *Porites porites*), microscopically presented hyperplasia of the epidermal cells, that could be an attempt of the coral to avoid the advance of the algal filaments through the tissues (Peters, 1984). Changes in the number of zooxanthellae in tissues near invasive algae have been observed in laboratory assays. For instance, in *P. verrucosa*, the dissolved compounds produced by algae generate hypoxic conditions from increased microbial activity, which cause coral mortality (Smith et al., 2006). Prior to coral death, the most important alteration of tissues was linked to the loss of symbionts from the gastrodermal cells located close to the region of algal–coral interaction (Rasher and Hay, 2010; Smith et al., 2006).

The presence of cell-associated microbial aggregates (CAMA) in tissues of a dominant coral genus in the region fits patterns seen elsewhere in the Pacific, where these aggregates tend to predominate in dominant genera. Cell-associated bacterial aggregate are thought to play an important role in the physiology of certain coral genera. The size ranges of aggregates seen here were very similar to those observed in *Pocillopora* spp. from the Central Pacific. Finally, others have noted a geographic heterogeneity in prevalence of infection by these aggregates with more of them observed in the western versus eastern Pacific (Work and Aeby, 2014). That there is also localized heterogeneity in their distribution in Western Mexico, exemplified by their absence in Marietas and Isabel, is intriguing. Exploring this localized difference in distribution of CAMA in *Pocillopora* may shed further light on the ecology of these microbial symbionts.

Influence of human activities in coastal areas is thought to be an important factor that has promoted decline of reef communities globally (Carpenter et al., 2008). We found a higher prevalence of lesions in the offshore islands reefs, Isabel and Marietas, than in coastal sites such as Bahías de Huatulco and Manzanillo. Other ecological and biological factors must be considered to completely understand the presence and distribution of diseases in coral communities. A baseline study such as ours is critical if we are to comprehend the underlying mechanisms and temporal status and trends of coral reef health so as to better manage reefs in the region. Future investigations should focus on determining how or if lesions progress over time at the gross and microscopic level. For example, tissue loss in *Montipora* waxes and wanes and microscopic appearance changes over time (Work et al., 2012). Clarifying the role of senescence in bleaching of *Pocillopora* would also be informative. Eastern Pacific reefs also provide a natural laboratory to contrast the ecology of cold- versus warm-water-induced bleaching. Finally, confirming whether or not reefs with high prevalence of fish bites are an index of healthy reefs might be useful for management.

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