Proceedings of the 11th International Coral Reef Symposium, Ft. Lauderdale, Florida, 7-11 July 2008
Session number 7

Coral and crustose coralline algae disease on the reefs of American Samoa

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Abstract. Surveys for lesions in corals were conducted at seven sites around Tutuila in June 2004 and January 2005. The objectives of the study were to document the distribution and prevalence of disease in the major genera of corals and crustose coralline algae, systematically describe gross and microscopic morphology of lesions in reef corals and determine whether there are seasonal differences in prevalence of disease. We documented 12 different coral disease states from the reefs of Tutuila and two diseases of crustose coralline algae (CCA). *Acropora* white syndrome, *Acropora* growth anomalies and coralline lethal orange disease were the most common diseases on the reefs of Tutuila. No seasonal differences were found in overall prevalence of coral or abundance of CCA disease. Histological analyses of coral lesions revealed that microscopic changes in tissues can be used to distinguish tissue loss due to trauma from changes due to disease, detect micro-organisms associated with certain types of discolorations and found that hyperplasia of the basal body wall was the hallmark microscopic appearance of *Acropora* growth anomalies regardless of gross morphology of tumors.

Key words: American Samoa, baseline disease surveys, coral disease, crustose coralline algae disease

Introduction

Global climate change and human activities are placing coral reef ecosystems at risk. The effects of over fishing and pollution from agriculture and land development have been a major force accelerating decreases in abundance of coral reef species (Pandolfi et al. 2003). Global climate change is compounding these anthropogenic stressors with increased frequency of bleaching episodes and increases in incidence of coral disease predicted (Hoegh-Guldberg 1999, Hoegh-Gulberg et al. 2007). The reefs of American Samoa have been affected by both natural disturbances (crown-of-thorns starfish invasion, hurricanes and mass bleaching events) and human-induced impacts (pollution and over fishing) (Craig et al 2005). The reefs suffered mass bleaching in 1994 (Goreau and Hayes 1994, Birkeland et al. 2000) with reports of bleaching also occurring in both 2002 and 2003 followed by outbreaks of coral disease (Peter Craig, pers. comm.). Earlier qualitative surveys revealed that diseases are present on coral reefs in American Samoa (Work and Rameyer 2005) but no quantitative baseline coral disease surveys had been conducted. A critical component of monitoring the health of reefs is to have baseline 'before' data with which to compare 'after' conditions (Porter et al. 2001, Santavy et al. 2001). We did baseline disease surveys at seven sites around Tutuila in June 2004 and January 2005. These were prompted by anectodal reports of coral disease outbreaks by local conservation agencies in American Samoa. The objectives of the study were to: 1) document the distribution and prevalence of disease in the major genera of corals and distribution and abundance of disease in crustose coralline algae; 2) systematically describe gross and microscopic morphology of lesions in corals 3) determine whether there are seasonal differences in prevalence of disease.

Methods

The distribution and prevalence of diseased corals and crustose coralline algae were documented at seven sites in American Samoa: Vatia, Tafeu, Fagaitua, Faga'alu, Fagatele Bay, Leone and Maloata. To examine potential seasonal differences in disease levels these sites were surveyed in June 2004 and re-surveyed in January 2005. We documented levels of coral and coralline algae disease at each of the sites using two 25 m belt transects with visual counts. The two transect lines were laid end to end along depth contours separated by approximately 5 meters. A team of two divers swam along the transect, with one diver identifying corals to genus and enumerating colonies, while the other diver recorded presence of

disease. Width of the transect was 1-2 m for colony counts and 6 m for disease assessment. Diseased corals and coralline algae were photographed and a general description of the condition recorded (Work and Aeby 2006, Work and Rameyer 2005). Samples of diseased coral (and healthy portions for controls) were collected for histopathological analyses.

Enumeration of individual CCA colonies was not possible so CCA cover was determined using the point-intercept method whereby the substratum underlying the tape measure was recorded at set intervals. At each site one to two stations were surveyed depending on time availability.

For histopathology, corals were processed using standard techniques and stained with hematoxylin and eosin. Special stains were used as appropriate to identify fungi, bacteria, algal filaments, or protozoa. On histology, lesions were classified as depletion of zooxanthella, atrophy, uncomplicated necrosis, necrosis associated with fungi, algae, protozoa, or metazoa, and hyperplasia of basal body wall.

For corals, prevalence of lesions was calculated by extrapolating colony counts within the 25 X 1 m transect to the wider 25 X 6 m disease survey area and using this as the denominator of prevalence calculations, e.g. (number of colonies with lesions/total number of estimated colonies)* 100. Abundance of CCA disease was determined by calculating the number of CCA lesions per square meter of CCA surveyed. Frequency of disease occurrence (FOC) was defined as the number of sites having corals or CCA with disease (within or outside of transects)/ total number of sites surveyed. FOC reflects the spatial distribution of diseases on reefs. Data were not normally distributed even with transformation so a Wilcoxon signed rank test was used to test for seasonal differences in coral disease prevalence and CCA disease abundance. For each survey period (2004 and 2005), a chi square homogeneity test was used to look for differences in the distribution of the number of diseased vs. healthy colonies among the six most common scleractinian genera (*Acropora, Pavona, Porites, Pocillopora, Montipora, Leptastrea*)

Results

Overall occurrence of disease

During surveys in 2004 and 2005, twelve different coral disease states (or lesion types) were documented from eight coral genera on the reefs of Tutuila (Table 1). Coral disease was found at all seven sites each year but the overall proportion of colonies examined that had lesions (prevalence) was low (avg.=0.18%) (range=0.04-0.36%) (Table 1). There were two diseases of crustose coralline algae (CCA) found: coralline lethal orange disease (CLOD) (Littler and Littler 1995) and CCA black fungal disease (Littler and Littler 1998).

Distribution, frequency of occurrence and prevalence of each disease state

Distribution and prevalence of diseases in corals and CCA varied among sites (Table 1). *Acropora* white syndrome (AWS) and *Acropora* growth anomalies (AGA) were the most widespread diseases. AWS was found at 5 of 7 sites (FOC=71.4%) and AGA was found at 4 of 7 sites (FOC=57.1%). The overall prevalence (all sites combined) of each disease was averaged between years and revealed that *Porites diffuse* tissue loss, *Acropora* growth anomalies and *Acropora* tissue loss to be the most commonly encountered lesions (Table 1). Prevalence of *Lobophyllia* diffuse tissue loss, *Pavona* growth anomalies and *Goniastrea* growth anomalies were not calculated as the diseases were present at the study site but not within the belt transects.

CLOD was a common CCA disease found at 4 of the 7 sites (57%) in 2004 and 43% of the sites in 2005 whereas CCA black fungal disease was only found within Fagatele Bay. The number of CLOD infections per m^2 of CCA ranged from 0 to 0.37 and the number of CCA black fungal disease within Fagatele Bay was 0.003 infections per m^2 of CCA.

Difference in disease levels among coral genera

Prevalence of disease differed among the coral genera within each year surveyed (2004: X^2=113.0 df=5, p=0.00; 2005: X^2=29.06, df=5, p=0.00). Of the six most abundant scleractinian coral genera found within our transects, *Acropora* had the highest average prevalence (0.85%) followed by *Pavona* (0.14%), *Porites* (0.11%), *Montipora* (0.06%), *Leptastrea* (0.06%) and *Pocillopora* (0%). One colony with growth anomalies was found in *Goniastrea* and a single colony with diffuse tissue loss in *Lobophyllia* but no signs of coral disease were found in any of the other coral genera that were surveyed.

Seasonal differences in coral and CCA disease on Tutuila

There were no seasonal differences found in coral disease prevalence or CLOD abundance. Average coral disease prevalence was 0.21% (SE$\pm$0.08) in Winter 2004 as compared to 0.15% (SE$\pm$0.05) in Summer 2005 (Wilcoxon signed rank, n=14, p=0.29). CLOD levels were 0.039 (SE$\pm$0.03) CLOD/m^2 CCA in winter as compared to 0.049 (SE$\pm$0.03) in summer (Wilcoxon signed rank, n=14, p=0.88).

Table 1. Prevalence and distribution of lesions in corals and distribution and abundance of lesions in crustose coralline algae. 'X' indicates presence of a lesion at a site outside of the survey area. Data show average prevalence (%) of coral lesions or abundance of lesions for CCA (# lesions/m^2) from surveys in 2004 & 2005 in Tutuila, American Samoa.

	avg. overall prevalence (±SE)	SITES						
		Maloata	Tafeu	Vatia	Leone	Fagatele	Faga'alu	Fagaitua
Acropora growth anomalies	0.47 (0.24)	2.10		1.32	2.90	X		
Acropora ciliate disease	0.04 (0.04)			0.14				
Acropora white syndrome	0.40 (0.24)		1.05	0.81		0.21	0.54	0.27
Lobophyllia diffuse tissue loss	0							X
Porites diffuse tissue loss	0.55 (0.55)					1.00		
Porites multi-focal tissue loss	0.013 (0.013)				0.11			
Pavona endolithic hypermycosis	0.14 (0.14)		0.75					
Pavona growth anomalies	0						X	
Montipora growth anomalies	0.02 (0.02)	0.49						
Montipora endolithic hypermycosis	0.04 (0.04)	0.11	0.12					
Leptastrea growth anomalies	0.06 (0.06)		0.17					
Goniastrea growth anomalies	0				X			
CLOD (# lesions/m^2 CCA)		0.01		0.09	0.02	0.12	0.37	0.04
CCA black fungus (# lesions/m^2 CCA)						0.003		

Histology

We examined tissue specimens from 100 colonies comprising at least 30 species of corals. In some instances, tissue loss in corals was explained by presence of feeding fish (fish bites) or encrusting organisms (barnacles or sponges). Histology in such cases, usually showed abrupt cessation of tissue. In contrast, tissue loss of unexplained etiology (potential disease lesions) usually showed necrosis sometimes associated with algae or sponges. A single plating *Acropora* with a distinct circular pattern of tissue loss revealed microscopic evidence of ciliate invasion into tissues. The gross lesion, as well as the microscopic evidence (ciliates), were both distinct from AWS. Discoloration in corals took two forms (pale and dark purple). The pale discoloration found in *Favia/Favites* was, on microscopy, attributed to mucus sheathing and not considered abnormal. Presence of diffuse, irregular dark purple discoloration in *Pavona* and *Pssamocora* were attributed to overgrowth of skeleton by endolithic fungi leading to necrosis of overlying tissues a disease we term endolithic hypermycosis (Work et al. 2008b). Growth anomalies in both acroporids and montiporids showed proliferation (overgrowth) of the basal body wall. .

Discussion

From surveys in June 2004 and January 2005 we documented 12 different coral disease states from the reefs of Tutuila and two diseases of crustose coralline algae (CCA). *Acropora* white syndrome (AWS), *Acropora* growth anomalies (AGA) and CLOD were the most common diseases on the reefs of Tutuila. White syndrome (defined as focal to multifocal to diffuse, acute to subacute, tissue loss) in *Acropora* is widespread across the reefs of the Indo-Pacific, and has been found from Australia (Willis et al. 2004) out to the remote reefs of the Northwestern Hawaiian Islands (Aeby 2006a, Aeby 2006b). In some regions, white syndrome in acroporids and other coral genera is presumably caused by bacteria within the family Vibrionacae (Sussman et al. 2008). Whether this is the case in other regions remains to be confirmed and further studies are currently underway to examine potential causes of AWS within American Samoa which should shed light on whether the underlying etiology of AWS is similar across regions within the Indo-Pacific.

Acropora growth anomalies (AGA) were common in Tutuila, which is in contrast to the GBR where they are seldom found even though acroporids are the dominant coral (Willis et al. 2004). Work et al. (2008a) compared AGA levels in American Samoa, Johnston Atoll and the northwestern Hawaiian Islands and suggested that occurrence of AGAs may be influenced by environmental factors. Similarly, the occurrence and rate of progression of black band disease are influenced by reduced water quality (Kaczmarsky et al. 2005, Voss et al. 2006).

CLOD, a bacterial disease that kills coralline algae, was originally reported from the Cook Islands in 1993 and subsequently spread throughout the South Pacific including American Samoa where it was first observed in 1995 (Littler and Littler 1995). We found that it is still common on the reefs off Tutuila suggesting it is a chronic disease on these reefs. The black fungal disease found on CCA has a limited distribution in the Indo-Pacific and has only been reported from Tutuila, American Samoa (Littler and Littler 1998).

Prevalence of coral disease was found to be low (avg. <1%) on the reefs of Tutuila, which contrasts with other studies. Dalton et al. (2006)

found disease prevalence ranging from 6.21% to 13.6% on reefs in the Solitary Islands, Australia. Raymundo et al. (2005) surveyed two regions in the Philippines and found the mean disease prevalence to be 8.3%. However, Haapkyla et al. (2007) surveyed reefs in southeast Sulawesi, Indonesia and reported overall prevalence to be 0.57%. Why some regions within the Indo-Pacific seem to be more affected by coral disease than others is still unknown. Coral disease research is in its infancy in the Indo-Pacific and more information is needed on the ecology and causes of coral diseases in order to properly interpret the patterns encountered in the field.

We found *Acropora* to be disproportionately affected by disease in Tutuila. *Acropora* appear to be particularly susceptible to disease and this same pattern is emerging Indo-Pacific wide being found on the GBR (Willis et al. 2004), Sulawesi, Indonesia (Haapkyla et al. 2007) and the northwestern Hawaiian Islands (Aeby 2007). *Acropora* are also highly susceptible to bleaching (Marshall and Baird 2000) and so should be monitored closely in the Indo-Pacific as we face future problems associated with global climate change. Increased sea surface temperatures as well as ocean acidification are predicted with global climate change as well as the resultant increases in coral bleaching and disease (Hoegh-Guldberg 1999, Hoegh-Gulberg et al. 2007).

Seawater temperature can influence disease occurrence. For example, black band disease in the Florida Keys is much more common in the warm summer months and virtually disappears from the reefs during the colder winter months (Kuta and Richardson 1996). In contrast, we found no seasonal differences in overall level of coral or CCA disease for any of the diseases in American Samoa. In Hawaii, the coral disease *Porites* trematodiasis also showed no seasonal variation (Aeby 2007). Differences in seasonal variation among coral diseases may be partially explained by degree of temperature fluxuation within the different regions. For example, the sea surface temperature (SST) in America Samoa only varies between seasons by approximately 2 degrees Celsius whereas in the Florida Keys, SST can vary up to 10 degrees Celsius. Perhaps the small seasonal differences in SST in American Samoa do not affect disease levels as strongly as found in other regions. During the two time periods that we conducted our surveys, the average SST varied by less that one degree Celsius. However, the manner in which a disease responds to a specific environmental stressor will also depend on what is causing the disease (etiology) and the host affected by the disease.

Histology proved to be a critical tool in understanding disease processes and allowed for the the detection of potential causative agents of lesions, particularly in cases where gross lesions had no obvious explanation in the field. For example, tissue loss due to trauma (either through predation or other insult) could be differentiated from tissue loss due to disease based on microscopic changes in tissues such as necrosis and presence or absence of associated factors such as algae, sponges, fungi, or protozoa. We also found that the mottled discoloration observed in faviids was associated with normal mucus sheathing. In some cases, histology also proved useful in understanding the potential etiology of a gross lesion. Dark purple discoloration observed in *Montipora* and *Pavona* was found to be associated with overgrowth of endolithic fungi (Work et al. 2008b). On the other hand, some corals manifesting different types of lesions grossly, had similar microscopic manifestations. For example, six distinct morphological types of growth anomalies were found in *Acropora*, yet on histology, hyperplasia of the basal body wall with absence of or reduced polyp formation was found to be the consistent microscopic change for all types of GA types suggesting perhaps a similar underlying etiology (Work et al. 2008a). In contrast, Domart-Coulon et al. (2006) found normal polyp development in *Porites* growth anomalies emphasizing the utility of histopathological analyses in understanding coral lesions. Comparing in situ photographs with follow-up histological analyses on lesions also provides the opportunity for a more accurate interpretation of lesions in corals in the field.

Future efforts should concentrate on elucidating the mechanisms and potential causes of major diseases on reefs from American Samoa. Given their frequency of occurrence, two candidate diseases for such studies would include growth anomalies and white syndrome in *Acropora* sp.

Acknowledgements The American Samoa coral reef advisory group funded this work. The National Park Service of American Samoa and the American Samoa Department of Marine and Wildlife Resources provided critical logistical support.

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