# ASSESSING THE EFFECTS OF SEWAGE ON CORAL REEFS: DEVELOPING TECHNIQUES TO DETECT STRESS BEFORE CORAL MORTALITY

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One issue coral reef researchers and managers are faced with is assessing the impact of sewage outfalls on coral reefs. Questions regarding how much and what kind of sewage can be tolerated on coral reefs are frequently asked (e.g., Should the level of treatment be secondary or tertiary?, Should the sewage pipe be extended into deeper water?). These questions are further complicated by financial considerations. Monitoring and assessing coral reefs usually consist of coral coverage and species diversity measurements. These data are useful in providing a snapshot of the coral reef, but fail to provide predictive data in a timely fashion as required for decision making. Techniques to detect stress before mortality and with predictive value are needed to conserve coral reefs (Brown, 1988).

Sewage pollution on coral reefs has been reviewed by Pastorak and Bilyard (1985). Sewage consists of nutrients, sediments and toxic substances, all of which may impact coral reef ecosystems. Case studies of coral reefs subject to sewage outfalls exist from the Pacific (e.g., Hawaii, Maragos and Chave, 1973; Smith et al., 1981; Evans et al. 1986) as well as the Caribbean (e.g., Barbados, Tomascik and Sander, 1985; 1987). The effects of sewage on coral reefs are debated (Dollar, 1994; Grigg and Dollar, 1994; Brown, 1997).

Typically, data are lacking for periods prior to effluent discharge, making application of the statistically powerful 'Before-After-Control-Impact-Paired' design impossible (BACIP; Stewart-Oaten et. al., 1986). Relaxation experiments are an alternative, if it is possible to stop the activity (i.e., sewage effluent) and use the 'affected' observations as the baseline as in the Kanehoe Bay, Hawaii Sewage Case Study (Maragos et al., 1973; Smith et al., 1981; Evans et al. 1986). However, this is not possible in most cases. Recognizing that limited before data exist, it is still possible to obtain meaningful data by using a variety of physiological and ecological tests on carefully selected and appropriate species. A practical alternative may be to transplant indicator species coupled with simultaneous physiological measurements at affected and reference sites.

Techniques for assessing growth rates of corals over various time scales and in relation to several water parameters have been described by several authors (e.g., Dodge and Vaisnys, 1977; Neudecker, 1983; Spencer-Davies, 1990; Davies, 1995). Along a eutrophication gradient in Barbados, a reduction in reproductive effort was found (Tomascik and Sander, 1987) for *Porites porites* (Pallas, 1766). In Guam, we are testing techniques that use survivorship, growth, fecundity and other population parameters to quantify sublethal effects of sewage stress in corals. Here we describe these techniques and report initial data. The well studied, common Pan-Pacific coral, *Pocillopora damicornis* (Linnaeus, 1758) has been chosen as our experimental species for its reproductive characteristics (monthly production of brooded planulae larvae) and wide distribution pattern (from the Red Sea to the eastern Pacific). Further, *P. damicornis* can be readily transplanted with no observed stress effects (Birkeland et al. 1979; Neudecker, 1979).

#### Methods

STUDY SITES .--- To assess the effect of sewage on coral survivorship, growth and fecundity, field experiments were set up at four reef sites on the north west coast of Guam (Fig. 1). These four reef sites include the Tanguisson Sewage Outfall site (TSO), Haputo Point (HP), Shark's Hole (SH), and the Piti Intake Channel (PIC). As the name implies, TSO is subject to effluent of primary-treated domestic sewage discharged at a rate of three to four million gallons per day. The outfall pipe extends 50 m offshore. At the end of the pipe, 17 diffusers (spaced at 10 m intervals) are oriented parallel to shore (Richmond, 1990). HP and SH are located north approximately 1.6 km and 0.8 km, respectively, from the outfall and were chosen as reference sites. The prevailing current flows southerly and offshore along the northwest coast of Guam so that the sewage effluent is carried offshore and in a southerly direction (Jones and Randall, 1973). The first three study sites (TSO, HP, SH, depth  $\sim 10$  m) are located on the seaward slope of fringing reefs that are similar in habitat with the exception that the TSO site is subject to sewage outfall (Jones et al., 1976). P. damicornis occurs at these three sites mostly on the reef flat and reef margin (Jones et al., 1976; Neudecker, 1977). At depths of 10m and greater, there is little to no colonies of P. damicornis due to fish predation (Neudecker, 1977). Although P. damicornis can grow well and survive in deeper habitats, it is generally not found in deeper areas of the reef in the Indo-Pacific (Neudecker, 1977; 1979). PIC is a manmade waterway which supports abundant populations of P. damicornis on patch reefs (depth 3-5 m) and was used for collection of colonies and as a 'control' site for the fecundity study. Naturally occurring populations of P. damicornis at TSO, HP, SH and PIC appear healthy with no observable signs of stress (e.g., mucus secretion, bleaching). At each of the study sites, standard methods (Parsons et al., 1984) are being used to monitor, on a bi-monthly basis, the environmental variables of phosphate, nitrate, nitrite, chlorophyll a, flow, light, sedimentation and temperature.

SURVIVORSHIP AND GROWTH.—To determine if survivorship and growth of *P. damicornis* decreases with environmental quality, planulae were collected (Richmond and Jokiel, 1984) from five different adult colonies and grown out in the laboratory for 1 mo. This allows us to obtain 1 mo-old coral replicates while removing some degree of genetic variability. Coral branches (length 10 cm) were gently removed at the base from the adult colonies or parents. Adult colonies are defined as sexually mature (more than 2 yrs old). The two age classes (1 mo and adult) were attached to the surface of concrete cement blocks (40 cm  $\times$  20 cm  $\times$  10 cm) using a non-toxic cement mixture (seven parts Portland cement to one part Plaster of Paris). One day prior to being secured to the cement block and transferred to the field the adult colonies were alizarin red stained for 8 h while the 1 mo replicates were measured directly with calipers. There were five replicates per each age class per cement block with a total of 30 cement blocks (10 blocks for each study site).

All cement blocks were transferred to the field in coolers and transplanted to one of the study sites. Ten cement blocks were chosen at random and secured with the non-toxic cement mixture to non-living reef substrate (depth  $\sim 10$  m) at one of the three sites (HP, SH, TSO). Cement blocks were caged to prevent the confounding factor of fish predation on the corals (Neudecker, 1979). Cages were rectangular (40 cm  $\times$  20 cm  $\times$  23 cm) and constructed of black plastic aquaculture netting (gridmesh 4 cm  $\times$  4.5 cm). The experimental design is a randomized complete block. No signs of stress were observed for either coral class (adult and recruit) at the three sites (TSO, HP, and SH) before, during, and immediately after the transplantation process.

Each site is visually inspected bi-monthly and any mortality found among the replicates of each age class is noted. After 8 mo, all coral blocks will be collected from each study site and transported back to laboratory to determine growth rates. The growth rate of the 1 mo age class will be determined by increases in their diameter according to the methods of Maragos (1974). Growth rate of the adults will be determined by direct linear measurements of skeletal increase from the alizarin red stain line on the central top branches (Kinzie and Sarmiento, 1986). A two-way ANOVA will be used to test for significant differences in survivorship and in growth rates among the study sites and the each age class per site (TSO, HP, and SH) as of 6 mo.



Figure 1. Map of Guam indicating location of study sites. The reference sites of Haputo Point and Shark's Hole are located north of the impact site of Tanguisson Sewage Outfall. Piti Intake Channel is located south of the impact site and serves as the "control" study site. All adult colonies of *Pocillopora damicornis* used in the study were collected from Piti Intake Channel.

FECUNDITY.—To determine if fecundity decreases with environmental quality, 48 *P. damicornis* colonies ranging in size from 15 to 20 cm in diameter, were collected from Piti Intake Channel (PIC) (depth 3–5 m). All colonies appeared healthy and were transported in coolers to one of three reef sites (HP, SH, TSO). Sixteen colonies were assigned randomly to a reef site for transplantation. In addition, 16 *P. damicornis* colonies transplanted back into PIC and transplanted back into the channel. Ninety-four percent of the colonies transplanted back into PIC survived. Each coral colony was cemented to non-living reef substrate at one of the four reef sites (depth 10 m for HP, SH, TSO and 5 m for PIC) with the non-toxic mixture of cement. To prevent the confounding factor of fish predation on the corals (Neudecker, 1979), all colonies were caged. Circular cages (height 18.5 cm,

diameter 28 cm) were constructed out of black plastic aquaculture netting (gridmesh 4 cm to 4.5 cm) and attached to the substrate surrounding each coral with cable ties and concrete nails. The experiment was set up in March 1999.

After 5 mo, the adult coral colonies will be collected and transported back to the lab where fecundity will be measured by direct counts of the number of planulae produced (Richmond and Jokiel, 1984). The number of planulae produced will be standardized by volume (ml) of the coral as determined by water displacement. Only portions of the coral colony covered in live tissue were included in volume measurement. An one-way analysis of variability will be used to determine if there are significant differences in number of planulae produced per coral volume among the four sites.

Here, we report fecundity data from a pilot study which compares fecundity of *P. damicornis* adult colonies (treated as described by the methods above) from TSO, HP, and SH. The adult colonies were placed at the three experimental sites in December 1998 and were collected in May 1999. Homogeneity of variance was violated even after data transformation, therefore a Kruskal-Wallis non-parametric (KWNP) ANOVA was used to test for significant differences in fecundity among the sites.

#### RESULTS

SURVIVORSHIP.—Experimental replicates were lost. At SH, replicates were detached from the surface of the cement blocks while at HP entire cement blocks containing replicates were dislodged from the reef substrata. This was most likely the result of a heavy storm swell and resulted in the loss of 15 replicates from both age classes at HP and five replicates from both age classes at SH. Percent mortality among the 1 mo age class was approximately 33 to 66% and was greater than that found for the adult age class (Fig. 2). Among the 1 mo age class, HP had twice an order of magnitude greater percentage of mortality than SH and TSO. Percent mortality among the adult age class was similar among the three sites with the greatest percentage occurring at HP.



Figure 2. Percent mortality after six months at reference sites (Sharks Hole and Haputo Point) and Sewage Site (Tanguisson Sewage Outfall) for the two age classes (1 mo and adult) of *Pocillopora damicornis*. Open bars represent the 1 mo age class while shaded bars represent the adult age class. Number of replicates per age class are reported above the bars.



Figure 3. Number of planulae produced per volume for adult *Pocillopora damicornis* coral colonies at the two reference sites (Haputo Point and Shark's Hole) and at the sewage site (Tanguisson Sewage Outfall) after a 5 mo period. Number of planulae were determined by direct counts while volume was determined by water displacement of coral skeleton covered in live tissue. Bars indicate standard error. Number of replicates are reported above corresponding bar. No data for the Piti Intake Channel is available at this time.

FECUNDITY.—Severe weather resulted in reduction of replicates at all sites for the pilot fecundity study. The circular cages came off the substrate due to a heavy storm surge. The number of replicates lost at each site was 13 for HP, 8 for SH, and 6 for TSO. The remaining replicates at the three sites were collected and used to determine fecundity. No significant differences were detected for fecundity by volume for coral colonies among sites for the pilot study (Fig 3, KWNP P < 0.05).

### DISCUSSION

The higher percent mortality of the 1 mo age class in comparison to the adult age class suggests that juvenile corals are more susceptible to stress. Others have reported that a juvenile coral's susceptibility to stress varies with age (Harriot, 1983). As corals less than 1 yr old may be more sensitive to environmental stress than adult colonies (Birkeland, 1976; Babcock, 1985), the use of corals less than 1 yr (recruits) may be recommended to increase sensitivity when assessing or monitoring environmental quality. The high percent mortality of recruits at the reference site of HP may be attributed to high wave energy that could have swept sediment or other debris onto the surfaces of the recruits and killed them. Extremely high wave energy was evident by the dislodgment and movement of three cemented concrete blocks on the reef at Haputo Point. Using coral recruits may provide data more expediently than adults and lessen the impact on natural populations if cultivated in the lab. Timely and useful data is critical for researchers and managers for decision making. An added advantage is that using laboratory cultivated corals for monitoring and assessing water quality minimizes impacts on 'healthy' reefs which are often used as collection sites.

The survivorship data will be augmented by growth rates for the two different age classes. Coral growth rates are a measurable parameter (Buddemeier and Kinzie, 1976) that integrates physiological processes into assessment and monitoring techniques

(Neudecker, 1983; Spencer Davies, 1990). If the impact site (TSO) stresses the coral then it is expected that those coral replicates will have significantly lower growth rates than the corals from the reference sites (HP and SH). However, if the impact site (TSO) favors coral growth then it is expected that those corals will have significantly greater growth rates than the reference sites (HP and SH).

Response of the coral growth rate to water quality parameters appears to be species specific. Along a eutrophication gradient in the coastal waters of Barbados, the growth rates (linear extension) of *Montastraea annularis* (Ellis and Solander, 1786) exhibited high correlation with several water quality parameters while *P. porites* showed no difference (Tomascik and Sander, 1985; Spencer Davies, 1990). Provided that coral growth rates of *P. damicornis* give a strong signal within either age class, then it may be used for monitoring and assessment techniques. However, if no significant differences are found for growth rates of any replicates then growth rates of this species may be disregarded for this technique.

No significant differences in fecundity of adult corals among the three sites may indicate several possibilities. Fecundity of *P. damicornis* may not be a useful or sensitive parameter to monitor or assess stress on coral reefs. Alternatively, the TSO site is not 'seen' as different by this coral species or does not effect reproductive output. Another explanation for the non-significant results is lack of replication as a result of severe weather. A trend for higher fecundity at the SH site in comparison to the TSO site is evident, however this cannot be substantiated at this time. Higher fecundity at the reference sites (HP, SH) and the 'control' site (PIC) in comparison to the impact site (TSO) may be confirmed upon completion of this study. This would indicate that the fecundity of *P. damicornis* (as determined by this methodology) could be used to detect sub-lethal stress among sites.

More results are forthcoming. Our initial findings suggest that survivorship of coral recruits instead of adults may be a more sensitive parameter to assess stress on reefs. Fecundity of *P. damicornis* shows potential as an indicator of sub-lethal stress.

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