THE ROLE OF CHEMICAL SIGNALS ON THE FEEDING BEHAVIOR OF THE CROWN-OF-THORNS SEASTAR, *ACANTHASTER PLANCI* (LINNAEUS, 1758)

BY
CIEMON FRANK CABALLES

A thesis submitted in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

IN

BIOLOGY

UNIVERSITY OF GUAM

SEPTEMBER, 2009

AN ABSTRACT OF THE THESIS of Ciemon Frank Caballes for the Master of Science in Biology presented 25 September, 2009.

Title: **The Role of Chemical Signals on the Feeding Behavior of the Crown-of-Thorns Seastar, *Acanthaster planci* (Linnaeus, 1758)**

Approved: _____
Peter J. Schupp, Chairman, Thesis Committee

Coral reefs are among the world's most diverse and productive ecosystems, but at the same time also one of the most threatened. Increasing anthropogenic pressure has limited the resilience of reefs to natural disturbances, such as outbreaks of crown-of-thorns seastar, *Acanthaster planci*. *A. planci* is a corallivore known to inflict large-scale coral mortality at high population densities and continues to be a reef management problem despite previous control efforts. There has been no active control of *A. planci* populations on Guam since the 1970's despite recent surveys showing that *A. planci* outbreaks continue to damage large areas of reef and is one of the primary sources of coral mortality around Guam. Large aggregations of up to 522 individuals/ha of reef were observed to feed mainly on Acroporids, especially encrusting *Montipora* and branching *Acropora*. Preferential feeding by *A. planci*, even at moderate densities, causes differential mortality among coral species, which can exert a major influence on community structure. Despite this, the underlying mechanisms involved in this feeding

behavior are still poorly understood. The role of chemical signals from the coral prey and chemoreception by *A. planci* is important in understanding prey location, prey discrimination, and the formation of aggregations. Several experiments were set-up to test these stages of *A. planci* feeding. Choice assays were conducted in a Y-maze and *in situ*, using extracts or compounds incorporated into agar-based gels at natural concentrations. Y-maze assays in the laboratory showed that *A. planci* individuals were attracted to crude extracts of *Montipora* sp., *Acropora surculosa*, and *Pocillopora eydouxi*, but did not show preference for extracts of *Porites rus*, *Porites cylindrica*, and *Diploastrea heliopora*. Further analyses of extracts reveal that the variability of betaine concentrations between coral species was not consistent with observed feeding preferences. Moreover, the 90% aqueous MeOH fraction of *A. surculosa* was the most active in terms of chemoattraction. Previously identified feeding attractants (*i.e.* a-linolenic acid and betaine) also elicited chemotaxis in Y-maze assays. In field experiments, betaine was shown to be effective in attracting *A. planci* from longer distances, while a-linolenic acid was more effective in short distances. In a natural setting, chemoreception by *A. planci* is largely influenced by the solubility of these compounds and the local hydrodynamic conditions. Furthermore, extraoral predation presumably results in tissue damage and decompartmentalization, which induces enzymatic cleavage of dimethylsulfoniopropionate in zooxanthellae to dimethyl sulfide and acrylic acid. Laboratory choice assays show that these cleavage products attract *A. planci* and could explain why individuals tend to aggregate toward colonies with partial mortality when feeding, rather than feed on intact colonies. Taken together, these results clearly indicate that chemical signals influence the feeding behavior of *A. planci* at

different stages: first, *A. planci* actively use chemical signals to locate prey from a distance and find favorable substrata; secondly, short-distance and contact chemoreception is used in prey discrimination and in determining which colonies are most palatable; and lastly, predation by *A. planci* facilitates chemical reactions that produce chemoattractive compounds and increase concentrations of attractants present in coral. Knowledge on the role of chemical signals on the feeding behavior of *A. planci* will be applicable in designing traps or bait stations as alternative tools in the control and management of outbreaks.

TO THE OFFICE OF GRADUATE SCHOOL AND RESEARCH

The members of the committee approve the thesis of Ciemon Frank Caballes presented 25 September, 2009.

_____

Dr. Peter J. Schupp, *Chairman*

_____

Dr. Laurie J. Raymundo, *Member*

_____

Dr. Alexander M. Kerr, *Member*

_____

Dr. Ross H. Miller, *Member*

Accepted:

_____ _____

Elise Ralph, Ph.D. Date
Dean, Graduate School and Research

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

General Introduction

1.1 Threats to coral reefs

Coral reefs are productive and diverse, yet fragile, ecosystems. The magnitude of coral reef degradation worldwide has been increasing at an alarming rate over the past several decades (Bruno & Selig, 2007; Gardner *et al*. 2003). Human activities such as overexploitation and destructive fishing practices, increased sedimentation resulting from poor land use, declining water quality caused by coastal pollution and eutrophication, habitat destruction through unsustainable development, and the spread of invasive species have been the major drivers of massive and accelerating decreases in coral abundance and diversity (Mumby & Steneck, 2008; Wilkinson, 2008). Attention is currently focused, to a greater extent, on potential coral reef damage brought about by phenomena associated with climate change: ocean acidification (Hoegh-Guldberg *et al*., 2007), sea-level rise (Blanchon & Shaw, 1995), bleaching events (Anthony *et al*., 2007; Glynn *et al*., 2001), and increasing frequency and intensity of tropical cyclones (Fabricius *et al*., 2008). Additionally, the rising prevalence of coral diseases (Bruno *et al*., 2007; Harvell *et al*., 2007), algal blooms resulting from overfishing (Hughes *et al*., 2007; Mumby *et al*., 2006) or mass mortalities of herbivores (Lessios, 1988), and outbreaks of coral predators (Baine, 2006; Kenyon & Aeby, 2009; Pratchett *et al*., 2009) have also caused significant, widespread coral mortality in recent years. Direct consumption of live corals, or corallivory, adversely affects coral fitness and accelerates coral decline (Rotjan & Lewis,

2008), yet little attention has been paid to the role corallivores might play in coral reef ecosystem dynamics. The most influential and perhaps the most devastating among these corallivores is the crown-of-thorns seastar, *Acanthaster planci* Linnaeus 1758.

1.2 General biology of *Acanthaster planci*

Acanthaster planci is a free-spawning, dioecious, sexually reproducing seastar (Moran, 1986) belonging to the genus *Acanthaster* Gervais 1841, the only genus under family Acanthasteridae Sladen 1889 (Birkeland & Lucas, 1990). Although *A. planci* has been regarded as a single species throughout its distribution (Nishida & Lucas, 1988), recent molecular analyses suggest that *A. planci* is a species complex consisting of four strongly diverged mitochondrial clades from the Red Sea, Pacific, Northern Indian Ocean and Southern Indian Ocean (Vogler *et al*., 2008). Other than its poisonous spines, *A. planci* resemble morphological traits of most asteroids: disk-shaped, multiarmed, pliable, prehensile, and have a high stomach surface area to biomass ratio (Birkeland & Lucas, 1990). Major internal components of the *A. planci* body include body wall, pyloric caeca, gonads, pyloric stomach, and the enormous cardiac stomach (Jangoux, 1982a). These anatomical and morphological characteristics offer several evolutionary advantages for *A. planci*, thus partly explaining why *A. planci* outbreaks occur and why predation damage to reefs has been extensive.

A. planci is a specialist coral feeder (Moran, 1986; Birkeland & Lucas, 1990) which feeds by everting its cardiac stomach through its mouth and spreading this over an area of coral tissue equal to that of the oral disc (Jangoux, 1982b). Several factors

influence its feeding preferences on certain species of corals (*reviewed by* Moran, 1986). The tube feet are used to position the stomach to fit the irregularities of the coral surface (Brauer *et al*., 1970). The stomach secretes enzymes, which digest coral tissues and products are then absorbed (Endean, 1973; Yomo & Egawa, 1978). *A. planci* can survive long periods of time without feeding (Sloan, 1980a).

The stomach surface area of *A. planci* is much larger than other asteroid corallivores (*i.e. Culcita novaeguineae*; Glynn & Krupp, 1986). This allows greater food intake and higher consumption rate, thereby allocating more nutrition for rapid growth out of the juvenile stage (Birkeland, 1989), which is more vulnerable to predation (Yamaguchi, 1973; Zann *et al*., 1987; Keesing & Halford, 1992). Like most multiarmed asteroids, *A. planci* are known for their early shift to adult feeding habits – from the juvenile diet of coralline algae to a carnivorous coral tissue diet (Sloan, 1980a), which allows small *A. planci* to outgrow predators by partitioning energy toward rapid somatic growth before partitioning energy into gonad development at the expense of body-wall maintenance (Kettle & Lucas, 1987). The multiarmed morphology of *A. planci* also means more pyloric caeca for increased digestive capacity and storage of nutrients, more gonads for increased fecundity, and more tube feet for improved locomotion and prehensile ability to attach to substratum or conform to the growth form of the coral it feeds, hence maximizing the surface area covered by the extruded stomach (Lawrence, 1987).

On the other hand, despite the abovementioned advantages, the fast growth rate, large size, high fecundity, and multiarmed morphology of *A. planci* are physiologically costly (Birkeland & Lucas, 1990). Therefore, to sustain this, *A. planci* need to have a

high level of food intake (Lawrence & Lane, 1987), which partly explains why their voracious predation cause large-scale devastation on coral communities during mass aggregations.

1.3 *A. planci* outbreaks: Causes and consequences

It is difficult to define "outbreaks" because each affected reef and each outbreak has its unique and distinctive characteristics. Some reefs may sustain a large *A. planci* population without irreversible degradation while others cannot. Despite this, several attempts had been made to define "outbreaking" and "normal" populations using different survey techniques, making it very difficult to make comparisons with each other (reviewed in Moran, 1986). This debate will go on until more is known on the interaction between *A. planci* and its coral prey and a standardized survey method is adopted. However, for management purposes, recent literature on *A. planci* control programs consider a population to be in active outbreak status when densities exceed 30 mature individuals per hectare of reef (Fraser *et al*., 2000, CRC Research Centre, 2003). This density estimate is considered as the threshold of what is "acceptable" rather than what is "normal" in most reefs. Outbreaks may arise; from a single mass recruitment event or from a progressive accumulation of starfish from multiple cohorts (Johnson, 1992). Outbreaks are generally classified into primary and secondary. Primary outbreaks involve increases in *A. planci* abundance associated with changes in local factors and have not originated from adjacent populations while secondary outbreaks have arisen from nearby outbreaks through larval input or adult migration (Endean, 1973).

The cause(s) of outbreaks has been a controversial issue and opposing ideas can be divided into two categories: one views outbreaks as natural phenomena that have occurred repeatedly in the past while the other is grounded on the premise that outbreaks are recent and novel events resulting from anthropogenic changes to the environment (Moran, 1986).

The former argues that spatial and temporal variability in *A. planci* population sizes are normal (Dana, 1970; Newman, 1970; Vine, 1970; Moore, 1978) since highly fecund mature females can produce more than a million fertilized eggs annually per individual under favorable conditions (Mundy *et al*., 1994). This view was initially supported by historical records, mostly from anecdotal information, suggesting that *A. planci* was abundant and had a high distribution in the past (Dana, 1970; Newman, 1970; Vine, 1973). However, Branham (1973) contends that these anecdotal accounts could have been in reference to normal spawning aggregations of *A. planci* rather than outbreaks. Birkeland (1981) and Flanigan & Lamberts (1981) also proposed that the incorporation of *A. planci* in Micronesian and Samoan cultures, respectively, could be indicative of its abundance in the past, but Moran (1986) argues that the occurrence of outbreaks in the past cannot be inferrred from the cultural importance of *A. planci* since this could just be a result of its sinister appearance and toxicity rather than its large population size. Another line of evidence that outbreaks have occurred in the past comes from the examination of *A. planci* skeletal elements found in abundance periodically in sediment cores over many thousands of years (Frankel, 1978; Walbran *et al*., 1989; Henderson, 1992; Henderson & Walbran, 1992). These attempts to establish a relationship between past outbreak events and the contribution of *A. planci* skeletal

elements to surface sediment have been criticized because of assumptions about post-outbreak mortality patterns, dispersion of skeletal ossicles, and dating methodology (Moran *et al*. 1986; Fabricius & Fabricius, 1992; Keesing *et al*., 1992; Moran, 1992; Pandolfi, 1992; Greenstein, 1995). Feeding scar patterns on the coral heads of massive colonies have also been used to infer past *A. planci* outbreaks, and the frequency of such scars appear to have substantially increased within the last half century compared to earlier decades (Devantier & Done, 2007). To further support the concept that outbreaks are a consequence of natural processes, Dana & Newman (1973) postulated that aggregation and migrations may be a behavioral response to the food limitation imposed by typhoon damage to coral reefs. Although this notion explains why individuals in outbreaks are all adult-sized, the amount of coral damage required to result in food limitation is much greater than that observed for most storms (Pearson, 1975). Furthermore, laboratory experiments by Lucas (1973) showed that lowered salinity and higher temperature improved the survival rate of *A. planci* larvae. These favorable conditions, which could be associated with runoff from rivers, may cause future outbreaks because a slight increase in the larval survival of a highly fecund species, such as *A. planci*, could lead to massive increases in recruitment (Lucas, 1975). This hypothesis is consistent with the observation that outbreaks in the Indo-Pacific region have occurred on reefs surrounding high islands or near continental land masses where freshwater runoff seasonally occurs (Tsuda, 1971; Pearson, 1975). While natural processes are seen to cause outbreaks in this hypothesis, the fact that anthropogenic activities may indirectly increase the occurrence of these processes should also be considered (Dana, 1970).

Supporters of the opposing view, which sees outbreaks as caused by anthropogenic changes to the environment, argue that the complexity of the coral reef community in the Great Barrier Reef and Guam could not have been attained if *A. planci* outbreaks occurred regularly in the past (Chesher, 1969a; Randall, 1972), and reefs would be primarily dominated by non-preferred coral species (Randall, 1972). With the frequency and intensity of recent outbreaks, doubts have been raised whether this could have been sustained over long periods of time throughout the history of coral reefs (Randall, 1972; Birkeland & Lucas, 1990). It has also been pointed out that almost all of the major outbreaks have occurred near centers of human populations (Chesher, 1969a; Randall, 1972; Nishihira & Yamazato, 1974). Therefore, the belief that the increased frequency and intensity of recent outbreaks are caused by anthropogenic alterations of natural environmental conditions cannot be discounted. Hypotheses supporting this view include: predator removal hypothesis (Endean, 1977), reef destruction hypothesis (Chesher, 1969a), pollution hypothesis (Fischer, 1969; Randall, 1972), and the terrestrial runoff hypothesis (Birkeland, 1982; Lucas, 1982; Brodie, 1992; Brodie *et al*., 2005). The predator removal hypothesis is based on the assumption that *A. planci* population dynamics are regulated by predation and that outbreaks are unique events which arise from the overharvesting of predators by humans (see Endean, 1973). While large *A. planci* appear to escape predation due to their poisonous spines and large size, predation pressure on smaller size classes could be high, as supported by the cryptic behavior of small individuals during the day, which indicates avoidance of visually searching predators (Birkeland & Lucas, 1990). Initially, this hypothesis referred to the triton snail, *Charonia tritonis*, as the major predator of *A. planci* (Endean, 1973). Since then, it has

been extended to include fish predators such as *Epinephelus lanceolatus* (Endean, 1977). Other reported predators of *A. planci* include, teleost species such as *Cheilinus undulatus*, *Balistoides viridescens*, *Pseudobalistes flavimarginatus* (Ormond & Campbell, 1974), *Arothron stellatus* (Keesing & Halford, 1992) and *Lethrinus miniatus* (Sweatman, 1995); other gastropod species such as *Cymatorium lotorium* (Ormond & Campbell, 1974) and *Bursa rubeta* (Alcala, 1974); crustacean species such as the shrimp, *Hymenocera picta* (Glynn, 1977) and the crab, *Dromidiopsis dormia* (Alcala, 1974); a polychaete worm, *Pherecardia striata* (Glynn, 1984); and a corallimorpharian, *Pseudocorynactis* sp. (Bos *et al*., 2008). Among these natural predators however, only *C. tritonis* and *C. undulatus* are known to be commercially exploited and there is no evidence to support that any of these proposed predators are present at high abundances. In line with the influence of predators on *A. planci* populations and the observed occurrence of outbreaks near centers of human populations, Fischer (1969) and Randall (1972) proposed that increased input of chemical pollutants, particularly chlorinated hydrocarbon pesticides (*e.g.* DDT, Dieldrin), into nearshore waters is responsible for reducing the abundance of predators of larval and juvenile *A. planci*. Nonetheless, no studies indicate that levels of chemical pollutants are abnormally high in animals near human populations. Alternatively, the reef destruction hypothesis proposed by Chesher (1969a) assumes that corals are the primary predators of *A. planci* larvae; therefore, decreasing coral abundance as a result of destructive human activities (*i.e.* dredging, blasting, and poor land use practices) subsequently leads to outbreaks. However, this does not explain the presence of outbreaks in areas that are not subject to these destructive activities (Endean, 1977). Lastly, the terrestrial runoff hypothesis pioneered by Birkeland (1982) states that

enhanced nutrient supply from river runoff, usually after periods of extremely heavy rainfall around high islands and continental land masses, elevates levels of primary production resulting in a phytoplankton bloom, which enhances the survival of *A. planci* larvae through decreased mortality from starvation (Lucas, 1982; Ayukai *et al*., 1997) or through more rapid larval development, decreasing exposure to other sources of mortality such as predation (Birkeland & Lucas, 1990). For a highly fecund species like *A. planci*, a slight increase in larval survival could lead to outbreaks of adults. Aside from river runoff, upwellings and sediment resuspension during storms (Furnas & Mitchell, 1986), and broad oceanographic features like the transition zone chlorophyll front (Houk *et al*., 2007) could also be responsible for enhanced phytoplankton levels. On the other hand, *in situ* culturing experiments by Olson (1987) showed that *A. planci* larvae develop at near-maximal rates in the absence of phytoplankton blooms, suggesting that fluctuation in larval food resources may be of little importance in explaining interannual variation in larval recruitment.

Given the complexity of biotic and abiotic interactions in coral reefs, it is more likely that outbreaks are caused by interacting processes that include some aspects of the proposed hypotheses above (Carpenter, 1997). Although this debate goes on, there is widespread consensus that the increasing intensity and frequency of outbreaks and the resulting coral mortality exerts more pressure on already stressed reefs. The dramatic effects of *A. planci* outbreaks in the Great Barrier Reef, the Indo-Pacific, and other parts of the world for the last half century have been well-documented (reviewed in Moran, 1986; Birkeland & Lucas, 1990). Feeding rates may be affected by several factors such as reef topography, extent of coral cover, coral species diversity, size class of feeding

individuals in outbreaks, and the abundance of *A. planci* in a given area (Endean, 1973). Adult *A. planci* individuals in Panama killed 5.2 m^2 of coral per year (Glynn, 1973). Chesher (1969a) observed that adult *A. planci* feed at rates twice the area of the central disk per day, *i.e.* approximately 378 cm^2 per day per animal. Field measurements of *A. planci* by Keesing and Lucas (1992) showed that feeding rates in summer (357 to 478 cm^2 per day per large adult seastar and 155-234 cm^2 per day per small seastar) were about twice that in winter (161 cm^2 per day per large adult seastar and 66 cm^2 per day per small seastar). On Guam, it was reported that in a 2 ½ year period, 90% of corals were killed along a 38-km shoreline (Chesher, 1969a). Damage of this magnitude has also been reported on the Great Barrier Reef (Done, 1985) and southern Japan (Yamaguchi, 1986). Although these reports point to the capability of *A. planci* to inflict extensive coral mortality across large areas of reef, not all outbreaks produce such destruction. Nevertheless, *A. planci* predation can have effects that cascade throughout the coral reef community (see Carpenter, 1997). Benthic macroalgae tend to immediately colonize newly available space following coral mortality (Belk & Belk, 1975), and on some reefs they are later displaced by sponges and soft corals (Birkeland & Lucas, 1990). Increased bioerosion of affected reefs could result in the damage of key features of reef community structure (Seymour & Bradbury, 1999) and the collapse of the general reef framework, consequently affecting reef fish assemblages (Sano *et al.*, 1984; Sano *et al*., 1987). Reef fish assemblages are also indirectly affected by *A. planci* predation. Increased algal abundance in affected reefs led to increases of herbivorous fish in some areas (Bouchon-Navaro *et al*., 1985), but not in other locations (Williams, 1986; Hart *et al*., 1996). Furthermore, decreased coral abundance led to a decrease in the population size of

obligate coral-feeding fishes, such as chaetodontids (Bouchon-Navaro *et al*., 1985; Williams, 1986; Sano *et al*., 1987).

Even at moderate densities, *A. planci* predation can still affect coral community structure. Selective feeding by *A. planci* on certain species of coral causes differential mortality among corals (De'ath & Moran, 1998b; Pratchett, 2007). The underlying mechanisms involved in this feeding behavior are still not well-studied. In the absence of vision, chemoreception is of great significance to relatively slow-moving predators like *A. planci*.

Although much of the "hype" generated by *A. planci* outbreaks during the late 1960's and the early 1970's has diminished through the years, there is still a general agreement that *A. planci* outbreaks are still very poorly understood phenomena and continue to be a major management problem of coral reefs despite previous control efforts. Therefore, this enigmatic predator warrants further multidisciplinary studies to have a deeper understanding of its biology and ecology.

1.4 Objectives

The purpose of this study was to describe and characterize the distribution of *A. planci* populations in relation to its feeding behavior and determine the role that chemical signals play in *A. planci* feeding preferences and in the formation of feeding aggregations. In greater detail, this study aimed to: (1) characterize the abundance and distribution of *A. planci* populations through broadscale and finescale surveys –*see* **Chapter 2**; (2) verify the role of chemical signals in the selectivity of *A. planci* for

certain coral prey – *see* **Chapter 3**; (3) characterize coral extracts and quantify betaine concentrations, and isolate attractant components based on choice assays. – *see* **Chapter 4**; (4) test the effectiveness of previously identified *A. planci* feeding attractant compounds via field and laboratory assays – *see* **Chapter 5**; and (5) present a mechanism for the formation of feeding aggregations by means of chemoattraction and determine the role of the cleavage products of DMSP as potential cues – *see* **Chapter 6**;

1.5 Significance of the study

Guam's coral reefs are home to more than 5000 species of marine flora and fauna (Paulay, 2003) and are both culturally and economically important, providing numerous goods and services to its residents (Burdick *et al*., 2008). The health of Guam's coral reefs are vital for these reasons. The sustained traditional use of coral reefs is essential in the cultural identity and social ties of the people of Guam (Guam Legislature, 1997). Moreover, coral reefs contribute approximately US$ 127 million to the island's tourism-driven economy per year (van Beukering *et al*., 2007). Despite the recognition of its critical importance, Guam's reefs continue to be subjected to threats ranging from sedimentation, freshwater runoff and associated pollutants, overharvesting of reef fishes, climate change impacts, and *A. planci* outbreaks (Burdick *et al*., 2008).

Natural disturbances are often an integral part of coral reef ecosystems (Connell, 1978) and coral reefs often recover under favorable conditions (Done, 1992). However, the increasing severity and frequency of anthropogenic disturbances have not allowed reefs to recover and significantly reduced reef resilience from natural threats, such as *A.*

planci outbreaks (Bellwood *et al*., 2004; Pandolfi *et al*., 2005). These anthropogenic threats should serve as an incentive to manage and mitigate against all other sources of coral mortality (*e.g. A. planci*), because minimizing coral loss is critical to maximizing their adaptive potential and resilience to future unknown disturbances (Marshall & Schuttenberg, 2006; Pratchett, 2009). Indeed, A. *planci* predation is undoubtedly one of the major sources of coral mortality on Guam. For this reason, further documentation on *A. planci* populations and information on their ecology is necessary to facilitate the formulation of active management and control programs. Data on the distribution of *A. planci* populations around Guam will be useful in identifying sites that require immediate control measures, particularly reefs with high ecological and socioeconomic value. Moreover, having a clearer understanding of localized outbreaks, in terms of the feeding behavior of individuals and the effects of this behavior on coral communities, is important in managing the *A. planci* populations. One crucial aspect in understanding the impact of *A. planci* predation on coral communities is defining the role of chemical signals in influencing *A. planci* feeding behavior. These information are vital in formulating management and control alternatives in the future.

CHAPTER 2

Distribution and Characteristics of *A. planci* Aggregations

2.1 Introduction

Information on the distribution and abundance of *A. planci* is essential for management (Birkeland & Lucas, 1990). Coral reefs in Micronesia and other neighboring islands had been extensively surveyed to assess *A. planci* populations in the early 1970's (Marsh and Tsuda, 1973). Between 1970 and 1972, the UOG Marine Laboratory surveyed the islands of Guam (Tsuda, 1970; Cheney, 1971; Marsh & Tsuda, 1973), Saipan and Tinian (Tsuda *et al*., 1970b; Marsh *et al*., 1971), Ponape (Tsuda *et al*., 1971b; Wass, 1972), Rota (Tsuda *et al*., 1970c), Yap (Tsuda *et al*., 1970a), Truk (Jones *et al*., 1970), Palau (Tsuda *et al*., 1971a; Marsh and Bryan, 1972), and the Caroline Islands (Bryan and Struck, 1971). As mentioned earlier, Guam's reefs were particularly hit hard by the outbreaks prior to this period (Chesher, 1969a).

Collectively, past surveys reveal that one of the main characteristics of *A. planci* populations is their patchiness in space and variability in time. This patchiness may be a result of; (1) social and reproductive mechanisms, (2) physical mechanisms, and (3) physiological and behavioral mechanisms related to predation and food supply (Valiela, 1995). Although response to other individuals rather than the environment have been known to occur in some echinoderms, true social behavior is rare and aggregations are mainly formed by the summation of individuals' reactions to environmental stimuli

(Reese, 1966). However, intraspecific attraction in relation to reproduction has been observed in *A.planci*. Cheney (1974) found that *A. planci* in aggregations exhibit uniform gonad state and size whereas isolated individuals show high variability. Moreover, Beach *et al*. (1975) suggested that a spawning pheromone could attract conspecifics to form reproductive aggregations. Physical mechanisms are also potential sources of patchiness, as habitat preference, in terms of depth, substratum, and shelter from wave action, is important to aggregations (Sloan, 1980a). Previous studies have shown that *A. planci* prefer sheltered environments (Ormond & Campbell, 1974) and avoids shallow or exposed locations where it is susceptible to wave action (Moran, 1986). Hydrodynamic models (Dight *et al*., 1990) and analysis of outbreak propagation (Reichelt *et al*., 1990) have been able to account for the distribution and patterns of *A. planci* outbreaks in the Great Barrier Reef. Other hydrodynamic influences, *e.g.* eddies and retention cells, may also affect the density of settlement at within-reef scales (Black & Moran, 1990). The behavior of *A. planci*, in terms of food availability, is similar to most corallivores, whereby areas of high coral damage are generally avoided (Dana & Newman, 1973). The availability of preferred coral prey species is also one of the major factors influencing the distribution of *A. planci* populations (Kenyon & Aeby, 2009; Pratchett, 2009). Accordingly, the distribution and abundance of preferred coral prey causes patchiness in the distribution of *A. planci* both spatially and temporally (Moran, 1986).

This chapter presents a broad estimate of the abundance of *A. planci* and describes the distribution of mass aggregations around Guam. Abundances were compared to identify temporal (*i.e.* between 2006 and 2008-2009 survey results) and spatial (*i.e.*

between windward and leeward sites, and between physiographic zones) variations in distribution. On a finer scale, two active outbreaks were characterized in terms of the abundance of *A. planci*, coral mortality resulting from *A. planci* predation, size class, and feeding preferences. The role of preferential feeding in the distribution of *A. planci* populations as well as in shaping community structure requires further understanding.

2.2 Methods

2.2.1 Study Sites

Guam is the southernmost island in the Mariana island chain. Broadscale surveys were primarily conducted at reefs along the northern and southern leeward side of the island facing the Philippine Sea, and along the windward side facing the Pacific Ocean (**Figure 1**). These regions were particularly selected since they have different amounts of rainwater runoff and levels of sedimentation, different intensities of disturbance in terms of storm-generated and monsoon-driven wave action (Kerr *et al*., 1993; Becerro *et al*., 2006), different coral communities (Burdick *et al*., 2008), and varying hydrodynamic patterns (Wolanski et *al*., 2003) .

The prevalent northeast tradewinds play a major role in generating the North Equatorial Current that sweeps by the island of Guam from east to west (Jones & Randall, 1973). This prevailing current is responsible for much of the energy that transports water along the coasts and makes the windward side more exposed to wave action. There are also existing localized eddies on the northwestern leeward side of the

island. Runoff is higher on the southern part of the island, brought by non-porous volcanic rock and stream discharge. The southwestern side typically has low coral cover caused by extremely high sedimentations rates (Richmond *et al*., 2008).

Northern leeward surveys in 2006 covered approximately 29.66 km of reef from Ypao Point up to Urunao Point while 26.85 km of reef from Gun Beach to Urunao Point were surveyed in 2008. Surveys on the southern leeward side in 2006 covered about 27.26 km of reef in Orote, Cocos, and along the southwest coastline from Dodi Beach to Shark's Pit and from Anae Island to Pinay Point. In 2008, a total of 24.58 km of reef from Anae Island to Sella Bay and from Agat Bay to Shark's Pit were surveyed. On the windward side, roughly 32.54 km of reef were surveyed in 2006; from Togcha Bay to Tagu'an Point, then from Pagat Point to Mati Point. In 2009, approximately 37.09 km of reef were surveyed from Asanite Bay to Fadian Point, then also from Pagat Point to Mati Point further north.

Finescale surveys were conducted on active outbreak sites at Tanguisson Reef (13.556097°N, 144.809399°E) on the northwest side of the island and Pago Bay (13.425079°N, 144.798274°E) on the east (**Figure 1**). These sites were selected based on the high density of *A. planci* observed during broadscale surveys.

2.2.2 Broadscale: Manta tow surveys

Broadscale surveys to document spatial and temporal variation in the abundance and distribution of *A. planci* populations and to identify outbreak sites around coral reefs on Guam were done using the manta tow technique (English *et al*., 1997). This method was appropriate in monitoring the identified impact sites because *A. planci* have highly

variable spatial distribution. While SCUBA surveys are more accurate, manta tows allowed observers to cover great distances and large areas of reef at a fairly short time with little fatigue. This technique had been extensively used for the same purpose by previous studies in Micronesia (Chesher, 1969a; Tsuda, 1970; Cheney; 1971), the Great Barrier Reef (Endean & Stablum, 1973; Moran *et al*., 1988), and the Red Sea (Ormond & Campbell, 1974) although details of the method varied.

Manta tow surveys required a minimum of 3 people: one boat driver, one time keeper and GPS coordinate recorder (usually done by the boat driver), and two observers, one on the left and one on the right, to cover a width of approximately 30-m (Tsuda, 1970; Cheney, 1971). Observers held on to a manta board connected to the boat by a 12-m rope and towed at approximately 2-4 knots every two minutes. Surveys were conducted at depths of approximately 5-m and 15-m, corresponding to two physiographic reef zones – the reef front and the submarine terrace, respectively (Jones & Randall,. 1973; Colgan, 1987). Both reef zones have relatively high coral cover compared to other reef zones (Randall, 1973) and were heavily damaged by *A. planci* predation in the past. Nevertheless, each zone has distinct characteristics in terms of water turbulence and coral composition (Randall & Jones, 1973). The time keeper marked the GPS coordinates at the end of each tow while the observers recorded the number of *A. planci*, average size of the *A. planci*, number of feeding scars on corals, which are indicative of *A. planci* predation (categories based on Bass and Miller, 1996). Other information (*e.g.* general description of benthic composition, common coral genera, and other sources of coral mortality) were also recorded.

Manta tow surveys were conducted in 2006 and 2008-2009 to look at short-term temporal variations in the distribution of *A. planci* populations and whether the direction of *A. planci* migrations are perpendicular (from one reef zone to the next) or parallel (from one reef to adjacent reefs) to the shoreline. Despite slight differences in the tow paths between surveys in each of these years (**Figure 1**), a total of 80 two-minute tows were done on each reef zone of each region for each year. Overall, 960 two-minute tows were done for both survey years.

2.2.3 Finescale: Belt transect surveys

Belt transect SCUBA surveys were done to examine identified major impact sites (*i.e.* Tanguisson and Pago Bay outbreak sites; see **Figure 1**) in greater detail than is possible with the manta tow technique and have a more accurate estimate of the number of individuals per unit area. Bass and Miller (1996) stated that SCUBA searches provide important additional information for *A. planci* surveys, enabling the detection of cryptic individuals and juveniles, which are not easily detected with the manta tow technique.

Three 25-m transects, 5-m apart, were laid parallel to the shore at a depth of approximately 10-15 m. The number of *A. planci* individuals within 1-m on each side of the transect line were counted. Twenty individuals from each transect were randomly selected for size-class determination by measuring the maximum whole body diameter (tips of opposite arms) and central disk diameter. Corals were also surveyed for feeding preferences. The abundance of each coral genus present within the belt transect was recorded and it was indicated whether each colony had a feeding scar. Since these surveys were conducted during active outbreaks in Tanguisson Reef (May, 2006) and

Pago Bay (July, 2006), most of the scars were characteristic of *A. planci* predation rather than partial mortality caused by *Drupella* spp., bleaching, or disease.

2.2.4 Data Analyses

Data from manta tow surveys were analyzed using Three-Factor Analysis of Variance (ANOVA) without replication (Sokal & Rohlf, 1995) to examine variation in *A. planci* densities between survey years (two levels), among regions (three levels), and between reef zones (2 levels). Density of *A. planci* (total count • total area covered^{-1}), expressed in individuals per hectare, was log-transformed prior to analysis to normalize data and improve homogeneity. Post-hoc comparisons between regions were done using the Tukey's Honestly Significant Difference (HSD) Method.

Feeding preference data, based on proportional mortality (number of colonies per species with *A. planci* feeding scars • total number of colonies per species^{-1}), for the 13 species present in all transects at each site were not normally distributed and did not show homogenous variance even after various transformations; thus data were analyzed using the Scheirer-Ray-Hare extension of the Kruskal-Wallis Test, a non-parametric analog of a Two-Factor ANOVA with replication (Sokal and Rohlf, 1995), using sites (Tanguisson Reef and Pago Bay) and coral species (13 most common species present in each transect at each site) as independent variables. This was followed by post-hoc comparisons using the Mann-Whitney U Test. A corrected α of 0.005 was used for each comparison to compromise between a large overall Type I error (which would happen if the same value of α is used, *i.e.* 0.05) and a large overall Type II error (which would arise with a strict application of the very conservative Bonferroni correction, *i.e.* $\alpha' = 0.05 \bullet 78^{-1} = 0.000641$). Variation in the total diameter of individuals between sites was analyzed

using Single-Factor ANOVA. Cluster analysis of a matrix using Bray-Curtis similarity indexes of proportional mortality of each species was also performed with Primer v.5 (Clarke & Gorley, 2001) to further analyze the feeding preference of *A. planci* for certain coral species.

2.2.5 Collection of A. planci

Collections of *A. planci* were conducted in sites with very high densities. Some of the removal dives were done in collaboration with government agencies (i.e. Bureau of Statistics and Plans – Guam Coastal Management Program, Department of Agriculture – Division of Aquatic and Wildlife Resources, and Guam Environmental Protection Agency). Seastars were collected using necessary protection such as gloves and tongs, and then placed inside plastic bin while underwater. As soon as these bins were full, the seastars were pulled up to the boat and placed in containers filled with seawater. The seastars were kept in concrete seawater tanks prior to being used for feeding experiments. Seastars were properly disposed after being used for the laboratory assays.

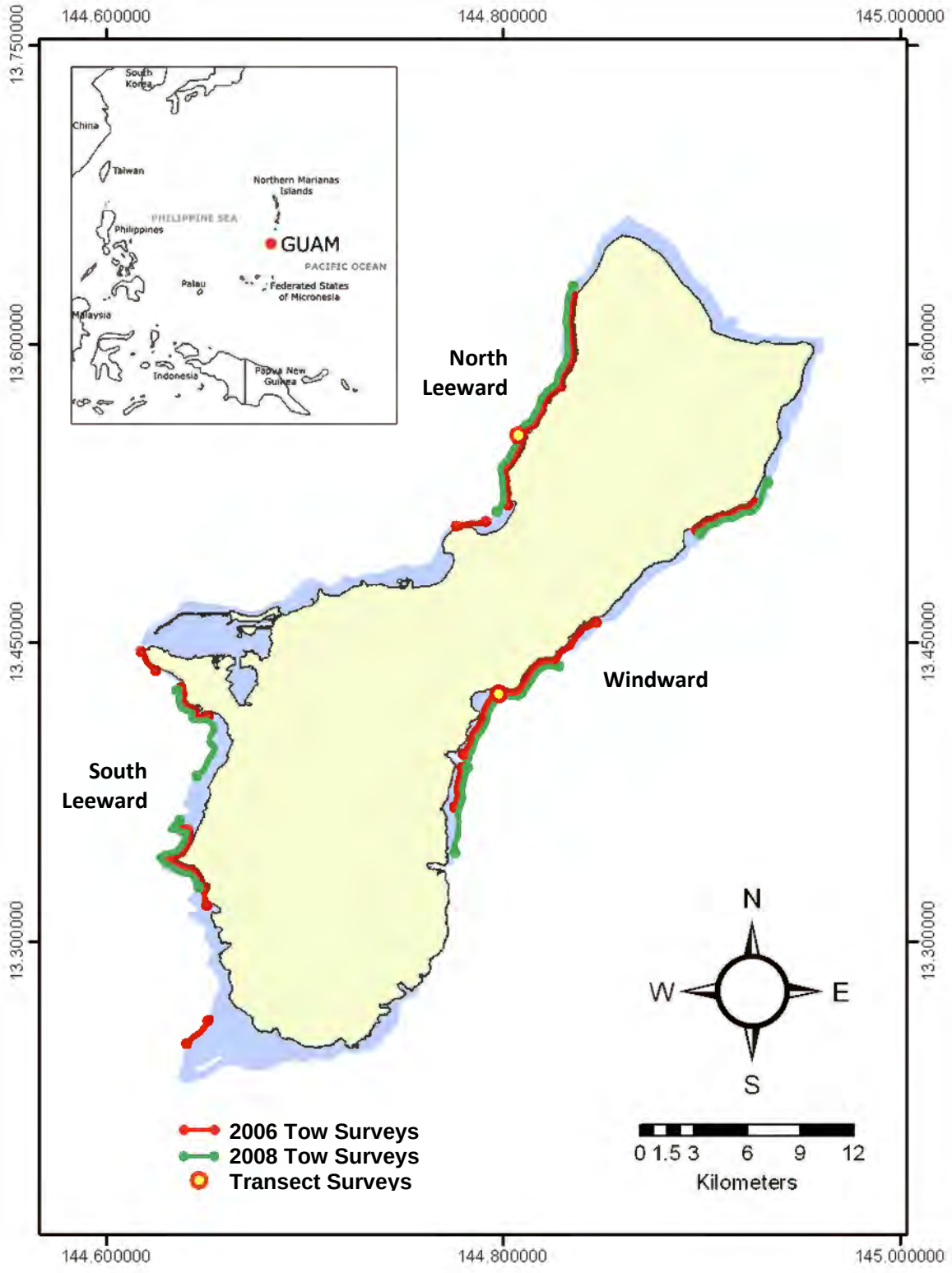


Figure 1. Sites of manta tow surveys and belt-transect surveys. Although tow paths in the 2008-2009 surveys were not exactly identical with those in the 2006 surveys, 80 two-minute tows were conducted on each region (island-side) for each survey period.

2.3 Results

2.3.1 Abundance and Distribution of A. planci

There was a significant difference in the densities of *A. planci* observed during surveys in 2006 and 2008-2009 ($F = 41.050$, $df = 1$, $p = 0.024$; see **Table 1**). More outbreaks were observed in 2006, with densities of up to 522 individuals per hectare (ind • ha^{-1}) observed during surveys from February to October, 2006 (**Figure 2**). No massive aggregations of *A. planci* were observed on the southern leeward side except for a localized outbreak in Tipalao Bay of up to 183 ind • ha^{-1}. On the other hand, several spots on the northern leeward and the windward side were affected by massive aggregations. Pago Bay was the most heavily affected area with densities of up to 430 ind • ha^{-1}, while Taga'chang Point, Ylig Bay, Lattes Point, and North of Fadian Point also had densities of more than 100 ind • ha^{-1}. The highest density (522 ind • ha^{-1}) recorded during these surveys was at Tanguisson Reef on the northern leeward side of the island. Most notably, 1569 individuals were counted during a 240-m swim search done on the same area. Densities of over 100 ind • ha^{-1} were also recorded at reefs facing NCS Beach and at Urunao Point. In most cases, aggregations were observed on the deeper (submarine terrace) tows, with the exception of aggregations of more than 100 ind • ha^{-1} on the reef front recorded at the North of Fadian Point on the windward side, and Tanguisson Reef and the reef facing Falcona Beach on the northern leeward side.

Compared to surveys in 2006, less massive aggregations were observed during surveys from October, 2008 to June, 2009 (**Figure 3**). Most aggregations were detected on the northern leeward side of the island, except for an aggregation of up to 351 ind • ha^{-}

[1] south of Mati Point on the northeast (windward) side. Aggregations ranging from 104 to 293 ind • ha^{-1} were recorded at Tanguisson Reef, Haputo Point, and south of Falcona Beach. No outbreaks were recorded within the survey path on the southern leeward side of the island. Furthermore, most massive aggregations were observed on the deeper submarine terrace zone compared to the shallower reef front zone, except for an aggregation of up to 147 ind • ha^{-1} at Haputo Point.

Overall, there was also a significant difference in density between the northern leeward, southern leeward, and windward sides of the island (F = 65.002, df = 2, p = 0.015; see **Table 1**). Post-hoc Tukey's HSD multiple comparisons showed that the northern leeward and windward sides were homogenous (q = 3.376, p=0.243) and significantly different from the southern leeward side (**Table 1[a])**. Between physiographic zones of the reef, the deeper submarine terrace had significantly higher densities of *A. planci* compared to the shallower reef front (F = 55.698, df = 1, p = 0.017; see **Table 1**). There were no significant interaction effects.

2.3.2 Coral Mortality

Coral mortality was estimated by looking at *A. planci* feeding scars on corals. Mortality, *per se*, cannot be fully determined because not all corals with feeding scars die, and some eventually recover. Regardless of whether a complete or partial mortality results from *A. planci* predation, feeding scars are useful in generally assessing the level of coral damage. During 2006 surveys, feeding scars were coincidentally common in areas where *A. planci* was abundant (**Figure 4**). On the northern leeward and windward sides, where *A. planci* was abundant, feeding scars were common (>10 feeding scars) in

18% and 22% of the tows, respectively. Feeding scars were less common during 2008-2009 surveys (**Figure 5**). Recent predation was mainly recorded on the northern leeward side, where it was common on 11% of the 160 tows conducted

2.3.3 Feeding Preferences

During manta tow surveys, mass aggregations of *A. planci* were mostly observed on reefs dominated by Acroporids (*i.e. Acropora* spp. and *Montipora* spp.). In contrast, no outbreaks or signs of recent predation were observed in reefs dominated by Poritids and Faviids. Belt-transect surveys provided more detailed observations on the feeding behavior of outbreak populations in Tanguisson Reef and Pago Bay. The most abundant coral during the outbreaks was *Montipora*, but at the same time, it also had the highest proportional mortality (**Figure 5**). Although *Galaxea* and *Porites* were relatively abundant in Tanguisson Reef (**Figure 5a**) and Pago Bay (**Figure 5b**), respectively, proportional mortality was generally low for both genera. There was no significant difference in the proportional mortality between these sites; but significant variation was observed in the proportional mortality of different coral genera ($H = 65.283$, $df = 12$, $p < 0.001$; see **Table 2**). Post-hoc comparisons revealed a well-ordered hierarchy, with *Montipora* and *Acropora* as the most preferred genera (highest proportional mortality), and *Millepora*, *Porites*, and *Goniopora* as the least preferred genera (**Figure 6**)

2.3.4 Size Class of Outbreak Populations

Almost all individuals counted during the manta tow surveys were adults (> 15 cm), except for Double Reef on the northwest leeward side of the island, where a low-

density (2-5 ind • ha^{-1}) population of juvenile *A. planci* was frequently observed. There was no significant variation in the sizes of *A. planci* between the two outbreak sites, Tanguisson and Pago ($F = 0.033$, $df = 1$, $p = 0.856$). Total diameter of individuals ranged from 248-390 mm in Tanguisson Reef and from 242-380 mm in Pago Bay (**Figure 7**). Overall, the mean size of *A. planci* in both outbreak sites is 309 ± 29 mm.

2.3.5 Collection of A. planci

A total of 1173 *A. planci* individuals were collected from April, 2006 to July, 2009 at various outbreak sites around Guam (**Table 3**). Most of the individuals collected were kept in concrete tanks at the UOG Marine Laboratory and used for several laboratory experiments, while some were processed and sent as samples to other researchers doing collaborative work.

Table 1. ANOVA Table of *A. planci* abundance and distribution. Three-Factor Analysis of Variance (ANOVA) without replication on log-transformed total density (ind • ha^{-1}) data and subsequent Tukey's HSD multiple comparisons. **Bold** values are significant at α=0.05.

SOURCE	*df*	*SS*	*MS*	*F*	*p-value*
Survey Year	1	0.537	0.537	41.050	**0.024**
Region (Island-side)[a]	2	1.702	0.851	65.002	**0.015**
Reef Zone	1	0.729	0.729	55.698	**0.017**
Survey Year x Reef Zone	1	0.000	0.000	0.001	0.978
Survey Year x Region	2	0.226	0.113	8.625	0.104
Reef Zone x Region	2	0.054	0.027	2.076	0.325
Error	2	0.026	0.013		
Total	11	3.274	0.298		

[a] Followed by Tukey's HSD multiple comparisons: north leeward vs. south leeward (q = 15.343, **p = 0.015**); north leeward vs. windward (q = 3.376, p = 0.243); south leeward vs. windward (q = 11.967, **p = 0.025**)

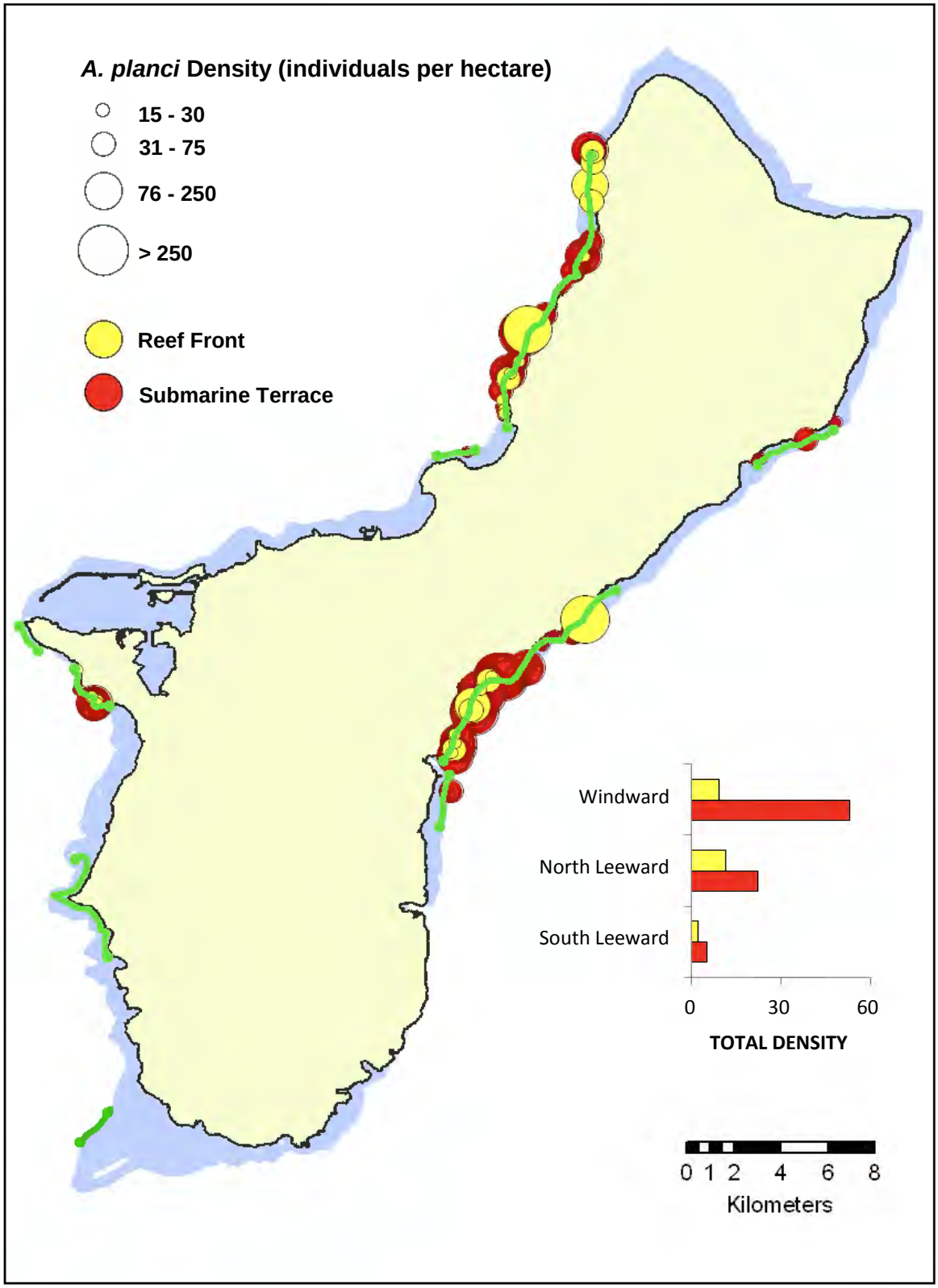


Figure 2. Distribution of *A. planci* around Guam recorded during manta tow surveys in 2006. High densities (individuals • ha^{-1}) of *A. planci* were observed on the north leeward and windward regions. Green lines indicate tow paths.

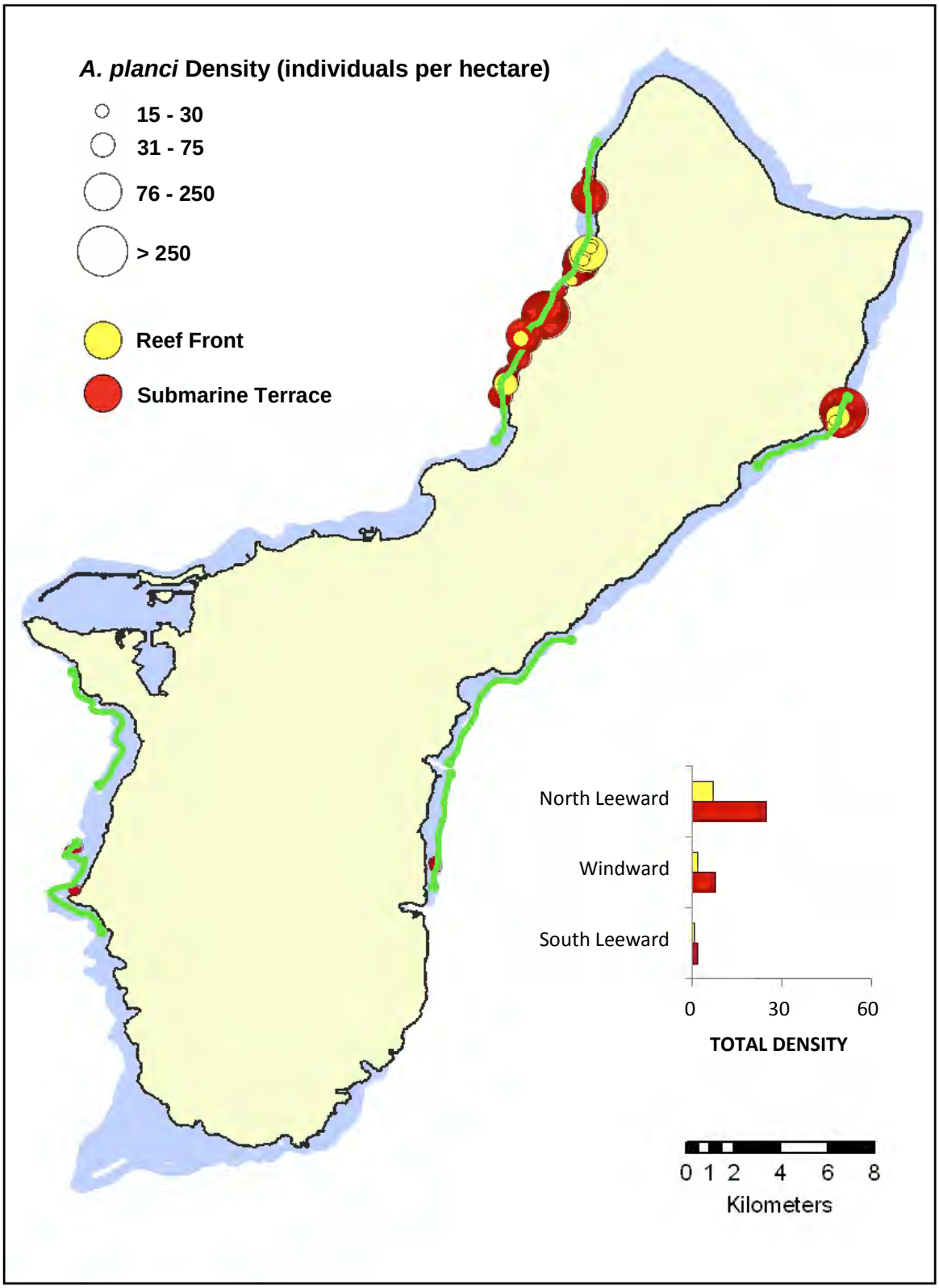


Figure 3. Distribution of *A. planci* around Guam recorded during manta tow surveys from 2008-2009. High densities (individuals • ha^{-1}) of *A. planci* were present on the submarine terrace at the north leeward region. Green lines indicate tow paths.

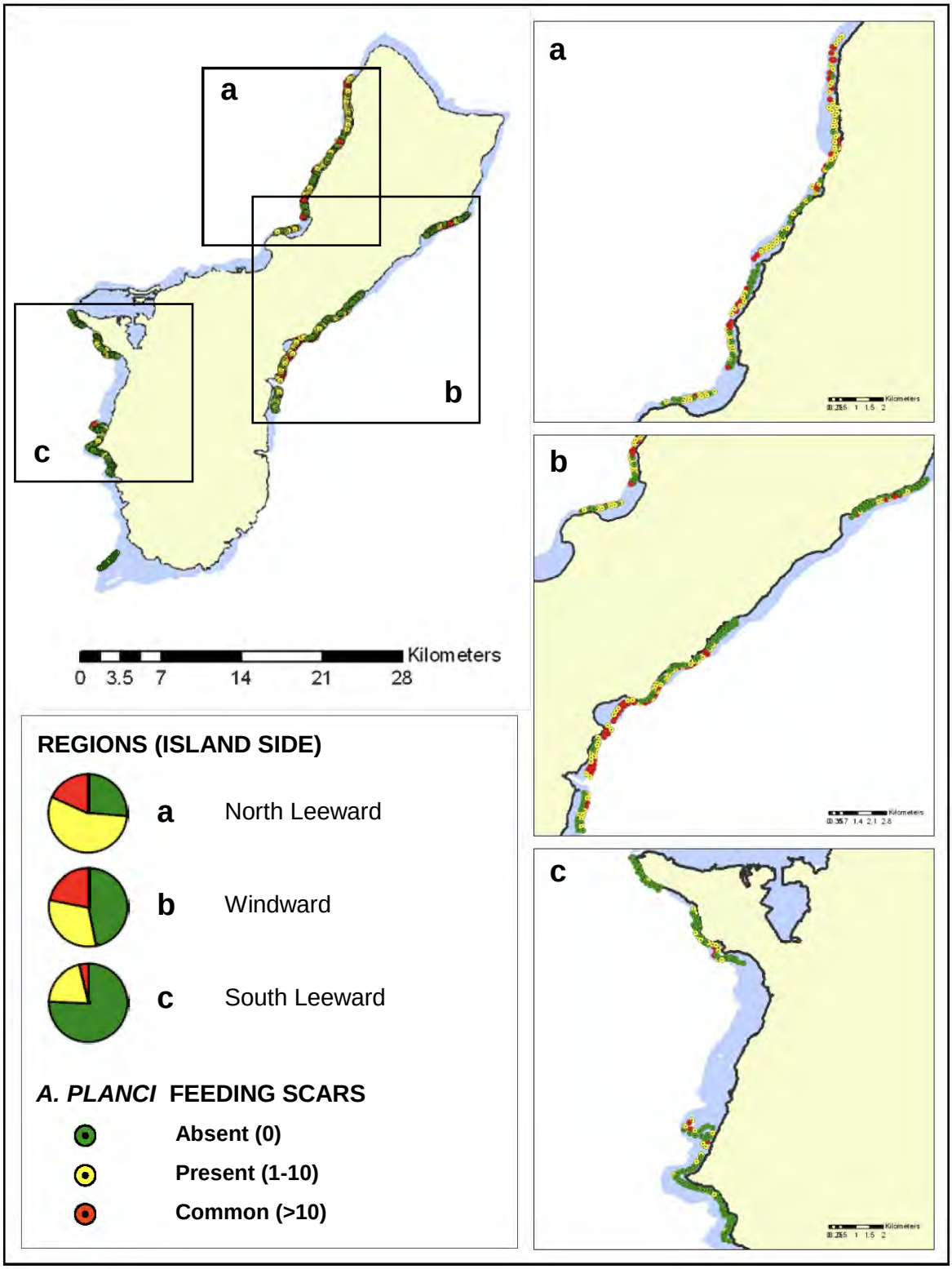


Figure 4. Distribution of *A. planci* feeding scars on corals around Guam in 2006. Pie charts show relatively high proportion of areas on the north leeward and windward regions where (partial) mortality was common or present.

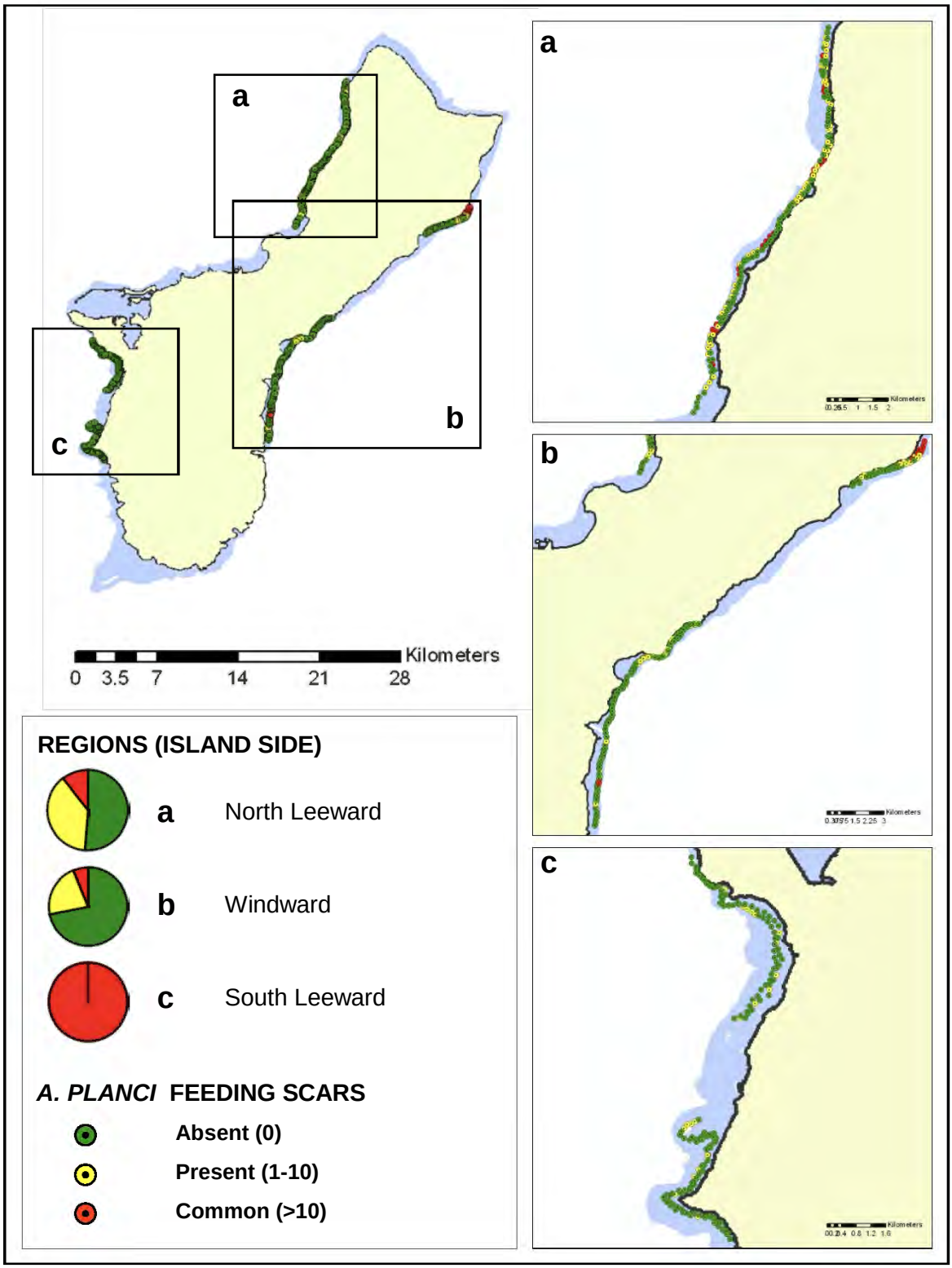


Figure 5. Distribution of *A. planci* feeding scars on corals around Guam from 2008-2009. Pie charts show low proportions of areas where (partial) mortality was common or present, with exception to some spots on the north leeward region.

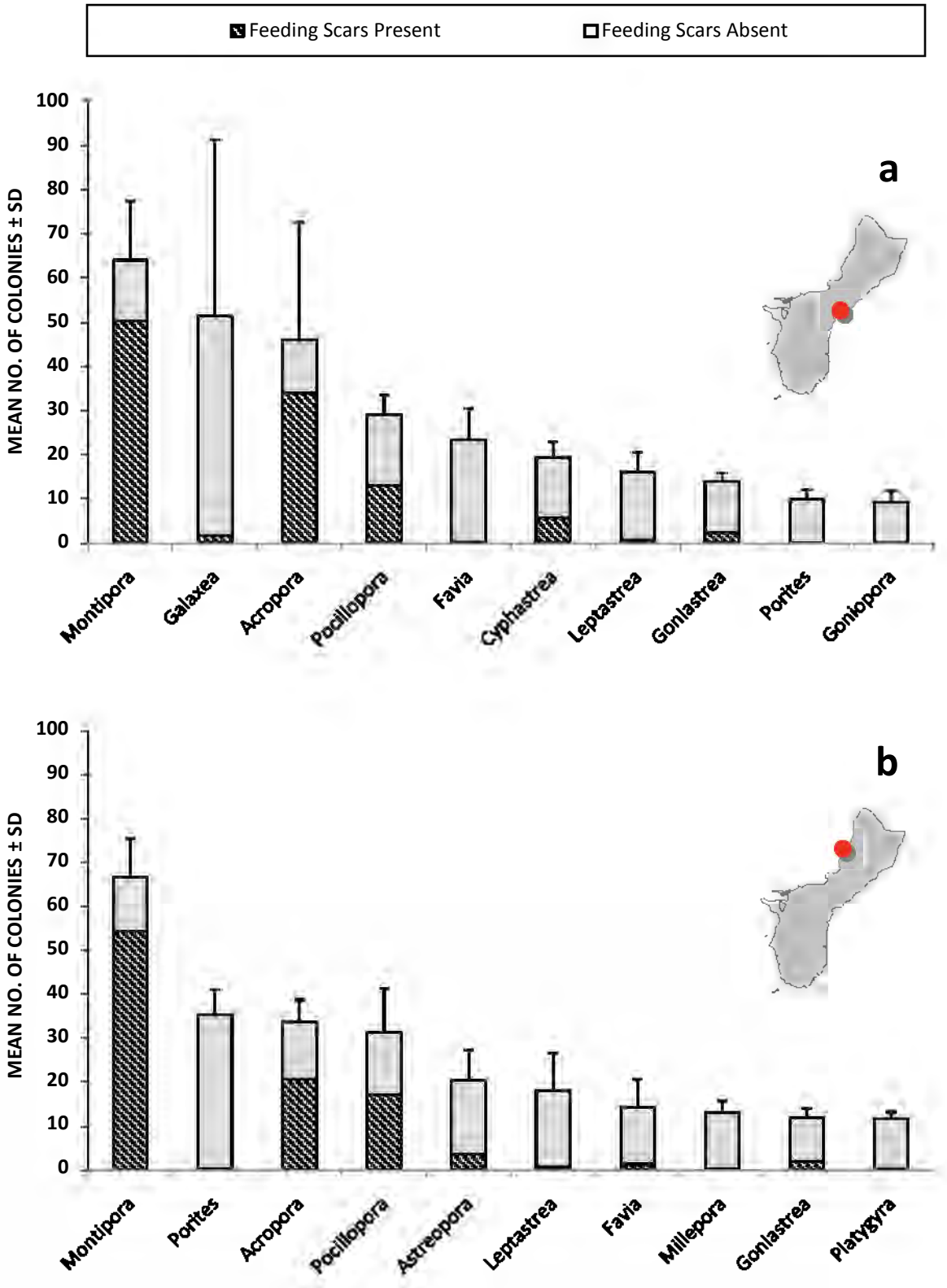


Figure 6. Proportional mortality of ten most abundant coral genera at each outbreak site: (**a**) Pago Bay, (**b**) Tanguisson Reef. Proportional mortality = mean number of colonies with feeding scars • mean no. of colonies^{-1}.

Table 2. SRH-ANOVA Table of field feeding preferences. Feeding preferences are based on number of colonies with feeding scars in relation to the total number of colonies. Scheirer-Ray-Hare extension of the Kruskal-Wallis Test with proportional mortality as dependent variable, and outbreak site (Tanguisson Reef vs. Pago Bay; $n = 3$ transects for each site) and coral genera ($n = 13$) as independent variables.

SOURCE	*df*	*SS*	*H*	*p-value*
Outbreak Site	1	108.513	0.224	0.636
Coral Genera	12	31610.833	65.283	<0.001
Outbreak Site x Coral Genera	12	465.654	0.962	1.000
Error	52	5099.500		
Total MS		484.214		

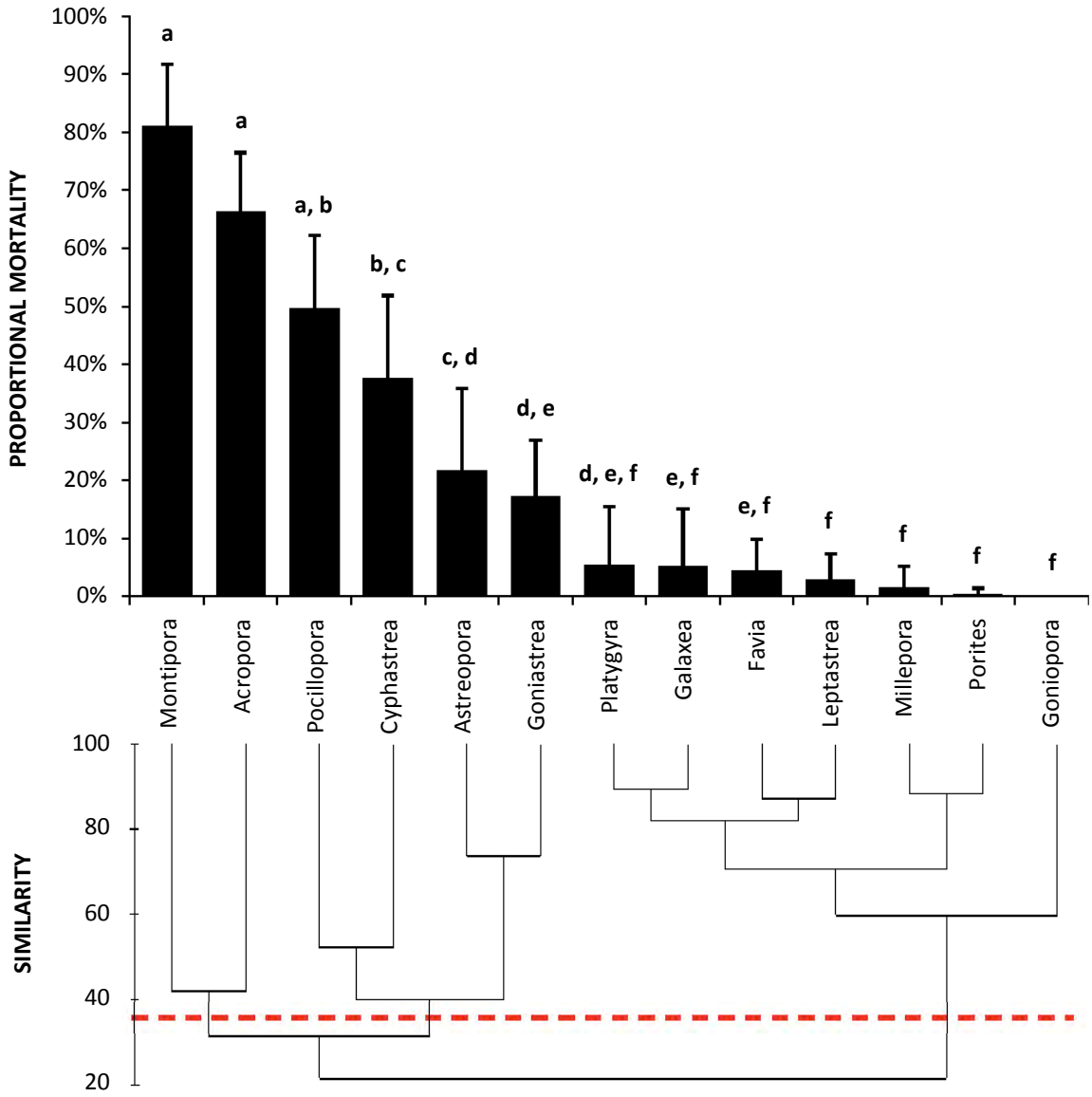


Figure 7. Hierarchy of proportional mortality (proportion of a particular coral eaten to its proportional availability) of each coral genera. Similar letters indicating no significant difference based on pairwise comparisons using Mann-Whitney U Test at $\alpha'=0.005$; and corresponding cluster analysis, with red broken lines showing preference gradient from most preferred, moderately preferred, to least preferred genera.

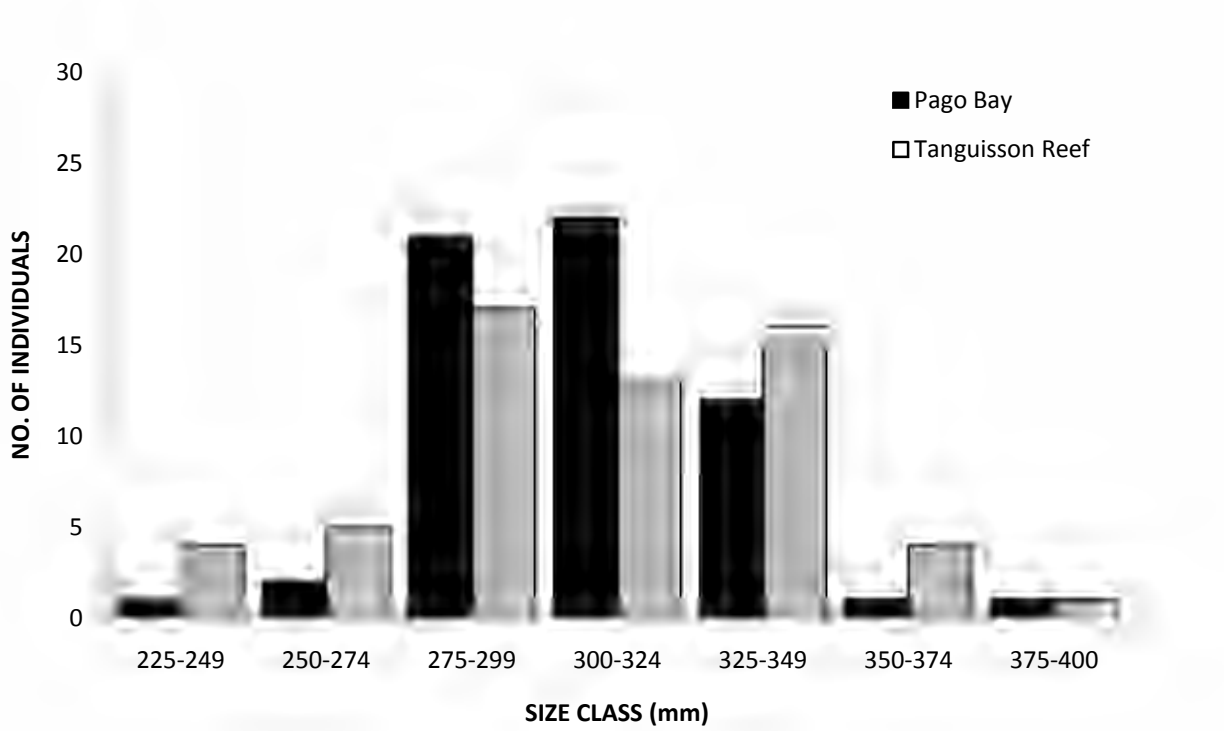


Figure 8. Size class structure of outbreak populations**:** Pago Bay and Tanguisson Reef (*n* = 60 individuals • site^{-1}).

Table 3. Collection of *A. planci* individuals from several outbreak sites around Guam.

SITE	***No. of Individuals Collected***	***Month, Year Collected***
Tanguisson Point	89	April, 2006
Tanguisson Point	133	May, 2006
Urunao Point	120	May, 2006
Taga'chang Point	71	June, 2006
North of Fadian Point	80	October, 2006
Gun Beach	74	July, 2007
Pago Bay	93	November, 2007
Gun Beach[a]	159	February, 2008
Faifai Beach[b]	182	May, 2008
Faifai Beach	167	May, 2008
South of Ague Point	55	October, 2008
Haputo Point	112	December, 2008
Haputo Point	109	March, 2009
Shark's Hole	37	July, 2009
Total No. of Individuals Collected	1481	April, 2006 to July, 2009

[a] Collection organized in collaboration with Bureau of Statistics and Plans - Guam Coastal Management Project (GCMP), Guam Department of Agriculture – Division of Aquatic and Wildlife Resources (DAWR), and Guam Environmental Protection Agency (GEPA)

[b] Collection done and organized by GCMP and GEPA with permission from DAWR

2.4 Discussion

Despite previous control efforts (Chesher, 1969b; Tsuda, 1971; Cheney, 1973), recent surveys show that high densities of *A .planci* are still present on reefs around Guam. From 2003 to 2009, population densities above 75 ind • ha^{-1} have been recorded on manta tow surveys in this study (**Figures 2** & **3**) and towed-diver studies during National Oceanic and Atmospheric Administration – Marianas Archipelago Rapid Assessment and Monitoring Program expeditions (NOAA-MARAMP, Burdick *et al*., 2008) on different locations around the island, resulting in high coral mortality on affected areas (**Figures 4** & **5**). The underlying causes of these outbreaks, however, are still unclear.

Temporal and spatial patchiness of *A. planci* populations may be a consequence of several environmental, physiological, and behavioral factors. Birkeland (1982) documented the occurrence of outbreaks of adult *A. planci* 2-3 years after intensive rains in numerous high islands in the Pacific and postulated that outbreaks originated from increased larval survival and recruitment facilitated by high nutrient levels from terrestrial runoff. The amount of runoff is influenced by rainfall, topography, soil type, and land use; in particular, runoff is typically high where rainfall is high and where rain falls on less permeable land surface (Gingerich, 2003). Mean annual rainfall on Guam is unevenly distributed (Lander & Guard, 2003) and hydrologic conditions of the southern and northern parts of the island are starkly different, *.i.e*. streams are only present on southern Guam where low-permeability volcanic rocks allow discharge of rainfall to drainage networks while northern Guam is made up of limestone, which allows virtually all rainfall to infiltrate and recharge aquifers within (Gingerich, 2003). Heavy rainfall

and high discharge flow rates at Pago Bay in late 2002 and mid-2004 (**Figure 9**), associated with typhoons Pongsona and Tingting, respectively, preceded the observation of high densities of *A. planci* on the windward side of Guam in 2006 (**Figure 2**) and in 2007 (Burdick *et al*., 2008), which is consistent with the mechanism proposed by Birkeland (1982). However, the persistence of high densities in the northern leeward side of the island despite relatively low terrestrial runoff, and the low frequency of *A. planci* in the southern leeward side despite the presence of numerous drainage networks to facilitate runoff, cannot be explained by this theory. In addition, terrestrial runoff (Birkeland, 1982; Brodie, 2005) and larval starvation (Lucas, 1982) hypotheses only suggest that high-biomass phytoplankton blooms enhance larval survival by providing required nutrition to food-limited *A. planci* to develop from bipinnaria to settlement larvae; but it does not explain the primary source of larvae.

Physical processes (*e.g.* hydrodynamic patterns) play a vital role in outbreak propagation through larval dispersal (Keesing & Halford, 1992). Rather than terrestrial runoff, Houk *et al*. (2007) suggest that a broad oceanographic feature, the transition zone chlorophyll front (TZCF), is primarily responsible for delivering enhanced phytoplankton levels ideal for *A. planci* spawning and larval survival in the northwest Hawaiian Islands. They argue that the annual southward migration of the TZCF during winter time is consistent with the reproductive biology of *A. planci* and triggers primary outbreaks in Hawaii, which are then followed by secondary outbreaks throughout the North Pacific Ocean, brought by currents associated with the North Pacific Gyre. This indicates that secondary *A. planci* outbreaks on Guam may have originated from TCZF-triggered primary outbreaks in the Hawaiian Islands through hydrodynamic-based larval transport.

(Houk *et al*., 2007). Analyses of hydrodynamic models and outbreak propagation, with emphasis on larval dispersal, have been able to account for most of the large-scale distributions and patterns of secondary outbreaks on the Great Barrier Reef (Dight *et al*., 1990; Reichelt *et al*., 1990; Black & Moran, 1991; Scandol & James, 1992). Moreover, Black *et al*. (1995) found that slow, low-frequency currents result in high local retention of *A. planci* larvae within eddy-induced well-mixed zones around reefs. On Guam, the interaction of the North Equatorial Current with the island mass results in three types of eddies: (1) intense vortices shed from Cocos Island down south, (2) a small, stationary eddy between Ritidian Point and Pati Point, and (3) a sluggish, island-size eddy in the northwest with an oceanic inflow toward the Faifai Beach area (Wolanski *et al*., 2003). These localized hydrodynamic patterns are sufficiently energetic to return eggs and larvae to their natal reefs (Richmond *et al*., 2008), and could therefore be linked to the chronic nature and close proximity of outbreaks in the northwest region of the island in the absence of terrestrial runoff. Outbreaks between Ritidian Point and Pati Point have also been reported in 2003 and 2005 (Burdick *et al*., 2008). Additionally, outbreaks on the backside of Cocos Island were also reported in 2007 and 2009 (Burdick *et al*., 2008; T.J. Donaldson, pers. comm.). The location of these outbreaks also coincide with the location of eddies described by Wolanski *et al*. (2003). However, these processes involve several stochastic components, which need to be considered in interpreting how they are associated with the distribution of *A. planci* populations.

As mentioned in Chapter 1, overexploitation of predators (Endean, 1973) and coastal pollution (Fischer, 1969; Randall, 1972) have also been associated with *A. planci* outbreaks. The higher frequency and intensity of outbreaks on Guam compared to

neighboring islands in FSM (George *et al*. 2008) and CNMI (Starmer *et al*. 2008) makes it tempting to associate outbreaks with elevated levels of overfishing and coastal development, and persistently poor water quality on Guam. Although these islands may have unique local hydrodynamic and hydrologic features, higher fishing pressure on Guam may have ultimately lead to a release from predation for adult, and more importantly, larval *A. planci*. The negative relationship between outbreaks and the abundance of potential fish predators (Dulvy *et al*., 2004) has led to the suggestion that no-take reserves indirectly protect coral reefs from *A. planci* predation (Sweatman, 2008). However, it would be hard to make conclusive correlations on this matter on Guam because there are only 5 marine protected areas (very small sample size), which were only fully implemented in 2001. In addition, outbreaks have also been recently reported within the Pati Point Marine Preserve (Burdick *et al*., 2008; this study) and Achang Reef Flat Marine Preserve (L. Raymundo, pers. comm..). Nevertheless, exploring the link between coastal pollution and enhanced survival of certain developmental stages of *A. planci* will be vital in further understanding the role of anthropogenic stressors in promoting outbreaks.

Habitat preference (*i.e.* depth, substratum, shelter from wave action) is also an important factor in the formation of mass aggregations (Sloan, 1980). It has been suggested that the distribution of *A. planci* is strongly correlated to shelter from prevailing winds and typhoon, since corallivores avoid areas of coral damage (Dana *et al*., ,1972; Dana & Newman, 1973). The low frequency of *A. planci* on the southwestern side of the island is most likely due to very low coral cover (= low food availability) on this region. Sedimentation rates, greatly exceeding published sediment-tolerance

thresholds, have ravaged southwestern reefs and significantly reduced species richness (Richmond *et al*., 2007). Large densities of *A. planci* on the more exposed leeward side, however, do not support this notion of avoiding winds and typhoons. On a local scale, *A. planci* has been observed to move along reefs in search of favorable substratum while avoiding wave action (Ormond & Campbell, 1974). The higher frequency of *A. planci* and higher coral mortality on the deeper submarine terrace compared to the more wave-washed, shallow reef front zone does follow this mechanism. Although it has been observed that the progression of mass aggregations is always from deep to shallow areas, it is not known whether *A. planci* proceed to adjacent reefs once they have reached certain depths where wave action becomes intolerable.

One common features of all *A. planci* populations observed around Guam is the size class structure of aggregating individuals (**Figure 8**). Individuals <250 mm in diameter are generally regarded as less than 2 yrs old, whereas seastars between 350 and 400 mm are generally considered as 3 yrs old or older (Stump, 1996). The unimodality of the size class structure of outbreak populations in Pago Bay and Tanguisson Reef, with the majority of individuals between 275 and 350 mm in diameter, represents an initial dominant cohort. These outbreaks, therefore, appear to have arisen mostly from a single massive recruitment event, which is typical of most outbreaks. However, the relatively higher frequency of smaller and larger size classes in Tanguisson Reef compared to Pago Bay may also be indicative of a population resulting from multiple successive recruitment events (Pratchett, 2005).

Another common feature of outbreak populations around Guam is the coral community structure of the substratum where aggregations were observed (**Figure 6**). All

heavily impacted reefs were dominated by corals from the family Acroporidae and areas dominated by non-preferred species, such as *Porites* spp. (Apra Harbor, Asan, Double Reef, Tumon) were generally avoided by adult *A. planci*. Variations in levels of coral mortality recorded for certain taxa (**Figure 7**) are consistent with patterns of prey preference known for *A. planci* on Guam and in the Great Barrier Reef (Chesher, 1969a; De'ath &Moran, 1998b; Pratchett 2001, 2007; Pratchett *et al*., 2009). It has been observed that the level of prey selectivity is higher in moderate densities compared to severe outbreaks due to food availability (Birkeland & Lucas, 1990). Less preferred species are often eaten when food becomes scarce as the outbreak progresses (Moran, 1986). However, despite densities of up to 3-4 ind • m^{-2} in Pago Bay and Tanguisson Reef, *A. planci* still exhibited a striking preference for *Acropora* spp., *Montipora* spp., and *Pocillopora* spp. (**Figure 7**). This indicates that outbreak populations were surveyed during its commencement stages based on high prey selectivity even at high densities.

The correspondence of the location of mass aggregations with the high availability of preferred prey may represent an actively directed aggregation, but long-term data on the movement of *A. planci* are essential in drawing conclusions regarding this matter. Nevertheless, short-term studies show that adult *A. planci* move at a rate ranging from 0.3 to 20 m • hr^{-1} based on observations on different substrata (reviewed by Moran, 1986). Keesing & Lucas (1992) found that movement rates are highest in areas of low coral cover. Tagged *A. planci* were observed to move at a rate of 2.8 m • day^{-1} when starfish density is low and coral cover is high, while at high starfish densities and low coral cover, they were observed to move at a rate of 10.3 m • day^{-1}. At these rates, however, annual surveys are insufficient in determining whether an outbreak population

from one location in a certain year is merely the same population that migrated to adjacent reefs. To date, there has been no study that investigated the long-term movement of *A. planci* populations and whether outbreak populations die-off once they have depleted food resources or migrate to other reefs with abundant coral prey. This lack of essential field data on longevity, growth rates, and movement are mainly due to the difficulty of tagging individuals for long-term monitoring (Birkeland and Lucas, 1990) and for lack of sufficient funding for long-term research. Nonetheless, recent advances in population genetics (Yasuda et al., 2006; Gérard *et al*., 2008; Vogler *et al*., 2008) may prove useful in establishing connectivity of *A. planci* populations.

Regardless of whether or not outbreaks are real, novel, or caused by anthropogenic stressors, there is a general consensus that the key issue is the consequences of repeated episodes of outbreaks on the integrity of reef ecosystems (Seymour & Bradbury, 1999). Although there are indications that initial recovery can occur rapidly (Colgan, 1987), more recent data from the Great Barrier Reef suggest that recovery has become progressively slower and long-term degradation of coral reefs is occurring (Seymour & Bradbury, 1999). Dead coral skeletons resulting from *A. planci* predation are immediately colonized by algae and cyanobacteria (Belk & Belk, 1975). The very low abundance of herbivorous fish (Burdick *et al*., 2008) and poor water quality (Richmond *et al*., 2008) on Guam makes matters worse in terms of coral recovery from *A. planci* predation. Coral recruitment has been observed to be steadily declining over the last 30 years on Guam (Porter *et al.* 2005). Furthermore, the present interval observed between outbreaks does not allow full recovery of affected reefs even with the most

favorable conditions based on published recovery rates (Moran *et al*., 1985; Done, 1988; Lourey *et al*., 2000; Ammar, 2005).

Although our collection efforts (**Table 3**) may have minimal impact, these types of activities are encouraged, since this is the only known management option to date. Organized collection programs in the past have indeed allowed affected corals reefs to recover (Cheney, 1973). The alarming frequency and intensity of these new wave of outbreaks around the island is an issue of concern that must be addressed by institutions tasked to manage coral reefs. Understanding the mechanisms involved in the choice of prey by *A. planci* is fundamental in determining its effect on coral communities (Pratchett, 2007). The chemical aspect of this topic is the subject of the succeeding chapters. This could help provide new insights and alternatives into the management of *A. planci* outbreaks in the future.

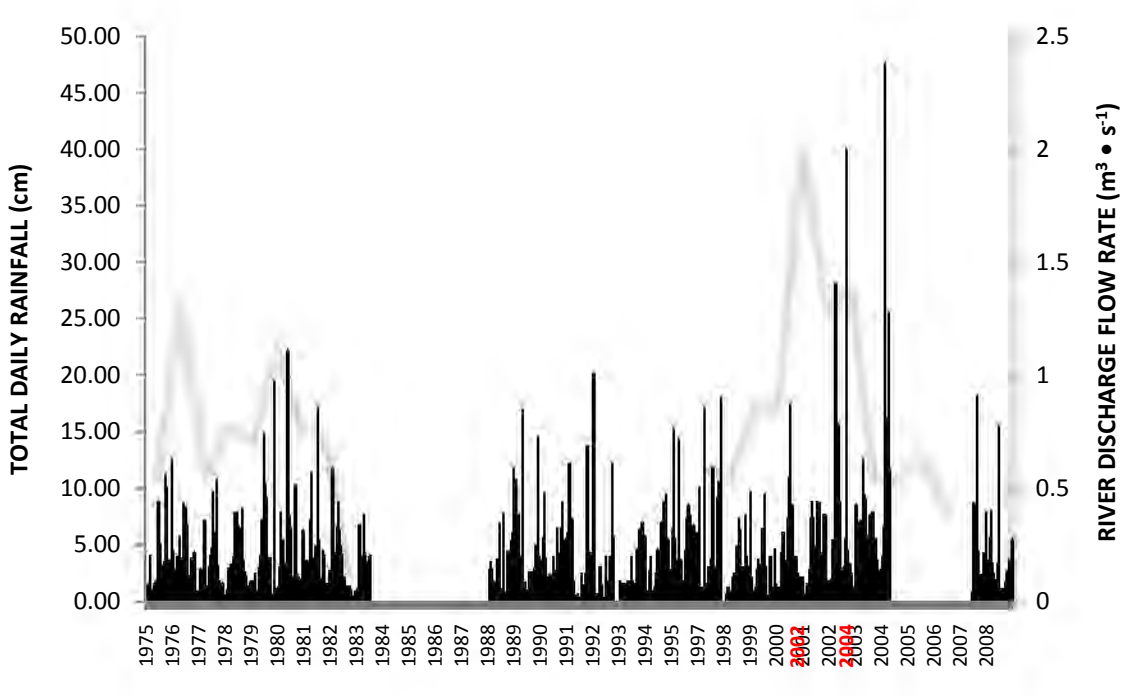


Figure 9. Rainfall and river-discharge records. Daily total rainfall data recorded at rain gauge in Yona, Guam and annual mean discharge flow rate at Pago Bay from 1975 to 2008. High river discharge flow rate usually coincides with heavy rainfall in southern Guam due to low soil permeability. Heavy rainfall and high stream flow rate recorded in 2002 and 2004 were associated with the passage of Super Typhoon Pongsona and Typhoon Tingting, respectively. Data from USGS Pacific Islands Water Science Center (http://hi.water.usgs.gov/).

CHAPTER 3

Prey Discrimination and Chemoreception

3.1 Introduction

Chemical signaling is of paramount importance to all animals, most especially as a means of connecting several life processes in aquatic environments. Chemicals are involved in mediating interspecific and intraspecific interactions, which strongly influence population structure, community organization, and ecosystem function (Hay, 2009). Benthic marine invertebrates use chemical signals to coordinate spawning (Beach *et al*., 1975, Watson *et al*., 2003) and select mates(Sato & Goshima, 2007), guide larval settlement on appropriate substrates (Morse & Morse, 1996), induce metamorphosis (Kitamura *et al*., 2007), and influence predator-prey interactions (Stachowicz, 2001; Paul & Ritson-Williams, 2008; Hay, 2009).

The predator-prey interaction between *A. planci* and coral is intimately linked and should not be studied in isolation (Moran, 1986). Both primary and secondary metabolites from marine organisms play a vital role in mediating various phases of predator-prey interactions (Stachowicz, 2001), from avoidance and escape responses (Campbell et al., 2001; Nishizaki and Ackerman, 2005), and defense against predators (Schupp *et al*., 1999; Ritson-Williams & Paul, 2007; Thoms & Schupp, 2008), to prey detection (Weissburg & Zimmer-Faust, 1994), feeding choices (Souza *et al*., 2008), prey capture (Biggs *et al*., 2008), and feeding stimulation (Sakata *et al*., 1986). Some studies

have suggested that *A.planci* exercise chemically-mediated prey location (Ormond & Campbell, 1974; Teruya *et al*., 2001), although it is still being debated whether these are mainly due to fortuitous encounter rather than sensory orientation. Nevertheless, the ability of *A. planci* to detect and orient to food from a distance is of considerable advantage, allowing it to detect prey over an area larger than they can profitably physically search.

Aside from foraging, chemical cues also potentially play a role in feeding (Collins, 1974; Hanscomb *et al*. , 1976; Huxley, 1976) and how *A. planci* determines its choice of prey (Brauer *et al*., 1970; Collins, 1975b). Preferential feeding on certain coral species by *A. planci* (De'ath and Moran., 1998b; Pratchett, 2007; Pratchett *et al*., 2009), even at moderate densities, causes differential mortality among coral species and can exert a major influence on coral community structure. In the eastern Pacific, preferential feeding by *A. planci* on rare species reduced diversity by increasing the dominance of *Pocillopora damicornis* (Glynn, 1976). On the other hand, *A. planci* can facilitate the growth and recruitment of rarer species by selectively feeding on more abundant and dominant corals, thus effectively increasing coral diversity (Porter, 1972). Understanding the factors that influence feeding preferences is fundamental in determining their effects on coral communities (Pratchett, 2007). Despite this, the role of chemical signaling in *A. planci*'s selectivity in their choice of prey is still poorly understood.

This chapter aims to establish the role of chemical signals in *A. planci* prey discrimination through laboratory feeding assays that control for the potential effects of coral growth form, release of nematocysts, density of prey, competition and environmental conditions such as hydrodynamics and substrate complexity. This chapter

also discusses the rate of extract diffusion in this bioassay set-up, which is important in determining the concentration of cues liberated to the water column and in setting the duration of experiments.

3.2 Methods

3.2.1 Study Organisms

Adult *A. planci* (~250- to 350-mm total diameter) were collected from major impact sites identified during manta tow surveys. Seastars were kept in 1.4-m^3 outdoor flow-through seawater tanks at the UOG Marine Laboratory with constant aeration and fed every two weeks with an artificial diet of catfish food and mashed seafood added to a mixture of agar and seawater, then poured into a plastic mold. Seastars were disposed after each feeding assay and seastars that were not used in the feeding assays within two months were also disposed.

Scleractinian corals extracted for the feeding assays were collected from different sites. Branching *Acropora surculosa* Dana 1846 and *Pocillopora eydouxi* Milne-Edwards & Haime 1860 colonies were collected from Pago Bay (13.426764°N, 144.798679°E); encrusting *Montipora* sp. colonies were collected from both Tanguisson Reef (13.556097°N, 144.809399°E) and Pago Bay. These corals were selected because they are known to be preferred by *A. planci* in the field (Colgan, 1987; De'ath and Moran, 1998b; Porter *et al*., 2005; Chapter 2 of this study). Columnar *P. rus* and branching *P. cylindrica* colonies were collected from Cocos Lagoon (coordinates) and

Fingers Reef, Apra Harbor (coordinates); massive *D. heliopora* colonies were collected from Western Shoals, Apra Harbor (coordinates). These species were chosen based on field studies indicating that these species are least preferred prey by *A. planci* (Colgan, 1987; De'ath and Moran, 1998b; Porter *et al*., 2005; Chapter 2 of this study). Coral colonies were stored at -20°C immediately after collection and prior to extraction.

3.2.2 Y-maze Specifications and Extract Diffusion Experiment

Laboratory feeding assays were carried out in a Y-maze olfactometer made of wood that is sealed with fiberglass (**Figure 10**). This system allowed equal currents to pass along each arm junction and the two entirely different water bodies become confluent in a non-turbulent manner, then move towards the base where it flows out of the system and allow seastars to move upstream and choose between discrete chemical signals from each arm (Davenport, 1950; Ormond *et al*. 1973, Teruya *et al*. 2001). The Y-maze is 400-cm long, and holds 424.5-L of seawater. A slow, steady stream of ambient, untreated seawater flowed through each arm of the Y-maze at a minimum restricted flow rate of 250 ml/s (**Figure 10b**). Tracking of chemical cues by aquatic animals is more efficient in calm flowing water and less in rough turbulent flows (Weissburg and Zimmer-Faust, 1994). Tests with fluorescent dye embedded in a gel matrix showed a laminar flow that delivered the pigment plume to the end of the Y-maze (Sec. C, **Figure 10a**) in 5 mins.

A time-series experiment was set-up to determine the diffusion of extracts from the agar matrix in the Y-maze using the flow rate specified above. Filtered seawater and agar were mixed and heated to boil in a microwave for 120 s. After the agar cooled down

to 60°C, *Montipora* sp. extract (see Section *3.2.2*for extraction procedures), dissolved in 3-ml MeOH, was added and poured into a 14.5-cm-diameter petri dish, where the agar and extract mixture was allowed to completely cool and harden. The natural concentration of *Montipora* sp. extract was used based on the surface area of the petri dish. Three replicate 1.5-cm samples were collected from the agar-based extract dish before placing it in the Y-maze to serve as baseline extract concentration. The same number of replicates was collected after 3 h, 9 h, and 24 h in the Y-maze. All samples were freeze-dried (Labconco, USA) and weighed. Three successive extractions in 8-ml MeOH/EtoAc 1:1 and filtrations were conducted. The solvent in the filtrate was removed with a rotary evaporator under low pressure. Each of the dried extracts was redissolved in 3-ml MeOH/EtoAc 1:1, sonicated, and then transferred to pre-weighed 25-ml scintillation vials. The mixed extraction solvent was concentrated to dryness under vacuum in a SpeedVac®. Extract weights were calculated by subtracting the weight of the empty vial from the weight of the extract and the vial. Each extract was dissolved in 2-ml MeOH, then 1 ml of each was transferred to 2-ml Eppendorf® tubes. All extracts were centrifuged (Eppendorf l5415C, Germany) at 14000 rpm for 10 mins, then 500 µl of the supernatant was transferred into an HPLC vial and diluted with 500-µl MEOH. Samples were injected into an HPLC-system, which consisted of a microprocessor-controlled Waters 1525 Binary HPLC Solvent Delivery Pump (Waters, USA), a Waters 717 plus Autosampler, a Waters In-line Degasser AT, a Waters 2996 Photodiode Array (PDA) Detector, a silica guard column (Alltech, USA), and a Rocket Platinum EPS C18 53 x 7 mm column (Alltech, USA). Separation was achieved by using a linear gradient from 90% water adjusted with trifluoroacetic acid (TFA) to 100% MeOH in 20 mins.

Flow rate was kept at 1-ml/min and detection was set at 230 nm. Three major peaks were chosen as basis for the diffusion of extracts (**Figure 12a**) and peak areas, which are indicative of compound concentration, were measured for each peak of interest by automatic electronic integration function of the Waters Empower Pro Software (Waters, USA).

3.2.3 Choice Assays

A known weight of each coral species (*Montipora* sp., *A. surculosa*, *P. eydouxi*, *P. rus*, *P. cylindrica*, *D. heliopora*) was subjected to four exhaustive extractions in MeOH-EtoAc (1:1). Evaporation of solvents in a rotary evaporator under low pressure yielded dark-colored extracts, which were subsequently redissolved in MeOH and concentrated to dryness in a SpeedVac®. Different amounts of extract (based on natural concentrations at surface area of petri dish; see **Table 4** for natural concentrations) were dissolved in 3-ml MeOH and incorporated into food gels (**Figure 13**, inset picture).

Experimental food gels were made by adding 2g of agar (Sigma-Aldrich, USA) to 100-ml filtered seawater in a glass beaker and stirring for 5 s. This mixture was heated in a microwave oven for 60 s and allowed to cool to 60°C before the extract dissolved in 3-ml MeOH was added and stirred using a magnetic stirrer (Corning, USA). The mixture was poured into a petri dish with a surface area of 77.5 cm^2 and left to cool and harden. Control food gels were treated in the same way, but only 3-ml MeOH was added without the coral extracts.

The control and treatment food gels in the petri dishes were placed in the Y-maze, as in **Figure 10a** (control plate denoted by “C”; treatment plate denoted by “T”). Based

on the result of the extract diffusion experiment, each food gel prepared was not used for more than 9 hrs and the sides where the experimental gels were placed was switched every time a new set of food gels was used. After each set, the seawater in the Y-maze was drained and the walls and floor of the Y-maze was rinsed with freshwater, then filled with seawater again. The Y-maze was shaded during feeding experiments to control for the potential effects of light (see Rosenberg, 1972). A new individual was used for each trial and each trial was scored as soon as the individual crossed Sec. 1 of the Y-maze (**Figure 10a**). If no choice was made after 90 mins, the trial was not scored and it as presumed that the individual was not hungry. Based on *a priori* power analysis test done, 36 individuals (85% statistical power) were used for each coral species tested.

3.2.4 Statistical Analyses

The diffusion of extracts in the Y-maze was measured by analyzing the chromatographic peak areas (μV • sec) of selected peaks at specific times in the Y-maze using Single-Factor Repeated Measures ANOVA. This was followed by multiple comparisons using *post hoc* Tukey's HSD Test.

To determine the appropriate sample size for feeding assays, an *a priori* power analysis test for goodness-of-fit was performed using G*Power v.3 (Erdfelder *et al*., 1996; Faul *et al*., 2007) with statistical power set at 85%, effect size at 0.5 (Cohen, 1992), and $df = 1$. Differences in the choice of individual *A. planci* between the tested extract and control were analyzed using G-test for goodness-of-fit, with Yates' correction for continuity, against a 1:1 null hypothesis. Planned, orthogonal comparisons between preferred and non-preferred species were also conducted by pooling unadjusted G-values

and calculating heterogeneity G-value (G_H) to test the difference in the frequency of preference for the two groups of species (Sokal & Rohlf, 1995). Cluster analysis of a Bray-Curtis similarity matrix based on proportions of preference frequency for each species was used to further assess feeding preferences (Primer v.5; Clarke & Gorley, 2001).

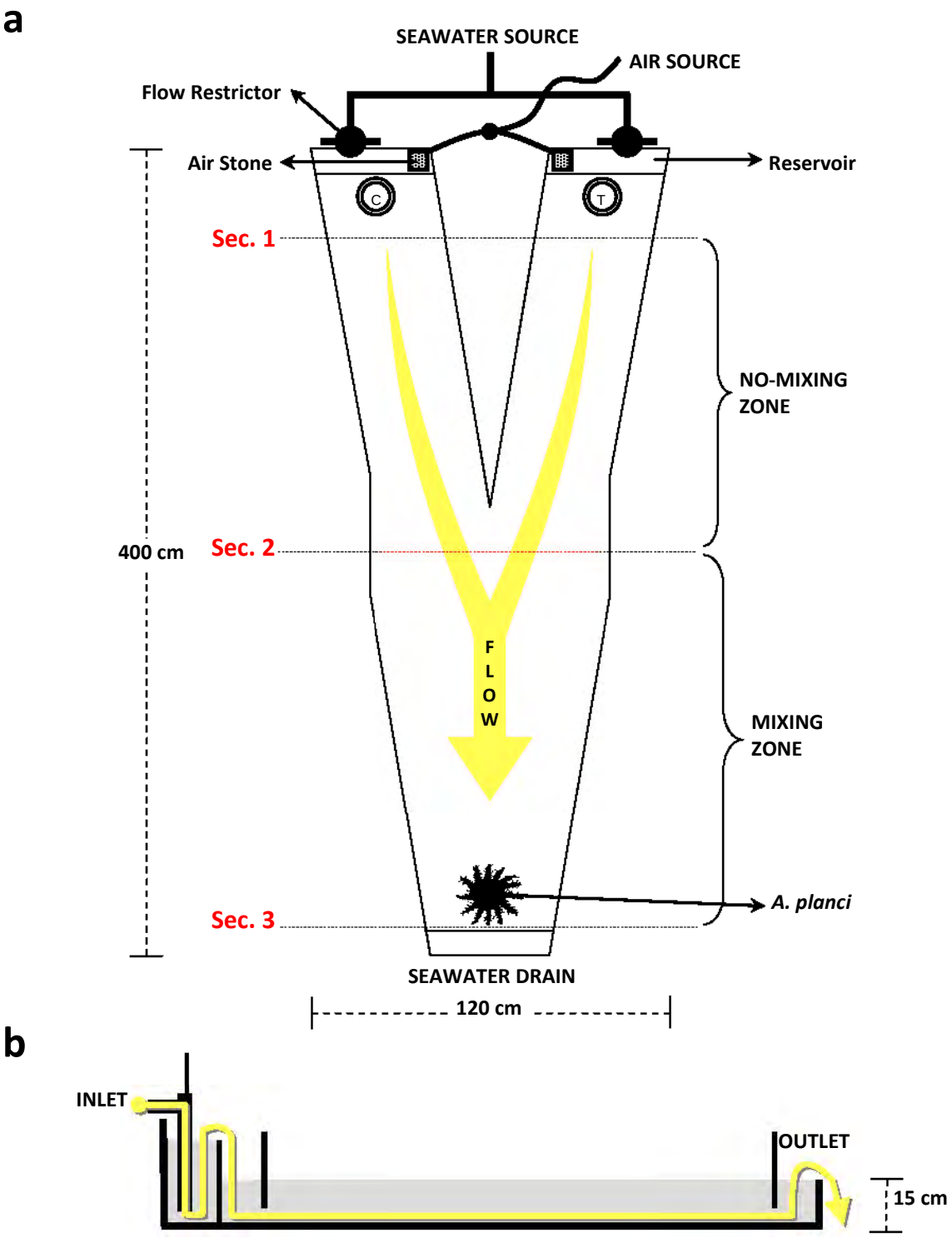


Figure 10. Diagrammatic representation of the Y-maze. (**a**) Top view of Y-maze showing flow direction from experimental cue source, Sec. 1, on each arm of the Y-maze to the start of the mixing zone (Sec. 2), then to the end of the Y-maze (Sec. 3); (**b**) longitudinal aspect with the yellow arrow showing laminar flow of seawater.

3.3 Results

3.3.1 Diffusion of Extracts in the Y-Maze

The extract diffusion experiment showed that there were still relatively high concentrations of extracts even after nine hours in the Y-maze, based on extract weight (**Figure 11**) and peak areas of three major peaks in chromatograms (**Figure 12b**). There was a significant variation in the extract weights (mg extract per g freeze dried gel mass) after each time interval ($F = 73.571$, $df = 3$, $p < 0.001$) and *post hoc* pairwise comparisons revealed that there were no homogenous means except for the time interval from 3 hrs to 9 hrs (Tukey HSD, $p = 0.278$). Furthermore, there was also a significant variation in peak areas after each time interval for Peak A ($F = 594.112$, $df = 3$, $p < 0.001$), Peak B ($F = 2495.400$, $df = 3$, $p < 0.001$), and Peak C ($F = 545.695$, $df = 2$, $p < 0.001$). All subsequent *post hoc* multiple pairwise comparisons showed a significant difference between all time intervals except for the peak areas of 0 hr and 3 hr samples from Peak A, which were homogenous (Tukey HSD, $p = 0.272$). Based on peak intensities from HPLC chromatograms, diffusion of extracts was relatively slow with 69%, 72%, and 56% retained from Peak A, Peak B, and Peak C, respectively. However, all compounds (= peaks) have been exhausted from the agar-based food gels after 24 hrs. This suggests that there was still a considerable concentration of compounds left on the gels even after being in the Y-maze for more than 6 h.

3.3.2 Coral Extract Choice Assays

Tested against a 1:1 null hypothesis, individual G-tests revealed significant differences in the frequency with which individual *A. planci* chose the Y-maze arm with

extracts of *A. surculosa*, *Montipora* sp., and *P. eydouxi* against controls (**Figure 13**). A total of 29 individuals preferred *A. surculosa* extracts compared to only 7 for solvent controls ($G_{Yates'}$ = 13.061, *df* = 1, $p < 0.001$). Extracts of *Montipora* sp. were preferred 27 times against 7 for control gels ($G_{Yates'}$ = 8.356, *df* = 1, $p = 0.004$); whereas *P. eydouxi* was preferred in 25 out of 36 assays over control gels ($G_{Yates'}$ = 4.802, *df* = 1, $p = 0.028$). In contrast, there were no significant differences in the frequency with which *A. planci* preferred *P. rus*, *D. heliopora*, *P. cylindrica* against controls (**Figure 13)**. Y-maze arms with extracts of *P. rus* were preferred 20 times, while control gels were preferred in 16 instances ($G_{Yates'}$ = 0.250, *df* = 1, $p = 0.617$). Extracts of *D. heliopora* were preferred in 19 out of 36 trials ($G_{Yates'}$ = 0.028, *df* = 1, $p = 0.868$), while *P. cylindrica* extracts were preferred 17 times in 36 trials ($G_{Yates'}$ = 0.028, *df* = 1, $p = 0.868$). Subsequent Replicated G-tests confirmed that the preference ratios of species preferred by *A. planci, i.e. A. surculosa*, *Montipora* sp., *P. eydouxi*, over controls were homogenous (G_H = 1.193, *df* = 2, $p = 0.551$), thus, pooling of values was appropriate. The same was true for non-preferred species, *i.e. P. rus*, *D. heliopora*, *P. cylindrica* (G_H = 0.520, *df* = 2, $p = 0.771$). Orthogonal comparisons between preferred and non-preferred species showed that there was a significant difference in the frequency with which extracts of preferred coral species were chosen over controls compared to those of non-preferred species (G_H = 12.637, *df* = 1, $p < 0.001$). In addition, cluster analysis of similarities of the proportion of the frequency with which extracts of each species were preferred over controls also show separation between preferred species and non-preferred species (**Figure 13**).

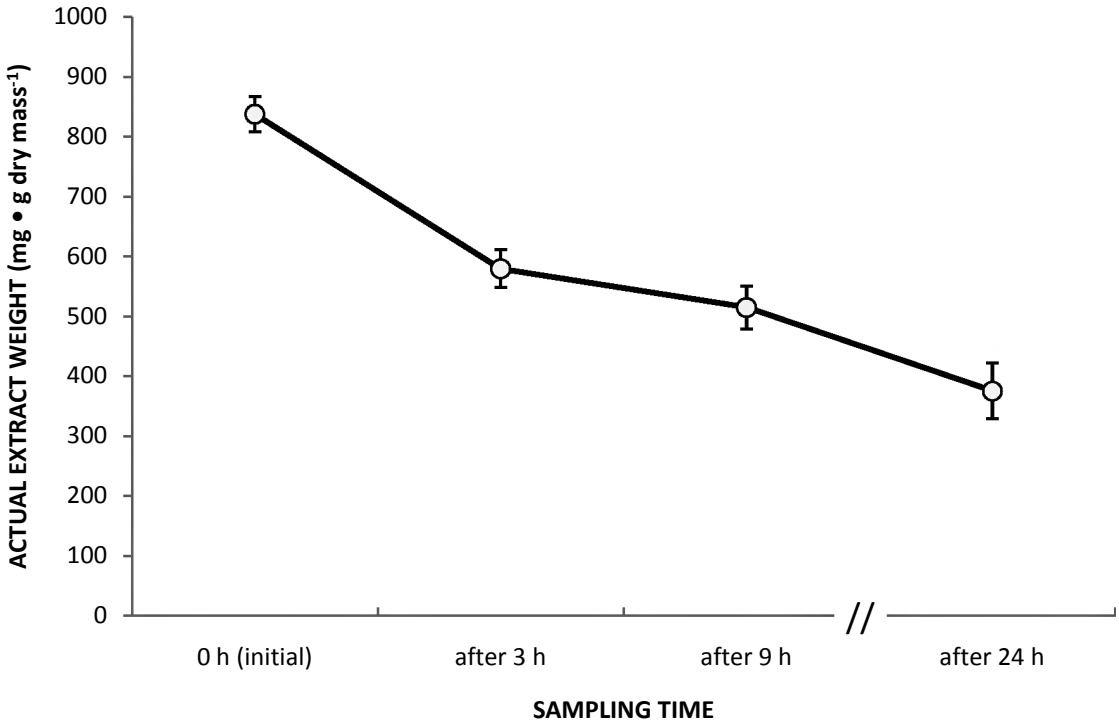


Figure 11. Diffusion of extracts based on extract weight. Freeze-dried samples for each sampling time (n = 3) were weighed to obtain dry mass. Extract yield was divided by the dry mass to get actual extract weight. Pairwise comparisons of means of each time segment showed significant differences except for time interval between 3 hrs and 9 hrs.

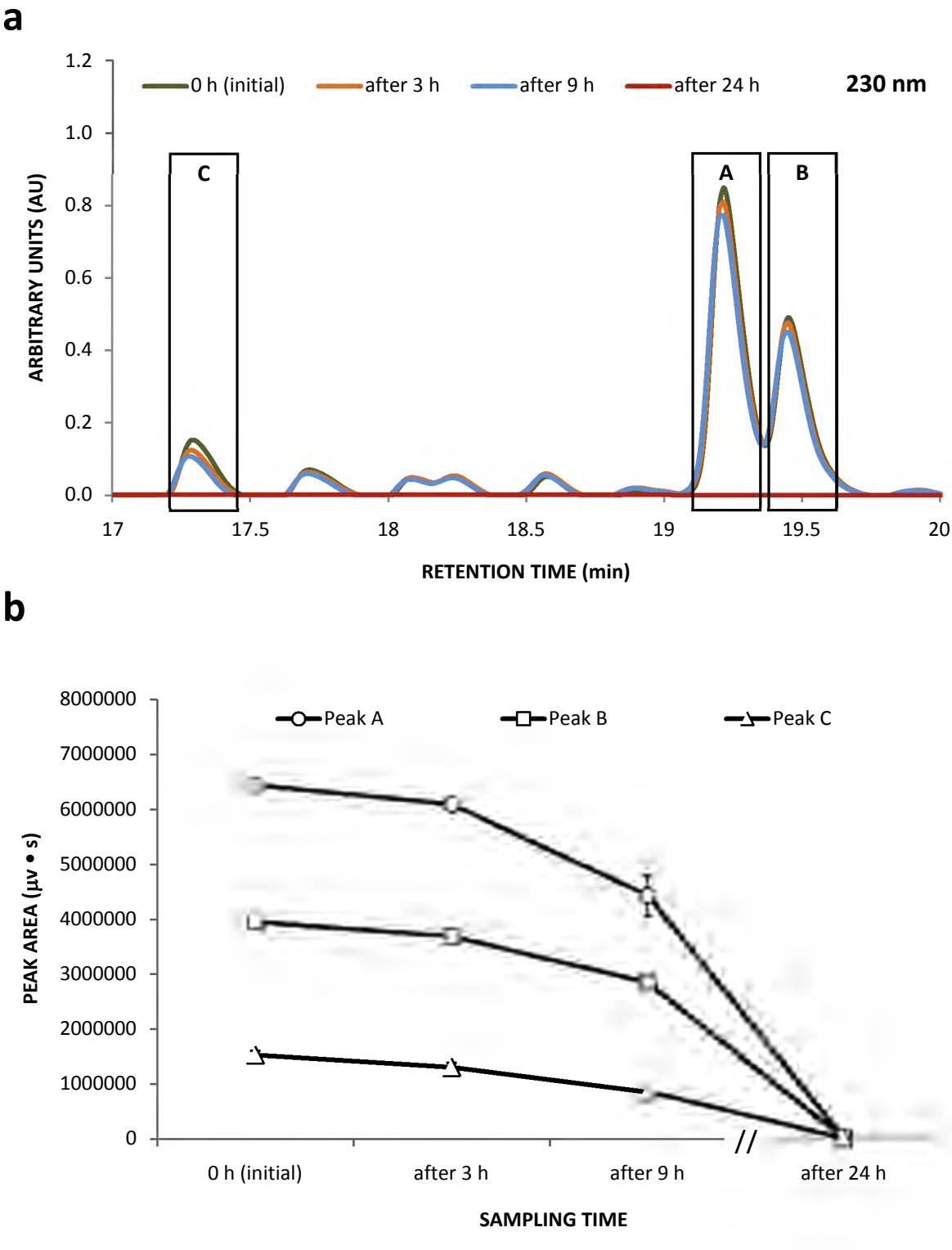


Figure 12. Diffusion of extracts based on chromatographic peak area. (**a**) Overlayed chromatograms showing three selected peaks; (**b**) time-series profile of peak areas of selected peaks showing minimal diffusion within 9 h.

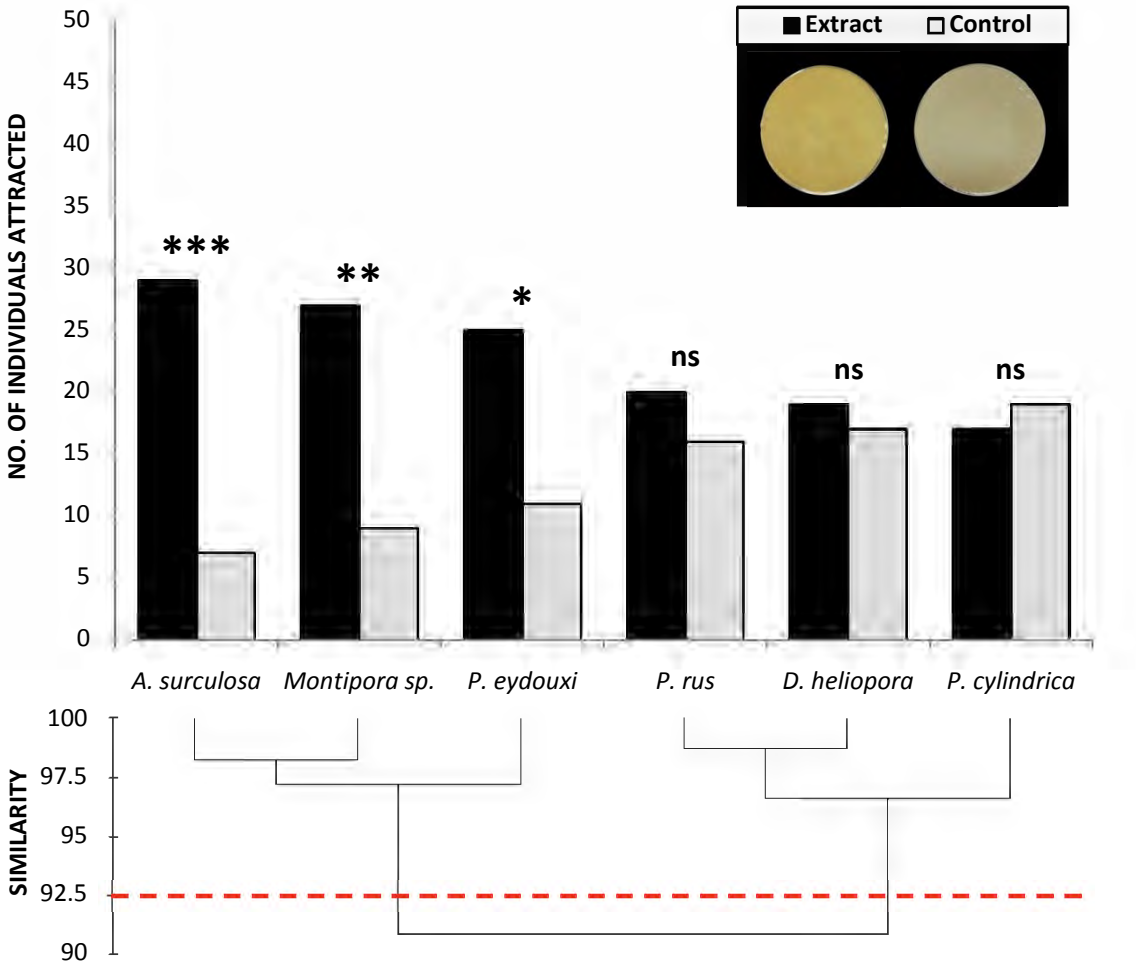


Figure 13. Choice assays testing coral extracts. Strong chemosensory preference for extracts of *Acropora surculosa*., *Montipora* sp., and *Pocillopora eydouxi* is evident. Cluster analysis showing two distinct groups: preferred species and non-preferred species (red dashed lines). Individual assays were analyzed using G-test for goodness-of-fit with Yates' correction at $\alpha = 0.05$ (*** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$; ^{ns} $p > 0.05$, therefore not significant).

3.4 Discussion

The Y-maze olfactometer has been an important tool for marine chemical ecologists to assess if cues impact distance chemoreception (Ormond *et al*., 1973; Teruya *et al*., 2001; Sorensen *et al*., 2005; Wyeth & Willows, 2006; Yambe *et al*., 2006). Despite its many advantages (*e.g.* allows regulation of water flow, permits isolation of tested cue from other background chemicals in the field, makes statistical analysis easier), the Y-maze also has its disadvantages, which were partly addressed in setting up feeding experiments. One obvious downside in using the Y-maze is that only one individual can be tested and only two choices can be offered each time, which may involve tedious experimentation and observation before enough data can be obtained for statistical analysis. Due to this constraint, only extracts of six coral species were tested and assays were conducted at different times, rather than exclusively at night where they are presumed to be most active. Observations of nocturnal activity, however, have mostly been qualitative (*see* Moran, 1986) and adult *A. planci* have been observed to exhibit diurnal feeding activity (De'ath & Moran, 1998a; personal observations during surveys). The Y-maze was also shaded to control for the possible effects of light on *A. planci* feeding behavior (Rosenberg, 1972). Another limitation of the Y-maze design was its smooth sides, which does not mimic the natural environment where *A. planci* usually forage – characterized by some water flow and topographic complexity that both increase turbulence (Weissburg & Zimmer-Faust, 1993). The steady flow in the Y-maze served as a laboratory imitation of slow tidal currents (**Figure 10b**) although topographic complexity was not incorporated into the design to facilitate the uniform delivery of stimuli to the seastars being tested. Another concern is the possibility that test animals

that move relatively fast may tend to overshoot into one or the other arm before it has time to make a choice (Bartel & Davenport, 1956). This was addressed by having a longer distance between the starting point and the point of juncture of the arms (200 cm; **Figure 10a**) to allow individuals to actually make a choice before proceeding into an arm of the Y-maze. Finally, it is very difficult to accurately determine the concentration of a certain stimulus that provokes activity because natural concentrations are known only before food gels are placed in the Y-maze and released to the water column and by the time the stimulus reaches the target organism, the concentration may be reduced (Hay *et al*., 1998). Although determining the amount of extract diffused ensures that individuals used in each trial continue to receive the stimulus, the difficulty of precisely determining active concentrations of stimulus upon contact with target organisms makes it hard to completely address this limitation.

The results of the time-series extract diffusion experiment demonstrates that there was still a considerable amount of extracts and potential compounds on the experimental food gel even after being placed in flowing water inside the Y-maze. At the same time, this also shows that agar-based gels were appropriate for choice assays since it gradually leached appropriate amounts of extract rather than restrict the delivery of extracts or leach significant amounts of extract too soon. However, the amount of extract released from the agar matrix cannot be actively regulated; therefore, individuals used in each trial did not receive equal amounts of extract even with equal and steady flow from both arms of the Y-maze. Despite the diffusion of extracts after some time (depending on how long a single food gel was used), *A. planci* still exhibited clear preferences for certain extracts even when gels contained less amounts of extract than they initially did at the beginning

of each set of assay. The Y-maze had been used for behavioral studies with *A. planci* in the past (Ormond *et al*., 1973; Teruya *et al*., 2001), but these studies did not test how much extract was retained or diffused from experimental plasters or gels as feeding experiments proceeded. This should be a prerequisite for choice assays, not only to determine how long to use each experimental gel (or other medium), but also to ensure that target organisms are making choices based on the hypothetical stimulus being tested.

The results of laboratory choice assays correspond with observations in the field (**Figure 7**, *see also* Porter *et al*., 2005) and demonstrate that *A. planci* use chemical signals in selecting prey. Laboratory conditions eliminated several factors that may influence prey preference in the field, including variation in abundance, distribution, and accessibility of corals (Barnes *et al*., 1970; Ormond *et al*., 1973; De'ath & Moran, 1998b), size and growth form of corals (Chesher, 1969a; De'ath & Moran, 1998b), coral defenses such as nematocysts and mesenteric filaments (Barnes *et al*., 1970; Goreau *et al*., 1972), potential role of coral symbionts (Glynn, 1976; 1980; Pratchett, 2001), and nutritional value of corals (Ormond *et al*., 1976). Although the potential role of these factors cannot be discounted, this study mainly aimed to isolate the effect of chemical signals from other confounding factors. Nevertheless, certain factors are still difficult to control. The physiological state of individual seastars used in assays cannot be controlled, as well as the effects of handling (Sloan, 1980). Although seastars were allowed to acclimate prior to experiments, it may not be enough to completely alter and normalize its internal physiological state. Learned behavior with regards to prior ingestive conditioning may also affect how individuals respond to certain feeding or foraging cues (Huxley, 1976; Ormond *et al*., 1976). This was addressed by feeding the

seastars with artificial diet every two weeks. However, the amount of food that each individual digests cannot be controlled.

These results are also mostly consistent with previous laboratory studies on the feeding preferences of *A. planci* (Brauer *et al*., 1970; Ormond *et al*., 1973; Sonoda & Paul, 1993; Pratchett, 2001; 2007). Whole corals, whole coral extracts, and chemical extracts have been used in these feeding experiments. Although Brauer *et al*. (1970) did not test distance chemoreception, they were able to establish that whole coral extracts of *Acropora formosa* and *Pocillopora eydouxi* evoke stomach eversion in *A. planci* while whole extracts of *Porites iwayamaensis* and *Porites lutea* causes withdrawal responses by the tube feet. Their results are indicative of the presence of cues in corals that stimulate *A. planci* feeding rather than foraging. Similarly, Ormond *et al*. (1973) showed that *A .planci* significantly preferred *Acropora* sp. over blank controls in Y-maze experiments. Moreover, in studying potential chemical defenses by corals, Sonoda &Paul (1993) found that whole, crushed, organic extracts, and aqueous extracts of *Acropora aspera* were mostly preferred by *A. planci* over *D. heliopora* and *P. rus.* However, they did not see a significant preference toward organic extracts of *A aspera* over solvent controls. Results obtained by Sonoda & Paul (1993) may have been confounded by the lack of a well-directed flow in the experimental set-up, which opened the possibility of fortuitous encounter rather than actual chemoreception. Nonetheless, their results suggested that non-preferred corals did not possess chemical defenses, but rather they released less chemical attractants compared to preferred species.

Although the potential influence of other factors, mentioned above, cannot be eliminated, these results clearly suggest that chemical signals play a vital role in how a

well-ordered hierarchy in coral mortality is formed via selective predation. These results also confirmed the active use of chemoreception by *A. planci* in searching for coral prey and some individuals were observed to move halfway into the control arm and move back to the arm juncture and proceed towards the arm with the coral extract. Chemical signals from the coral prey help determine the fate of coral species in terms of mortality through *A. planci* predation during moderate outbreaks. Preferential feeding by *A. planci* has a significant effect on coral community structure and these results improve the understanding as to why some coral species suffer higher mortality compared to other species. However, non-preferred species were not totally avoided by *A. planci* as well (**Figure 13**). This shows that *A. planci* may still feed on these species in the absence of more preferred species (Pratchett, 2007) and perhaps only possess or release less amounts of attractants or stimulants compared to preferred species as suggested by Sonoda & Paul (1993). Therefore, in severe and chronic outbreaks, immediate localized depletion of preferred species may be followed by consumption of less-preferred species, which could lead to extensive devastation across the entire coral community. The following chapter outlines efforts to isolate and characterize chemicals that are responsible for feeding attraction responses.

CHAPTER 4

Chemical Analysis of Coral Extracts

4.1 Introduction

Naturally produced chemicals mediate predator-prey interactions at many levels and different directions. Most research on the role of chemical signals in mediating ecological processes had been focused on identifying compounds that serve as defenses against consumers (see Hay & Fenical, 1988; Pawlik, 1993; Paul & Ritson-Williams, 2008). Although of equal importance, feeding attractants and stimulants are less thoroughly studied in an ecological context (Hay *et al*., 1998). Predators employ chemicals in all phases of their search for prey, from prey location to initiation of feeding (reviewed by Stachowicz, 2001). Feeding attractants or foraging cues are compounds used to locate, detect, and identify prey from a distance, while feeding stimulants are compounds that increase the probability of a certain prey to be consumed by promoting ingestion and continuation of feeding (Stachowicz, 2001). Biological studies of predator-prey interactions involving olfactory perception, receptor biochemistry, orientation behavior, and communication often require the use of purified chemical compounds (Byers, 1992). However, the isolation of specific compounds responsible for attracting predators to its prey has been elusive, and thus, rarely investigated. Different types of chemicals, including peptides, proteins, organic nitrogen bases, carbohydrates, and fatty acids may be involved in the mediation of these ecological processes, thereby slowing the progress in the isolation and identification of specific compounds because different

analytical approaches have been required on almost a case-by-case basis (see Zimmer & Butman, 2000). Nevertheless, the use of bioassays as a guide in the fractionation of extracts has led to the identification of bioactive metabolites and the discovery of unexpected ecological effects of well-known metabolites (van Alstyne & Paul, 1992; Cronin et al, 1995; Bolser & Hay, 1996; Teruya *et al*., 2001; de Oliveira *et al*., 2006; Kobayashi *et al*., 2007). Determining natural concentrations of extracts or specific attractant compounds are necessary in conducting ecologically relevant feeding assays (Hay *et al*., 1998). In addition, organisms which exhibit feeding preferences may be receptive to particular levels or spectra of specific chemical compounds (McClintock *et al*., 1984).

The physical and chemical properties of habitats are important in chemically-mediated interactions. In comparing terrestrial and aquatic environments, Zimmer & Butman (2001) stated that compounds with high vapor pressures facilitate chemical transport with air as the medium in terrestrial environments, while compounds with high aqueous solubility are more suitable as waterborne cues in aquatic environments. Certain amino acids (Zimmer *et al*., 1999) and peptides (Decho *et al*., 1998; Rittschof & Cohen, 2004) have been shown to attract certain predatory marine invertebrates and stimulate feeding. However, natural stimuli for foraging can be complex blends of different chemicals from multiple individuals and species whose combined activity may differ considerably from that of any component in isolation (Stachowicz, 2001). The role of mixtures as feeding attractants and stimulants further complicates bioassay-guided fractionation and identification of natural cues (Hay, 2009).

This chapter generally focuses on the chemical analysis of coral crude extracts to further understand and characterize chemical signals that influence the feeding behavior of *A. planci*. First, coral extracts were analyzed by determining the natural concentration of crude extracts in selected species. Next, choice assays were conducted to test which fractions were most chemoattractive to *A. planci.* Then, using the most appropriate partitioning system based on choice assay results, extracts were characterized by quantifying hydrophilic and lipophilic components. Finally, concentrations of betaine in 15 coral species were quantified to see whether the concentration of this known attractant compound (*see* **Chapter 5**) is important in determining the preferred prey of *A .planci*, following the mechanism, which Sonoda & Paul described as chemical crypsis.

4.2 Methods

4.2.1 Study Organisms

Collection and maintenance of adult *A. planci* followed the same protocol described in *Section 3.2.1* (**Chapter 3**). Fragments from the following Scleractinian coral species were collected from Pago Bay, Tanguisson Reef, and Apra Harbor to determine natural concentrations of extracts: Family Acroporidae – *Acropora humilis* Dana 1846, *A. irregularis* Brook 1892, *A. surculosa*, and *Montipora* sp. Blaineville 1830; Family Faviidae – *Diploastrea heliopora* Lamarck 1816, *Favia* sp. Oken 1815, *Goniastrea* sp. Milne-Edwards & Haime 1848, and *Platygyra* sp. Ehrenberg 1834; Family Merulinidae – *Hydnophora microconos* Lamarck 1816; Family Pocilloporidae –

Pocillopora damicornis Linnaeus 1758 and *P. eydouxi*; Family Poritidae – *Porites cylindrica* Dana 1846, *P. rus* Forskål 1775, and massive *Porites* spp.; and Family Sidastreidae – *Psammocora digitata* Milne-Edwards & Haime 1851.

4.2.2 Extraction Procedure

Fragments (~ 5 cm) were chiseled from the top, middle, and bottom portions of each coral colony and placed in Whirkpak® (Nasco, USA) specimen bags. This was done to average potential intracolony variation. These fragments were obtained from three different colonies of each of the 15 coral species to account for intercolony variation. All specimens were immediately stored at -20°C until extraction. The wet weights of the three fragments from each colony were determined and subjected to three successive one-hour extractions in a 1:1 combination of methanol (CH_3OH or MeOH) and ethyl acetate ($CH_3COOCH_2CH_3$ or EtoAc). Extracts were then filtered and the solvent from the filtrate was eliminated in a rotary evaporator (Büchi, Switzerland) under low pressure. The residue was redissolved in MeOH and then submitted to sonication in a Branson 1210 ultrasonic cleaner (Branson Ultrasonics Corp., USA) before being transferred to pre-weighed 25-ml scintillation vials. All extracts were concentrated to dryness under vacuum with an SPD 2010 SpeedVac® System (Thermo Savant, USA). The dried and concentrated crude extracts were weighed using an analytical AG Balance (Mettler-Toledo GmBH, Switzerland) and subtracted by the weight of the empty vial to calculate actual extract weight.

4.2.3 Determination of Natural Concentrations of Extracts

The dry, extracted fragments were weighed and the surface area of each fragment was determined following the foil-wrapping technique developed by Marsh (1970) with some modifications. Each piece of coral was tightly molded with heavy-duty aluminum foil to fit depressions and projections. Notches where cut where necessary and the excesses were trimmed to match the boundaries of the coral surface. Instead of using the surface area-to-mass calibrations (Marsh, 1970), the aluminum foil molds were flattened and digital photographs were taken using a ruler as scale. These pictures were analyzed by calculating the area (cm^2) of the flattened molds using Image J (Rasband, 2007), an image analysis software developed through the National Institutes of Health (NIH), USA.

Natural concentrations of extracts were calculated by dividing the extract weight (mg) with the surface area (cm^2). In scleractinian corals, surface area measurements are routinely used to allow indirect estimates of biomass and measures related important ecological processes (Bythell *et al*., 2001; Edmunds & Gates, 2002). It is also more ecologically relevant to use surface area measurements for corals instead of wet or dry weight. Nevertheless, the amount of extract per wet weight, as well as extract concentration per dry weight was also calculated to facilitate comparisons with other studies.

4.2.4 Choice Assays with Extract Fractions

Procedures for the preparation of crude extracts are discussed in *Section 4.2.2*. Experimental gels with crude extracts of *A. surculosa* were shown to be significantly preferred by *A. planci* over control gels (**Figure 13**). Following this behavioral response,

two sets of liquid-liquid partitioning systems were set-up: one between 90% aqueous MeOH (*Net Polarity Index* = 5.49) and hexane (*Polarity Index*, *P'* = 0.00), and the other between water (*P'* = 9.00) and EtoAc (*P'* = 4.40). This was done to cover a wider spectrum of polarities and narrow down choices in separating bioactive compounds. Solvents for each yielded layer were dried in a rotary evaporator at low pressure, reconstituted in corresponding solvents, and transferred to pre-weighed scintillation vials prior to concentration and evaporation in a SpeedVac system. Two sets of feeding assays were conducted to test these fractions. In the first assay, individual *A. planci* were given a choice between agar-based food gels incorporated with the 90% MeOH layer on one arm of the Y-maze and with the hexane layer on the other. In the second assay, individuals chose between gels with the water layer and with the EtoAc layer.

4.2.5 Extract Partitioning

In accordance with results of choice assays described above, one vial of crude extract of each species were subjected to liquid-liquid partitioning in a separation funnel (**Figure 14**) between 90% aqueous MeOH and hexane (C_6H_{14}) and yielded two distinct layers with different polarities, one more water-soluble and the other more lipid-soluble. Solvents from the two partitioned layers of each coral extract were separately evaporated using a rotary evaporator at reduced pressure. Samples were redissolved in corresponding solvents (*i.e.* MeOH/H2O 9:1 for polar fractions and hexane for nonpolar fractions) before being transferred into pre-weighed scintillation vials, and the concentrated to dryness in a SpeedVac sytem. Extract weights for both fractions of each

coral species were determined to calculate total percent yield proportion of polar and nonpolar fractions.

4.2.6 Quantification of Betaine Concentrations

Samples (10 mg) from the polar fractions of each coral species were placed in 1.5-mL Eppendorf® tubes. Because betaine has a weak UV chromophore, direct detection by low-wavelength UV was difficult to accomplish using HPLC alone, particularly when other substances, such as vitamins and amino acids are present in the sample (Shin *et al*., 1999). Instead, samples were shipped to Dr. Matthias Koeck of the Alfred Wegener Institute in Bremerhaven, Germany for screening of betaine concentrations using Liquid Chromatography – Mass Spectrometry (LC-MS).

4.2.7 Statistical Analyses

Natural crude extract concentrations of each coral species were log-transformed to improve normality and homogeneity and analyzed using Single-Factor ANOVA with Replication, followed by multiple comparisons using post hoc Tukey's HSD Test. Individual Y-maze bioassays were analyzed against a 1:1 null hypothesis using G-test for goodness-of-fit with Yates' correction for continuity at $\alpha = 0.05$. Variability of betaine concentrations (per mg extract, per g dry mass, per cm^2 coral tissue) between coral families were analyzed using Single-Factor ANOVA with unequal replication. Betaine concentrations per g dry mass and per cm^2 coral tissue were square-root transformed to improve normality.

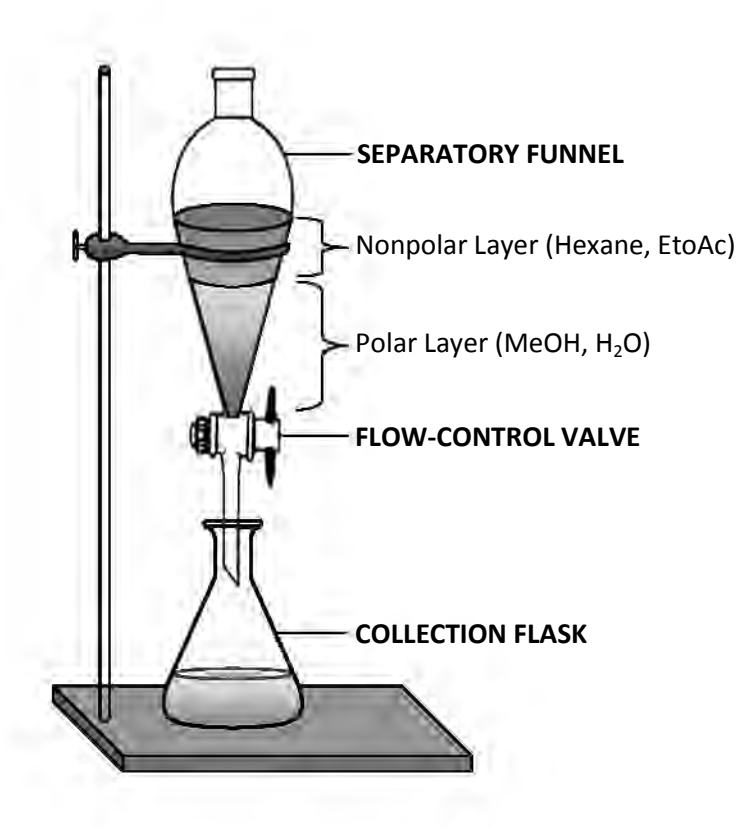


Figure 14. Laboratory set-up of liquid-liquid partitioning system.

4.3 Results

4.3.1 Natural Concentration of Extracts

Crude extract concentrations were highly variable among coral species (F = 45.469, df = 14, $p < 0.001$), ranging from 12.636 ± 1.237 mg/cm^2 in *Montipora* sp. to 1.272 ± 0.095 mg/cm^2 in *P. eydouxi*. Amounts of crude extract ± 1SD per unit surface area (cm^2) are summarized in **Table 4**. The pattern of crude extract concentration of each species was not consistent with the feeding preference patterns exhibited by *A. planci* in the field. In addition, *post hoc* multiple comparisons with Tukey's HSD test also show high variability in extract concentrations between and within families (**Table 4**; **Figure 15**). Interestingly, coral species with massive or encrusting growth forms had higher crude extract concentrations compared to branching or columnar growth forms (**Figure 15**).

4.3.2 Choice Assays with Extract Fractions

Feeding assays in Chapter 3 showed that crude extracts of *A. surculosa* were significantly preferred over solvent controls (**Figure 13**). Results of choice assays between different fractions of *A.* surculosa extracts are summarized in **Figure 16**. There was no significant difference in the choice of *A. planci* between agar gels with water layer fraction and agar gels with EtoAc layer fraction; water layer was preferred 21 times and EtoAc layer was chosen 15 times out of 36 trials ($G_{Yates'}$ = 0.697, df = 1, p = 0.404). On the other hand, when offered a choice between agar gels with 90% aqueous MeOH layer extract and agar gels with hexane layer extract, *A. planci* chose the 90% aqueous

MeOH layer 28 times, while only 8 individuals chose the gels with the hexane layer ($G_{Yates'}$ = 10.555, df = 1, p = 0.001). These bioassay results indicated that the moderately polar 90% aqueous MeOH layer was the active fraction, which potentially possesses the feeding attractant compound(s), and eliminated the highly polar water fraction, the moderately nonpolar EtoAc fraction, and the highly nonpolar hexane layer. Further extractions are currently being done to isolate and purify feeding attractant compounds.

4.3.3 Characterization of Extracts

Percentage yield after liquid-liquid separation were relatively high. Recovery was above 60% for all coral species (**Table 5**). Yield was lowest in *A. irregularis* at 63%and highest in *A. surculosa* at 93%. Crude extracts were partitioned based on polarity into two distinct layers, the hydrophilic 90% aqueous MeOH layer and the lipophilic hexane layer. The proportion of the two layers also varied between species, although the weight of the 90% aqueous MeOH fractions of each species were all higher than the hexane layer in all species. However the polar / nonpolar layer weight ratio in *P. eydouxi* was approximately 1:1. Moreover, when species were ordered from highest polar fraction proportion to lowest, the sequence did not correspond with the crude extract concentrations. Polar fractions of each species were used to quantify the concentration of betaine.

4.3.4 Betaine Concentration

Concentrations of betaine were highly variable between families and among species within each family. Betaine concentration of each species per mg extract, per g

coral dry mass, and per cm^2 coral tissue (*i.e.* skeletal surface area) are listed in **Table 6**. *Montipora* sp. had the highest betaine concentration (1.463 mg/cm^2) while *Favia* sp. had the lowest (0.048 mg/cm^2). There was no general pattern observed in the betaine concentrations of each species that resembled the well-ordered hierarchy of *A. planci* coral feeding preferences. In addition, when coral species were grouped into their respective families (*Hydnophora microconos* and *Psammocora digitata* combined as "Others"), there was no significant variability in betaine concentrations between families (per mg extract: $F = 794$, $df = 4$, $p = 0.555$; per g dry mass: $F = 0.454$, $df = 4$, $p = 0.768$; per cm^2 coral tissue: $F = 0.311$, $df = 4$, $p = 0.864$).

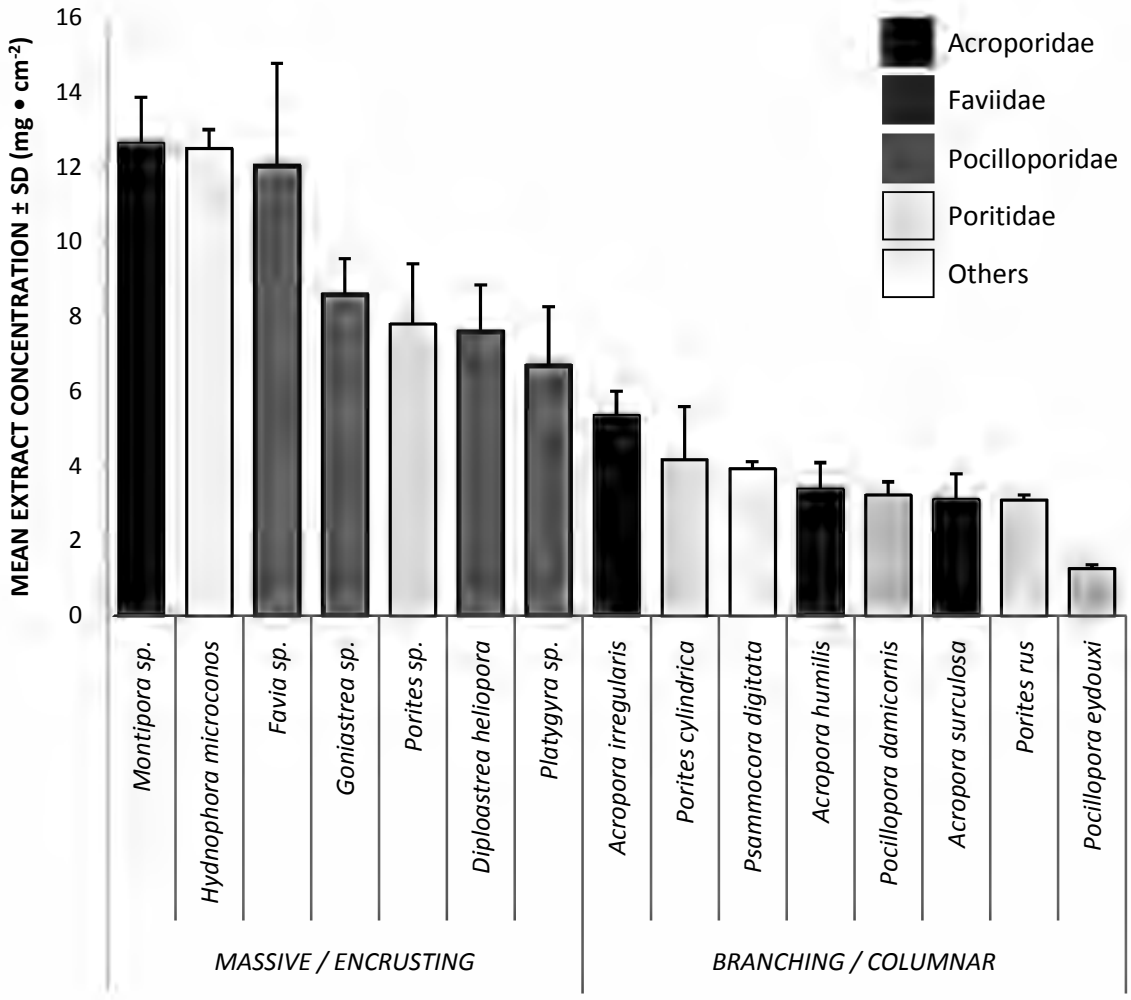


Figure 15. Natural concentration of crude extracts from selected coral species in descending order. High variability of extract concentrations between and within scleractinian coral families. In terms of growth form, massive or encrusting corals had higher concentrations than branching or columnar corals.

Table 4. Mean **c**rude extract concentrations of selected scleractinian corals. Values ± 1 SD are shown in mg / g dry mass[1] and mg / cm^2 to facilitate comparisons with other literature.

FAMILY	*SPECIES*	*CRUDE EXTRACT CONCENTRATION* Mean ± SD[a] (*mg / g dry mass*[1])	Mean ± SD[b] (*mg / cm*^{2})	*TUKEY'S HSD*
Acroporidae	*Acropora humilis*	7.200 ± 2.900	3.392 ± 0.711	e, f
	Acropora irregularis	11.693 ± 2.841	5.360 ± 0.659	c, d, e
	Acropora surculosa	10.254 ± 1.097	3.112 ± 0.686	f
	Montipora grisea	17.215 ± 1.406	12.636 ± 1.237	a
Faviidae	*Diploastrea heliopora*	4.135 ± 1.466	7.623 ± 1.238	b, c
	Favia	9.055 ± 1.576	12.061 ± 2.715	a, b
	Goniastrea	7.873 ± 2.381	8.620 ± 0.944	a, b, c
	Platygyra	5.616 ± 1.057	6.707 ± 1.572	c, d
Merulinidae	*Hydnophora microconos*	10.054 ± 2.230	12.502 ± 0.505	a, b
Pocilloporidae	*Pocillopora damicornis*	7.232 ± 0.848	3.234 ± 0.356	f
	Pocillopora eydouxi	2.563 ± 0.284	1.272 ± 0.095	g
Poritidae	*Porites cylindrica*	20.227 ± 6.356	4.185 ± 1.414	d, e, f
	Porites rus	7.214 ± 1.491	3.102 ± 0.143	f
	Porites sp.	9.850 ± 1.761	7.804 ± 1.623	a, b, c
Sidastreidae	*Psammocora digitata*	7.509 ± 0.573	3.933 ± 0.193	e, f

[a] Calculated by dividing the extract weight with the dry mass of the skeleton of extracted coral fragments (*n* = 3 for each species)

[b] Calculated by dividing the extract weight with the surface area measurements of extracted coral fragments (*n* = 3 for each species)

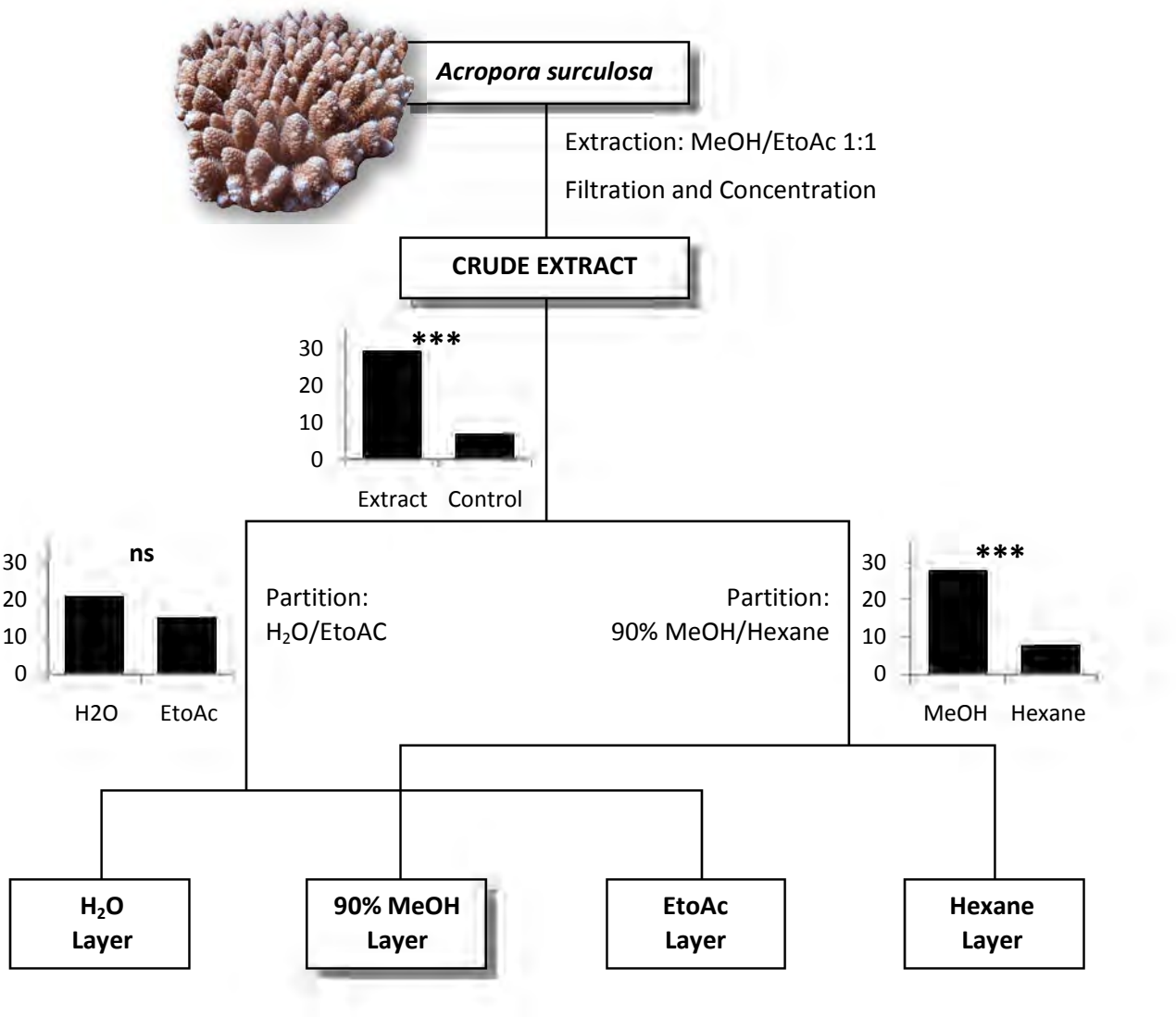


Figure 16. Bioassay-guided fractionation of *A. surculosa* extract. Y-axis of bar graphs refer to number of individuals attracted (n = 36; df = 1). Y-maze assays are analyzed using G-test for goodness-of-fit with Yates' correction (*** $p \leq 0.001$; ^{ns} $p > 0.05$).

Table 5. Proportion of hydrophilic and lipophilic components in selected coral species.

FAMILY	***SPECIES***	***TOTAL YIELD***[a] (%)	***FRACTION LAYER***[b]		***RATIO*** (*Polar* : *Nonpolar*)
			90% MeOH	***Hexane***	
Acroporidae	*Acropora humilis*	81%	0.846	0.154	5: 1
	Acropora irregularis	63%	0.702	0.298	2: 1
	Acropora surculosa	93%	0.921	0.079	12: 1
	Montipora	91%	0.804	0.196	4: 1
Faviidae	*Diploastrea heliopora*	68%	0.847	0.153	6: 1
	Favia	69%	0.803	0.197	4: 1
	Goniastrea	89%	0.878	0.122	7: 1
	Platygyra	87%	0.894	0.106	8: 1
Merulinidae	*Hydnophora microconos*	90%	0.818	0.182	4: 1
Pocilloporidae	*Pocillopora damicornis*	81%	0.798	0.202	4: 1
	Pocillopora eydouxi	72%	0.561	0.439	1: 1
Poritidae	*Porites cylindrica*	80%	0.895	0.105	9: 1
	Porites rus	71%	0.970	0.030	7: 1
	Porites sp.	67%	0.897	0.103	9: 1
Sidastreidae	*Psammocora digitata*	87%	0.815	0.185	4: 1

[a] Total Yield = [weight of crude extract / (weight of polar fraction + weight of nonpolar fraction)] x 100%

[b] Proportion of 90% aqueous MeOH and Hexane layer weights obtained from liquid-liquid separation of crude extracts

Table 6. Concentration of betaine in some scleractinian corals. Bars represent the value's overall proportion compared to the total betaine concentration of all species.

FAMILY	*SPECIES*	*BETAINE CONCENTRATION**		
		mg / mg extract	mg / g DM	mg / cm^2
Acroporidae	*Acropora humilis*	0.027	0.238	0.095
	Acropora irregularis	0.014	0.127	0.061
	Acropora surculosa	0.034	0.359	0.122
	Montipora	0.141	2.011	1.463
Faviidae	*Diploastrea heliopora*	0.036	0.177	0.276
	Favia	0.005	0.036	0.048
	Goniastrea	0.105	0.609	0.763
	Platygyra	0.065	0.283	0.314
Merulinidae	*Hydnophora microconos*	0.087	0.565	0.848
Pocilloporidae	*Pocillopora damicornis*	0.117	0.766	0.329
	Pocillopora eydouxi	0.077	0.102	0.055
Poritidae	*Porites cylindrica*	0.017	0.231	0.052
	Porites rus	0.058	0.425	0.166
	Porites sp.	0.104	1.097	0.892
Sidastreidae	*Psammocora digitata*	0.128	0.822	0.405

* Betaine concentration expressed in: (1) mg betaine per mg polar fraction from crude extract; (2) mg betaine per g coral skeleton dry mass; and (3) g betaine per cm^2 coral tissue, assumed as the skeletal surface area of the coral.

4.4 Discussion

Crude extract concentrations of coral species shown herein were not consistent with the patterns of coral mortality observed in the field. These results suggest that a higher concentration of constitutive crude chemicals alone does not determine whether a certain species is fed upon by *A. planci*. Instead, specific compounds or a mixture of compounds more likely influence prey selection by *A. planci*. Although extracting the three fragments together more or less standardized intracolony variation and extracting three replicate colonies of each species showed minimal intercolony variation, these values should still be interpreted with caution. Differences in geographical location (Barnes & Lough, 1992), season (Fitt *et al*., 2000), depth (Clayton, 1985), or levels of physiological stress (Anthony *et al*., 2002) may cause intercolony, and intracolony variability in coral tissue biomass. Moreover, despite being widely used to standardize physiological data, Edmunds & Gates (2002) argue that that the surface area of the coral skeleton has no clear relationship with the actual tissue area or biomass. Nevertheless, the trend of higher crude extract concentrations among massive or encrusting species compared to branching and columnar types is noteworthy. This pattern could be a consequence of variability in polyp or skeletal density, which affects the tissue biomass of different growth forms. It can be presumed that a higher tissue biomass per unit area yields higher concentrations of crude chemical extracts. Tricas (1989) showed that the polyps of the massive coral *Porites lobata* were higher in density and more uniformly dispersed compared to branching species, *Porites compressa* and *Pocillopora meandrina*. In addition, Hughes (1987) found that corals with massive growth forms had lower skeletal density while those with branching morphology had higher skeletal densities on

the base compared to the tips. Depending on skeletal depth (Barnes & Lough, 1992), a higher skeletal density would most likely result in reduced corallite size and increased ratio of wall thickness to corallite diameter (Highsmith, 1981), which would constrain the attainment of high tissue biomass.

Fractions obtained from liquid-liquid partitioning were tested by binary splitting since choice assays in the Y-maze can only test two experimental cues at the same time. The two separation systems (*i.e.* H_2O : EtoAc and 90% MeOH : Hexane) yielded different segments and covered a wide range of polarities. Of all the fractions tested, the 90% aqueous MeOH layer seems to be the most active (**Figure 16**). The absence of a significant preference for the highly polar (H_2O layer) fraction or the moderately nonpolar (EtoAc layer) fraction points toward the possibility that there might be more than one active compound acting in synergy, which were separated in this partitioning system. On the other hand, the clear preference by *A. planci* for the moderately polar (90% MeOH layer) fraction over the highly nonpolar (Hexane layer) fraction indicates that active components may be around the median of the 90% aqueous MeOH fraction. Similarly, Sonoda & Paul (1993) reported that *A. planci* were more attracted to aqueous extracts (extracted in MeOH / H_2O 1:1) compared to organic extracts (extracted in dichloromethane / MeOH 1:1)of *Acropora aspera* when tested against non-preferred species. Based on HPLC injections of betaine HCl standards (USB Corp., USA), betaine is most likely present in the most polar fraction (*i.e.* H_2O layer). Despite this, *A. planci* did not have a clear preference for the H_2O layer over the EtoAc layer, which further supports the notion that betaine alone does not determine prey selection by *A. planci*.

The 90% aqueous MeOH is almost certainly the solvent system that was able to pull together bioactive compounds.

Following aforementioned results, crude coral extracts were partitioned between 90% aqueous MeOH and hexane. Partitioning of crude extracts into hydrophilic and lipophilic fractions is a vital step in identifying feeding cues and quantifying concentrations of specific compounds (Hay *et al*., 1998). Aqueous solubility, imparted mainly by electronic charge, constrains the type of substances acting as waterborne cues, although lipophilic compounds may still be effective chemical signals when suspended and transported by appropriate fluid flow in the water column (Zimmer & Butman, 2000). Past attempts in isolating chemical attractants for *A. planci* have fractionated extracts based on molecular weight (Collins, 1975a; Hanscomb *et al*., 1976), but fractionation based on polarity or hydrophilicity is perhaps a more ecologically relevant approach in dealing with waterborne cues in aquatic systems (Zimmer & Butman, 2000).

Previous studies have confirmed the presence of betaine in corals (Teruya *et al*., 2001). Based on random testing of several low molecular weight compounds, mostly amino acids, Moore & Huxley (1976) reported that only betaine induced feeding at concentrations low enough to suggest its presence in coral as an active ingredient. Betaine concentrations as low as 10^{-4} M induced arm rearing responses in *A. planci* (Moore & Huxley, 1976) while aquarium experiments by Teruya *et al.* (2001) demonstrated that *A. planci* were attracted to concentrations above 0.8 mg per dish. Several studies have also shown that betaine is highly stimulatory to marine invertebrates that eat flesh (Crisp, 1967; Fuzessery & Childress, 1975; McClintock *et al*., 1984). Despite the dose-dependent nature of chemoreception, no studies have quantified the

concentration of betaine in corals. Results described herein show high variability in betaine concentration between coral species and between families (**Table 6**). In addition, preferred species, which usually suffer high mortality due to *A. planci* predation, did not necessarily possess high concentrations of betaine. Hence, betaine may play a role in active foraging by *A. planci*, based on laboratory (**Figure 21c**) and field experiments (**Figure 23**) shown in the following chapter, but it may not necessarily increase the possibility that a certain coral species is eaten. Betaine may facilitate the identification of prey (*i.e.* corals) from a distance, but may not be the sole coral component that aids *A. planci* in discriminating between coral species. In addition, betaine may also act in synergy with other attractant compounds to increase the potency of chemoattraction. It is interesting to note the high betaine concentration in *Montipora* sp., which was most preferred in the field, and the very low concentrations in Favia sp. and *P. cylindrica*, which were rarely eaten. As a component of fresh tissue, betaine is widespread among marine invertebrates (McClintock *et al*., 1984), and given that chemoreception is dependent on signal-to-background contrast (Atema, 1995), concentrations of betaine in corals should be compared with background levels to assess its overall influence in the search and selection of prey by *A. planci*. Furthermore, replication is also necessary to examine variations in betaine concentrations between and within colonies, locations, and under different environmental conditions, since mitochondrial betaine synthesis in marine invertebrates has been found to be influenced by changes in temperature and salinity (*see* Polat & Beklevik, 1999).

Further bioassays with active fractions can potentially yield unique compounds or identify combinations of compounds, even ordinary ones, which elicit selective

predation by *A. planci*. Still, it should be taken into consideration that repeated fractionation or certain extraction procedures may result in loss of bioactivity, because chemical conversions and instabilities can vary as a function of how samples are stored, extracted, and partitioned (*see* Hay *et al*., 1998). Nevertheless, the chemoattraction exhibited by *A. planci* towards the 90% aqueous MeOH fraction shows that bioactive compounds have been preserved even after extraction and partitioning. Evidence from chemoattraction studies with other asteroids suggest that amino acids, small peptides, and even proteins may also play a role in *A. planci* feeding behavior (Collins, 1975a). Further bioassay-guided fractionation and purification of active extracts are being done to isolate novel chemoattractant compounds and identify potent mixtures.

CHAPTER 5

Effectiveness of Identified Feeding Attractant Compounds

5.1 Introduction

The ability of asteroids to sense waterborne chemicals had been widely reported in the past. This ability aids marine invertebrates in detecting and discriminating potential food and is inherent in models of optimal foraging behavior (Hughes, 1980). Chemoreception is common in asteroids, although contact chemoreception is more well-studied compared to distance chemoreception or olfaction (see Sloan & Campbell, 1982 and references cited therein). Zimmer & Butman (2000) stated that distance chemoreception is particularly important to relatively slow-moving predators (*e.g.* seastars, snails, and worms). Many studies have examined the sensory basis for asteroid perception of chemical stimuli from live, dead, or injured prey, as well as whole and fractionated prey homogenates (reviewed by Sloan and Campbell, 1982). However, the isolation of specific compounds to which asteroids respond has been elusive, thus few studies have also been done on the effectiveness of specific compounds.

Few studies have attempted to determine the specific compounds responsible for inducing feeding in *A .planci*. Beach *et al*. (1975) were able to induce movement when they presented *A .planci* with live coral extracts, while Brauer *et al*. (1970) were able to trigger stomach eversion using extracts of *Acropora formosa* and *Pocillopora eydouxi*. Settlement (*i.e.* mounting and positioning on top of cue) and stomach eversion responses

were also induced using extracts of coral tissue (Collins, 1974), and further fractionation of live coral tissue yielded high (proteins) and low (amino acids and small peptides) molecular weight fractions (Collins, 1975a), which were both found to cause settlement and stomach eversion. Following through on these findings, Moore & Huxley (1976) randomly tested several low molecular weight compounds, which were known feeding attractants of other invertebrates, for arm-rearing activity and found that only betaine produced this response at concentrations low enough to suggest its presence in coral as an active ingredient. Teruya *et al*. (2001) were later able to isolate betaine from *Montipora* sp., as well as arachidonic acid, which was identified as another feeding attractant. In addition, α-linolenic acid was also isolated from the sea urchin *Toxopneustus pileolus* and field and Y-maze experiments revealed that this compound is also a feeding attractant for *A. planci*.

It has been demonstrated in the past that marine invertebrates, which are normally caught in traps baited with natural foods, can also be attracted by specific compounds and more so by mixture of different compounds. This chapter mainly focuses on the effectiveness of α-linolenic acid (ALA) and betaine, as feeding attractants and explores the potential of these compounds in developing artificial baits for traps or collection stations, which could serve as management alternatives in the future. Arachidonic acid was not included in these tests since it was not an economically feasible option. Since previous laboratory and field experiments have been conducted on ALA by Teruya *et al*. (2001), modified methodologies employed in this study was mainly confirmatory in nature as variation in responses may occur among individuals from different localities (*i.e*. Japan and Guam) and as a result of different environmental factors. In addition, it

was evaluated whether these compounds serve as cues for locating prey from a distance (foraging cue) or as feeding stimulants once *A. planci* comes in contact with its coral prey.

5.2 Methods

5.2.1 Chemical Reagents

The polyunsaturated fatty acid, α-linolenic acid ($C_{18}H_{30}O_2$), was purchased from Fluka (Buchs, Switzerland) and Kanto Chemicals (Tokyo, Japan). ALA **(Figure 19a)** is known to be essential for growth and development in some species of fish and molluscs (Tinoco, 1982) and has a molar mass of 278.430 g •/mol. Ultrapure grade betaine hydrochloride ($C_5H_{11}NO_2$•HCl) was purchased from USB Corporation (OH, USA) and Amresco (OH, USA). Betaine or trimethylglycine (**Figure 19b**) is a cheap and harmless quaternary ammonium alkaloid with a zwitterionic structure (Zhou *et al*., 2008) with a low molar mass (117.146 g /mol). Polyoxyethylene sorbitan monolaurate (Tween 20) was purchased from Sigma-Aldrich (MO, USA). This nonionic polysorbate emulsifier has a hydrophilic-lipophilic balance value of 16.7 (Narsimhan & Wang, 2008) and was used to form an oil-in-water emulsion to integrate ALA into the gel matrix.

5.2.2 Laboratory Feeding Assays

Adult *A. planci* (~250- to 350-mm total diameter) used for the feeding assays were maintained as in Section *3.2.1*. All feeding assays were conducted on the Y-maze

using the same set-up (*see* Section *3.2.4* for specifications). All experimental food gels were prepared by adding 2 g of agar (Sigma-Aldrich, USA) to 100-ml filtered seawater in a glass beaker and stirring for 5 s. This mixture was heated in a microwave oven until boiling. Below are specific treatments for each set of feeding assays.

ALA Feeding Assay. Preliminary trials with ALA showed that it was highly insoluble in water, thus it was not evenly incorporated into the agar gel matrix and was floating on the surface rather than being suspended in the water column of the Y-maze. For the purposes of developing artificial bait, an emulsifier was added to ALA so that it can be evenly integrated into the gel matrix and serve as a waterborne cue in the Y-maze. To achieve this, 0.2-ml Tween 20 surfactant was thoroughly mixed with 1 ml ALA. As soon as the agar and seawater mixture cooled to 60°C, ALA was added using a micropipette and stirred using a magnetic stirrer (Corning, USA). This solution with 1% v/v ALA concentration (ALA concentration herein and hereafter are expressed in percent volume per volume) was poured into a 10-cm diameter glass petri dish left to cool and harden. Control food gels were treated in the same way, but only 0.2-ml Tween 20 surfactant was added without ALA. Feeding assays were conducted using the same set-up and conditions described in Section *3.2.5*, however, each food gel prepared was not used for more than 6 hrs and only 15 individuals were used for this assay.

Betaine Feeding Assay. Like most zwitterions, betaine is highly soluble in water but poorly soluble in most organic solvents (IUPAC, 1997). Betaine HCl crystals (1 g) were dissolved in 10 ml seawater. Experimental food gels were made by adding 2-g agar to 90-ml filtered seawater. After heating for 90 mins and cooling down to 60°C, the dissolved betaine HCl crystals were stirred into the mixture to achieve even distribution.

This solution with a m/v concentration of 1% betaine (betaine concentration herein and hereafter are expressed in percent mass per volume) was poured into a 10-cm diameter glass petri dish to cool and harden. Feeding assays were conducted using the same set-up and conditions described for the ALA feeding assay.

ALA + Betaine Feeding Assay. To test whether responses to these compounds are enhanced in synergy, a mixture of ALA and betaine was compared with ALA. The same amount of ALA (1 ml with 0.2 ml Tween 20 surfactant) and betaine (1 g) were added and mixed to the heated and cooled 2-g agar and 100-mL seawater mixture. The ALA control was prepared as described above in the ALA feeding assay. Feeding assays were conducted in the same manner as the two preceding assays described.

5.2.3 Field Feeding Assays

Sites for field experiments were chosen based on the results of the manta tow surveys (*see* Chapter 2). Sites with relatively medium *A. planci* densities (< 100 ind • ha^{-1}) and well-dispersed individuals were preferred over high densities and clumping in massive outbreak sites and low densities in unaffected areas to control for the confounding effects of seastar density. Environmental conditions like water temperature, water flow and movement, coral reef complexity, hunger level of seastars, and other factors were not controlled but were noted in interpreting results. The set-up for these assays is illustrated in **Figure 18**.

ALA Feeding Assay. Feeding assays were conducted at Gun Beach (13.525291°N, 144.802459°E) on January, 2008 and at Faifai Beach (13.532359°N, 144.801159°E) on February, 2008. Each site had unique hydrodynamic regimes during

the field experiments, *i.e.* Gun Beach (10-m to 12-m depth)was calm, and had low wave action and turbulence while spur and groove areas at Faifai Beach (5-m to 7-m depth) had high wave action and turbulence during assays. Three sets of experimental food gels were prepared for each site, with three replicates each: control, 1 % ALA, and 2 % ALA. The gel matrix was prepared as in Section *4.2.2* and as soon as it cooled to 60°C, 1-ml ALA (mixed with 0.2-ml Tween 20 to emulsify with gel matrix) was added to the "1 % ALA Treatment", 2-ml ALA (mixed with 0.4-ml Tween 20 surfactant) to the "2 % ALA Treatment", and only 0.2-ml Tween 20 was added to the "Control". Each of these mixtures were poured into a 10-cm diameter petri dish; then after the gels cooled and hardened, each one was placed into separate sealed Ziploc plastic bags to avoid mixing once underwater. Each petri dish was placed in mesh pouches (1 mm^2 mesh size), then pouches were mounted on the substrate with concrete nails (**Figure 17a**) to avoid being carried by water current and surge. Each petri dish were positioned at least 8-m apart. The number of *A. planci* within a 1-m radius and 3-m radius from the experimental gels were recorded (**Figure 18**). After 2 hrs, the numbers of individuals within these radii were counted again. Individuals within 1-m radius were included in counting the number of *A. planci* within 3-m radius.

Betaine Feeding Assay. Field feeding experiments with 1% betaine concentration were conducted at Two Lovers' Point (13.534598°N, 144.801337°E) on June, 2008 at around 5-m to 7-m depth range. Three replicates each of control and betaine gels were prepared following methods used in the laboratory feeding assays with betaine in Section *4.2.2*. Each petri dish containing the gels were placed into mesh pouches and fixed to the substrate at least 8-m apart using concrete nails. The initial

number of *A. planci* within a 1-m radius and 3-m radius from the experimental gels were recorded (**Figure 18**) and counted again after 2 hrs. Field feeding assays using 10% betaine concentration were conducted at the southern end of Ague Point (13.565360°N, 144.819119°E) on October, 2008 around depths between 10-m to 12-m. The reef was mostly flat and dominated by recently killed *Montipora* sp. Gel preparation also followed methods described above, but 10 g of betaine was incorporated into the 100-ml agar and seawater mixture. Each of the petri dishes were separately placed into sealed Ziploc bags to avoid mixing. Each petri dish containing the gels were placed at least 8-m apart to avoid counting a single individual twice and fixed to the substrate using flagged concrete nails (**Figure 17b**). Gels were placed at 1200 h then the number of individuals within a 1-m and 3- m from each gel were recorded to get the initial count (**Figure 18**). The same counting procedure was carried out at 1500 h in the afternoon (after 3 h), nighttime at 1900 h (after 7 h), then on the following day at 1200 h (after 24 h). Two replicates each for "Control" amd "Betaine Treatment" were missing after 24 h, so, only 8 replicates of each were surveyed.

5.2.4 Field Trapping Experiments

Trapping of *A. planci* was attempted on May, 2008 at Faifai Beach (13.531427°N, 144.800231°E), where *A. planci* density was relatively moderate. Two wire mesh fish traps (120 cm X 90 cm X 60 cm; 1.25 cm^2 mesh size), with a single opening that tapered down to 20-cm, were modified by adding a plexiglass ramp to make it easier for *A. planci* to climb in. Concentrations of betaine were increased 10-fold and concentrations of ALA 5-fold. Gels were prepared by adding 4 g agar to 200 ml filtered seawater. This mixture

was heated to boil then cooled to 60°C before adding 20 g pre-dissolved betaine HCl and 10 ml ALA (pre-mixed with 2 ml Tween 20 surfactant) with constant stirring for 30 s. The mixture was poured into a 14.5-cm diameter plastic petri dish and left to cool and harden. Only 2 ml Tween 20 surfactant was added to the "Control" gel. Traps were installed approximately 5-m apart and gels were placed inside mesh pouches and secured inside the traps using cable ties. Traps were left for 24 h prior to retrieval.

5.2.5 Statistical Analyses

Frequency of moving upstream in the Y-maze towards stimulus-emitting arm versus control in each choice assay was analyzed using G-test for goodness-of-fit with Yates' correction for continuity against a 1:1null hypothesis. Variation in *A.planci* frequencies of preference (based on the difference of the number of individuals after 2 hrs from the initial count) within a radius of 1 m and 3 m from experimental food gels (2% ALA concentration, 1% ALA, and control) *in situ* were analyzed using Two-Factor ANOVA (Model I), with treatments and sites as fixed factors. Tukey's HSD Test was used for *post hoc* comparisons. A Student T-test was carried out to test differences in frequencies with which 1% betaine treatment and control were preferred within a radius of 1 m and 3 m from the experimental food gels. Data from field assays testing 10% betaine were also analyzed using Student's t-test at $\alpha = 0.05$, although its non-parametric analog, the Mann-Whitney U Test, was alternatively used in cases where assumptions of normality and homogeneity of variance were not met.

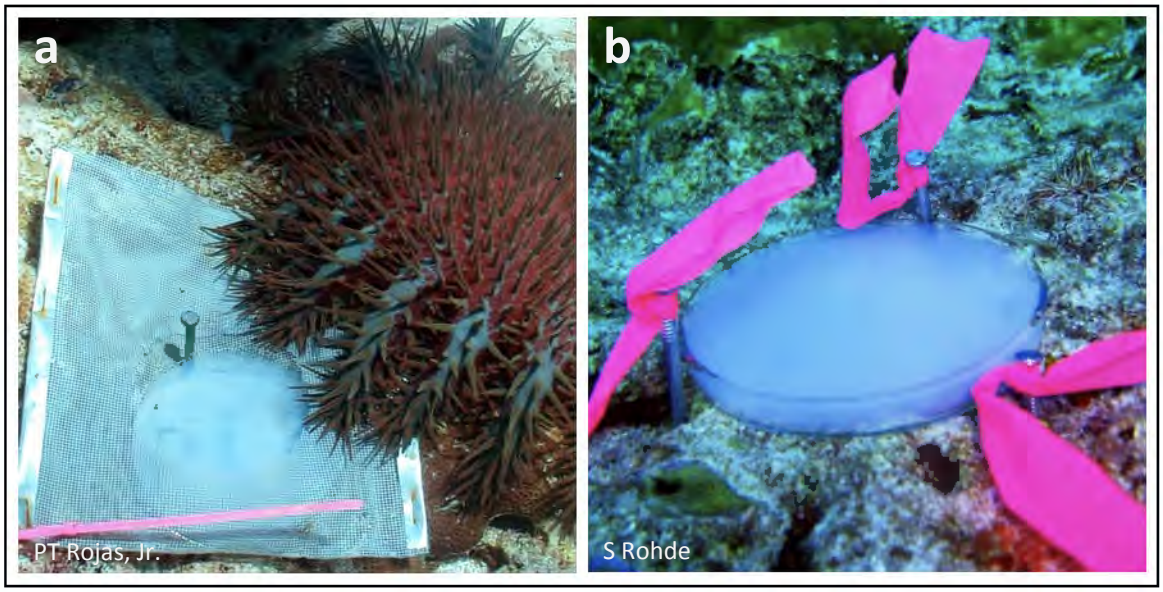


Figure 17. Experimental food gels used in field feeding assays. (a) ALA food gel inside mesh pouch and mounted using concrete nails; (b) Betaine food gel secured on the substrate using three flagged concrete nails.

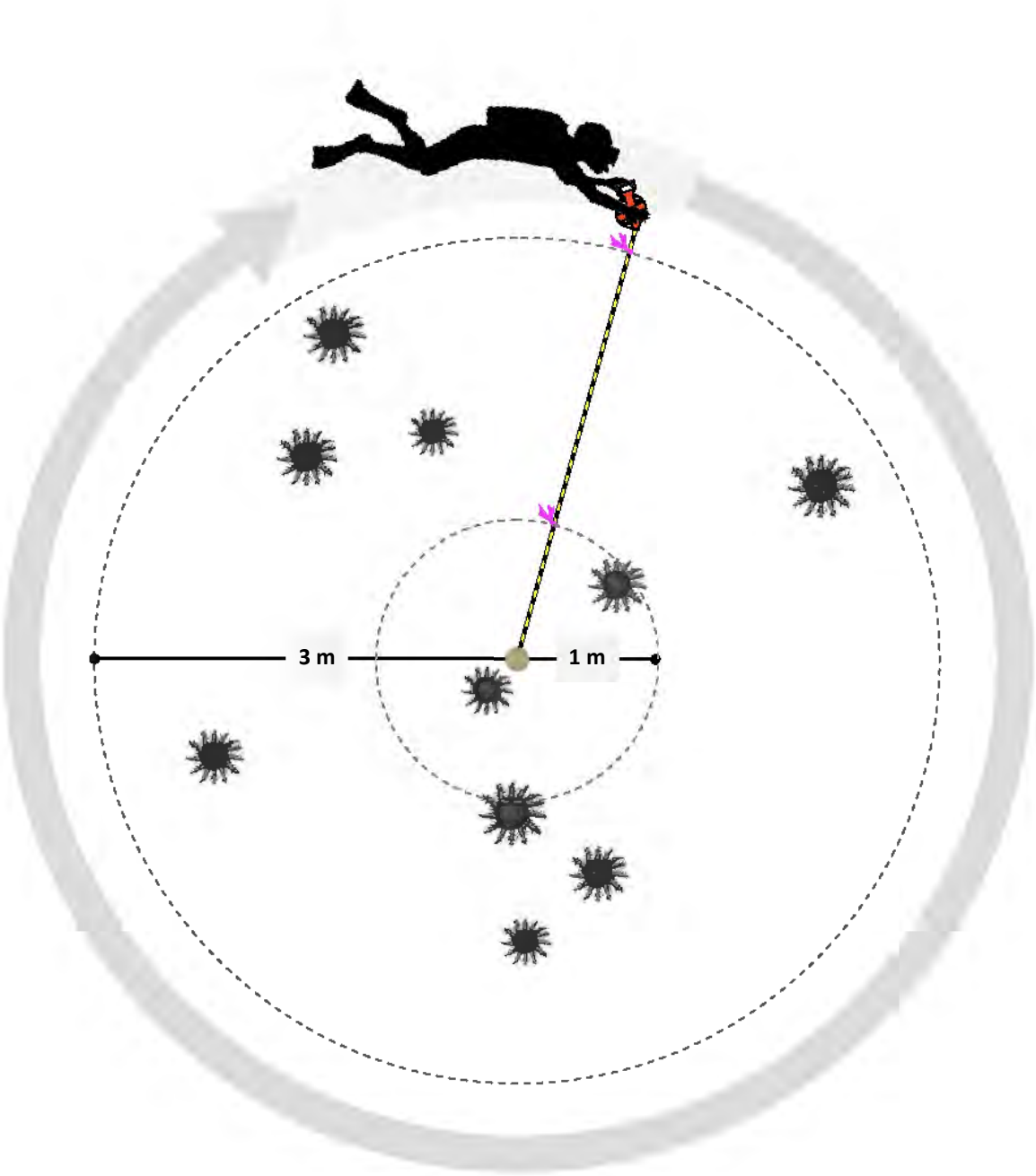


Figure 18. Set-up of field feeding assays. One end of the pre-marked tape measure was attached to the nail used to secure the gels and the observer held on to the other end while swimming a full circle around the petri dish and counting individuals within a 1-m radius and 3-m radius from the experimental food gel.

5.3 Results

5.3.1 Laboratory Feeding Assays

The frequency with which different food gel treatments were preferred over controls is summarized in **Figure 19c**. When *A. planci* were offered choices of control food gels without ALA and identical food gels with 1% ALA, 25 out of 30 individuals were more attracted to the food gels with ALA ($G_{Yates'}$ = 13.004, df = 1, $p < 0.001$). In Y-maze feeding experiments with 1% betaine, individuals significantly preferred food gels with betaine 23 out of 30 times over control gels ($G_{Yates'}$ = 8.992, df = 1, p = 0.003). However, when 1% betaine was combined with 1% ALA and compared to control food gels containing only ALA, there was no significant difference in the frequency with which experimental food gels were preferred ($G_{Yates'}$ = 0.837, df = 1, p = 0.360).

5.3.2 Field Feeding Assays

ALA Feeding Assay. Differences in the number of individuals from initial counts between sites and treatments are shown in **Figure 20**. The statistically significant interaction between the attractant and site factor (F = 6.857, df = 2, p = 0.010) indicates that the effectiveness of ALA as a short-distance (1-m radius) chemoattractant was dependent on whether experiments were conducted at the calmer Gun Beach site or at the more wave-washed Faifai Beach site (**Figure 20a**). Subsequent *post hoc* tests show that variation was mainly due to the differences in *A. planci* frequency net gain between 2% ALA and control gels at the Gun Beach site (Tukey's HSD, p = 0.002). On the other hand, there was no significant variation in the mean differences from initial counts within

3 m from the 2% ALA, 1% ALA, and control gels ($F = 2.083$, $df = 2$, $p = 0.167$) as well as between sites ($F = 4.083$, $df = 1$, $p = 0.066$), thereby indicating that ALA may not be as effective in long-distance chemoreception (**Figure 20b**).

Betaine Feeding Assay. Differences in the number of individuals from initial counts within a 1-m and 3-m radius from agar gels with betaine and control gels were used for statistical calculations and shown in **Figure 21**. In the field assay testing 1% betaine concentration against controls, there was no significant difference in the frequency with which *A. planci* were attracted to within 1m from the agar gels with 1% betaine and control gels (Student's $t = -0.707$, $df = 4$, $p = 0.519$; *see* **Figure 21a**). However, the difference of the initial number of *A. planci* from the count after 2 h was significantly higher in agar gels with 1% betaine compared to control gels within a 3-m radius (Student's $t = -4.899$, $df = 4$, $p = 0.008$; *see* **Figure 21b**). In the field assay testing 10% betaine concentration, the initial number of *A. planci* within 1-m from experimental food gels was subtracted from the number after 3h, after 7h, and after 24 h (**Figure 21c**). Calculated differences within the 1-m radius did not meet assumptions of normality and homogeneity of variance; hence medians were compared using non-parametric Mann-Whitney U Test instead of comparing means using Student's t-test. After 3 h, the difference from initial counts within 1-m from agar gels with 10% betaine and control gels did not vary significantly (Mann-Whitney $U = 45.000$, $p = 0.368$). Conversely, there was a significant difference in the median values between agar gels with 10% betaine and control gel after 7 h (Mann-Whitney $U = 15.000$, $p = 0.002$) and after 24 h (Mann-Whitney $U = 12.000$, $p = 0.038$). Similarly, the difference in the mean frequency with which *A. planci* were attracted to within 3 m (**Figure 21d**) from the agar gels with 10%

betaine and control gels were not significantly different after 3 h in the substrate (Student's $t = 0.507$, $df = 18$, $p = 0.618$), but were significantly different after 7h (Student's $t = -3.929$, $df = 18$, $p < 0.001$) and after 24 h (Student's $t = -2.581$, $df = 14$, $p = 0.022$).

5.3.3 Field Trapping Experiments

No *A. planci* were found inside the trap with betaine and ALA, as well as in the control trap after 24 h. Nonetheless, two individuals were observed close to the traps with agar gels containing betaine and ALA. New trap designs are under development to specifically take into consideration the behavior of *A. planci*.

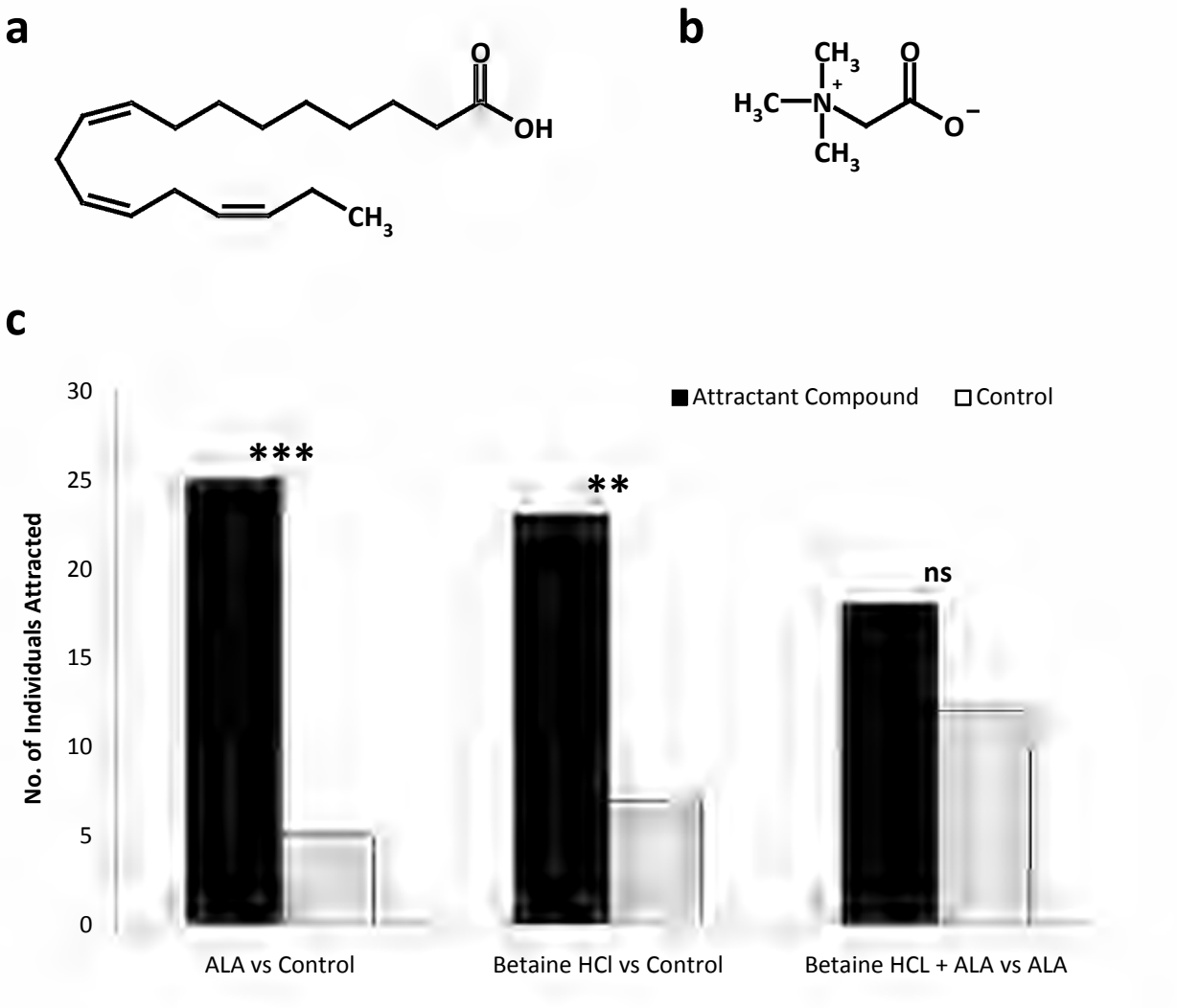


Figure 19. Laboratory feeding assays testing identified *A. planci* attractant compounds. (**a**) chemical structure of ALA; (**b**) chemical structure of betaine; (**c**) proportion of individuals attracted to test compound in the Y-maze. Each assay (n = 30) was analyzed using G-test for goodness-of-fit with Yates' correction (*** $p < 0.001$; ** $p < 0.01$; ^{ns} $p > 0.05$, therefore not significant) at $\alpha = 0.05$.

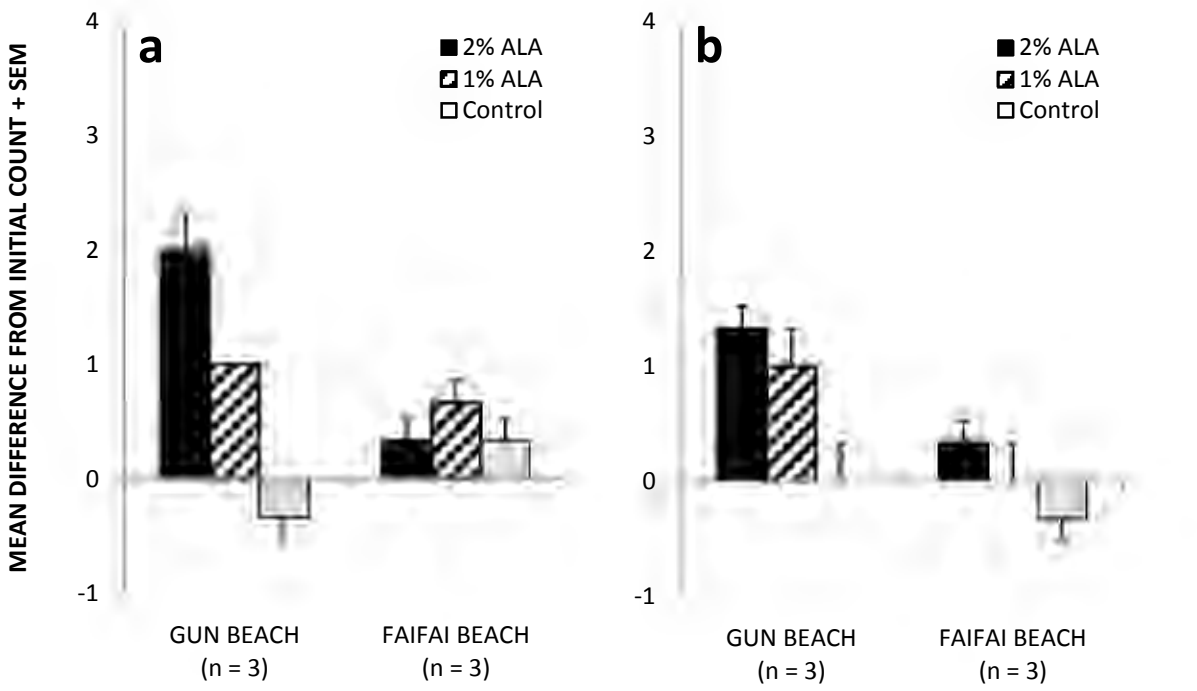


Figure 20. Field assays testing the effectiveness of ALA as feeding attractant. (**a**) Mean difference from initial count + SEM within a 1-m radius, *n* = 3 for each treatment; (**b**) mean difference from initial count + SEM within a 3-m radius, *n*= 3 for each treatment.

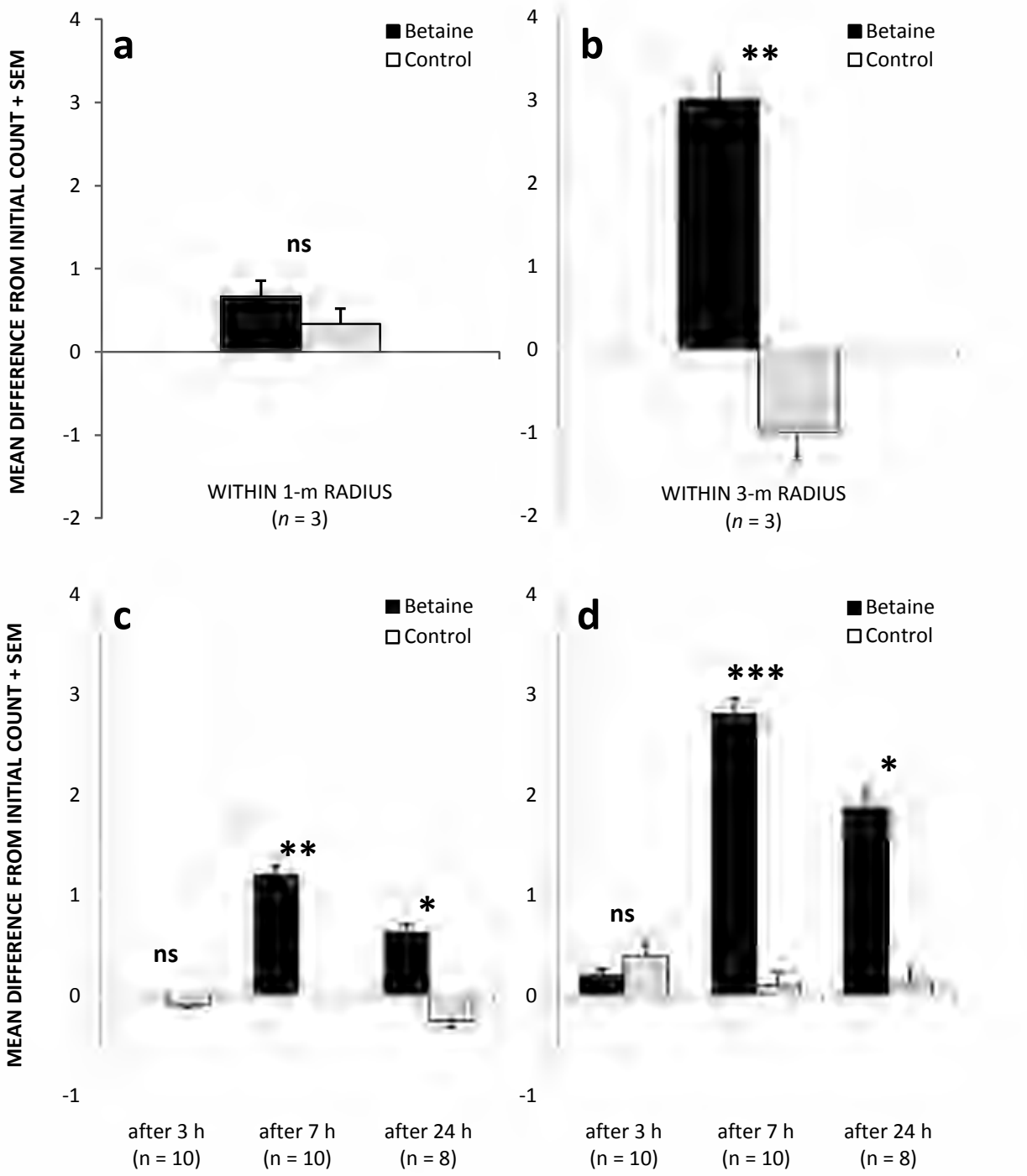


Figure 21. Betaine field experiments: (**a**) 1% betaine, within 1-m radius; (**b**) 1% betaine, within 3-m radius; (**c**) 10% betaine, within 1-m radius; (**d**) 10% betaine, within 3-m radius. Data analyzed using Student T-test at $\alpha = 0.05$, although Mann-Whitney U-Test was used whenever assumptions were violated (*** $p \leq 0.001$; ** $p < 0.01$; * $p \leq 0.05$; ^{ns} $p > 0.05$, therefore not significant).

5.4 Discussion

Results of laboratory and field assays described herein show that *A. planci* respond to specific chemical compounds. These are consistent with laboratory experiments by Moore & Huxley (1976), which found that betaine induced arm-rearing activity in *A. planci*; and with laboratory and field tests by Teruya *et al*. (2001), which showed that α-linolenic acid is a feeding attractant for *A. planci*. Chemoreceptive responses to specific compounds have also been observed in other asteroids. Anderson (1953) reported that acetylcholine elicited stomach eversion in the seastar *Asterias forbesi*, while adrenaline chloride caused retraction and closure of the stomach. Moreover, solutions containing L-glutamic acid, bacterial peptone, and a mixture of 10 amino acids acted as repellents and altered positive rheotactic behavior in *Asterias rubens* (Castilla, 1972). Conversely, Zafiriou *et al*. (1972) found that *A. rubens* was attracted to amino acids and their mixtures. A variety of chemical stimuli, including urea, lactic acid, and succinic acid were also shown to attract *Asterias vulgaris* (Zafiriou, 1972). Glucose and the amino acids, leucine and phenylalanine, were also found to simulate ciliary pumping in Tiedemann's pouches of *Echinaster echinophorus* (Ferguson, 1969). *Luida clathrata* exhibited a strong chemotactic response to betaine and amino acids such as L-cysteine, L-cystine, L-isoleucine, and L-glutamic acid (McClintock *et al*., 1984). Behavioral responses (*i.e.* feeding posture, stomach eversion, and locomotion of initially inactive animals) of *Marthasterias glacialis* were induced by lactic acid, amino acids, and acetylcholine (Valentinčič, 1985). Furthermore, Kidawa (2005) demonstrated that the Antarctic seastar *Odontaster validus* can detect amino acids, with glutamic acid being the most potent stimulant and mixtures being more stimulatory than single amino acids.

The ecological and physiological function of ALA in marine organisms is relatively unknown. Some studies have shown ALA to be essential for growth and development in several species of fish and molluscs (*see* Tinoco, 1982). The study on the role of ALA as a chemoattractant for *A. planci* (Teruya *et al*., 2001) was one of the first to propose an ecological function for ALA in marine invertebrates. However, Teruya *et al*. (2001) isolated ALA from the flower urchin *T. pileolus*, which is relatively rare and may not be subjected to predation in the presence of high coral densities (Moran, 1986). ALA may not be a significant factor in coral prey selection by *A. planci*. In fact, several studies on the lipid composition of corals consistently measure zero to very low concentrations of ALA (Meyers, 1979; Latyshev *et al*., 1991; Bachok *et al*., 2006; Zhukova & Titlyyanov, 2006). Moreover, these studies show that the most common fatty acids found at high concentrations in coral are palmitic acid (16:0), stearic acid (18:0), arachidonic acid (20:4 ω-6), EPA (20:5 ω-3),and DHA (22:6 ω-3). Among these, arachidonic acid has been identified as a feeding attractant for *A. planci* (Teruya *et al*., 2001); hence, this compound may play an important role in the discrimination between prey corals by *A. planci*.

Nevertheless, results of field and laboratory experiments indicate that ALA is an effective feeding attractant at favorable water flow conditions. Although the hydrophobicity of ALA limits its ability to be transported in the water column, a well-directed fluid flow from the cue source to the seastar (as demonstrated in the Y-maze) makes it a potent cue for short-distance location. Field assays also suggest that high levels of turbulence caused by waves during the experiment at Faifai Beach reduced chemoreception by *A. planci*. Asteroids in wave-washed environments that experience

high turbulence would not be able to use chemosensitivity as a means of locating prey (Dayton *et al*., 1977). Prey location is highly dependent on the hydrodynamic transport of attractants (Zimmer & Butman, 2000), thus, in the absence of bulk flow, *A. planci* will not have a coherent chemical trail to follow. Weissburg and Zimmer-Faust (1993) have shown that mechanisms governing the transport of chemical signals influence behavioral responses, which determine predatory success. In theory, it is more likely that ALA (if indeed present in corals at sufficient concentrations) or arachidonic acid are sequestered within coral tissues rather than released into the environment since both compounds are hydrophobic. Therefore, these fatty acids may be more important in short-distance chemoreception or may function as feeding stimulants identified via contact chemoreception rather than as foraging cues detected via olfaction.

Betaine, on the other hand, is found in large concentrations among marine invertebrates and is widely used as a feed additive in fish aquaculture and prawn farming to promote seawater adaptation and growth performance (Castro *et al*., 1998; Felix & Sudharsan, 2004; Genc et al., 2006; Shankar *et al*., 2008). Physiologically, betaine has been found to function as an osmoregulator and as a methyl donor in fish and marine invertebrates (*see* Polat & Beklevik, 1999; de Vooys & Geenevasen, 2002). As a component of fresh tissue, betaine has also been shown to be highly stimulatory to organisms which eat flesh (Crisp, 1967; Fuzessery & Childress, 1975; Moore & Huxley, 1976; Carr, 1978; McClintock *et al*., 1984). Results of laboratory and field assays confirm that betaine is an effective chemoattractant for *A. planci*. Moore & Huxley (1976) have shown that betaine strongly induced arm-rearing responses in *A. planci*. Teruya *et al*. (2001), likewise, demonstrated in aquarium experiments that *A. planci* was

attracted to betaine at 1.6 % m/v concentration. Consistent with the previous argument (Chapter 4: Discussion) that betaine plays a role in prey location but not in prey discrimination, field assays show that short-distance (1-m radius) chemoreception was only effective when the concentration was increased from 1% to 10%. However, betaine was an effective attractant for long-distance (3-m radius) chemoreception. The high water solubility of betaine facilitates its release and chemoreception by *A. planci* via molecular diffusion. In addition, a decrease of *A. planci* numbers in close proximity to attractant gels after 24 hrs may indicate a weaker signal due to diffusion of betaine from the gels. A low residence time in the water column, thus requires steady synthesis of betaine under natural conditions.

The effectiveness of betaine as a feeding attractant used for prey location by *A. planci* makes it a potential bait that may be incorporated into the design of traps or for collection stations. For this purpose however, higher concentrations of betaine may be required to increase the signal-to-noise ratio and cue residence time in the environment (Atema, 1995; Zimmer & Butman, 2000). This allows attractant gels to be effective for longer periods and attract *A. planci* from farther distances, depending on prevailing local hydrodynamic conditions. Failure to trap *A. planci* may partly be due to the moderate density of *A. planci* in the area and the design of the trap, which was originally a fish trap. An ideal trap is one that maximizes the probability of entry and minimizes probability of escape of the desired target species (Miller, 1990). Development of new trap designs should include easy and accessible entrances for *A. planci* on all sides while preventing its escape once captured. The use of an appropriate bait producing highly attractive signals and remaining active during the full soak time will also be crucial in

maximizing trap effectiveness (Hancock, 1974). Further isolation could yield new attractant compounds, which in addition to betaine, could be incorporated into bait gels to increase potency. Nevertheless, betaine may be used to set-up bait stations, which could reduce search efforts during *A. planci* collection.

Another important aspect in *A. planci* chemoreception, which certainly warrants further investigation, is whether or not responses are receptor-mediated, and if so, specific receptor sites for these chemical compounds should be identified. Receptors recognize chemical signals against a background of many other chemicals – a process that involves not only receptor specificity and diversity but also recognition of the intensity and time course of the signal to allow a receptor to distinguish a true signal from random events (Atema, 1995). Specific receptor sites for betaine have been proposed in the palatal taste system of the pufferfish *Fugu pardalis* (Kiyohara & Hidaka, 1991) and in the column ectoderm of sea anemones *Uriticina eques* and *U. felina* (Boothby & McFarlane, 1986). Unspecialized epithelial cells throughout the skin of *A. planci* (Pentreath & Cobb, 1972) and the tube feet, especially the terminal tentacle (Sloan, 1980b), are potential receptor sites for chemical signals.

CHAPTER 6

Chemoattraction and the Formation of Aggregations

6.1 Introduction

Aggregations are widely reported among benthic invertebrates and in the case of *A. planci*, as in most asteroids, it is most often related to feeding activities (Sloan, 1980). It has been observed previously (Ormond *et al*., 1973; Ormond & Campbell, 1974) and during surveys in this study that *A. planci* tend to feed on a coral colony that is being preyed upon or already preyed upon by other *A. planci*, bypassing a similar prey colony that is intact. This observed behavior lead to the formation of mini-aggregations of 3 to 20 individuals (Ormond & Campbell, 1974), thus suggesting some form of conspecific chemoattraction during feeding. Experiments by Ormond *et al*. (1973) using Y-cages on the sea bed and an aquarium in the form of a Y-maze have demonstrated preferential attraction by *A. planci* toward a partially eaten coral colony rather than intact corals. Although the source of cues eliciting a high degree of attraction to partially eaten coral was not determined, Collins (1974) suggested that this could be due to the greater release of normal constituents of coral once the cell structure of the animal has been disrupted, rather than to the release of breakdown products of digestion or components of the digestive juices of *A. planci*.

In the field, *A. planci* almost exclusively feed on scleractinian corals (see reviews by Moran, 1986; Birkeland & Lucas, 1990; Carpenter, 1997). These reef-building corals

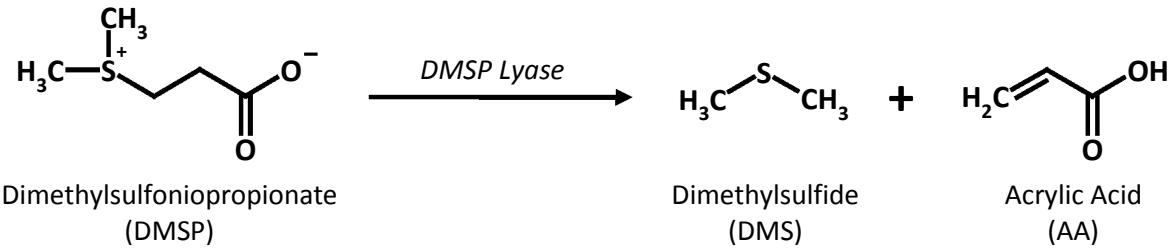
contain, within membrane-bound vacuoles in the gastrodermal cells (Kühlmann, 1988), small symbiotic dinoflagellates called zooxanthellae, with densities from 1-2.5 × 10^6 cells per cm^2 of coral tissue (Drew, 1972; Muscatine *et al*., 1989) under normal conditions. Dinoflagellates are large producers of dimethylsulfoniopropionate (DMSP) and coral zooxanthellae produce among the highest levels (Hill *et al*., 1995; Broadbent *et al*., 2002; Van Alstyne *et al*., 2006).

DMSP is a nonvolatile tertiary sulfonium compound that was first described as a precursor of volatile dimethylsulfide in *Polysiphonia fastigiata* (Challenger & Simpson, 1948). The DMSP cleavage pathway that yields dimethylsulfide (DMS) and acrylic acid was later described in *Enteropmorpha intestinalis* (Bywood & Challenger, 1953). This reaction is catalyzed by the enzyme DMSP lyase, which was identified from *Polysiphonia lanosa* by Cantoni and Anderson (1956). The conversion of DMSP to DMS and acrylic acid by DMSP lyase (**Figure 22**) is an important process because the DMS produced by this reaction plays an important role in oceanic sulfur cycles and global climate regulation (Lovelock *et al*. 1972; Charlson *et al*., 1987; Malin & Kirst, 1997; Yoch, 2002). Despite being extensively studied, the role of DMSP and its cleavage products in mediating ecological interactions is still poorly understood.

DMSP is believed to function primarily in osmoregulation (Vairavamurthy *et al*., 1985; Edwards, 1988), although other roles for DMSP and its cleavage products have also been proposed, including its functions as a cryoprotection for some coldwater species (Kirst et al., 1991), as an antioxidant (Sunda et al., 2002; Jones *et al*., 2007), and as an antibiotic (Sieburth, 1960). Several studies have also looked at its role as chemical defense of micro- and macroalgae (Wolfe & Steinke, 1996; Wolfe *et al*., 1997; Van

Alstyne *et al*., 2001; Steinke *et al*., 2002; Van Alstyne & Houser 2003; Wiesemeier *et al*., 2007). These studies propose an activated defense mechanism wherein grazing by herbivores convert the DMSP from the prey into DMS and acrylic acid by mixing with DSMP lyase once tissues are damaged, thereby acting as a feeding inhibitor. Aside from defense, DMSP also functions as a foraging cue for reef fishes (DeBose *et al*., 2008). Free-roaming pelagic fishes have also been found to associate with periodic elevations of DMSP in their natural habitat (DeBose & Nevitt, 2007). Moreover, behavioral tests also demonstrated that DMSP stimulates feeding in some marine fishes (Nakajima *et al*., 1990). However, the predation-induced release of DMSP cleavage products by corallivores is still poorly understood and its effect on the chemotaxis and aggregation behavior of these predators is essentially unknown.

This chapter explores the potential role of DMS and acrylic acid release during *A. planci* predation as potential cue in triggering the formation of mini-aggregations. Laboratory assays were conducted to determine the preference of *A.planci* between whole intact corals and injured corals, between DMS-acrylic acid mixture and control, and between intact coral and intact coral with DMS-acrylic acid mixture. This is the first study to examine the role of DMSP in the feeding behavior of a marine benthic corallivore.



Dimethylsulfoniopropionate (DMSP) | Dimethylsulfide (DMS) | Acrylic Acid (AA)

Figure 22. Enzymatic cleavage of DMSP. Reaction is catalyzed by DMSP lyase (dimethylpropiothetin dethiomethylase, EC 4.4.1.3) and yields DMS and acrylic acid (adapted from Wiesemeier & Pohnert, 2007; Van Alstyne, 2008)

6.2 Methods

6.2.1 Study Organisms

Adult *A. planci* (~250- to 350-mm total diameter) used for Y-maze feeding assays were maintained as in Section *3.2.1*. Although starving animals prior to food preference experiments has been shown to make some animals less selective (see Sloan, 1980a), individuals were not fed for two weeks before being used in assays involving DMS because DMS evaporates rapidly, thus it was necessary that *A. planci* were hungry enough so that it immediately approached the source of cues presented to it before DMS is completely depleted from experimental food gels. Ten colonies of *Acropora surculosa* (~ 15-cm diameter) were collected from Pago Bay (13.426764°N, 144.798679°E) and kept in 1.4-m^3 concrete tanks at the UOG Marine Laboratory with flow-through seawater and supplied with constant aeration. These corals were immediately used for feeding assays and intact colonies were returned to the collection site while partially injured colonies were extracted for further analysis (*see Section 6.2.3*). This species was selected because it is a preferred by *A. planci* and is relatively common around Guam.

6.2.2 Chemical Reagents

The volatile organosulfur, dimethylsulfide (C_2H_6S) and the second cleavage product, acrylic acid (C_3H_4O2) were purchased from Sigma-Aldrich (Steinheim, Germany). Both compounds were carefully sealed when not in use because of its characteristic foul odor and stored at -20°C. This odor is distinctly similar to the smell

that corals give off when stressed. Agar used to make experimental food gels were purchased from Sigma-Aldrich (MO, USA).

6.2.3 Choice Assays

Three sets of Y-maze assays were conducted with the following pairs of choices: **Experiment 1** – injured coral versus whole, intact coral control; **Experiment 2** – agar-based gels with DMS and acrylic acid versus blank control; and **Experiment 3** – intact coral with DMS and acrylic acid versus intact coral control. Y-maze specifications are identical with previously described assays.

Experiment 1. Two intact *A. surculosa* colonies were placed in the Y-maze, one on each arm, to acclimate for 1 hr. Each colony was enclosed by a 20-cm3 mesh (1.5 cm2 mesh size) cage so that *A. planci* cannot feed on them during assays. Partial damage due to sublethal predation by *A. planci* on corals was simulated on one colony by blasting tissues off the skeleton with a jet of compressed air and seawater from an artist's airbrush (*see* Szmant *et al*., 1990). The coral surface turned white as soon as tissues were washed off and the distinct sulfur-like smell was immediately noticeable. After five minutes (time for cues to reach the end of the Y-maze), one *A. planci* was placed on the end of the Y-maze and preference was scored once the individual reaches the cage. A sample size of 25 *A. planci* was used for this experiment. Simulation of predation was done every hour and colonies were replaced once half of the colony has been damaged by washing off tissues. The Y-maze was drained and washed, and the positions of intact and injured corals were reversed each time a new set of colonies was used to rule out any built-in bias of the Y-maze.

Experiment 2. Agar-based gels were prepared as described above, with 2 g agar dissolved in 100 ml filtered seawater and heated in a microwave oven for 60 s. This mixture was cooled to 40°C, then 5.41 µl DMS and 5.05 µl acrylic acid was added. Concentrations for DMS and acrylic acid are based DMSP concentrations from Van Alstyne *et al*. (2006) for *Acropora cerealis*, which had the highest concentration (fmol) per zooxanthellae among scleractinian corals. Calculations used molecular weight ($g \cdot mol^{-1}$) and density data ($g \cdot ml^{-1}$) of DMS and acrylic acid and assumed a zooxanthellae density of $1 \cdot 10^6$ cells per cm^2 coral surface (Drew, 1972; Muscatine *et al*., 1989). The mixture was poured into a 10-cm diameter glass petri dish and covered to minimize DMS evaporation before being left to cool and harden. Control gels were the same, except they lacked DMS and acrylic acid. Each set of gels were used for at most 2 h due to the volatility of DMS and positions of treatments were reversed after every three sets. The estimation of the duration of each set was based on Van Alstyne & Houser (2003), where approximately 25% of the iinitial DMS concentration remained after 2 h. A sample size of 35 *A. planci* was used for this experiment and preference was scored as soon as individuals pass Section 1 of the Y-maze (**Figure 10**).

Experiment 3. One intact coral colony, enclosed in a mesh cage (see Experiment 1) was placed in one arm of the Y-maze to acclimate for 1 h. At the start of the choice experiment, a petri dish with DMS and acrylic acid incorporated into the agar gel (see Experiment 2 for preparation procedures) was placed beside the cage. The intact coral control was also allowed to acclimate for 1 h prior to the experiment and a control gel (as in Experiment 2) was placed beside it at the start of the experiment. Each set of agar gels were used for a maximum of 2h in the Y-maze and positions were reversed for every 3

sets of agar gels. The Y-maze was drained and washed, and new coral colonies were also used each time positions were reversed. A total of 25 *A. planci* were used in this experiment and preference was scored as soon as individuals go beyond Section 1 of the Y-maze (**Figure 10**).

6.2.4 Statistical Analyses

Each choice experiment was analyzed using G-test for goodness-of-fit against a 1:1 preference ratio. Yates' correction for continuity was applied because $df = 1$ for each choice experiment.

6.3 Results

Choice assay results are summarized in **Figure 23**. In Experiment 1, where *A. planci* were given a choice between injured coral and intact coral, injured coral was preferred 20 out of 25 times (G_{Yates} = 8.312, df = 1, p = 0.004), even though *A. surculosa* is a highly preferred species. This suggests that damaged corals possibly release distinct compounds or increased concentrations of certain compounds, which trigger positive chemotaxis in *A. planci*. When offered choices of gels with DMS and acrylic acid versus identical gels without DMS and acrylic acid, *A.planci* significantly preferred gels with DMS and acrylic acid over controls in 24 out of 35 instances (G_{Yates} = 4.199, df = 1, p = 0.040). During gel preparation and choice assays, significant amounts of DMS are expected to be lost. In Van Alstyne and Houser (2003), 50 % of the DMS was lost in 1 h and 75% within 4 h. Thus as assays with each set of gels progressed, *A. planci* were making choices between gels with relatively smaller differences in DMS concentration making it conservative for the effect of DMS on chemoattraction (Van Alstyne & Houser, 2003). In Experiment 3, *A. planci* preferred the choice where DMS and acrylic acid were placed with intact corals over the intact coral with control gels in 18 out of 25 occasions (G_{Yates} = 4.114, df = 1, p = 0.043). Yet again, this experiment is also conservative for the effects of DMS in *A. planci* chemoattraction due to the volatility of DMS.

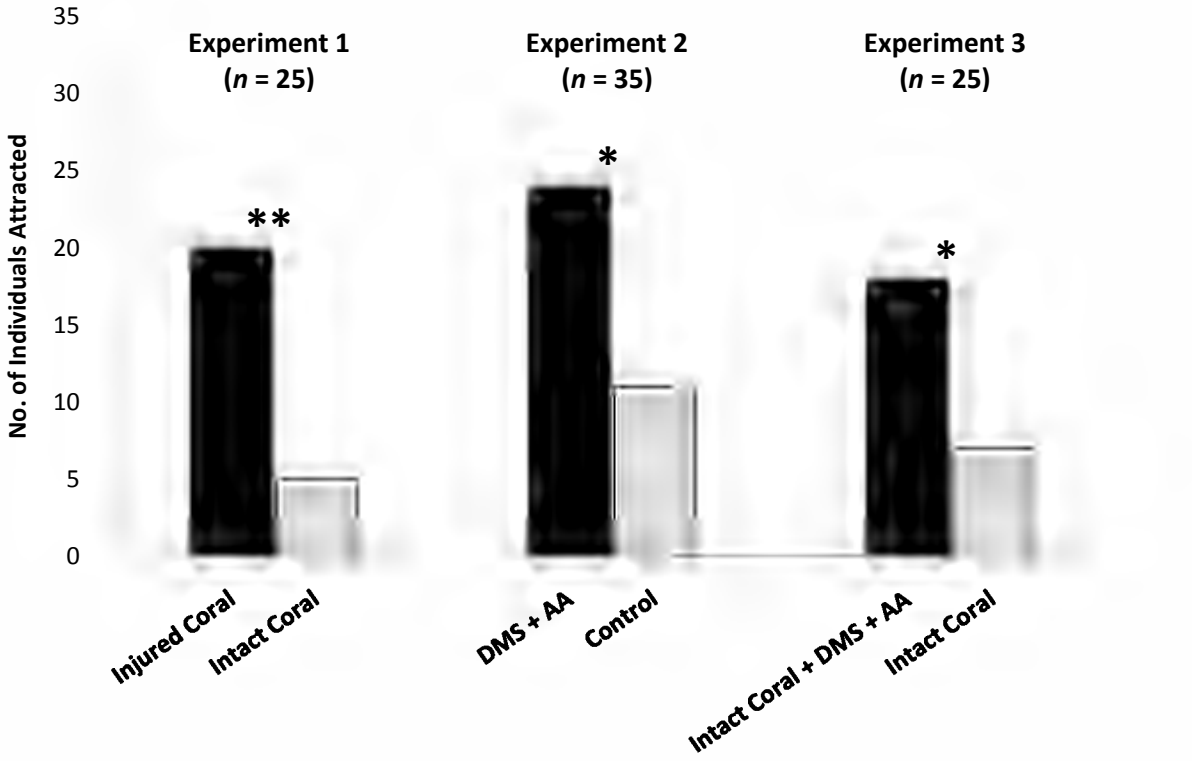


Figure 23. Laboratory choice assays on the mechanism of predation-induced conspecific chemoattraction. Different pairs of choices for each experiment are shown. Each experiment was analyzed using G-test for goodness-of-fit with Yates' correction (** $p < 0.01$; * $p < 0.05$ at $\alpha = 0.05$.

6.4 Discussion

These results once again suggest that *A. planci* are capable of navigating towards favorable cues by chemosensory means. In addition, these results provide further evidence that coral damage caused by sublethal predation releases material or promotes chemical reactions that stimulate other individuals towards its source. These data provide the strongest evidence yet that DMSP can function as substrate for a predation-induced chemoattraction system.

Although *A. surculosa* was found to be highly preferred based on field surveys (**Figure 7**) and laboratory assays (**Figure 13**), the first experiment (**Figure 23**) clearly demonstrates that *A. planci* exhibit more preference for damage or injured colonies. These results are consistent with field observations where *A. planci* were observed to feed on partially eaten colonies, bypassing similar intact colonies. Similarly, these results also agree with Y-maze experiments done by Ormond *et al*. (1973) using *Fungia* sp. However, *Fungia* sp. is not a highly preferred species, therefore, this behavior may be a response triggered by the damaged coral releasing larger than normal amounts of constitutive chemicals or by the release or destruction of nematocysts in the injured coral (Ormond & Campbell, 1974). Although the potential effects of digestive juices from conspecifics was not tested in this study, these results further support the argument that the coral prey is the source of chemoattractants, rather than feeding conspecifics (Collins, 1974).

Results of Experiment 2 (**Figure 23**) demonstrate that *A. planci* were attracted to the cleavage products of DMSP (*i.e.* DMS and acrylic acid). However, choice assays did

not partition the individual effects of each cleavage product. Nevertheless, this behavioral response is consistent with some marine organisms. For example, DMSP seems to be a potent foraging cue for planktivorous reef fishes (DeBose *et al*., 2008) and predatory pelagic fishes (DeBose & Nevitt, 2007). These imply that DMSP, closely linked to feeding activity and elevated primary productivity, rather than the presence of prey, serves as an indicator of potential foraging opportunities. In addition, DMS and acrylic acid were also found to stimulate feeding in the sea hare, *Scientific name* (Schupp, unpublished data) and the isopod, *Idotea wosnesenskii* (Van Alstyne *et al*., 2001). Conversely, the cleavage products of DMSP has been shown to be used by micro- and macroalgae in an activated defense system against herbivory by protozoans (Wolfe *et al*., 1997), sea urchins (Van Alstyne & Houser, 2003), and amphipods (Wiesemeier *et al*., 2007). Grazing by the protozoan, *Oxyrrhis marina*, on the unicellular alga, *Emiliania huxleyi*, causes lysis of ingested cells, which initiates mixing of algal DMSP and DMSP lyase, thus resulting in the conversion of DMSP to DMS and acrylic acid (Wolfe & Steinke, 1996). This reaction potentially produces high concentrations of acrylate, which presumably deterred further grazing by *O. marina* (Wolfe *et al*., 1997) because of its antimicrobial activity (Sieburth, 1960). Moreover, grazing by the sea urchin, *Strongylocentrotus droebachiensis*, on algae facilitated the enzymatic conversion of DMSP, which caused some herbivores to avoid DMSP-producing algal species. Similarly, Wiesemeier *et al*. (2007) demonstrated that wound-activated release of DMS and acrylate in the brown alga, *Dictyota dichotoma*, deters further grazing by the amphipod, *Amphithoe longimana*. Furthermore, DMS and acrylic acid have also been

observed to be avoided by the omnivorous fish, *Canthigaster solandri* and the herbivorous fish, *Siganus spinus* (Schupp, unpublished data).

Zooxanthellate cnidarians possess relatively high concentrations of DMSP, yet, the reasons for its production and accumulation of DMSP have not been well-studied (Van Alstyne *et al*., 2006; Van Alstyne & Puglisi, 2007). DMSP concentrations have been found to increase in response to environmental stress (Broadbent *et al*., 2002). Although still speculative, these results are the first to show a potential role for DMSP in the feeding behavior of a keystone coral predator. Constitutive concentrations of DMSP appear to be relatively higher among preferred Acroporid species, although values show high spatial, interspecific, and intraspecific variability (**Table 7**). Levels of the enzyme DMSP lyase were also highly variable between different strains of the symbiotic algae, *Symbiodinium microadriaticum* (Yost & Mitchelmore, 2009). Besides, DMSP was also found in non-preferred species, which suggests that *A. planci* may be chemoreceptive to the breakdown products of DMSP, rather than the DMSP itself. Following the context of higher bioactivity of DMSP breakdown products, macroalgae feeding experiments where DMSP, DMS, or acrylic acid were added to artificial diets showed that the sea urchins *Strongylocentrotus drobachiensis* and *S. purpuratus* avoided foods containing DMS and acrylic acid, but preferred foods containing DMSP over controls (Van Alstyne *et al*., 2001; Van Alstyne & Houser, 2003).

Although scleractinian corals are an abundant source of food on most reefs, they are relatively inaccessible to most predators because their tissues are like a thin veneer over a large amount of skeleton. However, the large eversible stomach and extraoral digestion exhibited by *A. planci* are well-adapted for feeding on this thin veneer of coral

tissue (Birkeland & Lucas, 1990). Assuming that DMSP and DMSP lyase are separately compartmentalized within zooxanthellae, *A. planci* feeding and extraoral digestion of coral tissues results in physical damage, which causes DMSP lyase to react with and cleave DMSP into DMS and acrylic acid locally at the site of tissue injury (Wolfe & Steinke, 1996; Van Alstyne *et al*., 2001). Digestive breakdown of DMS has also been observed in *S. droebachiensis* (Van Alstyne & Houser, 2003). Water flow then creates a chemical cue gradient of DMS and acrylic acid from the injured coral colony, which stimulates other individuals toward its source and initiates the formation of localized feeding aggregations.

Results of laboratory choice assays in this study support this proposed mechanism, but more studies are necessary. Although the strong odor of DMS was noted during *A. planci* predation and feeding simulation with an airbrush, levels of DMS and acrylic acid must be determined prior, during, and after grazing to confirm if *A. planci* predation indeed facilitates the breakdown of the DMSP precursor. Feeding experiments with gas tight chambers used by Van Alstyne & Houser (2003) to sample DMS in the headspace could be modified for the purpose of determining whether DMS levels increase during *A. planci* predation. Information on the concentrations of DMS and acrylic acid during tissue disruption are fundamental in designing future bioassays with greater ecological relevance. Moreover, an experiment to determine the amount of DMS lost during the preparation of agar food gels as well as when the experiments are run in the Y-maze is also necessary because of its volatility. The water-solubility of acrylic acid also warrants a separate diffusion experiment. In line with these, DMS and acrylic acid should be separately tested in order to partition the specific responses each one elicits

from *A. planci*. Theoretically, the water soluble acrylic acid is a more logical waterborne cue compared to the more volatile DMS (Zimmer & Butman, 2000). However, Wiesemeier *et al*. (2007) demonstrated that DMS, acrylate, and trimethylamine did not show repellent activity in isolation but significantly increased feeding deterrence when mixed together. Therefore, DMS and acrylic acid could potentially have a synergistic effect on *A. planci* behavior as well.

Like other metabolites, DMSP and its breakdown products could have a variety of functions (Kubanek *et al*., 2002; Van Alstyne & Puglisi, 2007). As mentioned above, these compounds may affect some consumers and function as feeding deterrents or attractants, but it may also have no effect at all on other organisms (Pawlik *et al*., 2002). The functions of DMSP and its byproducts are most likely species-specific and population-specific (Van Alstyne & Puglisi, 2007). Nevertheless, predation by *A. planci*, especially in outbreak numbers, could potentially cause pulses of exceptionally high DMS production, which increases biogenic sulfate aerosol release to the atmosphere that possible influences local climate (Andreae *et al*., 1983; Hill *et al*., 1995). Furthermore, a more direct consequence of local aggregations is that the increased proximity of potentially fecund individuals may enhance chances of fertilization, particularly if spawning is synchronized, and give rise to more population outbreaks. Understanding the underlying mechanisms in the formation of localized feeding aggregations is therefore crucial in management because the destructive influence of aggregated adult *A. planci* allows increased success in recruitment.

Table 7. Published DMSP concentrations in zooxanthellae of scleractinian corals.

SPECIES	*LOCATION*	*DMSP CONCENTRATION**		
		μmol • g^{-1} FM	μmol • g^{-1} DM	fmol • cell^{-1}
Acropora cerealis[c]	Gabgab Beach, GU, USA	47	236	950
Acropora digitifera[c]	Gabgab Beach, GU, USA	33	127	417
Acropora formosa[b]	Kelso Reef, GBR, Australia	-	-	641
Acropora formosa[b]	Nelly Bay Reef, GBR, Australia	3	-	235
Acropora formosa (bleached)[b]	Nelly Bay Reef, GBR, Australia	-	-	436
Acropora formosa (unbleached)[b]	Nelly Bay Reef, GBR, Australia	-	-	171
Acropora palifera[b]	Nelly Bay Reef, GBR, Australia	-	-	2831
Acropora palifera[b]	One Tree Reef, GBR, Australia	-	-	3831
Acropora pulchra[b]	One Tree Reef, GBR, Australia	-	-	40
Acropora valida[c]	Gabgab Beach, GU, USA	59	467	425
Favites sp.[b]	One Tree Reef, GBR, Australia	-	-	21
Heliopora coerulea[c]	Luminao Reef, GU, USA	1	9	310
Leptastrea purpurea[c]	Cocos Lagoon, GU, USA	3	12	211
Montipora verrucosa[a]	Kaneohe Bay, Oahu, HI, USA	-	-	73
Montipora verrucosa[a]†	cell culture	-	-	61-66
Pavona decusata[c]	Gabgab Beach, GU, USA	10	48	43
Pocillopora damicornis[a]	Kaneohe Bay, Oahu, HI, USA	-	-	117
Pocillopora damicornis[b]	Kelso Reef, GBR, Australia	-	-	179
Pocillopora damicornis[b]	Nelly Bay Reef, GBR, Australia	-	-	99
Pocillopora damicornis[b]	One Tree Reef, GBR, Australia	-	-	89
Pocillopora damicornis[a]†	cell culture	-	-	48
Pocillopora meandrina[c]	Gabgab Beach, GU, USA	3	10	80
Porites compressa[a]	Kaneohe Bay, Oahu, HI, USA	-	-	77
Porites cylindrica[c]	Gabgab Beach, GU, USA	3	18	107
Porites rus (decumbent)[c]	Gabgab Beach, GU, USA	3	18	88
Porites rus (upright)[c]	Gabgab Beach, GU, USA	2	14	69
Psammocora digitata[c]	Gabgab Beach, GU, USA	7	22	49

* DMSP Concentration (means or range of values): expressed in concentration per fresh mass (FM), per dry mass (DM), and per zooxanthella cell; some methods did not differentiate between DMSP and DMS

† DMSP quantified from cultured zooxanthella cell of corresponding coral species

[a] Hill *et al*. (1995)

[b] Broadbent *et al*. (2002)

[c] Van Alstyne *et al*. (2006)

CHAPTER 7

Synthesis and Implications

7.1 General Discussion and Summary

Broadscale and finescale surveys (**Chapter 2**) show that *A. planci* continues to be a coral reef management problem around Guam despite previous control efforts. Predation by *A. planci* can have adverse effects that range from organismal to community levels of organization. Partial mortality caused by *A. planci* predation consequently results in tissue loss, which requires neighboring polyps or the entire colony to spend energy on regeneration and repair (Meesters *et al*., 1994; Henry & Hart, 2005). This process involves reallocation of resources to tissue regeneration at the expense of colony growth and reproduction (*reviewed by* Rotjan & Lewis, 2008). The energetic cost of regeneration is related to lesion length and perimeter regardless of colony size (Meester *et al*., 1994; Oren *et al*., 1997); thus, relatively large scars (equal to seastar diameter) from *A. planci* predation should be costly for the colony. Lesion shape also affects regeneration rates, which is lowest in circular shapes (Bak & Steward-Van Es, 1980; Oren *et al*., 1997) like those of *A. planci* feeding scars. Moreover, Van Veghel & Bak (1994) found that colonies inflicted with artificial lesions had reduced fertility and fecundity in neighboring polyps. If regeneration does not occur immediately, post-predation algal colonization (Belk & Belk, 1975) could severely reduce the likelihood of coral re-growth (*see* Rotjan & Lewis, 2008 and references cited therein). Bentis *et al*. (2000) also found that regenerating acroporid and pocilloporid colonies were more

susceptible to fungal infections compared to intact colonies. Furthermore, Nugues & Bak (2009) observed brown-band syndrome on *A. planci* feeding scars on *Acropora cytherea* colonies, which suggests a potential role for *A. planci* in disease transmission. Taken together, these studies suggest that *A. planci* adversely impacts the overall physiology of the affected colony. The tendency of *A. planci* to aggregate toward partially eaten colonies (**Figure 24c**) further reduces the likelihood of colony recovery.

At the population and community levels, *A. planci* predation at outbreak densities results in drastic reduction of live coral cover (**Figure 24a** & **b**). Evidence also suggests that outbreak events may favor alternative communities dominated by benthic macroalgae, sponges, and alcyonacean corals (Birkeland & Lucas, 1990). Other consequences of *A. planci* outbreaks have been thoroughly discussed in **Chapter 1**. Even at moderate densities, preferential feeding by *A. planci* can have profound impacts on coral communities. Selective predation by *A. planci* could reduce diversity and increase the dominance of the most abundant species by feeding on rarer species (Glynn, 1976; Chess *et al*., 1997) or increase diversity by facilitating the growth and recruitment of rarer species through selective predation on more abundant species (Porter, 1972). One year after the 1969 outbreak of *A. planci* on Guam, Randall (1973) reported that coral species richness decreased from 146 to 96 species. Prior to outbreaks, affected reefs were dominated by *Acropora* and *Montipora*, but post-outbreak surveys revealed a shift in coral community structure towards non-preferred species, *e.g. Porites, Leptastrea* (Randall, 1973; Colgan, 1987). Predominance of non-preferred species was short-lived and by 1980, community structure shifted back to preferred species (Colgan, 1987). However, more recent outbreaks have once again resulted in high mortality of preferred

species and a shift in community composition towards non-preferred species (Porter *et al*., 2005). Similar changes were observed during resurvey of sites impacted by outbreaks in 2006. Differential mortality resulting from preferential feeding by *A. planci*, therefore drives changes in coral community structure.

Chemoreception by *A. planci* is an important feature in understanding mechanisms involved in selective predation (**Chapter 3 & 4**) and in the formation of aggregations (**Chapter 6**). Although *A. planci* responds to specific chemical compounds (**Chapter 5**) and chemoreception is well-known among asteroids, little is known about chemosensory organs and chemoreceptor sites. Despite the absence of a central ganglion in the asteroid nervous system, its radial symmetry and disk-like body covered with receptor units provide an ideal mechanism for gross chemosensory perception and simultaneous monitoring of stimulus intensity at different positions on its surface (Sloan & Campbell, 1982). The asteroid nervous system is composed of three neuronal networks, *i.e.* ectoneural, hyponeural, and entoneural systems (Brusca & Brusca, 2003). These nervous systems are integrated by a nerve net primarily derived from ectoneural and entoneural components (Smith, 1966). Unspecialized epithelial cells, innervated by a plexus of the ectoneural system, have been proposed to be receptive to a wide range of stimuli (Pentreath & Cobb, 1972). Reese (1966) stated that sensory cells are abundant in strategic regions such as the base of the spines and pedicellariae, in the ambulacral region, suckers of the podia (**Figure 24d**), and in the side and oral surface of the terminal tentacle (**Figure 24d, inset**). Furthermore, Sloan (1980b) also described chemically-mediated responses in the distal arm area, which includes the terminal or sensory tube feet. Since this area is the leading portion of a mobile asteroid, it is more likely that it

encounters and monitors changes in the surrounding environment (Sloan, 1980a). The implication of podia, especially the terminal tube feet, in chemoreception could partly be due to the fact that these are extensions of the body wall and covered with a basi-epithelial plexus (Sloan & Campbell, 1982). These chemosensory organs are particularly important when multiple cues are involved.

Based on results of surveys (**Chapter 2**) and laboratory and field feeding assays (**Chapter 3-6**), three chemoreception mechanisms, involving multiple cues, are proposed to influence prey location, prey discrimination, and aggregation by *A. planci* (**Figure 25**). Location of suitable substrate with abundant prey corals may be facilitated by water-soluble cues, such as betaine, which are easily diffused to the water column (**Figure 25a**). Since betaine is widespread among marine invertebrates, it is highly likely that changes in signal-to-background contrast are detected by predators, including generalists (Crisp, 1967; Fuzessery & Childress, 1975; Moore & Huxley, 1976; Carr, 1978; McClintock *et al*., 1984). Distance chemoreception by *A. planci* may be achieved by the sensory cells in the skin and terminal tentacles. Measurements of betaine concentration in different coral species (**Table 6**) suggest that the function of betaine may be primarily restricted to prey location and it may not be particularly important in prey discrimination. Nevertheless, this aspect is important in understanding the susceptibility of certain substrate types and reef areas to *A. planci* predation.

Results described herein show that there is a well-ordered hierarchy in terms of prey preference (**Figure 7**) and that chemoreception is involved in prey selection (**Figure 13**). It has been observed that *A. planci* walk on top of non-preferred corals without feeding en route to preferred species. This behavior indicates that contact

chemoreception by podia (**Figure 24c**) and short-distance olfaction by the terminal tube feet detect feeding stimulants from preferred prey (**Figure 25b**). Prey discrimination may involve chemoreception of different concentrations of attractant coral constituents or unique compounds present in preferred coral species. Previously described attractants, like arachidonic acid and α-linolenic acid (Teruya *et al*., 2001), are lipophilic, thus contact chemoreception might be a more effective mechanism for detection. In addition, fractions from the bioactive 90% aqueous MeOH layer (**Figures 16-18**) may yield unique compounds or normal coral constituents, such as amino acids, peptides, or proteins, which may be important in prey discrimination at appropriate concentrations. Aside from chemical signals, other factors may also influence prey selection by *A. planci*. General models of optimal diet theory would predict that *A. planci* would prefer to feed on corals with the highest nutritional value to maximize energetic return (Ormond, *et al*., 1976). Nutritional analyses of corals (Stimson, 1987; Tricas, 1989) showed that preferred pocilloporid species have higher lipid and caloric content than non-preferred poritids, but the number of species studied was very limited. Defensive mechanisms in corals may also deter *A. planci* predation. Barnes *et al*. (1970) partly attributed tube feet retraction upon contact with coral tissue to the presence of nematocysts and suggested that prey selection may be due to the variation in polyp size and arrangement between coral genera, which is important in the localization of nematocysts. The antagonistic behavior of coral symbionts, particularly trapeziid crabs, may also deter feeding by *A. planci* even on preferred genera such as *Acropora*, *Pocillopora*, and *Stylophora* (Glynn, 1976; 1980; Pratchett, 2001), but with minimal influence in the Indo-Pacific and Great Barrier Reef, as evidenced by high mortality of pocilloporids from *A. planci* predation in these

locations (Colgan, 1987; Chess *et al*., 1997, De'ath & Moran, 1998b). The size and morphology of corals may also influence prey choice by *A. planci*. Chess *et al*. (1997) and Kenyon & Aeby (2009) reported that *A. planci* choose to feed on smaller colonies of *Pocillopora*. Branching and encrusting growth forms are also more preferred over massive growth forms (De'ath & Moran, 1998). In Hawaii, *A. planci* tended to prefer the encrusting coral *Montipora patula*, presumably because it allowed easy access for the stomach to cover more coral surface area (Kenyon & Aeby, 2009). Similarly, surveys conducted in this study showed that encrusting *Montipora* colonies were also highly preferred (**Figures 6-7**). The abundance, distribution, and accessibility of corals may also explain differential mortality between species. Ormond *et al*. (1973) suggested that the *A. planci* consume coral in accordance with their relative abundance on reefs. Results of this study, however, demonstrate that some coral genera (*e.g*. *Galaxea, Porites*; see **Figure 6**) are not eaten despite their abundance. Chess *et al*. (1997) also observed that *Pocillopora meandrina* were preferred over the more dominant *Porites* spp. Furthermore, corals in shallow, wave-washed reefs (Moran, 1986) and in crevices (Barnes *et al*., 1970) are also often spared from *A. planci* predation. This is true in most reefs surveyed on Guam, where *A. planci* aggregations (**Figures 2-3**) and resulting mortality (**Figures 4-5**) were mostly observed on the deeper and less turbulent submarine terrace zone compared to the shallow and wave-washed reef front zone. All these factors may act in concert to aid *A. planci* in selecting prey, but results from this study nevertheless demonstrate that this behavioral response may be largely influenced by perception of favorable chemical signals.

Another important aspect of *A. planci* feeding behavior is the formation of feeding aggregations. Field observations by Ormond *et al*. (1973) indicated chemoattractive aggregation in *A. planci* by preferential feeding on colonies that are being fed by conspecifics or colonies that are already partially eaten. This behavior was also observed during field surveys and confirmed with Y-maze assays (**Figure 23**). It is proposed that this behavior is mediated by predation-induced chemoattractants (**Figure 25c**). As discussed in **Chapter 6**, extraoral predation results in tissue damage and decompartmentalization, which induces enzymatic cleavage of DMSP in zooxanthellae to DMS and acrylic acid. Laboratory choice assays in this study show that these cleavage products attract *A. planci* (**Figure 23**) and could explain why colonies with partial mortality are preferred over intact colonies. However, more experiments are required to confirm the compartmentalization of DMSP and DMSP lyase, to establish an increase in DMS and acrylic acid concentrations during *A. planci* predation, and to determine which cleavage product is responsible for chemoattraction or whether both compounds act synergistically. Additionally, extraoral predation by *A. planci* could also facilitate the release of increased concentrations of normal coral constituents (Collins, 1974) such as arachidonic acid (Teruya *et al*., 2001) or other undescribed feeding attractant compounds in coral (**Figure 25c**). The production of arachidonic acid from glycerophospholipids catalyzed by phospholipase A_2 (PLA_2) present in the body of *A. planci* (Shiomi *et al*., 1985) is also noteworthy in understanding conspecific chemoattraction. Improved understanding of mechanisms involved in the formation of aggregations is essential because the close proximity of individual seastars increases the likelihood of fertilization success, which consequently leads to enhanced larval recruitment and survival.

In summary, these results clearly indicate that chemical signals influence the feeding behavior of *A. planci* at different stages. First, *A. planci* actively use chemical signals to locate prey from a distance and find favorable substrata. Secondly, short-distance and contact chemoreception is also used in prey discrimination and in determining which colonies are most palatable. Lastly, predation by *A. planci* facilitates chemical reactions that produce chemoattractive compounds and increase concentrations of attractants present in coral. Still, caution should be taken in interpreting these results since variations in and interactions between hydrodynamic, chemical, and biological factors at numerous spatial and temporal scales does exist under natural conditions (Zimmer & Butman, 2000).

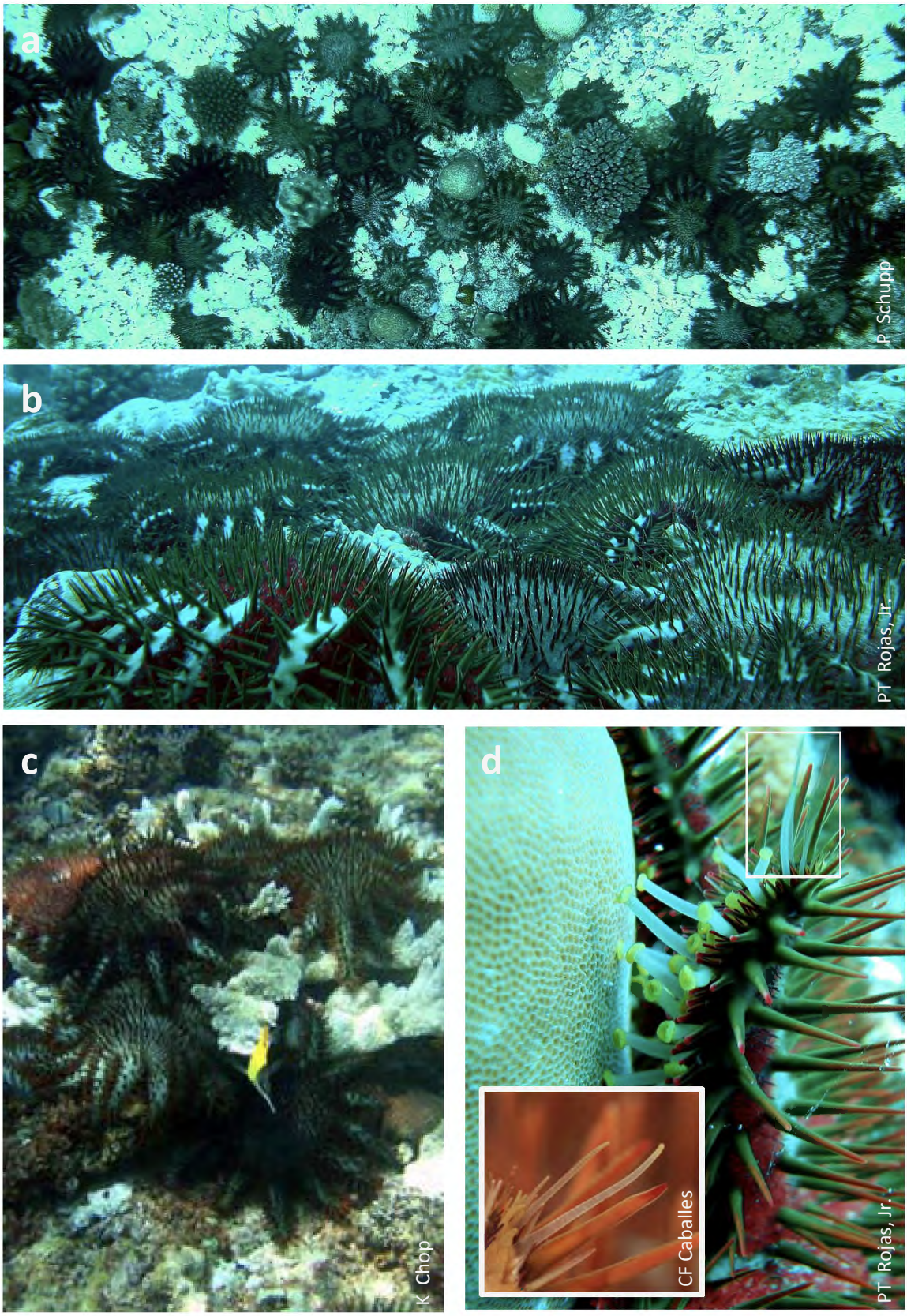


Figure 24. Aggregations of *A. planci* and consequences of predation.

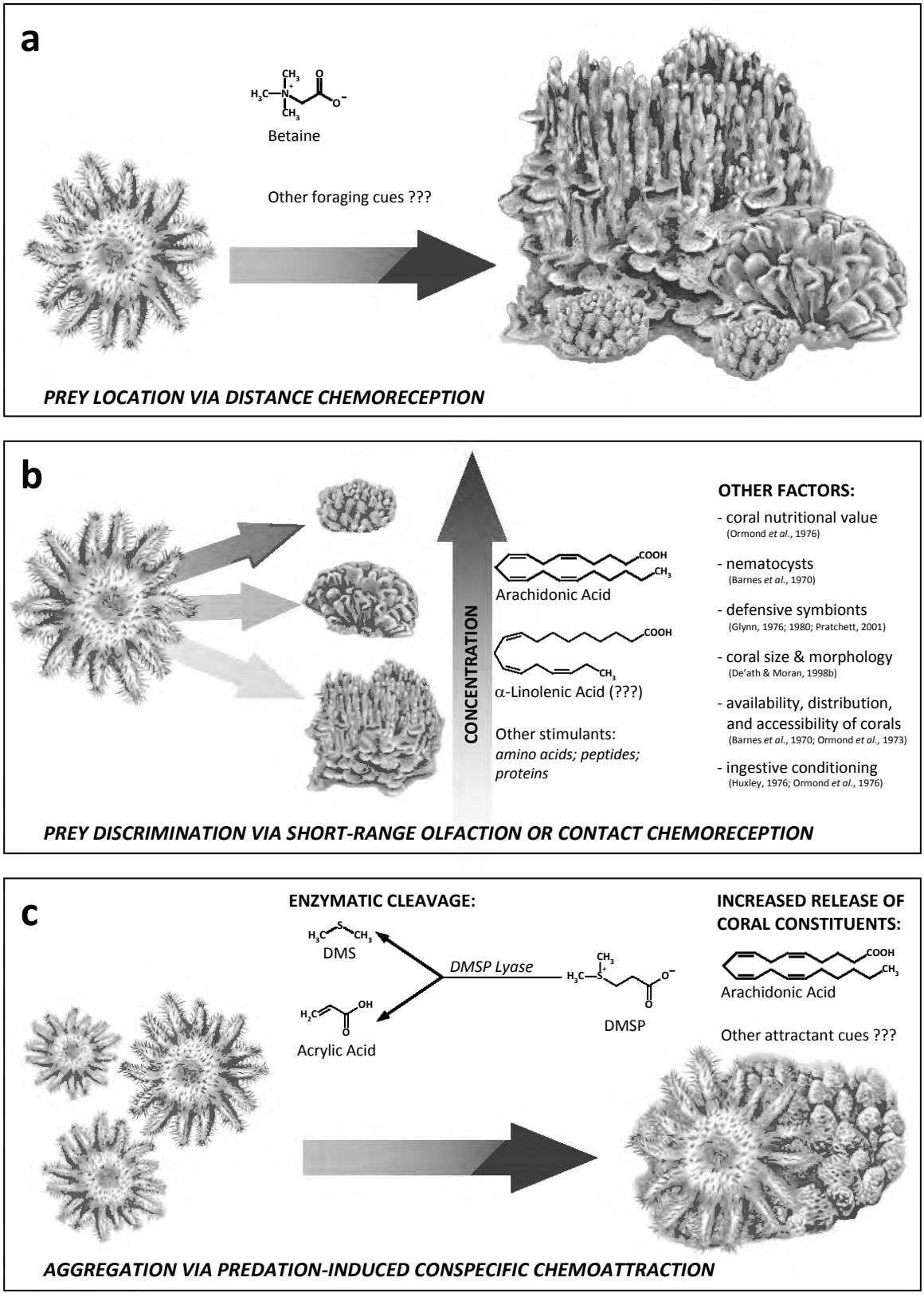


Figure 25. Diagrammatic representation of different chemoreception mechanisms that influence the feeding behavior of *A. planci*. (Illustrations by F. Macabenta)

7.2 Research Implications

Although these experiments may have improved the understanding of the biology and ecology of *A. planci*, several questions still remain unanswered. New techniques are being developed and new technologies are available to improve data quality and conduct experiments under environmentally realistic conditions. First of all, a standard survey method should be agreed upon to facilitate data comparisons between different localities and meta-analysis. Consolidation of these information is vital in assessing large-scale patterns. It is surprising that, despite the notoriety of *A. planci*, very few studies have described the pattern of change and abundance in the distribution and abundance of *A. planci* over a complete outbreak cycle. Knowledge on the movement, range, and post-outbreak fate of populations is still limited. The use of acoustic tags in studying fish and crustacean population dynamics present a new opportunity to investigate *A. planci* populations for longer periods. New techniques in population genetics may also prove useful in studying connectivity and large-scale dispersion of *A. planci*. The link between *A. planci* outbreaks and anthropogenic stressors also warrants more studies.

In the experimental aspect, new approaches are required to help identify controlling variables because the magnitude of turbulence usually covaries with flow speed, surface roughness, and animal size and because turbulent mixing dilutes waterborne cues (Zimmer & Butman , 2000). Although the Y-maze increased control over chemical stimulus environments, it potentially creates artificial patterns of contact between experimental subject and signal molecules. Zimmer & Butman (2000) thoroughly reviews innovative technologies and concepts that closely mimic natural hydrodynamic conditions and improve understanding of factors that constrain

chemosensory processes at the individual, population, and community levels of organization. Determination of background concentrations of betaine and other chemoattractants will also be important because the effectiveness of chemical cues mainly relies on signal-to-background contrast (Atema, 1995). In addition, the minimum concentration of a chemoattractant compound required to induce behavioral responses should also be determined because of the dose-dependent nature of chemoreception. Although tedious and time-consuming, a larger sample size in laboratory and field experiments will definitely yield more conclusive results. The small amounts of fractions left after repeated extractions also present a major challenge in setting up bioassays. The use of other chemically-induced behavioral criteria related to feeding such as stomach eversion (Brauer *et al*, 1970) and arm tip and terminal tube foot responses (Moore & Huxley, 1976) should be explored. Finally, despite the vast amount of literature on *A. planci*, the issues mentioned above need to be urgently addressed to improve management and control of *A. planci* predation on already stressed reefs.

7.3 Management Implications

Since Guam's reefs are already in poor condition due to several anthropogenic stressors (Burdick *et al*., 2008), other sources of coral mortality, particularly *A. planci* predation, should be managed and mitigated. A relatively high percentage of preferred prey, *e.g*. acroporid and pocilloporid corals, face elevated risks of extinction mainly due to climate change and local impacts, including *A. planci* predation (Carpenter *et al*., 2008). Surveys in this study confirm that *A. planci* is one of the major sources of direct

coral mortality on Guam and supports the urgent need for monitoring and control efforts. The creation of an outbreak response team, similar to that during the 1970's (Cheney, 1973), is proposed as an immediate measure to prevent further degradation of reefs with high ecological and socioeconomic value, reduce the likelihood of future mass recruitments, and facilitate recovery of affected reefs. Because reef management is a long-term commitment, the response team should ideally be spearheaded and funded by government agencies and involve university faculty and students, fishermen, hotel and dive shop operators, and other stakeholders. Dissemination of outreach materials and the formation of an outbreak reporting network has been initiated through a project funded by the National Oceanic and Atmospheric Administration (NOAA) but must be maintained by appropriate government agencies to ensure continuity. Financial and personnel resources are essential in the success of control programs (Fraser *et al*., 2000). While manual collection and injection of chemicals by SCUBA are effective during mass outbreaks, these methods are time-consuming and labor-intensive. It is also expensive due to the associated man-hours and boat time, especially if individuals are more dispersed. The use of feeding attractants offers time-saving alternatives, by using it in bait stations to attract *A. planci*, which could then be collected or killed without labor-intensive search. The use of chemoattractant-baited commercial traps or design of new ones would further reduce the manpower needed to collect or kill the starfish.

Although the causes of *A. planci* outbreaks are still subject to much debate, anthropogenic activities that potentially promote fertilization success and larval survival should be minimized or completely averted because these stressors also increase coral mortality. Better land use and management should be practiced to reduce sedimentation

and terrestrial runoff. Continued implementation of marine preserves will also be important in minimizing declines of herbivorous fish stocks, which are important in post-outbreak recovery. An ecosystem-based approach in management should be generally beneficial in maintaining the functional diversity of coral reefs and overall reef resilience.

LITERATURE CITED

Alcala, A.C. (1974). The sponge crab *Dromidiopsis dormia* as predator of the crown of thorns starfish. *Silliman Journal* **21**: 174-177.

Ammar, M.S.A. (2005). Recovery probabilities of coral reef communities after *Acanthaster planci* infestation: a case study at Shabror Umm Gam'ar, Hurghada, Red Sea, Egypt. *Egyptian Journal of Aquatic Research* **31**: 103-112.

Anderson, J. M. (1953). Structure and function in the pyloric caeca of *Astenias forbesi*. *Biological Bulletin* **105**: 47-61.

Andreae, M.O., W.R. Barnard & J.M. Ammons (1983). The biological production of dimethylsulfide in the ocean and its role in the global atmospheric sulfur budget. *Ecological Bulletins* **35**: 167-177.

Anthony K.R.N., S.R. Connolly & B.L. Willis (2002). Comparative analysis of energy allocation to tissue and skeletal growth in corals. *Limnology and. Oceanography* **47**: 1417-1429.

Anthony, K. R. N., S.R. Connolly & O. Hoegh-Guldberg (2007). Bleaching**,** energetics and coral mortality risk: Effects of temperature, light, and sediment regime. *Limnology and Oceanography* **52**:716-726.

Atema, J. (1995). Chemical signals in the marine environment: Dispersal, detection, and temporal signal analysis. *Proceedings of the National Academy of Sciences* **92**: 62-66.

Ayukai, T., K. Okaji & J. S. Lucas (1997). Food limitation in the growth and development of crown-of-thorns starfish larvae in the Great Barrier Reef. In: H.A. Lessios and I.G. Macintyre (Eds.), *Proceedings of the Eighth International Coral Reef Symposium*, Vol. 1., Panama City, June, 1996, (pp. 621-626). Panama: Smithsonian Tropical Research Institute.

Bachok, Z, P. Mfilinge, & M. Tsuchiya (2006). Characterization of fatty acid composition in healthy and bleached corals from Okinawa, Japan. *Coral Reefs* **25**: 545-554.

Baine, M.S.P. (2006). A major outbreak of crown-of-thorns starfish in Bootless Bay, Central Province, Papua New Guinea. *Coral Reefs* **25**: 607.

Bak, R.P.M. & Y. Steward-Van Es (1980). Regeneration of superficial damage in the scleractinian corals *Agaricia agaricites* f. *purpurea* and *Porites astreoides. Bulletin of Marine Science* **30**: 883-887.

Barnes D.J., & J.M. Lough (1992). Systematic variations in the depth of skeleton occupied by coral tissue in massive colonies of *Porites* from the Great Barrier Reef. *Journal of Experimental Marine Biology and Ecology* **159**: 113-128.

Barnes, D.J., R.W. Brauer & M.R. Jordan (1970). Locomotory response of *Acanthaster planci* to various species of coral. *Nature* **228**: 342-344.

Bartel, A.H. & D. Davenport (1956). A technique for the investigation of chemical responses in aquatic animals. *Animal Behaviour* **4**: 117-119.

Bass, D.K. & I.R. Miller (1996). Crown-of-thorns starfish and coral surveys using the manta tow and scuba search techniques. *Long-term Monitoring of the Great Barrier Reef Standard Operational Procedure 1*. Townsville, Australia: Australian Institute of Marine Science, 38 pp.

Beach, D.H., N.J. Hanscomb & R.F.G. Ormond (1975). Spawning pheromone in crown-of-thorns starfish. *Nature* **254**: 135-136.

Becerro, M., V. Bonito & V.J. Paul (2006). Effects of monsoon driven wave action on coral reefs of Guam and implications for coral recruitment. *Coral Reefs* **25**: 193-199.

Belk, M.S. & D. Belk (1975). An observation of algal colonisation on *Acropora aspera* killed by *Acanthaster planci*. *Hydrobiologia* **46**: 29-32.

Bellwood, D.R., T.P. Hughes, C. Folke & M. Nyström (2004). Confronting the coral reef crisis. *Nature* **429**: 827-833.

Bentis, C.J., L. Kaufman & S. Golubic (2000). Endolithic fungi in reef-building corals (Order: Scleractinia) are common, cosmopolitan, and potentially pathogenic. *Biological Bulletin* **198**: 254-260.

Biggs, J.S., B.M. Olivera & Y.I. Kantor (2008). α-Conopeptides specifically expressed in the salivary gland of *Conus pulicarius*. *Toxicon* **52**: 101-105.

Birkeland, C. (1981). *Acanthaster* in the culture of high islands. *Atoll Research Bulletin* **255**: 55-58.

Birkeland, C. (1982). Terrestrial runoff as a cause of outbreaks of *Acanthaster planci* (Echinodermata: Asteroidea). *Marine Biology* **69**: 175-185.

Birkeland, C. (1989). The faustian traits of the crown-of-thorns starfish. *American Scientist* **77**: 154-163.

Birkeland, C. and J.S. Lucas (1990). Acanthaster planci: *Major Management Problem of Coral Reefs*. Boca Raton: CRC Press, Inc. 257 pp.

Black, K.P. & P.J. Moran (1991). Influence of hydrodynamics on the passive dispersal and initial recruitment of larvae of *Acanthaster planci* (Echinodermata: Asteroidea) on the Great Barrier Reef. *Marine Ecology, Progress Series* **69**: 55-65.

Black K.P., P.J. Moran, D.M. Burrage & A.G. De'ath (1995). Association of low-frequency currents and crown-of-thorns starfish outbreaks. *Marine Ecology Progress Series* **125**: 185-194.

Blanchon, P. & J. Shaw (1995). Reef drowning during the last deglaciation: Evidence for catastrophic sea-level rise and ice-sheet collapse. *Geology* **23**: 4-8.

Bolser, R.C. & M.E. Hay (1996). Are tropical plants better defended? Palatability and defenses of temperate versus tropical seaweeds. *Ecology* **77**: 2269-2286.

Boothby, K.M. & I.D. McFarlane (1986). Chemoreception in sea anemones: Betaine stimulates the pre-feeding response in *Urticina eques* and *U. feline*. *Journal of Experimental Biology* **125**: 385-389.

Bos, A.R., G.S. Gumanao & F.N. Salac (2008). A newly discovered predator of the crown-of-thorns starfish. *Coral Reefs* **27**: 581.

Bouchon-Navaro, Y., C. Bouchon & M. Harmelin-Vivien (1985). Impact of coral degradation on a chaetodontid fish assemblage (Moorea, French Polynesia). In: M. Harmelin-Vivien and B. Salvat (Eds.), *Proceedings of the Fifth International Coral Reef Symposium*, Vol. 5, Tahiti, May-June, 1985 (pp. 427-432). Moorea, French Polynesia: Antenne Museum – Ecole Pratique des Hautes Etudes.

Branham, J.M. (1973). The crown-of-thorns on coral reefs. *Bioscience* **23**: 219-226.

Brauer, R. W., M. R. Jordan & D. J. Barnes (1970). Trigerring of the stomach eversion reflex of *Acanthaster planci* by coral extracts. *Nature* **220**: 344-346.

Broadbent, A. D., G. B. Jones & R. J. Jones (2002). DMSP in corals and benthic algae from the Great Barrier Reef. *Estuarine, Coastal and Shelf Science* **55**: 547-555.

Brodie, J. E. (1992). Enhancement of larval and juvenile survival and recruitment in *Acanthaster planci* from the effects of terrestrial runoff: A review. *Australian Journal of Marine and Freshwater Research* **43**: 539-554.

Brodie, J.E., K. Fabricius, G. De'ath & K. Okaji (2005). Are increased nutrient inputs responsible for more outbreaks of crown-of-thorns starfish? An appraisal of the evidence. *Marine Pollution Bulletin* **51**: 266-278.

Broom, D.M. (1975). Aggregation behaviour of the brittle-star *Ophiothrix fragilis*. *Journal of the Marine Biological Association of the U.K*. **55**: 191-197.

Bruno J.F., E.R. Selig, K.S. Casey, C.A. Page, B.L. Willis, C.D. Harvell, H. Sweatman & A.M. Melendy (2007). Thermal Stress and Coral Cover as Drivers of Coral Disease Outbreaks . *PLoS Biology* **5**: e124.

Bruno, J.F., & E.R. Selig (2007)**.** Regional decline of coral cover in the Indo-Pacific: timing, extent, and subregional comparisons. *PLoS One* **8**: e711.

Brusca, R.C. & G.J. Brusca (2003). *Invertebrates*, 2nd Ed. Sunderland, MA: Sinauer Associates, Inc., 936 pp.

Bryan, P.G. & M.R. Struck (1971). *Acanthaster planci*, crown-of-thorns starfish: Survey of islands and atolls in the Central Carolines, Yap and Truk Districts (*UOGML Miscellaneous Report*). Guam, USA: University of Guam Marine Laboratory, 22 pp.

Burdick, D., V. Brown, J. Asher, C. Caballes, M. Gawel, L. Goldman, A. Hall, J. Kenyon, T. Leberer, E. Lundblad, J. McIlwain, J. Miller, D. Minton, M. Nadon, N. Pioppi, L. Raymundo, B. Richards, R. Schroeder, P. Schupp, E. Smith & B. Zgliczynski (2008). *Status of the Coral Reef Ecosystems of Guam*. Guam, USA: Bureau of Statistics and Plans – Guam Coastal Management Program.

Byers, J.A. (1992) Optimal fractionation and bioassay plans for isolation of synergistic chemicals: The subtractive-combination method. *Journal of Chemical Ecology* **18**: 1603-1621.

Bythell**,** J.C., P. Pan, & J. Lee (2001). Three-dimensional morphometric measurements of reef corals using underwater photogrammetry techniques. *Coral Reefs* **20**:193-199.

Bywood, R. & F. Challenger (1953). The evolution of dimethyl sulphide by *Enteromorpha intestinalis*. Isolation of dimethyl-β-carboxyethyl sulphonium chloride from the alga. *Biochemical Journal* **53**(4): xxvi.

Campbell A.C., S. Coppard, N. D'Abreo & T. Tudor-Thomas (2001). Escape and aggregation responses of three enchinoderms to conspecific stimuli. *Biological Bulletin* **201**: 175-185.

Cantoni G.L. & D.G. Anderson (1956). Enzymatic cleavage of dimethylpropiothetin by *Polysiphonia lanosa*. *Journal of Biological Chemistry* **222**: 171-177.

Carpenter, K.E., M. Abrar, G. Aeby, R.B. Aronson, S. Banks, A. Bruckner, A. Chiriboga, J. Cortés, J.C. Delbeek, L. DeVantier, G.J. Edgar, A. J. Edwards, D. Fenner, .H.M. Guzmán, B. W. Hoeksema, G. Hodgson, O. Johan, W.Y. Licuanan, S.R. Livingstone, E.R. Lovell, J.A. Moore, D.O. Obura, D.Ochavillo, B.A. Polidoro, W.F. Precht, M.C. Quibilan, C. Reboton, Z.T. Richards, A.D. Rogers, J. Sanciangco, A. Sheppard, C. Sheppard, J. Smith, S. Stuart, E. Turak, J.E.N. Veron, C. Wallace, E. Weil & E. Wood (2008). One-third of reef-building corals face elevated extinction risk from climate change and local impacts. *Science* **321**: 560-563.

Carpenter, R.C. (1997). Invertebrate predators & grazers. In: C. Birkeland (Ed.), *Life and Death of Coral Reefs* (pp. 198-229). NY: Chapman & Hall.

Castilla, J.C. (1972). Avoidance behaviour of *Asterias rubens* to extracts of *Mytilus edulis*, solutions of bacteriological peptone and selected amino acids. *Marine Biology* **15**: 236-245.

Castro, H., J. Battaglia & E. Virtanen (1998). Effects of FinnStim on growth and sea water adaptation of Coho Salmon. *Aquaculture* **168**: 423-429.

Challenger, F. & M.I. Simpson (1948). Studies on biological methylation. Part XII. A precursor of the dimethyl sulphide evolved by *Polysiphonia fastigiata*. Dimethyl-2-carboxyethylsulphonium hydroxide and its salts. *Journal of the Chemical Society* **3**: 1591-1597.

Chambers, S.T., C.M. Kunin, D. Miller, & A. Hamada (1987). Dimethylthetin can substitute for glycine betaine as an osmoprotectant molecule for *Escherichia coli*. *Journal of Bacteriology* **169**: 4845-4847.

Charlson, R.J., J.E. Lovelock, M.O. Andreae, & S.G. Warren (1987). Oceanic phytoplankton, atmospheric sulphur, cloud albedo and climate. *Nature* **326**: 655-661.

Cheney, D. P. (1973). An analysis of the *Acanthaster* control programs in Guam and the Trust Territory of the Pacific Islands. *Micronesica* **9**: 171-180.

Cheney, D. P. (1974). Spawning and aggregation of *Acanthaster planci* in Micronesia. In: A.M. Cameron, B.M. Campbell, A.B. Cribb, R.Endean, J.S. Jell, O.A. Jones, P. Mather & F.H. Talbot (Eds.), *Proceedings of the Second International Coral Reef Symposium*, Vol. 1, Brisbane, June-July, 1974 (pp. 591-594). Brisbane, Australia: The Great Barrier Reef Committee.

Cheney, D.P. (1971). *Acanthaster planci*, crown-of-thorns starfish: Resurvey of Guam (*UOGML Miscellaneous Report*). Guam, USA: University of Guam Marine Laboratory, 7 pp.

Chesher, R. H. (1969a). Destruction of the Pacific Corals by the Sea Star *Acanthaster planci*. *Science* **165**: 280-283.

Chesher, R. H. (1969b). Divers wage war on the killer star. *Skin Diver Magazine* **18**(3): 34-35, 84-85.

Chess, J.R., E.S. Hobson & D.F. Howard (1997). Interactions between *Acanthaster planci* (Echinodermata, Asteroidea) and scleractinian corals at Kona, Hawai'i. *Pacific Science* **51**: 121-133.

Clarke K.R. & R.N. Gorley (2001). *PRIMER v5: User manual/tutorial*. Plymouth, UK: PRIMER-E, 91 pp.

Clayton, W.S. (1985). Polyp density variability among colonies of the reef coral *Pocillopora damicornis*. *Bulletin of Marine Science* **36**: 716-719.

Cohen, J. (1992). A power primer. *Psychological Bulletin* **112**: 155–159.

Colgan, M. W. (1987). Coral reef recovery on Guam (Micronesia) after catastrophic predation by *Acanthaster planci*. *Ecology* **68**: 1592-1605.

Collins, A. R. S. (1974). Biochemical investigation of two responses involved in the feeding behaviour of *Acanthaster planci* (L.) - I. Assay methods and preliminary results. *Journal of Experimental Marine Biology and Ecology* **15**: 173-184.

Collins, A. R. S. (1975a). Biochemical investigation of two responses involved in the feeding behaviour of *Acanthaster planci* (L.) - II. Isolation and characterization of chemical stimuli. *Journal of Experimental Marine Biology and Ecology* **17**: 69-86.

Collins, A. R. S. (1975b). Biochemical investigation of two responses involved in the feeding behaviour of *Acanthaster planci* (L.) - III. Food preferences. *Journal of Experimental Marine Biology and Ecology* **17**: 87-94.

Connel, J.H. (1978). Diversity in tropical rain forest and coral reefs. *Science* **199**: 1302-1309.

CRC Reef Research Centre (2003). *Crown-of-thorns starfish on the Great Barrier Reef: Current State of Knowledge* (November, 2003 Ed.). Townsville, Australia: CRC Reef Research Centre Ltd.

Crisp, D.J. (1967). Chemoreception in cirripedes. *Biological Bulletin* **133**: 128-140.

Cronin, G., M.E. Hay, W. Fenical & N. Lindquist (1995). Distribution, density, and sequestration of host chemical defenses by the specialist nudibranch *Tritonia hamnerorum* found at high densities on the sea fan *Gorgonia ventalina*. *Marine Ecology Progress Series* **119**: 177-189.

Dana, T.F. & W.A. Newman (1973). Massive aggregation and migration of *Acanthaster*: Behavioral responses to severe food limitation. *Micronesica* **9**: 205 (Abstract).

Dana, T.F. (1970). *Acanthaster*: A rarity in the past? *Science* **169**: 894.

Dana, T.F., W.A. Newman & E.W. Fager (1972). Acanthaster aggregations: interpreted as primarily responses to natural phenomena. *Pacific Science* **26**: 355-372.

Davenport, D. (1950). Studies in the physiology of commensalism: 1. The polynoid genus *Arctonoë*. *Biological Bulletin* **98**: 81-93.

Dayton, P.K., R.J. Rosenthal, L.C. Mahen & T. Antezana (1977). Population structure and foraging biology of the predaceous Chilean asteroid *Meyenaster gelatinosus* and the escape biology of its prey. *Marine Biology* **39**: 361-370.

de Oliveira M.F., J.H. de Oliveira, F.C. Galetti, A.O. de Souza, C.L. Silva, E. Hajdu, S. Peixinho & R.G. Berlinck (2006). Antimycobacterial brominated metabolites from two species of marine sponges. *Planta Medica* **72**: 437-441.

de Vooys, C.G.N. & J.A.J. Geenevasen (2002). Biosynthesis and role in osmoregulation of glycine-betaine in the Mediterranean mussel *Mytilus galloprovincialis* LMK. *Comparative Biochemistry and Physiology B*: *Biochemistry and Molecular Biology* **132**: 409-414.

De'ath, G. & P. J. Moran (1998a). Factors affecting the behavior of crown-of-thorns starfish (*Acanthaster planci* L.) on the Great Barrier Reef: 1: Patterns of activity. *Journal of Experimental Marine Biology and Ecology* **220**: 83-106.

De'ath, G. & P. J. Moran (1998b). Factors affecting the behaviour of crown-of-thorns starfish (*Acanthaster planci* L.) on the Great Barrier Reef: 2: Feeding preferences. *Journal of Experimental Marine Biology and Ecology* **220**: 107-126.

DeBose, J.L. & G.A. Nevitt (2007). Investigating the association between pelagic fish and dimethylsulfoniopropionate in a natural coral reef system. *Australian Journal of Marine and Freshwater Research* **58**: 720-724.

Debose, J.L., S.C. Lema & G.A. Nevitt (2008). Dimethylsulfoniopropionate as a foraging cue for reef fishes. *Science* **319**: 1356.

Decho, A. W., K. A. Browne, and R. K. Zimmer-Faust (1998). Chemical cues: why basic peptides are signal molecules in marine environments. *Limnology and Oceanography* **43**: 1410-1417.

DeVantier, L.M. & T. J. Done (2007). Inferring Past Outbreaks of the Crown-of-Thorns Seastar from Scar Patterns on Coral Heads. In: R.B. Aronson (Ed.), *Geological Approaches to Coral Reef Ecology* (pp. 85-125). NY: Springer Verlag.

Dight, I.J., Bode, L. & M.K. James (1990). Modeling the larval dispersal of *Acanthaster planci* I: Large scale hydrodynamics, Cairns Section, Great Barrier Reef Marine Park. *Coral Reefs* **9**: 115-123.

Done, T.J. (1985). Effects of two *Acanthaster* outbreaks on coral community structure - the meaning of devastation. In: M. Harmelin-Vivien and B. Salvat (Eds.), *Proceedings of the Fifth International Coral Reef Symposium*, Vol. 5, Tahiti, May-June, 1985 (pp. 315-320). Moorea, French Polynesia: Antenne Museum – Ecole Pratique des Hautes Etudes.

Done, T.J. (1988). Simulation of recovery of pre-disturbance size structure in populations of *Porites* spp. damaged by crown of thorns starfish *Acanthaster planci*. *Marine Biology* **100**: 51-61.

Done, T.J. (1992). Phase shift in coral communities and their ecological significance. *Hydrobiologia* **247**: 121-132.

Drew, E. A. (1972). The biology and physiology of algal-invertebrate symbiosis. II. The density of algal cells in a number of hermatypic hard corals and alcyonarians from various depths. *Journal Experimental Marine Biology and* Ecology **9**: 71-75.

Dulvy, N., R. Freckleton, & N. Polunin (2004). Coral reef cascades and the indirect effects of predator removal by exploitation. *Ecology Letters* **7**: 410-416.

Edmunds, P. J. & R.D. Gates (2002). Normalizing physiological data for scleractinian corals. *Coral Reefs* **21**: 193-197.

Edwards D.M., R.H. Reed & W.D.P. Stewart (1988). Osmoacclimation in *Enteromorpha intestinalis*: long-term effects of osmotic stress on organic solute concentration. *Marine Biology* **98**: 467-476.

Endean, R. (1973). Population explosions of *Acanthaster planci* and associated destruction of hermatypic corals in the Indo-west Pacific region. In: O.A. Jones & R. Endean (Eds.), *Biology and Geology of Coral Reefs*, Vol. 2: Biology 1 (pp. 389-438). NY: Academic Press.

Endean, R. & W. Stablum (1973). A study of some aspects of the crown-of-thorns starfish (*Acanthaster planci*) infestations of reefs of Australia's Great Barrier Reef. *Atoll Research Bulletin* **167**: 1-62.

Endean, R. (1977). *Acanthaster planci* infestations of reefs of the Great Barrier Reef. In: D.L. Taylor (Ed.), *Proceedings of the Third International Coral Reef Symposium*, Vol. 1: Biology, Miami, May, 1977 (pp. 185–191). Miami, FL: University of Miami – Rosenstiel School of Marine and Atmospheric Science.

English, S., C. Wilkinson & V. Baker (1997). *Survey Manual for Tropical Marine Resources*, 2nd Ed. Queensland: Australian Institute of Marine Science, 390 pp.

Erdfelder, E., F. Faul & A. Buchner (1996). GPOWER: A general power analysis program. *Behavior Research Methods, Instruments, and Computers* **28**: 1-11.

Fabricius, K.E. & F.H. Fabricius (1992). Re-assessment of ossicle frequency patterns in sediment cores: rate of sedimentation related to *Acanthaster planci*. *Coral Reefs* **11**: 109-114.

Fabricius, K.E., G. De'ath, M.L. Puotinen, T. Done, T. Cooper & S.C. Burgess (2008). Disturbance gradients on inshore and offshore coral reefs caused by a severe tropical cyclone. *Limnology and Oceanography* **53**: 690-704.

Faul, F., E. Erdfelder, A.-G. Lang & A. Buchner (2007). G*Power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behavior Research Methods, Instruments, and Computers* **39**: 175-191.

Felix, N. & M. Sudharsan (2004). Effect of glycine betaine, a feed attractant affecting growth and feed conversion of juvenile freshwater prawn *Macrobrachium rosenbergii*. *Aquaculture Nutrition* **10**: 193-197.

Ferguson, J.C. (1969). Feeding activity in *Echinaster* and its induction with dissolved nutrients. *Biological Bulletin* **136**: 374-384.

Fisher, J.L. (1969). Starfish infestation hypothesis. *Science* **165**: 645.

Fitt W.K., F.K. McFarland, M.E. Warner & G.C. Chilcoat (2000). Seasonal patterns of tissue biomass and densities of symbiotic dinoflagellates in reef corals and relation to coral bleaching. *Limnology and Oceanography* **45**: 677–685.

Flanigan, J.M. & A.E. Lamberts (1981). *Acanthaster* as a recurring phenomenon in Samoan history. *Atoll Research Bulletin* **255**: 59-62.

Frankel, E. (1978). Evidence from the Great Barrier Reef of ancient *Acanthaster* aggregations. Atoll Research Bulletin **220**: 75-93.

Fraser, N., B. Crawford & J. Kusen (2000). *Best Practices Guide for Crown-of-Thorns Clean-Ups* (Proyek Pesisir Special Publication: Coastal Management Report #2225). Narragansett, Rhode Island USA: University of Rhode Island – Coastal Resources Center.

Furnas, M.J. & A.W. Mitchell (1987). Phytoplankton dynamics in the central Great Barrier reef–II. Primary production. *Continental Shelf Research* **7**: 1049-1061.

Fuzessery, Z.M. & J.J. Childress (1975). Comparative chemosensitivity to amino acids and their role in the feeding activity of bathypelagic and littoral crustaceans. *Biological Bulletin* **149**: 522-538.

Gardner, T. A., I. M. Côté, J. A. Gill, A. Grant, & A. R. Watkinson (2003). Long-term region wide declines in Caribbean coral reefs. *Science* **301**: 958-960.

Genc, M.A., N. Tekelioglu, E. Yilmaz, A.O. Hunt & Y. Yanar (2006). Effect of dietary betaine on growth performance and body composition of *Oreochromis aureus r*eared in fresh and sea water: A comparative study. *Journal of Animal and Veterinary Advances* **5**: 1185-1188.

George, A., M. Luckymis, S. Palik, K. Adams, E. Joseph, D. Mathias, S. Malakai, M.R. Nakayama, C. Graham, K. Rikim, A. Marcus, J. Albert, V. Fread, M. Hasurmai, C. Fillmed, W. Kostka, A. Takesy, T. Leberer & S. Slingsby (2008). The State of the Coral Reef Ecosystems of the Federated States of Micronesia. In: J.E. Waddell & A.M. Clarke (Eds.), *The State of Coral Reef Ecosystems of the United States and Pacific Freely Associated States: 2008* (pp. 419-436). Silver Spring, MD: NOAA/NCCOS Center for Coastal Monitoring and Assessment's Biogeography Team. Silver Spring, MD.

Gerard, K.,C. Roby, N. Chevalier, B. Thomassin, A. Chenuil & J.P. Feral (2008). Assessment of three mitochondrial loci variability for the crown-of-thorns starfish: a first insight of *Acanthaster* phylogeography. *Comptes Rendus Biologies* **331**: 137-143.

Gingerich, S. B. (2003). Hydrologic resources of Guam (*USGS Water-Resources Investigations Report 03-4126*). Hawaii: US Geological Survey.

Glynn, P.W. (1973). *Acanthaster*: Effect on coral reef growth in Panama. *Science* **180**: 504-506.

Glynn, P.W. (1976). Some physical and biological determinants of coral community structure in the Eastern Pacific. *Ecological Monographs* **46**: 431-456.

Glynn, P.W. (1977). Interactions between *Acanthaster* and *Hymenocera* in the field and laboratory. In: D.L. Taylor (Ed.), *Proceedings of the Third International Coral Reef Symposium*, Vol. 1: Biology, Miami, May, 1977 (pp. 209-216). Miami, FL: University of Miami – Rosenstiel School of Marine and Atmospheric Science.

Glynn P.W. (1980). Defense by symbiotic crustacea of host corals elicited by chemical cues from predator. *Oecologia* **47**: 287-290.

Glynn, P.W. (1984). An amphinomid worm predator of the Crown-of-Thorns Sea Star and general predation on asteroids in Eastern and Western Pacific coral reefs. *Bulletin Marine Science* **35**: 54-71.

Glynn, P.W. & D.A. Krupp (1986). Feeding biology of a Hawaiian sea star corallivore, *Culcita novaeguineae* Müller and Troschel. Journal of Experimental Marine Biology and Eco1ogy **96**: 75-96.

Glynn, P.W., J.L. Mate, A.C. Baker, & M.O. Calderon (2001). Coral bleaching and mortality in Panama and Ecuador during the 1997-1998 El Nino-Southern Oscillation event: Spatial/temporal patterns and comparisons with the 1982-1983 event. *Bulletin of Marine Science* **69**: 79-109.

Goreau, T.F., J.C. Lang, E.A. Graham, & P.D. Goreau (1972). Structure and ecology of the Saipan reefs in relation to *Acanthaster planci* (Linnaeus). *Bulletin of Marine Science* **22:** 113-152.

Greenstein, B.J., J.M. Pandolfi, & P.J. Moran (1995). Taphonomy of crown-of-thorns starfish: implications for recognizing ancient population outbreaks. *Coral Reefs* **14**: 91-97.

Guam Legislature (1997). *Public Law 24-21*. Twenty-fourth Guam Legislature (May 12, 1997). Retrieved from: http://guamlegislature.com/public_laws.htm

Hancock, D.A. (1974). Attraction and avoidance in marine invertebrates - their possible role in developing an artificial bait. *Journal du Conseil International pour l'Exploration de la Mer* **35**: 328-331.

Hanscomb, N.J., J.P. Bennett & G. Harper (1976). Biochemical stimuli for feeding in *Acanthaster planci* (L.). *Journal of Experimental Marine Biology and Ecology* **22**: 193-197.

Hart, A.M., D. W. Klumpp & G.R. Russ (1996). Response of herbivorous fishes to crown-of-thorns starfish *Acanthaster planci* outbreaks: II. Density and biomass of selected species of herbivorous fish and fish-habitat correlations. *Marine Ecology Progress Series* **132**: 21-30.

Harvell, C.D., E. Jordan-Dahlgren, S. Merkel, L. Raymundo, E. Rosenberg, G. Smith, E. Weil & B. Willis (2007). Coral disease, environmental drivers and the balance between coral and microbial associates. *Oceanography* **20**: 58-81.

Hay, M.E. (2009). Marine chemical ecology: Chemical signals and cues structure marine populations, communities, and ecosystems. *Annual Review of Marine Science* **1**:193–212.

Hay, M.E. & W. Fenical (1988). Marine plant-herbivore interactions: the ecology of chemical defense. *Annual Review of Ecology and Systematics* **19**: 111-145.

Hay, M.E., J.J. Stachowicz, E. Cruz-Rivera, S. Bullard, M.S. Deal & N. Lindquist (1998). Bioassays with marine and freshwater macroorganisms. In: K.F. Haynes and J.G. Millar (Eds.), *Methods in Chemical Ecology*, Vol. 2, *Bioassay Methods* (pp.39-141). NY: Chapman and Hall.

Henderson, R.A. & P.D. Walbran (1992). Interpretation of the fossil record of *Acanthaster planci* from the Great Barrier Reef: A reply to critcism. *Coral Reefs* **11**: 95-101.

Henderson, R.A. (1992). Assessment of crown-of-thorns skeletal elements in surface sediment of the Great Barrier Reef. *Coral Reefs* **11**: 103-108.

Henry, L. & M. Hart (2005). Regeneration from injury and resource allocation in sponges and corals—a review. *International Review of Hydrobiology* **90**: 125-158.

Highsmith, R.C. (1981). Coral bioerosion: damage relative to skeletal density. *American Naturalist* **117**: 193-198.

Hill R.W., J.W.H. Dacey, & D.A. Krupp (1995). Dimethylsulfoniopropionate in reef corals. *Bulletin of Marine Science* **57**: 489-494.

Hoegh-Guldberg, O., P.J. Mumby, A.J. Hooten, R.S. Steneck, P. Greenfield, E. Gomez, C.D. Harvell, P.F. Sale, A.J. Edwards, K. Caldeira, N. Knowlton, C.M. Eakin, R. Iglesias-Prieto, N. Muthiga, R.H. Bradbury, A. Dubi & M.E. Hatziolos (2007). Coral Reefs Under Rapid Climate Change and Ocean Acidification. *Science* **318**: 1737-1742.

Houk, P., S. Bograd & R. van Woesik (2007). The transition zone chlorophyll front can trigger *Acanthaster planci* outbreaks in the Pacific Ocean: Historical confirmation. *Journal of Oceanography* **63**: 149-154.

Hughes, R N. (1980). Optimal foraging in the marine context. *Oceanography and Marine Biology*: *An Annual Review* **18**: 423-481.

Hughes, T.P., M.J. Rodrigues, D.R. Bellwood, D. Ceccarelli, O. Hoegh-Guldberg, L. McCook, N. Moltschaniwskyj, M.S. Pratchett, R.S. Steneck & B. Willis (2007). Phase shifts, herbivory, and the resilience of coral reefs to climate change. *Current Biology* **17**: 360-365.

Hughes, T.P. (1987). Skeletal density and growth form of corals. *Marine Ecology Progress Series* **35**: 259-266.

Huxley, C. J. (1976). Response of *Acanthaster planci* (L.) to partial stimuli. *Journal of Experimental Marine Biology and Ecology* **22**: 199-206.

IUPAC (1997). *Compendium of Chemical Terminology*, 2nd ed. Oxford: Blackwell Scientific Publications.

Jacobsen, H.P. & O.B. Stabell (2004). Antipredator behaviour mediated by chemical cues: the role of conspecific alarm signalling and predator labelling in the avoidance response of a marine gastropod. *Oikos* **104**: 43-50.

Jangoux, M. (1982a). Digestive systems: Asteroidea. In: M. Jangoux & J.M. Lawrence (Eds.), *Echinoderm Nutrition* (pp. 235-272). Rotterdam: A.A. Balkema Publishers.

Jangoux, M. (1982b). Food and feeding mechanisms: Asteroidea. In: M. Jangoux & J.M. Lawrence (Eds.), *Echinoderm Nutrition* (pp. 117-159). Rotterdam: A.A. Balkema Publishers.

Johnson, C. (1992). Settlement and recruitment of *Acanthaster planci* on the Great Barrier Reef: questions of process and scale. *Australian Journal of Marine and Freshwater Research* **43**: 611-627.

Jones, G.B., M. Curran, A. Broadbent, S. King, E. Fischer & R.J. Jones (2007). Factors affecting the cycling of dimethylsulfide and dimethylsulfoniopropionate in coral reef waters of the Great Barrier Reef. *Environmental Chemistry* **4**: 310-322.

Jones, R.S., & R.H. Randall. (1973). A study of biological impact caused by natural and man-induced changes on a tropical reef (*UOGML Technical Report No. 7*). Guam, USA: University of Guam Marine Laboratory.

Jones, R.S., R.T. Tsuda & M.R. Struck (1970). *Acanthaster planci*, crown-of-thorns starfish: Resurvey of Truk Lagoon, Truk District (*UOGML Miscellaneous Report*). Guam, USA: University of Guam Marine Laboratory, 13 pp.

Keesing , J.K., R.H. Bradbury, L.M. De Vantier, M.J. Riddle, & G. De'ath (1992). The geological evidence for recurring outbreaks of the crown-of-thorns-starfish: a reassessment from an ecological perspective. *Coral Reefs* **11**: 79-85.

Keesing, J.K. & A.R. Halford (1992). Importance of postsettlement processes for the population dynamics of *Acanthaster planci* (L.). *Australian Journal of Marine and Freshwater Research* **43**: 635-651.

Keesing, J.K. & J.S. Lucas (1992). Field measurement of feeding and movement rates of the crown-of-thorns starfish *Acanthaster planci* (L.). *Journal of Experimental Marine Biology and Ecology* **156**: 89-104.

Kenyon, J.C. & G.S. Aeby (2009). Localized outbreak and feeding preferences of the crown-of-thorns seastar *Acanthaster Planci* (Echinodermata, Asteroidea) on reefs off Oahu, Hawaii. *Bulletin of Marine Science* **84**: 199-209.

Kerr, A.M., E. Stoffel, & R.L. Yoon (1993). Abundance distribution of holothuroids (Echinodermata: Holothuroidea) on a windward and leeward fringing reef, Guam, Mariana Islands. *Bulletin of Marine Science* **52**: 780-791.

Kettle, B.T. & J.S. Lucas. Biometric relationships between organ indices, fecundity, oxygen consumption and body size in *Acanthaster planci* (L.) (Echinodermata: Asteroidea). *Bulletin of Marine Science* **41**: 541-551.

Kidawa, A. (2005). The role of amino acids in phagostimulation in the shallow-water omnivorous Antarctic sea star *Odontaster validus*. *Polar Biology* **28**: 147-155.

Kirst G.O., C. Thiel, H. Wolff, J. Nothnagel, M. Wanzek & R. Ulmke (1991). Dimethylsulfoniopropionate (DMSP) in ice-algae and its possible biological role. *Marine Chemistry* **35**: 381-388.

Kitamura, M., T. Koyama, Y. Nakano, D. Uemura (2007). Characterization of a natural inducer of coral larval metamorphosis. *Journal of Experimental Marine Biology and Ecology* **340**: 96-102.

Kiyohara, S. & Hidaka, I. (1991). Receptor sites for alanine, proline and betaine in the palatal taste system of the puffer, *Fugu pardalis*. *Journal of Compartive Physiology A* **169**: 523-530.

Kobayashi H., K. Kitamura, K. Nagai, Y. Nakao, N. Fusetani, R.W.M. van Soest & S. Matsunaga (2007). Carteramine A, an inhibitor of neutrophil chemotaxis, from the marine sponge *Stylissa carteri*. *Tetrahedron Letters* **48**: 2127-2129.

Kubanek, J., K.E. Whalen, S. Engel, S.R. Kelly, T.P. Henkel, W. Fenical, & J.R. Pawlik (2002). Multiple defensive roles for triterpene glycosides from two Caribbean sponges. *Oecologia* **131**: 125-136.

Kühlmann, D.H.H. (1988). The sensitivity of coral reefs to environmental pollution. *Ambio* **17**: 13-21.

Lander, M.A. & C.P. Guard (2003). Creation of a 50-year rainfall database, annual rainfall climatology, and annual rainfall distribution map for Guam (*UOG Technical Report. No. 102*). Guam, USA: University of Guam - Water and Energy Research Institute of the Western Pacific, 26 pp.

Latyshev, N.A., N.V. Naumenko, V.I. Svetashev & Y.Y. Latypov (1991). Fatty acids of reef-building corals. *Marine Ecology Progress Series* **76**: 295-301.

Lawrence, J. (1987). Functional consequences of the multiarmed condition in asteroids. In: Burke, R.D., P. V. Mladenov, P. Lambert, & R. L. Parsley (Eds.), *Echinoderm Biology: Proceedings of the Sixth International Echinoderm Conference*, Victoria, 1987 (pp. 597-602). Rotterdam: A.A. Balkema Publishers.

Lawrence, J.M. & Lane, J.M. (1982). The utilization of nutrients by post-metamorphic echinoderms. In: M. Jangoux & J.M. Lawrence (Eds.), *Echinoderm Nutrition* (pp. 331-371). Rotterdam: A.A. Balkema Publishers.

Lessios, H.A. (1988). Mass mortality of *Diadema antillarum* in the Caribbean: What have we learned? *Annual Review of Ecological Systematics* **19**: 371-393.

Lourey M.J., D.A.J. Ryan & I.R. Miller (2000). Rates of decline and recovery of coral cover on reefs impacted by, recovering from and unaffected by crown-of-thorns starfish *Acanthaster planci*: a regional perspective of the Great Barrier Reef. *Marine Ecology Progress Series* **196**:179-186.

Lovelock J.E., R.J. Maggs, & R.A. Rasmussen (1972). Atmospheric dimethyl sulfide and the natural sulfur cycle. *Nature* **237**: 452-453.

Lucas, J.S. (1973). Reproductive and larval biology of *Acanthaster planci* (L.) in Great Barrier Reef waters. *Micronesica* **9**: 197-203.

Lucas, J.S. (1975). Environmental influences on the early development of *Acanthaster planci* (L.). In: *Crown-of-thorns starfish seminar proceedings*, Brisbane, 6 September, 1974 (pp. 103-121). Canberra: Australian Government Publishing Service.

Lucas, J. S. (1982). Quantitative studies of feeding and nutrition during larval development of the coral reef asteroid *Acanthaster planci* (L.). *Journal of Experimental Marine Biology and Ecology* **65**: 173-193.

Malin, G. & G.O. Kirst (1997). Algal production of dimethyl sulfide and its atmospheric role. *Journal of Phycology* **33**: 889-896.

Marsh, J.A. (1970). Primary productivity of reef-building calcareous red algae. *Ecology* **51**: 255-263.

Marsh, J.A. Jr. & P.G. Bryan (1971). *Acanthaster planci*, crown-of-thorns starfish: Resurvey of Palau, Palau District (*UOGML Miscellaneous Report*). Guam, USA: University of Guam Marine Laboratory, 8 pp.

Marsh, J.A. Jr. & R.T. Tsuda (1973). Population levels of *Acanthaster planci* in the Mariana and Caroline Islands 1969-1972. *Atoll Research Bulletin* **170**: 1-16.

Marsh, J.A. Jr., R.T. Tsuda, M.R. Struck, & F.A. Cushing (1971). *Acanthaster planci*, crown-of-thorns starfish: Resurvey of Saipan, Tinian, and Aguijan, Mariana District (*UOGML Miscellaneous Report*). Guam, USA: University of Guam Marine Laboratory, 10 pp.

Marshall, P.A. & H. Schuttenberg (2006). Adapting coral reef management in the face of climate change. *Coastal and Estuarine Studies* **61**: 223–241.

McClintock, J.B., T.S. Klinger & J.M. Lawrence (1984). Chemoreception in *Luidia clathrata* (Echinodermata: Asteroidea): qualitative and quantitative aspects of chemotactic responses to low molecular weight compounds. *Marine Biology* **84**: 47-52.

Meesters, E.H., M. Noordeloos & R.P.M. Bak (1994). Damage and regeneration: links to growth in the reef-building coral *Montastrea annularis. Marine Ecology Progress Series* **112**: 119-128.

Meyers, P.A. (1979). Polyunsaturated fatty acids in coral: indicators of nutritional sources. *Marine Biology Letters* **1**: 69-75.

Miller, R.J. (1990). Effectiveness of crab and lobster traps. *Canadian Journal of Fisheries and Aquatic Sciences* **47**: 1228-1251.

Moore, R. J. (1978). Is *Acanthaster planci* an *r*-strategist? *Nature* **271**: 56-57.

Moore, R.J. & C.J. Huxley (1976). Aversive behaviour of crown-of-thorns starfish to coral evoked by food-related chemicals. *Nature* **263**: 407-409.

Moran, P.J. (1986). The *Acanthaster* phenomenon. *Oceanography and Marine Biology*: *An Annual Review* **24**: 379-480.

Moran, P.J. (1992). Preliminary observations of the decomposition of crown-of-thorns starfish, *Acanthaster planci* (L). *Coral Reefs* **11**: 115-118.

Moran, P.J., R.H. Bradbury, & R.E. Reichelt (1985). Mesoscale studies of the crown-of-thorns/coral interaction: a case history from the Great Barrier Reef. In: M. Harmelin-Vivien and B. Salvat (Eds.), *Proceedings of the Fifth International Coral Reef Symposium*, Vol. 5, Tahiti, May-June, 1985 (pp. 321-326). Moorea, French Polynesia: Antenne Museum – Ecole Pratique des Hautes Etudes.

Moran, P.J., R.E. Reichelt, & R.H. Bradbury (1986). An assessment of the geological evidence for previous *Acanthaster* outbreaks. *Coral Reefs* **4**: 235-238.

Moran, P.J., R.H. Bradbury, & R.E. Reichelt (1988). Distribution of recent outbreaks of the crown-of-thorns starfish (*Acanthaster planci*) along the Great Barrier Reef' 1985-86. *Coral Reefs* **7**: 125-137.

Morse, A.N.C. & D.E. Morse (1996). Flypapers for coral and other planktonic larvae. *BioScience* **64**: 254-262.

Mumby, P.J., C.P. Dahlgren, A.R. Harborne, C.V. Kappel, F. Micheli, D.R. Brumbaugh, K.E. Holmes, J.M. Mendes, K. Broad, J.N. Sanchirico, K. Buch, S. Box, R.W. Stoffle, & A.B. Gill (2006). Fishing, trophic cascades, and the process of grazing on coral reefs. *Science* **311**: 98-101.

Mumby, P.J. & R.S. Steneck (2008). Coral reef management and conservation in light of rapidly evolving ecological paradigms. *Trends in Ecology and Evolution* **23**: 555-563.

Mundy, C., R. Babcock, I. Ashworth, J. Small (1994). A portable,discrete-sampling submersible plankton pump and its use in sampling starfish eggs. *Biological Bulletin* **186**: 168-171.

Muscatine, L., P.G. Falkowski, Z. Dubinsky, P.A. Cook, & L.R. McCloskey (1989). The effect of external nutrient resources on the population dynamics of zooxanthellae in a reef coral. *Proceedings of the Royal Society of London B* **236:** 311-324.

Nakajima, K., A. Uchida & Y. Ishida (1990). Effect of a feeding attractant, dimethyl-β-propiothetin, on growth of marine fish. *Nippon Suisan Gakkaishi* **56**: 1151-1154.

Narsimhan, G. & Z. Wang (2008). Guidelines for processing emulsion-based foods. In: G.L. Hasenhuettl & R.W. Hartel (Eds.), *Food Emulsifiers and their Applications* (p. 390). Gaithersburg: Springer.

Newman, W.A. (1970). *Acanthaster*: A disaster? *Science* **167**: 1274.

Nishida, M. & J.S. Lucas (1988). Genetic differences between geographic populations of the crown-of-thorns starfish throughout the Pacific region. *Marine Biology* **98**: 359-368.

Nishihira, M. & K. Yamazato (1974). Human interference with the coral reef community and *Acanthaster* infestation of Okinawa. In: A.M. Cameron, B.M. Cambell, A.B. Cribb, R.Endean, J.S. Jell, O.A. Jones, P. Mather & F.H. Talbot (Eds.), *Proceedings of the Second International Coral Reef Symposium*, Vol. 1, Brisbane, October, 1974 (pp. 577-590). Brisbane, Australia: The Great Barrier Reef Committee.

Nishizaki, M.T. and J.D. Ackerman (2005). A secondary chemical cue facilitates juvenile–adult postsettlement associations in red sea urchins. *Limnology and Oceanography*.**50**: 354-362.

Nugues, M.M. & R.P.M. Bak (2009). Brown band syndrome on feeding scars of the crown-of-thorn starfish *Acanthaster planci*. *Coral Reefs* **28**: 507-510.

Olson, R.R. (1987). In situ culturing as a test of the larval starvation hypothesis for the crown-of-thorns starfish, *Acanthaster planci*. *Journal of Limnology and Oceanography* **32**: 895-904.

Oren, U., Y. Benayahu & Y.Loya (1997). Effect of lesion size and shape on regeneration of the Red Sea coral *Favia favus. Marine Ecology Progress Series* **146**: 101-107.

Ormond, R.F.G. & A. C. Campbell (1974). Formation and breakdown of *Acanthaster planci* aggregations in the Red Sea. In: A.M. Cameron, B.M. Cambell, A.B. Cribb, R.Endean, J.S. Jell, O.A. Jones, P. Mather & F.H. Talbot (Eds.), *Proceedings of the Second International Coral Reef Symposium*, Vol. 1, Brisbane, June-July, 1974 (pp. 595-619). Brisbane, Australia: The Great Barrier Reef Committee.

Ormond, R. F. G., A. C. Campbell, S. H. Head, R. J. Moore, P. R. Rainbow & A. P. Saunders (1973). Formation and breakdown of aggregations of the crown-of-thorns starfish, *Acanthaster planci* (L.). *Nature* **246**: 167-169.

Ormond R.F.G., N.J. Hanscomb, & D.A. Beach (1976). Food selection and learning in the crown-of-thorns starfish *Acantaster planci* (L.). *Marine and Freshwater Behaviour and Physiology* **4**: 93-105.

Pandolfi, J.M. (1992). A paleobiological examination of the geological evidence for recurring outbreaks of the crown-of-thorns starfish, *Acanthaster planci* (L). *Coral Reefs* **11**: 87-93.

Pandolfi, J.M., J.B.C. Jackson, N. Baron, R.H. Bradbury, H.M. Guzman, T.P. Hughes, C.V. Kappel, F. Micheli, J.C. Ogden, H.P. Possingham, & E.Sala (2005). Are U.S. coral reefs on the slippery slope to slime? *Science* **307**: 1725-1726.

Paul, V.J. & R. Ritson-Williams (2008). Marine Chemical Ecology. *Natural Product Reports* **25**: 662-695.

Paulay, G. (2003). Marine biodiversity of Guam and the Marianas: Overview. *Micronesica* **35-36**: 3-25.

Pawlik, J.R. (1993). Marine invertebrate chemical defenses, *Chemical Reviews* **93**: 1911-1922.

Pawlik, J.R., G. McFall & S. Zea S (2002). Does the odor from sponges of the genus *Ircinia* protect them from fish predators? *Journal of Chemical Ecology* **28**: 1103-1115.

Pearson, R.G. (1975). Coral reefs, unpredictable climatic factors and *Acanthaster.* In: Crown-of-thorns starfish seminar proceedings, Brisbane, 6 September, 1974 (pp. 131-134). Canberra: Australian Government Publishing Service.

Pentreath, V.W. & J.L.S. Cobb (1972). Neurobiology of Echinodermata. *Biological Reviews* **47**: 362-392.

Polat, A. & G. Beklevik (1999). The importance of betaine and some attractive substances as fish feed additives. *Chairs, Options, Mediterraneennes: Feed Manufacturing in the Mediterranean Region* **37**: 217-220.

Porter, J.W. (1972). Predation by *Acanthaster* and its effects on coral species diversity. *The American Naturalist* **106**: 487-492.

Porter, V., T. Leberer, M. Gawel, J. Gutierrez, D. Burdick, V. Torres & E. Lujan (2005). Status of the coral reef ecosystems of Guam (*UOGML Technical Report 113*). Guam, USA: University of Guam Marine Laboratory, 69 pp.

Pratchett, M. S. (2001). Influence of coral symbionts on feeding preferences of crown-of-thorns starfish *Acanthaster planci* in the Western Pacific. *Marine Ecology Progress Series* **214**: 111-119.

Pratchett, M. S. (2005). Dynamics of an outbreak population of *Acanthaster planci* at Lizard Island, northern Great Barrier Reef (1995-1999). *Coral Reefs* **24**: 453-462.

Pratchett, M. S. (2007). Feeding preferences of *Acanthaster planci* (Echinodermata: Asteroidea) under controlled conditions of food availability. *Pacific Science* **61**: 113-120.

Pratchett, M.S., T.J. Schenk, M. Baine, C. Syms, & A.H. Baird (2009). Selective coral mortality associated with outbreaks of *Acanthaster planci* L. in Bootless Bay, Papua New Guinea. *Marine Environmental Research* **67**: 230–236.

Randall, J. E. (1972). Chemical pollution in the sea and the crown-of-thorns starfish (*Acanthaster planci*). *Biotropica* **4**: 132-144.

Randall, R. H. (1973). Distribution of corals after *Acanthaster planci* (L.) infestation at Tanguisson Point, Guam. *Micronesica* **9**: 213-222.

Rasband, W.S. (2007). *ImageJ*. U. S. National Institutes of Health, Bethesda, Maryland, USA (http://rsb.info.nih.gov/ij/).

Reese, E. S. (1966). The complex behavior of echinoderms. In: R.A. Boolootian (Ed.), *Physiology of Echinodermata* (pp. 157-218). NY: Interscience Publishers.

Reichelt, R. E., R. H. Bradbury & P. J. Moran (1990). The crown-of-thorns starfish, *Acanthaster planci*, on the Great Barrier Reef. *Mathematical and Computer Modelling* **13**: 45-60.

Richmond R.H., P.H. Houk, M. Trianni, E. Wolanski, G. Davis, V. Bonito, & V. Paul (2008). Aspects of biology and ecological functioning of coral reefs in Guam and the Commonwealth of the Northern Mariana Islands. In: B. Riegl & R.E. Dodge (Eds.), *Coral Reefs of the World*, Vol. 1: Coral Reefs of the USA (pp. 715-735). NY: Springer.

Richmond, R.H., T. Rongo, Y. Golbuu, S. Victor, N. Idechong, G. Davis, W. Kostka, L. Neth, M. Hamnett, & E. Wolanski (2007). Watersheds and coral reefs: Conservation Science, Policy, and Implementation. *Bioscience* **57**: 598-607.

Ritson-Williams, R. & V. J. Paul (2007). Marine benthic invertebrates use multimodal cues for defense against reef fish. *Marine Ecology Progress Series* **340**: 29-39.

Rittschof , D. & J.H. Cohen (2004). Crustacean peptide and peptide-like pheromones and kairomones. *Peptides* **25**: 1503-1516.

Rosenberg, D. L. (1972). Behavioral aspects of photosensitivity and spectral sensitivity in *Acanthaster planci* (L.). *M.Sc. in Biology Thesis*. Guam, USA: University of Guam Marine Laboratory.

Rotjan, R.D. & S.M. Lewis (2008). The impact of coral predators on tropical reefs. *Marine Ecology Progress Series* **367**: 73-91.

Sakata, K., M. Tsuge, K. Ina (1986). A simple bioassay for feeding-stimulants for the young seahare *Aplysia juliana*. *Marine Biology* **91**: 509-511.

Sano, M., M. Shimizu, & Y. Nose (1984). Changes in structure of coral reef fish communities by destruction of hermatypic corals: observational and experimental views. *Pacific Science* **38**: 51-79.

Sano, M., M. Shimizu, & Y. Nose (1987). Long term effects of destruction of hermatypic corals by *Acanthaster planci* infestation on reef fish communities at Iriomote Island, Japan. *Marine Ecology Progress Series* **37**: 191-199.

Sato, T. & S. Goshima (2007). Female choice in response to risk of sperm limitation by the stone crab, *Hapalogaster dentate*. *Animal Behaviour* **73**: 331-338.

Scandol, J. P. & M. K. James (1992). Hydrodynamics and larval dispersal: a population model of *Acanthaster planci* on the Great Barrier Reef. *Australian Journal of Marine and Freshwater Research* **43**: 583-596.

Schupp, P., C. Eder, V. Paul, & P. Proksch (1999). Distribution of secondary metabolites in the sponge *Oceanapia* sp. and its ecological implications. *Marine Biology* **135**: 573-580.

Seymour, R. M. & R. H. Bradbury (1999). Lengthening reef recovery times from crown-of-thorns outbreaks signal systemic degradation of the Great Barrier Reef. *Marine Ecology Progress Series* **176**: 1 – 10.

Shankar R., H.S. Murthy, P. Pavadi, & K. Thanuja (2008). Effect of betaine as a feed attractant on growth, survival, and feed utilization in fingerlings of the Indian Major Carp, *Labeo rohita*. *The Israeli Journal of Aquaculture – Bamidgeh* **60**: 95-99.

Shin Y.G., K.H. Cho, J.M. Kim, M.K. Park, & J.H. Park (1999). Determination of betaine in *Lycium chinense* fruits by liquid chromatography-electrospray ionization mass spectrometry. *Journal of Chromatography A* **857**: 331-335.

Shiomi, K., K. Itoh, H. Yamanaka & T. Kikuchi (1985). Biological activity of crude venom from the crown-of-thorns starfish *Acanthaster planci*. *Nippon Suisan Gakkaishi* **51**: 1151-1155.

Sieburth, J.M. (1960). Acrylic acid, an 'antibiotic' principle in *Phaeocystis* blooms in Antarctic waters. *Science* **132**:676-677.

Sloan, N. A. (1980a). Aspects of the feeding biology of asteroids. *Oceanography and Marine Biology: An Annual Review* **18**: 57-124.

Sloan, N. A. (1980b). The arm curling and terminal tube-foot responses of the asteroid *Crossaster papposus* (L.). *Journal of Natural History* **14**: 469-482.

Sloan, N.A. & A.C. Campbell (1982). Perception of food. In: M. Jangoux & J.M. Lawrence (Eds.), *Echinoderm Nutrition* (pp. 3-23). Rotterdam: A.A. Balkema Publishers.

Smith, J.E. (1966). The form and functions of the nervous system. In: R.A. Boolootian (Ed.), *Physiology of Echinodermata* (pp. 503-511). NY: Interscience Publishers.

Sokal, R.R. & F.J. Rohlf (1995). *Biometry: the principles and practice of statistics in biological research.* 3rd Ed. NY: W. H. Freeman and Co.,887 pp.

Sonoda, K. & V. J. Paul (1993). Effect of stony coral extracts on feeding by *Acanthaster planci*. *Marine Ecology Progress Series* **102**: 161-168.

Sorensen,P.W., J. M. Fine, V. Dvornikovs, C. S. Jeffrey, F. Shao, J. Wang, L. A. Vrieze, K. R. Anderson & T. R. Hoye (2005). Mixture of new sulfated steroids functions as a migratory pheromone in the sea lamprey. *Nature Chemical Biology* **1**: 324-328.

Souza C.F., A.S. de Oliveira, R.C. Pereira (2008). Feeding preference of the sea urchin *Lytechinus variegatus* (Lamarck, 1816) on seaweeds. *Brazilian Journal of Oceanography* **56**(3): 239-247.

Stachowicz J.J. (2001). Chemical ecology of mobile marine invertebrates: predators and prey, allies and competitors. In: J. McClintock & B. Baker (Eds.), *Marine Chemical Ecology* (pp. 153-190). Boca Raton: CRC Press.

Stachowicz, J.J. (2001). Chemical Ecology of Mobile Marine Invertebrates: predators and prey, allies and competitors. B. Baker and K. McClintock (Eds.), *Marine Chemical Ecology* (pp. 153-190). Boca Raton: CRC Press.

Starmer, J., J. Asher, F. Castro, D. Gochfeld, J. Gove, A. Hall, P. Houk, E. Keenan, J. Miller, R. Moffit, M. Nadon, R. Schroeder, E. Smith, M. Trianni, P. Vroom, K.

Wong & K. Yuknavage (2008). The State of the Coral Reef Ecosystems of the Commonwealth of the Northern Mariana Islands. In: J.E. Waddell & A.M. Clarke (Eds.), *The State of Coral Reef Ecosystems of the United States and Pacific Freely Associated States: 2008* (pp. 437-463). Silver Spring, MD: NOAA/NCCOS Center for Coastal Monitoring and Assessment's Biogeography Team. Silver Spring, MD.

Steinke, M., G. Malin & P.S. Liss (2002). Trophic interactions in the sea: An ecological role for climate relevant volatiles? *Journal of Phycology* **38**: 630-638.

Stimson, J. (1987). Location, quantity and rate of change in quantity of lipids in tissue of Hawaiiian hermatypic corals. *Bulletin of Marine Science* **41**: 889-904.

Stump, R. J. W. (1996). An investigation of the methods to describe the population dynamics of *Acanthaster planci* (L.) around Lizard Island, northern Cairns Section, GBR (*Technical Report No. 10*). Townsville, Australia: CRC Reef Research and Great Barrier Reef Marine Park Authority.

Sunda, W., D.J. Kieber, R.P. Kiene & S. Huntsman (2002). An antioxidant function for DMSP in marine algae. *Nature* **418**: 317-320.

Sweatman, H. (2008). No-take reserves protect coral reefs from predatory starfish. *Current Biology* **18**: R598-R599.

Sweatman, H.P.A. (1995). A field study of fish predation on juvenile crown-of-thorns starfish. *Coral Reefs* **14**: 47-53.

Szmant, A.M., L.M. Ferrer & L.M. Fitzgerald (1990). Nitrogen excretion and O:N ratios in reef corals: evidence for conservation of nitrogen. *Marine Biology* **104**: 119-127.

Teruya, T., K. Suenaga, T. Koyama, Y. Nakano & D. Uemura (2001). Arachidonic acid and □-linoleic acid, feeding attractants for the crown-of-thorns sea star *Acanthaster planci*, from the sea urchin *Toxopneustes pileolus*. *Journal of Experimental Marine Biology and Ecology* **266**: 123-134.

Thoms, C. & P. Schupp (2008). Activated Chemical Defense in Marine Sponges—a Case Study on *Aplysinella rhax*. *Journal Chemical Ecology* **34**:1242–1252.

Tinoco, J. (1982). Dietary requirements and functions of □-linolenic acid in animals. *Progress in Lipid Research* **21**: 1–45.

Tricas, T.C. (1989). Prey selection by coral-feeding butterflyfishes: strategies to maximize the profit. *Environmental Biology of Fishes* **25**: 171-185.

Tsuda, R.T. (1970). *Acanthaster planci*, crown-of-thorns starfish: Resurvey of Guam (*UOGML Miscellaneous Report*). Guam, USA: University of Guam Marine Laboratory, 7 pp.

Tsuda, R.T. (Compiler). (1971). Status of Acanthaster planci and coral reefs in the Mariana and Caroline Islands, June 1970 to May 1971 (*UOGML Technical Report No. 2*). Guam, USA: University of Guam Marine Laboratory.

Tsuda, R.T., D.P. Cheney, J.A. Marsh, Jr., & M.R. Struck (1970a). *Acanthaster planci*, crown-of-thorns starfish: Resurvey of Yap, Yap District (*UOGML Miscellaneous Report*). Guam, USA: University of Guam Marine Laboratory, 13 pp.

Tsuda, R.T., R.S. Jones, R.H. Randall, & M.R. Struck (1970b). *Acanthaster planci*, crown-of-thorns starfish: Resurvey of Saipan and Tinian, Survey of Aguijan, Mariana District (*UOGML Miscellaneous Report*). Guam, USA: University of Guam Marine Laboratory, 11 pp.

Tsuda, R.T., M.R. Struck, R.J. Lujan, & A.J. Fortin (1970c). *Acanthaster planci*, crown-of-thorns starfish: Resurvey of Rota, Mariana District (*UOGML Miscellaneous Report*). Guam, USA: University of Guam Marine Laboratory, 10 pp.

Tsuda, R.T., P.G. Bryan, R.J. Lujan, & A.J. Fortin (1971a). *Acanthaster planci*, crown-of-thorns starfish: Resurvey of Palau, Palau District (*UOGML Miscellaneous Report*). Guam, USA: University of Guam Marine Laboratory, 11 pp.

Tsuda, R.T., P.G. Bryan, D.L. Rosenberg, & N.E. Vas (1971b). *Acanthaster planci*, crown-of-thorns starfish: Resurvey of Ponape and Ant, Ponape District (*UOGML Miscellaneous Report*). Guam, USA: University of Guam Marine Laboratory, 9 pp.

Vairavamurthy, A., M.O. Andreae & R.L. Iverson (1985). Biosynthesis of dimethyl sulfide and dimethylpropiothetin by *Hymenomonas carterae* in relation to sulfur sources and salinity variations. *Limnology and Oceanography* **30**: 59-70.

Valentincic, T. (1985). Behavioral study of chemoreception in the sea star *Marthasterias glacialis*: structure-activity relationships of lactic acid, amino acids, and acetylcholine. *Journal of Comparative Physiology A* **157**: 537-545.

Valiela, I. (1995). *Marine Ecological Processes*. 2nd Ed. NY: Springer-Verlag, 712 pp.

Van Alstyne, K.L. (2008). Ecological and physiological roles of dimethylsulfoniopropionate (DMSP) and its products in marine macroalgae. In: C. Amsler (Ed.), *Algal Chemical Ecology* (pp. 173-194). Heidelberg: Springer.

Van Alstyne, K.L. & L.T. Houser (2003). Dimethyl sulfide release during macroinvertebrate grazing and its role as an activated chemical defense. *Marine Ecology Progress Series* **250**: 175-181.

Van Alstyne, K.L. & V.J. Paul (1992). Chemical and structural defenses in the sea fan *Gorgoriia ventalina*: effects against generalist and specialist predators. *Coral Reefs* **11**: 155-159.

Van Alstyne, K.L. & M.P. Puglisi (2007). DMSP in marine macroalgae and macroinvertebrates: distribution, functions, and ecological impacts. *Aquatic Sciences* **69**: 394-402.

Van Alstyne, K. L., P. Schupp & M. Slattery (2006). The distribution of dimethylsulfoniopropionate in tropical Pacific coral reef invertebrates. *Coral Reefs* **25**: 321-327.

Van Alstyne, K.L., G.V. Wolfe, T.L. Freidenburg, A. Neill & C. Hicken (2001). Activated defense systems in marine macroalgae: evidence for an ecological role for DMSP cleavage. *Marine Ecology Progress Series* **213**: 53-65.

Van Beukering, P., W. Haider, M. Longland, H. Cesar, J. Sablan, S.Shjegstad, B. Beardmore, Y. Liu and G.O. Garces (2007). The economic value of Guam's coral reefs (*UOGML Technical Report No. 116*). Guam, USA: University of Guam Marine Laboratory.

Van Veghel, M.L.J. & R.P.M. Bak (1994). Reproductive characteristics of the polymorphic Caribbean reef building coral *Montastrea annularis*. III. Reproduction in damaged and regenerating colonies. *Marine Ecology Progress Series* **109**: 229-233.

Vine, P.J. (1970). Densities of *Acanthaster planci* in the Pacific Ocean. *Nature* **228**: 341.

Vine, P. J. (1973). Crown of thorns (*Acanthaster planci*): The natural causes theory. *Atoll Research Bulletin* **166**: 1-10.

Vogler, C., J. Benzie, H.A. Lessios, P. Barber, & G. Wörheide (2008). A threat to coral reefs multiplied? Four species of crown-of-thorns starfish. *Biology Letters* **4**(6): 696-699.

Walbran, P.D., R.A., Henderson, A.J.T. Jull, M.J. Head (1989). Evidence from sediments of long-term *Acanthaster planci* predation on corals of the Great Barrier Reef. *Science* **245**: 847–850.

Wass, R.C. (1972). 1972 Resurvey of Ponape for *Acanthaster planci*. In: Tsuda, R.T. (Ed.), *Proceedings of the University of Guam - Trust Territory Acanthaster planci (crown-of-thorns starfish) Workshop*, Guam, 1972 (*UOGML Technical Report 3*). Guam, USA: University of Guam Marine Laboratory, 25 pp.

Watson, G.J., M.G. Bentley, S.M. Gaudron, J.D. Hardege (2003). The role of chemical signals in the spawning induction of polychaete worms and other marine invertebrates. *Journal of Experimental Marine Biology and Ecology* **294**: 169-187.

Weissburg, M. J. & R. K. Zimmer-Faust (1993). Life and death in moving fluids: Hydrodynamic effects on chemosensory-mediated predation. *Ecology* **74**(5): 1428-1443.

Weissburg, M. J. & R. K. Zimmer-Faust (1994). Odor plumes and how blue crabs use them in finding prey. *Journal of Experimental Biology* **197**: 349-375.

Wiesemeier T., M.E. Hay & G. Pohnert (2007). The potential role of wound-activated volatile release in the chemical defence of the brown alga *Dictyota dichotoma*: blend recognition by marine herbivores. *Aquatic Sciences* **69**: 403-41.

Wiesemeier, T. & G. Pohnert (2007). Direct quantification of dimethylsulfoniopropionate (DMSP) in marine micro- and macroalgae using HPLC or UPLC/MS. *Journal of Chromatography B* **850**: 493-498.

Wilkinson, C. (Ed.) (2008). *Status of coral reefs of the world: 2008*. Townsville, Australia: Global Coral Reef Monitoring Network and Reef and Rainforest Research Centre. 296 pp.

Williams, D.McB. (1986) Temporal variation in the structure of reef slope fish communities (central Great Barrier Reef): short term effects of *Acanthaster planci* infestation. *Marine Ecology Progress Series* **28**: 157-l 64.

Wolanski, E., R.H. Richmond, G. Davis, E. Deleersnijder & R.R. Leben (2003). Eddies around Guam, Mariana Island Group. *Continental Shelf Research* **23**: 991-1003.

Wolfe G.V., M. Steinke & G.O. Kirst (1997). Grazing-activated chemical defense in a unicellular marine alga. *Nature* **387**: 894-897.

Wolfe, G.V. & M. Steinke (1996). Grazing-activated production of dimethyl sulfide (DMS) by two clones of *Emiliania huxleyi*. *Limnology and Oceanography* **41**: 1151-1160.

Wyeth, R.C. & A.O.D. Willows (2006). Odours detected by rhinophores mediate orientation to flow in the nudibranch mollusc, *Tritonia diomedea*. *Journal of Experimental Biology*. **209**: 1441-1453.

Yamaguchi, M. (1973). Early life histories of coral reef asteroids, with special reference to *Acanthaster planci* (L.). In: O.A. Jones & R. Endean (Eds.), *Biology and Geology of Coral Reefs*, Vol. 2: Biology 1 (pp. 369-387). NY: Academic Press.

Yamaguchi, M. (1986). *Acanthaster planci* infestations of reefs and coral assemblages in Japan: a restrospective analysis of control efforts. *Coral Reefs* **5**: 23-30.

Yambe, H., S. Kitamura, M. Kamio, M. Yamada, S. Matsunaga, N. Fusetani & F. Yamazaki. l-Kynurenine, an amino acid identified as a sex pheromone in the urine of ovulated female masu salmon. *Proceedings of the National Academy of Sciences USA* **103**: 15370-15374.

Yasuda, N., S. Nagai, M. Hamaguchi, C. L. Lian & K. Nadaoka (2006). Development of microsatellite markers for the crown-of-thorns starfish *Acanthaster planci*. *Molecular Ecology Notes* **6**: 141-143.

Yoch, D. C. (2002). Dimethylsulfoniopropionate: Its sources, role in the marine food web, and biological degradation to dimethylsulfide. *Applied and Environmental Microbiology* **68**: 5804-5815.

Yomo, H. & Y. Egawa (1978). Collagenase in stomach of crown-of-thorns starfish *Acanthaster planci*. *Agricultural and Biological Chemistry* **42**: 1591.

Yost, D.M. & C.L. Mitchelmore (2009). Dimethylsulfoniopropionate (DMSP) lyase activity in different strains of the symbiotic alga *Symbiodinium microadriaticum*. *Marine Ecology Progress Series* **386**: 61-70.

Zafiriou, O. (1972). Response of *Asterias vulgaris* to chemical stimuli. *Marine Biology* **17**: 100- 107.

Zafiriou, O., K.J. Whittle, & M. Blumer (1972). Response of *Asterias vulgaris* to bivalves and bivalve tissue extracts. *Marine Biology* **13**: 137-145.

Zann, L., J. Brodie, C. Berryman, & M. Naqasima (1987). Recruitment, ecology, growth and behaviour of juvenile *Acanthaster planci* (L.) (Echinodermata: Asteroidea). *Bulletin of Marine Science* **41**: 561-575.

Zhou, Y., S. Hu, X.Ma, S. Liang, T. Jiang & B. Han. (2008). Synthesis of cyclic carbonates from carbon dioxide and epoxides over betaine-based catalysts. *Journal of Molecular Catalysis A: Chemical* **284**: 52-57.

Zhukova, N.V. & E.A. Titlyanov (2006). Effect of light intensity on the fatty acid and composition of dinoflagellates symbiotic with hermatypic corals. *Botanica Marina* **49**: 339-346.

Zimmer, R. K. & C. A. Butman (2000). Chemical signaling processes in the marine environment. *Biological Bulletin* **198**: 168-187.

Zimmer, R. K., J. E. Commins, & K. A. Browne (1999). Regulatory effects of environmental chemical signals on search behavior and foraging success. *Ecology* **80**: 1432-1446.