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Assessing the Health of Coral Reef Ecosystems in the Florida Keys at Community, Individual, and Cellular Scales

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Assessing the Health of Coral Reef Ecosystems in the Florida Keys at Community, Individual, and Cellular Scales

by

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of the requirements for the degree of
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Dedication

This is dedicated to my fiancé Chris, my parents and family for their constant patience, love and support.

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ABSTRACT

Coral reefs are threatened in Florida and worldwide. Successful resource management requires rapid identification of anthropogenic sources of stress before they affect the reef community. I tested a multi-scale approach for assessing reef condition at seven reefs within the Florida Keys National Marine Sanctuary and Biscayne National Park between 2001 and 2003. I examined multiple environmental parameters to identify potential sources of stress. I utilized the Atlantic and Gulf Rapid Reef Assessment Biotic Reef Index to assess benthic community structure and an indicator species of Foraminifera (*Amphistegina gibbosa*) to determine if environmental conditions were suitable for calcareous organisms that host algal endosymbionts. Small tissue samples were extracted from colonies of *Montastraea annularis* species complex to assay a suite of cellular biomarkers to elucidate possible mechanisms of the coral stress response. I monitored regeneration rates of the resultant lesions to determine if the coral colonies were capable of recovering from damage. Multivariate data analyses indicated that corals at all study sites were experiencing stress with different degrees of response and decline. On reefs with coarse grain sediments that are adjacent to an intact mangrove shoreline, the Cellular Diagnostic System indicated that corals were responding to a xenobiotic stress but appeared to be compensating as evidenced by consistently high lesion regeneration rates, a high percentage of healed lesions, low coral mortality and high abundances of *A. gibbosa*. On reefs with silt-sized sediments adjacent to developed coastlines, corals also were responding to xenobiotic stresses, but were negatively affected as evidenced by low regeneration rates, a low percentage of healed lesions, high coral mortality, and low abundances of *A. gibbosa*. Corals at an 18 m offshore site

exhibited abnormally low biomarker levels and some died during the study, indicating that sampled colonies were incapable of upregulating necessary protective proteins. Further research will be required to determine stressor sources. This study demonstrates that a multiple-indicator approach, spanning scales from cellular to community, can provide marine resource managers with data linking decline of coral populations to specific environmental conditions and events, thereby providing potential for early detection of stressors allowing for preventive management.

1. Introduction

1.1. Reef Degradation

Coral reefs are threatened resources in Florida and many coastal regions worldwide (Bryant et al. 1998, Hoegh-Guldberg 1999, Hughes et al. 2003, Bellwood et al. 2004, Waddell 2005, many others). Yet these resources are rapidly being degraded or lost to a combination of global and local stressors. The economic value of reefs is $7.7 billion per year in goods and services for South Florida (Johns et al. 2001, Andrews et al. 2005) and $375 billion worldwide (Costanza et al. 1997).

On global scales, rising sea-surface temperatures (especially during El Niño) and increasing ultraviolet radiation (due to ozone thinning) (Hoegh-Guldberg 1999, Dustan 2000, Hallock 2001, Buddemeier et al. 2004, many others) were implicated as in bleaching and disease in corals (Porter et al. 2001, Sutherland et al. 2004, Marshall & Schuttenberg 2006, many others), as well as in other reef organisms. Coral bleaching has become common since 1983, affecting every region worldwide and in many cases resulting in significant coral mortality (Marshall & Schuttenberg 2006). The 1997-1998 bleaching event resulted in 90% mortality to 16% of reefs worldwide. Moreover, 97% of Caribbean reefs were impacted by disease (Green & Bruckner 2000). In the Florida Keys National Marine Sanctuary (FKNMS), the number of locations exhibiting disease increased by a factor of four between 1996 and 1998 (Porter et al. 2001), and disease prevalence only recently leveled off or began to decrease (Beaver et al. 2005). Changes in water chemistry and increasingly rapid sea-level rise also threaten the reef-building capacity of corals (Kleypas et al. 2001, Guinotte et al. 2003, Buddemeier et al. 2004, Hallock 2005, Pelejero et al. 2005). Intensified African dust storms and the microbes they transport also may contribute to the decline of Caribbean reefs (Shinn et al. 2000, Hayes et al. 2001). Local impacts on reefs increase with increasing urbanization and growing human populations (Bryant et al. 1998, Causey et al. 2000, Dustan 2000).

Although substantial effort is being expended to monitor coral communities and water quality associated with reefs (e.g., SEAKEYS/C-MAN Project; Jaap et al. 2000, Boyer and Jones 2002), why corals are dying at unprecedented rates (Wilkinson 2000, Porter et al. 2001) still is not well known. To better understand how to conserve coral reef ecosystems, scientists must determine their status on community scales and understand the underlying mechanisms on population, individual, and cellular scales (Jameson et al. 2001, 2002, Downs 2005, Downs et al. 2005b).

1.2. Florida Reef Tract

Reef degradation has been attributed to coastal development and associated stressors, with > 70% of coral reefs worldwide directly threatened by human-associated activities (Bryant et al. 1998, Waddell 2005). Florida has 12 of the top 100 fastest growing counties in the United States (US Census 2000). Miami-Dade County's population grew from 298 in 1889 to 495,047 in 1950 to over 2 million in 2000 (US Census). The upper and lower Florida Keys, particularly Key Largo and Key West, have experienced substantial increases in human population growth and urban development over the past four decades without adequate increases in supporting infrastructure to control runoff, groundwater pollution and sewage (Causey et al. 2000, Dustan 2000). Within the last 40 years, Monroe County's human population has increased by 40% to approximately 79,000 people (US Census 2000). This does not include the substantial tourist population (> 25,000 people), which results in more than 100,000 people in the Keys any given time during winter months (Kruczynski & McManus 2002).

Patterns of coral decline generally correspond with human population centers, with the largest declines documented in the upper and lower Keys (Jaap et al. 2000). Paradoxically, despite being closer to potential coastal impacts, inshore patch reefs on average appear to be in better condition and have higher coral cover relative to offshore reefs (Beaver et al. 2005). Reasons for these differences are not well understood but may be related to inshore corals being adapted to more variable environments, which allows them to tolerate anthropogenic stressors better than colonies in historically more stable offshore environments (e.g., Soto 2006).

Water quality in the Florida Keys declined over the last thirty years due to changes in water flow patterns from Florida Bay, sedimentation (from boat traffic and development) and increased near-shore nutrient concentrations (from local wastewaters, freshwater upwelling, fertilizers and industrial pollutants; Szmant & Forrester 1996, Lang et al. 1998, Causey et al. 2000, Porter et al. 2001, Andrews et al. 2005). Over the same time period, live coral cover decreased throughout the Florida Keys with 50 – 70 % loss in live coral cover since the 1970s (Dustan & Halas 1987, Lang et al. 1998, Causey et al. 2000, Porter et al. 2002, Palandro et al. 2003, Beaver et al. 2005, Palandro 2006). Significant loss occurred in major reef-building corals such as the *Montastraea annularis* complex, *Acropora palmata* and *A. cervicornis* (Beaver et al. 2005). Mats of sediment-trapping turf algae are gradually overgrowing corals and restricting settlement of new recruits (Lang et al. 1998, Petersen et al. 2005, Nugues & Szmant 2006). Large storms produce runoff laden with heavy metals (Glynn et al. 1989, Cantillo et al. 1997), pesticides and herbicides (Gardinali et al. 2002, Owen et al. 2002, 2003, Downs et al. 2006), and microbial pathogens associated with local sewage (Paul et al. 1995a, b, Paul et al. 1997, Griffin et al. 1999, Lipp et al. 2002). Pesticides and herbicides such as dibrom, which is heavily used in South Florida to control mosquitoes, induced stress responses in corals in the Upper Keys (Morgan & Snell 2002, Owen et al. 2003). Such toxins can interfere with chemical signals or larval behavior, thereby inhibiting coral reproduction and recruitment (e.g., Richmond 1993, 1997, Peters 1997, McKenna et al. 1999, Reichelt-Brushett & Harrison 2005).

Prevalence of coral diseases and bleaching also dramatically increased over the past three decades (Lang et al. 1998, Causey et al. 2000, Jaap et al. 2000, Green & Bruckner 2000, Harvell et al. 2004, Sutherland et al. 2004, Santavy et al. 2005). Some diseases appear most prevalent in populations stressed by anthropogenic pollution (Goreau et al. 1998, Richardson 1998, Kaczmarsky et al. 2005). For example, the causal agent of white pox disease was identified as the human fecal bacterium *Serratia marcescens* (Patterson et al. 2002). Nutrient enrichment increased severity of aspergillosis in a sea fan (*Gorgonia ventalina*) and yellow band disease in the *M.*

annularis complex (Bruno et al. 2003). White plague and black band disease were more prevalent in proximity to sewage effluent (Kaczmarsky et al. 2005).

1.3. Need for New Methodologies

Monitoring changes in species composition, abundance and coverage are important for 1) determining change in community dynamics and detecting patterns and trends over long time periods, 2) providing baseline data to compare present conditions, and 3) predicting how human activities might affect ecosystems (Rogers et al. 1994, Hughes & Connell 1999, Jaap et al. 2000). However, these traditional methods can only detect a disturbance after the community is altered, and often do not provide sufficient evidence of cause for managers to take specific actions. Therefore, more sensitive techniques are needed to detect stress responses before impacts begin to degrade a community (Brown 1988, Depledge et al. 1993, Risk 1999, Jameson et al. 2001, Downs 2005, Downs et al. 2005). Early detection of stressors enables implementation of preventive management rather than depending on post-damage restoration. Community-based bioindicators are needed for effective reef assessment, which underlies policy, legislation and management (Risk 1999, McCarty et al. 2002). There is a need for integrating monitoring with research designed to identify stressors and determine causality (Brown 1988, Risk 1999). Existing reef-monitoring programs commonly do not fulfill this need.

1.4. Overview of Dissertation Research

1.4.1. Approach

This dissertation directly addresses the deficiency described by Brown (1988) and Risk (1999) by integrating monitoring of environmental conditions and responses of reef populations with cellular data, which has the potential to identify specific stressors before they cause community degradation. The goal of my project is to determine if a suite of cellular, physiological, and community parameters can (1) distinguish between levels of physiological condition (e.g., nominal vs. diseased state), (2) identify types of stressors, and (3) elucidate mechanisms of stress response. My project also evaluates the strengths

and caveats of using individual bioindicators to detect differences among sites, times and types of stressors.

To accomplish these goals, this project took a multi-scale approach (Fig. 1.1), which was carried out over two years. Drawing on multiple assessment endpoints across hierarchical levels provides a mechanistic understanding of the causes of reef degradation (Downs 2005), which allows researchers to determine whether (1) an organism is responding to a stress and (2) that stress has resulted in reduced physiological function (Downs 2005). I assessed the condition of a reef ecosystem, including traditional community assessment (i.e., the Atlantic and Gulf Rapid Reef Assessment protocol of Lang 2003) and monitoring of selected environmental parameters, populations of a "surrogate" indicator (i.e., Hallock et al. 2004), coral-colony condition (Williams 1994, Meesters et al. 1997a), and cellular physiological responses of coral colonies as indicated by a diagnostic profile of cellular parameters (Downs 2005, Downs et al. 2000, 2002, 2005, 2006). With this approach, I characterized both reef and environmental conditions, while quantifying temporal changes in populations and individual coral colonies. I compared coral responses (e.g., regeneration rates) with results from Cellular Diagnostic System (CDS) assessments of the same colonies. The CDS has the potential of detecting deviations in cellular function before they alter physiological functions (e.g., regeneration, growth or reproduction) and degrade the community (Downs 2005).

I applied a diagnostic approach (Jameson et al. 2001, Downs et al. 2005) to assess reef condition at my study sites including (1) examination and preliminary classification of each reef (e.g., community and environmental assessments), (2) characterization of reef condition based on comparison with reference values of key organisms, (3) if an altered state is apparent, developing hypotheses explaining deviations from the nominal state, including investigating appropriate environmental parameters, (4) implementing relevant methodology to test hypotheses and build evidence for the greatest likelihood explaining the phenomenon, and (5) diagnostic interpretation based on 'weight of evidence' or reevaluation of hypotheses and methods if necessary. A diagnostic method requires knowledge of baseline or reference values. However, these values should not be considered fixed and can continually be modified as new information is gained.

1.4.2. Description of Sampling Sites

I sampled four patch reefs and two depths on one forereef within FKNMS, near Key Largo (KL), and one patch reef in Biscayne National Park (BNP), which is along the Northern Florida Reef Tract (Fig. 1.2). These sites were chosen in consultation with John Halas, Resource Manager (now Upper Keys Regional Manager), Upper Keys Region of FKNMS, and Richard Curry, Science Coordinator, BNP. Biomarker sampling had been conducted along the depth gradient within FKNMS near Molasses Reef since March 1999 to determine if levels of oxidative-damage products, antioxidant enzymes, and specific components of cellular structural integrity in the star coral (*Montastraea annularis* species complex) varied with coral bleaching, seasonal and increased SST, and water depth (Downs et al. 2002). Downs et al. (2002) chose these sites (Fig. 1.2, KL 3 m – KL 18 m) because they were near long-term monitoring locations, including those of the EPA-FKNMS Coral Reef Evaluation and Monitoring Project (e.g., Wheaton et al. 1998) and the Molasses Reef SEAKEYS Program C-MAN buoy, which records hourly weather and water quality parameters (http://coral.aoml.noaa.gov/cman/). Sampling at these sites continued during my project. Algae Reef (AR) was added because of potential groundwater contamination, as indicated by a cyanobacterial outbreak in the early 1990s (e.g., Kuta & Richardson 1997) and White Banks (WB) was added because it was considered to be a relatively pristine site. Alina's Reef (BNP) was chosen for its proximity to Miami and to coincide with other long-term monitoring projects. Previous research indicated that a quarterly sampling design was sufficient to detect changes in coral physiology resulting from seasonal and stressor variation, therefore sampling was conducted in March/April, June, August, and October/November of 2001 and 2002, plus February 2003 (Table 1.1).

1.4.3. Biology and Ecology of *Montastraea annularis* complex

My study focused on corals of the *Montastraea annularis* species complex (Fig. 1.3), which is an important reef-building coral found throughout the Caribbean over a range of depths (approx. 1 – 50 m). The *M. annularis* complex is made up of three morphotypes/species including *M. annularis, M. faveolata* and *M. franksi.* Taxonomic

differences among these morphologies remain uncertain (Weil & Knowlton 1994, Szmant et al. 1997, Severance et al. 2004a,b, Fukami & Knowlton 2005, Severance & Karl 2006). The corallite structure is similar, suggesting that they may be one species that exhibits different morphologies with changes in environmental conditions. However, all three morphotypes can be found in the same habitat and some physiological differences (e.g., aggression, growth) are known (Weil & Knowlton, 1994). *M. annularis* grows in columns with living polyps restricted to the top of each column. *Montastraea faveolata* grows in large mounds with bumps aligned in regular rows that extend down the mound, and its corallites are evenly extended. *Montastraea franksi* is usually found in deeper water and tends to grow in smaller mounds or flattened plates with irregular bumps and it has unevenly distributed and extended corallites. Recently, molecular techniques attempted to differentiate among the three morphotypes. Lopez et al. (1999) did not detect comparable differences between *M. franksi* and *M. annularis* with either AFLPs or a microsatellite locus, while Fukami and Knowlton (2005) found low genetic variability among the three members of the *M. annularis* complex using complete mitochondrial DNA sequences.

Members of the *M. annularis* complex are protogynous hermaphroditic, broadcast spawners (Fadlallah 1983). Spawning occurs annually in late summer (mid-August to mid-September; usually immediately after the full moon) releasing approximately 720-2016 eggs/cm^2 of coral tissue (Mergner 1971, Szmant 1986, 1991, Mendes & Woodley 2002). Colonies <100 cm^2 are rarely reproductive (Szmant 1986). Oogenesis begins in mid-May and spermatogenesis in mid-July (Szmant 1986). Juvenile recruitment of *M. annularis* from sexual reproduction is apparently infrequent, as there are typically fewer juveniles than adults in a population. Asexual reproduction accounts for most recruitment of new colonies of *M. annularis* through fission or separation resulting from mortality.

Growth rates of *M. annularis* are approximately 0.50 – 1.2 cm/yr in the Florida Keys (Hudson et al. 1976). Growth rates vary with depth, water clarity and temperature, and historically have been greatest in midshore reef areas of the FKNMS, where water is shallow and consistently clear with low temperature variability (Hudson 1981).

1.4.4. Specific Objective of Dissertation

The specific objectives of my study were to 1) assess reef condition using a hierarchical approach including selected environmental, community, population, colony and cellular parameters; 2) evaluate the ability of individual indicators to distinguish differences among sites, times and stressors; and 3) diagnose the physiological state of selected reefs based on 'weight of evidence' through the integration of multiple indicators. The overall scope of this project is outlined below (also see Fig. 1.1).

I. Environmental assessments of study sites (Chapter 2), including water temperature, turbidity, nutrient concentrations, and sedimentation, addressed these three questions:
   A. Were potential environmental stressors detected during the study period?
   B. What time periods were most stressful?
   C. Were there any environmental differences among sites?

II. Characterization of reef condition at hierarchical scales:
   A. I assessed community-scale condition of selected patch reefs using the Atlantic and Gulf Rapid Reef Assessment (AGRRA) protocol (Lang 2003) in March 2002. This assessment determined the condition of reefs by evaluating major benthic taxa: corals, fish and algae (Chapter 3), addressing these four questions:
      A. Does reef structure indicate whether conditions in the recent past were suitable for reef growth and development?
      B. Are significant differences evident among the reefs examined?
      C. Is there evidence of recent change (e.g., recent mortality)?
      D. How do data from these reefs compare with Caribbean-wide AGGRA data sets?
   B. I monitored population densities and visually assessed bleaching and shell breakage in a surrogate indicator group, symbiont-bearing ('larger') benthic Foraminifera, which lived near the corals (Chapter 4) to address two questions:

A. Are water quality and other environmental conditions suitable for calcifying organisms that host algal endosymbionts?
B. Do foraminifers indicate exposure to chronic or acute photic stress?

C. I monitored sampling-induced lesions and assessed overall condition (e.g., bleaching, disease, overgrowth, etc.) of individual colonies of *Montastraea annularis* species complex (Chapter 5) to address these two colony-scale questions:
    A. Can corals at the study sites recover from mechanically-induced lesions?
    B. Is there evidence for compromised physiological function of corals (e.g, reduced regeneration rates or increased mortality) at any site?
D. I used indicators of cellular physiology (Chapter 6) acquired by collaborators at MUSC/NOAA and Envirtue Biotechnology using a Cellular Diagnostic System (CDS) to address four questions:
    A. Did corals deviate from a nominal cellular state?
    B. Did cellular profiles indicate that corals were stressed and if so, where and when?
    C. To what types of stress were the corals responding?
    D. What were likely mechanisms of stress?

III. Diagnosis based on 'weight of evidence' (Chapter 6) addressed these two questions:
A. Where did sampled corals fall on a physiological scale of nominal to diseased state?
B. Can potential stressors be linked to physiological function?

IV. Conclusions (Chapter 7) examines the following:
A. evaluation of strengths and caveats of individual indicators,
B. summary of diagnosis, and
C. recommendations for future research.

Table 1.1. Sampling dates for 2001 – 2003 at Biscayne National Park (BNP), Algae Reef (AR), White Banks (WB) and Key Largo (KL) depth gradient (3, 6, 9 and 18 m).

Sampling period	Sampling Date	Site Sampled
March/April 2001	3/30/01	BNP
	3/31/01	KL 6 m, WB, AR
	4/1/01	KL 3 m, KL 9 m, KL 18 m
June 2001	6/26/01	BNP
	6/27/01	KL 6 m, WB, AR
	6/28/01	KL 3 m, KL 9 m, KL 18 m
August 2001	8/29/01	KL 6 m, WB, AR
	8/30/01	KL 3 m, KL 9 m, KL 18 m
	8/31/01	BNP
October 2001	10/23/01	BNP
	10/24/01	KL 6 m, WB, AR
	10/25/01	KL 3 m, KL 9 m, KL 18 m
March 2002	3/22/02	BNP
	3/24/02	KL 6 m, WB, AR
	3/25/02	KL 3 m, KL 9 m, KL 18 m
June 2002	6/23/02	KL 6 m, WB, AR, KL 3 m, KL 9 m, KL 18 m
	6/25/02	BNP
August 2002	8/19/02	KL 6 m, WB, AR, KL 3 m, KL 9 m, KL 18 m
	8/21/02	BNP
November 2002	11/1/02	KL 6 m, WB, AR
	11/2/02	BNP
	11/3/02	KL 3 m, KL 9 m, KL 18 m
February 2003	2/8/03	KL 6 m, WB, AR
	2/9/03	KL 3 m, KL 9 m, KL 18 m
	2/10/03	BNP

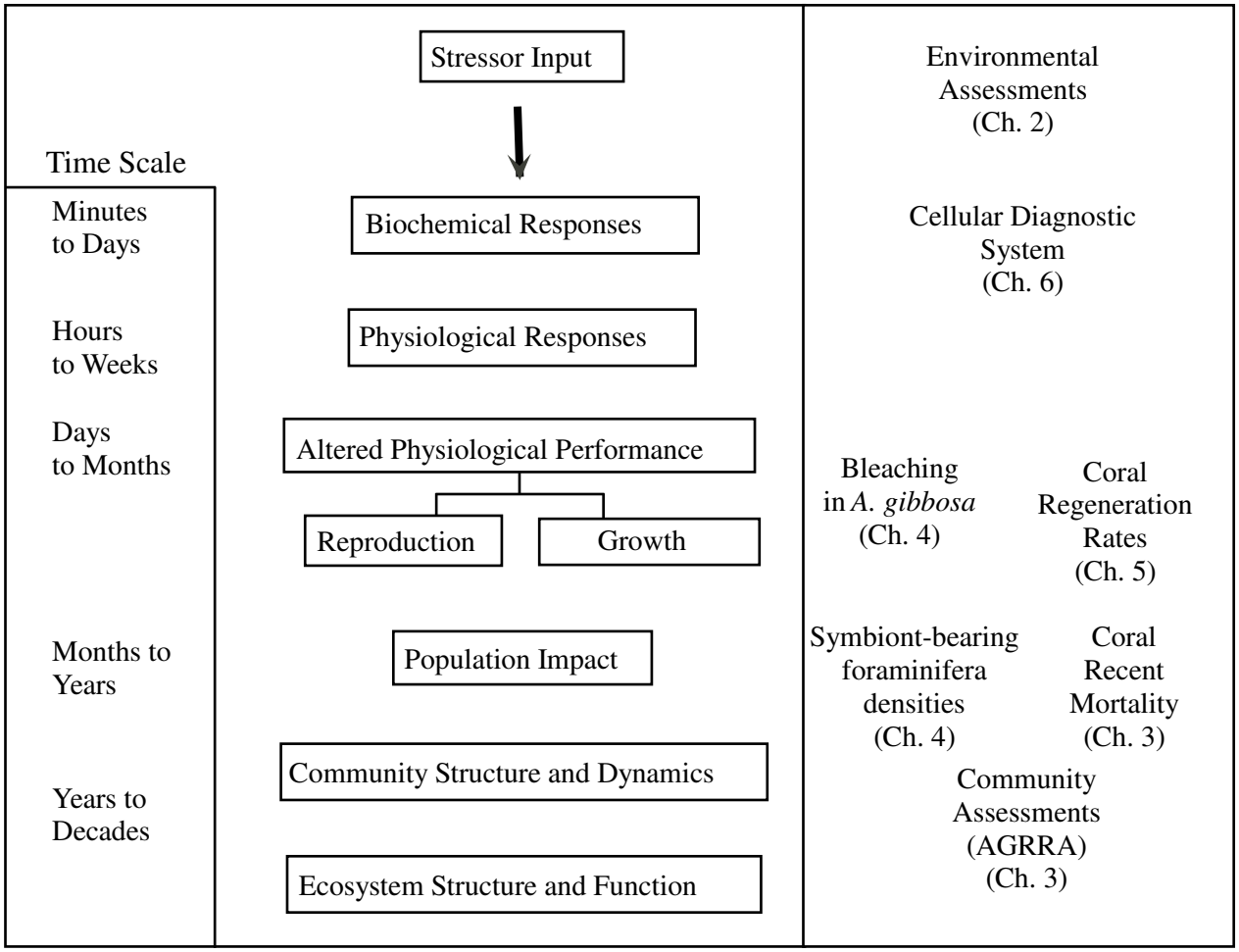


Figure 1.1. Multi-scale approach to study the effects of stress on the marine environment.

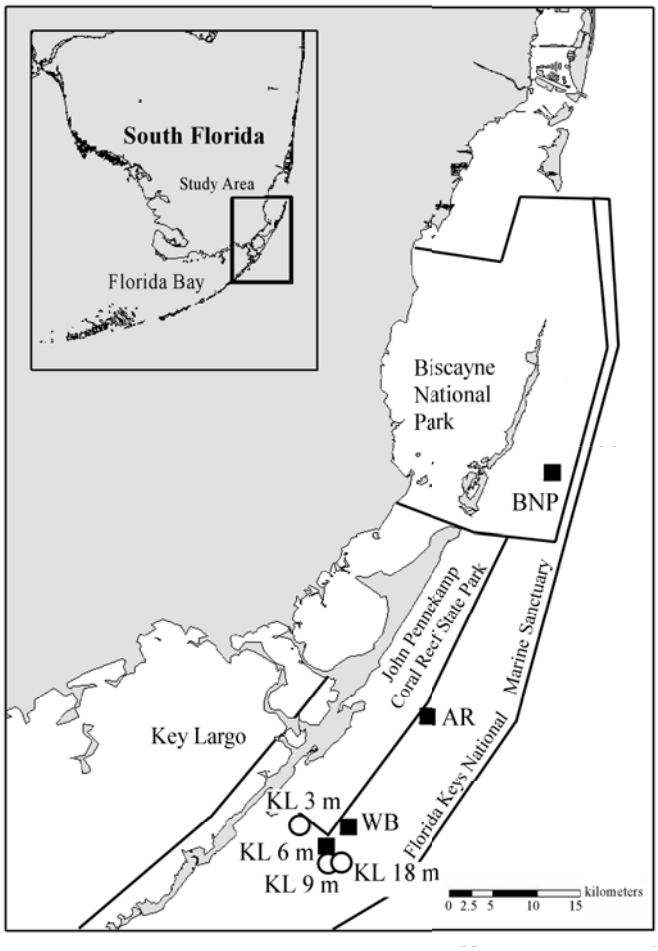


Figure 1.2. Chart of seven sampling sites in the Florida Keys National Marine Sanctuary and Biscayne National Park. ■ - squares designate the 6 m sites, including the four patch reefs Key Largo (KL) 6 m (25° 0192' N, 80° 23.844' W), White Banks (WB) (25° 02.232' N, 80° 22.496' W), Algae Reef (AR) (25° 08.799' N, 80° 17.579' W), and Alina's Reef (BNP) (25° 23.185' N, 80° 09.775' W). ○ - circles designate sites along the depth gradient, including two patch reefs, KL 3 m (25° 02.447' N, 80° 25.442' W) and KL 6 m, and two depths on one forereef, KL 9 m (25° 00.146' N, 80° 23.626' W) and KL 18 m (25°00.206' N, 80° 23.023' W).

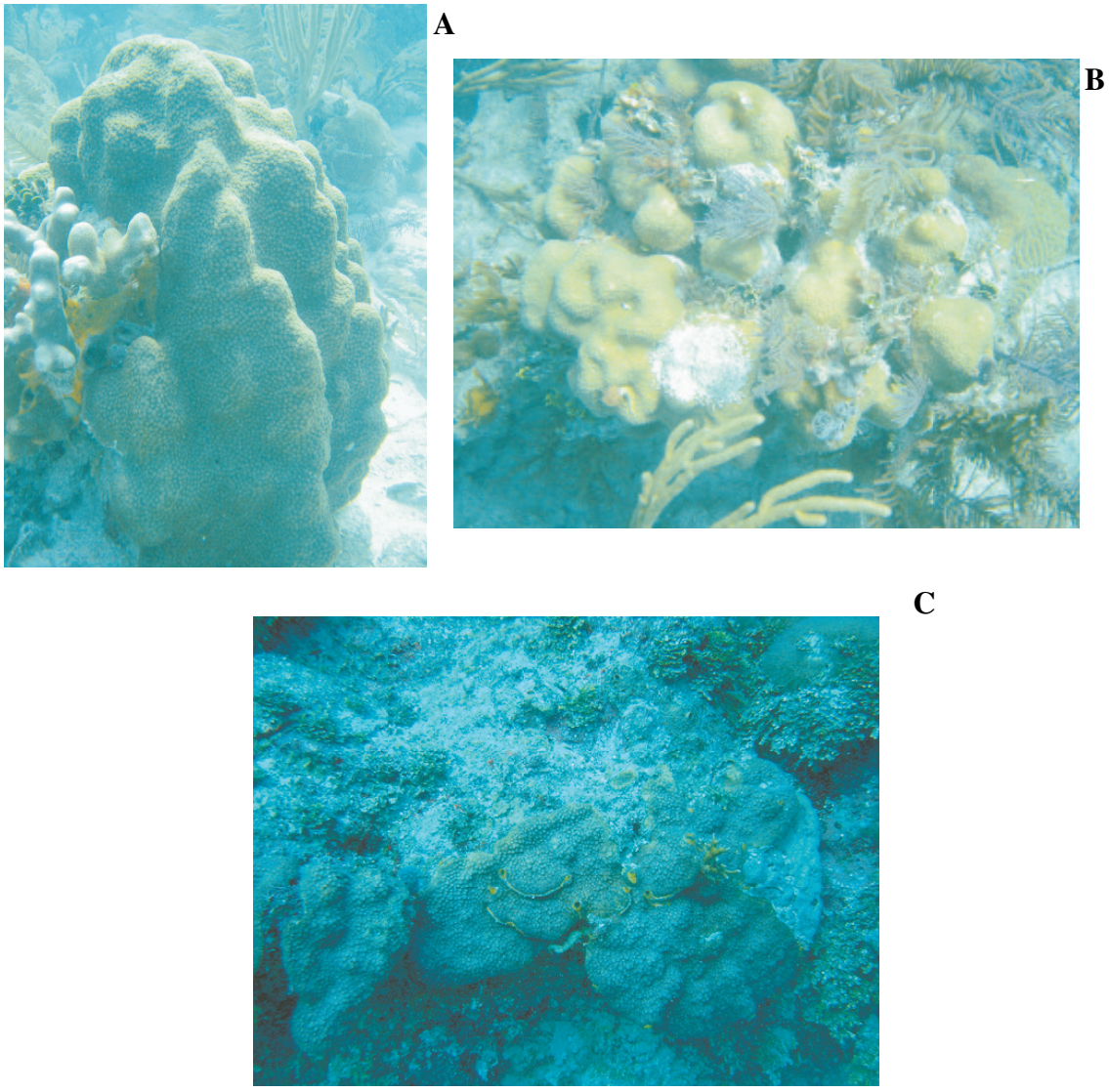


Figure 1.3. Representative colonies of (A) *Montastraea faveolata* (B) *M. annularis* and (C) *M. franksi.* Pictures taken by Roy Price.

2. Environmental Assessments

2.1. Introduction

Florida reefs have undergone severe degradation over the past several decades (Andrews et al. 2005), though reasons for coral loss are not fully understood. Diagnosing reef condition requires knowing reef history and potential stressors to which the reef was exposed (Depledge et al. 1999, Jameson et al. 2001, Downs et al. 2005b). Managers of the Florida Keys National Marine Sanctuary have requested information on the relationship between water quality and the incidence of coral disease and mortality (Bruckner 2002). Effective management also requires identifying indirect and direct stressors on reefs (Bruckner 2002). Coral reef degradation has been linked to changes in the natural coastline and increases in sedimentation, turbidity, temperature, light and nutrients (Waddell 2005). The objective of this chapter is to characterize these major environmental parameters, using data collected at my study sites and datasets available from other sources collected in the vicinity of my sites.

2.1.1. Coastal Wetlands

Coastal wetlands filter runoff, stabilize sediments and absorb nutrients, thereby helping maintain the clear, relatively nutrient-poor coastal waters required for coral reefs (Yentsch et al. 2002). Coastal waters off developed areas of the Florida Keys have an estimated 42% higher nitrogen and 79% higher phosphorus load due to stormwater than undeveloped areas (Kruczynski & McManus 2002). Coastal development increases turbidity through increased erosion and runoff, reduces filtering by wetlands and destabilizes sediments (Kruczynski & McManus 2002). Higher turbidity follows high rainfall during the wet season (May through November), reducing solar energy for photosynthesis by coral zooxanthellae, while lower turbidity occurs during the dry season (December through April), leaving zooxanthellae more susceptible to photo-oxidative stress from solar radiation.

Since 1960, development and construction of canals has resulted in > 15 % (approx. 1278 ha) loss of coastal mangroves in the Upper Keys (Strong & Bancroft 1994). Mangroves and seagrasses are important producers of colored dissolved organic matter (CDOM), which has a variety of benefits to coastal organisms (Coble et al. 2004). Alteration and destruction of watershed and coastal wetlands reduce natural and consistent sources of CDOM that are tidally flushed into reef waters. For example, waters overlying Algae Reef, which lies off a mangrove coastline, have a consistent source of CDOM, whereas waters at sites along highly developed portions of Key Largo, such as KL 6 m, have more variable concentrations of CDOM (Ayoub et al. 2006). CDOM acts as a scavenger of a variety of trace metals and organic pollutants including polyaromatic hydrocarbons (PAHs), removing them from solution and decreasing their toxicity to marine organisms (Coble et al. 2004). CDOM also acts as a sunscreen by rapidly absorbing shorter wavelengths such as ultraviolet (UV) light. Shorter, higher energy wavelengths (including blue, violet, UV-A and UV-B) can result in photo-oxidative stress in zooxanthellae (e.g, Lesser et al. 1990, Lesser 1996), which has been linked to coral bleaching (Downs et al. 2002). Therefore, decreases in natural suncreens, such as CDOM, can result in an increase in bleaching of corals and other reef-dwelling, symbiont-bearing organisms (Williams 2002, Hallock et al. 2006a,b).

2.1.2. Sedimentation and Turbidity

As human populations have increased in the Florida Keys, the combination of dredging and coastal runoff has increased sediment loads and turbidity on reefs. The average underwater visibility dropped from 175 ft. to approximately 35 ft. following the construction of finger-fill canals in the early 1970s (Krucynski & McManus 2002), and since then has increased slightly to between 50 and 80 ft. depending on location (Yentsch et al. 2002).

Sedimentation rates between 1 to 10 mg cm^{-2} d^{-1} and suspended sediment concentrations <10 mg l^{-1} are considered average for Caribbean reefs; values above these are considered potentially stressful to corals (Rogers 1983, 1990). Sedimentation limits coral reef development because sediments may block sunlight needed for photosynthesis,

abrade coral tissues, change nutrient supply, and increase the energy corals must expend to rid themselves of sediment (Woolfe & Larcombe 1999). Nutrient-rich sediments can smother corals by increasing microbial activity and creating anoxic conditions (Weber et al. 2006).

Experimentally increased sedimentation and turbidity significantly increased coral respiration rates (Telesnicki & Goldberg 1995, Abdel-Salem & Porter 1998), negatively affected growth and calcification rates (Dodge et al. 1974, Bak 1978, Kendall et al. 1983, Tomascik & Sander 1985, Hubbard 1986), reproduction (Kojis & Quinn 1984), and recruitment (Wittenberg & Hunte 1992, Richmond 1997). Meesters et al. (1992) found that the regeneration rate of boulder coral, *Montastraea annularis,* was lower in areas of higher sedimentation. Laboratory experiments with *M. annularis* have shown variable results, with some suggesting it is an inefficient sediment rejecter (7.5 - 15 mg h^{-1}) (Bak and Elgershuizen 1976) and others showing that these corals have relatively high clearing rates (28 - 66 mg cm^{-2} h^{-1}; Abdel-Salem and Porter 1988).

Sediment properties, including grain-size and organic and nutrient content, play key roles in determining sedimentation stress in corals and their ability to remove sediments (Weber et al. 2006). Silt-sized (< 63 μm), organic-rich sediments can stress corals after a short-term exposure, whereas sandy, organic-poor sediments have little effect (Weber et al. 2006).

Although prior research classified sedimentation and turbidity as major stressors to corals, in the Florida Keys many inshore patch reefs experience relatively high sedimentation and turbidity (Boyer & Briceño 2005) and still have higher coral cover and diversity than offshore reefs (Beaver et al. 2005). Organic matter associated with sediment may provide a food source for corals (Rosenfeld et al. 1999, Anthony & Fabricius 2000, Anthony 2006) but assimilation abilities and effects on physiology vary with coral species (Anthony 1999, Mills & Sebens 2004, Anthony 2006). High turbidity generally limits reef growth to <10 m depth (Yentsch et al. 2002, Fabricius 2005).

2.1.3. Temperature and Light

Coral bleaching is considered one of the main causes of degradation of coral reefs worldwide, resulting in 'destruction' of an estimated 16% of the world's reefs (Hughes et al. 2003, Wilkinson 2004, Marshall & Schuttenberg 2006). Bleaching is a process whereby corals and other invertebrates that host symbiotic algae (e.g., some foraminifers and bivalves) lose or experience degradation of their algal symbionts due stressors such as disease, sedimentation, pollutants, and changes in salinity, temperature or light (Brown 1997a).

In situ observations in the Caribbean, Indian Ocean and South Pacific coral reefs suggest a correlation between coral bleaching and high sea-surface temperatures (SST) (Goreau et al. 1992, Goreau & Hayes 1994, Brown et al. 1996, Wilkinson 1998, Hoegh-Guldberg 1999, Fitt et al. 2001). Large-scale bleaching events appear to primarily be caused by heat stress (Brown 1997b, Hoegh-Guldberg 1999, Marshall & Schuttenberg 2006), which results in photo-oxidative stress in the organism (Lesser et al. 1990, Downs et al. 2002). Bleaching stress can leave corals vulnerable to other stressors, including disease. For example, in the US Virgin Islands, corals that were severely bleached and later regained pigmentation died the following spring from infection by White Plague disease, resulting in a 26 – 48% loss of coral cover (Miller et al. 2006). Increased sea-surface temperatures, combined with other means of coral degradation (e.g., sedimentation, pollution, photic stress, disease, predation, etc.) threaten the health and vitality of coral reef ecosystems worldwide (Dustan 1999, Marshall & Schuttenberg 2006).

Bleaching of corals (Lesser et al. 1990, Gleason & Wellington 1993, Glynn 1993, Jones et al. 1998, Lesser & Farrell 2004) and other symbiont-bearing marine organisms (Jokiel 1980, Williams & Hallock 2004, Hallock et al. 2006a, b) also has been attributed to high levels of solar radiation, particularly shorter, higher energy wavelengths (blue to UV, 290 – 490 nm). Bleaching events usually coincide with periods of calm winds, resulting in increased penetration of solar radiation (Gleason & Wellington 1993, Glynn 1996, Wilkinson 1998). Stratospheric ozone depletion of 10 – 15% in mid-latitudes, caused by anthropogenic inputs of chlorofluorocarbons (Shick et al. 1996) increased

harmful UVB (290-320 nm) reaching the Florida Reef by roughly 20 – 30% in spring and early summer months (Shick et al. 1996, Moran & Sheldon 2000, Hallock et al. 2006b). Negative impacts of UVB on reef organisms range from death (Jokiel 1980), to depressed reproduction (Gleason & Wellington 1993), decreased calcification and growth (Roth et al. 1982), and increased susceptibility to other stressors (Drohan et al. 2005, Hallock et al. 2006b).

The first two widespread coral bleaching events, in 1982-83 and 1987-88, coincided with ENSO events but also followed accelerated ozone depletion associated with major volcanic eruptions (Hallock et al. 1993). Global ozone depletion of approximately 4%, which occurred following the Mt. Pinatubo eruption in May-June 1991, did not trigger mass coral bleaching (Shick et al. 1996), probably because the massive eruption also caused temporary global cooling. However, widespread bleaching in reef-dwelling foraminifers began shortly after the eruption; these foraminifers are more sensitive to photic stress than temperature stress (Talge & Hallock 2003, Williams & Hallock 2004).

2.1.4. Nutrients

Nutrification can be defined as an increase in nutrient input to an environment that results in a detectable change in community structure (Hallock 2001, Fabricius 2005). A coral-dominated reef community can shift to a mixed coral-algal dominated communities following limited increase in nutrient input and to domination by non-symbiotic filter-feeders and bioeroders following a substantial nutrient increase (Hallock 1988). How much nutrient input can induce community change is difficult to quantify because so many environmental variables are involved, including how nutrient depleted offshore waters are, rates of exchange with nutrient-depleted offshore waters, and respiration rates which are a function of temperature (Hallock 1988, Hallock et al. 1991, 1993.

Mangrove and seagrass detritus provide natural sources of nutrients to coastal reefs. Presently, numerous point sources of nutrients exist throughout the Florida Keys (e.g., septic discharges, water-treatment plant discharges), as well as non-point sources (e.g., Everglades, storm-water and agricultural run-off, groundwater contamination).

Existing wastewater facilities in Monroe County include about 23,000 private onsite systems (66% permitted septic tanks, 31% unknown systems and 3% aerobic treatment units) and 246 small wastewater treatment plants (WWTPs) (Monroe County 2000). About 2800 of the 7200 unknown systems are suspected illegal cesspools (Monroe County 2000). These systems provide minimal treatment and nutrient removal and, together with other non-point sources, impact coastal waters in the Florida Keys.

Due to the extremely porous limestone structure of the Keys, wastewater from onsite systems can be detected in adjacent canals within hours and along coastal surface waters within days (Paul et al. 2000). Twelve hot spots in Key Largo were designated by the USEPA (1993) as having known or suspected degraded water quality as a result of poorly designed canals, use of septic tanks or cesspits, and untreated runoff (Krucyznski & McManus 2002). Loading from the canal drainage system and inshore groundwater led to elevated concentrations of nitrate and other nutrients (e.g., 1 μg/l chl a; 1 μM NO_3) in Biscayne Bay and the Upper Florida Keys (Szmant & Forrester 1996, Boyer & Jones 2002).

Nevertheless, nutrient concentrations measured in offshore waters in the Upper Keys tend to be relatively low (e.g., chl a $\leq$ 0.25 μg/l; $NO_3 \leq 0.25$ μM; $NH_4 \leq 0.10$ μM; Szmant & Forrester 1996, Boyer & Jones 2002). Nutrients and contaminants also can be transported offshore (approximately 8 km) by surface-water and groundwater movement (Shinn et al. 1994, Reich et al. 2002). However, measuring dissolved inorganic nutrient concentrations in the water to determine possible nutrification can be misleading because nutrients are rapidly incorporated into reef and plankton biomass (Laws & Redalje 1979). Therefore, more sensitive and quantifiable biological indicators are needed to quantify the affects of nutrients on reefs. Koop et al. (2001) found that increased nutrients caused increased mortality and reduced reproduction in corals, but that nutrients alone did not result in a shift from a coral-dominated to algal-dominated community. The Florida Keys reefs are unusual for their very low coral cover and high algal abundance despite having abundant herbivorous fish (Szmant 2001). It is possible that nutrients and other stresses increase coral mortality and open substrate for algae to colonize (Koop et al. 2001, Szmant 2001).

2.2. Methods

2.2.1. Site Descriptions

An ARC-GIS map was produced to show the location of my study sites, Southeast Research Center's Water Quality Monitoring Network (SERC-WQMN) sites, and area coverage of mangrove forest and wetlands (Fig. 2.1). Wetland data were obtained from the National Wetlands Inventory (http://wetlands.fws.er.usgs.gov/NWI/Index.html).

2.2.2. Sedimentation/Turbidity

I used sediment traps to quantify sedimentation at my study sites. Each trap was 5.1 cm in diameter and 0.61 m long, constructed from PVC pipe capped at the bottom, and secured to rebar with stainless steel hose clamps. The rebar was secured into the bottom substrate. In early March 2001, three traps were placed at Algae Reef (AR) and White Banks (WB) sites (Fig. 2.1) next to the corals to be sampled. During sampling in late March, three traps were emplaced at the KL 6 m and BNP sites. In August 2001, three traps were deployed at the KL 3 m site. In March 2002, two more traps were added at each 6 m site, and one trap was placed at each of the 9 m and 18 m sites (Table 2.1). During each sampling, the pipe was capped at the top, swapped with a new trap, and brought to the surface (Table 2.1). Then at the onshore lab, sediment was filtered with a 2.00 mm sieve to remove seagrass and coarse sediment, washed with distilled water to remove salts, dried at 70° C, and weighed. Data were converted to sedimentation rates (mg cm^{-2} d^{-1}) by dividing dry sample weight by trap area and the number of days the trap was deployed.

Beginning in October 2001, during each sampling five water samples were taken by SCUBA divers in the proximity of the corals for turbidity analysis using a portable turbidimeter (Orbeco-Hellige model 966, Farmingdale, NY). Data were recorded as nephelometric turbidity units (NTU).

2.2.3. Temperature

Temperature was measured at hourly intervals throughout the 2-year study using temperature sensors (HOBO Data Logger H08-001-02 series; accuracy ± 0.7 °C) placed

at each coral colony sampled at each site. Due to failures of some sensors, sea-surface temperature (SST) data also were obtained from external databases such as NOAA SEAKEYS C-MAN (http://www.coral.noaa.gov/cman/) at Molasses Reef (for 2001 - 2003.

2.2.4. Nutrients

Water quality data (Table 2.2) for the Florida Reef tract have been collected by the Southeast Research Center's Water Quality Monitoring Network (SERC-WQMN) (http://serc.fiu.edu/wqmnetwork/FKNMS-CD/index.htm) quarterly since 1995. I matched my study sites to WQMN sites (Fig. 2.1, Table 2.3) using methods similar to those described by Callahan (2005), using an ARC view query tool and based on the following criteria: 1) proximity to study sites, 2) depth similarity, 3) distance to shore, and 4) benthic cover similarity under the WQNP station. Due to proximity, KL 6 m and WB were assigned the same water quality station. I compared surface samples for all water quality parameters (Table 2.1) during the time frame of my study.

2.2.5. Additional Environmental Data

Miami rainfall data were obtained from Florida State University's Florida Climate Center (http://www.coaps.fsu.edu/climate_center/). For comparison with quarterly data, I summed total precipitation (cm) for the two months prior to each sampling date. Maximum wind speeds were obtained from the NOAA SEAKEYS C-MAN at Molasses Reef for 2001 - 2003. Data on intensity of ultra-violet (UV) radiation were obtained from UV sensors (Brewster UV radiometers) (www.epa.gov/uvnet/) at Everglades National Park. Daily dose is defined as total energy from sunlight at 287 to 363 nm reaching one square meter of ground surface over an entire day. Ozone data were obtained from NASA's Total Ozone Mapping Spectrometer (http://toms.gsfc.nasa.gov/ozone/ozone.html).

2.2.6. Data Analysis

I performed repeated-measures MANOVA to determine if sites differed significantly in sedimentation or turbidity. To interpret effects detected by MANOVA, I used one-way ANOVA followed by Tukey-Kramer HSD method. Statistical analyses were performed using JMP statistical software (SAS Institute, Inc., Cary, NC, USA).

2.3. Results

2.3.1. Coastal Wetlands

Algae Reef, which was offshore from John Pennekamp Coral Reef State Park, was along the coastline with the highest density of intact mangrove forest and wetlands (Fig. 2.1). Sites along the KL depth gradient (KL 3, 6, 9 and 18 m) were off a developed portion of Key Largo with low densities of mangrove forest and wetlands (Fig. 2.1). Some mangrove forest occurs along the barrier islands near BNP but these forests and associated wetlands are not as extensive as those found in John Pennekamp Coral Reef State Park (Fig. 2.1).

2.3.2. Sedimentation

Sedimentation rates ranged from 2 to 187 mg $cm^{-2}d^{-1}$ with a mean (± SE) of 35 (± 3) mg cm^{-2} d^{-1} (n = 128) at the 6 m sites (Fig. 2.2). Sedimentation varied significantly among 6 m sites (repeated measures MANOVA: site effect $F_{3,8} = 37.9$, $p < 0.001$), with time ($F_{7,56} = 125.6$, $p < 0.001$), and time x site interactions ($F_{21,56} = 4.0$, $p < 0.001$; Fig. 2.2). Mean sedimentation rate was highest at AR relative to the other 6 m sites throughout the year (67 ± 9 vs. 24 ± 2 mg cm^{-2} d^{-1}; Fig. 2.2). Throughout the entire study period, AR had significantly higher mean sedimentation rate than BNP, KL 6 m (except between June – August 2001) and WB (except between June 2001 and October 2001) (Tukey HSD). White Banks also had significantly higher mean sedimentation rates throughout the study period than BNP (except between March and June 2001, August and October 2001 and June and August 2002) and KL 6 m (except between June and October 2001) (Tukey HSD). BNP had significantly higher mean sedimentation

rates than KL 6 m between March and June 2001 and June and August 2002 (Tukey HSD).

2.3.3. Turbidity

Turbidity ranged from 0.13 to 1.4 NTU with a mean (± SE) of 0.65 (± 0.03) NTU (n = 71) for the 6 m sites and from 0.26 to 1.36 with a mean of 0.65 (± 0.03) NTU (n = 71) along the depth gradient. No correlation was found between turbidity and sedimentation rates.

Turbidity differed significantly among the 6 m sites (repeated measures MANOVA: site effect $F_{3,7} = 10.0$, $p < 0.007$), with time ($F_{5,35} = 27.7$, $p < 0.0001$), and the time x site interactions ($F_{15,35} = 11.5$, $p < 0.0001$; Fig. 2.3A). Turbidity was highly variable at BNP, where turbidity was significantly lower than all other 6 m sites in October 2001 (BNP: 0.17 ± 0.02 vs. KL 6 m: 0.58 ± 0.02, WB: 0.55 ± 0.02, AR: 0.62 ± 0.06 NTU; ANOVA: $F_{3,8} = 32.0$, $p < 0.0001$) and significantly higher than all other 6 m sites in August 2002 (BNP: 1.17 ± 0.19 vs. KL 6 m: 0.49 ± 0.01, WB: 0.55 ± 0.02, AR: 0.50 ± 0.01 NTU; ANOVA: $F_{3,8} = 12.3$, $p < 0.003$). Turbidity was significantly lower at both BNP and KL 6 m than AR and WB in March 2002 (BNP: 0.62 ± 0.01, KL 6 m: 0.69 ± 0.04 NTU vs. WB: 1.08 ± 0.03, AR: 1.04 ± 0.05 NTU; ANOVA: $F_{3,8} = 48.6$, $p < 0.0001$). In June 2002, turbidity at KL 6 m was significantly lower than all other 6 m sites and significantly higher at BNP than AR (KL 6 m: 0.57 ± 0.03 NTU vs. WB: 0.98 ± 0.04, AR: 0.83 ± 0.07, BNP: 1.03 ± 0.02 NTU; ANOVA: $F_{3,8} = 22.9$, $p = 0.0003$). Turbidity at BNP also was significantly higher than turbidity at KL 6 m in February 2003 (BNP: 0.57 ± 0.03 vs. KL 6 m: 0.37 ± 0.03 NTU; ANOVA: $F_{3,8} < 4.7$, $p = 0.04$).

Turbidity also significantly differed among sites along the depth gradient (repeated measures MANOVA: site effect $F_{3,7} = 91.8$, $p < 0.001$), with time ($F_{5,3} = 37.0$, $p < 0.007$), and the time x site interactions ($F_{15,8.7} = 11.2$, $p < 0.007$; Fig. 2.3B). Turbidity was significantly higher at KL 3 m and KL 6 m than at KL 9 m and KL 18 m in October 2001 (KL 3 m: 0.60 ± 0.02, KL 6 m: 0.58 ± 0.02 NTU vs. KL 9 m: 0.46 ± 0.03 and KL 18 m: 0.46 ± 0.03 NTU; ANOVA: $F_{3,8} = 9.3$, $p < 0.006$). Turbidity was significantly higher at KL 3 m than at all other sites along the depth gradient in June 2002

(KL 3 m: 1.08 ± 0.01 vs. KL 6 m: 0.57 ± 0.03, KL 9 m: 0.59 ± 0.06, KL 18 m: 0.56 ± 0.01 NTU; ANOVA: $F_{3,8} = 57.7$, $p < 0.0001$), August 2002 (KL 3 m: 0.88 ± 0.11 vs. KL 6 m: 0.49 ± 0.01, KL 9 m: 0.44 ± 0.03, KL 18 m: 0.48 ± 0.03 NTU; ANOVA: $F_{3,8} = 12.7$, $p < 0.003$), November 2002 (KL 3 m: 1.33 ± 0.02 vs. KL 6 m: 0.51 ± 0.08, KL 9 m: 0.38 ± 0.04, KL 18 m: 0.95 ± 0.08 NTU; ANOVA: $F_{3,8} = 62.8$, $p < 0.001$) and February 2003 (KL 3 m: 1.07 ± 0.03 vs. KL 6 m: 0.37 ± 0.03, KL 9 m: 0.30 ± 0.01, KL 18 m: 0.29 ± 0.02 NTU; ANOVA: $F_{3,8} = 357.2$, $p < 0.001$). Turbidity also was significantly higher at KL 18 m than at KL 6 m and 9 m in November 2002.

2.3.4. Temperature

Temperature ranged from 15 – 32º C with a mean of 27º C at the 6 m sites. Temperature differences among the 6 m sites during my study period were within the precision of the instrument; ± 0.7 º C (Fig. 2.4A). Mean temperature also was 27º C along the depth gradient. The highest (33º C) and lowest (15º C) temperatures were observed at KL 3 m in August 2001 and January 2003, respectively (Fig. 2.4B).

2.3.5. Nutrients

While nutrient concentrations were generally low, elevated concentrations of dissolved inorganic nitrogen (Fig. 2.5A-C, 2.6B) and organic phosphorus (Fig. 2.7A) at SERC stations in proximity to AR and BNP followed heavy rainfall in October 2001 (Fig. 2.8). These increases were associated with decreases in salinity (Fig. 2.9A), and increases in turbidity (Fig. 2.9B) and chlorophyll a (Fig. 2.7D). Elevated turbidity (Fig. 2.9B and Fig. 2.10B) and organic nitrogen (Fig. 2.6C and Fig. 2.11C) concentrations at SERC stations in proximity to the KL depth gradient and WB were observed in February 2002 and April 2002, respectively, which corresponds with high winds (Fig. 2.12) that may have caused sediment resuspension (Fig. 2.2A, B). Organic nitrogen concentrations and dissolved oxygen were highest at the offshore site in proximity to KL 9 and 18 m (Fig. 2.11C). High TN:TP (Fig. 2.13A) and DIN:TP (Fig. 2.13B) suggest that phosphorus limited algal growth in that area during that time. Nitrite concentrations were elevated near BNP site in August 2002, following heavy summer rainfall in Miami (Fig.

2.8) but other nutrient concentrations remained low. Elevated inorganic phosphorus concentrations were observed at AR (Fig. 2.7A, C) and along the KL depth gradient (Fig. 2.14A, C) in late October 2002 but did not correspond with stormy weather. Nitrate also was elevated along the KL depth gradient at that time (Fig. 2.15A).

2.3.6. Additional Environmental Data

Rainfall was higher in 2001 than in 2002, with the highest rainfall in September 2001 (46 cm) and lowest in February 2001 (0.13 cm) (Fig. 2.8). Rainfall in 2002 was highest in July 2002 (39 cm) and lowest in January 2002 (0.56 cm) (Fig. 2.8). Mean and maximum wind speeds were highest in November 2001 (15 and 38 knots, respectively; Fig. 2.13) over the two year study period.

The ratio of ozone to daily dose UV was higher between 2001 and 2003 compared to 1998 (Fig. 2.16A), when coral bleaching was high. Additionally, daily dose of UV (J m^{-2} d^{-1}) between 2001 and 2003 was low in comparison to 1998 (Fig. 2.16A). UV-A irradiance was highest in 1998 and relatively low in 2001 (Fig. 2.16B). High UV-B irradiance was observed in 1999 and 2002 (Fig. 2.16C) but overall was temporally variable.

2.4. Discussion

Sedimentation was consistently highest at AR throughout the entire study period and was consistently above "threshold" stress levels (>10 mg cm^{-2} d^{-1}; Rogers 1983) at AR and WB. However, sediment collected in my traps was a combination of settled suspended sediment and resuspended sediments, and sedimentation stress varies with sediment characteristics (Weber et al. 2006). Sediments at AR and WB were dominated by coarse carbonate sands (particularly *Halimeda*), whereas KL 6 m and BNP were dominated by smaller grain sizes (i.e., muds). Sedimentation rates at KL 6 m and BNP also frequently exceeded "threshold" levels in 2001. Along the depth gradient, sedimentation rates were generally low and dominated by silty sediments. Silt-sized and organic-rich sediments are more stressful to corals than sandy sediments or organic-poor

silts (Weber et al. 2006), therefore sediments at KL 6 m and BNP are likely more detrimental to coral physiology than those at AR and WB.

Higher sedimentation rates followed peaks in rainfall and maximum wind speeds in 2001. These peaked in early fall as a result of Hurricane Gabrielle that made landfall on September 14, 2001 as a tropical storm near Venice, FL, and then passed over the peninsula exiting near Titusville, FL. The highest sedimentation rates were observed in winter months between October 2001 and March 2002, when precipitation was low, and were likely the consequence of resuspension of sediments during winter storms. There was no correlation between sedimentation rates and turbidity, which may be a result of different sampling frequencies or differences in sediment type among sites. In 2002, sedimentation rates were again lowest in summer and early fall (June through November), but in general sedimentation rates were considerably lower in 2002 than in 2001 and showed no relationship to mean precipitation. No hurricane or tropical storm activity affected South Florida in 2002; the combination of less rain and lower winds in 2002 may explain lower sedimentation rates that year.

Turbidity was highly variable at BNP, with the highest turbidity in the summer and lowest in winter. Turbidity was consistently highest at KL 3 m, where sediments were muddy. High turbidity can block sunlight essential for photosynthesis in symbiont-bearing organisms such as corals (Yentsch et al. 2002). On the other hand, corals and symbiont-bearing organisms on reefs with low turbidity (e.g., KL 6 m) may be more susceptible to photic stress (Williams 2002, Lesser & Farell 2004). Turbidity at KL 6 m was the most consistent and relatively low throughout the year.

Temperature did not vary among the 6 m sites but small differences were seen along the depth gradient, with the shallow inshore patch reef (KL 3 m) showing the largest range in temperature. Due to the high ratio of ozone to daily UV between 2001 and 2003 and infrequent high temperatures (>30° C), the probability of coral bleaching as a consequence of high levels of oxidative response (due to temperature and light) were low during my study. No bleaching was observed in 2001-2002, unlike 1998 when bleaching was observed Caribbean-wide including Florida (Beaver et al. 2005), and in 1999 when bleaching was observed at KL 9 m and 18 m (Fauth et al. 2003).

In the Upper Florida Keys, elevated nitrogen and chlorophyll concentrations occur near marinas and canals (e.g., 1 μg/l chl a; 1 μM NO_3) but generally decrease to oligotrophic concentrations (e.g., chl a ≤ 0.25 μg/l; NO_3 ≤ 0.25 μM; NH_4 ≤ 0.10 μM) within 0.5 km of the coast (Szmant & Forrester 1996). However, following heavy rainfall and high wind events, elevated levels of total dissolved phosphorus (0.30 μM; within days) and elevated concentrations of ammonia and chlorophyll a (4.0 μM and 0.45 μg/l, respectively; within 1 - 3 wks) can reach offshore reef sites (Lapointe & Matzie 1996). High rainfall in September 2001 was followed by decreased salinity and increased turbidity, inorganic nitrogen (NO_3, NO_2, NH_4), organic phosphorus (TP, APA) and cholorophyll a in October 2001 near my study sites. Elevated chlorophyll a indicates increased phytoplankton abundance and biomass. Phytoplankton growth in the FL Keys is typically limited by phosphorus (Boyer & Briceño 2005). Alkaline phosphatase enables phytoplankton to use organic phosphate for growth when dissolved inorganic phosphate concentrations are low (Dyhrman & Ruttenberg 2006) and inorganic nitrogen is available.

In 2002, rainfall was highest in the early summer (June) but it is difficult to determine if it had an affect on nutrient concentrations due to the low sampling frequency. Higher nutrient concentrations did not always correspond with increased rainfall. The predominant form of nutrients in the Florida Keys water column is organic (Szmant & Forrester 1994, Boyer & Briceño 2005). Elevated organic nitrogen in proximity to the Key Largo depth transect in June 2002 may result from high wind speeds during that time and sediment resuspension, as previously observed during winter storms in the Florida Keys (Szmant & Forrester 1996) and along the Great Barrier Reef (Ullman & Sandstorm 1987). Periodic upwelling along the shelf edge is another source of elevated phosphorus (≥ 0.2 μM PO_4) to offshore reefs (Szmant & Forrester 1996) and may account for elevated concentrations at AR and along the KL depth gradient in October 2002. In this case, inorganic nitrogen may actually be limiting to phytoplankton growth as shown by low ratios of nitrogen to phosphorus.

In June 2002, the State of Florida created the Key Largo Wastewater Treatment District with the goal to eliminate septic tanks and illegal cesspits and decrease nutrient

loads to coastal waters. By 2010, designated Priority Hot Spots including coastal areas between KL 6 m and AR in South Key Largo will receive community wastewater collection with advanced wastewater treatment (Monroe County 2000), which will potentially reduce the nutrient load and associated contaminants to these reefs. I recommend continued monitoring at these study sites to determine if advances in water quality result in improved reef condition.

2.5. Conclusions

Overall, large differences were observed among 6 m sites in sedimentation rates and turbidity but not in temperature. Sites along highly developed coastlines (KL 3 m, 6 m and BNP) were dominated by silt-sized sediments, which are potentially more stressful to corals. Algae Reef, offshore a mangrove lined coastline, had the highest sedimentation rates, which were sand-dominated and likely resulted from resuspension. Precipitation and wind speeds were generally higher in 2001 than 2002, resulting in decreased salinity and higher sedimentation rates, turbidity and nutrient loads near most study sites in the fall and winter 2001. Environmental conditions potentially were stressful during that time. No extreme temperatures or irradiance levels and no coral bleaching were observed during my study period. Relationships among the environmental data discussed above and other indicators are examined in Chapter 6.

Table 2.1. Deployment and collection of sediment traps in 2001 and 2002; NA indicates no traps added; NC indicates no traps collected and swapped; A indicates number of traps added; C indicates number of traps collected and swapped.

	March 2001	June 2001	August 2001	October 2001	March 2002	June 2002	August 2002	November 2002
KL 3m	NA/NC	NA/NC	8/30/01 (3A)	10/25/01 (3C)	3/25/02 (2A & 3C)	6/24/02 (5C)	8/19/02 (5C)	11/1/02 (5C)
KL 6m	3/31/02 (3A)	6/27/01 (3C)	8/29/01 (3C)	10/24/01 (3C)	3/24/02 (2A & 3C)	6/23/02 (5C)	8/20/02 (5C)	11/2/02 (5C)
KL 9m	NA/NC	NA/NC	NA/NC	NA/NC	3/25/02 (1A)	6/23/02 (1C)	8/19/02 (1C)	11/1/02 (1C)
KL 18m	NA/NC	NA/NC	NA/NC	NA/NC	3/25/02 (1A)	NA/NC	8/19/02 (1C)	11/1/02 (1C)
WB	3/14/01 (3 A) 3/31/01 (3C)	6/27/01 (3C)	8/29/01 (3C)	10/24/01 (3C)	3/24/02 (2A & 3C)	6/24/02 (5C)	8/20/02 (5C)	11/2/02 (5C)
AR	3/14/01 (3 A) 3/31/01 (3C)	6/27/01 (3C)	8/29/01 (3C)	10/24/01 (3C)	3/24/02 (2A & 3C)	6/24/02 (5C)	8/20/02 (5C)	11/2/02 (5C)
BNP	3/30/01 (3A)	6/26/01 (3C)	8/31/01 (3C)	10/23/01 (3C)	3/22/02 (2A & 3C)	6/25/02 (5C)	8/21/02 (5C)	11/3/02 (5C)

Table 2.2. SERC Water Quality Monitoring Network sampling list including summary statistics for all surface water quality variables for all FKNMS outer reef stations between 1995 and 2005 (Boyer & Jones 2002, Boyer & Briceño 2005).

Water Quality Parameters	**Median**	**Min**	**Max**
Salinity (practical scale salinity)	36.2	26.7	37.8
Temperature (°C)	26.9	16.3	32.2
Dissolved oxygen (DO, mg/l)	5.9	0.1	13.5
Turbidity (NTU)	0.33	0.00	10.14
Nitrate (NO_3^-, µM)	0.06	0.00	2.30
Nitrite (NO_2^-, µM)	0.03	0.00	0.71
Ammonium (NH_4^+, µM)	0.24	0.00	2.73
Total organic nitrogen (TON, µM)	8.95	0.00	67.72
Total nitrogen (TN, µM)	9.42	1.00	67.85
Soluble reactive phosphate (SRP, µM)	0.02	0.00	0.23
Total organic carbon (TOC, µM)	144.2	18.4	1054.8
Total phosphorus (TP, µM)	0.17	0.00	1.22
Chlorophyll a (CHL a, µg/L)	0.21	0.00	3.12
Alkaline phosphatase activity (APA, µM/h)	0.04	0.01	0.79

Table 2.3. Study sites and associated Southeast Research Center (SERC) water quality stations. SERC sampling dates for KL depth gradient and WB are the same. SERC sampling dates at KL 6 m, KL 9 m, KL 18 m and WB are the same as KL 3 m.

Study Site	SERC Station	SERC Station #	Distance from Study Site (m)	Depth (m)		Distance from Shore (m)		SERC Sampling Dates
				Study site	SERC	Study site	SERC	
KL 3 m	Mosquito Bay	223	2100	3	3.5	4200	2800	1/26/2001, 6/19/2001, 7/27/2001, 11/16/2001, 2/6/2002, 4/25/2002, 8/29/2002, 10/23/2002, 2/5/2003
KL 6 m	Molasses Reef Channel	224	1400	6	7.5	8000	7100	
KL 9 m	Molasses Reef	225	2400	9	36	9700	10700	
KL 18 m	Molasses Reef	225	1400	18	36	10100	10700	
WB	Molasses Reef Channel	224	2200	6	7.5	8000	7100	
AR	White Banks	218	1400	6	3.5	6200	5800	1/25/2001, 6/14/2001, 7/20/2001, 11/7/2001, 2/6/2002, 4/24/2002, 8/28/2002, 10/23/2002, 2/3/2003
BNP	Ajax Reef	206	6000	6	8	6000	3800	1/25/2001, 6/13/2001, 7/20/2001, 11/7/2001, 2/5/2002, 4/23/2002, 8/27/2002, 10/22/2002, 1/31/2003

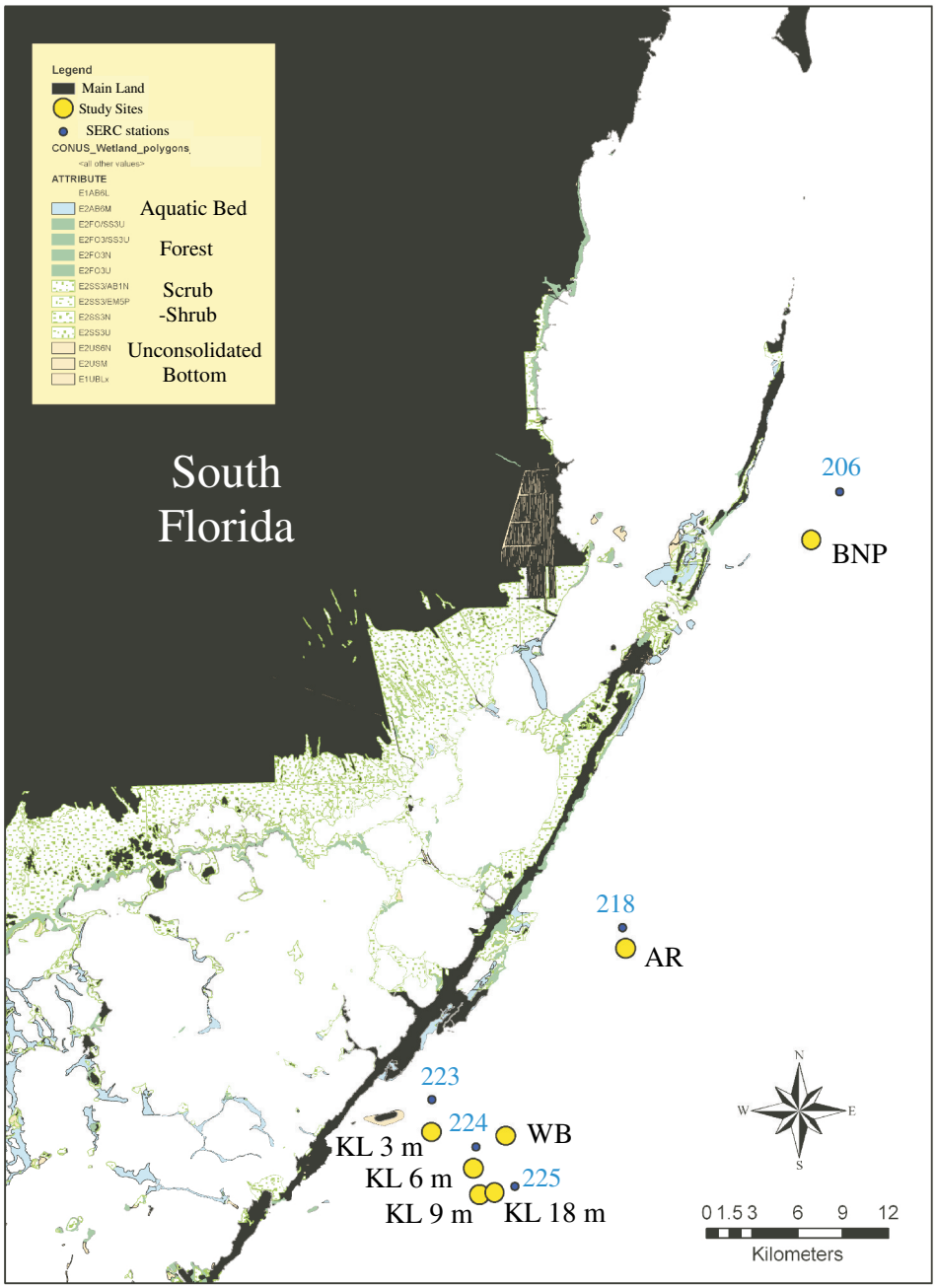


Figure 2.1. Map showing location of study sites (large yellow circles), Southeast Research Center Water Quality monitoring stations (small blue circles and blue station numbers) and area coverage of mangrove and wetlands in the study region.

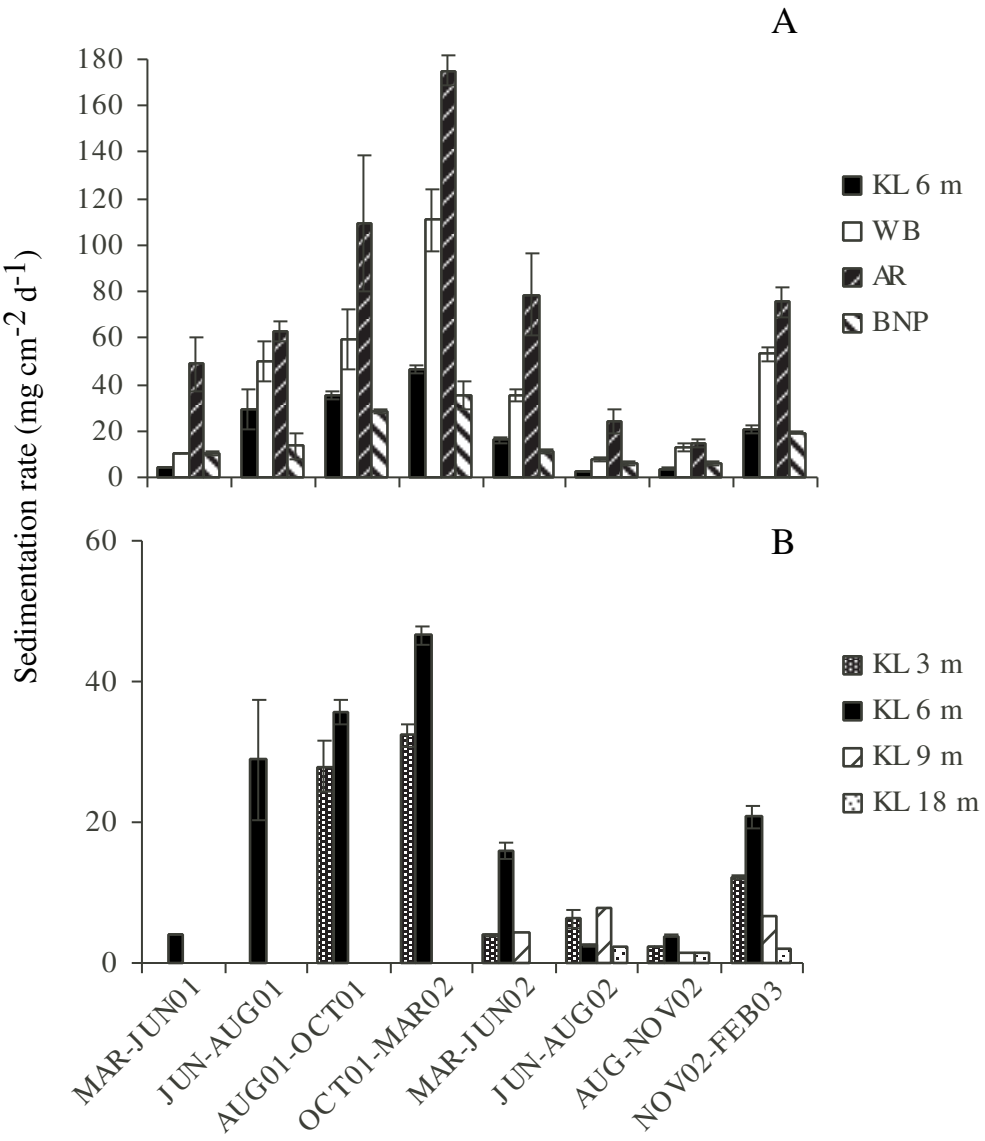


Figure 2.2. Mean (± SE) sedimentation rate (mg cm^{-2} d^{-1}) between March 2001 and February 2003 at (A) the 6 m sites and (B) along the depth gradient. The KL 6 m site is included in both panels. Note smaller scale along depth gradient.

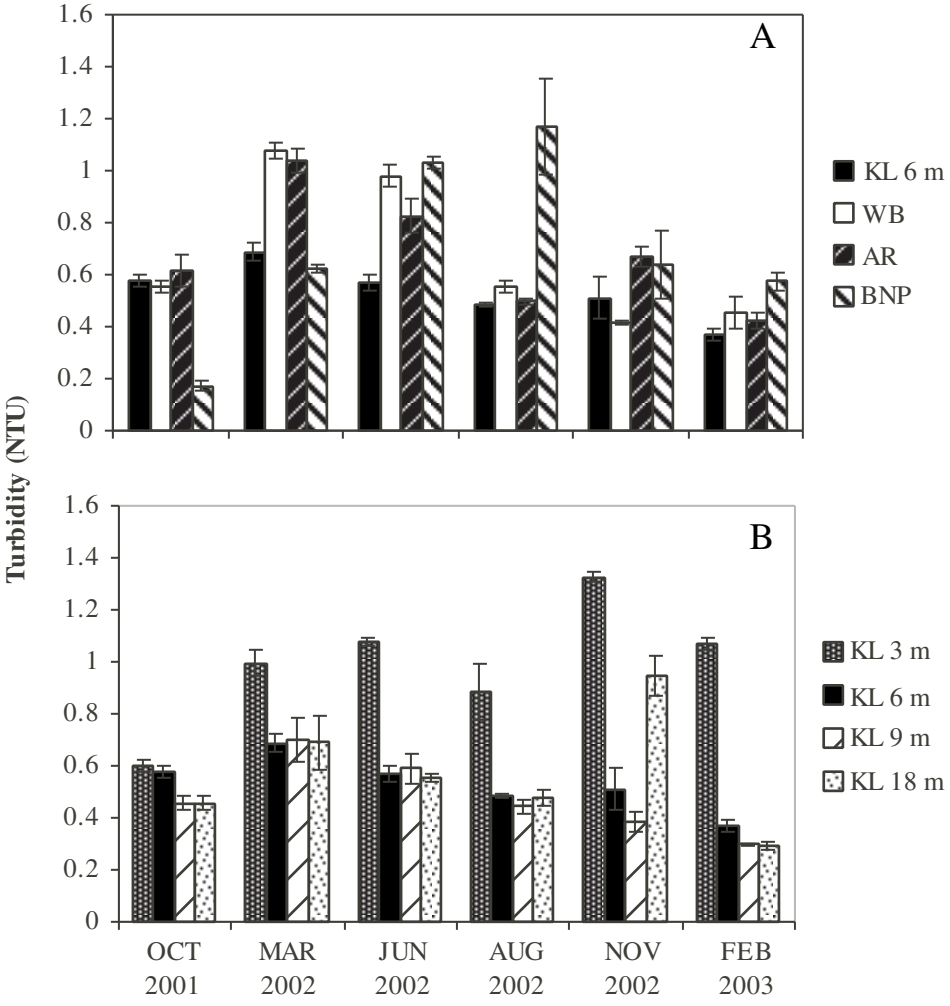


Figure 2.3. Mean (± SE) turbidity (nephelometric turbidity units: NTU) between October 2001 and February 2003 at (A) the 6 m sites and (B) along the depth gradient.

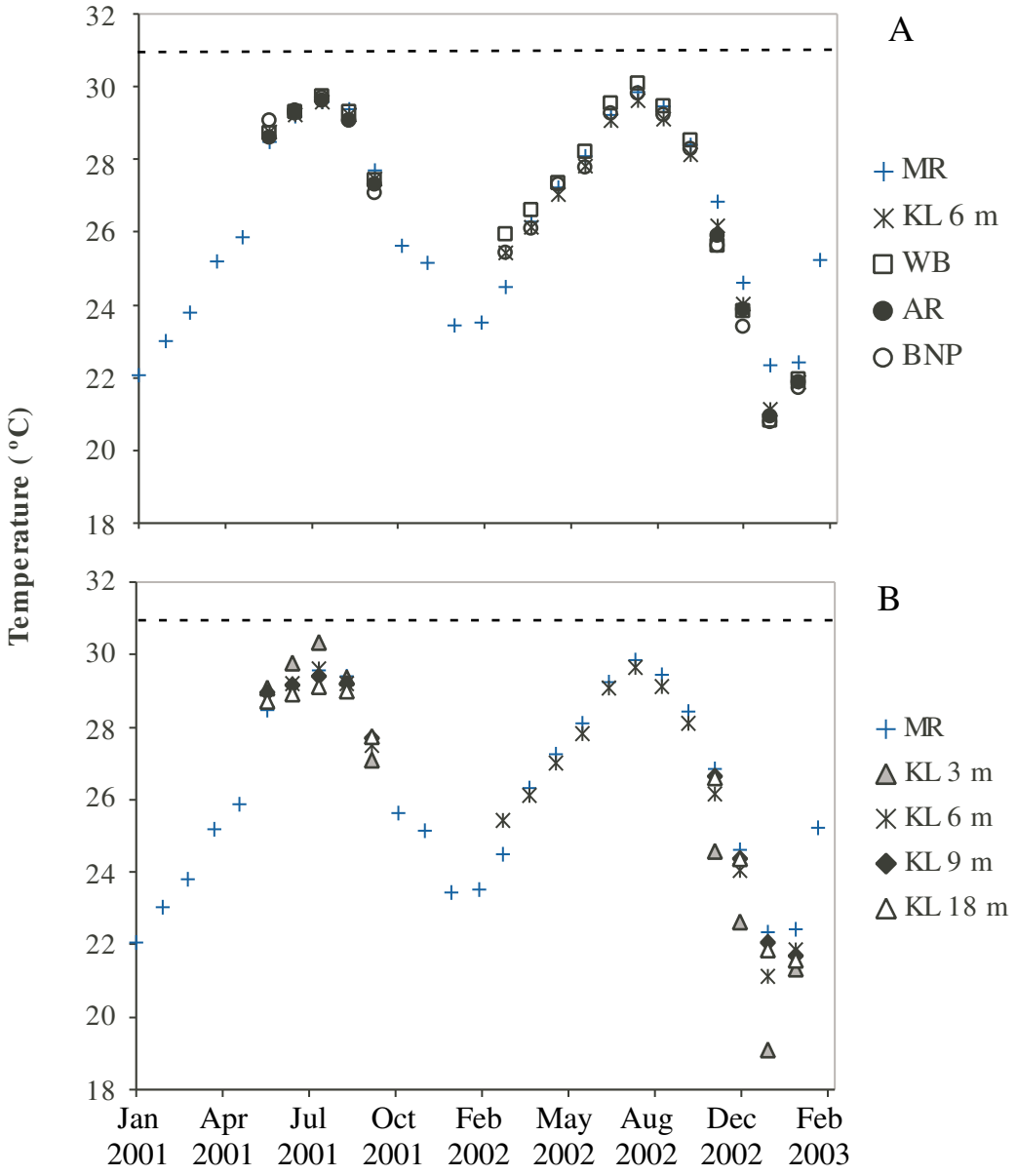


Figure 2.4. Mean temperature (°C) between January 2001 and February 2003 at (A) the 6 m sites and (B) along the depth gradient. Molasses Reef (MR) data from the SEAKEYS C-MAN buoy also is plotted. The dotted line represents the temperature where corals typically begin to bleach (31°C; Andrews et al. 2005).

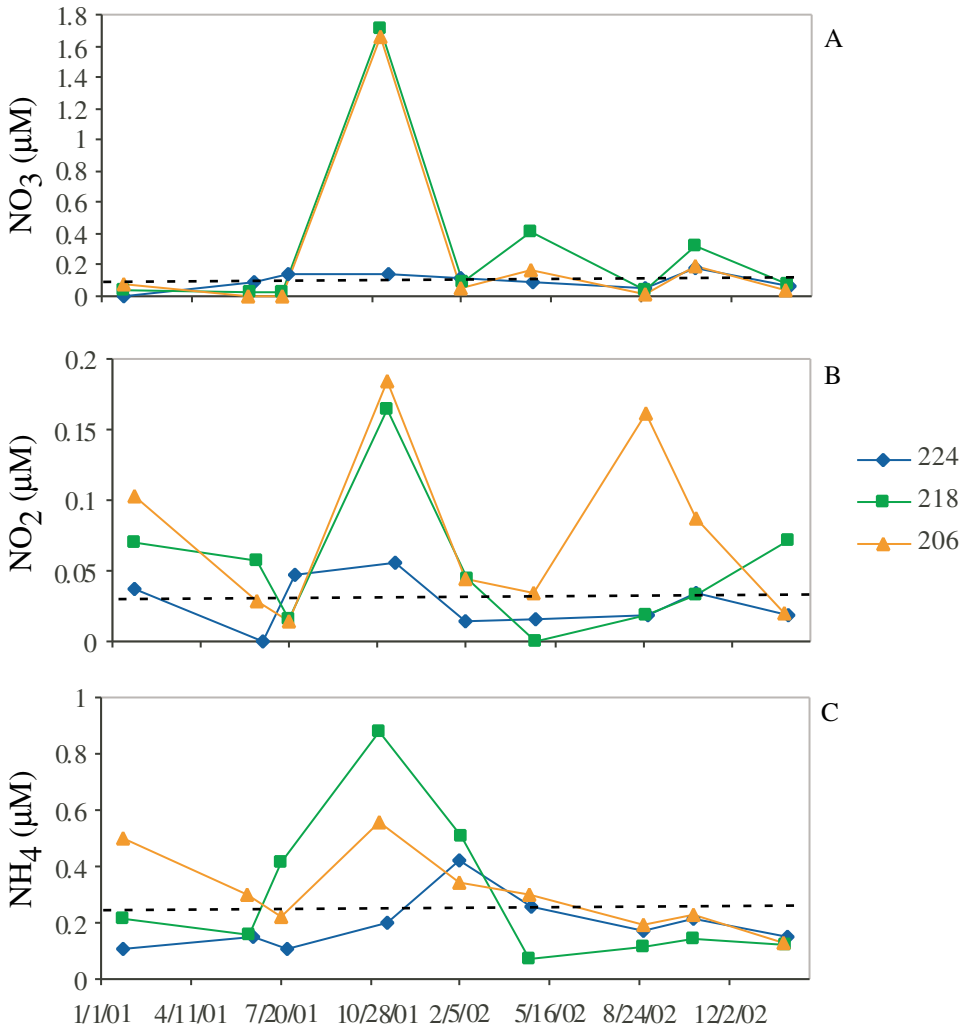


Figure 2.5. (A) Nitrate (NO_3), (B) nitrite (NO_2), and (C) ammonium (NH_4) for SERC stations between 2001 and 2003; Station 224 is closest to KL 6 m and WB, Station 218 is closest to AR, and 206 is closest to BNP; dashed line represents median values for FKNMS reef stations between 1995 and 2005.

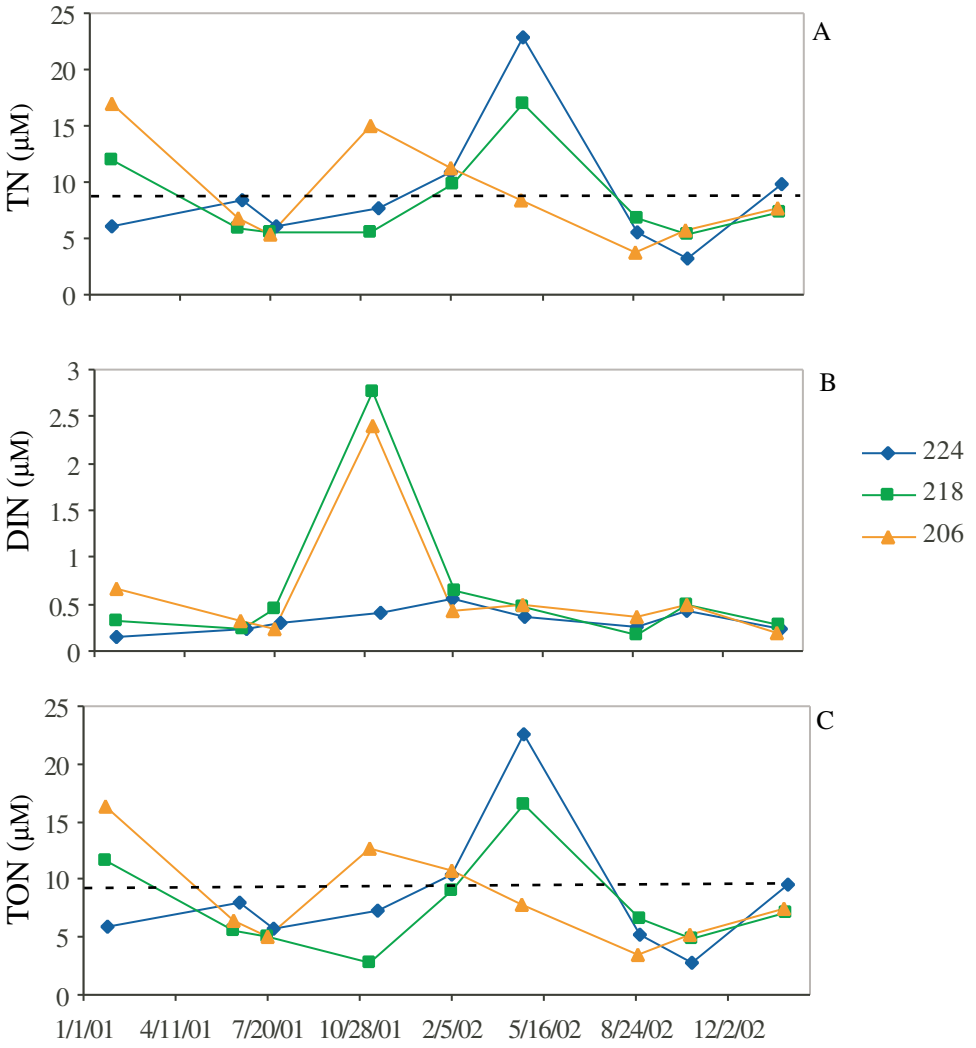


Figure 2.6. (A) Total nitrogen (TN), (B) dissolved inorganic nitrogen (DIN), and (C) total organic nitrogen (TN) for SERC stations between 2001 and 2003; Station 224 is closest to KL 6 m and WB, Station 218 is closest to AR, and 206 is closest to BNP; dashed line represents median values for FKNMS reef stations between 1995 and 2005.

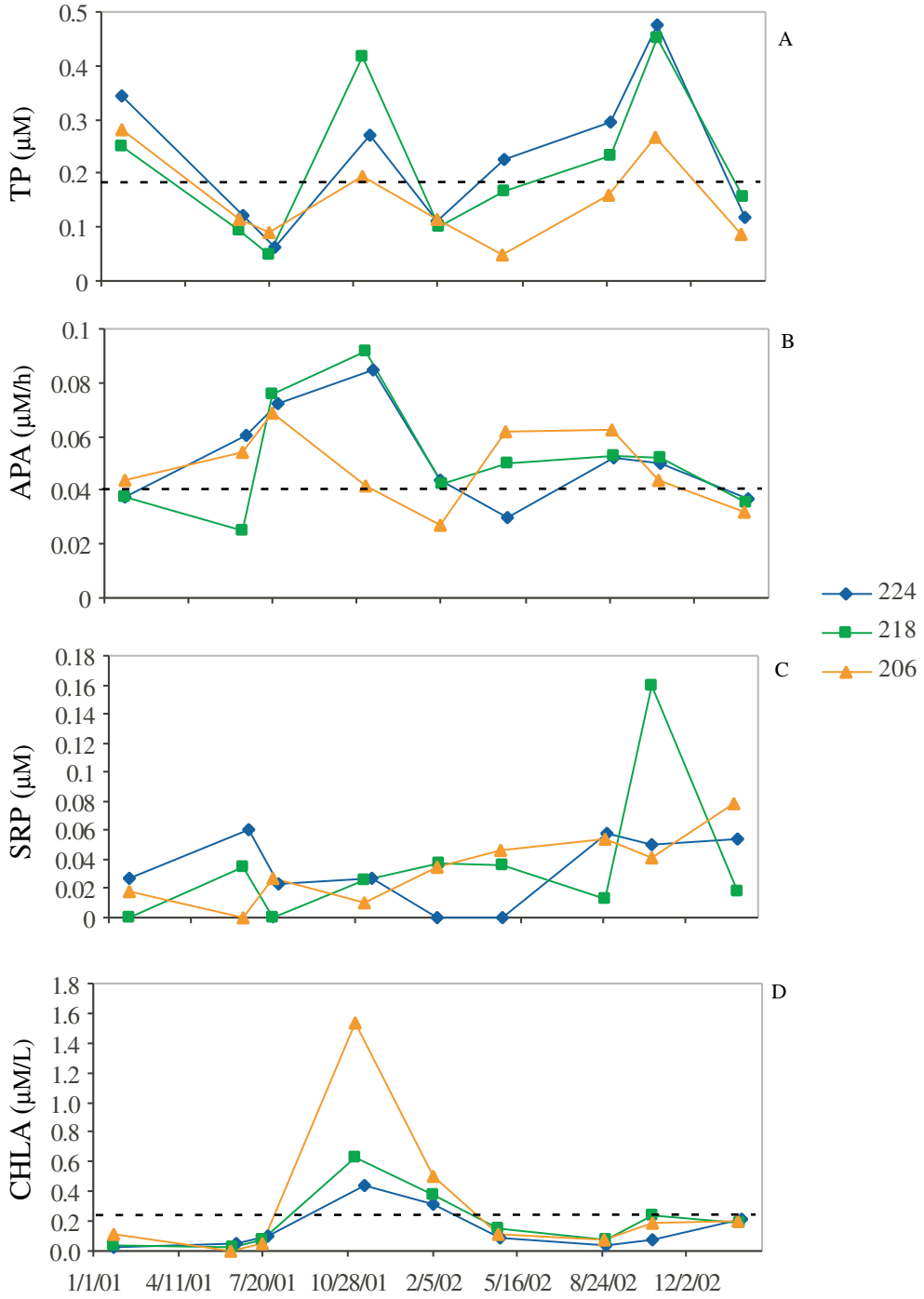


Figure 2.7. (A) Total phosphorus (TP), (B) alkaline phosphatase activity (APA), (C) soluble reactive phosphorus (SRP) and (D) chlorophyll-a (CHLA) for SERC stations between 2001 and 2003; Station 224 is closest to KL 6 m and WB, Station 218 is closest to AR, and 206 is closest to BNP; dashed line represents median values for FKNMS reef stations between 1995 and 2005

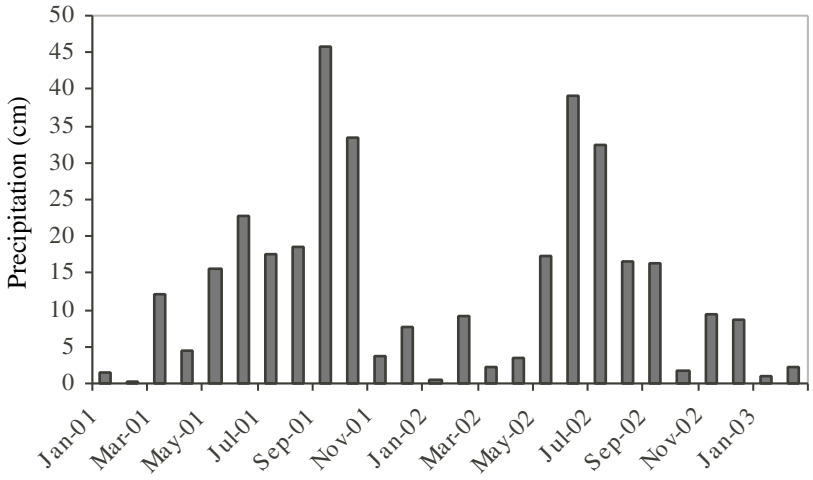


Figure 2.8. Total precipitation (cm) in Miami between January 2001 and February 2003.

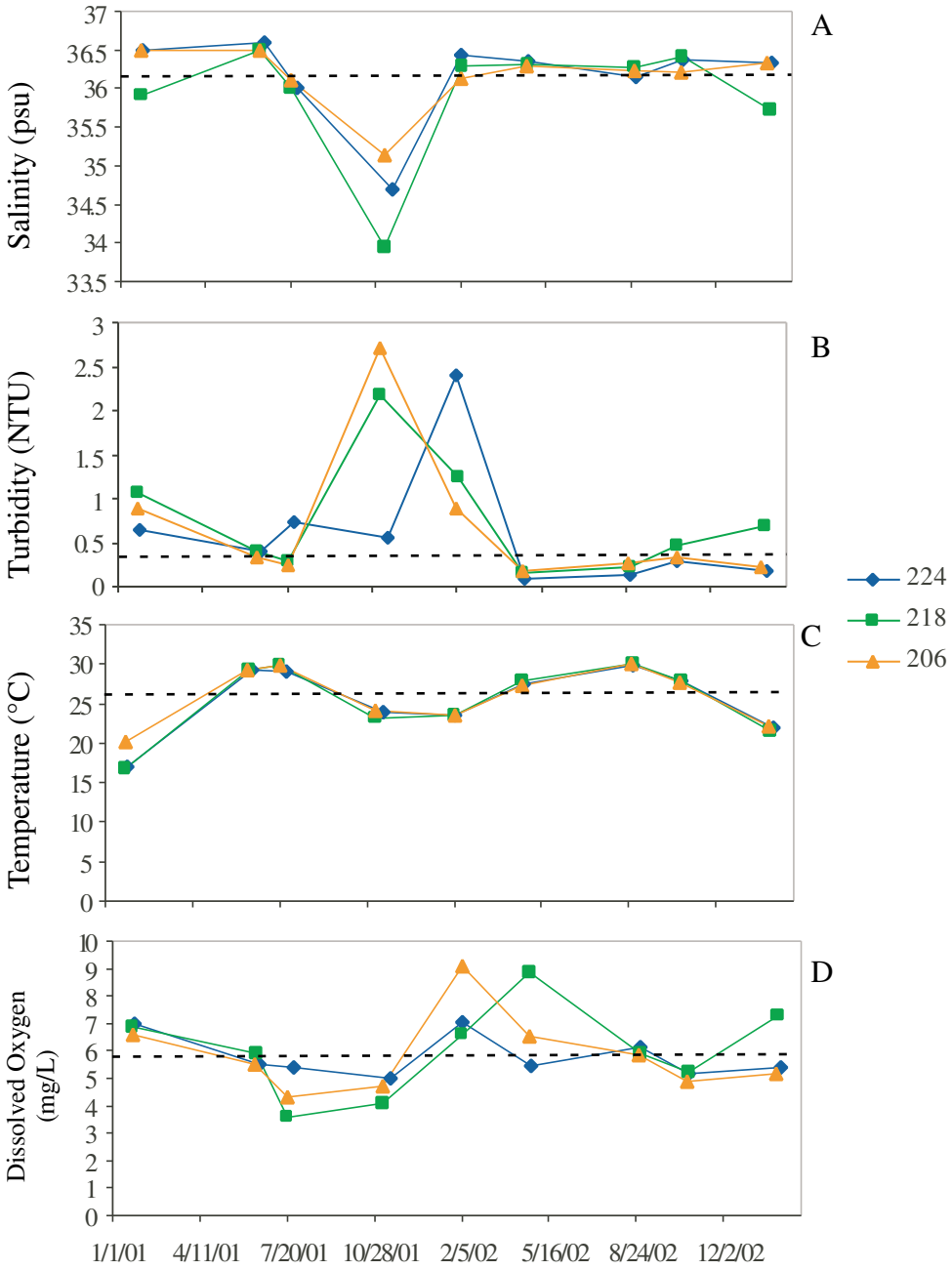


Figure 2.9. (A) Salinity, (B) turbidity, (C) temperature, and (D) dissolved oxygen for SERC stations between 2001 and 2003; Station 224 is closest to KL 6 m and WB, Station 218 is closest to AR, and 206 is closest to BNP; dashed line represents median values for FKNMS reef stations between 1995 and 2005.

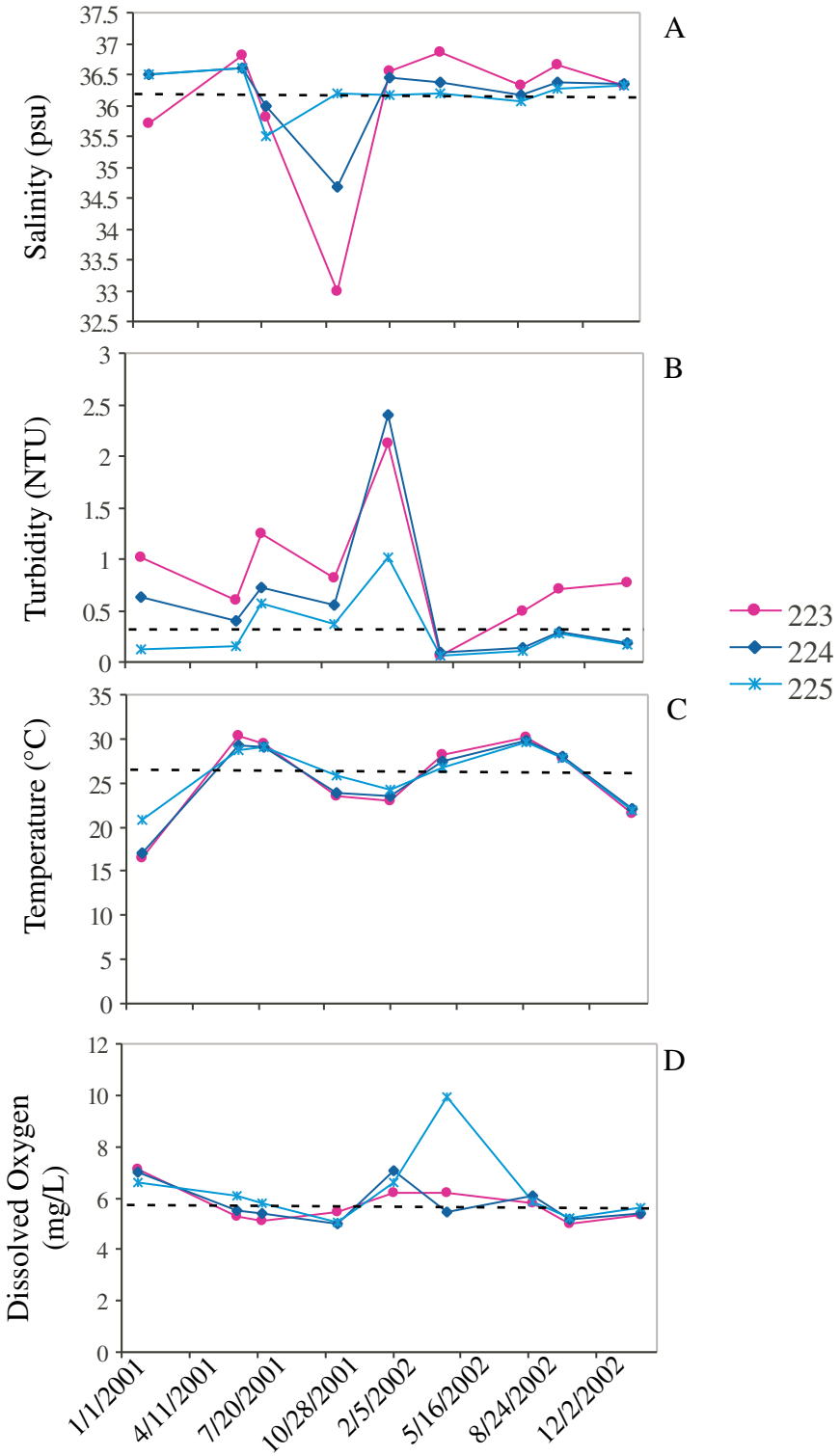


Figure 2.10. (A) Salinity, (B) turbidity, (C) temperature, and (D) dissolved oxygen for SERC stations along a Key Largo depth gradient between 2001 and 2003; Station 223, 224, and 225 is closest to KL 3 m, KL 6 m and KL 9/18 m, respectively; dashed line represents median values for FKNMS reef stations between 1995 and 2005.

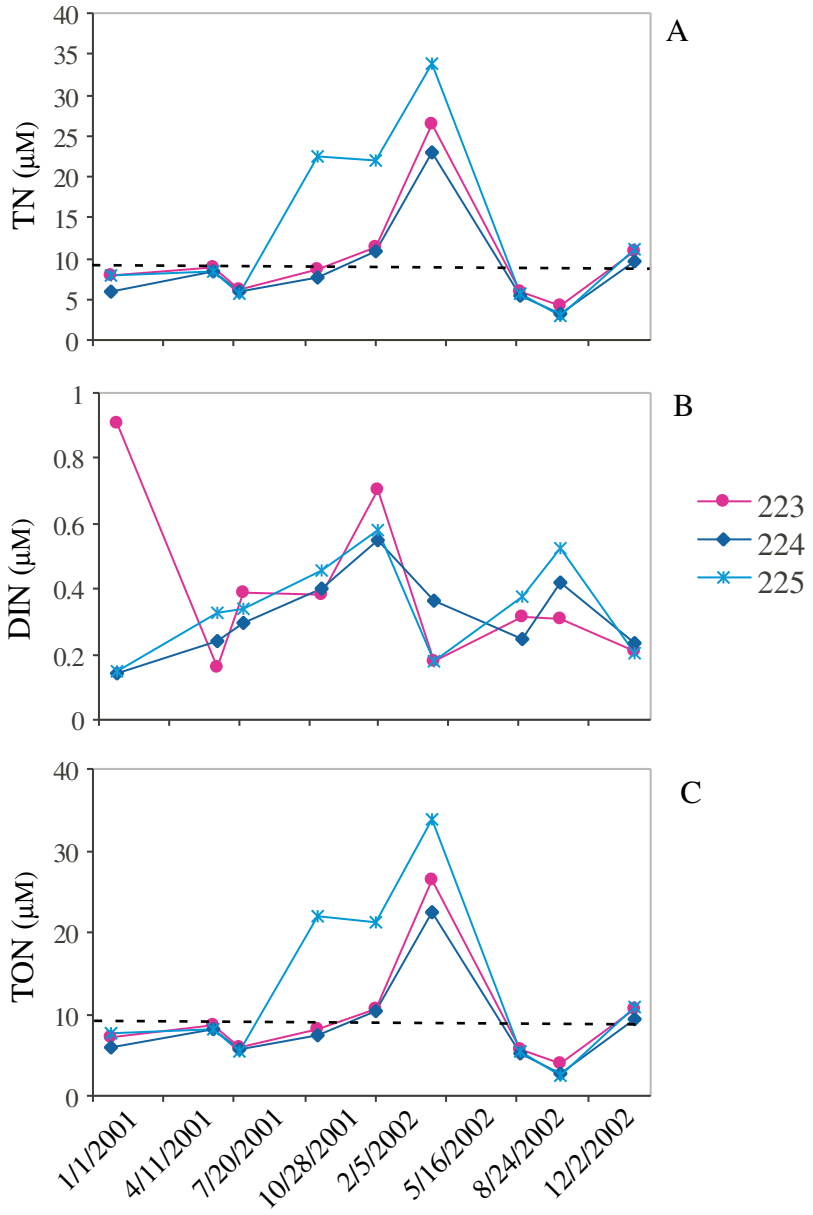


Figure 2.11. (A) Total nitrogen (TN), (B) dissolved inorganic nitrogen (DIN), and (C) total organic nitrogen (TON) for SERC stations along a Key Largo depth gradient between 2001 and 2003; Station 223, 224, and 225 is closest to KL 3 m, KL 6 m and KL 9/18 m, respectively; dashed line represents median values for FKNMS reef stations between 1995 and 2005.

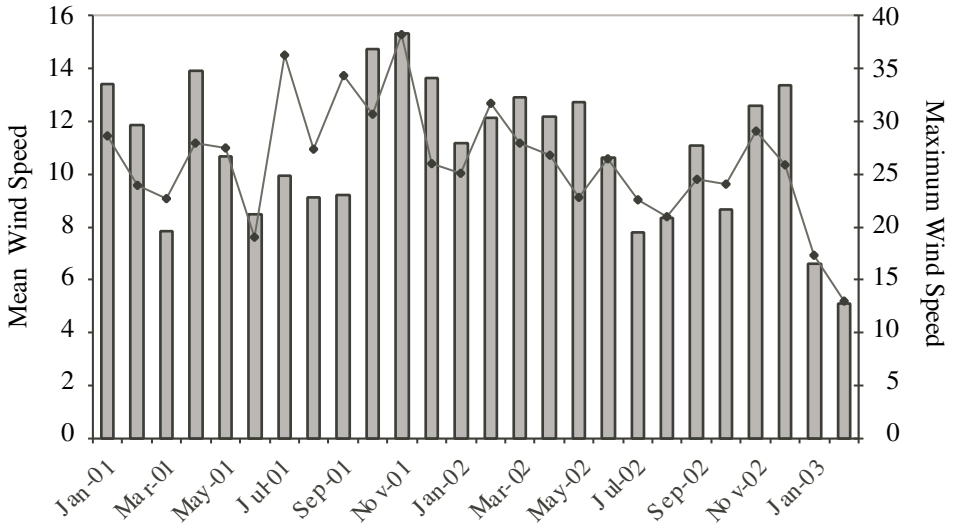


Figure 2.12. Mean (bars) and maximum (line) monthly wind speeds (knots) recorded by SEAKEYS C-MAN buoy at Molasses Reef between January 2001 and February 2003.

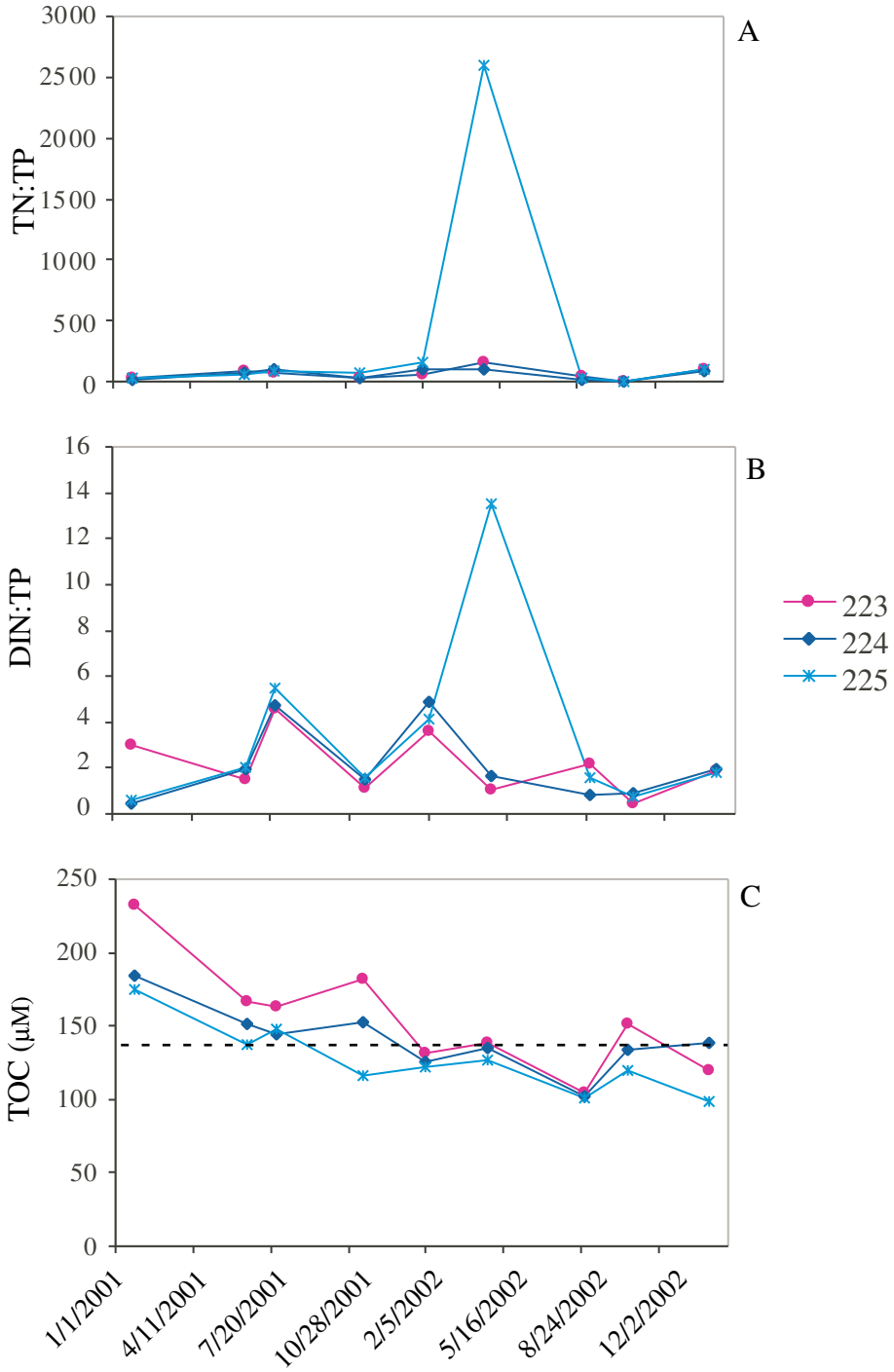


Figure 2.13. (A) Total nitrogen to total phosphorus (TN:TP), (B) dissolved inorganic nitrogen to total phosphorus (DIN:TP), and (C) total organic carbon (TOC) for SERC stations along a Key Largo depth gradient between 2001 and 2003; Station 223, 224, and 225 is closest to KL 3 m, KL 6 m and KL 9/18 m, respectively; dashed line represents median values for FKNMS reef stations between 1995 and 2005.

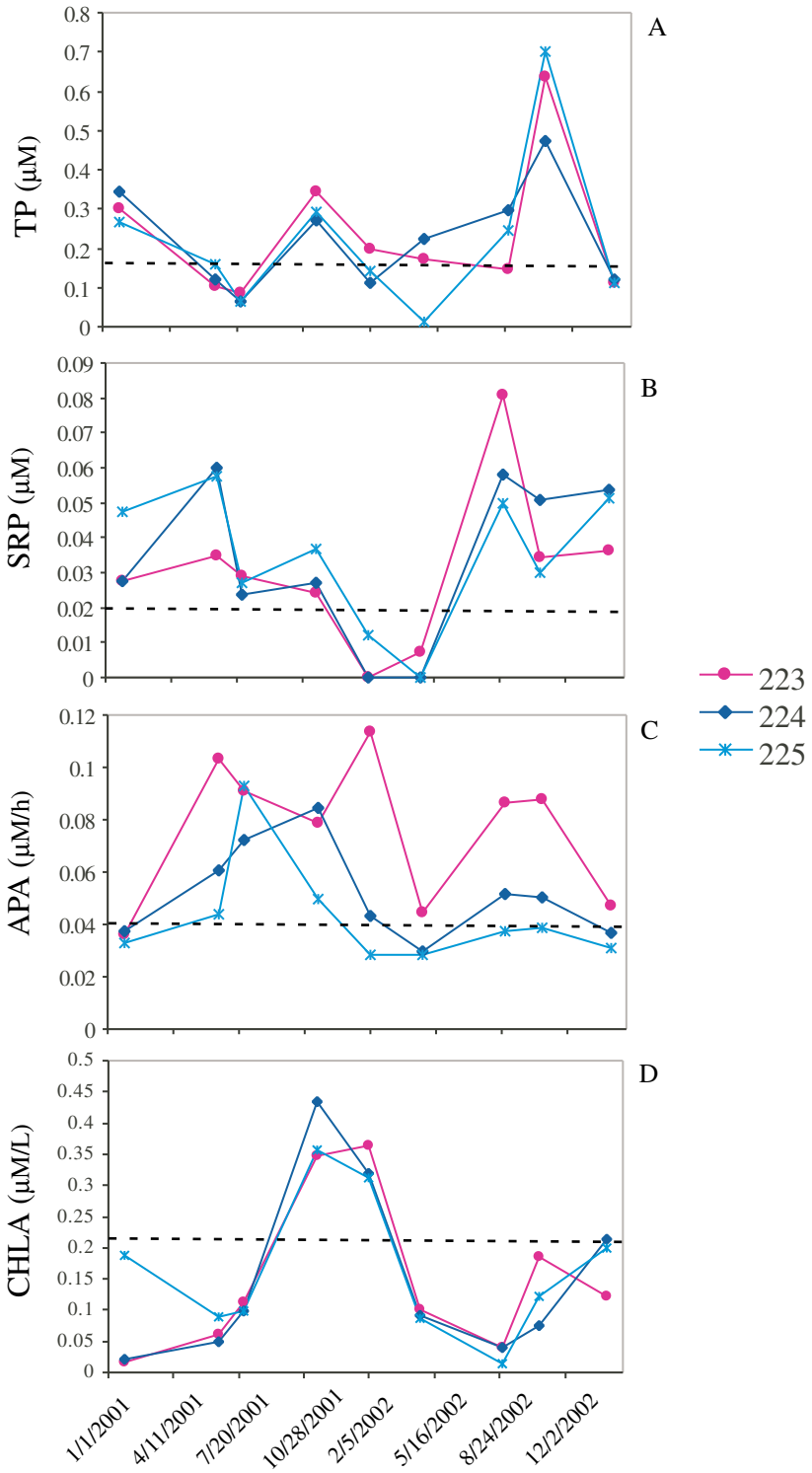


Figure 2.14. (A) Total phosphorus (TP) (B) soluble reactive phosphorus (SRP), (C) alkaline phosphatase activity (APA) and (D) chlorophyll-a (CHLA) for SERC stations along a Key Largo depth gradient between 2001 and 2003; Station 223, 224, and 225 is closest to KL 3 m, KL 6 m and KL 9/18 m, respectively; dashed line represents median values for FKNMS reef stations between 1995 and 2005.

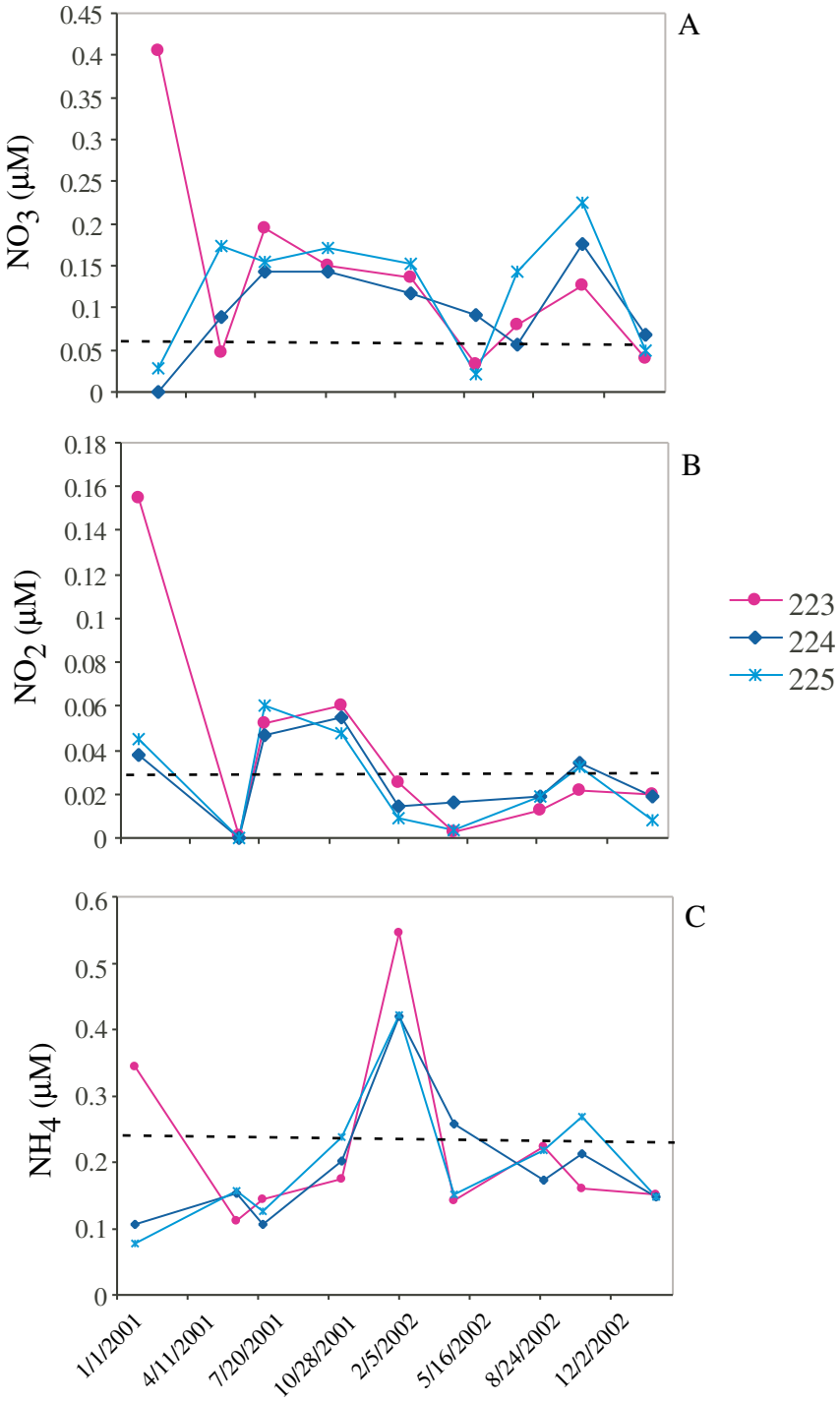


Figure 2.15. (A) Nitrate (NO_3), (B) nitrite (NO_2), and (C) ammonium (NH_4) for SERC stations along a Key Largo depth gradient between 2001 and 2003; Station 223, 224, and 225 is closest to KL 3 m, KL 6 m and KL 9/18 m, respectively; dashed line represents median values for FKNMS reef stations between 1995 and 2005.

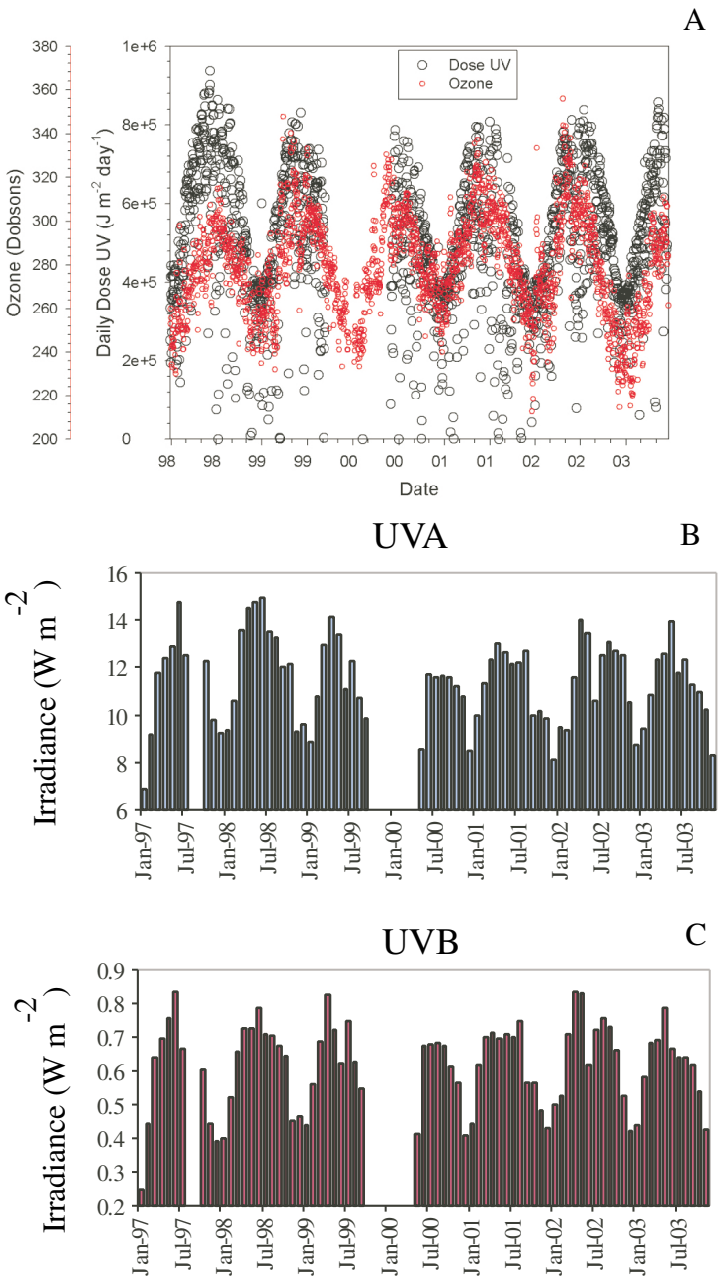


Figure 2.16. (A) Ozone (dobsons) and daily dose UV (J m^{-2} d^{-1}) between 1998 and 2003. Figure taken from Ivey, J. (B) Mean irradiance (W m^{-2}) of UVA between 1997 and 2003. (C) Mean irradiance of UVB (W m^{-2}) between 1997 and 2003.

3. Community Assessments

3.1. Introduction

Coral reefs have declined rapidly over the past several decades, with dramatic changes in the structure and composition of these dynamic ecosystems (Byrant et al. 1998, Hughes et al. 2003, Bellwood et al. 2004, Waddell 2005). Coral cover in the Florida Keys and Caribbean-wide was reduced 50 - 90% since the 1970s (Porter et al. 2002, Gardner et al. 2003, Palandro et al. 2003, Palandro 2006). Between 1996 and 2004, local, regional and global stressors caused declines in abundance of stony coral species at 79% of the Coral Reef Evaluation and Monitoring Project (CREMP) stations and decreased mean live stony coral cover from 12% to 7% on Florida Keys reefs (Beaver et al. 2005). Patch reefs have higher remaining mean stony coral cover than offshore reefs in Biscayne National Park (10-20% versus 2%; Miller et al. 2000) and in the Florida Keys (15% compared to <5%; Beaver et al. 2005). Therefore, it is important to understand what shapes community dynamics of these reefs. The structural complexity of coral reefs protects shorelines (e.g., Kunkel et al. 2006) and provides habitat for associated organisms, which benefit fisheries, tourism, and pharmaceuticals. The annual economic value of South Florida reefs is $7.7 billion dollars (Andrews et al. 2005) and depends on the diversity and abundance of reef organisms.

Ecosystem assessments and monitoring provide baseline information on reef condition, which can be used to improve resource management. Monitoring detects and quantifies change in the reef community, which can help explain underlying dynamic processes and characterize how they are disrupted by anthropogenic and natural stresses (Williams 1994, Jameson et al. 2001, Porter et al. 2001, Hallock et al. 2004). To better understand the diverse factors affecting reefs, scientists need to understand responses of coral communities to environmental variation and how population dynamics differ between degraded and undegraded reefs (Done 1992, Bak & Meesters 1999, Jameson et al. 2001, 2003).

Reef vitality depends on complex relationships among corals, fish, algae, and other organisms, whereby changes in one component can influence and even disrupt dynamics of another. For example, if abundance of herbivorous fish decreases, algal abundance often increases and subsequently further contributes to decreased coral cover (Hughes 1994). The Atlantic and Gulf Rapid Reef Assessment (AGRRA) protocol focuses on assessing the condition of principal scleractinian and hydrozoan corals that contribute most to the three-dimensional structure and complexity of reefs (Lang 2003). This protocol has been used throughout the Caribbean to assess over 400 reefs (see www.agrra.org) and provides a "snapshot" characterization based on selected structurally or functionally important benthic and fish indicators (Lang 2003 and references therein). Structural indicators include abundance of key species, benthic cover and rugosity (community structure). Functional indicators include coral and fish size-frequency distributions and coral recruitment (community dynamics, recruitment); recent and old partial mortality, prevalence of disease, bleaching, and predation (coral condition); and density of herbivorous fish and urchins, and abundance of functional algal groups (herbivory).

This chapter characterizes the community-scale condition of four 6 m deep patch reefs (Fig. 1.1) in March 2002 using data collected with the AGRRA protocol (Lang 2003). I address four questions. (1) Does reef structure indicate whether conditions in the recent past were suitable for reef growth? (2) Are significant differences evident among the reefs examined? (3) How do these reefs compare with "norms" established by Caribbean-wide AGRRA datasets? (4) Is there evidence of recent change? To answer these questions, I evaluated selected parameters for coral, fish and algae.

3.1.1. Common Coral Species

Understanding variation in coral abundance is important for understanding why some coral species may be better adapted to certain conditions than others (Bak & Meesters 1999). Corals with high rates of recruitment (e.g., *Porites* spp.) are favored in shallow, disturbed environments over robust, massive species with low recruitment rates (e.g., *Montastraea annularis* complex; Hughes & Jackson 1985, Bythell et al. 1993). The density and size of *Montastraea*, an important framework-building coral, provides

information on environmental stability. High densities of large (>1 m) *Montastraea* are indicative of a stable environment (e.g., Flower Gardens Bank) whereas low densities are indicative of a marginal reef environment (e.g., Costa Rica; Kramer 2003).

3.1.2. Coral Colony Condition and Mortality

Percent live coral cover is used as an indicator of reef health by many traditional reef-monitoring protocols (e.g., Coral Reef Evaluation and Monitoring Project (CREMP) and Global Coral Reef Monitoring Network (GCRMN)). The long-term integrity of reefs depends on recruitment, survival and growth of structure-producing scleractinian and hydrozoan corals (Dustan & Halas 1987, Done 1997, Kramer 2003). By quantifying the amount of recent mortality, and therefore the extent of damage, predictions can be made about whether corals are likely to recover. Corals with large amounts of damage are unlikely to have sufficient energy to fully recover (Lang 2003). In general, a "healthy" reef is expected to have relatively low coral mortality, so high levels of recent coral mortality indicate a major disturbance occurred in the previous days to months (Lang 2003).

Colonies are expected to have some old mortality, which represents an integration of polyp loss over time (Hughes & Connell 1999, Kramer 2003). Large, long-lived, broadcast-spawning corals such as the *Montastraea annularis* complex tend to exhibit higher amounts of mortality, while smaller, short-lived brooding species (e.g. *Porites*) tend to exhibit either complete or no mortality (Bythell et al. 1993, Kramer 2003). Partial mortality also sometimes varies with coral-colony size and morphology (Hughes & Jackson 1980) and with predator distribution (Babcock 1985). Size frequencies of corals depend on the processes of settlement, growth, survival, reproduction and mortality, and therefore also can be affected by environmental variation (Hughes & Jackson 1980, 1985, Bak & Meesters 1999). Smaller colonies typically have lower partial mortality but are more susceptible to complete mortality (Hughes & Jackson 1985, Bythell et al. 1993). Estimates of colony size also can provide information on rugosity and architectural complexity of the reef because large colonies of branching and boulder corals typically provide more 3-dimensional structure than do small colonies (Kramer 2003).

3.1.3. Fish Assemblage Structure

My study was concerned with fish species that influence benthic-community dynamics and have key roles in reef ecology (e.g., herbivorous fish, predatory fish, corallivorous fish) or are commercially important. Presence of large coral heads, amount of relief on reefs, or condition of coral colonies (% live coral cover) sometimes are correlated with aspects of fish-population dynamics, such as density or diversity (Carpenter et al. 1981, Bell & Galzin 1984, Kuffner et al. 2007 but also see Roberts & Ormond 1987, Bellwood et al. 2004). Chabanet et al. (1997) found fish density only was correlated with coral cover or coral diversity on disturbed reef sites. All Florida reef sites are disturbed to some degree (Andrews et al. 2005), so such measures are particularly appropriate.

3.1.4. Algal Biomass and Herbivory

Herbivorous fishes can affect the distribution and abundance of algae (Brock et al. 1979, Morrison 1988, Miller & Hay 1998, Chabanet et al. 1997), so disrupted reef fish assemblages can contribute to increased algal abundances and decreased coral cover. Over the past two decades, many Caribbean reefs have dramatically changed from coral-dominated communities to algal-dominated communities (Hallock et al. 1992, Hughes 1994, Lapointe 1997, 1999, Ostrander et al. 2000, Szmant 2001, Littler et al. 2006). A biologically intact reef is expected to have a low macroalgae to crustose coralline ratio, whereas declining reefs have high abundances of fleshy macroalgae, sometimes associated with a high abundance of *Halimeda* (Steneck & Detheir 1994, Kramer 2003). The cause of dramatic changes in community composition and population density continues to be debated (e.g., Szmant 2001). The "top-down" hypothesis contends that significant declines in herbivores, such as *Diadema antillarum* and herbivorous fish, are primarily responsible for increased algal abundance (e.g., Hughes 1994, Williams & Polunin 2001). The alternative, "bottom-up" hypothesis is that increased nutrient flux to reef waters drives algal blooms and shifts in community composition (e.g., Lapointe 1997, 1999). Moreover, these processes are not mutually exclusive and can act together to change community structure.

3.1.5. Comparisons with regional Caribbean values for the AGRRA Biotic Reef Index (Kramer 2003)

Natural variation in community dynamics over spatial and temporal scales makes it difficult to establish baselines for a "healthy" coral reef. However, comparisons with Caribbean-wide AGRRA values for means, and for best and worst cases, can be made using data collected using similar protocols as an indicator of deviations from the "norm." Kramer (2003) chose selected AGRRA parameters for a biotic health index to evaluate overall reef condition. They characterized a functional coral reef as having at least some of the following key attributes: high coral cover; high densities of coral > 25 cm; mid to high coral recruitment; low percentages of recent mortality; low abundance of macroalgae and high relative abundance of crustose coralline algae; low occurrence of bleaching and disease; and complex trophic webs including high densities of key herbivores (fish and *Diadema* urchins) and carnivores. I evaluated all these reef components at my 6 m deep sites.

3.2. Methods

3.2.1. Benthic Assessment

Dives were made at each 6 m site (Fig. 1.1) in early March 2002 to assess the benthic community, focusing on corals and algae, using the rapid assessment methods (Table 3.1) as described by Lang (2003). At each site, I haphazardly placed a 10-m transect line just above the reef surface, then estimated live coral cover using a 1 m measuring device to estimate the proportion of the line underlain by living coral. Then swimming back along the transect line, for each coral >10 cm in diameter (Table 3.2), I recorded the following information: species, maximum diameter, maximum height, percent recent mortality, percent old mortality, and any apparent conditions including bleaching, disease, or overgrowths. I estimated size (maximum diameter including live and dead areas) in planar view perpendicular to the axis of growth to the nearest cm. Partial mortality was visually quantified by estimating the percentage of dead area from above in planar view (see Lang 2003). "Recently dead" was defined as any non-living parts of the coral in which corallite structures were white and either still intact or covered by a thin layer of algae or fine mud (Lang 2003). "Long dead" was defined as any non-

living parts of the coral in which corallite structures were either gone or covered by organisms that were not easily removed (e.g., certain algae and invertebrates; Lang 2003). While each colony was examined, I recorded the number of territorial gardening damselfish (*Stegastes diencaeus*, *S. fuscus*, *S. planifrons* and *S. variabilis*), total number of damselfish gardens on each head, and the number of planktivorous damselfish, *Stegastes planifrons* (bicolor damselfish). I also recorded the number of *Diadema* along each transect. I estimated % abundance and height of macroalgae (fleshy and calcareous) and % abundance of crustose coralline algae within 25 X 25 cm quadrats located 1, 3, 5, 7, and 9 m along the transect. I also recorded the number and species of coral recruits (<2 cm maximum diameter) within the quadrat. I measured the maximum relief (rugosity) as the highest and lowest point within a meter radius of each quadrat.

3.2.2. Fish Assessment

I used belt transects at each reef to assess densities and sizes of selected key reef-fish families including Acanthuridae, Balistidae, Chaetodontidae, Haemulidae, Lutjanidae, Pomacanthidae, Scaridae, and Serranidae (Table 3.3). Divers swam a total of ten, 30 m transects, recording fish found within a 2 m wide, visually estimated belt transect. Size of each fish was estimated and assigned to a category (<5, 5-10, 10-20, 20-30, 30-40, >40 cm) using a one meter T-bar marked with 5 cm increments.

I estimated fish biomass using the power function: $W = aL^b$, where W is the mass (grams), L is the length (cm), and *a* and *b* are parameters estimated by a linear regression of logarithmically transformed length-mass data (Marks & Klomp 2003; Table 3.3).

3.2.3. Data Analysis

I tested specific hypotheses using non-parametric statistics, including an analysis of similarities (ANOSIM) test, to determine if sites differed significantly based on a group of benthic or fish parameters (Clarke & Warwick 2001). I used group average cluster analysis to determine how sites grouped based on coral or fish abundances, followed by SIMPER (similarity percentages) analyses to determine which species were primarily responsible for grouping of sites (Clarke & Warwick 2001). Herbivorous fish included all Acanthuridae, all Scaridae, *Microspathodon chrysurus* and *Melichthys niger*.

Carnivorous fish included all Haemulidae, all Lutjanidae, all Serridae and *Sphyraena barracuda*.

I used a one-way ANOVA followed by the Tukey-Kramer Honestly Significant Difference (HSD) method to determine if sites differed significantly in live coral cover and coral colony density. When data did not meet the normality assumptions of ANOVA, I used Kruskal-Wallis followed by Wilcoxon rank-sum tests to test for differences among sites (Sokal & Rohlf 1995). These tests and correlation analyses were run using JMP statistical software (SAS Institute, Inc., Cary, NC).

I used cluster analysis and SIMPER to assess how my study sites compared with regional means, and the best and worst regional values for a set of biotic health indices, based on data collected on Caribbean reefs >5 m depth (Lang 2003). For these comparisons, colonies <25 cm diameter along the transects were excluded from analyses to allow for comparisons with regional means. The biotic health indices used in this cluster analysis included live coral cover, density of corals with >25 cm diameter, density of coral recruits (<2 cm diameter), maximum diameter of *Montastraea* spp., mean recent and old mortality, % diseased corals, macroalgal index (both fleshy and calcareous algae), relative abundance of crustose coralline algae, *Diadema* density, and densities of herbivorous, carnivorous and total fish. For the biotic index, herbivorous and carnivorous fish were defined as above, with the exception that Haemulidae were not included in the carnivorous fish data to allow for comparisons with regional means. ANOSIM, cluster analysis and SIMPER were performed using PRIMER-e v. 5.2.8 statistical software.

3.3. Results

3.3.1 Community Structure

Porites astreoides, *P. porites*, and *Siderastrea siderea* comprised >50% of the coral species at all sites combined (Fig. 3.1). Sites differed significantly in coral assemblages (ANOSIM Global R = 0.21, p = 0.002, Table 3.4). *Porites astreoides* was the most common species at Algae Reef and WB (n = 39 and 11, respectivily), *Porites porites* at KL 6 m (n = 25), and *Montastraea annularis* at BNP (n = 15) (Fig. 3.1). Key Largo 6 m had a significantly different coral assemblage than AR (ANOSIM R = 0.60, p

= 0.001) and WB (ANOSIM R = 0.21, $p < 0.05$) due to differences in abundance of *Porites* spp. (Table 3.4). Algae Reef also had a significantly different coral assemblage than BNP (ANOSIM R = 0.19, $p < 0.05$) due to differences in abundance of *Porites* spp. and *Montastraea annularis* (Table 3.4).

Percent live coral cover was low at all sites (Table 3.5) ranging from 3 – 18% with a mean (hereinafter ± SE) of 10 ±1 % (n = 17). Mean coral cover at AR (16%) was statistically higher than at WB, BNP and KL 6 m (ANOVA $F_{3,13} = 11.4$, $p < 0.0007$; Table 3.5). Density of colonies (> 10 cm) ranged from 0.4 – 1.4 colonies/m with a mean of 0.72 ±0.04 (n = 31). Rugosity (maximum relief) did not differ significantly among sites, averaging 10 ± 1 cm^2.

3.3.2. Coral Mortality and Condition

Old mortality ranged from 0 to 67% of a coral colony with a mean of 12 ± 2% (n = 31 transects). Mortality differed by coral genus, with the *Montastraea annularis* complex having the highest percentage of mortality and *Porites* spp. tending to have a low percentage of mortality (old mortality: $\chi^2 = 61.8$, df = 4, $p < 0.0001$; recent mortality: $\chi^2 = 13.6$, df = 4, $p < 0.01$; Fig. 3.2). All sites were dominated by small colonies but mean colony size (maximum diameter) was significantly larger at BNP and AR, as a few large colonies were recorded at these sites ($\chi^2 = 36.5$, df = 3, $p < 0.001$; Table 3.5, Fig 3.3). Old mortality was positively correlated with colony size (maximum diameter: Spearman's Rho = 0.46, $p < 0.0001$; maximum height: Spearman's Rho = 0.47, $p < 0.0001$) but no relationship was found between colony size and recent mortality (Fig. 3.3). Very little disease (<4 %) and no bleaching were observed at any of the study sites. Evidence of fish predation on corals was observed at all sites except for AR.

3.3.3. Fish Community Structure

Haemulon sciurus, *Sparisoma viride* and *H. plumieri* comprised > 50% of fish biomass surveyed at my sites (Fig. 3.4). However, fish assemblages differed significantly among sites (ANOSIM Global R = 0.33, p = 0.001), with assemblages at WB differing significantly from those at KL 6 m, AR and BNP (ANOSIM; Table 3.6). Fish assemblages at AR also differed significantly from those at KL 6 m and BNP (ANOSIM;

Table 3.6). White Banks and AR were dominated by grunts (Haemulidae), KL 6 m by parrotfish (Scaridae), and BNP by a mix of parrotfish and grunts (Fig. 3.4).

Biomass of Haemulidae, Lutjanidae and Scaridae differed significantly among sites (Kruskal-Wallis ANOVA: Haemulidae: $\chi^2 = 23.4$, df = 3, $p < 0.0001$; Lutjanidae: $\chi^2 = 16.4$, df = 3, $p < 0.002$; Scaridae: $\chi^2 = 11.2$, df = 3, $p < 0.02$; Fig. 3.4); biomass of all three families was significantly lower at KL 6 m than at all other sites (Table 3.7). Haemulid biomass also was significantly lower at BNP than AR and WB (Table 3.7), while scarid biomass was significantly lower at WB than AR and BNP (Table 3.7).

Densities of herbivorous and carnivorous fish differed significantly among sites (Kruskal-Wallis ANOVA: herbivores: $\chi^2 = 10.0$, df = 3, $p < 0.02$; carnivores: $\chi^2 = 23.0$, df = 3, $p < 0.0001$; Fig. 3.5). Algae Reef and KL 6 m had significantly higher densities of herbivorous fish (predominantly Scaridae and Acanthuridae) than WB, which had a significantly higher density of carnivorous fish than the other three reefs (Table 3.8). Algae Reef also had a significantly higher density of carnivorous fish than KL 6 m and BNP (Table 3.8). Carnivorous fish at WB and BNP were predominantly grunts with a mean size of about 15 cm (Fig. 3.6); mean size of carnivorous fish at KL 6 m was very small (approx. 7 cm) compared to the other sites, resulting in a low biomass (Fig. 3.4).

The parrotfish assemblage at all four reefs consisted mainly of three species: Striped (*Scarus croicensis*), Stoplight (*Sparisoma viride*) and Redband Parrotfish (*S. aurofrenatum*). The grunt assemblage consisted primarily of four species, including Bluestriped (*Haemulon sciurus*), White (*H. plumieri*) and French Grunt (*H. flavolineatum*) plus Tomtate (*H. aurolineatum*). Although there were few snappers at any reef, those at WB consisted mainly of Schoolmaster (*Lutjanus apodus*), at AR of Gray Snappers (*L. griseus*), and at BNP of Yellowtail Snappers (*Ocyurus chrysurus*). Acanthurids were relatively equally mixed between Ocean Surgeonfish (*Acanthurus bahianus*), Doctorfish (*A. chirurgus*), and Blue Tang (*A. coeruleus*) except at AR, which was dominated by Blue Tang. No relationship was found between site relief and fish densities or biomasses.

3.3.4. Algal Abundance and Herbivory

Macroalgae were abundant at all sites, with the highest relative abundance at KL 6 m (56%) and lowest at AR (43%) (Table 3.9). Relative abundance of coralline algae ($\chi^2 = 8.2$, df = 3, $p < 0.05$) and fleshy macroalgae ($\chi^2 = 17.7$, df = 3, $p < 0.0005$) differed significantly among sites. Algae Reef had significantly more coralline algae than KL 6 m and WB (Table 3.9). Key Largo 6 m had significantly more fleshy macroalgae than WB and AR, and BNP also had significantly more fleshy algae than AR (Table 3.9). The fleshy macroalgal index (estimate of algal biomass) differed significantly among sites ($\chi^2 = 38.0$, df = 3, $p < 0.001$; Fig. 3.6). Algae Reef had a significantly lower, and KL 6m a significantly higher, fleshy macroalgal index than all the other sites (Table 3.9). The ratio of macroalgae to crustose coralline algae also differed significantly among sites ($\chi^2 = 33.0$, df = 3, $p < 0.001$). KL 6 m had a significantly higher index than all other sites and AR had a significantly lower index than all other sites (Table 3.10). The number of herbivorous or bicolor damselfish did not differ significantly among sites (Table 3.9). No *Diadema* were found along transects at any of the study sites. No relationship was found between herbivore abundance and algal abundance. However, % coral cover was negatively correlated with fleshy macroalgae biomass (Spearman Rho = -0.53, $p < 0.04$).

3.3.5. AGRRA Biotic Index

Kramer (2003) recommended using selected AGRRA parameters to create a biotic health index to evaluate overall reef condition (Table 3.10). A group-averaged cluster analysis based on a Bray-Curtis similarity matrix (Fig. 3.7) showed the greatest similarity between KL 6 m and regional worst values (78%), which clustered with WB (66%). High similarity also existed between AR and Caribbean mean values (77%), which together clustered with BNP (73%). Low similarity (44%) was observed between the study sites and best values for the Caribbean region (Fig. 3.7).

Major contributors to differences among my sites included maximum size of the *Montastraea* complex, macroalgal index, and fish densities (Table 3.11). Algae Reef and BNP were most similar to Caribbean mean values (Table 3.12). Several dissimilarities separated the 6 m sites from regional best values, including high macroalgal index values, low densities of fishes and *Diadema*, and low live coral cover (Table 3.13). Members of

the *Montastraea* complex also were smaller at all sites (except AR) than the regional best values. No colonies of *Montastraea* were >25 cm at either WB or KL 6 m. Key Largo 6 m showed a high similarity with the regional worst values except for the lack of *Montastraea* >25 cm diameter, lack of coral disease, and higher densities of herbivorous fish (Table 3.14). Compared to regional worst values, macroalgal index values were low at WB, AR and BNP. Densities of fishes were high at WB and AR relative to the regional worst values.

3.4. Discussion

3.4.1. Community Structure

Reefs in FKNMS are not stony coral-dominated communities (<7% of benthic cover) but rather are algal-dominated (~76% of benthic cover, of which 14% is macroalgae; all CREMP sites, Beaver et al. 2005). Explanations for algal dominance of reefs include loss of key herbivorous fish and urchins (Hughes et al. 1999), increases in nutrient flux to reef waters (Lapointe 1997), and increased available substrate due to coral loss to other ailments (e.g., disease, bleaching; Szmant 2001). Coral cover at my study sites (3-18%) was comparable to other reefs throughout the Florida Keys but low compared to other Caribbean reefs (e.g., Kramer 2003). Mean living stony coral cover at reefs deeper than 5 m in the Caribbean was 26 ± 13%, with a range of 3 to 58% (Lang 2003). Overall mean density of corals > 25 cm at my study sites was approximately half (0.46 colonies/m) that of Caribbean sites (0.93 colonies/m), indicating recruitment was limited.

The most commonly encountered corals were small brooding species (e.g., *Porites* spp.) and stress-tolerant species (e.g., *Siderastrea siderea*) at my FKNMS study sites and *M. annularis* complex at BNP. These observations are consistent with 2004 observations at CREMP sites, where *Montastraea annularis* complex, *M. cavernosa*, *Siderastrea siderea*, *Porites astreoides*, *Colpophyllia natans* and *Millepora complanata* were most common (Beaver et al. 2005). Significant declines in percent coral cover in the Florida Keys has occurred through losses in reef-building corals, *M. annularis* and *Acropora palmata* (Beaver et al. 2005). Density of *M. annularis* was very low at my FKNMS sites. The *Montastraea annularis* complex continues to be most common

species in deeper reef (> 5 m) assemblages throughout the Caribbean, with *M. annularis* as the most common, followed by *M. faveolata*, *M. cavernosa*, and *M. franksi* (Lang 2003). However, the *Montastraea annularis* complex comprised only 32% of coral density at BNP and <15% at the other sites.

Most coral colonies at my study sites were in the 10 – 29 cm size class (maximum diameter) compared to Caribbean-wide, where the most frequent coral size class was 30-40 cm. This could indicate that juveniles are not capable of surviving or accreting past a certain size (Miller et al. 2000) or that larger colonies have died or been partly bioeroded. While values are not directly comparable because my study only counted colonies >10 cm, similar trends were reported by Beaver et al. (2005) throughout the Florida Keys where 70% of coral colonies within CREMP value-added sites (VAS) sites were <11 cm and only 5% were >50 cm. Algae Reef and BNP had the highest frequency of coral colonies ≥ 30 cm, with 0.41 and 0.42, respectively. Nevertheless, <10% of coral colonies were larger than one meter, indicating that conditions were only marginal for reef growth (Lang 2003).

3.4.2. Mortality and Coral Colony Condition

Old mortality at BNP was high relative to other sites, with the highest percentage of mortality in intermediate-sized colonies (30 – 130 cm). The *Montastraea annularis* complex, which was predominantly found at BNP, had the highest percentage of mortality. Mortality was low compared to Caribbean-wide values (~22%), but small colonies (<30 cm) are less likely to have partial mortality and more likely to experience complete mortality because they are susceptible to colony edge (i.e., bottom-associated) mortality (Meesters et al. 1996, Kramer 2003). Decreases in live coral cover may depend more on rates of coral regeneration and recruitment than on mortality rates (Hughes & Connell 1999, Kramer 2003). Recent mortality was low at all of my sites. However, while monitoring recent mortality (Chapter 5), I observed overgrowth of lesions by algae and other bioeroders occurred relatively quickly (<1 year), possibly making recent mortality less evident. Mortality from predation and tissue necrosis also is higher in the *M. annularis* complex than other coral species (Bythell et al. 1993). Thus, if regeneration

were reduced at my study sites, it could lead to permanent patches of old mortality on corals.

3.4.3. Recruitment

Abundance of coral recruits was proportional to adult abundance at the study sites, i.e., dominated by *Porites* and *Siderastrea*. The most abundant recruit species were *Porites porites* (brooder), *Siderastrea siderea* (broadcast spawner) and *Stephanocoenia intersepta* (broadcast spawner). White Banks had the highest density of recruits (6.8 recruits/m^2) and other sites (4.3 recruits/m^2) were comparable to the regional Caribbean mean for reefs > 5 m depth (4.4 recruits/m^2). High juvenile mortality rather than low recruitment may be the dominant process affecting community structure on offshore bank reefs in BNP (Miller et al. 2000).

3.4.4. Fish Assemblage Structure

The most abundant fish families at my study sites were haemulids and scarids; other surveyed fish families were relatively scarce. Carnivore density (mainly invertivores) was higher or equivalent to the density of herbivores at all sites except KL 6 m, which had the highest density of herbivores. The highest densities and biomass of fish were found at WB, primarily due to large schools of haemulids. No relationship was found between the density or biomass of fish and the structural complexity of the sites (rugosity or coral-colony size). Other possible factors influencing fish assemblage structure at my sites could include fishing, environmental conditions and contaminants (Downs et al. 2006), predator-prey interactions (Hixon 1991), larval supply and recruitment (Cowen et al. 2000), history of disturbance (Syms & Jones 2000), quality and intactness of adjacent habitats (e.g., Munday 2002), and natural spatial variation within species (Kramer 2003).

3.4.5. Algal Biomass and Herbivory

No clear relationship was observed between macroalgae and herbivory. Macroalgal biomass was lowest at AR, which had the highest biomass of herbivorous fish. Macroalgal biomass was highest at KL 6 m, which had the highest density and

second highest biomass of herbivorous fishes. However, herbivorous fishes at KL 6 m were relatively small, suggesting that smaller fish may have less effect on macroalgae. Fleshy macroalgae found at these sites may not be palatable to herbivorous fish, which tend to prefer turf algae (Choat 1991). Also, AGRRA methods do not include algal cover estimates, but rather relative abundances of functional algal groups. Sites such as KL 6 m and BNP, which had higher macroalgal biomass also had a lower percentage of live coral cover. The macroalgal index at these two sites was considerably higher than the regional mean of 82, particularly at KL 6 m. High abundance of macroalgae at my study sites may be explained by low abundance of the long-spined sea urchin, *Diadema antillarum*, which is an indiscriminate herbivore, or by nutrient inputs (Lapointe 1997, 1999, Shinn et al. 2002, others).

3.5. Conclusions

All study sites showed signs of decline and stress as evidenced by high dissimilarity with regional best values. Low coral cover (<20%), relatively small colonies and the low abundance of framework corals such as the *Montastraea annularis* complex at all study sites indicates marginal reef development. These sites likely have experienced declines in coral cover over the past three decades, as have many reefs in the Upper Florida Keys (Dustan 1999, Porter et al. 2001, Beaver et al. 2005, Andrews et al. 2005).

The three FKNMS sites (AR, WB and KL 6 m) represent a clear gradient from "best" to "worst" based on the biotic reef health index. Based on AGRRA survey methods, WB and KL 6 m were similar in coral community structure, with a high similarity between KL 6 m and worst regional conditions. Fish assemblages at these two sites were very different, with WB dominated by larger invertivores and KL 6 m by smaller herbivores. Low biomass of macroalgae and abundant coral recruits at WB indicates a better chance for juvenile coral survival at WB than KL 6 m. Low fish biomass, particularly of top predators, indicates overall poor condition of reef fish, possible overfishing, or unsuitable habitat. The number of top predators was low at all sites, particularly at KL 6 m. Topographic relief did not correlate with fish biomass; what is controlling these differences remains unknown. Algae Reef and BNP appeared to

be similar to each other and to the Caribbean-wide regional mean based on coral assemblage structure (colony size). Index values for Algae Reef were most similar to regional best values and therefore this reef appears to be in the best condition among study sites. High macroalgal abundance, particularly at KL 6 m and BNP, is an indication of poor reef condition. Coralline algae, which are important contributors to reef structure and facilitate coral recruitment, were most abundant at AR. However, relatively low coral cover and fish densities indicate suboptimal conditions at AR for continued reef accretion.

Prevalence of large colonies of the *Montastraea annularis* complex combined with high recent mortality and high macroalgal abundance indicates that BNP is experiencing a recent decline, with low potential for juvenile survival. However, the cause of stress is not apparent from community assessments and requires further investigation. Recent exposure to stress in corals at BNP in 2000 and throughout this study were evident by increased protein turnover and oxidative and metabolic stress in response to a xenobiotic (Downs et al. 2005a, also see Chapter 6). Colonies at this site were generally large in size, with substantial contiguous areas of living tissue. Therefore if stressors can be identified and alleviated, these colonies may survive. White Grunt (*Haemulon plumieri*) also experienced an endocrine-disrupting stress presumably in response to a pesticide at BNP during this study (Downs et al. 2006).

Table 3.1. Benthic parameters measured and calculated at each site

Site Information	Depth (m)
Community Structure	Coral colony/meter (> 10 cm)
	Live coral cover (%)
	Mean colony diameter (all >10 cm)
	Mean colony height (all > 10 cm)
Coral Colony Condition	% old mortality
	% recent mortality
	Prevalence of disease (%)
	Prevalence of bleached or pale corals (%)
Community dynamics/	Coral recruits (<2 cm) (#/m^2)
Recruitment	Colony size distributions (max diameter)
Algae/Herbivory	% crustose coralline algae
	% calcareous macroalgae
	Macroalgae/Crustose coralline algae
	Fleshy macro height (cm)
	Calcareous macro height (cm)
	Fleshy macroalgae index
	Calcareous macroalgae index
	Macroalgae index
	Diadema (#/10m^2)
	Herbivorous damselfish density
	Planktivorous damselfish density

Table 3.2. Coral species assessed by Atlantic and Gulf Rapid Reef Assessment

Acropora cervicornis	AC	*Meandrina meandrites*	MEAN
Acropora palmata	AP	*Millepora complanata*	MILC
Agaricia agaricites	AGA	*Montastraea annularis*	MILA
Agaricia lamarcki	AGL	*Montastraea cavernosa*	MC
Agaricia tenuifolia	AGT	*Montastraea faveolata*	MAF
Colpophyllia natans	CN	*Montastraea franksi*	MFR
Dendrogyra cylindrus	DEN	*Porites astreoides*	PA
Dichocoenia stokesii	DIC	*Porites furcata*	PF
Diploria clivosa	DC	*Porites porites*	PP
Diploria labyrinthiformis	DL	*Siderastrea sidereal*	SS
Diploria strigosa	DS	*Solenastrea bournoni*	SB
Madracis decacitis	MAD	*Solenastrea Hyades*	SH
Madracis mirabilis	MM	*Stephanocoenia intersepta*	SI

Table 3.3. Fish species included in AGRRA assessments and established length and mass relationships for Caribbean fishes (Marks & Klomp 2003). Fish biomass was calculated using the power function: $W = aL^b$, where W is the mass (grams), L is the length (cm), and a and b are parameters estimated by linear regression of logarithmically transformed length-mass data.

Scientific Name	Common Name	A	b
Pomacanthidae (Angelfishes)			
Holacanthus ciliaris	Queen Angelfish	0.0337	2.9004
Holacanthus tricolor	Rock Beauty	0.0428	2.8577
Pomacanthus arcuatus	Gray Angelfish	0.0344	2.9680
P. paru	French Angelfish	0.0203	3.1264
Centropyge argi	Cherubfish	0.0601	2.6920
Stromateidae (Butterflyfishes)			
Chaetodon aculeatus	Longsnout Butterflyfish	0.0220	3.1897
Chaetodon capistratus	Foureye Butterflyfish	0.0220	3.1897
Chaetodon ocellatus	Spotfin Butterflyfish	0.0318	2.9838
Chaetodon sedentarius	Reef Butterflyfish	0.0252	3.0760
Chaetodon striatus	Banded Butterflyfish	0.0222	3.1395
Haemulidae (Grunts)			
Anisotremus surinamensis	Black Margate	0.0059	3.3916
Anisotremus virginicus	Porkfish	0.0148	3.1674
Haemulon album	White Margate	0.0167	3.0423
Haemulon aurolineatum	Tomtate	0.0100	3.2077
Haemulon carbonarium	Caesar Grunt	0.0147	3.0559
Haemulon chrysargyreum	Smallmouth Grunt	0.3971	2.1567
Haemulon flavolineatum	French Grunt	0.0127	3.1581
Haemulon macrostomum	Spanish Grunt	0.0244	3.0295
Haemulon parra	Sailors choice	0.0199	2.9932
Haemulon plumieri	White Grunt	0.0121	3.1612
Haemulon sciurus	Bluestriped Grunt	0.0194	2.9996
Scaridae (Parrotfishes)			
Scarus coelestinus	Midnight Parrotfish	0.0153	3.0618
Scarus coeruleus	Blue Parrotfish	0.0124	3.1109
Scarus croicensis	Striped Parrotfish	0.0147	3.0548
Scarus guacamaia	Rainbow Parrotfish	0.0155	3.0626
Scarus taeniopterus	Princess Parrotfish	0.0335	2.7086
Scarus vetula	Queen Parrotfish	0.0250	2.9214
Sparisoma atomarium	Greenblotch Parrotfish	0.0121	3.0275
Sparisoma aurofrenatum	Redband Parrotfish	0.0046	3.4291
Sparisoma chrysopterum	Redtail Parrotfish	0.0099	3.1708
Sparisoma rubripinne	Redfin Parrotfish	0.0156	3.0641
Sparisoma viride	Stoplight Parrotfish	0.0250	2.9214

Table 3.3 (cont.). Fish species included in AGRRA assessments and established length and mass relationships for Caribbean fishes (Marks & Klomp 2003). Fish biomass was calculated using the power function: $W = aL^b$, where W is the mass (grams), L is the length (cm), and a and b are parameters estimated by linear regression of logarithmically transformed length-mass data.

Serranidae (Groupers)			
Epinephelus adscensionis	Rock Hind	0.0111	3.1124
Epinephelus cruentatus	Graysby	0.0135	3.0439
Epinephelus fulvus	Red Grouper	0.0175	3.0000
Epinephelus guttatus	Red Hind	0.0111	3.1124
Epinephelus striatus	Nassau Grouper	0.0065	3.2292
Mycteroperca bonaci	Black Grouper	0.0068	3.2051
Mycteroperca interstitialis	Yellowmouth Grouper	0.0068	3.2051
Mycteroperca tigris	Tiger Grouper	0.0094	3.1200
Mycteroperca venenosa	Yellowfin Grouper	0.0069	3.1400
Lutjanidae (Snappers)			
Lutjanus analis	Mutton Snapper	0.0162	3.0112
Lutjanus apodus	Schoolmaster	0.0194	2.9779
Lutjanus cyanopterus	Cubera Snapper	0.0151	3.0601
Lutjanus griseus	Gray Snapper	0.0232	2.8809
Lutjanus jocu	Dog Snapper	0.0308	2.8574
Lutjanus mahogoni	Mahogany Snapper	0.0429	2.7190
Lutjanus synagris	Lane Snapper	0.0295	2.8146
Ocyurus chrysurus	Yellowtail Snapper	0.0405	2.7180
Acanthuridae (Surgeonfishes)			
Acanthurus bahianus	Ocean Surgeonfish	0.0237	2.9752
Acanthurus chirurgus	Doctorfish	0.0040	3.5328
Acanthurus coeruleus	Blue Tang	0.0415	2.8346
Balistidae/Monocanthidae (Leatherjackets)			
Aluterus scriptus	Scrawled Filefish	0.8230	1.8136
Balistes vetula	Queen Triggerfish	0.0267	2.9903
Cantherhines macroceros	Whitespotted Filefish	0.0562	2.6534
Cantherhines pullus	Orangespotted Filefish	0.0684	2.5632
Canthidermis sufflamen	Ocean Triggerfish	0.0176	3.0554
Melichthys niger	Black Durgon	0.0562	2.6534
Xanthichthys ringens	Sargassum Triggerfish	0.0562	2.6534
Other fishes			
Bodianus rufus	Spanish Hogfish	0.0144	3.0532
Caranx ruber	Bar Jack	0.0074	3.2370
Lachnolaimus maximus	Hogfish	0.0203	2.9880
Microspathodon chrysurus	Yellowtail Damselfish	0.0239	3.0825
Sphyraena barracuda	Great Barracuda	0.0050	3.0825
Kyphosus secatator	Bermuda Chub	0.0174	3.0800

Table 3.4. Identification of key coral species that discriminated among sites; -- represents species that did not contribute to 90% of dissimilarity between specific sites. Bold values represent species primarily responsible for differences between sites. Abbreviations of coral species are as shown in Table 3.2.

	AR/ KL 6 m	WB/ BNP	AR/ BNP	WB/ KL 6 m	KL 6m/ BNP	AR/ WB
Mean	78	72	72	71	69	68
Dissimilarity						
PA	**22**	10	**18**	9	7	**15**
PP	15	9	9	**13**	**16**	7
SS	6	9	8	9	9	8
SI	6	8	7	7	9	5
DIC	4	6	2	6	5	5
MA	4	**11**	11	4	13	3
MC	3	5	2	5	--	5
AC	3	4	4	3	2	4
DC	2	--	--	--	--	--
MILA	2	4	--	4	--	4
MAF	2	--	2	--	--	2
MEAN	--	--	--	--	--	2
CN	--	--	--	--	2	--
AGA	--	--	--	3	--	--

Table 3.5. Comparison of benthic parameters; values represent mean (± SE) at four 6 m patch reefs from 10 m transects using Atlantic and Gulf Rapid Reef Assessment protocol. Means not connected by the same letter differed significantly ($p < 0.05$).

Site	n	Colonies (#/m)	Live Coral Cover (%)	Coral Height (cm)	Coral Diameter (cm)	Recent Mortality (%)	Old Mortality (%)	Herbivorous Damselfish (#/m)	Bicolor Damselfish (#/m)
KL 6 m	7	0.64	7 ^{A}	12 ^{A}	21 ^{A}	3 ^{A}	11 ^{A}	0.06 ^{A}	0.04 ^{A}
		(0.07)	(1)	(3)	(5)	(2)	(4)	(0.04)	(0.04)
WB	8	0.71	9 ^{A}	13 ^{A}	19 ^{AB}	2 ^{A}	8 ^{A}	0.05 ^{A}	0.01 ^{A}
		(0.07)	(1)	(1)	(2)	(1)	(2)	(0.04)	(0.01)
AR	8	0.88	16 ^{B}	23 ^{B}	40 ^{C}	2 ^{A}	12 ^{A}	0.07 ^{A}	0.00 ^{A}
		(0.10)	(1)	(4)	(4)	(1)	(3)	(0.04)	(0.00)
BNP	8	0.64	8 ^{A}	25 ^{AB}	37 ^{BC}	4 ^{A}	19 ^{A}	0.03 ^{A}	0.03 ^{A}
		(0.05)	(1)	(6)	(6)	(2)	(8)	(0.02)	(0.03)

Table 3.6. Pairwise comparison of 6 m sites based on ANOSIM of fish composition; significant R values are in bold (significance level = 0.1%)

	WB	AR	BNP
KL 6 m	**0.47**	**0.37**	0.07
WB		**0.21**	**0.48**
AR			**0.45**

Table 3.7. Kruskal-Wallis (χ^2) and Wilcoxon pairwise comparison of 6 m sites based on biomass of fish families; n.s., not significant ($p > 0.05$). No significant differences were found for the groups Acanthuridae, Balistidae, Pomacanthidae, Serranidae, Stromateidae and other.

	Kruskal-Wallis	Wilcoxon pairwise comparison					
	6 m Sites	KL 6 m/WB	KL 6 m/AR	KL 6 m/BNP	WB/AR	WB/BNP	AR/BNP
Haemulidae	23.4	12.4	13.4	5.8	n.s.	9.2	4.2
Lutjanidae	16.4	13.3	10.4	5.1	n.s.	n.s.	n.s.
Scaridae	11.2	n.s.	n.s	n.s.	7.9	10.0	-
Herbivores	11.5	n.s.	n.s.	n.s.	8.3	9.6	n.s.
Carnivores	24.1	11.4	12.4	9.8	n.s.	8.7	6.6

Table 3.8. Kruskal-Wallis (χ^2) and Wilcoxon pairwise comparison of 6 m sites based on densities of fish families; n.s., not significant ($p > 0.05$). No significant differences ($p > 0.05$) were found for the groups Acanthuridae, Balistidae, Pomacanthidae, Scaridae, Serranidae, Stromateidae and other.

	Kruskal-Wallis	Wilcoxon pairwise comparison					
	6 m Sites	KL 6 m/WB	KL 6 m/AR	KL 6 m/BNP	WB/AR	WB/BNP	AR/BNP
Haemulidae	20.7	13.8	5.9	n.s.	7.9	9.3	n.s.
Lutjanidae	16.0	13.3	10.4	4.6	n.s.	4.2	n.s.
Herbivores	10.0	6.3	n.s.	n.s.	5.3	n.s.	n.s.
Carnivores	23.0	15.3	9.4	n.s.	5.4	9.8	4.0

Table 3.9. Characterization of functional algal groups and density of coral recruits; values represent mean (± SE). *Algal index = % relative abundance of macroalgae x canopy height. Means not connected by the same letter differed significantly ($p < 0.05$).

Site Name	# Quadrats	% Coralline algae	% Fleshy algae	% Calcareous algae	Fleshy height (cm)	Calcareous Height (cm)	Fleshy algal index*	Calcareous algal Index*	Macro: Crustose	# Coral Recruits/ m^2
KL 6 m	35	9 ^{A}	42 ^{A}	12 ^{A}	4.9 ^{A}	2.3 ^{A}	210 ^{A}	33 ^{A}	28 ^{C}	4 ^{A}
		(3)	(5)	(3)	(0.4)	(0.2)	(30)	(10)	(5)	(1)
WB	40	12 ^{BC}	24 ^{A}	14 ^{A}	2.4 ^{BC}	2.6 ^{A}	79 ^{B}	44 ^{A}	12 ^{B}	7 ^{A}
		(3)	(3)	(3)	(0.3)	(0.3)	(12)	(9)	(3)	(2)
AR	40	16 ^{C}	20 ^{B}	13 ^{A}	1.0 ^{C}	2.2 ^{A}	40 ^{C}	29 ^{A}	6 ^{A}	4 ^{A}
		(3)	(4)	(3)	(0.2)	(0.2)	(9)	(5)	(2)	(1)
BNP	40	10 ^{B}	31 ^{A}	12 ^{A}	2.9 ^{B}	3.2 ^{B}	91 ^{B}	46 ^{A}	16 ^{B}	4 ^{A}
		(3)	(3)	(2)	(0.3)	(0.3)	(12)	(9)	(4)	(1)

Table 3.10. Comparisons of study sites with AGRRA regional baselines (modified from Kramer et al. 2003) based on indicators selected for biotic reef health index for corals >25 cm maximum diameter

AGRRA Parameter	Regional mean	Regional (best value): optimal	Regional (worst value): suboptimal	KL 6 m	WB (this study)	AR (this study)	BNP (this study)
Live Coral Cover (%)	26	56	3	7	9	16	8
Large (>25 cm) Coral Density	9	18	4	1	1	4	3
Small Coral (<2 m) Density	4	15	2	4	7	5	5
Max Diameter of MA complex	71	115	49	0	0	128	89
Mean Recent Mortality (%)	4	1	18	8	2	1	5
Mean Old Mortality (%)	22	8	31	17	9	15	29
Diseased corals (%)	5	0	18	0	0	0	0
Macroalgal index	82	12	215	194	116	45	129
% Crustose coralline algae	29	42	11	10	12	16	10
Diadema Density	2	23	0	0	0	0	0
Total Fish Density	49	123	21	39	60	48	28
Herbivorous Fish Density	31	54	15	29	9	22	17
Carnivorous Fish Density	6	26	0.4	0.3	4.1	5.8	1.2

Table 3.11. SIMPER results of Biotic Reef Index; - represents parameters that did not contribute to 90% of dissimilarity among sites. Large and small coral density, diseased corals, *Diadema* density and carnivorous fish density did not contribute to differences among sites.

	KL 6 m/WB	KL 6 m/AR	KL 6 m/BNP	WB/AR	WB/BNP	AR/BNP
Average Dissimilarity	27	53	31	46	31	29
Maximum Diameter						
Montastraea annularis complex	-	21	14	24	16	6
Macroalgal Index	14	24	10	13	2	13
Total Fish Density	4	1	-	2	6	3
Old Mortality	2	-	2	-	4	2
Herbivorous Fish Density	4	-	2	2	1	-
Live Coral Cover	-	1	-	-	-	1
Recent Mortality	1	-	-	-	-	-
% Crustose Coralline	-	-	-	-	-	1

Table 3.12. SIMPER results of Biotic Reef Index showing dissimilarities among study sites and Caribbean means; - represents parameters that did not contribute to 90% of dissimilarity among sites. Small coral density, recent mortality, diseased corals, *Diadema* density and carnivorous fish density did not contribute to differences among sites.

	KL 6 m/Mean	WB/Mean	AR/Mean	BNP/Mean
Average Dissimilarity	40	36	23	25
Macroalgal Index	17	6	6	7
Max Diameter				
Montastraea annularis complex	11	12	9	3
Live Coral Cover	3	3	2	3
% Crustose Coralline	3	3	2	3
Total Fish Density	2	2	-	3
Herbivorous Fish Density	-	4	1	2
Large Coral Density	1	1	1	1
Old Mortality	-	2	1	1

Table 3.13. SIMPER results of Biotic Reef Index showing dissimilarities among study sites and Caribbean best values; - represents parameters that did not contribute to 90% of dissimilarity among sites. Small coral density, recent mortality and diseased corals did not contribute to differences among sites.

	KL 6 m/Best	WB/Best	AR/Best	BNP/Best
Average Dissimilarity	71	67	37	52
Macroalgal Index	22	14	4	14
Total Fish Density	10	8	9	11
Max Diameter				
Montastraea annularis complex	14	15	-	3
Live Coral Cover	6	6	5	6
Herbivorous Fish Density	3	6	4	4
% Crustose Coralline	4	4	3	4
Diadema Density	3	3	3	3
Carnivorous Fish Density	3	-	2	3
Old Mortality	-	3	2	-
Large Coral Density	-	-	2	-

Table 3.14. SIMPER results of Biotic Reef Index showing dissimilarities among study sites and Caribbean worst values; - represents parameters that did not contribute to 90% of dissimilarity among sites. Large and small coral density, % crustose coralline, *Diadema* density and carnivorous fish density did not contribute to differences among sites.

	KL 6 m/Worst	WB/Worst	AR/Worst	BNP/Worst
Average Dissimilarity	22	42	52	29
Macroalgal Index	3	17	25	12
Max Diameter				
Montastraea annularis complex	7	8	12	6
Total Fish Density	3	7	4	1
Diseased Corals	3	3	3	3
Recent Mortality	1	3	3	2
Old Mortality	1	-	-	3
Live Coral Cover	-	1	2	-
Herbivorous Fish Density	2	-	-	-

Table 3.15. SIMPER results of Biotic Reef Index showing dissimilarities among Caribbean mean, best and worst values; - represents parameters that did not contribute to 90% of dissimilarity among regional values. Large coral density and diseased corals did not contribute to differences among regional values.

	Mean/Worst	Mean/Best	Worst/Best
Average Dissimilarity	42	39	71
Macroalgal Index	19	8	23
Total Fish Density	4	9	12
Max Diameter			
Montastraea annularis complex	3	5	8
Live Coral Cover	3	4	6
Herbivorous Fish Density	2	3	4
% Crustose Coralline	3	2	4
Old Mortality	2	-	3
Diadema Density	-	2	3
Carnivorous Fish Density	-	2	3
Recent Mortality	2	-	-
Small Coral Density	-	1	-

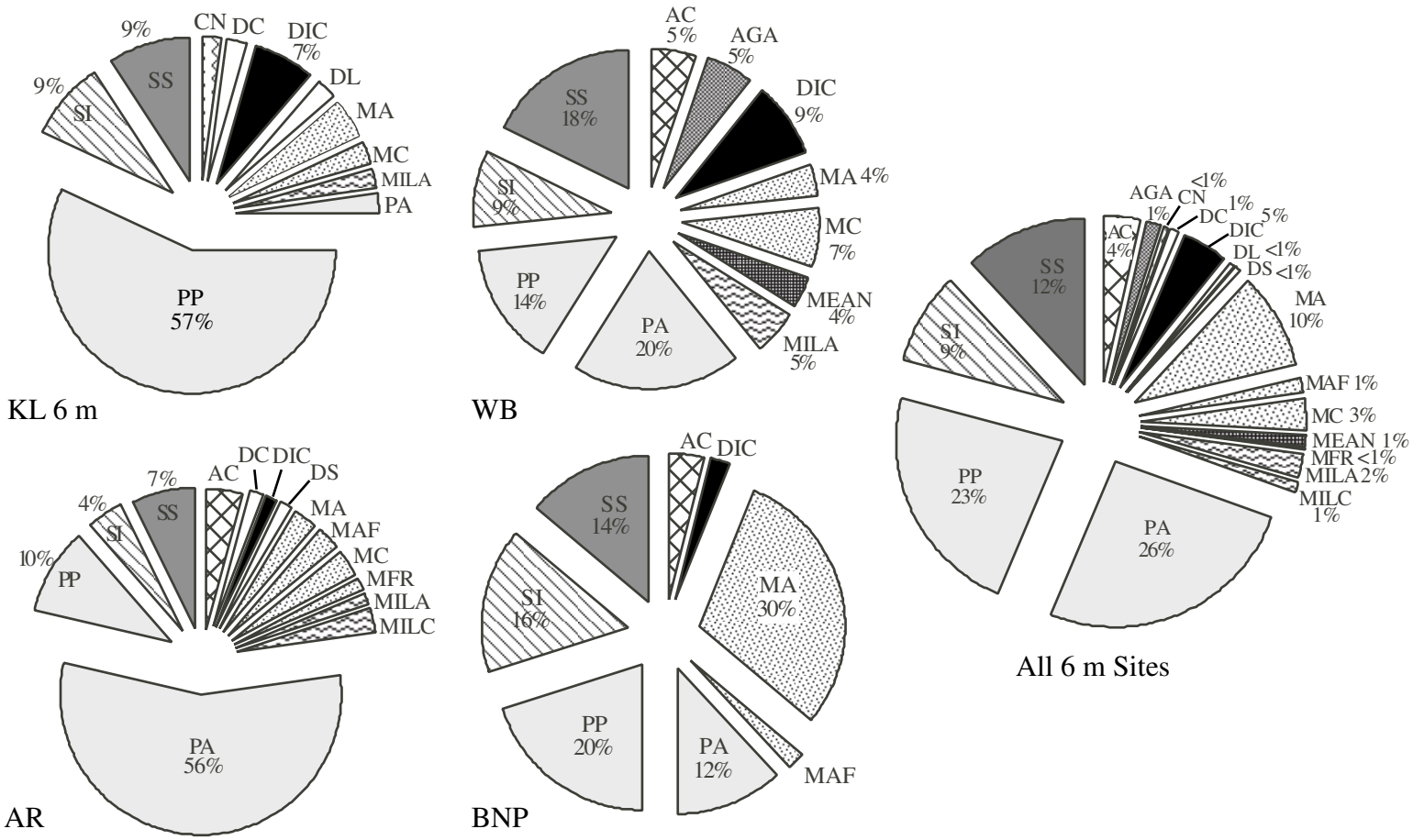


Figure 3.1. Relative abundance of coral species >10 cm maximum diameter.

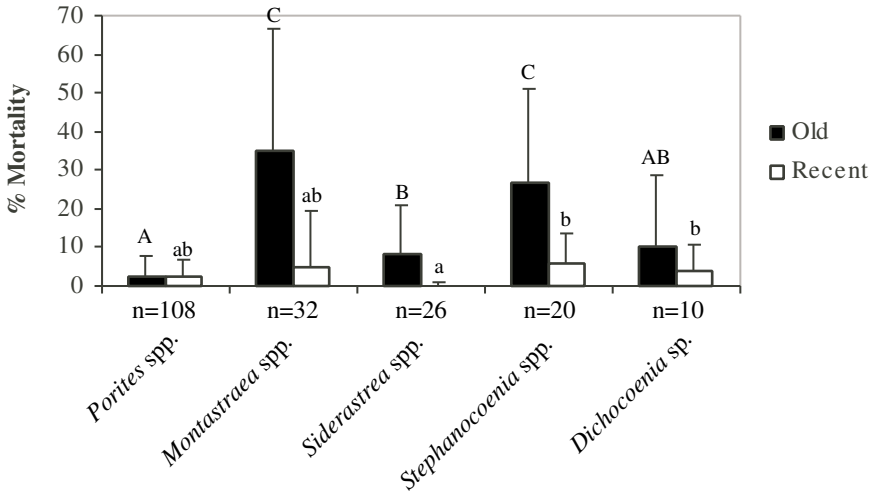


Figure 3.2. Mean (± SE) recent and old mortality for dominant coral species at the four study sites. Means not connected by the same letter (capital and lowercase for old and recent mortality, respectively) were not significantly different by Wilcoxon's test ($p < 0.05$).

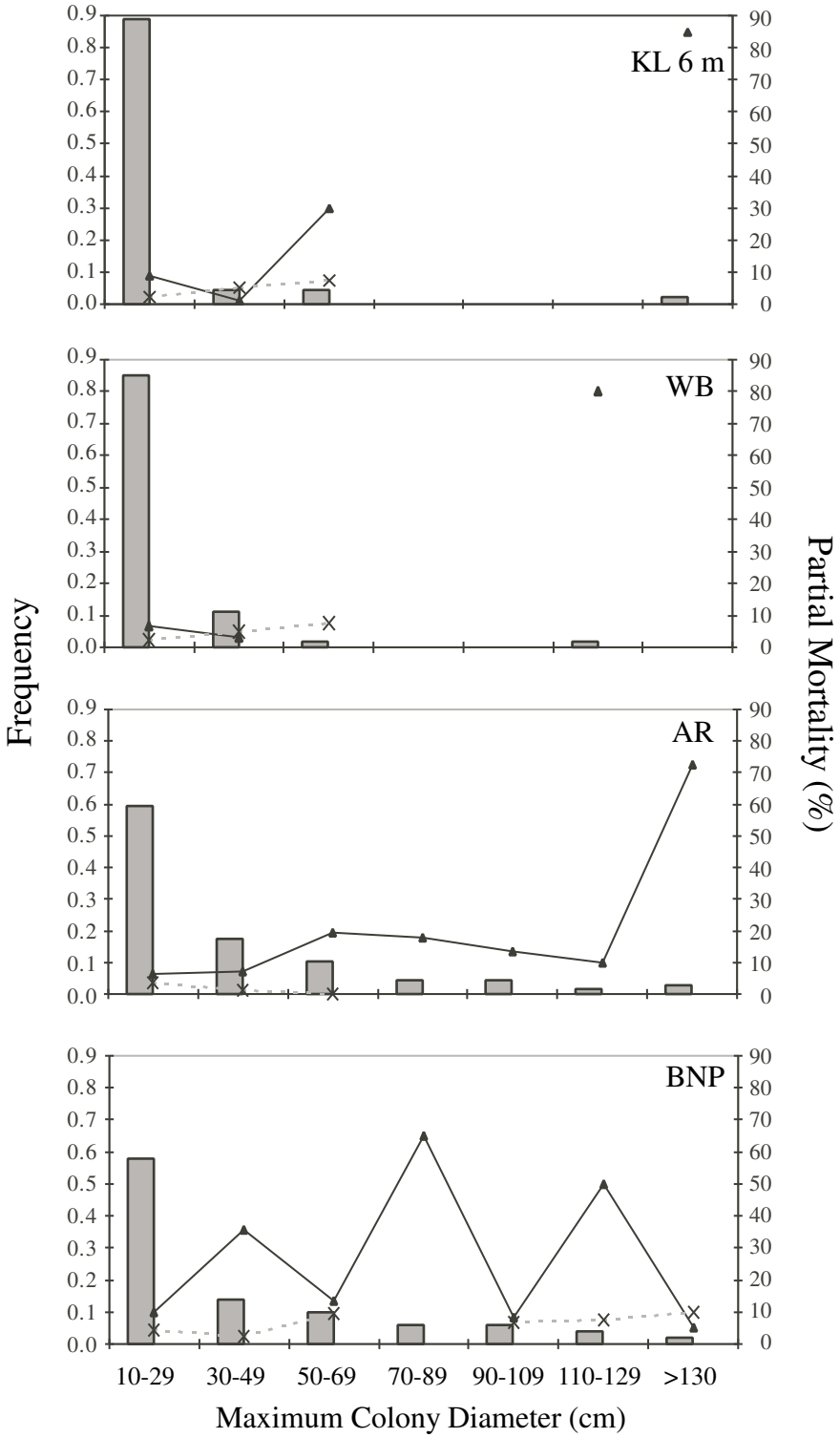


Figure 3.3. Frequency of coral colonies by size class (maximum diameter) and the relationship of colony size with recent and old mortality. Solid line represents old mortality (p) and the dashed line represents recent mortality (x).

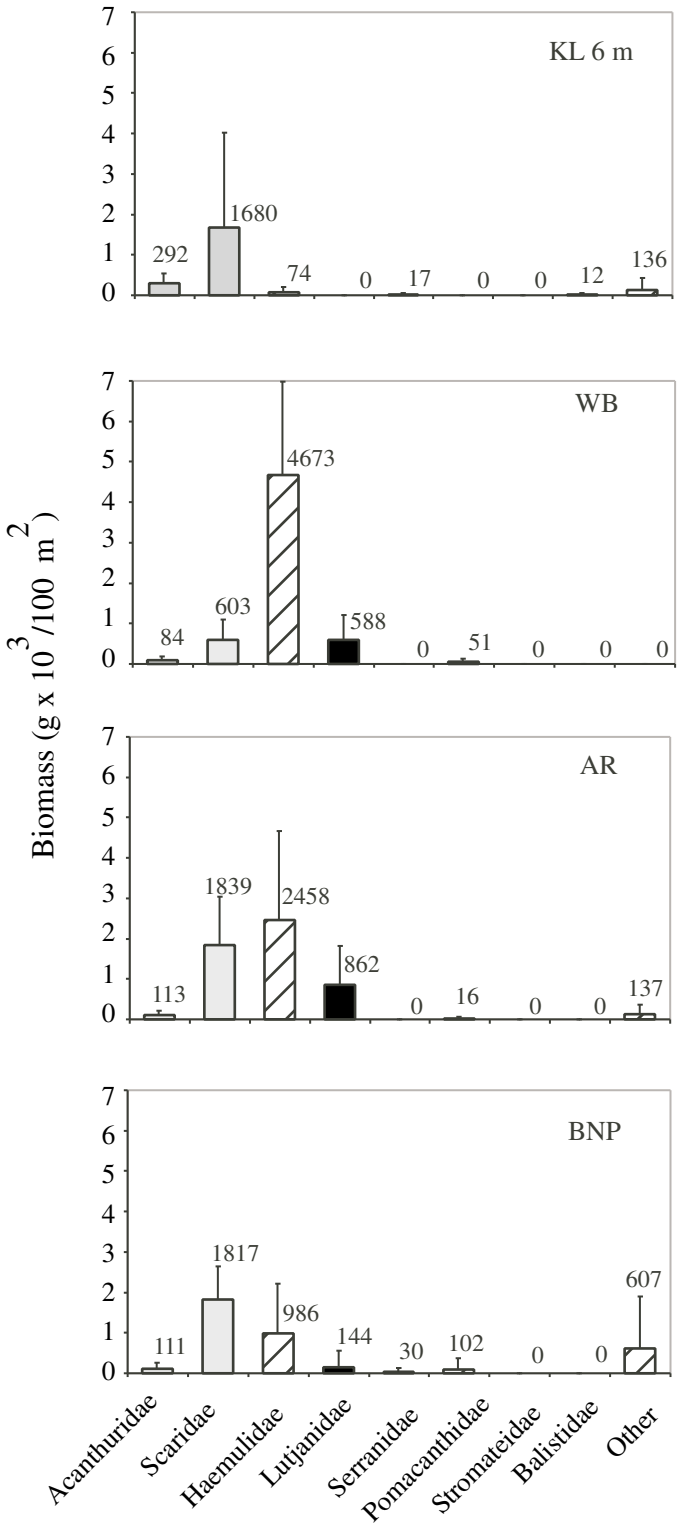


Figure 3.4. Fish biomass (g x 10^3/100 m^2) by family.

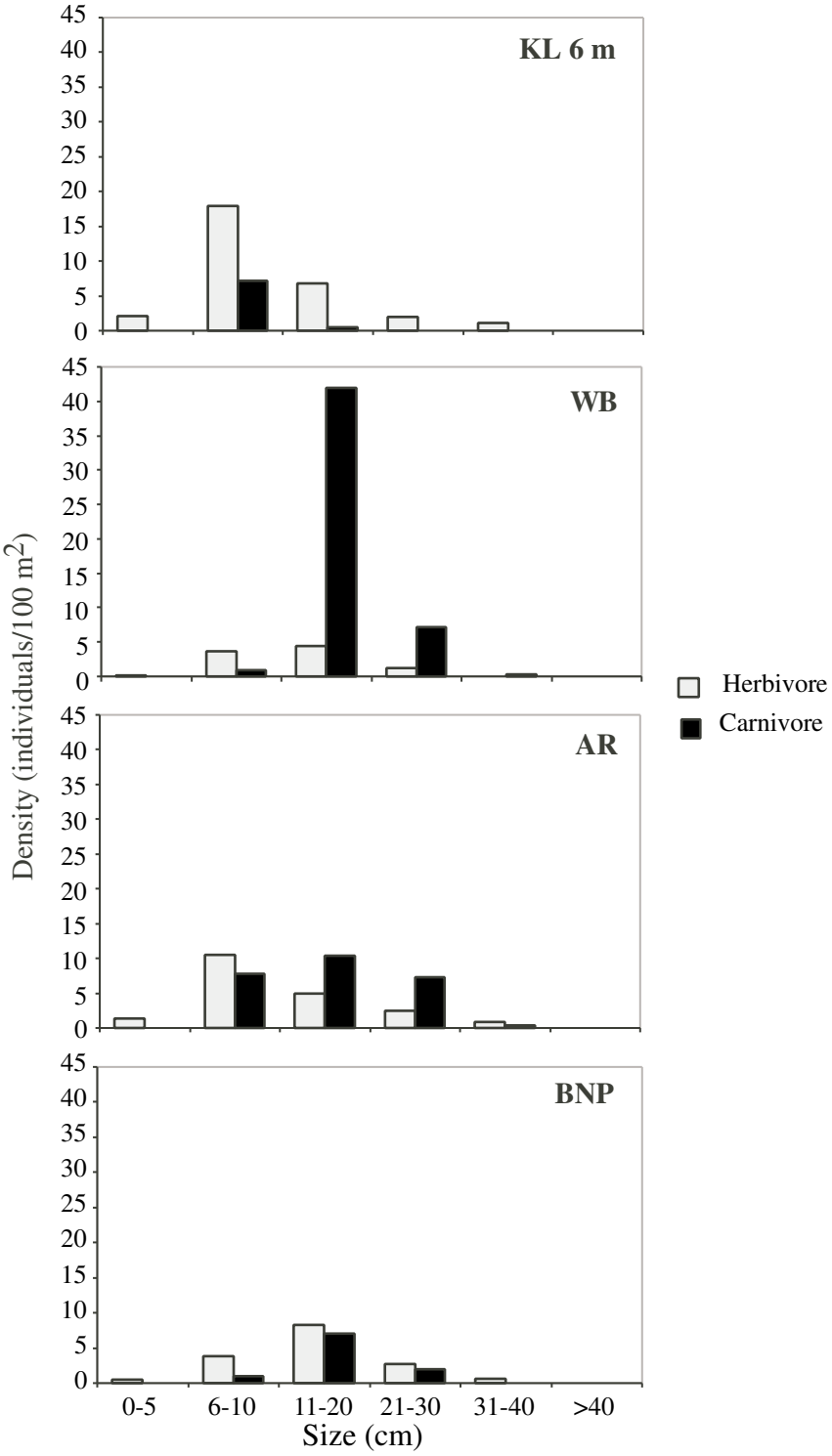


Figure 3.5. Density (individuals/100m^2) of herbivorous and carnivorous fish by size class (cm)

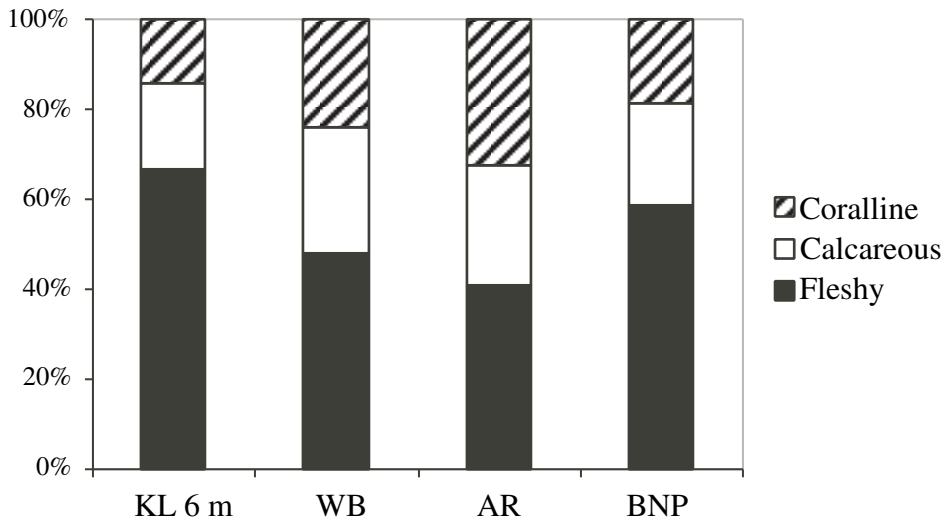


Figure 3.6. Relative abundance of functional algal groups

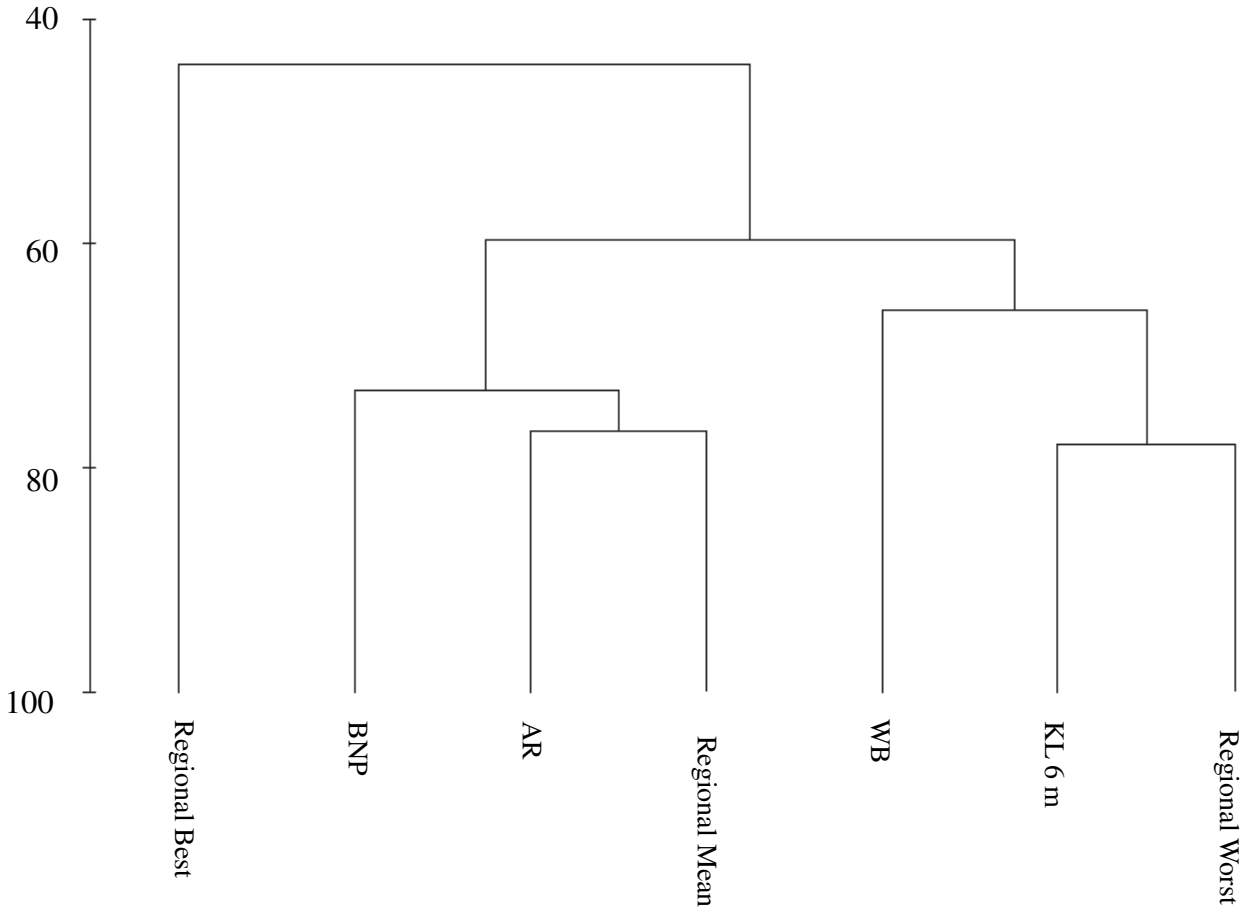


Figure 3.7. Cluster analysis based on Atlantic Gulf and Rapid Reef Assessment (AGRRA) biotic health indicators comparing study sites to regional AGRRA values for Caribbean reefs >5 m.

4. Symbiont-bearing Foraminifera as Indicators of Reef Health

4.1. Abstract

Symbiont-bearing ('larger') benthic Foraminifera (LBF) assemblages were examined at four 6 m deep patch reefs within Biscayne National Park (BNP) and the upper Florida Keys National Marine Sanctuary (FKNMS), and along a 3 – 18 m depth transect in FKNMS between August 2001 and February 2003. Populations of *Amphistegina gibbosa*, the dominant LBF, were assessed based on densities, size distributions, prevalence and severity of bleaching, and shell damage. These criteria were used in conjunction with physical and environmental data to assess the suitability of these reef sites to support growth and reproduction of calcifying organisms that host algal endosymbionts (i.e., "reef health").

Densities of *A. gibbosa* and other LBF were typically higher at Algae Reef (AR), a reef adjacent to an intact mangrove shoreline, than at Key Largo 6 m, which is closer to developed shoreline. Biscayne National Park had the lowest densities of all LBF, suggesting that water quality there was generally unsuitable for survival to maturation. Concurrent studies of lesion recovery on colonies of the coral *Montastraea annularis* species complex showed the same ranking of 6 m sites as LBF abundances, with both lesion recovery rates and LBF abundances highest at AR and lowest at BNP. Bleaching and breakage of *A. gibbosa* indicated chronic stress at all sites, with no evidence for acute photic stress during the study period. Similarly, no coral bleaching was observed during the study. Evaluation of LBF populations can provide managers with a relatively quick, low-cost method for determining presence and relative intensity of stressors influencing calcifying organisms that host algal symbionts.

4.2. Introduction

4.2.1. Larger Benthic Foraminifera as Indicators of Reef Condition

The biogenic constituents in reef sediments reflect the makeup of the benthic community and thereby characterize coral reef health. For example, reef sediments that favor calcifying organisms dependent on algal endosymbionts (i.e., mixotrophic organisms) over autotrophs (e.g., macroalgae) and heterotrophs (e.g., mollusks, sponges) are typically dominated by shells of larger benthic foraminifers (LBF) and physically degraded coral debris (e.g., Hallock 1988, 2005). Benthic foraminifers that host algal endosymbionts are useful bioindicators for reef studies because they 1) have physiological analogies with zooxanthellae corals and therefore similar water-quality requirements, 2) are abundant in healthy reef ecosystems and collected with minimal effort and effect on reef resources, and 3) have relatively short life spans and therefore comparably rapid responses to environmental stressors (Cockey et al. 1996, Hallock 2000, Hallock et al. 2003). Responses of populations of *Amphistegina* can indicate the presence and intensity of photo-oxidative stress, as well as general water quality suitability on times scales of weeks to months (Hallock et al. 2006a,b). My project provided the opportunity to assess LBF assemblages, including detailed assessments of populations of *Amphistegina*, at the same time coral assemblages and health of individual coral colonies were being assessed. This provided another line of evidence about coral reef health and also the opportunity to determine which LBF parameters had responses similar to coral parameters.

4.2.2. Rationale for Assessing Populations *of Amphistegina*

Amphistigina is the dominant, algal symbiont-bearing foraminiferal genus found on reefs worldwide (Langer & Hottinger 1997, Hallock 1999), commonly living on coralline and filamentous algae on reef substrate, as well as on some macrophytes. The two most similar species, *A. gibbosa* in the Caribbean and *A. lessonii* in the Indo Pacific, can be found from the shallowest subtidal zones to >100 m depth, and tend to be most abundant between 15 m and 40 m depth (Hallock 1999). The distribution of *Amphistegina* appears to be controlled regionally by temperature (~12-33^{o} C) and locally by hydrodynamics, water quality, light and substrate (Hallock 1999). Stress symptoms

similar to those reported for corals and coral-reef communities (e.g., bleaching, predation, and algal infestation) have been observed in populations of *Amphistegina* (Hallock et al. 2006b). *Amphistegina* are sensitive to water quality and bleach in response to excess radiant energy (Hallock et al. 2006b). These protists respond to environmental conditions within days to weeks and provide a low-cost method to quickly distinguish between local environmental conditions (e.g., water quality) and photo-oxidative stress at my study sites.

Amphistegina host diatom endosymbionts in a relationship analogous to coral-zooxanthellae symbioses (e.g., Lee & Anderson, 1991). Therefore, light is necessary for growth and calcification. However, high intensities of solar radiation, particularly shorter wavelengths (blue, violet and ultraviolet: 290-490 nm; $\geq$ 0.10 W m^{-2}), can induce photoinhibition (Muller 1978, Lee et al. 1980, Williams & Hallock 2004), loss of symbionts (Hallock et al. 1986b, 1995, Talge & Hallock 2003, Williams & Hallock 2004) and suppressed growth rates (Williams & Hallock 2004). Bleaching has been attributed to high levels of solar radiation, particularly shorter, higher energy wavelengths (UV-B, 290 – 320 nm) in corals (Lesser et al. 1990, Gleason & Wellington 1993, Glynn 1993, Jones et al. 1998, Lesser & Farrell 2004) and other symbiont-bearing marine organisms (Jokiel 1980, Williams & Hallock 2004, Hallock et al. 2006b). Bleaching was first documented in *Amphistegina* in laboratory experiments (Hallock et al. 1986b) and in field populations on the Florida reef tract beginning in summer 1991 (see Hallock et al. 2006a, b and references therein).

The abrupt onset of bleaching in *A. gibbosa* in late June 1991 was postulated to be associated with stratospheric ozone depletion following eruptions of Mt. Pinatubo in May-June 1991 (Hallock et al. 1992, 1995). Symbiont color loss is caused by degradation and digestion of the diatom endosymbionts, and partial bleaching induced by photoinhibition in laboratory experiments was identical to that seen in field-collected specimens (Talge & Hallock 1995, 2003). Populations of *Amphistegina* in the Florida Keys were monitored between 1991 and 1999 (Hallock et al. 1995, Williams et al. 1997, Hallock et al. 2006b) for changes in size distributions and condition (i.e., visible [with stereo microscope] color changes and shell damage) providing information (Table 4.1) on these protists for comparison with this study.

While thermal stress has been linked to coral bleaching (Marshall & Schuttenberg 2006), bleaching in *Amphistegina* is clearly not influenced by temperatures currently experienced along the Florida reef tract (Hallock et al. 2006b). Salinity also was ruled out as a potential cause of bleaching in *A. gibbosa* (Williams 2002). Bleaching incidence and intensity in these foraminifers typically increases in March, when water temperatures are near their lowest, peaking in July following the summer solstice, and declining in late summer when water temperatures are warmest (Hallock et al. 1995, Williams et al. 1997, Williams 2002). In culture, exposure to 32° C at either 6-8 or 13-15 µmol photon m^{-2} s^{-1} of photosynthetically active radiation (PAR) 12 hr d^{-1} for 4 weeks induced significant symbiont loss (Talge & Hallock 2003). However, in the same experiments, 4 weeks at 25° C and 13-15 µmol photon m^{-2} s^{-1} PAR induced twice as much symbiont loss as exposure to 32° C at 6-8 µmol photon m^{-2} s^{-1} PAR for the same duration. The trials at 25° C and 6-8 µmol photon m^{-2} s^{-1} PAR induced no symbiont loss. Sea surface PAR intensities in summer are approximately 1200-1500 umol photon m^{-2} s^{-1}, so *Amphistegina* normally avoid photic stress by phototaxic behavior (Lee et al. 1980). Hallock (2001) and Williams (2002) concluded that bleaching in *A. gibbosa* was linked to solar radiation, based on laboratory experiments (Hallock et al. 1986b, Talge & Hallock 2003, Williams & Hallock 2004), timing of onset, seasonal and latitudinal trends in bleaching in field populations (Hallock et al. 1995, Williams et al. 1997), and significantly higher bleaching prevalence at field sites with significantly higher water transparency. Coral bleaching events usually coincide with periods of unusually warm sea surface temperatures (SST) and calm winds, resulting in increased penetration of solar radiation (Gleason & Wellington 1993, Glynn 1996, Wilkinson 1998). Bleaching in *A. gibbosa* indicates that corals are likely being exposed to photoinhibitory stress and, along with SST anomalies, can predict susceptibility of corals to bleaching and disease (Hallock et al. 2006b).

Bleaching in *A. gibbosa* is typically progressive and degenerative; severity of bleaching typically increases with increasing size of individual foraminifers (Talge & Hallock 1995, Williams et al. 1997). Prior to the onset of stress in 1991, all adult individuals observed were normally colored. Since then in affected populations, individuals smaller than 0.5 mm in diameter seldom exhibited symbiont loss whereas specimens larger than 1.0 mm were seldom normally colored. Therefore, for this study

we only evaluated bleaching in adult *A. gibbosa* (> 0.6 mm) as recommended by Williams (2002) and Hallock et al. (2006a, b).

Bleaching also affects reproductive success and recruitment of *A. gibbosa* (Hallock et al. 1995, Williams 2002), and therefore population densities and their seasonality. Detailed studies, especially Williams (2002) and others summarized by Hallock et al. (2006b), demonstrated normal seasonality in population densities, size distributions, and other parameters, and changes in those parameters with increased prevalence and intensity of bleaching (Table 4.1). Medium to high population densities indicate environments suitable to support growth and reproduction of these protists over recent weeks to months. Size-frequency distributions of individuals making up the populations require context-dependent interpretations, based on season and population densities. For example, high population densities with high proportions of juveniles in summer months indicate favorable environmental conditions, while very low population densities dominated by juveniles indicate environmental conditions suited temporarily for survival of juveniles carried into the environment by currents, but unsuitable for their growth and survival to reproduction. Similarly, high population densities and infrequent bleaching indicates that water quality is suitable and photo-oxidative stress is limited. High population densities and high incidences of bleaching indicate acute photo-oxidative stress that occurred after reproduction peaked. Chronic stress from bleaching also is associated with increased shell breakage (Toler & Hallock 1998, Toler 2002), susceptibility to predation (Hallock & Talge 1994) and endolithic infestation (Hallock 2000). Therefore, damage assessment indicates whether chronic stress is affecting the population (Table 4.1).

4.2.3. Other Larger Benthic Foraminifers

Along a Molasses Reef-Rodriguez Key transect (upper Florida reef tract), foraminiferal tests shifted from those dominated by LBF in 1959-61 (Lidz & Rose 1989), to those dominated by smaller herbivorous and detrivorous taxa in 1991 (Cockey et al. 1996). This assemblage shift is consistent with ecological/sedimentological models of community response to increased nutrient flux (e.g., Hallock 1988, Hallock 2001). I

assessed abundances of live LBF to determine environmental suitability for supporting these mixotrophic calcifying organisms.

4.2.4. Study Goals

The purpose of my study was to use a suite of parameters (Table 4.1) to determine if (1) water quality and other environmental conditions were suitable for calcifying, symbiont-bearing organisms based on densities of live symbiont-bearing foraminifers and (2) foraminifers indicated exposure to chronic or acute photic stress, expressed as bleaching or shell damage in *A. gibbosa*. My study provided a unique opportunity to monitor LBF populations at sites where morphological and physiological conditions of scleractinian corals and other reef organisms also were being monitored (Downs et al. 2005a, 2006, Fisher et al. in press, Ch. 6, Fisher et al. in prep), an essential step for refining protocols for using foraminifers as bioindicators in reef monitoring and risk assessment.

4.3 Methods

4.3.1. Study Sites

I collected foraminifers at one patch reef in Biscayne National Park (BNP) and four patch reefs plus two depths on one forereef in the upper Florida Keys National Marine Sanctuary (FKNMS). These seven sites comprised both a latitudinal transect with four sites at 6 m depth and a depth transect from Key Largo (KL) 3 m to KL 18 m (Fig. 1.1). The 6 m sites represented a spectrum of possible anthropogenic influence based on distance from urbanized coastal development. Locations of all sites ≥ 6 m were suitable for LBF with respect to salinity and hydrodynamics.

The Key Largo (KL) sites were located offshore from the most urbanized coastline of Key Largo, where natural vegetation was removed (see Fig. 2.1), natural topography altered to maximize waterfront properties and the coastline was armored with seawalls. These sites also were positioned along the route taken by recreational boaters and commercial dive operators to reach Molasses Reef and other heavily used outer reefs, so pollutants such as hydrocarbon combustion products may be more prevalent. In

addition, larger boats regularly stirred up sediments, potentially remobilizing nutrients and chemical pollutants (Kruczynski & McManus 2002).

Algae Reef (AR) also was offshore from Key Largo but situated mid-way between the Key Largo and the BNP sites, adjacent to John Pennekamp Coral Reef State Park. Most of the extensive natural coastline was native, intact and relatively vegetated with coastal hammock mangroves and seagrass beds (see Fig. 2.1). White Banks (WB) was close to the KL sites, but still adjacent to the state park (Fig. 1.1). Alina's Reef (BNP) in Biscayne National Park was closest to the urban Miami area, including a large landfill, a nuclear power plant, and watershed canals that drain into Biscayne Bay. This site also may be influenced by extensive agricultural area south and west of Miami and associated nutrients and chemicals that enter watershed canals. However, the patch reefs of Biscayne National Park are somewhat protected from anthropogenic influence by distance, including Biscayne Bay and by uninhabited barrier islands. For example, Carnahan et al. (in press) reported that heavy metal concentrations in Biscayne Bay sediments decline with distance from urban Miami, the landfill and agricultural areas to the south.

4.3.2. Sampling and Assessment of Symbiont-bearing Foraminifers

Between August 2001 and February 2003, three (August and October 2001) or five (March 2002 – February 2003) reef rubble samples were collected quarterly except sampling along the depth gradient began in October 2001. Methods of sampling LBF and populations of *Amphistegina* assessment are described in detail elsewhere (Hallock et al. 1995, Williams et al. 1997, Hallock et al. 2006a). Reef rubble, although not the only habitat available to LBF, is easily compared among study sites. Suitable rubble for LBF was readily available at all sites except KL 3 m, where rubble was both hard to find and often partially buried in fine sediments.

Rubble was placed in plastic bags, which were brought to the surface and placed in a shaded bucket for transport to shore. Onshore, rubble was scrubbed using a small brush to detach associated sediment, algae and meiofauna, including foraminifers. I traced the outline of the rubble to determine its approximate area of bottom cover. The surface area of the rubble outline was determined using image analysis software to permit

density estimates. I distributed resultant residue among 150 mm diameter petri dishes and placed them in a culture chamber maintained between 23 and 28° C (depending on the time of year) on a 12-hour light/dark schedule at ~10 μmol photon m^{-2} s^{-1} PAR. I removed all LBF whose behavior or color indicated a high probability of being alive (Hallock et al. 1986a) and placed them in a 100 mm diameter petri dish of seawater. I identified to species and counted each specimen verified to be alive based on pseudopodial activity. Maximum diameter of *A. gibbosa* was measured to the nearest 0.05 mm, and each specimen was characterized according to symbiont color and presence of damage, either from breakage, predation, endolithic infestation or deformation (Fig. 4.1; methods similar to Hallock et al. 1995). Individual *Amphistegina* were visually characterized as "unbleached" (uniform brown color), "partly bleached" (specimens possessing white patches with at least half retaining some color), or "bleached" (specimens with <50% brown color remaining). If a sample contained larger numbers of individuals (> 150), all were counted, but a subsample of 150 - 200 individuals was haphazardly selected, measured and characterized. Other LBF (see list in Table 4.2) encountered live in each sample were counted but not measured or otherwise assessed.

4.3.3. Data Analysis

Data were analyzed in two groups: (1) by sites of 6 m depth along the northeast – southwest traverse (BNP, AR, WB and KL 6 m), and (2) by sites along the depth gradient: KL 3, 6, 9 and 18 m. The 6 m site was common to both groups (Fig. 1.1). I calculated mean density of all LBF, and, for *A. gibbosa* only, percent juveniles (individuals < 0.6 mm in diameter), mean (maximum) diameter and percent of adults that exhibited bleaching (e.g., were partly bleached or bleached; Williams, 2002). I also calculated the percentage of specimens that exhibited shell damage (i.e., chipped, broken, or deformed; Toler & Hallock 1997, Toler 2002) in samples between March 2002 and February 2003.

I used repeated-measures MANOVA to determine if significant differences existed in % juveniles and % shell damage among sites. I checked model assumptions (e.g., sphericity, homogeneity of variances, normality, and independence) using residual plots. Density and diameter data were $\log_{10}$ transformed to meet these assumptions. In

cases where the sphericity assumption was not met, I applied a univariate (unadjusted epsilon) approach. To interpret differences detected by MANOVA, I used one-way ANOVA followed by the Tukey-Kramer Honestly Significant Difference (HSD) method.

I used ANOSIM2 (two-way analysis of similarities) to determine if LBF assemblages differed significantly among sites (averaged over the entire study period) and times (averaged across all sites). To do this, I first calculated Bray-Curtis similarity matrices for all log-transformed LBF densities. For each sampling period, I used ANOSIM (one-way analysis of similarities) to determine if sites differed significantly based on assemblages of all LBF. I used MDS (multi-dimensional scaling) to determine how sites clustered based on densities of all LBF followed by SIMPER (similarity percentages) analyses to determine which species were primarily responsible for grouping of sites (Clarke and Warwick, 2001). I performed ANOSIM2, ANOSIM, MDS and SIMPER using PRIMER v.5 (Plymouth Routines in Multivariate Ecological Research PRIMER-E Ltd., Plymouth). All other statistical analyses used JMP v.3.2. (SAS Institute Inc., Cary, NC, USA), with $\alpha = 0.05$ for all hypothesis tests.

4.4. Results

4.4.1. 6 m Sites

4.4.1.1. Responses of *Amphistegina*

Live densities of *Amphistegina gibbosa* ranged from 4.8×10^2 to 4.37×10^4 individuals m^{-2} with a mean (± SE hereinafter) of 1.2×10^4 ($\pm 8.5 \times 10^2$) m^{-2} (n = 124). Densities differed significantly among the 6 m sites (repeated measures MANOVA: site effect $F_{3,8} = 20.6$, $p < 0.0004$) and sampling dates (time effect $F_{6,3} = 38.1$, $p < 0.007$; Fig. 4.2A), but there were no site x time interactions. Algae Reef had significantly higher densities than KL 6 m and BNP, and WB also had significantly higher densities than BNP (ANOVA: $F_{3,120} = 16.5$, $p < 0.0001$). Population densities were significantly higher in June and August 2002 than in August 2001 and February 2003 (ANOVA: $F_{6,117} = 4.8$, $p = 0.0002$).

Percentages of juvenile *A. gibbosa* ranged from 25 to 92%, with a mean of 62 ± 1 % (n = 122). The percentage of juveniles changed significantly with time (repeated measures MANOVA: time effect $F_{6,48} = 7.6$, $p < 0.0001$; Fig. 4.3A) but not among sites

or with site x time interactions. Percentage of juveniles was significantly higher in June and August 2002 than in February 2003 (ANOVA: $F_{6,115} = 11.4$, $p < 0.0001$).

Mean diameter of *A. gibbosa* ranged from 0.40 to 0.77 mm with an overall mean for all samples of 0.53 ± 0.01 mm (n = 124). Mean diameter changed significantly with time (repeated measures MANOVA: time effect $F_{6,48} = 11.2$, $p < 0.0001$; Fig. 4.4A), but not among sites or with site x time interactions. Mean diameter was significantly lower in summer 2002 (June and August) than all other months and significantly highest in February 2003 (ANOVA: $F_{6,117} = 14.7$, $p < 0.0001$).

Percentage of adults exhibiting any degree of bleaching ranged from 0 to 100 %, with an overall mean of 24 ± 1 % (n = 124). Incidence of bleaching differed significantly among the 6 m sites (repeated measures MANOVA: $F_{3,8} = 7.4$, $p < 0.02$; Fig. 4.5A) but not with time or time x site interactions. Incidence of partial bleaching at AR was significantly higher than at KL 6 m (ANOVA: $F_{3,80} = 3.3$, $p < 0.03$).

The percentage of the population with damaged tests ranged from 0 to 43 % with an overall mean of 17 ± 1 % (n = 100). The percentage of damaged *A. gibbosa* differed significantly among sites and with time (repeated-measures MANOVA: site effect $F_{3,16} = 7.0$, $p < 0.004$; time effect $F_{4,13} = 4.3$, $p < 0.02$; Fig. 4.6A) but their interactions were not significant. Alina's Reef had a significantly higher percentage of damaged *A. gibbosa* tests than WB (ANOVA: $F_{3,96} = 4.2$, $p < 0.01$). Percentage of test damage was significantly lower in August 2002 than in November 2002 and February 2003 (ANOVA: $F_{4,95} = 3.9$, $p < 0.006$).

4.4.1.2. Other Symbiont-bearing Foraminifera

Densities of LBF ranged from 6.7×10^2 to 9.97×10^4 individuals m^{-2} with a mean of $2.2 \times 10^4 \pm 1.6 \times 10^3$ m^{-2} (n = 130; Fig. 4.7). Geographic location was the primary factor affecting LBF densities (averaged across all time periods; ANOSIM2: Global R = 0.28, p = 0.10%) with significant differences among all sites (Table 4.3). The highest dissimilarity was between WB and BNP and the lowest between WB and AR (Table 4.3). Densities of LBF were highest at AR and lowest at BNP (Fig. 4.7A, 4.8A-C). Densities of LBF differed significantly with time (averaged across all 6 m sites; ANOSIM2: Global

R = 0.23, p = 0.10%). The highest dissimilarity was between October 2001 and June 2002 (Table 4.4).

Similarity percentages analyses (SIMPER) revealed that *Amphistigena gibbosa* was the dominant LBF at all sites and therefore the best indicator among 6 m sites (Table 4.5; Fig. 4.8). *Archaias angulatus* was the second most abundant and second most diagnostic, followed by *Laevipeneroplis proteus, Heterostegina depressa,* and *Broekina orbitolitoides.* Together with *A. gibbosa*, these taxa were the dominant contributors to differences among 6 m sites (Table 4.5). Based on LBF, Algae Reef and BNP were the most dissimilar, while White Banks represented a midpoint between AR (dissimilarity 36.8%) and KL 6 m (dissimilarity 36.4%). Alina's Reef was more dissimilar to all FKNMS sites than they were to each other.

Densities of *Archaias angulatus* ranged from 0 to 3.10 x 10^4 individuals m^{-2} with an overall mean of 3.98 x 10^3 ± 514 individuals m^{-2} (n = 124). Densities of *Archaias angulatus* differed significantly among sites (repeated measures MANOVA: $F_{3,8} = 35.0$, $p < 0.0001$); BNP had significantly lower densities than the other three sites (474 ± 122 vs. 5150 ± 640 individuals m^{-2}, respectively; ANOVA: $F_{3,120} = 14.7$, $p < 0.0001$; Fig. 4.9A).

Densities of *Laevipeneropolis proteus* ranged from 0 to 1.68 x 10^4 individuals m^{-2} at the 6 m sites with an overall mean of 2.53 x 10^3 ± 264 individuals m^{-2} (n = 124). Densities of this LBF differed significantly among sites, seasons and their interactions (repeated measures MANOVA: site effect $F_{3,8} = 4.1$, $p < 0.05$; time effect $F_{6,48} = 2.7$, $p < 0.03$; site x time interaction $F_{18,48} = 2.5$, $p < 0.006$; Fig. 4.9B). Densities of *L. proteus* were significantly lower at BNP than the other sites in August 2001 (207 ± 53 vs. 2139 ± 437 individuals m^{-2}, respectively; ANOVA: $F_{3,8} = 17.6$, $p = 0.0007$) and October 2001 (54 ± 28 vs. 2826 ± 882 individuals m^{-2}, respectively; ANOVA: $F_{3,8} = 10.9$, $p < 0.004$; Fig. 4.9B).

Densities of *Heterostegina depressa* ranged from 0 to 6.94 x 10^3 individuals m^{-2} with an overall mean of 645 ± 88 individuals m^{-2} (n = 124). Densities of this shallow-water species differed significantly among sites and site x time interactions (repeated measures MANOVA: site effect $F_{3,8} = 4.7$, $p < 0.04$; site x time effect $F_{18,9} = 3.6$, $p < 0.03$; Fig. 4.9C). Densities of *H. depressa* were significantly higher at AR than BNP in

August 2001 (1717 ± 700 vs. 90 ± 90 individuals m^{-2}, respectively; ANOVA: $F_{3,8} = 4.8$, $p < 0.04$). Densities of *H. depressa* were significantly higher at AR and WB than KL 6 m and BNP in October 2001 (2189 ± 498 and 164 ± 30 vs. 26 ± 26 and 0 ± 0 individuals m^{-2}, respectively; ANOVA: $F_{3,8} = 21.4$, $p = 0.0004$). Algae Reef had significantly higher densities of *H. depressa* than BNP in November 2002 (1755 ± 507 vs. 105 ± 53 individuals m^{-2}, respectively; ANOVA: $F_{3,16} = 4.1$, $p < 0.03$; Fig. 4.9C).

Densities of *Broekina orbitolitoides* ranged from 0 to 5.31 x 10^3 individuals m^{-2} with an overall mean of 452 ± 86 individuals m^{-2} (n = 124). Densities of this LBF differed significantly different among sites, seasons and their interactions (repeated measures MANOVA: site effect $F_{3,8} = 5.1$, $p < 0.03$; time effect $F_{6,3} = 220.0$, $p = 0.0005$; site x time interaction $F_{18,9} = 4.2$, $p < 0.02$; Fig. 4.9D). In August 2001, densities of *B. orbitolitoides* were significantly higher at AR than at KL 6 m and BNP, and significantly higher at WB than at BNP (1465 ± 410 vs. 83 ± 42 and 0 ± 0 individuals m^{-2}, respectively; ANOVA: $F_{3,8} = 14.6$, $p < 0.002$). In October 2001, densities of *B. orbitolitoides* were significantly higher at AR and WB than at KL 6 m and BNP (3287 ± 738 and 336 ± 32 vs. 32 ± 32 and 0 ± 0 individuals m^{-2}, respectively; ANOVA: $F_{3,8} = 21.9$, $p = 0.0003$; Fig. 4.9D). Densities of *B. orbitolitoides* dropped in June 2002 at all sites except BNP, where densities were consistently low (Fig. 4.9D).

4.4.2. Depth Gradient

4.4.2.1. Responses of *Amphistegina*

Along the KL depth gradient, densities of live *Amphistegina gibbosa* ranged from 5.5 x 10^2 to 1.1 x 10^5 individuals m^{-2} with an overall mean of 1.6 x 10^4 ± 1.7 x 10^3 individuals m^{-2} (n = 111). Densities of *Amphistegina gibbosa* and differed significantly among depths (repeated measures MANOVA: $F_{3,8} = 12.9$, $p < 0.002$; Fig. 4.2 B) but not with time or their interactions. Key Largo 9 m and KL 18 m had significantly higher densities than KL 3 m and KL 6 m (ANOVA: $F_{3,107} = 14.9$, $p < 0.0001$).

The percentage of juvenile *A. gibbosa* ranged from 9 to 100 % with an overall mean of 52 ± 2 % (n = 108). Percentage juveniles differed significantly with depth and time (repeated measures MANOVA: site effect $F_{3,7} = 29.2$, $p = 0.0002$; time effect $F_{5,3} = 10.6$, $p < 0.04$; Fig. 4.3B). Key Largo 3 m and KL 6 m had higher percentages of

juveniles than KL 9 m and KL 18 m (ANOVA: $F_{3,104} = 11.1$, $p < 0.0001$). Juveniles were more prevalent in June and August 2002 than in February 2003 (ANOVA: $F_{5,102} = 4.2$, $p < 0.0002$).

Mean diameter of *A. gibbosa* ranged from 0.36 to 0.96 mm with an overall mean of 0.61 ± 0.01 mm (n = 112). Mean diameter differed significantly with depth and time (repeated measures MANOVA: site effect: $F_{3,8} = 61.5$, $p < 0.0001$; time effect $F_{5,4} = 13.4$, $p < 0.02$; Fig. 4.4B) but not their interactions. Mean diameter of *A. gibbosa* was significantly larger at Key Largo 9 m and KL 18 m than at KL 3 m and KL 6 m (ANOVA: $F_{3,108} = 24.8$, $p < 0.0001$). Significantly larger *A. gibbosa* were found in February 2003 than in June, August, and October 2002 (ANOVA: $F_{5,106} = 5.1$, $p < 0.0004$).

The percentage of adult *A. gibbosa* that were partly bleached ranged from 0 to 73% with a mean of 28 ± 2 % (n = 112). Percentage bleached differed significantly with depth (repeated measures MANOVA: $F_{3,8} = 21.6$, $p < 0.0003$; Fig. 4.5B) but not with time or their interactions. Key Largo 9 m and 18 m had significantly higher percentages of partly bleached adult *A. gibbosa* than at KL 3 m and KL 6 m (ANOVA: $F_{3,108} = 13.2$, $p < 0.0001$).

The percentage of *A. gibbosa* with damaged tests ranged from 0 to 53 % with a mean of 16 ± 1 % (n = 100). Percentage of *A. gibbosa* with damaged tests differed significantly with depth (repeated-measures MANOVA: site effect $F_{3,16} = 10.9$, $p < 0.0005$; Fig. 4.6B) but not with time or their interactions. Key Largo 3 m had a significantly lower percentage of damaged *A. gibbosa* tests than other depths (ANOVA: $F_{3,96} = 9.3$, $p < 0.001$).

4.4.2.2. Other Symbiont-bearing Foraminifera

Densities of LBF ranged from 1.8×10^3 to 4.39×10^5 individuals m^{-2} along the depth gradient with a mean of $4.34 \times 10^4 \pm 6.1 \times 10^3$ individuals m^{-2} (n = 125). Depth was the primary factor affecting LBF densities (averaged across all time periods; ANOSIM2: Global R = 0.53, p = 0.10%) with significant differences among all depths (Table 4.6). The highest dissimilarity was between KL 3 m and KL 9 m and the lowest between KL 9 m and KL 18 m (Table 4.6). Densities of LBF were highest at KL 9 m and

lowest at KL 3 m (Fig. 4.7B, 4.10). LBF densities differed significantly with time (averaged across depths; ANOSIM2: Global R = 0.18, p = 0.10%) with the highest dissimilary between October 2001 and June 2002 (Table 4.7).

While *Amphistegina gibbosa* generally was the dominant LBF at all sites, SIMPER analyses revealed that *A. gibbosa* did not contribute highly to dissimilarities along the depth gradient (Table 4.8). *Cyclobiculina compressus* was the primary discriminating species along the depth gradient, followed by *Asterigerina carinata*, *Heterostegina depressa* and *Broekina orbitolitoides* (Table 4.8, Fig. 4.11 A-D). Based on LBF, the largest differences were between KL 3 m and the deeper sites: KL 9 m (51.2%) and KL 18 m (46.9%). The lowest dissimilarity (19.8%) was between the two deep sites, KL 9 m and KL 18 m.

4.5. Discussion

Over the last thirty years, water quality has declined in the Florida Keys due to changes in water flow patterns from Florida Bay, sedimentation (from boat traffic and development), and increased near-shore nutrient concentrations (from local wastewaters, freshwater upwelling, fertilizers, and industrial pollutants; Szmant and Forrester 1996, Lang et al. 1998, Causey et al. 2000, Porter et al. 2001, Andrews et al. 2005). Changes in water quality corresponded with significant decreases in live coral cover throughout the Florida Keys (Dustan and Halas 1987, Lang et al. 1998, Causey et al. 2000, Beaver et al. 2005) and a shift from symbiont-bearing foraminifers to heterotrophic foraminifers (Cockey et al. 1996). In this study, I evaluated LBF densities and populations of *Amphistigina gibbosa* at seven coral reefs and related this information to concurrent studies on environmental assessments, community condition and coral physiological condition.

The pattern of LBF densities among my study sites is consistent with susceptibility to reduced water quality (e.g., Cockey et al. 1996, Hallock et al. 2003). If conditions were optimum at all sites, LBF densities should exceed 10^4 individuals/m^2, with highest densities (approaching 10^5) in summer months and lowest in winter or early spring (i.e., December through March; Hallock et al. 1986a). Mean densities of both *A. gibbosa* and total LBF at my study sites were approximately 10^4 individuals/m^2,

indicating generally marginal to suitable conditions. Densities of LBF were highest at AR, a site adjacent to John Pennekamp Coral Reef State Park, which is characterized by extensive mangrove and seagrass flats that separate this site from urban development and provide a consistent source of colored dissolved organic matter (CDOM; Ayoub et al. 2006), which preferentially absorbs higher energy wavelengths of solar radiation (e.g., Kirk 1996). In contrast, KL 6 m had significantly lower densities of *A. gibbosa* and the other LBF. This site is off a highly developed coastline and has more variable CDOM concentrations (Ayoub et al. 2006). White Banks, which is physically closer to the KL 6 m site than AR but still adjacent to state park waters, had intermediate densities of *A. gibbosa* and other LBF. Densities of *A. gibbosa* and all other symbiont-bearing foraminifers were lowest in BNP, offshore of Miami, FL, indicating that some aspect of water quality there was unsuitable.

Seasonality would be expected along the depth transect, with increasing densities of *A. gibbosa* with depth over the depth range studied. Relative proportions of other taxa also would be expected to change with depth, since some species, e.g., *Archaias angulatus* and *Laevipeneroplis proteus*, tend to be shallower dwelling than others, e.g., *Cyclorbiculina compressa* and *Broeckina orbitolitoides* (see Table 4.2). Along the depth gradient, densities of *A. gibbosa* and other LBF were higher at KL 9 m and KL 18 m than at the shallower sites (KL 3 m and KL 6 m), which was anticipated because these protists prefer depths of 15 – 40 m (Hallock 1999). Densities of LBF were higher at KL 9 m than at KL 18 m, suggesting better conditions at KL 9 m relative to KL 18 m. Key Largo 9 m and KL 18 m were more comparable to sites monitored by Williams (2002): Conch (CR 10 m, CR 18 m) and Tennessee (TN 8 m, TN 20 m) Reef. Densities at KL 9 m and KL 18 m were lower than densities observed at CR and TN during periods of low stress (> 10^4 individuals m^{-2}) but higher than densities at CR and TN following acute bleaching events (10^3 to 10^4 individuals m^{-2}) in 1991 and 1998 (Williams 2002). No acute stress events occurred during my study (Ch. 2) but chronic stress presumably limited population densities as indicated by intermediate densities and bleaching.

Healthy *Amphistigina* populations typically reproduce by alteration of semelparous asexual and sexual generations (Harney et al. 1998). Asexual reproduction commonly occurs in the spring and each large individual can produce broods of

approximately 100 to 300 offspring (Williams 2002). Thus, population densities can increase dramatically in summer. Sexual reproduction by gamete broadcasting commonly occurs in the fall. Under ideal conditions, percentages of juvenile *A. gibbosa* should be highest (on the order of 40-60%) in early summer (May-July) and lowest, generally <40% in winter and early spring (December-March; Williams 2002). Similarly, mean individual diameters should be highest (approaching 1 mm) in late winter-early spring (February-March) and lowest (~0.6-0.7 mm) in mid summer. Increases in both population densities and percentages of juveniles at my study sites indicate successful reproduction. The percentage of juveniles at all sites was relatively high (> 40% all year). Williams (2002) typically observed 20 – 40% percentage juveniles during spring and winter months and between 40 – 60% during summer months in low-stress years at nearby Conch Reef, which is located immediately south of the area shown in Fig. 1.1. The high percentage of juveniles year-round at my study sites may indicate that zygotes or juveniles were consistently carried to my sites by currents, yet relatively few grew to adult sizes (> 0.6 mm in diameter). For example, Williams (2002) typically observed a mean diameter > 0.7 mm, whereas most *A. gibbosa* in this study were approximately 0.5 mm in diameter. In addition, summer abundances at BNP were comparable to the lowest densities at AR and WB, so conditions at BNP maybe unsuitable for survival of *A. gibbosa* throughout its life cycle.

In the absence of photo-oxidative stress, no bleaching and minimal breakage (<10% of the *A. gibbosa* individuals) would be anticipated. With strong seasonal photo-oxidative stress, one expects frequent bleaching in early summer and lowest percentages in winter to early spring. Because breakage is cumulative though the seasonal cycle of a bleaching-stressed population, highest percentages of shell damage are expected in the fall or winter (Toler 2002). Alina's Reef (BNP), which had the lowest LBF densities, also had the highest percentage of *A. gibbosa* with shell damage. Low-level bleaching stress increases susceptibility of these protists to predation and infestation (Toler 2002). Along the depth gradient, shell damage was highest at sites that also had higher percentages of bleached adults (KL 9 m and KL 18 m). In the Florida Keys, shell breakage, breakage and repair, and incidences of shape anomalies of *A. gibbosa* increased approximately 3-fold even in unbleached individuals after 1992, following the onset of

bleaching (Hallock et al. 1995, Toler 2002). During this time, profoundly damaged asexual broods defined as producing fewer than 50 offspring, at least 10% of which were malformed were frequently observed (Hallock et al. 1995). Breakage incidences in samples collected before 1990 from Hawaii, Cayman Islands, Puerto Rico and Florida Keys were consistently low (between about 5 and 6 %; Hallock 1995), relative to breakage observed following bleaching events (10 – 40 %; Williams 2002) and during my study. Breakage tends to be more prevalent under chronic rather than acute stress, perhaps because chronically-stressed individuals survive longer and have a higher probability of encountering predators or experiencing physical damage (Toler 2002). Shell damage during my study was consistent with chronic stress affecting *A. gibbosa*, with the largest affect on populations at KL 9 m, KL 18 m and BNP.

Amphistigina bleach in response to excess solar energy, particularly higher-energy (blue, violet, and ultraviolet) wavelengths. Bleaching was unknown in field populations of *Amphistigina* before 1988; since 1992 it has been observed annually on the Florida reef tract and in all oceans (Hallock 2000, Hallock et al. 2006b). Prevalence and severity of bleaching declined in Florida reef tract populations between 1992 and 1997, with a sharp increase in summer 1998 (Williams 2002, Hallock et al. 2006b). In summers of 1997 and 1999, bleaching prevalence and severity did not vary significantly with season but observations of bleaching in about 20% of adults indicated that photo-inhibitory stress was still chronic (Williams 2002, Hallock et al. 2006b). I frequently observed partly bleached individuals, but the percentage of the population exhibiting bleaching was generally low (~ 25%) and showed no clear seasonal trends. Thus, bleaching during my study likely was caused by a chronic rather than an acute stress, similar to observations made in 1997 and 1999 (Williams 2002).

Percentage of bleached *A. gibbosa* was highest at AR and at the deeper sites (KL 9 m and KL 18 m). Damage accumulates with test size (Williams et al. 1997, Williams 2002) because bleaching in *A. gibbosa* progresses as a degenerative disease, with permanent symbiont loss in affected chambers. Larger individuals at AR, KL 9 m and KL 18 m therefore had more time to accumulate damage. A paradox of chronic bleaching in these foraminifers is that incidences tend to be highest at sites otherwise suitable for growth and reproduction. At sites where juveniles recruit but other stressors

limit survival, percentage of juveniles will be higher, but both abundance and incidences of bleaching will be lower.

Successful reproduction, as shown by increases in juveniles during summer months, indicates that bleaching stress was not affecting reproduction. No coral bleaching was observed during my study, supporting this conclusion. However, the prevalence of smaller sized individuals indicated that some unidentified stressors limited growth and survival. Chronic bleaching stress may result from oxidative damage passed onto offspring or increased photosensitivity from exposure to photoinhibitors (e.g., herbicides, PAHs) acting alone or synergistically affecting *A. gibbosa* at these sites. Further investigation of UV and PAR levels, CDOM concentrations and concentrations of known photoinhibitors, along with cellular biomarker data, would help elucidate mechanisms and causes of bleaching in *A. gibbosa* at these sites.

4.6. Conclusions

Densities and conditions of symbiont-bearing foraminifera support conclusions based on other indicators (e.g., coral lesion regeneration rates; Fisher et al., in press; Ch. 5) that the relatively favorable AR site contrasted most sharply with the unfavorable BNP site. All LBF parameters except bleaching prevalence indicated that *A. gibbosa* were most stressed at the BNP site, likely due to unfavorable water quality (Cockey et al. 1996, Hallock et al. 2006a, b). Downs et al. (2005a, 2006) found evidence for a toxic response in both corals (*Montastraea annularis*) and white grunts (*Haemulon plumieri*) at BNP. Reef community assessments (Ch. 2) indicate that coral decline at BNP likely began relatively recently (within the last 10 years) based on high recent coral mortality and high macroalgal abundance. Densities of LBF were higher at KL 9 m than at KL 18 m suggesting that KL 9 m provides slightly better habitat than KL 18 m, which corroborates conclusions based on coral lesion regeneration rates (Ch. 5; Fisher et al. in press) and biomarker profiles (Ch. 6; Fisher et al. in prep).

Abundances of *Amphistegina* were critical to distinguishing among the 6 m sites, supporting use of LBF as indicators of reef condition. Incidences of bleaching and breakage in *A. gibbosa* indicated chronic stress at all sites during the study, with no evidence for acute photic stress during the study period. Similarly, no coral bleaching

was observed during my study. Using LBF, I determined that water quality was least favorable for calcifying organisms at BNP and less than optimal at all study sites. However the stressor(s) remain unknown. Further assays (e.g., cellular diagnostic biomarkers, histological studies, contaminant analysis) may be able to provide further insight into potential stressors that are compromising water quality at BNP and thereby direct management actions.

Along the depth gradient, KL 3 m appeared to be in good condition based on high coral cover and lesion regeneration rates. However, densities of *A. gibbosa* were low indicating that these foraminifers are not good indicators at such nearshore shallow environments, due to their preference for sandy sediments. *Amphistegina* are most suitable for assessing reef environments 10 – 20 m deep but can be used to compare among shallow reefs that are neither too high-energy nor too low-energy, e.g, very shallow, nearshore reef environments such as KL 3 m that have very silty sediments. Densities of *A. gibbosa* also were low at KL 6 m and BNP, both of which are dominated by silty sediments (see Ch. 2). *Archaias angulatus, Sorites marginalis* and *Laevipeneroplis proteus* may be very abundant in low energy, nearshore environments where water quality is suitable (Fujita & Hallock 1999) and therefore may be more useful bioindicators species there. Low densities of all LBF (especially *Archaias angulatus*) at KL 3 m, KL 6 m, and BNP indicates something is limiting LBF populations, because LBF occur in high densities at some low-energy sites in the Florida Keys (e.g., Hallock et al. 1986a, Fujita & Hallock 1999).

Table 4.1. Population parameters of *Amphistegina* (late spring – early autumn unless otherwise noted) and their interpretive value

Parameter(s)	Range	Interpretation
Density (#/m^{-2})	High (>$5x10^4$)	Environmental conditions very good
	Medium (1-$5x10^4$)	Environmental conditions suitable
	Low (5-$10x10^3$)	Environment marginal
	Very low (<$5x10^3$)	Environment stressed
	Absent	Environment unsuitable
Bleaching prevalence	High (>50%)	Acute photo-oxidative (photic) stress
	Medium (10-50%)	Chronic photic stress
	Low (<10%)	Minimal chronic photic stress
	Absent	No photic stress
Density/bleaching	High/high	Acute photic stress post-reproduction
	High/Medium	Chronic photic stress post-reproduction
	Medium/Medium	Chronic photic stress impacting reproduction; may include other stress
	Low/low	Environmental stress probably not photic
	Low/high	Ongoing, acute photic stress
Shell damage	High (>30%)	Highly susceptible to predation/infestation
	Medium (10-30%)	Chronically susceptible to pred/infestation
	Low (<10%)	Minimally susceptible to pred/infestation
Juveniles	High (>50%)	With high density, conditions good
		With low density, unsuitable at time scales of weeks to months
	Medium (25-50%)	Interpret in context of density and bleaching
	Low (<25%)	Reproduction impacted or suppressed
Mean diameter early summer	~0.6-0.7 mm	With medium to high densities, indicates reproductive success
	>0.8 mm	With bleaching and low densities, indicates suppressed reproduction
late winter	>0.9 mm	Large individuals available for reproduction

Table 4.2. Habitats of common larger benthic foraminifers found on Florida reefs (adapted from Levy 1991, Hallock & Peebles 1993, Hallock 1999, and Hallock pers. comm.)

Taxon	Reef Habitat	Depth (optimum)
Amphistegina gibbosa	Backreef, open shelf, reef margin	<100 m (10-40 m)
Asterigerina carinata	Open shelf, reef margin	<40 m (not known)
Gypsina spp.	Highly variable	not known
Heterostegina depressa	Deep shelf or reef margin	<100 m (30-50 m)
Archaias angulatus	Backreef, reef and open shelf	<40 m (<10 m)
Borelis pulchra	Backreef, reef and open shelf	<40 m (not known)
Broekina orbitolitoides	Backreef, reef, open shelf	<40 m (10-30 m)
Cyclorbiculina compressus	Backreef, reef and open shelf	< 40 m (5-30 m)
Laevipeneroplis bradyi	Backreef, reef and open shelf	<40 m (10-30 m)
Laevipeneroplis proteus	Backreef, reef and open shelf	<40 m (0-20 m)
Peneroplis pertusus	Backreef, reef and open shelf	<40 m (0-20 m)
Sorites marginalis	Backreef, reef and open shelf	<40 m (0-20 m)

Table 4.3. ANOSIM2 results for differences among 6 m sites (averaged across all sampling periods); Global R = 0.28, significance level = 5%

	KL 6 m	WB	AR	BNP
KL 6 m		0.24	0.30	0.37
WB			0.14	0.40
AR				0.36

Table 4.4. ANOSIM2 results for differences among sampling periods (averaged across all 6 m sites); Global R = 0.23, significance level = 5%

	Aug-01	Oct-01	Mar-02	Jun-02	Aug-02	Nov-02	Feb-03
Aug-01		n.s.	n.s.	0.46	0.36	n.s.	0.23
Oct-01			n.s.	0.51	0.33	n.s.	0.4
Mar-02				0.22	0.33	0.24	n.s.
Jun-02					0.25	0.38	0.34
Aug-02						0.17	0.26
Nov-02							0.24

Table 4.5. Identification of Key Discriminating Larger Benthic Foraminifers among the 6 m sites between August 2001 and February 2003

	AR/BNP	WB/BNP	KL 6 m/BNP	KL 6 m/AR	WB/AR	KL 6 m/WB
Mean dissimilarity	52.4	49.3	46.5	41.5	36.8	36.4
Amphistegina gibbosa	13.7	12.0	10.6	9.4	7.9	7.6
Archias angulatus	9.5	10.4	9.2	7.3	6.8	6.7
Asterigerina carinata	--	--	2.5	--	--	1.7
Borelis pulchra	--	--	2.2	1.7	1.4	1.4
Broekina orbitolitoides	4.5	3.2	--	3.7	3.4	2.4
Cyclobiculina compressus	2.3	2.0	1.9	1.9	1.8	1.7
Heterostegina antillarium	4.7	3.7	3.7	3.7	2.8	2.6
Gypsina sp.	2.9	--	1.8	2.4	2.3	--
Laevipeneroplis proteus	7.2	6.8	6.5	5.1	4.9	4.9
Peneroplis pertusus	2.3	2.5	2.9	2.1	2.0	2.3
Sorites marginalis	1.7	1.9	2.3	1.6	--	1.8

Table 4.6. ANOSIM2 results for differences among depths (averaged across all sampling periods); Global R = 0.53, significance level = 5%

	KL 3 m	KL 6 m	KL 9 m	KL 18 m
KL 3 m		0.53	0.82	0.71
KL 6 m			0.66	0.45
KL 9 m				0.32

Table 4.7. ANOSIM2 results for differences among sampling periods (averaged across all depths); Global R = 0.18, significance level = 5%

	Oct-01	Mar-02	Jun-02	Aug-02	Nov-02	Feb-03
Oct-01		n.s.	0.36	0.22	n.s.	0.34
Mar-02			n.s.	0.23	0.14	n.s.
Jun-02				0.25	0.25	0.19
Aug-02					0.2	0.32
Nov-02						n.s.

Table 4.8. Identification of Key Discriminating Larger Benthic Foraminifers along the depth gradient between October 2001 and February 2003

	KL 3m/6m	KL 3m/9m	KL 3 m/18 m	KL 6 m/9 m	KL 6 m/18m	KL 9 m/18 m
Mean dissimilarity	40.7	51.2	46.9	33.5	32.8	36.4
Amphistegina gibbosa	--	--	--	--	7.9	7.6
Archias angulatus	4.6	4.7	3.5	1.8	6.8	6.7
Asterigerina carinata	3.4	6.8	6.7	3.8	--	1.7
Borelis pulchra	3.7	4.1	--	1.7	.4	1.4
Broekina orbitolitoides	4.0	3.7	3.7	4.3	3.4	2.4
Cyclobiculina compressus	2.5	7.6	6.2	5.5	.8	1.7
Heterostegina antillarium	4.7	5.0	5.4	3.7	2.8	2.6
Gypsina sp.	2.9	--	3.3	2.2	2.3	--
Laevipeneroplis bradyi	--	4.6	5.5	3.4	4.9	4.9
Laevipeneroplis proteus	3.4	2.7	3.1	--	4.9	4.9
Peneroplis pertusus	4.0	3.2	3.3	2.3	2.0	2.3
Sorites marginalis	3.9	4.9	3.2	2.8	--	0.8

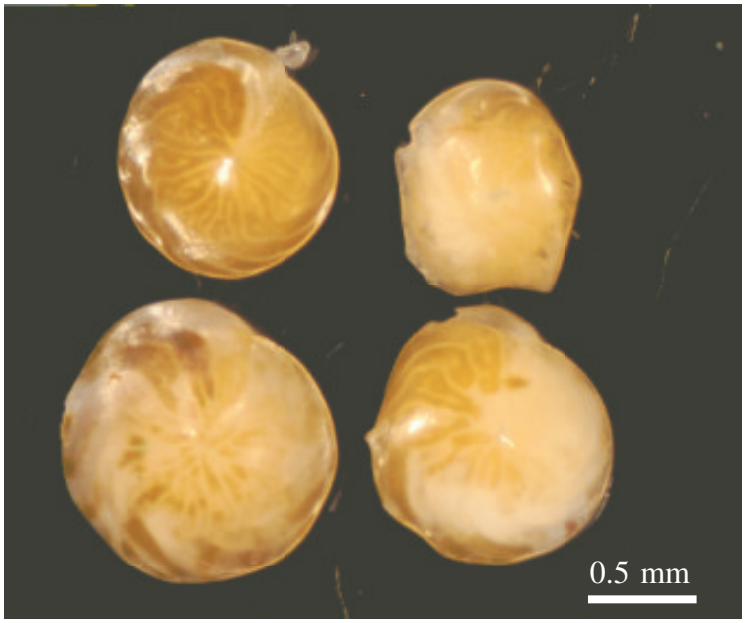


Figure 4.1. Pictures of bleaching and damage in *A. gibbosa*. Top left: normal color with no damage; Bottom left: partly bleached with no damage; Top right: pale and broken; Bottom right: partly bleached and chipped.

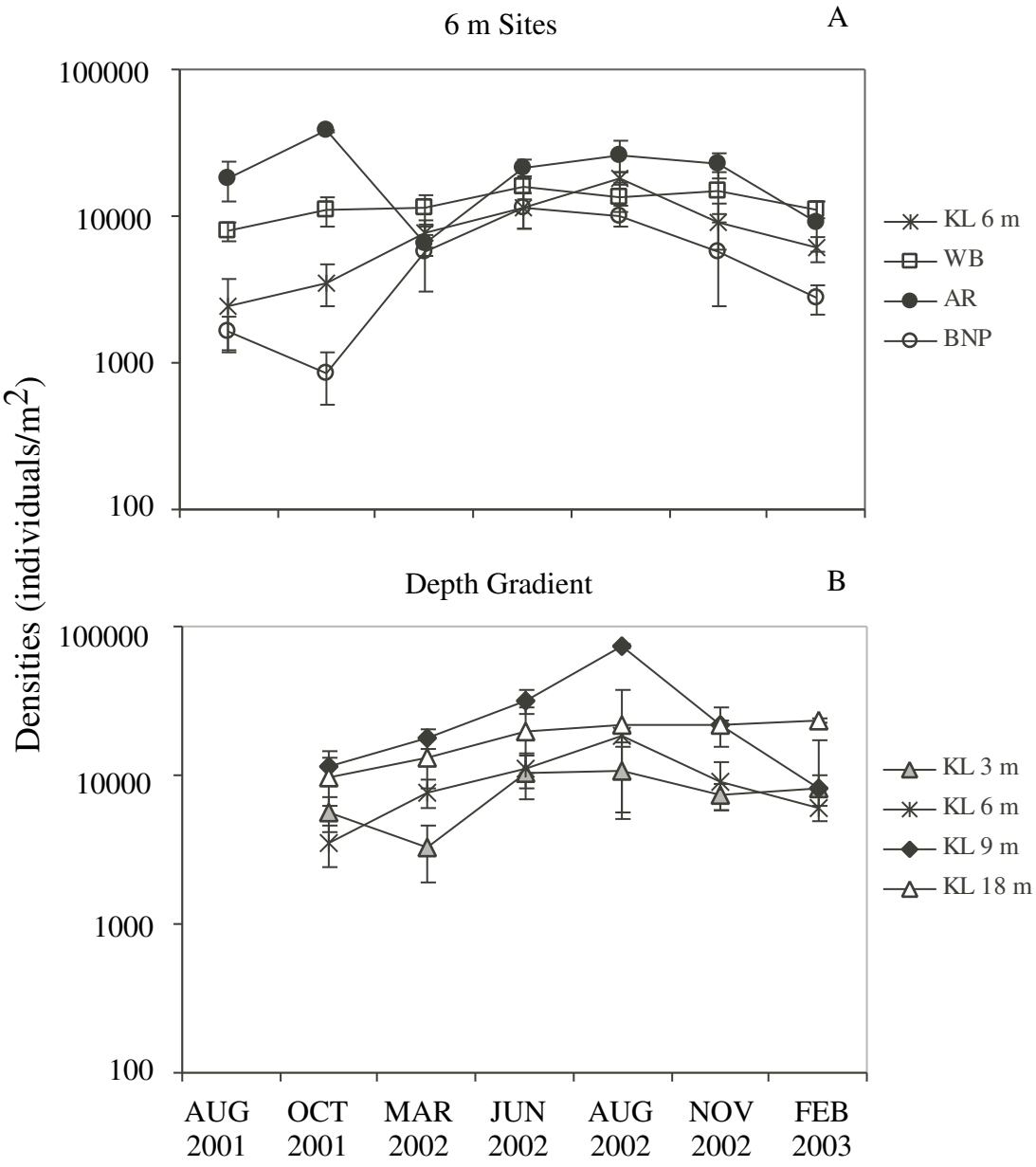


Figure 4.2. Mean (± SE) densities of *Amphistegina gibbosa* from August 2001 to February 2003 at (A) the 6 m sites and (B) along the depth gradient. Densities are plotted on a log scale.

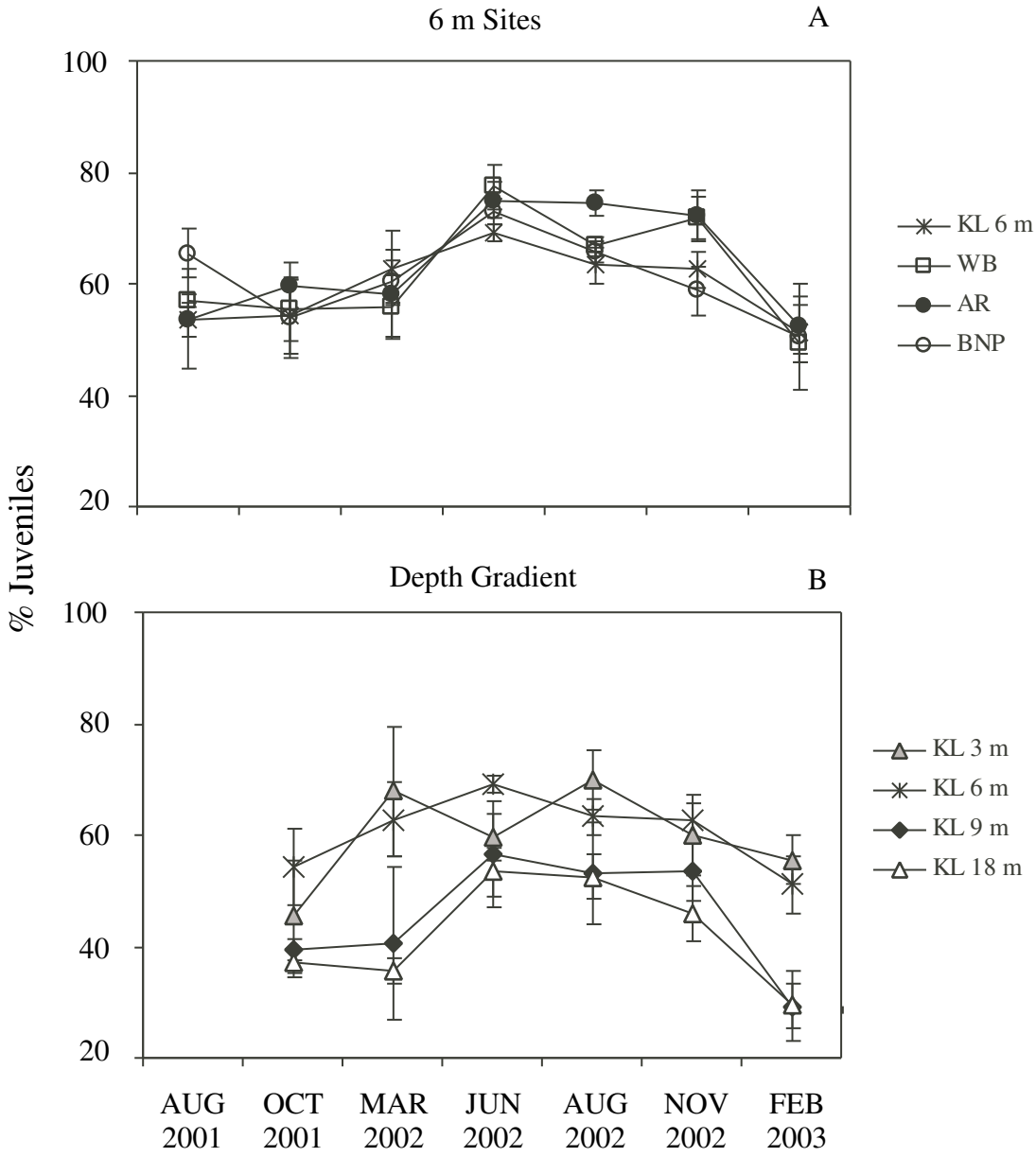


Figure 4.3. Mean (± SE) percentage of juvenile *A. gibbosa* from August 2001 to February 2003 at (A) the 6 m sites and (B) along the depth gradient.

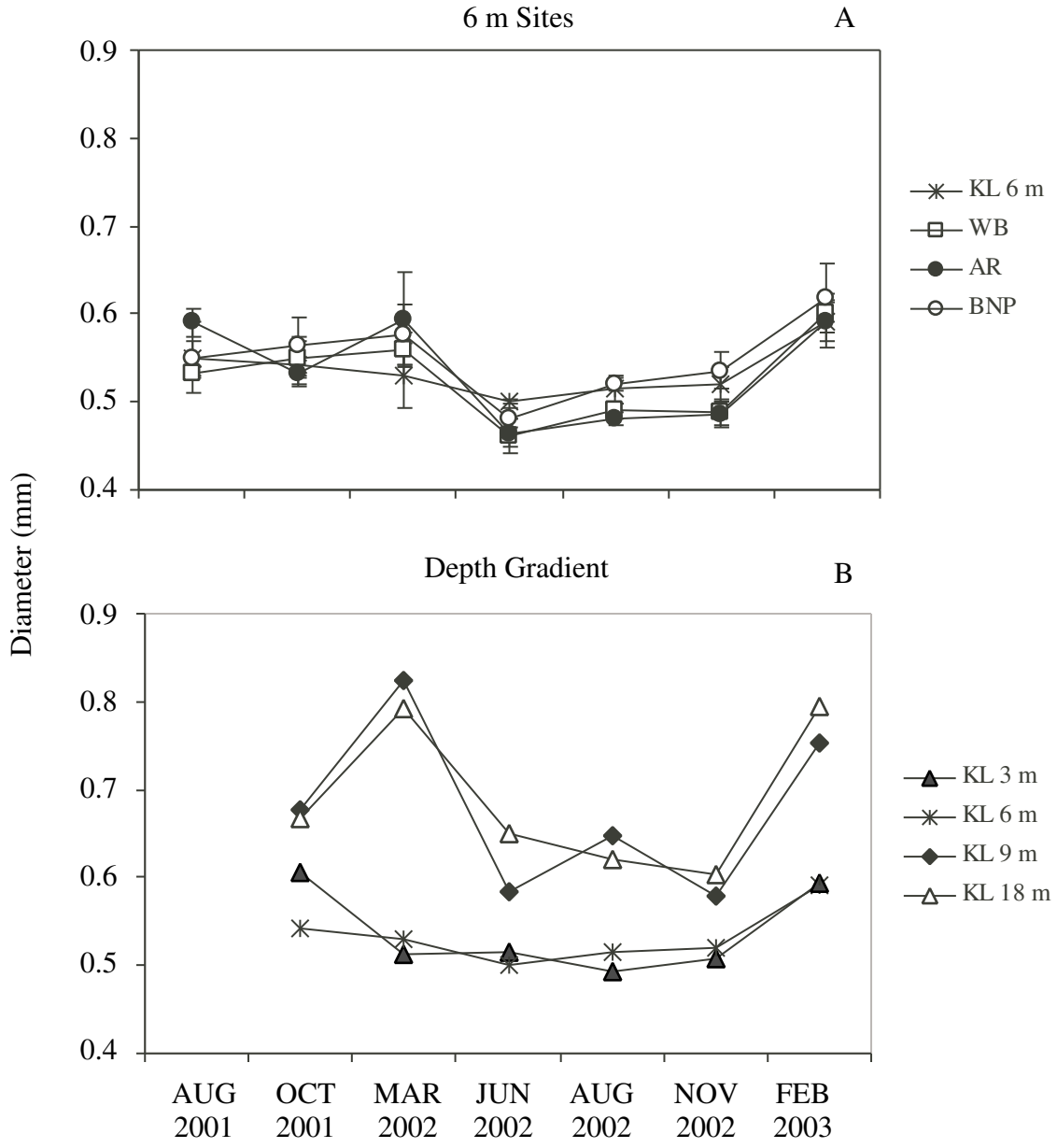


Figure 4.4. Mean (± SE) diameters of *A. gibbosa* from August 2001 to February 2003 at (A) the 6 m sites and (B) along the depth gradient.

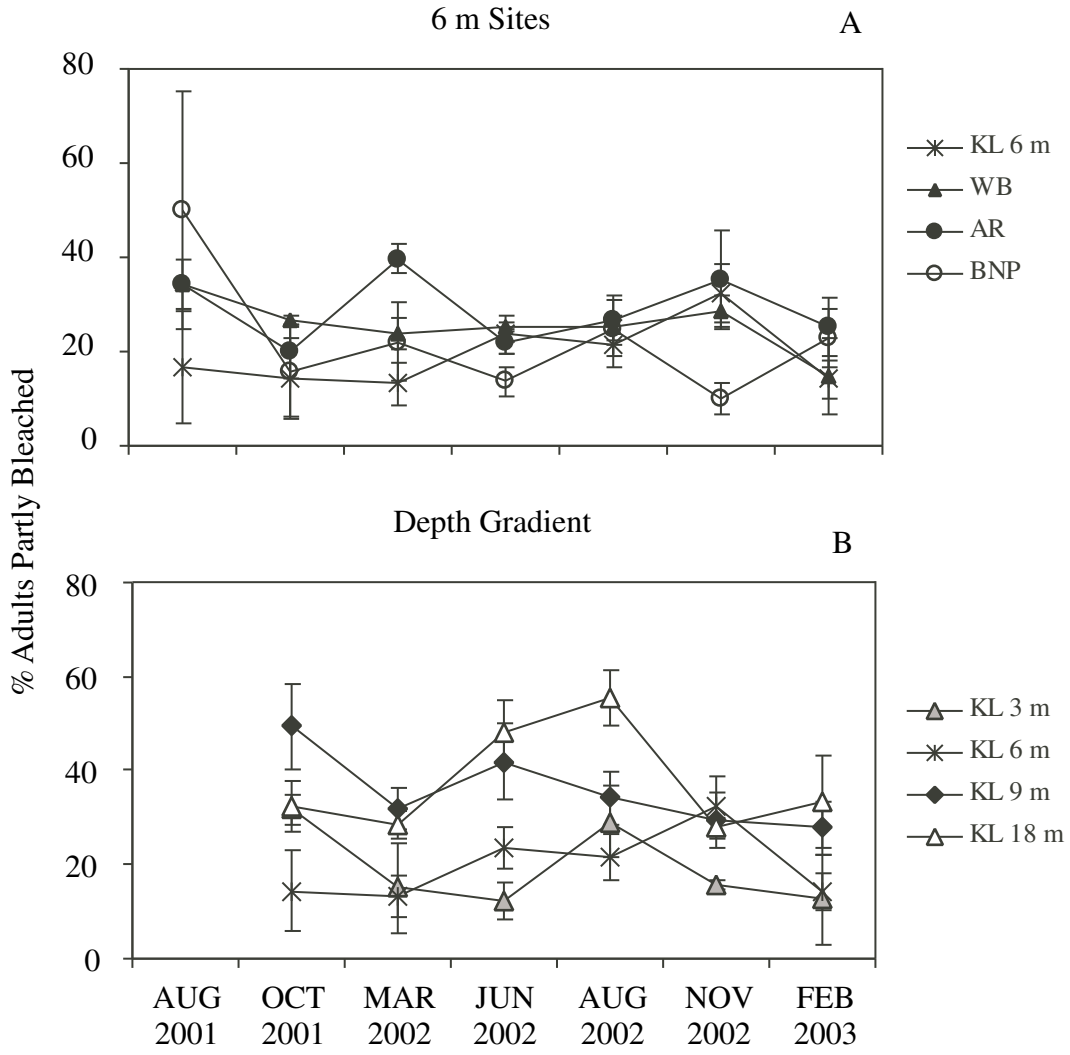


Figure 4.5. Mean (± SE) percentages of adult *A. gibbosa* exhibiting any degree of bleaching from August 2001 to February 2003 at (A) the 6 m sites and (B) along the depth gradient.

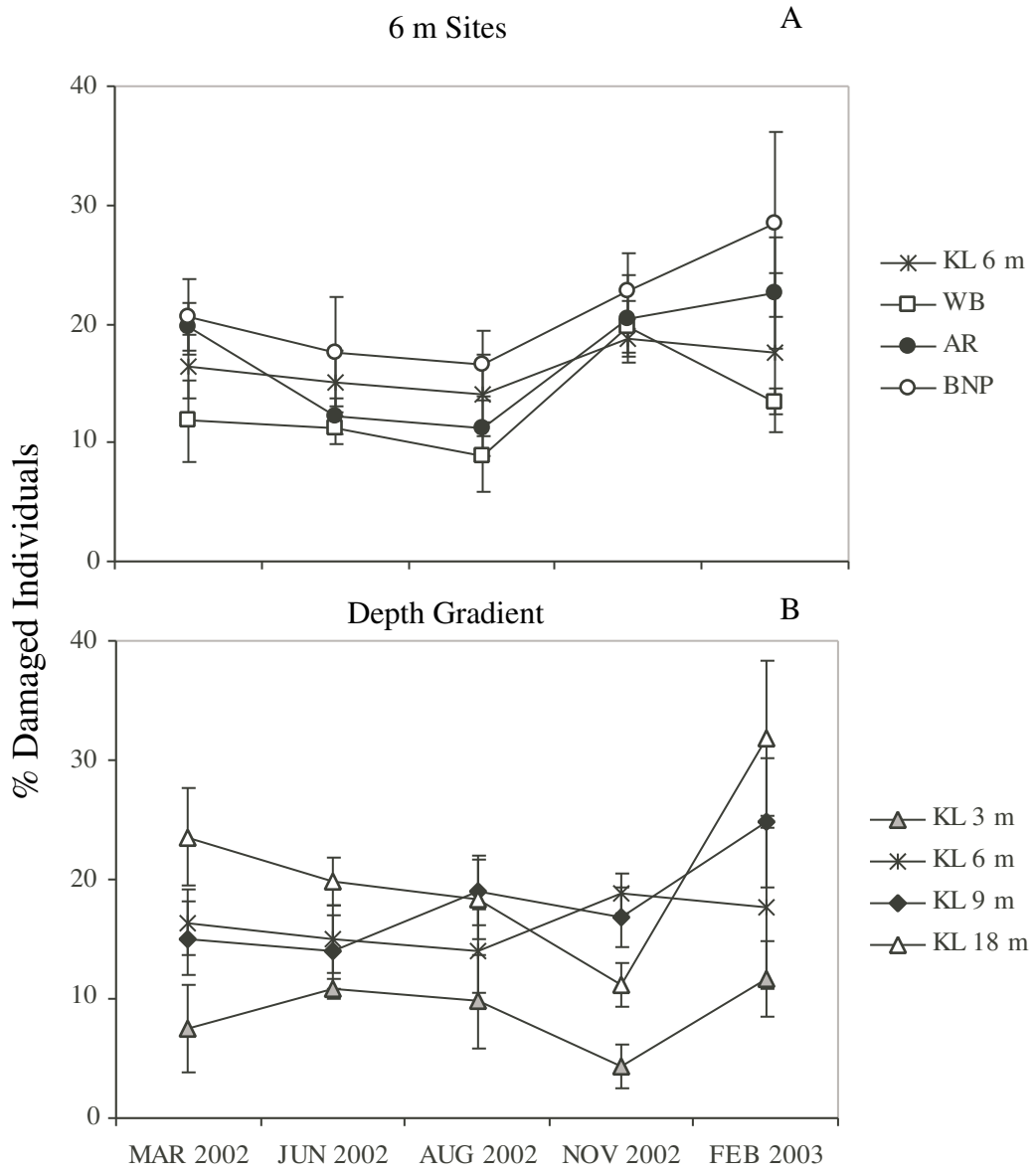


Figure 4.6. Mean (± SE) percentages of damaged tests in populations of *A. gibbosa* from August 2001 to February 2003 at (A) the 6 m sites and (B) along the depth gradient.

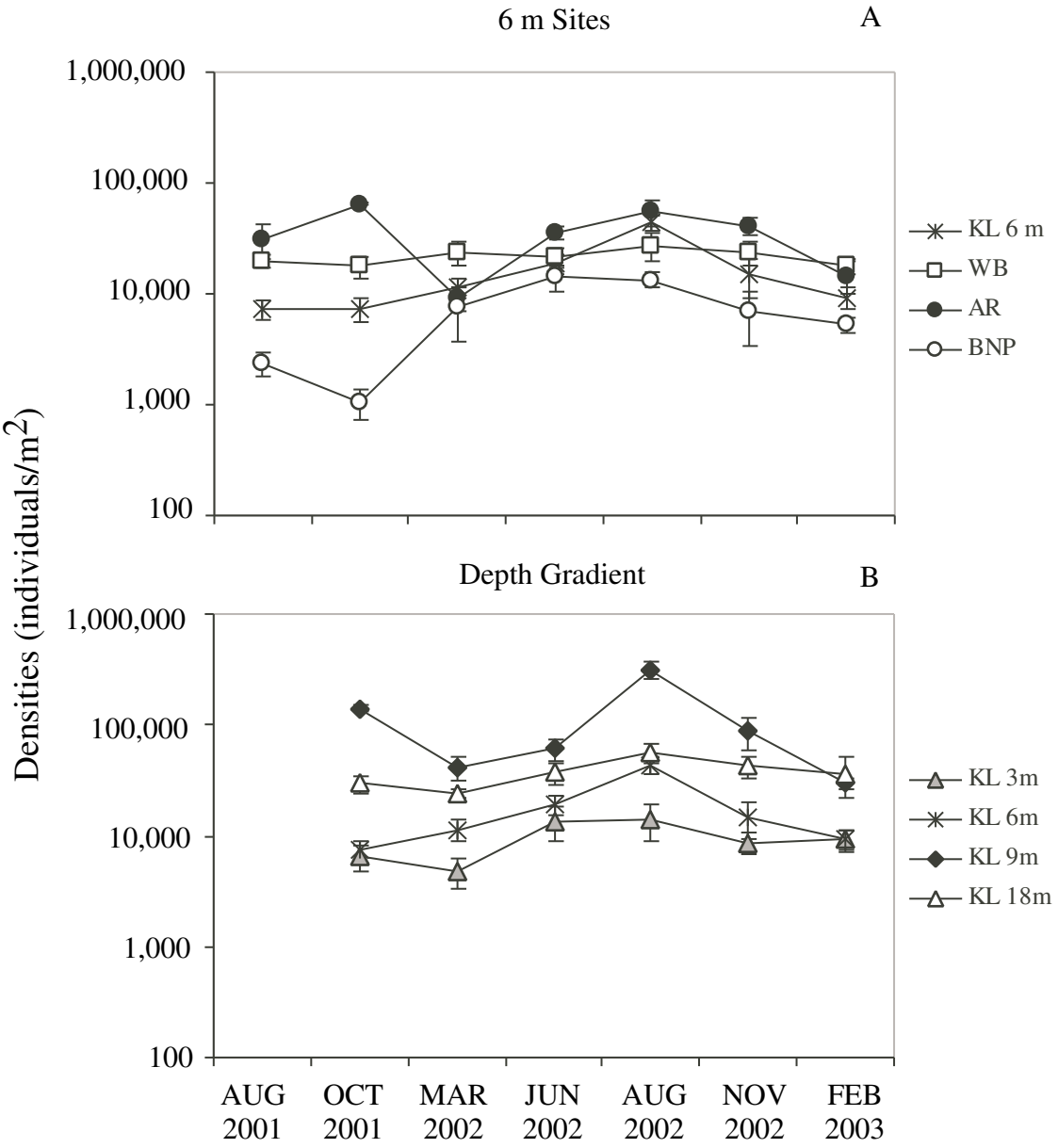


Figure 4.7. Mean (± SE) densities of all symbiont-bearing ('larger') foraminifera from August 2001 to February 2003 at (A) the 6 m sites and (B) along the depth gradient. Densities are plotted on a log scale.

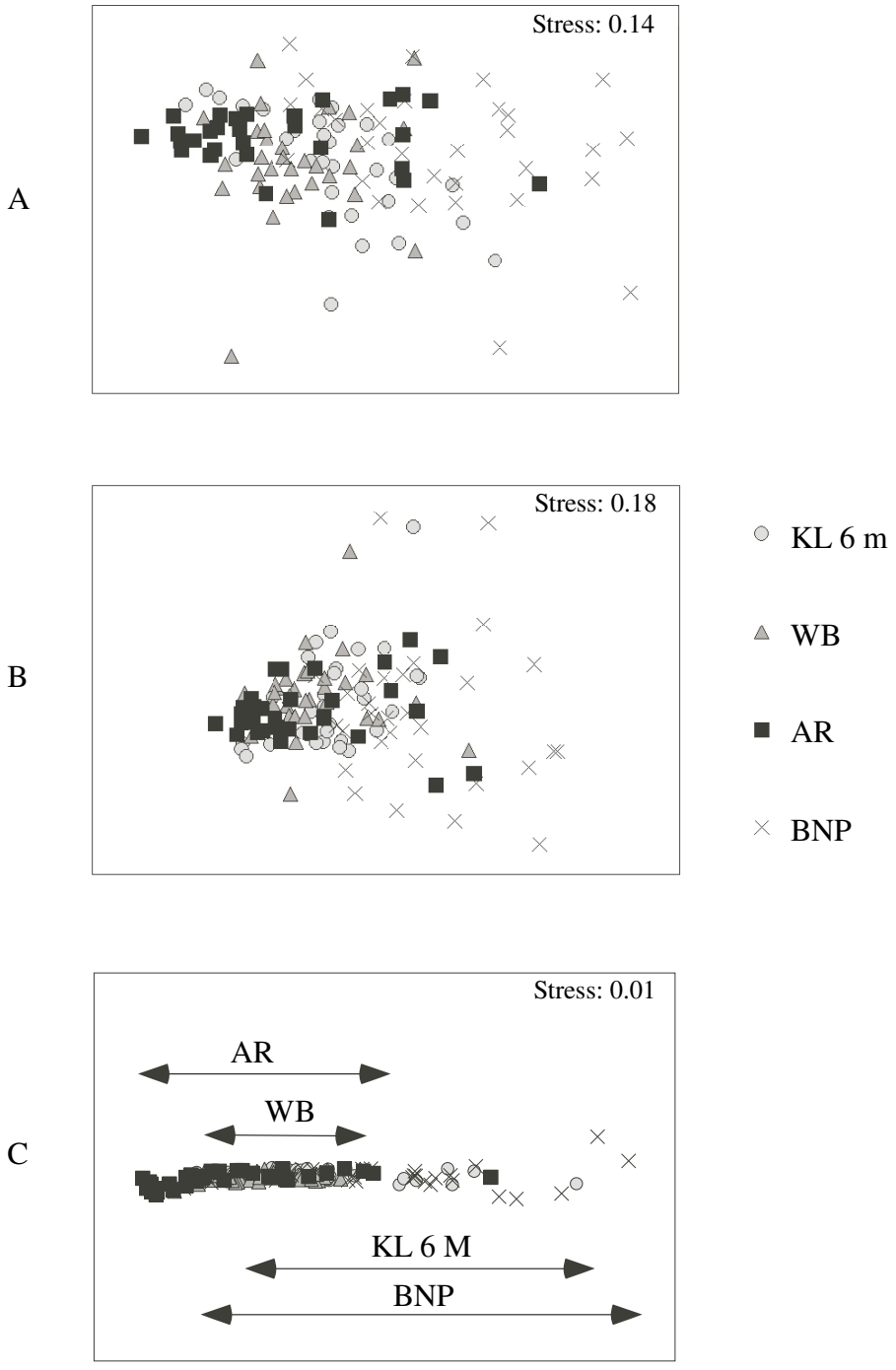


Figure 4.8. Multi-dimensional scaling plots (MDS) illustrate the ordination of samples collected between August 2001 and February 2003 based on (A) the entire assemblage of LBF, (B) the assemblage with *A. gibbosa* removed, and (C) *A. gibbosa* alone.

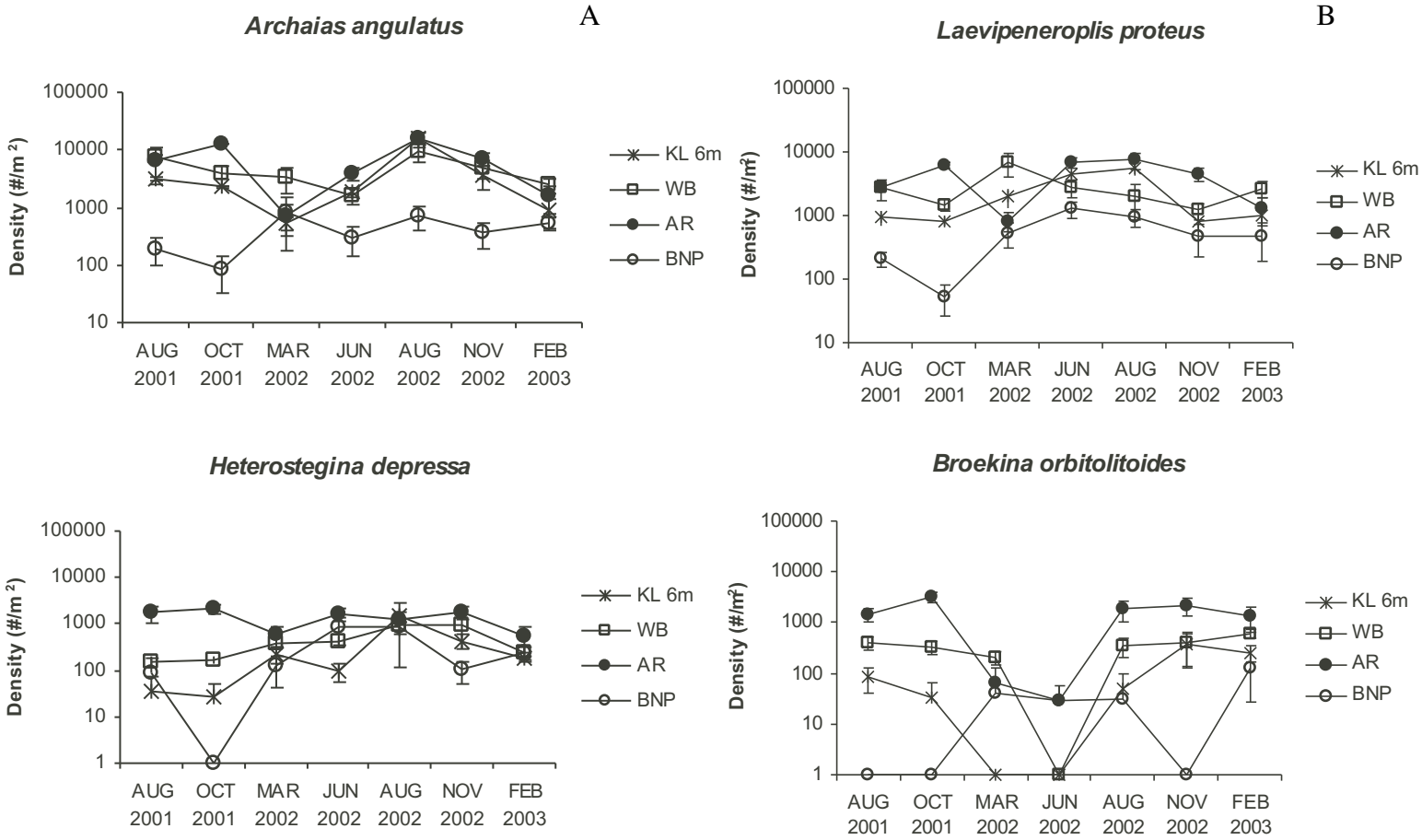


Figure 4.9. Mean (± SE) densities of other dominant symbiont-bearing foraminifera at the 6 m sites (A) *Archaias angulatus*, (B) *Laevipeneroplis proteus*, (C) *Heterostegina depressa*, and (D) *Broekina orbitolitoides*.

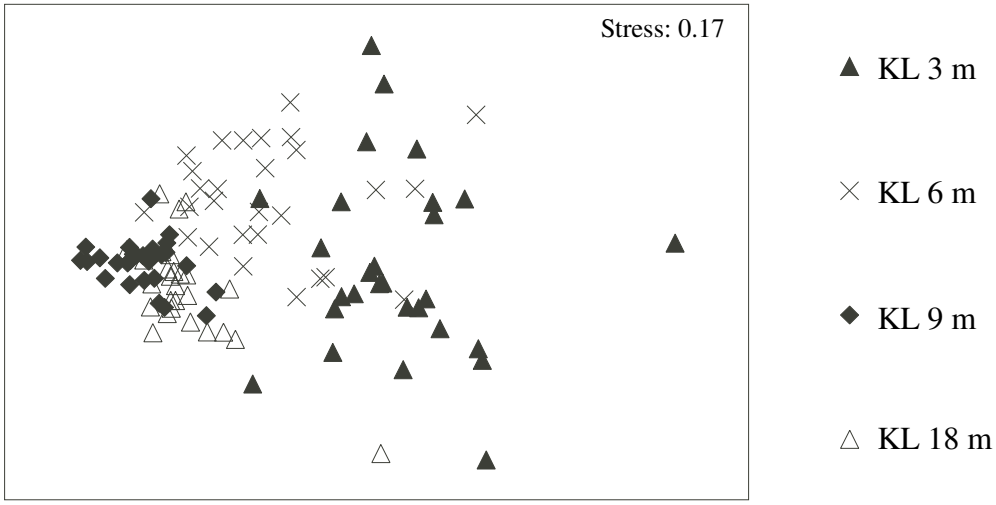


Figure 4.10. Multi-dimensional scaling plots (MDS) illustrate the ordination of samples collected along the depth gradient between October 2001 and February 2003 based on the entire assemblage of LBF

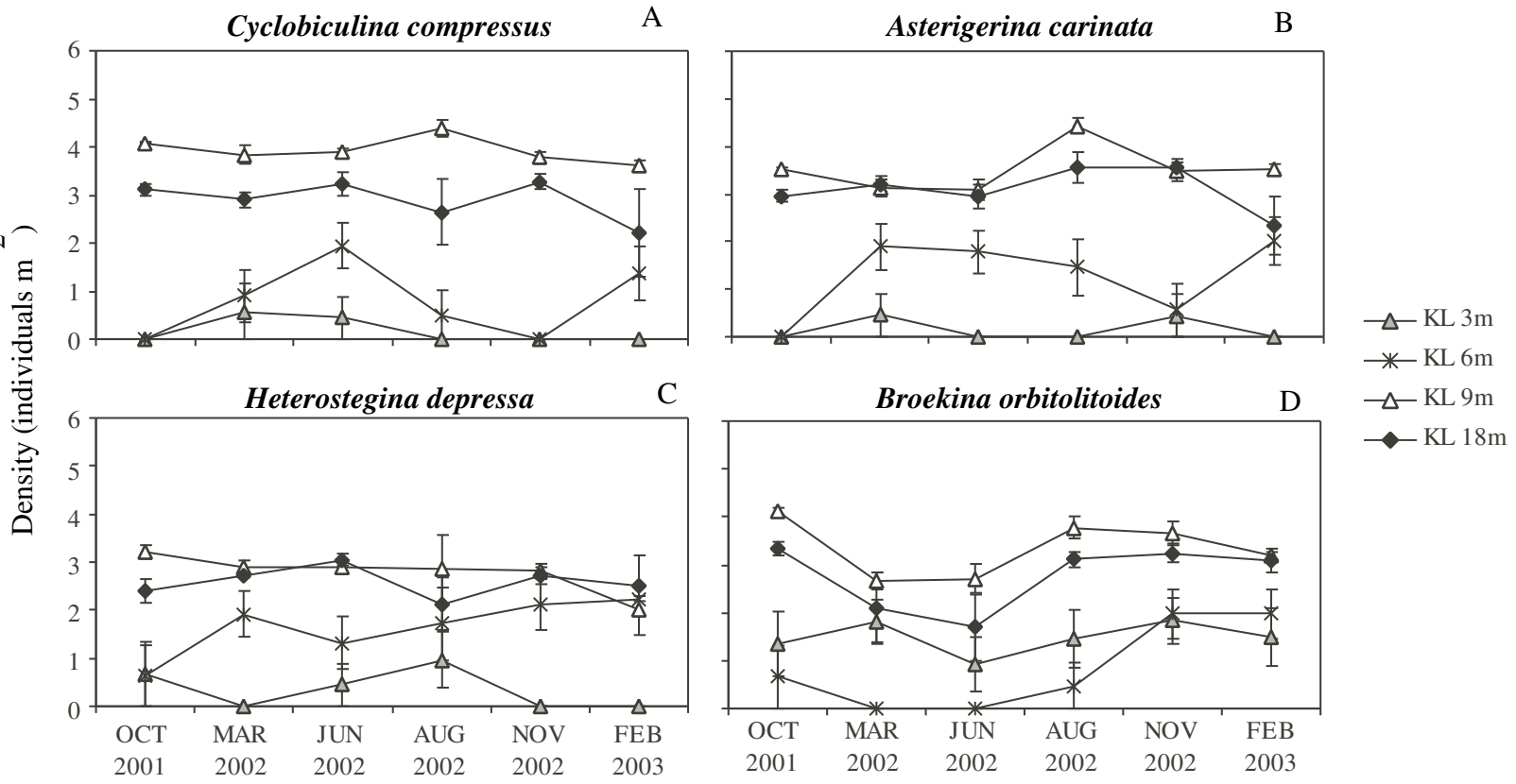


Figure 4.11. Mean densities (individuals m^{-2} ± SE) of other dominant symbiont-bearing foraminifera along the depth gradient (A) *Cyclobiculina compressus*, (B) *Asterigerina carinata*, (C) *Heterostegina depressa* and (D) *Broekina orbitolitoides*. Densities are plotted on a log scale.

5. Lesion Regeneration Rates in Reef-building Corals (*Montastraea* spp.) as Indicators of Colony Condition*

5.1. Abstract

Regeneration rates of coral lesions reflect the ability of colonies to repair damage and therefore can be useful indicators of coral health and environmental conditions. I quantified regeneration rates of boulder coral (*Montastraea* spp.) at four, 6 m deep patch reefs within Biscayne National Park (BNP) and the upper Florida Keys National Marine Sanctuary (FKNMS), and along a 3 – 18 m depth transect in FKNMS. Coral lesions (approx. 2 cm^2) created during sampling for cellular-diagnostic analysis were monitored quarterly in 2001 and 2002, and in February 2003. Regeneration was a dynamic process, continuing longer than previously reported (>300 d after lesion formation). Geographic location was the strongest factor affecting regeneration rate at my study sites. Lesion regeneration differed significantly among 6 m deep sites; sites offshore from John Pennekamp Coral Reef State Park (Algae Reef and White Banks) consistently had the highest regeneration rates, with colonies exhibiting exponential declines in lesion size and a high percentage of completely-healed lesions. Along the depth gradient, corals at the 3 m site regenerated significantly faster than corals at 6, 9 and 18 m. Colonies at the latter sites had highly variable and overall low regeneration rates, a low percentage of healed lesions, and a high occurrence of breakage or Type II lesions - lesions that increased in size by merging with areas of denuded tissue on the colony. These results suggest that corals sampled at FKNMS 6, 9 and 18 m sites and BNP were in poor physiological condition or were exposed to sub-optimal environmental conditions

* This chapter is in press in Marine Ecology Progress Series as Fisher EM, Fauth JE, Hallock P, Woodley CM (in press) Lesion regeneration rates in reef-building corals (*Montastraea* spp.) as indicators of colony condition. Mar Ecol Prog Ser

5.2. Introduction

Most corals are colonial organisms; a colony can experience partial mortality in which part remains alive while another portion dies. When a disturbance produces a lesion (partial mortality), the exposed coral skeleton becomes vulnerable to invasion by sessile organisms such as algae, resulting in the lesion increasing in size. Alternatively, healing can occur if tissue regeneration is not impeded (Kawaguti 1937). Lesions that fail to heal completely within about two months are likely to become permanent patches of mortality (Meesters et al. 1994).

Percent mortality of coral colonies is a useful gauge of reef condition (Ginsburg et al. 2001) because it can reveal a recent or chronic disturbance (Lang 2003) and influence colony growth and reproduction (Meesters et al. 1994, Van Veghel & Bak 1994, Lirman 2000a). Williams (1994) proposed using coral lesions as indicators of environmental stress because they are a generalized response to a range of disturbances, are independent of reef type, and can be monitored by managers easily and inexpensively. Williams noted that the frequency of coral lesions varies among sites, with polluted sites having more lesions than relatively unpolluted sites. Quantifying colony damage and recovery rates also are essential for predicting demographic changes in coral populations (Bak & Meesters 1999).

In corals, lesion regeneration begins with growth of an undifferentiated tissue layer created by the coenenchyme and polyps surrounding the lesion (Bak et al. 1977). After about two weeks, polyps begin to develop in the new tissue (Meesters et al. 1994) and secrete thecal walls and a basal plate. These give rise to numerous radially arranged calcareous partitions (septa), which project inward and support the polyp mesenteries. Pigmentation and zooxanthellae return at the end of the regeneration process (Bak et al. 1977, Kramarsky-Winter & Loya 2000). Coral regeneration rates can vary with species (Kawaguti 1937, Bak et al. 1977, Nagelkerken & Bak 1998) and are influenced by lesion characteristics including the type of injury and its initial size, perimeter and shape (Meesters et al. 1994, Meesters et al. 1997b, Oren et al. 1997, Lirman 2000b, Hall 2001), and colony characteristics such as size (Kramarsky-Winter & Loya 2000, Oren et al. 2001). Under normal conditions, lesion size decreases exponentially; deviations from

this response suggest resource limitation (Meesters et al. 1997b), stress due to environmental conditions (Lester & Bak 1985, Meesters et al. 1992, Meesters & Bak 1993, Mascarelli & Bunkley-Williams 1999, Croquer et al. 2002, Fine et al. 2002) or competition (Hall 2001).

The present study was part of a long-term project in the Florida Keys testing the use of an integrated molecular biomarker system in corals (Downs et al. 2000, 2005a, Fauth et al. 2003). Here I compare the ability of star boulder corals (*Montastraea* species complex) within two marine protected areas to regenerate biopsy-induced lesions. Lesion regeneration rates were assessed to characterize coral condition at these sites. Three specific questions were addressed: (1) Do regeneration rates differ among sites, seasons or years? (2) Do regeneration rates vary with depth? (3) Do regeneration rates vary with lesion parameters (e.g., initial lesion size, perimeter, shape) or colony characteristics (e.g., morphotype/species, size, % mortality)? In chapters 6 and 7, I will relate coral regeneration rates to ecological and cellular indicators to further identify potential sources of stress at my study sites.

5.3. Methods

I assessed reef condition at community and colony scales at one patch reef in Biscayne National Park (BNP), and four patch reefs and two fore reef sites in the upper Florida Keys National Marine Sanctuary (FKNMS), as part of an ongoing study of coral ecophysiology (e.g., Downs et al. 2000, 2005a, Fauth et al. 2003). These seven sites (Fig. 1.1) comprised both a latitudinal transect with four sites at 6 m depth and a depth transect (Key Largo (KL) 3 m – KL18 m) and were chosen in consultation with resource managers to reflect gradients in environmental conditions. Algae Reef (AR) and White Banks (WB) were adjacent to the extensive John Pennekamp Coral Reef State Park, with intact coastal hammock, mangroves and seagrass beds. Key Largo (KL) 6 m was located offshore from the most urbanized coastline of Key Largo, from which natural vegetation has been removed, natural topography has been altered to maximize waterfront properties and coastlines are lined with seawalls. This site lies along the route that recreational boaters and commercial dive operators take to reach popular Molasses Reef and other

outer reefs in the Upper Keys reef tract. Molasses Reef is “the most heavily visited reef in the Upper Keys for diving” (FKNMS website). Alina’s Reef, which is in Biscayne National Park (BNP) is offshore from urban Miami, FL. This site is potentially influenced by the extensive agricultural area south and west of Miami that drains into Biscayne Bay. Biscayne National Park also is near a nuclear power plant and major landfill.

5.3.1. Benthic Community Assessments

In March 2002, a dive was made at each 6 m site to assess the benthic organisms using the rapid assessment methods described by Lang (2003). At each site, a 10-m transect line was placed just above a haphazardly selected area of reef surface and live coral cover was determined by estimating the amount of living coral directly beneath the line. For each coral >10 cm in diameter lying beneath the transect, I recorded species, maximum diameter and height, and percent recent and old mortality. "Recently dead" was defined as any non-living parts of the coral in which the corallite structures were still intact or covered by a thin layer of algae or fine mud (Lang 2003). "Long dead" was defined as any non-living parts of the coral in which the corallite structures either were gone or covered by organisms that were not easily removed (Lang 2003). Due to differences in reef types, I could not use this method for comparisons along the depth gradient.

5.3.2. Lesion Regeneration

Between June 2001 and February 2003, I collected tissue samples that created standard-sized lesion) approximately quarterly (February/March, June, August, October/November) from the same five colonies at each site. Previous studies showed that quarterly sampling was adequate to detect changes in coral physiology as a result of seasonal and stressor variation (Downs et al. 2000, Fauth et al. 2003). I preferentially chose *Montastraea faveolata* for this study but sampled the morphotypes *M. annularis* and *M. franksi* when *M. faveolata* was not available. A single morphotype was not found at all study sites: I sampled *M. faveolata* at all sites except KL 18 m, *M. annularis* at KL

6 m and WB, and *M. franksi* at KL 9 m, KL 18 m, WB, and AR. I estimated size (maximum diameter) and percent partial mortality of each colony at the start of the study. I measured diameter (live and dead areas) to the nearest cm in planar view perpendicular to the axis of growth using a meter stick. Partial mortality was visually quantified by estimating the percentage of dead area from above in planar view as recommended by Lang (2003).

I removed coral tissue and skeleton using a leather punch, which created a circular lesion 1-2 cm^2 in area and 3 mm deep. Experimental lesions always were completely surrounded by live tissue. I immediately filled the hole with clay (Roma plastilina, medium grey; Blick Art Materials, Galesburg, IL) to fill the void produced by removing the underlying skeleton and limit intrusion of fouling and bioeroding organisms (Fig. 5.1). Use of clay filler was a decision made by park managers when permitting biopsy of these corals for molecular biomarker analysis. Clay provided corals with a flat surface over which to regenerate tissue but, as seen in this study, did not prevent fouling or bioerosion. However, regeneration rates reported here may represent maximal rates due to a possible reduction in biofouling. I then photographed each lesion using a Nikonos V 35mm camera with a close-up adapter and frame, calibrating measurements with a 4.5 mm long bar. I re-photographed each lesion during subsequent quarterly samplings to observe changes in size over time (Fig. 5.1). I scanned photographs to digital images and used image-analysis software (Image Pro™) to calculate area (A) and perimeter (P) of all lesions that remained completely surrounded by live tissue (Type I lesions: Meesters et al. 1997a).

If a lesion enlarged, thereby merging with an area of the colony that lacked tissue (Type II lesions: Meesters et al. 1997a), I conservatively assumed no change in lesion size for that sampling date and removed it from further analyses because subsequent changes in area were unconstrained. When lesions merged with other sampling lesions (Fig. 5.1B), I calculated their area as $A_L = A_T/n$; where A_L is the area of the lesion used for further analyses, A_T is the total area of all lesions joined together and n is the total number of lesions joined together. This calculation provided a conservative estimate of lesion area increase. In the few cases where initial lesion size was unavailable due to

camera malfunctions, I substituted mean initial lesion size for that sampling period. When photographs of final lesion size were unavailable, I used *in situ* measurements to calculate lesion area and perimeter using the equation for an ellipse, $A_L = \pi ab$, and $P_L = 2\pi * sqrt[(a^2 + b^2)/2]$, where A_L is the area of the lesion, P_L is the perimeter of the lesion, and a and b are one-half of lesion length and width, respectively. Larger lesions resulted from breakage of the coral skeleton, which often was highly bioeroded. Due to the effect of initial lesion size on regeneration, I removed lesions >3.4 cm^2 from further analyses.

5.3.3. Data Analysis

5.3.3.1. Benthic Community Assessments

I used one-way ANOVA followed by the Tukey-Kramer Honestly Significant Difference (HSD) method to determine if sites differed significantly in live coral cover and coral colony density. Data on coral diameter and height, and recent and old mortality did not meet the normality assumptions of ANOVA. For these data, I tested for differences among sites using Kruskal-Wallis followed by Wilcoxon rank-sum tests.

5.3.3.2. Lesion Regeneration

Data were analyzed in two groups: (1) by sites at 6 m depth along the northeast - southwest traverse (BNP, AR, WB and KL 6 m), and (2) by sites along the depth gradient (KL 3, 6, 9 and 18 m). The KL 6 m site was common to both groups (Fig. 1.1). I examined lesion changes in three different ways to answer specific questions.

(1) Did lesion size decrease exponentially with time ($cm^2\ d^{-1}$) and did this differ among sites and seasons? Can deviations from this model be used as an indicator of stress? I used least-squares regression to fit an exponential model of regeneration with an asymptote as recommended by Meesters et al. (1994, 1997b):

$$y = y_o + ae^{-b*time}$$

where y_o is the asymptote, *a* is the amount of tissue regenerated, and *b* is the slope of the curve. I only applied the exponential model to lesions with a minimum of one year of observations.

(2) Did regeneration rates differ among sites and seasons? I calculated rates for two different periods: short-term (45-154 d) and quasi-annual (319 – 376 d). The shortest period monitored reflects a time frame similar to previous regeneration studies (e.g., Meesters et al. 1994, 1997b, VanVeghel & Bak, 1994). I calculated the quasi-annual rates to determine how lesion size changed over multiple seasons. For each lesion, I calculated the amount of tissue regenerated or lost (ΔT) as ΔT = % change in lesion size*initial lesion size/time. I standardized regeneration to initial lesion perimeter (P) because this influenced regeneration rates. I used repeated-measures MANOVA to determine whether standardized regeneration rates (Δ T/P) differed among sites, seasons and their interactions. I checked model assumptions (e.g., sphericity, homogeneity of variances, normality and independence) using residual plots. In cases where the sphericity assumption was not met, I applied a univariate (unadjusted epsilon) approach. To interpret effects detected by MANOVA, I used one-way ANOVA followed by the Tukey-Kramer HSD method. I regressed residuals of the regeneration-rate model against lesion (A, P and shape (P/A)) and colony (species, size, % mortality) parameters to determine if they affected regeneration rates. All colony characteristics that explained significant variation in residuals were used as covariates in the MANOVA model. I also regressed quasi-annual regeneration rates against short-term rates to determine if monitoring for short time periods could be used to predict quasi-annual trends.

(3) Were lesions among all sites capable of completely healing and did the number of Type II Lesions differ among sites and seasons? I used G-tests of independence with William's correction (Sokal & Rohlf 1995) to determine if the number of lesions that closed completely or progressed into Type II lesions differed among sites.

I performed non-linear regression using SigmaPlot 2000 (Systat Software, Inc.) and all other statistical analyses using JMP v.3.2. (SAS Institute Inc., Cary, NC, USA), with $\alpha = 0.05$ for all hypothesis tests.

5.4. Results

5.4.1. Community Data

Mean percent live coral cover and coral colony density were low at all 6 m sites; both were highest at AR and lowest at KL 6 m (coral cover: ANOVA $F_{3,13} = 11.4$, $p < 0.0007$; coral colony density: ANOVA $F_{3,26} = 3.7$, df = 3, $p < 0.03$; Table 5.1). Colonies at AR were significantly larger (maximum diameter) than colonies at WB and KL 6 m; colonies at BNP also were significantly larger than colonies at KL 6 m ($\chi^2 = 9.2$, df = 3, $p < 0.03$; Table 5.1).

5.4.2. Regeneration Model

After removing lesions >3.4 cm^2 from further analyses, initial lesion area ranged from 0.75 to 3.02 cm^2 with a mean (± SE hereinafter) of 1.75 ± 0.04 cm^2 (n = 136) for the 6 m sites and from 0.68 to 3.32 cm^2 with a mean of 1.80 ± 0.05 cm^2 (n = 128) along the depth gradient.

Lesion size decreased exponentially over time at AR, WB and KL 3 m (Fig. 5.2) as indicated by large r^2 values and slopes (Table 5.2). With few exceptions, lesion size at BNP, KL 6 m, KL 9 m and KL 18 m either changed little or in some cases increased over time (Fig. 5.2). Lesions on corals at these sites deviated from the expected decay model and fit either an exponential growth (increase in lesion size) model or a reduced model as indicated by low r^2 values and slopes (Table 5.2).

5.4.3. Short-term (45 - 154 d) Regeneration Rates

Short-term regeneration rates (Δ T/P) ranged from –40 to 65 x 10^{-4} cm d^{-1} with a mean of 13 ± 1 x 10^{-4} cm d^{-1} (n = 136) at 6 m sites and from –43 to 91 x 10^{-4} cm d^{-1} with a mean of 13 ± 2 x 10^{-4} cm d^{-1} (n = 127) along the depth gradient.

Mean short-term regeneration rates differed significantly among the 6 m sites (repeated measures MANOVA: site effect $F_{3,10} = 10.6$, $p < 0.002$; Fig. 5.3A), but not among species, seasons or their interactions. Mean short-term regeneration rates at AR were significantly higher than at the other 6 m sites, and short-term regeneration rates at WB were significantly higher than KL 6 m and BNP (AR: 23 ± 2 cm d^{-1} x 10^4, WB: 15 ±

2 cm d^{-1} x 10^4, KL 6 m: 7 ± 2 cm d^{-1} x 10^4, BNP: 6 ± 2 cm d^{-1} x 10^4, Tukey's HSD Test). Short-term regeneration rates also differed significantly along the depth gradient (repeated measures MANOVA: site effect $F_{3,8} = 4.4$, $p < 0.05$; Fig. 5.3B) but not with season or depth x season interactions. Short-term regeneration rates at KL 3 m were significantly faster than at KL 6 m (19 ± 3 cm d^{-1} x 10^4 vs. 7 ± 2 cm d^{-1} x 10^4, respectively, Tukey's HSD Test) but not at KL 9 m and KL 18 m (Fig. 5.3B).

Species differences partially explained variation (<7 %) in the residuals of the regeneration-rate model for the 6 m sites (period: 45 - 154 days), with *M. annularis* having regeneration rates higher than *M. franksi* (1.3 ± 0.3 cm d^{-1} x 10^4 vs. 0.8 ± 0.4 cm d^{-1} x 10^4, respectively). However, this largely resulted from uneven distributions of morphotypes among sites, and the species effect was not significant when added as a covariate to the regeneration-rate model. Species differences did not significantly explain variation in the residuals along the depth gradient. Initial lesion size, perimeter and shape explained less than 10% of the residual error in the regeneration (T/P) model at the 6 m sites ([A] $r^2 = 0.09$, $p = 0.002$; [P] $r^2 = 0.06$, $p < 0.01$; [P/A] $r^2 = 0.09$, $p = 0.002$). Residuals were positively correlated with both initial lesion size and perimeter, and negatively correlated with P/A. Along the depth gradient, lesion size, perimeter and shape were independent of model residuals**.**

5.4.4. Quasi-Annual Regeneration Rate (319 - 376 days)

At the 6 m sites, mean quasi-annual regeneration rates differed among sites (repeated measures MANOVA: site effect $F_{3,12} = 14.8$, $p = 0.0002$), season ($F_{3,36} = 11.2$, $p < 0.0001$), and with the site x season interactions ($F_{9,36} = 4.2$, $p < 0.0009$). Corals at AR and WB regenerated significantly faster than corals at KL 6 m and BNP between June 2001 and 2002 (ANOVA: $F_{3,15} = 7.3$, $p < 0.003$; 9 ± 1 cm d^{-1} x 10^4 and 9 ± 0 cm d^{-1} x 10^4 vs. 3 ± 1 cm d^{-1} x 10^4 and 2 ± 3 cm d^{-1} x 10^4, respectively, Tukey HSD, Fig. 5.4A). Corals at BNP regenerated significantly slower than corals at WB between August 2001 and 2002 (ANOVA: $F_{3,14} = 3.4$, $p < 0.05$; -4 ± 5 cm d^{-1} x 10^4 vs. 8 ± 1 cm d^{-1} x 10^4, respectively, Tukey HSD) and corals at AR between October 2001 and 2002 (ANOVA: $F_{3,14} = 4.8$, $p < 0.01$; 0 ± 3 cm d^{-1} x 10^4 vs. 8 ± 1 cm d^{-1} x 10^4, respectively, Tukey HSD).

Corals at AR also regenerated significantly faster than colonies at KL 6 m and BNP between March 2002 and February 2003 (ANOVA: $F_{3,15} = 5.3$, $p < 0.01$; Fig. 5.4A; 21 ± 5 cm d^{-1} x 10^4 vs. 7 ± 1 cm d^{-1} x 10^4 and 2 ± 5 cm d^{-1} x 10^4, respectively Tukey HSD). Short-term regeneration rates were significantly correlated with quasi-annual trends ($r^2 = 0.37$, $p = 0.0001$; regression equation: $\Delta T/P$ (annual) = 0.29 $\Delta T/P$ (short) + 2.3).

Mean regeneration rates varied significantly along the depth gradient (repeated measures MANOVA: site effect $F_{3,10} = 4.1$, $p = 0.04$), with season ($F_{3,8} = 38.3$, $p < 0.0001$), and the season x site interactions ($F_{9,19.6} = 5.6$, $p < 0.0007$; Fig. 5.4B). Regeneration rates at KL 3 m exceeded those at all other sites between March 2002 and February 2003 (ANOVA: $F_{3,15} = 5.3$, $p < 0.02$; 18 ± 4 cm d^{-1} x 10^4 vs. 7 ± 1 cm d^{-1} x 10^4, 6 ± 2 cm d^{-1} x 10^4 and 4 ± 2 cm d^{-1} x 10^4, respectively Tukey HSD). Along the depth gradient, short-term regeneration rates explained little variation in quasi-annual trends ($r^2 = 0.10$, $p = 0.007$; regression equation: $\Delta T/P$ (annual) = 0.12 $\Delta T/P$ (short) + 3.3) due to high variability, especially among colonies at the KL 9 m and KL 18 m site.

5.4.5. Healed and Type II lesions

Coral colonies at AR completely healed significantly more lesions (30%) than colonies at the other 6 m sites ($G_{adj} = 15.8$, df = 3, $p < 0.005$). Along the depth gradient, significantly more lesions healed completely at 3 m depth (31%) than at other depths ($G_{adj} = 12.8$, df = 3, $p < 0.01$; Fig. 5.5). These results indicate significant heterogeneity among sites in healing (Table 5.3).

Of a total of 170 lesions created at the KL 3 m and all 6 m sites combined, only two merged with other lesions to become Type II lesions (Table 5.3). In contrast, at the deepest sites (KL 9 m and KL 18 m combined), 26% of lesions merged to become Type II lesions ($G_{adj} = 12.2$, df = 3, $p < 0.01$; Table 5.3). In two cases, lesions joined with another sampled lesion before merging with partial mortality on other parts of the colony, becoming Type II lesions. In all other cases, lesions joined with partial mortality that occurred naturally on the colony, often associated with increases in algae.

5.5. Discussion

Following recommendations of Williams (1994) and utilizing the extensive work of Bak, Meesters and coworkers, I evaluated lesion regeneration as an indicator of coral-colony condition at seven reefs in BNP and FKNMS. Using this bioindicator, I detected significant differences among sites in mean lesion regeneration rates. While most previous studies of regeneration monitored lesions for 60 -150 days; I followed regeneration for up to 595 days, which allowed me to observe changes in recovery trends that might be missed by a study of shorter duration. Short-term regeneration rates were useful predictors of longer-term regeneration rates among 6 m sites, but explained little variation along the depth gradient. Monitoring long-term regeneration appears necessary when comparing coral colonies living in different reef types/depths. Long-term regeneration rates were time dependent whereas short-term regeneration rates were not.

Coral lesions regenerate at a rate determined by the number of polyps surrounding each lesion (Meesters et al. 1997b, Oren et al. 1997, Lirman 2000b) and normally follow an exponential-decay model with an asymptote at full healing (Meesters et al. 1994, 1997a, Lirman 2000b). I found that changes in lesion size were dynamic and site-dependent and often deviated from the expected exponential-decay model. Some lesions that initially increased in size later regenerated, and other lesions that initially began to regenerate later increased in size, especially at sites with high algal growth (e.g., BNP, KL 9 m, KL 18 m). If lesions with a P/A ratio >2 cm^{-1} should be able to fully regenerate (Meesters et al. 1997a), then most lesions in my study should have healed completely. However, only 14% (n = 228) fully regenerated. The largest lesion (2.0 cm^2) that fully regenerated did so after 243 days; after 151 days, this lesion had regenerated 78% of its area to a size of 0.45 cm^2. Most lesions that healed completely regenerated most (>70%) of their area within 151 days, but complete healing often required a year or longer. One lesion that completely healed after 270 days increased 66% in size in the first 56 days before beginning to regenerate. My study confirms that regeneration can continue for a year or more and that lesions that do not initially regenerate (or even increase in size) can regenerate later if conditions become favorable.

5.5.1. 6 m Sites

Mean lesion regeneration rates varied significantly among sites at the same depth, suggesting that lesion regeneration may be a useful indicator of variation in environmental conditions. Of the 6 m sites, corals consistently had the highest regeneration rates at AR, which is adjacent to the extensive John Pennekamp Coral Reef State Park. Colonies at AR had significantly more completely healed lesions than the other 6 m sites. AR also had the highest live coral cover with relatively large colonies. Lesions at the other site adjacent to the state park (WB) also regenerated exponentially but many failed to heal completely, leaving those corals susceptible to fouling organisms. Partial coral mortality of the community was lowest at this site. In contrast, KL 6 m, located offshore from the most urbanized coastline of Key Largo, had low regeneration rates and low overall live coral cover. Corals from the site in Biscayne National Park, offshore from urban Miami, FL, had the lowest regeneration rates; lesions there often increased in size. Large increases in lesion size at BNP often were associated with seasonal increases in algae (e.g., June 2002), which sometimes resulted in lesions merging together. During my study, BNP corals had poor lesion recovery and also exhibited mortality elsewhere on the colonies. Mean partial mortality estimated along transects also was highest at this site. In 2000, *Montastraea* colonies at BNP experienced a severe oxidative and protein denaturing stress, likely due to chemical contaminant exposure (Downs et al. 2005a). The colonies I sampled were generally large in size, with substantial contiguous areas of living tissue, suggesting that the stressor(s) causing poor lesion recovery and partial mortality likely were recent, within the last 10 - 15 years or less. Therefore, if stresses can be identified and alleviated at this site, these large coral colonies may survive.

Responses of other reef organisms (e.g., white grunts and foraminifers) at these sites are consistent with observations of lesion regeneration. Downs et al. (2006) compared biomarker levels in white grunts (*Haemulon plumieri*) at BNP, WB and KL 6 m, finding evidence for a toxic response to a xenobiotic at BNP. Concentrations of pesticides in grunt livers were highest at KL 6 m and lowest at WB (Downs et al. 2006). Hallock (2000) proposed using abundances of reef-dwelling foraminifers that host algal

symbionts to indicate whether environmental conditions support calcifying organisms dependent upon algal symbioses; Fisher and others (in prep.) found densities of such foraminifers lowest at BNP and KL 6 m and highest at WB and AR (see Ch. 4).

5.5.2. Depth Gradient

Mean regeneration rate also varied among depths, but qualitative results depended on how long lesions were monitored. When monitored for <1 year (45 – 154 d), shallow-water (3 m) corals regenerated significantly faster than corals at 6 m but not those at 9 and 18 m. Key Largo 3 m also had significantly more healed lesions than other sites along the depth gradient. For lesions sampled in March 2002 and monitored for approximately 1 year, shallow-water corals (3 m) showed higher regeneration rates than all deeper water corals (6 – 18 m) along the Key Largo transect. Deeper-water corals typically receive less radiant energy and therefore may have lower carbon reserves than corals in shallow water (Nagelkerken et al. 1999). However, this does not explain why KL 9 m and KL 18 m had mean short-term regeneration rates similar to the shallowest site. Also, differences in mean regeneration rates were not seen between the KL 6 m and the deeper sites.

Regeneration rates of corals from KL 9 m and KL 18 m were highly variable. Lesions that initially decreased in size often later increased in size, and overall live coral cover at these sites was low (<7%). These *Montastraea* colonies were bioeroded by clionid sponges, making them susceptible to breakage and resulting in greater patchiness of live tissue, possibly reducing the coral's ability to recover from damage. Type II lesions developed more frequently in corals from these deeper sites. Many lesions joined with dead regions that were unrelated to my sampling. In two cases, the entire colony died; one each at KL 9 m and KL 18 m.

Lesion growth often was associated with increased algal turf, particularly thick turfs mixed with fine sediments. Hall (2001) reported that regeneration was negatively correlated with algal settlement and cover (particularly macroalgae), which requires large energy expenditure by corals to overgrow. In my study, algal turfs and macroalgae fluctuated in abundance, possibly associated with seasonal changes (as in Lirman &

Biber 2000). Some lesions at my sites regenerated when algal biomass declined but later increased in size as algae grew, shading and possibly killing polyps surrounding the lesions. Particularly in spring and summer, I observed dark reddish cyanobacterial blooms that formed thick mats on the bottom and overgrew portions of these corals.

5.5.3. Comparisons among All Study Sites

All sites I sampled had relatively low coral cover (<20%) and appeared to be experiencing stress (Fisher et al. unpublished data). Connell (1997) observed that chronically stressed reefs were less likely to recover from acute or physical disturbances than reefs that were not chronically stressed. I observed that coral colonies along developed portions of the coastline (i.e., BNP, KL 6 m, KL 9 m, and KL 18 m) were least capable of recovering from damage and mortality. Colonies at sites offshore from John Pennekamp State Park (AR and WB) recovered from damage despite exposure to potential stressors (e.g., photic stress, contaminants). Although KL 3 m is along the same portion of coastline as KL 6, 9, and 18 m, lesion recovery and coral condition (36 ± 13 % live coral cover) at this site was good. Other studies also found that Florida's inshore patch reefs appear to be in better condition and have higher coral cover relative to offshore reefs (Beaver et al. 2005). For example, corals at KL 9 m and KL 18 m bleached in 1999, while those at KL 3 m and KL 6 m did not (Fauth et al. 2003).

5.5.4. Effect of Colony and Lesion Characteristics

Colony size and previous partial tissue mortality did not affect regeneration rates, probably because colony size was not small enough to limit resources allocated to regeneration (Oren et al. 2001). Once regeneration rate was standardized to perimeter, which is a measure of coral tissue available for regrowth in the surrounding margin (Meesters et al. 1994), lesion area explained only a small percentage of the variation in regeneration rate. Colony morphotype did not affect regeneration rate but the three types were not evenly sampled among sites, which could influence the results. However, low variation among colonies within sites containing different species suggests that morphotype was not a major factor affecting regeneration. Taxonomic differences

between these morphologies remain uncertain (Lopez et al. 1999, Fukami & Knowlton 2005).

5.6. Conclusions

Based on observations of lesion regeneration rates, coral colonies under relatively favorable environmental conditions (e.g., AR, WB, and KL 3 m) consistently have: high regeneration rates, where lesion sizes decrease exponentially over time; a high percentage of healed lesions; and infrequent Type II lesions. Likewise, under less favorable conditions (e.g., KL 6 m, KL 9 m, KL 18 m, BNP) lesions exhibit little regeneration, or high variability including increases in lesion size (overall low regeneration rates); low percentage of healed lesions; frequent Type II lesions; and a high percentage of breakage (indicative of bioerosion). Causes of differences in coral regeneration at small spatial scales deserve further investigation.

To standardize comparisons of lesion regeneration rates, I recommend (1) monitoring lesions of a similar size and perimeter, (2) comparing sites similar in depth and habitat type (e.g., patch reef, fore reef), and (3) monitoring lesions for more than one year because many lesions may require >200 days to heal. I also recommend recording the percentage of healed lesions and the occurrence of Type II lesions. Regeneration rates of coral lesions reflect the ability of colonies to repair damage and therefore can be useful, inexpensive indicators of reef coral condition or of environmental conditions. A caveat of this bioindicator is that it is not capable of separating effects of coral health versus external environmental factors on lesion regeneration rate. More expensive assays can then be applied to distinguish between stressor types at sites where coral regeneration is compromised.

Table 5.1. Comparison of benthic parameters (mean ± SE) along 10 m transects at four 6 m-patch reefs. Methods followed the Atlantic Gulf and Rapid Reef Assessment protocol. Data not connected by the same superscript letter differed significantly ($p < 0.05$).

Site	n	Colonies Density (#/m)	Live Coral Cover (%)	Coral Height (cm)	Coral Diameter (cm)	Recent Mortality (%)	Old Mortality (%)
KL 6 m	7	0.64 ^{A}	7 ^{A}	11 ^{A}	21 ^{A}	3 ^{A}	11 ^{A}
		(0.07)	(1)	(3)	(5)	(2)	(4)
WB	8	0.71 ^{AB}	9 ^{A}	13 ^{A}	19 ^{AB}	2 ^{A}	8 ^{A}
		(0.07)	(1)	(1)	(2)	(1)	(2)
AR	8	0.94 ^{B}	16 ^{B}	23 ^{A}	40 ^{C}	2 ^{A}	12 ^{A}
		(0.10)	(1)	(4)	(9)	(1)	(3)
BNP	8	0.64 ^{A}	8 ^{A}	25 ^{A}	37 ^{BC}	4 ^{A}	19 ^{A}
		(0.05)	(1)	(6)	(8)	(2)	(8)

Table 5.2. Mean (± SE) r^2 values of the five colonies for the regression decay model, $y = y_o + ae^{-b*time}$. A zero r^2 value was assumed for all lesions that did not fit this model. The last column includes overall mean (± SE) slope (b) ($cm^2\ d^{-1} \times 10^2$). Site abbreviations as in Fig. 1.

Location	JUN 2001-JUN 2002	AUG 2001-AUG 2002	OCT 2001-OCT 2002	MAR 2002-FEB 2003	OVERALL MEAN	SLOPE
KL 3 m	0.95 (0.03)	0.52 (0.22)	0.94 (0.05)	0.98 (0.01)	0.85 (0.07)	2.1 (0.7)
KL 6 m	0.55 (0.18)	0.61 (0.15)	0.33 (0.18)	0.88 (0.07)	0.59 (0.09)	0.3 (0.1)
KL 9 m	0.58 (0.19)	0.39 (0.24)	0.55 (0.23)	0.72 (0.24)	0.55 (0.11)	0.1 (0.1)
KL 18 m	0.56 (0.22)	0.37 (0.22)	0.72 (0.24)	0.71 (0.24)	0.59 (0.11)	0.4 (0.2)
WB	0.97 (0.01)	0.98 (0.01)	0.98 (0.02)	0.90 (0.07)	0.96 (0.02)	1.2 (0.2)
AR	0.96 (0.01)	0.80 (0.20)	1.00 (0.00)	1.00 (0.00)	0.93 (0.05)	1.7 (0.3)
BNP	0.23 (0.23)	0.36 (0.22)	0.37 (0.22)	0.21 (0.20)	0.30 (0.10)	0.2 (0.2)

Table 5.3. Percentage of healed and Type II lesions (no longer enclosed by living tissue) at each site. Total number of lesions was <35 at KL 9 m, KL 18 m, AR and BNP due to breakage during sampling (as discussed in methods).

Site	Total #	# Healed	% Healed	# Type II	% Type II
KL 3 m	35	11	31	0	0
KL 6 m	35	2	6	1	3
KL 9 m	29	4	14	6	21
KL 18 m	29	1	3	9	31
WB	35	1	3	0	0
AR	33	10	30	0	0

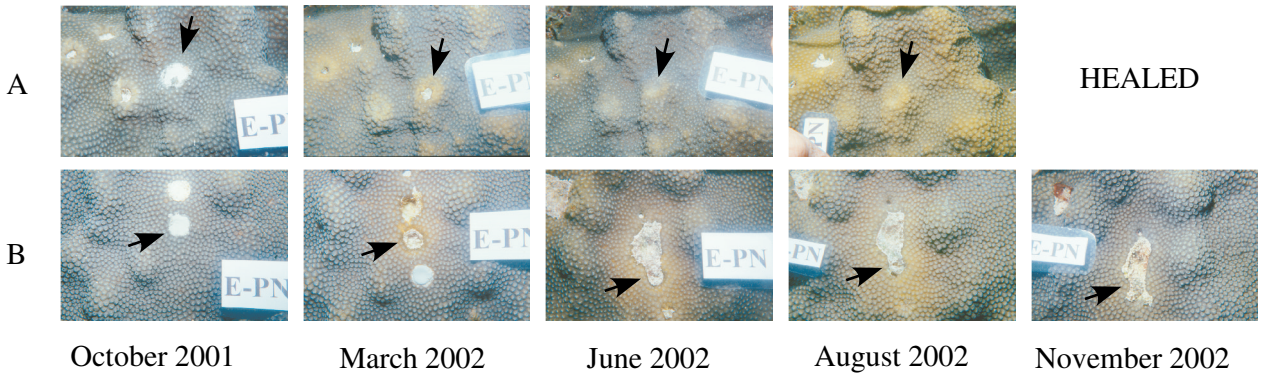


Figure 5.1. Examples of lesions at 6 m sites between October 2001 and November 2002 showing two extremes. (A) Algae Reef (AR) - lesion completely healed by June 2002. (B) Alina's Reef (BNP) - lesion joined with other sampling lesions in June 2002 and became covered with turf algae. Black arrow points to the lesion of interest.

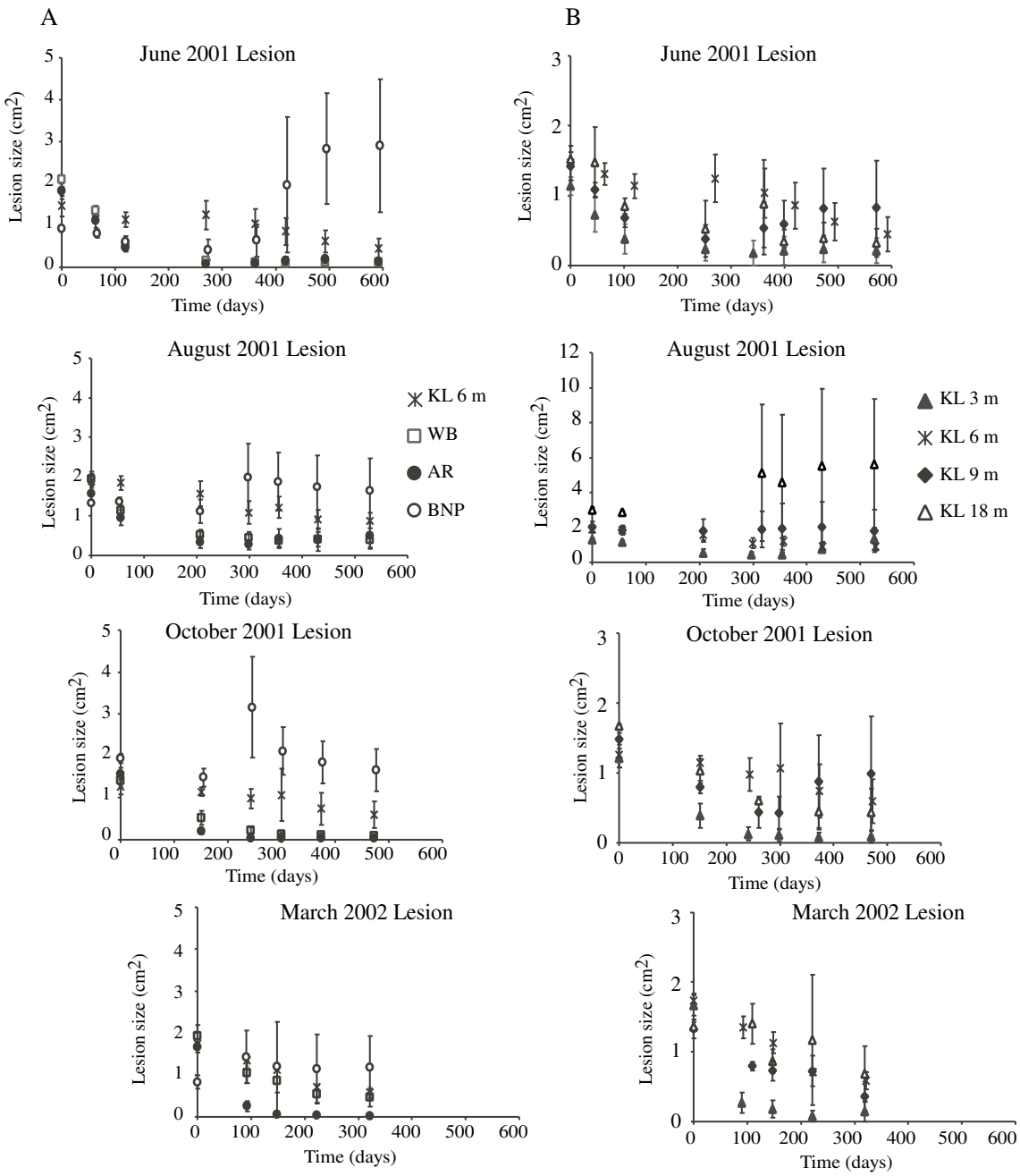


Figure 5.2. Mean lesion size (± SE) through time for each season between June 2001 and March 2002 at (A) the 6 m sites and (B) along the depth gradient. Axes staggered to align sampling dates. Note expanded y-axis in panels showing lesion regeneration along depth gradient in August 2001. Merging of two sampling-induced lesions occurred at KL 9 m (in March 2002), at KL 18 m (in February 2003) and at BNP (in June 2002 and August 2002). An additional lesion joined with the previously merged lesions at BNP in October 2002. Lesions that progressed into Type II lesions or data removed for other reasons (as discussed in methods: breakage or initial size >3.4 cm^2) were not included in means.

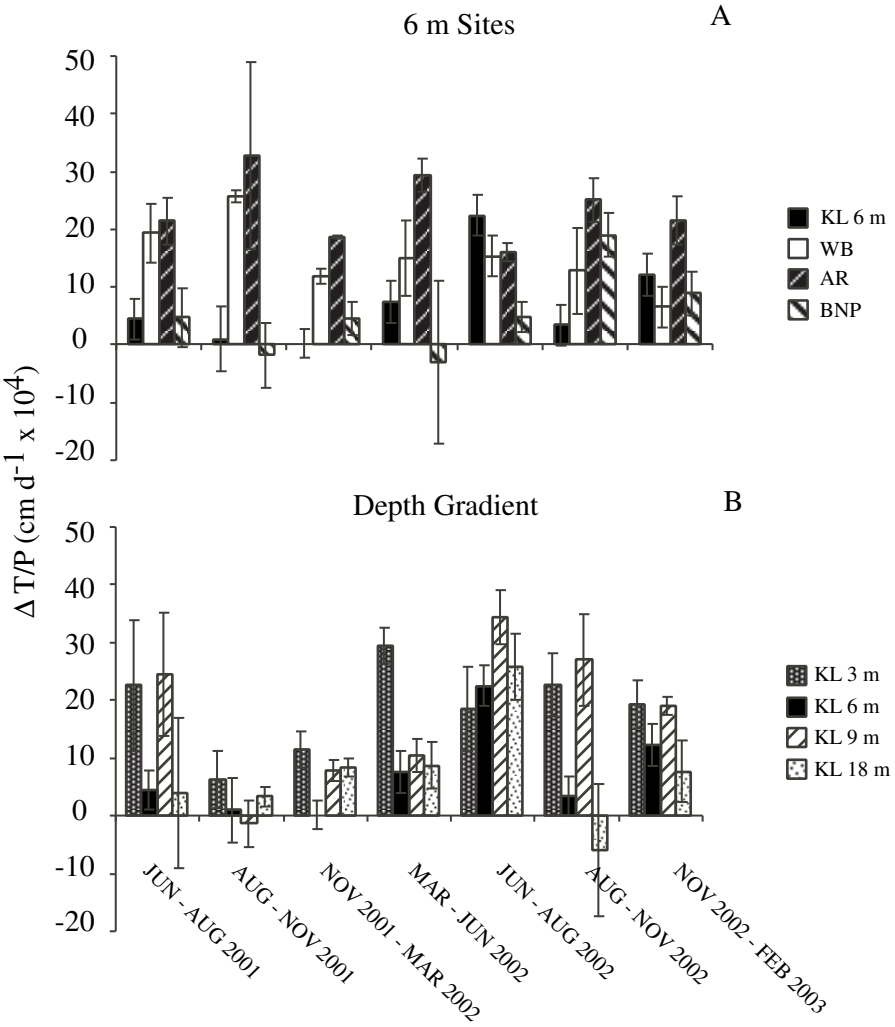


Figure 5.3. Regeneration rates standardized to initial lesion perimeter (mean ΔT/P ± SE) for each season from one sampling event until the next. Comparisons (A) among 6 m sites and (B) along depth gradient. Regeneration rates were calculated between June and August 2001 (54 ± 13 d), August and October 2001 (56 d), October 2001 and March 2002 (153 ± 2 d), March and June 2002 (91 ± 1 d), June and August 2002 (48 ± 13 d), August and November 2002 (74 d) and November 2002 and February 2003 (99 ± 1 d).

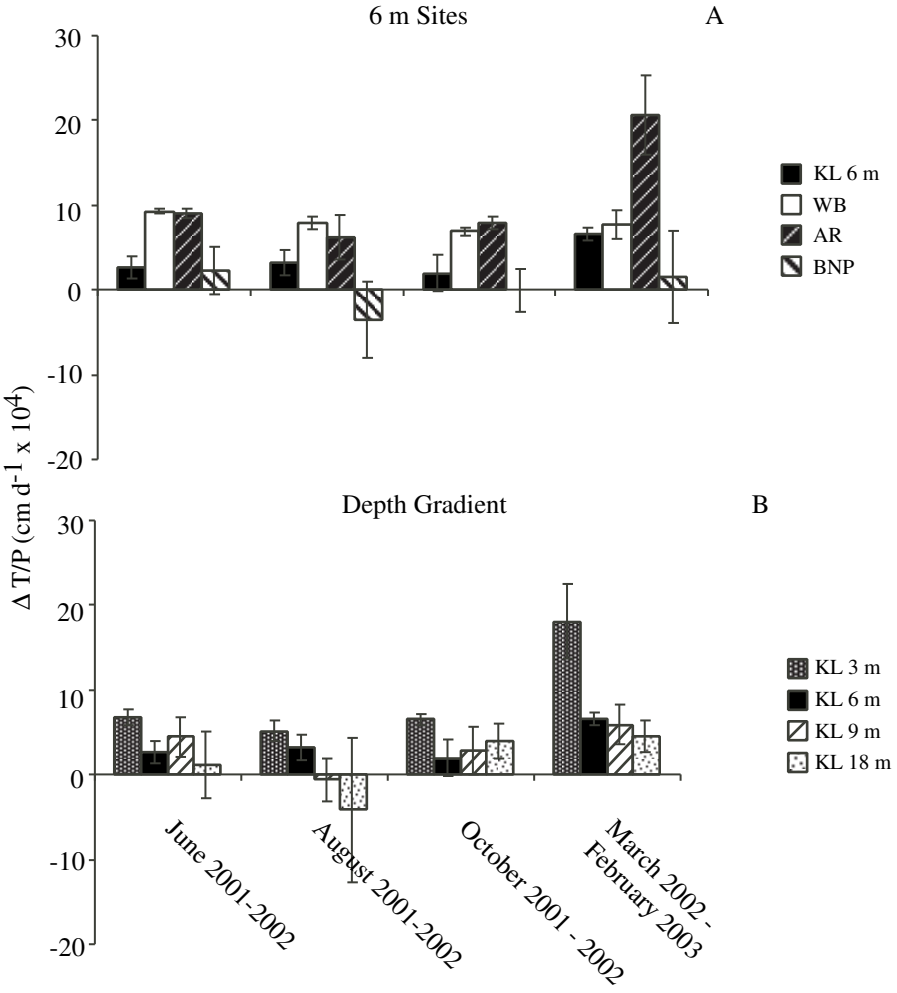


Figure 5.4. Regeneration rates standardized to initial lesion perimeter (mean Δ T/P ± SE) for each season between June 2001 and March 2002 from the time of sampling until the following year. Compared (A) among the 6 m sites and (B) along the 3 - 18 m depth gradient. Regeneration rates were calculated between June 2001 and 2002 (357 ± 10 d), August 2001 and 2002 (355 ± 1 d), October 2001 and November 2002 (374 ± 2 d) and March 2002 and February 2003 (321 ± 2 d).

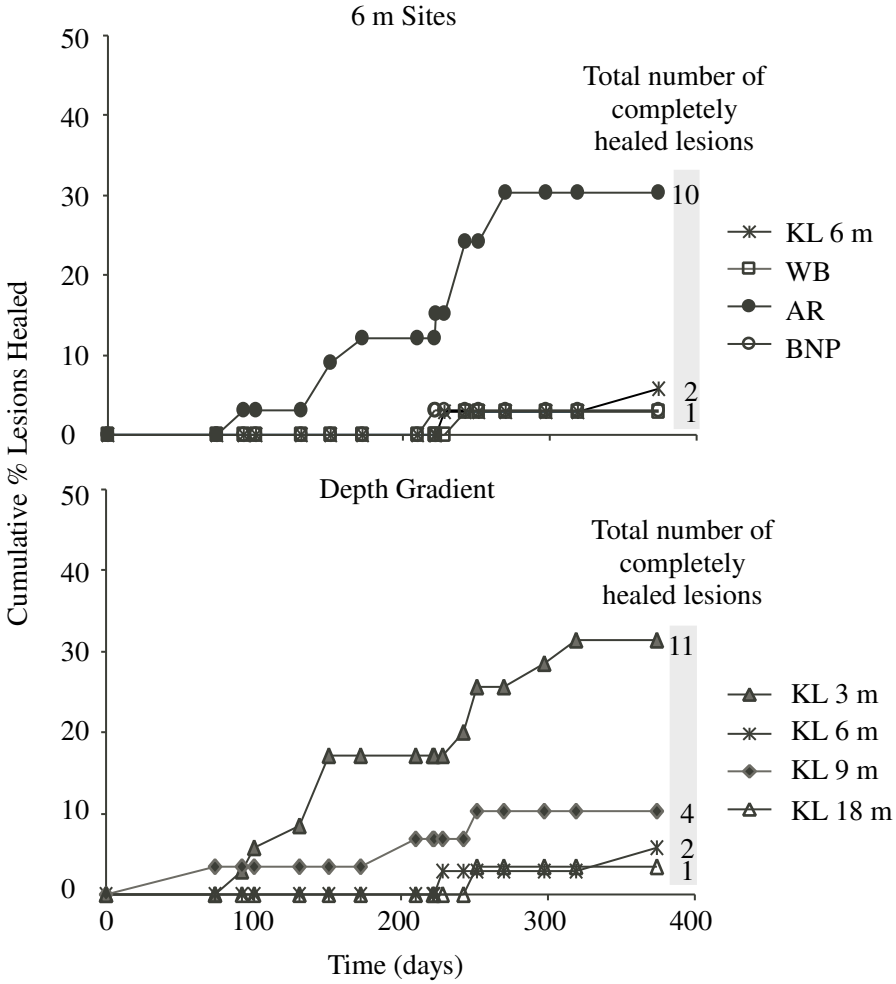


Figure 5.5. Cumulative percentage (%) of lesions completely healed with time (days) at (A) the 6 m sites and (B) along the depth gradient. Numbers adjacent to lines in the shaded area are total numbers of completely healed lesions at each site.

6. Environmental Links to Coral Stress Response

6.1. Introduction

6.1.1. Linking Potential Stressors to Organism Responses

Florida reefs have undergone severe degradation over the past several decades (Andrews et al. 2005), with a documented Keys-wide loss of 45% live coral cover between 1996 and 2004 (Beaver et al. 2005) and 50 – 90% since the 1970s (Porter et al. 2002, Gardner et al. 2003, Palandro et al. 2003 Palandro 2006). Reef decline has been attributed to a number of global and regional stressors including climate change and bleaching, disease, tropical storms, coastal development and runoff, over-harvesting and pollution (Porter et al. 1999, Bellwood et al. 2004, Waddell 2005). Current monitoring tools have limited ability to differentiate among these stressors and are incapable of determining mechanisms of decline (Downs et al. 2005b). My study examined effects of selected stressors on corals' ability to heal by comparing lesion regeneration rates (Ch. 5) with environmental data sets (Ch. 2) and cellular diagnostic data (this chapter). Regeneration rates reflect a coral's ability to heal from a disturbance and have been used as indicators of coral physiological condition (Fauth et al. 2005, Fisher et al. in press). Regeneration can affect coral fitness by competing for energy with other critical processes such as growth (Bak 1983, Guzman et al. 1994, Meesters et al. 1994) and reproduction (Guzman et al. 1994, VanVeghel & Bak 1994, Kramarksy & Loya 2000, Lirman 2000a, Oren et al. 2001, Kramarsky-Winter 2004).

Causal inference can be used to link effects of stressors to responses in corals (Suter et al. 2002). Causal inference is defined as analyzing available information, which may include spatial or temporal associations of potential cause and effect, field or lab results, and diagnostic evidence from affected organisms to generate evidence against a particular stressor (Bro-Rasmussen & Løkke 1984, Fox 1991, Suter et al. 2002). If the cause cannot be identified with sufficient confidence, effects are re-evaluated and the process starts over. A better understanding of the causes of reef decline provides managers with greater confidence when targeting remediation efforts against stressors

and determining when and what type of action is necessary (Jameson et al. 2002, Suter et al. 2002, Adams 2005).

6.1.2. Potential Candidates of Stress

The first step of causal inference is listing potential stress candidates based on available information (Suter et al. 2002). Regeneration rates are reduced with increases in nutrients (Koop et al. 2001), turbidity (Croquer et al. 2002), sedimentation and resuspension rates (Meesters et al. 1992, Croquer et al. 2002), and enhanced with increases in water temperature (Lester & Bak 1985, Kramarsky-Winter & Loya 2000, Paz-García & Reyes-Bonilla 2006). Other factors known to stress corals that may negatively affect regeneration rates include algal competition (Lirman 2001), high irradiance (Lesser & Farrell 2004, Lesser 2006), and marine pollutants (e.g., heavy metals, pesticides; Guzman & Jimenez 1992, Morgan & Snell 2002, Owen et al. 2002, Downs et al. 2005a). Reduced availability of food or autotrophically derived energy also can reduce regeneration rates, as evidenced by decreased regeneration rates with reduced light levels (with increasing depth; Nagelkerken et al. 1999) and with symbiont loss during bleaching (Meesters & Bak 1993, Fine et al. 2002, Mascarelli & Bunkley-Williams 1999). In some cases, energy may be gained heterotrophically (Rinkevich 1996, Henry & Hart 2005) or by reallocating energy from other life history processes (Guzman et al. 1994, Kramarsky-Winter 2004, Henry & Hart 2005).

6.1.3. Metabolic Costs of Stress on Corals

An animal usually functions in a homeostatic state and has a limit of compensation for changes in any environmental factor (Sindermann 1996). Stress is defined as any environmental altercation that extends homeostatic or protective processes into a compensatory state beyond the normal limits of an organism (Seyle 1955, Bayne et al. 1985, Moore 2002). If compensatory limits are exceeded, the organism experiences increased energy expenditure and disabilities begin to appear (Depledge et al. 1993, Sindermann 1996). Corals in stressed environments are likely to increase energy expenditure as cellular defenses neutralize or dissipate the effects of stress and restore cellular or tissue damage, thereby reducing resources (Koehn & Bayne 1989, Williams

1994, Morgan & Snell 2002) available for regeneration, growth and reproduction. Small changes in protein/maintenance metabolism can have major effects on energy status (Hawkins 1991, Coustau et al. 2000). Consequently, stress renders the individual, and ultimately the population, at a disadvantage through reduced growth, impaired reproduction, and increased susceptibility to disease and mortality (Bayne et al. 1985, Adams 2005, Downs 2005, Downs et al. 2005b). The extent of population response (collective individual responses) and the ability of the population to recover depend on the intensity and duration of environmental change (Sindermann 1996). There are three phases of physiological responses to stress: alarm, resistance and exhaustion (Sindermann 1996). The alarm phase includes immediate or short-term behavioral, biochemical, or physiological responses to non-optimum changes in the environment. The resistance or adaptation phase includes longer-term biochemical/ physiological responses that improve the likelihood of survival in the non-optimal environment; and the exhaustion phase includes failure of critical biochemical functions, leading to physiological and morphological disorders and death (Sindermann 1996). This study provided an opportunity to determine where coral colonies at my study sites fit within these phases of physiological stress response by examining both coral cellular parameters and colony responses (e.g., regeneration rates).

6.1.4. Cellular Diagnostic System

Environmental stressors affect organisms by overwhelming defenses at lower levels of the biological hierarchy: molecular, cellular, and organismal-level homeostatic processes (Moore 2002, Downs 2005). The Cellular Diagnostic System (CDS) is a systematic approach to defining and integrating cellular biomarkers based on their functionality within the cell and how deviations in their behavior may reflect overall cellular operation or performance (Downs 2005). Cellular Diagnostic parameters can be split into functional groups including (1) protein metabolic condition, (2) oxidative damage and response, (3) metabolic condition and integrity and (4) xenobiotic detoxification (Appendix A; Downs 2005). Protein metabolic condition involves the process of protein synthesis, maturation and degradation. Oxidative damage and response involve antioxidant pathways that allow the cell to function in an oxygen-rich

environment. Metabolic condition and integrity involve a number of sub-processes or metabolic pathways that maintain the cell in a differentiated state from its environment. Xenobiotic detoxification involves the process of preventing or reducing the adverse effects of exposure to foreign chemicals (e.g., pesticides).

The CDS diagnoses organismal health by (1) quantifying cellular and physiological condition (e.g., protein metabolism, genomic integrity), (2) characterizing types of cellular physiological stress (e.g., oxidative stress, xenobiotic stress), and (3) determining if defenses have been built up against a particular stress (e.g., pesticide, heavy metal, or polycyclic aromatic hydrocarbons; Downs 2005, Downs et al. 2000, 2001a,b, 2005a, 2006). The CDS was used in this study to determine (1) if coral biomarker profiles differed among study sites or sampling periods, (2) if coral biomarker profiles reflected stressed conditions in the coral, and (3) what stressor(s) the corals likely experienced (e.g., pollutants vs. increased ocean temperatures) and possible mechanisms of stress. Shifts in the steady-state biomarker levels indicate a shift in the equilibrium of the sub-systems that they represent. Deviation of the behavior of a specific parameter from the reference is an altered state, which is defined as a pathology or 'diseased condition' if that phenotype is associated with conditions that adversely affect performance (e.g., reduced regeneration rates or growth; Peters 1997, Downs 2005).

Unlike traditional ecological monitoring methods, a cellular diagnostic approach can distinguish among different stressors because they elicit a specific biological response in exposed organisms (Depledge et al. 1993) and the cellular function of each biomarker is well understood (Downs 2005). Through examination of multiple parameters, CDS also can elucidate cellular mechanisms of stress. For example, in the Florida Keys, concentrations of specific diagnostic markers (e.g., lipid peroxide and chloroplast small heat shock protein) were linked to elevated water temperatures, disruption of homeostatic mechanisms and bleaching of the coral, *Montastraea annularis* (Downs et al. 2002). Certain diagnostic markers (e.g., chloroplast small heat shock protein [ChlpsHsp]) taken in the context of other cellular parameters could predict which coral colonies would bleach six months in advance (Fauth et al. 2003). Corals with high levels of antioxidant enzymes (e.g., Cu/Zn superoxide dismutase, Mn superoxide dismutase) were less likely to bleach than those with low levels (Fauth et al. 2003).

Downs et al. (2002) used this evidence to form the oxidative theory of coral bleaching based on the following evidence: (1) pigment loss and bleaching followed oxidative damage; (2) increased oxidative damage products (protein carbonyl and lipid peroxide) and protein turnover activity were highly correlated with bleaching; (3) cellular integrity was compromised by oxidative stress; (4) cellular defenses (e.g., antioxidant defenses and stress proteins) were capable of providing protection from bleaching to corals; and (4) negative relationships between ChlpsHsp and oxidative damage indicated that breakdown of photosystem II was the primary generator of reactive oxygen species and therefore the underlying source of oxidative stress and temperature-associated coral bleaching. Downs et al. (2002) proposed that bleaching is the coral's final defense against oxidative stress.

Some caveats of using a biomarker approach include: (1) natural populations often are exposed to multiple stressors, many of which can act synergistically, making interpretations difficult, (2) different organisms can have variable biomarker responses to similar stresses (e.g., Downs et al. 2001a,b), and (3) biomarker responses fluctuate seasonally and with changes in nutritional state, and in developmental or reproductive stages (Depledge et al. 1993). Use of an integrated biomarker system in addition to laboratory studies can elucidate the synergistic effects of multiple stressors. For example, CDS showed that normal photosynthetically active radiation (PAR) increased effects of heat stress on *Montastraea faveolata* by increasing levels of oxidative stress, overwhelming antioxidant defenses, and resulting in high levels of protein denaturation (Downs et al. 2000).

6.1.5. Linking Cellular Biomarkers to Higher Order Processes

Understanding how multiple stressors effect reefs requires a hierarchical, mechanistic approach based on multiple lines of evidence (Adams 2005, Downs 2005, Yeom & Adams in press). This allows researchers to determine whether (1) an organism is responding to a stressed condition and (2) that stress resulted in reduced physiological function (Downs 2005, Moore et al. 2006). Lower levels of biological organization (e.g., cellular biomarkers) can provide information on the mechanism of decline, whereas higher levels of biological organization (e.g., coral regeneration rates, community

condition) provide information on the effect of stress on overall organismal fitness and function (Fig. 1.1).

A major challenge in biomarker work is connecting cellular processes with higher order processes (Moore et al. 2006, Yeom & Adams in press). Molecular and cellular biomarkers have the potential to provide early distress signals of reduced performance, impending pathology and diminished health (Moore 1990, Moore & Simpson 1992, Depledge et al. 1993, Downs 2005, Downs et al. 2005b). For example, lysosomal stability was a predictive tool of cellular injury and pathology in marine mussels (Moore et al. 2006). Accumulations of biomarkers of xenobiotic response and decreased protein turnover were associated with decreased regeneration rates in the mustard hill coral, *Porites astreoides* (Fauth et al. 2005). Ubiquitin, cytochrome P450 2-class (CYP-2) and cytochrome P450 6-class (CYP-6) explained 24% of variation in regeneration rates of *P. astreoides*. Corals with high levels of ubiquitin and low levels of CYP-2 and CYP-6 had the highest regeneration rates, indicating that exposure to a xenobiotic resulted in reduced physiological condition. My study further examines the relationship between changes in coral cellular condition and changes in physiological condition (e.g., regeneration rates and mortality).

6.2. Objectives

My study is a continuation of biomarker studies in the Upper Florida Keys that began in 1999 (e.g., Downs et al. 2000, 2005a, Fauth et al. 2003). Objectives of this chapter include (1) to differentiate between global (e.g., temperature) versus local (e.g., pollutants) stressors, (2) to detect subtle and chronic effects of environmental stress on corals and (3) to diagnose coral condition at my study sites based on parameters of the cellular diagnostic system and coral colony responses (e.g., regeneration rates). My hypothesis was that a coral colony, for which the CDS indicated stress (e.g., xenobiotic, oxidative, etc.), was less likely to regenerate than a coral with a lower stress signal, due to allocation of resources to cellular maintenance. I worked in collaboration with C. M. Woodley, C. A. Downs, and J. E. Fauth to interpret these data.

6.3. Methods

6.3.1 Study Sites

Coral condition was assessed using the cellular diagnostic system at one patch reef in Biscayne National Park (BNP), and four patch reefs plus two depths on one forereef in the upper Florida Keys National Marine Sanctuary (FKNMS). These seven sites (Fig. 2.1) comprised both a latitudinal transect with four sites at 6 m depth and a depth transect [Key Largo (KL) 3 m – KL18 m] and were chosen in consultation with resource managers to reflect a spectrum of possible anthropogenic influence, based on distance from urbanized coastal development. The study was carried out approximately quarterly between March 2001 and February 2003. The Key Largo (KL) depth transect is located offshore from the most urbanized coastline of Key Largo, from which natural vegetation has been removed and natural topography altered to maximize waterfront properties; the coastline is lined with seawalls. These sites lay along the route used by recreational boaters and commercial dive operators to reach Molasses Reef and other heavily used outer reefs in the Upper Keys reef tract, so pollutants such as hydrocarbon combustion products may be more prevalent. In addition, larger boats regularly stir up sediments, potentially remobilizing nutrients and chemical pollutants (Kruczynski & McManus 2002). Algae Reef (AR) also is offshore from Key Largo but is adjacent to John Pennekamp Coral Reef State Park and situated mid-way between the Key Largo and the BNP sites. The natural coastline is native, intact and relatively vegetated with coastal hammock mangroves and seagrass beds (see Fig. 2.1). White Banks (WB) is close to the KL 6m site, but still adjacent to the state park (Fig. 2.1). Biscayne National Park (BNP) is closest to urban Miami, FL. This site is potentially influenced by the extensive agricultural area to the south and west that drains into Biscayne Bay through a series of watershed canals. BNP also is located relatively near Turkey Point nuclear power plant and a major landfill in the Black Point area.

6.3.2 Cellular Diagnostic Sampling

For cellular diagnostic sampling, a 1-2 cm^2 plug of coral tissue was obtained using a leather punch and hammer from each of five separate colonies of the *Montastraea annularis* complex (Fig. 5.1). Samples were kept in the dark by placing them in opaque

film canisters underwater. On deck, water was removed from the canisters and samples were immediately transferred to a liquid nitrogen dry shipper. Samples were stored at -80° C until analyses were conducted by EnVirtue Biotechnologies (Winchester, VA, USA). Frozen coral samples were ground with a liquid nitrogen-chilled mortar and pestle. A suite of 20 biomarkers (Table 6.1) was assayed during each sampling period. Concentrations of all biomarkers were determined using enzyme-linked immunosorbent assays (ELISA). Samples were assayed in triplicate. Detailed description of the ELISA assays are in Downs (2005) and Downs et al. (2005a).

6.3.3. Data Analysis

Data used in the analysis for this chapter also include those presented in Chapter 2 (environmental assessments) and Chapter 5 (lesion regeneration). Sampling sites, dates and methods, as well as sample processing methods, were detailed previously in those chapters.

6.3.3.1. Cellular Diagnostic System

Data were analyzed in two groups: by sites at 6 m depth along the northeast – southwest traverse, and by sites along the depth gradient. The KL 6 m site was common to both groups. For each cellular biomarker, I calculated descriptive statistics: mean, standard error, range and coefficient of variation. For descriptive statistics, biomarkers were organized based on their grouping in one of the four subsystems (Table 6.1). I compared mean levels of selected biomarkers with "stressed" and "basal" levels as defined by Downs et al. (2005a) (Table 6.2). I used these values as a reference for comparison but they should be adjusted as additional information is obtained.

I used repeated-measures MANOVA on each biomarker for the entire study period (March 2001 to February 2003) to determine if individual biomarker concentrations varied with site, time and their interactions. I checked model assumptions (e.g., sphericity, homogeneity of variances, normality, and independence) using residual plots. Biomarker data were log transformed to meet these assumptions. To interpret effects detected by MANOVA, I used one-way ANOVA followed by the Tukey-Kramer HSD method.

Similarity matrices were constructed using Euclidean distance for cellular diagnostic data to allow for comparison of samples. Cellular diagnostic data were normalized and log-transformed to minimize the effect of using parameters measured at different scales. To determine if the entire suite of biomarkers differed among sites or sampling periods, I used ANOSIM2 and ANOSIM analyses. I used ANOSIM2 (two-way analysis of similarities) to determine if all biomarkers differed significantly among sites (averaged over the entire study period) and time (averaged across all sites). I used ANOSIM (one-way analysis of similarities) to determine if sites differed significantly for each sampling period (i.e., there were site x time interactions) based on all biomarkers. I used Principle Components Analysis (PCA) to interpret the differences found in ANOSIM2 and ANOSIM.

6.3.3.2. Relating Coral Cellular Biomarkers to Coral Regeneration Rates

I compared regeneration rates with biomarker concentrations among sites for a given time period using the BEST routine to determine which biomarkers reflected observed trends in coral regeneration. This routine selects the biomarkers that best explain regeneration rates, by maximizing a rank correlation between their respective resemblance matrices (Clarke & Gorley 2006). The BEST routine is similar to a stepwise regression but does not assume independence or normality, is non-additive (R^2 does not increase with addition of parameters to the function), and can handle data sets with a high number of parameters and low sample size (Clarke & Gorley 2006). This procedure is based on the weighted Spearman rank coefficient (ρ_w) between the ranked regeneration and CDS similarity matrices. I then used the RELATE routine to determine the significance of the relationship between the two similarity matrices based on the set of markers chosen using BEST. Visualizations of this relationship were presented by superimposing the cellular biomarker concentrations on to the regeneration rate multi-dimensional scaling (MDS) plot and comparing this with the bubble plot of the regeneration rates.

6.3.3.3. Linking Environmental Data to Coral Regeneration Rates

Regression analyses were used to determine if sedimentation rates influenced regeneration rates among the 6 m sites. Sedimentation rates were log transformed to meet assumptions of normality and homoscedasticity. I used data obtained from the Southeast Research Center's Water Quality Monitoring Network (SERC-WQMN) to examine the effects of changes in environmental parameters on regeneration rates. Due to the overlap in environmental datasets for some study sites (see Table 2.3), comparisons between regeneration rates and SERC water quality parameters (Table 2.2) were made within sites to determine which parameters affected changes in regeneration rates during the study period. I first tested for collinearity by calculating Pearson correlation coefficient between all pairs of environmental parameters. I then selected parameters that accounted for the majority of the variation based on PCA and were not duplicative. I used the BEST routine to determine which environmental parameters best reflected trends seen in regeneration at each site. Similarity matrices used in this analysis were constructed using Euclidean distance for regeneration and environmental data. The sets of parameters with the largest ρ_w were considered to provide the best match with regeneration rates. Visualization of this relationship was presented through bubble plots of selected environmental parameters on the regeneration rate multidimensional scaling (MDS) plot.

6.3.3.4. Analysis Routines

ANOSIM2, ANOSIM, PCA, BEST, RELATE, MDS and Pearson correlations were performed using PRIMER v. 6 (Plymouth Routines in Multivariate Ecological Research, PRIMER-E Ltd., Plymouth). All other analyses were performed using JMP v.3.2. (SAS Institute Inc., Cary, NC, USA) except repeated-measures MANOVA, which was performed using SAS 9.1 (SAS Institute Inc., Cary, NC, USA) with $\alpha = 0.05$ for all hypothesis tests.

6.4. Results and Discussion

I utilized data acquired from the cellular diagnostic system (Fig. 6.1 – 6.8) to address these three objectives (1) differentiate between global (e.g., temperature) versus

local (e.g., pollutants) stressors, (2) detect subtle and chronic effects of environmental stress on corals and (3) diagnose coral condition at my study sites based on parameters of the cellular diagnostic system and coral colony responses (e.g., regeneration rates).

6.4.1. Evidence for Temperature or Light Stress?

The CDS was applied to distinguish between global stressors (e.g., sea-surface temperatures; Downs et al. 2000) and local stressors (e.g., pesticides; Downs et al. 2005a). Characterization of candidate stressors includes elimination, diagnosis and strength of evidence (Suter et al. 2002). One of the criteria in causal inference is temporal association (e.g., time order), therefore it is important to determine when corals were stressed and how that relates to environmental conditions. Time of sampling was the dominant factor affecting the entire suite of cellular biomarker levels among the 6 m sites (time: averaged across all 6 m sites; ANOSIM2: Global R = 0.38, p = 0.10%; Table 6.3; site: averaged across all time periods; ANOSIM2: Global R = 0.13, p = 0.10%; Table 6.4; time x site; ANOSIM; Table 6.5) and along the depth gradient (time: averaged across depth gradient; ANOSIM2: Global R = 0.32, p = 0.10%; Table 6.6; site: averaged across all time periods; ANOSIM2: Global R = 0.27, p = 0.10%; Table 6.7; time x site; ANOSIM; Table 6.8). At the 6 m sites, all individual biomarker levels between 2001 and 2003 were significant with time except for Cn Hsp 60 and Catalase (Repeated Measures MANOVA; Table 6.9). Most individual biomarker levels along the depth gradient also were significantly different with time, except for CYP-3, ChlpsHsp, Heme and Catalase (Repeated-measures MANOVA; Table 6.10).

A moderate increase in protein, oxidative and metabolic markers is expected in the summer months because coral metabolic rates increase with warmer water temperatures. For example, Hsp 70 and ubiquitin levels in bivalves increased in summer months, with temperatures normally experienced by these organisms (Hofmann & Somero 1995). Several coral biomarkers (e.g., heat shock proteins, antioxidant enzymes) upregulate further in response to temperature stress (Black et al. 1995, Lesser 1996, 1997, Fang et al. 1997, Downs et al. 2000, 2002, Brown et al. 2002a,b). For example, in 1999 along the KL depth gradient, high levels of antioxidants and oxidative damage products were observed in *Montastraea annularis* between May and September. Increases in

biomarkers corresponded with increases in water temperatures and preceded bleaching that September (Fauth et al. 2003).

During my study, high biomarker levels, particularly those indicative of protein denaturation and oxidative stress, occurred along the depth gradient at KL 3 m and KL 9 m in August 2001 (Fig. 6.5 – 6.6) but did not appear to be responses to temperatures, which were not abnormally high at that time (30º and 29º C, respectively; Fig. 2.4) and no coral bleaching was observed at any of my study sites throughout my study. Biomarkers levels also were generally not as high as in March and October 2001 (Table 6.11, Fig. 6.9B) when temperatures are cooler. Biomarker levels were highest at KL 9 m (Fig. 6.10C), with high levels of all biomarkers. Cnidarian GST reached "stressed" levels at KL 3 m (Fig. 6.8D) and Dn GST reached "stressed" levels at KL 3m and KL 9m (Fig. 6.9E), suggesting that corals were responding to a xenobiotic stress. Xenobiotic markers do not increase in response to a temperature stress. Potential sources of xenobiotic stressors in summer months include increased use of insecticides to control mosquito populations (e.g., dibrom in August; Morgan & Snell 2002) and increased boat use (May through August). High wind speeds were observed at the Molasses Reef buoy in August 2001 (Fig. 2.12), which could have resuspended sediments and exposed corals to associated contaminants.

In 2000, no sea surface temperature anomalies occurred and biomarker levels no longer correlated with temperature (Downs et al. 2005a). Instead, corals at BNP responded to a xenobiotic stress in March that resulted in a severe oxidative, metabolic, and protein-denaturing stress (Downs et al. 2005a). Similar biomarker profiles were observed at the 6 m sites during my study; low levels of all biomarkers were observed in summer months June 2001, August 2001 and August 2002, whereas all biomarker levels were high in the spring and fall months of March 2001, October 2001 and February 2003 (Table 6.12, Fig. 6.9B).

Temperature can be eliminated as a potential cause of stress because (1) no significant differences in temperature were observed among sites and therefore it could not account for intersite differences in cellular diagnostic markers or regeneration rates, (2) no relationship was observed between changes in temperature during the study and changes in regeneration rates within sites (Table 6.13), and (3) temperatures did not

exceed known stress thresholds during the period of the study. Limited information on light levels (PAR and UV) did not allow for comparisons among sites. However, stressors were generally highest in winter months and no extreme light levels were observed during my study (Fig. 2.16), indicating that excess light was most likely not a major cause of stress. Consistent methodology in cellular diagnostic sampling will allow for future comparisons among studies and long time-series comparison to further elucidate seasonal differences in biomarkers and establish baseline values for biomarkers. Experimental setups with corals will allow for comparison of biomarker levels during routine metabolism versus defenses against specific stressors (particularly contaminants such as pesticides and herbicides).

6.4.2. Evidence for Local Xenobiotic Stress

Higher biomarker levels during cooler months (March 2001, February 2003) suggest that local conditions rather than temperature were the source of stress. The highest stress levels at the 6 m sites were observed in March 2001 (Table 6.12) with no significant differences among sites (Table 6.8). High protein turnover and denaturation affecting both the cnidarian host and the symbiont (dinoflagellate) occurred in March 2001 at all 6 m sites as indicated by significant increases in Cn Hsp70, Dn Hsp70, Dn Hsp60 and ubiquitin (Fig. 6.1; Table 6.12). Cnidarian Hsp70 reached "stressed" levels and Dn Hsp70 exceeded "basal" levels at all 6 m sites (Fig. 6.1). Mean Dn Hsp60 reached "stressed" levels at BNP and "basal" levels at all 6 m sites (Fig. 6.1). Oxidative stress, particularly in the dinoflagellate symbionts, was evident in March 2001 at all 6 m sites as indicated by significant elevations in Dn Cu/Zn SOD, Cn Mn SOD, Dn Mn SOD and Dn GPx (Fig. 6.2; Table 6.12). Cnidarian Mn SOD and Dn MnSOD reached "stressed" levels at BNP in March 2001 (Fig. 6.2). Metabolic condition was compromised in March 2001 as indicated by elevated concentrations of HO and Cn sHsp (Fig 6.3; Table 6.12). Elevations of CYP-2, Cn GST, Dn GST and MXR in March 2001 indicated that corals were responding to a xenobiotic, particularly at KL 6 m and BNP. Cnidarian and dinoflagellate GST reached "stressed" levels at KL 6 m (Fig. 6.4).

In February 2003, the highest biomarker levels were found at BNP and the lowest at AR (Fig. 6.11). High protein turnover and denaturation occurred at BNP as indicated

by "stressed" levels of Cn Hsp70 and higher ubiquitin levels (Fig. 6.1). Weaker oxidative stress was evident in both the cnidarian host and dinoflagellate symbiont as indicated by significant increases in Dn Cu/Zn SOD, Cn Mn SOD, Cn GPx and Dn GPx; "basal" levels of Cn Mn SOD, Dn Mn SOD and Dn GPx were exceeded at all 6 m sites (Fig. 6.2; Table 6.12). Metabolic condition was compromised as indicated by elevated ferrochelotase, ChlpsHsp and metallothionein in February 2003 (Fig. 6.3; Table 6.12), most likely in response to oxidative stress in both the cnidarian and dinoflagellate symbiont. A xenobiotic response also was apparent as indicated by elevated concentrations of MXR, Cn GST, Dn GST, CYP-2 and CYP-6 (Fig. 6.4; Table 6.12). Lower biomarker levels at AR in February 2003 suggest that stress levels were lowest at this site. Regeneration rates were highest at AR relative to other sites between November 2002 and February 2003 (Fig. 5.3), supporting this interpretation.

Potential stressors during South Florida winters include increased resuspension of bottom sediments (Fig. 2.2) and associated contaminants due to higher wind speeds (Fig. 2.11) during winter storms (Kruczyinski & McManus 2002); increased pesticide use by agriculture (Miles & Pfeuffer 1997, Pfeuffer & Rand 2004); and increased tourism (November through April; Krucyznski & McManus 2002). Sedimentation was consistently highest at AR (Fig. 2.2), but was unrelated to regeneration rates when compared among sites; therefore sediment loads alone are an unlikely stressor. Sediment properties, including grain-size and organic and nutrient content, play a key factor in determining sedimentation stress in corals (Weber et al. 2006). Silt-sized (< 63 μm) and organic-rich sediments can stress corals after a short-term exposure and are more likely to bind with pollutants including heavy metals and pesticides (Wu et al. 2004, Weber et al. 2006), whereas sandy, organic-poor sediments have little effect (Weber et al. 2006). Sediments at Key Largo 6 m and BNP were dominated by finer sands and muds, whereas WB and AR are characterized by coarse sediments. Therefore sediments at KL 6 m and BNP are likely more detrimental to coral physiology than at AR and WB. Microbial communities, particularly pathogens, found in the suspended sediment can have detrimental effects on corals' ability to repair damage (Hodgson 1990, Kramarsky-Winter 2004). Organic pollutants can bind with fine marine sediments that are ingested by benthic organisms such as corals and snails (Anthony et al. 2006).

Maximum pesticide detections (dominated by atrazine, ametryin, bromacil, simazine and norflurazon) in South Florida typically occur in winter to late spring with peaks in February and March (Miles & Pfeuffer 1997). Atrazine concentrations in South Florida canals also are typically high in November but highly variable (Harman-Fetcho et al. 2005). The largest number of endosulfan detections (a chemical of concern in Miami Dade County) corresponded with the agricultural growing season (October/November and March/April; Pfeuffer & Rand 2004, Harman-Fetcho et al. 2005). Concentrations of Irgarol 1051, a herbicide commonly found in anti-fouling paints, were higher in March 2001 than July 2001 in the Miami River (Gardinali et al. 2004).

In summary, cellular stress was highest in winter months and was observed at all study sites. Corals were responding to a xenobiotic stress that resulted in increased protein denaturation and turnover, as well as oxidative and metabolic stress. The largest effect was observed at sites with silty sediments (e.g., BNP and KL 6 m), most likely due to the resuspension of sediments and associated contaminants during winter storms. Concurrent contaminant analysis of sediments with future studies should be conducted at these sites to narrow down potential xenobiotics (e.g., endosulfan, Igrarol 101) that may be affecting corals. Often concentrations of any one contaminant are lower than what is typically thought to have a biological effect but a better understanding of chronic exposure to low dose contaminants (e.g., Owen et al. 2003) and the synergistic effect of multiple stressors (e.g., Porter et al. 2001) is needed.

6.4.3. Enhanced Local Effects with Heavy Rainfall

High rainfall also can increase input of stressors (e.g., nutrients, viruses, chemicals) to offshore reefs (Lapointe & Matzie 1996, Lapointe 1997, Paul et al. 2000). Large increases in nutrients and sedimentation followed large rainfall events in September 2001 and June 2002 (see Ch. 2). Higher biomarker levels in October 2001 and June 2002 may correspond with high rainfall prior to sampling (see Ch. 2, Fig. 2.8).

Rainfall was highest in September 2001 as a result of Hurricane Gabrielle that passed over central Florida and was associated with decreased salinity and increased turbidity, inorganic nitrogen, organic phosphorous and chlorophyll-a, particularly in areas near BNP and AR (Fig. 2.5 – 2.7; 2.9). Increases in nutrients during this study indicate

that terrigenous stressors reached my study sites after large rainfall events and therefore nutrients or other land-based stressors may have influenced these corals. High levels of all biomarkers separated BNP and AR from WB and KL 6 m, with the highest stress found at the most northern site (BNP) and the least stress at the most southern site (KL 6 m) (Fig. 6.12A). High protein turnover and denaturation affecting both the cnidarian and symbiont occurred in October 2001, particularly at BNP and AR, as indicated by significant increases in Cn Hsp70, Dn Hsp70 and ubiquitin (Table 6.5). Mean Cn Hsp70 reached "stressed" levels at all 6 m sites and mean Dn Hsp70 exceeded "basal" levels at WB, AR and BNP (Fig. 6.1). Oxidative stress also was evident in October 2001 as indicated by significant increases in Dn Cu/Zn SOD, Dn Mn SOD and Dn GPx (Fig. 6.2; Table 6.12). Mean Dn Mn SOD reached "stressed" levels at BNP and AR (Fig. 6.2). Cnidarian Cu/Zn SOD was significantly higher in October 2001 at AR relative to previous months (3.98 ± 0.69 vs. 1.45 ± 0.14 Eunits/ng TSP; ANOVA: $F_{3,16}$ = 15.0, p <0.0001; Fig. 6.3). Elevated concentrations of Heme, metallothionein and Cn sHsp in October 2001 (Fig. 6.3; Table 6.12), indicated that an oxidative or xenobiotic stress was compromising host mitochondria. Corals were responding to a xenobiotic stress as indicated by elevations in CYP-2, Dn GST and MXR (Fig. 6.4; Table 6.12).

Regeneration rates between October 2001 and March 2002 were best explained by October 2001 levels of Cn Cu/Zn SOD and metallothionein (Table 6.14). High levels of Cn Cu/Zn SOD in October 2001 positively correlated with regeneration rates between October 2001 and March 2002 (Fig. 6.13C; separating coral colonies at AR from all colonies at KL 6 m and most colonies at BNP (Fig. 6.13B-C). This indicates that while corals at AR were stressed, increased levels of antioxidants protected these corals from further damage. This also was observed in August 2001 when high levels of Dn Hsp 60 and Dn Cu/Zn SOD were found in the corals with the highest regeneration rates (Fig. 6.14). While most colonies at KL 6 m did not appear to be responding to stress in October 2001, high metallothionein in the colony with the lowest regeneration rates (Fig. 6.13D) suggest that a heavy metal exposure reduced colony performance.

Rainfall also was high in June 2002 but was not associated with an increase in inorganic nutrients. High concentrations of Cn Hsp 60, Ubiquitin, Dn Cu/Zn SOD, Dn MnSOD, Cn GPx, Cn MnSOD, Cn GST, CYP-6 and low concentrations of Dn Hsp60,

ChlpsHsp, CYP-2, CYP-3 separated KL 6 m from all other sites in June 2002 (Fig. 6.12B). Corals were responding to a xenobiotic, particularly at KL 6 m, where elevated concentrations of Cn GST indicated a "stressed" condition (Fig. 6.4). Cytochrome P450 2-class and 3-class were negatively correlated with concentrations of CYP-6 in June 2002 at KL 6 m (Fig. 6.12B). Cytochrome P450 6-class is specific to invertebrates and is recognized as a major contributor to insecticide resistance (Feyereisen 1999, Široká & Drastichová 2004); it oxidizes (and thus can be upregulated by exposure to) pesticides such as aldrin, dieldrin, diazinon, chlorpyrifos, deltamethnin and a wide range of pyrethrin-like compounds. Cytochrome P450 2-class and 3-class play a role in steroidogenesis (both sex steroids and cholesterol) and prostaglandin synthesis, but also are important monooxygenases for detoxifying different xenobiotics. Cytochrome P450 3-class was significantly depressed in corals at KL 6 m compared to corals from AR and BNP in June 2002 (0.26 ± 0.07 vs. 0.54 ± 0.07 and 0.51 ± 0.07 relative units/ng TSP, respectively; ANOVA: $F_{3,16} = 3.70$, $p < 0.04$; Fig. 6.4) indicating that steroid metabolism may have been disrupted. High protein turnover and denaturation occurred at KL 6 m in June as indicated by "stressed" levels of Cn Hsp70 and higher ubiquitin levels (Fig. 6.2). Metabolic condition was compromised as indicated by elevated concentrations of FC and ChlpsHsp (Fig. 6.3; Table 6.12).

The strongest relationship (Rho = 0.43, s.l. = 0.4%) between biomarkers and regeneration rates was observed in June 2002, when mean regeneration rates at KL 6 m were at their highest for the entire study period (Table 6.14). The combination of five biomarkers (cnidarian Hsp70, sHsp, metallothionein, cnidarian GST, CYP-6) clearly separated colonies at KL 6 m and BNP from those at WB and AR (Fig. 6.15). However, interpretation becomes difficult because the highest biomarker concentrations were observed both at the site with the highest (KL 6 m) and lowest (BNP) regeneration rates for that period (Fig. 6.15), with the exception of Cn GST, which was high and variable at KL 6 m only. While corals at KL 6 m and BNP generally both had low regeneration rates, in June 2002 regeneration rates at KL 6 m were highest among the 6 m sites (Fig. 5.3).

Chemicals can interfere with the basic cellular mechanisms of regeneration, which are likely controlled by steroid hormones. Prolonged exposure to low

concentrations of polychlorinated biphenyls (PCBs) affected the physiology of the crinoid (*Antedon mediterranea)* by interacting with cellular mechanisms that regulate growth and cell proliferation/turnover, which resulted in accelerated but abnormal arm regeneration (Candia Carnevali et al. 2001, Barbaglio et al. 2006). High contaminant levels, on the other hand, suppressed growth and increased mortality (Barbaglio et al. 2006).

Scleractinian corals produce a variety of steroids (Tarrant et al. 2001, 2003, 2004, Tarrant 2004, 2005). Abnormal regeneration rates at KL 6 m in June 2002 may be a result of endrocrine disrupting (EDs) contaminants such as PCBs, as evidenced by (1) increased levels of Cn GST, CYP-6, and MXR; (2) inhibition of CYP-2 and CYP-3, which are known for steroid metabolism; (3) increased levels of protein turnover indicated by increased ubiquitin and Cn Hsp60; and (4) oxidative stress as indicated by Cn GPx and Dn Cu/Zn SOD. The corallivorous snail (*Coralliophora abbreviata*), which feeds on these corals, also showed evidence of endocrine disruption at KL 6 m in June 2002 as evidenced by severe metabolic and oxidative distress, high levels of protein turnover and evidence of DNA damage (Woodley et al. unpublished data). Xenobiotic stress is implicated by high levels of GST, CYP-3, CYP-6, and MXR (Bard 2000). While White Grunts (*Haemulon plumieri*) did not show cellular evidence of endocrine disruption at KL 6 m, pesticide concentrations in livers of these fish were highest at this site relative to WB and BNP (Downs et al. 2006).

High rainfall in June 2002 could have led to an increase in pollutant exposure, particularly at KL 6 m, which lies off a developed coastline. Insecticide application is highest in late spring to summer months (Pierce 1998) during the warm, wet season when mosquitoes and other insects are most prevalent. Colored dissolved organic matter (CDOM), which scavenges various organic pollutants and thereby decreases their toxicity to marine organisms (Coble et al. 2004), tends to be lower at KL 6 m (Ayoub et al. 2006) relative to the AR and WB sites. Ultraviolet (UV-B) radiation levels also peaked in April and May 2002 (Ch 2), which can leave corals and other organisms more sensitive to chemical pollutants (Shick et al. 1996, Owen et al. 2003, Jones 2005).

6.4.4. Are Coral Regeneration Rates and Protein Production Energy-limited?

Another explanation for increased regeneration rates and biomarker levels in June 2002 at KL 6 m could be related to increased food availability in the form of organic nitrogen (Table 6.13; Fig. 6.16). Unusually high regeneration rates also were observed at KL 9 m and 18 m, where organic nitrogen also was high (Table 6.13; Fig. 6.17 and 6.18). Organic nitrogen increased in the area of the WB and the Key Largo depth gradient prior to the peak in rainfall in April 2002 (Fig. 2.6A and 2.10A). Individual corals can grow heterotrophically by feeding on dissolved and particulate organic matter (Edinger et al. 2000). Alternatively, these corals may have higher zooxanthellae and chloroplast densities and grow autotrophically using enhanced zooxanthellae photosynthesis fertilized by increased dissolved organic nutrients (Edinger et al. 2000). Stable isotopes have shown that corals can consume terrigenous organic matter or sewage-derived inorganic nitrogen (Mendes et al. 1997). Increases in inorganic nitrogen leads to increased protein synthesis in zooxanthellae (Muscantine et al. 1989). Nitrogen availability also influences heat shock protein production in higher plants, suggesting that Hsp production might be resource-limited (Heckathorn et al. 1996).

Unusually high biomarker concentrations at KL 18 m in November 2002 (Fig. 6.20) followed increases in total phosphorus and nitrate (NO_3) in October 2002 (Fig. 2.14A, 2.16A). Periodic upwelling along the shelf edge is a periodic source of phosphorus to offshore reefs (Szmant & Forrester 1996). Increased nutrients did not correspond to an increase in regeneration rates at KL 18 m in November 2002 (Fig. 6.18), which were lowest between August and November 2002 (Fig. 5.3). High levels of protein denaturation and turnover were evident by increased levels of Cn Hsp60 (Fig. 6.5; Table 6.11). While Cn Hsp70 was generally low in corals at KL 18 m, "stressed" levels were indicated at KL 18 m in November 2002 (Fig. 6.5). At that time, samples from both KL 3 m and KL 18 m had significantly higher ubiquitin levels than from KL 6 m (521 ± 21 and 613 ± 14 vs. 371 ± 40 fmols/ng TSP, respectively; ANOVA: $F_{3,16} = 8.8$, $p < 0.002$). Oxidative stress was also apparent at KL 18 m by significantly higher concentration of Cn GPx in corals from KL 18 m than in corals from KL 6 m and KL 9 m (55.5 ± 1.5 vs. 32.8 ± 3.7 and 38.9 ± 2.7 pmol/ng TSP, respectively; ANOVA: $F_{3,16} = 9.3$, $p < 0.001$). Ferrochelotase at KL 18 m was five times higher in November 2002 than

June 2001 (0.50 ± 0.08 vs. 0.10 ± 0.05 Eunits/ng TSP; ANOVA: $F_{8,45}$ = 2.6, p <0.03), likely in response to a need for increased heme oxygenage production for either antioxidant or xenobiotic detoxification pathways. Xenobiotic stress was evident in corals from KL 18 m, with significantly elevated CYP-6 in November 2002 compared to June 2002 (0.53 ± 0.12 vs. 0.10 ± 0.04 relative units/ng TSP; ANOVA: $F_{4,19}$ = 3.0, p < 0.05). "Stressed" levels of Dn GST were exceeded at KL 18 m in November 2002 (Fig. 6.8). Key Largo 3 m and KL 18 m had significantly higher levels of MXR than KL 6 m (0.74 ± 0.04 and 0.78 ± 0.04 vs. 0.53 ± 0.06 Eunits/ng TSP, respectively; ANOVA: $F_{3,16}$ = 6.7, p < 0.004). In November 2002, MXR was elevated at KL 3 m relative to June 2001 (0.74 ± 0.04 vs. 0.26 ± 0.10 Eunits/ng TSP, respectively; ANOVA $F_{8,35}$ = 2.9, p < 0.02) at KL 9 m relative to June 2001 (0.67 ± 0.03 vs. 0.35 ± 0.05 Enits/ng TSP, respectively; ANOVA $F_{8,36}$ = 5.2, p < 0.0003) and at KL 18 m relative to March 2001, June 2001 and August 2002 (0.78 ± 0.04 vs. 0.23 ± 0.12, 0.16 ± 0.02 and 0.24 ± 0.07 Eunits/ng TSP, respectively; ANOVA $F_{8,35}$ = 2.9, p < 0.02). Cytochrome P450 2-class was significantly depressed in corals from KL 18 m relative to corals from KL 3 m and KL 9 m in November 2002 (0.12 ± 0.07 vs. 0.57 ± 0.03 and 0.50 ± 0.04 Eunits/ng TSP, respectively; ANOVA: $F_{3,16}$ = 9.8, p < 0.001). Cytochrome P450 2-class also was significantly higher at KL 3 m in November 2002 than June 2001 (0.57 ± 0.03 vs. 0.27 ± 0.08 Eunits/ng TSP, respectively; ANOVA: $F_{8,36}$ = 8..2, p <0.0001) but was not as high as concentrations in March and October 2001 (Fig. 6.8).

In summary, periodic increases in nutrients preceded increases in regeneration rates and protein production along the depth gradient in June 2002 and protein production at KL 18 m in November 2002. These results suggest that regeneration and protein production may be energy-limited along the Key Largo depth gradient. While increased nutrient supply can explain increases in protein production, it does not explain why corals at these sites were responding to a xenobiotic stress.

6.4.5. Diagnosing Reef Condition

Integrating biomarkers and organism responses can be valuable tools by providing insight into where an organism lies on the health status curve (Fig. 6.22); that is, whether an organism is healthy and exhibiting homeostasis or has initiated compensatory

responses associated with a decline in health (Depledge et al. 1993). Organisms are generally more sensitive to environmental changes during certain stages of development. Candia Carnevali et al. (2001) recommended monitoring adult developmental stages (e.g., regeneration) of crinoids as an indicator of pollution-induced stress. In this study, I compared lesion regeneration rates of the coral, *Montastraea annularis*, with selected environmental parameters and cellular diagnostic tools of stress. Cellular biomarker levels indicate whether or not an organism is responding to a stress. Monitoring of regeneration rates and densities of symbiont-bearing ('larger') benthic foraminifera (LBF; Chapter 4) indicate if organisms are responding to a stress at a colony level and a population level, respectively.

6.4.5.1. 6 m Sites

Biomarker levels differed significantly among 6 m sites with significant differences among all sites (Table 6.4). Overall, the highest dissimilarity was between WB and BNP, while biomarker profiles for WB and AR were the most similar (Table 6.5). Among the 6 m sites, samples from BNP had the highest overall means for all biomarkers except Dn Hsp70, Cn GST and CYP-6 (Table 6.15). Samples from WB and KL 6 m tended to have the lowest overall means for biomarkers, however, variability as indicated by the coefficient of variance was highest in samples from KL 6 m (Table 6.16).

At the 6 m sites, the relationship between regeneration rates and biomarkers was generally low (Table 6.14). This inconsistency is a result of high biomarker concentrations at a site that had the lowest regeneration rates (BNP) and also at a site that had the highest regeneration rates (AR) (see Ch 5 and 6). On the other hand, the sites with the lowest biomarker concentrations had low (KL 6 m) to intermediate (WB) regeneration rates.

A need for increased protein synthesis or denaturation of damaged proteins was evident at AR and BNP due to significantly higher Dn Hsp60 than at KL 6 m (Table 6.15). Dn Hsp60 exceeded "basal" levels at all 6 m sites throughout the study (Fig. 6.1). Ubiquitin is a 76-residue polypeptide that is conjugated to proteins slated for degradation by the 26S proteosome. Mean ubiquitin was above "basal" levels at all 6 m sites

throughout the study period but never reached "stressed" levels (Fig. 6.1). Significantly higher ubiquitin levels at BNP than KL 6 m and WB (Table 6.15) indicates that more proteins were being damaged and targeted for rapid degradation. Oxidative stress in the dinoflagellate symbiont at BNP was high as evidenced by significantly higher Dn GPx at BNP than KL 6 m throughout the study (Table 6.15).

Corals at KL 6 m, which is located offshore from the most urbanized coastline in Key Largo, tended to have low biomarker concentrations throughout the study period, except for March 2001 and June 2002 when several biomarkers (including Cn GST, Ubiquitin, Cn Hsp 60 and sHsp) increased, indicating a xenobiotic response resulting in protein denaturation and turnover. Biomarker concentrations also were generally low in white grunts (*Haemulon plumieri*) at KL 6 m and WB, while liver concentrations of numerous pesticides (including DDT and its metabolites DDD and DDE, oxadiazon, heptachlor, endosulfan sulfate and others) were highest at KL 6 m relative to WB and BNP (Downs et al. 2006). While biomarker levels of corals were not consistently high at KL 6 m, periodic exposures to xenobiotic(s) may have created relatively unfavorable conditions for reef organisms (Fig. 6.22) as evidenced by low coral cover (Ch. 3), low densities of symbiont-bearing foraminifera (Ch. 4) and low regeneration rates (Ch. 5). Increased biomarker levels at this site followed winter storms (March 2001 and February 2003), when sediments and associated contaminants were likely resuspended, and high rainfall in June 2002.

Corals at WB generally had low to intermediate levels of all biomarkers throughout the study, with the highest stress evident in March 2001. Biomarkers at WB were generally above "basal" levels but below "stressed" levels as defined by Downs et al. (2005); exceptions include Cn Hsp70 (2001) and ChlpsHsp (2002). Corals at WB appeared to be compensating for stress (Fig. 6.22) as indicated by low coral mortality (Ch. 3), intermediate to high densities of symbiont-bearing foraminifera (Ch. 4) and intermediate to high coral regeneration rates (Ch. 5).

Corals at AR, which is adjacent to John Pennekamp Coral Reef State Park, had intermediate to high levels of biomarkers, particularly in March and October 2001, with biomarker levels indicating reduced stress throughout 2002 and 2003. Reef organisms appeared to tolerate occasional high stress conditions, as occurred in 2001, as evidenced

by relatively high coral cover (Ch. 3), high densities of symbiont-bearing foraminifera (Ch. 4) and consistently high coral regeneration rates and percentage of healed lesions (Ch. 5). Unlike KL 6 m, where biomarker and regeneration rates were variable, both regeneration rates and biomarkers were consistently high at AR. Therefore, colonies at AR appear to be stressed in response to a xenobiotic stress but protective cellular pathways are allowing the colonies to function normally at this site (Fig. 6.22). Contaminant exposures may be too low to cause overt effects, but over longer time periods may manifest into adverse conditions and mortality (Depledge et al. 1993). Therefore, management should identify and alleviate potential stressors at this site before they result in further degradation of the reef community. This provides an example of where CDS can be used to detect stress prior to a perceived affect on the organism at higher levels. This site lies along the most protected portion of coastline, with intact mangroves and wetlands, that provide a consistent source of colored dissolved organic matter (CDOM) (Ayoub et al. 2006). Colored DOM acts as a scavenger to a variety of trace metals and organic pollutants (Coble et al. 2004) and acts as a sunscreen to ultraviolet radiation. Further investigation is needed to determine the influence of CDOM or other factors that may have contributed to higher regeneration rates at this site relative to the others.

Corals from BNP, offshore from urban Miami, FL, tended to have the highest biomarker levels, particularly those indicative of high protein denaturation and turnover (ubiquitin and Dn Hsp60) and oxidative stress (Dn Cu/Zn SOD and Dn GPx) in the symbiotic dinoflagellates. Small changes in protein metabolism may result in a negative energy balance and greater variability in physiological performance (Koehne & Bayne 1989, Hawkins 1991). Refolding a protein with the help of stress proteins may cost in excess of 100 ATP molecules; therefore, increased synthesis of stress proteins can greatly increase energetic costs (Werner & Hinton 1999). Colonies at BNP had considerable recent coral mortality (Ch. 3), low densitities of symbiont-bearing foraminifera (Ch. 4) and low coral regeneration rates (Ch. 5) indicating that conditions were unsuitable for reef organisms during the study period. Biomarker levels at BNP indicate that these colonies are likely being stressed by a chemical pollutant, which is starting to overwhelm cellular defenses, resulting in physiological decline of the organisms (Fig. 6.22).

Although stress levels were generally lower in summer months, some biomarkers (e.g., Cn Hsp 70, Dn MnSOD) remained at "stressed" levels at BNP in June and August 2001. In August 2001, BNP showed evidence of oxidative stress with significantly higher Cn Cu/Zn SOD than WB and KL 6 m. Corals at BNP and AR both responded to a xenobiotic in October 2001. At the same time, BNP also experienced oxidative stress. High rainfall in South Florida (associated with Tropical Storm Gabrielle in September 2001) decreased salinity and increased turbidity and nutrients at water quality stations near AR and BNP (Ch. 2). Watershed canals flowing into Biscayne and Florida Bay potentially carry large loads of nutrients and pesticides (Harman-Fetcho et al. 2005).

In March 2000, corals at BNP experienced a protein-denaturing stress (as indicated by increased ubiquitin and Hsp70), likely in response to a xenobiotic (e.g., a fungicide, an organometalloid, endosulfan) affecting both the cnidarian host and the symbiotic zooxanthellae (Downs et al. 2005a). Corals likely responded to the xenobiotic by conjugating glutathione to it by glutathione-s-transferase (GST), and cellular exclusion of the GSH-conjugated xenobiotic by a P-glucoprotein 140/160 pump action (a.k.a. MXR; Downs et al. 2005a). The CDS also indicated that White Grunt (*Haemulon plumieri*) from BNP were exhibiting a xenobiotic response in August 2002 that adversely affected heme metabolism, resulting in endocrine disruption and elevated protein turnover (Downs et al. 2006). Contaminant levels in fish livers interpreted in the context of biomarker response profiles led to four probable suspects including the pesticides hexachlorobenzene, endrin, PCB 105 and Mirex (Downs et al. 2006).

6.4.5.2. Depth Gradient

Biomarker levels differed significantly along the depth gradient with significant differences among all depths (Table 6.7). Larger differences in biomarker levels were observed along the depth gradient during my study than in 2000, when no difference was seen along the KL depth gradient and overall biomarker levels were low compared to 2001-2003 (Downs et al. 2005a). However, trends in biomarker levels did not follow a straightforward depth trend. Instead, KL 3 m and KL 9 m tended to have higher biomarker levels, whereas KL 6 m and KL 18 m tended to have lower but more variable levels (Table 6.17, Table 6.18). Along the depth gradient, the highest dissimilarity was

between KL 9 m and KL 18 m and the lowest between KL 9 m and KL 3 m (Table 6.7). Key Largo 18 m had the lowest overall means for all biomarkers except for Dn Hsp60, which was low at both KL 6 m and KL 18 m. Pairwise comparisons of the biomarker suite (Table 6.8) revealed that KL 3 m and KL 9 m were the most similar, differing significantly only in August 2001. These two sites differed significantly from KL 18 m on at least two-thirds of the sample dates, with no significant differences in June 2001, October 2001, and March 2002.

A similar pattern was observed in 1999 in response to a temperature stress. Corals at KL 3 m had high levels of antioxidant enzymes between May and September, which protected corals at this site from oxidative damage, as evidenced by low levels of oxidative damage products (lipid peroxide and protein carbonyl) and no bleaching (Fauth et al. 2003). Lipid peroxide (LPO) is a product of oxidative damage to cell membranes and protein carbonyl is a product of oxidative damage to proteins. Corals at KL 6 m, KL 9 m and KL 18 m had low levels of antioxidant enzymes despite exposure to temperature stress as evidenced by high levels of oxidative damage products (LPO and protein carbonyl) and bleaching (Fauth et al. 2003). In my study, corals did not appear to be experiencing a temperature stress and no bleaching was observed, but low regeneration rates and increased mortality at KL 6 m, KL 9 m and KL 18 m indicate that these corals were stressed (Fisher et al. in press; Ch. 5). The relationship between cellular biomarkers and regeneration rates along the depth gradient were generally low (Table 6.19).

During my study, KL 3 m and KL 9 m both had high biomarker concentrations indicative of oxidative and metabolic stress and high protein turnover likely as a result of a xenobiotic stress, particularly in March, August and October 2001. However, physiological status at these two sites was very different. Despite exposure to high stress conditions, corals at KL 3 m had consistently high regeneration rates and a large percentage of completely healed lesions (Fig. 6.22). In contrast, corals from KL 9 m had highly variable regeneration rates, few completely healed lesions, extensive mortality and one colony died during the study period. Live coral cover at KL 3 m (36 %) was approximately five times that of KL 9 m (< 7%). Corals in deeper water typically receive less radiant energy and therefore may have lower carbon reserves than those in shallower water (Nagelberken et al. 1999). Therefore, increased protein production at KL 9 m

might have drained energy reserves for other physiological processes (e.g., regeneration; Fig. 6.22)

Corals at the deepest site, KL 18 m, consistently had very low biomarker levels (except for November 2002) and showed signs of physiological stress, indicating that these corals were in a diseased state (Fig. 6.22; Moore et al. 2006); stressors overwhelmed allostatic defenses and reduced the ability of corals to recover from damage (Downs 2005). Catalase, Dn GPx and CYP-3 were depressed in corals at KL 18 m compared to corals at other depths (Fig. 6.20; Table 6.17), despite high coral mortality and low regeneration rates, indicating that something is limiting production of these proteins. The coral, *Madracis mirabilis*, had depressed levels of catalase and cnidarian GPx following exposure to elevated concentrations of Igrarol 2051, an herbicide commonly used in antifouling paint (Downs & Downs 2007). Concentrations of Igrarol 1051 are a function of boat density and activity, with concentrations as high as 182 ng/L in the Key Largo Harbor canals in September 2001 (Gardinali et al. 2004). Concentrations as low as 63 ng/L can affect carbon uptake in coral symbionts (Owen et al. 2002), however, concentrations <2 km offshore were generally below detection limits (1 ng/L; Gardinali et al. 2004).

Other biomarkers concentrations remained low at KL 18 m, particularly CYP-2, ChlpsHsp, Dn Hsp 60 and Cn MnSOD. Down-regulation (as well as up-regulation) of stress proteins may indicate stress (Werner & Hinton 1999). Mechanisms leading to reduced protein levels may involve disruption of protein synthesis at the transcription, translational or post-translational (e.g., phosphorylation/dephosphorylation events) level, or may result from pathological effects or reduced energy (ATP) availability (Werner & Hinton 1999). When energy reserves are depleted, organisms may use proteins for additional energy and glucose supplies. Increased synthesis of stress proteins would represent an additional energy burden. This may explain why increases in total nitrogen at this site were positively correlated with increased regeneration rates.

Exposure to hypoxia in clams resulted in either no change or reduced levels of antioxidant enzymes and heat shock proteins even though this stress ultimately resulted in mortality (Joyner-Matos et al. 2006). Indirect exposure to turf algae can lead to coral mortality through enhanced microbial activity leading to hypoxic conditions (Smith et al.

2006). Corals along the Key Largo depth gradient, particularly KL 6 m, KL 9 m and KL 18 m, experienced increased mortality as a result of overgrowth by turf algae. In many cases, corals paled in color prior to death and overgrowth by these algae. Low levels of antioxidant enzymes and stress proteins indicates that either oxidative stress did not play a role in mortality at KL 18 m or severe oxidative stress may have left corals incapable of responding to stress conditions (Werner & Hinton 1999, Joyner-Matos et al. 2006). Probes for hypoxic conditions in the mucus layer of these corals will help test this hypothesis.

Additional biomarker assays of damage products (e.g., proteins, membranes, DNA adducts), in conjunction with studies of physiological status (e.g., growth, reproduction, histological examination), can help distinguish where corals sit along the stress gradient (healthy vs. stressed vs. diseased, Moore et al. 2006). Based on the evidence above, it would be expected that levels of damage products would be high at KL 18 m as observed in 1999 (Downs et al. 2002, Fauth et al. 2005).

6.5. Conclusions

Local stressors, specifically xenobiotics, impacted corals at my study sites, with the highest stress levels in the winter months and following heavy rainfall. No evidence for temperature or light stress was observed during this study.

This study emphasized the importance of using a hierarchical, mechanistic approach to assessing reef condition. Reefs with high regeneration, high densities of LBF and intermediate cellular biomarker levels can be considered "healthy." None of my study sites were considered to be in a wholly "healthy" state.

If biomarker profiles indicate stressed conditions but no changes are seen at the organismal or population level, this indicates corals are responding to stress but it is not affecting their performance; colonies effectively are "compensating" to stress. All of the biomarkers measured in this study are involved in pathways that protect the cell from further damage. Corals at both AR and KL 3 m were compensating to a xenobiotic stress but this did not appear to affect regeneration rates during the study period.

Low coral regeneration rates, high biomarker levels, and high mortality, with low LBF densities at the sites, indicate stress is negatively affecting organisms, overwhelming protective cellular pathways and resulting in reduced performance. Corals at both BNP and KL 9 m were responding to a stress that reduced regeneration rates and increased mortality. Stressors were likely recent at BNP as indicated by community assessments, specifically high recent morality and macroalgal biomass.

Where both cellular biomarker levels and regeneration rates were low, corals have been severely damaged physiologically and have reached an incurable state (Allen & Moore 2004). Abnormally low cellular biomarkers, low regeneration rates and high mortality at KL 18 m indicated that corals at this site were responding to severe stress, which has left these colonies incapable of recovering from damage.

Table 6.1. List of biomarkers assayed, representing four cellular subsystems including the sampling period and organism (cnidarian – host or algal symbiont) tested for each biomarker. See Appendix 6.1 for a description of each biomarker.

Biomarker	Sampling Period	Cnidarian/Algae
Protein Metabolic Condition		
Heat-shock protein (Hsp70)		
• Cnidarian (Cn) Hsp70	2001 – 2003	Cnidarian
• Dinoflagellate (Dn) Hsp70	2001	Algae
Heat-shock protein (Hsp60)		
• Cnidarian (Cn) Hsp60	2001 – 2003	Cnidarian
• Dinoflagellate (Dn) Hsp60	2001 – 2003	Algae
Ubiquitin	2001-2003	Cnidarian/Algae
Oxidative damage and response		
Copper/Zinc Superoxide Dismutase (Cu/Zn SOD)		
• Cnidarian (Cn) Cu/Zn SOD	2001	Cnidarian
• Dinoflagellate (Dn) Cu/Zn SOD	2001 – 2003	Algae
Manganese Superoxide Dismutase (Mn SOD)		
• Cnidarian (Cn) MnSOD	2001 – 2003	Cnidarian
• Dinoflagellate (Dn) MnSOD	2001 – 2003	Algae
Glutathione Peroxidase (GPx)		
• Cnidarian (Cn) GPx	2001 – 2003	Cnidarian
• Dinoflagellate (Dn) GPx	2001 – 2003	Algae
Catalase	2002 – 2003	Cnidarian
Metabolic Condition		
Heme Oxygenase (HO)	2001	Cnidarian
Ferrochelatase (FC)	2001 – 2003	Cnidarian
Metallothionein (Met)	2001 – 2003	Cnidarian
Chloroplast small heat-shock protein (ChlpsHsp)	2001 – 2003	Algae
Invertebrate small heat-shock protein isoforms (sHsp)	2001 – 2003	Cnidarian
Xenobiotic detoxification		
Cytochrome P450 2E homologue (CYP P450)		
• CYP 450 2 class (CYP-2)	2001- 2003	Cnidarian
• CYP 450 3 class (CYP-3)	2002 – 2003	Cnidarian
• CYP 450 6 class (CYP-4)	2002 – 2003	Cnidarian
Glutathione-S-transferase (GST)		
• Cnidarian (Cn) GST	2001 – 2003	Cnidarian
• Dinoflagellate (Dn) GST	2001 – 2003	Algae
Multiple Xenobiotic Resistance Protein (MXR)	2001 – 2003	Cnidarian/Algae

Table 6.2. Descriptive statistics for each cellular biomarker from 2000 (Downs et al. 2005a). Mean values from March 2000 at Biscayne National Park represent a "stressed" condition and overall mean values from pooled Key Largo sites represent "basal" condition; - indicates reference conditions for this biomarker are unavailable.

Biomarker	units	March 2000 BNP "Stressed"	2000 KL sites "Basal"
Protein Metabolic Condition			
Ubiquitin	fmol/ng TSP	968	67
Cnidarian Hsp70	pmol/ng TSP	0.50	0.14
Dino Hsp 70	pmol/ng TSP	1.59	0.18
Cnidarian Hsp60	pmol/ng TSP	16.2	8.8
Dino Hsp60	pmol/ng TSP	0.126	0.014
Oxidative Response			
Cnidarian Cu/Zn SOD	Eunits/ng TSP	-	-
Dino Cu/Zn SOD	Eunits/ng TSP	-	-
Cnidarian MnSOD	pmol/ng TSP	0.086	0.024
Dino MnSOD	fmol/ng TSP	2283	553
Cnidarian GPx	pmol/ng TSP	171	70
Dino GPx	pmol/ng TSP	3.30	0.33
Catalase	pmol/ng TSP	-	-
Metabolic Condition			
Heme oxygenase	Eunits/ng TSP	-	-
Ferrochelatase	Eunits/ng TSP	-	-
Metallothionein	Eunits/ng TSP	-	-
Cnidarian sHsp	Eunits/ng TSP	-	-
ChlpsHsp	Eunits/ng TSP	-	-
Xenobiotic Response			
MXR	Eunits/ng TSP	-	-
Cnidarian GST	pmol/ng TSP	2.36	0.82
Dino GST	pmol/ng TSP	9.72	6.39
CYP450 2-class	Eunits/ng TSP	-	-
CYP450 3-class	relative units/ng TSP	-	-
CYP450 6-class	relative units/ng TSP	-	-

Table 6.3. ANOSIM2 results for differences between sampling periods based on all cellular biomarkers (averaged across all 6 m sites); Global R = 0.38, 0.1% significance level; Multivariate Dispersion Indices (MVDISP) shown in shaded area for each sampling period.

	Mar-01	Jun-01	Aug-01	Oct-01	Mar-02	Jun-02	Aug-02	Nov-02	Feb-03
MVDISP	1.24	1.18	1.31	1.53	0.91	0.82	0.96	0.49	0.67
Mar-01		0.36	0.39	n.s.	0.48	0.61	0.63	0.68	0.65
Jun-01			n.s.	0.23	0.39	0.55	0.44	0.61	0.61
Aug-01				0.21	0.43	0.53	0.44	0.58	0.60
Oct-01					0.34	0.52	0.51	0.54	0.51
Mar-02						0.23	0.33	0.39	0.11
Jun-02							0.42	0.16	0.41
Aug-02								0.31	0.36
Nov-02									0.51

Table. 6.4. ANOSIM2 results for differences among 6 m sites based on all cellular biomarkers (averaged across all sampling periods); Global R = 0.13, 0.1% significance level; Multivariate Dispersion Indices (MVDISP) shown in shaded area for each site.

	KL 6 m	WB	AR	BNP
MVDISP	1.13	0.98	0.95	0.97
KL 6 m		0.13	0.17	0.17
WB			0.06	0.18
AR				0.12

Table 6.5. Global R values of ANOSIM significance tests for differences among 6 m sites based on all cellular biomarkers during each sampling period. No significant differences among sites were observed in March 2001, June 2001, August 2001 or March 2002. The R statistic for pairwise comparison of 6 m sites based on ANOSIM of all biomarkers during each sampling period also is shown; n.s. represents not significant (> 5%).

	OCT 2001	JUN 2002	AUG 2002	NOV 2002	FEB 2003
Global R	0.15	0.27	0.26	0.19	0.11
KL 6 m vs. WB	n.s.	0.53	0.38	0.20	n.s.
KL 6 m vs. AR	0.39	0.44	n.s.	n.s.	n.s.
KL 6 m vs. BNP	0.37	0.31	n.s.	n.s.	n.s.
WB vs. AR	n.s.	n.s.	n.s.	n.s.	0.23
WB vs. BNP	n.s.	0.17	0.73	0.50	n.s.
AR vs. BNP	n.s.	0.26	0.34	n.s.	0.20

Table 6.6. ANOSIM2 results for differences between sampling periods based on all cellular biomarkers (averaged across all depths); Global R = 0.32, 0.1% significance level; Multivariate Dispersion Indices (MVDISP) shown in shaded area for each sampling period.

	Mar-01	Jun-01	Aug-01	Oct-01	Mar-02	Jun-02	Aug-02	Nov-02	Feb-03
MVDISP	1.45	1.08	1.31	1.47	0.98	0.96	0.67	0.64	0.49
Mar-01		0.24	0.15	n.s.	0.32	0.49	0.49	0.57	0.49
Jun-01			n.s.	0.25	0.34	0.49	0.57	0.69	0.58
Aug-01				n.s.	0.33	0.47	0.48	0.61	0.50
Oct-01					0.28	0.49	0.46	0.50	0.47
Mar-02						0.22	0.21	0.41	0.13
Jun-02							0.16	0.35	0.32
Aug-02								0.28	0.11
Nov-02									0.50

Table 6.7. ANOSIM2 results for differences along depth gradient based on all cellular biomarkers (averaged across all sampling periods); Global R = 0.27, 0.1% significance level; Multivariate Dispersion Indices (MVDISP) shown in shaded area for each depth.

	KL 3 m	KL 6 m	KL 9 m	KL 18 m
MVDISP	0.97	1.05	0.92	1.07
KL 3 m		0.21	0.14	0.46
KL 6 m			0.20	0.18
KL 9 m				0.47

Table 6.8. Global R values of ANOSIM significance tests for differences among depths based on all cellular biomarkers during each sampling period. No significant differences among depths were observed in March 2002. The R statistic for pairwise comparison of depths based on ANOSIM of all biomarkers during each sampling period also is shown; n.s. represents not significant (> 5%).

	MAR 2001	JUN 2001	AUG 2001	OCT 2001	JUN 2002	AUG 2002	NOV 2002	FEB 2003
Global R	0.32	0.21	0.37	0.24	0.46	0.30	0.31	0.16
KL 3 m/6 m	0.43	n.s.	0.28	n.s.	0.52	n.s.	0.34	n.s.
KL 3 m/9 m	n.s.	n.s.	0.31	n.s.	n.s.	n.s.	n.s.	n.s.
KL 3 m/18 m	0.62	0.33	n.s.	n.s.	0.83	0.68	0.61	0.52
KL 6 m/9 m	n.s.	n.s.	0.77	0.45	0.25	n.s.	n.s.	n.s.
KL 6 m/18 m	n.s.	n.s.	n.s.	n.s.	0.83	0.30	0.32	0.46
KL 9 m/18 m	0.46	n.s.	0.67	n.s.	0.52	0.45	0.58	0.36

Table 6.9. Repeated measures MANOVA results for individual cellular biomarker levels among 6 m sites; bold values significant $p<0.05$ (Note: degrees of freedom for site effect: numerator = 3, denominator = 16; degrees of freedom for time and site x time effect change with sampling frequency of each biomarker; see Table 6.2)

	Site		Time				Site x Time			
Protein Metabolic Condition	F	p	Num DF	Den DF	F	p	Num DF	Den DF	F	p
Ubiquitin	**3.99**	**0.0269**	8	124	**2.8**	**0.007**	24	124	1.28	n.s.
Cnidarian Hsp70	2.7	n.s.	8	124	**2.72**	**0.0086**	24	124	0.68	n.s.
Dino Hsp 70	2.46	n.s.	3	44	**18.72**	**<0.0001**	9	44	1.95	n.s.
Cnidarian Hsp60	1.32	n.s.	8	124	1.72	n.s.	24	124	0.85	n.s.
Dino Hsp60	**3.57**	**0.0377**	8	123	**6.93**	**<0.0001**	24	123	1.21	n.s.
Xenobiotic Response and Damage										
MDR	1.55	n.s.	8	122	**15.89**	**<0.0001**	24	122	1.4	n.s.
CYP450 2-class	2.01	n.s.	8	122	**10.65**	**<0.0001**	24	122	1.35	n.s.
Cnidarian GST	1.75	n.s.	8	124	**6.08**	**<0.0001**	24	124	1.00	n.s.
Dino GST	0.86	n.s.	8	124	**2.36**	**0.0212**	24	124	0.32	n.s.
CYP450 3-class	2.7	n.s.	4	64	**3.06**	**0.0227**	12	64	**2.16**	**0.0243**
CYP450 6-class	0.55	n.s.	4	64	**11.29**	**<0.0001**	12	64	0.62	n.s.
Metabolic Condition										
Ferrochelatase	2.66	n.s.	8	124	**5.96**	**<0.0001**	24	124	0.72	n.s.
Metallothionein	1.50	n.s.	8	124	**3.28**	**0.002**	24	124	0.46	n.s.
sHsp	**6.08**	**0.0058**	8	124	**17.2**	**<0.0001**	24	124	**3.13**	**<0.0001**
ChlpsHsp	2.02	n.s.	8	123	**5.51**	**<0.0001**	24	123	1.09	n.s.
Oxidative Damage and Response										
Heme oxygenase	1.87	n.s.	3	44	**6.62**	**0.0009**	9	44	1.89	n.s.
Cnidarian Cu/Zn SOD	2.64	n.s.	3	44	**9.44**	**<0.0001**	9	44	**3.99**	**0.0009**
Dino Cu/Zn SOD	**3.43**	**0.0424**	8	124	**4.54**	**<0.0001**	24	124	0.86	n.s.
Cnidarian MnSOD	1.41	n.s.	7	109	**4.17**	**0.0004**	21	109	0.52	n.s.
Dino MnSOD	3.14	n.s.	8	124	**5.63**	**<0.0001**	24	124	1.08	n.s.
Cnidarian GPx	2.55	n.s.	8	124	**3.83**	**0.0005**	24	124	0.65	n.s.
Dino GPx	**4.00**	**0.0266**	8	124	**6.53**	**<0.0001**	24	124	1.02	n.s.
Catalase	0.41	n.s.	4	64	0.62	n.s.	12	64	0.49	n.s.

Table 6.10. Repeated measures MANOVA for individual cellular biomarker levels along depth gradient. Bold values significant p<0.05; (Note: degrees of freedom for site effect: numerator = 3, denominator = 16; degrees of freedom for time and site x time effect change with sampling frequency of each biomarker; see Table 6.2)

	Site		Time				Site x Time			
Protein Metabolic Condition	F	p	Num DF	Den DF	F	p	Num DF	Den DF	F	P
Ubiquitin	**6.92**	**0.0034**	8	122	**2.85**	**0.0061**	24	122	**1.61**	**0.0487**
Cnidarian Hsp70	**10.00**	**0.0006**	8	122	**2.30**	**0.0246**	24	122	**1.79**	**0.0211**
Dino Hsp 70	**5.19**	**0.0108**	3	44	**9.02**	**<0.0001**	9	44	**2.20**	**0.04**
Cnidarian Hsp60	**11.38**	**0.0003**	8	122	**2.53**	**0.0139**	24	122	1.58	n.s.
Dino Hsp60	**8.70**	**0.0012**	8	122	**7.16**	**<0.0001**	24	122	1.29	n.s.
Xenobiotic Response and Damage										
MDR	**10.83**	**0.0004**	8	121	**11.63**	**<0.0001**	24	121	**2.10**	**0.0047**
CYP450 2-class	**15.26**	**<0.0001**	8	121	**8.40**	**<0.0001**	24	121	**1.90**	**0.0125**
Cnidarian GST	**11.54**	**0.0003**	8	122	**5.72**	**<0.0001**	24	122	**2.22**	**0.0025**
Dino GST	**4.76**	**0.0148**	8	124	**2.76**	**0.0077**	24	124	1.11	n.s.
CYP450 3-class	**21.75**	**<0.0001**	4	62	1.20	n.s.	12	62	1.60	n.s.
CYP450 6-class	**10.54**	**0.0005**	4	62	**4.01**	**0.0059**	12	62	**2.13**	**0.0272**
Metabolic Condition										
Ferrochelatase	**9.93**	**0.0006**	8	122	**4.39**	**0.0001**	24	122	**1.96**	**0.0094**
Metallothionein	**12.03**	**0.0002**	8	122	**2.34**	**0.0224**	24	122	1.59	n.s.
sHsp	**5.19**	**0.0108**	8	122	**18.86**	**<0.0001**	24	124	**2.16**	**0.0034**
ChlpsHsp	**7.94**	**0.0018**	8	122	1.79	n.s.	24	123	1.56	n.s.
Oxidative Damage and Response										
Heme	**5.17**	**0.0109**	3	44	2.05	n.s.	9	44	**2.15**	**0.0447**
Cnidarian Cu/Zn SOD	**6.77**	**0.0037**	3	44	**3.21**	**0.0321**	9	44	**2.37**	**0.0279**
Dino Cu/Zn SOD	**5.27**	**0.0102**	8	122	**5.18**	**<0.0001**	24	122	**1.63**	**0.0443**
Cnidarian MnSOD	**13.97**	**<0.0001**	8	115	**2.85**	**0.0064**	22	115	**2.15**	**0.0049**
Dino MnSOD	**12.22**	**0.0002**	8	122	**4.38**	**0.0001**	24	122	**2.38**	**0.0011**
Cnidarian GPx	**10.03**	**0.0006**	8	122	**5.25**	**<0.0001**	24	122	**1.8**	**0.0207**
Dino GPx	**8.67**	**0.0012**	8	122	**6.41**	**<0.0001**	24	122	**2.11**	**0.0045**
Catalase	**14.53**	**<0.0001**	4	62	0.94	n.s.	12	62	1.22	n.s.

Table 6.11. Overall mean (± SE) biomarker concentrations (averaged across all depths) for each sampling period. Shaded values represent biomarkers that did not vary significantly with time or time x site interactions; * represent biomarkers that varied significantly with time but not time x site interactions; means for each biomarker that are not connected by the same letter differed significantly based on Tukey HSD test ($p < 0.05$); - represents biomarkers that were not sampled during that time period.

	F	Num	Den	p-value	Mar-01	Jun-01	Aug-01	Oct-01	Mar-02	Jun-02	Aug-02	Nov-02	Feb-03
Protein Metabolic Condition													
Ubiquitin					616	317	322	452	344	416	388	491	410
					(125)	(80)	(51)	(135)	(48)	(33)	(25)	(24)	(20)
Cnidarian Hsp70					0.85	0.44	0.69	0.74	0.38	0.36	0.35	0.46	0.39
					(0.13)	(0.09)	(0.11)	(0.13)	(0.04)	(0.04)	(0.03)	(0.02)	(0.03)
Dino Hsp 70					0.26	0.13	0.18	0.25	-	-	-	-	-
					(0.03)	(0.03)	(0.03)	(0.04)	-	-	-	-	-
Cnidarian Hsp60*	2.3	8	163	0.0224	40^{AB}	22^{A}	29^{AB}	36^{AB}	27^{AB}	35^{AB}	30^{AB}	39^{B}	31^{AB}
					(4)	(2)	(5)	(3)	(4)	(3)	(2)	(2)	(2)
Dino Hsp60*	9.8	8	166	<0.0001	0.137^{C}	0.071^{AB}	0.096^{BC}	0.096^{BC}	0.027^{A}	0.024^{A}	0.029^{A}	0.026^{A}	0.029^{A}
					(0.027)	(0.016)	(0.019)	(0.020)	(0.003)	(0.004)	(0.002)	(0.003)	(0.002)
Oxidative Damage and Response													
Cnidarian Cu/Zn SOD					2.8	1.5	2.1	2.2	-	-	-	-	-
					(0.5)	(0.2)	(0.4)	(0.3)	-	-	-	-	-
Dino Cu/Zn SOD					0.44	0.22	0.29	0.33	0.39	0.50	0.42	0.50	0.43
					(0.07)	(0.04)	(0.05)	(0.06)	(0.05)	(0.05)	(0.02)	(0.02)	(0.02)
Cnidarian MnSOD					0.076	0.046	0.082	0.055	0.056	0.045	0.051	0.041	0.061
					(0.010)	(0.011)	(0.016)	(0.011)	(0.007)	(0.006)	(0.003)	(0.005)	(0.004)
Dino MnSOD					2310	786	2105	1632	642	785	872	820	756
					(381)	(189)	(561)	(274)	(83)	(59)	(142)	(50)	(42)
Cnidarian GPx					32	19	25	26	30	36	34	43	36
					(4)	(3)	(3)	(3)	(4)	(4)	(2)	(2)	(2)
Dino GPx					2.00	0.73	1.14	1.33	0.71	0.33	0.57	0.46	0.81
					(0.37)	(0.30)	(0.30)	(0.25)	(0.09)	(0.05)	(0.04)	(0.02)	(0.04)
Catalase					-	-	-	-	16.7	15.9	15.2	16.1	18.0
					-	-	-	-	(2.2)	(2.6)	(1.7)	(2.3)	(1.1)

Table 6.11. (cont.) Overall mean (± SE) biomarker concentrations (averaged across all depths) for each sampling period. Shaded values represent biomarkers that did not vary significantly with time or time x site interactions; * represent biomarkers that varied significantly with time but not time x site interactions; means for each biomarker that are not connected by the same letter differed significantly based on Tukey HSD test ($p < 0.05$); - represents biomarkers that were not sampled during that time period.

	F	Num	Den	p-value	Mar-01	Jun-01	Aug-01	Oct-01	Mar-02	Jun-02	Aug-02	Nov-02	Feb-03
Metabolic Condition													
Heme oxygenase					0.48	0.26	0.37	0.40	-	-	-	-	-
					(0.07)	(0.05)	(0.08)	(0.06)	-	-	-	-	-
Ferrochelatase					0.41	0.22	0.50	0.41	0.34	0.42	0.38	0.48	0.42
					(0.05)	(0.05)	(0.12)	(0.07)	(0.05)	(0.04)	(0.03)	(0.03)	(0.02)
Metallothionein*	2.2	8	166	0.0310	0.54^{AB}	0.26^{A}	0.40^{AB}	0.86^{B}	0.36^{AB}	0.29^{AB}	0.35^{AB}	0.33^{AB}	0.41^{AB}
					(0.09)	(0.06)	(0.07)	(0.36)	(0.05)	(0.03)	(0.02)	(0.01)	(0.02)
sHsp					0.055	0.026	0.045	0.045	0.010	0.006	0.005	0.014	0.011
					(0.008)	(0.006)	(0.006)	(0.005)	(0.001)	(0.001)	(0.001)	(0.001)	(0.001)
ChlpsHsp					0.45	0.23	0.30	0.60	0.80	0.68	0.72	0.72	0.85
					(0.06)	(0.05)	(0.05)	(0.17)	(0.10)	(0.12)	(0.10)	(0.11)	(0.07)
Xenobiotic Response and Damage													
MXR					0.72	0.24	0.37	0.49	0.50	0.59	0.51	0.68	0.59
					(0.11)	(0.04)	(0.05)	(0.08)	(0.07)	(0.04)	(0.05)	(0.03)	(0.04)
Cnidarian GST					2.71	0.66	1.42	1.58	1.20	1.89	1.26	1.59	1.41
					(0.59)	(0.13)	(0.37)	(0.27)	(0.16)	(0.49)	(0.10)	(0.07)	(0.08)
Dino GST*	2.7	8	165	0.0086	12.0^{B}	9.6^{AB}	10.9^{AB}	12.4^{B}	8.0^{A}	9.7^{AB}	9.8^{AB}	11.7^{B}	9.8^{AB}
					(0.8)	(0.7)	(1.0)	(1.0)	(1.2)	(0.8)	(0.5)	(0.7)	(0.6)
CYP450 2-class					0.92	0.19	0.28	0.74	0.37	0.33	0.39	0.38	0.41
					(0.29)	(0.04)	(0.05)	(0.14)	(0.05)	(0.05)	(0.03)	(0.05)	(0.03)
CYP450 3-class					-	-	-	-	0.50	0.47	0.58	0.54	0.58
					-	-	-	-	(0.07)	(0.08)	(0.05)	(0.08)	(0.05)
CYP450 6-class					-	-	-	-	0.54	0.42	0.48	0.49	0.66
					-	-	-	-	(0.07)	(0.05)	(0.03)	(0.04)	(0.03)

Table 6.12. Overall mean (± SE) cellular biomarker concentrations (averaged across all 6 m sites) for each sampling period. Shaded values represent biomarkers that did not vary significantly with time or time x site interactions. * represent biomarkers that varied significantly with time (ANOVA statistics shown) but not time x site interactions; means for each biomarker that are not connected by the same letter differed significantly based on Tukey HSD ($p < 0.05$). - represents biomarkers that were not sampled during that time period; units as shown in Table 6.2.

	F	Num	Den	p-value	Mar-01	Jun-01	Aug-01	Oct-01	Mar-02	Jun-02	Aug-02	Nov-02	Feb-03
Protein Metabolic Condition													
Ubiquitin*	3.9	8	164	0.0003	620^{C}	320^{AB}	309^{A}	494^{BC}	396^{BC}	434^{BC}	295^{ABC}	381^{ABC}	436^{BC}
					(96)	(38)	(56)	(76)	(46)	(25)	(29)	(12)	(27)
Cnidarian Hsp70*	6.2	8	167	<0.0001	0.73^{BC}	0.48^{AB}	0.54^{ABC}	0.83^{BC}	0.41^{A}	0.41^{A}	0.36^{A}	0.32^{A}	0.44^{AB}
					(0.08)	(0.08)	(0.07)	(0.14)	(0.04)	(0.02)	(0.02)	(0.02)	(0.03)
Dino Hsp 70*	9.9	3	72	<0.0001	0.28^{B}	0.12^{A}	0.14^{A}	0.28^{B}	-	-	-	-	-
					(0.03)	(0.02)	(0.02)	(0.04)	-	-	-	-	-
Cnidarian Hsp60					38	27	27	36	32	38	28	31	34
					(3)	(3)	(3)	(4)	(4)	(1)	(2)	(2)	(2)
Dino Hsp60*	15.2	8	167	<0.0001	0.115^{C}	0.066^{B}	0.054^{AB}	0.082^{BC}	0.029^{A}	0.030^{A}	0.029^{A}	0.029^{A}	0.032^{AB}
					(0.012)	(0.016)	(0.008)	(0.011)	(0.003)	(0.002)	(0.001)	(0.002)	(0.001)
Oxidative Damage and Response													
Cnidarian Cu/Zn SOD					2.1	1.4	1.2	2.8	-	-	-	-	-
					(0.3)	(0.2)	(0.2)	(0.4)	-	-	-	-	-
Dino Cu/Zn SOD*	6.3	8	167	<0.0001	0.48^{B}	0.22^{A}	0.25^{A}	0.40^{B}	0.47^{B}	0.53^{B}	0.37^{AB}	0.43^{B}	0.49^{B}
					(0.05)	(0.03)	(0.06)	(0.08)	(0.05)	(0.02)	(0.03)	(0.01)	(0.02)
Cnidarian MnSOD*	5.8	7	149	<0.0001	0.070^{C}	0.034^{A}	0.042^{AB}	-	0.065^{BC}	0.052^{ABC}	0.052^{ABC}	0.044^{ABC}	0.074^{C}
					(0.009)	(0.010)	(0.007)	-	(0.007)	(0.002)	(0.003)	(0.003)	(0.003)
Dino MnSOD*	5.3	8	164	<0.0001	2050^{C}	1602^{ABC}	706^{A}	1924^{BC}	748^{ABC}	771^{AB}	673^{AB}	714^{AB}	820^{AB}
					(221)	(540)	(110)	(225)	(82)	(34)	(38)	(24)	(38)
Cnidarian GPx*	8.6	8	167	<0.0001	31^{AB}	23^{AB}	20^{A}	31^{AB}	35^{CD}	41^{CD}	32^{BC}	35^{CD}	43^{D}
					(2)	(3)	(2)	(3)	(4)	(2)	(2)	(1)	(2)
Dino GPx*	11.8	8	167	<0.0001	2.19^{D}	0.70^{AB}	0.79^{AB}	2.27^{CD}	0.81^{ABC}	0.40^{A}	0.65^{AB}	0.40^{AB}	0.93^{BC}
					(0.28)	(0.12)	(0.19)	(0.91)	(0.09)	(0.03)	(0.04)	(0.01)	(0.04)
Catalase					-	-	-	-	18.8	20.0	15.8	17.7	20.4
					-	-	-	-	(2.2)	(1.4)	(1.1)	(1.1)	(1.3)

Table 6.12 (cont.). Overall mean (± SE) cellular biomarker concentrations (averaged across all 6 m sites) for each sampling period. Shaded values represent biomarkers that did not vary significantly with time or time x site interactions. * represent biomarkers that varied significantly with time (ANOVA statistics shown) but not time x site interactions; means for each biomarker that are not connected by the same letter differed significantly based on Tukey HSD ($p < 0.05$). - represents biomarkers that were not sampled during that time period; units as shown in Table 6.2.

	F			p-value	Mar-01	Jun-01	Aug-01	Oct-01	Mar-02	Jun-02	Aug-02	Nov-02	Feb-03
Metabolic Condition													
Heme oxygenase*	5.5	3	72	0.0019	0.45^{B}	0.27^{AB}	0.22^{A}	0.42^{B}	-	-	-	-	-
					(0.05)	(0.04)	(0.04)	(0.07)	-	-	-	-	-
Ferrochelatase*	5.9	8	167	<0.0001	0.40^{BC}	0.26^{AB}	0.19^{A}	0.38^{A}	0.40^{BC}	0.60^{C}	0.36^{ABC}	0.41^{BC}	0.48^{C}
					(0.04)	(0.05)	(0.03)	(0.06)	(0.05)	(0.14)	(0.02)	(0.02)	(0.02)
Metallothionein*	5.0	8	167	<0.0001	0.43^{ABC}	0.23^{A}	0.24^{A}	0.60^{C}	0.42^{ABC}	0.31^{ABC}	0.36^{ABC}	0.30^{AB}	0.49^{BC}
					(0.06)	(0.03)	(0.04)	(0.15)	(0.05)	(0.01)	(0.02)	(0.01)	(0.02)
sHsp					0.039	0.017	0.026	0.047	0.010	0.006	0.006	0.007	0.014
					(0.006)	(0.003)	(0.003)	(0.006)	(0.001)	(0.002)	(0.001)	(0.001)	(0.001)
ChlpsHsp*	15.9	8	167	<0.0001	0.40^{AB}	0.22^{A}	0.15^{A}	0.42^{AB}	0.92^{C}	0.84^{C}	0.58^{BC}	0.84^{C}	0.90^{C}
					(0.04)	(0.05)	(0.02)	(0.07)	(0.10)	(0.08)	(0.09)	(0.06)	(0.08)
Xenobiotic Response and Damage													
MXR*	11.7	8	167	<0.0001	0.61^{B}	0.19^{A}	0.25^{A}	0.53^{B}	0.59^{B}	0.58^{B}	0.47^{B}	0.55^{B}	0.69^{B}
					(0.08)	(0.03)	(0.04)	(0.07)	(0.07)	(0.03)	(0.04)	(0.02)	(0.03)
Cnidarian GST*	6.6	8	167	<0.0001	1.92^{D}	0.70^{A}	0.81^{AB}	0.95^{ABC}	1.40^{BCD}	1.96^{D}	1.17^{ABCD}	1.39^{CD}	1.51^{CD}
					(0.49)	(0.12)	(0.15)	(0.14)	(0.15)	(0.48)	(0.09)	(0.05)	(0.09)
Dino GST*	3.5	8	167	0.0008	12.3^{B}	11.0^{B}	8.6^{AB}	11.5^{B}	9.3^{A}	10.1^{AB}	9.3^{AB}	9.9^{AB}	11.3^{B}
					(0.6)	(0.5)	(0.6)	(0.9)	(1.1)	(0.5)	(0.5)	(0.3)	(0.5)
CYP450 2-class*	7.6	8	167	<0.0001	0.53^{D}	0.23^{AB}	0.20^{A}	0.64^{D}	0.43^{BCD}	0.40^{BCD}	0.29^{ABC}	0.40^{BCD}	0.50^{CD}
					(0.06)	(0.06)	(0.04)	(0.12)	(0.05)	(0.03)	0.04	(0.02)	(0.02)
CYP450 3-class					-	-	-	-	0.60	0.60	0.47	0.61	0.73
					-	-	-	-	(0.07)	(0.05)	(0.06)	(0.04)	(0.03)
CYP450 6-class*	12.1	4	95	<0.0001	-	-	-	-	0.65^{B}	0.47^{AB}	0.51^{AB}	0.42^{A}	0.82^{C}
					-	-	-	-	(0.07)	(0.02)	(0.03)	(0.02)	(0.04)

Table 6.13. BEST results for coral regeneration rates and SERC environmental parameters. Abbreviations include alkaline phosphatase activity (APA), chlorophyll-a (CHLA), dissolved inorganic nitrogen (DIN), dissolved oxygen (DO) nitrate (NO_3), nitrite (NO_2), soluble reactive phosphate (SRP), total nitrogen (TN), total organic carbon (TOC), total organic nitrogen (TON) and total phosphorus (TP)

Site	Rho	# variables	Selected environmental parameters
KL 3 m	0.60	5	NO_3, DIN, TOC, salinity, DIN:TP
KL 6 m	0.81	1	TN
KL 9 m	0.75	4	NO_2, CHLA, turbidity, TN:TP
KL 18 m	0.80	5	NO_2, SRP, CHLA, TOC, TN:TP
WB	0.68	1	TP
AR	0.38	4	TON, TOC, TN:TP DIN:TP
BNP	0.19	3	NO_2, APA, DO

Table 6.14. BEST and RELATE results for coral regeneration rates and biomarker concentrations during a given time period at the 6 m Sites; n.s. represents not significantly (> 5%)

Regeneration Rate	Biomarker	Rho	Significance level	# variables	Selected Biomarkers
JUN – AUG 2001	JUN 2001	0.16	n.s.	-	-
AUG – OCT 2001	AUG 2001	0.30	1%	2	Dn Hsp60, Dn Cu/Zn SOD
OCT 2001 – MAR 2002	OCT 2001	0.24	3.1%	2	Met, Cn Cu/Zn SOD
MAR – JUN 2002	MAR 2002	0.09	n.s.	-	-
JUN – AUG 2002	JUN 2002	0.43	0.4%	5	Cn Hsp70, sHsp, Met, Cn GST, CYP-6
AUG – NOV 2002	AUG 2002	0.17	3.6%	2	Ubiquitin, Cn MnSOD
NOV 2002 – FEB 2003	NOV 2002	0.11	n.s.	-	-

Table 6.15. Descriptive statistics for each cellular biomarker at the 6 m sites over the entire study period between March 2001 and February 2003. * represents biomarkers that differed significantly among sites but not with time x site interactions. Means of biomarkers not connected by the same letter differed significantly based on Tukey HSD test ($p < 0.05$)

		Overall (all 6 m sites)						Overall mean (± SE)			
Biomarker	**units**	**n**	**Max**	**Min**	**Mean**	**SE**	**Median**	**KL 6 m**	**WB**	**AR**	**BNP**
Protein Metabolic Condition											
Ubiquitin*	fmol/ng TSP	176	2121	-59	409	18	399	382^{A}	359^{A}	374^{AB}	517^{B}
								(52)	(25)	(28)	(32)
Cnidarian Hsp70	pmol/ng TSP	176	2.40	-0.01	0.50	0.02	0.42	0.47	0.44	0.48	0.60
								(0.06)	(0.04)	(0.04)	(0.06)
Dino Hsp 70	pmol/ng TSP	76	0.62	0.01	0.21	0.02	0.19	0.13	0.18	0.26	0.25
								(0.03)	(0.03)	(0.03)	(0.03)
Cnidarian Hsp60	pmol/ng TSP	176	86.4	-3.7	32.5	0.9	33.9	31.1	30.4	32.3	35.9
								(2.6)	(1.8)	(1.7)	(1.4)
Dino Hsp60*	pmol/ng TSP	176	0.313	-0.001	0.051	0.003	0.034	0.033^{A}	0.040^{AB}	0.061^{B}	0.065^{B}
								(0.004)	(0.005)	(0.009)	(0.007)
Oxidative Damage and Response											
Cnidarian Cu/Zn SOD	Eunits/ng TSP	76	6.62	0.08	1.84	0.16	1.49	1.24	1.44	2.08	2.49
								(0.37)	(0.25)	(0.31)	(0.26)
Dino Cu/Zn SOD	Eunits/ng TSP	176	1.34	-0.07	0.41	0.02	0.42	0.37	0.36	0.43	0.46
								(0.04)	(0.03)	(0.04)	(0.03)
Cnidarian MnSOD	pmol/ng TSP	157	0.186	-0.007	0.054	0.002	0.054	0.048	0.052	0.056	0.060
								(0.005)	(0.005)	(0.005)	(0.004)
Dino MnSOD	fmol/ng TSP	176	10104	-61	1096	78	815	910	920	1128	1412
								(126)	(96)	(124)	(230)
Cnidarian GPx	pmol/ng TSP	176	59	-3	32	1	35	30	30	34	35
								(3)	(2)	(2)	(1)
Dino GPx*	pmol/ng TSP	176	17.98	-0.08	1.01	0.12	0.73	0.71^{A}	0.78^{AB}	1.04^{AB}	1.48^{B}
								(0.14)	(0.09)	(0.12)	(0.40)
Catalase	pmol/ng TSP	100	30.6	-2.5	18.5	0.7	19.4	17.9	18.7	17.8	19.8
								(1.8)	(1.2)	(1.2)	(1.1)

Table 6.15 (cont.). Descriptive statistics for each cellular biomarker at the 6 m sites over the entire study period between March 2001 and February 2003. * represents biomarkers that differed significantly among sites but not with time x site interactions. Means of biomarkers not connected by the same letter differed significantly based on Tukey HSD test ($p < 0.05$)

		Overall (all 6 m sites)						Overall mean (± SE)			
Biomarker	**units**	**n**	**Max**	**Min**	**Mean**	**SE**	**Median**	**KL 6 m**	**WB**	**AR**	**BNP**
Metabolic Condition											
Heme oxygenase	Eunits/ng TSP	76	1.01	0.01	0.34	0.03	0.30	0.24 (0.06)	0.29 (0.04)	0.37 (0.05)	0.45 (0.05)
Ferrochelatase	Eunits/ng TSP	176	3.27	-0.04	0.39	0.02	0.39	0.35 (0.03)	0.32 (0.02)	0.38 (0.02)	0.50 (0.07)
Metallothionein	Eunits/ng TSP	176	2.93	-0.04	0.38	0.02	0.35	0.38 (0.07)	0.35 (0.03)	0.38 (0.02)	0.39 (0.03)
Cnidarian sHsp	Eunits/ng TSP	176	0.134	-0.001	0.019	0.002	0.013	0.018 (0.004)	0.015 (0.002)	0.020 (0.003)	0.023 (0.003)
ChlpsHsp	Eunits/ng TSP	176	1.35	-0.11	0.59	0.03	0.56	0.51 (0.07)	0.56 (0.06)	0.60 (0.06)	0.68 (0.06)
Xenobiotic Response and Damage											
MXR	Eunits/ng TSP	176	1.65	-0.06	0.50	0.02	0.54	0.52 (0.05)	0.44 (0.04)	0.50 (0.03)	0.54 (0.04)
Cnidarian GST	pmol/ng TSP	176	11.04	-0.08	1.32	0.09	1.37	1.56 (0.34)	1.15 (0.09)	1.13 (0.06)	1.45 (0.10)
Dino GST	pmol/ng TSP	176	17.00	-0.92	10.34	0.23	10.53	9.74 (0.56)	10.09 (0.46)	10.31 (0.38)	11.18 (0.42)
CYP450 2-class	Eunits/ng TSP	176	2.21	-0.03	0.40	0.02	0.42	0.33 (0.04)	0.39 (0.04)	0.39 (0.03)	0.49 (0.05)
CYP450 3-class	relative units/ng TSP	100	1.01	-0.06	0.60	0.02	0.66	0.55 (0.06)	0.55 (0.05)	0.62 (0.04)	0.68 (0.04)
CYP450 6-class	relative units/ng TSP	100	1.14	-0.04	0.57	0.02	0.56	0.58 (0.04)	0.53 (0.04)	0.60 (0.05)	0.58 (0.05)

Table 6.16. Coefficient of variance for each cellular biomarker at the 6 m sites over the entire study period between March 2001 and February 2003.

Biomarker	units	n	KL 6 m	WB	AR	BNP
Protein Metabolic Condition						
Ubiquitin*	fmol/ng TSP	176	87	47	50	42
Cnidarian Hsp70	pmol/ng TSP	176	79	61	56	63
Dino Hsp 70	pmol/ng TSP	76	100	61	58	48
Cnidarian Hsp60	pmol/ng TSP	176	53	38	35	26
Dino Hsp60*	pmol/ng TSP	176	79	88	93	72
Oxidative Damage and Response						
Cnidarian Cu/Zn SOD	Eunits/ng TSP	76	119	77	68	46
Dino Cu/Zn SOD	Eunits/ng TSP	176	68	50	56	43
Cnidarian MnSOD	pmol/ng TSP	157	58	54	55	45
Dino MnSOD	fmol/ng TSP	176	88	70	74	109
Cnidarian GPx	pmol/ng TSP	176	53	43	32	29
Dino GPx*	pmol/ng TSP	176	128	77	80	180
Catalase	pmol/ng TSP	100	50	32	33	27
Metabolic Condition						
Heme oxygenase	Eunits/ng TSP	76	100	62	65	56
Ferrochelatase	Eunits/ng TSP	176	57	50	42	92
Metallothionein	Eunits/ng TSP	176	121	57	45	46
Cnidarian sHsp	Eunits/ng TSP	176	133	107	105	83
ChlpsHsp	Eunits/ng TSP	176	90	73	65	57
Xenobiotic Response and Damage						
MXR	Eunits/ng TSP	176	63	55	42	50
Cnidarian GST	pmol/ng TSP	176	139	51	37	44
Dino GST	pmol/ng TSP	176	37	30	25	25
CYP450 2-class	Eunits/ng TSP	176	82	67	49	65
CYP450 3-class	relative units/ng TSP	100	53	49	29	26
CYP450 6-class	relative units/ng TSP	100	38	42	43	41

Table 6.17. Descriptive statistics for each cellular biomarker along the depth gradient over the entire study period between March 2001 and February 2003. * represents biomarkers that differed significantly among sites but not with time x site interactions. Means of biomarkers not connected by the same letter differed significantly based on Tukey HSD test ($p < 0.05$)

		Overall (along depth gradent)						Overall Mean			
Biomarker	**units**	**n**	**Max**	**Min**	**Mean**	**SE**	**Median**	**KL 3 m**	**KL 6 m**	**KL 9 m**	**KL 18 m**
Protein Metabolic Condition											
Ubiquitin	fmol/ng TSP	175	2690	-62.5	417	24	402	482 (44)	382 (52)	516 (55)	282 (33)
Cnidarian Hsp70	pmol/ng TSP	175	2.06	-0.02	0.51	0.03	0.44	0.68 (0.06)	0.47 (0.06)	0.60 (0.06)	0.29 (0.04)
Dino Hsp 70	pmol/ng TSP	76	0.66	0.01	0.20	0.02	0.19	0.29 (0.04)	0.13 (0.03)	0.26 (0.02)	0.127 (0.03)
Cnidarian Hsp60*	pmol/ng TSP	175	108.1	-5.0	32.1	1.1	34.1	36.6^{C} (1.6)	31.1^{B} (2.6)	37.3^{BC} (2.1)	23.1^{A} (2.2)
Dino Hsp60*	pmol/ng TSP	175	0.424	-0.001	0.059	0.005	0.033	0.072^{B} (0.010)	0.033^{A} (0.004)	0.093^{B} (0.015)	0.035^{A} (0.008)
Oxidative Damage and Response											
Cnidarian Cu/Zn SOD	Eunits/ng TSP	76	6.18	0.08	2.13	0.19	1.86	3.07 (0.36)	1.24 (0.37)	3.08 (0.25)	0.96 (0.21)
Dino Cu/Zn SOD	Eunits/ng TSP	175	1.03	-0.07	0.39	0.02	0.42	0.43 (0.03)	0.37 (0.04)	0.49 (0.02)	0.28 (0.03)
Cnidarian MnSOD	pmol/ng TSP	166	0.284	-0.009	0.057	0.003	0.056	0.074 (0.007)	0.048 (0.005)	0.071 (0.004)	0.035 (0.006)
Dino MnSOD	fmol/ng TSP	175	9162	-82	1188	97	837	1583 (247)	910 (126)	1592 (215)	630 (89)
Cnidarian GPx	pmol/ng TSP	175	60.0	-4.1	31.4	1.1	34.0	36.0 (1.6)	30.0 (2.6)	36.5 (1.2)	22.3 (2.4)
Dino GPx*	pmol/ng TSP	175	5.83	-0.09	0.89	0.08	0.62	1.28 (0.19)	0.71 (0.14)	1.05 (0.14)	0.51 (0.08)
Catalase*	pmol/ng TSP	99	29.0	-3.1	16.4	0.9	19.6	20.2 (1.2)	17.9 (1.8)	20.6 (1.0)	6.37 (1.6)

Table 6.17 (cont.). Descriptive statistics for each cellular biomarker along the depth gradient over the entire study period between March 2001 and February 2003. * represents biomarkers that differed significantly among sites but not with time x site interactions. Means of biomarkers not connected by the same letter differed significantly based on Tukey HSD test ($p < 0.05$)

		Overall (along depth gradient)						Overall Mean			
Biomarker	**units**	**n**	**Max**	**Min**	**Mean**	**SE**	**Median**	**KL 3 m**	**KL 6 m**	**KL 9 m**	**KL 18 m**
Metabolic Condition											
Heme oxygenase	Eunits/ng TSP	76	1.55	0.01	0.38	0.03	0.35	0.48 (0.07)	0.24 (0.06)	0.57 (0.04)	0.20 (0.05)
Ferrochelatase	Eunits/ng TSP	175	2.29	-0.06	0.40	0.02	0.41	0.53 (0.05)	0.35 (0.03)	0.46 (0.03)	0.24 (0.03)
Metallothionein*	Eunits/ng TSP	175	6.75	-0.06	0.42	0.04	0.36	0.59^{B} (0.14)	0.38^{AB} (0.07)	0.49^{B} (0.03)	0.22^{A} (0.03)
Cnidarian sHsp	Eunits/ng TSP	175	0.134	-0.001	0.024	0.002	0.013	0.026 (0.004)	0.018 (0.004)	0.033 (0.005)	0.017 (0.003)
ChlpsHsp*	Eunits/ng TSP	175	3.03	-0.11	0.60	0.04	0.60	0.83 (0.08)	0.51 (0.07)	0.77 (0.05)	0.27 (0.05)
Xenobiotic Response and Damage											
MXR	Eunits/ng TSP	175	1.65	-0.07	0.52	0.02	0.57	0.59 (0.04)	0.52 (0.05)	0.65 (0.03)	0.33 (0.04)
Cnidarian GST	pmol/ng TSP	175	11.04	-0.14	1.53	0.11	1.43	1.93 (0.21)	1.56 (0.34)	1.73 (0.10)	0.87 (0.13)
Dino GST*	pmol/ng TSP	175	19.64	-1.07	10.42	0.29	10.80	11.6^{AB} (0.50)	9.74^{AB} (0.56)	11.87^{B} (0.41)	8.38^{A} (0.65)
CYP450 2-class	Eunits/ng TSP	175	4.56	-0.06	0.44	0.04	0.51	0.50 (0.04)	0.33 (0.04)	0.73 (0.13)	0.19 (0.03)
CYP450 3-class*	relative units/ng TSP	99	0.96	-0.09	0.53	0.03	0.63	0.68^{B} (0.04)	0.55^{B} (0.06)	0.68^{B} (0.03)	0.21^{A} (0.05)
CYP450 6-class	relative units/ng TSP	99	0.98	-0.05	0.51	0.02	0.56	0.54 (0.03)	0.58 (0.04)	0.58 (0.03)	0.36 (0.05)

Table 6.18. Coefficient of variance for each cellular biomarker along the depth gradient over the entire study period between March 2001 and February 2003.

Biomarker	units	n	KL 3 m	KL 6 m	KL 9 m	KL 18 m
Protein Metabolic Condition						
Ubiquitin	fmol/ng TSP	175	61	87	71	79
Cnidarian Hsp70	pmol/ng TSP	175	57	79	63	103
Dino Hsp 70	pmol/ng TSP	76	59	100	38	98
Cnidarian Hsp60	pmol/ng TSP	175	28	53	37	65
Dino Hsp60	pmol/ng TSP	175	94	79	104	146
Oxidative Damage and Response						
Cnidarian Cu/Zn SOD	Eunits/ng TSP	76	52	119	36	98
Dino Cu/Zn SOD	Eunits/ng TSP	175	47	68	33	68
Cnidarian MnSOD	pmol/ng TSP	166	62	58	42	114
Dino MnSOD	fmol/ng TSP	175	104	88	91	94
Cnidarian GPx	pmol/ng TSP	175	30	53	22	73
Dino GPx	pmol/ng TSP	175	103	128	89	104
Catalase	pmol/ng TSP	99	29	50	23	123
Metabolic Condition						
Heme oxygenase	Eunits/ng TSP	76	67	100	33	105
Ferrochelatase	Eunits/ng TSP	175	68	57	37	75
Metallothionein	Eunits/ng TSP	175	163	121	45	86
Cnidarian sHsp	Eunits/ng TSP	175	100	133	94	106
ChlpsHsp	Eunits/ng TSP	175	64	90	40	119
Xenobiotic Response and Damage						
MXR	Eunits/ng TSP	175	47	63	35	79
Cnidarian GST	pmol/ng TSP	175	74	139	38	102
Dino GST	pmol/ng TSP	175	29	37	23	51
CYP450 2-class	Eunits/ng TSP	175	48	82	121	105
CYP450 3-class	relative units/ng TSP	99	29	53	19	110
CYP450 6-class	relative units/ng TSP	99	30	38	26	69

Table 6.19. BEST and RELATE results for coral regeneration rates and biomarker concentrations during a given time period along the depth gradient, n.s. represents not significantly (> 5%)

Regeneration Rate	Biomarker	Rho	Significance level	# variables	Selected Biomarkers
JUN – AUG 2001	JUN 2001	0.08	n.s.	-	-
AUG – OCT 2001	AUG 2001	0.25	n.s.	-	-
OCT 2001 – MAR 2002	OCT 2001	0.24	1.1%	1	Cn Hsp70
MAR – JUN 2002	MAR 2002	-0.05	n.s.	-	-
JUN – AUG 2002	JUN 2002	0.06	n.s.	-	-
AUG – NOV 2002	AUG 2002	0.27	2.4%	1	Cn GPx
NOV 2002 – FEB 2003	NOV 2002	0.07	n.s.	-	-

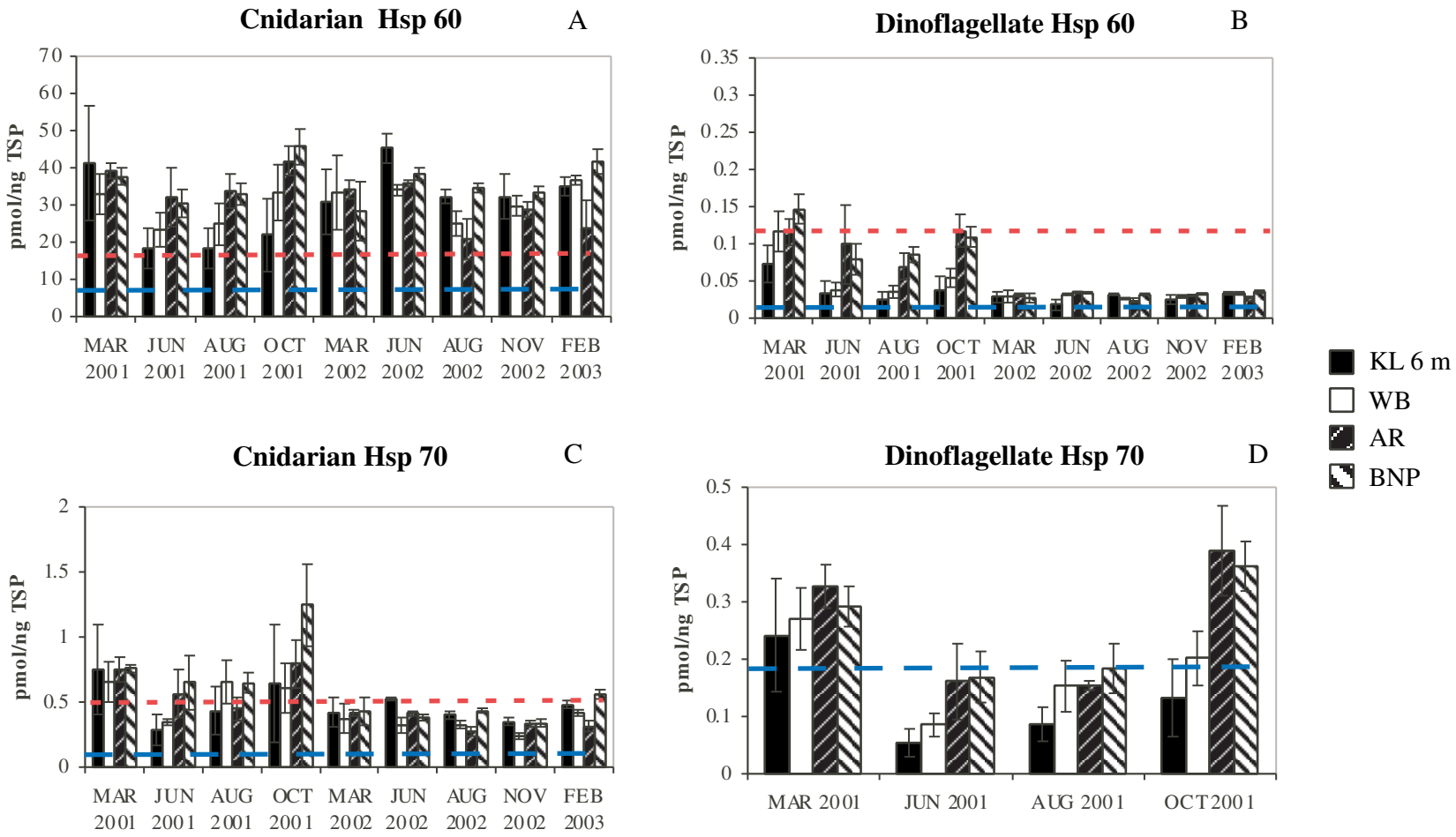


Figure 6.1. Protein Metabolic Condition at the 6 m sites including (A) cnidarian heat shock protein (Hsp) 60, (B) dinoflagellate heat shock protein 60, (C) cnidarian heat shock protein 70, (D) dinoflagellate heat shock protein 70 and (E) ubiquitin. Data presented as means (± SE) in pmol/ng TSP for cnidarian Hsp 60 and 70, dinoflagellate Hsp 60 and 70 and in fmol/ng TSP for ubiquitin. The red and blue dashed line represents "stressed" and "basal" levels, respectively as defined by Downs et al. 2005a. Means for dinoflagellate Hsp 70 were all below "stressed" levels (1.68 pmol/ng TSP).

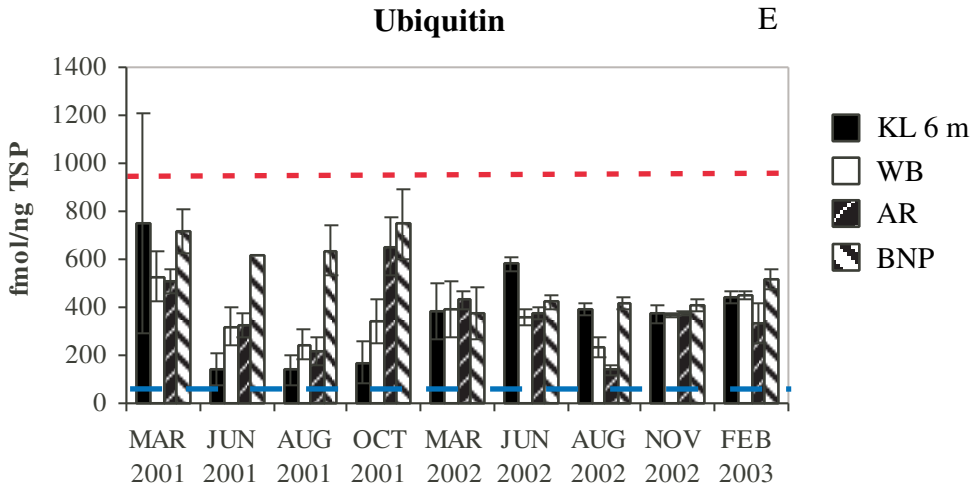


Figure 6.1 (cont.). Protein Metabolic Condition at the 6 m sites including (A) cnidarian heat shock protein (Hsp) 60, (B) dinoflagellate heat shock protein 60, (C) cnidarian heat shock protein 70, (D) dinoflagellate heat shock protein 70 and (E) ubiquitin. Data presented as means (± SE) in pmol/ng TSP for cnidarian Hsp 60 and 70, dinoflagellate Hsp 60 and 70 and in fmol/ng TSP for ubiquitin. The red and blue dashed line represents "stressed" and "basal" levels, respectively as defined by Downs et al. 2005a. Means for dinoflagellate Hsp 70 were all below "stressed" levels (1.68 pmol/ng TSP).

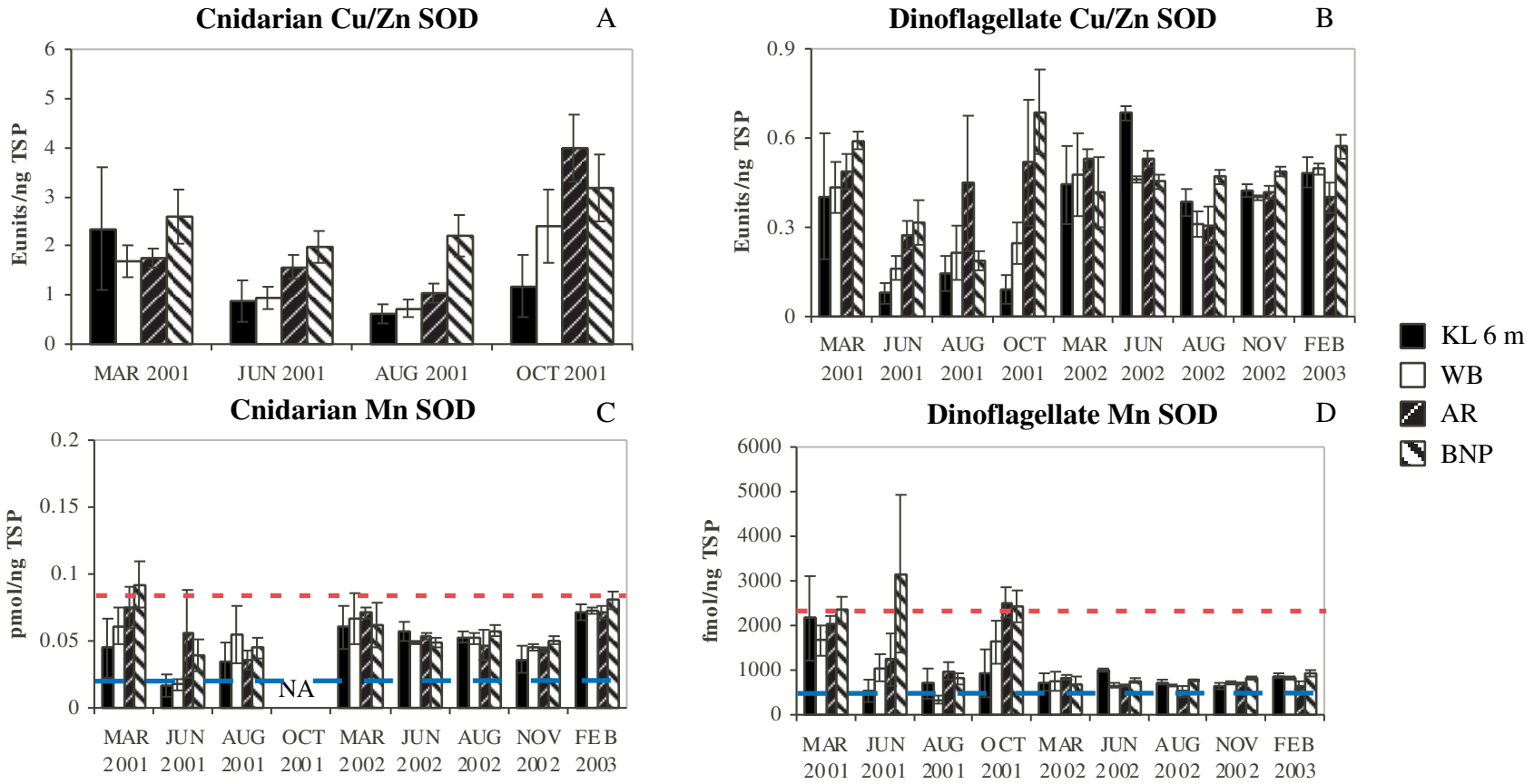


Figure 6.2. Oxidative damage and response at the 6 m sites including (A) cnidarian copper/zinc superoxide dismutase (Cu/Zn SOD), (B) dinoflagellate Cu/Zn SOD, (C) cnidarian manganese superoxide dismutase (MnSOD), (D) dinoflagellate Mn SOD, (E) cnidarian glutathione peroxidase (GPx), (F) dinoflagellate GPx and (G) catalase. Data presented as means (± SE) in pmol/ng TSP. The red and blue dashed line represents "stressed" and "basal" levels, respectively as defined by Downs et al. 2005. Means for cnidarian GPx were below both "basal" and "stressed" levels at all sites (70 and 171 pmol/ng TSP, respectively). "Stressed" or "basal" levels are not available for cnidarian and dinoflagellate Cu/Zn SOD or catalase.

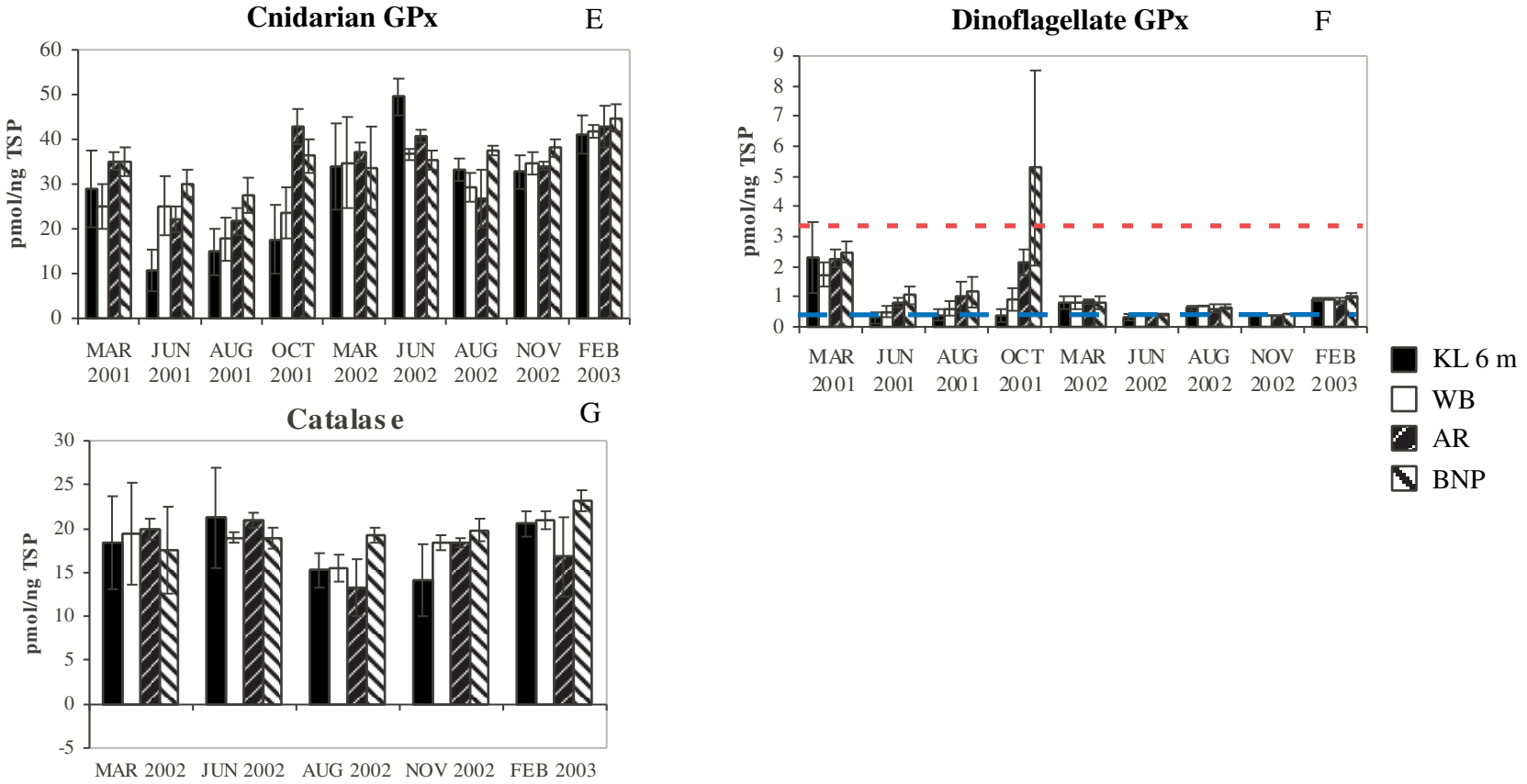


Figure 6.2 (cont.). Oxidative damage and response at the 6 m sites including (A) cnidarian copper/zinc superoxide dismutase (Cu/Zn SOD), (B) dinoflagellate Cu/Zn SOD, (C) cnidarian manganese superoxide dismutase (MnSOD), (D) dinoflagellate Mn SOD, (E) cnidarian glutathione peroxidase (GPx), (F) dinoflagellate GPx and (G) catalase. Data presented as means (± SE) in pmol/ng TSP. The red and blue dashed line represents "stressed" and "basal" levels, respectively as defined by Downs et al. 2005. Means for cnidarian GPx were below both "basal" and "stressed" levels at all sites (70 and 171 pmol/ng TSP, respectively). "Stressed" or "basal" levels are not available for cnidarian and dinoflagellate Cu/Zn SOD or catalase.

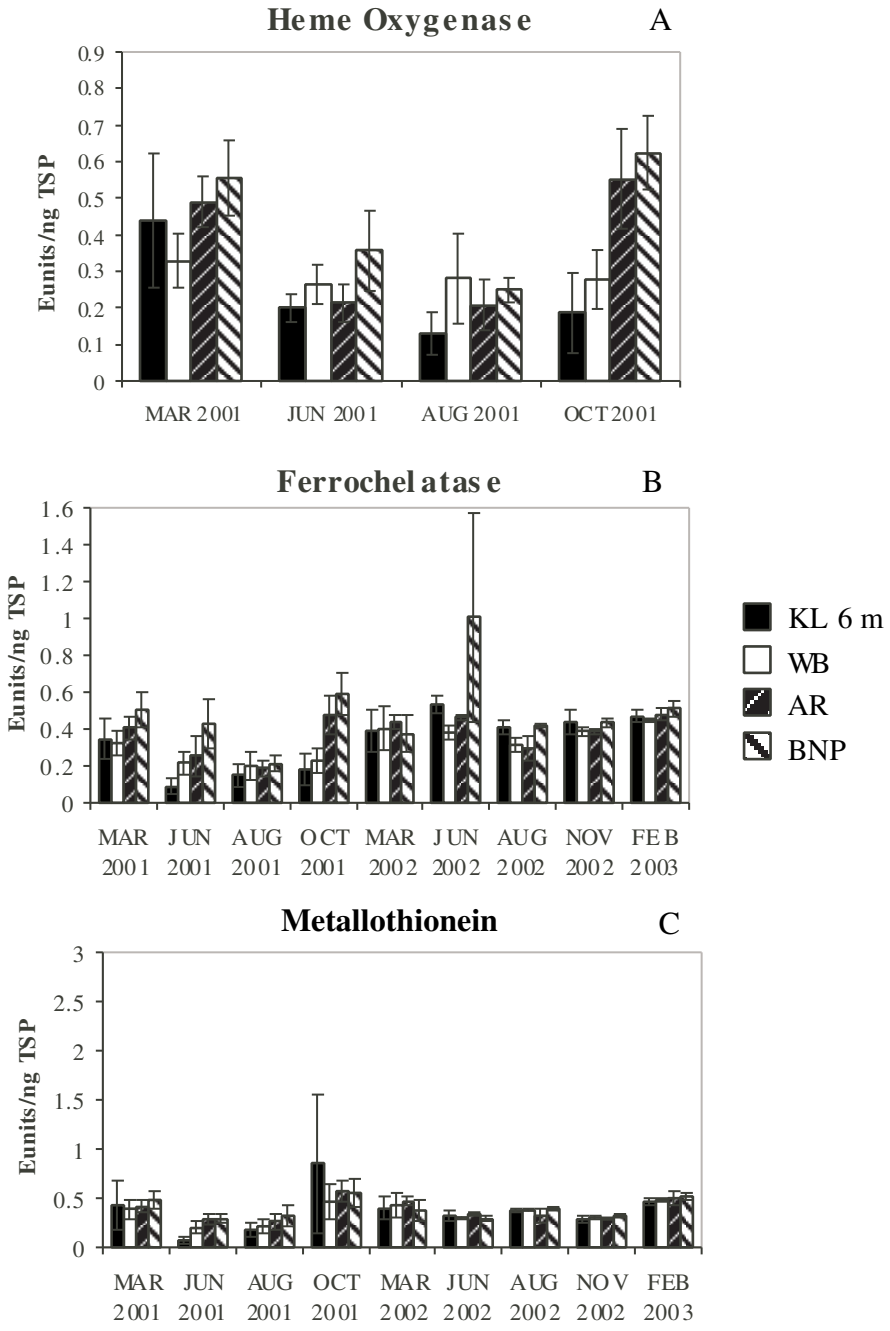


Figure 6.3. Metabolic Condition at the 6 m sites including (A) heme oxygenase, (B) ferrochelatase, (C) metallothionein, (D) cnidarian small heat shock protein (sHsp) and (E) chloroplast small heat shock protein (ChlpsHsp). Data presented as means (± SE) in Eunits/ng TSP. Baseline condition is not available for these biomarkers.

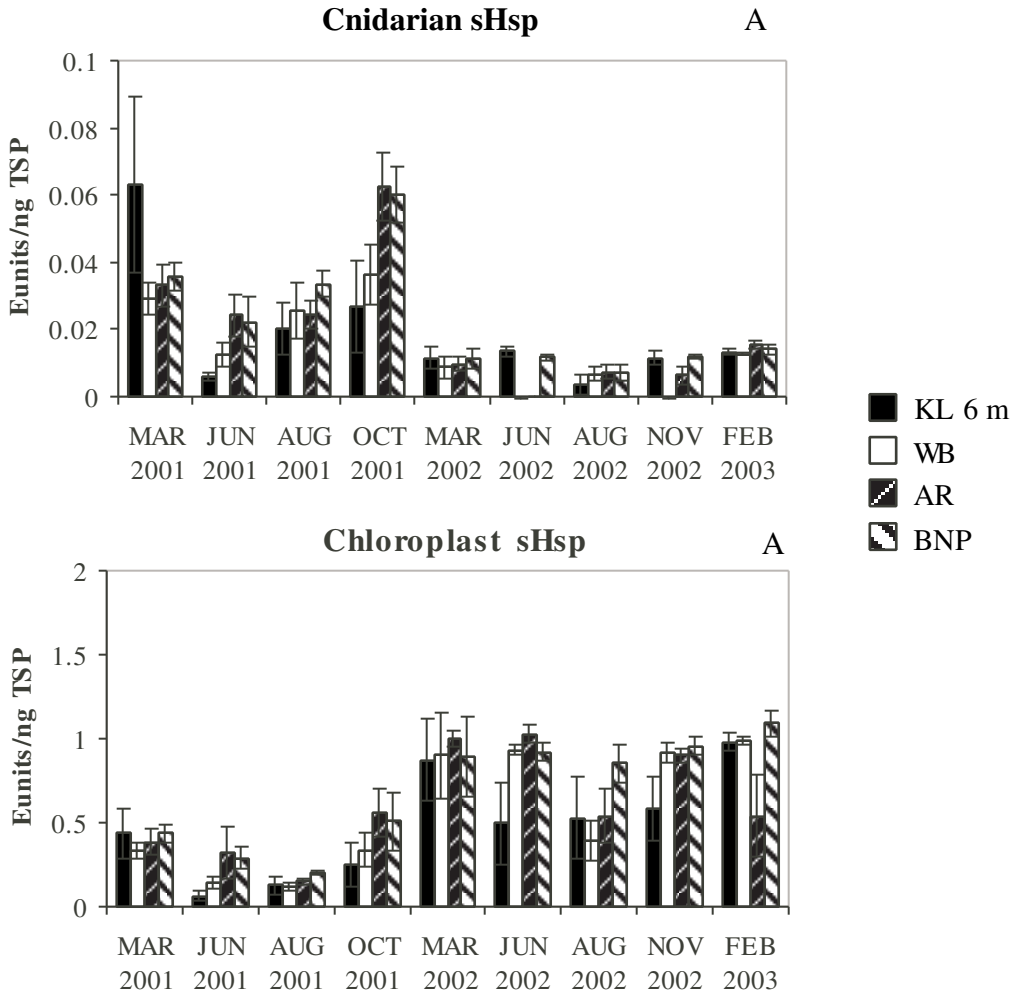


Figure 6.3 (cont.). Metabolic Condition at the 6 m sites including (A) heme oxygenase, (B) ferrochelatase, (C) metallothionein, (D) cnidarian small heat shock protein (sHsp) and (E) chloroplast small heat shock protein (ChlpsHsp). Data presented as means (± SE) in Eunits/ng TSP. Baseline condition is not available for these biomarkers.

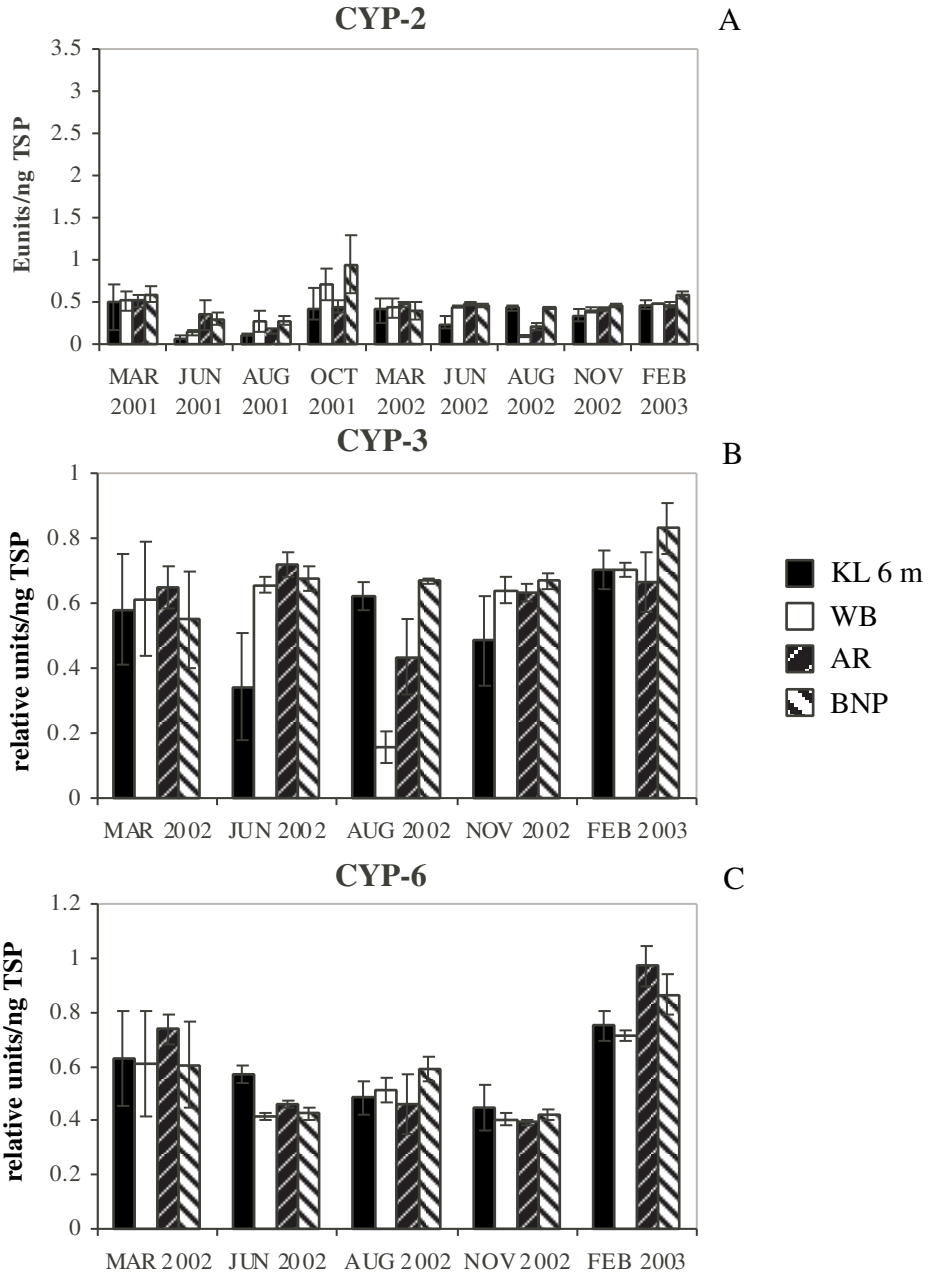


Figure 6.4. Xenobiotic Detoxification and Response at the 6 m sites including (A) cytochrome P450 2-class (CYP-2), (B) cytochrome P450 3-class (CYP-3), (C) cytochrome P450 6-class (CYP-6), (D) cnidarian glutathione-S-transferase (Cn GST), (E) dinoflagellate GST and (F) multixenobiotic resistance protein (MXR). Data presented as means (± SE) in Eunits/ng TSP for CYP-2 and MXR; relative units/ng TSP for CYP-3 and CYP-6; and pmol/ng TSP for Cn and Dn GST. Baseline information is not available for any of the cytochrome P450 classes or for MXR. The red and blue dashed line represents "stressed" and "basal" levels, respectively as defined by Downs et al. 2005. Baseline information is not available for CYP-2, CYP-3, CYP-6 or MXR.

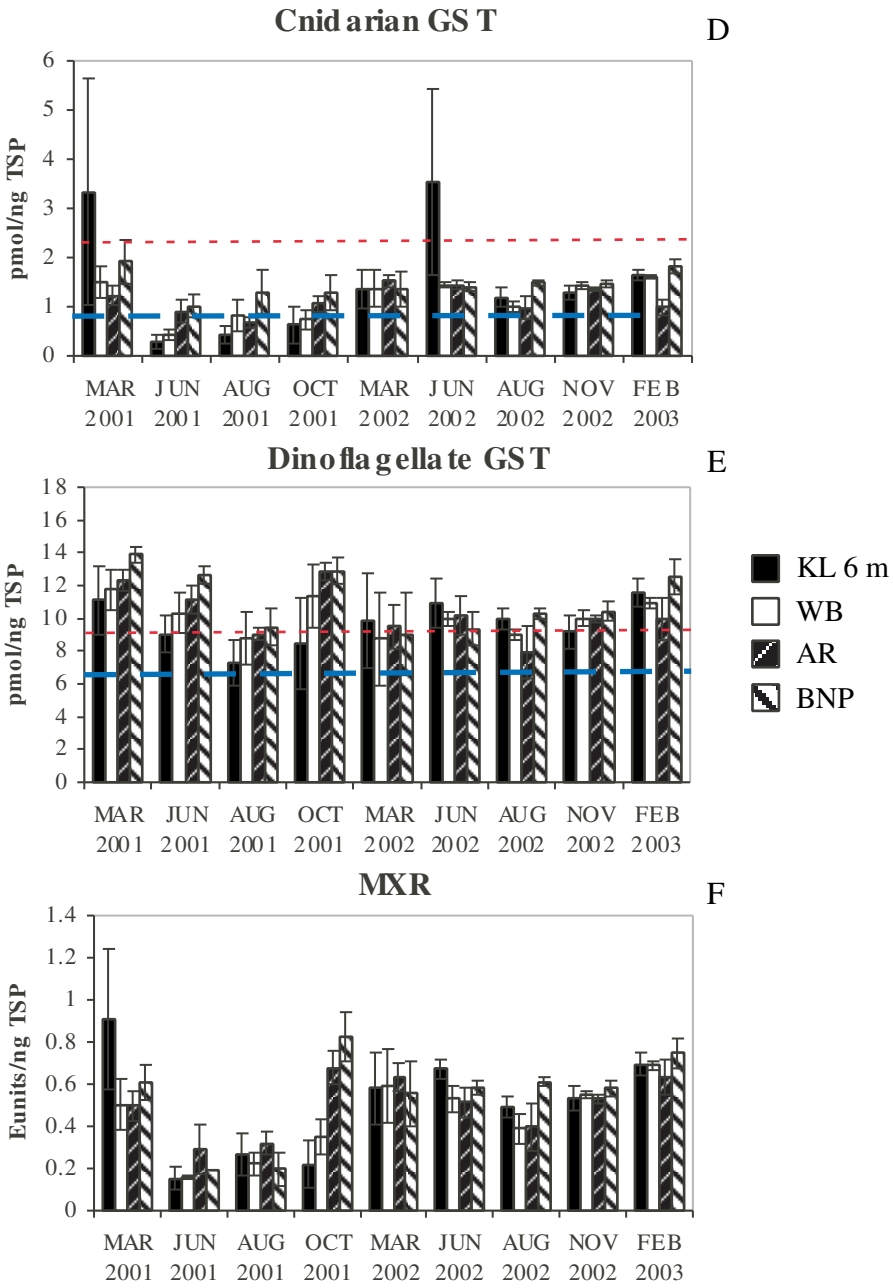


Figure 6.4 (cont.). Xenobiotic Detoxification and Response at the 6 m sites including (A) cytochrome P450 2-class (CYP-2), (B) cytochrome P450 3-class (CYP-3), (C) cytochrome P450 6-class (CYP-6), (D) cnidarian glutathione-S-transferase (Cn GST), (E) dinoflagellate GST and (F) multixenobiotic resistance protein (MXR). Data presented as means (± SE) in Eunits/ng TSP for CYP-2 and MXR; relative units/ng TSP for CYP-3 and CYP-6; and pmol/ng TSP for Cn and Dn GST. Baseline information is not available for any of the cytochrome P450 classes or for MXR. The red and blue dashed line represents "stressed" and "basal" levels, respectively as defined by Downs et al. 2005. Baseline information is not available for CYP-2, CYP-3, CYP-6 or MXR.

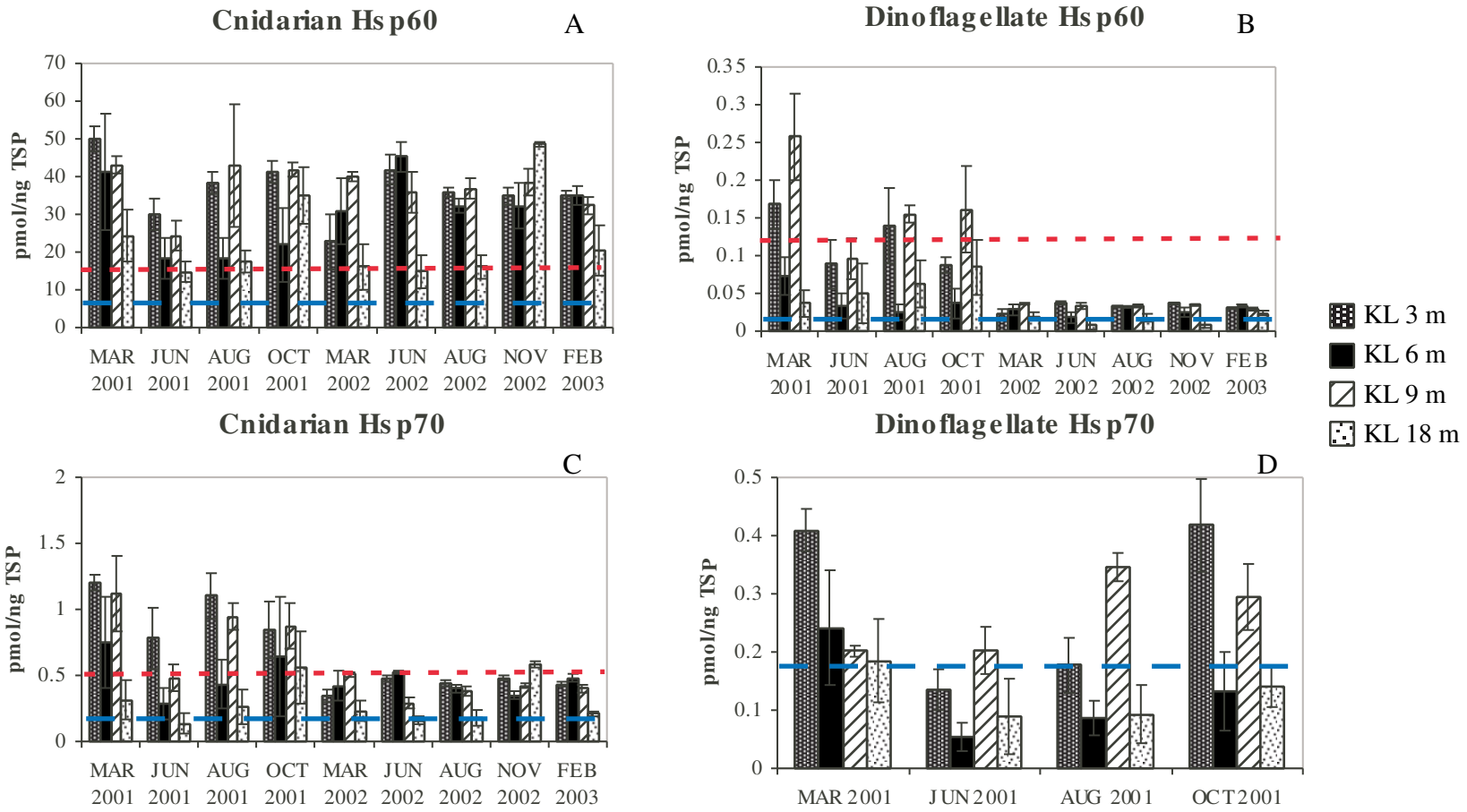


Figure 6.5. Protein Metabolic Condition along the depth gradient including (A) cnidarian heat shock protein (Hsp) 60, (B) dinoflagellate heat shock protein 60, (C) cnidarian heat shock protein 70, (D) dinoflagellate heat shock protein 70 and (E) ubiquitin. Data presented as means (± SE) in pmol/ng TSP for cnidarian Hsp 60 and 70, dinoflagellate Hsp 60 and 70 and in fmol/ng TSP for ubiquitin. The red and blue dashed line represents "stressed" and "basal" levels, respectively as defined by Downs et al. 2005a. Means for dinoflagellate Hsp 70 were all below "stressed" levels (1.68 pmol/ng TSP).

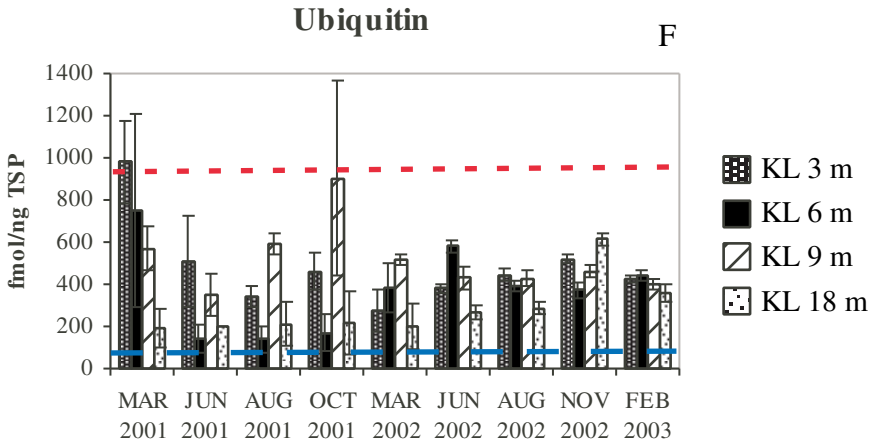


Figure 6.5 (cont.). Protein Metabolic Condition along the depth gradient including (A) cnidarian heat shock protein (Hsp) 60, (B) dinoflagellate heat shock protein 60, (C) cnidarian heat shock protein 70, (D) dinoflagellate heat shock protein 70 and (E) ubiquitin. Data presented as means (± SE) in pmol/ng TSP for cnidarian Hsp 60 and 70, dinoflagellate Hsp 60 and 70 and in fmol/ng TSP for ubiquitin. The red and blue dashed line represents "stressed" and "basal" levels, respectively as defined by Downs et al. 2005a. Means for dinoflagellate Hsp 70 were all below "stressed" levels (1.68 pmol/ng TSP).

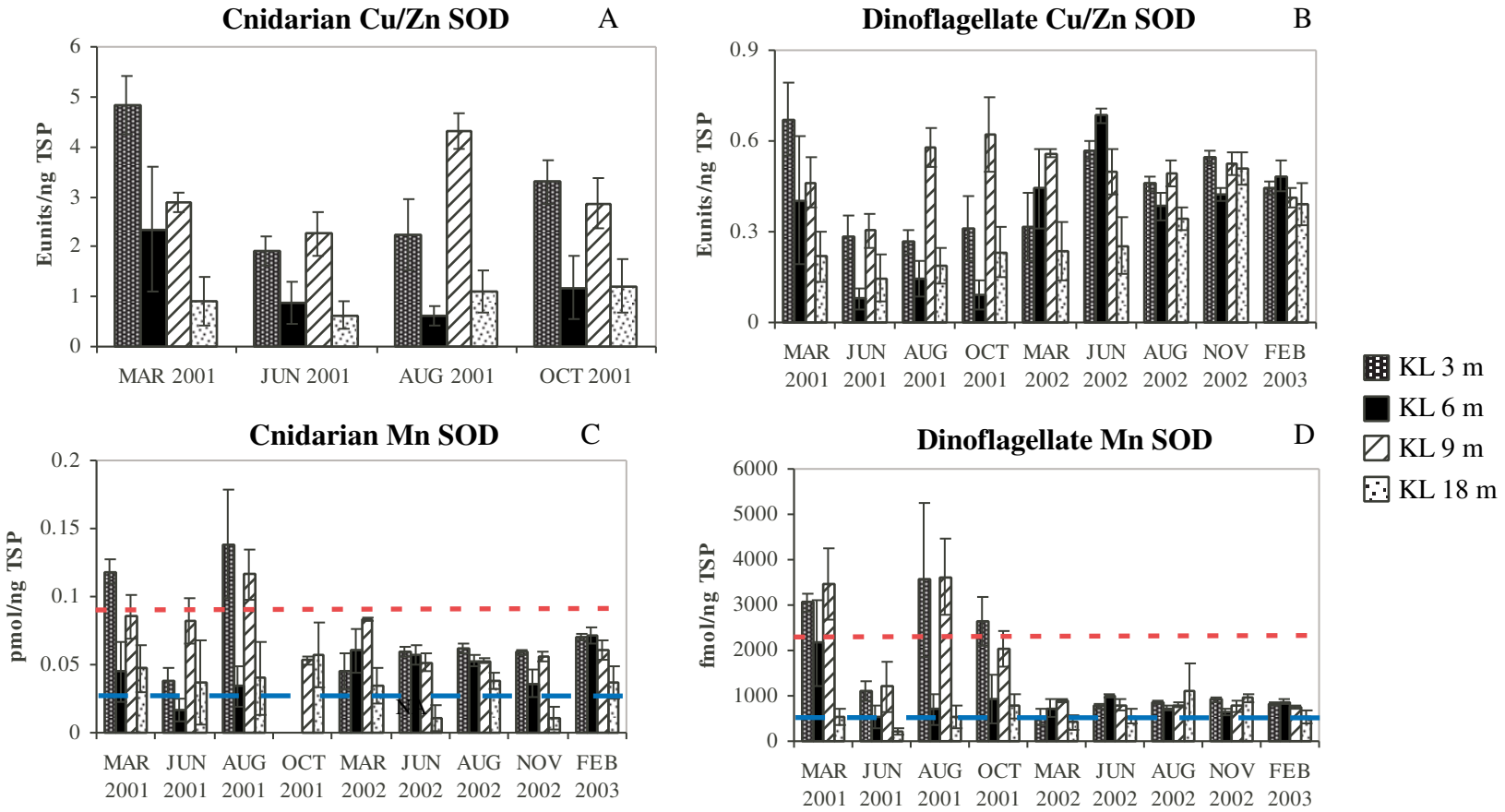


Figure 6.6. Oxidative damage and response along the depth gradient including (A) cnidarian copper/zinc superoxide dismutase (Cu/Zn SOD), (B) dinoflagellate Cu/Zn SOD, (C) cnidarian manganese superoxide dismutase (MnSOD), (D) dinoflagellate Mn SOD, (E) cnidarian glutathione peroxidase (GPx), (F) dinoflagellate GPx and (G) catalase. Data presented as means (± SE) in pmol/ng TSP. The red and blue dashed line represents "stressed" and "basal" levels, respectively as defined by Downs et al. 2005. Means for cnidarian GPx were below both "basal" and "stressed" levels at all sites (70 and 171 pmol/ng TSP, respectively). "Stressed" or "basal" levels are not available for cnidarian and dinoflagellate Cu/Zn SOD or catalase.

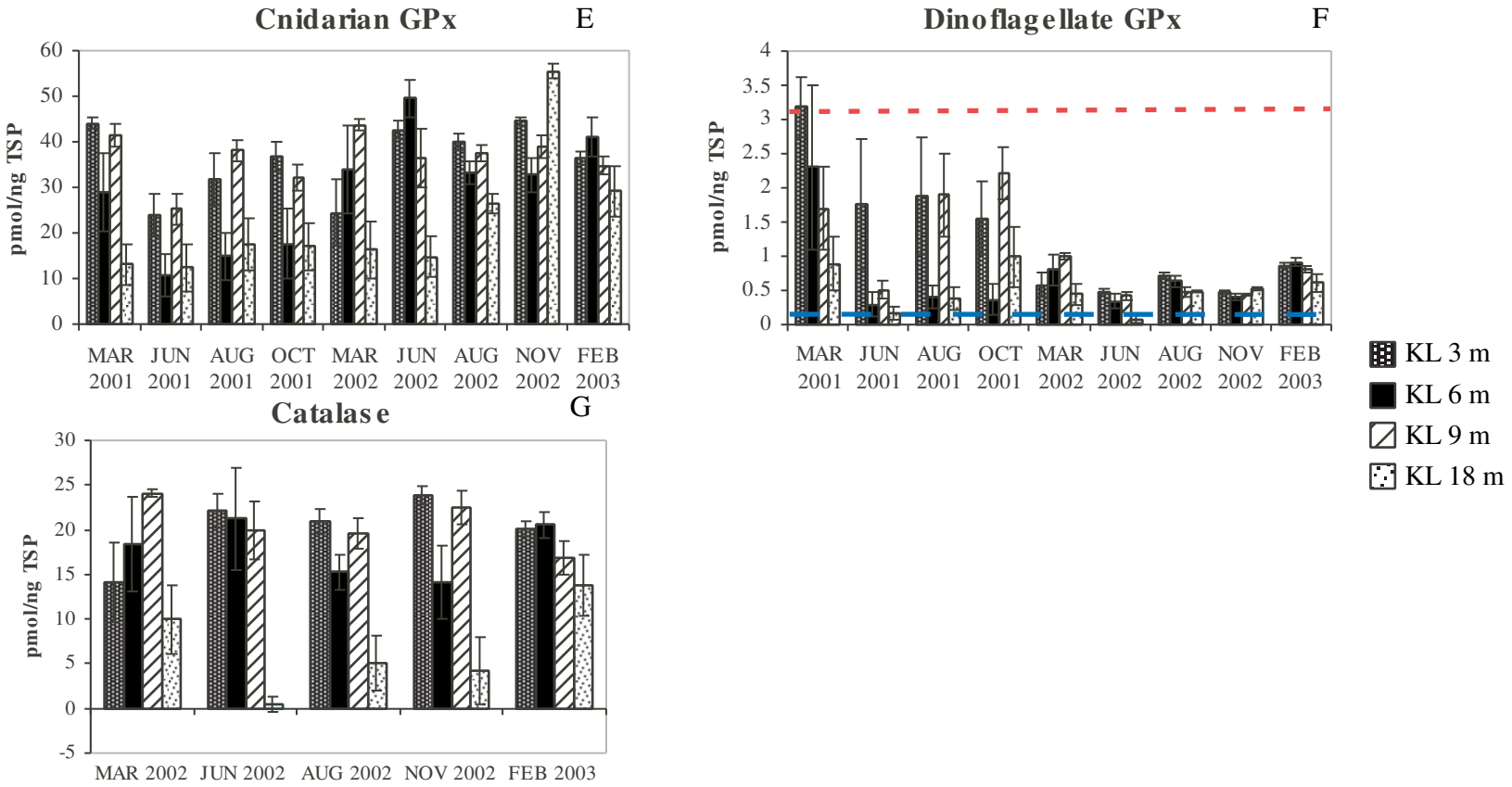


Figure 6.6 (cont.). Oxidative damage and response along the depth gradient including (A) cnidarian copper/zinc superoxide dismutase (Cu/Zn SOD), (B) dinoflagellate Cu/Zn SOD, (C) cnidarian manganese superoxide dismutase (MnSOD), (D) dinoflagellate Mn SOD, (E) cnidarian glutathione peroxidase (GPx), (F) dinoflagellate GPx and (G) catalase. Data presented as means (± SE) in pmol/ng TSP. The red and blue dashed line represents "stressed" and "basal" levels, respectively as defined by Downs et al. 2005. Means for cnidarian GPx were below both "basal" and "stressed" levels at all sites (70 and 171 pmol/ng TSP, respectively). "Stressed" or "basal" levels are not available for cnidarian and dinoflagellate Cu/Zn SOD or catalase.

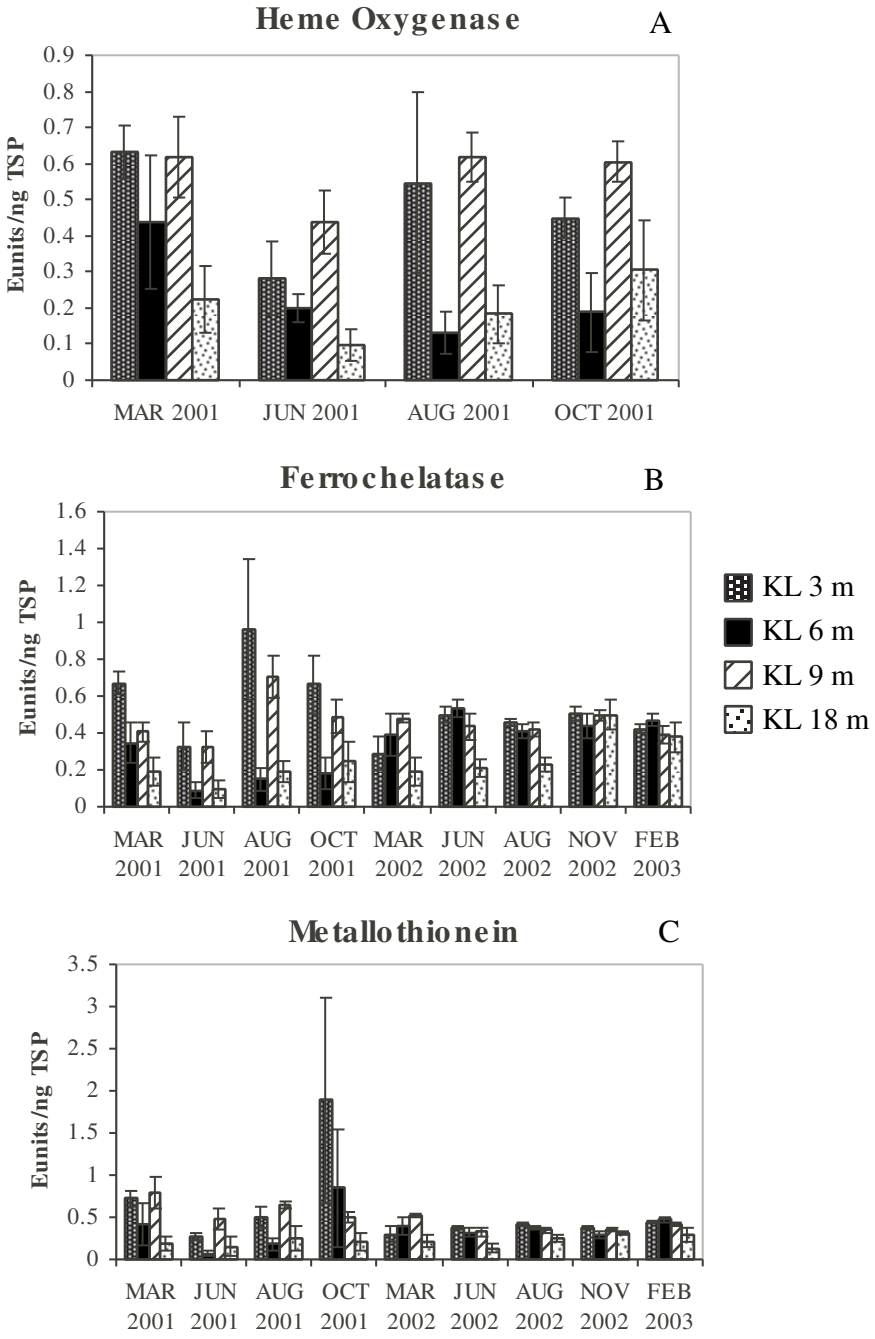


Figure 6.7. Metabolic Condition along the depth gradient including (A) heme oxygenase, (B) ferrochelatase, (C) metallothionein, (D) cnidarian small heat shock protein (sHsp) and (E) chloroplast small heat shock protein (ChlpsHsp). Data presented as means (± SE) in Eunits/ng TSP. Baseline condition is not available for these biomarkers.

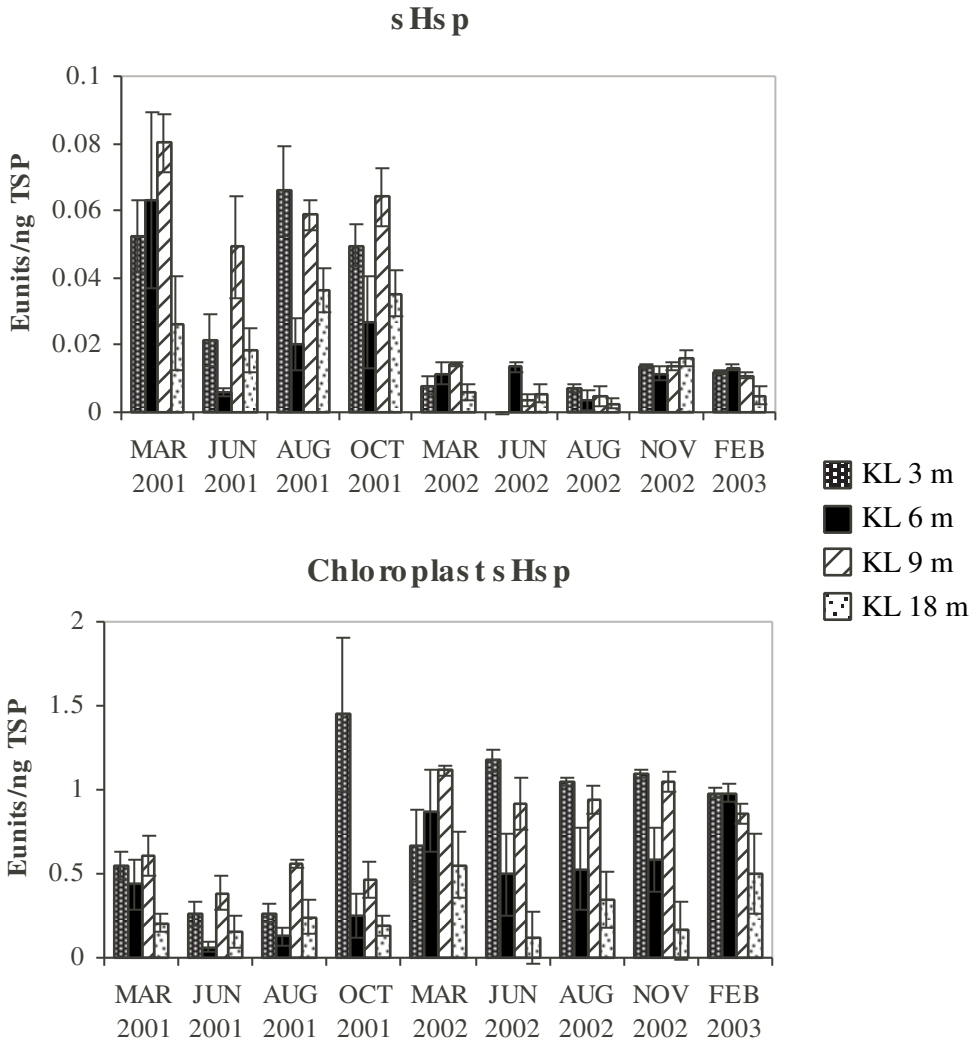


Figure 6.7 (cont.). Metabolic Condition along the depth gradient including (A) heme oxygenase, (B) ferrochelatase, (C) metallothionein, (D) cnidarian small heat shock protein (sHsp) and (E) chloroplast small heat shock protein (ChlpsHsp). Data presented as means (± SE) in Eunits/ng TSP. Baseline condition is not available for these bio-markers.

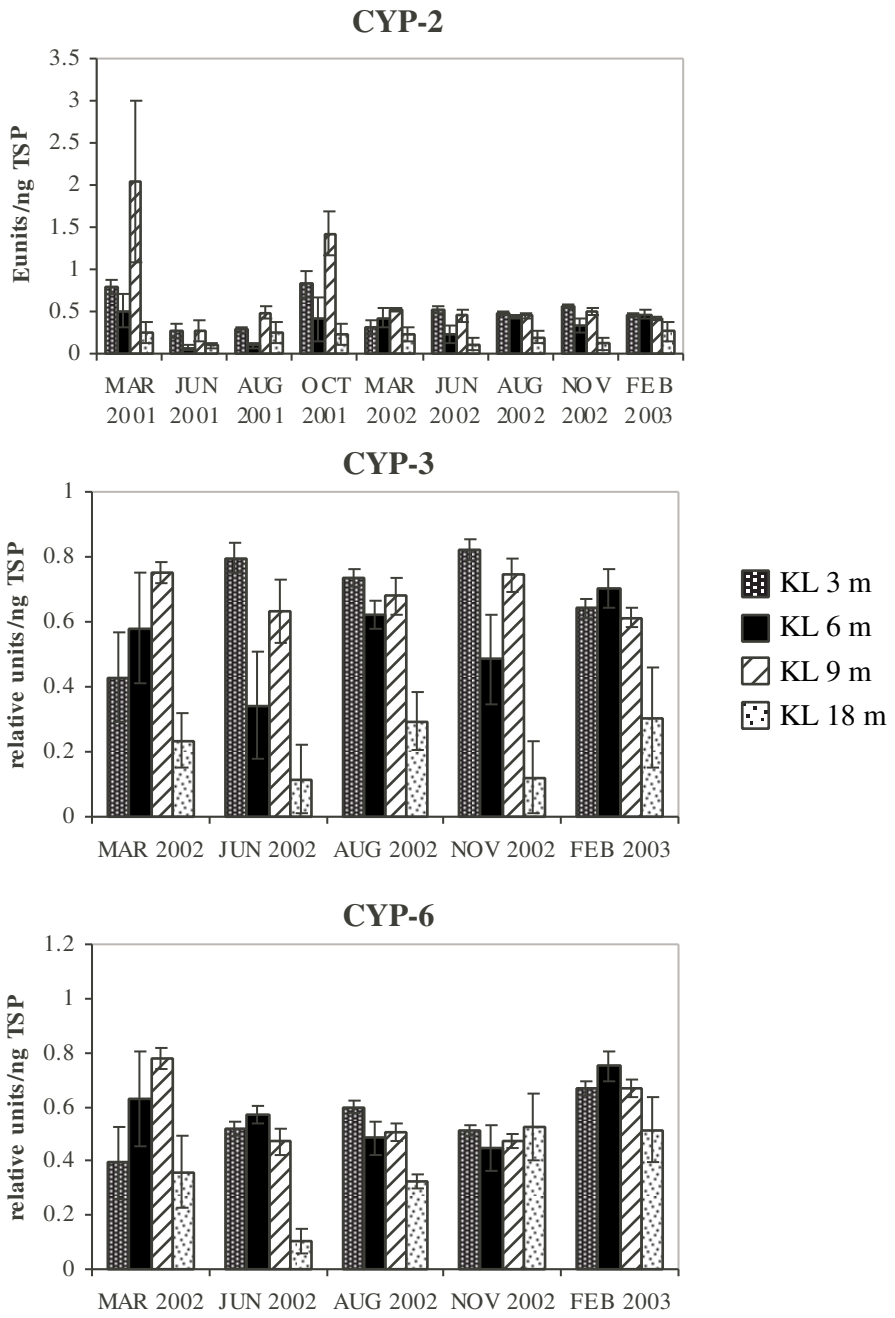


Figure 6.8. Xenobiotic Detoxification and Response along the depth gradient including (A) cytochrome P450 2-class (CYP-2), (B) cytochrome P450 3-class (CYP-3), (C) cytochrome P450 6-class (CYP-6), (D) cnidarian glutathione-S-transferase (Cn GST), (E) dinoflagellate GST and (F) multixenobiotic resistance protein (MXR). Data presented as means (± SE) in Eunits/ng TSP for CYP-2 and MXR; relative units/ng TSP for CYP-3 and CYP-6; and pmol/ng TSP for Cn and Dn GST. Baseline information is not available for any of the cytochrome P450 classes or for MXR. The red and blue dashed line represents "stressed" and "basal" levels, respectively as defined by Downs et al. 2005. Baseline information is not available for CYP-2, CYP-3, CYP-6 or MXR.

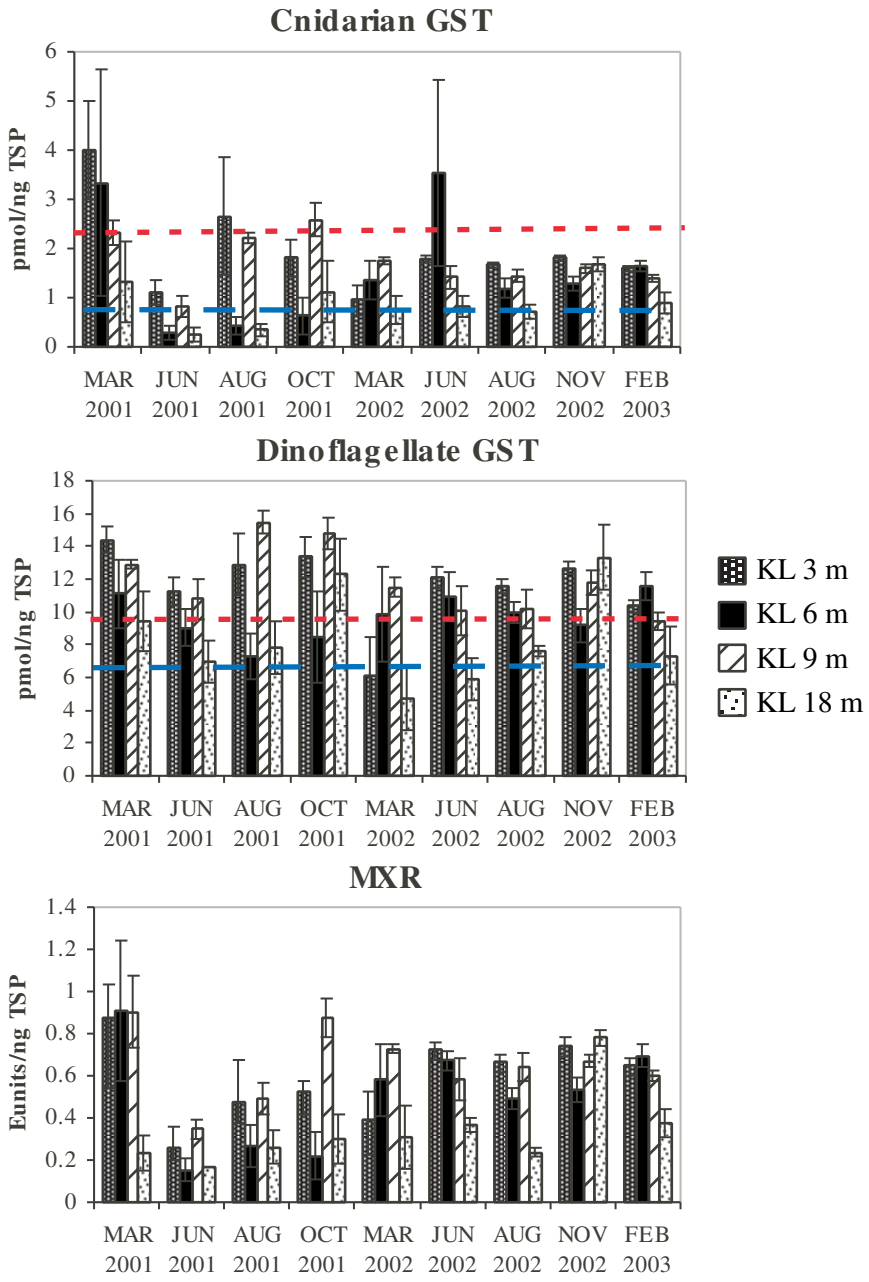


Figure 6.8 (cont.). Xenobiotic Detoxification and Response along the depth gradient including (A) cytochrome P450 2-class (CYP-2), (B) cytochrome P450 3-class (CYP-3), (C) cytochrome P450 6-class (CYP-6), (D) cnidarian glutathione-S-transferase (Cn GST), (E) dinoflagellate GST and (F) multixenobiotic resistance protein (MXR). Data presented as means (± SE) in Eunits/ng TSP for CYP-2 and MXR; relative units/ng TSP for CYP-3 and CYP-6; and pmol/ng TSP for Cn and Dn GST. Baseline information is not available for any of the cytochrome P450 classes or for MXR. The red and blue dashed line represents "stressed" and "basal" levels, respectively as defined by Downs et al. 2005. Baseline information is not available for CYP-2, CYP-3, CYP-6 or MXR.

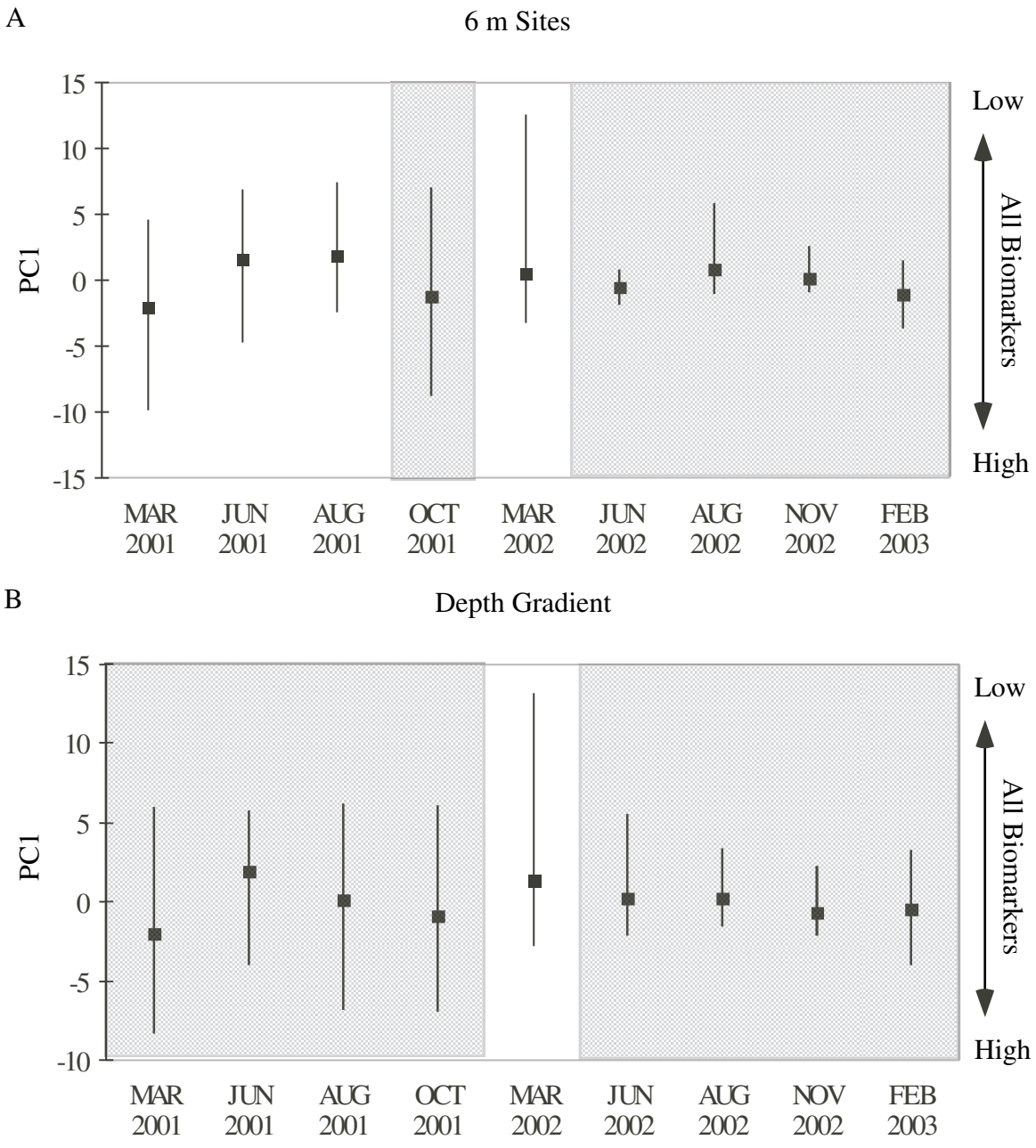


Figure 6.9. Plots of Principle Component (PC1) scores for each sampling period at (A) the 6 m sites and (B) along the depth gradient. Vertical bars show the range of values for each sampling period, squares indicate the sample mean for each period. Shaded areas represent sampling periods when there were significant differences among sites based on ANOSIM (6 m sites: Table 6.5; depth gradient: Table 6.8).

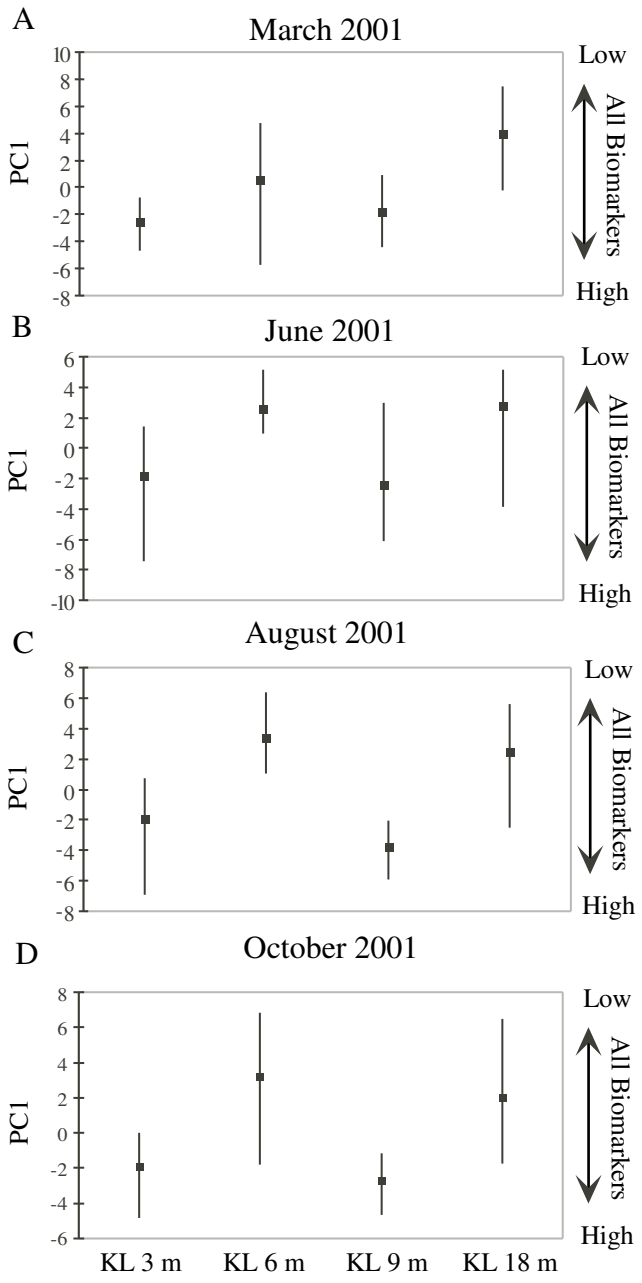


Figure 6.10. Plots of Principle Component (PC1) scores at each site for (A) March 2001, (B) June 2001, (C) August 2001 and (D) October 2001. Vertical bars show the range of values for each site, squares indicate the sample mean for each site. Eigenvalues and eigenvectors as shown in Appendix B.

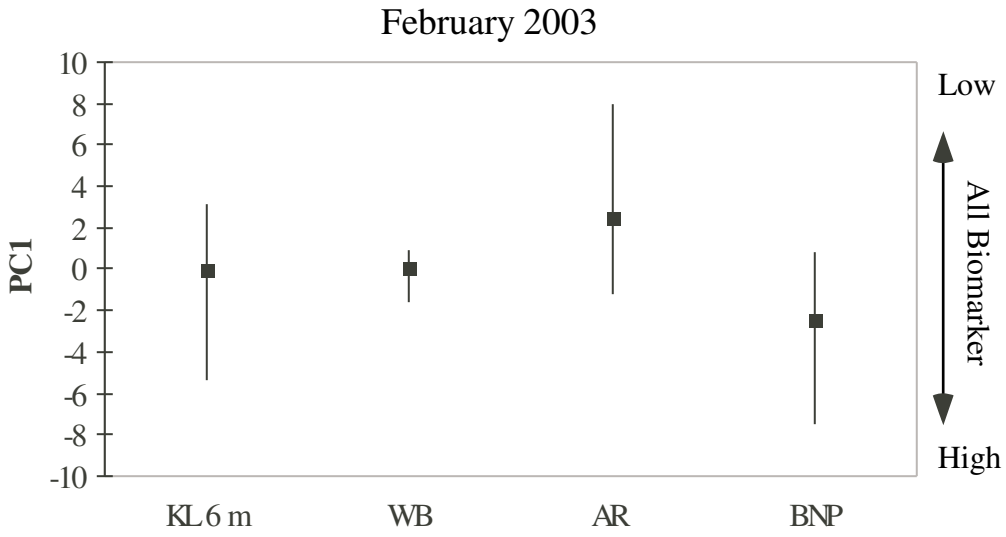


Figure 6.11. Plots of Principle Component (PC1) scores at each site in February 2003. Vertical bars show the range of values for each site; squares indicate the sample mean for each site. Eigenvalues and eigenvectors as shown in Appendix C.

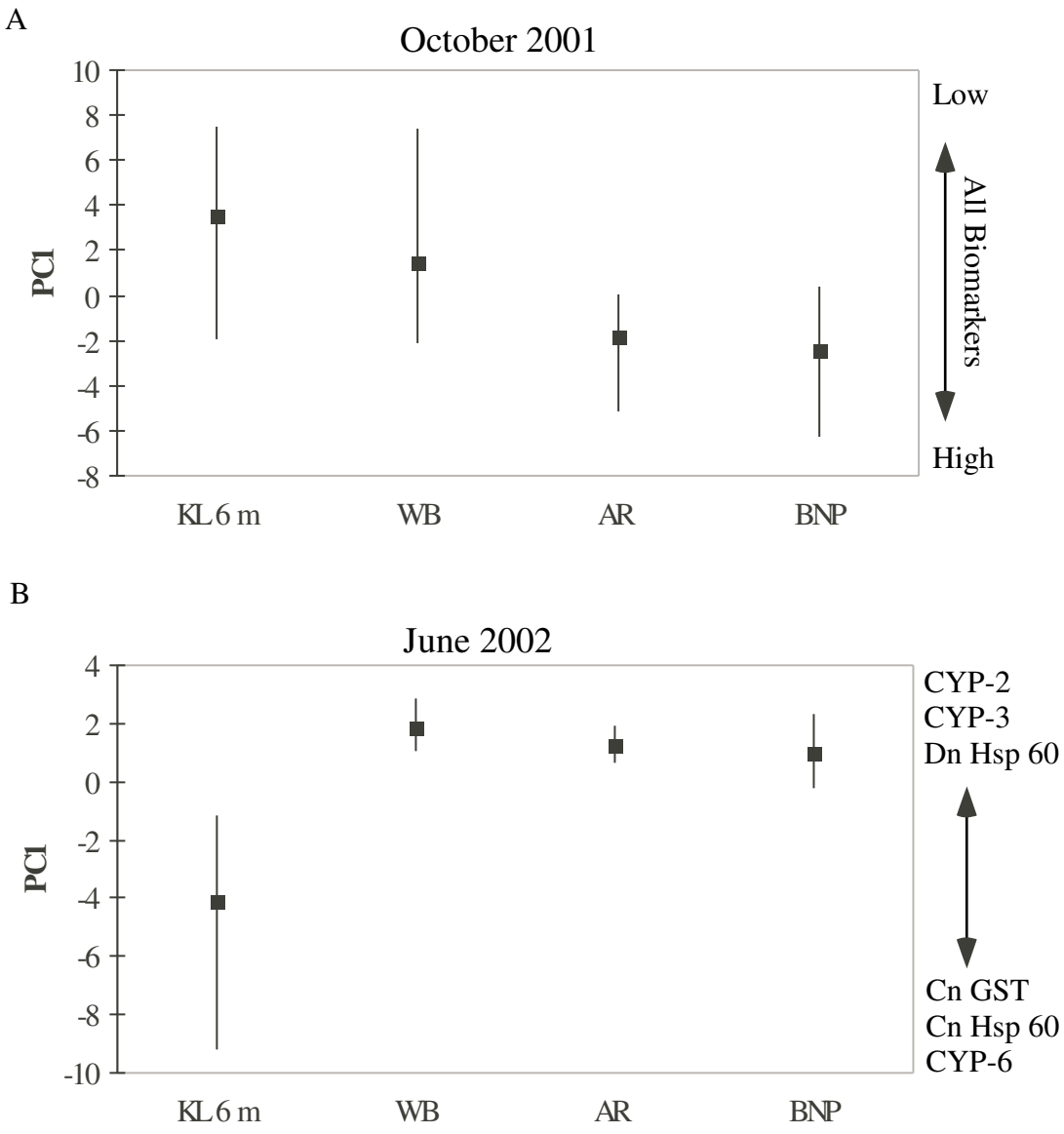


Figure 6.12. Plots of Principle Component (PC1) scores at each site for (A) October 2001 and (B) June 2002. Vertical bars show the range of values for each site, squares indicate the sample mean for each site. Eigenvectors and eigenvalues as shown in Appendix C.

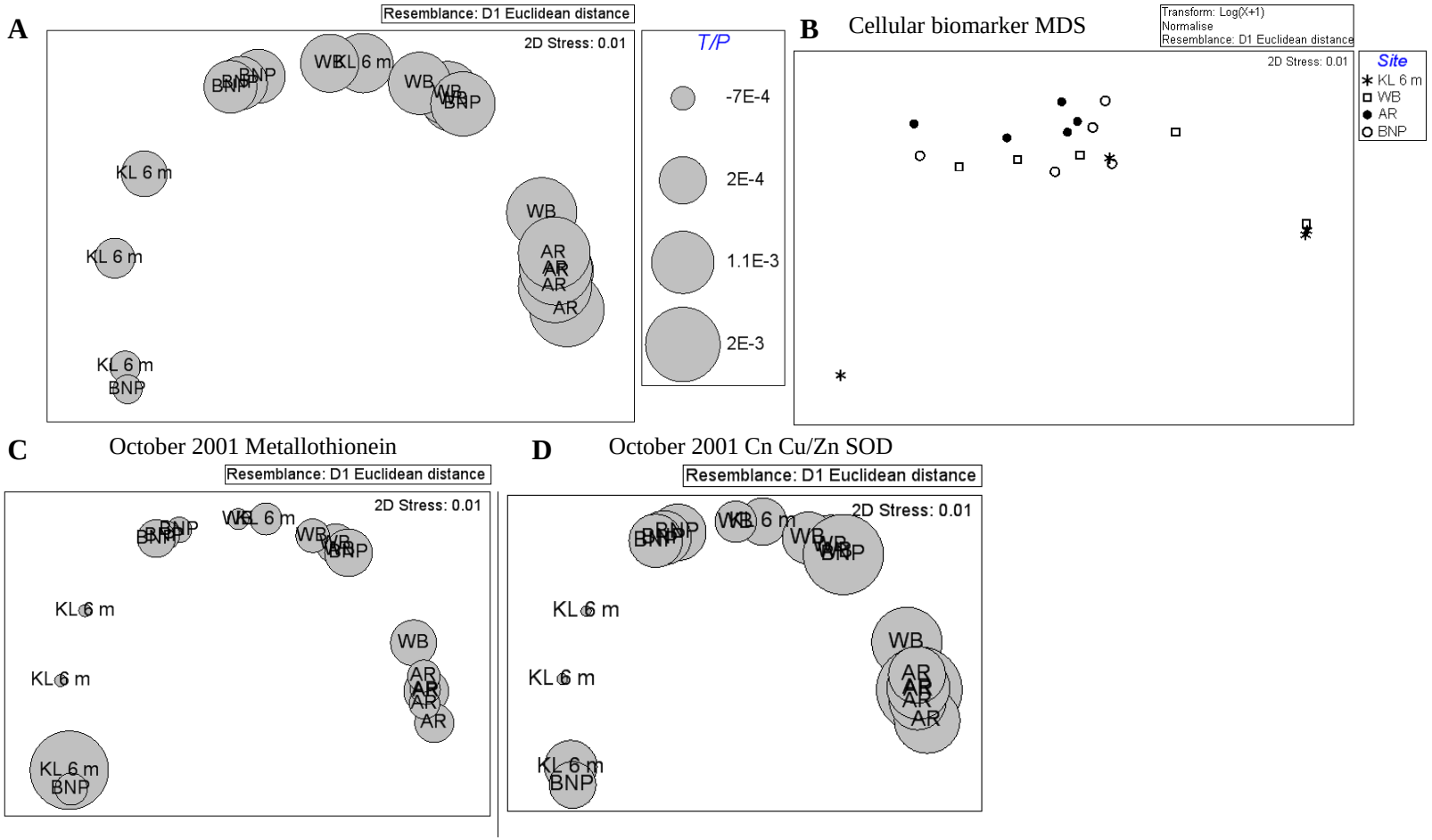


Figure 6.13. (A) Multi-dimensional scaling (MDS) plot for regeneration rates (T/P) between October 2001 and March 2002 at the 6 m sites; larger circles represent higher regeneration rates. (B) MDS plot of October 2001 cellular biomarkers (CDS) selected by BEST routine (Table 6.14), which included metallothionein and cnidarian copper/zinc superoxide disputase (Cn Cu/Zn SOD). Regeneration rate MDS superimposed by individual CDS biomarkers (C) metallothionein and (D) Cn Cu/Zn SOD; circle size increases with increasing concentration.

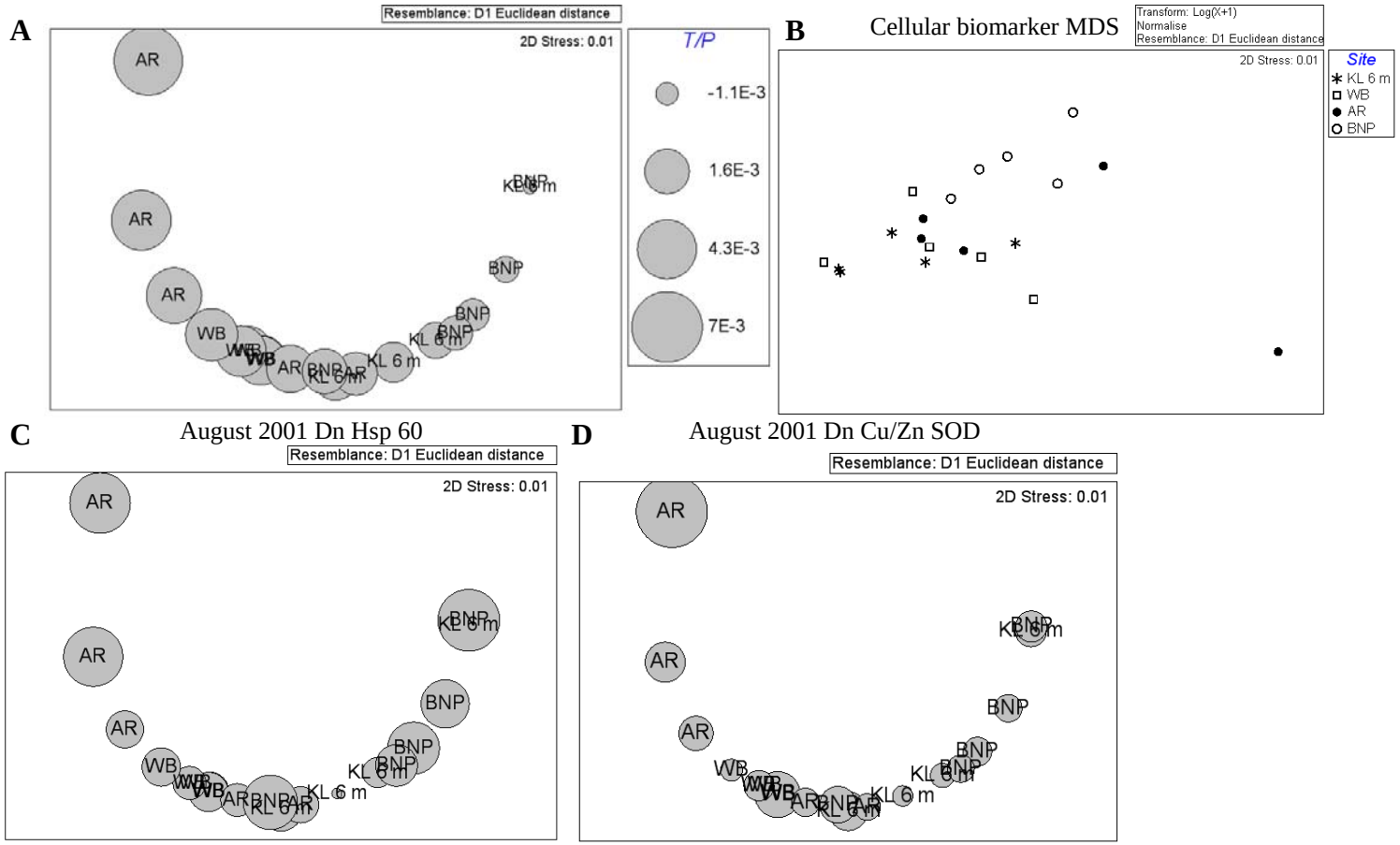


Figure 6.14. (A) Multi-dimensional scaling (MDS) plot for regeneration rates (T/P) between August and October 2001 at the 6 m sites; larger circles represent higher regeneration rates. (B) MDS plot of August 2001 cellular biomarkers (CDS) selected by BEST routine (Table 6.14), which included dinoflagellate heat shock protein 60 (Dn Hsp 60) and dinoflagellate copper/zinc superoxide dismutase (Dn Cu/Zn SOD). Regeneration rate MDS superimposed by individual CDS biomarkers including (C) Dn Hsp 60 and (D) Dn Cu/Zn SOD; circle size increases with increasing concentration.

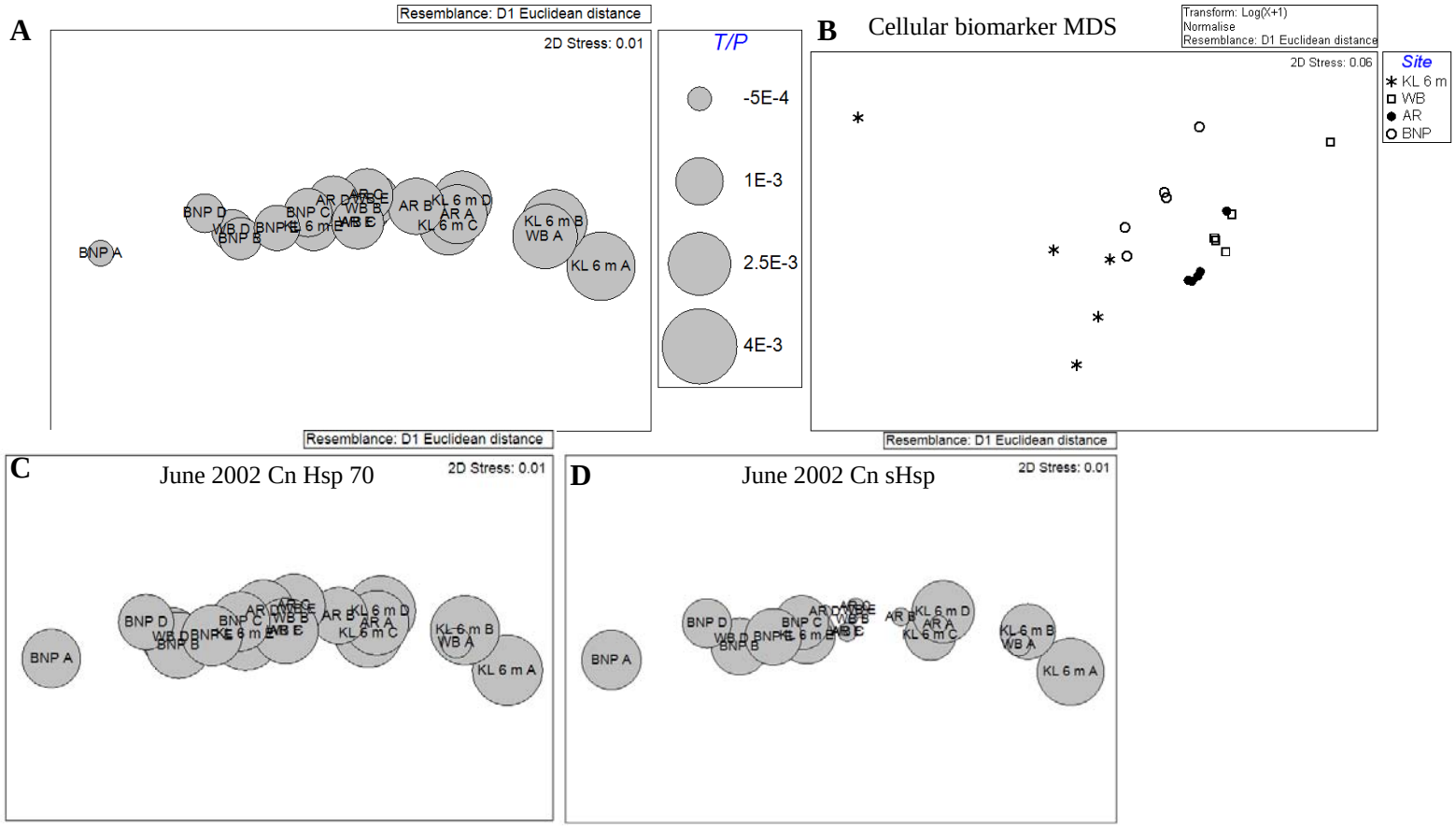


Figure 6.15. (A) Multi-dimensional scaling plots for regeneration rates (T/P) between June and August 2002 at the 6 m sites; larger circles represent higher regeneration rates. (B) MDS plot of June 2002 cellular biomarkers (CDS) selected by BEST routine (Table 6.14), which included cnidarian heat shock protein (Cn Hsp 70), cnidarian small heat shock protein (Cn sHsp), metallothionein, cnidarian glutathione-S-transferase and cytochrome P450 6-class. Regeneration rate MDS superimposed by individual CDS biomarkers (C) Cn Hsp 70, (D) Cn sHsp, (E) metallothionein, (F) Cn GST and (G) CYP-6; circle size increases with increasing concentration.

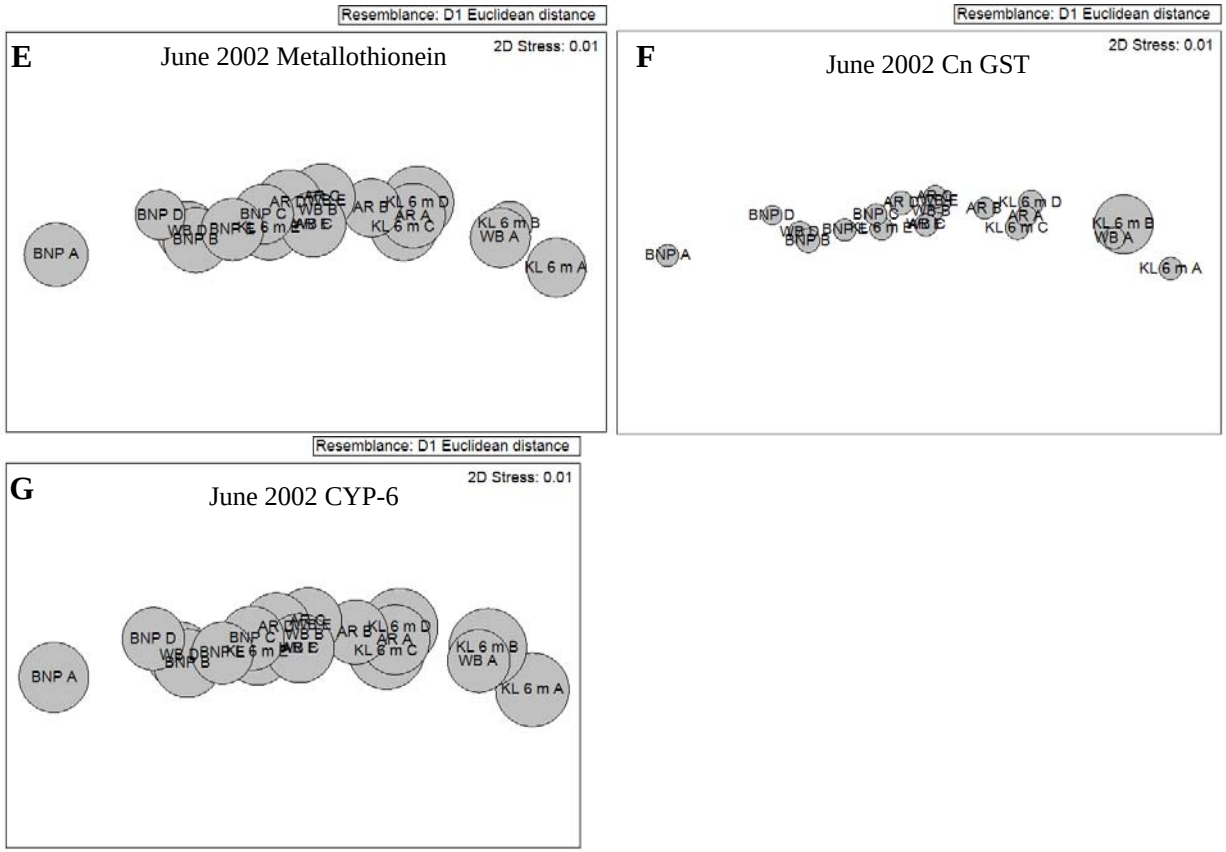


Figure 6.15 (cont.). (A) Multi-dimensional scaling plots for regeneration rates (T/P) between June and August 2002 at the 6 m sites; larger circles represent higher regeneration rates. (B) MDS plot of June 2002 cellular biomarkers (CDS) selected by BEST routine (Table 6.14), which included cnidarian heat shock protein (Cn Hsp 70), cnidarian small heat shock protein (Cn sHsp), metallothionein, cnidarian glutathione-S-transferase and cytochrome P450 6-class. Regeneration rate MDS superimposed by individual CDS biomarkers (C) Cn Hsp 70, (D) Cn sHsp, (E) metallothionein, (F) Cn GST and (G) CYP-6; circle size increases with increasing concentration.

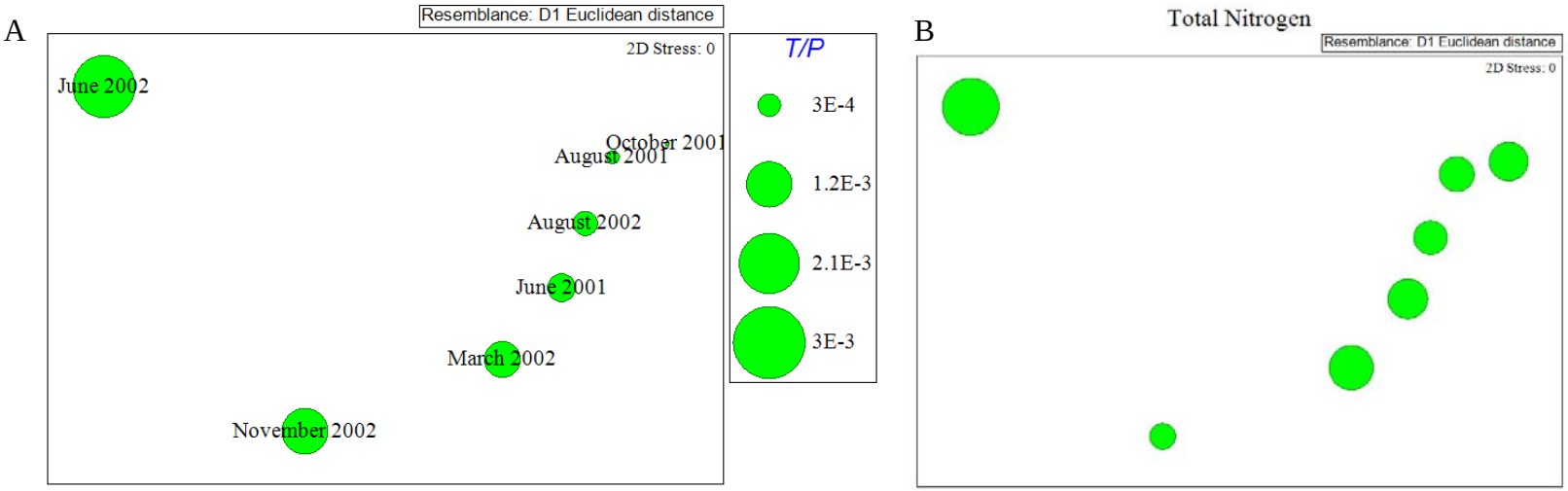


Figure 6.16. (A) Multi-dimensional scaling (MDS) and bubble plot of Key Largo 6 m regeneration rates (T/P); larger circles represent higher regeneration rates. (B) Regeneration rate MDS superimposed by total nitrogen, which was selected by the BEST routine (Table 6.13); circles increase in size with increasing concentration.

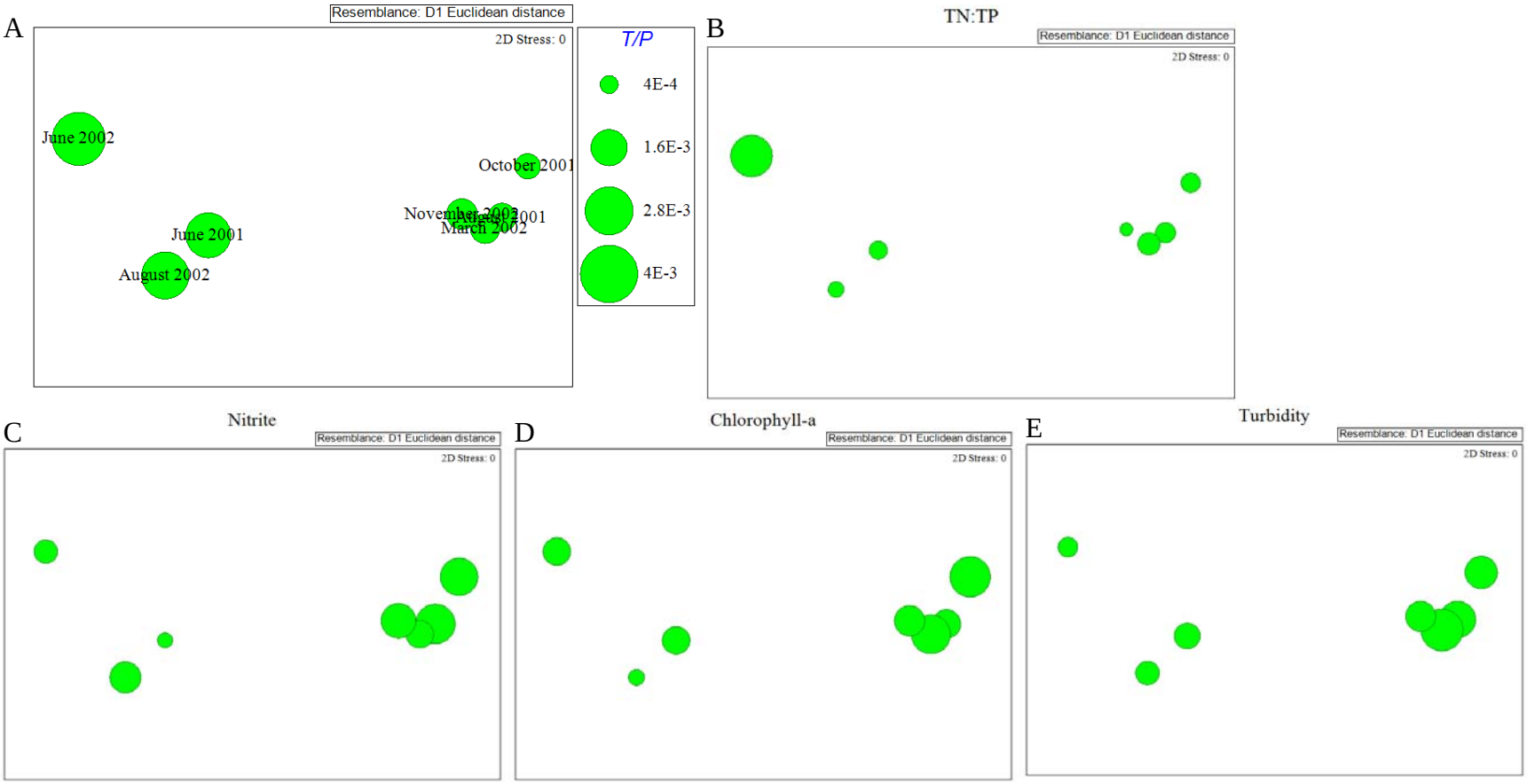


Figure 6.17. (A) Multi-dimensional scaling (MDS) and bubble plot of Key Largo 9 m regeneration rates (T/P); larger circles represent higher regeneration rates. Regeneration rate MDS superimposed by environmental variables selected by BEST routine (Table 6.13) including (B) ratio of total nitrogen to total phosphorus (TN:TP), (C) nitrite, (D) chlorophyll-a and (E) turbidity; circles increase in size with increasing concentration.

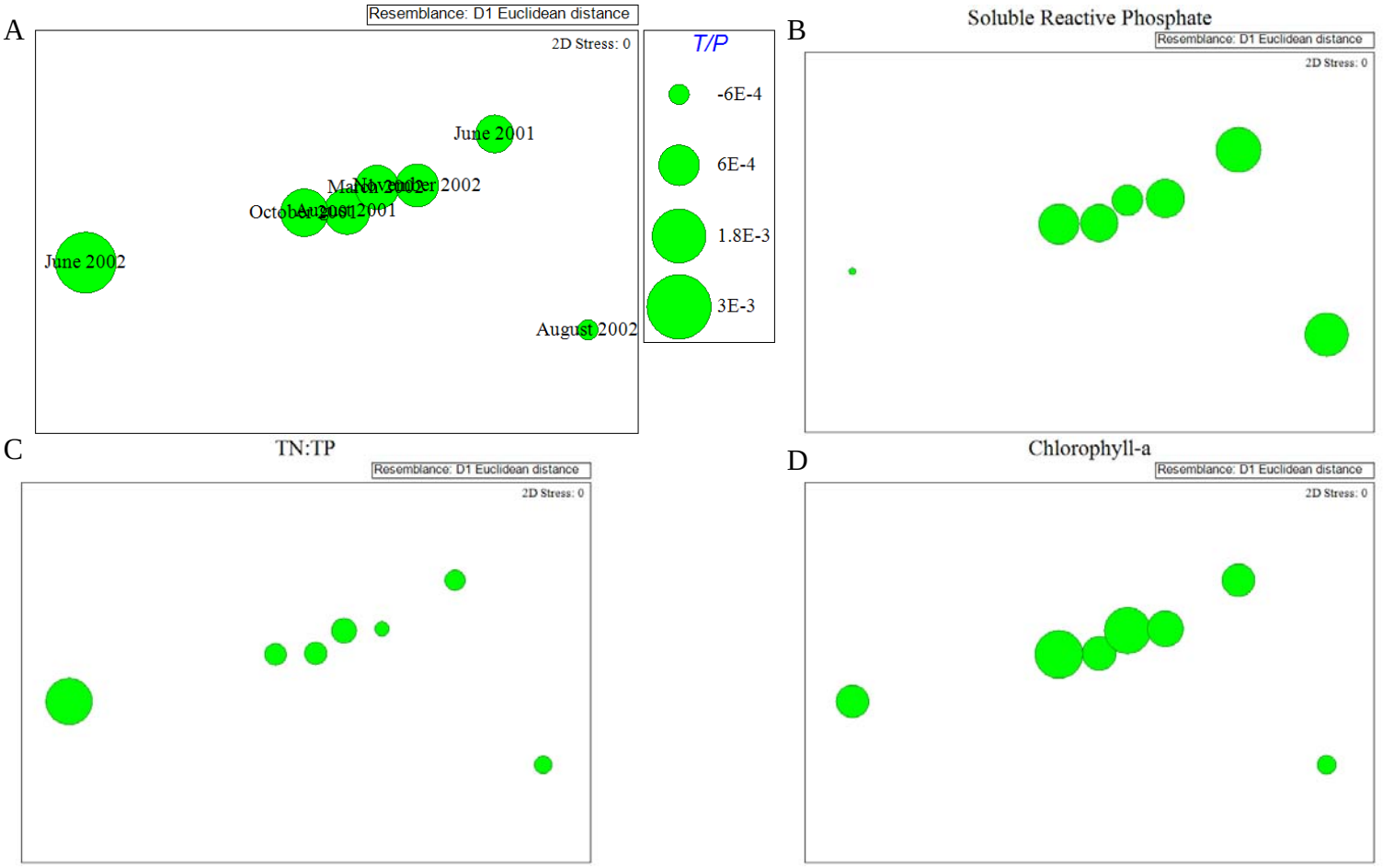
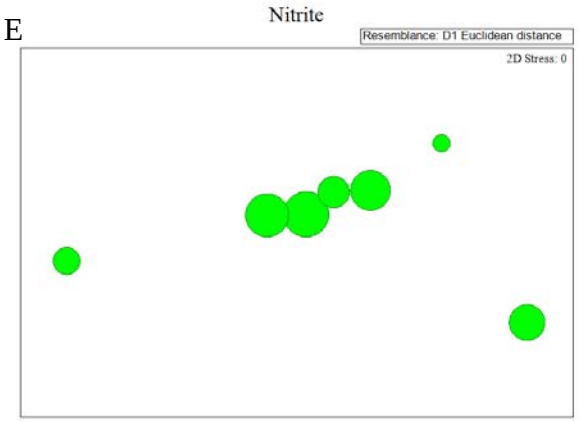


Figure 6.18. (A) Multi-dimensional scaling (MDS) and bubble plot of Key Largo 18 m regeneration rates (T/P); larger circles represent higher regeneration rates. Regeneration rate MDS superimposed by environmental variables selected by BEST routine (Table 6.13) including (B) soluble reactive phosphate, (C) ration of total nitrogen to total phosphorus (TN:TP), (D) chlorophyll-a (E) nitrite and (F) total organic carbon; circles increase in size with increasing concentration.

E



F

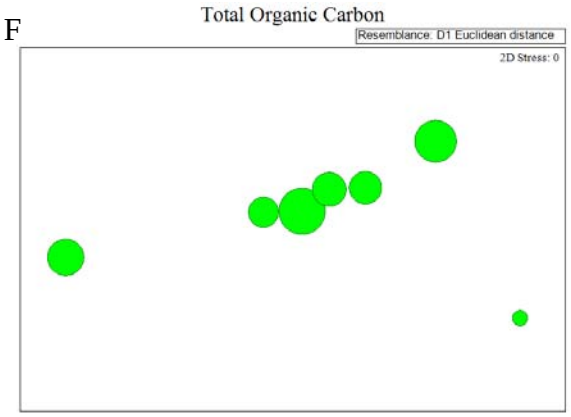


Figure 6.18 (cont.). (A) Multi-dimensional scaling (MDS) and bubble plot of Key Largo 18 m regeneration rates (T/P); larger circles represent higher regeneration rates. Regeneration rate MDS superimposed by environmental variables selected by BEST routine (Table 6.13) including (B) soluble reactive phosphate, (C) ration of total nitrogen to total phosphorus (TN:TP), (D) chlorophyll-a (E) nitrite and (F) total organic carbon; circles increase in size with increasing concentration.

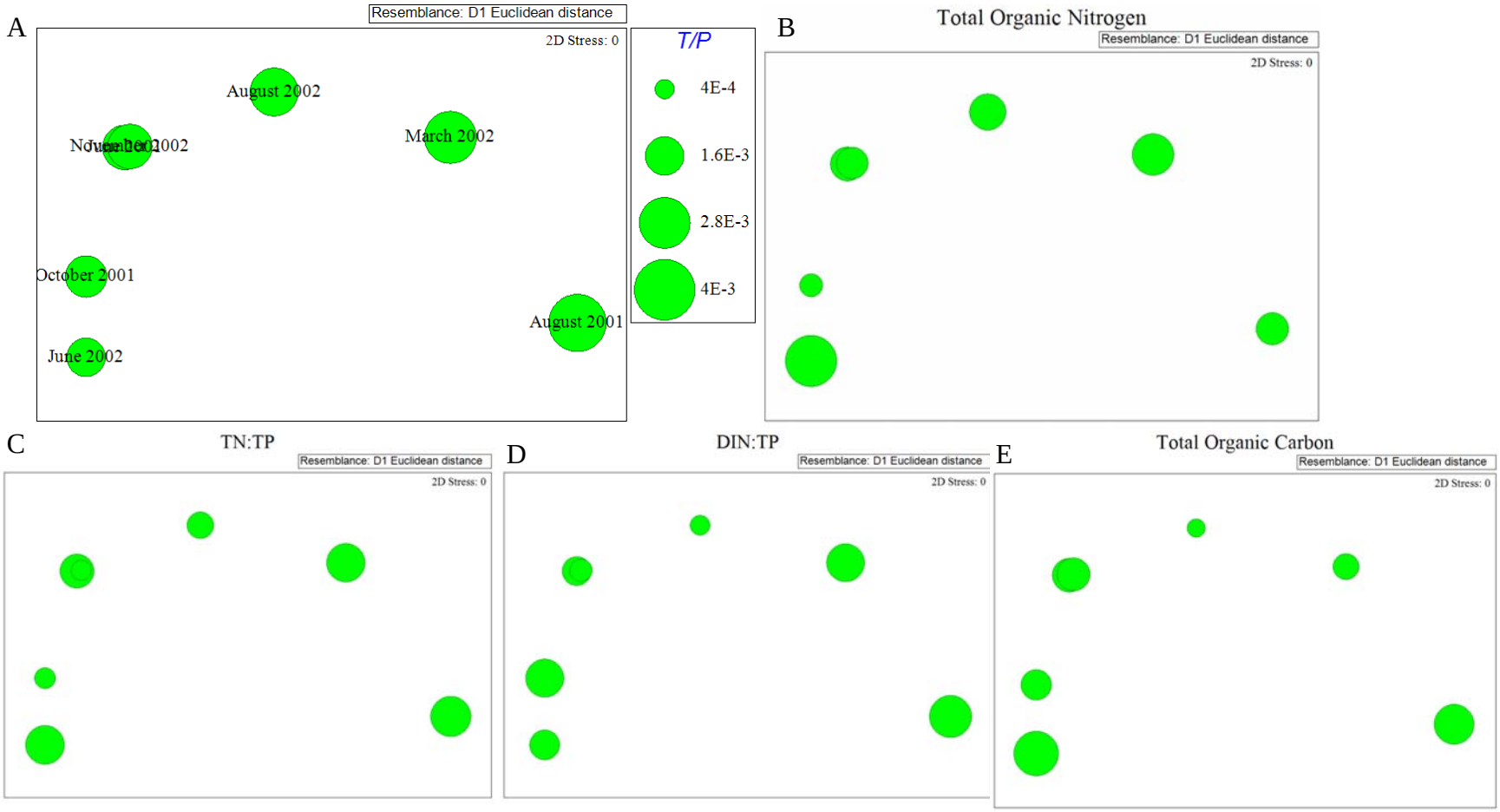


Figure 6.19 (A) Multi-dimensional scaling (MDS) and bubble plot of Algae Reef regeneration rates (T/P); larger circles represent higher regeneration rates. Regeneration rate MDS superimposed by environmental variables selected by BEST routine (Table 6.13) including (B) total organic nitrogen, (C) ratio of total nitrogen to total phosphorus (TN:TP), (D) ratio of dissolved inorganic nitrogen to total phosphorus (DIN:TP) and (E) total organic carbon; circles increase in size with increasing concentration.

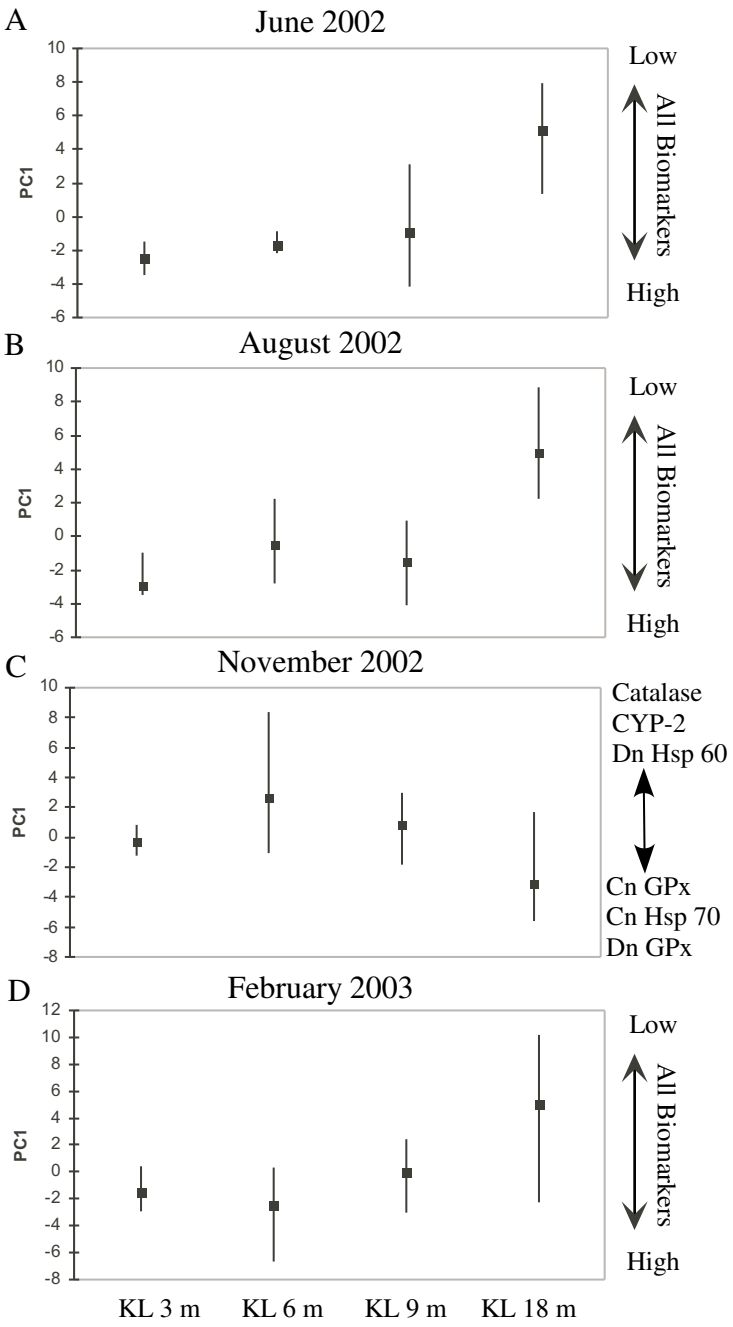


Figure 6.20. Plots of Principle Component (PC1) scores at each site for (A) June 2002, (B) August 2002, (C) November 2002 and (D) February 2003. Vertical bars show the range of values for each site; squares indicate the sample mean for each site. Eigenvalues and vectors as shown in Appendix B.

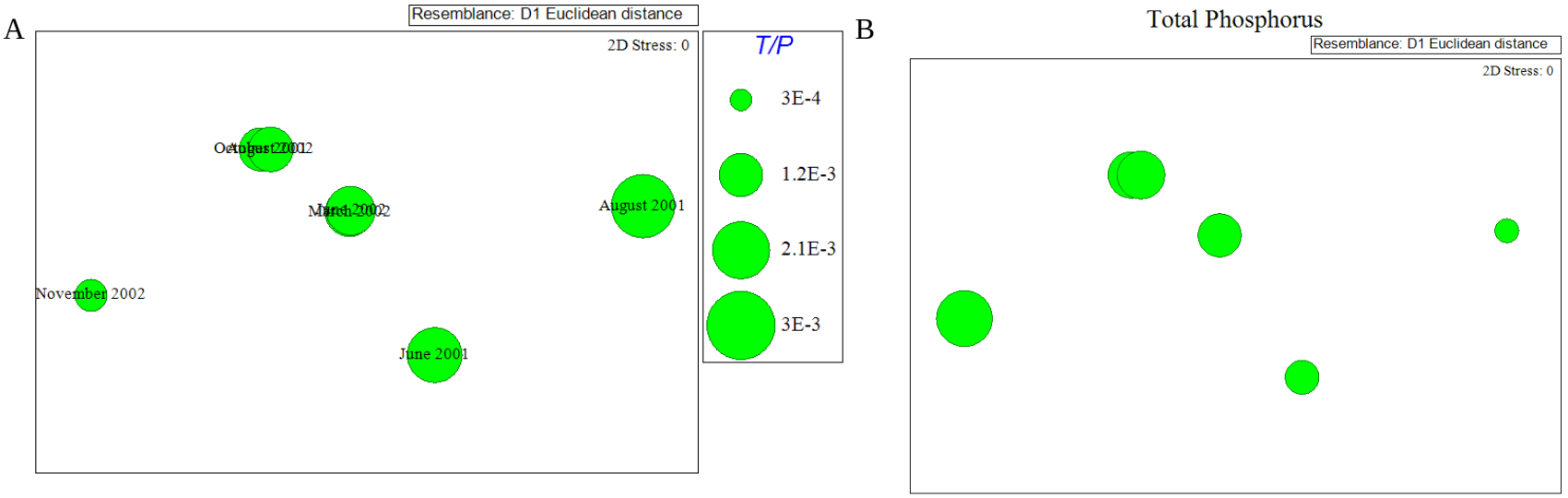


Figure 6.21. (A) Multi-dimensional scaling (MDS) and bubble plot of White Banks regeneration rates (T/P); larger circles represent higher regeneration rates. (B) Regeneration rate MDS superimposed by total nitrogen, which was selected by BEST routine (Table 6.13); circles increase in size with increasing concentration.

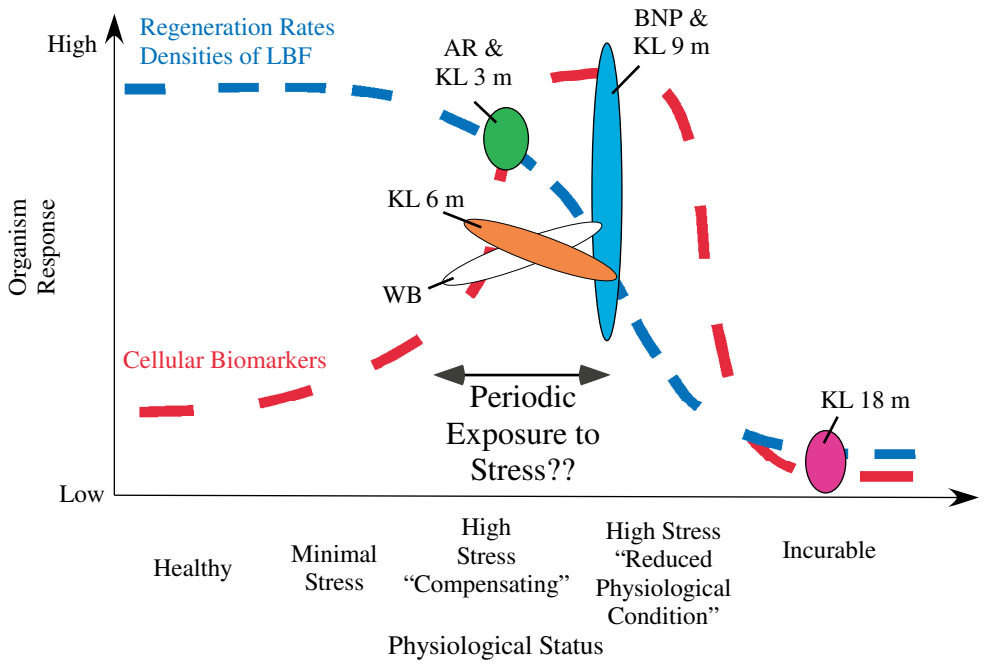


Figure 6.22. Physiological status of corals at each study site based on the relationship between regeneration rates, a surrogate indicator and cellular diagnostic markers (modified from Allen & Moore 2004). Regeneration rates and densities of symbiont-bearing foraminifera (LBF) are represented by the dashed blue line and cellular biomarker levels are represented by the dashed red line. The position of each site is represented by where the circle intersects these two lines. Note: regeneration rates and densities of LBF follow similar trends with the exception of KL 3 m, where densities are low but regeneration rates are high, and with KL 9 m and KL 18 m where densities are high but regeneration rates are low. In these cases, density of LBF were not considered due to the caveats of this indicator with depth. Site abbreviations are the same as those used in Fig. 1.1.

7. Conclusions and Future Research

7.1. Multivariate Approach to Assessing Reef Condition

No single metric is adequate to study the complex and inherently variable effects of environmental change on marine ecosystems (Adams 2005). A mechanistic understanding of the effects of multiple stressors on reefs requires a hierarchical approach based on multiple lines of evidence (Downs 2005), which allows researchers to determine whether an organism is responding to a stressed condition and, if so whether that stress has resulted in reduced physiological function (Downs 2005, Moore et al. 2006). Lower levels of biological organization can provide information on the mechanism of decline, whereas higher levels of biological organization provide information on the effect of stress on the overall fitness and function of the organism, population, community or ecosystem.

The specific objectives of my study were to 1) evaluate the ability of individual indicators to distinguish differences among sites, times and stressors; 2) assess reef condition using a hierarchal, multi-scale approach including selected environmental, community, population, colony and cellular parameters; and 3) diagnose the physiological state of selected reefs based on 'weight of evidence' through the integration of multiple indicators. In this chapter, I address the strengths and caveats of individual bioindicators used in this study, summarize the major conclusions of this study, and make recommendations for management of these ecosystems and future research.

7.2. Strengths and Caveats of Individual Indicators

All indicators used in this study were capable of distinguishing among study sites. Cellular biomarkers were the most sensitive to changes in environmental conditions with time. Each indicator provided a different perspective into reef condition. However, each indicator also had its limitations (Table 8.1).

Community assessments provided insight into past conditions. For example, low coral cover, small coral-colony size and low fish biomass indicated that past reef conditions were sub-optimal for reef growth and development. An advantage of using the Atlantic Gulf and Rapid Reef Assessment (AGRRA) for community assessment is that it has been widely used throughout the Caribbean, which provided valuable baseline data for comparison. Similar methods have been used Keys-wide, providing information on long-term changes in the Florida Keys. Algae Reef (AR) showed the highest similarity to Caribbean regional "best" values and Key Largo (KL) 6 m showed the highest similarity to Caribbean regional "worst" values. Alina's Reef (BNP) had community characteristics similar to both AR and regional means, but high recent mortality and high abundances of macroalgae indicate that this site has experienced decline that began relatively recently.

Changes at the community scale can occur over years to decades and often the stressor remains unknown. For example, the cause for increased macroalgae on reefs has been widely debated, with reduced herbivory (Hughes 1994, Williams & Polunin 2001), increased nutrients (Lapointe 1997, 1999) and increased available substrate due to coral mortality (Szmant 2001) among the postulated factors. Methods such as AGRRA are unsuitable for detecting interannual changes in reef communities. Long term and chronic exposure to environmental stress, including chemical pollutants or other anthropogenic factors, rarely result in rapid and catastrophic change. Instead, the effects are most likely gradual, subtle and difficult to separate from the effects of natural environmental change (Moore et al. 2004).

Foraminiferal assemblages and condition provided insight into the suitability of the environment for symbiont-bearing calcifying organisms (e.g., nutrient-depleted, minimal pollution) and the presence of photic stress (e.g., Hallock et al. 2003, 2006). An advantage of monitoring symbiont-bearing ('larger') benthic foraminifera (LBF) is that these foraminifera have short life spans (approx. 1 yr) and therefore population changes respond quickly to environmental changes in comparison to long-lived species such as corals and fish. By monitoring LBF, I was able to detect a reduction in stress, particularly at BNP, between 2001 and 2002. Monitoring of LBF also has been used

throughout the Florida Keys and in other regions providing baseline data for comparison (e.g., Hallock et al. 1986, Williams 2002; Hallock et al. 2006). Population densities of LBF were low in the vicinity of sampled corals at KL 6 m and BNP, indicating sub-optimal water quality or other environmental conditions at those sites. Intermediate population densities and bleaching in *Amphistegina gibbosa*, the dominant LBF species, at AR, KL 9 m and KL 18 m indicated that chronic photic stress and possibly other chronic stresses were effecting these sites. This indicator was unable to identify the environmental parameter(s) or aspect(s) of water quality that were impacting population densities. Previous research has shown that bleaching in *A. gibbosa* is a response to photic stress but not to temperature (Williams 2002, Hallock et al. 2006). Further investigation is needed to determine if other stressors (e.g., chemical pollutants) make these organisms more susceptible to photic stress. Another caveat of using LBF as an indicator is that they are not suitable in low-energy, near shore shallow environments such as KL 3 m. These foraminifera also prefer deeper depths, limiting comparisons among different depths. While a strong relationship was seen between densities of LBF and regeneration rates among 6 m sites, a poor relationship was found along the depth gradient. Thus, my research demonstrated that comparisons should be restricted to sites of similar depths.

Lesion regeneration provided insight into the physiological condition of the framework-producing corals, *Montastraea annularis* complex. Colony-scale studies indicated significant differences among sites in the ability of *M. annularis* complex (Ch 5) to recover from damage. Low regeneration rates, increases in mortality and high breakage indicated that physiological condition of corals along developed portions of the coastline at KL 6 m, BNP, KL 9 m and KL 18 m was compromised. Reefs along undeveloped portions of the coastline (e.g., AR and WB) and the nearshore patch reef (KL 3 m) had consistently high regeneration rates. Regeneration rates of coral lesions reflect the ability of colonies to repair from damage, providing a useful, inexpensive indicator of coral condition or environmental conditions. A caveat of this indicator is that it is not capable of separating effects of coral health versus external environmental factors (e.g., sedimentation, temperature, pollution) on lesion regeneration rates.

The cellular diagnostic system (CDS) indicated that corals from all sites deviated from a nominal cellular physiological state, with the highest stress observed in winter months and following high rainfall. Certain cellular parameters assayed indicated that corals were responding to a xenobiotic stress, connecting coral condition to local stressors (e.g., pesticides or herbicides). Most cellular parameters also respond to changes in the environment within days to weeks, although elevated levels of some proteins may persist for longer periods of time, complicating interpretations of the data. Among the 6 m sites, corals at BNP and AR tended to have higher levels of biomarkers, whereas those at WB and KL 6 m tended to have lower levels of biomarkers. Along the depth gradient, corals at KL 3 m and KL 9 m tended to have higher levels of biomarkers, whereas, those at KL 6 m and KL 18 m tended to have lower levels of biomarkers. When selecting a set of cellular parameters, an investigator must consider the questions that they are interested in addressing. For example, additional cellular parameters indicative of a xenobiotic response or cellular damage can be used to help narrow down the list of potential stressors and provide a more thorough understanding of the mechanisms of stress. A couple caveats of this indicator is that it can be cost-prohibitive, requires consumptive sampling and the relative novelty of this indicator means that information on baselines are limited. Data collected in this and previous studies (e.g., Downs et al. 2005) provide a basis for comparison where actual concentrations are known. Further development of biomarker and bioindicator baselines is needed to gain a better understanding of what is "normal" versus "stressed." These baselines can be further defined through controlled field and laboratory experiments. Use of additional cellular biomarkers indicative of specific damage, along with targeted functional studies (e.g., histology), might provide definitive evidence that corals are in a diseased or incurable state.

Environmental assessments provided the opportunity to connect responses in reef organisms to changes in the reef environment. Environmental assessments indicated that sedimentation was highest in the winter months, associated with high winds. Increased nutrients at my study sites followed heavy rainfalls, indicating that land-based stressors were reaching these reefs. Algae Reef and WB overall had higher sedimentation rates

than KL 6 m and BNP, though sediments at the latter sites tended to be finer and therefore potentially more damaging. No significant differences were observed in temperature and no bleaching was observed at my study sites during this project.

Both AGRRA and monitoring of LBF are relatively low cost and low impact indicators providing information on the suitability of the environment for reef inhabitants. Monitoring of coral-regeneration rates is another low cost indicator, which requires minimal training. Because this method requires creation of small lesions on corals, it is readily paired with cellular diagnostic sampling, which requires sampling of small amounts of coral tissue. The cellular diagnostic methods are costly and require technical training and biochemical background to process samples and interpret results.

By drawing from multiple lines of evidence at multiple scales, I was able to diagnose the physiological condition of coral colonies at these reefs (Fig. 6.22). None of our study sites was considered to be in a "healthy" state. Corals at both AR and KL 3 m were compensating to a xenobiotic stress but this did not appear to affect regeneration rates. Corals at both BNP and KL 9 m were responding to a stress that reduced regeneration rates and increased mortality. Stressors were likely recent at BNP as indicated by community assessments, specifically high recent morality and macroalgal biomass. Abnormally low cellular biomarkers concentrations, low regeneration rates and high mortality at KL 18 m indicated that corals at this site were responding to severe stress, which has left these colonies incapable of recovering from damage.

7.3. Recommendations for Management and Future Research

Reefs are both ecologically and economically important resources. Yet the inability to identify stressors has left management incapable of preventing or alleviating stressors that have resulted in drastic coral loss since the 1970s. Inclusion of these reef sites in a marine protected area (Florida Keys National Marine Sanctuary and Biscayne National Park) has not adequately prevented their deterioration (Jameson et al. 2002). One of the major objectives of my study was to test the use of an integrated Cellular Diagnostic System in the characterization of coral condition. The CDS was capable of detecting subtle stress conditions due to periodic events (e.g., following heavy rainfall)

and chronic stress conditions (e.g., sustained high biomarker levels at BNP and KL 9 m). Using CDS, I was able to determine that local stressors, specifically xenobiotics, were affecting corals at my sites. Possible mechanisms of stress included an endocrine disrupting stress in June 2002 at KL 6 m and depressed protein levels at KL 18 m either due to hypoxic conditions or exposure to contaminants. Therefore, this study was an important contribution in the process of diagnosing reef condition by providing avenues for future research to help narrow down the identification of stressors to these reefs. Further investigations in contaminant exposure and organism responses at these sites are needed to help managers identify and alleviate these stressors, which included temperature (e.g., Downs et al. 2002, Fauth et al. 2005) and pesticides (e.g., Downs et al. 2005, Downs et al. 2006). Exposure to xenobiotic stressors may make these corals more susceptible to predicted climate changes (e.g., increased temperatures, reduced pH). While all sites experienced stress, my approach distinguished between reefs that were compensating for stress (e.g., Algae Reef and Key Largo 3 m) and those that appeared beyond repair (e.g., Key Largo 18 m), as defined by Moore et al. (2006). This information also can help managers target their efforts to reefs that are capable of recovery, as recommended by Jameson et al. (2002).

Further investigation is needed to determine what factors contribute to better conditions (i.e., higher regeneration rates, higher densities of LBF) at some sites relative to others. For example, sites along a less developed portion of coast (e.g., AR and WB) were in better condition than those along developed coastlines (KL sites and BNP), indicating the importance of intact coastlines and wetlands. Currently, the Florida Department of Transportation is removing up to 106 acres of coastal wetlands to widen the 18 mile stretch of highway between the mainland and the Upper Florida Keys, potentially increasing sediment loads and reducing inputs of colored dissolved organic matter to these KL reefs in future years. I recommend continued monitoring of this area to determine if removal of mangroves and other natural vegetation affects reef condition.

Transplant experiments can help determine if organisms can acclimate to stress conditions through increased production of proteins involved in protective pathways (e.g., heat shock proteins and antioxidants) allowing them to compensate for the stress.

For example, KL 3 m had consistently high regeneration rates and biomarkers levels. Shallow nearshore patch reefs are generally exposed to a wider range of natural and anthropogenic stressors and have likely become adapted to these conditions through the consistent upregulation of protective enzymes and chaperone proteins (Moore et al. 2006). For example, echinoderms from a variable (intertidal) environmental showed a distinctly sustained expression pattern of Hsp72 compared with animals from a stable (benthic) environment, suggesting a functionally adaptive and dynamic stress response (Patruno et al. 2001).

My study also provides opportunities for preventive management by pinpointing areas of high stress where corals still appear to be physiologically healthy. High stress levels at Algae Reef and Key Largo 3 m could be an indication of early stages of physiological change so that slight increases in stress loads at these sites may result in reduced colony function (e.g., reduced regeneration rates, growth, reproduction). Contaminant exposures may be too low to cause overt effects, but over longer time periods may manifest into adverse conditions and mortality (Depledge et al. 1993). Thresholds could be tested experimentally in the lab and in the field by gradually applying an additional stressor (e.g., pesticide) to colonies and monitoring changes in cellular diagnostics and colony function (e.g., regeneration rates). Therefore, management should identify and alleviate potential stressors at these sites before they result in further degradation of the reef community.

Table 7.1. Indicators of reef condition

Indicator	Description	Spatial Scale	Time Scale	Training required for analyses	Cause of stress readily identified (Y/N)	Seasonally dependent (Y/N)	Recommended frequency of sampling	Sampling Cost (High/Low)
Atlantic and Gulf Rapid Reef Assessment (AGRRA)	Assessment of coral, fish and algal community	Community	Years – Decades	Species identification and calibration among observers	N	Macroalgal abundance can change seasonally	Once every five years	Low
Symbiont-bearing foraminifera	Density of symbiont-bearing organisms and assessment of bleaching and condition	Population	Weeks – Months	Species identification	Can identifiy if photic stress is present	Best to sample in late spring or late summer	Biannually	Cost of stereo microscope
Regeneration rates	Ability of coral to heal from damage over time	Colony	Weeks - Months	Basic photography and computer analysis	N	Short- term (54 -154 d): N Quasi-annual (319 – 376 d): Y	Biannually/ Quarterly	Cost of underwater camera and image analysis software
Cellular Diagnostic System	Monitors changes in concentrations of stress proteins	Cellular	Days - Weeks	Lab analyses and knowledge of biochemical pathways	Can distinguish between types of stress and mechanisms of stress	Y	Monthly or Quarterly	High

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Appendices

Appendix A. Parameters measured by Cellular Diagnostic System and their biological significance

Parameter	Description
Protein Metabolic Condition	
Heat-shock protein (Hsp60) and Heat-shock protein (Hsp70)	Heat shock proteins 60 and 70 are molecular chaperones. Chaperones regulate protein structure and function under normal physiological conditions as well as during and following stress by renaturing denatured proteins into active states in an ATP-dependent manner. Both Hsp60 and Hsp70 are found in all phyla of life and are essential components for correct conformation of protein structure. Heat shock proteins 60 and Hsp70 levels increase in response to stress, specifically in response to increased protein synthesis and denaturation. These two chaperones are indicators that the "house-keeping" proteins in the cell are experiencing denaturing conditions.
Ubiquitin	Ubiquitin is a 76-residue protein found in most phyla of life that is conjugated to proteins slated for degradation by the 26S proteosome. Proteins, during stress, are targeted for degradation usually because these proteins have undergone an irreversible denaturation. Increases in ubiquitin levels are an indication of increased levels of protein degradation, and hence, increased protein turnover. Consequently, to compensate for decreased functional protein levels due to stress, the cell will increase production of these same proteins. Thus measurement of levels of ubiquitin is an index of the structural integrity of the protein component of the superstructure of the cell. Increased ubiquitin levels indicates: (1) a protein denaturing stress is occurring; (2) increased expenditure of energy is required to compensate for this stress-induced protein turnover; and (3) in comparison to baseline data of this parameter for a particular species, may act as an indicator of individual fitness.

Appendix A (Continued). Parameters measured by Cellular Diagnostic System and their biological significance

Oxidative Damage and Response	
Superoxide dismutases (SODs)	Superoxide dismutases (SOD) play a large role in cellular antioxidant defenses by catalyzing a reaction of superoxide ions and two protons to form hydrogen peroxide and O_2, thereby reducing the harmful effects of oxidants. Copper/zinc SOD is an enzyme involved with antioxidant defenses localized in the cytosol of animal cells and in the cytosol and chloroplast in plants and algae. Manganese SOD is localized in the mitochondria of eukaryotic cells and is therefore a specific index that the mitochondria are experiencing an oxidative stress.
Glutathione Peroxidase (GPx)	Glutathione peroxidase is another important antioxidant enzyme with the majority of activity in the cytoplasm but also is involved in mitochondrial function. This selenoprotein catalyzes the reaction that detoxifies hydroperoxides and organic peroxides to their corresponding alcohol by oxidizing glutathione to glutathione disulphide and water.
Catalase	A heme-containing enzyme that catalyzes the breakdown of hydrogen peroxide into water and oxygen.
Metabolic Condition	
Heme Oxygenase (HO)	Heme oxygenase, also known as Hsp32, is an enzyme that catalyzes decomposition of heme to biliverdin, ferrous iron, and carbon monoxide. Biliverdin is further catalyzed to bilirubin, which is a powerful lipophic antioxidant. Heme production can increase in response to an increased demand for 1) membrane associated antioxidants and 2) the breakdown of hemin as a result of CYP P450 “suicide reactions” and the production of N-alkyl porphyrins.

Appendix A (Continued). Parameters measured by Cellular Diagnostic System and their biological significance

Metabolic Condition (cont.)	
Ferrochelatase (FC)	Ferrochetalase is an enzyme that catalyzes the final step in heme synthesis by inserting inserts ferrous iron into protoporphyrin IX to form heme. Both cellular detoxification pathways and essential cellular metabolism require heme or porphoryn-based substrates. For example, cytochrome c uses a form of heme in order to become an active electron carrier. Further, the class of monooxygenases, cytochrome P450, requires heme to function. As an organism up-regulates metabolic or xenobiotic detoxification pathways, it increases heme production; thus, ferrochetalase is up-regulated too.
Metallothionein	Metallothioneins are cysteine-rich, low-molecular-weight proteins that will bind a variety of metals depending on the class of metallothionein. Metallothionein often is used as a biomarker of heavy metal exposure because it accumulates in response to exposure to different heavy metals, such as cadmium (Tang 1999, Downs et al. 2001a, b). However, metallothionein also can hyper-accumulate in response to bacterial infection, exposure to some types of mitochondrial inhibitors (e.g., pesticides), oxidative stress, developmental changes, and growth factors. For example, metallothionein type 1 localizes to the mitochondrial inter-membrane space and can help mitigate superoxide production by controlling aspects of oxidative phosphorylation (Simpkins et al. 1994, Ye et al. 2001, Downs et al. 2006).

Appendix A (Continued). Parameters measured by Cellular Diagnostic System and their biological significance.

Metabolic Condition (cont.)	
Cnidarian small heat-shock protein (Cn sHsp)	Total small heat-shock proteins includes αβ-crystallin, Hsp22, Hsp23, Hsp26, and Hsp28. In most cases, the small heat-shock proteins are not present during optimal growing conditions and are only elicited by stress. αβ-crystallin is a small heat-shock protein found only in the cytosol of animals, where it protects the cytoskeletal elements during stress. The presence and concentration of different small heat-shock proteins helps determine the physiological status of several metabolic processes.
Chloroplast small heat-shock protein (ChlpsHsp)	The ChlpsHsp is a small heat-shock protein found only in the choloroplast in response to a stressed condition. The ChlpsHsp specifically associates with the oxygen evolving complex of photosystem II thereby protecting photosystem II activity during heat stress, ultraviolet radiation exposure, dehydration, and oxidative stress, most likely via a recycling anti-oxidant mechanism. These proteins also will upregulate in response to some herbicides (e.g., atrazine).

Appendix A (Continued). Parameters measured by Cellular Diagnostic System and their biological significance.

Xenobiotic Response and Detoxification	
Xenobiotic detoxification involves a three-phase process including multixenobiotic resistance proteins (MXR), cytochrome P450s (CYP) and glutathione-s-transferase (GST) that either prevents or reduces the adverse effects of xenobiotic exposure. Phase I of this process involves the enzymatic adduction of polar groups (e.g., hydroxyl) to the xenobiotic via cytochrome P450s. In Phase II, these new polar metabolites are conjugated with endogenous substrates by enzymes that include glutathione-s-transferase. Phase III involves the export of these water-soluble products either to the lysosomes for further metabolism, lysosome-like structures for containment or out of the cell through active diffusion transporters, such as ATP-binding cassette transporters (e.g., MXR; Borst & Elfrink 2002).	
Multiple Xenobiotic Resistance Protein (MXR)	Multixenobiotic resistance proteins (MXR), also known as P-glycoproteins, play a role in xenobiotic detoxification by actively transporting certain xenobiotics out of the cell (Bard 2000). The level of P-glycoproteins increases with a sustained exposure to certain xenobiotics (Downs et al. 2005a, 2006). If this process becomes overwhelmed or if the xenobiotic is not recognized by MXR, it can be metabolized into a hydrophilic compound that can be easily removed from the cell in Phase I or Phase II.

Appendix A (Continued). Parameters measured by Cellular Diagnostic System and their biological significance.

Xenobiotic Response and Detoxification (cont.)	
Cytochrome (CYP) P450	Cytochrome P450 oxidizes ethanol to acetaldehyde via a monooxygenase mechanism, as well as other xenobiotics. Cytochrome P450 2E has both physiologically relevant oxidative and reductive reactions and associates and catalyzes as many as 60 xenobiotic-based substrates. One of the primary reasons for using the 2E class of cytochrome P450s is that it is not induced by heat stress, but can respond to hypoxia/reperfusion events in mammals. CYP-2 and CYP-3 are involved with drug and steroid metabolism and detoxification of electrophilic carcinogens, drugs and environmental pollutants. CYP-6 has been implicated in the evolution of pesticide resistance, including DDT.
Glutathione-S-transferase (GST)	Glutathione transferases are usually associated with detoxificaiton by conjunction of genotoxic and cytotoxic xenobiotic electrophiles derived from drugs, carcinogens, and environmental pollutants. During a xenobiotic challenge, glutathione may be conjugated to a xenobiotic by glutathione S-transferase and represent a major detoxification pathway. Additionally, glutathione-S-transferase (GST) may detoxify DNA hydroperoxides, and thus may play an important role in DNA repair.

Appendix B. Eigenvalues and eigenvectors for principle components analysis on cellular biomarkers along the depth gradient for selected sampling periods.

	March 2001	June 2001	August 2001	October 2001	June 2002	August 2002	November 2002	February 2003
PC	PC1	PC1	PC1	PC1	PC1	PC1	PC1	PC1
Eigenvalue	14.6	15.9	15.3	13.8	12.5	13.6	9.9	15.5
% Variation	77.1	79.5	76.3	72.4	62.7	67.8	49.2	77.3
Variables	Eigenvectors							
Cnidarian Hsp70	-0.242	-0.226	-0.229	-0.224	-0.213	-0.248	-0.287	-0.208
Dino Hsp70	-0.149	-0.233	-0.196	-0.217	n/s	n/s	n/s	n/s
Cnidarian Hsp 60		-0.227	-0.214	-0.233	-0.266	-0.26	-0.161	-0.233
Dino Hsp60	-0.2	-0.232	-0.222	-0.203	-0.201	-0.253	0.162	-0.244
Ubiquitin	-0.247	-0.242	-0.235	-0.245	-0.199	-0.232	-0.274	-0.186
Cnidarian Cu/ZnSOD	-0.243	-0.236	-0.241	-0.252	n/s	n/s	n/s	n/s
Dino Cu/ZnSOD	-0.243	-0.238	-0.224	-0.231	-0.25	-0.203	-0.193	-0.197
Cnidarian MnSOD	-0.221	-0.202	-0.223		-0.256	-0.243	0.149	-0.242
Dino MnSOD	-0.243	-0.215	-0.24	-0.254	-0.206	0.02	-0.247	-0.245
Cnidarian GPx	-0.244	-0.234	-0.24	-0.252	-0.267	-0.188	-0.285	-0.227
Dino GPx	-0.232	-0.195	-0.234	-0.242	-0.229	-0.103	-0.292	-0.24
Catalase	n/s	n/s	n/s	n/s	-0.21	-0.252	0.184	-0.227
Heme oxygenase	-0.251	-0.218	-0.239	-0.242	n/s	n/s	n/s	n/s
Ferrochelatase	-0.229	-0.233	-0.222	-0.216	-0.259	-0.254	-0.245	-0.168
Metallothionein	-0.243	-0.223	-0.249	-0.168	-0.237	-0.244	-0.17	-0.249
Cnidarian sHsp	-0.201	-0.195	-0.206	-0.241	0.032	-0.119	-0.281	-0.238
Chloroplast sHsp	-0.245	-0.24	-0.219	-0.151	-0.208	-0.224	0.153	-0.212
MXR	-0.23	-0.189	-0.104	-0.238	-0.251	-0.256	-0.26	-0.229
Cnidarian GST	-0.234	-0.238	-0.233	-0.247	-0.147	-0.257	-0.265	-0.246
Dino GST	-0.239	-0.224	-0.247	-0.246	-0.246	-0.209	-0.27	-0.227
CYP 450-2	-0.195	-0.221	-0.215	-0.227	-0.203	-0.247	0.159	-0.209
CYP-3	n/s	n/s	n/s	n/s	-0.204	-0.253	0.166	-0.227
CYP-6	n/s	n/s	n/s	n/s	-0.264	-0.235	-0.124	-0.199

Appendix C. Eigenvalues and eigenvectors for principle components analysis on cellular biomarkers at the 6 m sites for selected sampling periods.

Sampling Period	October 2001	June 2002	August 2002	November 2002	February 2003
PC	PC1	PC1	PC1	PC1	PC1
Eigenvalue	15.1	9.3	13	9.6	10.9
% Variation	79.2	46.4	65.2	47.9	54.6
Variable	Eigenvectors				
Cnidarian Hsp70	-0.23	-0.191	-0.162	-0.172	-0.251
Dino Hsp70	-0.248	n/s	n/s	n/s	n/s
Cnidarian Hsp60	-0.242	-0.272	-0.249	-0.216	-0.149
Dn Hsp60	-0.234	0.284	-0.262	-0.134	-0.238
Ubiquitin	-0.235	-0.233	-0.198	-0.306	-0.19
Cnidarian Cu/ZnSOD	-0.245	n/s	n/s	n/s	n/s
Dn Cu/ZnSOD	-0.209	-0.246	-0.251	-0.228	-0.236
Cnidarian MnSOD	n/s	-0.251	-0.245	-0.164	-0.275
Dn MnSOD	-0.244	-0.241	-0.258	-0.302	-0.283
Cnidarian GPx	-0.231	-0.215	-0.265	-0.308	-0.208
Dino GPx	-0.217	0.077	-0.177	-0.235	-0.283
Catalase	n/s	0.178	-0.247	-0.069	-0.117
Heme oxygenase	-0.243	n/s	n/s	n/s	n/s
Ferrochelatase	-0.235	-0.003	-0.248	-0.232	-0.254
Metallothionein	-0.173	0.031	-0.241	-0.31	-0.202
Cnidarian sHsp	-0.249	-0.188	-0.109	-0.093	-0.065
Chloroplast sHsp	-0.227	0.283	-0.183	-0.153	-0.169
MXR	-0.21	-0.19	-0.213	-0.276	-0.258
Cnidarian GST	-0.238	-0.257	-0.243	-0.299	-0.216
Dino GST	-0.236	-0.158	-0.247	-0.287	-0.269
CYP450-2	-0.198	0.292	-0.163	-0.121	-0.269
CYP450-3	n/s	0.284	-0.196	-0.116	-0.277
CYP450-6	n/s	-0.281	-0.236	-0.172	-0.039

About the Author

Elizabeth M. Fisher received her Bachelor's degree in Marine Science and Biology with a minor in Chemistry from the University of Miami in 1999. While at the University of Miami, she worked with Dr. Robert Ginsburg and Dr. Philip Kramer conducting coral reef assessments in South Florida, Mexico, Jamaica and Bahamas using the Atlantic Gulf and Rapid Reef Assessment protocol.

She started her graduate work at the University of South Florida with Dr. Pamela Hallock Muller in the College of Marine Science in 2000 focusing on indicators of reef condition. While at USF she received the Sanibel-Captiva Shell Club Endowed Fellowship and a teaching assistantship in Environmental Science. She has been actively involved in education outreach and received the National Science Foundation GK-12 OCEANS Fellowship and was the National Ocean Sciences Bowl Regional Coordinator. She also is an active diver and served on the USF Diving Safety and Control Board.