THE ECOLOGICAL RELATIONSHIP BETWEEN THE TUMOR-PROMOTING DINOFLAGELLATE, *PROROCENTRUM* SPP., AND FIBROPAPILLOMATOSIS IN GREEN TURTLES (*CHELONIA MYDAS*) IN HAWAII AND FLORIDA

By

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The etiology of green turtle (*Chelonia mydas*) fibropapillomatosis (FP) is still unknown, although the cause is circumstantially linked to a virus. Many potential cofactors may play a role in the etiology of this disease. Benthic dinoflagellates, *Prorocentrum* spp., produce the toxin okadaic acid (OA). Okadaic acid has been demonstrated experimentally to induce papillomas in mice. A preliminary study in the Hawaiian Islands found a positive correlation between the distribution of these potential tumor-promoting dinoflagellates, turtle food substrates (macroalgae and seagrass) with epiphytic *Prorocentrum* spp., presumptive OA in turtle tissues, and FP prevalence.

On a more expansive geographic scale, a study comparing abundance and distribution of *Prorocentrum* spp. at two locales isolated from one another, the Pacific and the Atlantic/Gulf of Mexico region, has been completed. Nine research sites in the Hawaiian Islands and four sites around Florida have been sampled seasonally for the

presence and abundance of epiphytic, benthic *Prorocentrum* spp. Previous research has shown that these sites demonstrate a marked FP distribution, with areas having high prevalence of FP, and "control" areas with no FP. Environmental factors that influence *Prorocentrum* abundance are considered, including seasonal variation and substrate preferences. Land use/land cover (LULC) within the drainage basins of the study sites was also investigated.

Results indicate that there is a trend between *Prorocentrum* abundance and FP. However, three study sites (Mosquito Lagoon in Florida, Maui and Molokai in Hawaii) with high FP prevalence had overall low *Prorocentrum* abundance when compared to other sites, although they each had several samples with high densities of toxic *Prorocentrum* spp. Geographic location and environmental variables are important, as many dinoflagellates, including *Prorocentrum* spp., are known to vary their toxin production depending upon their distribution. There are a number of variables in toxin production, and hence the exposure of green turtles to varying concentrations of OA. Land use/land cover also appears to play a role in FP prevalence, although the relationship may not be direct. *Prorocentrum* abundance and LULC are not directly related in either Hawaii or Florida. However, LULC types can contribute nutrients and pollutants to coastal areas. Factors including flushing rates and point-source pollution, which were not included in this analysis, can have a large impact on coastal ecosystems where green turtles forage.

Confirmation of the presence of *Prorocentrum* species known to produce OA in areas of FP is important. These results clarify that in two major areas in the U.S. where FP is highly prevalent, *Prorocentrum*, a known tumor-promoter, is a common risk factor.

CHAPTER 1
INTRODUCTION AND BACKGROUND

Introduction

Fibropapillomatosis (FP) is a debilitating and life-threatening disease of the endangered green turtle (*Chelonia mydas*) and has been documented worldwide (Balazs 1991, Herbst 1994, Hirth 1997). First reported in 1938 in Key West, Florida (Smith and Coates 1938), there has been a dramatic increase of the prevalence of FP in Hawaii, Florida, and the Caribbean, with a clearly defined distribution of percent prevalence FP in green turtle populations in these areas (Landsberg et al. 1999). The tumors are often "benign," but growths can adversely affect locomotion, feeding, breathing, eyesight, body functions and overall physiological status of green turtles (Herbst 1994, Landsberg et al. 1999, Work and Balazs 1999). The etiology of FP is unknown, although there are several hypotheses linking viruses, parasites, pollutants, or combinations of these agents to the disease (Hirth 1997). Herbst et al. (1995) have shown that FP can be experimentally transferred to disease-free recipient turtles via a virus. It is also thought that there is a cofactor (Herbst 2000), possibly from the environment, that contributes to the spreading of FP in sea turtles. Potential cofactors include the biotoxins found in sea turtle habitats (Landsberg et al. 1999).

The chronic effects of biotoxins produced by microalgae on marine organisms are unknown (Steidinger et al. 1999). *Prorocentrum lima, P. concavum*, *P.* cf *hoffmannianum,* and *P. belizeanum* are benthic dinoflagellates that produce the tumor-

promoter Okadaic Acid (OA) (Murakami et al. 1982, Dickey et al. 1990, Aikman et al. 1993, Morton et al. 1998). Okadaic acid has been experimentally shown to induce skin tumors in mice (Suganuma et al. 1989, Fujiki and Suganuma 1993, Landsberg et al. 1999). Benthic *Prorocentrum* have a worldwide distribution and are epiphytic on macroalgae and seagrasses (Bomber et al. 1989, Steidinger and Tangen 1996), which are major components of green turtle diets. Green turtles may be consuming *Prorocentrum* while grazing on macroalgae and seagrass, and are potentially exposed to OA (Steidinger et al. 1999).

The goal of this study is to establish if a potential relationship exists between *Prorocentrum* and FP in the green turtle populations of Florida and Hawaii. These two locations were chosen to represent geographically isolated green turtle populations, in the Pacific and the Atlantic. This study examines possible correlations between abundance and distribution of *Prorocentrum* and FP prevalence in these two isolated green turtle populations. In addition, land use/land cover (LULC) at the study sites is compared to *Prorocentrum* abundance and FP prevalence.

Land use/land cover within a watershed has been positively correlated with water quality of rivers and coastal areas (Beaulac and Rechhow 1982, Tufford et al. 1998). Higher nutrient levels (N, P) of waters within a watershed are associated with land uses such as agriculture and urban areas, while lower nutrient levels are linked to forested or non-developed/utilized lands (Basnyat et al. 1999). With remote sensing and GIS, it was possible to quantify percent land cover types (e.g. agriculture, urban, forest) for a comparison amongst drainage basins. These results were compared to FP and

Prorocentrum abundance and potential relationships were identified. This study provides further insight into impacts of land use on coastal environments.

Background

Biology and Ecology of the Green Turtle

Green turtles (*Chelonia mydas*) are found in tropical and subtropical seas and oceans around the world, in general between 40º N and 40º S latitudes (Hirth 1997). They are listed as endangered in Florida and threatened in Hawaii under the U. S. Endangered Species Act (Balazs and Pooley 1991). Over-harvesting, habitat destruction, incidental capture and mortality by fisheries, and inadequate regulations and enforcement have historically caused serious population declines (Balazs and Pooley 1991).

Green turtles are characterized by slow growth, delayed sexual maturity, high fecundity, high predation rates on eggs and hatchlings, and a long reproductive life (Hirth 1997). There are several distinct metapopulations of green turtles, including recognized subspecies, which can be identified by their nesting beaches (Hirth 1997).

Green turtles occur in the coastal waters or on the nesting beaches of at least 139 countries and territories (Hirth 1997). In the continental USA the main nesting grounds are in Florida, with the northernmost nesting record on the Atlantic coast in North Carolina (Peterson et al. 1985). In Hawaii, the majority of green turtles nest in the Northwest Hawaiian Islands (Balazs 1980).

It is believed that female green turtles nest on the same beach where they hatched. Re-nestings during a nesting season usually occur on the same sector of beach, and most females tend to return to the same nesting area on their reproductive migrations in their

lifetime (Balazs 1980, Hirth 1997). Some of these migrations between feeding grounds and nesting areas can encompass thousands of km (Hirth 1997). Green turtles show high site fidelity for both their feeding and nesting grounds (Balazs 1980, Balazs et al. 1994a, 1994b, 1998, 2000a, Russell and Balazs 1994, Hirth 1997).

Feeding ecology of green turtles varies by growth stages, with hatchlings (believed to be up to five years old) thought to be omnivorous and/or carnivorous, and living in pelagic rafts consisting of communities of Sargassum, fish, and invertebrates (Hirth et al. 1997). Juvenile, sub-adult, and adult green turtles live in near-shore habitat and are herbivorous, primarily feeding on macroalgae and seagrasses. As green turtles grow, some appear to go through a series of "developmental feeding habitats" (Carr and Caldwell 1956, Ehrhart and Witherington 1992, Hirth 1997). The majority of a green turtle's lifetime is spent in these coastal feeding grounds, with a much smaller proportion of time spent on nesting migrations and in inter-nesting habitat (Balazs 1980). These life cycle characteristics are important for identifying threats to green turtle populations.

The main predators of large green turtles in coastal waters are sharks (Hendrickson 1958, Balazs 1980, Witzell 1987). Predation of nesting turtles by jaguars in Suriname (Autar 1994) has also been recorded. Predators of turtle eggs include multiple species of crabs and mammals, and hatchlings are preyed upon by birds, fish, mammals, and crabs (Hirth 1997). Green turtles and their eggs also have a long history of human exploitation (Parsons 1962, Balazs 1980, Hirth 1997).

Competitors of green turtles in their feeding grounds are other herbivores, including dugongs, fish spp., sea urchins, amphipods, and gastropods (Hirth 1997). Humans are the largest competitors for nesting beach space.

The list of parasites, commensal symbionts, and diseases found in green turtles worldwide is extensive (Hirth 1997). However, none have been of the extent, magnitude, or have caused as much concern as the disease fibropapillomatosis in green turtle populations worldwide.

Disease in Wildlife

Ecologists define ecological communities in terms of current species distributions and interactions, and try to integrate the roles of both biotic and abiotic factors influencing species distributions (Andersen 1995). Within community ecology is the importance of disease and its relationship to the community structure, its cycle within the community, and the populations that are directly impacted by it.

One definition of disease is "any impairment that interferes with or modifies the performance of normal functions, including responses to environmental factors such as nutrition, toxicants, and climate; infectious agents; inherent or congenital defects, or combinations of these factors" (Wobeser 1994, p. 4). Disease in wildlife is often of multiple causal factors, and the effects of many diseases on wildlife are poorly understood (Wobeser 1994). Any single factor may be a necessary component but may not be sufficient, in and of itself, to produce disease without the presence of cofactors. Cofactors, in addition to infectious agents, could be described as environmental and host risk factors contributing to the occurrence of a disease, including inter-specific and intra-specific competition, human harassment, poor habitat quality, pollution, and toxic chemicals (naturally occurring and unnatural) (Wobeser 1994).

A major part of defining any disease is identification of its cause. Associations should be identified, particularly where they represent a causal relationship (Wobeser

1994). This requires distinguishing between situations where factor X and Y simultaneously occur together in time or place and those in which they occur together because X caused Y (Wobeser 1994). One example of a factor X could be a virus, and Y an infectious disease.

Viruses are infectious obligatory intracellular parasites, and contain either RNA or DNA in a nucleic acid core surrounded by a protein coat. Viruses are often considered one of the most important disease agents in wildlife populations. In wildlife, transmission of viruses between individuals within a population includes vectors such as: blood-sucking arthropods, saliva exchange (bites), direct contact, or contaminated food and water. An example of a common viral disease in wildlife is rabies. Papillomaviruses have long been known as the cause of papillomas (commonly known as warts), fibropapillomas, and fibromas (Sundberg 1991). Many species of mammals and other vertebrates are infected with this type of virus (Sundberg 1991). Poxviruses are associated with the development of tumors, such as the avian poxvirus found in mourning doves in the U.S. (Locke 1961). Herpesviruses also are associated with proliferative diseases. Tumors have been linked to herpesvirus infections in other vertebrates, including fish (Anders and Yoshimizu 1994), frogs (McKinnell 1984), lizards (Raynaud and Adrian 1976), and birds (Locke 1961, Herbst 1994). Chemical carcinogens induce papillomas and squamous cell carcinomas independently or in conjunction with papillomaviruses (Sundberg 1991). For example, environmental pollutants have been implicated in papillomas in fish and eels (Sundberg 1991).

To verify that an agent is the cause of an infectious disease, Koch's postulate provides a set of four criteria (Luria et al. 1978). These four criteria are as follows: 1) the

pathogen may be isolated from the diseased host; 2) the pathogen may be grown in pure culture or identified under a microscope; 3) the pathogen causes the disease when inoculated into a laboratory animal; 4) the agent can be re-isolated from artificially-infected hosts (Luria et al. 1978). Past research on the disease fibropapillomatosis in green turtles has been unable to fulfill all four of these criteria (Landsberg et al. 1999).

Fibropapillomatosis

Fibropapillomatosis (FP, Fig. 1-1) in marine turtles has been documented since the 1930's (Lucké 1938, Smith and Coates 1938), but the exact route of transmission in the wild is still unknown. In the past decade, FP has been documented worldwide, with a prevalence ranging from 0% to 92% in some areas. There is a difference in FP between the Pacific and the Atlantic, with a high incidence of tumors in oral cavities of turtles in Hawaii, which has not been observed in Florida or elsewhere (Balazs et al. 1997). This could imply differences in the etiology of the disease in different locations. Turtles with FP have been found to have a significantly slower growth rate than turtles without FP in Hawaii (Balazs et al. 1998). Also, tumor severity is a factor, with a significant difference in growth rates and physiological status among turtles with different tumor scores (Balazs et al. 1998, Work and Balazs 1999). Scientists are trying to elucidate the complex etiology of FP.

An infectious virus has been linked to FP (Jacobson et al. 1991, Herbst 1994, Herbst et al. 1994, Herbst et al. 1995), but the cofactors in the etiology of FP have yet to be found. A herpesvirus associated with lung-eye-trachea disease in green turtles is still infectious after 120 hours exposure to seawater at 23 ºC (although not at 30 ºC) under laboratory conditions (Curry et al. 1999). The herpesvirus linked with FP may also be

stable in the environment for a period of time (Curry et al. 1999), increasing exposure risk to green turtles.

A recent study has demonstrated the presence of the herpesvirus associated with green turtle FP within snout, gill, and liver tissue of the cleaning wrasse, *Thalassoma duperrey*, in the Hawaiian Islands (Lu et al. 2000a). *T. dupperay* is regularly observed "cleaning" green turtles in Hawaii. Lu et al. (2000a) suggest the possibility of *T. duperrey* being the mechanical vector in the spread of FP between green turtles via transmission of the herpesvirus from one turtle to another while "cleaning." Lackovich et al. (1999) found the green turtle herpesvirus only in tumor tissue and skin tissue within two centimeters of a fibropapilloma, and found no herpesvirus present in any other tissue of a turtle infected with FP. However, two other studies did find the green turtle herpesvirus in the majority of tissues of turtles infected with FP (using a different methodology than the previous study), although the herpesvirus was in much lower quantity in non-tumored tissue than in tumored tissue (Lu et al. 2000b, Quackenbush et al. 2001). Zamzow (1999) observed an apparent preference of *T. duperrey* for feeding on non-tumored tissue of green turtles in Kaneohe Bay (Oahu), a site with >50% FP prevalence (Balazs 1991). The hypothesis of the cleaning fish, *T. duperrey* as the mechanical vector of FP is not conclusive, and further research is required to isolate the exact transmission route of FP.

Studies attempting to connect FP to parasites and pollution have been inconclusive (Dailey and Morris 1995). Many scientists agree that the unknown cofactor in the spread of FP is found within the green turtle's habitat.

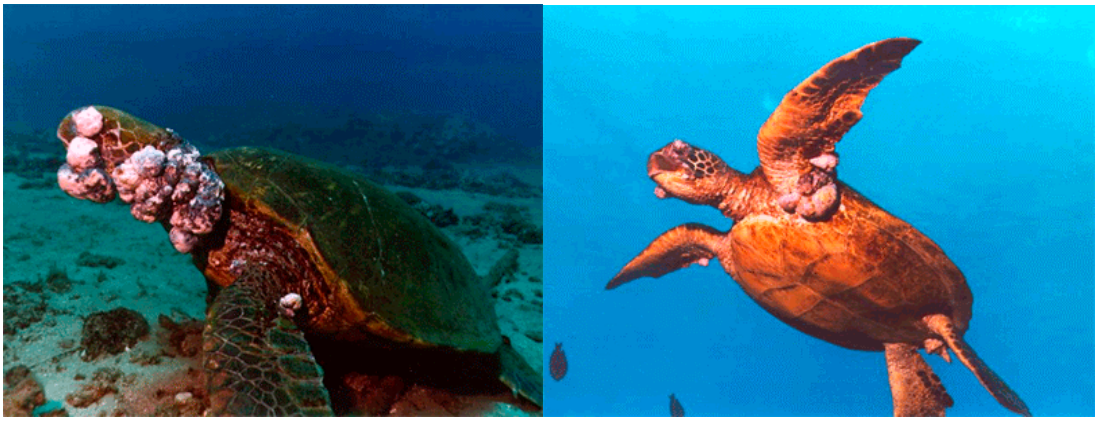


Figure 1-1. Two Hawaiian green turtles with FP at Honokowai, Maui. Photos courtesy of Ursula Keuper-Bennett and Peter Bennett.

Figure 1-2. Two Hawaiian green turtles feeding on macroalgae (left – *Acanthophora spicifera*, right- *Hypnea musciformes*) at Honokowai, Maui. Photos courtesy of Ursula Keuper-Bennett and Peter Bennett.

Seagrass ecosystems, found in relatively shallow waters worldwide, are among the most productive in the world (McRoy and McMillan 1977). Under natural conditions, the high population densities that sea turtles can attain make them major predators and grazers in their ecosystems, which include the seagrass systems in Florida (Bjorndal 1997). Green turtles show high fidelity toward their feeding sites, where they graze on specific macroalgal and seagrass substrates (Balazs et al. 1994a, 1994b, 1998, 2000a, Russell and Balazs 1994, Fig. 1-2). The community interactions within this

feeding habitat of the green turtle are important to consider when developing conservation strategies.

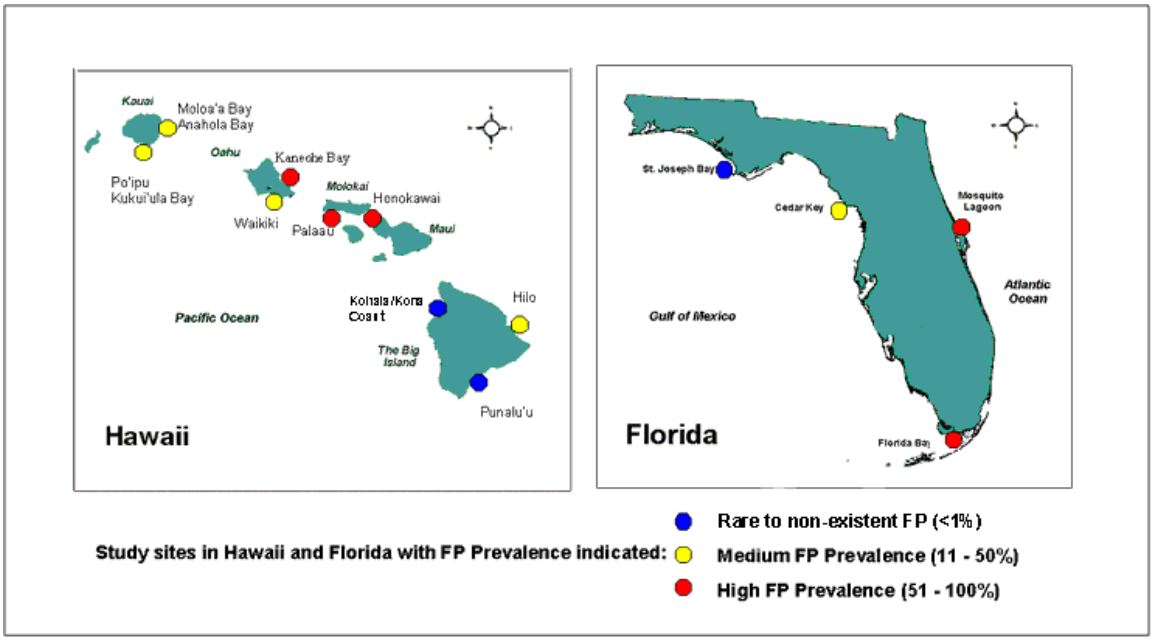


Figure 1-3. FP Distribution in Florida (Fick et al. 2000) and Hawaii (Balazs 1991, unpublished data, Balazs et al. 1994a, 1994b, 2000a, and Landsberg et al. 1999).

In Florida, the distribution of FP has been recorded from information based on stranded turtles. FP mostly occurs in central and south Florida, below 29'00 latitude (south of the Cape Canaveral National Seashore area on the east coast) (Fick et al. 2000), although recently the first two cases of FP were recorded in the panhandle. Two out of 400 cold-stunned green turtles exhibited low-severity tumors in St. Joseph Bay in January 2001 (A. Foley, Florida Marine Research Institute, pers. comm., 2001). In general, the distribution of seagrass and macroalgal species also follows a boundary defined by Cape Canaveral, with temperate species to the north and tropical species to the south (Stephenson and Stephenson 1952, Briggs 1974, Searles 1984, Landsberg et al. 1999). Within the Hawaiian Islands, there is also a distinct pattern of FP distribution in green

turtles (Balazs 1991, Fig. 1-3). This distribution pattern of FP suggests there is an environmental cofactor involved in the etiology of the disease. If the disease was spread by co-occurrence of turtles at high densities, why is it not found in some locations where high densities occur, e.g. Kona/Kohala Coast in Hawaii or in St. Joseph Bay, Florida?

Harmful Algal Blooms

Harmful Algal Blooms (HABs) are the increase of harmful microalgae that harm natural resources or humans and occur worldwide (Steidinger et al. 1999). "The term 'bloom' indicates an increase in abundance above normal background numbers of the species in a specific geographic area" (Steidinger et al. 1999, p. 1).

One of the possible causal factors leading to HABs is nutrient loading (Steidinger et al. 1999). One argument is that coastal ecosystems have been altered due to the increase in anthropomorphic activity, resulting in elevated nutrient levels (eutrophication) in coastal waters. These activities include nutrient and sediment runoff from agriculture, industrial outflow, sewage treatment facilities, wastewater from urban areas, and alteration of estuarine circulation (Steidinger et al. 1999). For example, red tides have coincided with population growth in Tolo Harbor, Hong Kong (Lam and Ho 1989, Steidinger et al. 1999). Eutrophication can increase duration of HABs beyond normal compared to areas of low nutrients, increase substrate for epiphytic toxic species, and increase phytoplankton prey and organic matter for toxic heterotrophic species. Biological indicators of the presence of HABs include fish kills, fish health problems, wildlife kills, human health problems, alteration of the food chain, and reduction in shellfish growth (Steidinger et al. 1999). Eutrophication may increase frequency and intensity of HABs that have historically occurred in the area, but the relationship is

ecologically complex. HABs research have found no direct link with nutrient loading and blooms, and have shown that many blooms begin in nutrient-poor areas (Steidinger et al. 1999).

HABs are naturally occurring, so it can be difficult to isolate the factors leading to the blooms. There are many microalgae species that comprise HABs, including a range of organisms that produce cancerous compounds (Burkholder 1968, Steidinger et al. 1999).

It is unknown what effect HAB-produced presumptive cancer-inducing compounds have on marine systems (Landsberg et al. 1999). There is little information concerning chronic, lethal, or sub-lethal effects on animals caused by bioaccumulated or biomagnified algal toxins, nor do we know whether such effects render animals susceptible to disease. Toxic microalgae produce some of the most potent toxins known (Landsberg et al. 1999).

Prorocentrum

Prorocentrum lima, P. concavum, *P.* cf *hoffmannianum*, and *P. belizeanum* produce the tumor-promoter Okadaic Acid (OA) (Murakami et al. 1982, Dickey et al. 1990, Aikman et al. 1993, Morton et al. 1998, Fig. 1-4), which significantly affects cellular processes (Bialojan and Takai 1988, Fujiki et al. 1989, Haystead et al. 1989, Herschman et al. 1989, Yamashita et al. 1990, Sakai and Fujiki 1991, Fujiki and Suganuma 1993, Schonthal and Feramisco 1993, Landsberg et al. 1999). OA has been experimentally shown to induce skin papillomas and carcinomas in mice (Suganuma et al. 1989, Fujiki and Suganuma 1993, Landsberg, unpublished). The potential tumor-

promoting effects of OA on aquatic animals are unknown (Steidinger et al. 1999), as are the risks of disease susceptibility due to OA exposure through consumption of benthic dinoflagellates (Landsberg et al. 1999). "The potential role of protein phosphatase inhibitors such as OA in tumor development in marine turtles should be further explored, either for direct tumorigenic effects, as cofactors, or as sublethal immunosuppressive factors that render animals susceptible to oncogenic viruses or other pathogens" (Landsberg et al. 1999, p. 207). One working hypothesis is that "the etiology of FP involves a tumor promoter such as OA that operates in conjunction with a tumor initiator such as a herpesvirus or retrovirus" (Landsberg et al. 1999, p. 206, Fig. 1-5).

Preliminary studies in Hawaii have documented the distribution of OA-producing *Prorocentrum* species and found a relationship with the distribution of FP in Hawaiian green turtles (Landsberg et al. 1999). High FP prevalence areas (up to 90% of the local green turtle population) in Hawaii also supported high densities of *Prorocentrum,* and control areas with 0% FP prevalence had corresponding low densities of toxic *Prorocentrum* spp. (Landsberg et al. 1999).

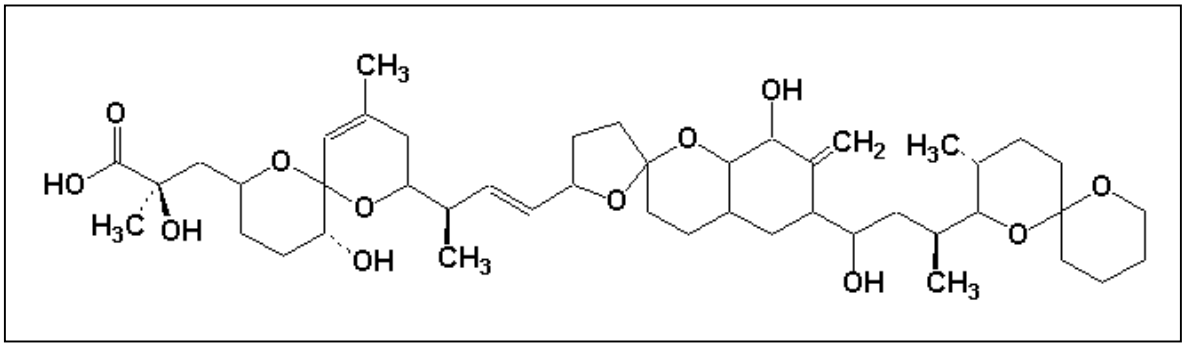


Figure 1-4. The chemical structure of Okadaic Acid (from http://vm.cfsan.fda.gov/).

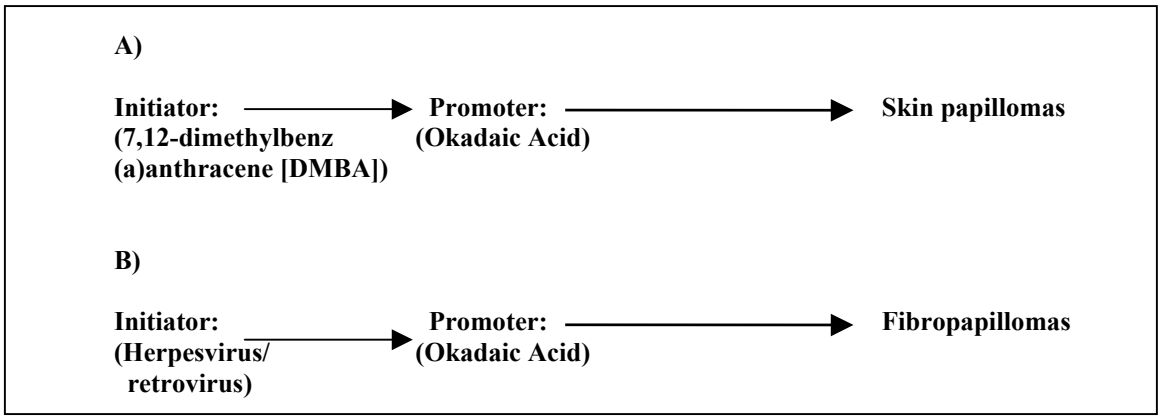


Figure 1-5. Proposed hypothesis of OA involvement in FP. A) Two-stage carcinogenesis model for development of skin papillomas in mice (Fujiki and Suganuma 1993; Landsberg, unpublished) B) Hypothetical model for development of fibropapillomas in sea turtles (Landsberg, unpublished).

Seasonality

Seasonality influences *Prorocentrum* growth, reproduction, and abundance. *P. lima* annually varies in population levels around the Florida Keys (Bomber et al. 1985). Highest abundance of *P. lima* occurs during the cool-water season when water temperatures are less than 26° C (October through May). *P. hoffmannianum* can grow from 21° C to 36° C, with the optimal growth occurring at 27° C and has a salinity preference of 34‰ for maximum growth rate (Morton et al. 1994). Seasonal changes in water temperature and salinity can indirectly influence *Prorocentrum* populations by impacting growth of the macroalgal species that act as substrate for epiphytic *Prorocentrum* species, varying host plant availability.

Substrate preference

Epiphytic *Prorocentrum* spp. have nutritional requirements satisfied by macroalgal substrate. Macroalgae species that maintain the highest densities of *Prorocentrum* cells are the preferred hosts (Bomber et al. 1989). One potential regulating growth factor of *P. lima* is macroalgal surface area (cm^2/g) (Bomber et al. 1985). High surface-area plants provide more space for cell division and attachment (Bomber et al. 1985). A negative correlation between % ash content in substrate and *P. lima* density indicates that plants with low ash content are less rigid, allowing more movement in the water column and thereby increasing exposure to nutrients and suspended cells (Bomber et al. 1985). Substrate preference in *Prorocentrum* combined with diet preference in green turtles can influence turtles potential exposure to OA.

Turtle Exposure to Okadaic Acid

Green turtles feed on multiple species of macroalgae and/or seagrasses. Often, as green turtles do demonstrate a level of site fidelity for grazing areas (Balazs et al. 1994a, 1994b, 1998, 2000a, Russell and Balazs 1994), these preferences may be limited by forage availability. For example, in this study, one species of macroalgae in Hawaii was collected at all of the sample sites for a cross-site comparison. However, even though the selected species (*Acanthophora spicifera*) is considered an ubiquitous species, it is very difficult to find on the Big Island. Due to geographic distribution of macroalgae and seagrass species, turtles will have very different options while foraging, depending on geographic location. Green turtles are known to demonstrate forage preferences amongst locations (Balazs 1980, Balazs et al. 1994a, 1994b, Redfoot 1997, Rice et al. 1998).

When *Prorocentrum* prefers substrates that turtles favor as food, the potential for green turtles to be exposed to OA is high, particularly in locations where *Prorocentrum* is

abundant. In areas where *Prorocentrum* abundance is low, and in particular if *Prorocentrum* do not prefer the substrates that turtles consume, risk of turtle exposure to OA is reduced. The risk to turtles depends on species composition of macroalgae and seagrasses consumed, and the abundance and toxicity of *Prorocentrum* on these substrates. There are additional factors that influence *Prorocentrum* abundance and substrate composition (substrates for *Prorocentrum* and forage sources for turtles) at a particular geographic location, including nutrient levels.

Land Use/ Land Cover (LULC)

Nutritional input from coastal sources is an important factor that could contribute to the abundance of *Prorocentrum*. Increased concentrations of dissolved inorganic nitrogen and soluble reactive phosphorus often exceed nutrient thresholds in areas of coastal input and can cause macroalgal growth to replace coral reefs (Lapointe 1997). Increased nitrogen supply can increase macroalgal nitrogen uptake rates, nitrogen content in tissues, photosynthesis levels, and frond growth, resulting in higher macroalgal biomass levels and phytoplankton division rates (Valiela et. al. 1997). This can provide additional substrate for *Prorocentrum*, by supporting higher division rates and densities. Elevated nitrogen levels from anthropogenic nutrient loading has not been considered for its potential effect on toxin production in natural *Prorocentrum* communities, although ambient concentrations of phosphorus has been linked to OA production in *Prorocentrum* (Morlaix and Lassus 1992, Tomas and Baden 1993, Sohet et al. 1995, Landsberg et al. 1999).

LULC has repeatedly been linked to water quality in coastal environments. Agriculture and urban lands in riparian zones contribute nutrients and decrease water

quality, while native grasslands, wetlands, and forests absorb nutrients and increase water quality (Beaulac and Reckhow 1982). FP is more prevalent in near-shore habitats with low flushing rates and drainage basins with agricultural, industrial, and urban lands (Herbst 1994). Environmental degradation may play a role in disease expression (Herbst 1994). Comparing LULC with FP prevalence and *Prorocentrum* abundance at the study sites may highlight a potential contributing factor. The link between LULC and FP may be direct or indirect, and LULC may influence *Prorocentrum* distribution and abundance. For example, if the preferred macroalgal substrate of *Prorocentrum* flourishes in areas of nutrient loading, the *Prorocentrum* population could by affected, as well. Areas of high substrate abundance could support larger densities of *Prorocentrum*. Mapping and quantifying LULC within the drainage basin of each study site, and comparing the results to FP and *Prorocentrum* abundance can identify potential relationships.

Goals of this Study

Hypothesis. In this study, two hypotheses have been tested: 1) the toxic dinoflagellates of the genus *Prorocentrum* are a potential cofactor in the spread of FP in green turtle populations of Florida and Hawaii; and 2) higher proportions of agriculture and/or urban land in drainage basins will result in higher densities of *Prorocentrum*, and therefore FP. To accomplish this, the following main objectives were addressed:

1. Distribution and abundance of *Prorocentrum* around the Hawaiian Islands and Florida were determined. The study sites sampled for *Prorocentrum* were chosen based on distribution of green turtle populations and FP prevalence.

2. Substrate preferences of *Prorocentrum* were established. There may be high concentrations of *Prorocentrum* in the same areas as high prevalence of FP, but are they present on the macroalgae species which green turtles are known to feed on?

3. Potential relationships between LULC, FP, and *Prorocentrum* were identified.

Predictions. In the context of this study, if the hypotheses are valid, the following outcomes are predicted:

1. At the study sites where there is high FP prevalence, there will also be high densities of *Prorocentrum* present on the macroalgae species that are eaten by green turtles.

2. At the control sites with rare (<1%) FP prevalence, there will be significantly lower densities of *Prorocentrum*.

3. A relationship exists between LULC, FP, and *Prorocentrum*.

Assumptions. It will be assumed that all *Prorocentrum* observed and accounted for are producing equal amounts of the toxin Okadaic Acid (OA). Morton et al. (1994) did find a variation in OA production in *P. hoffmanianum* depending on environmental factors, although presence alone of *Prorocentrum* in an area indicates that OA production could be prevalent. It will also be assumed that there is an epiphytic preference of *Prorocentrum* for substrate, which will be addressed directly by taking five different macroalgal species (when available) during each sampling versus only one. Also, the macroalgae substrate that is targeted for sampling for the presence of the epiphytic *Prorocentrum* are species determined from previous dietary studies of green turtles, the assumption being that all green turtles share the same preferences for certain macroalgal species, depending on availability, at the study sites. Tumor prevalence in the study areas will be based on publications of observations, examinations of turtles captured for tagging and health screening, and stranding data. The final assumption is that green turtles do exhibit a level of site fidelity while feeding, as reported in past research (Balazs et al. 1994a, 1994b, 1998, 2000a, Russell and Balazs 1994).

Inference. The strength and type of inference about the hypotheses that can be achieved with this design is strong, but not causal. The alternative hypotheses including parasitism, pollution, cleaner fish, and the horizontal spreading of an infectious agent (e.g. virus) acting alone in the etiology of FP have been studied and have yet to be found conclusive.

Therefore, this study provides meaningful conclusions by supplying evidence that the geographic distribution of FP in green turtles may be linked to the distribution of harmful microalgae. This is an important step in the identification of cofactors of the disease, fibropapillomatosis, in the endangered green turtle. The results of this study indicate that we may be closer to identifying the cause of this epizootic disease, and it supports the necessity of further research in these directions.

CHAPTER 2
PROCENTRUM IN FLORIDA AND HAWAII

Introduction and Literature Review

Prorocentrum spp. have been studied for the past 150 years, but have only recently been identified as a prominent component in the benthic community. Toxin production is still being studied amongst *Prorocentrum* spp., and there are many newly discovered species that have not been tested for toxicity. Past research on *Prorocentrum* demonstrates how much has been discovered, and how much is still unknown.

The dinoflagellate genus, *Prorocentrum,* contains over 70 species, both benthic and pelagic, with new species being described annually. Several species of *Prorocentrum* have been found to produce toxins, and in some instances, they are linked to diarrhetic shellfish poisoning (DSP) and Ciguatera fish poisoning. Benthic *Prorocentrum* spp. have a worldwide distribution, occasionally form red tides, and are associated with sediments, detritus, sand, coral rubble, macroalgal surfaces, and drift algae (Faust et al. 1999). Several benthic *Prorocentrum* species, including *P. lima, P. concavum*, *P. hoffmannianum*, and *P. belizeanum* produce the toxin OA (Murakami et al. 1982, Dickey et al. 1990, Aikman et al. 1993, Morton et al. 1998). OA has experimentally induced skin papillomas and carcinomas in mice (Suganuma et al. 1989, Fujiki and Suganuma 1993, Landsberg et al. 1999), is linked to ciguatera and DSP (Faust et al. 1999), and is important in public health where *Prorocentrum* occurs. Because of

public health implications, the production of OA has been a point of interest in biological research communities.

Okadaic acid is not only important as a potential public health hazard, but is also economically valuable in biological research. The primary, most efficient source of OA is from dinoflagellates, particularly *Prorocentrum* spp. (Morton et al. 1994). *Prorocentrum* may produce variable levels of OA under different environmental conditions. Research has focused on combining environmental factors to obtain optimum OA production from different species of *Prorocentrum*. The results of these studies can be utilized in the public health sector when considering environmental factors in natural environments where *Prorocentrum* and aquaculture are co-occurring, or in ecological studies where *Prorocentrum* may be linked to disease in wildlife or fish populations.

Background and Biology

Dinoflagellates are important among eukaryotic (cells containing a distinct membrane-bound nucleus) algae because they impact the carbon cycle and coastal fisheries production (Graham and Wilcox 2000). They are found in both benthic and pelagic environments, from arctic to tropical seas and estuaries as well as fresh to hypersaline waters (Steidinger and Tangen 1996). Most are unicellular flagellates, with two distinctive flagella and a characteristic rotary mode of locomotion (Graham and Wilcox 2000). At the ultrastructural level, dinoflagellates have a common thecal- or cell-covering structure that, along with their flagellar and nuclear characters, differentiates them from other algal groups (Steidinger and Tangen 1996). Although dinoflagellate nuclei are not characteristically eukaryotic because they lack histones, nucleosomes, and maintain continually condensed chromosomes during mitosis, this group of microalgae

does have typical eukaryotic organelles such as chloroplasts, mitochondria, and golgi bodies (Steidinger and Tangen 1996).

"Toxic marine dinoflagellates, consisting of less than 60 of nearly 2000 extant species, vary little from nontoxic free-living dinoflagellates except (1) the majority are photosynthetic estuarine or neritic forms; (2) most probably produce benthic, sexual resting stages; (3) most are capable of producing monospecific or near monospecific populations above background levels; and (4) all produce bioactive water-soluble and/or lipid-soluble substances that are cytolytic, hemolytic, hepatotoxic, or neurotoxic in activity" (Steidinger and Tangen 199, p. 389). The dinoflagellate genus *Prorocentrum* has both toxic and non-toxic species.

Ehrenberg discovered the genus *Prorocentrum* in 1833, and more than 70 species have since been described (Faust et al. 1999). The majority of described *Prorocentrum* species are pelagic, but *Prorocentrum* is increasingly recognized as an important part of the benthic microalgae community (Faust 1990 a, b, c, Faust et al. 1999). In the natural world, many factors contribute to the abundance and fluctuation of *Prorocentrum* populations.

As epiphytes, benthic *Prorocentrum* spp. will prefer specific substrates where they can obtain necessary micronutrients for growth, reproduction, and cellular requirements. Epiphytic, benthic *P. lima* prefers NH_4^+ as its primary nitrogen source (Pan et al. 1999). Previous experiments with *P. lima* indicate a positive correlation between growth rates and NH_4^+ concentration (Pan et al. 1999). The preferential use of NH_4^+ over NO_3 is consistent with the N-utilization strategy of most dinoflagellates, including other *Prorocentrum* species such as benthic *P. hoffmannianum* (Aikman et al.

1993) and planktonic *P. micans* (Pan et al. 1999). The favored consumption of NH_4^+ over NO_3 appears to be common among epiphytic, benthic dinoflagellates (Aikman et al. 1993), perhaps due to the sustained high levels of non-oxidized nitrogen sources available in their natural habitats (Pan et al. 1999). For certain phytoplankton, the bioenergetic advantage of NH_4^+ versus NO_3 -uptake and assimilation is balanced by the lethal effect of NH_4^+ toxicity at high levels.

In general, life-cycle events appear to vary among different *Prorocentrum* species studied thus far (Faust et al. 1999). The cell cycle is used to measure species-specific growth rates, and for unicellular organisms, completion of the cycle directly causes population growth (Weiler and Chisholm 1976, Antia et al. 1990). Nutrient availability impacts life-cycle stages and growth rates of *P. minimum* by altering the duration of cell-cycle phases (Anita et al. 1990). Increasing nutrient levels shorten cell cycle phases, which increase growth rates. Therefore, *Prorocentrum* could grow to higher numbers at a faster rate in areas with high nutrient levels. However, it is important to note that nutritional requirements of dinoflagellates are complex, requiring a certain ratio of nutrients (e.g. Redfield Ratio, or 106C: 16N: 1P by atomic weight) depending on other environmental factors (e.g. light and temperature; E. Phlips, University of Florida, pers. comm. 2001), so an increase in one nutrient (e.g. nitrogen) may or may not influence growth rate.

Morphology of the Genus *Prorocentrum*

Species in the genus *Prorocentrum* have two laterally compressed valves, anteriorly inserted flagella, and cell shapes ranging from ovate to rotundate and pyriform (Faust et al. 1999). Faust et al. (1999) has proposed taxonomic importance in the surface

morphology of the valves and architecture of the flagellar pore area and intercalary band. *Prorocentrum* spp. possess 5 to 14 apical platelets that surround the flagellar and apical pores (Faust et al. 1999).

Ornamentation of the apical area has been the most prominent feature in identifying species of *Prorocentrum* (Faust et al. 1999). Some *Prorocentrum* species, such as *P. mexicanum*, have a spine extending from the apical collar, and *P. belizeanum* has the posterior collar extending above the anterior collar. These features are useful when identifying otherwise similar benthic species (Faust 1990a, Faust et al. 1999).

Resting-cyst data for *Prorocentrum* are limited (Faust et al. 1999), and early reports indicate two types. One type is a brown, spherical resting cyst of *P. micans* and *P. lima* (Bergh 1881, Breemen 1905, Faust et al. 1999). The second type is a thin cyst of *P. lima*, in which the development of two daughter cells was recorded (Lebour 1925, Wood 1954), enlarging in length and width inside the cyst (Faust et al. 1999).

Public Health Concerns and *Prorocentrum*

Although several planktonic species of *Prorocentrum* may form blooms or "red tides" (Lassus 1988), few are reported to be harmful (Faust et al. 1999). However, several benthic species do produce toxins. *Prorocentrum lima* produces several different toxins (including OA) that can cause human illness such as DSP (Yasumoto 1990, Jackson et al. 1993). OA has been isolated from the benthic species *P. concavum* (Dickey et al. 1990), *P. hoffmannianum* (Aikman et al. 1993), *P. lima* (Murakami et al. 1982), *P. belizeanum* (Morton et al. 1998), *P. maculosum* (Hu et al. 1992 – previously thought of as *P. concavum*), *P. faustiae* (Morton 1998), and *P. arenarium* (Ten-Hage et

al. 2000). Out of these, the species quantified in this study include *P. concavum, P. hoffmannianum, P. lima*, and *P. belizeanum* (Fig. 2-1).

Toxins produced by dinoflagellate populations cause ciguatera and DSP (Steidinger 1983, Tindall et al. 1984, Yasumoto et al. 1984). Ciguatera is a tropical fish-borne poisoning (Banner 1976, Withers 1982, Juranovic and Park 1991, Faust et al. 1999) caused by toxins accumulated through the marine food web, and bio-concentrated in the soft tissue of fishes (Yasumoto et al. 1977). The first causative organism of ciguatera was identified as *Gambierdiscus toxicus*, but as mentioned previously, *Prorocentrum* species have also been implicated as sources of these toxins (Nakajima et al. 1981, Tindall et al. 1984, Faust et al. 1999). Ciguatera poisoning has more than 175-recorded symptoms in humans (Halstead 1967, Faust et al. 1999), and can affect each individual differently.

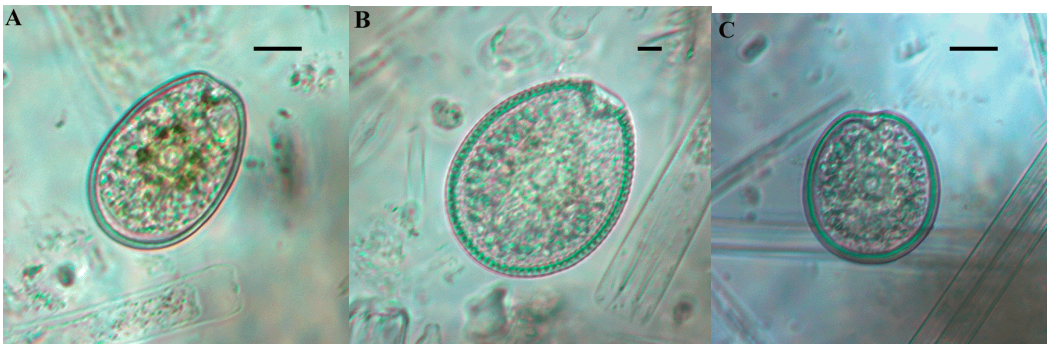


Figure 2-1. OA-toxic *Prorocentrum* Species: A – *Prorocentrum lima* from Waikiki, Oahu, Hawaii, B – *P.* cf *hoffmanianum* from St. Joseph Bay, Florida; C – *P. concavum* from St. Joseph Bay, Florida. Scale = 10 µm. (*P. belizeanum* not shown.)

DSP has been a recognized problem in Europe and Japan since the early 1980's. DSP symptoms are easily confused with those of bacterial gastroenteritis and result from the consumption of shellfish contaminated with DSP toxin-producing microalgae (Marr et al. 1992). In August 1990, the first confirmed incident occurred in North America

when several people developed symptoms consistent with DSP (diarrhea, nausea, and vomiting) following the consumption of cultured mussels from Mahone Bay on the Atlantic coast of Nova Scotia, Canada (Marr et al. 1992). Extracts of mussel samples were toxic to mice as demonstrated by intraperitoneal (ip) injection, and subsequent analyses, using liquid chromatography-mass spectrometry (LC-MS) and LC with fluorescence detection showed the presence of the DSP toxin, dinophysistoxin –1 (DTX1) (Marr et al. 1992).

DSP cases have been linked to shellfish cultivation sites and could become a major aquaculture problem in North America (Marr et al. 1992). In the past, DSP toxins in mussels have been generally correlated with a relative abundance of photosynthetic species of *Dinophysis* in associated plankton samples. However, samples are usually not taken and analyzed at DSP sites to confirm the source of toxins (Marr et al. 1992), and *Prorocentrum* could also be a factor. Both the water column and mussel substrate need to be tested for DSP-toxin producing species, such as benthic *P. lima*. Isolates of *Prorocentrum* need to be sampled from aquaculture sites to determine if DSP toxins are being produced.

Toxin production in planktonic *P. minimum* from the French Mediterranean and English Channel is also of concern (Grzebyk et al. 1997), although *P. minimum* has rarely been associated with toxic effects. Venerupin Shellfish Poisoning (VSP) has caused illness as early as 1942 in Japan, where 114 people living around a coastal lagoon died after consuming oysters and clams. The toxin venerupin was isolated in the shellfish and was associated with *P. minimum* (also known as *Exuviaella* mariae-lebouriae Parke and

Ballantine, Grzebyk et al. 1997). *P. minimum* has been linked to shellfish poisoning in Portugal and Norway.

Toxin Production in *Prorocentrum*

The primary toxin associated with *Prorocentrum* spp. is OA, and toxin-production research within the genus has focused on isolating factors that influence the production of this toxin.

OA is a toxic polyether fatty acid that was first isolated from two sponges of the genus *Halichondria: H. okadaii* and *H. Melanodocia* (Tachibana et al. 1981, Morton et al. 1994). OA in the sponges resulted from the dinoflagellates *Prorocentrum* and *Dinophysis*, which accumulate in the sponges through filter feeding (Kat 1979, Murakami et al. 1982, Lee et al. 1989, Dickey et al. 1990, Morton et al. 1994). OA is a potent, non-phorbol ether tumor-promoter and is a potent inhibitor of serine/thronine-specific protein phosphatases 1 and 2A (and also 2B at very high concentrations) (Morton et al. 1994). "Inhibition of these enzymes increases protein phosphorylation that:

1. Affects intracellular processes including metabolism, contractility, gene transcription, maintenance of cytoskeletal structure, receptor-mediated signal transduction, and cellular division;
2. Stimulates the expression of certain proto-oncogenes;
3. Activates H1 kinase in vitro;
4. Induces various mitosis-specific events (Herschman et al. 1989, Sakai and Fujiki 1991, Fujiki and Suganuma 1993, Honkanen et al. 1994)" (Landsberg et al. 1999, p. 200).

OA has become an important and valuable molecular probe in biological research (Cohen et al. 1990, Morton et al. 1994). Since OA is valuable, and many steps make the chemical synthesis of OA impractical, *Prorocentrum* spp. are the only renewable sources

of OA (Morton et al. 1994). *Dinophysis* spp. have not been successfully cultivated in the laboratory, which makes *Prorocentrum* the obvious choice for producing OA (Morton et al. 1994). Because of this, environmental factors influencing OA- production in *Prorocentrum* have been assessed within laboratory environments.

Optimal environmental conditions for the highest OA- production levels in *P. hoffmannianum* include specific ranges in light intensity and temperature (Morton et al. 1994). Maximum biomass, total harvestable OA, growth rate, and OA content per cell are all independent variables, with each maximized or minimized with different environmental parameters (Morton et al. 1994).

Toxin production in benthic *P. lima* is related to the cell cycle (Pan et al. 1999). *P. lima* not only produces OA, but also three other DSP-associated toxins: OA C8-diol-ester (OA-D8), dinophysistoxin-1 (DTX1), and dinophysistoxin-4 (DTX4) (Pan et al. 1999). DTX4 synthesis is initiated in the G1 phase of the cell cycle and persists into S phase ("morning" of the photoperiod), whereas OA and DTX1 are produced later during S and G2 phases ("afternoon"). No toxin production occurs during cytokinesis, which happens early in the dark period. Evidence indicates toxin synthesis is restricted to the light period and is coupled to cell cycle events. Biosynthesis of these toxins occurs sequentially, and the labile water-soluble DTX4 component is the precursor for lipophilic toxins such as OA and acts as a potent (and auto-toxic) phosphatase inhibitor (Wright and Cembella 1998, Pan et al. 1999). Ultimately, a biosynthetic cascade occurs, with the synthesis of toxin derivatives in different cell-cycle stages (Pan et al. 1999).

Grzebyk et al. (1997) took eight *P. minimum* clones isolated from natural environments along French coasts and cultured them for toxicity studies. The existence

of toxic as well as non-toxic clones of *P. minimum* accounts for the different observations concerning the toxicity and non-toxicity of blooms of *P. minimum*. The new form of *P. minimum* toxin produced neurotoxic symptoms that appeared rapidly in mice. However, if a lesser dose was administered, effects were not as apparent, potentially accounting for under-estimations of the toxicity of *P. minimum* blooms in the past (Grzebyk et al. 1997). Toxin production can vary among *Prorocentrum* populations at different locations. Variation in OA production in *P. lima* clones from different locations in Spain and the Southwest Indian Ocean has been observed (Boraïcha et al. 2001, Bravo et al. 2001). Distribution alone of *Prorocentrum* does not necessarily indicate the presence of toxins where *Prorocentrum* occurs.

Toxin production may be beneficial for *Prorocentrum* for several reasons. One theory suggests that OA inhibits growth of microalgae, providing a competitive advantage for *Prorocentrum* (Windust et al. 1996). OA and other toxins may also protect *Prorocentrum* against predation (Hu et al. 1995).

Landsberg et al. (1999) found a positive correlation between *Prorocentrum* abundance and FP around the Hawaiian Islands. Florida, on the other hand, has not had any extensive statewide surveys of the populations of *Prorocentrum*. The present study compares *Prorocentrum* populations around both the Hawaiian Islands and Florida to FP, representing two isolated green turtle populations in the Pacific and Atlantic within the same time-period.

Study Sites

The basic design of this study is a comparison of distribution and abundance of *Prorocentrum* to known FP prevalence around Florida and Hawaii. Samples of

macroalgae were collected at four study sites around the state of Florida and at nine study sites around the state of Hawaii on a seasonal basis. These study sites were chosen by the criteria of the presence of grazing green turtle populations and differences in FP prevalence among the locations.

Florida

The following four study sites were sampled quarterly to account for seasonality fluxes in the population of *Prorocentrum* within Florida (Fig. 2-2). These sites were chosen for known green turtle grazing populations and salinity ranges that are greater than 26 ppt on average. Since *Prorocentrum* prefers a higher salinity, choosing sites with comparable salinity levels reduced the importance of salinity as a covariate in the statistics.

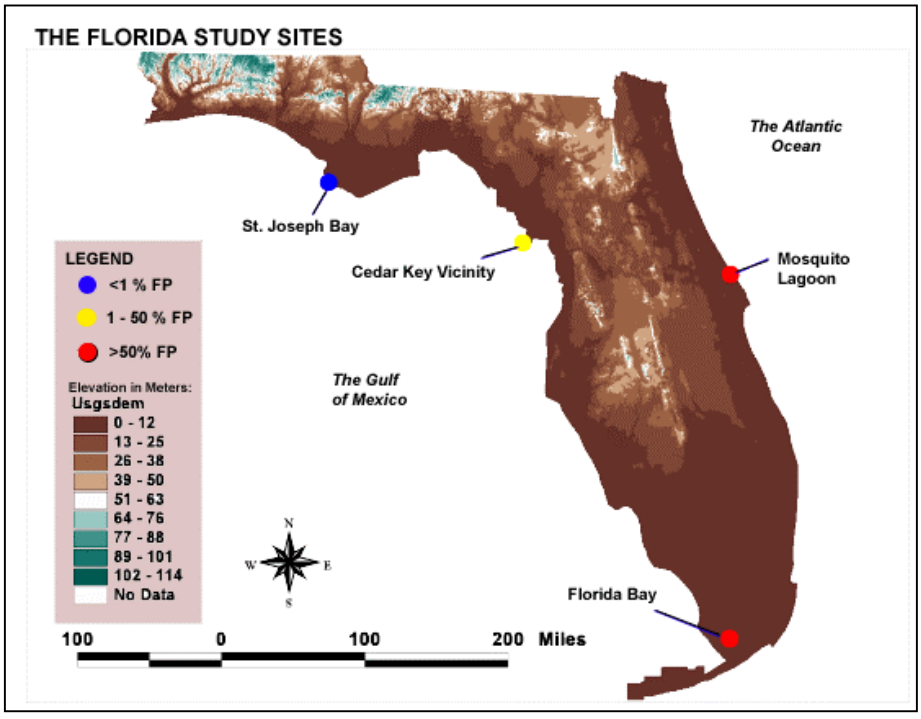


Figure 2-2. The location and FP prevalence of the Florida study sites.

Study site 1: St. Joseph Bay (Panhandle, Florida)

As the Florida control site for this study, St. Joseph Bay (Fig. 2-3) stranding and observation data of the local green turtle population has indicated that FP prevalence is less than 1% in this area. In December 2000, a stranding of 389 green turtles occurred in St. Joseph Bay. Only two green turtles had tumors. One had only a small (<1cm) tumor on its chin and the other had numerous small to moderate-sized tumors (1-4cm) on the flippers, tail, and neck (A. Foley, Florida Marine Research Institute, pers. comm. 2001), indicating only light afflictions in tumor severity.

St. Joseph Bay may be more appropriately called a lagoon. It is located on the panhandle portion of the Florida West Coast of Gulf County. The boundary spit of land enclosing the bay is about 17.6 km long, with an approximately 6.4 km wide opening in the north of the bay into the Gulf of Mexico. Almost 118-km^2 lie within the lagoon. St. Joseph Bay is unique in being the only large embayed body of water in the Eastern Gulf of Mexico not influenced by inflowing fresh water, thereby maintaining a salinity level equal to the Gulf (Wetherwell 1997). It is relatively shallow, with a mean depth of 7 m. In the most extensive shallow reaches of the lower bay (where the majority of benthic algae occurs and therefore where most samples were collected), there is little current except for the effects of tide. Because of this, St. Joseph Bay functions as an almost closed ecosystem. Seagrass communities cover about one-sixth of the bay bottom (Wetherwell 1997).

St. Joseph Bay was designated by the state of Florida as an Aquatic Preserve in 1969 to maintain and preserve the natural condition of the bay and all of the biological

resources within (Wetherwell 1997). The human population around St. Joseph Bay is growing slowly, and the Bay is known for its abundance of shrimp, fish, and shellfish.

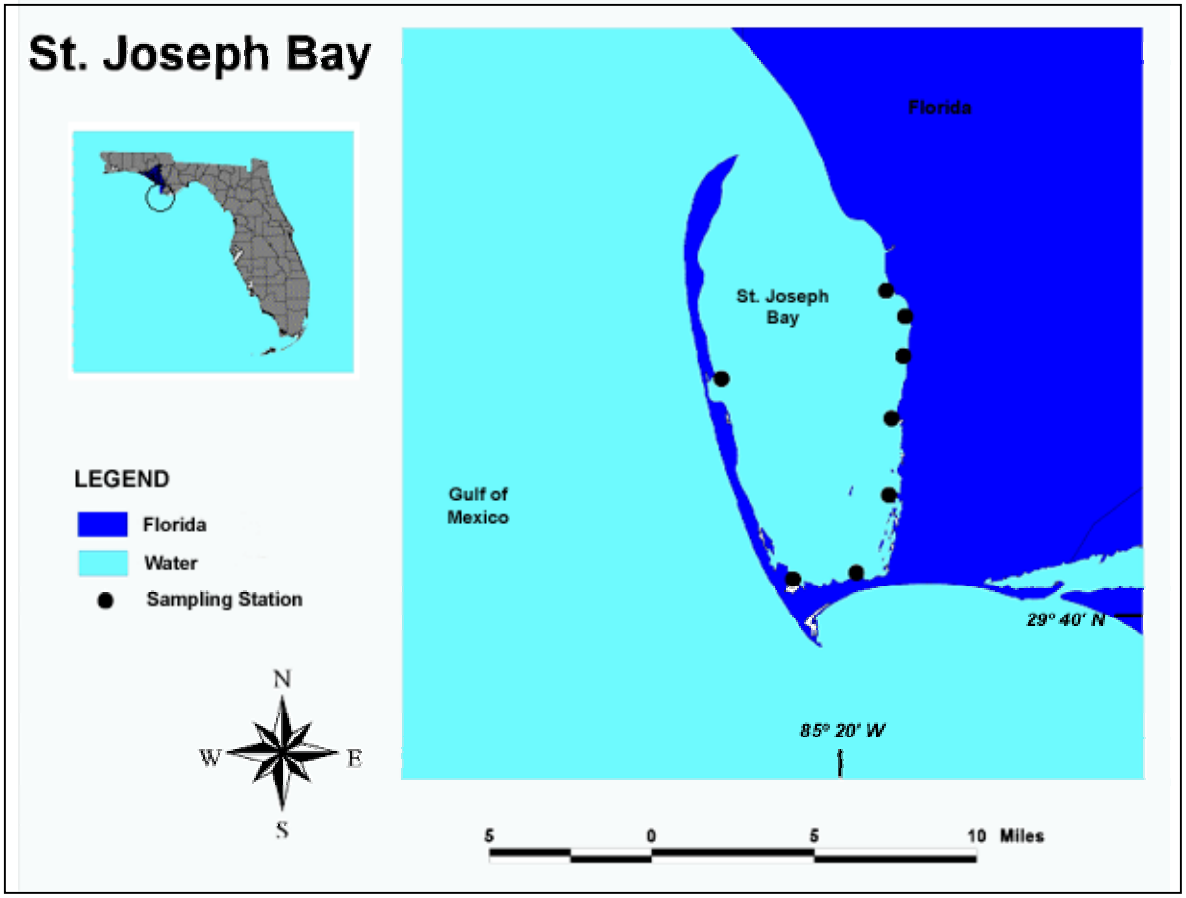


Figure 2-3. Sampling stations in St. Joseph Bay, Florida.

Study site 2: Cedar Key vicinity/ Seahorse Key (Northwest Florida)

The Cedar Key vicinity (Fig. 2-4) has a history of research on green turtles, and it is located in the latitude where the transition between >10% FP and <1% FP in green turtle populations is observed within Florida. A 1955 study on the green turtle population of the vicinity did not report the presence of FP (Carr and Caldwell 1956). Prior to 1998, FP was not observed, and in the past two years, up to 15 - 30% green turtles caught or observed in the vicinity have had FP, although not severely (J. Barichivich, University of

Florida, pers. comm. 1999, J. Schmidt, National Marine Fisheries Service, pers. comm. 2000).

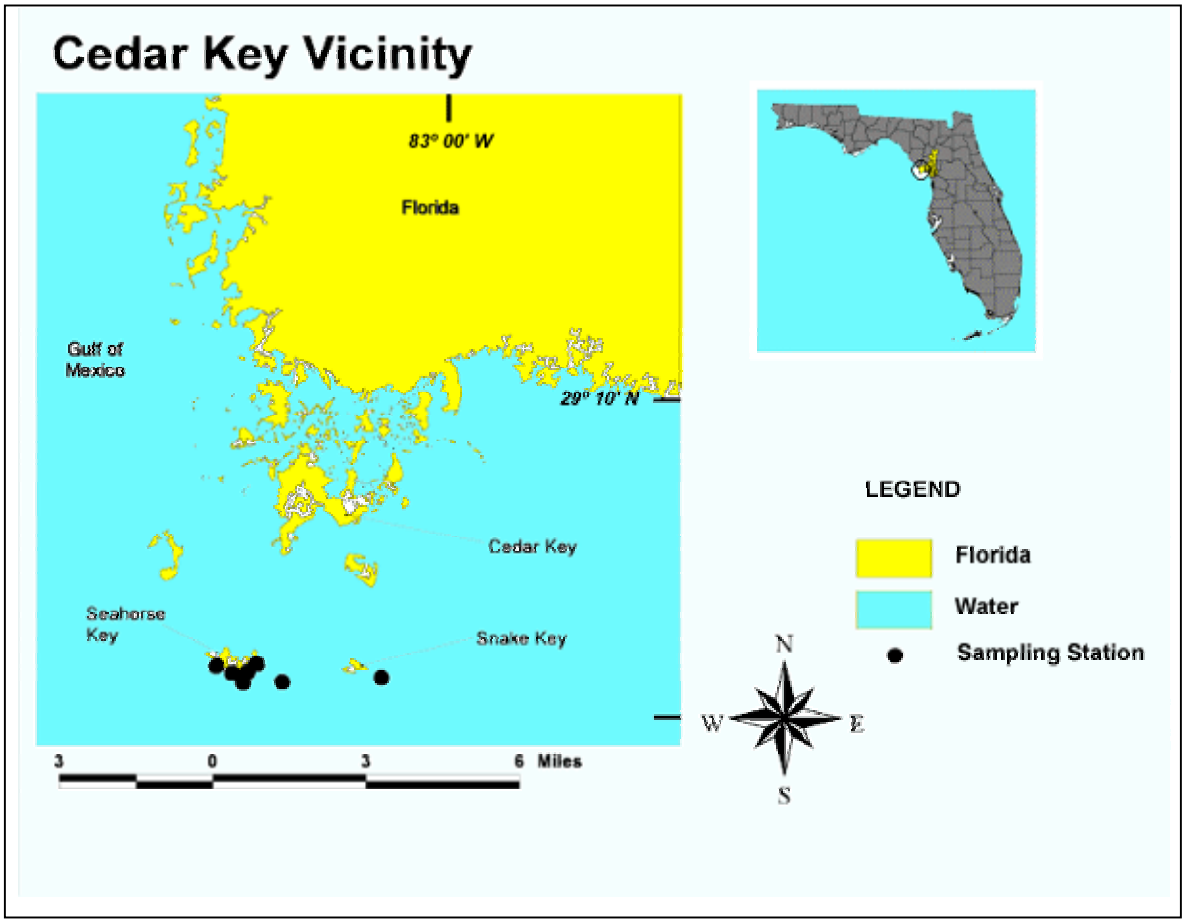


Figure 2-4. Sampling stations in the Cedar Key vicinity.

Study site 3: Mosquito Lagoon (East-Central Florida)

The prevalence of FP in Mosquito Lagoon, based on netting data for the past three years, is 57% (J. Provancha, Dynamac, pers. comm. 2000). This site (Fig. 2-5) has had extensive research on green turtle diet at different locations within the area, which was very useful in targeting macroalgae species for the present study. The location was also chosen for its high salinity levels, which was an important consideration for the comparison among all Florida sites.

Mosquito Lagoon is located in both Brevard and Volusia counties, on the central east coast of Florida. The lagoon is connected to other bodies of water by two narrow passages: the Ponce de Leon Inlet, which connects the northern end of the lagoon to the

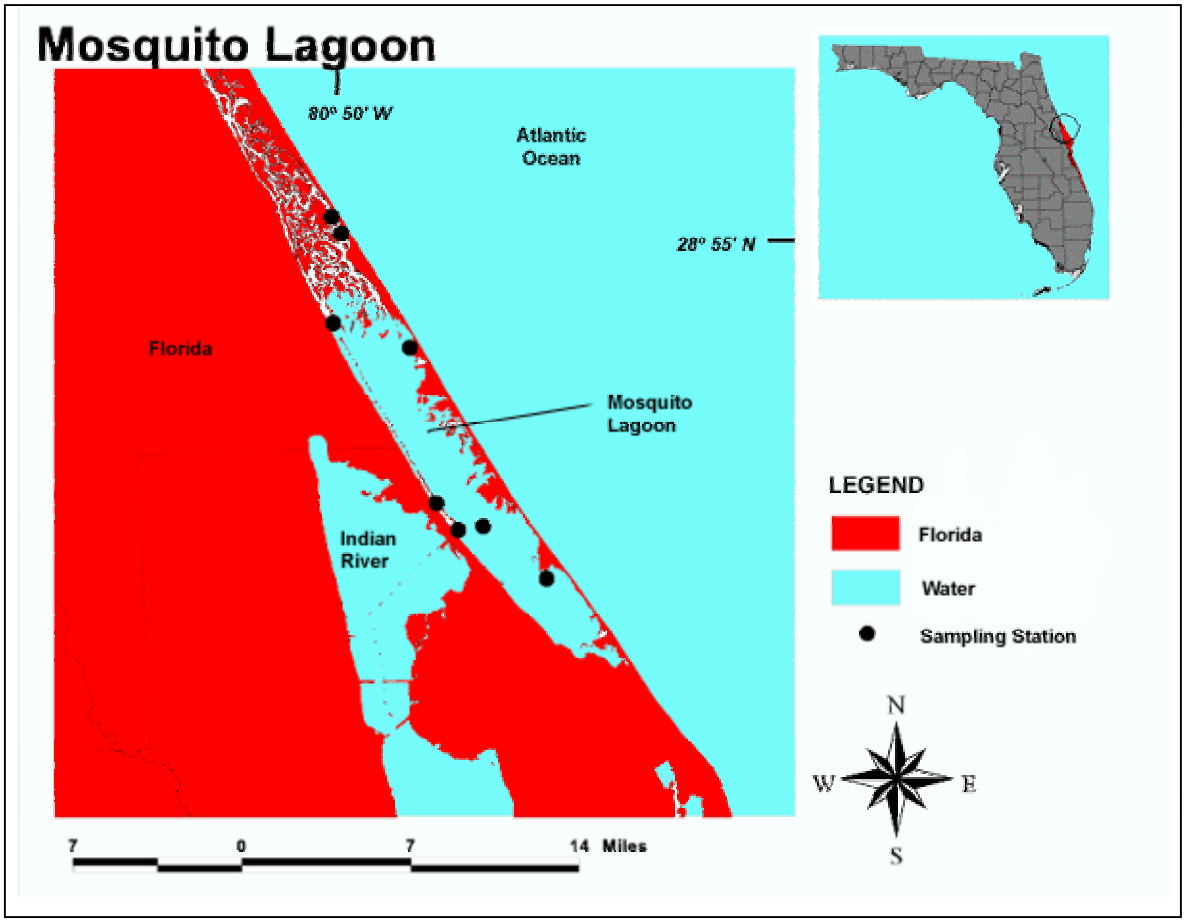


Figure 2-5. Sampling stations in Mosquito Lagoon.

Atlantic Ocean, and the man-made Haulover Canal, which connect the southwestern portion of the lagoon to the adjacent Indian River. The lagoon itself is 54 km long and four km wide at its widest point (Mendonça 1983). The northern portion of the lagoon includes numerous closely-spaced islands. The southern portion is open water. The average depth of the lagoon is 1.5 m, with an area greater than 3 m deep confined to the dredged intercoastal waterway (Mendonça 1983). Tides do not influence the lagoon

(except near the ocean inlet), but considerable wind-driven water movement occurs (Mendonça 1983). The northern half of the lagoon is highly developed and urban, and the southern half is protected, bordering Merritt Island National Wildlife Refuge (NWR) and Cape Canaveral National Seashore.

Seagrasses made up of 88% of the diet of 18 green turtles sampled in Mosquito Lagoon in 1978 - 1981 (Mendonça 1983).

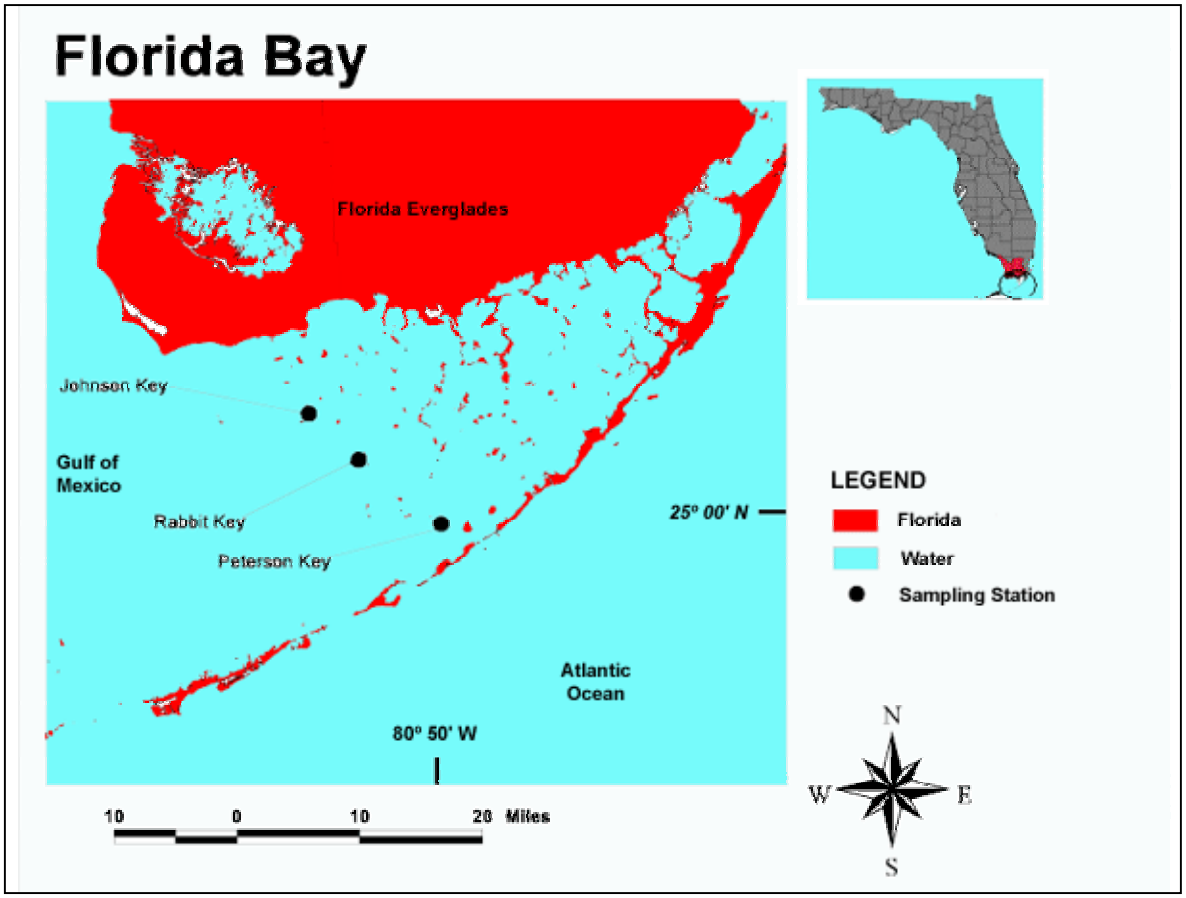


Figure 2-6. Sampling stations in Florida Bay.

Study site 4: Florida Bay (South Florida)

Florida Bay (Fig. 2-6) has one of the highest prevalence's of FP in green turtles in the state of Florida. Florida Bay is a large body of water approximately 2200 km^2 located

at the southern terminus of the Florida peninsula (Schroeder and Foley 1995). 69.2% of 26 green turtles captured in Florida Bay in 1997 exhibited FP (Schroeder and Foley 1995). Strandings and sightings of severely afflicted green turtles with FP are not uncommon in the Bay (B. Peterson, Florida Marine Research Institute, pers. comm. 2000). Several study sites within Florida Bay were chosen for accessibility and the presence of grazing green turtles to obtain an overview of the distribution and abundance of *Prorocentrum*.

Florida Bay is a shallow, triangular lagoon bordered by Everglades National Park to the north, the Florida Keys to the southeast, and the Gulf of Mexico to the west. Shallow carbonate mud banks divide the bay into basins, restrict circulation, and attenuate the Gulf's lunar tidal influence (Robblee et al. 1991). Water salinities throughout the bay fluctuate between brackish and hypersaline, due to rainfall and freshwater inflow.

Three basins within Florida Bay were sampled for *Prorocentrum* species: Johnson Key basin, Rabbit Key basin and Peterson Key basin. All three basins are in the western portion of Florida Bay where there are higher salinity ranges and seagrass cover than the Eastern portion of the bay.

Hawaiian Islands

In the Hawaiian Islands, the following sites were sampled for the comparison. The initial study by Landsberg et al. (1999) discovered a correlation between FP prevalence and *Prorocentrum* densities at these locations (Fig. 2-7).

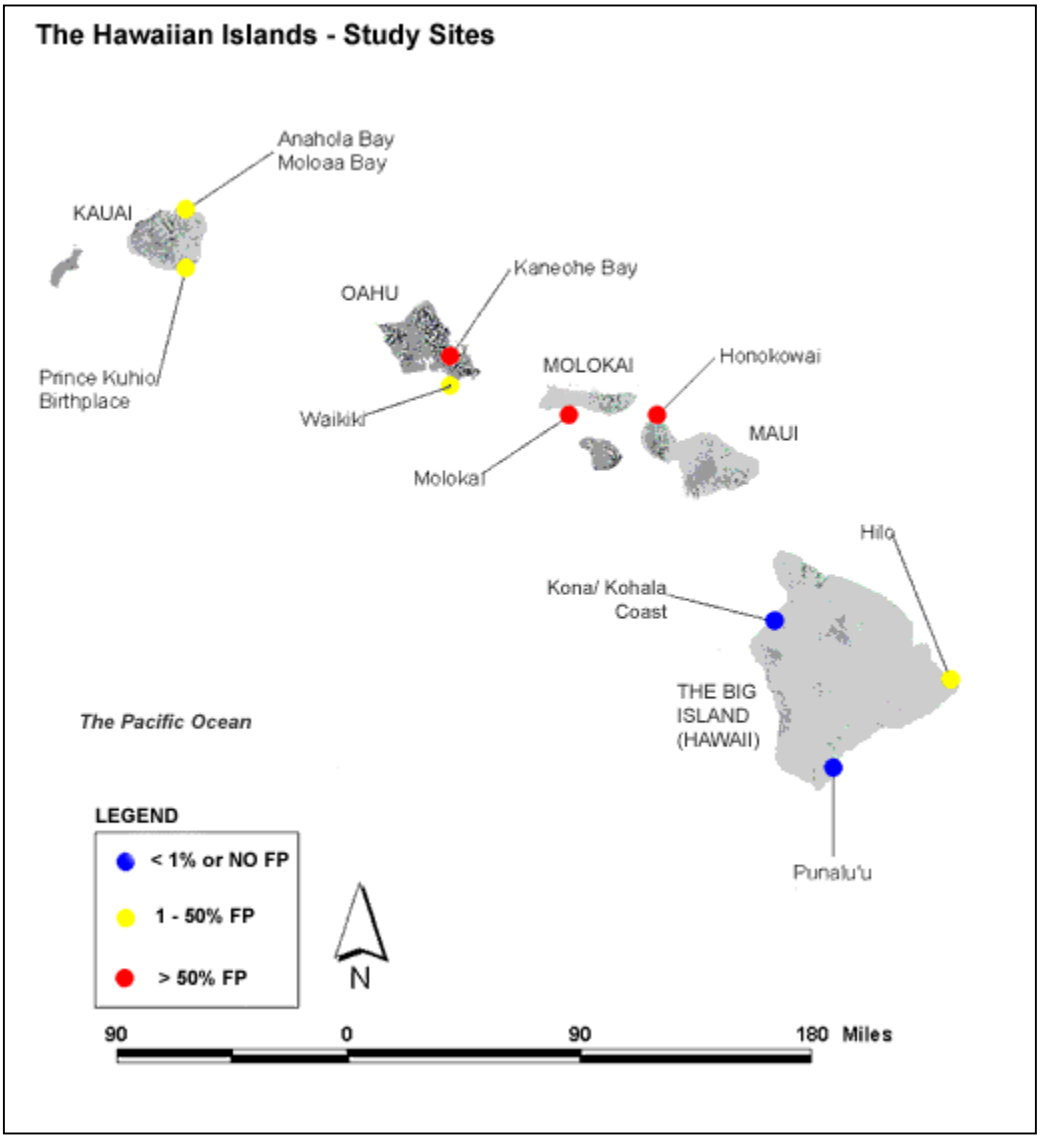


Figure 2-7. Location and FP prevalence of the study sites in the main Hawaiian Islands.

Study site 1: Punalu'u, Hawaii (East coast of the Big Island)

Although the northeast coast of the Big Island does have FP in the green turtle population, this particular site (Fig. 2-8) in the southeast has <1% prevalence of FP (Landsberg et al. 1999), and was considered a control site. Balazs et al. (1994a) recorded 2 green turtles out of 183 with FP at this location.

The green turtles at Punalu'u forage on benthic algae extensively in a depth of 1 – 2 m (Balazs et al. 1997, Rice et al. 1998). Their primary food source is *Pterocladiella capillacea*, an intertidal red alga that is only accessible to the foraging turtles during mid- to high-tide (Rice et al. 1998). Turtles are often observed feeding with their carapaces exposed in shallow waters (Rice et al. 1998, personal observation 2000).

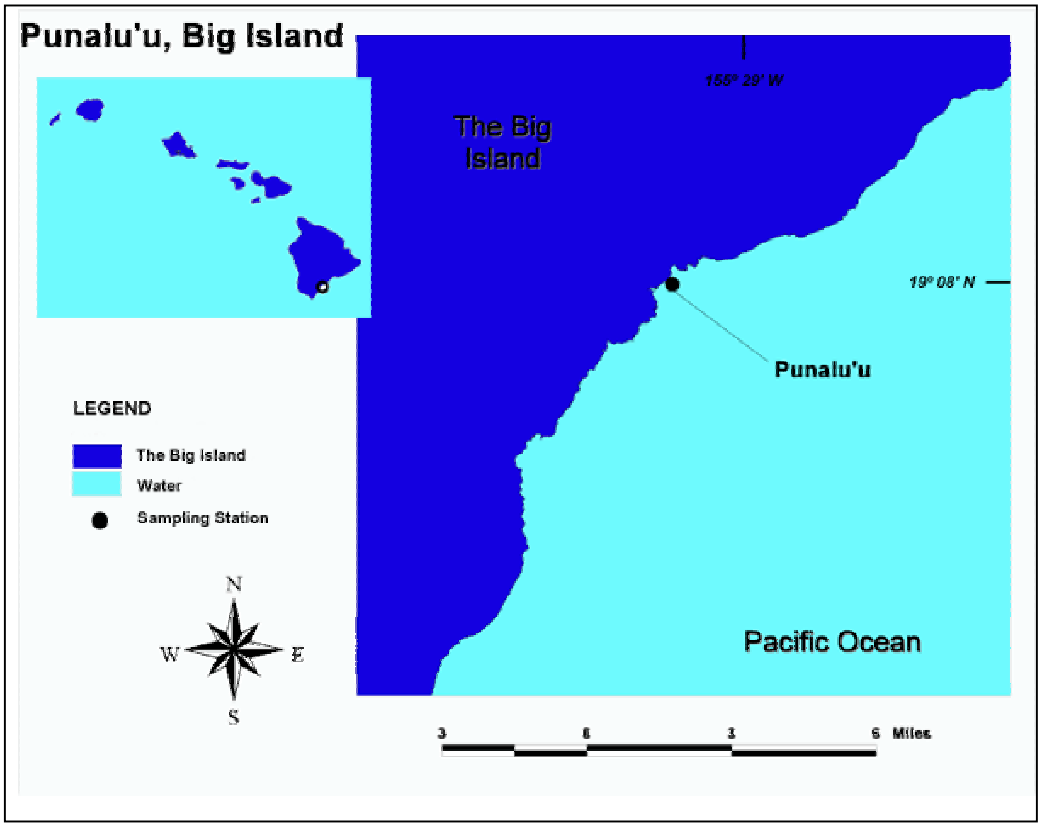


Figure 2-8. Sampling station in Punalu'u Bay, Big Island.

Study site 2: Kona/Kohala Coast, Hawaii (West coast of the Big Island)

The West coast of the Big Island in Hawaii has had < 1% prevalence of FP in turtle strandings (Murakawa et al. 2000). None of the turtles captured for tagging and health screening during the past 15 years have had FP (Murakawa et al. 2000). This site represents a valuable, firm control site for the Hawaii portion of this study. Low seasonal

rainfall, desert-like conditions, lava fields separated by areas of vegetation, and a very low-density population characterize this area. Many green turtles (>40) were observed foraging along this coast while collecting samples, with no visible signs of FP. Many turtles were feeding in the shallows on *Gelidium*-like turf at three of the study sites (Puako, Kiholo, and Kaloko-Honokohau). Kawaihae was deeper (~3 m), and turtles were observed feeding on *P. capillacea* on the rocky substrate. At this latter site, *P. capillacea* was collected from the underside of rocks where it was not heavily grazed.

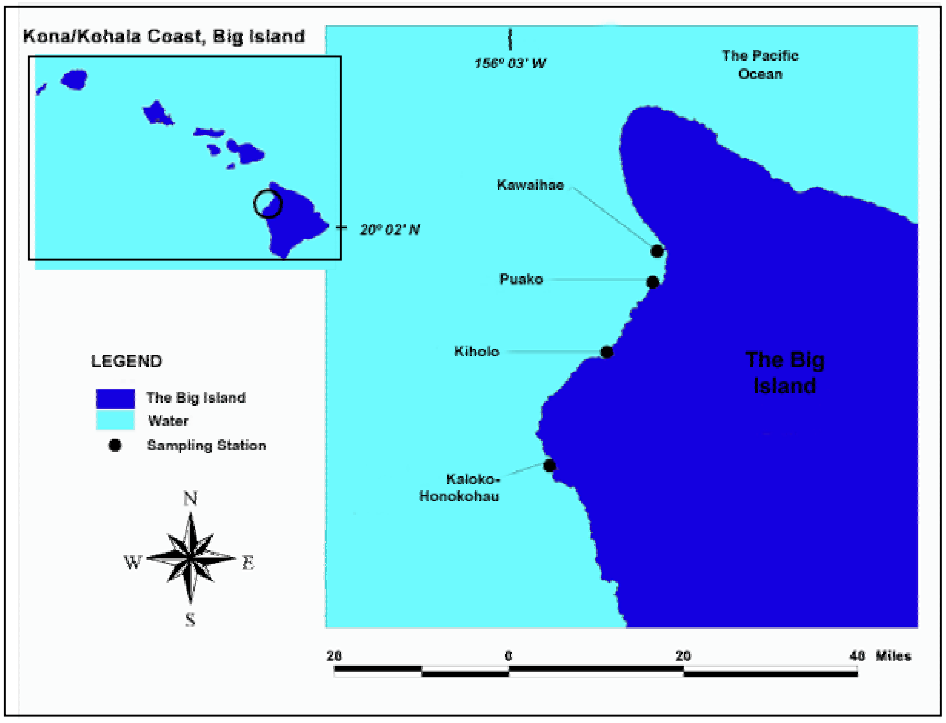


Figure 2-9. Sampling stations on the Kona/Kohala Coast, Big Island.

Study site 3: Hilo Bay Vicinity (East Coast of the Big Island)

Hilo Bay has a green turtle population in the mid-range of FP prevalence at 11-50% (Balazs 1991 unpublished data, Balazs et al. 1994a, 1994b, 1998, 2000a, Landsberg

et al. 1999). The two sample sites in the Hilo area that were sampled are Richardson Beach Park and Puhi Bay (Fig. 2-10).

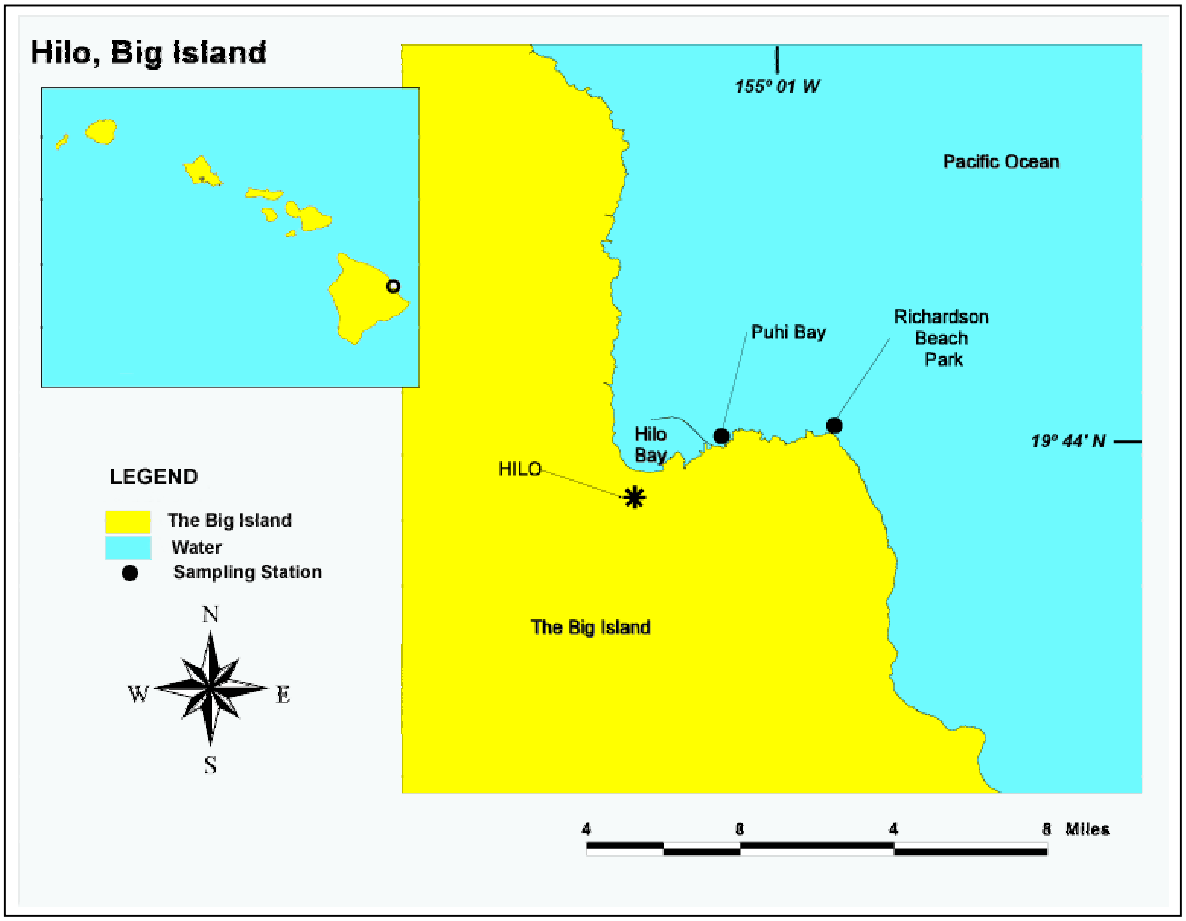


Figure 2-10. Sampling stations in the Hilo Bay vicinity.

The Hilo area is the most populated area of the Big Island, and has the highest prevalence of FP on the Big Island. Both study sites are relatively protected, with shallow areas of relatively diverse algal species. Puhi Bay is of special interest. When Landsberg et al. (1999) sampled here in 1998 there was a sewage plant adjacent to the bay, contributing treated sewage outflow to the local environment and resulting in water turbidity. In 2000, however, the sewage plant was closed, and the water appeared to be clear.

Study site 4: Southeast Kauai (Poipu)

Another study site in the mid-range of FP prevalence at 11-50% (Balazs 1991 unpublished data, Balazs et al. 1994a, 1994b, 1998, 2000a, Landsberg et al. 1999), Poipu (Fig. 2-11) consists of three sub-sites.

This site consists of two semi-sheltered bay areas, rocky with strong wave action. It is a relatively populated area, surrounded by hotels and residential areas.

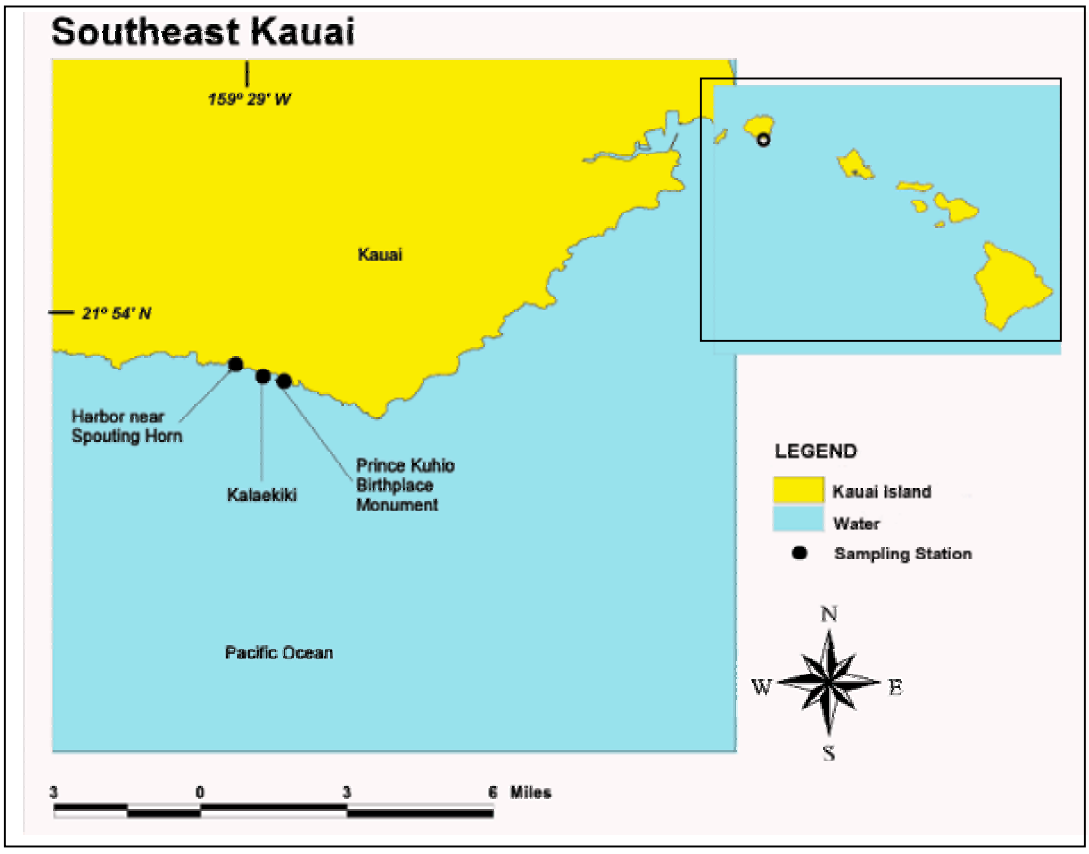


Figure 2-11. Sampling stations in Southeast Kauai.

Study site 5: Northeast Kauai (Moloaa Bay and Anahola Bay)

Also in the mid-range of FP prevalence at 11-50% (Balazs 1991 unpublished data, Balazs et al. 1994a, 1994b, 1998, 2000a, Landsberg et al. 1999), these two bays are two sub-sites representing northeast Kauai (Fig. 2-12).

Both bays consist of rocky tidal areas intermixed with sandy areas. Although both areas are protected, wave action can be strong in the shallow areas, particularly at low tide. Both vicinities appeared to be less populated than SE Kauai, with some residential areas mixed with undisturbed forest.

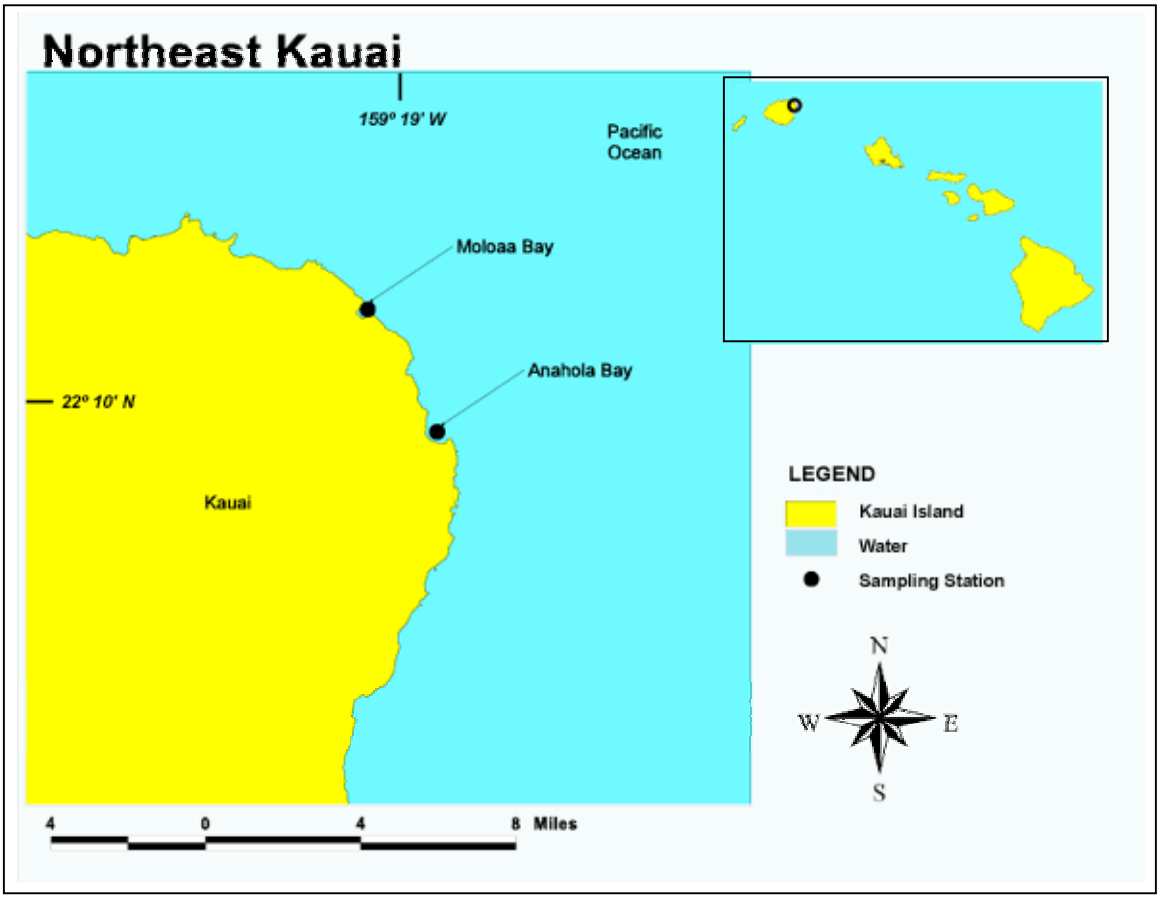


Figure 2-12. Sampling stations in Northeast Kauai.

Study site 6: Waikiki, Oahu

Waikiki (Fig. 2-13) has a green turtle population with FP prevalence within 11 - 50% (Balazs 1991 unpublished data, Balazs et al. 1994a, 1994b, 1998, 2000a, Landsberg et al. 1999). Waikiki is a very urban, high-density resort area, with little agriculture within its drainage basin, and little freshwater inflow.

Waikiki consists of a beach of re-nourished sand. Macroalgae can be found on the jetties and rocky areas around them. Two green turtles were observed feeding near the site where samples were collected.

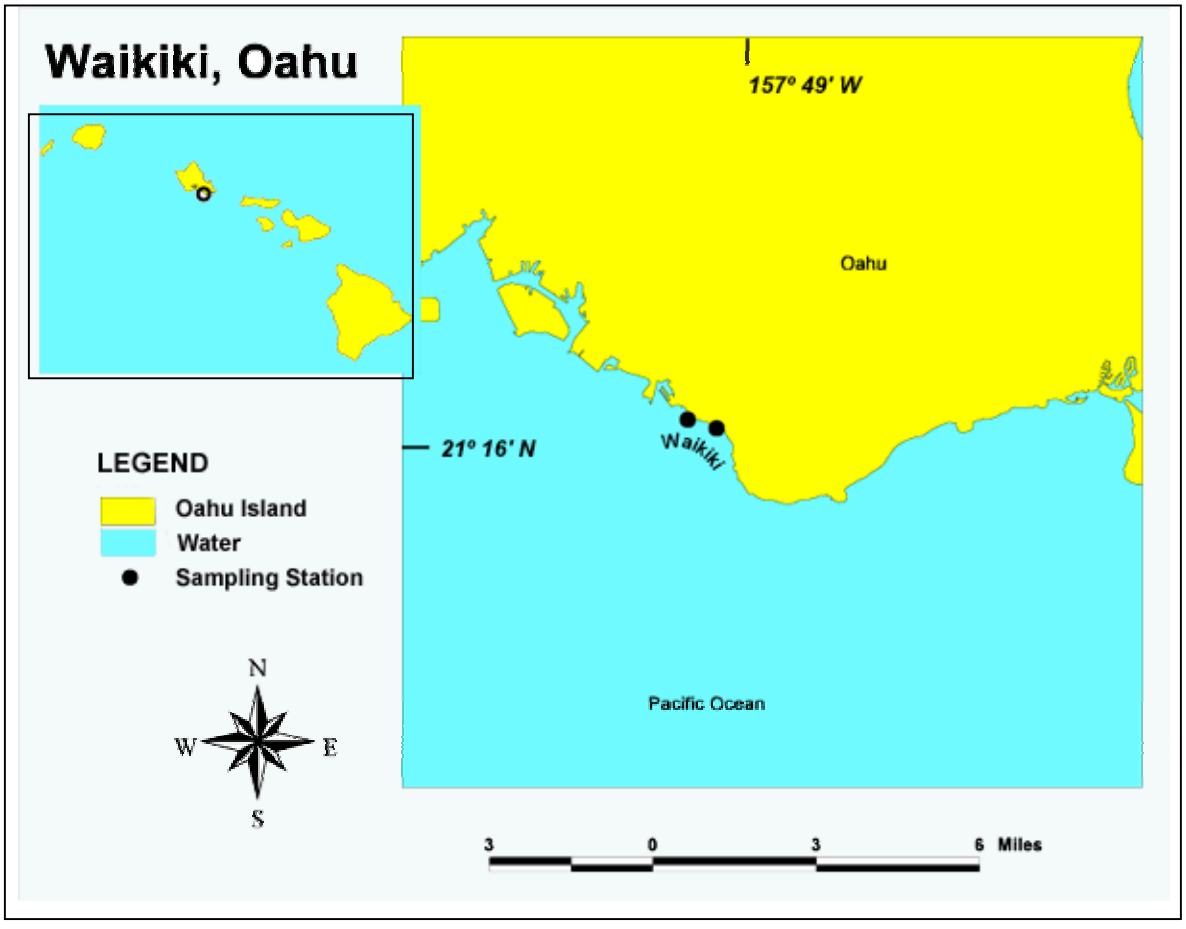


Figure 2-13. Sampling stations along Waikiki Beach, Oahu.

Study site 7: Kaneohe Bay, Oahu

Kaneohe Bay (Fig. 2-14) has a mean FP prevalence of >50% (Balazs 1991, Landsberg et al. 1999), and is the largest bay in the Hawaiian Islands. A relatively large assemblage of green turtles (>500 individuals) inhabits Kaneohe Bay, utilizing the area for foraging and resting purposes (Balazs et al. 1993).

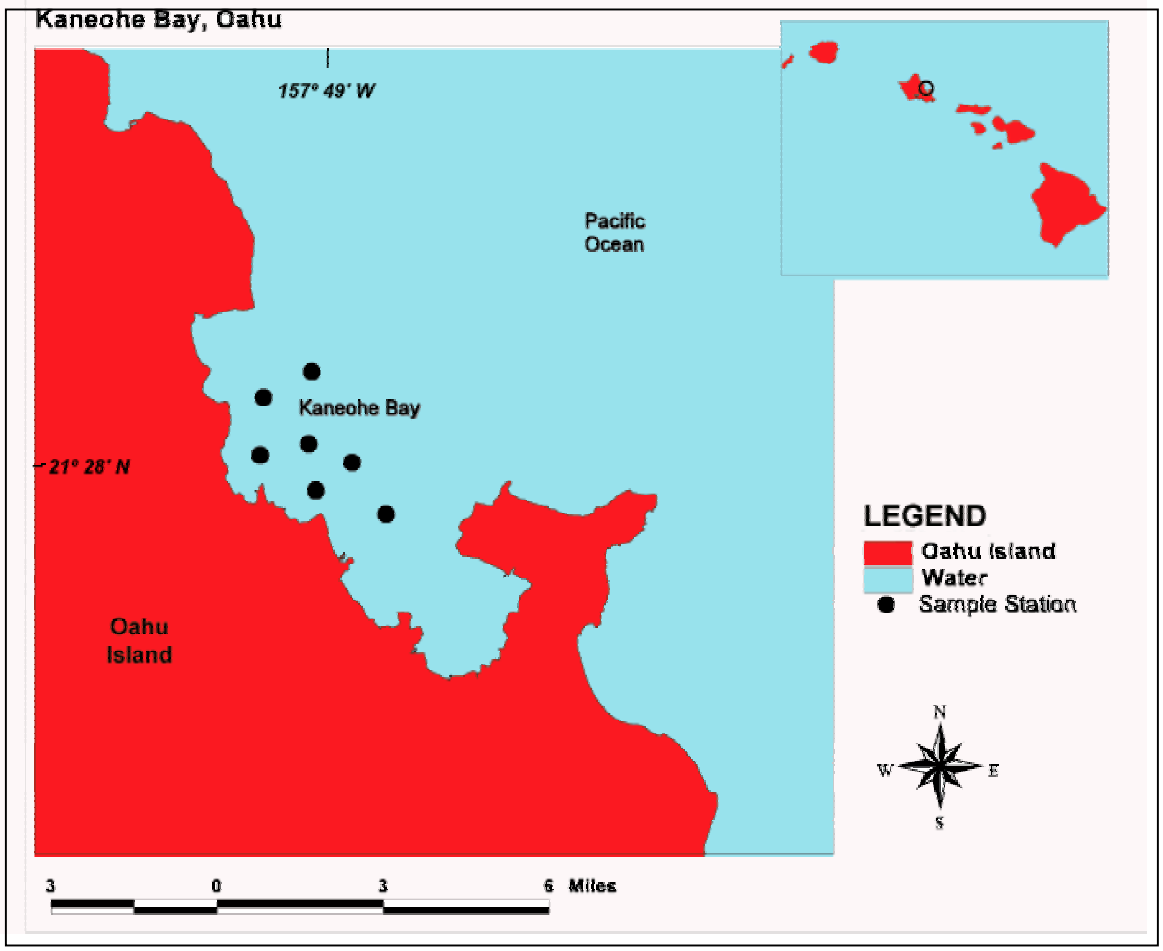


Figure 2-14. Sampling stations within Kaneohe Bay, Oahu.

Kaneohe Bay is a semi-enclosed embayment on the northeast coast of Oahu. The landward boundary of the Kaneohe Bay watershed is bordered by a nearly vertical mountain range, and the seaward boundary of the bay is a barrier reef extending across

the bay mouth. The bay is a unique combination of estuarine and coral reef ecosystems, and is influenced by freshwater inflow, sediments and nutrients from within its watershed (Smith et al. 1981). The shape of the bay is roughly a rectangle 13 km long and 4 km wide. The land use within the watershed of Kaneohe Bay is developed urban/residential land, with some agriculture.

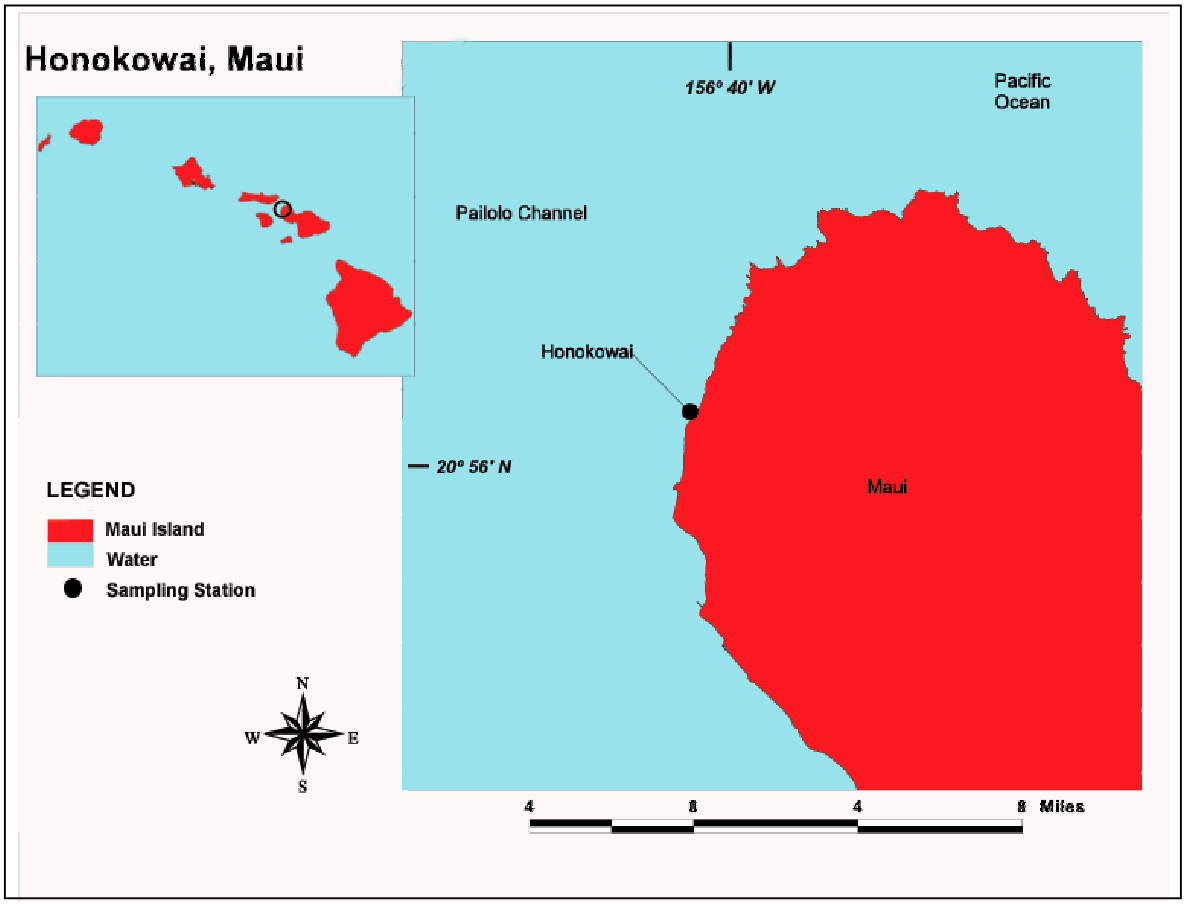


Figure 2-15. Sampling station(s) of Honokowai, Maui.

Study site 8: Honokowai, Maui

Honokowai, Maui has a high prevalence of FP (>50%), and the green turtle population in this area has been monitored closely since 1990 (Fig. 2-15). Out of 247

green turtles, 64% (n = 158) have had FP, with disease regression for 21 of the FP–afflicted turtles recorded (Bennett et al. 2000).

Honokowai is located on the western portion of the West Maui volcano (Fig. 2-15). The watershed where Honokowai is located consists of steep interior section giving away to more gently sloping land of the coastal plain. This area has a history of macroalgae blooms including *Cladophora hemisphaerica* and the introduced species *Hypnea musciformis* (the latter was introduced from Florida).

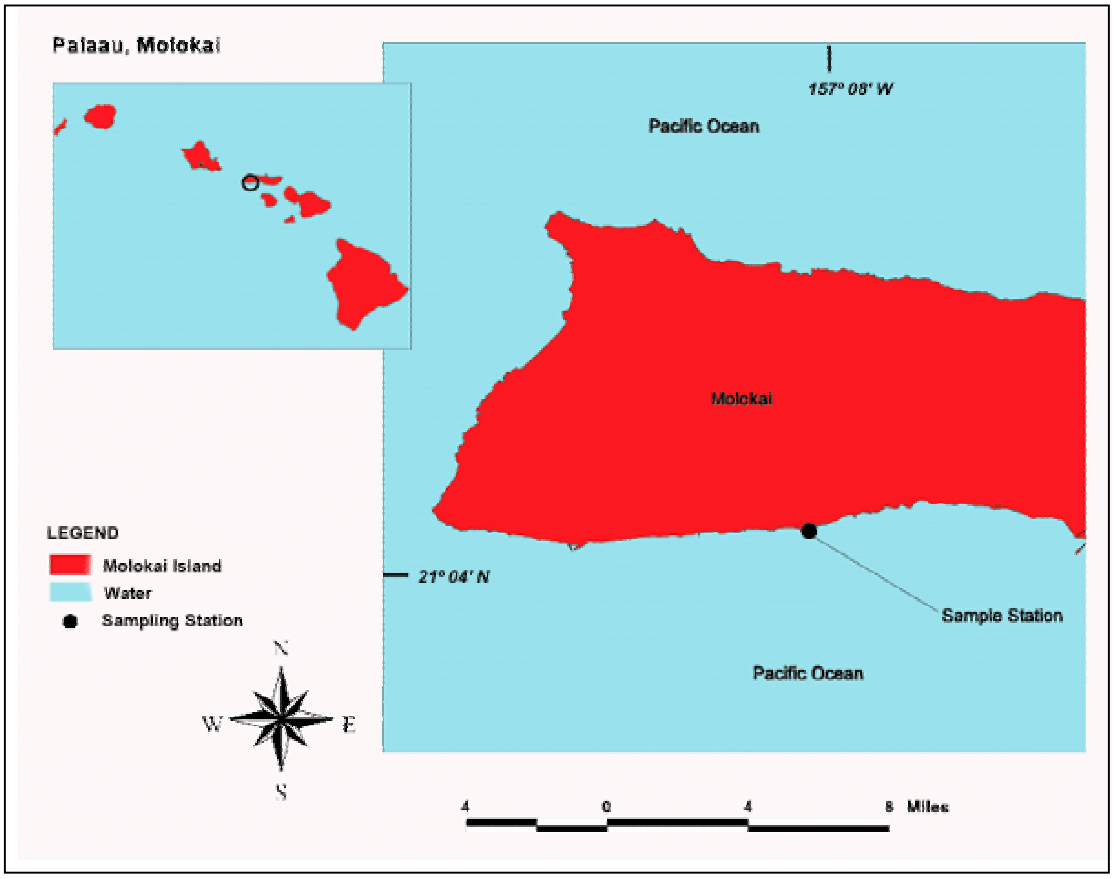


Figure 2-16. Sampling station at Palaau, Molokai.

Study site 9: Palaau, Molokai

Southwest Molokai is another high FP (>50%) prevalence study site (Fig. 2-16). The first green turtle to be observed with FP in Molokai occurred in 1982 (Balazs et al. 1997). Between 1982 and 1996, 1458 green turtles were captured, and a massive increase in FP was recorded, peaking at 61% prevalence in 1995 (Balazs et al. 1997).

The study site consists of very shallow (<1 m deep) flats, which are very silty (see Fig. 16 for location of sample site). Silt coated the macroalgae substrates collected in this area.

Methods

For each study site, multiple species of macroalgae and seagrass were targeted for collection on a basis of identified species grazed by green turtles. For the Hawaiian Islands, the alga *Acanthophora spicifera* was targeted, and in Florida, the seagrass *Halodule beaudettei* was targeted for collection at every site for cross-site comparisons. These species were chosen because they were the most likely to be found at all the study sites within each state, and green turtles are known to consume both species at their respective locations.

In Florida, samples were collected four times (every three months) over a year, to account for seasonality. Due to logistical constraints, the Hawaii sites were not sampled on a quarterly basis, but were sampled twice in one year: summer and winter (based on water temperature highs and lows). Since there was less temporal sampling in Hawaii, additional study sites were added. This expanded the number and range of samples for a more comprehensive study of Hawaii within the limited time frame.

For both Hawaii and Florida, the samples were collected within the time period of February 2000 through February 2001. For comparative purposes, the seasons were characterized as: Spring (May), Summer (August), Fall (November), and Winter (February), based on average water temperatures in both Florida and Hawaii.

For the purpose of this study, FP prevalences were categorized as follows (from Landsberg et al. 1999): rare (<1%), low (1-10%), medium (11-50%), and high (51-100%). Not only does this ranking account for % FP prevalence, but it also incorporates tumor severity. None of the study sites fall into the low FP prevalence category, so the rare ranking is called "low" for comparative purposes, although it remains to be <1% FP prevalence.

The control areas in this study were the locations where there was <1% prevalence of FP in the green turtle population frequenting that area, e.g. St. Joseph Bay in Florida and the Kona coast and Punalu'u Black Sand Beach in Hawaii.

Within each of the study sites, there were at least two stations (independent experimental units to account for local variation), and five species of macroalgae and/or seagrass collected (depending on availability), two replicates each. At locations where five species were not found, additional stations were added when available. For Florida, the three study sites of St. Joseph Bay, Cedar Key, and Mosquito Lagoon had different stations targeted in different seasons (e.g. for spring, station 1 and 2 were sampled, and for summer, station 3 and 4 were sampled, etc.). There were several reasons for this. First, there was no data available for these sites about specific locations where green turtles are known to forage, although it is known there are green turtles present and foraging within these study sites. Second, since the study sites are relatively large areas,

it is statistically more significant to sample throughout these areas to give an overall representation of *Prorocentrum* abundance. The best option would be to sample all the stations for each collection period, but it would not be possible to analyze the amount of samples this would entail in the time frame of the study. The obvious drawback to this sampling method is that there would be inherent variation between stations, as well as between seasons. For these three study sites in Florida, at least one station was sampled within two or more seasons. The stations were chosen based on access, transportation, and substrate availability, while attempting to cover as much of the study site as possible.

Florida Bay was sampled repeatedly at the same two stations for all four seasons, with additional *H. beaudettei* samples from an additional station in winter. This was due to transportation restraints, with the stations chosen for frequent observations of green turtles and comparative salinity levels (>26ppt).

For the Hawaiian Islands, stations were the same as the preliminary study on *Prorocentrum* by Landsberg et al. (1999). These were sites with specific observations of foraging green turtles (G. H. Balazs, National Marine Fisheries Service, pers. comm. 2000, personal observation). The only exception was Kaneohe Bay, which is a large area with a large green turtle population, and therefore samples were collected at different stations for summer and winter, with one station sampled both seasons.

At each station substrates were collected, the geographic coordinates were taken using a Global Positioning System (GPS) unit (Magellan GPS 3000 XL). This was performed to make it possible to return to the exact location at a later time if needed, and to also construct accurate maps of sampling locations in ArcView 3.2. Also, salinity and temperature readings of the water at the sample location were taken using a YSI Model

30 SCT handheld conductivity meter. This data was important in making accurate statistical comparisons between sample sites. It was therefore possible to compare between samples collected in an identified temperature and salinity range.

The presence, abundance, and species composition of *Prorocentrum* was evaluated from these macroalgal samples via microscope counts and identification.

Collection Methods

For collecting, processing, and analyzing samples, the protocol by Landsberg et al. (1999) was followed. Approximately 30g (wet) of each substrate species was carefully handpicked with minimal disturbance to avoid dislodging any *Prorocentrum* present. Substrates were collected by scuba diving, snorkeling, or wading and placed directly into plastic ziplock bags, with ambient seawater to maintain moisture. The water that came with the macroalgae was poured out into a 100mL-graduated cylinder, and seawater was added to the graduate cylinder to make up to 100mL. The 100mL was added to the 30g of macroalgae/seagrass in the ziplock bag.

Ziplock bags were shaken vigorously 30 times to dislodge attached microepiphytes (Ballantine et al. 1985, 1988) and the total volume was decanted and measured. From this volume, 50 mL was fixed with 5 mL of 10% buffered formalin to prepare for analysis.

Analysis of Samples

To analyze collected samples for *Prorocentrum* species and abundance, a 2-mL aliquot was placed in a settling chamber and left to settle for 12 hours. The bottom of the settling chamber was screened using a 200X-inverted microscope. Two replicates from

each sample were assessed. Toxic and non-toxic species of *Prorocentrum* cells were identified and counted. The species were visually identified by physical characteristics including cell shape, size, and apical collar features (Steidinger and Tangen 1996).

The number of cells per gram wet weight of macroalgal or seagrass substrate was estimated by determining the volume to weight ratio of the individual samples (Landsberg et al. 1999).

Samples of the macroalgae and seagrass substrates species were collected and dried to determine dry weight and ash-free dry weight for each species. Samples were dried for 24 h at 105˚C, cooled with a dessicant for 2 h, and the dried biomass was weighed to the nearest 0.01 g. The samples were then ashed for 24 h at 500˚C, cooled with dessicant, and weighed to the nearest 0.01 g to determine ash-free dry weight. The dry weights were compared to the wet weight of each substrate species, and *Prorocentrum* cells per gram dry substrate and per gram ash-free dry substrate were determined and compared among study sites in both Hawaii and Florida for the majority of substrate species collected (see Appendices E and F).

Statistical Analyses

Statistical analyses were completed using Minitab 11.2 statistical software. All statistical analyses were completed using toxic cells per g/wet substrate, for the purpose of allowing comparisons among previous studies that used the same measures. For each site, cross-site comparisons were made using the same substrates (*Halodule beaudettei* for the Florida comparison, and *Acanthophora spicifera* for Hawaii) to eliminate substrate preference as a variable. For both Florida and Hawaii, mean toxic *Prorocentrum* cells per g/wet substrate within a specified salinity and temperature range

were compared among sites, among FP rankings (low, medium, high), by season, by temperature, and by salinity levels by one-way ANOVAs with Tukey's pairwise comparison.

Results

Prorocentrum Abundance, FP, and Substrate Preference

Prorocentrum species were identified and counted for all the substrates collected in both Florida and Hawaii. For each study site, *Prorocentrum* species were calculated by station, by season, and by substrate. Toxic *Prorocentrum* was present on all 29 substrates collected in Florida and all 33 substrates in Hawaii, although there was variation in substrate preference between study sites.

Substrate preference of *Prorocentrum* was observed at each of the study sites, depending on geographical location, and varies by toxic cells per gram wet substrate, toxic cells per gram dry substrate, and toxic cells per gram ash-free dry substrate.

Florida

There were nine *Prorocentrum* species identified from samples collected in Florida and included in the results. Four species are known OA-producers including *P. lima, P. concavum*, *P.* cf *hoffmannianum*, and *P. belizeanum*, and five are not known to produce OA including *P. mexicanum, P. emarginatum*, *P.* cf *taylori, P. micans*, and very small cells of *Prorocentrum* that for the purpose of this study have been called "*Small Prorocentrum*".

Study Site 1: St. Joseph Bay (Panhandle, Florida)

All fifteen substrates and all samples collected in St. Joseph Bay had *Prorocentrum* present. Eight of nine species of *Prorocentrum* were found in St. Joseph Bay, with only *P. micans* not present (Fig. 2-17). *P. mexicanum* outnumbers all other species exponentially (Fig. 2-18). There is variation in numbers of species by season (Figs. 2-19, 2-20, 2-21, 2-22), which also could be attributed to variances between stations. *P. belizeanum* was present in samples collected in winter and fall (stations 1, 5, 6, 7, 8), but not in spring or summer samples (stations 1, 2, 3, 4). In spring (stations 1 and 2), the substrates *Gracilaria* sp., *H. musciformes* and *T. testudinum* had the highest densities of *Prorocentrum* (Fig. 2-19). For summer (stations 3 and 4), *H. beaudettei* and *T. testudinum* maintained highest densities of *Prorocentrum,* with an overall decrease in *Prorocentrum* numbers compared to the other three seasons (Fig. 2-20). Fall (stations 5, 6, and 7) demonstrated a *Prorocentrum* substrate preference for *H. beaudettei, S. filamentosa*, and *T. testudinum* (Fig. 2-21). During winter season (stations 1 and 8), living *T. testudinum* was not present, and a shift in substrate preference of *Prorocentrum* was observed, with *Chondria* sp., *Enteromorpha* sp., *Heterosiphonia* sp., and *Lyngbya* sp. maintaining the highest abundances (Fig. 2-22), which also corresponded with a drop in salinity. The majority of toxic *Prorocentrum* cells in St. Joseph Bay are found within a salinity of 28 – 36 ppt (Fig. 2-23) and a temperature range of 20 – 30º C (Fig. 2-24). Overall, the highest numbers of toxic *Prorocentrum* in St. Joseph Bay were on the substrate *Gracilaria* sp. (n = 2), followed by *Dasycladus vermicularis* (n = 4) and *T. testudinum* (n = 14, Fig. 2-25).

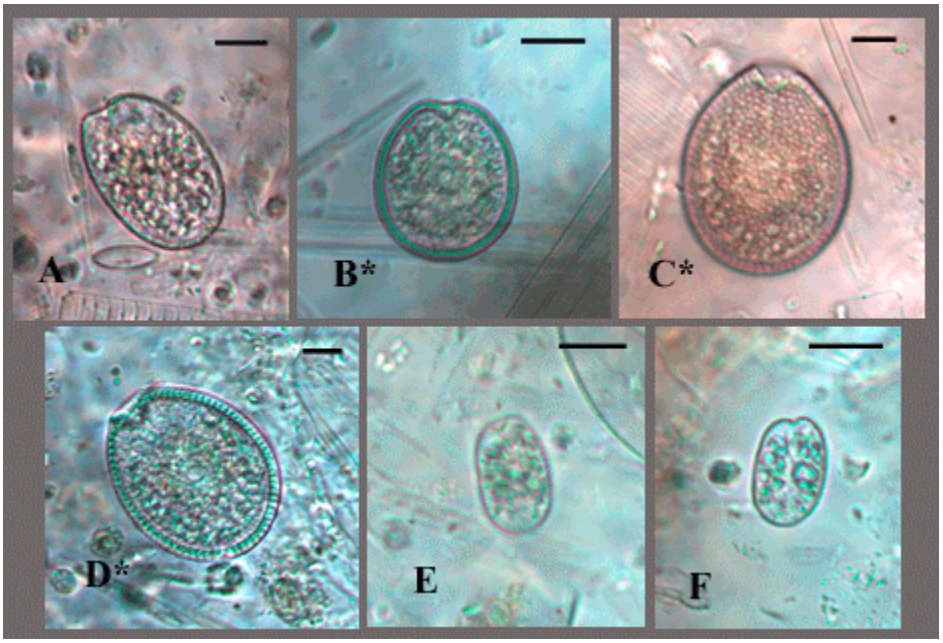


Figure 2-17. *Prorocentrum* spp. from St. Joseph Bay: A) *P. mexicanum*, B) *P. concavum**, C) *P.* cf *hoffmannianum**, D) another *P.* cf *hoffmannianum**, E) "Small *Prorocentrum"*, F) another "Small *Prorocentrum"*. *P. lima**, *P. emarginatum, P. belizeanum**, and *P. taylori* not shown. Scale = 10 µm, * - OA producing species.

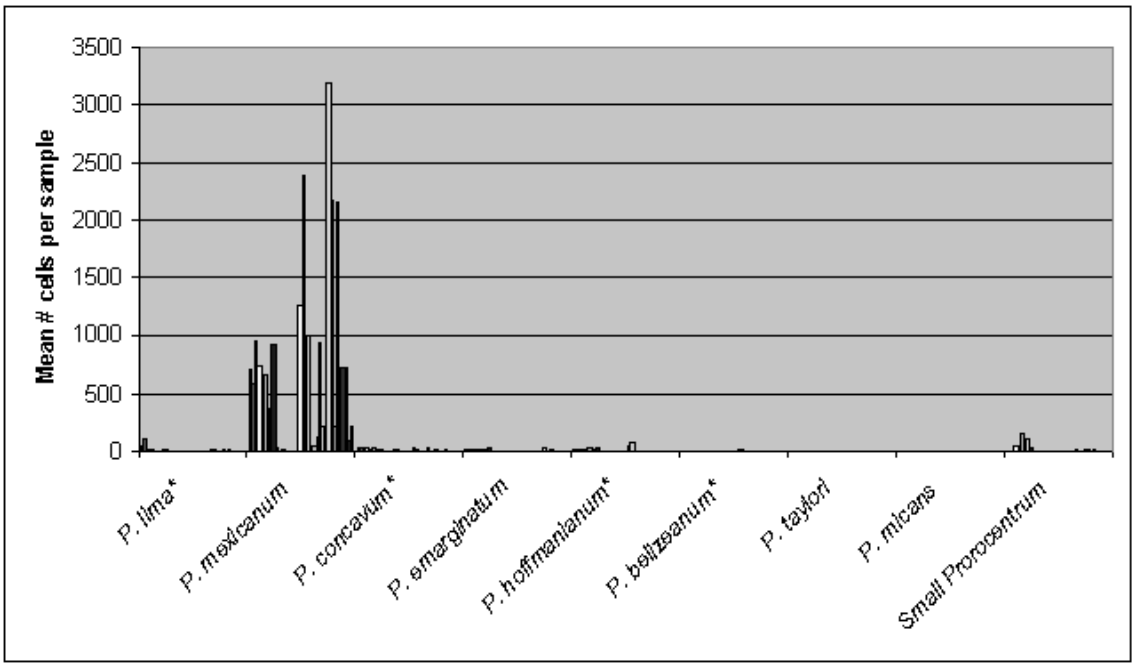


Figure 2-18. *P. mexicanum* (a non-OA producing species) exceeds numbers of all other *Prorocentrum* spp. in St. Joseph Bay (over all substrates and all seasons).

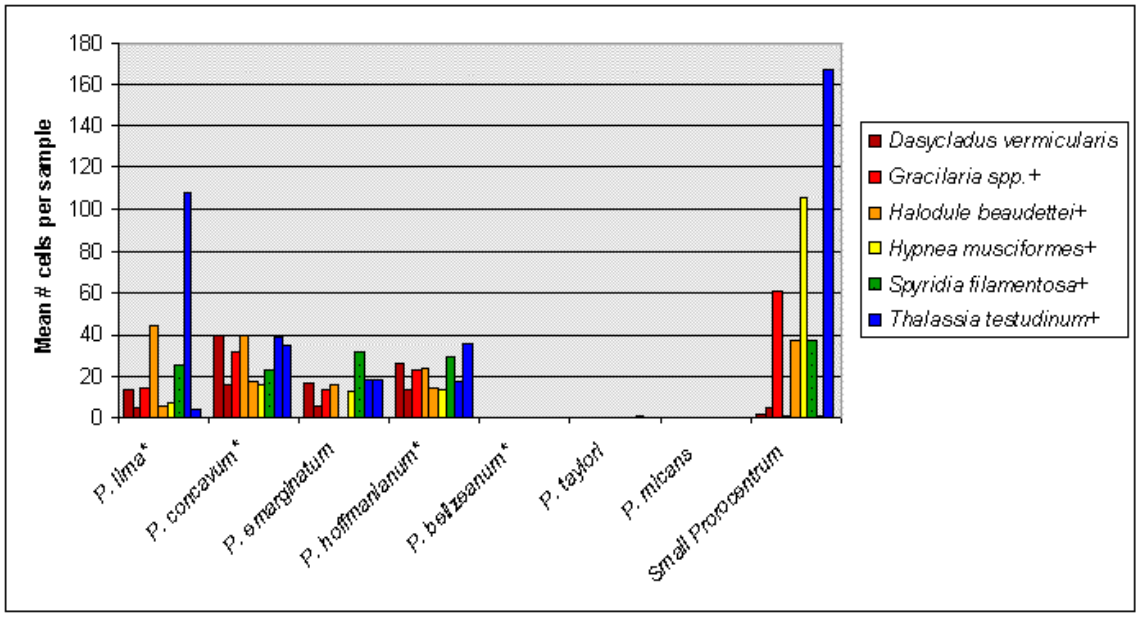


Figure 2-19. *Prorocentrum* spp. found on substrates collected in spring (May) in St. Joseph Bay (stations 1 and 2), excluding *P. mexicanum*. *- OA-producing *Prorocentrum* species, + - found in green turtle diet.

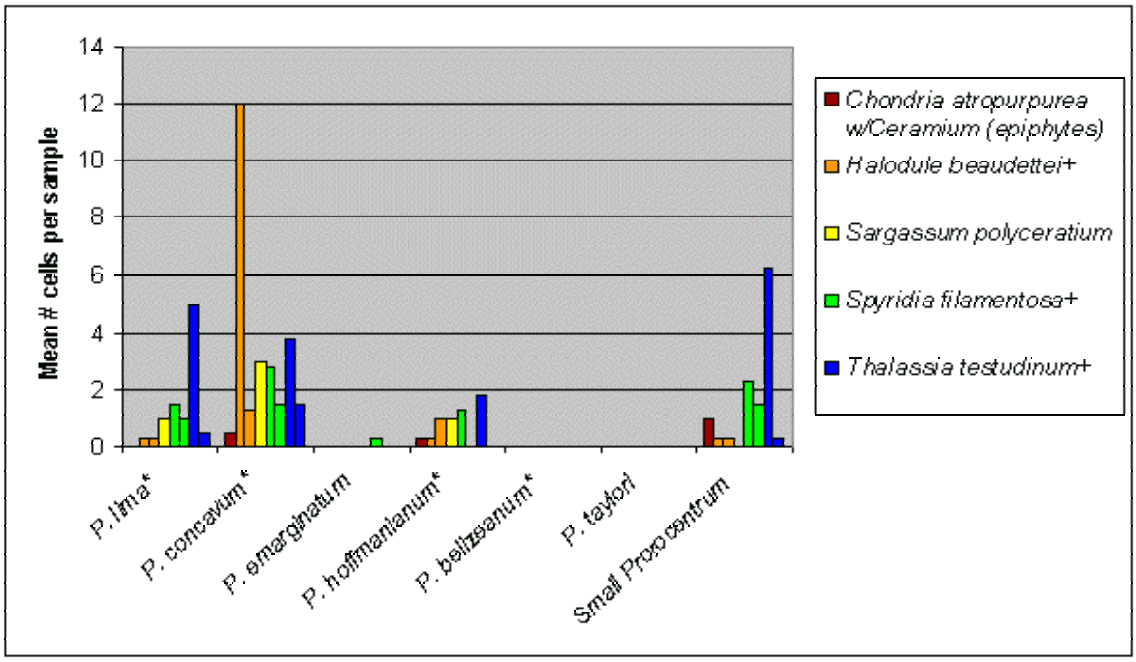


Figure 2-20. *Prorocentrum* spp. found on substrates collected in summer (August) in St. Joseph Bay (stations 3 and 4), excluding *P. mexicanum*. * - OA-producing *Prorocentrum* species, +- found in green turtle diet.

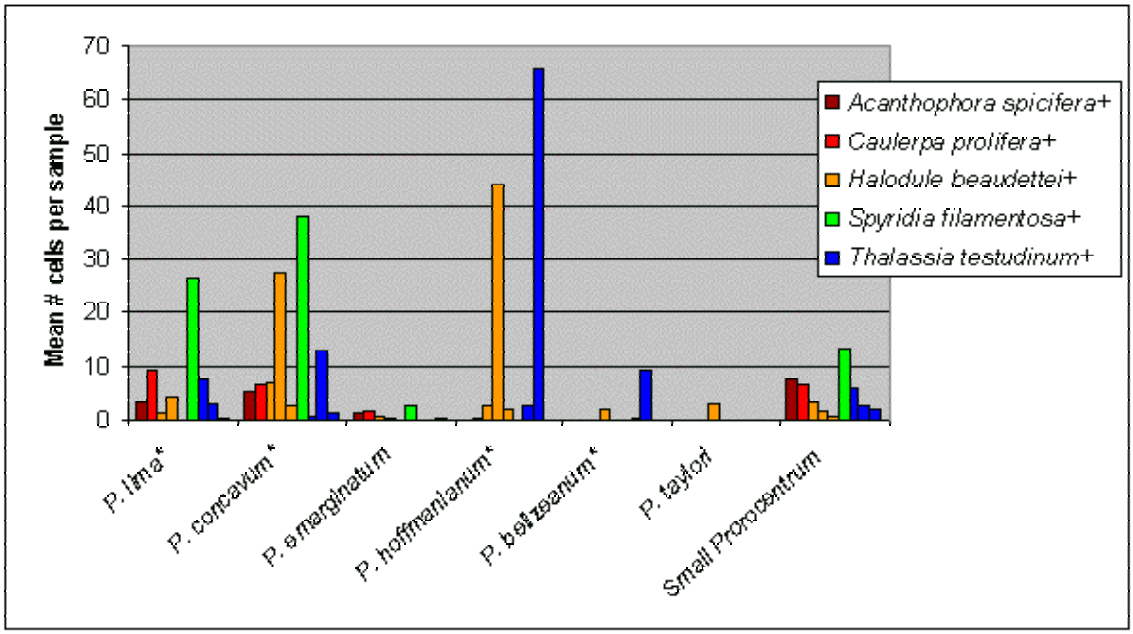


Figure 2-21. *Prorocentrum* spp. found on substrates collected in fall (November) in St. Joseph Bay (stations 5, 6, and 7), excluding *P. mexicanum*. * - OA-producing *Prorocentrum* species, +- found in green turtle diet.

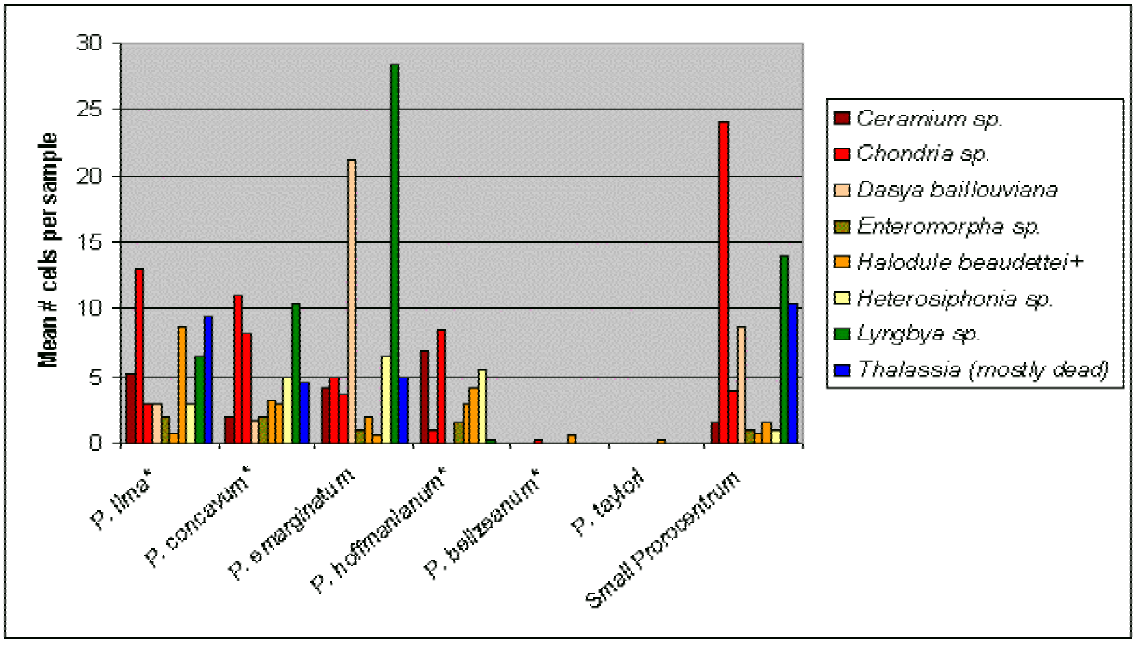


Figure 2-22. *Prorocentrum* spp. found on substrates collected in winter (February) in St. Joseph Bay (stations 1 and 8), excluding *P. mexicanum*. * - OA-producing *Prorocentrum* species, +- found in green turtle diet.

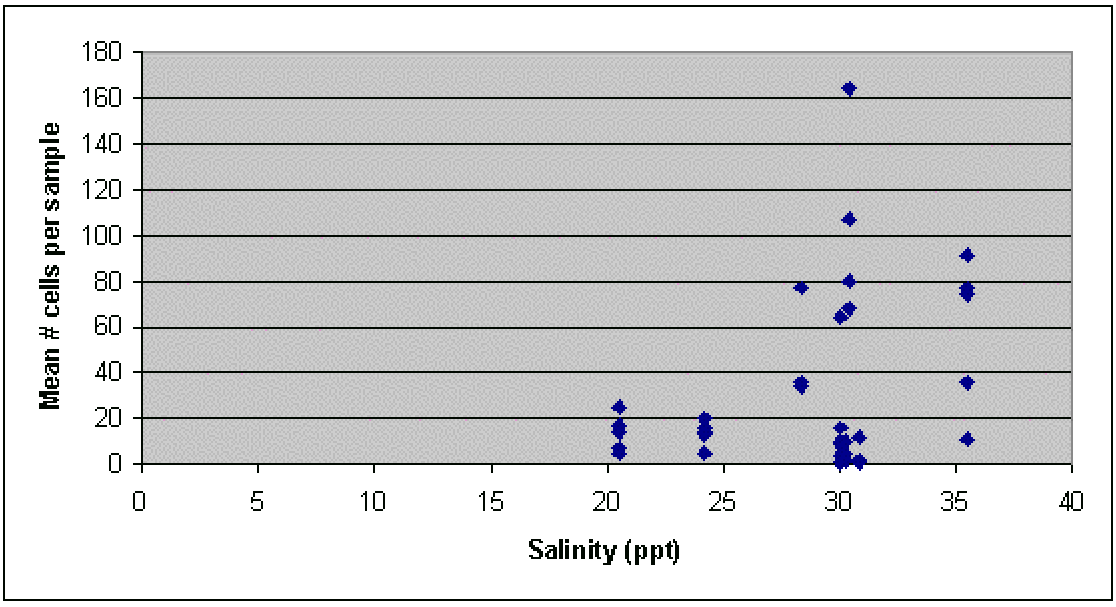


Figure 2-23. Toxic *Prorocentrum* vs. salinity at St. Joseph Bay.

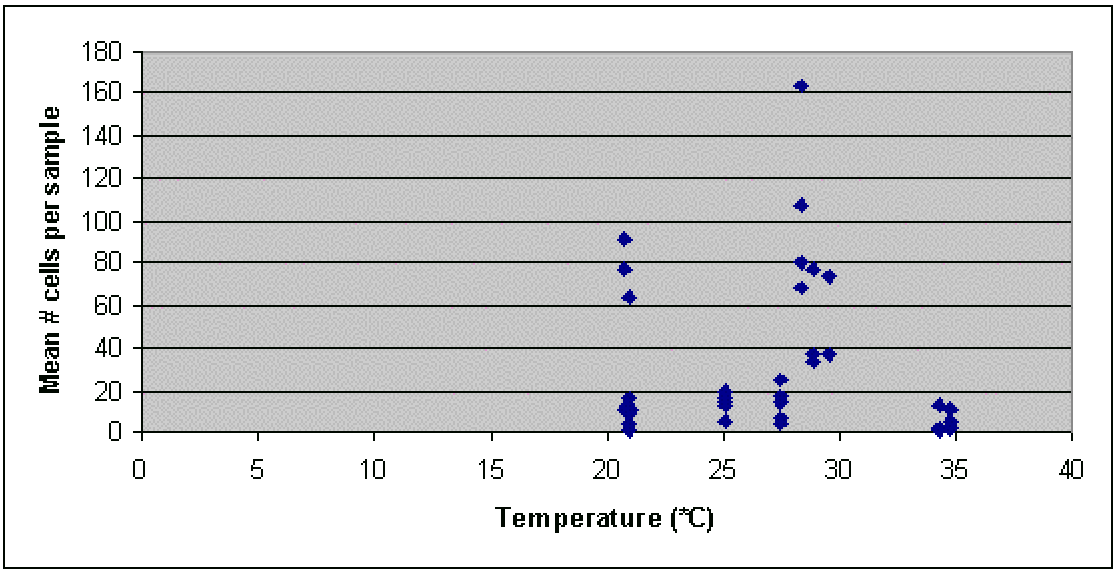


Figure 2-24. Toxic *Prorocentrum* vs. temperature in St. Joseph Bay.

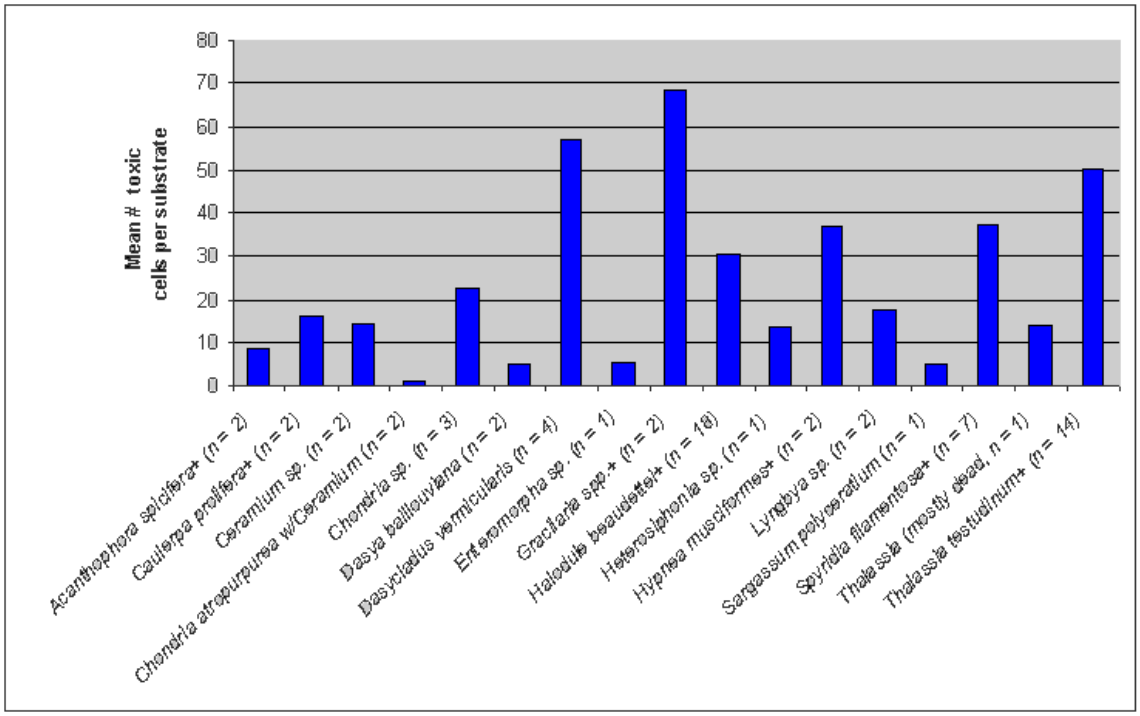


Figure 2-25. Mean toxic *Prorocentrum* cells by substrate in St. Joseph Bay.

Study Site 2: Cedar Key vicinity / Seahorse Key (Northwest Florida)

All samples collected in the Cedar Key vicinity had *Prorocentrum*, with a total of 11 substrates. *P. belizeanum* was the only species of the nine *Prorocentrum* spp. not found at this site, although *P. micans* and *P. taylori* were present in very low numbers (Figs. 2-26, 2-27, 2-28, and 2-29). *Prorocentrum* abundance was much higher in spring and summer (stations 1, 2, 3, and 4). *P. lima* was the prevalent toxic species present in all seasons except for winter, when *P. concavum* reached similar numbers (Fig. 2-26, 2-27, 2-28, and 2-29). The majority of toxic *Prorocentrum* cells were found within a salinity range of 28 – 32 ppt (Fig. 2-30) and a temperature range of 24 – 30.2º C (Fig. 2-31). Overall, the three substrates with the highest number of toxic *Prorocentrum* in the

Cedar Key vicinity were seagrasses: *Syringodium filiforme* mix (n = 2), followed by *Halophila englemannii* (n = 2) and *T. testudinum* (n = 12, Fig. 2-32).

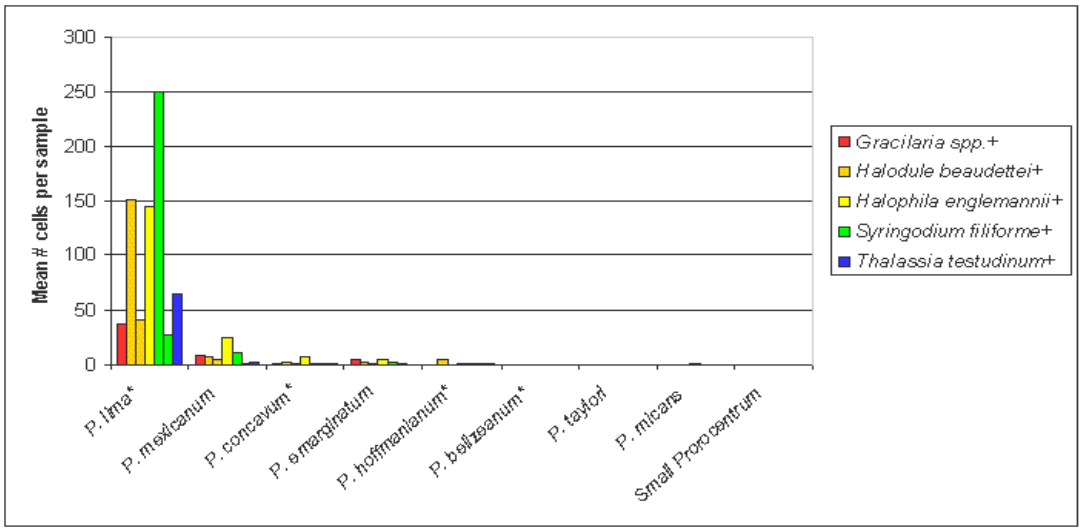


Figure 2-26. *Prorocentrum* spp. found on substrates collected in spring (May) in the Cedar Key vicinity (stations 1 and 2). * – OA-producing *Prorocentrum* sp., + – found in green turtle diet.

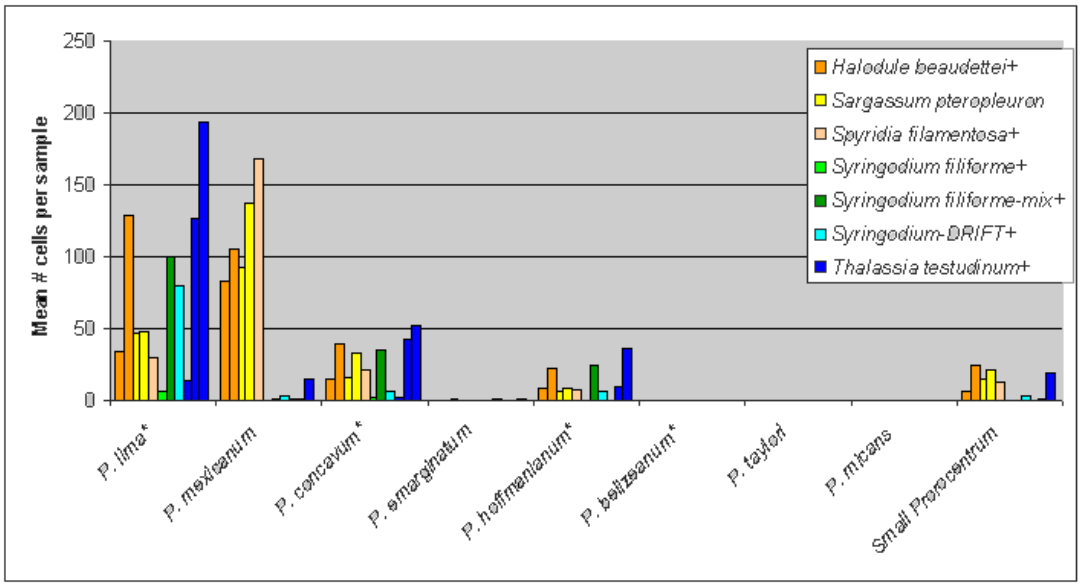


Figure 2-27. *Prorocentrum* spp. found on substrates collected in summer (August) in the Cedar Key vicinity (stations 2, 3, and 4). * – OA-producing *Prorocentrum* sp., + – found in green turtle diet.

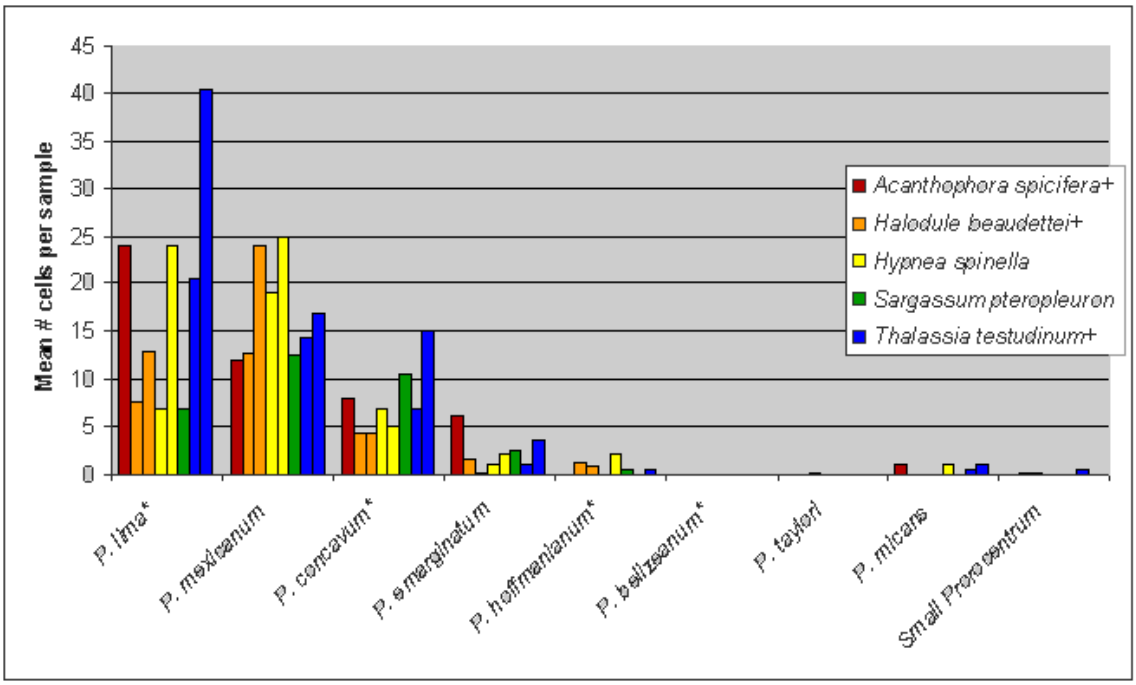


Figure 2-28. *Prorocentrum* spp. found on substrates collected in fall (November) in the Cedar Key vicinity (stations 5 and 6). * – OA-producing *Prorocentrum* sp., + – found in green turtle diet.

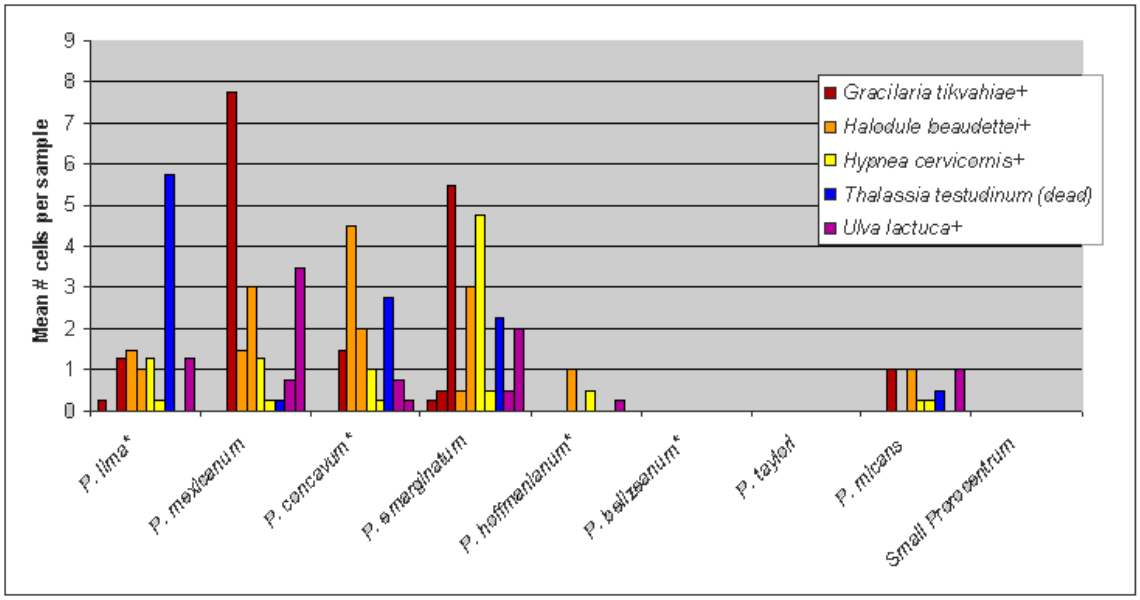


Figure 2-29. *Prorocentrum* spp. found on substrates collected in winter (February) in the Cedar Key vicinity (stations 7 and 8). * – OA-producing *Prorocentrum* sp., + – found in green turtle diet.

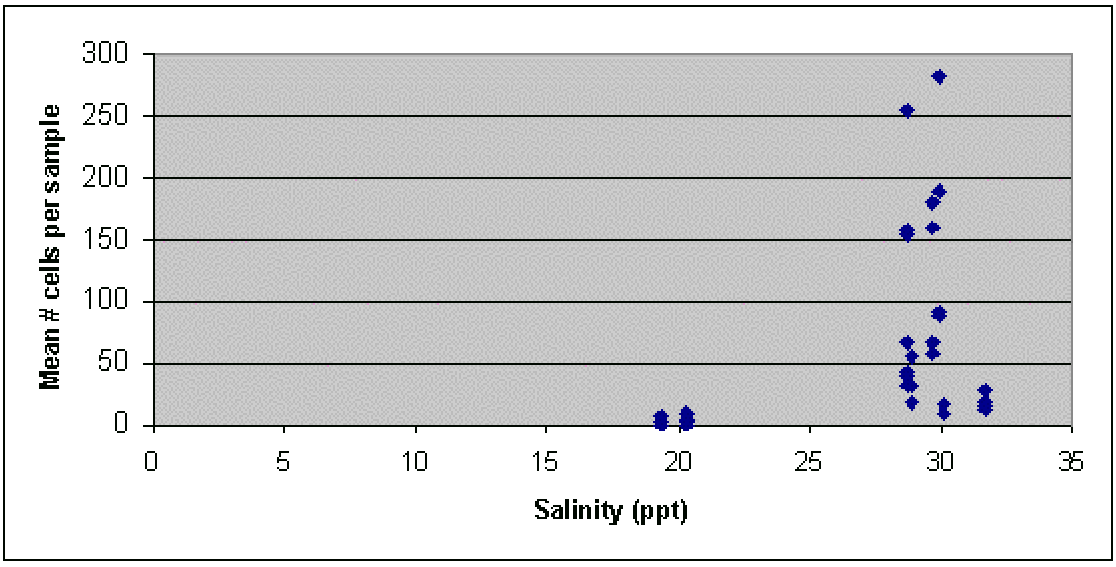


Figure 2-30. Toxic *Prorocentrum* vs. salinity in the Cedar Key vicinity.

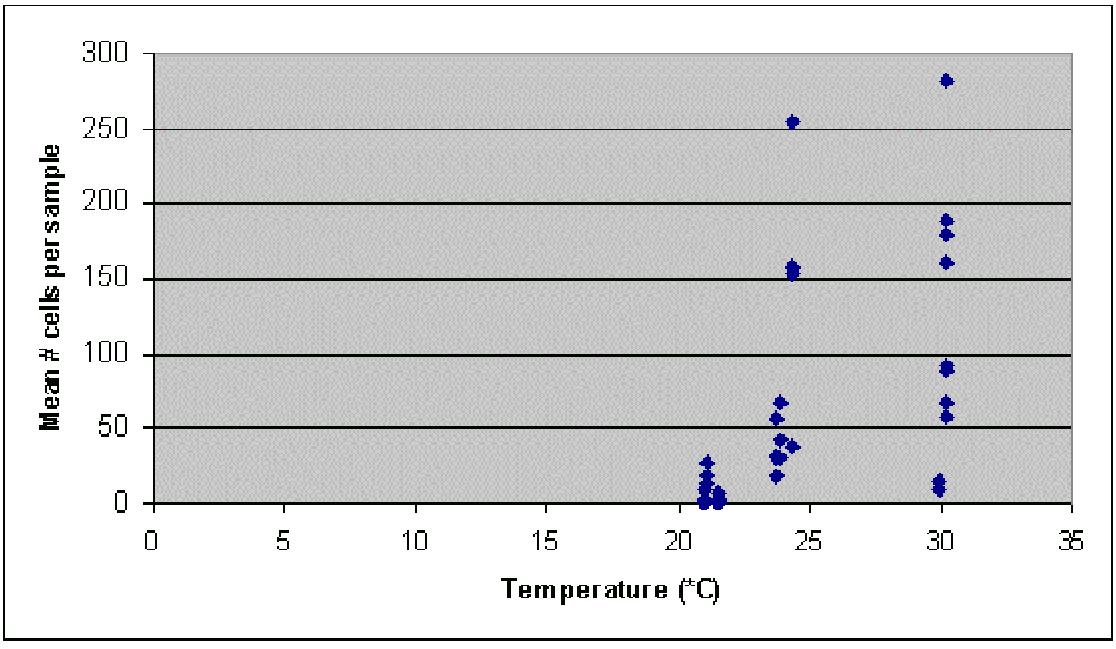


Figure 2-31. Toxic *Prorocentrum* vs. temperature in the Cedar Key vicinity.

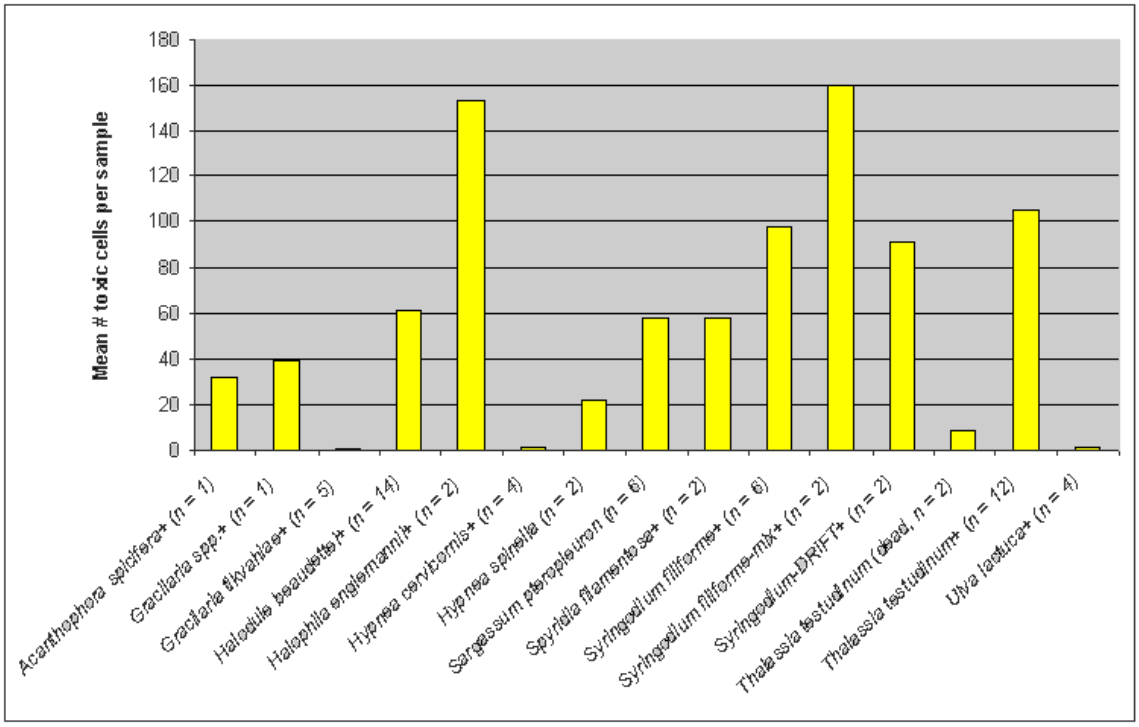


Figure 2-32. Mean toxic *Prorocentrum* by substrate in the Cedar Key vicinity.

Study Site 3: Mosquito Lagoon (East-Central Florida)

All 13 substrates collected in Mosquito Lagoon had *Prorocentrum*. The highest abundance of *Prorocentrum* spp. was found in the spring (stations 1 and 2, Fig. 2-33, 2-34, 2-35, and 2-36). *P. mexicanum* is the most abundant species for all seasons and stations, while *P. belizeanum* was the only Florida species not found at this site. *P. taylori* had only one cell found within all the samples (n = 72).

The majority of toxic *Prorocentrum* cells were found within a salinity range of 28 – 33 ppt (Fig. 2-37) and a temperature range of 25 – 35º C (Fig. 2-38). Overall, the highest numbers of toxic *Prorocentrum* were found on the substrate *H. beaudettei*, followed by *Hypnea musciformes* (n = 6) and *A. spicifera* (n = 5, Fig. 2-39). Two samples of *Halodule beaudettei* from station 3 collected in the summer had very high

numbers of both toxic (predominantly *P. concavum*) and non-OA toxic (*P. mexicanum*) cells. This is significant, for it indicates that *Prorocentrum* abundance, and hence potential turtle exposure to toxic *Prorocentrum*, can be very high within Mosquito Lagoon.

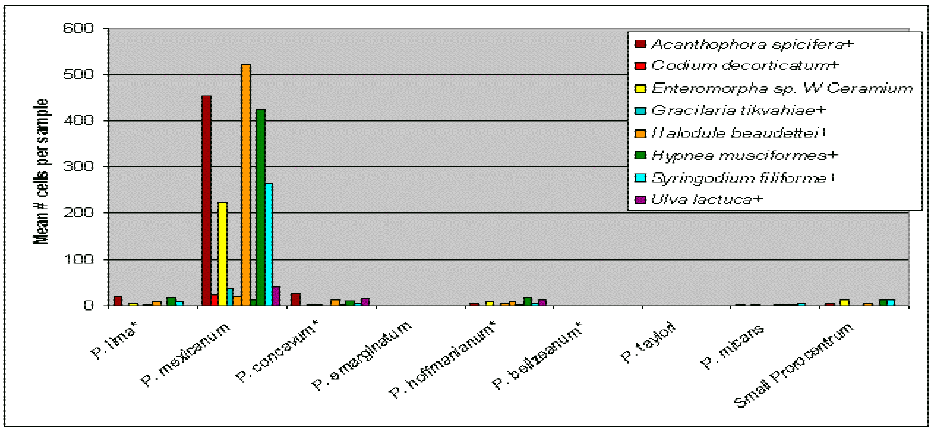


Figure 2-33. *Prorocentrum* spp. found on substrates collected in spring (May) in Mosquito Lagoon (stations 1 and 2). * – OA-producing *Prorocentrum* sp., + – found in green turtle diet.

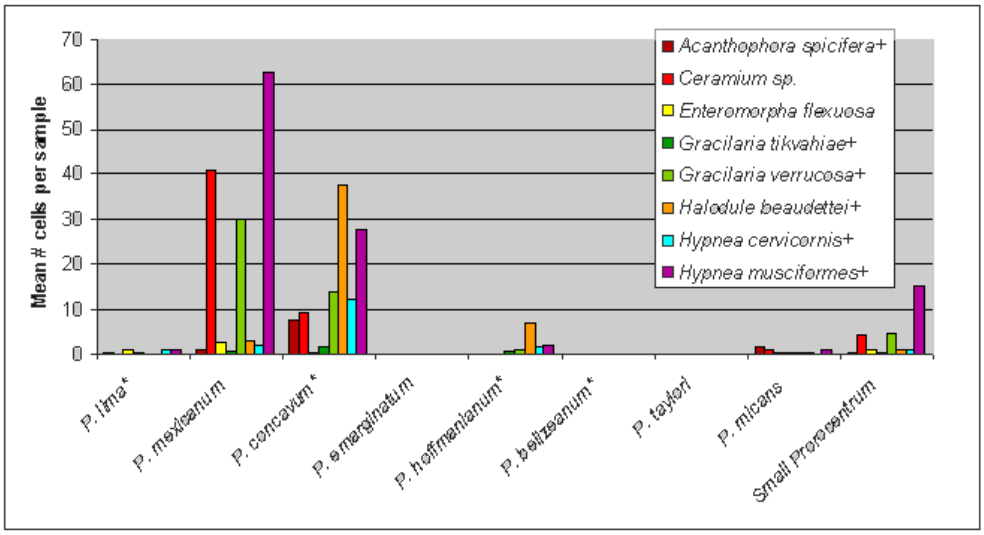


Figure 2-34. *Prorocentrum* spp. found on substrates collected in summer (August) in Mosquito Lagoon (stations 3, 4, and 5), excluding one *H. beaudettei* sample (>3000 *P. mexicanum*, 340 *P. concavum*). * – OA-producing *Prorocentrum* sp., + – found in green turtle diet.

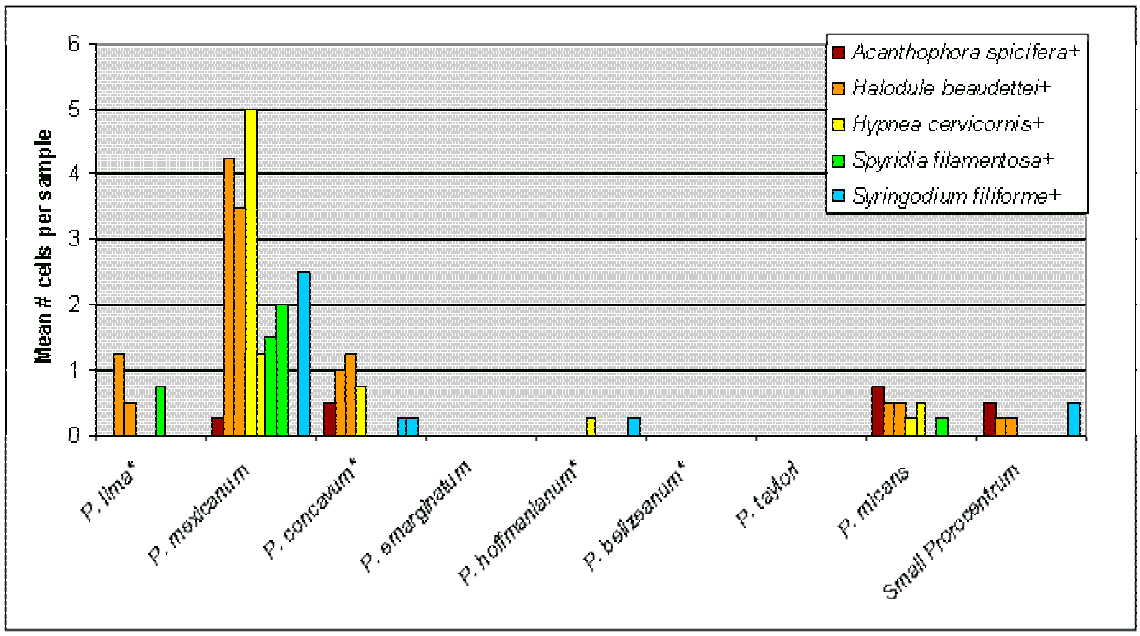


Figure 2-35. *Prorocentrum* spp. found on substrates collected in fall (November) in Mosquito Lagoon (stations 6 and 7). * – OA-producing *Prorocentrum* sp., + – found in green turtle diet.

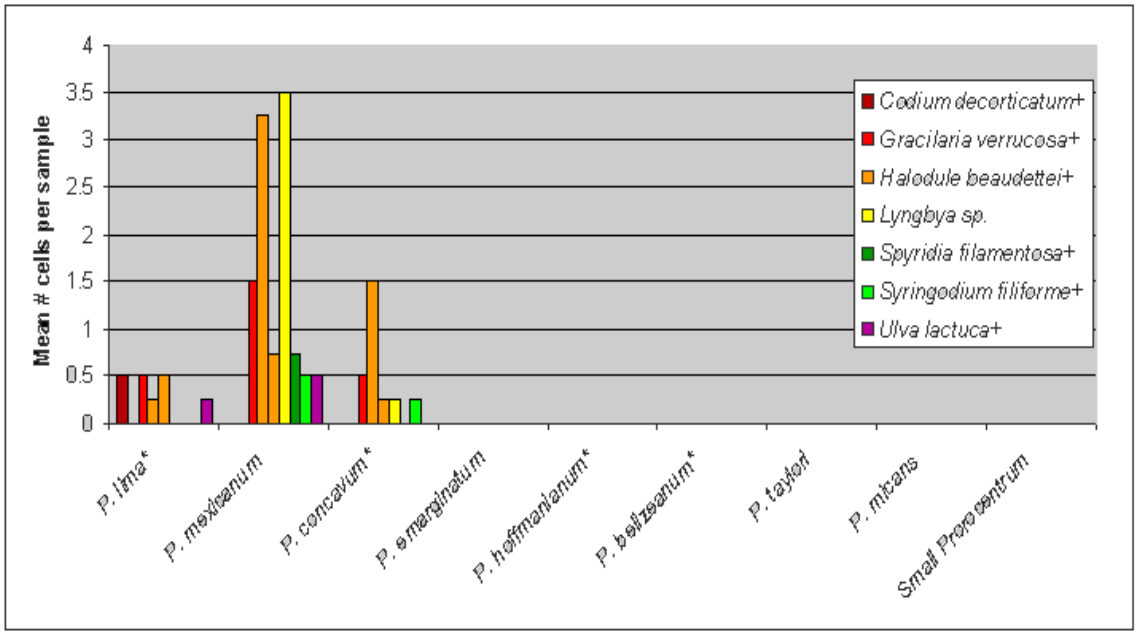


Figure 2-36. *Prorocentrum* spp. found on substrates collected in winter (February) in Mosquito Lagoon (stations 1, 2, and 8). * – OA-producing *Prorocentrum* sp., + – found in green turtle diet.

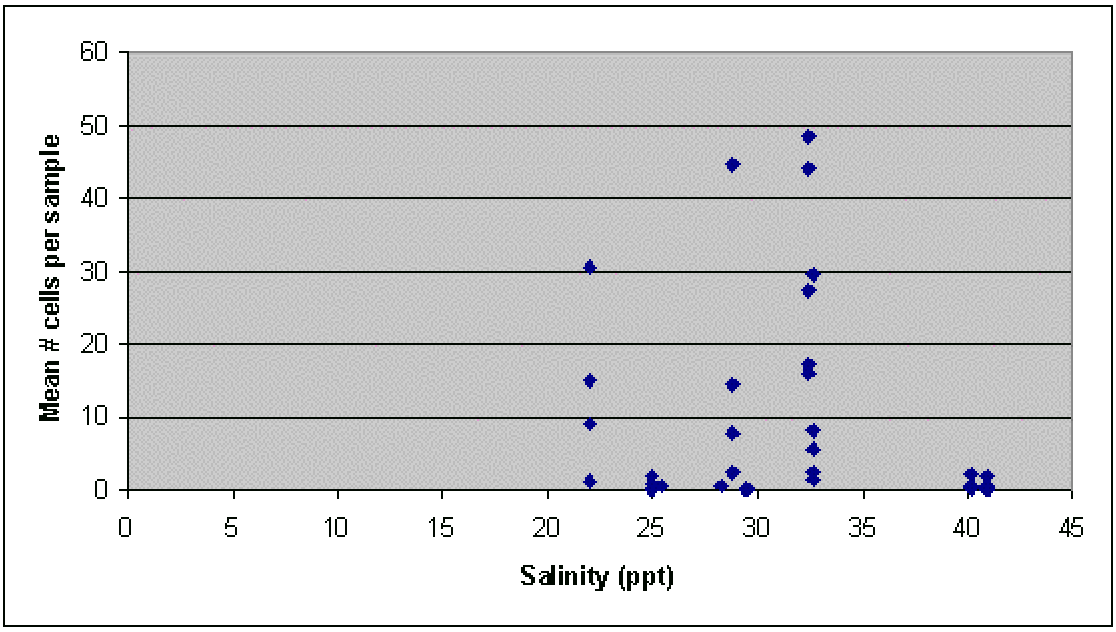


Figure 2-37. Toxic *Prorocentrum* vs. salinity in Mosquito Lagoon.

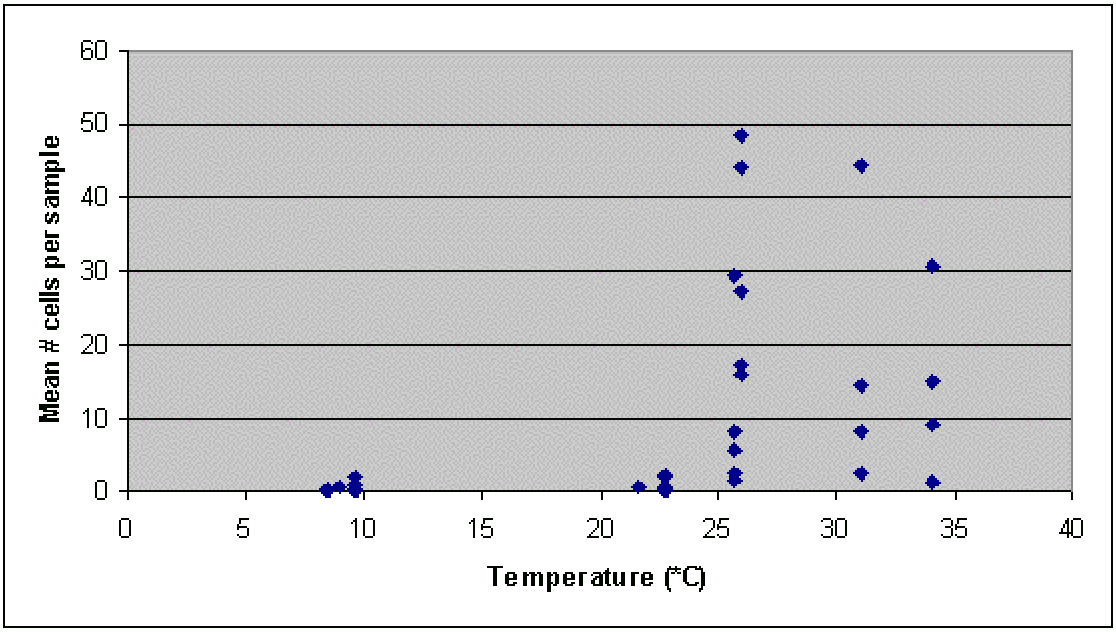


Figure 2–38. Toxic *Prorocentrum* vs. temperature in Mosquito Lagoon.

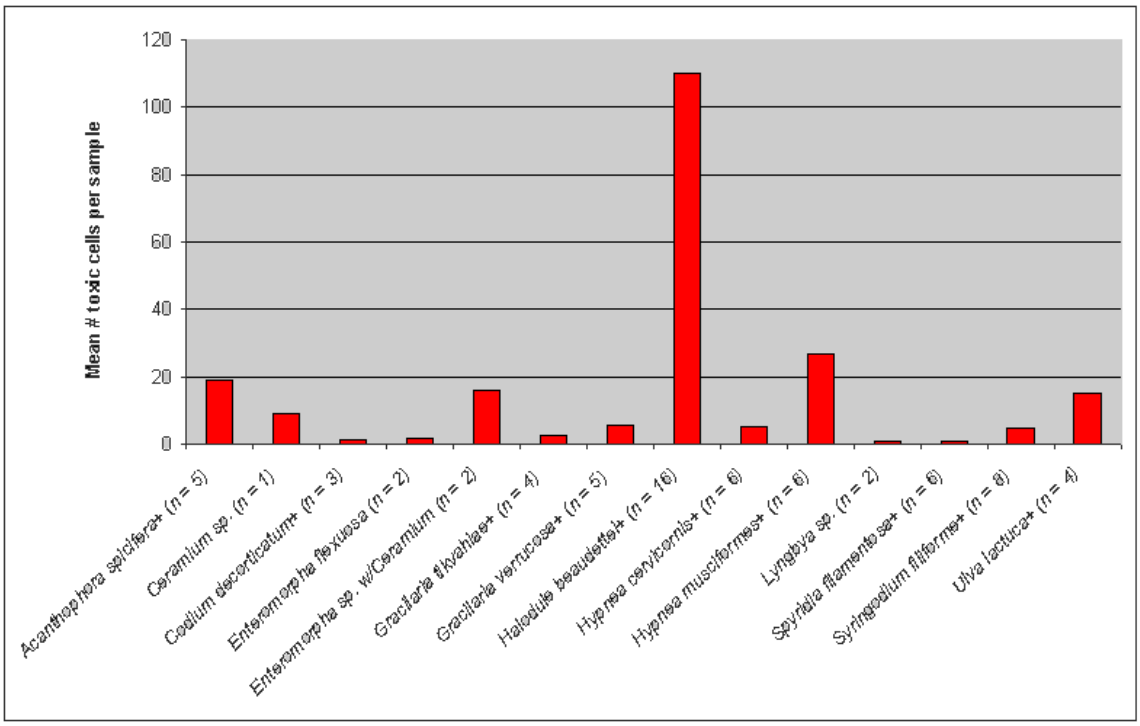


Figure 2-39. Mean toxic *Prorocentrum* by substrate in Mosquito Lagoon.

Study Site 4: Florida Bay (South Florida)

All 12 substrates collected in Florida Bay had *Prorocentrum*. All 9 species of *Prorocentrum* (Fig. 2-40) identified in this study are represented, with *P. taylori* and *P. micans* least abundant. For the spring season, the majority of *Prorocentrum* species were <140 cells per sample, with the substrates *C. tenuissima* and *T. testudinum* maintaining the highest densities (Fig. 2-41). For summer, the numbers of *Prorocentrum* species were <240 cells per sample (with the majority <100 cells per sample), with *H. beaudettei*, *P. dumetosus*, and *T. testudinum* substrates maintaining the highest densities (Fig. 2-42). For fall, the numbers were similar to summer results, with *H. beaudettei, S. filiforme*, and *T. testudinum* maintaining highest densities of *Prorocentrum* (Fig. 2-43). For winter, the densities of *Prorocentrum* species decreased to <50 cells per sample, with the substrates

C. occidentalus, D. cervicornis, H. beaudettei, and T. testudinum maintaining the highest densities (Fig. 2-44).

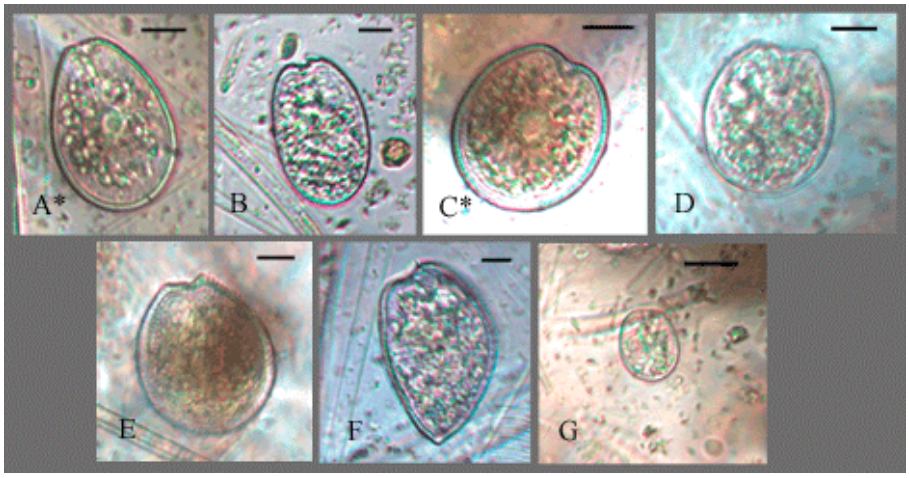


Figure 2-40. *Prorocentrum* spp. from Florida Bay. A) *P. lima**, B) *P. mexicanum*, C) *P. concavum**, D) *P. emarginatum*, E) *P.* cf *taylori,* F) *P. micans,* G) "Small *Prorocentrum*". Not shown: *P.* cf *hoffmannianum** and *P. belizeanum**. * - OA-producing species. Scale = 10 µm.

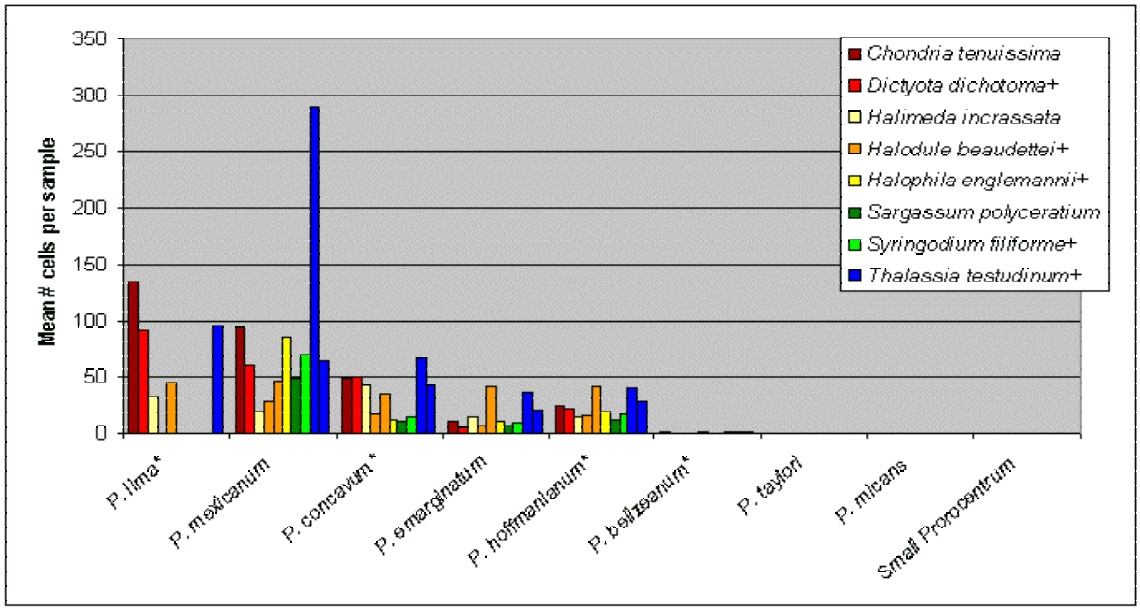


Figure 2-41. *Prorocentrum* spp. found on substrates collected in spring (May) in Florida Bay (stations 1 and 2). * – OA-producing *Prorocentrum* sp., + – found in green turtle diet.

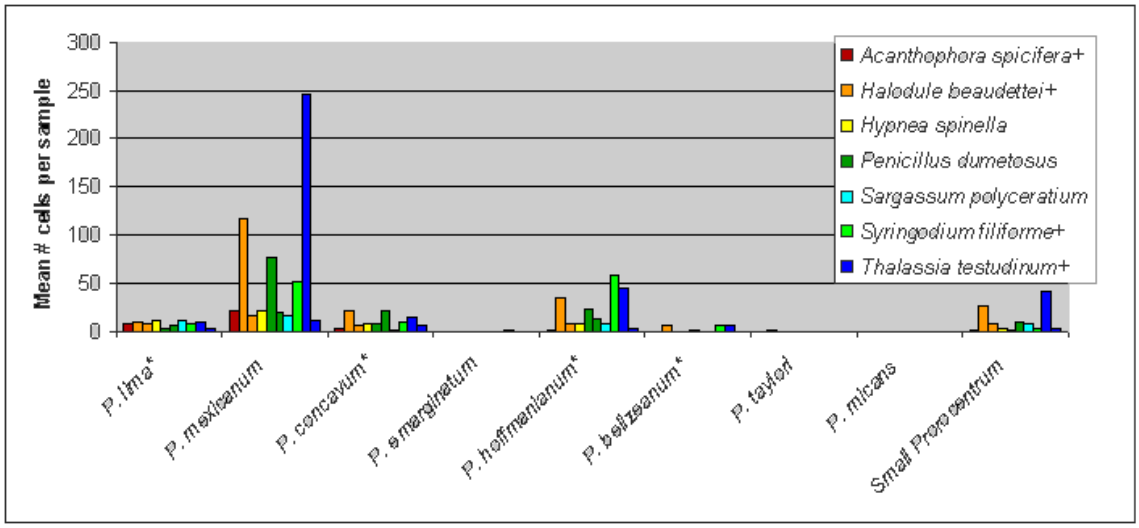


Figure 2-42. *Prorocentrum* spp. found on substrates collected in summer (August) in Florida Bay (stations 1 and 2). * – OA-producing *Prorocentrum* sp., + – found in green turtle diet.

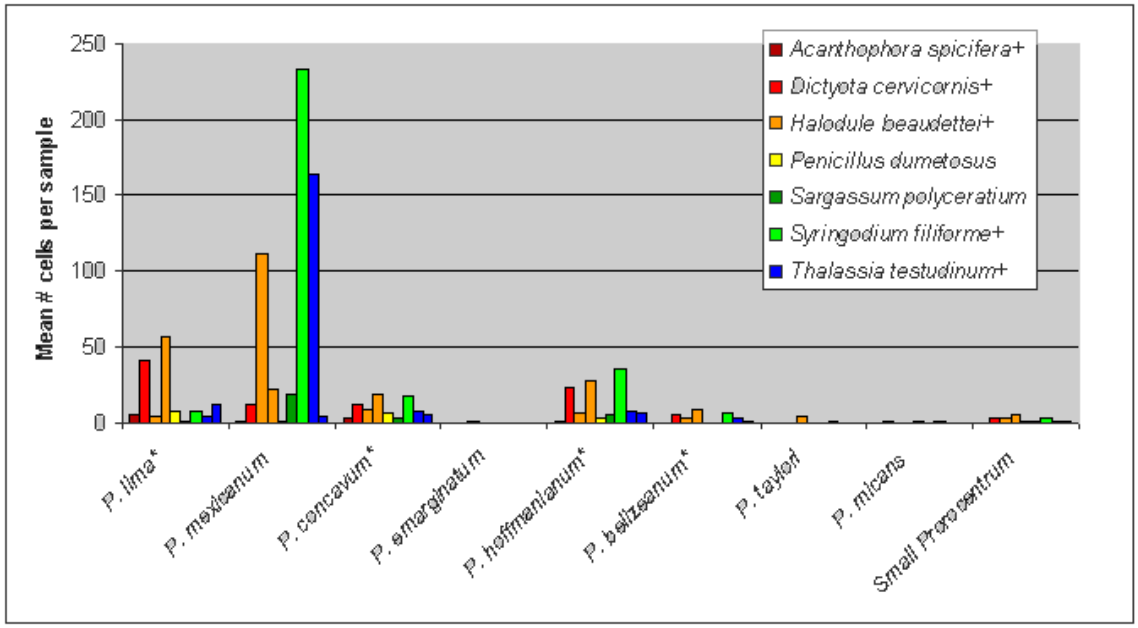


Figure 2-43. *Prorocentrum* spp. found on substrates collected in fall (November) in Florida Bay (stations 1 and 2). * – OA-producing *Prorocentrum* sp., + – found in green turtle diet.

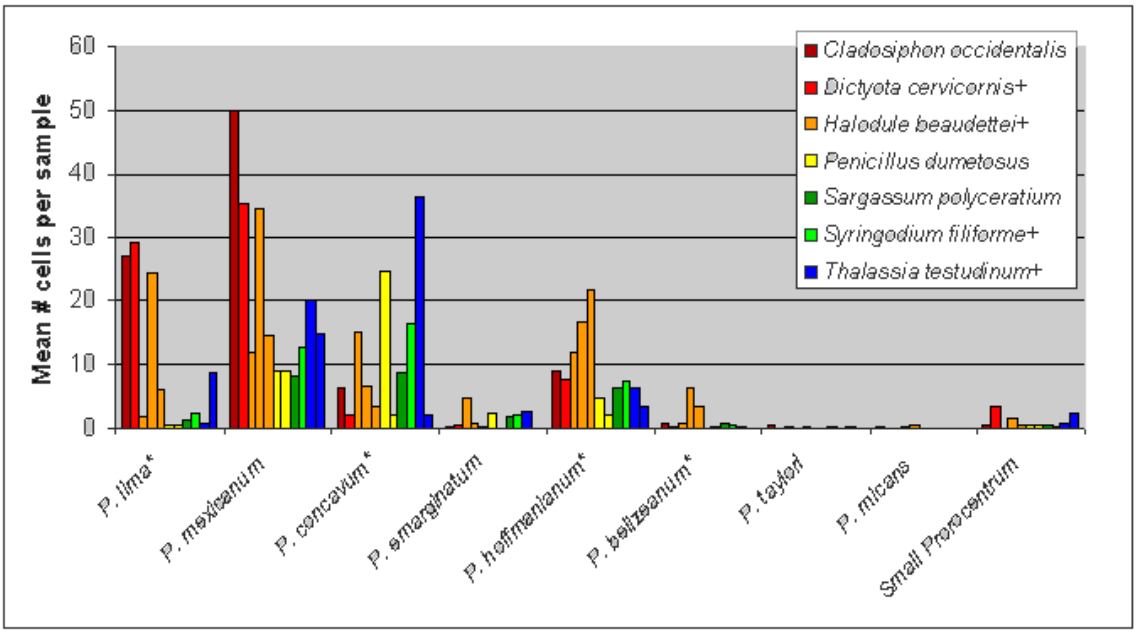


Figure 2-44. *Prorocentrum* spp. found on substrates collected in winter (February) in Florida Bay (stations 1, 2, and 3). * – OA-producing *Prorocentrum* sp., + – found in green turtle diet.

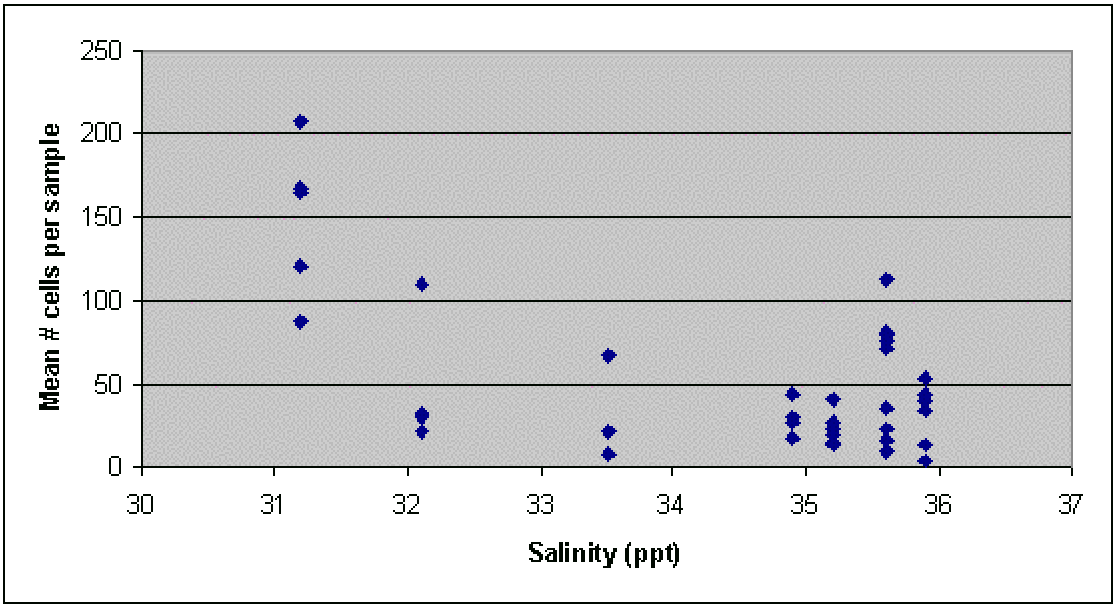


Figure 2-45. Toxic *Prorocentrum* vs. salinity in Florida Bay.

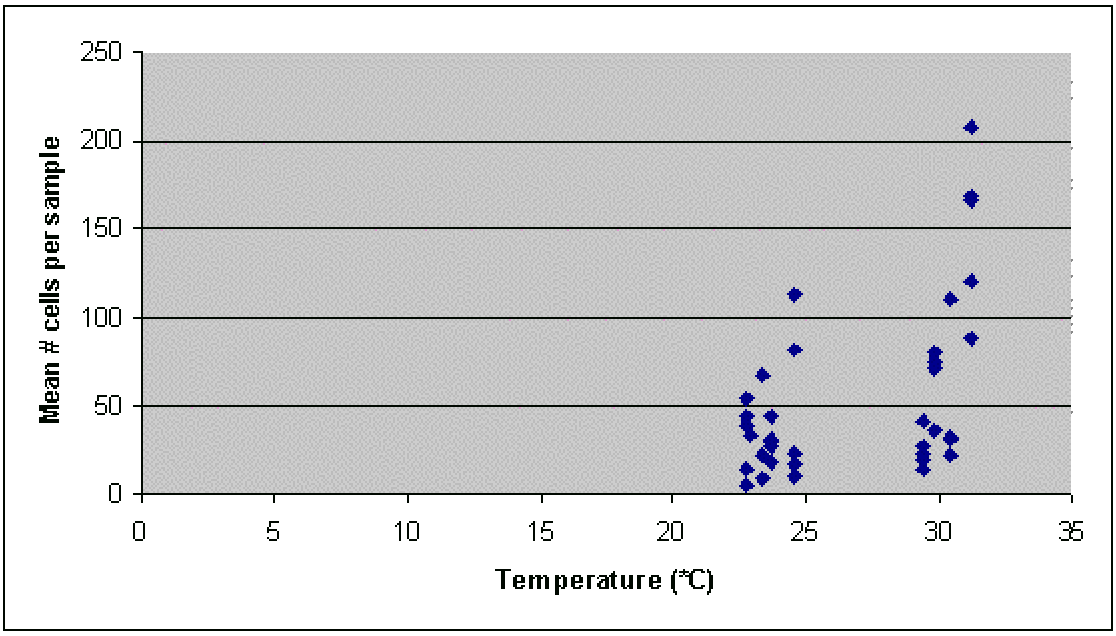


Figure 2-46. Toxic *Prorocentrum* vs. temperature in Florida Bay.

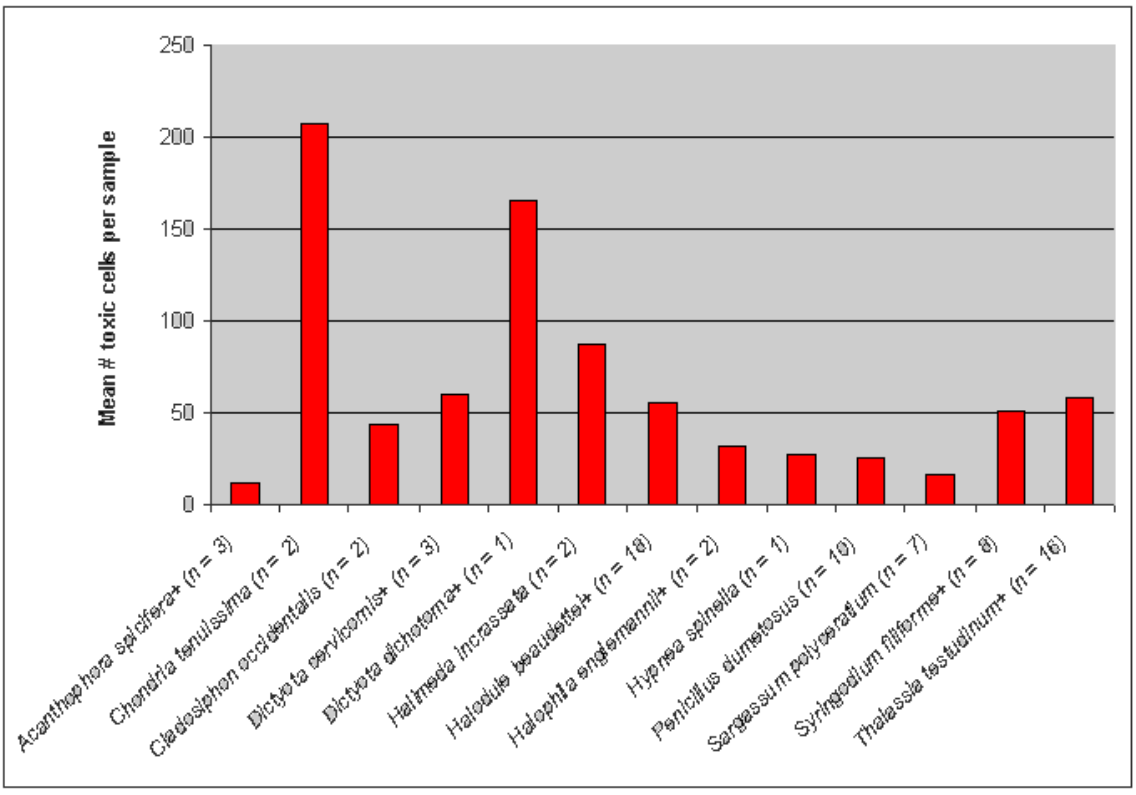


Figure 2-47. Mean toxic *Prorocentrum* by substrate in Florida Bay.

The salinity range of 31 – 36 ppt (Fig. 2-45) and temperature range of 24 – 32º C (Fig. 2-46) encompass all the samples collected, with relatively high numbers found throughout both ranges. Overall, the highest number of toxic *Prorocentrum* was found on the substrate *Chondria tenuissima* (n = 2), followed by *Dictyota dichotoma* (n = 1) and *Halimeda incrassata* (n = 2, Fig. 2-47).

Substrate preference in Florida. Toxic *Prorocentrum* were present on all 29 species of substrate evaluated in Florida. However, variation was seen amongst sites (Table 2-1), and there were samples collected with no *Prorocentrum* present. Mosquito Lagoon, a site with high FP prevalence, had the smallest percent of samples with toxic *Prorocentrum* but the largest range of toxic *Prorocentrum* cells per g/wet substrate.

Table 2-1. Summary of Florida results (over all four seasons).

	St. Joseph Bay	Cedar Key	Mosquito Lagoon	Florida Bay
Samples w/*Prorocentrum*	98.5% (n = 68)	97.0% (n = 65)	75.3% (n = 73)	97.4% (n = 77)
Substrate spp. w/*Prorocentrum*	100% (n = 16)	100% (n = 11)	100% (n = 13)	100% (n = 12)
Range of toxic cells per g/wet substrate	0 – 305.0	0 – 524.2	0 – 693.3	0 – 455.8
Mean (toxic cells per g/wet substrate)	60.4 (n = 68)	108.9 (n = 65)	33.1 (n = 73)	83.7 (n = 77)

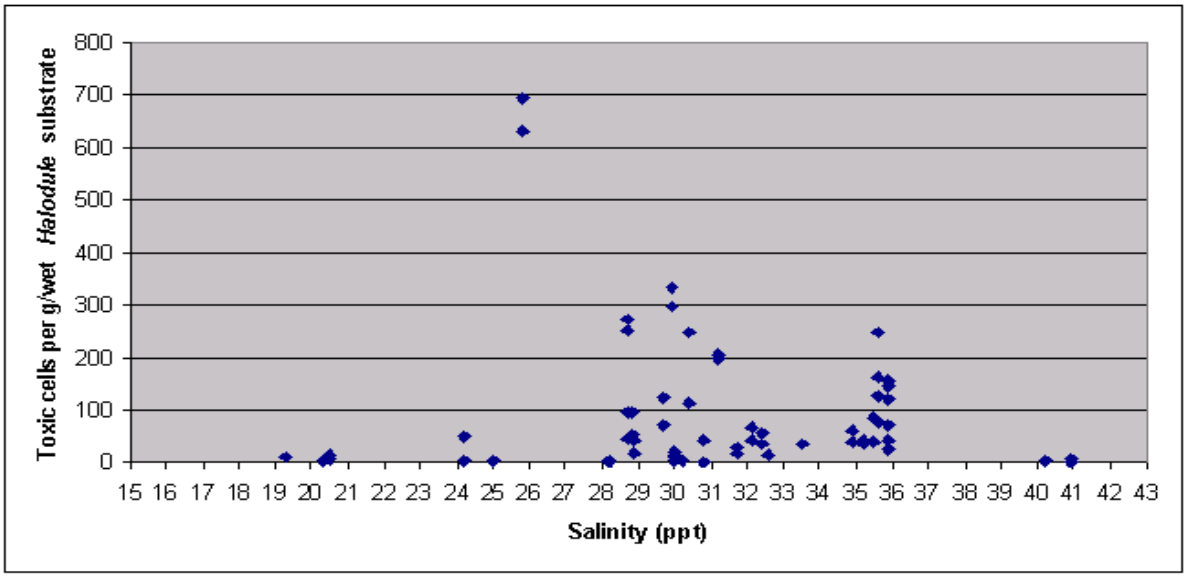


Figure 2-48. Salinity vs. toxic *Prorocentrum* on g/wet *Halodule* substrate in Florida.

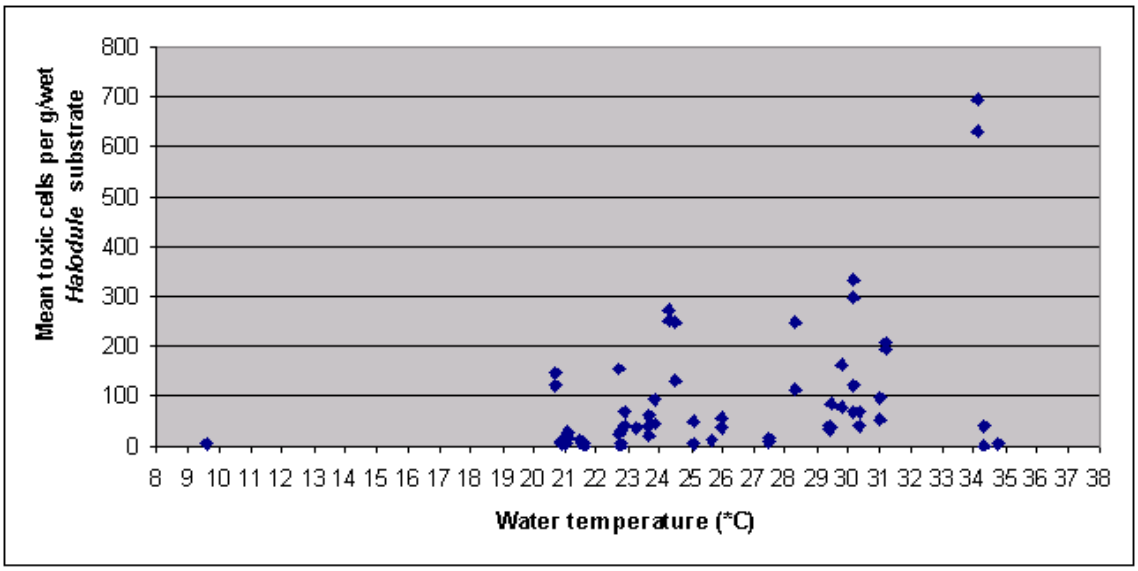


Figure 2-49. Temperature vs. toxic *Prorocentrum* on g/wet *Halodule* substrate in Florida.

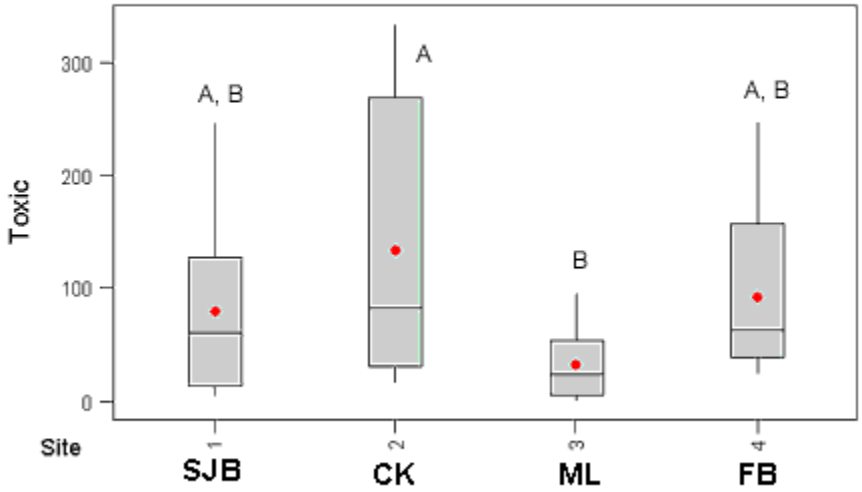


Figure 2-50. *Prorocentrum* cells per g/wet *Halodule* substrate amongst the Florida study sites, within the designated salinity and temperature range (1 – St. Joseph Bay, 2 – Cedar Key, 3 – Mosquito Lagoon, 4- Florida Bay). Means bearing the same alphabet letter (A, B) are not significantly different at p = 0.10. The boxes represent the 75th percentile, with the line at the median, the red dot at the mean, and asterisks indicating outliers (no outliers in this particular graph).

Comparing toxic *Prorocentrum* abundance requires the definition of salinity and temperature ranges for an equivalent comparison amongst the sites of Florida. For salinity, the range of 27.5 – 36 ppt has the largest proportion of toxic *Prorocentrum* for all of the Florida sites (Fig. 2-48). For temperature, the range of 20 - 32 ºC is optimum (Fig. 2-49). Samples collected within these ranges will be used for the comparison amongst sites and amongst FP rates. This did reduce the sample size of *H. beaudettei* samples all four sites: St. Joseph Bay (n = 10), Cedar Key (n = 12), Mosquito Lagoon (n = 8), and Florida Bay (n = 18).

Comparison among sites. Within the salinity range of 27.5 - 36 ppt and temperature range of 20 - 32 ºC, mean toxic *Prorocentrum* abundance on *Halodule* substrate (over all four seasons) in Cedar Key is significantly different than Mosquito Lagoon (at p = 0.10, Fig. 2-50). St. Joseph Bay and Florida Bay are not significantly different than one another, Cedar Key or Mosquito Lagoon.

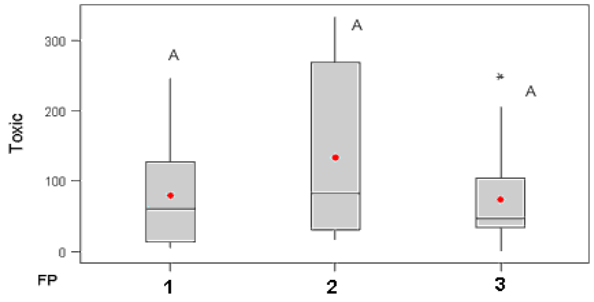


Figure 2-51. Toxic *Prorocentrum* on *Halodule* vs. FP prevalence in Florida (within the designated salinity and temperature range). FP 1 – low, 2 – medium, and 3 – high. Means bearing the same alphabet letter (A, B) are not significantly different at p = 0.10.

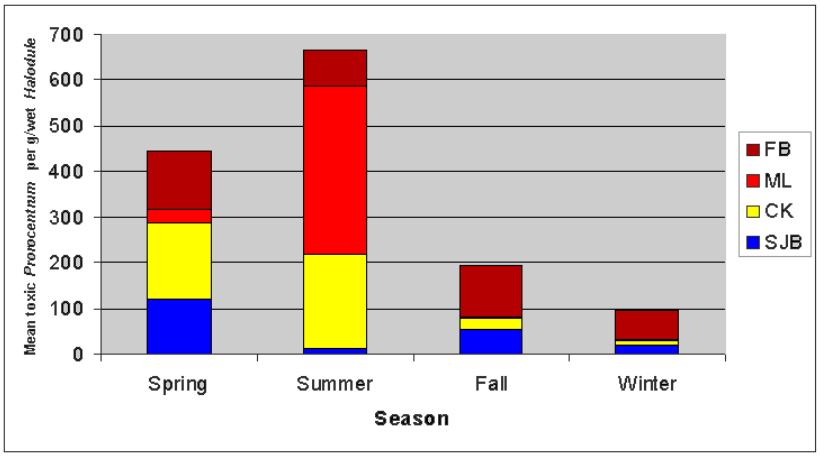


Figure 2-52. Mean toxic *Prorocentrum* cells per g/wet *Halodule* by season and study site. Average water temperatures: Spring (27.8 ˚C, May, n = 16), Summer (31.6 ˚C, August, n = 16), Fall (22.4 ˚C, November, n = 18), and Winter (22.2 ˚C, February, n = 16).

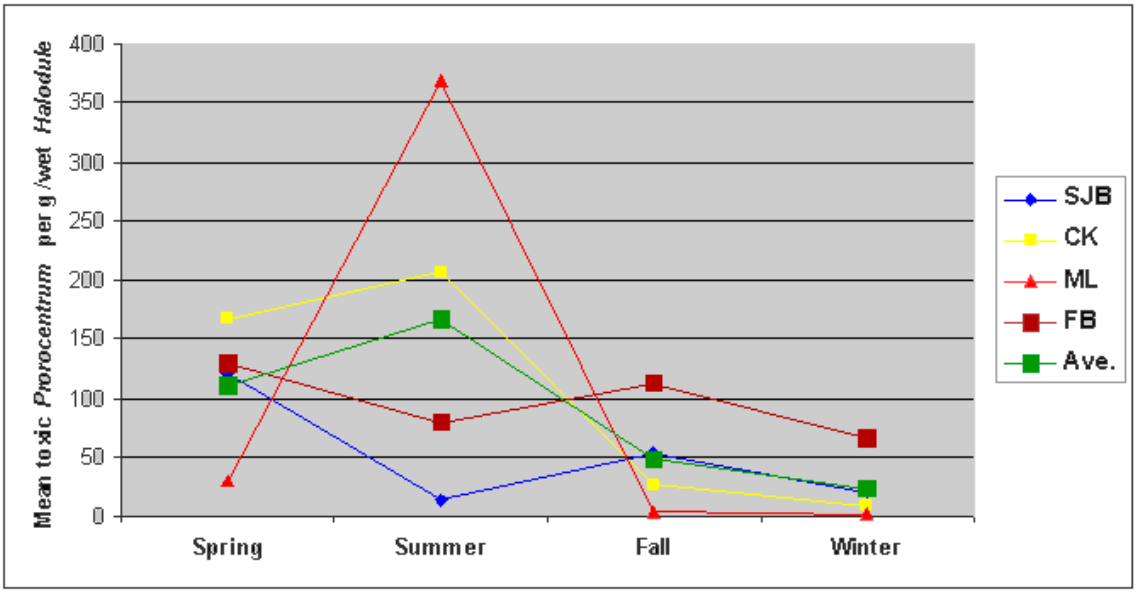


Figure 2-53. Mean toxic *Prorocentrum* on *Halodule* substrate by season and study sites in Florida.

FP. Comparing mean toxic *Prorocentrum* cells per g/wet *Halodule* substrate within the designated salinity and temperature range (over all four seasons) among FP rankings (Mosquito Lagoon and Florida Bay, with their FP rankings of high, were combined) found no significant difference between FP location prevalences (Fig. 2-51).

Season. The trends of mean *Prorocentrum* cells per g/wet *Halodule* substrate over the seasons can be seen on Fig. 2-52. An overall trend can be observed for all the study sites combined, although the trend is not as clear amongst individual sites. Summer is significantly higher than fall and winter seasons when all the sites are combined ($p = 0.10$). Seasonal difference by study site also demonstrates a general trend (Fig. 2-53), with the highest ranges of *Prorocentrum* among study sites found in summer. When season is compared within the designated salinity and temperature range, there is no significant difference between seasons (at $p = 0.10$).

Salinity. The only relationship between salinity and *Prorocentrum* around Florida is a significant difference between numbers of toxic *Prorocentrum* per g/wet *Halodule* substrate between the salinity range 25 – 30 ppt vs. 30 – 35 ppt (p = 0.10, Fig. 2-54).

Temperature. There is a significant difference between toxic *Prorocentrum* abundance at 20 – 30 ºC vs. 30 – 35 ºC (p = 0.10) in Florida (Fig. 2-55). Toxic *Prorocentrum* in Florida is overall more abundant at temperatures greater than 30 ºC.

Maximum abundance per site. Turtles feed on a variety of substrates, and can potentially ingest incidental substrates that may be mixed with the substrate they are targeting as food. Among sites, the highest number of toxic *Prorocentrum* per g/wet substrate is compared to determine the highest number of toxic *Prorocentrum* turtles

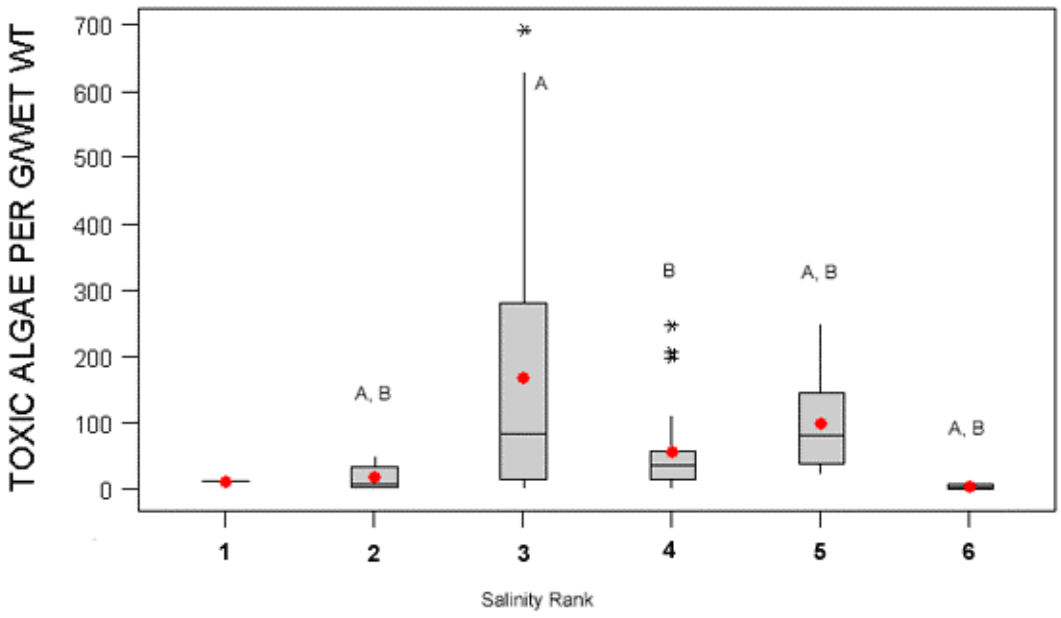


Figure 2-54. Mean toxic *Prorocentrum* per g/wet *Halodule* substrate by salinity ranking, 1: 15-19.9 ppt (n = 1), 2: 20-24.9 ppt (n = 5), 3: 25-29.9 ppt (n = 17), 4: 30-34.9 ppt (n = 24), 5: 35- 39.9 ppt (n = 14), and 6: 40 – 45 ppt (n = 4) at all the Florida sites. Means bearing the same alphabet letter (A, B) are not significantly different at p = 0.10.

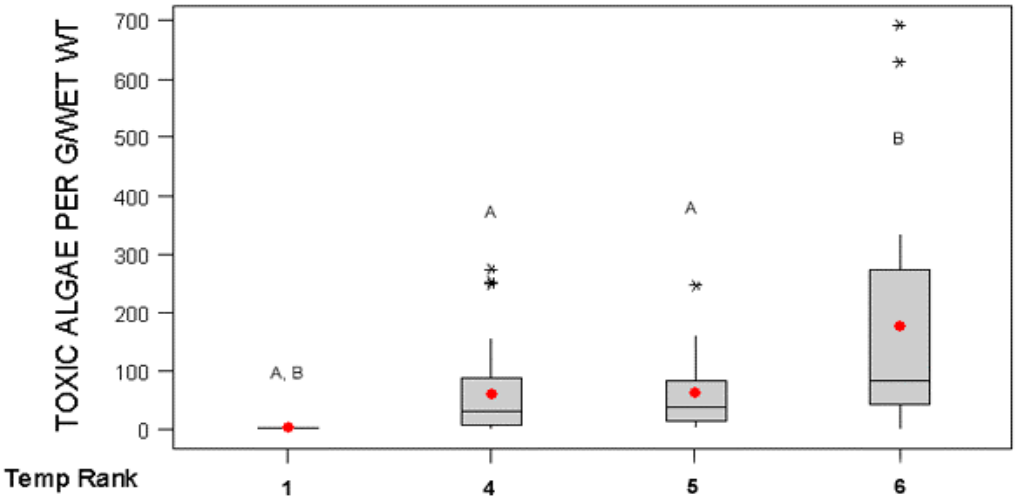


Figure 2-55. Mean toxic *Prorocentrum* per g/wet *Halodule* substrate by temperature ranking 1: 5 – 9.9 ºC (n = 2), 2: 10-14.9 ºC (n = 0), 3: 15-19.9 ºC (n = 0), 4: 20-24.9 ºC (n = 32), 5: 25- 29.9 ºC (n = 16), and 6: 30 – 35 ºC (n = 16) at all the Florida sites. Means bearing the same alphabet letter (A, B) are not significantly different at p = 0.10.

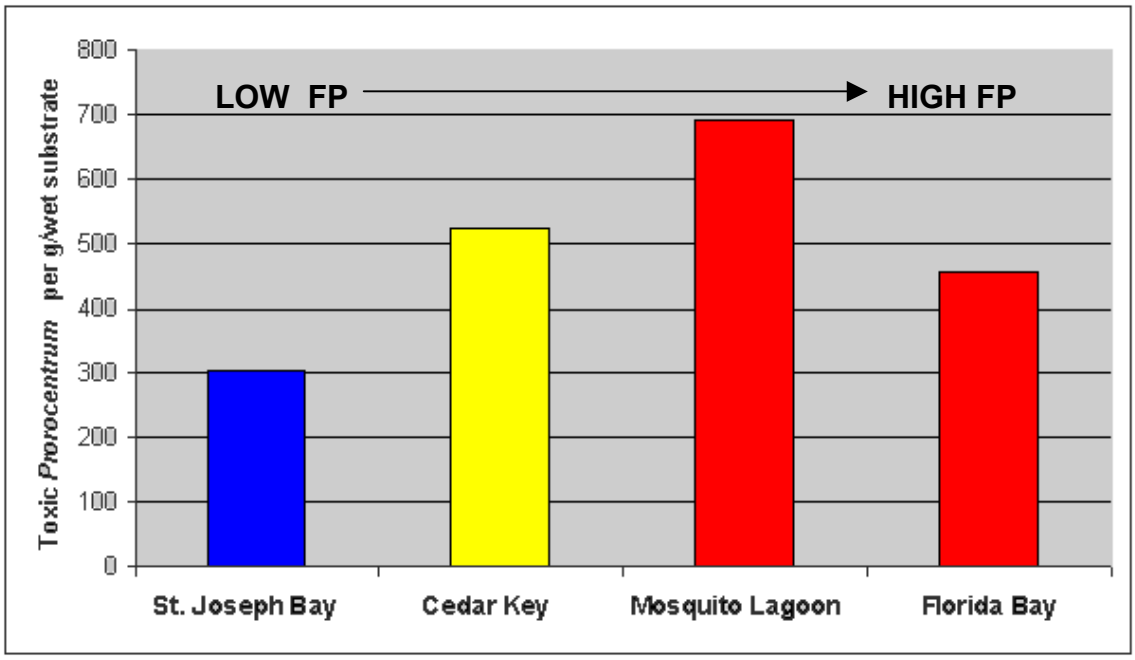


Figure 2-56. Maximum number of toxic *Prorocentrum* per g/wet substrate from the Florida Study sites (SJB – *T. testudinum*, CK- *T. testudinum*, ML – *H. beaudettei*, and FB – *C. tenuissima*).

could potentially be exposed to (Fig. 2-56). Mosquito Lagoon had the sample with the highest number of toxic *Prorocentrum* per g/wet substrate, followed by Cedar Key, Florida Bay, and St. Joseph Bay.

The Hawaiian Islands

There were six *Prorocentrum* species identified from samples collected throughout the Hawaiian Islands. Three species are OA-producers, including *P. lima, P. concavum*, and *P.* cf *hoffmannianum*, and three are not known to produce OA, including *P. mexicanum, P. emarginatum*, and very small cells of *Prorocentrum* that are called "*Small Prorocentrum*".

Study Site 1: Punalu'u Bay (East Coast of Big Island)

Results from this site found *Prorocentrum* present on the four substrates species collected only in the summer season. Out of four substrates, the only substrate with no *Prorocentrum* present was *Pterocladiella capillacea*. The only *Prorocentrum* species found at Punalu'u were *P. lima, P. concavum*, and small *Prorocentrum* (Fig. 2-57). The substrates *U. rigida, A. concinna*, and *E. paradox* had equal mean abundances of toxic *Prorocentrum,* which amounted to a mean of less than 1 cell per sample (Fig. 2-58).

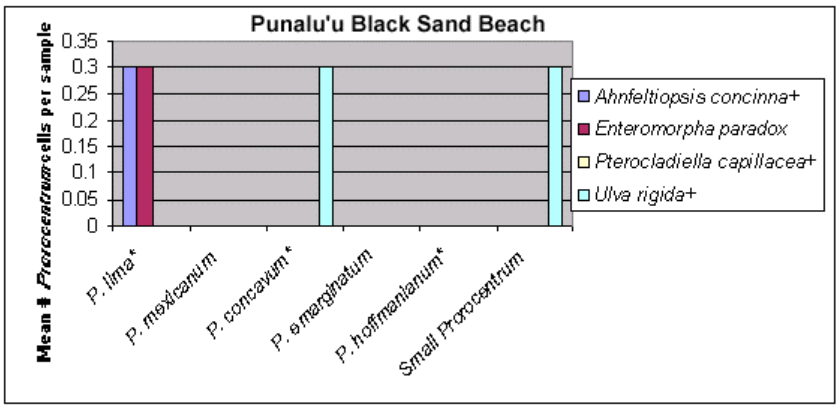


Figure 2-57. Mean number of *Prorocentrum* cells by substrate in summer collection at Punalu'u. * - OA-producing species, + – found in green turtle diet.

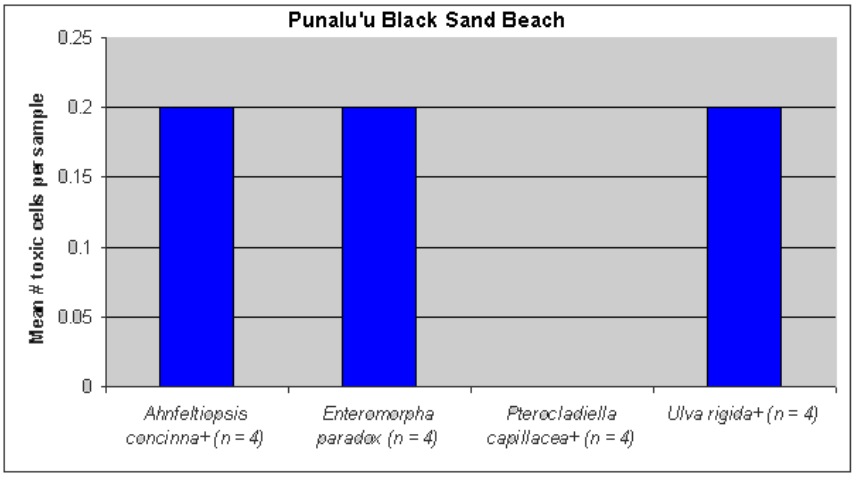


Figure 2-58. Mean toxic *Prorocentrum* by substrates in Punalu'u.

Study Site 2: Kona/Kohala Coast (West Coast of the Big Island)

There were four stations where substrate was collected on the Kohala/Kona Coast. *Prorocentrum* were found in very low numbers at the first two stations, and not found at all on the substrates collected at stations 3 and 4. A total of eight substrates were collected and five substrates had *Prorocentrum* (*Enteromorpha paradox, Gelidium-like wiry turf, Melanamansia glomerata, Polysiphonia hawaiiensis,* and *Rhizoclonium*

riparium). Seasonality is observed at this site, with higher numbers and greater diversity of *Prorocentrum* spp. found in the summer (Figs. 2-59 and 2-60).

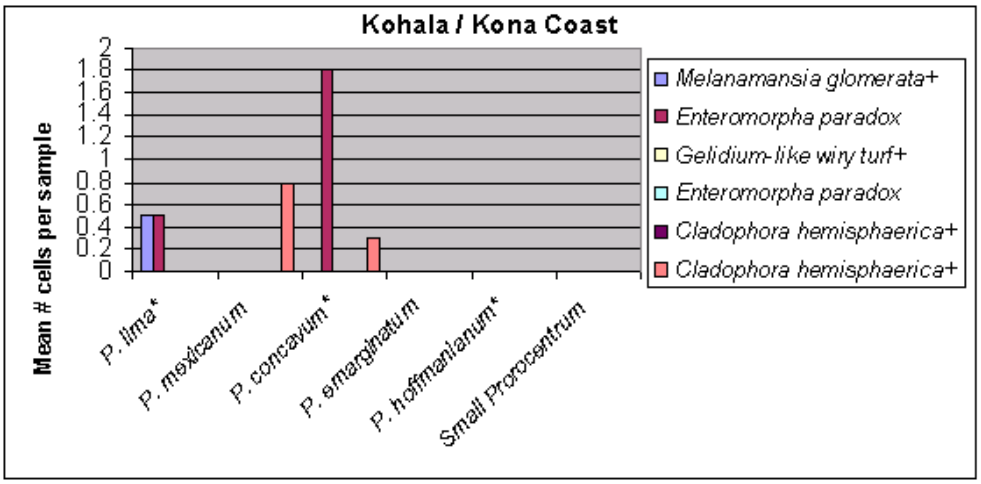


Figure 2-59. Mean *Prorocentrum* cells per sample by substrate for samples collected in winter (February) on the Kona/Kohala Coast.

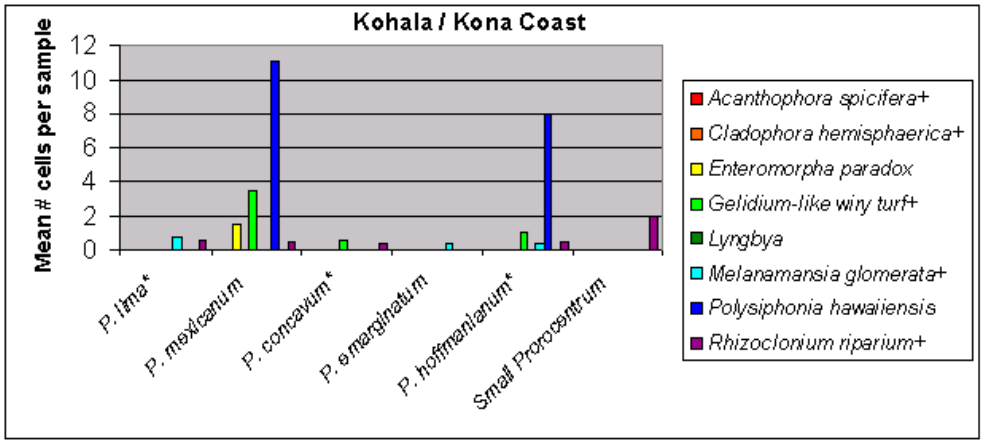


Figure 2-60. Mean *Prorocentrum* cells per sample by substrate for samples collected in summer (August) on the Kona/Kohala Coast.

Toxic *Prorocentrum* spp. were most abundant within the salinity range of 31 - 33 ppt (Fig. 2-61). All the toxic *Prorocentrum* were found in a temperature range of 34 – 30 ºC (Fig. 2-62). The substrate with the highest abundance of toxic *Prorocentrum* on the

Kona Coast was *P. hawaiiensis* (n = 1, collected only in summer), followed by *M. glomerata* (n = 4) and *Gelidium-like wiry turf* (n = 3, Fig. 2-63).

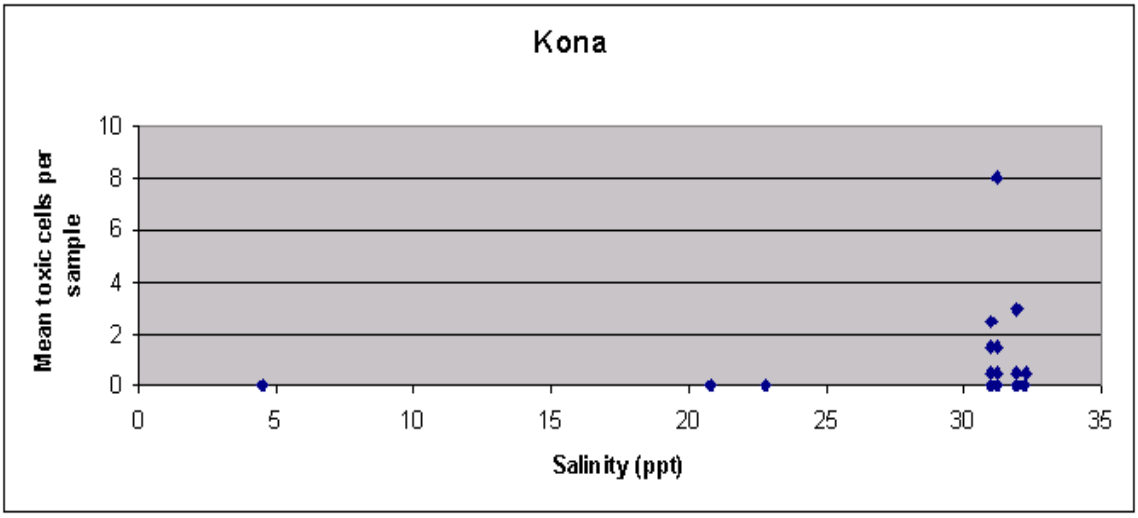


Figure 2-61. Toxic *Prorocentrum* spp. vs. salinity on the Kona/Kohala Coast.

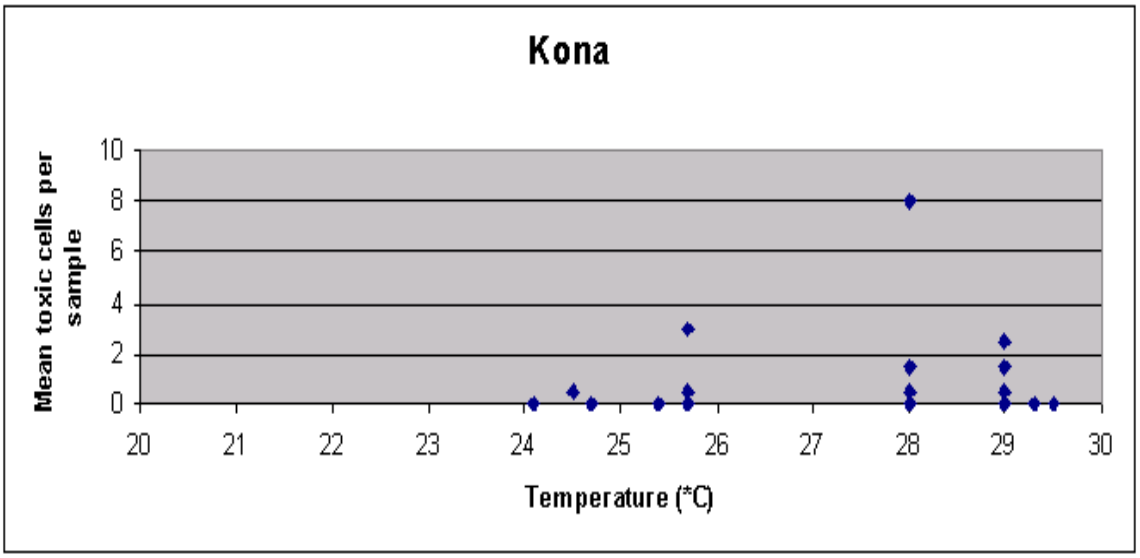


Figure 2-62. Toxic *Prorocentrum* spp. vs. temperature on the Kona/Kohala Coast.

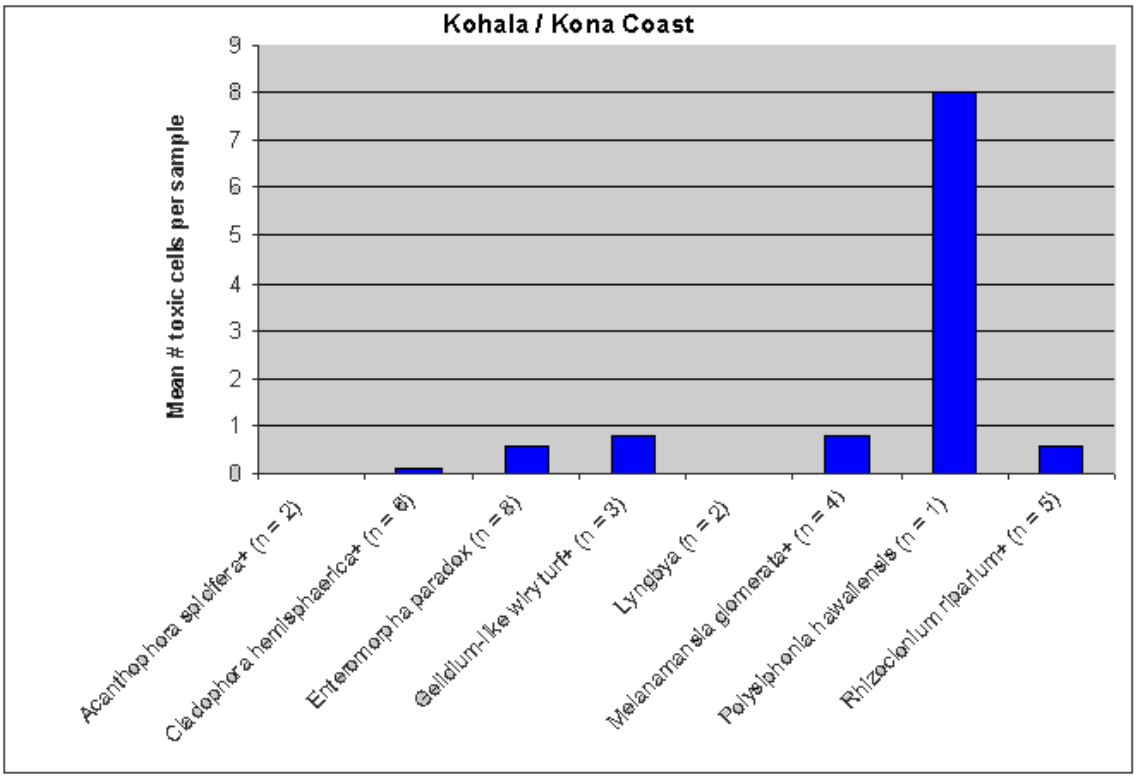


Figure 2-63. Mean toxic *Prorocentrum* by substrate on the Kona/Kohala Coast.

Study Site 3: Hilo Bay Vicinity (East Coast of the Big Island)

There were two stations at the Hilo Bay study site, one near an abandoned sewage treatment plant, and another at Richardson Beach Park. Of the seven substrates collected in winter (February 2000), only *Colpomenia sinuosa* (n = 2) had *Prorocentrum* (Fig. 2-64). However, out of seven substrates collected in summer (August 2000), six had *Prorocentrum*, with only the substrate *Ahnfeltiopsis concinna* (n = 2) without *Prorocentrum* cells (Fig. 2-65). The summer substrates of *Melanamansia glomerata* (n = 2) and *Ulva rigida* (n = 2) had *P. mexicanum* present, but no OA-producing spp. Toxic *Prorocentrum* in Hilo were found in a salinity range of 21 – 30 ppt (Fig. 2-66) and a temperature range of 17 – 25 ºC (Fig. 2-67). Mean toxic *Prorocentrum* were most

abundant on the substrate *C. sinuosa* (n = 3), followed by *A. spicifera* (n = 2) and *P. capillacea* (n = 6, Fig. 2-68).

Unique to the Hilo area, amongst all of the study sites, was the presence and abundance of diatoms species in many of the samples collected.

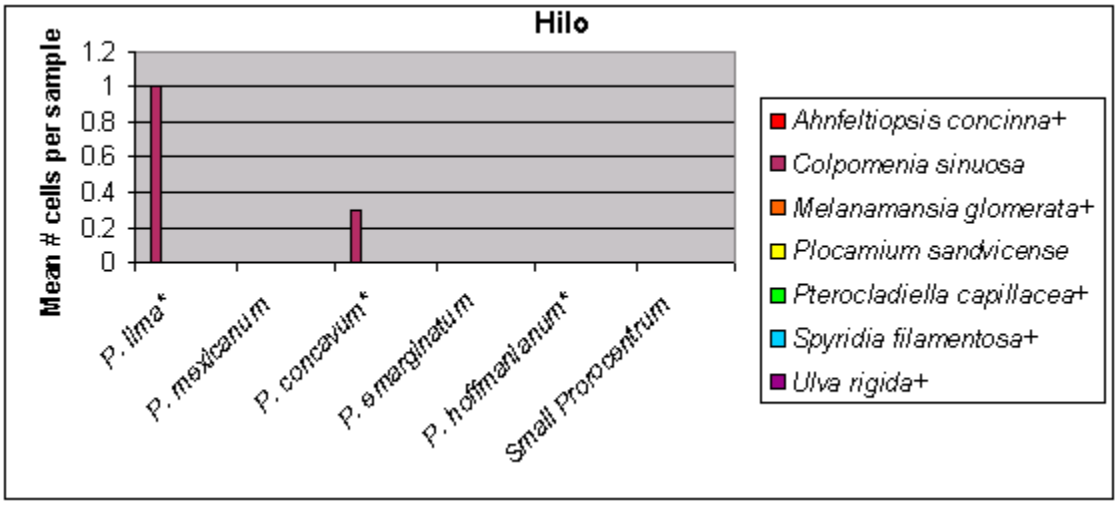


Figure 2-64. Mean *Prorocentrum* cells per sample by substrate collected in winter (February) in Hilo Bay.

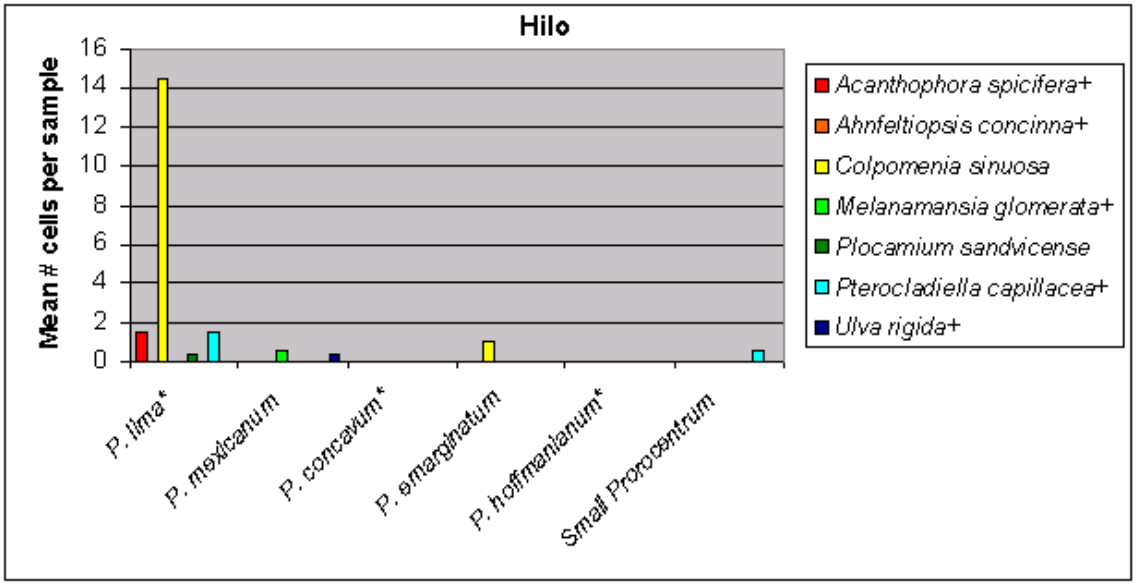


Figure 2-65. Mean *Prorocentrum* cells per sample by substrate collected in summer (August) in Hilo Bay.

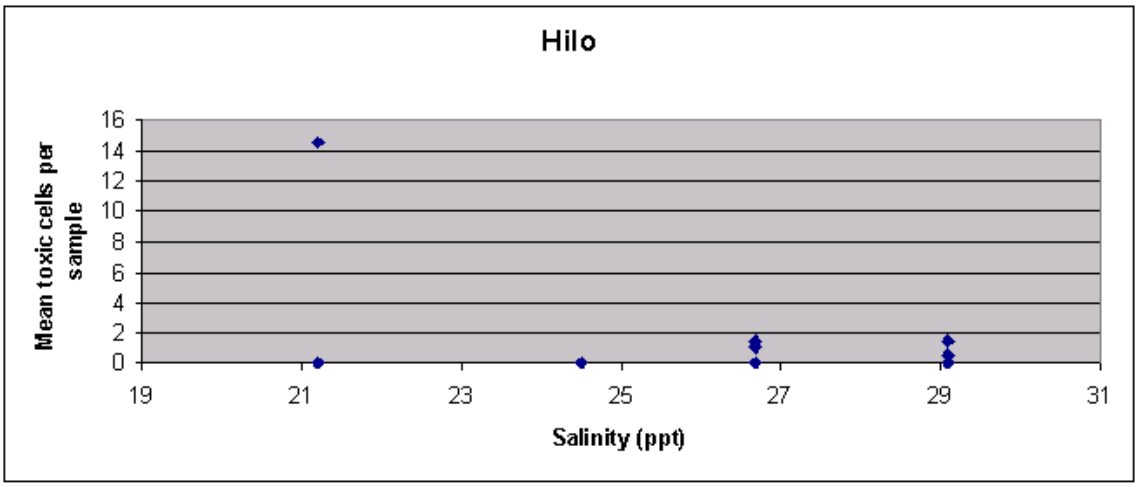


Figure 2-66. Toxic *Prorocentrum* vs. salinity at Hilo.

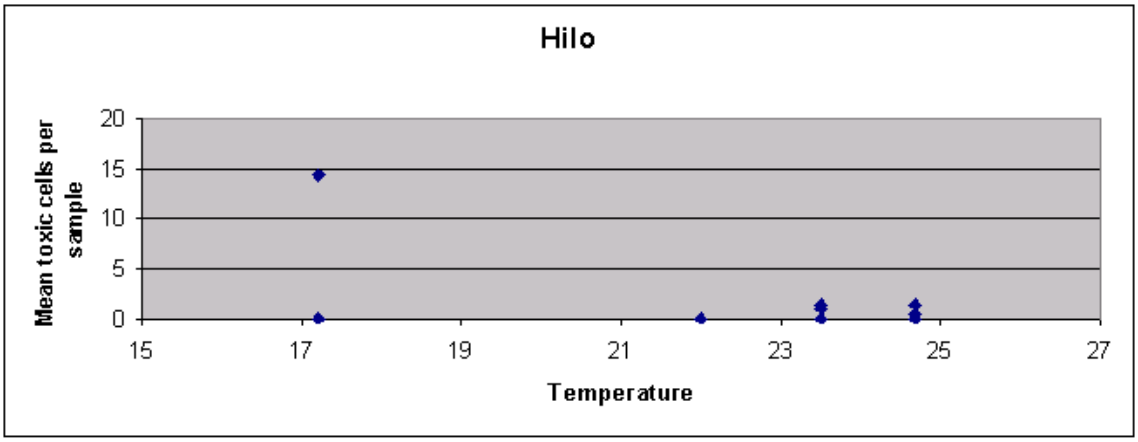


Figure 2-67. Toxic *Prorocentrum* vs. temperature (ºC) at Hilo.

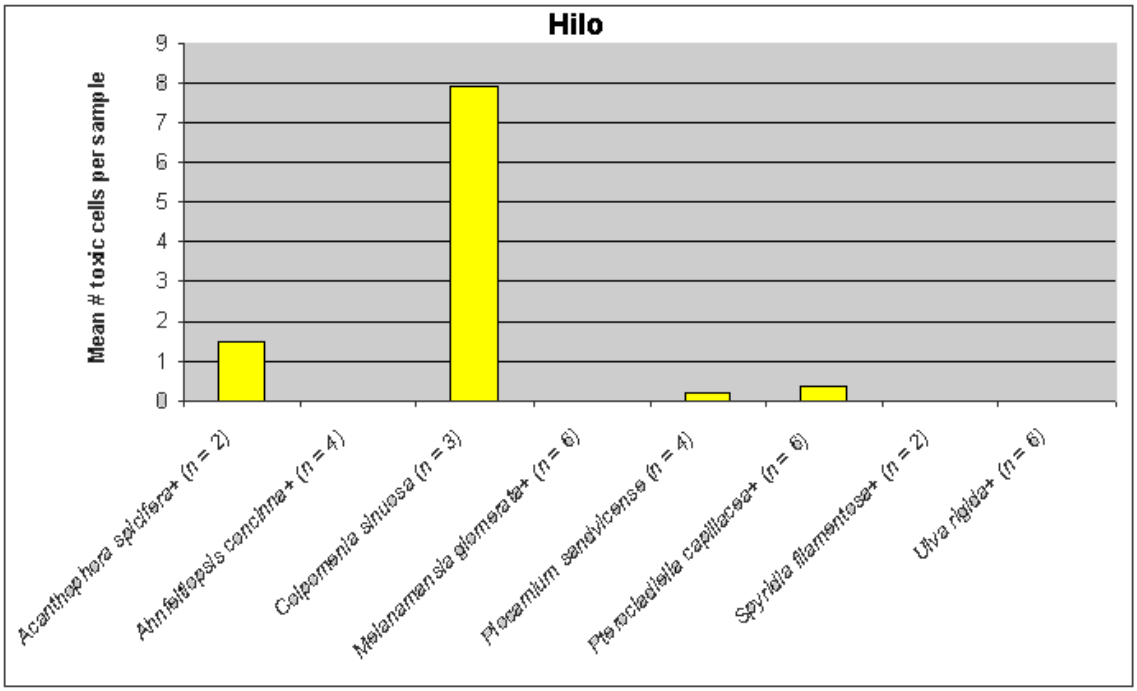


Figure 2-68. Mean toxic *Prorocentrum* by substrate in Hilo.

Study Site 4: Southeast Kauai (Poipu)

There were three stations in SE Kauai. Station one was within the small bay in front of the Prince Kuhio Birthplace monument, where samples were collected in both winter and summer. The second station was on tidal flats approximately ½ mile to the West of the Prince Kuhio Birthplace, where samples were collected only during winter. In the summer, a 3rd station in a harbor near Spouting Horn was sampled instead of Station 2, to have a broader representation of SE Kauai. Of eight substrates collected in winter, only one did not have *Prorocentrum* (*Ahnfeltiopsis concinna*, Fig. 2-69). All nine substrates collected in summer had *Prorocentrum* spp (Fig. 2-70). All the samples were collected in a salinity range between 32.0 and 36.5 ppt (Fig. 2-71), and a temperature range of 23.5 – 26 ºC (Fig. 2-72). The highest abundance of toxic *Prorocentrum*

occurred on the substrate *P. crassa* (n = 2), followed by *C. sinuosa* (n = 2) and *S. echinocarpum* (n = 4, Fig. 2-73).

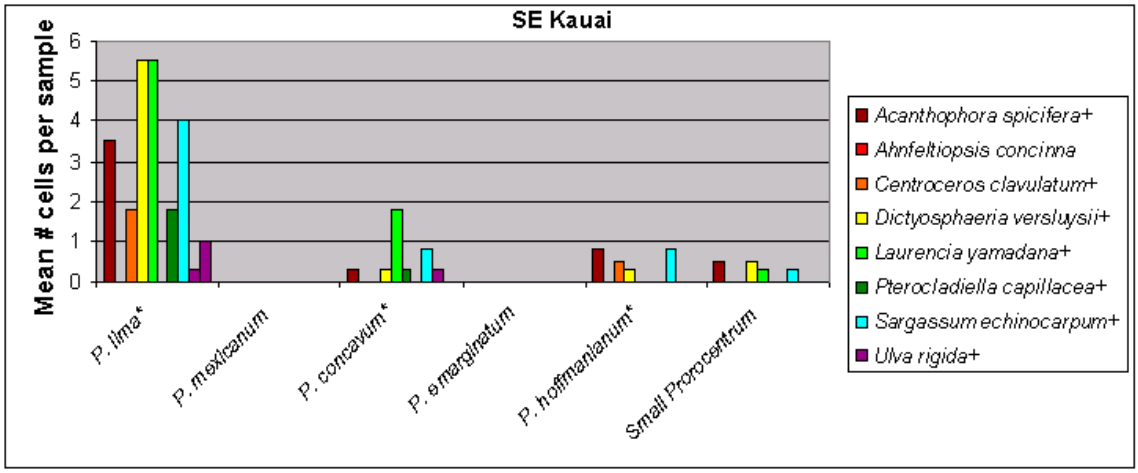


Figure 2-69. Mean *Prorocentrum* cells per sample by substrate collected in winter (February) at the SE Kauai stations 1 and 2.

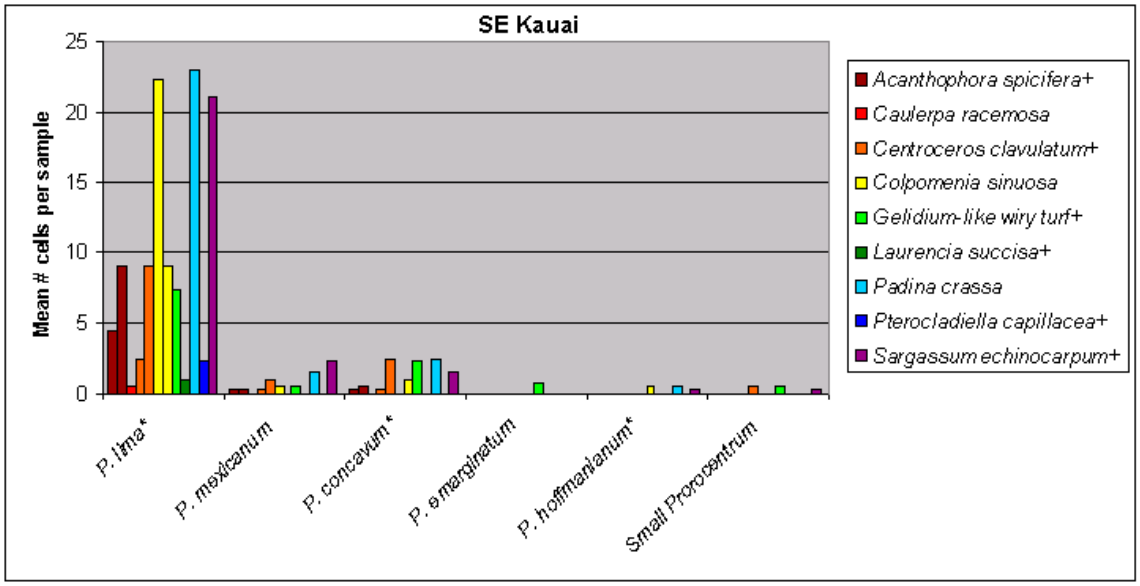


Figure 2-70. Mean *Prorocentrum* cells per sample by substrate collected in summer (August) at the SE Kauai stations 1 and 3.

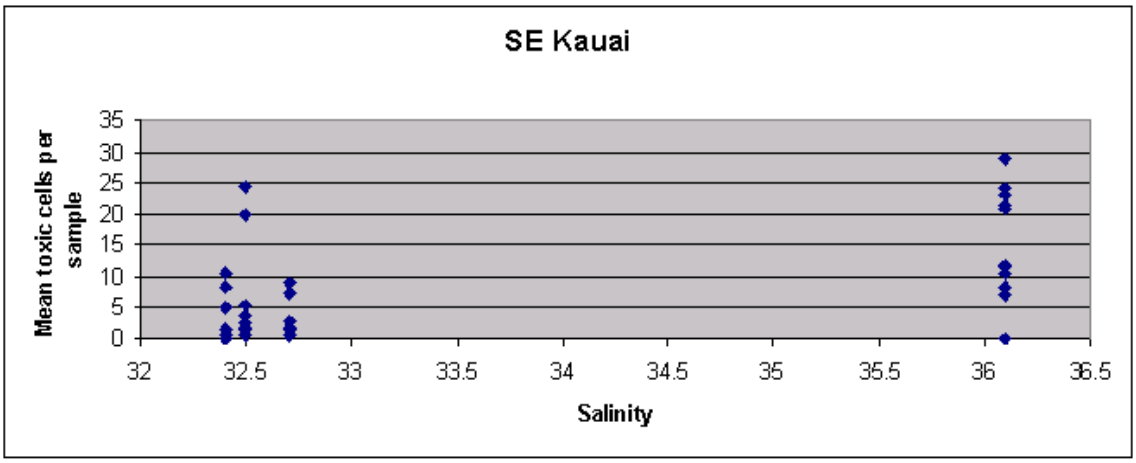


Figure 2-71. Toxic *Prorocentrum* vs. salinity in SE Kauai.

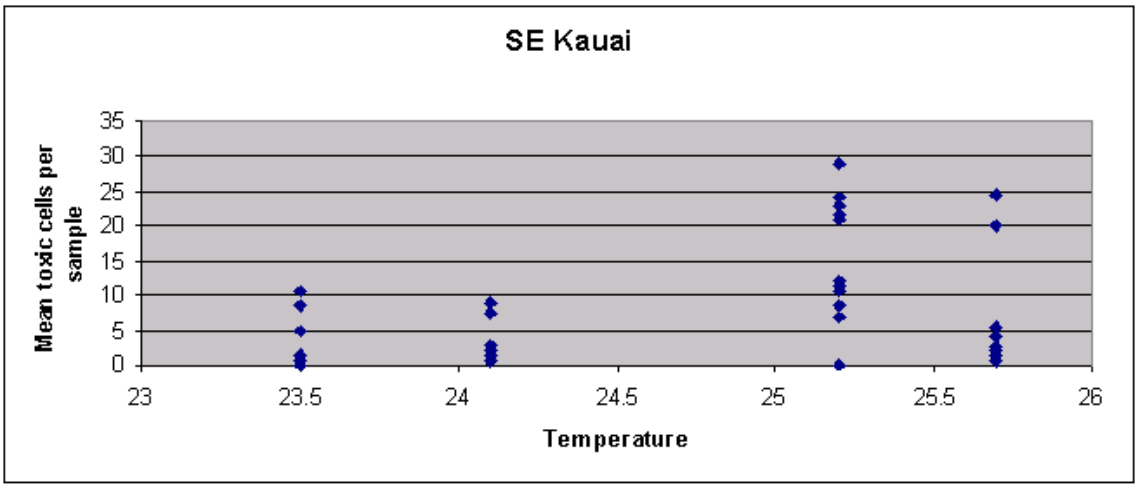


Figure 2-72. Toxic *Prorocentrum* vs. temperature (ºC) in SE Kauai.

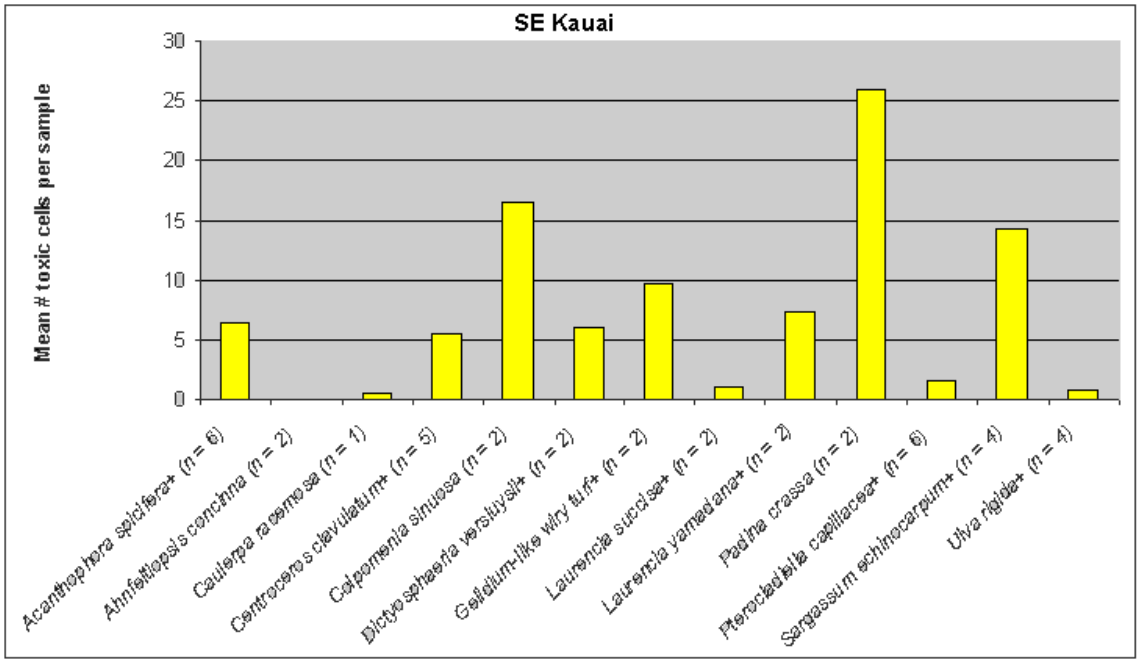


Figure 2-73. Mean toxic *Prorocentrum* by substrate in SE Kauai.

Study Site 5: Northeast Kauai (Moloaa Bay and Anahola Bay)

Two stations were sampled in summer and winter in NE Kauai: Moloaa Bay and Anahola Bay. All 9 substrates collected in winter (Fig. 2-74) and all 11 substrates collected in summer (Fig. 2-75) in NE Kauai had *Prorocentrum*. *P. lima* is the most common species found on all the substrates collected in NE Kauai. All samples were collected in a salinity range of 28 – 39 ppt (Fig. 2-76), and a temperature range of 24.5 – 27.5 ºC (Fig. 2-77). The highest abundance of toxic *Prorocentrum* occurred on the substrate *Jania* sp. (n = 2), followed by *H. chordacea* (n = 2) and *D. cavernosa* (n = 2, Fig. 2-78).

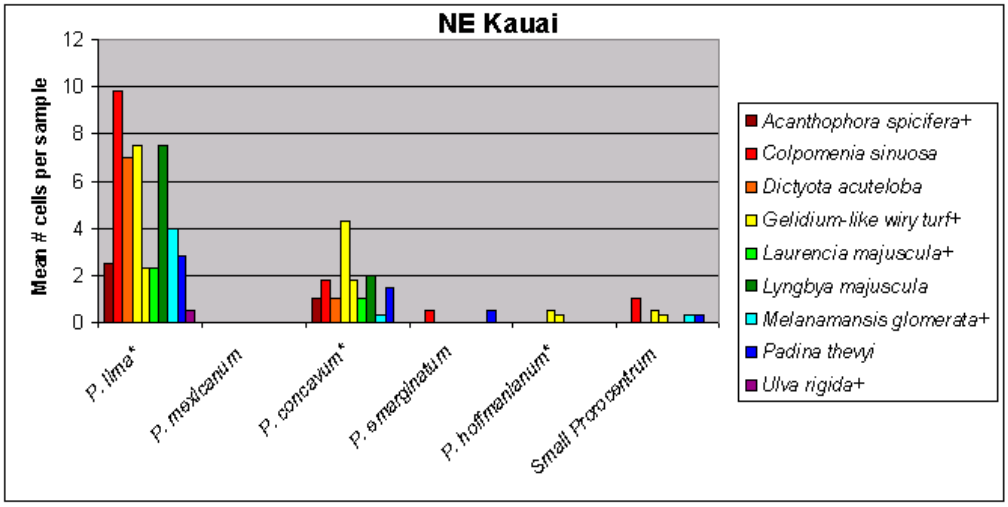


Figure 2-74. Mean *Prorocentrum* cells per sample by substrate collected in winter (February) at the NE Kauai stations.

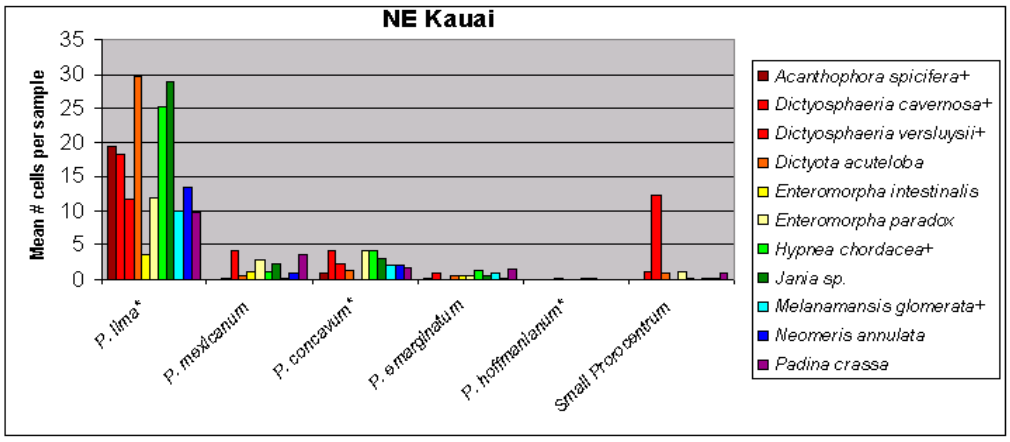


Figure 2-75. Mean *Prorocentrum* cells per sample by substrate collected in summer (August) at the NE Kauai stations.

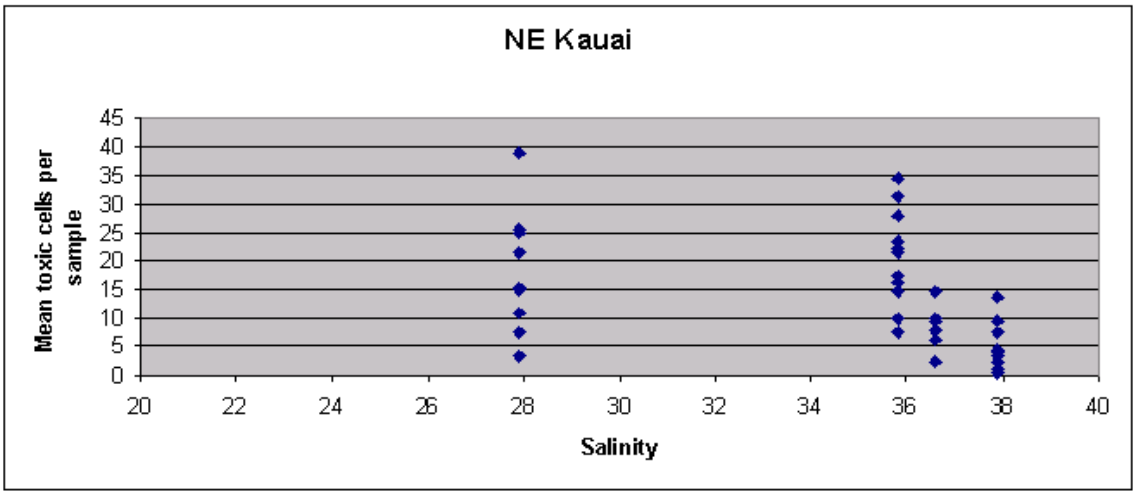


Figure 2-76. Toxic *Prorocentrum* vs. salinity (ppt) in NE Kauai.

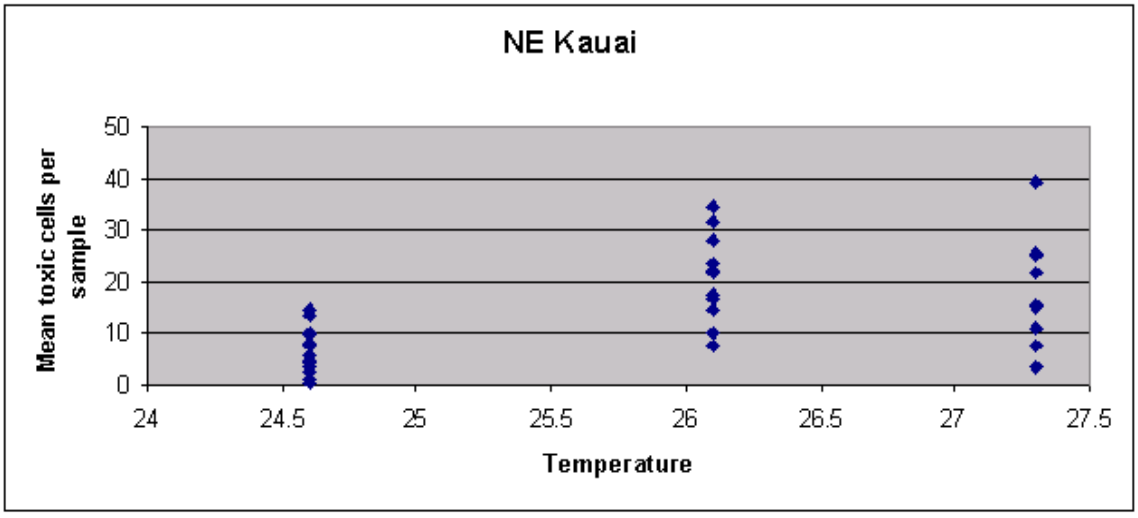


Figure 2-77. Toxic *Prorocentrum* vs. temperature (ºC) in NE Kauai.

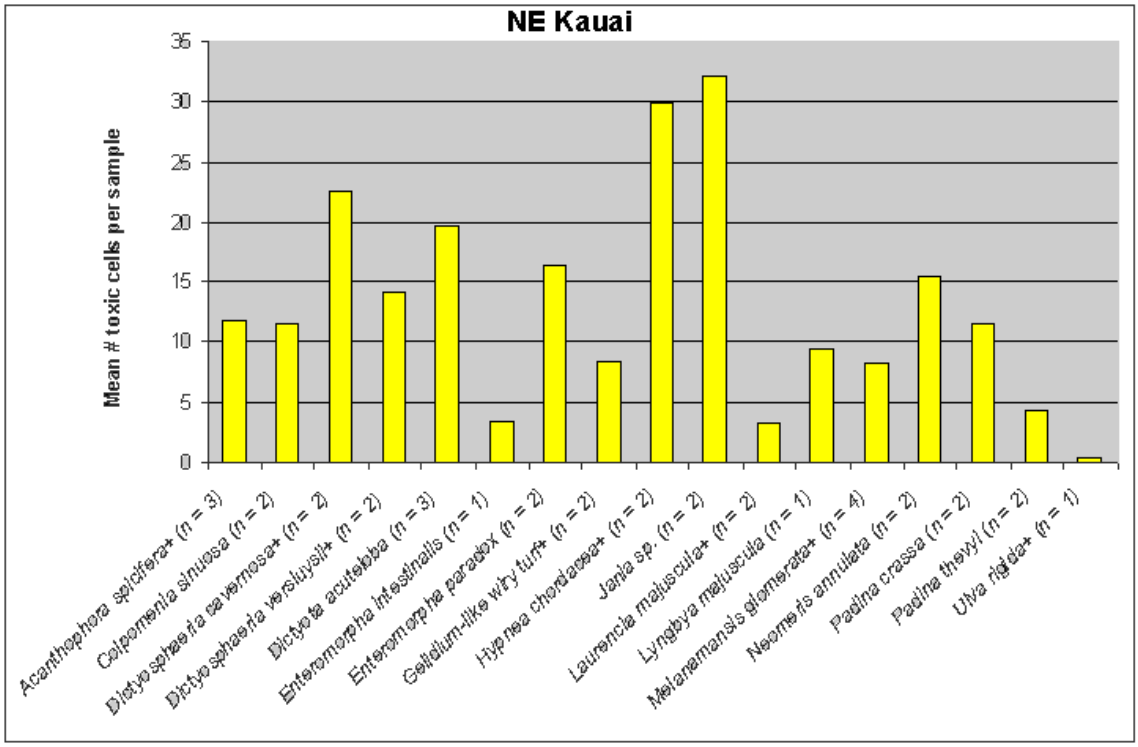


Figure 2-78. Mean toxic *Prorocentrum* by substrate in NE Kauai.

Study Site 6: Waikiki, Oahu

Substrates were collected at two stations in Waikiki in both summer and winter. *P. lim*a is the most abundant *Prorocentrum* species at Waikiki (Fig. 2-79). All six substrates collected in winter had P*rorocentrum* present (Fig. 2-80). Seven of eight substrates collected in summer had *Prorocentrum*, with only *Gelidiopsis scoparia* (n = 1) with no *Prorocentrum* (Fig. 2-81).

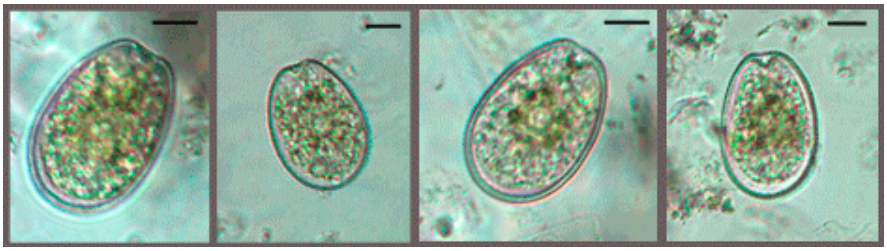


Figure 2-79. Different *P. lima* cells from Waikiki. Scale = 10 µm.

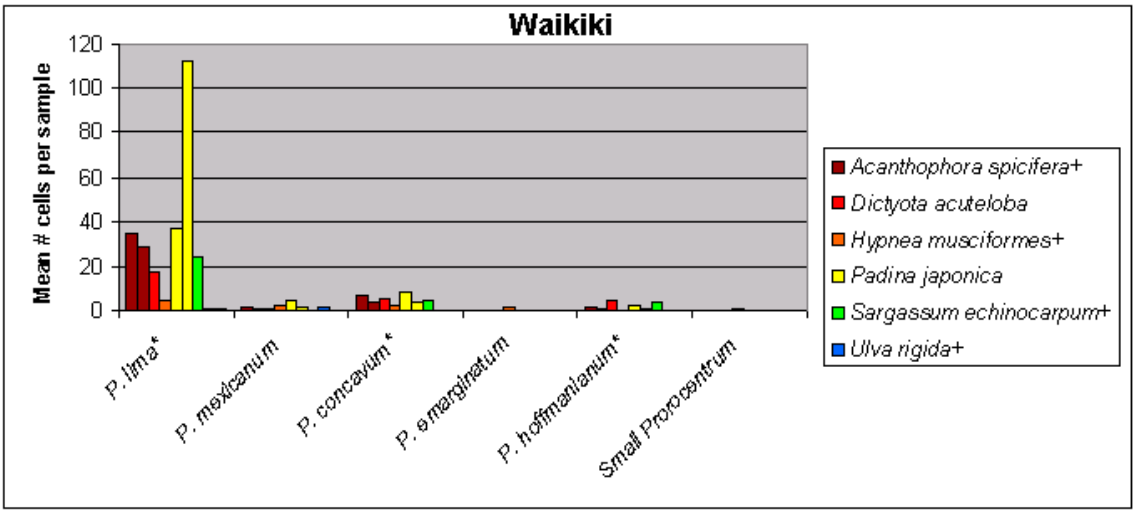


Figure 2-80. Mean *Prorocentrum* cells per sample by substrate collected in winter (February) at the Waikiki stations.

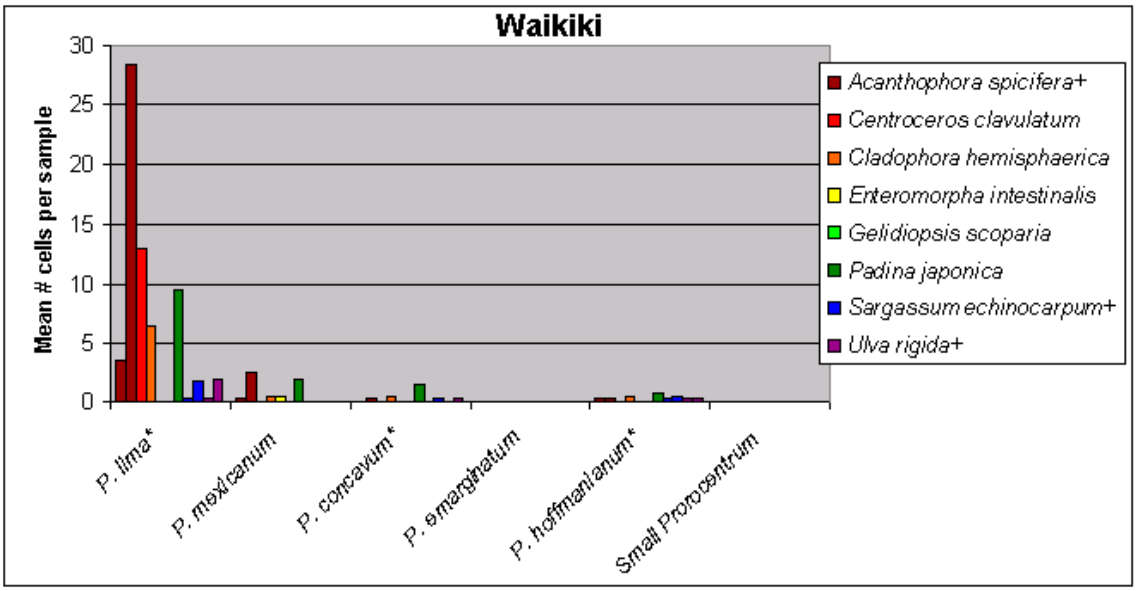


Figure 2-81. Mean *Prorocentrum* cells per sample by substrate collected in summer (August) at the Waikiki stations.

All the samples were collected within a salinity range of 29–38 ppt (Fig. 2-82), and a temperature range of 25.5–26.5 ºC (Fig. 2-83). The substrate with the highest

abundance of toxic *Prorocentrum* was *P. japonica* (n = 6), followed by *A. spicifera* (n = 9) and *D. acuteloba* (n = 2, Fig. 2-84).

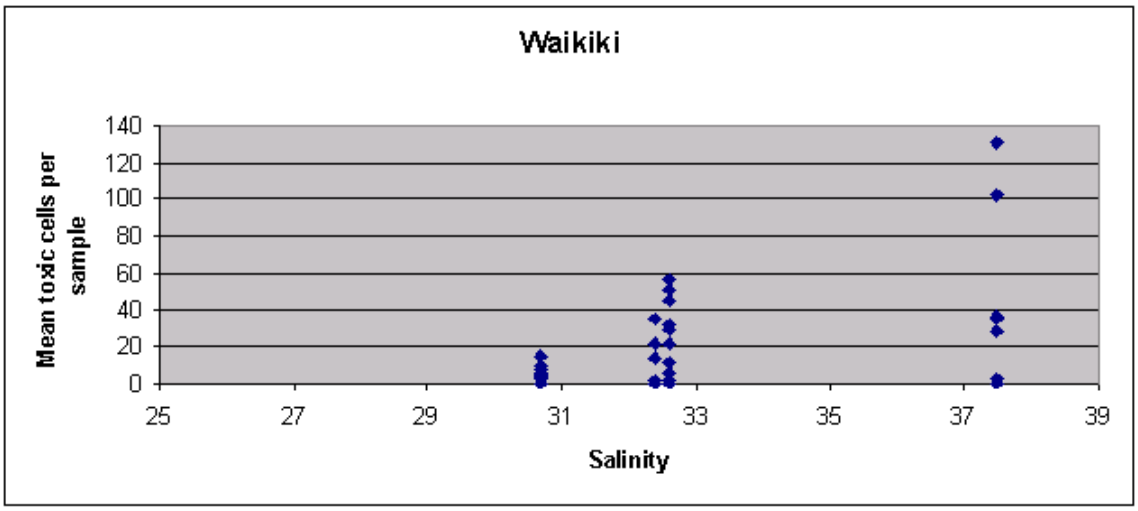


Figure 2-82. Toxic *Prorocentrum* vs. salinity (ppt) at Waikiki.

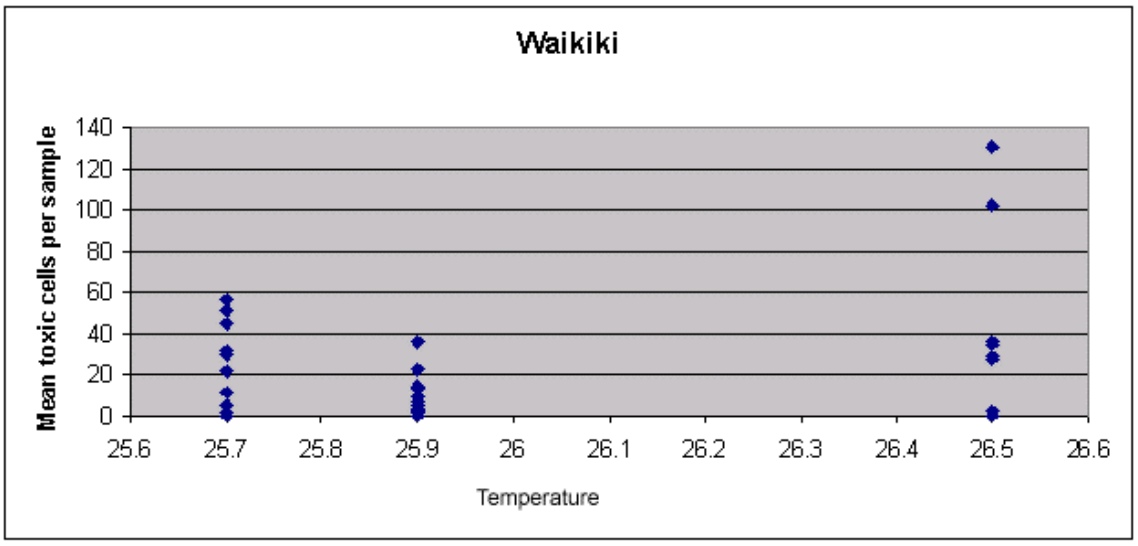


Figure 2-83. Toxic *Prorocentrum* vs. temperature (ºC) at Waikiki.

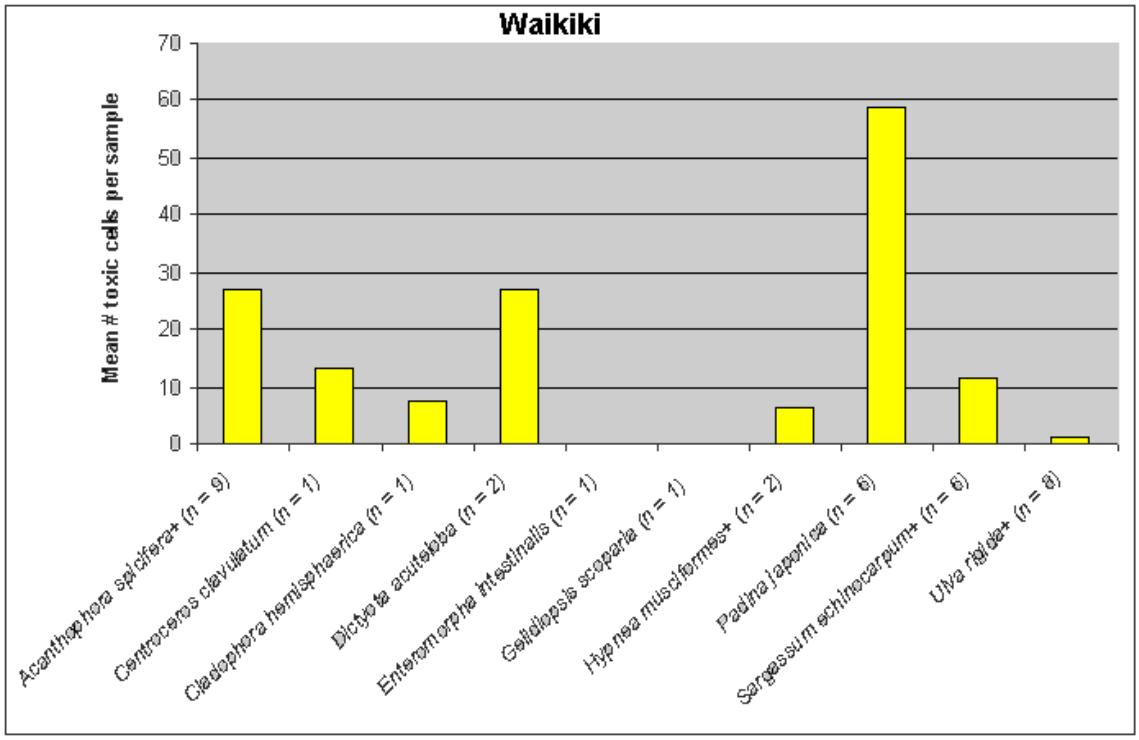


Figure 2-84. Mean toxic *Prorocentrum* by substrate in Waikiki.

Study Site 7: Kaneohe Bay, Oahu

Substrates were collected at seven stations throughout Kaneohe Bay. Stations 1 – 3 were sampled in winter, and stations 1 and 4 – 7 were sampled in summer. All ten substrates collected in winter had *Prorocentrum* (Fig. 2-85), as did all nine substrates collected in summer (Fig. 2-86). All the samples were collected in a salinity range of 32 – 37 ppt (Fig. 2-87), and a temperature range of 24.5 – 28.5 ºC (Fig. 2-88). The substrate with the highest abundance of toxic *Prorocentrum* was *D. acuteloba* (n = 3), followed by *Hypnea* spp. (n = 1) and *A. spicifera* (n = 12, Fig. 2-89).

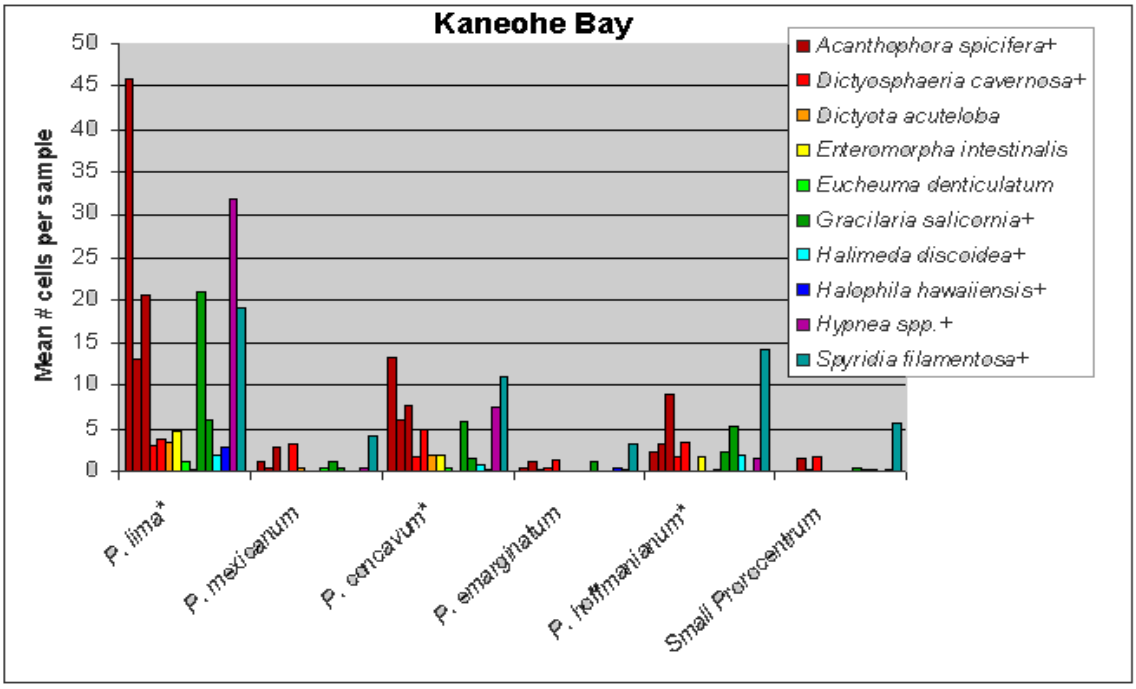


Figure 2-85. Mean *Prorocentrum* cells per sample by substrate collected in winter (February) at the Kaneohe Bay stations 1 - 3.

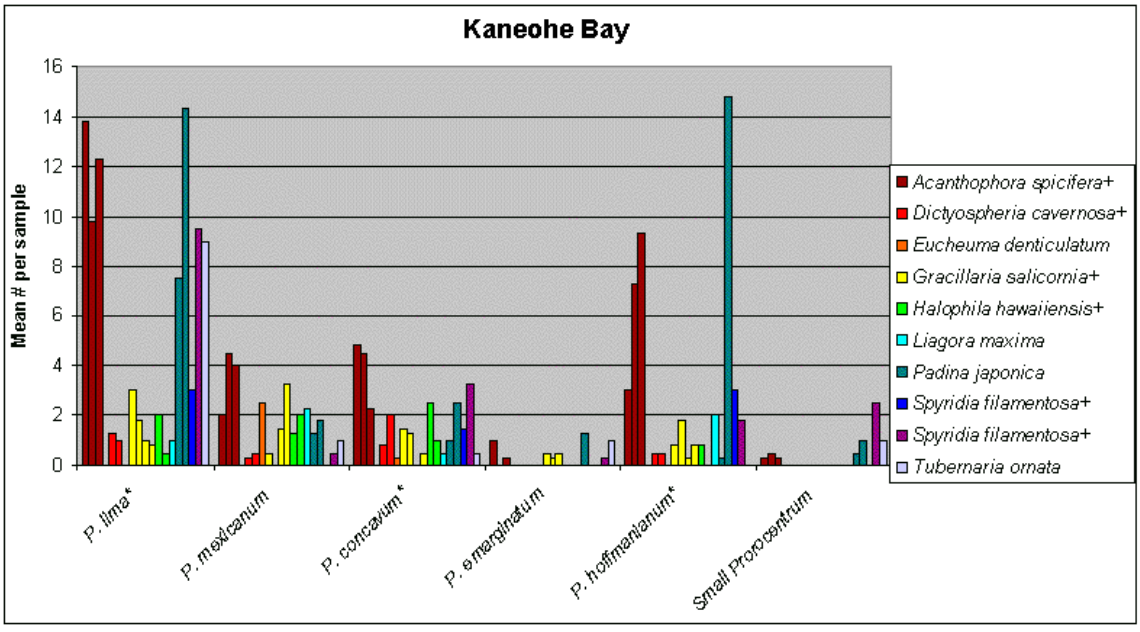


Figure 2-86. Mean *Prorocentrum* cells per sample by substrate collected in summer (August) at the Kaneohe Bay stations 1, 4 - 7 (excluding the *Dictyota acuteloba* sample with the very high numbers of *P. lima*).

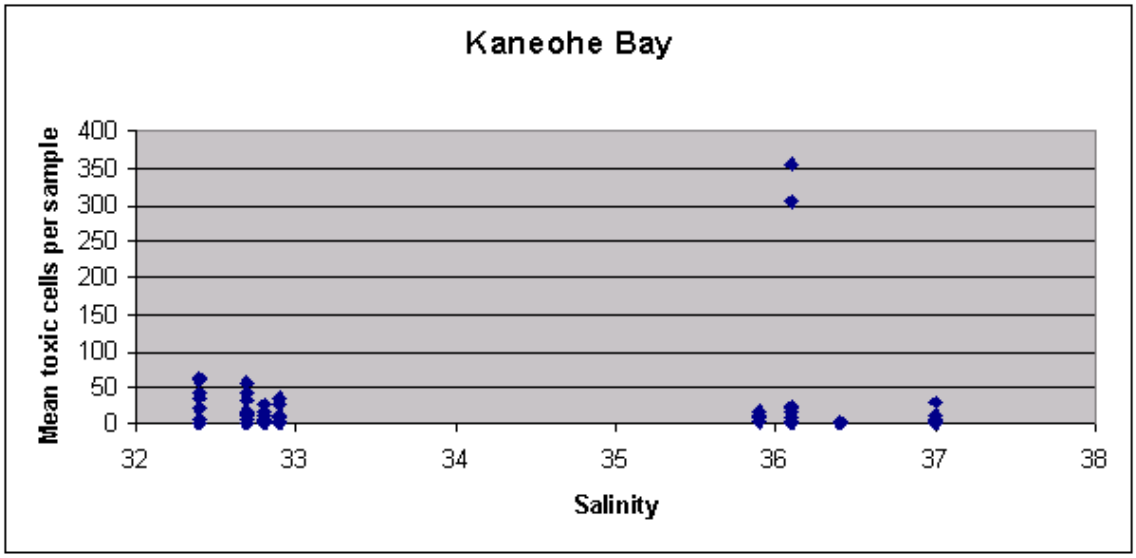


Figure 2-87. Toxic *Prorocentrum* vs. salinity (ppt) in Kaneohe Bay.

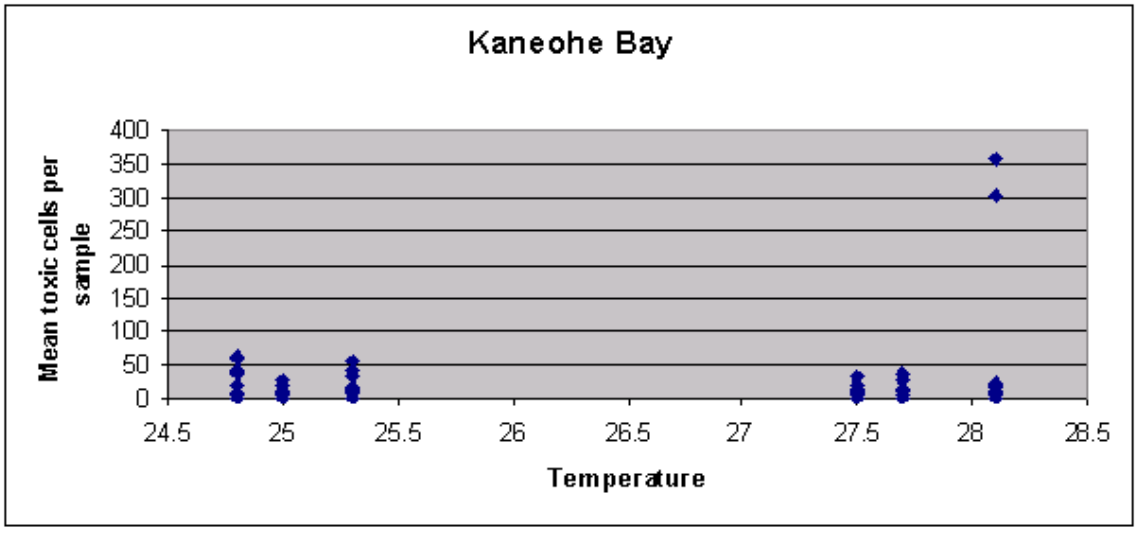


Figure 2-88. Toxic *Prorocentrum* vs. temperature (ºC) in Kaneohe Bay.

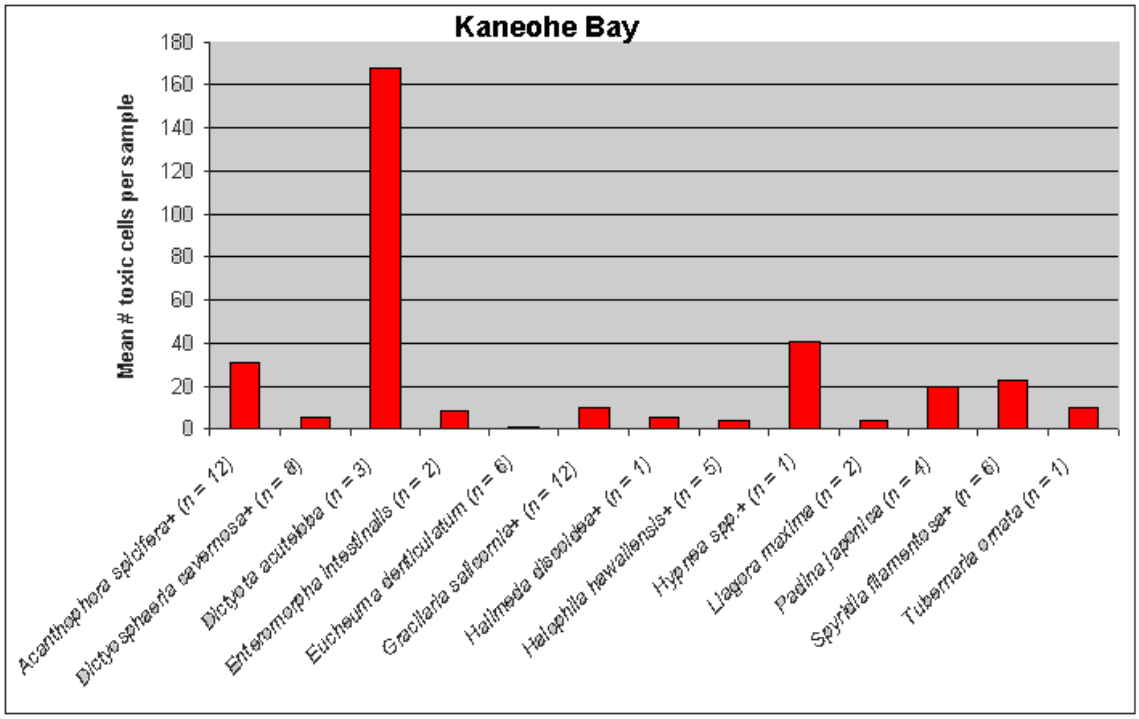


Figure 2-89. Mean toxic *Prorocentrum* by substrate in Kaneohe Bay.

Study Site 8: Honokowai, Maui

There were seven stations sampled at Honokowai, Maui (the stations were at various locations and depth within the vicinity). The winter samples were collected <3 m deep via snorkeling (designated as station 1), but in the summer, samples were collected both shallow (station 1) and at depth via scuba diving (stations 2 – 7). Seven substrates were collected in the winter, and all except for *Ulva rigida* had *Prorocentrum* (Fig. 2-90). All eight substrates collected in summer had *Prorocentrum* present (Fig. 2-91). Substrates collected at various depths in the summer season had more toxic spp. of *Prorocentrum* present than non-toxic spp., and substrates collected at depths at <3 m had very few non-toxic spp. present (Fig. 2-92). The highest abundance of toxic *Prorocentrum* occurred within a salinity range of 30 – 35 ppt (Fig. 2-93). All samples

with toxic *Prorocentrum* present were collected within a temperature range of 25 – 27 ºC (Fig. 2-94). The substrate with the highest abundance of toxic *Prorocentrum* was *Cladophora hemisphaerica* (n = 2), followed by *Spyridia filamentosa* (n = 4) and *Melanamansia glomerata* (n = 4, Fig. 2-95).

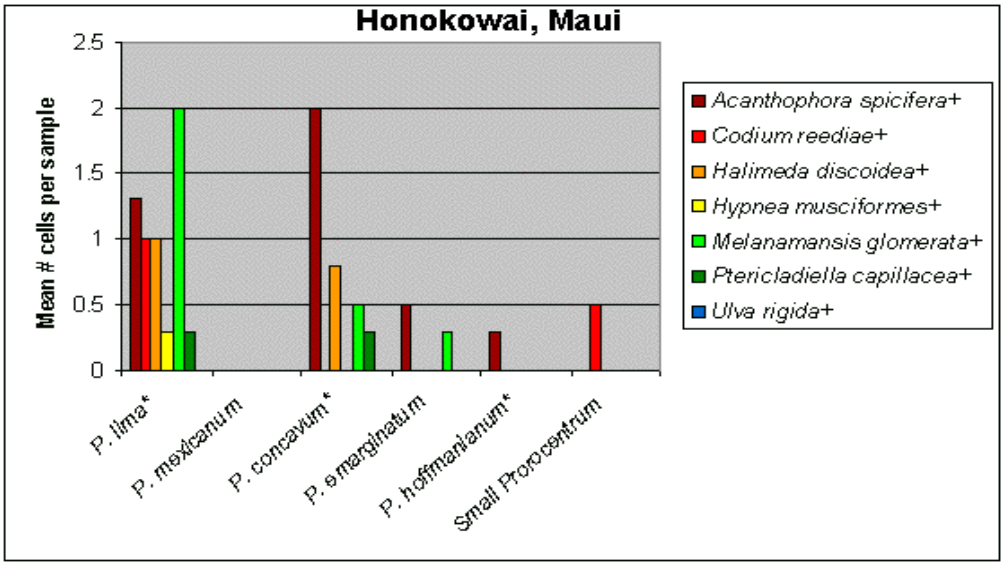


Figure 2-90. Mean *Prorocentrum* cells per sample by substrate collected in winter (February) at the Honokowai station 1.

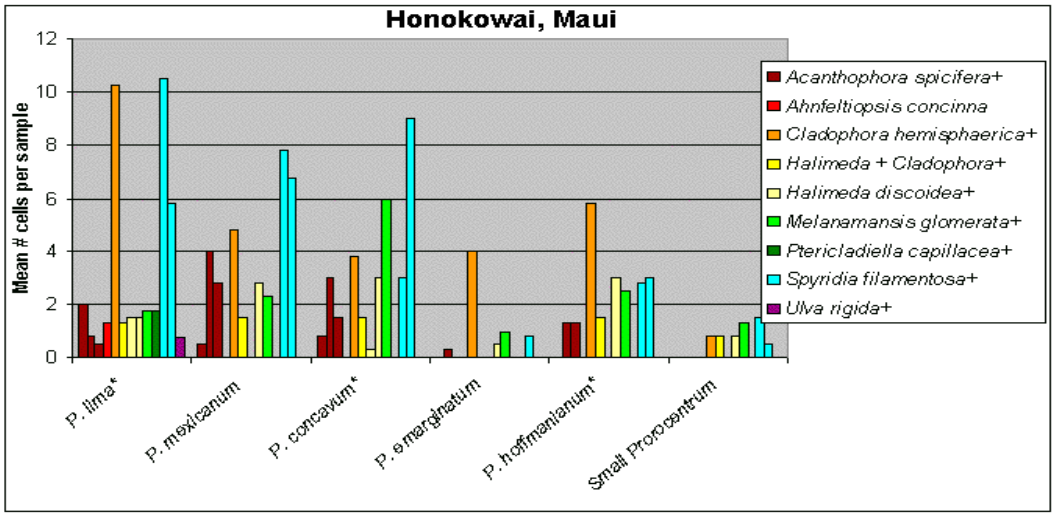


Figure 2-91. Mean *Prorocentrum* cells per sample by substrate collected in summer (August) at the Honokowai stations 1 - 7.

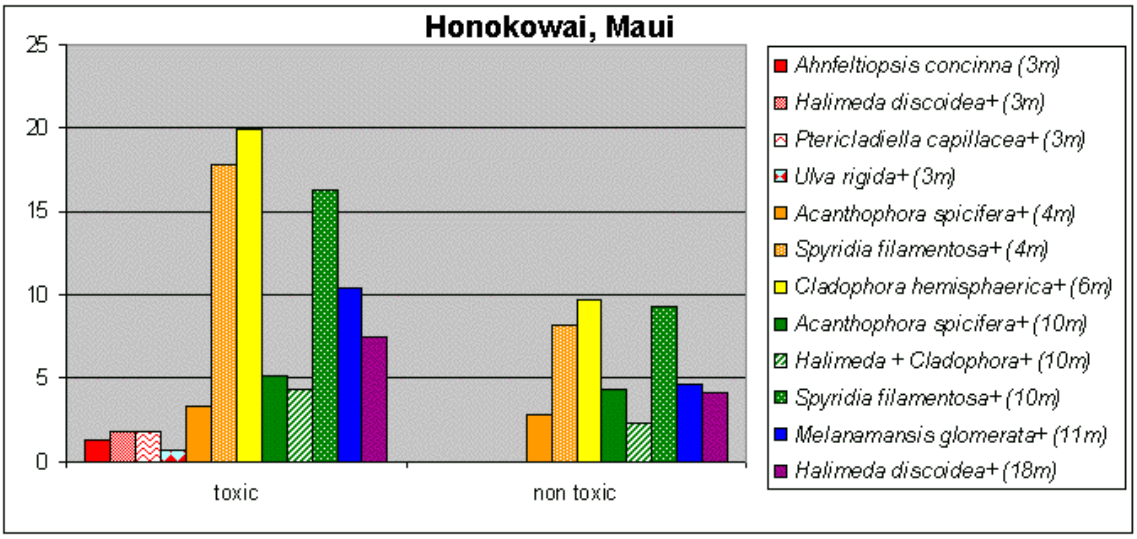


Figure 2-92. Mean *Prorocentrum* cells per 2 mL sample (toxic spp. combined vs. non-toxic spp. combined) at Honokowai by substrate and depth. Red tones – 3 m depth, orange – 4 m, yellow – 6 m, green – 10 m, blue- 11 m, and purple –18 m.

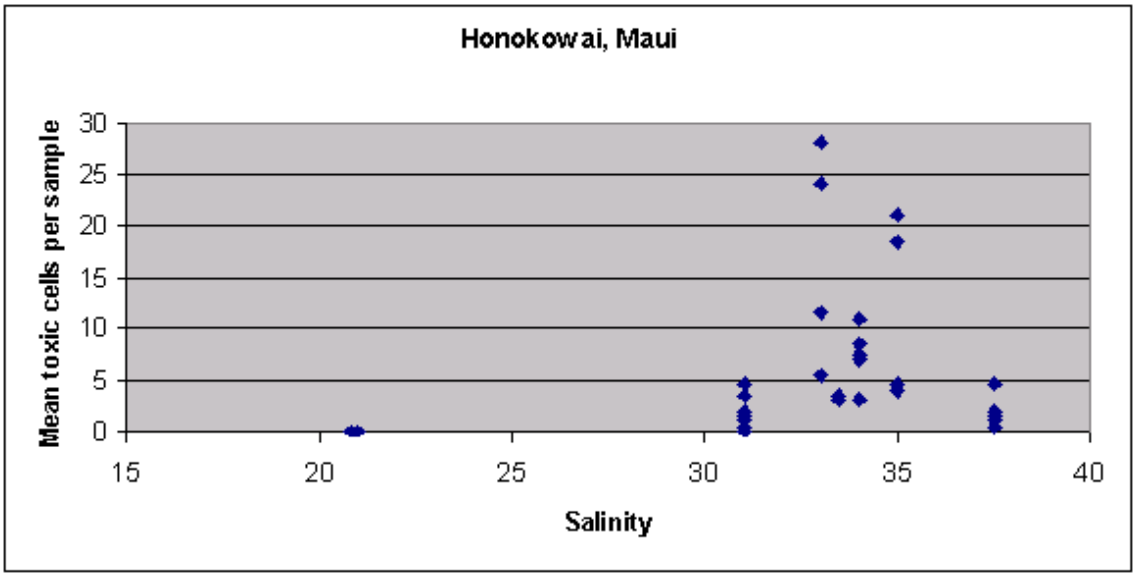


Figure 2-93. Toxic *Prorocentrum* vs. salinity (ppt) at Honokowai.

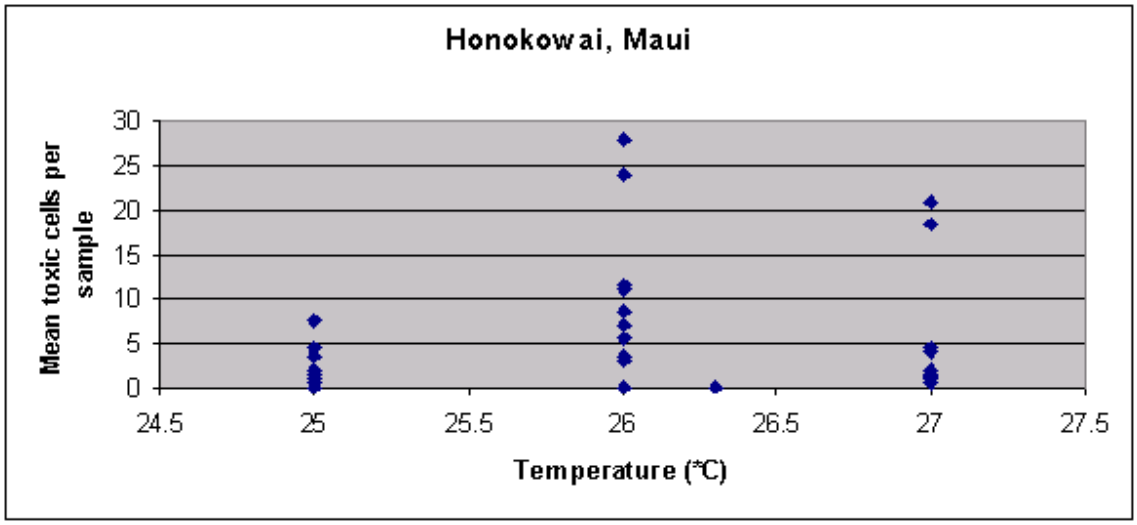


Figure 2-94. Toxic *Prorocentrum* vs. temperature (ºC) at Honokowai.

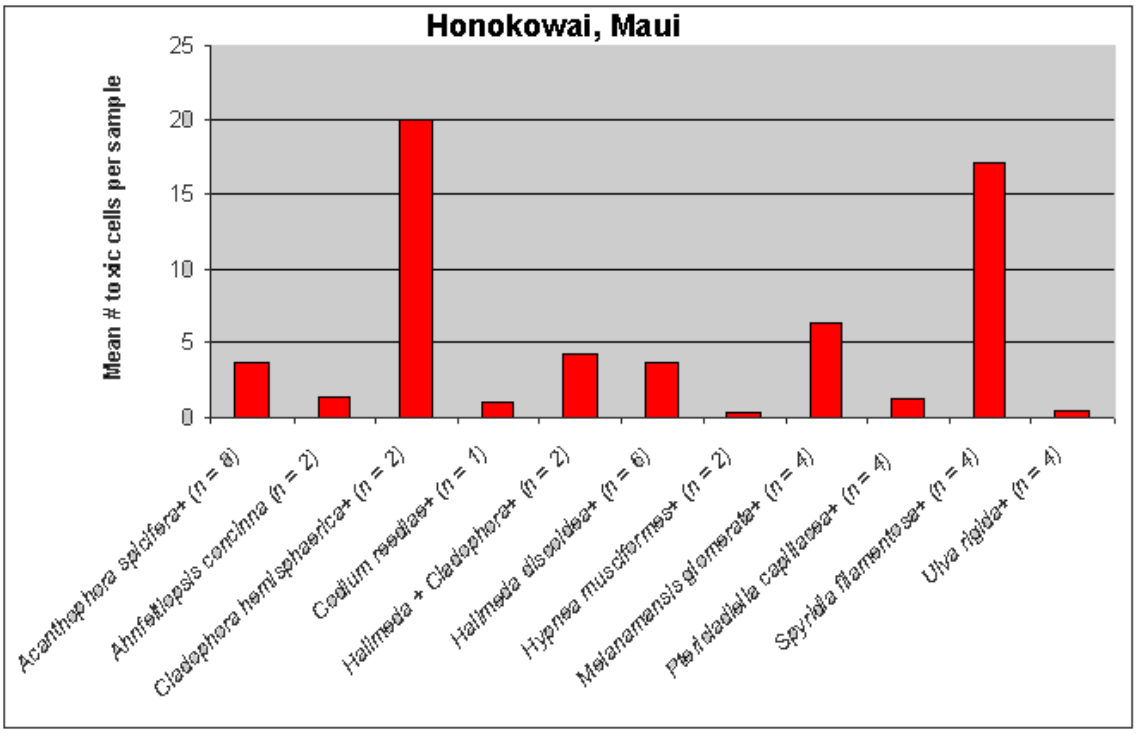


Figure 2-95. Mean toxic *Prorocentrum* by substrate at Honokowai, Maui.

Study Site 9: Palaau, Molokai

One station was sampled on the Southeast Molokai coast, a known green turtle grazing area. All seven substrates collected in winter had *Prorocentrum* present (Fig. 2-96). Four of six substrates collected at this site in summer had *Prorocentrum*, with no cells found on *Hypnea musciformes* (n = 2) and *Gracilaria bursapastoris* (n = 2, Fig. 2-97). These two substrates were not collected during winter. *P. lima* is the most common and abundant *Prorocentrum* spp. in Molokai. Highest abundance of toxic *Prorocentrum* occurred at the salinity level of 34 ppt (in winter, Fig. 2-98), and a temperature of 28.2 ºC (Fig. 2-99). The substrate with the highest abundance of toxic *Prorocentrum* was *S. filamentosa* (n = 2), followed by *P. crassa* (n = 4) and *R. riparium* (n = 1, Fig. 2-100).

An unusual occurrence between winter and summer sampling occurred at this site, *Halophila hawaiiensis*, the only seagrass found in Hawaii, disappeared from this location.

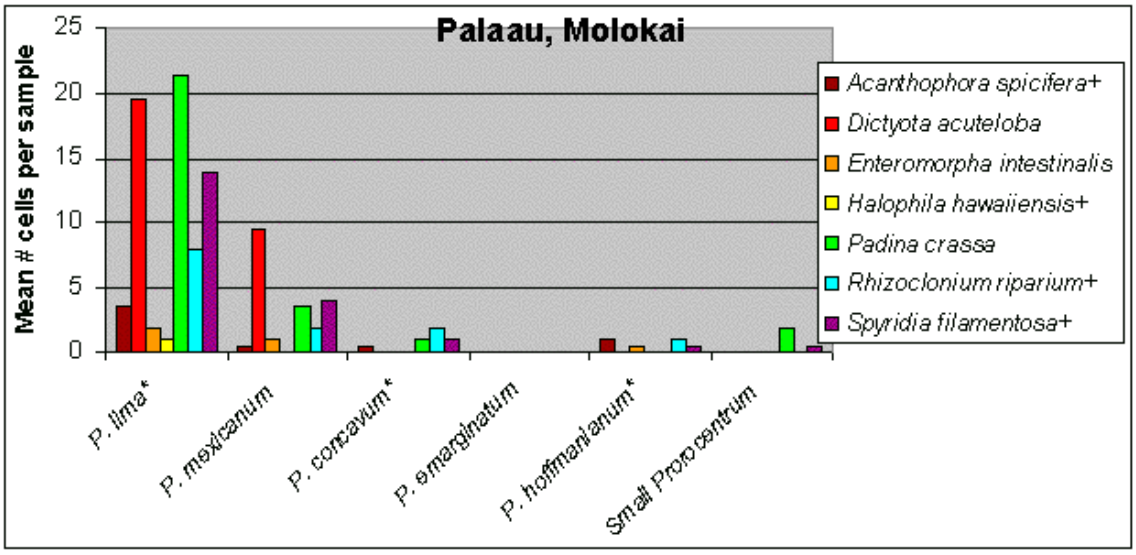


Figure 2-96. Mean *Prorocentrum* cells per sample by substrate collected in winter (February) at the Palaau, Molokai station.

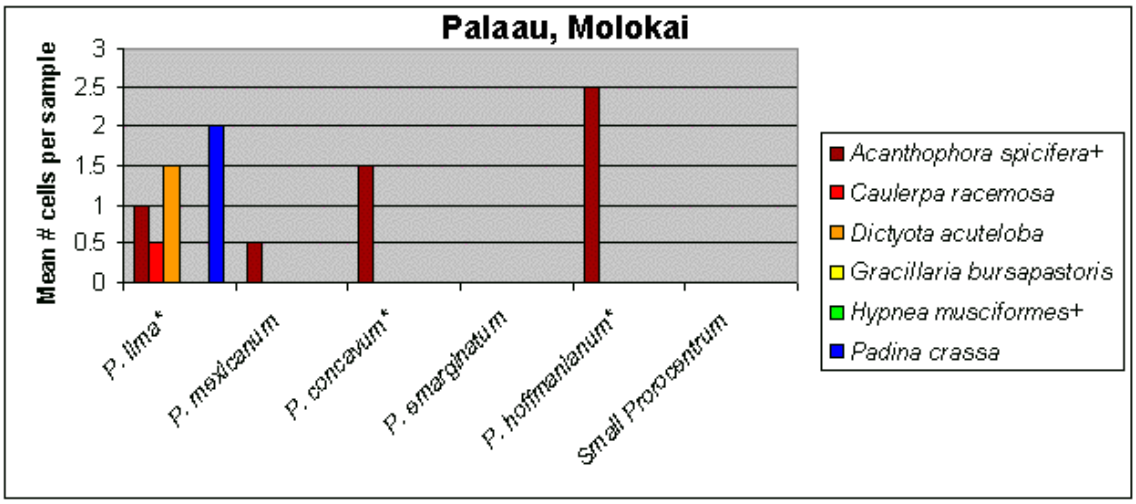


Figure 2-97. Mean *Prorocentrum* cells per sample by substrate collected in summer (August) at the Palaau, Molokai station.

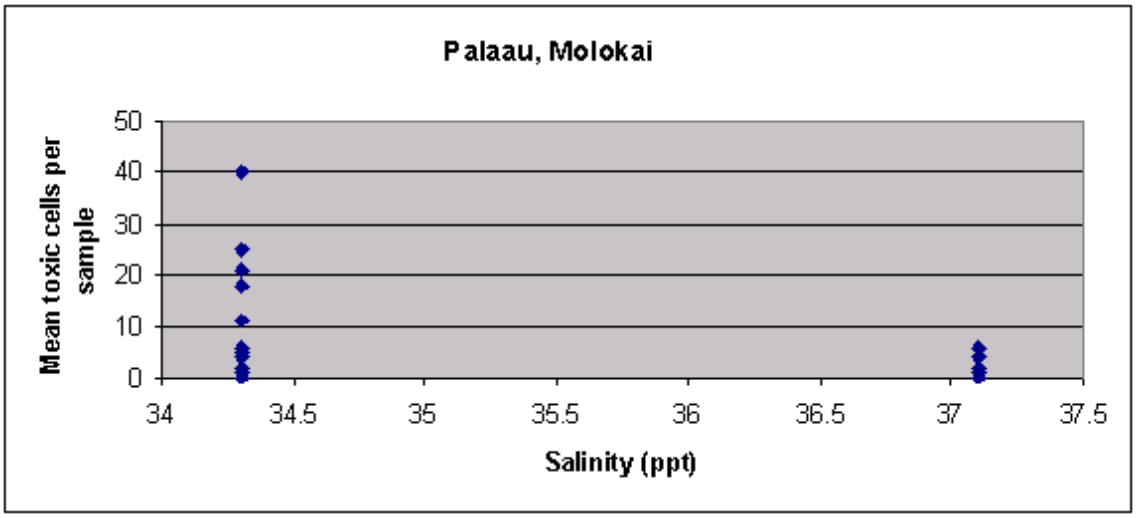


Figure 2-98. Toxic *Prorocentrum* vs. salinity at the Palaau, Molokai site.

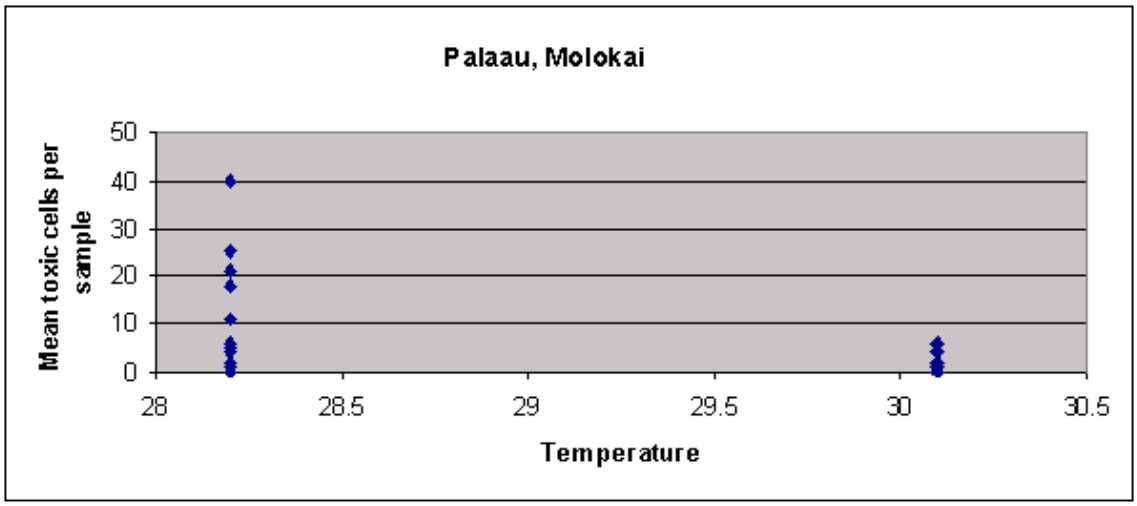


Figure 2-99. Toxic *Prorocentrum* vs. temperature (ºC) at the Palaau, Molokai site.

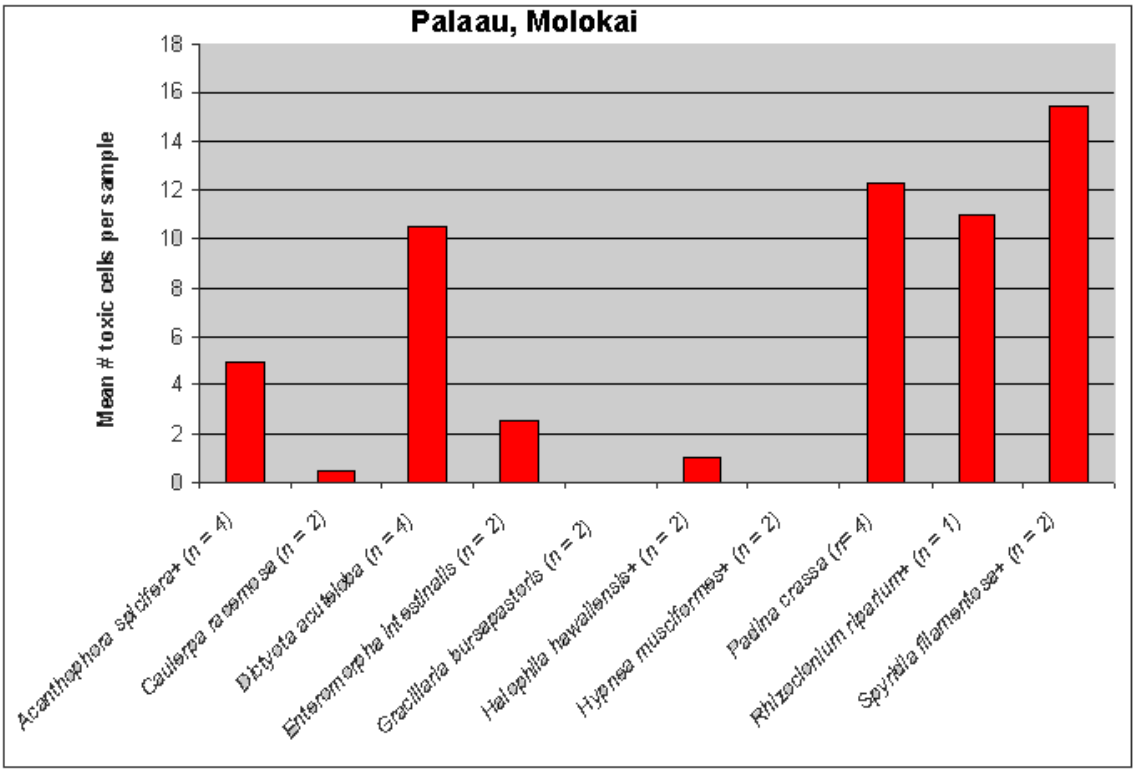


Figure 2-100. Mean toxic *Prorocentrum* and substrates in Palaau, Molokai.

Substrate preference in the Hawaiian Islands. All thirty-three species of substrate collected in Hawaii had toxic *Prorocentrum*. However, site variation was observed (Table 2-2). There were substrates with no *Prorocentrum* at one site, but had *Prorocentrum* at other sites. For example, *Melanamansia glomerata* (n = 6) and *Spyridia filamentosa* (n = 2), both preferred forages of green turtles, did not have any *Prorocentrum* at the Hilo site, but had significant proportions of *Prorocentrum* at Honokowai.

Table 2-2. Summary of the Hawaiian Islands data (across seasons).

	Kona	Punalu'u	Hilo	SE Kauai	NE Kauai	Waikiki	Kaneohe Bay	Palaau, Molokai	Maui
Samples w/*Prorocentrum*	35.5% (n = 31)	19.0* (n = 16)	30.0 (n = 33)	90.5 (n = 42)	100.0 (n = 37)	88.9 (n = 36)	92.3 (n = 65)	72.0 (n = 25)	89.7 (n = 39)
Substrate spp. w/*Prorocentrum*	75.0% (n = 8)	75.0 (n = 4)	62.5 (n = 8)	92.3 (n = 13)	100.0 (n = 17)	90.0 (n = 10)	100 (n = 13)	80.0 (n = 10)	100 (n = 10)
Range of toxic cells per g/wet substrate	0–4.2	0–0.8	0–24.2	0-48.3	0.8–65.0	0–217.5	0–594.2	0–66.7	0–46.7
Mean toxic cells per g/wet substrate	0.8 (n = 31)	0.2 (n = 16)	1.2 (n = 33)	13.1 (n = 42)	23.4 (n = 37)	34.4 (n = 36)	39.9 (n = 65)	10.7 (n = 25)	8.6 (n = 39)

* Only samples from the summer season had *Prorocentrum*.

Comparing toxic *Prorocentrum* spp. abundance requires the definition of salinity and temperature ranges as well as a substrate (*A. spicifera*) for an equivalent comparison amongst the sites of Hawaii. For salinity, the range of 30 – 38 ppt has the largest proportion of toxic *Prorocentrum* for the Hawaii sites (Fig. 2-101). For temperature, the range of 24 – 29 ºC is optimum (Fig. 2-102). Samples collected within these ranges will be used for the comparison amongst sites and amongst FP prevalence. This did reduce the sample sizes from the individual sites: Kona Coast (n = 2), Hilo (n = 0), SE Kauai (n

= 6), NE Kauai (n = 1), Waikiki (n = 9), Kaneohe Bay (n = 12), Honokowai (n = 8), and Molokai (n = 4).

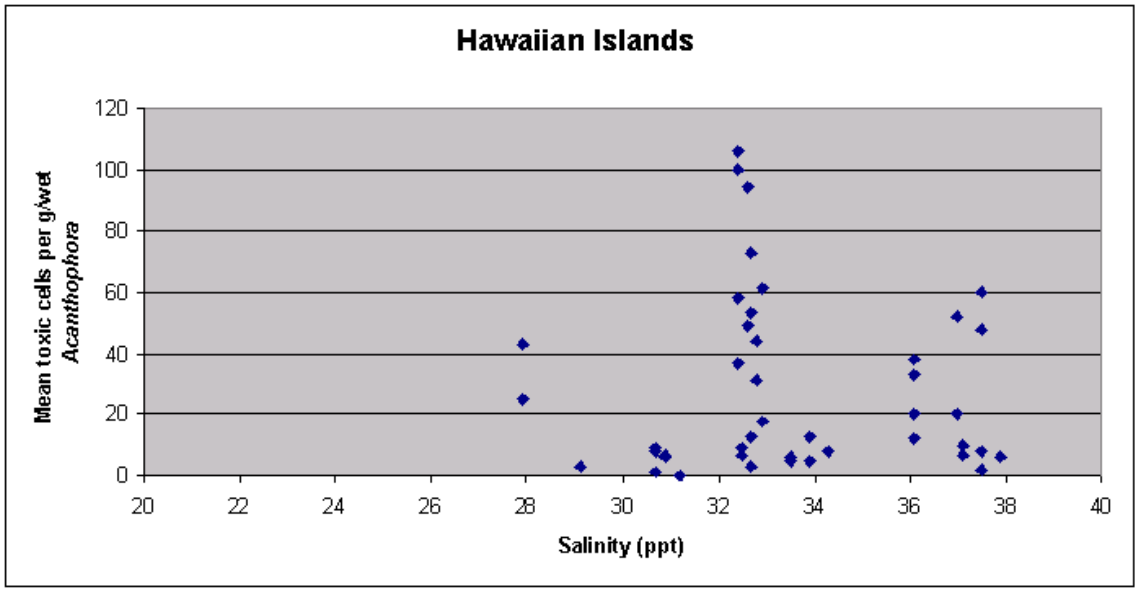


Figure 2-101. Toxic *Prorocentrum* on *Acanthophora* vs. salinity in Hawaii.

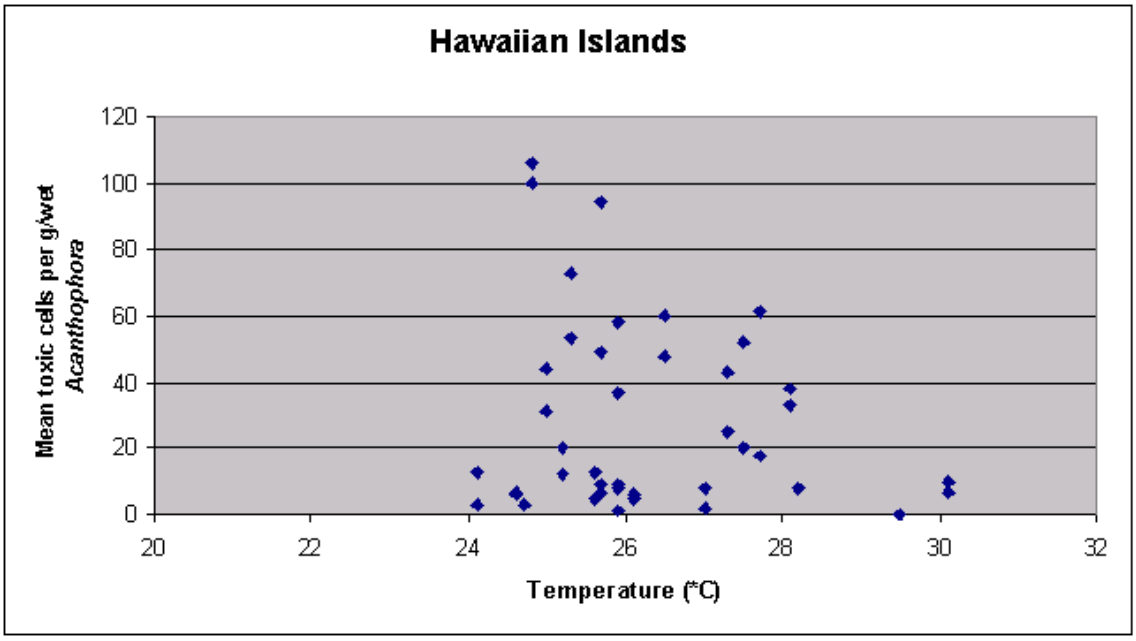


Figure 2-102. Toxic *Prorocentrum* on *Acanthophora* vs. temperature in Hawaii.

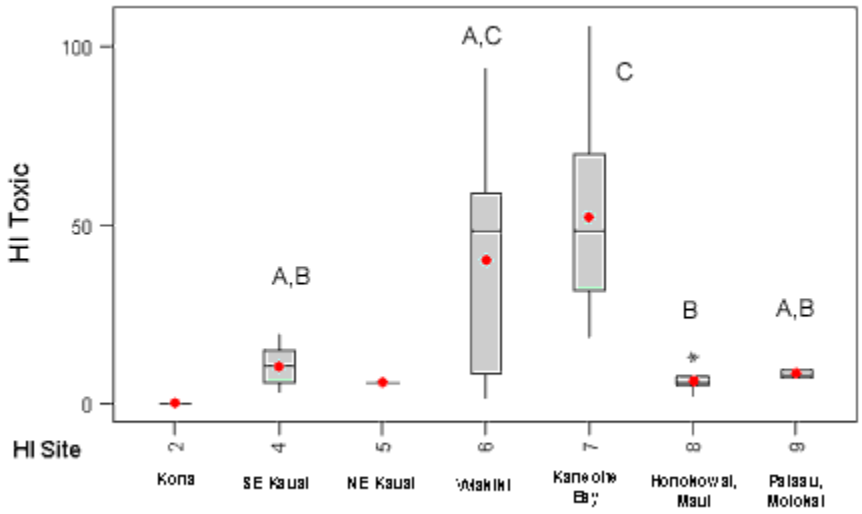


Figure 2-103. Toxic *Prorocentrum* on *Acanthophora* substrate among study sites in Hawaii within the range of 32-38 ppt and 24-28 ºC. Sites: 2 – Kona (n = 2, not significant in analysis) 4 – SE Kauai (n = 6), 5 - NE Kauai (n = 1, not significant in analysis), 6 – Waikiki (n = 9), 7 – Kaneohe Bay (n = 12), 8 – Honokowai (n = 8), and 9 – Molokai (n = 4). Means bearing the same alphabet letter (A, B) are not significantly different at p = 0.10.

Site comparison. When *Prorocentrum* abundance is compared among Hawaii study sites within the designated salinity and temperature ranges and substrate, Kona, Hilo, and NE Kauai drop out of the analysis (no or too few samples within the criteria). SE Kauai and Waikiki are not significantly different from one another at p = 0.10, SE Kauai, Honokowai, and Molokai are not significantly different from one another, and Waikiki and Kaneohe Bay are not significantly different (Fig. 2-103).

FP. Comparing mean toxic *Prorocentrum* per g/wet *Acanthophora* substrate (within the designated salinity and temperature range) to FP prevalence in the Hawaiian sites shows no significant difference between low, medium, and high FP sites (p = 0.10, Fig. 2-104).

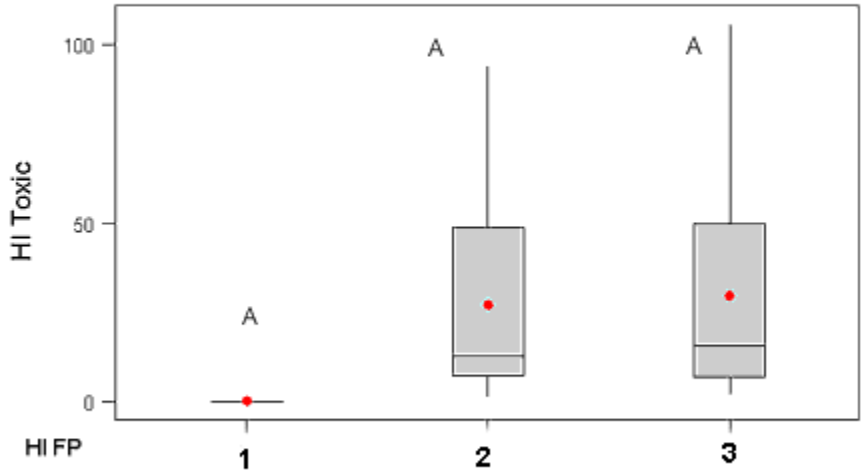


Figure 2-104. Toxic *Prorocentrum* vs. FP in the Hawaiian Islands. FP: 1 – low, 2 – medium, and 3 – high. There is no significant difference at p = 0.10.

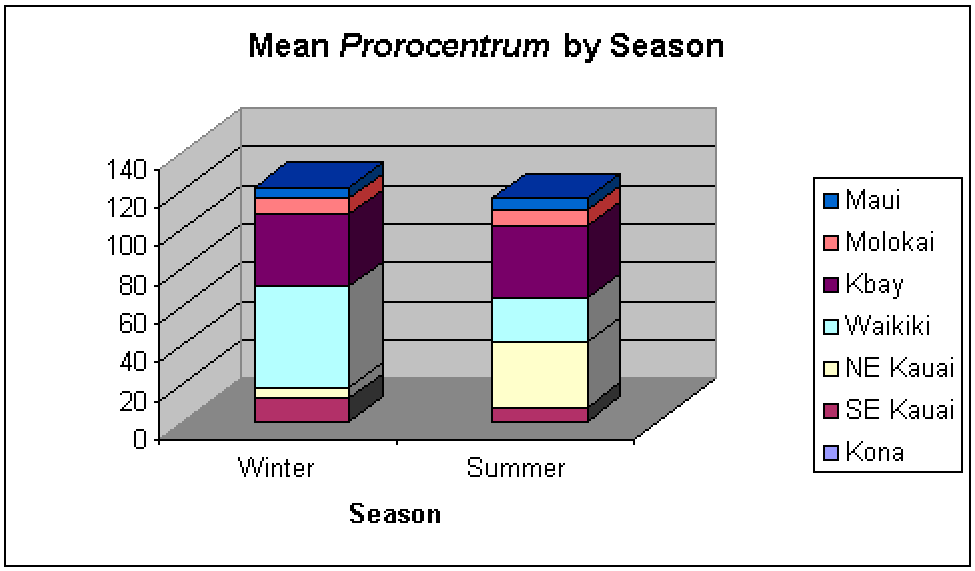


Figure 2-105. Toxic *Prorocentrum* by season and study site (on *Acanthophora* substrate). Average water temperatures: summer (27.43 ˚C, n = 31) and winter (26.12 ˚C, n = 16). There is no significant difference at p = 0.10.

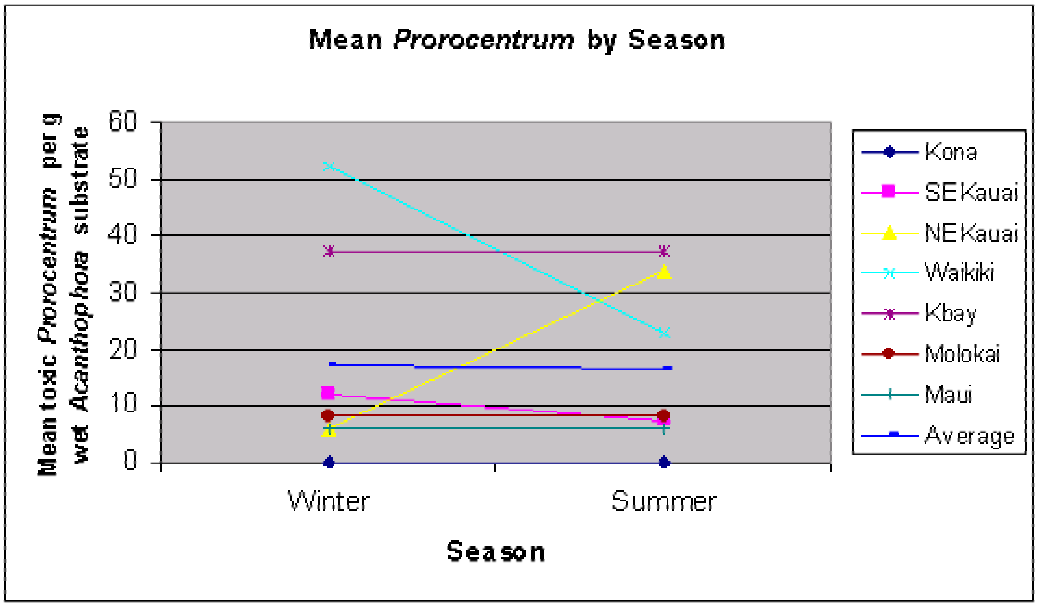


Figure 2-106. Mean toxic *Prorocentrum* on *Acanthophora* substrate by season and study sites.

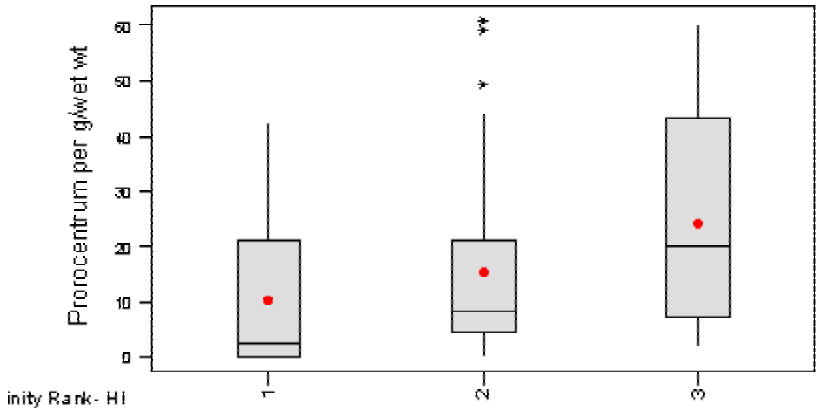


Figure 2-107. Mean toxic *Prorocentrum* vs. salinity rankings (1: 25 – 29.9 ppt, 2: 30–34.9 ppt, 3: 35 – 40 ppt) for the Hawaiian Islands. There if no significant difference at p = 0.10.

Season, Salinity, and Temperature. No significance was found between *Prorocentrum* abundance and season, salinity ranking, or temperature ranking for all the Hawaii study sites (Fig. 2-105, 2-106, 2-107, and 2-108). However, seasonal variation was observed at the NE Kauai sites and Waikiki. *Acanthophora spicifera* was not found at Punalu'u, so it is not included in the cross-site comparison, but *Prorocentrum* were only present on the summer samples (although in very low numbers), indicating seasonal variation occurs at this location.

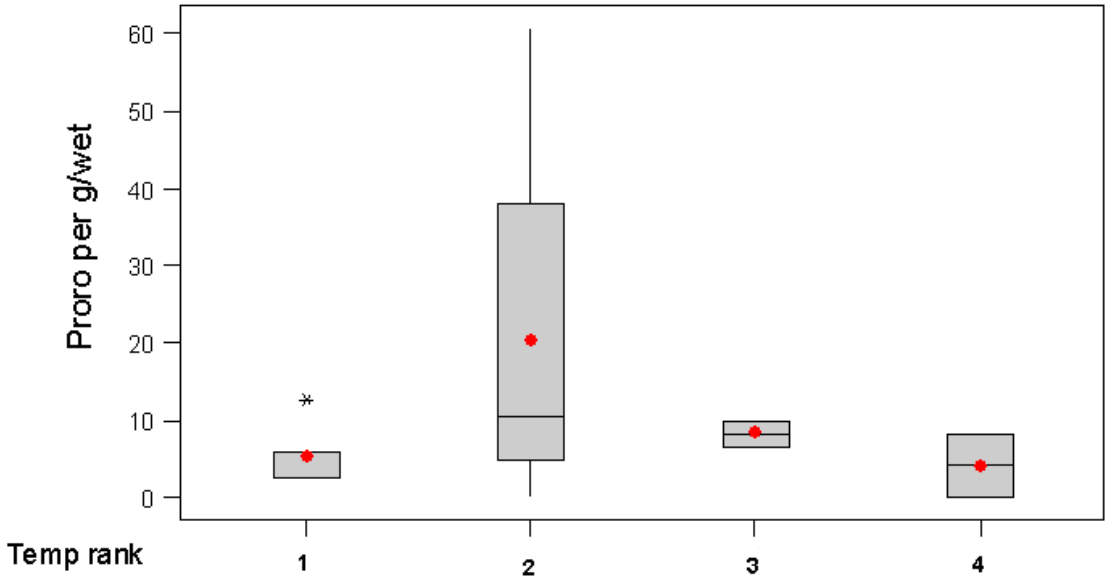


Figure 2-108. Toxic *Prorocentrum* on *Acanthophora* substrate by temperature ranking in Hawaii: 1: 20-24.9 ºC (n = 7), 2: 25-29.9 ºC (n = 36), 3: 30-34.9 ºC (n = 2), 4: 35-40 ºC (n = 2). There is no significant difference amongst the rankings (p = 0.10).

Maximum abundance per site. Amongst sites, the highest number of toxic *Prorocentrum* per g/wet substrate is compared to determine the highest number of toxic cells turtles can potentially be exposed to at each site (Fig. 2-109). A trend is observed,

with higher abundances of toxic *Prorocentrum* at sites with medium or high prevalences of FP. This demonstrates a similar pattern as found by Landsberg et al. (1999).

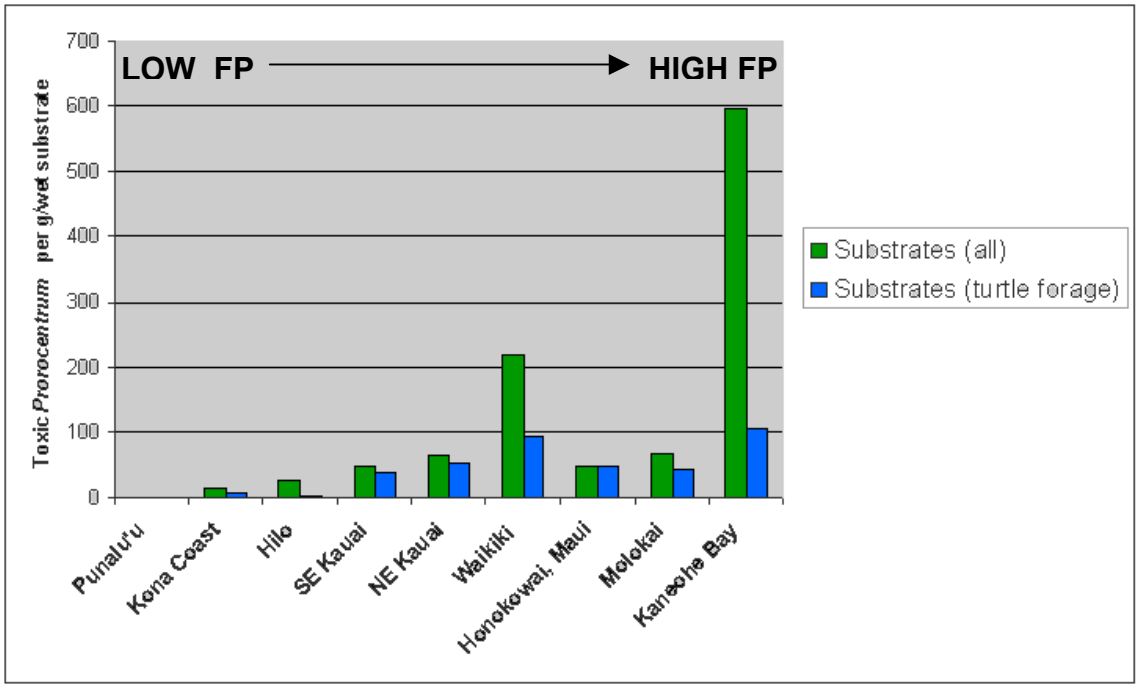


Figure 2-109. Maximum number of toxic *Prorocentrum* per g/wet substrate found at each Hawaii site, amongst all substrates and known turtle forage.

Discussion

Prorocentrum abundance and FP in Florida and the Hawaiian Islands

A trend between toxic *Prorocentrum* abundance and FP was observed in this study. Areas with medium and high FP prevalences had higher densities of OA-producing *Prorocentrum* species than areas with <1% FP prevalence in both Florida and Hawaii. Toxic *Prorocentrum* species were found in all the study sites where FP occurs, in both the Atlantic and Pacific, confirming that they could be a factor in the etiology of FP.

As in all ecological studies, there are multiple factors influencing *Prorocentrum* abundance, many of which were not accounted for in a study of this scope. Epiphytic dinoflagellates are affected not only by temperature, salinity, seasonal variation, and substrate, but also by nutrient levels within the water column, light intensity, turbidity, and community structure (both micro and macro).

Prorocentrum abundance varies, not only amongst sites, but also amongst substrates at different geographic locations. This, in part, is due to different macroalgae species compositions amongst sites. Some macroalgae species are known to contain toxins that dissuade epiphytes from attaching, while others may have components that stimulate epiphyte growth (Grzebyk et al. 1994). Brown and red macroalgae may release "growth factors", or substances such as vitamins, nutrients, or other chemicals that would stimulate growth (Morton and Faust 1997). Substrate preference in previous studies has linked high dinoflagellate biomass with red or brown algae substrates (Ballentine et al. 1988), and low epiphytic dinoflagellate biomass with green algae and seagrasses (Yasomoto et al. 1979, Morton and Faust 1997). The Hawaii results of this study demonstrate a similar pattern, with the highest abundances of *Prorocentrum* on red or brown macroalgae species, although seagrasses are uncommon and are only present at two of the sites (Kaneohe Bay and Molokai). The Florida study sites, however, maintained the highest densities of *Prorocentrum* on seagrasses.

Prorocentrum, as an epiphyte, will also obtain some nutrients and other cellular requirements from the water column. In theory, *Prorocentrum* will seek substrates with dietary components that they cannot obtain from the ambient environment. Depending on the geographic location, there will be differences in water column nutrient

composition, which will influence substrate preference of *Prorocentrum*. This is particularly important to note in this study, for one substrate was chosen to compare among all sites (within each location) to account for variability amongst substrates. However, *Acanthophora spicifera* and *Halodule beaudettei* may vary in their appeal as substrate to *Prorocentrum* at different locations, depending on local conditions. The factor of different ambient conditions (e.g. nutrients) at each of the sites is very difficult to quantify (particularly variation over time), and not included in this comparison.

Surface area of a substrate can also be a limiting factor for *Prorocentrum* (Bomber et al. 1985), but it is very difficult to quantify accurately with the numerous branching algal species and is not addressed in this study. A 20 – 45% increase in error per sample has been recorded in determining surface area for macroalgae and seagrasses in past studies (Morton and Faust 1997).

Seasonality can be observed in *Prorocentrum* in Florida, with higher overall densities during spring and summer (May and August, with higher water temperatures). This contradicts other studies, which have found *Prorocentrum* preferring cooler seasons, with temperatures less than 26˚C (Bomber et al. 1985). In this study, Florida Bay results are the opposite, with a significantly higher mean abundance of *Prorocentrum* on samples collected at temperatures above 26˚C than collected at lower temperatures ($p = 0.10$). Of course, temperature alone does not encompass seasonality. Other factors including ambient light, nutrients, and water movement can change with the season, and from year to year. Seasonality in Hawaii is not as apparent as in Florida, although it was observed at several of the sites. This implies that other location-specific factors are involved.

In the context of FP, seasonality may or may not have a large role. Green turtles show high site fidelity for feeding areas (Balazs et al. 1994a, 1994b). Seasonality in green turtles moving between foraging areas has not been recorded in Hawaii or Florida, although turtle movement has been linked to water temperature (Mendonça 1983). At temperatures greater than 25 ºC, immature green turtles in Mosquito Lagoon adopted a home range for foraging, and would leave their area only when temperatures dropped below 25 ºC (Mendonça 1983). These turtles would return to their home range once the temperature was above 25 ºC (Mendonça 1983). In theory, although *Prorocentrum* abundance may fluctuate by season at a location, the turtles feeding there will be exposed to some mean abundance throughout the year. On the other hand, seasonality may influence the exposure of turtles to OA. Higher water temperatures appear to enhance *Prorocentrum* abundance, and turtles move into warmer waters where *Prorocentrum* numbers are likely to be higher. This coincides with the distribution of FP prevalence in Florida, for example, where there are generally higher disease prevalences in warmer areas. OA exposure is an unknown factor, since we do not know how much toxin or exposure time is required if it is a part of the etiology of FP.

The exposure of green turtles to *Prorocentrum* (and OA) is linked to diet preference. Bjorndal (1980) hypothesized that microbial populations in the digestive tracts of green turtles may affect diet selection. This theory is supported by several studies, for example, in areas where there is both seagrasses and macroalgae present, turtles may only feed on one or the other, not a mixture (Mortimer 1982). This was observed in a study on Mosquito Lagoon green turtles, where approximately 90% of what they consumed was seagrasses, although macroalgae species were abundant in the area

(Mendonça 1983). In another site on the east coast of Florida, the Trident submarine basin, juvenile green turtles fed on macroalgae and not seagrasses although they were present (Redfoot 1997). Other studies in Florida have not observed this delineation between seagrass or macroalgae preference within green turtle diet as clearly, but have seen differences in substrates found in diets depending on geographic location (Ehrhart 1991, Ehrhart et al. 1996). These differences in seagrass and/or macroalgae diet in green turtles at different locations, along with variation in substrate preference of *Prorocentrum,* may be significant in the potential exposure of Florida green turtles to OA.

In Hawaii, green turtles feeding at different sites will have dissimilar options for feeding substrates, and vary their diets depending on location (Balazs et al. 1994a, 1994b), and will also have different potential exposure rates to OA. Substrate species are not ubiquitous amongst all of the study sites. Different sites have different macroalgae communities, depending on factors such as abiotic substrate, water movement, nutrients, and salinity. For example, in Honokowai the substrate *Spyridia filamentosa* has one of the highest proportions of toxic *Prorocentrum* cells per g/wet substrate, and it is a forage item of green turtles (Russell and Balazs 1994). But on the Kona Coast and at Punalu'u, *S. filamentosa* is not available, and *Pterocladiella capillacea* comprises a high percentage of green turtle diet (Balazs et al. 1994a). *P. capillacea* is not a preferred substrate of *Prorocentrum,* with very low densities at every site where it was found. Therefore, diet preference in green turtles and macroalgae distribution in Hawaii can also influence the number of *Prorocentrum* consumed by turtles, depending on location.

Green turtles feed on a variety of substrates (Mendonça 1983, Ehrhart 1991, Balazs et al. 1994a, Russell and Balazs 1994, Ehrhart et al. 1996, Redfoot 1997), e.g. the green turtles in Hawaii do not feed on only *Acanthophora spicifera.* Substrates may be selected by availability and abundance, and some may be incidentally ingested while turtles are grazing. Given all the variables accounted for in this study (salinity, temperature, substrate), what is the maximum potential exposure of green turtles to OA at each site? For both Hawaii and Florida, the maximum potential OA exposure (highest density of toxic *Prorocentrum* found) for each site parallels FP prevalence (Fig. 2-56 and Fig. 2-109).

As mentioned previously, toxin production (and in particular, OA production) is the unknown factor in this study. A major assumption is that known toxic *Prorocentrum* species are producing equal amounts of OA, regardless of geographic location, environmental variables, genetics, or species specificity. OA production does vary greatly between *Prorocentrum* populations and species, and factors influencing toxin production have been identified in laboratory experiments (Murakami et al. 1982, Dickey et al. 1990, Aikman et al. 1993, Morton et al. 1994, Morton et al. 1998).

Two recent studies have shown variation in OA production between *P. lima* isolates from within and between different geographical locations. In the Southwest Indian Ocean, OA content within *P. lima* varies amongst clones isolated from four islands (Boraïcha et al. 2001). Toxin production, toxin composition, and toxin profile vary significantly amongst 19 clones of *P. lima* from seven sites in Northwest Spain, with geographic location a significant component (Bravo et al. 2001). Toxin production

within *Prorocentrum* species is complex and varies within and between populations from different geographic locations.

There are multiple species of *Prorocentrum*, with four OA-producing species accounted for in this study. Toxin production may vary among species, e.g. *P. lima* may be toxic at one site but the *P. concavum* present at the same site may not be producing OA. Each species may have different combinations of environmental variables that trigger toxin production in their natural environment, or vary in their genetic strain of toxicity amongst species at one location. Being epiphytes, it is possible that toxin production may also be influenced by substrate, although this has not been shown. For example, in one location *P. lima* on *Acanthophora spicifera* may be producing more OA than the *P. lima* cells on the adjacent *Spyridia filamentosa* substrate. *P. lima* cells do vary in toxin production within the same location (Boraïcha et al. 2001, Bravo et al. 2001). There are still many unknown factors. Sterile, highly organized laboratory experiments cannot account for all possible combinations of variables occurring in natural environments, and can therefore be misleading. There are also many new species of *Prorocentrum* being discovered, some of which may be toxic (Landsberg and Steidinger, unpublished data), and known species, such as *P. mexicanum*, which does not produce OA but produces other toxins (Faust et al. 1999).

P. mexicanum produces ciguatera-like toxins (Tindall et al. 1989, Faust et al. 1999), is the most prevalent *Prorocentrum* species at the Florida study sites, and is found throughout Hawaii. In St. Joseph Bay and Mosquito Lagoon, *P. mexicanum* is found in much higher densities than any other *Prorocentrum* species. Although *P. mexicanum*

does not produce OA, it should be recognized as a toxic species. How do these other toxins affect foraging turtles?

To quantify OA amongst green turtle foraging grounds, *Prorocentrum* spp. have to be tested for the presence, quantity, and potency of OA and other toxins amongst sites. If *Prorocentrum* is a part of the etiology of FP, as this study has re-confirmed the possibility, it is very important to understand toxin production and identify the factors that trigger it. For example, if *P. lima* and *P. concavum* do not presently produce OA in St. Joseph Bay (<1% FP), but produce high amounts in Mosquito Lagoon (>50% FP), is it because environmental variables in Mosquito Lagoon are different than in St. Joseph Bay? Or could it be that different strains of *Prorocentrum* vary in toxicity, as found in *P. minimum* in the Mediterranean Sea (Grzebyk and Berland 1996)? A combination of genetics and local conditions play a role, although we do not know what the specific factors are at this point. If toxin production in *Prorocentrum* spp. in natural environments is better understood, we may be able to pinpoint causation factors, e.g. nutrient loading high in phosphorus, and develop management recommendations.

Eutrophication may play a large role in *Prorocentrum* abundance. Past studies have linked high dinoflagellate density with areas of human disturbance (Bagnis et al. 1987, Morton and Faust 1997). Land uses such as urban/residential and agriculture contribute larger amounts of phosphorus to nearby waters than other land use types (Beaulac and Reckhow 1982, Dauer et al. 2000). Ambient concentrations of phosphorus in the water column can influence OA production by *Prorocentrum* (Morlaix and Lassus 1992, Tomas and Baden 1993, Sohet et al. 1995, Landsberg et al. 1999). Nutrient loading can also alter micro- and macroalgae community structure, and may be linked to

algae blooms (Hallegraeff 1993). The Maui site of Honokowai has been plagued by algae blooms, which may be linked to eutrophication. The Oahu study sites (Waikiki and Kaneohe Bay) have the highest densities of *Prorocentrum* amongst all the sites in Hawaii. Oahu has the highest population and is the most developed of the Hawaiian Islands, and the most likely to be affected by eutrophication. Eutrophication and land use is discussed further in Chapter 3.

The time period of this study was one year. There will be natural variation in *Prorocentrum* and macroalgae/seagrass abundance between years, and there may be high fluctuations within a 5- to 10-year period. There may also be a specific exposure time of green turtles to OA before it may affect them, e.g. 3 – 5 years. This would correspond to some reports of regression in the disease (Bennett et al. 2000), if the FP-afflicted turtles exposure to OA were reduced over time.

High FP Sites with Overall Low *Prorocentrum* Abundance: Maui, Molokai, and Mosquito Lagoon

Comparing *Prorocentrum* abundance amongst sites, using the same substrate and designated salinity and temperature range, found Maui, Molokai, and Mosquito Lagoon with significantly low numbers.

In Florida, Mosquito Lagoon does not fit the hypothesized linear trend between *Prorocentrum* abundance and FP. Mosquito Lagoon has higher salinity levels than the other sites, elevated to over 40 ppt in places, although *Prorocentrum* abundance is low in Mosquito Lagoon even where salinity is comparable to the other Florida sites. *P. hoffmannianum* has a salinity preference of 34 ppt for optimal growth (Morton et al. 1994). *Prorocentrum* abundance is affected by salinity in Florida (Fig. 2-54). However,

no other sites in Florida or in Hawaii had salinities >40 ppt, so it is difficult to confirm this on a large scale within the context of this study.

In Hawaii, the results for Maui and Molokai vary from the trend observed within the other Hawaii study sites. The Molokai site consists of an extensive, shallow (<1m at low tide) tidal flat where siltation covers all the macroalgae present. This siltation is likely caused by anthropomorphic activities such as agriculture and land use change on Molokai. The silty conditions found on the south coast of Molokai have not always been present, and have been observed to worsen with population increases and land use changes (W. Puleloa, Division of Aquatic Resources, State of Hawaii, pers. comm. 2001). These silty conditions reduce light, which can inhibit growth of epiphytes, including *Prorocentrum*. The fact that the seagrass *Halophila hawaiiensis* disappeared from this site over the study period is an indicator of the occurrence or presence of a stressful environmental variable at this location, which may also be affecting *Prorocentrum* abundance.

The Maui study site can have very high wave action and surf, which can directly impact epiphytes, particularly fragile dinoflagellates. Berdalet and Estrada (1993) found a negative correlation between dinoflagellate reproduction and high turbulence. Maui also has a history of algae blooms, an indicator of a variation within the environment that is affecting macroalgae/microalgae community dynamics.

However, *Prorocentrum* are present at the Mosquito Lagoon, Molokai, and Maui study sites. It has been demonstrated that *Prorocentrum* can be more toxic when stressed (Morton et al. 1994). High salinity, low light, and strong water movement may be

stressful to *Prorocentrum* cells, and although there may be fewer densities at these sites, they may be more toxic.

Comparing the maximum toxic *Prorocentrum* abundance found at each site, regardless of substrate, salinity, and temperature, indicates that Mosquito Lagoon had samples with the highest numbers of toxic *Prorocentrum* amongst the four Florida study sites (Fig. 2-56). Maui and Molokai also had samples with high numbers of toxic *Prorocentrum*, indicating that potential exposure of turtles to OA can be quite high at these locations (Fig. 2-109).

Florida vs. Hawaii

The higher densities of *Prorocentrum* around Florida versus Hawaii could result from multiple factors. The Florida study sites, as locations where green turtles feed around the state, are sheltered bays and lagoons with protection against waves and currents. Epiphytes were observed by the naked eye on the substrates collected at all of the Florida study sites. The sites around Hawaii are the opposite of the Florida sites, exposed to high wave action, currents, and surf. The substrates collected around Hawaii appeared "clean", with the exception of the silty samples from the Palaau, Molokai site. Overall, there were more epiphytes in the Florida samples than in the Hawaii samples.

Molokai and *Halophila hawaiiensis*

Halophila hawaiiensis is the only seagrass found in the Hawaiian Islands. *H. hawaiiensis* spreads by rhizomes and has leaves that extend a few cm above the substrate. In this study, it was collected in two locations where green turtles are known to graze on

it: Kaneohe Bay and Palaau, Molokai. Only the Molokai bed of *H. hawaiiensis* disappeared within this study's timeframe.

There are many possible explanations for the disappearance of this seagrass, and this site should be monitored to see if it reappears. Potential stressful factors include thermal events, elevated or decreased light, osmotic stress, parasites, smothering by biofouling, disease, or combinations of these factors, which can have an additive effect (Ralph 1999). Within a six-month period, some factor(s) caused the disappearance of *H. hawaiiensis*.

In Galveston Bay, Texas, submerged vegetation (including *Halophila* spp.) disappeared in the late 1980s into the early 1990s, and identified causes included turbidity from shoreline erosion and dredging, and water quality changes from the impacts of waterfront development (Pulich and White 1991). Construction on the shoreline results in erosion and redistribution of dredged sediments, excessive nutrient loading from wastewater discharges, non-point source runoff, and toxic spills from shipping and industry (Pulich and White 1991). Molokai and the islands near it are under strong land-use pressure for agriculture and development purposes.

High siltation occurs at the Molokai study site. Land use practices such as agriculture on Molokai are likely the source. The introduction of ungulates along with intensive crop-row agriculture has coincided with high siltation on Molokai's coastal reefs since the early 1900s (W. Puleloa, Division of Aquatic Resources, State of Hawaii, pers. comm. 2001). A study in Southeast Asia found that *Halophila ovalis* was one of the most sensitive to siltation of seven individual seagrass species tested (Terrados et al. 1998). They identified *Halophila* as a good indicator species to provide an early warning

of detrimental siltation loads to SE Asian seagrass beds (Terrados et al. 1998). The effects of light deprivation from turbidity were investigated on the survival, morphology and physiology of *Halophila ovalis* in Australia. *H. ovalis* displayed little tolerance to light deprivation, with plant death occurring after 38 days in the dark (Longstaff and Dennison 1999).

A plasmodiophorid fungal parasite has been linked to *H. stipulacea* (Marziano et al. 1995), another potential source of mortality. Periodic exposure to high wave action, high currents, or storm activity can also harm seagrass beds (Pulich and White 1991).

However, *Halophila* seeds can remain dormant in sediments for several years (McMillan 1981, 1991), so there is a good chance that *Halophila hawaiiensis* will reappear at this site, if the cause of its disappearance is no longer present.

Mosquito Lagoon and *Thalassia testudinum*

After collecting substrates amongst eight sample stations around Mosquito Lagoon, it was noted that *Thalassia testudinum*, a dominant seagrass at the other three study sites in Florida, was not found or observed at any of the stations. Since 1983, six nearshore seagrass transects have been monitored in the southern end of Mosquito Lagoon (Provancha et al. 1992). Three species of seagrass have been recorded: *Halodule wrightii* (also called *Halodule beaudettei*), *Ruppia maritima*, and *Syringodium filiforme*, with *Halodule* the dominant species. Unlike *Halophila hawaiiensis* at the Molokai, Hawaii site, *T. testudinum* does not appear to have occurred in Mosquito Lagoon for at least the past twenty years.

T. testudinum is not mentioned in underwater vegetation surveys of the lagoon from the early 1980's, or in dietary studies of green turtles within Mosquito Lagoon

(although seagrasses were dominant within diet composition, Mendonça 1983). The range of *T. testudinum* on the east coast of the United States is described as from the Carolinas to the Caribbean (Stuckey and Gould 2001). However, *T. testudinum* is not found north of Sebastian Inlet (except for a few ephemeral patches), which is located approximately fifty miles south of Mosquito Lagoon (C. White, Brevard County, pers. comm. 2001). It is unknown why it does not occur in Mosquito Lagoon, and there are no credible hypotheses. Temperature and turbidity do not appear to be factors, since *T. testudinum* occurs in colder, warmer, and more turbid areas in Florida than Mosquito Lagoon. The factors influencing the range of *T. testudinum* on the East coast of Florida appear to be a mystery discussed by many prominent seagrass researchers (C. White, Brevard County, pers. comm. 2001).

Conclusion

It is recognized that there are a number of variables in toxin production, and hence the exposure of green turtles to OA. Little is known about toxin production in *Prorocentrum* in the natural environment, and further research is recommended. But confirmation of the presence of *Prorocentrum* species known to produce OA in areas of FP is important. In this study it was clarified that in two major areas in the U.S. where FP is highly prevalent, *Prorocentrum*, a known tumor-promoter, is a common risk factor.

CHAPTER 3
LAND USE/LAND COVER AND THE STUDY SITES

Introduction and Literature Review

Land-water interactions, particularly the matrix of land-use and its effects on water quality within a watershed, have been a "hot" topic in ecology in the past three decades. Since there is correlation between nutrient loading and land use (Perry and Vanderklein 1996), water quality is an issue that directly relates to its terrestrial counterpart in the landscape. Water quality can dictate the overall health of coastal and estuarine ecosystems, including the biological communities that live there. Water quality and aquatic ecosystems are impacted by nutrient flow, which is inexorably linked to watershed dynamics. Many land-water interaction models have been developed using the technology of remote sensing and Geographic Information Systems (GIS) to demonstrate the relationship between land use/land cover (LULC), nutrient flow, and water quality. Factors influencing nutrient flow and water quality include not only LULC, but also: soil, hydrology, climate, geology, topography, coastal processes, and management practices. GIS is a tool that can combine these factors to predict nutrient flow in a given watershed, and its potential consequences on water quality. A GIS model used to compare the drainage basins of the study sites in Hawaii and Florida to FP and *Prorocentrum* abundance not only looked at LULC at these areas, but also incorporated the other components that are linked to nutrient movement in the landscape.

Landscape Components

Land use/ land cover

Land cover is the landscape classified into surface components such as forest, water, wetlands, and urban. Land cover can be determined with methods including surveying, air photography, or spectral signatures of satellite imagery (http://www.csc.noaa.gov/crs/lca/). Land use is the documentation of human uses of the landscape: residential, commercial, agricultural, etc. Land use can be inferred but not explicitly derived from satellite imagery. There is no spectral basis for land use determination in satellite imagery. The imagery used in this study was first classified into land cover by computer analysis of spectral signatures of satellite remote sensing data, and then re-classified into land use by further analysis. Land use and land cover combined are an important component in analyzing a landscape to identify the factors influencing water quality.

LULC affects water quality by altering sediment, chemical loads, and watershed hydrology (Wang and Yin 1997, Basnyat et al. 1999). Multiple studies have produced models and correlated land use types with nutrient export (Table 3-1). These export values vary depending on the particular location under study (Mattson and Isaac 1999).

Basin characteristics such as LULC, slope, and soil attributes affect water quality by regulating sediment and chemical concentrations (Basnyat et al. 2000). LULC types can serve as nutrient detention media or as nutrient transformers as dissolved nutrients flow towards a stream or coastal area.

Table 3-1. North American annual average nutrient export (kg/ha/yr) data summary (from Beaulac and Reckhow, 1982), as an example of correlating LULC directly with nutrient export rates.

Broad land use type	Phosphorus range	Phosphorus Median	Nitrogen Range	Nitrogen Median
Urban	0.7 - 2.8	1.2	4.0 - 11.5	5.5
Pasture	0.3 - 2.8	0.9	2.0 - 11.0	5.0
Mixed Agriculture	0.5 - 1.5	1.0	9.0 - 25.5	14.0
Row Crops	1.0 - 5.3	2.3	4.0 – 22.5	8.5
Non-row Crops	0.7 - 1.6	0.8	4.0 – 6.5	6.0
Forest	0.1 – 0.4	0.25	2.0 – 3.5	2.5

Numerous studies have found strong correlations between water quality and LULC (Knisel 1980, Johnes et al. 1996, Mattikalli and Richards 1996, Allan et al. 1997, Tufford et al. 1998, Basnyat et al. 1999, McFarland and Hauck 1999, Rodda et al. 1999). In general, urban/residential and agriculture lands are the strongest contributors of nutrients and sediments, resulting in negative impacts on water quality (Basnyat et al. 1999, Dauer et al. 2000). Urban/residential lands are associated with wastewater runoff, sediment contaminants, high phosphorus contributions, and inducing low dissolved oxygen in adjacent waters (Dauer et al. 2000). Agriculture contributes nutrients from fertilizers and livestock waste, and it contributes sedimentation from altered vegetation and management practices (Mattikalli and Richards 1996). Wetlands, grassland/scrub, and forests usually act as nutrient sinks and improve water quality (Tufford et al. 1998, Basnyat et al. 1999).

LULC within riparian zones are better indicators of water quality than LULC within whole drainage basins (Allan et al. 1997, Tufford et al. 1998, Basnyat et al. 1999, Dauer et al. 2000). LULC within 150 m of water is a much better indicator of water quality than LULC >150 m away (Tufford et al. 1998). Riparian zones ranging between 0.1 to 6 km wide have been identified as good predictors of water quality, depending on topography and runoff conditions (Soranno et al. 1996). Locations and patterns of

different LULCs within the basin are very important. Nitrogen and phosphorus have different flow pathways from terrestrial to aquatic systems (Tufford et al. 1998), and the matrix of LULC can impact one differently than the other. For example, if a drainage basin has a large proportion of livestock agriculture within riparian zones, nitrogen runoff from waste will likely have a large influence on water quality. If urban or residential lands border streams or coastline, phosphorus could be a problem. If a drainage basin is heavily used as residential and/or agricultural land, but has large, forested riparian zones (>150 m), water quality within that basin will likely be good.

LULC has been repeatedly linked to nutrient levels and flow in past studies. However, factors such as geographic location variables (e.g. soils, hydrology) play an important role. Quantifying nutrient flow is complex, and requires the consideration of other landscape variables in context with LULC.

Soils

Soil type affects nutrient export in a number of ways. The nutrient content of the soil determines the potential for nutrient export, with native phosphorus content being dependent on parent geology type (Young et al. 1996). Soil types differ in their nutrient absorption capacity, which affects the amount of nutrients the soil can store (Young et al. 1996). Nutrient properties of broad soil types have been generalized in Table 3-2. For example, nutrient export from sandy soils is likely to be lower than clay soils. While general classification can be useful, many soils can be a mixture, making their properties difficult to define.

"Land form" has been characterized as the most important characteristic in nutrient export (Sonzogni et al. 1980). The term includes soil texture, soil chemistry, soil

type (mineral or organic), and surface geology and physiography (slope, drainage density). Of these, soil texture (soil particle size distribution) was identified as the most important factor (Sonzogni et al. 1980).

Table 3-2. General properties of broad soil types (Beaulac and Reckhow 1982).

SOIL TYPE	GENERAL PROPERTIES
Sandy/gravel soils	• low cation content (low adsorption) • low erodibility • high infiltration capacity
Clay soils	• high cation content • high erodibility • low infiltration capacity
Organic soils	• high nutrient content • limited nutrient retention capacity • low infiltration capacity

Soil pH is also an indicator of nutrient availability (Young et al. 1996). Correlations have been made between soil pH and nutrient export, with the higher loadings coming from areas with low acidity soils (Young et al. 1996). However, the levels of minerals such as iron, aluminum, and calcium in the soil also influence nutrient availability (Young et al. 1996).

Therefore, many soil type variables influence nutrient export. For most soil types, it is unclear how these variables will combine to influence nutrient transport, unless they are homogenous clay, sandy or organic soils. Soil type is a variable important in nutrient flow.

Geology

Past studies in the 1970s suggested a link between geology and eutrophication of lakes in North America (Dillon and Kirchner 1975, Omernik 1977, Young et al. 1996). These studies were not conclusive, however. The high phosphorus content of some rock types indicates a potential source for high nutrient export rates, although the studies mentioned above suggest the influence on export rates may not be great (Young et al. 1996).

Geology is not directly used in this study, although it is represented in the soil data.

Hydrology

Hydrological processes are affected by the spatial variability of all the other factors discussed here: LULC, soils, topography, climate, and management (Vieux 1991). Hydrology is at the core of water quality and quantity concerns, and it is dependent on spatially distributed attributes of the catchment or watershed (Vieux 1991).

Water flow rates have been linked to nutrient loading and water quality. This is integrally tied to climate, since water flow can change by rain and other climatic factors.

Climate

Nutrients are transported by surface and sub-surface flows, and by wind (Young et al. 1996). Surface runoff has been monitored for nutrient export data and is considered important due to particle-bound nutrients. Sub-surface flows are significant with associated phosphorus movement in some soil landscapes (Chittleborough et al. 1994,

Young et al. 1996). It should be noted that in certain environments, wind can have an impact on movement of nutrients and soils across a landscape, but can be very difficult to quantify.

Surface runoff is primarily determined by rainfall, though influenced by soil-plant systems. Rainfall intensity, rainfall depth, rainfall drop size, and time since last rainfall are all variables important to consider. One study found a positive relationship between nitrogen loss and rainfall intensity (Young et al. 1996). Natural variation in rainfall intensity with soil detachability and soil surface exposure to rainfall are important variables. Rainfall is strongly linked to nutrient transport in urban areas, where there is a larger occurrence of wash-off of unattached deposited material.

Although climate is an important variable, it is integrally linked to hydrology, topography, and LULC. It is very difficult to quantify, and will not be incorporated in the model for this study. Its importance is emphasized, however, and will be represented in the other variables.

Topography

Elements of topography include land slope, drainage density, and catchment size (Young et al. 1996). Slope dictates the velocity of surface runoff, which affects the erosive power and the transport capacity of the flow (Young et al. 1996). In previous studies in the early 1990s, Triangular Irregular Network (TIN) models were used in GIS applications to provide land surface slope to estimate overland flow. Slope is major factor influencing non-point source pollution, water quality and quantity (Vieux 1991).

Drainage density is important, for as streams and rivers increase in density, run-off distances decrease, and nutrients have less of a distance to flow to the waterways

(Young et al. 1996). Increasing catchment size affects nutrient export due to the increasing capacity for sediment (and associated nutrient storage in both channel bars and floodplain deposits).

Coastal processes

All of this discussion on nutrient flow in watersheds is of particular importance when these nutrients filter into coastal waters. Nitrogen loading to coastal watersheds is of great interest, because loading is increasing, and rates of primary production in many coastal waters are largely limited by nitrogen supply (Valiela et. al 1997). Eutrophication of coastal waters, linked to the increasing nitrogen loading from watersheds, is arguably the largest anthropogenic alteration to coastal ecosystems (Valiela et al. 1997).

Mixing of nutrients within an estuary or coastal area is a function of residence time and flushing rates (Balls 1994). The growing interest in nutrient levels entering coastal zones is often associated with concern over algal blooms. In general, multiple factors influence phytoplankton populations, including nitrogen, phosphorus, silica, the ratio of nutrients (e.g. the Redfield ratio), the rate of nutrient turnover, and turbidity.

Multiple studies have linked algal blooms with nutrient loading as related to land use. Severe algal blooms off of Lahaina and other areas of West Maui, Hawaii, in 1989 and 1991 initiated interest in land use and a potential nutrient loading relationship. Nutrients are released into the coastal waters at elevated levels due to sugarcane and pineapple agriculture, treated domestic sewage affluent, and seasonal temporary streams (Soicher and Peterson 1997). Conversely, golf courses were shown to have negligible impacts on nutrient and sediment loading (Soicher and Peterson 1997). No causal

relationship was determined between the algal blooms and the elevated nutrients, but the study did demonstrate that nutrient loading is a concern in Maui's coastal waters.

Multiple nutrients are involved in phytoplankton dynamics, and to focus on one, such as nitrogen or phosphorus, can be misleading and even irrelevant in the coastal area under study.

Coastal processes are not quanitified in this study, but their potential impacts on coastal ecosystems, *Prorocentrum,* and FP are discussed.

Point-source pollution

Point-source pollution, e.g. from sewage and waste water treatment plants or industrial outflow, can also have a significant impact on water quality at a particular location. For example, soluble reactive phosphorus concentrations were significantly linked to wastewater treatment plant locations in a drainage basin in Southeast Michigan (Castillo et al. 2000). The same study found a relationship between nitrate concentration and LULC type (Castillo et al. 2000). Concentrations of phosphorus are not as linked to LULC types as concentrations of nitrates are (Castillo et al. 2000). Although point-source pollution may be impacting water quality in several of the drainage basins in this study, it is very difficult to quantify at the scale of multiple drainage basins within two states. Therefore, it is not included in this analysis. However, potential impacts are discussed.

Management

Management refers to any attempts to reduce nutrient or sediment exports, including source controls in urban areas, fertilizer application rates, erosion controls

(structure or procedure), or interception techniques (e.g. buffer strips, artificial wetlands) (Young et al. 1996). While these may play a large role in nutrient export rates, they may prove to be difficult to quantify.

Summary

Estimating water quality from LULC requires consideration of many other landscape factors. In this study, the variables of LULC, soil, hydrology, and topography will be quantified within a GIS model. Other factors, including climate, coastal processes, point-source pollution, and management practices will not be quanitifed within the model, but will be discussed.

Methods

The Model

A model that has successfully identified significant relationships between LULC within a drainage basin and water quality (nutrient levels from water samples taken throughout the basin) is used in this study. Basnyat et al. (1999) found a statistically significant relationship between water quality and LULC within the "contributing zones" of the streams in which they sampled for nutrient levels. Contributing zones are the areas, or "buffer" or "riparian" zones, around streams and coastlines defined uniquely for each location within a drainage basin based on soil characteristics, slope, and LULC types. It is important to note that in the same study, no significant relationship was found between water quality and LULC for the entire basin according to their data, indicating

that the land closest to streams (within the riparian zone) impacts water quality more than overall LULC within the entire basin.

The following LULC classifications are used in this study: forest, residential/urban, agriculture, grasslands/scrub, wetlands, forests, and barren land (the latter in Hawaii only, where it is significant). The definitions, equations and theory behind the model linking LULC and nutrient loading described in the following text are from Basnyat et al. (1999).

Contributing zones

A contributing zone is defined as "the buffer zone or riparian zone surrounding a stream or coastline that contributes nutrients and other non-point source pollutants to surface and subsurface waters due to land-use practices" (Basnyat et al. 1999). This definition recognizes that soil, slope, vegetation types, and spatial positioning of each LULC type within a drainage basin affect the assimilation and detention of nutrients. Using landscape characteristics such as slope, soil characteristics, and vegetation can provide information needed to define the dimensions of contributing zones within a drainage basin. The zone has to be large enough to assimilate ~90% of the nutrients it receives from land uses outside the zone.

To determine contributing zone dimensions using topography, land cover, and hydrological features, the riparian buffer delineation equation (RBDE) can be used (Phillips 1989, Basnyat et al. 1999):

1. $$B_b/B_r = (n_b/n_r)^{0.6} (L_b/L_r)^2 (K_b/K_r)^{0.4} (s_b/s_r)^{-0.7} (C_b/C_r)$$

Subscribt *b* – proposed contributing zone;

Subscript *r* – reference-contributing zone;

B_b/B_r – contributing zone effectiveness ratio;

n– Manning roughness coefficient;

L – contributing zone width (m);

K – saturated hydraulic conductivity (cm/h) (equal to permeability as given in US Soil Surveys);

s – slope (%);

C – soil moisture storage capacity (cm) (equal to available water capacity * profile thickness above confining layer or seasonal high water table from US soil surveys).

"The RBDE considers relative detention time over the conditions slope, soil characteristics, and vegetation. It compares the ability of a given vegetative contributing zone to retain runoff to that of a user's defined reference contributing zone- resulting in a quantitative, dimensionless index of contributing zone effectiveness" (Basnyat et al. 1999). To determine the width of the contributing zone, the terms can be rearranged:

2. $$L_b = p^{0.5} L_r [(n_b/n_r)^{0.6} (K_b/K_r)^{0.4} (s_b/s_r)^{-0.7} (C_b/C_r)]^{0.5}$$

p – contributing zone effectiveness ratio (e.g. $p = B_b/B_r$)

L_b – proposed width of the contributing zone

The assumptions in this study will be the same as in Basnyat et al. (1999). *p* will be set equal to 1 to match the assimilation/detention capability of the contributing zone to that of the reference zone. Since forest cover is assumed to be most efficient at nutrient assimilation, the Manning roughness coefficient ($n_r = n_b = 0.46$) for riparian forest will be used. This assumes that the defined contributing zone width, if it were forested, is large enough to absorb a significant proportion of the nutrients from outside of the zone before they filter to the water. Based on these assumptions, equation 2 can be re-written:

3. $L_b = L_r\,[(K_b/K_r)^{0.4}\,(s_b/s_r)^{-0.7}\,(C_b/C_r)]^{0.5}$

The area generated using the width (L_b) is a nutrient-contributing zone because nutrients entering this area (contributing zone) from the LULC outside of the zone will be assimilated or detained before reaching the stream water if it were forest.

Reference contributing zones

"A reference-contributing zone should provide effective filtration under average runoff conditions (Phillips 1989)" (Basnyat et al. 1999), and be a distance that represents a good indication of water quality. For this study, the reference zone width will be set at 150 m (L_r), a riparian distance demonstrated to indicate overall water quality by Tufford et al. (1998).

The soil and topography factors (K_r, s_r, and C_r) are derived by taking the mean of the values within the 150 m riparian reference zone, and the other factors (K_b, s_b, and C_b) are the means from within the whole drainage basin.

***Prorocentrum* abundance and FP prevalence**

To compare potential impacts of LULC on *Prorocentrum* abundance and FP prevalence between drainage basins of the study sites within Florida and Hawaii, the contributing zone model, as describe above, was used. For each *Prorocentrum* sampling site, the basin incorporating the sampling site was analyzed. A total of four basins around Florida and nine basins around the Hawaiian Islands were considered. The drainage basin for the Molokai, Hawaii study site was not included due to lack of current LULC data.

The following GIS data were used (for both states) in the analyses: LULC, soils, hydrology, Digital Elevation Model (DEM), and drainage basin boundaries (Table 3-3). Smaller drainage basins were used versus overall watersheds, as the contributing zone model is meant for a drainage basin-scale study (150 to 6000 ha in size, Basnyat et al. 1999). A drainage basin is defined for the purpose of this study as a subset of a larger watershed, the basin that is adjacent to/flows into the coastal area under study.

The LULC data were generalized into the following categories: urban/residential, agriculture, forest, scrub/grasslands, wetlands, barren land (only significant for Hawaii), and water. The soil data were obtained from the USDA Natural Resource Conservation Service in digital form (STATSGO), which provided the variables: water table depth, soil permeability, and soil water capacity (the STATSGO data set was chosen over the more finely detailed SSURGO data because the average was taken over the defined areas, so fine detail was not required). Slope information was derived for each watershed by a surface analysis of USGS Digital Elevation Model (DEM) information.

Florida. For LULC layers, a combination of available LULC data from the water management districts were used for three of the sites (St. Joseph Bay, Cedar Key, and Mosquito Lagoon) and a classified coverage from the Gap Analysis Project (GAP) was used for Florida Bay (South Florida Water Management District has not classified LULC for the Florida Keys, which are the southern boundary of Florida Bay, whereas the GAP image included them).

Hawaii. For LULC layers, satellite (Landsat TM) imagery that has been classified into LULC by NOAA according to the Coastal Change Analysis Program (C-CAP) protocol was used.

Table 3-3. GIS data used in the analysis.

Attribute	Layer name	Scale	Date	Source	Feature
FLORIDA					
Basin boundaries	Basins	1:24,000	N/A	FL DEP	Polygon
Coastline	Coast	1:40,000	1993	FMRI	Line
Soils	Gsoils	1:250,000	1991	USDA	Polygon (w/ tables comp & layer)
Hydrology	HY24L (Hydro)	1:24,000	1994	USGS	Line
LULC (St. Joe)	NWLU95	1:24,000	1994	FL DEP	Polygon
LULC (Fbay)	SFLU95	1:24,000	1994	GAP	Grid
LULC (Mosquito)	SJLU95	1:40,000	1994	SJWMD	Polygon
LULC (Cedar Key)	SRLU95	1:40,000	1994	SRWMD	Polygon
DEM	DEM	1:250,000	1983	USGS	Grid
HAWAII					
Basin boundaries	WTRSHDPY	1:24,000	1995	GDSI	Polygon
Coastline	Coast	1:24,000	1983	USGS	Polygon
Soils	STATSGO	1:250,000	1994	USDA	Grid
Hydrology	DLGHYDLN	1:24,000	1983	USGS	Line
LULC	C-CAP	1:24,000	1999-2001	NOAA	Image/Grid
DEM	DEM	1:250,000	1983	USGS	Grid

Analysis. All analyses were conducted with ArcView 3.2 software. Using the equations provided earlier, with soil data and average slope values, the contributing zones of each stream and the coastline were determined within each of the basins of the individual *Prorocentrum* sampling sites in Florida and Hawaii. With these results, the streams and coastlines were buffered to the contributing zone distance, and the area and proportion of each LULC within each contributing zone and basin was determined (Fig. 3-1).

These LULC proportions were compared amongst basins, to FP prevalence, and to *Prorocentrum* abundance. The hypothesis of a relationship existing between LULC and *Prorocentrum* abundance and/or FP prevalence was tested. For comparative purposes in several of the analyses, nutrient-retaining LULC types (forest, wetland,

scrub/prairie) were combined, as were nutrient-contributing LULC types (urban and agriculture).

Results

Florida

Proportions of LULC within contributing zones of the study site drainage basins (Fig. 3-2) indicate there is a trend amongst sites (Fig. 3-3, Table 3-4). The results indicate there is a relationship between FP prevalence and LULC (Fig. 3-4 and 3-5) in Florida.

There is a positive relationship with FP prevalence and increasing proportion of urban and agriculture LULC, and a negative relationship with FP prevalence and proportion of forests, grasslands, and wetlands within the contributing zones of the basins (Fig. 3-4). Proportions of LULC correspond with FP prevalence around Florida.
The results of the LULC analysis comparison with toxic *Prorocentrum* at the Florida study sites is not as clear, although a similar trend to FP and LULC can be observed (Fig. 3-5), particularly with maximum abundance of toxic *Prorocentrum*. There are other factors besides LULC influencing *Prorocentrum* abundance in Florida.

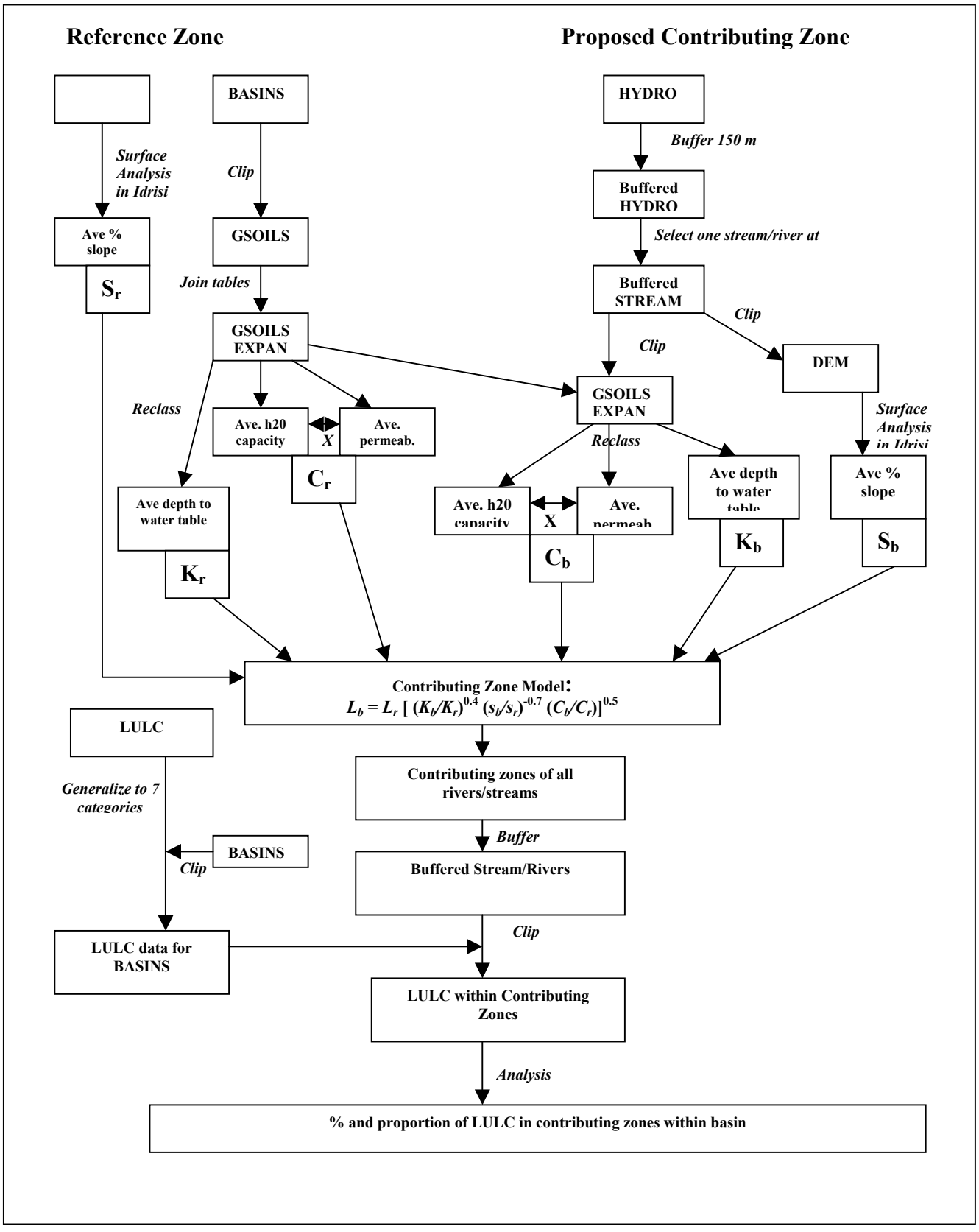


Figure 3-1. Flow chart of method used in analysis.

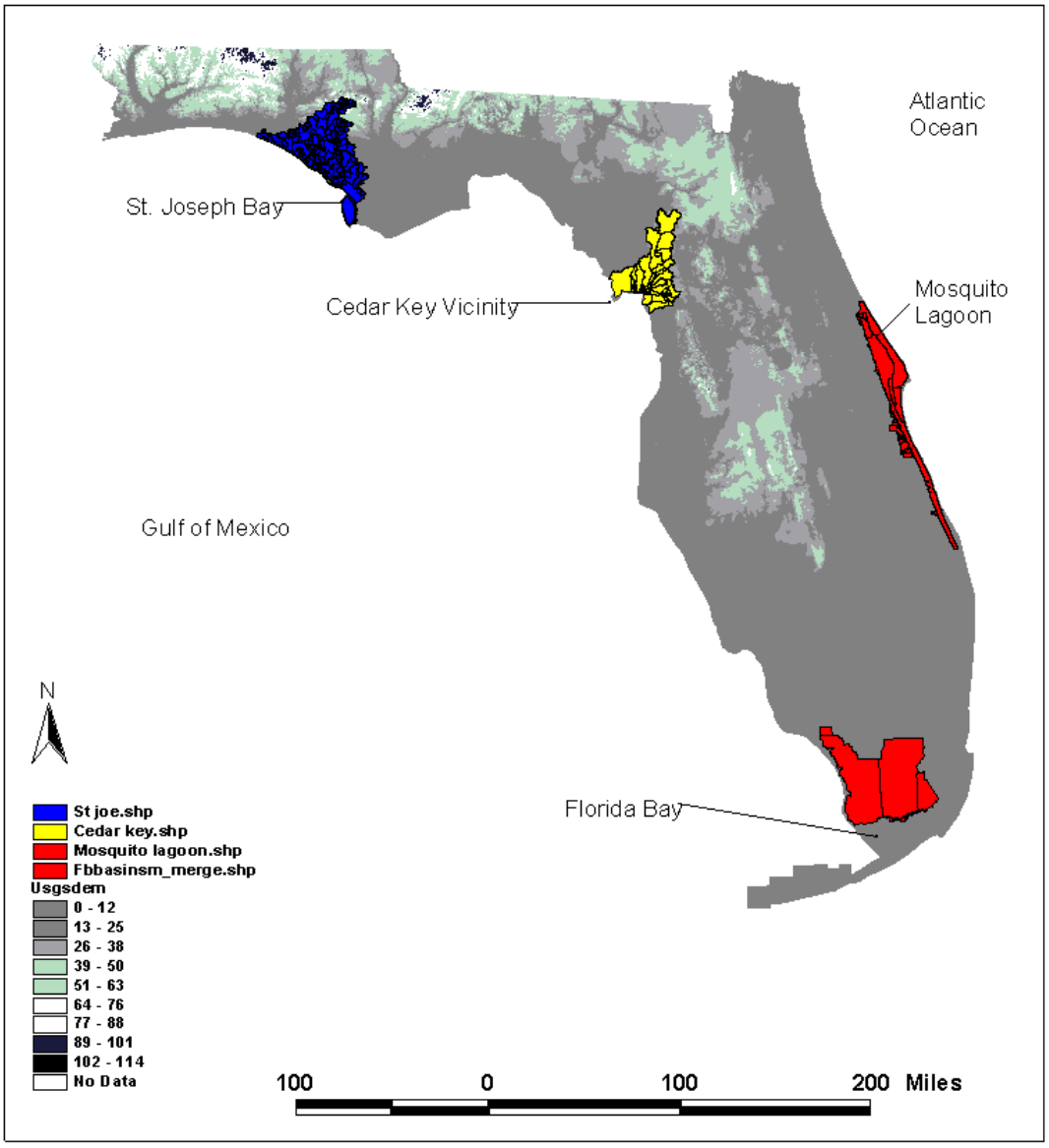


Figure 3-2. A map of the drainage basins of the study sites in Florida under analysis.

Table 3-4: Resulting proportion LULC of the four study sites in Florida.

Basin	% Urban	% Agriculture	% Forest	% Wetland	% Prairie/ Scrub
St. Joe Bay (low FP)	2.82	0.25	93.97	1.98	0.99
Cedar Key (medium FP)	3.08	2.80	55.88	37.53	0.71
Mosquito Lagoon (high FP)	5.58	14.89	58.37	17.43	3.72
Florida Bay (high FP)	19.28	43.60	0.95	32.44	3.73

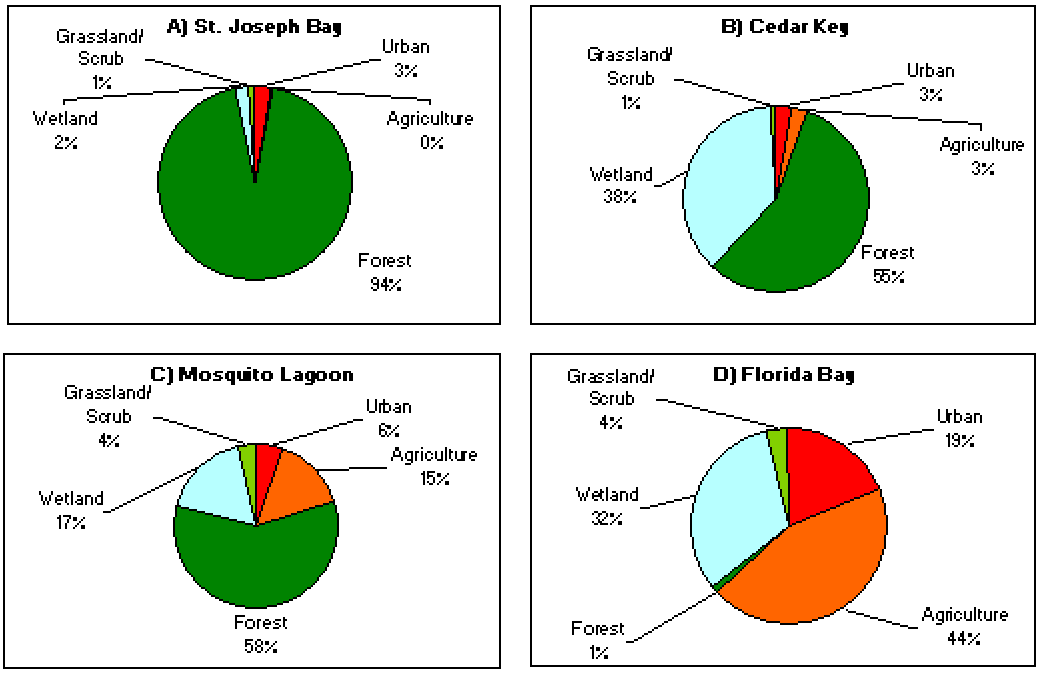


Figure 3-3. Proportion of LULC in contributing zones in the drainage basins of the Florida study sites: A) St. Joseph Bay; B) Cedar Key; C) Mosquito Lagoon; and D) Florida Bay.

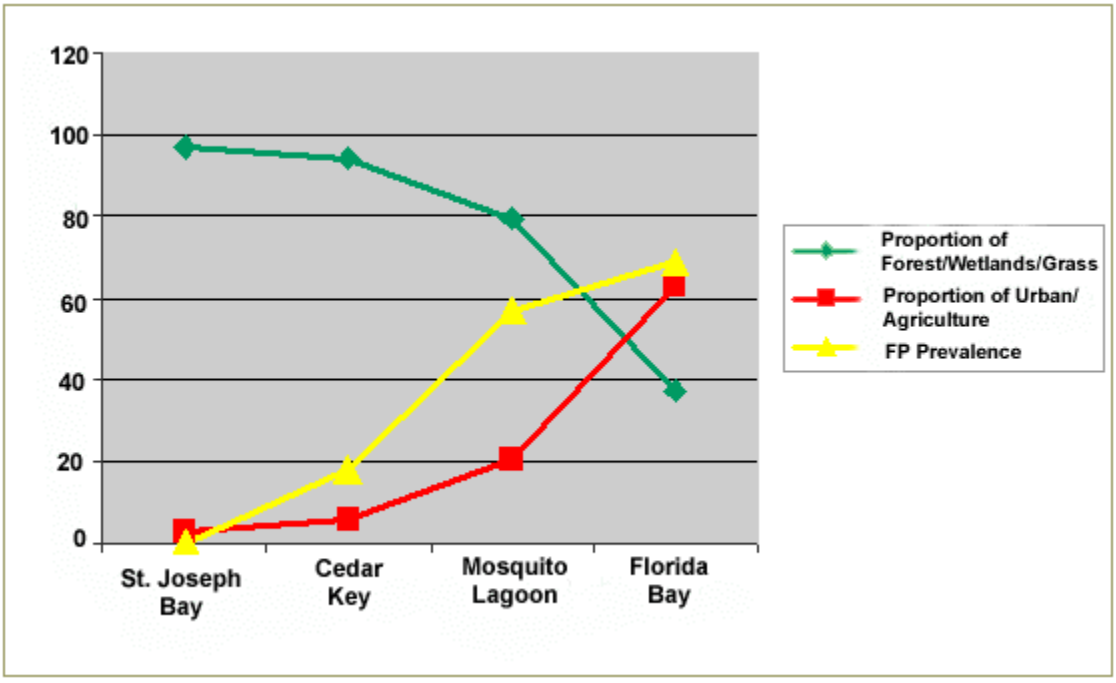


Figure 3-4. FP vs. LULC at the four study sites in Florida

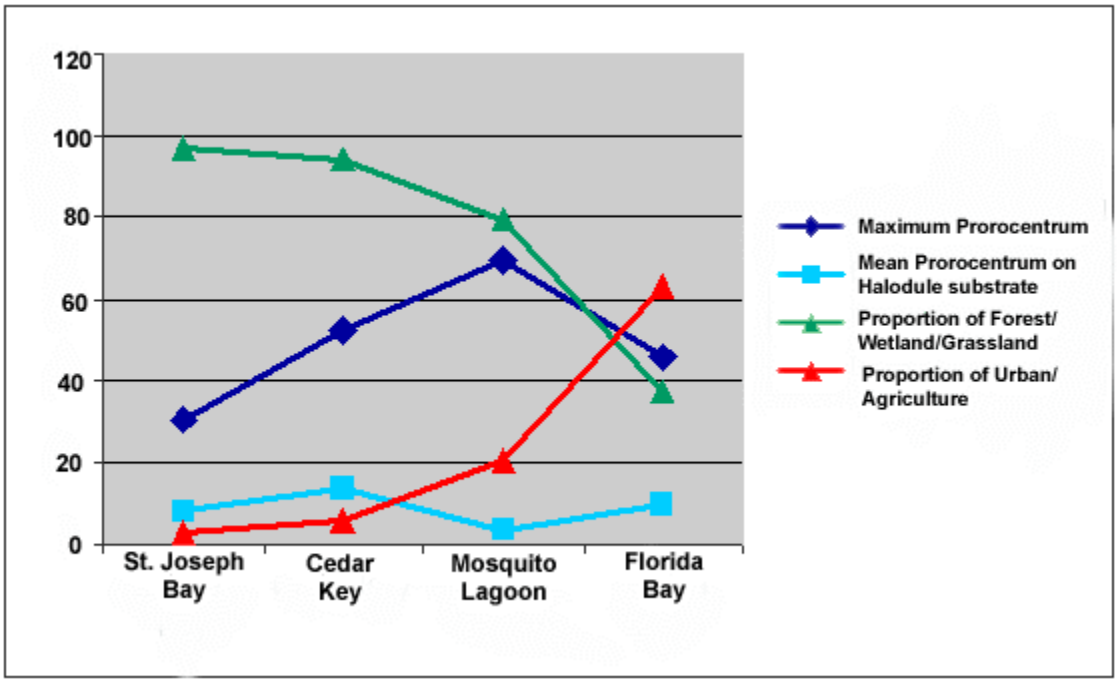


Figure 3-5. Mean toxic *Prorocentrum* on *Halodule* substrate and Maximum *Prorocentrum* (divided by 10) vs. LULC at the four study sites in Florida.

Hawaii

The proportions of LULC within contributing zones of the drainage basins of Hawaii (Fig. 3-6) demonstrate an interesting trend (Table 3-5, Fig. 3-7). The results of a comparison among Hawaii study sites do not indicate a distinct relationship between FP prevalence and LULC (Fig. 3-8), using the contributing zone model. However, a general trend can be observed: there is a higher percentage of urban and agriculture LULC within the contributing zones of study sites with medium or high FP vs. study sites with no FP.

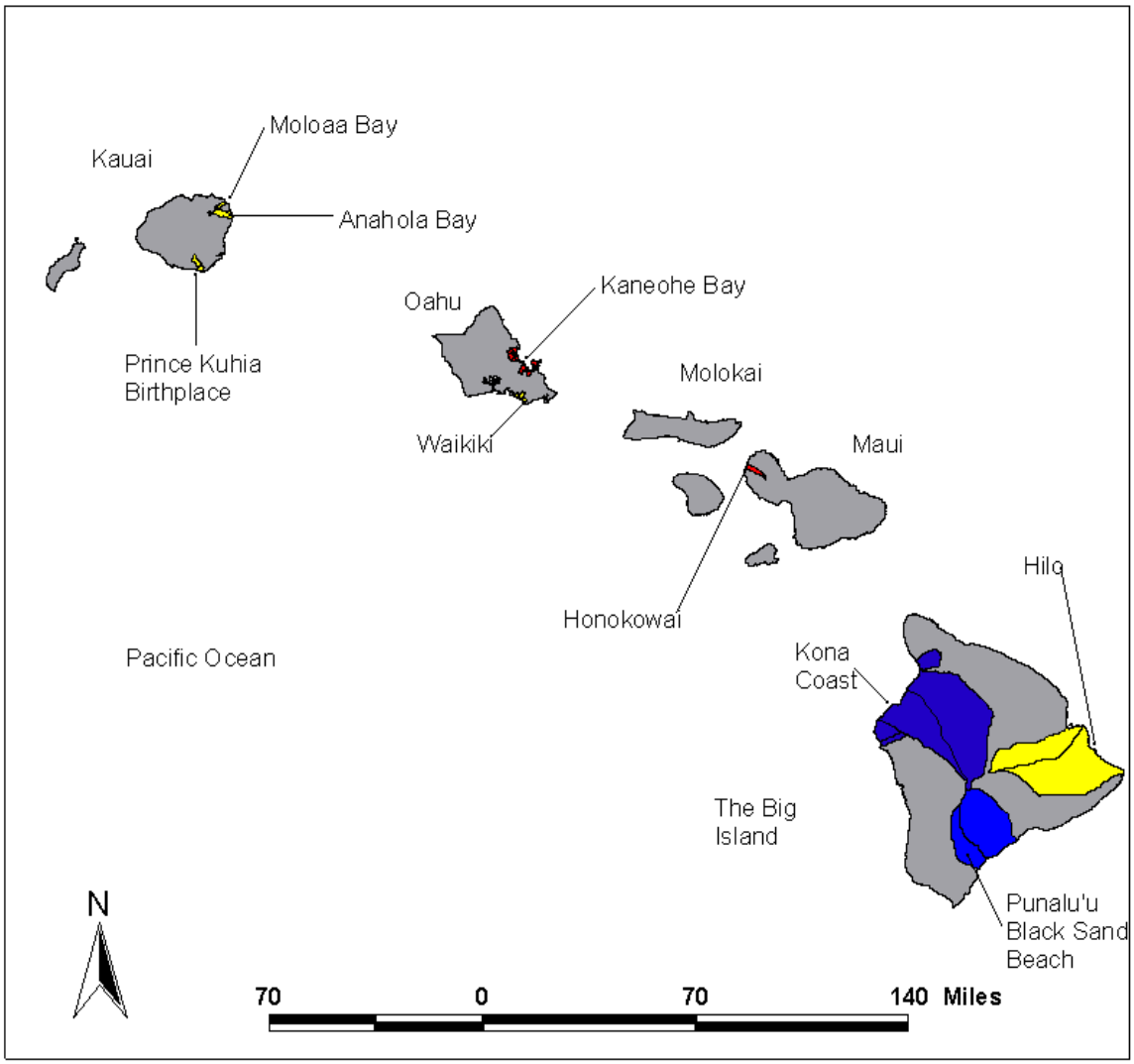


Figure 3-6. Drainage Basins of study sites (used in the LULC analysis) in Hawaii.

Table 3-5. Resulting proportion LULC of eight study sites in the Hawaiian Islands.

BASIN	Urban	Agri-culture	Grasslands/ Scrub	Forest	Wetland
Kona/Kohala, Big Island	0.93	0.00	80.15	6.02	0.00
Punalu'u, Big Island	0.46	6.37	28.00	60.91	0.00
Hilo, Big Island	7.61	2.78	37.91	48.43	0.06
Anahola, NE Kauai	0.36	0.55	62.21	33.41	3.34
Moloaa, NE Kauai	0.52	0.03	63.79	33.31	1.85
SE Kauai	19.00	0.56	48.40	30.19	0.20
Waikiki, Oahu	73.39	0.00	16.83	0.68	0.00
Kaneohe Bay, Oahu	13.95	1.60	45.91	36.49	1.37
Honokowai, Maui	1.79	26.05	28.54	43.22	0.00

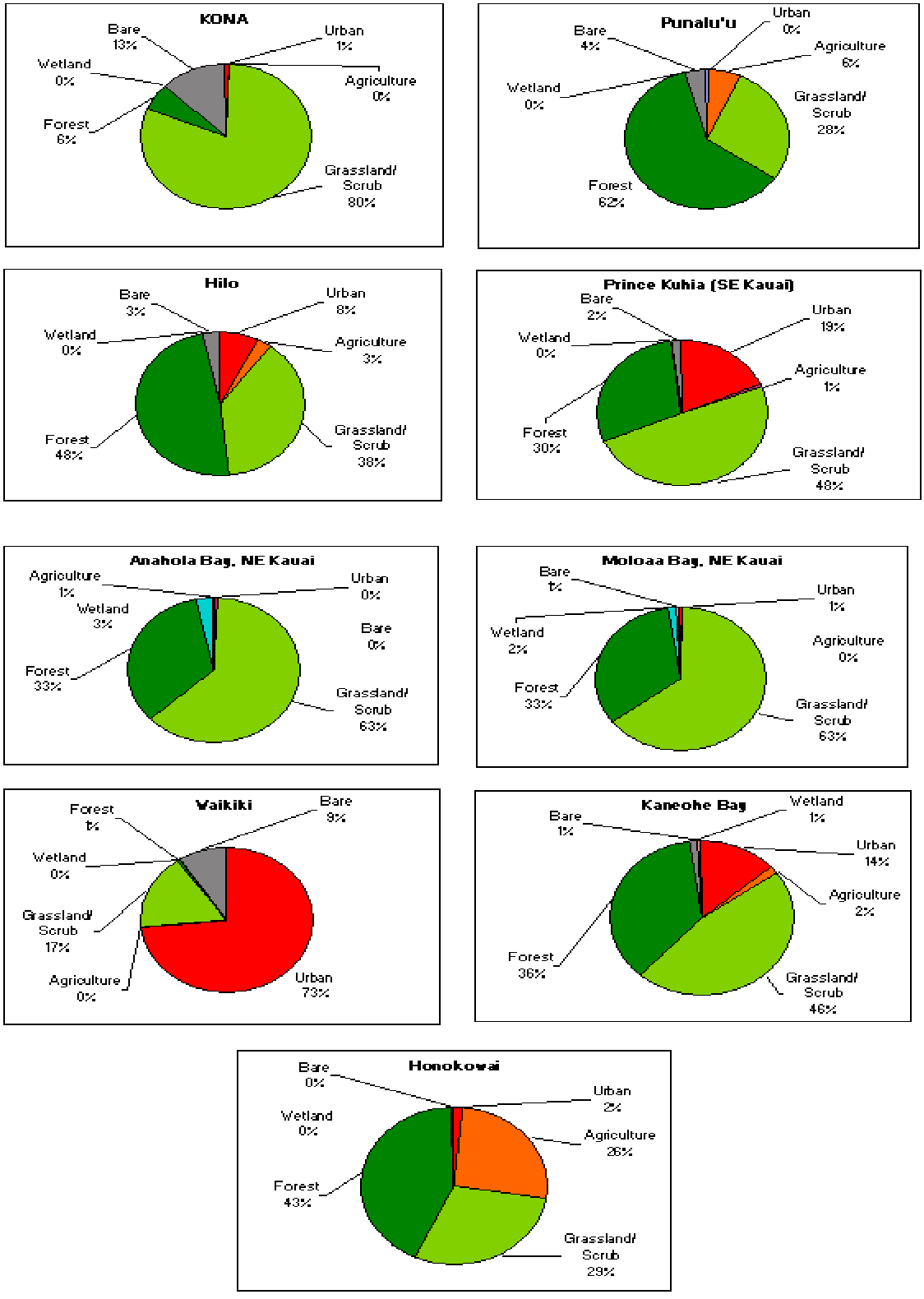


Figure 3-7. Proportions of LULC within contributing zones in the drainage basins of the Hawaii study sites.

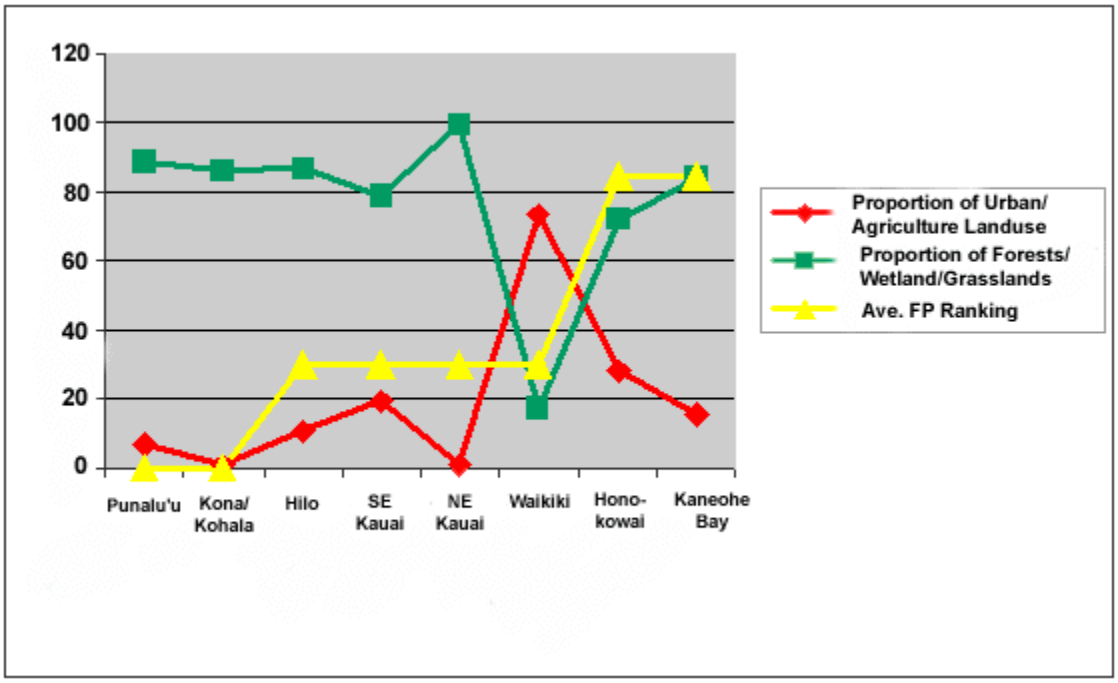


Figure 3-8. FP prevalence vs. LULC at the study sites in Hawaii.

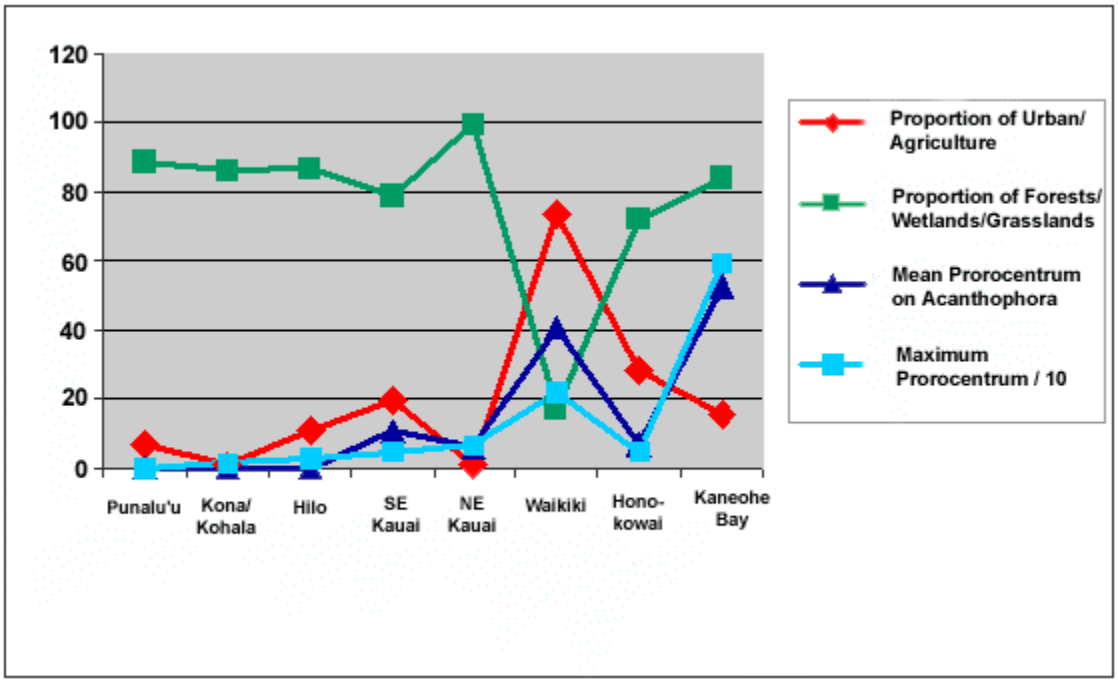


Figure 3-9. LULC vs. proportion toxic *Prorocentrum* spp. on *Acanthophora* substrate and maximum toxic *Prorocentrum* (divided by 10) among the study sites in Hawaii.

Comparing average toxic *Prorocentrum* on *Acanthophora* substrate to LULC among all the sites indicates a slight trend, but no definite relationship (Fig. 3-9). As in Florida, there are other factors besides LULC influencing *Prorocentrum* abundance in Hawaii. However, it is important to note that the proportion of forests/wetlands/ scrub/grasslands is greater than 80% within contributing zones of study sites that have low abundances of *Prorocentrum*.

Discussion

A clear trend is observed between FP prevalence in LULC in Florida, but the trend is not as clear in Hawaii, although it is present. The implications of these results are important. LULC is directly linked to water quality, and water quality is connected to disease.

Disease within a natural community can result from direct toxic pollutants or from the conditions induced by nutrient loading or pollution, e.g. the promotion of infectious agents or naturally occurring toxins. Pollutants such as heavy metals, pesticides, and estrogenic compounds have been demonstrated to suppress the immune system in mammals (including humans, Davidson 2001). There are hypotheses incorporating immunosuppression as a potential factor in the etiology of FP (Herbst 1994), although recently it has been shown that immunosuppression may not be a prerequisite of FP (Work et al. 2001). Also, anthropogenic changes to coastal environments can produce a pathogen-friendly environment (Davidson 2001). The distribution of FP appears to be linked to human activity, and is more prevalent in areas with agriculture, industry, and urban development versus areas that are undisturbed (Herbst 1994). This study

quantified LULC to compare directly to FP prevalence in two areas representing the Pacific and the Atlantic, within the context of land-water interactions.

Land-water interactions are not always considered in coastal studies, but they play a large role in the ecosystem dynamics. Anthropogenic uses of land have a direct impact on water quality, some of which can be observed readily, e.g. siltation at the Molokai study site. One of the sample sites in Hilo had a working sewage treatment plant adjacent to it when Landsberg et al. (1999) were collecting samples, and the water was murky with low visibility (G. Balazs, National Marine Fisheries Service, pers. comm. 2000). During February and August 2000 collections at the same sample station, the sewage plant was closed, and the water appeared very clear. This is an example of how coastal areas can potentially recover quickly when the source of disturbance is removed, particularly point sources.

This analysis focused on LULC only, and did not include nutrient loading from point-source pollution, such as sewage treatment plants. Point-source pollution is very difficult to quantify at the scale of multiple drainage basins (particularly if they vary in output over time, as many do), but they can have a very large influence on water quality and the benthic condition in their vicinity. However, a relationship is evident between LULC and FP, even though point-source pollution output was not included in this analysis.

FP appears to be most prevalent in coastal areas with LULC of urban and agriculture lands. This can be said of both Hawaii and Florida, with the higher FP prevalence areas adjacent to lands with higher densities of people or agriculture. The

Florida results of this study are a good example of a strong, clear trend between LULC and FP.

In this study, LULC is taken in context of soil properties and topography. It is complex. Different agriculture types use different fertilizer application rates for different areas, or have different types and quantities of livestock (some livestock, e.g. pigs, may have integrated systems which funnel waste to nearby water sources). Urban/residential areas can vary from one another as well. In some areas residents may apply more fertilizers than in others, and paved urban areas can provide wastewater runoff that varies in phosphorus content (Dauer et al. 2000). However, generalizing and quantifying agriculture and urban LULC types is valuable in obtaining an overview of the nutrient-loading potential of a drainage basin.

Soil chemistry (including soil pH, cation exchange capacity, and nutrient content) is a factor not included within the model used in this study. Soil chemistry may help explain the different results between Hawaii and Florida. Florida soils vary widely, and have different chemical properties in different areas (Brown et al. 1990), but the overall general soil types found within Florida are not known to retain nutrients, so LULC type will impact water quality directly. In Hawaii, the volcanic soils and rock are very porous, and water (and nutrients) sink into streambeds and produce less discharge into coastal areas (Carlquist 1980). These characteristics may influence the results of the Hawaiian drainage basin analysis, which do not demonstrate the clear trend between LULC type and FP that is apparent in Florida.

Coastal processes such as water residence time and flushing rates were also not included in this analysis. They can vary by season, and from year to year. Unfortunately,

these data were not available for the specific sites under study. But coastal processes are important in the context of nutrient retention in a coastal area. For example, areas that have high flushing rates may not be influenced as drastically by nutrient loading from LULC as places with low flushing rates. High-flush rate areas may not retain the nutrients that enter into the coastal area. For example, St. Joseph Bay and Cedar Key are considered to have very slow flushing rates (E. Phlips, Dept. of Fisheries and Aquatic Sciences, University of Florida, pers. comm. 2001). Even though both of these locations have low proportion urban and agriculture land uses, they have relatively high abundances of *Prorocentrum*. Nutrients may be retained and build up slowly in these systems and provide *Prorocentrum* and its substrates required nutrients. Tides, upwelling, and currents are also examples of coastal processes not quantified in this study, particularly currents that may bring additional nutrients in an area, or remove them before they can become integrated into the ecosystem. Nutrient loading and algae-community dynamics is undeniably complex, as well, and very difficult to quantify on a large scale.

In addition, there are multiple factors that influence *Prorocentrum* growth and reproduction. For example, surf and high-energy wave activity in the shallow areas of the Hawaii study sites will have a greater impact on *Prorocentrum* abundance vs. areas that are protected and/or deeper. As mentioned previously, dinoflagellates are not as productive and successful in areas of high wave action or turbulence because they affect cell division processes and migratory behavior (Berdalet and Estrada 1993).

The unknown etiology of FP is a factor. The etiology is relatively complex, as indicated by years of research and the present level of knowledge. *Prorocentrum* may

play a role in this etiology, as indicated in this study by the confirmation of the presence of toxic *Prorocentrum* spp. in the foraging areas of two isolated green turtle populations. However, factors behind toxin production and how OA affects turtles still have to be determined. There also may be other components linked to water quality that may play a role in the etiology, including pollutants from LULC types and/or point sources.

As a recommendation for future management and conservation practices, riparian areas around rivers and coastlines should be protected to maintain high water quality, either as forests, grassland/scrub, or as wetlands. Further research is recommended, including incorporating point-source pollution sources, coastal processes, and quantification of nutrient levels, sedimentation, and water quality parameters in parallel to FP and/or *Prorocentrum* studies.

Conclusion

This analysis confirms that there is a relationship between LULC and FP prevalence in the coastal areas where green turtles are foraging. As in all ecological studies, there are multiple factors influencing FP prevalence and *Prorocentrum* abundance. Point-source pollution, soil chemistry, and coastal processes were not included in the model used for this analysis, although they may also have an impact on coastal ecosystems. However, even without these factors, a relationship between LULC and FP is observed, and the results indicate that LULC types within riparian zones may also influence *Prorocentrum* abundance. Although many previous studies have noted the apparent correlation between the distribution of FP and anthropogenic factors, this is one of the first studies to quantify LULC to compare to FP prevalence. The results of this

study do indicate a relationship exists between anthropogenic land use and the health of green turtle populations of Hawaii and Florida.

CHAPTER 4
SUMMARY AND CONSERVATION SIGNIFICANCE

This study provides support for the hypothesis of a relationship between *Prorocentrum* spp. and FP by geographic confirmation of the presence of toxic *Prorocentrum* within the feeding habitats of green turtles in the Atlantic and the Pacific Oceans. A geographic correlation between *Prorocentrum* abundance and FP prevalence is also evident. The etiology of FP is complex and still unknown, as is OA production in benthic *Prorocentrum* spp. in different locales.

There are numerous factors that can contribute to turtles exposure to OA, including variances in toxin production among species of *Prorocentrum*, substrate variability, substrate choices, and where turtles are feeding. In this study, one factor was focused upon: distribution of *Prorocentrum*, within the context of environmental variables and seasonality. Although this study compared density of *Prorocentrum* versus FP, in reality the etiology of FP is much more complex.

The four *Prorocentrum* species discussed herein are known to produce OA, but usually under specific environmental conditions (Murakami et al. 1982, Dickey et al. 1990, Aikman et al. 1993, Morton et al. 1998). Optimal environmental conditions for the highest OA-production levels in *P.* cf *hoffmannianum* include specific ranges in light intensity and temperature (Morton and Bomber 1994). Maximum biomass, total OA

from a group of cells, growth rate, and OA content per cell are all independent variables, with each maximized or minimized with different environmental parameters (Morton and Bomber 1994). Geographic location is also important. Many dinoflagellates are known to vary their toxin production depending on their distribution, including *Prorocentrum* spp. In other words, even if *Prorocentrum* is present at high or low numbers in an area, the total amount of OA produced among the sites may be independent of the number of *Prorocentrum* present. There are also several new species of *Prorocentrum* that produce OA that are not accounted for in this study (Steidinger and Landsberg, unpublished data). Further research on OA production levels among *Prorocentrum* species should be completed, and contributing environmental factors to toxin production within *Prorocentrum* populations and among different populations should be examined within the context of FP prevalence.

Controlled laboratory studies of the direct effects of OA on green turtles and/or green turtle cell lines (Moore et al. 1998) are recommended. Additionally, we need to determine the exposure of turtles to OA in the wild. Comparisons need to be made in parallel with *Prorocentrum* abundances and presumptive OA exposure. There is sufficient justification to further explore the relationship between *Prorocentrum* and FP.

There are other tumor-promoting biotoxins in green turtle habitats besides OA. For example, the cyanobacteria, *Lyngbya* spp., produces a toxin (Lyngbyatoxin) that, similar to OA, induces papillomas in two-stage mouse carcinogenesis experiments (Fujiki et al. 1984, Landsberg et al. 1999). *Lyngbya* is found in Hawaii, Florida, and there are problematic blooms in Australia in turtle grazing areas where FP is prevalent (Limpus

2001). *Lyngbya* could also be linked to FP in areas such as these FP sites in Australia (Landsberg et al. 1999, Limpus 2001).

FP was not recorded in Florida until 1938 (Smith and Coates 1938, Lucké 1938), and in Hawaii it was noted twenty years later (Balazs 1991). People have been catching and harvesting green turtles for food for centuries (e.g. Emory 1947, Balazs et al. 2000b), and only in the past century, coinciding with the rise of the industrial revolution, large-scale agriculture, and population growth, has FP become more prevalent in green turtle populations worldwide.

In this study, a link has been found between LULC and FP, although LULC is not clearly related to *Prorocentrum* abundance. It is highly suspected that multiple environmental variables play roles in the etiology of FP and in *Prorocentrum* abundance (and toxin production), and many of these variables are unknown at this time, including the impact of nutrient loading by coastal lands.

Land use and landscape factors, including soil characteristics and topography, play a large role in land-water interactions in coastal ecosystems where green turtles reside for the majority of their lives. Water quality is impacted by LULC in coastal systems, although point-source pollution and coastal processes such as flushing rates are also influences. Anthropogenic factors do impact surrounding environments, and often have a negative toll on native animal and plant populations and communities, sometimes indirectly. Nutrient and sediment loading, alteration of the benthos, and temperature changes not only influence water quality, but they can all contribute to providing a pathogen-friendly environment (Davidson 2001).

Conservation of the endangered green turtle first requires the identification of threats to its populations around the world. These threats include, but are not limited to, illegal and excessive harvesting of turtles and eggs, accidental take in fishing gear (including drowning in trawls and hooking in long-line fishing), land development of nesting habitat, reef and coastal habitat degradation, and disease (Ehrenfield 1979). Secondly, these threats have to be addressed with management practices based on solid research results to lead to population recovery. The disease of FP is increasing in green turtle populations around the world, and it promotes premature mortality (Herbst 1994). FP is a disease that is now significant on a global scale.

This study has addressed two hypotheses related to the etiology of FP in green turtles, and the results have provided support to endorse the necessity of further research. OA-producing *Prorocentrum* spp. may play a key role in the etiology of FP, which only additional studies can confirm.

Determining the etiology of FP will allow us to work towards reducing the disease in marine turtle populations worldwide. With knowledge of the disease etiology we can address new outbreaks in the disease when they occur in previously unaffected areas. It may also provide more insight on how closely anthropogenic factors are linked to the natural communities around us. Human alteration of marine habitat is the one characteristic shared by all the areas of high FP prevalence (Davidson 2001). If we, as combined societies worldwide, place value on endangered species, we need to recognize how our land and resource management practices have far-reaching consequences in our coastal environments and the inhabitants who live there.

APPENDIX A
MACROALGAE AND SEAGRASS SPECIES UTILIZED BY GREEN TURTLES

Table A-1. Forage utilized by green turtles in the Hawaiian Islands and Florida.

The Hawaiian Islands		Florida	
Acanthophora spicifera[3]	(Rhodophyta)	*Acanthophora spicifera*[3]	(Rhodophyta)
Bryopsis pennata[3]	(Chlorophyta)	*Amphiroa rigida*[2]	(Rhodophyta)
Caulerpa racemosa[1,3]	(Chlorophyta)	*Caulerpa sp.*[7]	(Chlorophyta)
Cladophora sericea[3]	(Chlorophyta)	*Centroceras clavulatum*[2]	(Rhodophyta)
Codium sp. • *C. arabicum*[1,3] • *C. edule*[1,3] • *C. phasmaticum*[1,3] • *C. reediae*[3]	(Chlorophyta)	*Codium sp.*[7]	(Chlorophyta)
Dictyosphaeria sp. • *D. cavernosa*[3] • *D. versluysii*[3]	(Chlorophyta)	*Dictyota sp.*[2]	(Phaeophyta)
Gracilaria sp.[3] • *G. salicornia* • *G. tikvaehae*	(Rhodophyta)	*Gelidium americanum*[2]	(Rhodophyta)
Halophila hawaiiensis[3] *	(Anthophyta)	*Gracilaria sp.*[7]	(Rhodophyta)
Hypnea musciformes[1,3]	(Rhodophyta)	*Halodule beaudettei*[4,5,6] *	(Anthophyta)
Melanamansia glomerata[1,3]	(Rhodophyta)	*Halophila engelmanni*[4,5,6] *	(Anthophyta)
Pterocladiella capillacea[1,3]	(Rhodophyta)	*Hypnea sp.* • *H. musciformes*[1] • *H. cervicornes*[2]	(Rhodophyta)
Sargassum echinocarpum[3]	(Phaeophyta)	*Sargassum sp.*[2]	(Phaeophyta)
Spyridia filamentosa[1,3]	(Rhodophyta)	*Syringodium filiforme*[4,5,6] *	(Anthophyta)
Turbanaria ornata[1,3]	(Phaeophyta)	*Thalassia testudinum*[6] *	(Anthophyta)
Ulva sp. • *U. fasciata*[1] • *U. reticulata*[3] • *U. rigida*[3]	(Chlorophyta)	*Ulva lactuca*[2]	(Chlorophyta)

* - Seagrass species

[1] Balazs, G.H. 1980.
[2] Redfoot, W. R. 1997.
[3] Russell, D. J. and G. Balazs. 2000.
[4] Mendonca, M. T. 1983.
5 Bjorndal, K. 1997.
6 Mortimer, J. 1982.
7 Hirth, H. F. 1997.

APPENDIX B
FLORIDA DATA

Table B-1. Summary of St. Joseph Bay results. Cell numbers are per 2 mL sample (mean over replicates).

Station #	Date of Collection	GPS Coordinates of Station	Salinity (ppt)	Temp (*C)	Substrate (+ - found in green turtle diet)	Season: 1- winter 2- spring 3 - summer 4 - fall	n (reps)	MEAN toxic Prorocentrum	MEAN *Prorocentrum* (toxic + non-toxic)	*P. lima**	*P. mexicanum*	*P. concavum**	*P. emarginatum*	*P. hoffmannianum**	*P. belizeanum**	*P. taylori*	*P. micans*	*Small Prorocentrum*
1	5/1/2000	29 47.40N 85 18.12W	30.4	28.3	*Halodule beaudettei*	2	2	107.5	834.5	45	711	40	15	24	0	0.3	0	1
1	5/1/2000	29 47.40N 85 18.12W	30.4	28.3	*Thalassia testudinum*	2	2	164	771	108	587	39	18	17	0	0.8	0	1.3
1	5/1/2000	29 47.40N 85 18.12W	30.4	28.3	*Dasycladus vermicularis*	2	2	80.25	1054.5	14	956	40	17	26	0	0	0	1.5
1	5/1/2000	29 47.40N 85 18.12W	30.4	28.3	*Gracilaria spp.*	2	2	68.5	887.75	14	745	32	13	23	0	0.3	0	60.5
2	5/1/2000	29 45.20N 85 18.26W	35.5	29.5	*Halodule beaudettei*	2	2	36.5	459.75	5	386	17	0.3	15	0	0	0	37.5
2	5/1/2000	29 45.20N 85 18.26W	35.5	29.5	*Thalassia testudinum*	2	2	73.75	905.5	3.8	647	35	18	35	0	0	0	167
2	5/1/2000	29 45.20N 85 18.26W	28.3	28.8	*Dasycladus vermicularis*	2	2	33.75	300.25	4.5	257	15	5.8	14	0	0	0	4.3
2	5/1/2000	29 45.20N 85 18.26W	28.3	28.8	*Hypnea musciformes*	2	2	36.5	520	7.5	366	16	12	14	0	0	0	105.5
2	5/1/2000	29 45.20N 85 18.26W	28.3	28.8	*Spyridia filamentosa*	2	2	76.75	1061.25	25	915	23	32	29	0	0	0	37.5
3	8/4/2000	29 48.66N 85 18.32W	30.2	34.8	*Thalassia testudinum*	3	2	10.5	58.5	5	41.8	3.8	0	1.8	0	0	0	6.3
3	8/4/2000	29 48.66N 85 18.32W	30.2	34.8	*Halodule beaudettei*	3	2	2.5	6.5	0.3	3.75	1.3	0	1	0	0	0	0.3
3	8/4/2000	29 48.66N 85 18.32W	30.2	34.8	*Spyridia filamentosa*	3	2	5.5	33.25	1.5	25.5	2.8	0	1.3	0	0	0	2.3
3	8/4/2000	29 48.66N 85 18.32W	30.2	34.8	*Sargassum polyceratium*	3	1	5	27.5	1	22.5	3	0	1	0	0	0	0
4	8/4/2000	29 48.01N 85 18.07W	30.8	34.3	*Halodule beaudettei*	3	2	12.5	13	0.3	0.25	12	0	0.3	0	0	0	0.3
4	8/4/2000	29 48.01N 85 18.07W	30.8	34.3	*Thalassia testudinum*	3	2	2	3.25	0.5	1	1.5	0	0	0	0	0	0.3
4	8/4/2000	29 48.01N 85 18.07W	30.8	34.3	*Spyridia filamentosa*	3	2	2.5	9.25	1	5	1.5	0.3	0	0	0	0	1.5
4	8/4/2000	29 48.01N 85 18.07W	30.8	34.3	*Chondria atropurpurea*	3	2	0.75	4	0	2.25	0.5	0	0.3	0	0	0	1
5	11/10/2000	29 40.74N 85 21.87W	30	21	*Halodule beaudettei*	4	2	10.5	1262.5	1	1248	6.8	0.8	2.8	0	0	0	3.3

Table B-1 (continued).

Station #	Date of Collection	GPS Coordinates of Station	Salinity (ppt)	Temp (*C)	Substrate (+ - found in green turtle diet)	1- winter 2- spring 3 - summer 4 - fall	n (reps)	MEAN toxic Prorocentrum	MEAN *Prorocentrum* (toxic + non-toxic)	*P. lima**	*P. mexicanum*	*P. concavum**	*P. emarginatum*	*P. hoffmannianum**	*P. belizeanum**	*P. taylori*	*P. micans*	*Small Prorocentrum*
6	11/10/2000	29 41.19N 85 18.89W	35.9	20.7	*Thalassia testudinum*	4	2	11.25	2399.75	7.5	2383	0.8	0	2.8	0.3	0	0	6
6	11/10/2000	29 41.19N 85 18.89W	35.9	20.7	*Halodule beaudettei*	4	2	77.5	966.5	4	884	27	0.3	44	2	3	0	1.5
6	11/10/2000	29 41.19N 85 18.89W	35.9	20.7	*Thalassia testudinum*	4	2	90.75	1094.5	3	1001	13	0	66	9.3	0	0	2.8
7	11/10/2000	29 47.49N 85 18.10W	30.0	20.9	*Halodule beaudettei*	4	2	4.5	40.5	0	35.3	2.5	0	2	0	0	0	0.8
7	11/10/2000	29 47.49N 85 18.10W	30.0	20.9	*Thalassia testudinum*	4	2	1.25	50.25	0.3	46.8	1	0.3	0	0	0	0	2
7	11/10/2000	29 47.49N 85 18.10W	30.0	20.9	*Acanthophora spicifera*	4	2	8.75	134	3.5	116	5.3	1.3	0	0	0	0	7.8
7	11/10/2000	29 47.49N 85 18.10W	30.0	20.9	*Spyridia filamentosa*	4	1	64.5	1022.5	27	942	38	2.5	0	0	0	0	13.5
7	11/10/2000	29 47.49N 85 18.10W	30.0	20.9	*Caulerpa prolifera*	4	2	16	233.75	9.3	210	6.5	1.5	0.3	0	0	0	6.5
1	3/1/2001	29 47.40N 85 18.12W	20.5	27.5	*Halodule beaudettei*	1	2	7	136	0.8	126	3.3	2	3	0	0.3	0	0.8
1	3/1/2001	29 47.40N 85 18.12W	20.5	27.5	*Lyngbya sp.*	1	2	17.25	3234.5	6.5	3175	11	28	0.3	0	0	0	14
1	3/1/2001	29 47.40N 85 18.12W	20.5	27.5	*Thalassia (mostly dead)*	1	1	14	2206	9.5	2177	4.5	5	0	0	0	0	10.5
1	3/1/2001	29 47.40N 85 18.12W	20.5	27.5	*Dasya baillouviana*	1	2	4.75	240.75	3	206	1.8	21	0	0	0	0	8.8
1	3/1/2001	29 47.40N 85 18.12W	20.5	27.5	*Chondria sp.*	1	1	25	2202	13	2148	11	5	1	0	0	0	24
8	3/1/2001	29 46.54N 85 24.12W	24.2	25.1	*Halodule beaudettei*	1	2	16.5	149.75	8.8	131	3	0.5	4.3	0.5	0	0	1.5
8	3/1/2001	29 46.54N 85 24.12W	24.2	25.1	*Chondria sp.*	1	2	20	750.75	3	723	8.3	3.8	8.5	0.3	0	0	4
8	3/1/2001	29 46.54N 85 24.12W	24.2	25.1	*Ceramium sp.*	1	2	14.25	740.25	5.3	720	2	4.3	7	0	0	0	1.5
8	3/1/2001	29 46.54N 85 24.12W	24.2	25.1	*Enteromorpha sp.*	1	1	5.5	94	2	86.5	2	1	1.5	0	0	0	1
8	3/1/2001	29 46.54N 85 24.12W	24.2	25.1	*Heterosiphonia sp.*	1	1	13.5	239.5	3	219	5	6.5	5.5	0	0	0	1

Table B-2. Summary of Cedar Key results. Cell numbers are per 2 mL sample (mean over replicates). Stations: 1 – East of Snake Island, 2 – South of Snake Island, 3 – 8 are off of Seahorse Key.

Station #	Date of Collection	GPS Coordinates of Station	Salinity (ppt)	Temp (*C)	Substrate (+ - found in green turtle diet)	1- winter 2- spring 3 - summer 4 - fall	n (reps)	MEAN toxic Prorocentrum	MEAN *Prorocentrum* (toxic + non-toxic)	*P. lima**	*P. mexicanum*	*P. concavum**	*P. emarginatum*	*P. hoffmannianum**	*P. belizeanum**	*P. taylori*	*P. micans*	*Small Prorocentrum*
1	5/4/2000	29 05.95N 83 01.79W	28.7	24.3	*Halodule beaudettei+*	2	2	157.8	167.5	151	7	2.8	2.8	3.8	0	0	0	0
1	5/4/2000	29 05.95N 83 01.79W	28.7	24.3	*Syringodium filiforme+*	2	2	253.8	268.3	250	11	2	3.5	1.5	0	0	0	0

Table B-2 (continued)

1	5/4/2000	29 05.95N 83 01.79W	28.7	24.3	*Halophila englemannii*+	2	2	153.3	181.8	145	23.8	6.5	4	2	0	0	0.8	0
1	5/4/2000	29 05.95N 83 01.79W	28.7	24.3	*Gracilaria spp.*+	2	1	39	51.5	38	8	1.5	4.5	0	0	0	0	0
2	5/4/2000	29 05.68N 83 01.66W	28.7	23.9	*Halodule beaudettei*+	2	2	42.5	49.3	41	5	1.3	1.5	0	0	0	0.3	0
2	5/4/2000	29 05.68N 83 01.66W	28.7	23.9	*Thalassia testudinum*+	2	2	67	71.3	64	3.3	1.8	0.5	1.3	0	0	0.5	0
2	5/4/2000	29 05.68N 83 01.66W	28.7	23.9	*Syringodium filiforme*+	2	2	30.8	33.8	28	1	2	1.8	1	0	0	0.3	0
2	8/2/2000	29 05.68N 83 01.66W	30.1	29.9	*Thalassia testudinum*+	3	2	16	18.3	13	1.8	3	0.3	0	0	0	0.3	0
2	8/2/2000	29 05.68N 83 01.66W	30.1	29.9	*Syringodium filiforme*+	3	2	9	9.3	6.3	0.3	2.3	0	0.5	0	0	0	0
3	8/2/2000	29 05.66N 83 03.84W	29.7	30.2	*Thalassia testudinum*+	3	2	179.3	182.3	127	1.3	43	0.3	9.8	0	0	0	1.5
3	8/2/2000	29 05.66N 83 03.84W	29.7	30.2	*Syringodium filiforme-mix*+	3	2	160	162.3	100	1.8	36	0	25	0	0	0	0.5
3	8/2/2000	29 05.66N 83 03.84W	29.7	30.2	*Halodule beaudettei*+	3	2	57.75	147	35	82.8	15	0	8	0	0	0.3	6.3
3	8/2/2000	29 05.66N 83 03.84W	29.7	30.2	*Sargassum pteropleuron*	3	2	67.5	176.5	46	93	16	0.8	6.3	0	0	0.3	15
3	8/2/2000	29 05.66N 83 03.84W	29.7	30.2	*Spyridia filamentosa*+	3	2	58	240	29	169	21	0	7.8	0	0	0.5	13
4	8/2/2000	29 05.75N 83 04.20W	29.9	30.2	*Thalassia testudinum*+	3	2	282.3	316.8	194	14.5	52	0.8	36	0	0	0	19
4	8/2/2000	29 05.75N 83 04.20W	29.9	30.2	*Syringodium-DRIFT*+	3	2	91.5	99	79	3.3	6.3	0.8	6.3	0	0	0	3.5
4	8/2/2000	29 05.75N 83 04.20W	29.9	30.2	*Halodule beaudettei*+	3	2	189	318.5	128	105	39	0	22	0	0	0.3	24
4	8/2/2000	29 05.75N 83 04.20W	29.9	30.2	*Sargassum pteropleuron*	3	2	88.8	247	47	137	33	0	9	0	0	0.5	21
5	11/15/2000	29 05.75N 83 03.87W	31.7	21.1	*Halodule beaudettei*+	4	2	13.3	28	7.5	12.8	4.5	1.8	1.3	0	0	0	0.3
5	11/15/2000	29 05.75N 83 03.87W	31.7	21.1	*Thalassia testudinum*+	4	2	27.5	44	21	14.5	7	1	0	0	0	0.5	0.5
5	11/15/2000	29 05.75N 83 03.87W	31.7	21.1	*Sargassum pteropleuron*	4	2	18	33	7	12.5	11	2.5	0.5	0	0	0	0
5	11/15/2000	29 05.75N 83 03.87W	31.7	21.1	*Hypnea spinella*	4	1	14	34	7	19	7	1	0	0	0	0	0
6	11/15/2000	29 05.79N 83 03.78W	28.9	23.7	*Halodule beaudettei*+	4	2	18.3	43	13	24	4.5	0.3	0.8	0	0.3	0	0.3
6	11/15/2000	29 05.79N 83 03.78W	28.9	23.7	*Thalassia testudinum*+	4	2	56	77.5	41	17	15	3.5	0.5	0	0	1	0
6	11/15/2000	29 05.79N 83 03.78W	28.9	23.7	*Hypnea spinella*	4	1	31	59	24	25	5	2	2	0	0	1	0
6	11/15/2000	29 05.79N 83 03.78W	28.9	23.7	*Acanthophora spicifera*+	4	1	32	51	24	12	8	6	0	0	0	1	0
7	2/8/2001	29 05.79N 83 04.16W	19.3	21.5	*Halodule beaudettei*+	1	1	7	9	1.5	1.5	4.5	0.5	1	0	0	0	0
7	2/8/2001	29 05.79N 83 04.16W	19.3	21.5	*Gracilaria tikvahiae*+	1	2	0.3	0.5	0.3	0	0	0.3	0	0	0	0	0
7	2/8/2001	29 05.79N 83 04.16W	19.3	21.5	*Ulva lactuca*+	1	2	1	2.3	0	0.8	0.8	0.5	0.3	0	0	0	0
7	2/8/2001	29 05.79N 83 04.16W	19.3	21.5	*Hypnea cervicornis*+	1	2	2.8	9	1.3	1.3	1	4.8	0.5	0	0	0.3	0
7	2/8/2001	29 05.79N 83 04.16W	19.3	21.5	*Gracilaria tikvahiae*+	1	1	0	0.5	0	0	0	0.5	0	0	0	0	0
8	2/8/2001	29 05.75N 83 03.95W	20.3	21	*Halodule beaudettei*+	1	1	3	10	1	3	2	3	0	0	0	1	0
8	2/8/2001	29 05.75N 83 03.95W	20.3	21	*Thalassia testudinum (dead)*	1	2	8.5	11.5	5.8	0.3	2.8	2.3	0	0	0	0.5	0
8	2/8/2001	29 05.75N 83 03.95W	20.3	21	*Hypnea cervicornis*+	1	2	0.5	1.5	0.3	0.3	0.3	0.5	0	0	0	0.3	0
8	2/8/2001	29 05.75N 83 03.95W	20.3	21	*Ulva lactuca*+	1	2	1.5	8	1.3	3.5	0.3	2	0	0	0	1	0
8	2/8/2001	29 05.75N 83 03.95W	20.3	21	*Gracilaria tikvahiae*+	1	2	2.8	17	1.3	7.8	1.5	5.5	0	0	0	1	0

Table B-3. Summary of Mosquito Lagoon results. Cell numbers are per 2 mL sample (mean over replicates). Stations: 1 – Outside of Cape Canaveral gate, 2 – Culdesac, 3 – S. of canal, 4 – in canal (to Indian River), 5 – Oak Hill Trailer Park, 6 – Sandbar, 7 – Turtle netting station, 8 – Boat landing in West.

Station #	Date of Collection	GPS Coordinates of Station	Salinity (ppt)	Temp (*C)	Substrate (+ - found in green turtle diet)	1- winter 2- spring 3 - summer 4 - fall	n (reps)	MEAN toxic Prorocentrum	MEAN *Prorocentrum* (toxic + non-toxic)	*P. lima**	*P. mexicanum*	*P. concavum**	*P. emarginatum*	*P. hoffmannianum**	*P. belizeanum**	*P. taylori*	*P. micans*	*Small Prorocentrum*
1	5/9/2000	28 56.40N 80 49.97W	32.6	25.7	*Halodule beaudettei*+	2	2	8.25	29	1.5	20.3	1	0.5	5.8	0	0	0	0
1	5/9/2000	28 56.40N 80 49.97W	32.6	25.7	*Ulva lactuca*+	2	2	29.5	71	0.3	40	16	0	14	0	0	1.3	0.3
1	5/9/2000	28 56.40N 80 49.97W	32.6	25.7	*Hypnea musciformes*+	2	2	5.5	18.5	0.3	11.5	2.3	0	3	0	0	1.5	0
1	5/9/2000	28 56.40N 80 49.97W	32.6	25.7	*Gracilaria tikvahiae*+	2	2	2.5	41	0.5	37.5	1.8	0	0.3	0	0	0.8	0.3
1	5/9/2000	28 56.40N 80 49.97W	32.6	25.7	*Codium decorticatum*+	2	2	1.5	23.5	1	21.8	0.5	0	0	0	0	0	0.3
2	5/9/2000	28 51.47N 80 46.64W	32.4	26	*Halodule beaudettei*+	2	2	27.25	556.5	6.5	522	14	1	7	0	0.3	2.3	4
2	5/9/2000	28 51.47N 80 46.64W	32.4	26	*Acanthophora spicifera*+	2	2	48.5	511.25	20	454	25	0.3	4.3	0	0	2.8	5.5
2	5/9/2000	28 51.47N 80 46.64W	32.4	26	*Enteromorpha sp. w/Ceramium*	2	2	16	254.25	6	225	2.5	0	7.5	0	0	2	12
2	5/9/2000	28 51.47N 80 46.64W	32.4	26	*Syringodium filiforme*+	2	2	17.25	301.5	7.3	266	4.5	0	5.5	0	0	5.5	13
2	5/9/2000	28 51.47N 80 46.64W	32.4	26	*Hypnea musciformes*+	2	2	44	483.25	18	424	9	0	18	0	0	3	12
3	8/1/2000	28 44.13N 80 44.19W	25.8	34.1	*Halodule beaudettei*+	3	2	397	4161	20	3689	347	0.5	30	0	0	2	73
4	8/1/2000	28 44.33N 80 45.13W	28.8	31	*Halodule beaudettei*+	3	2	44.5	49	0	3	38	0	7	0	0	0.5	1
4	8/1/2000	28 44.33N 80 45.13W	28.8	31	*Hypnea cervicornis*+	3	2	14.5	17.5	1	2	12	0	1.5	0	0	0	1
4	8/1/2000	28 44.33N 80 45.13W	28.8	31	*Gracilaria tikvahiae*+	3	2	2.5	4	0.3	0.75	1.5	0	0.8	0	0	0.3	0.5
4	8/1/2000	28 44.33N 80 45.13W	28.8	31	*Acanthophora spicifera*+	3	1	8	11	0.5	1	7.5	0	0	0	0	1.5	0.5
5	8/1/2000	28 51.95N 80 49.99W	22	34.1	*Enteromorpha flexuosa*	3	2	1.25	5	1	2.5	0.3	0	0	0	0	0.3	1
5	8/1/2000	28 51.95N 80 49.99W	22	34.1	*Hypnea musciformes*+	3	2	30.5	109.5	1	63	28	0	2	0	0	1	15
5	8/1/2000	28 51.95N 80 49.99W	22	34.1	*Gracilaria verrucosa*+	3	2	15	50	0	30	14	0	1	0	0	0.5	4.5
5	8/1/2000	28 51.95N 80 49.99W	22	34.1	*Ceramium sp.*	3	1	9	55	0	41	9	0	0	0	0	1	4
6	11/13/2000	28 44.33N 80 43.65W	40.2	22.8	*Halodule beaudettei*+	4	2	2.25	7.25	1.3	4.25	1	0	0	0	0	0.5	0.3
6	11/13/2000	28 44.33N 80 43.65W	40.2	22.8	*Syringodium filiforme*+	4	2	0.25	0.25	0	0	0.3	0	0	0	0	0	0
6	11/13/2000	28 44.33N 80 43.65W	40.2	22.8	*Spyridia filamentosa*+	4	2	0.75	2.25	0.8	1.5	0	0	0	0	0	0	0
6	11/13/2000	28 44.33N 80 43.65W	40.2	22.8	*Hypnea cervicornis*+	4	2	0.75	6	0	5	0.8	0	0	0	0	0.3	0
7	11/13/2000	28 42.51N 80 41.27W	40.9	22.8	*Halodule beaudettei*+	4	2	1.75	6	0.5	3.5	1.3	0	0	0	0	0.5	0.3
7	11/13/2000	28 42.51N 80 41.27W	40.9	22.8	*Syringodium filiforme*+	4	2	0.5	3.5	0	2.5	0.3	0	0.3	0	0	0	0.5
7	11/13/2000	28 42.51N 80 41.27W	40.9	22.8	*Acanthophora spicifera*+	4	2	0.5	2	0	0.25	0.5	0	0	0	0	0.8	0.5

Table B-3 (continued).

Station #	Date of Collection	GPS Coordinates of Station	Salinity (ppt)	Temp (*C)	Substrate (+ - found in green turtle diet)	1- winter 2- spring 3 - summer 4 - fall	n (reps)	MEAN toxic Prorocentrum	*P. lima** MEAN *Prorocentrum* (toxic + non-toxic)	*P. mexicanum*	*P. concavum**	*P. emarginatum*	*P. hoffmanianum**	*P. belizeanum**	*P. taylori*	*P. micans*	*Small Prorocentrum*
7	11/13/2000	28 42.51N 80 41.27W	40.9	22.8	*Spyridia filamentosa*+	4	2	0	2.25	0	2	0	0	0	0	0	0.3	0
7	11/13/2000	28 42.51N 80 41.27W	40.9	22.8	*Hypnea cervicornis*+	4	2	0.25	2	0	1.25	0	0	0.3	0	0	0.5	0
1	2/16/2001	28 56.44N 80 50.00W	29.4	8.5	*Ulva lactuca*+	1	2	0.25	0.75	0.3	0.5	0	0	0	0	0	0	0
1	2/16/2001	28 56.44N 80 50.00W	29.4	8.5	*Lyngbya sp.*	1	2	0.25	3.75	0	3.5	0.3	0	0	0	0	0	0
1	2/16/2001	28 56.44N 80 50.00W	29.4	8.5	*Gracillaria verrucosa*+	1	2	0	0	0	0	0	0	0	0	0	0	0
1	2/16/2001	28 56.39N 80 49.96W	25.4	9	*Codium decorticatum*+	1	1	0.5	0.5	0.5	0	0	0	0	0	0	0	0
2	2/16/2001	28 51.46N 80 46.65W	25	9.6	*Halodule beaudettei*+	1	2	1.75	5	0.3	3.25	1.5	0	0	0	0	0	0
2	2/16/2001	28 51.46N 80 46.65W	25	9.6	*Syringodium filiforme*+	1	2	0.25	0.75	0	0.5	0.3	0	0	0	0	0	0
2	2/16/2001	28 51.46N 80 46.65W	25	9.6	*Spyridia filamentosa*+	1	2	0	0.75	0	0.75	0	0	0	0	0	0	0
2	2/16/2001	28 51.46N 80 46.65W	25	9.6	*Gracillaria verrucosa*+	1	1	1	2.5	0.5	1.5	0.5	0	0	0	0	0	0
8	2/16/2001	28 45.35N 80 45.94W	28.2	21.6	*Halodule beaudettei*+	1	2	0.75	1.5	0.5	0.75	0.3	0	0	0	0	0	0

Table B-4. Summary of results from Florida Bay. Stations: 1–Johnson Key Basin, 2–Rabbit Key Basin, 3–Peterson Key Basin. Season: 1–Spring, 2-Summer, 3–Fall, 4–Winter.

Station #	Date of Collection	GPS Coordinates of Station	Salinity (ppt)	Temp (*C)	Substrate (+ - found in green turtle diet)	spring Season	n (reps)	MEAN toxic Prorocentrum	*Prorocentrum* (toxic + non-toxic)	*P. lima**	*P. mexicanum*	*P. concavum**	*P. emarginatum*	*P. hoffmanianum**	*P. belizeanum**	*P. taylori*	*P. micans*	*Small Prorocentrum*
1	6/3/2000	25 02.95N 80 54.62W	32.1	30.4	*Halodule beaudettei*+	2	2	32.8	66	0.3	27.8	17	5.5	16	0	0	0	0
1	6/3/2000	25 02.95N 80 54.62W	32.1	30.4	*Thalassia testudinum*+	2	2	109.5	434.8	0	290	67	36	41	1.8	0	0	0
1	6/3/2000	25 02.95N 80 54.62W	32.1	30.4	*Syringodium filiforme*+	2	2	30.5	109.3	0	70	13	8.8	17	0.8	0	0	0
1	6/3/2000	25 02.95N 80 54.62W	32.1	30.4	*Halophila englemannii*+	2	2	31.8	126.8	0	84.5	13	11	19	0.5	0	0	0
1	6/3/2000	25 02.95N 80 54.62W	32.1	30.4	*Sargassum polyceratium*	2	2	22	76.5	0	48.8	10	5.8	12	0.3	0	0	0
2	6/3/2000	24 58.97N 80 50.30W	31.2	31.2	*Halodule beaudettei*+	2	2	120.8	208.8	45	46.3	34	42	41	1	0	0	0
2	6/3/2000	24 58.97N 80 50.30W	31.2	31.2	*Thalassia testudinum*+	2	2	167.8	252.5	95	64.5	43	20	29	1.3	0	0	0
2	6/3/2000	24 58.97N 80 50.30W	31.2	31.2	*Chondria tenuissima*	2	2	207.8	311.5	134	93.3	49	11	24	0.8	0	0	0
2	6/3/2000	24 58.97N 80 50.30W	31.2	31.2	*Dictyota dichotoma*+	2	1	165.5	232	93	61.5	51	5	22	0	0	0	0

Table B-4 (continued).

Station #	Date of Collection	GPS Coordinates of Station	Salinity (ppt)	Temp (*C)	Substrate (+ - found in green turtle diet)	Season	n (reps)	MEAN toxic Prorocentrum	*Prorocentrum* (toxic + non-toxic)	*P. lima**	*P. mexicanum*	*P. concavum**	*P. emarginatum*	*P. hoffmannianum**	*P. belizeanum**	*P. taylori*	*P. micans*	*Small Prorocentrum*
2	6/3/2000	24 58.97N 80 50.30W	31.2	31.2	*Halimeda incrassata*	2	2	88	120.3	32	18.8	43	14	13	0.3	0	0	0
1	8/26/2000	25 02.95N 80 54.62W	35.6	29.8	*Halodule beaudettei*+	3	2	71.8	217.8	11	118	22	0.3	35	5.3	1	0	27
1	8/26/2000	25 02.95N 80 54.62W	35.6	29.8	*Thalassia testudinum*+	3	2	75	363	10	246	16	0	44	5.5	0.5	0	42
1	8/26/2000	25 02.95N 80 54.62W	35.6	29.8	*Penicillus dumetosus*	3	2	36	116	4.3	77.5	7.8	0.3	22	1.8	0.3	0	2
1	8/26/2000	25 02.95N 80 54.62W	35.6	29.8	*Syringodium filiforme*+	3	2	80	137.5	7	51.3	9.8	1	58	5	0.8	0	4.5
2	8/26/2000	24 58.97N 80 50.30W	35.2	29.4	*Halodule beaudettei*+	3	2	22.8	48.5	8.3	17.8	5.5	0	8.5	0.5	0.3	0	7.8
2	8/26/2000	24 58.97N 80 50.30W	35.2	29.4	*Thalassia testudinum*+	3	2	13.8	29.5	4.3	12.3	6.5	0	3	0	0	0	3.5
2	8/26/2000	24 58.97N 80 50.30W	35.2	29.4	*Penicillus dumetosus*	3	2	41	69.3	6.3	19	21	0	14	0	0	0	9.3
2	8/26/2000	24 58.97N 80 50.30W	35.2	29.4	*Sargassum polyceratium*	3	1	19.5	45.5	12	18	1	0	7	0	0	0	8
2	8/26/2000	24 58.97N 80 50.30W	35.2	29.4	*Hypnea spinella*	3	1	27	51.5	12	21	8.5	0.5	7	0	0	0	3
2	8/26/2000	24 58.97N 80 50.30W	35.2	29.4	*Acanthophora spicifera*+	3	1	14.5	38	7.5	21	4.5	0	2.5	0	0	0	2.5
1	11/4/2000	24 58.97N 80 50.30W	33.5	23.3	*Halodule beaudettei*+	4	2	21.8	137.3	4	112	8.8	1.3	7	2	0	0.3	2.3
1	11/4/2000	24 58.97N 80 50.30W	33.5	23.3	*Thalassia testudinum*+	4	2	22.3	188.5	3.8	165	8.3	0.3	8.3	2	0	0.3	1.3
1	11/4/2000	24 58.97N 80 50.30W	33.5	23.3	*Sargassum polyceratium*	4	2	8.8	28.3	1	19	2.3	0	5	0.5	0	0.3	0.8
1	11/4/2000	24 58.97N 80 50.30W	33.5	23.3	*Syringodium filiforme*+	4	2	67.8	307.3	7.5	234	18	0.5	36	6.3	1.3	1.3	2.8
2	11/4/2000	24 58.97N 80 50.30W	35.6	24.5	*Halodule beaudettei*+	4	2	112.5	143.8	57	22	19	0.3	29	8.8	4	0	5
2	11/4/2000	24 58.97N 80 50.30W	35.6	24.5	*Thalassia testudinum*+	4	2	23.5	29	11	3.8	5.3	0	6.5	0.8	0	0.3	1.5
2	11/4/2000	24 58.97N 80 50.30W	35.6	24.5	*Penicillus dumetosus*	4	2	17	20.8	7.3	1.8	6.5	0.3	3	0.3	0	1	0.8
2	11/4/2000	24 58.97N 80 50.30W	35.6	24.5	*Acanthophora spicifera*+	4	2	10	11.3	5.5	0.8	2.8	0	1.8	0	0	0.3	0.3
2	11/4/2000	24 58.97N 80 50.30W	35.6	24.5	*Dictyota cervicornis*+	4	1	81.5	97	42	11	11	0	24	5.5	0.5	1	3
1	2/3/2001	25 02.92N 80 54.64W	34.9	23.7	*Halodule beaudettei*+	1	2	30	47	1.8	12	15	4.8	12	1	0.3	0	0
1	2/3/2001	25 02.92N 80 54.64W	34.9	23.7	*Thalassia testudinum*+	1	2	44	68	1	20	36	2.8	6.5	0.3	0.3	0	1
1	2/3/2001	25 02.92N 80 54.64W	34.9	23.7	*Syringodium filiforme*+	1	2	27	42	2.5	12.8	17	2	7.3	0.8	0	0	0.3
1	2/3/2001	25 02.92N 80 54.64W	34.9	23.7	*Sargassum polyceratium*	1	2	17.5	28.5	1.3	8.25	8.8	1.8	6.5	1	0.3	0	0.8
1	2/3/2001	25 02.92N 80 54.64W	34.9	23.7	*Penicillus dumetosus*	1	2	30.5	42.8	0.8	9	25	2.5	5	0	0	0	0.8
2	2/3/2001	24 58.97N 80 50.29W	35.9	22.7	*Halodule beaudettei*+	1	2	54	91.3	24	34.5	6.8	1	17	6.3	0	0.3	1.5
2	2/3/2001	24 58.97N 80 50.29W	35.9	22.7	*Thalassia testudinum*+	1	2	14	31.5	8.8	15	2	0	3.3	0	0	0	2.5
2	2/3/2001	24 58.97N 80 50.29W	35.9	22.7	*Dictyota cervicornis*+	1	2	39.5	78.8	29	35.3	2.3	0.8	7.8	0.3	0	0	3.3
2	2/3/2001	24 58.97N 80 50.29W	35.9	22.7	*Penicillus dumetosus*	1	2	4.8	14.5	0.5	9.3	2	0	2	0.3	0	0	0.5
2	2/3/2001	24 58.97N 80 50.29W	35.9	22.7	*Cladosiphon occidentalis*	1	2	43.5	95	27	50	6.5	0.3	9	1	0.5	0.3	0.5
3	2/3/2001	24 55.07N 80 44.80W	35.9	22.9	*Halodule beaudettei*+	1	2	34	50	6	14.5	3.3	0.3	22	3.3	0.3	0.5	0.5

APPENDIX C
HAWAIIAN ISLANDS DATA

Table C-1. Summary of Punalu'u Bay, Big Island results. Cell numbers are per 2 mL sample (mean over replicates).

Station #	Date of Collection	GPS Coordinates of Station	Salinity (ppt)	Temp (*C)	Substrate (+ - found in green turtle diet)	Season: 0- winter 1 - summer	n (reps)	MEAN - all Prorocentrum	MEAN- all Prorocentrum	*P. lima**	*P. mexicanum*	*P. concavum**	*P. emarginatum*	*P. hoffmanianum**	*Small Prorocentrum*
1	2/17/2000	19 08.08N 155 30.29W	22.1	22.6	*Ahnfeltiopsis concinna*+	0	2	0	0	0	0	0	0	0	0
1	2/17/2000	19 08.08N 155 30.29W	22.1	22.6	*Ulva rigida*+	0	2	0	0	0	0	0	0	0	0
1	8/11/2000	19 08.08N 155 30.29W	19	22.5	*Ahnfeltiopsis concinna*+	1	2	0.3	0.3	0.3	0	0	0	0	0
1	8/11/2000	19 08.08N 155 30.29W	19	22.5	*Enteromorpha paradox*	1	2	0.3	0.3	0.3	0	0	0	0	0
1	8/11/2000	19 08.08N 155 30.29W	19	22.5	*Pterocladiella capillacea*+	1	2	0	0	0	0	0	0	0	0
1	8/11/2000	19 08.08N 155 30.29W	19	22.5	*Ulva rigida*+	1	2	0.3	0.6	0	0	0.3	0	0	0.3
2	2/17/2000	19 08.12N 155 30.28W	17.6	21.6	*Enteromorpha paradox*	0	2	0	0	0	0	0	0	0	0
3	2/17/2000	19 40.35N 156. 01.59W	3.4	20.2	*Pterocladiella capillacea*+	0	2	0	0	0	0	0	0	0	0

Table C-2. Summary of the Kona/Kohala Coast results. Cell numbers are per 2 mL sample (mean over replicates). Stations: 1 – Kawaihae, 2 – Puako, 3a – Kiholo (beach), 3b – Kiholo (connected pond), 4 – Kaloko-Honokohau Historic Park.

Station #	Date of Collection	GPS Coordinates of Station	Salinity (ppt)	Temp (*C)	Substrate (+ - found in green turtle diet)	Season: 0- winter 1 - summer	n (reps)	MEAN - toxic Prorocentrum	MEAN- all Prorocentrum	*P. lima**	*P. mexicanum*	*P. concavum**	*P. emarginatum*	*P. hoffmanianum**	*Small Prorocentrum*
1	2/16/2000	20 01.82N 155 49.82W	32.3	24.5	*Melanamansia glomerata*+	0	2	0.5	0.5	0.5	0	0	0	0	0
1	8/10/2000	20 01.83N 155 49.81W	31.2	28	*Gelidium-like wiry turf*+	1	1	1.5	5	0	3.5	0.5	0	1	0
1	8/10/2000	20 01.83N 155 49.81W	31.2	28	*Melanamansia glomerata*+	1	2	1.1	1.4	0.8	0	0	0.3	0.3	0
1	8/10/2000	20 01.83N 155 49.81W	31.2	28	*Polysiphonia hawaiiensis*	1	1	8	19	0	11	0	0	8	0
1	8/10/2000	20 01.83N 155 49.81W	31.2	28	*Rhizoclonium riparium*+	1	1	0	0	0	0	0	0	0	0
2	2/16/2000	19 58.30N 155 50.53W	31.9	25.7	*Enteromorpha paradox*	0	2	2.3	2.3	0.5	0	1.8	0	0	0
2	2/16/2000	19 58.30N 155 50.53W	31.9	25.7	*Gelidium-like wiry turf*+	0	2	0	0	0	0	0	0	0	0

Table C-2 (continued).

Station #	Date of Collection	GPS Coordinates of Station	Salinity (ppt)	Temp (*C)	Substrate (+ - found in green turtle diet)	Season: 0- winter 1 - summer	n (reps)	MEAN - toxic Prorocentrum	MEAN- all Prorocentrum	*P. lima**	*P. mexicanum*	*P. concavum**	*P. emarginatum*	*P. hoffmanianum**	*Small Prorocentrum*
2	8/10/2000	19 58.31N 155 50.49W	31	29	*Enteromorpha paradox*	1	2	0	1.5	0	1.5	0	0	0	0
2	8/10/2000	19 58.31N 155 50.49W	31	29	*Rhizoclonium riparium*+	1	4	1.2	3.6	0.5	0.4	0.3	0	0.4	2
3a	2/16/2000	19 51.33N 155 55.35W	20.8	25.4	*Cladophora hemisphaerica*+	0	2	0	0	0	0	0	0	0	0
3a	8/10/2000	19 51.31N 155 55.36W	31.2	29.5	*Acanthophora spicifera*+	1	2	0	0	0	0	0	0	0	0
3a	8/10/2000	19 51.31N 155 55.36W	31.2	29.5	*Cladophora hemisphaerica*+	1	2	0	0	0	0	0	0	0	0
3b	2/16/2000	19 51.33N 155 55.35W	4.5	24.7	*Cladophora hemisphaerica*+	0	2	0	0	0	0	0	0	0	0
4	2/16/2000	19 40.38N 156 01.56W	22.8	24.1	*Enteromorpha paradox*	0	2	0	0	0	0	0	0	0	0
4	8/10/2000	19 40.29N 156 01.56W	32.2	29.3	*Enteromorpha paradox*	1	2	0	0	0	0	0	0	0	0
4	8/10/2000	19 40.29N 156 01.56W	32.2	29.3	*Lyngbya sp.*	1	2	0	0	0	0	0	0	0	0

Table C-3. Summary of results from Hilo Bay. Cell numbers are per 2 mL sample (mean over replicates). Stations: 1 - By former sewage treatment plant, 2 - Richardson Beach Park.

Station #	Date of Collection	GPS Coordinates of Station	Salinity (ppt)	Temp (*C)	Substrate (+ - found in green turtle diet)	Season: 0- winter 1 - summer	n (reps)	MEAN toxic Prorocentrum	MEAN- all Prorocentrum	*P. lima**	*P. mexicanum*	*P. concavum**	*P. emarginatum*	*P. hoffmanianum**	*Small Prorocentrum*
1	2/17/2000	19 43.90N 155 02.81W	24.5	22	*Ahnfeltiopsis concinna*+	0	2	0	0	0	0	0	0	0	0
1	2/17/2000	19 43.90N 155 02.81W	24.5	22	*Pterocladiella capillacea*+	0	2	0	0	0	0	0	0	0	0
1	2/17/2000	19 43.90N 155 02.81W	24.5	22	*Ulva rigida*+	0	2	0	0	0	0	0	0	0	0
1	8/12/2000	19 43.90N 155 02.81W	21.2	17.2	*Ahnfeltiopsis concinna*+	1	2	0	0	0	0	0	0	0	0
1	8/12/2000	19 43.90N 155 02.81W	21.2	17.2	*Melanamansia glomerata*+	1	2	0	0.5	0	0.5	0	0	0	0
1	8/12/2000	19 43.90N 155 02.81W	21.2	17.2	*Pterocladiella capillacea*+	1	1	0	0	0	0	0	0	0	0
1	8/12/2000	19 43.90N 155 02.81W	21.2	17.2	*Ulva rigida*+	1	2	0	0.3	0	0.3	0	0	0	0
2	2/17/2000	19 44.20N 155 00.80W	26.7	23.5	*Colpomenia sinuosa*	0	2	1.3	1.3	1	0	0.3	0	0	0
2	2/17/2000	19 44.20N 155 00.80W	26.7	23.5	*Melanamansia glomerata*+	0	2	0	0	0	0	0	0	0	0
2	2/17/2000	19 44.20N 155 00.80W	26.7	23.5	*Plocamium sandvicense*	0	2	0	0	0	0	0	0	0	0
2	2/17/2000	19 44.20N 155 00.80W	26.7	23.5	*Pterocladiella capillacea*+	0	2	0	0	0	0	0	0	0	0
2	2/17/2000	19 44.20N 155 00.80W	26.7	23.5	*Spyridia filamentosa*+	0	2	0	0	0	0	0	0	0	0
2	2/17/2000	19 44.20N 155 00.80W	26.7	23.5	*Ulva rigida*+	0	2	0	0	0	0	0	0	0	0
2	8/12/2000	19 44.15N 155 00.81W	29.1	24.7	*Acanthophora spicifera*+	1	2	1.5	1.5	1.5	0	0	0	0	0

Table C-3 (Continued)

Station #	Date of Collection	GPS Coordinates of Station	Salinity (ppt)	Temp (*C)	Substrate (+ - found in green turtle diet)	Season: 0- winter 1 - summer	n (reps)	MEAN - toxic *Prorocentrum*	MEAN- all *Prorocentrum*	*P. lima**	*P. mexicanum*	*P. concavum**	*P. emarginatum*	*P. hoffmanianum**	*Small Prorocentrum*
2	8/12/2000	19 44.15N 155 00.81W	29.1	24.7	*Colpomenia sinuosa*	1	1	14.5	15.5	14.5	0	0	1	0	0
2	8/12/2000	19 44.15N 155 00.81W	29.1	24.7	*Melanamansia glomerata+*	1	2	0	0	0	0	0	0	0	0
2	8/12/2000	19 44.15N 155 00.81W	29.1	24.7	*Plocamium sandvicense*	1	2	0.3	0.3	0.3	0	0	0	0	0
2	8/12/2000	19 44.15N 155 00.81W	29.1	24.7	*Pterocladiella capillacea+*	1	1	1.5	2	1.5	0	0	0	0	0.5

Table C-4. Summary of results from Southeast Kauai. Cell numbers are per 2 mL sample (mean over replicates). Stations: 1 - Prince Kuhia's Birthplace, 2 - Tidal flats 1/2 mile west of 1, and 3 - Harbor near Spouting Horn.

Station #	Date of Collection	GPS Coordinates of Station	Salinity (ppt)	Temp (*C)	Substrate (+ - found in green turtle diet)	Season: 0- winter 1 - summer	n (reps)	MEAN - toxic *Prorocentrum*	MEAN- all *Prorocentrum*	*P. lima**	*P. mexicanum*	*P. concavum**	*P. emarginatum*	*P. hoffmanianum**	*Small Prorocentrum*
1	2/21/2000	21 52.83N 159 28.45W	32.4	23.5	*Ahnfeltiopsis concinna*	0	2	0	0	0	0	0	0	0	0
1	2/21/2000	21 52.83N 159 28.45W	32.4	23.5	*Dictyosphaeria versluysii+*	0	2	6.1	6.6	5.5	0	0.3	0	0.3	0.5
1	2/21/2000	21 52.83N 159 28.45W	32.4	23.5	*Laurencia yamadana+*	0	2	7.3	7.6	5.5	0	1.8	0	0	0.3
1	2/21/2000	21 52.83N 159 28.45W	32.4	23.5	*Pterocladiella capillacea+*	0	2	0.3	0.3	0	0	0.3	0	0	0
1	2/21/2000	21 52.83N 159 28.45W	32.4	23.5	*Ulva rigida+*	0	2	0.6	0.6	0.3	0	0.3	0	0	0
1	8/13/2000	21 52.88N 159 28.44W	32.5	25.7	*Acanthophora spicifera+*	1	2	4.8	5.1	4.5	0.3	0.3	0	0	0
1	8/13/2000	21 52.88N 159 28.44W	32.5	25.7	*Caulerpa racemosa*	1	1	0.5	0.5	0.5	0	0	0	0	0
1	8/13/2000	21 52.88N 159 28.44W	32.5	25.7	*Centroceros clavulatum+*	1	2	2.8	3.1	2.5	0.3	0.3	0	0	0
1	8/13/2000	21 52.88N 159 28.44W	32.5	25.7	*Colpomenia sinuosa*	1	2	22.3	22.8	22.3	0.5	0	0	0	0
1	8/13/2000	21 52.88N 159 28.44W	32.5	25.7	*Laurencia succisa+*	1	2	1	1	1	0	0	0	0	0
1	8/13/2000	21 52.88N 159 28.44W	32.5	25.7	*Pterocladiella capillacea+*	1	2	2.3	2.3	2.3	0	0	0	0	0
2	2/21/2000	21 52.92N 159 28.67W	32.7	24.1	*Acanthophora spicifera+*	0	2	4.6	5.1	3.5	0	0.3	0	0.8	0.5
2	2/21/2000	21 52.92N 159 28.67W	32.7	24.1	*Centroceros clavulatum+*	0	2	2.3	2.3	1.8	0	0	0	0.5	0
2	2/21/2000	21 52.92N 159 28.67W	32.7	24.1	*Pterocladiella capillacea+*	0	2	1.8	1.8	1.8	0	0	0	0	0
2	2/21/2000	21 52.92N 159 28.67W	32.7	24.1	*Sargassum echinocarpum+*	0	2	5.6	5.9	4	0	0.8	0	0.8	0.3
2	2/21/2000	21 52.92N 159 28.67W	32.7	24.1	*Ulva rigida+*	0	2	1	1	1	0	0	0	0	0
3	8/13/2000	21 53.06N 159 29.23W	36.1	25.2	*Acanthophora spicifera+*	1	2	9.5	9.8	9	0.3	0.5	0	0	0
3	8/13/2000	21 53.06N 159 29.23W	36.1	25.2	*Centroceros clavulatum+*	1	1	11.5	13	9	1	2.5	0	0	0.5
3	8/13/2000	21 53.06N 159 29.23W	36.1	25.2	*Colpomenia sinuosa*	1	2	10.5	10.5	9	0	1	0	0.5	0
3	8/13/2000	21 53.06N 159 29.23W	36.1	25.2	*Gelidium-like wiry turf+*	1	2	9.6	11.4	7.3	0.5	2.3	0.8	0	0.5
3	8/13/2000	21 53.06N 159 29.23W	36.1	25.2	*Padina crassa*	1	2	26	27.5	23	1.5	2.5	0	0.5	0
3	8/13/2000	21 53.06N 159 29.23W	36.1	25.2	*Sargassum echinocarpum+*	1	2	22.8	25.4	21	2.3	1.5	0	0.3	0.3

Table C-5. Summary of Northeast Kauai results. Cell numbers are per 2 mL sample (mean over replicates). Stations: 1 – Anahola Bay, 2 – Moloaa Bay.

Station #	Date of Collection	GPS Coordinates of Station	Salinity (ppt)	Temp (*C)	Substrate (+ - found in green turtle diet)	Season: 0- winter 1 - summer	n (reps)	MEAN - toxic Prorocentrum	MEAN- all Prorocentrum	*P. lima**	*P. mexicanum*	*P. concavum**	*P. emarginatum*	*P. hoffmannianum**	*Small Prorocentrum*
1	2/21/2000	22 09.84N 159 18.54W	36.6	24.6	*Dictyota acuteloba*	0	1	8	8	7	0	1	0	0	0
1	2/21/2000	22 09.84N 159 18.54W	36.6	24.6	*Gelidium-like wiry turf+*	0	2	12.3	12.8	7.5	0	4.3	0	0.5	0.5
1	2/21/2000	22 09.84N 159 18.54W	36.6	24.6	*Lyngbya majuscula*	0	1	9.5	9.5	7.5	0	2	0	0	0
1	2/21/2000	22 09.84N 159 18.54W	36.6	24.6	*Melanamansia glomerata+*	0	2	4.3	4.6	4	0	0.3	0	0	0.3
1	8/14/2000	22 08.78N 159 18.13W	35.8	26.1	*Dictyosphaeria cavernosa+*	1	2	23	24.7	18.3	0.3	4.3	0.8	0	1
1	8/14/2000	22 08.78N 159 18.13W	35.8	26.1	*Dictyosphaeria versluysii+*	1	2	14	30.6	11.8	4.3	2.3	0	0	12.3
1	8/14/2000	22 08.78N 159 18.13W	35.8	26.1	*Dictyota acuteloba*	1	2	31	33.2	29.8	0.5	1.3	0.5	0.3	0.8
1	8/14/2000	22 08.78N 159 18.13W	35.8	26.1	*Hypnea chordacea+*	1	2	30	32.5	25.3	1	4.3	1.3	0.3	0.3
1	8/14/2000	22 08.78N 159 18.13W	35.8	26.1	*Melanamansia glomerata+*	1	2	12	13.4	10	0.3	2	0.8	0	0.3
1	8/14/2000	22 08.78N 159 18.13W	35.8	26.1	*Neomeris annulata*	1	2	15.5	17.4	13.5	0.8	2	0.3	0	0.3
2	2/21/2000	22 11.53N 159 19.86W	37.9	24.6	*Acanthophora spicifera+*	0	1	3.5	3.5	2.5	0	1	0	0	0
2	2/21/2000	22 11.53N 159 19.86W	37.9	24.6	*Colpomenia sinuosa*	0	2	11.6	13.1	9.8	0	1.8	0.5	0	1
2	2/21/2000	22 11.53N 159 19.86W	37.9	24.6	*Gelidium-like wiry turf+*	0	2	4.4	4.7	2.3	0	1.8	0	0.3	0.3
2	2/21/2000	22 11.53N 159 19.86W	37.9	24.6	*Laurencia majuscula+*	0	2	3.3	3.3	2.3	0	1	0	0	0
2	2/21/2000	22 11.53N 159 19.86W	37.9	24.6	*Padina thevyi*	0	2	4.3	5.1	2.8	0	1.5	0.5	0	0.3
2	2/21/2000	22 11.53N 159 19.86W	37.9	24.6	*Ulva rigida+*	0	1	0.5	0.5	0.5	0	0	0	0	0
2	8/14/2000	22 11.65N 159 20.02W	27.9	27.3	*Acanthophora spicifera+*	1	2	20.3	20.6	19.5	0	0.8	0.3	0	0
2	8/14/2000	22 11.65N 159 20.02W	27.9	27.3	*Enteromorpha intestinalis*	1	1	3.5	5	3.5	1	0	0.5	0	0
2	8/14/2000	22 11.65N 159 20.02W	27.9	27.3	*Enteromorpha paradox*	1	2	16.3	20.6	12	2.8	4.3	0.5	0	1
2	8/14/2000	22 11.65N 159 20.02W	27.9	27.3	*Jania sp.*	1	2	32	35	28.8	2.3	3	0.5	0.3	0
2	8/14/2000	22 11.65N 159 20.02W	27.9	27.3	*Padina crassa*	1	2	11.6	17.4	9.8	3.5	1.8	1.5	0	0.8

Table C-6. Summary of results from Waikiki, Oahu. Cell numbers are per 2 mL sample (mean over replicates). Stations: 1 – Breakwater, 2 - Sheraton Breakwater.

Station #	Date of Collection	GPS Coordinates of Station	Salinity (ppt)	Temp (*C)	Substrate (+ - found in green turtle diet)	Season: 0- winter 1 - ummer	n (reps)	MEAN - toxic Prorocentrum	MEAN- all Prorocentrum	*P. lima**	*P. mexicanum*	*P. concavum**	*P. emarginatum*	*P. hoffmanianum**	*Small Prorocentrum*
1	2/18/2000	21 16.66N 157 50.05W	32.6	25.7	*Acanthophora spicifera*+	0	2	43.4	45.3	34.8	1.3	6.8	0.3	1.8	0.3
1	2/18/2000	21 16.66N 157 50.05W	32.6	25.7	*Dictyota acuteloba*	0	2	26.8	27.9	17.3	0.8	5.5	0.3	4	0
1	2/18/2000	21 16.66N 157 50.05W	32.6	25.7	*Hypnea musciformes*+	0	2	6.3	11.6	4	2.8	2.3	2	0	0.5
1	2/18/2000	21 16.66N 157 50.05W	32.6	25.7	*Padina japonica*	0	2	48.1	52.7	36.8	4.3	8.8	0.3	2.5	0
1	2/18/2000	21 16.66N 157 50.05W	32.6	25.7	*Ulva rigida*+	0	2	1	2.8	1	1.8	0	0	0	0
1	8/9/2000	21 16.67N 157 50.02W	30.7	25.9	*Acanthophora spicifera*+	1	3	3.8	4.1	3.5	0.3	0	0	0.3	0
1	8/9/2000	21 16.67N 157 50.02W	30.7	25.9	*Cladophora hemisphaerica*	1	1	7.5	8	6.5	0.5	0.5	0	0.5	0
1	8/9/2000	21 16.67N 157 50.02W	30.7	25.9	*Padina japonica*	1	2	11.8	13.8	9.5	2	1.5	0	0.8	0
1	8/9/2000	21 16.67N 157 50.02W	30.7	25.9	*Sargassum echinocarpum*+	1	2	0.6	0.6	0.3	0	0	0	0.3	0
1	8/9/2000	21 16.67N 157 50.02W	30.7	25.9	*Ulva rigida*+	1	2	0.6	0.6	0.3	0	0	0	0.3	0
2	2/18/2000	21 16.61N 157 49.79W	37.5	26.5	*Acanthophora spicifera*+	0	2	32.3	33.1	28.5	0.5	3.3	0.3	0.5	0
2	2/18/2000	21 16.61N 157 49.79W	37.5	26.5	*Padina japonica*	0	2	116.8	118.4	113	1.3	3.8	0.3	0.5	0
2	2/18/2000	21 16.61N 157 49.79W	37.5	26.5	*Sargassum echinocarpum*+	0	2	31.3	31.9	24	0.3	4	0	3.3	0
2	2/18/2000	21 16.61N 157 49.79W	37.5	26.5	*Ulva rigida*+	0	2	1	1	1	0	0	0	0	0
2	8/9/2000	21 16.62N 157 49.78W	32.4	25.9	*Acanthophora spicifera*+	1	2	28.9	31.4	28.3	2.5	0.3	0	0.3	0
2	8/9/2000	21 16.62N 157 49.78W	32.4	25.9	*Centroceros clavulatum*	1	1	13	13	13	0	0	0	0	0
2	8/9/2000	21 16.62N 157 49.78W	32.4	25.9	*Enteromorpha intestinalis*	1	1	0	0.5	0	0.5	0	0	0	0
2	8/9/2000	21 16.62N 157 49.78W	32.4	25.9	*Gelidiopsis scoparia*	1	1	0	0	0	0	0	0	0	0
2	8/9/2000	21 16.62N 157 49.78W	32.4	25.9	*Sargassum echinocarpum*+	1	2	2.6	2.6	1.8	0	0.3	0	0.5	0
2	8/9/2000	21 16.62N 157 49.78W	32.4	25.9	*Ulva rigida*+	1	2	2.6	2.6	2	0	0.3	0	0.3	0

Table C-7. Summary of Kaneohe Bay, Oahu results. Cell numbers are per 2 mL sample (mean over replicates). Stations: 1 - Ahu'o-laka Sandbar, 2 - Patch Reef 13, 3 - Patch Reef 27, 4 - See GPS Coordinates, 5 - Reef #42, 6 - Near E. Shore, 7 - Near Tower #25.

Station #	Date of Collection	GPS Coordinates of Station	Salinity (ppt)	Temp (*C)	Substrate (+ - found in green turtle diet)	Season: 0- winter 1 - ummer	n (reps)	MEAN - toxic Prorocentrum	MEAN- all Prorocentrum	*P. lima**	*P. mexicanum*	*P. concavum**	*P. emarginatum*	*P. hoffmannianum**	*Small Prorocentrum*
1	2/15/2000	21 28.02N 157 48.88W	32.4	24.8	*Acanthophora spicifera*+	0	2	61.6	63.1	46	1	13.3	0.5	2.3	0
1	2/15/2000	21 28.02N 157 48.88W	32.4	24.8	*Gracilaria salicornia*+	0	2	29.1	31.6	21	1	5.8	1	2.3	0.5
1	2/15/2000	21 28.02N 157 48.88W	32.4	24.8	*Halimeda discoidea*+	0	1	4.8	5.1	2	0	0.8	0	2	0.3
1	2/15/2000	21 28.02N 157 48.88W	32.4	24.8	*Halophila hawaiiensis*+	0	2	3.1	3.6	2.8	0	0.3	0.5	0	0
1	2/15/2000	21 28.02N 157 48.88W	32.4	24.8	*Hypnea spp.*+	0	1	40.8	41.9	31.8	0.5	7.5	0.3	1.5	0.3
1	8/16/2000	21 27.96N 157 48.73W	36.1	28.1	*Acanthophora spicifera*+	1	2	21.6	24.9	13.8	2	4.8	1	3	0.3
1	8/16/2000	21 27.96N 157 48.73W	36.1	28.1	*Dictyota acuteloba*	1	2	330.1	340.6	322	9	4.3	0.3	4.3	0.9
1	8/16/2000	21 27.96N 157 48.73W	36.1	28.1	*Gracilaria salicornia*+	1	2	5.3	5.8	3	0.5	1.5	0	0.8	0
1	8/16/2000	21 27.96N 157 48.73W	36.1	28.1	*Halophila hawaiiensis*+	1	2	5.3	6.6	2	1.3	2.5	0	0.8	0
1	8/16/2000	21 27.96N 157 48.73W	36.1	28.1	*Padina japonica*	1	2	8.8	11.9	7.5	1.3	1	1.3	0.3	0.5
2	2/15/2000	21 27.29N 157 48.22W	32.8	25	*Acanthophora spicifera*+	0	2	22.3	23.8	13	0.5	6	1	3.3	0
2	2/15/2000	21 27.29N 157 48.22W	32.8	25	*Dictyosphaeria cavernosa*+	0	2	6.6	7.4	3	0	1.8	0.5	1.8	0.3
2	2/15/2000	21 27.29N 157 48.22W	32.8	25	*Dictyota acuteloba*	0	1	5.5	6	3.5	0.5	2	0	0	0
2	2/15/2000	21 27.29N 157 48.22W	32.8	25	*Enteromorpha intestinalis*	0	2	8.5	8.5	4.75	0	2	0	1.75	0
2	2/15/2000	21 27.29N 157 48.22W	32.8	25	*Eucheuma denticulatum*	0	2	1.5	1.5	1	0	0.5	0	0	0
3	2/15/2000	21 27.94N 157 49.17W	32.7	25.3	*Acanthophora spicifera*+	0	2	37.3	41.9	20.5	2.8	7.8	0.3	9	1.5
3	2/15/2000	21 27.94N 157 49.17W	32.7	25.3	*Dictyosphaeria cavernosa*+	0	2	12.3	18.7	3.8	3.3	5	1.3	3.5	1.8
3	2/15/2000	21 27.94N 157 49.17W	32.7	25.3	*Eucheuma denticulatum*	0	2	0.6	1.1	0.3	0.5	0	0	0.3	0
3	2/15/2000	21 27.94N 157 49.17W	32.7	25.3	*Gracilaria salicornia*+	0	2	12.8	13.6	6	0.5	1.5	0	5.3	0.3
3	2/15/2000	21 27.94N 157 49.17W	32.7	25.3	*Spyridia filamentosa*+	0	2	44.5	57.3	19	4	11.3	3.3	14.3	5.5
4	8/16/2000	21 27.59N 157 49.35W	32.9	27.7	*Acanthophora spicifera*+	1	2	21.6	26.6	9.8	4.5	4.5	0	7.3	0.5
4	8/16/2000	21 27.59N 157 49.35W	32.9	27.7	*Dictyosphaeria cavernosa*+	1	1	0	0	0	0	0	0	0	0
4	8/16/2000	21 27.59N 157 49.35W	32.9	27.7	*Gracilaria salicornia*+	1	2	4.9	5.4	1.8	0	1.3	0.5	1.8	0
4	8/16/2000	21 27.59N 157 49.35W	32.9	27.7	*Padina japonica*	1	2	31.6	34.4	14.3	1.8	2.5	0	14.8	1
4	8/16/2000	21 27.59N 157 49.35W	32.9	27.7	*Spyridia filamentosa*+	1	2	7.5	7.5	3	0	1.5	0	3	0
5	8/16/2000	21 28.62N 157 49.55W	35.9	27.5	*Dictyosphaeria cavernosa*+	1	2	2.6	2.9	1.3	0.3	0.8	0	0.5	0
5	8/16/2000	21 28.62N 157 49.55W	35.9	27.5	*Spyridia filamentosa*+	1	2	14.6	17.9	9.5	0.5	3.3	0.3	1.8	2.5
5	8/16/2000	21 28.62N 157 49.55W	35.9	27.5	*Turbinaria ornata*	1	1	9.5	12.5	9	1	0.5	1	0	1
6	8/16/2000	UK	36.4	27.7	*Gracilaria salicornia*+	1	2	1.3	3.1	1	1.5	0	0.3	0.3	0

Table C-7 (continued).

Station #	Date of Collection	GPS Coordinates of Station	Salinity (ppt)	Temp (*C)	Substrate (+ - found in green turtle diet)	Season: 0- winter 1 - ummer	n (reps)	MEAN - toxic Prorocentrum	MEAN- all Prorocentrum	*P. lima**	*P. mexicanum*	*P. concavum**	*P. emarginatum*	*P. hoffmannianum**	*Small Prorocentrum*
7	8/16/2000	21 27.93N 157 50.00W	37	27.5	*Acanthophora spicifera*+	1	2	23.9	28.5	12.3	4	2.3	0.3	9.3	0.3
7	8/16/2000	21 27.93N 157 50.00W	37	27.5	*Dictyosphaeria cavernosa*+	1	1	3.5	4	1	0.5	2	0	0.5	0
7	8/16/2000	21 27.93N 157 50.00W	37	27.5	*Eucheuma denticulatum*	1	2	0.3	2.8	0	2.5	0.3	0	0	0
7	8/16/2000	21 27.93N 157 50.00W	37	27.5	*Gracilaria salicornia*+	1	2	2.1	5.9	0.8	3.3	0.5	0.5	0.8	0
7	8/16/2000	21 27.93N 157 50.00W	37	27.5	*Halophila hawaiiensis*+	1	1	1.5	3.5	0.5	2	1	0	0	0
7	8/16/2000	21 27.93N 157 50.00W	37	27.5	*Liagora maxima*	1	2	3.5	5.8	1	2.3	0.5	0	2	0

Table C-8. Summary of results of Honokowai, Maui. Cell numbers are per 2 mL sample (mean over replicates). Stations: 1 - Near Breakwater (<3m), 2 - Graveyard (6m), 3 - Shredder's Ridge (10m), 4 - Outback (18m), 5 - Broken Reefs (11m), 6 - Tip of the finger (10m), 7 - Coral Garden (4m).

Station #	Date of Collection	GPS Coordinates of Station	Salinity (ppt)	Temp (*C)	Substrate (+ - found in green turtle diet)	Season: 0- winter 1 - ummer	n (reps)	MEAN - toxic Prorocentrum	MEAN- all Prorocentrum	*P. lima**	*P. mexicanum*	*P. concavum**	*P. emarginatum*	*P. hoffmannianum**	*Small Prorocentrum*
1	2/22/2000	20 57.38N 156 41.18W	30.9	24.6	*Acanthophora spicifera*+	0	2	3.6	4.1	1.3	0	2	0.5	0.3	0
1	2/22/2000	20 57.38N 156 41.18W	30.9	24.6	*Codium reediae*+	0	1	1	1.5	1	0	0	0	0	0.5
1	2/22/2000	20 57.38N 156 41.18W	30.9	24.6	*Halimeda discoidea*+	0	2	1.8	1.8	1	0	0.8	0	0	0
1	2/22/2000	20 57.38N 156 41.18W	30.9	24.6	*Hypnea musciformes*+	0	2	0.3	0.3	0.3	0	0	0	0	0
1	2/22/2000	20 57.38N 156 41.18W	30.9	24.6	*Melanamansia glomerata*+	0	2	2.5	2.8	2	0	0.5	0.3	0	0
1	2/22/2000	20 57.38N 156 41.18W	30.9	24.6	*Ptericladiella capillacea*+	0	2	0.6	0.6	0.3	0	0.3	0	0	0
1	2/22/2000	20 57.38N 156 41.18W	30.9	24.6	*Ulva rigida*+	0	2	0	0	0	0	0	0	0	0
1	8/17/2000	20 57.38N 156 41.18W	37.5	27	*Acanthophora spicifera*+	1	2	2.8	3.3	2	0.5	0.8	0	0	0
1	8/17/2000	20 57.38N 156 41.18W	37.5	27	*Ahnfeltiopsis concinna*	1	2	1.3	1.3	1.3	0	0	0	0	0
1	8/17/2000	20 57.38N 156 41.18W	37.5	27	*Halimeda discoidea*+	1	2	1.8	1.8	1.5	0	0.3	0	0	0
1	8/17/2000	20 57.38N 156 41.18W	37.5	27	*Pterocladiella capillacea*+	1	2	1.8	1.8	1.8	0	0	0	0	0
1	8/17/2000	20 57.38N 156 41.18W	37.5	27	*Ulva rigida*+	1	2	0.75	0.75	0.75	0	0	0	0	0
2	8/17/2000	20 57.38N 156 41.18W	35.3	26.67	*Cladophora hemisphaerica*+	1	2	19.85	29.45	10.3	4.8	3.8	4	5.8	0.8
3	8/17/2000	20 57.38N 156 41.18W	35.4	26.67	*Halimeda + Cladophora*+	1	2	4.3	6.6	1.3	1.5	1.5	0	1.5	0.8
4	8/17/2000	20 57.38N 156 41.18W	34.3	25	*Halimeda discoidea*+	1	2	7.5	11.6	1.5	2.8	3	0.5	3	0.8

Table C-8 (continued).

Station #	Date of Collection	GPS Coordinates of Station	Salinity (ppt)	Temp (*C)	Substrate (+ - found in green turtle diet)	Season: 0- winter 1 - ummer	n (reps)	MEAN - toxic Prorocentrum	MEAN- all Prorocentrum	*P. lima**	*P. mexicanum*	*P. concavum**	*P. emarginatum*	*P. hoffmanianum**	*Small Prorocentrum*
5	8/17/2000	20 57.38N 156 41.18W	33.9	25.56	*Melanamansia glomerata*+	0	2	10.3	14.9	1.8	2.3	6	1	2.5	1.3
6	8/17/2000	20 57.38N 156 41.18W	33.9	25.56	*Acanthophora spicifera*+	1	2	5.1	9.4	0.8	4	3	0.3	1.3	0
6	8/17/2000	20 57.38N 156 41.18W	32.9	25.56	*Spyridia filamentosa*+	1	2	16.3	25.6	10.5	7.8	3	0	2.8	1.5
7	8/17/2000	20 57.38N 156 41.18W	33.5	26.11	*Acanthophora spicifera*+	1	2	3.3	6.1	0.5	2.8	1.5	0	1.3	0
7	8/17/2000	20 57.38N 156 41.18W	32.9	26.11	*Spyridia filamentosa*+	1	2	17.8	25.9	5.8	6.8	9	0.8	3	0.5

Table C-9. Summary of results from Palaau, Molokai. Cell numbers are per 2 mL sample (mean over replicates). There was one station.

Station #	Date of Collection	GPS Coordinates of Station	Salinity (ppt)	Temp (*C)	Substrate (+ - found in green turtle diet)	Season: 0- winter 1 - ummer	n (reps)	MEAN - * toxic Prorocentrum	MEAN- all Prorocentrum	*P. lima**	*P. mexicanum*	*P. concavum**	*P. emarginatum*	*P. hoffmanianum**	*Small Prorocentrum*
1	2/19/2000	21 05.59N 157 08.79W	34.3	28.2	*Acanthophora spicifera*+	0	2	5	5.5	3.5	0.5	0.5	0	1	0
1	2/19/2000	21 05.59N 157 08.79W	34.3	28.2	*Dictyota acuteloba*	0	2	19.5	29	19.5	9.5	0	0	0	0
1	2/19/2000	21 05.59N 157 08.79W	34.3	28.2	*Enteromorpha intestinalis*	0	2	2.5	3.5	2	1	0	0	0.5	0
1	2/19/2000	21 05.59N 157 08.79W	34.3	28.2	*Halophila hawaiiensis*+	0	2	1	1	1	0	0	0	0	0
1	2/19/2000	21 05.59N 157 08.79W	34.3	28.2	*Padina crassa*	0	2	22.5	28	21.5	3.5	1	0	0	2
1	2/19/2000	21 05.59N 157 08.79W	34.3	28.2	*Rhizoclonium riparium*+	0	1	11	13	8	2	2	0	1	0
1	2/19/2000	21 05.59N 157 08.79W	34.3	28.2	*Spyridia filamentosa*+	0	2	15.5	20	14	4	1	0	0.5	0.5
1	8/15/2000	21 05.59N 157 08.79W	37.1	30.1	*Acanthophora spicifera*+	1	2	5	5.5	1	0.5	1.5	0	2.5	0
1	8/15/2000	21 05.59N 157 08.79W	37.1	30.1	*Caulerpa racemosa*	1	2	0.5	0.5	0.5	0	0	0	0	0
1	8/15/2000	21 05.59N 157 08.79W	37.1	30.1	*Dictyota acuteloba*	1	2	1.5	1.5	1.5	0	0	0	0	0
1	8/15/2000	21 05.59N 157 08.79W	37.1	30.1	*Gracilaria bursapastoris*	1	2	0	0	0	0	0	0	0	0
1	8/15/2000	21 05.59N 157 08.79W	37.1	30.1	*Hypnea musciformes*+	1	2	0	0	0	0	0	0	0	0
1	8/15/2000	21 05.59N 157 08.79W	37.1	30.1	*Padina crassa*	1	2	2	2	2	0	0	0	0	0

APPENDIX D
PROROCENTRUM COMPARISON DATA

Table D-1. *Prorocentrum* counts on *Acanthophora spicifera* substrate in the Hawaiian Islands (Site #: 1 –Kona/Kohala, 2 – Hilo Bay, 3 – SE Kauai, 4 – NE Kauai, 5 – Waikiki, 6 – Kaneohe Bay, 7 – Honokowai, Maui, 8 – Palaau, Molokai).

FP Rank: 1 – low, 2 – med 3 - high	Site #	Date	Salinity (ppt)	Temp (*C)	Toxic *Prorocentrum* per g/wet substrate
1	1	8/10/2000	31.2	29.5	0
1	1	8/10/2000	31.2	29.5	0
2	2	8/12/2000	29.1	24.7	3
2	2	8/12/2000	29.1	24.7	3
2	3	2/21/2000	32.7	24.1	13
2	3	2/21/2000	32.7	24.1	3
2	3	8/13/2000	32.5	25.7	7
2	3	8/13/2000	32.5	25.7	9
2	3	8/13/2000	36.1	25.2	12
2	3	8/13/2000	36.1	25.2	20
2	4	2/21/2000	37.9	24.6	6
2	4	8/14/2000	27.9	27.3	25
2	4	8/14/2000	27.9	27.3	43
2	5	2/18/2000	32.6	25.7	49
2	5	2/18/2000	32.6	25.7	94
2	5	2/18/2000	37.5	26.5	60
2	5	2/18/2000	37.5	26.5	48
2	5	8/9/2000	32.4	25.9	37
2	5	8/9/2000	32.4	25.9	58
2	5	8/9/2000	30.7	25.9	9
2	5	8/9/2000	30.7	25.9	8
2	5	8/9/2000	30.7	25.9	1
3	6	2/15/2000	32.4	24.8	100
3	6	2/15/2000	32.4	24.8	106
3	6	2/15/2000	32.8	25	31
3	6	2/15/2000	32.8	25	44
3	6	2/15/2000	32.7	25.3	73
3	6	2/15/2000	32.7	25.3	53
3	6	8/16/2000	36.1	28.1	38
3	6	8/16/2000	36.1	28.1	33

Table D-1 (continued).

FP Rank	Site #	Date	Salinity (ppt)	Temp (*C)	Toxic *Prorocentrum* per g/wet substrate
3	6	8/16/2000	37	27.5	52
3	6	8/16/2000	37	27.5	20
3	6	8/16/2000	32.9	27.7	18
3	6	8/16/2000	32.9	27.7	61
3	7	2/22/2000	30.9	24.6	7
3	7	2/22/2000	30.9	24.6	6
3	7	8/17/2000	37.5	27	8
3	7	8/17/2000	37.5	27	2
3	7	8/17/2000	33.9	25.6	5
3	7	8/17/2000	33.9	25.6	13
3	7	8/17/2000	33.5	26.1	5
3	7	8/17/2000	33.5	26.1	6
3	8	2/19/2000	34.3	28.2	8
3	8	2/19/2000	34.3	28.2	8
3	8	8/15/2000	37.1	30.1	10
3	8	8/15/2000	37.1	30.1	7

Table D-2. *Prorocentrum* counts on *Halodule beaudettei* from Florida (Sites specified by FP: 1 – St. Joseph Bay, 2 – Cedar Key, 3 – Mosquito Lagoon, 4 – Florida Bay).

FP Rank	Site #	Date	Salinity (ppt)	Temp (*C)	Toxic *Prorocentrum* per g/wet substrate
1	1	5/1/2000	30.4	28.3	247
1	1	5/1/2000	30.4	28.3	113
1	1	5/1/2000	35.5	29.5	38
1	1	5/1/2000	35.5	29.5	84
1	1	8/4/2000	30.2	34.8	4
1	1	8/4/2000	30.2	34.8	4
1	1	8/4/2000	30.8	34.3	1
1	1	8/4/2000	30.8	34.3	41
1	1	11/10/2000	30	21	14
1	1	11/10/2000	30	21	21
1	1	11/10/2000	30.0	20.9	10
1	1	11/10/2000	30.0	20.9	5
1	1	11/10/2000	35.9	20.7	144
1	1	11/10/2000	35.9	20.7	122
1	1	3/1/2001	20.5	27.5	16
1	1	3/1/2001	20.5	27.5	8
1	1	3/1/2001	24.2	25.1	5

Table D-2 (continued)

FP Rank	Site #	Date	Salinity (ppt)	Temp (*C)	Toxic *Prorocentrum* per g/wet substrate
1	1	3/1/2001	24.2	25.1	50
2	2	5/4/2000	28.7	24.3	253
2	2	5/4/2000	28.7	24.3	273
2	2	5/4/2000	28.7	23.9	95
2	2	5/4/2000	28.7	23.9	47
2	2	8/2/2000	29.7	30.2	123
2	2	8/2/2000	29.7	30.2	70
2	2	8/2/2000	29.9	30.2	297
2	2	8/2/2000	29.9	30.2	334
2	2	11/15/2000	31.7	21.1	17
2	2	11/15/2000	31.7	21.1	28
2	2	11/15/2000	28.9	23.7	18
2	2	11/15/2000	28.9	23.7	43
2	2	2/8/2001	19.3	21.5	12
2	2	2/8/2001	20.3	21	5
3	3	5/9/2000	32.6	25.7	13
3	3	5/9/2000	32.6	25.7	14
3	3	5/9/2000	32.4	26	35
3	3	5/9/2000	32.4	26	56
3	3	8/1/2000	25.8	34.1	693
3	3	8/1/2000	25.8	34.1	630
3	3	8/1/2000	28.8	31	97
3	3	8/1/2000	28.8	31	52
3	3	11/13/2000	40.2	22.8	4
3	3	11/13/2000	40.2	22.8	3
3	3	11/13/2000	40.9	22.8	6
3	3	11/13/2000	40.9	22.8	0
3	3	2/16/2001	25	9.6	3
3	3	2/16/2001	25	9.6	3
3	3	2/16/2001	28.2	21.6	0
3	4	2/16/2001	28.2	21.6	3
3	4	6/3/2000	32.1	30.4	68
3	4	6/3/2000	32.1	30.4	43
3	4	6/3/2000	31.2	31.2	206
3	4	6/3/2000	31.2	31.2	197
3	4	8/26/2000	35.6	29.8	78
3	4	8/26/2000	35.6	29.8	162
3	4	8/26/2000	35.2	29.4	34
3	4	8/26/2000	35.2	29.4	42
3	4	11/4/2000	33.5	23.3	36
3	4	11/4/2000	33.5	23.3	37
3	4	11/4/2000	35.6	24.5	128

Table D-2 (continued)

FP Rank	Site #	Date	Salinity (ppt)	Temp (*C)	Toxic *Prorocentrum* per g/wet substrate
3	4	11/4/2000	35.6	24.5	248
3	4	2/3/2001	34.9	23.7	61
3	4	2/3/2001	34.9	23.7	40
3	4	2/3/2001	35.9	22.7	24
3	4	2/3/2001	35.9	22.7	156
3	4	2/3/2001	35.9	22.9	43
3	4	2/3/2001	35.9	22.9	71

APPENDIX E
DRY WEIGHTS AND ASH-FREE DRY WEIGHTS OF SUBSTRATES

Table E-1. Mean dry weights (g) and ash-free dry weights (g) per g wet weight macroalgae and seagrass substrates in Hawaii.

Substrate spp.	n =	Mean g dry wt per g wet wt	Mean g ash-free dry wt per g wet wt
Acanthophora spicifera+	5	7.76	16.99
Ahnfeltiopsis concinna+	2	6.27	17.36
Caulerpa racemosa+	1	4.16	6.15
Centroceros clavulatum	2	4.86	9.7
Cladophora hemisphaerica+	2	3.7	6.43
Colpomenia sinuosa	1	4.09	11.46
Dictyosphaeria versluysii+	1	15.68	22.75
Dictyosphaeria cavernosa+	2	4.37	10.12
Dictyota acuteloba	2	6.02	14.8
Enteromorpha paradox	3	3.78	5.13
Eucheuma denticulatum	1	7.58	19.47
Gelidium-like wiry turf+	2	3.99	6.22
Gracilaria salicornia	1	9.21	18.2
Halimeda discoidea+	1	2.57	7.77
Halophila hawaiiensis+	1	5.46	9.78
Hypnea chordacea+	1	9.55	17.85
Hypnea musciformes+	1	4.45	13.97
Jania sp.	1	5.62	11.29
Laurencia succisa+	1	7.27	12.82
Liagora maxima	1	3.54	5.74
Lyngbya sp.	1	5.13	16.39
Melanamansia glomerata+	3	6.12	14.74
Neomeris annulata	1	5.47	7.85
Padina crassa	2	4.59	7.07
Plocamium sandvicense	1	5.93	14.72
Polysiphonia hawaiiensis	1	3.7	4.26
Pterocladiella capillacea+	1	4.74	12.36
Rhizoclonium riparium+	2	7.23	10.5
Sargassum echinocarpum+	1	7.09	19.68
Spyridia filamentosa+	2	4.5	8.35
UK thread-like branching brown	1	4.09	7.5
Ulva rigida+	2	5.51	14.05

+ - found in green turtle diet

Table E-2. Mean dry weights (g) and ash-free dry weights (g) per gram wet weight macroalgae and seagrass substrates in Florida.

Substrate spp.	n =	Mean g dry wt per g wet wt	Mean g ash-free dry wt per g wet wt
Acanthophora spicifera+	5	7.76	16.99
Dictyota cervicornis+	1	8.38	18.33
Enteromorpha spp.	3	3.78	5.13
Gracilaria spp.+	1	8.41	22.11
Halimeda incrassata	1	2.94	3.62
Halodule beaudettei+	4	6.52	22.02
Hypnea cervicornis+	2	9.75	23.45
Hypnea musciformes+	2	4.45	13.97
Hypnea spinella	2	8.46	15.37
Lyngbya sp.	1	5.13	16.39
Penicillus dumetosus	2	3.74	6.07
Sargassum pteropleuron	4	7.56	18.42
Spyridia filamentosa+	1	9.03	21.09
Syringodium filiforme+	2	8.76	24.45
Thalassia testudinum+	4	7.21	21.69
Ulva spp.+	2	5.51	14.05
UK - fa-CK8	1	12.39	22.99

+ - found in green turtle diet

APPENDIX F
PROPORTION OF TOXIC *PROROCENTRUM* PER GRAM WET WEIGHT, DRY WEIGHT AND ASH-FREE DRY WEIGHT SUBSTRATE

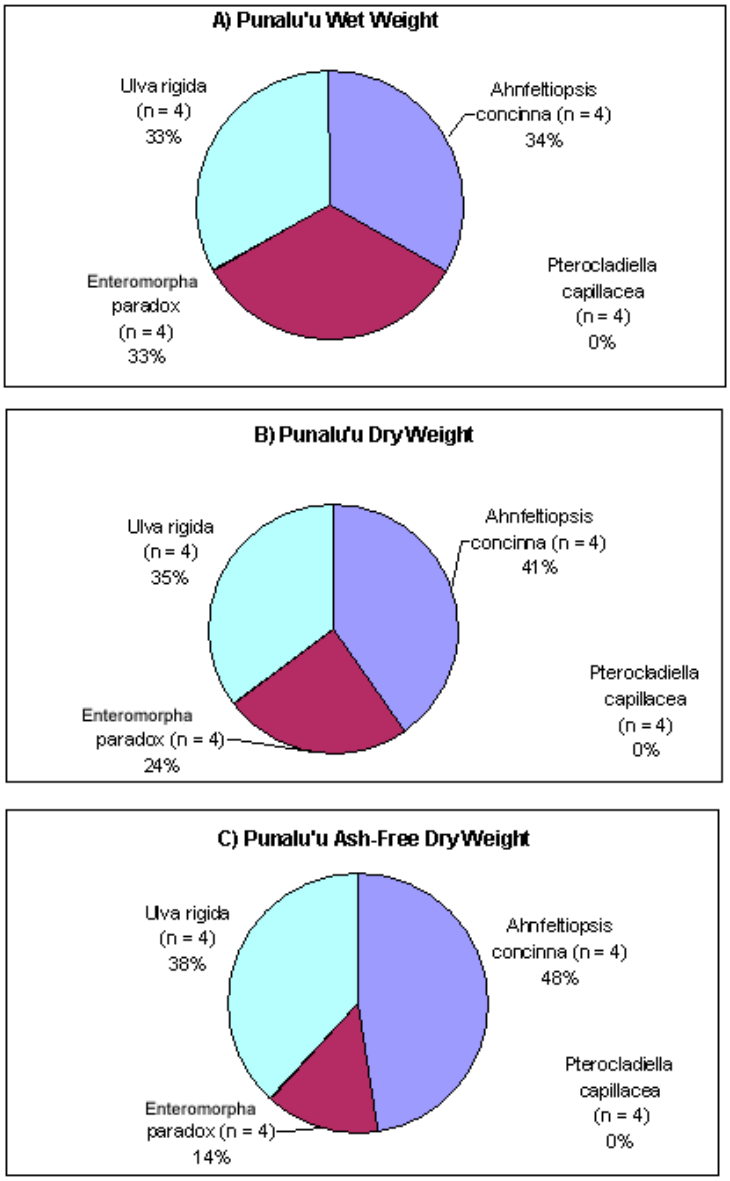


Figure F-1. Proportion of Toxic *Prorocentrum* spp. found on substrates at Punalu'u, Big Island: A) per g/wet wt substrate, B) per g/dry wt substrate, C) per g/ash-free dry wt.

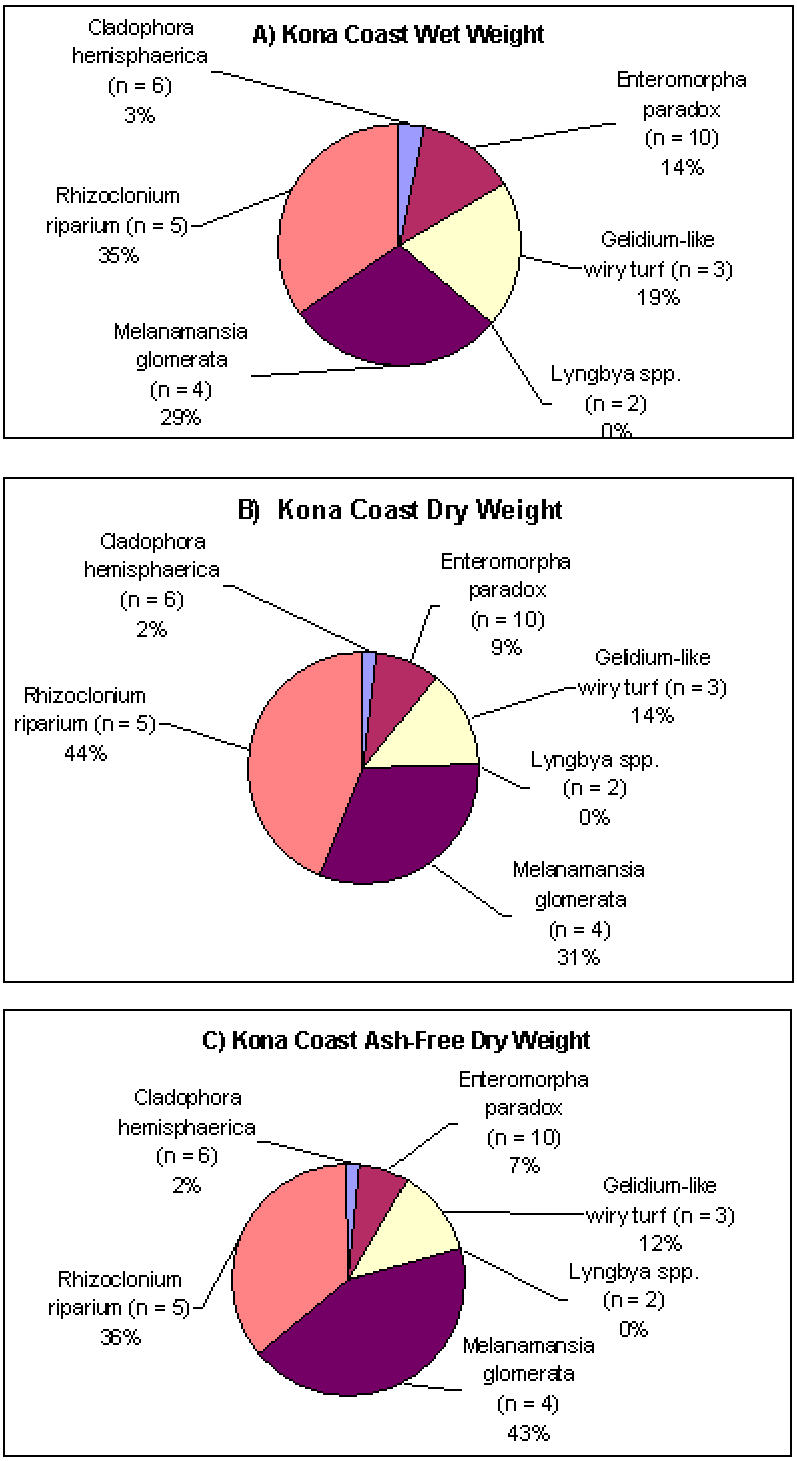
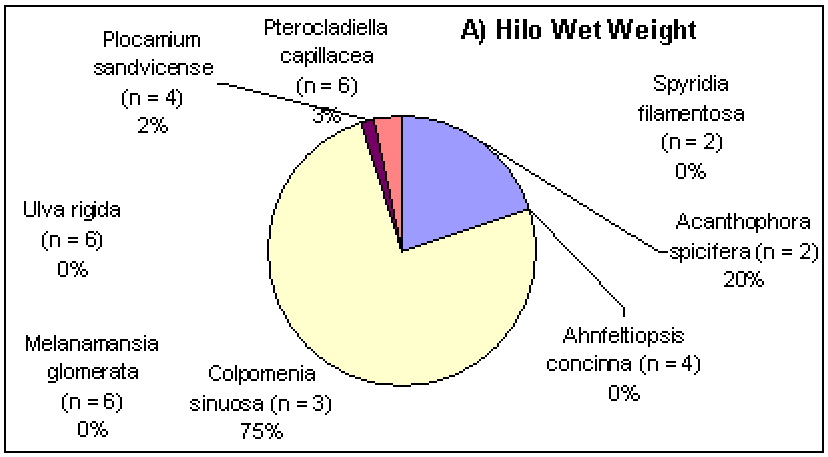
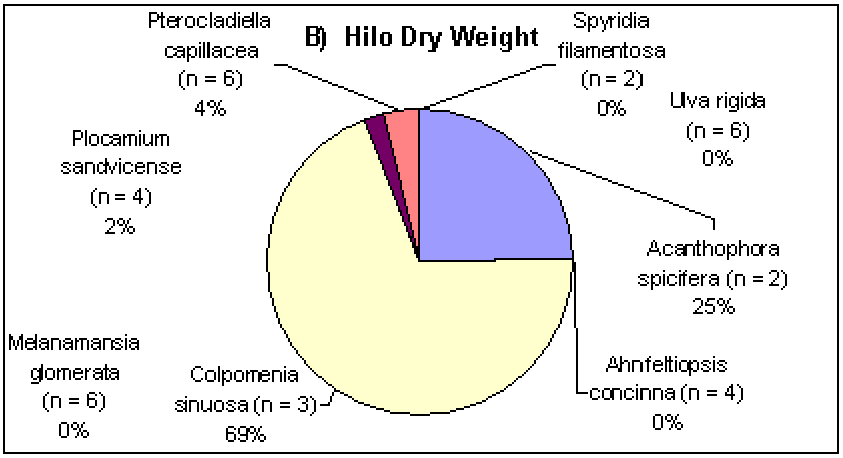


Figure F-2. Proportion of Toxic *Prorocentrum* spp. found on substrates from Kona/Kohala Coast, Big Island: A) per g/wet wt substrate, B) per g/dry wt substrate, C) per g/ash-free dry wt.





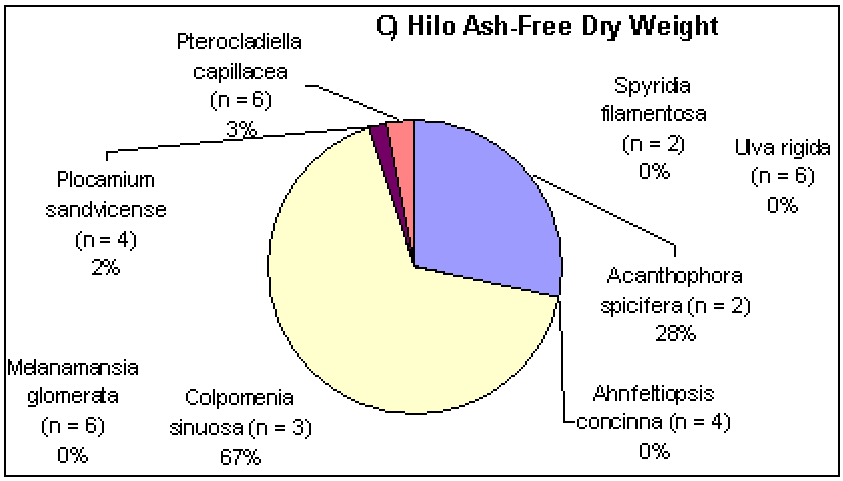


Figure F-3. Proportion of Toxic *Prorocentrum* spp. found on substrates from Hilo Bay, Big Island: A) per g/wet wt substrate, B) per g/dry wt substrate, C) per g/ash-free dry wt.

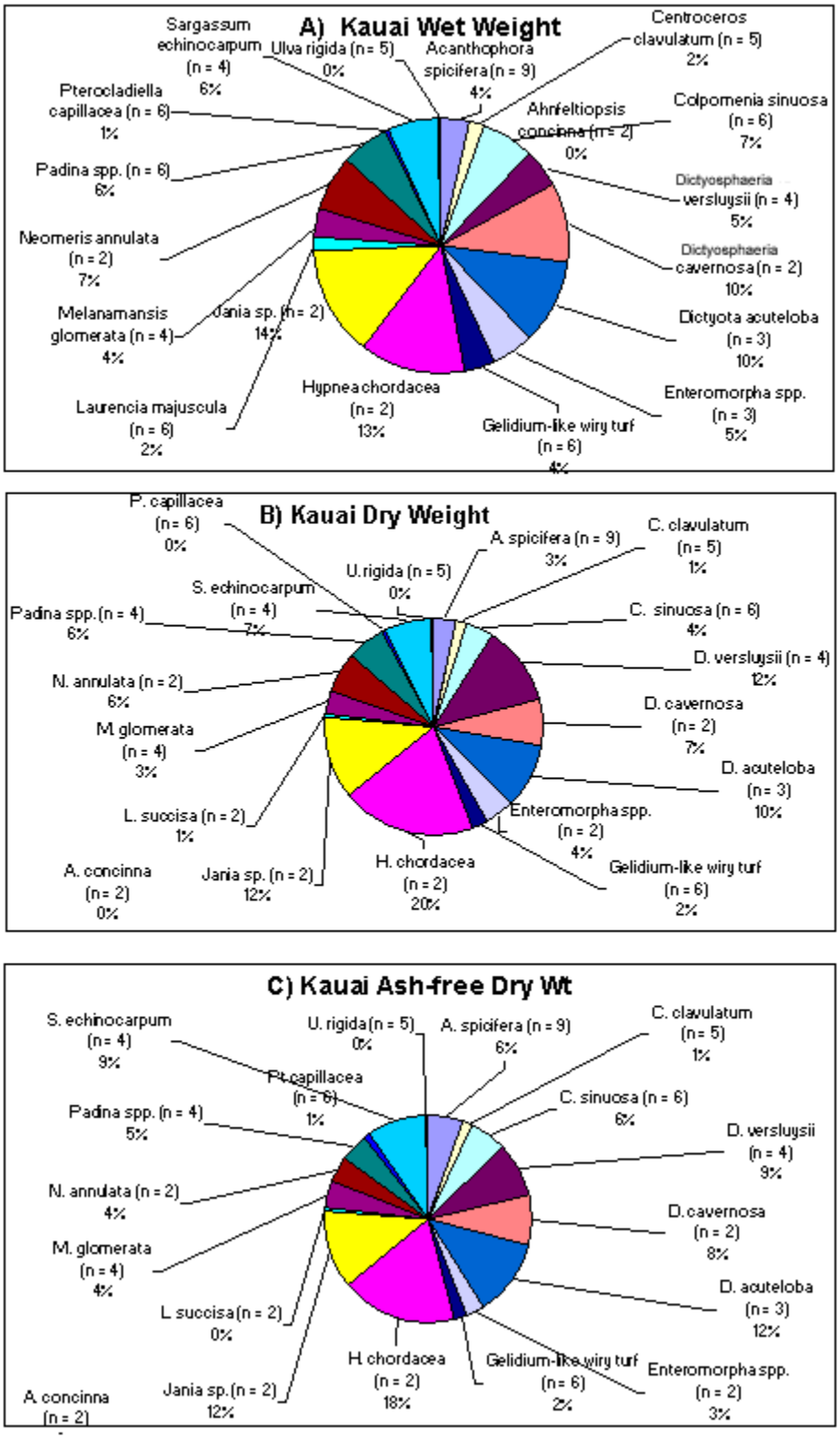


Figure F-4. Proportion of Toxic *Prorocentrum* spp. found on substrates from Kauai: A) per g/wet wt substrate, B) per g/dry wt substrate, C) per g/ash-free dry wt.

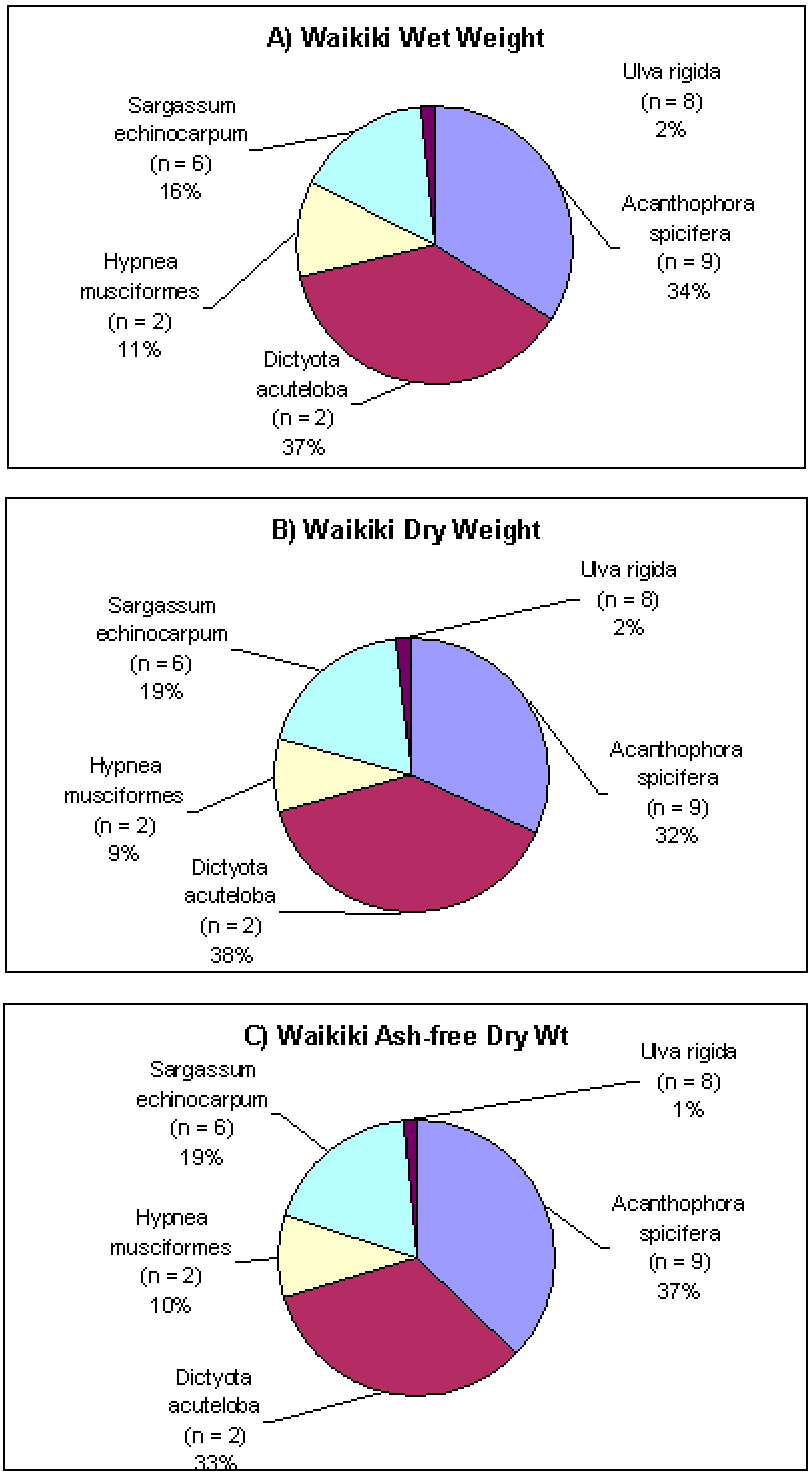


Figure F-5. Proportion of Toxic *Prorocentrum* spp. found on substrates from Waikiki, Oahu: A) per g/wet wt substrate, B) per g/dry wt substrate, C) per g/ash-free dry wt.

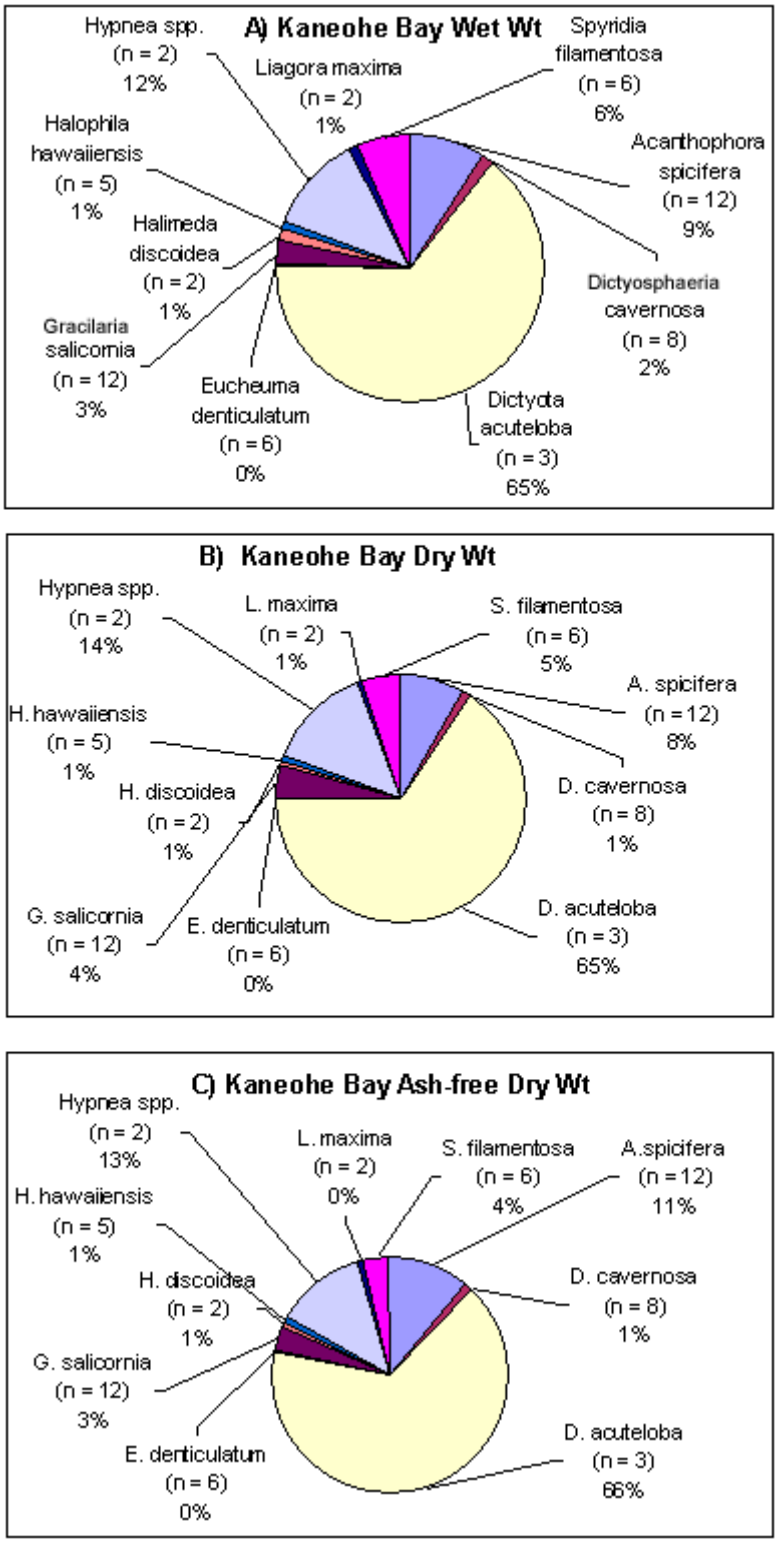


Figure F-6. Proportion of Toxic *Prorocentrum* spp. found on substrates from Kaneohe Bay, Oahu: A) per g/wet wt substrate, B) per g/dry wt substrate, C) per g/ash-free dry wt.

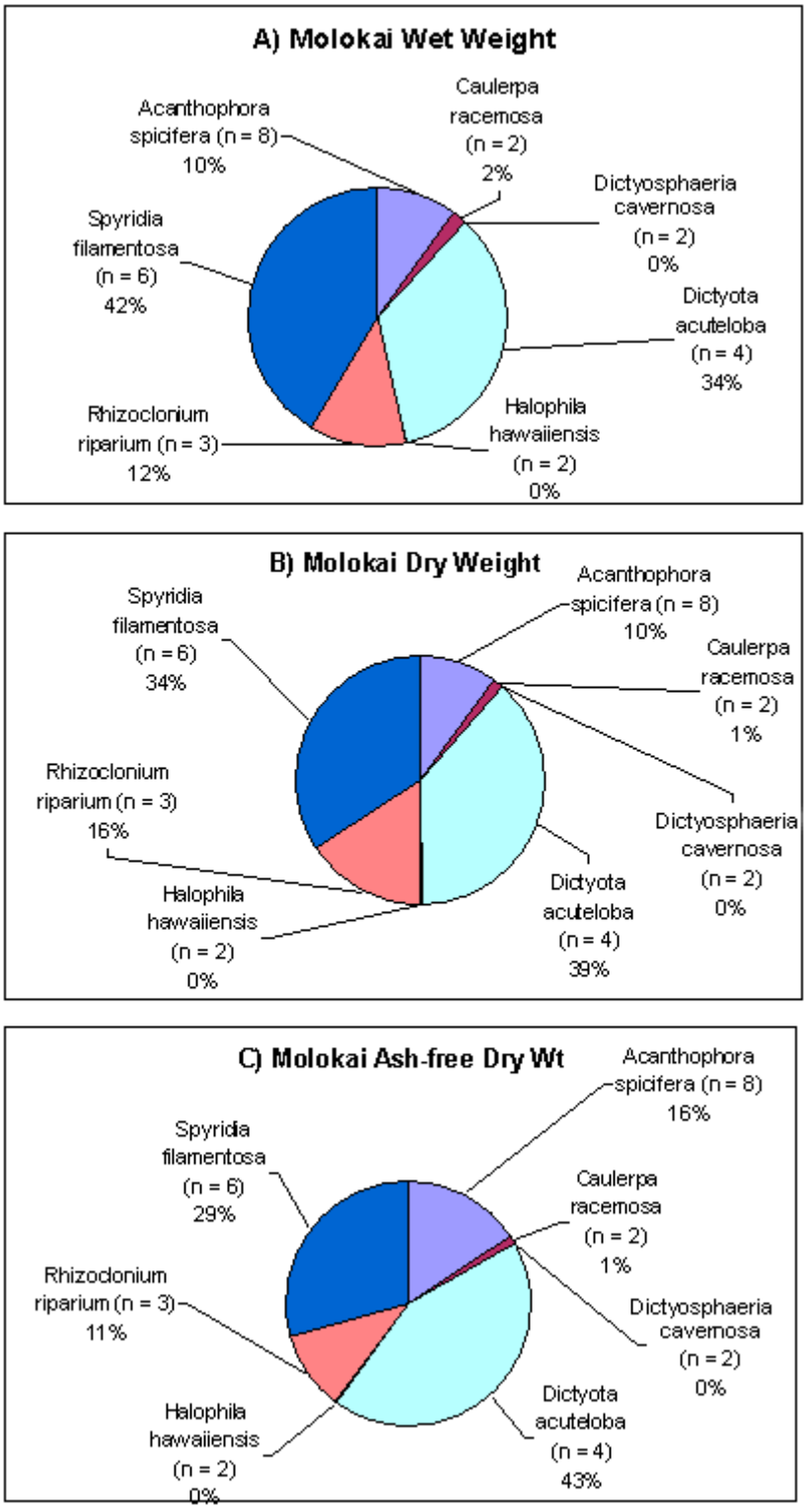
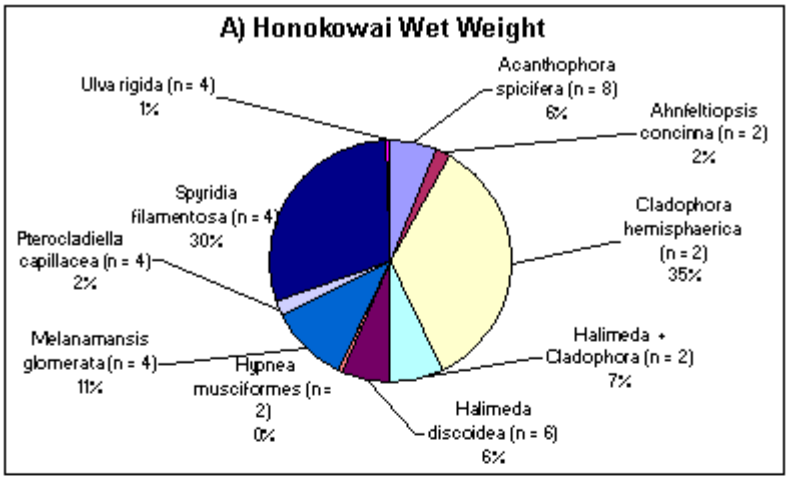
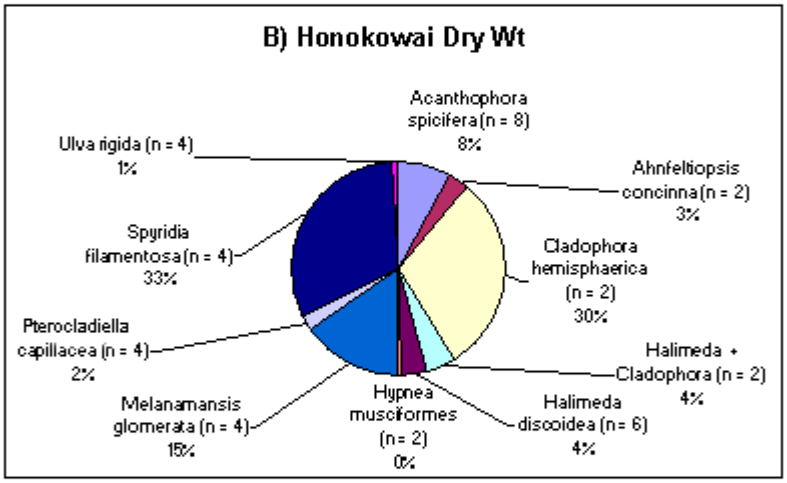


Figure F-7. Proportion of Toxic *Prorocentrum* spp. found on substrates from Palaau, Molokai: A) per g/wet wt substrate, B) per g/dry wt substrate, C) per g/ash-free dry wt.





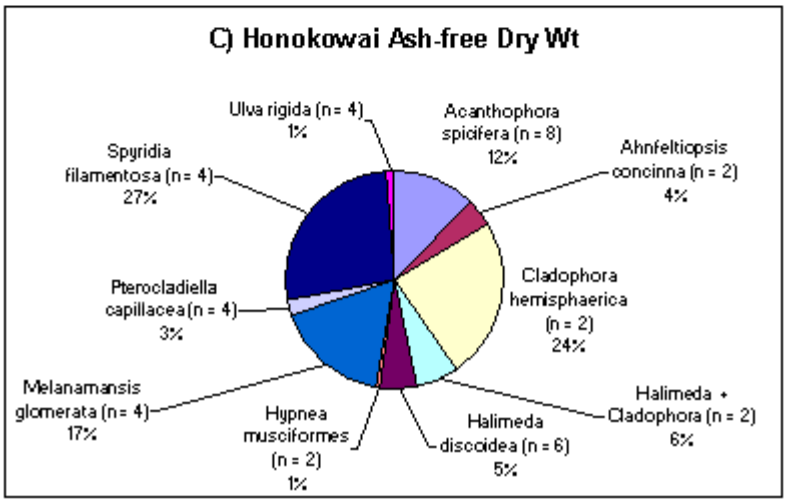


Figure F-8. Proportion of Toxic *Prorocentrum* spp. found on substrates from Honokowai, Maui: A) per g/wet wt substrate, B) per g/dry wt substrate, C) per g/ash-free dry wt.

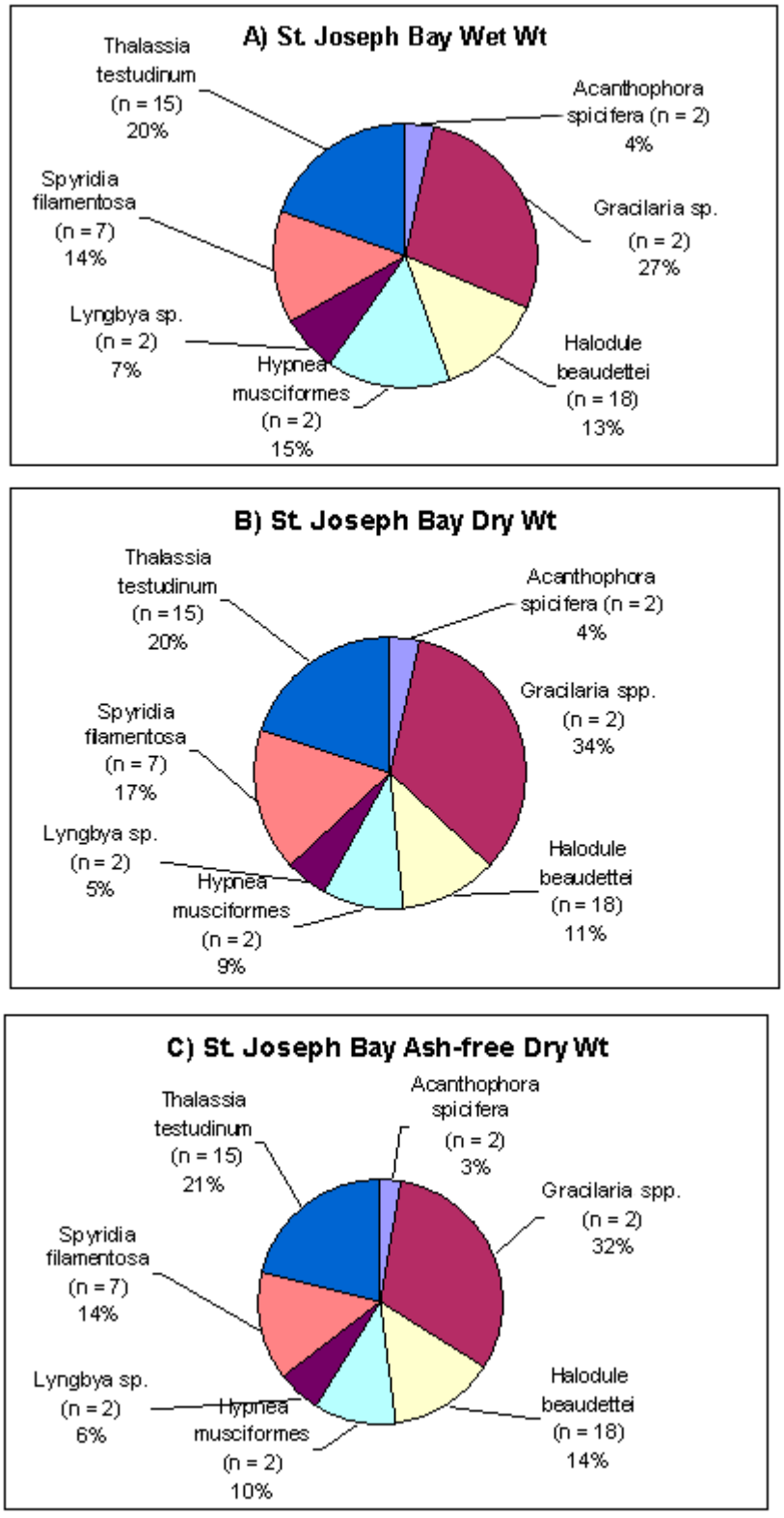


Figure F-9. Proportion of Toxic *Prorocentrum* spp. found on substrates from St. Joseph Bay, Florida: A) per g/wet wt substrate, B) per g/dry wt substrate, C) per g/ash-free dry wt.

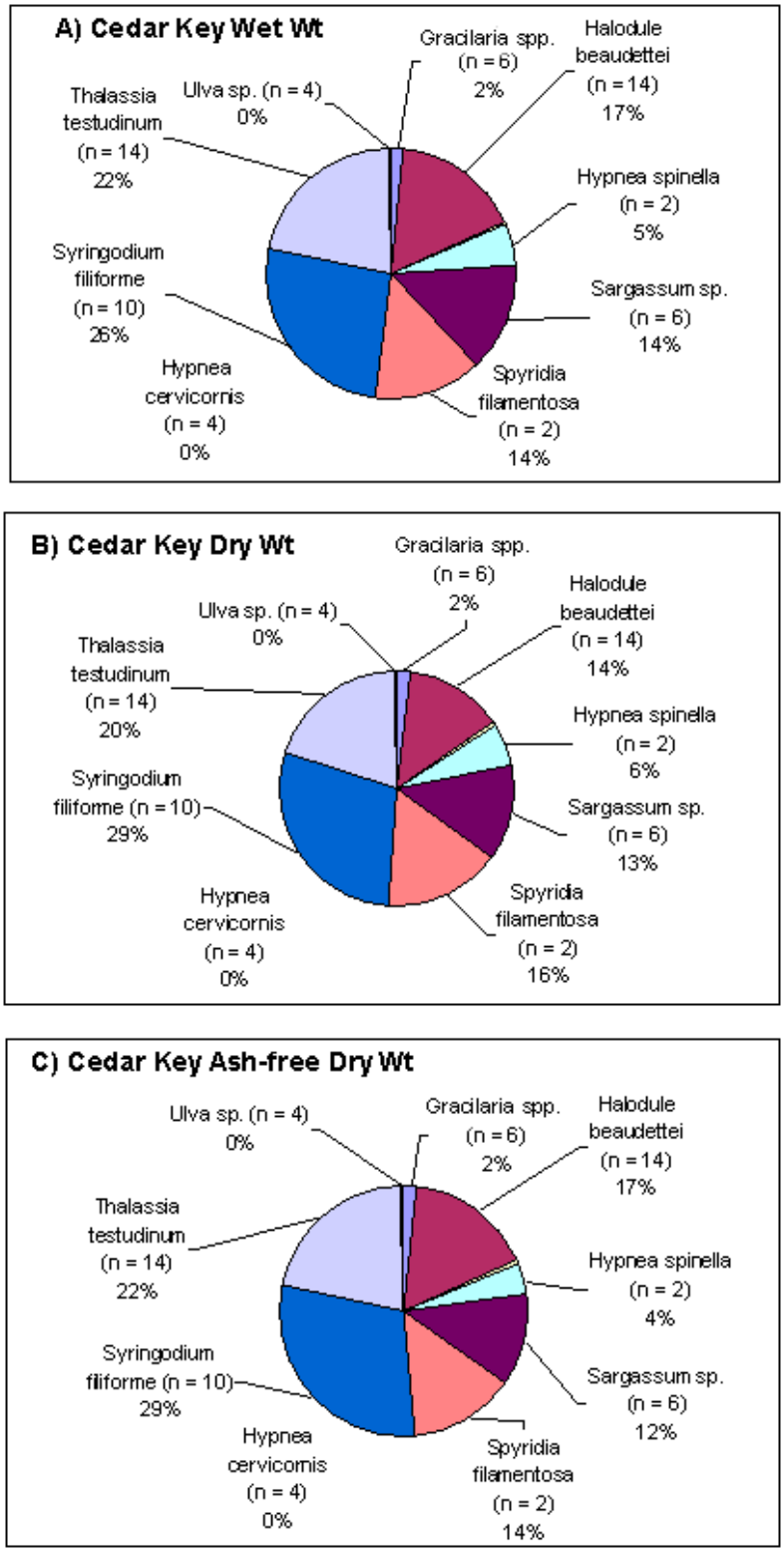


Figure F-10. Proportion of Toxic *Prorocentrum* spp. found on substrates from Cedar Key, Florida: A) per g/wet wt substrate, B) per g/dry wt substrate, C) per g/ash-free dry wt.

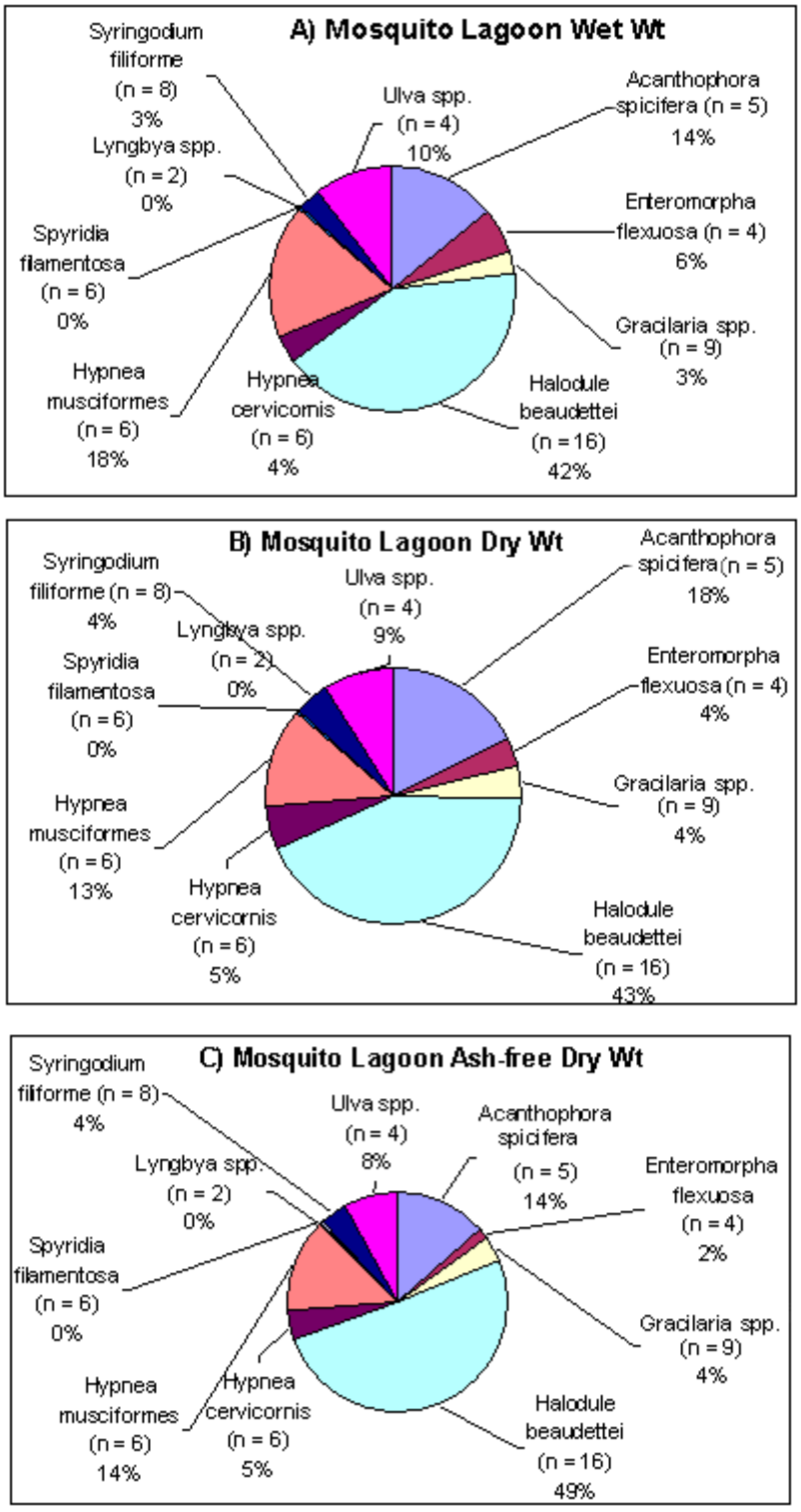


Figure F-11. Proportion of Toxic *Prorocentrum* spp. found on substrates from Mosquito Lagoon, Florida: A) per g/wet wt substrate, B) per g/dry wt substrate, C) per g/ash-free dry wt.

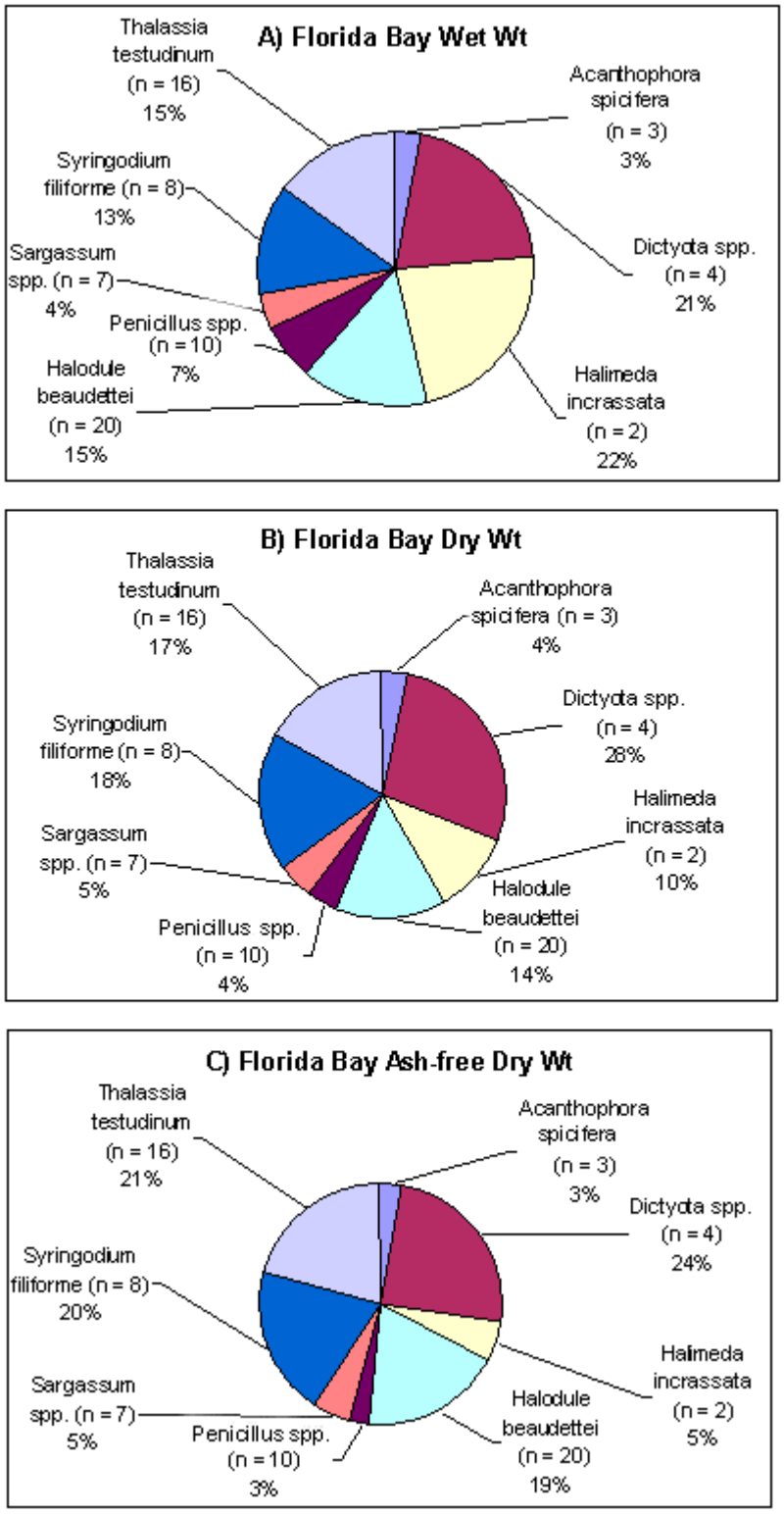


Figure F-12. Proportion of Toxic *Prorocentrum* spp. found on substrates from Florida Bay, Florida: A) per g/wet wt substrate, B) per g/dry wt substrate, C) per g/ash-free dry wt.

APPENDIX G
DATA USED TO DETERMINE CONTRIBUTING ZONES

Table G-1. Data used to determine Florida contributing zones.

Location	Basin/stream	Permh	Awcl	Watertable	Slope	Contrib.Zone
St. Joe	Basin	14.74	0.07	2.4	0.30	
St. Joe	River	20.2	0.04	0.2	0.99	980.31
St. Joe	Stream1	11.0	0.07	2.4	0.05	83.51
St. Joe	Stream2	13.7	0.06	2.4	0.08	104.55
St. Joe	Stream3	13.91	0.06	2.3	0.13	125.94
St. Joe	Stream4	15.17	0.06	2.3	0.32	168.65
St. Joe	Stream5	16.90	0.07	1.9	0.19	139.15
St .Joe	Coast	20.0	0.03	2.6	0.16	165.65
Cedar Key	Basin	14.99	0.08	2.0	0.13	
Cedar Key	Streams1	13.00	0.06	2.6	0.06	117.24
Cedar Key	Streams2	17.17	0.06	1.7	0.12	180.97
Cedar Key	Streams3	14.75	0.12	1	0.08	145.06
Cedar Key	Streams4	8.50	0.09	2.6	0.06	109.27
Cedar Key	Coast	10.75	0.06	3.1	0.09	130.42
Mosquito L	Basin	17.59	0.05	1.0	0.07	
Mosquito L	Streams1	19.13	0.04	0.7	0.03	145.49
Mosquito L	Streams2	19.26	0.05	0.9	0.03	107.08
Mosquito L	Streams3	13.00	0.06	0.6	0.08	191.75
Mosquito L	Coast	19.33	0.04	0.6	0.01	86.53
Florida Bay	Basin	15.47	0.06	0.4	0.02	
Florida Bay	Canals1	13.67	0.09	0.5	0.08	183.53
Florida Bay	Canals2	14.90	0.05	0.8	0.03	142.78
Florida Bay	Canals3	17.26	0.05	0.4	0.02	169.55
Florida Bay	Canals4	16.63	0.08	0.1	0.02	250.55
Florida Bay	Canals5	14.83	0.05	0.4	0.03	191.54
Florida Bay	Canals6	16.08	0.06	0.4	0.01	127.15
Florida Bay	Canals7	18.76	0.05	0.4	0.01	117.06
Florida Bay	Streams1	15.57	0.05	0.4	0.03	192.98
Florida Bay	Streams2	19.39	0.05	0.2	0.01	155.65
Florida Bay	Streams3	14.23	0.06	1.1	0.06	134.73
Florida Bay	Streams4	18.46	0.07	0.5	0.02	113.67
Florida Bay	FBCoast	20.00	0.04	0.2	0.01	140.02
Florida Bay	Keys-coast	20.00	0.01	0.9	0.04	297.09

Table G-2. Data used to determine the Hawaiian Islands contributing zones.

Location	Basin/stream	Permh	Awcl	watertable	slope	Cont. Zone (m)
BI- Hilo	Basin	13.79	0.20	6.0	4.36	
BI- Hilo	Streams1	14.89	0.21	6.0	4.47	145.36
BI- Hilo	Streams2	14.67	0.24	6.0	4.49	136.67
BI- Hilo	Streams3	11.0	0.50	6.0	4.36	99.26
BI- Hilo	Coast	12.29	0.40	6.0	4.36	108.54
BI- Punaluu	Basin	13.56	0.12	6.0	11.63	
BI- Punaluu	Streams1	16.26	0.17	6.0	13.41	127.75
BI- Punaluu	Streams2	13.66	0.16	6.0	13.10	135.24
BI- Punaluu	Streams3	8.64	0.22	6.0	14.96	132.40
BI- Punaluu	Coast	7.53	0.33	6.0	11.63	101.75
BI- Kona	Basin	9.57	0.17	6.0	8.67	
BI- Kona	Streams1	7.11	0.04	6.0	14.84	396.04
BI- Kona	Streams2	5.38	0.20	6.0	6.28	138.61
BI- Kona	Streams3	8.20	0.29	6.0	5.65	101.97
BI- Kona	Streams4	8.00	0.54	6.0	24.75	125.93
BI- Kona	Coast	2.00	0.47	6.0	8.67	123.38
Maui- Hono	Basin	4.40	0.12	6.0	16.79	
Maui- Hono	Streams1	4.40	0.12	6.0	10.09	156.00
Maui- Hono	Streams2	4.67	0.13	6.0	18.78	119.17
Maui- Hono	Coast	2.00	0.10	6.0	1.80	88.01
Molokai	Basin	8.00	0.07	6.0	7.06	
Molokai	Streams1	11.00	0.06	6.0	7.07	152.03
Molokai	Streams2	11.00	0.06	6.0	7.44	154.79
Molokai	Streams3	8.00	0.07	6.0	7.21	151.08
Molokai	Streams4	8.00	0.07	6.0	6.73	147.44
Molokai	Streams5	11.00	0.04	6.0	7.38	189.02
Molokai	Coast	11.00	0.04	6.0	2.05	120.84
OA- Waikiki	Basin	0.81	0.06	5.0	1	
OA- Waikiki	Stream1	3.0	0	3.0	1	117.56
OA- Waikiki	Canals	3.0	0	3.0	1	117.56
OA- Waikiki	Coast	0	0	0	0.95	150.00
OA- KBay	Basin	7.45	0.05	4.2	12.99	
OA- Kbay	Streams1	13.00	0.05	4.0	14.72	143.65
OA- Kbay	Streams2	9.50	0.06	5.0	18.27	134.70

Table G-2 (continued)

Location	Basin/stream	Permh	Awcl	watertable	slope	Contrib. Zone (m)
OA- Kbay	Streams3	4.07	0.10	6.0	6.39	78.11
OA- Kbay	Coast	8.42	0.06	3.6	1.98	74.72
Kauai- Prince	Basin	2.15	0.06	6.0	4.43	
Kauai- Prince	Streams1	1.84	0.07	6.0	6.45	163.40
Kauai- Prince	Coast	0.60	0	6.0	4.43	193.62
KA- Anahola	Basin	3.20	0.10	5.2	9.15	
KA- Anahola	Streams1	2.50	0.10	5.0	8.73	158.04
KA- Anahola	Coast	2.00	0.14	4.0	9.15	158.79
KA- Moloaa	Basin	3.50	0.09	6.0	6.92	
KA- Moloaa	Streams1	3.50	0.09	6.0	6.60	147.53
KA- Moloaa	Coast	2.00	0.14	6.0	6.92	134.51

KEY

Permh – Soil permeability
Awcl - Water capacity
W**atertable** – Profile thickness above seasonal high water table

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BIOGRAPHICAL SKETCH

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