INTRODUCTION

Coral reef habitats are noted for intense levels of predation (Huston 1985). Sponges are conspicuous members of the reef community and, as sessile soft-bodied animals, appear to be vulnerable to predation. Sponges are preyed upon by gastropods (Dayton et al 1974), polychaetes (Fauchald and Jumars 1979, Pawlik 1983), asteroids (Dayton et al 1974, McClintock 1987), echinoids (Ayling 1981), turtles (Meylan 1988), and fish (Bakus 1964, 1966, Randall and Hartman 1968, Ayling 1981). Large, exposed sponges living on coral reefs can suffer major physical damage due to fish predation (Randall and Hartman 1968, Reiswig 1973, Hoppe 1988). Randall and Hartman (1968) analyzed the gut contents of 212 species of reef fishes from the West Indies and found sponge in the stomachs of 21 species. Sponge tissue comprised more than 6% of the gut contents of 11 species of fishes. The species that concentrated on sponges as a food source were angelfishes of the genus *Holacanthus* and *Pomocanthus* (>95% and >70% stomach content of sponge, respectively) and the filefish *Cantherines macrocerus* (>85%). Parrotfishes, most noteably of the genus *Sparisoma*, have frequently been seen grazing sponges (Dunlap and Pawlik 1996, Chanas and Pawlik 1997). The results of the foregoing studies demonstrate that two groups of fishes exist on reefs, the small number of spongivorous fish species and the much larger number of fish species that do not eat sponges. The persistence of sponges on reefs and the small numbers of fishes that concentrate on sponges as food, suggest that sponges have highly effective methods for discouraging predation (Randall and Hartman 1968).

Sponges deter predators by producing chemicals that are unpalatable to fishes (Pawlik et al 1995, Uriz et al 1996, Chanas and Pawlik 1997, Pawlik et al 2002). Sponges have yielded the highest numbers and greatest diversity of secondary metabolites isolated from marine organisms (Faulkner 1994). Secondary metabolites are structurally complex and are often found in high concentrations in sponge tissue (Pawlik et al 1995). Synthesis of secondary metabolites is likely at a high metabolic cost, and therefore, should provide some benefit to the organism (Paul 1992, Pawlik 1993, McClintock and Baker 2001). Secondary metabolites function in antifouling, anti-overgrowth, and UV protection but the most common role that has been studied is that of predator deterrence (Pawlik 1993, Pawlik et al 1995, Chanas et al 1996, McClintock 1997, Wilson et al 1999, Waddell and Pawlik 2000a,b). Pawlik et al (1995) surveyed 71 species of Caribbean demosponges, of which, 69% yielded deterrent crude extracts in aquarium assays with a generalist fish predator. Sponges that are preferred as prey items by sponge-eating fishes lack chemical deterrency and live in mangrove habitats, in refugia under rock ledges or coral rubble, or exposed on the reef (Pawlik 1997, 1998). Undefended sponges that live exposed on the reef appear to be continually grazed. The fourth most common sponge in the Caribbean, *Callyspongia vaginalis*, a sponge lacking chemical defenses, was the most abundant sponge found in the stomachs of reef fishes (Randall and Hartman 1968). Lack of strong chemical deterrence in sponges is not compensated for by structural or nutritional defenses (Chanas and Pawlik 1995, Uriz et al 1996). But, sponges lacking chemical anti-predatory defenses may direct energy, otherwise used for production of secondary metabolites, to increased reproduction, growth and regeneration, or wound healing (Pawlik, 1998).

Fish predation on sponges results in a breach of the defensive outer layer of sponges by creating a wound that can become invaded or infected by microbes and pathogens (Sara and Vacelet 1973, Smith and Hildemann 1986). Predation also affects the physical structure of the sponge, altering the flow of water through the sponge. Flagellated chambers greatly increase the surface area that incurrent water passes across, resulting in increased flow through the sponge. The velocity of the excurrent stream through the osculum may be several hundred times that at the level of the choanocytes (Vogel 1974). Due to the physical structure of a sponge, choanocyte chambers and oscular chimneys, water can passively flow unidirectionally from ostia to oscula without the choanocytes actively pumping (Vogel 1974). A constant flow is essential for maximizing feeding rates and nutrient exchanges in sponges (Vogel 1974, 1977, Bell 2002). A wound could disrupt the natural flow through the sponge possibly causing a complete structural failure or collapse of the animal (Jackson and Palumbi 1979, Bak 1983, Wahle 1983). It may be important for sponges to rapidly heal wounds to prevent infection and re-establish feeding currents and structural stability.

Sponges repair tissue damage by either an infiltration of archaeocytes and other mesohyl cells to the wound area or, by local increases in mitosis that results in ingrowth (Smith and Hildemann 1986). Sponges heal wounds at much faster rates than normal growth rates (Ayling 1983, Smith and Hildemann 1986, Hoppe 1988, Bell 2002). Wound healing could be a large drain on the energy budget of sponges (Bak 1983, Wahle 1983). Sponges with anti-predatory chemical defenses may not be able to heal wounds at the same rates as sponges lacking chemical defenses because they allocate energy for the synthesis and storage of secondary metabolites.

Considering the foregoing, the following questions were addressed in this study. Do different species of sponges heal similar wounds at different rates? Is there a relationship between healing rates and the chemical deterrency of sponge tissue? Does sponge morphology affect the healing rates of individual sponges within a species?

METHODS

Wound healing experiments were conducted to determine if differences exist in the healing rates of sponges. To account for growth differences that may occur between sponges with different morphologies, only sponge species that grow in tubular or vasiform shapes were used. The first experiment was conducted at 10 m depth at North Dry Rocks Reef (25º07.850N, 80º17.521W) in the northern Florida Keys in May 2002. Sponge species used in the first experiment were *Callyspongia plicifera* (n=16, undefended), *Callyspongia vaginalis* (n=14, undefended), *Ircinia campana* (n=7, chemically defended), and *Niphates digitalis* (n=14, undefended). Individual sponges were numbered and tagged using flagging tape. A scalpel was used to cut a circular hole in each sponge approximately 2 cm^2 in area and 3 cm from the lip of the sponge. The wound was intended to simulate an area of tissue that is lost when a predator bites into the sponge. The wound location was used because the tissue of vasiform sponges is most uniform in thickness approximately 3 cm from the lip of the sponge. A photograph of the wound was taken after cutting using a digital camera. The experiment ran 10 days and a photograph of the wound was taken at the end of this experiment to determine the amount of healing by each sponge. The digitizing software ImageTool was used to measure initial and final wound areas in square centimeters (2- dimensional healing). Percentage healing was calculated as the change in wound area (area of regenerated tissue) divided by the initial wound area, multiplied by 100. An estimate of daily healing rates for each sponge species were calculated by dividing the total percentage of area healed by the number of days the experiment ran. A linear regression was calculated to assess the

relationship between healing rate and levels of chemical defense using data from Pawlik et al (1995) (and see below).

The first experiment showed a difference in healing rates of sponges with high chemical deterrencies and sponges lacking anti-predatory chemical defenses. A second experiment was conducted to replicate the first in a different location and with additional sponge species. The second experiment was conducted at 5 m depth for *C. vaginalis* and 15 m depth for all other species at Sweetings Cay (26º25.212N, 77º53.347W) in the Bahamas in June 2002. Sponge species used in this experiment were *C. vaginalis* (n=16), *Cribrochalina vasculum* (n=10, chemically defended), *N. digitalis* (n=14), *Verongula gigantea* (n=5, chemically defended), and *Xestospongia muta* (n=18, undefended). The same protocol used in the first experiment was used to measure healing rates of sponges in this experiment. The experiment ran 8 days for *V. gigantea*, 9 days for *C. vasculum*, *N. digitalis*, and *X. muta*, and 12 days for *C. vaginalis*.

A third set of experiments was conducted in the northern Florida Keys in October 2002 at 15 m depth at Pickles Reef (24º59.272N, 80º17.521W) for *X. muta* and at 10 m depth at North Dry Rocks Reef (25º07.850N, 80º17.521W) for all other sponge species. This experiment repeated the methods of the first two experiments, but included multiple photos of healing wounds over the experiment time period. Experiments were also conducted to determine if healing rates were affected by the orientation of wounds relative to the tidal current and to determine if changes in sponge shape affected the healing rates of sponges within a species. Sponge species used in these experiments were *C. plicifera* (n=17), *C. vaginalis* (n=16), *I. campana* (n=10), *N. digitalis* (n=20), and *X.*

muta (n=17). The sponges were wounded in the manner previously described and a picture was taken of the wound. The experiments ran for 12 days.

To investigate patterns in daily healing rates, multiple pictures were taken of four species of sponges. Three individuals of *N. digitalis*, *C. plicifera*, and *C. vaginalis* at North Dry Rocks Reef were photographed at days 0, 3, 6, 10, and 12. Three individuals of *X. muta* at Pickles Reef were photographed at days 0, 3, 9, and 12. Daily healing rates were calculated for each block of time that a picture was taken. A one-way analysis of variance was calculated to see if significant differences existed between healing rates at different times during the experiment.

An experiment was conducted to look for differences in the healing rates of wounds relative to the tidal current. Ripples in the sand were used to assess the direction of the tidal current. Five individuals of each species were cut with a wound facing the direction of the tidal current and another wound in a direction perpendicular to the tidal current. A paired t-test comparing the healing rates of the two wounds facing opposing directions was calculated for each species.

Pictures were also taken from the side and the top of each sponge. Sponge heights and oscular diameters were measured using ImageTool. The oscular diameter was divided by the sponge height and multiplied by 100 to give a shape ratio for each sponge. The shape ratios of the sponges were used to investigate the healing rates of intraspecific sponge individuals with different morphologies. A linear regression of sponge ratios and healing rates was calculated for each species.

Individuals of *Callyspongia vaginalis* are often found growing as a cluster of tubes. To investigate the healing rates of *C. vaginalis* clusters, multiple tubes of

individual sponges were wounded. Five individuals, each with a cluster of five tubes, were wounded as follows: one individual with one tube wounded, one individual with two tubes wounded, one individual with three tubes wounded, one individual with four tubes wounded, and one individual with five tubes wounded. A one-way analysis of variance was calculated to determine if differences existed in the healing rates of the five different sponges. Four individuals of *C. vaginalis* were wounded with two holes in one tube. The sponges wounded in this manner were individuals with clusters of two tubes, four tubes, five tubes, and seven tubes. A one-way analysis of variance was calculated to determine if differences existed in the healing rates of the four sponges tested.

Spicules were identified and spicule lengths were measured to determine if differences existed between spicules in regenerated tissue and spicules in original sponge tissue. Wound plugs were collected from each sponge and frozen in ziploc bags filled with seawater. At the end of the experiment the region of the sponge that encompassed the wound was cut from the regenerated sponge and frozen in a ziploc bag filled with seawater. All samples were thawed at room temperature. The regenerated tissue was excised from the sponge using a scalpel. Wound plugs and regenerated tissue were placed in separate test tubes and filled with bleach. After the tissue dissolved, the spicules were pipetted out and mounted on a depression slide. A light microscope with an ocular micrometer was used to identify and measure the lengths of ten spicules from each sponge sample. A paired t-test was calculated to compare spicule sizes of regenerated tissue and original wound plug tissue for each sponge species.

This study used the results from experiments conducted by Pawlik et al (1995) to determine anti-predatory chemical defenses in Caribbean sponges. Pawlik et al (1995)

made crude extracts from volumetric tissue samples of sponges. Extracts were incorporated into a food mixture that was fed to a generalist fish predator in aquarium assays. For each sponge species, an extract was considered deterrent if the mean number of food pellets eaten was less than or equal to 6. This protocol was replicated by Chanas and Pawlik (1997), Pawlik et al (2002), and Neilson (unpublished) in experiments conducted to determine the chemical deterrency of *Xestospongia muta*, *Ircinia campana*, and *Niphates digitalis,* respectively. We used data from these experiments as well as Pawlik et al (1995) to investigate the relationship between chemical defense and wound healing.

RESULTS

Sponge species with highly deterrent anti-predatory chemical defenses healed wounds at significantly slower rates than sponges that lack chemical defenses (ANOVA $p<0.001$) (Figure 1). The three sponges that were chemically deterrent, *C. vasculum* (2% average wound area regenerated per day), *I. campana* (2%), and *V. gigantea* (0%), had the lowest daily healing rates of the sponges tested in this study. Wound healing in *C. vasculum* and *I. campana* only sealed the edge of the wound whereas in the sponges that were less chemically deterrent, wound healing was much more substantial, regrowth tissue quickly sealed the edge of the wound then filled in the wound hole. Undefended sponges healed more rapidly: *C. plicifera* (8% average wound area regenerated per day), *C. vaginalis* (6%), *N. digitalis* (6%), and *X. muta* (6.5%). Multiple individuals of undefended sponges sealed the entire hole with tissue before the experiment was completed.

In *C. plicifera, C. vaginalis*, *N. digitalis,* and *X. muta* the greatest percentage of growth occurred the first few days after the wound was cut. The daily healing rates of the four species monitored were significantly greater at day 3, [ANOVA $p<0.001$ (Tukey) $p<0.05$], and healing rates appeared to level off after the third day (Figure 2). Many individuals of *C. plicifera*, *C. vaginalis*, and *N. digitalis* grew a thin membrane across the entire wound area during the first few days of the experiment. This membrane was often found torn on subsequent days, however, the membrane sustained the duration of the experiment in some individuals. The membrane was not used in measurements for

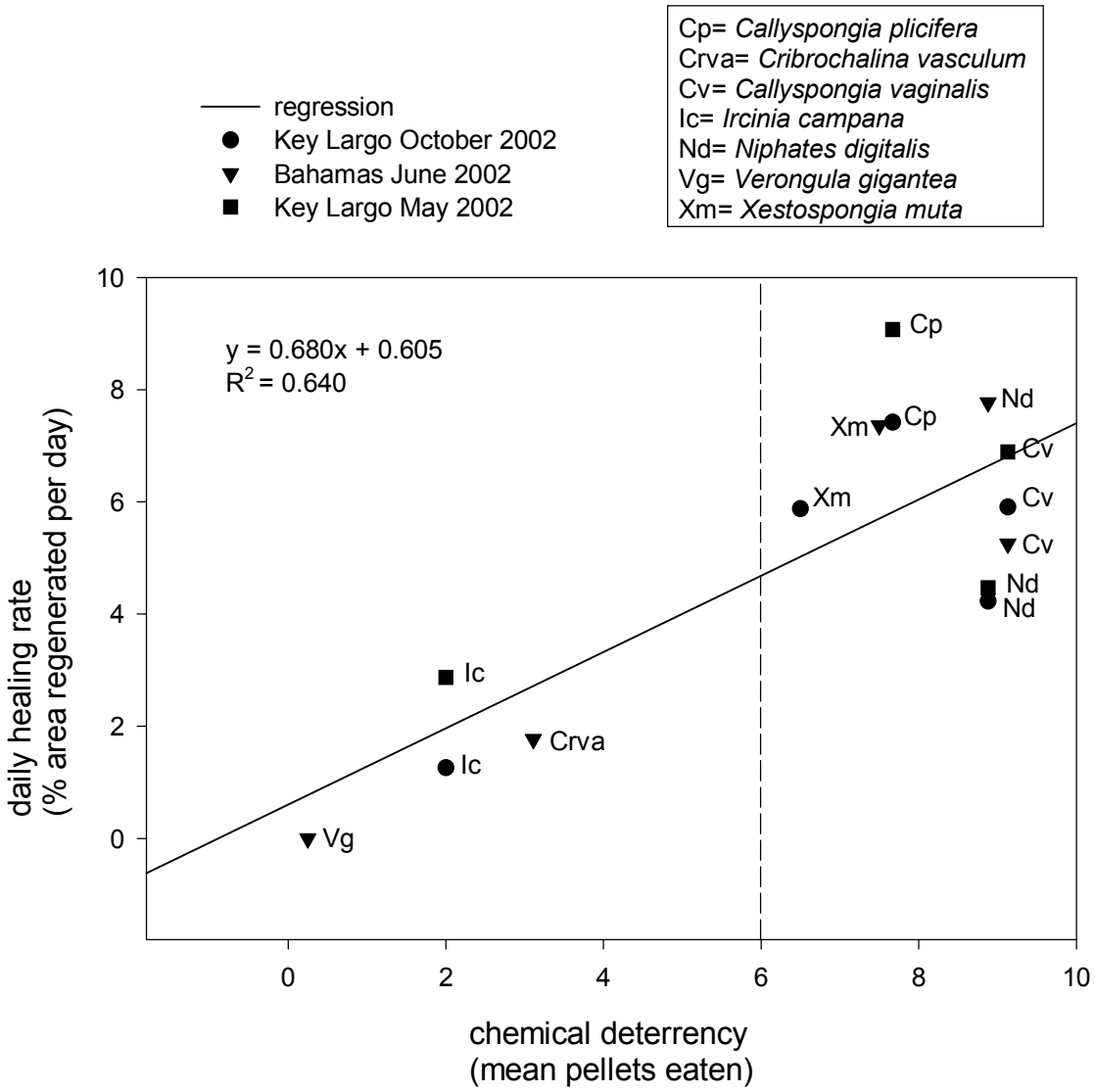


Figure 1: Chemical deterrencies and healing rates for all sponges. Sponge extracts were considered deterrent if number of pellets eaten was less than or equal to 6, as indicated by the vertical dashed line. A sponge species with a chemical deterrency value of 0 is the most deterrent and a sponge with a value of 10 is the least deterrent (Pawlik et al, 1995, 2002).

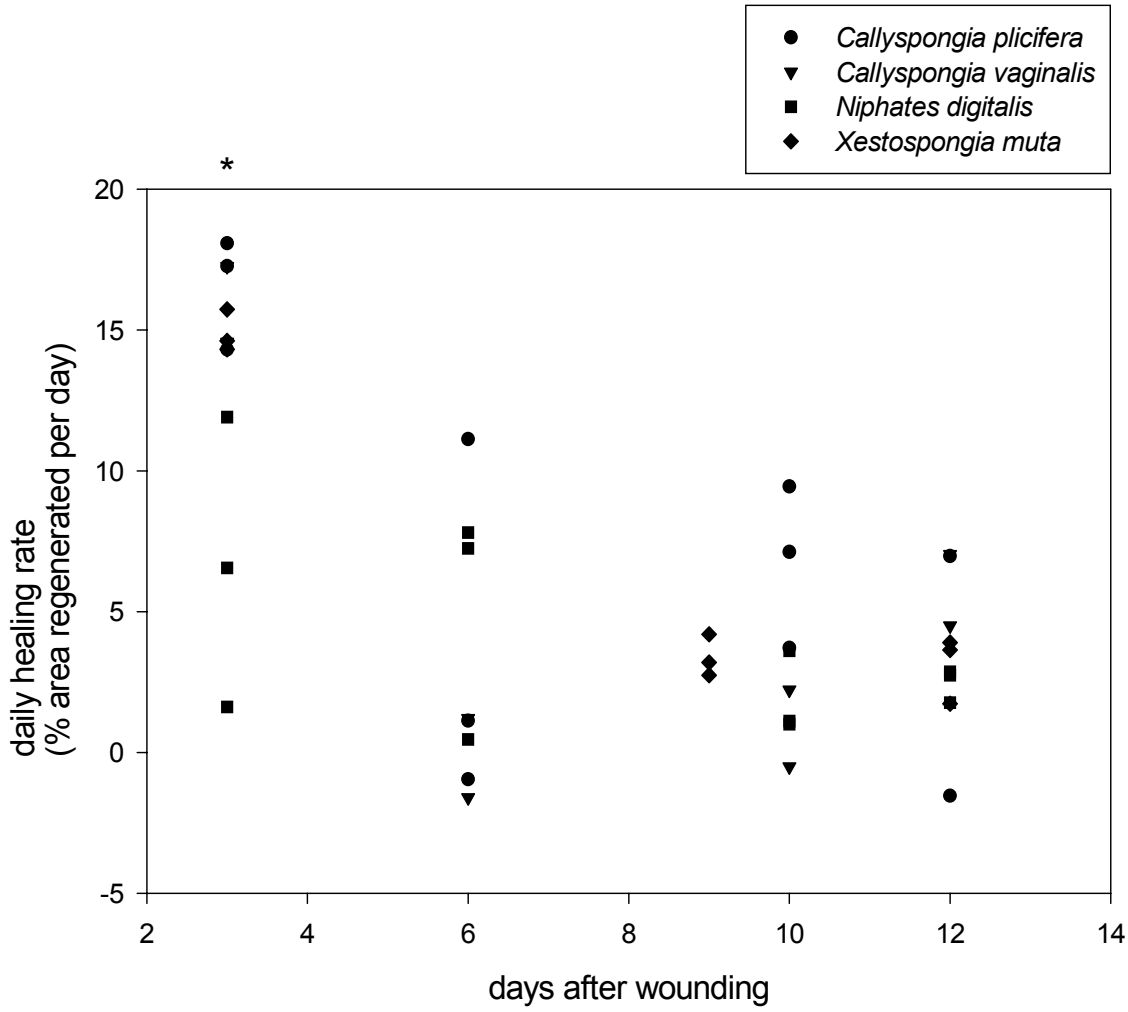


Figure 2: Rates of healing. Approximate daily healing rates of three individuals of each sponge species were calculated at different time intervals throughout the experiment. The star represents a day that was significantly different from the other days [ANOVA $p<0.001$ (Tukey) $p<0.05$].

wound healing calculations. Only regenerated sponge tissue was used to calculate the wound area regenerated per day. Individuals with membranes did not appear to heal wounds at different rates than other individuals of the same species without membranes.

The orientation of wounds relative to the tidal current did not significantly affect the healing rates of sponges. Sponge individuals that were used in this experiment had wounds facing the tidal current and perpendicular to the tidal current. The healing rates of the differently positioned wounds were not significantly different in any of the sponge species tested (Figure 3).

To investigate the effects of sponge morphology on healing rates, we wounded individual sponges of the same species growing in shapes ranging from tubular to bowl shaped. Sponges of *N. digitalis* growing in tubular forms (low shape ratio) had faster healing rates than sponges that grew in vasiform shapes (ANOVA $p=0.024$) (Figure 4). For *C. vaginalis*, a significant relationship existed in the linear regression ANOVA test ($p= 0.025$), however, individuals of *Callyspongia vaginalis* were only found growing in tubular shapes, therefore, the relationship expressed may not be indicative of an effect due to sponge morphology.

Wound healing in individuals of *Callyspongia vaginalis* with multiple wounds was not significantly different. The one-way analysis of variance for the healing rates of sponges with five tubes had a p-value of 0.102. The one-way analysis of variance for the healing rates of sponges with two holes had a p-value of 0.114.

Identifications of spicules revealed that original wound plug tissue and regenerated tissue contained the same types of spicules for all the species tested.

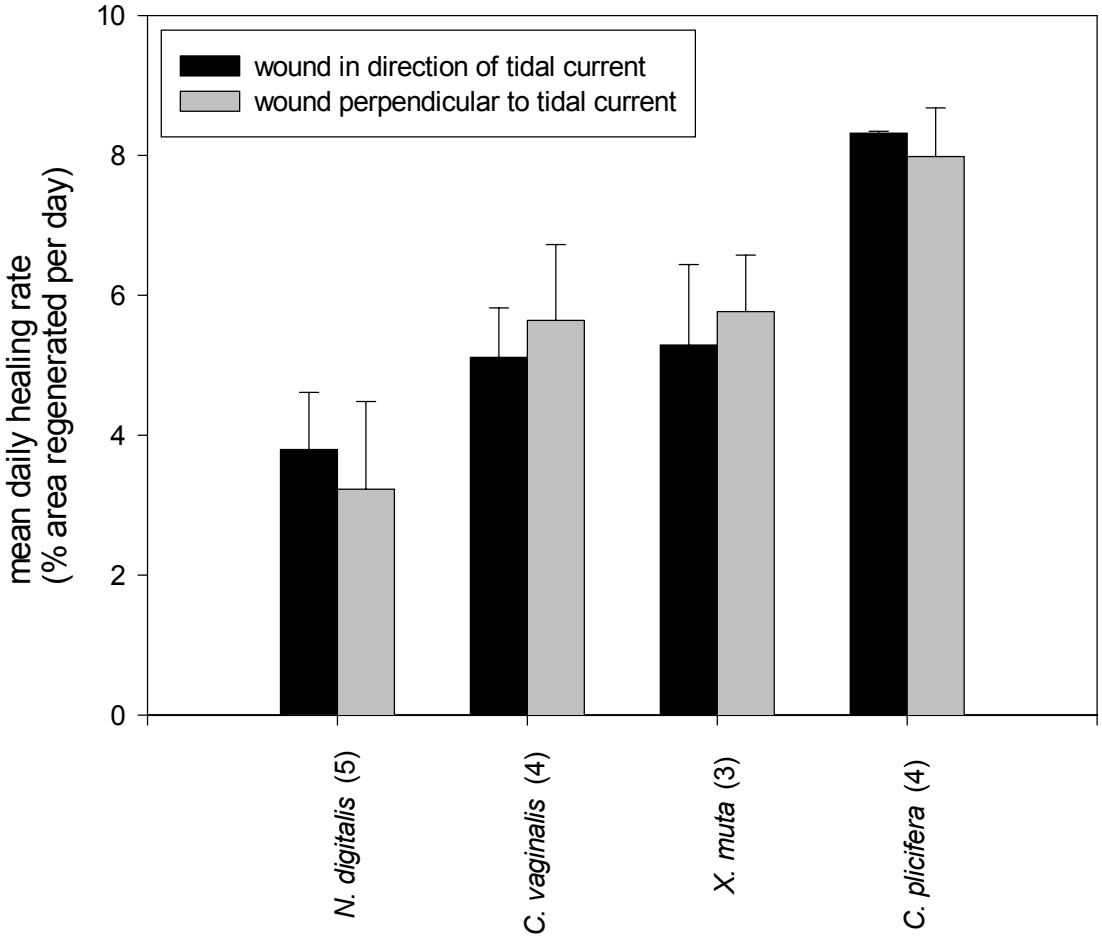


Figure 3: Orientation of wounds relative to the tidal current. Number of individual sponges are shown in parentheses.

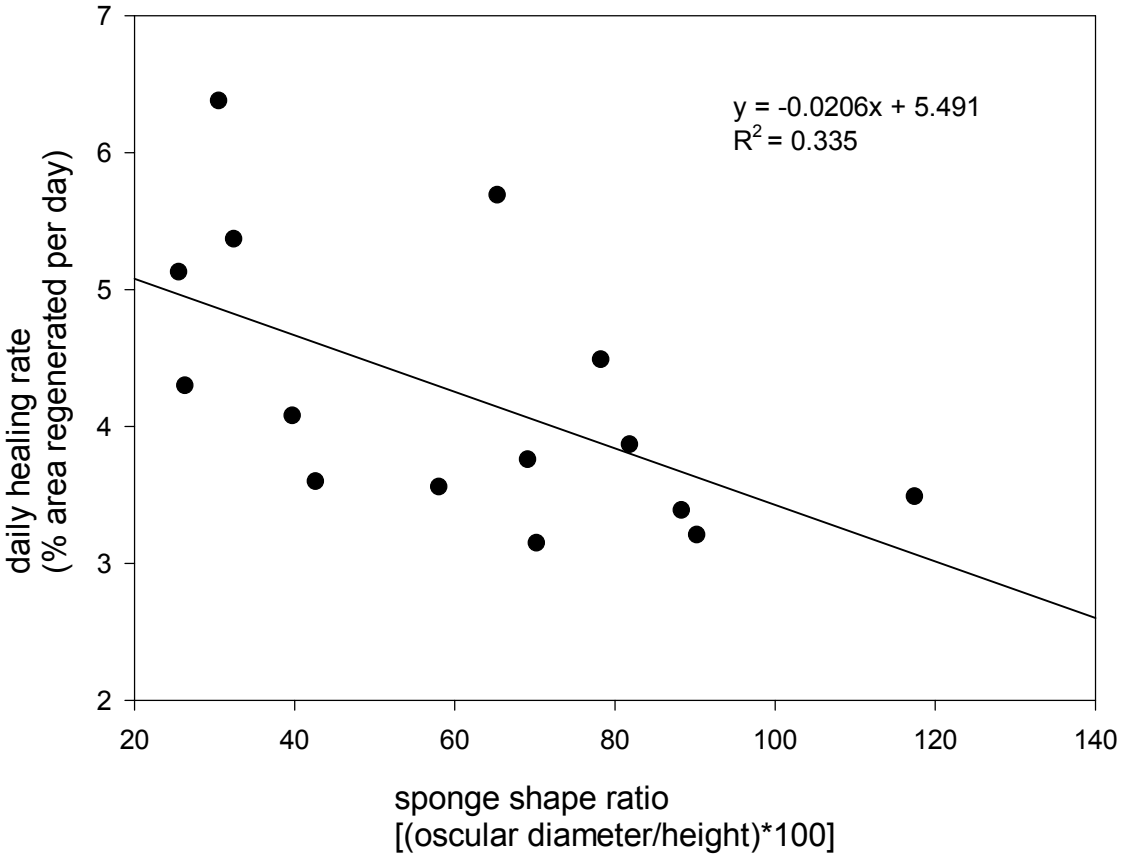


Figure 4: Sponge morphology of *Niphates digitalis* correlated with healing rate. Sponges with low sponge shape ratios are more tubular in form and sponges with higher ratios have vasiform shapes.

Megascleres, all of oxea shape, were used to measure spicule lengths in the different sponges. No significant differences existed between spicule lengths of original wound plug tissue and spicule lengths of regenerated tissue for any of the sponge species tested (paired t-tests: *C. vaginalis* P = 0.32, *N. digitalis* P = 0.96, *C. plicifera* P = 0.089, *X. muta* P = 0.14).

DISCUSSION

Metabolic energy is used to pump water through the body of a sponge and pumping rates can affect the amount of energy that a sponge uses (Reiswig, 1974). The idea of entrained water flow determines the induced flow through the body cavity of a sponge during fast flow conditions and acts to reduce the energy for pumping water through the sponge body (Vogel 1974). This passive flow may allow more metabolic energy to be used for growth and regeneration. Sponge feeding and survival may be enhanced by morphology and any deviation from the optimal shape may change localized feeding currents. It may be essential for sponges to heal wounds to re-establish natural flow rates. In the present study, the undefended sponges *Callyspongia plicifera, C. vaginalis*, and *Niphates digitalis* that created a thin membrane during initial wound healing may be using the membrane as a temporary wall to quickly restore flow rates. *Niphates digitalis* healed wounds faster when in tubular than in vasiform shape, which may indicate the need for more erect sponges to rapidly rebuild their structures. Rapid structural repair may be important for erect sponges because damage, if not repaired quickly, may cause a complete structural failure or collapse of the animal (Jackson and Palumbi 1979, Bak 1983, Wahle 1983). *Cliona celata*, a slow growing sponge, had rapid regeneration rates after wounding to quickly return the sponge to its original size and shape (Bell 2002).

The interaction between plants and herbivores and the ecology of chemical defense has been well studied in both terrestrial and marine ecosystems. To persist in a community, plants must escape, deter, or tolerate herbivory. Plants have adapted two

major strategies for dealing with herbivory, either to grow fast or to produce defensive chemicals (Coley et al 1985). Resource allocation within the plant determines which strategy a particular species uses (Bazzaz et al 1987). Fast growing species invest a large proportion of their resources into processes and structures further enhancing resource acquisition, resulting in high relative growth rates. Slow growing species invest a large proportion of resources into non-growth processes and structures required to retain and make efficient use of resources under intense herbivory, resulting in low relative growth rates (Herms and Mattson 1992). The resource investment in slow growing species is used for anti-herbivory chemical defenses in the form of secondary metabolites. The production of secondary metabolites incurs a high metabolic cost for the organism. Costs are due to the raw materials needed to make the compounds, the production and storage of the secondary metabolites, and the prevention of autotoxicity within the organism (Van Alstyne et al 2001). Producing and maintaining the enzymes required for the synthesis and safe management of toxic compounds could be much more costly than the energy invested in the compounds themselves (Hay and Fenical 1988). Metabolites are often in high concentrations and have low turnover rates in slow growers and may have dosage dependent effects on herbivores (Coley et al 1985). Fast growers suffer high rates of damage from herbivores and have both lower amounts and different types of defensive chemicals than slow growers. Defenses of fast growers are attributable to diverse chemicals that are present and effective in lower concentrations and have high turnover rates (Coley et al 1985). The dilemma inherent within plants is that they must grow fast enough to compete, yet maintain the defenses necessary to survive in the presence of pathogens and herbivores. Environmental changes can alter the amounts of materials and

the energy available for defensive production and how those resources are allocated to growth, reproduction, and defense (Van Alstyne et al 2001). When environmental conditions are favorable, vegetative growth receives resource priority over secondary metabolite synthesis (Herms and Mattson 1992). Growth processes demand high levels of limited plant resources, therefore during periods of intense growth, secondary metabolites may be substrate and or energy limited (Herms and Mattson 1992). In highly defended plants, growth rate is a balance between growth reduction due to defense costs and a growth increase due to better protection from herbivores. Compensatory growth may represent an evolved alternative to chemical defense as a strategy for reducing net losses to herbivores.

The two strategies used by plants to compensate for herbivory may be translatable to sponges and their responses to predation. In sponges there appears to exist a group of fast wound healing species and a group of slow healing, chemically defended species. Predation may act as a key factor in determining the life history strategies of sponges. In the Antarctic, sponge eating invertebrate predators appear to be generalists which feed on the more abundant, non- to mildly-toxic sponge species (McClintock 1987). *Callyspongia* spp., undefended sponges that are among the most common found on Caribbean coral reefs, were the most abundant sponges found in the stomachs of reef fishes (Randall and Hartman 1968). Uriz et al (1996) found the sponge *Crambe crambe* to have much slower growth rates than *Dysidea avara*, a sponge that is grazed by sea urchins, suggesting that slow growth rates favor large investments in anti-grazing defenses. Resources are limiting and reproduction, growth, and defense interact within individuals and compete for these limited resources so that an additional investment in

one function must be at the expense of the remainder (Uriz et al 1996). Chemically defended sponges are assumed to expend a large proportion of their resources on the synthesis and storage of secondary metabolites (Paul 1992, Pawlik 1993, McClintock and Baker 2001). This strategy is effective at inhibiting predation by generalist fishes (Pawlik et al 1995). But, anti-predatory chemical defenses may come at the expense of the wound healing capacities of defended sponges. *Ircinia campana*, a chemically defended sponge, exhibited tissue necrosis surrounding the wound at the end of the experiment in 3 out of 10 individuals. Chemically defended sponges may not have the resources available for wound healing. Because fish predators do not feed on highly chemically defended sponges, these sponges may not have developed the adaptation for efficient wound healing (Randall and Hartman 1968, Pawlik et al 1995). Undefended sponges, which appear to be frequently grazed, may compensate with faster wound healing rates.

The study conducted by Pawlik et al (1995) calculated numerical values corresponding to the levels of chemical deterrency for each sponge species tested. Sponges with values of 6 or less were considered deterrent to a generalist fish predator. Sponges with values closest to 0 were the most chemically deterrent species, and sponges with values closest to 10 were the least deterrent species. In the present study, *Xestospongia muta* [6.5 (Keys) and 7.5 (Bahamas) chemical deterrency values] and *Callyspongia plicifera* (7.7) were the sponges with the most intermediate levels of chemical deterrency. Individuals of *X. muta* have been found with variable levels of chemical deterrency (Pawlik et al 1995, Pawlik et al 2001). Intermediate levels of defense cause maximum growth rates in plants (Coley 1985). *Xestospongia muta* (6.5 %

wound area regenerated per day) and *Callyspongia plicifera* (8 %) had the fastest rates of wound healing of the sponge species tested in this study. These sponges may be better adapted to dealing with the effects of predation by using high rates of wound healing and intermediate levels of chemical deterrence.

Future experiments could answer more questions about resource allocation in sponges. In plants, the optimum level of defense investment increases as the potential growth rate of the plant decreases. As the organism gets older, replacement of resources lost to herbivory becomes more costly, a given rate of herbivory represents a larger fraction of net production of slow growers than fast growers, and a percentage reduction in growth rate due to the cost of producing defenses represents a greater absolute growth reduction for fast growers (Coley et al 1985). The cost of defense increases as growth rate increases, therefore sponges that are larger or older may not heal wounds as fast as sponges that are younger and have more resources available. An investigation into the types of chemicals and their concentrations within sponge tissues in chemically deterrent sponges and undefended sponges may show higher concentrations of dosage dependent metabolites within defended sponges. In seaweeds, individuals within the same species from habitats with high densities of herbivores are sometimes better defended chemically than individuals from areas with fewer herbivores (Hay and Steinberg 1992). Differences may exist in healing rates of sponges living in habitats with low levels of predation and sponges that live exposed on coral reefs.