

Comparison of anti-predatory defenses of Red Sea and Caribbean sponges. I. Chemical defense

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ABSTRACT: Among marine invertebrates, sponges produce the largest number and diversity of novel secondary metabolites, some of which are used as defenses against predation. Seventeen abundant Red Sea sponge species were assayed for their ability to deter predation by 2 common Red Sea generalist predators: the fish *Thalassoma klunzingeri* and the sea urchin *Diadema setosum*. In addition, 17 common Caribbean sponge species were tested against both the Caribbean wrasse *T. bifasciatum* and the Red Sea wrasse *T. klunzingeri*. Among the 17 Red Sea species tested, 7 (41%) deterred *T. klunzingeri*. Extracts from the 17 Caribbean sponge species spanned a range of activities, from highly deterrent to highly palatable, when tested against both fish predators. Extracts were either deterrent to both fish species or palatable to both, suggesting that there are general responses by fish predators to deterrent sponge metabolites, regardless of geographic origin. Of the 17 Red Sea sponge species, 11 (65%) produced extracts deterrent to the sea urchin *Diadema setosum*. The same 7 species that deterred fish also deterred sea urchins, with 4 additional sponge species affecting only the sea urchins. Sea urchins were either deterred by different metabolites than fish or are more sensitive and thus are deterred by the same metabolites but at lower concentrations. Extracts from endosome and ectosome layers of 6 Red Sea sponges showed no difference in their ability to deter predation by *T. klunzingeri* and *D. setosum*, contrary to the prediction of the optimal defense theory.

KEY WORDS: Porifera · Secondary metabolites · Fish · Sea urchins · Intraspecimen variation · Optimal defense theory

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INTRODUCTION

Marine sponges are known to constitute the single richest source of novel marine natural products (Faulkner 2000 and reviews cited therein). These compounds exhibit high pharmacological potential as anti-tumor agents, antibiotics, anti-inflammatory agents, etc. (Fenical 1997, Kerr & Kerr 1999), but only in recent years have scientists focused on revealing their ecological roles.

Due to the high structural and stereochemical complexity characteristic of many marine chemical compounds (Fenical 1997), it was suggested that they are metabolically expensive and therefore probably have an adaptive purpose and play a role in ecological func-

tions (Paul 1992, Pawlik 1993). Among the suggested roles, sponge secondary metabolites have been implicated in sponge-sponge allelopathic interactions (Engel & Pawlik 2000), settlement inhibition of fouling larvae (Hirota et al. 1998), and antiviral, antifungal and antibacterial defenses (Rinehart et al. 1981).

By far the most prevalent theory regarding the function of sponge secondary metabolites is that they act to deter predation (e.g. Pawlik et al. 1995, Wilson et al. 1999, Assmann et al. 2000). Sponges, being soft-bodied sessile organisms, appear to be defenseless and thus helpless in facing predation. Despite this, spongivory on tropical reefs is largely restricted to a few fish species (Randall & Hartman 1968, Wulff 1994, Dunlap & Pawlik 1996) and hawksbill turtles (Meylan 1988),

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which feed on a diversity of sponge species, and nudibranchs, which usually specialize on only one or a few sponge species (Thompson 1976). Echinoderms also may graze on sponges (Dayton 1989, Birenheide et al. 1993). Studies have shown that sea urchins occasionally reduce the diversity and coverage of sponges (Vance 1979, Ayling 1981) and thus may have an overall effect on sponge populations. The limited number of sponge predators is especially surprising because predation pressure in tropical reefs is known to be very intense (Hay 1991). The question thus arises: what do reef sponges use to defend themselves from predation?

In a survey of chemical anti-predatory defenses, 49 out of 71 Caribbean sponge species (69%) yielded secondary metabolites deterrent to fish predators (Pawlik et al. 1995). It would seem, therefore, that many sponge species avoid predation by relying on the production of deterrent secondary metabolites that are distasteful to potential predators. Small pieces of the 71 sponge species examined were offered to a generalist predator, the Caribbean blue-headed wrasse *Thalassoma bifasciatum*. In no case were the pieces eaten, even in species that yielded palatable extracts (such as *Callyspongia vaginalis*, the 4th most abundant sponge on Caribbean reefs). In most cases the pieces were ignored, suggesting that the fish have learned from visual cues to avoid sponge tissue (Pawlik et al. 1995). These results suggest that chemical defense is commonly used by Caribbean sponges against predation, but that alternative tactics may exist as well.

In addition to chemical defense, physical defenses (i.e. spicules and spongin contribution to predator deterrence) and low nutritional quality of tissue constitute anti-predatory defense mechanisms previously discussed regarding sponges. Chanas & Pawlik (1995, 1996) studied the contribution of these mechanisms in defense against predation in Caribbean sponges. None of the 8 species tested for physical defenses were found to be defended by spicules or spongin fibers. In addition, sponges were also found to have high protein content (mean of 20.7 mg soluble protein per ml sponge tissue, N = 71), excluding low nutritional quality as a defense mechanism. It was concluded that chemical defense, rather than physical defense or low nutritional quality, was the most important defensive mechanism used by sponges on Caribbean reefs. The physical defense of Red Sea sponges and aspects of their nutritional quality are discussed separately in a companion paper (Burns & Ilan 2003, this issue).

Knowledge of intraspecimen variation of secondary metabolites may increase our understanding of predator-prey relationships (Becerro et al. 1998). Secondary metabolites may be concentrated in the vulnerable body parts that are first encountered by predators

(Avila & Paul 1997, Gillor et al. 2000). Studies involving intraspecimen chemical variation in marine organisms have been mainly restricted to algae, soft corals and molluscs (e.g. Meyer & Paul 1992, Van Alstyne & Paul 1992, Avila & Paul 1997). Only a few studies have addressed this issue regarding sponges (e.g. Becerro et al. 1998, Swearingen & Pawlik 1998, Schupp et al. 1999), mainly because sponges lack distinct tissues and organs and may exhibit irregular growth forms, hindering distinction of separate body parts. According to the optimal defense theory first suggested by McKey (1974), an organism channels defenses to body parts that are most vulnerable or have high value in a way that will maximize its fitness. The theory assumes that resources are limited and that the production of defenses is costly. Previous studies conducted on various marine invertebrates (Van Alstyne & Paul 1992, Avila & Paul 1997), including sponges (Chanas & Pawlik 1997, Swearingen & Pawlik 1998, Schupp et al. 1999), elaborated on this theory and hypothesized that those body parts that are first encountered and are easily accessible to predators should be the more heavily defended. Intraspecimen variation in secondary metabolite distribution may be the result of seasonal change, physical stress or induction by a prior attack (reviewed by Hay 1996). Some studies of marine organisms support the optimal defense theory (e.g. Hay et al. 1988) while others are either inconclusive (Zangerl & Nitao 1998) or do not support the theory (Cronin & Hay 1996, Swearingen & Pawlik 1998).

For sponges, Schupp et al. (1999) studied the distribution of secondary metabolites in the Micronesian sponge *Oceanapia* sp. They concluded that distribution of the 2 major deterrent metabolites kuanoniamine C and D in this sponge supports the optimal defense theory by showing the highest metabolite concentrations in the parts most visible to predators. In contrast, Chanas & Pawlik (1997) and Swearingen & Pawlik (1998) found no evidence that deterrent compounds were concentrated in the surface tissue of the common Caribbean sponges *Xestospongia muta* and *Chondrilla nucula*, respectively.

The 2 geographically distinct reefs in question are very different from one another. They differ, among other things, in their architecture and dominant taxa (e.g. soft coral and sponges in the Caribbean reef vs stony corals in the Red Sea). Sponge biomass in the Caribbean reef is by far greater than that in the Red Sea. We thus assumed Caribbean sponges are more susceptible to predation and therefore different evolutionary processes regarding predator-prey relationships may be expected.

This is the first study to examine the anti-predatory defense mechanisms in Red Sea sponges. We examined the chemical anti-predatory defense mechanism

of 17 abundant Red Sea sponge species by extracting their tissue and presenting food containing the crude organic extracts to generalist predators. Specifically, our goals were to determine: (1) whether organic extracts of sponges deter feeding by the Red Sea reef fish *Thalassoma klunzingeri* and the Red Sea sea urchin *Diadema setosum*; (2) whether organic extracts of Caribbean sponges affect fish from the Red Sea in the same way as fish from Caribbean reefs; and (3) whether secondary metabolites are concentrated near the sponge surface in support of the optimal defense theory. Data obtained in this study was compared with data known for the Caribbean in an attempt to broaden our understanding as to what regional contrasts may tell us about the evolution of predator-prey relationships, and the predictions that can be made about the susceptibility of sponges to naïve versus native predators.

MATERIALS AND METHODS

Sponge collection and identification. Sponges were collected in Elat, northern Red Sea, Israel, between October 1997 and December 1999, and from the Bahamas during 2 research expeditions on board RV 'Edwin Link' and RV 'Seward Johnson' during August 1998 and July 1999, respectively. Samples were collected from reefs by SCUBA diving, put in separate bags and immediately stored at -20°C for later use in subsequent extractions. Seventeen abundant Red Sea sponge species and 17 Caribbean species were collected. Sponges were identified on the basis of morphology, spicule and tissue preparations (Row 1911, Levi 1965, Wiedenmayer 1977, Hartman 1982, Zea 1987).

Tissue extraction. Frozen sponge tissues were cut into $\sim 1\text{ cm}^3$ cubes. Total sample volume was measured by water displacement. The samples were then covered with a 1:1 mixture of dichloromethane (DCM) and methanol. The mixture was kept at 4°C for a 24 h extraction period and shaken every few hours. During this period, water mixed with the methanol, and the resulting methanol:water phase was separated from the DCM phase. By shaking the mixture periodically, the sponge tissue was alternately exposed to both phases. After extraction, both phases were filtered through filter paper (Whatman), evaporated to dryness on a rotary evaporator at low heat ($<50^{\circ}\text{C}$), and then combined. This process was repeated twice. The remaining tissue was extracted a third time with methanol for 6 to 10 h at room temperature, and the resulting extract filtered, evaporated, and then combined with all prior extracts. The crude extract was stored at -20°C until further use. The natural concen-

tration of the metabolites extracted from the sponge was calculated by dividing the dry weight of the crude extract by the volume of the tissue extracted as measured by water displacement.

Separation of sponge layers. Six Red Sea sponge species in which the external layer (ectosome) could be easily differentiated and separated from the inner mass (endosome) were chosen. The layers were separated using a razor blade. The volume of each layer was measured separately by water displacement and the tissue was extracted as explained above.

Fish feeding experiments. Aquarium assays were performed as described in Pawlik et al. (1995) at the Interuniversity Institute (IUI) Marine laboratory in Elat and on board RV 'Edwin Link' and RV 'Seward Johnson' in the Bahamas.

Food preparation: Crude organic extract from 10 ml of each sponge species was added to a mixture of 0.3 g alginate acid, 0.5 g of freeze dried powdered squid mantle and distilled water to yield a final volume of 10 ml. The mixture was vigorously stirred until the extract was homogeneously distributed in the alginate acid matrix. The mixture was then loaded into a 10 ml syringe, the syringe tip was submerged in a 0.25 M solution of calcium chloride, and the content of the syringe was emptied to form a long, spaghetti-like strand. After a few minutes, the hardened strand was removed, rinsed in seawater, and cut into 3 mm long pellets using a razor blade. Control pellets were made the same way, but without addition of the extracts. However, food coloring was added if necessary to match the color of extract-treated pellets.

Feeding assays: Control and treatment pellets were presented to groups of 2 to 3 Red Sea wrasses *Thalassoma klunzingeri*, in experiments conducted in Elat, and groups of 3 or more Caribbean blue-headed wrasses *T. bifasciatum* in experiments conducted in the Bahamas. The fish groups were held in 10 separate, opaque-sided compartments in laboratory aquaria, as described in Pawlik et al. (1995). The 2 fish species are both abundant reef predators. The merits of using *T. bifasciatum* in aquarium bioassays conducted in the Caribbean have been previously described (Pawlik et al. 1987), as have the reasons for using a generalist fish, sensu Pawlik et al. (1995). In addition to *T. klunzingeri* being an abundant generalist fish in Red Sea reefs, it was chosen for bioassays conducted in Elat in order to reduce to a minimum the potential variation in predator behavior between the 2 study sites due to taxonomic differences of the fish. The general biology (diet, habitat etc.) of the 2 species is very similar.

Fish were presented with a control pellet to insure that they were feeding. Fish that did not eat the control pellets were not used in the experiment. After the con-

trol pellet, the fish were presented with treated food pellet. If the pellet was rejected another control pellet was offered to determine whether the fish were satiated with food. A pellet was considered rejected if not eaten after a minimum of 3 attempts by 1 or more fish to take it into their mouth cavity, or if the pellet was approached and ignored after 1 such attempt. The significance of differences in the consumption of treated versus control pellets was evaluated using Fisher's exact test. For any single assay of 10 replicates, a treatment was considered significantly deterrent if 4 or more pellets were rejected ($p \leq 0.043$, 1-tailed test). Therefore, in judging the mean deterrence of 10 trials with the same sponge species, a treatment was considered deterrent if the mean number of pellets eaten was less than or equal to 6.

The deterrence of extracts from the 17 Caribbean sponges was examined against *Thalassoma klunzingeri* as well as against *T. bifasciatum* in order to compare results obtained against the natural potential predator with those obtained against a naïve predator. The goal of these experiments was to determine whether sponge chemical defenses have specific effects on sympatric predators.

Sea urchin experiments. Assays using *Diadema setosum*, the most abundant sea urchin species in the reef of Elat (Rogel 2001), were performed as described in Pennings & Paul (1992) and in Hay et al. (1994). Sea urchins are mainly grazers that feed on algae but are usually referred to as generalist predators that also feed on a variety of invertebrates (Barnes 1987). *D. setosum* (as well as other echinoderms) is known to occasionally feed on sponges in the Red Sea (Meroz & Ilan 1995). For each experiment, 20 medium sized sea urchins (3 to 5 cm in diameter) were collected after sunset. Collections were made in different areas of the IUI Marine Laboratory in order to reduce the chances of collecting the same sea urchin for separate experiments. Collected sea urchins were starved for 24 h prior to the experiment and were released at the same place from which they had been collected, immediately after the experiment was over.

Food preparation: Artificial food was based on freeze-dried and finely powdered green alga *Ulva* sp., which is commonly consumed by a variety of herbivores. For the preparation of treatment food, 0.72 g agar was added to 20 ml distilled water and heated in a microwave to boiling. Sponge extract, at a weight equivalent to 36 ml live sponge, was suspended in 2 ml ethanol and added to the agar solution after cooling to $\sim 50^\circ\text{C}$. The solution was mixed vigorously until the extract was homogeneously distributed in the agar, and poured into a mixture of 14 ml distilled water and 2 g *Ulva* sp. powder, to yield a total of 36 ml. The total mixture was again stirred to homogeneity. The control

food was prepared in the same way, without addition of the sponge extract. The final hot gel was poured into the mold and onto a fiberglass window screen to cool. More detailed explanation on the fiberglass mold structure and the food preparation protocol is given in Hay et al. (1994). When hardened, food strips were cut so that each grid unit contained a control food strip and a treatment food strip, both of which had been weighed separately prior to use in the experiment.

Feeding assays: Starved sea urchins were put separately in aquaria with running seawater. Each assay consisted of 20 separate aquaria that were monitored every 20 to 30 min. Food strips were removed whenever it appeared that $\frac{1}{2}$ or more of either the treatment or the control food strips had been consumed, or when the experiment terminated (at 07:00 h, the morning after the assay had begun). In cases where the sea urchin either completely consumed both food strips or did not eat at all, the replicate produced no information of relative palatability of the 2 strips and thus was excluded from the statistical analysis. When all replicates were completed, the remaining food strips were collected, separated, dried on a paper towel and weighed. A preliminary test showed that neither grid nor foods absorbed any water during immersion. Results were analyzed using the Mann-Whitney test.

The sea urchin assays were conducted only in Elat because of the low abundance of *Diadema antillarum* in the Caribbean due to the species mass mortality during the 1980s (Lessios et al. 1984).

RESULTS

Fish experiments

Interspecific variation in sponge chemical deterrence

Seventeen abundant Red Sea reef sponges were examined for the existence of deterrent secondary metabolites in their tissue. Seven species (41%) were found to be deterrent when tested against a naturally occurring generalist fish—the Red Sea wrasse *Thalassoma klunzingeri* (Fig. 1). Most extracts showed definitive activity and were either highly deterrent (e.g. *Ophlitaspongia arbuscula* and *Amphimedon viridis*) or palatable (e.g. *Petrosia* sp. and *Diacarnus erethrianus*).

No obvious pattern was found between sponge taxonomy and degree of deterrence. Species belonging to the same family yielded both palatable and deterrent extracts (e.g. *Diacarnus erethrianus* vs *Negombata magnifica* and *Amphimedon viridis* vs *Niphates* sp.). Interestingly, the aspiculated sponges in this survey, *Dysidea* sp., *Hyrtios erecta* and *Hyrtios nigra* (Order: Dictyoceratida), yielded palatable extracts.

Extracts of the 17 Red Sea sponge species were also examined in feeding experiments using the Caribbean wrasse *Thalassoma bifasciatum* during a research expedition to the Bahamas. All extracts, including those that were deterrent when tested against *T. klunzingeri* in Elat, were palatable to *T. bifasciatum*. We wanted to make sure that the extracts did not decompose during transport from Elat to the Bahamas. To check this, a 'double blind' feeding experiment was performed with extracts from 17 Caribbean sponge species using both the Caribbean wrasse *T. bifasciatum* and the Red Sea wrasse *T. klunzingeri*. Following tests in the Bahamas, subsamples from the same extracts were shipped to Israel under nitrogen gas to be assayed against *T. klunzingeri*. The extracts were sent numbered, so that the identity was unknown during the feeding experiment. The results obtained for both predators are presented in Fig. 2. Only 1 replicate was made with each predator species and, when examined in Israel, only 8 aquaria were used.

Feeding behavior was similar for fish from both geographical areas. Sponge extracts from species such as *Smenospongia aurea*, *Aplysina insularis* and *Ircinia felix* that were found to be highly deterrent against the naturally occurring predator, *Thalassoma bifasciatum*, also deterred *T. klunzingeri*.

Endosome versus ectosome deterency

No significant difference was found in the palatability of extracts of the endosome and the ectosome of 6 Red Sea sponge species (Fig. 3). Furthermore, no difference existed between the ability of each layer to deter predation in comparison to the total crude extract (as shown in Fig. 1).

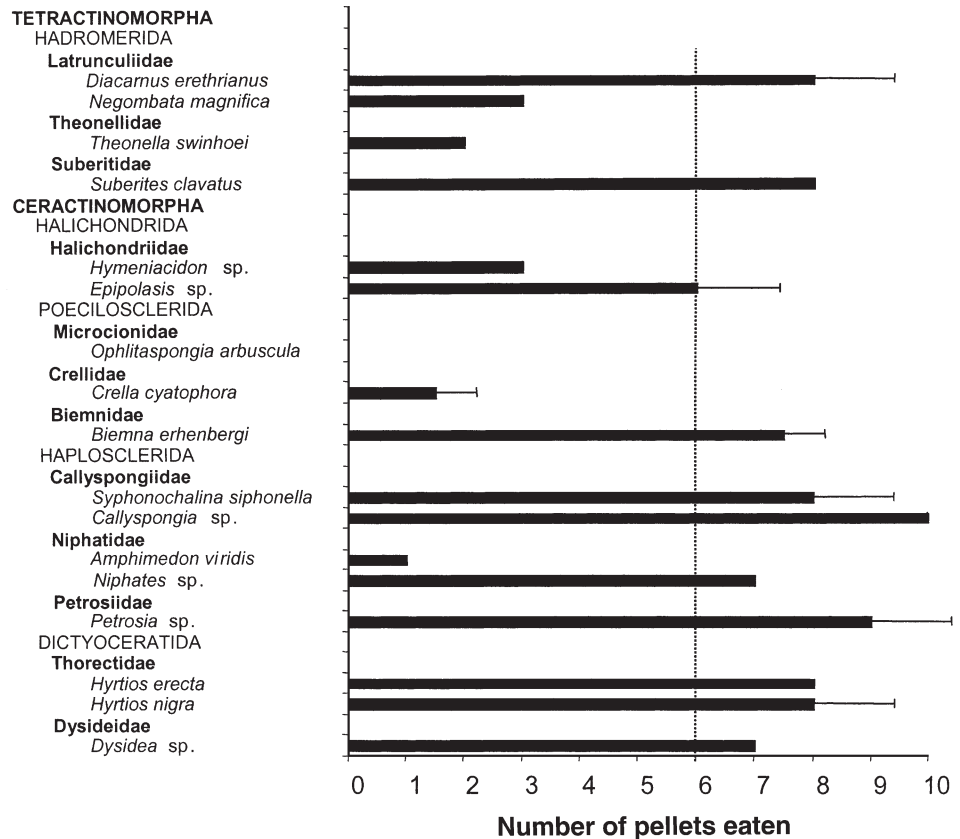
Sea urchin experiments

Interspecific variation in sponge chemical deterency

The ability of 17 abundant Red Sea sponge species to deter predation by the common sea urchin *Diadema setosum* was examined. Results are shown in Fig. 4. A high proportion (65%) of the sponge extracts examined were found to deter *D. setosum* feeding.

There was also a high degree of interspecific variability in the ability of extracts to deter predation. Among the most deterrent species were *Hymeniacion* sp., *Crella cyatophora*, and *Theonella swinhoei*. Other sponge species were either moderately deterrent (e.g. *Diacarnus erethrianus*) or palatable (e.g. *Callyspongia* sp. and *Hyrtios erecta*). Treatment food

Fig. 1. Effect of crude sponge extracts from 17 Red Sea sponges on predation by *Thalassoma klunzingeri* (mean + SD). Food pellets contained organic extracts at natural concentrations. Fish consumed all 10 control pellets in all cases (2 replicate assays for every species). For any individual assay, an extract was considered deterrent if the number of pellets eaten was ≤ 6 ($p \leq 0.043$, Fisher's exact test, 1-tailed) as indicated by the dotted line on the figure



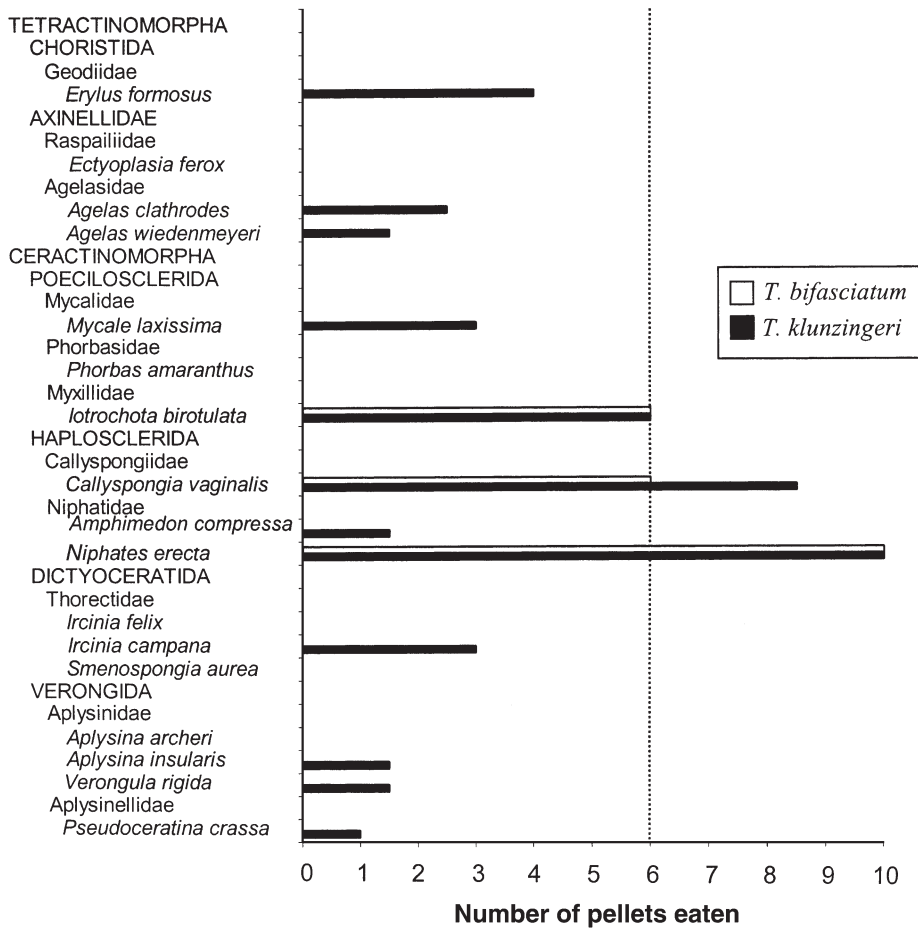


Fig. 2. *Thalassoma klunzingeri* and *T. bifasciatum*. Consumption of food pellets containing organic extracts of 17 Caribbean sponges at natural concentrations. Fish consumed all control pellets in all cases. For any individual assay, an extract was considered deterrent if the number of pellets eaten was ≤ 6 ($p \leq 0.043$, Fisher's exact test, 1-tailed) as indicated by the dotted line on the figure. In order to show the results from both locations on the same scale, we have standardized the results as if 10 aquaria had been used (which explains the fractionated values, even though only 1 replicate was made)

strips containing extracts of 4 sponges (*Biemna erhenbergi*, *Dysidea* sp., *Niphates* sp., and *Suberites clavatus*) seemed to be preferred by the sea urchins in comparison to the control, but results were not statistically significant.

Endosome versus ectosome detergency

The 6 Red Sea sponge species tested for differences in endosome/ectosome deterrence against fish were also tested against sea urchins. Here too, there was no significant difference between the detergency of the endosome, the ectosome (Fig. 5) and the total crude extract (as shown in Fig. 4), with 1 exception—the extract from the endosome of *Diacarnus erethrianus* was significantly deterrent ($p < 0.05$) while the ectosome extract from this sponge was palatable.

DISCUSSION

This is the first survey to examine the scope of the chemical anti-predatory defense mechanism in Red

Sea sponges. Crude organic extracts of Red Sea sponges were shown to have a wide range of effects on food consumption by the Red Sea wrasse *Thalassoma klunzingeri* and the sea urchin *Diadema setosum*. These effects are similar to those shown with extracts from Caribbean sponges on the Caribbean blue-headed wrasse *T. bifasciatum* (Pawlik et al. 1995). On Caribbean reefs, fish represent the dominant predator (Randall & Hartman 1968, McClintock et al. 1997). In the Red Sea, in addition to fish, sea urchins also represent major sponge predators (Meroz & Ilan 1995) with the most abundant species being *D. setosum* (Rogel 2001). Thus, we examined the ability of sponge metabolites to inhibit predation by these 2 predators.

Of the Red Sea sponges examined, 41% (7 species) were found to produce secondary metabolites that deterred feeding by *Thalassoma klunzingeri* (Fig. 1). In the Bahamas 69% (49 species) of the sponge species tested were found to deter predation by the Caribbean wrasse *T. bifasciatum* (Pawlik et al. 1995). As mentioned above, predation pressure on Caribbean reefs is caused mainly by fish, and Caribbean sponges may address their defense mainly against fish. Red Sea sponges, in addition to defense against fish, also have

to defend themselves against potential predation by sea urchins, especially *Diadema setosum*. The Caribbean *D. antillarum* was common on Caribbean reefs until an epidemic in 1983 (Lessios et al. 1984). It

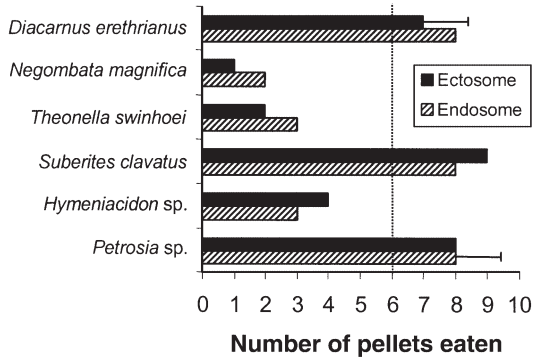


Fig. 3. *Thalassoma klunzingeri*. Consumption of food pellets (mean + SD) containing organic extracts from endosome and ectosome layers of 6 Red Sea sponges at natural concentrations. Fish consumed all 10 control pellets in all cases (2 replicate assays for every species). For any individual assay, an extract was considered deterrent if the number of pellets eaten was ≤ 6 ($p \leq 0.043$, Fisher's exact test, 1-tailed) as indicated by the dotted line on the figure

is therefore possible that Caribbean sponges may also chemically deter predation by sea urchins, but we did not examine this point because of the low abundance of *D. antillarum* on the Caribbean reefs at the present time.

No consistent pattern of deterency was found in sponge species within the same order. The only order in which all the examined species were palatable to fish was the Dictyoceratida. This is especially interesting because the latter is an order in which sponges lack the silicate spicules usually occurring in other demosponges. Most Caribbean aspiculated sponges tested by Pawlik et al. (1995) for their chemical defense (from the orders Dictyoceratida and Verongida) deterred predation by *Thalassoma bifasciatum*. The possible defense mechanisms used by such sponges that lack both physical and chemical defense are discussed elsewhere (Burns & Ilan 2003).

Some of the Red Sea sponge species collected in this study belong to genera also found in the Bahamas. In comparing our results with Pawlik et al. (1995), we can state that related species usually showed the same activity when each was tested against the naturally occurring wrasse species. The Caribbean *Amphimedon compressa* and the Red Sea *A. viridis* were both

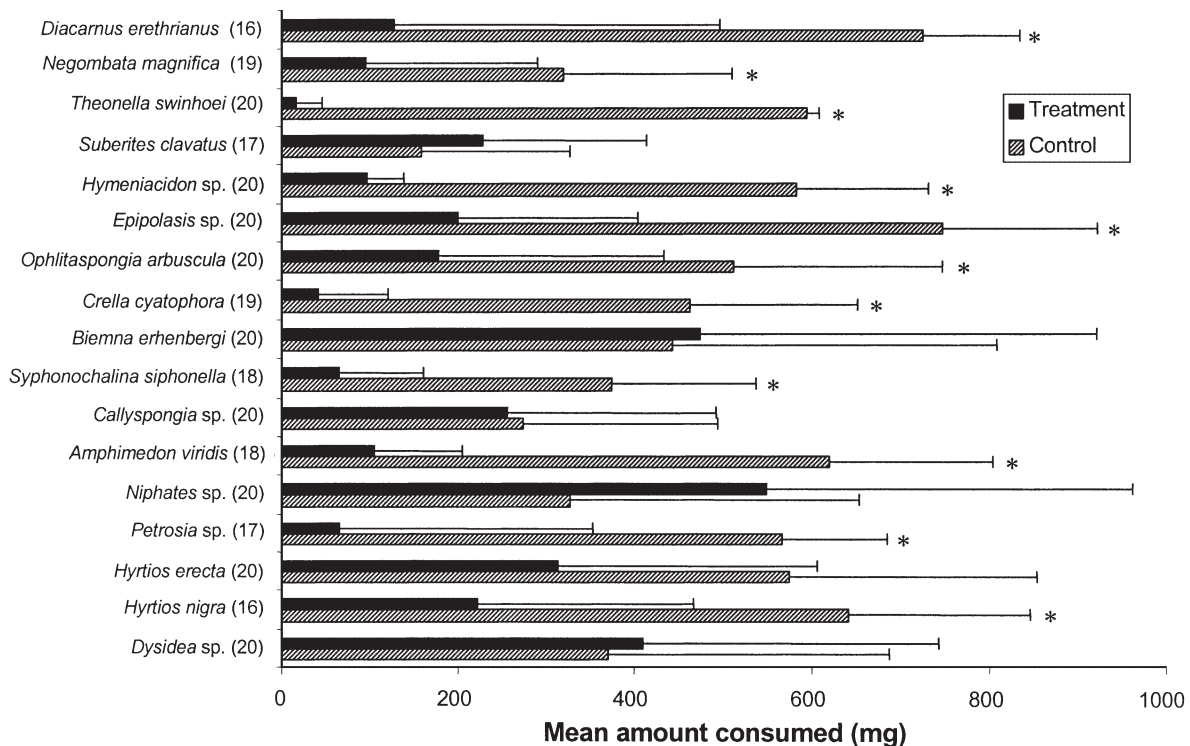


Fig. 4. Effect of crude sponge extracts from 17 Red Sea sponges on predation by *Diadema setosum* (mean + SD). Artificial treatment food contained organic extracts at natural concentration. Number of replicates is shown in parentheses after each sponge species. Results were analyzed using the Mann-Whitney test. * Statistically significant ($p < 0.05$)

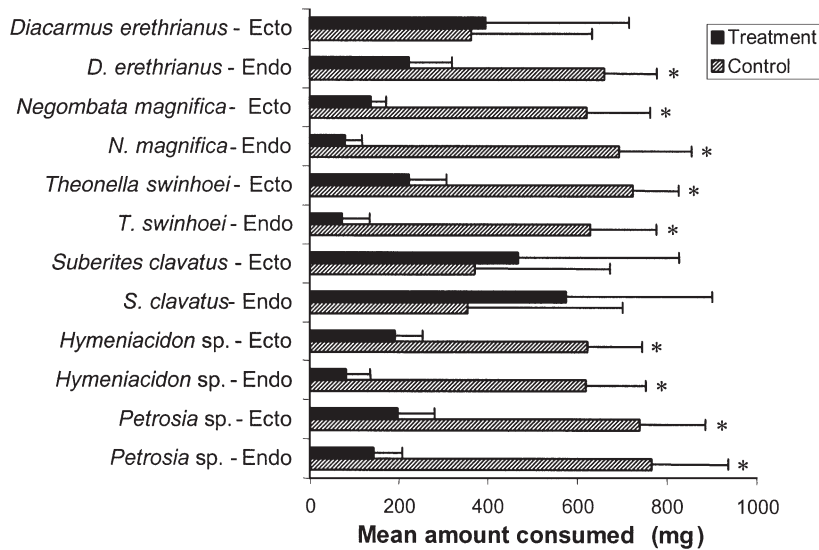


Fig. 5. *Diadema setosum*. Consumption of artificial food (mean + SD) containing organic extracts of endosome and ectosome layers from 6 Red Sea sponges at natural concentrations. Number of replicates for each species was 20 except for the endosome of *Suberites clavatus* in which there were 17 replicates. Results were analyzed using the Mann-Whitney test. *Statistically significant ($p < 0.05$)

highly deterrent against *Thalassoma bifasciatum* and *T. klunzingeri* respectively. *Biemna* and *Callyspongia* species from both reefs were palatable. In some cases, results are only partially compatible. *Dysidea* sp. from the Red Sea is palatable to fish, as is the Caribbean *D. janiae*, but the Caribbean *D. etheria* is deterrent. The Red Sea *Niphates* sp. and the Caribbean *Niphates erecta* are both palatable, but the Caribbean *N. digitalis* shows deterrent activity.

Crude extracts from Caribbean sponges, tested against both the Red Sea wrasse *Thalassoma klunzingeri* and the Caribbean wrasse *T. bifasciatum* (Fig. 2), showed similar activities. These findings supported our assumption that the Red Sea sponge extracts decomposed during transport from Elat to the Bahamas. Further support was given when, in experiments held in the Bahamas a year later, the crude extract of the Red Sea sponge *Crella cyatophora* was found to deter predation by *T. bifasciatum* as opposed to it being palatable in this first experiment (Burns & Ilan 2003). From these data we conclude that the chemical defenses of sponges affect fish predators in a generalized manner.

Although a few sea urchin species are known to graze on sponges (Karlson 1978, Vance 1979), this is the first study to survey the ability of sponge extracts to deter sea urchin grazing. Of the Red Sea sponge species tested, 65% (11 species) deterred grazing by the sea urchin *Dysidea setosum* (Fig. 4). Among these 17 species, 14 grow on hard substrates while 3 live on soft sandy bottoms. These 3 species (*Biemna erhenbergi*,

Dysidea sp. and *Suberites clavatus*) all yielded extracts palatable to *D. setosum*. Because sea urchins graze on hard substrates such as rocks and corals, it is possible that these sponge species do not have to invest energy in chemical means in order to deter predation by sea urchins. *Niphates* sp. and *Callyspongia* sp. also produced palatable extracts. These species may survive predation by investing their energy in reproduction, as the reproductive period of these 2 sponges is long (Ilan & Loya 1988, 1990).

The ability of sponge extracts to deter predation varied between the 2 types of predators used in our assays. Sea urchins were deterred by 65% of sponge extracts whereas fish were deterred by only 41%. The 7 sponge species deterrent to fish were also deterrent to sea urchins, while 4 additional sponge species yielded extracts deterrent only to sea urchins (Table 1). The differences in responses of predators to sponge extracts may be the result of different secondary metabolites

targeted to different predators, or the result of sea urchins being more sensitive to metabolites than fish.

Secondary metabolites that provide anti-predatory chemical defenses have been shown to be concentrated in the body parts that are first encountered by predators in some studies (Avila & Paul 1997). Our

Table 1. Deterrency of Red Sea sponge extracts towards the fish *Thalassoma klunzingeri* and the sea urchin *Diadema setosum*. -: deterrent; +: palatable

Sponge species	Predator	
	<i>Thalassoma klunzingeri</i>	<i>Diadema setosum</i>
<i>Diacarnus erethrianus</i>	+	-
<i>Negombata magnifica</i>	-	-
<i>Theonella swinhoei</i>	-	-
<i>Suberites clavatus</i>	+	+
<i>Hymeniacion</i> sp.	-	-
<i>Epipolasis</i> sp.	-	-
<i>Ophlitaspongia arbuscula</i>	-	-
<i>Crella cyatophora</i>	-	-
<i>Biemna erhenbergi</i>	+	+
<i>Siphonochalina siphonella</i>	+	-
<i>Callyspongia</i> sp.	+	+
<i>Amphimedon viridis</i>	-	-
<i>Niphates</i> sp.	+	+
<i>Petrosia</i> sp.	+	-
<i>Hyrtios erecta</i>	+	+
<i>Hyrtios nigra</i>	+	-
<i>Dysidea</i> sp.	+	+
Total number of deterring species	7	11

results do not support the theory that chemical defenses are optimized by differential allocation. Extracts from both the endosome and ectosome layers of 6 Red Sea sponges showed no difference in their ability to deter either *Thalassoma klunzingeri* (Fig. 3) or *Dysidea setosum* (Fig. 5). Fish bite into the sponge tissue and thus encounter both the ectoderm and endoderm sponge layers. Sea urchins, on the other hand, graze on the outer surface of the sponge and thus encounter mainly the ectoderm layer. If the deterrent secondary metabolites were optimally distributed, one would expect *D. setosum* be deterred by ectoderm extracts and not by endosome extracts. Our results do not show such a distinction. In addition to having the same level of deterrence, the endosome and ectosome extracts also had the same effect as the crude organic extract from the whole sponge tissue taken from each of these sponge species. The only sponge in which the extracts of the 2 layers had a different effect was *Diacarnus erethrianus*. Treatment food containing ectosome extract of *D. erethrianus* were palatable to *D. setosum*, while food containing the endosome extract of this sponge was deterrent. If anything, this result contradicts the optimal defense theory. Therefore we can conclude that in the 6 species tested, deterrent secondary metabolites were not concentrated in the outer layer of the sponge.

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