Chemical defense strategies in sponges: a review

Carsten Thoms, Peter J. Schupp

University of Guam Marine Laboratory, UOG Station, Mangilao, Guam 96923, USA. cthoms@guam.uog.edu, pschupp@guam.uog.edu

Abstract: Sponges, as well as other sessile marine invertebrates, share numerous ecological features with plants and have evolved similar strategies to defend themselves against threats from their biotic environment. Chemical defense plays a preeminent role in this context. General concepts from plant chemical ecology applied to sponges have revealed interesting parallels in regard to resource investment, defense compound allocation, and synergism between chemical and structural defenses. However, these concepts often cannot be generalized in sponges since also numerous contradictory examples exist. Sponges frequently use their compounds as "multi-purpose tools", with concurrent activity against various threats (e.g., predation, pathogens, and biofouling). Apparently, a latitudinal gradient in chemical deterrence, as it was previously shown for marine algae, is lacking in sponges. Recently, facultative defenses (i.e., activated and inducible defenses; immune reactions) have received increased attention in sponge chemical ecology. Although there are examples that clearly demonstrate that these strategies exist in sponges, their number is still very low. One focus of this review is laid on the discussion of the various difficulties inherent to experiments in regards to facultative defenses in sponges that may explain why, as yet, only few studies have found compelling evidence for their existence.

Keywords: activated defense, chemical ecology, growth-differentiation balance hypothesis, inducible defense, optimal defense theory

Introduction

The ecological factors structuring sponge communities resemble in many respects that of plants. Sponges are often abundant and apparent in the habitat they grow in (Bergquist 1978, McClintock *et al*. 2005), lack behavioral defenses, and in many cases they are autotrophic (due to photosynthetic symbionts) (Arillo *et al*. 1993, Usher *et al.* 2001, Hentschel *et al.* 2006). Similar to plants, they are most of the times non-fatally grazed by predators. It is, therefore, not surprising that sponges also have evolved defense strategies similar to those known from terrestrial and aquatic plants. Already in the early 1950s it was discovered that sponges yield secondary metabolites with pronounced bioactivity (Bergmann and Feeney 1950). Since then, more than 5000 compounds have been isolated from sponges (Blunt and Munro 2003). Numerous ecological studies have shown that they often serve defensive purposes to protect the sponges from threats such as predator attacks, microbial infections, biofouling, and overgrowth by other sessile organisms (reviewed in McClintock and Baker 2001, Paul and Puglisi 2004, Paul *et al.* 2006). However, there is also a large number of sponge secondary metabolites with no apparent ecological function. One theory addressing this issue suggests that some secondary metabolites simply do not have any ecological function, but rather represent evolutionary baggage (Jones and Firn 1991, McClintock and Baker 1998). One the other hand, one has to acknowledge that marine natural products research has been and still is driven by pharmacological screening programs, which aim at the discovery of new chemical structures with pharmacological activity rather than investigating possible ecological functions of these compounds.

The results of recent ecological studies indicate that in addition to the simple storage of "chemical weapons" in their tissues sponges have evolved mechanisms to increase the efficiency of their chemical defense, to save metabolic energy invested in their defense, and to protect themselves from cell damage caused by their own bioactive defense compounds. In the following, we review studies that describe such chemical defense strategies in sponges.

Constitutive defense

Optimal defense theory and growth-differentiation balance hypothesis in sponges

The first reports on predation on sponges appeared in the 1960s (Bakus 1966, Randall and Hartman 1968). Nowadays, it is generally accepted that predation has a major impact on sponge ecology, and that sponge populations can be significantly reduced by predators if they are not sufficiently protected (e.g., Hill 1998, Pawlik 1998, Hill *et al*. 2005, Wulff 2006). Chemical defense undoubtedly ranges among the most important anti-predator strategies of sponges (e.g., Pawlik *et al*. 1995, Uriz *et al*. 1996, Wright *et al*. 1997). This holds especially true in habitats with high predation pressure, such as tropical coral reefs (Pawlik 1998), where highly mobile predators such as fishes or turtles can quickly remove substantial biomass, compared to more urchin dominated grazing in temperate regions, or starfish dominated grazing in polar regions (Wright *et al*. 1997, McClintock and Baker 2001, Davis *et al*. 2003).

For plants, there are two prominent hypotheses to explain spatial and temporal variation in defense expression, the growth-differentiation balance hypothesis (GDBH) and the optimal defense theory (ODT). The GDBH assumes that a balance must be maintained between resources invested in growth and in differentiation (which includes the production of defense compounds) (Stamp 2004, Barto and Cippolini 2005). A key premise of this hypothesis is that defense is costly. Due to the high structural complexity of defense compounds in sponges it can be assumed that in many cases their biosynthesis is, indeed, metabolically expensive (Paul 1992, Pawlik 1993). Whereas few studies have assessed the metabolic costs of sponge chemical defense so far, several authors have investigated the interrelations between sponge growth and the investment in chemical defense. Turon *et al*. (1998) reported seasonal patterns in growth rate and toxicity level of the Mediterranean sponge *Crambe crambe* and observed a significant negative correlation between these parameters. Moreover, they found that *C. crambe* growing in shaded areas had lower growth rates but invested more resources in chemical defense than individuals growing in well-illuminated habitats (Turon *et al*. 1998). Wulff (2005) reported a positive correlation between growth rates and palatability to fish predators of twelve reef and mangrove sponge species. Walters and Pawlik (2005) found that sponge species with a pronounced chemical protection had slower wound-healing rates than chemically unprotected species. The authors ascribed this to a trade-off between investment of resources in chemical defense and tissue growth (Walters and Pawlik 2005). However, it has to be noted that growth rates and regeneration capabilities in sponges may not necessarily be equated but can differ substantially in the same species (Reiswig 1973, Ayling 1983). This, in turn, raises interesting questions about differences in resource allocation to each of these processes in relation to investment in anti-predator defenses.

The optimal defense theory (ODT) postulates that defenses are primarily allocated to plant parts of high fitness value (e.g., reproductive tissues) and / or that have a higher risk of predation (Rhoades 1976). By restricting metabolite allocation to these areas rather than distributing them over the entire plant, biosynthesis costs may be lowered. Numerous, but not all studies on terrestrial plants support the ODT (see Baldwin and Ohnmeiss 1994, Zangerl and Rutledge 1996, Heil *et al.* 2002 in support of the ODT, and Zangerl 1986, Zangerl and Nitao 1998 contradicting the theory). Similarly, many but not all studies on marine organisms, such as algae (Cronin and Hay 1996, van Alstyne *et al.* 1999, Pavia *et al.* 2002, Toth *et al.* 2005), sea fans (Dube *et al.* 2002), mollusks (Avila and Paul 1997, Thoms *et al.* 2006a) and brachiopods (Mahon *et al.* 2003) are conform with this hypothesis. This also holds true for sponges, where no clear pattern regarding defense compound allocation has emanated so far.

The Micronesian sponge *Oceanapia* sp. is an example that supports predictions of the ODT, as the sponge allocates the highest concentrations of the pyridoacridine alkaloids kuanoniamine C and D in tissue parts that are most apparent to predators and that most likely play a role in reproduction (Eder *et al*. 1998, Schupp *et al*. 1999). Schupp *et al*. (1999) demonstrated in a series of field and laboratory experiments using different predators that both alkaloids were deterrent at natural concentrations towards generalist reef fish and the spongivorous angelfish *Pomacanthus imperator*. In detailed field experiments using the two major predators Becerro *et al*. (1998) found intracolonial variation of crude organic extracts containing the sesterterpenes scalaradial and desacetylscalaradial in the tropical sponge *Cacospongia* sp. The concentrations were highest at the sponge tips and in the ectosome. However, when tested against fish predators, even the lowest concentration of the extract found in the sponge tissue was already effective. The specialized nudibranch *Glossodoris pallida*, on the other hand, preferred pieces of *Cacospongia* base over tips, thereby selecting the chemically less defended sponge parts (Becerro *et al*. 1998). *Latrunculia apicalis*, a spherically shaped Antarctic sponge, is protected against the keystone spongivorous sea star *Perknaster fuscus* by the sequestration of discorhabdin G. Consistent with the ODT the concentration of this alkaloid decreases rapidly from the surface tissue of the sponge towards its core (Furrow *et al*. 2003). In the tropical sponge *Ectyoplasia ferox* the concentrations of defensive triterpene glycosides were found to be approximately twice as high in the outer 2 mm layer than in the deeper tissue layers of this sponge (Kubanek *et al*. 2002), a finding that again supports the ODT. However, the same study reported that in the sponge *Erylus formosus* concentrations of the defensive triterpene glycoside formoside were only about one-third as high in the outer 1 mm layer of the sponge as in its more interior layers (Kubanek *et al*. 2002). Swaeringen and Pawlik (1998), studying chemical gradients in sponge tissue as well as differences in antifeeding properties against predators in the field, found no evidence that deterrent compounds were concentrated in the surface tissues of the sponge *Chondrilla nucula* collected from the Bahamas and Florida. Becerro *et al*. (1995) found no differences in toxicity between the periphery and the central parts of the Mediterranean sponge *Crambe crambe*. However, in this study toxicity was only evaluated by a Microtox bioassay and no feeding experiments with predators in the field were conducted. Burns *et al*. (2003) reported no difference in deterrence towards the wrasse *Thalassoma klunzingeri* and the sea urchin *Diadema setosum* when they compared extracts of ectosome and endosome layers of six sponges from the Red Sea. Furrow *et al*. (2003) offer a possible explanation for the discrepancy between studies comparing inner and outer tissue layers of sponges in regard to the ODT: they suggest that sequestration of anti-predatory metabolites primarily to the outermost layers in Antarctic sponges such as *L. apicalis* could be highly adaptive because of the ubiquity of sea star sponge predators in Antarctic marine benthic environments. Other than fish, whose bites penetrate well below the sponge surface, sea stars feed on sponges by extrusion of the cardiac stomach for external digestion. This feeding behavior could be a particularly strong selective force for surface sequestration of chemical defenses (Furrow *et al*. 2003). Thus, differential distribution of defensive secondary metabolites to outer compared to inner layers may reflect the feeding behavior of the predominant predators in the respective habitat (i.e., surface feeding affecting only the ectosome versus biting larger pieces including both ecto- and endosomal layers), and may, therefore, not always be present. Moreover, the often amorphous morphology and anatomy of sponges as well as their extraordinary ability to rapidly regenerate lost tissue after wounding may complicate the assignment of high fitness value to distinct parts of their body. This may further explain why defensive metabolite allocation in accordance with the ODT is less apparent in sponges than in other sessile organisms.

It is conceivable that the efficiency of chemical defense can also be optimized by utilizing the same compound for different ecological purposes. Biosynthesis costs may be saved, if instead of producing several compounds for multiple purposes, only one metabolite is sequestered that is active against a variety of target organisms and other threats. However, Schmitt *et al*. (1995) pointed out that multiple uses of defensive compounds could limit adaptive changes following the evolution of resistance to these compounds by the affected organisms (e.g., predators). The first study to assess this topic in sponges was conducted by Thompson *et al*. (1985). They tested 28 compounds isolated from eight sponge species for a broad range of bioactivities including antimicrobial properties, inhibition of larval settlement, fish toxicity, inhibition of sexual reproduction, and anti-predator activity. Most of the compounds tested showed activity in at least one assay, but usually they were active in several of these tests. Bobzin and Faulkner (1992) tested the metabolites manool and cholesterol endoperoxide isolated from the Bahamian sponge *Aplysilla glacialis* for their feeding deterrent and antifouling properties. Whereas the compounds significantly deterred feeding by fish, they actually increased the rate of fouling. Becerro *et al.* (1997) tested three fractions of different polarity of crude extracts from the sponges *Crambe crambe* and *Hemimycale columella* for their inhibitory activity against cell division, photosynthesis, and settlement of organisms growing in the same habitat. They found that several compounds in these fractions displayed multiple activities and concluded that secondary metabolites may be "multi-purpose tools". Thacker *et al.* (1998) reported that 7-deacetoxyolepupuane, a secondary metabolite isolated from *Dysidea* sp., caused necrosis in the competing sponge *Cacospongia* sp., and additionally showed feeding-deterrent activity against fish. Newbold *et al*. (1999) observed that certain sponge crude extracts with anti-feeding activity against fishes (Pawlik *et al*. 1995) at the same time inhibited growth of marine bacteria. In a follow-up study, several of these extracts were also tested for activity against bacterial attachment (Kelly *et al*. 2003). Seven compounds from different sponges were isolated and identified that proved to be active in both deterring predators and inhibiting bacterial attachment (Kelly *et al*. 2003). Kubanek *et al*. (2002) reported multiple defensive roles for triterpene glycosides isolated from *Erylus formosus* and *Ectyoplasia ferox*, two Caribbean sponges belonging to different taxonomic orders. Formoside and other triterpene glycosides from *Erylus formosus* concurrently deterred predators, inhibited microbial attachment and prevented fouling by invertebrates and algae, whereas triterpene glycosides from *Ectyoplasia ferox* had both antipredatory and allelopathic activities (Kubanek *et al*. 2002).

For terrestrial plants it has been reported that concentrations of chemical defenses are significantly higher in species growing in tropical than in temperate forests (Levin and York 1978, Coley and Aide 1991). This has been interpreted as an evolutionary response to greater herbivory in the tropics (Coley and Aide 1991). Similar observations have been made for marine algae: tropical algae yield higher numbers and more deterrent secondary metabolites (Faulkner 1984, Hay and Fenical 1988, Hay 1996, Bolser and Hay 1996) and, thus, seem to be better defended than temperate species. Again, this has been attributed to the higher number of herbivorous fish on tropical compared to temperate reefs (Bolser and Hay 1996, Meekan and Choat 1997). Since sessile invertebrates in tropical coral reefs do, indeed, suffer greater predation pressure than in any other marine environment (Vermeij 1978, Carpenter 1997), it seemed not surprising that Bakus and Green (1974) found an inverse relationship between latitude and ichthyotoxicity in sponges. However, several subsequent studies did not find support for this latitudinal gradient theory (e.g., McCaffrey and Endean 1985, McClintock 1987, van de Vyver *et al*. 1990). This motivated Becerro *et al*. (2003) to test this theory by directly comparing chemical defenses from tropical and temperate sponges (collected from Guam and the Mediterranean Spanish coast respectively). Contrary to their predictions, they found the chemical defenses of tropical and temperate sponges to be equally effective against both sympatric (i.e., co-occurring with the sponges) and allopatric (i.e., not sharing habitat with the prey sponges) predatory fish. However, the authors point out that their results may be due to a response of the sponges and their predators to specific traits of the areas they investigated. They advise to be cautious with generalizing their results until they are confirmed by studies in other geographic areas (Becerro *et al*. 2003).

Interactions of chemical and structural features in sponge defense

It has been well documented that structural features in plants can also act as a defense against predators (e.g., McNaughton *et al*. 1985, Pennings and Paul 1992). In sponges inorganic spicules can amount for up to 75% of the total dry mass (Rützler and Macintyre 1978) and are often arranged with their sharp end towards or protruding the sponge surface (Uriz *et al*. 2003). Thus, it was hypothesized already early on that these skeletal components of sponges provide anti-predator defense, too (Randall and Hartman 1968, Sarà and Vacelet 1973). The most likely mechanism of action for sponge spicules is abrasion or injury of feeding structures (e.g., mouth parts, lining of the digestive system), as has been observed in the gut of the hawksbill turtle (Meylan 1988). Nevertheless, several studies on the antipredatory properties of sponge spicules found results contrary to this assumption and concluded that sponges may have evolved spicules solely for structural purposes (Chanas and Pawlik 1995, 1996, Waddell and Pawlik 2000). However, an additive or even synergistic feeding deterrent effect between sponge spicules and secondary metabolites is conceivable if it is assumed that spicules act as an abrasive while passing through the gut of a potential predator. This way, they may facilitate or enhance the action of defense compounds (Hill *et al*. 2005).

Similar synergistic effects have been observed in plants (e.g., Pennings 1996). In recent studies, Hill *et al*. (2005) as well as Jones *et al*. (2005) analyzed interactions between sponge spicules and secondary metabolites in the context of predator deterrence. Both studies found examples of synergistic or additive effects in 1 of 4 and 4 of 8 tested sponge species, respectively. However, both studies came to the conclusion that synergism between structural and chemical defense cannot be considered the general rule in sponges. Moreover, differential results of various studies dealing with the effectiveness of structural defenses in sponges suggest that the results cannot be extended to all predators and different predator species can be affected differently by sponge spicules in their diets (Paul and Puglisi 2004).

Inducible defense and immune reactions

Inducible defenses were defined by Harvell (1990) as "responses activated through a previous encounter with a consumer or competitor that confer some degree of resistance to subsequent attacks". Inducible defenses are most common if levels of disturbing impacts are unpredictable and display a high spatial or temporal variability (Harvell 1990, Zangerl and Rutledge 1996, Toth and Pavia 2007). Under these circumstances they can be more economical and effective against herbivores than constitutive defenses (Karban *et al*. 1997, Heil 2002, Toth and Pavia 2007). In contrast to plants, sponge chemical defense has largely been considered static. Only in recent years a small number of studies has looked into facultative, inducible defense mechanisms in this phylum. Thacker *et al*. (1998) investigated changes in the chemical profiles of the Indopacific sponges *Dysidea* sp. and *Cacospongia* sp. in the process of the former overgrowing the latter. They found no changes in the chemistry of *Dysidea* sp., but observed an increase in quantity of organic extract in portions of *Cacospongia* sp. that were covered by agar strips containing *Dysidea* crude extract, suggesting an induced defense against overgrowth. Richelle-Maurer *et al*. (2003) detected a sharp increase in the concentrations of the alkaloids oroidin and sceptrin in the Caribbean sponge *Agelas conifera* after experimental simulation of predator bites. Both compounds deterred feeding when tested at near natural concentrations against the predatory reef fish *Stegastes partitus* (Richelle-Maurer *et al*. 2003). A mixture of the compounds also proved to be active against the coral *Madracis mirabilis*, a potential competitor for space. Addition of the two compounds to ambient seawater at 0.0125% of the natural sponge concentration resulted in closure and retraction of the coral polyps. However, forced confrontation of *A. conifera* with the corals did not yield measurable changes in oroidin and sceptrin concentrations in the sponge tissue (Richelle-Maurer *et al*. 2003).

An explanation for the low number of studies on inducible defenses in sponges may be that changes in chemical profiles most likely are a function of numerous biotic and abiotic factors influencing secondary metabolite biosynthesis (Thompson *et al*. 1987, Becerro *et al*. 1995, Turon *et al*. 1996). Moreover, unfavorable influences from the environment can do both increase defense compound metabolism (as a defensive response to the influencing factor) or decrease investment in the secondary metabolite production, if energy is preferentially invested in cell repair (Agell *et al*. 2001, Walters and Pawlik 2005). As induced reactions in the chemical profile often are observable only after days or even weeks following the inducing event (Taylor *et al*. 2002, Richelle-Maurer 2003), this severely complicates interpretations on interrelations between observed secondary metabolite changes and assumed inducing factors.

Müller and coworkers approached this problem by investigating adaptive antibacterial responses in sponges at the genetic level (Müller and Müller 2003). They found various immune reactions, primarily in *Suberites domuncula*, and described the signal transduction pathways as well as the defensive agents involved. The sponge responded to treatment with the bacterial endotoxin lipopolysaccharide (LPS) (Müller *et al*. 2004) with increased biosynthesis of two alkyl-lipid derivatives, 1-O-hexadecyl-sn-glycero-3-phosphocholine and 1-O-octadecyl-sn-glycero-3-phosphocholine. Both compounds showed pronounced activity in an antibacterial assay. In order to prove that the compounds were indeed produced by *S. domuncula*, a key enzyme of their biosynthetic pathway was cloned from the sponge.

In a subsequent study, Wiens *et al*. (2005) discovered a receptor for LPS at the surface of cells from *S. domuncula*. They identified a signal transduction pathway that is induced upon elevated LPS levels and resulted in the enhanced expression of a perforin-like protein primarily at the sponge surface. The protein eliminates Gram-negative bacteria, whereas it is inactive against Gram-positive species. Based on these findings the authors concluded that the sponge *S. domuncula* possesses an innate immune system against Gram-negative bacteria (Wiens *et al*. 2005). Thakur *et al*. (2005) were able to show that *S. domuncula* also exhibits immune reactions against Gram-positive bacteria. The sponge reacts to exposure to peptidoglycan – the characteristic cell wall component of Gram-positive bacteria – with activation of endocytosis and release of lysozyme. Activation of endocytosis was determined by differential expression of an adaptor gene (AdaPTin-1) isolated from the sponge that encodes for a putative protein involved in endosome formation (Thakur *et al*. 2005). The release of lysozyme results in digestion and, thus, in elimination of the bacteria. Immunofluorescence studies with antibodies raised against lysozyme revealed that this immune reaction is targeted exclusively against extracellular bacteria in the sponge mesohyl and not against potentially symbiotic bacteria located in sponge bacteriocytes (Thakur *et al*. 2005).

These examples clearly demonstrate that sponges, indeed, have inducible defenses and immune reactions. The application of biomolecular techniques to analyze the responses of sponges toward predator or pathogen attacks on the gene expression level may help to unravel mechanisms that otherwise are concealed by the complexity of factors influencing the sponge secondary chemistry.

Activated defense

Rapid wound-induced conversions of stored precursors to potent defensive compounds have been referred to with various terms, including "short-term inducible defense

(STID)" (Haukioja 1980, Clausen *et al*. 1989), "dynamic defense" (Reichardt *et al*. 1990) and "induced direct defense" (van Hulten *et al*. 2006). Paul and van Alstyne (1992), when reporting the first defense of this type in the marine environment, termed this process "activated defense" to clearly distinguish it from the predator-induced biosynthesis of defensive metabolites (see "inducible defense"). By converting inactive or less active precursors to defense metabolites with pronounced activity only upon tissue damage and locally restricted to the wounded tissue area, the risk of autotoxicity caused by the defensive conversion products can be alleviated (Saunders *et al*. 1977, Frehner and Conn 1987, Poulton 1988). Typically, the rapid activated defense reactions are catalyzed by enzymes that – upon disruption of tissue compartments – get into contact with the precursors and facilitate the conversion reactions (Matile 1984, Wittstock and Gershenzon 2002).

Activated chemical defenses are widespread in terrestrial vascular plants. The most prominent example is the conversion of cyanogenic glycosides to HCN (e.g., Jones 1988, Seigler 1991, Wajant and Effenberger 1996, Gleadow and Woodrow 2002, and references cited therein). Numerous analogous defense mechanisms in the terrestrial environment involve other molecules such as glucosinolates, phenolic glycosides, and sesquiterpenes (Sterner *et al*. 1985, Clausen *et al*. 1989, Stoewsand 1995, Fahey *et al*. 2001).

In aquatic habitats, activated defenses so far have predominantly been found in plants [see reviews by Paul and Puglisi (2004) and Pohnert (2004)]. Reported examples include numerous macroalgal species (e.g., Paul and van Alstyne 1992, Cetrulo and Hay 2000, Jung *et al*. 2002, van Alstyne and Houser 2003) as well as planktonic diatoms and dinoflagellates (Pohnert 2005, Strom *et al*. 2003). In contrast, there are very few reports on analogous defense mechanisms in sessile marine invertebrates. It is yet unresolved whether this is due to a limited distribution of this strategy among invertebrates or rather reflects the fact that the majority of ecological studies so far have focused on constitutive defenses. When discussing the possibility of activated defenses in sponges, it is interesting to note that secondary metabolites in sponges are often stored in specialized cells (e.g., spherulous cells, choanocytes), which may provide the necessary compartments to separate precursors from converting enzymes – a prerequisite for activated defense reactions (e.g., Thompson *et al.* 1983, Turon *et al.* 2000, Richelle-Maurer *et al.* 2003).

To our knowledge, to date only two examples of activated defenses in sessile marine invertebrates have been reported – one occurring in the sponge genus *Aplysina* (Teeyapant and Proksch 1993, Weiss *et al.* 1996, Ebel *et al.* 1997, Thoms *et al.* 2004, 2006b), the other in the marine hydroid species *Tridentata marginata* (Lindquist 2002). Ettinger-Epstein *et al.* (2007) recently observed a deacetylation of acetylated sesterterpenes in *Luffariella variabilis* to the corresponding alcohols when they thawed frozen tissue of the sponge. Since the compounds were stable when isolated from the tissue, the authors proposed that the conversion to the alcohols may be enzyme-mediated. Further, they speculated about a role of this reaction as an activated defense, but have not tested this hypothesis yet (Ettinger-Epstein *et al.* 2007). Recently, we found another example of an activated defense in the sponge *Aplysinella rhax*, which we presented at the 7th International Sponge Symposium (Thoms and Schupp 2006). Despite these reported examples, the existence of activated defenses in sponges has been a controversially discussed topic (see Puyana *et al.* 2003 and Thoms *et al.* 2006b). Here, we highlight the various aspects that need to be considered when examining chemical profiles of sponges for changes that may be interpreted as activated defenses. Moreover, we point out various methodological constraints (some being more and some less sponge-specific) that complicate interpretations in this context and, therefore, resulted in this controversy.

Sample handling and preservation

Due to the circumstances involved in marine sampling (e.g., wave action and currents during sampling, transport and storage during dive and on the boat, etc.) sponge samples are at high risk of unintentional damage before they are finally preserved. Thus, to gain insight into the chemistry of an intact sponge, it is necessary to minimize damage as well as transportation times (e.g., by using sturdy sample containers instead of plastic bags; by on-board preservation of the samples, etc.). Further, the method of preservation can considerably impact the "intactness" of the analyzed chemical profile. Freezing wet sponge tissue results in the formation of intracellular ice crystals that may cause decompartmentalization by disrupting cellular membranes (Hällgren and Öquist 1990). Upon thawing, enzymes may be reactivated and catalyze conversion reactions (Gahan 1981, Ettinger-Eppstein *et al.* 2007). Interestingly, a similar effect can be caused by extraction or preservation of wet sponge tissue with organic solvents (Teeyapant and Proksch 1993, Thoms and Schupp unpublished). This, at first, may seem surprising, since enzymes usually are considered sensitive to contact with organic solvents. However, sustained catalytic activity of enzymes in aqueous solvents, as it may occur when wet sponge tissue gets gradually soaked during the extraction or preservation procedure, is a known phenomenon (see Klibanov 2001 and references cited therein). If the disintegrating effect of organic solvents on biomembranes (Jones 1989, Weber and deBont 1996) causes decompartmentalization within the sponge tissue, contact between the active enzymes and the precursors may be facilitated and the conversion reactions can take place. To ensure enzyme inactivation in the sponge tissue, samples should, therefore, be processed by flash-freezing and subsequent lyophilization.

The existence of activated chemical defenses in sponges is a rather recent concept and, thus, earlier studies did not necessarily have possible enzymatic reactions in sponge tissue in mind. This may explain why several compounds that originally were considered constitutive in sponges (Fattorusso *et al.* 1970, Kernan *et al.* 1987, Shin *et al.* 2000) later revealed to be conversion products (Thoms *et al.* 2006b, Ettinger-Epstein *et al.* 2007, Thoms and Schupp unpublished).

Natural variability of the sponge chemistry

Many sponge species display pronounced variability in their chemical profiles. Not only do individuals of the same species show considerable quantitative and qualitative differences in their secondary metabolite chemistry, but even within single individuals vast divergences are observed (Schupp *et al.* 1999, Furrow *et al.* 2003, Thoms *et al.* 2006b). This variability can do both conceal activated defense reactions as well as falsely hint to them. To avoid misinterpretations, each study on activated defenses should be preceded by a survey on the chemistry of intact individuals under various natural conditions.

Interestingly, all activated defenses in sessile marine invertebrates discovered so far involve components that are easily detectable by HPLC-UV and are present in the organisms' tissues in extraordinarily high concentrations (Lindquist 2002, Thoms *et al.* 2006b, Thoms and Schupp unpublished). However, secondary metabolites can possess pronounced activity and mediate ecological interactions even at minute concentrations (Paul and Puglisi 2004, Paul *et al.* 2006). Moreover, due to specific chemical characteristics (e.g., lack of chromophores) compounds involved in defense reactions may not be readily detectable by standard chemoanalytical techniques. Therefore, changes in the chemical profiles may not always become apparent, even if they have major ecological effects.

Pronounced natural variability also entails difficulties for data analysis of wounding experiments. Substantial fluctuations of compound concentrations may impede validation of observed wound-activated changes. It may be reasonable to analyze shifts in relative compound proportions rather than measuring changes in their absolute concentrations if the relative pattern of the sponge's chemistry turns out to be more uniform. Further, if there is more than one assumed precursor and/or product, pooling their respective concentrations can help to identify wound-activated changes. High intensities of wounding – even if ecologically irrelevant – can help to initially observe wound-activated chemical reactions. By gradually decreasing wounding intensity in a series of samples and analyzing the resulting chemical profiles, a causal link between wounding and the reactions in the chemical profiles can be investigated.

Field experiments versus laboratory experiments

Generally, field experiments are clearly to be favored over laboratory experiments when investigating ecological phenomena. However, investigations on activated defenses in sponges in the field entail several experimental constraints. If wounding is caused to sponges *in situ*, i.e., in their natural habitat, usually a time-consuming sampling procedure has to follow (underwater bagging, transportation to the surface, etc.) before the samples can be preserved and enzymatic reactions can be stopped. Underwater handling of the samples entails the risk of elution of the conversion products from the sponge tissue, especially if hydrophilic compounds are formed. This can be minimized if after wounding samples are immediately sealed underwater in small containers and if the ambient seawater in these containers is analyzed as well. Since wound-activated reactions often occur within seconds (Paul and van Alstyne 1992, Pohnert 2000, Jung and Pohnert 2001, Thoms *et al.* 2006b, Thoms and Schupp unpublished), a prolonged sampling procedure precludes monitoring the conversion event over time. Thus, only start and end points of the conversion reactions can be appropriately analyzed in field experiments. Moreover, it is difficult to determine the effect of defined wounding intensities in field experiments, since unintentional damage in the course of sample handling are likely to occur.

Due to these difficulties, laboratory experiments conducted in seawater tanks with carefully handled, entire sponge individuals may be a reasonable alternative that allows for more controllable conditions. By ensuring the healthy condition of the sponges (e.g., by using individuals without any signs of damage and with open oscules indicating metabolic activity) and by comparing their chemical profiles to those of intact sponges in the field, bias caused by the laboratory conditions can be minimized.

Determining ecological relevance and target organisms

Determining wound-activated reactions in chemical profiles of sponges is a matter of careful investigation and use of appropriate analytical techniques (allowing for compound identification, description of reaction kinetics, and determination of enzymatic catalysis). Considerably more ambiguous are interpretations on the ecological relevance of such reactions.

To provide evidence for an activated defense, the conversion of less active precursors into defensive agents with higher activity has to be shown (Paul and van Alstyne 1992). Thus, precursors and products have to be compared in bioassays in their respective naturally occurring concentrations. Since the product concentrations are a function of the wounding caused to the sponge tissue, an ecologically relevant manner of wounding needs to be applied. Often tissue grinding has been employed to elicit wound-activated reactions and the compound concentrations thereof have been used in bioassays to assess the defensive function of the conversion products (e.g., Paul and van Alstyne 1992, Cetrulo and Hay 2000, Jung and Pohnert 2001). Yet, if large tissue pieces are bitten off from the sponge and immediately swallowed by the predator, measurements in ground tissue are likely to overestimate the naturally formed concentrations. Puyana *et al.* (2003) chose stabbing of sponge tissue with a scalpel as an alternative to grinding. This likely resulted in the disruption of tissue compartments at the surface of the scalpel cuts, but left the tissue underneath unaffected. Subsequently, the extracts from entire sponge pieces bearing the scalpel cuts were analyzed (Puyana *et al.* 2003). However, if the ratio of damaged to undamaged tissue in the sampled sponge pieces is low, concentrations of conversion products might become too low for detection. In our recent study on an activated defense in the sponge *Aplysinella rhax* we picked tissue pieces with forceps in order to mimic predator bites and elicit conversion reactions (Thoms and Schupp unpublished). Compound concentrations were analyzed in the picked tissue pieces. At best, these approaches will imitate feeding behavior of one predator type, only. The actual predator may bite off larger or smaller pieces, cause less or more tissue squeezing, or may abrade the surface layers instead (Toth and Pavia 2007). Thus, a reliable comparison of the bioactivity before and after wound-activated conversion is only possible if the actual predator is known and its feeding behavior can appropriately be mimicked. But even if this is feasible, effects on compound concentrations occurring after wounding, such as dilution by seawater or leakage from the sponge tissue, may impede their proper assessment.

Water solubility of the defensive metabolites also poses a challenge to the design of bioassays testing their anti-predator effect. Compound loss from the experimental food needs to be minimized in order to keep the assay conditions constant over the experiment course. However, under natural conditions the defensive agents may be exuded from the tissue directly into the predator's mouth when the tissue gets squeezed and cells disrupt (Thoms and Schupp unpublished) – an effect that can hardly be imitated with food designed to retain the compounds efficiently.

Further, it has to be taken into account that predators may learn to link sensing the precursors with the formation of repellent conversion products (see Chivers and Smith (1998), Rochette *et al.* (1998), and Larson and McCormick (2005) on learning and recognition of chemical cues in potential sponge predators). Thus, a comparative bioassay may not necessarily reveal any difference between the compounds if the predator stops feeding already upon contact with the non-repellent precursors.

The above considerations are based on the assumption that the target organism of an activated defense is a predator and, by this, also represents the elicitor of the defense reactions. Providing evidence for the ecological relevance of an observed wound-activated chemical reaction gets even more intricate if the eliciting organisms and the target organisms are not identical. This, for example, is the case in sponges of the genus *Aplysina* (Thoms *et al.* 2004, 2006b). Here, the conversion precursors possess a pronounced repellent effect against potential fish predators (Thoms *et al*. 2004). If despite this chemical protection the sponge gets wounded, these compounds are enzymatically converted into agents with considerably enhanced antimicrobial properties, presumably providing a barrier against microbial pathogens and protecting the wounded sponge tissue against infection (Thoms *et al.* 2006b). To reveal such functions of activated defense reactions, the search for potential targets has to be based broadly, including both macro- and microorganisms. Moreover, it has to be taken into account that defensive compounds may be active at various scales (e.g., in quantities high enough to overcome dilution effects en route to a predator's olfactory organs, or at only locally arising concentrations that form a barrier against microbes).

Taken together, various methodological constraints as well as inherent limitations on the interpretability of the results considerably complicate the accumulation of evidences for activated defense mechanisms in sponges. This, on the one hand, may explain the low number of reports in this context – with respect to sponges, but also with respect to sessile marine invertebrates in general. On the other hand, this is an exciting challenge for future studies aiming to shed light on the question whether in this group of animals activated defenses are, indeed, isolated phenomena, or may represent a common but as yet largely overlooked strategy.

Conclusions

While considerable work has been done on sponge chemical ecology over the last decade and we are seeing some trends emerging from the multitude of studies, it has to be acknowledged that often ecological concepts can not be generalized. For instance, there are numerous examples that support the optimal defense theory and the growth-differentiation balance hypothesis – but almost as many contradicting them. Many tropical sponge species have a stronger protection against predators than their temperate relatives, however, a general proof for the "latitudinal gradient theory" failed – as a whole, the defensive chemistry of tropical sponges is apparently not more repellent than that of temperate species. Sponges do make use of synergisms between structural and chemical defenses – but not all sponge species do and this strategy is not equally effective against all types of predators.

It is obvious that single concepts are unlikely to be valid for all the numerous sponge species in the multitude of habitats they live in. However, from the studies reviewed it becomes apparent that in many cases evidences for these concepts may be obscured by methodological constraints as well as by the complexity of parameters affecting sponges and their secondary metabolisms. Secondary metabolite profiles of sponges often are characterized by pronounced variability. In fact, sponges have been described as "dynamic multicellular systems" that undergo constant changes in adaptation to altering external factors (Gaino and Magnino 1999). This versatility undoubtedly complicates seeing clear patterns in sponge traits. Moreover, there is evidence that microbial symbionts often contribute substantially to both nutrition and secondary metabolite biosynthesis of sponges (Taylor *et al.* 2007). Still very little is known about these interactions in most sponge species, which severely complicates answering such basic questions as to whether chemical defense is costly for a sponge. Further complexity is added by sponges employing chemical defenses simultaneously against various threats on various sizes of scale (e.g., against predators, competitors, biofouling, pathogens), and using metabolites in multiple ways, being concurrently active against several of these threats ("multi-purpose tools"). The resulting interferences may obscure links between single effects and single causes and, this way, complicate discerning clear defense concepts.

This is similar for facultative defenses. While parallels between sponge and plant ecology make it rational to search for such defenses in sponges (i.e., for activated and inducible defenses), so far only few examples have been identified. Here as well, experimental constraints and interfering parameters complicate investigations on effects and causes. Hence, the question whether facultative defenses in sponges are isolated phenomena or common but as yet largely overlooked strategies remains to be resolved.

In many cases it will be inevitable to evaluate the ecological concepts and defense strategies at the species or even at the individual level to be able to contemplate all the factors that potentially impact their outcome. To break down the complexity of parameters, investigation of certain processes in artificial systems may be necessary. Biomolecular approaches similar to those currently employed to elucidate innate immune reactions in sponges on the genetic level may, in the future, prove useful also to study other aspects of sponge chemical ecology. However, to draw legitimate conclusions on ecological interrelations, findings from *in vitro* experiments should always be validated in natural systems.

Acknowledgements

We thank Claudia Kohlert-Schupp and several colleagues for interesting discussions and three anonymous reviewers for their comments that helped to improve this review. Carsten Thoms gratefully acknowledges a Feodor Lynen Fellowship by the Alexander von Humboldt-Foundation, Bonn, Germany. Peter Schupp acknowledges funding by NIH MBRS SCORE S06-GM44796 and NIH SCORE S06-GM044796-16A1. This is contribution number 608 of the University of Guam Marine Laboratory.

References

Agell G, Uriz MJ, Cebrian E, Marti R (2001) Does stress protein induction by copper modify natural toxicity in sponges? *Environ Toxicol Chem* 20: 2588-2593

Arillo A, Bavestrello G, Burlando B, Sará M (1993) Metabolic integration between symbiotic cyanobacteria and sponges: a possible mechanism. *Mar Biol* 117: 159-162

Avila C, Paul VJ (1997) Chemical ecology of the nudibranch *Glossodoris pallida*: Is the location of diet-derived metabolites important for defense? *Mar Ecol Prog Ser* 150: 171-180

Ayling AL (1983) Growth and regeneration rates in thinly encrusting Demospongiae from temperate waters. *Biol Bull* 165: 343-352

Bakus GJ (1966) Some relationships of fishes to benthic organisms on coral reefs. *Nature* 210: 280-284

Bakus GJ, Green G (1974) Toxicity in sponges and holothurians: a geographic pattern. *Science* 185: 951-953

Baldwin IT, Ohnmeiss TE (1994) Coordination of photosynthesis and alkaloidal responses to damage in uninducible and inducible *Nicotiana sylvestris*. *Ecology* 75: 1003-1014

Barto EK, Cipollini D (2005) Testing the optimal defense theory and the growth-differentiation balance hypothesis in *Arabidopsis thaliana*. *Oecologia* 146: 169-178

Becerro MA, Paul VJ, Starmer J (1998) Intracolonial variation in chemical defenses of the sponge *Cacospongia* sp. and its consequences on generalist fish predators and the specialist nudibranch predator *Glossodoris pallida*. *Mar Ecol Prog Ser* 168: 187-196

Becerro MA, Thacker RW, Turon X, Uriz MJ, Paul VJ (2003) Biogeography of sponge chemical ecology: comparisons of tropical and temperate defenses. *Oecologia* 135: 91-101

Becerro MA, Turon X, Uriz MJ (1995) Natural variation of toxicity in encrusting sponge *Crambe crambe* (Schmidt) in relation to size and environment. *J Chem Ecol* 21: 1931-1946

Becerro MA, Turon X, Uriz MJ (1997) Multiple functions for secondary metabolites in encrusting marine invertebrates. *J Chem Ecol* 23: 1527-1547

Bergmann W, Feeney RJ (1950) The isolation of a new thymine pentoside from sponges. *J Am Chem Soc* 72: 2809-2810

Bergquist PR (1978) *Sponges*. University of California Press, Berkeley

Blunt J, Munro M (2003) *MarinLit. A database of the literature on marine natural products prepared and maintained by the Marine Chemistry Group*. Department of Chemistry, University of Canterbury, Canterbury

Bobzin SC, Faulkner DJ (1992) Chemistry and chemical ecology of the Bahamian sponge *Aplysilla glacialis*. *J Chem Ecol* 18: 309-332

Bolser RC, Hay ME (1996) Are tropical plants better defended? Palatability and defenses of temperate vs. tropical seaweeds. *Ecology* 77: 2269-2286

Burns E, Ifrach I, Carmeli S, Pawlik JR, Ilan M (2003) Comparison of anti-predatory defenses of Red Sea and Caribbean sponges. I. Chemical defense. *Mar Ecol Prog Ser* 252: 105-114

Carpenter RC (1997) Invertebrate predators and grazers. *In:* Birkeland C (ed). *Life and death of coral reefs*. Chapman and Hall, New York. pp. 198-229

Cetrulo GL, Hay ME (2000) Activated chemical defenses in tropical versus temperate seaweeds. *Mar Ecol Prog Ser* 207: 243-253

Chanas B, Pawlik JR (1995) Defenses of Caribbean sponges against predatory reef fish. 2. Spicules, tissue toughness, and nutritional quality. *Mar Ecol Prog Ser* 127: 195-211

Chanas B, Pawlik JR (1996) Does the skeleton of a sponge provide a defense against predatory reef fish? *Oecologia* 107: 225-231

Chivers DP, Smith RJF (1998) Chemical alarm signalling in aquatic predator-prey systems: a review and prospectus. *Ecoscience* 5: 338-352

Clausen TP, Reichardt PB, Bryant JP, Werner RA, Post K, Frisby K (1989) Chemical model for short-term induction in quaking aspen (*Populus tremuloides*) foliage against herbivores. *J Chem Ecol* 15: 2335-2346

Coley PD, Aide TM (1991) Comparison of herbivory and plant defenses in temperate and tropical broad-leaved forests. *In:* Price PW, Lewinsohn TM, Fernandes GW, Benson WW (eds). *Plant-animal interactions: evolutionary ecology in tropical and temperate regions*. Wiley, New York. pp. 25-49

Cronin G, Hay ME (1996) Within plant variation in seaweed palatability and chemical defenses: optimal defense theory versus the growth differentiation balance hypothesis. *Oecologia* 105: 361-368

Davis AR, Fyfe SK, Turon X, Uriz MJ (2003) Size matters sometimes: wall height and the structure of subtidal benthic invertebrate assemblages in south-eastern Australia and Mediterranean Spain. *J Biogeogr* 30: 1797-1807

Dube D, Kim K, Alker AP, Harvell CD (2002) Size structure and geographic variation in chemical resistance of sea fan corals *Gorgonia ventalina* to a fungal pathogen. *Mar Ecol Prog Ser* 231: 139-150

Ebel R, Brenzinger M, Kunze A, Gross HJ, Proksch P (1997) Wound activation of protoxins in marine sponge *Aplysina aerophoba. J Chem Ecol* 23: 1451-1462

Eder C, Schupp P, Proksch P, Wray V, Steube K, Muller CE, Frobenius W, Herderich M, van Soest RWM (1998) Bioactive pyridoacridine alkaloids from the Micronesian sponge *Oceanapia* sp. *J Nat Prod* 61: 301-305

Ettinger-Epstein P, Motti CA, de Nys R, Wright AD, Battershill CN, Tapiolas DM (2007) Acetylated sesterterpenes from the Great Barrier Reef sponge *Luffariella variabilis*. *J Nat Prod* 70: 648-651

Fahey JW, Zalcmann AT, Talalay P (2001) The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. *Phytochemistry* 56: 5-51

Fattorusso E, Minale L, Sodano G (1970) Aeroplysinin-1, a new bromo-compound from *Aplysina aerophoba*. *J Chem Soc D-Chem Commun* 12: 751-753

Faulkner DJ (1984) Marine natural products: metabolites of marine algae and herbivorous marine mollusks. *Nat Prod Rep* 1: 251-280

Frehner M, Conn EE (1987) The linamarin beta-glucosidase in Costa Rica wild bean (*Phaseolus lunatus* L.) is apoplastic. *Plant Physiol* 84: 1296-1300

Furrow FB, Amsler CD, McClintock JB, Baker BJ (2003) Surface sequestration of chemical feeding deterrents in the Antarctic sponge *Latrunculia apicalis* as an optimal defense against sea star spongivory. *Mar Biol* 143: 443-449

Gahan PB (1981) Cell senescence and death in plants. *In:* Bowen ID, Lockshin RA (eds). *Cell death in biology and pathology*. Chapman & Hall, London. pp. 145-169

Gaino E, Magnino G (1999) Dissociated cells of the calcareous sponge *Clathrina*: a model for investigating cell adhesion and cell motility *in vitro*. *Microsc Res Tech* 44: 279-292

Gleadow RM, Woodrow IE (2002) Constraints on effectiveness of cyanogenic glycosides in herbivore defense. *J Chem Ecol* 28: 1301-1313

Hällgren J, Öquist G (1990) Adaptations to low temperatures. *In:* Alscher RG, Cumming JR (eds). *Stress responses in plants: adaptation and acclimation mechanisms*. Wiley-Liss, New York. pp. 265-293

Harvell CD (1990) The ecology and evolution of inducible defenses. *Q Rev Biol* 65: 323-340

Haukioja E (1980) On the role of plant defenses in the fluctuation of herbivore populations. *Oikos* 35: 202-213

Hay ME (1996) Marine chemical ecology: what´s known and what´s next? *J Exp Mar Biol Ecol* 200: 103-134

Hay ME, Fenical W (1988) Marine plant - herbivore interactions: the ecology of chemical defense. *Annu Rev Ecol Syst* 19: 111-145

Heil M (2002) Ecological costs of induced resistance. *Curr Opin Plant Biol* 5: 345-350

Hentschel U, Usher KM, Taylor MW (2006) Marine sponges as microbial fermenters. *FEMS Microbiol Ecol* 55: 167-177

Hill MS (1998) Spongivory on Caribbean reefs releases corals from competition with sponges. *Oecologia* 117: 143-150

Hill MS, Lopez NA, Young KA (2005) Anti-predator defenses in western North Atlantic sponges with evidence of enhanced defense through interactions between spicules and chemicals. *Mar Ecol Prog Ser* 291: 93-102

Jones AC, Blum JE, Pawlik JR (2005) Testing for defensive synergy in Caribbean sponges: Bad taste or glass spicules? *J Exp Mar Biol Ecol* 322: 67-81

Jones CG, Firn RD (1991) On the evolution of plant secondary chemical diversity. *Phil Trans R Soc Lond B Biol Sci* 333: 273-280

Jones DA (1988) Cyanogenesis in animal-plant interactions. *In:* Evered D, Harnett S (eds). *Cyanide compounds in biology*. Wiley, Chichester. pp. 151-170

Jones RP (1989) Biological principles of the effects of ethanol: a review. *Enzyme Microb Technol* 11: 130-152

Jung V, Pohnert G (2001) Rapid wound-activated transformation of the green algal defensive metabolite caulerpenyne. *Tetrahedron* 57: 7169-7172

Jung V, Thibaut T, Meinesz A, Pohnert G (2002) Comparison of the wound-activated transformation of caulerpenyne by invasive and noninvasive *Caulerpa* species of the Mediterranean. *J Chem Ecol* 28: 2091-2105

Karban R, Agrawal AA, Mangel M (1997) The benefits of induced defenses against herbivores. *Ecology* 78: 1351-1355

Kelly SR, Jensen PR, Henkel TP, Fenical W, Pawlik JR (2003) Effects of Caribbean sponge extracts on bacterial attachment. *Aquat Microb Ecol* 31: 175-182

Kernan MR, Faulkner DJ, Jacobs RS (1987) The luffariellins, novel antiinflammatory sesterterpenes of chemotaxonomic importance from the marine sponge *Luffariella variabilis*. *J Org Chem* 52: 3081-3083

Klibanov AM (2001) Improving enzymes by using them in organic solvents. *Nature* 409: 241-246

Kubanek J, Whalen KE, Engel S, Kelly SR, Henkel TP, Fenical W, Pawlik JR (2002) Multiple defensive roles for triterpene glycosides from two Caribbean sponges. *Oecologia* 131: 125-136

Larson JK, McCormick MI (2005) The role of chemical alarm signals in facilitating learned recognition of novel chemical cues in a coral reef fish. *Anim Behav* 69: 51-57

Levin DA, York BM (1978) The toxicity of plant alkaloids: an ecographic perspective. *Biochem Syst Ecol* 6: 61-76

Lindquist N (2002) Tridentatols D-H, nematocyst metabolites and precursors of the activated chemical defense in the marine hydroid *Tridentata marginata* (Kirchenpauer 1864). *J Nat Prod* 65: 681-684

Mahon AR, Amsler CD, McClintock JB, Amsler MO, Baker BJ (2003) Tissue-specific palatability and chemical defenses against macropredators and pathogens in the common articulate brachiopod *Liothyrella uva* from the Antarctic Peninsula. *J Exp Mar Biol Ecol* 290: 197-210

Matile P (1984) The toxic compartment of plant cells. *Naturwissenschaften* 71: 18-24

McCaffrey EJ, Endean R (1985) Antimicrobial activity of tropical and subtropical sponges. *Mar Biol* 89: 1-8

McClintock JB (1987) Investigation of the relationship between invertebrate predation and biochemical composition, energy content, spicule armament and toxicity of benthic sponges at McMurdo Sound, Antarctica. *Mar Biol* 94: 479-487

McClintock JB, Amsler CD, Baker BJ, van Soest RWM (2005) Ecology of Antarctic marine sponges: an overview. *Integr Comp Biol* 45: 359-368

McClintock JB, Baker BJ (1998) Chemical ecology in Antarctic Seas: chemical interactions can lead to unusual arrangements between species. *Am Sci* 86: 254-264

McClintock JB, Baker BJ (2001) Marine chemical ecology. CRC, Boca Raton

McNaughton SJ, Tarrants JL, McNaughton MM (1985) Silica as a defense against herbivory and a growth promotor in African grasses. *Ecology* 66: 528-535

Meekan MG, Choat JH (1997) Latitudinal variation in abundance of herbivorous fishes: a comparison of temperate and tropical reefs. *Mar Biol* 128: 373-383

Meylan A (1988) Spongivory in hawksbill turtles: a diet of glass. *Science* 239: 393-395

Müller WEG, Klemt M, Thakur NL, Schröder HC, Aiello A, D'Esposito M, Menna M, Fattorusso E (2004) Molecular/chemical ecology in sponges: evidence for an adaptive antibacterial response in *Suberites domuncula*. *Mar Biol* 144: 19-29

Müller WEG, Müller IM (2003) Origin of the metazoan immune system: identification of the molecules and their functions in sponges. *Integr Comp Biol* 43: 281-292

Newbold RW, Jensen PR, Fenical W, Pawlik JR (1999) Antimicrobial activity of Caribbean sponge extracts. *Aquat Microb Ecol* 19: 279-284

Paul VJ (1992) Chemical defense of benthic marine invertebrates. *In:* Paul VJ (ed). *Ecological roles of marine natural products*. Cornell University Press (Comstock), Ithaca. pp. 164-188

Paul VJ, van Alstyne KL (1992) Activation of chemical defenses in the tropical green algae *Halimeda* spp. *J Exp Mar Biol Ecol* 160: 191-203

Paul VJ, Puglisi MP (2004) Chemical mediation of interactions among marine organisms. *Nat Prod Rep* 21: 189-209

Paul VJ, Puglisi MP, Ritson-Williams R (2006) Marine chemical ecology. *Nat Prod Rep* 23: 153-180

Pavia H, Toth GB, Aberg P (2002) Optimal defense theory: elasticity analysis as a tool to predict intraplant variation in defenses. *Ecology* 83: 891-897

Pawlik JR (1993) Marine invertebrate chemical defenses. *Chem Rev* 93: 1911-1922

Pawlik JR (1998) Coral reef sponges: do predatory fishes affect their distribution? *Limnol Oceanogr* 43: 1396-1399

Pawlik JR, Chanas B, Toonen RJ, Fenical W (1995) Defenses of Caribbean sponges against predatory reef fish. I. Chemical deterrency. *Mar Ecol Prog Ser* 127: 183-194

Pennings SC (1996) Testing for synergisms between chemical and mineral defenses - a comment. *Ecology* 77: 1948-1950

Pennings SC, Paul VJ (1992) Effect of plant toughness, calcification, and chemistry on herbivory by *Dolabella auricularia*. *Ecology* 73: 1606-1619

Pohnert G (2000) Wound-activated chemical defense in unicellular planktonic algae. *Angew Chem Int Ed* 39: 4352-4254

Pohnert G (2004) Chemical defense strategies of marine organisms. *In:* Schulz S (ed). *Topics in current chemistry, volume 239: the chemistry of pheromones and other semiochemicals I*. Springer, Berlin, Heidelberg. pp. 179-219

Pohnert G (2005) Diatom/copepod interactions in plankton: The indirect chemical defense of unicellular algae. *Chem Biochem* 6: 946-959

Poulton JE (1988) Localization and catabolism of cyanogenic glycosides. *In:* Evered D, Harnett S (eds). *Cyanide compounds in biology*. John Wiley & Sons, Chichester. pp. 67-91

Puyana M, Fenical W, Pawlik JR (2003) Are there activated chemical defenses in sponges of the genus *Aplysina* from the Caribbean? *Mar Ecol Prog Ser* 246: 127-135

Randall JE, Hartman WD (1968) Sponge-feeding fishes of the West Indies. *Mar Biol* 1: 216-225

Reichardt PB, Bryant JP, Mattes BR, Clausen TP, Chapin FS, Meyer M (1990) Winter chemical defense of Alaskan balsam poplar against snowshoe hares. *J Chem Ecol* 16: 1941-1959

Reiswig HM (1973) Population dynamics of three Jamaican Demospongiae. *Bull Mar Sci* 23: 191-226

Rhoades DF, Cates RG (1976) Toward a general theory of plant antiherbivore chemistry. *Recent Adv Phytochem* 10: 168-213

Richelle-Maurer E, de Kluijver MJ, Feio S, Gaudencio S, Gaspar H, Gomez R, Tavares R, van de Vyver G, van Soest RWM (2003) Localization and ecological significance of oroidin and sceptrin in the Caribbean sponge *Agelas conifera*. *Biochem Syst Ecol* 31: 1073-1091

Rochette R, Arsenault DJ, Justome B, Himmelman JH (1998) Chemically-mediated predator-recognition learning in a marine gastropod. *Ecoscience* 5: 353-360

Rützler K, Macintyre I (1978) Siliceous sponge spicules in coral reef sediments. *Mar Biol* 49: 147-159

Sarà M, Vacelet J (1973) Écologie des démosponges. *In:* Grassé P (ed). *Spongiaires. Traité de zoologie, vol. 3*. Masson, Paris. pp. 462-576

Saunders JA, Conn EE, Lin CH, Stocking CR (1977) Subcellular localization of the cyanogenic glycoside of *Sorghum* by autoradiography. *Plant Physiol* 59: 647-652

Schmitt TM, Hay ME, Lindquist N (1995) Constraints on chemically mediated coevolution – multiple functions for seaweed secondary metabolites. *Ecology* 76: 107-123

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

Seigler DS (1991) Cyanide and cyanogenic glycosides. *In:* Rosenthal GA, Berenbaum MR (eds). *Herbivores: their interactions with secondary plant metabolites*. Academic Press, San Diego. pp. 35-78

Shin J, Lee HS, Seo Y, Rho JR, Cho KW, Paul VJ (2000) New bromotyrosine metabolites from the sponge *Aplysinella rhax*. *Tetrahedron* 56: 9071-9077

Stamp N (2004) Can the growth-differentiation balance hypothesis be tested rigorously? *Oikos* 107: 439-448

Sterner O, Bergman R, Kihlberg J, Wickberg B (1985) The sesquiterpenes of *Lactarius vellereus* and their role in a proposed chemical defense system. *J Nat Prod* 48: 279-288

Stoewsand GS (1995) Bioactive organosulfur phytochemicals in *Brassica oleracea* vegetables – a review. *Food Chem Toxicol* 33: 537-543

Strom S, Wolfe G, Slajer A, Lambert S, Clough J (2003) Chemical defense in the microplankton II: Inhibition of protist feeding by beta-dimethylsulfoniopropionate (DMSP). *Limnol Oceanogr* 48: 230-237

Swearingen DC, Pawlik JR (1998) Variability in the chemical defense of the sponge *Chondrilla nucula* against predatory reef fishes. *Mar Biol* 131: 619-627

Taylor RB, Sotka E, Hay ME (2002) Tissue-specific induction of herbivore resistance: seaweed response to amphipod grazing. *Oecologia* 132: 68-76

Taylor MW, Radax R, Steger D, Wagner M (2007) Sponge-associated microorganisms: evolution, ecology, and biotechnological potential. *Microbiol Mol Biol Rev* 71: 295-347

Teeyapant R, Proksch P (1993) Biotransformation of brominated compounds in the marine sponge *Verongia aerophoba*. Evidence for an induced chemical defense? *Naturwissenschaften* 80: 369-370

Thacker RW, Becerro MA, Lumbang WA, Paul VJ (1998) Allelopathic interactions between sponges on a tropical reef. *Ecology* 79: 1740-1750

Thakur NL, Perovic-Ottstadt S, Batel R, Korzhev M, Diehl-Seifert B, Müller IM, Müller WEG (2005) Innate immune defense of the sponge *Suberites domuncula* against gram-positive bacteria: induction of lysozyme and AdaPTin. *Mar Biol* 146: 271-282

Thompson JE, Barrow KD, Faulkner DJ (1983) Localization of two brominated metabolites, aerothionin and homoaerothionin, in spherulous cells of the marine sponge *Aplysina fistularis*. *Acta Zool Stockh* 64: 199-210

Thompson JE, Murphy PT, Bergquist PR, Evans EA (1987) Environmentally induced variation in diterpene composition of the marine sponge *Rhopaloeides odorabile*. *Biochem Syst Ecol* 15: 595-606

Thompson JE, Walker ND, Faulkner DJ (1985) Screening and bioassays for biologically active substances from forty marine sponge species from San Diego, California, USA. *Mar Biol* 88: 11-21

Thoms C, Ebel R, Proksch P (2006a) Sequestration and possible role of dietary alkaloids in the sponge-feeding mollusk *Tylodina perversa*. *In:* Cimino G, Gavagnin M (eds). *Molluscs. Progress in molecular and subcellular biology, vol. 43*. Springer, Berlin. pp. 261-275

Thoms C, Ebel R, Proksch P (2006b) Activated chemical defense in *Aplysina* sponges revisited. *J Chem Ecol* 32: 97-123

Thoms C, Schupp PJ (2006) Activated chemical defense in *Aplysinella rhax*. *In:* Custódio MR, Lôbo-Hajdu G, Hajdu E, Muricy M (eds). *7th International Sponge Symposium - Book of Abstracts (Armação dos Búzios. Brazil)*. Museu Nacional, Série Livros, vol. 16. pp. 143

Thoms C, Wolff M, Padmakumar K, Ebel R, Proksch P (2004) Chemical defense of Mediterranean sponges *Aplysina cavernicola* and *Aplysina aerophoba*. *Z Naturforsch* 59c: 113-122

Toth GB, Langhamer O, Pavia H (2005) Inducible and constitutive defenses of valuable seaweed tissues: consequences for herbivore fitness. *Ecology* 86: 612-618

Toth GB, Pavia H (2007) Induced herbivore resistance in seaweeds: a meta-analysis. *J Ecol* 95: 425-434

Turon X, Becerro MA, Uriz MJ (1996) Seasonal patterns of toxicity in benthic invertebrates: the encrusting sponge *Crambe crambe* (Poecilosclerida). *Oikos* 75: 33-40

Turon X, Becerro MA, Uriz MJ (2000) Distribution of brominated compounds within the sponge *Aplysina aerophoba*: coupling of X-ray microanalysis with cryofixation techniques. *Cell Tissue Res* 301: 311-322

Turon X, Tarjuelo I, Uriz MJ (1998) Growth dynamics and mortality of the encrusting sponge *Crambe crambe* (Poecilosclerida) in contrasting habitats: correlation with population structure and investment in defence. *Funct Ecol* 12: 631-639

Uriz MJ, Turon X, Becerro MA, Agell G (2003) Siliceous spicules and skeleton frameworks in sponges: origin, diversity, ultrastructural patterns, and biological functions. *Microsc Res Tech* 62: 279-299

Uriz MJ, Turon X, Becerro MA, Galera J (1996) Feeding deterrence in sponges. The role of toxicity, physical defenses, energetic contents, and life-history stage. *J Exp Mar Biol Ecol* 205: 187-204

Usher KM, Kuo J, Fromont J, Sutton DC (2001) Vertical transmission of cyanobacterial symbionts in the marine sponge *Chondrilla australiensis* (Demospongiae). *Hydrobiologia* 461: 15-23

van Alstyne KL, Houser LT (2003) Dimethylsulfide release during macroinvertebrate grazing and its role as an activated chemical defense. *Mar Ecol Prog Ser* 250: 175-181

van Alstyne KL, McCarthy JJ, Hustead CL, Kearns LJ (1999) Phlorotannin allocation among tissues of northeastern pacific kelps and rockweeds. *J Phycol* 35: 483-492

van de Vyver G, Huysecom J, Braekman JC, Daloze D (1990) Screening and bioassays for toxic substances in sponges from western Mediterranean Sea and North Brittany. *Vie Milieu* 40: 285-292

van Hulten M, Pelser M, van Loon LC, Pieterse CMJ, Ton J (2006) Costs and benefits of priming for defense in *Arabidopsis*. *Proc Natl Acad Sci USA* 103: 5602-5607

Vermeij GJ (1978) *Biogeography and adaption patterns of marine life*. Harvard University Press, Cambridge

Waddell B, Pawlik JR (2000) Defenses of Caribbean sponges against invertebrate predators. I. Assays with hermit crabs. *Mar Ecol Prog Ser* 125: 125-132

Wajant H, Effenberger F (1996) Hydroxynitrile lyases of higher plants. *Biol Chem* 377: 611-617

Walters KD, Pawlik JR (2005) Is there a trade-off between wound-healing and chemical defenses among Caribbean reef sponges? *Integr Comp Biol* 45: 352-358

Weber FJ, deBont JAM (1996) Adaptation mechanisms of microorganisms to the toxic effects of organic solvents on membranes. *Biochim Biophys Acta-Rev Biomembr* 1286: 225-245

Weiss B, Ebel R, Elbrächter M, Kirchner M, Proksch P (1996) Defense metabolites from the marine sponge *Verongia aerophoba*. *Biochem Syst Ecol* 24: 1-12

Wiens M, Korzhev M, Krasko A, Thakur NL, Perovic-Ottstadt S, Breter HJ, Ushijima H, Diehl-Seifert R, Müller IM, Müller WEG (2005) Innate immune defense of the sponge *Suberites domuncula* against bacteria involves a MyD88-dependent signaling pathway – Induction of a perforin-like molecule. *J Biol Chem* 280: 27949-27959

Wittstock U, Gershenzon J (2002) Constitutive plant toxins and their role in defense against herbivores and pathogens. *Curr Opin Plant Biol* 5: 300-307

Wright JT, Benkendorff K, Davis AR (1997) Habitat associated differences in temperate sponge assemblages: the importance of chemical defence. *J Exp Mar Biol Ecol* 213: 199-213

Wulff JL (2005) Trade-offs in resistance to competitors and predators, and their effects on the diversity of tropical marine sponges. *J Anim Ecol* 74: 313-321

Wulff JL (2006) Ecological interactions of marine sponges. *Can J Zool* 84: 146-166

Zangerl AR (1986) Leaf value and optimal defense in *Pastinaca sativa* L. (Umbelliferae). *Am Midl Nat* 116: 432-436

Zangerl AR, Nitao JK (1998) Optimal defense, kin conflict and the distribution of furanocoumarins among offspring of wild parsnip. *Evolut Ecol* 12: 443-457

Zangerl AR, Rutledge CE (1996) The probability of attack and patterns of constitutive and induced defense: a test of optimal defense theory. *Am Nat* 147: 599-608