Vol. 23: 129–137, 2015
doi: 10.3354/ab00614

AQUATIC BIOLOGY
Aquat Biol

Published online January 22

Chemical versus structural defense against fish predation in two dominant soft coral species (Xeniidae) in the Red Sea

Ben Xuan Hoang[1,2,*], Yvonne Sawall[1], Abdulmohsin Al-Sofyani[3], Martin Wahl[1]

[1]Helmholtz Centre for Ocean Research, GEOMAR, Wischhofstrasse 1-3, 24148 Kiel, Germany
[2]Institute of Oceanography, Vietnam Academy of Science and Technology, 01 Cada, Nha Trang, Vietnam
[3]Faculty of Marine Science, King Abdulaziz University, PO Box 80207, Jeddah 21589, Saudi Arabia

ABSTRACT: Soft corals of the family Xeniidae are particularly abundant in Red Sea coral reefs. Their success may be partly due to a strong defense mechanism against fish predation. To test this, we conducted field and aquarium experiments in which we assessed the anti-feeding effect of secondary metabolites of 2 common xeniid species, *Ovabunda crenata* and *Heteroxenia ghardaqensis*. In the field experiment, the metabolites of both investigated species reduced feeding on experimental food pellets in the natural population of Red Sea reef fishes by 86 and 92% for *O. crenata* and *H. ghardaqensis*, respectively. In the aquarium experiment, natural concentration of soft coral crude extract reduced feeding on experimental food pellets in the moon wrasse *Thalassoma lunare* (a common reef fish) by 83 and 85% for *O. crenata* and *H. ghardaqensis*, respectively. Moon wrasse feeding was even reduced at extract concentrations as low as 12.5% of the natural crude extract concentration in living soft coral tissues. To assess the potential of a structural anti-feeding defense, sclerites of *O. crenata* (*H. ghardaqensis* lacks sclerites) were extracted and mixed into food pellets at natural, doubled and reduced concentration without and in combination with crude extract at 25% of natural concentration, and tested in an aquarium experiment. The sclerites did not show any effect on the feeding behavior of the moon wrasse, indicating that sclerites provide structural support rather than anti-feeding defense. We conclude that the conspicuous abundance of xeniid soft coral species in the Red Sea is likely a consequence of a strong chemical defense, rather than physical defenses, against potential predators.

KEY WORDS: Chemical defense · Feeding deterrence · Sclerites · Soft coral · *Xeniidae* · *Ovabunda* · *Heteroxenia* · Red Sea

INTRODUCTION

Soft corals (Cnidaria, Alcyonacea) are a major component of the sessile coral reef benthos and are highly diverse in tropical Indo-Pacific coral reefs (Dinesen 1983, Fabricius & Alderslade 2001), including the Red Sea (Benayahu & Loya 1977, 1981, Benayahu 1985, Reinicke 1997). Some soft corals of the families Xeniidae and Alcyoniidae contribute to the diet of coral reef fishes (Gohar 1940). Secondary metabolites of some soft corals have been shown to possess ecological functions including anti-predatory protection (La Barre et al. 1986), allelopathy (Sammarco et al. 1983, 1985) and anti-fouling activity (Changyun et al. 2008, Limna Mol et al. 2010).

These chemical defenses may be as effective as biomineralized skeletons in that they protect hermatypic corals from predation by most reef fishes (Sammarco & Coll 1992). For example, some Alcyoniidae species (e.g. *Sinularia polydactyla, Rhytisma fulvum fulvum*) were shown to possess secondary metabolites, which protected the soft corals against predation by carnivorous fish (Wylie & Paul 1989, Van Alstyne et al.1994, Kelman et al. 1999). A survey by Coll et al.

*Corresponding author: bhoang@geomar.de

Publisher: Inter-Research · www.int-res.com

(1982) showed a high prevalence of toxic species among the soft coral order Alcyonacea (>50% of the species) in the central Great Barrier Reef, suggesting that secondary metabolites, which are active against predators, are common in the Alcyonacea. In an extensive study by La Barre et al. (1986), it was found that the majority of soft coral taxa in the Great Barrier Reef possess defense mechanisms against fish predation, although toxicity and repellence are not always related to each other.

Chemical defense against predation may already be present in eggs, embryos or larvae of some soft corals (Coll et al. 1989, Kelman et al. 1999, Slattery et al. 1999, Lindquist 2002) indicating the importance of chemical anti-feeding defense throughout the life history of soft corals. In addition to their well-studied anti-feeding role, secondary metabolites of soft corals may also serve to combat fouling on the surface (Bhosale et al. 2002, Limna Mol et al. 2010) and may protect corals against viral infections (Ahmed et al. 2013). The conspicuous richness of chemical defenses in soft corals (Rocha et al. 2011) may therefore contribute to their remarkable invasion potential (Lages et al. 2006, Fleury et al. 2008).

In soft corals, chemical defense can be supplemented by mechanical defense such as mucus secretion (La Barre et al. 1986, Sammarco et al. 1987, Harvell & Fenical 1989) or elevated spicule concentration (Van Alstyne et al. 1992). Calcium carbonate spicules are common attributes in Octocorallia, as well as in Porifera, Echinodermata and Ascidiacea (Kingsley 1984). The size and shape of the spicules are often species-specific and can be used as taxonomic tools (e.g. Bayer et al. 1983). In soft corals, they are assumed to mainly function as structural support for the polyps and colonies (Lewis & Von Wallis 1991, Van Alstyne et al. 1992, O'Neal & Pawlik 2002), however, they can also function as defensive structures. This was demonstrated for some soft coral species, where fishes rejected sclerites containing artificial food (Van Alstyne et al. 1992, 1994), but not for others (Kelman et al. 1999, O'Neal & Pawlik 2002). The anti-feeding defense by sclerites may be effective only in those parts of the colony where their concentration is particularly high (Puglisi et al. 2000). Where sclerites do play a defensive role, their shape, size and abundance determine their protective efficiency, traits which may differ throughout a coral colony (Sammarco et al. 1987, Van Alstyne et al. 1992, Koh et al. 2000).

The family Xeniidae is composed of 34 species and is one of the most common and widely distributed octocoral families in the Red Sea (Reinicke 1997). It can cover up to 50% of the substrate in some shallow reef areas (~4 m depth), forming extensive carpets (Benayahu & Loya 1981, Reinicke 1997). Xeniid 'colonies' consist of numerous conspecific individuals occurring side by side (Gohar 1940). The family differs from all other Octocorallia due to the soft, fleshy consistency of the colony and its non-retractile polyps (Ashworth 1899). Some xeniid species lack stinging nematocysts (Janes 2008), which might reduce their capacity for protection against predators (Vermeij 1978, Bakus 1981, McIlwain & Jones 1997). Nevertheless, their competitiveness is high, presumably due to their motility as adults, their rapid asexual reproduction (Benayahu & Loya 1981), and the widespread allelopathy against space competitors and hard coral recruitment (Sammarco et al. 1983, Atrigenio & Alino 1996). Secondary metabolites with antimicrobial (Kelman et al. 1998, 2006) and anti-fouling activity (König et al. 1989) also seem to be common in xeniid soft corals.

Indeed, xeniids, like many other soft coral taxa, are remarkably rich in bioactive secondary metabolites (König et al. 1989, El-Gamal et al. 2005). Some chemical compounds that have been isolated from xeniid species are considered to be useful candidates in the field of medicine, particularly against cancer cells. These include the compounds blumiolide A, B and C of the species *Xenia blumi* (El-Gamal et al. 2005), different umbellacins of the species *X. umbellata* (El-Gamal et al. 2006) and different xeniolide of the species *X. blumi, X. novaebrittanniae and X. umbellata* (Bishara et al. 2006). To the best of our knowledge, their potential chemical defense against fish predation, which could contribute to their high abundance in the Red Sea, has not been investigated so far.

In this study, we investigated the chemical defense against fish predation of 2 particularly abundant xeniid species in the Red Sea, *Ovabunda crenata* and *Heteroxenia ghardaqensis*. We further studied whether or not chemical defense is enhanced by the presence of sclerites. To this purpose, artificial food was prepared and charged with crude extract of soft coral (1) at natural concentration and fed to the reef fish community *in situ*, and (2) at natural and reduced concentrations, with and without the addition of sclerites, and fed to the moon wrasse *Thalassoma lunare* in aquaria.

MATERIALS AND METHODS

Sample collection and identification

Soft coral samples were collected near the city of Jeddah, Saudi Arabia, in the central Red Sea. Coral (hard and soft) cover in this area ranged from 36 to 61%, of which the family Xeniidae comprised 7.5 to 14% (determined by line intercept transects at 3 to 4 m depth). The 2 xeniid soft coral species were collected by SCUBA diving in 3 to 6 m depth. *Ovabunda crenata* was collected at off-shore reefs (10 km from the coast) while *Heteroxenia ghardaqensis* was collected near-shore (50 m from the coast), where the respective species dominated the soft coral populations in the reefs. Five replicate samples of each species were collected at ~0.5 kg wet weight sample^{-1}. The samples were brought to the laboratory, the volume was determined immediately by water displacement (live colonies), and their identity was verified under the microscope following the identification criteria of Reinicke (1997).

Chemical extraction

Extraction was carried out in 2 steps in order to guarantee maximum metabolite extraction of a wide polarity spectrum. For the first extraction, fresh samples (whole colonies) were immersed in ethyl acetate for 24 h at room temperature (Lages et al. 2006). The gained crude extract was filtered through a paper filter and the solvent was removed with a rotary evaporator. The extracted coral tissue was stored in a freezer at −20°C until further processing. A second extraction followed, in which the frozen coral sample was freeze-dried, chopped into small pieces of about 0.5 cm^3 and weighed (dry weight). The tissue was then immersed in a solvent consisting of a 1:1 (v/v) mixture of dichloromethane and methanol (DCM: MeOH) for 24 h at room temperature (Wylie & Paul 1989). This second crude extract was also filtered through filter paper, and the solvent was evaporated until dry. The first and second crude extract were combined, weighed and stored at −20°C until assays were performed. The calculation of the natural concentration of crude extract was based on the volume of samples. The values were 32 mg ml^{-1} for *O. crenata* and 35 mg ml^{-1} for *H. ghardaqensis*, and were used as a reference for the preparation of the food pellets.

Sclerite preparation

We prepared sclerite samples from *O. crenata* only, as *H. ghardaqensis* does not contain sclerites. In order to obtain pure samples, each colony of *O. crenata* was cut into small pieces and immersed in 12% sodium hypochlorite to dissolve the tissue and leave the sclerites. After 12 h, the supernatant was carefully decanted and fresh sodium hypochlorite was added. This process was repeated until the tissue was completely dissolved and the sclerites remained on the bottom of the tube. Sclerites were collected and rinsed 3 times with distilled water, dried in an oven at 80°C until completely dry, and weighed. The natural concentration of sclerites was calculated by dividing the dry weight of sclerites by the dry weight of the colony.

Field assay

The frozen crude extract was re-dissolved in ethanol. Food pellets were produced following Pawlik & Fenical (1992) with some modifications: the basis of the food pellets was made by mixing and boiling 1.30 g phytagel (Sigma-Aldrich), 1.38 g of freeze-dried powdered squid and 30 ml distilled water. After the mixture cooled to ~40°C, the crude extract dissolved in ethanol (1.1 ml of *O. crenata* or 1.08 ml of *H. ghardaqensis*) was added at the natural concentration found in the soft coral tissue. The viscous mixture was poured into a plastic mould containing a piece of mosquito net with a mesh size of 1 mm^2. After the matrix cooled down, the solidified gel was removed from the mould and cut into pieces of 3 different sizes: 1, 2 and 3 cm^2. From each extract (n = 5), 3 pellets were made, resulting in a total of 15 pellets (replicate and sub-replicate) for each species.

The feeding assay was conducted at the same offshore reef and at the same depth where the xeniid samples had been collected. The procedure was similar to the method described by Van Alstyne et al. (1992, 1994), where pellets were individually weighed and fixed to a fishing line. Each size class (1, 2 or 3 cm^2) was represented as a pair with one pellet containing crude extract and the other pellet (of identical size) containing ethanol only. The distance between the pellets within a pair was 5 cm and the distance between pairs was 25 cm. A buoy at one end and a weight at the other end held the rope in a vertical position in the reef. The lowest pair was 1 m above the ground. The feeding activity of the reef fish was observed by SCUBA divers from a distance of ~3 m. The ropes were re-collected after one of either the control or treatment pellets on each rope were eaten completely by reef fishes. The pellets were re-weighed to determine percentage consumed.

Aquarium experiment

The food pellets for the aquarium experiment were made following Pawlik et al. (1995). The crude extract, dissolved in ethanol, was mixed with 0.3 g alginic acid and 0.5 g powdered squid. Distilled water was added to obtain a final volume of 10 ml. The mixture was stirred until it was homogeneous, and then loaded into a 10 ml syringe. The tip of the syringe was immersed into a 0.25 M $CaCl_2$ solution and the content of the syringe was slowly expelled into the $CaCl_2$ solution to form noodle-like food pellets. After several minutes, the solidified 'noodles' were rinsed with sea water and cut into pieces 2 to 5 mm long.

The effectiveness of the anti-feeding activity was tested with different concentrations of crude extract in the food pellets. This was done to determine the efficiency of secondary metabolites, which may vary in concentrations within the soft coral tissue seasonally, among populations, among organs and/or among life stages (Slattery et al. 1999, 2001). Thus, we produced pellets with 100, 50, 25, and 12.5% of the natural extract concentration. In order to assess the potential anti-feeding effect of the sclerites, sclerites were added to the food pellets (without extract) in their natural concentration (0.13 g sclerites g^{-1} soft coral dry weight). Additionally, sclerites were added to food pellets containing reduced concentrations of crude extract (25% of the natural concentration) in different concentrations (50, 100, and 200% of natural sclerite concentration) to determine the potential interactive effect of sclerites and secondary metabolites. We used 25% extract concentration so as not to mask any potential sclerite effect by a dominant chemical effect.

The feeding experiments in aquaria were carried out in Kiel, Germany, using the climate rooms of the GEOMAR institute. The moon wrasse *Thalassoma lunare* (purchased from Aqua Inspiration), was chosen because it is an abundant species in the central Red Sea and known to be a generalist feeder on a wide assortment of benthic invertebrates including soft corals (Randall 1983, Rotjan & Lewis 2008). Furthermore, this species has been used frequently for aquarium bioassays (Pawlik et al. 1987, Harvell et al. 1988, Kelman et al. 1999, Epifanio et al. 2007) due to its wide prey spectrum, its fast adaptation to aquarium conditions, and slow satiation (Pawlik et al. 1987). Each fish (n = 9) was placed in a separate aquarium filled with 40 l artificial sea water with 35 psu salinity, temperature 25°C and a 12 h light:12 h dark rhythm. The feed choice test was conducted by alternatingly feeding the fish control and treatment pellets loaded with extract and/or sclerites. In case the fish ignored the treatment pellet, another control pellet was offered in order to discriminate between the repellence of the treatment pellet and satiation. A pellet was considered rejected when it was ignored or spit out by the fish and the fish consumed a control pellet thereafter. The feeding tests were repeated with 10 control and 10 treatment pellets at once with each of the 9 fish, and the number of pellets consumed or rejected was recorded. Different treatments were tested on different days, with 3 to 5 d rest between each test. During resting time, fish were fed with artificial fish fodder.

Analyses

The feeding deterrence in the field assays was assessed using paired *t*-tests, by comparing the consumption rates on the pairwise deployed pellets containing or not containing extract. The consumption of pellets containing extracts from either of the 2 species was compared by *t*-test as well. Because the data of the extract-loaded pellets were used twice, the alpha-level was Bonferroni-corrected to $\alpha = 0.025$. Only the data of the largest food pellets were used because the control pellets were not entirely consumed at the end of the experiment i.e. the fishes could choose between extract-loaded and extract-free pellets throughout deployment.

The learning capacity of fishes in the aquaria experiment, which were repeatedly (10×) fed an identical extract pellet (intermittently with a control pellet), was assessed as the % decrease of acceptance between successive offerings during a given test day (i.e. increasingly experienced fish) relative to the acceptance at the first offering of an extract-loaded pellet (i.e. naive fish). These slopes were calculated for the pellets containing 25% of the natural extract concentration, because with full concentration the acceptance in most cases reached zero too early to calculate reliable slopes, and at concentrations below 25%, repellence and learning were almost absent. Because the fish showed some learning capacity, only the acceptance or rejection of the first treatment pellet (i.e. the reaction of a naive fish) was used for the statistical assessment of the extract defense strength.

The discrimination between control and extract pellets was tested by Fisher's exact test for the 2 soft coral species separately. Replication was done on the extract side (n = 5 colonies extracted) and on the consumer side (n = 9 individual fish tested). This procedure assessed the difference in proportion of consumed relative to rejected pellets between pellets with versus without extracts for the 5 replicate extracts per soft coral species offered to 9 fish. Analyses were performed with the software Statistica v.8 (StatSoft).

RESULTS

In the field experiment, a mean (±SE) of 97 ± 2.5 and 92 ± 2.9% of the control pellets were eaten by the reef fishes, while only 14.4% (±3.9) and 8.7% (±3.2) of the extract containing pellets of *Ovabunda crenata* and *Heteroxenia ghardaqensis* were eaten, respectively (Fig. 1). The repellent effect was significant for both species ($p < 0.001$; Table 1). The repellency between the food pellets containing extracts of the 2 coral species at natural concentration did not differ ($p = 0.53$; Table 1). The main fish species observed feeding on the pellets were *Thalassoma lunare, T. rueppellii, Pomacentrus sulfureus, Sufflamen albicaudatum, Oxycheilinus digramma*, and *Cephalopholis argus.* In addition, some allegedly herbivorous fishes such as parrot fishes and surgeon fishes occasionally fed on the pellets.

In the aquarium experiment, the fish quickly adapted to the new condition and readily accepted the control food pellets (without extract). For most concentration levels of both crude extracts, most fish individuals learned to recognize and avoid the deterrent pellets during the series of 10 subsequent encounters with a given pellet type. For the 25% natural concentrations, we assessed the slope of decreasing acceptance by increasingly experienced fish. The average slopes of the learning curves of the 9 fish i.e. increasing rejection with increasing experience, were −3.3 (±1.6 SE) and −8.6 (±2.7) for *H. ghardaqensis* and *O. crenata* extracts, respectively. This meant that each time the fish faced a further extract-loaded pellet they accepted it on average 3.3 to 8.6% less often than at the preceding encounter. Between the 1st and the 10th encounter, the acceptance thus decreased by 33 and 86% for *H. ghardaqensis* and *O. crenata* extracts, respectively. The difference in learning speed of fish with regard to the 2 potential prey species was, however, not significant ($t = 1.6$, df = 11, $p = 0.13$).

At the first encounter between a pellet and a naive moon wrasse, the control pellets were always eaten, while the treatment pellets containing the natural concentration of crude extracts were rejected to different degrees. On average, only 21 ± 6.4% of the pellets containing crude extract of *O. crenata* and 26 ± 8% of the pellets containing crude extract of *H. ghardaqensis* were consumed by naive moon wrasses (Fig. 2), which in both cases was significantly less than the feeding on control pellets (Fisher's exact test, $p < 0.001$).

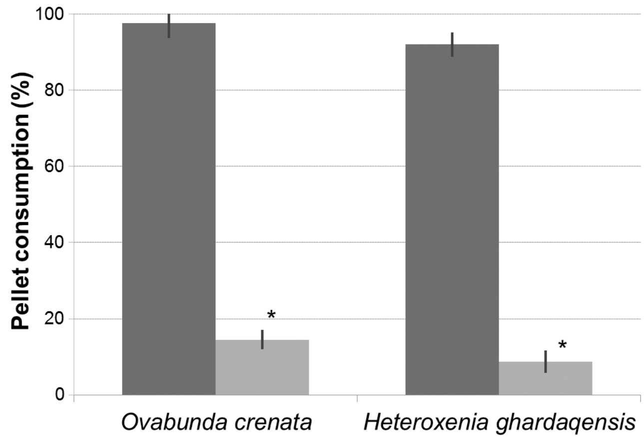

Fig. 1. Field assay, showing mean (±SE) percentage of pellets consumed by reef fishes. Dark grey columns: control (pellets without crude extract); light grey columns: pellets containing crude extracts of *Ovabunda crenata* and *Heteroxenia ghardaqensis* at natural concentrations. Significant differences ($p < 0.001$) between pellets without and with crude extract are indicated by (*). Consumption of extract-loaded pellets did not differ between species ($p > 0.05$)

Table 1. Results of *t*-tests comparing the consumption of *Ovabunda crenata* and *Heteroxenia ghardaqensis* extract-loaded versus extract-free pellets, and the consumption of extract-loaded pellets between species

	Mean	Variance	df	*t*	p (T ≤ *t*) 2-tail	*t* Critical 2-tail
H. ghardaqensis						
Extract-loaded pellet	0.136	0.019	4	−15.677	<0.001	2.776
Extract-free pellet	1.566	0.042				
O. crenata						
Extract-loaded pellet	0.232	0.054	4	−14.949	<0.001	2.776
Extract-free pellet	1.6	0.012				
Inter-species						
H. ghardaqensis	0.136	0.019	4	−0.684	0.531	2.776
O. crenata	0.232	0.054				

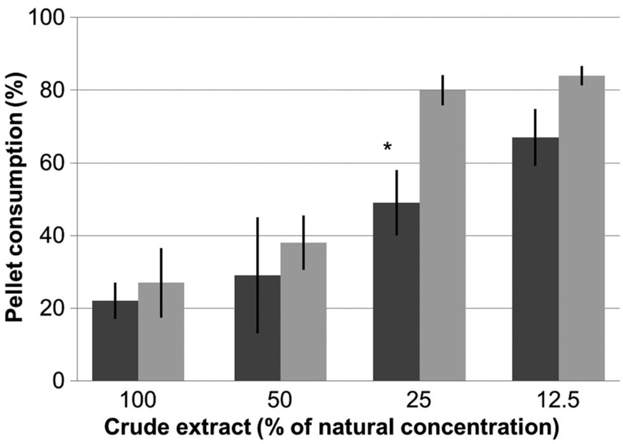

Fig. 2. Aquarium experiment, showing mean percentage of pellets containing different natural concentrations of crude extract of *Ovabunda crenata* (dark grey) and *Heteroxenia ghardaqensis* (light grey) consumed by moon wrasse *Thalassoma lunare*. Pellets without coral extract (controls) were consumed completely in all tests (data not shown). Differences between crude extract and control of the 2 xeniid species were highly significant ($p < 0.001$; data not shown). Significant difference ($p < 0.01$) between pellets containing *O. crenata* and *H. ghardaqensis* extracts are indicated by (*)

The deterrent activity decreased with decreasing crude extract concentration for both soft coral species (Fig. 2). This trend appeared to be slightly stronger for the *H. ghardaqensis* extract compared to *O. crenata*, but both species were significantly distasteful even at the lowest tested concentration of crude extract (12.5%). Comparing only the treatment pellets of the 2 species (Fig. 2), *O. crenata* extracts appeared to be less repellent than *H. ghardaqensis* extracts. However, this difference was significant at only the 25% natural concentration (Fisher's exact test, $p < 0.01$).

Sclerites of *O. crenata* did not affect the feeding behavior of moon wrasses at any sclerite concentration (50, 100 or 200% of natural sclerites) when added to food pellets without coral extract or in combination with 25% of crude extract concentration (Fig. 3).

DISCUSSION

Our results show that the crude extracts from 2 highly abundant soft coral species in the Red Sea, *Ovabunda crenata* and *Heteroxenia ghardaqensis*, strongly deter reef fishes from feeding on their polyps. This protective effect was not only detected at natural concentrations but even at 4-fold reduced concentrations, highlighting the efficiency of the involved secondary metabolites. Consequently, these soft coral species are likely to be well-defended against fish consumption, even if the defense metabolite concentration fluctuates to some extent among individuals, populations, life history stages or seasons. This anti-feeding defense most likely contributes to the success and remarkable abundance of these soft coral species in the reefs along the Saudi Arabian Red Sea coast. Sclerites, in contrast, did not show any deterring effect against fish predation in *O. crenata*. A negative relationship between sclerite armament and chemical defense, suggestive of a defensive role of the sclerites, had previously been reported by Sammarco et al. (1987) for some soft coral taxa (*Sinularia, Lemnalia, Heteroxenia*).

The similarity of the results found in both the field and aquarium assays suggests that the secondary metabolites of xeniid soft corals are broadly distasteful repellents that are effective against predation by

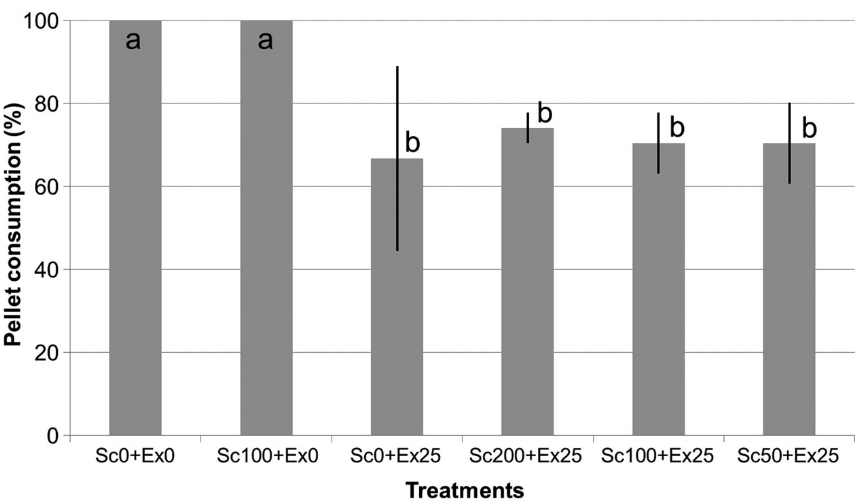

Fig. 3. Aquarium experiment, showing mean percentage of pellets containing different crude extract and sclerite concentrations of *Ovabunda crenata* consumed by the moon wrasse. Sc = sclerite, Ex = *Ovabunda crenata* extract; numbers behind abbreviations indicate concentration of extract and sclerites (in % of natural concentration). Letters above bars indicate significant difference ($p < 0.01$) between treatments

various fishes, rather an against only a specific species (e.g. *Thalassoma lunare*). Xeniids are known to be a rich source of terpenoids with anti-fouling activity (König et al. 1989, Bishara et al. 2006) or substances with potential anti-tumor activity (El-Gamal et al. 2005, 2006). Although the chemical composition of the crude extracts in our study species is not yet known, the fact that they inhibited feeding by a wide variety of fish species in the field, and even at strongly reduced concentrations by the moon wrasse in the aquarium indicates an impressive activity. Similar chemical anti-feeding defenses have been described for other soft coral species such as *Sinularia polydactyla, S. maxima* (Wylie & Paul 1989) and *Rhytisma fulvum fulvum* (Kelman et al. 1999). Such effective predator deterrence may be due to the combined effect of several different compounds (Van Alstyne et al. 1994), and it might therefore be rewarding, in a follow-up study, to assess the composition of effective metabolites and whether they act additively or synergistically.

Although the crude extract of both soft coral species was similarly repellent at natural concentrations, repellence decreased slightly faster with decreasing extract concentration for *H. ghardaqensis* than for *O. crenata* extracts. Concomitantly, fish seemed to learn to avoid pellets faster when they were loaded with *O. crenata* extracts than with *H. ghardaqensis* extracts. Whether this was attributable to the stronger chemical activity or a more characteristic or stronger olfactory cue in *O. crenata* extracts is not clear. It should be noted that our extraction procedure did not capture the most polar metabolites of the soft corals, and it is possible that highly water-soluble cues were missed as a consequence. If not, the slightly more active repellence and the more pronounced recognition value might confer a better anti-feeding protection to *O. crenata* compared to *H. ghardaqensis*. In the field, we also observed fish behavior suggestive of learned avoidance when certain chemicals of the treatment pellets were sensed. Thus, some fishes approached the treatment pellets but did not take a bite. In contrast to the fish in the laboratory, the natural reef population of fishes was presumably not naive. Given the abundance of the 2 soft coral species in these reefs, the fishes likely encounter them regularly and may have learned how to recognize them by chemical cues. The prevalence of these highly deterrent chemical cues in soft corals is not necessarily related to toxicity, as described by La Barre et al. (1986). A capacity of fishes to use olfactory or visual cues to avoid unpalatable organisms has been suggested (Pawlik et al. 1995, Miller & Pawlik 2013).

It is conceivable that the chemical repellence is complemented by other morphological or behavioral protective adaptations in these soft coral species. In both regards, the 2 species differ to a certain degree. Colonies of *O. crenata* reach a total height of 3 cm, while *H. ghardaqensis* colonies can reach 12 cm in height. *O. crenata* polyps do not show any pulsating activity, whereas the polyps of *H. ghardaqensis* feature continuous pulsation (Gohar 1940, Reinicke 1997). Whether these traits increase or decrease the species' susceptibility to fish consumption is presently unknown. Furthermore, many xeniid species are known to release mucus upon mechanical stress (Gohar 1940, Ducklow & Mitchell 1979). If this mucus bears olfactory signals it might enhance the avoidance behavior of reef fishes. Another distinctive property with potential relevance to predation is that *O. crenata*, in contrast to *H. ghardaqensis*, possesses sclerites. These, however, did not affect fish feeding in the aquarium experiments, even at double the natural concentration. In contrast, the presence of calcareous sclerites in other prey species was reported to enhance the efficiency of chemical anti-feeding defenses by neutralizing the digestive enzymes in the stomachs of various consumers, including fishes (Hay & Kappel 1994). Similarly, high concentrations of sclerites (31 to 82% of total tissue dry weight) of some Octocorallia (*S. maxima, S. polydactyla, Annella mollis*) were found to deter fish feeding (Van Alstyne et al. 1992, Puglisi et al. 2000). On the other hand, the soft coral *R. f. fulvum,* which contains sclerite concentrations of almost 80% of tissue dry weight, did not deter feeding (Kelman et al. 1999). Reasons for the lack of anti-feeding activity of the *O. crenata* sclerites may be (1) that the natural (13% of coral dry weight) and even the doubled sclerite concentration is too low to affect the predator's enzymatic functionality, and/or (2) that the sclerite size and shape may be harmless to predator fishes (Van Alstyne et al. 1992). The latter reason is supported by results from Burns & Ilan (2003), who found that sponge spicules deterred fish only when larger than ~250 µm. The size of *O. crenata* sclerites in this study were below 50 µm in length and the sclerite morphologies were simple flat discs of round to oval shape (Reinicke 1997, Halász et al. 2014), and might, therefore, only play a role as structural support (Lewis & Von Wallis 1991, Van Alstyne et al. 1992). In *H. ghardaqensis,* structural support is provided by the mesoglea, which is particularly strong and well-developed compared to the mesogloea of other xeniid species (G. B. Reinicke pers. comm.).

In conclusion, the chemical defense of the 2 xeniid species clearly prevents fish-feeding, while the sclerites, where present, seem to serve only as structural support or have other functions unrelated to defense. The high anti-feeding efficiency of the metabolites most certainly contributes to the robustness, perseverance and considerable abundance of xeniid species in the Red Sea. The chemical repellency of the soft corals may be enhanced by the capacity of the fish to learn.

Data archive. Data sets to this article are available under http://doi.pangaea.de/10.1594/PANGAEA.841563

Acknowledgements. This work was conducted within the Jeddah Transect Project, in collaboration with the King Abdulaziz University (KAU) Jeddah, Saudi Arabia. The project was funded by KAU under grant no. T-065/430-DSR. The authors thank the technical staff of KAU for technical and logistic support as well as the technical staff of the Benthic Ecology group of GEOMAR for experimental and laboratory assistance. We thank Götz B. Reinicke for helpful suggestions that improved the quality of the manuscript.

LITERATURE CITED

Ahmed S, Ibrahim A, Arafa AS (2013) Anti-H5N1 virus metabolites from the Red Sea soft coral, *Sinularia candidula.* Tetrahedron Lett 54:2377–2381

Ashworth JH (1899) The structure of *Xenia hicksoni* nov. sp., with some observations on *Heteroxenia elizabethae* Kölliker. Q J Microsc Sci 42:245–304

Atrigenio MP, Alino PM (1996) Effects of the soft coral *Xenia puertogalerae* on the recruitment of scleractinian corals. J Exp Mar Biol Ecol 203:179–189

Bakus GJ (1981) Chemical defense mechanisms on the Great Barrier Reef, Australia. Science 211:497–499

Bayer FM, Grasshoff M, Verseveldt J, Brill EJ (1983) Illustrated trilingual glossary of morphological and anatomical terms applied to Octocorallia. EJ Brill, Leiden

Benayahu Y (1985) Faunistic composition and patterns in the distribution of soft corals (Octocorallia, Alcyonacea) along the coral reefs of Sinai Peninsula. Proc 5th Int Coral Reef Cong, Tahiti 6:255–260

Benayahu Y, Loya Y (1977) Space partitioning by stony corals, soft corals and benthic algae on the coral reefs of the northern Gulf of Eilat (Red Sea). Nature 382:362–382

Benayahu Y, Loya Y (1981) Competition for space among coral-reef sessile organisms at Eilat, Red Sea. Bull Mar Sci 3:514–522

Bhosale SH, Nagle VL, Jagtap TG (2002) Antifouling potential of some marine organisms from India against species of *Bacillus* and *Pseudomonas.* Mar Biotechnol 4:111–118

Bishara A, Rudi A, Goldberg I, Benayahu Y, Kashman Y (2006) Novaxenicins A-D and xeniolides I-K, seven new diterpenes from the soft coral *Xenia novaebrittanniae.* Tetrahedron 62:12092–12097

Burns E, Ilan M (2003) Comparison of anti-predatory defenses of Red Sea and Caribbean sponges. II. Physical defense. Mar Ecol Prog Ser 252:115–123

Changyun W, Haiyan L, Changlun S, Wang-Yanan W, Liang L, Huashi G (2008) Chemical defensive substances of soft corals and gorgonians. Acta Ecol Sin 28:2320–2328

Coll JC, La Barrel S, Sammarco PW, Williams WT, Bakus GJ (1982) Chemical defences in soft corals (Coelenterata: Octocorallia) of the Great Barrier Reef: a study of comparative toxicities. Mar Ecol Prog Ser 8:271–278

Coll JC, Bowden BF, Heaton A, Scheuer BJ and others (1989) Structures and possible functions of epoxypukalide and pukalide: diterpenes associated with eggs of sinularian soft corals (Cnidaria, Anthozoa, Octocorallia, Alcyonacea, Alcyoniidae). J Chem Ecol 15:1177–1191

Dinesen ZD (1983) Patterns in the distribution of soft corals across the central Great Barrier Reef. Coral Reefs 1: 229–236

Ducklow HW, Mitchell R (1979) Composition of mucus released by coral reef coelenterates. Limnol Oceanogr 24:706–714

El-Gamal AAH, Chiang CY, Huang SH, Wang SK, Duh CY (2005) Xenia diterpenoids from the formosan soft coral *Xenia blumi.* J Nat Prod 68:1336–1340

El-Gamal AAH, Wang SK, Duh CY (2006) Cytotoxic xenia diterpenoids from the soft coral *Xenia umbellata.* J Nat Prod 69:338–341

Epifanio RA, Maia LF, Pawlik JR, Fenical W (2007) Antipredatory secosterols from the octocoral *Pseudopterogorgia americana.* Mar Ecol Prog Ser 329:307–310

Fabricius KE, Alderslade P (2001) Soft corals and sea fans: a comprehensive guide to the tropical shallow water genera of the Central-West Pacific, the Indian Ocean and the Red Sea. Australian Institute of Marine Science, Townsville

Fleury BG, Lages BG, Barbosa JB, Kaiser CR, Pinto AC (2008) New hemiketal steroid from the introduced soft coral *Chromonephthea braziliensis* is a chemical defense against predatory fishes. J Chem Ecol 34:987–993

Gohar HAF (1940) Studies on the Xeniidae of the Red Sea: their ecology, physiology, taxonomy and phylogeny. Publ Mar Biol Stn Gharadaqa 2:25–118

Halász A, McFadden CS, Aharonovich D, Toonen R, Benayahu Y (2014) A revision of the octocoral genus *Ovabunda* (Alderslade, 2001) (Anthozoa, Octocorallia, Xeniidae). ZooKeys 373:1–41

Harvell CD, Fenical W, Greene CH (1988) Chemical and structural defenses of Caribbean gorgonians (*Pseudopterogorgia* spp.). I. Development of an *in situ* feeding assay. Mar Ecol Prog Ser 49:287–294

Harvell CD, Fenical W (1989) Chemical and structural defenses of Caribbean gorgonians (*Pseudopterogorgia* spp.): intracolony localisation of defense. Limnol Oceanogr 34: 382–389

Hay ME, Kappel QE (1994) Synergisms in plant defenses against herbivores: interactions of chemistry, calcification, and plant-quality. Ecology 75:1714–1726

Janes MP (2008) A study of the Xeniidae (Octocorallia, Alcyonacea) collected on the 'Tyro' expedition to the Seychelles with a description of a new genus and species. Zool Meded 82:599–626

Kelman D, Kushmaro A, Loya Y, Kashman Y, Benayahu Y (1998) Antimicrobial activity of a Red Sea soft coral, *Rhytisma fulvum fulvum*: reproductive and developmental considerations. Mar Ecol Prog Ser 169:87–95

Kelman D, Benayahu Y, Kashman Y (1999) Chemical defence of the soft coral *Rhytisma fulvum fulvum* (Forskäl) in the Red Sea against generalist reef fish. J Exp Mar Biol Ecol 238:127–137

Kelman D, Kashman Y, Rosenberg E, Kushmaro A, Loya Y

(2006) Antimicrobial activity of Red Sea corals. Mar Biol 149:357–363

Kingsley RJ (1984) Spicule formation in the invertebrates with special reference to the gorgonian *Leptogorgia virgulata.* Am Zool 24:883–891

Koh LL, Goh NKC, Chou LM, Tan YW (2000) Chemical and physical defenses of Singapore gorgonians (Octocorallia: Gorgonacea). J Exp Mar Biol Ecol 251:103–115

König GM, Coll JC, Bowden BF, Gulbis JM, MacKay MF, La Barre SC, Laurent D (1989) The structure determination of a xenicane diterpene from *Xenia garciae.* J Nat Prod 52:294–299

La Barre SC, Coll JC, Sammarco PW (1986) Defensive strategies of soft corals (Coelenterata: Octocorallia) of the Great Barrier Reef. II. The relationship between toxicity and feeding deterrence. Biol Bull (Woods Hole) 171: 565–576

Lages BG, Fleury BG, Ferreira CEL, Pereira RC (2006) Chemical defense of an exotic coral as invasion strategy. J Exp Mar Biol Ecol 328:127–135

Lewis JC, Von Wallis E (1991) The function of surface sclerites in gorgonians (Coelenterata, Octocorallia). Biol Bull 181:275–288

Limna Mol VP, Raveendrana TV, Parameswaran PS, Kunnath RJ, Sathyan N (2010) Antifouling sesquiterpene from the Indian soft coral *Sinularia kavarattiensis* Alderslade and Prita. Indian J Mar Sci 39:270–273

Lindquist N (2002) Chemical defense of early life stages of benthic marine invertebrates. J Chem Ecol 28:1987–2000

McIlwain JL, Jones GP (1997) Prey selection by an obligate coral-feeding wrasse and its response to small-scale disturbance. Mar Ecol Prog Ser 155:189–198

Miller AM, Pawlik JR (2013) Do coral reef fish learn to avoid unpalatable prey using visual cues? Anim Behav 85: 339–347

O'Neal W, Pawlik JR (2002) A reappraisal of the chemical and physical defenses of Caribbean gorgonian corals against predatory fishes. Mar Ecol Prog Ser 240:117–126

Pawlik JR, Fenical W (1992) Chemical defense of *Pterogorgia anceps*, a Caribbean gorgonian coral. Mar Ecol Prog Ser 87:183–188

Pawlik JR, Burch MT, Fenical W (1987) Patterns of chemical defense among Caribbean gorgonian corals: a preliminary survey. J Exp Mar Biol Ecol 108:55–66

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

Puglisi MP, Paul VJ, Slattery M (2000) Biogeographic comparisons of chemical and structural defenses of the Pacific gorgonians *Annella mollis* and *A. reticulata.* Mar Ecol Prog Ser 207:263–272

Randall JE (1983) Red Sea reef fishes. IMMEL Publishing, London

Reinicke GB (1997) Xeniidae (Coelenterate: Octocorallia) of the Red Sea, with descriptions of six new species of *Xenia.* Fauna of Saudi Arabia 16:5–62

Rocha J, Peixe L, Gomes NCM, Calado R (2011) Cnidarians as a source of new marine bioactive compounds—an overview of the last decade and future steps for bioprospecting. Mar Drugs 9:1860–1886

Rotjan RD, Lewis SM (2008) Impact of coral predators on tropical reefs. Mar Ecol Prog Ser 367:73–91

Sammarco PW, Coll JC (1992) Chemical adaptations in the Octocorallia: evolutionary considerations. Mar Ecol Prog Ser 88:93–104

Sammarco PW, Coll JC, La Barre SC, Willis B (1983) Competitive strategies of soft corals (Coelenterata, Octocorallia): allelopathic effects on selected scleractinian corals. Coral Reefs 1:173–178

Sammarco PW, Coll JC, La Barre SC (1985) Competitive strategies of soft corals (Coelenterata: Octocorallia). II. Variable defensive responses and susceptibility to scleractinian corals. J Exp Mar Biol Ecol 91:199–215

Sammarco PW, La Barre SC, Coll JC (1987) Defensive strategies of soft corals (Coelenterata: Octocorallia) of the Great Barrier Reef. III. The relationship between ichthyotoxicity and morphology. Oecologia 74:93–101

Slattery M, Hines GA, Starmer J, Paul VJ (1999) Chemical signals in gametogenesis, spawning, and larval settlement and defense of the soft coral *Sinularia polydactyla.* Coral Reefs 18:75–84

Slattery M, Starmer J, Paul VJ (2001) Temporal and spatial variation in defensive metabolites of the tropical Pacific soft coral *Sinularia maxima* and *S. polydactyla.* Mar Biol 138:1183–1193

Van Alstyne KL, Wylie CR, Paul VJ, Meyer K (1992) Antipredator defenses in tropical Pacific soft corals (Coelenterata: Alcyonacea). I. Sclerites as defenses against generalist carnivorous fishes. Biol Bull (Woods Hole) 182:231–240

Van Alstyne KL, Wylie CR, Paul VJ (1994) Antipredator defenses in tropical Pacific soft corals (Coelenterata: Alcyonacea). II. The relative importance of chemical and structural defenses in three species of *Sinularia.* J Exp Mar Biol Ecol 178:17–34

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

Wylie CR, Paul VJ (1989) Chemical defenses in three species of *Sinularia* (Coelenterata, Alcyonacea): effects against generalist predators and the butterflyfish *Chaetodon unimaculatus* Bloch. J Exp Mar Biol Ecol 129:141–160

Editorial responsibility: Paul Sammarco, Chauvin, Louisiana, USA

Submitted: January 20, 2014; Accepted: November 6, 2014
Proofs received from author(s): December 22, 2014