Mar Biol (2008) 156:141–153
DOI 10.1007/s00227-008-1071-3

ORIGINAL PAPER

# Rapid tissue reduction and recovery in the sponge *Aplysinella* sp.

**Carsten Thoms · Ute Hentschel · Susanne Schmitt · Peter J. Schupp**

Received: 29 January 2008 / Accepted: 30 September 2008 / Published online: 4 November 2008


**Abstract** We observed a pronounced, yet reversible tissue reduction in the tropical sponge *Aplysinella* sp. under non-experimental conditions in its natural habitat, after transfer into seawater tanks, as well as after transplantation from deep to shallow water in the field. Tissue reduction resulted in the formation of small "reduction bodies" tightly attached to the sponge skeleton. Although volume loss and gain were substantial, both tissue reduction and regeneration were often remarkably rapid, occurring within few hours. Microscopic analysis of the reduction bodies revealed morphological similarities to previously described sponge primmorphs, with densely packed archaeocytes and spherulous cells enclosed by a thin layer of epithelial-like cells. Denaturing gradient gel electrophoresis (DGGE) revealed pronounced changes in the sponge-associated microbial community upon tissue reduction during laboratory and field experiments and following changes in ambient conditions after transplantation in the field. Generally, the microbial community associated with this sponge proved less stable, less abundant, and less diverse than those of other, previously investigated Verongid sponges. However, one single phylotype was consistently present in DGGE profiles of *Aplysinella* sp. This phylotype clustered with $\gamma$-proteobacterial sequences found previously in other sponge species of different taxonomic affiliations and geographic provenances, as well as in sponge larvae. No apparent changes in the total secondary metabolite content (per dry weight) occurred in *Aplysinella* sp. upon tissue reduction; however, comparative analysis of intact and reduced tissue suggested changes in the concentrations of two minor compounds. Besides being ecologically interesting, the tissue reduction phenomenon in *Aplysinella* sp. provides an experimentally manipulable system for studies on sponge/microbe symbioses. Moreover, it may prove useful as a model system to investigate molecular mechanisms of basic Metazoan traits in vivo, complementing the in vitro sponge primmorph system currently used in this context.

Communicated by M. Kühl.

C. Thoms (✉)
Jena School for Microbial Communication (JSMC),
Friedrich Schiller University Jena, Jenergasse 8,
07743 Jena, Germany
e-mail: carsten.thoms@gmail.com

U. Hentschel · S. Schmitt
Research Center for Infectious Diseases,
University of Wuerzburg, Roentgenring 11,
97070 Wuerzburg, Germany

P. J. Schupp (✉)
University of Guam Marine Laboratory,
UOG Station, Mangilao, Guam 96923, USA
e-mail: peter.schupp@gmail.com

## Introduction

The organization of sponges has been described as "modular" (Dyrynda 1986) and as a "dynamic multicellular system" with a high degree of cell motility and a low degree of cell differentiation (Gaino and Magnino 1999). The sponge surface towards the ambient seawater is formed by adjacent cells (Bergquist 1978). However, the bulk of the sponge body, named mesohyl, is a collagenous matrix harboring motile, amoeboid sponge cells, so-called archaeocytes. The archaeocytes represent stem cells for all other sponge cell types present (Müller 2006). The mesohyl is permeated by a highly branched water channel system that includes specialized chambers lined with flagellated cells (choanocytes). The uptake of food (dissolved and particulate

organic matter) takes place mainly in these chambers (Bergquist 1978). Organic particles (including bacteria) are phagocytized by the choanocytes and transferred into the mesohyl.

Many sponge species undergo constant anatomical rearrangements, considerably changing their morphology within few hours (Bond 1992). Next to versatility in morphology, the modular body plan of sponges entails high regenerative capabilities. Two interesting phenomena in the context of tissue regeneration in sponges are the formations of gemmules and primmorphs: gemmules are formed in high numbers predominantly by freshwater sponges to outlast adverse environmental conditions (e.g., low temperature, desiccation, starvation, hypoxia). They consist of numerous totipotent archaeocytes encapsulated by a rough shell, mainly of spongin and siliceous gemmuloscleres (Schill et al. 2006). Once the sponge disintegrates, the gemmules are released and subsequently dispersed. Settling of the gemmules under more favorable conditions can trigger hatching and regeneration to functional sponges. Thus, gemmulation is also a form of asexual reproduction. Primmorphs are formed in vitro by archaeocytes from artificially dissociated sponge tissue (Custodio et al. 1998). In an appropriate medium, these cells independently aggregate and start to proliferate. After a short time, an epithelial cell layer is formed and eventually, the primmorphs can redevelop to fully functional, structured sponges (Custodio et al. 1998).

Sponges are often associated with highly abundant and diverse microbial communities. In many sponge species, microorganisms can comprise in excess of 40% of the total "sponge" biomass (Hentschel et al. 2003). Remarkably, microbial communities in sponges often differ considerably from those in the ambient seawater. Striking similarities between microbial communities associated with phylogenetically and geographically distant sponge species were observed (Hentschel et al. 2002; Taylor et al. 2007b). Moreover, sponge/microbe associations are often highly resistant to external disturbance (Friedrich et al. 2001; Thoms et al. 2003). However, due to the complexity of most sponge-associated microbial communities, unraveling the actual modes of interaction in these associations has proven highly challenging. Various attempts have been made to gain a better understanding of these symbioses [see reviews by Hentschel et al. (2006) and Taylor et al. (2007a)]. These attempts partly have been motivated by commercial interests, since one possible symbiotic function of sponge-associated microbes is a contribution to the pharmaceutically highly interesting "sponge chemistry".

This study employs a multidisciplinary approach to describe a pronounced, yet reversible tissue reduction in the sponge *Aplysinella* sp. (order Verongida, family Aplysinellidae). Tissue shrinking was observed on undisturbed individuals in their natural habitat, following transfer of *Aplysinella* sp. into seawater tanks, and after experimental transplantation of the sponge in the field. We investigated the morphological changes *Aplysinella* sp. underwent in the course of tissue reduction and recovery, as well as the effects of these processes on the sponge-associated microbial community and on the chemical profile of *Aplysinella* sp.

## Materials and methods

### Sponge material

The sponge *Aplysinella* sp. (phylum Porifera, class Demospongiae, order Verongida, family Aplysinellidae) grows abundantly on coral reefs in Apra Harbor, Guam (13°27′078′′N,144° 39′382′′E). Occasionally, patches of small colonies can be found at depths as low as 3 m on reef flats close to the reef edge, but their abundance is highest on reef slopes between 12 and 18 m. A voucher specimen of the sponge has been deposited at the Museum for Natural History in Leiden, the Netherlands. Review of the literature indicates that there might have been several publications on this species under the species name "*Aplysinella rhax*" (Shin et al. 2000; Tabudravu et al. 2002; Pham et al. 2000; Thoms and Schupp 2008) and *Pseudoceratina purpurea* (Jimenez and Crews 1991; Pina et al. 2003). When compared with the vouchers of the taxonomic relevant type specimens of *Dysidea rhax* de Laubenfels 1954 and *Aplysinella strongylata* Bergquist 1980 *Aplysinella* sp. the sponge of this study resembled neither of the two species. Chemical analysis of the sponge revealed high concentrations of bromotyrosine alkaloids structurally similar to the alkaloids of Verongida sponges (Shin et al. 2000; Tabudravu et al. 2002; Pham et al. 2000; Thoms and Schupp 2008 this study), indicating affiliation to this sponge order. Therefore we recommend, in accordance with Dr. Nicole J. de Voogd at the National Museum of Natural History in Leiden, the Netherlands, to use *Aplysinella* sp. as taxonomic entity until a proper revision of the species is completed.

### Tank experiments

Sponge individuals were collected within Apra Harbor at Western Shoals by scuba diving at depths around 15 m during May 2005. Fourteen sponges were carefully detached from coralline substrate, transported to the laboratory in large wide mouth bottles and transferred into a seawater tank of approximately 6,600 L volume. Here, they were loosely tied to tiles. During the entire procedure, air contact was avoided and mechanical damage was kept at a minimum. The sponges usually attached themselves to the new substrate within few days. The tank was partly shaded by

50% shading cloth (Aquatic Eco-Systems, Apopka) and a constant flow of fresh, untreated seawater was provided. Water temperatures ranged from 29–31°C.

### Field transplantation experiment

Ten *Aplysinella* sp. individuals were transplanted from the reef slope of Western Shoals, Apra Harbor from depths of 12–18 to 3–4 m on the reef flat in November 2006. The sponges were removed from the reef together with a piece of substrate and were individually transported to the shallower areas, thus avoiding mechanical damage. The new location was in close vicinity (~10 m apart) to a patch of *Aplysinella* sp. individuals that naturally grew in 3–4 m depth on the reef edge. The transplanted sponges were checked for morphological changes after 1 week, 1 month, and 3 months. Samples for microscopical, microbiological, and chemical analysis were taken before and 3 months after transplantation. Small pieces of tissue were cut with a sterile scalpel and transferred into 50 mL centrifuge tubes. The tubes were sealed underwater and once at the surface they were transported to the laboratory on ice. All samples were washed three times with sterile-filtered seawater. For DGGE and chemical analysis they were frozen at −80°C and subsequently lyophilized.

### Light and transmission electron microscopy

Sample preparation was performed as described in Thoms et al. (2003). Briefly, small tissue samples were preserved in 2.5% glutaraldehyde in sterile-filtered seawater. In brief, after washing in cacodylate buffer samples were post-fixed in 2% osmium tetroxide and incubated overnight in 0.5% uranyl acetate. Following dehydration, samples were embedded in Epon 812 (Serva). Semithin and ultrathin sections were prepared with an ultramicrotome (OM U3, C. Reichert, Austria). Semithin sections were stained with methylene blue for examination with a Leica DM-LB2 (Leica Microsystems, Bannockburn, IL) light microscope. Ultrathin sections were examined with a Zeiss EM 10 (Zeiss, Jena, Germany) transmission electron microscope.

### Denaturing gradient gel electrophoresis (DGGE)

DGGE of bacterial 16S rRNA was performed essentially as described by Thoms et al. (2003). Microbial DNA was extracted with the Fast DNA Spin kit for soil (MP Biomedicals, Solon, OH). For PCR, universal primers 341f-GC and 907r (Muyzer et al. 1998) were used. DGGE was performed with a Bio-Rad DCode system (Bio-Rad, Hercules, CA) on 7% (wt/vol) polyacrylamide gels in 1X TAE, using a 10–65% denaturing gradient [100% denaturant corresponds to 7 M urea and 40% (vol/vol) formamide]. Electrophoresis time was 340 min at 180 V and 60°C. Gels were stained for 30 min with SYBR green II (Invitrogen, Carlsbad, CA) and photographed with a Biorad GelImager FluorS system (Bio-Rad). Similarity matrices of the DGGE banding patterns were calculated using the Dice coefficient method implemented in the Quantity One Gel Analysis software (Bio-Rad). The distance matrices were then used in an unweighted pair group method using arithmetic averages (UPGMA) cluster analysis. PCR products eluted from excised bands were re-amplified with the primers 341f and 907r, purified with the QIAquick PCR Purification Kit (Qiagen, Valencia, CA), and cloned with the pGEM cloning kit (Promega, Madison, WI). The correct insert size was verified on 2% agarose gels after PCR amplification with the primers T7 and SP6. Plasmid DNA was isolated by standard miniprep procedures (Sambrook and Russell 2001). Sequencing was performed on an ABI 377XL automated sequencer (Applied Biosystems, Foster City, CA). Sequences were checked for amplification and sequencing artifacts with ChromasPro (Technelysium, Helensvale, Australia) and for chimeras with Pintail (Ashelford et al. 2005).

Sequences obtained in this study together with reference sequences downloaded from NCBI BLAST (Altschul et al. 1990) were automatically aligned with ClustalX (Thompson et al. 1997) and manually refined with Align (Hepperle 2004). Phylogenetic trees were constructed with the ARB software package (Ludwig et al. 2004). Initially, neighbor-joining (Jukes-Cantor correction) and maximum parsimony trees were calculated with nearly full length sequences (>1,200 bp) and 100 pseudoreplicates. Partial sequences were added to the trees without changing the topology by the use of the parsimony-interactive tool in ARB. Finally, 50% majority rule consensus trees were constructed. The 16S rRNA gene sequences obtained in this study were deposited in the EMBL/GenBank/DDBJ database under accession numbers EU352739-EU352747.

### Chemical analysis

Extraction of the sponge tissue was performed using a standardized procedure as described by Thoms and Schupp (2008). Fifty milligrams of lyophilized and ground material of each sample was extracted by vortexing with 1.5 mL methanol for 60 s. After 2 min, vortexing was repeated for 30 s. Samples were centrifuged (13,000 rpm, 2 min) and the diluted supernatants were analyzed by high-performance liquid chromatography (HPLC) using an Alltech Rocket Platinum EPS C18, 53 × 7 mm column (Alltech, Deerfield, IL). The HPLC system was coupled to a photodiode-array detector (Waters, Milford, Ireland). Routine detection was performed at 254 and 280 nm. Additionally, the chromatograms were inspected with the MaxPlot

function of the Waters Empower Pro software, covering wavelengths between 210 and 400 nm. The compounds psammaplin A sulfate and psammaplin A were identified based on their retention times and UV spectra by direct comparison with previously isolated standards (Thoms and Schupp 2008).

## Results

### Morphological changes

Directly after transfer into the seawater tank all 14 sponge individuals displayed closed oscules, which is probably attributable to the mechanical stress by handling. Within one day, all of them opened their oscules and appeared healthy by visual judgment. No sponge showed any signs of tissue reduction (e.g., tubercular surface, protruding spongin skeleton) (Fig. 1a). The first signs of tissue reduction occurred 10 days after transfer into the tank. Within 13 days after transfer, the tissue of all 14 individuals was reduced to small, irregular lumps ("reduction bodies", 2–4 mm in diameter) encasing the by now easily visible spongin skeletons (Fig. 1b). Roughly estimated, the reduction accounted for more than half of the original volume of each sponge.

Three days later, 11 of the 14 sponges showed first signs of recovery, i.e., the reduction bodies had regained size and covered visibly larger areas of the spongin skeletons. One week after onset of recovery, three individuals had fully regenerated to their original volume and condition (Fig. 1c). After three more days, 10 of the 14 sponges were fully regenerated, with most of them having open oscules, indicating active pumping. Another 2 weeks later (48 days after transfer into the seawater tank), most sponges were in the reduced state again. Only three sponges were still in a recovered condition, two of which had undergone another tissue reduction in the meantime but were regenerated again.

We made similar observations on numerous other individuals of *Aplysinella* sp. collected in different contexts (data not shown). Tissue reduction always occurred only

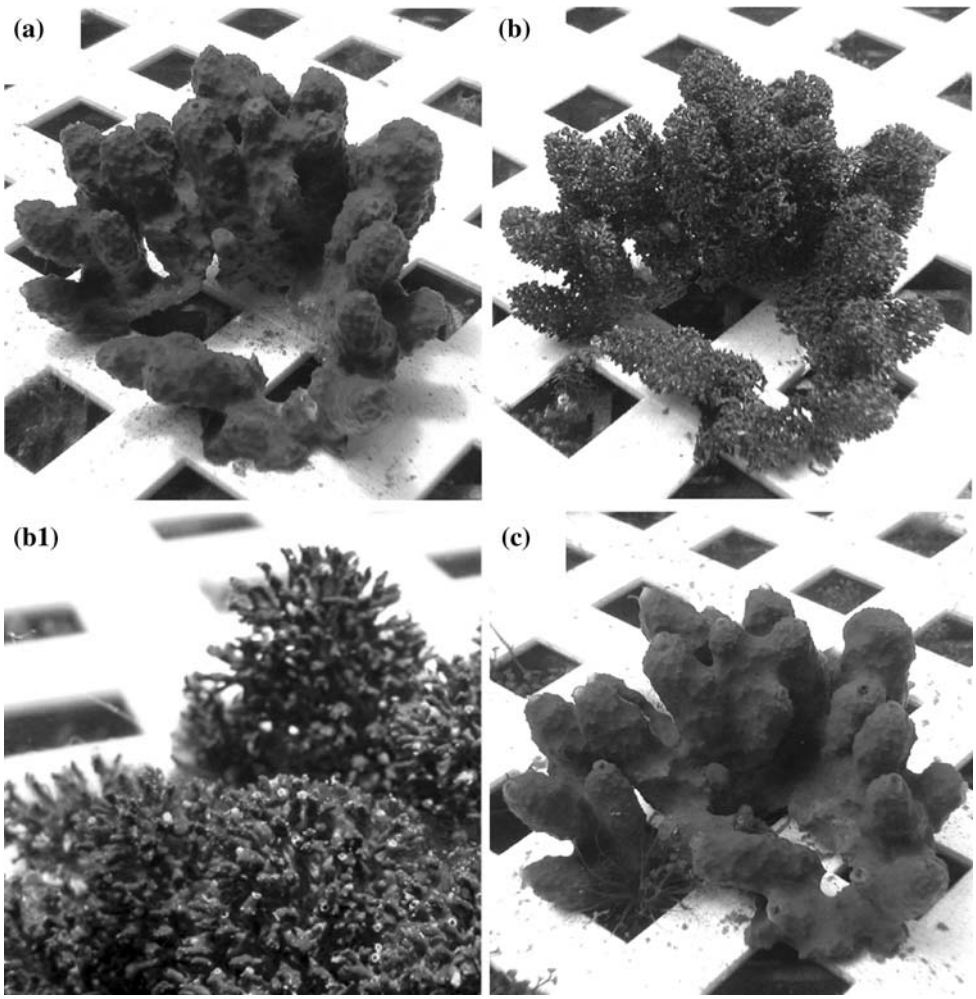


**Fig. 1** Photos of an *Aplysinella* sp. individual undergoing tissue reduction and recovery in the tank experiment. **a** Intact ("*int*") state, one day after transfer from the natural habitat into the tank. **b** Reduced ("*red*") state. **b1** Close-up of sponge tubes in the reduced state. **c** Regenerated ("*reg*") sponge

several days after transfer into the tanks. Once reduction started, the morphological changes often were very rapid. The fastest change from the intact to the completely reduced condition occurred within less than a day. The fastest recovery from small reduction bodies back to the original condition occurred within 7 h.

No signs of tissue reduction were observed on the transplanted sponges in the field 1 week and 1 month after transplantation. After 3 months, the tissue of one individual was substantially reduced, whereas all other transplanted sponges were still intact. Morphological changes in the reduced sponge were similar to those observed in the tank experiment (Fig. 1), and on sponges in the field without experimental manipulation.

## Microscopic observations

Cross-sections of tissue of intact individuals revealed numerous, yet loosely distributed sponge cells (predominantly archaeocytes and spherulous cells) (Fig. 2a). The distribution of cells in the mesohyl was heterogeneous, with areas high in archaeocyte density, areas high in spherulous cell density, areas with both cell types in about equal numbers, and collagenous areas free of sponge cells. Numerous large water channels and choanocyte chambers were observed. Compared with closely related sponges of the genus *Aplysina,* the microbial abundance in *Aplysinella* sp. tissues was considerably lower. Moreover, the characteristic microbial morphotypes described for other Verongid species (Vacelet 1975; Friedrich et al. 1999; Thoms et al. 2003) were not found in *Aplysinella* sp.

Reduced tissue of *Aplysinella* sp. had a very different appearance (Fig. 2b). The entire cross-section of the reduction bodies was densely packed with archaeocytes and spherulous cells, with hardly any space in-between. No choanocyte chambers were found. In the periphery of the reduction bodies spherulous cells were particularly abundant and both spherulous cells and archaeocytes were considerably larger than in intact *Aplysinella* sp. tissue. Microorganisms were not detected, possibly because small structures were no longer discernible in the tight gaps between the sponge cells.

Regenerated tissue (Fig. 2c) had largely regained the appearance of the original, intact state (Fig. 2a). In some areas, the sponge cells were still more densely packed and structures such as choanocyte chambers and channels were not as abundant or large as in intact tissue before reduction. Nevertheless, the overall impression, with heterogeneous distribution of cells, few extracellular microorganisms, relatively large distances between cells, and cell free areas was that of the intact tissue prior to the reduction event.

## Microbial community changes

Tissue reduction in the tank experiment resulted in a change in the overall impression of the DGGE profiles, very similar in all three sponge individuals analyzed (individuals T1–T3, Fig. 3a, "T" for tank experiment): Whereas before reduction ("int" for intact condition; Fig. 3) one to three bands in the high G + C region dominated the pattern, following tissue reduction ("red" for reduced condition, Fig. 3) their intensity decreased in favor of numerous other bands, predominantly towards the lower G + C region of the gel (i.e., towards the top of the gel). This change was not reversed in the course of tissue recovery ("reg1" for condition immediately after regeneration; "reg2" for regenerated condition 3 days after "reg1", Fig. 3) within the time frame of the tank experiment. Generally, DGGE profile variability was high among individuals and conditions in this experiment, with similarities as low as 26.7% between individual samples (for example "T1 int" versus "T1 reg1"; value from similarity matrix). Cluster analysis of the banding patterns (Fig. 3b) revealed a clustering of all intact samples ("int") separate from the samples taken after tissue reduction ("red") and recovery ("reg1" and "reg2"). Based on the similarity matrix values, the DGGE profiles of the three sponge individuals continuously became more similar over time (from an initial mean similarity of 54.6 ± 17.3% between the "int" samples to 79.2 ± 12.2% between the samples "reg2").

Also in the field experiment the DGGE profile of *Aplysinella* sp. proved highly variable (Fig. 4). Similarities between patterns of individual samples were as low as 11.8% (for example sample "F1 15 m" versus "F3"; value from similarity matrix; "F" for field experiment). Tissue reduction in the transplanted sponge "F2" coincided with a change in the overall impression of the DGGE profile analogous to that observed in the individuals of the tank experiment. Here, as well, the dominance of a band in the high G + C region of the DGGE gel decreased in favor of bands towards the lower G + C region (F2, Fig. 4a). In all individuals that had been transplanted in the field experiment (exemplified by individual "F1") no tissue reduction was observed. Nevertheless, the DGGE profile of individual "F1" had changed considerably within the 3 months following transplantation, resulting in a clustering with the profiles of the sponges "F3" and "F4" that originally had grown in the shallow water habitat (Fig. 4b).

However, one band in the DGGE profiles of *Aplysinella* sp. resisted all changes in ambient conditions (caused by transplantation of the sponge and by tissue reduction) and was present in all samples analyzed (Figs. 3a, 4a). For identification of the bacterial phylotype represented by this band, PCR products eluted from excised bands b and f (Fig. 4a) were sequenced. Closest hits in BLAST for the

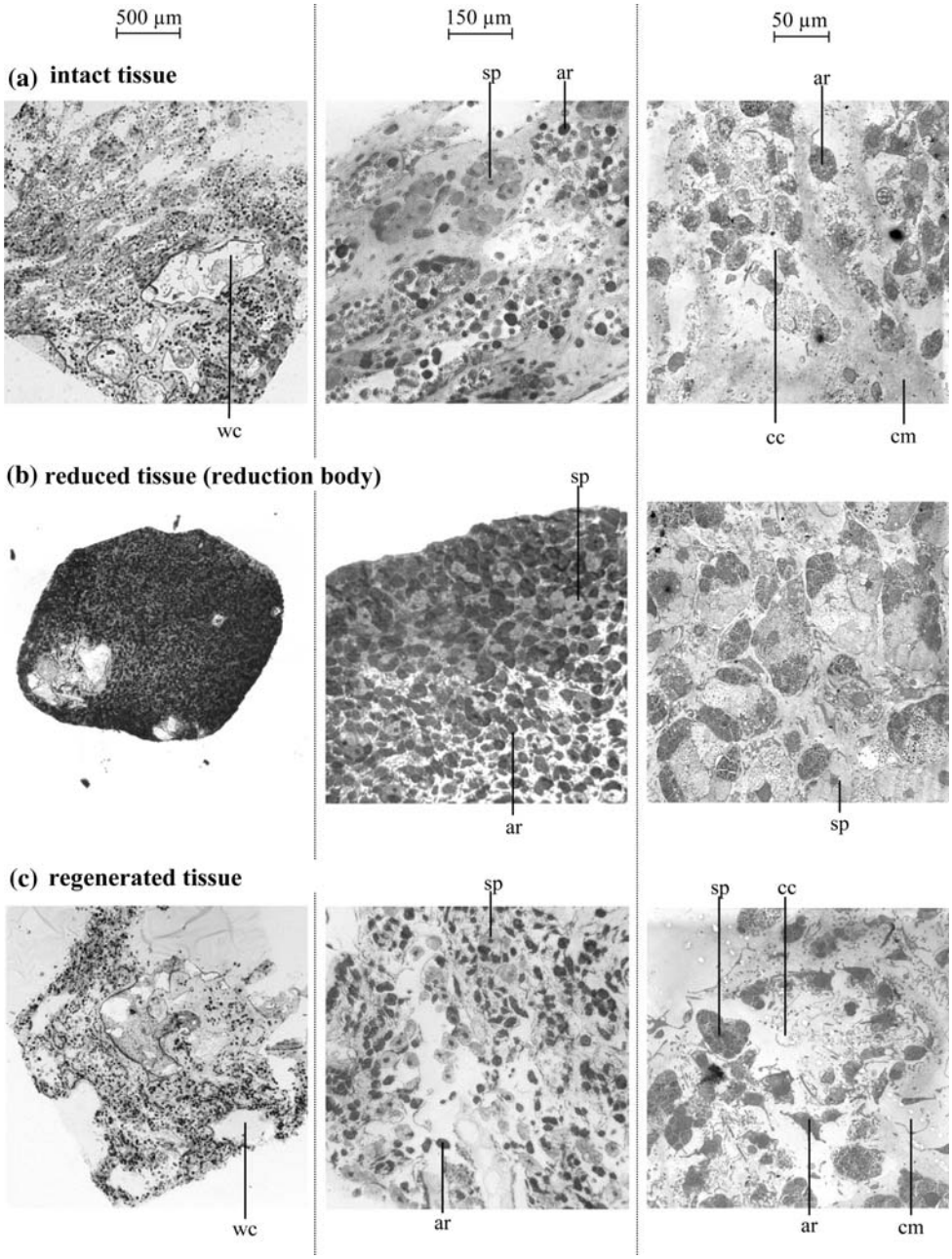


**Fig. 2** Cross-sections of *Aplysinella* sp. tissue in three magnifications (*left and middle column*: light micrographs; *right column*: transmission electron micrograph). All micrographs represent tissue of the same individual in the respective tissue states and are representative for all individuals analyzed. **a** Intact tissue before reduction. **b** Reduced tissue, cross-section of an entire reduction body. **c** Tissue after regeneration ("*reg*2"). *ar* archaeocyte, *cc* choanocyte chamber, *cm* collagenous matrix, *sp* spherulous cell, *wc* water channel

obtained sequences were γ-proteobacterial sequences previously derived from other sponge species. Phylogenetic analysis resulted in a cluster within the γ-Proteobacteria of sequences derived from sponges and marine sediments (Fig. 5). Sources of the sponge-derived bacterial sequences included larvae of the Caribbean sponge *Ircinia felix* as well as adults of the two Mediterranean sponges *Chondrilla nucula* and *Tethya aurantium* (Fig. 5). Additionally, various examples of transient bands were selected for sequencing (bands a, c–e, g–i, Fig. 4a). Bands a, c, d, and e represented sequences within the phylum Bacteroidetes and the class α-Proteobacteria with closest similarities to either seawater-derived bacterial sequences or to sequences of bacteria associated with marine organisms other than sponges. The sequence represented by band i was most similar to algal chloroplast sequences. The sequences from bands g and h were closely related to the γ-proteobacterial sequences derived from bands b and f.

## Chemical profile changes

The chemical profiles of the morphological states *Aplysinella* sp. was found in were compared by HPLC. Nine individuals of the tank experiment were examined in all three states (intact, reduced, regenerated). Additionally, the four sponges of the field transplantation experiment were

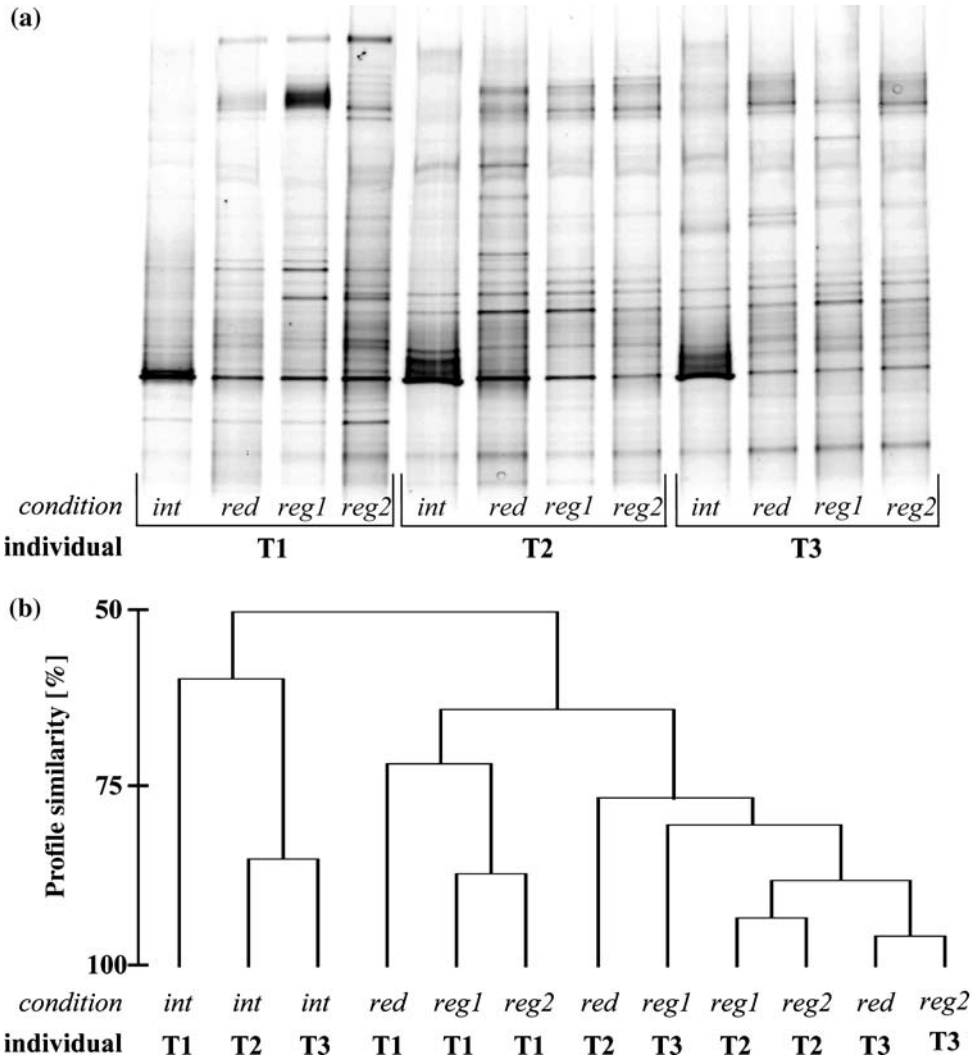


**Fig. 3** **a** DGGE profiles three *Aplysinella* sp. individuals (T1–T3) in the tank experiment in different tissue states. **b** *Dendrogram* showing banding pattern similarities. Tissue states: *int* intact tissue, *red* reduced tissue; *reg1* regenerated tissue, *reg2* regenerated tissue sampled 3 days later than *reg1*

analyzed. The HPLC profiles (Fig. 6) were examined for shifts in peak sizes and possible links of these shifts to the observed morphological changes. Peak size comparison revealed no major changes in the total secondary metabolite content (per gram dry weight of sponge tissue). However, changes in the proportions of the compounds psammaplin A sulfate and psammaplin A as well as in the peak sizes of two compounds tentatively named compound 3 and compound 4 were observed. Compound 3 was present in the intact state of seven of the nine sponges analyzed in the tank experiment. After tissue reduction, it was consistently non-detectable. Following tissue regeneration; however, the peak of compound 3 was found in the HPLC profiles of all nine sponges (Fig. 6). In contrast, this compound was never detected in any of the tissue states of the sponges from the field experiment (data not shown). Compound 4 was present in all samples analyzed (both from the tank and from the field experiment). Its concentration consistently decreased upon tissue reduction and increased when the sponges regenerated their tissues (Fig. 6).

## Discussion and conclusions

### Morphological changes

Under environmental stress such as starvation, desiccation, low temperatures, and oxygen deprivation sponges, particularly freshwater species but also some marine species, form internal buds, so-called gemmules (Simpson and Fell 1974; Reiswig and Miller 1998). These small, ball-shaped structures can remain intact under such adverse conditions, even if the other sponge tissue dies and disintegrates. The tissue reduction we observed in *Aplysinella* sp. was in at least two aspects similar to gemmule formation: (1) it was triggered by (potentially adverse) changes in ambient conditions and (2) resulted in a substantial reduction of the sponge volume. However, various distinct differences to gemmulation were observed: (1) Gemmules are usually encapsulated by a rough shell consisting of spongin and/or siliceous scleres (Devos and Rozenfeld 1974); no such shell formation was observed on the reduction bodies formed by *Aplysinella* sp.

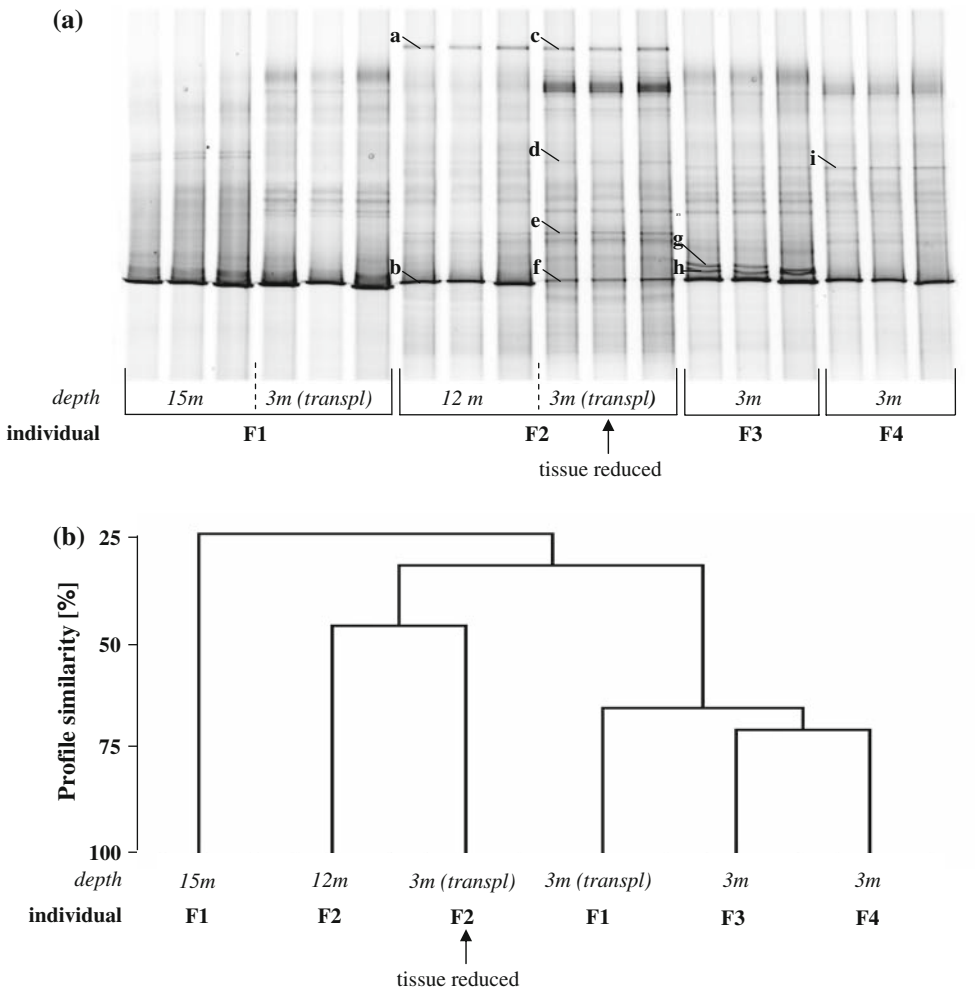


**Fig. 4** **a** DGGE profiles of four *Aplysinella* sp. individuals (F1–F4) of the transplantation experiment in the field. For each sample, three independent PCRs were run. *Letters a–i* on the gel indicate excised and sequenced bands. **b** *Dendrogram* showing banding pattern similarities. *(transpl)* samples from transplanted sponges collected together with F3 and F4 3 months after transplantation

(2) Unlike gemmules (which in most cases are released from maternal dead tissue), the tissue bodies were not released, but regenerated the original sponge by regaining size. (3) Hatching of gemmules usually requires a change back to more favorable ambient conditions (Simpson and Fell 1974; Reiswig and Miller 1998); no such change was apparent when the *Aplysinella* sp. individuals started to recover.

Remarkably, when examined under the microscope, the cross-sections of the reduction bodies formed by *Aplysinella* sp. (Fig. 2, "reduced") strongly resembled those of sponge primmorphs in early reorganizing stages (Custodio et al. 1998). Artificially produced sponge primmorphs are currently in use as a model system to study molecular processes involved in basic Metazoan traits such as differentiation and bodyplan formation (Wiens et al. 2003; Müller et al. 2004; 2006). Most striking about the tissue recovery in *Aplysinella* sp.—next to its rapidness—was that, irrespective of altered external factors, the sponge virtually regenerated its original morphology. In the course of this process cells differentiated and morphological structures such as water channels, choanocyte chambers, epithelial layers and oscules were reconstituted. The remarkable rapidness and the external inducibility of these processes may render the tissue reduction and recovery of *Aplysinella* sp. an expedient in vivo model to complement the existing in vitro system of sponge primmorphs for studies on Metazoan morphogenetic factors.

Interesting, but not yet completely resolved, are the processes that allowed *Aplysinella* sp. to reduce and regenerate its tissue within the very short time of only a few hours. Apoptosis and subsequent cell divisions are unlikely to explain this phenomenon, in particular, since we observed cell density in the tissue to actually increase in the course of the reduction and to decrease upon recovery (Fig. 2). However, we observed the sponge cells to change in size. In particular, cells in the peripheral layers of the reduction bodies appeared larger than in the intact and in the regenerated tissue. In this regard it is interesting to note that we did not observe any major changes in the total concentration of HPLC-UV-detectable metabolites in *Aplysinella* sp. per dry weight of sponge tissue (Fig. 6). In sponges of the genus *Aplysina* that, like *Aplysinella* sp., belong to the order Verongida, brominated compounds structurally and

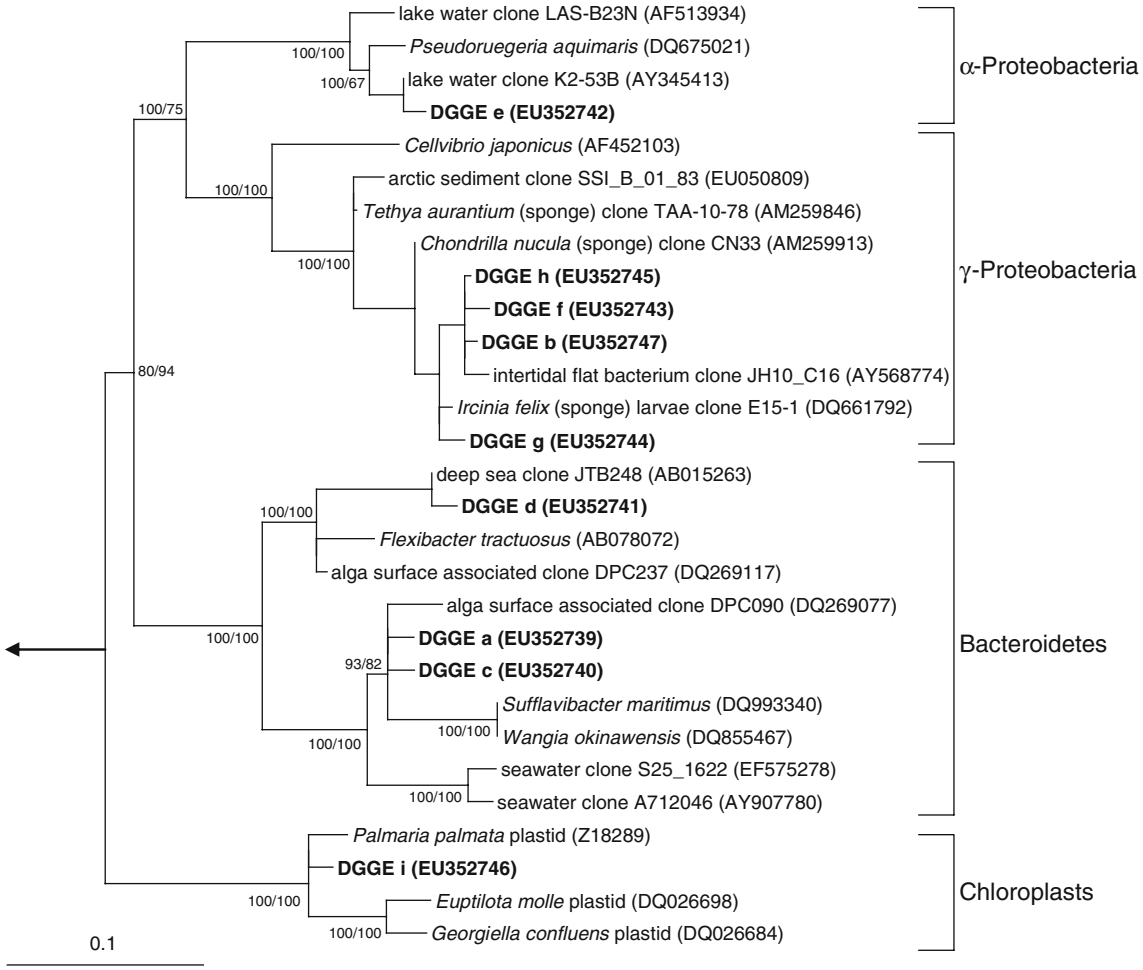


**Fig. 5** Phylogenetic distance tree calculated with 16S rRNA gene bacterial DGGE sequences recovered from *Aplysinella* sp. individuals of field experiment. Neighbor-joining and maximum parsimony (100 pseudoreplicates) bootstrap values are provided. DGGE sequences obtained in this study are in *bold*, *letters a–i* refer to respective excised bands marked in Fig. 4. *Scale bar* indicates 10% divergence. *Arrow*, to outgroup (*Pyrobaculum calidiformis* AB078332, *Sulfolobus metallicus* D85519). GenBank accession numbers are shown in *parentheses*

biogenetically similar to the *Aplysinella* sp. metabolites are stored in spherulous cells (Thompson et al. 1983; Turon et al. 2000). Thus, one could have expected that the considerably higher density of spherulous cells in the reduced tissue correspondingly resulted in a higher concentration of brominated compounds per gram sponge tissue. The observation that this was not the case may indicate that the tissue reduction was not actually associated with a loss of material. A possible explanation could be that the collagenous spongin that constitutes the matrix of the intact sponge mesohyl (Simpson 1984; Garrone 1985) was resorbed by the sponge cells in the course of the reduction and secreted again upon recovery. This could have resulted in a volume, but not weight loss during reduction. Indeed, the spongin fibers that usually fill the spaces in-between sponge cells were no longer observed in the reduced tissue (Fig. 2). Moreover, the larger sponge cell size in the reduced tissue could be interpreted as a result of spongin resorption.

Still unresolved is the question of whether the tissue reduction and recovery in *Aplysinella* sp. can be explained from an ecological standpoint. Even though it could be experimentally triggered by transferring the sponges into the tank, it was also repeatedly observed on undisturbed *Aplysinella* sp. individuals in the field; thus, representing a naturally occurring phenomenon. All sponges transferred into the tank displayed tissue reduction, whereas individuals in vicinity of the original habitat of these sponges at Western Shoals did not show any apparent changes in their morphology. In the natural environment, under non-experimental conditions, tissue reduction was predominantly observed on individuals in shallow areas on the reef flat. Following transplantation to the reef flat, at least one sponge showed tissue reduction after 3 months exposure to the altered conditions. Abiotic parameters such as temperature, current regime, and sun exposure are usually considered more fluctuant in shallower than in deeper water

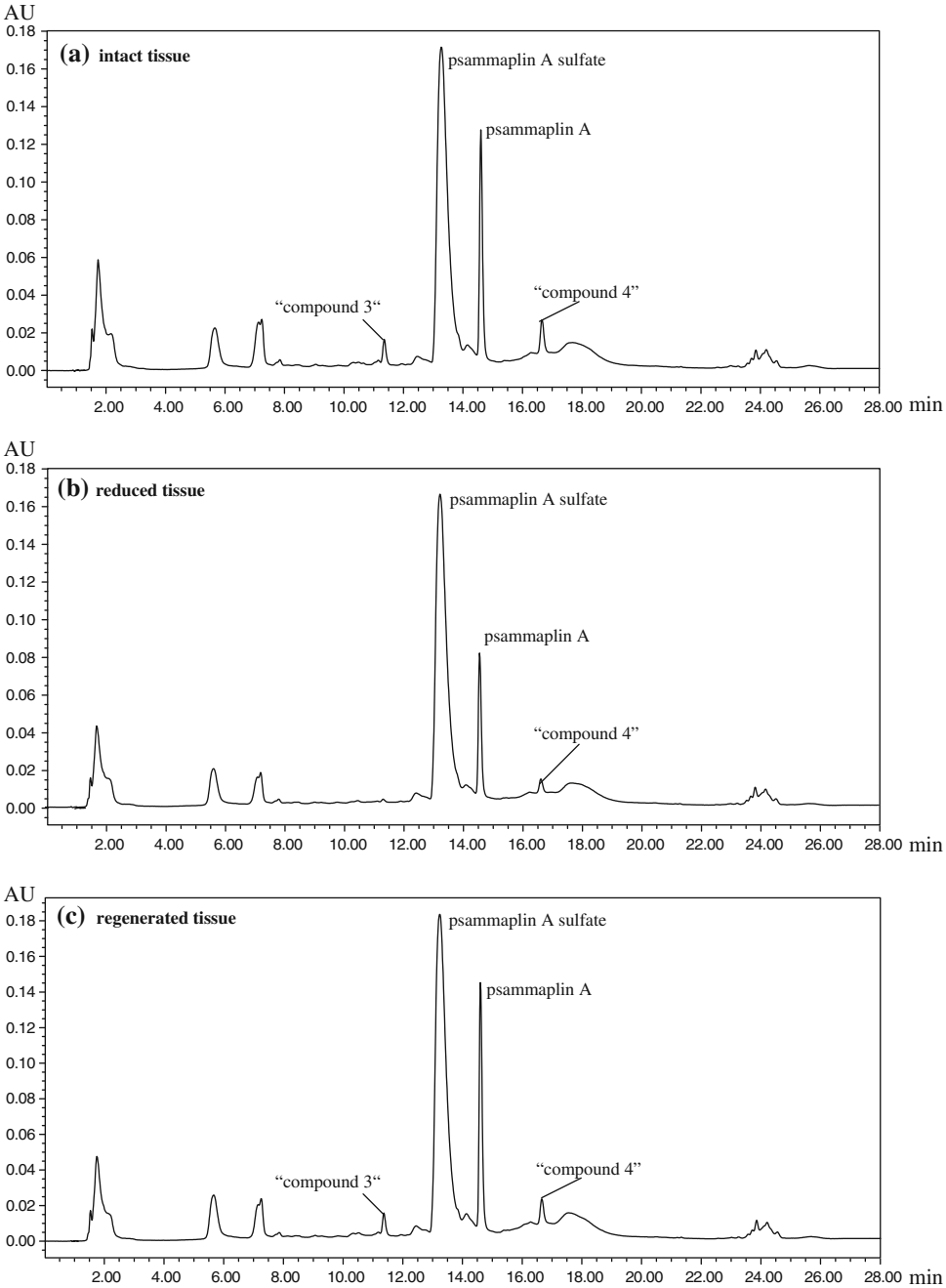


**Fig. 6** HPLC chromatograms of MeOH extracts of *Aplysinella* sp. in the tank experiment. All three chromatograms represent the same sponge individual in the respective tissue states and are representative for most individuals analyzed. **a** Intact tissue (compound 3 was present in seven of nine individuals analyzed). **b** Reduced tissue (reduction bodies) **(c)** Regenerated tissue

(Hirose et al. 2006; Monismith et al. 2006; de Voogd and Cleary 2007). Based on these observations and considerations, we propose that the tissue reduction in *Aplysinella* sp. is a response to changes in ambient conditions. However, other than for the tissue reduction, for the regeneration of the sponges in the tank no external trigger was apparent. In fact, we never observed any link between the onset of regeneration and changes in ambient conditions. This could indicate that the tissue reduction and subsequent regeneration represent an adaptation mechanism to altered ambient conditions rather than a reaction to outlast adverse conditions.

## Microbial community changes

Microbial abundance in intact *Aplysinella* sp., estimated based on extensive electron microscopical inspections of the sponge tissue (Fig. 2), was considerably lower than in other, previously analyzed members of the order Verongida (Vacelet 1975; Friedrich et al. 2001; Thoms et al. 2003). *Aplysinella* sp. clearly does not represent a "high microbial abundance sponge", as defined by Hentschel et al. (2003). In fact, microorganisms were only rarely observed in cross-sections of the sponge, irrespective of the state of the sponge tissue. Characteristic microbial morphotypes,

typically present in high abundances in other Verongida species (Vacelet 1975; Friedrich et al. 1999; Thoms et al. 2003), were not found in *Aplysinella* sp.

Tank maintenance of three *Aplysinella* sp. individuals and consequential tissue reduction coincided with noticeable changes in the DGGE profiles of the sponges (Fig. 3). Comparison of DGGE banding patterns revealed that compared to the original states the three sponge-associated microbial communities had become more similar to each other. Moreover, a similar shift in relative intensity of bands was observed in the DGGE profiles of all three sponges. Apparently, the microbial communities in the different sponge individuals adapted in a similar way to the now more similar ambient conditions in the seawater tank, indicating a strong impact of external parameters on the bacterial composition in *Aplysinella* sp.

Low stability of the microbial community associated with *Aplysinella* sp. and a high dependence on external factors was also revealed in the field transplantation experiment (Fig. 4). Similar to the sponges of the tank experiment, sponge "F2" showed tissue reduction after being transplanted. Also the changes in its DGGE profile were similar to those previously observed in the reduced sponges of the tank experiment, indicating a direct impact of the reduction phenomenon on the microbial composition in *Aplysinella* sp. (Fig. 4a, see Fig. 3a for comparison). Sponge "F1" did not display tissue reduction 3 months after transplantation into the shallow-water habitat. Nevertheless, its DGGE profile showed considerable changes. Banding pattern comparison revealed that it had become more similar to the profiles of the non-transplanted sponges "F3" and "F4" that originally had grown in the shallow-water habitat (Fig. 4b). Apparently, here as well, more similar conditions in the environment of the sponges led to higher similarities in the microbial community compositions.

The observation of high susceptibility of the microbial community associated with *Aplysinella* sp. to external factors does not conform to previous findings on the stability of bacterial compositions associated with other Verongida species. In fact, experiments have shown that their microbial communities are extremely stable even under severely altered ambient conditions (Friedrich et al. 2001; Thoms et al. 2003). The low permanence of the bacterial assemblage in *Aplysinella* sp. may, at least partly, be explained by the lack of bacteria with particular surface structures (e.g., slime capsules and additional membrane layers) that usually are present in other Verongida in high abundances. Since these structures are believed to prevent phagocytosis by sponge cells (Wilkinson 1978; Wilkinson et al. 1984), they may be necessary to maintain long-term associations between the bacteria and their hosts.

Despite the high variability of the microbial community composition in *Aplysinella* sp., one band was constantly found in all DGGE profiles of the sponge (Figs. 3a, Fig. 4a). In fact, in altogether 25 specimens of *Aplysinella* sp. collected at various locations in Apra Harbor, Guam, the $\gamma$-proteobacterium represented by this band was always present, whereas it was not found in the ambient seawater (Thoms, unpublished data). Remarkably, phylogenetical analysis revealed that this bacterium clusters with several other sponge-derived bacteria (Fig. 5). It has to be acknowledged that the cluster also includes two bacteria from other marine sources and, thus, is not exclusively sponge-specific, as defined by Hentschel et al. (2002). Yet, (1) the repeated occurrence of this bacterial phylotype in distantly related sponges, growing in different oceans, (2) its presence in sponge larvae, suggesting vertical transmission (Schmitt et al. 2007), and (3) the high stability of its association with *Aplysinella* sp., irrespective of pronounced changes in external parameters, indicates a symbiotic relation that potentially goes beyond coincidental filtration from seawater. Interestingly, while tissue reduction never completely eliminated this bacterium in *Aplysinella* sp., it repeatedly coincided with a decrease in intensity of the bands representing it in the DGGE profiles (Figs. 3a, Fig. 4a). Whether these shifts in band intensities actually reflect changes in the microbial community will have to be investigated in future studies.

## Chemical profile changes

The total content of secondary metabolites in *Aplysinella* sp. remained largely unaffected by the tissue reduction and recovery as revealed by comparison of peak sizes in the HPLC profiles of the respective morphologic states. Repeatedly observed shifts in the proportions of the two compounds psammaplin A sulfate and psammaplin A most likely can be attributed to a previously described wound-activated conversion of the former compound into the latter (Thoms and Schupp 2008). This reaction inevitably occurs when samples are cut from fresh sponge tissue. Usually, these shifts were not linked to the morphological changes but rather reflected differences in wounding intensity unintentionally caused by sampling the tissue. However, it was repeatedly observed that psammaplin A was present in lower concentrations in samples of reduced tissue than in samples of the two other tissue states (Fig. 6). Other than the tissue from intact and regenerated individuals, the reduction bodies could be sampled with little damage by cutting through the thin skeleton ligaments that connected them to the remaining sponge. This, most likely, explains the lesser extent of wound-activated psammaplin A formation in samples of reduced *Aplysinella* sp. tissue. However, it cannot be ruled out that the pronounced concentration shifts due to the wound-activated conversion could have concealed minor changes in the concentrations of psammaplin

A sulfate and psammaplin A caused by tissue reduction and recovery.

The changes, we observed in the peak sizes of compounds 3 and 4 (Fig. 6) are, to our knowledge, not related to wound-activated conversion reactions in *Aplysinella* sp. Here, links to the morphological changes of the sponge seem possible, particularly since compound 3 was consistently non-detectable in reduced sponges and the peak size of compound 4 consistently decreased and increased, respectively, in the course of the reduction and recovery processes. However, factors other than tissue reduction affecting the concentrations of these two compounds possibly were involved, and further investigation is needed to clarify this point. Particularly since compound 3 was not detected in any intact *Aplysinella* sp. individuals analyzed and the extents of the chemical changes could not clearly be correlated to the extents of morphological changes, based on the current set of data, it seems premature to us to suggest a link between these two events. However, this may deserve further attention in future studies.

## Conclusions and outlook

This study employs a multidisciplinary approach to describe in detail the tissue reduction and recovery processes in *Aplysinella* sp. To our knowledge, this phenomenon, being different from gemmulation in various regards, has not been reported in living sponges in their natural habitat. We observed changes in all characteristics of the sponge investigated, including its morphology, microbiology, and chemistry. Other than the microbial communities in previously analyzed species of the order Verongida, the microbial community in *Aplysinella* sp. proved highly variable. Yet, one $\gamma$-proteobacterial phylotype that co-occurs in various sponge species proved consistently associated with *Aplysinella* sp. indicating a close symbiotic relationship to sponges.

Due to the inducibility and rapidness of the morphological changes, the tissue reduction phenomenon in *Aplysinella* sp. may prove a useful in vivo system to complement the sponge primmorph system currently employed in studies on morphogenetic factors in Metazoa. Moreover, it may represent an expedient model to advance research on symbioses between sponges and bacteria. The simplicity of this system, together with the observation that it is experimentally manipulable, may facilitate systematic manipulative approaches with the aim to gain insights on the impact of the presence of bacteria on other features of the sponge. Existing parallels in chemical and microbiological features of *Aplysinella* sp. to other species may, in turn, allow for the extrapolation of the insights gained to other, more complex sponge/bacteria systems.

**Acknowledgments** LC/MS analyses were conducted by A. Grube (Alfred Wegener Institute, Bremerhaven, Germany). We thank N. J. de Voogd (National Museum of Natural History in Leiden) for advice on the taxonomy of the sponge species studied. M. Taylor and an anonymous reviewer gave valuable comments on the manuscript. C. Thoms gratefully acknowledges support with a Fedodor Lynen Fellowship and a "Rückkehrstipendium" from the Alexander von Humboldt Foundation. This research was supported by NIH MBRS SCORE grant SO6-GM-44796 and NCI grant 5U56CA096278 to P. J. Schupp and BMBF BiotecMarin grant# 03F0414E to U. Hentschel. This is University of Guam Marine Laboratory contribution number 616. The experiments of this study comply with the current laws of the country in which they were performed.

## References

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. J Mol Biol 215:403–410

Ashelford KE, Chuzhanova NA, Fry JC, Jones AJ, Weightman AJ (2005) At least 1 in 20 16S rRNA sequence records currently held in public repositories is estimated to contain substantial anomalies. Appl Environ Microbiol 71:7724–7736. doi:10.1128/AEM.71.12.7724-7736.2005

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

Bond C (1992) Continuous cell movements rearrange anatomical structures in intact sponges. J Exp Zool 263:284–302. doi:10.1002/jez.1402630308

Custodio MR, Prokic I, Steffen R, Koziol C, Borojevic R, Brümmer F, Nickel M, Müller WEG (1998) Primmorphs generated from dissociated cells of the sponge *Suberites domuncula*: a model system for studies of cell proliferation and cell death. Mech Ageing Dev 105:45–59. doi:10.1016/S0047-6374(98)00078-5

de Voogd NJ, Cleary DFR (2007) Relating species traits to environmental variables in Indonesian coral reef sponge assemblages. Mar Freshw Res 58:240–249. doi:10.1071/MF06125

Devos L, Rozenfeld F (1974) Ultrastructure of collagen shell of sponge gemmules. J Microsc (Oxf) 20:15–28

Dyrynda PEJ (1986) Defensive strategies of modular organisms. Philos Trans R Soc Lond Ser B 313:227–243. doi:10.1098/rstb.1986.0035

Friedrich AB, Merkert H, Fendert T, Hacker J, Proksch P, Hentschel U (1999) Microbial diversity in the marine sponge *Aplysina cavernicola* (formerly *Verongia cavernicola*) analyzed by fluorescence *in situ* hybridization (FISH). Mar Biol (Berl) 134:461–470. doi:10.1007/s002270050562

Friedrich AB, Fischer I, Proksch P, Hacker J, Hentschel U (2001) Temporal variation of the microbial community associated with the Mediterranean sponge *Aplysina aerophoba*. FEMS Microbiol Ecol 38:105–113. doi:10.1111/j.1574-6941.2001.tb00888.x

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. doi:10.1002/(SICI)1097-0029(19990215)44:4<279::AID-JEMT7>3.0.CO;2-E

Garrone R (1985) The collagen of Porifera. In: Bairati A, Garrone R (eds) Biology of invertebrate and lower vertebrate collagens. Plenum Press, London, pp 157–175

Hentschel U, Hopke J, Horn M, Friedrich AB, Wagner M, Hacker J, Moore BS (2002) Molecular evidence for a uniform microbial community in sponges from different oceans. Appl Environ Microbiol 68:4431–4440. doi:10.1128/AEM.68.9.4431-4440.2002

Hentschel U, Fieseler L, Wehrl M, Gernert C, Steinert M, Hacker J, Horn M (2003) Microbial diversity of marine sponges. In: Müller WEG (ed) Marine molecular biotechnology: Sponges (Porifera). Springer, Berlin, pp 59–88

Hentschel U, Usher KM, Taylor MW (2006) Marine sponges as microbial fermenters. FEMS Microbiol Ecol 55:167–177. doi:10.1111/j.1574-6941.2005.00046.x

Hepperle D (2004) Align Ver.07/04©. Multisequence alignment-editor and preparation/manipulation of phylogenetic datasets. Win32-Version, 2004. [Distributed by the author via: http://www.sequentix.de]

Hirose E, Hirabayashi S, Hori K, Kasai F, Watanabe MM (2006) UV protection in the photosymbiotic ascidian *Didemnum molle* inhabiting different depths. Zoolog Sci 23:57–63. doi:10.2108/zsj.23.57

Jimenez C, Crews P (1991) Novel marine sponge derived amino-acids, 13 additional psammaplin derivatives from psammaplysilla-purpurea. Tetrahedron 47:2097–2102. doi:10.1016/S0040-4020(01)96120-4

Ludwig W, Strunk O, Westram R, Richter L, Meier H, Yadhukumar Buchner A, Lai T, Steppi S, Jobb G, Forster W, Brettske I, Gerber S, Ginhart AW, Gross O, Grumann S, Hermann S, Jost R, Konig A, Liss T, Lussmann R, May M, Nonhoff B, Reichel B, Strehlow R, Stamatakis A, Stuckmann N, Vilbig A, Lenke M, Ludwig T, Bode A, Schleifer KH (2004) ARB: a software environment for sequence data. Nucleic Acids Res 32:1363–1371. doi:10.1093/nar/gkh293

Monismith SG, Genin A, Reidenbach MA, Yahel G, Koseff JR (2006) Thermally driven exchanges between a coral reef and the adjoining ocean. J Phys Oceanogr 36:1332–1347. doi:10.1175/JPO2916.1

Müller WEG (2006) The stem cell concept in sponges (Porifera): Metazoan traits. Semin Cell Dev Biol 17:481–491. doi:10.1016/j.semcdb.2006.05.006

Müller WEG, Wiens M, Adell T, Gamulin V, Schröder HC, Müller IM (2004) Bauplan of Urmetazoa: basis for genetic complexity of Metazoa. Int Rev Cytol 235:53–92. doi:10.1016/S0074-7696(04)35002-3

Müller WEG, Müller IM, Schroder HC (2006) Evolutionary relationship of Porifera within the eukaryotes. Hydrobiologia 568:167–176. doi:10.1007/s10750-006-0318-6

Muyzer G, Brinkhoff T, Nübel U, Santegoeds C, Schäfer H, Wawer C (1998) Denaturing gradient gel electrophoresis (DGGE) in microbial ecology. In: Akkermans ADL, van Elsas JD, de Bruijn FJ (eds) Molecular microbial ecology, manual 344. Kluwer, Dordrecht, pp 1–27

Pham NB, Butler MS, Quinn RJ (2000) Isolation of Psammaplin A 11¢-Sulfate and Bisaprasin 11¢-Sulfate from the Marine Sponge *Aplysinella rhax*. J Nat Prod 63:393–395. doi:10.1021/np990335y

Pina IC, Gautschi JT, Wang G, Sanders ML, Schmitz FJ, France D, Cornell-Kennon S, Sambucetti LC, Remiszewski SW, Perez LB, Bair KW, Crews P (2003) Psammaplins from the Sponge *Pseudoceratina purpurea:* inhibition of both histone deacetylase and DNA methyltransferase. J Org Chem 68:3866–3873. doi:10.1021/jo034248t

Reiswig HM, Miller TL (1998) Freshwater sponge gemmules survive months of anoxia. Invertebr Biol 117:1–8. doi:10.2307/3226846

Sambrook J, Russell DW (2001) Molecular cloning: a laboratory manual, 3rd edn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor

Schill RO, Pfannkuchen M, Fritz G, Köhler H-R, Brümmer F (2006) Quiescent gemmules of the freshwater sponge, *Spongilla lacustris* (Linnaeus, 1759), contain remarkably high levels of hsp70 stress protein and hsp70 stress gene mRNA. J Exp Zool 305A:449–457. doi:10.1002/jez.a.281

Schmitt S, Weisz JB, Lindquist N, Hentschel U (2007) Vertical transmission of a phylogenetically complex microbial consortium in the viviparous sponge *Ircinia felix*. Appl Environ Microbiol 73:2067–2078. doi:10.1128/AEM.01944-06

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. doi:10.1016/S0040-4020(00)00761-4

Simpson TL (1984) Collagen fibrils, spongin, matrix substances. In: Simpson TL (ed) The cell biology of sponges. Springer, New York, pp 216–254

Simpson TL, Fell PE (1974) Dormancy among the Porifera: gemmule formation and germination in freshwater and marine sponges. Trans Amer Micros Soc 93:544–577

Tabudravu JN, Eijsink VGH, Gooday GW, Jaspars M, Komander D, Legg M, Synstad B, Van Alten DMF (2002) Psammaplin A, a chitinase inhibitor isolated from the Fijian marine sponge *Aplysinella rhax*. Bioorg Med Chem 10:1123–1128. doi:10.1016/S0968-0896(01)00372-8

Taylor MW, Radax R, Steger D, Wagner M (2007a) Sponge-associated microorganisms: Evolution, ecology, and biotechnological potential. Microbiol Mol Biol Rev 71:295–347. doi:10.1128/MMBR.00040-06

Taylor MW, Thacker RW, Hentschel U (2007b) Genetics—Evolutionary insights from sponges. Science 316:1854–1855. doi:10.1126/science.1144387

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 Zoologica 64:199–210

Thoms C, Horn M, Wagner M, Hentschel U, Proksch P (2003) Monitoring microbial diversity and natural product profiles of the sponge *Aplysina cavernicola* following transplantation. Mar Biol (Berl) 142:685–692

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res 25:4876–4882. doi:10.1093/nar/25.24.4876

Thoms C, Schupp PJ (2008) Activated chemical defense in marine sponges—a case study on *Aplysinella* sp. J Chem Ecol 34:1242–1252. doi:10.1007/s10886-008-9518-z

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. doi:10.1007/s004410000233

Vacelet J (1975) Electron-microscope study of association between bacteria and sponges of genus *Verongia* (Dictyoceratida). J Microsc Biol Cell 23:271–288

Wiens M, Mangoni A, D'Esposito M, Fattorusso E, Korchagina N, Schroder HC, Grebenjuk VA, Krasko A, Batel R, Müller IM, Müller WEG (2003) The molecular basis for the evolution of the metazoan bodyplan: extracellular matrix-mediated morphogenesis in marine demosponges. J Mol Evol 57:S60–S75. doi:10.1007/s00239-003-0008-1

Wilkinson CR (1978) Microbial associations in sponges. III. Ultrastructure of the in situ associations in coral reef sponges. Mar Biol (Berl) 49:177–185. doi:10.1007/BF00387117

Wilkinson CR, Garrone R, Vacelet J (1984) Marine sponges discriminate between food bacteria and bacterial symbionts: electron microscope radioautography and in situ evidence. Proc R Soc Lond B Biol Sci 220:519–528