

Influence of spatial competitor on the growth and regeneration of the marine sponge *Cinachyrella cf. cavernosa* (Porifera, Demospongiae)

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Abstract

Availability of suitable substratum is often a limiting factor for sessile organisms. We studied growth and regeneration of golf ball sponge *Cinachyrella cf. cavernosa* with and without its aggressive neighbour, soft coral (*Zoanthus sansibaricus*) in rocky inter-tidal area of Anjuna (Goa), India to understand impact of spatial competition on these life history processes. Specific growth rate of *C. cf. cavernosa* ranged from $3.38 \pm 0.47 \% y^{-1}$ to $24.57 \pm 1.99 \% y^{-1}$. A wound-healing experiment demonstrated 50-100 times faster regeneration, compared to their average growth rate. Both growth and regeneration decreased as sponge size increased. The sponges exhibited 36-69% higher growth and 30-76% faster regeneration rate in the absence of the competitor species, *Z. sansibaricus*. The protein synthesis ability (RNA to DNA ratio), which is an index for physiological activity was adversely affected by sponge size and competitor abundance. Results suggest that in a space-limited region, presence of an aggressive neighbour acts as one of the crucial factors affecting the primary functions (such as growth and regeneration) of the sponge. This study also highlights the remarkable regenerative ability of the slow growing sponge *C. cf. cavernosa* which aids in maintaining its abundance under stressful conditions of rocky inter-tidal region.

Keywords: Inter-tidal sponge, Soft coral, Growth, Regeneration, Competitive stress, RNA to DNA ratio.

Introduction

Sponges are the simplest animals with high totipotency (Müller, 2006). They are soft-bodied, sessile and filter feeding organisms that a) take part in benthic–pelagic coupling (Hadas et al., 2009; Riisgård & Larsen, 2010), b) act as a habitat for other organisms (Henkel & Pawlik, 2005; Hultgren & Duffy, 2010), c) determine benthic community structure by ecological interactions (Engel & Pawlik, 2000) and d) produce bioactive molecules of therapeutic importance (Thakur & Müller, 2004; Sipkema et al., 2005). In spite of their great ecological and biotechnological importance as listed above, the basic information on sponge growth and regeneration, especially those from the Indo-pacific regions is still lacking (Sivaramakrishnan; Bell, 2002; Bremner et al., 2003).

Sponges are mostly slow growing but they possess remarkable ability to regrow their lost tissue (Simpson, 1984). Growth and regeneration in sponges are highly variable and differ within the species depending on ecological factors (Ayling, 1981; Becerro et al., 1994). Previous studies predicted that growth of the sessile organisms (such as sponges, corals, bryozoans, etc) was indeterminate, that is, the individual (or colony) size would increase exponentially with time (Jackson, 1977). However, recent studies have proved that growth and regeneration in the sponges are influenced by various abiotic (such as ambient water temperature, sedimentation, physical disturbance) and biotic (size, age, morphology, food availability and ecological interactions) factors (Stone, 1970; Elvin, 1976; Turon et al., 1998; Duckworth, 2000; Duckworth & Battershill, 2001; Garrabou & Zabala, 2001; Bell, 2002; Duckworth, 2003; Henry & Hart, 2005; Wulff, 2006a; De Caralt et al., 2008; Walker et al., 2008). However, the influence of ecological interactions (especially competition for space) on growth and regeneration has not been studied in detail (Aerts, 1999).

Intertidal sponges are exposed to highly variable and stressful environmental conditions. The acquisition of a suitable substratum is necessary for proper development and growth of the sessile organisms such as sponges and corals (Wulff, 2006a; Wulff, 2006b; Chadwick & Morrow, 2011). The interacting organisms adopt various offensive and defensive strategies to obtain a suitable substratum and protect the acquired space from other encroaching neighbours (Chadwick & Morrow, 2011). The sponge maintains its living space either by overgrowing (especially in case of encrusting sponges) and / or by producing toxic chemicals (allelochemicals)(Wulff, 2006b). However, the energy reservoir of the sponge, like of any other organism, is limited and resources are needed to be adequately allocated to other biological functions (such as growth, regeneration, reproduction and defences) (Rinkevich, 1996;

Zera & Harshman, 2001). In space-limited environment such as intertidal regions, sponges have to devote a portion of their energy to defend the space against encroaching neighbours, which might hamper their growth and regeneration. To determine the possible impact of aggressive neighbour on growth and regeneration of a sponge, we chose an intertidal globular species *Cinachyrella* cf. *cavernosa* (*sensu* BURTON, 1959), which was growing in close contact with the aggressive, colonial soft coral *Zoanthus sansibaricus* (CARLGREN, 1900). Soft corals such as *Palythoa caribaeorum* and *Zoanthus sociatus* are aggressive organisms which provide tough competition for space (Karlson, 1980; 1988; Bastidas & Bone, 1996; Rabelo et al., 2013). Zoanthids are known to produce bioactive compounds which have important role in defence mechanisms (Harper et al., 2001). They can acquire the substratum by overgrowth (Rabelo et al., 2013) and /or using allelochemicals to inhibit growth of neighbouring fauna (Sammarco et al., 1985). However, the sponge under present investigation grows in close contact with *Z. sansibaricus*, which reveals its ability to protect itself from encroaching neighbour. Sponges of the genus *Cinachyrella* are a rich source of unusual steroids and phospholipids (Aiello et al., 1991; Barnathan et al., 1992; Barnathan et al., 2003; Li et al., 2004; Xiao et al., 2005). Two new ceramides and tetillapyrone have been isolated from an Indian specimen of *C. cavernosa* (Lakshmi et al., 2008). Recently, the study sponge *C. cf. cavernosa* from Anjuna, Goa has been reported to contain rare sterols (4 α -methyl gorgostanol and 16-dehydropregnenolone), which can be useful in its chemotaxonomic studies (Wahidullah et al., 2015). As a part of our research program on the bioactive metabolites from marine invertebrates (particularly the sponges), we investigated *C. cf. cavernosa* exhibiting 20% antiviral activity against Semiliki Forest Virus (SFV) and high cytotoxicity against Hela cell lines (IC₅₀= 5 nM) and PC-3 cell lines (IC₅₀= 9 nM). Moreover, we have isolated the cytotoxic compound ‘ β -sitosterol’ from *C. cf. cavernosa* which showed a temporal variation depending on the abundance of *Z. sansibaricus*, thereby suggesting its possible role as an allelochemical (Singh & Thakur, unpubl. data). The use of allelochemicals in competition for space has been observed in several ecological interactions among sponges and corals (Sullivan et al., 1983; Porter & Targett, 1988; Aceret et al., 1995; Maida et al., 1995; Russell et al., 2003).

Although there are potential biomedical applications of this sponge species, almost no data is available on its life-history processes and ecology. To the best of our knowledge, this is the first long-term study on the growth and regeneration in *C. cf. cavernosa*. This study could provide the information on the factors affecting growth and regeneration in the study sponge which can be utilized to 1) design of mariculture conditions and 2) explore whether this sponge species can be used as a sustainable source

for isolation of novel bioactive compounds. Growth and regeneration studies were conducted on tagged sponges of different sizes for a period of 18 months through non-manipulative observation (control condition) and a manipulative experiment (test condition) by scraping off the aggressive neighbour, *Z. sansibaricus*. In addition, RNA to DNA ratio of the sponges from both conditions (control and test) was compared to estimate the effect of aggressive neighbour on their physiological conditions. It is assumed that the primary genetic material 'DNA' does not vary with environmental condition or growth rate (Bulow, 1970) whereas amount of RNA which is directly related to the protein synthesis, varies with age, life-stage, organism size, and environmental conditions. The RNA to DNA ratio is a measure of eco-physiological index of activity in many marine organisms such as microbes, sponges, crustaceans, fishes (Robinson & Ware, 1988; Anger & Hirche, 1990; Chicharo et al., 1998; Anil et al., 2001; Singh & Thakur, 2015).

Materials and methods

The study site and specimens

Field studies and sample collection were carried out on rocky beach of Anjuna, Goa, India (15° 34' 34.986"N; 73° 44' 23.895" E). The sponge under present investigation is *Cinachyrella cavernosa sensu* BURTON, 1959 (in part) (not: *Cinachyrella cavernosa* (LAMARCK, 1813)), and it is likely to be a new species (JNA Hooper & KA Hall, pers. comm.). The representative specimen has been deposited at CSIR-NIO Repository and Taxonomic Center under the reference code (NIO-41). *Cinachyrella cf. cavernosa* is abundantly present in low inter-tidal region of the rocky shore, which is affected by high water movement and exposed to high solar radiation during low tide. The sponge was found to be surrounded by aggressive neighbour *Zoanthus sansibaricus*. The soft coral *Zoanthus sansibaricus* was identified using taxonomical keys (Uchida & Soyama, 2001; Ryland & Lancaster, 2003). Further, we validated the identity of *Zoanthus sansibaricus* using molecular markers (mitochondrial 16S and 12S rRNA genes), according the PCR method described by (Sinniger et al., 2005) and the sequences have been submitted to NCBI with GenBank Accession no.KT266275 and KT266276 respectively.

Estimation of sponge growth rate

Fifteen sponges of varying sizes (representing the entire size range of the present population, except new recruits of size < 2 cm) were selected and each sponge was marked with four stainless steel nails forming a quadrat (15 cm × 15 cm). In the manipulative experiment, additional fifteen sponges of various sizes were tagged in a similar way and polyps of *Z. sansibaricus* which surrounded the tagged sponges

were carefully removed using a scalpel (**Fig. 1**). For a period of 18 months (from October, 2013 to March, 2015), photographs of the marked areas were taken every month with a digital camera (Canon Power shot A650) fixed on a photo-quadrat stand to capture images at the same angle and distance. These photographs were analysed using Image J software (developed at the National Institutes of Health, USA). The body size of the individual sponge was measured by taking multiple reading (4 readings per sponge) for the diameter and mean diameter was calculated. Then, the volume was calculated from the mean diameter using the formula for the volume of sphere since the sponge was globular in shape. The specific growth rate (SGR) was calculated using the following equation for globular sponges (Di Bari et al., 2014): $SGR = (\log_e V_2 - \log_e V_1) / (t_2 - t_1) \times 100$, where \log_e is the natural logarithm, V_2 and V_1 are the volume at the two different times (t_2 and t_1), and $(t_2 - t_1)$ the time interval in months. We also calculated the abundance of competitor *Z. sansibaricus* as total of its polyps which surrounded each sponge in the marked quadrat (n=15) from the photographs. After taking photographs of the tagged sponges, the physical and chemical parameters (temperature, salinity, pH, and dissolved oxygen) of the seawater in rock pool were measured on the same day on a monthly basis, using the waterproof multi parametric probe Oakton PCD 650 (OAKTON Instruments, Vernon Hills, IL, USA). The water samples (n=3) were collected monthly from the rock pool containing the tagged sponges and nutrient analysis (nitrate, nitrite, ammonia, phosphate and silicate) was carried out using a continuous flow analyzer (San++, SKALAR, Holland). The concentration of nitrate, nitrite, ammonia and phosphate was expressed in terms of N: P ratio. The average values of environmental parameters were used in the final analysis. The data were log-transformed and also checked for multicollinearity by examining tolerance and variance inflation factors (VIF) for each predictor variable. We found multicollinearity in some of the predictor variables; therefore, a stepwise multiple regression was used to establish the relationship between ecological factors (such as pH, temperature, salinity, DO, silicate, N: P ratio and competitor) and the specific growth rate of the tagged sponges, with 'size' as the co-variate. Two approaches (e.g., forward selection and backward elimination) were followed to select the predictive variables.

Estimation of regeneration rate

The regeneration in *C. cf. cavernosa* was estimated in the presence and the absence of its neighbour *Z. sansibaricus* 1) from October, 2013 to January, 2014 (winter) and 2) from March, 2014 to June, 2014 (summer). For each season, a separate set of 18 sponges of different sizes was tagged as described earlier. From those, in 9 tagged quadrats, polyps of *Z. sansibaricus* were completely removed with

scalpel whereas the remaining 9 quadrats contained both *C. cf. cavernosa* and *Z. sansibaricus*. A cork borer was used to cut a core of approximately 1 cm in diameter and 1cm in depth into each sponge to experimentally simulate predators' bites. The core from each sponge was collected and carefully preserved in absolute ethanol (MP Biomedicals) for analysis of RNA: DNA ratio. Wounds similar to these are frequently seen on *C. cf. cavernosa* in our study (Singh and Thakur, personal observation). The depth and diameter of the wound were measured by vernier callipers. Damaged areas were photographed at the start of the experiment and after every 2 weeks for entire study period and percentage regeneration was measured in terms of percentage of volume change using equation: $\% \text{ Regeneration} = (\Delta V/V \times 100)$, where V was initial wound volume and ΔV was change in the volumes of the wound at the end of the experiment. Seasonal variation in regeneration of the sponges with and without competitor was calculated by taking mean of the % regeneration values and represented as mean with 95% confidence intervals. Multiple regression analysis was performed to determine the impact of sponge body size and competitor (*Z. sansibaricus*) on its regeneration rates. Additional 10-15 sponges were tagged for both the growth and the regeneration experiments in the beginning which served as backup in case of loss of any tagged experimental sponges. In final calculations of growth and regeneration, only those tagged sponges were chosen which had not suffered any damage from predators or other physical disturbances (strong wave or sediment burial).

Estimation of RNA to DNA ratio

For analysis of RNA to DNA ratio, the tissue samples (n=36) were obtained from the same tagged sponges which were used for the wound healing experiments as described earlier. Nucleic acids were extracted from 25 mg of each of sponge tissue samples using GenElute Mammalian Genomic DNA Minipreps (Sigma), following the manufacturer's instructions. The spectroscopic method was followed for estimation of RNA to DNA ratio using EBproK (fluorophor: EB ethidium bromide, Sigma and proteinase K, Sigma) (Caldarone et al., 2006). Total nucleic acid content was determined using a NanoDrop ND-1000 Spectro-photometer (NanoDrop, Wilmington, DE, USA). Each sample was treated with ribonuclease A, incubated at 37°C for 30 minutes and then cooled to room temperature (25°C). The fluorescence due to total RNA was calculated as the difference of the fluorescence before and after ribonuclease A treatment. It was assumed that the fluorescence before ribonuclease A treatment was due to total concentration of nucleic acids (DNA and RNA), whereas the fluorescence after ribonuclease A was only due to DNA. The auto-fluorescence of ribonuclease A was also calculated and factored into

final calculations. Multiple regression analysis was performed to determine the variability in RNA to DNA ratio as a function of sponge body size and competitor (*Z. sansibaricus*). All statistical analyses were completed by using the Statistica 8 software (StatSoft, Poland).

Results

Abiotic parameters

The temperature of seawater in the rock pools varied from 31.2° C in March, 2014 to 27.6° C in August, 2014. The mean salinity was 33.06‰ which dropped dramatically to 26.82–29.27 ‰ during monsoon season (June–September) due to the influx of freshwater. Dissolved oxygen ranged from 5.76 mg L⁻¹ (December) to 2.19 mg L⁻¹ (May), while the mean pH value was 7.92. N: P ratio of the rock pool water varied from 2.32 (August) to 16.76 (October) whereas silicate content (Si) ranged from 2.76 μM (October) to 19.12 μM (June). The cover of competitor *Zoanthus sansibaricus* (expressed in terms of total number of polyps) showed a seasonal pattern and was the highest for October (Mean± SE = 1170± 76, n=15 quadrats) and the lowest for March (Mean± SE= 289 ± 18, n=15 quadrats) as shown in **Fig.2**.

Growth rates

The overall growth of *Cinachyrella cf. cavernosa* was found to be very slow. However, there was measurable increment in size over the 18 months of study period. The specific growth rate (SGR) was highly variable among the different sizes of *C. cf. Cavernosa*, ranging from 3.38 ± 0.47 % y⁻¹ to 24.57 ± 1.99 % y⁻¹ (**Fig. 3**). In the manipulative experiment involving removal of aggressive neighbour *Z. sansibaricus* from the vicinity of the tagged sponges, the specific growth of small-sized sponges (body size up to 3 cm in mean diameter) increased by 68.62 % whereas that of larger sponges (mean diameter > 3 cm) rose by 35-38%. Stepwise multiple regression analysis yielded two significantly different models (p=0.041). The forward selection MRA resulted in a minimal model (Model I), comprised of four predictor variables (size, temperature, silicate and DO) whereas the backward elimination MRA gave an alternative model (Model II) by removing the effect of pH on SGR. Model II explained greater variability in SGR by taking into account another significant variable (competitor), in addition to the other four variables of the minimal model. The rest of the environmental parameters did not show any significant influence on SGR (**Table 1**).

Regeneration rate

The damaged sponges exhibited 50 to 100 fold faster regeneration than the actual growth. The regeneration in *C. cavernosa* followed three distinctive phases (**Fig. 4**): 1) Phase A involved formation of a thin layer of undifferentiated tissue (scar tissue) which was darker in colour, over the wound area. 2) During phase B, the scar tissue became lighter in colour and thicker due to accumulation of net of transparent fibres and spicules. Undifferentiated tissue grew from the edges of the artificially induced lesion, thereby leaving only a depression in the middle of the wound. 3) Phase C was the final phase of regeneration, which was achieved by thickening of the overlying tissue to fill the depression. All tagged individuals of *C. cf. cavernosa* (n = 18/season) which were experimentally damaged, showed initial signs of regeneration within one week time. However, the sponges demonstrated different timings for complete wound healing depending on their size. The first phase of regeneration 'Phase A' took place in less than a week in smaller sponges (3 cm in mean diameter), whereas it took 10-15 days in larger sponges (> 3 cm in mean diameter). The subsequent phases (Phase B and C) followed the same pattern. In smaller sponges, 30-50% of lesion volume was filled within 13 days of the wound induction time. The recovery from artificially inflicted wound (calculated as mean of % regeneration) was 10-15 % faster in summer months (March to June) as compared to winter months (October to January). In absence of *Z. sansibaricus*, sponges of different sizes showed 30-75 % increase in regeneration capacity, irrespective of environmental conditions (**Fig. 5**). Multiple regression analysis demonstrated the regeneration rate in *C. cf. cavernosa* was negatively influenced by its body size and competitor ($R^2=0.54$; $p < 0.0001$) (**Table 2 A**).

RNA to DNA ratio

The DNA content in sponges of different sizes was constant, without any significant difference ($p = 0.18$). The RNA content was inversely proportional to the body size of the sponge ($R = 0.67$, $p < 0.001$). The multiple regression analysis showed a negative influence of 'body size' and competitor on the RNA: DNA ratio of the sponges ($R^2 = 0.52$; $p < 0.00001$) (**Table 2 B**).

Discussion

The sponge *C. cf. cavernosa* is an important component of the benthic community in the central west coast of India. The present investigation demonstrated that *C. cf. cavernosa* grows at a very slow rate (with an increase in mean diameter = $0.1 - 0.2 \text{ cm y}^{-1}$) as compared to the growth rates of other tropical and temperate sponges which range from 2.4 cm y^{-1} to 102.2 cm y^{-1} (Reiswig, 1973; Ayling, 1983;

Hoppe, 1988; Pansini & Pronzato, 1990; Turon et al., 1998; Garrabou & Zabala, 2001; Tanaka, 2002). Slower and irregular growth rates have been recorded for many other sponges, especially thinly encrusting sponges from temperate subtidal waters (Ayling, 1981; Ayling, 1983). For instance, a thinly encrusting sponge *Eurypon* sp. grew very slowly, with mean rate of $0.0003 \text{ mm}^2 \text{ cm}^{-1} \text{ border day}^{-1}$ (Ayling, 1983). In contrast to the sponge *Microciona* sp., which showed asynchronous and overall negative growth rate (Ayling, 1983), the study sponge *C. cf. cavernosa* showed positive growth rate, thus making it possible to examine the variation in its growth rate in relation to seasonal variation in environmental parameters. The specific growth rate of *C. cf. cavernosa* varied with its body size and was found to be positively regulated by temperature. Seasonal pattern in growth rate has also been reported in other sponges (Johnson, 1979; Turon et al., 1998; Garrabou & Zabala, 2001; Tanaka, 2002; De Caralt et al., 2008; Leong & Pawlik, 2010). In most of the cases, higher growth rates in warmer months have been linked to increase in primary productivity which results in availability of sufficient food (Duckworth et al., 2004; Koopmans & Wijffels, 2008; McMurray et al., 2008; Di Camillo et al., 2012). Interestingly, decrease in concentration of silicate in the ambient sea water of the rock pool resulted in higher SGR in *C.cf. cavernosa* for that particular month which is suggestive of incorporation of large amount of silica (Si) by the sponge for skeletal development. The localized depletion of Si from the surrounding water have been reported in case of many sponge species which makes them as ‘Si sink’ (Maldonado et al., 2005). On the other hand, higher concentration of DO in the rock pool water showed increase in SGR. The silica content and DO of seawater are among the important limiting factors which affect the growth of the sponge, especially during in vitro-cultivation (Osinga et al., 1999). However, the role of silica in regulating the growth rates of sponges under natural environment needs to be validated through a separate set of experiment by monitoring the actual intake of silica by the sponges during filter-feeding.

The growth rate of *C. cf. cavernosa* decreases with increase in its body size, which is in accordance with various other reports on sponge growth (Reiswig, 1973; Hoppe, 1988; Leys & Lauzon, 1998; Garrabou & Zabala, 2001; De Caralt et al., 2008; McMurray et al., 2008; Teixidó et al., 2009). Field studies on the newly settled sponge buds of *Tethya citrina* demonstrated that growth rate was seasonal and it decreased with sponge age (Cardone et al., 2010). In our recent work on the asexual reproduction (budding) of the same sponge species, *C. cf. cavernosa*, from the intertidal region, Mhapan, we found that budding occurred only in larger sponges (mean diameter > 3 cm), whereas newly recruited or small-sized sponges (mean diameter $\leq 3\text{cm}$) were not involved in any reproduction process (Singh & Thakur,

2015). A similar trend was observed in tagged specimens of *C. cf. cavernosa* from the Anjuna region, which suggests that lower SGR in the larger sponges might be due to their active participation in reproduction (Singh and Thakur unpubl. data). Many authors have reported trade-off between growth and sexual reproduction, as the reproduction process involved conversion of feeding choanocytes into reproductive cells (sperm cells), thereby hampering sponge' feeding efficiency and ultimately its growth rate (Turon et al., 1998; Duckworth et al., 2004). Recent mariculture attempts on the sponge *Tethya citrina* showed a significant reduction in its size under ex-situ conditions, followed by massive production of asexual buds, which is suggestive of possible trade off among life history processes such as growth and asexual reproduction (Di Bari et al., 2014).

In comparison to the growth, the regeneration process in *C. cf. cavernosa* is very fast. If the tissues of the sponges were artificially inflicted, the regeneration process was activated at rates 50-100 times faster than the undisturbed growth rate of the species which depicts that growth and regeneration are entirely different cellular processes. The regeneration in the sponges *Hippiospongia lachne* and *Spongia barbara* involved formation of ectosome tissue without the formation of new biomass (Storr, 1964), which further supports our contention that regeneration and growth are different phenomenon. In a recent study on the sponge *Halisarca caerulea*, it was found that regeneration involves collagen production and migration of existing amoeboid cells to the mesohyl tracts of the damaged area, rather than proliferation of mesohyl cells (Alexander et al., 2015). However, when major portion of tissue is lost, the organisms exhibit both regeneration (reorganization of existing differentiated cells) and growth (production of newly differentiated cells from archaeocytes) to fully recover the lost structures (Simpson, 1984). Recently, it has been demonstrated that development of a fully functional individual from sponge cell aggregates is an adaptive trait which passes through certain checkpoints such as the sorting of cells, adhesion to a substrate (by developing polarity) and cell differentiation; followed by organization of cells into tissue and regionalization of tissues to create three-dimensional structure (Eerkes-Medrano et al., 2014). In various wound healing experiments, it was found that the regeneration rate was much faster than the actual growth rate (Ayling, 1981; Ayling, 1983; Hoppe, 1988; Bell, 2002; Walters & Pawlik, 2005). The rapid regeneration rate is advantageous for slow growing sponges in areas subjected to high predation pressure, frequent storms and strong wave actions (Ayling, 1981). High regenerative capacity offers various advantages to the sponges : 1) it aids in rapid recovery from wounds, thus avoiding microbial infection on wounded parts (Leys & Lauzon, 1998), 2) it plays important role in recolonization of sponges (Kay & Keough, 1981), 3) it maintains optimal feeding rate by a regenerating

damaged canal system (Bell, 2002; Walters & Pawlik, 2005) and 3) it mitigates mortality due to predators' attacks or physical disturbances (Battershill & Bergquist, 1990; Wulff, 2006a) and overgrowth by other organisms (Turon et al., 1998). We found that even very small sponge tissue fragments (10-20% of total sponge body) of *C. cf. cavernosa* could recover after almost all living tissue was scraped from the rock. A high threshold for recovery (>90%) has been also documented in two temperate subtidal massive sponges *Latrunculia wellingtonensis* and *Polymastia croceus* (Duckworth, 2003). In contrast, the threshold percentage is only 20-30% in other massive sponges (Dayton et al., 1974; Sheild & Witman, 1993). The regeneration rates vary among different species of sponges (Dayton et al., 1974; Ayling, 1981; Ayling, 1983; Hoppe, 1988; Sheild & Witman, 1993; Duckworth, 2003). The sponges *L. wellingtonensis* and *P. croceus* exhibit different regeneration rate depending on their internal structures (presence of regular or irregular reticulation in 'choanosome' =inner layer of sponge), rather than external morphology (Duckworth, 2003). An inverse relationship between regeneration rate and complexity of the structure has been established for many species of coral reef sponges (Wulff, 2006a). The faster regeneration rate in the study sponge *C. cf. cavernosa* can be attributed to its structural homogeneity i.e. absence of differentiated cortical layer in all species of genus *Cinachyrella* (Hooper & Van Soest, 2002). Thus, no extra energy needs to be invested in differentiation of primary cells into various types to form new tissue. Comparable to *C. cf. cavernosa*, the regeneration rate in the thinly encrusting sponge *Stylopus* sp. was recorded to be 200 times higher than the normal growth rate of the species (Ayling, 1983). Interestingly, the encrusting sponge *Eurypon* sp. which showed slower growth rate could rapidly regenerate 2,900 times faster than the growth rate of the species (Ayling, 1981). The regeneration process in *C. cf. cavernosa* followed three distinctive phases (A, B, C), similar to those described for other sponge species such as *Agelas clathrodes*, *Neofibularia nolitangere* and *Ircinia strobilina* (Hoppe, 1988). Different species of sponges follow a comparable sequence of regeneration phases but the timing of each phase differs widely among them (Hoppe, 1988). In our study, we found that the timing of each phase of regeneration was affected by the size of the sponge. Compared to larger sponges (mean diameter >3 cm), damaged tissue was rapidly healed in the smaller sponges (mean diameter ≤ 3cm), which is probably in line with the high protein synthetic capability (RNA: DNA) found in these specimens. The RNA: DNA ratio was found to be inversely proportional to the size of the sponges which has also been reported in case of clupeids larva and marine fishes (Fonseca et al., 2006; Vinagre et al., 2008). Low regeneration capacity in the larger-sized sponges (mean diameter >3 cm) might be attributed to their reproductive investment (Uriz et al., 1995) and ageing which is a general

trend observed in many organisms (Fleming et al., 2007). Comparisons of similar wounds in *C. cf. cavernosa* of different sizes have demonstrated that variation in regeneration may be tied to differences in energy reservoirs which determine the physiological condition of the sponge. During our field studies, we did not find any sponges larger than 6 cm in mean diameter which indicates that the sponges are subjected to frequent grazing and other physical disturbances (strong waves and sedimentation), thus leading to partial and complete loss of tissue.

Interaction with other organisms can hamper the physiological functioning of the sponges (Wulff, 2005). In this investigation, the effect of aggressive neighbour *Zoanthus sansibaricus* on growth and regenerative ability of the sponge *C. cf. cavernosa* is reported for the first time. The sponge showed a significant reduction in its growth and regeneration in the presence of *Z. sansibaricus*. Aggressive soft coral species including zoanthids have been reported to deploy various physical (nematocyst, sweeper tentacles) and/or chemical defences (toxic secondary metabolites) against competitors and predators (Moore & Scheuer, 1971; Karlson, 1980; Sammarco et al., 1985; Hines & Pawlik, 2012; Rabelo et al., 2013). Various wound healing experiments on 10 species of Caribbean coral reef sponges showed trade-off between regeneration rate and chemical defences, as the defended sponges' demonstrated slower rate of healing than the undefended sponges (Walters & Pawlik, 2005). Some sponges with low growth and regeneration rates utilize their energy in various other ecological functions (such as competition for resources, chemical defences etc.) (Turon et al., 1998; Aerts, 1999; Walters & Pawlik, 2005; Wulff, 2005; Pawlik et al., 2008; Leong & Pawlik, 2010; Pawlik, 2011). Thus, slower growth and regeneration rate in *C. cf. cavernosa* could be attributed to lesser resource allocation to these primary functions in presence of spatial competitors. Competition reduces growth and regeneration, as the competitive interactions use energy which otherwise is reserved for these primary processes. This was further validated by the significant increase in growth and regeneration of *C.cf. cavernosa* in the manipulative experiment which involved the complete removal of competitor *Z. sansibaricus*.

In spite of its slow growth rate, the sponge *C. cf. cavernosa* is abundantly present in the stressful conditions of intertidal regions of Anjuna. The existence of the sponge *C.cf. cavernosa* in the vicinity of the aggressive soft coral *Z. sansibaricus* depicts its ability to utilize the limited resources for the successful settlement in intertidal environments. Over the entire study period, the interactions between *C.cf. cavernosa* and *Z. sansibaricus* showed an apparent equilibrium without any overgrowth of any of interacting species, which indicates the possible role of allelochemicals for such "stand-off" interactions. Interestingly, from the manipulative experiment, we can conclude that removal of *Z. sansibaricus*

increases both growth and regeneration in *C.cf. cavernosa* by releasing the competitive pressure, thereby permitting it to allocate more resources to the primary processes. The “standoff” strategy displayed by the sponge and the soft coral permits them to create a balance in their use of space. High threshold value (80-90%) for recovery of *C.cf. cavernosa* allows it to regain after a major tissue loss. The slow growing *C.cf. cavernosa* maintains high regeneration as one of the strategies to thrive successfully in highly diverse intertidal regions, with fluctuating environmental conditions. Knowledge of sponge ecology is restricted to only a few species, and a holistic approach involving multidisciplinary studies is needed to improve our understanding of the trade-off among various life history functions of these organisms.

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References

- Aceret, T., P. Sammarco & J. Coll, 1995. Effects of diterpenes derived from the soft coral *Sinularia flexibilis* on the eggs, sperm and embryos of the scleractinian corals *Montipora digitata* and *Acropora tenuis*. *Marine biology* 122: 317-323.
- Aerts, L. A. M., 1999. Sponge-coral interactions on Caribbean reefs. PhD thesis, University of Amsterdam, Netherlands.
- Aiello, A., E. Fattorusso, S. Magno, M. Menna & M. Pansini, 1991. Steroids of the marine sponge *Cinachyra tarentina*: Isolation of cholest-4-ene-3, 6-dione and (24R)-24-ethylcholest-4-ene-3, 6-dione. *Journal of Natural Products* 54: 281-285.
- Alexander, B. E., M. Achlatis, R. Osinga, H. G. van der Geest, J. P. Cleutjens, B. Schutte & J. M. de Goeij, 2015. Cell kinetics during regeneration in the sponge *Halisarca caerulea*: how local is the response to tissue damage? *PeerJ* 3: e820.
- Anger, K. & H.-J. Hirche, 1990. Nucleic acids and growth of larval and early juvenile spider crab, *Hyas araneus*. *Marine biology* 105: 403-411.

- Anil, A. C., D. Desai & L. Khandeparker, 2001. Larval development and metamorphosis in *Balanus amphitrite* Darwin (Cirripedia; Thoracica): significance of food concentration, temperature and nucleic acids. *Journal of Experimental Marine Biology and Ecology* 263: 125-141.
- Ayling, A., 1981. The role of biological disturbance in temperate subtidal encrusting communities. *Ecology* 62: 830-847.
- Ayling, A. L., 1983. Growth and regeneration rates in thinly encrusting demospongiae from temperate waters. *The Biological Bulletin* 165: 343-352.
- Barnathan, G., E. Genin, N. E. Velosaotsy, J.-M. Kornprobst, S. Al-Lihaibi, A. Al-Sofyani & R. Nongonierma, 2003. Phospholipid fatty acids and sterols of two *Cinachyrella* sponges from the Saudi Arabian Red Sea: comparison with *Cinachyrella* species from other origins. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology* 135: 297-308.
- Barnathan, G., J. Miralles, J.-M. Njinkoue, A. Mangoni, E. Fattorusso, C. Debitus, N. Boury-Esnault & J.-M. Kornprobst, 1992. Sterol composition of three marine sponge species from the genus *Cinachyrella*. *Comparative Biochemistry and Physiology Part B: Comparative Biochemistry* 103: 1043-1047.
- Bastidas, C. & D. Bone, 1996. Competitive strategies between *Palythoa caribaeorum* and *Zoanthus sociatus* (Cnidaria: Anthozoa) at a reef flat environment in Venezuela. *Bulletin of marine science* 59: 543-555.
- Battershill, C. & P. Bergquist, 1990. The influence of storms on asexual reproduction, recruitment, and survivorship of sponges. Smithsonian Institution Press, Washington, DC.
- Becerro, M., M. Uriz & X. Turon, 1994. Trends in space occupation by the encrusting sponge *Crambe crambe*: variation in shape as a function of size and environment. *Marine biology* 121: 301-307.
- Bell, J. J., 2002. Regeneration rates of a sublittoral demosponge. *Journal of the Marine Biological Association of the UK* 82: 169-170.
- Bremner, J., S. Rogers & C. Frid, 2003. Assessing functional diversity in marine benthic ecosystems: a comparison of approaches. *Marine Ecology Progress Series* 254: 11-25.
- Bulow, F. J., 1970. RNA-DNA ratios as indicators of recent growth rates of a fish. *Journal of the Fisheries Board of Canada* 27: 2343-2349.
- Caldarone, E. M., C. Clemmesen, E. Berdalet, T. J. Miller, A. Folkvord, G. Holt, M. Olivar & I. M. Suthers, 2006. Intercalibration of four spectrofluorometric protocols for measuring RNA/DNA ratios in larval and juvenile fish. *Limnology and Oceanography: Methods* 4: 153-163.
- Cardone, F., E. Gaino & G. Corriero, 2010. The budding process in *Tethya citrina* Sarà & Melone (Porifera, Demospongiae) and the incidence of post-buds in sponge population maintenance. *Journal of Experimental Marine Biology and Ecology* 389: 93-100.

- Chadwick, N. E. & K. M. Morrow, 2011. Competition among sessile organisms on coral reefs. In Dubinsky, Z. & N. Stambler (eds), *Coral Reefs: An Ecosystem in Transition*. Springer, New York: 347-371.
- Chícharo, A., L. Chícharo, L. Valdés, E. Lopez-Jamar & P. Re, 1998. Estimation of starvation and diet variation of the RNA/DNA ratios in field-caught *Sardina pilchardus* larvae off the north of Spain. *Marine Ecology Progress Series* 164: 273-283.
- Dayton, P. K., G. A. Robilliard, R. T. Paine & L. B. Dayton, 1974. Biological accommodation in the benthic community at McMurdo Sound, Antarctica. *Ecological Monographs* 44: 105-128.
- De Caralt, S., M. J. Uriz & R. H. Wijffels, 2008. Grazing, differential size-class dynamics and survival of the Mediterranean sponge *Corticium candelabrum*. *Marine Ecology Progress Series* 360: 97-106.
- Di Bari, G., F. Cardone, E. Gaino, G. Liuzzi, C. N. Marzano, F. Scoccia & G. Corriero, 2014. Biological variations in a long-term ex situ cultivation: a Mediterranean demosponge as model system. *Mediterranean Marine Science* 16: 73-81.
- Di Camillo, C., M. Coppari, I. Bartolucci, M. Bo, F. Betti, M. Bertolino, B. Calcinai, C. Cerrano, G. De Grandis & G. Bavestrello, 2012. Temporal variations in growth and reproduction of *Tedania anhelans* and *Chondrosia reniformis* in the North Adriatic Sea. *Hydrobiologia* 687: 299-313.
- Duckworth, A. R., 2000. Aquaculture of sponges for production of bioactive metabolites. PhD thesis, University of Canterbury, Christchurch, New Zealand.
- Duckworth, A. R., 2003. Effect of wound size on the growth and regeneration of two temperate subtidal sponges. *Journal of Experimental Marine Biology and Ecology* 287: 139-153.
- Duckworth, A. R. & C. N. Battershill, 2001. Population dynamics and chemical ecology of New Zealand Demospongiae *Latrunculia* sp. nov. and *Polymastia croceus* (Poecilosclerida: Latrunculiidae: Polymastiidae). *New Zealand Journal of Marine and Freshwater Research* 35: 935-949.
- Duckworth, A. R., C. N. Battershill & D. R. Schiel, 2004. Effects of depth and water flow on growth, survival and bioactivity of two temperate sponges cultured in different seasons. *Aquaculture* 242: 237-250.
- Eerkes-Medrano, D., C. J. Feehan & S. P. Leys, 2014. Sponge cell aggregation: checkpoints in development indicate a high level of organismal complexity. *Invertebrate Biology*.
- Elvin, D. W., 1976. Seasonal growth and reproduction of an intertidal sponge, *Haliclona permollis* (Bowerbank). *Biological Bulletin* 151: 108-125.
- Engel, S. & J. R. Pawlik, 2000. Allelopathic activities of sponge extracts. *Marine Ecology Progress Series* 207: 273-281.
- Fleming, P. A., D. Muller & P. W. Bateman, 2007. Leave it all behind: a taxonomic perspective of autotomy in invertebrates. *Biological Reviews* 82: 481-510.

- Fonseca, V., C. Vinagre & H. Cabral, 2006. Growth variability of juvenile soles *Solea solea* and *Solea senegalensis*, and comparison with RNA: DNA ratios in the Tagus estuary, Portugal. *Journal of Fish Biology* 68: 1551-1562.
- Garrabou, J. & M. Zabala, 2001. Growth dynamics in four Mediterranean demosponges. *Estuarine, Coastal and Shelf Science* 52: 293-303.
- Hadas, E., M. Shpigel & M. Ilan, 2009. Particulate organic matter as a food source for a coral reef sponge. *Journal of Experimental Biology* 212: 3643-3650.
- Harper, M., T. Bugni, B. Copp, R. James, B. Lindsay, A. Richardson, P. Schnabel, D. Tasdemir, R. Vanwagoner & S. Verbitski, 2001. Introduction to the chemical ecology of marine natural products. In McClintock, J. & B. Baker (eds), *Marine Chemical Ecology, Marine Biology*. CRC Press, Boca Raton, Fla. USA.: 3-69.
- Henkel, T. & J. Pawlik, 2005. Habitat use by sponge-dwelling brittlestars. *Marine biology* 146: 301-313.
- Henry, L. A. & M. Hart, 2005. Regeneration from injury and resource allocation in sponges and corals—a review. *International Review of Hydrobiology* 90: 125-158.
- Hines, D. E. & J. R. Pawlik, 2012. Assessing the antipredatory defensive strategies of Caribbean non-scleractinian zoantharians (Cnidaria): is the sting the only thing? *Marine biology* 159: 389-398.
- Hooper, J. N. & R. W. Van Soest, 2002. *Systema Porifera. A guide to the classification of sponges*. Springer, US.
- Hoppe, W. F., 1988. Growth, regeneration and predation in three species of large coral reef sponges. *Marine Ecology Progress Series Oldendorf* 50: 117-125.
- Hultgren, K. M. & J. E. Duffy, 2010. Sponge host characteristics shape the community structure of their shrimp associates. *Marine Ecology Progress Series* 407: 1-12.
- Jackson, J., 1977. Competition on marine hard substrata: the adaptive significance of solitary and colonial strategies. *American Naturalist* 111: 743-767.
- Johnson, M. F., 1979. Recruitment, growth, mortality and seasonal variations in the calcareous sponges *Clathrina coriacea* (Montagu) and *C. blanca* (Miklucho-Maclay) from Santa Catalina Island, California. *Biologie des Spongiares* 291: 325-334.
- Karlson, R. H., 1980. Alternative competitive strategies in a periodically disturbed habitat. *Bulletin of marine science* 30: 894-900.
- Karlson, R. H., 1988. Size-dependent growth in two zoanthid species: a contrast in clonal strategies. *Ecology* 69: 1219-1232.
- Kay, A. M. & M. J. Keough, 1981. Occupation of patches in the epifaunal communities on pier pilings and the bivalve *Pinna bicolor* at Edithburgh, South Australia. *Oecologia* 48: 123-130.

- Koopmans, M. & R. H. Wijffels, 2008. Seasonal growth rate of the sponge *Haliclona oculata* (Demospongiae: Haplosclerida). *Marine Biotechnology* 10: 502-510.
- Lakshmi, V., R. Raghuram & P. Gupta, 2008. New ceramides from the sponge *Cinachyra cavernosa*. *Journal of Asian Natural Products Research* 10: 747-751.
- Leong, W. & J. R. Pawlik, 2010. Evidence of a resource trade-off between growth and chemical defenses among Caribbean coral reef sponges. *Marine Ecology Progress Series* 406: 71-78.
- Leys, S. P. & N. R. Lauzon, 1998. Hexactinellid sponge ecology: growth rates and seasonality in deep water sponges. *Journal of Experimental Marine Biology and Ecology* 230: 111-129.
- Li, L., Z. Deng, J. Li, H. Fu & W. Lin, 2004. [Chemical constituents from Chinese marine sponge *Cinachyrella australiensis*]. *Beijing da xue xue bao Yi xue ban= Journal of Peking University Health sciences* 36: 12-17.
- Maida, M., P. W. Sammarco & J. C. Coll, 1995. Preliminary evidence for directional allelopathic effects of the soft coral *Sinularia flexibilis* (Alcyonacea: Octocorallia) on scleractinian coral recruitment. *Bulletin of marine science* 56: 303-311.
- Maldonado, M., M. C. Carmona, Z. Velásquez, A. Puig, A. Cruzado, A. López & C. M. Young, 2005. Siliceous sponges as a silicon sink: an overlooked aspect of benthopelagic coupling in the marine silicon cycle. *Limnology and Oceanography* 50: 799-809.
- McMurray, S., J. Blum & J. Pawlik, 2008. Redwood of the reef: growth and age of the giant barrel sponge *Xestospongia muta* in the Florida Keys. *Marine biology* 155: 159-171.
- Moore, R. E. & P. J. Scheuer, 1971. Palytoxin: a new marine toxin from a coelenterate. *Science* 172: 495-498.
- Müller, W. E., 2006. The stem cell concept in sponges (Porifera): metazoan traits. *Seminars in cell & developmental biology* 17: 481-491.
- Osinga, R., J. Tramper & R. H. Wijffels, 1999. Cultivation of marine sponges. *Marine Biotechnology* 1: 509-532.
- Pansini, M. & R. Pronzato, 1990. Observations on the dynamics of a Mediterranean sponge community. In Rützler, K. (ed), *New perspectives in sponge biology*. Smithsonian Institution Press, Washington, DC: 404-415.
- Pawlik, J. R., 2011. The chemical ecology of sponges on Caribbean reefs: natural products shape natural systems. *Bioscience* 61: 888-898.
- Pawlik, J. R., T. P. Henkel, S. E. McMurray, S. López-Legentil, T.-L. Loh & S. Rohde, 2008. Patterns of sponge recruitment and growth on a shipwreck corroborate chemical defense resource trade-off. *Marine Ecology Progress Series* 368: 137-143.

- Porter, J. W. & N. M. Targett, 1988. Allelochemical interactions between sponges and corals. *The Biological Bulletin* 175: 230-239.
- Rabelo, E. F., M. d. O. Soares & H. Matthews-Cascon, 2013. Competitive interactions among zoanthids (Cnidaria: Zoanthidae) in an intertidal zone of Northeastern Brazil. *Brazilian Journal of Oceanography* 61: 35-42.
- Reiswig, H., 1973. Coral Reef Project—Papers in Memory of Dr. Thomas F. Goreau. 8. Population Dynamics of Three Jamaican Demospongiae. *Bulletin of marine science* 23: 191-226.
- Riisgård, H. U. & P. S. Larsen, 2010. Particle capture mechanisms in suspension-feeding invertebrates. *Marine Ecology Progress Series* 418: 255-293.
- Rinkevich, B., 1996. Do reproduction and regeneration in damaged corals compete for energy allocation? *Marine Ecology Progress Series Oldendorf* 143: 297-302.
- Robinson, S. & D. Ware, 1988. Ontogenetic Development of Growth Rates in Larval Pacific Herrings, *Clupea harengus pallasi*, Measured with RNA-DNA Ratios in the Strait of Georgia, British Columbia. *Canadian Journal of Fisheries and Aquatic Sciences* 45: 1422-1429.
- Russell, B., B. Degnan, M. Garson & G. Skilleter, 2003. Distribution of a nematocyst-bearing sponge in relation to potential coral donors. *Coral Reefs* 22: 11-16.
- Ryland, J. S. & J. E. Lancaster, 2003. Revision of methods for separating species of *Protopalythoa* (Hexacorallia: Zoanthidea) in the tropical West Pacific. *Invertebrate Systematics* 17: 407-428.
- Sammarco, P. W., J. C. Coll & S. La Barre, 1985. Competitive strategies of soft corals (Coelenterata: Octocorallia). II. Variable defensive responses and susceptibility to scleractinian corals. *Journal of Experimental Marine Biology and Ecology* 91: 199-215.
- Sheild, C. J. & J. D. Witman, 1993. The impact of *Henricia sanguinolenta* (OF Müller)(Echinodermata: Asteroidea) predation on the finger sponges, *Isodictya* spp. *Journal of Experimental Marine Biology and Ecology* 166: 107-133.
- Simpson, T. L., 1984. *The cell biology of sponges*. Springer-Verlag, New York.
- Singh, A. & N. L. Thakur, 2015. Field and laboratory investigations of budding in the tetillid sponge *Cinachyrella cavernosa*. *Invertebrate Biology* 134: 19-30.
- Sinniger, F., J.-I. Montoya-Burgos, P. Chevaldonne & J. Pawlowski, 2005. Phylogeny of the order Zoantharia (Anthozoa, Hexacorallia) based on the mitochondrial ribosomal genes. *Marine biology* 147: 1121-1128.
- Sipkema, D., M. C. Franssen, R. Osinga, J. Tramper & R. H. Wijffels, 2005. Marine sponges as pharmacy. *Marine Biotechnology* 7: 142-162.
- Sivaramakrishnan, V., 1951. Studies on early development and regeneration in some Indian marine sponges. *Proceedings of the Indian Academy of Sciences-Section B*. Springer, India. 34: 273-310

- Stone, A., 1970. Seasonal variation in the gross biochemical composition of *Hymeniacidon perleve*(Montagu). *Journal of Experimental Marine Biology and Ecology* 5: 265-271.
- Storr, J. F., 1964. Ecology of the Gulf of Mexico commercial sponges and its relation to the fishery, Special Scientific Report. US Fish and Wildlife Service Washington, DC 466: 1-73.
- Sullivan, B., D. J. Faulkner & L. Webb, 1983. Siphonodictidine, a metabolite of the burrowing sponge *Siphonodictyon* sp. that inhibits coral growth. *Science* 221: 1175-1176.
- Tanaka, K., 2002. Growth dynamics and mortality of the intertidal encrusting sponge *Halichondria okadae* (Demospongiae, Halichondrida). *Marine biology* 140: 383-389.
- Teixidó, N., M. Pineda & J. Garrabou, 2009. Decadal demographic trends of a long-lived temperate encrusting sponge. *Marine Ecology Progress Series* 375: 113-124.
- Thakur, N. L. & W. E. Müller, 2004. Biotechnological potential of marine sponges. *Current Science* 86: 1506-1512.
- Turon, X., I. Tarjuelo & M. Uriz, 1998. Growth dynamics and mortality of the encrusting sponge *Crambe crambe* (Poecilosclerida) in contrasting habitats: correlation with population structure and investment in defence. *Functional Ecology* 12: 631-639.
- Uchida, H. & I. Soyama, 2001. Sea anemones in Japanese waters (In Japanese). *Britannica*, Tokyo, Japan.
- Uriz, M. J., X. Turon, M. A. Becerro, J. Galera & J. Lozano, 1995. Patterns of resource allocation to somatic, defensive, and reproductive functions in the Mediterranean encrusting sponge *Crambe crambe*(Demospongiae, Poecilosclerida). *Marine Ecology Progress Series Oldendorf* 124: 159-170.
- Vinagre, C., V. Fonseca, A. Maia, R. Amara & H. Cabral, 2008. Habitat specific growth rates and condition indices for the sympatric soles *Solea solea* (Linnaeus, 1758) and *Solea senegalensis* Kaup 1858, in the Tagus estuary, Portugal, based on otolith daily increments and RNA-DNA ratio. *Journal of Applied Ichthyology* 24: 163-169.
- Wahidullah, S., B. Naik & A. A. Al-Fadhli, 2015. Chemotaxonomic study of the demosponge *Cinachyrella cavernosa* (Lamarck). *Biochemical Systematics and Ecology* 58: 91-96.
- Walker, S., B. Degnan, J. Hooper & G. Skilleter, 2008. Will increased storm disturbance affect the biodiversity of intertidal, nonscleractinian sessile fauna on coral reefs? *Global Change Biology* 14: 2755-2770.
- Walters, K. D. & J. R. Pawlik, 2005. Is there a trade-off between wound-healing and chemical defenses among Caribbean reef sponges? *Integrative and Comparative Biology* 45: 352-358.
- Wulff, J., 2006a. Resistance vs recovery: morphological strategies of coral reef sponges. *Functional Ecology* 20: 699-708.

Wulff, J. L., 2005. Trade-offs in resistance to competitors and predators, and their effects on the diversity of tropical marine sponges. *Journal of Animal Ecology* 74: 313-321.

Wulff, J. L., 2006b. Ecological interactions of marine sponges. *Canadian Journal of Zoology* 84: 146-166.

Xiao, D., X. Peng, S. Deng, W. Ma & H. Wu, 2005. Structure elucidation of (3E)-cholest-4-en-3, 6-dione-3-oxime in marine sponge *Cinachyrella australiensis* from the south china sea. *Chinese Journal of Organic Chemistry* 25: 1606-1609.

Zera, A. J. & L. G. Harshman, 2001. The physiology of life history trade-offs in animals. *Annual review of Ecology and Systematics* 32: 95-126.

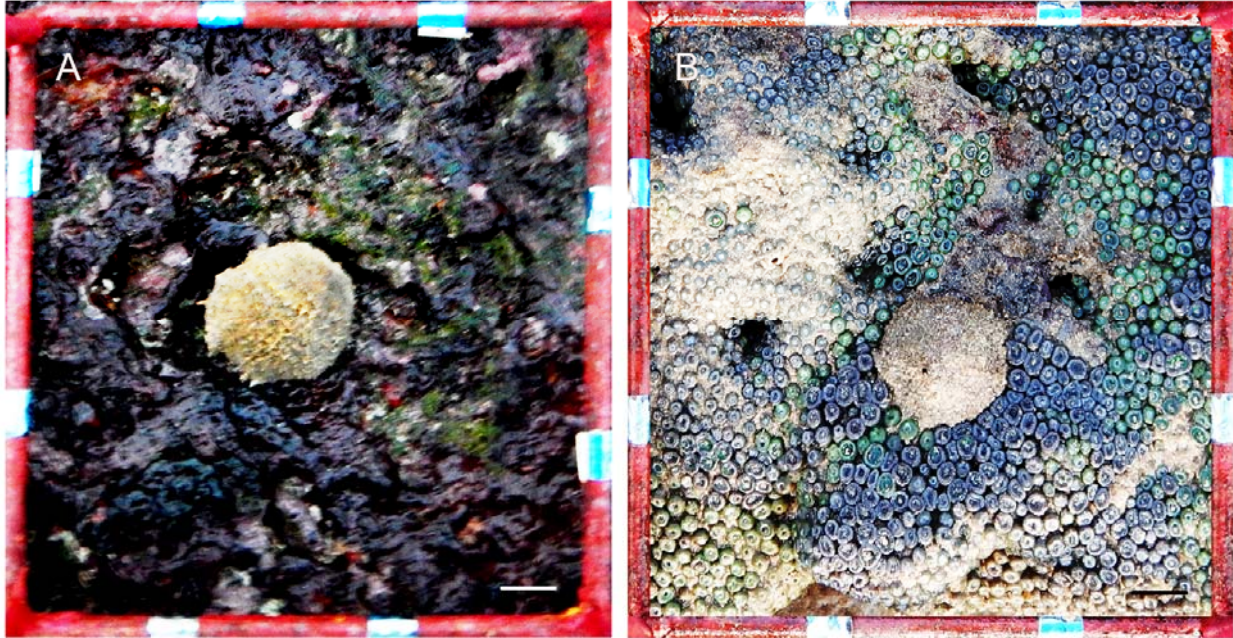


Fig.1 *C. cf. cavernosa* growing A) along with competitor *Z. sansibaricus* and B) without competitor (Scale 1: cm)

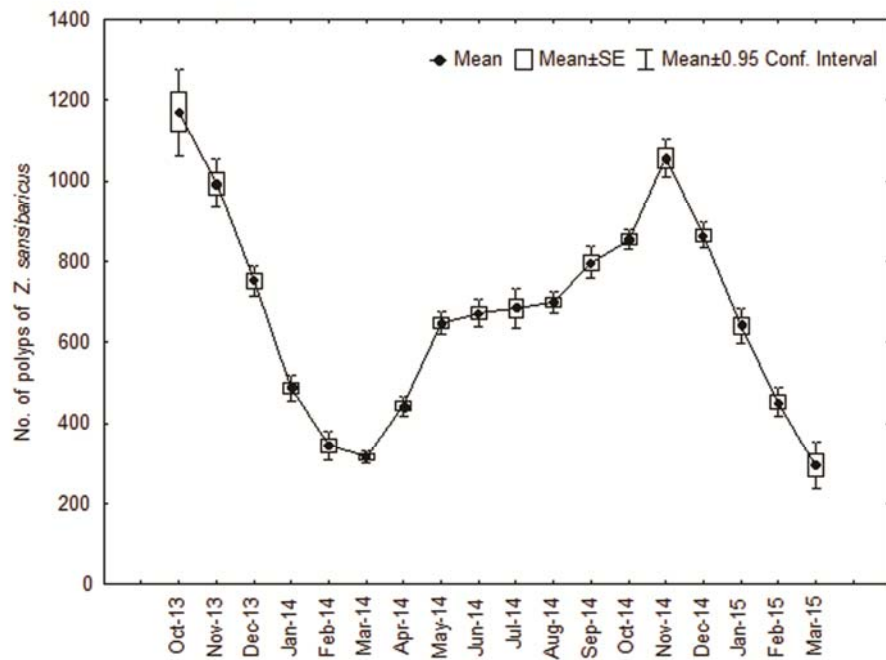


Fig. 2 Abundance of competitor *Z. sansibaricus* (represented as no. of polyps)

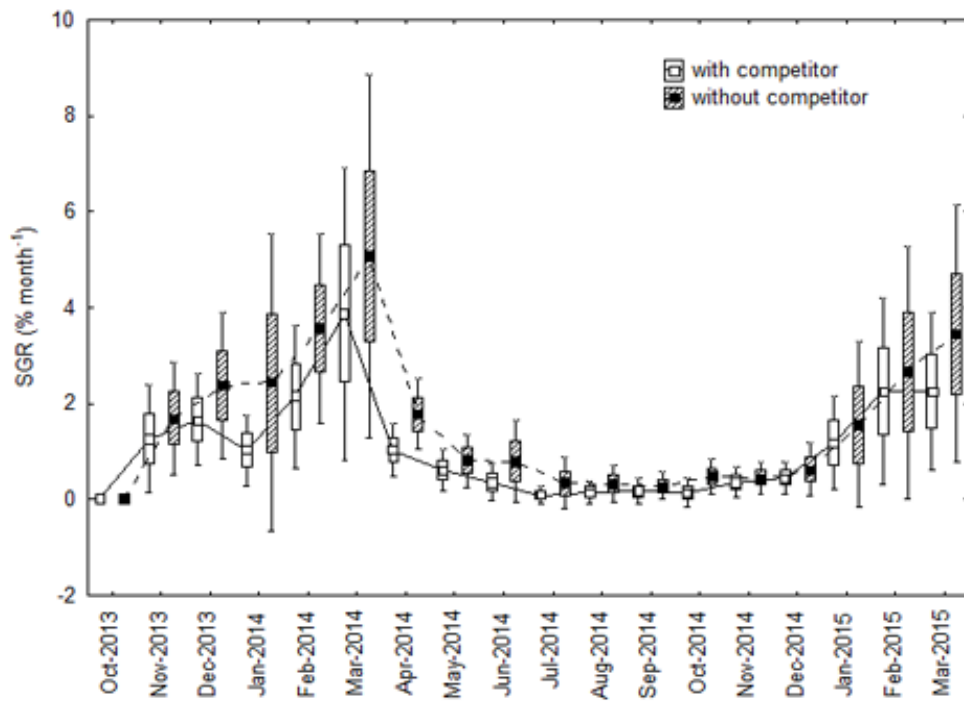


Fig. 3 Specific growth rate (SGR) of the sponges with and without the spatil competitor *Z. Sansibaricus*



Fig. 4 Phases of regeneration. Phase A: Closure of lesion by scar tissue; Phase B: Recovery of tissue to normal appearance and Phase C: Leveling of lesion depression (Scale: 1 cm)

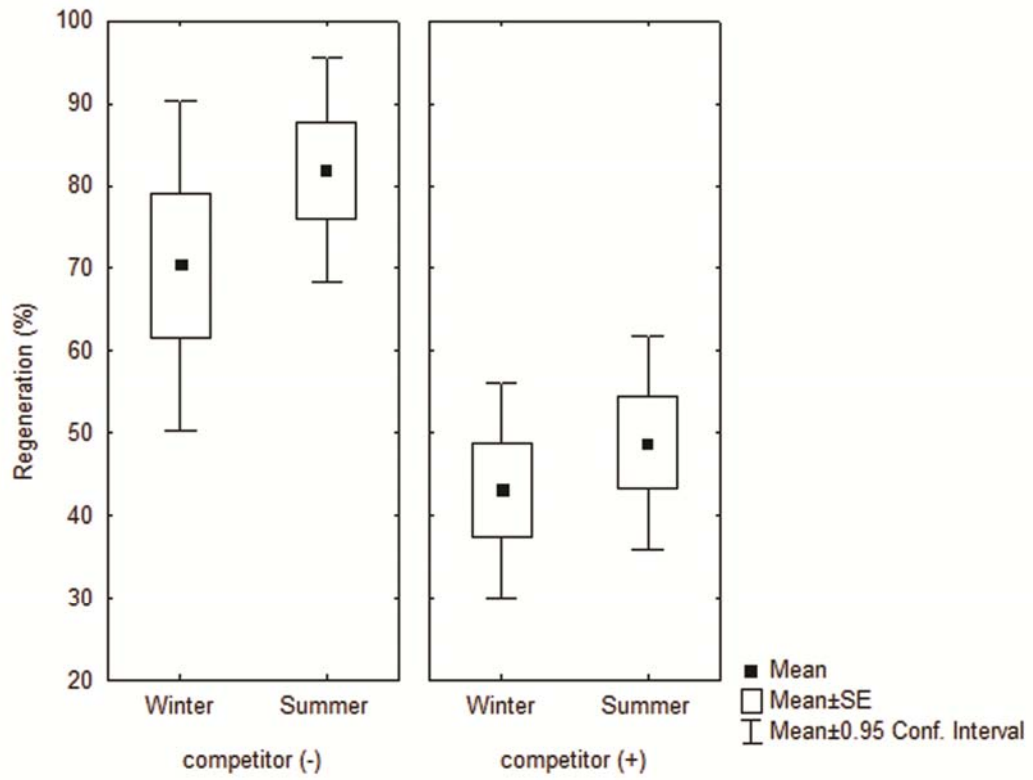


Fig.5 Variation in volume of newly regenerated area (in percentage). Symbol (+/-) represent the presence or absence of the spatital competitor *Z. sansibaricus*

Table 1 Estimated coefficients, standard errors, and p-values of multiple regression models of “Specific growth rate” as predicted by the covariate ‘size of the sponge’ and other environmental variables using a forward stepwise selection procedure (Model A, four explanatory variables, Multiple R= 0.74, R² = 0.53, adjusted R² = 0.52, p < 0.000001) and a backward stepwise elimination procedure (Model B, five explanatory variables, R = 0.84, R² = 0.68, Adjusted R² = 0.65, p < 0.0001).

	Estimate	Standard Error	p
Model I (Forward Selection)			
Intercept	-13.92	3.45	0.0000
Size	-1.13	0.12	0.0000
Temperature	0.65	0.11	0.0000
Competitor	-0.00	0.00	0.0010
Silicate	-0.07	0.03	0.0149
DO	0.22	0.12	0.0773
Model II (Backward Elimination)			
Intercept	-15.51	3.76	0.0000
Size	-1.13	0.12	0.0000
Temperature	0.68	0.12	0.0000
Salinity	0.16	0.08	0.0562
DO	0.41	0.17	0.0161
N/P	-0.07	0.04	0.0916
Silicate	-0.12	0.03	0.0007
Competitor	-0.00	0.00	0.0018

Table 2 Regression analysis showing the influence of competitor *Zoanthus sansibaricus* and body size of the sponge on its A) Regeneration, Multiple R= 0.75, R²=0.57 and Adjusted R²= 0.54; (p < 0.0001) and B) RNA: DNA ratio, R= 0.74, R²= 0.54 and Adjusted R² = 0.52; (p < 0.00001).

A)

	Estimate	Standard Error	p-level
Intercept	3595.87	622.37	0.0000
competitor	-31.68	5.63	0.0000
size	-9.23	2.46	0.0006

B)

	Estimate	Standard Error	p-level
Intercept	1.67	0.21	0.0000
competitor	-0.02	0.01	0.0144
size	-0.77	0.13	0.0000