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Growth and survival of marine sponges, *Stylissa massa* (Carter, 1887) and *Liosina paradoxa* (Thiele, 1899) in sea and land based culture systems

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ABSTRACT

Two species of marine sponges *Stylissa massa* and *Liosina paradoxa* were cultured in cages (*in situ)* and in land based aquaria *(ex situ*) over a period of 120 days. Growth and survival were evaluated using four substrates *viz.*, tile, block, coral rock and rope) under both *in situ* and *ex situ* conditions. *L. paradoxa* in cages recorded significant increase ($p<0.05$) on day 90 *i.e*., by 70% compared to initial volume whereas at 120 days there was a significant ($p<0.05$) decrease (54.22%). *S. massa* showed significant increase ($p<0.05$) in growth by 95.6% at 120 days compared to initial volume. Negative growth was recorded in *S. massa* under aquarium conditions while *L. paradoxa* recorded good growth as well as survival and performed exceptionally well in aquarium during the entire experimental period of 120 days. In cages, 90.62% survival was recorded for *S. massa* in 120 days. Among the different substrates used, coral rocks gave 100% survival whereas other substrates such as tiles, blocks and ropes showed 87.5% survival. Under aquarium conditions, *S. massa* showed overall survival of 81.25% and among the different substrates, 100% survival was obtained with coral rocks, 87.5% with blocks, 75% with ropes and the lowest survival of 62.5% was recorded with tiles.

Keywords: Aquarium, Cage, Growth, Mariculture, Metabolite, Sponges, Survival

Introduction

Sponges are known for presence of rich variety of bioactive metabolites (Sipkema *et al.,* 2005). Since 1950s, interest in sponges increased due to the discovery of bioactive secondary metabolites (Bergmann and Feeney, 1950). The importance of marine sponges and its application to mankind has been gaining importance globally. More than 5300 different products are recorded from sponges and their associated microorganisms and every year around 200 new metabolites are reported (Lipton and Shine, 2009). Owing to their high potential and demand, there are chances of overexploitation of the natural stocks. Since the discovery of pharmacologically interesting compounds in sponges many attempts have been made to culture sponges as renewable sources of these metabolites. In order to obtain sufficient amounts of bioactive compounds, a sustainable production technique is required. Sponge aquaculture is one possible method of supplying sufficient and sustainable quantities of sponge metabolites that have pharmaceutical potential (Osinga *et al.,* 1999). The ability of sponges to reproduce from small fragments/explants make them attractive for commercial farming (Colin and Arneson, 1995; Macmillan, 2002). The first scientific report on methods for cultivating sponges were published in the late 19th and early 20th centuries (Smith, 1897; Cotte, 1908). These early studies generally concentrated on *in situ* cultivation experiments with species of the family Spongiidae, which were and still are of commercial importance as bath sponges. Many species of sponges were found to be slow growing organisms (Dayton, 1979; Ayling, 1983; Garrabou and Zabala, 2001). In order to get requisite quantity of sponges without affecting the natural population, mariculture is the best available option which may be either sea based or land based.

Andaman Islands are bestowed with rich marine resources among which sponges are one of the most widely distributed resource. The present study evaluated the growth and survival of two sponge species *viz., Stylissa massa* (Carter, 1887) and *Liosina paradoxa* Thiele, 1899 in the sea in fabricated cages as well as in aquarium tanks, in the Andaman Islands. Eight alkaloids having anti-tumour potential were derived from *S. massa* (Tasdemir *et al.,* 2002*).* Several compounds like hymenialdisine, an alkaloid with potential against human neurodegenerative diseases were found from *S. massa*. *L. paradoxa* contains sterols and its main steroidal constituents were found to be Delta[5] sterols and

5α, 8α epidoxy sterols. (Aknin *et al.,* 2010). In the present study four different substrates *viz*., tiles, block, coral rock and ropes were evaluated for culture of *S. massa* and *L. paradoxa* in cages as well as in aquaria for a period of 120 days.

Materials and methods

The *in situ* culture experiments in cages were conducted in the Andaman Islands, near the Marine Hill shore, Port Blair, South Andaman (11°40'38.77''N; 92°44'20.11''E) at 3-5 m depth on a rocky bottom. *Ex situ* culture in aquaria was conducted at the marine research laboratory of ICAR-Central Island Agriculture Research Institute, Port Blair. Sponges were collected by SCUBA diving from North Bay and transported to the laboratory. As sponges require substrates for attachment, growth and survival, sponge explants were cut underwater using sterile knife and were pierced with nylon threads from side to side and tied on to the substrates *viz.,* tile, concrete blocks, coral rocks (natural substrate) and ropes. The cages (1x1x0.5 m) were fabricated with four compartments in order to accommodate the four substrates. The substrates were tied to the cages to avoid toppling or dislodgement. Cages were placed at depth of 3-5 m and tied on to the rocks to avoid disturbances due to waves. The cages were then overlaid with a polythene coated steel mesh of 1.5 cm mesh size, to prevent any possible predation. In total, 16 cages (8 cages per species) were used for the experiment (Fig. 1a). For *ex situ* cultivation experiment, 16 aquarium tanks (8 tanks per species) filled with unfiltered seawater without any additional nutrients were used. Sponge explants prepared were tied to the four different substrates (tile, concrete blocks, coral rocks and ropes) and placed in the tanks (Fig. 1b-c).

Growth was measured once in 15 days, as increase in the surface volume estimated from length, breadth and height of explants and expressed in cm^3 following Schifenhovel and Kunzmann (2012). Initiation of attachment and survival of both the species were noted during the first fortnight. When mortality was observed, dead explants were removed at first notice to avoid damage to other sponge explants. Survival rate of sponges were calculated using the formula (Schifenhovel and Kunzmann, 2012) as:

$$\text{Survival, S \%} = N_T/N_0 \times 100$$

where, N_0 - number of sponges at start of the experiment and N_T - number of sponges on the day of sampling

Data analysis was carried out using one way ANOVA with SPSS version16.0.

Results and discussion

In situ cultivation in sea cages

Water quality parameters recorded in the cages were: pH - 7.9 to 8.2, temperature - 26 to 28°C, salinity - 33.5 to 35‰ and dissolved oxygen -5 to 5.3 mg l^{-1}. Growth of *L. paradoxa* explants varied significantly ($p<0.05$) between the sampling days. On an average, *L. paradoxa* explants grew from initial size of 6.27cm^3 to 9.67 cm^3 on termination of the experiment. Positive growth rate was recorded during the first 90 days ($p<0.05$) of the culture period followed by a decrease in volume. Explant volume increased by 72% on 90th day (10.67±0.31 cm^3) while significant growth reduction ($p<0.05$) was recorded on 105th and 120th day of sampling (Fig 2a). Among the four substrates used for cultivation of sponges in cages, coral rock gave significantly ($p<0.01$) better growth compared to other substrates and there was no significant difference between other three substrates. Growth of explants was lowest in the block substrate (Fig 2b). In case of *L. paradoxa* culture in cages, out of 32 explants 27 explants survived with overall survival rate of 84.37%.

S. massa in cages showed increasing growth trend throughout the experimental period, with highest growth recorded on 120 days of rearing (14.30±2.04 cm^3). However, on day 105 of rearing a reduction in growth was observed (13.34±1.83 cm^3) (Fig. 2c). Performance of *S. massa* on all the substrates was good but coral rock substrate (12.05±0.18 cm^3) showed the highest ($p<0.05$) followed by concrete block (11.84±0.19 cm^3), tile (10.85 ± 0.19 cm^3) and rope (10.95 ±0.19 cm^3) (Fig. 2d).

(a) (b) (c)

Fig. 1. a. Sponge explants in cages; b - c: Sponge explants in aquaria

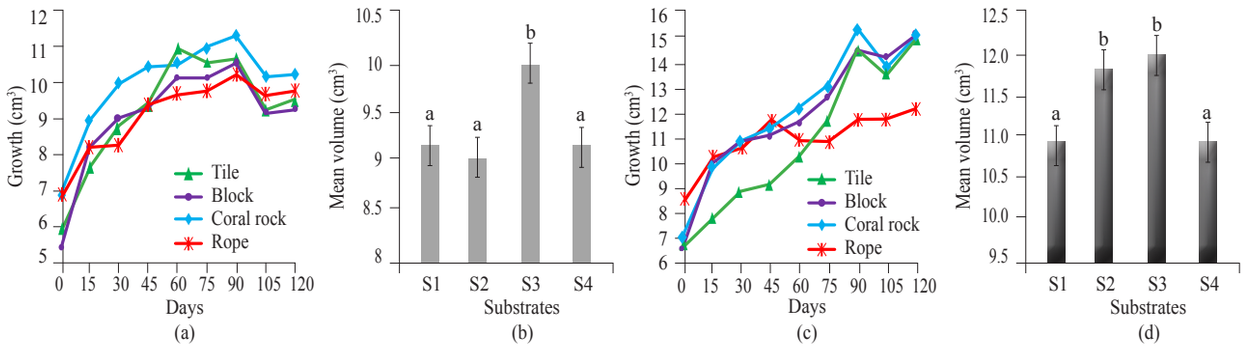

Fig. 2. Growth of *Liosina paradoxa* (a-b) and *Stylissa massa* (c-d) on different substrates in cages

Out of the 32 explants of *S. massa,* 29 explants survived at 120 days showing 90.62% survival. Among the different substrates, coral rocks showed 100% survival rate whereas other substrates (tile, block and ropes) showed only 87.5% survival.

For sponge culture to be commercially viable explants should attain at least double the size each year (Crawshay, 1939) and should have survival greater than 90% (Verdanel and Vacelet, 1990). In cages, *S. massa* with mean initial volume of 7.31 cm^3 attained 14.30 cm^3 on termination of the experiment. *L. paradoxa* in cages recorded maximum increase in volume at day 90 by 70% compared to initial volume whereas at 120 days it decreased to 54.22%. *S. massa* showed 95.6% increase in volume at 120 days compared to initial volume with no shrinkage in growth. Culture environment could be a major discriminating factor for growth and survival in sponges. Our cage site was prone to high water flow and sediments, where in many a time sponges were covered by silt and deposits which were cleaned prior to measurements. If sediments affect the inflow of water in sponges it might probably lead to occasional shrinkage since they are filter feeders. Marine sponge *Callyspongia subargimera* grew in open sea conditions at 99.94 mg day^{-1} in Kerala, India (Lipton and Shine, 2009). Our study had limitation with reference to growth measurement as we measured increase in volume using length, breadth and height. Most advanced methodologies like 3D-photogrammatry techniques are not much used in Indian context.

Survival rate of sponges is an important aspect which indicates the potential of sponges for mariculture. Survival rate mainly depends on environmental conditions, initial damage during explant preparation and type as well as availability of substrates. Survival of *S. massa* with coral rock substrates was 100%. In cages, mean volume of *S. massa* and *L. paradoxa* were higher in coral rock substrates compared to the other substrates. Observations made by Decaralt *et al.* (2010) when studying *in situ* culture of *Dysidea avara* in North-west Mediterranean comparing growth in cage, glue and rope methods, indicated that cage method was best for survival, glue method being best for growth and rope method for bioactivity. From Indonesia (close to Andamans in geographical location), high survival rates of 80% for the sponge *Amphimedon paravirdis*, and 92% for *Ircinia ramose* were reported (Devoogd, 2005). In our study, mortality occurred during first 15 days and hardly any mortality was noticed further as mostly sponges were acclimatised. Similar observations showed that morality mostly occurs in the initial days of transplantation (Pronzato *et al.,* 1999; van Treeck, 2003) and hence survival rates will give good impression on the sensitivity of the species (Devoogd, 2005).

Sediment load in cages is also one of the important issue which might have influenced the survival and growth of sponges. Bakus (1967) in experiments conducted at Fanning Island, Central Pacific indicated that many sponges and ascidians are adversely affected by sediment deposition by burial and clogging of canals and chambers. During the present study, sponges were mostly covered with sediments which were cleaned every time prior to morphometric measurements. Cages were cleaned regularly to remove possible sediment accumulation at the site. Though survival was not high, *i.e.*, above 90% as described by Verdenal and Vacelet (1990), survival might improve if initial damage is minimal. Site selection of cages is also important factor for growth and survival. Our site was chosen in close proximity for easy monitoring and maintenance and it's not a protected bay. UV radiation in the sunlight is also believed to limit the growth of sponges (Duckworth *et al.,* 1997). As the cages were placed at shallow waters in depth <5 m, sunlight also could have influenced the growth of sponges.

Ex situ cultivation

Under aquarium culture, negative growth was recorded in *S. massa* after 15 days of culture (Fig. 3a).

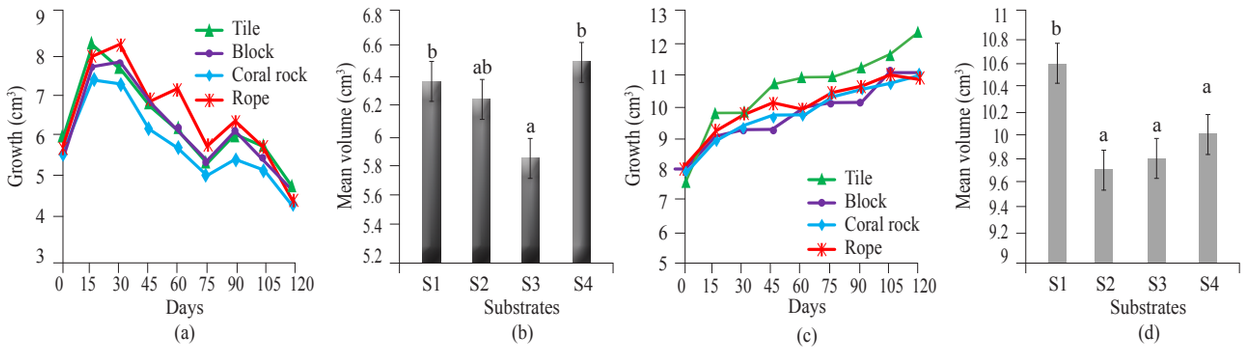

Fig. 3. Growth of *Stylissa massa* (a-b) and *Liosina paradoxa* (c-d) in different substrates in aquarium

Mean volume of *S. massa* in aquarium on day 15 (7.76±1.10 cm^3) and day 30 (7.70±1.24 cm^3) was at par and significantly higher than that of other days. After 30th day, reduced growth was observed till the end of the culture period. Lowest volume was observed on day 120 (4.61±0.76 cm^3). The volume decreased significantly (4.61±0.76 cm^3) by 25% compared to the initial volume of 5.77±0.56 cm^3. Significantly higher growth (6.497±130 cm^3) was recorded with rope and tile substrates as compared to that of coral rock (Fig. 3b). Out of the 32 explants of *S. massa* used at the start of the experiment, only 26 explants survived at the end of the experiment with an overall survival of 81.25%. Survival however varied among the substrates with 100% survival for coral rock. Coral rock, a naturally available substrate, showed good survival and attachment. Survival was also dependent on attachment. Sponge explants did not attach well on tiles and therefore, lowest survival (62%) was observed with tiles. In case of *Liosina paradoxa* highest mean growth of 11.33±0.21 cm^3 (44% increase in volume), was recorded during 120 days of culture. The trend of growth in four substrates is shown in Fig 3c. Among the four substrates (tile, block, coral rock and rope), explants cultured on tiles showed significantly higher growth (10.58±0.15 cm^3) than that of the other three substrates (Fig. 3c). There was no significant difference among other three substrates during 120 days of culture of *L. paradoxa* (Fig. 3d). Out of the 32 explants of *L. paradoxa,* 30 explants survived at the end of the experiment showing highest survival of 93.75% with block and coral rock showing 100% survival. Sponges in tile and rope substrates showed only 87.5% survival as the explants failed to attach on the substrates initially leading to mortality.

In India reports on land based culture of sponges are reported by Lipton and Shine (2009) for culturing *Echinodictyum gorgonoides* in aquarium tanks using recirculated seawater. There is no previous report on *ex situ* cultivation of sponges in India. In another study, three specimens of the marine sponge *Spirastrella inconstans* cultured in captivity in 2 t FRP tanks showed height increments of 26,12 and 11 mm in one month (Vinod *et al.,* 2009). Studies on growth of *L. paradoxa* has not been reported elsewhere whereas Schifenhovel and Kunzmann (2012) have reported on culture of *S. massa* in Indonesia. Interestingly *L. paradoxa* performed well in aquarium compared to *S. massa* which exhibited a negative growth. The growth patterns were likely to be the result of several factors like food limitation and behavioural responses in explants (Duckworth, 2009). Water exchange was done every day or on alternate days in order to maintain aquarium conditions suitable for the optimum growth and survival of sponges. Aquarium cultivation using running, unfiltered seawater seemed to be the most successful system with respect to long term *in vitro* cultivation of marine sponges (Osinga *et al.,* 1999). It was assumed that the natural seawater would be good enough to provide nutrients required for the growth of sponges and no supplementation was done during the present study. The dead sponges were immediately removed from the aquaria as they could easily pollute the water which might influence the growth and survival of other sponges. Survival rates were critical as explained by Verdenal and Vacelet (1990), as a minimum survival of 90% was essential for mariculture. High survival of 93.75% was recorded for *L. paradoxa* in aquarium, while for *S. massa,* 81% survival was recorded in aquarium. In India, Lipton and Shine (2009) reported that *E. gorgonoides* survived for 80 days in aquarium culture of sponges.

Ex situ approaches of marine sponge cultivation have shown encouraging growth rates (Osinga *et al.,* 1999; 2001; 2003). Several attempts have been made to culture sponges on land. Several works have been conducted for studying growth of sponges like *Cliona celata* using unfiltered seawater (Waburton, 1958), *Ephydatia fluviatilis* using *E. coli* (Porrier *et al.,* 1981), *Halichondria panacea* using unfiltered seawater (Barthel and Theede, 1986), *Microciona prolifera* fed on *Dunaliella euchlora* (Simpson, 1968) and *Ophlitaspongia seriata* fed with *Isochrysis galbana* (Fry, 1971) as reviewed by Osinga *et al.* (1999). Studies using alternate feedings with unfiltered

seawater could give more idea on the comparative growth and influence of feed on growth and survival of sponges. Despite the ability of sponges to adjust to changes in the environment, only a low number of successful *ex situ* systems were developed (Koopmans, 2009).

Substrates and attachment are also important factors used to evaluate the growth of sponges. In case of *Crambe crambe* cultured in closed systems, it was found that the highest growth rate for all explants was observed in first 10 days of culture just after the cuttings were planted, as stress might have triggered regeneration process and biomass production (Belarbi *et al.,* 2003). In case of *Chondrosia reniformis* also higher cellular proliferation was reported only in first 10 days compared to the latter period (Nickel and Brummer, 2003). In our study also *S. massa* exhibited good growth until first 15 days with a decreasing trend subsequently.

In our study, there were limitations in measurement of growth of sponges as methods for determination of growth of sponges like 3D-photogrammatry and underwater weighing were not used. Simple measurement was used for calculation of growth. Nutrient requirement of sponges is another interesting area of work which could be specifically taken up for sponges which might augment growth and survival. The growth and survival studies of *S. massa* and *L. paradoxa* showed that these species can be taken up for mariculture to harness their bioactive potential. In India, sponge farming is most primitive and relatively unexplored compared to other marine fauna like finfishes and shellfishes. Though sponge cultivation is one of the most challenging task when compared to other marine fauna owing to their irregular growth pattern, feeding and slow growth, they could be one of the most potential organisms for mankind since these organisms possess enormous potential for drug development.

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