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An assessment of sponge mariculture potentials in the Spermonde Archipelago, Indonesia

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In the present study done in the Spermonde Archipelago, South Sulawesi, Indonesia an assessment is presented of the farming potential of six sponge species (out of nine candidates considered) possessing pharmacologically promising compounds, i.e. *Aaptos suberitoides*, *Amphimedon paraviridis*, *Callyspongia* (*Euplacella*) *biru*, *Hyrtios reticulatus*, *Ircinia ramosa* and *Pseudoceratina* cf. *verrucosa*. A total of 350 cuttings were threaded on a polyethylene rope and attached to horizontal mooring structures at three experimental sites. Growth and survival was monitored for a period of six months and for some explants for an additional period of ten months. All cuttings were photographed at regular time intervals and growth was measured as increase in length and dry weight. With the exception of *P.* cf. *verrucosa*, survival rates were high (80–96%) during the mariculture period. Size increases were too little to be measured in *A. suberitoides* and *P.* cf. *verrucosa* cuttings. Significant growth was only observed for *C. biru*. The high growth potential and high survival rate of this sponge species suggest that it is a promising candidate for further mariculture development.

INTRODUCTION

In recent years, there has been a dramatic increase in the interest of marine organisms as sources of compounds with pharmacological and other bioactive properties (Munro et al., 1999). Although a large amount of pharmaca is derived from organisms in the terrestrial environment, oceans are also being explored with an increasing rate. Since 1950, 14,000 novel compounds have been described mainly from marine invertebrates (Blunt & Munro, 2003). However, since the discovery of Caribbean sponge-derived antiviral and anticancer drugs, not many compounds have actually made it to the pharmacist (Dumdei et al., 1998; Munro et al., 1999; Proksch et al., 2003). The problem here rests not only with the lengthy road from discovery of a novel secondary metabolite to preclinical and clinical trials, but also with the supply side of the compound. Of all marine invertebrates, sponges, are by far the most promising producers of potential pharmaceuticals (e.g. Munro et al., 1999). A number of promising bioactive compounds have been selected for further preclinical evaluation, such as the anti-tumor compound halichondrin B isolated from *Lissodendoryx* sp., and anti-inflammatory compounds isolated from *Petrosia hoeksemai* (initially identified as *Petrosia contignata*, see de Voogd & van Soest, 2002; Newman & Cragg, 2004).

The supply-problem has been extensively discussed by Faulkner (2002) and Proksch et al. (2003), who concluded that incredibly large amounts of target organisms are needed in order to supply sufficient quantities of bioactive compounds even to pass only the preclinical trials. Although the total synthesis of a pharmaceutically active compound in some cases is economically feasible, it is unlikely to be mass-produced before a compound passes all preclinical trials, even though tons of the target sponges are needed to pass these stages (Munro et al., 1999). Moreover, the molecular structure of many compounds is so complicated, that synthesis is not even possible (Munro et al., 1999). Other supply methods such as tissue culture (Pomponi & Willoughby, 1994; Nickel et al., 2001), genome transfer (Osinga et al., 1999b; Pomponi, 1999), *in vitro* culture (Osinga et al., 1999a; Duckworth et al., 2003; Richelle-Maurer et al., 2003) and aquaculture (Müller et al., 1999; Duckworth & Battershill, 2003a, 2003b; van Treeck et al., 2003; Page et al., 2005) are currently being investigated. So far, mariculture remains the most reliable and cost-effective method to provide the large quantities of sponge biomass needed for further drug development until more advanced techniques such as genome transfer and tissue culture have been realized and optimized (van Treeck et al., 2003; Sipkema et al., 2005).

The exploitation of sponges as a bathing tool has a long history and tradition in the Mediterranean and the Caribbean and ample studies have assessed the farming of bath sponges worldwide (Cotte, 1908; Crawshay, 1939; Storr, 1964; Corriero et al., 2004). Although these were constrained by the size and shape of commercial bath sponges, all belonging to the family Spongiidae, the culture of sponges for the production of bioactive chemicals has met with a novel challenge. In recent years, substantial work focused on the farming success of sponge species in different geographical regions with respect to explant size (Duckworth et al., 1997) farming method (Duckworth et al., 1997; Belarbi et al., 2003; Duckworth & Battershill, 2003b; Van Treeck et al., 2003; Corriero et al., 2004), depth and water flow (Duckworth & Battershill, 2003b; Duckworth et

al., 2004), and compound concentration (Mendola, 2003; Page et al., 2005). Most of these studies were in temperate or subtropical regions, where sponges often grow seasonally; which implies dying off during cold seasons and rapid growth in warm seasons (Duckworth et al., 1997).

Although bath sponges have been cultured in tropical waters (MacMillan, 1996), few studies have focused on the farming of tropical sponges for their chemicals (Mendola, 2003). A strong option for countries such as Indonesia, with a wealth of bioactive sponges, is a sustainable culture of bioactive sponges under field conditions with a minimum of investment and little environmental stress. It is expected that sponge diversity is highest in the Indo-Pacific, but 'only' about 60 different papers have discussed secondary metabolites isolated from Indonesian sponges by various research groups worldwide (Supriyono et al., 1995; Roy et al., 2002; Salmoun et al., 2002; Yousaf et al., 2004). Although the chemical defence and the biomedical potential of marine sponges have been investigated for certain geographic regions such as China (Zhang et al., 2003), the Caribbean (Waddell & Pawlik, 2000; Burns et al., 2003) Brazil (Berlinck et al., 2004), the Mediterranean (Müller et al., 1999; Beccero et al., 2003; van Treeck et al., 2003), and Guam (Beccero et al., 2003), the mariculture potential of marine sponges has not yet been discussed for any geographic region.

For the present study, several abundant sponge species were selected for mariculture trials in the Spermonde Archipelago, south-western Sulawesi. This barrier reef area is well known for its high marine biodiversity (Cleary et al., 2005) and its sponge fauna is well documented (de Weerdt & van Soest, 2001; de Voogd & van Soest, 2002; Alvarez et al., 2003; de Voogd et al., 2006). Although only one of the proposed sponge species is known to have biomedical potential, they all produce compounds, which are strongly bioactive or are known to be in active in specific bioassays (de Voogd & Cleary, 2007). Since the demand for species will probably be variable, in order to make a future sponge culture successful, it is important to know how different species will react and grow using different farming methods.

In this paper I highlight to what extent and how many sponge species possibly may be cultured in the Spermonde Archipelago based on the monitoring of growth and survival of some species over a period of several months. My goal was to select one or two species with high survival and growth rates for further optimization of the methods for mariculture.

MATERIALS AND METHODS

Study site

The fieldwork was carried out in the Spermonde Archipelago, Indonesia from June 2002 to September 2003 (see de Voogd, 2007). The Spermonde is a well-documented carbonate shelf just off the coast of southwest Sulawesi. The rivers Maros and Tallo in the north and Jenebarang in the south deposit nutrients and silt predominantly during the monsoon period, so that the coastal inshore reefs are more affected than the offshore reefs bordering the clear waters of the Makassar Strait. The reef sites near Samalona island and the shoal Bone Lola were selected because of their high sponge densities and their good accessibility from Makassar (de Voogd et al., 2006). Not all species were farmed at all locations due to time limitation. At Samalona island explants were farmed at the exposed northwestern side and the sheltered southeastern side. This is important, since water flow differs substantially between these sites (Cleary et al., 2005) and sponge growth generally increases with increasing water flow with only a few exceptions (Duckworth & Battershill, 2003b).

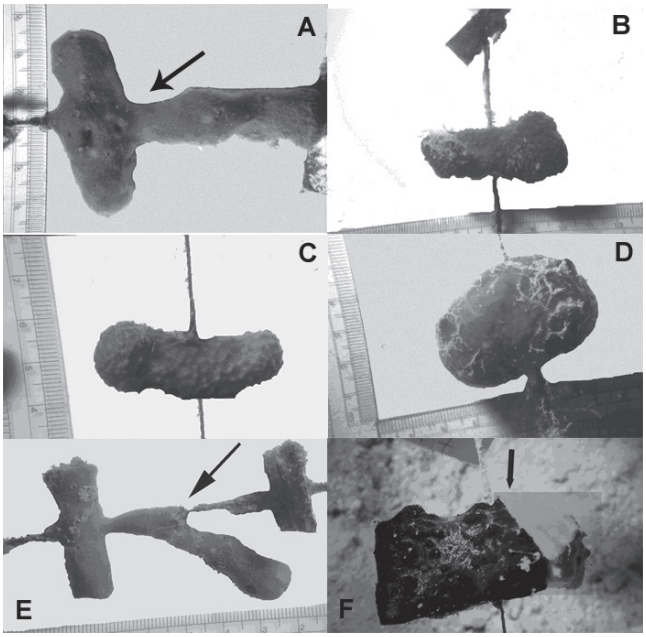

Figure 1. Explants with time intervals between brackets (A) *Amphimedon paraviridis* after 138 days, arrow indicating growth across rope at arrow; (B) *Hyrtios reticulatus* after 154 days, no growth; (C) *Ircinia ramosa* after 126 days, thickening at the both ends; (D) *Aaptos suberitoides* after 168 days, explant has developed two new oscules; (E) *Callyspongia biru* after 106 days showing new branch from crust grown across rope; (F) *Pseudoceratina* cf. *verrucosa*, after 4 days, inset shows healthy tissue, while the rest is blackened.

Culture methods

Sponges were collected by SCUBA diving at a depth of 10–14 m, and carefully cut *in situ* with a razor blade into standard sized explants of 30 and 40 mm in length, depending on the sponge morphology, with at least one side provided with an exopinacoderm (after Pronzato et al., 1999).

Initially a number of trials were performed with a variety of mariculture methods. These included growing sponges on a rope attached to horizontal mooring system, a net attached to a horizontal mooring, and a vertical rope in the water column. Only the first method proved feasible for the use of all species in the present study. The third method which was performed close to the reef area, failed because of the prevalent high water turbulence, causing most of the sponge cuttings to drop from the rope. The freak stranding of a cargo ship destroyed a second attempt.

The herein used mariculture method was executed in the following way: a polyethylene fishing rope (diameter=1 mm) was inserted in a large needle and carefully pushed through the sponge tissue. A knot and plastic label was placed after each explant. Each rope carrying approximately nine

Table 1. *Experimental design. Number of explants per site.*

Species	SE	SW	BL
Aaptos suberitoides		25	25
Amphimedon paraviridis	25	50*	25
Callyspongia biru	25	50*	50*
Hyrtios reticulatus			25
Ircinia ramosa			25
Pseudoceratina cf. *verrucosa*			25

*S*E, Samalona sheltered; SW,Samalona exposed; BL, Bone Lola.
*, 25 explants each of 30 and 40-mm.

explants was attached to a 70×100 cm^2 PVC frame (see de Voogd, 2007). These frames were then placed horizontally approximately 20 cm above the reef bottom at a depth of 12–15 m and secured with iron pegs. The frames were placed on the reef at the exposed (05°07'19.6"S 119°20'24.6"E) and the sheltered (05°07'35.8"S 119°20'41.1"E) side of Samalona island and the exposed side of the submerged Bone Lola reef (05°03'08.7"S 119°21'12.4"E).

Sponge species selected

The target species used in the present study were selected based on surveys in 1997 and 2000 (de Voogd et al., 2006, 2007). During these surveys, 151 larger reef sponge species were recorded from 34 sites. For the present study we only selected species that were abundant (N>100 observed), wide-spread, and are known to metabolize bioactive or pharmaceutically interesting compounds (de Voogd, 2007). Only a few species fulfilled all of these criteria and a short description of each is given below; *Aaptos suberitoides* Brøndsted, 1934 (Hadromerida: Suberitidae); *Acanthostrongylophora ingens* Thiele, 1899 (Haplosclerida: Petrosiidae); *Amphimedon paraviridis* Fromont, 1993 (Haplosclerida: Niphatidae); *Callyspongia* (*Euplacella*) *biru* de Voogd, 2004 (Haplosclerida: Callyspongiidae); *Hyrtios reticulatus* Thiele 1899 (Dictyoceratida: Thorectidae); *Ircinia ramosa* Keller, 1889 (Dictyoceratida: Irciniidae); *Petrosia* (*Petrosia*) *hoeksemai* de Voogd and van Soest, 2002 (Haplosclerida: Petrosiidae); *Petrosia* (*Petrosia*) *nigricans* Lindgren, 1867 and *Pseudoceratina* cf. *verrucosa* Bergquist, 1995 (Verongida: Pseudoceratidae).

1. *A. suberitoides* (Figure 1D, Table 1) forms masses of globular osculiferous lobes. The ectosome is rubbery and dark colored, while the interior is canary yellow, but turns dark brown when exposed to air. The consistency is fleshy, tough, but compressible. The skeleton is radiate with tracts and single spicules issuing from the center of the lobe. Sponges of the genus *Aaptos* and *Suberites* are known to produce aaptamine-type alkaloids (van Soest & Braekman, 1999), and these compounds were isolated from *Aaptos suberitoides* collected in the Spermonde Archipelago. The species is occasionally consumed and rarely overgrown by other organisms (personal observation). It is widespread, and has been reported from the Red Sea to Australia. The sponges were cut into explants of approximately 30×30×30 mm^3 with at least one side of the pinacoderm; 25 sponge cuttings each were placed at the exposed side of Samalona (10 June–21 September 2003) and Bone Lola (12 June–November, 2002).

2. *A. paraviridis* (Figure 1A) is an olive green thickly encrusting to ramose sponge. The texture varies from elastic to firm crumbly. 3-Alkylpiperidine alkaloids have been recorded in all five families of the Haplosclerida, including Niphatidae, and are considered as a chemical marker for the order (Andersen et al., 1996; van Soest & Braekman, 1999). Pharmaceutical properties of these compounds include antifungal (Albrizio et al., 1995; Nicholas & Molinksi, 2000), antimicrobial (Kelman et al., 2001) and anticancer (Pettit et al., 1992). Recently, amphitoxin was isolated from this species and several *Callyspongia* spp., including *C. biru* de (de Voogd et al., 2005). Symbiotic barnacles and bivalves occasionally inhabit this sponge. It exudes copious mucus when cut. Branches were cut in fragments of 30 and 40-mm length and farmed at the sheltered and exposed sides of Samalona and Bone Lola. A total of 100 explants were farmed; (25 explants each of 40-mm at the sheltered and exposed sides of Samalona, and Bone Lola and 25 explants of 30-mm at the exposed side of Samalona) between 12 June–25 November 2002).

3. *C. biru* (Figure 1E) is a bright blue sponge, bassally encrusting, but forming single erect branches or masses of rounded, occasionally bifurcating branches. It has numerous slightly elevated small oscules. Symbiotic barnacles and bivalves often inhabit this species. The species, furthermore, exudes copious slime when handled. Branches were cut in explants of 30 and 40-mm length and set out at the exposed and sheltered sides of Samalona and Bone Lola. A total of 125 explants were farmed (25 explants of 40-mm at the exposed and sheltered side of Samalona and Bone Lola, 25 explants of 30-mm at the exposed side of Samalona and Bone Lola) between 10 June 2002 and 21 September 2003).

4. *H. reticulatus* (Figure 1B) forms erect dark brown firm branches and is very similar to *H. erectus*. Bioactive 5 hydroxytryptamine-derived alkaloids have been isolated from the species *H. erectus* and *H. reticulatus* from the Spermonde Achipelago (Salmoun et al., 2002). Although *H. erectus* is more abundant than *H. reticulatus* in the Spermonde Archipelago, the specimens are in general much smaller or too small to be useful as a 'source' population for the present study. A total of 25 explants of approximately 40-mm in length were farmed at the reef of Bone Lola between 17 June–18 November 2002.

5. *I. ramosa* (Figure 1C) forms yellowish rubbery-tough anastoming branches. The anticancer compounds irciniastatins has been isolated from the Indo-Pacific I. ramosa (Pettit et al., 2004). It is a widespread species in the Indo-Pacific region. A total of 25 explants of approximately 30-mm in length were farmed at Bone Lola reef between 15 July and 19 November 2002.

6. *P.* cf. *verrucosa* (Figure 1F) forms rubbery yellow-brown irregular branches or masses. It turns black when handled. Sponges belonging to the order Verongida are known to synthesize bromotyrosine derivatives which show a variety of biological activities, including antifouling, antibacterial and cytotoxicity (Fusetani et al., 2001; van Soest & Braekman, 1999). A total of 25 explants of approximately 30×30×30 mm^3 were farmed at the exposed side of Samalona reef.

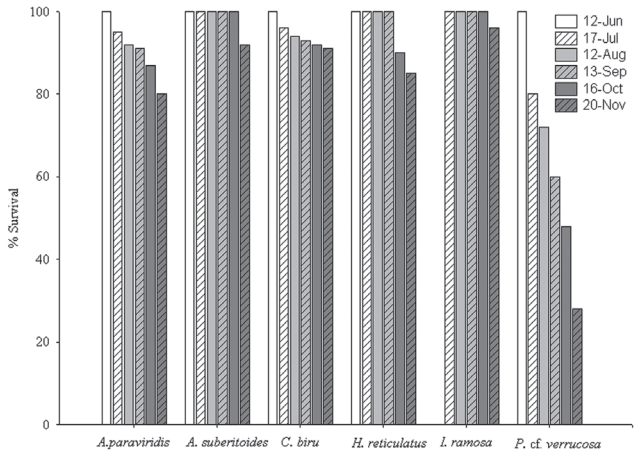

Figure 2. Percent survival rates over time for all species.

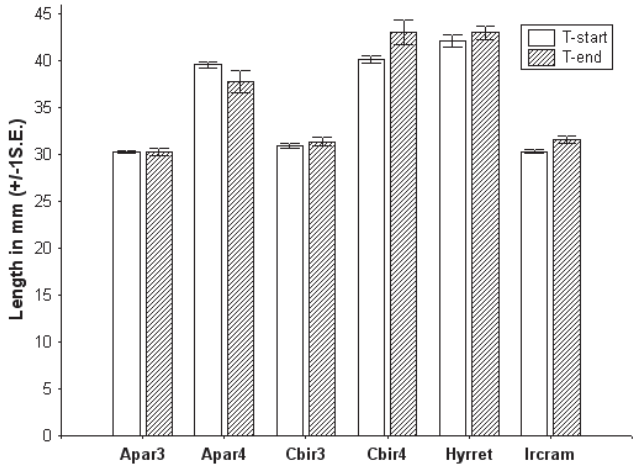

Figure 3. Growth in length for all species. Apar, *Amphimedon paraviridis*; Cbir, *Callyspongia biru*; Hyrret,= *Hyrtios reticulatus*; Ircam, *Ircinia ramosa*. The numbers 3 and 4 refer to the explant sizes 30 and 40-mm.

Acanthostrongylophora ingens, *P. hoeksemai* and *P. nigricans* were either too brittle or too stony to make suitable cuttings were omitted from the present study.

Monitoring

Each month, all sponge cuttings were photographed, checked for survival, and size of the explants to the nearest mm. However, sponges grew in a multidirectional manner, and increase in length could not only accurately be deduced for all explants. In the present study, growth was measured with different methods depending on the morphology of the species in question. Wet weighing of the samples was not a viable option because the explants were often inhabited by barnacles and bivalves, besides, most explants were attached to the PE-rope, which made wet weighing impossible. Increase in length could easily and accurately be determined for branch-forming species such as *A. paraviridis*, *C. biru*, *H. reticulatus* and *I. ramosa*. The increase of sponge tissue could easily be deduced for the sponge cuttings of *C. biru* and *A. paraviridis*, and the dry weights at the start and end of the mariculture trials were calculated as $(W_{end}-W_{start})\times 100/W_{start}$.

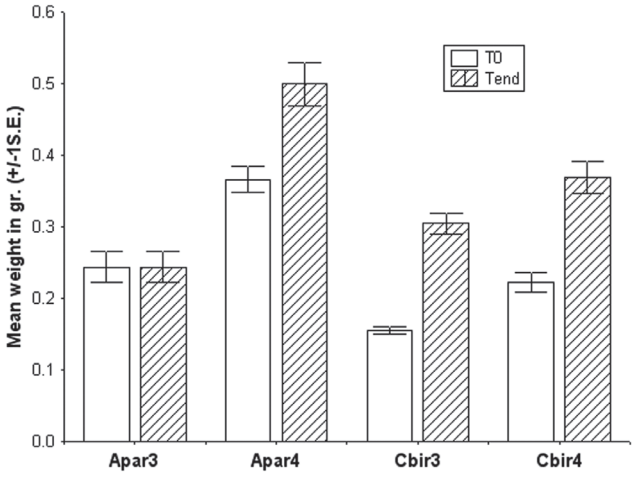

Figure 4. Mean dry weight at the start (T_0) and the end (T_{end}) of the mariculture trial for *Amphimedon paraviridis* and *Callyspongia biru*. See Figure 3 for abbreviations.

In addition, fouling organisms and settled sediment was removed from the explants and PVC-frames.

RESULTS

Mariculture trials

Of the original nine species considered, six were selected for use in the mariculture trials. Survival rates gave a first overall impression of the sensitivity of sponges to cutting and transplantation. The survival rates were, with the exception of *Pseudoceratina* cf. *verrucosa*, high for all sponge species (Figure 2). Survival rates were 80% for *Amphimedon paraviridis* and 92% for *Ircinia ramosa*. All but one of the explants of *I. ramosa*, survived during the mariculture trial. Also, few explants of *Aaptos suberitoides* died off during the course of the trial period, although some explants disappeared from the frame; whether they had dropped or fell prey to consumers remains unclear. The highest mortality was observed for *P.* cf. *verrucosa*. The freshly cut explants all turned completely black, and parts of the blackened tissue eventually detached itself from the remaining healthy tissue (Figure 1F). The explants of *A. paraviridis* were occasionally preyed on during the course of the experiment. The partly eaten leftovers of the smaller-sized remaining explants were rapidly overgrown by algae and eventually died. Mortality observed for *Callyspongia biru* was mostly caused by pressure exerted on the sponge tissue during threading. This species is extremely spongy and elastic and the smallest amount of pressure caused damage, necrosis and eventually death to the explants. Interestingly, the explants threaded first had the highest mortality rates. All explants of *A. suberitoides* and *C. biru* survived the additional period of 10 months in culture.

Growth during the culture experiment

Although three sides of the lobe-shaped *A. suberitoides* were measured, no measurable growth was observed during the course of the experiment. The standard-sized cubes slowly transformed into lobe-shaped explants

Table 2. *Dependent t-test for increase in length and weight.*

Species	P Length	P weight
Amphimedon paraviridis	0.1463	5.6×10^{-14}*
Callyspongia biru	0.0025*	5.3×10^{-9}*
Hyrtios reticulatus	0.0827	
Ircinia ramosa	0.0050*	

*, Significant

during the experiment. Also, the exposed yellow-coloured tissue gradually turned brown. Growth could also not be determined for *P.* cf. *verrucosa* because of the high mortality and loss of tissue of the remaining explants (Figure 1F).

All species, with exception of *A. paraviridis*, increased slightly in length from the beginning to the end of the trials, although this was only significant for *C. biru* and *I. ramosa* (dependent *t*-test, $P=0.0025$; $P=0.005$ respectively, Table 2, Figure 3). Explants of *I. ramosa* showed a change of shape, although this could not be measured accurately (Figure 1C). The mean change in weight for *C. biru* and *A. paraviridis* is reflected in Figure 4. The increase in weight was significant for both species, but not for the smaller explants class of *A. paraviridis*.

DISCUSSION

Survival rate

Based on the natural abundances observed in a survey of the target reef, nine sponges were initially selected for mariculture trials (de Voogd et al., 2006). Three of these were quickly omitted from further study because of deficient transplantation properties; both *Petrosia* species were too compact and stony to make successful cuts, whereas the tissue of *Acanthostrongylophora ingens* crumbled to pieces while cutting. For the remaining sponge species a culture period of approximately six months was chosen to monitor survival and growth. Explant survival was in general high, only *Pseudoceratina* cf. *verrucosa* had low survival rates and is thus unsuitable for further farming with the herein used farming method. For the other sponges, a low mortality rate occurred gradually over the experimental period. Other studies have shown that mortality mostly occurs in the first days to months after transplantation (Pronzato et al., 1999; van Treeck et al., 2003), thus survival rates will give a good first impression of the sensitivity of the selected species after transplantation.

Growth and culture methods

During the experimental period horizontal growth was observed but proved only significant for the larger sized explants of *Callyspongia biru*. Although survival rates were high for *Ircinia ramosa*, only few explants grew during the trial period. Although *Aaptos suberitoides* was able to reattach to an artificial substrate, growth could not be measured after five months and also not after an additional period of nine months. The shape of the explants changed over time, but the surface area of the sponge remained the same. Moore (1908) remarked that some commercial sponge cuttings could survive for years without an increase in volume, and that approximately seven years were needed in order to gain commercial size. In the present study, only growth rates have been measured for farmed sponges, whereas natural growth may be completely different. Also Reiswig (1973) failed to determine the growth rates of a Jamaican *Verongia* (= *Aplysina*) sp. because of non-measurable size change, although regeneration took place rapidly. Hoppe (1988) was able to determine the growth and regeneration efficiency of three large non-branching Caribbean sponges and observed irregular and unpredictable growth. The volumes of massive sponges are probably better-measured using UW-photography and image analysis systems (van Treeck et al., 2003). Müller et al. (1999) observed no growth in the initial month after transplantation, but excessively high growth rates in the third and sixth months. They contributed the high growth rates to extra nutrients released by a proximate mussel and fish culture. The road towards success in the artificial cultivation of both terrestrial and marine organisms lies in maximizing growth by adding fertilizers and food pellets. Although the food uptake of sponges cannot be forced, sponges grow bigger and faster in nutrient rich environments (Verdenal & Vacelet, 1985).

Notwithstanding, growth might have been hampered by the present culture method. After damage, specimens of *C. biru* quickly regenerated by spreading a thin layer of tissue over the wounded areas; explants were also able to attach to the artificial substrate within a few days. In contrast, explants of *Amphimedon paraviridis* had more difficulty attaching to the rope and some of them never reattached. The skeleton of *C. biru* is composed of a flexible almost elastic structure of unispicular spongin fibres. *Amphimedon paraviridis* has a more rigid, crumbly skeleton which is composed of dense multispicular fibres. Thus, spongin probably plays an important role in regeneration and attachment potential. Also, the explants of the densely spicular sponges of *A. ingens*, *Petrosia hoeksemai* and *Petrosia nigricans* fell off the rope line, indicating the poor ability of these species to attach. Thus, other modes of attachments or other farming methods are required for such sponges.

Duckworth & Battershill (2003b) mentioned that a major obstacle to sponge farming is the lack of a suitable large-scale farming method. Most methods were not suitable for commercial application because the farmed explants were not able to attach to the substrate but grew away and were eventually lost. Mesh arrays are probably suitable for soft, fleshy sponges, which require a secure support on which to attach and grow (Duckworth & Battershill, 2003b; van Treeck et al., 2003), although fouling can be a major problem with this method.

The rope method used here appeared to be unsuitable for the species *P.* cf. *verrucosa*. The surviving explants of *P.* cf. *verrucosa* never reattached to the rope lines. Besides, after cutting the wound would turn purple-black and the tissue partly died. Species belonging to the order Verongida show a very fast pigment change upon death or damage, which is a result of oxidation of tyrosine derived brominated compounds (Bergquist & de Cook, 2002). Thus, species, which produce such compounds, are probably all unsuitable for sponge farming. *Aaptos suberitoides* exhibits a similar rapid

colour change when exposed to air, but this never happened after cutting sponge explants. Although, the present method has been used successfully elsewhere (Pronzato et al., 1999), the horizontal mooring system must be protected from destruction due to cage fishing in the Spermonde archipelago. Fishermen throw bamboo fish cages to the sea bottom near the reef and collect these after a certain period of time with a large anchor. The anchor often drags the fish cages over the sea bottom. During the experiments some frames were dislodged from the bottom and were deposited on top of the coral reef. Such fishing practices also present a threat to sponge divers and successful prevention must be part of the process to install a commercial sponge farm.

Differences in biomass structure may also have contributed to the differences in growth rates and final sizes between the two sponge species. Sponges with a low spicule density and unstructured mesohyl exhibited higher growth rates (Duckworth and Battershill 2003b). Although the sponges *C. biru* and *A. paraviridis* showed growth during the short mariculture period, there was a large variability in growth between the explants. Some explants doubled in size and weight, while other explants exhibited no growth at all. Duckworth & Battershill (2001) noticed the large variability of sponge growth in temperate regions due to fluctuating water temperatures, seasonal differences of food availability and reproductive investment. Storr (1964), Stevely et al. (1978) and Verdenal & Vacelet (1985) already commented on the variable growth rates of sponge cuttings, even under the same conditions and originating from the same mother sponge. Kaandorp & de Kluijver (1992) only observed growth at the tips of transplanted branch-forming sponges, whereas secondary growth was observed at the position where the sponge was attached to artificial substrate. Sponges probably first invest their energy in healing and regeneration.

Explant size

Explants of *A. paraviridis* and *C. biru* were set out at various locations and in various sizes. Explants of *A. paraviridis* had in general lower survival rates than explants of *C. biru*. Survival rates were lowest at the exposed side of Samalona for the larger-sized explants, although these were the first to be transplanted. *Callyspongia biru* is extremely spongy and elastic and the smallest amount of pressure caused damage, necrosis and eventually death to the explants. After the threading of the first 25 explants, a smaller-sized needle was needed for this species. Mendola (2003) cultured the Indo-Pacific sponge *Acanthella cavernosa* in an *in situ* culture and surival rates were 81% after seven months, although all explants lost cellular material during this period. Van Treeck et al. (2003) observed high survival rates of three out of four sponges in the Mediterranean. Although both explant sizes of *C. biru* grew, this was not the case for the smaller-sized explants of *A. paraviridis*. Also these explants exhibited lower survival rates. Reiswig (1973) remarked that growth rates of sponges may be greater in younger than in older specimens, assuming that older specimens are in general larger specimens. Importantly, damaged sponges would quickly recover and growth rates were much greater than the growth rates of undamaged sponges (Ayling, 1983). Thus, transplanted small-sized explants would in general grow faster than natural sponges. However, Garrabou & Zabala (2001) noted that all specimens of the Mediterranean sponge Crambe crambe that died during their observations were the smallest. In general smaller individuals have fewer reserves to repair wounds after disturbance events (Jackson, 1979). Pronzato et al. (1999) showed that some sponge species are not suitable for culture due to damage exerted on the sponge body (*Ircinia variabilis* and *Axinella damicornis*) or dripping of tissue from the rope (*Chondrosia reniformis*) and that commercial bath sponges showed the lowest mortality rates, due to the fibrous layer.

In conclusion, although Indonesia probably harbours the highest diversity of sponge species and consequently a multitude of bioactive compounds with pharmaceutical properties, most species occur in relatively low densities compared with temperate regions. The realization of a large-scale sponge culture for bioactive chemicals is not only hampered by the limited availability of sponge species and low growth rates, but also by the effects of fishing and poor access of remote coral reefs. It is thus important, prior the setting up a sponge culture to make an inventory of the prevalent sponge species and abundances in a specific region. Surprisingly few species appeared to be suitable for culture based on their natural abundance in the Spermonde Archipelago. The high overall survival rates and growth potential of *C. biru*, makes this species the only real candidate for further mariculture and quantification of bioactive compounds (de Voogd, 2007). For the large-scale production of bioactive compounds through mariculture, large quantities of the target species are needed. Other regions within Indonesia might have higher densities of certain suitable sponges. However, with the exception of the Spermonde Archipelago, no quantitative data is available of sponge assemblages in Indonesia. Some papers have discussed species richness in various regions within Indonesia (Bell & Smith, 2004; van Soest, 1989), but these contain insufficient detail for selection of locations for sponge farming. Several of the herein used species used, *A. suberitoides*, *I. ramosa*, *H. reticulatus*, *P. hoeksemai* and *P. nigricans* are widespread and common in Indonesia (van Soest, 1989), thus these species could probably be cultured elsewhere. It also stands to reason that a multispecies culture is the most preferred method. Preferably, sponge cultures should be combined with shrimp or fishponds and in the proximity of terrestrial nutrient runoff to promote maximum growth (Müller et al., 1999). However more suitable farming methods must be explored first, because the farming method applied here is found to be unsuitable for cultivating these species. Long-term monitoring, quantification of the bioactive compounds, and up scaling of explants is needed to give more conclusive evidence.

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