Indonesian sponges : biodiversity and mariculture potential

de Voogd, N.J.

Citation for published version (APA):

General rights
It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations
If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: https://uba.uva.nl/en/contact, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.

UvA-DARE is a service provided by the library of the University of Amsterdam (http://dare.uva.nl)
CHAPTER 8

AN ASSESSMENT OF SPONGE MARICULTURE POTENTIALS IN THE SPERMONDE ARCHIPELAGO, INDONESIA

Nicole J. de Voogd
ABSTRACT

Sessile marine invertebrates are sources for substances with potential as pharmaceuticals or as biochemical tools for a variety of applications. Alternative ways to obtain bulk quantities of these compounds are currently under study by several research groups. In the present study (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 purpurea. A total of 340 cuttings was threaded on a PE rope and attached to horizontal mooring structures at three experiment sites. Growth and survival was monitored for a period of six months and for some explants for an additional period of 10 months. All cuttings were photographed at regular time intervals and growth was measured as increase in volume, length and ultimately dry weight. With the exception of Pseudoceratina purpurea, survival rates were high (80-96%) during the mariculture period. Size increases were too little to be measured in Aaptos suberitoides and P. purpurea cuttings. Those of most other species also increased only very slowly, and significant growth was only observed in Callyspongia (E.) 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. The world’s oceans harbor an enormous diversity of organisms, belonging to many more phyla than on land, which in turn produce a vast amount of secondary metabolites with pharmacological potential. 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 actual made it to the pharmacist (Dumdei et al., 1998; Munro et al., 1999; Proksch et al., 2003). The problem herein 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 (a.o. 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). Sponges are usually patchily distributed, in low densities, with the exception of some hexactinellid sponges, which reach high concentrations in specific environments.
An assessment of sponge mariculture potentials

(Barthel & Gutt, 1992; Leys et al., 2004). Unfortunately, hexactinellids are not known to produce pharmacologically interesting compounds. Moreover, most of their secondary metabolites (bioactive compounds) are present in only minute concentrations.

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. Mendola (2003) calculated that 1000 kg of the tunicate Ecteinascidia turbinata was needed to obtain 1 gram of the anticancer agent ET-743. The same amount of wet weight was needed from the sponge Lissodendoryx sp. to supply only 300 mg of halichondrin (Hart et al., 2000). 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, 1998; Nickel et al., 2001), genome transfer (Pomponi, 1999; Osinga et al., 1999), in-vitro culture (Osinga et al., 1999; Duckworth et al., 2003; Richelle-Maurer et al., 2003) and aquaculture (Müller et al., 1999; Munro et al., 1999; Duckworth & Battershill, 2003a, 2003b; van Treeck et al., 2003) 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., 2004).

The exploitation of sponges as a bathing tool has a long history and tradition in the Mediterranean and the Caribbean. Pronzato et al. (1999) remarked that the incredibly high densities of commercial bath sponges in the 1930's of 300 ind/100 m² has been drastically reduced to 50 ind/100 m² at present, due to over-harvesting and disease. The depletion of the world's commercial sponge beds, and the need for a sustainable supply was already discussed in the early 1900s (Moore, 1908a, 1908b), and since then ample studies have assessed the farming of bath sponges worldwide (Cotte, 1908; Crawshay, 1939; Storr, 1964; Verdenal & Vacelet, 1990; Pronzato et al., 1999; Wolff et al., 2002). 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. Substantial work focused on the farming success of sponge species in different geographical regions with respect to explant size (Duckworth et al., 1997; Duckworth et al., 1999) farming method (Duckworth et al., 1999; Duckworth & Battershill, 2003b; Belarbi et al., 2003; Van Treeck et al., 2003; Corriero et al., 2004), depth and water flow (Duckworth et al., 1999, 2004; Duckworth & Battershill, 2003b), and compound concentration (Mendola, 2003). 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
(e.g., 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. Although it is expected that sponge diversity is highest in the Indo-Pacific, "only" about 60 different papers have discussed secondary metabolites isolated from Indonesian sponges by various research groups worldwide (e.g., Supriyono et al., 1995; Salmoun et al., 2002; Yousaf et al., 2002; Roy et al., 2002), and so far no published results are available on sponge mariculture in this region. Although the chemical defense 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., 2002) Brazil (Berlinck et al., 2004), the Mediterranean (Beccero et al., 2003; van Treeck et al., 2003; Müller et al., 2004), 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 in the Spermonde Archipelago, SW Sulawesi, several abundant sponge species were selected for mariculture trials. This barrier reef area is well known for its high marine biodiversity (Hoeksema, 1990; Verheij, 1993, Renema 2002) and its sponge fauna is well documented (de Voogd et al., 1999, 2004; de Weerd & van Soest, 2001, de Voogd & van Soest, 2002, Alvarez et al., 2003). 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. 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. It 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).

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 and the enhanced production of bioactive compounds.

**Materials and Methods**

**Location**
The fieldwork was carried out in the Spermonde Archipelago, SW Sulawesi, Indonesia from June 2002 to September 2003. 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 target species used in the present study were selected based on surveys in 1997 and 2000 (see also de Voogd et al., 1999, 2004). During these surveys, 151 larger reef sponge species were recorded from 34 sites. For the present study we only selected species that were abundant
An assessment of sponge mariculture potentials

$n > 100$ observed (de Voogd et al., submitted), wide-spread, and are known to metabolize bioactive or pharmaceutically interesting compounds. Only a handful of species fulfilled all of these criteria. 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. Not all species were set out at all locations due to time limitation. At Samalona island some explants were set out at the exposed NW side and the sheltered SE side. This is important, since water flow differs substantially between these sites (de Rooij & van Bruijnsvoort 1997) and sponge growth generally increases with increasing water flow with only a few exceptions (Verdenal & Vacelet, 1990; Duckworth & Battershill, 2003b).

**Culture methods**

Sponges were collected using SCUBA at a depth of 10-14 metre, and carefully cut in situ with a razor blade into standard sized (c. 3-4 cm long) explants, 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 (1) a rope attached to horizontal mooring system, (2) a net attached to a horizontal mooring, and (3) a vertical rope in the water column (see Duckworth et al., 1999). Only method 1 proved feasible for the use of all species in the present study. Method 3, 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 cargoship destroyed a second attempt.

Method (1) 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 explants was attached to a 70 x 100 cm² PVC frame (Fig. 1).

![Underwater image of the horizontal mooring system.](image-url)
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 and the sheltered side of Samalona island (SW = 05°07'19.6"S, 119°20'24.6"E; SE = 05°07'35.8"S 119°20'41.1"E) and the exposed side of the submerged Bone Lola reef (05°03'08.7"S, 119°21'12.4"E).

**Sponge species**
The following species were selected for the study:

1. *Aaptos suberitoides* Brondsted, 1934 (Hadromerida: Suberitidae): 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 (van Soest, 2002). Sponges of the genus *Aaptos* and *Suberites* are known to produce aaptamine-type alkaloids (van Soest & Braekman, 1999), and these compounds were isolated from *A. suberitoides* collected in the Spermonde Archipelago (unpublished ‘Symbiosponge’ results). The species is occasionally consumed and rarely overgrown by other organisms (personal observations). It is widespread, and has been reported from the Red Sea to Australia. The maximum observed densities at Bone Lola, and the exposed sides of Samalona were 55 and 132 /100m$^2$ respectively. The sponges were cut into explants of approximately 3 x 3 x 3 cm$^3$ with at least one side of the pinacoderm; 25 sponge cuttings each were placed at the exposed side of Samalona (June 10 – September 21, 2003) and Bone Lola (June 12 – November, 2002). Fig. 2D.

2. *Acanthostrongylophora ingens* Thiele, 1899 (Haplosclerida: Petrosina: Petrosiidae): has variable growth forms from encrusting to large tubes. The species is known to produce manzamine alkaloids, (Yousaf et al., 2004; unpublished ‘Symbiosponge’ results) and is frequently inhabited by polychaete worms and occasionally consumed by fish predators. While cutting, individuals often crumbled to pieces, and the species was therefore omitted from the present study.

3. *Amphimedon paraviridis* Fromont, 1993 (Haplosclerida: Haplosclerina: Niphatidae): 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 (Nicholas & Molinks, 2000; Albrizio et al., 1995), antimicrobial (Schmitz et al., 1978; Kelman et al., 2001) and anticancer (Pettit et al., 1992). Recently, amphitoxin was isolated from this species and several *Callyspongia* spp., including *Callyspongia (Euplacella) biru* de Voogd, 2004 (unpublished ‘Symbiosponge’ data). Symbiotic barnacles and bivalves occasionally inhabit this sponge. It exudes copious mucus when cut. The maximum observed densities at Bone Lola reef, and the exposed and sheltered sides of Samalona island were 117, 303, and 20 inds/100m$^2$ respectively. Branches were cut in fragments of 3 and 4 cm length and farmed at the sheltered and exposed sides of Samalona and Bone Lola. Growth was expressed as increase in length, in addition, growth could accurately be deduced, because the diameter did not increase during the mariculture period.
Fig. 2. 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 (Euplacella) biru after 106 days showing new branch from crust grown across rope (F) Pseudoceratina purpurea, after 4 days; inset shows healthy tissue, while the rest is blackened.

The dry weights at the start and the end of the mariculture trial were calculated as $W_{\text{end}} - W_{\text{start}} \times 100$ (see fig. 2E, F). A total of 100 explants was farmed (25 each at SW4, BL4, SE4 and SW3 between June 12 – November 25, 2002). Fig. 2A.

4. Callyspongia (Euplacella) biru de Voogd, 2004 (Haplosclerida: Haploslerina: Callyspongiidae) is a thinly encrusting to elastic branch-forming bright blue sponge. It has numerous slightly elevated small oscules. Symbiotic barnacles and bivalves occasionally inhabit individuals. The species, furthermore, exudes copious slime when handled. Maximum observed densities at Bone Lola, and the exposed and sheltered sides of Samalona were 14, 40, and 38 inds/100m² respectively. Branches were cut in explants of 3 and 4 cm length and set out at the exposed and sheltered sides of Samalona and Bone Lola.
Growth was expressed as increase in length and weight (see A. paraviridis) total of 125 explants were cultured (25 each at SW4, SW3, SE4, BL4, and BL3 between June 10, 2002, and September 21, 2003). Fig. 2E.

5. *Hyrtios reticulatus* Thiele 1899 (Dictyoceratida: Thorectidae) 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 Archipelago (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. The highest observed abundance for the latter species was 20 inds/100m2 at 9 m at Samalona. The branches were cut in 20 standard-sized explants of approximately 4 cm and placed on PVC-frames at the reef of Bone Lola between June 17 – November 18, 2002. Growth was expressed as increase in length and in volume based on changes in length and radius of the explants according to the formula of a cylinder:

\[
\frac{1}{3} \pi \cdot L_{\text{END}} \cdot \left( (r_{1,\text{END}}^2 \cdot r_{2,\text{END}}^2) + (r_{1,\text{END}}^2 \cdot r_{2,\text{END}}^2) \right) - \frac{1}{3} \pi \cdot L_{\text{START}} \cdot \left( (r_{1,\text{START}}^2 \cdot r_{2,\text{START}}^2) + (r_{1,\text{START}}^2 \cdot r_{2,\text{START}}^2) \right)
\]

Whereby L refers to length of explant, \( r_1, r_2 \) the radii of explant. The suffix END refers to values of L and \( r_1, r_2 \) at the end of the trial and START at the beginning of the trial. Fig. 2B.

6. *Ircinia ramosa* Keller, 1889 (Dictyoceratida: Irciniidae) forms yellowish rubbery-tough anastomosing 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. Maximum observed density at Bone Lola was 34 inds / 100m2. Collected branches were cut into 25 smaller pieces of approximately 3 cm length and placed on PVC-frames at Bone Lola reef between July 15 and November 19, 2002 (Fig. 3 I, J). Growth was expressed as increase in volume based on changes in length and radius of the explants according to the formula of a cylinder (see formula for *Hyrtios reticulatus*). Fig. 2C.

7. *Petrosia* (*Petrosia*) *hoeksemai* de Voogd & van Soest, 2002 (Haplosclerida: Petrosina: Petrosiidae): forms dark-brown arm-thick creeping branches. The texture is stony, although the ectosome is often brittle and crumbly. This species has been observed from a variety of habitats and regions within Indonesia and has been chemically analysed under the name *P. contignata* (in Burgoyne & Andersen, 1992). Subsequent examination of the voucher material confirmed that it is conspecific with the later described *P. (P.) hoeksemai* and not with *P. contignata* Thiele, 1899. The isolated contignasterol and its derivate, IPL576092 are highly effective inhibitor of histamines and the latter compound is currently in Phase II of clinical trials (Newman & Cragg, 2004). The highest density was observed at a depth of 15 m (53 inds / 100m2). During cutting it became clear that the species was too rough to be cut without damaging the tissue excessively, thus it was omitted from the present study.

8. *Petrosia* (*Petrosia*) *nigricans* Lindgren, 1897 (Haplosclerida: Petrosina: Petrosiidae) is a chocolate-dark brown flabelliform to cup-shaped sponge. The texture
An assessment of sponge mariculture potentials

is stony, but the ectosome may be brittle. This species is distributed throughout the Indonesian archipelago and inhabits shallow to deep water. Cyclopropene sterols, such as contignasterol have been reported from a variety of Petrosia spp. (van Soest & Braekman, 1999). Although the compounds of this particular species appear not to have been analysed, the crude extract exposed to Artemia larvae showed moderate bioactivity (see appendix chapter 5). The highest observed density of 32 inds / 100m² species was observed at the reef of Kudingareng Keke at a depth of 9 m (de Voogd, submitted). This species is very similar in texture to P. hoeksemai and omitted from the present study because suitable cuttings could not be made.

9. Pseudoceratina purpurea Carter, 1880 (Verongida: Pseudoceratidae) 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 and cytotoxicity (van Soest & Braekman, 1999; Fusetani et al., 2001). The highest observed abundance of 32 inds / 100m² was observed at 9 m at Samalona island. This species was cut into 25 standard size cubes of approximately 3 x 3 x 3 cm³ and placed at the exposed side of Samalona reef. Fig. 2F.

Table 1. Experimental design. Number of explants per site. SE = Samalona sheltered; SW = Samalona exposed; BL = Bone Lola.

<table>
<thead>
<tr>
<th>Species</th>
<th>SE</th>
<th>SW</th>
<th>BL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aaptos suberitoides</td>
<td>25</td>
<td></td>
<td>25</td>
</tr>
<tr>
<td>Amphimedon paraviridis</td>
<td>25</td>
<td>50</td>
<td>25</td>
</tr>
<tr>
<td>Calysspongia (Euplacella) biru</td>
<td>25</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Hyrtios reticulatus</td>
<td></td>
<td></td>
<td>25</td>
</tr>
<tr>
<td>Ircinia ramosa</td>
<td></td>
<td></td>
<td>25</td>
</tr>
<tr>
<td>Pseudoceratina purpurea</td>
<td></td>
<td></td>
<td>25</td>
</tr>
</tbody>
</table>

Monitoring

The frames were monthly photographed, the explants were checked for survival and growth was measured to the nearest mm. Growth was determined as increase in length for the branch-forming species, and increase in volume for the lobe-shaped species. In addition, growth could accurately be deduced from the original explants for C. biru and A. paraviridis, because their diameter did not increase during the mariculture period, unlike in the other species (see Fig. 2A, E). Duckworth (1999) successfully wet-weighed the cuttings at the start and the end of his experiment, but I refrained from doing this because I did not want to exert additional stress on my sponge cuttings. Besides, the sponge fragments were almost invariably inhabited by barnacles and bivalves, which substantially contributed to the dry weights (20 barnacles made up 200 % of the total weight). Oisinga et al. (1999) developed an underwater scale for in vivo culture, but this is not a feasible method in the open sea due to excessive water movement, and changes in salinity of the sea water.
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 P. purpurea, high for all sponge species (Fig. 3). Survival rates were 80% for A. paraviridis and 92% for I. ramosa. All but one of the explants of I. ramosa, survived during the mariculture trial. Also, few explants of A. 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. purpurea. The freshly cut explants all turned completely black, and parts of the blackened tissue eventually detached itself from the remaining healthy tissue (Fig. 2F). Remaining explants often dropped down, whereas others never reattached themselves to the rope. 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 C. 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.

Fig 3. Percent survival rates over time for all species.
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 during the experiment (fig. 2D). Also, the exposed yellow-coloured tissue gradually turned brown. Growth was also not determined for *P. purpurea* because of the high mortality and loss of tissue of the remaining explants (fig. 2F).

![Graph showing growth in length for all species.](image)

**Fig. 4.** Growth in length for all species. Abbreviations: Apar3 = *Amphimedon paraviridis*; Cbir = *Callyspongia biru*; Hyret = *Hyrtios reticulatus*; Irca = *Ircinia ramosa*. The number 3 and 4 refer to the explant sizes.

![Graph showing mean dry weight at the start (T0) and the end (T-end) of the mariculture trial.](image)

**Fig. 5.** Mean dry weight at the start (T0) and the end (T-end) of the mariculture trial for *Amphimedon paraviridis* and *Callyspongia (Euplacella) biru*. 

107
All species, with exception of *A. paraviridis*, increased slightly in length from the beginning to the end of the trials (Fig. 4), although this was only significant for *C. biru* and *I. ramosa* (dependent t-test, $p = 0.0025$; $p = 0.005$ respectively, table 2). Also, *I. ramosa* and *H. reticulatus* increased slightly (dependent t-test, $p = 0.0385$ and $p = 0.0655$) in volume during the mariculture period (Fig. 6). Explants of *I. ramosa* showed a change of shape, although this could not be measured accurately (Fig. 3C). Fig. 5 shows the mean change in weight for *C. biru* and *A. paraviridis*. Although, the explants of the latter species lost tissue, an increase in weight did occur. Some explants lost tissue, while others grew across the rope, which is not reflected in Fig. 4. Furthermore, explants of *A. paraviridis* with a dimension of 4 cm weighed more than the same sized explant of *C. biru*. Thus, the smallest increase of tissue would result in a weight increase. Growth seemed unpredictable for those species, while some explants did not grow at all, others doubled their size and weight during the mariculture trial. The orientation of growth also differed drastically among species. The branch-forming species would either grow in length or across the rope, thickening and eventually new branches grew from the rope for *C. biru* (Fig. 2E).

**Table 2.** Dependent $t$-test for increase in length, volume and weight. * Significant.

<table>
<thead>
<tr>
<th>Species</th>
<th>$P$ Length</th>
<th>$P$ Volume</th>
<th>$P$ Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Amphimedon paraviridis</em></td>
<td>0.1463</td>
<td></td>
<td>$5.6 \times 10^{-14}$</td>
</tr>
<tr>
<td><em>Callyspongia (Euplacella) biru</em></td>
<td>0.0025*</td>
<td></td>
<td>$5.3 \times 10^{-7}$</td>
</tr>
<tr>
<td><em>Hyrtios reticulatus</em></td>
<td>0.0827</td>
<td>0.0385</td>
<td></td>
</tr>
<tr>
<td><em>Ircinia ramosa</em></td>
<td>0.0050*</td>
<td>0.0655</td>
<td></td>
</tr>
</tbody>
</table>
An assessment of sponge mariculture potentials

**Discussion**

**Survival rate**

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

**Explant size**

Explants of *A. paraviridis* and *C. biru* were set out at various locations and in various sizes. Survival rates were lowest at the exposed side of Samalona for the larger-sized explants, although these were the first to be transplanted. *C. 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. The survival rates varied between 75% (SW4) and 100%. Explants of *A. paraviridis* had in general lower survival rates than explants of *C. biru*. Mendola (2003) cultured the Indo-Pacific sponge *Acanthella cavernosa* in an *in situ* culture and survival 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.

**Growth and culture methods**

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 *A. 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 spongine fibres. *A. paraviridis* has a more rigid, crumbly skeleton which is composed of dense multispicular fibres. Thus, spongine probably plays an important role in regeneration and attachment potential. Also, the explants of the densely spicular sponges of *P. hoeksemaei* and *P. 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 (2003a) 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, 2003a; 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 \( P. \text{purpurea} \) and \( A. \text{ingens} \). The surviving explants of \( P. \text{purpurea} \) 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. \( A. \text{suberitoides} \) 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; Sipkema, 2004), 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 our 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. Another mishap occurred during a trial culture attempt with a vertical rope line anchored to the bottom and buoyed at 5 m below the water surface; this was destroyed by a drifting cargo ship, which stranded on the reef of Samalona.

Sponges are in general slow-growing animals, and only limited data is available on the growth and population dynamics of sponges (Garrabou & Zabala, 2001). Sponge growth rates under natural and experimental conditions have been measured using many different techniques, but appear very difficult to define. For instance Wilkinson & Vacelet (1979) used the mean doubling time of volume of 19-369 weeks for different Mediterranean sponge species. Hoppe (1988) measured linear and vertical growth in cm per year for large Caribbean sponges. Osinga (1999) developed an underwater scale, which can only be used under laboratory conditions due to inaccurate results from water movement. Duckworth (1999) wet-weighed the cuttings after a standard dripping period of half an hour. In order to accurately measure growth, methods should ideally be combined and compared. For instance, Van Treeck et al. (2003) compared the wet weighing with photographs using a professional image analysis system and these two methods were positively correlated. However, the regression quotient differed per species and was lower for a spherically growing species than for a sponge showing a branching shape.

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 \( A. \text{paraviridis}, C. \text{biru}, H. \text{reticulatus} \) and \( I. \text{ramosa} \). During the experimental period horizontal growth was observed but proved only significant for the larger sized explants of \( C. \text{biru} \). Although survival rates were high for \( I. \text{ramosa} \), only few explants grew during the trial period. Moore (1908) remarked that some
An assessment of sponge mariculture potentials

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. Reiswig (1973) distinguished specialist and opportunistic sponges in the Caribbean, with the latter functional group having low life spans, but also high growth and turnover rates, whereas specialists often have relatively large population densities, but low growth rates. Although terrestrial plants have been extensively categorised into functional groups, no such subdivision exists for marine invertebrates with respect to turnover and growth rates. However, relating natural sponge growth rates to transplanted sponges can also be useful. In the present study, only growth rates have been measured for cultured sponges, whereas natural growth may be significantly different. Although A. 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. 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 (Duckworth, 2003; van Treeck et al., 2003). Brümmer & Nickel (2003) used CT scan to analyse the volume of live specimens of Suberites domuncula, although this is not a very cost-effective method. Notwithstanding, growth might have been hampered by the present culture method. 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, 1990).

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 (Aylling, 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). Prior to death, explants of A. paraviridis were overgrown by algae, while mortality of C. biru was mostly caused by the amount of pressure exerted on the sponge body. 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 damiconis) or dripping of tissue from the rope (Chondrosia reniformis) and that commercial bath sponges showed the lowest
mortality rates, due to the fibrous layer. Also some species grew better, because of minor reorganization of the canal system, as is the case with branching species which possess oscules and pores in all the explants (Verdenal & Vacelet 1985). The explants of the herein used branch-forming species all possessed oscules. This was not, however, the case for A. suberitoides and P. purpurea, although the high mortality rate of the latter species probably has a different origin.

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 & 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. With the exception of reproductive investment, these factors are relatively unimportant in the tropics. 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. Although, specimens of H. reticulatus and I. ramosa did not grow significantly during the explant period, the observed growth did not per definition occur at the parts that were taken from the tips of the parent specimen. The spreading of tissue of C. biru occurred in a multidirectional manner. Explants would grow in length but also across the artificial substrate; spreading tissue across the rope, thickening, and eventually forming new branches from thickened crusts. Due to the high overall survival rates and growth potential, this species is the only real candidate for further mariculture and quantification of bioactive compounds.

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. 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), but these contain insufficient
An assessment of sponge mariculture potentials

detail for selection of locations for sponge farming. Several of the species used here, 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 with different farming methods. The commonly used rope 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. Large quantities need to be delivered before a compound will ever make it to preclinical trials (Munro et al., 1999). Importantly, the only sponge-derived pharmaceutical currently on the market is provided through synthesis of the product and illustrates the supply-problem of sponge compounds.

Acknowledgements

This research was funded by the Netherlands Foundation for the Advancement of Tropical research (WOTRO grant W84-474). I am grateful to the Indonesian Institute of Sciences (LIPI) for sponsoring the research in Indonesia. Prof Dr Alfian Noor acted as supervisor at Hasanuddin University, Makassar (UNHAS). Chris Battershill, Libby Evans-Illidge and Carsten Wolff (Australian Institute of Marine Science) provided valuable advice on how to start a sponge culture. Karin Didderen and Chris Sperduto are thanked for their cutting and threading expertise in the field. Daniel F.R. Cleary, Bert W. Hoeksema and Rob W.M. van Soest are thanked for comments on earlier versions of this manuscript.