Indonesian sponges: biodiversity and mariculture potential

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CHAPTER 9

THE MARICULTURE POTENTIAL OF THE INDONESIAN REEF-DWELLING SPONGE CALLYSPONGIA (EUPLACELLA) BIRU: GROWTH, SURVIVAL AND BIOACTIVE COMPOUNDS

NICOLE J. DE VOOGD


**ABSTRACT**

Before sponge mariculture can be used as a commercially viable means to supply pharmaceutically interesting bioactive compounds, it needs to be demonstrated that it can produce sufficient sponge biomass and compounds. In the present study, the Indonesian reef-dwelling sponge *Callyspongia (Euplacella) biru* de Voogd, 2004 (Demospongiae, Callyspongiidae) was cultured for a period of six months using two attachment methods at three sites in the Spermonde Archipelago, Indonesia. A total of 250 sponge cuttings of two size classes were threaded on either polyethylene or nylon rope and attached to horizontal mooring systems. All sponge cuttings were photographed at regular time intervals. Growth was measured as the increase in length and in dry weight. Survival rates were high (82%-100%) for all methods and sizes used. Growth rates were unpredictable and differed significantly among experiments, but did not differ significantly per location, farming method, explant size and explant position obtained from the parent sponge. The concentration of the bioactive compound amphitoxin in the cultured explants was significantly lower than in the natural population, but the concentration did not vary significantly between explants used in the different experiments. Although survival, growth rates and yield of amphitoxin were relatively high, a mariculture of *C biru* is probably not feasible on a commercial scale, due to the lack of sufficient seed individuals that need to be obtained from a wild population.

**INTRODUCTION**

Marine sponges produce numerous bioactive compounds with promising pharmaceutical properties. Since the discovery in the late 1950s of two sponge-derived drugs from the Caribbean sponge *Cryptotethya crypta (=Tectitethya)*, many compounds have faltered on the lengthy road to manufacturing via preclinical and clinical trials (Newman & Cragg, 2004). The major obstacle is not, however, simply due to the side effects of the toxicity of the bioactive compounds, but lies primarily in their limited supply. Bioactive compounds are often present in minute concentrations in often patchily distributed or rare species. Harvesting natural populations will without doubt lead to a depletion of the target organism, and sustainable development of these organisms is a vital requirement. Regular harvesting of sponges could present a feasible option, although re-growth and recovery of the population is a lengthy process and certainly not an option for rare species or species that grow in extreme habitats (van Treeck et al., 2003). For instance, a survey of the abundance of a deepwater sponge species, *Lissodendoryx* sp., resulted in an estimated mean biomass of 289,000 kilos and it was maintained that this would never supply enough of the target compound halichondrin required for commercial use (Dumdei et al., 1998). Although 1000 kilos (wet weight) of sponge were taken from the wild population to isolate the compound required exclusively for preclinical trials, the need for a sustainable and environmentally friendly method to obtain halichondrin was obvious. Currently, *Lissodendoryx* sp. is cultured on a small scale, which produces 1-5 kilos of halichondrin on an annual basis. If halichondrin proves...
to be a promising anticancer drug, the compound will most likely be manufactured through biotechnological production of a genetically modified bacterial strain and subsequent chemical synthesis (Sipkema, 2004).

Although mariculture is currently the most cost-effective method to obtain the large quantities needed all clinical trials, future trends will consist of the optimization and realization of high-tech methods such as tissue culture (Pomponi & Willoughby 1998, Nickel et al. 2001), genome transfer (Pomponi 1999; O singa et al., 1999) and in-vitro culture (O singa et al., 1999; Duckworth et al., 2003). However, before sponge mariculture is applied it must be demonstrated that adequate production of the relevant bioactive compound can be achieved (Duckworth & Battershill, 2003). Although there is a long tradition of farming bath sponges in the Mediterranean, Florida and Cuba, commercial development of the culture of sponges for their bioactive chemicals is still very rare (Dumdei et al., 1998; Duckworth & Battershill, 2003; Hadas et al., 2005). Currently two main farming techniques are being investigated: the vertical rope techniques used in the open sea and horizontal structures moored close to the sea bottom in lagoon areas (Duckworth, 1999; Corriero et al., 2004; van Treeck et al., 2003). Sponges of various species respond very differently to alternative farming techniques with respect to growth and survival. While some species are very flexible and can be manipulated in various ways, others do not survive transplantation at all (Duckworth et al., 1997, 1999; Pronzato et al., 1999). Lissodendoryx sp., for instance, has been successfully cultured when attached to vertical ropes buoyed near the surface; other species, however, were unable to survive transplantation because of an inability to attach to the artificial substrate. The explant procedure is clearly constrained by the morphology and texture of the different species. Duckworth et al. (2003) remarked that fragile and digitate species are optimally cultured in mesh arrays, while amorphous species can be cultured threaded through cable ties. The key factor behind sustainable sponge mariculture consists of the ability of many sponge species to survive and regenerate lost tissue (e.g. due to predator attack, dislodgement due to excessive water movement during storms, Ayling, 1983). Sponge cuttings or explants are experimentally transplanted from a parent stock to designated areas and farmed on a regular basis, while the original sponge cuttings are left to regenerate and re-grow. Thus, a prerequisite of farming success is not only positive growth and high survival of explants, but also strong potential to regenerate. As a rule, small sponge cuttings are capable of extremely fast growth and therefore grow faster than the full-grown parent sponge (Hart et al. 2000; Duckworth, 2002). Ideally, the regular partial harvesting of a farmed sponge should promote sponge growth.

In an earlier study (chapter 8), an assessment was made of the mariculture potential of sponges in the Spermonde Archipelago. Based on their abundance and pharmaceutical properties, nine sponge species out of 151 species observed during a quantitative survey were selected for mariculture trials. Only one of these showed high survival and growth rates, the haplosclerid Callyspongia (Euplacella) biru, which was subsequently selected for further mariculture trials, the primary objective of which was to assess whether sustainable mariculture for the production of promising bioactive compounds is feasible in the Spermonde Archipelago. Sponge cuttings were explanted in two size classes using two attachment methods and positioned
at three sites with different environmental conditions (e.g. sedimentation levels). Survival and growth rates were monitored for a period of six months and 20 explants were left for an additional period of ten months. Sponge cuttings were farmed until the explants had doubled their original explant size. *C. biru* produces amphitoxins (3-alkylpiperidine alkaloids) which have antifungal (Nicholas & Molinksii, 2000; Albrizio et al., 1995), antimicrobial (Schmitz et al., 1978; Kelman et al., 2001) and anticancer (Pettit et al., 1992) properties. In addition, the amphitoxin concentration of farmed explants and samples taken from a wild population were quantified and compared.

**Materials and Methods**

**Experimental design**

Fieldwork was carried out in the Spermonde Archipelago, SW Sulawesi, Indonesia from June 2002 until September 2003. *Callyspongia (Euplacella) biru* de Voogd, 2004 was selected for mariculture trials based on a preliminary assessment of nine sponge species (chapter 8). The high growth potential and survival rate indicated that this sponge species is a promising candidate for further mariculture development. Recently, amphitoxin (3-alkylpiperidine alkaloids) was isolated from several *Callyspongia* species, including *C. biru*, and the compound is hypothesized to be responsible for the overall pronounced bioactivity of this particular species (unpublished 'Symbiosponge' data). Moreover, this type of compound has been recorded in all five families of the Haplosclerida, including the Callyspongiidae, and is thought to be a true sponge compound rather than having a bacterial origin (Andersen et al., 1996; van Soest & Braekman, 1999). Amphitoxin has promising pharmaceutical properties, is active on a variety of test organisms and in various bioassays (Schmitz et al., 1978; Pettit et al., 1992; Albrizio et al., 1995; Nicholas & Molinksii, 2000; Kelman et al., 2001). So far, other bioactive compounds have not been isolated from this species, and quantification is facilitated by the presence of a high concentration of amphitoxin. Sponges were collected by SCUBA at a depth of 10-14 m, and carefully cut in situ with a razor blade into standard-sized explants of 3 and 4-cm in length and subsequently attached to two general attachment methods: (1) a rope and (2) a net, both attached to a horizontal mooring system. The rope method has already been used successfully to culture bath sponges (Pronzato et al., 1999) and *Dysidea avara* for the production of avarol (Sipkema, 2004) in the Mediterranean. For the rope method, 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 were placed after each explant. Each rope carried approximately nine explants and was attached to 70 x 100 cm² PVC frames (Fig. 1a). The net method was developed after the first batch of threaded explants showed a high mortality rate due to the pressure exerted on the sponge tissue during threading and explants were threaded on a very thin nylon rope. Frames were made of PVC tubes mounted in a rectangular shape (70 x 100 cm²) over which nylon fishing net was tightened.
Fig. 1. Underwater image of the horizontal mooring system (A) rope-method (B) net-method.

A very thin nylon rope was threaded to the base of the sponge explant and subsequently attached to the fishing net (Fig. 1b). 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. Separate ‘rope’ and ‘net’ 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). Each experiment (eight at Samalona, two at Bone Lola) consisted of 25 sponge explants. A total of 80 parent sponges were used to culture 250 sponge fragments (table 1).

Table 1. Experimental design for Callyspongia biru explants. Abbreviations SW = Samalona exposed; SE = Samalona sheltered; BL = Bone Lola. * 19 explants of CB-G were cultivated for an additional period of 297 days (10 months).

<table>
<thead>
<tr>
<th>Code</th>
<th>Location</th>
<th>Method</th>
<th>No. of explants</th>
<th>Explant size (mm)</th>
<th>Days in culture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cb</td>
<td>SW</td>
<td>Rope</td>
<td>25</td>
<td>40</td>
<td>171</td>
</tr>
<tr>
<td>Cb-A</td>
<td>BL</td>
<td>Rope</td>
<td>25</td>
<td>40</td>
<td>171</td>
</tr>
<tr>
<td>Cb-B</td>
<td>SW</td>
<td>Net</td>
<td>25</td>
<td>40</td>
<td>159</td>
</tr>
<tr>
<td>Cb-C</td>
<td>SE</td>
<td>Net</td>
<td>25</td>
<td>40</td>
<td>152</td>
</tr>
<tr>
<td>Cb-D</td>
<td>BL</td>
<td>Net</td>
<td>25</td>
<td>40</td>
<td>149</td>
</tr>
<tr>
<td>Cb-E</td>
<td>SE</td>
<td>Rope</td>
<td>25</td>
<td>40</td>
<td>149</td>
</tr>
<tr>
<td>Cb-F</td>
<td>BL</td>
<td>Rope</td>
<td>25</td>
<td>30</td>
<td>136</td>
</tr>
<tr>
<td>Cb-G</td>
<td>SW</td>
<td>Rope</td>
<td>25</td>
<td>30</td>
<td>128, 125*</td>
</tr>
<tr>
<td>Cb-H</td>
<td>SW</td>
<td>Net</td>
<td>25</td>
<td>30</td>
<td>126</td>
</tr>
<tr>
<td>Cb-I</td>
<td>SE</td>
<td>Net</td>
<td>25</td>
<td>30</td>
<td>125</td>
</tr>
</tbody>
</table>
Each month, the frames were photographed, checked for survival, and sponge growth of the explants was measured to the nearest mm. In addition, fouling organisms and settled sediment was removed from the explants and PVC-frames. Growth was determined as increase in length and growth rates (gram per day). Growth could accurately be deduced from the original explants, thus the dry weight at the start and the end of the mariculture trial was calculated as final weight – initial weight / days of culture. After approximately four months, explants that had doubled in size were ‘farmed’. The explants were photographed, measured to the nearest mm, and the newly grown tissue was carefully removed with a razor blade. After the experimental period, the growth rates before and after farming were compared.

**EXTRACTION AND TLC**

At the end of the mariculture period, 29 explants of *C. biru* from four different experiments were chemically analyzed by HPLC quantification. The sponge fragments were measured (length in cm) and extracted in pure methanol for 24 hours at room temperature immediately upon collection. In addition, three branches of *C. (E.) biru* were collected from different locations and habitats and subsequently cut into samples of 4-cm length, yielding 13 samples. These were used to determine the natural variation of amphitoxin concentration. The sponge samples were washed twice in succession in solvent to ensure an exhaustive extraction. All the filtrates were combined and placed in a cooled evaporator. The crude extract was collected after approximately 24 hours of evaporation and stored at 7°C. Detection of the 3-alkylpiperidine alkaloids was achieved with the aid of Thin Layer Chromatography. Although 3-alkylpiperidine alkaloids have previously been isolated from *C. biru*, the presence of these alkaloids was verified for several sponge fragments prior to HPLC quantification. A capillary was held in the methanolic sponge extract and carefully spotted five times onto 0.25-mm Si gel coated sheets (5 x 2 cm²). The plates were eluted with a solvent mixture containing water / acetonitrile (7:3) + 0.1% trifluoroacetic acid. After completion of the chromatography, the dried plates were sprayed with a solution of Dragendorff reagent; 3-Alkylpiperidine alkaloids can be detected as one of the major compounds present in the extract and appear as a bright orange spot.

**QUANTIFICATION OF SECONDARY METABOLITES BY HPLC**

Analysis of amphitoxin was performed by high-performance liquid chromatography (HPLC) with a Waters 600E system controller equipped with a Waters 717 plus auto sampler and a Waters 848 Tunable Absorbance Detector. Crude extracts from the sponge fragments were dissolved in a mixture (70:30) of water and acetonitrile + 0.1% trifluoroacetic acid, after which 10-μl was injected by auto-sampling into a HPLC system. The column used was a Lichrospher RP18 (125 by 2.0-mm with 3-μm pore size) and the mobile phase consisted of acetonitrile (70%) and nanopure water (30%) with trifluoroacetic acid (0.1%) at a flow rate of 1 ml/min. Amphitoxin was detected at its ultraviolet maximum of 266-nm. Quantitative analysis was based on peak area calibration using previously purified amphitoxin; data was compared with spectral data reported in the literature (Albrizio et al., 1995). Amphitoxin concentration was expressed as a mean percentage of the total dry weight. After extraction, symbiotic
barnacles and bivalves were carefully removed from the samples and the tissue was subsequently dried for 48 hours. Because of the costs involved, it was not possible to analyze replicate explants and to test all samples.

**DATA ANALYSIS**

One-way ANOVA's were used within Statistica 6.1 (Statsoft, Tulsa, USA) to examine the relative effect of location (Samalona SE, Samalona NW and Bone Lola), explant size (30-mm and 40-mm), farming method (rope and net), and explant position obtained from the parent sponge (tip or non-tip). To meet ANOVA assumptions, the growth rates were square root-transformed. Finally, Pearson's Product-Moment correlation within Statistica 6.1 was used to test for an association between growth rate and amphitoxin concentration.

**RESULTS**

**Survival**

In general, survival rates were high for all methods used. The mean survival rates varied from 82% for the larger sized explants with the rope method on the exposed side of Samalona island to 100% for the smaller sized explants with the rope method (Fig. 2). All explants of experiment Cb-G (same site) survived the additional culture period of 271 days.

![Fig. 2. Mean percentage survival of Callyspongia (Euplacella) biru cultured in two size classes and two farming methods. The number of explants present per method are given in the bars.](image-url)
Growth

Growth (Figs. 3, 4) was expressed as increase in length (growth occurring at the tips of the explants) and weight (growth rate in grams per day). Growth differed considerably per experiment. Since not all samples increased in length during the mariculture period, increase in weight gave more accurate results. The highest growth rates were observed for experiment Cb-C (152 days, SE, net method 40-mm) and experiment CB-G (128 days, SW, rope method, 30-mm), while the lowest growth rates were observed for experiment Cb-B (159 days, SW, net method, 40-mm) and Cb-I (125 days, SE, net method, 30-mm) (Fig. 4). Statistical analysis showed that growth rates differed significantly different among experiments ($F_{3,178} = 7.2615, p < 0.001$). The lowest growth rates were observed for the smaller-sized explants and the highest for the largest explants on the sheltered side of Samalona island. Overall growth rates for the different sized explants (Fig. 5A) did not, however, differ significantly ($F_{1,186} = 0.0416, p = 0.838640$). Although the rope method had a slightly lower growth rate than the net method (Fig. 5B), there was no significant difference between both methods ($F_{1,186} = 2.4856, p = 0.1166$). Among localities, the lowest growth rates were observed at Bone Lola (Fig. 6A), but growth rates did not differ significantly ($F_{2,185} = 0.4250, p= 0.0654$).

![Graph](image)

**Fig. 3.** Average length of *Callyspongia (Euplacella) biru* explants for all experiments at the start and at the end of the mariculture trial.
Fig. 4. Mean growth rates of Callyspongia (Euplacella) biru explants for all experiments.

Fig. 5. Different types of growth of Callyspongia (Euplacella) biru. (A) Cb-7G at SW after 106 days; growth at both sides of explants, the upper arrow indicate the encroachment of a bivalve (B) Cb-10G at SW after 106 days; 200% growth at one side of explants, arrows indicates the end of the original explant (C) Cb-21G at SW after 106 days; New branch is formed from crust growing across rope (D) Cb-8C at SE after 145 days; Original explant is indicated by inset. Arrow indicates new branch formed from crust.
The explants obtained from the tip of the parent sponges (Fig. 6B) had higher growth than the explants taken from below the tips, but again the difference was not significant (F_{1,180} = 1.8137, p = 0.1798). Not all explants grew during the experimental period, while others doubled in size. But, increase in length was observed for all experiments, with the exception of the small sized rope explants at Bone Lola reef (Fig. 3). Growth was often observed as spreading of the tissue across the artificial substrate and new branches arose from those crusts (Fig. 7C, D). Most rope-cultured explants grew horizontally in one direction and across the rope in both directions (Fig. 7A, B), while the net-cultured explants could only grow in two directions, in height or across the net.

![Fig. 6](image.png)

**Fig. 6.** Mean growth rates of *C. biru* explants (A) farming method and (B) explant size. The number of explants are given in the bars.

![Fig. 7](image.png)

**Fig. 7.** Mean growth rates of *C. biru* explants (A) per location. WS= Samalona NW; SE = Samalona east; BL= Bone Lola (B) explant position obtained from parent sponge. T= tip and NT= beneath tip of branch.
Farming

Tissue from 17 sponge explants from five experiments were farmed after approximately four months from the original explants to investigate regeneration potential and tissue growth after farming (Fig. 8A-E). All explants regenerated after a few days, and sponge growth was observed for all explants (Fig. 8F). Only tissue of the initially horizontally growing explants could be removed. Dependent t-tests showed no significant difference (t = 1.199128, p=0.26949) between growth rates before and after harvesting (Table 2).

Fig. 8. Explant Cb- 3A of Callyspongia (Euplacella) biru at Bone Lola reef at different time intervals. The original size of the explant is shown by the arrows (A) 62 days (B) 79 days (C) 130 days (D) 140 days (E) 140 days after removal of tissue ('farming') (F) 21 days after farming, arrows show bi-directional growth.
Table 2. Growth rates in g/day before and after harvesting of *Callyspongia (Euplacella) biru* explants. Between brackets the number of days until and after harvesting.

<table>
<thead>
<tr>
<th>Explant (no. of days)</th>
<th>Before farming</th>
<th>After farming</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Growth rate (gr./d)</td>
<td>Growth rate (gr./d)</td>
</tr>
<tr>
<td>Cb-1A (143/21d)</td>
<td>0.00112</td>
<td>0.001</td>
</tr>
<tr>
<td>Cb-2A (143/21d)</td>
<td>0.00100</td>
<td>0.0018</td>
</tr>
<tr>
<td>CB-23A (143/21d)</td>
<td>0.00112</td>
<td>0.0085</td>
</tr>
<tr>
<td>Cb-21C (127/20d)</td>
<td>0.00200</td>
<td>0.0014</td>
</tr>
<tr>
<td>Cb-25C (127/20d)</td>
<td>0.00160</td>
<td>0.0021</td>
</tr>
<tr>
<td>Cb-2E (117/21)</td>
<td>0.00170</td>
<td>0.0023</td>
</tr>
<tr>
<td>Cb-3E (117/21)</td>
<td>0.00140</td>
<td>0.0012</td>
</tr>
<tr>
<td>Cb-18E (117/21)</td>
<td>0.00220</td>
<td>0.0026</td>
</tr>
</tbody>
</table>

**Compound quantification**

All samples analyzed contained amphitoxin. Analysis of variance showed that the amphitoxin concentration in the cultured explants was significantly lower than that in the natural population ($F_{1,40} = 13.261, P < 0.001$), but the amphitoxin concentration of the different experiments did not differ significantly ($F_{3,25} = 3.1438, p=0.4290$).

![Image of mean amphitoxin per dry weight for different farming techniques](image-url)

Fig. 9. Mean amphitoxin per dry weight of sponge fragments for the different farming techniques (Cb-E, Cb-C, Cb-G, Cb-H, see table 1) for *Callyspongia (Euplacella) biru*. Mean natural concentrations are indicated as a straight line; the dotted lines are is the +/- SE.)
The amphitoxin concentration varied from 0.03 - 0.17% in the cultured explants to 0.04 - 0.22% for the natural variation (Fig. 9). The highest amphitoxin concentration was observed in the explant taken from the sheltered side of Samalona island. These samples were also from larger-sized explants. The lowest amphitoxin percentage was observed in the smaller-sized samples cultured with the net method. A Pearson's Product-Moment correlation showed a positive but not significant association between growth rate and amphitoxin concentration ($r^2 = 0.016, p = 0.61$).

The yield of amphitoxin from 500 explants can be deduced from the mean growth rates, weight of explant, amphitoxin concentration with a mortality rate of 9% per year (mean mortality taken from all experiments). Thus, the yield of amphitoxin of a hypothetical mariculture composed of 500 explants would be 14.82 g per year compared to 25.66 g per harvestable amphitoxin per year from a similar sized wild population. This represents a 38% lower amphitoxin production if a farming solution is chosen over harvesting the wild population.

**Table 3.** Yield of amphitoxin calculated obtained from 500 explants of *Callyspongia biru* with a mortality rate of 8% after one year for a cultured and wild population. * Growth rates and mean weight per explants are derived from the cultured population.

<table>
<thead>
<tr>
<th>Unit</th>
<th>Cultured population</th>
<th>Wild population*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean growth rate (gr./day)</td>
<td>0.00129</td>
<td>0.00129</td>
</tr>
<tr>
<td>Mean dry weight of explant (gr.)</td>
<td>0.2130</td>
<td>0.2130</td>
</tr>
<tr>
<td>Mean concentration amphitoxin /dry weight</td>
<td>0.06897%</td>
<td>0.1194%</td>
</tr>
<tr>
<td>Yield of amphitoxin per year (gr.)</td>
<td>14.82</td>
<td>25.66</td>
</tr>
</tbody>
</table>

**DISCUSSION**

In the present study survival, growth, and amphitoxin production concentration were determined for an experimental mariculture of the sponge *C. biru* using two different attachment methods and under different environmental conditions. The results were compared with a simulated harvest from the wild population.

**Survival**

Survival rates were generally high for all methods used, although the first threaded explants on the ropes had the highest observed mortality due to the amount of pressure exerted on the sponge tissue. The remaining 19 explants left to grow for an additional period of 276 days all survived. Duckworth & Battershill (2003a) mentioned that a major obstacle to sponge farming is the lack of a suitable large-scale farming method. They noted that most methods were unsuitable for commercial applications because, for example, the farmed explants were unable to attach
themselves to the artificial substrate but rather grew in the opposite direction from the rope 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).

**Growth**

Growth rates were unpredictable and varied substantially among experiments and explants. Statistical analysis showed no substantial variation among sites, farming method and explant size, which can be attributed to differences between the parent individuals from which the explants were obtained. In situ individuals of *C. biru* tend to be larger on the sheltered side of Samalona island (unpublished pers. obs.). Reefs of the Spermonde are usually cay crowned (Umbgrove 1929, 1930; Renema 2002); the west sides of these cays are exposed to oceanic swell and coral covered; the east side generally consists of carbonate sand with isolated coral patches. The SE side of Samalona island is sheltered from wave action, thus sponge species present at this side of the reef might be less prone to damage or fragmentation caused by storms. High water movement might favour high growth rates, but is probably less favorable for smaller sized individuals. Although non-significant, the highest growth rates were observed for the larger sized cuttings of *C. biru*, thus environmental conditions with pronounced hydrodynamic energy might therefore be favourable to larger sized individuals.

Duckworth et al. (1997) remarked that although water movement may be favourable by removing sediment from the culture and supplying ample amounts of food to the sponge cuttings, excessive water movement may dislodge explants from the rope. It is likely that the high water movement prevents rapid attachment to the substrate and that the fish net is a better substrate than the rope in areas with greater hydrodynamic energy. On the other hand, the PVC-frames covered by a fish net resulted in higher sedimentation on the sponge frames and cuttings. During each check, the settled sediment had to be removed from the frames.

The submerged Bone Lola reef showed intermediate growth rates. Growth rates were more or less similar, although the smaller sized explants with the net method had the lowest overall growth rates. Corriero et al. (2004) also observed variable growth among the different sponge cuttings, and attributed this to the fact that the explants were taken from a source population of individuals of different ages. In the present study, an average of four explants were used from the same parent sponge. Growth rates varied among explants of the same parent sponge, but also among the orientation of sponge cuttings. The highest growth was not always observed for explants originating from the tips of the branches, or in those originating from the same parent sponge, but rather growth seemed somewhat unpredictable. Moreover, explants grew in a multidirectional manner. Although Kaandorp & de Kluijver (1992) only observed growth at the tips of transplanted branch-forming sponges, secondary growth was observed at the position where the sponge was attached to the artificial substrate. They concluded that sponges probably invest energy first in healing and regeneration. Indeed, in the present study, most explants grew in a multidirectional orientation. In addition to growth at the tips of the explants, growth was also observed at the positions where the sponges were attached to the artificial
substrate. Verdenal & Vacelet (1990) also reported variable growth rates of sponge cuttings, even under the same conditions and even if the cuttings were taken from the same parent sponge. Reiswig (1973) remarked that the growth rate of sponges may be higher in younger than older specimens, assuming that older specimens are generally also larger specimens. Importantly, damaged sponges tend to recover quickly and growth rates much higher than the growth rates of undamaged sponges (Ayling, 1983). Thus, transplanted small-sized explants in general are expected to grow faster than natural sponges, and sponge cuttings should ideally be taken from younger smaller wild sponges as opposed to full-grown large specimens. However, Garrabou & Zabalta (2001) noted that all specimens of the Mediterranean encrusting sponge *Crambe crambe* that died during their observations were the smallest. In general, smaller individuals seem to have fewer reserves to repair wounds after disturbance events (Jackson, 1979).

Growth of sponges is a very slow process, and it takes several years for a small sponge cutting to grow to commercial size (von Lendenfeld, 1889). In most cases the sea is a nutrient-poor environment, but sponges can efficiently filter out food particles in such systems. 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 there are no known means to enhance the food uptake of sponges, sponges grow bigger and faster in nutrient-rich environments (Verdenal & Vacelet 1990). Müller et al. (1999) observed high growth rates of the cultured *Geodia* sp. in the Mediterranean due to nutrient influx of a proximate fish and mussel farm. Although locally abundant in the Spermonde, *C. biru* was absent from the most perturbed sites and preferred areas with good visibility, low human settlement and low rates of sedimentation (unpublished results).

**Farming**

In the present study, several explants were harvested after approximately four months in culture. Tissue of rope or net-spreading explants could not initially be removed. Although growth rates may appear high for explants spreading across the artificial substrate, these explants could not be efficiently farmed because of loss by "leaching" of the bioactive compound due to the handling pressure exerted on the sponge tissue during cutting. Harvested explants of *C. biru* regenerated and growth was observed within an additional culture period of 20 days, but because of the short time frame after harvesting no conclusive evidence can be given on growth rates of the new explants. Nevertheless, growth rates appeared similar or higher (although not significantly so). Duckworth & Battershill (2003) remarked that farmed explants had growth rates similar to or higher than non-harvested explants. Also, Ayling (1983) noted high growth rates and fast recovery of damaged sponges compared to undamaged sponges. This is an important observation, because sponge explants have to be farmed frequently in order to deliver a sufficient yield of compounds. Unfortunately due to time constraints, the present study followed the recovery and regeneration of only a few explants after only a single harvesting event. Upscaling and multiple harvesting of the sponge cuttings will show whether sponges can sustain frequent damage and regeneration.
Bioactive compounds

A prerequisite for a commercial sponge culture, in addition to high growth and survival, is a sufficient production of bioactive compounds. The yield of amphitoxin was significantly lower in the cultured explants than that obtained from sponges in their natural environment. It is likely that "wounded" individuals invest their limited energy in healing and growth rather than in the production of secondary metabolites. Importantly, all samples tested in the present study contained amphitoxin. Richelle-Maurer et al. (2003a) remarked that there seems to be a minimal production of bioactive compounds at the boundary of efficiency. Contrary to our results, Duckworth & Battershill (2003b) observed an on average elevated bioactivity in the cultured New Zealand *Polymastia croceus* compared to the bioactivity of wild sponges. However, *Lissodendoryx* sp. produced significantly lower concentrations of halichondrin than wild sponges (Munro et al., 1999). One would expect that explants deprived of spatial competitors or predators would produce lower concentrations of secondary metabolites. It is feasible, that removal of explants from their natural habitat also freed them from predators and spatial competitors, thus removing the main inducing factors for compound production. However, Thacker et al. (1998) found no evidence that the production of secondary metabolites was induced by the presence of a spatial competitor; rather they suggested that the constant threat of predators might maintain high concentrations of the compound. Richelle et al. (2003b) did not detect differences in metabolite concentration in sponges that were submitted to a prolonged protection (by caging) from predators and uncaged individuals.

In another study (chapter 6), a forced confrontation of *C. biru* specimens with individuals of the scleractinian *Fungia fungites* showed a consistently lower amphitoxin concentration compared to the natural population. It was concluded that other ecological factors besides spatial competition interact in regulating the production of amphitoxin.

Yield of amphitoxin

In spite of high sponge survival and growth rates, it is vitally important to evaluate the potential of sponge mariculture to actually deliver the required bulk amounts of the target secondary metabolites. Sipkema (2004) evaluated the economic value of halichondrin isolated from *Lissodendoryx* sp. and avarol from *Dysidea avara*. Wild populations of *D. avara* generally have an avarol concentration of 2 g/kg wet weight of sponge. He calculated that 75,000 kilos or $1.19 \times 10^5$ explants were needed in order to supply the required amount of 150 kg avarol per annum. Although halichondrin concentrations are generally much lower, one kg wet weight of *Lissodendoryx* sp. yields 0.0004 g. Sipkema calculated that "only" 7000 tons of wet weight or $73.6 \times 10^6$ explants were needed to deliver the required amount of 2.8 kg halichondrin per annum. The difference here lies in the different quantities required to produce the desired pharmaceutical. Such an evaluation cannot be made for amphitoxin at present because it is unknown whether amphitoxin can actually be processed into a pharmaceutically active compound, and which concentrations are needed for drug production, but a comparison can be made based on the above-mentioned values. It was calculated in this study that the mean wet mass is approximately 14 times
higher than the mean dry weight. Thus, one kilo of wet weight would produce 4.92 grams of amphitoxin, whereas this would be 8.53 grams from a wild population, which is a great deal higher than the natural concentrations of halichondrin or even avarol. Hypothetically, if a required amount of 50 kg/year of amphitoxin is needed, then 10,000 kg wet weight or 42,258 explants of 40-mm in size have to be cultured. A survey of 34 sites in the Spermonde Archipelago yielded a total of 373 specimens of the sponge *C. biru*, with an average size of 75 cm² (pers. obs.). An average individual of *C. biru* can deliver four explants, thus these individuals would hypothetically deliver (373 × 4) or 1492 explants, and this is clearly not enough for the annual required supply of amphitoxin (10,564 individuals are needed to deliver 42,258 explants). Notwithstanding, the possible detrimental effects, such as diseases, of the high number of frames (at least 1600) that will have to be positioned at suitable locations along the reef. Besides, an average mortality rate of 9% must be taken into account as well (mean mortality rate was derived from all experiments, see Fig. 5). On an annual basis, 122 explants have to be added to replace the losses due to mortality. Although survival growth rates are high, mariculture of the locally abundant *C. biru* is probably not feasible, even if mariculture conditions and amphitoxin concentrations were to be optimized, due to the supply problem of the stock population and the limited growth rate. However, other sponge species might have higher population sizes in other regions within Indonesia.

Tropical sponges and other reef organisms in general are present in low densities. Occasionally some organisms may reach high population densities due to unnatural outbreaks as a result of anthropogenic factors, such as the direct effects of eutrophication or the indirect effects of over-fishing or dynamite fishing, or asexual reproduction (Seymour & Bradbury, 1999; Hoeksema, 2004). To date it is even questionable whether the successful mariculture of the bryostatin-producing bryozoan *Bugula neritina* will ever be embraced by a pharmaceutical company due to the high risk implementation of this methodology. Notwithstanding the fact, that this cosmopolitan organism was an excessive fouling organism on boats and jetties (Mendola, 2003). Thus, it can generally be concluded that the mariculture potential of the prevalent sponges in the Spermonde Archipelago demands high investment and is threatened by relatively low overall population sizes of the more suitable species. Mariculture is probably only a viable option when low quantities of the target compounds are needed or when growth can be maximized when combined with other cultured organisms. An alternative for sponge mariculture is the harvesting of tissue of target organisms from wild populations. Lasker (2004) assessed the colony growth of the anticancer pseudopterin-producing *Pseudopterogorgia elisabethae* in the Caribbean. Although harvesting was constrained by the combined effects of trained collectors, use of SCUBA and seasonally adverse weather conditions, growth appeared high. Although, this species is a conspicuous reef organism and is easily recognized, the population density is also too low for this method to become feasible. Besides, natural growth rates have to be assessed before wild harvesting is deemed feasible.
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