Growth and regeneration in cultivated fragments of the boreal deep water sponge *Geodia barretti* bowerbank, 1858 (Geodiidae, Tetractinellida, Demospongiae)

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Abstract

A cultivation method has been developed for the boreal deep-water sponge *Geodia barretti* (Demospongiae, Geodiidae), a species which is common in the deep Norwegian fjords. The species is known to contain secondary metabolites which are biologically active. Choanosomal fragments of 2–4 cm\(^3\) (\(\approx3–7\) g) were kept in half-open systems. Cicatrisation and regeneration processes were surveyed by histological examination during 8 months of cultivation. During the first weeks, the weight of the fragments decreased. However, after about 6 weeks the weight equalled the original weight, and after 1 year the weight had increased by about 40% compared to the original weight. The initial decrease was due to complex healing processes and the regeneration of the cortex, a sterrastral layer typical for the family of the Geodiidae. We document, for the first time, the complete cortex reconstruction in an adult *G. barretti*, as well as the development of egg cells during cultivation. Our study represents the first attempt at biotechnological production of boreal sponge tissue. For successful farming of *G. barretti* and other boreal and arctic sponges, however, further investigation is needed on factors stimulating growth and secondary metabolite production in the target species.

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Keywords: *Geodia barretti*; Cicatrisation; Reproduction; Regeneration; Cortex; Half-open system

1. Introduction

During the past few years, interest in biotechnological production of sponge tissue has increased due to the increasing interest in sponge secondary metabolites (Pomponi and Willoughby, 1994; Duckworth et al., 1999; Müller et al., 1999). Most research, however, was directed towards tropical and Mediterranean species, and no attempt has been made so far at biotechnological production of boreal sponge tissue. Previous cultivation experiments on boreal species have been short-time scale studies for fundamental
research, as for Thenea muricata (Witte, 1995) or Halichondria panicea (Barthel and Theede, 1986).

Though many Antarctic and boreal marine species are known to have antibacterial and antiviral activity (Andersson et al., 1983; McClintock and Baker, 1997), only a few studies have been made on isolation and further investigation of secondary metabolites. Water- and petroleum ether extracts of the northern deep-water sponge *G. barretti* show antibacterial and antiviral activity (Andersson et al., 1983), and an indole alkaloid has been isolated from this sponge (Lidgren and Bohlin, 1986; Lieberknecht and Griesser, 1987). *G. barretti* is especially common in the deep Norwegian fjords, growing on hard bottom slopes below 40 m depth. Sponges are known to possess strong regenerative capacities (reviewed in Simpson, 1984), and pieces of living sponge tissue are able to grow and regenerate into healthy sponges. This potency has been used for cultivation of sponge tissue samples in both half-open systems and open sea aquaculture on a broad range of sponge species (reviewed in Osinga et al., 1999). In this study, we describe a cultivation technique in half-open systems for tissue samples of *G. barretti*, with parallel surveying of regeneration processes in tissue, skeleton and canal system.

2. Material and methods

2.1. Sampling

Sponges were sampled near the city of Bergen on the west coast of Norway, between 100 and 150 m depth on a hard bottom slope in Korsfjord at 60°09'12"N; 05°08'52"E (Fig. 1). Samples were taken in July 2000 and in March and May 2001 with the Norwegian research vessels ‘Hans Brattström’ and ‘Aurelia’ by dredging with a triangular dredge. Sampling is one of the most critical factors in sponge cultivation experiments, as many sponges are sensitive to air exposure and shifts in water temperature. To minimize exposure to air, the wire was stopped when the dredge appeared at the water’s surface. An outboard-working person placed the sponges in buckets under water, which were subsequently transported to deck and emptied into larger vessels filled with running seawater. Later experience in the lab showed that *G. barretti* tolerates short exposures to air, and sampling in 2001 was done by emptying the dredge directly in water-filled vessels. The described sampling method should, however, be applied to extremely air-sensitive species if sampling by SCUBA diving or submersibles is not possible.

After sampling, the sponges were transported immediately to the cultivation site.

2.2. Cultivation of tissue samples

The cultivation experiments were performed at the Marine Biological Station of the University of Bergen, Norway. The tissue of *G. barretti* is divided into the cortex, a superficial region reinforced by special spicules, and the choanosome, the interior part of the sponge where choanocyte chambers are located. The cortex is about 500 μm thick and consists of star- and ball-shaped micro-scleres, small spicules called euasters and sterrasters, respectively. Megascleres, larger spicules, are radially arranged directly beneath the cortex as well as chaotically in the choanosome (see Fig. 5a, b, and Soest et al. (2000) for details). For this...
experiment, only tissue from the choanosome, at least 2 cm below the cortex, was used. Tissue samples of $2-4 \text{ cm}^3$ ($\approx 3-7 \text{ g}$) were placed in cultivation tanks of 50 l which were connected in an open circulation system with seawater from 40 m depth. Chemical and physical conditions in both sampling site and cultivation site water were determined by standard methods (Grasshoff et al., 1983) and are shown in Table 1. No food except that from the unfiltered seawater was added to the cultivation tanks. The explants were placed on fine mesh plastic grids (3-mm mesh) without any artificial connection. The weight of the samples ($n = 30$ for batch 1, $n = 300$ for batch 2 and $n = 100$ for batch 3) was determined as drip dry wet weight at day 0, 15, 45, 100 (batch 1–3), and 350 (batch 1). Only the explants that were still alive at the time of measurement were included.

2.3. Unsuccessful cultivation methods

Different approaches were made to find a successful cultivation technique. Tissue samples considerably smaller than 2 or larger than 4 cm$^3$ died within the first week, as well as samples including old cortex. Fragments placed on stones (natural substrate), hung on plastic-coated threads, or glued to the substrate with silicone also failed to survive. New explants died when the water temperature was raised above 10 $^\circ$C, while explants with a partly developed cortex survived temperatures up to about 15 $^\circ$C.

2.4. Histology

For histological examination, sponge fragments were fixed in 2% formaldehyde/0.04% glutaraldehyde in filter sterilized seawater at day 0, 2, 6, 10, 20, 27 and after 8 months. Fixed samples were subsequently dehydrated in ethanol series (15%, 30%, 50% EtOH in artificial seawater) and stored in 70% EtOH.

To obtain microscopical sections including silicate spicules, tissue samples were dehydrated in 90 and 99% EtOH, pre-infiltrated with LR-White: EtOH 1:2 and 2:1 for at least 18 h, finally embedded in LR-White (Plano W. Plannet, Wetzlar, Germany) and hardened for 20 h at 60 $^\circ$C. Sections from 500 to 20 $\mu$m were cut with a circular saw (Leica 1600), mounted in Biomount balsam (Plano) and viewed under a Zeiss axioplan microscope (Zeiss, Oberkochen, Germany).

To obtain thinner sections, the spicules were removed with 5% hydrofluoric acid, and tissue samples were embedded in paraffin after standard protocol (Romeis, 1989). Sections of 5 $\mu$m were cut with a steel knife (Microm microtome HM 340 E). After removal of the paraffin, sections were stained according to Goldner (Romeis, 1989).

Microscopical pictures were taken with a CCD Camera and processed with MetaMorph.

3. Results

3.1. Growth

The explants showed a decrease in weight the first weeks after transplantation into the cultivation tanks. However, after approximately 6 weeks the weight equalled the original weight, and within 1 year it increased by approximately 40% compared to the original weight (Fig. 2).

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Water analysis</th>
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<tbody>
<tr>
<td></td>
<td>Temperature ($^\circ$C)</td>
</tr>
<tr>
<td>Sampling site</td>
<td>7–10$^a$</td>
</tr>
<tr>
<td>Cultivation site</td>
<td>6–15</td>
</tr>
</tbody>
</table>

$^a$ Bakke and Sands (1977).
$^b$ Matthews and Sands (1973).
From batch 1, 33% of the explants were still alive after 1 year, while in batch 2 and 3, 89% and 97% respectively, died within the first 3 months (Table 2).

3.2. Cicatrisation and regeneration

During cultivation, the shape of the explants changed from cubic to spherical (Fig. 3). Already after 2 days, the canals at the surface were closed (observation by eye and microscopic control), the edges of the fragments were rounded, and the entire surface was covered by a transparent 'skin'. After 8 months, the canals within the fragments were considerably smaller in diameter (Fig. 3c), and a new cortex had developed.

Closing of the canals at the cut surface was due to initiation growth at the canal walls (Fig. 4a and b) by a growing front of motile sponge cells, followed by cells with inclusions in an otherwise cell- and bacteria-poor mesohyl. We observed the same process at canal walls in the endosome, diminishing the diameter of the canals. Torn tissue at the cut surfaces was covered by a closed line of motile sponge cells (Fig. 4c). Remodelling, defined as spontaneous disorganisation and reorganisation of the tissue (Simpson, 1984) occurred in the superficial region of the explants (ectosome), where choanocyte chambers had degenerated, and cells with inclusions accumulated (Fig. 4d). After 10–20 days, sterrasters began to accumulate in the ectosome, and after 8 months, both sterraster and euaster layers of the new cortex had developed (Fig. 5). Microscleres of all age classes in the subcortical tissue showed that at this time, the new cortex was still under construction. Broken choanosomal megascleres were incorporated in the new cortex, whereas radially arranged triaene megascleres were lacking. Subcortical spaces and ostia started to form.

Table 2
Survival with time of explants of *G. barretti* from batch 1 to 3 (in %).

<table>
<thead>
<tr>
<th>Batch</th>
<th>Day 0</th>
<th>Day 15</th>
<th>Day 45</th>
<th>Day 100</th>
<th>Day 350</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (n = 30)</td>
<td>100</td>
<td>100</td>
<td>33</td>
<td>33</td>
<td>33</td>
</tr>
<tr>
<td>2 (n = 300)</td>
<td>100</td>
<td>100</td>
<td>89</td>
<td>11</td>
<td>–</td>
</tr>
<tr>
<td>3 (n = 100)</td>
<td>100</td>
<td>95</td>
<td>5</td>
<td>3</td>
<td>–</td>
</tr>
</tbody>
</table>
3.3. Reproduction

In explants of batch 2, fixed in April 2001, both oogenesis (2/4-01, see Fig. 4e) and mature oocytes (10/04-01, Fig. 4f) could be observed. The sponges used for initiation of batch 3 (sampled in May 2001) contained numerous spermatocysts (not shown). No reproductive cells, however, could be found in samples from batch 1.

4. Discussion

4.1. Cultivation methods

In contrast to previous studies on Geodia cydonium (Müller et al., 1999), Tethya lyncurium (=Tethya aurantium Pallas) (Connes, 1966a), and Chondrosia reniformis (Bavastrello et al., 1998) our explants derived from choanosomal
Fig. 4. Histological sections of explants. (a–e) Paraffin embedding, Goldner staining, spicules removed. (f) LR-White embedding. (a) Cut surface of a 2 days old explant of *G. barretti*. A growing front of motile sponge cells (Cm) is accompanied by cells with inclusions (Ci) in an otherwise cell- and bacteria-poor mesohyl. Bar = 500 μm. (b) Detailed view of a. (c) Torn tissue at the cut surface of a 2 days old explant is covered by a closed line of motile sponge cells (Cm). Bar = 30 μm. (d) During cultivation, the (new) ectosome is remodelled: choanocyte chambers are regenerated, and cells with inclusions (Ci) accumulate (27 days old explant). Bar = 100 μm. (e) Oogenesis in cultivated explants sampled 2nd of April 2001. Bar = 30 μm. (f) Mature oocytes in cultivated explants sampled 10th of April 2001. Bar = 30 μm.
tissue regenerated into healthy sponges, while explants including old cortex failed to survive. Connes (1966a) demonstrated that choanosomal fragments of *T. lyncurium* died within a few days. The other studies only regarded ectosomal fragments. Cultivation methods which have been successfully applied to a broad range of sponges (e.g. Verdenal and Vacelet, 1985; Duckworth et al., 1999; Pronzato et al., 1999) did not succeed with *G. barretti*. These contrasting results show once more that 'there will probably never be a standard method for sponge cultivation' (Oisinga et al., 1999).

### 4.2. Growth rates

The weight increase of 40% within 1 year is lower than described for cultivation of warm-water species. Explants of the commercial bath sponge *Spongia officinalis* double their weight in about 1 year in open-sea aquacultures in the Mediterranean Sea (Verdenal and Vacelet, 1985). For *G. cydonium*, Müller et al. (1999) observed weight increases of 53 and 90% after 3 and 6 months, respectively, both in open-sea aquacultures and half-open systems in the North Adriatic Sea. The highest growth rates ever reported (5000% in 1 month for *Lissodendoryx* sp.) were found in an aquaculture in New Zealand by Battershill and Page (1996).

So far, almost nothing is known about growth rates of *G. barretti* in the field. A specimen of *G. barretti* has been observed in situ by scuba diving in the Trondheimsfjord, Mid-Norway, over a 2-year period, but no measurable change in size or shape was noticed (Rapp, personal observation).

Since weight increase starts parallel to intensive spicule production in the explants of *G. barretti*, it remains an open question how much of the increase is due to the production of organic material. However, though not measured, an increase in size of the explants was observed during the cultivation period.

### 4.3. Cicatrisation and regeneration

The role of healing and regeneration processes for the success of a sponge aquaculture is largely unknown. Few studies are available on this subject. Cicatrisation has been described in cultivated fragments of *Hippospongia communis*, *S. officinalis*, *Agelas oroides*, *Axinella damicornis* and *Petrosia ficiformis* in the Mediterranean Sea (Pronzato et al., 1999). Regenerative processes started immediately after transplantation. Within 2–3 days the sponges rebuilt their external protective layer, and after 24 h a thin, transparent cell layer covered the cut surfaces. After 1 month, the sponge fragments developed a rounded shape. In the studies on *T. lyncurium* (Connes, 1966a,b), and *C. reniformis* (Bavastrello et al., 1998), the old cortex was observed to surround the entire surface of the explant after a few weeks. Unfortunately, none of these studies related cicatrisation to growth rates. The 'presence of a cuticle' on explants of *S. officinalis* is suspected to limit further growth (Verdenal and Vacelet, 1985).

Cells with inclusions, also called spherulous cells, possess nutritive-metabolic or storage functions, and are found in a broad range of sponge species (see Simpson (1984) for review). Such cells are often connected to growth and secretion processes (Reitner and Gautret, 1996). The generally high amount of these cells in the tissue of *G. barretti* may be the reason for the good regenerative capacity of this sponge.

Regeneration of all functional units from a random piece of the choanosome requires totipotency of cells, information transfer, and orientation in the given piece of sponge tissue. Cultivation experiments with parallel histological examinations may be most enlightening for fundamental research on these still poorly understood aspects of sponge biology. It was suggested by Simpson (1984) that the formation of a highly structured, definitive cortex may completely limit further growth and thus may act as a signal for senescence. We demonstrated that tissue from an adult Geodia, though the whole sponge may have reached senescence, never looses its ability to grow and regenerate. These findings may encou-
rage starting successful aquacultures of other sponge species with pronounced cortex.

The decrease in weight during the first weeks is most probably due to remodelling of the tissue, i.e. degeneration of choanocyte chambers and torn tissue in the ectosome, and by consumption of storage material. Weight increases when cicatrisation is finished, and the new cortex starts to develop. We consider the first 1–2 months as the most sensitive phase in aquaculture initiation of *G. barretti*, and recommend to avoid any disturbance during this time.

Fig. 5. Cortex of an adult *G. barretti* (a, b) compared to the cortex of an 8-months old explant (c, d). In cultivated fragments, the sterraster (st) layer is considerably thinner, and subcortical spaces (sp) are smaller. Old choanosomal megascleres are occasionally included in the new cortex, but radial triaene megascleres are lacking. Both sterraster (st) and euaster (eu) layers are developed in the new cortex. Ostia (os) begin to form. Bar = 200 μm (a, c); bar = 50 μm (b, d).
4.4. Reproduction and seasonality

The development of egg cells shows that cultivated fragments of *G. barretti* are able to continue their reproductive cycle. This observation may be used for harvesting of egg cells to raise this species in culture, as first described by Wilson (1898). The reproductive cycle for *G. barretti* is unknown. Tetractinellid sponges are oviparous: they release eggs and sperm to the water, where free-swimming larvae develop (Bergquist, 1978). *G. cydonium* from the Mediterranean (Adriatic, Italy) is described as a gonochoristic, oviparous species with reproductive period from June to October (Liaci and Sciscioni, 1969 in Simpson, 1984). Boreal and arctic deep-water sponges usually have seasonal reproduction (Witte, 1996; Ereskovsky, 2000) with reproductive periods between February and July. We did not find any gametes in *G. barretti* sampled in March, July and August from the Korsfjord, and assume that *G. barretti* is a seasonal breeder with reproductive period in early summer (April–June). The high mortality of explants from batch 2 and 3 may imply that the reproductive period is a bad time for initiation of a sponge aquaculture. Tissue regeneration, spicule production, and sexual reproduction are energy-consuming processes, and may be difficult to perform at the same time. Fröhlich and Barthel (1997) showed seasonal variations in rates of silica uptake in *H. panicea* from the Baltic Sea. During the most intense phase of reproduction activity, female specimens showed a significant drop in their silica uptake, and obviously did not produce spicules during this time. However, more experimental data are needed to elucidate a correlation between reproductive season and cultivation success.

4.5. Aquaculture of boreal sponges?

Increasing interest in secondary metabolites from boreal and arctic sponges may cause the same ‘supply problem’ as was pointed out for tropical species (Munro et al., 1999; Pomponi, 1999; Faulkner, 2000). Boreal and arctic sponges usually live in deep waters in remote, sometimes ice-covered areas, which makes sampling even more difficult than for tropical shallow-water species. Intensive harvesting may cause serious damage in sensitive cold-water benthic ecosystems. Therefore, scientific exploration must go hand-in-hand with sustainable production of this promising marine resource. We have shown that at least for *G. barretti*, growth could be achieved during long-time cultivation in a half-open system, though low growth rates and high mortalities make the described method not profitable at present. *G. barretti*, as most cold-water Demosponges, is extremely rich in silicate spicules (Barthel, 1995). Spicule production will cost the sponge a lot of energy, which will not be available for growth and production of secondary metabolites. This energy cost and the low in situ temperature may be the reason for the low growth rates observed. However, high growth rates do not always correlate with high amounts of secondary metabolites. Battershill (1990) and Battershill et al. (1998) showed that sponges growing least well at certain locations were found to elicit the highest concentrations of metabolites of interest. They suggested that it is possible to artificially enhance the yield of target metabolite prior to harvest.

Half-open systems allow optimisation of cultivation conditions, which may help to overcome the limitations described above. For successful farming of boreal and arctic sponges, factors stimulating growth and secondary metabolite production in the target species need to be specified.

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