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Rearing cuttings of the soft coral *Sarcophyton glaucum* (Octocorallia, Alcyonacea): towards mass production in a closed seawater system

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Abstract

The octcoral *Sarcophyton glaucum* has a wide Indo-Pacific distribution and is known for its diverse content of natural products. The aim of the current study was to establish a protocol for rearing miniature cuttings of *S. glaucum* in a closed seawater system. In order to determine the optimal conditions for rearing, the survival, average dry weight, percentage of organic weight and development of the cuttings were monitored under different temperature, light, salinity and feeding regimes. At 26 °C, the highest dry weight was obtained, and at 20 °C, the highest percentage of organic weight. The dry weight of the cuttings increased with the light intensity, while under 35–130 $\mu E\, m^{-2}\, s^{-1}$, survival was high. Salinity did not affect any of the colonies' features. Feeding intervals of 7 and 30 days yielded a better result than of 2 days. A comparison of the colonies derived from the closed system with the colonies reared in a flow-through system, those reared in the sea and with field-collected colonies revealed the importance of environmental conditions in determining the features of the colonies. The study emphasizes the advantages of a closed seawater system in controlling the conditions needed for rearing cuttings of *S. glaucum* for targeted farming.

Keywords: soft corals, closed seawater system, *Sarcophyton glaucum*, coral farming, Red-Sea

Introduction

A large variety of reef invertebrates, including soft corals (Octocorallia), have long been used as a source for diverse natural products with pharmaceutical or cosmetic value (e.g., Blunt, Copp, Munro, Northcote & Prinsep 2005; Slattery, Gochfeld & Kamel 2005; Sipkema, Osinga, Schatton, Mendola, Tramper & Wijffels 2005), as well as for the reef-aquarium trade (Wabnitz, Taylor, Grenn & Razak 2003). The increased demand for these organisms has led to their massive harvesting (Castanaro & Lasker 2003) and has raised the need for efficient farming methodologies (Ellis & Ellis 2002; Mendola 2003).

Coral propagation has been commonly used for the production of daughter colonies, rather than harvesting naturally grown ones (e.g., Soong & Chen 2003; Fox, Mous, Pet, Muljadi & Caldwell 2005). This practice has been based on the ability of corals to reproduce asexually (e.g, Delbeek 2001; Ellis & Ellis 2002). Captive-grown colonies, or field-collected ones, are used for the production of cuttings or fragments. The latter are usually glued or attached by various means to bases or stubs, until natural attachment is achieved (Delbeek 2001).

Over the last decade, coral propagation in closed artificial seawater systems has been commonly used, making the process more simple and cost effective (Borneman & Lowrie 2001; http://www.reefkeeping.com). The advantage of these systems lies in their ability to control abiotic and biotic parameters (Wheeler 1996; Borneman & Lowrie 2001; Abramovitch-Gottlib, Katoshevski & Vago 2002). Such systems have been proposed as a source by which to obtain corals and other invertebrates with commercial value (e.g., Wabnitz *et al.* 2003; Sipkema *et al.* 2005), as well as for restoration and conservation purposes (Becker & Mueller 1999; Petersen, Laterveer, Van Bergen, Hatta, Hebbinghaus, Janse, Jones,

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Richter, Ziegler, Visser & Schuhmacher 2006; Okuzawa, Maliao, Quinitio, Buen-ursua, Lebata, Gallardo, Garcia & Primavera 2008).

Species of the soft coral genus *Sarcophyton* (Octocorallia) have a wide Indo-Pacific distribution and are known for the diverse content of their natural products (e.g., Look, Fenical, Matsumoto & Clardy 1986; Tanaka, Yoshida & Benayahu 2005; Nguyen, Tran, Phan, Chau, Eun & Young 2008). *S. glaucum* (Quoy & Gaimard, 1833) is the most common species of the genus, also on the northern Red Sea reefs, where it was found to be a dioecious broadcaster with the onset of reproduction at the age of 6–10 years (Benayahu & Loya 1986). *S. glaucum* is zooxanthellate and extremely rich in natural products, which have been studied extensively (e.g., Fridkovsky, Rudi, Benayahu, Kashman & Schleyer 1996; Badria, Guirguis, Perovic, Steffen, Muller & Schroder 1998; Tanaka *et al.* 2005).

The aim of the current study was to establish the scientific ground for a protocol suitable for rearing miniature cuttings of *S. glaucum* in a closed seawater system. Our objectives were to study the effect of temperature, light intensity, salinity and feeding regimes on the survival, average dry weight, percentage of organic weight and development of the cuttings. In addition, colonies reared in a closed seawater system were compared with those reared in a flow-through seawater system in the sea and with field-collected ones.

Materials and methods

In a preliminary work, we examined attachment of the *S. glaucum* cuttings to stubs made of ceramic and plastic materials, using a cyanoacrylate adhesive (3M) and rubber bands. We also examined the minimal size of cuttings yielding the highest survivorship (10–400 mm^2) (Sella 2007). Consequently, in the current study, we decided to use cuttings measuring 36 mm^2 (6 × 6 mm) with an average dry weight of 0.0077 ± 0.0024 g and 8.864 ± 2.461 average percentage of organic weight ($n = 30$ random cuttings from colonies used in the experiments; see 'Collection of colonies, preparation of cuttings and experimental setup'). The cuttings were glued using a cyanoacrylate adhesive to cylindrical ceramic (Fig. 1a) stubs that we produced ourselves (clay WBB Fuchfs GmbH & Co R2502, burnt at 1200 °C for 12 h) at a cost of ~4 US cents (without labour). The stubs measured 5 cm in length and 1 cm in diameter, with a hexagonal indentation (ca. 3 mm deep and 7–8 mm wide) at one end, into which the cuttings were attached.

Experimental closed seawater system

We constructed a closed seawater system at Tel Aviv University (TAU). The system comprised three 1 m^3 PVC tanks, each filled with artificial seawater (Red Sea salt©) and 200 kg of live rocks obtained from Eilat (Gulf of Aqaba, northern Red Sea). Each tank was provided with 40 *Trochos dentatus* snails for algal grazing. Every 10 days, their faeces were removed from the bottom of the tanks, together with ca. 5% of the water volume, which was then replaced. A set of 24 glass aquaria (30 L each) was connected to the tanks by 26-mm-diameter pipes. The aquaria were illuminated by neon bulbs (22 W, 400–800 nm, JEBO daylight, 12 L:12 D, 35 $\mu E\ m^{-2}\ s^{-1}$ on the colony surface). The water circulated from the aquaria to the tanks, which were equipped with protein skimmers (JEBO 3500), a chiller (JEBO 2000, Zhongshan Zhenhua Aquarium Accessories, Zhongshan, China) and a 5000 L per hour circulation pump (JEBO). The water temperature in each aquarium was controlled using an individual heater (60 W). Salinity, temperature and pH were monitored daily, and the nutrient levels weekly (nitrite < 0.05 ppm, nitrate < 10 ppm, ammonia 0 ppm and 8.1–8.3 pH) (see Delbeek 2001).

Collection of colonies, preparation of cuttings and experimental set-up

Colonies of *S. glaucum* with a polypary diameter of 5–7 cm (the polyp-bearing part of the colony) were collected by SCUBA from three reef sites in Eilat (5–7 m, August 2004–January 2007). For each experiment, we collected 10–15 colonies, growing at least 5 m apart in order to avoid the use of asexually produced colonies (Benayahu & Loya 1986). In order to prevent possible bias in the study, only colonies that did not contain gonads were used for formation of the cuttings (see Okubo, Motokawa & Omori 2007). At the Interuniversity Institute for Marine Science (IUI), the colonies were placed in a flow-through seawater system for 3 days and all epibiotic organisms were removed. The colonies were flown to TAU in plastic containers filled with seawater and placed in thermally isolated boxes. They were then maintained in the experimental system for a 1-month quarantine period, under conditions resembling the average ambient Eilat ones: salinity 40 ppt, pH 8.2 and 26 °C (IUI Data Base, http://www.iui-eilat.ac.il/NMP/Default.aspx).

For preparation of the cuttings, the colonies of *S. glaucum* were handled with latex gloves. The perimeter of the polypary was removed using a scalpel

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(see section 'General observations' ahead), and the remaining part was then kept for future regeneration (Sella 2007). The perimeter of the polypary was thoroughly rinsed with artificial seawater for removal of mucus and cellular debris, and 36 mm^2 (6×6 mm) cuttings were prepared using autoclaved scissors, scalpels and forceps and paper blotted. An adhesive was applied to the cuttings' lower epidermal surface, and they were mounted on the stubs and kept in the air for 30 s to dry. Notably, the cuttings retrieved for the different treatments of each experiment were randomly derived from the same parent colonies and therefore represented similar genotypes. The stubs were then inserted into test tube stands (Fig. 1a), and kept in artificial seawater for 20 min in order to wash away any adhesive residue before introduction into the aquaria. During the following week, if mortality did not exceed 15%, dead cuttings were replaced with new ones and only then the 60-day experiments were started; otherwise, all cuttings were discarded and a new experiment was set up as it occurred once. In all the experiments, each treatment comprised three aquaria and a control, which was maintained under Eilat average environmental conditions. Unless stated otherwise, the aquaria were supplied every 25 days with 5000 nauplii of *Artemia salina* (Sorgeloos & Persoone 1975; Sella 2007).

Development and transformation of the cuttings into small colonies with a stalk and a polypary was monitored weekly by digital photography and their survival was calculated at the end of the experiments (day 60). The cuttings were then removed with a scalpel from the stubs and placed individually in aluminium dishes (5 cm in diameter). Their dry weight was determined by drying (Binder oven, 60 °C) for 24 h and then weighing to an accuracy of four decimal points (Mettler balance, Toledo AB54-S, Mettler-Toledo, Columbus, OH, USA). Subsequently, they were burnt (Carbolite furnace, 450 °C) for 6 h and their inorganic weight was determined. The organic weight of each cutting was calculated by subtracting the inorganic from the dry weight, and the result was used to calculate the percentage of organic weight of each cutting. The stubs were reused after burning (800 °C, 4 h) for removal of fouled material.

Effect of environmental parameters

In order to determine the effect of temperature on the cuttings of *S. glaucum*, they were reared at 20, 23, 26 and 29 °C, falling within the range representing Eilat annual seawater temperatures $\pm$ 1 °C (IUI Data Base, http://www.iui-eilat.ac.il) (10–15 cuttings per aquaria: March–May 2005). Seawater at 20 °C was circulated from the tanks to all the aquaria. The 23, 26 and 29 °C seawater temperatures were obtained using 60–300 W heaters with thermostats. The temperature was routinely monitored using an alcohol thermometer.

To determine the effect of illumination on the cuttings, they were reared at light intensities of 20, 35, 130 and 250 μE ($\mu E\ m^{-2}\ s^{-1}$), as measured on their surface, falling within the prevailing Eilat values along the depth gradient (Winters, Loya & Beer 2006) (20–30 cuttings per aquaria: December 2005–January 2006). In order to supply 20 μE, a PVC filter (PLASON UV+) was placed between the light source and the surface of the respective aquaria, while for 130 and 250 μE, one or two additional neon bulbs were added. Light intensity was monitored daily using an underwater-light photometer (Bio-sciences, Light 250L Li-CO).

To determine the effect of salinity on the cuttings, the aquaria were disconnected from the tanks. The cuttings were reared under salinities of 30, 34, 37 and 40 ppt, representing the salinity within the geographic distribution of the species (Levitus 1982) (15–25 cuttings per aquaria: February–March, 2006). Each aquarium had a 60 W heater with a thermostat, a filter (JEBO Eco) and an air stone. Water from the 1 m^3 tanks (40 ppt) was diluted with distilled water in order to obtain the lower salinities. Ten litres of seawater was changed daily in each aquarium and the salinity was monitored using a light refractometer and an electronic conductivity meter (MRC Salinity 1, Lutron YK-315A, Lutron Electronic Enterprise, Taipei, Taiwan).

To determine the effect of feeding, cuttings were fed at intervals of 2, 7 and 30 days, where a 2-day interval is commonly used by aquarists (http://www.reefkeeping.com) (12–20 cuttings per aquaria: April–May 2006). Five thousand nauplii were supplied to each aquarium for each feeding episode, which lasted 3 h, and was terminated by replacing the water.

Organic weight in field-collected colonies

To examine the percentage of organic weight in *S. glaucum* colonies of different sizes, samples were removed from colonies of three polypary diameters: 5–7, 10–15 and 20–30 cm ($n = 6$ for each size group: August 2006). Each sample constituted a longitudinal section comprising ca. 10% of each colony, and included respective portions of the polypary and the stalk. Their dry weight, inorganic weight and percentage of organic weight were determined.

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Comparison between cuttings reared in the reef, flow-through seawater system and closed seawater system

Fifteen colonies of *S. glaucum* were collected from the IUI reef and placed in a flow-through seawater system at the IUI for 3 days for acclimatization (November 2006). Following this, 320 mounted cuttings were produced, divided into three groups and reared in three settings. The first setting consisted of 90 cuttings placed on a table-like structure (galvanized steel, 220 × 80 × 80 cm) at the IUI reef (5 m depth). The second setting consisted of 110 cuttings placed in three flow-through seawater tanks at the IUI with 20 complete water changes per 24 h (each tank 80 × 80 × 40 cm, 35–38 cuttings per tank) and shaded by a net reducing 40% sun radiation. For the third setting, 120 cuttings were placed in the TAU closed seawater system (six aquaria, 20 cuttings per aquaria). Based on the results obtained from the environmental parameter experiments (see 'Results'), the following conditions were chosen for the latter setting: 26 °C, 250 μE and weekly feeding intervals with 5000 nauplii as well as Eilat salinity of 40 ppt. The cuttings were monitored weekly and their survivoral was determined at the end of the experiment (60 days, January 2007). Fifty cuttings from each setting were then randomly retrieved and their dry, inorganic and percentage of organic weight were determined.

Statistics

In each experiment, results from aquaria of the same treatment were tested using one-way ANOVA, and because no significant differences were found, the results were pooled. Between treatments, the average dry weight and percentage of organic weight were tested using one-way ANOVA and *post hoc* Tukey test. Log and arcsinus transformations were used to normalize distribution when needed. Survival of the cuttings at the end of each experiment was tested by χ^2. The deviation around means is presented by standard error.

Results

During the experiments, the miniature cuttings of *S. glaucum* regenerated and acquired the structure of naturally growing juvenile colonies with a mushroom-shaped morphology (Fig. 1). Between days 5 and 18 of the experiments, the rectangular cuttings

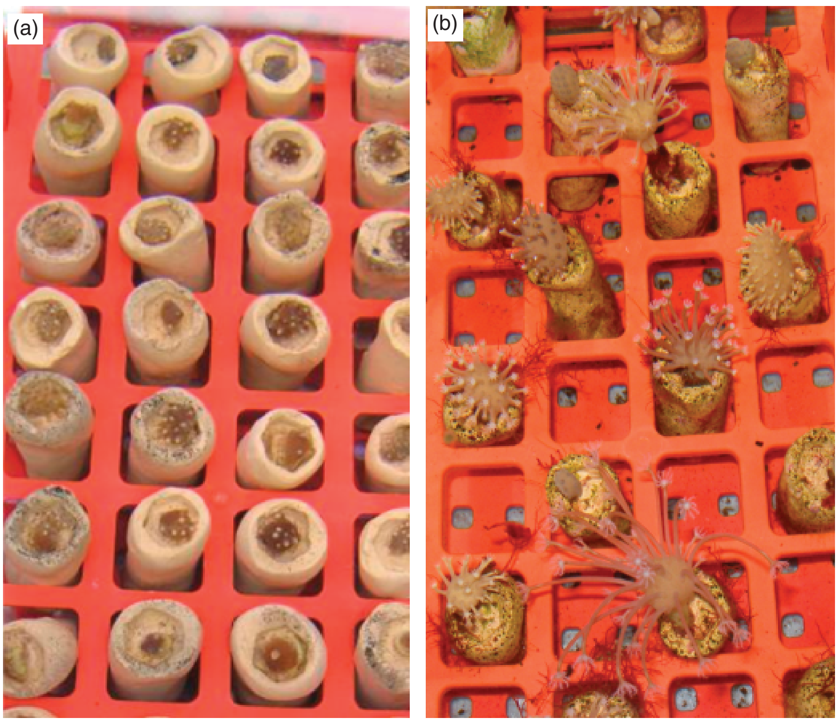

Figure 1 Rearing cuttings of *Sarcophyton glaucum* in a closed seawater system: (a) cuttings of 36 mm^2 attached to ceramic stubs by a cyanoacrylate adhesive, and placed in PVC test tube holders at day one of the experiment and (b) cuttings at day 60.

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became rounded and natural attachment occurred. A distinct stalk then developed and by days 30–37, the colonies had attained the typical *S. glaucum* shape.

Table 1 Survivorship of *Sarcophyton glaucum* cuttings in a closed seawater system under different environmental parameters and in different settings on day 60 of the experiments

Treatments	Initial # of cuttings	% survivorship on day 60
Environmental parameters		
Temperature (°C)		
20	18	66 (12)
23	35	65 (23)
26	42	88 (37)
29	35	88 (31)
Light (μE)		
20	65	62 (42)
35	65	89 (58)
130	80	94 (75)
250	65	62 (42)
Salinity (ppt)		
30	40	80 (32)
34	42	78 (33)
37	40	100 (40)
40	40	67 (27)
Feeding intervals (days)		
2	60	25 (15)
7	43	44 (19)
30	36	44 (16)
Settings		
Reef	90	96 (87)
Flow-through	110	69 (76)
Closed system	120	96 (116)

Number of cuttings on day 60 is given in parentheses.

Effect of environmental parameters

There were significant differences in the average dry weight and percentage of organic weight of *S. glaucum* cuttings reared under the tested temperatures (Fig. 2, one-way ANOVA, $P<0.05$). The average dry weight of cuttings at 26 °C was significantly higher than those at 23, 20 and 29 °C (Tukey test, $P<0.05$). Cuttings reared at 20 °C showed higher average percentage of organic weight than those at 23, 26 and 29 °C (Tukey test, $P<0.05$), while no significant differences were noted among the other temperatures ($P>0.05$). The survival of cuttings at the end of this experiment ranged between 65% and 88%. There were no significant differences in the survival of the cuttings among the tested temperatures (Table 1, χ^2 0.05, d.f. 3, $P>0.05$).

Significant differences in the average dry weight of the cuttings were found among the different light

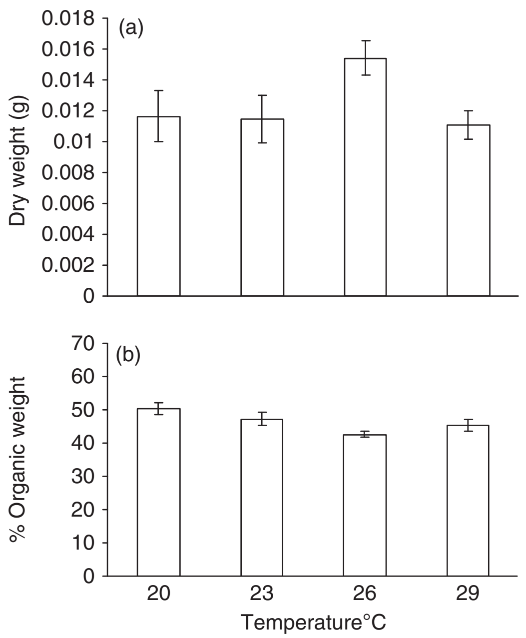

Figure 2 Rearing cuttings of *Sarcophyton glaucum* in a closed seawater system: (a) average dry weight (g) and (b) average percentage of organic weight at different temperatures on day 60 of the experiment (± SE, number of cuttings indicated in Table 1).

intensities tested (Fig. 3a, one-way ANOVA, $P<0.05$). Under the highest intensity of 250 μE, the dry weight was significantly higher compared with all the other treatments, and under 20 μE, it was the lowest (Tukey test, $P<0.05$). The average percentage of organic weight significantly differed among treatments (Fig. 3b, one-way ANOVA, $P<0.05$). Cuttings reared under a light intensity of 130 μE had a significantly higher average percentage of organic weight than in all the other treatments (Tukey, test $P<0.05$), while under 250 μE, it was the lowest (Tukey test, $P>0.05$). The survival of cuttings at the end of the experiment ranged between 62% and 94% (Table 1). Under both the lowest (20 μE) and the highest (250 μE) light intensity tested, a significantly lower survival was obtained than under the intermediate intensities (35 and 130 μE) (χ^2 0.05, d.f. 4, $P<0.05$).

No significant differences were found in the average dry weight (Fig. 4a) and average percentage of organic weight (Fig. 4b) in cuttings reared under the different salinities (one-way ANOVA, $P>0.05$). The survival of cuttings at the end of this experiment ranged

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between 67% and 100%. There were no significant differences in the survival of the cuttings under the different salinities (Table 1, χ^2 0.05, d.f. 3, $P>0.05$). Natural attachment of the cuttings did not occur simultaneously and was observed during days 5 and 6.

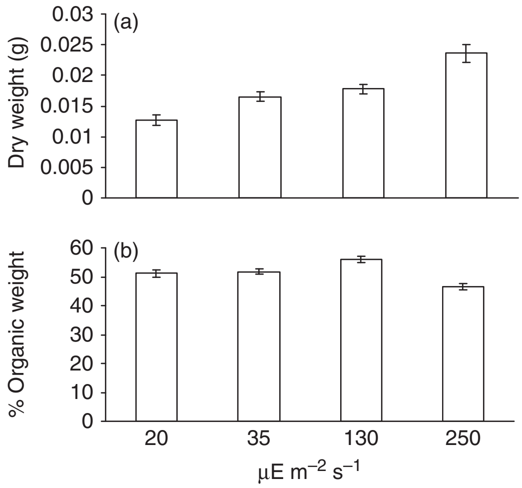

Figure 3 Rearing cuttings of *Sarcophyton glaucum* in a closed seawater system: (a) average dry weight (g) and (b) average percentage of organic weight under different light intensities on day 60 of the experiment (± SE, number of cuttings indicated in Table 1).

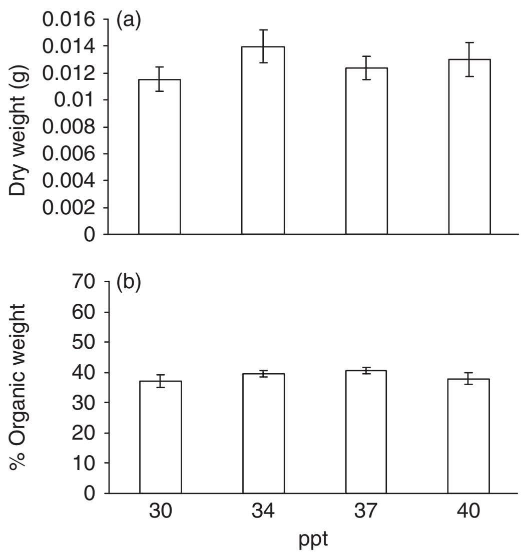

Figure 4 Rearing cuttings of *Sarcophyton glaucum* in a closed seawater system: (a) average dry weight (g) and (b) average percentage of organic weight under different salinities on day 60 of the experiment (± SE, number of cuttings indicated in Table 1).

There were no significant differences in the average dry weight of cuttings obtained from the different feeding intervals (Fig. 5a, one-way ANOVA, $P>0.05$), whereas the average percentage of their organic weight did significantly differ among them (Fig. 5b, one-way ANOVA, $P<0.05$). Under the two-day feeding interval, there was a significantly lower average percentage of organic weight than under the 7- and 30-day intervals (Tukey test, $P<0.05$). There was a similar average percentage of organic weight in cuttings reared under the latter two intervals (one-way ANOVA, $P>0.05$). Survival of cuttings at the end of the experiment ranged between 25% (2-day feeding interval) and 44% (7- and 30-day intervals) (Table 1, χ^2 0.05, d.f. 3, $P>0.05$) and was lower than that in all the other experiments (Table 1).

Organic weight in field-collected colonies

Significant differences were found in the average percentage of organic weight among the *S. glaucum* colonies of different size groups (Fig. 6, one-way ANOVA, $P<0.05$). The smallest colonies had a lower percentage of organic weight compared with the larger ones (Tukey test, $P<0.05$).

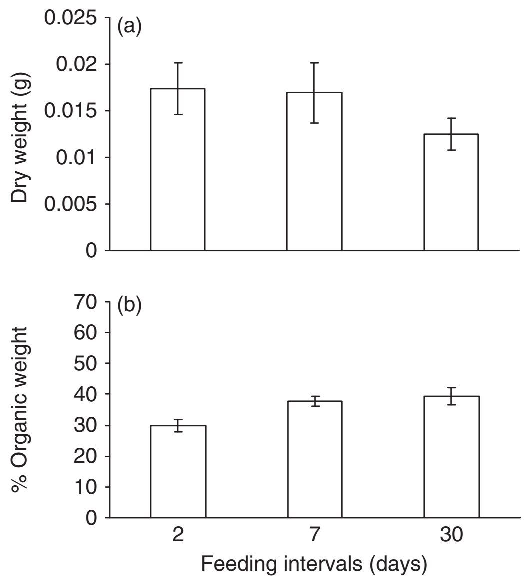

Figure 5 Rearing cuttings of *Sarcophyton glaucum* in a closed seawater system: (a) average dry weight (g) and (b) average percentage of organic weight under different feeding regimes on day 60 of the experiment (± SE, number of cuttings indicated in Table 1).

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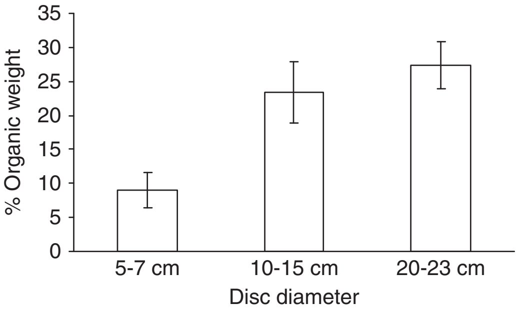

Figure 6 Samples removed from reef-collected *Sarcophyton glaucum* colonies of different size classes (disc diameter) average percentage of organic weight (± SD, $n = 6$ samples for each size group).

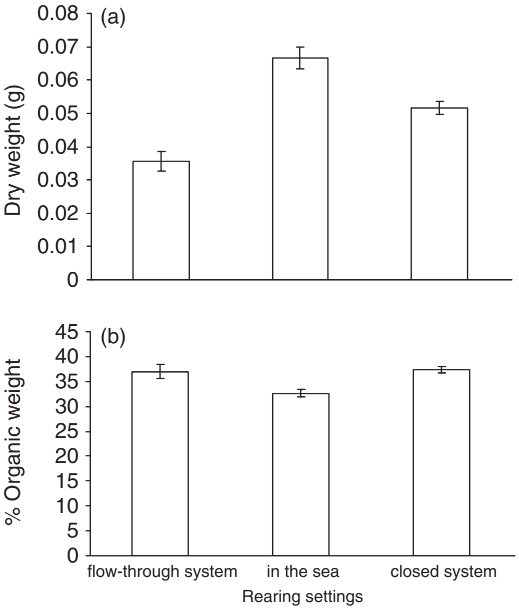

Figure 7 Rearing of cuttings of *Sarcophyton glaucum* in a closed seawater system, flow-through seawater system and in the sea (a) average dry weight (g) and (b) average percentage of organic weight on day 60 of the experiments (± SE, $n = 50$ colonies for each group).

Comparison between cuttings reared in the reef, flow-through seawater system and closed seawater system

Significant differences were found in the average dry weight of *S. glaucum* cuttings among the three settings (Fig. 7a, one-way ANOVA, $P < 0.01$). The average dry weight of cuttings reared in the reef was the highest, while that of those reared in the flow-through seawater system was the lowest (Tukey test, $P < 0.01$). The average percentage of organic weight of the cuttings significantly differed among the three settings (one-way ANOVA, $P < 0.01$). Those reared in the reef had a lower average percentage of organic weight than in the other two systems (Fig. 7b, Tukey test, $P < 0.01$). A similar average percentage of organic weight was obtained in cuttings reared in the flow-through system and the closed system ($P > 0.05$). Survival of cuttings at the end of this experiment ranged between 69% and 96%. Both cuttings reared in the reef and in the closed seawater system had a significantly higher survival rate than those reared in the flow-through system (Table 1, χ^2 0.05, d.f. 2, $P < 0.05$).

General observations

During the course of preliminary work, we noted that cuttings derived from the periphery of the polypary survived better than those taken from its inner part. Although the cuttings from both areas were similar in size (36 mm^2), the former had more polyps per unit area. We noted that cuttings that consisted of > 5 polyps survived better than those with fewer polyps. It is interesting to note that in all the experiments, except the salinity one, natural attachment of cuttings was synchronized among the aquaria over a 12-h period that took place during days 14–16. In the salinity experiment, natural attachment of the cuttings already started on day 5 and ended on day 8. Low water temperature (20°–23 °C) during the first 4–5 days of the experiment minimized necrosis among the cuttings. Foraging of the *T. dentaus* snails larger than 1 cm disturbed the freshly introduced cuttings and prevented their attachment, and therefore only small snails were used. Nonetheless, after natural attachment of the cuttings had taken place, larger snails were successfully used.

Cuttings reared in the reef achieved a hemispheric polypary, short polyps and were more rigid than those in the closed system. The cuttings reared in the closed system were characterized by a soft flattened polypary, long polyps and an elongated stalk. Those reared in the flow-through seawater system developed both the above-mentioned morphologies, with some intermediate shapes.

Discussion

The current study examined the practice and conditions required in order to develop a protocol for rearing miniature cuttings removed from colonies of *S. glaucum* in a closed seawater system. We compared the

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derived colonies with those reared in a flow-through seawater system, with those reared in the seas and with field-collected colonies. The ability of these cuttings to successfully regenerate and form juvenile colonies was facilitated by examining the effect of various environmental parameters on their development.

The ceramic stubs enabled the natural attachment of the cuttings and their further development (see Sella 2007). The durability of clay (ceramic) in the sea is well known from archaeological findings (Haldane 1993), and its malleability, low cost, tendency to be fouled and to support coral growth (see Baine 2001) made it suitable for the purpose of the present study. Ceramic can endure high temperatures and therefore can be sterilized and reused. The current study is the first to use clay-made stubs for rearing soft coral cuttings, and to suggest clay as a suitable material for large-scale soft coral farming.

Effect of environmental parameters

Notably, there were significant differences in the average dry weight and percentage of organic weight of *S. glaucum* cuttings reared under the different temperatures (Fig. 2a). Cuttings reared at 26 °C showed the highest average dry weight while cuttings at 20 °C showed the highest percentage of organic weight (Fig. 2b). Many studies have indicated that the calcification rate in corals is enhanced with increased temperature (Carricart-Ganivet 2004). Thus, it is suggested that the differences in the average dry weight between treatments are a result of different calcification rates. In addition, it seems that at low temperature, there is some kind of a trade-off, as calcification decreased, and the percentage of organic weight increased (see also Rodolfo-Metalpa, Richard, Allemand & Ferrier-Pages 2006). This finding is valuable for the farming of soft corals for natural product purposes, which requires maximizing the yield of organic weight. We also noted that, at the low water temperature, necrosis of the cuttings took place only during the first seven days of the experiment and was minimal compared with all the other experiments, most probably due to inhibition of bacterial spread (Bourne, Lone, Webster, Swan & Hall 2006). It is suggested that the use of anti microbial methods, such as an Ozone reactor or UV, may further decrease necrosis in the system (Delbeek 2001).

S. glaucum cuttings reared under the highest light intensity (250 μE) had a significantly higher dry weight than at all the other light intensities tested, but the lowest percentage of organic weight (Fig. 3). These results further indicate the trade-off between calcification rate and percentage of organic weight of the cuttings. At a high light intensity, photosynthesis increased and enhanced calcification (e.g., Allemand, Tambutte, Girard & Jaubert 1998; Tentori & Allemand 2006), yielding cuttings with higher dry weight and lower percentage of organic weight. Cuttings reared under intermediate light intensities (35–130 μE) had considerably higher survival than those reared under the extreme intensities. It can be assumed that photo-inhibition at the high light intensity (see Smith, Suggett & Baker 2005) and photo-adaptation at the low intensity (see Muller-Parker & D'Elia 1997) caused high mortality during the first 14 days of the experiment, and that only those cuttings that managed to adapt to these intensities survived. We suggest that gradual light acclimatization should be performed in order to increase survival.

Salinity did not affect the survival, average dry weight or organic weight of the *S. glaucum* cuttings. This finding is economically important, because salt is considered to constitute a significant expense for coral farming in closed seawater systems. (Borneman & Lowrie 2001; Calfo 2003). Interestingly, in this experiment, natural attachment of the cuttings was not synchronized and occurred earlier compared with the other experiments (see 'Results'). Notably, in the salinity experiment, the cuttings were reared in 30 L aquaria that were disconnected from the 1 m^3 tanks. Therefore, we suggest that by maintaining the cuttings in a small water volume, natural attachment was achieved earlier than in the other experiments of this study, possibly due to a particular substance released by the cuttings, whose greater concentration in the water was consequently more effective. Benthic marine organisms such as mollusks, echinoderms and arthropods synchronized processes such as courtship and mating, aggregation behaviour and habitat selection through the secretion of certain chemicals (Zimmer & Butman 2000). Undoubtedly, future studies are still needed in order to verify our suggestion, which has obvious advantages for commercial coral farming.

The cuttings of *S. glaucum* that were fed most frequently (every 2 days) had the lowest percentage of organic weight while those fed every 7 or 30 days had the highest one. The digestion process of *A. salina* nauplii by *Sarcophyton* sp. may take up to 24 h (Lewis 1982). We suggest that the time required for digestion may determine the rate of food capture. Furthermore, the low number of polyps of the developed small

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colonies limited their food-capturing abilities. Therefore, frequent feeding does not necessarily benefit the cuttings and may even harm them. The higher mortality of cuttings in this experiment can be explained as a result of the introduction of microbial fauna associated with the *A. salina* nauplii, and the decomposition of organic debris in the aquaria, as the water change after each feeding episode did not completely remove debris from the colonies. It is suggested that the use of decapsulating methods for the *A. salina* cysts (Lim, Cho, Dhert, Wong, Nelis & Sorgeloos 2002), with anti-microbial water filtration as indicated above, may decrease mortality. Venn, Loram and Douglas (2008) indicated that a large number of soft corals possess a high density of zooxanthellae in their tissues and obtain most of their energetic requirements from photosynthetic assimilates. It seems that plankton feeding plays only a partial role in the energy demands of the cuttings. Minimizing food quantity and optimizing its sterility can increase survival and development of the cuttings. This finding has practical implications as live food is a costly component in aqua farming (Kyewalyanga 2003).

Organic weight in field-collected colonies

The percentage of organic weight in all three size groups of *S. glaucum* colonies was significantly lower compared with those reared in the closed seawater system. Interestingly, the lowest percentage of organic weight (10%) was found in the small-size group (5–7 cm polypary diameter), which was used for the preparation of the cuttings. At the end of the different experiments conducted in the closed system, the resulting colonies had three to four times more organic weight (Fig. 6). Further studies are still needed in order to determine the percentage of organic weight in colonies reared in a closed seawater system beyond the 60-day period studied by us. It is likely that the large reef colonies might possess more calcareous sclerites, which are required to contend with conditions of strong water currents, wave action and predation pressure, as was found for the octocorals *Junceella fragilis* (see Chang, Chi, Fan, & Dai 2007) and *Renilla muelleri* (see Clavico, De Souza, Da Gama & Pereira 2007). We suggest that colonies of *S. glaucum* quickly adapt to a new suite of environmental conditions by changing their relative organic vs. inorganic weight. This finding may also explain the wide distribution of this species in a variety of reef habitats (Benayahu & Loya 1986; Tanaka *et al.* 2005).

Comparison between cuttings reared in the reef environment, closed and flow-through seawater systems

This comparison revealed that environmental conditions determine the percentage of organic weight and morphology of *S. glaucum* colonies. Because the cuttings used for each rearing system were derived from the same parent colonies and were genetically identical (see 'Materials and methods'), those reared in the closed seawater system revealed a higher percentage of organic weight than those in the reef, possessed long polyps, a long stalk and a flat polypary and differed from those in the reef. These findings provide further evidence that the conditions in the closed rearing system can determine the particular features of the developed cuttings, as desired for the reef aquaria trade or for the extraction of natural products (large and robust colonies vs. high percentage of organic weight).

We noted that cuttings derived from the perimeter of the polypary survived better than those taken from its inner part (Sella 2007). Although all cuttings were of the same size, the former possessed more polyps per unit area. Oren, Benayahu and Loya (1997) found that lesions in the stony coral *Favia favus* with a relatively long perimeter obtained a higher energetic allocation from the colony, probably due to the larger colony portion associated with their recovery. In addition, regeneration from injury in corals was found to be faster when more tissue was available to supply the damaged area (Henry & Hart 2005). It is suggested, therefore, that the cuttings from the periphery of the colonies supplied the energy needed for the regeneration and growth of these cuttings, either by photosynthesis of zooxanthellae harvested in the polyps (Muller-Parker & DiElia 1997) or by capture of zooplankton (Lewis 1982).

Conclusions

The results of the current study emphasize the advantages of a closed seawater system for rearing cuttings of *S. glaucum* for various purposes. Our findings indicate those conditions of the rearing system that will determine the desired particular features of the developed cuttings, as required for purposes such as the reef aquaria trade and restoration or for the extraction of natural products (large and robust colonies vs. colonies with a high percentage of organic weight).

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Throughout the entire rearing period, water quality must be maintained, with the following nutrient concentration values : NO_2 0–0.05 ppm, NO_3 0–10 ppm, NH_3 0 ppm and under a pH of 8.2. During the first week, the cuttings should be maintained at 20 °C, light intensities of 35–130 μE (400–800 nm), salinity of 30–37 ppt and no feeding. Water circulation in the rearing aquaria should be moderate in order to avoid detachment of the cuttings from the stubs. A routine removal of tissue debris and decayed cuttings from the aquaria during the first week is important in order to reduce the spread of necrosis to other cuttings. After the first week, it is possible to adjust the rearing conditions for the different purposes. For the aquarium trade and restoration purposes, cuttings should be maintained at 26–29 °C, a light intensity of 250 μE and salinity of 30–37 ppt. Weekly feeding by *A. salina* nauplii should be conducted. For natural products' oriented rearing, cuttings should be maintained at 20–23 °C, a light intensity of 130 μE, salinity of 30–37 ppt and a feeding regime as stated above.

The ability to control the environmental conditions undoubtedly makes such a system an efficient means for targeted soft coral farming. Our study has established the scientific ground for a protocol suitable for large-scale soft coral farming, and presents a platform for future joint endeavours by researchers and the commercial sector engaged in coral farming.

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