Sponge Aquaculture Trials in the East-Mediterranean Sea: New Approaches to Earlier Ideas

Ronald Osinga[*,1,2], Marzia Sidri[1], Erdener Cerig[3], Suha Z. Gokalp[4] and Mert Gokalp[4]

[1]*Porifarma BV, Poelbos 3, 6718 HT Ede, The Netherlands*

[2]*Wageningen University, Aquaculture & Fisheries, P.O. Box 338, 6700 AH Wageningen, The Netherlands*

[3]*Labrax Fish Farm, Fırkateyn Bodrum, Turkey*

[4]*GPO Sponge Culture, Aklaryar, Turkey*

Abstract: Aquaculture trials were conducted in the East Aegean Sea with *Dysidea avara* and *Chondrosia reniformis* to test the possibility of growing these sponges in the vicinity of sea-based fish farms. Culturing sponges in the vicinity of fish farms may have two benefits: the sponges may grow faster due to an increased availability of organic food and the pollution caused by the fish farms is remediated by the filtering activities of the sponges. An initial trial was conducted to compare growth of the two sponge species under floating fish cages to growth in a natural, pristine environment. Explants of *D. avara* were grown suspended on nylon threads, explants of *C. reniformis* were grown in cages constructed of stainless steel. After being one year in culture, nearly 100% of all explants of *D. avara* survived. Growth was highest underneath the fish cages, but growth rates were low compared to earlier studies. For *C. reniformis* survival at the pristine site was 100%, and growth was estimated at 800% per year. All explants cultured underneath the fish cages died due to smothering with sediment. After the initial trial, a new, cost-saving and growth promoting method for *D. avara* was tested at the fish farm location. Explants were grown on PVC pins that were mounted into a metal frame. Growth of the sponges on the pins was eight times faster than that of sponges growing on threads. We conclude that culturing *D. avara* under floating fish cages is feasible when using the new methodology.

Keywords: Sponges, aquaculture, dysidea avara, chondrosia reniformis.

INTRODUCTION

Sponge culture in the Mediterranean started already more than two centuries ago with the early work of Cavolini [1], who made the first attempt to aquaculture natural bath sponges. Since then, many studies have been conducted to develop suitable methods for sponge culture, both in the Mediterranean [e.g. 2-7] and beyond [e.g. 8-13]. The interest in sponge culture increased due to the discovery of many natural products in sponges: the focus shifted from production of natural bath sponges to sustainable supply of marine natural products.

In the late nineties of the last century, the idea was developed to combine the production of sponges with remediation of pollution, particularly pollution caused by sea-based fish farming [14,15]. Sponges are very efficient filter feeders. Hence, a large-scale sponge culture may have a profound effect on the water quality in the vicinity of fish farms. Conversely, the organic enrichment originating from the fish may stimulate sponge growth, thus making sponge aquaculture more efficient.

Although the idea of integrated sponge/fish aquaculture has been discussed in several papers [3, 16-19], until now it has not been applied on a commercial scale. Here, we describe preliminary attempts to combine the mariculture of fish (sea bass) with the culture of two Mediterranean Demospongiae species: *Dysidea avara* (Schmidt, 1862) and *Chondrosia reniformis* (Nardo, 1847). Both species have potential commercial interest. *D. avara* is known for its secondary metabolite avarol [20], which has been in clinical trials as a potential HIV inhibitor [21]. Later, it was discovered that avarol can also be effective against skin diseases such as psoriasis [22]. *C. reniformis* is of interest as a producer of collagen, particularly collagen for cosmetic and biomedical applications [23]. Since both avarol and collagen are produced in relatively large quantities by the sponges, aquaculture appears to be a feasible alternative for production of the compounds through chemical synthesis or recombinant production.

In this study, the survival and growth of cultured fragments of these two sponge species was measured in the Aegean Sea (East-Mediterranean), hereby comparing an organically polluted fish farm site to different pristine, natural sites. For *D. avara*, the influence of water depth on culture success was studied as well. In the Aegean Sea, *D. avara* is hardly found in shallow water. In deeper waters, the species is more abundant and the individuals are larger. This suggests that the growth of this species is inhibited in shallow water, either by light, or by high water temperatures that occur in the summer season at lower depth.

*Address correspondence to this author at the Porifarma BV, Poelbos 3, 6718 HT Ede, The Netherlands; Tel: +31 6 45460582; E-mail: ronald.osinga@porifarma.com

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Fig. (1). Stainless steel frame hosting ten explants of *D. avara*.

In addition to comparative studies for site selection, a new, successful technique for the large scale culture of *D. avara* was developed, which may be suitable for the economically feasible production of avarol.

MATERIALS AND METHODOLOGY

Comparison of Sites

Comparative culture trials were executed on four locations in the area around Turgutreis (Bodrum Peninsula, Turkey) with different characteristics: a pristine, rocky site, depth: 38 m; a pristine rocky site, depth 28 m; a pristine site covered with seagreass (*Posidonia*), depth 18 m; a polluted muddy sediment below a floating cages fish farm, depth 21 m.

Mendola *et al*. [24] related distribution patterns to flow regimes and argued that *D. avara* preferred sites with average flow velocities below 10 cm s^{-1}. The culture sites used in this study were also characterized by low flow: even during stormy weather conditions, the water around the cultures was nearly stagnant, the average flow velocity being estimated at less than 2 cm s^{-1} (visual observation).

Table-like stainless steel frames (Fig. **1**) were used to culture *D. avara*. *D. avara* explants were held on nylon threads, conforming to earlier studies on this and other species [3,25]. One frame was moored on each of the four locations to study both the effect of depth (18-28-38 m) and the effect of fish farm effluent. Cages of stainless steel grids (Fig. **2**) were used for *C. reniformis*. For *C. reniformis*, we compared a pristine site (28 m) to the fish farm site. The two cages were moored on these sites and explants of *C. reniformis* were positioned on the cage bottom without artificial attachment. This method was applied because previous studies on *C. reniformis* [3,4] and the related species *Chondrilla nucula* [26] indicated that these species cannot be grown suspended on threads or meshes.

For each trial, ten explants of each species were used. To prepare the explants, parent specimens were taken from hard substrate in the vicinity of the deeper culture site (at 28 and 38 m). Some of the materials were transported from the deeper sites to the shallow sites (22 m seagrass meadow and fish farm), where there was no standing stock of this species available.

The four culture sites were revisited two times, in October 2006 and in June 2007 (additional attempts failed due to bad weather conditions and/or limited availability of ship-time). Pictures of the explants were taken while holding a ruler next to the explant as a reference. Growth was

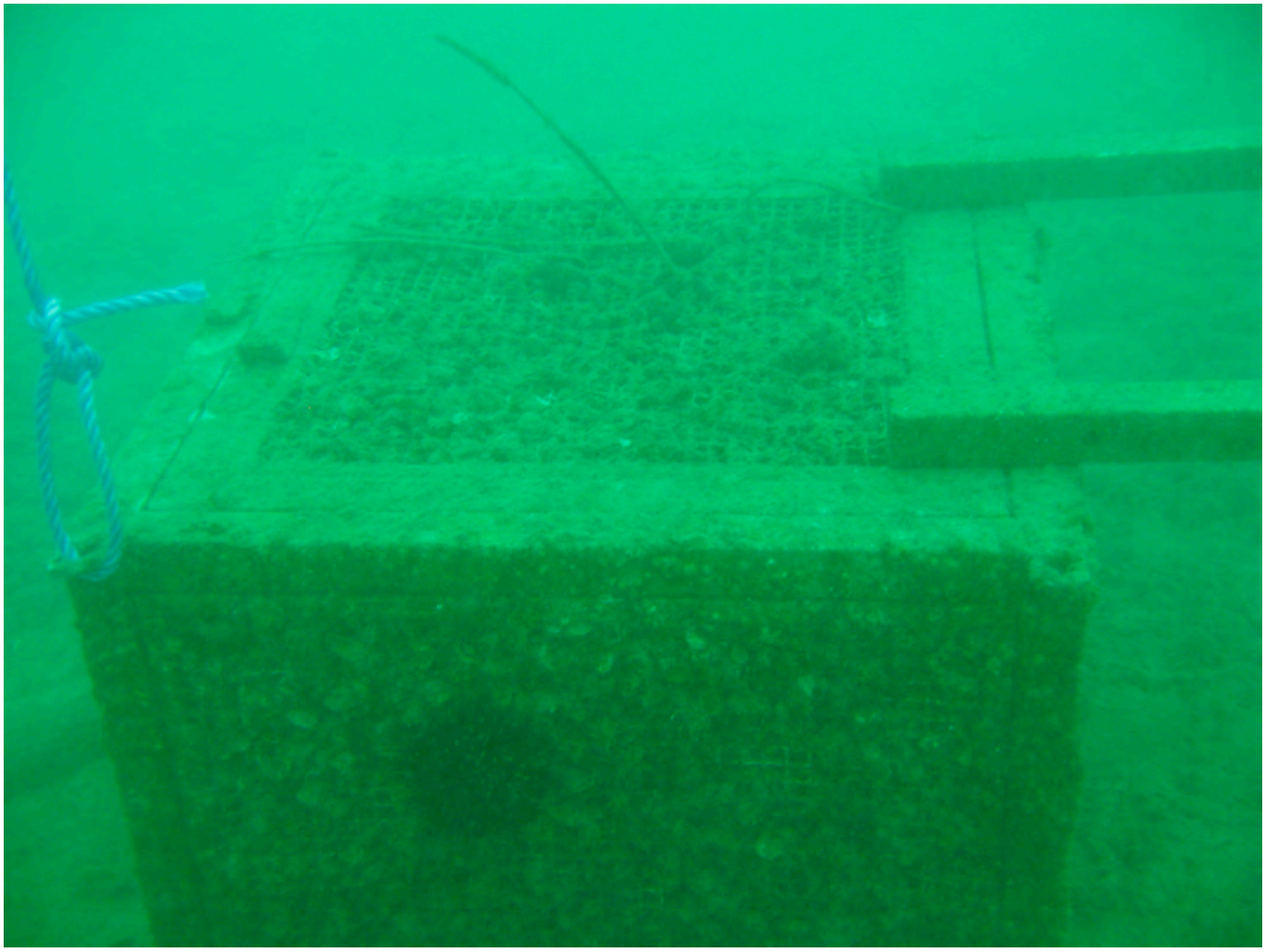


Fig. (2). Stainless steel cage hosting explants of *C. reniformis* on the inside.

determined as the increase in surface area, estimated from the length and width measurements of each explant.

Scale-Up

In June 2007, i.e. at the end of the first trials, a first scale-up was done for *D. avara*. A new method was designed to promote sponge growth and reduce labor.

The new method uses plastic (PVC) pins as a solid carrier for sponge explants. The pins have a sharp end to accommodate easy positioning of sponge fragments on the pin. After adding the fragments, a rubber stopper is mounted on the sharp end of the pin. A four meter long metal frame was constructed that consisted of two stainless steel bars through which 20 holes were drilled. These bars were mounted in parallel on the two existing culture structures (a frame and a cage; Fig. **3**), thus creating a four meter long rack with 20 slots for sponge pins (the rubber stoppers fit into the holes in the steel bars). Because of its resemblance to a local Turkish dish, the new method was termed "Shish Kebab Method" (Fig. **4**).

Specimens of *D. avara* were harvested from a vertical wall at a depth of 28 m, in a pristine area 30 kms away from the culture site. The sponges were transported immediately

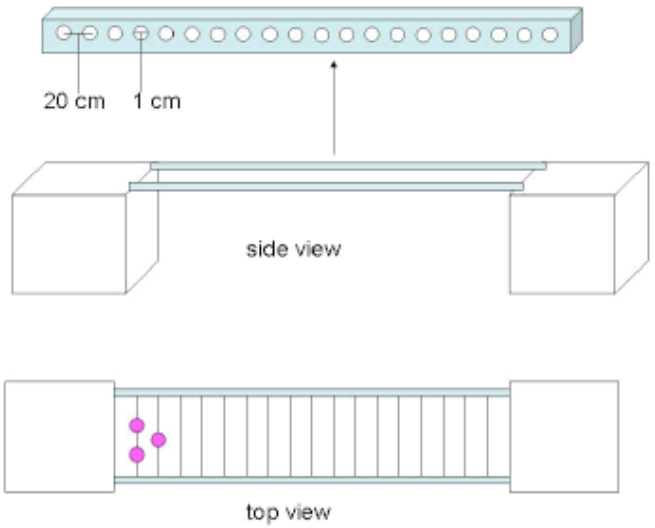


Fig. (3). Schematic drawing of the frame used for the Shish Kebab method for *Dysidea avara* aquaculture.

to the culture site in cool-containers. Twenty sponge kebabs (each holding 4 to5 sponge fragments) were prepared underwater just below the sea surface by SCUBA divers and immediately thereafter transported to, and mounted into the

Fig. (4). Overview (left) and detail (right) of Shish Kebab method for aquaculture of *D. avara*.

culture frame at 21 m depth. The total number of explants was 86.

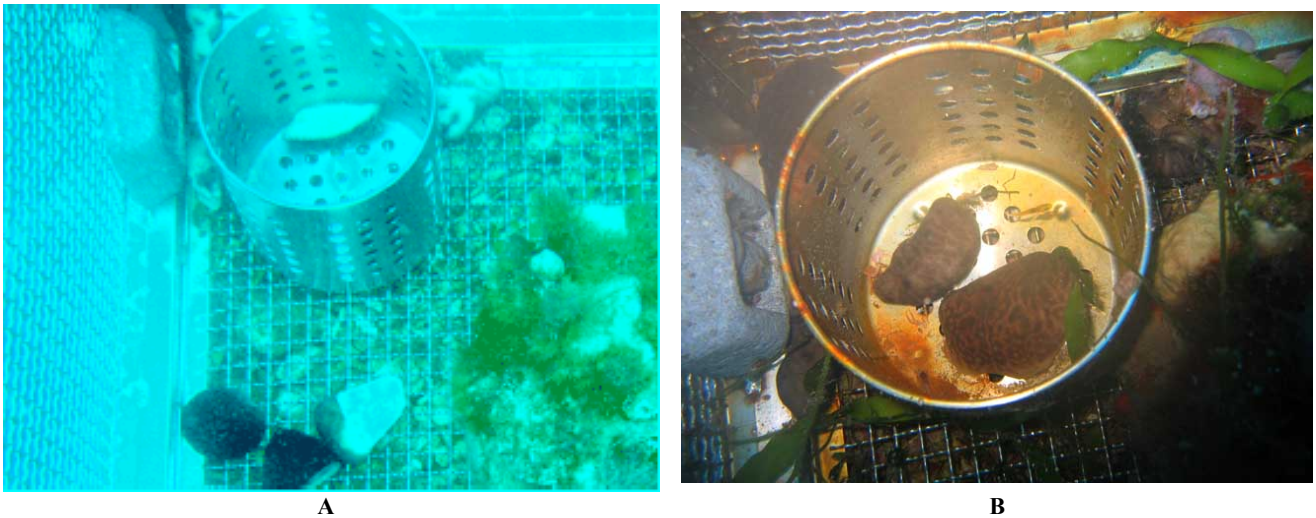
Pictures of each explant were taken 4 times using a Canon Ixus 750 digital camera with underwater housing: at the day of preparation, after 2 months in culture, after 4 months in culture and after one year. A ruler was held next to the explants as a reference for its size. The horizontal surface of the explants was assessed from the pictures, by multiplying the average length and height of each explant. Volume was estimated by assuming that the average width of an explant was equal to the average length and height measurements.

RESULTS

Comparison of Sites

Survival rates of the two species were good (90 to 100 %), except for *C. reniformis* at the fish farm location. These explants had become very soft and were covered with a layer of deposited organic matter, which caused them to die (smothering). *Chondrosia reniformis* grew well in the cage at 28 m (Fig. **5**; growth was estimated to be a 2 to 3 times increase in size per year, i.e. up to 700 % per year, survival was 100 %). There was no difference in growth of explants growing on the walls/floor of the cage and explants growing in small iron boxes positioned on the bottom of the cage.

Survival of *D. avara* was high at all locations, although some explants fell of the culture threads. Four months after being fragmented, all explants had recovered well and had several pumping oscula. The sponges did not attach very well to the nylon lines. In particular at the fish farm location, the explants formed big holes around the threads (Fig. **6**) instead of attaching themselves to the threads. Despite this lack of attachment, growth of *D. avara* was most pronounced at the fish farm location (Table **1**). No increase in size was observed in explants grown at 28 m and 18 m, explants grown at 38 m had increased in size slightly.



A **B**

Fig. (5). Explants of *C. reniformis* cultured in a cage at 28 m depth (pristine site). **A**: Initial fragments. **B**: the same fragments, after 4 months in culture. The tissue completely recovered and the explants had doubled their size.

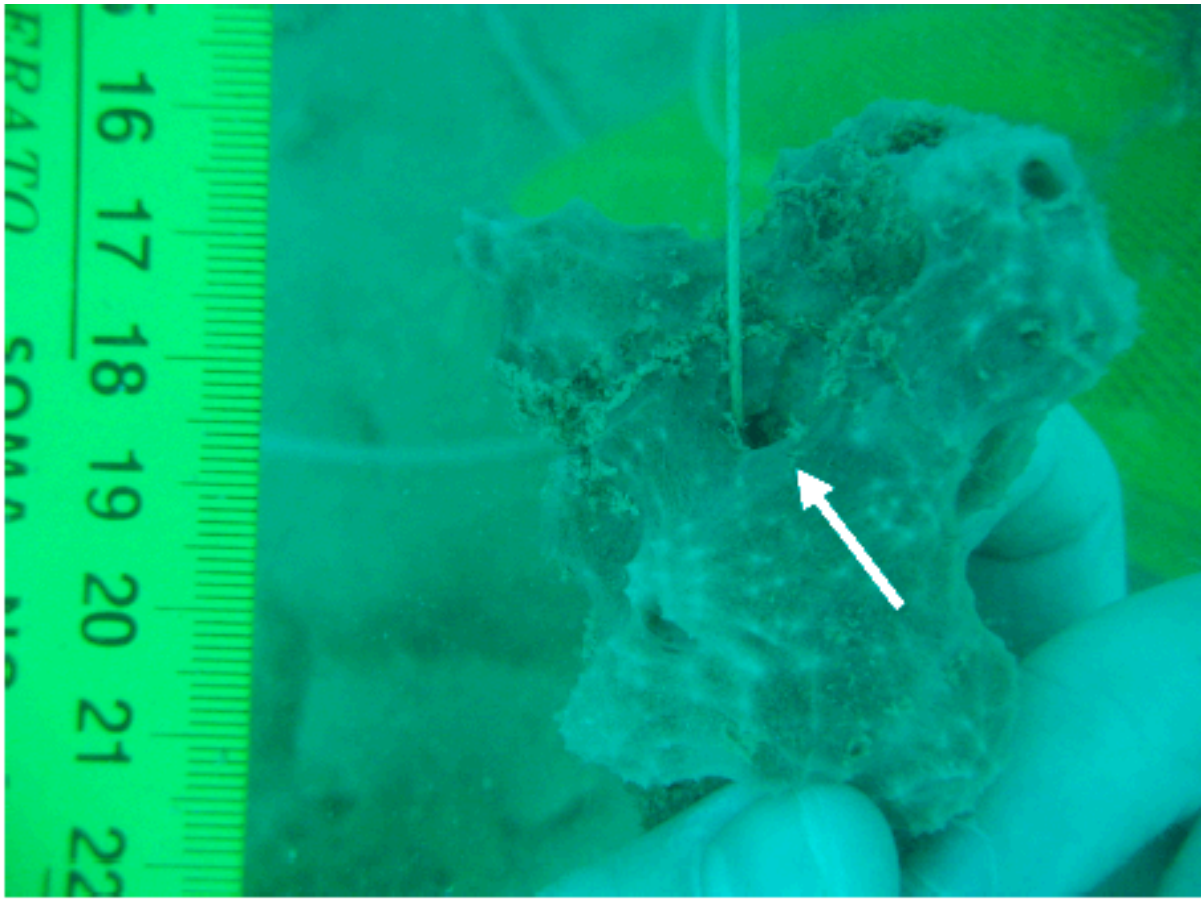


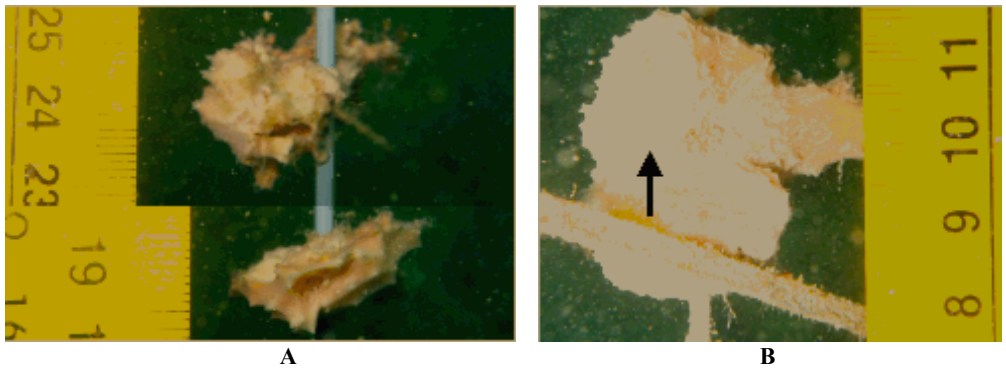
Fig. (6). Explant of *D. avara* cultured on a nylon thread at the fish farm site, pictured after being in culture for one year. A hole had developed around the nylon thread (see arrow).

Table 1. Growth Rates of *D. avara* During First Trial (Expressed as the Percentage of Newly formed Projected Surface Area Related to the Initial Explant Size)

Culture Site	Growth (% increase year-1)
Pristine, rocky site; 38 m	20-50
Pristine, rocky site: 28 m	0
Pristine, Posidonia-covered site: 22 m	0
Floating cages fish farm: 22 m	100

Scale-Up

Explants of *D. avara* cultured using the Shish Kebab Method showed 100 % survival after being in culture for four months. Recovery was remarkable, given the fact that the explants had been prepared by pulling pieces of sponge from the parent sponges by hand and that they experienced a heat shock during transport and further explanting procedures. Hence, at the start of the culture trial, the explants were in bad condition (Fig. **7a**). Within a time frame of two months, all 86 explants had transformed into healthy looking, actively pumping sponges (Fig. **7b**).



A **B**

Fig. (7). Recovery of explants of *D. avara* cultured using the Shish Kebab method. **A**: initial fragments. **B**: the same fragments, 2 months in culture (the explants had fused and developed pumping oscules - see arrow). The scale of the images is equal.

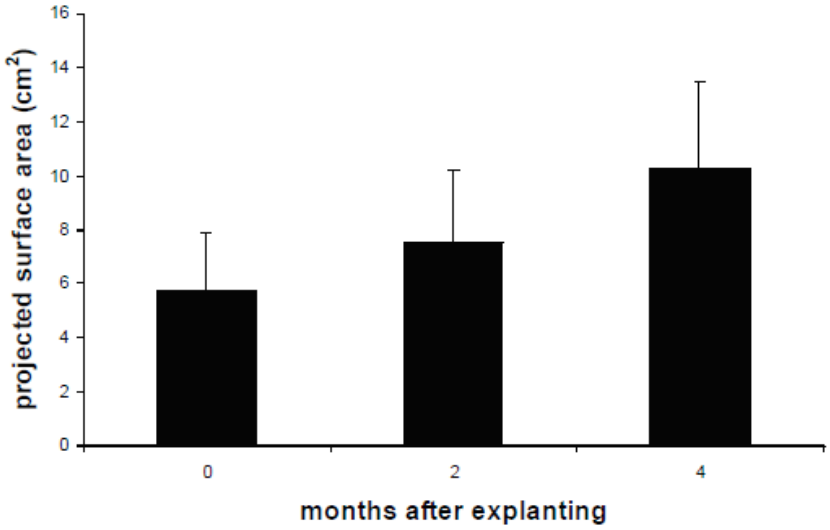


Fig. (8). Growth of *D. avara* at the fish farm site using the Shish Kebab method. Error bars indicate standard deviations, n = 86.

The sponges grew faster than during the initial trial. After four months in culture, the average projected surface area was 1.78 times the initial average projected surface area (Fig. **8**). This increase was significant (paired t-test, n = 86, t = 10.88, $p < 0.001$). The extrapolated volume was 2.37 times the starting volume, hence biomass more than doubled within a period of four months. Unfortunately, when we revisited after one year, the culture was lost: the frame was disrupted and some of the pins with the explants were found in the sediment around the frame. Therefore, it was not possible to assess the annual growth rate. However, analysis of the data obtained during the first four months (Fig. **8**) shows that growth was exponential: in both time intervals measured, the estimated volume had increased approximately 1.5 times. When extrapolating this trend, an annual growth rate of more than 1100 % can be assumed.

DISCUSSION AND CONCLUSIONS

Our aquaculture trials gave promising results and represent significant progress towards feasible aquaculture of *Dysidea avara* and *Chondrosia reniformis*. The good growth of *C. reniformis* in the cage at 28 m depth supports earlier observations in the Aegean Sea (W. Schatton, personal communication) that this species is easy to grow in clear water environments and that it grows well on solid substrata. Growth rates were higher than those reported in literature for this species [27, 28]. When assuming first order exponential growth kinetics as described by Sipkema *et al*. [29], a growth rate of 700 % per year equals a daily growth rate of 0.57 %. Garrabou and Zabala [27] reported a rate of 0.084 % day^{-1}, Sipkema *et al*. [29] calculated that the highest growth rates reported by Wilkinson and Vacelet [28] corresponded to a daily growth of 0.18 %, which is three times lower than the growth rate estimated in this study.

The technique applied here to grow *C. reniformis* appeared not to be suitable for turbid environments such as the fish farm location. However, we made an interesting visual observation that may gives new clues for designing cultures at such sites. At the polluted site, the downside of the lid of the cage was completely covered with invertebrate life (molluscs, sponges, tunicates, etc.). Growing in this position, the organisms are prevented from being covered by depositions of solids, while maintaining their access to the high suspended food levels. For comparison, the lid of the cage at the pristine location was completely covered, mainly with algae. It is likely that culture of *C. reniformis* will be more successful at the fish farm location when this sponge is grown in a more suspended way. Analogous to this view is the successful culture of corals that has been achieved in the vicinity of fish cages in the Gulf of Eilat (Israel). Corals growing on an open nursery construction that was positioned half way between the surface and the seafloor exhibited higher growth rates than corals growing in pristine areas [30]. Concurrently, reefs on the seafloor in the vicinity of floating fish cages suffer from smothering by high loads of organically rich sediments [31].

Our study sheds new light on the factors determining vertical distribution of *D. avara* in natural waters. During the initial trial, the explants grew fastest at the fish farm location. This site was relatively shallow (22 m) and warm (in summer, the temperature rose above 25 °C). Irradiance was low (considerably lower than at 38 m in clear water) due to the high turbidity at the fish farm site (2-3 meters visibility, compared to 15-25 m at the pristine sites). Hence, light rather than temperature appears to be inhibiting the growth of *D. avara* in shallow water. This is consistent with the distribution patterns of *D. avara* in the Western Mediterranean. Here, *D. avara* is often found in shallow waters, but its presence there is limited to caves and crevices [24, 32].

Despite their high survival rate and fast regeneration, growth rates of *D. avara* explants during the initial trial were low (0-100 % per year). Previous estimations of growth of this species made by Sipkema *et al*. [25] using similar methodologies resulted in much higher growth rates (more than five-fold increase per year). Probably, the low growth was caused by a lack of solid substratum. In contrast to the study by Sipkema *et al*. [25], we used soft separators (artificial sponges) instead of corks or plastic to separate the individual explants. Hardy separators may act as additional substrates, thus helping to keep the sponges continuously in

the same position. *Dysidea avara* was found to reorganize its internal structure as a result of being put into another position ("re-plumbing") [33]. The sponges in this study failed to attach to the threads and the separators. As a result of this, the explants may face a continuous change of their position on the thread due to water flow. Better attachment (i.e. more support by solid substratum) will keep the sponge permanently in the same position, so that it will not put too much metabolic energy in processes such as "re-plumbing". Indeed, the results obtained using the Shish Kebab method (plastic pins) were much better than the results of the nylon line cultures. This is in contrast to a study by Duckworth *et al.* [12], who did not obtain good results when using a similar technique (termed "spike-method") to culture the tropical bath sponge species *Rhopaloides odorabile* and *Coscinoderma* sp. This emphasizes that different species require different culture techniques.

The Shish Kebab method was designed to promote growth, but also to reduce costs. Indeed, installing the culture proved to be tenfold faster than the nylon line-cultures. During one dive, two divers were able to prepare 20 sponge kebabs, to mount them into the culture frame at 22 meters and to take pictures of each individual explant. Based on this experience, the costs of the materials and the growth rate of the sponges, we estimated a production cost of 36 euro per kg sponge (wet weight). Following the economic analysis made by Sipkema *et al.* [25], production of *D. avara* becomes profitable below a cost price of 50 euro/kg. An earlier estimation of production costs for *D. avara* [25] was 1.5 times lower (24 euro/kg), but this value was based on a growth rate that was four times higher than the growth rate reported here. This shows that the Shish Kebab method effectively reduced labor costs.

It is important to note that the sponges in the culture had an avarol content that did not differ from naturally growing specimens (W. Schatton and R. Osinga, unpublished data). Therefore, it can be concluded that commercial production of avarol is possible using the methodology described in this paper. Using new approaches, the earlier idea of combined fish/sponge culture has come one step closer to reality.

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