Mar. Biotechnol. 3, 544–554, 2001
DOI: 10.1007/s1012601-0078-2

MARINE BIOTECHNOLOGY



# Development of In Vivo Sponge Cultures: Particle Feeding by the Tropical Sponge *Pseudosuberites* aff. *andrewsi*

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**Abstract:** The rate of food particle uptake of the tropical sponge *Pseudosuberites* aff. *andrewsi* was studied in relation to particle concentrations and particle size. A range of different concentrations of either the marine microalga *Dunaliella tertiolecta* (~5–8 μm) or the marine cyanobacterium *Synechococcus* sp. (~1 μm) was supplied to the sponges. *D. tertiolecta* had a pronounced effect on the filtration activity of the sponges: at concentrations higher than approximately $4 \times 10^5$ cells/cm$^3$, the filtration rates dropped dramatically. Such a clear effect was not found for *Synechococcus* sp. The results further showed that the maximal amount of food (when expressed in organic carbon) that can be taken up per cubic centimeter of sponge volume per unit of time should in principle be sufficient to enable growth (irrespective of the food particle type). At the maximal food particle concentration that did not affect the filtration rates, the uptake of organic carbon is already highly in excess of the amount of organic carbon that the sponges need to cope with their respiratory demand. Based on these findings, a series of growth experiments was carried out in which the sponges were subjected to a constant concentration of different types of food particles (*Synechococcus* sp. and the microalgae *Chlorella sorokiniana* and *Nannochloropsis* sp). Although initial growth was sometimes observed, continuous growth at a constant rate could not be obtained. It is concluded that qualitative aspects of feeding rather than quantitative aspects are the key to successful in vivo sponge culture.

**Key words:** *Pseudosuberites* aff. *andrewsi,* sponges, filtration, in vivo culture.

## INTRODUCTION

Sponges have been recognized as a rich source of natural products that are of potential interest to society. As a consequence, research efforts on the cultivation of marine sponges for biomedical purposes have increased in recent years. Different approaches to produce sponge biomass are under study, such as aquaculture (Duckworth et al., 1999; Müller et al., 1999a; Pronzato et al., 1999), suspended cell culture (Ilan et al., 1996; Pomponi et al., 1998), and primmorph culture (Custodio et al., 1998; Müller et al., 1999b). In our laboratory, research has focused primarily on development of in vivo cultures, i.e., cultivation of functional sponges under controlled conditions (Osinga et al., 1998, 1999a,b).

Received December 20, 2000; accepted March 26, 2001
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Our attempts to design an in vivo sponge culture are based on the strategy suggested by Osinga et al. (1999c): first, the minimal amount of food that is needed by the sponge per unit of time is determined by measuring the respiration rate of the sponge. Subsequently, the amount of organic carbon needed to cope with this respiratory demand is calculated. Since sponges are assumed to be efficient filter feeders, food is supplied as live organic particles, such as microalgae and bacteria. The food supply per unit of time is set at a rate that is always considerably higher than the calculated minimal carbon demand, on the assumption that an excess of food particles will not negatively affect the sponges; however, there is no experimental evidence to support this assumption. Information on effects of particle concentrations on the physiology of marine sponges is scarce.

It seems reasonable to assume that the rate at which particles can be processed (i.e., taken up, transferred from cell to cell, and digested) will have a maximal value. When the amount of particles entering the aquiferous system (i.e., the system of channels and chambers inside the sponge body) exceeds this maximal uptake rate, the excess particles may either be released through the exhalant channel or accumulate inside the aquiferous system. In the latter case, they may block the water current. Gerrodette and Flechsig (1979) reported that increased concentrations of nonfood particles (such as clay and silt) caused a drop in the pumping activity of three tropical sponge species, suggesting that these particles block the sponge pump.

In this study, we investigated whether digestible particles have similar effects on sponge physiology. Sponges have different uptake mechanisms for smaller and bigger particles (Bergquist, 1978). Hence, effects of increasing particle concentrations may change with particle size. Effects of both particle size and particle concentration on the particle filtration rate of the tropical Indopacific sponge *Pseudosuberites* aff. *andrewsi* were studied. The sponges were subjected to a series of increasing particle concentrations and filtration rates were measured at every concentration. The green microalga *Dunaliella tertiolecta* (size ~5–8 μm) and the marine cyanobacterium *Synechococcus* sp (size ~1 μm) were selected as target particles.

Tropical waters are usually rather oligotrophic, and the available plankton is dominated by relatively small organisms (<2 μm), such as bacteria, cyanobacteria, and prochlorophytes. It has been demonstrated that these small particles are indeed the main food source for tropical sponges (Pile, 1997, 1999). Hence, the aquiferous system of tropical sponges might be adapted to processing large amounts of water containing low concentrations of predominantly small food particles. Therefore, our hypothesis was that *Pseudosuberites* aff. *andrewsi* will feed efficiently on *Synechococcus,* while its capability to process *Dunaliella* cells will be limited.

In addition to the filtration experiments, we describe a series of growth experiments that were done with *P.* aff. *andrewsi.* Three microphototrophic species with different sizes were used as food particles: *Chlorella sorokiniana* (~3 μm), *Nannochloropsis* sp. (~2 μm), and *Synechococcus* sp. (~1 μm). The freshwater Chlorophycea *Chlorella sorokiniana* (previously classified as *Chlorella vulgaris*) was used by Barthel and Theede (1986) to enrich natural seawater for culturing *Halichondria panicea. Nannochloropsis* sp. is a related marine Chlorophycea that is rich in polyunsaturated fatty acids, especially EPA. It is therefore considered to be an excellent species for use in aquaculture feeds (Chini Zittelli et al., 1999). *Synechococcus* sp. is the same strain that was used for the filtration experiments. It was selected because of its small size and its worldwide abundance in marine waters, thus being an important food source for many sponge species (Pile, 1999; Pile et al., 1996). Previous experiments showed that these food particle species were indeed taken up by the sponge cells (Osinga et al., 1999b and unpublished data). The results of the growth experiments will be discussed within the context of the preceding studies on particle filtration.

## Materials and Methods

### Sponges and Microorganisms

Fresh sponge material was obtained from Artis Zoo, Amsterdam, The Netherlands, and was maintained in our laboratory in 80-$dm^3$ aquaria. The material was classified as *Pseudosuberites* aff. *andrewsi* and was identical to the material used in previous work (Osinga et al., 1999b). The sponge colonies were cut into several smaller pieces using a razor-sharp knife. The volume of each piece was determined by water replacement of the drip-dry piece in a graded cylinder, following the procedures described by Osinga et al. (1999a). After determining the volume, the pieces were tied onto a solid carrier (either Perspex slides or plastic meshes) with nylon fishing line. These fixed pieces of

**Table 1.** Composition of Growth Medium Used for Marine Microphototrophs

| | Amount (g/dm$^3$) |
|---|---|
| Major salts | |
| NaCl | 24.5 |
| $MgCl_2 \cdot 6H_2O$ | 9.8 |
| $Na_2SO_4$ | 3.2 |
| $K_2SO_4$ | 0.85 |
| $CaCl_2 \cdot 2H_2O$ | 0.53 |
| Trace elements | |
| $FeCl_3$ | $1.9 \times 10^{-3}$ |
| $Na_2EDTA$ | $4.36 \times 10^{-3}$ |
| $MnCl_2 \cdot 4H_2O$ | $0.18 \times 10^{-3}$ |
| $ZnSO_4 \cdot 7H_2O$ | $0.022 \times 10^{-3}$ |
| $CuSO_4$ | $0.01 \times 10^{-3}$ |
| $CoCl_2 \cdot 6H_2O$ | $0.01 \times 10^{-3}$ |
| $Na_2Mo_4 \cdot 2H_2O$ | $0.006 \times 10^{-3}$ |
| Vitamins | |
| Thiamine HCl | $20 \times 10^{-6}$ |
| Cyanocobalamine | $0.8 \times 10^{-6}$ |
| Biotin | $0.04 \times 10^{-6}$ |
| Nutrients | |
| $NaHCO_3$ | 0.84 |
| $KNO_3$ | 0.1011 |
| $NaH_2PO_4 \cdot H_2O$ | 0.0138 |

sponge are termed explants and were used for both filtration and growth experiments.

Four species of phototrophic microorganisms were used in this study: *Chlorella sorokiniana, Dunaliella tertiolecta, Nannochloropsis* sp., and *Synechococcus* sp. *Chlorella sorokiniana* was cultured according to Osinga et al. (1999b). The other species were cultured in a simple growth medium for marine phototrophs based on Guillard's F2 medium (Guillard, 1975). The composition of this medium is given in Table 1. Batch cultures of all organisms were grown at a temperature that varied between 17 and 20°C under a light/dark cycle of 14 hours light (light intensity between 30 and 120 μmol/m$^2$/s) and 10 hours darkness.

Batch cultures of *Chlorella sorokiniana, Nannochloropsis* sp., and *Synechococcus* sp. were used to feed the sponges in the maintenance aquaria.

## Filtration Experiments

Filtration experiments were carried out in a 100-cm$^3$ glass beaker with continuous stirring. An explant was placed in the beaker with a known volume of filtered (0.2 μm filter) artificial seawater. Preliminary studies showed that the concentration of algae decreases during the first 10 minutes after addition, presumably due to adsorption of the algae to the glass. Therefore, a known volume of algal culture was added to the artificial seawater 10 minutes before the introduction of the explant.

Sponge explants and algae were incubated for 30–60 minutes at a temperature of 25°C. During the incubation, small samples (2 ml) were taken every 5 minutes for analysis of particle density on a Coulter Multisizer type II automatic particle counter. After taking a sample, 2 cm$^3$ of 0.2-μm filtered seawater was added to the beaker glass to replace the sample volume. This dilution was taken into account in the calculations of filtration rates.

A sponge that exhibits a continuous pumping and filtration activity will cause a logarithmic decrease of the concentration of particles in the surrounding water, which will appear as a straight line if plotted on a semilogarithmic scale. This exponential decrease, also termed clearance or filtration, can be described by the following equation (Thomassen and Riisgård 1995):

$$F = V_{\text{water}}/V_{\text{sponge}}\ t^{-1}\ \ln(C_0/C_t) \qquad (1)$$

in which $F$ is the filtration rate, $V_{\text{water}}$ is the volume of the surrounding water in the beaker glass, $V_{\text{sponge}}$ is the volume of the sponge, $t$ is the incubation time, $C_0$ is the initial concentration of particles, and $C_t$ is the concentration of particles at time $t$.

The minimal size of particles that can be distinguished by the Coulter Multisizer is 0.8 μm, which means that many *Synechococcus* cells are too small to be enumerated. Hence, filtration rates for *Synechococcus* were determined using an internal standard of *Dunaliella* cells. In addition to the varying concentrations of *Synechococcus,* a fixed low number of *Dunaliella* cells ($1 \times 10^5$ cells/ml) was added to the beaker. This concentration was assumed to be too low to have an effect on the pumping rate of the sponge, so that possible effects could be ascribed solely to changes in the concentration of *Synechococcus.* It was further assumed that the clearance rate for *Dunaliella* was equal to that of *Synechococcus.* This was verified by counting the *Synechococcus* cells under a microscope before and after the incubations. Cells were counted using a Bürker Türk hemacytometer. The percentual difference found by counting was compared to the measured decrease in *Dunaliella* cells. This test was done

**Table 2.** Design of Growth Experiments

| Exp. | Food source | Mode of culture | $V_{reactor}$ ($cm^3$) | Shape |
|---|---|---|---|---|
| 1 | *C. sorokiniana* | continuous | 1600 | cylindrical |
| 2 | *Synechococcus* sp. | batch | 63 | rectangular |
| 3 | *Nannochloropsis* sp/*C. sorokiniana* | batch | 880 | rectangular |

for both a low and a high concentration of *Synechococcus.*

A series of filtration experiments was carried out for both target particles. The initial concentrations of *Dunaliella* that were applied varied between $1 \times 10^4$ and $1.3 \times 10^6$ cells/$cm^3$ while the initial concentrations of *Synechococcus* varied between $1 \times 10^6$ and $6.5 \times 10^7$ cells/$cm^3$. A high variability in filtration activity is not unusual for sponges (e.g., Reiswig, 1971a; Frost, 1980; Riisgård et al., 1993). To keep the effect of this temporal variation on the experimental results low, measurements of filtration rates under increasing particle densities were always done on the same day.

*Dunaliella* and *Synechococcus* differ considerably in size. In terms of nutritive/energetic value for the sponge, the uptake of a few *Dunaliella* cells will be equal to the uptake of many *Synechococcus* cells. We will use the carbon content of both particle types to compare the overall efficiency of food uptake. For *Dunaliella tertiolecta,* an average carbon content of 19 pg C per cell has been reported (Koski et al., 1998). There is also literature available that provides data on *Synechococcus* spp. Because the strain used in this study is not classified on the species level, it is difficult to adapt a value "at random" from literature. Therefore, the organic carbon content of our *Synechococcus* strain was determined by measurement of the organic carbon concentration in a *Synechococcus* culture from which the cell density had been counted by hemacytometer. A wet oxidation method was applied, using an OIC 700 Total Organic Carbon analyzer.

## Growth Experiments

Growth experiments were designed following the strategy described by Osinga et al. (1999c) as outlined in the introduction. The oxygen consumption rate of *Pseudosuberites* aff. *andrewsi* was measured by placing a piece of sponge of known volume into a small enclosure. The decrease in oxygen concentration in the enclosure was then followed with a WTW EO 90 oxygen probe (Osinga et al., 1998). The oxygen consumption rate was converted into a carbon demand assuming a C:$O_2$ molar ratio of 1. The calculated carbon demand was converted into a minimal amount of food particles needed to cope with the sponges' respiratory demand using the following equation:

$$[\text{Particles}] = R/(F \times 0.35\ C_p) \qquad (2)$$

in which $R$ is the respiration (carbon demand) (mg C/$cm^3$ sponge tissue/day), $F$ is the particle filtration rate of the sponges ($cm^3$/$cm^3$ sponge tissue/day, and $C_p$ is the carbon content of a particle (mg). To fill in the equation, the carbon content per food particle ($C_p$) must be known; it is assumed that 35% of the carbon in the microorganisms is readily available for respiration (Osinga et al., 1999c).

In the growth experiments, particles (microorganisms) were continuously fed to a sponge bioreactor containing a few sponge explants. Either batch cultures or continuous cultures of microorganisms were used. Batch cultures were kept refrigerated after five days of cultivation and were continuously supplied to the sponge bioreactor from the refrigerator using a peristaltic pump. When continuous cultures were used, the overflow of the culture was pumped directly into the sponge bioreactors. Different sizes and types of sponge bioreactors were used throughout this study. Table 2 summarizes the characteristics of the reactors for each experiment.

For all types of particles used in the growth experiments, the minimal concentration needed to cope with the sponges' respiratory demand was calculated using equation 2. Values for the carbon content per cell ($C_p$) were determined for *C. sorokiniana* and *Nannochloropsis* sp. using the same methodology that was described for *Synechococcus* sp.

The particle concentration in the sponge bioreactors was always set at a concentration that was higher than the calculated minimal value based on the respiration rate. In order to calculate the amount of particles to be supplied

with the inflowing stream to obtain this setpoint, the following balance equation was used:

$$C = C_{in}\ \varphi\ (\varphi + F\ V_{sp})^{-1} \quad (3)$$

in which $C$ is the desired concentration of particles in the reactor, $C_{in}$ is the particle concentration in the inflowing stream, $\varphi$ is the flow rate ($cm^3$/day), $F$ is the particle filtration rate of the sponges ($cm^3/cm^3$ sponge tissue/day), and $V_{sp}$ is the total volume of sponges in the bioreactor ($cm^3$). When $F$ and $V_{sp}$ are known, different combinations of $C_{in}$ and $\varphi$ can be chosen to obtain the desired value for $C$. It must be noted here that in all calculations we used an average value for $F$ that was based on all filtration rate experiments that were carried out.

A series of three experiments was done with either one food particle species or combinations of two. Experiments were run for at least 14 days. Inflowing cultures were always diluted with seawater containing dissolved silicate ($Na_2SiO_3$) in order to supply silicate for spicule formation (Osinga et al., 1999c). Table 2 summarizes the experimental details of the growth experiments.

As a control (simulating a natural growth environment), growth measurements were also done with four explants in the central filtration system of the marine aquaria in Artis Zoo, from where the sponge material had been obtained. To investigate the availability of food for the sponges in this aquarium system, bacteria in the aquarium water were enumerated using the acridine orange direct counting technique (Hobbie et al., 1977) following the procedures described by Van Duyl and Kop (1990). In addition, the amount of organic carbon in the water was quantified with the OIC 700 Total Organic Carbon analyzer.

Growth of the sponge explants was followed by determination of the underwater weight, which was measured by hanging the explants under an A&D HR300 analytical balance while keeping the explant underwater (Osinga et al., 1999a). The methodology described by Osinga et al. (1999a) was slightly modified: during weighing, the explants were placed into a glass cylinder filled with artificial seawater (made from Instant Ocean Reef Crystals artificial sea salt) at a depth of 25 cm. Increasing the depth of the water column increases the accuracy of the method. We found the depth of 25 cm to be suitable for our measurements. It must be noted here that underwater weight is not an absolute measure: all measurements should be done under exactly the same conditions (water depth, salinity) to obtain comparable results.

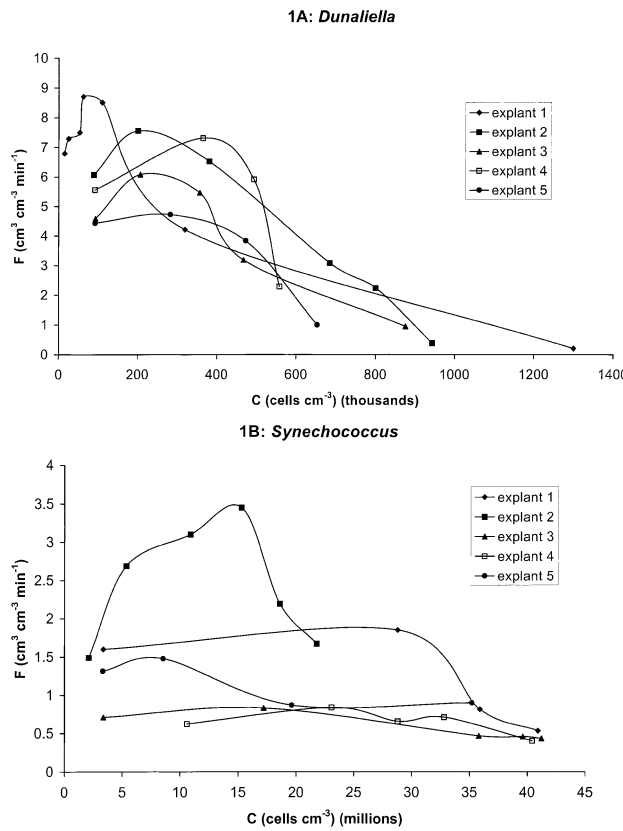


**Figure 1.** Relationship between the particle concentration in the surrounding water ($C$) and the particle filtration rate ($F$) of *Pseudosuberites* aff. *andrewsi,* using *Dunaliella tertiolecta* **(A)** and *Synechococcus* sp. **(B)** as the target particle.

## Results and Discussion

### Filtration Experiments

The filtration rate of *Pseudosuberites* aff. *andrewsi* was strongly affected by the concentration of *Dunaliella* cells in the surrounding water (Figure 1a). The filtration rates were relatively constant when the initial cell concentration was lower than $4 \times 10^5$ cells/$cm^3$. Increasing the initial concentration to values higher than ~$4 \times 10^5$ cells/$cm^3$ caused a dramatic drop in the filtration rate. At a concentration of $1 \times 10^6$ cells/$cm^3$, filtration activity could hardly be detected, because the decrease in particle concentration in time was no longer logarithmic at this concentration, making accurate estimations of the filtration rate difficult.

These results are in agreement with previous findings of Frost (1980), who studied food particle uptake by the freshwater sponge *Spongilla lacustris.* The filtration rate of yeast cells of that sponge decreased almost 10-fold when the

applied concentration was increased from $1.3 \times 10^4$ to $1.7 \times 10^6$ cells/cm$^3$ (Frost 1980).

The observed drop in filtration activity at concentrations higher than $4 \times 10^5$ cells/cm$^3$ may have been caused by a reduced retention efficiency (i.e., the percentage of particles retained by the sponge filter). In that case, the rate of water pumping by the sponge is not affected, but the excess particles are released into the exhalant current. However, if only the particles that are in excess are released, the total number of cells taken up by the sponge will not change at increasing particle concentrations. In our experiments, this overall cell uptake decreased dramatically at concentrations above $5 \times 10^5$ cells/cm$^3$. Therefore, it is more likely that the high concentrations of *Dunaliella* cells blocked the aquiferous system, leading to a reduction in pumping activity. The findings of Gerrodette and Flechsig (1979), who found a reduction in pumping rate under increasing concentrations of nonfood particles (clay), support this view.

Apart from food uptake, oxygen transport and removal of metabolic waste products are also dependent on the pumping activity of a sponge. Therefore, a continuous supply of excess food may be harmful for a sponge, as it will lead to long periods of reduced pumping activity, thus causing oxygen stress and accumulation of waste products in the interior parts of the sponge body. Hence, in addition to determining a minimal concentration of food that should be supplied to sponges (based on oxygen consumption measurements), a maximal concentration also must be defined based on studies such as presented in Figure 1a.

Clearance rates for *Synechococcus* (Figure 1b) could accurately be measured using *Dunaliella* as an internal standard. Both for high and low concentrations of *Synechococcus,* the clearance rate for *Synechococcus* determined by microscopic counting was similar to the clearance rate for the internal standard. Therefore, we used the internal standard method for the remaining series of experiments.

The experiments with *Synechococcus* differed from those with *Dunaliella* with respect to the accuracy of the measurements. Very low filtration rates could still be measured accurately, the decrease in particle concentration being slow, but usually clearly logarithmic.

A particle concentration-dependent decrease in filtration activity, as was found for *Dunaliella,* could not be found for *Synechococcus* (Figure 1b). At the highest concentration applied ($4 \times 10^7$ cells/cm$^3$), filtration could still be measured, and for two explants, the rate measured at this particle concentration did not differ much from the rate measured at the lowest concentration that was applied. When concentrations of *Synechococcus* higher than $4 \times 10^7$ cells/cm$^3$ were supplied, the internal standard of *Dunaliella* cells could no longer be counted accurately (probably due to aggregation of *Synechococcus* cells).

It is noted here that the average filtration rate in the experiments with *Synechococcus* (1.9 cm$^3$/cm$^3$/min) was significantly lower (Student's $t$ test, $q > 0.9995$) than in the experiments with *Dunaliella* (6.0 cm$^3$/cm$^3$/min; only measurements done at concentrations below $4 \times 10^5$ cells/cm$^3$ were used to calculate this average, due to the finding that the filtration rate was affected by higher cell concentrations). Because *Dunaliella* and *Synechococcus* are likely to be taken up by different filtration mechanisms, their retention efficiency may be different. However, the difference in our experiments can not have been caused by a difference in retention efficiency between the two particles, since filtration of *Synechococcus* was measured using an internal standard of *Dunaliella,* which was shown to exhibit the same filtration rate. Frost (1978, 1980) did also not find differences between filtration rates for small and big particles measured in freshwater sponges in situ. Hence, we assume that the difference in our experiments is caused by the physiological condition of the sponge explants used. The explants used for the experiments with *Synechococcus* had been kept in our lab for a period of several weeks, while the experiments with *Dunaliella* were done with material that was freshy harvested from Artis Zoo. The freshly harvested material seemed to be more viable, showing a better performance in both filtration experiments and growth experiments (see also "Growth Experiments" below). Therefore, only preliminary conclusions can be drawn about the filtration efficiency for *Synechococcus.*

In Figure 2, the total number of particles ingested per minute by 1 cm$^3$ of sponge biomass ($F \times C$) is plotted against the particle concentration ($C$). The measured filtration rate ($F$) at each particle concentration ($C$) that was applied was used to calculate the corresponding total particle ingestion ($F \times C$). For uptake of *Dunaliella* cells, parabolic curves were found that show an optimal ingestion rate at particle concentrations around $4 \times 10^5$ cells/cm$^3$. For *Synechococcus,* the pattern is not so clear: two explants (1 and 2) showed a parabolic curve similar to the curves found for *Dunaliella,* but the decrease in total uptake at higher particle concentrations was not so steep. For explants 3 and 4, the total particle ingestion increased gradually until a more or less constant value was reached. Explant 5 showed the highest uptake rate at the highest particle density.

Multiplication of the numbers of particles in Figure 2

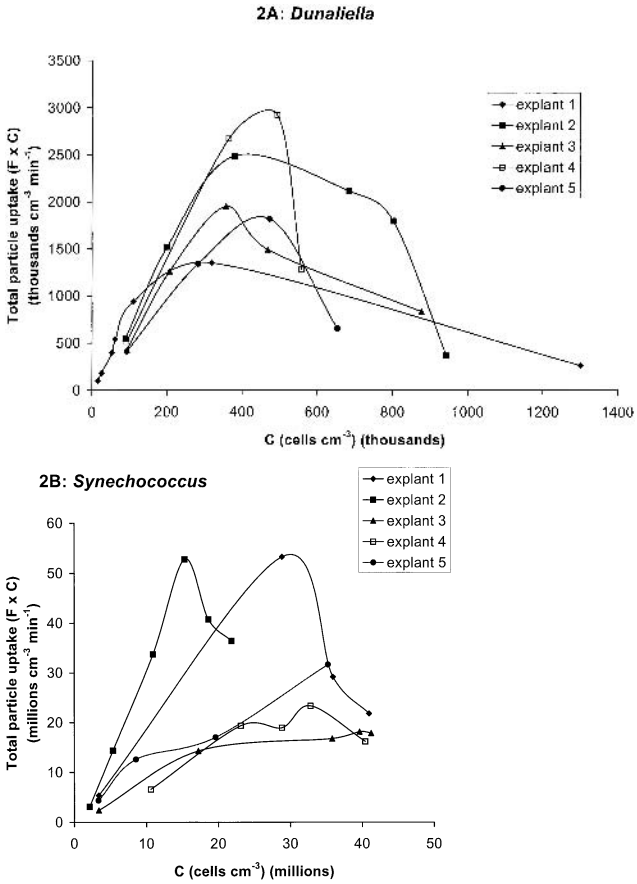


**Figure 2.** Relationship between the total number of particles taken up by *Pseudosuberites* aff. *andrewsi* per cubic centimeter of sponge volume per minute ($F \times C$) and the particle concentration ($C$) in the surrounding water. **(A)** *Dunaliella tertiolecta,* **(B)** *Synechococcus* sp.

by their respective carbon contents allows direct comparison of the food uptake efficiency for both particle types. In Figure 3, the total amount of carbon ingested by 1 $cm^3$ of sponge volume per minute is plotted against the carbon concentration in the surrounding water. In our experiments, feeding with *Dunaliella* led to a more efficient uptake of carbon than feeding with *Synechococcus.*

Although further research is needed, the results with *Synechococcus* do not indicate that the sponge *P.* aff. *andrewsi* feeds more efficiently on smaller particles than on bigger particles. Similar to the bigger *Dunaliella* cells, high concentrations of *Synechococcus* had a negative effect on the filtration rate, especially in those explants that exhibited the highest filtration rates at low particle concentrations. In addition, when the total amount of organic carbon taken up by the sponges was compared (Figure 3), much more carbon was taken up when *Dunaliella* was applied as a food particle.

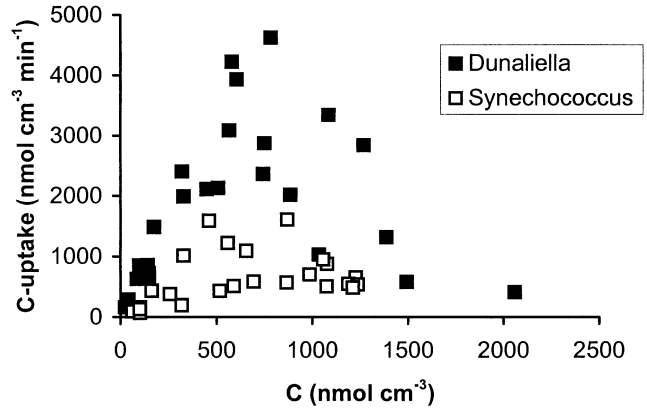


**Figure 3.** Total amount of organic carbon taken up by *Pseudosuberites* aff. *andrewsi* (nmol C/$cm^3$ sponge volume/min) plotted against the total amount of organic carbon (nmol C/$cm^3$) present in the surrounding water. Open squares: *Synechococcus* sp; black squares: *Dunaliella tertiolecta.*

It seems reasonable to assume that the negative effect of high concentrations of *Synechococcus* on the filtration rate is caused by a reduced pumping rate rather than by a reduced retention efficiency, since these filtration rates were measured indirectly (as a filtration rate of the internal standard, which was a low concentration of *Dunaliella* cells). If high concentrations of *Synechococcus* had caused a decrease in retention efficiency for *Synechococcus,* this would not have affected the measured filtration rate for the internal standard. Hence, our preliminary conclusion is that high concentrations of small particles will also lead to a reduced pumping activity. It remains to be investigated whether this reduced pumping activity is a simple physical effect (clogging of the aquiferous system, thus reducing the potential of the sponge to process water) or a behavioral response of the animal.

## Growth Experiments

An average oxygen consumption rate for *Pseudosuberites* aff. *andrewsi* of 53 nmol $O_2$/$cm^3$ sponge biomass/min ($N$ = 4, SD = 12) was measured. The average filtration rate $F$ of *Pseudosuberites* aff. *andrewsi,* was 3.5 $cm^3$/$cm^3$/min (range = 0.4–8.5, standard deviation = 2.3). Oxygen consumption rate, filtration rate, and the carbon contents of the microorganisms were used to calculate the minimal concentration of each microorganism to be applied in the growth experiments (equation 2). The measured carbon contents were 6 pg C/cell for *Chlorella sorokiniana,* 3 pg C/cell for *Nannochloropsis* sp., and 0.2 pg C/cell for *Synechococcus* sp. For

**Table 3.** Design of Growth Experiments: Values Used for Calculations*

| Food source | Exp | $C_{\text{minimal}}$ | $V_{sp}$ | φ | $C_{in}$ | Expected C |
|---|---|---|---|---|---|---|
| *C. sorokiniana* | 1 | 230 | 1.5 | 0.15 | 10.000 | 280 |
| *Synechococcus* sp. | 2 | 3850 | 1.5 | 10.75 | 15.000 | 9300 |
| *Nannochloropsis* sp./ | 3 | 460/230 | 1.5 | 0.56 | 30.000/14.000 | 770 |
| *C. sorokiniana* | | | | 0.56 | | 360 |

*Cell concentrations ($C_{\text{minimal}}$, $C_{in}$, and expected $C$) are expressed in cells/cm$^{-3}$ × 10$^3$, sponge volume ($V_{sp}$) in cm$^3$, and the flow rate (ϕ) in cm$^3$/min.

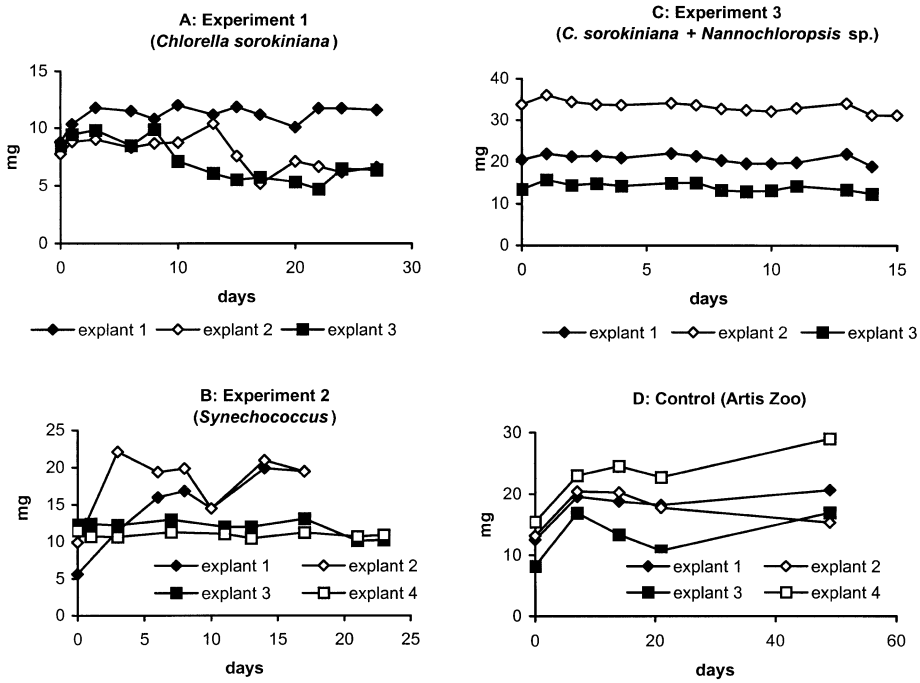


**Figure 4.** Results of the growth experiments. The underwater weight (mg) of the sponge explants is plotted against time. (**A**) Explants fed with *Chlorella sorokiniana,* (**B**) explants fed with *C. sorokiniana* and *Nannochloropsis* sp., (**C**) explants fed with *Synechococcus* sp., (**D**) explants grown at Artis Zoo.

*Dunaliella tertiolecta,* a value of 19 pg C/cell was adapted from Koski et al. (1998). Subsequently, equation 3 was used to calculate the values for φ and $C_{in}$ needed to establish a $C$ that was higher than these minimal concentrations. The calculated minimal concentrations and the applied values for φ, $C_{in}$, and $C$ are summarized in Table 3.

The results of the growth experiments are presented in Figure 4, showing the growth curves of each individual explant. In experiment 1 (*Chlorella sorokiniana,* Figure 4a), an initial increase in underwater weight was observed (especially for explant 1), but this was followed by a long period in which the underwater weight hardly changed (explant 1), or even decreased (explants 2 and 3). In experiment 2 (*Synechococcus,* Figure 4b), two explants showed an initial increase in underwater weight during the first days of the experiment. Thereafter, growth did not continue at the same rate: the biomass of explant 1 further increased, and explant 2 did not change much in underwater weight after day 3. Explants 3 and 4 in experiment 2 hardly changed at all throughout the experiment. It is interesting to note that these explants had been prepared from material that had been maintained in our laboratory aquaria for a few months. Explants 1 and 2 (and also the explants in experiment 1) were made a few days after collection of the fresh material from Artis Zoo. Explants 1 and 2 had to be discarded from the experiment 2 at day 17 due to an infection.

No growth was observed in experiment 3 (*Chlorella sorokiniana* + *Nannochloropsis* sp., Figure 4c). These explants were also made of "old" sponge material that had been kept in the lab for several months.

These results show that we are still unable to establish a continuously growing culture of sponges in a controlled,

closed system. The amount of food that was supplied in our experiments was, in principle, sufficient to allow growth, but the applied food particle species apparently led to unknown deficiencies in the sponge. Hence, we state that qualitative aspects of feeding (finding an appropriate food particle species or a combination of species) is the main key to successful culture.

A clear indication that deficiencies occurred in our experiments is the observation that fresh explants often showed an initial increase in weight while old explants that were cultured under the same conditions did not grow at all. Probably, fresh explants still have reserve nutrients stored in their tissue that enable them to grow for a short period. Thereafter, the condition of the sponge deteriorate, growth stops, and pumping acticity is reduced. Notwithstanding this, the observed initial growth confirms our previous finding (Osinga et al. 1999b) that *Pseudosuberites* aff. *andrewsi* is in principle able to grow relatively fast.

The control explants that were left in Artis Zoo (Figure 4d) showed considerable growth, although also in this experiment, growth was fastest in the early phase of the experiment. The measured average concentration of bacteria in the seawater used in Artis Zoo was $2 \times 10^5$ cells/cm$^3$, which is approximately 0.04 μg C/cm$^3$ (A.J. Kop, personal communication). This was only a small fraction of the measured total organic carbon concentration in this seawater, which was 2.95 μg C/cm$^3$.

The fact that the control sponges in Artis Zoo did continue growing (showing an average increase of 70% in 49 days), in water that hardly contained living nano- and picoplankton (bacteria) obviously leads to the following question: what did the sponges eat in Artis Zoo? Interestingly, the measured total organic carbon concentration in the Artis seawater (2.95 μg C/cm$^3$) was within the range applied in our experiments (1.68–4.47 μg C/cm$^3$). Most of the organic matter in Artis Zoo seawater may consist of dissolved compounds and detritus (particulate dead organic matter), probably remainders of the food that is supplied to the animals in the overlying aquaria (dead fish and shrimps).

The observation that the available nano- and picoplankton in the Artis seawater is not sufficient to satisfy the nutritional requirements of the sponges is consistent with earlier studies in natural waters (Von Pütter, 1914; Reiswig, 1971b). More recently, Pile (1999) quantified the availability of nano- and picoplankton in Caribbean waters. The detected numbers of bacteria, cyanobacteria, prochlorophytes, and microalgae were extremely low and are as such not likely to sustain the large sponge population in this area. Therefore, the sponges must also feed upon alternative food sources such as dead organic particles (Reiswig, 1971b), dissolved organic compounds, or get their additional nutrition from photosynthetic microsymbionts (e.g., Wilkinson, 1983).

With respect to these findings, the question arises as to what extent sponges can obtain their food from dissolved organic compounds. Some examples of uptake of dissolved compounds can be found in the literature. Ferguson (1982) measured uptake of amino acids by *Cliona celata.* Recently, Krasko et al. (2000) demonstrated that primmorph cultures of *Suberites domuncula* were able to take up ethylene from seawater. The uptake of ethylene stimulated cell division, which indicates that it is used by the cells as a substrate. Reiswig (1990) even found that the hexactinellid sponge *Rhabdocalyptus dawsoni* depends almost completely on dissolved organic food.

Probably, the textbooks that say sponges are filter feeders that feed predominantly on small organic particles should be rephrased as follows: sponges are sessile animals that process large volumes of water, from which they take up dissolved and particulate organic matter. Further hypothesizing, sponges may even prefer dissolved food over particulate food. The capacity to process particles and discard the undigestible parts (such as cell walls) could have been developed as an evolutionary strategy to survive circumstances in which the availability of dissolved substrates is limited. This would give a new perspective to studies that focus on controlled culture of sponges: development of a nonparticulate growth medium.

## Conclusions

The hypothesis that the tropical sponge *Pseudosuberites* aff. *andrewsi* would feed more efficiently on smaller particles (*Synechococcus* sp., ~1 μm) than on larger particles (*Dunaliella tertiolecta,* ~5–8 μm) could not be confirmed. Particle uptake experiments showed that both *Dunaliella* and *Synechococcus,* if supplied in sufficient quantities, could be taken up at rates that are in excess of the minimal amount of carbon that the sponges need for respiration. High concentrations of *Dunaliella* in the surrounding water led to a reduced water pumping activity in the sponges, implying that food should not be supplied to sponges in too much excess.

Our growth experiments confirmed the old experience (e.g., Arndt, 1933) that in vivo sponge culture is difficult; we

did not succeed in establishing an in vivo sponge culture that exhibited continuous growth. However, we have excluded the possibility that the lack of success is caused by quantitative aspects of feeding. It is therefore concluded that the persisting difficulties with in vivo sponge culture are caused by qualitative aspects of feeding.

## Acknowledgments

We thank Artis Zoo for supplying us with sponge material.

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