Original Article

MARINE BIOTECHNOLOGY

Hypothesized Kinetic Models for Describing the Growth of Globular and Encrusting Demosponges

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Abstract

The marine sponges *Dysidea avara* and *Chondrosia reniformis* (globular forms) were cultured in the laboratory on a diet of viable *Phaeodactylum tricornutum* cells and dissolved nutrients (algae and fish powders). Our growth data were combined with literature data for *Pseudosuberites andrewsi* (a globular sponge) and for the encrusting sponges *Oscarella lobularis*, *Hemimycale columella*, and *Crambe crambe*. The suitability of three growth models—linear, exponential, and radial accretive—for describing the growth of globular and encrusting sponges was assessed. Radial accretive growth was determined to be the best model to describe growth of both encrusting and globular sponges. Average growth rates of 0.051 ± 0.016 and 0.019 ± 0.003 mm/day (calculated as the increase of the radius of the sponge per day) were obtained experimentally for *D. avara* and *C. reniformis*, respectively.

Keywords: Growth — kinetics — morphology — sponge

Introduction

The discovery of many secondary metabolites from marine sponges with interesting pharmaceutical properties (e.g., Faulkner 2000, 2001, 2002) has been followed by a number of attempts to culture sponges for their chemical constituents. Cultivation of sponges in situ has been moderately successful, with many species having been cultured. However, the results have shown that growth rates are generally low and mortality is high (Duckworth et al., 1997; Duckworth and Battershill, 2003; Van Treeck et al., 2003). Other researchers have tried to culture sponges, but optimum conditions for maintaining sponges in the laboratory have not been completely determined to date (Barthel and Theede, 1986; Belarbi et al., 2003; de Caralt et al., 2003; Duckworth et al., 2003; Mendola, 2003; Osinga et al., 2003). Marine sponges appear to be very sensitive to environmental, and therefore also experimental, conditions (De Vos et al., 1991; Nickel et al., 2000). For example, it is striking to see a thriving sponge population on basalt blocks of a dike and just a few hundred meters away (in apparently similar environmental conditions) a total absence of sponges. It is most likely that the lack of sufficient understanding of environmental subtleties and their effects on sponge settlement and growth, together with the inherently slow growth rates (which necessitate long-duration growth experiments, usually with small numbers of replicates) have resulted in only a small number of successful *ex situ* culture experiments to date.

The use of different methods to define or measure sponge growth [e.g., two-dimensional photography (Garrabou and Zabala, 2001), drip dry weight (Thomassen and Riisgård, 1995), underwater weight (Osinga et al., 2003), or qualitative indications (Nickel, 2001)], and different ways to express growth [e.g., %/day (Belarbi et al., 2003), cm^2/cm border per day (Ayling, 1983), or per day (Garrabou and Zabala, 2001)], make it difficult to compare data from different experiments and sponges and have led to fragmented knowledge about the growth kinetics of sponges.

The aim of this study was to develop a mechanistic model for describing the growth of globular (clump-shaped) and encrusting (flattened) sponges, in order to compare growth rates between different

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species of these selected morphotypes. Branching forms such as *Haliclona oculata*, vase-shaped sponges, or other morphotypes were therefore not included in this study. Such a growth model can be used to study the quantitative effect of factors such as pressure, light, current, temperature, nutrition source, nutrient concentration, or age on the growth rate of sponges. Moreover, it could prove valuable as a tool to predict sponge growth in maricultures. For this work, we used growth data from experiments conducted in our laboratory with two Mediterranean demosponges, *Dysidea avara* and *Chondrosia reniformis* (both globular forms). In addition, we used literature growth data (for *in situ* growth where a relationship between sponge size and growth rate could be extracted) to test the fit of the model, and to obtain additional insight into the growth kinetics of the two morphotypes.

Growth Kinetics

The suitability of the following three mechanistic growth models has been assessed for data obtained from the literature and for our own data (Figure 1): linear growth (zero-order kinetics) exponential growth (first-order kinetics); and growth related to the surface/circumference for globular/encrusting sponges, respectively.

Linear Growth. Linear growth occurs at the peripheral end (or a fixed number of growth tips) of a sponge. It is comparable to growth of the apical meristem of a plant stem or root. The growth rate of *Crambe crambe* explants held in closed systems has been assumed to be linear (Belarbi et al., 2003). It is described by the equation:

$$\frac{dX}{dt} = k \quad (\text{ml/day or mm}^2\text{/day})$$

where X is the volume or area of globular or encrusting sponge, respectively, and k is the linear growth rate constant. Integration leads to:

$$X_t = X_0 + k \cdot t \quad \left(\text{ml or mm}^2\right) \qquad (1)$$

Fig. 1. The different growth models assessed for encrusting sponges. (**A**) Linear growth, (**B**) exponential growth, and (**C**) circumference-dependent growth of the sponge.

Exponential Growth. Exponential growth implies that the proliferation rate of cells is independent of their location within the sponge. For example, in demosponges the largest fraction of totipotent archeocytes are scattered through the mesohyl, which typically contains a large number of open spaces (Bergquist, 1978). Conceivably, therefore, each archeocyte could divide without being impeded by the other cells. In the process, the new cells differentiate and move through the mesohyl to their ultimate destination within the sponge, clearing space for the next group of daughter cells. The process repeats itself over and over again to produce an *exponential* increase in the number of sponge cells. An approximation of exponential growth has been used in a number of publications (e.g., Garrabou and Zabala, 2001; de Caralt et al., 2003). Exponential growth is described by:

$$\frac{dX}{dt} = \mu \cdot X \quad [\text{mL/day or mm}^2\text{/day}]$$

where μ is the specific growth rate constant. Integration leads to:

$$X_t = X_0 \cdot e^{\mu \cdot t} \quad \left(\text{ml or mm}^2\right) \qquad (2)$$

Surface-Dependent Growth. Surface-dependent growth implies that growth occurs only at the outer surface of a globular sponge. Given that the influx of nutrient-containing seawater is dependent on the number of ostia (correlated with the surface area), the surface-dependent model can be used to describe growth of a sponge in relation to the increase of its surface area per unit time. Some support for a surface-dependent growth model can be found in the work of Kaluzhnaya and colleagues (2005), who discovered that the genes involved in spiculogenesis (required for support of newly added sponge cells) are expressed more at the periphery of the sponge than at its basal region. Surface-dependent growth is described by:

$$\frac{dV_r}{dt} = c \cdot A_r \quad (\text{ml/day})$$

where V and A are the volume and surface area of a globular sponge, respectively. Both V and A are dependent on the radius r, and c is the surface-dependent growth rate constant. Integration leads to:

$$V_t = \left(\sqrt[3]{V_0} + c \cdot \left(\sqrt[3]{2/3\pi}\right) \cdot t\right)^3 \quad (\text{ml}) \qquad (3)$$

if it is assumed that the sponge is a hemisphere.

Circumference-Dependent Growth. Circumference-dependent growth of encrusting sponges is the two-dimensional equivalent of surface-dependent growth of globular sponges. Ayling (1983) used this type of growth to express the growth rate of a number of encrusting sponges. Circumference-dependent growth is described by:

$$\frac{dA_r}{dt} = c \cdot O_r \quad (\text{mm}^2/\text{day})$$

where O is the circumference of the encrusting sponge, and both A and O are dependent on r. Integration leads to:

$$A_t = \left(\sqrt{A_0} + c \cdot \sqrt{\pi} \cdot t\right)^2 \quad (\text{mm}^2) \qquad (4)$$

if the sponge is assumed to be circular.

The surface- or circumference-dependent growth rate constant c applies to both surface-dependent growth of globular sponges and circumference-dependent growth of encrusting sponges, and is expressed as:

$$c = \frac{r_t - r_0}{t} \quad (\text{mm/day})$$

where r is the radius of the sponge.

Thus, it can be concluded that both surface-dependent growth and circumference-dependent growth assume radial accretive growth of the sponge.

Each of these growth models assumes that a sponge can grow to infinite size, which of course does not occur in nature. Therefore, one would expect a sigmoidal-shaped growth curve with the final size of the sponge indicated as the horizontal asymptote in a "size" (y-axis) vs. "time" (x-axis) graph. The sponge explants used for this study were still far from attaining their maximum size and therefore it is assumed that maximum size is not an important parameter.

Materials and Methods

Sponges. *Dysidea avara* specimens (which were originally collected in the northern Adriatic Sea near Rovinj, Croatia) were obtained from Professor W.E.G. Müller, who kept them in an aquarium at his institute in Mainz (Germany) at 16°C. They were transported from Mainz to our laboratory in an isolated vessel at a constant temperature of 16°C. The seawater in the transport vessel was aerated without exposing the sponges to air bubbles and it was continually purified in transit by means of an activated carbon filter. In Wageningen, the sponges were maintained in an aquarium at 16.5°C (which is comparable to the natural summer temperature of the Adriatic at a depth of 15 m). Explants were prepared by cutting pieces of 1 to 4 cm^3 from the "parent" sponges by use of a razor-sharp knife. The cuttings were tied onto terracotta tiles with tie-raps. The explants attached to the tiles, usually after 2 to 4 weeks, and thereafter the tie-raps were removed. Only attached explants were used for cultivation experiments and then only after they had been attached for several weeks so that any growth observed could not be attributed to wound healing (following cutting of the explants) or the attachment process. *Chondrosia reniformis* specimens were collected by scuba divers in the Mediterranean near Kalymnos (Greece) and Blanes (Spain). Transport and preparation of explants were carried out in the same manner as described for *D. avara* specimens.

Chemicals and Substrates

- Artificial sponge seawater was prepared from 33 g/L instant ocean reef crystals (Aquarium systems, Sarrebourg, France) and 7.1 mg/L sodium metasilicate in demineralized water. After preparation, the artificial sponge seawater was aerated for at least 1 week to stabilize the chemical reactivity before it was used for the culture of sponges.
- Artificial algae seawater was prepared by enrichment of artificial sponge seawater with 0.013 g/L of $NaH_2PO_4{}^{\star}H_2O$, 0.101 g/L of KNO_3, 2×10^{-5} g/L of thiamine*HCl, 4×10^{-8} g/L of biotin, 8×10^{-7} g/L of cyanocobalamin, 9.5×10^{-4} g/L of $FeCl_3$, 9×10^{-5} g/L of $MnCl_2{}^{\star}4H_2O$, and 1.21 g/L of Tris.
- Fish powder (GVP) was purchased from Snick Ingredients (Beernem, Belgium). It was prepared from the meat of frozen coalfish that was boiled, dehydrated, and micromilled (composition: 73% protein, 4.5% fat, 21% ash, and 1% NaCl). Sixty-six percent of the fish powder was present as dissolved organic carbon (DOC), while the rest were particles ranging from 1 to 200 μm.
- Algae powder was prepared from two batches of *Phaeodactylum tricornutum* that were cultured in a 70-L bubble column. The 140-L microalgal suspension was concentrated to a volume of 14 L by use of a "supercentrifuge" (Sharples, Rueil, France). Subsequently, the centrifuged suspension was mildly spray dried (T_{inlet} ~73°C and T_{outlet} ~46°C) with a laboratory minispray dryer (Büchi, Flawill, Swit-

zerland) and the algal powder was stored in the refrigerator. Seventy-four percent of the algae powder was present as DOC, while the remainder was present in dead algae cells.
- A stock solution of Complete Nutrition Mix was made up of 2.5 g/L of fish powder, 25 ml/L of newborn calf serum (Life Technologies, Paisley, UK), and 12.5 ml/L of minimum essential medium (MEM) vitamin solution (Sigma).

***Cultivation of* Dysidea avara.** A three-step reactor system was built to provide a constant environment for the cultivation of *D. avara* explants on live food (cultured *Phaeodactylum tricornutum*) and Complete Nutrition Mix (Figure 2). The system consisted of 2.5 L of photobioreactor (22°C) for the continuous cultivation of the microalga *Phaeodactylum tricornutum* (length ~12 μm, width ~1μm). This microalgal strain was selected because previous research showed its suitability for feeding sponges in tank cultures (Osinga et al., 2003). The effluent of the photobioreactor overflowed into a 8.5-L dilution reactor where the algal suspension was diluted with artificial sponge seawater to the desired concentration. The dilution reactor was shaded from ambient light to inhibit growth of the microalgae. For the same reason, a short residence time in the dilution reactor was chosen (0.3 day). A continuous flow was pumped from the dilution reactor to the three 7-L bioreactors containing the sponges (16.5°C). The dilution reactor was included in the system only for the practical reason that pumping directly from the algal photobioreactor to the bioreactors containing the sponges would result in extremely low flows of a dense algae suspension, which in testing was observed to clog the tubing. Each of the bioreactors containing sponges was mixed with a magnetic stirrer to create a current within the reactor. The *D. avara* explants, which were attached to the terracotta tiles, were placed on a fired clay brick placed in the bioreactor to raise the explants from the bottom of the container to prevent contact with the stirrer and to limit sedimentation of detritus onto the explants. The bioreactors were covered to obtain a light level comparable to that of the natural environment, namely low light during the day and complete darkness during the night. The overflow of bioreactors 1 and 3 was recycled via a three-step filtration unit. The seawater was first passed through a biological stone filter, then a cotton wool filter, and finally through a 0.2-μm filter before it was reused in the dilution reactor. A weighing column was placed in the circulation path and used for the determination of the underwater weight of the sponges. This method prevented thermo- or salinity shocks for the sponges during the underwater weight determination and increased the accuracy of the measurements.

Experimental Procedures. Two *D. avara* explants (1 and 2) were cultured in bioreactor 1. They were fed solely with live *P. tricornutum* by a continuous flow from the dilution reactor (7 L/day), containing 1×10^5 cells/mL (based on Osinga et al., 2003). Explants 3 and 4 were cultured in bioreactor 2 and received an additional inflow of Complete Nutrition Mix stock solution (concentration in the reactor: 0.1 g/L fish extract, 1 ml/L serum, and 0.5 ml/L of vitamins). After day 40, explants 5 and 6

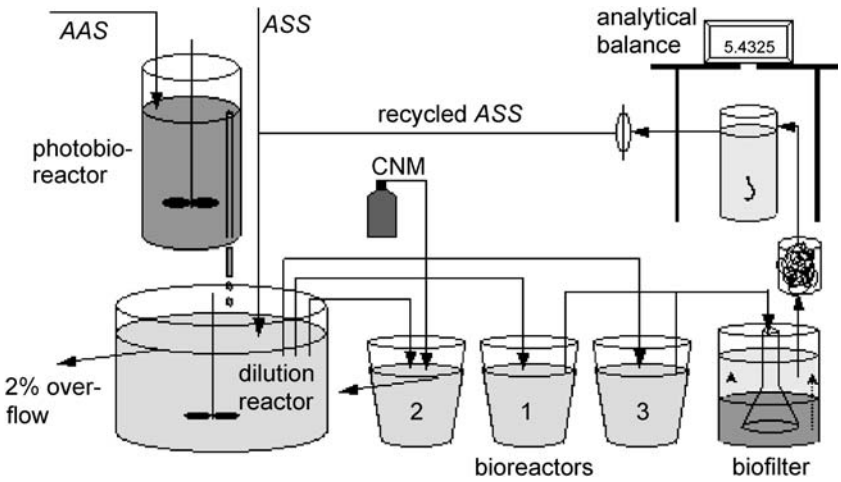

Fig. 2. The *D. avara* continuous cultivation system was composed of a photobioreactor, an algae dilution reactor, and three bioreactors for the cultivation of sponges. The effluent from bioreactors 1 and 3 was reused after filtration through three filters: a biofilter, a cotton wool filter, and a 0.2-µm bacterial filter. The explants in bioreactor 2 were initially continuously supplied with a complete nutrition mix. AAS, Artificial algae seawater; ASS, artificial sponge seawater; CNM, complete nutrition mix.

were cultured in bioreactor 2 under exactly the same conditions as in bioreactor 1. Bioreactor 3 was operated as a control reactor to examine the filtration rate of the sponges. No sponges were cultured in this reactor, while it received the same continuous flow of algae cells as bioreactor 1.

Chondrosia reniformis *cultivation system.* *C. reniformis* explants were cultured in two 7-L bioreactors in a continuously operating system at a temperature of 20°C (comparable to the natural ambient summer temperature in the Mediterranean at a depth of 5 to 10 m). Mixing light and the positioning of the explants were the same as in the cultivation of *D. avara*. Explants 1, 2, and 3 were placed in bioreactor 1 and received a continuous flow of 7 L of artificial sponge seawater per day containing an amount of algae powder equivalent to approximately 2.5×10^4 cells/ml. The overflow of this reactor was recycled via a filtration system and the weighing column was similar to the system used to culture *D. avara*. Explants 4 and 5 were placed in bioreactor 2 and received a continuous flow of 7 L/day of artificial sponge seawater containing algae powder (equivalent to 2.5×10^4 cells/ml) and fish powder (0.02 g/L). The overflow of this bioreactor was discarded. Based on total organic carbon (TOC) measurements of the algae-and-fish powder, the TOC concentrations in bioreactor 1 and 2 were expected to be approximately 1 and 2 mg/L, respectively. The two vessels containing the seawater enriched with nutrients were placed in the refrigerator and continuously stirred to prevent sedimentation of the substrates. These vessels were cleaned daily and refilled with fresh solutions.

Analyses. *Microalgae:* Throughout the *D. avara* cultivation experiment, the *P. tricornutum* cell concentrations in the growth, dilution, and sponge reactors were determined daily using a Coulter Multisizer type II automatic particle counter.

Organic carbon: The total organic carbon (TOC) concentration in the sponge reactors was determined regularly by subjecting samples to wet oxidation, using an OIC 700 Total Organic Carbon analyzer. The carbon content of *P. tricornutum* cells was determined by a duplicate measurement of the TOC concentration in artificial seawater containing 7.5×10^4 cells/ml and subtracting the TOC concentration of artificial seawater.

Size Determination of Explants. The size of the explants plus tile was measured as the underwater weight using an A&D HR300 analytical balance with underweighing device (Figure 2). The sponge explants were moved from the bioreactors to the weighing column without exposing them to the air. The salinity (33%) and temperature (16.5° or 20°C) in the weighing column were always the same as in the bioreactors. The underwater weight of the empty tiles was subtracted from the measured weights to obtain the underwater weight of the explants.

Statistical Methods. The suitability of the three different models to describe growth of globular and encrusting sponges was assessed via the following steps:

1. Determination of the growth rate constants (k, μ, and c) for all specimens for which the best fit of the models (equations 1 to 4) could be obtained (lowest sum of squares).

2. Normalization of all calculated growth rate constants for each species and model. The growth constants must first be normalized to be able to make a sound comparison between the different model equations, as follows:

$$\mu_{n,Da,1} = \frac{\mu_{Da,1}}{\mu_{Da,\,\max}}$$

in which:

μ = growth rate constant of the exponential model

μ_n = normalized μ

Da, 1 = Dysidea avara specimen 1

Da, max = Dysidea avara specimen with the highest exponential growth rate constant

3. Plotting the normalized growth rate constants per species per growth model and determination of the variance with the best horizontal fit of the data (i.e., sponge size does not affect its growth rate constant).

4. A t-test was used to determine the significance of differences in variance between the models.

Results and Discussion

Cultivation of Dysidea avara. *D. avara* explants were cultured in bioreactors for a period of 37 to 83 days. All explants supplied solely and continuously with live *P. tricornutum* displayed growth (Figure 3). The relatively fast growth of explant 2 during the first 20 days is remarkable if one takes into account

that the algae concentration during this period was only a fraction of the desired concentration of 1×10^5 cells/ml. An organic carbon content of 36 pg C/cell was determined for *P. tricornutum*, which implies that the average organic carbon concentration in the bioreactors during the first 20 days was only 0.24 mg C/L. This is much lower than concentrations that are usually found in the Mediterranean Sea, which range from 0.6 to 3.4 mg/L (Ribes et al., 1999; Seritti et al., 2003; our data: Kos (Greece), 1.29 mg/L; Kalymnos (Greece), 3.43 mg/L; and Rovinj (Croatia), 1.89 mg/L). During this period of nutrition-poor conditions, explant 1 decreased in size. The resumption of growth of explant 1 after day 40 may be correlated with the presence of sufficient *P. tricornutum* cells in the bioreactors after that date. The average concentration between day 35 and day 60 was 5.65×10^4 cells/ml, which corresponds to approximately 1 mg C/L. At day 41 explants 5 and 6 were introduced in bioreactor 2, and between days 41 and 60 all explants displayed growth. No significant differences between the *P. tricornutum* concentrations in bioreactors 1 and 2 and the control bioreactor 3 without sponges were observed, which indicates that algal consumption by the sponges was low compared to the continuous supply of fresh algae. Because of an unfortunate occurrence (failure of the cooling water bath) the experiments came to an abrupt end at day 76. The failure caused the temperature to rise from 16.5 to 20°C during the night, and led to disintegration and significant weight loss of all explants (Figure 3). No significant changes were measured for the control dishes (dishes without a sponge), indicating that the increase in the underwater weight of the experimental sponges was truly related to growth.

Explants 3 and 4 (which were initially cultured in bioreactor 2) received a continuous extra nutrition flow composed of fish powder, serum, and vitamins. These explants died after 1 week, probably owing to strong microbial growth within the culture vessel, and subsequent dissolved oxygen depletion. The total organic carbon (TOC) concentration in this bioreactor was 38 mg/L, which is much higher than natural occurring concentrations. The high concentration of easily metabolized carbon sources was obviously more favorable for growth of bacteria and fungi (which were visible as long slimy threads) than it was for growth of the sponges.

Cultivation of Chondrosia reniformis. Five *C. reniformis* explants were cultured in two bioreactors with either a continuous supply of algae powder or a mixture of algae and fish powder. The underwater weight of all explants increased during the experiment (Figure 4). An empty tile was included in both bioreactors to correct for measurement errors due to slight changes in temperature and salinity during the determination of the underwater weight. No significant differences were found between the average growth rates in bioreactors 1 and 2. This may have been the result

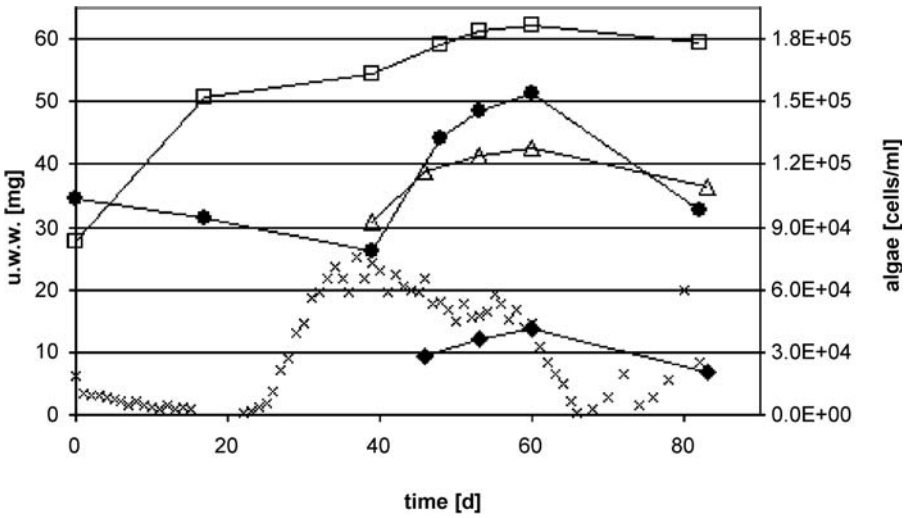

Fig. 3. The underwater weight (uww) of *D. avara* explants 1 (●), 2 (□), 5 (△), and 6 (♦) during 83 days of cultivation. The underwater weight of explant 2 must be multiplied by four (real *x*–*y* coordinates are: (0, 111), (17, 203), (39, 217), (48, 236), (53, 244), (60, 249), (82, 238). The actual data were divided by four to obtain a clearer view in the graph. The *P. tricornutum* cell concentration (×), which was present in the bioreactors as nutrition for the explants, is depicted on the secondary *y*-axis. For clarity of the graph only the cell concentration in bioreactor 1 is shown; however, the cell concentrations in all of the bioreactors were almost equivalent throughout the experimental period.

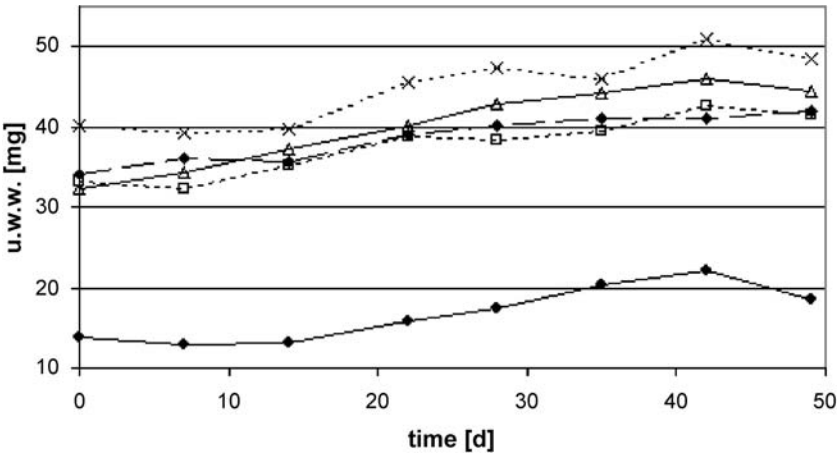

Fig. 4. The underwater weight (uww) of *C. reniformis* explants. Explants 1 (●), 2 (□), and 3 (△) were cultured in bioreactor 1 and were supplied solely with algae powder. Explants 4 (♦) and 5 (×) were cultured in bioreactor 2 and were fed with a mixture of algae and fish powder.

of comparable average TOC concentrations in bioreactors 1 and 2 (1.43 ± 1.23 and 1.43 ± 1.29 mg C/L, respectively). It was unexpected that the TOC concentrations would be the same in both bioreactors, as bioreactor 2 was supplemented with additional fish extract. The TOC concentration in bioreactor 2 may have been lower than expected because of observed sedimentation of floccules onto the sponges and onto the bottom of the reactor. Following this initial observation of flocculation, the two bioreactors and the sponges were carefully cleaned once during every subsequent week of the experiment. For most sponges the major increase in underwater weight occurred between days 14 and 42, except explant 3, which increased weight throughout the entire 42-day period. Growth for all explants halted after day 42 and the underwater weight of all explants remained constant for more than 1 month (not in graph) before the experiment ended.

For *C. reniformis*, some growth data derived from the sea are available, and from these data it can be concluded that *C. reniformis* is very slow growing compared to most other sponges (Wilkinson and Vacelet, 1979; Garrabou and Zabala 2001). Garrabou and Zabala found a growth rate of $7.2 \times 10^{-4} \pm 6.2 \times 10^{4}$/day, which was calculated via an equation that approximates exponential growth. Wilkinson and Vacelet (1979) tested the effect of different light and current conditions on the growth of *C. reniformis* in the sea. If the exponential model is used to estimate the growth rate for their specimens (cultured under optimized conditions with respect to light and current) a growth rate of 1.8×10^{-3}/day is obtained. For the explants that were cultured in our bioreactors an average growth rate of $6.7 \times 10^{-3} \pm 1.3 \times 10^{-3}$/day is obtained via the exponential model. This is four to nine times higher than in the sea, and may give an indication of the potential advantages of *ex situ* cultivation of marine sponges. However, since our measured *ex situ* growth rate is based on only five explants (and as reproducibility is a general problem in sponge culture experiments) the actual growth rates that can be reached in *ex situ* culture systems remain elusive, to be determined in an expanded set of future growth experiments using higher numbers of replicates per growth trial.

In addition, we consider that the exponential model may not be the best model to describe sponge growth. Therefore a comparison between growth rates derived via the exponential model may result in an incorrect assumption as to the suitability of *ex situ* cultivation of sponges. For *C. reniformis* it was not possible to compare growth rates reported for in situ growth with our *ex situ* growth data using the linear or surface-dependent growth model, because size is reported in mm^2 (while it is a globular species) or no size could be deducted from the data reported (Wilkinson and Vacelet, 1979; Garrabou and Zabala, 2001).

Growth Kinetics. To determine the best model to describe the growth of sponges the growth data of the globular sponges *D. avara* and *C. reniformis* were fit with the equations of the linear, exponential, and surface-dependent growth models (equations 1, 2, and 3, respectively). In addition, literature data available for the marine sponge *Pseudosuberites andrewsi* (Osinga et al., 2003) were compared to the three models to expand the data set. The growth kinetics of encrusting sponges was studied based on field data for *Oscarella lobularis*, *Hemimycale columella* (Garrabou and Zabala, 2001) and *Crambe crambe* (Turon et al., 1998; Garrabou and Zabala, 2001). For *O. lobularis* and *C. crambe*, which display clearly seasonal growth, only data for the period of growth were used. The model equations (1, 2, and 3/4) were used

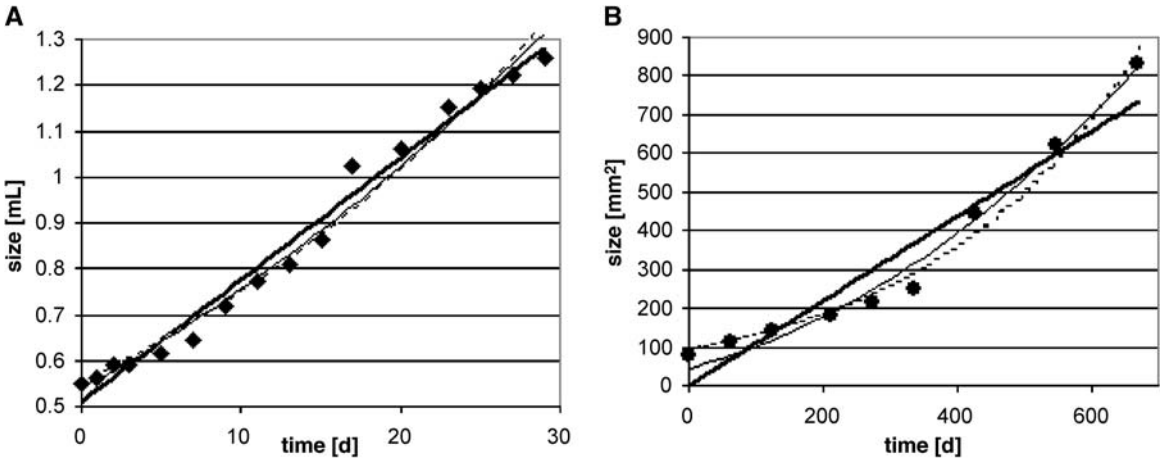

Fig. 5. (**A**) Example of the growth curve of a globular specimen (*P. andrewsi*) with best fits of the linear (▬), exponential (- - -), and surface-dependent (—) models. (**B**) Example of the growth curve of an encrusting specimen (*H. columella*) with best fits of the linear (▬), exponential (- - -), and circumference-dependent (—) models.

to determine the growth rate constants (k, μ, and c) for which the best fits of the data could be obtained (Figure 5).

It can be seen that especially for very short-term cultivations (ca. 30 days; Figure 5A) the differences between the three models are smaller than for longer-term cultivations of more than 600 days (Figure 5B). The calculated growth rate constants for all explants according to the three models were related to their initial size (Figure 6). Theoretically, if a model appropriately describes growth there should be no correlation between the growth rate constant and the size of the sponge. From Figure 6, no quantitative information to support determina-

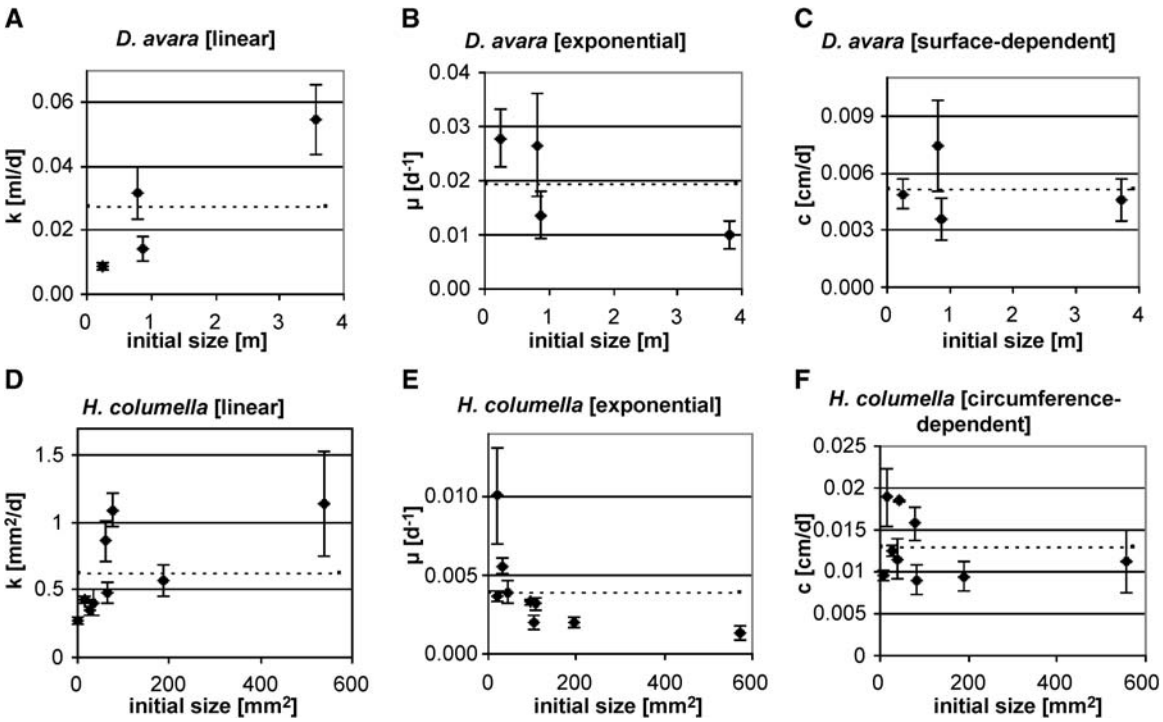

Fig. 6. The relationship between the size of the sponge and the growth rate constant according to the three different models for a globular and an encrusting sponge with their standard errors. The linear (**A**), exponential (**B**), and surface-dependent (**C**) models describe the growth of *Dysidea avara* specimens (globular). The linear (**D**), exponential (**E**), and circumference-dependent (**F**) models describe the growth of *Hemimycale columella* specimens (encrusting). The dotted line represents the constant growth rate with the smallest sum of squares. To compare the three models, these data were normalized so that the variance of the three different models could be compared.

tion of the best growth model can be derived. Therefore the data depicted in Figure 6 were normalized, and the variance of the normalized calculated growth rate constants of the three models was used to determine which model best described the data (Figure 7). For all *ex situ* cultivated globular sponges [*D. avara*, *C. reniformis,* and *P. andrewsi* (Osinga et al., 2003)] the surface-dependent model was determined to be the best model to describe the growth of globular morphotypes (Figure 7). For two encrusting sponges (*H. columella* and *C. crambe*) the circumference model proved to be the best model to describe growth. For *O. lobularis* the exponential model best simulated the growth observed. However, for *O. lobularis* the variance between the model and the calculated data was higher than for the other sponges. This indicates that the reliability of the models used to describe the growth for this species is lower than with the other sponges. This is most likely the result of the relatively small amount of data available for this sponge.

These data lead us to the conclusion that in general: $r_t = r_0 + c \cdot t$ (where r is the radius, and c is the surface-dependent or circumference-dependent growth rate constant) is currently the best kinetic model to describe growth of both encrusting and globular sponges. The surface-dependent or circumference-dependent growth rates of all sponges assessed in this study are summarized in Table 1. Also included in Table 1 are the data of Ayling (1983), who expressed growth of a number of encrusting sponges as increase of the area per centimeter border per day (which is the same as radial accretive growth). It can be seen from these data that *D. avara* and *P. andrewsi* grow faster than *C. reniformis*.

Table 1. The Calculated Surface-Dependent and Circumference-Dependent Growth Rates (c) and Their Standard Deviations for Globular (g) and Encrusting (e) Species, Respectively.

Sponge	*c (mm/d)*
Pseudosuberites andrewsi (g)	0.068 ± 0.038
Dysidea avara (g)	0.051 ± 0.016
Oscarella lobularis (e)	0.036 ± 0.019
Aplysilla rosea (e)[a]	0.028 ± 0.019
Stylopus sp. (e)[a]	0.023 ± 0.009
Chondrosia reniformis (*ex situ*) (g)	0.019 ± 0.003
Hemimycale columella (e)	0.013 ± 0.004
Chondropsis sp. (e)[a]	0.013 ± 0.009
Crambe crambe (e)	0.011 ± 0.007
Tedania sp. (orange) (e)[a]	0.008 ± 0.005
Stylopus sp. (e)[a]	0.008 ± 0.006
Chelonaplysilla sp. (e)[a]0.006 ± 0.005	
Hymedesmia sp. (red) (e)[a]	0.005 ± 0.003
Hymedesmia sp. (orange) (e)[a]	0.002 ± 0.003
Anchinoe sp. (e)[a]	0.001 ± 0.006
Eurypon sp. (e)[a]	0.0003 ± 0.031
Microciona sp. (e)[a]	–0.001 ± 0.0003

[a]Sponge growth rates determined by Ayling (1983).

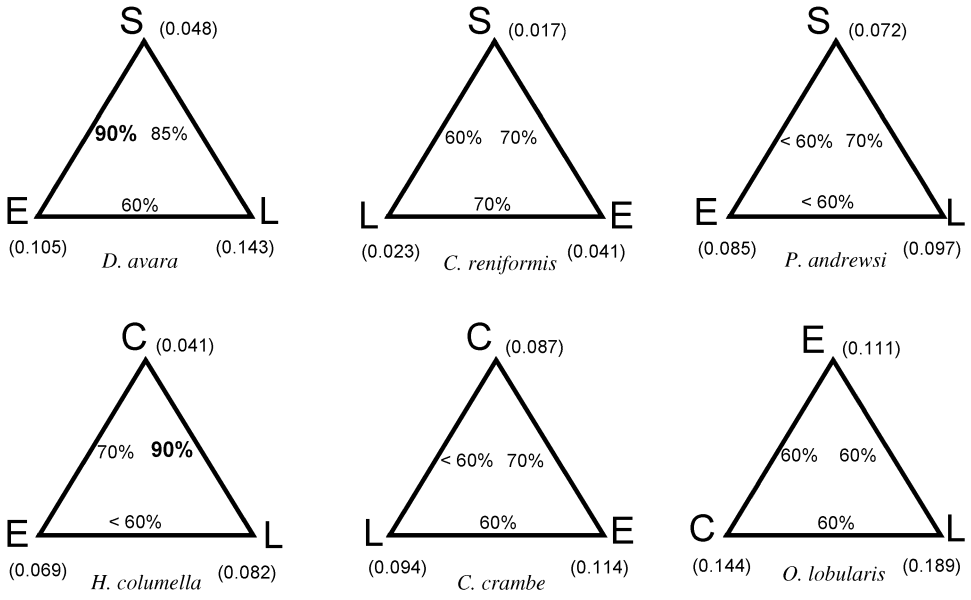

Fig. 7. The variance of the normalized calculated growth constants in relation to the three growth models. The three upper triangles show the result for the globular sponges, while the three lower triangles present the result for the encrusting species. The best model (with the lowest variance) is always depicted at the top of the triangle. The second-best and third-best models are depicted in the left and right corners of the triangles, respectively. **L**, linear; **E**, exponential; **S**, surface-dependent (for globular sponges); and **C**, circumference-dependent (for encrusting sponges). The variance of the different models is given in parentheses. The confidence intervals for significant differences between the models are presented along the sides of the triangles.

mis. However, *C. reniformis* has been characterized as a very slow grower by Garrabou and Zabala (2001) and by Nickel and Brümmer (2003). The radial accretive growth rates of the globular sponges are generally higher than those of the encrusting sponges. However, these differences may be at least partially caused by the different conditions to which the sponges were exposed (encrusting sponges in the sea and globular sponges in the laboratory).

It is also concluded that even when the data available in the literature are combined with our own data, this is still not sufficient to obtain highly significant differences between the three growth models assessed (Figure 7). Only for *H. columella* is the circumference-dependent model significantly better than the linear model, and for *D. avara* the surface-dependent model is significantly better than the exponential model (90% confidence interval with *t*-test). However, the significance levels (based on differences between normalized calculated growth constants and the model) do not take into account a trend that can be discerned in the data. In Figure 6 it can be seen that especially for the linear and exponential models there appears to be correlation between the initial size of the sponge and the growth rate. This trend is not as obvious for the circumference- or the surface-dependent growth models, which suggests that the linear and exponential models are not appropriate to describe the growth of globular or encrusting sponges.

The lack of significant differences between the three growth models may be further explained as follows. First, the data sets were based on a relatively small number of individuals per species: *O. lobularis* (4), *H. columella* (9), *C. crambe* (11), *D. avara* (4), *C. reniformis* (5), and *P. andrewsi* (7). This problem of a low number of replicates persists even with the incorporation of literature data, as references containing information about both growth rates and size of the sponge are few. Second (and especially for the specimens that were cultured in the laboratory), time in culture was relatively short, and because of the inherent slow growth rate of sponge in general, the differences observed between the three models remained small. Third, the globular sponges were modeled as hemispheres for the calculation of the surface-dependent growth rate, and therefore every deviation of this ideal morphology leads to a larger surface when related to its volume. The *C. reniformis* explants resembled a hemisphere quite well whereas the *D. avara* and *P. andrewsi* explants were more erratically shaped (Figure 8). Given that surface area is the major factor that determines sponge growth, a more accurate estimation of the surface of globular-shaped sponges is required. Accuracy in determining the actual circumference of encrusting sponges modeled as circles is likewise important, but for encrusting sponges it is easier to determine the exact circumference by use of underwater photography. Fourth, it is probable that additional factors such as pressure, light, nutrition concentration, and current speed affect the growth rate of sponges, for which terms are not included in the simplistic equations derived in this study. For example, ambient current profiles may cause a nutritional gradient around and over the sponge. In recent research it has been found that such factors can significantly affect growth rate and the morphology of the sponge (McDonald et al., 2003). Kaandorp (1995) has developed elegant morphological models to describe the growth pattern of sponges based on simulated nutrition profiles around a sponge. Using these models, Kaandorp found that the morphological growth pattern of the sponge could be predicted quite well. Combining the morphological models of Kaandorp, with the simple kinetic model described in this article could lead to a more accurate prediction of the growth rates of sponges under differing conditions.

It is interesting to examine the implications of applying growth models to mariculture or *ex situ* cultivation of sponges. Assuming radial accretive growth, the highest absolute increase of biomass is supported by the use of small explants, as the surface

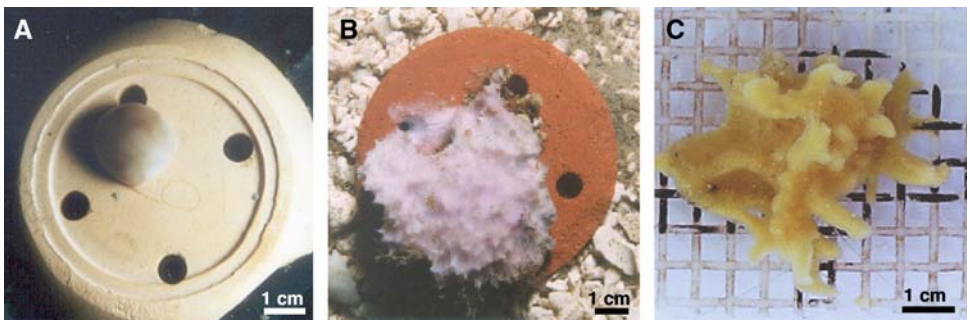

Fig. 8. Differences in the morphology of *C. reniformis* (**A**), *D. avara* (**B**), and *P. andrewsi* (**C**) explants.

area/volume and circumference/area ratios are highest for small globular and encrusting sponges, respectively. However, small explants generally have a higher mortality than larger explants (Duckworth et al., 1997; Turon et al., 1998). Therefore the optimal size and number of explants to be used to start and scale up a mariculture or an *ex situ* tank culture operation will be a trade-off between an acceptable mortality and a predicted growth rate for the species of choice. These choices will in turn be governed by the prevailing environmental and/or laboratory conditions and the desired outcomes of the project, based on desired yields and acceptable economics.

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