MEASUREMENT OF SPONGE GROWTH BY PROJECTED BODY AREA AND UNDERWATER WEIGHT

RONALD OSINGA, DAVID REDEKER, PETER B. DE BEUKELAER AND RENÉ H. WIJFFELS

Osinga, R., Redeker, D., De Beukelaer, P.B. & Wijffels, R.H. 1999 06 30: Measurement of sponge growth by projected body area and underwater weight. *Memoirs of the Queensland Museum* **44**: 419-426. Brisbane. ISSN 0079-8835.

In vitro growth rates of the Indo-Pacific demosponge *Pseudosuberites andrewsi* (Kirkpatrick) were measured using two alternative techniques to estimate biomass: determination of projected body area, and determination of underwater weight. Four small explants of *P. andrewsi* were fed regularly with the microalgae *Rhodomonas* sp. and *Chlorella sorokiniana*, and growth was monitored over a period of 24 days. Three explants showed considerable increase in both projected body area and underwater weight, but the growth pattern was irregular. Although the observed trends in growth were similar for both methods, the absolute values were not in general agreement, which may be due to the fact that photographic data were two-dimensional. It was concluded that determination of underwater weight is a promising method for measuring growth of sponges if the size of the explants used is sufficiently large. Measuring projected body area has a higher precision when explants are smaller than 10mg and is a preferred method when small explants are used. ❐ *Porifera, Pseudosuberites andrewsi, growth monitoring, projected body area, underwater weight.*

Ronald Osinga (e-mail: ronald.osinga@algemeen.pk.wau.nl), David Redeker, Peter B. de Beukelaer & René H. Wijffels, Wageningen Agricultural University, Food and Bioprocess Engineering Group, PO Box 8129, 6700 EV Wageningen, The Netherlands; 17 March 1999.

Due to the rich potential of marine sponges as producers of interesting natural compounds, there is a growing need for methods to produce large amounts of sponge biomass (Munro et al., 1994; Osinga et al., 1998a). *In vitro* cultivation of sponges in bioreactors may be an interesting option for mass production of sponge metabolites, because such systems can easily be manipulated and optimised. An essential prerequisite for studying and optimising *in vitro* growth of sponges, is to have a good method to monitor this growth.

Sponge growth can be monitored by measuring increases in biomass. Thus, to detect slight changes in growth rates, a method is required to precisely measure sponge biomass. This method should not negatively affect the survival or growth of sponges, and in this respect it is important to keep sponges continuously underwater. Although some sponge species can tolerate short exposure to air, it is generally assumed that exposure to air can be harmful to living sponge tissue. Air entering the aquiferous system can irreversibly damage choanocyte chambers (Fosså & Nilsen, 1996). Therefore, determination of sponge volume (by water replacement), wet weight, or drip dry wet weight, although often applied (e.g. Barthel, 1986; Thomassen & Riisgård, 1995), are not preferred methods to measure living sponge biomass and to simultaneously maintain viable experimental populations.

In some studies, growth rates are determined by measuring the area of two-dimensionally projected images of the sponge body. Ayling (1983) used this technique to measure *in situ* growth and regeneration rates of several encrusting sponge species in the coastal water of New Zealand. Series of photographs were taken underwater over a period of time and the images were projected on graph paper. Poirrier et al. (1981) used similar methods to measure *in vitro* growth of the freshwater sponges *Ephydatia fluviatilis* and *Spongilla alba*. Although these methods may be suitable to measure growth in almost two-dimensionally growing encrusting species, or for regularly shaped species such as the sphere-shaped *Cinachyrella* spp. and *Tethya* spp., problems may occur when the two-dimensional surface area data are converted into three-dimensional volumes. Especially with more irregularly shaped species, increases in surface area can easily under- or overestimate increases in body volume, especially in more irregularly shaped species.

FIG. 1. A 200dm^3 air-lift bioreactor for maintaining the culture of *P. andrewsi*.

In this study, we introduce a new, three-dimensional measure of sponge biomass: underwater weight. Determination of underwater weight is used to measure *in vitro* growth rates of the Indo-Pacific demosponge *Pseudosuberites andrewsi* (Kirkpatrick). These results are compared with two-dimensional growth rates obtained from projected body areas. The value of underwater weight as a measure of sponge biomass is further evaluated by correlating these data to other biomass parameters (volume, wet weight, dry weight and ash-free dry weight).

MATERIALS AND METHODS

SPONGES. On the basis of previous results (Osinga et al., 1998b), *P. andrewsi* was selected as a model species for further experiments to improve the methodology for *in vitro* sponge culture. Living material of *P. andrewsi* was obtained from Blijdorp Zoo (Rotterdam, The Netherlands), where it was growing in a large, shallow basin, in which a strong water current was generated to simulate an intertidal environment. We are uncertain about the location were these sponges originally came from. They had been introduced in the zoo coincidentally on so called 'living stones', which were presumably collected from Indonesian coastal waters. In our laboratory, we have been able to maintain small colonies of this species for more than a year under the conditions described below.

Sponges were held in a 200dm^3 airlift bioreactor (Fig. 1) containing artificial seawater (using Instant Ocean Reef Crystals artificial sea salt) with a salinity of ~32‰. This water was replaced continuously ($D=0.033d^{-1}$). The temperature in the bioreactor varied between 25-29°C. In order to provide the sponges with a source of silica, 0.25mM $Na_2O_3Si\ 9H_2O$ was added to the artificial seawater. Measurements of the silica concentration in outflow water showed that this addition was sufficient to cope with sponge demands. Non-axenic batch-cultures of two species of microalgae were regularly added as a food source for the sponges. Twice a week, 1dm^3 of a culture of the freshwater alga *Chlorella sorokiniana* (Chlorophyceae, average size ~3µm) was added, containing ~$1x10^7$ cells cm^{-3}. In addition, 1dm^3 of a culture of marine *Rhodomonas* sp. (Cryptophyceae, average size ~6µm), containing ~$1x10^6$ cells cm^{-3}, was added weekly. The algae were cultured at a temperature varying between 17-20°C. A light-dark cycle of 14hrs light and 10hrs darkness was applied. The growth media for the algae are given in Table 1. When the cultures were added to the sponge reactor, the algae were usually near the end of their logarithmic growth phase.

The choice to use these two algae in current experiments was based on the literature. Additions of *Chlorella sorokiniana* were used successfully to enhance the growth of the temperate sponge *Halichondria panicea* in semi-controlled cultures (Barthel & Theede, 1986). *Rhodomonas* sp. was used by Thomassen & Riisgård (1995) to feed *in vitro* cultures of *H. panicea*.

Growth experiment. Comparative growth rate measurements were performed on four explants colonies of *P. andrewsi*. Explants were prepared using razor-sharp knifes. Pieces of sponge tissue were tied onto perspex slides with nylon fishing-line. Explants were placed in temperature controlled, 1.58dm^3 bioreactors, equipped with a

TABLE 1. Growth media for algae (a freshwater medium for *Chlorella sorokiniana* and a seawater medium for *Rhodomonas* sp.). The freshwater medium was based on the A9 medium described by Lee & Pirt (1981). Concentrations are given in mM, unless indicated otherwise.

Component	Freshwater medium concentration	Seawater medium concentration
$NaHCO_3$	10.0	5.00
KNO_3	1.00	0.50
NaH_2PO_4	0.10	0.05
Instant Ocean Reef Crystals artificial seasalt		~ 33 g dm^{-3}
$MgSO_4 \cdot 7H_2O$	4.99	
$CaCl_2 \cdot 2H_2O$	0.272	
$EDTANa_2 \cdot 2H_2O$	0.391	
$FeCl_3$	0.148	
$Na_2B_4O_7 \cdot 10H_2O$	$4.72 \cdot 10^{-2}$	
$ZnSO_4 7H_2O$	$3.13 \cdot 10^{-2}$	
$CuSO_4 \cdot 5H_2O$	$3.20 \cdot 10^{-2}$	
$MnSO_4 \cdot H_2O$	$3.59 \cdot 10^{-2}$	
$Na_2MoO_4 \cdot 2H_2O$	$2.07 \cdot 10^{-2}$	
$NiSO_4 \cdot 6H_2O$	$2.85 \cdot 10^{-3}$	
$NaVO_3$	$2.85 \cdot 10^{-3}$	
thyamin-HCl		$5.93 \cdot 10^{-5}$
cyanocobalamin		$5.90 \cdot 10^{-6}$
biotin		$1.64 \cdot 10^{-6}$

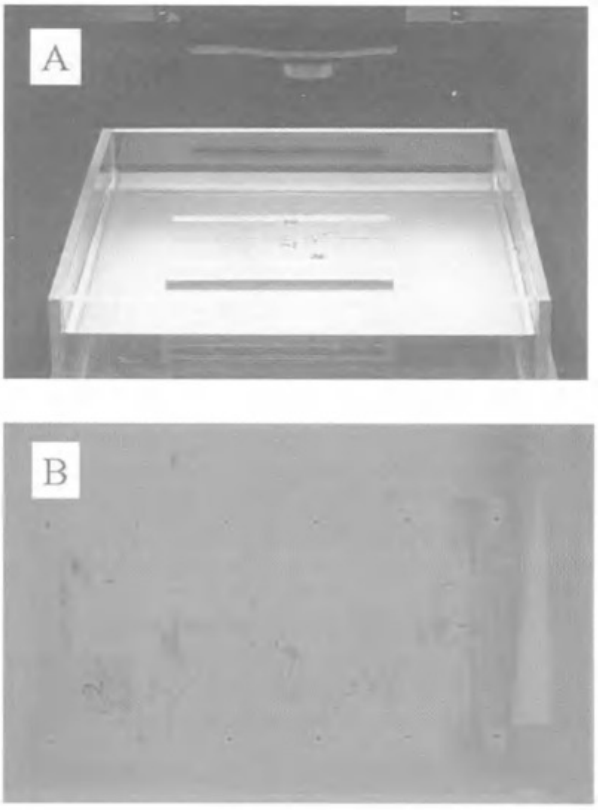

FIG. 2. Photographic method. A, Overview of the system. B, Detail of the explants laying on the perspex rack. The black dots on the rack indicate a known distance.

sparger for air-supply and a magnetic stirrer to keep food particles in suspension.

Sponges were fed with *C. sorokiniana* (twice a week, 50cm^3) and *Rhodomonas* sp. (once a week, 50cm^3) using material from batch-cultures described in the previous section. Temperature and salinity in the bioreactors were kept constant at 25°C and 33‰, respectively. The experiment was run for a period of 24 days. Monitoring of the growth of the explants was performed according to the procedures described below.

Determination of projected body area. During the experiment, several photographs of the explants were made to determine changes in the projected body area. To take photographs, explants were removed from the bioreactor (kept underwater, in a beaker glass) and placed onto a rack (also underwater) on which black dots were painted to indicate a known distance (Fig. 2). Photographs were taken under a straight angle with a digital camera (Hewlett Packard Photo-Smart Model C5340A). Digital images were printed, the areas of sponges were cut out with scissors and these cuttings were weighed. Weights of these cuttings were converted to areas by comparing them to the weight of a cutting of a known area. These values were converted to real body areas using the marked distances on the rack as a reference. Photographs were taken at Days 1, 7, 10, 20 and 22.

Determination of underwater weight. Underwater weight was measured using a A&D HR300 analytical balance (weighing range: 0.0001-300g) equipped with an underweighing-possibility. A hanger was connected to the balance, in which the slides with the explants could be placed. This balance was placed over a small basin filled with artificial seawater in such a manner that the part of the hanger containing the explant would remain underwater (Fig. 3). It is important to keep the level and salinity of the seawater in the basin constant. Changes in salinity will change the density of the seawater. Since sponge tissue is not much denser than seawater, a slight change in salinity will affect the underwater weight of sponges. The salinity of the seawater in the basin was always maintained at 33‰.

FIG. 3. Underwater weight measurement. Detail of an explant placed in the hanger under the balance.

The underwater weight was calculated by subtracting the weight of the carrier slide from the combined weight of the explant + carrier slide. It was therefore important that the weight of the carrier slide remained constant throughout the experiment, especially since the slides in this study were much heavier than the explants. Therefore, an inert material was required to be used as carrier, and consequently we chose to use perspex slides instead of the commonly used glass slides (e.g. Simpson, 1963; Poirrier et al., 1981; Vethaak et al., 1982), because glass was found to dissolute slowly in seawater, causing a slow, but steady decrease of the underwater weight of the carrier slide.

Explants were transported from the bioreactors to the weighing basin underwater in a beaker glass. Measurements were performed on Days 1, 2, 5, 9, 12, 22 and 24.

Determination of volume, wet weight, dry weight and organic carbon and nitrogen content. In order to evaluate the utility of underwater weight as a measure of biomass, underwater weight data were correlated to other non-destructive biomass parameters, volume and wet weight (WW).

To determine volume and WW, sponge-explants were removed from the water and firmly shaken until they no longer dripped. Volume was then determined by putting an explant into a graded cylinder filled to a certain reference level with artificial seawater. After addition of the explant, all water in excess of this reference level was removed with a syringe and transferred into a $1cm^3$ glass pipette, in which the volume of excess water could be determined. In this way, sponge volumes of about $0.1cm^3$ could be determined with reasonable precision (the methodological error was less than 10%). The corresponding WW of explants was measured on an analytical balance. For these measurements, it was imperative that explants were not attached to carrier materials, and hence, volume and WW determinations of experimental explants were undertaken immediately prior to growth experiments. Some additional explants were measured to obtain more reliable correlations/ conversion factors.

Dry weight (DW) organic carbon content and organic nitrogen content of sponge tissue were also determined, but only for a single sample, since these measurements are destructive and only limited amounts of sponge material were available. For the determination of DW, pieces of sponge were dried for 24hrs in an oven at 80°C and weighed. The dried material was ground and analysed for organic carbon and nitrogen on a Fisons EA 1108 Elemental Analyser.

RESULTS AND DISCUSSION

GROWTH RATES AND KINETICS. Results of growth experiments are presented in Figure 4, showing changes in surface area and underwater weight. Three of the four explants showed growth during the experimental period, both when measured with two-dimensional photography and with the underwater weighing technique. The fourth explant did not show obvious changes in projected body area or underwater weight. This explant failed to attach to the perspex slide, while the other three explants firmly attached within a few days. Explants used for the experiment were made shortly before the experiment started. In future work, only healthy (attached) explants should be used, demonstrating viability after a period of acclimatisation

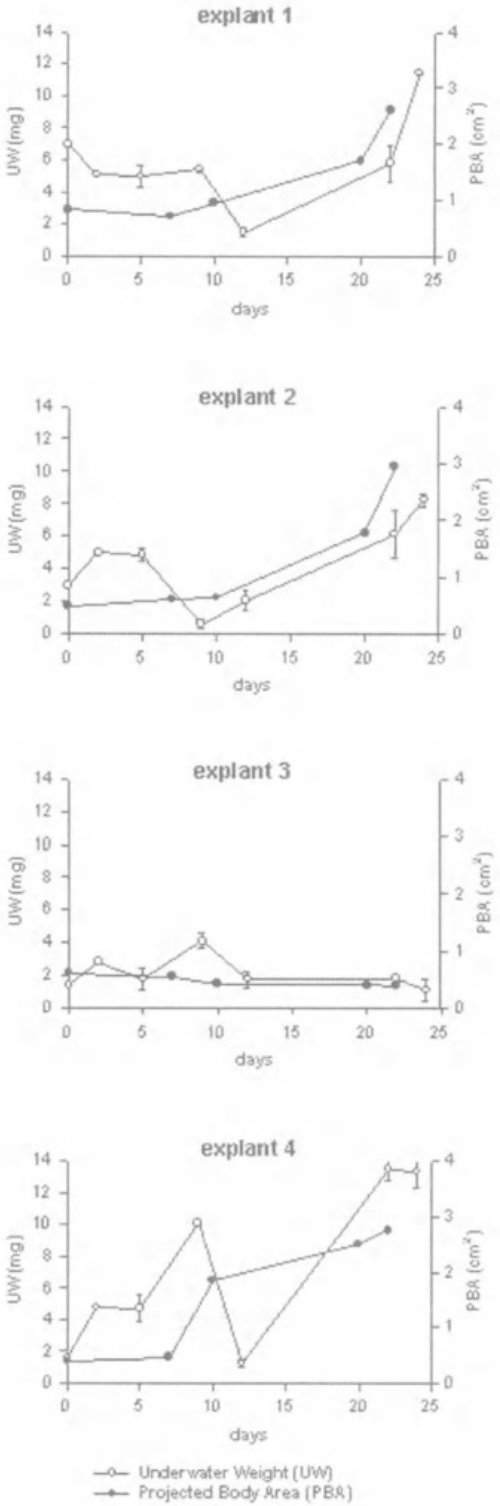

FIG. 4. Results of the growth experiment. Changes in underwater weight (mg, open symbols) and projected body area (dm^2, black symbols) of the four explants. Error bars for underwater weight data indicate the standard deviation of two replicate measurements.

in aquaria. However, some explants (e.g. explants in Fig. 3) attach to the glass only at one point, and start to form lateral processes that are not attached. These explants do grow, but this growth is difficult to quantify using photographic techniques. As these processes can easily break off from the explant, such explants are also not very suitable for growth experiments using determination of underwater weight or other weight parameters.

It is not easy to deduce general statements about the kinetics of growth in *P. andrewsi* from the data presented in Figure 4. Explants 1 and 2 seem to exhibit a kind of lag-phase, followed by a period of exponential growth after Day 12. The lag-phase may be some kind of response to cutting sponge tissue: the tissue must rearrange and attach to the substratum before growth can start. This process may have also caused the observed decrease in underwater weight of explants 1, 2 and 4 that occurred around Day 10-12. Rearrangement of the body into a functional, pumping sponge will cost energy that is probably obtained from respiring sponge tissue. More data are needed to give a reliable description of sponge growth in this species.

It is difficult to estimate a specific growth rate for *P. andrewsi* under the given experimental conditions, due to the strong variability in our data. We calculated specific growth rates only for explants 1 and 2, based on data measured after the lag-phase. These data tend to show exponential growth, which justifies calculation of a specific growth rate μ according to the formula:

$$\mu = t^{-1} \cdot \ln C_0/C_t$$

where C_0 is sponge biomass at the start of the exponential growth, C_t is sponge biomass at the end of the experiment, and t is the number of days between the start of the exponential growth and the end of the experiment. The calculated specific growth rates (Table 2) were between 0.08-0.10d^{-1} for data of projected body area and 0.16d^{-1} for underwater weight. These values are considerably higher than previously reported growth

TABLE 2. Specific growth rates (d^{-1}) for explants 1 and 2 during the period of exponential growth. Calculations are performed with data for projected body area (PBA) and underwater weight (UW).

Explant	Period used for calculation	μ(PBA)	μ(UW)
1	Days 7-22	0.08	
1	Days 12-24		0.16
2	Days 7-22	0.10	
2	Days 9-24		0.16

rates, which range from 0.01-0.058 (see Table 2, in Thomassen & Riisgård, 1995). Hence, *P. andrewsi* is able to grow faster under the applied conditions.

PROJECTED BODY AREA VS. UNDERWATER WEIGHT. Although general trends in results are similar between the two methods, some differences are apparent. The calculated specific growth rates for explants 1 and 2 (Table 2) are almost twice as high when underwater weight is compared to projected body area. Furthermore, data of underwater weight show a more irregular pattern than data of projected body area. The steep decrease in underwater weight for explants 1, 2 and 4 around Day 12 was not reflected in surface area. Shrinking of more massive body parts may not be reflected in changes in projected body area. Finally, the absolute growth after 22 days of the explants in projected body area is different from the growth in underwater weight (Table 3). These differences could be caused by the projection of three-dimensional growth onto two dimensional body areas. Explants 1 and 2 may have spread out horizontally without a corresponding increase in body mass, leading to an overestimation of actual growth. In contrast, explant 4 may have formed vertical outgrows that are difficult to quantify as increase in body area on a two-dimensional image, thus leading to an underestimation of growth.

A possible improvement for the photography method would be to use so-called 'sandwich-cultures', flat sponge tissue cultures growing in a narrow space between a glass slide and a cover slip. This method, introduced by Ankel & Eigenbrodt (1950) to study development of freshwater sponges, was successfully applied to seawater sponges by Langenbruch (1983) and Sanchez-Moreno (1984). Sandwich-cultures can be viewed as forced two-dimensional explants, and may thus be very suitable for growth rate measurements based on changes in projected body area. However, growth of sandwich-cultures may not mimic that of normal explants, which could be a major drawback when using this type of culture.

Despite the precision of 0.1mg provided by the analytical balance, the methodological error in the weighing technique (expressed in Fig. 4 as the standard deviation of two replicate measurements) is usually around 1mg. Hence, the precision of the weighing method for determining growth rates decreases when small explants are used. A better stabilised weighing device could probably improve this precision, but it is probably more practical to work with bigger explants with an underwater weight of at least 10mg. The methodological error in photographic measurements is not shown in Figure 4. A previous study in our lab (D. Redeker, unpublished data), set up to develop the photographic method, showed that this error is generally less than 10%, even when small explants are used. Images can be easily enlarged without losing too much contrast, which makes the photographic method more favourable over the weighing method when small explants are used.

VOLUME AND WEIGHT PARAMETERS. Volume (V), wet weight (WW) and underwater weight were compared in order to determine conversion factors for these parameters and to evaluate the utility of underwater weight as a measure of sponge biomass. Correlations are shown in Figure 5, and the corresponding conversion factors can be found in Table 4. Both V and WW of *P. andrewsi* showed a moderate positive correlation (Fig. 5A; r=0.78), that is highly significant (n=11, α=0.001). In a study on *Halichondria panicea*, Barthel (1986) also found that the correlation between V and WW was not very strong, probably due to variability in the water- and spicule-content of sponge tissue. In contrast, we found considerably stronger

TABLE 3. Growth of the sponge explants after 22 days (projected body area and underwater weight) and 24 days (underwater weight). Growth is defined as the newly formed sponge biomass, expressed as a percentage of the initial projected body area (PBA) or underwater weight (UW).

Explant	Increase in PBA after 22 days	Increase in UW after 22 days	Increase in UW after 24 days
1	215 %	- 30 %	63 %
2	500 %	105 %	175 %
3	- 37 %	7 %	- 21 %
4	605 %	745 %	735 %

TABLE 4. Wet Weight (WW), Underwater Weight (UW) and Dry Weight (DW) of 1cm^3 tissue of *P. andrewsi*, and the percentages of Organic Carbon Content (OCC) and Organic Nitrogen Content (ONC) in the dried sponge material. Key: 1, Not significant; 2, Reliability unknown (one sample only).

WW(mg)	UW(mg)	DW(mg)	OCC(% of DW)	ONC(% of DW)
0.68	0.044[1]	0.01[2]	13.9	3.15

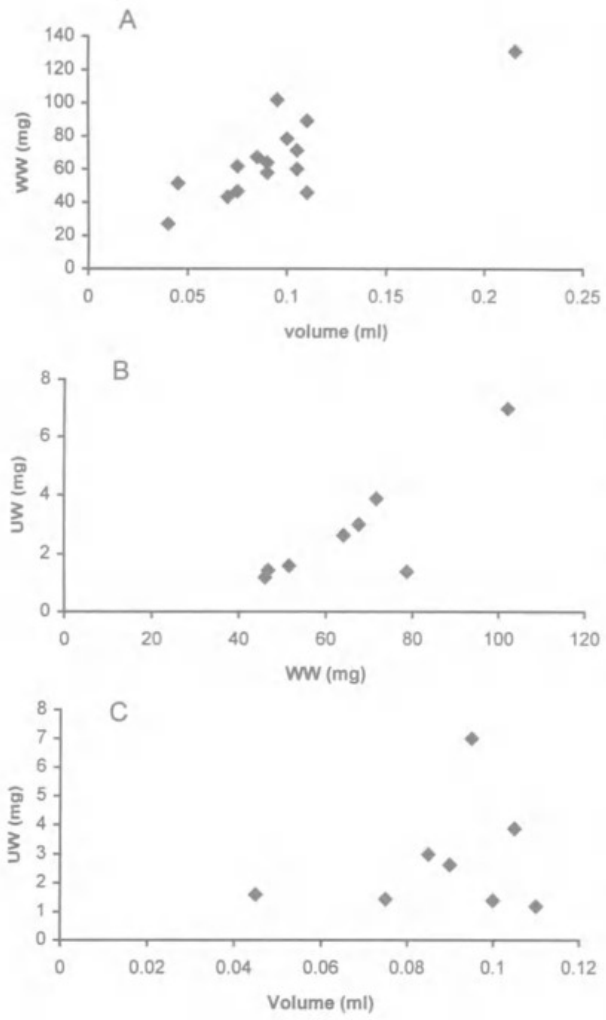

FIG. 5. Comparison between the three techniques for measuring biomass. A, correlation between WW and V. B, correlation between WW and underwater weight (UW). C, correlation between V and UW.

correlations for *Axinella polycapella* (0.98) and *Cinachyrella apion* (0.99) (R. Osinga & E. Planas Muela, unpublished results), and for these species conversion factors are much more reliable.

To evaluate the use of underwater weight as a measure of sponge biomass, the underwater weight data were compared to corresponding measurements of WW and V (Fig. 5B-C). Here, WW showed a significant ($r=0.73$; $n=8$; $\alpha=0.025$) correlation with underwater weight. Hence, our underwater weight data may be converted to WW, using the conversion factor given in Table 4, and underwater weight therefore seems to be an acceptable measure of sponge biomass. However, no significant relation between underwater weight and V could be detected, despite the weak, but significant correlation found for volume and WW. This indicates that tissue of *P. andrewsi* might be subject to a large intraspecific variation in density, which implies that the other data in Table 4 (DW, organic carbon content, and organic nitrogen) must be seen as a first indication only.

CONCLUSIONS

We found that under the applied food regimen (batches of the microalgae *Rhodomonas* sp. and *Chlorella sorokiniana*), the sponge *Pseudosuberites andrewsi* is able to grow rapidly. However, we have not yet succeeded in creating artificial circumstances that enable a constant growth rate; fluctuations in time and intraspecific differences between explants were large. In further studies, this may be improved by using only those explants that have already shown the ability to grow and by adding food particles continuously using continuous cultures of algae.

The two methods used in this study to determine growth have both proven their value in studying sponges. Photography of the body area is the most suitable technique when the availability of sponge material is limited (i.e. when small explants are used). Determination of underwater weight is a promising alternative for photography. Underwater weight has the advantage of being a direct measure of biomass, and therefore, the accuracy of this method to measure growth may be better. The method has a detection limit of ~1mg, which makes it less suitable for small explants.

More data are needed to provide a reliable picture of the relation between volume and weight parameters for tissue of *P. andrewsi*, as this species seems to exhibit a high variability in tissue density.

ACKNOWLEDGEMENTS

We thank Michael Laterveer (Blijdorp Zoo, Rotterdam) for providing us with sponge material and Dr Rob W.M. van Soest (University of Amsterdam) for identifying sponge material. Ellen M. Meijer and Boudewijn van Veen are acknowledged for their technical support and Marcel Janssen for his valuable suggestion to use underwater weighing as a method to determine sponge biomass.

LITERATURE CITED

ANKEL, W.E. & EIGENBRODT, H. 1950. Über die Wuchsform von *Spongilla* in sehr flachen Räumen. Zoologischen Anzeiger 145: 195-204.

AYLING, A.L. 1983. Growth and regeneration rates in thinly encrusting Demospongiae from temperate waters. Biologial Bulletin 165: 343-352.

BARTHEL, D. 1986. On the ecophysiology of the sponge *Halichondria panicea* in Kiel Bight. I. Substrate specificity, growth and reproduction. Marine Ecology Progress Series 32: 291-298.

BARTHEL, D. & THEEDE, H. 1986. A new method for the culture of marine sponges and its application for experimental studies. Ophelia 25: 75-82.

FOSSÅ, S.A. & NILSEN, A.J. 1996. Korallenriff-Aquarium, Band 5. Einzellige Organismen, Schwämme, marine Würmer und Weichtiere im Korallenriff und für das Korallenriff-Aquarium. (Birgit Schmettkamp Verlag: Bornheim).

LANGENBRUCH, P.-F. 1983. Untersuchungen zum Körperbau von Meeresschwämmen. II. Das wasserleitungssystem von *Halichondria panicea*. Helgoländer Meeresuntersuchungen 36: 337-346.

LEE, Y.-K. & PIRT, S.J. 1981. Energetics of photosynthetic algal growth: influence of intermittent illumination in short (40 s) cycles. Journal of General Microbiology 124: 43-52.

MUNRO, M.H.G., BLUNT, J.W., LAKE, R.J., LITAUDON, M., BATTERSHILL, C.N., PAGE, M.J. 1994. From seabed to sickbed: what are the prospects? Pp. 473-484. In Soest, R.W.M. van, Kempen, T.M.G. van & Braekman, J.C. (eds), Sponges in time and space. (Balkema: Rotterdam).

OSINGA, R., TRAMPER, J. & WIJFFELS, R.H. 1998a. Cultivation of marine sponges for metabolite production: applications for biotechnology? Trends in Biotechnology 16:130-134.

OSINGA, R., PLANAS MUELA, E., TRAMPER, J. & WIJFFELS, R.H. 1998b. *In vitro* cultivation of four marine sponge species. Determination of the nutritional demands. Pp. 121-127. In LeGal, Y. & Muller-Feuga, A. (eds) Marine microorganisms for industry. Actes des colloques 21. (IFREMER: Plouzané).

POIRRIER, M.A., FRANCIS, J.C. & LABICHE, R.A. 1981. A continuous-flow system for growing fresh-water sponges in the laboratory. Hydrobiologia 79: 255-259.

SANCHEZ-MORENO, H. 1984. Cultivo experimental de dos esponjas marinas en condiciones de laboratorio. Anales Instituto de Investigaciones Marinas Punta de Betin 14: 17-28.

SIMPSON, T.L. 1963. The biology of the marine sponge *Microciona prolifera* (Ellis and Solander) I. A study of cellular function and differentiation. Journal of experimental Zoology 154: 135-152.

THOMASSEN, S. & RIISGÅRD, H.U. 1995. Growth and energetics of the sponge *Halichondria panicea*. Marine Ecology Progress Series 128: 239-246.

VETHAAK, A.D., CRONIE, R.J.A. & SOEST, R.W.M. VAN 1982. Ecology and distribution of two sympatric, closely related sponge species, *Halichondria panicea* (Pallas, 1766) and *H. bowerbanki* (Burton, 1930)(Porifera, Demospongiae), with remarks on their speciation. Bijdragen tot de Dierkunde 52: 82-102.

PREDATION ON CARIBBEAN SPONGES: THE IMPORTANCE OF CHEMICAL DEFENSES. *Memoirs of the Queensland Museum 44: 426. 1999*:- The conventional view has been that the impact of predation on Caribbean reef sponges is minimal: generalist predatory fishes are deterred by sponge spicules and chemistry, while the few spongivorous fishes are 'smorgasbord' feeders that circumvent chemistry by eating small amounts of many different sponge species. New data suggest that this traditional view needs to be re-examined. Generalist predatory fishes are deterred by chemistry, but not by structural elements, toughness, or nutritional quality of sponge tissue. Spongivorous fishes are not smorgasbord feeders, but instead choose to eat chemically undefended sponge species. Transplantation experiments reveal that the grazing activity of spongivorous fishes restricts certain sponge species to refugia, including cryptic habitats on the reef and mangrove and grassbed environments, where these fish are absent. Chemical defense plays an important role in the ecology of sponges on Caribbean reefs. ❐ *Porifera, chemical defense, predation, Caribbean, ecology.*

Joseph R. Pawlik (email: pawlikj@uncwil.edu), Biological Sciences, UNC-Wilmington, Wilmington, NC 28403-3297 USA; 1 June 1998.