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Sea ranching of the marine sponge *Negombata magnifica* (Demospongiae, Latrunculiidae) as a first step for latrunculin B mass production

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Abstract

Marine sponges are a rich source of bioactive metabolites that can potentially be used as lead compounds to treat various human diseases. Due to the difficulty in obtaining a steady supply of such metabolites, few of these compounds have reached the clinical trials stage. Sponge sea ranching is one of the most promising methods to overcome this "supply problem". The Red Sea sponge *Negombata magnifica* is a potential source of the cytotoxin, latrunculin B (lat-B). The aim of the research was to compare the effect of different sea ranching culture methods on *N. magnifica* growth and survival rates and to identify factors that might enhance these rates. Since the ultimate goal of the project is to produce bioactive metabolites, we measured the lat-B concentration in the sponge tissues. Ninety-eight fragments were cut from nine different individuals and attached to PVC plates or threaded on fishing lines. The PVC-attached sponges were fastened to plastic nets and the threaded sponges were suspended on horizontal ropes. Each of these treatments was stocked at 10 or 20 m depth. Within 177 days, the sponges' average size increased from 11.6±0.7 to 24.5±2 gr and the survival weighted mean of all treatments was 71.4%. The specific growth rate (SGR) of sponges that were suspended on nylon threads was significantly higher ($P<0.05$) than the SGR of sponges that were attached to the net (0.9±0.08% day^{-1} and 0.5±0.05% day^{-1}, respectively). No significant difference ($P>0.05$) in growth rate was found between the two stocking depths. Fouling organisms significantly ($p<0.05$) reduced the sponge SGR compared to that of sponges grown free of fouling organisms (0.26±0.07% day^{-1} and 0.55±0.09% day^{-1}, respectively). The amount of latrunculin B found within body of sponges cultured for a year in the sea ranching system (1.2±0.16 mg lat-B gr dry matter sponge^{-1}) was higher than the concentration previously reported for this species (0.35 mg lat-B gr dry matter sponge^{-1}). The present results demonstrate that *N. magnifica* could be cultured in sea ranching systems for the production of lat-B with relatively good survival and growth rates.

Keywords: Sponge culture; Latrunculin B; Sea ranching; *Negombata magnifica*

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1. Introduction

Marine organisms are a rich source of bioactive metabolites, and as of 2001 over 12,000 new compounds had been isolated from marine sources (Mendola, 2003; Donia and Hamann, 2003). Of all marine organisms, members of the phylum Porifera are the most productive source of such natural products (Faulkner, 2000); out of 677 new compounds reported in the year 2002, 247 (36%) originated from sponges (Blunt et al., 2004).

Despite the considerable amount of new bioactive metabolites that originated from marine sponges, very few compounds have reached advanced stages of clinical trials (Newman et al., 2000). One of the reasons for this status is that many of the sponge bioactive metabolites are very toxic, thus leading to a low therapeutic index (Donia and Hamann, 2003).

A second major obstacle hampering the pharmaceutical use of sponge natural products is the "supply problem" (Munro et al., 1999). Approval of any new compound for human therapeutic use requires a steady supply of material. Sponge tissues usually bear only trace amounts of the secondary metabolites of interest (Kuramoto et al., 1996). Therefore, obtaining sufficient amounts of the bioactive metabolites from natural populations requires collection of vast amounts of sponge biomass. It is clear that for most sponge species, natural populations are unable to sustain such heavy exploitation (Munro et al., 1999; Pomponi, 1999).

Different methods have been proposed to overcome the supply problem. The most direct method is simply by chemical synthesis of the target compound. Most natural products are not amenable to such synthesis due to the high complexity of their chemical structure. Even for those compounds that can be synthesized in laboratory settings, the scaling up of their production is usually not feasible (Munro et al., 1999).

Sponges possess certain features that make them good candidates for developing immortal cell lines (Sipkema et al., 2004), although such attempts were unsuccessful, and only primary cell lines have been established (Ilan et al., 1996; De Rosa et al., 2003). Sponge tissue culture (primmorphs) is another method that has been examined (Müller et al., 2000), but as with cell culture, much research needs to be done. Possibly the emerging new field of marine biotechnology may enable production of bioactive metabolites by cloning the relevant genes into fermentable microbes (Salomon et al., 2004). In vivo marine sponge culture has been tested by several researchers (Osinga et al., 2001; Mendola, 2003). In these experiments, sponges were kept in recirculated systems with full control over water conditions and quality. These studies reported high sponge survival but poor growth rates.

Of all methods aimed at sponge biomass production, sea ranching appears to be the most promising and applicable method to date. The method most commonly used for sponge culture is the suspension method. This method was already applied for bath sponge culture in the 1860's by Schmidt and Buccich in the Mediterranean Sea, and later by Moore in Florida [reviewed by Duckworth and Battershill (2003a)]. More recently Pronzato et al. (1999), MacMillan (1996), and Verdenal and Vacelet (1990) cultured bath sponges as well, using the same principles. The suspension method is based on threading sponge fragments on ropes. The sponge fragments are kept in position by tying them to a mooring system.

Duckworth et al. (1999) and Duckworth and Battershill (2003a) presented three new techniques for farming New Zealand sponge species as a source for bioactive metabolites. In the first method, sponges were cultured in individual mesh bags. The advantage of this method is that it is not stressful to the sponges compared to other culture methods; the sponges are simply placed in the mesh bag without any further physical damage to their tissues as a result of the attachment procedure. On the other hand, the fouling organisms that grow on the mesh might block water flow to the sponges. In the second method, the researchers attempted to fasten sponge fragments onto thick ropes. In the third method, sponges were suspended by wrapping them with thin ropes (instead of threading them). The two last methods were reported to be unsuitable for these sponges, since the sponges grew away from the ropes, leading to their dislodgement and loss. Sponges were also cultured on tray batteries that were moored to the sea floor (Müller et al., 1999; van Treeck et al., 2003). The latter method is also described for the farming of other sessile inverte-

brates such as the bryozoan *Bugula neritina* (Mendola, 2003).

In addition to culture method, environmental conditions in which the sponges are maintained, such as water temperature (MacMillan, 1996), sedimentation and pollution (Gerrodette and Flechsing, 1979; Verdenal and Vacelet, 1990), light and depth (Barthel, 1986; Duckworth et al., 1997) and currents (Duckworth and Battershill, 2003b) should be considered as well.

This research is the first to report successful culture of the Red Sea sponge *Negombata magnifica*. This branching red sponge is bathymetrically distributed between 3 to at least 60 m (Ilan, 1995, and personal observation) usually in exposed areas of the coral reef, but could be found attached to rocks in sandy areas as well. The sponge contains two bioactive metabolites, latrunculin A and latrunculin B (lat-B) (Kashman et al., 1980), with specimens in the northern Gulf of Aqaba containing only latrunculin B (lat-B) (Groweiss et al., 1983). The latrunculins are compounds of high cytotoxicity that exert various effects on cell cycle, such as inhibition of microfilament-mediated process (Gillor et al., 2000).

The aims of the present study were to evaluate two sea ranching culture methods for *N. magnifica* and to identify environmental factors that might improve the sponges' biomass production and survival rates, along with production of the bioactive metabolite lat-B.

2. Methods

2.1. Sponge collection and preparation

Negombata magnifica branches were cut from nine different sponge individuals growing at a depth of about 10 m in the north tip of the Israeli part of the Gulf of Aqaba, during July 2002. The sponge fragments were transported submerged in seawater to the laboratory, where they were further cut into 108 pieces. To avoid exposure of more than 20% of the sponge inner layer, the cuts followed the sponge morphology; hence the initial size and shape varied between fragments. Excess water was removed from the sponges by paper towel, and only then their wet weight (WW) was determined by using an electronic balance (mean±SE was 11.6±0.7 gr).

Following WW determination, ten fragments were kept aside to analyze the biochemical composition of sponges and the rest of the fragments were divided randomly into two groups. Those in the first group were attached to pre-weighed PVC plates, one sponge for each plate. The purpose of the PVC plates was to attach the sponge fragments onto a portable substrate and thereby enabling their transition as a whole intact organism, i.e. for weighing them in the laboratory after a growth period in the sea. The attachment procedure was initiated by fastening the sponges to the plates with a 0.5 mm fishing line; after two weeks in the sea the sponges grew and started adhering to the plates themselves. The sponges of the second group were threaded on 30 cm long, 0.5 mm nylon fishing line for later suspension, one sponge on each line. Such a thin nylon thread was used because inserting a thicker line through the sponge fragment damages its tissue beyond repair (MacMillan, 1996). The sponge preparation process in the laboratory lasted less than 24 h in order to reduce negative effects related to the laboratory conditions.

2.2. Location and arrangement of culture units

The culture system was located in the north tip of the Gulf of Aqaba, Israel, in the vicinity of a sea bream fish farm. The study site was located at a distance of 1 km offshore where the sea bottom is at a depth of 44 m. The sea is clear and calm during most of the year and water temperature fluctuates between 21 °C in the winter and 27 °C in the summer.

2.3. Evaluation of culture methods

We compared the sponge growth rate and survival in two culture methods at two depths each, from July 2002 to January 2003. In the first method, sponge fragments were attached to 1.2 m×1 m plastic nets with a mesh size of 20 mm; the nets were stretched over a PVC frame. The sponges were not attached directly to the net but through the PVC plates. Culture units were positioned at the desired depths by tying them to concrete sinkers; on top of the frames two four liter buoys were mounted to keep the units floating (Fig. 1a). In the second culture method, sponge fragments were suspended on nylon threads,

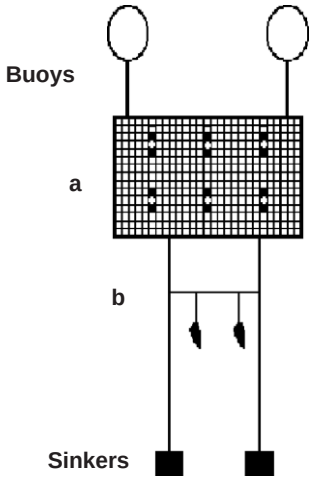

Fig. 1. Scheme of the different *N. magnifica* culture methods. (a) represents the method in which sponges were attached to a plastic nets. (b) represents the method in which the sponges were suspended on nylon threads.

which were tied to ropes stretched horizontally between the mooring ropes (Fig. 1b).

The fifty-eight sponge fragments that were previously attached to the PVC plates were connected to two plastic nets, one net at 10 m and the other at 20 m. Twenty-nine sponge fragments were placed at each depth. The other forty fragments, which had been threaded, were also divided into two groups of twenty sponges each and tied on horizontal ropes at the same respective depths.

After 177 days in the sea, the PVC plates bearing the sponges were detached from their positions on the culture units and taken to the laboratory for measurements. All fouling organisms (mostly attached to the PVC plates) were cleaned off, and each individual's WW was measured and its growth rate calculated. After all sponges were measured, ten sponge individuals (four sponges from the net-attached population at 20 m and two sponges from the other three treatments) were randomly pulled from the total sponge population, rinsed with fresh water to remove salt and put at −20 °C for later biochemical composition analysis of the sponge.

The average daily specific growth rate (SGR) was calculated using the following equation:

$$SGR = 100\left(W_t - W_0\right)*W_0^{-1}*D^{-1} \quad (1)$$

where W_t and W_0 are the final and the initial weight, respectively, and D is the duration of the experiment in days. Since there are no data indicating a logarithmic growth curve in sponges, we used a linear function for SGR calculation.

2.4. Fouling effect experiment

Based on the results of the culture method experiment, we hypothesized that the fouling organisms that grow on the nets of the culture units negatively influenced the sponge growth rate. Hence after completion of the first experiment, we tested this assumption from January 2003–June 2003. The sponges used in the latter experiment were the fifty-eight individuals remaining from the first experiment that were randomly re-divided into three treatments. In the first treatment, sponges were attached to the same plastic netting as in the previous experiment and were cleaned of the fouling organisms weekly. The second treatment was similar to the first treatment except that the fouling was not removed. In the third treatment, sponges were suspended by tying a rope to the PVC plate. This change in the suspension method was necessary because the thin fishing line used in the first experiment proved unsuccessful in holding the sponges for the duration of the experiment.

After 159 days, sponges were detached from the culture systems and weighed. Following the weighing, seventeen sponge individuals were randomly pulled out of the entire population, rinsed in fresh water and ten of them were used for the biochemical composition analysis (three from the cleaned net, three from the uncleaned net and four from the suspended sponges), and seven sponges (three from the cleaned net, two from the control and two from the suspended sponges) were analyzed for latrunculin B content.

2.5. Sponge weighing method and validity

We based our growth estimation on changes in sponge WW [or the 'drip-dry wet weight' as termed by Barthel (1986)]. To estimate possible variations in *N. magnifica* WW we carried out two repeated measurements of sponge weights. Seventeen sponges were collected randomly from the sea based exper-

imental system, cleaned of fouling organisms, and their WW was then measured. After the weighing procedure the sponges were kept for 24 h in flow-through aquaria. The following day these sponges were re-weighed and the differences between the two weights were analyzed by paired *t*-test.

2.6. Analysis of Latrunculin B content

The seven sponge fragments prepared for the lat-B analysis were rinsed with distilled water to remove salt and epibionts from the sponge surface, followed by freeze-drying. The dry samples were weighed and kept in −20 °C until further analysis. The lat-B extraction procedure was adapted from Kashman et al. (1980). Briefly, sponges were immersed in dichloromethane (DCM) for 50 h in extraction. The crude extraction was filtered through a filter paper (Whatman 2V folded filter, 150 ∅) and the filtrate was dried using a vacuum rotor evaporator (Büchi Rotavapor R-114). The crude extract was diluted in ethanol (1:100) and 20 μL of sample were injected into a HPLC (Merck) equipped with a L-4200 UV-VIS detector set on 220 nm. The initial medium used for the separation was methanol:water (1:1) gradually replaced to 100% ethanol. Lat-B was detected after 18.4 min and the pick area was calculated by the software BORWIN™ (v-1.5). The samples were run in duplicates and the amount of lat-B in the samples was calculated by applying the standard curve (four points, r^2=0.99) on the mean area of the duplicates.

2.7. Sponge biochemical composition

To detect possible seasonal variations in the *N. magnifica* growth rate, we analyzed the sponge body composition. The assumption behind this measurement is that changes in the biochemical composition can be correlated to the growth rate potential; e.g. a decrease in sponge total energy is an indicator to negative energy balance, thus growth is not expected at this situation. Ten sponges were randomly pulled out of the entire cultured sponge population. The sampling schedule was at the end of each experiment, i.e. in the winter (January) and in the summer (June). The samples were washed in distilled water to remove salt and epibionts, and kept in −20 °C until analysis. The sponge biochemical composition was determined using standard procedures (Lupatsch et al., 1997). Briefly, sponge dry matter and ash content were calculated from the weight loss after 24 h drying at 105 and 550 °C, respectively. Crude protein content was measured using the Kjeldahl method and multiplying nitrogen amount by 6.25. Crude lipid content was measured gravimetrically after chloroform–methanol extraction and evaporation under nitrogen. Crude carbohydrates were determined using the phenol–sulphuric acid method, utilizing glucose light absorbance at 490 nm as a standard. Gross energy content was measured by combustion in a Parr semimicro bomb calorimeter using benzoic acid as the standard. The first two measurements did not include the carbohydrate analysis because of insufficient sponge material.

2.8. Ambient conditions

Data on seawater temperature and chlorophyll-a (Chl-a) concentrations were obtained from the Israeli National Monitoring Program of the northern Gulf of Aqaba (REF) and from the Inter University Institute (IUI) (http://www.iui-eilat.ac.il).

The temperature was measured every hour and data were saved automatically in a data-logger. Water samples for Chl-a determination were collected monthly from the site of study. Chl-a concentration (μg L^{-1}) in seawater was resolved using the IUI standard protocol. Briefly, water samples were filtered and the chlorophyll was extracted using acetone 90%. Chlorophyll-a concentration was determined by reading the fluorescence in a TD-700 fluoremeter (Turner Designs, Sunnyvale, CA, USA).

2.9. Statistic analysis

Statistic analyses were performed using JMPIN (v 5.0.1a, SAS Institute). Bartlett's test was used to test the variance's homogeneity and Shapiro–Wilk test was used to test for normality. The parametric tests used in this study included *t*-test, one way and two way ANOVA and linear regression (model I). Data sets were log transformed when necessary to meet the requirement of normality and homogeneity of variance (Sokal and Rohlf, 1995).

3. Results

3.1. Growth and survival in different culture conditions

The sponge average final WW in all treatments (Fig. 2) was significantly higher than the average initial WW (24.52 gr and 11.60.7 gr, respectively; data were log transformed to meet normality, student's *t*-test, t=6.1, df=120, P;0.001). The sponges suspended on nylon threads had a significantly higher SGR (P<0.05, Table 1) than those attached to the net (0.91±0.09% day^{-1} and 0.54±0.05% day^{-1}, respectively). However, no significant difference (P>0.05, Table 1) in SGR was found between the two stocking depths; the SGR of sponges stocked at 10 m depth was 0.65±0.07% day^{-1} whereas the SGR of sponges stocked at 20 m was 0.68±0.07% day^{-1} (Table 2).

Seventy out of the ninety-eight sponges survived the 177 days of experiment and the survival weighted mean in all treatments was 71.4% (Table 3). In three of the treatments the survival was about 80%; the lowest survival (40%) was recorded in the sponges suspended on nylon threads at a depth of 20 m. Most of the threaded sponges were lost because of disintegration of the thin rope on which they were suspended, rather than mortality.

3.2. Effect of fouling on growth rate

Fifty-one sponges out of initial number of fifty-eight sponges (88%) survived to the end of the experiment. A one way ANOVA model applied to the data revealed a significant effect of the treatment. ($F_{2,48}$=0.75, P<0.001). By using a Tukey Kramer multiple comparison test it was found that the SGR of the suspended sponges did not differ significantly from that of fragments cultured in a net free of fouling (0.55±0.09% day^{-1} and 0.56±0.08% day^{-1}, respectively, Fig. 3). On the other hand, sponges cultured attached to an uncleaned net (control) had a significantly lower SGR (0.26±0.07% day^{-1}, P<0.05) than those in the other two treatments.

Although the culture conditions in the control treatment were comparable to those of the net-attached sponges from the first experiment, the SGR of the two groups significantly differed (0.54±0.05% day^{-1} and 0.26±0.07% day^{-1}, respectively, Student's *t*-test, data were log transformed to equalize variances, t=6.5, df=58, P<0.001). Moreover, the SGR of the whole sponge population (regardless of the experimental treatments) was higher in the first experiment than in the second one (0.66±0.05% day^{-1} and 0.44±0.05% day^{-1}, respectively, Student's *t*-test, data were log transformed, t=5.2, df=111, P<0.001).

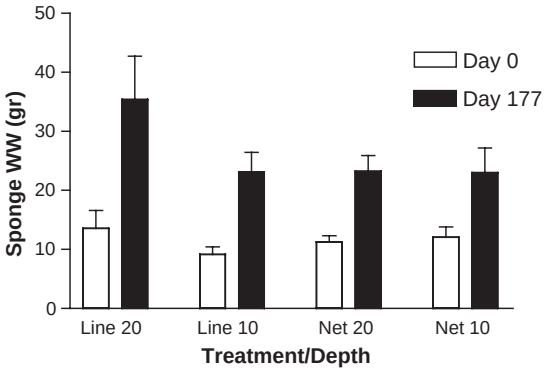

Fig. 2. The effect of attachment method and depth on *N. magnifica* average weights, at the beginning of the experiment and after 177 days. Line, Net—indicates that the sponge individuals were suspended on nylon or attached to a plastic net. The numbers indicate the depth of culture. Error bars indicate SE (n=70).

Table 1
A two way ANOVA model comparing the *Negombata magnifica* SGR between two attachment methods and two depths

Source	*df*	SS	MS	*F* ratio	*P*
Analysis of variance					
Model	3	2.3	0.77	5.25	0.002
Error	66	9.73	0.14		
Effect test					
Attachment	1	2.1		14.07	0.0004
Depth	1	0.07		0.5	0.48
Attachment×depth	1	0.02		0.15	0.7

No transformation was needed in this analysis. SGR was calculated as: 100×(final weight−initial weight)×initial weight^{-1}×days of experiment^{-1}. Duration of experiment was 177 days. n=70.

Table 2
SGR of *N. magnifica* fragments cultured in two depths and two attachment methods

	Suspended on rope	Attached to net	Average
10 m	0.9±0.12	0.49±0.07	0.65±0.06
20 m	0.94±0.17	0.6±0.06	0.68±0.07
Average	0.91±0.1	0.54±0.05	

SGR values (see Eq. (1)) are expressed as average±SE. Duration of experiment was 177 days. n=70.

Table 3
N. magnifica survival in the sea after 177 days of experiment

Attachment method	Depth (m)	No. initial individuals	No. final individuals	% Survival
Suspended	10	20	15	75
Attached to net	10	29	24	83
Suspended	20	20	8	40
Attached to net	20	29	23	79

3.3. Ambient parameters

Mean water temperature during the first and the second experiments averaged 25.3 and 22.5 °C, respectively (Fig. 4). For a better comparison between the water temperatures in the two experiments, we summed the daily temperatures [day degrees, D°, (Seed, 1976)]; it was found that the sponges of the first experiment were exposed to higher degree-days than the sponges of the second experiment (3864 and 3360 D°, respectively, over a 150-day period).

Annual Chl-a concentration ranged among 0.12 μg L^{-1} in July to a maximum of 0.38 μg L^{-1} in August (Fig. 4). In general, the data correspond with the Chl-a dynamics in the northern part of the Gulf of Aqaba; relatively low concentration during the whole year and elevated concentrations during the spring algal bloom (Lindell and Post, 1995). The unexpected high Chl-a concentration in August was supported by a low Secchi disk number (data not shown), thus this measurement cannot be considered as a measurement error. Instead, it indicates a relatively large temporal variation in chlorophyll concentrations at the study site. No significant difference (Student's *t*-test, t=0.78, df=10, P=0.45) in Chl-a concentrations as a consequence of these variations was found between the two experimental periods.

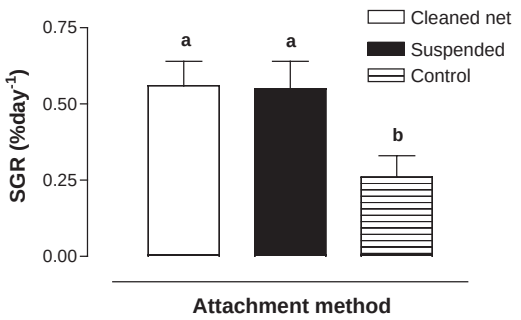

Fig. 3. Average SGR of *N. magnifica* individuals in three treatments testing the influence of biofouling. Different letters indicate significant difference (P<0.05, Tukey Kramer). Error bars indicate SE (n=51).

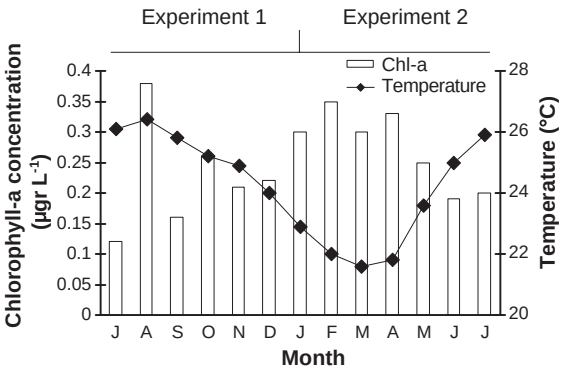

Fig. 4. Yearly profile of Chlorophyll-a concentrations (μg L^{-1}) and temperatures in the study site. Each bar represents one monthly measurement of Chl-a concentration. The dots in the line represent the average monthly temperature. The first experiment started in mid July 2002 and ended in mid January 2003. The second experiment started in mid January 2003 and ended in mid June 2003. SE was less than 0.1 thus not shown in the figure.

3.4. Sponge biochemical composition

Sponge biochemical composition in the samples taken at the end of each experiment did not differ significantly. However, significant differences in protein, lipid and ash content were found between the freshly collected sponges at the experiment outset and the experimentally cultured sponges (Table 4). There were no significant variations in the sponges' energy content between the three measurements (one way ANOVA, $F_{2,22}$=1.71, P=0.2). Summation of all sponge biochemical components reveals that not all the organic components were resolved in the methods applied in this research. We assume that the missing material is part of the carbohydrate fraction, since the phenol-sulphuric acid method does not detect the total carbohydrates, whereas the other parameters are measured in full.

3.5. Latrunculin B content

The average lat-B amount found in sponges that were kept for a year in a sea ranching system was 1.2±1.6 mg lat-B gr dry matter $sponge^{-1}$.

Table 4
Body composition of *N. magnifica* in winter and summer

Date (m/y)	Dry matter (% of WW)	Protein (%)	Lipid (%)	Carbohydrate (%)	Ash (%)	Phosphorus (%)	Energy (cal/gr DM)
7/02	15.3±0.7	44.7±1	11.4±0.5		27.8±1.3	0.4±0.01	4417.8±96.4
1/03	14.6±0.2	50.8±0.6	10.2±0.2		22.6±0.9	0.5±0.02	4415.0±46.6
6/03	15.1±0.4	50.1±0.6	10.6±0.2	6.2±0.07	22.0±0.2	0.3±0.01	4526.5±27.4

For each measurement 10 sponge individuals were collected randomly from the sea experiment.
Data are presented as the average percent from dry matter (DM)±SE. Carbohydrates were not measured in the first two samplings because of insufficient sponge material.

3.6. Validation of the wet-weighing method

The average difference between the initial sponge weight and the repeated measurement was −0.6±0.3 gr, which is an average decrease of 1.15%.The maximal deviation between the two measurements was 6%. Paired *t*-test analysis revealed that the decrease in sponge weight between the initial and final measurements was significant ($t=-1.8$, $df=16$, $P_{(t<-1.8)}=0.04$).

4. Discussion

The method of determining WW is relatively simple to apply and it directly measures the sponge biomass while not being harmful to *N. magnifica*. The method applied in this research is useful particularly for the three-dimensional branching sponges where indirect methods, such as the two-dimensional projections of body area (Ayling, 1983), are ineffective. Stone (1970) stated that sponge WW cannot be used as an indicator for growth due to irregular water retention by the sponges' aquiferous system. We demonstrated that although the decrease in sponge weight was statistically significant, it was minute (average decrease of 1.15%) and practically insignificant. Thus the WW method was applicable for our experimental settings of an extended period during which body weight changed considerably. This method, however, could not be applied to detect minuscule weight changes. It is important to note that variation in WW due to hydration state is common to all organisms and is not characteristic only of sponges.

Successful sponge aquaculture requires the development of efficient culture systems. Because of the relatively low growth rates, low bioactive metabolites yield, and the complexity of constructing underwater culture systems, research should concentrate on finding sponge culture techniques and environmental conditions that would promote the highest yields.

We demonstrated that *N. magnifica* could be cultured in sea ranching systems for the production of lat-B, with relatively high survival and growth rates. The highest growth rates were obtained when sponges were threaded on a thin nylon line, so they were free of the negative effect of the fouling organisms, as was proposed by Duckworth and Battershill (2003b) for *Latrunculia wellingtonensis* and *Polymastia croceus*. Nevertheless, the suspension method was technically non-applicable since the thin nylon threads used (see Methods) did not endure the environmental conditions and started to disintegrate after a few months, leading to loss of sponge fragments. However, cleaning the nets off fouling organisms resulted in a sponge growth rate similar to the suspension method.

Both stocking depths tested were found suitable for *N. magnifica* culture and no significant difference was found between them in growth rate and survival. Since *N. magnifica* has no photosynthetic obligate symbionts (E. Hadas, personal observations), light intensity cannot affect the sponge growth, unless the excess light catalyzes damaging algal growth on and around the sponge surface, as was found for *Psammocinia hawere* (Duckworth et al., 1997) or causes deleterious effects from UV. Because of the open sea features of the study site and its distance from the sea bottom, we could not observe any depth related factor other than light that might affect the sponges' growth rate.

Growth rates of the suspended sponges in the first experiment were nearly double those of sponges in the

second experiment (0.91% day^{-1} and 0.55% day^{-1}, respectively). Since we used the same sponge fragments in these two consecutive experiments, the possibility of any casual genetic variability is unlikely. For that same reason, the average sponge size in the second experiment was larger than in the first experiment (24.5±2, 11.6±0.7 gr, respectively). The relation between body mass (W) and metabolic rate (R) is nonlinear and usually described by the equation: $R=aW^x$ (Kleiber, 1975). In homeothermic organisms the mass exponent (x) is about 0.75. The mass exponent for aquatic invertebrates is less documented and is proposed to be between 0.47 to 1.28 (Patterson, 1992). If the mass exponent for *N. magnifica* is less than one, we could expect a decrease in growth rate in the second experiment relative to the first one. We plotted, separately for each experiment, the sponge initial sizes versus the SGR (Fig. 5) and no linear regression was found between the two parameters in the first and second experiments (regression coefficients were not significantly different from zero, t=0.25, n=70, P=0.8; t=−1.21, n=51, P=0.2, respectively). These data suggest that the different average sponge size between the two experiments cannot explain the difference in *N. magnifica* growth rates (at least in the size range tested). Published data regarding the correlation between SGR and sponge size is controversial, where for some sponges such correlation was found (Dayton, 1979) while for others it was not (Turon et al., 1998). Interestingly, van Treeck et al. (2003) proposed an optimal *Ircinia variabilis* yield when initial sponge explants sizes were between 9.95 and 16.32 gr.

Because very small *N. magnifica* explants are expected to show a low rate of biomass gain, we estimate that stocking a grow-out system with small explants such as sexual reproduction products is not feasible. The best culture option for *N. magnifica* might be harvesting a standing sponge stock, a method that was found to be nonharmful to the sponges (Hadas, E., unpublished data).

Since differences in sponge size could not clarify the discrepancy in growth rates between the two experiments, we tested the possibility of seasonal fluctuations as a result of changes in seawater temperature and food availability (Barthel, 1986; Turon et al., 1998; Duckworth and Battershill, 2003a,b). Most of the Chl-a in the Gulf of Aqaba

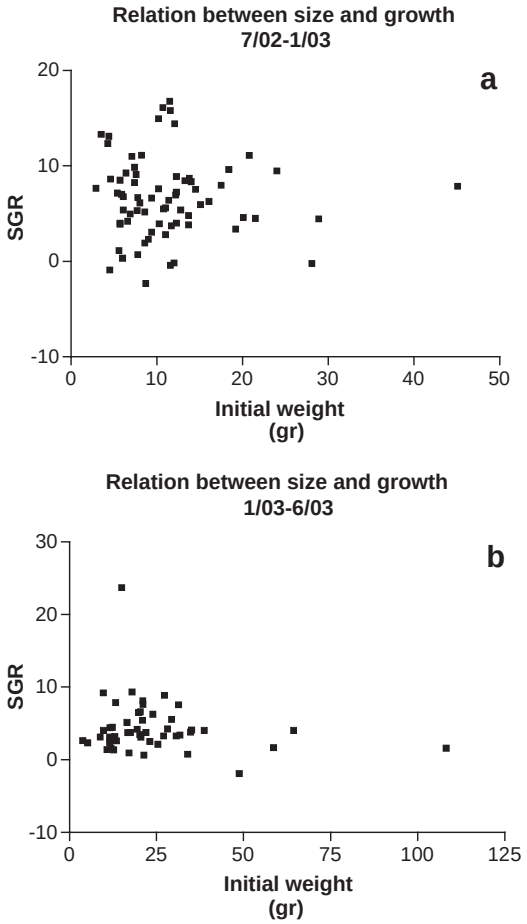

Fig. 5. *N. magnifica* SGR plotted against the initial sponge size. (a) first experiment and (b) second experiment. Regression analysis of the two experiments indicated that regression coefficient was not significantly different from zero (P>0.05, data in text).

(over 80%) is attributed by ultraphytoplankton (Yahel et al., 1998) that is an important food source of many marine sponges (Ribes et al., 1999; Hadas, E., unpublished data); therefore the Chl-a concentration is a good indicator to the sponge natural food availability. Generally, the north tip of the Red Sea is characterized by an adverse relation between water temperature and Chl-a concentration (Lindell and Post, 1995). This general pattern can be seen in Fig. 4, though the Chl-a concentration shows high daily and spatial variation, as reflected from the high Chl-a level detected in August. Moreover, in addition to their natural food, by using $\delta^{15}N$ as a marker, it was demonstrated that sponges growing near fish farms

feed on organic matter released from the fish cages (Lojen et al., 2004, personal communication). These findings, combined with the fact that there were no significant differences in sponge body composition between measurements, might indicate that food was not a limiting factor for *N. magnifica* growth; instead, the higher water temperature during the first experiment promoted the sponge's growth rate in the first experiment, relative to the second, as was found for temperate sponges *Halichondria panicea* (Barthel, 1986) and *Crambe crambe* (Turon et al., 1998).

N. magnifica cultured in our growth system produced lat-B comprising 1.2% of the sponge dry weight (DW). This amount is relatively high compared to the bioactive metabolites found in other sponges, such as 0.48% kahilinanes in dry *Acanthella cavernosa* (Mendola, 2003) and 1.5 ppm of halichondrin in *Lissodendoryx* n. sp. 1 (Munro et al., 1999). According to the present study, annual production of 1 kg lat-B requires the extraction of 555 kg (WW) *N. magnifica* (1/0.012/0.15=555). Assuming a conservative growth rate of 200% year^{-1}, the *N. magnifica* standing stock should be of about 280 kg WW (including 20% sponge mortality, this study). To culture such sponge amount a huge infrastructure is required, but we estimate it is feasible.

The cultured sponges produced higher amounts of lat-B than those previously reported for wild *N. magnifica* [up to 0.35% of the sponge dry weight (Groweiss et al., 1983)]. There is no information about the factors that dictate the lat-B production rate. Culture site, environmental conditions, sponge strain, culture conditions, or seasonal variations (as was found for the toxicity of *Crambe crambe* by Turon et al., 1996), should be considered.

Our results demonstrated that the presence of fouling organisms had a great effect on *N. magnifica* growth rate, whereas the depth of culture had no effect at all. We also attained a lat-B concentration exceeding what has been previously reported (Groweiss et al., 1983). However, we did not identify the factors contributing to the increased lat-B production, which is, after all, the ultimate goal in producing *N. magnifica*.

Future studies should concentrate on finding possible variations in lat-B production rate and defining factors that might regulate these rates. Integration of these two functions will specify the optimal sea ranching culture system needed for the most efficient lat-B production.

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