Relative importance of bacteria, microalgae and yeast for growth of the sponge *Halichondria melanadocia* (De Laubenfels, 1936): A laboratory study

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Abstract

Bacteria, microalgae and yeast less than 10 μm in size are the primary food source of sponges but their relative contribution to somatic growth is poorly understood. In a laboratory study, the sponge *Halichondria melanadocia* was fed for 6 weeks a diet consisting solely of four bacterial strains, or a mixed diet consisting of bacteria, microalgae and yeast. Both diets were fed at three concentrations, based on the natural concentration (NC) of particles available to sponges: 1/5, 1 and 5NC. Mean final size of *H. melanadocia* was 40% greater on a mixed diet than on the bacteria diet, probably because of the greater supply of carbon and other essential nutrients in microalgae and yeast. Cell concentration also significantly affected the growth of *H. melanadocia*, with greatest growth for sponges fed at the highest cell concentration. The estimated carbon requirement for *H. melanadocia* to meet metabolic costs was 0.356 mg C l^{-1} or 103 μg C h^{-1} gDW^{-1}. Many *H. melanadocia* appeared to be optimizing their surface area for food uptake.

Keywords: Bacteria; Clearance rates; Sponge; Ultraplankton

1. Introduction

Bacteria, microalgae and yeast numerically dominate the planktonic community in coastal waters and are an essential food source for many suspension feeders (Stuart and Klumpp, 1984; Bak et al., 1998). Sponges are common active suspension feeders in many benthic habitats and their consumption of suspended cells provides an important coupling between the pelagic and benthic communities (Reiswig, 1971; Pile et al., 1996; Ribes et al., 1999). Sponges are generally nonselective feeders and capture food particles by pumping seawater into their internal canal system. Water entering through incurrent pores, or ostia, covering the sponge surface passes through diverging incurrent canals into flagellated choanocyte chambers which drive the water current. Water exits

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the sponge through excurrent pores, or oscula. Cells < 5 μm are generally captured by choanocytes while larger cells are primarily engulfed through phagocytosis by pinacocytes lining the incurrent canals (Reiswig, 1971; Weissenfels, 1992). Using this unique pumping system, sponges can filter large volumes of water with high retention rates of small microorganisms such as bacteria and mircoalgae (Reiswig, 1971; Huysecom et al., 1988; Pile et al., 1996; 1997). Sponges may also remodel their shape to promote feeding efficiency, by increasing water flow and thus food supply through the individual (Vogel, 1974). Morphological changes result from cellular rearrangement and it allows the sponge to better adapt to its local environment (Bond and Harris, 1988).

The relative importance of bacteria and other microorganisms to supply the necessary nutrients, such as carbon, for sponge growth and metabolism varies greatly among the few investigated species. Bacteria contribute approximately 75% of the daily carbon uptake in sponges (Pile et al., 1997; Ribes et al., 1999), which represents from 17% (Stuart and Klumpp, 1984) to 100% (Reiswig, 1975) of the total nutritional requirements. In addition, several culture studies have found that bacteria only promote sponge growth when fed at unnaturally high cell concentrations (Francis and Poirrier, 1986; Huysecom et al., 1988; Francis et al., 1990). Therefore, bacteria may numerically dominate the < 10 μm size fraction (Reiswig, 1971; Ribes et al., 1999), yet their relative importance as a food source to supply nutrition for sponge growth and metabolism is poorly understood. Because the mechanism of particle capture in a sponge depends on particle size (Reiswig, 1971; Weissenfels, 1992), clearance rates may vary between bacteria and the comparatively larger microalgae and yeast cells. Differences in clearance rate between cell sizes could therefore affect the nutritional contribution of each food type (e.g. bacteria, microalgae) to the sponge.

In this study, *Halichondria melanadocia* (Demospongiae, Halichondrida, Halichondriidae), common to coastal areas of the southeastern USA, was fed either a diet consisting of bacteria strains or a mixed diet consisting of bacteria, microalgae and yeast. The bacterial strains (*Marinococcus halophilus*, *Vibrio alginolyticus*, *Escherichia coli*, and *Bacillus subtilis*), microalgae (*Isochrysis galbana*) and yeast (*Saccharomyces cerevisiae*) were chosen as food types in this study because they have been used before in sponge culture (Francis and Poirrier, 1986; Duckworth et al., 2003) and their nutritional properties are well understood (Phillips, 1984). *H. melanadocia* for this study was collected from the Indian River Lagoon, Florida, which is a restricted estuarine ecosystem subject to spatial and temporal variation in seston composition (Badylak and Phlips, 2004). The food types used in this study do not naturally occur in the Indian River Lagoon (Douding, 1987; Garland, 1995). However, sponges are generally considered unselective feeders (Pile et al., 1996), for example retaining similar sized latex beds and bacterial cells at similar rates (Francis and Poirrier, 1986), and grow well on cultured microbes (Thomassen and Riisgård, 1995). Both bacteria and mixed diets were fed for 6 weeks at differing concentrations, based on the natural seston concentration available to *H. melanadocia*. Microbial analysis has found that the sponge contains no photosynthetic symbionts that could provide additional energy for growth (unpublished data). The main aim of this study was to determine the relative importance of bacteria and the larger celled microalgae and yeast for growth of *H. melanadocia*, with the null hypothesis being that growth will be similar between sponges fed bacteria and mixed diets. In addition, feeding both diets at different concentrations will provide further information on the nutritional requirements of *H. melanadocia*. Lastly, a clearance rate experiment will examine the effect of particle size on the filtering of *H. melanadocia*, and thus help determine the relative contribution of each diet to sponge growth and metabolism. This study was done during spring, when seston concentration is high in the Indian River Lagoon (Phlips et al., 2002). Growth and abundance of *H. melanadocia* in the Indian River Lagoon vary among seasons, being both high during spring and summer and low in autumn and winter (personal observation).

2. Material and methods

2.1. Sponge collection and cutting

Ten sponges of *H. melanadocia*, approximately 20 × 20 × 5 cm in size, were collected at a depth of

10–50 cm off polyethylene buoys, which were supporting a floating dock. The sponges were cut in aerated seawater using sharp scalpels into approximately 80 cube-shaped and smaller sponges, to provide sufficient replicates for both the feeding and clearance rate experiments. All "new" sponges had at least one side covered in pinacoderm. After cutting, the 80 sponges were separated and placed into three 80 l aquaria for 1 week to allow time for them to reorganize their canal system before being used. Each aquarium contained mechanical and biological filters to maintain high water quality.

2.2. Feeding experiment setup

Eighteen aquaria were used in this experiment, each containing 15 l of seawater. Total water changes done five times each week removed any uneaten food cells and detrital matter, and reduced ammonia levels reaching high and possibly damaging levels (average = 0.06 mg l^{-1}, S.E. = 0.02). The fresh seawater added to the aquaria was first passed through a series of filters (down to 1 μm pore size) and UV lights to remove or kill any particles. Water temperature, salinity and pH were kept constant throughout the experiment at 25 °C, 31‰ and 8.1, respectively. These represented the environmental conditions in the Indian River Lagoon at the time of collection. Because silicon is an essential skeletal component for Demospongiae and may affect sponge growth (Osinga et al., 1999), 50 μl of 227 M sodium metasilicate (SIGMA, S-4392) was added to each aquarium after each water change to a final concentration of 0.76 mM.

Into each aquarium, three sponges were randomly chosen from the eighty cut sponges and placed onto a horizontal polyethylene mesh support, >5 cm apart to prevent possible inter-sponge interference. Sponges remained in the same place throughout the experiment so that individual progress could be monitored. For statistical analysis, the data for the three sponges in each aquarium were averaged. The mesh support in each aquarium was wedged off the bottom to promote water circulation and feeding around each sponge and to prevent possible smothering from detritus. The seawater was aerated constantly from two air-stones located in opposite corners of each aquarium at a rate of 2×20 ml s^{-1}. This provided O_2 for respiration and promoted good water circulation keeping the added food particles in suspension.

2.3. Feed types and concentrations

To determine the appropriate cell concentrations to feed *H. melanadocia*, the natural concentration (NC) of seston present in the Indian River Lagoon was first examined. Over several days, water samples were collected next to sponges and then analyzed using a Multisizer 3 Coulter Counter (Beckman). Particles of two size classes were counted: 0.8–3 μm, mean NC = 2.2×10^6 cell ml^{-1}; and 3–10 μm, mean NC = 4×10^4 cell ml^{-1}. It was not possible to accurately count cells <0.8 μm. Microscopic analysis determined that the 0.8–3 μm size class consisted mostly of bacteria, while the larger size class was dominated by microalgae and yeast. The cell concentrations reported here are within the range expected for the Indian River Lagoon (Garland, 1995).

Sponges were fed a multispecific diet, with food types grouped into the two size classes. The smallest size class consisted of four bacterial strains, *M. halophilus*, *V. alginolyticus*, *E. coli*, and *B. subtilis*, while the 3–10 μm size class consisted of the microalga *I. galbana* and the yeast *S. cerevisiae*. The mean cell diameter in the small and large size classes was approximately 1.5 and 5 μm, respectively. The carbon content per cell was: bacteria, 0.025 pg C $cell^{-1}$ (Fukuda et al., 1998; Posch et al., 2001); *I. galbana*, 10 pg C $cell^{-1}$ (Flynn et al., 1992); *S. cerevisiae*, 8 pg C $cell^{-1}$, determined from the equation pg C = $0.433 \times [\text{volume } (\mu m^3)]^{0.866}$ (Pile et al., 1997), where average cell volume = 29 μm^3.

The four bacterial strains were cultured individually in 200-ml Pyrex flasks containing 50 ml of marine broth (Difco, 0791-01-2) or nutrient broth (Difco, 0003-01-6). The first cultures were inoculated by adding 2 ml cryopreserved stock culture. The subsequent cultures were inoculated using 2 ml from the previous culture. To reduce microbial contamination, new cultures were started from cryopreserved stock every 2 weeks. The yeast *S. cerevisiae* (0.5 g of Red Star active dry yeast) was cultured in 50 ml of sugar–water (1 g sucrose and 50 ml water). All bacterial strains and *S. cerevisiae* were incubated in an environmental shaker (Innova 4900) at 210 rpm and 25 °C for 1–2 days. Because the microbial culture medium might have

influenced sponge growth it was removed by centrifugation prior to feeding the sponges. The bacteria and *S. cerevisiae* cultures were poured into two separate vials, and centrifuged at 1400×*g* for 20 min. The culture broth was removed and the cell pellets of either bacteria or *S. cerevisiae* were resuspended in large flasks containing 1000 ml and 100 ml of seawater, respectively. Each week, *I. galbana* was cultured in a 20-l vessel, containing F/2 enriched seawater (Hoff and Snell, 1987), for >5 days at 24 °C on 24-h light. Each feeding day, 900 ml of *I. galbana* was added to the 100 ml *S. cerevisiae* suspension; the percentage of each food in the 1000 ml solution was, on average, 76% and 24%, respectively. Average cell concentration of each food suspension (0.8–3 and 3–10 μm) was determined by counting three subsamples using the Multisizer 3 Coulter Counter.

2.4. Feeding regimes

This study examined the effect of food diet and concentration. Sponges were fed either a bacteria diet consisting of a mixture of the four bacterial strains, or a mixed diet consisting of the four bacterial strains plus the larger *I. galbana* and *S. cerevisiae*. The two diets were fed at three concentrations, thus the effects of six feeding regimes were tested in this study (Table 1). Each feeding regime had three aquaria, randomly distributed; each regime cultured nine sponges. Sponges were fed after each water change, five times each week, for a total of 6 weeks. The examination of water samples after food addition determined that initial cell concentrations were close to the desired concentration (range over all treatments: 91 to 113%), and that <20% of food cells remained in suspension after 24 h.

Table 1
Cellular concentration of each food size class in each feeding regime

Diet	Concentration	0.8–3 μm	3–10 μm
Bacteria	1/5NC	440	
Bacteria	1NC	2200	
Bacteria	5NC	11,000	
Mixed	1/5NC	440	8
Mixed	1NC	2200	40
Mixed	5NC	11,000	200

The 0.8–3 μm size class consisted of the bacterial strains *M. halophilus*, *V. alginolyticus*, *E. coli*, and *B. subtilis;* and the 3–10 μm class consisted of *I. galbana* and *S. cerevisiae*. Concentrations: $\times 10^3$ cells ml^{-1}. NC—natural seston concentration.

The growth of *H. melanadocia* was examined by measuring the weight of each sponge (to 0.1 g) in a container of water resting on a scale at the start and end of the experiment. There was no significant difference in initial weight among the six feeding regimes (One-Way ANOVA: $F_{df(5,12)}=0.46$, $P=0.80$), averaging 8.5 g (1S.E.=0.2). Growth examined both final weight and specific growth rate, the latter determined using the formulae:

$$\mathrm{SGR} = \ln\left(W_f W_i^{-1}\right)\ \mathrm{day}^{-1}$$

where W_f and W_i are the final and initial weight, respectively.

To test for possible differences in sponge shape between the feeding regimes, all surviving sponges at the end of the experiment were digitally photographed: each sponge was placed with their largest side facing up on a light background and next to a ruler to provide a scale. Using an image analysis program (Scion Image), sponge surface area and perimeter were recorded and the circularity index calculated. The circularity index of each sponge is the surface area/area of a circle of equal perimeter (Turon and Becerro, 1992) with increasing irregularity in shape as the circularity index approaches zero.

2.5. Clearance rates

In a separate experiment, we examined the effect of food type (bacteria versus microalgae and yeast) and cell concentration on the clearance rate (ml h^{-1} gDW^{-1}) of *H. melanadocia*. For both food types, each cell concentration (1/5, 1 and 5NC) had three replicate aquaria with one sponge each and three aquaria with no sponges (controls). All treatments and replicates were distributed randomly. Small 2 l aquaria were used with sponges resting on a horizontal mesh support so that each sponge was situated in midwater. The seawater was filtered to 1 μm, with temperature, salinity and pH being 25 °C, 31‰ and 8.1, respectively. Air was bubbled into each aquarium to provide O_2 for respiration and to keep the added food cells in suspension. At intervals of 0 (initial), 1, 2, 3, 4 and 5 h after cell addition, a 1-ml water sample was collected from each aquarium and counted on the Multisizer 3 Coulter Counter. At the end of the experiment, the 18 sponges

were placed in an oven set at 100 °C in order to determine their dry weights. Regression analysis determined that for *H. melanadocia*, dry weight = 0.0646 wet weight + 0.279 ($r^2 = 0.679$). Clearance rate (c) of food cells per gram dry weight (gDW) of sponge was determined by the formula:

$$c = \left[\left(V_w t^{-1}\right)\ln\left(C_0 C_t^{-1}\right)\right]\mathrm{gDW}^{-1}$$

where V_w represents the volume of water in the flasks (2 l), and C_0 and C_t are the food cell concentrations at time 0 and time t respectively (Riisgård et al., 1993). Paired T Tests determined that for the control treatments there was no significant difference between initial and final cell concentrations, and therefore any changes in concentration in the sponge treatments would have resulted from filtration.

2.6. Data analysis

Two-way analysis of variances (ANOVA) were used to statistically test for differences of final weight, survival, circularity (shape) and clearance rate among diets and cell concentrations. Both diet and cell concentrations were considered fixed factors. Data with unequal variances were log-transformed to meet the assumption of ANOVA. Where factors were significant ($\alpha = 0.05$), the Tukey–Kramer Multiple Comparison Test was used to determine what treatment means were significantly different. Analyses were preformed using Number Crunching Statistical Systems version 2003.

3. Results

3.1. Feeding experiment

Final weight of *H. melanadocia* varied significantly between the two diets (Table 2). For each concentration, growth was greater on a mixed diet than on a diet consisting solely of bacteria (Table 3), thus we reject the null hypothesis that growth is similar for sponges fed bacteria and mixed diets. At natural cell concentrations (1NC), for example, sponges fed a mixed diet changed little in weight, while sponges fed a diet consisting solely of bacteria shrank by approximately one-third of their initial weight over 6 weeks. Overall, final weight of *H. melanadocia* was 40% greater on average on the mixed diet than on the bacteria diet. Final weight also varied significantly among cell concentrations (Table 2). The Tukey–Kramer Multiple Comparison Test found that sponges fed diets at 5NC were significantly larger than sponges fed at 1NC and 1/5NC. Although there was no significant difference of final weight between the two lower concentrations, weight loss was greater for sponges fed 1/5NC diets (Table 3). Sponges fed a mixed diet at 5NC grew the most overall, with an average specific growth rate of 0.6% day^{-1} (Table 3).

Table 2
Two-way ANOVA to test for differences in final weight of *H. melanadocia* among diets and cell concentrations

Factor	DF	SS	MS	F-ratio	Prob.
Diet	1	21.66	21.66	10.42	**
Concentration	2	56.38	28.19	13.56	**
Diet × Concentration	2	2.64	1.32	0.63	NS
Residual	12	20.79	2.08		

Modified-Levene Equal-Variance test: test value = 2.68, $P > 0.05$. Probability: ** <0.01; NS—nonsignificant.

Table 3
Estimated available carbon, average final weights and specific growth rates (SGR) of *H. melanadocia* among feeding regimes

Diet	Concentration	Carbon (mg C l^{-1})	Final weight (g)	SGR (% day^{-1})
Bacteria	1/5NC	0.011	4.9 (0.8)	−1.39 (0.21)
Bacteria	1NC	0.055	5.0 (0.3)	−1.22 (0.24)
Bacteria	5NC	0.275	9.3 (0)	0.08 (0)
Mixed	1/5NC	0.087	6.3 (1.1)	−0.74 (0.37)
Mixed	1NC	0.436	8.2 (1.0)	−0.16 (0.58)
Mixed	5NC	2.179	12.4 (0.9)	0.60 (0.09)

The carbon content per cell was: bacteria, 0.025 pg C cell^{-1} ; *I. galbana*, 10 pg C cell^{-1} ; *S. cerevisiae*, 8 pg C cell^{-1} . The average initial weight of *H. melanadocia* was 8.5 g (1S.E. = 0.2). Standard errors are in parentheses.

Statistical analysis determined that the circularity index was similar between the two diets, but differed greatly among the three cell concentrations (Table 4). The Tukey–Kramer Multiple Comparison Test determined that shape was similar for sponges fed 1NC and 5NC diets, but significantly different for the 1/5NC diet. Sponges fed at the two highest concentrations had the most circular shape (approaching 1), while sponges fed 1/5NC diets had the most irregular shape, with many sponges having 1–2 cm long tissue outgrowths. These "outgrowths" were observed to grow out over time from the sponge, and were not a result of tissue left over as the rest of the sponge shrank.

Table 4
Two-way ANOVA to test for differences in final shape (circularity index) of *H. melanadocia* among diets and cell concentrations

Factor	*df*	SS	MS	*F*-ratio	Prob.
Diet	1	0.00034	0.00034	0.07	NS
Concentration	2	0.1009	0.0504	9.76	**
Diet × Concentration	2	0.0035	0.0018	3.39	NS
Residual	12	0.0052	0.0005		

Modified-Levene Equal-Variance test: test value=2.35, $P>0.05$. Probability: ** <0.01; NS—nonsignificant.

Final survival was similar between bacteria and mixed diets but varied significantly among cell concentrations (Table 5). All sponges fed at low and natural concentrations survived, while half of the sponges fed 5NC diets died. Sponges died during the first 2 weeks of the study only. Dead sponges were covered with a white unidentified biofilm, and were removed immediately from the aquaria.

3.2. Clearance rates

Clearance rates of *H. melanadocia* were similar among cell concentrations but differed significantly between the two food types (Table 6). The mean clearance rates for bacteria and microalgae/yeast were 367 ml h^{-1} gDW^{-1} (S.E.=60) and 220 ml h^{-1} gDW^{-1} (S.E.=36), respectively, and varied little over the 5-h experimental period.

4. Discussion

Growth of the demosponge *H. melanadocia* in this laboratory study was greatly affected by both diet and cell concentration. Final weight was on average 40% greater on a mixed diet, consisting of bacteria, microalgae and yeast, than on a diet consisting solely of bacteria. This large variation in growth between the two diets probably resulted from variation in supply and composition of essential nutrients. Carbon content per cell is very low in bacteria (0.025 pg C $cell^{-1}$) compared with the larger celled *I. galbana* and *S. cerevisiae* (10 and 8 pg C $cell^{-1}$, respectively). Consequently the bacteria diet in this study supplied approximately 13% of the carbon present in the mixed diet at a given cell concentration (Table 3). Therefore, it is likely that the relatively poor growth of *H. melanadocia* fed only bacteria resulted from the comparatively low carbon content. In addition to having low amounts of carbon, bacteria are generally considered to be deficient in polyunsaturated fatty acids and sterols, nutrients that are vital for somatic growth in marine invertebrates (reviewed in Phillips, 1984). These nutrient deficiencies would have further reduced growth. Both polyunsaturated fatty acids and sterols are also rare in yeasts, but their high content in microalgae (Phillips, 1984) would have offset any deficiencies in the mixed diet. The variation in the growth of *H. melanadocia* between the two diets supports the findings of other studies that have shown that mixed diets generally promote greatest growth of marine invertebrates (Helm, 1977; Brown et al., 1998; Parrish et al., 1998) because they better meet the nutritional requirements of the organism.

Reiswig (1975), examining the feeding of the temperate sponge *Haliclona permollis*, suggested that natural concentrations of bacteria (7.4×10^5 cells ml^{-1}) could meet the basic requirements of metabolism. Similarly, the freshwater sponges *Baikalospongia bacillifera* and *Baikalospongia intermedia*, and *Dysidea avara* from the Mediterranean Sea could obtain most of their carbon requirements from prokaryotes, particularly *Synechococcus* strains (Pile et al., 1997; Ribes et al., 1999). In contrast, Stuart and Klumpp (1984) estimated that a bacterial concentra-

Table 5
Two-way ANOVA to test for differences in final survival of *H. melanadocia* among diets and cell concentrations

Factor	*df*	SS	MS	*F*-ratio	Prob.
Diet	1	0.5	0.5	0.75	NS
Concentration	2	9.0	4.5	6.75	*
Diet × Concentration	2	1.0	0.5	0.75	NS
Residual	12	8	0.67		

Modified-Levene Equal-Variance test: test value=0.44, $P>0.05$. Probability: * <0.05; NS—nonsignificant.

Table 6
Two-way ANOVA to test for differences in clearance rates (ml h^{-1} gDW^{-1}) of *H. melanadocia* among food types and concentrations

Factor	*df*	SS	MS	*F*-ratio	Prob.
Food type	1	0.224	0.224	4.96	*
Concentration	2	0.209	0.105	2.31	NS
Food type × Concentration	2	0.171	0.085	1.88	NS
Residual	12	0.543	0.045		

The data were log transformed to meet ANOVA assumptions. Probability: * <0.05; NS—nonsignificant.

tion of 5×10^5 cells ml^{-1} provided only 17% of the carbon requirements of the sponge *Haliclona anonyma*. In addition, several culture studies have found that bacteria only promote growth of *Ephydatia fluviatilis* and *Spongilla alba* when fed at unnaturally high cell concentrations ($\geq 10^7$ cell ml^{-1}; Francis and Poirrier, 1986; Huysecom et al., 1988; Francis et al., 1990). In this study, *H. melanadocia* required 5 times the natural concentration of bacteria (11×10^6 cells ml^{-1}) to maintain stable weight. These studies suggest that at normal concentrations, bacteria alone do not provide sufficient nutrition to meet metabolic and growth requirements for many sponge species. Sponges with symbiotic bacteria may also obtain much of their energy from DOC (Yahel et al., 2003), but this is unlikely for nonsymbiotic species (Ribes et al., 1999). Microbial analysis suggests that symbiotic bacteria are probably too few in number to be significant in the metabolic balance for *H. melanadocia* (unpublished data), but direct DOC absorption by sponges is poorly investigated and could also occur. If the results of this laboratory study correspond to natural feeding processes, then it suggests that although cells 3–10 μm in size represent only a small fraction (2%) of the total cell abundance in the Indian River Lagoon, they are important for the growth and metabolism of *H. melanadocia*.

Cell concentration also greatly influenced the growth of *H. melanadocia*, with greatest growth for sponges fed at the highest cell concentration. Better growth at higher levels of food abundance has been recorded for other active suspension feeders (Lesser et al., 1994; Lenihan et al., 1996) and results from increased levels of nutrition. There is not always a general relationship between sponge growth and food abundance, however, as some species have reduced or static weight at very high cell concentrations (Huysecom et al., 1988; Duckworth et al., 2003). High particle concentration can block the aquiferous system of sponges (Gerrodette and Flechsig, 1979; Huysecom et al., 1988), which may reduce their ability to feed efficiently. However, similar clearance rates among cell concentrations suggest that high food abundance does not impede filtration in *H. melanadocia*. The maximum specific growth rate of 0.60% per day, obtained on the 5NC mixed diet, is comparable to growth reported for other sponge species (Thomassen and Riisgård, 1995; Table 2).

H. melanadocia fed two feeding regimes, 1NC mixed and 5NC bacteria, had relatively constant weights during the experiment. This indicates that these two regimes provided sufficient carbon (mean = 0.356 mg C l^{-1}) to meet metabolic costs only. This carbon requirement determined for *H. melanadocia* is within the range, 0.07–0.462 mg C l^{-1}, determined for other sponge species (Table 7). Factoring in the lower clearance rates of the larger more nutritious *I. galbana* and *S. cerevisiae* cells shows that estimated carbon uptake of *H. melanadocia* fed 1NC mixed and 5NC bacteria regimes was 104 and 101 μg C h^{-1} gDW^{-1}, respectively. This is similar to the carbon uptake required for the sponge *H. anonyma* and other suspension feeders (Stuart and Klumpp, 1984).

Many sponges have a plastic morphology, where shape is influenced by biotic and physical factors such as competition (Becerro et al., 1994) and water flow (Kaandorp and de Kluijver, 1992). The final shape of *H. melanadocia* varied greatly among food concentrations, probably resulting from sponges optimizing their surface area for food uptake. Sponges fed diets at low concentrations (1/5NC) had the most irregular shape (low circularity index) with many having 1–2 cm long tissue outgrowths. These tissue outgrowths would greatly increase the surface area of the sponge for the uptake of cells, and may compensate for the low food abundance fed to them. Jones (1994) called this morphological response to starvation "process formation" and suggested that sponges may also de-

Table 7
The amount of carbon needed (mg C l^{-1}) to meet metabolic costs for various sponge species

Species	Carbon (mg C l^{-1})	Source
Halichondria panicea	0.17	Thomassen and Riisgård, 1995
Axinella polycapella	0.158a	Osinga et al., 1997
Axinella waltonsmithi	0.207a	Osinga et al., 1997
Cinachyrella apion	0.186a	Osinga et al., 1997
Pseudosuberites andrewsi	0.462a	Osinga et al., 1997
Isodictya kerguelensis	0.07	Kowalke, 2000
Mycale acerata	0.22	Kowalke, 2000
Halichondria melanadocia	0.356b	This study

Codes: a—determined from multiplying *Dunaliella* sp. (microalgae) concentration by carbon content per cell (Osinga et al., 1997); b—average of 1NC mixed and 5NC bacteria diets.

velop tissue outgrowths to find more favorable sites. In contrast, *H. melanadocia* fed 1NC and 5NC diets were circular in shape and had no tissue outgrowths.

The survival of *H. melanadocia* was significantly affected by cell concentration with low survival of sponges fed bacteria and mixed diets at the highest concentration. In contrast, all sponges fed diets at low or natural concentrations survived. It therefore appears that *H. melanadocia* can normally survive the traumatic process of being cut into smaller sponges, and has a high ability to heal wounds similar to other species (Ayling, 1983; Duckworth, 2003). Although the mass mortality of sponges has been blamed on plankton blooms (Bulter et al., 1995), Lynch and Phlips (2000) found no relationship between bloom concentration (5×10^6 cells ml^{-1}) and sponge mortality for several species including *H. melanadocia*. Mortality of sponges in the 5NC treatments occurred only during the first 2 weeks of the study, and while they were still healing their cut surfaces. This suggests that high cell concentration is potentially lethal for *H. melanadocia* when individuals are recovering from tissue damage and may be less able to phagocytose or clear particles. High cell concentration does not affect the filtration of *H. melanadocia* (Lynch and Phlips, 2000; this study) and thus it is unlikely that sponge mortality resulted from blockage of the aquiferous system. Although sponges fed the highest concentration had the lowest survival, these sponges had the best growth in each diet. Therefore, survival does not appear to be correlated to growth for *H. melanadocia*.

The clearance rates of sponges are generally unaffected by particle concentration (Francis and Poirrier, 1986; Ribes et al., 1999), except at very high levels (10^8 cell ml^{-1}; Huysecom et al., 1988). A similar clearance rate among concentrations for *H. melanadocia* indicates that it does not compensate for low food abundance by ingesting more food cells. Clearance rates of *H. melanadocia*, however, varied between the two food types, being greatest for the comparatively small bacterial cells. Several species show a similar pattern with highest clearance rate or retention efficiency for small prey items such as bacteria (Stuart and Klumpp, 1984; Ribes et al., 1999; Kowalke, 2000), and it probably results from the mechanism of particle capture depending on particle size (Reiswig, 1971; Weissenfels, 1992).

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