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Filtering activity of *Spongia officinalis* var. *adriatica* (Schmidt) (Porifera, Demospongiae) on bacterioplankton: Implications for bioremediation of polluted seawater

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ABSTRACT

A study on the filtering activity has been carried out on reared specimens of the demosponge *Spongia officinalis* var. *adriatica* coming from an off-shore farm displaced off the Apulian coast (Ionian Sea). The experience was carried out under laboratory conditions, by using natural seawater collected from the sponge environment. The study demonstrates a high efficiency of the sponge in removing bacteria. Bacterial concentration significantly decreases in presence of the sponge, with a marked drop after 2 h from the start of the experience. The maximum clearance rate was 210 ml h^{-1} g^{-1} DW at 60 min. Retention efficiency reached the highest value of 61% at 120 min. The bacterial density removed by the *S. officinalis* filtering activity was $12.3 \pm 1.8 \times 10^4$ cells ml^{-1} corresponding to a biomass of about 11.7 ± 1.4 μg C l^{-1}. The sponge fed preferentially large- and medium-size bacteria, whereas the small ones are fed after the removal of the largest size categories. The results obtained suggest that *S. offcinalis* is a suitable species for marine environmental bioremediation.

1. Introduction

Demospongiae (Porifera) are ubiquitous organisms comprising species living in freshwater or marine ecosystems, where they constitute the dominant active suspension feeding macroinvertebrates in many hard bottom communities (Sarà and Vacelet, 1973). Since the former research performed by Reiswig (1974) it has been demonstrated the ability of Demospongiae to unselectively filter organic particles within a size range 0.1–50 μm (i.e., heterotrophic bacteria, heterotrophic eukaryotes, phytoplankton and detritus), processing the water column within 24 h, and retaining up to 80% of the suspended particles. However, apart from some seasonal variations (Ribes et al., 1999) or local cases typically occurring in very oligotrophic environments (Vacelet and Boury-Esnault, 1995), demosponges feed primarily on picoplankton (<2 μm), with high removal efficiency (Pile et al., 1996). Therefore, abundant sponge populations may exert an important grazing impact on the water near the bottom, significantly reducing the picoplanktonic component (Savarese et al., 1997). The impact of grazing on bacteria may be crucial especially in oligotrophic marine ecosystems where heterotrophic bacteria constitute a major biomass component and the microbial loop represents a biological force

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driving the energy flux and the functioning of ecosystems (Fuhrman et al., 1989; Cho and Azam, 1990).

The utilization of bacteria by sponges also suggests an applicative role for bioremediation purposes, considering that bacteria are usually abundant in waters with high amount of organic matter, reaching high densities in harbours and in areas subjected to aquaculture activities (Barg and Phillips, 1998). In this farming scenario the use of sponges for marine bioremediation has been recently suggested (Richelle-Maurer et al., 1988; Manconi et al., 1999; Milanese et al., 2003; Corsi et al., 2004).

This work constitutes the first study on the filtering activity of the demosponge *Spongia officinalis* var. *adriatica* (Schmidt, 1892), the most common Mediterranean 'bath sponge', with high commercial value, successfully employed in experimental aquaculture experiences (Corriero et al., 2004). The availability of reared specimens allowed us to investigate on biological material homogeneous in size and age, a feature very suitable for experimental purposes but infrequent in the research on marine sponge filtering activity.

The study has been carried out by the application of the clearance test which gives an indirect evaluation of the volume of water cleared of particles per unit of time and weight. Sponge clearance tests have been previously performed using living microorganisms, such as microbes (Milanese et al., 2003), unicellular algae (Riisgard et al., 1993) or artificial latex beads (Willenz and van de Vyver, 1982; Turon et al., 1997) added to filtered, artificial seawater. We report here results on the filtering activity of *S. officinalis* var. *adriatica* on the bacterioplankton present in the seawater from the sponge sampling site.

2. Materials and methods

2.1. Species studied

S. officinalis var. *adriatica* (order Dictyoceratida) is a massive sponge, highly variable in shape and size, with small conules and large oscules on its surface, black or deep grey in colour. It lives in the Mediterranean, on hard substrates, between the surface and about 60 m of depth. Very common until the past century, it has been recently subjected to a strong depletion due to uncontrolled fishing together with some massive and wide events of mortality (Gaino and Pronzato, 1989; Gaino et al., 1994; Vacelet et al., 1994; Pronzato, 1999).

2.2. Sponge sampling

Samples were collected by SCUBA diving in December 2003 from an off-shore rearing farm in the Northern Ionian Sea (Porto Cesareo, Italy) (Fig. 1). They consisted of sub spherical specimens, about 7–8 cm in maximum diameter, obtained by cutting into explants different sponges from a wild population, and rearing the fragments at 30 m of depth for about 2 years (Corriero et al., 2004). Soon after collection they were transported within cooled bags to the laboratories of the University of Lecce and acclimated in aquarium at 22 °C for 1 day. Before the experiment, the samples were kept in sterilized and filtered (0.45 μm pore) seawater for 1 h to ensure the reduction of pre-existing bacteria before the analyses.

2.3. Filtering experiment

In order to examine the retention efficiency and the clearance rate of *S. officinalis* on bacterioplankton, 42 beakers were each filled with 1 l of seawater constantly aerated by an air pump collected from the same site where the sponges were sampled. One specimen was placed, suspended at 5 cm from the bottom, in each of the 21 beakers utilized as treatments, the remaining 21 beakers were left with only seawater and employed as controls. The experiment was carried out in 3 replicates at a temperature of 22 °C over a 5 h. This temperature corresponds to the annual mean value observed at the sampling site. Observations were performed every 15 min for the first hour and every 60 min for the last 4 h, for a total of 9 sampling times. The retention of bacterioplankton by *S. officinalis* was studied measuring the removal of bacterial cells from seawater by the aseptical collection of seawater aliquots from each treatment and control beaker (10 ml at each interval). For estimating bacterioplankton abundance, sub-samples were preserved with buffered formaldehyde at a final concentration of 2% and kept at 4 °C until analysis.

At the end of the experiment the volume of each specimen was measured (mean value = 91.42 ± 24.61 ml), then sponges were dried in pre-weighted aluminium foil at 100 °C for 24 h and weighted to determine the dry weight (mean value = 10.02 ± 2.77 g).

Retention efficiency (R) was calculated as percent difference in bacterial abundances within each time interval:

$$R(\%) = 100 \times [(C_0 - C_t)/C_0],$$

where C_0 is the initial bacterial concentration at t_0 and C_t is the bacterial concentration at each considered time (15, 30, 45, 60, 120, 180, 240, 300 min).

The clearance rate (c) was estimated by measuring the bacteria removal from the experimental container as a function of time using the equation given by Coughlan (1969).

Data were reported as both weight- and volume-specific clearance rates, expressed as millilitres per gram of sponge dry tissue per hour, and millilitres per millilitre of sponge per hour, respectively. Since the mean clearance rate predictably includes periods of sponge inactivity or transition between feeding and inactivity, for each specimen the clearance rate within each time interval was calculated and data were reported as mean ± standard deviation (SD) within each sampling time. Moreover, according to Navarro and Widdows (1997), to avoid the inclusion of periods of inactivity, maximum clearance rates were calculated based on the maximum values recorded during the entire period of observation for each individual in each experiment.

Differences between controls and treatments and among bacterial concentrations at different time intervals were determined by the analysis of variance (ANOVA).

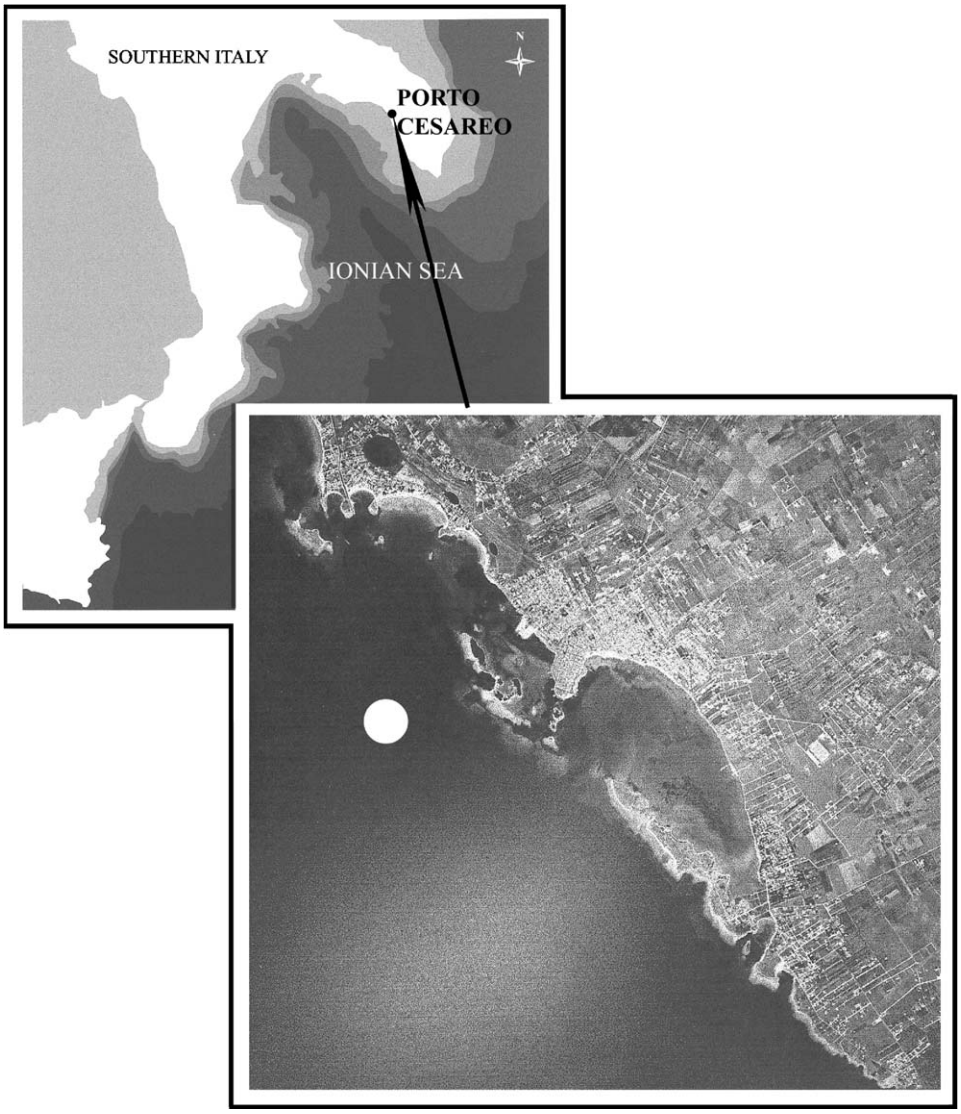

Fig. 1 – Map of the Northern Ionian Sea showing the sampling site where the off-shore rearing farm is located.

2.4. *Bacteriological methods*

Bacterioplankton counts were performed using a Zeiss Standard Axioplan microscope equipped with a halogen 1A (Hg 100) light.

Duplicate slides were prepared from each sample by filtering 1 ml of seawater onto a Millipore filter (0.2 μm pore), using DAPI (4,6-diamidino-2 phenyl-indole) as fluorochrome (Porter and Feig, 1980). AG 365 excitation filter, an FT 395 chromatic beam splitter and an LP 420 barrier filter were used. At least 40 microscopic fields were counted for each preparation at 1000 × magnification.

Cell size of bacterioplankton was estimated by epifluorescence microscopy using microphotographs. Each cell size was determined after projection on a screen and at least 60 cells per filter were measured manually. Bacterioplankton cells were subdivided into three size classes: small, medium and large (<0.065, 0.065–0.320 and 0.320–0.780 μm^3) (Danovaro et al., 1998). Bacterioplankton biovolume was converted into biomass assuming a carbon content of 310 fg C μm^{-3} (Fry, 1990).

3. Results

The results obtained demonstrated the efficiency of *S. officinalis* in removing bacteria from the surrounding environment.

The trends of bacterial abundance during the exposure period, in the controls and treatments, are shown in Fig. 2. Bacterial concentrations decreased markedly with time in presence of sponges ($p<0.001$). The mean bacterial density in the controls was 12.3 ± 1.8 cells $\times 10^4$ ml^{-1}. In treated samples these values decreased after 15 min from the beginning of the experiment, reaching a value of 10.7 ± 1.3 cells $\times 10^4$ ml^{-1}. Bacterial concentration further decreased after 1 h (2.9 ± 0.91 cells $\times 10^4$ ml^{-1}) and dropped drastically after 2 h until the end of the experiment. Statistical analysis revealed a significant

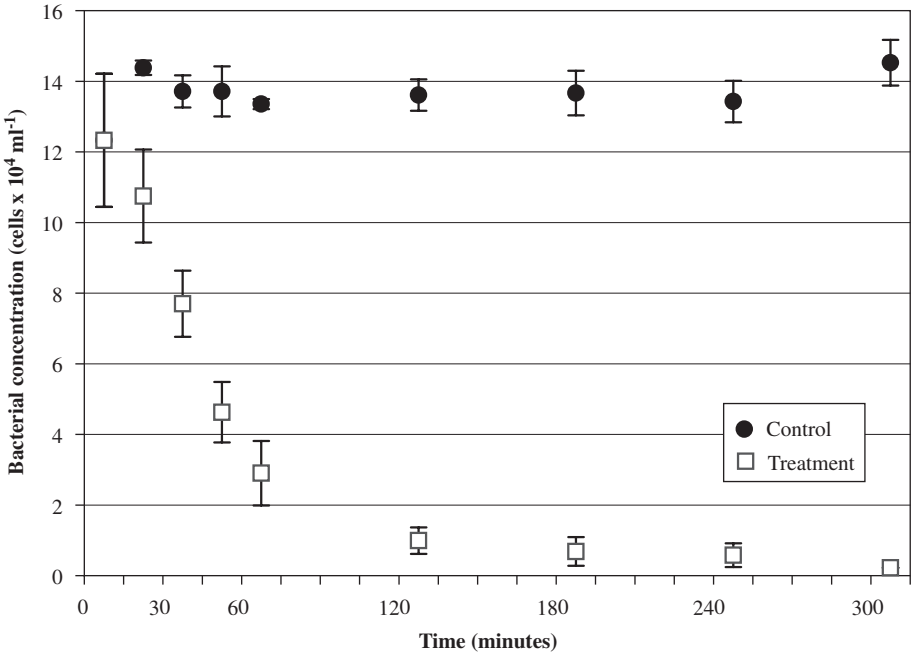

Fig. 2 – Temporal trend of bacterial concentrations (mean values ± SD) in the control (*C*) and treatment (*T*).

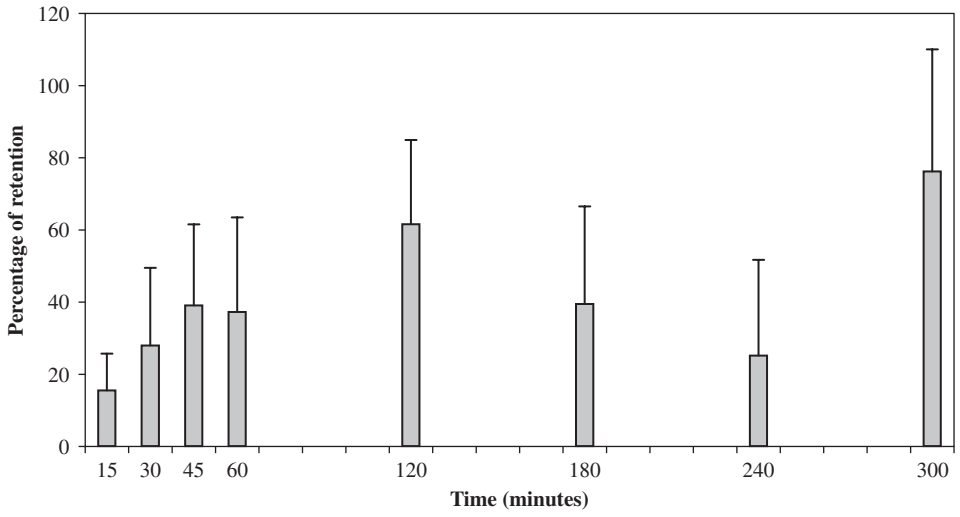

Fig. 3 – Retention efficiency (mean values ± SD) of bacterioplankton by *Spongia officinalis* var. *adriatica* over time.

difference between the bacterial densities in the controls and the treatments ($p < 0.001$).

Retention efficiency gradually increased during the first 2 h, reaching the highest value of 61% at 120 min, then it was subjected to moderate oscillations, probably reflecting the variations in bacterioplankton concentration in the water (Fig. 3).

Clearance values calculated considering the sponge volumes (Fig. 4), ranged between 0.53 and 2.7 $ml\,h^{-1}\,ml^{-1}$ of sponge at 240 and 60 min, respectively. The maximum clearance rate expressed taking into account the sponge dry weights (Fig. 4) was 210 $ml\,h^{-1}\,g^{-1}$ DW at 60 min while the minimum was 34 $ml\,h^{-1}\,g^{-1}$DW at 240 min. Considering the carbon equivalent of the mean numbers of cells removed per ml^{-1} we estimated the bacterial biomass filtered by *S. officinalis* (Fig. 5). Bacterial biomass in the seawater employed was 11.7 ± 1.4 μg Cl^{-1} and decreased of about 3 folds during the first 15 min in the treatments (7.6 ± 2.6 μg Cl^{-1}). After 30 min the bacterial biomass was less than a half in the treatments (4.3 ± 1.5 μg Cl^{-1}). As observed for the bacterial densities also the bacterial biomass felt down after 2 h.

Analysing the cell size distribution in the seawater (Fig. 5), medium-size bacteria prevailed followed by large-size and finally by small-size ones. Sponges filtered preferentially large- and medium-size bacteria, in fact after 15 min the former were reduced of about a half (from 4.0 ± 0.9 to 2 ± 1.5 μg Cl^{-1}) and the latter decreased from 7.0 ± 0.3 to 4.7 ± 1.0 μg Cl^{-1}.

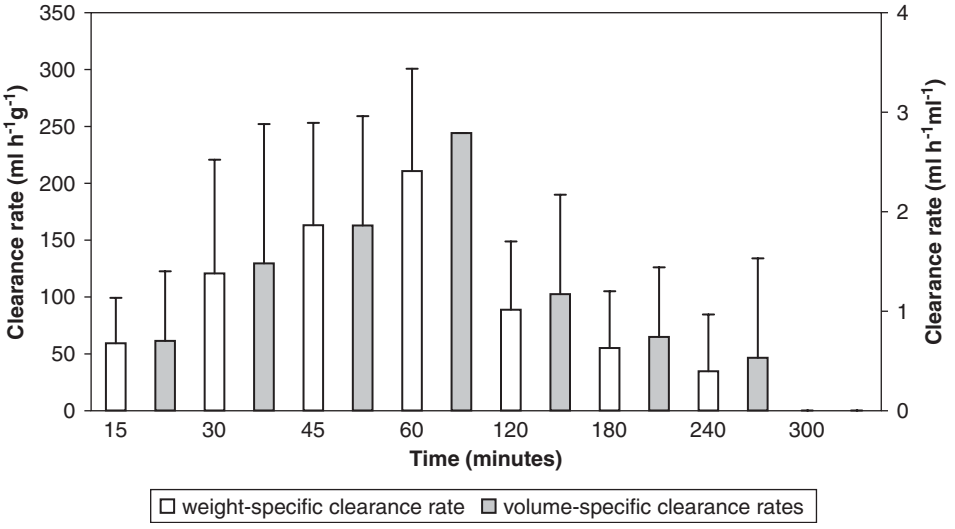

Fig. 4 – Clearance rates (mean values ± SD) of *Spongia officinalis* var. *adriatica* during the experimental period expressed as ml h^{-1} ml^{-1} of sponge and as ml h^{-1} g^{-1} dry weight of sponges.

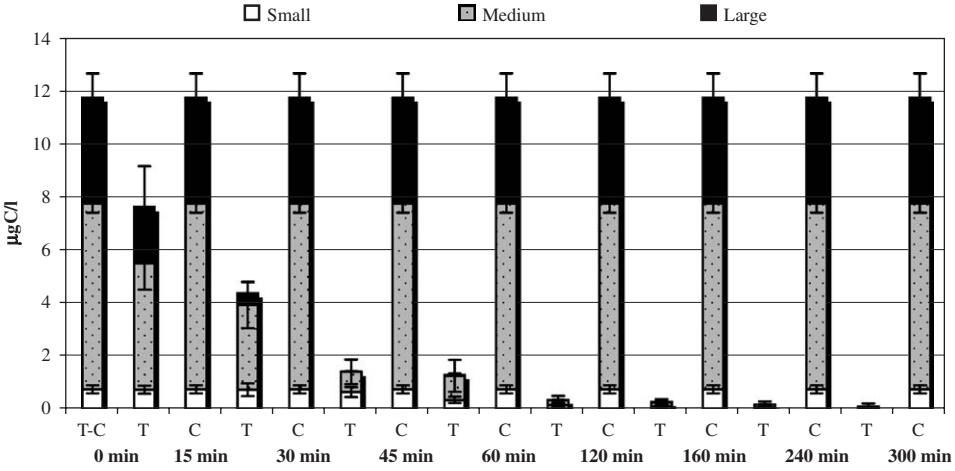

Fig. 5 – Temporal trend of bacterial biomass (mean values ± SD) in the control (*C*) and treatment (*T*). Bacterial biomass is expressed taking into account the size of bacteria (small, medium, large).

Only after 45 min sponges removed also the small-size bacteria when the large-size bacteria were completely absent and the medium-size bacteria densities were low.

4. Discussion

Available food for filter-feeders in the water column is continuum from DOC to both the largest forms of particulate organic carbon, the detrital organic carbon and the live carbon including bacteria, protozoa, phytoplankton and zooplankton (Ostroumov, 2003, 2005). Grazing on bacterio-plankton has been documented in some demospongiae (*Dysidea avara*, *Mycale lingua*, *Mycale* sp., *Tethya crypta* and *Verongia gigantea*) (Pile et al., 1996; Ribes et al., 1999; Fu et al., 2006), and generally this fraction of plankton is considered as the main sources of carbon for sponges (Pile et al., 1996). Ribes et al. (1999) reported that the Mediterranean *D. avara* obtained 85% of its ingested carbon from the fraction of plankton smaller than 5 µm, while it did not fed significantly on POC of detrital origin or DOC. Bacteria alone can constitute a major food source for demosponges and could potentially fulfil their total dietary requirements, especially in coastal waters with high organic content (Becerro et al., 1994; Kefalas et al., 2003; Fu et al., 2006). Referring to our results, *S. officinalis* utilizes POC of bacterial origin; indeed, bacterial biomass in the seawater was 11.7 ± 1.4 µg C l^{-1} whilst in the treatments at the end of the experiment bacterial biomass was reduced to about 0.009 µg C l^{-1}.

Estimates of clearance rate calculated in this study are near to the range of values reported by Ribes et al. (1999) for the Mediterranean *D. avara*, which varies between 104 and 2046 ml h^{-1} g^{-1} DW, whereas is lower than that obtained for the same species by Turon et al. (1997), (1391 and

3806 ml h^{-1} g^{-1} DW) by using latex beads of 0.2 μm in laboratory experimental conditions. The clearance rate of *S. officinalis*, calculated considering the sponge volume, is about 2 times higher than that reported for *Chondrilla nucula* by Milanese et al. (2003), ranging between 0.2 and 1.4 ml h^{-1} cm^3 of living sponge (assuming 1 cm^3 of sponge = 1 ml).

These values, however, are markedly lower than 5660 ml h^{-1} g^{-1} DW reported for the tropical *Haliclona anonyma* (Stuart and Klump, 1984). Perhaps to obtain energy requirements tropical species might need to process larger volumes of water than species living in temperate seas with higher quantities of food.

Moreover, comparison of clearance rate values among different species is difficult as also suggested by Ribes et al. (1999) due to the variability within each species and the effect of sponge size. Variable clearance rates, decreasing with sponge size, have been previously observed for several sponge species (Reiswig, 1974; Riisgard et al., 1993). It has been suggested that the decrease of the clearance rate with the increase of the sponge size could be the result of fewer living choanocytes per unit weight in large sponges (Riisgard et al., 1993).

Our data show a variability in bacterial depletion and clearance rates of sponges with time, consistent with reports by other authors (Frost, 1980; Willenz et al., 1986). It is well known the ability of sponges in regulate their pumping rates and even stop filtering, according to physiological and/or behavioural needs (Kilian, 1952; Frost, 1980; Simpson, 1984). This feature could explain the variability in the filtering activity observed among the individuals of *S. officinalis* during our study. Our results show that in *S. officinalis* the retention efficiencies gradually increase during the first hour when the bacterial density was high, and reaches the highest value at 120 min, when the bacterial concentration was decreased. This suggests that *S. officinalis* could regulate at the best its filtering activity at different bacterial concentrations on the basis of the availability of preys in the water column. Such variation in the retention efficiency could be related solely to the variation in the bacterial concentration and not to a depletion in oxygen concentration since the aeration in the small volumes utilized was ensured.

Several studies estimating clearance rates based on the uptake of different prey sizes have observed that clearance rates varied according to the particle sizes, showing different retention efficiencies, and indicating that sponges actively select particles (Frost, 1980). In *D. avara* clearance rates varied among different prey types, suggesting an inverse relationship between mean prey size and clearance rate (Ribes et al., 1999). Our results show a selection with regard to the size of bacteria. In fact, the large-sized bacteria in the treatments were filtered firstly and the smaller ones only at the end of the experiment when the large-sized bacteria were completely disappeared from the seawater. The large-size bacteria retained with high efficiency by *S. officinalis* correspond in size to the preys (1 μm) captured most efficiency from *D. avara* and *Crambe crambe* (Turon et al., 1997; Ribes et al., 1999). As suggested by Turon et al. (1997), the diversity of structure and complexity of aquiferous systems in sponges may influence their efficiency in food capture over a range of particle sizes, especially when considering sizes near the threshold values. Particle size about 0.2 μm corresponds to the lower limit that is usually considered to be retained by the choanal microvilli. Maybe the fraction $<0.065\,\mu m^3$ analysed in our study is too small to be retained from the choanal microvilli, if we considered that the microvilli spaces ranged between 0.09 and 0.1 μm in *C. crambe* and 0.14–0.23 μm in *D. avara* (Turon et al., 1997). There is little information on the feeding behaviour related to biological and structural characteristics of Demospongiae and in this framework our study represents a first contribute relative to the selective retention efficiency of *S. officinalis* on bacteria of different size. In order to confirm our hypothesis and to exclude possible differentiations in the filtering activity due to the "taste" of bacteria, further investigations will be accomplished by employing inert particles of defined sizes in the range of the bacterial sizes. In artificially enriched Mediterranean seawater mesocosms Servais et al. (1999) and Bernard et al. (2000) have shown that medium and large-sized bacteria have higher growth rates and a larger contribution to the activity of bacterial communities than smaller cells. In our study, the medium and large-sized bacteria dominated the bacterioplankton community of the Northern Ionian Sea and these size-classes were significantly affected by sponge filtration. Furthermore, studies on size-selective ingestion by grazers indicate that very small and large bacteria are partially or totally protected from protozoan grazing which selectively feed on the medium-sized fraction of bacterioplankton, inducing morphological shifts of natural communities toward small and large cells (Jürgens and Güde, 1994; Posch et al., 1999). The removal of both the medium and large-sized bacteria by *S. officinalis* suggests that this species may influence the bacterial size distribution and indirectly the diversity and activity of natural bacterioplankton.

Finally, the ability of *S. officinalis* to retain high quantities of suspended bacteria could also include pathogenic microrganisms usually occurring in coastal seawaters. This feature could be coherent with the employment of this species as bioremediator of waters discharged from aquaculture farms and urban sewage discharges as already suggested by Richelle-Maurer et al. (1988) for *Ephydatia fluviatilis*, Milanese et al. (2003) for *C. nucula* and by Fu et al. (2006) for *Hymeniacidon perleve*. Aquaculture systems, in fact, contain also microbial organisms comprising both autotrophic and heterotrophic flora (Leonard et al., 2000) including potential pathogenic bacteria for the reared species. Considering the resistance against antibiotics developed by bacteria (Skjermo and Vadstein, 1999) and that the already utilized biological filters do not solve the problem of the bacterial growth in the cultivation ponds, the research of alternative strategies for control of the microbial conditions is essential for further progress in aquaculture such as the employment of filter-feeders as bioremediators. Few studies have considered macroinvertebrates as bioremediators of microbial pollution. The idea of employing filter-feeders for applied purposes has been suggested by several authors (Ostroumov 2002, 2004; Licciano et al., 2005; Stabili et al., 2006). Thus, further studies will be accomplished by considering the filtration ability of *S. officinalis* on bacteria potentially pathogenic to man and marine organisms such as vibrios in order to confirm this hypothesis.

Obviously this report represents the starting point of investigation concerning the bioremediation topic. In this framework, further studies are in progress to evaluate if the filtration process of *S. officinalis* up to now characterized in the laboratory conditions, will be also efficient in the field where chemical physical parameters, e.g., water flow, temperature, depth and light conditions as well as the presence of other filtering organisms act simultaneously.

5. Conclusions

This work constitutes the first study on the filtering activity of the demosponge *S. officinalis* var. *adriatica* (Schmidt, 1892), the most common Mediterranean 'bath sponge', with high commercial value, successfully employed in experimental aquaculture experiences. Our study demonstrates a high efficiency of the sponge *S. officinalis* in removing bacterioplankton. Bacterial concentration significantly decreases in presence of the sponge, with a marked drop after 2 h from the start of the experience. Referring to our results, *S. officinalis* utilizes POC of bacterial origin; indeed, bacterial biomass in the seawater was $11.7 \pm 1.4\,\mu g\,C\,l^{-1}$ whilst in the treatments at the end of the experiment bacterial biomass was reduced to about $0.009\,\mu g\,C\,l^{-1}$. The sponge fed preferentially large- and medium-size bacteria, whereas the small ones are fed after the removal of the largest size categories. The utilization of bacteria by sponges suggests an applicative role for bioremediation purposes, considering that bacteria are usually abundant in waters with high amount of organic matter, reaching high densities in harbours and in areas subjected to aquaculture activities.

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