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Evaluation of microbiological accumulation capability of the commercial sponge *Spongia officinalis* var. *adriatica* (Schmidt) (Porifera, Demospongiae)

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ABSTRACT

This study was carried out to evaluate the microbiological accumulation capability of the demosponge *Spongia officinalis* var. *adriatica*. Six microbiological parameters were researched in two sampling periods in the water and in reared sponge samples coming from sites with different degrees of microbial contamination: an off-shore fish farm displaced off the Apulian coast (Southern Adriatic Sea) and a no-impacted area displaced into the Marine Protected Area of Porto Cesareo (Apulian coast-Ionian Sea). We detected the density of culturable heterotrophic bacteria by spread plate on marine agar, total culturable bacteria at 37 °C on Plate Count Agar and vibrios on thiosulphate–citrate–bile–sucrose–salt (TCBS) agar. Total and fecal coliforms as well as fecal streptococci concentrations were detected by the MPN method. Bacterial densities were always higher in the sponge homogenates compared with the corresponding seawater in the sampling points and in both sampling periods. As regard vibrios, total culturable bacteria at 37 °C and fecal streptococci concentrations, the highest values were observed in the sponge samples coming from the off-shore fish farm during the summer period. The ability of *Spongia officinalis* var. *adriatica* to accumulate the microbial pollution indicators suggests that this species can be employed as a bioindicator for monitoring water quality.

1. Introduction

During the last years the significant expansion of off-shore mariculture activities, has generated increasing interest in their potential impact on the aquatic environment (Rana, 1998; Trovar et al., 2000). While a lot of studies have demonstrated the effects of fish farming on sediments and meiofauna (Karakassis et al., 2000; Mazzola et al., 2000; Mirto et al., 2000; La Rosa et al., 2001; Vezzulli et al., 2002), knowledge of the influence of aquaculture activities on microbial abundance is still scarce (La Rosa et al., 2001; Caruso et al., 2003). Available data demonstrated the impact of organic inputs associated to fish feeding on the nutritional load in coastal areas (La Rosa et al., 2002). The introduction of organic matter in large amounts provides bacteria, including those potentially pathogenic to humans and marine organisms, with the complex of dissolved and particulate substrates available as a food source that may determine changes

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in their community structure and dynamics (Bedwell and Goulder, 1996). In this context, studies on the ability of filter-feeding marine macroinvertebrates to remove from the water free-living bacteria have a large interest for bioremediation purpose. Most of the studies concerning the removal of bacterioplankton by filter-feeders focus on molluscs (Birkbeck and Mc Henry, 1982; Kaspar and Tamplin, 1993; Cavallo and Stabili, 2002; Ostroumov, 2005; Stabili et al., 2005). Recently, some studies have demonstrated the ability of the polychaete *Sabella spallanzanii* to accumulate microorganisms under natural and experimental conditions (Licciano et al., 2007; Stabili et al., 2006a).

Demospongiae (Porifera) constitute the dominant active suspension feeding macroinvertebrates in many hard bottom communities. They are able 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 (Reiswig, 1971). However, demosponges feed primarily on bacterioplankton (<2 μm), with high removal efficiency (Reiswig, 1971; van de Vyver et al., 1990; Ribes et al., 1999), which could potentially fulfil their total dietary requirements, especially in coastal waters where bacteria are abundant (Kefalas et al., 2003; Fu et al., 2006). In light of this, the use of sponges for bioremediation purposes has been repeatedly suggested (Richelle-Maurer et al., 1988; Manconi et al., 1999; Milanese et al., 2003).

The demosponge *Spongia officinalis* var. *adriatica* (Schmidt, 1892) is one of the most common Mediterranean 'bath sponges', with high commercial value; it inhabits hard substrata, between the surface and about 60 m of depth. Very common until the past century, it has been subjected to a strong depletion due to uncontrolled fishing together with some recent and widespread mortality events (Vacelet et al., 1994; Pronzato, 1999). In the last years this species was successfully employed in experimental aquaculture experiences (Verdenal and Vacelet, 1990; Corriero et al., 2004). Concerning the sponge's filter-feeding activity, laboratory experiments have just demonstrated the high efficiency of this species in removing bacterioplankton from the natural surrounding environment (Kefalas et al., 2003; Stabili et al., 2006b).

The present study represents a further contribution to the knowledge on the ability of *S. officinalis* var. *adriatica* to remove from the surrounding water environment and concentrate bacteria including those potentially pathogenic to humans and marine organisms, such as vibrios, by comparing specimens reared in sites with different degrees of microbial contamination.

2. Materials and methods

2.1. Sampling sites

This study was carried out in two sites located along the coast of Apulia (Italy): a no-impacted area in the Marine Protected Area of Porto Cesareo (Apulian coast-Ionian Sea) and an offshore fish farm off the Bisceglie coast (Bari-Southern Adriatic Sea) (Fig. 1). At Porto Cesareo the sampling point was located 1 mile off the coast at 20 m depth. The Panittica fish farm, consists of a system of 12 rearing cages placed over a soft bottom at 20 m depth. Cages are located 1 mile off the coast of Bisceglie and contain basses and black sea basses, with a density of about 60 fishes m^{-3}. At this site, in order to evaluate the ability of the sponge to remove bacteria at different distances from the source of pollution, two sampling points were used: one located under a fish cage (Bisceglie-C), the other one on a long line located at 25 m of distance from the cages (Bisceglie-LL). Both sampling points were at 20 m depth. Thereafter, a total of three sampling points were considered named, respectively, Porto Cesareo, Bisceglie-C and Bisceglie-LL.

Fig. 1 – Map of the Apulian coast showing the locations of the sampling sites.

2.2. Sponge and water sampling

In December 2004, sponge cuttings, subspherical in shape, with a maximum diameter of about 7–8 cm, were obtained by 30 adult sponge specimens coming from a wild population located in the Northern Ionian Sea at 30 m of depth. Fragments were divided into 18 groups each consisting of 10 cuttings. Six groups of sponges were transplanted on rearing structures placed at 20 m of depth at the Marine Protected Area of Porto Cesareo. Twelve groups were transplanted at the fish farm of Bisceglie, six of them at Bisceglie-C and the last ones at Bisceglie-LL. Sponge cuttings and rearing techniques were according to Corriero et al. (2004).

Samples of sponge cuttings together with the surrounding seawater ($n = 3$) were collected in December 2005 (*T*1), and in July 2006 (*T*2) from the three sampling points (3 sponge groups at *T*1 and 3 at *T*2, for a total of 6 groups for each sampling point). Samples were collected by SCUBA diving. Water samples were obtained aseptically in presterilized 1000 ml glass bottles submerged to a depth of 50 cm. Soon after collection, fragments and water samples were transported within cooled bags to the laboratories of the University of Lecce. Both water and sponge samples were processed for bacteriological analysis.

2.3. Abiotic parameters

Temperature and salinity were measured *in situ* using a multiparametric sounding-line "Ocean Seven 401" (Jolzonant, Italy).

2.4. Bacteriological methods

In order to obtain a total of three sponge samples for each site and sampling time in the laboratory all the 10 individuals of each sponge group were pooled into one sample.

Each sponge sample was gently squeezed with a glass stick and then the sponge pieces were homogenized in a sterile Waring blender. The homogenates were subjected to a series of dilutions (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}). In order to enumerate the culturable vibrios in seawater, 1, 5 and 10 ml of seawater were filtered on 0.22 μm pore size filters; then the filter disks were aseptically placed onto triosulphate–citrate–bile–sucrose–salt (TCBS) agar. Incubation was carried out at 20–25 and 35 °C for 2 days and the colonies of presumptive vibrios were counted according to the colony-forming unit (CFU) method. The incubation temperature of 35 °C was chosen to estimate the fraction of vibrios potentially pathogenic to humans. The incubation temperature of 20–25 °C was chosen since some *Vibrio* spp., such as *Vibrio anguillarum*, do not grow well at higher temperature (Høi et al., 1998). Mean values for three replicate samples were determined and expressed as CFU g^{-1} taking into account of the dilution factor. For the vibrios quantitative research in sponges 0.1 ml of each sponge homogenate or appropriate decimal dilution of the homogenate (using a sterilized water sample from the collection area as diluent) was plated onto TCBS agar. After incubation for 2 days, the culturable vibrios were counted according to the CFU method.

For enumeration of heterotrophic bacteria, the number of CFU was determined by plating 100 μL of undiluted seawater and serial dilutions of each water sample as well as sponge extract in triplicates on Bacto Marine Agar 2216 (Difco). The plates were incubated at 22 °C for 7 days. At the end of the incubation period all colonies were counted in terms of CFU method through a 10× magnification lens.

Counts of culturable bacteria at 37 °C (including human potential pathogens) in seawater and sponge samples were determined by the spread plate method, using Bacto Plate Count Agar medium (seeding with 0.1 ml of sample). The plates were incubated at 37 °C for 48 h.

For all the microbiological parameters counted according to the CFU method, bacterial densities were expressed as CFU ml^{-1} for seawater and CFU g^{-1} for sponge samples.

Total and fecal coliforms as well as fecal streptococci densities were determined to evaluate the degree of water pollution and sponges' microbiological accumulation of the classical microbial pollution indicators. Fecal coliforms and total coliforms were enumerated by the most probable number (MPN) method using the standard five-tube method of 10-fold dilution in the case of seawater and the three-tube MPN series in the case of sponges (American Public Health Association, 1992). Results were expressed as MPN 100 ml^{-1} and MPN 100 g^{-1} for seawater and sponge samples, respectively. For total and fecal coliforms determination, lactose broth and brilliant green-lactose broth were used as culture media in the presumptive and confirmative test, respectively (incubation at 37 °C for 24–48 h). For fecal streptococci the presumptive probe was performed using Azide and the confirmative one using ethyl violet azide broth.

2.5. Statistical analysis

In order to evaluate differences in the mean abundance of the bacteriological groups at *T1* and *T2* between seawater and sponge samples, as well as among the three sampling points, analysis of variance (ANOVA) was performed by using STATSOFT STATISTICA v. 6.0.

3. Results

3.1. Abiotic parameters

At Porto Cesareo the water temperature was 15.1 °C in December and 26.3 °C in July; the salinity was 37.2‰ in December and 38.5‰ in July. At Bisceglie the water temperature was 15.4 °C in December and 26 °C in July; the salinity was 37.3‰ in December and 38.1‰ in July.

3.2. Bacteriological parameters

Culturable heterotrophic bacteria, culturable *Vibrio* spp., culturable bacteria at 37 °C and microbial pollution indicators were enumerated in the water and the *S. officinalis* samples collected from the three sampling points.

Culturable heterotrophic bacterial abundances in seawater and sponge samples collected from the three sampling points are shown in Fig. 2. In seawater samples, culturable heterotrophic bacterial densities were higher in *T2* than in *T1*, as well as in sponge samples. Moreover the abundances of these bacteria in the sponge samples were significantly higher than in the surrounding seawater both in *T1* ($p<0.05$) and *T2* ($p<0.05$). Particularly, bacterial abundances were two orders of magnitude higher in sponges than in seawater at *T1* and four orders of magnitude higher than in seawater at *T2*.

As regards culturable vibrios (Fig. 3) the lowest densities were observed at *T1* both in seawater and sponge samples, even though *S. officinalis* concentrated vibrios in comparison of the surrounding seawater in both the sampling periods. Statistical analysis revealed a significantly lower vibrio concentration in sponge samples from Porto Cesareo with respect to those from Bisceglie-C ($p<0.01$) and Bisceglie-LL ($p< 0.05$) both at *T1* and *T2*. Moreover, at Bisceglie-C, vibrio densities were also higher than at Bisceglie-L ($p<0.01$). The highest vibrios concentration was recorded at *T2* in the sponge samples collected at Bisceglie-C (1.8×10^5 CFU g^{-1}).

The trends of culturable bacteria at 37 °C are given in Fig. 4. As observed for culturable heterotrophic bacteria and vibrios, the sponges significantly concentrated this bacterial group in comparison to the surrounding seawater ($p<0.05$). The highest concentrations (8.5×10^6 CFU g^{-1}) were monitored at Bisceglie-C and Bisceglie-LL in *S. officinalis* (*T2*) as well as the seawater of Bisceglie-C (5.3×10^4 CFU ml^{-1}).

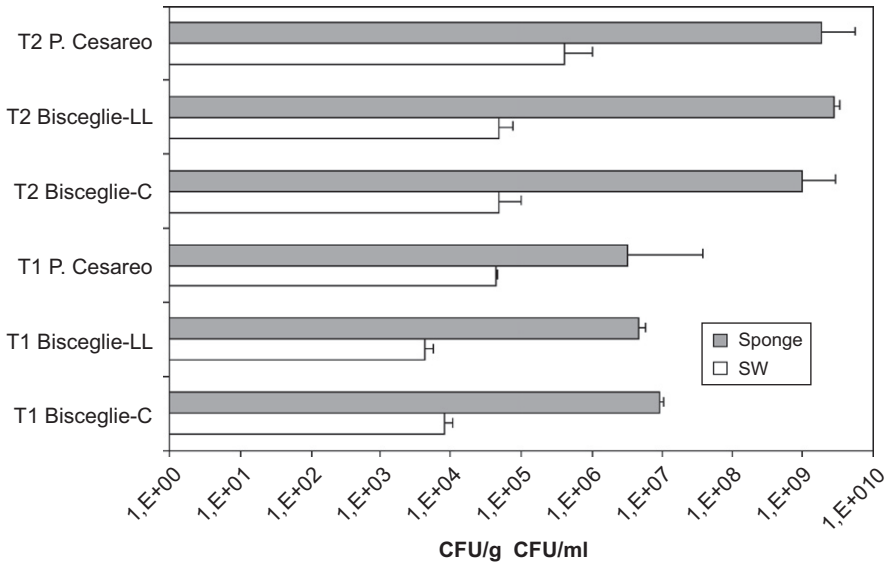

Fig. 2 – Averaged culturable heterotrophic bacterial abundances over the two sampling periods in seawater and sponge samples collected in the three sampling points: Porto Cesareo, Bisceglie C (under a fish-cage) and Bisceglie-LL (at 25 m of distance from the cages). Each column represents the mean value ± standard deviation.

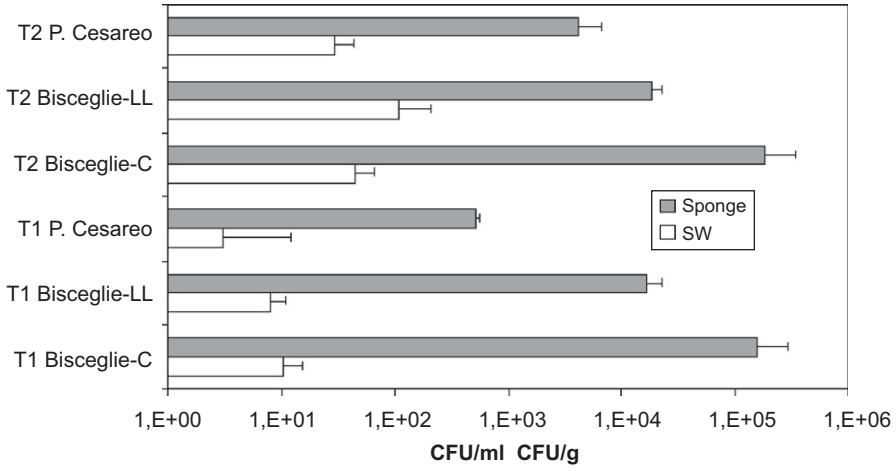

Fig. 3 – Averaged vibrios abundances over the two sampling periods in seawater and sponge samples collected in the three sampling points: Porto Cesareo, Bisceglie C (under a fish-cage) and Bisceglie-LL (at 25 m of distance from the cages). Each column represents the mean value ± standard deviation.

The results concerning the main microbiological pollution indicators in *S. officinalis* and seawater are reported in Fig. 5. Values for total coliforms (Fig. 5A) and fecal coliforms (Fig. 5B) were low or zero in the water samples collected at Porto Cesareo, both at *T1* and *T2*. By contrast, in Bisceglie-C and Bisceglie-LL the densities of these bacteria in seawater samples were low at *T2* and reached 17 MPN 100 ml^{-1} and 11 MPN 100 ml^{-1}, respectively at *T1*. The abundances of total and fecal coliforms were always significantly higher in the sponge sample then in the surrounding seawater in the three considered sampling points ($p < 0.05$).

Concerning fecal streptococci (Fig. 5C), the highest concentrations in seawater were observed both at *T1* and *T2* at Bisceglie under the fish cage (11 and 46 MPN 100 ml^{-1} respectively). Sponges concentrated fecal streptococci with respect to the surrounding seawater. The highest values were observed at Bisceglie: 4600 MPN 100 g^{1} for both Bisceglie-LL (*T1* and *T2*) and Bisceglie-C (*T2*). The densities of this bacterial group were significantly higher in sponge samples from Bisceglie-C and Bisceglie-LL than Porto Cesareo ($p < 0.01$).

4. Discussion

A previous study on *S. officinalis* was undertaken by Kefalas et al. (2003) in order to evaluate the sponge-associated bacteria. Among the isolated bacteria, *Escherichia coli* was recovered at higher concentration in sponge extract compared with the proximal seawater. Moreover, laboratory experiments have already demonstrated the high efficiency of this species in removing bacterioplankton from the natural surrounding environment (Stabili et al., 2006b). The results of

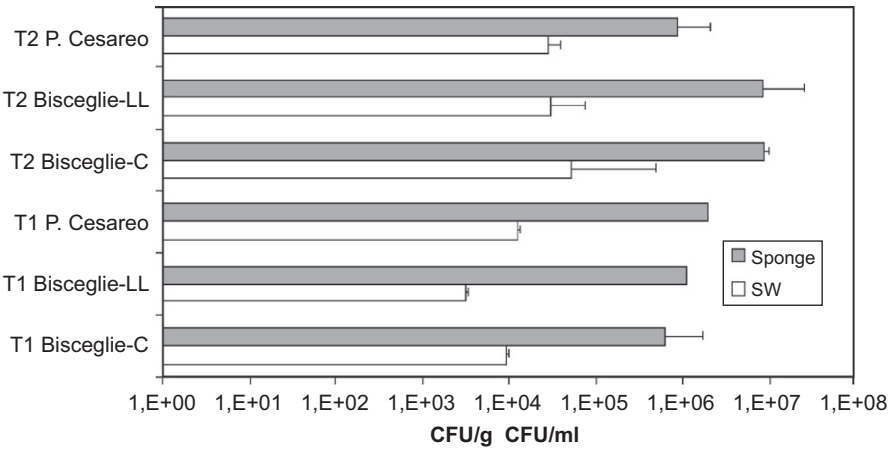

Fig. 4 – Averaged culturable bacteria at 37 °C abundances over the two sampling periods in seawater and sponge samples collected in the three sampling points: Porto Cesareo, Bisceglie C (under a fish-cage) and Bisceglie-LL (at 25 m of distance from the cages). Each column represents the mean value ± standard deviation.

the present study, showing the capability of *S. officinalis* to filter and accumulate several bacterial groups, represent a significant advance in the knowledge of this species filter-feeding on bacteria.

As regards heterotrophic bacteria abundances, the highest values were observed in *T2* both in sponge and seawater samples from Porto Cesareo and Bisceglie. The increased bacterial densities in seawater in the summer period could also depend, besides from the increase of water temperature (Pomeroy and Wiebe, 2001), on the substrate supply due to Adriatic superficial waters (ASW) reaching Apulian coasts in this period. The ASW, coming from an area heavily supplied by rivers, are characterized by high levels of dissolved nutrients and suspended matter concentrations (Fonda Umani et al., 1992; Franco and Michelato, 1992, Stabili et al., 2006a). Due to its high filtering capability, *S. officinalis* concentrated heterotrophic bacteria and reflected the bacterial pattern abundance in the surrounding environment, showing the highest density values in the summer period, just when the highest values of seawater bacterial abundances occur.

The seasonal pattern of culturable heterotrophic bacteria was also recorded for vibrios densities, which show the highest values in summer both in the sponge and water samples, reflecting a significant positive relationship with water temperature (Kaspar and Tamplin, 1993). The values of vibrios concentrations in sponge and water samples were significantly higher at Bisceglie than at Porto Cesareo where oligotrophic conditions prevail (Alabiso et al., 2001; Fraschetti et al., 2005). Literature data indicated that different ecological parameters such as nutrient availability, temperature and salinity determine the occurrence of different *Vibrio* species in the sea (Kaspar and Tamplin, 1993). It has been previously demonstrated that organic inputs associated with fish feeding have a substantial impact on the nutrient load in coastal areas (Handy and Poxton, 1993; Karakassis et al., 2000). La Rosa et al. (2001) reported an increase in vibrios density at a Tyrrhenian station close to a mussels farm, suggesting that vibrios are good indicators of organic enrichment. Thus, the higher trophic availability occurring at Bisceglie, presumably related to the fish farm, is reflected in the higher vibrios densities in comparison with Porto Cesareo. This hypothesis seems to be confirmed by the differences in vibrios concentrations between Bisceglie-C and Bisceglie-LL. Indeed, the highest densities of these bacteria were observed just under the fish cages.

Our results showed that *S. officinalis* concentrated culturable vibrios from the surrounding environment. Indeed vibrios concentrations were always higher in sponge samples than in seawater ones. The high vibrios abundances in *S. officinalis,* also detected during winter, lead us to suppose that vibrios can adopt, as a survival strategy to overcome unfavorable environmental conditions, the association with sponges, as already reported for phytoplankton, zooplankton, crustaceans and molluscs (Montanari et al., 1999; Wai et al., 1999).

Total and fecal coliform concentrations in seawater were very low both at *T1* and *T2*, particularly at Porto Cesareo. The low densities of these microbial pollution indicators suggest that the studied coastal areas are subjected to low microbial pollution due to human discharges. However, in *S. officinalis* the abundances of these bacteria were always higher than those of the surrounding environment. Moreover, the densities of fecal streptococci were significantly higher in sponge samples from Bisceglie-C and Bisceglie-LL than those from Porto Cesareo, reflecting the same bacterial pattern abundance of the surrounding seawater. The higher concentrations of this bacterial group in the seawater and sponge samples from Bisceglie reflect the presence in this area of the fish farm as a source of microbial pollution. In fact, fecal streptococci are not so ubiquitous as coliforms, but always present in the feces of warm-blooded animals and their die-off is less rapid than the removal of coliforms (APHA, 1992). The usefulness of fecal streptococci as microbiological indicators is generally accepted because of their inability to multiply in sewage effluents (Slanetz and Bartley, 1965). As a consequence, their concentration should reflect the real degree of fecal contamination. Moreover, the capability of *S. officinalis* to concentrate microbial pollutants by removing them from the surrounding environment allows the detection of these bacteria even when they are present in the water

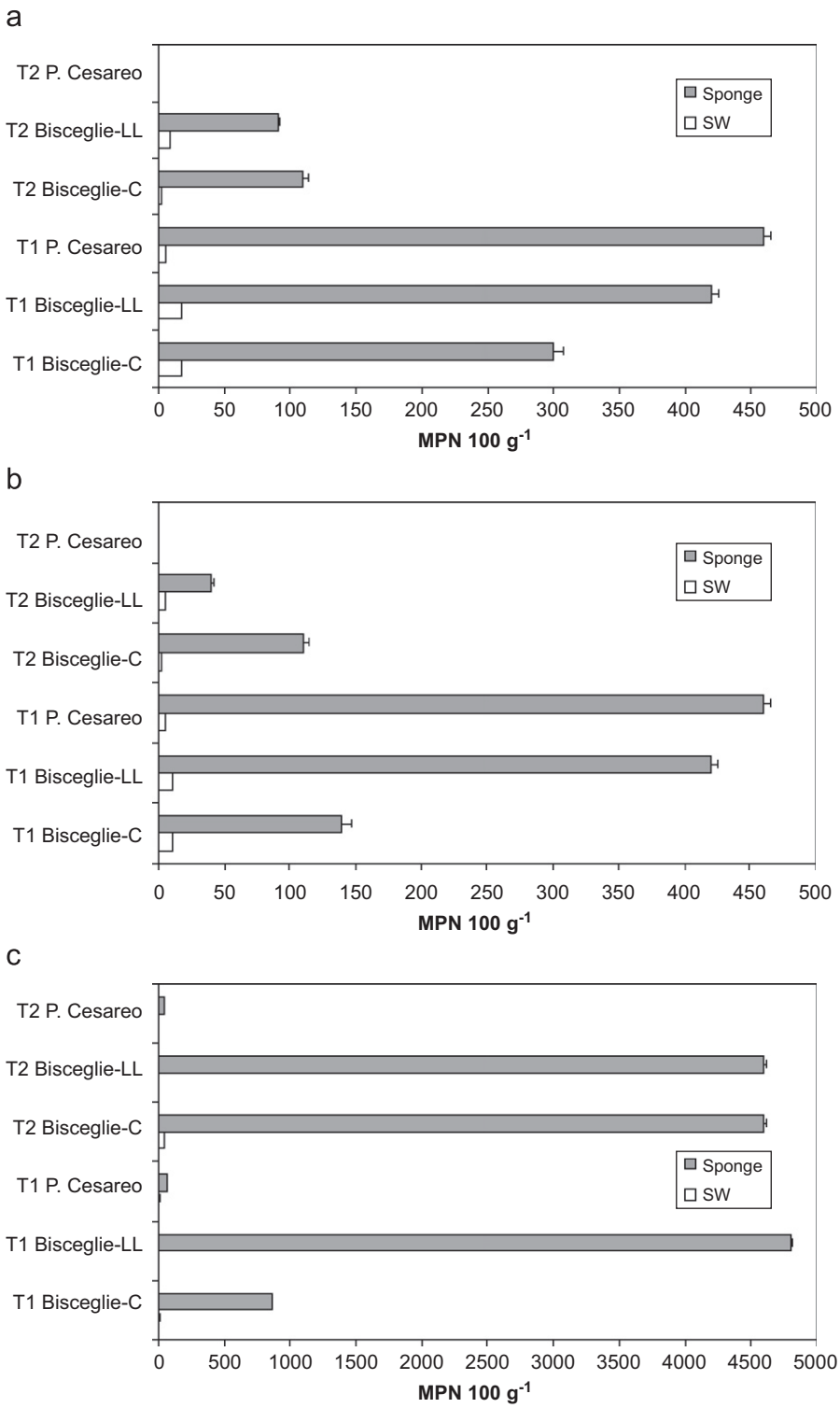

Fig. 5 – Averaged pollution microbial parameters over the two sampling periods in seawater and sponge samples collected in the three sampling points: Porto Cesareo, Bisceglie C (under a fish-cage) and Bisceglie-LL (at 25 m of distance from the cages): (a) total coliforms, (b) fecal coliforms, (c) fecal streptococci. Each column represents the mean value ± standard deviation.

column at very low concentrations, suggesting the possibility to use this sponge as a bioindicator in monitoring microbial pollution in the marine environment. Indeed, bacteriological monitoring is of great importance in areas suitable for aquaculture activities, as these areas must possess optimal quality levels.

Taking into account that bacteria, including pathogenic microrganisms for human and reared species, are usually abundant in areas subjected to high amounts of organic matter, the demonstrated ability of *S. officinalis* to accumulate bacteria encourage the employment of this sponge as a bioremediator. Such a hypothesis was already suggested by

Stabili et al. (2006b) on the basis of the high clearance rate values recorded for this species under laboratory conditions. The idea of employing sponges for bioremediation purposes has been already suggested for other species such as *Ephydatia fluviatilis* (Richelle-Maurer et al., 1988), *Chondrilla nucula* (Milanese et al., 2003) and *Hymeniacidon perleve* (Fu et al., 2006). Considering the resistance against antibiotics developed by bacteria (Skjermo and Vadstein, 1999) and the need to control fish and shellfish diseases due to bacterial infections, including vibriosis (Bordas et al., 1998), the possibility to use filter feeder macroinvertebrates capable of concentrating and controlling bacterial communities represents a very important step for bioremediation of waste, ensuring the safety of both aquaculture farms and seafood. Thus surely the development of such alternative strategies for control of microbial conditions is essential for further progress in aquaculture.

5. Conclusions

We demonstrate the *S. officinalis* accumulation capability of different bacterial groups (culturable heterotrophic bacteria, total culturable bacteria at 37 °C, culturable vibrios, total and fecal coliforms and fecal streptococci) by comparing specimens reared in sites with different degree of microbial contamination. This ability could facilitate the detection of microbial pollution, even when they are present in the water column at very low concentrations, and lead to the employment of this species as a bioindicator for monitoring water quality. Moreover, the demonstrated ability of *S. officinalis* to accumulate bacteria, including vibrios, encourage the employment of this sponge as a bioremediator. This could enhance the safety of sea-farming and seafood production.

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