Bacterial accumulation by *Branchiomma luctuosum* (Annelida: Polychaeta): A tool for biomonitoring marine systems and restoring polluted waters

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Abstract

In this study, we examined the bacterial accumulation in the filter feeder polychaete *Branchiomma luctuosum* GRUBE (Sabellidae). Analyses were performed on worm homogenates from 'unstarved' and 'starved' individuals, and seawater from the same sampling site (Gulf of Taranto, Western Mediterranean, Italy). Densities of culturable heterotrophic bacteria (22 °C), total culturable bacteria at 37 °C and halophilic vibrios at 22 and 35 °C were measured on Marine Agar 2216, Plate Count Agar and thiosulphate-citrate-bile-salt-agar (TCBS) plus 2% NaCl, respectively. Total and faecal coliforms as well as faecal streptococci were determined by the Most Probable Number method. Results showed that *B. luctuosum* is able to accumulate all the considered six microbiological groups with a higher efficiency for autochthonous bacteria. The analysis also indicated that bacterial groups differ in their resistance to digestion by *B. luctuosum*. Our data suggest the potential role of *B. luctuosum* as an useful bioindicator to assess low levels of microbiological water pollution as well as bioremediator of microbial polluted waters.
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1. Introduction

Filter-feeding is a trophic strategy widely represented among benthic marine invertebrates and plays a crucial role in energy transfer processes in marine systems for the pelagic and benthic coupling (Cloern, 1982; Officer et al., 1982; Kimmerer et al., 1994; Gili and Coma, 1998; Widdows et al., 1998; Widdows et al., 2000, 2004).

Polychaetes are one of the best represented taxa of marine invertebrates in benthic assemblages and in various groups are able to collect particles suspended in the water column. Species belonging to the family Sabellidae are easily recognizable for the presence of an elaborated tentacular crown that expands out of the tube they inhabit. A number of studies on Sabellidae has provided descriptions of filtering organs and particle capture mechanisms (Wells, 1952; Fitzsimons, 1965; Lewis, 1968; Bonar, 1972) but there are very few accounts of the qualitative and quantitative aspects of the filtration process (Dales, 1957; Shumway et al., 1988; Riisgård and Ivarsson, 1990; Clapin, 1996; Licciano et al., 2005; Stabili et al., 2006). Sabellids are able to sort collected material of different sizes: small particles are swallowed, while large ones are pushed away from the mouth and ejected into the water.

As a consequence of the filter feeding activity, sabellids may accumulate and concentrate many pollutants in seawater, particularly those which are particulate or associated with particles (Cavallo and Giangrande, 2002; Bocchetti et al., 2004; Fattorini et al., 2005). This ability to accumulate materials may facilitate the detection and measurement of pollutants at very low environmental concentration. The use of bioindicators for monitoring water quality, in fact, is essentially based on the filter-feeders' ability to accumulate contaminants in their tissues to a degree proportional to their bioavailability (Widdows and Donkin, 1992; Andral et al., 2004).

Field and laboratory studies on filter feeder polychaetes have been performed employing phytoplankton as a feeding source (Shumway et al., 1988; Clapin, 1996), even though the nature of suspended matter may be either non-living (detrital organic carbon) or living (bacteria, phytoplankton and zooplankton) (Jørgensen, 1966, 1975; Wotton, 1994; Riisgård and Larsen, 1995, 2001a,b; Silverman et al., 1995, 1997; Gili and Coma, 1998). Many studies dealing with different benthic filter feeder organisms feeding on marine and terrestrial bacteria have been carried out (Jørgensen et al., 1984; Prieur et al., 1990; Bak et al., 1998; Ribes et al., 1999). Special attention was paid to molluscs, particularly bivalves. The potential of bivalves in microbiological surveys has been intensively investigated in relation to their capability to concentrate particles, bacteria and viruses and also in relation to potential human health risks related to their physiology, production and consumption (Wood, 1957; Ayres et al., 1978; Bostock, 1979; Bolinches et al., 1986; Prieur et al., 1990; Yam et al., 1999). Quantitative data on sabellid filtration of bacterioplankton are very scant and limited to *Sabella spallanzanii* (Licciano et al., 2003; Stabili et al., 2006), with the exception of one paper dealing with the clearance rate of *Branchiomma luctuosum* on a pure culture of *Vibrio alginolyticus* (Licciano et al., 2005).

The present study is focused on the filter-feeder sabellid *B. luctuosum* GRUBE, which forms dense populations on hard substrata in heavy eutrophic sheltered marine areas of the Mediterranean basin (Licciano et al., 2002). The species is a lessepsian migrant (from the Red Sea) recorded in the Mediterranean, since 1983 (Bianchi, 1983; Knight-Jones and Ergen, 1991). The aim of this work is to provide evidence for its ability to filter and consequently concentrate several bacterial groups, and to assess if this species may substan-

tially contribute to the removal of bacteria from the water column by its potential accumulation capability thus constituting a potential candidate for bioremediation of microbial polluted waters.

2. Materials and methods

2.1. Samples collection

Adult specimens of *B. luctuosum* were collected along a dock wall by SCUBA divers in the Gulf of Taranto (Ionian Sea, Italy). Water samples were collected from the same sampling site with 5 l sterilized Niskin bottles and processed within 4 h of sampling to enumerate bacteria. Immediately upon return to the laboratory, worms were cleaned of any tube epibionts and randomly divided into two sets. Specimens from one set (90 individuals) were separated in 9 groups each consisting of 10 individuals and immediately extracted from the tubes and utilized for the analysis ('unstarved' condition). The second set (90 individuals) was divided and placed in 9 aquaria each containing 10 individuals. Each aquarium was filled with filtered (0.22 μm pore size filters, Millipore) seawater and acclimatized in a temperature controlled room (22 °C) for 48 h to ensure the reduction of pre-existing microorganisms before the beginning of the experiment ('starved' condition).

2.2. Bacteriological analyses

The bacteriological analyses were performed on seawater from the worm sampling site and on both starved and unstarved worms. Each group of 10 worms (9 groups of starved and 9 of unstarved worms) was processed separately for the enumeration of bacteria. After their extraction from the tubes and washing several times with sterile seawater, worms were homogenized for 9 s in a Waring blender. The homogenates (ca. 100 g) were filtered through sterile gauze and diluted with filtered (0.22 μm) seawater to obtain a 1:10 (w/v) dilution.

Quantitative analyses of culturable heterotrophic bacteria (22 °C), total culturable bacteria at 37 °C, culturable halophilic vibrios at 22 and 35 °C, faecal and total coliforms as well as faecal streptococci were performed on seawater and on worm homogenates.

To enumerate the culturable halophilic vibrios in seawater, 1, 5 and 10 ml were filtered on 0.45 μm pore size filters that were aseptically placed onto thiosulphate-citrate-bile-salt-agar (TCBS) plus 2% NaCl. After incubation for 48 h at 22 and 35 °C, the colonies grown were counted in terms of colony forming units (CFU). For the enumeration of vibrios in polychaetes, 0.1 ml of each worm homogenate and of appropriate decimal dilutions were plated on TCBS agar and after incubation of 48 h at 22 and 35 °C, the culturable vibrios developed on each plate were counted. Results were referred as mean value recorded at 22 and 35 °C.

Final bacterial concentrations were obtained taking into account the dilution factors.

Total and faecal coliforms as well as faecal streptococci were determined by the most probable number (MPN) method using the standard five-tube method of 10-fold dilutions for seawater samples and three-tube MPN series for worm homogenates (APHA, 1998). For total and faecal coliforms determination, lactose broth and brilliant-green-lactose broth were used as cultural media in the presumptive and confirmative test, respectively. For faecal streptococci the presumptive test was performed using Azide broth and the con-

firmative one using Ethyl violet azide broth. Results were referred as MPN 100 ml^{-1} or 100 g^{-1}, for water and worm samples, respectively.

Culturable heterotrophic bacteria abundance was determined by spreading 100 μl of undiluted sea water or worm homogenates and the respective serial dilutions in triplicate on plates of Marine agar 2216 (Difco, USA). The plates were incubated at 22 °C over 7 days, then the colonies were counted.

Total culturable bacterial densities growing at 37 °C, indicating the fraction of bacterial potentially pathogenic to humans, were determined as previously described for the heterotrophic bacteria but using plate count agar (PCA) as the culture medium.

2.3. Statistical analysis

The experimental design consisted of two factors: concentration (Co), with two levels (i.e. seawater and polychaetes, fixed) and time (Ti), with two levels (i.e. before and after worm starvation, fixed and orthogonal to concentration). Analysis of variance (ANOVA) was used to assess differences in the mean abundance of the bacteriological groups between seawater and worms before (Ti_0) and after (Ti_1) worm starvation. Prior to analysis, the homogeneity of variance was tested using Cochran's test and, if necessary, data were $\ln(x+1)$ transformed to remove heterostochasticity. The Student–Newman–Keuls test (SNK) was used for post-hoc comparisons among means (Underwood, 1997). The analysis was done using GMAV 5 computer program (University of Sidney, Australia).

3. Results

Culturable heterotrophic bacteria (22 °C) densities (Fig. 1a) found in *B. luctuosum* differed from those found in the surrounding seawater ($8.8 \pm 1.5 \times 10^4$ CFU g^{-1} and $3.1 \pm 0.2 \times 10^3$ CFU ml^{-1}, respectively). Starved individuals concentrated this kind of bacteria of about 1 order of magnitude ($9.6 \pm 1.2 \times 10^5$ CFU g^{-1}) with respect to the values detected in unstarved animals.

Analysis of variance performed on the abundance of this bacterial group in worm homogenate and seawater samples revealed a significant Co × Ti interaction ($P < 0.001$) (Table 1). *Post hoc* comparisons suggested that heterotrophic bacterial density was significantly lower in unstarved than in starved worms ($P < 0.01$) and that the bacterial concentrations did not vary significantly in seawater samples over time.

Fig. 1b reports the densities of halophilic vibrios growing at 22 and 35 °C detected in *B. luctuosum* homogenates and the surrounding seawater. Starved animals showed the highest vibrios densities with a mean value of $2.4 \pm 0.4 \times 10^5$ CFU g^{-1}. Analysis of variance (Table 1) revealed a significant Co × Ti interaction at $P < 0.001$. Bacteria were significantly more concentrated in unstarved and starved polychaetes in comparison to the seawater, as established by SNK test ($P < 0.01$). Halophilic vibrios concentrations in unstarved animals were significantly lower than starved ones ($P < 0.01$) whilst no significant differences in vibrios abundance were evidenced in seawater samples at Ti_0 and Ti_1.

The abundance of total culturable bacteria at 37 °C (about 1.8×10^4 CFU g^{-1}) did not differ significantly in starved and unstarved *B. luctuosum* (Table 1, Fig. 1c) whereas the bacterial concentration measured at Ti_0 and Ti_1 in polychaetes was significantly higher ($P < 0.001$) than the sampling site seawater where the density was $7.4 \pm 1.3 \times 10^2$ CFU ml^{-1}.
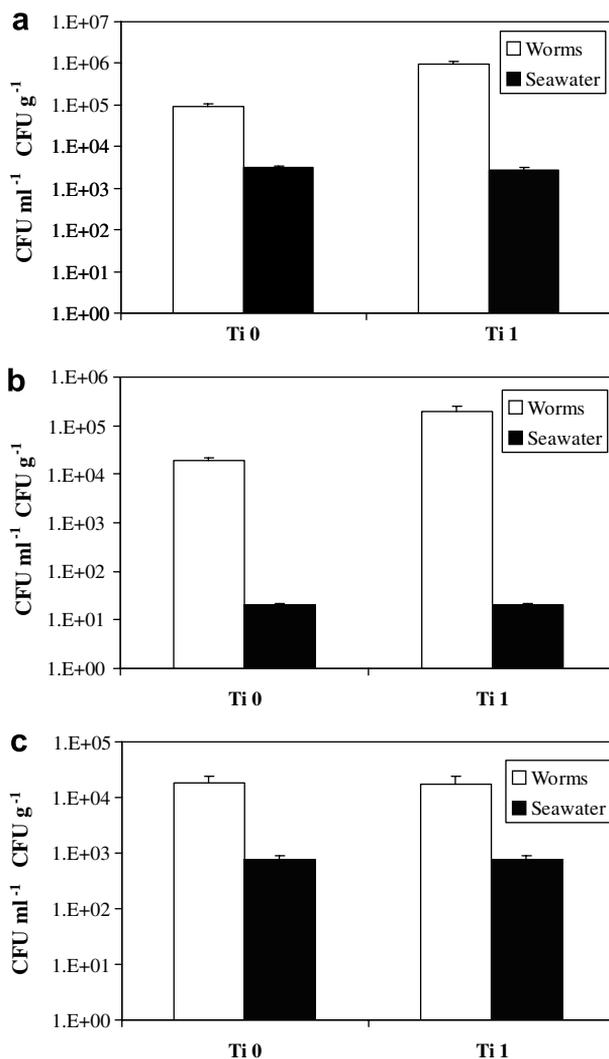
Fig. 1. Mean abundance and relative standard deviations of bacterial densities in seawater and worm samples at Ti_0 (unstarved) and Ti_1 (starved): (a) culturable heterotrophic bacteria at 22 °C; (b) vibrios; (c) total culturable bacteria at 37 °C. (y-axes are logged).

To evaluate the accumulation capability of *B. luctuosum* with respect to the classical microbial pollution indicators, we analyzed the densities of total and faecal coliforms as well as faecal streptococci.

Total and faecal coliforms concentration trends were similar (Fig. 2a) and in both cases bacterial densities were about 5 times lower in the unstarved worms than in the surrounding environment. Particularly, total coliform were 40 ± 4 MPN 100 g^{-1} in the worms and 131 ± 18.5 MPN 100 ml^{-1} in the seawater. Faecal coliform concentrations were 40 ± 4 MPN 100 g^{-1} in *B. luctuosum* and 94 ± 6.56 MPN 100 ml^{-1} in seawater. When the animals were subjected to starvation these indicators were not detected in the homogenates. The SNK test performed on the significant Co $\times$ Ti interaction ($P < 0.01$), revealed

Table 1
Summaries of ANOVAs testing for differences in average bacterial abundances measured at Ti_0 (before starvation period) and Ti_1 (after starvation) in both polychaete and seawater samples

Source of variation	df	Heterotrophic bacteria (22 °C)			Halophilic vibrios (22 and 35 °C)			Total culturable bacteria (37 °C)			CT			CF			SF		
		MS	F	P	MS	F	P	MS	F	P	MS	F	P	MS	F	P	MS	F	P
Concentration = Co	1	12.9			11.7			0.02	0.28	NS	3600.0			3600.0	93.35		0.0002	0.00	NS
Time = Ti	1	183.5			572.6			88.3	1056.32	***	110,889.0			49,284.0	1278.03		20.14	5554.39	***
CoxTi	1	12.9	84,593 ***		11.7	255.70 ***		0.02	0.28	NS	3600.0	18.11 ***		3600.0	93.35 ***		0.0002	0.00	NS
Residual	32	0.01			0.5			0.09			198.8			38.6			0.04		
Total	35																		
Cochran's test		NS			NS			0.5322			NS			NS			NS		
Transform		ln(x + 1)			ln(x + 1)			ln(x + 1)			None			None			ln(x + 1)		
SNKtest																			
Co(Ti)		Pol Ti_0 < Pol Ti_1 SW Ti_0 = SW Ti_1			Pol Ti_0 < Pol Ti_1 SW Ti_0 = SW Ti_1						Pol Ti_0 > Pol Ti_1 SW Ti_0 = SW Ti_1			Pol Ti_o > Pol Ti_1 SW Ti_0 = SW Ti_1					
Ti(Co)		Pol Ti_0 > SW Ti_0 Pol Ti_1 > SW Ti_1			Pol Ti_0 > SW Ti_0 Pol Ti_1 > SW Ti_1			Pol > SW			Pol Ti_0 < SW Ti_0 Pol Ti_1 < SW Ti_1			Pol Ti_0 < SW Ti_0 Pol Ti_1 < SW Ti_1			Pol > SW		

Reported are CT total coliforms, CF fecal coliforms, SF fecal streptococci, SW Ti_0 bacterial concentration measured at Ti_0 in seawater samples, SW Ti_1 bacterial concentration on measured at Ti_1 in seawater samples, Pol Ti_0 bacterial concentration in unstarved polychaetes, Pol Ti_1 bacterial concentration in starved polychaetes; *** $P < 0.001$, NS not significant.
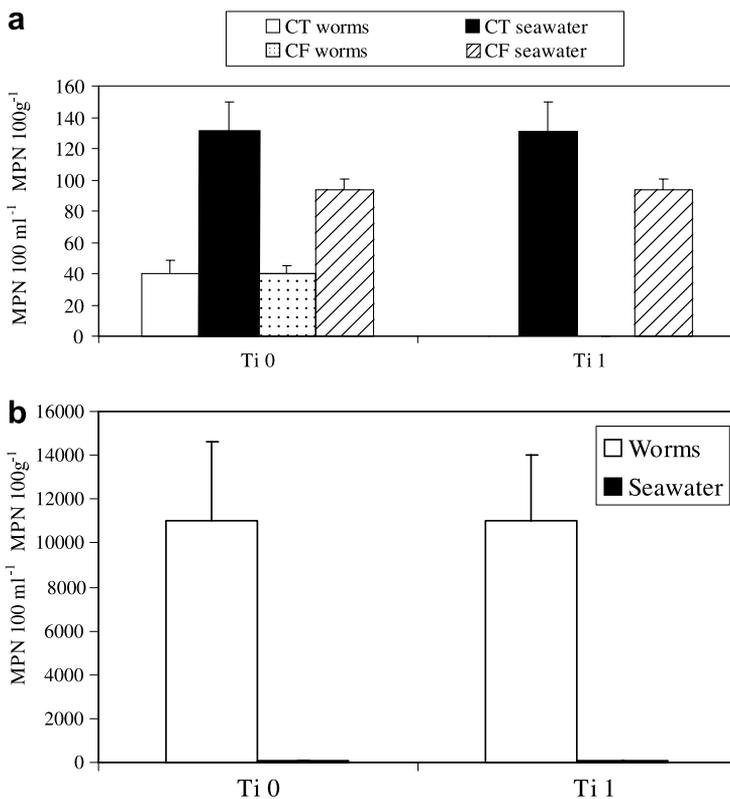
Fig. 2. Mean abundance and relative standard deviations of microbial pollution indicators in seawater and worm samples at Ti_0 (unstarved) and Ti_1 (starved): (a) total and faecal coliforms; (b) faecal streptococci.

that both total and faecal coliforms concentrations in polychaetes were significantly lower than in the surrounding environment over time ($P < 0.01$). Moreover, significant higher values occurred in unstarved *B. luctuosum* with respect to starved animals ($P < 0.01$), whereas no significant difference in seawater samples were detected.

Regarding to faecal streptococci concentrations (Fig. 2b), the values recorded (about 11,000 MPN 100 g^{-1}) did not differ significantly between the unstarved and starved animals whilst a significant difference ($P < 0.001$) was observed between seawater and *B. luctuosum* with a density of 4 orders of magnitude higher in the worms both at Ti_0 and Ti_1 (Table 1).

4. Discussion

Results showed that *B. luctuosum* is able to accumulate all the six considered microbiological groups, thus reducing the microbial concentration in the surrounding environment. A similar microbiological accumulation capability has been demonstrated for the closely related species *Sabella spallanzanii* (Licciano et al., 2003; Stabili et al., 2006). The impact of grazing on bacteria may be crucial especially in coastal marine ecosystems where these sabellids could form dense populations and heterotrophic bacteria constitute a major biomass component. In such a case, the microbial loop can represent a biological

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

A selectivity in digesting was inferred from the results indicating:

- No significant differences between starved and unstarved *B. luctuosum* in the accumulation of the total culturable bacteria at 37 °C and faecal streptococci. This means that these allochthonous bacteria do not find a suitable environment for their accumulation inside the worms, as observed in *S. spallanzanii* which was unable to digest faecal streptococci (Stabili et al., 2006). It has been demonstrated that Gram positive bacteria may be more resistant than Gram negative bacteria to digestion by marine invertebrates (Plante and Shriver, 1998).
- A significant increase of total culturable heterotrophic bacteria as well as halophilic vibrios abundances was instead observed between starved worms and the unstarved ones. The same trend was recorded for vibrios. This could suggest that such bacteria, being typically marine, may be members of the worm microflora some of which could also be symbiotic, and that *B. luctuosum* could represent an ecological reservoir for survival and growth of these bacteria.
- Finally, the study also revealed the occurrence of total and faecal coliforms only in unstarved worms since in the starved ones these bacteria were completely absent. This finding indicates a lower efficiency of the worms, compared to seawater, to concentrate and accumulate microbial pollution indicators. To explain this, we hypothesize that in *B. luctuosum* the degradation processes prevailed over the filtering ones, thus masking the bacterial accumulation ability of this worm.

The selectivity of digestion of microorganisms has been studied in sponges and the bacterial adherence properties have been postulated to play a key role in the selective retention rate (Willenz and van de Vyver, 1984; van de Vyver et al., 1985). Earlier studies on detritivorous polychaetes showed that some taxonomic groups of bacteria seem to fare well with gut throughput when compared to other groups (Plante and Mayer, 1994). Moreover, studies on filter feeders have demonstrated that bacterial species differ in their resistance to digestion (Prieur et al., 1990). Our data were merely descriptive, therefore the mechanisms utilized by *B. luctuosum* to degrade bacteria were not analysed during this study and more studies are needed to establish the exact mechanisms involved in the bacterial digestion exerted by the investigated species. These mechanisms, as observed in other invertebrates, could include ciliary movements and mucus secretion which restrict microbial growth (Garland et al., 1982; Canicattì and D'Ancona, 1990). Alternatively, the activity of antimicrobial compounds, especially lysozyme, could be hypothesized (Canicattì and Roch, 1989; Stabili and Canicattì, 1994; Stabili et al., 1996).

High microbial concentrations and the high accumulation efficiency inside the worms in comparison to the surrounding environment, especially regarding halophilic vibrios, suggest *B. luctuosum* as a useful bioindicator, particularly when low pollution levels of marine water pollution make environmental impact assessment procedures difficult. On the other hand, as suggested by Colwell (1984) and confirmed by other authors (Caruso et al., 1996; Cavallo and Stabili, 2002; Zaccone et al., 2002), vibrios may represent an additional parameter to faecal coliforms in determining water quality. Moreover, monitoring for microorganisms other than coliforms is also recommended by various guidelines developed for estuarine waters (Neill, 2004), which generally apply to bathing or recreational

or shellfish harvesting areas. Specimens of *B. luctuosum* were collected in a harbour area in the Gulf of Taranto subjected to anthropogenic pollution. Therefore, the detection of elevated microbial pollution indicators leads us to consider this species as a suitable bioindicator, particularly because it is able to tolerate high levels of marine pollution and environmental instability.

According to the results about coliforms digestion, another applicative aspect of our research suggests the potential use of *B. luctuosum* as biofilter for sewage, such as urban waste or animal slurry, where faecal coliforms can provide information on the possible occurrence of enteric pathogens and the general level of the microbiological quality of water. The application of biotechnologies to the marine environment is a fairly "hot topic" addressed by the scientific community worldwide (Colwell, 1998, 2002) and in the recent years, several studies have been carried out to assess the potential of bioremediation for the recovery of impacted ecosystems (Bragg et al., 1994; Vezzulli et al., 2004). The increasing eutrophication of coastal aquatic ecosystems resulting from human activities (i.e. agriculture runoff, direct wastewater discharge, aquaculture and mariculture impacts, disturbance of natural water flows) (Mendez and Comin, 2000), requires the recovery of the coastal polluted environments and the identification of bioremediator organisms. In the previous studies on *S. spallanzanii* (Licciano et al., 2003; Stabili et al., 2006), we suggested the use of this species in restoring water quality in aquaculture farms, based on its high capability to remove and digest vibrios from the surrounding environment. In comparison, our results demonstrate that *B. luctuosum* is more effective than *S. spallanzanii* in removing and digesting coliforms, supporting the possibility of its use as biofilter of domestic sewage.

The idea of employing polychaetes for applied purposes has been already suggested by several authors (Tenore et al., 1974; Ryther et al., 1975; Kurihara, 1983; Gambi et al., 1994; Giangrande et al., 2005), who considered only the application of some detritivorous polychaetes to reduce microbial and organic pollution in marine sediments. Therefore, the possibility of using filter feeder polychaetes as biofilters for the water column treatment, provides an innovative approach in the biological treatment of polluted sea water.

Acknowledgements

References

Andral, B., Stanisiere, J.Y., Sauzade, D., Damier, E., Thebault, H., Galgani, F., Boissery, P., 2004. Monitoring chemical contamination levels in the Mediterranean based on the use of mussel caging. Marine Pollution Bulletin 49, 704–712.

APHA, AWWA, WEF, 1998. US Standard Methods for the Examination of Water and Wastewater, twentieth ed. ISBN 0-87553-235-7. American Public Health Association.

Ayres, P.A., Burton, H.W., Cullum, M.L., 1978. Sewage pollution and shellfish. In: Davies, L. (Ed.), Techniques for the study of mixed populations. Academic Press, New York, pp. 51–62.

Bak, R.P.M., Joenje, M., Lambrechts, D.Y.M., Nieuwland, G., 1998. Bacterial suspension feeding by coral reef benthic organisms. Marine Ecology Progress Series 175, 285–288.

Bianchi, C.N., 1983. Serpuloidei (Annelida, Polychaeta) delle lagune costiere laziali e campane. Annali del Museo Civico di Storia Naturale di Genova 84, 231–243.

Bocchetti, R., Fattorini, D., Gambi, M.C., Regoli, F., 2004. Trace metal concentrations and susceptibility to oxidative stress in the polychaete *Sabella spallanzanii* (Gmelin) (Sabellidae): potential role of antioxidants in revealing stressful environmental conditions in the Mediterranean. Archives of Environmental Contamination and Toxicology 46, 353–361.

Bolinches, J., Toranzo, A.E., Silva, A., Barja, J.L., 1986. Vibriosis as the main causative factor of heavy mortalities in the oyster culture industry in North-western Spain. Bulletin of the European Association of Fish Pathologists 6, 1–4.

Bonar, D.B., 1972. Feeding and tube construction in *Chone mollis* Bush (Polychaeta, Sabellidae). Journal of Experimental Marine Biology and Ecology 9, 1–18.

Bostock, A.D., 1979. Hepatitis A infection associated with the consumption of mussel. Journal of Infectious Diseases 1, 171–177.

Bragg, J.R., Prince, R.C., Harner, E.J., Atlas, R.M., 1994. Effectiveness of bioremediation for the Exxon Valdez oil spill. Nature 368, 413–418.

Canicattì, C., D'Ancona, G., 1990. Biological protective substances in *Marthasterias glacialis* (Asteroidea) epidermal secretion. Journal of Zoology, London 222, 445–454.

Canicattì, C., Roch, P., 1989. Studies on *Holothuria polii* (Echinodermata) antibacterial proteins. I. Evidence for an activity of a coelomocyte lysozyme. Experientia 45, 756–759.

Caruso, G., Zaccone, R., Crisafi, E., 1996. Distribution and numerical taxonomy of Vibrionaceae in the waters of the Straits of Messina. Microbiologica 19, 155–166.

Cavallo, A., Giangrande, A., 2002. Prove di sperimentazione ai fini dell'utilizzazione di Sabella spallanzanii (Polychaeta) come biorimediatore in acquicoltura. Biologia Marina Mediterranea 10 (1), 438–440.

Cavallo, R.A., Stabili, L., 2002. Presence of vibrios in seawater and *Mytilus galloprovincialis* (Lam.) from the Mar Piccolo of Taranto (Ionian Sea). Water Research 36, 3719–3726.

Cho, B.C., Azam, F., 1990. Biogeochemical significance of bacterial biomass in the oceans' euphotic zone. Marine Ecology Progress Series 63, 253–259.

Clapin, G., 1996. The filtration rate, oxygen consumption and biomass of the introduced polychaete *Sabella spallanzanii* Gmelin within Cockburn Sound: can they control phytoplankton level and is it an efficient filter feeder? Honours Thesis. Edith Cowan University, Joondalup, Western Australia, p. 90.

Cloern, J.E., 1982. Does the benthos control phytoplankton biomass in the South San Francisco estuary. Marine Ecology Progress Series 9, 191–202.

Colwell, R.R., 1984. Vibrios in the environment. Wiley, New York.

Colwell, R.R., 1998. Environmental biotechnology: the biotechnology revolution comes down to earth. Current Opinion in Biotechnology 9, 245–246.

Colwell, R.R., 2002. Fulfilling the promise of biotechnology. Biotechnology Advances 20, 215–228.

Dales, R.P., 1957. Some quantitative aspects of feeding in sabellid and serpulid fan worms. Journal of Marine Biological Association of the United Kingdom 36, 309–316.

Fattorini, D., Notti, A., Halt, M.N., Gambi, M.C., Regoli, F., 2005. Levels and chemical speciation of arsenic in polychaetes: a review. Marine Ecology 26, 255–264.

Fitzsimons, G., 1965. Feeding and tube-building in *Sabellastarte magnifica* (Shaw) (Sabellidae: Polychaeta). Bulletin of Marine Science 15, 642–671.

Fuhrman, J.A., Sleeter, T.D., Carlson, C., Proctor, L.M., 1989. Dominance of bacterial biomass in the Sargasso Sea and its ecological implications. Marine Ecology Progress Series 57, 207–217.

Gambi, M.C., Castelli, A., Giangrande, A., Lanera, P., Prevedelli, D., Zunarelli Vandini, R., 1994. Polychaetes of commercial and applied interest in Italy: an overview. Mémoires du Muséum National d'Histoire Naturelle 162, 593–603.

Garland, C.D., Nash, G.V., Mc Mukin, T.A., 1982. Absence of surface associated microorganisms in adult oysters (*Crassostrea gigas*). Applied and Environmental Microbiology 44, 1205–1211.

Giangrande, A., Cavallo, A., Licciano, M., Mola, E., Pierri, C., Trianni, L., 2005. Utilization of the filter feeder *Sabella spallanzanii* as bioremediator in aquaculture. Aquaculture International 13, 129–136.

Gili, J.M., Coma, R., 1998. Benthic suspension feeders: their paramount role in littoral marine food webs. Trends in Ecology and Evolution 13 (8), 316–321.

Jørgensen, C.B., 1966. Biology of suspension feeding. Pergamon Press, Oxford, p. 357.

Jørgensen, C.B., 1975. Comparative physiology of suspension feeding. Annual Review of Physiology 37, 57–79.

Jørgensen, C.B., Kiørboe, T., Møhlenberg, F., Riisgård, H.U., 1984. Ciliary and mucus-net filter feeding, with special reference to fluid mechanical characteristics. Marine Ecology Progress Series 15, 283–292.

Kimmerer, W.J., Gartside, E., Orsi, J.J., 1994. Predation by an introduced clam as the likely cause of substantial declines in zooplankton in San Francisco Bay. Marine Ecology Progress Series 113, 81–93.

Knight-Jones, P., Ergen, Z., 1991. Sabelliform polychaetes, mostly from Turkey's Aegean coast. Journal of Natural History 25, 837–858.

Kurihara, I., 1983. Study of domestic sewage waste treatment by the polychaetes *Neanthes japonica* and *Perinereis nuntia* on artificial tidal flat. Internationale Revue der Gesamten Hydrobiologie 68, 649–670.

Lewis, D., 1968. Feeding and tube-building in the Fabriciinae (Annelida, Polychaeta). Proceedings of the Linnean Society of London 179 (1), 37–49.

Licciano, M., Giangrande, A., Gambi, M.C., 2002. Reproduction and simultaneous hermaphroditism in *Branchiomma luctuosum* (Polychaeta, Sabellidae) from the Mediterranean Sea. Invertebrate Biology 121 (1), 55–65.

Licciano, M., Stabili, L., Cavallo, R.A., Giangrande, A., 2003. Prove di stabulazione per la valutazione della capacità di accumulo microbiologico con il sabellide filtratore *Sabella spallanzanii* (Annelida: Polychaeta). Biologia Marina Mediterranea 10 (2), 1105–1107.

Licciano, M., Stabili, L., Giangrande, A., 2005. Clearance rates of *Sabella spallanzanii* and *Branchiomma luctuosum* (Annelida: Polychaeta) on a pure culture of *Vibrio alginolyticus*. Water Research 39 (18), 4375–4384.

Mendez, M., Comin, F.A., 2000. Spring and summer proliferation of floating macroalgae in a Mediterranean coastal lagoon (Tancada Lagoon) Ebro Delta, NE Spain. Estuarine Coastal and Shelf Science 51, 215–226.

Neill, M., 2004. Microbiological indices for total coliform and *E. coli* bacteria in estuarine waters. Marine Pollution Bulletin 49, 752–760.

Officer, C.B., Smayda, T.J., Mann, R., 1982. Benthic filter feeding: a natural eutrophication control. Marine Ecology Progress Series 9, 203–210.

Plante, C.J., Mayer, L.M., 1994. Distribution and efficiency of bacteriolysis in the gut of *Arenicola marina* and three additional deposit feeders. Marine Ecology Progress Series 109, 183–194.

Plante, C.J., Shriver, A., 1998. Differential lysis of sedimentary bacteria by *Arenicola marina*: examination of cell wall structure and exopolimeric capsules as correlates. Journal of Experimental Marine Biology and Ecology 229, 35–52.

Prieur, D., Mével, G., Nicolas, J.L., Plusquellec, A., Vigneulle, M., 1990. Interactions between bivalve molluscs and bacteria in the marine environment. Oceanography and Marine Biology: An Annual Review 28, 277–352.

Ribes, M., Coma, R., Gili, J.M., 1999. Natural diet and grazing rate of the temperate sponge *Dysidea avara* (Demospongiae, Dendroceratida) throughout an annual cycle. Marine Ecology Progress Series 176, 179–190.

Riisgård, H.U., Ivarsson, N.M., 1990. The crown-filament pump of the suspension feeding polychaete *Sabella penicillus*: filtration, effects of temperature, and energy cost. Marine Ecology Progress Series 62, 249–257.

Riisgård, H.U., Larsen, P.S., 1995. Filter-feeding in marine macro-invertebrates: pump characteristics, modelling and energy cost. Biological Reviews of the Cambridge Philosophical Society (London) 70, 67–106.

Riisgård, H.U., Larsen, P.S., 2001a. Minireview: ciliary filter feeding and bio-fluid mechanics – present understanding and unsolved problems. Limnology Oceanography 46 (4), 882–891.

Riisgård, H.U., Larsen, P.S., 2001b. Comparative ecophysiology of active zoobenthic filter-feeding, essence of current knowledge. Journal of Sea Research 44, 169–193.

Ryther, J.H., Goldman, J.C., Gifford, C.E., Huguenin, J.E., Wing, A.S., Clarner, P.J., Williams, L.D., Lapointe, B.E., 1975. Physical integrated waste-recycling-marine polyculture systems. Aquaculture 5, 163–177.

Shumway, S.E., Bogdanowicz, C., Dean, D., 1988. Oxygen consumption and feeding rates of the sabellid polychaete, *Myxicola infundibulum* (Renier). Comparative Biochemistry and Physiology 90A (3), 425–428.

Silverman, H., Achberger, E., Lynn, J.W., Dietz, T.H., 1995. Filtration and utilization of laboratory-cultured bacteria by *Dreissena polymorpha*, *Corbicula fluminea*, and *Carunculina texasensis*. Biological Bulletin 189, 308–319.

Silverman, H., Nichols, S.J., Cherry, J.S., Achberger, E., Lynn, J.W., Dietz, T.H., 1997. Clearance of laboratory-cultured bacteria by freshwater bivalves: differences between lentic and lotic unionids. Canadian Journal of Zoology 75, 1857–1866.

Stabili, L., Canicattì, C., 1994. Antibacterial activity of the seminal plasma of *Paracentrotus lividus*. Canadian Journal of Zoology 72, 1211–1216.

Stabili, L., Pagliara, P., Roch, P., 1996. Antibacterial activity in the coelomocytes of the sea urchin *Paracentrotus lividus*. Comparative Biochemistry and Physiology 113 (B3), 639–644.

Stabili, L., Licciano, M., Giangrande, A., Fanelli, G., Cavallo, R.A., 2006. *Sabella spallanzanii* filter-feeding on bacterial community: Ecological implications and applications. Marine Environmental Research 61 (1), 74–92.

Tenore, K.R., Browne, M.G., Chesney, E., 1974. Polyspecies aquaculture systems: the detrital trophic level. Sears Foundation. Journal of Marine Research 32, 425–432.

Underwood, A.J., 1997. Experiments in ecology: their logical design and interpretation using analysis of variance. Cambridge University Press, Cambridge, p. 504.

van de Vyver, G., Vray, B., Belaouane, S., Toussaint, D., 1985. Efficiency and selectivity of microorganism retention by *Ephydatia fluviatilis*. Third International Sponge Conference, pp. 511–515.

Vezzulli, L., Pruzzo, C., Fabiano, M., 2004. Response of the bacterial community to in situ bioremediation of organic-rich sediments. Marine Pollution Bulletin 49, 740–751.

Wells, G.P., 1952. The respiratory significance of the crown in the polychaete worms *Sabella* and *Myxicola*. Proceedings of the Royal Society B: Biological Sciences 140, 70–82.

Widdows, J., Donkin, P., 1992. Mussels and environmental contaminants: bioaccumulation and physiological aspects. In: Gosling, E.M. (Ed.), The Mussel Mytilus: Ecology, Physiology, Genetics and Culture, Developments in Aquaculture and Fisheries Science, vol. 25. Elsevier Science Publisher, Amsterdam, pp. 383–424.

Widdows, J., Brinsley, M.D., Salkeld, P.N., Elliott, M., 1998. Use of annular flumes to determine the influence of current velocity and bivalves on material flux at the sediment–water interface. Estuaries 21, 552–559.

Widdows, J., Brinsley, M.D., Salkeld, P.N., Lucas, C.H., 2000. Influence of biota on spatial and temporal variation in sediment erodability and material flux on a tidal flat (Westerschelde, The Netherlands). Marine Ecology Progress Series 194, 23–37.

Widdows, J., Blauw, A., Heip, C.H.R., Herman, P.M.J., Lucas, C.H., Middelburg, J.J., Schmidt, S., Brinsley, M.D., Twisk, F., Verbeek, H., 2004. Role of physical and biological processes in sediment dynamics of a tidal flat in Westerschelde Estuary, SW Netherlands. Marine Ecology Progress Series 274, 41–56.

Willenz, P., van de Vyver, G., 1984. Ultrastructural localization of lysomila digestion in the freshwater sponge *Ephydatia fluviatilis*. Journal of Ultrastructure Research 87, 13–22.

Wood, P.C., 1957. Factors affecting the pollution and self-purification of molluscan shellfish. Journal du Conseil International pour l'Exploration de la Mer 22, 202–208.

Wotton, R.S., 1994. Methods for capturing particles in benthic animals. In: Wotton, R.S. (Ed.), The biology of particles in aquatic systems. Lewis Publishers, London, pp. 183–204.

Yam, W.C., Chan, C.Y., Ho Bella, S.W., Tam, T.Y., Kueh, C., Lee, T., 1999. Abundance of clinical enteric bacterial pathogens in coastal waters and shellfish. Water Research 34 (1), 51–56.

Zaccone, R., Caruso, G., Calì, C., 2002. Heterotrophic bacteria in the northern Adriatic Sea: seasonal changes and ectoenzyme profile. Marine Environmental Research 54, 1–19.