

Clearance rates of *Sabella spallanzanii* and *Branchiomma luctuosum* (Annelida: Polychaeta) on a pure culture of *Vibrio alginolyticus*

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Abstract

The influence exerted by filter-feeding activity on bacterial density by two sabellid species from the Mediterranean Sea (Ionian Sea, Italy), *Branchiomma luctuosum* Grube and *Sabella spallanzanii* Gmelin (Annelida: Polychaeta) was investigated. Clearance rates and retention efficiencies were estimated utilizing the species *Vibrio alginolyticus* selected on account of previous field studies and its importance in fish culture pathogenicity. The C_{\max} was $43.2 \pm 2.63 \text{ L h}^{-1} \text{ g}^{-1}$ DW for *B. luctuosum* and $12.4 \pm 2.22 \text{ L h}^{-1} \text{ g}^{-1}$ DW for *S. spallanzanii*. The Retention efficiency was 98% corresponding to a removed bacterial biomass of $44.8 + 7.88 \mu\text{gC L}^{-1} \text{ g}^{-1}$ DW for *B. luctuosum* and 70% corresponding to a bacterial biomass of $23.8 + 2.95 \mu\text{gC L}^{-1} \text{ g}^{-1}$ DW for *S. spallanzanii*. Maximum retention was recorded after 20 min for the first species and after 30 min for the second one. Present laboratory experiments represent a contribution to the knowledge of the filtration activity of the two polychaetes, characterizing the filtration process on bacterioplankton. Both species resulted extremely efficient in removing *V. alginolyticus* from seawater in experimental tanks, thus confirming the previous data from the field studies and suggesting their employment as biofilters of microbially contaminated waters in intensive aquaculture.

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1. Introduction

The filter-feeding trophic strategy is widely represented among marine invertebrate taxa which must filter large volumes of waters for their food requirements and they have evolved a great number of convergences to

solve this common basic problem also among taxonomically distant species (Riisgård and Larsen, 2001a, b).

Investigations on clearance rates of these organisms were performed employing mainly phytoplankton as food source, even though it is well-known that some filter feeders such as sponges, ascidians and bivalves remove also bacterioplankton (Jørgensen et al., 1984; Wotton, 1994; Riisgård and Larsen, 1995; Silverman et al., 1995, 1997; Bak et al., 1998; Gili and Coma 1998; Ribes et al., 1999; Riisgård and Larsen, 2001a, b). Because heterotrophic bacteria are an important

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component of planktonic systems (Cole et al., 1988), the filter-feeders with powerful water filtration mechanisms may potentially affect the levels of the bacterioplankton, thus the relationship between bacteria and benthic filter feeders may be functionally important to aquatic ecosystems (Kautsky, 1981; Prins et al., 1998). Studies dealing with the interactions between filter feeders and marine bacteria have been carried out with special attention to molluscs (Wood, 1957; Ayres et al., 1978; Bostock, 1979; Birkbeck and McHenry, 1982; Bollenches et al., 1986; Prieur et al., 1990; Yam et al., 1999). By contrast, data relative to bacterioplankton retention are at present scant as regards filter feeder polychaetes, one of the best-represented taxa of marine invertebrates in benthic assemblages. Species belonging to the Sabellidae family are easily recognizable for the presence of an elaborated tentacular crown, that expands out of the tube they inhabit, to collect and sort material of different sizes; small particles are swallowed, while large ones are pushed away from the mouth and dropped into the water (Nicol, 1930; Mayer, 1994). A number of studies on sabellids provide descriptions of filtering organs and particle capture mechanisms (Wells, 1952; Fitzsimons, 1965; Lewis, 1968; Bonar, 1972) but very few accounts on the qualitative and quantitative aspects of the filtration process are available (Dales, 1957; Shumway et al., 1988; Riisgård and Ivarsson, 1990; Clapin, 1996). Field and laboratory studies on filter feeder polychaetes have been performed mainly employing phytoplankton as feeding source (Shumway et al., 1988; Riisgård and Ivarsson, 1990; Clapin, 1996), even though recent studies have shown that they also feed on suspended matter either nonliving (detrital organic carbon) or living (bacteria, phytoplankton) (Clapin, 1996; Cavallo and Giangrande, 2002; Licciano et al., 2003; Cavallo et al., 2005; Stabili et al., 2005; Licciano et al., submitted).

In the present paper we investigated the filtration process on bacteria of two sabellid species: *Branchiommma luctuosum* Grube, and *Sabella spallanzanii* Gmelin (Annelida: Polychaeta) on account of the above-reported sabellid trophic strategies (Giangrande et al., 2000; Licciano et al., 2002). Up to now laboratory experiences conducted on these species demonstrated only their positive role on solid removal from water column (Cavallo and Giangrande, 2002; Giangrande et al., 2005). The aim of this study was to characterize the filtration process estimating clearance rate and retention efficiency utilizing the bacterial species *Vibrio alginolyticus*. Vibrios are Gram-negative, curved, halophilic, nonspore forming bacteria, autochthonous inhabitants of the marine and estuarine environments, which constitute a considerable part of marine heterotrophic bacterial population (Cavallo and Stabili, 2002). *V. alginolyticus* was selected on account of both previous data from the field studies and its importance in fish

culture pathogenicity. In this framework a multidisciplinary project for an employment of the investigated polychaete species as bioremediators in aquaculture is in progress in our laboratory.

2. Materials and methods

2.1. Polychaete sampling

Adult specimens of *B. luctuosum* and *S. spallanzanii* were collected along a dock wall by SCUBA diving in the Gulf of Taranto (Ionian Sea, Italy) and transferred to the laboratory. After removal of tube epibionts, worms were separated and for each species, 18 individuals of similar tube length were selected and placed for 48 h in an aerated aquarium containing 25 L of sterile-filtered sea water (SFSW) (0.22 µm pore size filters, Millipore) daily replaced and kept in a temperature controlled room (22 °C). At the end of the starvation period, individuals of *S. spallanzanii* and *B. luctuosum* have been separately utilized for the filtration experiments.

2.2. *Vibrio alginolyticus* retention and clearance rate calculation

In order to examine the clearance rate and the retention efficiency of *Vibrio alginolyticus* two experiments were separately conducted on *S. spallanzanii* and *B. luctuosum*. For each species, a total of 36 tanks each containing 1 L of SFSW were prepared. Eighteen starved worms were individually placed in 18 single tanks (treatment tanks) whilst the remaining 18 tanks filled with SFSW but without worms were employed as controls.

Just prior to the beginning of experiments a *V. alginolyticus* suspension of a known concentration (10^8 cells mL⁻¹) was added to both experimental and control beakers so that the final concentration was about 25×10^3 cells mL⁻¹ for *S. spallanzanii* and 5×10^3 cells mL⁻¹ for *B. luctuosum*. *V. alginolyticus* cultures were previously prepared in Marine Broth (DIFCO) and incubated for 24 h at 30 °C. The bacterial density in the culture medium was then diluted and monitored spectrophotometrically at 700 nm to obtain absorbance values of about 0.174 corresponding to a bacterial concentration of about 10^8 cells mL⁻¹.

The retention of *V. alginolyticus* cells by *S. spallanzanii* and *B. luctuosum* was studied measuring the removal of bacterial cells from SFSW over 4 h at a temperature of 22 °C. Aliquots of SFSW (1 mL) were aseptically collected from each of the treatment and control tanks (for a total of 11 sampling times for each species): every 15 min within the first hour and every 30 min during the following 3 h for *S. spallanzanii*; every 20 min within the

first 2 h and every 30 min during the remaining 2 h for *B. luctuosum*.

The differences in the experimental variables between the two experiments (initial bacterial concentration and sampling times) account for the different body sizes of the two polychaete species leading to a different gut capacity too, *B. luctuosum* being smaller than *S. spallanzanii* (mean dry weights of 0.182 g and 1.227 g, respectively, calculated at the end of the experiments).

To evaluate the density of *V. alginolyticus*, 1 mL of both undiluted samples and serial dilutions in SFSW (10^{-1} , 10^{-2} , 10^{-3} and 10^{-4}) were filtered on 0.45 µm pore size filters (Millipore) that were aseptically placed onto thiosulphate-citrate-bile-salt-agar (TCBS) plus 2% NaCl. After incubation at 30 °C for 24 h, the emerging colonies of vibrios were counted according to the colony-forming unit (CFU) method. At each time, a mean value from the three replicates were computed and expressed as CFU mL⁻¹ taking account of the dilution factor.

For each species, the retention efficiency *R* was calculated as a percentage for the difference in bacterial abundances at each sampling time, by the following equation:

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

where C_0 is the initial bacterial concentration in the experimental tanks and C_t is the bacterial concentration at the end of time *t*.

At the end of the experiments worms were extracted from the tubes and dried in pre-weighed aluminum foil at 60 °C for 24 h and then weighed to determine the dry weights (g).

Clearance rates (*C*) were estimated by measuring the bacteria removal from the experimental tanks as a function of time using the equation given by Coughlan (1969). Data were reported as weight-specific clearance rates and expressed in liters per hour per gram of worm dry tissue (L h⁻¹ g⁻¹ DW). Since the mean clearance rate predictably includes periods of inactivity or transition between feeding and inactivity by worms, due to the intrusion of the branchial crown within the tube, for each species *C* was reported for each time point as mean ± standard deviation (SD) of all the *C* values calculated for each specimen within each sampling time. Moreover, according to Navarro and Widdows (1997), for each species the maximum clearance rate (C_{\max}) based on the highest clearance rate value recorded for each of the 18 individuals during the entire experimental period (4 h) were also calculated and reported as mean ± SD.

2.3. Bacterial biomass

Bacterial biovolume was determined by using a Zeiss Standard Axioplan microscope equipped with a halogen

lamp (Hg 100) light. Duplicate slides were prepared by filtering 1 mL of the bacterial suspension onto a Millipore filter (0.2 µm pore), using DAPI (4,6-diamidino-2 phenyl-indole) as fluorochrome (Porter and Feig, 1980). A G 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. *V. alginolyticus* biovolume was converted into biomass assuming a carbon content of 310 fg C µm⁻³ (Fry, 1990).

2.4. Statistical analysis

The experimental design consisted of three factors; Polychaetes (Po, two levels, i.e. absent and present, fixed); Time (Ti, 11 levels, fixed and crossed with Po); Tank (Ta, three levels, random and nested in Po), with *n* = 6 per combination of factors, for a total of 396 observation units. Analysis of variance was used to assess significant variations in the bacterial concentration temporal trend in treatment and control tanks. The occurrence of a significant “tank effect” accounting for a variability due to the experimental containers was also evaluated. Prior to analysis, the homogeneity of variance was tested using the Cochran’s test and, if necessary, data were Sqrt(*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 Sydney, Australia).

3. Results

3.1. *Branchiomma luctuosum*

In Fig. 1a the mean concentrations of *V. alginolyticus* at any sampling time in the treatment and control tanks are shown. The slope of the line ($y = 21,139 e^{-2.1422x}$; $R^2 = 0.9331$) demonstrates the exponential reduction in bacterial concentration as function of time in the treatment tanks, whilst no significant differences in the controls were observed. Particularly, *V. alginolyticus* concentration in presence of *B. luctuosum* rapidly decreased in time reaching a mean value of $119.2 + 21.5$ CFU mL⁻¹ just after 20 min from the beginning of the experiment and a minimum value of $6.67 + 5.16$ CFU mL⁻¹ after 60 min. At the following sampling time (80 min), bacterial cells were no more detected in the treatment tanks. Analysis of variance revealed a significant Ti × Po interaction (Table 1) and the following post-hoc comparison showed that *V. alginolyticus* concentration in the treatment tanks was always significantly lower than in the controls ($P < 0.001$). Moreover, no significant “tank effect” due

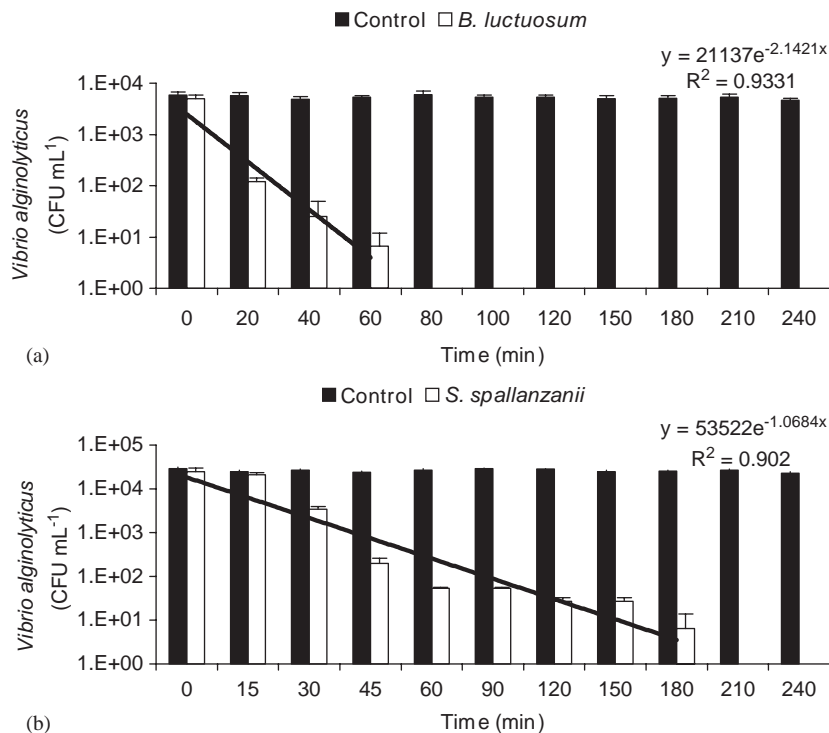


Fig. 1. Changes in *V. alginolyticus* abundances measured in the control and treatment tanks with *B. luctuosum* (a) and *S. spallanzanii* (b).

Table 1

Branchiomma luctuosum: summaries of ANOVAs testing for differences in average *V. alginolyticus* abundances measured at the different sampling times in the control and treatment tanks

Source of variation	DF	MS	F	P	F versus
Po	1	4,04,929			
Ti	10	4395			
Ta(Po)	4	25.65	1.46	NS	Residual
Ti × Po	10	3473	485.55	0.00001	TiXTa(Po)
Ti × Ta(Po)	40	7.15	0.41	NS	Residual
Residual	330	17.54			
TOT	395				
Cochran's test	$C = 0.0628$ (NS)				
Transformation	Sqrt($X + 1$)				
SNK test					
Po(Ti)	$T < C$				

Terms already involved in significant higher order interactions were not analyzed (Underwood, 1997).

Reported are: Po, Polychaetes; Ti, Time; Ta, Tank; T, Bacterial concentration in the treatments; C, Bacterial concentration in the controls; NS, Not significant.

to experimental containers, as source of variability, was established by statistical analysis.

The maximum removal of *V. alginolyticus* by filtering activity of *B. luctuosum* occurred after 20 min, when $R = 98\%$ corresponding to a removed bacterial biomass of $44.8 + 7.88 \mu\text{gCL}^{-1} \text{g}^{-1} \text{DW}$ (Fig. 2a).

The C values measured for *B. luctuosum* within each sampling time (Fig. 3a) significantly differed ($P < 0.001$). The highest value $43.2 \pm 2.63 \text{ L h}^{-1} \text{g}^{-1} \text{DW}$ was observed after 20 min from the beginning of the experiment. After this first sampling point, C decreased at the following times with values ranging from 16.4 ± 11 to

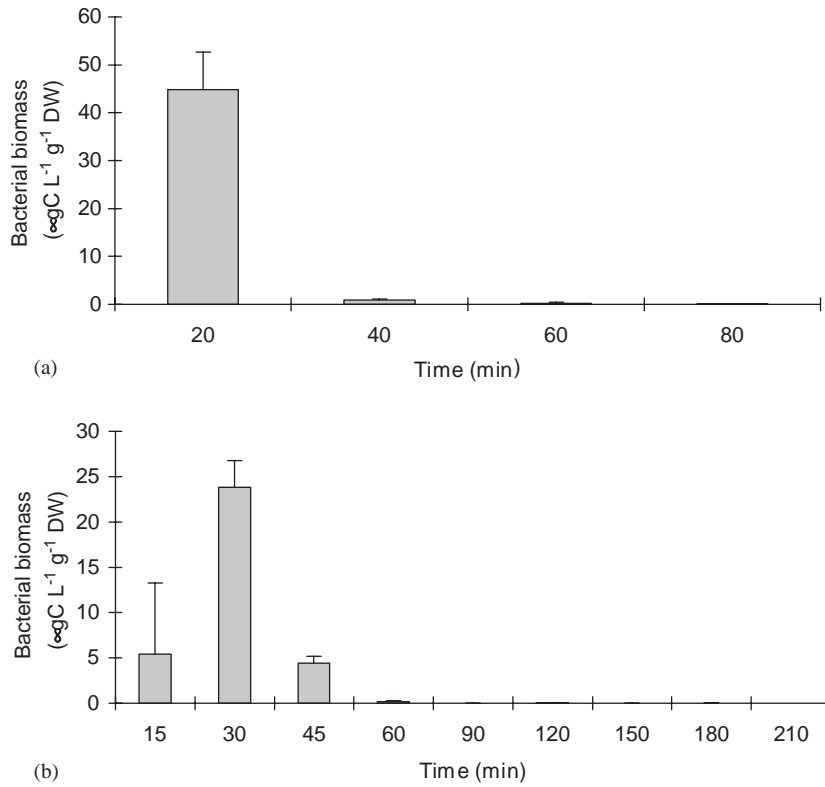


Fig. 2. Removal of bacterial biomass by *B. luctuosum* (a) and *S. spallanzanii* (b).

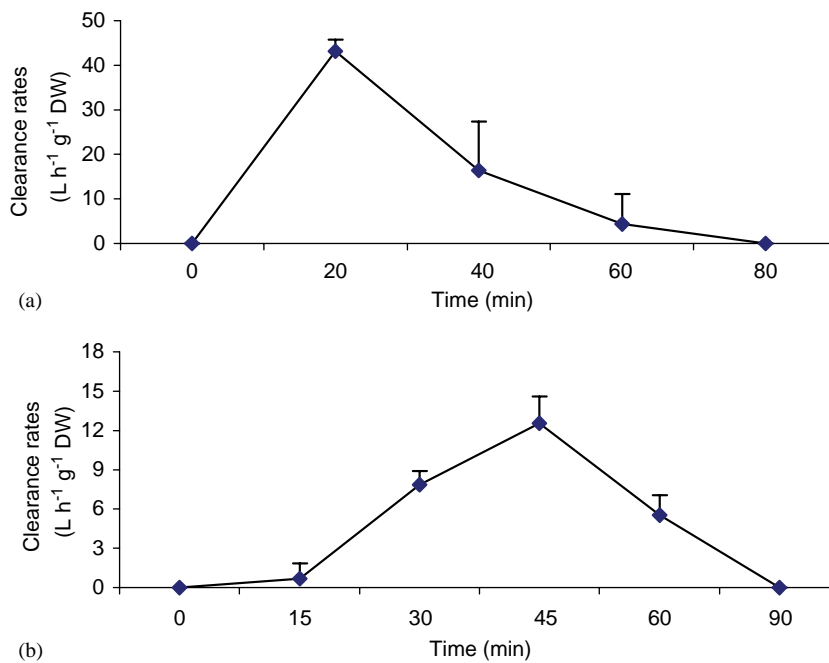


Fig. 3. Clearance rates calculated within each sampling time for *B. luctuosum* (a) and *S. spallanzanii* (b).

$4.37 \pm 6.74 \text{ L h}^{-1} \text{ g}^{-1} \text{ DW}$, recorded at 40 and 60 min, respectively. The C_{max} , calculated as mean value of all the single maximal values recorded for each individual, was $43.2 \pm 2.63 \text{ L h}^{-1} \text{ g}^{-1} \text{ DW}$.

3.2. *Sabella spallanzanii*

For *S. spallanzanii*, the bacterial concentration temporal trend in the control and treatment tanks is shown in Fig. 1b. An exponential reduction of *V. alginolyticus* density in the treatments can be inferred from the slope of the line ($y = 53522 e^{-1.0684x}$; $R^2 = 0.902$). By contrast, bacterial concentration in the controls did not vary significantly during the entire period of the experiment. In the treatment tanks the bacterial density rapidly decreased through time reducing significantly to $3458 + 493 \text{ CFU mL}^{-1}$ already 30 min after the beginning of the experiment and reaching the lowest value of $6.5 + 7.01 \text{ CFU mL}^{-1}$ after 180 min. The resulting seawater was completely cleared from bacteria at the following time point (210 min). The analysis of variance revealed a significant $\text{Ti} \times \text{Po}$ interaction (Table 2). The SNK test established significantly lower bacterial concentration in the treatment tanks in comparison with the controls for all the sampling times ($P < 0.001$). Moreover, as stated in Table 2, the experimental containers did not act as sources of variability and no significant “tank effect” was evidenced.

The *V. alginolyticus* CFU mL^{-1} removal by filter activity of *S. spallanzanii* at each sampling time allowed to compute the highest R value of 70%, corresponding to a bacterial biomass of $23.8 + 2.95 \mu\text{g CL}^{-1} \text{ g}^{-1} \text{ DW}$, after 30 min from the beginning of the experiment (Fig. 2b).

C values calculated within each sampling time for *S. spallanzanii* are shown in Fig. 3b. Significant differences among values were enhanced over time ($P < 0.001$). The lowest value of $0.69 \pm 1.16 \text{ L h}^{-1} \text{ g}^{-1} \text{ DW}$, computed within the first 15 min, increased to $7.88 \pm 1 \text{ L h}^{-1} \text{ g}^{-1} \text{ DW}$ at the 30 min time point. After this period C values further increased to $12.5 \pm 2.04 \text{ L h}^{-1} \text{ g}^{-1} \text{ DW}$ at 45 min, but decreased at 60 min when a value of $5.52 \pm 1.56 \text{ L h}^{-1} \text{ g}^{-1} \text{ DW}$ was reached and lowered to zero at 90 min time. The C_{max} calculated for *S. spallanzanii* was $12.4 \pm 2.22 \text{ L h}^{-1} \text{ g}^{-1} \text{ DW}$.

4. Discussion

The relative importance of bacteria as a nutrient used by suspension feeders has been commonly found in sponges and reported also for ascidians, corals and many different bivalve species (Jørgensen et al., 1984; Prieur et al., 1990; Bak et al., 1998; Ribes et al., 1999). However, often laboratory experiments do not test the ability of the filter feeders in selecting particles from mixed seston and results from these experiments do not imply that organisms actually feed on free-living individual bacteria, because a large part may be attached to particulate matter that could facilitate bacterial intake (Silverman et al., 1997; Bak et al., 1998). Laboratory studies indicate that bacteria in the $1 \mu\text{m}$ size range can be cleared and actually assimilated by some bivalves, specially within the genus *Mytilus* (McHenery and Birbeck, 1985; Prieur et al., 1990; Silverman et al., 1997) even though not all the investigated molluscs are able to efficiently retain picoplankton (Dupuy et al., 2000).

Table 2

Sabella spallanzanii: summaries of ANOVAs testing for differences in average *V. alginolyticus* abundances measured at the different sampling times in the control and treatment tanks

Source of variation	DF	MS	F	P	F versus
Po	1	15,39,613			
Ti	10	32,690			
Ta(Po)	4	93.22	1.64	NS	Residual
Ti \times Po	10	30,317	1178.49	0.00001	Ti \times Ta(Po)
Ti \times Ta(Po)	40	25.72	0.45	NS	Residual
Residual	330	56.73			
TOT	395				
Cochran's test	C = 0,1029 (P < 0.01)				
Transformation	Sqrt(X + 1)				
SNK test					
Po(Ti)	T < C				

Terms already involved in significant higher order interactions were not analyzed (Underwood, 1997).

Reported are: Po, Polychaetes; Ti, Time; Ta, Tank; T, Bacterial concentration in the treatments; C, Bacterial concentration in the controls; NS, Not significant.

Like *Mytilus* also *S. spallanzanii* and *B. luctuosum* typically live in eutrophic and polluted environments, such as harbour areas, where they form very dense populations. Both species are opportunistic from a trophic point of view (Clapin, 1996; Cavallo and Giangrande, 2002; Licciano et al., 2003) but the role of the pelagic bacteria as food source in their diet is still largely unknown. Moreover, no data relative to filtering activity on bacterioplankton of other filter-feeding polychaetes are up to now available, and clearance rates were always estimated by using phytoplankton in laboratory experiments (Dales, 1957, 1961; Buhr, 1976; Klockner, 1978; Shumway et al., 1988; Riisgård, 1989, 1991; Riisgård and Ivarsson, 1990; Riisgård et al., 1992; Clapin, 1996). Field studies conducted in a coastal area of the Northern Ionian Sea (South Mediterranean) on *S. spallanzanii* and *B. luctuosum* showed that both species are able to accumulate and concentrate bacteria from the surrounding environment with a higher efficiency for autochthonous bacteria (Stabili et al., 2005; Licciano et al., submitted). Moreover, laboratory experiments carried out on both sabellid species demonstrated that worms are able to digest most of the retained bacteria, particularly vibrios (Licciano et al., 2003; Licciano et al., submitted). On the basis of these previous observations, in order to test and characterize the filter-feeding of *S. spallanzanii* and *B. luctuosum* on bacteria, we selected the species *V. alginolyticus*, one of the most widely distributed in the Mediterranean Sea (Ortigosa et al., 1994, 1995; Pujalte et al., 1999; Cavallo and Stabili, 2002, 2004) and causative agent of vibriosis for fish and shellfish (Bordas et al., 1998).

Present laboratory experiments, showed that both species are extremely efficient in removing *V. alginolyticus* from seawater in experimental tanks, thus confirming the previous data from the field studies. Both species showed very high retention efficiency already in the first sampling times. *B. luctuosum* showed an intense feeding activity within the first 20 min, with a retention efficiency of 98%, while *S. spallanzanii* seems to have a lower efficiency, removing 70% of the bacteria within the first 30 min. The high efficiency of *B. luctuosum* within a short time is only apparently in contrast with preliminary observations indicating that this polychaete delayed to establish a regular filtration (Stabili et al., 2003). Since these previous experiments had been performed utilizing seawater from a highly eutrophic area in the Gulf of Taranto (Northern Ionian Sea), it may be suggested that the reduced gut capacity of *B. luctuosum*, linked to the small body size, exceeded on account of the wider availability of several trophic sources (Riisgård and Ivarsson, 1990). In our experiment, instead, the presence of bacteria as only food source in SFSW allowed the worms to efficiently filter and retain bacterial cells.

Differences in retention efficiency between the two sabellids could be due to the different initial bacterial concentration. Translating the *R*-values into bacterial biomass, we can conclude that *B. luctuosum* and *S. spallanzanii* are able to remove $44.8 + 7.88$ and $23.8 + 2.95 \mu\text{gC L}^{-1} \text{g}^{-1} \text{DW}$, respectively. Although the filtration process was studied *in vitro*, we can assume that the grazing of the two polychaetes on bacteria is remarkable taking into account that the bacterial biomass in the Mediterranean Sea is about $50 \mu\text{gC L}^{-1}$ (Krstulovic and Solic, 1988; Precali et al., 1989; Turk, 1991; Fonda Umani et al., 1992; Maugeri et al., 1992; Stabili et al., 2002).

The maximum clearance rate was higher in *B. luctuosum* than in *S. spallanzanii*, probably owing to the different metabolism of the two sabellids, the first species being smaller than the second one. This is also confirmed by the higher clearance values found in smaller individuals of *S. spallanzanii* (Licciano, 2004). Few data concerning the filtration process are at present available relative to the genus *Sabella*, while the present paper represents the first study on *B. luctuosum*. Present clearance values found for *S. spallanzanii* and *B. luctuosum* under laboratory conditions are considerably lower than that reported by Riisgård and Ivarsson (1990) for *Sabella penicillus* (= *pavonina*) feeding on phytoplankton ($114.5 \text{ L h}^{-1} \text{g}^{-1} \text{DW}$ at 17°C), but higher than the clearance value calculated by Shumway et al. (1988) for *Myxicola infundibulum* ($2.78 \text{ L h}^{-1} \text{g}^{-1} \text{DW}$).

The only data concerning the filtration process of *S. spallanzanii* are referred to a field study carried out on a polychaete population inhabiting the bare sediment in the Southern Flats of Cockburn Sound (Australia) (Clapin, 1996). In this study the feeding efficiency (volume of water filtered per metabolic demand) of $13 \text{ L mg}^{-1} \text{O}_2$ consumed is reported.

Our results are particularly interesting considering that the two sabellids could be utilized as biofilters. Bioremediation in marine systems is a new and sustainable tool to be applied in waters subjected to high charges of organic pollutants, such as fish farms and urban sewage discharges. Until now, mainly molluscs have been employed as filter-feeding organisms and recently the sponge *Chondrilla nucula* also was proposed for the same purpose (Milanese et al., 2003).

Aquaculture optimization through the employment of the filter-feeder polychaetes *S. spallanzanii* and *B. luctuosum* as bioremediators in wastewater treatment has been recently suggested, on account of the feeding on dissolved organic matter (Gambi et al., 1994; Giangrande et al., 2005). In fact, as a consequence of the filter-feeding activity, these sabellids may accumulate and concentrate the particulate as nourishment, thus transferring the organic matter from the water column to the sediment as faeces and pseudofaeces (Cavallo and

Giangrande, 2002). In a study where worms fed on water wastes from an intensive fish-growing tank, the particulate removed from the water column by *S. spallanzanii* was estimated of about 60.4 mg L^{-1} (Giangrande et al., 2005).

The rapid expansion of aquaculture in coastal areas, particularly fish and mussel farming, is generating an increasing concern over also the microbial control (Msuya and Neori, 2002). Aquaculture systems in fact can contain microbial organisms comprising both autotrophic and heterotrophic micro-organisms (Leonard et al., 2000) mainly including vibrios as potential pathogenic bacteria for the reared species. In an attempt to control bacteria, prophylactic use of antibiotics has become a frequently used strategy (Gatesoupe, 1982, 1989). Considering the resistance against antibiotics developed by bacteria (Skjermo and Vadstein, 1999) and that biological filters do not solve the problem of bacterial growth in the cultivation, alternative strategies are needed. Data from the present paper further support the previous hypothesis of using the studied filter-feeding polychaetes as bio-filters of also microbially contaminated waters in intensive aquaculture tanks where *Vibrio* species represent also a common economic problem. Many commercially important food fish and shellfish are, in fact, susceptible to *Vibrio* infections in natural environment as well as in aquaculture intensive farms (Bordas et al., 1998).

Obviously this report represents the starting point of investigation concerning the bioremediation topic. In this framework, in our laboratory further studies are in progress to evaluate if the filtration process of *S. spallanzanii* and *B. luctuosum*, up to now characterized in laboratory conditions on a pure culture of *V. alginolyticus*, will be also efficient when the two polychaete species are subjected directly to genuine-unfiltered aquaculture effluent.

5. Conclusions

Present paper represents the first study on *B. luctuosum* filtration process contributing also to better characterization of the filter-feeding process of *S. spallanzanii*. Here, the role of bacteria as food source has been firstly investigated for both polychaetes.

Clearance rates and filtration efficiencies calculated for *S. spallanzanii* and *B. luctuosum* feeding on *V. alginolyticus*, showed that these species filtered bacteria with high efficiency already within the first period of observation. Highest values were measured for the latter small-sized species.

On account of the filtering features described in the present paper, and on the basis of the capability shown by this species to accumulate culturable vibrios, the two investigated sabellids seem to be suitable for a their

employment as biofilters in aquaculture, also considering their heavy action in removing suspended solids in waste waters from intensive fish farms to which bacteria can also be attached.

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