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Functional role of biofouling linked to aquaculture facilities in Mediterranean enclosed locations

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ABSTRACT: Biofouling is generally considered a serious threat for human coastal activities such as aquaculture, and the ecological role of fouling organisms associated with fish-farm cages remains one of the most debated topics in the ecological field. However, although biofouling may cause significant problems related to human health, environmental impact and financial losses, in the past decade there has been an increasing interest in developing methods to promote the growth of biofouling on artificial structures as a strategy to mitigate human impacts and reduce the organic enrichment caused by net-cage fish farming. Here we investigated the filtration activity of biofouling assemblages colonizing artificial substrata located within a harbor. The main objective of the study was to determine if and how changes in composition and functioning of biofouling may be affected by hypoxic conditions that periodically occur within the port site selected for this study. To this purpose, artificial panels were used as biofouling collectors and were brought back to the laboratory seasonally where they were divided in 3 subgroups and acclimated at 3 different oxygen levels to mimic the naturally occurring oxygenic conditions. Clearance and respiration rates of each community were measured 6 and 24 h after the beginning of each treatment. Regardless of experimental conditions, performance of the communities was affected by the seasonality and the amount of biomass recruiting on the panels, mainly composed of crustaceans, ascidians, polychaetes, seaweeds and several introduced species. Our study demonstrated that, in particular cases, fouling assemblages linked to aquaculture facilities may contribute to reducing environmental impact and at the same time may serve as input for their re-use in different disciplines.

KEY WORDS: Bioremediation · Coastal aquaculture · Fouling · Hypoxia

1. INTRODUCTION

A major constraint in Mediterranean aquaculture is the access to coastal space and, although offshore aquaculture may offer a solution, in several countries aquaculture is still most common at sheltered sites, such as lagoons or semi-enclosed bays. These sites bring large advantages by being close to coastal infrastructures, but the likelihood to generate detrimental local ecological effects with severe repercussions on habitat quality is high. Water column and sediment quality and benthic biota can be impaired at a local scale (i.e. beneath the cages and in the surroundings; Price et al. 2015, Gentry et al. 2017) due to depletion of dissolved oxygen (DO) and nutrient enrichment (Neofitou et al. 2010). Additionally, aquaculture facilities and other artificial structures work as attachment substrata facilitating the establishment and the spread of fouling communities, i.e. the undesirable accumulation of different organisms on wetted artificial structures, which, in turn, could impair shipping industry and industrial aquatic operations such as aquaculture (Sarà et al. 2007).

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Generally, biofouling consists of multi-specific assemblages of microorganisms, algae and animals belonging to different trophic levels which may fulfill several ecological functions. Biofouling is prevalent in marine environments, causing significant problems related to health risks, environmental impact and financial losses (e.g. Wang et al. 2017). Detrimental effects are highly variable and depend on the extent, type and ecological roles of biofouling which are linked to many factors such as the biological traits of the species comprising the assemblage, aquaculture rearing techniques (subtidal, intertidal or suspended longlines), geographic location and local hydrodynamics (e.g Rivero et al. 2013). The presence of biofouling may change the hydrodynamic regimes affecting the ecological functioning of surrounding ecosystems (Mazouni et al. 2001, Wahl 2008) by altering the interactions within local assemblages (e.g. directly competing for resources with cultured organisms) and generating extra-economic costs. The direct economic cost of controlling biofouling in aquaculture is estimated conservatively at around 5–10% of the industry value (Fitridge et al. 2012), and is mainly related to the increased maintenance of overloaded infrastructure and production loss due to low growth and/or poorer quality of farmed species (Lane & Willemsen 2004).

Recent research has, however, emphasized the role of biofouling as an environmental mitigator of local detrimental impacts in that it is able to reduce the organic enrichment generated by fish farming at micro- and mesoscales, particularly where the aquaculture sites are within enclosed locations (Hughes et al. 2005, Floerl et al. 2016).

Indeed, the most common and abundant taxa among fouling organisms are usually suspension feeders and detritivorous (Mangano et al. 2019) and which thus may be able to exert a strong 'cleaning' (Sarà & Mazzola 2004) effect due to their effective pumping performance.

Thus, the debate on the functional role of biofouling is still unresolved, and there is an urgent need to increase our understanding of the potential positive role of biofouling in reducing local detrimental impact caused by aquaculture in enclosed locations. Reassessing the role of fouling in more positive terms may bring scientists to re-think management measures in enclosed habitats and to inform stakeholders about other options in managing biofouling in aquaculture. While it is crucial to gather data on if and how the ability of biofouling to retain fish-derived organic matter is spatially and temporally constant and persistent over time, in order to design effective management measures, it is necessary to understand whether local hypoxia events (highly frequent under enclosed shallow conditions; e.g. Bravo & Montañes 2001) can impair the rate of pumping in biofouling communities. Hypoxia can reduce the filtration efficiency of suspension feeders, diminishing their ability to retain organic particles (e.g. Sebens et al. 2016, Tang & Riisgård 2018); in the case of aquaculture, fish feces and uneaten food.

Here we present a study designed to (1) investigate the filtration activity of fouling assemblages colonizing artificial substrata mounted on finfish cages located in shallow enclosed waters within a Mediterranean harbour and (2) study how changes in the composition, and thus in the functioning of biofouling — as expressed by the filtration potential — may be affected by suboptimal environmental conditions (i.e. levels of DO in a range where physiological performances may be reduced without causing the death of aquatic organisms) that periodically occur within coastal shallow sites such as those chosen in this study.

2. MATERIALS AND METHODS

2.1. Study area, experimental design and sampling

The study was performed in a system of aquaculture fish cages (Ittica San Giorgio s.r.l.) located in southern Sicily (Licata harbour, 37.087° N, 13.943° E) from March 2014 to March 2015. The farm covers a total surface of ~8000 m^2 and encompasses 23 floating cages arranged in 2 rows containing sea bass *Dicentrarchus labrax* (Linnaeus, 1758) and sea bream *Sparus aurata,* Linnaeus, 1758, with a total annual production exceeding 300 t. The farm is located in a semi-enclosed and sheltered area characterized by limited hydrodynamic circulation and shallow depth (max depth ~10 m). Consequently, a large amount of organic matter, in the form of uneaten food and feces of the reared fish, accumulates on the sea floor under the cages, a phenomenon that appears to cause a progressive transformation of the benthic substrate into a muddy black sediment (Ape et al. 2019). In addition, the area is periodically (from August to October) affected by hypoxia phenomena, with oxygen concentration dropping within the sublethal (sensu Sokolova et al. 2012) range (~2 mg l^{-1}) in aquatic invertebrates (Giomi & Pörtner, 2013). In March 2014 (T0), 180 cement fiber panels (10 × 10 cm), 5 to 10 mm thick, were placed close to 2 fish cages within the fish farm and divided in 2 groups.

The first group (Group A) was positioned to follow the successional stages of biofouling and was composed of 144 panels. The colonization stages of biofouling were monitored throughout the study period by collecting 36 panels every 3 mo (T1: June 2014; T2: September 2014; T3: December 2014; T4: March 2015). The second group of panels (Group B) was used to investigate seasonal recruitment processes and was composed of 36 panels which were replaced every 3 mo with new (i.e. uncolonized) ones. This allowed us to investigate whether seasonality plays a role in the response of the assemblage (see Table 1 for an overview of sampling times). Cement fiber was chosen as it appears to be more suitable for settlement of natural fouling than more common substrata (e.g. glass or PVC; Chase 2015) traditionally used in monthly or annual samplings (Terlizzi & Faimali 2010). Six panels were attached to 200 cm long vertical nautical ropes and the distance between panels on a single rope was 30 cm. A total of 48 ropes were used for the whole sampling period.

Table 1. Timetable for sampling of biofouling growing on artificial substrates in an aquaculture facility in southern Sicily, Italy. X: panels were first put in the water, n = 180 (Groups A and B); +: 36 new panels were put in the field; –: 72 panels (Groups A and B) were removed

2014										2015			
T0			T1			T2			T3			T4	
Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	
X			+			+			+			+	
			–			–			–			–	

Water temperature (°C) and DO (mg l^{-1}) were continuously monitored before and during the whole sampling period by means of thermo and DO loggers (Type 22 iButton and HOBO U26 Dissolved Oxygen Data Logger, respectively). Loggers were placed in each cage at a depth of ca. 1 m and data recorded were used to set laboratory experimental treatments (see Text S1 and Figs. S1–S4 in the Supplement at www.int-res.com/articles/suppl/q012p011_supp.pdf). Every time we removed panels, water samples were collected and the amount of total suspended matter and its fraction were quantified to characterize the trophic condition of the target system (Table S1; Figs. S5–S6).

2.2. Ecophysiology and community composition of biofouling

Every 3 months (i.e. T1, T2, T3 and T4), 36 panels from each group (A and B) were removed from the rope and brought back to the laboratory (Laboratory of Ecology, University of Palermo, Italy), where they were maintained undisturbed for 24 h to reduce the stress due to manipulation. Then the panels were randomly divided into 6 groups of 6 panels and conditioned to 3 different DO concentrations (i.e. normoxic, intermediate [4 mg l^{-1}] and hypoxic [2 mg l^{-1}] conditions), mimicking the natural DO profile occurring within the study site, for a period of 6 and 24 h. Additionally, measurements of community clearance rate (CR) and metabolic rate, by using respiration rate (RR) as a proxy, were performed at 6 and 24 h after the beginning of each treatment.

Both CR and RR were measured to get values at community level using methods described in several studies designed for the individual analysis of functional traits (e.g. Sarà et al. 2000, 2008, 2013, 2014, Ezgeta-Balić et al. 2011, Giomi et al. 2016). In short, we placed 1 panel in a beaker containing 2.5 l of filtered seawater. Beakers were placed on heated stirring base plates to keep the water thermo-regulated, mixed and oxygenated throughout the experimental sessions. For CR measurements, algal cells (*Isochrysis galbana,* Parke 1949) were added to each beaker at an initial concentration of 25 000 cells ml^{-1}, and aliquots of 20 ml were sampled from every beaker at 30 min intervals over a period of 2h. The choice of the algal species, concentration and timing have been demonstrated to be adequate to measure filtration rate not only in bivalves (e.g. Widdows & Staff 2006), but also in other active filter-feeders such as ascidians and bryozoans (e.g. Pascoe et al. 2007, Montalto et al. 2017). The decline in *I. galbana* cell concentration was monitored using a Model Z2 Coulter Counter (Beckman Coulter). Before starting with measurements of RR, beakers were completely wrapped in black cellophane in order to make the contribution of primary production negligible. The decline in oxygen concentration was measured using a calibrated oxygen fibreglass sensor connected to a data logger (PyroScience Firesting O_2) and continuously recorded for at least 1 h after waiting 10 min, during which a more rapid decline in oxygen caused by a disturbance of the sensor's temperature equilibration is usually recorded (Svendsen et al. 2016).

Once physiological measurements on panels treated with different levels of oxygen had been performed, encrusting benthic communities were gently scraped from the panel surface and the animal component separated from the algal component and fixed in

70% ethanol. Then, benthic specimens were gently washed over a 500 µm sieve, sorted from the abiotic components (mostly calcareous tubes) and identified to the lowest taxonomic level. Both algae and animals were separately weighted to determine the biomass (expressed as g wet weight [WW]) and to standardize physiological measurements. Specifically, CR measurements have been standardized by taking into account only the animal component, while the whole community biomass is considered in estimates of metabolic performances (Sarà et al. 2000, 2008, 2013, 2014, Ezgeta-Balić et al. 2011, Giomi et al. 2016).

2.3. Statistical analysis

Due to the loss of some panels in both groups (a total of 10 in Group A and 27 in Group B) during the autumn season (T3), the experimental design became unbalanced, and therefore data were analysed through PERMANOVA (Anderson 2001) using Euclidean distance and 9999 permutations. Further, the sets of panels collected at the first sampling period (T1) from both groups were excluded from the analysis because they were characterized by the presence of algae only and, as a consequence, filtration activity was unable to be measured. Thus, season (3 levels: T2, T3, T4), O_2 treatment (3 levels: control, 4 mg l^{-1}, 2 mg l^{-1}), and exposure time (2 levels: 6 h, 24 h) were treated as fixed factors in the experimental design, and with the exception of T3, 6 replicates for each level (i.e. oxygen treatments and exposure times) were used (i.e. a total of 36 replicates for each group for each sampling time). PERMANOVA was carried out by using the PRIMER software (version 6.0).

3. RESULTS

3.1. Ecophysiological measurements: CR and RR

Overall, standardized CRs for Group A ranged between 1.22 and 3.01 l h^{-1} g WW^{-1}, showing significant differences among seasons (Table 2a), while in Group B, measurements varied between 0.50 and 5.62 l h^{-1} g WW^{-1} (Fig. 1). Despite differences found in both biomass and composition of benthic fauna (see Section 3.2) recruited on panels belonging to Group B, results showed similar CR of communities when both oxygen levels and exposure time varied ($p > 0.05$; Table 2b). On the contrary, when the algal components were included, there were significant differences in RRs of the whole communities among different seasons (Table 2b). Specifically, as showed in Fig. 2, communities recruited at T2 showed significantly higher RRs than those measured in communities settled at T3 and T4, with average (± SE) RRs of 6.79 ± 0.65 µmol O_2 h^{-1} g WW^{-1}, 2.94 ± 0.36 µmol O_2 h^{-1} g WW^{-1} and 4.27 ± 0.50 µmol O_2 h^{-1} g WW^{-1}, respectively. Analogously, in Group A panels, both CR and RR were significantly different among seasons (Table 2). Accordingly, CR and RR in assemblages recruited after 6 mo of submersion were higher than those in communities after 9 and 12 mo submersion.

No panels from either group showed significant differences in physiological measurements when oxygen availability and/or exposure time varied (Table 2). Exceptions were represented by CRs measured at T2 and T3 ($p < 0.05$) under normoxic conditions, at T2 and T4 ($p < 0.001$) under the hypoxic conditions and the treatments performed at intermediate oxygen concentration (i.e. 4 mg l^{-1}), where CRs of communities collected at T2 were significantly higher than in assemblages collected at T3 ($p < 0.01$) and T4 ($p < 0.05$) (Table 3).

3.2. Fouling communities: biomass and composition

Crustaceans (mainly barnacles and amphipods), tunicates (mainly ascidians), polychaetes and seaweeds were the components of fouling commonly observed on the panels (Fig. 3). Overall results showed that the average biomass of Group A was higher than that of Group B measured at each period, with values ranging, respectively, between 15.45 and 46.01 g WW and 4.85 and 18.96 g WW. Also, as shown in Fig. 4, the average % biomass of algae in Group B was higher (more than 50% of the total biomass on each panel) than animal biomass when compared with that measured on panels of Group A. Indeed, in the latter group, longer submersion times resulted in a reduction of algal biomass up to 30% of the mean total wet weight after 1 yr of deployment. PERMANOVA showed significant differences in the biomass of communities recruited during the different seasons in both groups of panels. On the other hand, there were no significant differences in panels of both groups used during every experimental treatment (i.e. when both oxygen levels and exposure time varied; Table 3).

The pairwise comparison (Table 3) for panels colonized quarterly (i.e. Group B) showed that benthic biomass (animal + algae) recruited between T3 and T4 was significantly lower than that measured in the other 2 periods, with mean (± SE) values of 4.12 ± 0.98 g

Table 2. PERMANOVA of results and group analysis for standardized (st) clearance rate (CR, l h^{-1} g WW^{-1}) and respiration rate (RR, µmol l^{-1} h^{-1} g WW^{-1}) during different sampling periods. (a) Panels collected after 6, 9 and 12 mo (Group A); (b) = 3 mo old panels submerged during different seasons (Group B). SE: season; TREAT: O_2 treatment, EXP: exposure time; RES: residuals. See Table 1 for further definitions

Source	df	SS	MS	Pseudo-*F*	P(perm)	Unique perms	Groups	*t*	P(perm)	Unique perms
(a)										
CRst										
SE	2	53.93	26.97	12.24	0.0002	9952	T2, T3	3.67	0.0007	9848
TREAT	2	11.85	5.92	2.69	0.0712	9951	T2, T4	3.76	0.0001	9839
EXP	1	0.68	0.68	0.31	0.5963	9860	T3, T4	0.15	0.1502	9863
SE × TREAT	4	22.32	5.58	2.53	0.0466	9951				
SE × EXP	2	1.54	0.77	0.35	0.7132	9944				
TREAT × EXP	2	5.84	2.92	1.32	0.266	9964				
SE × TREAT × EXP	4	10.5	2.63	1.19	0.3232	9957				
RES	77	169.64	2.2							
RRst										
SE	2	307.52	153.76	22.21	0.0001	9941	T2, T3	4.25	0.0001	9861
TREAT	2	14.21	7.1	1.03	0.3716	9940	T2, T4	5.36	0.0001	9861
EXP	1	26.27	26.27	3.8	0.0514	9851	T3, T4	1.52	0.1328	9831
SE × TREAT	4	44.92	11.23	1.62	0.165	9950				
SE × EXP	2	26.43	13.21	1.91	0.1553	9957				
TREAT × EXP	2	34.49	17.24	2.49	0.0795	9951				
SE × TREAT × EXP	4	46.7	11.67	1.69	0.157	9946				
RES	74	512.2	6.92							
(b)										
CRst										
SE	2	194.21	97.1	1.74	0.1619	9937				
TREAT	2	46.87	23.44	0.42	0.5381	9931				
EXP	1	9.25	9.25	0.17	0.6432	9800				
SE × TREAT	4	66.09	16.52	0.3	0.8455	9942				
SE × EXP	2	176.15	88.07	1.58	0.1693	9940				
TREAT × EXP	2	9	4.5	0.08	0.8923	9929				
SE × TREAT × EXP	4	186.25	46.56	0.83	0.3882	9939				
RES	53	2959.7	55.84							
RRst										
SE	2	169.54	84.77	8.33	0.002	9961	T2, T3	3.18	0.0053	9808
TREAT	2	18.65	9.32	0.92	0.4081	9943	T2, T4	2.8	0.0073	9845
EXP	1	0.01	0.01	0	0.9828	9829	T3, T4	1.98	0.0620	9834
SE × TREAT	4	15.68	3.92	0.39	0.8136	9945				
SE × EXP	2	26.24	13.12	1.29	0.2839	9948				
TREAT × EXP	2	22.23	11.12	1.09	0.3448	9948				
SE × TREAT × EXP	4	26.27	6.57	0.65	0.63	9950				
RES	56	569.97	10.18							

WW (T2), 7.89 ± 1.95 g WW (T3) and 0.37 ± 0.09 g WW (T4). Within the animal component, the largest number of taxonomic groups (i.e. 7; Fig. 3b) were identified in the post-summer panels (T2), where the prevailing biomass comprised bryozoa, polychaetes, ascidians and crustaceans, whereas after the autumn and winter periods (T3 and T4), panels were mainly colonized by polychaetes and crustaceans, which in both cases accounted for more than 90% of mean animal biomass.

In Group A, wet mass of animal assemblages increased with increasing time of submersion, with post-summer WW significantly lower ($p < 0.001$; Table 3) and corresponding to a recruitment period of 6 mo (i.e. T2). Similarly, the number of taxonomic groups varied with length of submersion, with a total of 6 groups (Fig. 3a) identified in 6 and 9 mo old panels (at T2 and T3) and of 9 groups distinguished in panels submersed for 1 yr (at T4) (Fig. 3b). After this time of exposure, the benthic community settling on the panels reached the highest number of species and was mainly dominated by epibenthic incrusting species such as the tunicate *Styela plicata* (Lesueur, 1823), bryozoans (*Cryptosula* spp.), sponges (*Halicondria* spp.), barnacles and serpulid tubeworms (*Hydroides* spp.; see Table S2 for more details). High numbers of both errant (*Nereis pelagica* Linnaeus, 1758) and

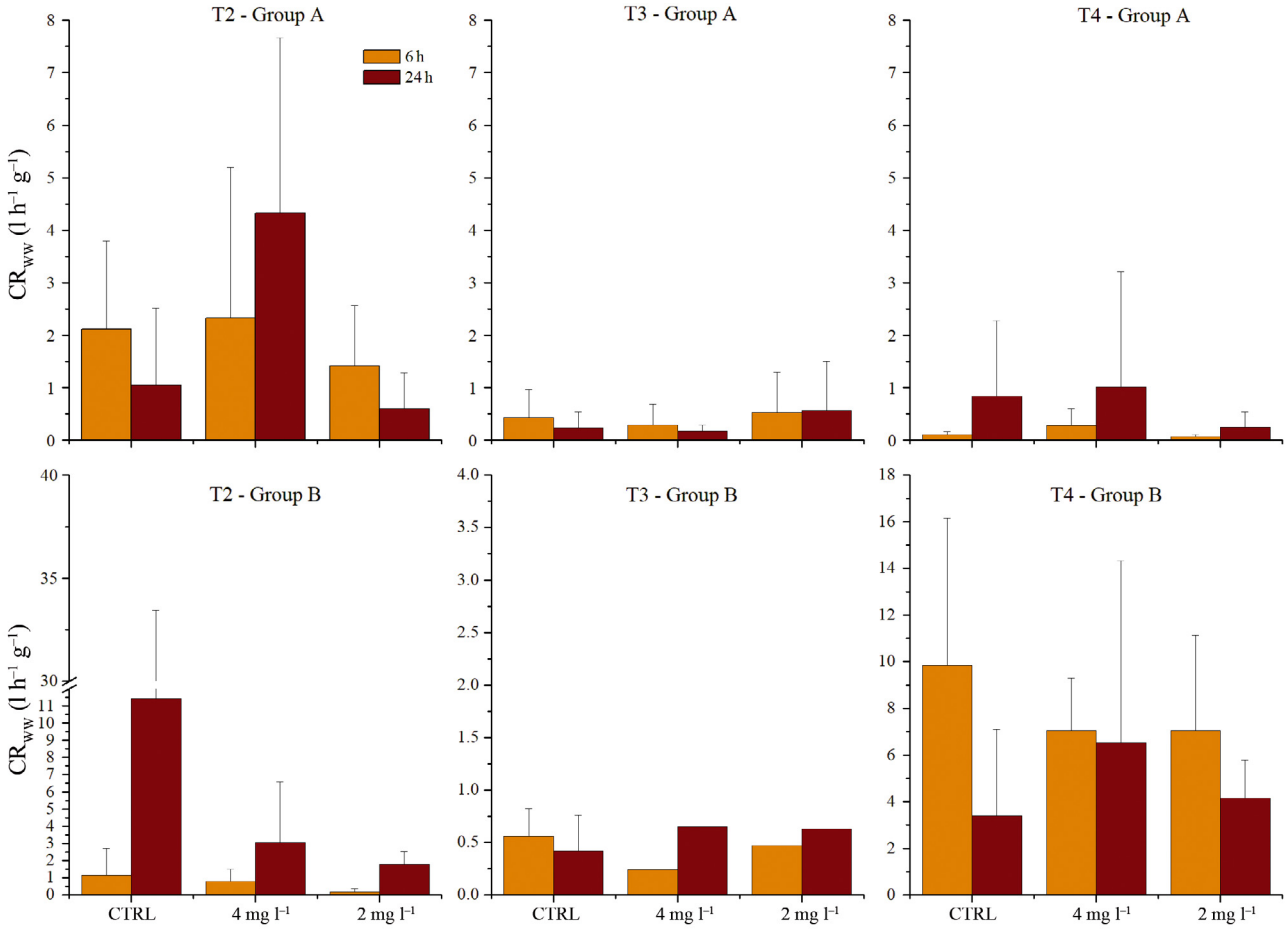

Fig. 1. Standardized clearance rates (CR) of biofouling communities at varying levels of oxygen availability after 6 and 24 h of treatment (CTRL [well-saturated], 4 mg l^{-1} and 2 mg l^{-1}) for panels collected after 6, 9 and 12 mo submersion (Group A) (at T2, T3 and T4, respectively) and panels submersed for 3 mo and collected at T2, T3 and T4 (Group B). WW: wet weight. See Table 1 for dates

sedentary polychaetes (e.g. *Spirobranchus* sp.) were observed. The filter feeder tubiculous Sabellariidae *Branchiomma bombyx* was the most frequent sedentary polychaete found on the panels. Among the vagile fauna, crustacean species (mainly amphipods and tanaids) and the echinoderm *Ophiothrix fragilis* were most abundant (Mangano et al. 2019). Tube-dwelling amphipods and several introduced species were also recorded (see Text S2 and Table S2). Algal biomass in both groups of panels was significantly different among seasons (Table 3) except for algal biomass in panels of Group A submersed for 6 and 12 mo, where the pairwise comparison yielded no significant differences ($p > 0.05$; Table 3).

4. DISCUSSION

Biofouling is a recurring issue in aquaculture worldwide, especially when activities are geographically located in suboptimal sites such as enclosed sites, where the assimilative capacity (sensu Chopin 2010) can be frequently exceeded. Nevertheless, our results show that biofouling can also have an ecologically facilitating role, thanks to the active (and massive) filtering capacity of assemblages encrusting artifical substrata. Indeed, regardless of the composition of the species succeeding in the panels, the positive clearance rates confirmed a continuous activity of particle removal by structured communities. Accordingly, the high clearance rates measured in recruitment panels collected at T2 (summer) and T4 (winter), compared to those collected at T3, i.e. autumn, suggested that the environmental physical-chemical conditions were able to play a role in affecting particle removal dynamics. In fact, other than crustaceans, biofouling in both groups of panels was mainly composed of the ascidian *Styela plicata*, whose recruitment may be promoted by an optimal combination of thermal (22.23 ± 0.03°C) and trophic

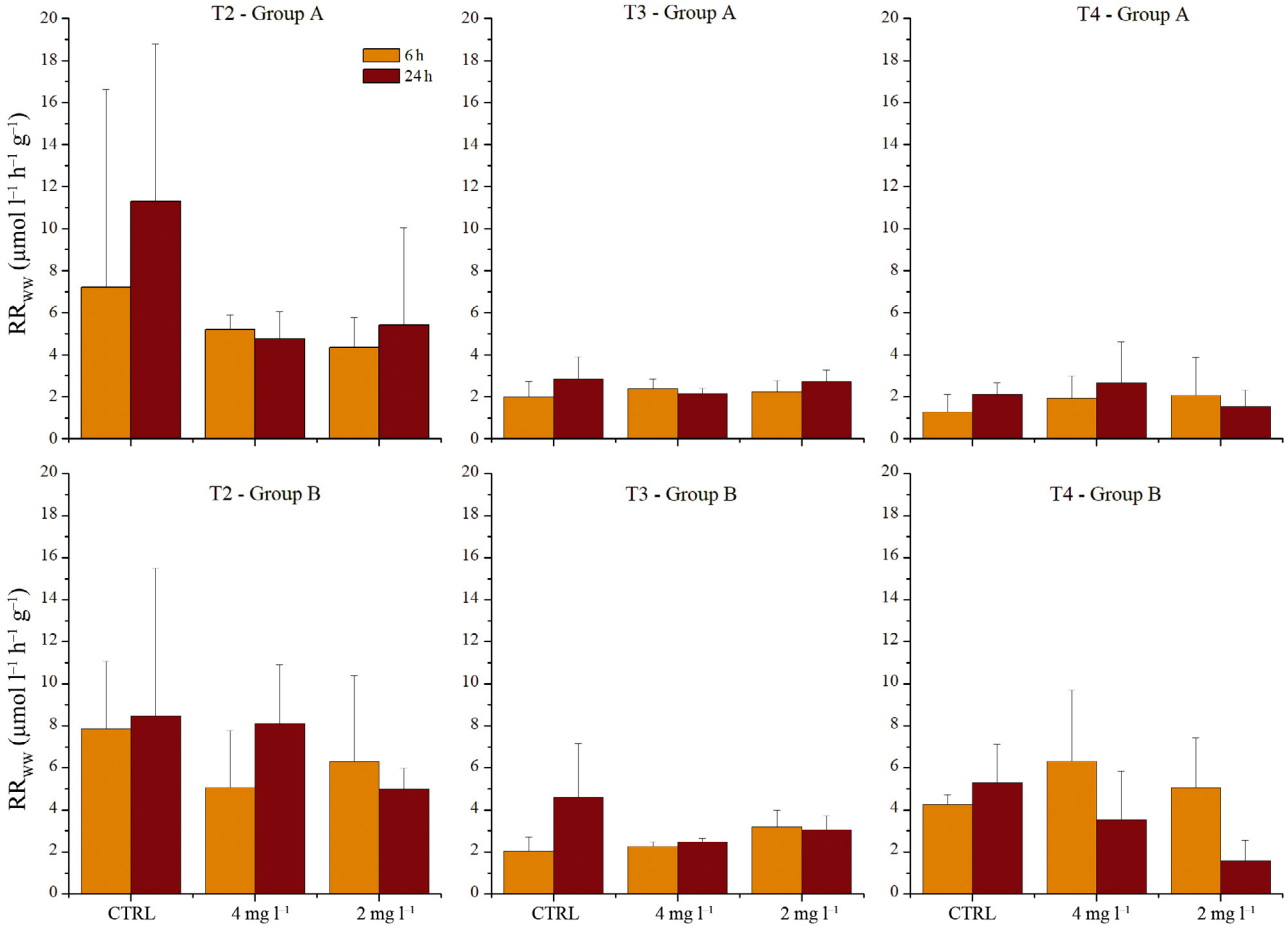

Fig. 2. Standardized respiration rates (RR) of biofouling communities at varying levels of oxygen availability. See Fig. 1 and Table 1 for further details

conditions, together with a favourable relationship between oxygen uptake and body size (sensu Montalto et al. 2017), by disavantaging the presence of other filter-feeders such as hydrozoans and porifers. Ascidians thus seem to take an advantage of local conditions, and this makes them 'winners' among other filter feeders. The inhibition of settlement or recruitment by resident adults has been described by several authors (e.g. Tyrrell & Byers 2007) as a result of competition for space (altering the possibility of adhesion by the larvae) and/or chemical inhibition mechanisms (e.g. Davis 1991, Paul & Puglisi 2004).

Surprisingly, oxygen consumption was also affected by factors other than environmental oxygen availability. As resulted from the PERMANOVA carried out on RR, the experimental oxygen treatments did not impair assemblage response, while the analysis suggests that variations in the magnitude of the response are a function of the biomass recruiting at different seasons. Nonetheless, the sequence in which fouling organisms recruit is influenced primarily by seasonal temperature fluctuations, as temperature affects reproductive cycle and subsequent larval release and development of marine organisms (Duarte 2007). However there are several data showing that some stages of succession can be influenced by the previously established taxa (Lezzi et al. 2018). Hydroids, for example, seem be able to prepare a micro-environment suitable for bryozoans (Menon & Nair 1971, Khalaman 2001) and mussels can be more successful on substrates already occupied by ascidians and hydroids (Dean & Hurd 1980), while the presence of filter feeder worms seems to be more related to a high availability of food in the environment. As shown in Fig. 3, indeed, there was an increase in biomass during autumn, which corresponds at these latitudes to the period of highest concentrations of both chl *a* and suspended organic matter (Table S1, Figs. S5 & S6). Finally, some groups like ascidians, amphipods and polychaetes, being able to efficiently use the organic fraction of suspended matter and aquaculture wastes, are known for reducing

Table 3. PERMANOVA of results and group analysis for wet biomass (WW, g); animal (WW an), algal (WW veg) and total (WW tot); during different sampling periods. (a) Panels collected after 6, 9 and 12 mo (Group A); (b) 3 mo old panels submerged during different seasons (Group B). SE: season; TREAT: O_2 treatment, EXP: exposure time; RES: residuals. See Table 1 for further definitions

Source	df	SS	MS	Pseudo-*F*	P(perm)	Unique perms	Groups	*t*	P(perm)	Unique perms
(a)										
WW an										
SE	2	12010	6005.2	21.75	0.0001	9958	T2, T3	6.4098	0.0001	9838
TREAT	2	53.32	26.66	0.1	0.9103	9946	T2, T4	6.1745	0.0001	9854
EXP	1	83.89	83.89	0.3	0.5976	9844	T3, T4	1.8811	0.0702	9842
SE × TREAT	4	1890.5	472.61	1.71	0.1571	9961				
SE × EXP	2	519.44	259.72	0.94	0.4034	9955				
TREAT × EXP	2	309.01	154.51	0.56	0.5815	9950				
SE × TREAT × EXP	4	544.81	136.2	0.49	0.7486	9949				
RES	77	21259	276.08							
WW veg										
SE	2	1680.8	840.42	23.18	0.0001	9950	T2, T3	6.9567	0.0001	9821
TREAT	2	68.13	34.07	0.94	0.3898	9947	T2, T4	1.5111	0.1368	9830
EXP	1	31.43	31.43	0.87	0.3528	9825	T3, T4	5.3018	0.0001	9822
SE × TREAT	4	57.39	14.35	0.4	0.8114	9962				
SE × EXP	2	194.93	97.47	2.69	0.0723	9951				
TREAT × EXP	2	97.7	48.85	1.35	0.2644	9958				
SE × TREAT × EXP	4	127.73	31.93	0.88	0.4814	9955				
RES	77	2792.3	36.26							
WW tot										
SE	2	17507	8753.5	27.4	0.0001	9933	T2, T3	9.0983	0.0001	9833
TREAT	2	126.74	63.37	0.2	0.8269	9937	T2, T4	6.4547	0.0001	9813
EXP	1	91.96	91.96	0.29	0.6024	9856	T3, T4	0.2922	0.7726	9841
SE × TREAT	4	1264.5	316.13	0.99	0.4185	9952				
SE × EXP	2	227.79	113.9	0.36	0.7114	9954				
TREAT × EXP	2	327.45	163.73	0.51	0.6194	9955				
SE × TREAT × EXP	4	500.82	125.21	0.39	0.8169	9944				
RES	74	23645	319.53							
(b)										
WW an										
SE	2	360.68	180.34	7.49	0.0057	9936	T2, T3	1.3413	0.1891	9815
TREAT	2	47.84	23.92	0.99	0.3502	9956	T2, T4	3.0348	0.003	9818
EXP	1	0.13	0.13	0.01	0.939	9832	T3, T4	6.1396	0.0007	9833
SE × TREAT	4	23.44	5.86	0.24	0.882	9952				
SE × EXP	2	3.84	1.92	0.08	0.9159	9947				
TREAT × EXP	2	36.3	18.15	0.75	0.4219	9950				
SE × TREAT × EXP	4	91.05	22.76	0.94	0.4228	9936				
RES	53	1276.9	24.09							
WW veg										
SE	2	328.38	164.19	31.38	0.0001	9960	T2, T3	3.2475	0.0021	9850
TREAT	2	16.08	8.04	1.54	0.2261	9943	T2, T4	5.6918	0.0001	9834
EXP	1	3.19	3.19	0.61	0.4391	9850	T3, T4	10.963	0.0001	9860
SE × TREAT	4	12.36	3.09	0.59	0.6689	9957				
SE × EXP	2	10.11	5.06	0.97	0.3876	9944				
TREAT × EXP	2	24.96	12.48	2.39	0.1036	9944				
SE × TREAT × EXP	4	8.86	2.22	0.42	0.7935	9954				
RES	53	277.3	5.23							
WW tot										
SE	2	1513.7	756.87	18.36	0.0001	9941	T2, T3	2.2301	0.0313	9809
TREAT	2	35.61	17.81	0.43	0.6479	9935	T2, T4	4.3201	0.0002	9840
EXP	1	11.71	11.71	0.28	0.6056	9849	T3, T4	9.8455	0.0001	9839
SE × TREAT	4	110.18	27.55	0.67	0.6219	9937				
SE × EXP	2	7.52	3.76	0.09	0.9118	9953				
TREAT × EXP	2	104.02	52.01	1.26	0.2919	9948				
SE × TREAT × EXP	4	98.01	24.5	0.59	0.6727	9958				
RES	56	2308.8	41.23							

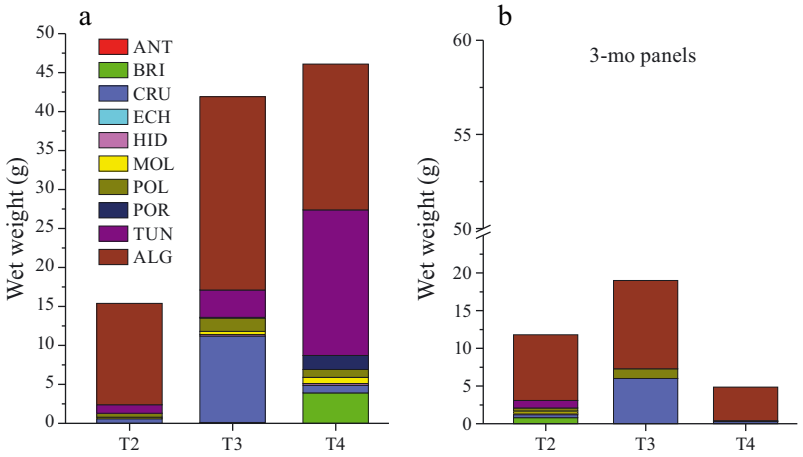

Fig. 3. Mean wet weight and relative composition of biofouling communities on submersed panels. (a) Assemblages recruited on panels left to colonize for 6, 9 and 12 mo (Group A) (collected at T2, T3 and T4, respectively) and (b) panels submersed for 3 mo and collected at T2, T3 and T4 (Group B). ANT: Anthozoa; BRI: Bryozoa; CRU: Crustacea; ECH: Echinodermata; HID: Hydrozoa; MOL: Mollusca; POL: Polychaeta; POR: Porifera; TUN: Tunicata; ALG: Algae. See Table 1 for dates

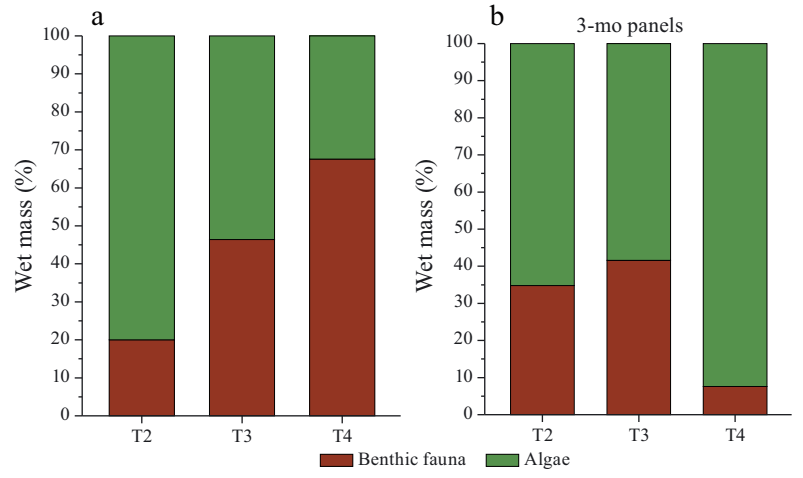

Fig. 4. Percentage contribution of macrofaunal community and algae assemblages recruited on submersed panels. (a) Assemblages recruited on panels left to colonize for 6, 9 and 12 mo (Group A) (collected at T2, T3 and T4, respectively) and (b) panels submersed for 3 mo and collected at T2, T3 and T4 (Group B). See Table 1 for dates

competition for other types of resources such as phytoplankton (Bracken et al. 2012). This in turn could positively affect the presence of bivalves, which rely on a diet of fresh organic matter such as phytoplankton, may represent an additional set of organisms contributing to particle removal (Newell 2004) with a consequent improvement of water quality (Manganaro et al. 2009, Sarà et al. 2009, Troell et al. 2009).

However, in the last decade, the optimization of environmental conditions and aquaculture activities has led to the need to investigate and propose alternative solutions in order to advance in the sustainability of aquaculture (Troell et al. 2003, Chopin 2010). Within this context, many of the species found on the panels in our study are by-products of aquaculture and could be employed in aquaculture systems as a novel bioremediation technology to reduce waste materials and restore water quality. On the other hand, while higher macroalgae (e.g. *Gracilaria* sp.) and mussels are well known co-cultured species for the removal of nitrogen and phosphorus, phytoplankton and suspended particle with the advantage that they are subsequently reused for food purposes, other organisms may find a further application in other disciplines that are not directly linked to a food trade (e.g. Hamed et al. 2015). To date, few studies have provided evidence for the capability of biofouling to remove aquaculture waste and employed different organisms as potential candidates for bioremediation in an aquaculture farming scenario. For example, several authors have demonstrated the potential of amphipod cultures associated with integrated multi-trophic aquaculture systems, demonstrating the suitability of the amphipod-based product for use as a natural ingredient in aquafeed compositions (e.g Fernandez-Gonzalez et al. 2018). Further, Licciano et al. (2005) estimated a filtration capacity of 0.28 m^3 g dry weight $(DW)^{-1}$ d^{-1} in *Sabella spallanzanii*, while Pierri (2007) reported a filtered volume of 1.1 m^3 g DW^{-1} d^{-1} in *Branchiomma luctuosum*; both polychaetes have been proposed with the dual purpose of acting as bioremediators and subsequently serving as food for aquaculture species (*S. spallanzanii*; Stabili et al. 2013) or as a bioindicator for the surrounding environment, particularly when low levels of pollution make impact assessment extremely complex (Licciano et al. 2007). More recently, filtration estimates performed on *S. plicata* fed with the microalgae *Isochrysis galbana* showed values ranging between 1.0 and 1.53 l h^{-1} g DW^{-1} when chlorophyll concentration varied between 0.3 and 3.1 µg l^{-1} (Montalto et al. 2017). Thus, if we assume an average density of 200 ind. m^{-2} (which corresponds to the density

of ascidians present on the panels collected every 3 mo) and an average clearance rate equal to 1.01 l h^{-1} g DW^{-1}, the mean filtering capacity in *S. plicata* could be about 4.85 m^3 g DW^{-1} d^{-1} m^{-2} ; such a species, although it is still poorly used for bioremediative purposes, has been frequently used in the pharmaceutical field. Several examples show how this species can be used for extraction of compound like heparin, with potential uses in treating inflammation (Wang et al. 2002), thrombosis (Myers et al. 2005) and metastasis (Borsig et al. 2001). In addition, aided by a growing interest for functional food ingredients, e.g. nutraceuticals, probiotics, prebiotics and various dietary supplements (Shahidi 2009) that provide health and medical benefits (including the prevention and/or treatment of disease), much of recent research has addressed this important issue. The possibility to extract numerous compounds such as enzymes, proteins, peptides, polysaccharides, polyunsaturated fatty acids, phenolics, pigments and other secondary metabolites from various sources such as prokaryotes, micro- and macroalgae, seaweeds, crustaceans, sponges and other invertebrates as well as various vertebrates, may indeed represent a great potential for biotecnological applications and may be useful to the food industry in a number of applications (Holdt & Kraan 2011, Freitas et al. 2012, Murray et al. 2013, Boziaris 2014, Dewapriya & Kim 2014).

In summary, our study supports the novel idea that in suboptimal aquatic conditions such as those occurring in sheltered sites (e.g. hypoxya and/or organic enrichment), important ecosystem services may be supported by fouling communities. In fact, while species that are commonly co-cultivated within aquaculture farms may exhibit signs of disturbance, potentially affecting the whole food web, fouling assemblages in particular cases, such as enclosed locations, may contribute to reduce the environmental impact and at the same time serve as inputs for their re-use in different disciplines. Despite the effect of fouling communities being commonly seen as being negative, there are several studies highlighting the important role of filtration exerted by foulers (e.g. Hughes et al. 2005) as well as how their presence in aquaculture facilities would benefit productions lead to economic savings (Lacoste & Gaertner-Mazouni 2015) and support the the sustainability of aquaculture in a changing climate (Sarà et al. 2018).

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