



Two cases study of fouling colonization patterns in the Mediterranean Sea in the perspective of integrated aquaculture systems

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ABSTRACT

Fouling assemblage colonizing fish-farms is considered mostly to produce negative impacts causing financial loss. By contrast, large evidences emerged on the bioremediation role by biofouling associated to aquaculture facilities, even if the fouling assemblages thriving in the water column was poorly investigated. The aim of the present work was to investigate the macrofouling assemblages over one year of immersion, in order to single out the fouling species, which play the most remarkable role for the bioremediation of the marine areas affected by aquaculture activities. With this in mind, we studied the fouling community dynamics in two Mediterranean maricultural facilities, respectively in the Mar Grande of Taranto (Ionian Sea) and in the Gulf of Gaeta (Tyrrhenian Sea), using the same experimental design and time frame. Two experiments were carried out using artificial panels anchored to two finfish cages. The one-year old fouling communities in the two sites were compared at four seasons of immersion, four submersion durations and three depths, both communities from structural and functional points of view. Notwithstanding the quite similar species composition of fouling of the two sites, the biofouling showed the highest biodiversity in the Mar Grande of Taranto. In the Gulf of Gaeta mussels, *Mytilus galloprovincialis*, dominated at all the times and depths, whilst in the Mar Grande of Taranto they were especially abundant at the surface with the deepest panels being largely colonized by polychaete sabellids. The co-occurrence of the filter-feeders *Mytilus*-sabellids recorded in the Gulf of Taranto also highlighted the highest filtration capability. Our results suggest two different fouling assemblages as candidates for bioremediation in integrated multitrophic aquaculture facilities: both a monospecific system dominated by mussels and a multi-specific system with sabellids and mussels as most abundant filter-feeders.

1. Introduction

The biofouling assemblage colonizing fish farms artificial structures is conditioned by the floating nature of the substrate and by the high nutrient levels deriving from the aquaculture wastes (Cook et al., 2006; Perkol-Finkel et al., 2008). For this reason, fouling communities often are dominated by suspension-feeding macroinvertebrates (Fernandez-Gonzalez and Sanchez-Jerez, 2017; Fitridge et al., 2012; Lezzi et al., 2017; Pica et al., 2019). To date, most of the knowledge on biofouling related to aquaculture facilities is about its direct settlement on the net

cages, highlighting its negative impact on farm structures. Indeed, the thriving fouling increases weight and drag of the cages, it reduces water flow and negatively affects production, especially because of the periodical mechanical cleaning actions required for biofouling removal (Bannister et al., 2019; Baxter et al., 2012; Braithwaite and McEvoy, 2005; Edwards et al., 2015; Fitridge et al., 2012). Fouling is also considered to produce also negative effects for the longline mussels culture, where it acts as competitor for space for the growth of *Mytilus galloprovincialis* (Antoniadou et al., 2013; Khalaman, 2001; Lacoste and Gaertner-Mazouni, 2015; Sievers et al., 2013; Watts et al., 2015).

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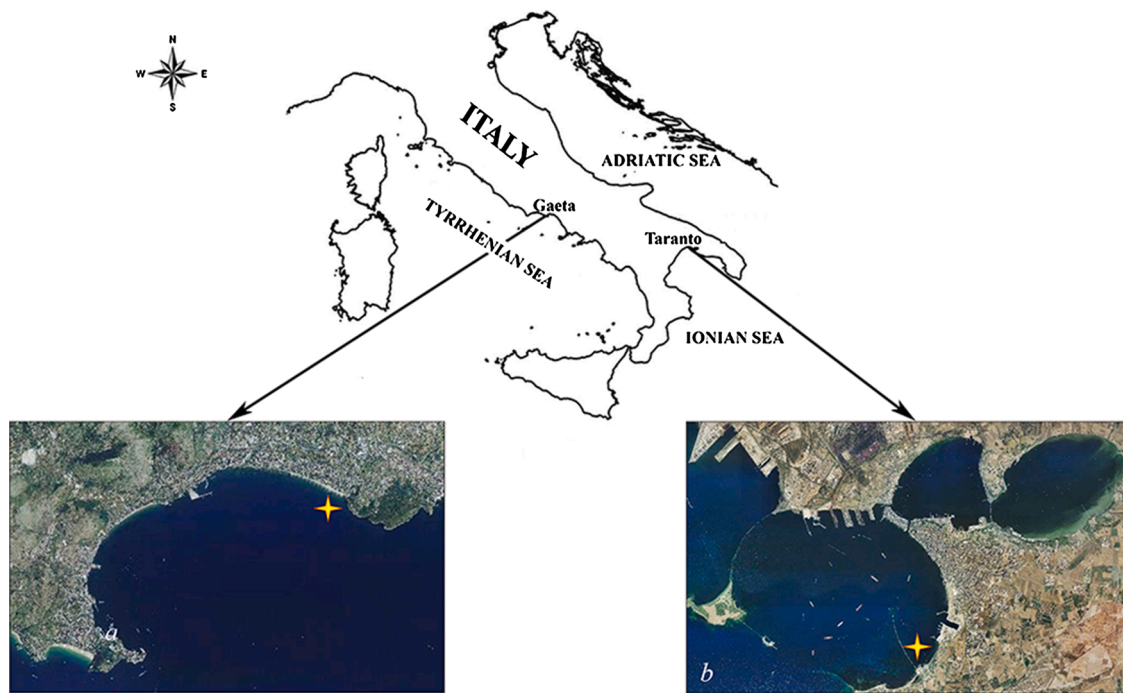


Fig. 1. Map of the two study areas. Stars indicate the investigated sites in the Gulf of Gaeta (a) and in the Mar Grande of Taranto (b).

In contrast with such negative impacts, some authors highlighted the effectiveness of the biofouling in reducing water organic enrichment caused by intensive mariculture (Angel et al., 2001, 2002; Angel and Spanier, 2002; Black, 2001; Chopin et al., 2008, 2012; Gonzalez-Silvera et al., 2015; Hughes et al., 2005). Indeed, some invertebrates, named bioremediators, can be used within the Integrated Multi-Trophic Aquaculture (IMTA) (Hughes and Black, 2016; Rena et al., 2012), which is a practice combining the cultivation of feed aquaculture species with other benthic filter-feeder species. These organisms are able to remove organic and inorganic particles from the enriched seawater acting as bioremediators and creating balanced systems for environmental sustainability, economic stability and social acceptability (Barrington et al., 2009). The organic load produced by farming activities is recorded both in the water column (Sarà et al., 2006, 2007) and in the underlying sediments (see the review of Kalantzi and Karakassis, 2006; Sarà et al., 2006). Therefore, bioremediators are both detritivore organisms, which act on the sediment, and filter feeders species, which act in the water column (Dafna et al., 2019). Moreover, in the areas influenced by lateral drift, the organic load has been noted to be efficiently reduced by the bioremediators that are present in the vertically suspended fouling in the water column (Cromey et al., 2002a, b; Sarà et al., 2004, 2007).

To promote a circular economy, edible filter feeder like mussels and other species of economical interest, are commonly cultured in IMTA fish farm systems (Badalamenti et al., 1992; Hughes and Black, 2016; Rena et al., 2012; Sarà et al., 2004, 2009). Recently, other filter feeder species have been tested as biofilters in the IMTA systems, resulting in the production of possible valuable biomass to the producer as well. They use mostly porifera (Longo et al., 2016) and polychaete sabellids (Giangrande et al., 2014), which are very efficient in removing bacteria and organic matter from the water column (Licciano et al., 2005; Stabili et al., 2006).

The study of the local distribution of the fouling communities is a basic requirement for promoting the selection of species which can be successfully reared in IMTA systems located in different geographical areas. On the other hand, available data on the functional aspects of filter feeders fouling components are very scanty (Angel and Spanier, 2002; Hughes et al., 2005; Woods et al., 2012) and coming from

laboratory experiments (Montalto et al., 2020). With the purpose to shed light on such issue, we investigated the fouling colonization pattern in two aquaculture facilities located in different Mediterranean areas along the Italian coast.

The aims of the present study are (i) to analyse the fouling community settled on experimental substrata after one year of exposure in two sites along the Italian coast; (ii) to compare the fouling communities using the same experimental design and time of exposure in different geographical areas; (iii) to assess the filtration capability of the fouling communities.

2. Material and methods

2.1. Study sites

Two sites were investigated. The first is located in the south-west side of the Mar Grande of Taranto ($40^{\circ}25'56''N$ $17^{\circ}14'19''E$), which is a semi-enclosed basin with an area of 35.5 km^2 and a maximum depth of 42 m, located in the Ionian Sea (Central Mediterranean Sea) (Fig. 1a). Water temperature fluctuates according to a seasonal pattern and ranges between 14° and $28^{\circ}C$ with an average annual value of $19^{\circ}C$, accordingly with the typical fluctuations occurring in the coastal Ionian region; the average salinity is 38 PSU and it remains quite constant throughout the year. The investigation was carried out in the aquaculture plant “Maricoltura mar grande”, which is located in a particularly confined area of the basin with a maximum depth of 14 m and includes 15 cages. Annual trends of water quality indicators (dissolved oxygen, Chlorophyll and dissolved organic matter) and current regimes highlighted highly eutrophic conditions of the area (De Serio and Mossa, 2016; D’Ambrosio et al., 2019). In such facility an innovative IMTA system is currently under experimentation within the Life project “REMEDIATION”.

The second study site is located in the Gulf of Gaeta, a large inlet placed in the Central Tyrrhenian Sea (Central Mediterranean Sea), that extends between Punta Stendardo ($41^{\circ}12'35''N$ / $13^{\circ}35'24''E$) and the mouth of the Garigliano river within 20 m maximum depth ($41^{\circ}13'22.5''$ / $13^{\circ}45'44''E$) (Fig. 1b). The Gulf’s hydrology is influenced by currents with SE-NW direction, with the intensity of the coastal components being mainly affected by persistent E-W winds. The area is also

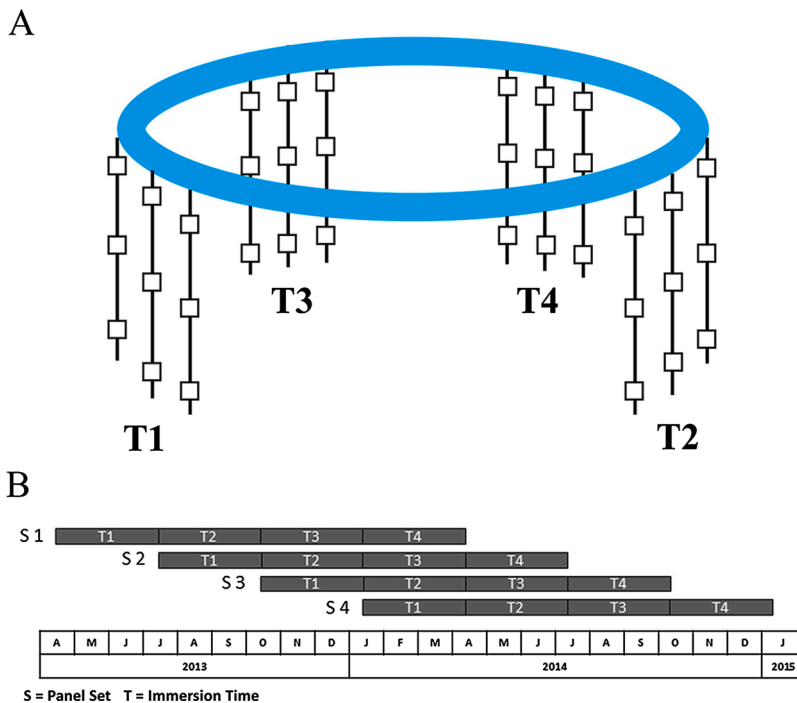


Fig. 2. Experimental units consisting in artificial panels: (A) panels location pattern anchored to the rearing fish cage; (B) temporal chart template of experimental field activities. Grey bars are the times of immersion (T1 = 3 months, T2 = 6 months, T3 = 9 months, T4 = 12 months); the series of panels immersed at different times are marked by “S” (S1, immersed in April, S2 immersed in July, S3 immersed in October, S4 immersed in January). The period of experiment is showed in the white bars with the indication of months and years.

affected by the freshwater inputs coming from the Garigliano river, which discharges terrigenous inputs into the sea, which in Autumn and Winter spread through the entire water column due to the absence of the thermocline (Ferretti et al., 1989). Nutrient inputs and dynamics in the Gulf derive both from *in situ* marine primary production and from freshwater terrigenous inputs (Careddu et al., 2015). The off-shore hydrodynamic regime return dissolved and particulate matter towards the shore and makes the coastal area eutrophic and rich of nutrients and fine sediment particles suspended. The annual average salinity is 38 PSU; the annual average temperature is 18 °C, ranging from the minimum and maximum value of 13° and 27 °C respectively. The investigate plant is a large facility containing 40 cages placed in a sheltered area with an average depth of 15 m.

In both the facilities, European sea bass *Dicentrarchus labrax* (Linnaeus, 1758) and Gilthead sea bream *Sparus aurata* Linnaeus, 1758 are reared for production.

2.2. Field sampling and experimental layout

Two cages were selected for this study: one located in the Mar Grande of Taranto site and one in the Gulf of Gaeta site, where the same experimental layout was performed. In both maricultural facilities the cages were located in similar places, which were characterized by very low current velocities and affected by high water content of organic matter. The fouling colonization was studied using 15 × 15 × 0.5 cm PVC artificial panels, as macrofouling collectors. Each panel surface was previously roughened with sandpaper (grain 60) to facilitate larval settlement and prevent the detachment of sessile organisms. In each study site, the experimental unit consisted of three panels tied to a vertical ballasted nylon rope at 0.3, 3 and 6 m depth respectively with three replicates at each depth and anchored to the floating outer edge of the cage. The cages were circular (20 m diameter) floating structures with submerged flexible net. The panels were vertically distributed at regular depth intervals in the water column subjected to the most variable light conditions and according to the cage depth. No relevant differences in current velocity emerged at the different investigated depths. The units were positioned at intervals of approximately 6 m between them along the cage edge (Fig. 2a).

The study began in April 2013 when the experimental modules were firstly immersed and the final samples were collected in April 2015. The outline of the entire procedure is showed in Fig. 2b. Four series of panels were immersed respectively in April (S1), July (S2), October (S3), and January (S4), as temporal series throughout one year, in order to investigate the seasonal variation of the fouling recruitment and settlement. For each temporal series, the experimental units were collected after four progressively longer time periods of immersion: after 3 months (T1), after 6 months (T2), after 9 months (T3) and after 12 months (T4) of immersion. This latter was the end point of the experiment. In the present paper, the fouling communities present at the end points, e.g. after one year of immersion, are considered. At each site four temporal series starting at four different periods of immersion (April, July, October, January) were analysed and compared, for a total of 72 panels throughout the study.

2.3. Data acquisition and processing

In the laboratory, each panel was photographed *in vivo* before fixation in a solution of 4% formaldehyde-seawater and subsequently preserved in 70 % alcohol. Organisms were then identified by specialists to the lowest taxonomic level as possible using stereo-microscopes. Biomass was measured by drying the species at 60 °C for 48 h, considering only the soft tissue of species.

The BV-STEP stepwise routine, a multivariate analysis based on combined biomass data from different taxonomic groups, was used to select subsets of species of the experiment end points by the highest degree of association with the entire assemblage set variability. This analysis uses Spearman rank correlation to detect the minimum number of variables (taxa) which results in the highest possible correlation with the environmental data (Clarke and Gorley, 2006).

The clearance rate was computed for the resulted subset of species and referred to the biomass measurements ($L h^{-1} g^{-1} DW$) of each species. Following the methodology already performed by other Authors, e.g. Lemmens et al. (1996) and Woods et al. (2012), the clearance rate was estimated for each species relatively to each module (3 replicates at each depth), combining literature data of species clearance rate with the dry weight values measured for each species. Only in the cases of species

Table 1

Macrofouling species collected in the fish farming at Taranto (Jonian Sea) and Gaeta (Tyrrhenian Sea). * marks indicate the non-indigenous species; in bold the species occurring at the end points.

TAXA	TARANTO	GAETA
PORIFERA		
* <i>Paraleucilla magna</i> Klautau, Monteiro & Borojevic, 2004	+	+
<i>Clathrina coriacea</i> (Montagu, 1814)	+	
CNIDARIA		
<i>Aglaophenia picardi</i> (Svoboda, 1979)	+	
<i>Aglaophenia octodonta</i> (Heller, 1869)		+
<i>Aiptasia diaphana</i> (Rapp, 1829)	+	
<i>Bougainvillia muscus</i> (Allman, 1863)	+	
<i>Clytia hemisphaerica</i> (Alder, 1862)	+	
<i>Eudendrium racemosum</i> (Cavolini, 1785)	+	+
<i>Halecium petrosum</i> (Stechow, 1919)	+	
<i>Halecium pusillum</i> (M. Sars, 1857)	+	
<i>Kirchenpaueria halecioides</i> (Alder, 1859)	+	+
<i>Obelia dichotoma</i> Linnaeus, 1758	+	+
<i>Sagartia troglodytes</i> (Price in Johnston, 1847)		+
<i>Sertularella ellisii</i> (Deshaye and Milne-Edwards, 1863)	+	
<i>Turritopsis dohrnii</i> (Weismann, 1883)	+	
<i>Plumularia setacea</i> (Linnaeus, 1758)		+
<i>Ectopleura larinx</i> (Ellis & Solander, 1786)	+	+
<i>Pennaria disticha</i> (Goldfuss, 1820)		+
MOLLUSCA		
<i>Anomia ephippium</i> Linnaeus, 1758	+	+
<i>Arca noae</i> Linnaeus, 1758	+	+
<i>Fissurella nubecula</i> (Linnaeus, 1758)	+	+
<i>Hiatella rugosa</i> (Linnaeus, 1767)	+	+
<i>Limaria hians</i> (Gmelin, 1791)	+	+
<i>Limaria tuberculata</i> (Olivi, 1792)	+	+
<i>Mimachlamys varia</i> (Linnaeus, 1758)	+	+
<i>Modiolus barbatus</i> (Linnaeus, 1758)	+	+
<i>Musculus subpictus</i> (Cantraine, 1835)	+	+
<i>Mytilaster minimus</i> (Poli, 1795)	+	+
<i>Mytilus galloprovincialis</i> Lamarck, 1819	+	+
<i>Ostrea edulis</i> Linnaeus, 1758	+	+
<i>Vermetus triquetrus</i> Bivona-Bernardi, 1832	+	+
ANNELIDA		
<i>Amphitrite rubra</i> (Risso, 1826)	+	
* <i>Branchiomma luctuosum</i> (Grube, 1870)	+	+
* <i>Branchiomma bohollense</i> (Grube, 1878)	+	+
<i>Branchiomma bombyx</i> (Dalyell, 1853)	+	
<i>Dasybranchus gajolae</i> Eisig, 1887	+	+
<i>Filograna implexa</i> Berkeley, 1835	+	+
* <i>Hydroides dianthus</i> (Verrill, 1873)	+	+
* <i>Hydroides dirampha</i> (Morch, 1863)	+	+
* <i>Hydroides elegans</i> (Haswell, 1883)	+	+
<i>Hydroides stoichadon</i> Zibrowius, 1971	+	+
<i>Hydroides helmatus</i> (Iroso, 1921)	+	+
<i>Hydroides nigra</i> Zibrowius, 1971	+	+
<i>Nicolea venustula</i> (Montagu, 1818)	+	+
<i>Parasabella langerhansi</i> (Knight-Jones, 1983)	+	+
<i>Parasabella tenuicollaris</i> (Grube, 1861)	+	+
<i>Pista cristata</i> (Muller, 1776)	+	+
<i>Sabella discifera</i> Grube, 1874	+	+
<i>Sabella pavonina</i> Savigny, 1822	+	+
<i>Sabella spallanzanii</i> (Gmelin, 1791)	+	+
<i>Serpula concharum</i> Langerhans, 1880	+	+
<i>Serpula vermicularis</i> Linnaeus, 1767	+	+
<i>Simplaria pseudomilitaris</i> (Thiriot-Quievreux, 1965)	+	+
<i>Spirobranchus triqueter</i> (Linnaeus, 1758)	+	+
<i>Spirobranchus polytrema</i> (Philippi, 1844)	+	+
<i>Spirobranchus lamarki</i> (Quatrefages, 1866)	+	+
<i>Timarete filigera</i> (Delle Chiaje, 1828)	+	+
<i>Terebella lapidaria</i> Linnaeus, 1767	+	+
<i>Vermiliopsis striaticeps</i> (Grube, 1862)	+	+
CRUSTACEA		
<i>Amphibalanus amphitrite</i> (Darwin, 1854)	+	+
* <i>Balanus trigonus</i> Darwin, 1854	+	+
<i>Chthamalus depressus</i> (Poli, 1791)	+	+

Table 1 (continued)

TAXA	TARANTO	GAETA
<i>Perforatus perforatus</i> (Bruguiere, 1789)	+	+
<i>Microdeutopus gryllotalpa</i> A. Costa, 1853		+
<i>Microdeutopus sporadhi</i> Myers, 1969		+
<i>Apocorophium acutum</i> (Chevreux, 1908)		+
<i>Elasmopus pecteniscus</i> (Bate, 1862)		+
<i>Monocorophium sextonae</i> (Crawford, 1937)		+
<i>Monocorophium acherusicum</i> (A. Costa, 1851)		+
<i>Jassa marmorata</i> Holmes, 1903		+
<i>Jassa slatteryi</i> Conlan, 1990		+
<i>Stenothoe gallensis</i> Walker, 1904		+
<i>Stenothoe tergestina</i> (Nebeski, 1880)		+
<i>Caprella dilatata</i> Krøyer, 1843		+
<i>Caprella penantis</i> Leach, 1814		+
<i>Tanais dulongii</i> (Audouin, 1826)		+
ASCIDIACEA		
<i>Aplidium coeruleum</i> Lahille, 1890	+	+
<i>Aplidium densum</i> (Giard, 1872)	+	
<i>Ascidia conchilega</i> Muller, 1776	+	+
<i>Ascidia malaca</i> Traustedt, 1883	+	+
<i>Ascidella aspersa</i> (Muller, 1776)	+	+
<i>Botrylloides leachii</i> (Savigny, 1816)	+	+
<i>Botryllus schlosseri</i> (Pallas, 1766)	+	+
<i>Ciona intestinalis</i> (Linnaeus, 1767)	+	+
<i>Clavelina lepadiformis</i> (Muller, 1776)	+	+
<i>Diplosoma listerianum</i> (Milne Edwards, 1841)	+	+
* <i>Distaplia bermudensis</i> Van Name, 1902	+	
<i>Ecteinascidia turbinata</i> Herdman, 1880	+	
<i>Lissoclinium perforatum</i> (Giard, 1872)	+	+
<i>Lissoclinium weigelei</i> Lafargue, 1968	+	+
<i>Microcosmus polymorphus</i> Heller, 1877	+	+
* <i>Microcosmus squamiger</i> Michaelsen, 1927	+	+
* <i>Perophora multiclathrata</i> (Sluiter, 1904)	+	+
<i>Phallusia ingeria</i> Traustedt, 1883	+	+
<i>Phallusia mammillata</i> (Cuvier, 1815)	+	+
* <i>Polyandrocarpa zorritensis</i> (Van Name, 1931)	+	+
* <i>Styela canopus</i> (Savigny, 1816)	+	+
* <i>Styela plicata</i> (Lesueur, 1823)	+	+
<i>Trididemnum cereum</i> (Giard, 1872)	+	+
<i>Trididemnum inarmatum</i> (Drasche, 1883)	+	+
<i>Didemnum maculosum</i> Milne-Ewards, 1842	+	+
BRYOZOA		
* <i>Amathia verticillata</i> (delle Chiaje, 1822)	+	
<i>Bugula neritina</i> (Linnaeus, 1758)	+	+
<i>Bugulina calathus</i> (Norman, 1868)	+	+
* <i>Celleporaria brunnea</i> (Hincks, 1884)	+	+
<i>Crisia denticulata</i> (Lamarck, 1816)	+	+
<i>Conopeum reticulum</i> (Linnaeus, 1767)	+	+
<i>Crisia fistulosa</i> (Heller, 1867)	+	+
<i>Crisularia aperta</i> (Hincks, 1886)	+	+
<i>Cryptosula pallasiana</i> (Moll, 1803)	+	+
<i>Savignyella lafontii</i> (Audouin, 1826)	+	+
<i>Schizobrachiella sanguinea</i> (Norman, 1868)	+	+
<i>Schizoporella errata</i> (Waters, 1878)	+	+
<i>Scrupocellaria bertholletii</i> Audouin in Savigny, 1826	+	+
<i>Scrupocellaria scruposa</i> (Linnaeus, 1758)	+	+
* <i>Watersipora subtorquata</i> (d'Orbigny, 1852)	+	+

lacking information on clearance rate, the available data referring to the closest taxonomic level as possible were considered (*i.e.* *Botryllus/Botrylloides*, *Didemnidae*, *Paraleucilla magna/Myxilla* sp., *Porifera*) (Denis et al., 1999; Frost, 1980; Gardner, 2002; Licciano et al., 2005; Riisgård et al., 2016; Simpson, 1984; Stabili et al., 2014). For both the fouling communities investigated, the relationship between total biomass and clearance rate ($L h^{-1} g^{-1} DW$) was analysed by means of Pearson correlation and regression analysis using the software StatSoft, Inc. STATISTICA (2001) Data Analysis Software System, Version 6. <http://www.statsoft.com>.

The non-parametric permutational analysis of variance, PERMANOVA was used to test for differences between fouling community clearance rates in the two sites, Gulf of Gaeta and Mar Grande of

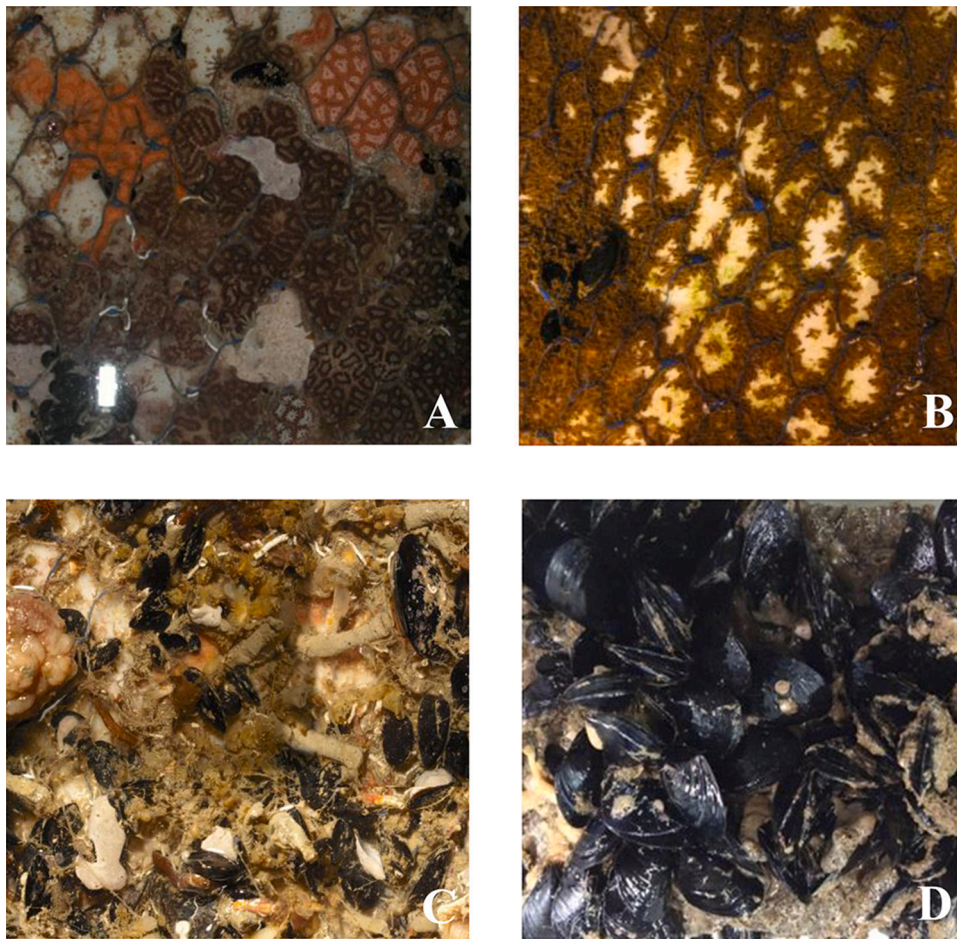


Fig. 3. Examples of panels at fouling colonization starting point (three months of immersion) from the Mar Grande of Taranto (A) and from the Gulf of Gaeta (B); examples of panels at the fouling colonization end point (twelve months of immersion) from the Mar Grande of Taranto (C) and from the Gulf of Gaeta (D). The panels are parts of the Series immersed in July and were collected at 3 m depth.

Taranto, in two geographical areas (Ar, 2 levels), at four immersion periods (S, 4 levels) and at three depths (D, 3 levels) as factors. Bray–Curtis similarity matrix under 9999 permutations was used to perform the analyses.

The Canonical Analysis of Principal Coordinates (CAP) (Anderson and Willis, 2003) was carried out in order to investigate (dis) similarity in clearance rates of the end point fouling communities, concerning differences between sites, Gulf of Gaeta and Mar Grande of Taranto, and depths (0.3 m, 3 m, 6 m). The species were added as vectors overlaying on the CAP plot to detect species which best characterize the sample-points ordination in the plot. The statistical analysis was performed with PRIMERv6 software.

3. Results

3.1. End point fouling comparison

In both study areas all the panels appeared to be largely dominated by filter feeder macroinvertebrates, while macroalgae were not considerably detected, even in the starting phases of the colonization (T1 of all the temporal series). Throughout the period of investigation, a total of 117 taxa were recorded, 93 of which in the Mar Grande of Taranto and 75 in the Gulf of Gaeta (Table 1). At the end points, i.e. the final succession stage after one year of immersion, the highest diversity of the fouling community was observed in the site of the Mar Grande of Taranto with 71 taxa, whilst 57 taxa were found in the site of the Gulf of Gaeta (Table 1). A partial communality of species between the

communities of the two study sites resulted, according to the similarity degree revealed by the Sørensen index value of 0.62. A considerable number of allochthonous species occurred on the panels in the Mar Grande of Taranto (16 species), representing 17 % of the assemblage; among them the most abundant were *Branchiomma boholense*, *B. luctuosum*, *Hydroides elegans*, *H. dirampha*, *Paraleucilla magna*, *Polyandrocarpa zorritensis* and *Celleporaria brunnea*. By contrast, on the panels in the Gulf of Gaeta, a low number of non-indigenous species was recorded, i.d. *H. elegans*, *H. dirampha*, *Balanus trigonus*, *Elasmopus pectenicrus*, *Styela plicata* and *P. magna*.

Regarding the temporal development of the fouling communities, the first succession times (T1) diverged between the two sites: the panels of the Mar Grande of Taranto were largely colonized by colonial ascidians in all the temporal series (Fig. 3A), contrary to the panels of the Gulf of Gaeta which were extensively covered by hydroids colonies and muddy-tubes built and inhabited by amphipods (Fig. 3B). Particularly, the main differences were observed between the two studied sites at the end point of the experiment (T4, one year of immersion). In the Mar Grande of Taranto diverse assemblages occurred, with various large species as dominant according to the four temporal series of immersion and different depth. In fact, only the panels immersed at 0.3 m depth were largely colonized by *M. galloprovincialis*, whereas the sabellid polychaetes were dominant on the deeper panels (Fig. 3C). The fouling succession pattern occurred on the panels in the site of Mar Grande of Taranto was previously described in detail (Lezzi et al., 2017; Lezzi and Giangrande, 2018). Here we report the species, selected by means of the BV-STEP routine, which showed the best correlation with the end-points

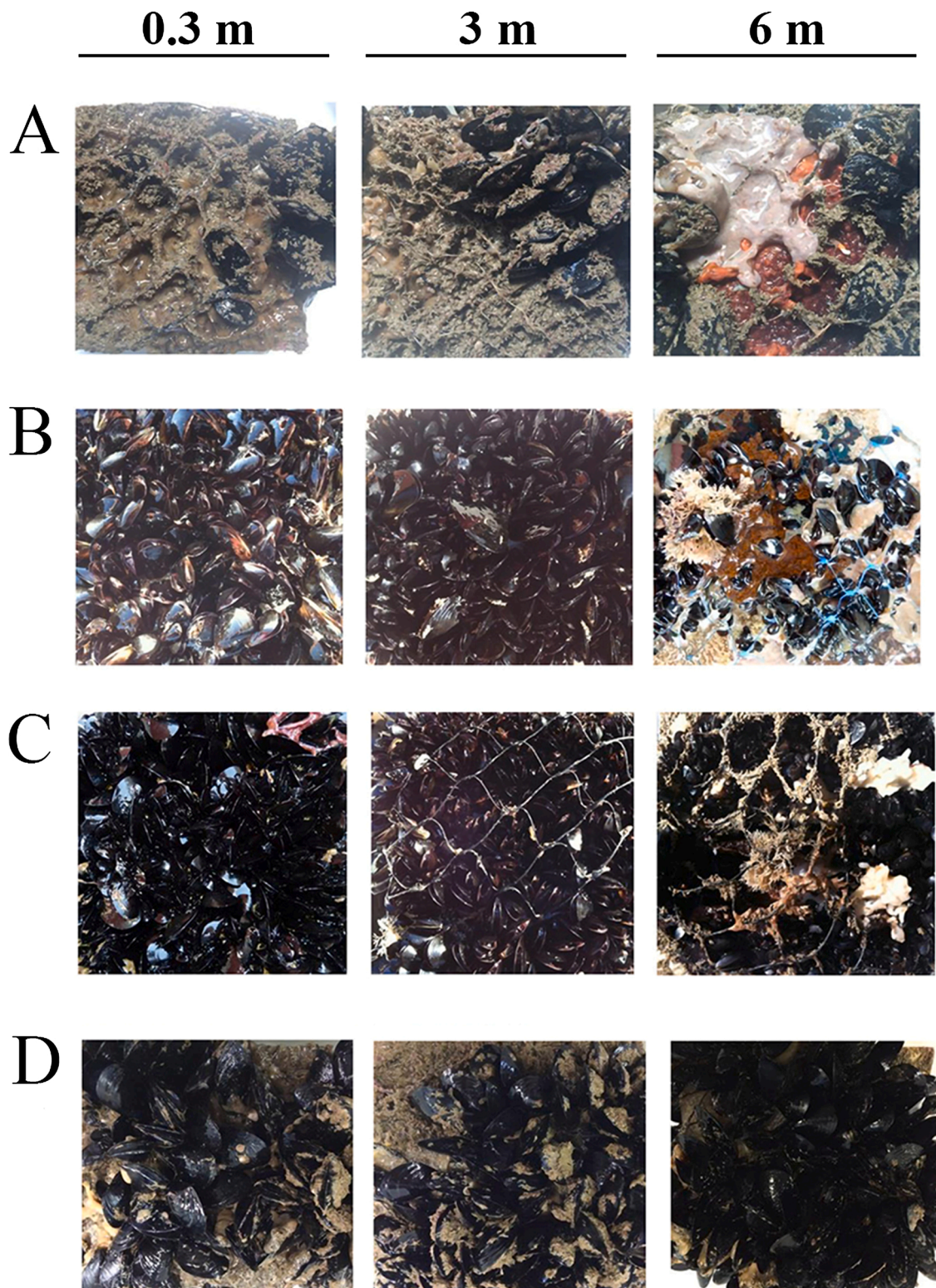


Fig. 4. Examples of panels collected in the Gulf of Gaeta at the end points of fouling colonization (twelve months on immersion). A, panels immersed in April; B, panels immersed in July; C, panels immersed in October; D, panels immersed in January. Depth (0.3 m, 3 m, 6 m) of the panels is showed at the top.

of the entire community: they are *M. galloprovincialis*, *Nicolea venustula*, *Limaria hians*, *Crisularia aperta*, *Branchiomma boholense*, *Mimachlamys varia*, *Sabella spallanzanii*, *Branchiomma luctuosum*, *Paraleucilla magna*, *Anomia ephippium*, *Phallusia mammillata*, *Myxilla* sp., *Trididemnum inar-matum*, *Schizoporella errata*, *Clavelina lepadiformis*, *Botrylloides leachii*, *Exaiptasia diaphana*.

By contrast, in the Gulf of Gaeta the final stages (T4, one year of immersion) of the fouling community were marked by the dominance of *M. galloprovincialis* at all the depths (Fig. 3D), with little differences in abundance according to the time series of immersion. Unfortunately, detailed results of the fouling colonization at the site of the Gulf of Gaeta have not been published elsewhere, so we consider appropriate to report

Table 2
Specific clearance rate values ($L\ h^{-1}\ g^{-1}DW$) relative to the taxa found in the macrofouling community at Gaeta and Taranto.

Taxon	Mean clearance rate ($L\ h^{-1}\ g^{-1}DW^{-1}$)	Particle size (μm)	References
Chordata			
Ascidacea (<i>Botrylloides</i> sp.; <i>Clavelina lepadiformis</i> ; <i>Phallusia mammillata</i> ; <i>Trididemnum inarmatum</i>)	2.5 ± 3.0	1–500	Carlisle, 1966; Comeau et al., 2015; Fiala-Medioni, 1973, 1978; Hily, 1991; Petersen et al., 2002; Randlov and Riisgård, 1979; Whalen et al., 2017
Bryozoa (<i>Schizoporella errata</i> ; <i>Crisularia aperta</i>)	1.6 ± 0.5	1–30	Bullivant, 1968; Lisbjerg and Petersen, 2000.
Mollusca			
<i>Mytilus galloprovincialis</i>	2.8 ± 2.1	2–63	Denis et al., 1999; Gardner, 2002
Other bivalves (<i>Anomia ephippium</i> ; <i>Limaria hians</i> ; <i>Mimachlamys varia</i>)	1.9 ± 0.7	5–11	Hily, 1991; Petersen et al., 2003.
Polychaeta			
<i>Branchiommia</i> spp.	43.2 ± 2.6	0.45	Licciano et al., 2005.
<i>Sabella spallanzanii</i>	12.4 ± 2.2	0.45	Licciano et al., 2005.
Porifera spp.	0.6 ± 0.7	1–50	Duckworth et al., 2003; Riisgård et al., 1993; Stabili et al., 2006; Turon et al., 1997

here the characteristic aspects of the colonization pattern of this latter site, in order to extensively show the differences between the fouling colonization of the two sites.

In the Gulf of Gaeta at the temporal series starting in April (S1) many species of amphipods, such as *Jassa slatteryi*, *J. marmorata*, *Stenothoe tergestina*, *S. gallensis*, *Microdeutopus sporadhi*, occurred, constructing their muddy-tubes inside the cavities and interstices among the mussel valves, which largely covered the panel surface at all the depths; other amphipods like *Caprella dilatata* and *C. penantis* were found in association with hydroids, bryozoans and sponges, especially covering the surface of the deep panels (Fig. 4A). At the temporal series starting in July (S2) large mussels extensively covered all the panels, together with colonies of sponges and encrusting bryozoans particularly at 6 m depth (Fig. 4B). The largest mussels occurred at the temporal series starting in October (S3) and their valves were quite clean, except for spotted epibionts, such as the sponge *Paraleucilla magna* especially recorded at 6 m depth (Fig. 4C). Finally, at the series starting in January (S4) the mussels were dominant at all the depths with their valves covered by dense assemblages of amphipod tubes, ascidian colonies, barnacles and the sponge *P. magna* (Fig. 4D). The species selected by the BV-STEP routine were: *M. galloprovincialis*, *C. aperta*, *S. spallanzanii*, *P. magna*, *T. inarmatum*, *S. errata*, *B. leachii*, *E. diaphana*.

3.2. Functional analysis

The clearance rate data of the species recorded at the end point of the fouling communities in the two study sites are reported in Table 2, with the corresponding references from the available literature. The clearance rate was computed for each species selected by the BV-STEP analysis, considering their respective biomass recorded on the panels at each temporal series (S1-S4) in the two study sites. The clearance rate was higher in the Mar Grande of Taranto, particularly at 6 m depth, where the highest diversity of fouling was also detected (Fig. 5A). In this site, the contribution of *M. galloprovincialis* to the total clearance rate was only considerable at every depth in the temporal series starting in April (S1). By contrast, in the other temporal series (S2-S4) the contribution of mussels to the total clearance was remarkable only in the panels at 0.3 m depth, in contrast with other species, particularly sabellids, which showed their highest contribution in the deep panels, especially in the S2 and S4 series. Different results characterized the fouling panels of the Gulf of Gaeta site, where *M. galloprovincialis* mostly contributed to the total clearance rate at all the temporal series and at all depths (Fig. 5B). The results of the correlation analysis between total biomass and clearance rate were significant for the two fouling communities, with $r = 0.98$ ($p < 0.001$) and $r = 0.44$ ($p = 0.007$) respectively for the Gulf of Gaeta and the Mar Grande of Taranto, and the

regression lines ($R^2 = 0.99$ and $R^2 = 0.2$ respectively) are plotted in Fig. 6. The highest correlation was exhibited in the Gulf of Gaeta, where the highest contribution of mussels to the total biomass and the resulting fouling clearance were reported. In the Mar Grande of Taranto the lower correlation matched with the contribution of a higher number of filtering species to the total biomass.

These results were confirmed by the Canonical Analysis of Principal Coordinates (CAP) carried out on the clearance rate of the species recorded at the end points of the fouling community in the two sites (Fig. 7): in the model, the sample-points of 0.3 m depth of the Mar Grande of Taranto are close to those of the Gulf of Gaeta, both being characterized by the dominance of the mussels. On the contrary, the other sample-points of the Mar Grande of Taranto are placed separately because of the differences in terms of fouling composition and functioning. The vectors in the plot correspond to the species more dominant on the sample-points ordination: *M. galloprovincialis* clearly marks the sample-points of the Gulf of Gaeta and those of the Mar Grande of Taranto at 0.3 m depth; other different taxa, e.g. *Branchiommia* spp., *Sabella spallanzanii*, Bryozoa, mostly characterize the sample-points of the Mar Grande of Taranto at 6 m and 9 m depth. The described pattern is also supported by the results of the PERMANOVA, that show significant differences in the clearance rate between the fouling communities of the Mar Grande of Taranto and the Gulf of Gaeta, relatively to different immersion periods ($p = 0.001$) and depths ($p = 0.001$) (Table 3).

4. Discussion

In the context of the fouling colonization problem in aquaculture facilities, our study suggests several novelties. Firstly, the investigation has been planned to cover a one-year period and the settlement seasonal variations of fouling on artificial substrates have been studied until an end point of community development was reached. Long-term data concerning the colonization of artificial structures placed close to the cages are very scant (Bloecher et al., 2013; Cook et al., 2006; Lezzi et al., 2017; Manganò et al., 2019; Martell et al., 2017; Pica et al., 2019). Indeed, most of the previous studies have been carried out on fouling communities directly settled on fish nets where, due to the periodic mechanical cleaning, only the early stages of the fouling colonization were possible to investigate (see e.g. Baxter et al., 2012; Bosch-Belmar et al., 2017; Braithwaite and McEvoy, 2005; Braithwaite et al., 2007; Edwards et al., 2015; Fernandez-Gonzalez and Sanchez-Jerez, 2017; Fernandez-Gonzalez et al., 2016, 2018; Fitridge et al., 2012; Lacoste and Gaertner-Mazouni, 2015; Sarà et al., 2007; Sievers et al., 2013; Sliskovic et al., 2011;). Secondly, the use of the same planned experimental design allowed us to analyse and compare the fouling colonization patterns in

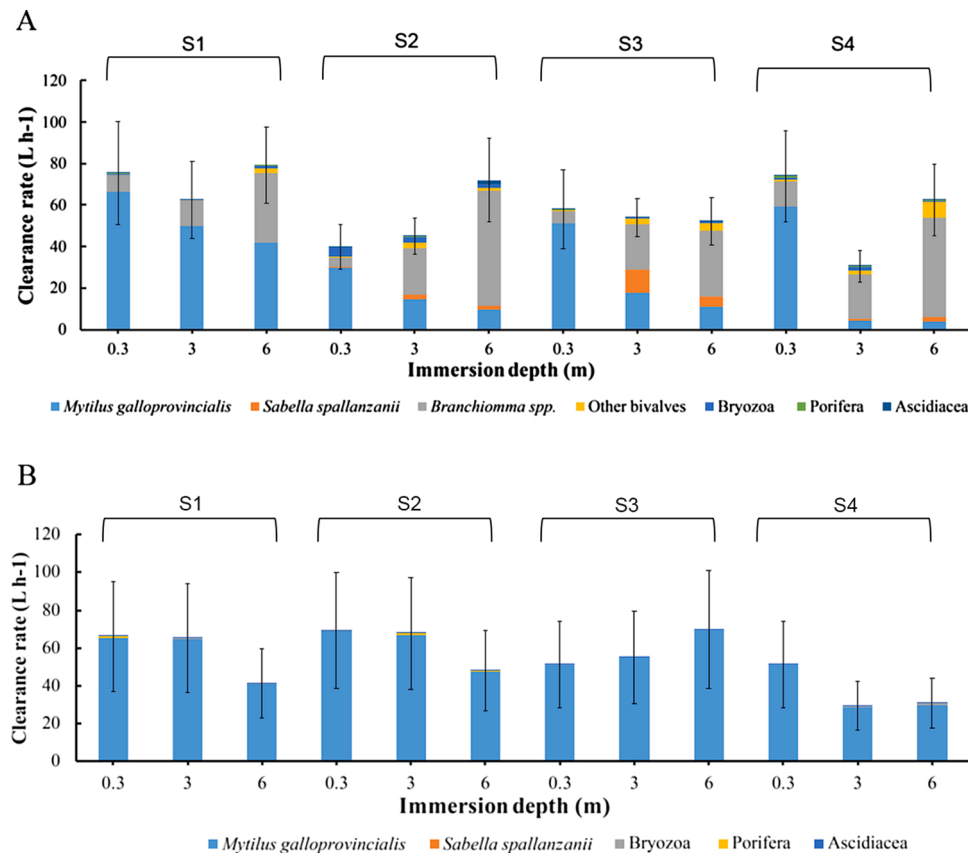


Fig. 5. Clearance rate computed for the fouling species found on the panels at the end points (twelve months of immersion) of the four temporal series: S1 (immersed in April), S2 (immersed in July), S3 (immersed in October), S4 (immersed in January) in the two study sites (A) Mar Grande of Taranto (B) Gulf of Gaeta.

two Mediterranean areas along Italian coast.

On the one hand, macrobenthic communities settled on the experimental substrates in the Mar Grande of Taranto and in the Gulf of Gaeta exhibited a similarity in species composition, with a pool of species typical of Mediterranean fouling assemblages (Relini and Faimali, 2003). On the other hand, the communities of the two sites showed some differences: in the site of the Mar Grande of Taranto the community was characterized by the highest species richness and diversity, this fact being mainly supported by a higher number of alien species and mainly confirming the area as a hotspot of alien biodiversity (Cecere et al., 2016; Lezzi et al., 2017; Lezzi and Giangrande, 2018).

Other similarities and differences emerged from the comparison between the fouling succession patterns: in the two areas the end points of the communities were both dominated by large filter feeders macroinvertebrates, but with different species compositions. The abundance of filter feeders is explained by the location of the two aquaculture facilities in semi-enclosed and sheltered areas, which are characterized by reduced hydrodynamic forces and high levels of organic particles, mostly derived from uneaten food and faeces coming from the fish rearing cages. Indeed, filter feeders macroinvertebrate are commonly found to be dominant in aquaculture fouling communities (Fernandez-Gonzalez and Sanchez-Jerez, 2017; Fitridge et al., 2012; Lezzi et al., 2017; Pica et al., 2019) and in our study a high abundance of *M. galloprovincialis* was observed in both sites, with special incidence in the Gulf of Gaeta. This result is in agreement with the observations of other fouling communities climax stages (Hughes et al., 2005), where mussels were dominant along the Mediterranean (Ardizzone et al., 1989; Pierrri et al., 2010) and the Atlantic coast (Bloecher et al., 2013; Cook et al., 2006; Greene and Grizzle, 2007; Hodson et al., 2000; Khalaman, 2001). The abundance of *M. galloprovincialis* recorded in the Gulf of Gaeta, and supported by the large amount of juveniles in spring and of

adults in autumn and winter, is in agreement with the life cycle and reproduction time of this species along the Italian coast. Here, *M. galloprovincialis* spawns twice a year, in winter and autumn, with most of the recruitment occurring in spring and late autumn (Ceccherelli and Rossi, 1984; Gravina et al., 2018; Tursi et al., 1990). The abundance of *M. galloprovincialis* found on our experimental panels in the two sites was also affected by the proximity of mussel breeding rearing plants, which acted as source of propagule. However, notwithstanding the rearing plants are present in both areas, the end points of the fouling succession were different. In the Gulf of Gaeta the mussels monopolized the experimental substrata at all the depths and at all the temporal series end points, whereas in the Mar Grande of Taranto a “multi-climax stage” with different succession end points was observed (Lezzi et al., 2017; Lezzi and Giangrande, 2018). Indeed, in such site the mussels dominated the end points at all the depths only in the temporal series starting in April (S1), whereas the end points of the other temporal series were characterized by an increase in the number of species and in the assemblage diversity. These latter end points were mainly composed by the polychaete sabellids *B. bohollense*, *B. luctuosum*, *S. spallanzanii*, the ascidians *C. lepadiformis*, *B. leachii*, the bryozoans *S. errata*, the bivalves *A. ephippium*, *M. varia* and the sponge *P. magna* at the S2 series and *Branchiometta* spp., *A. ephippium*, *L. hians*, *S. spallanzanii* at the S3 and S4 series.

The different end point assemblages observed in the two study sites were reached through different succession patterns. The typical environmental constraints of the two geographical areas drove the entire course of the fouling development during the year conditioning the succession process from the early stages of colonization (3 months) to the resulting end points. In the Gulf of Gaeta, the allochthonous high terrigenous freshwater inputs strongly affected the sedimentation rate that, in turn, conditioned the fouling settlement. In particular, this fact

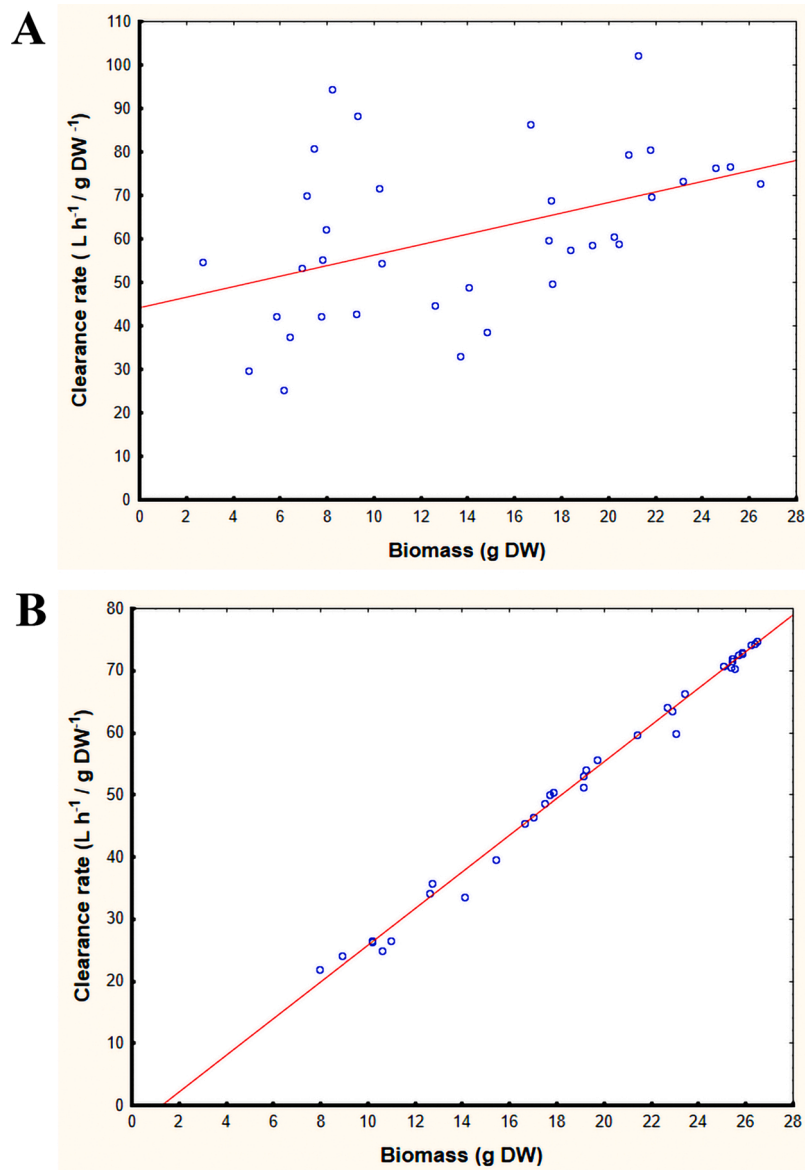


Fig. 6. Regression lines between biomass and clearance rate relative to the site of the Mar Grande of Taranto (A), with $r = 0.44$, $p = 0.007$, and the site of the Gulf of Gaeta, with $r = 0.98$, $p < 0.001$ (B).

resulted in the extensive colonization by hydroids as pioneer organisms, which settled on the abundant shells of *A. ephippium*; so they facilitated the settlement of the mussel pediveligers and this fact entailed the monopolization by *M. galloprovincialis* of all available space. Moreover, the large quantity of sedimentary particles in the water column also facilitated the tubicolous amphipods settlement. The resulting increase of amphipods abundance on the panels was evident until after one year of immersion in all the temporal series end points, but particularly in Spring and Winter. By contrast, in the area of the Mar Grande of Taranto, that is not directly affected by allochthonous terrigenous inputs, the colonial ascidians dominated the fouling pioneer stages (Lezzi et al., 2017). The relationship between differences in invertebrate species abundance and different trophic levels of the waters was already highlighted by Pierri et al. (2010) for the fouling development in the Mar Piccolo of Taranto, where *M. galloprovincialis*, sabellids and ascidians respectively dominated in environmental conditions of different organic load levels. In particular, ascidians were the dominant component in the most eutrophic site, as well as they were very abundant at the fouling succession end point in another aquaculture plan located along the Southern Adriatic coast (Apulia) (Pica et al., 2019). In this latter study

case, *M. galloprovincialis* and sabellids resulted with a lower contribute to the fouling assemblages than those observed in the present study. Our idea on this regard is that in the sheltered coastal areas, where aquaculture facilities are harboured, the fouling colonization dynamics turned up different end points, which are marked by different dominant filter feeder invertebrates.

In comparison with other geographical areas, our results are in line with other published studies, which reported a variation on the biofouling species composition associated to aquaculture facilities (Bloecher et al., 2013; Cook et al., 2006; Hodson and Burke, 1994; Hughes et al., 2005; Madin et al., 2009; Salama et al., 2018). Particularly in different Mediterranean areas, different dominant taxa were recorded, such as macroalgae in North Adriatic Sea (Sliskovic et al., 2011), hydroids, mussels and amphipods along the Spanish coast (Fernandez-Gonzalez and Sanchez-Jerez, 2014; Fernandez-Gonzalez et al., 2016; Fernandez-Gonzalez and Sanchez-Jerez, 2017; Fernandez-Gonzalez et al., 2016, 2018), ascidians along Southern Adriatic coast (Pica et al., 2019). As regards macroalgae, it is worth to note that they didn't represent an important part of the biofouling community in our study and this is possibly due both to the nature of the experimental

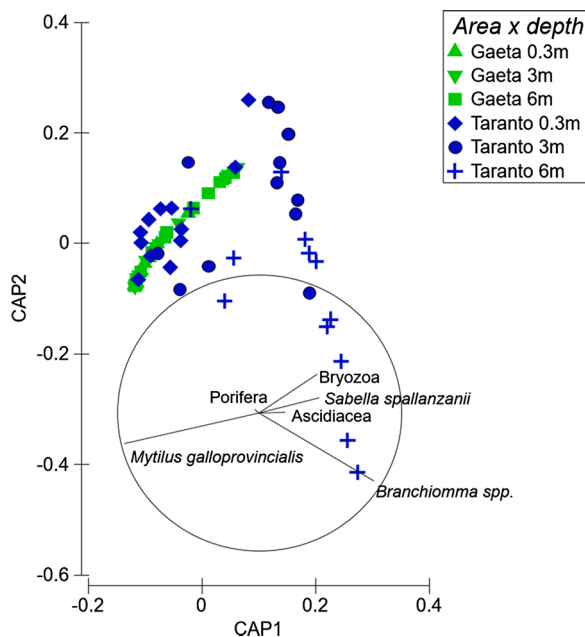


Fig. 7. Ordination model resulted from the Canonical Analysis of Principal Coordinates carried out on clearance data of each species/taxon found at the fouling end points in the sites of Mar Grande of Taranto (Taranto) and Gulf of Gaeta (Gaeta). Vectors correspond to the species/taxa mostly characterizing the sample-points ordination.

Table 3

Results from multivariate (PERMANOVA), based on Bray-Curtis similarity matrix to test differences for Areas (Ar), Immersion period (S) and Depth (D) on the clearance rate efficiency of the studied macrofouling communities. Df: degrees of freedom, MS: mean square, Pseudo-F: F-distribution, P: levels of significance.

(A)	Source of variation	df	MS	Pseudo-F	P(perm)
	Ar	1	15595.0	71.2	0.001
	S	3	2338.4	10.7	0.001
	D	2	5104.2	23.3	0.001
	Ar*S	3	1509.2	6.9	0.001
	Ar*D	2	2615.5	11.9	0.001
	S*D	6	656.2	3.0	0.006
	Ar*S*D	6	420.2	1.9	0.037
	Residual	48	219.0		
	Total	71			

substratum (Guarnieri et al., 2009) and to the high turbidity level of the water, as recorded in other our studies (Ardizzone et al., 1989). Our results are also consistent with data obtained in other studies regarding the fouling settlement on collectors placed around the cages in other European coastal areas: for example hydroids were proved to enhance the settlement of *Mytilus edulis* in the Northern Sea (Bloecher et al., 2013) and the sabellid *Sabella pavonina* was a notable component of the fouling along the Southern UK coast (Cook et al., 2006).

In both the investigated sites, notwithstanding the observed differences in terms of species composition, the fouling communities were dominated by large macroinvertebrate filter-feeders, which acted as ecosystem engineers. They intercepted the organic matter coming from the aquaculture activity, preventing its accumulation on the sea floor and so notably altering the habitat features. Moreover, in the Mar Grande of Taranto the highest species diversity of the fouling end points is possibly related to the diversity in filtration capability: in fact a high number of species can elaborate a wider range of suspended particles, in terms of nature and sizes (Whalen and Stachowicz, 2017). Our results highlighted that the higher clearance rate recorded at the site of Mar Grande of Taranto is mainly due to the contribution of sabellids, whose

specific filtration rates are even higher than that of *M. galloprovincialis*.

Finally, the dominance of sabellids in the Mar Grande of Taranto did not negatively impact the mussel rearing, because the bivalves are cultivated at the surface level in floating systems, whilst the largest amount of other biofilters, such as sabellids and ascidians, grew in suitable conditions below the molluscs, where they were able to intercept bacteria, particulate organic matter and pseudofaeces (Giangrande et al., 2014).

5. Conclusions

The knowledge of the macrofouling assemblages development is a prerequisite for pinpointing the best strategy for species selection, to be possibly exploited in a IMTA system. It is also worth to take in consideration that the negative impact of the macrofouling can be driven positively towards bioremediation activity and production of useful biomass as a by-product. In fact, we are currently experimenting the possibility of rearing various species of filter feeders, such as sabellids, within an innovative IMTA system in the Gulf of Taranto. This includes organisms, as sponges, sabellids and macroalgae, chosen during several years of studies concerning their physiology, life cycle and filtering efficiency (Giangrande et al., 2014; Licciano et al., 2005; Longo et al., 2016). The employment of the sabellids in the new proposed IMTA would give an added value to the plant, not only because of their high bioremediation capability (Licciano et al., 2005), but also because their biomass could be used for different purposes (Giangrande et al., 2014; Stabili et al., 2019). Finally, the fouling colonization pattern observed in the Gulf of Gaeta, characterized by the monopolization by the mussels, suggests that only such filter feeder may be efficiently reared close to the fish-cages in such IMTA system. However, even in this area, the very abundant muddy-tube builder amphipods, which settled among the mussel valves, may have an active role in removing the suspended sediment and the organic particles from water and so may be efficient contributors to bioremediate the seawater.

CRedit authorship contribution statement

A. Giangrande: Conceptualization, Experimental design planning, Data curation, Investigation, Methodology, Taxonomical and Formal analysis, Writing - original draft, Writing - review & editing, Supervision, Funding acquisition. **M. Lezzi:** Data curation, Investigation, Taxonomical and formal analysis, Writing - review & editing. **M. Del Pasqua:** Field methodology, Data curation and Formal analysis, Writing - review & editing. **C. Pierri:** Field methodology, Writing - review & editing. **C. Longo:** Investigation, Taxonomical analysis, Writing - review & editing. **M.F. Gravina:** Conceptualization, Data curation, Investigation, Methodology, Taxonomical and Formal analysis, Writing - original draft, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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