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THE PROBLEM OF BIOFOULING FOR HUMAN ACTIVITIES: INSIGHTS FROM ITALIAN MARINAS AND AN ADRIATIC FINFISH FARM

PhD candidate

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A Daniele,

con tutto il mio amore…

ABSTRACT

Marine biofouling is defined as the community of organisms encrusting the submerged hard substrates and whose composition is influenced by several environmental factors. Only a few information is available about spatial and temporal changes of biofouling within Italian marinas and biofouling communities associated with Italian aquaculture farm activities. Moreover, there is also a lack of knowledge about the boat owners' perception of biofouling problem.

In order to fill this gap, the PhD thesis aims to study the spatial and temporal changes of fouling community in a recreational harbor in the north Adriatic Sea and in a finfish farm in the south Adriatic Sea. Moreover, a social investigation through an online survey is presented, with the purpose to investigate the boaters' perception of biofouling as a challenge, quantifying the expenses related to biofouling management, assessing their awareness of negative impacts of antifouling paints on the marine environment and their knowledge of silicone coatings.

Results shows that the community composition of biofouling in the recreational harbor is influenced by temporal and spatial variability, in terms of number of species, species composition, cover percentage and biomass. Similarly, the composition and biomass of biofouling community present in the finfish farm is strongly influenced by time of immersion, season and water column depth.

Finally, the results of the survey suggest that biofouling is considered a problem for vessels and navigation, and although antifouling paints with biocides are considered a problem for the environment by most the participants, the survey indicates a lack of information on more environmental friendly alternatives such as silicone coatings.

Overall, these results provide new insights on biofouling community dynamics and new data useful for the sustainable management of biofouling in Italian recreational navigation and aquaculture farm.

The PhD thesis is structured as follow:

- **Introduction.** It is an overview about biofouling and problems related to human activities, especially recreational navigation and finfish aquaculture. Moreover, the aims of the work are listed and briefly described.

The other chapters are presented in the form of scientific papers.

- **Chapter 1.** It deals with the spatial and temporal changes of biofouling community in the recreational harbor of Ancona (Italy).
- **Chapter 2.** It reports the outputs of an on-line survey submitted to Italian boaters to know their perception of the biofouling problem, quantify the expenses they must face to manage it and to assess their awareness about antifouling paints and foul-release coatings as eco-friendly alternative.
- **Chapter 3.** It reports the results of an investigation about spatial and temporal changes of biofouling community in an aquaculture facility located in the south Adriatic Sea in Mattinata (Foggia, Italy).
- **Conclusions and future works.** The conclusions of the work are here summarized, together to future works.

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Again…Grazie! Alessandra

Sommario

1. INTRODUCTION

1.1 BIOFOULING IN THE MARINE ENVIRONMENT

Marine biofouling is defined as an association of marine organisms settled on hard artificial substrate, or any natural substrate artificially submerged, irrespective of the place, time and duration of immersion; this kind of association cannot be defined, from a biocenotic point of view, as a distinct and univocal entity, because it varies according to the different environmental conditions (Relini & Faimali 2003).

The term "fouling" means "dirt" or "filth" and it has a negative connotation, due to the damages and alteration that occur on the colonised substrate (Figure 1).

Biofouling can be divided in *microfouling* and *macrofouling* according to size of the organisms, despite the limit between the two categories is sometimes different (Relini & Faimali 2003).

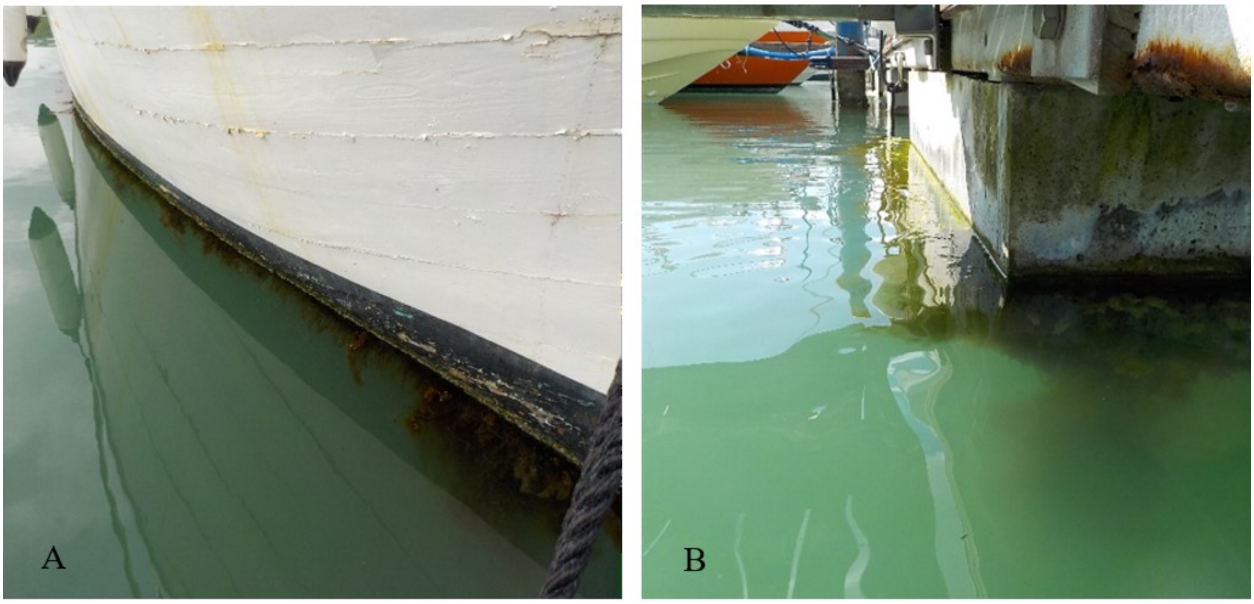


Figure 1 Fouling encrusting the hull of the ship (A) and pontoons (B) inside the recreational harbour of Marina Dorica (Ancona)

There are more than 4000 marine biofouling species (Arai 2009; Bai et al. 2013; Buskens et al. 2013), including both hard e soft organisms. All artificial substrates submerged in the sea are immediately covered by a layer of biofilm, which is the base and the first step of the biological colonisation (Relini et al. 1974): it represents the condition for the following colonisation by macro-organisms. The term macrofouling refers to the community composed by macro-organisms and the antifouling methods aim to prevent their settlement.

Persoone (1971), studying the ecology of biofouling community encrusting artificial structures submerged in a polluted harbour, identified three different stages during the colonization process:

1) Primary film: it's mainly composed by bacteria and detritus developed during the first days of immersion of the substrate;

2) Primary growth: after more or less fifteen days, where the community composition is strongly influenced by temperature and season. Depending on season, it's composed by protozoan and diatoms during winter, while in spring and summer by peritrich ciliates followed by cirripeds, tubeworms, copepods and nematods;

3) Secondary growth: after one month of immersion, the organisms of macrofouling dominate the community (mainly cirripeds, polychaetes and bivalves).

Many biofouling species naturally settle in different zones and have a different pattern of substrate colonisation (Prendergast 2010) (Figure 2). Three hypotheses were firstly proposed to explain this mechanism of selection: 1) larvae settle randomly and they can attach to the substrate when they find the right conditions; 2) species that have adult motile stage choose the suitable substrate only after a first step of random settlement; 3) when larvae are ready to settle they congregate in a specific and suitable depth in the water column. But soon it was realized that the larvae of many species can actively choice the substrate, and settle only when they find the perfect combination of biotic and abiotic factors, such as surface topography, water flow and chemical properties (Prendergast 2010). In addition, other factors have proven to be important, such as the signals from the conspecific adults, the prey organisms and the type of substrate (Nobuhiro 2004).

For this reason, to prevent the species settlement, seasonal and site-specific variations in marine biofouling need scientific attention (Vedaprakash et al. 2013) and it is important to identify the factors that influence and structure the community (Pierri et al. 2010). Moreover, it's crucial to investigate the ecological needs of foulers, their temporal and spatial distribution and when their spawning events occur (Prendergast 2010).

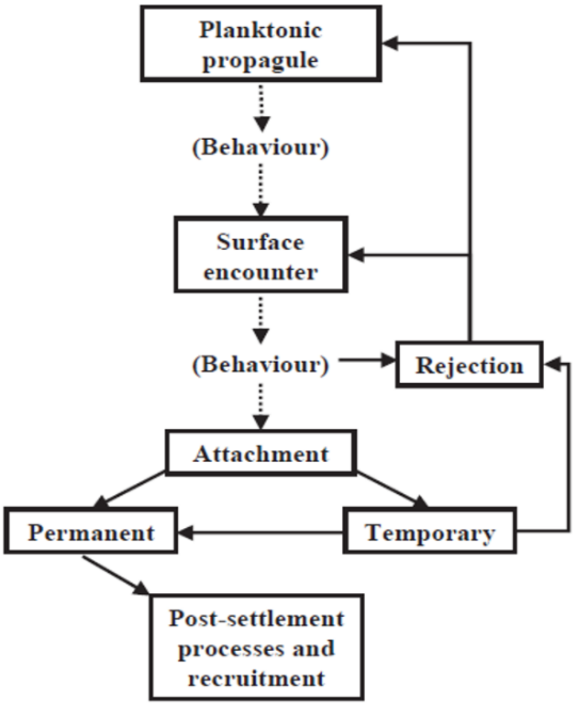


Figure 2 The process of settlement (from Prendergast, 2010)

1.2 MAIN FACTORS INFLUENCING THE PATTERN OF BIOFOULING COMMUNITIES

The severity of biofouling incrustation is related to several factors and many environmental parameters, all together driving the process of colonization of the substrate. Most important environmental factors involved in that biological process were summarized by Cowie (2010) and include light, temperature, pressure, food supply and water current.

The light is the main parameter that influence the fouling community structure. In the photic zone, algae represent the main fouling group, because there is high availability of light for photosynthesis. The amount of light decreases with depth: at a lowest light level coralline algae represent the seaweed growth form (Cowie 2010) and in deep water photosynthetic species are absent in the fouling community. Since long time it was noticed that usually the shaded undersides of surfaces often have more rich growth than vertical or unshaded surfaces (Visscher 1927; Coe & Allen 1937; Glasby & Connell 2001).

The temperature follows the same trend of the light, decreasing with the increasing of depth, while for the pressure it is exactly the opposite. In general, fouling process is more severe in temperate zone (Cao et al. 2011). Temperature appears to be the principal factor limiting the geographical distribution of marine animals, and determining their reproductive periods (Woods Hole Oceanographic Institution 1952).

Water current is an important factor for two main reasons: 1) it can prevent or interfere with the process of adhesion to the substrate and 2) it drives planktonic larval dispersion (Cowie 2010). Moreover, biofouling is known to be more severe in shallow waters along the coast and in harbour basins, where a high supply of nutrients is available (Cao et al. 2011).

Many other factors can influence and have an effect on the composition of biofouling community on artificial substrates, such as size (Keough 1984), colour (James & Underwood 1994), orientation (Glasby 2001; Glasby and Connell 2001) and movement (Glasby 2001), surface material and texture of the substrates (James & Underwood 1994), together with the various interactions between all these components (Marraffini et al. 2017).

1.3 BIOFOULING AND BIOLOGICAL INVASIONS: THE MEDITERRANEAN CASE

Many efforts of the scientific community are currently focused to prevent the spreading of Non Indigenous Species (NIS). This is considered the most important ecological issue of our century (Savini et al. 2006), and the problem is mentioned by the Marine Strategy Framework Directive of the European Union (European Commission 2008), as it’s one of the major problems for EU policy (Zanetos et al. 2012).

Anthropogenic activities, such as shipping, recreational boating, shellfish culture and live seafood trade, are well known to be one of the main vectors for the spreading of alien species (Marchini et al. 2015).

According to Boero (2002), a biological invader must present the following characteristics:

1) the possibility to be transported on ship for a long distance;
2) the capacity to pass through the bottleneck and to be a “lucky” founder;
3) pre-adaptive capacity;
4) the capacity to be a strong competitor;
5) high reproductive rate.

The Mediterranean Sea is an important model to study the phenomenon of bioinvasions.

Recently Marchini et al. (2015) updated the number NIS of the lagoon of Venice. Since long time it's known that this basin represents a very important hotspot for marine NIS introduction due to the presence of numerous recreational and commercial harbours, as well as aquaculture facilities (Occhipinti-Ambrogi et al. 2011) (Figure 4).

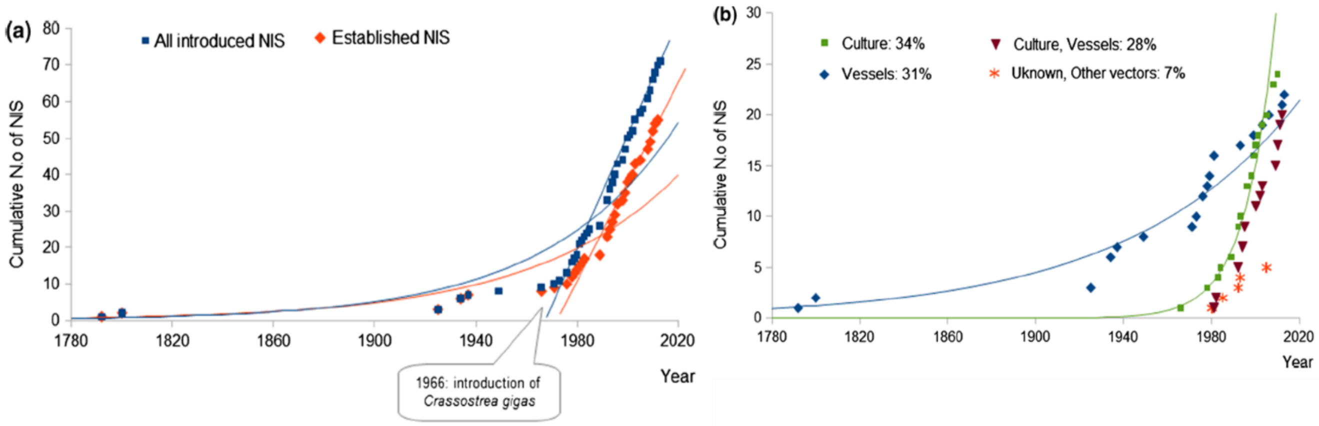


Figure 4 Graphs showing NIS accumulation and their vectors in the Venice Lagoon (from Marchini et al. 2015)

All the marine infrastructures are potential substrates for the settlement of invasive species, that are known to compete for space with autochthonous organisms. As suggested by Dafforn (2017), there are some simple procedures to decrease the possible colonisation of the substrates by NIS: 1) manipulating the substrates in order to favour the settlement of native species over NIS (for example, natural substrates favour native recruitment); 2) facilitate the spreading and the access to structures to native grazers and predators; 3) consider the timing of cleaning/construction operations, in order to avoid the period in which the fouling pressure is at maximum level.

Moreover, world is facing the fastest rise in temperature according to the Intergovernmental Panel on Climate Change (IPCC) Fourth Assessment Report (IPCC 2007) and the climate change can seriously alter the benthic communities also influencing the success of alien species.

1.4 BIOFOULING AND RECREATIONAL NAVIGATION

"Of some protective and anti-fouling compositions in use by the Navy,
it is no exaggeration to say that, as far as speed is concerned, one
half of our fleet would be useless before one year had elapsed, from
the accumulation of rust, weed and shell"
Quoted by Townsin (2003).

Biofouling process affects all the human activities that take place in marine environment. Damages are mainly related to navigation, aquaculture activities, cooling systems and scientific sensors submerged in the sea.

The problem of biofouling incrusting the boats leads to relevant economic and operational costs to the boat owners (Figure 5). In fact, biofouling can interfere with the activity of submerged equipment, increase the weight to the submerged part of the boat, accelerates corrosion of materials, and adversely affects the performance of ships by strongly increasing hydrodynamic drag with consequent high fuel consumption. In absence of antifouling treatment, the fuel consumption increases up to 40% and the speed decrease up to 10% (Kohli 2007; Schultz et al. 2011). The costs associated to fouling issue comprehends also the money spent to clean and coat the ship hull (Schultz et al. 2011; Stanley et al. 2016), that have to be done frequently by the owners of the boat.

Commercial harbours are the first recipient for introduction of NIS, but recreational ports may facilitate their secondary spread due to the intense traffic of small boats towards different recreational harbour (Floerl et al. 2009; Marchini et al. 2015; Ferrario et al. 2017). Moreover, the fouling community in the marinas was found to be more variable than that found in commercial harbours, due to the lower level of contamination (that favour not only the more tolerant species as in the large ports), variability of substrates and different routes of pleasure crafts (Connell 2001; Holloway & Connel 2002; Dafforn et al. 2009; Megina et al. 2016; Ferrario et al. 2017).

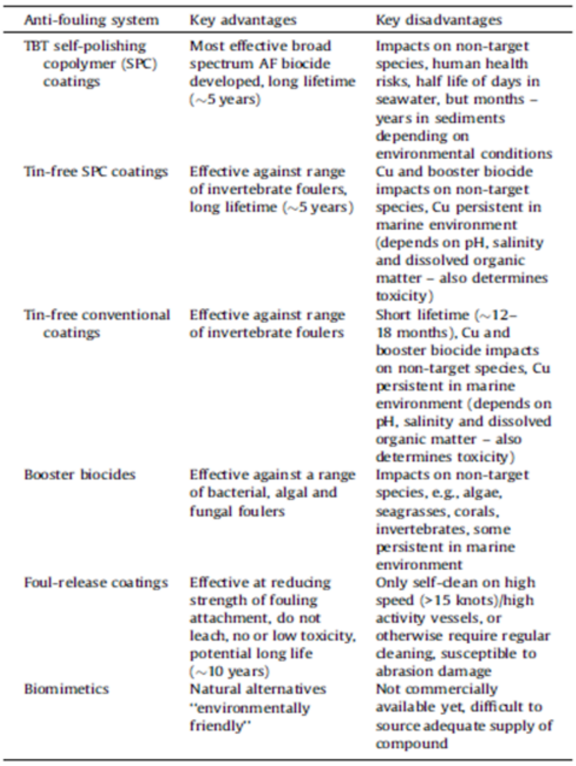
Figure 5 Cleaning procedures of the ship hull of the boat (A) and incrustations on the propeller and helm of the vessel (B)

1.4.1 ANTIFOULING STRATEGIES IN NAVIGATION: PAST AND PRESENT

The development of antifouling paints has an old origin, and it's associated with the increase of the maritime traffic. The ancient Phoenicians and Carthaginians (1500-300 B.C.) used copper sheets in their wooden ships, and later also coating containing sulphur and arsenic compounds, while Romans and Greeks introduced lead sheathing (Howell & Behrends 2010).

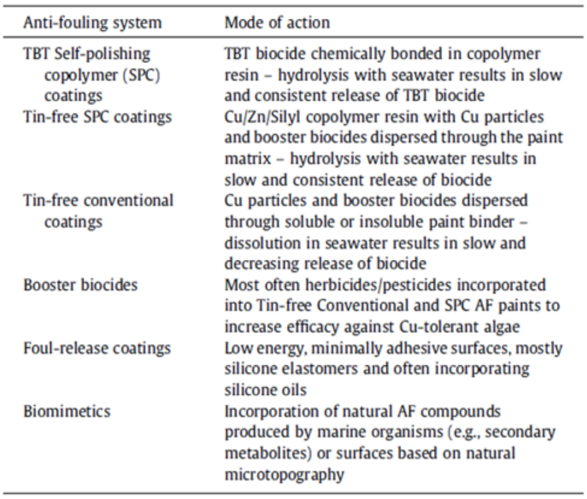
During late 18th and 19th centuries copper was the most used and effective coating, but the life of this product was short due to the galvanic corrosion and it must be reapplyed every 18 months (Lewis, 1998). Since this period, additional solutions have been continuosly proposed (Table 1).

Table 1 Description of the major AF strategies past and present (A) and advantages and disadvantages of past and present AF systems (from Dafforn et al. 2011).



Anti-fouling system	Key advantages	Key disadvantages
TBT self-polishing copolymer (SPC) coatings	Most effective broad spectrum AF biocide developed, long lifetime (~5 years)	Impacts on non-target species, human health risks, half life of days in seawater, but months – years in sediments depending on environmental conditions
Tin-free SPC coatings	Effective against range of invertebrate foulers, long lifetime (~5 years)	Cu and booster biocide impacts on non-target species, Cu persistent in marine environment (depends on pH, salinity and dissolved organic matter – also determines toxicity)
Tin-free conventional coatings	Effective against range of invertebrate foulers	Short lifetime (~12–18 months), Cu and booster biocide impacts on non-target species, Cu persistent in marine environment (depends on pH, salinity and dissolved organic matter – also determines toxicity)
Booster biocides	Effective against a range of bacterial, algal and fungal foulers	Impacts on non-target species, e.g., algae, seagrasses, corals, invertebrates, some persistent in marine environment
Foul-release coatings	Effective at reducing strength of fouling attachment, do not leach, no or low toxicity, potential long life (~10 years)	Only self-clean on high speed (>15 knots)/high activity vessels, or otherwise require regular cleaning, susceptible to abrasion damage
Biomimetics	Natural alternatives "environmentally friendly"	Not commercially available yet, difficult to source adequate supply of compound

A



Anti-fouling system	Mode of action
TBT Self-polishing copolymer (SPC) coatings	TBT biocide chemically bonded in copolymer resin – hydrolysis with seawater results in slow and consistent release of TBT biocide
Tin-free SPC coatings	Cu/Zn/Silyl copolymer resin with Cu particles and booster biocides dispersed through the paint matrix – hydrolysis with seawater results in slow and consistent release of biocide
Tin-free conventional coatings	Cu particles and booster biocides dispersed through soluble or insoluble paint binder – dissolution in seawater results in slow and decreasing release of biocide
Booster biocides	Most often herbicides/pesticides incorporated into Tin-free Conventional and SPC AF paints to increase efficacy against Cu-tolerant algae
Foul-release coatings	Low energy, minimally adhesive surfaces, mostly silicone elastomers and often incorporating silicone oils
Biomimetics	Incorporation of natural AF compounds produced by marine organisms (e.g., secondary metabolites) or surfaces based on natural microtopography

B

Tributyl tin (TBT)

In 1970s, Milne and Hails patented a new coating, a self- polishing copolymers (SPCs) loaded with Tributyl Tin (TBT) as the biocide (Milne & Hails 1971), that signed a great revolution on performance and history of antifouling paints (Townsin 2003; Finnie & Williams 2010). TBT antifouling coatings has proven to be a very good tool to solve the problem of fouling (de La Court 1984), inhibiting the growth of organisms up to five years (Townsin 2003; Lejars et al. 2012).

Unfortunately, the TBT was proven to be very toxic for a high number of organisms, and was categorized as the more dangerous compound ever used in marine environment (Dafforn et al. 2011; Faimali 2014). A lot of harmful effects were associated with the introduction of TBT, since even a low concentration of this compound has an effect on the health of marine organisms that are able to accumulate tin (Terlizzi et al. 2001; Lejars et al. 2012), such as imposex in gastropods and shell deformation in oysters, larval anomalities and lower recruitment (Alzieu et al. 1986).

France was the first country to recognise the effects of TBT, and since 1982 banned the application of this toxic coat on vessels less than 25 metres long (Alzieu et al. 1986; Lejars et al. 2012). But the ban of TBT from the International Maritime Organisation (IMO) occurred only in November 2001 with the adoption of the “AFS Convention”, that banned the application of this paint on vessels after 1st January 2003 and the complete removal from the ship hull after 1st January 2008 (IMO 2001).

From the restriction on the use of TBT, a lot of efforts were focused to search an alternative antifouling technology, leading mainly to the use of copper-based paints or paints with copper as a biocide (Lejars et al. 2012; Gittens et al. 2013).

Copper

After the ban of TBT, copper is becomed the main antifouling biocide for navigation; nevertheless, its impact on the marine environment are monitored in many countries (Dafforn et al. 2011). Copper represents a natural resource but it starts to be toxic when it exeeds the tolerance capacity of the organisms (Xie et al. 2005). Nowaday, the main copper compounds used as antifouling include metallic copper, cuprous thiocyanate, and cuprous oxide (Cao et al. 2011).

When release from antifouling paints, copper can be immediately bound to the bottom and sediments where its toxic effects impact the benthic community, diminishing the larval recruitment and settlement (Dafforn et al. 2011).

Furthermore, copper is worlwide recognised to facilitate aliens over the local species (Piola et al. 2009; Dafforn 2017), because only most tolerant species can survive to biocides.

Booster biocides

Despite the large use of copper or zinc as additives, the antifouling action is not effective enough to prevent the growing of some organisms, such as some tolerant algal groups. Therefore, the need of additional biocides named “booster biocides” (Batista-Andrade et al. 2016). For this reason, some products such as chlorothalonil, Igarol 1051, diuron, zinc- and copper pyrithione and many others are usually incorporated in antifouling coats all over the world (Yebra et al. 2004; Dafforn et al. 2011). Moreover, many of these products are also used in agriculture as herbicides and due to this extensive use, they are becomed common pollutants in marine anvironment, persisting from one month to one year (Giacomazzi & Cochet 2004).

Foul-release coatings

Different types of coating have been developed in the last decades, and among them foul-release coatings were considered in several studies for their application in marine environment (Buskens et al. 2013), receiving increasing attention year after year.

The foul-release coatings are low surface energy (Watson et al. 2015), and they are usually applied to the boat by airless spray. This technology allows not only to reduce the environmental impact, but also smoke stack emissions due to its inherent smoothness (Anderson et al. 2003; Watson et al. 2015). The coatings belonging to this group includes silicones, fluoropolymers, hybrids and hydrogel silicones (Yebra et al. 2004; Dafforn et al. 2011). The functioning of foul-release coatings, namely the low surface energy (Anderson et al. 2003), consists not to prevent the attachment of the organisms, but in reducing the strength of this interaction with surfaces (Terlizzi et al. 2001; Dafforn et al. 2011; Faimali 2014), saving time and effort during the cleaning operations. The surface energy represents the capacity of the substrate to create connections and interact with all the other materials, and the relationship between relative adhesion and surface energy is described by the “Baier curve” (Anderson et al. 2003) (Figure 6).

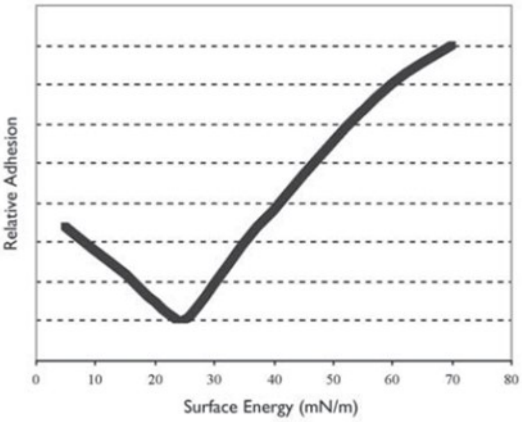


Figure 6 The Baier Curve (from Anderson et al. 2003)

The silicone polymers shown the lower surface energy, due to the extremely durable structure. In this way, it owns the perfect characteristic of longevity (beyond five years) requested from the market (Anderson et al. 2003), proving that silicone technology is a good environmental friendly alternative to antifouling paints containing biocides (Swain et al. 1992; Buskens et al. 2013).

Despite they are very promising, these coatings have some limits: they require usually a minimum of speed to be truly effective (10-20 knots typically) (Dafforn et al. 2011; Watson et al. 2015), despite the organisms can also be easily removed by light brushing (Watson et al. 2015). Moreover the costs for the application is higher than the other coatings (Dafforn et al. 2011; Watson et al. 2015), and it's generally estimated that the foul-release coatings are three times more expensive than tin-free SPC coatings (Bressy & Lejars, 2014). Moreover, further studies are needed to assess the toxicity on species of the compounds released from silicone coatings (Feng et al. 2012).

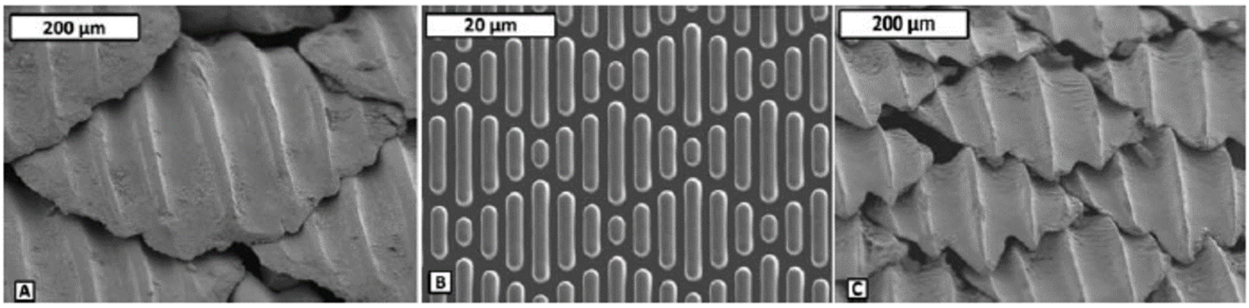
Biomimetic approach

Nature offers a great number of ideas to control biofouling, using both physical and chemical methods (Davis et al. 1989; Wahal 1989; Chambers et al. 2006). Marine organisms have achieved a good protection against biofouling changing continuously their defence strategies (short-time and renewable defences) (Ralston & Swain 2009). These defences can inspire humans to solve problems in human installations (Scardino & de Nys 2011), signing a new era in fouling control (Faimali et al. 2014). Moreover, despite there are still many aspects to be investigated, the natural compounds produced for example from algae, cnidarians and sponges seem to be a good alternative to common biocides (Hellio et al. 2009). As reviewed by Scardino & de Nys. (2011), many are the solutions inspired to nature and to marine organisms.

Cnidarians, especially gorgonians and corals are well known to produce antibacterial substances, specific for the bacterial in surrounding water and substrates (Ralston & Swain 2009). Over their surfaces they produce mucus containing different secondary metabolites (Brown & Bythell 2005), and together with the tentacular action they maintain the surfaces free from sediments (Stafford-Smith & Ormond 1992)

The skin of cetaceans seems to be clean and perfectly protected from fouling incrustations due to the microtopography and the surface enzymes that protect them from the adhesion process; moreover the skin is similar to oral mucosa and, for instance, the process of jump in dolphins seems to renew and sloughing the skin surface (Baum et al. 2003).

Also the shark skin received special attention from the researchers for its complex and very particular topography (Figure 7). The skin surface of these animals is characterised by micro-ridges very variable in relation to species (Bechert et al. 2000). Carman et al. (2006) demonstrated that Sharklet AF™, a new surface inspired to shark skin, has the potential to reduce the settlement of *Ulva* by 86%.



Fiure 7 Shark skin topography (from Scardino & de Nys 2011)

Echinoderms are a taxonomic group well known to have the surfaces free of fouling. They hold pedicellaria as antifouling strategies that, together with a complex structure of the surface, help these organisms to maintain the surfaces free of biofouling (McKenzie & Grigolava, 1996). However, these defences are not efficient enough and some studies suggest that in some species there are combined mechanical-chemical strategies (Scardino & de Nys, 2011).

Algae are the lonely group where chemical defences are not only supported, but also well documented and unequivocally established by a high number of studies and for a high number of species (Scardino & de Nys, 2011). They can produce metabolites that deter fouling, as the red alga *Delisea pulchra*, that has shown to be very effective in deter the settlement and adhesion of barnacles (de Nys et al. 1995).

The group of molluscs is also well known to have antifouling properties, but not all the molluscs are affected by fouling at the same way (Scardino & de Nys 2011). Bai et al. (2013) observed that the microtopography of the shell surface has some effects on the recruitment of fouling and this ability is species specific: the structure of the shells of *Dosinia japonica* and *Gafrarium pectinarum* can avoid the settlement of organisms, while the shell of *Mimachlamys nobilis* is a suitable substrate for their growth (Figure 8). The topography of a shell is due to the proteinaceous layer, the periostracum and the surface parameters, such as texture and roughness, are important to prevent the incrustations and boring by biofoulers (Scardino & de Nys 2011).

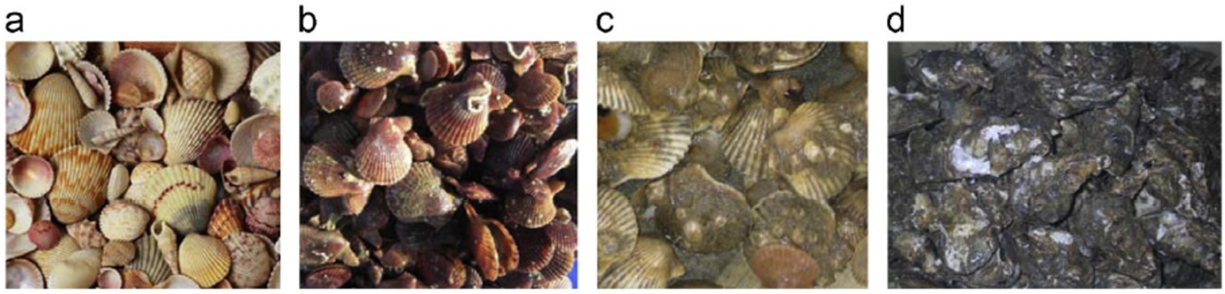


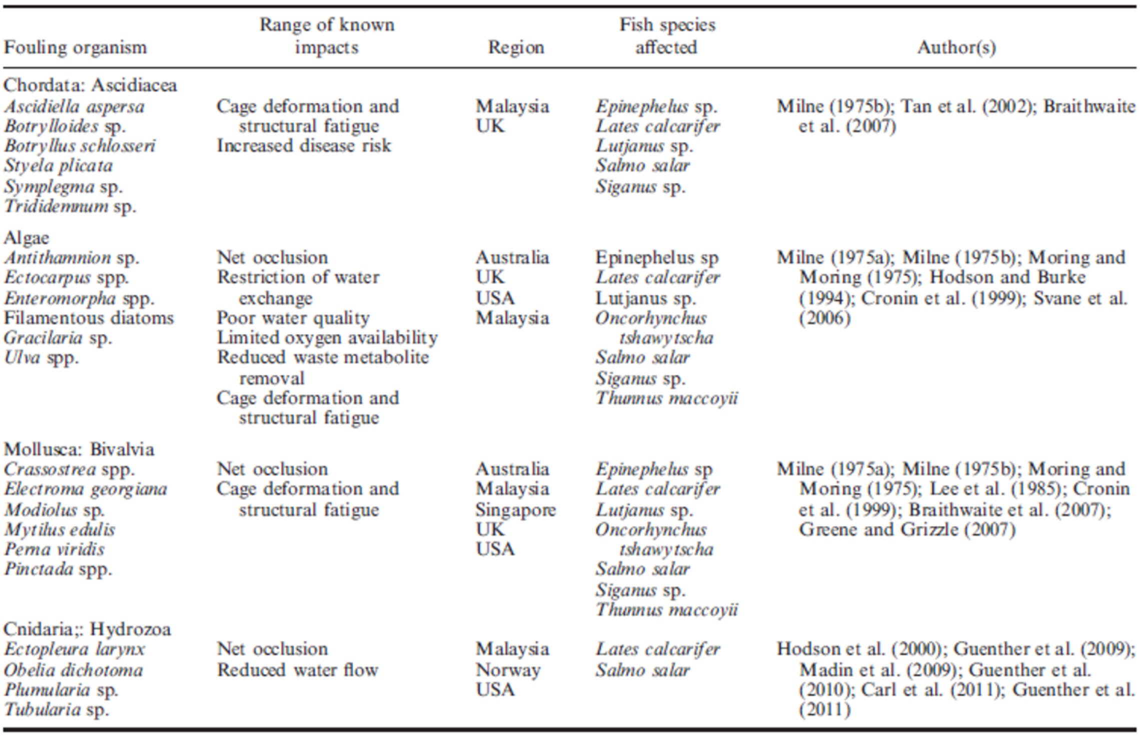
Figure 8 Bivalves with different levels of fouling incrustations (from Bai et al. 2013)

Despite the great potential of marine organisms, many problems still persist in the production of AF using this approach (Rittschof, 2000): the structure of the compounds in most cases is unclear and they require high costs and efforts for the production. Despite the combination of the surface chemistry and topography seems the right way to direct future researches (Scardino & de Nys 2011), more studies are still needed to better understand this topic and to demonstrate the efficacy of these compounds (Webster & Chisholm 2010).

1.5 BIOFOULING AND AQUACULTURE

Biofouling process affects all the aquaculture structures and has a negative impact on farmed species (reviewed in Braithwaite & McEvoy 2005; Willemsen 2005; de Nis & Guenther 2009; Dürr & Watson 2010; Fitridge et al. 2012). The costs to solve the problem of biofouling for aquaculture industry is high, usually 20-30% of the total amount of management expenses (Claereboudt et al. 1994; Dürr & Watson 2010). For Europe, the cost to solve the fouling-related problems amount up to 260 million euros per year and in general the biofouling can decrease the value of the products until the 90% (Dürr & Watson 2010).

Table 2 List of fouling organisms in world finfish farms and their impacts on farmed species (reviewed by Fitridge et al. 2012)



Fouling organism	Range of known impacts	Region	Fish species affected	Author(s)
Chordata: Ascidiacea *Ascidiella aspersa* *Botrylloides* sp. *Botryllus schlosseri* *Styela plicata* *Symplegma* sp. *Tridideminum* sp.	Cage deformation and structural fatigue Increased disease risk	Malaysia UK	*Epinephelus* sp. *Lates calcarifer* *Lutjanus* sp. *Salmo salar* *Siganus* sp.	Milne (1975b); Tan et al. (2002); Braithwaite et al. (2007)
Algae *Antithamnion* sp. *Ectocarpus* spp. *Enteromorpha* spp. Filamentous diatoms *Gracilaria* sp. *Ulva* spp.	Net occlusion Restriction of water exchange Poor water quality Limited oxygen availability Reduced waste metabolite removal Cage deformation and structural fatigue	Australia UK USA Malaysia	Epinephelus sp *Lates calcarifer* Lutjanus sp. *Oncorhynchus tshawytscha* *Salmo salar* *Siganus* sp. *Thunnus maccoyii*	Milne (1975a); Milne (1975b); Moring and Moring (1975); Hodson and Burke (1994); Cronin et al. (1999); Svane et al. (2006)
Mollusca: Bivalvia *Crassostrea* spp. *Electroma georgiana* *Modiolus* sp. *Mytilus edulis* *Perna viridis* *Pinctada* spp.	Net occlusion Cage deformation and structural fatigue	Australia Malaysia Singapore UK USA	*Epinephelus* sp *Lates calcarifer* *Lutjanus* sp. *Oncorhynchus tshawytscha* *Salmo salar* *Siganus* sp. *Thunnus maccoyii*	Milne (1975a); Milne (1975b); Moring and Moring (1975); Lee et al. (1985); Cronin et al. (1999); Braithwaite et al. (2007); Greene and Grizzle (2007)
Cnidaria;: Hydrozoa *Ectopleura larynx* *Obelia dichotoma* *Plumularia* sp. *Tubularia* sp.	Net occlusion Reduced water flow	Malaysia Norway USA	*Lates calcarifer* *Salmo salar*	Hodson et al. (2000); Guenther et al. (2009); Madin et al. (2009); Guenther et al. (2010); Carl et al. (2011); Guenther et al. (2011)

All the structures submerged by the farmers, as ropes and nets, are hypothetic substrates for the instauration and development of fouling community (Sarà et al. 2007; Fitridge et al. 2012; Fernandez Gonzalez & Sanchez-Jerez 2017).

The damages to the structures and stock species due to this phenomenon are numerous and can vary from deformation of cages and structures to the occlusion of the nets (that lead to less oxygen exchange and consequently increase of disease risk for fishes) in the finfish farms (Table 2), while in the shellfish cultures main problems are related to shell disruption, competition for food and space and recession of shell growth (reviewed from Fitridge et al. 2012). Control of fouling is particularly difficult due to the high spatial and community variations, especially in tropical regions were the growth rate is high during the whole year (de Nys and Guenther 2009).

Many studies have been carried out to characterise the biofouling community associated to aquaculture installations, both in shellfish and finfish farms (Braithwaite et al. 2007; Baxter et al. 2012; Fitridge et al. 2012; Peteiro & Freire 2013; Sievers et al. 2013; Velmurugan et al. 2013; Edwards et al. 2015; Lacoste & Gaertner-Mazouni 2015; Bosch-Belmar et al. 2017). The most common organisms are algae, hydroids, mussels and ascidians (Sarà et al. 2007; Guenther et al. 2010; Fitridge et al. 2012).

During the last decades, the global aquaculture production is constantly increasing in many countries (FAO, 2016), due to the increased demand of production (Figure 9), therefore it is of crucial importance to solve the problem of biofouling.

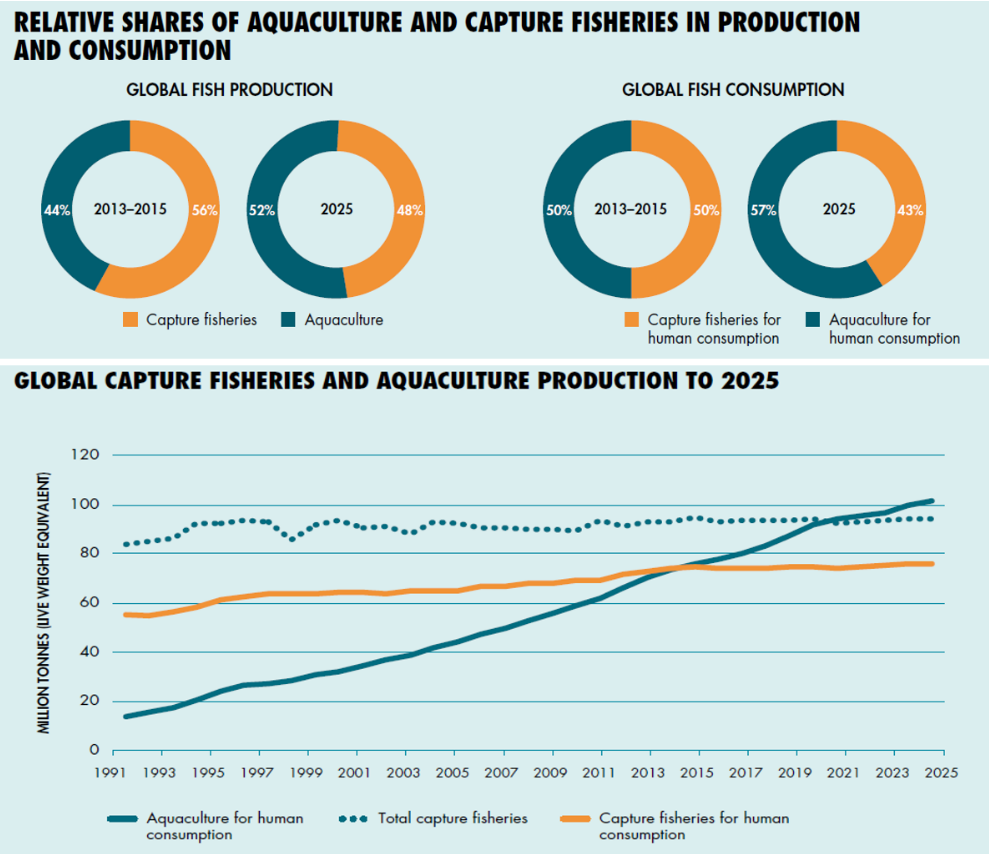
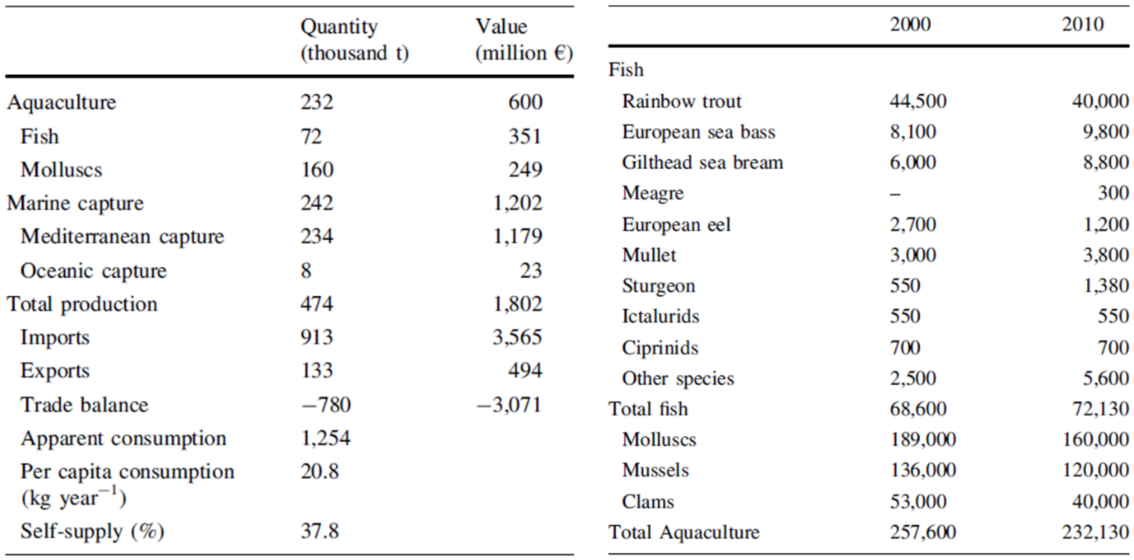


Figure 9 Aquaculture and fisheries production/consumption (from FAO, 2016)

1.5.1 BIOFOULING IN MEDITERRANEAN AQUACULTURE FARMS

As reviewed by Parisi et al. (2014), Italian aquaculture has decreased the general production from the 2000 to 2010; however, the production of sea bream and sea bass has increased, going from 8.100 t to 9.800 tons for the sea bass, and from 6.000 tons to 8.800 for the sea bream (Table 3).

Table 3 Aquaculture production (from Parisi et al. 2014)



	Quantity (thousand t)	Value (million €)
Aquaculture	232	600
Fish	72	351
Molluscs	160	249
Marine capture	242	1,202
Mediterranean capture	234	1,179
Oceanic capture	8	23
Total production	474	1,802
Imports	913	3,565
Exports	133	494
Trade balance	−780	−3,071
Apparent consumption	1,254	
Per capita consumption (kg year^{-1})	20.8	
Self-supply (%)	37.8	

	2000	2010
Fish		
Rainbow trout	44,500	40,000
European sea bass	8,100	9,800
Gilthead sea bream	6,000	8,800
Meagre	–	300
European eel	2,700	1,200
Mullet	3,000	3,800
Sturgeon	550	1,380
Ictalurids	550	550
Ciprinids	700	700
Other species	2,500	5,600
Total fish	68,600	72,130
Molluscs	189,000	160,000
Mussels	136,000	120,000
Clams	53,000	40,000
Total Aquaculture	257,600	232,130

Usually Mediterranean aquaculture facilities consist of structures submerged between 0,5 to 3 km from the coast and between 10 to 50 m depth (Parisi et al. 2014).

In Mediterranean Sea, the studies concerning the biofouling problems showed a regional variability of organisms. The fouling communities of fish farms are mainly composed by hydroids, bryozoans and bivalves, together to the vagile organisms as amphipods, which represent a large part of the epifauna associated with aquaculture facilities (Fernandez-Gonzalez & Sanchez-Jerez 2017).

In a recent work done in Croatia, a biofouling community of finfish farm was studied (Sliskovic et al. 2011). For that site, it was found a domination of algae over the animal component.

Antoniadou et al. (2013) studied the biofouling community in a mussel farm in Greece: *Mytilus galloprovincialis* was affected by a high level of incrustations.

1.5.2 ANTIFOULING STRATEGIES IN AQUACULTURE

Different antifouling strategies can be adopted in shellfish and finfish farms, and can be used alone or combined (Dürr & Watson 2010), increasing their effectiveness and specificity for each site of farming.

Human-induced techniques

Farm staff usually uses different methods to reduce fouling pressure in aquaculture installations, depending on the type and location of the farms.

In shellfish farm a simple method consist to expose the net to air, use a disk cleaner in situ or transport the nets on the ground and clean them using washing machines (Dürr & Watson 2010).

A very used technique in shellfish farm is the physical removal of fouling, by scrubbing and brushing the organisms or through the use of coatings and sprays (reviewed by Fitridge et al. 2012). Since several studies showed that the fouling is more severe in shallow water and decreases with the depth (Cronin et al. 1999; Guenther et al. 2010; Fitridge et al. 2012), so it's common solution to change the depth at which the farm structures are submerged. Bloecher et al. (2013) showed that acetic acid and high temperature have some positive effects against the hydroids *Ectopleura larynx*, that cause the occlusion of the nets in Norwegian salmon farms and is one of the main fouler in that region (Figure 10). Also heat treatment is resulted successful against biofouling both in aquaculture and in other human activities (Rajagopal et al. 1995).

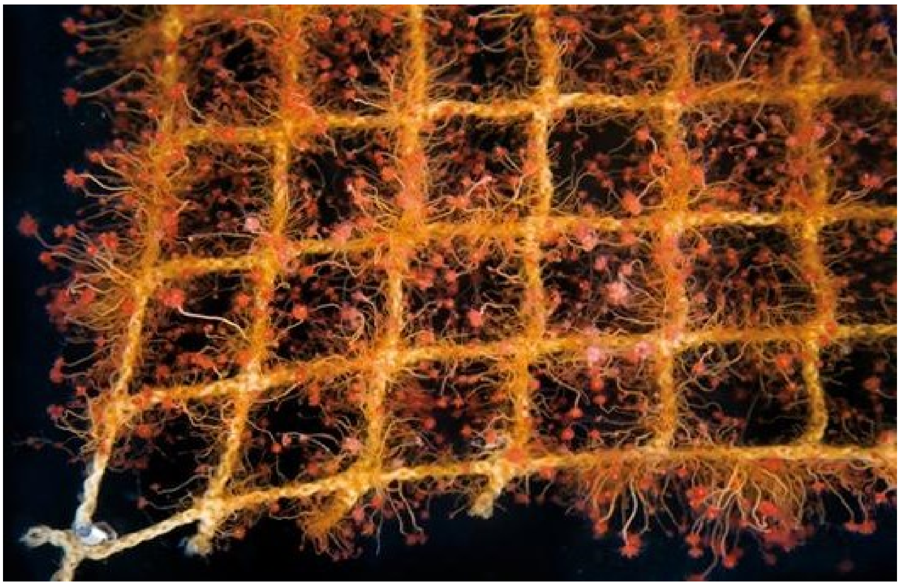


Figure 10 Net of a Salmon farm in Norway, fouled by E. larynx *(picture by SINTEF)*

Antifouling coatings

It has been demonstrated that the use of antifouling paints as a strategy to prevent biofouling is an important source of pollution for marine environment: the biocides spread in the water can have a strong impact to the farmed species (Castritsi-Catharios et al. 2015).

The antifouling paints applied to the nets are generally the same used for other industries (for details see chapter 1.4.1) (Fitridge et al. 2012), usually low-tech version of coating for boats. After the ban of TBT, the paints are usually composed of copper-oxide (Cu_2O) (Figure 11) and last only one season (Willemsen 2005). Sometimes also inorganic zinc or other kind of booster biocides such as Irgarol 1051, Sea Nine 211, dichlofluanid, chlorothalonil, zinc pyrithione, and Zineb are added to copper, to increase the performance and effectiveness of the antifouling paints (Parks et al. 2010).

Generally, two are the main risks associated to the use of biocides in aquaculture: 1) bioaccumulation of the pollutants that, through the trophic chain, arrive to predator and human, and 2) increase in number of bacteria resistant to antibiotic treatments (Guardiola et al. 2012).

Nanotechnology materials for coatings may be an additional possible solution to preserve both environmental and food safety (Dürr & Watson 2010).

Figure 11 Net coated with copper oxide in a salmon farm in Norway

Biological control

Biological control is an environmental friendly and non-toxic method to prevent the growth of biofouling (Fitridge et al. 2012). It consists to introduce different predators and grazers in aquaculture farms, to control and reduce the growth of biofouling (Dürr & Watson 2010) (Figure 12).

Some examples of this strategy are the use of herbivorous fishes and sea cucumbers in finfish farms, or sea urchins and crabs in shellfish farm (Ross et al. 2004).

Lodeiros and Garcia (2004) evaluated the antifouling capacity of two sea urchin species as biocontrol in the culture *Pinctada imbricata,* confirming that the use of sea urchins can be adopted as an effective strategy in bivalve farms.

Unfortunately, all these methods are really useful and effective only in a small scale (Fitridge et al. 2012) and not applicable for the big farms and cages.

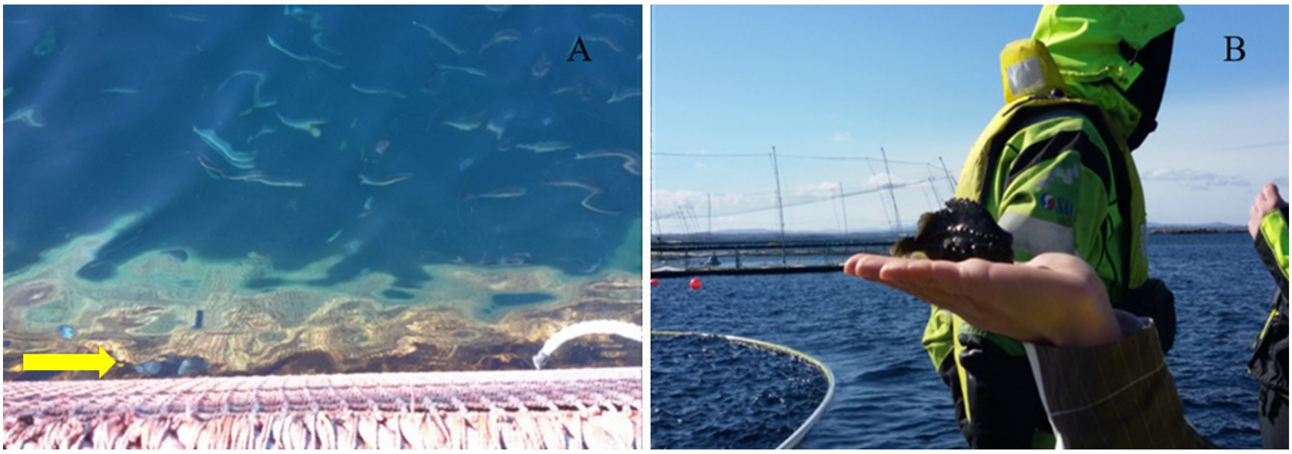


Figure 12 Fishes used in salmon farm in Norway to clean the nets (fig. A-B)

New solutions to control biofouling in aquaculture

There is an urgent need to solve the problem of biofouling in aquaculture: the solution must be simple, low cost and low time consuming. The knowledge of spawning events of foulers and how to limit them could represent a method to save both money and efforts (Dürr & Watson 2010).

Another possible solution is the use of silicone coatings, not toxic for the farmed species and that facilitates the removal of biofouling, but the costs for the application are relevant. In fact, the cost of the copper and silicone increases the raw material costs of the net by about 1.5 and 6.0 times respectively. However, silicone coatings with improved formulation may be, in a near future, a not toxic solution against biofouling (Swain & Shinjo 2014).

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2. AIMS OF THE STUDY

The main aims of the present PhD thesis are:

1) to investigate the succession and recruitment of biofouling in the recreational harbour of Marina Dorica in Ancona (Italy), through use of artificial panels. The spatial and temporal changes of biofouling have been studied to test the hypothesis that the location inside the marina basin influences the biofouling composition and biomass accumulation. **(Chapter 1)**;

2) to i) improve the knowledge about the Italian boater's habits and outline their social and economic profile, ii) increase the knowledge on boater's perception about biofouling problem and estimate their economic loss, iii) assess the boater's knowledge about antifouling paints and their awareness to the problems related to them, iv) explore the open-mindedness of boat owners to take in account alternative eco-friendly technologies and assess the possible need of specific information campaigns. **(Chapter 2)**;

3) to improve the knowledge about the biofouling community and its variation over time and depth in a Mediterranean finfish aquaculture in order to provide insights useful for the sustainable management of biofouling in fish farms and for the implementation of environmental monitoring programme guidelines for marine finfish cage farming. **(Chapter 3)**.

CHAPTER 1

Spatial and temporal changes of biofouling community within a marina of the North Adriatic Sea (Mediterranean Sea)

Spatial and temporal changes of biofouling community within a marina of the North Adriatic Sea (Mediterranean Sea)

Abstract

Biofouling represents a problem for navigation and causes heavy money loss to boaters. To manage this problem, a good local knowledge of dominant foulers and their temporal and spatial variation is a key requirement. In this work, we investigated the spatial and temporal changes of biofouling in the recreational harbour of Ancona (Italy), through the submersion of static panels in two sites of the marina. A total of 60 taxa were identified during the study period. The biofouling community was dominated by algae and the animal component was largely represented by filter-feeding invertebrates. The number of species, species composition, percentage of coverage and biomass were characterised not only by major temporal changes, but also changes at small spatial scale. These findings provide new insights on biofouling community dynamics and represent a starting point to better address the control methods used to minimise the biofouling problems within marinas of the North Adriatic Sea.

Introduction

Every substrate submerged in the sea represents a new habitat that is rapidly colonised by a biofilm followed by the settlement of a macroscopic community of marine organisms: this process is known as biofouling (Gittenberger & van der Stelt 2011).

More than 4000 species have been described as part of biofouling communities (Arai, 2009; Bai et al. 2013; Buskens et al. 2013), including hard and soft organisms. Most biofouling species disperse via planktonic larvae, spores and other propagules, which are often released with seasonal patterns. Therefore, the time at which the substrates are submerged in sea water plays a primary role in structuring the fouling community and influences the subsequent interactions between early and later arrivals (Lin & Shao 2002). For this reason, seasonal and site-specific variation of marine biofouling requires scientific attention (Vedaprakash et al. 2013), and it is important to identify the factors that influence and structure the community (Pierri et al. 2010).

Biofouling causes several problems for navigation and maritime industry: in boating activity, it is responsible for heavy economic losses, due to increased fuel consumption up to 40% and decreased speed up to 10% (Kohli 2007; Schultz et al. 2011). Marinas and commercial harbours are considered vector hubs for biofouling organisms, but marinas show a greater variability of substrates and a lower level of water contamination (Dafforn et al. 2011). The submerged structures typical of marinas represent ideal substrates for the settlement of biofouling species (Lin & Shao 2002) and many studies demonstrated that unstable substrates such as floating piers host different biofouling communities compared to fixed substrates typical of commercial harbours (Connell 2001; Holloway & Connell 2002; Dafforn et al. 2009; Megina et al. 2016). Moreover, the routes of pleasure crafts are different and in general more variable than for the commercial ship ones (Megina et al. 2016; Ferrario et al. 2017).

Marinas are semi-enclosed basins usually built in coastal areas where the currents and water turbulence are low; if these conditions are not naturally available, artificial barriers are built to protect the area and create them. However, artificial barriers can affect the recruitment of species due to modified wave propagation (Floerl & Inglis 2003). Although many studies have been carried out for touristic harbours in the Mediterranean Sea, most of them are descriptive and focus on Non-Indigenous Species (NIS) (Marchini et al. 2015; Steen et al. 2016; Ferrario et al. 2017). Recent papers concerning the spatial and temporal variability of biofouling within Italian marinas are scarce (Pierri et al. 2010, Lezzi et al. 2017), especially for the Adriatic basin. To control biofouling and succeed in preventing its damages, a general background of basic information on the diversity, abundance, biomass accumulation and seasonality of common local biofouling species is essential (Berntsson & Jonsson 2003). All these factors are important to understand the biofouling pressure in a studied area (Pati et al. 2015).

The aim of this study is to investigate the succession and recruitment of biofouling in the recreational harbour of Marina Dorica in Ancona (Italy), through the use of artificial panels. We examined the spatial and temporal variation of biofouling to test the hypothesis that the location inside the marina basin influences the biofouling composition and biomass accumulation. The results represent a baseline of information necessary for the recommendation of good management practices and optimization of cleaning procedures of boats and submerged structures.

Materials and Methods

Experimental design

The experiments were carried out at Marina Dorica (43°36’ N, 13°28’ E), the recreational harbour of Ancona (Italy) (Figure 1) active since September 2000. It is characterised by a sandy bottom with depth ranging from 2.5 to 4 m. The marina can host 1300 vessels with a maximum length of 27 m (www.marinadorica.it).

Two experiments were conducted from 21 September 2015 to 21 September 2016. Succession and recruitment of biofouling organisms to forex panels (15 x 15 cm) were compared between two sites, in the inner harbour and the outer harbour (near the entrance) (Figure 1). In the succession experiment 36 panels, attached vertically to a metal frame on piers, were submerged at 1 m depth at each site. Every month, 3 panels were randomly retrieved from each set to be analysed. For the recruitment experiment, a set of 3 panels was submerged at 1 m depth in each site. They were collected every three months and replaced with a new set of panels. The trimesters were regarded as seasons: September-December 2016 as autumn, December-March 2017 as winter, March-June 2017 as spring and June-September 2017 as summer.

The collected samples were labelled and transported to the laboratory in plastic bags filled with seawater for analysis.

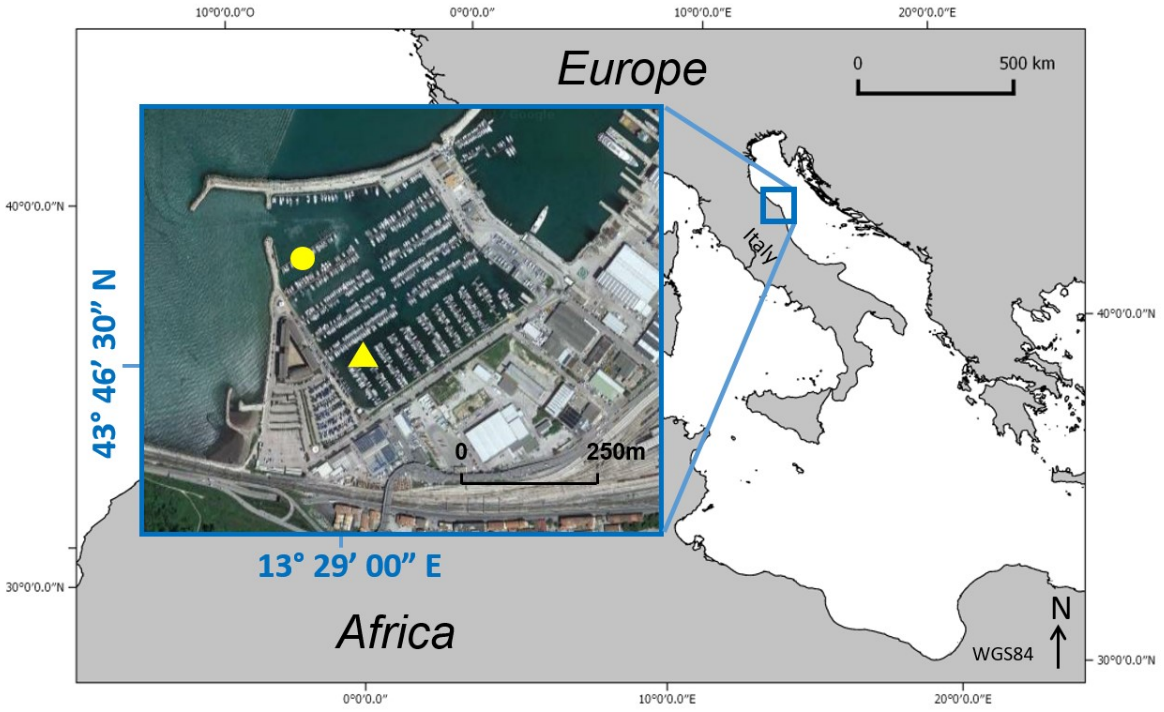

Figure 1 Location of the study, indicating the outer site (dot) and inner site (triangle) of sampling within the harbour

Laboratory analysis

In the laboratory, the panels were analysed firstly under a stereomicroscope before being preserved in 70% alcohol.

The sessile organisms settled on the front of each panel (the side not shaded by the pier) were identified to the lowest taxonomic level possible by microscopic observation. The abundance of biofouling was calculated as percent coverage of each taxonomic unit. These data were obtained by superimposing a reference grid of 144 equally-sized squares of 1x1 cm over the panel. To avoid an effect of the panels edge, the outer 1.5 cm were excluded from the analysis. The coverage of organisms was recorded for each of the 144 squares and ranked in categories following a modified Dethier et al. (1993) method: zero (absent), 0.25 (6.25%), 0.5 (12.5%), 1 (25%), 2 (50%), 3 (75%), and 4 (100% of the square). Total cover per panel was then estimated as the sum of the categorical values of all squares and then expressed as percentage of 144 cm^2.

As one organism can overgrow other ones, it is possible to obtain overall coverage values that exceed 100% cover. Therefore, all the layers were ideally compressed to one level, and the coverage was estimated from the top view, taking only into account species visible in the top layer (Pierri et al. 2010).

The biofouling biomass was calculated as wet weight of the organisms settled on front side of the panels. It was obtained by subtracting the wet weight of cleaned panels from the wet weight of fouled panels, after the elimination of biofouling settled on the back side; the values are reported as mean per panel (= 0.0225 m^{-2}) ± standard error (SE).

Statistical analysis

Differences in biofouling community composition were analysed using permutational multivariate analysis of variance (PERMANOVA, PRIMER 7) (Anderson 2001). The factors included in the model were Location (L, two levels, fixed) and Age (A, 12 levels, fixed) for the succession study, and Location (L, two levels, fixed) and Season (S, four levels, fixed) for the recruitment study. Analyses were based on Bray-Curtis dissimilarities, using untransformed data. P-values were obtained by 9999 permutations of residuals under a reduced model. Pairwise comparisons were used to examine differences where main or interaction terms associated with the tested factors were significant. Where the number of unique permutations was ≤100, the Monte-Carlo asymptotic pMC-value was consulted (Anderson 2008). Graphical representations of community composition were obtained by non-metric multi-dimensional scaling (nMDS).

Results

Taxonomy and diversity

Considering all the 96 panels from both experiments, 56 species and 4 multi species groups were identified. Among them, 8 species identified for the marina are considered NIS for the Mediterranean basin (Table 1).

The most abundant taxon was macroalgae, which represented 34% of the identified taxa. It was followed by annelids (18%), bryozoans (14%), molluscs (10%), ascidians (10%), cnidarians (8%), sponges (3%) and crustaceans (3%) (Figure 2a).

The most common organisms were the algae *Cladophora vagabunda,* Ectocarpales spp., *Ulva* cf. *intestinalis, Ulva rigida,* the bryozoan *Bugula neritina,* the mussel *Mytilus galloprovincialis,* the anellids *Hydroides* spp*., Janua heterostropha, Pileolaria* cf. *militaris,* the barnacles *Amphibalanus* spp. and the ascidian *Botryllus schlosseri.* They were recorded from at least 50% of the panels.

Nineteen species were exclusively observed in the outer harbour site (e.g. cnidaria; Table 1). Contrastingly, all taxa collected in the inner harbour were also observed in the outer harbour (Table 1). In both study sites, panels showed a high amount of silt during the entire study period.

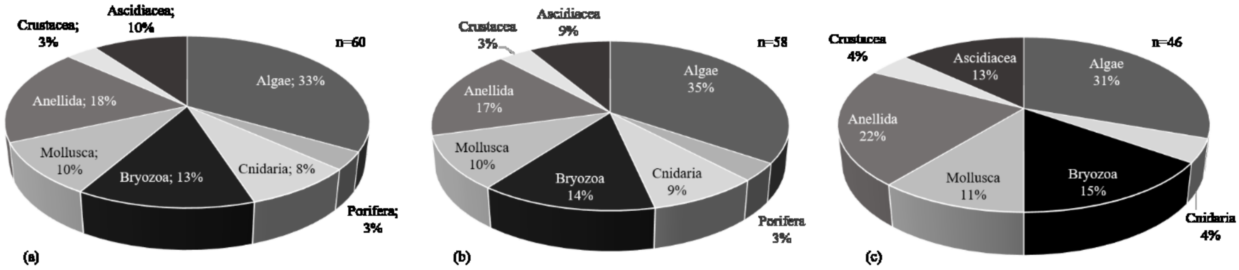

Figure 2 Taxon groups represented in the fouling community of the Marina Dorica: a) general results, b) succession c) recruitment

*Table 1 List of taxa identified in the harbour Marina Dorica during the study; s = succession, r = recruitment; *= NIS*

	IN	OUT		IN	OUT
ALGAE			Cheilostomatida spp.	sr	sr
Antithamnion cruciatum	s	sr	*Cryptosula* sp.	sr	sr
Antithamnionella spirographidis		s	*Cellepora* cf. *adriatica*	sr	sr
Brown algae n.i.	sr	r	Encrusting bryozoan n.i.		s
Bryopsis plumosa	s	s	*Schizoporella errata*		sr
Ceramium sp.		s	MOLLUSCA		
Chaetomorpha sp.	s	s	*Anadara transversa**		s
Cladophora vagabunda	sr	sr	*Anomia ephippium*	sr	sr
Colaconema daviesii	s	s	*Arcuatula senhousia**		sr
Corallinaceae n.i.		s	*Hiatella arctica*	s	sr
Cutleria multifida	sr	sr	*Magallana gigas**		sr
Cutleria sp. (sporophyte “Aglaozonia”)	sr	sr	*Mytilus galloprovincialis*	sr	sr
Ectocarpales spp.	sr	sr	ANELLIDA		
Polysiphonia cf. *scopulorum*	sr	sr	*Amphiglena* sp.		r
*Polysiphonia morrowii**	sr	sr	*Filograna* sp.	sr	sr
Polysiphonia sp.	sr	sr	*Hydroides* spp.	sr	sr
*Scytosiphon dotyi**	sr	sr	*Janua heterostropha*	sr	sr
Stictyosiphon soriferus	sr	r	*Pileolaria* cf. *militaris*	sr	sr
Ulva cf. *clathrata*	sr	s	*Sabella spallanzanii*		s
Ulva cf. *intestinalis*	sr	sr	*Sabellaria spinulosa*		sr
Ulva rigida	sr	sr	*Spirobranchus lamarcki*		sr
PORIFERA			*Spirobranchus triqueter*	sr	sr
Halichondria sp.		s	Serpulidae n.i.	s	sr
Sycon sp.	s	s	*Vermiliopsis striaticeps*	sr	sr
CNIDARIA			CRUSTACEA		
Bougainvilliidae n.i.		s	*Aphibalanus* spp.	sr	sr
Clytia sp.		s	Balanidae n.i.	sr	s
*Ectopleura crocea**		s	ASCIDIACEA		
Hydrozoan n.i.		sr	*Ascidiella aspersa*	sr	s
Kirchenpaueria halecioides		sr	*Botryllus schlosseri*	sr	sr
BRYOZOA			*Ciona intestinalis*	sr	sr
Amathia gracilis	sr	sr	*Diplosoma* sp.1	sr	sr
*Amathia verticillata**	sr	sr	*Diplosoma* sp.2		r
Bugula neritina	sr	sr	*Styela plicata**	sr	sr

Spatial and temporal changes in the succession experiment

A total of 58 taxa, including multi species categories, were identified on the panels in the succession experiments. Among these, 56 taxa occurred on the panels in the outer harbour, while 41 were found on panels from the inner harbour (Table 1).

Macroalgae were the most abundant taxon (35% of the total identified taxa), followed by annelids and bryozoans (17% and 14%, respectively). In addition, molluscs, cnidarians, ascidians, sponges and crustaceans represented 10%, 9%, 9%, 3% and 3%, respectively (Figure 2b).

Seventeen species were exclusively found in the outer harbour: 5 cnidarians (*E. crocea, K. halecioides*, *Clytia* sp., Bougainvilliidae n.i., hydrozoan n.i.), 3 algae (*Antithamnionella spirographidis, Ceramium* sp*.,* unidentified Corallinaceae), 3 molluscs (*Anadara transversa*, *Arcuatula senhousia*, *Magallana gigas*), 3 annelids (*Sabella spallanzanii*, *Sabellaria spinulosa*, *Spirobranchus lamarcki*), 2 bryozoans (*Schizoporella errata* and an unidentified encrusting bryozoan) and 1 sponge (*Halichondria* sp.) (Table 1). In contrast, two species of algae (*Stictyosiphon soriferus* and a species of brown alga that could not be identified) were only observed in the inner harbour (Table 1).

The number of identified species was higher in the outer site than in the inner one, except for the months of August and September (Figure 3a). In both sites, the number of species increased with time, yet the maximum species abundance was reached at different times. Species numbers in the outer harbour peaked during February (23.7 ± 0.7 species per panel ± SE), while species numbers for the inner site reached a maximum in the month of July (18.3 ± 2.9). The biomass accumulated on the panels grew with time especially in the outer site, where it reached the maximum value at the end of the experiment (304.7 ± 91.2 g per panel ± SE) (Figure 3a). In the inner site, the biomass grew more slowly and reached the maximum value in June (59.3 ± 35.3) (Figure 3a).

The community composition was impacted significantly by both Time and Location (Time x Location: $f_{11,71}$= 5.07; p< 0,001). The community composition differed most of the months at the beginning of the study (pairwise comparison pMC < 0.05) in both sites, while during the later months of succession similarities between months were a more common situation. The community composition differed significantly between the two locations for all months with exception of December and August (pairwise comparison pMC < 0.05) (Figure 4).

The analysis of cover percentage identified macroalgae as the dominant group from October to March in both sites (Figure 5). In the outer harbour molluscs, mainly represented by *Mytilus galloprovincialis*, dominated the community from April to September, reaching the maximum coverage in July (53.6 ± 9.7%) (Figure 5a). In the inner site algae were still dominant in April and May, but in June ascidians, in particular the solitary species *Ciona intestinalis*, became dominant

(19.4 ± 12.1%) (Figure 5b). In July, August and September the community was dominated by algae, annelids (mainly *Hydroides* spp.) and ascidians (Figure 5b). The species that mainly contributed to this last group were in July the colonial ascidian *Botryllus schlosseri* (14.5 ± 8%) and in September *Styela plicata* (9.0 ± 4.5%).

Soft tubes, especially represented by amphipod tubes, were mainly observed in the outer site and reached a maximum during the months of March (17.3 ± 2.3%).

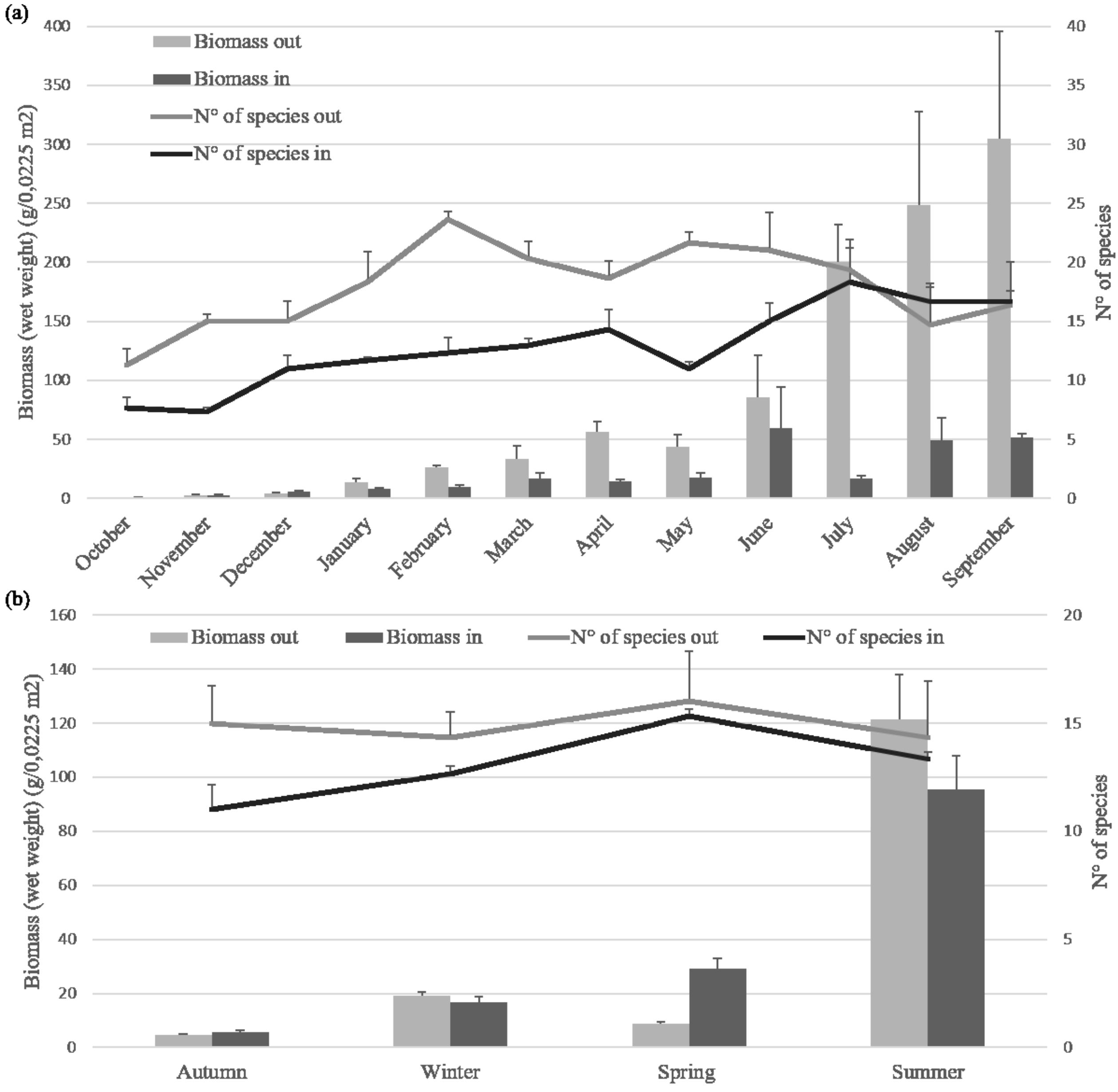

Figure 3 Average (±SE) biofouling biomass and number of identified species on panels from the outer (out) and inner (in) harbour during the succession (a) and recruitment (b) study

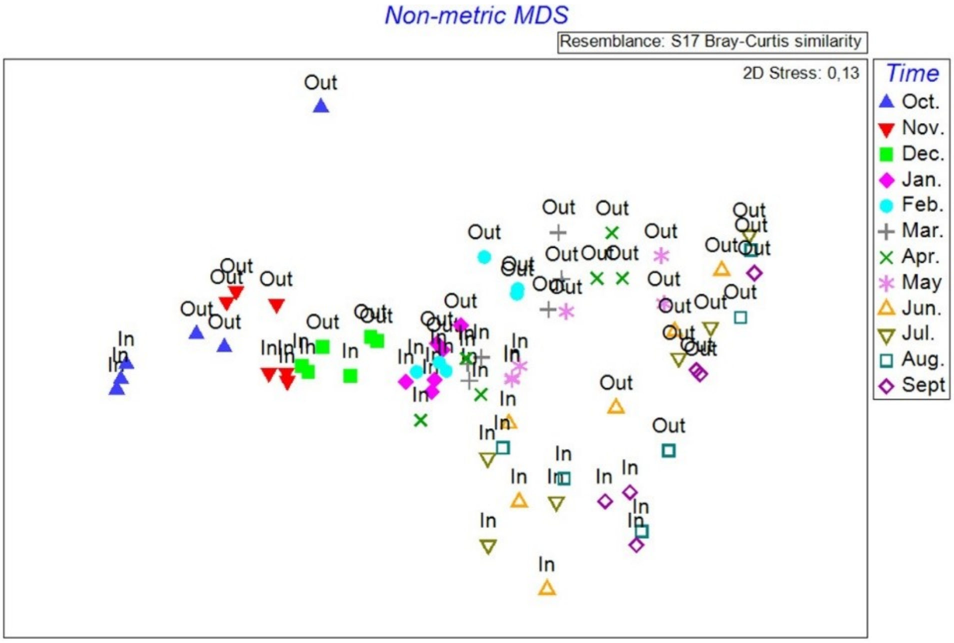

Figure 4 nMDS plot of the community composition resemblance between outer (out) and inner (in) harbour during the successional study

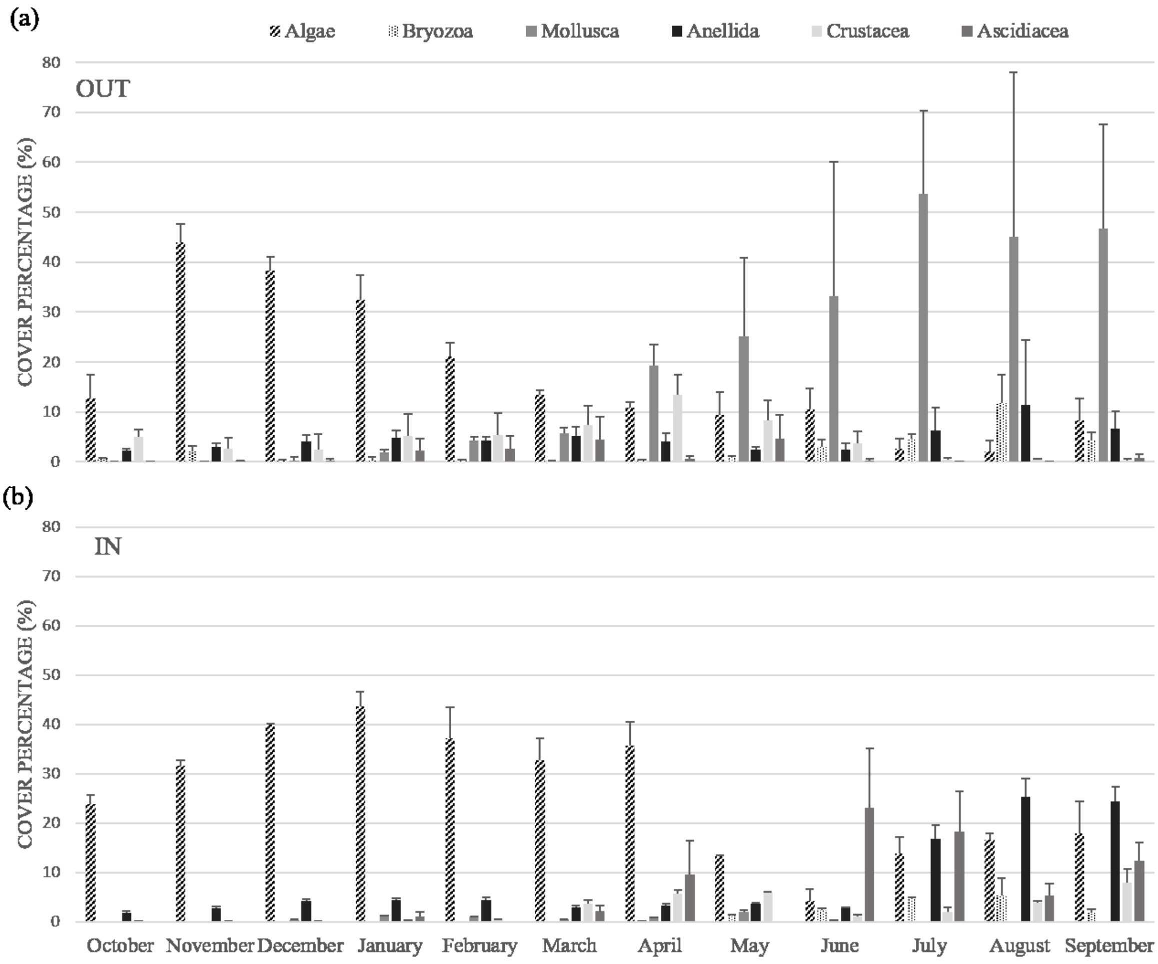

Figure 5 Mean cover percentage (±SE) of the main groups in the succession study: a) outer harbour, b) inner harbour

Spatial and temporal changes in the recruitment experiment

Forty-six taxonomic units were identified from the panels in the recruitment experiment; 43 taxa were found in the outer site and 33 in the inner one (Table 1). Macroalgae and annelids were the most represented taxa (31% and 22% of the total identified taxa respectively), followed by bryozoans (15%), ascidians (13%), molluscs (11%), cnidarians (4%) and crustaceans (4%) (Figure 2c).

Twelve species were exclusively present in the outer site: 4 annelids (*Sabellaria spinulosa*, *Spirobranchus lamarcki* and *Amphiglena* sp.), 3 molluscs (*Arcuatula senhousia, Hiatella arctica, Magallana gigas*), 2 cnidarians (*Kirchenpaueria halecioides* and an unidentified hydrozoan), 1 alga (*Antithamnion cruciatum*), 1 bryozoan (*Schizoporella errata*) and 1 ascidian (*Diplosoma* sp.2) (Table 1). The green alga *Ulva* cf. *clathrata,* an unidentified barnacle species and the ascidian *Ascidiella aspersa* were exclusively observed in the inner site (Table 1).

The number of species was constantly higher in the outer site than in the inner one (Figure 3b). The highest number was reached in spring both in the outer site (16.0 ± 2.3 specie per panel ± SE) and in the inner one (15.3 ± 0.3), while the minimum values were registered in winter and summer (14.3 ± 1.2 and 14.3 ± 2.6) in the outer site and during autumn in the inner one (11 ± 1.2) (Figure 3b). The analysis of the biomass showed the lowest values in autumn, both in the outer (4.6 ± 0.4 g per panel±SE) and inner sites (5.5 ± 0.8). In winter the values were similar in the two sites, while during spring, the biomass was higher in the inner site (29.1±3.8) than in the outer one (9.0 ± 0.4). In both sites the biomass peaked in summer with values higher in the outer site (121.4 ± 16.5) than in the inner one (95.6 ± 12.2) (Figure 3b).

The community composition was significantly impacted by both Season and Location (Season x Location: $f_{3,23} = 7,28$; $p < 0.001$).

Community composition differed for all seasons in both inner and outer harbour (pairwise comparisons, pMC < 0.05) (Figure 6). Furthermore, community composition differed significantly between inner and outer harbour in all seasons, except autumn (pairwise comparisons pMC < 0.05) (Figure 6).

The analysis of the cover percentage showed that in autumn algae dominated both the outer and the inner sites (Figure 7). The main species were *Cladophora vagabunda* (9.2 ± 0.9% in the outer site; 10.7 ± 2.7% in the inner site), Ectocarpales spp. (17.8±1.9% in the outer site; 17.5 ± 2.9% in the inner site) and *Ulva* cf. *intestinalis* (6.7 ± 0.7% in the outer site; 10.7 ± 1.0% in the inner site). In winter, crustaceans and algae dominated the community of both sites (Figure 7). Among crustaceans in the outer site the genus *Amphibalanus* is the main contributor (14.9 ± 0.4%). In spring and summer several taxa contributed to the coverage. In spring algae (mainly *Cutleria* sp.

13.4 ± 2.8%), followed by annelids and bryozoans (mainly *Cryptosula* sp., 12.6 ± 3.7%) were major contributors in the outer site (Figure 7a). In the inner site annelids (mainly *Hydroides* spp.) dominate the community (18.6 ± 2.6%), together with ascidians (mainly *B. schlosseri,* 11.8 ± 5.7%) (Figure 7b). In summer bryozoans strongly dominated the community, especially the incrusting *S. errata* (19.5 ± 2.8%), but crustaceans, ascidians (mainly *S. plicata*, 13.6 ± 3.8%) and annelids were also relevant in the outer site (Figure 7a). In the inner site, the crustaceans *Amphibalanus* spp. (33.0 ± 8.0%) and the annelids *Hydroides* spp. (22.2 ± 5.0%) were strongly dominant (Figure 7b).

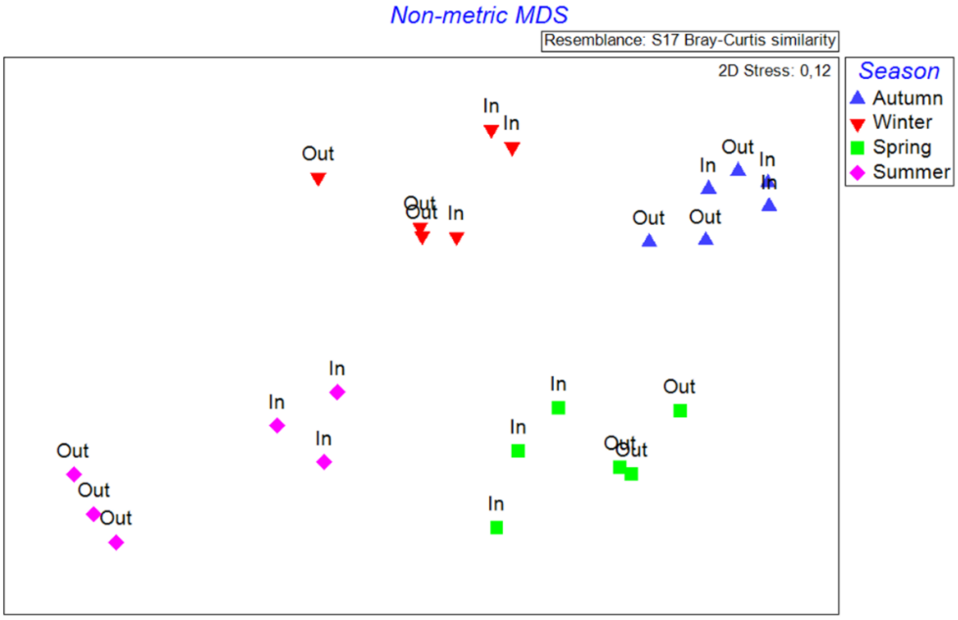

Figure 6 The nMDS plot showing differences of biofouling communities between sites during the seasonal study of biofouling

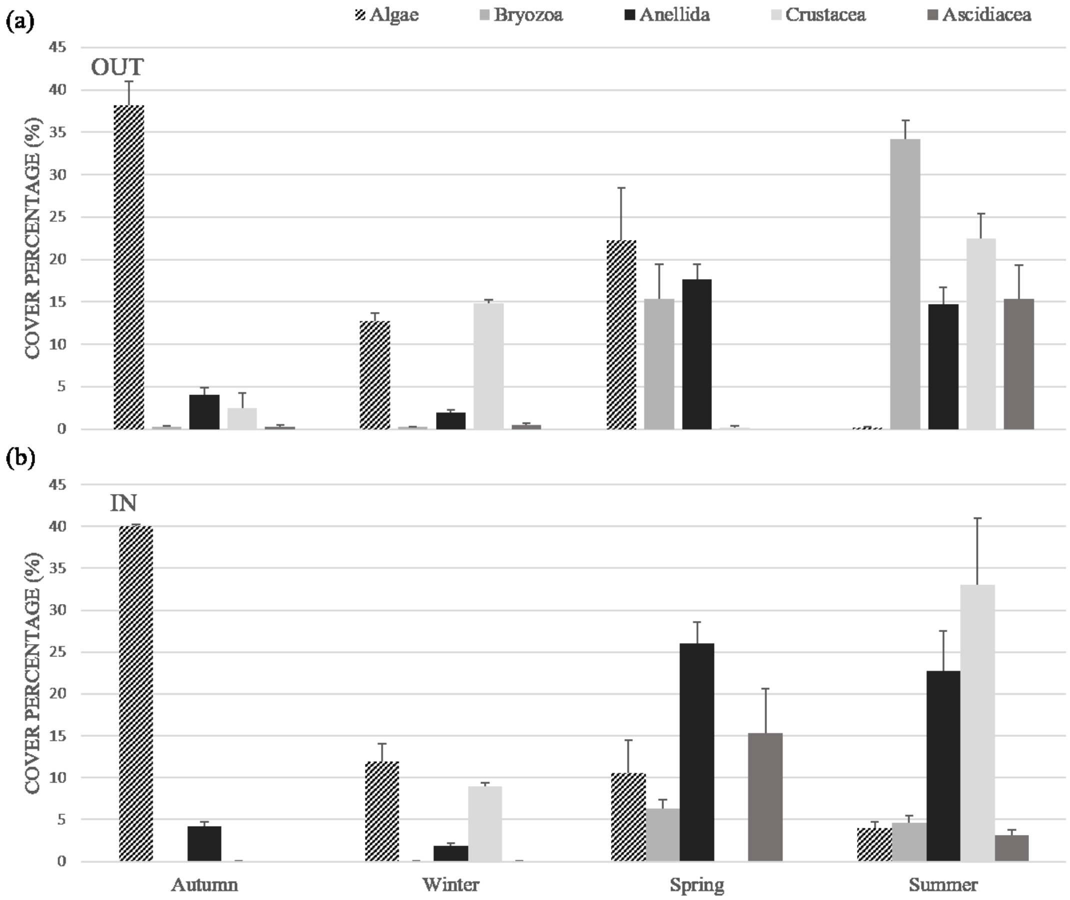

Figure 7 Mean coverage percentage (±SE) of the main groups found during the recruitment study: a) outer harbour, b) inner harbour

Discussion

Biofouling causes major problems for navigation and heavy money loss to boaters. As highlighted by Berntsson & Jonsson (2003), future antifouling strategies should be based on a detailed knowledge of dominant foulers, such as their temporal and spatial variation, for more focused actions. This underlines the importance of local knowledge of biofouling, which is specific for different study areas.

In this framework, the present investigation provides information on the succession and recruitment of the biofouling community in a recreational harbour located in the North Adriatic Sea.

The study was performed using artificial panels, which already showed to be a good and widely tested tool for the study and characterization of biofouling: in fact, they allow following the dynamic of colonisation, the modification of the community during time and performing quantitative analyses of biofouling community (Cornelio & Occhipinti-Ambrogi 2001).

The biofouling community present in the studied marina was dominated by macroalgae in terms of number of species, but these organisms were also dominant or at least relevant during the first period of the succession study and during three of the four seasons of the recruitment experiment, regardless of the study site. Considering the animal component, the community was dominated by filter feeders. In fact, annelids, bryozoans, molluscs, and ascidians were the most relevant taxa in term of number of species. Moreover, they were the dominant taxa in the second period of the succession experiment (this shift of the community was clear from April) and during spring and summer recruitment of both outer and inner study sites. Biofouling communities have frequently been reported to be dominated by filter feeders in several Mediterranean areas such as in Greece and South Italy (Antoniadou et al. 2013; Lezzi et al. 2017).

The present study shows that the community composition varies in relation to time and space, in terms of number of species, species composition, cover percentage and biomass, as already demonstrated in previous studies (Cornelio & Occhipinti-Ambrogi, 2001; Ramadan et al. 2006; Pierri et al. 2010, Toh et al. 2017; Lezzi et al. 2017).

Most of the recorded foulers, such as *Ulva* cf. *intestinalis*, *Mytilus galloprovincialis*, *Bugula neritina*, *Schizoporella errata* and *Styela plicata,* are organisms typical of biofouling communities (Relini & Faimali, 2003; Pierri et al. 2010; Lezzi et al. 2017). Moreover, some foulers known as NIS for the Mediterranean Sea have been recorded in the marina (Marchini et al. 2015; Ferrario et al. 2017; Lezzi et al. 2017; Ulman et al. 2017), such as *Amathia verticillata*, *Anadara transversa* and *Arcuatula senhousia*.

The number of identified taxa varied in time both in the succession and recruitment experiments and both in the inner and outer sites. Except for the last two months of the succession experiment, the outer site showed higher values of number of species. Comparing the peaks of number of species, the only match was recordable between the inner and outer sites in the spring period of the recruitment experiment, but the species composition was different. Moreover, numerous taxa identified in the marina, such as all cnidarians, several molluscs and annelids, were present only in the outer site. These results corroborate previous studies (Toh et al. 2017), which highlighted that the distance from harbour entrance can be the most important factor in determining the differences in the biofouling community. At the same time such differences can be dependent upon the lower number of larvae and less amount of food reaching the inner part of the harbour, as well as hydrodynamic conditions influencing larval recruitment and dispersal (Pierri et al. 2010).

The analysis of the biomass shows that the peaks of number of species do not match the peaks of biomass either in the succession and recruitment experiments. This observation suggests that the peaks of biomass are due to a limited number of species that are particularly abundant, or provided by heavy structures. As observed for the number of species, the biomass accumulated on panels is also highest in the outer site especially in the succession study, with maximum gap at the end of the experiment. The main contributor is the mussel *M. galloprovincialis,* which dominated the community from April to the last month of succession experiment. This pattern was not observed in the inner site of the harbour, where biomass peaked in June and the main contributor was the solitary ascidian *Ciona intestinalis*. The presence and growth of filter-feeding molluscs is controlled by food availability (Sarà & Pusceddu 2008) and may be one of the factors influencing the dominating presence of *M. galloprovincialis* at the outer harbour. Moreover, the outer harbour probably receives a more abundant supply of larvae than the inner site (Toh et al. 2017). In fact, the infralittoral bottom between the Ancona harbour and the Monte Conero Promontory (located few km far from the harbour) is characterized by rocky substrates with a community dominated by beds of *M. galloprovincialis* (Panfili et al. 2003, Froglia et al. 2006).

In the recruitment experiment the biomass reached the highest values during the summer period. This is a common trend related to environmental conditions and life strategies of sessile species (Kocak & Kucuksezgin 2000). In fact, in temperate waters, the productivity is generally reduced during winter and larval settlement is usually high during the summer period (Kocak & Kucuksezgin, 2000).

With very few exceptions (such as the corticated brown *Cutleria multifida*), the algae collected from the panels in both experiments are species with a simple morphological habit, i.e. foliose (*Ulva*), tubular (*Ulva* of *Entermomorpha*-type, *Scytosiphon*) or filamentous (*Cladophora*, *Chaetomorpha*, *Ceramium*, Ectocarpales, *Polysiphonia*). Seaweeds with such morphologies are ephemeral algae with high primary production, fast growth, short life cycles and high output of propagules, which typically act as rapid colonizers on newly cleared surfaces (Littler & Littler 1980; Steneck & Dethier 1994; Jänes et al. 2017). Green algae of the genera *Ulva*, *Cladophora* and *Chaetomorpha*, in particular, are well known as highly tolerant organisms capable to withstand large variations in environmental parameters (temperature, salinity, pH, nutrient load, concentration of chemical pollutants) and are often abundant in disturbed environments, especially when the nutrient load is high (Ballesteros et al. 2007; Kwon et al. 2017); their presence in the biofouling of harbours and marinas is nearly universal (Mathieson 2016; Neill & Nelson 2016; Rico et al. 2016; Kwon et al. 2017). In general, the stressful conditions of harbour environments tend to promote a homogenization of the algal communities, in which relatively few tolerant taxa become abundant; in algal communities described from these environments in different parts of the world, species of *Ulva*, *Cladophora*, *Ceramium*, Ectocarpales and *Polysiphonia* are almost constantly the main components (Terlizzi et al. 2000a, 2000b; Rico & Gappa 2006; Rico et al. 2012; Mathieson 2016;

Neill & Nelson 2016; Rico et al. 2016). Such a widespread distribution is promoted by the capacity of many of these algae to grow attached to the hulls of boats and ships (Mineur et al. 2007) and the wide dispersal of their propagules, which has been well documented for some species (Amsler & Searles 1980; Zechman & Mathieson 1985; Norton 1992). The capacity of these organisms to colonize rapidly newly available substrates is evident in the results of the succession experiment: algae were the fastest colonizers on the submerged panels and became the dominant group in the initial stages of the experiment, both in the inner and the outer site. This pattern, however, was not consistent in the recruitment study, where marked differences were observed between different seasons: algae were the most abundant recruiters in autumn and were still well represented in winter and spring, but their recruitment was almost inexistent in summer. We interpret these differences as a consequence of the vegetative and reproductive phenology of the most common species, which evidently have their periods of best growth and highest reproductive output in autumn and winter. The few comparable studies available for Mediterranean harbours reported algal communities with a composition similar to that of the present study, but showed some differences in temporal dynamics. In a similar experiment carried out for two years in the harbor of Ischia (that started in April 1996), Terlizzi et al. (2000b) reported ectocarpalean brown algae as pioneer taxa and remarked the significance of *Ectocarpus siliculosus* as a key species triggering the community and influencing its development. Differently from our results, on the exposed side of the control panels algae remained the dominant group for the whole period of the study. The dominance of algae observed in autumn and winter in our experiments is in agreement with the results provided by Kocak & Kucuksezgin (2000) from Turkish marinas, where they reported numerous algae during the colder period of the year.

The organisms of biofouling are usually categorised in “hard” and “soft” in relation to the presence of hard structures such as calcareous shells or skeletons (Zeinoddini et al. 2016). Hard biofouling is considered more problematic than soft one as it is heavier and more difficult to be removed from the substrate (Bressy & Lejars 2014). The biofouling settled on the panels of the succession experiment reveals that the community considered in this study is dominated by soft organisms (algae) during the early months, then hard organisms (mainly molluscs and annelids) become more relevant. The results of the recruitment experiment are in accord with these data: panels recruit soft biofouling in autumn, whereas the recruitment of hard biofouling becomes more substantial in spring and summer.

In conclusion, this study provides new insights on the biofouling communities colonising marinas of the North Adriatic Sea and their spatial and temporal dynamics. The data represent a starting point to better address the control methods used to overcome the biofouling problems associated with marinas. Findings reported in this study point out that specific investigations should be carried out for a better understanding of the dynamics of biofouling within marinas characterised by different environmental and ecological settings.

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CHAPTER 2

Italian boater’s perception of biofouling: a survey

Italian boater’s perception of biofouling: a survey

Abstract

Biofouling represents a critical problem for navigation and strongly affects the performance of boats. In 2016, an online survey was conducted among Italian boaters, investigating their perception of biofouling as a challenge, quantifying the expenses related to biofouling management, assessing their awareness of negative impacts of antifouling paints on the marine environment, and their knowledge of silicon coatings as eco-friendly alternative. Biofouling is perceived as an issue for boat and navigation, and to manage it the boaters pay on average 10-30% of total costs for boat maintenance. Although antifouling paints with biocides are considered a problem for the environment by most of the participants, the survey indicates a lack of information on more environmentally friendly alternatives such as silicon coatings. This indicates a need to increase the awareness of the boaters with specific information campaigns.

Introduction

Biofouling affects many maritime industries and has serious impact on boating activities. Biofouling leads to structural damages and increases drag and fuel emissions in vessels, causing operational challenges and increasing costs (Flemming 2002; Schultz et al. 2011; Stanley et al. 2014). In presence of biofouling on the boat hull, fuel consumption can increase up to 40% and the speed can decrease up to 10% (Kohli 2007; Schultz et al. 2011). Biofouling has been a critical problem for navigation since the beginning of shipping. As such to prevent biofouling accumulation, treatments based on specific paints are performed (Finnie & Williams 2010). At the beginning of ‘70s, Milne and Hail patented a new coating, the Tributyltin (TBT) (Milne & Hail 1971), which started a great revolution in the story of antifouling paints (Townsin 2003; Finnie & Williams 2010) and it was considered one of the most promising solutions against biofouling. However, TBT was proven to cause serious environmental problems, leading to negative effects on the health of bivalves and other marine organisms. After its complete ban by the International Maritime Organisation (IMO) from November 2001 (IMO, 2001), many studies were carried out to identify alternative antifouling technologies (Lejars et al. 2012; Gittens et al. 2013). Different types of coatings have been developed in the last decades and, among them, foul-release coatings were taken into account in several studies for their application in marine environment (Buskens et al. 2013). The coatings belonging to this family include silicones, fluoropolymers, hybrids and hydrogel silicones (Dafforn et al. 2011). Their function is not to prevent the adhesion of the organisms, but to reduce the strength of the adhesion without releasing biocides (Terlizzi et al. 2001; Dafforn et al. 2011; Faimali 2014), with the benefit to reduce costs and efforts during cleaning operations. For these reasons and their extremely durable structure (Anderson et al. 2003) silicone polymers are currently considered a good alternative to classic antifouling paints containing biocides (Swain et al. 1992; Buskens et al. 2013). Nevertheless, silicon coatings are not free of problems. Since they do not prevent the settlement of organisms, but decrease the strength of their adhesion, they increase the probability of their release during navigation (Bressy & Lejars 2014). Therefore, the risk for transfer of non-indigenous species (NIS) may be high (Piola et al. 2009). Moreover, more information is needed on the potential impact of compounds possibly released from silicone coatings (Feng et al. 2012).

Despite most research focussing on biofouling in commercial shipping, also marinas received attention from the scientific community during the last years, especially in studies concerning the problem of bioinvasions (Zabin et al. 2014; Anderson et al. 2015; Ferrario et al. 2017).

In Italy, recreational boating activity is widespread and represents a sector of strategic importance for the economic development of the country. According to the data provided by Italian Ministry of Infrastructures and Transports (MIT), the number of registered recreational boats has increased from 98 138 in 2007 to 101 055 in 2016 (MIT 2008, 2017). In some areas of the Italian peninsula, also the number of marinas has increased greatly during last years (Marchini et al. 2015), imposing to control this activity and make it as much sustainable as possible, from both economic and environmental points of view. The awareness of boat owners of biofouling, the travel history of their boat, and common biofouling maintaining practices have been investigated in New Zealand, Australia, Canada and California by means of questionnaires (Floerl et al. 2005; Floerl & Inglis 2005; Darbyson et al. 2009; Davidson et al. 2010; Clarke Murray et al. 2013). In the Mediterranean Sea, Mineur et al. (2008) carried out a preliminary survey to assess the role of recreational vessels as vectors for the macroalgal introduction in some marinas of France and Spain. Recently Ferrario et al. (2016) published the results of a questionnaire studying the spreading of NIS in relation to boater's habits that included Italian marinas. However, a sparse information is available on the perception of Italian boaters regarding the biofouling problem and the currently adopted solutions. Moreover, no data have been published about the costs sustained by Italian boaters related to biofouling management. To cope this gap, in this study we prepared and distributed an online questionnaire aiming to i) improve the knowledge about the Italian boater's habits related to their social and economic profile, ii) know their perception about the biofouling problem and estimate their economic loss, iii) assess the boater's knowledge about antifouling paints and their awareness to the problems related to them. In addition, the knowledge around and current use of foul-release coatings by Italian boaters was investigated to assess their attitude towards alternative eco-friendly technologies and to evaluate the need for a specific information campaign.

Materials and methods

An anonymous questionnaire in Italian language was created on the online platform Lime Survey and the link (https://servizi.scienze.univpm.it/surveys/index.php/153196?lang=it) was provided to Italian recreational boaters using two different methods: 1) mailing them directly through a cooperation with various boating clubs; 2) contacting them through a social network. The questionnaire was accompanied by a short description of the project and its purpose. Because of the way by which the questionnaire was distributed, it was not possible to measure the exact number of boaters that received the link or the response rate.

The questionnaire consists of 31 questions with 16 sub-questions, divided in 5 different sections (see Supplementary information, S1) and it was developed to obtain quantitative and qualitative information from the boaters. Respondents were asked to provide general information about the boat (e.g., type, age, temporal and spatial details of their trips over the past 12 months), about the knowledge on and management of biofouling (e.g., perception of biofouling as a problem for their activity, parts of the boat that are the most encrusted, mechanical cleaning of the boat hull), and about the use of antifouling paints (e.g., knowledge of antifouling paints, their impact on the environment, type of paint applied to their boat). In the last part questions about foul release coatings such as silicone coatings and their use by boaters (knowledge of silicone coatings, use of silicone coatings) were included. The final question was an open question, to explore possible solutions to the problem of biofouling proposed by boaters. The responses to the questionnaire were tabulated and then analysed. Since not all questionnaires were completed, the number of respondents to each question is given in parentheses.

Results

Profile of survey participants

A total of 211 questionnaires were returned, of which 170 were completed. The participants were 92% male and 8% female (n=211), and the age ranged between 20 to 78 years (64% were more than 50 years old). The largest group of participants (48%) had a high degree of education (n=210) (Figure 1a) and high professional profile (the 27% practices intellectual, scientific and high specialization professions) (n=209) (Table 1). The 54% of the respondents paid between 1000 and

5000 euros for total boat management expenses every year, while 17% paid between 5000-10000 euros (n=210) (Figure 1b).

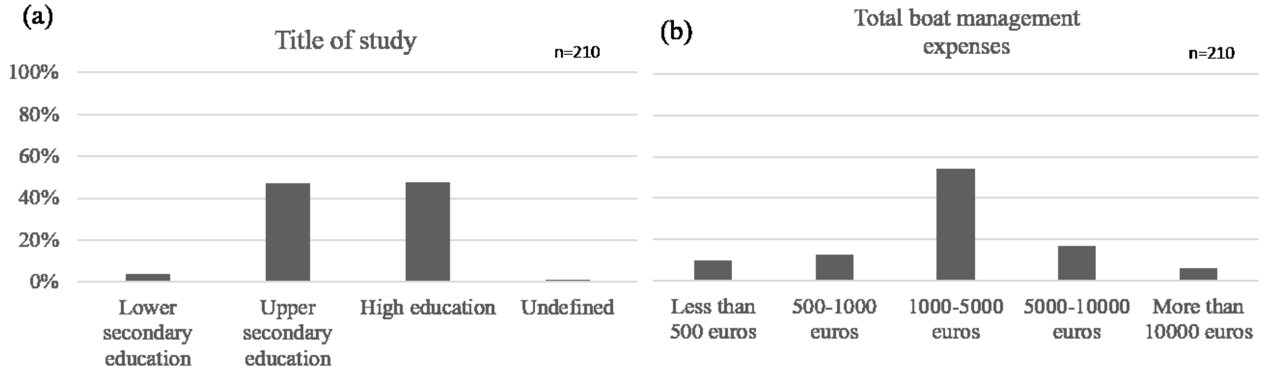

Figure 1 Summary of the general information

Table 1 Professions of the respondents (n=209) (ISTAT, 2011)

PROFESSION	% (n=209)
Intellectual professions, scientific and high specialization	27%
Executive professions in office work	14%
Pensioner	13%
Technical professions	11%
Legislators, entrepreneurs and high direction	9%
Artigians, specialized workers and agriculturals	4%
Qualified professions in commercial activities and services	3%
Armed forces	2%
Student	2%
Jobless	1%
Undefined	13%

General information

The home ports of the boats were distributed between the Tyrrhenian Sea (42%), the Adriatic Sea (40%), the Ligurian Sea (6%), and the Ionian Sea (3%) (n=195, Table 2a). Sailboats made up most of the crafts (77%) compared to powerboats (23%) (n=195) (Table 2b). The length of 50% of the boats ranged between 5 to 10 meters, while the 42% of them was 10 to 15 meters long (n=195) (Table 2c). Most boats were older than 10 years (69%) or 5-10 years old (23%) (n=195) (Table 2d), with the boat hull being almost exclusively made of fiberglass (95%). Of the participants, 57% (n=195) used the boat regularly (Figure 2a) and, when not in use, 81% stored the boat in the water, while the 19% stored it out of water (n=195). During the lasts 12 months, 49% of the interviewed used their boat for 1-10 trips (n=195) (Figure 2b); 25% of the boaters went 5-10 miles away from their home port, 24% more than 50 miles, 19% 10-25 miles, 18% 25-50 miles and 14% less than 5 miles (n=195) (Figure 2c). The maximum average cruising speed was less than 10 knots in most cases (79%) (n=195) (Figure 2d).

Most respondents did not moor the boat in other Italian harbours than their home port (75%) nor in foreign harbours (82%) (n=195).

Table 2 Data about: (a) boat types, (b) Location of the home port, (c) boat length and (d) boat age

(a) HOME PORT LOCATION	% (n=195)	(b) BOAT TYPES	% (n=195)
Tyrrenian Sea	42%	Sailboat	77%
Adriatic Sea	40%	Powerboat	23%
Ligurian Sea	6%		
Ionian Sea	3%		
Undefined	9%		
(c) BOAT LENGTH	% (n=195)	(d) BOAT AGE	% (n=195)
Less than 5 m	6%	Less than 1 year	1%
5-10 m	50%	1-3 years	2%
10-15 m	42%	3-5 years	5%
15-20 m	1%	5-10 years	23%
More than 20 m	1%	More than 10 years	69%

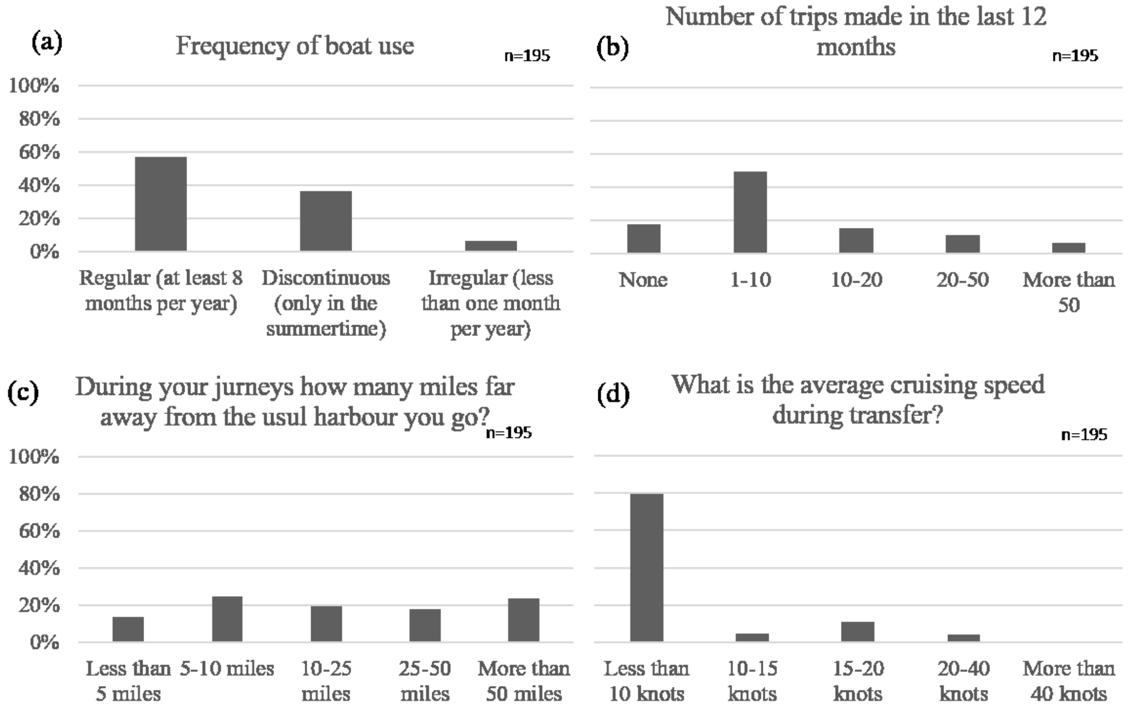

Figure 2 Overview on (a) frequency of boat use, (b) number of trips, (c) length of trips and (d) cruising speed

Knowledge and management of biofouling

Biofouling was considered an issue for the boats (53% strongly agree and 33% agree), and for navigation (52% strongly agree and 36% agree) by most interviewed boaters (n=181) (Figure 3a, b), while 42% consider biofouling also a problem for the environment (18% strongly agree and 24% agree). Problems due to biofouling were indicated by 28% of respondents (n=181), and the 51% of them reported the decrease in speed of their boat (n=51). Moreover, when questioned about the differences noted after hull cleaning, 92% of respondents (n=146, more than one answer was possible) indicate an increase in speed. The parts of the boat most encrusted or impacted by biofouling organisms were reported to be the ship hull (64%) and propeller (42%), followed by rudder and keel (23% and 19% respectively) (n=179, more than one answer was possible). The most frequent organisms on the boats, observed by 37% of the respondents, were barnacles (69%), followed by algae (42%) and calcareous polychaetes (37%) (n=65, more than one answer was possible). Restoration of antifouling paint was performed by 73% of the interviewed at least once since the purchase of the boat (n=180); among them, 48% usually did it once a year (n=180) (Figure 3c). Average yearly costs for antifouling paint were estimated between 500-1000 euros for 35% of the participants, while 25% estimated costs to be around 100-500 or 1000-2000 euros (n=131) (Figure 3d). For most the respondents (n=131) these costs represented 10-30% of the general maintenance expenses. In addition to the application of antifouling paints, hull cleaning was conducted by 61% of the boaters, using spatula or scrapers while diving, and most them (42%) do it two or more times every year (n=181) (Figure 4).

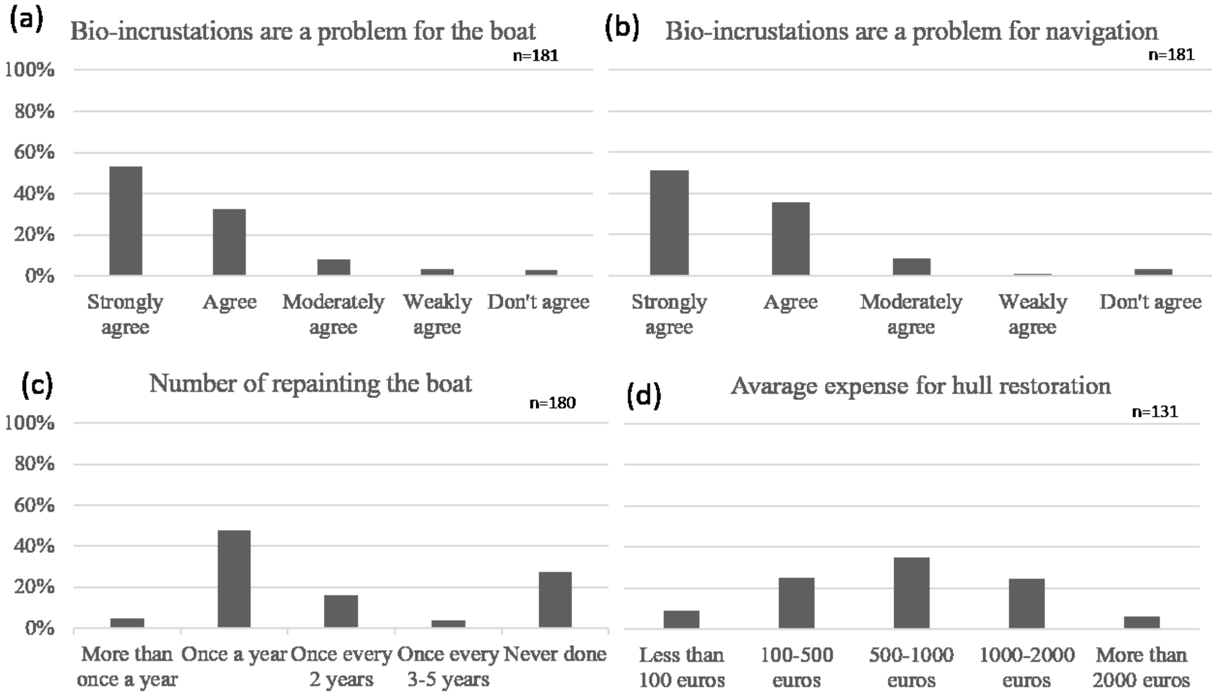

Figure 3 Overview on (a-b) perception of bio-incrustations, (c) frequency in the use of antifouling paints and (d) costs for the hull painting.

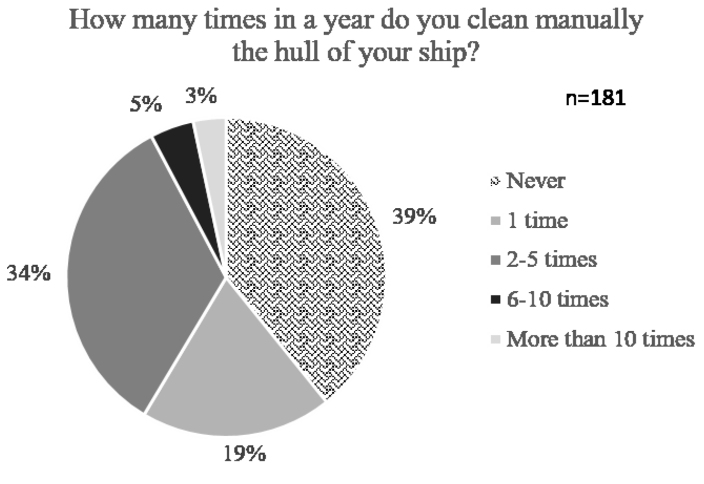

Figure 4 Number of manual hull cleaning events per year

Knowledge and use of antifouling coatings

Most interviewed considered the commercial antifouling paints containing biocides as useful and effective (48% agree and 17% strongly agree) (n=174) (Figure 5a). Although most were aware of the negative impact of biocides on the marine environment (49% agree and 24% strongly agree) (n=174) (Figure 5b), half of the boat owners did not know the type of paint applied to their own boat (50%) (n=174).

When question on their knowledge of alternative coatings, only 16% of the respondents knew silicone coatings (n=174) and most consider these coatings useful and effective (11% strongly agree and 54% agree) (n=28) (Figure 5c). Twenty-nine percent of them agree with the possibility of silicone coatings having a negative impact on the marine environment (15% strongly agree and 14% agree), while 32% moderately agree, 32% weakly agree and 7% did not agree (n=28) (Figure 5d). Most boaters that knew these coatings didn't used them (86%) (n=28), mainly because of their cost (46%), but also owing to the difficulty to find a supplier (21%) and their perceived poor efficacy (8%) (Figure 6). Twenty-one percent responded "other causes" indicating among them difficulty of application and removal of this type of coating (n=24) (Figure 6).

When interviewed about possible solutions to the biofouling problem, 41% of the respondents answered "I don't know", while 11% suggested solutions that could be categorised as eco-friendly, e.g., the use of products not harmful to the environment (n=169, more than one answer was possible) (Table 3). The category "other" included also the suggestion of a biomimetic approach.

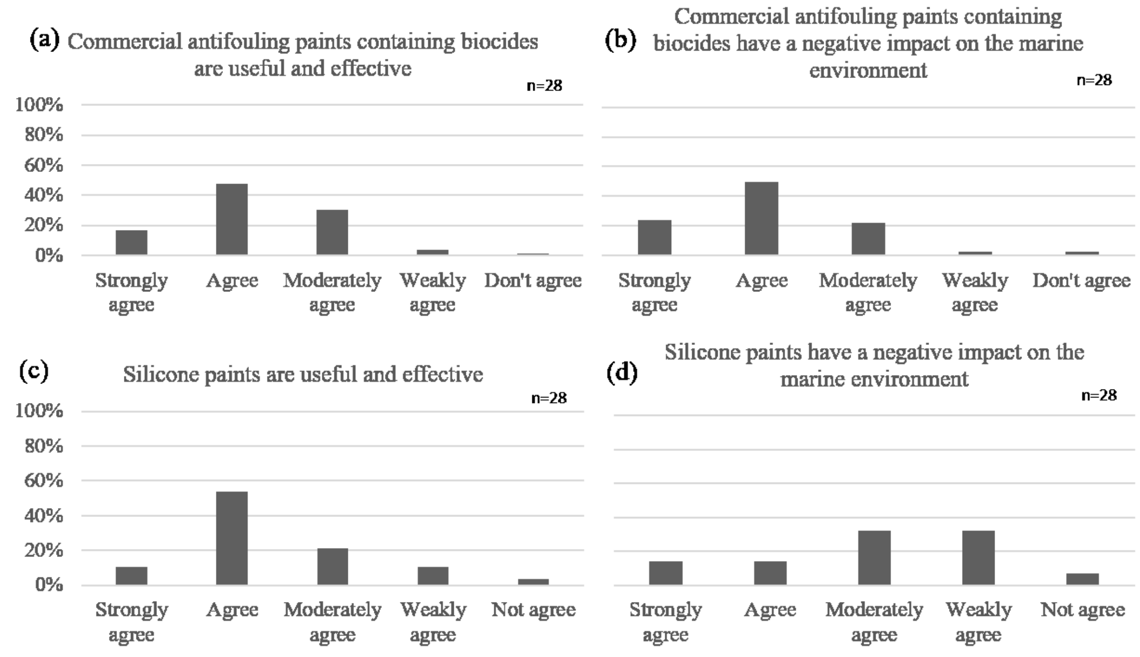

Figure 5 Opinions of Italian boaters about antifouling technology

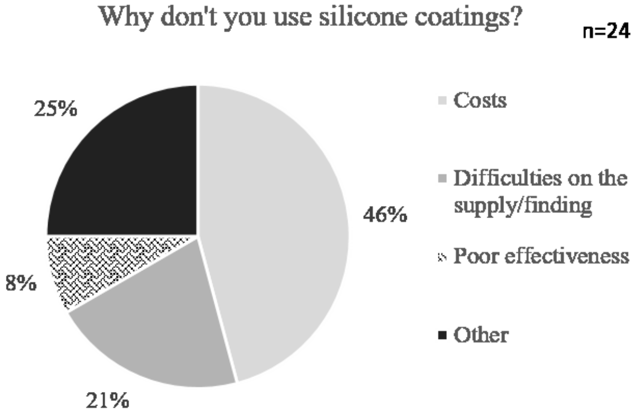

Figure 6 Reasons against the use of silicone coatings

Table 3 Possible solutions proposed by boaters to solve the problem of biofouling

POSSIBLE SOLUTION PROPOSED	% (n=169)
I don't know	41%
Antifouilng paints with biocides	17%
Eco-friendly solution	11%
Mechanical cleaning	8%
Implementation of the research	7%
More frequent use of the boat	3%
Drydocking of the boat	3%
Ultrasonic devices	2%
Other	8%
Undefined	5%

Discussion

Recreational boating and yachting activity has dramatically grown during last decades (Davidson et al. 2010). In the Mediterranean basin, more than 1.5 million recreational vessels are estimated, representing 70% of global charter boating traffic (Cappato 2011; Ulman et al. 2017). Nevertheless, information about the boaters and their habits and perceptions concerning biofouling and its management are lacking.

The survey results show that the typical Italian boater owns a sailboat, is male, older than 50 years, and has a high-level education or profession. These data appear to be representative from the geographic point of view, as the owner of the boat come from all the Italian Seas, and are in accord with the Italian boater profile defined by Ferrario et al (2016). The prevalence of sailboats has also been reported by other surveys carried out in California and this information has been considered representative of the local situation (Davidson et al. 2010; Zabin et al. 2014). However, the proportion of sailboats in the survey does not correspond to the number of registered recreational vessels under 24 meters length, where sailboats represent only 25.3%.

Sailboats are more likely to accumulate macrofouling due to their lower transit speeds (Clarke Murray et al. 2013). Boat owners reported that even small amounts of biofouling can impact the boat's speed and make navigation without the use of an auxiliary engine difficult. This may result in sailboat owners perceiving biofouling as a larger problem and therefore motivate them to participate in a survey. In fact, more sailboat than powerboat owners strongly agree with the affirmation that biofouling represents a problem for boat and navigation.

Biofouling is clearly perceived as a problem by boat owners, especially in terms of its impact on the vessel speed. However, they seem more confused about possible environmental problems. Most boat owners consider antifouling paints harmful for the marine environment, yet 30% of respondents seems unaware of problems directly related to the biofouling attached to the hull. This finding confirms observations by Ferrario et al. (2016) where 34% of respondents never heard about biological invasions or had misconceptions about the topic of NIS. Normal cleaning operations result to be demanding for boat owners. Most of the interviewees regularly clean the boat, e.g., by manually scrape it, and repaint it. The management of biofouling has large economic relevance as boat owners spend 10-30% of total maintenance costs on biofouling prevention and removal. Based on the 101055 recreational crafts hosted in Italian marinas (MIT 2017), and considering that most interviewees indicated an yearly average expense of 500 to 1000 euros for paint and/or restoration of the hull paint, the total yearly cost of biofouling management on Italian pleasure crafts can be estimated to range between 50 to 100 million euros.

Moreover, the survey suggests that boaters have limited interest in and knowledge about the type of paint applied to their boat, and that there is a strong lack of awareness about more eco-friendly solutions based on alternative products such as silicone coatings. Consequently, these products are little used in the field of recreational boating despite the fact that the few boat owners that use silicone coatings consider them useful, effective and eco-friendly. The main reason preventing the use of silicone coatings is the cost, as indicated by survey data. In addition, frequent cleaning is necessary to prevent the biofouling growth on vessels that do not have the speed to self-clean (Carson et al. 2009). The minimum speed necessary for full functionality of silicone coatings is between 10-20 knots (Dafforn et al. 2011; Watson et al. 2015). This survey shows that recreational boats usually travel with speeds less than 10 knots and may therefore not be able to fully exploit the potential of silicone paints. Therefore, this technology has long been exclusively used in military context to protect the submerged parts of ships and submarines that customary travel at higher speeds (Mazziotti et al. 2005; Townsin & Anderson, 2009). However, Watson et al. (2015) report that light brushing is sufficient to dislodge biofouling organisms attached to silicon coatings. Considering that boat owners manually scrape the hull several times a year to remove biofouling, the use of silicone coatings could result in easier cleaning of the boat. These coatings have an initial higher cost than classic antifouling paints containing biocides, but they may last 5-10 years (Bressy & Lejars 2014), leading to a compensation of the initially higher costs over time. However, this survey suggests that Italian boaters are not yet prepared to accept this technology, in fact only 11% of the respondents proposed eco-friendly products as a solution to the problem of biofouling. Therefore, these results emphasize the importance of a specific information campaign to increase the awareness of boat owners for possible alternative solutions.

In addition to the use on boats, foul release technology could also be suggested as a coating for submerged structures inside marinas, allowing easier cleaning. This prevention of biofouling on piers and buoys is important as they may represent hubs for larval dispersion i.e. to moored ships, and reservoirs and stepping stones for NIS.

In conclusion, this survey provides important knowledge about Italian boaters, showing that they perceive biofouling as a problem, which requires considerable costs for its management. Although Italian boat owners are largely aware that antifouling paints may impact the environment, the survey indicates a lack of information about alternative and more environmentally friendly coatings, highlighting the need to increase the awareness for such e.g., through specific information campaigns.

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Supplementary information

(S1)

PROGETTO BIOFOULING

(Anonymous questionnaire)

The questionnaire is part of a research project of the Polytechnic University of Marche, in collaboration with CNR Ismar of Genoa.

The aim of the project is to gather information about the problem of biofouling on boats and its impact on yachting. Our research can be used to improve the understanding of the marine environment, the management of biofouling and suggest potential solutions. Participation is voluntary and confidential. The questionnaire is anonymous. Our concern is merely scientific.

A) INTERVIEWED PROFILE

Age:
Sex:
Title of study:
Job:
How much are you paying per year? (total boat management expenses)

- Less than 500 euro
- 500-1000 euro
- 1000-5000 euro
- 5000-10000 euro
- More than 10000 euro

B) GENERAL INFORMATIONS

1_ Maritime compartments of the boat

……
……

2_ Boat type

- Sailboat
- Powerboat

2.1 If powerboat, please indicate the engine power:

………

3_ Boat length

- Less than 5 meters
- 5-10 meters
- 10-15 meters
- 15-20 meters
- More than 20 meters

4_ What is the boat hull made of?

- Fiberglass
- Wood
- Other (specify)……

5_ Boat age

- Less than 1 year
- 1-3 years
- 3-5 years
- 5-10 years
- More than 10 years

6_ Frequency of boat use

- Regular (at least 8 months per year)
- Discontinuous (only in the summertime)
- Irregular (less than one month per year)

7_ When not used, the boat:

- Continues to be moored in the harbour
- Is landed outside the water

8_ Number of trips made in the last 12 months with your boat

- None
- 1-10
- 10-20
- 20-50
- More than 50

9_ During your journeys, how many miles from the home port do you travel?

- Less than 5 miles
- 5-10 miles
- 10-25 miles
- 25-50 miles
- More than 50 miles

10_ Has the the boat been moored in other Italian harbours than the home port during the last 12 months?

- Yes
- No

If yes:

10.1 List them

……

10.2 Which was the maximum mooring time?

- 1 day
- 2-7 days
- 7-15 days
- 15-30 days
- More than 30 days

11_ In the last 12 months, has the boat been moored in foreign harbours?

- Yes
- No

If yes:

11.1 List them:

………

11.2 Which was the maximum mooring time?

- 1 day
- 2-7 days
- 7-15 days
- 15-30 days
- More than 30 days

12_ What is the average cruising speed during travel?

- Less than 10 knots
- 10-15 knots
- 15-20 knots
- 20-40 knots
- More than 40 knots

C) KNOWLEDGE AND MANAGEMENT OF BIOFOULING

1_ Bio-incrustations (also called biofouling) on the hull of ships are a problem for the boat

- Strongly agree
- Agree
- Somewhat agree
- Slightly agree
- Not agree

2_ Bio-incrustations (also called biofouling) on the hull of ships are a problem for navigation

- Strongly agree
- Agree
- Somewhat agree

- Slightly agree
- Not agree

3_ <u>Bio-incrustations (also called biofouling) on the hull of ships are a problem for the environment</u>

- Strongly agree
- Agree
- Somewhat agree
- Slightly agree
- Not agree

4_ <u>Have you ever had problems caused by bio-incrustation on your boat ?</u>

- Yes
- No

4.1 If yes, list them:

………
………
……

5_ <u>During the years owing this boat did you noticed organisms more frequent than other on the hull of your boat?</u>

- Yes
- No

5.1 If yes, specify what are the organisms and try to describe them, distinguishing between soft organisms (e.g., algae, sea-squirts) and calcareous one (e.g., tube worms, barnacles):

………
………
………

6_ <u>Which parts of the boat are the most encrusted and impacted by bio-fouling?</u>

………
………

7_ <u>Did you ever restore the original paint on the boat hull?</u>

- Yes
- No

If yes:

7.1 How many times have you repainted your boat?

- More than once a year
- Once a year
- Once every 2 years

- Once every 3-5 years
- Once every more than 5 years

7.2 What is the yearly average expense for painting and/or restoring hull paint?

- Less than 100 euro
- 100-500 euro
- 500-1000 euro
- 1000-2000 euro
- More than 2000 euro

7.3 How much does the maintenance expenses related to painting and/or restoration of paint on the boat hull weigh on general maintenance expenses of your boat? (Put a circle on a percentage value)
10% 20% 30% 40% 50% 60% 70% 80% 90% 100%

8_ Do you mechanically clean the hull of the boat during the year by diving below the boat, using spatulas and/or scrapers?

- Yes
- No

8.1 If yes, how many times in a year?

- Never
- 1 time
- 2-5 times
- 6-10 times
- More than 10 times

9_ Did you perceive differences in term of performance of your boat (in fuel consumption and navigation speed) before and after hull cleaning operations?

- Yes
- No
- I don't know

9.1 If Yes, indicate the variation that you perceive:

………
………
…….

D) ANTIFOULING PAINTS

1_ Commercial antifouling paints (also called antifouling paints) containing biocides are useful and effective

- Strongly agree
- Agree
- Somewhat agree
- Slightly agree
- Not agree

2_ Commercial anti-vegetative paints (also called anti-fouling paints) containing biocides have a negative impact on the marine environment

- Strongly agree
- Agree
- Somewhat agree
- Slightly agree
- Not agree

3_ Do you know the type of paint applied to your boat?

- Yes
- No

3.1 If yes, specify the type of paint (hard-core antifouling / self-polishing antifouling) or the specific product used, referring on the boat's area where they are applied:

Hull: …….

Metal parts (e.g. helm, propeller):

…….

4_ Do you know foul-release coatings such as silicone coatings?

- Yes
- No

If yes:

4.1 Silicone coatings are useful and effective

- Strongly agree
- Agree
- Somewhat agree
- Slightly agree
- Not agree

4.2 Silicone coatings have a negative impact on the marine environment

- Strongly agree
- Agree
- Somewhat agree
- Slightly agree
- Not agree

4.3 Are you using silicone coatings?

- Yes
- No

4.3.1 If not, explain why:

- Costs
- Difficulties with the application
- Difficulties with finding suppliers
- Poor efficacy
- Other (specify)

……….

E) POSSIBLE SOLUTIONS

1_ How do you think the biofouling problem can be solved?

……
……
……
……

Thank you for your contribution.
For more information, curiosity and clarification, please contact us at:

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CHAPTER 3

Biofouling in finfish aquaculture: a case study from the South Adriatic Sea (Mediterranean Sea)

Biofouling in finfish aquaculture: a case study from the South Adriatic Sea (Mediterranean Sea)

Abstract

Finfish aquaculture is a growing sector in the Mediterranean Sea that plays an important role in suppling food and reducing the dependence on the overexploited wild fish stocks. Biofouling, the growth of marine organisms on submerged surfaces, is one of the major challenges in finfish farming and may led to direct and indirect costs for the aquaculture industry, such as biofouling control, change of damaged net, detrimental effects on fish. However, only few studies have investigated fouling and the problems related to it in the Mediterranean Sea. The aim of this work was to improve this situation by investigating the temporal and spatial changes of biofouling community in finfish aquaculture farm located in the South Adriatic Sea. In particular, we analysed on an annual basis the composition and the biomass of biofouling colonising artificial substrates in relation to immersion period, season and water column depth. Our results indicate that these factors strongly influence the fouling composition and the biomass in this area. The high diversity of 111 recorded species shows that the finfish farms are able to harbour a wide range of taxa, potentially acting as stepping stones for the spreading of species. While filter/suspension-feeding organisms dominated the communities, the biomass composition differed with immersion period, season and depth. During the successional study the biomass was mainly driven by the ascidian *Styela plicata* demonstrating to be the supreme competitor for space and indicating its crucial role as structuring species of the climax stage. Overall, these results provided new insights for the sustainable management of biofouling in fish farms and for the implementation of environmental monitoring programme guidelines for marine finfish cage farming as required by the Blue Growth Initiative of the FAO.

Introduction

Marine aquaculture became globally important with production of great amount of biomass due to the decline in global fisheries (Fitridge et al. 2012; FAO 2016). The farm structures act as artificial habitats creating suitable settlement surfaces for the settlement of marine organisms (Fitridge et al. 2012). The aquaculture industry has to face economic costs for biofouling control and from the loss of reared organisms that usually are difficult to estimate (Claereboudt et al. 1994; Watson et al. 2009; Fitridge et al. 2012). Problems related to fouling and data concerning its composition and succession are well documented worldwide and they are related both to farm activities and reared organisms (Khalaman 2001; Braithwaite et al. 2007; Baxter et al. 2012; Fitridge et al. 2012; Park & Hwang 2012; Peteiro & Freire 2013; Sievers et al. 2013; Velmurugan et al. 2013; Edwards et al. 2015; Lacoste and Gaertner-Mazouni 2015; Bosch-Belmar et al. 2017). The species composition of the biofouling community depends on several biotic and abiotic factors (Callow and Callows 2002, 2009; Fitridge et al. 2012), and on geographical location although many biofouling species are considered cosmopolitan (Fitridge et al. 2012). Fouling variations are mainly driven by season, light availability and water flow. Moreover, it changes also in relation to the depth and orientation of infrastructure (e.g. Cronin et al. 1999; Greene and Grizzle 2007; Howes et al. 2007; Guenther et al. 2010; Bloecher et al. 2013). Usually a decrease of biomass and diversity with water depth is recorded (Cronin et al. 1999; Guenther et al. 2010; Fitridge et al. 2012). The biofouling communities are generally dominated by suspension-feeding organisms, including sponges, hydroids, molluscs, crustaceans and ascidians (de Nys and Guenther 2009; Dürr and Watson 2010; Fitridge et al. 2012, Bloecher et al. 2013), and when light is not limiting can be also dominated by algae (Sliskovic et al. 2011; Fernandez-Gonzalez and Sanchez-Jerez 2017). Fouling biomass is generally related to the faunal composition and its value changes along the water depth and with season with usually a maximum in the summer (Cronin et al. 1999; Greene and Grizzle 2007; Sliskovic et al. 2011; Bloecher et al. 2013). In the Mediterranean Sea the marine aquaculture started in the 80’s by the culture of several fish species (Grigorakis and Rigos 2011) and then this industry is rapidly grown with the opening of many farms (Dapueto et al. 2015; Squadrone et al. 2016; FAO 2016). Several studies have been conducted on biofouling in aquaculture and related problems in Mediterranean Sea (e.g. Tsiamis et al. 2008; Galil 2009; Grigorakis and Rigos 2011; Marchini at al. 2016) but few on finfish farming (Sliskovic et al. 2011; Fernandez-Gonzalez et al. 2016; Fernandez-Gonzalez and Sanchez-Jerez 2017). In the Mediterranean Sea, the fouling communities of fish farms are mainly composed by hydroids, bryozoans and bivalves with an abundant motile fauna associated (Fernandez-Gonzalez and Sanchez-Jerez, 2017). Regional variability in biofouling composition is observed in Mediterranean Sea. In Croatia, the algae can be the main taxon in fouling community (Sliskovic et al. 2011), while in Spain mussels and hydroids dominate (Fernandez-Gonzalez and Sanchez-Jerez, 2017). The aquaculture is a growing sector in the Mediterranean Sea having an important role in food supply reducing the dependence on the overexploited wild stocks. In this area, the aquaculture production is expected to reach over 4 600 000 tonnes in 2020-2030 highlighting the attention to the overall sustainability of the sector (FAO, 2017). In this context, the FAO has launched the Blue Growth Initiative with the aim of securing a productive and sustainable aquaculture in the Mediterranean Sea and Black Sea. The guiding principles of the aquaculture strategy are several and one regards the “best available information” that should be collect from the General Fisheries Commission for the Mediterranean (GFCM) (FAO, 2017). Information regarding biofouling communities and the related problems for finfish farm activities are scant in this area. This study aims to improve the knowledge about the biofouling community and its variation over time and depth in a Mediterranean finfish aquaculture in order to provide insights useful for the sustainable management of biofouling in fish farms and for the implementation of environmental monitoring programme guidelines for marine finfish cage farming. To archive this objective we have investigated 1) the succession of biofouling composition and biomass over a one-year study, 2) the effect of seasonality in the recruitment of species and in the development of the biofouling and biomass and 3) the effect of water column depth.

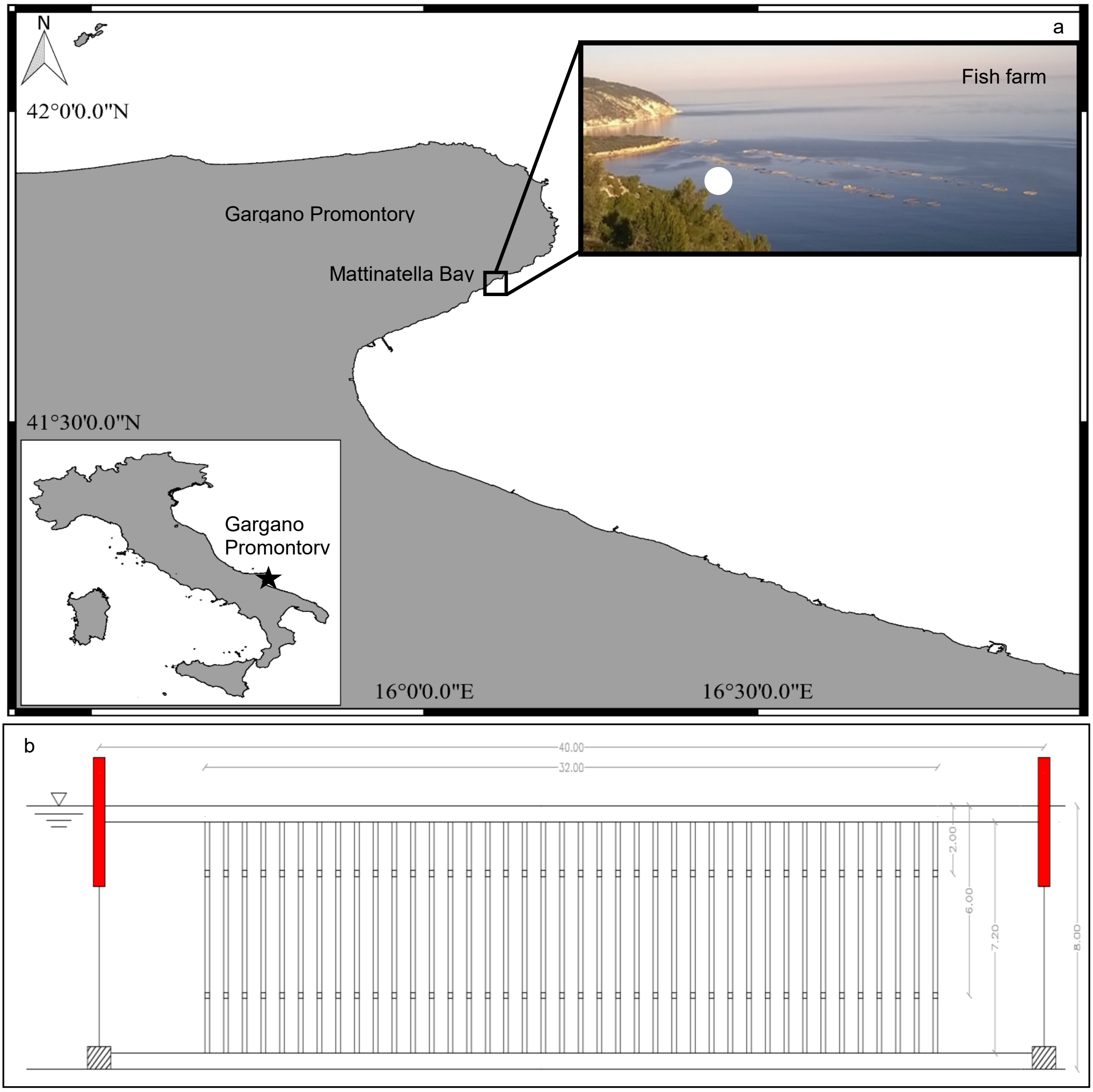
Materials and Methods

Study site

The field experiment was conducted in a commercial off-shore aquaculture farm (Maricoltura Mattinatese, www.maricoltura.com) in Mattinata (Foggia, Italy), located in front of the Gargano Promontory in the bay of Mattinatella (41°43'37.03"N, 16°6'36.52"E; Fig. 1a).

The study site is characterized by sand bottom with a depth ranging between 7-12m. The cages containing seabass (*Dicentrarchus labrax*) and seabream (*Sparus aurata*) are submerged at the mean depth of 8 m. The feed supply is manual and the commercial size of the fish (300-350 g) is reached during 16-18 months of farming for seabream and 18-20 months for seabass. In 2015, the farm had 48 large circular cages (diameter 17.2 m, volume 1200 m^3) and 18 small circular cages (diameter 11 m, volume 500 m^3). The cages are arranged in two rows, parallel to the coast and equipped with an uncoated polyethylene multifilament net (Dyneema, 1.5 x 1.5 cm mesh size).

Figure 1 a Location of the studied farms in Mattinatella Bay on the Gargano Promontory, south-east coast of Italy, in the Adriatic Sea; b. experimental structure positioned in front of the farm cages (with point in the a.).



Experimental design and sampling

A long line system of 40 m in length was installed parallel to the cages of the farm at 8 m depth (Fig. 1b). For the successional experiment, a set of 36 lines were installed in April 2015 while for the recruitment experiment a total of 12 lines in June 2015. The lines were positioned at 0.8 m each other. Each line carried two net panels, one located at 2m depth and the second at 6 m depth (Fig. 1b). The panels were made by the same net of the farm cages, polyethylene multifilament net, and had the dimension of 20 x 20cm. For the successional experiment, each month three lines were collected randomly for a total of three panels at 2 m depth and three panels at 6 m depth. For the recruitment experiment, three lines with a panel at 2 and 6m depth each were immersed in June 2015 and then sampled and replaced every three months. The trimester have been considered as seasons: June-July-August as summer, September-October-November as autumn, December-January-February as winter, and March-April-May as spring. Each panel was inserted in a labelled plastic bag and then cut from the ropes by scuba divers to avoid organisms loss (both sessile and mobile). The seawater were then filtered by means of a sieve with 500 µm mesh. All the organisms were collected in the bag with the panel and were preserved in alcohol 70%. Two panels from 2 m depth of the successional experiment were lost due to a storm in April 2016, and the analysis in this month was limited to the 6 m depth panels.

Samples analysis

In the laboratory, each net panel was carefully rinsed with fresh water on a sieve with a 500 µm mesh in order to collect all the mobile organisms. The panel was observed under a stereomicroscope. All the organisms were identified to the lowest taxonomic level possible and their presence/absence recorded. When clear species distinction was not possible, species were included in a multispecies category. Successively, species or group of taxa were considered separately on the basis of their abundance in the biofouling community and their biomass (>0.10 g) were recorded. Taxa with biomass less than 0.10 g were placed in the category “other”. Each group were then well-drained and the wet weigh determined. Results of wet weight were reported as mean weight (g) per m^2 ± standard error (SE).

Statistical analysis

Differences in total biomass and species composition (based on species wet weights) over time (month, Mo: fixed factor with 12 levels; season, Se: fixed factor with 4 levels) and between depths (De: fixed factor with 2 levels) were assessed with a permutational multivariate analysis of variance (PERMANOVA, PRIMER 7 with PERMANOVA+ add-on package; Anderson et al. 2008). The univariate analysis for the total biomass was based on Euclidean distance, while the multivariate analysis of the biomass composition was based on Bray–Curtis similarity. Both analyses were conducted with square root-transformed data and 9999 permutations of residuals under a reduced model. A significance level of 5% was used. When less than 100 unique values in the permutation distribution were available, asymptotical Monte Carlo P-values instead of permutational P-values was used. Significant interactions among main factors were investigated by post-hoc pairwise tests. A non-metric multidimensional scaling (nMDS) was used to explore differences in biomass composition in both the experiments.

Results

Considering all the 96 panels from both the experiments, 109 species and 2 multi species groups were identified (Table 1).

Successional study

On 72 net panels, 95 taxa were identified among the mobile (58) and sessile (37) fouling organisms. 52.6% of the species belonged to three taxa, the polychaetes (30 species), the amphipods (11 species) and the bivalves (9 species). The other fouling organisms were hydroids and bryozoans (7 species each), decapods and gastropods (6 species each), ascidiaceans (5 species), algae and isopods (3 species each), tanaidaceans (2 species) and sponge, anthozoans, nemerteans, platyhelminthes, cirripeds and ophiuroids (1 species each). The total diversity value varied with time, reaching the highest value in December (21.7 ± 2.7 species; Figure 2a).

At both depths, 74 species were recorded and polychaetes, amphipods and bivalves were the more diversified taxa (21, 10 and 8 species respectively at 2 m depth; 19, 11 and 8 species respectively at 6 m depth). The two depths do not differs in number of species for the analysed months of study (Figure 2a). Highest value of species richness at 2 m depth was observed in March (21.7 ± 5.7 species) while at 6 m depth in October (23.3 ± 5.5 species). The diversity increased with time of submersion until October, from when on the diversity in both depths oscillated simultaneously between 21.7 ± 3.3 / 11.0 ± 1.5 species (Figure 2a).

Table 1 List of taxa identified in the finfish farm during the study; s= succession; r= recruitment;

	2m	6m		2m	6m
ALGAE			*Lumbrineris* sp.		s
Ceramium sp.	r	r	*Marphysa fallax*	s	
Halimeda tuna	s		*Myrianida* sp.1	s	s
Polysiphonia sp.1	sr	sr	*Myrianida* sp.2		r
Polysiphonia sp.2	sr	sr	*Neanthes* cf *nubila*	s	
PORIFERA			*Nereis* cf *pelagica*		s
Chondrosia reniformis		s	*Nereis* cf *rava*	s	
ANTHOZOA			*Nereis perivisceralis*	s	s
Anemonia viridis	s	sr	*Nereis splendida*	s	sr
HYDROZOA			*Nereis zonata*	s	
Bougainvilliidae sp.1	sr	sr	*Orseis pulla*	s	s
Bougainvilliidae sp.2		r	*Oxydromus flexuosus*		s
Clytia hemisphaerica	r		*Paradoneis lyra*	s	
Ectopleura crocea	sr	sr	*Platynereis* cf *australis*	r	
Kirchenpaueria halecioides	sr	sr	*Platynereis coccinea*		r
Lafoeina tenuis		s	*Platynereis dumerilii*	sr	sr
Laomedea calceolifera	sr	sr	*Polyophthalmus pictus*	sr	r
Merona sp.	sr	sr	*Sabellaria spinulosa*		sr
Pennaria disticha	sr	sr	*Scoletoma fragilis*		s
NEMERTEA			*Sphaerosyllis pirifera*	sr	sr
Nemertea spp.	sr	sr	*Subadyte pellucida*		s
PLATYHELMINTHES			*Syllis gerlachi*	sr	sr
Platyhelminthes spp.	sr	sr	*Terebella lapidaria*	s	
BRYOZOA			*Websterinereis* cf *glauca*		s
Bugula neritina	sr	sr	AMPHIPODA		
Bugulina calathus	sr		*Apocorophium acutum*	sr	sr
Bugulina stolonifera	s	s	*Caprella dilatata*	sr	s
Conopeum reticulum		r	*Caprella equilibra*	sr	sr
Cradoscrupocellaria berthollet	sr	r	Dexaminidae	r	
Nolella sp.	r	s	*Elasmopus rapax*	sr	sr
Schizoporella cf *errata*	sr	sr	*Ericthonius didymus*	sr	sr
Scruparia ambigua	s	s	*Jassa marmorata*	s	s
GASTROPODA			*Jassa slatteryi*	sr	sr
Alvania sp.		s	*Lysianassa* cf *longicornis*	r	s
Bittium sp.		sr	*Monocorophium acherusicum*	sr	sr
Bolinus brandaris		sr	*Quadrimaera inaequipes*	sr	sr
Calma glaucoides	s		*Stenothoe tergestina*	sr	sr
Conus ventricosus	s		ISOPODA		
Rissoidae	sr	sr	*Cymodoce* sp.	s	s
BIVALVIA			*Gnathia* sp.	r	
Aequipecten sp.1	sr	sr	*Paranthura* cf *japonica*	sr	sr
Aequipecten sp.2	s		*Uromunna petiti*		sr
Anadara transversa	sr	sr	TANAIDACEA		
Anomia ephippium	s		*Chondrochelia savignyi*	s	s
Arca noae		s	Tanaidacea	s	
Gibbomodiola adriatica	r		DECAPODA		
Hiatella arctica	sr	s	*Athanas nitescens*	sr	s
Modiolus barbatus	s	sr	*Eualus occultus*	sr	s
Mytilus galloprovincialis	sr	sr	Inachidae	r	
Talochlamys multistriata	s		*Pachygrapsus marmoratus*	s	s
ANELLIDA			*Pilumnus hirtellus*	sr	s
Amphitrite sp.	sr		*Pisidia longicornis*	sr	sr
Amphitritides cf *kuehlmanni*	r		*Upogebia pusilla*		s
Ceratonereis costae	sr	sr	CIRRIPEDIA		
Cirratulidae sp.		r	Balanidae	s	
Dorvillea rubrovittata		r	OPHIUROIDEA		
Dorvilleidae		s	*Amphipholis squamata*	sr	sr
Eumida sanguinea	s	sr	Ophiuroidea ind.	r	
Eunice schizobranchia	s		ASCIDIACEA		
Exogone dispar		r	Colonial ascidian sp.1	sr	sr
Harmothoe cf *johnstoni*	s		Colonial ascidian sp.2	r	sr
Harmothoe sp.	s	r	*Didemnum* sp.	sr	sr
Hydroides elegans	sr	sr	*Polycarpa* sp.	s	s
Hydroidres nigra		s	*Styela plicata*	sr	sr

The total biomass changed significantly over time and depths (‘Mo’, ‘De’ and ‘MoxDe’: $p<0.001$; Figure 2b). In February, the maximum value of total biomass was reached with 5895.7 ± 1945.4 g/m^2 (Figure 2b). Considering the depth, at 2 m after four months of submersion the total biomass reached a value of 5798.8 ± 758.8 g/m^2 and the highest value is recorded in February (9922.5±1552.4 g/m^2) (Figure 2b), while at 6 m depth the maximum value was recorded in October (4104.4±271.8 g/m^2). In particular, total biomass was significantly different in the last months of the experiments and in September (pairwise tests, $p < 0.05$). The value of biomass increased greatly at 2m respect 6m (Figure 2b).

The biomass contribution of the taxa differed significantly over time and between depths (‘Mo’, ‘De’ and ‘MoxDe’: $p<0.001$; Figure 2b). In September, December, January and March, the total biomass was significantly higher at 2 m depth when compared with 6 m depth (pairwise tests, $p<0.05$; Figure 2b). The nMDS analysis, based on taxa biomass, showed a separation between the first three months and the following months, with no differences between the depths. Moreover, the factors depth (Figure 3a) mainly separated the last analysed months.

The main taxa that contributed to the total biomass were ascidians, polychaetes, bryozoans and molluscs. The biomass of other taxa did not exceed 0.01g (collected in the category “other” in Figure 2c). The taxon that mainly contributed to the total biomass from August to March were the solitary ascidians with the species *Styela plicata* (Figure 2c). Such a dominance was also recorded at 2 m depth. Independent from water depths, during the first months the total biomass was dominated by the soft tubes of amphipods and algae. In June together with several other taxa the mollusc increased and became dominant in term of biomass in July (on average 83.4% in total; 82.4% at 2m; 84.6% at 6m). At 6 m depth temporal changes of biomass were observed. In September the ascidians that dominate the previous month (59.8%) decreased (23.5%) and the polychaetes (mainly *H. elegans*) increased in weight (27.7%). The ascidians increased again their weight in October (62.7%) and then decreased until March (64.4%). In November the dominant taxon was the bryozoans (69.7%) with *B. neritina* and *Schizoporella* cf. *errata*. In December the biomass was composed in weight by the “others” (40.5%) and the polychaetes (37.5%) while in January by only the “others” (55.4%). In February, total biomass was ascribed for a large fraction to molluscs (40.1%).

Figure 2 Succession study. a. total diversity of the fouling community; b. total biomass trend vs. total diversity; c. Biomass composition of the main taxa with the value of the biomass (red line). Tot= total.

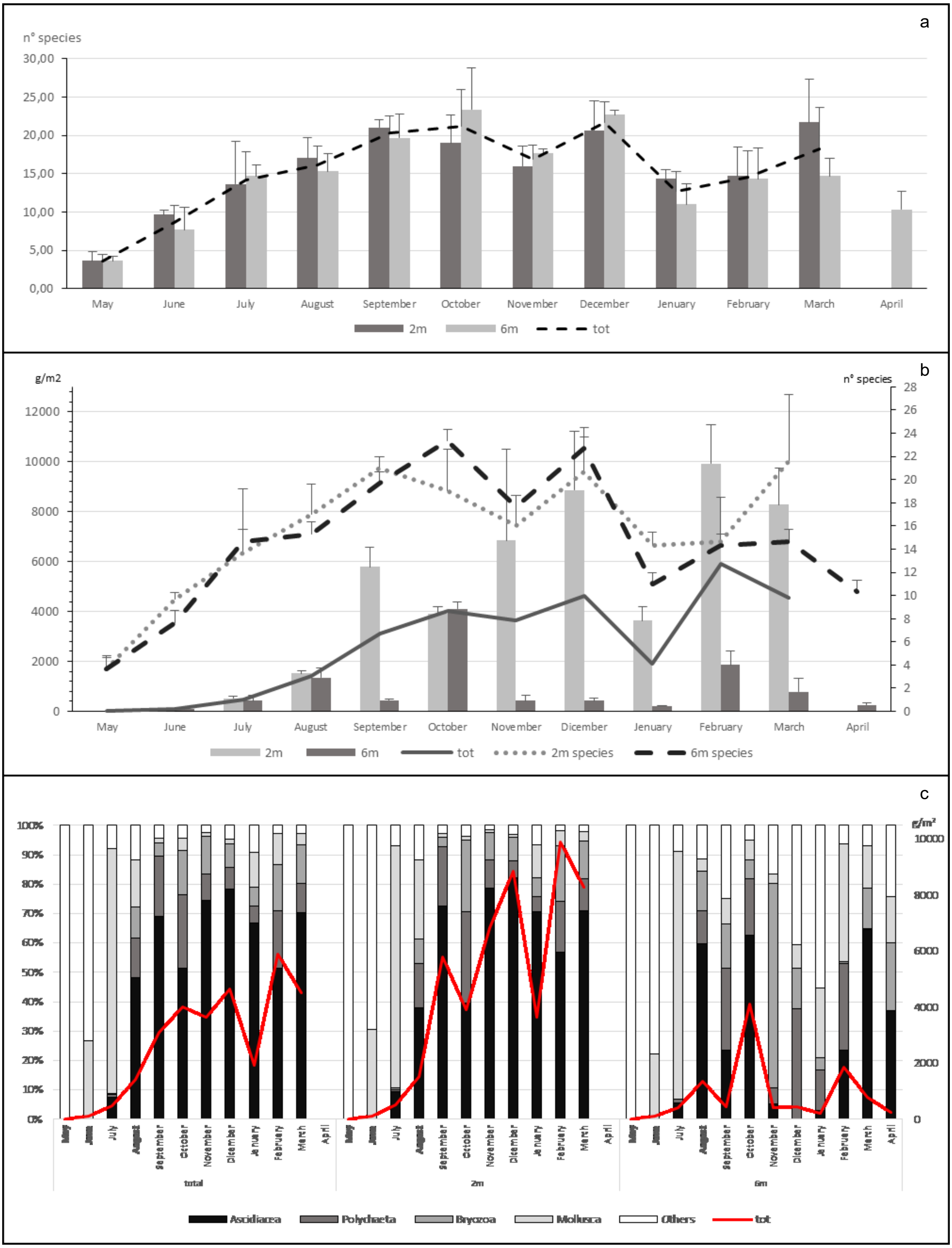
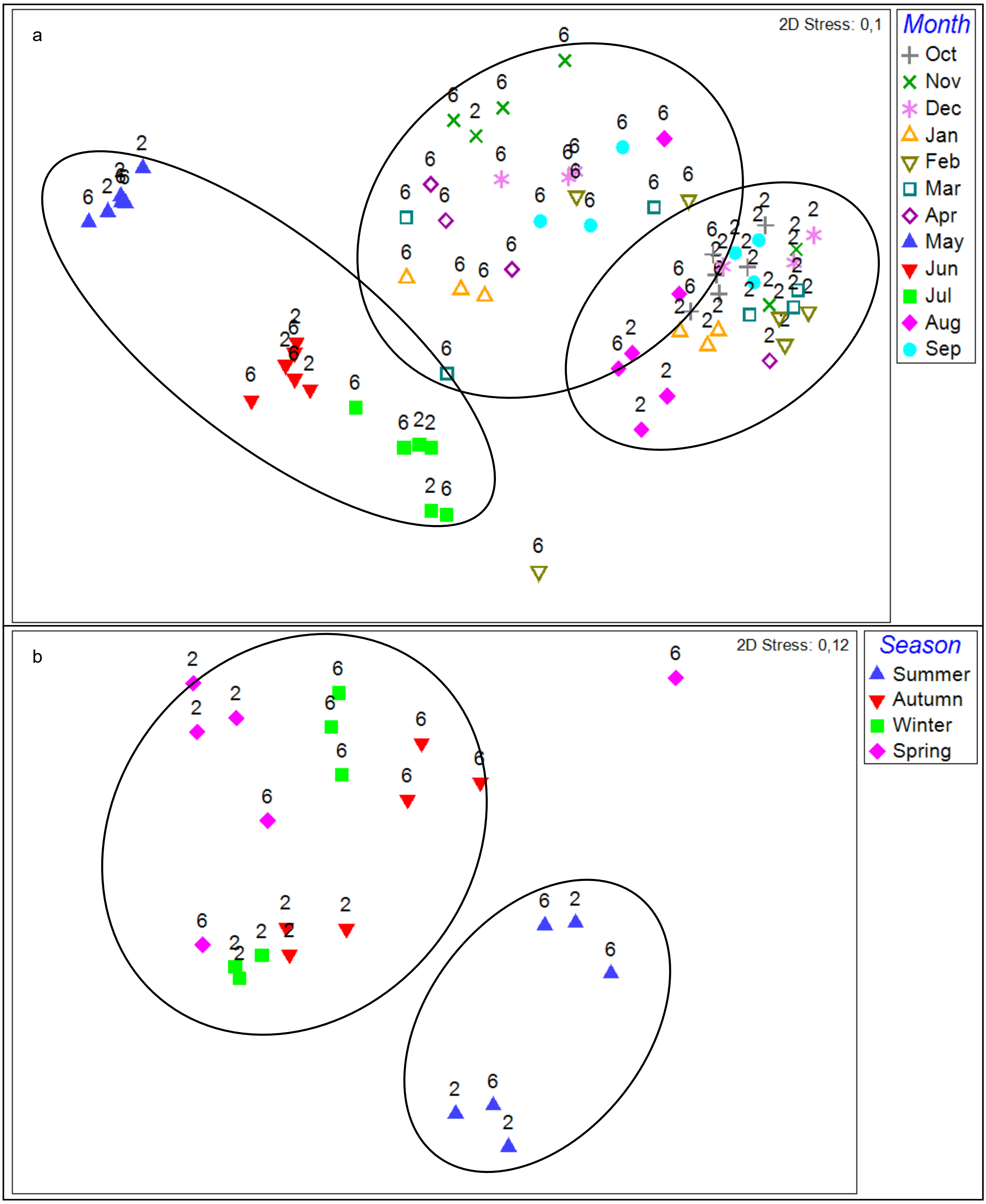


Figure 3 Non-metric multi-dimensional scaling (MDS) plot of biofouling biomass composition in a. the succession study and in b. recruitment study. Times (moth/season) are represented by colours, depths (2 and 6m) by numbers.



Recruitment study

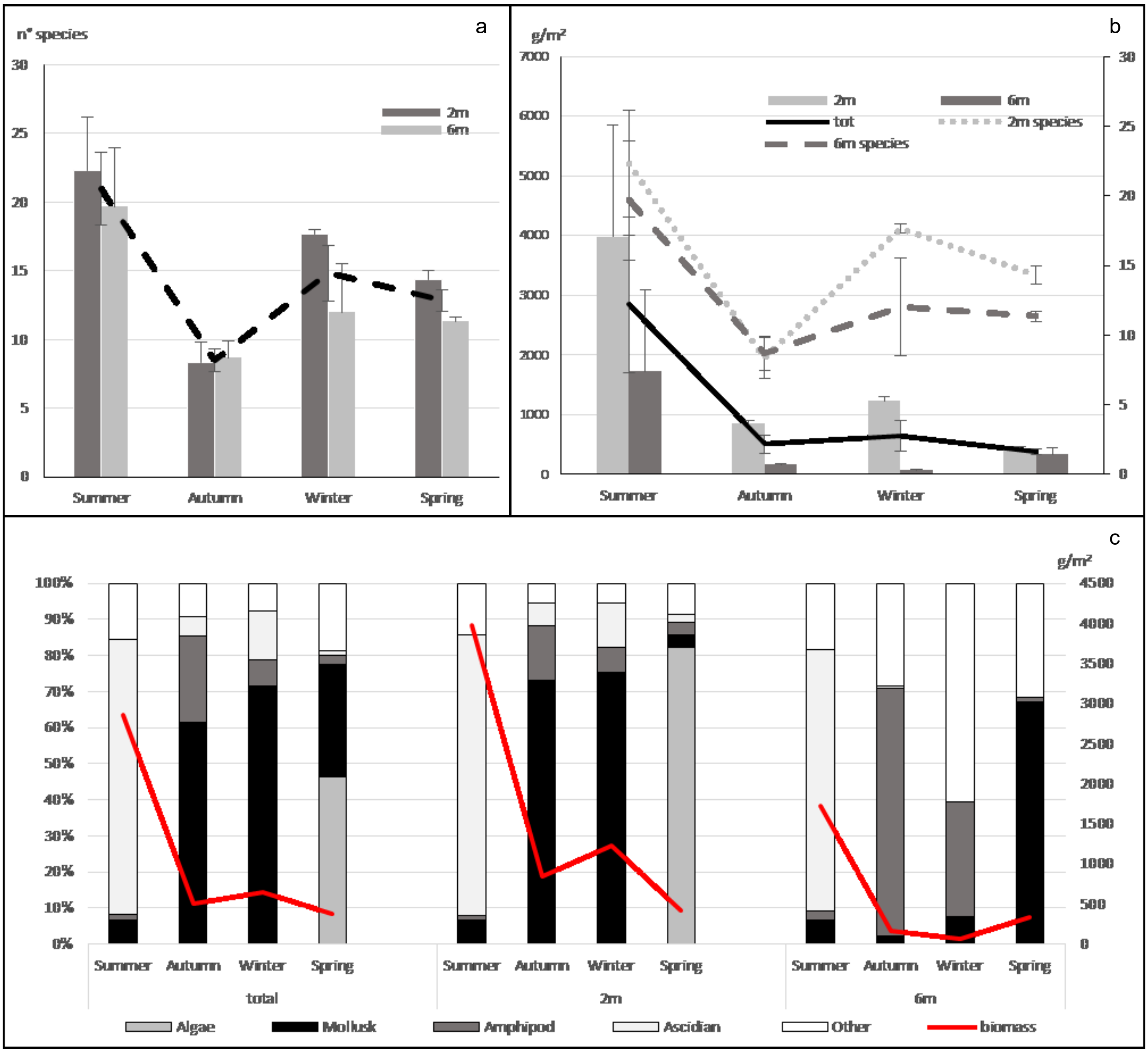
On 24 panels, 72 taxa were identified among the mobile (39) and sessile (33) organisms in the fouling community. The most representative taxa were the polychaetes (18 species), the amphipods (11 species) and the hydroids (8 species) representing the 51.4% of the total diversity. The other organisms were the bryozoans and bivalves (6 species each), decapods (5 species), the ascidians (4 species), algae, isopods and gastropods (3 species each), ophiuroids (2 species) and anthozoans, nermerteans and platyhelminthes (1 species each). The total diversity varied with season, with the higher value in summer (21.0±2.6 species) (Figure 4a). There was no difference in total number of taxa between the two depths (56 taxa at 2m; 55taxa at 6m). The most frequent taxa at both depths were the polychaetes (9 and 15 species respectively), the amphipods (11 and 8 species, respectively) and hydroids (7 and 7 species, respectively), representing the 48.2% and the 54.5% of the total diversity at 2 m and 6 m depth, respectively. At both depths, maximum diversity was recorded in summer (22.3±3.8 species at 2m and 19.7±4.3 species at 6m; Figure 4a).

The total biomass value changed significantly between seasons and depths (‘Se’, ‘De’: $p<0.05$). The maximum total biomass value, independently from the depth, was reached in summer with 2853.5±1150.6 g/m^2. Similar to the trend observed in species diversity was the highest biomass accumulated during the summer months in both depths (2m: 3982.3±1866.7 g m-2, 6m: 1724.7±1364.07 g m-2, Figure 4b). Biomass was generally higher on samples from 2m depth than from 6m depth (Figure 4b). The biomass contribution of the individual taxa varied with season and depths (‘Se’ and ‘De’: $p<0.05$), and differed between all seasons with the exception of winter and spring in 6 m depth (pairwise $p_{MC}<0.05$). The nMDS analysis showed a separation between summer and the other seasons (Figure 3b), and among the seasons a separation between the depths (Figure 3b).

The relevant groups contributing to seasonal changes of biomass changed with the season among algae, molluscs, amphipods and ascidians (Figure 4c). The biomass of other taxa did not exceed 0.01g (collected in the category “other” in Figure 4c). 76.1% of the total biomass in summer was due to the ascidian *S. plicata,* while in autumn was largely composed by molluscs (*M. galloprovincialis*) and amphipods (61.4% and 24.1% respectively) (Figure 4c). In winter the main contribution was due to the mollusc *M. galloprovincialis* (71.5%) while in spring to algae (*Ceramium* sp., *Polysiphonia* sp.1 and *Polysiphonia* sp.2) and molluscs (*M. galloprovincialis*) (46.2% and 31.3%, respectively) (Figure 4c). The contribution to the biomass was slightly different between the two depths considered. In summer at both the depths, the ascidians (mainly *S. plicata*) were the main component of the fouling community, accounting for more than 70% of the total biomass respectively. In autumn at 2 m depth the molluscs (mainly *M. galloprovincialis*) dominated

(73.1%) while at 6 m amphipods dominated (68.7%). In winter the molluscs remained the major biomass component (75.3%) at 2 m depth while at 6 m the group “others” accounted for more than 60%. In spring the algae (*Ceramium* sp., *Polysiphonia* sp.1 and *Polysiphonia* sp.2) represented the 82.1% of total biomass at 2 m depth while at 6 m is the molluscs were the more relevant taxon (62.7%) (Figure 4c).

Figure 4 Recruitment study. a. total diversity of the fouling community; b. total biomass trend vs. total diversity; c. Biomass composition of the main taxa with the value of the biomass (red line). Tot= total.



Discussion

This study shows that the immersion period, the seasonality and the depth are key factors strongly influencing biofouling community composition and biomass in a fish farm along the Apulian coast. The total diversity found in the present study considering both the experiments (111), was higher in comparison to similar study where 90 species were recorded in a Norwegian finfish farm (Bloecher et al. 2013). A study conducted in the Manfredonia harbour, close to the studied farm, revealed that less than 35 taxa were settled in the biofouling community during one-year study (Gherardi et al. 1974). The high diversity recorded in the present study may reflect local ecological conditions as well as the impact of organic enrichment by farm activities. Usually the effect of high organic enrichment determined by farming activities reflects in a strong decrease of biodiversity in the surrounding environment (Mannino & Sarà 2008). However, the South Adriatic Sea is an oligotrophic basin less influenced by the Adriatic Surface Waters limiting to the Italian coastal shelf (Cerino et al. 2012). In this environmental condition, the biofouling communities can positively use the surplus of organic matter derived from the fish feeding, as demonstrated in other studies (Gao et al. 2006; Gonzalez-Silvera et al. 2015). This can also explain why communities in our study were dominated by filter/suspension-feeders. The high diversity recorded indicates that the finfish farms are able to recruit a wide range of taxa and may also act as stepping stones for the spreading of species, such as the case for the non-indigenous marine species (Grigorakis & Rigos 2011; Fernandez-Gonzalez & Sanchez-Jerez 2014; Campbell et al. 2017).

The total diversity in term of number of taxa differed between the two studies even if both cover one year period. The increase in length of immersion during the succession experiment may allow the settlements of more diversified fauna, as observed in other study (Gherardi et al. 1974; Qiu et al. 2003). However, the larval recruitment and the succession patterns are dependent upon a more complex array of factors (Fraschetti et al. 2005; Greene & Grizzle 2007; Fitridge et al. 2012), so the duration of immersion can not be predictive of an higher diversified fauna colonizing artificial substrates. To this regards, it has been reported a much higher diversity of biofouling in the fish farms of the Atlantic Ocean after 3-months when compared to 12-months in which the dominance of *Mytilus edulis* was reported (Bloecher et al. 2013). The bivalve species *M. edulis* characterizes the final stage of the biofouling community in several temperate areas (Khalaman 2001; Greene and Grizzle 2007; Bloecher et al. 2013), competing sometimes with the solitary ascidians, as *Styela rustica* (Hodson et al. 2000; Khalaman 2001). In the present study, the biomass of the succession experiment was dominated by the ascidian *Styela plicata* already after 5 months. This species represented the major contributor to the biomass, mainly at 2 m depth, and was responsible for the biomass trend in the succession study. The contribution of *M. galloprovincialis* to the biomass, and bivalves in general, was less relevant despite this species are very abundant in the studied area and therefore it was expected to be a problem for the farm. However, it was recorded on the panels but it did not reach adult size maybe owing to an unstable attachment or predation/competition events. Despite individuals belonging to *M. galloprovincialis* in our successional experiment were relevant during the first stage of biofouling colonisation, they were progressively overwhelmed by ascidians. These results suggests that such bivalves are low efficient competitors if compared to the ascidians in this area. Moreover *S. plicata*, being able to form large aggregates after the colonisation of the substrate, has competitive advantages for resources exploitation compared to other organisms, also by removing through filtration processes other species' larvae (Greene et al. 1983). This result may reflect its biology as invasive species. This species can potentially spawn all over the year, although it seems to release gametes and recruit particularly in spring (Pineda et al. 2011). The results of the biofouling community in the successional experiment may be influenced by its starting time. In fact, the recruitment experiment shows that *S. plicata* settles and dominates the biomass only in summer, while it is not present in other seasons, where only colonial ascidians were recorded.

Additional studies are necessary to clarify if the “climax” community of this area, which is actually represented by *S. plicata* communities, and changing the starting time of the experiments the community probably could evolve in a different way.

Until now in the Mediterranean Sea, the few studies carried out on fish farm fouling have highlighted the dominance of algal species (Sliskovic et al. 2011; Fernandez-Gonzalez and Sanchez-Jerez 2017), hydroids and mussels (Fernandez-Gonzalez & Sanchez-Jerez 2017). Immersion period and season have a strong influence on the fouling composition and the biomass as observed in several tropical and temperate regions (Hodson & Burke, 1994; Madin et al. 2009; Bloecher et al. 2013; Salama et al. 2017). In the present study, we found that different species recruit in different seasons, leading to major changes also in terms of biomass. In particular, we observed higher value of diversity and biomass in summer. This temporal pattern of biomass has been frequently reported in temperate systems because of the increase of zooplankton (including larval stages of sessile/benthic organisms i.e. meroplankton) in the water column (Greene and Grizzle 2007; Sliskovic et al. 2011; Bloecher et al. 2013). The algae dominated only the panels in spring at 2 m depth, while they were scarce in the succession study. Moreover, their diversity was very low (i.e. only three species) despite this area (i.e. the Gargano Promontory) is characterised by high algal species diversity (more than 80 species; Cecere et al. 2001). Thus, conversely to the dominance of algae reported in other sectors of the North Adriatic Sea (Sliskovic et al. 2011), the fouling community in our study was almost completely composed by invertebrates. This is not surprising, since local differences in hydrodynamic regimes as well as in biological/ecological characteristics can have an important role in structuring the biofouling communities (Braithwaite & McEvoy 2005; Dürr and Watson 2010; Fitridge et al. 2012). The farm in the Apulian coast is located offshore in a site characterised by a north-southern current stronger during the summer (Artegiani et al. 1997). The general environmental conditions may promote the recruitment in the offshore farm of mainly filter/suspension-feeding organisms instead of algae. In spring also *M. galloprovincialis* was present, as newly recruited individuals with small size and biomass. *M. galloprovincialis* spawns in winter months (February-March) and a partial release of gametes were also observed during autumn (Ceccherelli & Rossi 1984). These spawning events coincide with recruitment periods in late spring and late autumn (Ceccherelli & Rossi 1984). These aspects can explain why we found *M. galloprovincialis* juveniles in spring (March-May) and a dominance during autumn and winter. Studies dealing with the abundance and diversity of larvae in the area surrounding the fish farm should be conducted to better clarify the recruitment dynamics of *Styela* vs. *Mytilus*. Moreover, other study has highlighted the importance of monitoring plankton dynamic and composition in finfish farm since evidence in connection between stinging plankton blooms and finfish pathology in fish farms have been recorded (Baxter et al. 2012; Bosch-Belmar et al. 2017).

Both in the succession and recruitment studies, we found that water depth is an important factor, influencing fouling biomass and composition. In both the experiments, biomass values were lower at 6 m than at 2 m depth. Such a depth-related pattern, which has been already reported in literature (Hodson & Burke 1994; Guenther et al. 2010; Abdelsalam & Abdel Wahab 2012; Salama et al. 2017), was mainly due to the settlement and growing of *S. plicata*. The total diversity was not different between the two depths, but at 6 m depth biomass was mainly composed by molluscs, amphipods, bryozoans and other smaller organisms instead of solitary ascidian as at 2 m depth. The taxa encountered at 6 m depth reached high biomass likely as a result of a reduced competition with ascidians. The studied finfish farm appears also to attract many non-sessile species such as amphipods, errant polychaetes, decapods, isopods, nemerteans, platyhelminthes, ophiuroids and gastropods. Similar results have been reported in other farms (Cook et al. 2006; Greene & Grizzle 2007; Guenther et al. 2010; Fernandez-Gonzalez & Sanchez-Jerez 2014; Gonzalez-Silvera et al. 2015; Fernandez-Gonzalez et al. 2017), suggesting to include also mobile fauna for an overall assessment of the effects of fish farms on marine biodiversity.

The analysis of biofouling dynamics is important to improve management strategies aiming at identifying cost effective and environmental-friendly solutions of biofouling control. Different kind of antifouling solutions are used to minimise this problem along with different methods of cleaning (Fitridge et al. 2012; Bloecher et al. 2013; Edwards et al. 2015). Different approaches should be used in relation to the characteristic of the fouling community to maximise the results of cleaning operations. However, the knowledge is sometimes scant and usually limited to the dominant species (Hodson & Burke 1994; Bloecher et al. 2013). The farm investigated in the present study does not use any net coating solutions to prevent biofouling (Deady et al. 1995; Ahlgren 1998; Callow & Callow 2011; Fitridge et al. 2012; Edwards et al. 2015). Every month, expert divers check the status of the nets and decide if it should be cleaned or not. The nets are generally cleaned every 3-4 months, with an increase in the frequency of cleaning operations between April to September (Pinto personal communication). Such cleaning operations reflect the dynamics of biofouling colonisation reported in the present study, with major foulers (in particular *S. plicata*) recruiting and developing between June-August. Moreover, the reproductive strategy and the ecology of ascidians pose significant challenges to find efficient and eco-compatible antifouling strategies and future studies should be conducted targeting this taxon (Aldred & Clare 2014).

Results from the present study corroborate previous findings of a high heterogeneity of biofouling communities present in different regions of the Mediterranean Sea (Sliskovic et al. 2011; Fernandez-Gonzalez and Sanchez-Jerez 2017). This information should be included to identify suitable areas when siting new fish farms (Dapueto et al. 2015). The knowledge of the fouling community that can develop in a specific area could provide important information about management efforts and costs that farmers should face. Moreover, information on fouling communities is also useful to implement aquaculture strategies for the Mediterranean Sea and for the improvement of environmental monitoring programme guidelines for marine finfish cage farming as required by the Blue Growth Initiative of the FAO.

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CONCLUSION AND FUTURE WORK

The present PhD thesis provides new insights on the biofouling communities of marinas and finfish farms of the Adriatic Sea and their spatial and temporal dynamics. It points out the importance of the knowledge of the biofouling communities that can develop in areas characterised by different environmental and ecological settings to better address the control methods used to overcome the biofouling problems. The specific knowledge of the biofouling assemblage is essential to find new and suitable antifouling solutions. Detailed data about the dominant foulers and the species composition are needed, in order to identify the period in which the biofouling organisms represent the major problem for the farmer and for the owners of the recreational vessels. Moreover, the outputs of this thesis improve the knowledge about Italian boater's profile, suggests that they perceive the biofouling as a problem and show that the expenses to manage it are relevant. In addition, the results indicate a lack of information especially about foul-release coatings as possible eco-friendly solution to the problem and the need to increase the awareness of the boaters with specific information campaigns.

Future work could include the use of experimental panels of different shape and material, the submersion in different months ad for longer periods to study possible changes in the biomass and composition of the biofouling communities. Moreover, a survey like that submitted to Italian boat owners could be proposed to Italian farmers to know their perception of biofouling and the currently used solutions. In addition, a survey could be also submitted to managers of Italian marinas to explore the possible use of foul-release coatings on submerged structures such as piers and buoys.