

Article

An Innovative IMTA System: Polychaetes, Sponges and Macroalgae Co-Cultured in a Southern Italian In-Shore Mariculture Plant (Ionian Sea)

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Abstract: In this paper, we report data from the first year of rearing of a set of filter feeder bioremediator organisms: macrobenthic invertebrates (sabellid polychaetes and sponges), coupled with macroalgae, realized in a mariculture fish farm. This innovative integrated multi-trophic aquaculture (IMTA) system was realized at a preindustrial level in the Gulf of Taranto (southern Italy, northern Ionian Sea), within the framework of the EU Remedia Life project. Long lines containing different collector typologies were placed around the fish breeding cages. Vertical collectors were utilized for both polychaetes and sponges, whilst macroalgae were cultivated in horizontal collectors. Data on the growth and mortality of the target species after the first year of rearing and cultivation are given together with their biomass estimation. Polychaete biomass was obtained from natural settlement on ropes previously hung in the system, while sponges and macroalgae were derived from explants and/or inocules inserted in the collectors. The description of the successional pattern occurring on collectors used for settling until reaching a “stable” point is also described, with indications of additional filter feeder macroinvertebrates other than polychaetes and sponges that are easily obtainable and useful in the system as bioremediators as well. The results demonstrate an easy, natural obtaining of large biomass of sabellid polychaetes settling especially from about a 4 to 10 m depth. Sponges and macroalgae need to be periodically cleaned from the fouling covering. The macroalgae cycle was different from that of invertebrates and requires the cultivation of two different species with about a 6-month cycle for each one. The present study represents one of the first attempts at IMTA in the Mediterranean area where invertebrates and macroalgae are co-cultured in an inshore fish farm. Possible utilization of the produced biomass is also suggested.

Keywords: integrated multi-trophic aquaculture; Mediterranean Sea; sponges; polychaetae; macroalgae; bioremediation

1. Introduction

The strong growth of the mariculture industry calls for the development of new strategies to control and minimize the impact of this activity on the environment [1,2]. The first step of this process should

be the replacement of monoculture with polyculture, and the integrated multi-trophic aquaculture (IMTA) technique fall into this philosophy [3–6]. The IMTA technique consists of combining the cultivation of fed aquaculture species associated with other species able to extract organic and/or inorganic substances from seawater. This practice is aimed to improve environmental quality, economic return and social suitability [7,8].

In the current literature, mariculture bioremediation focused especially on sediment restoration and on the role of the detritivore community [9–14]. However, according to recent practical approaches, a bioremediation effect on the water column can also be corroborated, especially in environments submitted to lateral drift, where nutrient loading can be efficiently exploited directly by the filter feeders' community [15–18]. For this purpose, in recent decades, there has been an increasing interest in promoting the growth of biofouling on devoted artificial structures [6,18–28]. Considering the extreme spatial and temporal variability of fouling, to reach good performances, the planned system should be based on knowledge of the environmental and biological characteristics of the area [29–33].

IMTA systems are not immune from criticisms deriving from the large number of collectors that should be placed to produce acceptable quantities of biomass for an efficient bioremediation. Moreover, to promote a circular economy, the IMTA approach is, today, based only on economically viable aquaculture species [7], such as edible filter feeders, particularly molluscs [3,6,34]. Moreover, it was proven that the polyculture of fin fish and bivalves does not represent an appropriate tool for reducing the environmental impact of fin fish aquaculture [29].

The present paper refers to an innovative IMTA rearing model, performed at a preindustrial level within the EU Remedia Life project (LIFE16 ENV/IT/000343) ongoing in the Gulf of Taranto (Ionian Sea), where a new set of bioremediators, such as polychaetes, sponges and macroalgae, coupled with mussels, has been proposed within a fish farm for the first time at the European level, with a tentative aim to also utilize the fouling natural settling on collectors, which is still considered a negative factor. Indeed, the fouling directly settled on the cages can reduce water flow, thus negatively affecting production, especially due to the periodical mechanical cleaning actions required for biofouling removal [35]. The main target organisms, e.g., sabellids, sponges and macroalgae, have been the object of numerous investigations by our research groups in relation to marine aquaculture waste treatment for about 20 years. Filter feeder polychaetes [36–40] and sponges [41–44] can act on organic particulate matter and bacteria. As concerns macroalgae, bioremediation of nutrient-rich aquaculture wastewater (ammonium, nitrate and phosphate) was demonstrated to be effective at a laboratory scale [45–48].

Sabellids are also ideal candidates for IMTA purposes due to the high density that they can reach on artificial substrates in eutrophic environments, where they can be easily obtained. Moreover, although further studies on the possible use of their biomass are still being performed, first investigations on their biochemical composition indicated their possible employment as a dietary supplement for fish nourishment [49]; this is also a very attractive suggestion considering the need for sustainable alternatives to fishmeal [50]. However, the possibility of using large quantities of biomass for these purposes must deal with the need not to deplete natural stocks. The first attempt of sabellid rearing was carried out in a land-based fish farm with recycled water [36], but it was also conducted at a small scale in the sea [51] and coupled with a mollusc farm [52].

As concerns sponges, findings obtained from about 20 years of field and laboratory research on more than 30 sponge species by Italian and international researcher teams suggest that in situ sponge-culture may be suitable for the eco-sustainable supply chain of sponge biomass for some target species [44,53–57]. The sponge biomass obtained in polyculture systems has considerable potential from a commercial point of view, having good appeal for cosmetic or hobbyist production of natural biocides, or for the pharmaceutical industry thanks to their bioactive compounds. Among marine invertebrates, sponges and their microbiome are one of the main producers of natural bioactive compounds, and, indeed, among about 7000 chemical compounds derived from marine organisms, more than 30% have been isolated from sponges [58].

Finally, the role of marine macroalgae in offering good ecosystem services is well known; they enrich seawater with oxygen, naturally mitigate nutrient excess and are increasingly used in the bioremediation of chemical pollution [59,60]. Their biomass is recognized as a good stock of bio-products [60,61], useful in different biotechnological applications in medicine, cosmetics or formulation of nutraceuticals and human food products [49,62–64]. However, to become marketable, biomass needs to be produced in considerable quantities, therefore, macroalgae are suitable candidates for mass production in sustainable multitrophic aquaculture plants [65].

A pilot attempt of IMTA with seaweed was carried out in the USA at the end of the 1970s [66], while in Israel, these organisms were introduced in IMTA from the mid-1980s [67,68]. Since then, most integrated polycultures were performed in Asia and Canada [8,59,69,70]. In Europe, a recent survey showed that several constraints of different kinds slowed down the application of such technology; only few sea-based experimental farms were set up with seaweeds, of which only two had a commercial perspective in Atlantic waters [71]. A first attempt of seaweed polyculture in the Mediterranean area refers to the Mar Piccolo of Taranto, where the species *Gracilaria dura* was co-cultured with *Mytilus galloprovincialis* and *Sabella spallanzanii* [72].

In the present paper, data on the first cycle of production of sabellids, sponges and seaweeds, obtained in the framework of the Remedia Life project, are reported. In particular, this early period of setting up this innovative rearing plant aimed to provide answers to several crucial points: (1) to investigate the most simple culturing methods compatible with fish production; (2) to determine which species could be obtained in a natural way and how macrobenthic settlers can interfere with target species; (3) to establish the amount of biomass that can be produced at large scale, to estimate the best cycle length of the production.

2. Material and Methods

2.1. Study Area

The study area is located on the south-west side of the Mar Grande of Taranto (40°25'56" N; 17°14'19" E) (Ionian Sea), which is part of one of the most important coastal marine ecosystems along the Apulian coast (Figure 1). The Mar Grande of Taranto is a semi-enclosed basin connected to the Gulf of Taranto through three artificial dams. The temperature shows seasonal variations typical of the coastal Ionian regions with an average annual value of about 18 °C, while the salinity is about 38‰ and is almost uniform over the year. The area is affected by intense *Mytilus galloprovincialis* farming, a species that finds, in the area, optimal conditions to dominate the final stage of the shallow local fouling communities, sometimes competing with solitary ascidians as well as with sabellid polychaetes [73–75].

The investigation was performed in the aquaculture plant Maricoltura del Mar Grande, which is a partner of the Remedia Life project hosting the experimentation of the innovative IMTA system. The plant covers a surface of 0.06 Km² and is located in a semi-confined area of the Mar Grande, positioned at about 600 m away from the coast. It consists of 15 cages (Ø 22 m), working at a depth ranging from 7 to 12 m and producing about 100 tons*year⁻¹ of European seabass *Dicentrarchus labrax* (Linnaeus, 1758) or sea bream *Sparus aurata*, Linnaeus, 1758.

2.2. Field Work: Sampling and Processing

With one of the goals of the Remedia Life project being the estimation of the mitigation of aquaculture wastes, the experimental design was planned after the realization of a large monitoring survey performed to individuate the best area for the placement of rearing structures [76]. During the *ex ante* survey, the first step concerned an analysis of the hydro dynamism of the area together with measurements of the physico-chemical parameters. Both water column (bacterial component and nutrients) and sediment (bacterial community and taxonomic structure of the benthic assemblages) parameters were also investigated to be compared to the situation after the bioremediation activity.

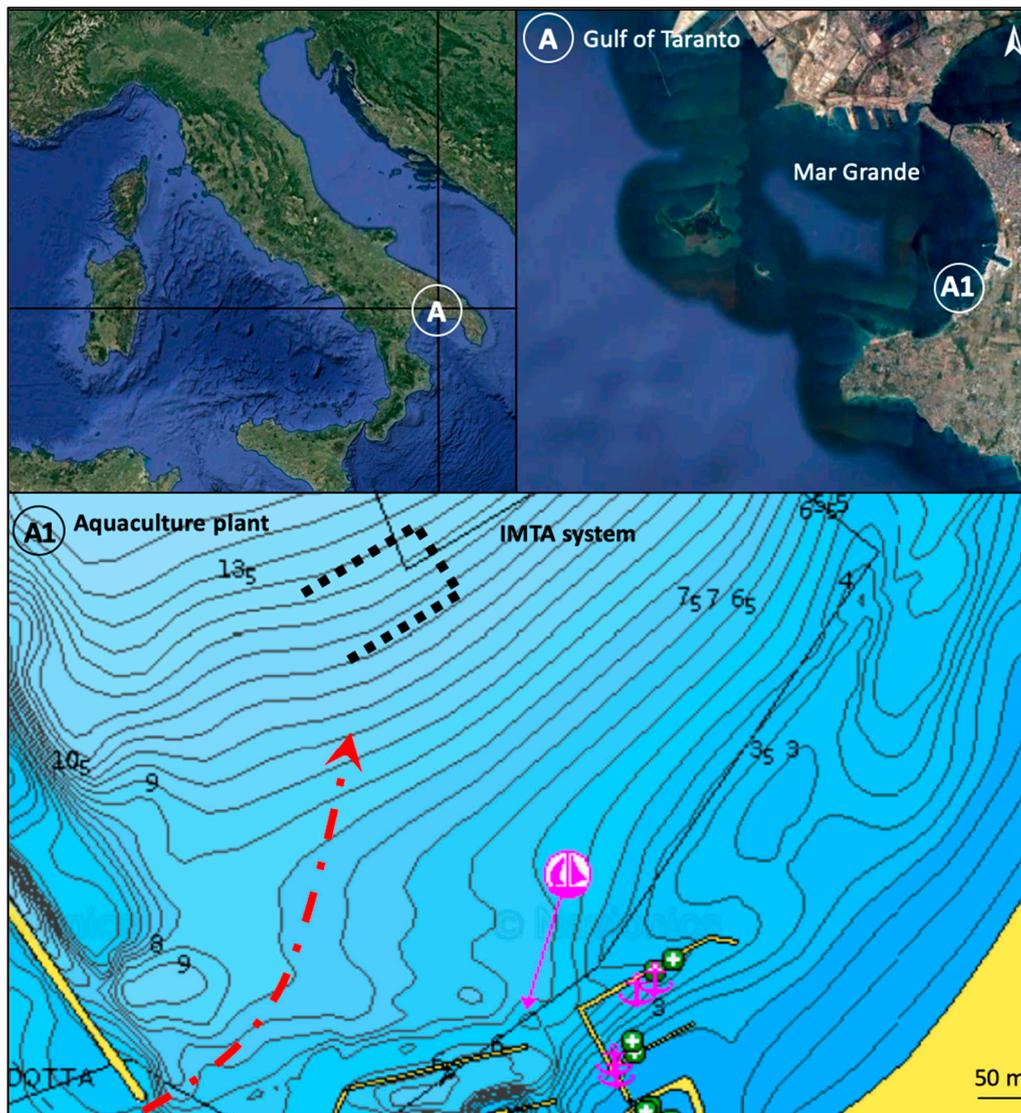


Figure 1. Map of the study site. The dashed red arrow indicates the direction of the dominant current on the bottom.

The hydrodynamic analysis has shown that in the area, the surface current is directed from the north-east to the south-west at a speed of about 3 cm s^{-1} . At the bottom, the direction of the current is inverted, proceeding from south-west to north-east at a speed of about 1.3 cm s^{-1} . The results of the ex ante monitoring revealed which was the more impacted site in terms of bacterial, inorganic and organic compound concentration, specific richness of soft-bottom macrobenthic communities and fouling taxonomic structure (unpublished data). Macroinvertebrates and seaweeds were added in this area, which is considered the treatment site (T). Here, three long lines, named LLA, LLB and LLC, were realized and placed around six cages. Another six cages, separated by an additional line of cages, acted as the control (C) (Figure 2A).

The Remedial Life plant, placed around the treatment (T) cages, was supported by buoys to avoid the sinking of the structure following the increase of the biomass raised (Figure 2B). The space between two consecutive buoys constituted a breeding “chamber” to house the invertebrates and macroalgae modules, represented by vertical collectors for macrobenthic rearing (bare collectors for the recruitment of polychaetes and modules with explant of sponges and macroalgae) (Figure 2C). In each chamber, several retinas of 5 m length containing molluscs were also placed alternating with polychaetes and

sponges in order to fill empty space, a relevant component of the local fouling. Macroalgae were instead placed at the surface within plastic socks.

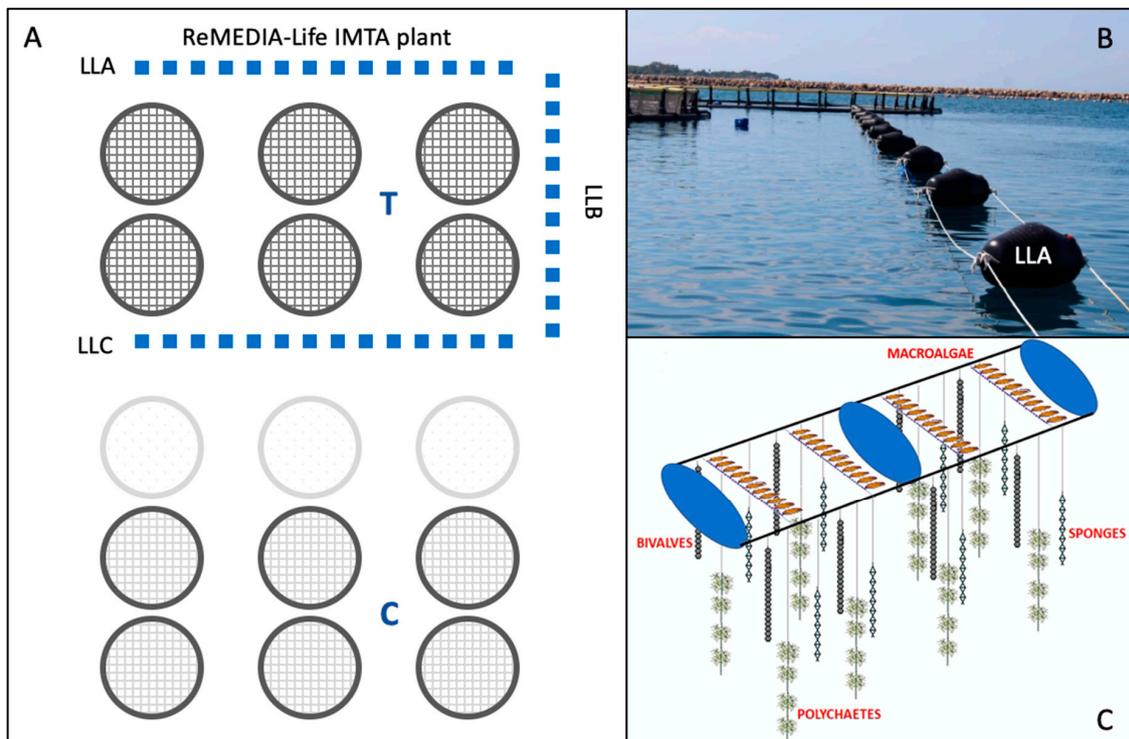


Figure 2. (A) Map of the plant with indications of the long lines and the two areas (T: treatment and C: control); (B) a photo of the external long line; (C) scheme of a rearing unit (chamber) with the disposition of invertebrates and macroalgae.

A total of 196 bare collectors (76 modules in the LLA, 54 in the LLB, 66 in the LLC) consisting of coconut fibre ropes, 2 cm wide and 10 m long, were placed for macroinvertebrate settlement and, in particular, for polychaete sabellid rearing. For this purpose, adult worms of the species *Sabella spallanzanii* collected around the area were inserted in the last meter of their length, acting as spawners and enhancing self-settlement on the collectors (Figure 3A). This species was chosen because it represents a natural and abundant element of the fouling communities all around in the area [73–75,77]. Of the 196 collectors, only 184 were followed until the end of the year cycle because some of them were utilized for a previous experimentation on *S. spallanzanii* biomass [78].

The sponge-rearing modules consisted of 7 m long ropes (Figure 3B) in which sponge explants were inserted within plastic nets at regular intervals every 40 cm, each counting a total of 12–15 explants. Among the species relatively common on the artificial and natural hard substrates of the area, the horny sponge *Sarcotragus spinosulus* Schmidt, 1862 (Porifera, Demospongiae, Keratosa, Dictyoceratida, Irciniidae) was tested due to its spread and potential for biomass exploitation [79–81]. The donor sponges were collected near the plant by scuba divers, according to Corriero et al. [55], from artificial substrates, such as iron poles and concrete boulders, avoiding picking up natural hard substrates. Once the donor specimens were collected, they were cut into fragments of uniform volume (about 150 mL) and fixed to the rearing modules with clamps. In total, 172 modules were inserted, containing about 2000 explants of sponge (72 modules in the LLA, 56 in the LLB, 44 in the LLC).

Macroalgae were collected among the most abundant unattached species living in the Mar Piccolo (Mediterranean Sea, northern Ionian Sea, Italy), a semi-enclosed basin with lagoon features [82]. Once collected, the macroalgae were transferred to the aquaculture farm to set up the cultivation

sockets, each consisting of seaweed enclosed into a net sack and hung with a festoon arrangement at about a 1 m depth.



Figure 3. Some phases of in situ retinas preparation: (A) placement of vertical bare collectors with adult polychaetes at the base; (B) a module with sponges; (C) part of a drifting mattresses of *Gracilaria bursa-pastoris*; (D) seaweed harvesting within the Mar Piccolo.

Two different species were tested, i.e., *Chaetomorpha linum* (Chlorophyta, Cladophorales) and *Gracilaria bursa-pastoris* (Rhodophyta, Gracilariales), in two different seasons of the year due to their diverse life cycle. A total of 186 cultivation sockets (99 for *C. linum* and 87 for *G. bursa-pastoris*) were allocated in the plant. In particular, *C. linum* cultivation lasted from October 2018 until March 2019, while *G. bursa-pastoris* was cultivated in the plant from April 2019 until September 2019. Since during the season of maximum growth, both *C. linum* (i.e., early autumn) and *G. bursa-pastoris* (i.e., early spring) commonly make large and thick drifting mattresses (Figure 3C), it was easy to hand-collect their thalli at a 50 cm depth by means of a rake (Figure 3D).

The study started in October 2018 when the natural fibre collectors, sponge-rearing modules and macroalgae collectors were immersed.

Macrofouling colonization has been monitored on natural fibre collectors beginning from December 2018 in the external (LLA) and internal (LLC) long lines. The monitoring was performed using a digital camera. Fifty-centimeter-long portions of collectors were photographed in vivo at two different depths (0 m and 10 m) and 3 replicates for each long-line were taken 5 different times, corresponding to the months of December, February, March, April and June. Twelve photographs were taken each time for a total of 60 photographs. The photographs were analyzed using the software ImageJ [83], which determined the percent coverage of the sessile organisms that were identified to species level

when possible. Thus, the coverage matrices of each species detected were obtained. The total area was 100 cm². A multi-layer coverage was considered, with a potential coverage area greater than 100%.

Starting from April 2019, when polychaete sabellids become easily recognizable, worm density was measured in situ along 1 m of the rope, considering three replicates randomly chosen from both the long lines. Moreover, about 50 individuals were randomly collected each time from each long line and taken to the laboratory for length and weight measurements. During this sampling phase, the health status of the worms was also controlled. At the end of the first annual cycle (December 2019), half of the coconut ropes were removed and worms were separated and extracted from the tube for biomass estimation. The other structures were maintained in the system in order to measure the growing parameters during the second year of life and to keep some adult worms to continue the bioremediation activity.

As concerns sponges, starting from January up to December 2019, the modules were monitored to check the well-being of the explants (assessed through visual observation of the healing of the cutting surface), the survival (living, dead or damaged) and the specific growth rate (SGR), valued as $[(V_f - V_i) \cdot V_i^{-1} / t] \cdot 100$ (V_i = initial Volume; V_f = final Volume; t = days of rearing), of randomly selected samples. In the case of wholly healed cuts and absence of stress factors (unhealed cutting surfaces, presence of areas with bacterial microfilm or exposed skeletal tissue), the individuals were defined as living; those with evident signs of stress were instead considered damaged. Starting from March 2019, the sponge rearing modules were affected by the recruitment of fouling, and, to avoid suffocation of the sponges' explants, the modules were regularly cleaned by operating in immersion to prevent stress.

Bimonthly monitoring of the well-being and growth of seaweeds was also performed. In particular, well-being was assessed both in situ, by observing the consistency and coloring of the algae in the field, and in the laboratory, by microscopic observation of cells and chloroplasts. When thalli fragmentation was observed, followed by a rapid decay, cultivation was stopped. Macroalgae biomass was measured every two weeks on three socks (one for each long line), randomly collected, and the increase was calculated as the specific growth rate (SGR), referring to the average percentage increase in weight/day according to the following formula: $SGR = [(FW_t / FW_0) / t] \times 100$. (FW_t = final Fresh Weight; FW_0 = initial Fresh Weight; t = days of immersion) [84].

3. Results

3.1. Collector Colonization and Growth of Sabellid Worms

During the starting phase of collector colonization, 40 macroinvertebrate taxa and four macroalgae were sampled, 3 of which formed the early algal turf. Among the collected taxa, the ascidiacea was the most represented taxon with 19 species, while sponges were represented by only the species *Paraleucilla magna*. Most of the recorded species were typical fouler species, such as *Mytilus galloprovincialis*, *Sabella spallanzanii*, *Hydroides elegans* and *Phallusia mammillata*.

The coverage percent value linearly increased, reaching 95% in June, after eight months of colonization (Figure 4A). By contrast, species richness increases were more irregular (Figure 4B). At the beginning, few early colonizing species, such as the bryozoan *Bugulina calathus* and the ascidian *Ascidia conchilega* growing on a considerable algal felt, contributed to the coverage at all depths and in both of the examined long-lines, leading to a high similarity among the long lines' colonization. After eight months, the community had reached its end point with 25 taxa covering the substrate, with *S. spallanzanii* and *M. galloprovincialis* as the dominant species, both of them showing a similar increasing trend from December to June, even if with different distributions (Figure 4C). *Sabella spallanzanii* coverage was only 1% at the surface, becoming dominant under 4 m depth (64%). A reverse trend was observed for *M. galloprovincialis* (65% at the surface and 2% deeper). Intermixed with *S. spallanzanii*, other sabellids, such as *Branchiomma luctuosum*, were also found, but with only about a 4% coverage. At this stage of the community's development, the solitary ascidians *P. mammillata* and *Styela plicata*, unlike the colonial ones, remained abundant, the first one in the deeper assemblages and the second in

the shallower assemblages. Moreover, some edible molluscs, such as *Ostrea edulis* and *Limaria hians*, were found in abundance along the whole collector.

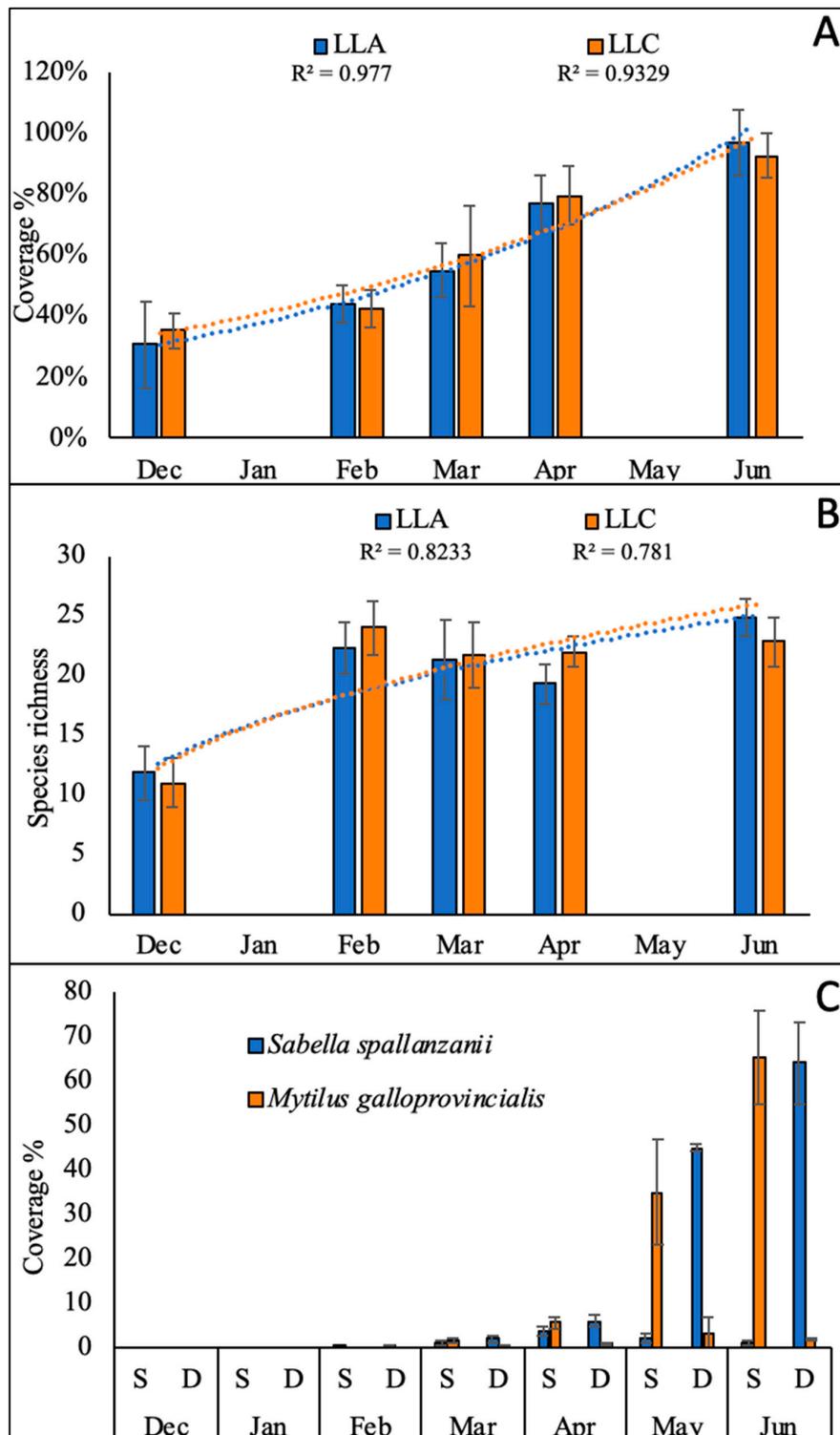


Figure 4. Mean trend over time of macrofouling: (A) percent coverage; (B) species richness; (C) percent coverage at different depths (D = deep, S = shallow) of *Sabella spallanzanii* and *Mytilus galloprovincialis* in relation with sampling time.

The successional pattern has similar results in both of the examined long lines, with neither variation in species composition nor in abundance; the main difference in the two investigated LLs was observed during the first and intermediate stage of colonization, with a conspicuous presence of colonial ascidians, mainly *Didemnum coriaceum*, *Diplosoma listerianum* and *Botrylloides leachii*, in LLC. The distribution present in June 2019, with the shallow part of the collectors composed of 90% molluscs (mainly mussels) and the more in-depth community (below 4 m depth) contained 80% sabellid polychaetes, among which 90% consisted of *S. spallanzanii* (Figure 5A), was maintained until the following month of December 2019.

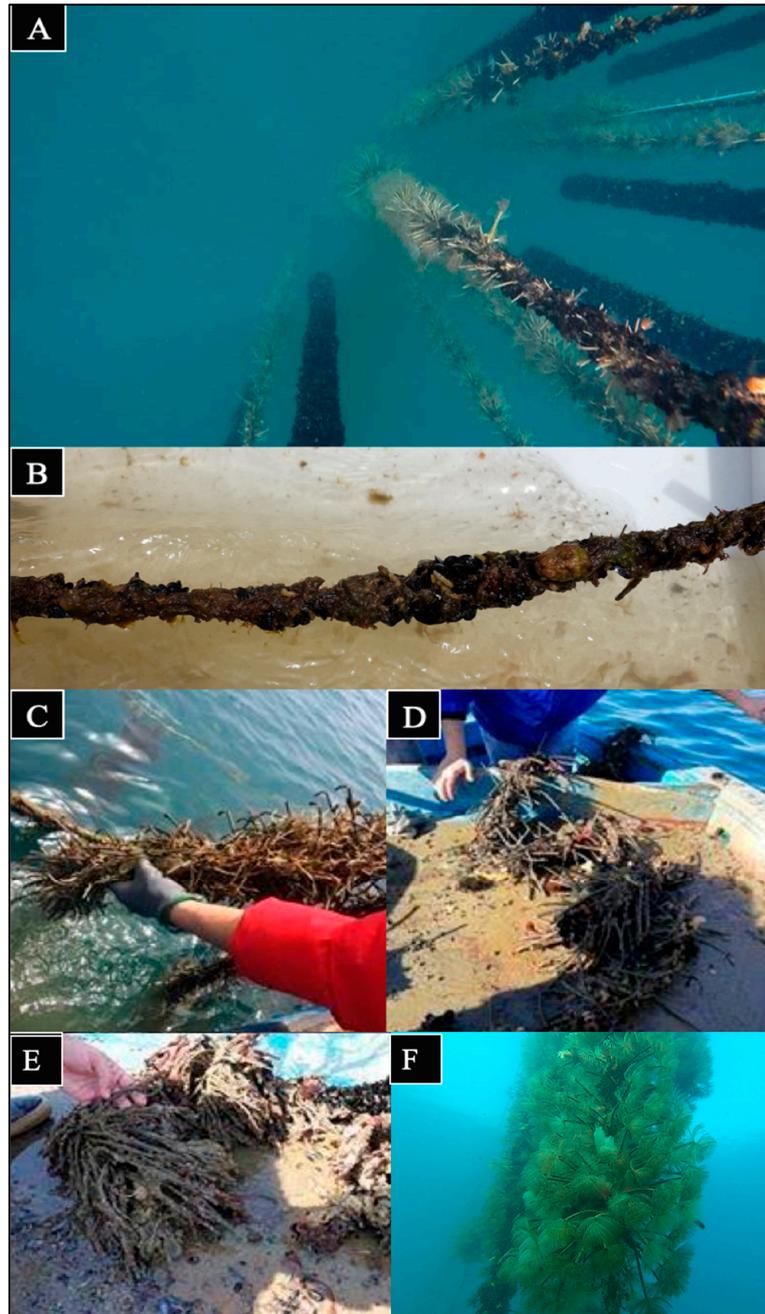


Figure 5. Collectors for fouling recruitment and *Sabella spallanzanii* rearing. (A) view of collectors from July 2019 showing the zonation of the two dominant species *S. spallanzanii* and *Mytilus galloprovincialis*; (B) collector from February 2019; (C,D) collectors from May and July 2019; (E) tube of *S. spallanzanii* covered by mud in August 2019; (F) photograph of a collector in December 2019.

Although recruits of *S. spallanzanii* were probably already present on the collectors starting from February [52], during the earlier stages of colonization, individuals were too small to be detected by photograph analysis (Figure 5B). Therefore, measurements of increasing length and weight of this species began starting from April 2019, when the specimens were more manageable (Figure 5C–E). Their growth was followed up until December 2019, when the species reached up to 10 cm in length and 2.5 g in wet weight (Figure 5F). The increase in size (cm ± SD) was quite linear (Figure 6A); by contrast, increases in weight (g ± SD) became more evident starting from October 2019 (Figure 6B). Both trends were very similar in the two long lines examined, even if the growth appeared slightly higher in the external one.

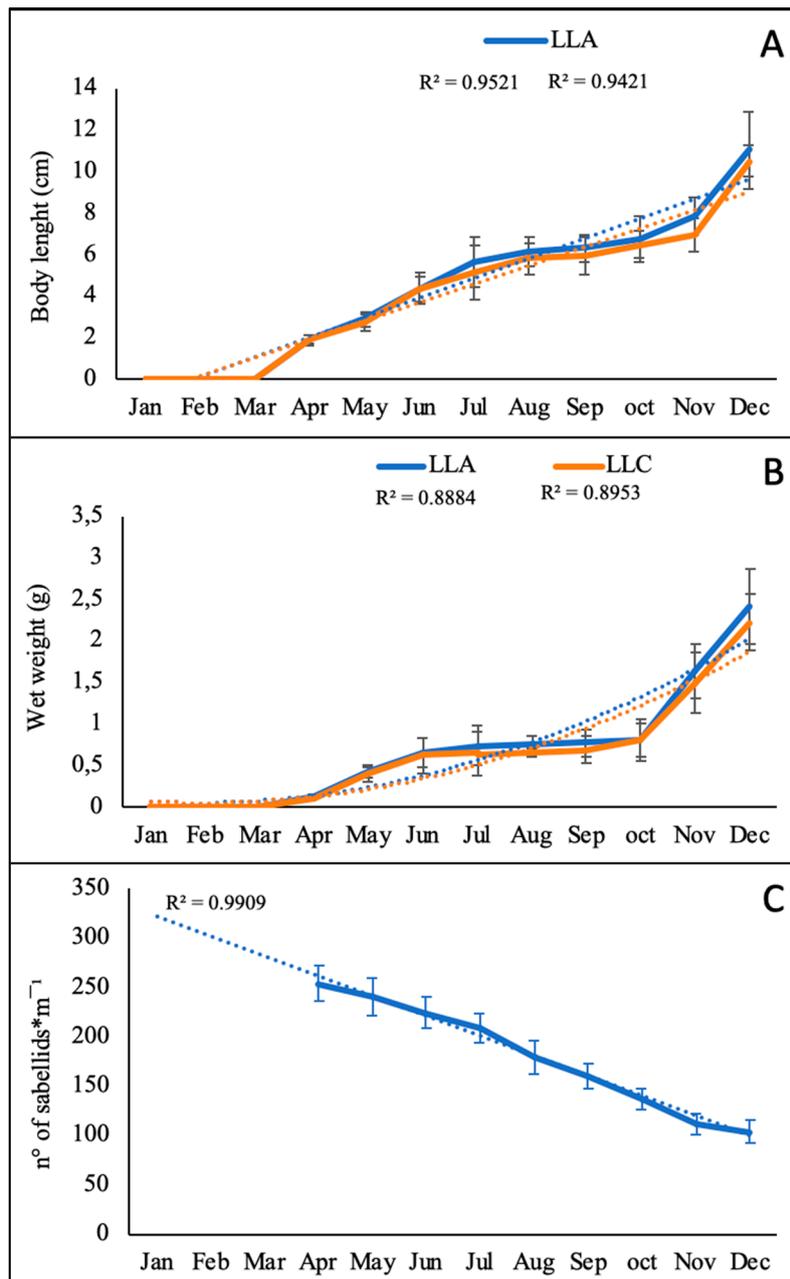


Figure 6. (A) body length increase; (B) biomass increase; (C) trend of density of *Sabella spallanzanii*.

The first density measurements were also relative to April 2019, which was the first month showing easily identifiable worms on the collectors. In this month, a density of about 250 worms per

linear meter occurred. This value remained relatively constant in July and then there was a decrease, leading to the number of 100 worms at the end of the first year (Figure 6C). Therefore, from June to December, a 50% mortality rate could be computed.

During this first year of measurements and control, the worms never showed signs of physiological discomfort and always had a withdrawal response in the tube. Moreover, even if other fouler organisms settled on their tubes, they never suffocated them. The only negative factor observed was the detachment of some tubes from the collectors because of the weight of the whole fouling. The worms remained healthy even during summer 2019, when the whole area faced a crisis related to high temperatures and the collectors were covered by a dense, muddy layer (Figure 5E), as well as during the conspicuous bloom of the red alga *Spyridia filamentosa* which occurred from late summer to early autumn. Even after these environmental stressing conditions, the whole fouling community maintained the above-described pattern.

From the values of biomass and density measured in half of the immersed structures, at the end of the first cycle (December 2019), a total production of about 147,000 worms was computed, with an estimated biomass of about 3.6 quintals, a production referring to the worms depleted from the tubes. During the harvesting of this first annual production, other than polychaetes, the biomass of other invertebrates, estimated at least 15 L for each collector, was also collected. This biomass was composed mainly of ascidians, especially *P. mammillata* and *S. plicata*, but also bryozoan and molluscs, especially the above reported *O. edulis* and *L. hians*, as well as some different sponges from the cultured species. Moreover, together with sessile filter feeder invertebrates, a conspicuous number of vagile taxa, such as echinoderms and crustaceans, were found colonizing the entire length of the collectors, especially when the macroalga *S. filamentosa* was highly developed.

Lastly, it is interesting to underline that when the worms were collected for biomass estimation at the end of the first annual cycle, the assemblage present on the collectors had trapped a large amount of mud, weighing at least 3 kg for each collector.

3.2. Growing of the Sponges

The monthly monitoring of the well-being and growth of the reared *S. spinosulus* explants showed high rearing performances, with increased survival values and noteworthy increases in biomass in short time intervals. During the entire period of observation, the mean value of living sponge explants ranged between 93% and 80.84% in January and December 2019, respectively. Besides, the highest mean value of damaged explants was registered during the first months of rearing (ranging between 23% and 16.66%) while it reduced drastically from April onwards, down to values close to zero (Figure 7A).

In March 2019, the percentage of living explants reached 87.18% and that of damaged or exposed skeleton areas reached 16.66%. In this month, the sponge-rearing modules began to be covered with abundant mucilage, particularly those placed in long line C. The percentage of living explants reached 87.23% and that of damaged ones dropped to 11.45%. Starting from May, the value of damaged explants dropped drastically, while live ones showed only a slight decrease, despite the algal bloom and the dystrophic crisis recorded during the summer months.

The sponge explant specific growth rate is shown in Figure 7B; the graph clearly shows that an initial volume loss occurred as a consequence of the stress due to the collection and cutting of donor sponges into uniform explants. Starting from February, a continuous positive trend in both LLA and LLC was registered. At the end of the observation period, the explants left in the rearing modules were more than double the initial volume, going from an initial value of about 100 ± 10 mL to about 240 ± 20 mL (average \pm SD). The overall volume (mL) of a pool of samples progressively increased, ranging from 1180 mL, recorded in January, to the value of 1540 mL. It must be noticed that, since April 2019, sponge rearing modules appeared covered by fouling, and recruits of *S. spallanzanii* and monthly cleaning operations were carried out until December 2019 (Figure 8).

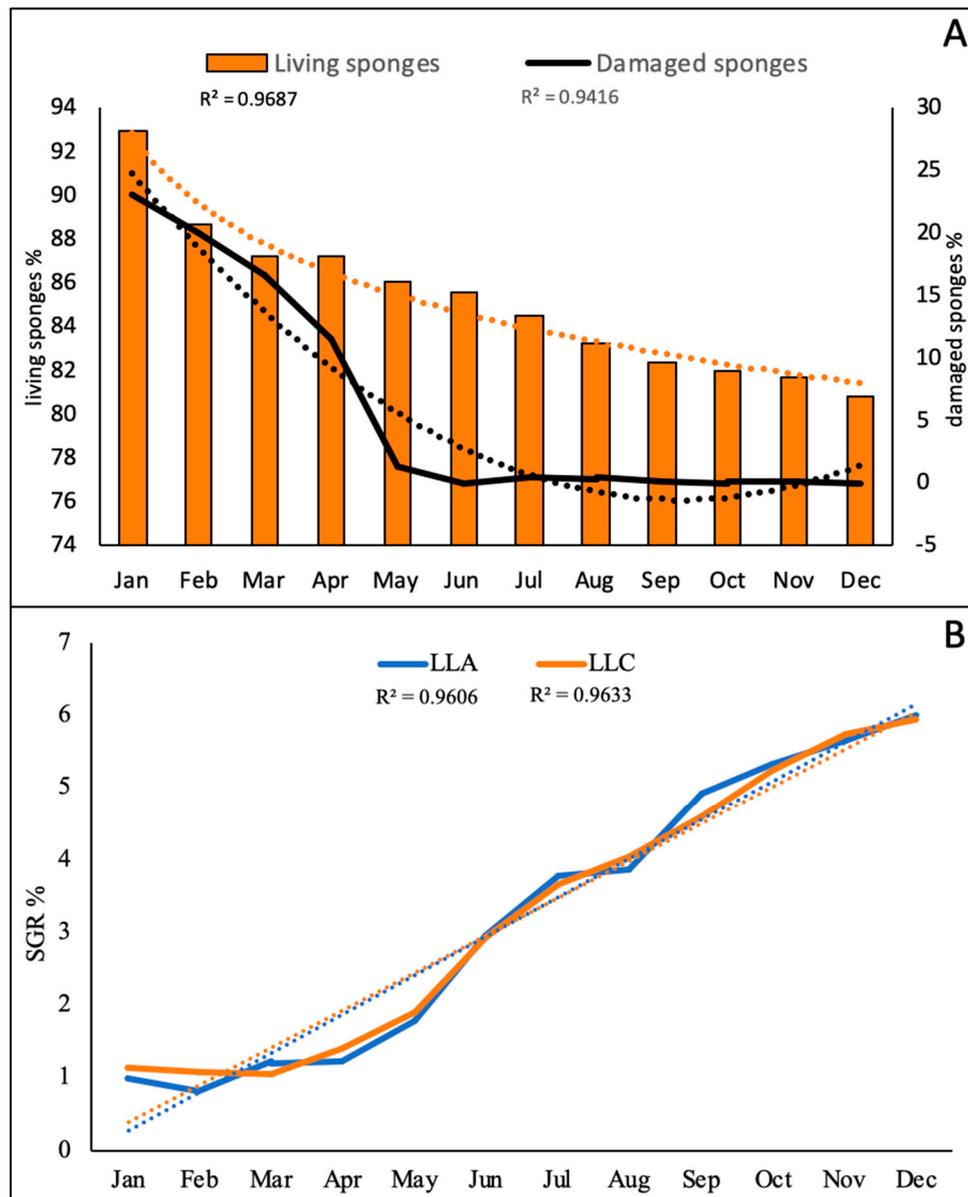


Figure 7. (A): Relationship between survival (histogram), damaged specimens (solid line) of reared sponges and month of observation. Survival is indicated by the percentage of live individuals observed in each month; individuals of damaged sponges are indicated with the solid line. (B): Relationship between the specific growth rate (SGR) of the reared sponges and the month of observation (from January to December 2019).

3.3. Growing of Macroalgae

The bimonthly monitoring of the well-being and growth of the macroalgae placed in the experimental plant showed interesting growth performances, with high survival rates and significant increases in the biomass produced in short time intervals (Figure 9A). As regards *C. linum*, from October 2018 until March 2019, low mortality values were recorded. Moreover, a medium specific growth rate (SGR) equal to 5% was calculated in the six-month trial. Interestingly, in the first 11 days of cultivation, an 11% SGR was measured for this species. At the end of March, *C. linum* thalli underwent fragmentation followed by a rapid decay, so cultivation was cut short and biomass was harvested. Subsequently, in April 2019, the red alga *Gracilaria bursa-pastoris* was tested and remained in the cultivation plant until the end of summer 2019, when a dystrophic crisis occurred in the basin and

problems with pathogens and epiphytes of seaweeds arose (Figure 9B). Such fouling led to extensive loss of biomass and considerable quality deterioration. However, it should be stressed that, until the end of August, this second selected algal species also increased considerably in biomass and a good increase in wet weight was recorded with a medium daily biomass increase of 4.4%.



Figure 8. A specimen of *Sarcotragus spinosulus* covered by sabellids, bryozoans and ascidians. The picture was taken during the April 2019 monitoring at the IMTA Remedia Life plant in Taranto.

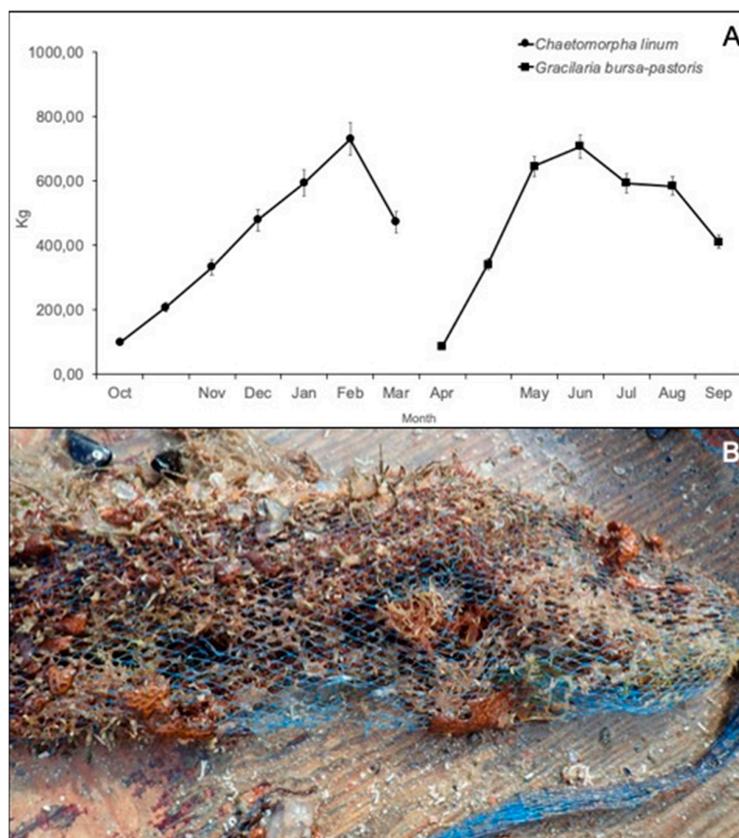


Figure 9. (A): trend of growth of the two cultivated seaweeds. (B): photograph of a retina from *Gracilaria bursa-pastoris* showing the fouling covering.

Interestingly, in the first 7 days of cultivation of *Gracilaria bursa-pastoris*, the mean SGR value was 11.1%, with a maximum of 14.6%. The interruption of the algal biomass production due to the dystrophic crisis and the development of epiphytes was particularly evident at the end of September. On account of these results, we calculated the possible production of a total algal biomass of about 1.4 tonnes, i.e., ca. 0.84 tonnes for *C. linum* and ca. 0.56 tons for *G. bursa-pastoris*.

4. Discussion and Conclusions

The experimentation of the polyculture system utilized within the Remedia Life project started about two years ago, and in this first cycle of production, we tried to solve some critical points, one of which was linked to the development of simple and useful practices to obtain a large amount of biomass of bioremediators. This was particularly relevant concerning the recruitment of polychaetes on collectors with the recovery of adults from adjacent artificial substrates, avoiding the removal from wild populations.

In the present paper, the results of the polyculture of three selected components (sponges, polychaetes and macroalgae) are reported. All these organisms, although needing different methodological approaches, were reared/cultivated in the same long lines. On the collectors utilized for rearing the sabellid *S. spallanzanii* (henceforth called *Sabella*), a macrofaunal colonization pattern was followed in order to understand the possibility of both a natural sabellid recruitment and the settlement of other fouler species that can cooperate in the bioremediation activity. For this reason, the collectors for polychaetes were never cleaned from the fouling, being also *Sabella*-protected by the covering through long tubes that run away from the substrate. By contrast, the collectors for sponge and macroalgae rearing suffered from fouling covering and needed periodical cleaning during the growth of the target species.

4.1. Performances of the Target Species

4.1.1. Polychaetes

Filter feeder polychaete-rearing for bioremediation purposes is a novelty all around the world. An experiment was carried out in an area of mussel farming in south-east Brazil, where the species *Branchiomma luctuosum* was obtained with a technique similar to that utilized in the present paper, obtaining settlers on vertically positioned floating structures [85]. However, the authors refer to rearing for ornamental purposes and do not mention their bioremediation activity which, instead, could be of paramount importance in the treatment of farming waters, and the biomass can be considered as a by-product of this activity. Excluding this paper, all experiences of sabellid rearing must be referred to our team's experience, the first one carried out in a land-based fish farm with recycled water [36], followed by another experiment conducted at a small scale in the sea [51] and coupled with a mollusc farm [52].

One of the aims of the first year of observations within the IMTA system was to analyze the possibility to obtain recruits in situ. Indeed, the techniques utilized in previous experiences proved unthinkable to be applied at larger scales since requiring a workforce leading to prohibitive costs [36,52]. The method presently utilized, focused on obtaining recruitment directly on bare collectors, is very simple and does not require any management cost after the immersion of collectors. The method is based on strong local knowledge on the fouling community settlement dynamic coupled with the knowledge of sabellid life cycles and their recruitment dynamics [43,44,75,86,87], and is linked to the massive presence in the environment of this species.

The pattern of community development observed on the bare vertical collectors utilized, containing, at the base, individuals of *Sabella*, agrees with previous studies on the fouling community obtained with the use of panels [75]. The macrofouling colonization investigated in the bare collector was followed until a "stable point" was reached after almost nine months; at this point, all the structures were characterized by a shallow assemblage (0–3 m), dominated by *M. galloprovincialis*, and a deeper

assemblage (3–10 m), dominated almost exclusively by sabellid worms. These final assemblages obtained are perfectly in line with the endpoint observed in the same period and in the same area by Lezzi and Giangrande [75], utilizing PVC panels, with depth and immersion durations having a significant effect on the evolution of fouling community structures on collectors.

Similar succession was observed in the two long lines examined, with no effect of the position on the variation in species composition nor on abundance. With both long lines, the assemblage converged in a “stable state” dominated by late successional species, namely *M. galloprovincialis* and *Sabella* dominating the shallower and deeper assemblages, respectively. This deeper assemblage appeared very interesting, showing a coverage due almost exclusively to worms, with the target species contributing up to 85%, whilst other species, such as the allochthonous *B. luctuosum* and *B. boholense*, occurred with sporadic presence throughout the year. The success of *Sabella* was due to the immersion time matching with the spawning period of the species in the Taranto sea [86], leading to a massive recruitment since January and preventing the settlement of mussels that commonly recruit later (April–June).

The growth of *Sabella* was monitored for up to a year, allowing to estimate the possible annual yield. Morphometric measurements started in April 2019 because, during the first life period, an in situ measurement of the worm density showed particularly dangerous results due the impossibility of handling the tubes without inducing stress phenomena capable of affecting their survival. After one year of their maintenance in the sea, each worm reached up to 10 cm in length and 2.5 g in wet weight. Collectors showed a density of about 100 worms per linear meter, with 50% of mortality occurring from June to December. However, it is probable that this decrease in density was mainly due to the detachment of the worms from the collector due to the weight of the fouling, because worms were observed to always be in good condition and never suffered from overgrowth of different organisms. From the performance of the first cycle, it a yearly production of about 0.36 tonnes of worms (wet weight) was estimated.

4.1.2. Sponges

Sponge mariculture has received increased attention over recent decades [55,88–93], and in the Mediterranean area, experiences using IMTA with fish and sponges have been recently performed for experimental purposes [42,44,54,94,95]. Laboratory and field experiments of sponge cultures have been conducted toward production of biomass for extraction of bioactive compounds, highlighting that the in situ sponge culture may be suitable for the eco-sustainable production of biomass for some target species [96]. The results of a life cycle assessment (LCA) conducted at a sea-based farm’s in situ cultivation of the sponge *Sarcotragus spinosulus* by Perez-Lopez et al. [81] demonstrated that the cultivation aiming to extract bioactive molecules had a more environmentally-friendly performance than the subsequent downstream processes (solvent extraction), which were the main cause of the impact.

The sponge rearing performances obtained during the first production cycle have highlighted the high capacity of *S. spinosulus* to grow and survive in vertical structures, increasing its biomass constantly and rather fast. The mortality values obtained during the monthly monitoring ranged between 7% and 19.16%, reaching around 20% at the end of the first year of observations. This was perfectly in line with the forecast and largely due to an initial death following collection, cutting and cultivation; subsequent mortality during the spring months was mainly due to competition phenomena with other filter feeder organisms, such as ascidians, mussels and polychaetes, which often outgrew the sponge, in part causing their detachment and partly suffocating it. For this reason, it was necessary to often intervene with removal of the fouling from the modules. This operation, normally carried out with *M. galloprovincialis*, exposing the structures to the air to cause the detachment of the epibionts, in reality, cannot be performed as it is for the sponges. The sponges, in fact, do not have a great chance of resisting dehydration for many hours and the cleaning operations must be carried out manually, often operating in immersion in the hottest periods.

The analysis of the sponges showing signs of stress but still living, such as not completely healed tissue or bacterial patina, suggests a great capacity for regeneration and recovery of the sponges grown in IMTA. In fact, the initial damage values, up to about 23%, when mortality was already 7%, did not translate into an increase in mortality which, on the contrary, showed a partially independent pattern.

The first cycle of sponge rearing has shown extremely promising results in all the long lines: in October 2018, about 300 L of sponges were placed in the multitrophic rearing plant, which, in the first months, shrunk to about 220 L. The biomass loss is essentially attributable to the initial mortality due to manipulation, and it had a completely exceptional value since no further decreases in volume were recorded, but, on the contrary, biomass regularly increased until the end. The evaluation of the rearing performance of the sponges after the initial stress has highlighted how, in December 2019, the total volume almost doubled, reaching a net production of about 200 L of sponge biomass, demonstrating its ability to adapt to the IMTA plant of Taranto. In addition to the good rearing performance, therefore, the reared sponge explants have shown good resistance to environmental stress, allowing to consider promising the use of this species in integrated mariculture systems.

4.1.3. Macroalgae

Seaweed polyculture has a recent history in Europe, mainly addressed to kelp species (e.g., *Alaria esculenta*, *Laminaria digitata*, and *Saccharina latissima*) reared with mussels [97]. Two of the main constraints to the development of IMTA with seaweeds are the seasonality of their life cycle and the scarcity of seed-stocks immediately available [98]. To overcome these problems, in the experimental IMTA plant set up in the Taranto sea, two species with different growth seasons, *Chaetomorpha linum* and *Gracilaria bursa-pastoris*, were maintained for the first time in co-culture with bioremediation macroinvertebrates during alternate seasons. To date, no information is available on *C. linum* cultivation either in monoculture or in an IMTA attempt anywhere, except a growth test performed in Canada, in tanks with different kinds of municipal wastewaters, in order to assess the bioremediation capacity. A SGR between 0.8–6.5% in 12 days of cultivation was calculated in that case [99]. Our values are included in this interval, but they are medium values calculated from a longer period of about six months. In the first 11 days of cultivation, an 11% SGR was determined, showing that the species has a moderate/high capacity of utilizing nutrients coming from the nearby fish cages for its own growth. Concerning *Gracilaria bursa-pastoris*, only in Israel has an experimental polyculture with *Ulva rigida* and *Sparus aurata* been attempted; the species reached a maximum SGR of 10% in specimens hung to the fish cages, and values of about 6% at the control site 100 m from the fish cages [100]. These values seem much higher in comparison with those calculated during the Life Remedia cultivation, which, conversely, showed results slightly higher than those recorded in autumn (i.e., 3.5%) and in spring (i.e., 3.2%) in a three-seaweed cultivation plant (i.e., *Chondracanthus teedei*, *Gracilariopsis longissimi* and *Gracilaria bursa-pastoris*) set up in an eutrophic embayment in southern Spain [101]. However, it should be considered that the Israel experiment lasted only 14 days, while the Spanish experiment was carried out for 3 weeks in autumn and for 6 weeks in spring and was not realized in an integrated polyculture. The medium value of *G. bursa-pastoris* SGR in the Taranto seas was calculated in an experiment lasting 163 days, which is about 5 months. However, in the first 7 days of cultivation, the mean measured SGR reached a value of 11.1% with a maximum of 14.6%. These high values suggest, also for *G. bursa-pastoris*, that eutrophic conditions, most conceivably nitrogen from the fish cages, foster thalli growth. However, considering the noticeable decrease of the growth rate that occurred for both species up to the end of the selected cultivation period, nitrogen may have become a limiting factor for their growth in the long run. Another obstacle to the development of cultured seaweed biomass could be the biofouling by epiphytes. In this respect, the abundant epiphyte blooming observed in September 2019 could have adversely affected *G. bursa-pastoris* growth, causing a limiting reduction in light penetration [102] as already observed in a preliminary IMTA experiment carried out in the Mar Piccolo of Taranto using mussels (*Mytilus galloprovincialis*), sabellids (*Sabella spallanzanii*) and seaweeds (*Gracilaria dura*) [72]. This phenomenon was also observed elsewhere. For example, in Norway, *Saccharina latissima* thalli

are often hugely covered in late spring/early summer by both invertebrates (e.g., bryozoans, blue mussels, hydroids) and macroalgae [103]. This, in the worst case, causes considerable damages to seaweed production, so farmers consider it necessary to harvest *S. latissima* crops in late spring, possibly replacing them with some other species more resistant to fouling [103].

On account of these considerations, we suggest the realization, throughout the year, of two cycles of seaweed cultivation, one related to *C. linum*, starting in October and ending in February, and the other related to *G. bursa-pastoris*, starting in April and ending in early July. Moreover, a shorter rearing time could be advisable for seaweed in this innovative system in order to maximize their biomass production. A biweekly harvesting of biomass returning to the original weight could be optimal in order to lighten the socks and make more space for a new production; this will be the strategy applied to the next production cycle.

4.2. Conclusive Remarks

The biological assemblage obtained in the IMTA system realized in the Gulf of Taranto represents an interesting tool to exploit for several reasons: ease of species cultivation, efficiency of the filtering system and crop diversification of valuable by-products which could increase the biomass production of an aquaculture site, opening new commercial horizons.

The method for sabellid breeding applied here demonstrated that a conspicuous amount of worm biomass can be obtained, coming mostly by filtering the wastes of the aquaculture farm. On the basis of some data from previous experiments [36], a requirement of five quintals of polychaetes to process the yearly quantity of wastes was computed; this amount is slight higher than that produced during the first year of observations. However, the presence on the collectors of other bioremediators must also be considered, as well as sponges and macroalgae having a high efficiency in bioremediation of seawater nutrient enrichment and removal of heavy metals [48,99–101,104–108]. Although mass balance with net removal of wastes is still under study, preliminary analyses of benthic communities under cages have shown relevant positive structural changes (unpublished data). In this context, the fouling community associated with the collectors is noteworthy. Sessile organisms were mainly ascidians, whose filtration efficiency has already been investigated by our group [109]. The presence of rearing structures increases indeed biodiversity, modifying the spatial structure of the original substrates with positive repercussions on the fauna composition of the underlying hard and inconsistent substrates. If, however, increasing the biomass of fouling leads to an advantage in the removal of organic substance, it should be emphasized that an accumulation of biomass could have negative effects if it remains for a long time in the area, or if it even had to detach and accumulate on the bottom. For this reason, it is necessary to periodically remove the biomass obtained, directing it towards commercial and processing chains that are not yet well defined, definitively removing the organic substance from the plant.

The different reared target species, sabellid, sponges and seaweeds, need different periods of maintenance in the system, from a very short period for macroalgae, where a biweekly harvesting of biomass was hypothesized as the best strategy, to at least one year for sabellid and sponges. After the first year, only half of the collectors were harvested, leaving the others operating in situ in order to evaluate the produced biomass after a two-year cycle. Moreover, the adults remaining in the system could also continue to act as reproducers for the new cycle.

Sponges open up new perspectives related to their use in different fields, representing a valuable by-product of the IMTA system due to the potential for new biomaterials, biomedical applications and pharmaceutical products [110]. Several natural products can be extracted from this species, and among them, polyphenylhydroquinones [111] drew particular attention due to their wide spectrum of bioactivities (antibacterial, antiviral, anti-inflammatory and cytotoxic) [79].

Macroalgae could be an important by product too, highlighting their capacity of producing bioactive compounds for multiple applications [49,78,112–114]. In particular, in the lipidic extract of *C. linum* from the Taranto sea, Stabili et al. [49] demonstrated the existence of antibacterial and antioxidant activity against common pathogens in aquaculture, such as *Vibrio ordalii* and *Vibrio vulnificus*. It is

likely that the antibacterial activity is linked to the high density of linolenic acid, while carotenoids and chlorophylls could be responsible for the antioxidant activity. Moreover, the presence of omega-3 and omega-6 fatty acids could make it an innovative ingredient in fortified food, and polyhydroxybutyrate could be used in the production of biodegradable bioplastics. Notwithstanding the proved utility of macroalgae cultivation and the possible setting up of new economic scenarios, Europe is still several steps behind regarding effective establishment of a seaweed industry. To this end, in order to create the right conditions for the development of a sustainable European bioeconomy founded on macroalgal biomass, Asian models, both technical and economic, have to be used as a point of departure, making aquaculture enterprises and policy makers acquainted with the risks and advantages related to seaweed cultivation in IMTA systems. The present study could represent a first step towards this direction.

Lastly, the utilization of *Sabella* and sponge biomass is still under investigation, including market analysis, those being actual by-products from IMTA systems.

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