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Growth Performance of *Mytilus galloprovincialis* Lamarck, 1819 under an Innovative Integrated Multi-Trophic Aquaculture System (IMTA) in the Mar Grande of Taranto (Mediterranean Sea, Italy)

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Abstract: The cultivation of the Mediterranean mussel, *Mytilus galloprovincialis* Lamarck, 1819, has been tested in an innovative Integrated Multitrophic Aquaculture system (IMTA) in the Mar Grande of Taranto, as part of the EU-funded Remedialife project. This farming method could solve several problems including the low growth rate in mesotrophic environments while reducing the environmental impact of fish mariculture. Three productive cycles have been carried out. The first (2018–2019, traditional experiment) was conducted in three long lines around six cages of the fish farm in order to evaluate total mussel production under the innovative IMTA system and quality for human consumption by analyzing the concentration of culturable heterotrophic bacteria, total and fecal coliforms, *Escherichia coli* and *Salmonella* spp. in mussel tissues. In addition, 17 polycyclic aromatic hydrocarbons (PAHs), including 16 EPA priority compounds and seven polychlorinated biphenyls (PCBs), which are indicators of PCB contamination in the environment, were analyzed using gas chromatography in conjunction with a mass spectrometer. The second cycle (2020–2021, horizontal distance experiment) aimed to test the influence of fish cages on mussel growth by placing mussels near and far from the fish cages. The third cycle (2021–2022, vertical distance experiment) aimed to overcome the phenomenon of "heat waves" that can occur in the Mar Grande of Taranto during summer by testing the growth performance of mussels at two different depths (1 and 12 m). The following parameters were measured: Shell Length, L (mm); Shell Dry Weight, SDW (g); Flesh Dry Weight, FDW (g); Condition Index, IC = FDW/SDW. The results showed that the best growth performance was obtained near the fish cages and at a depth of 12 m. Moreover, the indicators of microbial contamination and concentrations of chemical compounds analyzed in mussel tissues cultured under the innovative IMTA system were in compliance with the reference values of European regulations.

Keywords: mariculture; mussel farm; IMTA system; mesotrophic condition; heat waves

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1. Introduction

The Taranto Sea system is one of the most important and human-influenced coastal marine ecosystems in the Apulia region (southern Italy) [1]. It consists of two communicating basins characterized by different degrees of confinement: the Mar Grande, directly connected to the Gulf of Taranto (Ionian Sea) and the Mar Piccolo, the lagoon-like inland basin with limited water circulation and low hydrodynamics [1–3]. Taranto, also called "*Città dei due Mari*" (City of the two Seas) due to its location between the two basins, has a deep and ancient connection with the sea. Mussel farming has been practiced for centuries,

especially in the Mar Piccolo, and the local product was renowned worldwide for its quality. Taranto was one of the most important areas in Europe for the production of Mediterranean mussels, *Mytilus galloprovincialis* Lamarck, 1819, and mussel farming was one of the main economic activities of the local population [4]. However, in the last three decades, mussel production in Taranto has declined greatly, in line with the general European downward trend [5]. Previous studies from other Mediterranean regions have cited disease or a lack of mussel seed as the main causes of this decline [6,7], but global warming and low profitability may also have played a role [8–11]. In the era of climate change exceptional events such as "marine heat waves" (i.e., prolonged periods, >5 days, of abnormally high seawater temperatures) are greatly increasing in both frequency and magnitude [12]. Marine heat waves are detrimental to marine ecosystems and can lead to mass mortality of organisms when the individual thermal tolerance limits are exceeded [13]. Therefore, benthic sessile species are at high risk due to their immobility [11], and mussel mass mortality events that may be related to high temperatures were recently reported [14–17].

In this context, it is important to know the history of the development of mussel farming in Italy and Taranto. Traditionally, mussel farming was mainly carried out in lagoons and ponds. These highly productive environments were used for a long time because of their easy accessibility. However, when the spatial and biological capacity was exhausted, the need to move to the open sea became apparent [18]. The first offshore installations were built in the Gulf of Trieste more than 50 years ago, and the most significant offshore effort was made by France in the 1070s. However, several studies in the Adriatic Sea have shown that offshore mussel farms have a minimal negative impact on zoobenthic communities [19–22], and the oligotrophic nature of the Mediterranean Sea and the high maintenance costs of offshore farms have limited their expansion. Currently, most mussel farms off the Italian coast are located in the Adriatic Sea, which is still a rather eutrophic basin, especially in the northern part near the Po Delta.

Traditional mussel farming in Taranto was artisanal and mussels were suspended with wooden stakes. In the 1990s, the stakes were replaced with long lines, which improved mussel production. Mussel farming took place mainly in the inland basin, the Mar Piccolo. The presence of 34 underwater freshwater springs (known locally as "Citri") gives the Mar piccolo its lagoon-like characteristics and provides an ideal growing environment for mussels [1,4]. However, the Mar Piccolo is about half as deep as the Mar Grande and has less water exchange [2,3], therefore, it is more likely to experience recurrent algal blooms, hypoxic crises and marine heat waves, especially in summer [4,16]. During these events, mussels are temporarily relocated to the Mar Grande [4]. Since 2006, after the extension of the concessions to further expand the farming area, an increase in production would have been expected, but instead a decrease has been observed, including in the quality of the mussels. This was most likely due to the relocation of some sewage discharges out of the Mar Piccolo to reduce bacterial load, which, in turn, reshaped the trophism of the system [4,23] as well as the increasing pollution, especially by organic compounds, mainly due to navy and industrial activities [24]. Furthermore, in recent years (2012, 2015 and 2017), the Taranto area suffered several mass mortalities of mussels due to recurring summer heat waves [4,16]. In July 2015, for example, the water temperature peaked to 30.4 °C and remained high for a month, and mussels living near the surface were the first to die, followed by those that grew at higher depth [16].

Polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) are among the priority organic compounds widespread in the marine environment. They have attracted considerable attention due to their high toxicity, persistence in the environment and the ability to bioaccumulate in several organisms. Levels of these pollutants were found in benthic, demersal and pelagic fish. Fish is a suitable indicator for environmental pollution monitoring because they concentrate pollutants in their tissues directly from water, but also through their diet, thus enabling the assessment of the transfer of pollutants through the trophic web [25,26]. These xenobiotics have been also recorded in crustaceans and shellfish collected in many Mediterranean areas, especially in polluted coastal en-

vironments impacted by anthropogenic activities [27–32]. In particular, in the Southern Ionian Sea consistent levels of PAHs and PCBs were detected in edible organisms such as bivalves, gastropods, echinoderms and fish [33–35]. Mussels can represent a food at risk of contamination because are filter-feeding organisms with high bioaccumulation and low biotransformation potential for both organic and inorganic contaminants [36,37].

Since 2011, the Prevention Department of the Taranto Local Health Authority has been carrying out a monitoring plan to assess the contamination with organic compounds of *M. galloprovincialis* farmed in the Mar Piccolo and Mar Grande. The results of this study showed that results in the levels of PCDD/Fs and DL-PCBs in mussels from Mar Piccolo during summer were above regulatory levels. This prompted the Apulia region to adopt the Regional Ordinance n. 188/2016 to prohibit the removal and handling of commercial mussels in the Mar Piccolo, with the only possibility of transferring juvenile mussels to other basins such as the Mar Grande. Although the Mar Grande is a mesotrophic area, it is not suitable for the fast growth of mussels and is used mainly for the purification stage before marketing. This is causing mussel farmers to switch to more profitable fish farming, which, however, is more harmful to the environment [38,39], especially when practiced in confined areas. The negative impact on the environment mainly comes from fish waste (e.g., feces and uneaten feed), whose long-term accumulation enriches the environment around fish cages of both inorganic and organic matter [40]. Therefore, it would be desirable to combine the co-culture of mussels and fish in an Integrated Multitrophic Aquaculture system (IMTA), where the filter feeders can mitigate the effects of fish monoculture while assimilating fish waste for their own growth [41,42]. Bivalves are the most commonly used extractive species in IMTA systems, due to their economic value, especially in northern Europe [43], while other highly efficient filter feeders are underexploited [44]. Although IMTA systems are less developed in the Mediterranean Sea, several studies carried out recently report the integration of mussel farming to IMTA also in Mediterranean areas [45–48]. However, the exclusive use of bivalves is not considered an appropriate tool to reduce the environmental impact of fish aquaculture [49].

On account of these considerations, we tested the cultivation of the Mediterranean mussel, *M. galloprovincialis*, under an innovative IMTA system combining fish, mussels and a new group of bio-remediators such as sponges, polychaetes and macroalgae to improve the overall bio-remediating performance [44]. The aim was to study the growth performance of mussels in such a mariculture scenario and to evaluate the possibility of obtaining a healthy product for human consumption, overcoming the mesotrophic conditions and summer heat waves that affect the growth of *M. galloprovincialis*, while reducing the environmental impact of fish farming.

2. Materials and Methods

2.1. Study Area

The study area is located on the southwestern 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 ranging between 14 °C in winter and 28 °C in summer, while the salinity is about 38 and is almost uniform over the year. The investigation was performed in the aquaculture plant Maricoltura Mar Grande, hosting the experimentation of the innovative IMTA system.

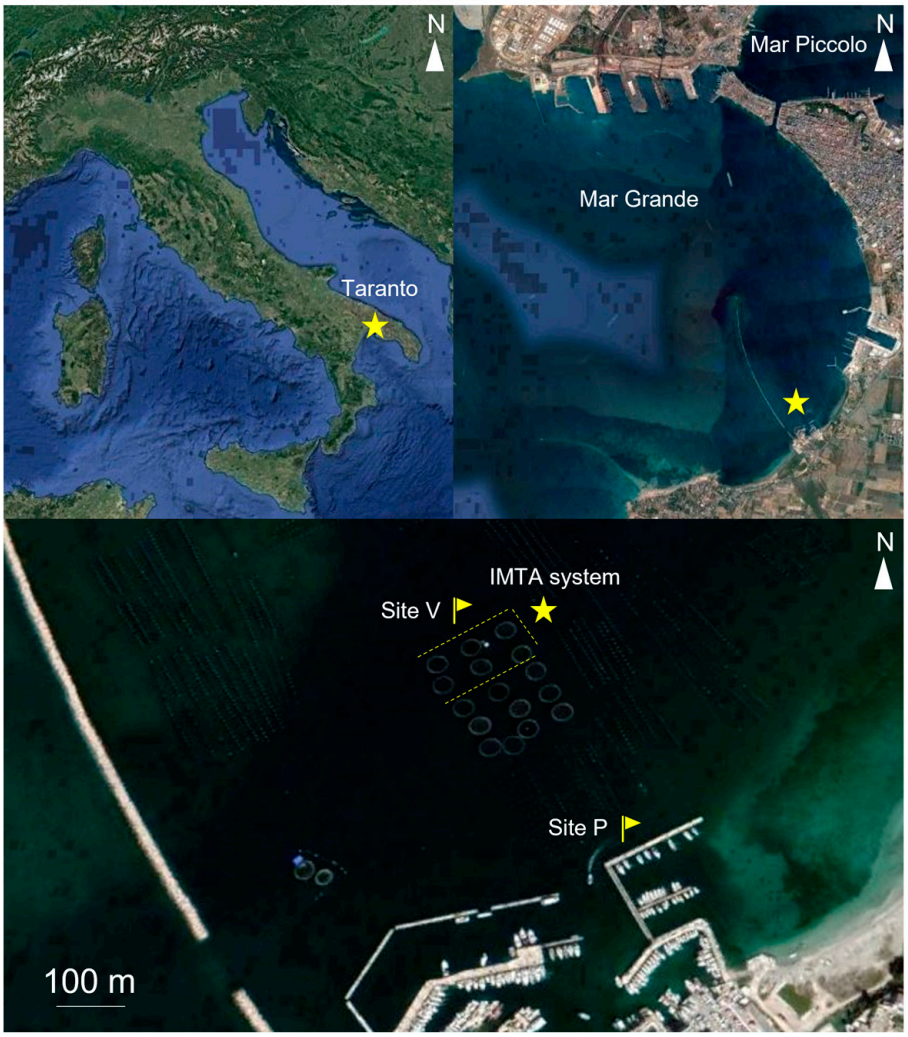

Figure 1. Map of the study area showing the location of the IMTA system and mussel farming sites.

The aquaculture plant is in a semi-confined area of the Mar Grande, covering a surface of 0.06 Km^2 and 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.

The Mar Grande of Taranto is affected by intense *M. galloprovincialis* farming, but mainly for the purification stage before marketing. The species is also naturally abundant in all the artificial hard bottoms in the area and mussel spats are abundant each year.

2.2. Field Work: Sampling and Processing

One of the goals of the Remedia Life project was to estimate the mitigation of aquaculture waste using bioremediating organisms. The results of the *ex ante* monitoring carried out in the study area (Table 1) showed which part of the aquaculture plant was most impacted in terms of bacterial, inorganic and organic compounds, specific richness of soft-bottom macrobenthic communities and fouling taxonomic structure [50].

Table 1. Environmental indices and bacterial contamination values measured in the study area during the *ex ante* monitoring [50].

Site	AMBI (Status)	M-AMBI (Status)	Microtox STI (%Bioluminescence Inhibition)	*Escherichia coli* (MPN/g)	*Salmonella* spp. (+/−)
IMTA-converted	4.81 (Poor)	0.41 (Moderate)	0.33 ± 0.01 (Hormensis)	40.0 ± 9.4	Absent
Control	2.78 (Good)	0.95 (High)	0.13 ± 0.01 (Hormensis)	40.0 ± 9.4	Absent

Notes: Reported abbreviations: AMBI, AZTI's Marine Biotic Index; M-AMBI, Multivariate-AZTI's Marine Biotic Index; STI, Sediment Interstitial Water; MPN/g, Most Probable Number/1 g of sediment.

The most impacted part of the aquaculture plant was converted to the IMTA system (Table 1) by adding macroinvertebrates and seaweed as bio-remediating organisms on three long lines around fish cages (Figure 1). The long lines were supported by buoys to prevent the structure from sinking as the biomass grew. Each space (3 m long) between two successive buoys formed the culture "chamber" that housed the extractive species modules: the macroinvertebrate modules were suspended vertically, while the macroalgal modules were arranged horizontally (see [44] for a complete description of the IMTA scheme).

Three productive cycles were conducted. In the first cycle (2018–2019), referred to as the "traditional experiment", a total of 307 mussel nets (4 m long) were attached to the three long lines around the fish cages. Juveniles from the 2018 mussel seed collection were placed in the nets in November 2018 (T0-1) and suspended on long lines at 1 m depth. The traditional farming method was followed for mussel growth until the end of the production cycle (July 2019, T7-1).

A second cycle, called the "horizontal distance experiment", was planned for the years 2020–2021 to determine whether the growth rate would be affected by the presence of fish cages (greater food availability), and to determine whether the mussels would reach the required size for marketing. In this case, the experiment was conducted on a small scale, considering only 16 mussel nets at two different sites. In November 2020 (T0-2), 16 nets (1 m long) were placed at site V, near the aquaculture plant (Figure 1). The growth performance of these mussels was compared to that of mussels from the same initial stock placed in 16 nets at site P, approximately 300 m from the aquaculture plant (Figure 1).

A third cycle (2021–2022), called the "vertical distance experiment", was then planned to test the hypothesis of higher growth performance in relation to the water depth, combined with obtaining the required size for sale. As in the second cycle, the experiment was tested on a small scale but only at site V, near the aquaculture plant. In November 2021 (T0-3), 16 nets (1 m long) were placed at 1 m depth (Surface), as in the traditional farming method, whilst 16 were maintained at 12 m (Deep). An overview of the three experiments is reported in Table 2.

Table 2. Overview table of the mussel growth experiments.

Production Cycle	Experiment	Number of Mussel Nets	Study Period	Site	Distance from the Cages	Depth
2018/2019	Traditional	307	T0–T7 (1)	V	1 m	1 m
2020/2021	Horizontal distance	16	T0–T6 (2)	V/P	1 m/300 m	1 m
2021/2022	Vertical distance	16	T0–T7 (3)	V	1 m	1 m/12 m

2.3. Microbiological Analyses

Samples for microbiological analysis were collected in July 2019 at the end of the first cycle of production. *M. galloprovincialis* samples (three replicates each consisting of 40 specimens) were received in the microbiology laboratory within 4 h of collection (stored at about 4 °C) and then subjected to microbiological analyses. In particular, mussels were

washed, scrubbed free of dirt and shucked with a sterile knife [51]. Meats and liquors of each replicate were homogenized for 90 s in a sterile blender (Waring Commercial, Stamford, CT, USA) then filtered through sterile gauze and diluted with sterile seawater (filtered through 0.2 µm filters, Millipore, Burlington, MA, USA) to obtain a 1:10 (*w*/*v*) dilution immediately added to the appropriate medium.

For enumeration of culturable heterotrophic bacteria, mussel homogenate and serial dilutions of each replicate were plated in triplicates onto Bacto Marine Agar 2216 (Difco, Detroit, MI, USA) (seeding with 0.1 mL). The plates were incubated at 22 °C over 7 days. At the end of the incubation period, all colonies were counted through a 10× magnifying glass. Total culturable bacteria at 37 °C (including human potential pathogens) in the samples were determined by plating 0.1 mL of each sample and serial dilutions in triplicates on Bacto Plate Count Agar (Difco, Detroit, MI, USA). After incubation for 48 h at 37 °C, the growing CFU were counted.

The enumeration of *Escherichia coli* in bivalve samples was performed by using the Most Probable Number (MPN) method in accordance with the EU reference methods [51,52]. Briefly, 75–100 g of flesh and intervalvular liquid were added to 2 parts of Peptone water (Oxoid, Basingstoke, UK) and homogenized by a Stomacher for 2.5 min. The homogenate was added to Peptone water to reach a final 1:10 dilution. Aliquots from this diluted homogenate were transferred to tubes with Mineral Modified Glutamate Medium (MMGB) (Oxoid, Basingstoke, UK) [53] by employing the method using the standard five-tube method of 10-fold dilution. The tubes were incubated aerobically at 37 ± 1 °C for 24 ± 2 h. Positive MMGB tubes changed color from purple to yellow and subcultures from these tubes were plated on chromogenic Tryptone Bile X-Glucuronide Agar (TBX) plates (Oxoid, Basingstoke, UK) incubated aerobically at 44 °C for 20 h. At the end of incubation, the grown blue-green colonies were recognized as presumptive *E. coli* [54]. The concentration of *E. coli*/100 g was estimated by counting the number of positive tubes giving the growth of blue-green colonies on TBX agar by using the MPN table reported in [52].

Coliform bacteria (total and fecal coliforms) concentrations were determined by using the Most Probable Number (MPN) method and the three-tube MPN series in accordance with the EU reference methods [55] by using Lauryl sulfate tryptose broth (Oxoid, Basingstoke, UK) in the presumptive test (incubation at 37 °C for 24–48 h). All presumptive positive (gas production) tubes were transferred to tubes containing brilliant green lactose bile broth and incubated for 24–48 h at 37 °C (confirmatory test). The number of test tubes giving positive results (gas production) was recorded and a table for determination of Most Probable Numbers was used.

Salmonella spp. were determined in accordance with [56]. Briefly, 25 g of each sample were homogenized in 225 mL of buffered peptone water (BPW) (Oxoid, Basingstoke, UK) and incubated for 18 ± 2 h at 37 ± 1 °C. Thereafter, an aliquot of the pre-enrichment was inoculated into two selective broths, Rappaport–Vassiliadis medium with soya (RVS broth) (Oxoid, Basingstoke, UK) and Muller-Kauffmann tetrathionate/novobiocin broth (MKTTn broth) (Oxoid, Basingstoke, UK), incubated at 41.5 ± 1 °C for 24 h ± 3 h and 37 ± 1 °C for 24 ± 3 h, respectively. Then, after incubation, sub-cultured from RVS and MKTTn broths were plated onto the surface of one Xylose-Lysine-Desoxycholate (XLD) (Oxoid, Basingstoke, UK) agar plates and incubated at 37 °C for 24 h. Suspected grown colonies were confirmed biochemically (triple sugar iron [TSI] agar, urea agar, l-lysine decarboxylation medium and indole reaction) and by serological tests.

2.4. Chemical Analyses

As for the microbiological analysis, chemical analysis was relative to the first productive cycle. The whole mussel tissues (30 specimens for each of the 3 replicates) were homogenized (T 25 basic ULTRA-TURRAX (IKA®—Werke GmbH & Co. KG, Staufen, Germany) and freeze-dried (LIO 5P Cinquepascal S.r.L., Milan, Italy). Before lyophilization, an aliquot of the sample was used for dry weight calculation by oven drying at 105 °C until constant weight. For polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls

(PCBs) determinations, 2 g of freeze-dried mussels were extracted by a microwave system (MARS-X C EM Corporation, Matthews, NC) with an appropriate solvent mix solution (cyclohexane/acetone,1:1 *v*/*v*) and purified by Gel Permeation Chromatography Clean-up system (AZURA, Knauer, Berlin, Germany). Analyses were performed by gas chromatography coupled with a mass spectrometer (Agilent 7890A gas chromatograph—Agilent 975C inert mass spectrometer, Agilent Technologies, inc. Santa Clara, CA, USA). A total of 17 PAHs included 16 Priority EPA compounds (naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, benzo[g,h,i]perylene, indeno[1,2,3-c,d]pyrene and dibenz[a,h]anthracene) more benzo[J]fluoranthene and seven PCBs congeners (IUPAC No. 28, 52, 101, 118, 138, 153 and 180), considered as indicators of environmental PCB contamination, were analyzed according to USEPA Method 8270E [57]. Major information on analytical procedures was reported in [58]. PAHs and PCBs standards, deuterated internal and surrogate standards, were purchased from Merck© as well as all chromatographic grade solvents (Merck s.p.a., Milan, Italy). The recoveries, determined by spiking the appropriate number of standard mixtures to mussel, were 60–110 and 70–120% for PAHs and PCBs, respectively. The method detection limit (MDL), based on a signal-to-noise ratio of 3:1, ranged from 0.6 to 2 µg/kg and 0.2 to 0.4 on a dry weight basis for PAHs and PCBs, respectively.

2.5. Growth Parameter Analysis

At the end of the traditional experiment (July 2019), an estimate of total mussel production was calculated. During the horizontal and vertical distance experiments, measurements of growth performance were taken. In the horizontal distance experiment, 4 mussel ropes were randomly sampled each time (T1-2, January 2021; T2-2, February 2021; T4-2, April 2021; T6-2, June 2021) for each site (V, P) and 5 individuals were considered for each mussel rope. The following parameters were measured: Shell Length, L (cm); Shell Dry Weight, SDW (g); Flesh Dry Weight, FDW (g); Condition Index, IC = FDW/SDW [59]. The same sampling design was followed during the vertical distance experiment (T2-3, February 2022; T4-3, April 2022; T6-3, June 2022; T7-3, July 2022) for each depth (Surface, Deep).

Shapiro–Wilk test (Horizontal distance experiment, L: W = 0.91, p = 0.08; SDW: W = 0.97, p = 0.78; FDW: W = 0.96, p = 0.64; CI: W = 0.92, p = 0.09; Vertical distance experiment, L: W = 0.95, p = 0.44; SDW: W = 0.95, p = 0.34; FDW: W = 0.95, p = 0.43; CI: W = 0.94, p = 0.23) and Levene's test were performed to verify normal distribution of data and homogeneity of variances, respectively. When the hypothesis of homogeneity of variances was not met, the Welch *t*-test was applied. Student's *t*-test (n = 20, df = 38, *t*-value = 2.02) was performed to test for differences in mussel growth performance between the P and V sites during the horizontal distance experiment and between the "Surface" and "Depth" groups during the vertical distance test by analyzing the increase in Shell Length (L), Shell Dry Weight (SDW) and Flesh Dry Weight (FDW) between T6-2/T7-3 and T0-2/T0-3, while only the final stage (T6-2/T7-3) was considered for the Condition Index (IC). Significance was set at a critical level of 95% ($p < 0.05$). All statistical tests were performed using STATISTICA 10.0 software package.

3. Results

3.1. Growth Performance

The total macroinvertebrate biomass for the three productive cycles (Table 2) was about 800 kg for polychaetes, 258 kg of sponges and about 1300 kg of macroalgae. For the traditional experiment, the mussel production at the end of the cycle was about 3.5 tons including the weight of the shells. However, no measurements of growth performance were made during the first cycle of production, so no information on the condition index can be provided. After these apparently good results, we started with the second cycle in November 2020 to test the hypothesis of higher growth performance near fish cages.

Figure 2 shows the time trend of the mussel growth parameters during the horizontal distance experiment.

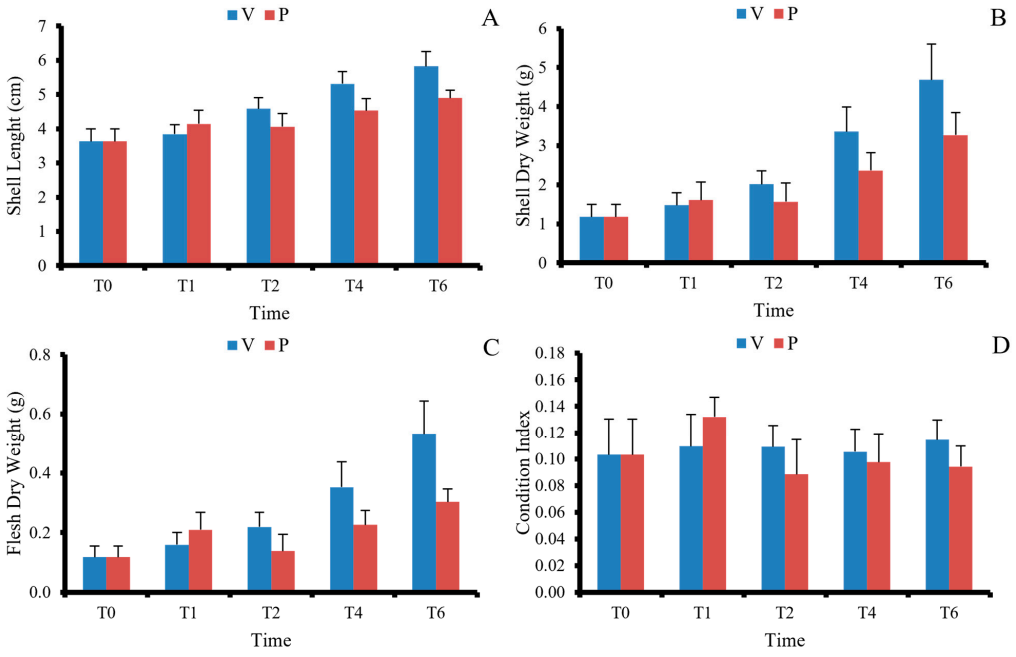

Figure 2. Time trends of mussel growth parameters Shell Length (**A**), Shell Dry Weight (**B**), Flesh Dry Weight (**C**) and Condition Index (**D**) at sites V and P, respectively, near and far from the fish cages.

The mussels cultured at site P (far) were found to have smaller values of L, SDW, FDW and IC than those at site V (near) at all sampling times after T1-2 (Figure 2). Moreover, the increase in L, SDW and FDW between T6-2 and T0-2 was significantly higher (Table 3) in mussels grown at site V than in those at site P. The same was true for the IC at T6-2 (Table 3). However, mussels from both sites did not reach the required IC value for sale as they died due to the high temperatures recorded in the Mar Grande in July 2021 (Figure 3).

Table 3. Results of Student *t*-test on Condition Index (CI) in June 2021 and July 2022 (T6-2, T7-3) and increase in Shell Length (L), Shell Dry Weight (SDW) and Flesh Dry Weight (FDW) between T6-2/T7-3 and T0-2/T0-3 in mussels, grown near (Site V) and far (Site P) from the fish cages during the horizontal distance experiment and at site V at 1 m (Surface) and 12 m (Depth) depth during the vertical distance experiment.

Growth Parameter	Horizontal Distance					Vertical Distance				
	Site V	Site P	df	*t*	*p*	Surface	Depth	df	*t*	*p*
L increase (cm)	2.19 ± 0.48	1.27 ± 0.39	37	6.64	*<0.001*	2.06 ± 0.52	2.44 ± 0.34	32	2.77	0.009
SDW increase (g)	3.51 ± 0.94	2.09 ± 0.68	35	5.47	*<0.001*	4.03 ± 1.53	4.59 ± 1.10	34	1.33	0.190
FDW increase (g)	0.42 ± 0.12	0.19 ± 0.06	27	7.85	*<0.001*	0.45 ± 0.24	0.74 ± 0.17	34	4.31	*<0.001*
CI	0.11 ± 0.02	0.09 ± 0.02	38	4.13	*<0.001*	0.11 ± 0.03	0.15 ± 0.03	38	5.08	*<0.001*

Note: Significant *p*-values are given in italics.

Figure 3. Empty shells left after experimental mussels died due to high temperatures during the second cycle.

The experiment to test the possibility of obtaining a good condition index for mussels was repeated in the third cycle only at site V near the fish cages, and mussel growth was compared in relation to water depth (1 m vs. 12 m). During the colder months (T2-3), mussels cultured at surface showed higher values than deep-grown mussels in all the parameters under consideration (Figure 4). After April 2022 (T4-3), when only SDW and FDW values remained higher in surface-grown mussels, a reverse trend was observed in all parameters being higher in deep-grown mussels (Figure 4). Moreover, during warmer months (T6-3, T7-3), a slight reduction in all parameters was observed in surface-grown mussels preventing them from reaching the required IC for market (Figure 4). By contrast, at T7-3 deep-grown mussels reached the required IC for sale, which was also found to be significantly higher than that of surface-grown mussels (Table 3). Furthermore, mussels placed at higher depth had significantly higher values; also, L and FDW increase the mussels placed at more surface depth, while the increase in SDW showed no significant differences (Table 3).

3.2. Microbiology

The quality of the product was determined on mussels produced during the first cycle of production by analyzing microbiological parameters as well as PAHs and PCBs concentrations. Data on heavy metals are available in [60].

M. galloprovincialis samples contained a mean bacterial concentration of $17 \pm 1.2 \times 10^3$ CFU g^{-1} at 22 °C and $2.3 \pm 0.2 \times 10^3$ CFU g^{-1} at 37 °C. The values of the measured microbial pollution indicators are shown in Table 4. Total coliforms and fecal coliforms reached the value of 430 MPN/100 g. The concentration of *Escherichia coli* was very low and *Salmonella* spp. was absent.

Table 4. *E. coli* (MPN/100 g) and *Salmonella* spp. (presence/absence) results in mussel samples.

Samples	Total Coliforms	Fecal Coliforms	*Escherichia coli*	*Salmonella* spp.
	MPN/100 g	MPN/100 g	MPN/100 g	Presence/Absence
Mytilus galloprovincialis	430	430	18	Absence

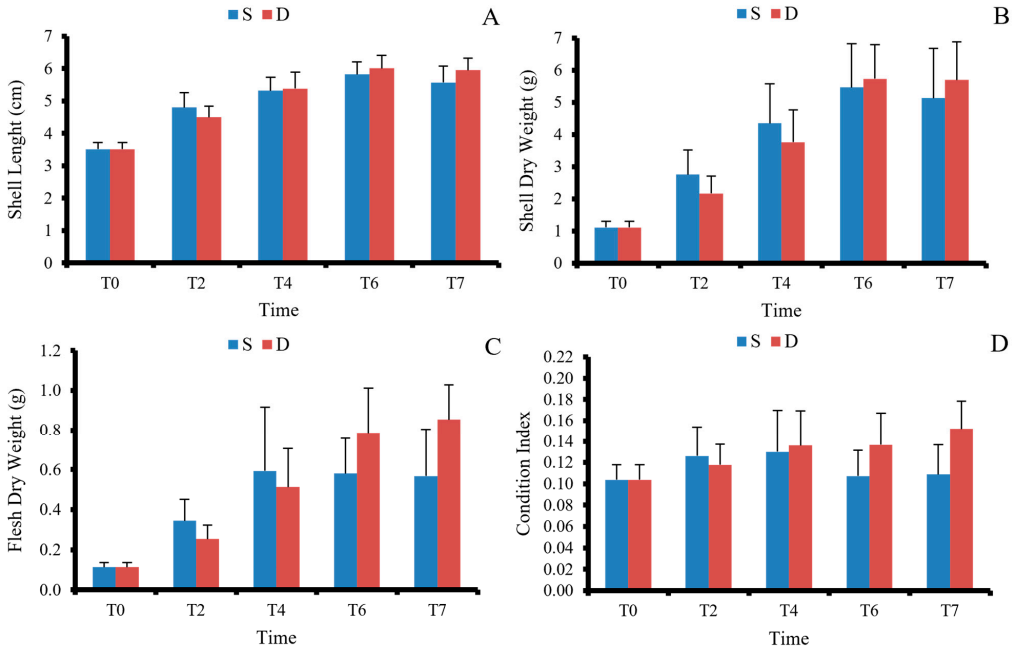

Figure 4. Time trends of mussel growth parameters Shell Length (**A**), Shell Dry weight (**B**), Flesh Dry Weight (**C**) and Condition Index (**D**) at 1 m (S) and 12 m (**D**) depth at site V.

3.3. PAHs and PCBs Concentration

PAHs and PCBs compounds in mussels reached, respectively, a total concentration of 46 ± 6.5 (Mean value ± SD) and 11 ± 1.4 µg/kg wet weight (ww). In Figure 5, the distributions of all PAHs and PCBs are reported. In particular, B[a]P and the sum of B[a]A, Cry, B[b]F and B[a] (∑4PAHs), considered markers for the occurrence and effect of carcinogenic PAHs in food, showed concentrations of 2.9 ± 0.3 and 17.4 ±1.7 µg/kg ww, respectively, while the sum of PCB 28, 52, 101, 138, 153 and 180 (∑6 PCB), representative of non-dioxin-like PCBs contamination in food, was 9.6 ±1.2 µg/kg ww.

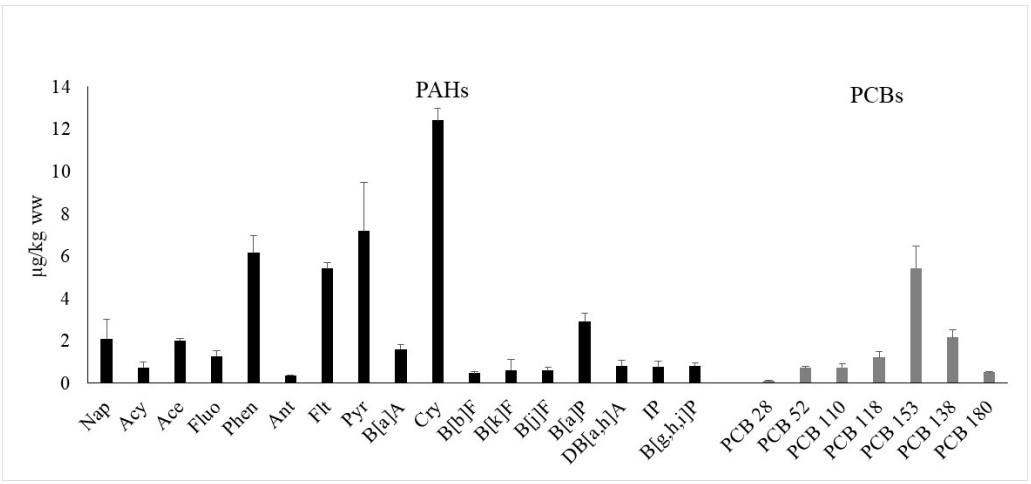

Figure 5. PAH and PCB compounds in mussels. PAHs (polycyclic aromatic hydrocarbons); PCBs (polychlorobiphenyls compounds); Nap = naphthalene, Acy = acenaphthylene, Ace = acenaphthene, Fluo = fluorene, Phen = phenanthrene, Ant = anthracene, Flt = fluoranthene, Pyr = pyrene, B[a]A = benzo[a]anthracene, Cry = chrysene, B[b]F = benzo[b]fluoranthene, B[k]F = benzo[k]fluoranthene, B[j]F = benzo[j]fluoranthene, B[a]P = benzo[a]pyrene, D[a,h]A = dibenz[a,h]anthracene, IP = indeno[1,2,3-c,d]pyrene and B[g,h,i]P = benzo[g,h,i]perylene. Results are expressed on a wet weight (ww) basis considering a wet/dry ratio of 5.0.

4. Discussion and Conclusions

In the present study, we report the results on the growth and quality of the Mediterranean mussel, *M. galloprovincialis*, cultured together with fish and a new group of bioremediators such as sponges, polychaetes and macroalgae under an innovative multitrophic aquaculture system (IMTA).

In the traditional experiment, we obtained a higher biomass of mussels than the other bio-remediating organisms [44] and observed the first signs of environmental restoration, as both the hard-bottom [61] and the soft-bottom communities [62] under the fish cages showed a recovery in species number and diversity.

Following these encouraging results, in the horizontal distance experiment, we compared the growth of mussels near and far from the fish cages (sites V and P, respectively). The data obtained show that the mussel growth was influenced by the distance from the fish cages, yet mussels from both sites did not reach the condition index value (i.e., 0.15) required for marketing [59]. This was due to a severe heat wave in July, which caused mass mortality of up to 70–80% of mussels farmed throughout the Taranto basin. However, mussels grown near the floating cages (site V) showed significantly higher values for all growth parameters than those grown at a distance of 300 m (site P). It is, therefore, likely that mussels from the experimental site benefited from the organic load from the fish cages. In addition, according to the mussel farmers, mussels in the vicinity of the fish cages are not apparently eaten by sea bream.

M. galloprovincialis is quite a generalist suspension feeder. Although it prefers to feed on phytoplankton, it is capable of ingesting such a wide variety of particle types and sizes that fish waste can provide an additional food source. Indeed, laboratory and field studies using stable isotopes and fatty acids as biomarkers have confirmed that Mediterranean mussels are able to ingest and assimilate organic waste from fish farms [63–65], suggesting that aquaculture activities play an important role in nutrient cycling. However, dilution of particulate fish waste increases with distance from fish cages [66], so the best growth performance of extractive species in IMTA systems is achieved when mussels are cultured near fish farms. This implies that production increases are generally greatest at relatively small spatial scales [43], although the growth rate varies greatly depending on the hydrodynamic characteristics of the area under consideration. In addition, nutrient dispersal near fish farms is influenced by hydrodynamics, subsurface geographic features and fish cage structure [67]. It appears that mussels are better able to take up particulate fish waste when farmed in areas with low current velocity [68,69]. In addition, stable isotopes used to study the dispersal area of fish farm wastes have shown that sediments surrounding cages can be organically enriched up to about 1000 m from the cages [65,70,71].

Improved growth performance of mussels near fish cages has been observed in several previous works [43,45–48,72]. It was not possible to compare the condition index values obtained in the present study with those obtained in other IMTA systems of other Mediterranean regions [46–48], as in the latter the wet weight was used to determine the condition index, making the values subject to higher variability. However, mussels grown in the aforementioned IMTAs showed higher CI values than mussels grown under monoculture or natural conditions in the respective reference areas.

Finally, the results of the third experiment (vertical distance) were quite interesting as the achievement of the condition index required for marketing (i.e., 0.15) [59] was observed only in mussels grown at 12 m depth and not in those grown at the surface, as the traditional farming method requires. Mussels grown at a depth of 12 m may have benefited to a greater extent from the organic load of fish cages, which tend to settle or they may have found temporary shelter to surface temperatures. Further studies with stable isotopes and fatty acid content are planned to explain the determining factors for the different growth at the surface and at depth. However, mussels cultured near fish cages showed condition index values among the highest measured in the Taranto area in the last twenty years [4,16].

It is known that bivalves can concentrate fecal-associated pathogenic bacteria from the surrounding water in their bodies, so their consumption poses a risk to human

health [73–76]. Each year, the consumption of contaminated seafood, including shellfish, is implicated in outbreaks of food poisoning caused by pathogenic microorganisms. In this scenario, evaluation of the microbiological quality of shellfish cultured in the IMTA system was critical in determining whether the recovered shellfish were suitable for human consumption. Fecal coliform bacteria, including Escherichia coli, are useful indicators of fecal contamination for assessing the bacterial quality of a mussel farming area and the mussels within it [77–79]. To protect public health, several countries, including Korea, the United States (US), the EU and New Zealand, have established regulatory criteria and monitoring programs using fecal indicators for bivalves and their growing areas [80–84].

According to the present results, mussels grown under traditional surface conditions were safe from a microbiological point of view. All monitored parameters were very low. In particular, the concentration of Escherichia coli was 18 MPN/100 g. This value is lower than the limit for Class A areas according to EU Regulation No. 854/2004 [85], which classifies production areas into A, B, C or restricted areas depending on the *E. coli* content in the soft tissues and shellfish water of the harvested mussels. For a Class A area, an upper limit of 230 *E. coli*/100 g of sample material, is measured as fresh weight and such mussels can be used directly for human consumption. Our results suggest that *M. galloprovincialis* farmed in the innovative IMTA system could also be marketed directly under the EU Regulation No. 2285/2015 [86], which specifies that 20% of samples may contain *E. coli* between 230 and 700/100 g of the sample material, while the remaining 80% of samples must be below 230/100 g of sample material. In addition, in the present study, *Salmonella* spp. were not detected in any of the samples analyzed (Table 4), as required by EU Regulation No. 2073/2005 [80], which stipulates the absence of *Salmonella* spp. in 25 g of meat and intervalval liquid. The microbiological analyses were also confirmed by chemical analyses, which showed that the levels of PAHs and PCBs were below the European maximum levels for food (EU Regulation No. 835/2011) [87], as these compounds in the tested bivalves did not exceed the maximum levels for B[a]P, ∑4 PAHs and ∑6 PCBs, which were set at 5, 30 and 75 µg/kg weight, respectively, for bivalves. In the samples with the composition of PAH and PCBs, all analyzed compounds were detectable. Among the light PAHs (2–3 aromatic rings), phenanthrene was the most abundant compound, while chrysene, fluoranthene and pyrene were the predominant heavy PAHs (4–6 aromatic rings), accounting for 35–42% of the total PAHs. The value of the ratio between light and heavy PAHs was less than one, indicating that the PAHs originated from pyrogenic sources. This distribution pattern is consistent with other studies on *M. galloprovincialis* from the Mediterranean Sea [31]. Among PCB profiles, PCB-153 (hexachlorobiphenyl) was the predominant contaminant (about 50%), followed by PCB-138 (hexachlorobiphenyl), while the least chlorinated congeners such as PCB-28 (trichlorobiphenyl) and PCB-52 (tetrachlorobiphenyl) were found in lower proportions. Highly chlorinated compounds are often the most abundant PCBs detected in biota and marine sediments because their molecular structure makes them lipophilic, stable and persistent [29]. These results were comparable to PCB distribution in other marine organisms [30,88–91]. Compared to the PAH values reported for *M. galloprovincialis* from other Mediterranean regions, the total PAH values obtained in this study were similar to those found in mussels from different areas of the Adriatic Sea [92,93] and the Mar Grande e Mar Piccolo (First Bay) of Taranto [34], but higher than those reported from the northern Apulian coast [34]. Moreover, the levels of PAH in our results were higher than those detected by other authors [31] in the coastal waters of the Adriatic and Ionian Seas during active mussel monitoring.

Regarding PCBs, the results of the present study show that the concentrations of the seven target compounds were lower than those found in mussels from the western and southern Mediterranean coasts [30,94], the Tyrrhenian Sea [29], the Venice Lagoon [95] and the Mar Piccolo of Taranto [33]. These concentrations were higher than those found by colleagues [93] in mussels from the central Adriatic Sea, but always below the established European maximum levels. Finally, a previous report [60] presented data on heavy metals

that showed no accumulation of hazardous compounds in mussel tissues, indicating that the mussels produced in the IMTA system are suitable for human consumption.

In summary, mussel farming remains one of the most sustainable methods of animal origin food production in relation to a number of global/regional anthropogenic indicators such as eutrophication, acidification, climate change, land use, energy demand and biotic depletion [96–98], resulting in net carbon sequestration [38,99–102], opening a potential new market where mussel farmers can issue 'green' certificates for the amount of CO2 captured. Moreover, mussel farming can be combined with other species farming in IMTA systems, which is also considered a highly sustainable aquaculture practice [41]. The present study demonstrated that mussel growth was improved by the proximity of fish cages in the implemented IMTA system. Moreover, the condition index and survival increased at a depth of 12 m, providing shelter from potentially harmful heat waves. Moreover, the mussels grown under the innovative IMTA system showed values of indicators of microbial contamination and PHA and PCB concentrations that were in line with the reference values of European regulations. Considering that the Regional Ordinance n. 188/2016 prohibits commercial mussel farming in the Mar Piccolo of Taranto, the results of the present work offer the mussel farmers of Taranto the opportunity to increase mussel production in the Mar Grande by cultivating mussels under IMTA systems with a perspective of economic, environmental and social sustainability.

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