

Article

Bioremediation Capabilities of *Hymeniacidon perlevis* (Porifera, Demospongiae) in a Land-Based Experimental Fish Farm

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Abstract: The expansion of aquaculture practices in coastal areas can alter the balance of microbial communities in nearby marine ecosystems with negative impacts on both farmed and natural species, as well as on human health through their consumption. Among marine filter-feeder invertebrates, poriferans are known as effective microbial bioremediators, even though they are currently still underutilized in association with fish mariculture plants. In this study, we investigate the microbial bioremediation capability of the demosponge *Hymeniacidon perlevis* in an experimental land-based fish farm where this species occurred consistently in the drainage conduit of the wastewater. Microbiological analyses of cultivable vibrios, total culturable bacteria (37 °C), fecal and total coliforms, and fecal enterococci were carried out on the fish farm wastewater in two sampling periods: autumn and spring. The results showed that *H. perlevis* is able to filter and remove all the considered bacterial groups from the wastewater, including human potential pathogens, in both sampling periods. This finding sustains the hypothesis of *H. perlevis* use as a bioremediator in land-based aquaculture plants as well.

Keywords: bacterial bioremediation; *Hymeniacidon perlevis*; land-based fish farm



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1. Introduction

Porifera, or sponges, are the oldest metazoans that are exclusively aquatic, mainly marine, that are still extant. They are primitive pluricellular animals whose functions are based on a flexible differentiation system characterized by a high plasticity at the cellular, structural, and reproductive levels [1,2]. These features make them able to adopt a wide range of adaptive strategies for population survival and support great success in benthic communities. Sponges are effective filter-feeders that are able to process a huge water volume and retain up to 99% of the particulate and dissolved organic matter [3–5]. These sessile metazoans combine sexual and asexual reproduction [6]. This latter reproductive strategy, carried out by means of fragmentation, budding, and gemmulae, allows for the spread of numerous well-adapted individuals with a higher chance of survival. Their cellular and reproductive plasticity give them a characteristic regenerative ability that allows for the healing of damaged or lost parts, as well as the complete regeneration of an adult from fragments or even single cells.

The ability of sponges to grow from fragments excised from wild donor sponges, and then seed a range of standard aquaculture infrastructure, makes them ideal candidates for aquaculture [7–10].

Recently, in order to develop methods for “artificial seed” production and sustainable supply in sponge aquaculture, an intensive study on *Hymeniacidon perlevis* (Montagu, 1818) larval release and settlement under controlled conditions has been carried out [11–13]. Nevertheless, harvesting from wild donor stock and a subsequent culture of explants is currently the most feasible method to obtain valuable quantities of sponge [14].

Several sponge species show commercial potential use, including as biomaterials. In this framework, there is increased interest in investigating sponge features, not only for their biological relevance per se but also for the contributing information that can be used in the context of applied research [15].

Bacteria are important components of sponge holobionts (Ref. [16] and references therein) and shifts in the composition of the microbiota can compromise the health of the whole animal [17,18]. The maintenance of a characteristic microbial community appears to be a complex trait, suggesting that hosts deprived by their normal microbiota should be at a disadvantage [2].

The excessive release of microbial pathogens, such as bacteria, viruses, and protozoans that come from human and animal waste, has become a major concern with increasing coastal urbanization and aquaculture practices because of their potential impact on coastal marine environments [19,20]. First, pathogenic bacteria represent a significant health hazard to aquatic species and can cause recurrent occurrences of marine diseases. Second, seafood products are severely contaminated by pathogenic bacteria and represent a biohazard to human health through human consumption [21].

In this scenario, scientists all over the world are working towards the development of remediation technologies, including physical, chemical, and biological remediation (named bioremediation). Bioremediation is a potential technique for the biological treatment of industrial waste and contaminated environments [22–24]. Recent research has shown that, apart from the utilization of microorganisms for the biodegradation of target pollutants, aquatic ecosystems are home to several invertebrate species who deserve the definition of zooremediators [25] due to their ability to remove, hyperaccumulate, stabilize, or degrade pollutants. Among them, marine sponges have demonstrated the capability to remediate aquatic microbial pollution [26–36] and accumulate or degrade metals [37–43]. In addition, sponges host massive consortia of microorganisms within the mesohyl matrix that can amount for up to 60% of their total biomass [44], exceeding that of seawater by two to three orders of magnitude [16] and significantly contributing to the host metabolism [45].

Among poriferans, *H. perlevis* has been shown to be an effective bioremediator that is able to remove potential pathogenic bacteria from the cultured media by retaining and digesting them by phagocytosis [26,27,31,32,34,46]. In addition, recent research [40] has shown that *H. perlevis* has impressive bioaccumulation properties for different organic and inorganic pollutants (particularly metallic trace elements and polycyclic aromatic hydrocarbons). Moreover, their associated microbiota have been demonstrated to have capability in lindane pesticide degradation [47,48]. These features make *H. perlevis* suitable for the bioremediation of polluted seawater by microbial, organic, and inorganic pollutants, such as in aquaculture farming plants.

Most of the known studies are detailed laboratory-based studies that were not performed in situ in a true aquaculture system, except those of Longo et al. [32] and Zhang et al. [27].

The occurrence of large amounts of the demosponge *H. perlevis* consistently living in the drainage conduit of the wastewater flowing out from an experimental land-based fish farm offered the opportunity to investigate its ability for microbial reduction in such conditions, and to evaluate the possibility to use this species in integrated multitrophic farming systems for wastewater treatment, which is in contrast to traditional technologies with high negative impacts on the whole ecosystem.

2. Materials and Methods

2.1. Studied Species

The Demospongiae, *Hymeniacidon perlevis*, family Halichondriidae Gray, 1867, is extremely plastic in shape and can form encrusting or massive specimens that are able to survive prolonged periods of air exposure [49,50]. This sponge is typically characterized by orange to red or pale green specimens that can reach up to 30 cm in diameter. Its surface is characterized by emerging short papillae and small digitations.

H. perlevis is one of the most common species of Europe [51], it has a wide distribution, is considered cosmopolitan [52], and currently is the most common and widely-distributed exotic sponge [53].

H. perlevis, predominantly reported from temperate and colder waters, lives in shallow subtidal and intertidal zones where it can colonize both hard substrates and soft bottoms, and where it is able to live buried into the sediment with its oscules projecting outwards [51,54]. This species is one of the most common in the central Mediterranean lagoon systems and has recently been recorded in a Turkish lagoon [50,55], where it inhabits an environment deeply affected by anthropogenic impact. *H. perlevis* is able to survive both air exposure and strong environmental parameter changes [49,55]. Despite local seasonal variations in growth and population size [49,56,57], this species shows a peculiar adaptive strategy, thereby allowing it to face critical environmental conditions.

2.2. In-Situ Bioremediation Study

Bioremediation studies were performed at the COISPA, a land-based experimental fish farm station (Torre a Mare, southern Italy; www.coispa.it (accessed on 23 June 2022)) where *Hymeniacidon perlevis* lives on the bottom of the drainage conduit for wastewater (Figure 1). This conduit receives wastewater from the fish farming tanks and conveys it into the sea. The drainage conduit considered in this study consists of a cement canal that is 4.0 m long, 0.57 m wide, and 0.15 m deep, and is closed by a walkable inspection protection that makes this part of the conduit completely dark (Figure 1). The water used in this land-based fish farm is sea-groundwater and its temperature is constant throughout the year (18 °C). The wastewater from the fish tanks flows out through the drainage conduit and passes through the area with sponges. Each tank has an overflow system and also a tap at the bottom. To eliminate feed and feces residues that accumulate at the bottom of the tank, the tap of each tank is opened daily and approximately one-third of the entire volume of the tank is discharged. The bioremediation experiment was performed during two sampling periods: spring and autumn. The fish biomass in the system was similar in both sampling periods, ranging between 590 and 660 kg in spring and autumn, respectively. The water volume of the fish tanks was 27,990 and 35,190 L in spring and autumn, respectively. The volume of the sponges living inside the drainage conduit during the bioremediation experiment was similar in spring and autumn (mean value \pm SD 7457.50 ± 53.03 cm³). The water flow in the drainage conduit ranged between 15 and 28 L sec⁻¹ before and after the tank discharge, respectively.

2.3. Microbiological Analysis

Bacteriological analyses were performed on wastewater flowing in the drainage conduit where the sponge lives.

Wastewater was collected in triplicates at two sampling periods (spring and autumn) for three collection times—immediately before (T0) and after (T1) the discharge of fish tank wastewater and five minutes later (T2)—at two collection points: upstream (UP) and downstream (DOWN) of the sponge biomass (Figure 1). Bacteriological analyses included the quantitative analyses of cultivable vibrios, total culturable bacteria (37 °C), fecal and total coliforms, and fecal enterococci.

Enumeration of the culturable vibrios in seawater was achieved by filtering volumes of 1, 5, and 10 mL of seawater on 0.22 µm pore size filters and aseptically placing the filter disks onto thiosulphate-citrate-bile salt-sucrose agar (TCBS) plus 2% NaCl, a selective

medium for halophilic vibrio. Incubation was carried out at 25 °C and 35 °C for two days and the colonies of presumptive vibrios were counted according to the colony-forming unit (CFU) method. The fraction of vibrios that were potentially pathogenic to humans was estimated with the incubation temperature of 35 °C. An incubation temperature of 20–25 °C was chosen because some *Vibrio* spp., such as *Vibrio anguillarum*, do not grow well at higher temperatures [58]. Mean values for three replicate samples were determined.

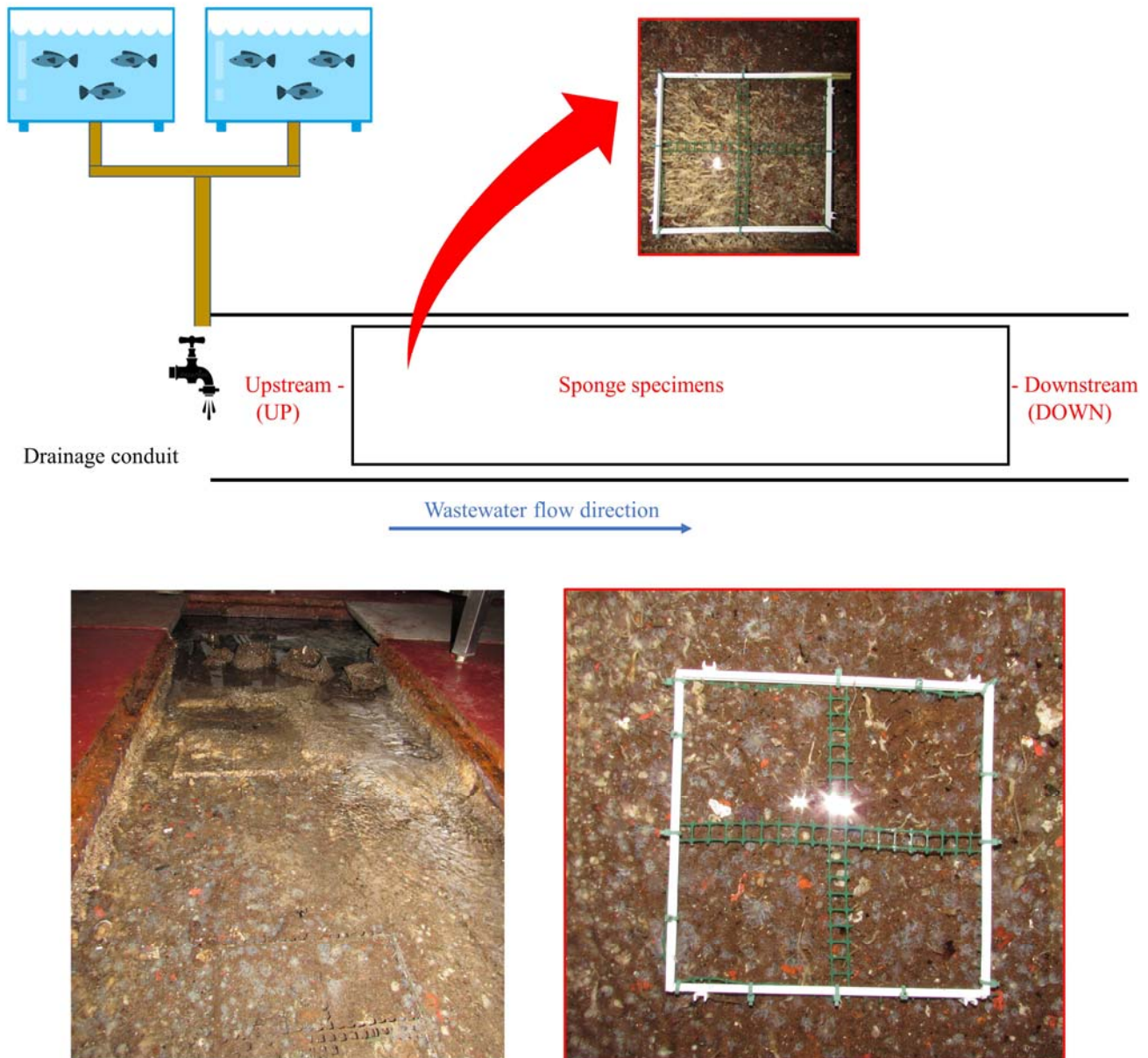


Figure 1. Schematic drawing (not in scale) and real picture of the wastewater drainage conduit where the sponge, *Hymeniacidon perlevis*, lives, as well as an indication of the wastewater collection points: upstream (UP) and downstream (DOWN). In the red box, picts of *H. perlevis* with a polyvinyl chloride (PVC) frames utilized for sponge volume measurement.

The total culturable bacterial densities that grew at 37 °C (indicating the fraction of bacteria potentially pathogenic to humans) were determined in the number of CFU by plating 100 µL of undiluted and serial dilutions of seawater in triplicate on a plate count agar (PCA). The plates were incubated at 37 °C for seven days [31]. All of the colonies were counted using the CFU method through a 10x magnification lens at the end of the

incubation period. For each microbiological parameter counted according to the CFU method, the bacterial densities were expressed as CFU/mL.

Total and fecal coliforms as well as fecal enterococci were determined by the most probable number (MPN) method using the standard five-tube method of 10-fold dilutions for seawater samples [59]. For total and fecal coliform determination, lactose broth and brilliant-green-lactose broth were used as cultural media in the presumptive and confirmative tests, respectively. For fecal enterococci, the presumptive test was performed using azide broth and the confirmative test was performed using ethyl violet azide broth. The results are reported as MPN/100 mL.

2.4. Statistical Analysis

All analyses were performed using the Primer software and at a level of significance of 95%. All experimental data were computed as dependent variables using PERMANOVA in an approach similar to parametric ANOVA. Univariate PERMANOVA tests were run on Bray–Curtis similarity matrices with 9999 permutations [60]. Season (S; 2 levels: autumn and spring), Station (St; 2 levels: UP and DOWN), and Time (T, 3 levels: T0, T1, and T2) factors were used to detect the differences in the removal efficiency of Total Vibrio, Total CFU, Fecal coliforms, Fecal enterococci, and Total coliforms. Each factor and its interactions were individually analyzed using Univariate PERMANOVA tests with the same experimental design. If necessary, square root transformed data in a Bray–Curtis similarity matrix with 9999 permutations were used to perform the analyses [60]. If it was impossible to obtain enough permutations for PERMANOVA analysis, the reference *p* was obtained using a permutation simulation test (MONTECARLO test). The PAIR-WISE test was applied to discover statistically significant differences in each pair of factor levels based on the significance value of PERMANOVA/MONTECARLO tests.

3. Results

Microbiological analysis showed that *Hymeniacidon perlevis* in the drainage conduit is able to reduce all the considered bacterial categories during both the spring and autumn sampling period (Figures 2 and 3). With regards to culturable Vibrios, this category seems to be the least favorite one by *H. perlevis*. In fact, in the upstream wastewater (T0_UP, T1_UP, T2_UP; before the sponge) its value differs from that found in the corresponding downstream wastewater (T0_DOWN, T1_DOWN, T2_DOWN; after the sponge), with only a few hundred of CFU/mL—almost at each sampling time (T0, T1 and T2). The vibrios concentration reduce at least an order of magnitude at T1 and T2, particularly during the autumn sampling, when their concentration is the highest recorded in the whole experiment (e.g., Autumn: T1_UP $8.90 \pm 1.1 \times 10^4$ and T1_DOWN $4.50 \pm 0.25 \times 10^4$; T2_UP $1.75 \pm 0.10 \times 10^4$ and T2_DOWN $4.55 \pm 0.97 \times 10^3$ CFU mL⁻¹; Spring: T2_UP $1.50 \pm 0.11 \times 10^3$ and T2_DOWN $8.50 \pm 0.55 \times 10^2$ CFU mL⁻¹) (Figure 2). PERMANOVA analyses showed significant differences in bacterial concentration before (UP) and after (DOWN) the passage through the sponge community in the interaction between the three factors considered (PERMANOVA, SE × ST × T, Pseudo-F = 12.591, *p* (perm) < 0.001). The PAIR-WISE posterior test revealed significant differences in all three observation times in the autumn period, while no significant domination was recorded in the spring for any T factor level.

Total culturable bacteria at 37 °C values in the downstream wastewater were always lower than that found in the upstream wastewater, with an impressive reduction during the autumn collection with a reduction of three and two orders of magnitude at T1 and T2, respectively (Figure 2). The PERMANOVA analyses, carried out on the same experimental design, also showed significant differences between UP and DOWN in the interaction between the three factors considered for this bacterial category (PERMANOVA, SE × St × T, Pseudo-F = 69.264, *p* (perm) = 0.001). According to the PAIRWISE test, these differences were significant for all levels of the analyzed factors, with the exception of T1 in the spring.

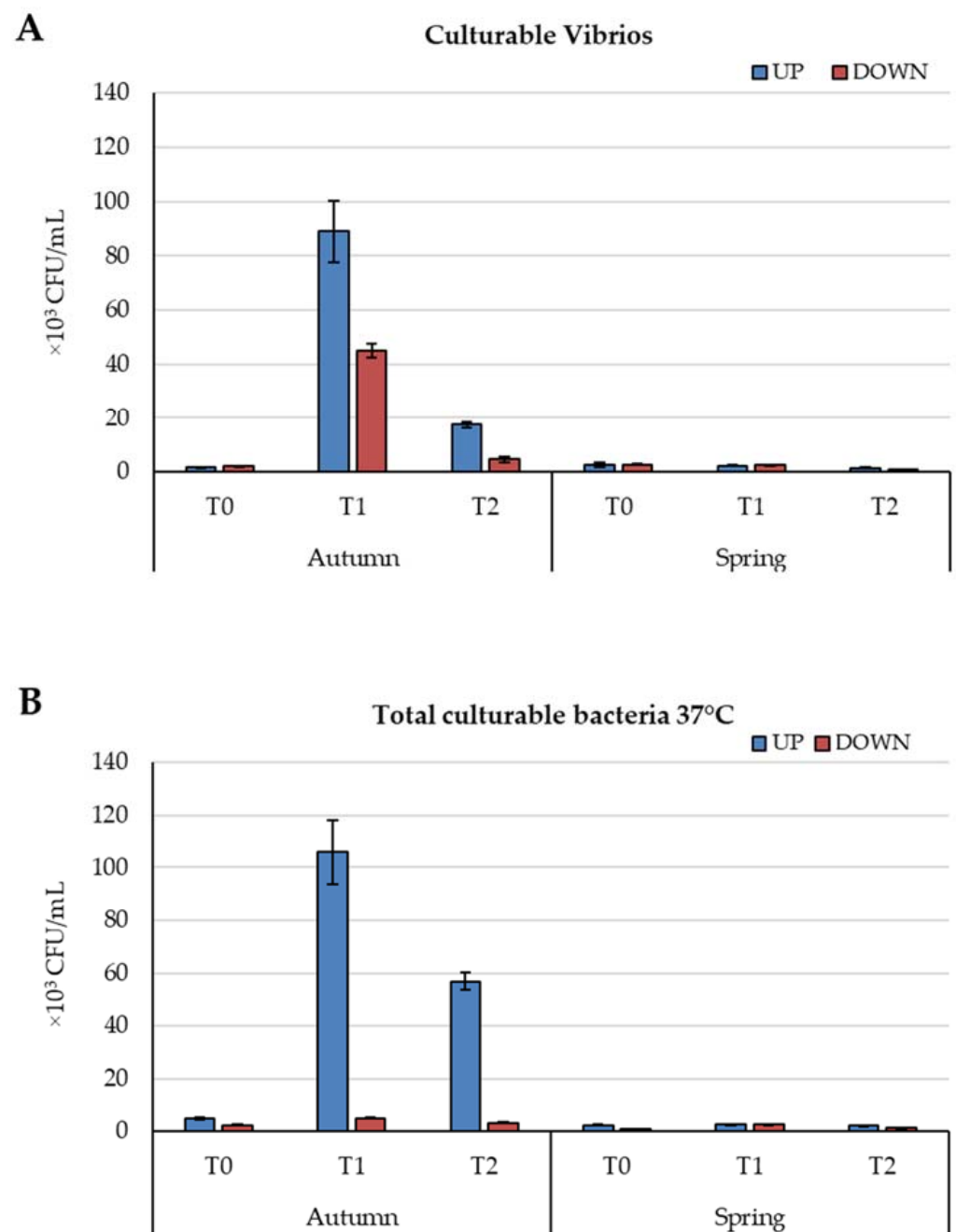


Figure 2. Culturable Vibrios (**A**) and total culturable bacteria at 37 °C (**B**) mean values \pm standard errors in wastewater upstream (UP) and downstream (DOWN) of *Hymeniacidon perlevis* at each sampling time (T0, T1, T2) during both sampling periods (autumn and spring).

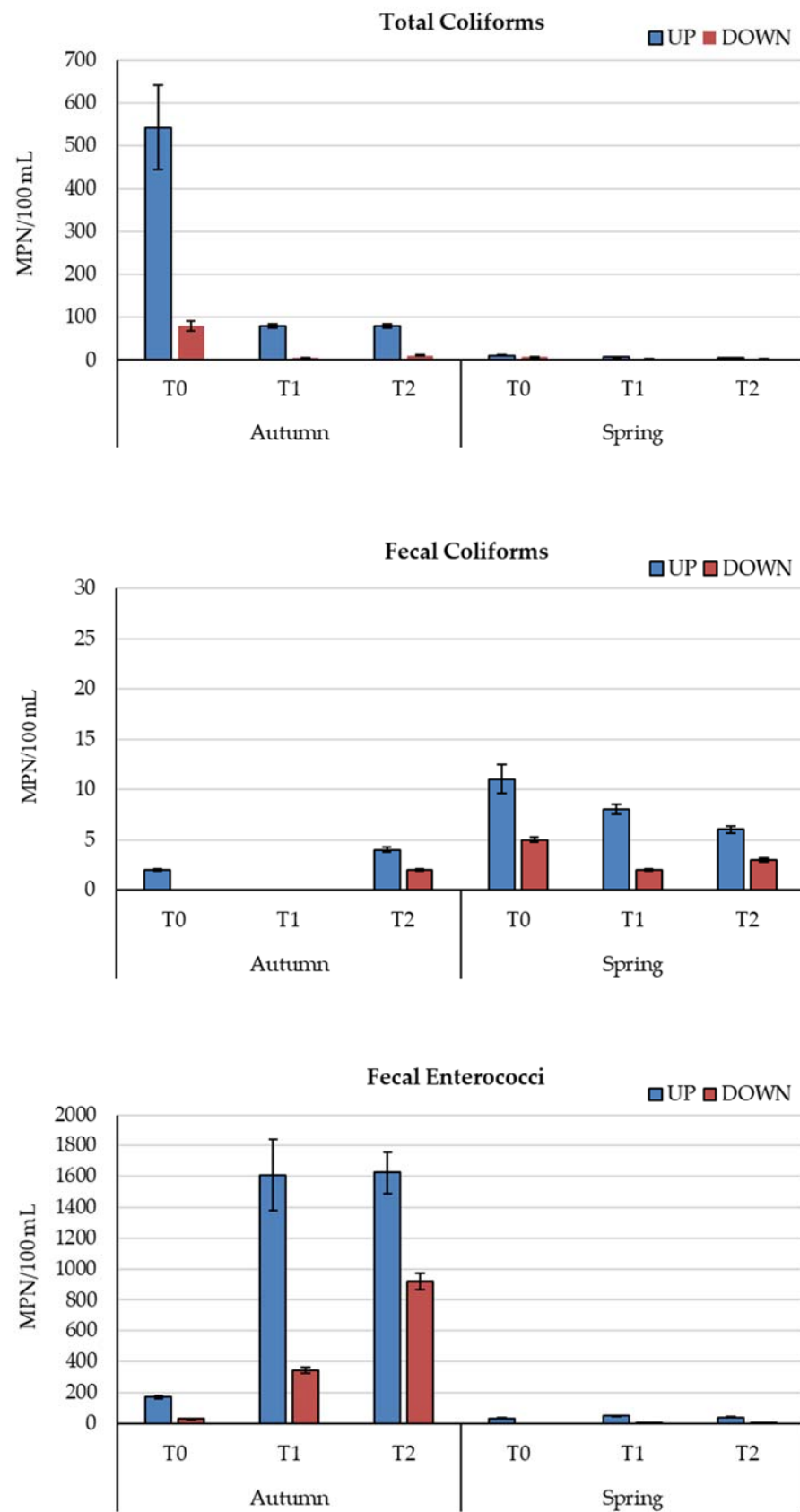


Figure 3. Fecal contamination indicators (mean values \pm standard errors) in wastewater upstream (UP) and downstream (DOWN) of the sponge *Hymeniacidon perlevis* at each sampling time (T0, T1, T2) during both sampling periods (autumn and spring).

A similar trend was found regarding the fecal contamination indicators, where those values were lower in the downstream wastewater compared with that in the upstream wastewater for all three bacterial categories considered (total coliforms, fecal coliforms, fecal enterococci, Figure 3). PERMANOVA highlighted a level of significance in the interaction of the three factors (PERMANOVA, $SE \times ST \times T$, Pseudo-F = 13.258, p (perm) < 0.001). The a posteriori test on the interaction of the three factors showed a significant reduction in the concentration of coliforms at all times in the two seasons, with the exception of T2 in the spring. In particular, in the autumn sampling, the total coliforms at T0 in the upstream wastewater were $5.4 \pm 0.98 \times 10^2$ MPN 100 mL⁻¹, while in the downstream wastewater they reduced to $7.9 \pm 1.1 \times 10$ MPN 100 mL⁻¹. Regarding fecal coliforms, statistical analyses have highlighted a significance in the interaction between seasons and stations (PERMANOVA, Pseudo-F = 5.2483, p (perm) = 0.0204) with significant differences in the spring during the times T0 and T1. (Figure 3). Also, fecal enterococci in the upstream wastewaters were always higher than the downstream ones with values that were lower by, at least, an order of magnitude at each time analyzed (T0, T1, T2) (e.g., autumn: T1_UP $1.61 \pm 0.23 \times 10^3$ MPN 100 mL⁻¹, and T1_DOWN $3.45 \pm 0.19 \times 10^2$ MPN 100 mL⁻¹; spring: T1_UP $4.9 \pm 0.28 \times 10$ MPN 100 mL⁻¹, and T1_DOWN 6.0 ± 0.17 MPN 100 mL⁻¹ (Figure 3). These differences are clear in the PERMANOVA analysis, which highlights a significance level in the interaction of the three factors (PERMANOVA, pseudo-F = 7.2591, p (perm) < 0.001) and a significant decrease in the enterococci concentration at any time for each season.

4. Discussion and Conclusions

Aquaculture plays a critical role in global food production, but with its explosive expansion, considerable negative environmental impacts become obvious [61–63]. FAO cautioned that such an increase will mainly be restricted by two factors, environmental impacts and aquaculture diseases. One of the most prominent issues is that the water-borne bacterial pathogens represent a significant biohazard for both aquaculture species and human health [64]. To prevent pathogen-related aquaculture diseases and the potential contamination of aquaculture products by pathogens, bioremediation is an attractive option in aquaculture ecosystems. In particular, filter-feeder invertebrates, such as sponges, polychaetes, and ascidians, due to their ability to remove pathogenic bacteria through the filtration process, have recently been proposed as potential bioremediators [31,65–74].

The present study represents a significant advance on this topic since the demosponge *Hymeniacidon perlevis* was employed in a land-based experimental fish farm as a novel bioremediation technology to reduce the bacterial concentration and restore water quality.

Field and laboratory studies conducted in the Mediterranean area showed the ability of some sponge species to remove, accumulate, and concentrate bacteria from the surrounding environment with higher efficiency for autochthonous bacteria, both under oligotrophic and eutrophic conditions [29–33,35,36,75–77]. *H. perlevis* is able to remove bacteria even in eutrophic environments thanks to its ability to tolerate high levels of microbial pollution [32]. For this reason, this species was indicated as a potential bioremediator of microbial pollution in impacted semi-enclosed basins [32].

The filtering activity of *H. perlevis* was also studied in laboratory experiments in which its capability to remove and accumulate bacteria from sewage flowing into the Northern Ionian Sea was investigated [31].

Present experiments showed that *H. perlevis* is able to filter and remove all the considered bacterial groups from the wastewater and this was reflected in the observed reduced bacterial abundances in the out-flowing wastewater in comparison to those recorded in the waste in-flowing system. Thus, *H. perlevis* can effectively control the growth of potential pathogenic bacteria, including human pathogens culturable at 37 °C, and vibrios in the wastewater, with important epidemiological implications since its accumulation by these filter-feeders may reduce their abundance in seawater and their risk as a biohazard to human health.

The diffusion of filter-feeders, such as *H. perlevis*, in an aquaculture scenario could allow fish a better rearing environment, with lower risks related to the presence of pathogenic bacteria such as vibrios, which are one of the main causes of infections and mass deaths of farmed fish. Furthermore, this would also reduce human health risks and the financial losses resulting from the loss of farmed fish products [78–80].

H. perlevis was particularly efficient in removing all the examined microbial pollution indicators from the surrounding environment. The proven bioremediation capability of the sponges when reared in an integrated system could be strategic since *H. perlevis* lives in environments subjected to strong anthropogenic pollution. Thus, our results demonstrated that in the presence of the sponge, the quality of seawater is improved, thereby reaching low values of coliform bacteria and leading to new realms of bioremediation for coastal communities.

The *H. perlevis* biomass living in the drainage conduit of the experimental fish farm found in the present study seem to be able to reduce the bacterial concentration at both wastewater flow values with an average percentage abatement for all bacterial categories that ranges between 52% and 89% in the autumn and 35% and 93% in the spring. It has been demonstrated that food availability affects the filtering physiology of sponges: With the increase of food concentration, sponges respond with a decrease in pumping rate as a protection against overloading the sponge feeding system, but the number of filtered particles remains constant over time [81]. The results obtained in the present research are in agreement with the abovementioned findings; in fact, in autumn, all the bacterial categories considered, except for fecal coliforms, showed a higher concentration and a lower abatement percentage with respect to those measured in spring.

The employment of *H. perlevis* in aquaculture waste treatment represents a more attractive option to reduce bacterial loads in comparison to other technologies with high negative impacts on the whole ecosystem. In particular, antibiotics-supplemented feeds are commonly used in farms [82,83]. The massive use of antibiotics to control infections in aquaculture has resulted in the development of resistant strains, which have rendered antibiotic treatments ineffective.

In addition, the observation of biomass recovery of *H. perlevis* in the drainage conduits of the fish farm indicated a surprising resilience of this species, opening a new scenario on sponge rearing applications in land-based aquaculture facilities. We observed that *H. perlevis* is ubiquitous in the drainage conduits of the experimental fish farm. Moreover, although the drainage conduits were periodically cleaned by removing the sponge biomass, it reaches impressive volume even after a few months (authors' personal observation).

The use of marine sponges as bioremediators in appropriate environments is attractive due to their effectiveness in reducing microbial pollution and the potential valorisation of their biomass. Indeed, *H. perlevis* is known as a cytotoxic sponge and its associated marine bacteria show antimicrobial and anticancer properties [84–86]. In addition, it has recently been demonstrated that culturable bacteria associated with this sponge are promising sources of antibacterial compounds of great pharmaceutical interest against pathogenic multi-drug resistant strains (e.g., *Staphylococcus aureus*), which new antibiotics are in urgent need of [87].

The *H. perlevis* rearing in land-based aquaculture facilities with bioremediation purposes is also supported by the potential marketable value of the obtainable sponge biomass, suggesting a novel application of zooremediation methodologies with potential commercial gain. Thus, we propose an aquaculture farm model that is ecologically and economically self-sustaining where, in one embodiment, it involves fish cultivation and sponge rearing.

Future studies should be aimed at investigating the effects of bioaccumulation of microbial pollutants in *H. perlevis* and determine the tolerance limits for the selected species, as well as the detection of biohazards in sponge tissues. The investigation of the capacity for this sponge to utilise coliform bacteria may also lead to new realms of bioremediation for coastal communities.

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