Journal of *Marine Science and Engineering*

MDPI

Article

Seasonal Dynamics of Marine Bacterial Communities in Aquaculture Farms: The case of the Northern Ionian Coastal Ecosystem (Mediterranean Sea)

Maria Kyritsi [1], Antiopi Tsoureki [1], Konstantinos Koukaras [1], Nikolaos Kamidis [2], Grigorios Krey [2], Sofia Michailidou [1] and Anagnostis Argiriou [1,3,*]

[1] Institute of Applied Biosciences, Centre for Research and Technology Hellas, 57001 Thessaloniki, Greece; kyritsimaria@certh.gr (M.K.); adatsoureki@certh.gr (A.T.); kkoukaras@certh.gr (K.K.); sofia_micha28@certh.gr (S.M.)
[2] Fisheries Research Institute, Hellenic Agricultural Organization—DEMETER, 64007 Kavala, Greece; nikkami@inale.gr (N.K.); krey@inale.gr (G.K.)
[3] Department of Food Science and Nutrition, University of the Aegean, 81400 Myrina, Greece
* Correspondence: argyriou@aegean.gr

Abstract: Coastal aquaculture systems are complex environments with multiple microbial interactions that affect fish health and productivity. High-throughput amplicon sequencing is a valuable tool for identifying such bacterial communities and investigating the relationship between bacterial diversity and sustainability in these systems. In the present study, the seasonal dynamics of marine bacterial communities were assessed, in terms of diversity and composition, in three marine aquaculture farms in the northern Ionian Sea (western Greece) and a distant control site unaffected by aquaculture activities, using 16S rRNA amplicon metabarcoding. Results revealed that Proteobacteria, Bacteroidota, Cyanobacteria, and Verrucomicrobiota were the dominant phyla in the bacterial communities. Alpha diversity was significantly lower in the aquaculture farms compared to the control site. Season was the major factor driving bacterial community fluctuations. Comparative analysis between seasons revealed the presence of differentially abundant amplicon sequence variants (ASVs) in all pairwise comparisons, with the majority of them belonging to the phyla Bacteroidota (families *Flavobacteriaceae*, *Cryomorphaceae*) and Proteobacteria (family *Rhodobacteraceae*). Our study provides the first detailed description of bacterial communities present in Greek coastal aquaculture farms using amplicon metabarcoding analysis and expands our understanding of the impact of seasonality and environmental variables on marine bacterial community diversity and composition.

Keywords: 16S rRNA; amplicon metabarcoding; marine aquaculture; seasonal variation; bacterial diversity; Ionian Sea; gilthead seabream; European seabass

Citation: Kyritsi, M.; Tsoureki, A.; Koukaras, K.; Kamidis, N.; Krey, G.; Michailidou, S.; Argiriou, A. Seasonal Dynamics of Marine Bacterial Communities in Aquaculture Farms: The case of the Northern Ionian Coastal Ecosystem (Mediterranean Sea). *J. Mar. Sci. Eng.* **2023**, *11*, 1332. https://doi.org/10.3390/jmse11071332

Academic Editor: Casotti Raffaella

Received: 7 June 2023
Revised: 25 June 2023
Accepted: 27 June 2023
Published: 29 June 2023

1. Introduction

Marine bacteria play essential roles in global biogeochemical systems, including nutrient cycling, photosynthesis, and the biological pump [1]. Marine environmental factors cause alterations in microbial communities that can herald changes in energy transfer pathways through the food web, biogeochemical cycles, and ecosystem biodiversity in general [2–4]. Thus, it is important to explore how changes in the marine environment may affect bacterial composition and functionality, taking also into consideration the fact that the majority of marine bacteria belong to uncultured taxa, posing major restrictions to traditional microbiological methodologies [5,6].

Aquaculture is one of the fastest-growing sectors worldwide and is expected to play a notable role in the global food system. Over the past decades, marine aquaculture farming systems have progressed in response to growing global demand [7,8] and the increasing pressure of overfishing on natural stocks [9,10], but at the same time, they are exposed to

changing environmental factors. The recent exponential evolution of aquaculture farming is critical for achieving the targets set in the 2030 Agenda for the Sustainable Development Goals, such as stock management sustainability and biodiversity conservation [11].

Coastal aquaculture systems provide crucial habitats for microorganisms, which are essential for biogeochemical processes and nutrient cycling [12]. Yet, aquafarms also represent hot spots for fish disease outbreaks, mostly of bacterial, but also of viral and parasitic natures, which can result in a significant decrease in production and economic losses. Since the dynamics of microbial communities' structure in seawater have a significant impact on fish health and productivity [13], it has been proposed that monitoring the prokaryotes' composition in aquatic habitats can aid in identifying microorganisms associated with fish health [14]. At the same time, marine microbiomes are strongly affected by environmental fluctuations in seawater, such as temperature and nutrient levels, which alter their biogeochemistry and potentially create pools of pathogens and toxic metabolites affecting, in turn, fish and human health [15–17]. Hence, understanding the structure and temporal alterations of marine aquaculture microbial communities will allow for improved aquaculture productivity and enhancement of fish welfare.

To this end, mass amplicon-based sequencing has revolutionized the field of microbial ecology and provided valuable information on the phylogenetic diversity in aquatic ecosystems [18–20]. The assessment of bacterial diversity and the respective interactions in aquaculture systems can be achieved by next-generation sequencing (NGS) technologies, particularly with the use of 16S rRNA gene amplicon sequencing [21,22]. This technology has been effectively used to study the temporal dynamics of marine microbial communities in aquaculture farms [23], identify the presence of potential pathogens [24,25], and assess the impact of environmental stressors, such as climate change, mainly in ecosystems like the Mediterranean Sea where large variations in future temperature levels are expected [26].

According to the European Commission, the Mediterranean Sea will be one of Europe's primary sites for marine aquaculture development in the following years. Concerning finfish aquaculture, during the last few decades, there has been a steady growth and diversification of their production in the Mediterranean Sea [27]. Greece is one of the main finfish-producer countries in the Mediterranean basin, with a substantial and well-organized marine aquaculture industry, primarily in coastal waters [28]. Annual fish production in Greece ranges from 131,250 to 151,372 tons, with an estimated value of aquaculture production output of 0.59 to 0.64 billion euros during the period 2019–2021 [29]. In Greece, 98% of the farmed fish volume comes from marine aquaculture systems, with the European seabass (*Dicentrarchus labrax*) and gilthead seabream (*Sparus aurata*) dominating the sector's production [29]. In terms of economic value, these two species rank second and third in the EU, respectively [30]. In particular, the Thesprotia regional unit, located in northwestern Greece, is an area where many fish farming units are operating. Sagiada, which is the area under investigation in this study, is a narrow strip of land along the Greek–Albanian border region (~10 km in length). This region is of high economic importance, since in its coastal area, a total of 22 aquaculture units are active, producing about 12% of the national aquaculture production (approximately 14,000 tons) [31]. Hence, the aim of this study was the assessment of bacterial communities in coastal aquaculture farms located in Greece, using amplicon metabarcoding analysis. Composition of prokaryotes in seawater was monitored throughout a one-year period, to surveil fluctuations in bacterial populations. Results reported here are, to our knowledge, the first investigation of seasonal bacterial microbiome profiling using genomic tools in Greek coastal finfish farms, aiming to broaden the current knowledge on the impact of seasonality and aquaculture practices on bacterial populations.

2. Materials and Methods

2.1. Experimental Design and Sample Collection

Seawater samples were collected from three marine aquaculture farms (namely Lorida, Skaloma, and Bastia), located in the northwestern area of the Sagiada strip (Thesprotia,

Ionian Sea, Western Greece). All farms grew both gilthead seabream (*S. aurata*) and European seabass (*D. labrax*) during the sampling period. Sampling was conducted once a month from June 2020 to May 2021. Within each farm, seawater samples were collected from two different locations among the fish cages (approximately 60 m apart) to capture the spatial variation of the bacterial communities. A control sampling site was included in the study, to compare the aquaculture bacterial communities with those derived from an unexploited region (Supplementary Materials, Figure S1). The control site was located at a distance of more than 6 km from the aquaculture farms and close to the northern end of the Sagiada strip. In each sampling site, seawater samples were collected at different depths of the water column (middle and bottom depths) using a standard Niskin water sampler (sample volume 5 L; Hydro-Bios Apparatebau GmbH, Kiel, Germany). Water samples in the middle depth were collected close to the cage bottom at the aquaculture farming sites. Depending on the bathymetry of each aquaculture farm, the middle and bottom depths were 10 m and 30 m from the surface, respectively, for both Lorida and Skaloma, 10 m and 20 m for Bastia, and 20 m and 40 m for the control site. The samples from the different depths were mixed together, and 1 L of the composite water samples was filtered through 0.22 μm pore Sterivex-GV Pressure Filter Units (Merck KGaA, Darmstadt, Germany). Filters were immediately frozen in liquid nitrogen, kept on dry ice during transportation to the laboratory, and stored at −80 °C until further processing. In total, 100 samples were collected from the aquaculture farms and the control site and were further processed. Samples were named using the pattern of "one letter (L, S, B, or C) + the serial number of the sample (1 to 15) + one letter (B or E)", with the first letter representing the sampling site (L: Lorida aquaculture farm, S: Skaloma aquaculture farm, B: Bastia aquaculture farm, or C: Control site) and the last letter representing the two different locations within each farm (B: Beginning, E: End). Numbers 1 to 5 correspond to the samples collected in summer and numbers 6 to 8 to those collected in autumn, while numbers 9 to 11 and 12 to 15 denote samples collected in winter and spring, respectively (Table S1).

Temperature and salinity data were acquired from the entire water column at the center of each aquaculture farming system, using a Seabird 19plus CTD (Sea-Bird Scientific, Bellevue, WA, USA). Nutrient (nitrate—NO_3^-, nitrite—NO_2^-, and ammonium—NH_4^+) and chlorophyll-a levels were measured at the surface and bottom depths. For nutrient analyses, 0.5 L of water samples were vacuum-filtered through 0.45 μm preweighted nitrocellulose filters and frozen at −20 °C. The methods provided by Parsons et al. [32] were used to determine nutrient levels. An amount of 1 L of each water sample was filtered through 47 mm diameter GF/F glass fiber filters for chlorophyll-a analysis. Filters were diluted in 10 mL of 90% acetone, stored overnight at 4 °C in the dark, and then examined according to [33]. A HITACHI U-2001 spectrophotometer was used for all analyses.

2.2. Microbial DNA Extraction

Microbial DNA was extracted from Sterivex™ filters, as described in Cruaud et al. (2017) [34]. In brief, the whole filter was removed from its casing and dissected into smaller pieces using aseptic techniques. To achieve high DNA yields, all the dissected pieces were placed into lysis solution and DNA was immediately extracted following the protocol of the ZymoBIOMICS™ DNA Miniprep Kit (Zymo Research, Irvine, CA, USA). Sample concentration was measured on a Qubit™ 4 Fluorometer, using the Qubit™ dsDNA BR Assay Kit (Invitrogen, Carlsbad, CA, USA), and its integrity was evaluated using 1% agarose gel electrophoresis. DNA samples were stored at −20 °C until library construction.

2.3. Library Construction and Sequencing

Total bacterial composition in seawater samples was assessed through 16S ribosomal RNA amplicon sequencing, following the instructions of Illumina's "16S Metagenomic Sequencing Library Preparation" (15044223 B) protocol, with some modifications. Briefly, the V3–V4 hypervariable regions of the 16S rRNA gene were amplified (≈460 bp) using primers D-Bact-0341-b- S-17 and D-Bact-0008-a-S-16 [35] with Illumina overhang adapter

nucleotide sequences added at their 5′ end. The forward primer sequence was 5′-TCG TCG GCA GCG TCA GAT GTG TAT AAG AGA CAG CCT ACG GGN GGC WGC AG-3′, and the reverse primer sequence was 5′-GTC TCG TGG GCT CGG AGA TGT GTA TAA GAG ACA GGA CTA CHV GGG TAT CTA ATC C-3′. Amplicon PCR reactions were performed on an Applied Biosystems StepOnePlus™ Real-Time PCR System (Thermo Fisher Scientific, Waltham, MA, USA), using KAPA HiFi Hot Start Ready Mix (Kapa Biosystems, Woburn, MA, USA), 0.8 µM forward and reverse primer mix, 0.625 µM SYTO9 (Thermo Fisher Scientific, Waltham, MA, USA), and 12.5 ng/µL template DNA, in a final volume of 20 µL. PCR conditions included a 3 min hold at 95 °C, followed by 30 cycles of 30 s at 95 °C, 30 s at 55 °C, and 30 s at 72 °C, and a 5 min hold at 72 °C. Dual index barcodes were added to the amplified products for multiplexing, through an 8-cycle PCR reaction, with the same PCR conditions as above. PCR products were purified, using AMPure XP magnetic beads (Beckman Coulter, Brea, CA, USA), to remove unincorporated primers, primer dimers, and salts. Library concentration was measured using fluorometric quantification with the Qubit™ dsDNA BR assay kit, and the quality and average size were determined using capillary electrophoresis on a Fragment Analyzer 5200 system with the DNF-474-0500 High Sensitivity NGS Fragment Analysis Kit (Agilent Technologies, Santa Clara, CA, USA). The final molarity was calculated based on a quantitative PCR using the KAPA Library Quantification kit for Illumina sequencing platforms (Kapa Biosystems, Woburn, MA, USA). Paired-end sequencing was performed on a MiSeq platform using the MiSeq® reagent kit v3 (2 × 300 cycles) (Illumina, San Diego, CA, USA).

2.4. Bioinformatics Analysis

Raw, demultiplexed paired-end reads were processed using the QIIME2 microbiome bioinformatics platform (v2021.11) [36]. The 'denoised-paired' method of the DADA2 (Divisive Amplicon Denoising Algorithm 2) plugin was applied for sequence quality control, including denoising and dereplicating the sequences and removing chimeras [37]. The inferred amplicon sequence variants (ASVs) were aligned to the SILVA database version 138, at 99% identity, using the 'classify-consensus-blast' function of the q2-feature-classifier plugin [38] for taxonomic classification. Nonbacterial ASVs, including those assigned to mitochondria and chloroplasts, or unassigned sequences were removed from the dataset. A FastTree-based rooted tree was constructed using a MAFFT sequence alignment [39], through the 'align-to-tree-mafft-fasttree' function of the q2-phylogeny plugin [40].

2.5. Diversity and Composition Analysis

The final ASV abundance table along with the taxonomic classification (.biom file) and the phylogenetic tree (.nwk file) for the samples were exported and further processed in R (v4.1.1) [41]. The phyloseq package (v1.40.0) [42] was used for barplot visualizations and alpha diversity analysis. Bacterial community composition was depicted as the relative abundance percentage of bacterial species in each sample. Species richness and diversity across different groups were evaluated by calculating the observed ASVs, Shannon, Simpson, and Inverse Simpson diversity indices with the 'estimate_richness' function, after rarefying to the smallest library size. Statistical significance was tested with the Wilcoxon rank-sum test, and *p*-values were adjusted with the Bonferroni method, using the package ggpubr (v0.6.0) [43]. Rarefaction curves were created with the metagMisc (v0.5.0) [44] and iNEXT (v3.0.0) [45,46] packages. To assess changes in bacterial relative abundance in the aquaculture farms across seasons, nonmetric multidimensional scaling (NMDS) based on the Hellinger-transformed Bray–Curtis dissimilarity matrix was performed, using the ampvis2 package (v2.7.31) [47]. Beta diversity analysis was also conducted based on the phylogenetic distance between ASVs, through Principal Coordinates Analysis (PCoA) on the weighted UniFrac metric [48] with the ampvis2 package. Permutational multivariate analysis of variance (PERMANOVA) with 1000 permutations was conducted for comparison of multiple groups, using the function 'adonis2' of the vegan package (v2.6.4) [49],

and for pairwise comparisons, using the 'pairwise.adonis2' function of the pairwiseAdonis package (v0.4.1) [50].

2.6. Differential Abundance Analysis

Differential abundance analysis was performed on the data from the three aquaculture farms combined, to identify differentially abundant ASVs (DA ASVs) between seasons, using the R package DESeq2 (v1.36.1) [51]. The 'poscounts' method was used for size factors' calculation to account for ASVs with zero counts. The 'apeglm' shrinkage estimator was used to adjust for low counts and highly dispersed features [52]. *p*-values were calculated with the Wald significance test and corrected for multiple testing, using the Benjamini–Hochberg method. In total, six pairwise comparisons between summer, autumn, winter, and spring were conducted, and ASVs with an absolute log2 fold-change ≥ 2 and an adjusted *p*-value < 0.01 were deemed differentially abundant.

3. Results

3.1. Environmental Variables Characteristics

The average seasonal measurements of environmental parameters in the water column including temperature, salinity, nitrogen compounds (nitrate—NO_3^-, nitrite—NO_2^-, and ammonium—NH_4^+), and chlorophyll-a (Chl-a) are presented for the three aquaculture farms on average (±SD) and the control site in Tables 1 and 2, respectively. The water temperature ranged from 16.59 °C (±0.09 °C) in spring to 22.18 °C (±0.10 °C) in autumn in the aquaculture farms. Salinity levels ranged from 38.04 psu (±0.08 psu) to 38.82 psu (±0.01 psu), with winter presenting the lowest levels and summer and autumn the highest. Similar variations in temperature and salinity were observed in the control site (Table 2). Nitrate levels were higher during summer (5.41 µM ± 2.91 µM), gradually decreasing as the seasons progressed, and reaching the lowest in spring (1.14 µM ± 0.78 µM) in the aquaculture farms. Ammonium appeared higher during autumn and winter, 2.89 µM (±1.11 µM) and 3.05 µM (±0.49 µM), respectively, and lower in spring (1.24 µM ± 0.55 µM), whereas nitrite levels were slightly higher in autumn and winter compared to the other seasons, ranging from 0.10 µM (±0.01 µM) in spring to 0.15 µM (±0.03 µM) in autumn. The chlorophyll-a levels were highest in autumn, 1.53 µg/L (±0.46 µg/L), and lowest in winter, 0.38 µg/L (±0.07 µg/L). On the other hand, nitrogen compounds were higher in summer and winter in the control site, and chlorophyll-a was higher in winter and spring compared to the other seasons, but these concentrations were lower than those in the aquaculture farms. ANOVA analysis revealed statistically significant differences across seasons for all environmental parameters, except for nitrite and ammonium in aquacultures and all nitrogen compounds and chlorophyll-a in the control site.

Table 1. Seasonal average variations in physicochemical parameters (temperature and salinity), nitrogen compounds (nitrate—NO_3^-, nitrite—NO_2^-, and ammonium—NH_4^+), and chlorophyll-a (Chl-a) in the seawater of aquaculture farming systems. Values represent the average (±SD) of all seasonal measurements from the three aquaculture systems.

Season	Temperature (°C)	Salinity (psu)	NO_3^- (µM)	NO_2^- (µM)	NH_4^+ (µM)	Chl-a (µg/L)
Summer	20.77 ± 0.32	38.80 ± 0.01	5.41 ± 2.91	0.12 ± 0.03	2.66 ± 0.56	0.66 ± 0.03
Autumn	22.18 ± 0.10	38.82 ± 0.01	2.22 ± 0.89	0.15 ± 0.03	2.89 ± 1.11	1.53 ± 0.46
Winter	16.62 ± 0.07	38.04 ± 0.08	1.28 ± 0.52	0.14 ± 0.02	3.05 ± 0.49	0.38 ± 0.07
Spring	16.59 ± 0.09	38.49 ± 0.04	1.14 ± 0.78	0.10 ± 0.01	1.24 ± 0.55	0.88 ± 0.11
ANOVA	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p > 0.05$	$p > 0.05$	$p < 0.05$

Table 2. Seasonal average variations in physicochemical parameters (temperature and salinity), nitrogen compounds (nitrate—NO_3^-, nitrite—NO_2^-, and ammonium—NH_4^+), and chlorophyll-a (Chl-a) in the seawater of the control site.

Season	Temperature (°C)	Salinity (psu)	NO_3^- (μM)	NO_2^- (μM)	NH_4^+ (μM)	Chl-a (μg/L)
Summer	19.87	38.86	3.45	0.13	1.08	0.09
Autumn	21.71	38.91	0.87	0.06	1.22	0.15
Winter	16.77	38.52	2.12	0.09	2.63	0.25
Spring	16.44	38.65	1.25	0.06	0.42	0.39
ANOVA	$p < 0.05$	$p < 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$

3.2. Sequencing Overview and Alpha Diversity Analysis of Bacterial Communities

The seasonal bacterial composition in three aquaculture farms and one control site was examined. Sequencing of the 16S rRNA V3–V4 hypervariable regions in 100 samples yielded 5,923,000 high-quality sequences. After the DADA2 quality filtering, denoising, merging, chimera removal, and taxonomy-based filtering of nontarget sequences, 3,511,516 sequences remained, corresponding to 2739 ASVs. An average of 35,115 sequences per sample were obtained after filtering, ranging from 14,512 to 133,544 (Table S1). Rarefaction curves reached a plateau for all samples, displaying that the sequencing depth was sufficient to capture most of the bacterial communities' diversity (Figure S2).

Analysis of alpha diversity indices (observed ASVs, Shannon, Simpson, and Inverse Simpson) revealed statistically significant differences between the control site and aquaculture farms (Figure 1A). The Simpson and Inverse Simpson indices also revealed significant differences between the Skaloma and Bastia farms. On average, the lowest within-group species diversity was observed in the Skaloma fish farm (Shannon = 4.35 ± 0.36), while the highest was in the control site (Shannon = 5.03 ± 0.22). Seasonal variation was observed in the total bacterial diversity of the three farms, with higher diversity, on average, in winter (Shannon = 4.77 ± 0.50), and gradual reduction during the following seasons (spring Shannon = 4.63 ± 0.34, summer Shannon = 4.42 ± 0.26, autumn Shannon = 4.15 ± 0.16) (Figure 1B). Alpha diversity indices values per sample are presented in Table S2.

3.3. Beta Diversity Analysis and Bacterial Community Composition

To evaluate the clustering of samples based on bacterial communities, NMDS (Bray-Curtis) and PCoA (weighted UniFrac) plots were created. Both analyses revealed a seasonal successive transition of samples in a circular pattern (Figure 2). In both plots, samples collected during the same season were placed closer to each other, and in particular, those collected during the same month formed small, although loose, clusters, indicating a more similar bacterial composition compared to samples collected at different time points. More specifically, in NMDS, season was the variable with the strongest effect on the bacterial community composition (PERMANOVA: $F = 24.24$, $R^2 = 0.38$, $p < 0.001$), while at the same time, samples collected from the control site were differentiated from those collected from the three fish farms (PERMANOVA: $F = 8.36$, $R^2 = 0.13$, $p < 0.001$) (Figure 2A). Statistically significant differences were observed in all pairwise comparisons between seasons (Table S3). A similar pattern was observed in the PCoA plot of the weighted UniFrac metric. In particular, the season strongly affected the microbiome (PERMANOVA: $F = 25.12$, $R^2 = 0.38$, $p < 0.001$), while the samples collected in the different months formed more discrete clusters. The exception to this was the cluster formed by the samples collected during May (samples with the number "15" in their name), June (numbers "1" and "2"), and July (number "3"), as well as the cluster formed by samples collected during late August (number "5") and September (number "6"). PCo1 (23.3%) and PCo2 (12.5%) accounted for 35.8% of the total genetic variation in the samples (Figure 2B).

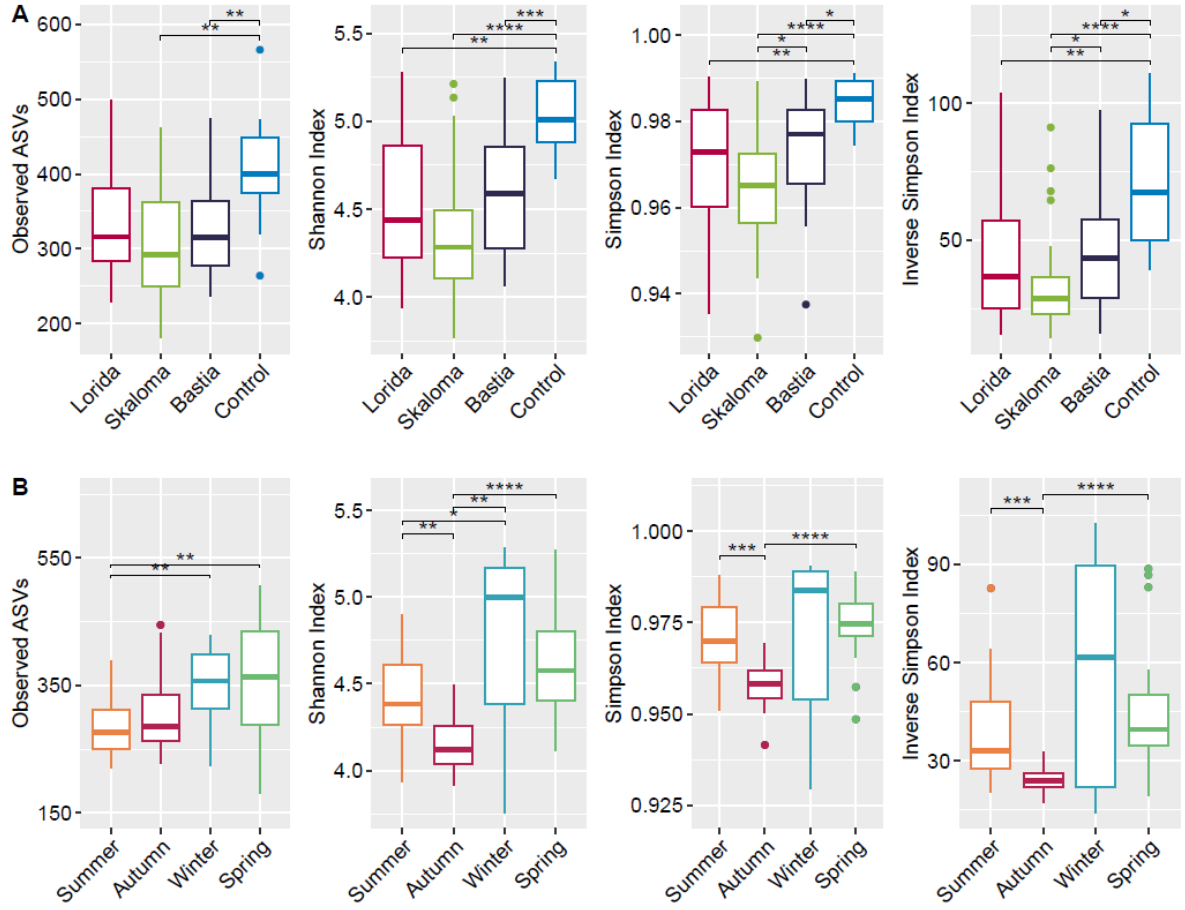

Figure 1. Alpha diversity indices for (**A**) bacterial communities in the three different aquaculture farms and the control site and (**B**) bacterial communities present in the three aquaculture farms (excluding the control site) by season. Minimum and maximum values are represented by the whiskers in the boxplots, the median by the midline, and outliers by dots. Asterisks indicate statistically significant differences in pairwise comparisons (*: *p*-adjusted < 0.05; **: *p*-adjusted < 0.01; ***: *p*-adjusted < 0.001; ****: *p*-adjusted < 0.0001).

Overall, the ASVs identified in all samples were classified into 32 phyla, 64 classes, 261 families, and 482 genera. Bacterial communities were dominated by Proteobacteria (50.8%), Bacteroidota (28.5%), Cyanobacteria (9.3%), and Verrucomicrobiota (4.3%) (Figure S3). Proteobacteria comprised almost exclusively Alphaproteobacteria (29.5%) and Gammaproteobacteria (21.4%), while Bacteroidota, Cyanobacteria, and Verrucomicrobiota were dominated by the classes Bacteroidia (28.0%), Cyanobacteriia (9.3%), and Verrucomicrobiae (4.1%), respectively (Figure S4). Bacterial abundances in the control site presented notable differences compared to the aquaculture farms. *Flavobacteriaceae* (19.5%), *Rhodobacteraceae* (12.2%), *Alteromonadaceae* (8.9%), *Cyanobiaceae* (8.4%), and *Cryomorphaceae* (5.0%) were the dominant bacterial families in the aquaculture samples. In the control samples, *Cyanobiaceae* (15.0%) was the most abundant family, followed by the Gammaproteobacteria SAR86 clade (9.2%), *Flavobacteriaceae* (7.8%), and the Alphaproteobacteria groups of the SAR116 clade (7.7%), AEGEAN-169 marine group (7.4%), and Clade I (7.0%) (Figure 3).

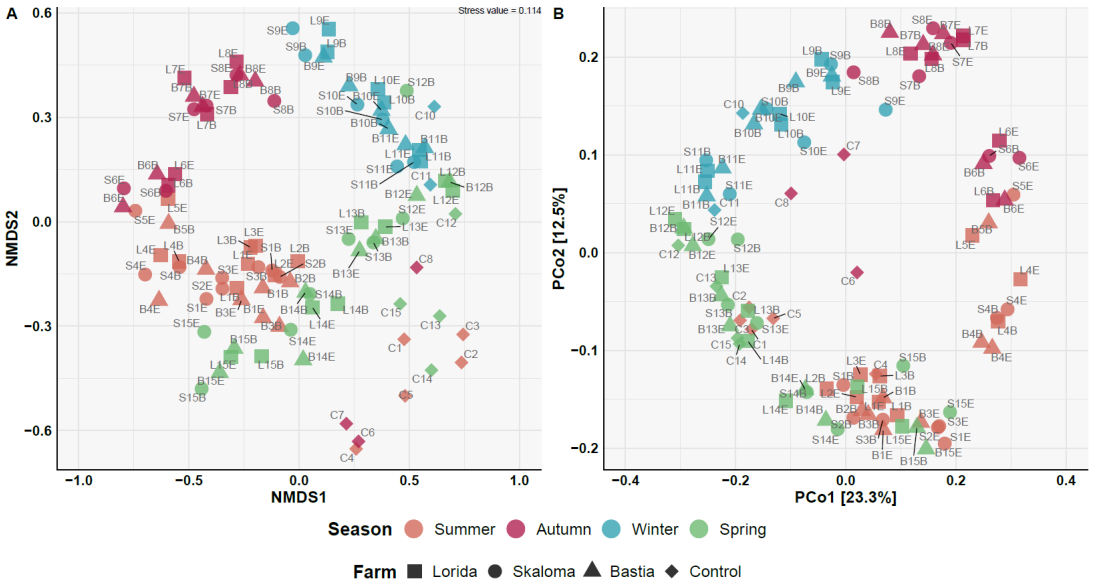

Figure 2. Beta diversity ordinations for the 100 seawater samples. (**A**) NMDS plot based on a Hellinger-transformed Bray–Curtis dissimilarity matrix. (**B**) PCoA plot based on the weighted UniFrac distances. Shapes depict the different sampling sites. Colors indicate the sampling seasons.

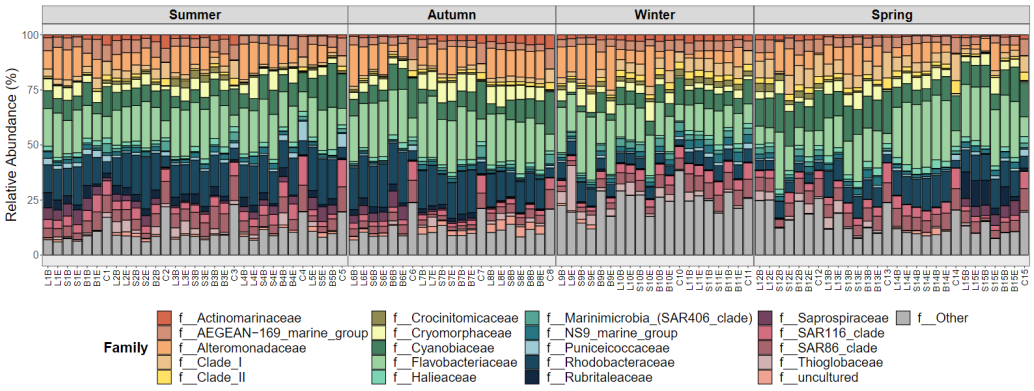

Figure 3. Barplot of relative abundance per sample at the family taxonomic level. The 20 most abundant families in the seawater samples from the aquaculture farms and the control site are presented. The remaining families are included under the "Other" category.

Among the most abundant families, *Flavobacteriaceae* dominated across seasons in the three aquaculture farms, with decreased abundance mostly in winter and early spring. This trend was reflected in most of the identified genera in the *Flavobacteriaceae* (NS3a and NS5 marine groups and uncultured bacteria). Bacterioplankton of the NS3a group was the most abundant group in the aquaculture sites, which showed higher abundance levels in autumn (7.3%) and spring (5.2%). The NS4 and NS5 groups were also present in high abundance in all sampling sites. The genus *Formosa* was detected in overall low abundances (2.1% on average for all seasons), presenting temperature-related variation, with an increase in summer, autumn, and spring (2.3% ± 1.5%, on average), except in June and March, and a significantly lower percentage from November to March (0.4% ± 0.4%, on average). On the other hand, *Aurantivirga* showed a sharp increase in abundance in December (7.8%) which gradually decreased in late winter and spring, while it dropped below 1.0% in August and remained at that abundance level throughout autumn. These two genera, along with the NS3a group, were almost absent from the control site (Figure 4).

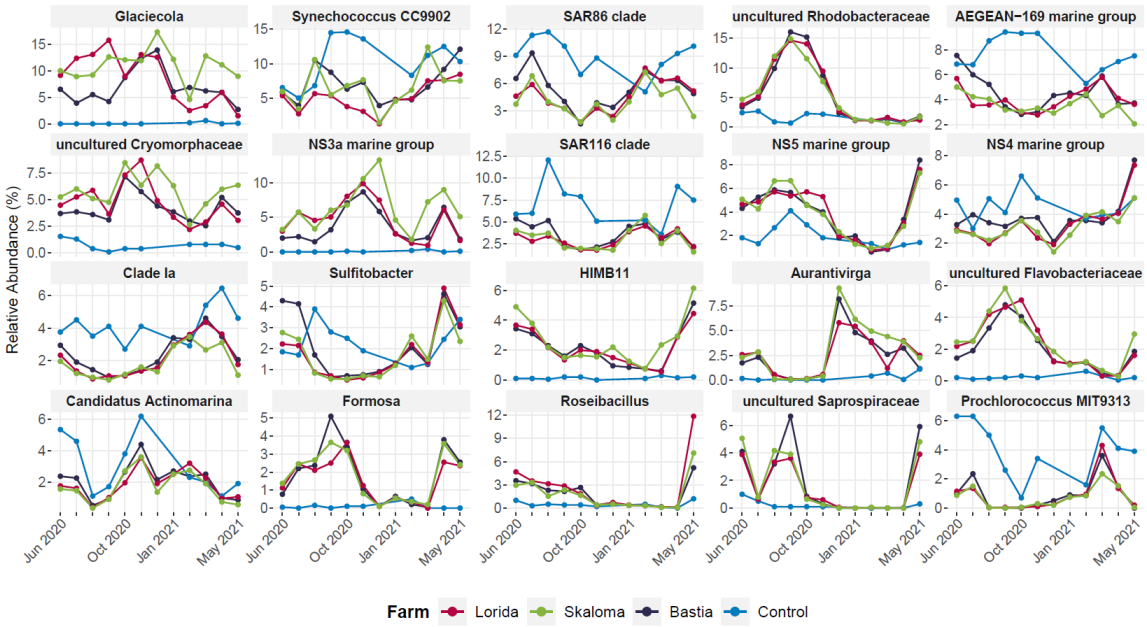

Figure 4. Time-series plots showing the changes in bacterial relative abundance (%) from June 2020 to May 2021. The major genera are presented in descending order of relative abundance, with colored lines indicating the sampling sites (Lorida—red, Skaloma—green, Bastia—purple, Control site—light blue).

Among the most prevalent in the aquaculture farms were also uncultured bacteria of the *Rhodobacteraceae* family, which showed higher relative abundances during summer and autumn (6.8% and 12.5%, respectively) compared to winter and spring (1.7% and 1.1%, respectively). Uncultured *Cryomorphaceae*, on the contrary, exhibited consistent relative abundance levels during winter, spring, and summer (4.3%, 4.6%, and 4.8%, respectively), with an increase in autumn (6.8%). These uncultured bacteria were observed at consistently lower abundances in the control site (1.5% ± 1.1% and 0.8% ± 0.5% on average, respectively) (Figure 4).

Alteromonadaceae, which was the third most abundant family in the aquaculture farms, comprised almost exclusively of members of the *Glaciecola* genus. *Glaciecola* dominated the aquaculture farms, with higher abundance in summer till mid-winter (10.0% ± 4.3%, on average), and lower in late winter and spring (6.4% ± 3.7%, on average). However, it was only detected in traces in the control sampling site and only during winter and spring (0.2% ± 0.1% and 0.2% ± 0.3%, on average, respectively). The most abundant bacteria in the control site were those belonging to the *Cyanobiaceae* family. Specifically, the genus *Synechococcus* CC9902 ranged from 6.3% in summer (C1–5) to 14.2% in autumn (C6–8), and was the second most abundant taxon in the aquaculture farms, reaching its maximum and minimum abundance levels in spring (8.6%) and winter (4.1%), respectively. The Gammaproteobacteria SAR86 clade ranged from 6.8% in winter (C10–11) to 10.6% in summer in the control site, while its relative abundance was constant during winter (4.9%), spring (5.6%), and summer (5.3%) but decreased in autumn (3.0%) in the aquacultures. The Alphaproteobacteria SAR116 and AEGEAN-169 groups showed higher relative abundances during spring and summer with peaks in April and August and lower abundances in late autumn and winter in all sampling sites (Figure 4).

Furthermore, at the genus level, *Sulfitobacter* was detected in the aquaculture farms at low abundances in autumn and winter (1.1% ± 0.6%, on average), with a sharp increase in April (4.6%), whereas in the control site, its abundance remained stable throughout the one-year study period at 2.4% ± 0.9% on average, except for a peak at 3.9% in August. In the aquaculture farms, the genus *Roseibacillus* was found in higher abundance in summer (3.2%), which gradually decreased in autumn (1.6%) and reached undetectable levels in

winter and spring (<0.4%), but showed an abrupt peak again in May (8.1%). In the control site, *Roseibacillus* was found in trace abundance levels (0.3–0.7%) in all seasons. The same was observed for uncultured bacteria of the *Saprospiraceae* family, whose relative abundance dropped to zero levels from winter to April. While *Prochlorococcus* was detected in low abundance in the aquacultures' samples (ranging from 0.1% in autumn to 1.6% in spring), it was one of the dominant genera in the control site, with higher abundance in summer (5.8%) and spring (4.4%), and lower in autumn (2.2%) and winter (1.7%). Moreover, since the majority of the ASVs found were classified as uncultured bacteria of their respective genera, the taxonomic classification at the species level could not be clarified; consequently, these results are not discussed further.

3.4. Seasonal Patterns of Differentially Abundant Taxa in the Marine Aquaculture Systems

Differential abundance analysis revealed the effect of seasonal changes on bacterial communities' composition in the three aquaculture systems. Most of the DA ASVs were those belonging to the phyla Proteobacteria, Bacteroidota, Verrucomicrobiota, Planctomycetota, Actinobacteriota, and Marinimicrobia. DA Chloroflexi ASVs were found only in summer and winter, while Cyanobacteria and Desulfobacterota were found only in summer and spring (Figure 5 and Table S4). The number of enriched ASVs in winter in all pairwise comparisons was higher than that of the depleted ASVs for this season, while the opposite was true for autumn. Furthermore, when summer was compared to spring, there were more enriched than depleted ASVs in summer (Table S4 and Figure S5).

The comparison of spring against autumn revealed 118 and 31 DA ASVs in the two seasons, respectively. The family *Flavobacteriaceae*, along with the SAR86 clade (Proteobacteria) and *Rhodobacteraceae*, had the highest number of DA ASVs between the two seasons. Those families, as well as *Cryomorphaceae*, *Vibrionaceae*, and *Actinomarinaceae*, contained DA ASVs in both seasons, with most of them being enriched during spring, except for *Actinomarinaceae*, in which most ASVs were depleted in spring. In addition, DA ASVs belonging to families such as *Cyanobiaceae*, the SAR116 clade (Proteobacteria), Clade I (Alphaproteobacteria), the NS9 marine group (Bacteroidota), the AEGEAN-169 marine group (Proteobacteria), and *Halieaceae* were enriched only in spring (Figure 5A).

When winter was compared to autumn, the enriched ASVs in each season amounted to 95 and 33, respectively. The families with the greatest number of DA ASVs were *Flavobacteriaceae* and *Rhodobacteraceae*, which included ASVs enriched in both seasons. Families *Microtrichaceae*, *Vibrionaceae*, the SAR116 clade, and the NS9 marine group also included ASVs enriched in both seasons, while the *Marinimicrobia* family (SAR406 clade), the AEGEAN-169 marine group, and the Clade I group comprised only ASVs depleted in autumn, to name a few (Figure 5B).

When winter was compared to summer, the number of DA ASVs was the highest among all pairwise comparisons, 160 in total, of which 48 and 112 were enriched in summer and winter, respectively. *Flavobacteriaceae*, *Rhodobacteraceae*, the SAR116 clade, the AEGEAN-169 marine group, *Cryomorphaceae*, the SAR86 clade, the NS9 marine group, and *Puniceicoccaceae* were among the families with enriched ASVs in both seasons, with *Flavobacteriaceae* having the highest number of enriched ASVs in both summer and winter, as well as the highest number of DA ASVs in total between the two seasons compared to other families. Moreover, members of the Marinimicrobia family (SAR406 clade) and the SAR202 clade were found enriched only in winter, while members of the NS11–12 marine group (Bacteroidota) were enriched only in summer (Figure 5C).

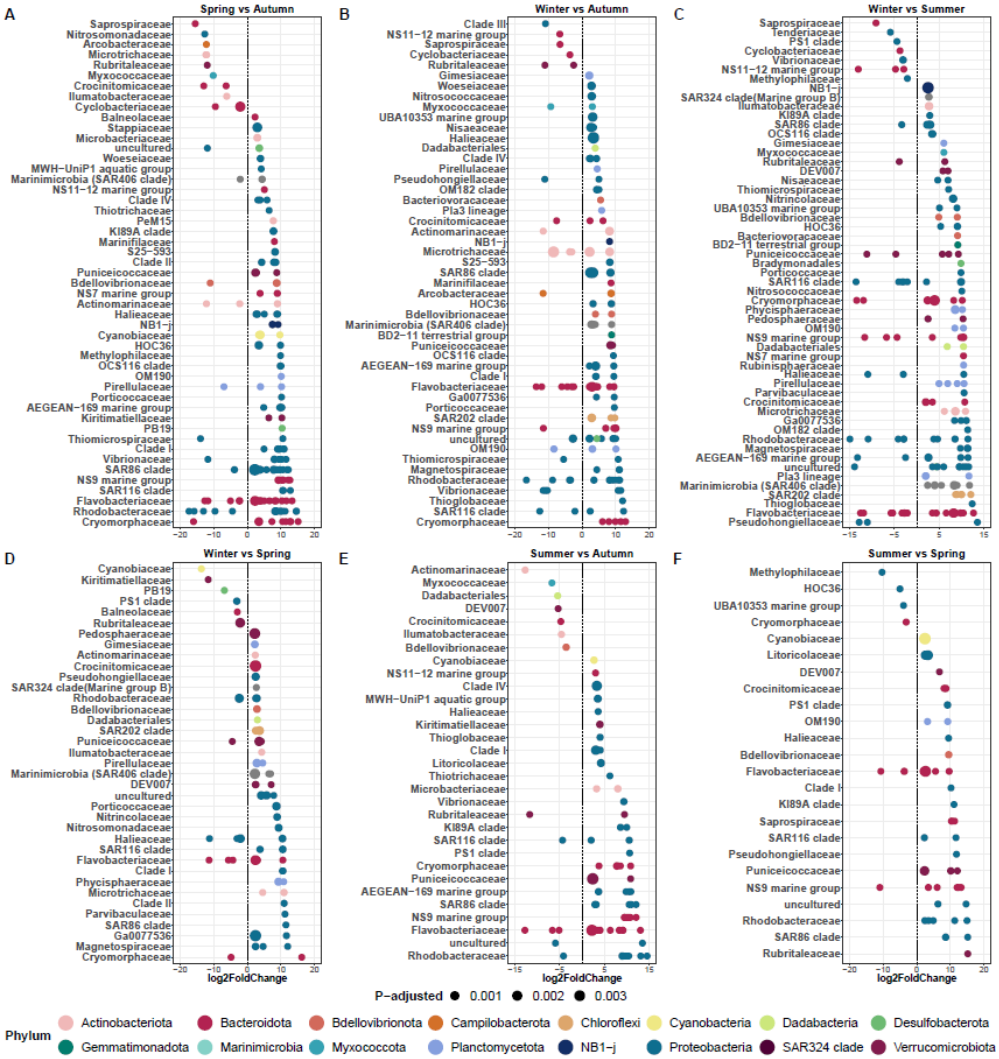

Figure 5. Dotplots for the DA ASVs identified in pairwise comparisons between the four seasons in the aquaculture farms. Positive values correspond to the first season in each comparison; (**A**) spring versus autumn, (**B**) winter versus autumn, (**C**) winter versus summer, (**D**) winter versus spring, (**E**) summer versus autumn, and (**F**) summer versus spring. Each dot represents a DA ASV with adjusted *p*-value < 0.01 and absolute $\log_2$(fold-change) ≥ 2, and ASVs are presented at the family (*y*-axis) and phylum (color) taxonomic levels. Dot size reflects adjusted *p*-values and color denotes taxonomy at the phylum level.

In the winter versus spring comparison, 69 ASVs were found to be differentially abundant in either of the seasons, with 15 of them being enriched during spring and 54 during winter. These 69 ASVs corresponded to 40 families, 6 of which contained only one ASV enriched during spring (e.g., *Cyanobiaceae*), while another 29 contained only ASVs enriched during winter (e.g., *Magnetospiraceae*, Marinimicrobia—SAR406 clade, Chloroflexi—SAR202 clade, the SAR116 clade, and Clade I). Finally, families *Flavobacteriaceae*, *Halieaceae*, and *Rhodobacteraceae*, which were those with the highest number of enriched ASVs, along with *Puniceicoccaceae*, included enriched ASVs in both seasons (Figure 5D).

In the summer versus autumn comparison, 59 and 14 ASVs were found to be enriched, respectively. Of those, members of *Flavobacteriaceae*, *Rhodobacteraceae*, and the SAR116 clade were enriched in both seasons, while members of the SAR86 clade, AEGEAN-169 marine group, *Cryomorphaceae*, and *Puniceicoccaceae* were enriched only in summer (Figure 5E).

The summer versus spring comparison resulted in the fewest DA ASVs, 47 in total, with seven enriched during spring and forty depleted. ASVs from *Flavobacteriaceae* and

the NS9 marine group were enriched in both seasons, but more so in summer than in spring. Furthermore, DA ASVs belonging to families such as *Rhodobacteraceae*, *Puniceicoccaceae*, the SAR116 clade, and the SAR86 clade, were exclusively enriched during summer, whereas ASVs enriched during spring belonged to families such as *Cryomorphaceae* and *Methylophilaceae* (Figure 5F).

4. Discussion

Aquaculture is a crucial food sector that has the potential to grow into a sustainable and secure global food supply system. Microorganisms are the most numerous components of marine ecosystems and have been shown to respond to environmental changes and anthropogenic activities. In aquaculture systems, bacteria in the water column interact constantly with farmed fish and are critical drivers of fish health and wellbeing. Thus, understanding marine bacterial communities' dynamics is critical in addressing challenges such as the increased transmission of pathogens inside the farms causing outbreaks and significant production losses [53], the introduction of antimicrobial resistance in marine environments due to excessive use of antibiotics [54], and the release of excessive amounts of nutrients into the marine environment, leading to seawater and sediment disturbances [55,56]. The seasonal dynamics of bacterial communities in Greek coastal aquaculture farming systems have yet to be thoroughly investigated. Here, as a first step in our effort to connect the effect of biotic and abiotic environmental factors on the bacterial community structure, as well as disease appearance in marine pisciculture, we performed 16S rRNA metabarcoding analysis on seawater samples collected monthly from three aquaculture farms in the Sagiada strip (Ionian Sea, northwestern Greece), over the course of one year.

Marine aquaculture farming systems, which are characterized by high fish biomass concentrations and strong nutrient fluxes, provide an ideal environment for studying bacterial community interactions. Our results revealed significant differences concerning the structure of bacterial communities between the aquaculture farms and the control site. The control site was located further from the coast, representing an undisturbed environment in terms of industrial and agriculture water discharge or aquaculture activities, and had statistically significantly higher species richness, on average, than the aquaculture farms. Several studies have reported increased nutrient loading, especially for nitrogen and phosphorus, in aquaculture farming systems due to the release of organic waste, uneaten feed, and fish excrement [57–59]. In terms of nutrient concentrations, we have observed increased nitrogen compounds such as nitrate, nitrite, and ammonium in summer and autumn and decreases in spring, which could be attributed to the increased feeding that takes place in the farming systems during these periods. Furthermore, nutrient and chlorophyll-a concentrations were lower in the control site than in the aquaculture systems. Thus, differences in terms of species richness in the aquaculture systems compared to a distant unexploited region could be attributed to selective nutrient enrichment in these systems, which favors the growth of certain bacterial groups, leading to a less diverse bacterial community. Fodelianakis et al. (2014) found that alpha diversity was reduced in a near-shore aquaculture farm in Crete compared to the control site in their study [60]. They also reported a decrease in the abundance of the SAR86 clade and an increase in the abundance of *Alteromonadaceae*, *Rhodobacteraceae*, and *Cryomorphaceae* in the aquaculture site, which is in agreement with our findings. Our results indicate that the impact of aquaculture activities is reflected in the diversity of the bacterial community; thus, microbiome diversity in the water column can be a powerful tool in monitoring environmental disturbances in aquaculture farming.

The Sagiada strip is a zone of intensive aquaculture farming in the Ionian Sea, and it is an oligotrophic environment with seasonal variability [61]. Indeed, our findings indicate a strong relationship between the presence of bacterial communities and the season of the year, suggesting that seasonality, in addition to several environmental factors, is the major driver shaping bacterial community composition and diversity, rather than the locations of aquaculture farms. Similar dynamics were observed in the Mediterranean Sea in either

coastal areas [59,62] or coastal aquaculture farms [63,64]. Furthermore, there appear to be no significant differences in the bacterial communities between or within farms, based on our findings. It is worth mentioning that, although the aquaculture farming systems have their own different environments and hence different bacterial community structures compared to the control site, the influence of seasonality is apparent in both areas, though it may involve different genera in certain cases. As a result, seasonality, and specifically temperature, is the most important factor influencing communities' abundance, at least at the genus taxonomic level.

The microbial community in aquaculture systems is vital for several key functions, including nutrient cycling, water quality maintenance, and disease appearance. Many of the identified bacterial groups in our study have been linked to such processes; the most prevalent bacterial phyla in the ocean, Proteobacteria and Cyanobacteria, participate in the carbon and nitrogen cycles [65–67], followed by Bacteroidota, which have a role in breaking down complex organic matter [68], and Verrucomicrobiota, which are suggested to be polysaccharide degraders [69]. The presence of these groups has also been reported in aquaculture areas in the Mediterranean Sea [70,71], while the increased abundance of Proteobacteria and Bacteroidota has been linked to fish excrement or the introduction of feed components [72].

Several Proteobacteria taxa perform significant functions in the seawater associated with marine aquaculture systems, with Alpha- and Gamma-proteobacteria often found in high abundances [70]. In our study, *Glaciecola* (Gammaproteobacteria), a genus of diverse marine bacteria with unique properties that allow them to thrive in marine environments, predominated in the three aquaculture farms. These bacteria have been isolated from a variety of maritime environments and exhibit a cold adaptation strategy [73]. This genus was found to be the most prevalent in autumn and early winter in coastal seawater samples in Korea [74], and in autumn in aquaculture waters in the Adriatic Sea [64]. In our study, its relative abundance was high from summer until mid-winter, confirming the results of previous studies. Moreover, the Cyanobacteria and Bacteroidota phyla comprise multiple families that perform important roles in seawater ecosystems in marine aquaculture systems [75,76]. Kolda et al. (2020) also found *Synechococcus* CC9902 and the NS4 and NS5 marine groups of the Flavobacteriales to be among the most abundant taxa during spring and autumn in coastal areas of the southern Adriatic Sea [64], which is in accordance with our findings for the adjacent north Ionian Sea.

Due to the observed strong impact of seasonality on the bacterial communities' structure in the three aquaculture farming systems, we further investigated the existence of statistically significant differences across seasons. The water column bacterial composition varied significantly between seasons, with ASVs from the most prevalent families, such as *Flavobacteriaceae* and *Cryomorphaceae*, being constantly enriched across all seasons. *Flavobacteriaceae*, which had the highest number of DA ASVs in all comparisons, represents a major family of the Bacteroidota phylum, with its marine clade (e.g., NS4 and NS5 groups) being among the most common bacterioplankton on the ocean's surface [76], justifying their enrichment in all seasons. They are known to become dominant in response to the input of organic matter [77], which is a typical outcome of aquaculture activity, as mentioned above. Moreover, they comprise species that cause infectious diseases in fish, which is a primary concern in the aquaculture industry [78], rendering their study crucial for disease monitoring and prevention in such systems. *Cryomorphaceae*, another Bacteroidota family with DA ASVs throughout the year, has also been demonstrated to be prevalent in surface waters of coastal sites [60,79].

Furthermore, several Alphaproteobacteria ASVs were differentially abundant among seasons. In all comparisons, uncultured bacteria of the *Rhodobacteraceae* family, which was among the dominant families in our samples, were the group with the second-highest number of DA ASVs. *Rhodobacteraceae* also thrive in marine environments and are key players in sulfur and carbon biogeochemical cycling [80]. Moreover, members of the Alphaproteobacteria SAR11 clade (e.g., Clade I and IV) and the AEGEAN-169 group were depleted in all

pairwise comparisons regarding autumn, contrary to the findings of Kolda et al. (2020), who showed the SAR11 clade to be one of the most abundant taxa in autumn. Uncultured bacteria of the SAR116 clade were also enriched in all seasons, with the exception of spring, in which they were only enriched when compared to autumn. This group consists largely of bacteria that have been implicated in the metabolism of inorganic and organic sulfur compounds [81]. Another group of bacteria associated with sulfur cycling was the Desulfobacterota PB 19, which was enriched exclusively in spring versus autumn and winter. Previous research has found an increase in sulfur cycling in sediments beneath aquaculture farms [82], and another study has pointed out sediment-associated bacterial taxa linked to the sulfur cycle as useful indicators for assessing aquaculture practices' impact on marine sediment [59]. The changes in sulfur-cycle-associated bacteria in seawater observed in this study suggest that the influence of organic waste from aquaculture farming can be monitored in the water column as well.

ASVs within the family *Rubritaleaceae*, specifically the genus *Roseibacillus*, and uncultured *Saprospiraceae* ASVs were enriched in summer and autumn and depleted in the other seasons. These two taxa have been rarely mentioned in studies of the coastal seawater bacterial communities in the Mediterranean [83]. However, our results indicate that the relative abundance of *Roseibacillus* and uncultured *Saprospiraceae* is positively associated with late spring, summer, and early autumn, indicating a preference for warmer temperatures. Another major bacterial group identified as significantly enriched in warmer temperatures (spring and summer), Cyanobacteria, plays a vital role as oxygenic photosynthetic bacteria and as nitrogen-fixing agents in aquatic environments [84]. These bacteria are generally more prevalent in oligotrophic environments and exhibit optimal growth rates in warmer seasons (25–35 °C) [85,86]. Indeed, in our results, ASVs belonging to the picophytoplankton genera *Synechococcus* CC9902 and *Prochlorococcus* MIT9313 were enriched in summer and autumn, which showed the highest temperatures throughout the year, compared to the other seasons, which is in accordance with previous findings in adjacent coastal areas [64,87]. Autumn had the highest chlorophyll-a levels, followed by spring and summer, which appears to be related to the relative abundance levels of these Cyanobacteria genera.

Pairwise comparison of winter versus summer showed the greatest number of DA ASVs, as expected due to the significant differences in environmental conditions, in our case temperature, salinity, and nitrate levels, that are usually observed in the area under study [88]. Likewise, the comparison of spring against autumn demonstrated the second highest number of DA ASVs. These two seasons also presented quite different environmental conditions regarding temperature, salinity, nitrate, and ammonium compounds; the differences, however, are less extreme than those observed between summer and winter, partly justifying the slightly lower number of DA ASVs.

Undoubtedly, aquacultures establish unique ecosystems in coastal regions and promote considerable changes in marine environments. Incorporation of genomics into farm management practices can not only assist in monitoring bacterial populations, but also contribute towards the identification of species tolerant to increasing temperatures and changing water conditions or towards the detection and prevention of disease outbreaks.

5. Conclusions

This study presents a bacterial community profiling and seasonal pattern investigation of the water column of marine coastal aquaculture farming systems. Bacterial community diversity and composition differed significantly between the aquaculture systems and an unexploited region. Furthermore, season appeared to be the major factor shaping the bacterial communities in the three aquaculture systems. Capturing the temporal dynamics of bacterial communities will enable the application of more targeted and informed management practices, ultimately supporting the sustainable and responsible operation of coastal aquaculture. At the same time, bacterial community profiling can be used as a monitoring tool, which will provide feedback on the impact of aquaculture practices on the environment and the effectiveness of the farming strategies applied.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/jmse11071332/s1, Figure S1: Map of the study sites. The blue circle on the upper-right-corner map denotes the wider region of Greece in which the area under investigation is located. The three aquaculture farms' units and the control location are indicated by blue dots; B: Bastia aquaculture farm, S: Skaloma aquaculture farm, L: Lorida aquaculture farm, C: Control site; Figure S2: Alpha rarefaction curves of 16S rRNA amplicon sequences of bacterial communities in the whole dataset for each season; summer: 31 samples, autumn: 21 samples, winter: 20 samples, spring: 28 samples. The solid lines depict the observed accumulation with the number of reads sampled, whereas the dashed lines depict the extrapolated accumulation up to the double amount of reads; Figure S3: Barplot of relative abundance per sample at the phylum taxonomic level. The 10 most abundant phyla in the seawater samples from the aquaculture farms and the control site are presented. The remaining phyla are included under the "Other" category; Figure S4: Barplot of relative abundance per sample at the class taxonomic level. The 10 most abundant classes in the seawater samples from the aquaculture farms and the control site are presented. The remaining classes are included under the "Other" category; Figure S5: Volcano plots of the differentially abundant ASVs identified in the pairwise comparisons between seasons in the three aquaculture farms (Lorida, Bastia, Skaloma). Yellow and blue dots indicate differentially abundant ASVs (SA at absolute $\log_2$(fold–change) ≥ 2 and Benjamini–Hochberg adjusted p-value < 0.01 cutoffs), and not significant ASVs are presented with grey (NS); Table S1: Information on the season, month, and location within the farm for each sample, and the number of raw and filtered paired-end sequences per sample; Table S2: Alpha diversity indices per sample; Table S3: PERMANOVA results of the pairwise comparisons between each season using the Bray–Curtis dissimilarity matrix and the weighted UniFrac metric on the bacterial ASVs for all seawater samples. Significant codes: '***': 0; '**': 0.001; '*': 0.01; '.': 0.05; ' ': 0.1; Table S4: Number of enriched ASVs (En) in pairwise comparisons between the four seasons in aquaculture farms ($\log_2$(fc) ≥ 2, p-adjust < 0.01). Columns show the season to which the number of enriched ASVs corresponds, while rows show the season compared to which the ASVs were found enriched.

Author Contributions: Conceptualization, A.A.; methodology, M.K., S.M., K.K., N.K. and G.K.; validation, A.T.; formal analysis, M.K. and A.T.; investigation, M.K.; resources, A.A.; writing—original draft preparation, M.K.; writing—review and editing, M.K., A.A., S.M., K.K. and A.A.; visualization, M.K. and A.T.; supervision, A.A.; project administration, S.M.; funding acquisition, G.K. and A.A. All authors have read and agreed to the published version of the manuscript.

Funding: This research is implemented in the framework of the PROGNOSIS research project (project code MIS 5032517) which was co-funded by the European Union and Greek National Funds (Partnership Agreement 2014–2020) under the National Fisheries and Sea Operational Program.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Raw sequencing data are available through the Sequence Read Archive (SRA) of the National Center for Biotechnology Information (NCBI) under the BioProject Accession number PRJNA977998.

Acknowledgments: The authors wish to thank Achilleas Chatzopoulos from Bastia fish farm for his contribution to water sampling and the General Secretariat for Research and Innovation for continuous support.

Conflicts of Interest: The authors declare that they have no known competing financial interests or personal relationships which have or could be perceived to have influenced the work reported in this article.

References

1. Arrigo, K.R. Molecular diversity and ecology of microbial plankton. *Nature* **2005**, *437*, 343–348. [CrossRef]
2. Morris, R.; Nunn, B.; Frazar, C.; Goodlett, D.R.; Ting, Y.S.; Rocap, G. Comparative metaproteomics reveals ocean-scale shifts in microbial nutrient utilization and energy transduction. *ISME J.* **2010**, *4*, 673–685. [CrossRef]
3. Acinas, S.G.; Sánchez, P.; Salazar, G.; Cornejo-Castillo, F.M.; Sebastián, M.; Logares, R.; Royo-Llonch, M.; Paoli, L.; Sunagawa, S.; Hingamp, P.; et al. Deep ocean metagenomes provide insight into the metabolic architecture of bathypelagic microbial communities. *Commun. Biol.* **2021**, *4*, 604. [CrossRef] [PubMed]

4. Benedetti, F.; Vogt, M.; Elizondo, U.H.; Righetti, D.; Zimmermann, N.E.; Gruber, N. Major restructuring of marine plankton assemblages under global warming. *Nat. Commun.* **2021**, *12*, 5226. [CrossRef] [PubMed]
5. Staley, J.T.; Konopka, A.; Staley Allan, J.T.K. Microorganisms in Aquatic and Terrestrial Habitats. *Annu. Rev. Microbiol.* **1985**, *39*, 321–346. [CrossRef] [PubMed]
6. Steen, A.D.; Crits-Christoph, A.; Carini, P.; DeAngelis, K.M.; Fierer, N.; Lloyd, K.G.; Cameron Thrash, J. High proportions of bacteria and archaea across most biomes remain uncultured. *ISME J.* **2019**, *13*, 3126–3130. [CrossRef] [PubMed]
7. Hilborn, R.; Amoroso, R.O.; Anderson, C.M.; Baum, J.K.; Branch, T.A.; Costello, C.; De Moor, C.L.; Faraj, A.; Hively, D.; Jensen, O.P.; et al. Effective fisheries management instrumental in improving fish stock status. *Proc. Natl. Acad. Sci. USA* **2020**, *117*, 2218–2224. [CrossRef] [PubMed]
8. Melnychuk, M.C.; Kurota, H.; Mace, P.M.; Pons, M.; Minto, C.; Osio, G.C.; Jensen, O.P.; de Moor, C.L.; Parma, A.M.; Richard Little, L.; et al. Identifying management actions that promote sustainable fisheries. *Nat. Sustain.* **2021**, *4*, 440–449. [CrossRef]
9. Tsikliras, A.C.; Dinouli, A.; Tsiros, V.Z.; Tsalkou, E. The Mediterranean and Black Sea fisheries at risk from overexploitation. *PLoS ONE* **2015**, *10*, e0121188. [CrossRef]
10. Froese, R.; Winker, H.; Coro, G.; Demirel, N.; Tsikliras, A.C.; Dimarchopoulou, D.; Scarcella, G.; Quaas, M.; Matz-Lück, N. Status and rebuilding of European fisheries. *Mar. Policy* **2018**, *93*, 159–170. [CrossRef]
11. United Nations. Transforming Our World: The 2030 Agenda for Sustainable Development. In *A New Era in Global Health*; Springer Publishing Company: New York, NY, USA, 2018. [CrossRef]
12. Wang, Z.; Tsementzi, D.; Williams, T.C.; Juarez, D.L.; Blinebry, S.K.; Garcia, N.S.; Sienkiewicz, B.K.; Konstantinidis, K.T.; Johnson, Z.I.; Hunt, D.E. Environmental stability impacts the differential sensitivity of marine microbiomes to increases in temperature and acidity. *ISME J.* **2021**, *15*, 19–28. [CrossRef]
13. Nikouli, E.; Meziti, A.; Antonopoulou, E.; Mente, E.; Kormas, K.A. Host-Associated Bacterial Succession during the Early Embryonic Stages and First Feeding in Farmed Gilthead Sea Bream (*Sparus aurata*). *Genes* **2019**, *10*, 483. [CrossRef]
14. Yajima, D.; Fujita, H.; Hayashi, I.; Shima, G.; Suzuki, K.; Toju, H. Core species and interactions prominent in fish-associated microbiome dynamics. *Microbiome* **2023**, *11*, 53. [CrossRef]
15. Trainer, V.L.; Moore, S.K.; Hallegraeff, G.; Kudela, R.M.; Clement, A.; Mardones, J.I.; Cochlan, W.P. Pelagic harmful algal blooms and climate change: Lessons from nature's experiments with extremes. *Harmful Algae* **2020**, *91*, 101591. [CrossRef] [PubMed]
16. Wang, C.; Wang, Y.; Liu, P.; Sun, Y.; Song, Z.; Hu, X. Characteristics of bacterial community structure and function associated with nutrients and heavy metals in coastal aquaculture area. *Environ. Pollut.* **2021**, *275*, 116639. [CrossRef] [PubMed]
17. Jurelevicius, D.; Cotta, S.R.; Montezzi, L.F.; Dias, A.C.F.; Mason, O.U.; Picão, R.C.; Jansson, J.K.; Seldin, L. Enrichment of potential pathogens in marine microbiomes with different degrees of anthropogenic activity. *Environ. Pollut.* **2021**, *268*, 115757. [CrossRef]
18. Uyaguari-Diaz, M.I.; Chan, M.; Chaban, B.L.; Croxen, M.A.; Finke, J.F.; Hill, J.E.; Peabody, M.A.; Van Rossum, T.; Suttle, C.A.; Brinkman, F.S.L.; et al. A comprehensive method for ampliconbased and metagenomic characterization of viruses, bacteria, and eukaryotes in freshwater samples. *Microbiome* **2016**, *4*, 20. [CrossRef]
19. Debroas, D.; Domaizon, I.; Humbert, J.F.; Jardillier, L.; Lepére, C.; Oudart, A.; Taib, N. Overview of freshwater microbial eukaryotes diversity: A first analysis of publicly available metabarcoding data. *FEMS Microbiol. Ecol.* **2017**, *93*, fix023. [CrossRef] [PubMed]
20. Mahmoud, M.A.A.; Magdy, M. Metabarcoding profiling of microbial diversity associated with trout fish farming. *Sci. Rep.* **2021**, *11*, 421. [CrossRef]
21. Lee, D.E.; Lee, J.; Kim, Y.M.; Myeong, J.I.; Kim, K.H. Uncultured bacterial diversity in a seawater recirculating aquaculture system revealed by 16S rRNA gene amplicon sequencing. *J. Microbiol.* **2016**, *54*, 296–304. [CrossRef]
22. Kalaitzidou, M.P.; Alvanou, M.V.; Papageorgiou, K.V.; Lattos, A.; Sofia, M.; Kritas, S.K.; Petridou, E.; Giantsis, I.A. Pollution Indicators and HAB-Associated Halophilic Bacteria Alongside Harmful Cyanobacteria in the Largest Mussel Cultivation Area in Greece. *Int. J. Environ. Res. Public Health* **2022**, *19*, 5285. [CrossRef]
23. Jing, X.; Gou, H.; Gong, Y.; Ji, Y.; Su, X.; Zhang, J.; Han, M.; Xu, L.; Wang, T. Seasonal dynamics of the coastal bacterioplankton at intensive fish-farming areas of the Yellow Sea, China revealed by high-throughput sequencing. *Mar. Pollut. Bull.* **2019**, *139*, 366–375. [CrossRef] [PubMed]
24. Huang, H.; Zan, S.; Ming, H.; Li, M.; Xu, J.; Xie, Y.; Wang, W.; Fan, J. 16S Next-generation sequencing and quantitative PCR reveal the distribution of potential pathogens in the Liaohe Estuary. *Front. Mar. Sci.* **2022**, *9*, 1044260. [CrossRef]
25. Pavloudi, C.; Tsertou, M.I.; Antonopoulou, E.; Katharios, P. Investigation of systemic granulomatosis in cultured meagre, Argyrosomus regius, using clinical metagenomics. *Aquaculture* **2023**, *567*, 739249. [CrossRef]
26. Stavrakidis-Zachou, O.; Lika, K.; Anastasiadis, P.; Papandroulakis, N. Projecting climate change impacts on Mediterranean finfish production: A case study in Greece. *Clim. Change* **2021**, *165*, 67. [CrossRef]
27. Cascarano, M.C.; Stavrakidis-Zachou, O.; Mladineo, I.; Thompson, K.D.; Papandroulakis, N.; Katharios, P. Mediterranean aquaculture in a changing climate: Temperature effects on pathogens and diseases of three farmed fish species. *Pathogens* **2021**, *10*, 1205. [CrossRef]
28. Katselis, G.; Tsolakos, K.; Theodorou, J.A. Mapping of Greek Marine Finfish Farms and Their Potential Impact on the Marine Environment. *J. Mar. Sci. Eng.* **2022**, *10*, 286. [CrossRef]
29. HAPO. *Hellenic Organization of Aquaculture Producers—Aquaculture Annual Report*; HAPO: Peania, Greece, 2022; Available online: https://fishfromgreece.com/wp-content/flipbook/nov22/ (accessed on 28 June 2023).

30. Eurostat European Commission. *Agriculture, Forestry and Fishery Statistics: 2020 Edition*; Publications Office of the European Union: Luxembourg, 2020; ISBN 978-92-76-21521-9.
31. Konstantinidis, E.; Perdikaris, C.; Batzios, C.; Michaelidis, B.; Ganias, K. Estimating cage farming capacity of data-poor mariculture sectors in the Eastern Mediterranean. *J. Appl. Aquac.* **2021**, *33*, 111–125. [CrossRef]
32. Parsons, T.R.; Maita, Y.; Lalli, C.M. *A Manual of Chemical and Biological Methods for Seawater Analysis*; Pergamon Press: Oxford, UK, 1984. [CrossRef]
33. American Public Health Association (APHA). *Standard Methods for the Examination of Water and Wastewater*, 20th ed.; American Public Health Association, American Water Works Association and Water Environmental Federation: Washington, DC, USA, 1998.
34. Cruaud, P.; Vigneron, A.; Fradette, M.S.; Charette, S.J.; Rodriguez, M.J.; Dorea, C.C.; Culley, A.I. Open the SterivexTM casing: An easy and effective way to improve DNA extraction yields. *Limnol. Oceanogr. Methods* **2017**, *15*, 1015–1020. [CrossRef]
35. Klindworth, A.; Pruesse, E.; Schweer, T.; Peplies, J.; Quast, C.; Horn, M.; Glöckner, F.O. Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. *Nucleic Acids Res.* **2013**, *41*, e1. [CrossRef]
36. Bolyen, E.; Rideout, J.R.; Dillon, M.R.; Bokulich, N.A.; Abnet, C.C.; Al-Ghalith, G.A.; Alexander, H.; Alm, E.J.; Arumugam, M.; Asnicar, F.; et al. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nat. Biotechnol.* **2019**, *37*, 852–857. [CrossRef] [PubMed]
37. Callahan, B.J.; McMurdie, P.J.; Rosen, M.J.; Han, A.W.; Johnson, A.J.A.; Holmes, S.P. DADA2: High-resolution sample inference from Illumina amplicon data. *Nat. Methods* **2020**, *13*, 581–583. [CrossRef] [PubMed]
38. Bokulich, N.A.; Kaehler, B.D.; Rideout, J.R.; Dillon, M.; Bolyen, E.; Knight, R.; Huttley, G.A.; Gregory Caporaso, J. Optimizing taxonomic classification of marker-gene amplicon sequences with QIIME 2's q2-feature-classifier plugin. *Microbiome* **2018**, *6*, 90. [CrossRef] [PubMed]
39. Katoh, K.; Standley, D.M. MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. *Mol. Biol. Evol.* **2013**, *30*, 772–780. [CrossRef]
40. Price, M.N.; Dehal, P.S.; Arkin, A.P. FastTree 2—Approximately maximum-likelihood trees for large alignments. *PLoS ONE* **2010**, *5*, e9490. [CrossRef]
41. R Development Core Team. R: A Language and Environment for Statistical Computing. R 2011. Available online: https://www.r-project.org/ (accessed on 28 June 2023).
42. McMurdie, P.J.; Holmes, S. Phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. *PLoS ONE* **2013**, *8*, e61217. [CrossRef]
43. Kassambara, A. Ggpubr: "ggplot2" Based Publication Ready Plots. R Package version 0.6.0, 2020. Available online: https://CRAN.R-project.org/package=ggpubr (accessed on 28 June 2023).
44. Mikryukov, V. metagMisc: Miscellaneous Functions for Metagenomic Analysis. R Package version 0.5.0, 2020. Available online: https://github.com/vmikk/metagMisc (accessed on 28 June 2023).
45. Hsieh, T.; Ma, K.; Chao, A. iNEXT: Interpolation and Extrapolation for Species Diversity. *R Package version 3.0.0*, 2022.
46. Chao, A.; Gotelli, N.J.; Hsieh, T.C.; Sander, E.L.; Ma, K.H.; Colwell, R.K.; Ellison, A.M. Rarefaction and extrapolation with Hill numbers: A framework for sampling and estimation in species diversity studies. *Ecol. Monogr.* **2014**, *84*, 45–67. [CrossRef]
47. Andersen, K.S.; Kirkegaard, R.H.; Karst, S.M.; Albertsen, M. ampvis2: An R package to analyse and visualise 16S rRNA amplicon data. *bioRxiv* **2018**, 10–11. [CrossRef]
48. Lozupone, C.; Knight, R. UniFrac: A new phylogenetic method for comparing microbial communities. *Appl. Environ. Microbiol.* **2005**, *71*, 8228–8235. [CrossRef]
49. Oksanen, J.; Simpson, G.; Blanchet, F.; Kindt, R.; Legendre, P.; Minchin, P.; O'Hara, R.; Solymos, P.; Stevens, M.; Szoecs, E.; et al. vegan: Community Ecology Package. R Package version 2.6.4. 2022. Available online: https://CRAN.R-project.org/package=vegan (accessed on 28 June 2023).
50. Martinez Arbizu, P. pairwiseAdonis: Pairwise Multilevel Comparison Using. *R Package version 0.4.1*, 2020.
51. Love, M.I.; Huber, W.; Anders, S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol.* **2014**, *15*, 550. [CrossRef] [PubMed]
52. Zhu, A.; Ibrahim, J.G.; Love, M.I. Heavy-tailed prior distributions for sequence count data: Removing the noise and preserving large differences. *Bioinformatics* **2018**, *35*, 2084–2092. [CrossRef]
53. Bouwmeester, M.M.; Goedknegt, M.A.; Poulin, R.; Thieltges, D.W. Collateral diseases: Aquaculture impacts on wildlife infections. *J. Appl. Ecol.* **2021**, *58*, 453–464. [CrossRef]
54. Zhao, J.L.; Liu, Y.S.; Liu, W.R.; Jiang, Y.X.; Su, H.C.; Zhang, Q.Q.; Chen, X.W.; Yang, Y.Y.; Chen, J.; Liu, S.S.; et al. Tissue-specific bioaccumulation of human and veterinary antibiotics in bile, plasma, liver and muscle tissues of wild fish from a highly urbanized region. *Environ. Pollut.* **2015**, *198*, 15–24. [CrossRef]
55. Morsy, A.; Ebeid, M.; Soliman, A.; Halim, A.A.; Ali, A.; Fahmy, M. Evaluation of the water quality and the eutrophication risk in Mediterranean sea area: A case study of the Port Said Harbour, Egypt. *Environ. Chall.* **2022**, *7*, 100484. [CrossRef]
56. Wang, K.; Wei, Q.; Jian, H.; Gao, Z.; Yao, Q. Variations in the nutrient concentration and composition in Liaodong Bay under long-term human activities. *Mar. Pollut. Bull.* **2022**, *182*, 114016. [CrossRef]
57. Sarà, G. Ecological effects of aquaculture on living and non-living suspended fractions of the water column: A meta-analysis. *Water Res.* **2007**, *41*, 3187–3200. [CrossRef]

58. Bouwman, A.F.; Beusen, A.H.W.; Overbeek, C.C.; Bureau, D.P.; Pawlowski, M.; Glibert, P.M. Hindcasts and future projections of global inland and coastal nitrogen and phosphorus loads due to finfish aquaculture. *Rev. Fish. Sci.* **2013**, *21*, 112–156. [CrossRef]
59. Rubio-Portillo, E.; Villamor, A.; Fernandez-Gonzalez, V.; Antón, J.; Sanchez-Jerez, P. Exploring changes in bacterial communities to assess the influence of fish farming on marine sediments. *Aquaculture* **2019**, *506*, 459–464. [CrossRef]
60. Fodelianakis, S.; Papageorgiou, N.; Pitta, P.; Kasapidis, P.; Karakassis, I.; Ladoukakis, E.D. The pattern of change in the abundances of specific bacterioplankton groups is consistent across different nutrient-enriched habitats in crete. *Appl. Environ. Microbiol.* **2014**, *80*, 3784–3792. [CrossRef] [PubMed]
61. Kalimeris, A.; Kassis, D. Sea surface circulation variability in the Ionian-Adriatic Seas. *Prog. Oceanogr.* **2020**, *189*, 102454. [CrossRef]
62. Reich, I.; Ijaz, U.Z.; Gormally, M.; Smith, C.J. 16S rRNA sequencing reveals likely beneficial core microbes within faecal samples of the EU protected slug *Geomalacus maculosus*. *Sci. Rep.* **2018**, *8*, 10402. [CrossRef] [PubMed]
63. Pereira, C.; Salvador, S.; Arrojado, C.; Silva, Y.; Santos, A.L.; Cunha, Â.; Gomes, N.; Almeida, A. Evaluating seasonal dynamics of bacterial communities in marine fish aquaculture: A preliminary study before applying phage therapy. *J. Environ. Monit.* **2011**, *13*, 1053–1058. [CrossRef] [PubMed]
64. Kolda, A.; Gavrilović, A.; Jug-Dujaković, J.; Ljubešić, Z.; El-Matbouli, M.; Lillehaug, A.; Lončarević, S.; Perić, L.; Knežević, D.; Vukić Lušić, D.; et al. Profiling of bacterial assemblages in the marine cage farm environment, with implications on fish, human and ecosystem health. *Ecol. Indic.* **2020**, *118*, 106785. [CrossRef]
65. Deng, M.; Hou, J.; Song, K.; Chen, J.; Gou, J.; Li, D.; He, X. Community metagenomic assembly reveals microbes that contribute to the vertical stratification of nitrogen cycling in an aquaculture pond. *Aquaculture* **2020**, *520*, 734911. [CrossRef]
66. Fuchsman, C.A.; Palevsky, H.I.; Widner, B.; Duffy, M.; Carlson, M.C.G.; Neibauer, J.A.; Mulholland, M.R.; Keil, R.G.; Devol, A.H.; Rocap, G. Cyanobacteria and cyanophage contributions to carbon and nitrogen cycling in an oligotrophic oxygen-deficient zone. *ISME J.* **2019**, *13*, 2714–2726. [CrossRef]
67. Han, Y.; Zhang, M.; Chen, X.; Zhai, W.; Tan, E.; Tang, K. Transcriptomic evidences for microbial carbon and nitrogen cycles in the deoxygenated seawaters of Bohai Sea. *Environ. Int.* **2022**, *158*, 106889. [CrossRef]
68. Fernández-Gómez, B.; Richter, M.; Schüler, M.; Pinhassi, J.; Acinas, S.G.; González, J.M.; Pedrós-Alió, C. Ecology of marine bacteroidetes: A comparative genomics approach. *ISME J.* **2013**, *7*, 1026–1037. [CrossRef]
69. He, S.; Stevens, S.L.R.; Chan, L.-K.; Bertilsson, S.; Glavina del Rio, T.; Tringe, S.G.; Malmstrom, R.R.; Mcmahon, K.D. Ecophysiology of Freshwater Verrucomicrobia Inferred from. *mSphere* **2017**, *2*, e00277-17. [CrossRef]
70. Palladino, G.; Rampelli, S.; Scicchitano, D.; Musella, M.; Quero, G.M.; Prada, F.; Mancuso, A.; Seyfarth, A.M.; Turroni, S.; Candela, M.; et al. Impact of marine aquaculture on the microbiome associated with nearby holobionts: The case of patella caerulea living in proximity of sea bream aquaculture cages. *Microorganisms* **2021**, *9*, 455. [CrossRef]
71. Tsikopoulou, I.; Lampa, M.; Tsiola, A.; Pitta, P.; Tsapakis, M.; Karakassis, I. Functional adaptations of benthic communities to organic matter enrichment at the edge of an allowable zone of effect (AZE). *Estuar. Coast. Shelf Sci.* **2021**, *262*, 107596. [CrossRef]
72. Quero, G.M.; Ape, F.; Manini, E.; Mirto, S.; Luna, G.M. Temporal Changes in Microbial Communities Beneath Fish Farm Sediments Are Related to Organic Enrichment and Fish Biomass Over a Production Cycle. *Front. Mar. Sci.* **2020**, *7*, 524. [CrossRef]
73. Qin, Q.L.; Xie, B.B.; Yu, Y.; Shu, Y.L.; Rong, J.C.; Zhang, Y.J.; Zhao, D.L.; Chen, X.L.; Zhang, X.Y.; Chen, B.; et al. Comparative genomics of the marine bacterial genus Glaciecola reveals the high degree of genomic diversity and genomic characteristic for cold adaptation. *Environ. Microbiol.* **2014**, *16*, 1642–1653. [CrossRef]
74. Bae, S.; Yoo, K. Microplastic contamination and microbial colonization in coastal area of Busan City, Korea. *Front. Mar. Sci.* **2022**, *9*, 1030476. [CrossRef]
75. Sinden, A.; Sinang, S. Cyanobacteria in aquaculture systems: Linking the occurrence, abundance and toxicity with rising temperatures. *Int. J. Environ. Sci. Technol.* **2016**, *13*, 2855–2862. [CrossRef]
76. Gavriilidou, A.; Gutleben, J.; Versluis, D.; Forgiarini, F.; Van Passel, M.W.J.; Ingham, C.J.; Smidt, H.; Sipkema, D. Comparative genomic analysis of Flavobacteriaceae: Insights into carbohydrate metabolism, gliding motility and secondary metabolite biosynthesis. *BMC Genom.* **2020**, *21*, 569. [CrossRef]
77. Kirchman, D.L. The ecology of Cytophaga-Flavobacteria in aquatic environments. *FEMS Microbiol. Ecol.* **2002**, *39*, 91–100. [CrossRef] [PubMed]
78. Habib, C.; Houel, A.; Lunazzi, A.; Bernardet, J.F.; Olsen, A.B.; Nilsen, H.; Toranzo, A.E.; Castro, N.; Nicolas, P.; Duchaud, E. Multilocus sequence analysis of the marine bacterial genus Tenacibaculum suggests parallel evolution of fish pathogenicity and endemic colonization of aquaculture systems. *Appl. Environ. Microbiol.* **2014**, *80*, 5503–5514. [CrossRef]
79. Bowman, J.P. Out from the Shadows—Resolution of the Taxonomy of the Family Cryomorphaceae. *Front. Microbiol.* **2020**, *11*, 795. [CrossRef] [PubMed]
80. Pohlner, M.; Dlugosch, L.; Wemheuer, B.; Mills, H.; Engelen, B.; Reese, B.K. The majority of active *Rhodobacteraceae* in marine sediments belong to uncultured genera: A molecular approach to link their distribution to environmental conditions. *Front. Microbiol.* **2019**, *10*, 659. [CrossRef] [PubMed]
81. Roda-Garcia, J.J.; Haro-Moreno, J.M.; Huschet, L.A.; Rodriguez-Valera, F.; López-Pérez, M. Phylogenomics of SAR116 Clade Reveals Two Subclades with Different Evolutionary Trajectories and an Important Role in the Ocean Sulfur Cycle. *mSystems* **2021**, *6*, e00944-21. [CrossRef] [PubMed]

82. Asami, H.; Aida, M.; Watanabe, K. Accelerated sulfur cycle in coastal marine sediment beneath areas of intensive shellfish aquaculture. *Appl. Environ. Microbiol.* **2005**, *71*, 2925–2933. [CrossRef]
83. Needham, D.M.; Fichot, E.B.; Wang, E.; Berdjeb, L.; Cram, J.A.; Fichot, C.G.; Fuhrman, J.A. Dynamics and interactions of highly resolved marine plankton via automated high-frequency sampling. *ISME J.* **2018**, *12*, 2417–2432. [CrossRef] [PubMed]
84. Khalifa, S.A.M.; Shedid, E.S.; Saied, E.M.; Jassbi, A.R.; Jamebozorgi, F.H.; Rateb, M.E.; Du, M.; Abdel-Daim, M.M.; Kai, G.Y.; Al-Hammady, M.A.M.; et al. Cyanobacteria—From the oceans to the potential biotechnological and biomedical applications. *Mar. Drugs* **2021**, *19*, 241. [CrossRef] [PubMed]
85. Lürling, M.; Eshetu, F.; Faassen, E.J.; Kosten, S.; Huszar, V.L.M. Comparison of cyanobacterial and green algal growth rates at different temperatures. *Freshw. Biol.* **2013**, *58*, 552–559. [CrossRef]
86. Flombaum, P.; Gallegos, J.L.; Gordillo, R.A.; Rincón, J.; Zabala, L.L.; Jiao, N.; Karl, D.M.; Li, W.K.W.; Lomas, M.W.; Veneziano, D.; et al. Present and future global distributions of the marine Cyanobacteria Prochlorococcus and Synechococcus. *Proc. Natl. Acad. Sci. USA* **2013**, *110*, 9824–9829. [CrossRef]
87. Santic, N.; Solic, M.; Kuspilic, G.; Kuspilic, D. Distribution of Synechococcus and Prochlorococcus in the central Adriatic Sea. *Acta Adriat.* **2011**, *52*, 101–114.
88. Makris, C.; Galiatsatou, P.; Tolika, K.; Anagnostopoulou, C.; Kombiadou, K.; Prinos, P.; Velikou, K.; Kapelonis, Z.; Tragou, E.; Androulidakis, Y.; et al. Climate change effects on the marine characteristics of the Aegean and Ionian Seas. *Ocean Dyn.* **2016**, *66*, 1603–1635. [CrossRef]