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# How Much Recurrent Outbreaks of the Moon Jellyfish May Impact the Dynamics of Bacterial Assemblages in Coastal Lagoons?

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**Abstract:** The moon jellyfish *Aurelia coerulea* (Scyphozoa) is one of the most common and largest jellyfish inhabiting coastal lagoons, confined bays, and marinas of temperate and subtropical coastal waters. The annual population dynamics of *A. coerulea* along with some bacterial parameters (bacterial size and biomass, total coliforms, faecal coliforms, intestinal enterococci, culturable *Vibrio* spp., and culturable bacteria at 37 °C), sea surface temperature (SST), salinity, and an array of nutrients (ammonia, nitrites, nitrates, phosphates, silicates, total nitrogen, and total phosphorus) were assessed in the Varano lagoon (Adriatic Sea) that is subject to anthropogenic pollution. Statistical analyses revealed that jellyfish outbreaks and their consequent biomass deposition significantly correlated to seawater temperature, total nitrogen, phosphates, and ammonia concentrations while negative correlations appeared with nitrite and nitrate concentrations. In addition, bacterial biomass and *Vibrio* abundance correlated with each other and temperature, jellyfish density, and total nitrogen. These findings suggest that environmental changes could trigger the occurrence of jellyfish bursts in the lagoon which, in turn, may act as one of the central drivers of processes regulating some bacterial components. The positive relationship between jellyfish flush-and-crash dynamics and SST suggests that ongoing global warming will seemingly increase jellyfish outbreaks.

**Keywords:** jellyfish blooms; microbial community; coastal systems

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## 1. Introduction

Over the recent decades, growing evidence suggests gelatinous zooplankton is on the rise in many coastal areas, frequently with massive population outbreaks [1,2]. Possible explanations alternatively invoke possible causes, including climate change, eutrophication, overfishing or the removal of top predators from trophic webs, and invasions (e.g., [3–13]). Gelatinous predators consume zooplankton, especially crustaceans and ichthyoplankton (fish eggs and larvae) as well as juvenile fish, thus decreasing food availability for fish. Consequently, gelatinous plankton has an indisputable impact on fisheries [1,14]. Due to their total abundance and bloom-and-bust population dynamics, jellyfish are likely to affect carbon (C), nitrogen (N), and phosphorus (P) cycling in the ecosystems they live in, with direct and indirect effects on bacterioplankton [15–17]. Jellyfish obtain C, N, and P by assimilating organic nutrients from consumed prey, taking up small quantities of dissolved organic compounds, and some species actively take up dissolved inorganic ones. Some ingested elements become incorporated into their biomass, and the undigested amount is released via 'sloppy feeding' or egested as faeces. Organic quantities of C and N are processed into the environment as mucus, and both organic and inorganic metabolic compounds are

excreted [15]. During outbreaks, jellyfish populations act as a sink for C, N, and P as they increase in size, [18]. In particular, the biomasses of jellyfish produce a huge amount of colloidal and dissolved organic matter (jelly-DOM) [19], which is discharged into the seawater available for bacterioplankton [20,21], consequently impacting bacterial growth as well as extracellular enzymatic activities [16,22,23]. The biochemical features of the DOM released by jellyfish are poorly investigated, although it is likely to contain some polysaccharides and other C-rich compounds [24,25] highly bioavailable to heterotrophic bacteria [15,16,26]. Bacteria are the main consumers of organic matter in a marine environment, and the carbon cycle is largely affected by this remineralization of biomass [27–30]. Thus, the repackaging of gelatinous organic carbon due to the bacterial use of jelly-DOM could lead to reincorporation into the food web [31]. In addition, the gelatinous biomasses following bloom events lead to a rapid and massive release of dissolved inorganic and organic compounds which are ready to be consumed by pelagic microbial communities [23,32]. Moreover, decaying organic matter falls to the seafloor [33] and is used by both benthic suspension and detritus feeders [34,35]. Jellyfish carcasses, characterized by proteinaceous substances, a low C:N ratio, and the absence of a hard exoskeleton, represent a high-quality source of specific pelagic bacteria and alter the functioning of trophic webs [23,36,37]. Indeed, during jellyfish outbreaks or biomass depositions, changes in trophic relations with higher trophic levels have been observed, leading to implications for carbon, nitrogen, and phosphorus cycles [18,19,22,23,31,32,36–40]. Yet, the key players and degradation process of microbial jellyfish detrital organic matter (jelly-OM) and their effects on marine ecosystems remain unclear. However, few studies investigated the links between jellyfish and bacterioplankton metabolisms and structures. An experimental study was carried out to analyse the consequences of jellyfish outbreaks on the microbial planktonic assemblages in the marine lake of Mljet Island (Big Lake, Southern Adriatic), which is populated by the endemic scyphomedusa *Aurelia relicta*. The addition of gelatinous biomass to experimental in situ enclosures revealed that bacterial productivity doubled when the jellyfish were added, boosting large bacterial biomass [36,41]. In a recent study [42] on the biomass deposition of another alien jellyfish in the Adriatic Sea, *Aurelia solida*, it was estimated that about 100 mg of jelly-OM $L^{-1}$ became available. A conglomerate of bacteria belonging to the genera *Alteromonas*, *Pseudoalteromonas*, and *Vibrio* are jointly involved to degrade the labile jelly-OM. These bacteria represented >90% of all metabolically active jelly-OM consumers, displaying high bacterial growth efficiencies.

In 2000, the invasive jellyfish *Aurelia coerulea* (von Lendenfeld, 1884) entered the Adriatic coastal sound of Varano (Mediterranean Sea, Italy), turning into a key component of the present-day lagoonal biota. The coastal lake of Varano is a euryhaline, brackish ecosystem affected by anthropogenic stressors. During the recent decades, the growing increase in water temperatures has triggered frequent and massive jellyfish outbreaks [43]. In light of these events, we evaluated the annual proliferation of *Aurelia coerulea* medusae, the consequent deposition of their gelatinous biomass (jelly-fall) as well as some bacterial components of the coastal lagoon of Varano. In particular, over the course of a year, we assessed bacterial biomass and the abundances of total coliforms, faecal coliforms, intestinal enterococci, culturable vibrios, and culturable bacteria at 37 °C (including human potential pathogens) along with an array of seawater chemical variables (concentrations of ammonia, nitrites, nitrates, phosphates, silicates, total nitrogen, and total phosphorus) and abiotic factors (temperature and salinity). This work aims to provide a dataset for the evaluation of the potential dynamic impact of jellyfish biomass on some aquatic bacterial parameters (i.e., culturable bacteria at 37 °C, *Vibrio* spp., enterococci, faecal and total coliforms, and bacterial biomass). Contributing to the understanding of the coupling between bacterioplankton and jellyfish dynamics may provide a hint to better recognize changes in trophic pathways and unravel the overall impact of jellyfish outbreaks on the ecology of coastal areas.

## 2. Materials and Methods

### *2.1. Sampling Area*

Varano lagoon is the largest coastal lake in southern Italy. It is situated on the southern Adriatic coast (Apulia, Italy) to the north of the Gargano (Figure 1). This lagoon is characterized by brackish water, and it extends over a 60.5 $km^2$ area with a perimeter of 33 km. The average depth is 4 m with maximum value of 7 m in the central zone. Separated from the Adriatic Sea by a tongue of sand 10 km long, it is connected with open sea through two channels, respectively called Foce Varano and Foce Capoiale (Figure 1). Salinity values are relatively homogeneous for a lagoon, never dropping below 20 psu. Temperature range falls between 5 °C and 30 °C.

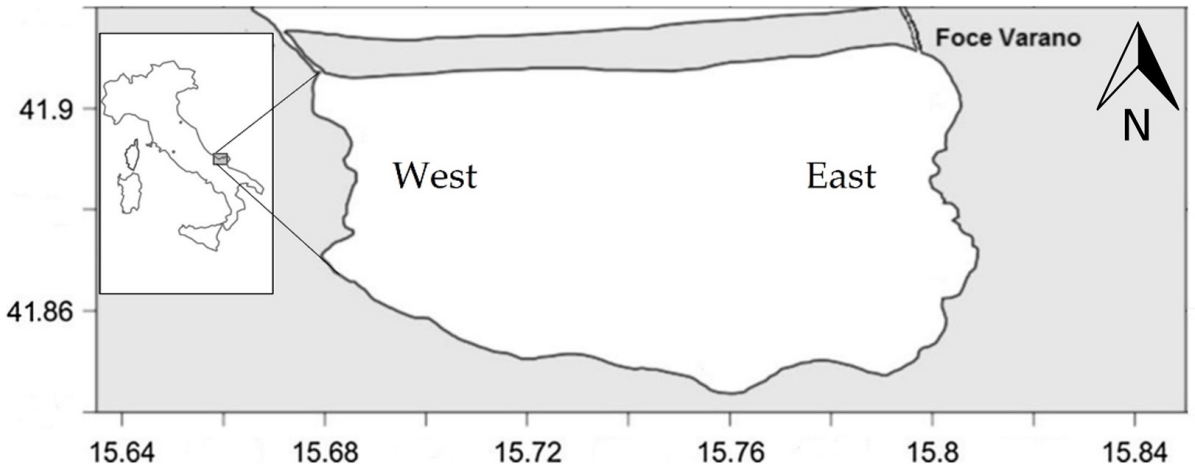


**Figure 1.** Study area: Varano lagoon with W and E zones.

### *2.2. Sample Collection*

Sampling was conducted over the course of a year in the Varano lagoon in four sampling times (February, May, September, and December). During this sampling period, a bloom of *Aurelia coerulea* occurred from February to May followed by the presence of jelly-falls from June to September. Water samples were collected in two zones of the lagoon (Figure 1), designated west (W) and east (E). In each sampling time, zone salinity and seawater temperature were recorded by using a multiparametric probe Ocean Seven 401 (Jolzonant, Italy). For the microbiological analyses, one-litre water sample was collected in each zone in triplicate by a Niskin bottle (General Oceanics, Inc., St. Miami, FL, USA) to a depth of about 50 cm from the surface. The samples were transported on ice and processed for enumeration and isolation of bacteria within 4 h of sampling.

### *2.3. Nutrients in the Water Column*

For the nutrient analysis, additional water samples were collected for each replica with a Niskin bottle of 1 L. For dissolved inorganic nutrients (ammonia $NH_3$-N, nitrite $NO_2$-N, nitrate $NO_3$-N, silicate Si-$SiO_2$, phosphate P-$PO_4$, total nitrogen N, and total phosphorus P), the samples were filtered onboard on precombusted Whatman GF/F filters and kept frozen (−20 °C) until laboratory analysis. Dissolved inorganic nutrients were determined with a segmented flow Bran + Luebbe AutoAnalyzer 3 following standard colorimetric methods [44].

### *2.4. Jellyfish Density*

To assess the jellyfish population density in the lagoon of Varano, individuals of *Aurelia coerulea* were collected using a net with diameter of 1 m and mesh of 1 cm, including a flowmeter model 438-110 (Hydro-Bios). The net was linked to the boat and dragged for 2 min. This operation was replicated 3 times in each zone (W and E). The jellyfish in the

net were counted, subsequently, in the laboratory; the density of population for both zones was calculated.

### *2.5. Total Bacterial Biomass*

Seawater samples for bacterial biomass were fixed with formaldehyde (0.2 µm prefiltered, 2% final concentration) and stored at 4 °C. Bacterioplankton counts were accomplished using a Zeiss Standard Axioplan microscope equipped with a halogen lA (Hg 100) light. Duplicate slides were displayed from each sample by filtering 1 mL of seawater into black polycarbonate filters Millipore (0.2 µm pore) using DAPI (4′,6-diamino-2-phenylindole, 1 µg $mL^{-1}$ final, Sigma) as fluorochrome [45]. AG 365 excitation filter, an FT 395 chromatic beam splitter, and an LP 420 barrier filter were used. At least 40 microscopic fields were counted for each preparation at 1000× magnification. Cell size of bacterioplankton was assessed by epifluorescence microscopy using microphotographs. Each cell size was estimated after projection on a screen, and at least 60 cells per filter were measured manually. Bacterioplankton cells were subdivided into three size classes: small, medium, and large (<0.065, 0.065–0.320, and 0.320–0.780 $\mu m^3$) [46]. Bacterioplankton biovolume was transformed into biomass assuming a carbon content of 310 fg C$\mu m^{-3}$ [47].

### *2.6. Culturable Bacteria*

#### 2.6.1. Culturable Vibrios

To count the culturable vibrios in seawater, 1, 5, and 10 mL of seawater were filtered through 0.22 µm pore size filters; then the filter disks were aseptically placed onto triosulphate–citrate–bile–sucrose–salt (TCBS) agar. Incubation at both 20–25 and 35 °C for 2 days was performed and *Vibrio* colonies were counted according to the colony-forming unit (CFU) method. The incubation temperature of 35 °C was chosen to assess vibrios potentially pathogenic to humans. The incubation temperature of 20–25 °C was chosen since some *Vibrio* spp. (e.g., *Vibrio anguillarum*) do not grow well at higher temperatures [48]. Mean values for three replicate samples were determined and expressed as CFU/mL considering the dilution factor.

#### 2.6.2. Culturable Bacteria at 37 °C

Estimations of culturable bacteria at 37 °C (including human potential pathogens) in seawater samples were performed by the spread plate method using Bacto Plate Count Agar medium (PCA, seeding with 0.1 mL of sample). The plates were incubated at 37 °C for 48 h.

#### 2.6.3. Microbial Pollution Indicators

To evaluate the microbial water quality, standard methods (e.g., ISO—the International Organization for Standardization) were performed. In particular, total coliforms and faecal coliforms as well as faecal enterococci were assessed by the Most Probable Number (MPN) method using the standard five-tube method of ten-fold dilutions for seawater samples [49]. Coliform bacteria concentration was calculated by using the miniaturized MPN [50]. Faecal enterococci were measured by using the miniaturized MPN method (incubation at 44 °C for 24–48 h) [51]. Results were expressed as MPN 100 $mL^{-1}$.

### *2.7. Statistical Analyses*

The differences in (i) environmental variables (i.e., temperature, salinity), (ii) nutrients (i.e., ammonia, nitrites, nitrates, phosphates, silicates, total nitrogen, and total phosphorus), (iii) jellyfish density, (iv) bacterial biomass, and (v) culturable bacteria (culturable bacteria at 37 °C, *Vibrio* spp., faecal enterococci, and coliforms) were assessed in W and E zones at several sampling times (T1 = February, T2 = May, T3 = September, T4 = December) by univariate and multivariate analyses. PERMANOVA analyses were based on Euclidean distances of untransformed data using 9.999 random permutations [52]. When significant differences were encountered ($p < 0.05$), posthoc pairwise tests for the fixed factor were

performed. Because of the restricted number of unique permutations in the pairwise tests, *p*-values were obtained from Monte Carlo samplings. Pearson's correlation matrices and *p*-values were calculated by performing linear regressions between investigated variables. Differences were plotted through multidimensional scaling (MDS) plot. All analyses were performed using the software PRIMER v. 6 including the PERMANOVA þ add-on package [53,54].

## 3. Results

### 3.1. Abiotic Parameters

The recorded temperatures ranged from 7 to 25 °C, varying significantly among the sampling times (Table 1) in the two zones of the lagoon designated W and E. September was the warmest sampling time with a mean value of 25 ± 0.03 °C (Figure 2) followed by May (17.41 ± 0.15 °C).

**Table 1.** Results of the permutational analyses (PERMANOVA) on water salinity, temperature, and jellyfish density in the W and E zones (Z) among sampling times (T). Abbreviations: Res—residual; Tot—total; df—degrees of freedom; MS—mean squares; Pseudo-F—F critic; P—permutational level of probability. *** $p < 0.001$; ns—not significant.

| | | **Temperature** | | | **Salinity** | | | **Jellyfish Density** | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Source | df | MS | Pseudo-F | *p* | MS | Pseudo-F | *p* | MS | Pseudo-F | *p* |
| T | 3 | 446.67 | 8847.70 | *** | 13.03 | 1004.20 | *** | 258.47 | 1391.10 | *** |
| Z | 1 | 0.01 | 0.10 | ns | 0.03 | 1.07 | ns | 60.34 | 1.07 | ns |
| T × Z | 3 | 0.11 | 2.09 | ns | 0.03 | 2.41 | ns | 56.33 | 303.17 | *** |
| Res | 16 | 0.05 | | | 0.01 | | | 0.19 | | |
| Tot | 23 | | | | | | | | | |

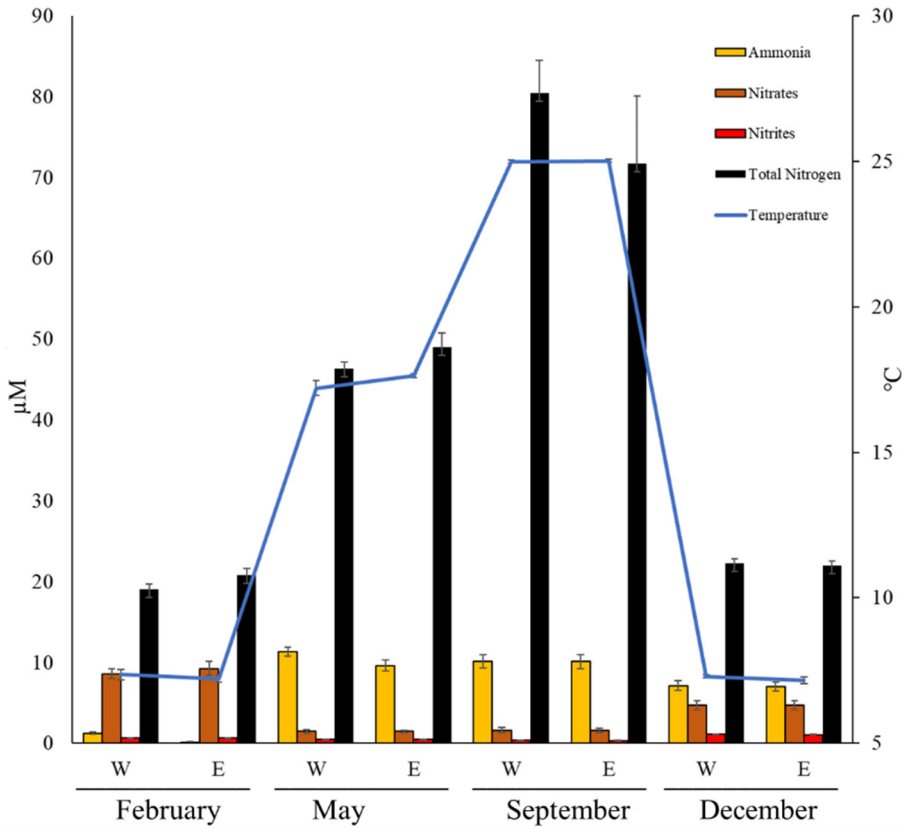


**Figure 2.** The seawater temperature and nutrient variables (ammonia, nitrites, nitrates, and total nitrogen) characterizing the water columns of the W and E zones during the sampling times.

Water salinity ranged from 25 to 28 ppm, varying significantly among the sampling times (Table 1), but no significant differences were found between the two examined zones (Figure 3). The highest values of salinity were observed in September (28.08 ± 0.03 ppm), and the lowest ones were in February (25.17 ± 0.09 ppm, Figure 3).

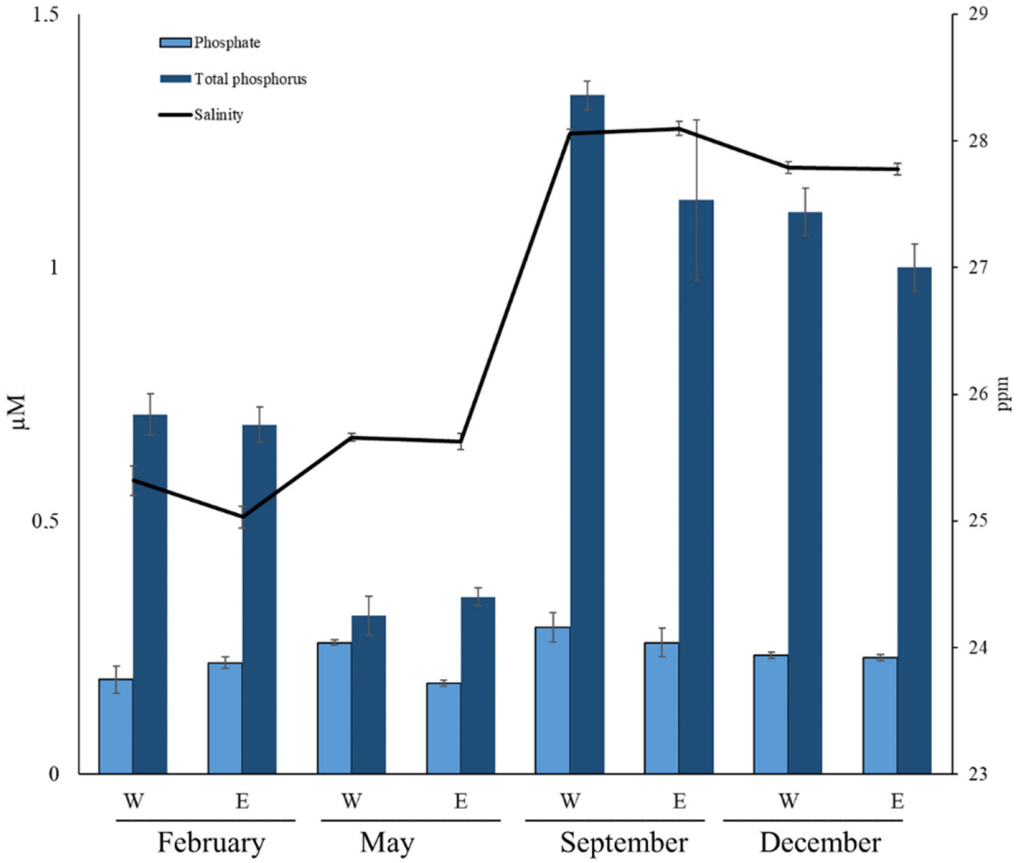


**Figure 3.** The seawater salinity and nutrient variables (phosphates and total phosphorus) characterizing the water column of the W and E zones during the sampling times.

### *3.2. Inorganic Nutrients*

The trends of the examined inorganic nutrients are shown in Figure 2, Figure 3, and Figure S1. The concentration of ammonia, nitrite, nitrate, total nitrogen, and total phosphorus varied significantly during the whole year, as shown by the univariate PERMANOVA (Table 2). High values of ammonia were recorded in May during the jellyfish bloom (10.46 ± 0.56 µM) whilst the lowest values were detected in February (0.69 ± 0.26 µM) (Figure 2). The highest concentrations of nitrite were observed in December with values of 1.09 ± 0.004 and the lowest in February. Nitrate concentrations were high in February (8.92 ± 0.49 µM) and December (4.71 ± 0.37 µM), and no differences were evidenced between the two investigated zones (Table 2, Figure 2). The highest total nitrogen concentrations were observed in September during the period of jelly-falls (Figure 2).

**Table 2.** Results of the permutational analyses (PERMANOVA) on ammonia, nitrates, nitrites, total nitrogen, phosphates, total phosphorus, and silicates in the water columns of W and E zones (Z) among sampling times (T). Abbreviations: Res—residual; Tot—total; df—degrees of freedom; MS—mean squares; Pseudo-F—F critic; P—permutational level of probability. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; ns—not significant.

| | | **Ammonia** | | | **Nitrites** | | | **Nitrates** | | | **Total Nitrogen** | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Source | df | MS | Pseudo-F | *p* | MS | Pseudo-F | *p* | MS | Pseudo-F | *p* | MS | Pseudo-F | *p* |
| T | 3 | 123 | 109.46 | *** | 0.66 | 221.69 | *** | 73.27 | 98.52 | *** | 4141.60 | 119.38 | *** |
| Z | 1 | 3.34 | 3.40 | ns | $4.59 \times 10^{-4}$ | 0.20 | ns | 0.12 | 0.16 | ns | 8.17 | 0.20 | ns |
| T × Z | 3 | 0.98 | 0.88 | ns | $2.32 \times 10^{-3}$ | 0.78 | ns | 0.17 | 0.22 | ns | 40.56 | 1.17 | ns |
| Res | 16 | 1.12 | | | $2.97 \times 10^{-3}$ | | | 0.74 | | | 34.69 | | |
| Tot | 23 | | | | | | | | | | | | |
| | | **Phosphates** | | | **Total Phosphorus** | | | **Silicates** | | | | | |
| Source | df | MS | Pseudo-F | *p* | MS | Pseudo-F | *p* | MS | Pseudo-F | *p* | | | |
| T | 3 | $5.63 \times 10^{-3}$ | 5.60 | ** | 0.96 | 73.355 | *** | 2235.30 | 2780.00 | *** | | | |
| Z | 1 | $2.50 \times 10^{-3}$ | 0.74 | ns | 0.03 | 1.9821 | ns | 2.09 | 4.28 | ns | | | |
| T × Z | 3 | $3.38 \times 10^{-3}$ | 3.37 | * | 0.02 | 1.2978 | ns | 0.49 | 0.61 | ns | | | |
| Res | 16 | $1.00 \times 10^{-3}$ | | | 0.01 | | | 0.80 | | | | | |
| Tot | 23 | | | | | | | | | | | | |

The univariate PERMANOVA revealed that the phosphate concentration varied significantly during the jellyfish bloom (May) between the investigated zones ($p < 0.05$, t = 9.80). The highest phosphate concentrations were found in September during the jelly-falls (0.27 ± 0.02 µM). The concentration of total phosphorus significant differences was recorded during the different sampling times (Table 2, Figure 3), and in particular, high concentrations were evidenced in September during the jellyfish deposition (1.24 ± 0.09 µM).

As reported in Table 2 and Figure S1, the concentration of silicates showed a marked difference throughout the year, as evidenced by the univariate PERMANOVA. The highest concentrations were found in September (46.34 ± 0.40 µM) while the lowest values were in May (0.87 ± 0.26 µM).

### *3.3. Jellyfish Density*

In February, specimens of *Aurelia coerulea* were present with a low density in the W zone. A jellyfish bloom was recorded in May when the highest density of *A. coerulea* was recorded as 12.39 ind $m^{-3}$ in the W zone (Figure S2). The abundance of jellyfish was so high that in the E zone, some individuals were also present. In September, there were a great number of jelly-falls in both the investigated zones while in December, there were no live jellyfish in the lagoon.

Significant positive correlations between the jellyfish density and seawater temperature (R = 0.86), total nitrogen (R = 0.85), phosphates (R = 0.67), and ammonia (0.65) concentrations were observed while negative correlations between the jellyfish density and nitrites (R= −0.70) and nitrates (R= −0.65) were recorded (Table 3).

**Table 3.** Pearson's correlation matrix for environmental, biochemical, and biological variables is presented. Number of samples = 24. In red, the correlations show statistical significance ($p < 0.05$).

| | Temperature | Salinity | Jellyfish Density | Ammonia | Nitrates | Nitrites | Total Nitrogen | Phosphates | Total Phosphorus | Silicates | Culturable Bacteria at 37 °C | *Vibrio* spp. | Enterococci | Total Coliforms | Faecal Coliforms |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Salinity | 0.38 | | | | | | | | | | | | | | |
| Jellyfish Density | 0.86 | 0.40 | | | | | | | | | | | | | |
| Ammonia | 0.73 | 0.50 | 0.65 | | | | | | | | | | | | |
| Nitrates | −0.78 | −0.47 | −0.65 | −0.91 | | | | | | | | | | | |
| Nitrites | −0.82 | 0.15 | −0.70 | −0.35 | 0.48 | | | | | | | | | | |
| Total Nitrogen | 0.97 | 0.44 | 0.85 | 0.69 | −0.74 | −0.76 | | | | | | | | | |
| Phosphates | 0.47 | 0.51 | 0.67 | 0.50 | −0.34 | −0.17 | 0.49 | | | | | | | | |
| Total Phosphorus | 0.20 | 0.81 | 0.26 | 0.10 | −0.01 | 0.20 | 0.28 | 0.52 | | | | | | | |
| Silicates | 0.09 | 0.57 | 0.20 | −0.27 | 0.27 | 0.08 | 0.18 | 0.34 | 0.86 | | | | | | |
| Culturable bacteria at 37 °C | 0.13 | 0.89 | 0.15 | 0.34 | −0.24 | 0.40 | 0.21 | 0.45 | 0.81 | 0.54 | | | | | |
| *Vibrio* spp. | 0.84 | 0.59 | 0.80 | 0.43 | −0.53 | −0.63 | 0.87 | 0.44 | 0.53 | 0.54 | 0.34 | | | | |
| Enterococci | −0.52 | 0.50 | −0.42 | 0.00 | 0.08 | 0.88 | −0.46 | 0.02 | 0.36 | 0.12 | 0.66 | −0.34 | | | |
| Total coliforms | −0.43 | −0.27 | −0.35 | −0.54 | 0.54 | 0.17 | −0.43 | −0.40 | −0.02 | 0.18 | −0.25 | −0.27 | −0.02 | | |
| Faecal coliforms | −0.43 | −0.28 | −0.35 | −0.54 | 0.54 | 0.17 | −0.43 | −0.40 | −0.02 | 0.18 | −0.25 | −0.27 | −0.02 | 1.00 | |
| Bacterial biomass | 0.86 | 0.54 | 0.83 | 0.44 | −0.47 | −0.66 | 0.87 | 0.58 | 0.58 | 0.58 | 0.33 | 0.94 | −0.41 | −0.22 | −0.22 |

### *3.4. Bacterial Biomass*

The bacterial biomass varied significantly throughout the year, as shown by the univariate PERMANOVA (Table 4). In May, a significant increase ($p < 0.05$; t = 6.96) in the bacterial biomass was observed in the W zone (Figure S3). In September, simultaneously with the jellyfish deposition in the lagoon, the bacterial biomass was 1404.03 ± 27.45 µgC $L^{-1}$. Low values were found in December (16.70 ± 0.39 µgC $L^{-1}$). The large-sized bacteria represented 40–57% of the total biomass while the medium and small bacterial sizes were 34–46% and 7–19%, respectively. Significant positive correlations between the bacterial biomass and the jellyfish density (R = 0.83), salinity (R = 0.54), temperature (R = 0.86), silicates (R = 0.58), total phosphorus (R = 0.58), total nitrogen (R = 0.87), phosphates (R = 0.58), and ammonia (R = 0.44) concentrations were observed while the bacterial biomass correlated negatively with nitrites (R = −0.66) and nitrates (R = −0.47) (Table 3).

**Table 4.** Results of the permutational analyses (PERMANOVA) on bacterial biomass, *Vibrio* spp., culturable bacteria at 37 °C, faecal enterococci, and faecal and total coliforms in the W and E zones (Z) among sampling times (T). Abbreviations: Res—residual; Tot—total; df—degrees of freedom; MS—mean squares; Pseudo-F—F critic; P—permutational level of probability. *** $p < 0.001$; ns—not significant.

| | | **Bacterial Biomass** | | | **Culturable Bacteria at 37 °C** | | | ***Vibrio* spp.** | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Source | df | MS | Pseudo-F | *p* | MS | Pseudo-F | *p* | MS | Pseudo-F | *p* |
| T | 3 | $2.50 \times 10^6$ | 1364.60 | *** | $2.66 \times 10^7$ | 34.96 | *** | $3.73 \times 10^5$ | 68.48 | *** |
| Z | 1 | 7203.10 | 1.83 | ns | $1.48 \times 10^5$ | 1.73 | ns | 620.17 | 2.26 | ns |
| T × Z | 3 | $3.93 \times 10^3$ | 2.14 | ns | 85698.00 | 0.11 | ns | 274.35 | 0.05 | ns |
| Res | 16 | 1835 | | | $7.62 \times 10^5$ | | | 5441.60 | | |
| Tot | 23 | | | | | | | | | |
| | | **Faecal Enterococci** | | | **Total Coliforms** | | | **Faecal Coliforms** | | |
| Source | df | MS | Pseudo-F | *p* | MS | Pseudo-F | *p* | MS | Pseudo-F | *p* |
| T | 3 | 4575.20 | 115.58 | *** | 95795 | 101.75 | *** | 94661 | 112.43 | *** |
| Z | 1 | 1.50 | 27.00 | ns | $1.19 \times 10^5$ | 1.03 | ns | $1.17 \times 10^5$ | 1.03 | ns |
| T × Z | 3 | 0.06 | $1.40 \times 10^{-3}$ | ns | $1.16 \times 10^5$ | 123.05 | *** | $1.14 \times 10^5$ | 135.35 | *** |
| Res | 16 | 217.08 | | | 941.46 | | | 841.92 | | |
| Tot | 23 | | | | | | | | | |

### *3.5. Culturable Bacteria*

#### 3.5.1. Culturable Bacteria at 37 °C

The trend of culturable bacteria at 37 °C, including potential pathogenic bacteria, is presented in Figure S4. Significant differences in the density of these bacteria were detected among the sampling times (Table 4). In particular, high abundances were recorded in December ($4.56 \pm 0.40 \times 10^3$ CFU/mL) and September during the jellyfish deposition ($3.81 \pm 0.91 \times 10^3$ CFU/mL). The potentially pathogenic bacteria abundance correlated positively with salinity (R = 0.89), silicates (R = 0.54), total phosphorus (R = 0.81), and phosphates (R = 0.45) concentrations (Table 3).

#### 3.5.2. Culturable Vibrios

*Vibrio* abundance varied significantly throughout the year (Table 4), reaching a value of $511.67 \pm 51.94$ CFU/mL in September (Figure S5). *Vibrio* spp. density correlated positively with jellyfish density (R = 0.80), salinity (R = 0.59), temperature (R = 0.84), silicates (R = 0.54), total phosphorus (R = 0.53) total nitrogen (R = 0.87) concentrations, and bacterial biomass (R = 0.94); by contrast, it correlated negatively with nitrite (R = −0.63) and nitrate (R = −0.53) concentrations (Table 3).

#### 3.5.3. Microbial Pollution Indicators

The concentrations of faecal enterococci in the sampling times are reported in Figure S6. The density of these microorganisms was low throughout the year, ranging from 0 to 5 MPN/100 mL except for December when a mean value of $57.17 \pm 4.51$ MPN/100 mL was recorded (Table 4).

The counting of faecal enterococci correlated positively with salinity (R = 0.50), nitrite concentration (R = 0.88), and culturable bacteria at 37 °C (R = 0.66); however, it correlated negatively with the temperature (R = −0.52), jellyfish density (R = −42), and the total nitrogen concentration (R = −0.46) (Table 3).

#### 3.5.4. Total and Faecal Coliforms

Total and faecal coliforms showed abundances significantly higher in the February zone (t = 20.68, $p < 0.05$ and t = 22.46; $p < 0.05$) in the W zone ($558.33 \pm 26.97$ and $553.67 \pm 24.63$ MPN /100 mL) when compared with the values found in the E zone (Figure 4, Table 4). Coliform densities correlated positively with nitrate (R = 0.54) and

negatively with the temperature (R = −0.43), ammonia (R = −54), and the total nitrogen concentration (R = −0.43) (Table 3).

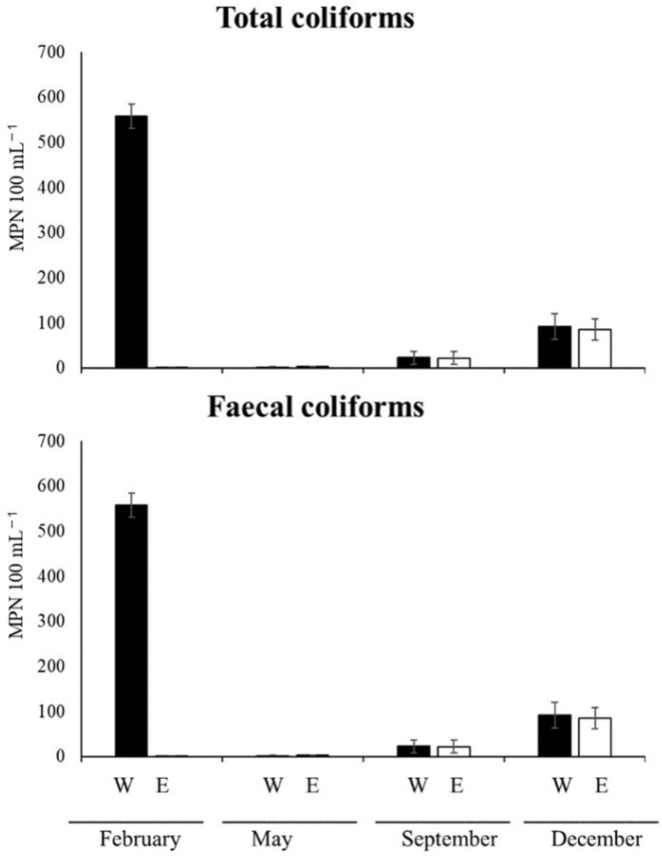


**Figure 4.** Density and size composition of *Aurelia coerulea* in W and E zones during the sampling times.

### *3.6. Multivariate Analysis*

Multivariate PERMANOVA showed significant differences in the composition of nutrients among sampling times (Table 5). As shown in Figure 5, the jellyfish outbreak in May appears to be related to a high concentration of ammonia while the jelly-fall in September is characterized by high phosphate values, high bacterial biomass, and high *Vibrio* spp. density.

**Table 5.** Results of the permutational analyses (PERMANOVA) on nutrient composition (ammonia, nitrates, nitrites, total nitrogen, phosphates, total phosphorus, and silicates) in the water columns of W and E zones (Z) among sampling times (T). Abbreviations: Res—residual; Tot—total; df—degrees of freedom; MS—mean squares; Pseudo-F—F critic; P—permutational level of probability. *** $p < 0.001$; ns—not significant.

| | | Nutrient Composition | | |
|---|---|---|---|---|
| Source | df | MS | Pseudo-F | *p* |
| T | 3 | 46.62 | 55.63 | *** |
| Z | 1 | 1.74 | 0.87 | ns |
| T × Z | 3 | 2.00 | 2.39 | ns |
| Res | 16 | 0.84 | | |
| Tot | 23 | | | |

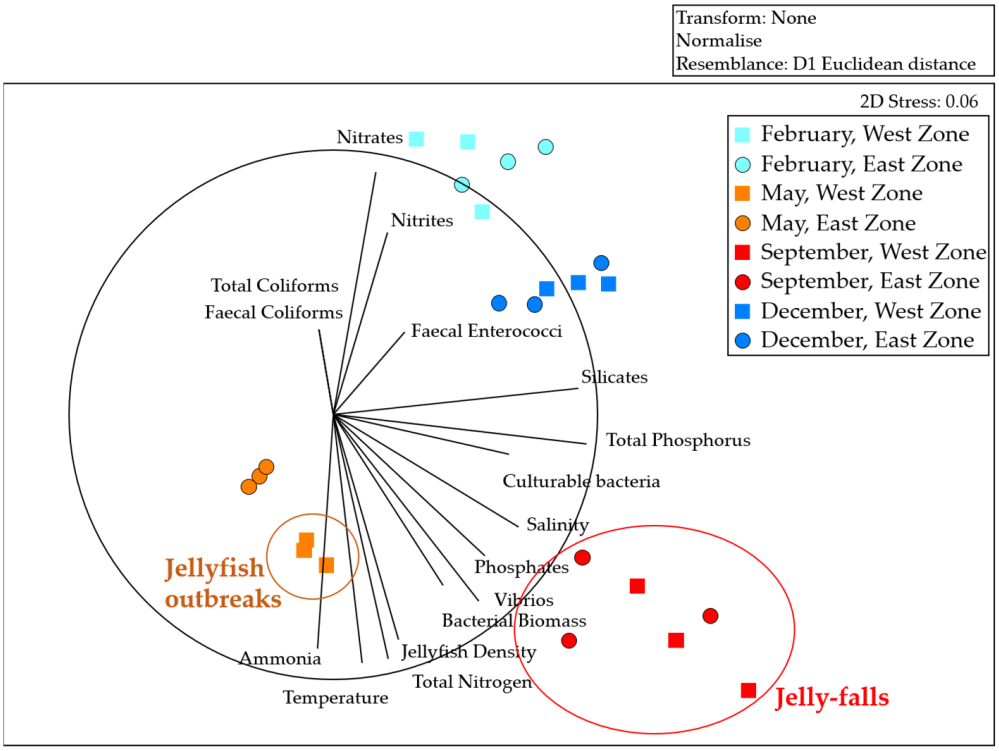


**Figure 5.** Multidimensional scaling (MDS) plot on Euclidean distances based on normalized data, showing nutrient concentrations in seawater columns along with environmental variables and bacteriological data from the W and E zones during the sampling times.

February and December were characterized by high values of nitrate and nitrite, paralleled by a high density of total/faecal coliforms (in February and faecal enterococci (in December). Culturable bacteria at 37 °C and total phosphorus showed high values both in September and December.

## 4. Discussion

In the present work, the occurrence of jellyfish proliferations and the subsequent deposition of gelatinous biomasses as well as some marine bacterial components and abiotic variables were investigated in a confined coastal environment (the brackish lake of Varano). Here, the links between jellyfish and bacterioplankton were investigated, contributing to improving the knowledge of their relationships and clarifying whether recurrent outbreaks of the moon jellyfish *Aurelia coerulea* could be one of the main drivers of bacterial assemblage changes in coastal lagoons.

This study corroborates the findings of a metagenomic study [38], showing that in the lagoon of Varano, the influence of jellyfish on the microbial compartment is mostly limited to the W zone, i.e., the area most populated by *A. coerulea* jellyfish. In fact, the greatest density of jellyfish was usually encountered in the western lagoonal area, first flanked by the northwestern coastal currents, corresponding to the W zone of the study area which is known as the most productive and populated area of the lagoon [55]. Seemingly, live jellyfish are not found in the eastern area because they can swim weakly against the eastward currents. Following a spring outbreak, senescence (a phase characterized by degenerating individuals with reduced bell pulsation rate and reduced and/or absent oral arms) occurs, and the medusae increasingly die (from June to September). Thus, organic carbon is released into the water, and nutrients from jelly-falls are carried eastward, then dispersed and homogenized from west to east across the lagoon. This phenomenon could explain the similar values of the bacterial biomasses, the culturable bacteria at 37 °C, and

the *Vibrio* and enterococci found in the W and E zones in September, as also indicated by the statistical analyses performed on some bacterial assemblages.

Further, the coastal lagoon of Varano is affected by seasonal temperature and salinity variations typical of confined coastal environments [56–58]. In the present work, a positive correlation between temperature and jellyfish density was found as well as between temperature, bacterial biomass, and *Vibrio* spp. densities. Temperature increases usually are directly correlated to an increase in bacterial density, biomass, and enzymatic activities [59,60]. Other environmental variables that covary with temperatures, such as nutrient concentration or primary productivity, may largely affect the bacterial abundance and activity [61,62]. The potential effects of these environmental factors on bacterial dynamics in the Varano lagoon have been particularly observed in September when an increase of bacterial biomass was observed in relation to a combination of high water temperatures and a conspicuous trophic load, also due to an increase of the recreational and touristic human activities throughout the summer months [63]. However, a large amount of gelatinous biomass from jelly-fall could be considered an additional input of organic matter that may contribute to the substantial bacterial biomass increase observed in September. Several studies indeed have demonstrated that show when jellyfish blooms settle on the seabed, jelly-OM becomes an abundant source of organic matter for microbial communities in coastal ecosystems [32,37,38,41]. Jelly-falls indeed represent a high-quality source for specific pelagic bacteria, modifying the diversity and functioning of marine trophic nets [23,36,37].

Moreover, live (healthy) jellyfish release abundant quantities of colloidal and labile-rich DOM through several mechanisms (i.e., sloppy feeding, egested material, mucus production, and excretion) [31,64,65]. However, it has been confirmed that DOM released by living jellyfish is fast respired rather than fuelled into biomass production by other species of the microbial community [31,38,66]. Moreover, the OM release following a massive jellyfish deposition should be even higher than the release from living jellyfish [18]. Interestingly, in accordance with these considerations, when *A. coerulea* was present at low density in the seawater (February) in the W zone, we also recorded low values of bacterial biomass. During the jellyfish outbreak (May) when the jellyfish density was higher in the W zone, the bacterial biomass was also significantly higher in this zone than in the E zone, as revealed by the pairwise test. In particular, in May, when *A. coerulea* reached a density of 12.39 individuals/$m^3$, a bacterial biomass of 235 μgC $L^{-1}$ was recorded in the W zone, suggesting that exudates (mucus) from jellyfish outbreaks might be one of the main key drivers of the microbial community. In addition, the highest bacterial biomass (1404 μgC $L^{-1}$) was recorded in September when jelly-falls were present in both the investigated zones.

Analysing the bacterial cell size distribution in the water of Varano lagoon, large-sized bacteria prevailed over medium-sized bacteria and small-size ones. This result is particularly remarkable since large bacteria are often metabolically active, suggesting that, as already reported by Tinta et al. [41,67], not all bacterial communities grew under the addition of jellyfish detrital matter. These authors demonstrated that in the jellyfish-degrading community, the opportunistic microbial species accounted for >90% of all metabolically active bacteria and quickly consumed almost the entire pool of jellyfish proteins (>98%), amino acids (70%), and jelly-DOC, showing a fast turnover of jellyfish-DOM and related soluble proteins. In particular, in their studies, other authors observed that the jellyfish-degrading bacterial conglomerate is composed of specific opportunistic bacteria. It is worth noting that the bacterial community rapidly shifted from a various coastal assemblage dominated by Alphaproteobacteria (a typical assemblage for the region [67]) to a community of low diversity made mainly of Gammaproteobacteria, Pseudoalteromonadaceae, Alteromonadaceae, and Vibrionaceae accounting for 86% of all Gammaproteobacteria. The observed structural shift was in accordance with previous research, consistently reporting a fast decrease of Alphaproteobacteria and an increase of Gammaproteobacteria developing on fresh and labile jellyfish detritus followed by a succession of Bacteroidetes growing

on complex and presumably less-labile jellyfish OM [36–38,66]. This suggests that a huge source of bioavailable DOM of high quality reduces the biodiversity of bacteria by favouring a small number of corticotrophs dominating the community [68]. By a metagenomic approach, in the lagoon of Varano [38], our research group has also identified changes in the planktonic microbial community under the influence of a jellyfish outbreak.

Significant differences between the two sampling areas designated as W and E differing in their jellyfish densities were particularly detected in the occurrence of 16 families, 22 genera, and 61 species of microbial taxa [38]. In the present study, we also observed in May in the W zone where jellyfish prevailed, a higher density of culturable *Vibrio* spp. was present compared to the E zone, suggesting that the growth of this bacterial genus is favoured by the increased availability of mucopolysaccharides and glycoproteins deriving from jellyfish tissues. However, the highest *Vibrio* spp. density was observed in September, in conjunction with the jellyfish deposition. Such a result is also in agreement with the data reported by Tinta et al. [36], who studied the response of the bacterial community to decomposing jellyfish biomass in two marine coastal environments, the Gulf of Trieste (northern Adriatic Sea) and Big Lake (Mljet Island, southern Adriatic Sea). The increased abundance of culturable *Vibrio* spp. in relation to jellyfish dynamics could have some epidemiological consequences for human and marine biota health since some bacteria, mainly belonging to the genus *Vibrio*, such as *V. alginolyticus*, *V. harveyi*, *Vibrio ordalii*, *V. parahaemolyticus*, *V. salmonicida*, and *V. vulnificus*, have been reported as the etiological agents in the most common shellfish and fish disease outbreaks, otherwise known as vibriosis [69–72]. Moreover, microbial accumulation in reared animal flesh may also become a severe threat to human health. In addition, wound infections, gastroenteritis, severe necrotizing infections of soft tissues, and fatal septicaemia (in debilitated patients) are known to be caused by some pathogenic vibrios in humans [73]. The increased density of culturable *Vibrio* spp., potentially in relation to jellyfish outbreaks, is also important considering that recent data have shown that *Vibrio*-associated illnesses are more frequent worldwide and mainly in Northern European countries after swimming/bathing activities in seawater [74–76]. In summary, our results suggest, as also reported by Basso et al. [15] and Tinta et al. [77], that jellyfish outbreaks and their subsequent deposition might change the functioning and community composition of marine food webs, ultimately also affecting human health via supporting the growth of potential pathogens.

In spite of the high domestic and agricultural pollution of the close terrestrial areas as well as the jellyfish outbreaks, the microbial pollution variables showed that the water quality of the Varano lagoon remains almost unaltered for several years, as previously observed by Caroppo [78]. In general, the recorded densities of the microbial pollution indicators are comparable with those observed in other lagoonal environments and previous studies on the Varano lagoon [78]. The total coliform and faecal coliform concentrations remained constant throughout the year except in February when the density of medusae was very low in the W zone; the observed coliform increase in that month could be related to meteorological phenomena and, in particular, to terrestrial runoff due to heavy rain precipitations in February. In this period, the highest concentrations of nitrates were recorded in both zones and, as already indicated by Specchiulli et al. [55], this increase may be related to terrestrial runoff during seasonal rain. Overall, jellyfish outbreaks in the lagoon are not linked to microbial pollution from land.

Regarding the seasonal dynamics and concentrations of inorganic nutrients in the investigated lagoon, the results obtained were in agreement with previous investigations of this area reporting fluctuations in nutrient concentrations and chemical–physical and biological parameters [55,58,79,80]. These studies on water columns, sediments, and biological features (organic matter, Chl-a, and phyto- and zooplankton) showed comparable conditions across the lagoon and, specifically, did not underline significant differences between E and W zones. Despite strong seasonal oscillations of most environmental variables (as is typical of confined coastal systems), the Varano lagoon seems to exhibit relatively homogeneous spatial, hydrological, and geomorphological features. However, the west-

ern area of the lagoon is characterized by numerous artificial structures for mussel seed farming. Artificial structures usually represent a preferential substrate for the settlement of planula larva followed by development into the polyp stage [81,82]. This could explain the spatial separation of jellyfish in the W zone in May, which might also be supported by the jellyfish conglomerate that was actively directed against the water flow [83]. In the present research, a significant positive correlation was found between jellyfish density and total nitrogen, phosphates, and ammonia. Jellyfish mucus excretions include dissolved free amino acids (Aas), dissolved organic nitrogen (DON), dissolved organic phosphorus, nucleosides, and purine compounds as well as inorganic nutrients, mainly ammonia (except for the urea) and phosphate [18]. Moreover, the total organic content deriving from jellyfish generally consists of proteins (72 ± 14%), lipids (22 ± 12%), and carbohydrates (7 ± 5%) [18,39,84,85]. In this framework, it is well-known that bacteria are capable of hydrolysing high-molecular-weight organic matter by means of extracellular enzymes, and the turnover rate of soluble proteins by bacteria in seawater is short [86,87] and similar to the turnover rate of free amino acids [88]. The final products are derived from the degradation of the protein flow into a pool of dissolved inorganic and organic nitrogen [89]. In relation to this suggested potential bacterial degradation of jellyfish, an increase in total nitrogen has been found in our study in September during the jellyfish deposition. Moreover, the highest values of ammonia concentrations were observed in May and September. Such a result is in agreement with the study of Tinta et al. [41], who investigated—under controlled laboratory conditions—the changes of pelagic microbial communities following the jellyfish biomass deposition as related to higher $NH_4$ and $PO_4^{3-}$ concentrations in jelly-OM treatments. In the present study, we recorded an increase of $PO_4^{3-}$ in the seawater in May and September in relation to high jellyfish numbers and the consequent occurrence of jelly-falls. This significant pulse of inorganic nutrients is a main source of perturbation for pelagic microbial assemblages, in particular, in oligotrophic or P-limited seawaters [90,91]. Accordingly, in the present study, a significant correlation was observed between *Vibrio* density and N and P concentrations, suggesting a potential shift in the bacterial community was driven by coastal eutrophication.

Conversely to ammonia, the highest values of nitrites and nitrates in the present investigation were recorded in the winter in the absence or low presence of jellyfish. High concentrations of nitrates in winter can be related to terrestrial runoff during seasonal rain precipitation [55]. A significant correlation was found only between faecal enterococci density and N-NO2. Particularly, high concentrations of nitrites and faecal enterococci in the December water samples revealed recent contamination from the land.

In conclusion, there is no correlation between faecal bacteria and jellyfish outbreaks and deposition. Instead, jellyfish outbreaks in coastal systems may represent an important but often overlooked source of organic carbon, exerting significant top-down control over the native microbial community and the functioning of aquatic ecosystems. Live jellyfish can drive changes in the structure of some bacterial components in the surrounding water column with the high production and release of DOM and inorganic nutrients, whereas the consequent gelatinous biomass deposition may locally represent a key detritus source controlling the phenology and dynamics of the lagoonal microbial community.

**Supplementary Materials:** The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/w14233908/s1, Figure S1: The silicates characterizing the water column of the W and E zones during the sampling times; Figure S2: Density of *Aurelia coerulea* or jelly-falls in the W and E, zones during the sampling times; Figure S3: Bacterial biomass and size composition in the seawater of the W and E zones during the sampling times; Figure S4: Culturable bacteria (at 37 °C) in W and E zones during the sampling times; Figure S5: *Vibrio* density in seawater column of W and E zones during the sampling times; Figure S6: Faecal enterococci in seawater column of W and E zones during the sampling times.

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**Data Availability Statement:** The data presented in this study are available on request from the authors.

**Conflicts of Interest:** The authors declare no conflict of interest.

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