

## Article

# Monitoring Portuguese Shellfish Continental Production Areas: Insights for Coastal Water Quality and Regeneration

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## Abstract

The Portuguese Water Quality Policy, aligned with the Water Framework Directive, requires the monitoring of shellfish production in coastal and brackish water bodies and is based on the EC Shellfish Waters Directive; it preserves the environmental conditions necessary for shellfish growth and contributes to the production of a safe, high-quality sustainable animal food source. Between 2017 and 2021, quarterly measurements of physico-chemical and microbial parameters were conducted at 11 coastal stations and 21 estuarine/coastal lagoons stations along Portugal, to evaluate shellfish water quality and identify the environmental drivers influencing it. Results showed that estuaries and coastal lagoons were more vulnerable to fluctuations in salinity, oxygen and suspended matter and consistently exhibited higher faecal contamination in bivalves than coastal waters. These patterns were shaped by freshwater inputs, climate-driven variability, and local anthropogenic pressures, with contamination often increasing during rainy periods or under stressful environmental conditions. The study highlights the sensitivity of transitional waters to both natural and human-induced changes and underscores the need for targeted management strategies, such as improved wastewater treatment and integrated coastal management, to reduce pollution pressures. Strengthening these measures is essential for the long-term sustainability of Portugal's shellfish industry and coastal ecosystems.

**Keywords:** WFD; shellfish waters; regeneration; faecal contamination; water quality; Portuguese coast; coastal ecosystem resilience; ecosystem-base management

## 1. Introduction

The quality of coastal and brackish waters is a complex issue, impacted by pollution, climate, human activities, and many other factors. As a result, water degradation is a threat, and several monitoring programmes or legislation have been implemented all over the world. Moreover, coastal and brackish waters need protection against pollution and degradation or improvement, notably those where bivalves grow. These marine invertebrates are valuable for ecosystems as they provide several services (such as, counteracting eutrophication and providing habitats for other species) and feed on natural nutrients and phytoplankton, representing a highly efficient and sustainable animal food source. Bivalves are predominantly filter-feeders; consequently, they can accumulate harmful substances that can undermine their viability and/or sanitary quality [1].

The Portuguese Water Quality Policy, outlined by the Water Framework Directive (WFD) [2], requires the monitoring of shellfish production areas in coastal and brackish



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water bodies. The WFD follows a river basin management cycle approach on a sextennial basis to permit an improved assessment of methodologies and plans. The latter monitoring plan is based on the Shellfish Waters Directive (SWD) [3] which protects or improves shellfish waters to preserve the environmental conditions necessary for shellfish growth, contributing to safe, high-quality shellfish for human consumption. It comprises the assessment of both chemical and microbial parameters. Regarding microbiological criteria, the SWD establishes a limit ( $\leq 300$  or  $\leq 2.5 \text{ Log}_{10}$  faecal coliforms per 100 g of bivalve flesh and intervalvar liquid) that should not be exceeded each year in 75% of samples.

Shellfish Waters in Portugal were evaluated by applying the criteria adopted by the SWD, whose classification was published in the official Portuguese WFD 3rd cycle of evaluation [4–11]. All coastal and estuarine/coastal lagoon shellfish waters were considered compliant, according to the physico-chemical parameters tested, except for Mondego Estuary, which was excluded as a shellfish water body [12]. However, according to microbiological parameters, most of these shellfish waters were considered non-compliant. Considering all the parameters, as required by the Shellfish Waters Directive, only two out of the 11 (18%) coastal waters, one out of six (17%) estuarine waters and one out of four (25%) coastal lagoon shellfish waters were considered compliant.

Therefore, the contamination of Portuguese shellfish water bodies by faecal microorganisms is clear, with consequences for the health of humans and those who live economically by harvesting or culturing bivalves. In fact, based on data until 2022, only approximately 15% of wastewater in Portugal underwent tertiary treatment (including a disinfection process), with 76% receiving secondary treatment [13]. This implies that most of the Portuguese bivalve production areas are not classified as category A, which corresponds to clean areas, with a low level of faecal contamination [14]. In higher sanitary categories (B and C classes) bivalves may be harvested, but only commercialised alive after treatment in a purification centre or relaying [15]. Production areas with higher sanitary categories represent an economic burden on the shellfish industry [16], as those treatments have additional costs. The sustainability of shellfish farming and harvesting is dependent on clean coastal waters. So, there is a need to assess the physico-chemical parameters associated with the occurrence and persistence of faecal contamination in Portuguese shellfish waters. Some of the environmental conditions favourable to the survival of faecal indicator organisms in seawater are low salinity and low temperatures, and high levels of nutrients and organic matter [17].

In this study, we aim to assess the spatial and temporal variability of the analysed parameters across three ecosystems (coastal zone, coastal lagoons, and estuaries) and to detect and understand relationships between the physico-chemical parameters and the occurrence and persistence of faecal contamination in bivalves in Portuguese shellfish waters. Possible measures for better management of production areas are also discussed.

## 2. Materials and Methods

### 2.1. Study Area Description

This study was focused on the Portuguese coast (from 36.0° N to 41.8° N and 7.2° W to 9.5° W), a highly heterogeneous region shaped by a continental margin divided into several sub-regions by the occurrence of seas, submarine canyons and abyssal plains. The area is characterised by a strong seasonal and inter-annual variability in the hydrology and circulation of the upper layers (0–200 m), largely driven by wind patterns and fluctuations in freshwater input from river discharge [18]. Wind-driven upwelling occurs typically in late spring and summer [19,20], while in winter, increased river runoff is responsible for the formation of plumes, especially on the northern coast, where a larger number of rivers

exists whereas southern areas below the Tejo estuary receive less freshwater input due to a significant decrease in the number of rivers.

The littoral coastal waters studied (Figure 1), cover the west coast in the areas of Carreço, Leça, Torreira, Leirosa, Peniche, Costa da Caparica, Comporta and Aljezur, and on the south coast the areas of Porto de Mós, Faro and Monte Gordo. The coastal water bodies in this study cover the coast from north to south (Figure 1), namely the estuaries: Lima, Ria de Aveiro, Mondego, Tejo, Sado and Mira, and the coastal lagoons: Lagoa de Óbidos, Lagoa de Albufeira, Lagoa de Alvor and Ria Formosa.

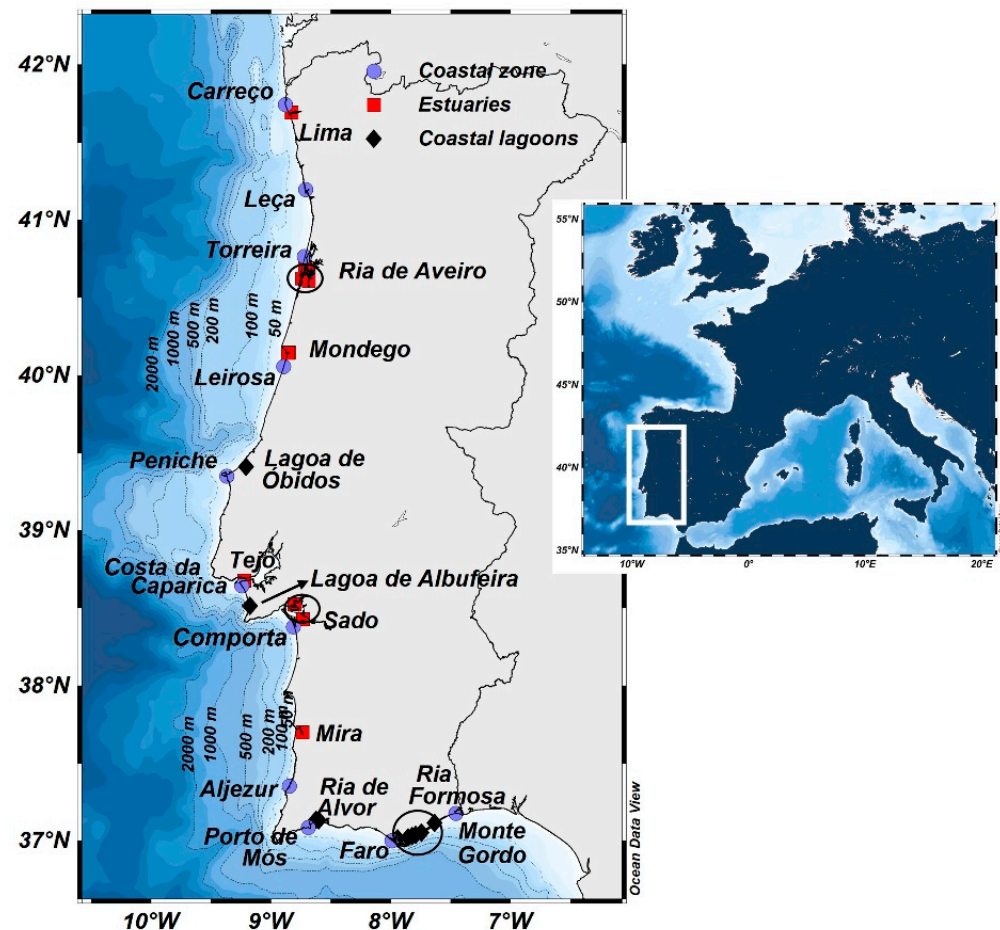


**Figure 1.** Sampling stations for the coastal zone, estuaries and coastal lagoons. Circles surrounding a group of stations correspond to the same water body.

According to Bettencourt et al. [21], regarding coastal water bodies, Lima is a mesotidal stratified estuary and is characterised by consistently high river flows throughout the year, which promote stratification of the estuary water column. Ria de Aveiro, Mondego, Tejo, Sado and Mira estuaries are mesotidal, well-mixed estuaries with river discharge that varies strongly with the season. Torrential flows typically occur during winter due to intense rainfall. These estuaries remain well-mixed for most of the year, with stratification occurring only rarely, usually during extreme flood events. The coastal lagoons are divided into two types. Lagoa de Óbidos and Lagoa de Albufeira, located on the west coast, are mesotidal semi-enclosed systems that are shallow and maintain a direct but intermittent connection with the ocean, often blocked by a sand bar. Artificial openings are performed primarily in the spring and summer [22]. The tidal influence within these lagoons is moderate and only present when the connection to the ocean is unobstructed. Ria de Alvor and Ria Formosa, located on the south coast, are mesotidal shallow lagoons with permanent communication with the sea maintained through several inlets distributed along the system. Their shallow

depth, strong tidal currents, and high rates of water renewal result in these lagoons being vertically well-mixed.

## 2.2. Sampling

The sampling programme was carried out quarterly (every three months) at 32 sampling stations distributed across the coastal area (11 stations), 6 estuaries (11 stations) and 4 coastal lagoons (10 stations) (Figure 1), from north to south of continental Portugal, between 2017 and 2021. Surface water samples were collected using a 5 L Niskin bottle or a polyethene 5 L container and subdivided into vials for salinity, pH, dissolved oxygen (DO) and suspended particulate matter (SPM) following the respective analytical protocols and transported to the laboratory. Live bivalve samples were collected from each representative sampling point of the official programme for monitoring bivalve production areas [23] and transported under refrigeration to the laboratory within 24 h.

## 2.3. Analytical Procedures

The temperature was determined in situ with a HANNA (HI98192) probe (Hanna Instruments, Smithfield, RI, USA). Practical salinity measurements were carried out using a salinometer Guildline Autosol 8400B (Guildline Instruments Limited, Smiths Falls, ON, Canada), calibrated with certified IAPSO Standard Seawater reference. pH was measured in situ using a HANNA probe calibrated with NIST standards (Merck KGaA, Darmstadt, Germany) for 7.00 and 10.00.

In the laboratory, the Winkler method [24] was used to determine dissolved oxygen in duplicate. The precision of the method was between 0.08 and 0.11 mg·L<sup>-1</sup>. For suspended particulate matter samples, 100 mL of water was filtered in duplicate through pre-dried and pre-weighed 0.45-µm cellulose acetate membranes. The filters were dried at 50 °C for at least 48 h and weighed again. The levels of faecal coliforms in live bivalves were quantified using a multiple tube fermentation technique followed by confirmation in chromogenic agar (Merck KGaA, Darmstadt, Germany), as described by standard protocols (adapted from ISO 16649-3:2015 [25]). Results were expressed as the most probable number (MPN) per 100 g of flesh and intervalvar liquid and, when appropriate, were log<sub>10</sub> transformed. This method presented a detection limit (DL) of 30 MPN/100 g or 1.5 Log<sub>10</sub> MPN/100 g.

## 2.4. Data Analysis

To better understand the relationships among the studied parameters, the sampling stations were grouped by water body type/ecosystem: Coastal zone, Estuaries and Coastal lagoons. This approach reflects the distinct morphology and hydrodynamic characteristics of the three ecosystem types. Faecal coliform levels were converted to a log scale for analysis. The non-parametric (quartiles, median, 10th and 90th percentile) statistics were calculated. Multivariate analysis using principal component analysis (PCA) was applied to the dataset to identify the major drivers of system variability. Physico-chemical and microbial parameters were included in the PCA, performed using Sigmaplot 14 (Systat Software, Inc., San Jose, CA, USA). Additionally, Kruskal–Wallis H (KW-H) and Mann–Whitney (U) non-parametric tests were used to assess differences among ecosystem types for the environmental and microbial parameters (salinity, temperature, DO, pH, SPM and faecal coliforms) due to the data non-compliance with the parametric ANOVA assumptions. A significance level of  $p < 0.05$  was used for all statistical analyses, which were performed using STATISTICA 6 (StatSoft GmbH, Hamburg, Germany).

### 3. Results and Discussion

#### 3.1. Spatial and Temporal Variability of the Coastal Zone

Over the five-year study period, surface water salinity ranged from 27.20 to 37.05, with an average value of 35.25 (Figure 2). The lowest salinity was observed at the Leça station, located near the mouth of the Douro River, one of Portugal's major rivers, where freshwater influence is strongest. Both Carreço and Leça stations showed the highest salinity variability, reflecting the high river discharge characteristics of this region. In contrast, the highest and least variable salinity values were observed at the Comporta station. Temperature ranged from 12.0 °C to 26.8 °C, with the lowest values observed in Torreira station (north) and the highest at Monte Gordo station (south). A clear north–south increase gradient was evident for both salinity and temperature (Figure 2), consistent with the climatic pattern of the Portuguese coast, where colder waters dominate in the north and warmer waters in the south. This gradient is further influenced by river plumes, which are more extensive in the north due to the greater number of river systems [26]. The DO concentrations ranged from 6.10 to 14.80 mg·L<sup>−1</sup>. The lowest values occurred during summer, particularly at southern stations, with the Aljezur station presenting the minimum concentration. In contrast, the highest DO levels were found at the Torreira station in the north. This pattern reflects the temperature dependence of oxygen solubility: warmer waters hold less oxygen and promote higher biological and chemical oxygen demand. Additionally, the northern coast experiences strong wave action, currents, and wind [27], which enhance water mixing and oxygenation. Under climate change scenarios, the southern Portuguese coast may face increasing challenges regarding ecosystem service management, as rising temperatures are expected to further reduce oxygen availability [28]. As water temperatures increase, oxygen solubility declines, and ocean stratification strengthens, limiting both the concentration of dissolved oxygen and the transfer of oxygen-rich surface waters to deeper layers [28,29]. SPM ranged from 0.02 to 64.80 mg·L<sup>−1</sup>, with a five-year average of 15.64 mg·L<sup>−1</sup>. The highest SPM value was observed at Leça station (north), while the lowest was at the Aljezur station (south). A decreasing north–south gradient was evident, driven by riverine inputs that deliver more turbid waters, particularly to the northern coast zone, and by sediment resuspension on the continental shelf, which is wider between Carreço and Comporta stations (western coast) and between Porto de Mós and Monte Gordo stations (south coast). Values for pH ranged from 7.10 to 8.44, with an average value of 7.92. The highest pH was observed at Faro station, while the lowest occurred at Carreço station. No clear latitudinal gradient was detected, and both north (Carreço) and south stations (Faro and Monte Gordo) showed wide pH ranges. This variability may be linked to discharges from submarine outfalls and river discharges in the north and the influence of the Ria Formosa and Guadiana estuary waters in the south. Temperature-driven pH fluctuations may also contribute to the observed patterns as well as salinity changes. Faecal contamination in coastal bivalves ranged from below detection value (<1.5 Log<sub>10</sub>) to 4.3 Log<sub>10</sub> faecal coliforms MPN/100 g. The highest values were observed at Leça station (north) and at Aljezur, Faro and Monte Gordo stations (south). Significantly, higher levels were observed at most stations compared with Comporta, Aljezur and Porto de Mós stations. Lower contamination at the latter stations may be associated with their location near smaller riverine systems and a narrower continental shelf, where hydrodynamic conditions favour the dispersion of chemical compounds and microorganisms.

Temporal variability was assessed at two different time scales, annually and seasonally (Figure 3). No annual trend was observed for any of the studied parameters. The interannual variability observed in salinity and temperature reflects the Portuguese climate pattern, which alternates between wet and dry, and cold and hot years. The years 2017, 2019, and 2021, which experienced higher rainfall [30] showed lower salinity values and

appeared as outliers. The SPM annual distribution followed the salinity pattern, with wider ranges and higher concentrations occurring in years with lower salinity.

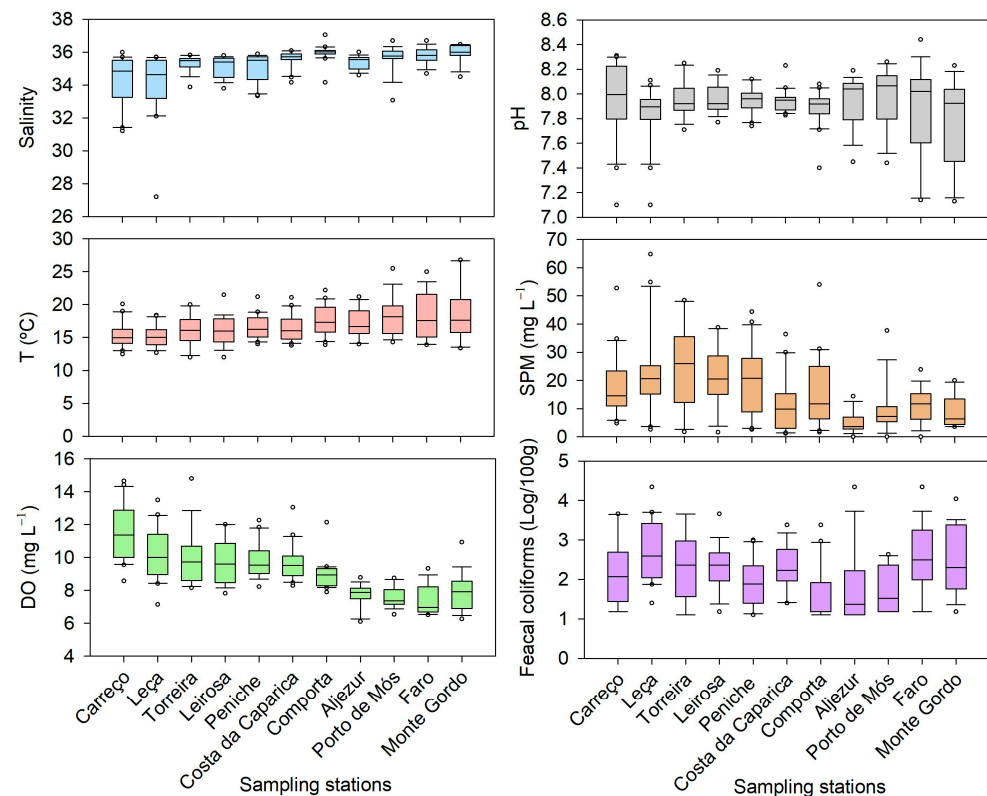


**Figure 2.** Spatial distribution of studied physicochemical and microbial parameters for coastal zones from 2017 to 2021. In the boxplots, the median is represented by the solid line, the 25% and 75% percentile by the lower and upper hinges, and the 10th and 90th percentile by the whiskers. Sample size for each parameter is  $n = 196$  for salinity,  $n = 200$  for temperature,  $n = 198$  for DO,  $n = 200$  for pH,  $n = 200$  for SPM and  $n = 208$  for faecal coliforms.

Seasonal patterns showed that salinity was the lowest and most variable during winter, reflecting stronger river influence, while higher and less variable values were observed in spring, summer, and autumn. Temperature followed the expected seasonal cycle, with colder conditions in winter and warmer conditions in summer. The wider summer range is consistent with the occurrence of upwelling in the summer months [20]. The DO also reflected two dominant coastal features, namely, elevated values in winter due to freshwater inputs and in summer due to the intrusion of cold upwelled waters. The seasonal distribution of pH appeared to follow the same pattern but with lower variability in winter and in summer. Faecal coliforms concentrations in coastal bivalves at coastal zones showed no temporal/seasonal trend although 2018 exhibited the highest median values. Winter showed the highest median values and a lower range, possibly reflecting the persistence of continuous sources of faecal contamination, during this typically rainy season linked to emergency outfall and water treatment facilities discharges.

Overall, the coastal zone displayed marked north–south environmental gradients driven largely by river influence and hydrodynamic conditions, with northern stations showing lower salinity and temperature, higher SPM and greater oxygenation, while southern stations exhibited warmer, less oxygenated and clearer waters. Temporal analyses revealed no consistent annual trends, with interannual variability in salinity, temperature, and SPM reflecting fluctuations between wet and drier years. Seasonally winter was characterised by strong river influence, lower salinity and higher DO, whereas summer conditions reflected warming and upwelling dynamics. The pH values showed no consistent spatial

or seasonal pattern but varied at stations influenced by river plumes and anthropogenic discharges. Faecal coliform levels in bivalves showed no clear seasonal trend though higher winter and spring values suggest persistent contamination sources during rainy periods. Together, these patterns highlight the combined effects of freshwater inputs, climate-driven variability and human pressures on coastal water quality, underscoring the need for the improvement of wastewater treatment, the reduction in stormwater-related contamination and the strengthening of ecosystem-based approaches.

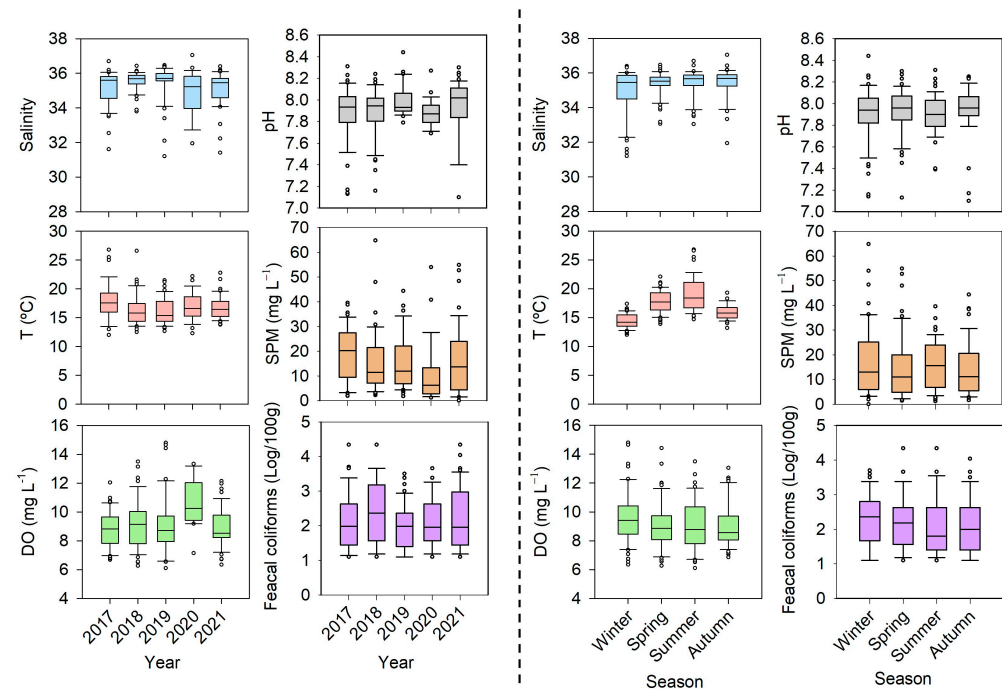


**Figure 3.** Temporal/seasonal distribution of studied physicochemical and microbial parameters for the coastal zone from 2017 to 2021. In the boxplots, the solid line represents the median, the lower and upper hinges are the 25% and 75% percentiles, and the whiskers represent the 10th and 90th percentiles. Sample size for each parameter is  $n = 196$  for salinity,  $n = 200$  for temperature,  $n = 198$  for DO,  $n = 200$  for pH,  $n = 200$  for SPM and  $n = 208$  for faecal coliforms.

### 3.2. Spatial and Temporal Variability of Estuaries

In estuaries, salinity ranged from 0.25 to 39.80, with an average value of 30.73. The lowest values ( $<0.33$ ) were observed in the Lima and Mondego estuaries, while the highest values were observed in the Sado estuary. The spatial distribution of salinity (Figure 4) showed that northern and central estuaries exhibited lower salinity and wider variability, particularly Lima and Mondego, reflecting their funnel-shaped morphology, high river flow and short water residence times ( $<2$  weeks) [31]. In contrast, Ria de Aveiro, Tejo and Sado showed higher and less variable salinity values, consistent with their lagoon-type characteristics, with more ocean water intrusion and longer water residence times (up to 1 month in Sado estuary [32]). Mira, a small southern river with small river flow, showed high and relatively stable salinity values due to strong oceanic water influence. Temperatures ranged from 11.0 °C to 27.9 °C, with the lowest value observed in the Lima estuary and the highest again in the Sado estuary, reflecting its shallow depth, extensive intertidal mudflats and long water residence time. The temperature patterns were estuary-specific and driven by local weather, morphology, and hydrodynamics rather than the north–south gradient observed in the coastal zone. The DO ranged from 6.39 to 14.35 mg·L<sup>-1</sup> with both extremes observed in Ria de Aveiro, indicating a distinct hydrodynamic regime. The mean DO concentration (9.65 mg·L<sup>-1</sup>) confirmed that estuar-

ine waters were generally well oxygenated. As expected, Lima and Mondego estuaries showed greater DO variability and higher values due to strong river flow, which enhances water aeration. Regarding pH, its values ranged from 6.9 to 8.33 with the lowest value observed in the Lima estuary and the highest in the Ria de Aveiro. Overall, pH distribution was similar across estuaries, although Lima and Sado estuaries exhibited wider ranges. These patterns appear linked to salinity fluctuations in the Lima estuary and temperature variability in the Sado estuary. SPM ranged from 1.40 to 110.80 mg·L<sup>-1</sup> with an average of 19.16 mg·L<sup>-1</sup>. The highest value was observed in the Sado estuary and the lowest in the Tejo estuary. SPM generally varied inversely with salinity, except for the Sado estuary. Estuaries with a lower and wider range of salinity (Lima and Mondego) showed higher and more variable SPM, while the opposite was observed for Tejo and Mira estuaries. Elevated SPM in the Sado estuary reflects shallow, lagoon-type estuary morphology with mudflats, which promotes sediment resuspension. Faecal contamination in bivalves ranged from 2.3 Log<sub>10</sub> MPN/100 g in Mira to 3.6 Log<sub>10</sub> MPN/100 g in Mondego. Spatially, Mondego presented significantly higher faecal contamination than the Mira and Sado estuaries, whereas Mira had significantly lower faecal contamination than Lima, Ria de Aveiro, Mondego and Tejo. The low contamination in the Mira estuary likely reflects its location in a sparsely populated region with fewer sources of faecal contamination.

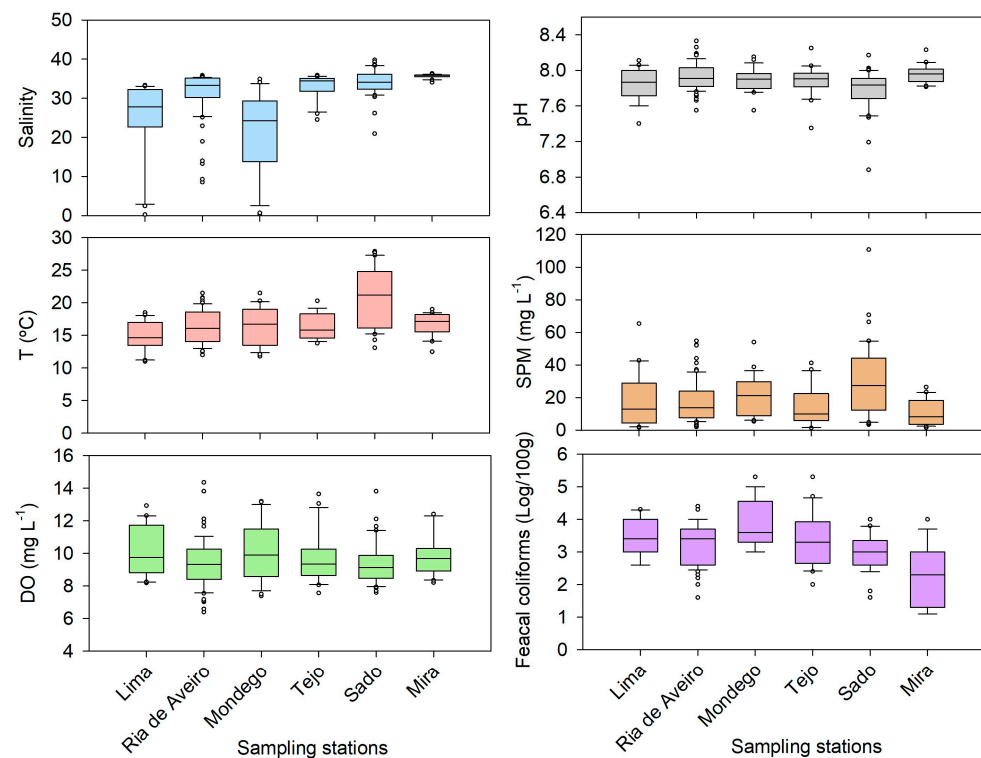


**Figure 4.** Spatial distribution of studied physicochemical and microbial parameters in estuaries from 2017 to 2021. In the boxplots, the median is represented by the solid line, the 25% and 75% percentile by the lower and upper hinges, and the 10th and 90th percentile by the whiskers. Sample size for each parameter is  $n = 194$  for salinity,  $n = 194$  for temperature,  $n = 189$  for DO,  $n = 194$  for pH,  $n = 194$  for SPM and  $n = 195$  for faecal coliforms.

No annual trend (Figure 5) was observed for any of the studied parameters, consistent with the pattern observed in the coastal zone. Interannual variability in salinity and temperature reflected the torrential regime of the Portuguese climate, with the rainier years 2017, 2019, and 2021 showing markedly lower salinity values in some cases up to 0. As in the coastal zone, SPM annual distribution followed the salinity pattern, with wider ranges and higher concentrations occurring in years of lower salinity due to increased river

runoff responsible for particle transport. Although faecal coliforms concentrations did not exhibit an annual trend, 2018 again showed the highest median contamination levels, while broader ranges were observed in the wetter years (2017, 2019 and 2021). This aligns with previous findings indicating that rainfall is one of the factors most frequently implicated with peak faecal contamination in shellfish waters [17].

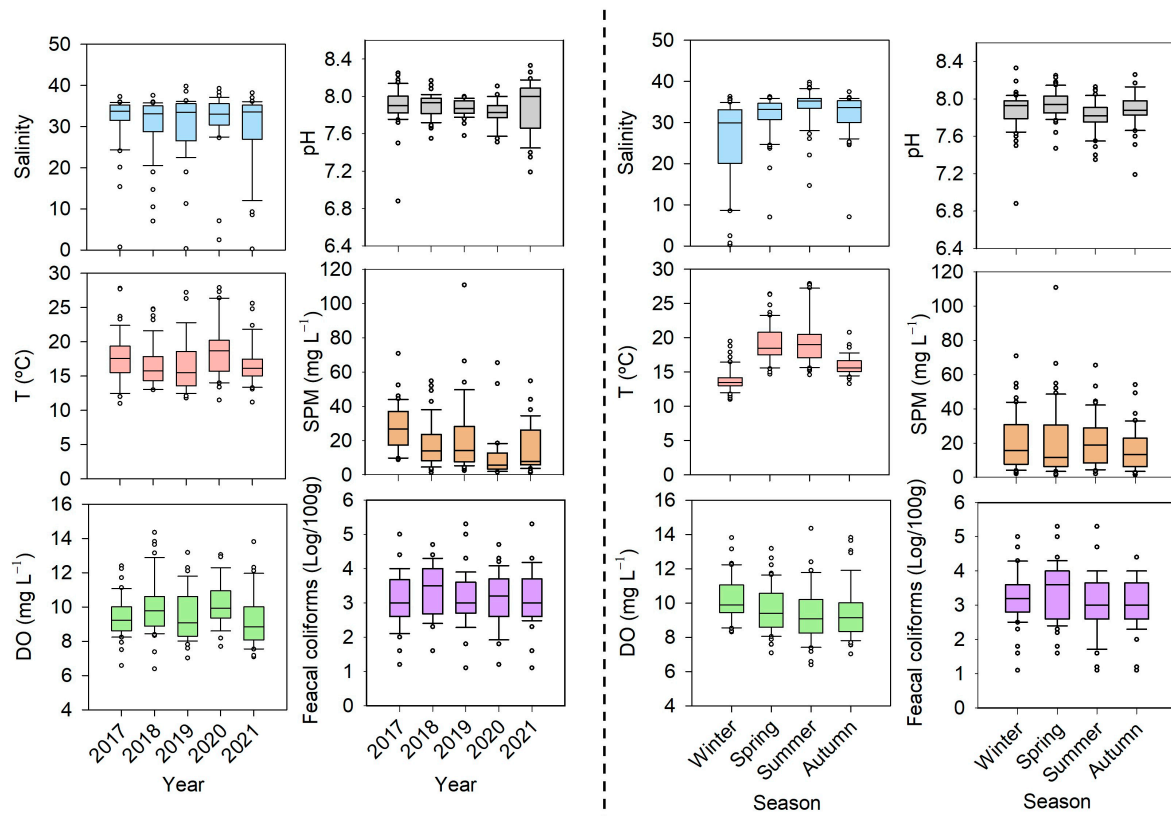


**Figure 5.** Temporal/seasonal distribution of studied physicochemical and microbial parameters for estuaries from 2017 to 2021. In the boxplots, the median is represented by the solid line, the 25% and 75% percentile by the lower and upper hinges, and the 10th and 90th percentile by the whiskers. Sample size for each parameter is  $n = 194$  for salinity,  $n = 194$  for temperature,  $n = 189$  for DO,  $n = 194$  for pH,  $n = 194$  for SPM and  $n = 195$  for faecal coliforms.

Seasonal patterns (Figure 5) showed that salinity was lowest and most variable in winter, consistent with the increase in precipitation and river discharge, and the highest in summer, when rainfall is minimal, and river flow decreases sharply, especially for smaller and lagoon-type estuaries. The temperature followed the expected seasonal cycle, with colder conditions in the winter and hotter conditions in the summer, closely followed by spring. This pattern aligns with global observations of climate-driven shifts towards less distinct seasonality, with a tendency toward two predominant seasons rather than four [28,33]. The DO also reflected these seasonal dynamics, with higher values in winter due to freshwater inputs and lower values in the summer when oxygen solubility decreases with rising temperatures. The seasonal pH distribution showed a different pattern, with higher values observed in spring and autumn, probably linked with estuarine primary production. The SPM showed an inverse pattern to pH and showed higher variability in the winter and spring, reflecting the influence of hydrodynamics and estuarine morphology. Faecal coliforms in bivalves showed no seasonal trend; nevertheless, spring exhibited the highest median contamination ( $3.6 \text{ Log}_{10} \text{ MPN}/100 \text{ g}$ ), possibly linked with runoff following rainfall events. The lowest median levels ( $3.0 \text{ Log}_{10} \text{ MPN}/100 \text{ g}$ ) occurred in summer and autumn.

Estuaries exhibited pronounced spatial and temporal variability, shaped by differences in river flow, morphology, hydrodynamics, and human pressures. Northern and central systems, such as Lima and Mondego, showed lower and more variable salinity, higher SPM and greater DO variability due to strong freshwater inputs, while lagoon-type estuaries like Ria de Aveiro, Tejo and Sado displayed higher and more stable salinity and distinct thermal regimes. Interannual patterns reflected the influence of Portugal's highly variable climate, with wetter years producing lower salinity, higher SPM and occasional peaks in faecal contamination. Seasonally, winter was marked by strong river influence, lower salinity, and higher DO, whereas summer brought warmer waters, reduced oxygen, and higher salinity. Values for pH, SPM and faecal contamination showed no consistent seasonal trend. Morphology, hydrodynamics, climate variability, and local anthropogenic pressures seem to be the main drivers for estuarine water quality and microbial contamination.

### 3.3. Spatial and Temporal Variability of Coastal Lagoons

In lagoons, salinity ranged from 1.28 to 37.70, with an average of 34.85. The highest value was observed in Ria de Alvor and the lowest in Lagoa de Óbidos. Temperature varied between 9.5 °C and 30.6 °C (average 18.9 °C). The north–south distribution (Figure 6) showed that lagoons on the western coast exhibited greater salinity variability due to episodic freshwater inputs, while southern lagoons displayed salinity values typical of seawater. This pattern reflects lagoon morphology and hydrology. Lagoa de Óbidos and Lagoa de Albufeira are lagoons that periodically lose connection with the sea [22,34], in contrast with Ria de Alvor and Ria Formosa, which maintain permanent inlets. The temperature was highest in Ria Formosa and lowest in Lagoa de Albufeira. A north–south increase was observed, but lower in range when compared to the coastal zone, likely because shallow systems respond strongly to atmospheric temperature and local water inflows. The DO ranged from 3.73 to 14.37 mg·L<sup>−1</sup>, with both the minimum and maximum values observed in the Lagoa de Albufeira lagoon during summer. Levels of DO were generally higher in western lagoons, reflecting elevated primary production associated with limited water renewal. The pH ranged from 6.9 to 8.53 (average 7.96), with both the lowest and highest values observed in Ria Formosa. The pH pattern mirrored the high productivity observed in these systems. SPM ranged from 0.02 to 91 mg·L<sup>−1</sup> although the average value was 9.81 mg·L<sup>−1</sup>. The highest SPM concentration occurred in Lagoa de Óbidos and the lowest in Ria Formosa. SPM showed an inverse relationship with salinity, whereas the western lagoons exhibited higher and more variable SPM values, while the southern lagoons had lower concentrations. This reflects the hydrodynamic differences between lagoons, as lagoons with limited exchange with the sea tend to accumulate suspended material. Median faecal coliform concentrations in bivalves were lower in the western lagoons (2.0 Log<sub>10</sub> MPN/100 g), but higher in the south (2.9 and 3.0 Log<sub>10</sub> MPN/100 g in Ria de Alvor and Ria Formosa, respectively). The elevated contamination in the south may be linked to greater tourism pressure and with under-dimensioned sanitary infrastructures.

No annual trend (Figure 7) was observed for any of the studied parameters in coastal lagoons, consistent with the patterns observed in the coastal zone and the estuaries. Variability in salinity and temperature reflected the Portuguese climatic regime, with the wetter years (2107, 2019 and 2021) appearing only as outliers with lower salinity values. As in the other systems, the annual distribution of SPM followed the salinity pattern, with the widest ranges and highest values occurring in 2019, likely due to prolonged periods of inlet closure in the western lagoons (particularly Óbidos and Albufeira). Faecal coliforms levels also showed no annual trend, although the highest median values (2.9 Log<sub>10</sub> MPN/100 g) were observed in 2017, a year marked by intense rainfall [30].

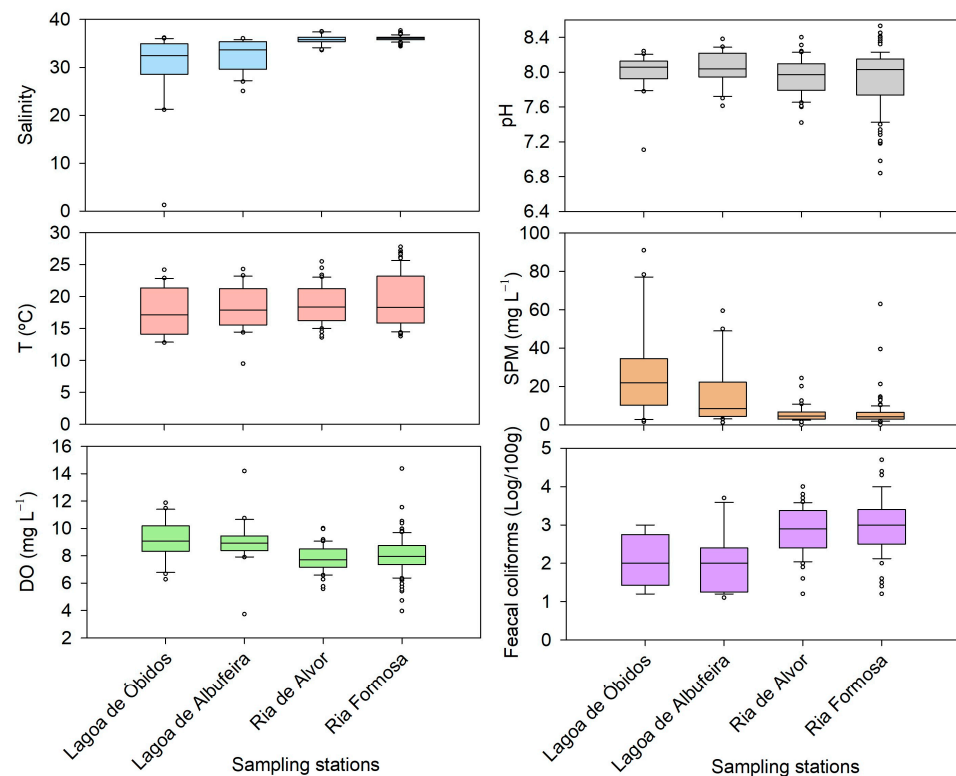


**Figure 6.** Spatial distribution of studied physicochemical and microbial parameters for coastal lagoons from 2017 to 2021. In the boxplots, the median is represented by the solid line, the 25% and 75% percentile by the lower and upper hinges, and the 10th and 90th percentile by the whiskers. Sample size for each parameter is  $n = 166$  for salinity,  $n = 174$  for temperature,  $n = 174$  for DO,  $n = 174$  for pH,  $n = 174$  for SPM and  $n = 183$  for faecal coliforms.

Seasonal patterns (Figure 7) showed that salinity was slightly lower and more variable in winter, reflecting increasing precipitation, and highest in summer, when rainfall is minimal and lagoon inlets are generally open to the sea. The temperature followed the expected seasonal cycle with colder conditions in winter and warmer conditions in summer [30]. Unlike estuaries, the four seasons were well defined in lagoons, likely due to regular water renewal through their ocean connections. DO followed the temperature patterns, with higher values during the cooling seasons when oxygen solubility is greater. The pH showed no seasonal trend, although variability was low in the autumn. The SPM also exhibited no consistent seasonal pattern, appearing instead to reflect lagoon morphology and hydrodynamics as previously noted for estuaries. Faecal coliforms levels in bivalves showed no seasonal trend, although the highest median values were observed in spring ( $2.8 \text{ Log}_{10} \text{ MPN}/100 \text{ g}$ ) accompanied by a wider range of values observed in spring and summer. These variations, including the occurrence of both high and low faecal contamination levels, may be linked to the intermittent periods of low oxygen and elevated seawater temperature (well above  $20^\circ\text{C}$ ), which can stress bivalves, causing gaping [35] and reduced filtration activity, thereby influencing particle and contaminant retention.

Coastal lagoons exhibited strong spatial and temporal variability shaped by their morphology and degree of connection with the sea. Western lagoons, which experienced intermittent seawater exchange, showed greater variability in salinity, DO, pH and SPM, while southern lagoons, permanently open to the ocean, displayed more stable, marine-like conditions and clear waters. Faecal contamination in bivalves was generally lower in western lagoons and higher in southern ones, likely reflecting greater tourism pressure and limitations in local sanitation infrastructure. No annual trends were detected for any

parameter, although wetter years occasionally showed low salinity outliers and higher SPM in lagoons with restricted ocean exchange. Seasonally, winter brought slightly lower salinity and higher oxygen levels, while summer reflected more marine-influenced conditions. Faecal contamination showed no consistent seasonal pattern, though higher and more variable levels in spring and summer may be linked to periods of low oxygen and elevated temperatures that can stress bivalves and alter filtration activity.

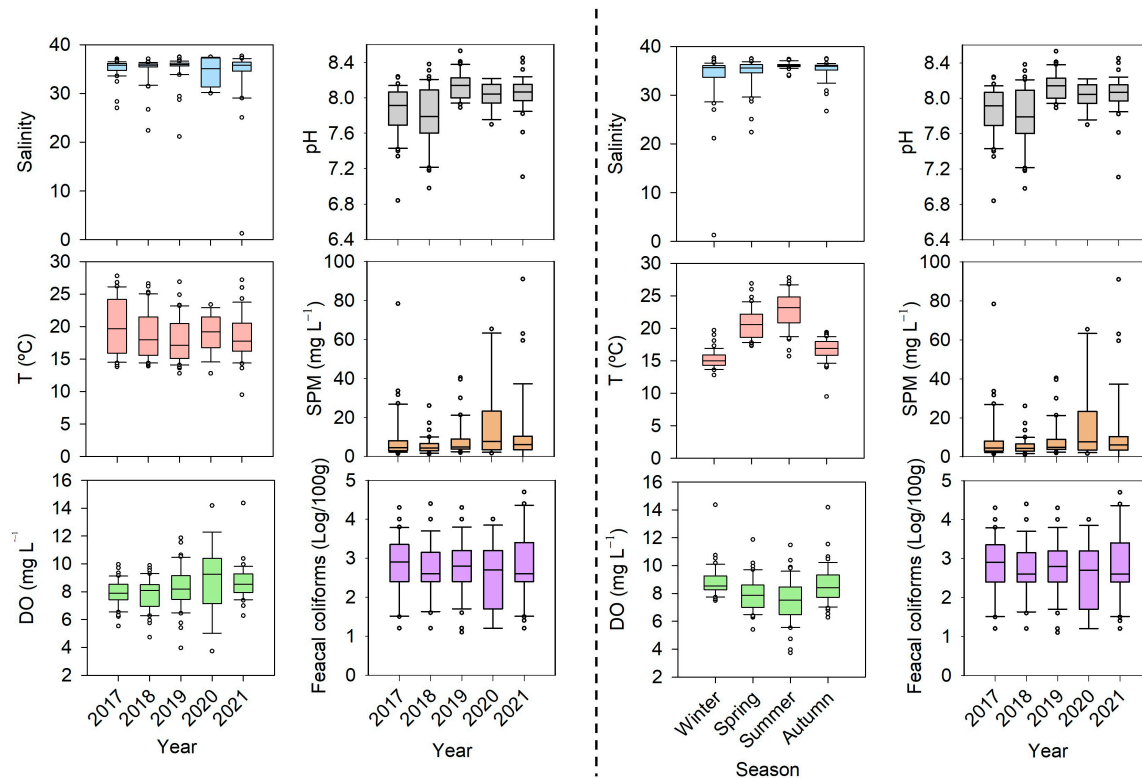


**Figure 7.** Temporal/seasonal distribution of studied physicochemical and microbial parameters for the coastal lagoons from 2017 to 2021. In the boxplots, the median is represented by the solid line, the 25% and 75% percentile by the lower and upper hinges, and the 10th and 90th percentile by the whiskers. Sample size for each parameter is  $n = 166$  for salinity,  $n = 174$  for temperature,  $n = 174$  for DO,  $n = 174$  for pH,  $n = 174$  for SPM and  $n = 183$  for faecal coliforms.

### 3.4. Relationships Between Physico-Chemical and Microbial Parameters

Principal component analysis (PCA) was used to assess the relationship between the physico-chemical and microbial parameters for coastal, estuarine and coastal lagoons areas (Figure 8). For the coastal zone, PCA explains 48% of the variance and PC1 (29%) separates salinity and temperature from the other variables while PC2 (19%) separates faecal coliforms from pH and SPM. However, there is evidence that SPM can facilitate the growth and persistence of faecal bacteria through the protection of microbes from biotic and abiotic stresses. Some of the findings suggest that waters with lower salinity and temperature are more oxygenated and carry more particles, and support that faecal coliforms have more favourable conditions at lower pH values, as stated by others [17]. No separation was observed for the sampling stations, meaning that PCA made with the studied parameters cannot fully explain the observed variability.

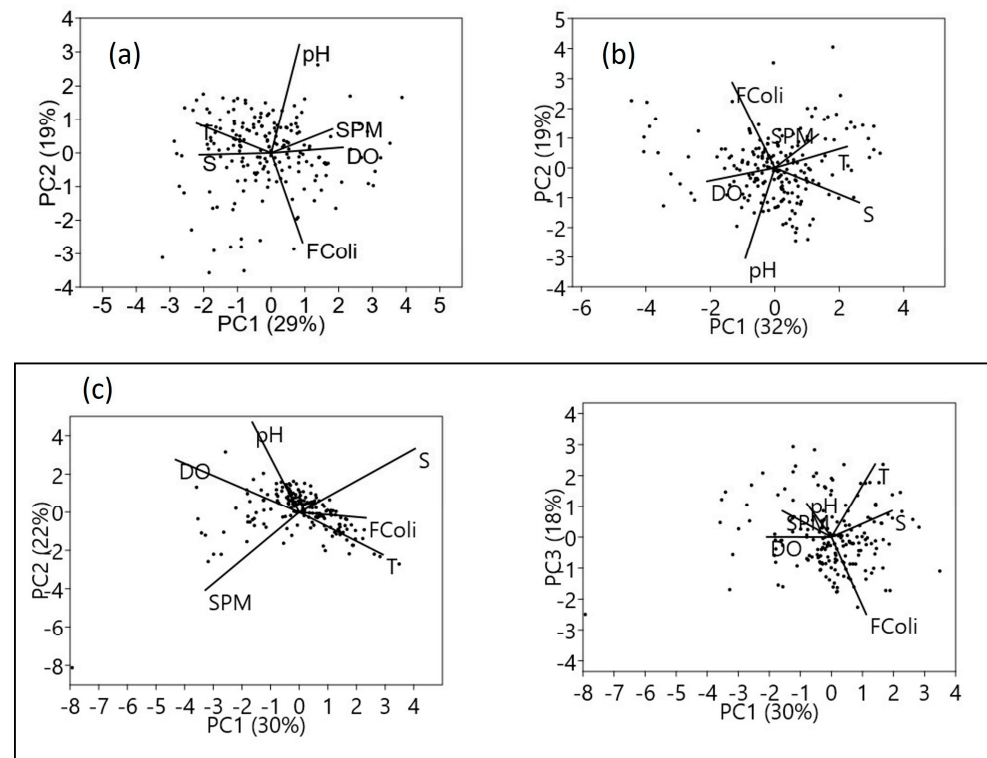


**Figure 8.** PCA for the three systems. (a) coastal zone, (b) estuaries, (c) coastal lagoons.

For estuaries, PCA explains 51% of the variance with two components. PC1 (32%) separates salinity and temperature from pH, DO, and faecal coliforms while PC2 (19%) separates faecal coliforms from pH. In estuaries, low salinity and temperature waters were more oxygenated, as observed for the coastal zone, and faecal coliforms were higher with lower values of pH, as was observed for the coastal zone. Again, no separation was observed for the sampling stations meaning that PCA made with the studied parameters cannot fully explain the observed variability.

PCA for the lagoons explains 70% of the variance and it has three components. PC1 accounts for 30%, PC2 for 22% and PC3 for 18%. PC1 separates DO and SPM from salinity, PC2 separates salinity and pH from SPM and temperature and PC3 separates temperature from faecal coliforms. So, in lagoons, water with less salinity was less oxygenated and had more particles and less pH and at the same time, water with lower temperature had lower faecal coliforms. As observed for the other systems, no separation was observed for the sampling stations although PCA can assess the main relations between parameters in the lagoons.

### 3.5. Ecosystems Comparison

Across the five-year study, coastal waters showed higher median salinity concentrations, whereas estuaries and coastal lagoons exhibited the widest salinity ranges (Figure 9). The temperature and pH varied similarly across all three environments. Although maximum dissolved oxygen values were comparable, the lowest concentrations were observed in coastal lagoons. Suspended particulate matter was generally highest in estuarine areas and lowest in coastal lagoons. As expected, faecal coliform concentrations in bivalves were greater in estuaries and coastal lagoons—ecosystems with more restricted water exchange and presenting higher availability of nutrients and organic material, as well as shelter from sunlight—while coastal zones showed lower contamination levels, with estuaries presenting the highest medians. Additionally, most estuaries and coastal lagoons are subject

to urban influences, related to several sources of faecal pollution—in particular, deficient sewage treatment and leaks due to wastewater treatment plant outflows [36].

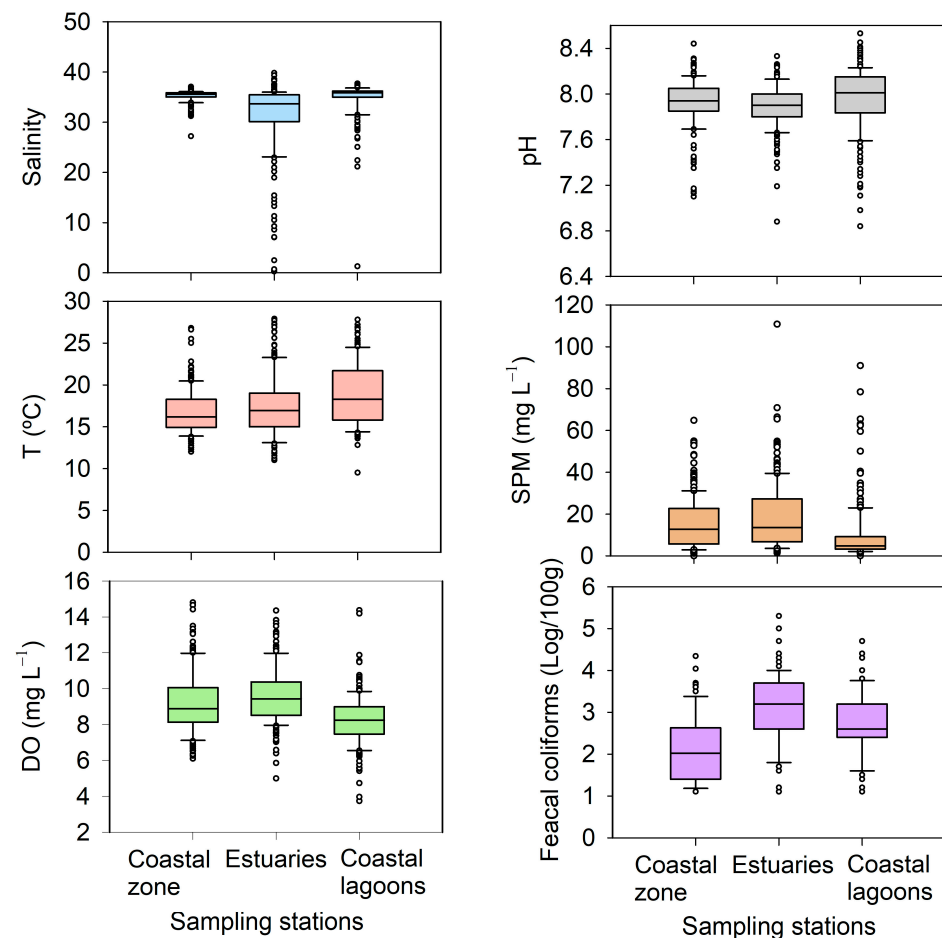


**Figure 9.** Distribution of studied physicochemical and microbial parameters according to ecosystem group from 2017 to 2021. In the boxplots, the median is represented by the solid line, the 25% and 75% percentile by the lower and upper hinges, and the 10th and 90th percentile by the whiskers. Sample size for each parameter is  $n = 556$  for salinity,  $n = 568$  for temperature,  $n = 561$  for DO,  $n = 568$  for pH,  $n = 568$  for SPM and  $n = 586$  for faecal coliforms.

This study reveals that Portuguese coastal waters, estuaries and coastal lagoons are ecosystems highly sensitive to freshwater inputs, climate-driven variability and human pressures—factors that directly influence their long-term sustainability. Spatial patterns showed that systems with restricted water exchange, such as estuaries and coastal lagoons, experience greater fluctuations in salinity, oxygen and suspended matter, making them more vulnerable to pollution and environmental stress. Coastal zones, though more open, were still shaped by river plumes, upwelling, and anthropogenic discharges. Interannual variability linked to wet and dry years further highlighted the dependence of these environments on river flow and the boarder Portuguese climate regime. Seasonal shifts reinforced this sensitivity, with winter bringing stronger freshwater influence and higher oxygen levels, whereas summer reflecting warming waters, reduced oxygen and increased susceptibility to contamination. Faecal pollution showed no consistent annual or seasonal trend but tended to rise during rainy periods or under stressful environmental conditions, underscoring persistent contamination sources and the vulnerability of filter-feeding species.

These findings emphasise the need for regenerative and sustainable management strategies that strengthen wastewater treatment processes, reduce stormwater-related

pollution, and adopt ecosystem-based approaches tailored to each system's hydrodynamics and morphology. The potential establishment of protected areas, restricting or regulating external activities, can help prevent or reduce the impacts of anthropogenic contamination. Improving water quality is essential not only for ecosystem health but also for sustaining shellfish harvesting and aquaculture, as well as the broader ecosystem services it supports, including nutrient removal, shoreline protection, carbon sequestration, and maintaining local biodiversity and fisheries productivity [37,38].

#### 4. Conclusions

Portuguese coastal waters, estuaries and coastal lagoons are highly dynamic and environmentally sensitive systems, strongly influenced by freshwater inputs, climate-driven variability and anthropogenic pressures. Microbial contamination plays a central role in determining the quality of Portuguese shellfish waters, as their classification also depends on this parameter. Although faecal pollution does not affect bivalve survival or growth, it directly constrains their suitability for human consumption. Among the physico-chemical variables analysed, none showed a consistent relationship with faecal contamination in bivalves. In some ecosystems, parameters, such as pH and salinity, may exhibit inverse associations with faecal coliform levels. However, these variables fluctuated seasonally and spatially, which limits their reliability as predictors. This variability hinders the management of shellfish production areas, making the identification and implementation of effective mitigation measures challenging. Reducing faecal contamination at its origin—through improvements in wastewater infrastructure to tertiary treatment (e.g., UV light water treatment, membrane filtration), reduction in intermittent discharges and enhanced stormwater management—remains essential for providing a safe and sustainable animal food source. Additional sustainability measures, including implementation of targeted monitoring programmes to detect earlier contamination events and support adaptive management, restoration of riparian buffers and wetlands, implementation of protected areas, promotion of nature-based wastewater treatment solutions (both in rural and tourism intensive areas), as well as adoption of integrated coastal zone management practices, can further support long-term water quality and ecosystem resilience. Furthermore, this study also indicates that the southern Portuguese coast may face higher challenges under future climate-change scenarios, reinforcing the urgency of proactive and regenerative management strategies.

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