

## Article

# Coastal Wetlands: Ecosystems Affected by Urbanization?

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**Abstract:** Coastal wetlands are ecosystems that provide multiple benefits to human settlements; nonetheless, they are seriously threatened due to both a lack of planning instruments and human activities associated mainly with urban growth. An understanding of their functioning and status is crucial for their protection and conservation. Two wetlands with different degrees of urbanization, Rocuant-Andalién (highly urbanized) and Tubul-Raqui (with little urbanization), were analyzed using temperature, salinity, dissolved oxygen, pH, turbidity, granulometry, fecal coliform, and macroinvertebrate assemblage variables in summer and winter. In both wetlands marked seasonality in salinity, temperature and sediment texture classification, regulated by oceanic influence and changes in the freshwater budget, was observed. In the Rocuant-Andalién wetland, the increases in pH, dissolved oxygen, gravel percentage, and coliform concentration were statistically significant. Urbanization generated negative impacts on macroinvertebrate assemblage structure that inhabit the wetlands; greater richness and abundance (8.5 times greater) were recorded in the Tubul-Raqui wetland than in the more urbanized wetland. The multivariate statistical analysis reflects the alteration of these complex systems.

**Keywords:** salt marshes; urban growth; anthropogenic stressors; aquatic fauna; bioindicators; Chile

## 1. Introduction

The development and use of coastal areas have increased in recent decades, resulting in significant environmental changes [1]. Thus, these areas have presented different population structure and growth patterns linked to global urbanization trends and demographic changes [2,3]. Coastal zones account for only 10% of the Earth's surface but are home to 54% of the world population [4,5] and have a population density 2.6 times greater than interior continental areas [6,7].

These areas offer multiple ecosystem services that contribute positively to human wellbeing, i.e., microclimates, hydrological regulation, tourism and natural resources, among others [8,9], connected to a variety of ecosystems, with wetlands standing out. Coastal wetlands are areas of transition

between terrestrial and oceanic ecosystems, saltmarshes and/or estuaries; they often border inner bays or low-energy intertidal zones [10,11]. Land-use changes have had a substantial impact on these ecosystems, associated mainly with their fragmentation, loss of area and degradation. Indeed, urban growth has been deemed the main anthropogenic stressor, directly responsible for the loss of more than 67% of coastal wetlands, exerting determinant influences on aquatic ecosystems by modifying habitat structure, altering water quality and other actions [2,12–14].

Coastal wetlands act as nutrient generators for the coastal zone, climate (temperature and humidity) stabilizers and protectors of human settlements from floods, storm surges and/or tsunamis [15,16]. They are defined as complex ecosystems as a result of their hydrodynamic characteristics, in which it is possible to connect freshwater unidirectionally through precipitation, groundwater or input from rivers and then bidirectionally through saline water influenced by the tide cycle, prevailing winds and local morphology, variables that determine salinity distributions and stratification. The foregoing influences the chemical characteristics of water [17,18] and therefore aquatic biota. Coastal wetlands are catalogued as biologically diverse and highly productive systems [19,20], as they are inhabited by a large variety of plant and animal species, including hydrophyte plants along with woody and herbaceous species. They provide a structural habitat for epiphyte bacteria, benthic algae, macroinvertebrates, and fish and generally present dominant species along their salinity and physical stress gradients [21,22]. The macroinvertebrates present in these areas provide a fundamental energy subsidy in sections of the food chain for vertebrates such as amphibians, fish and aquatic birds [23–25].

The carrying capacity or resilience of saltmarshes and/or estuaries is determined by interactions of hydrological regimes; sedimentation rates; biomass production; nutrient generation; and processes driven by runoff, salinization and sea level rise [11,26–29]. The seasonal changes in these ecosystems influence water residence time, flooding, pH, salinity, and temperature [30,31]. As coastal wetlands are highly complex, multifactorial and geographically dependent, human activities and climate change are having drastic effects on their functioning [32,33], affecting local vegetation patterns, increasing the number of and distance between patches [34], decreasing biodiversity [14], altering carbon flows or reserves [35,36], and exacerbating ecological vulnerability [37]. It is therefore necessary to contribute to the understanding of these ecosystems in order to design and implement suitable strategies aimed at sustainable management that ensures their preservation and/or conservation [38,39]. Thus, this investigation evaluates the relationship between urbanization and the integrity and health of wetlands in order to answer the following question: How do different degrees of urbanization affect the variables linked to the functioning of a coastal saltmarsh wetland?

Chile has an extensive coastal zone, with approximately 83,850 km of coastline and around 40,000 wetlands throughout the country. However, it has a weak planning policy, along with a lack of land-use planning and coastline zoning instruments that would protect ecosystems [16]. While a bill was recently passed to protect urban wetlands, the lack of such measures in the past allowed a significant reduction in wetland area, mainly due to urban growth and especially in the central part of the country (33–37.5° S), in which 73% of the national population is concentrated [40]. The case of the Concepción Metropolitan Area (CMA) is especially relevant; since the 1970s, more than 23% of total wetland area has been lost, with saltmarshes and estuaries the most affected [41]. However, wetlands in the CMA continue to exist amid different degrees of anthropogenic stressors. One of the systems under the most pressure is the Rocuant-Andalién saltmarsh (36°43′ S–73° W); 575 ha had been urbanized by 2004 and 725 ha by 2014 [16], which has led to the loss of 40% of the wetland, mainly due to housing, road and industrial projects. Since the 1980s it has been used for fishing industry operations and to receive wastewater [15,42,43]. In contrast, the Tubul-Raqui system (37°13′ S–73° W), located south of the CMA, is a wetland with a low degree of urbanization [44], where the greatest anthropogenic pressure is the discharge of little-treated water directly into the estuarine system.

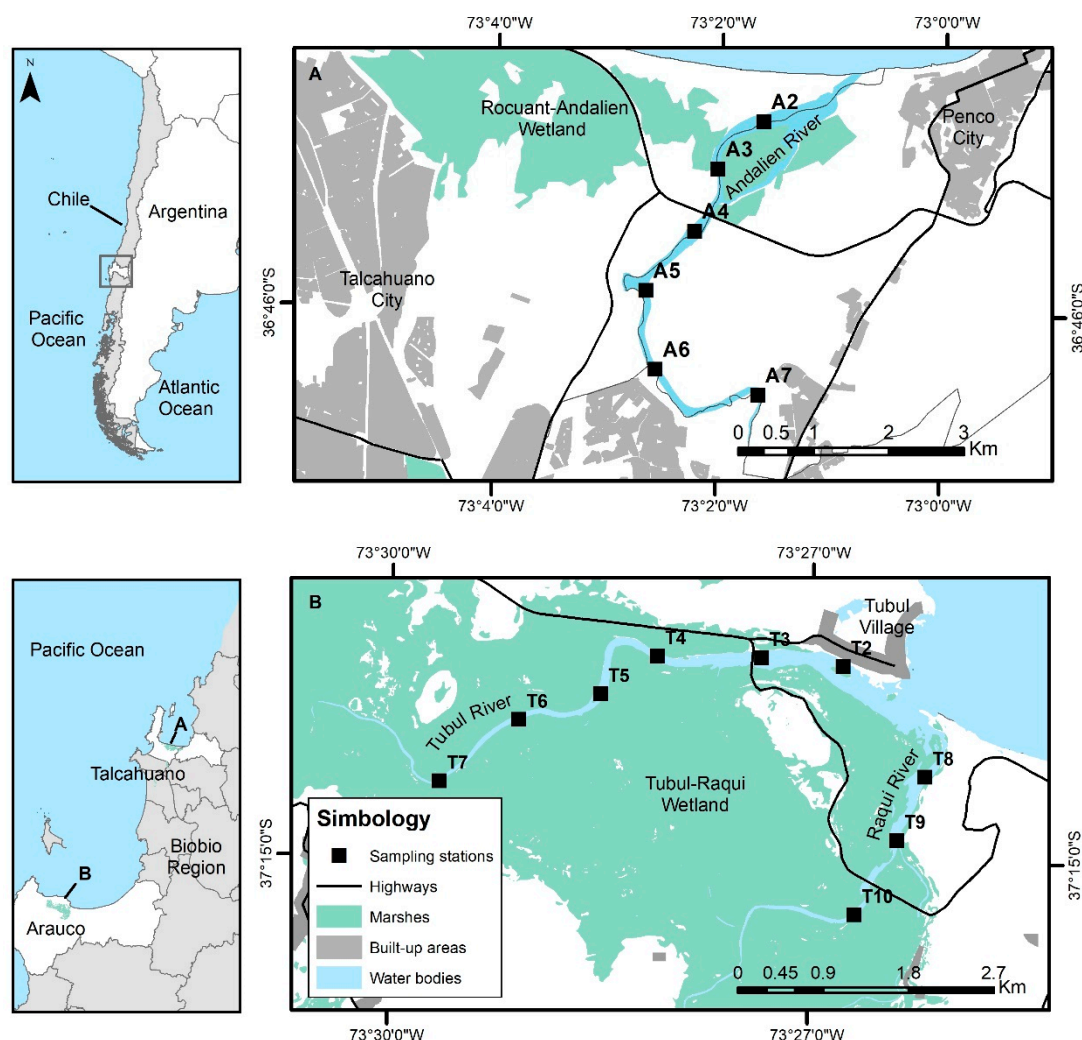
Assessment of the impacts of urbanization on coastal wetlands must include an understanding of the local environment and especially the factors that are responsible for the characteristics of the ecosystem. Therefore, the objective of this work was to comparatively analyze the functioning of

two wetlands with different degrees of urbanization that are located in the coastal zone of the CMA: the highly anthropized Rocuant-Andalién wetland and, as a reference, the Tubul-Raqui wetland, an ecosystem with a low level of urbanization. The investigation will allow these wetlands to be described and the impact of intervention in them on water quality, sediment and aquatic biota to be assessed, providing information that will allow the creation of protection and or/conservation tools for these ecosystems in urbanized environments.

## 2. Materials and Methods

### 2.1. Study Areas

The Rocuant-Andalién ( $36^{\circ}43' \text{ S}$ – $73^{\circ}60' \text{ W}$ ) and Tubul-Raqui coastal saltmarsh wetlands ( $37^{\circ}13' \text{ S}$ – $73^{\circ}26' \text{ W}$ ), located in the CMA of the Biobío Region, were studied. This area presents a mediterranean climate, with precipitation concentrated in the austral winter, resulting in higher streamflows in winter, while in summer streamflows are diminished. Both wetlands depend on the interaction of marine water from the Pacific Ocean and continental water from their respective drainage basins (Figure 1).



**Figure 1.** Study areas: (A) Rocuant-Andalién wetland, with locations of sampling stations ( $n = 6$ ) in the Andalién River, the main aquatic system of the wetland; and (B) Tubul-Raqui wetland, with sampling stations in the Tubul ( $n = 6$ ) and Raqui ( $n = 3$ ) rivers.

The Rocuant-Andalién wetland has a surface area of 767 ha, and its perimeter is under pressure from a consolidated urban area of 90,000 inhabitants, road and port infrastructure, industrial zones,

and infilling for future urban projects [16,40]. It is located in the Andalién River watershed (71,500 ha), where 4% of land use is urban (Table 1). Its main freshwater source is the Andalién River, which had average annual streamflows of 4.5 m<sup>3</sup>/s in 2016 and 6.6 m<sup>3</sup>/s in 2017, with minimum streamflows of 1.01 m<sup>3</sup>/s in summer and maximums of up to 565 m<sup>3</sup>/s in winter, according to General Water Directorate (DGA, for its initials in Spanish) records (2018). In its last 6 km, the river forms an estuary that sustains the vegetation of the wetland. In this section, the river is mostly channelized and undergoes frequent dredging, contributing to a highly modified regime or hydroperiods. The river drains into the Bay of Concepción, a shallow, semi-closed, highly productive system sustained by bottom water upswelling and intrusion from oceanic zone water with high nutrient content [45], along with nutrients and sediment that enter from the Rocuant-Andalién wetland.

The Tubul-Raqui wetland is the most important in the region due to its large surface area of 2238 ha. It is made up of a large saltmarsh lying on the coastal plain of fluvial-marine sediment, with sediment from the Quaternary [46,47]. Along its perimeter are forestry plantations and a small fishing village of about 2500 inhabitants that presents high poverty rates and lacks wastewater treatment [15]. The Tubul-Raqui River watershed (23,209 ha) has an urban land-use percentage of 0.1% (Table 1). The main rivers that form the estuary associated with the wetland are the Tubul and Raqui Rivers, which drain into the Gulf of Arauco, which is a highly productive system maintained by upswelling and entry of oceanic bottom water, along with the input of the Tubul-Raqui wetland, all of which are rich in nutrients [48].

**Table 1.** Land-use categories [49].

Land-Use or Land-Cover Category	Description	Andalién River Watershed		Tubul-Raqui River Watershed	
		Area (ha)	%	Area (ha)	%
Native forest	Forests of native species	11440	16	2685	11.6
Planted forest	Forests of exotic species	39325	55	10782	46.4
Farmland	Agricultural crops	7865	11	298	1.3
Meadows and scrubland	Dense herbaceous cover	10010	14	6572	28.3
Wetland	Coastal area covered by vegetation (salt marshes) and occasionally flooded	715	1	2551	11.0
Urban and industrial areas	Land covered with structures, urban areas with buildings and infrastructure	2860	4	33	0.1
Water bodies	Continental water bodies such as streams, lakes and lagoons and marine water bodies such as seas, oceans and estuaries	215	0.3	242	1.0

## 2.2. Physical-Chemicals Parameters of Wetland Water Quality

In the center of the channels of the main rivers of each wetland, from the mouth to the upper part of the estuary (defined by the salt tide), samplings of physical, chemical and biological variables were carried out in winter of 2016 and summer of 2017. For the Rocuant-Andalién wetland, there were six sampling stations, while for the Tubul-Raqui wetland, there were six stations in the Tubul River and three in the Raqui River.

In situ observations of salinity, temperature, pH, dissolved oxygen, and turbidity were obtained with a previously calibrated Hanna HI9829 multiparameter (In-situ Inc., Ft. Collins, CO, USA). Dissolved oxygen was automatically corrected by the device with respect to electrical conductivity with combined sondes. The measurement was carried out with a luminescent sensor, preventing the errors associated with membrane sensors.

At each of the sampling points, six sediment replicates were collected from the bed using a Van Veen manual dredge (0.025 m<sup>2</sup> capacity). Three replicates were used for granulometry analysis in the Sedimentology Laboratory of the Universidad de Concepción. The samples with high fine content (below 63 µm) were analyzed using laser diffraction with a Mastersizer, while the coarse material samples were analyzed by sieving with an AS-200 Control. The granulometric parameters were

obtained by integrating the coarse and fine fractions in Gradistat v8.0 [50], according to the method of Folk & Ward [51].

### 2.3. Biological Sampling

One liter of water per sampling point was collected in the center of the channels for the determination of microbiological parameters and was kept cold and in darkness and transported to the laboratory to measure total and fecal coliforms using the Standard Methods for the Examination of Water and Wastewater 9221 F, E, B (APHA, 1992; Anon, 2012), which were analyzed in the Microbiology Laboratory of the School of Biochemistry and Pharmacy of the Universidad de Concepción.

### 2.4. Macroinvertebrate Assemblages

The macroinvertebrates were sampled from the center of each channel using a Van Veen manual dredge (0.025 m<sup>2</sup> capacity), with three replicates per station. The samples were fixed in situ with 7% formalin and then transported to the laboratory, where the organisms were separated and preserved in 70% ethanol. All the individuals of each taxon were identified and counted under a stereomicroscope (Zeiss, model Stemi Dv4), and were identified to the lowest possible taxonomic resolution, using available taxonomic keys and reference collections [52–54].

### 2.5. Statistical Analysis

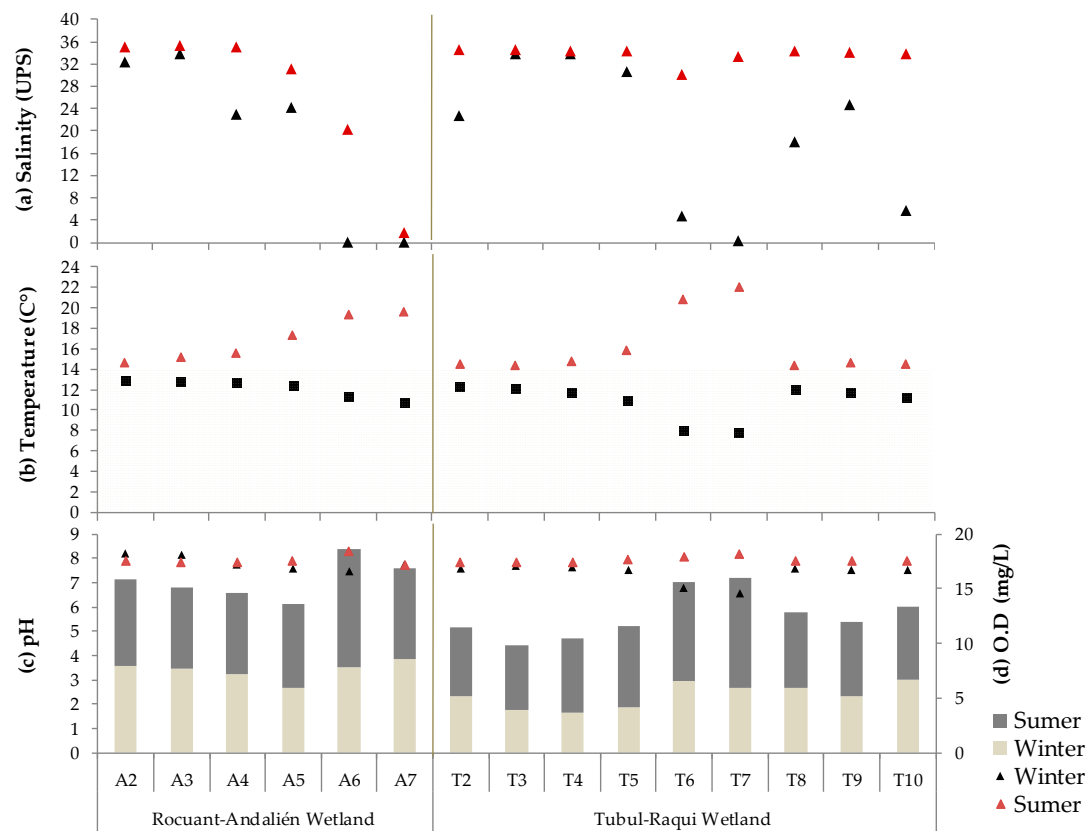
For the analysis of physical-chemical variables, a two-way repeated measures ANOVA model was fitted. In the cases where interaction was not significant, the main effects were analyzed; otherwise, contrasts were carried out, fixing the level of one factor and comparing the levels of the other factor. In addition, principal component analysis (PCA) and Spearman correlation analysis of the variables for both wetlands were carried out using the R program (R Core Team, 2016). Prior to the analyses, the physical-chemical data were normalized. To analyze the macroinvertebrate community structures of the wetlands, the richness (S) and abundance (N) indices were calculated, allowing the alpha ecological diversity to be determined; to this end, various indices were used comparatively to lend robustness to the results, i.e., the Shannon–Weiner index ( $H'$ ) (bits ind), Simpson index (C), Margalef index (Dmg), and Menhinick index (Dmn).

The macroinvertebrate assemblage data were used to identify differences between the sampling sites and/or seasonality. Canonical correlation analysis (CCA) was used to understand and analyze relationships in the assemblage structure and its distribution, along with the gradients of specific environmental factors. An analysis of variance was carried out to determine significant differences in abundance between the studied wetlands.

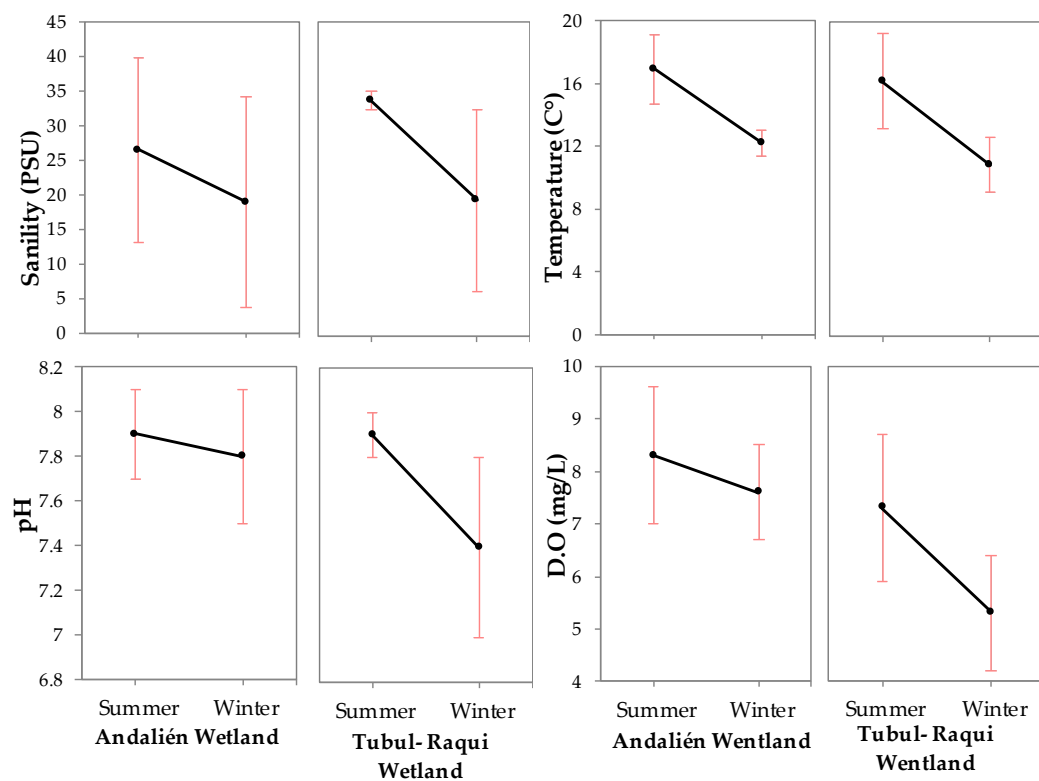
## 3. Results

### 3.1. Physical-Chemical and Bacteriological Parameters of Wetland Water Quality

Both wetlands maintained a marked marine and seasonality influence during the analyzed periods (winter, summer), with no significant differences between them in the variables of salinity ( $p = 0.448$ ) and temperature ( $p = 0.489$ ). The Rocuant-Andalién wetland presented significantly greater salinity levels in summer, with an average  $26.4 \pm 13.4$  PSU ( $p = 0.053$ ), until station A6 (20.15 PSU), while in winter, due to the fluvial input, the marine influence extended only to station A5 (4.2 km from the mouth), with 24.13 PSU at a depth of 1 m (Figures 2 and 3).



**Figure 2.** Parameters of (a) Salinity, (b) Temperature, (c) pH, and (d) DO at the observed stations in the Rocuant-Andalién and Tubul-Raqui wetlands, in summer and winter.



**Figure 3.** Graph of the model effects (Station × Wetlands effect plot).

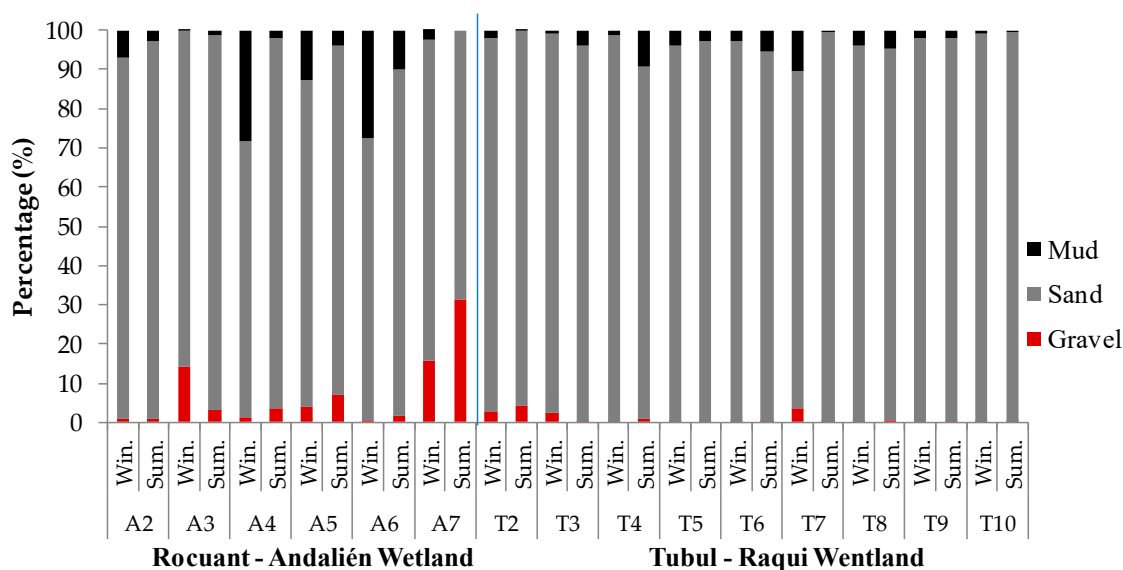


Likewise, in the Tubul-Raqui wetland, salinity was significantly greater in summer ( $33.7 \pm 1.4$  PSU) ( $p = 0.007$ ), with values  $> 30.02$  PSU until 6.8 km from the mouth of the Tubul River and until 3.8 km from the mouth of the Raqui River. At T2, a decrease in salinity (22.3 PSU) was observed, which is attributable to rainwater collector and sewer outfalls, while in winter the effect of salinity in both courses was lower, i.e., until 4.2 km from the mouth of the Tubul River and 2.7 Km from the mouth of the Raqui (Figure 2). Only in this wetland was salinity observed to be correlated with temperature ( $r = 0.623$ ;  $p = 0.004$ ) and pH ( $r = 0.641$ ;  $p = 0.006$ ).

The mean temperature in the Rocuant-Andalién saltmarsh was significantly higher in summer ( $17 \pm 2.2^\circ\text{C}$ ) ( $p = 0.011$ ), with the temperature gradient varying by approximately  $5.05^\circ\text{C}$  between the extreme stations; that is, near the mouth (A2 and A3) low temperatures were recorded ( $14.6$  and  $15.1^\circ\text{C}$ ), with temperatures increasing to  $19.6^\circ\text{C}$  in shallower areas with gentler currents (A7). The opposite was observed in winter, when the temperature decreased from the mouth to the innermost station (A7) (Figure 2). This was also the case in the Tubul-Raqui wetland, in which the summer temperature was significantly higher ( $16.2 \pm 3.0^\circ\text{C}$ ) ( $p = 0.0003$ ), with this variable correlated with pH ( $r = 0.944$ ;  $p = 0.001$ ) and DO ( $r = 0.706$ ;  $p = 0.001$ ).

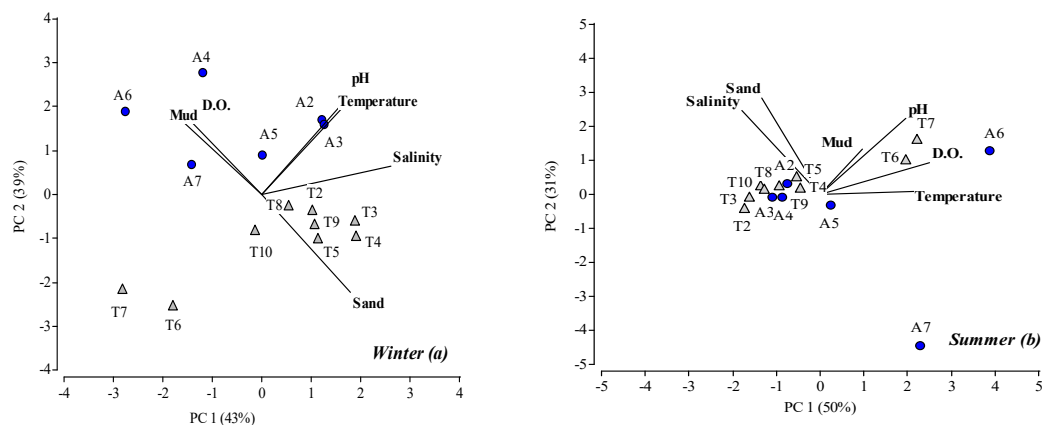
The pH and dissolved oxygen content variables presented clear differences between wetlands in accord with their degree of urbanization. The Rocuant-Andalién wetland reached significantly higher pH values in winter  $7.8 \pm 0.3$  ( $p = 0.05$ ) (Figure 3), while in Tubul-Raqui they were significantly greater in summer ( $7.9 \pm 0.1$ ) ( $p = 0.003$ ), a result of the variation and increase at stations T6 and T7 (Figure 2). Dissolved oxygen content was significantly greater in the Rocuant-Andalién wetland compared to the Tubul-Raqui wetland, a result of the increased concentration in winter (i.e.,  $7.6 \pm 0.3$  mg/L) ( $p = 0.066$ ) at stations A6 and T6 and T7 (Figures 2 and 3).

Regarding the granulometric composition of the Rocuant-Andalién wetland, it presented a significantly greater gravel percentage in winter and summer, i.e.,  $8.0 \pm 11.6\%$  and  $6.1 \pm 6.9\%$ , respectively ( $p = 0.008$ ), in contrast to what was observed in the Tubul-Raqui wetland, where the finer content fraction, mainly sand, was greater, i.e.,  $95.7 \pm 3.7\%$  and  $96.0 \pm 2.9\%$  ( $p = 0.04$ ) (Figure 4). In the Rocuant-Andalién wetland, the quantity of sand was correlated with the salt concentration ( $r = 0.825$ ;  $p = 0.001$ ). Regarding the recorded mud percentage, no significant differences between the wetlands in summer were observed ( $p = 0.990$ ), while in winter the quantity of mud increased significantly in the Rocuant-Andalién wetland, with  $13.1 \pm 12.2\%$  ( $p = 0.008$ ).



**Figure 4.** Granulometric analysis of the Rocuant-Andalién and Tubul-Raqui stations during summer and winter.

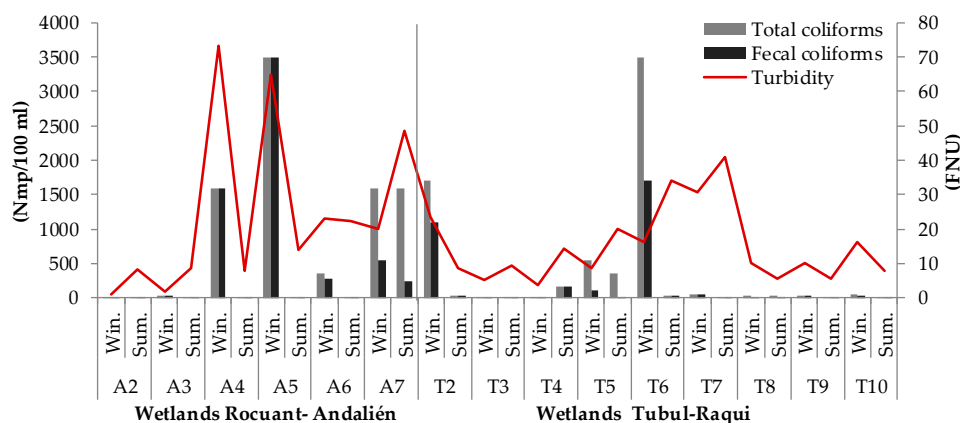
The principal component analysis allowed the functioning of the stations, along with their seasonality, to be compared on the basis of degree of urbanization and the recorded physical-chemical variables; it was observed that the first two components, i.e., PC1 and PC2, accounted for 82% of the total variance. In summer stations, T7, T6 and T10 were grouped together, as were A7, A6, A5 and A4. In winter, meanwhile, T7 and T6 were grouped together, along with A7 and A6 (tributary river sector), due to their greater continental influence (Figure 5).



**Figure 5.** Vector diagram that indicates the loading on PC1 and PC2 of the variables salinity, pH, temperature, dissolved oxygen content, mud, and sand during winter (a) and summer (b).

### 3.2. Biological Parameters

The microbiological water quality studies showed significant differences between the coastal wetlands in accord with their degree of urbanization. The total coliform concentration was greater in the Rocuant-Andalién wetland (more urbanized), with a mean of  $1179.1 \pm 84.4$  Mpn/100 in winter and  $270.1 \pm 162$  Mpn/100 in summer ( $p = 0.03$ ). The fecal coliform concentration was also higher, with an average in both periods of  $516.7 \pm 261.4$  Mpn/100, in comparison to the Tubul-Raqui wetland, which presented a concentration of  $184.1 \pm 114.1$  Mpn/100 ( $p = 0.001$ ). During summer, the concentrations at stations T6 and T2 in the latter wetland stood out, with a total coliform concentration of 3500 Mpn/100 mL and a fecal coliform concentration of 1700 Mpn/100 mL, respectively, which appear to be directly related to emissions from the urbanized sector (Figure 6). Both variables (fecal and total coliforms) in the Rocuant-Andalién wetland were observed to be correlated with pH ( $r = 0.68$ ;  $p = 0.015$ ) and turbidity ( $r = 0.75$ ;  $p = 0.005$ ). In addition, the observed turbidity was greater winter in the Rocuant-Andalién wetland ( $30.7 \pm 31.3$ ) and in summer in the Tubul-Raqui wetland ( $16.2 \pm 13.04$ ).

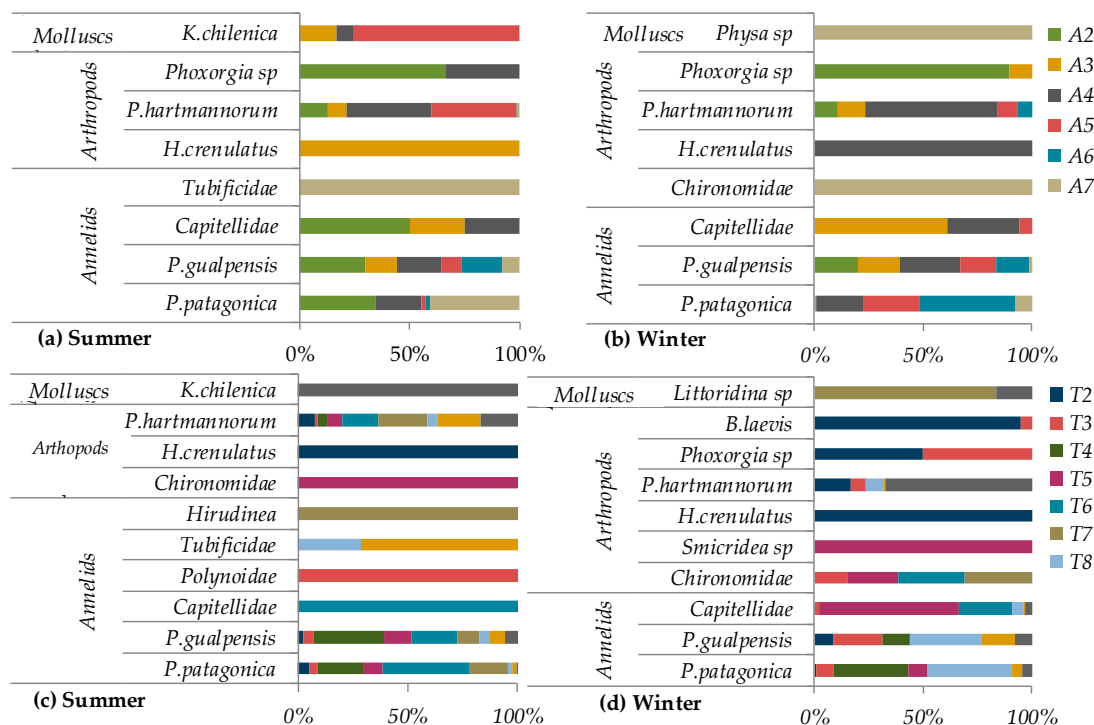


**Figure 6.** Diagram of coliform content and turbidity in both wetlands.



### 3.3. Macrovertebrate Taxonomic Composition, Abundance and Richness

In both study areas, a total of 15 taxa was observed, including six Annelida taxa, six Arthropoda taxa and three Mollusca taxa. Macroinvertebrate taxon richness was significantly greater in the Tubul-Raqui wetland compared to the Rocuant-Andalién wetland, with the Arthropod and Annelid phyla presenting the greatest number of taxa (Figure 7). Similarly, total macroinvertebrate abundance in the Tubul-Raqui wetland was significantly greater, i.e., 8.5 times greater, compared to that in the Rocuant-Andalién wetland (ANOVA  $F_{(1,28)} = 28.5$ ;  $p = 0.001$ ); in addition, abundance was greater in summer (7329 individuals per 0.025 m<sup>2</sup>), in contrast to the Rocuant-Andalién wetland, where it was higher in winter (2310 por 0.025 m<sup>2</sup>) (ANOVA  $F_{(1,28)} = 30.2$ ;  $p = 0.0007$ ) (Table 2, Figure 7).



**Figure 7.** Relative abundance of the macroinvertebrate community structure (a,b) Rocuant-Andalién wetland, (c,d) Tubul-Raqui wetland.

For both the Rocuant-Andalién wetland and the Tubul-Raqui wetland, the maximum Simpson's index values were recorded at the stations closest to freshwater, i.e., A7, A6, A5 and T10, T6. By contrast, the highest Shannon–Wiener diversity index values were recorded at the stations closest to the sea: A2 and A4 and T3. The Margalef- and Menhinick-specific richness indices showed low biodiversity values (<2) in both analyzed wetlands. The maximums were observed in intermediate stations, i.e., A4 and A3 and T3, T7 and T8. Absolute richness was greater in summer in the Rocuant-Andalién wetland ( $4.5 \pm 1.04$ ) and in winter in the Tubul-Raqui wetland ( $4.6 \pm 1.5$ ) (Table 2).

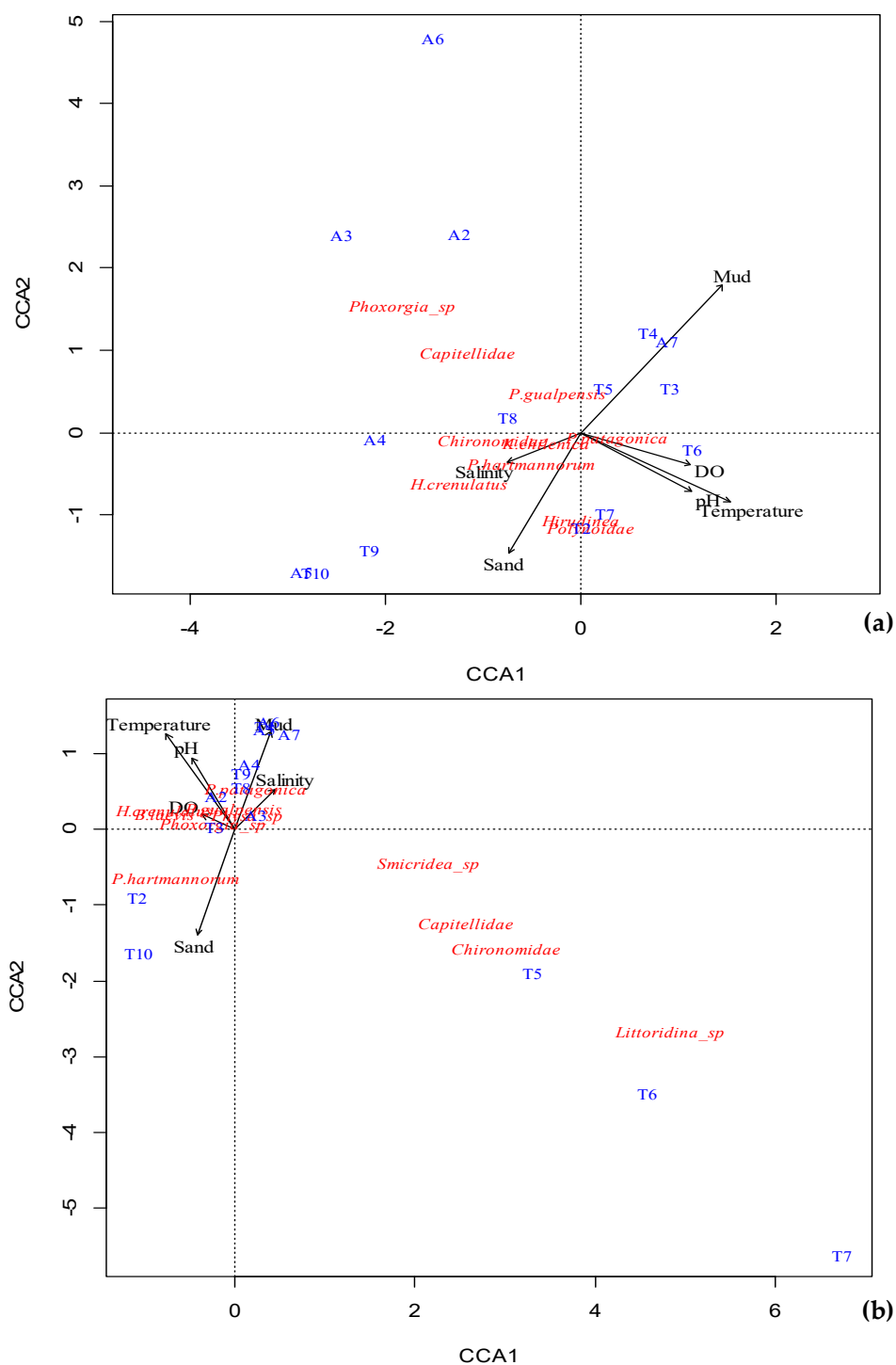
The canonical correspondence analysis showed that the first 2 components accounted for 92% of total macroinvertebrate variance throughout all the sites sampled in the summer. Mud was positively associated with axis 1, while sand was negatively associated. In general, all the Rocuant-Andalién wetland stations were positioned in the far left of axis 1, while the Tubul-Raqui stations were associated with the far right of axis 1. These latter stations were characterized by high mud content and temperature and low DO and pH. The greatest abundance of the polychaete *Prionospio patagonica* was associated with these sites. The amphipod *Phoxorgia* sp. and individuals of the polychaete family Capitellidae were found in greater abundance in the Rocuant-Andalién wetland, specifically at stations A3 and A2. Hirudinea and Polynoidae were observed in greater abundance at stations T7 and T2,

associated with a greater percentage of sand in the sediment. The other taxa, i.e., *Chironomidae* and *H. crenulatus*, along with *K. chilensis* and *P. hartmannorum*, were associated with high/low salinity.

**Table 2.** Total population abundance, Shannon–Wiener diversity index ( $H'$ ), Simpson's index ( $D$ ), richness of species according to the Margalef ( $D_{mg}$ ) and Menhinick indices ( $D_{mn}$ ) of the macroinvertebrates in the Rocuant-Andalién wetland ( $n = 7$ ) and Tubul-Raqui wetland ( $n = 10$ ) during summer and winter.

Rocuant-Andalién Wetland							Tubul-Raqui Wetland						
Station	Period	Total Abundance	Shannon	Simpson	Margalef	Menhinick	Station	Period	Total Abundance	Shannon	Simpson	Margalef	Menhinick
		N° of org.	(H')	(D)	(Dmg)	(Dmn)			N° of org.	(H')	(D)	(Dmg)	(Dmn)
A2	Summer	270	1.6	0.4	0.7	0.3	T2	Summer	388	1.4	0.4	0.5	0.2
	Winter	157	1.1	0.5	0.4	0.2		Winter	577	1.6	0.4	0.9	0.3
A3	Summer	113	1.3	0.5	0.8	0.5	T3	Summer	253	1.2	0.5	0.5	0.3
	Winter	144	1.3	0.6	0.8	0.4		Winter	432	1.9	0.3	1.0	0.3
A4	Summer	282	1.6	0.4	0.9	0.4	T4	Summer	1432	1.2	0.5	0.3	0.1
	Winter	585	1.4	0.4	0.6	0.2		Winter	529	0.8	0.7	0.3	0.1
A5	Summer	203	1.2	0.5	0.6	0.3	T5	Summer	669	1.4	0.4	0.5	0.2
	Winter	517	0.8	0.7	0.5	0.2		Winter	319	1.1	0.5	0.5	0.2
A6	Summer	95	0.3	0.9	0.4	0.3	T6	Summer	2220	1.1	0.6	0.4	0.1
	Winter	784	0.6	0.8	0.3	0.1		Winter	86	0.6	0.8	0.7	0.4
A7	Summer	214	1.6	0.4	0.6	0.3	T7	Summer	1234	1.4	0.4	0.4	0.1
	Winter	133	0.6	0.8	0.6	0.3		Winter	10	1.4	0.4	0.9	0.9
	Summer	1177					T8	Summer	236	1.6	0.3	0.5	0.3
	Winter	2320						Winter	937	1.6	0.4	0.4	0.1
							T9	Summer	512	1.4	0.5	0.5	0.2
								Winter	194	1.3	0.5	0.6	0.3
							T10	Summer	385	1.1	0.6	0.5	0.2
								Winter	1564	0.5	0.8	0.5	0.1
									Summer	7329			
									Winter	4648			

In winter, the first two components, CCA1 and CCA2, explained 76.6% of total macroinvertebrate variance throughout all the sampled sites. As in summer, mud and sand were associated positively and negatively with axis 1. In contrast to what occurred at the sampling sites in the two wetlands, mud and sand were not clearly separated by axes 1 and 2. *P. patagonica*, *P. gualpensis* and *Physa* sp. were associated with stations T9, A4, A5, T4, and A6, characterized by high salinity and mud content. *H. crenulatus*, *B. laevis* and *Phoxorgia* sp. species were associated with high/low temperature and pH and greater/lower dissolved oxygen content, conditions found at stations A2 and T3. Finally, *P. hartmannorum* was abundant at stations with high sand content (T2, T10) (Figure 8).



**Figure 8.** Canonical correspondence analysis (CCA) triplot for macroinvertebrates in summer (a) and winter (b). These plots illustrate the relationships between environmental variables and individual taxa for sampling stations in the Rocuant-Andalién and Tubul-Raqui wetlands.

#### 4. Discussion

Analysis of biophysical processes in wetland systems is highly complex because they involve dynamic interactions between physical–chemical variables and biotic communities; it is even more complex if the systems are modified and/or under pressure from human settlements, like those analyzed in this study [55]. The results of this investigation present an account of the negative impacts in a wetland system caused by urbanization.

#### 4.1. Physicochemical Parameters

Surface water temperature presented marked seasonality in both wetlands. In summer, freshwater varied between 8 °C and 14 °C, and was higher mainly in shallow areas, in contrast to winter, when the temperature was greater at stations close to the mouth due to the stabilizing effect of the sea. In addition, the increase in surface water temperature regulates energy flows and evaporation rate increases, thereby affecting the water level [20,34,56], as well as influencing the generation of secondary salinization processes, mainly in urban wetlands, as recently described in wetlands of western Australia [57], South Africa [58] and North America [59]. The increase in salinity due to evaporation can become a serious threat to ecological structure and functioning. It is feared that climate change, with an increase in average temperatures, and anthropogenic alterations could alter the fundamental physical–chemical nature of the soil–air environment, increasing ion concentrations and transforming the chemical equilibrium and the solubility of minerals in wetlands [32].

The effects of salinization on the biogeochemistry of wetlands could contribute to a decrease in dissolved oxygen content and nitrates [60]; a reduction in carbon storage [61]; greater sulfide and ammonium (reduced form of nitrogen) generation; alterations in the phosphorus, sulfur, iron, and silica cycle [62], and therefore alterations in the development of biota [63,64] and water quality and regulation processes.

The Rocuant-Andalién and Tubul-Raqui wetlands maintain a marked marine influence; however, in summer the saline influence reaches higher concentrations and a greater extension, i.e., over 5.3 km from the mouth, correlated with temperature. In addition, low depths in summer contribute to the entry of atmospheric oxygen due to the influence of the south wind, increasing the oxygenation capacity of the systems, in contrast to the clear fluvial influence during winter due to the microtidal condition and seasonal freshwater inputs [65].

In both watersheds, the rainfall regime determines the expansion of the flood area; thus, any extreme climate event, as well as urban growth, can drastically alter long-term hydrological and biogeochemical responses [66,67]. Likewise, pH responds to water level variations and increases at stations with freshwater influence, which is greater in summer in the Tubul-Raqui wetland. pH values over 7 indicate alkaline wetlands, thus regulating soil organic matter turnover, which is directly related to dissolved and oxidizable organic carbon [68,69], which is associated with the selective influence of pH during the organic matter decomposition process [70] and on multiple sediment parameters [71–75].

Sediment transported by freshwater or seawater causes substantial changes in the texture of fluvial sediment [76]. In winter, significant increases in mud content in the Rocuant-Andalién wetland and gravel content in the Tubul-Raqui wetland were observed. Likewise, the sand percentages are correlated with salinity values or oceanic influence. It has been described that sand predominates in areas subject to significant energy from estuarine waves or with greater erosion [77].

The high turbidity values observed in the Rocuant-Andalién wetland are characteristic of urban wetlands and could result from sediment resuspended by waves in situ, which is attributable to changes in the seasonal water regime, as indicated by Davis et al., [78]. However, turbidity can also be influenced by the salinity of the ecosystem, since divalent cations in saltwater (i.e.,  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$ ) add suspended matter prior to the flocculation process in sediment [79]. Improved flocculation increases water transparency [32,78]. Meanwhile, in the Tubul-Raqui wetland, greater turbidity is observed at stations T6 and T7, where the water level is lower (summer), possibly due to the increase in sediment resuspension caused by wave action and the south wind, coinciding with the maximum dissolved organic oxygen recorded in these areas.

#### 4.2. Biological Parameters

Coliforms in wetlands increase mainly as a (direct or indirect) result of urban runoff loading and resuspended sediment. Urban runoff controls concentrations and significantly influences bacteria distribution in the water column; therefore, the loading due to sediment resuspension explains the generation of this type of fecal indicator bacteria (FIB) in wetland systems [80,81]. Recent studies

demonstrate that sediment, sand and mud can be heavily colonized by FIB, as these colonies are associated with sediment particles and colloidal organic matter or are free-living in water when bottom sediments are disturbed or swept by tides. It is probable that the relative magnitude of the effects of resuspension versus the effects of sedimentation determines whether coastal wetlands are net generators or accumulators of colonies [82]. The results of this study help to determine the impact of urban runoff and its relationship with high FIB concentrations, as observed in the more anthropized wetland (Rocuant-Andalién), where total and fecal coliforms were also found to be related to turbidity (resuspension) and the pH of the ecosystem.

It has been observed in many urban aquatic systems that in the case of wetlands, elevated FIB levels are related to urban runoff such as the discharge or overflow of untreated or incompletely treated wastewater [83], and their contamination raises significant concerns regarding public health and their management for recreational uses. While wastewater from a point source can transport many types of contaminants (e.g., metals, excess nutrients, pharmaceutical products) to a receiving water body, the bacteria and viral pathogens associated with feces present the greatest risk of disease. The Rocuant-Andalién wetland reached maximum total and fecal coliform concentrations of 3500 (Mpn/100 mL). Although there is no specific regulation for wetland protection in Chile, these values drastically exceed those indicated in the secondary environmental quality standard for the protection of continental surface water, i.e., a fecal coliform concentration of 1000 Mpn/100 mL and a total coliform concentration of 2000 Mpn/100 mL, indicating microbiological water contamination and deterioration. This environmental quality indicator allows a more precise assessment of the ecosystem to aid in the development of more precise environmental management policies and the setting of limits.

#### 4.3. Macroinvertebrate Assemblages

The macroinvertebrate composition observed in Tubul-Raqui reference wetland was similar to those reported in estuaries and coastal lagoons with little human intervention [84,85]. Our study corroborates that urbanization has negative impacts on the assemblage structure of the macroinvertebrates that inhabit wetlands, as has been demonstrated in other freshwater wetlands in the region [86]. Specifically, we observed greater richness and abundance in the Tubul-Raqui wetland, which presents less human intervention than the Rocuant-Andalién wetland. It is known that habitat productivity and heterogeneity are determining factors in the community level component, along with energy availability [87,88]. However, species richness and abundance can also be heavily affected by other external disturbances such as pollution generated by anthropogenic pressures [89], as observed in the Rocuant-Andalién wetland (i.e., channelization, entry of surface water runoff, wastewater, etc.). Using CCA, it is possible to verify that the use of macroinvertebrates as bioindicators aids in differentiating or characterizing each wetland, also reflecting the influence of seasonality on the aquatic biota [90,91]. Seasonality has been shown to be a key factor in the structuring of the macroinvertebrate assemblage, suggesting that biomonitoring should be carried out in low-flow periods, when habitats are more stable [24].

Understanding of the distribution and abundance of these macroinvertebrate taxa is essential for assessing the functions of natural systems, as these organisms contribute to the decomposition of organic matter and support the food web, which are key in the productivity of upper trophic levels [92,93]. Expanding urban development has intensified anthropogenic pressures, leading to the destruction of coastal wetland equilibriums, with degraded water quality and loss of biodiversity observed, although the surface water at the sampled points of the wetlands does not present significant decreases in dissolved oxygen content or anoxic zones.

Finally, urban wetland management planning is a dynamic, participatory process unique to each place [94,95]. To identify and analyze the important particulars of fragile ecosystems such as wetland systems, it is necessary to propose clear objectives and agree on protection goals. Planning processes require joint learning among all actors and must be described and evaluated considering laws, land requirements for urbanization and the benefits of conserving a wetland.

## 5. Conclusions

Our results suggest that the Rocuant-Andalién and Tubul-Raqui wetlands maintain a marked marine influence. In summer, the saline influence reaches a greater area and concentration, although it does not seem to influence dissolved oxygen content given the low average depth of both systems, i.e.,  $\geq 1$  m. The sediment transported by freshwater causes substantial changes in the estuary channels; this is especially significant in the mud content and high turbidity values observed in the Rocuant-Andalién wetland, which is characteristic of urban wetlands and is also associated with high total and fecal coliform concentrations resulting from illegal discharges or possible leaks in the wastewater network of the city of Concepción.

In both watersheds, the rainfall regime determines the expansion of the flood area, such that any extreme climate event, along with urban expansion into both wetlands, can drastically alter long-term hydrological and biogeochemical responses. The benthic macroinvertebrate diversity and richness indices were lower in the highly urbanized Rocuant-Andalién wetland, which has undergone changes in the structure and composition of these organisms, compared to the wetland with less intervention, which presented an abundance 8.5 times greater and an increase in specific richness. Thus, the analysis of macroinvertebrate assemblage structure in a wetland can be a good indicator of these complex ecotones.

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