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Evidence of the Anthropic Impact on a Crustacean Zooplankton Community in Two North Patagonian Lakes

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Abstract: Lately, agriculture, livestock, forestry, and aquaculture activities have been greatly developed in Chilean North Patagonia, negatively impacting the balance of the environmental conditions in lakes and affecting the development and survival of several native species. The aim of this study was to assess the anthropic impact on a zooplankton community in two North Patagonian lakes. We collected samples from four sites belonging to Lake Icalma and Lake Llanquihue, including four replicates per site. Water samples were analyzed for physicochemical characteristics and zooplankton communities. We focused on the presence of *Daphnia pulex*, a species of zooplanktonic crustacean that performs a key role in capturing energy from primary producers to deliver it to final consumers such as fish. We found that Llanquihue showed higher total phosphorus, nitrogen, copper, iron, manganese, total dissolved solids (TDS), and conductivity (EC) than Icalma. Furthermore, ecological variables were greatly decreased due to total P, total N, manganese, copper, total dissolved solids, and conductivity, which changed the species dominance of the zooplankton community in Llanquihue, indicating some degree of anthropization. This study provides fundamental information on the anthropogenic impact on water quality, as well as on zooplankton diversity, highlighting the importance of monitoring the health of these North Patagonia freshwater ecosystems.

Keywords: impact assessment; water quality; North Patagonian lakes

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Citation: Norambuena, J.-A.; Poblete-Grant, P.; Beltrán, J.F.; De Los Ríos-Escalante, P.; Farías, J.G. Evidence of the Anthropic Impact on a Crustacean Zooplankton Community in Two North Patagonian Lakes. *Sustainability* **2022**, *14*, 6052. https://doi.org/10.3390/su14106052

Academic Editor: Tommaso Caloiero

Received: 8 March 2022
Accepted: 9 May 2022
Published: 17 May 2022

1. Introduction

Anthropogenic pressure associated with economic development and industrialization has generated pollution in freshwater bodies such as rivers and lakes [1]. It has been estimated that 54% of lakes in Asia and the Pacific, 53% of lakes in Europe, 28% of lakes in North America, and 41% of lakes in South America exhibit some degree of pollution [2,3]. This economic development and industrialization has led to the intensification of different anthropogenic activities located in watersheds, mainly agriculture, livestock, forestry and aquaculture, which affect water bodies [4,5]. In fact, Lake Bénit, in the French Alps, showed oligotrophic characteristics until the 20th century; however, due to the intensification of different activities, mainly agricultural and livestock farming, phosphorus (P) concentrations have increased, causing its eutrophication [6]. Eutrophication involves an increase in nutrients, mainly phosphorus (P) and nitrogen (N), in freshwater bodies, leading to a rapid expansion of algae that consequently alters their zooplankton community balance and water quality [3]. The level of eutrophication can be accelerated by the effect of pollution associated with industrial waste, negatively affecting aquatic habitat heterogeneity and leading to biodiversity loss [7]. In fact, lakes in the United States reduced their oligotrophic state from 25 to 7% in just 5 years, showing signs of eutrophication associated with anthropogenic effects [8].

In South America, specifically in Chilean North Patagonia, there are lakes that were characterized by their morphology, physicochemical properties, environmental conditions, and biodiversity as oligotrophic lakes in the 1980s and 1990s [9–16]. However, in Chilean North Patagonia, a significant expansion of different economic activities has been observed in recent decades, such as agriculture, livestock, forestry, and aquaculture [17]. These activities are justified by the existing irrigation infrastructure and crops, favorable climatic conditions, as well as the opening to external markets [4,18–23]. These anthropogenic activities have polluted the soil and aquatic environment due to their production processes not having considered sustainable resource management.

This anthropogenic pressure on freshwater bodies has been mainly characterized by records of high concentrations of N, P, changes in chlorophyll-a (Ch-a) concentrations associated with microalgae populations, low pH values and decreases in dissolved oxygen (DO), which can cause changes in zooplankton community structure, affecting the entire aquatic trophic web [24–29]. Zooplankton play a fundamental role in freshwater ecosystems as they carry the energy captured by primary producers to species of higher trophic levels such as fish [30]. This group of invertebrates is structured depending on the type of trophies that exist in the aquatic environment [31]. An example of this is *Daphnia pulex* (species of the order Cladocera), which feeds on microalgae, and its abundance is greater in mesotrophic lakes or lakes undergoing eutrophication, since the presence of microalgae is greater in such ecosystems [28,32]. The opposite is the case of oligotrophic lakes, which have low trophic levels reflected in their low concentrations of N and P, as well as low concentrations of chlorophyll-a, in which species of the order Copepoda, such as *Boeckella gracilipes* and *Mesocyclops longisetus*, are dominant [13,33].

Ecological studies analyzing the biodiversity and trophic role of zooplankton in oligotrophic freshwater ecosystems in Chile have described a low number of endemic and cosmopolitan species, where *Daphnia pulex* is located [11,33–35]. However, according to what was described above, in recent decades, anthropogenic activities could have changed the trophic status of some aquatic ecosystems in North Patagonia. Consequently, some evidence comprising increases in N and P levels as well as changes in the composition of phytoplankton and zooplankton communities could be found [4,12,36–39]. Changes in the composition of zooplankton communities have been observed in alpha and beta diversity variables, showing a decrease in the abundance and species richness of zooplankton as well as different values in the Shannon and Weaver diversity index, which is related to fluctuations of freshwater physical and chemical conditions [40–42]. In addition, this change in species composition, due to the effect of anthropogenic activities, has been observed in measurements of alpha and beta diversity variables on benthic macroinvertebrates of North Patagonian rivers, determining, together with the family biotic index, the biological quality of the water [43].

Thus, given the characteristics of North Patagonian lakes, the study of the impact of anthropogenic activities on the food web balance and water quality offers a scientific contribution to the monitoring of the health of the freshwater ecosystem. Comparing deep lentic ecosystems of the same glacial and volcanic origin at a different trophic level would provide some evidence of the degree to which the existing aquatic biodiversity is being affected by humans. Thus, the aim of this study was to assess the anthropic impact on the zooplankton community of two North Patagonian lakes.

2. Materials and Methods

2.1. Study Area

The study area is two Chilean North Patagonian lakes located in La Araucanía and Los Lagos Regions (Figure 1), Chile. The first is Lake Icalma (38°48′ S and 71°17′ W), an oligotrophic lake with very low anthropogenic interference (Figure 2a). The second is Lake

Llanquihue (41°08′ S and 72°47′ W) (Figure 2b). Both have been described as being under intense anthropogenic pressure due to economic activities such as agriculture, livestock, forestry, and aquaculture [9,18,34,44–47]. Both lakes are in a closer geographic unit, originated from glaciers, and they share similar environmental characteristics, being part of the North Patagonian lakes associated with the temperate Andean evergreen and deciduous forests of *Nothofagus obliqua*, *Araucaria araucana*, and *Fitzroya cupressoides* [15,47–51].

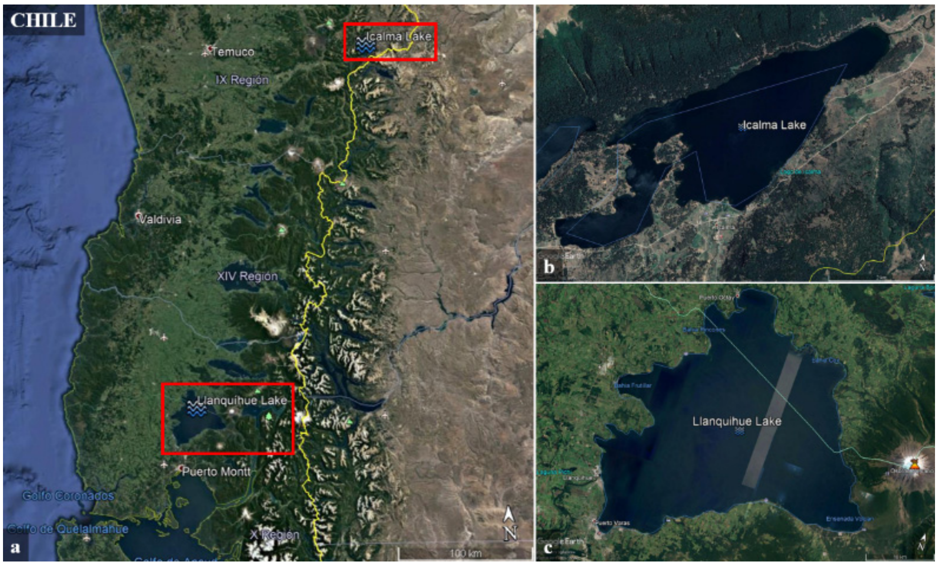

Figure 1. North Patagonian area of Chile (38°S, 71° W; 41° S, 72° W), area situated between the regions of La Araucanía and Los Lagos (**a**,**b**) Lake Icalma, and (**c**) Lake Llanquihue, where *Daphnia pulex* is found. Picture obtained from Google Earth Pro 7.3.2.5776.

(**a**) (**b**)

Figure 2. Sampling sites Lake Icalma (**a**) and Lake Llanquihue (**b**), geographic details are shown in Table 1. Picture obtained from Google Earth Pro 7.3.2.5776.

2.2. Water and Zooplankton Sampling

Sampling in the two North Patagonian lakes was carried out during the first weeks of March 2020, the period of the highest abundance of *Daphnia pulex* [52]. Water and zooplankton samples were taken at four sampling sites per lake (Figure 2a,b; Table 1) and four replicates at each sampling site for physicochemical analysis and chlorophyll-a determination. Water samples were collected using a Van Dorn device at 10 m depth [52,53]. In addition, one effluent was detected which directly discharged wastewater into Lake Llanquihue (Figure S1). Thus, a sample with three replicates of water from the effluent close to Puerto Varas bay was collected at the same time of water lake sampling to assess its physicochemical properties. All water samples were stored in 1 L Van Dorn bottles at 4 °C (±0.5) during sampling and transport, and then stored in the laboratory at −27 °C (±0.5).

Table 1. Sampling sites in the North Patagonian lakes Icalma and Llanquihue.

Lake	Sampling Site	Location	Detail
Icalma	I_1	38°47′42.2″ S; 71°16′10″ W	
	I_2	38°47′0.9″ S; 71°16′11″ W	
	I_3	38°48′21″ S; 71°17′0.7″ W	Close to Icalma
	I_4	38°48′53″ S; 71°18′24″ W	
Llanquihue	LL_1	41°17′42.2″ S; 72°52′46″ W	Close to Puerto Rosales
	LL_2	41°19′11.5″ S; 72°57′29.2″ W	
	LL_3	41°19′17.5″ S; 72°57′53.5″ W	Close to Puerto Varas bay
	LL_4	41°18′57.9″ S; 72°58′39.6″ W	Close to Puerto Varas bay

In addition, to characterize the crustacean zooplankton community, samples were collected at 20 m depth using a Nansen net of 20 cm diameter and 200 µm mesh, suitable for macro- and mesozooplankton, according to the methodology described by De Los Ríos-Escalante [52] and Meddeb et al. [54]. Each sample was collected in a 50 mL centrifuge tube (Falcon) fixed in 70% ethanol to preserve the zooplankton species, as described by Black and Dodson [55]. A total of four replicates per site was collected for the ecological analysis of zooplankton communities. The two lakes in this study do not belong to the National System of State Protected Areas, and the species *Daphnia pulex* is not classified as endangered according to the criteria of the International Union for Conservation of Nature (IUCN). Some of the analyses were carried out following [56] criteria, as well as according to Woelfl et al. [53], including sampling, water sample analysis, and zooplankton analysis.

2.3. Physicochemical Characterization of Water Samples

For each sampling site in the lakes as well as for the effluent samples, the following parameters were recorded in situ: water column transparency (m), temperature (°C), pH, conductivity (µS cm^{-1}), total dissolved solids (TDS) (mg L^{-1}), and dissolved oxygen (DO) (mg L^{-1}) using the WTW Multi 340i multiparameter probe following the Standard Methods for the Examination of Water and Wastewater (APHA, AWWA & WEF) [57]. In addition, water samples were taken from the water column for the analytical analysis of chlorophyll-a (µg L^{-1}), which was carried out according to Wetzel and Likens [58], and was based on extraction with acetone and measured in a spectrophotometer at 665 and 750 nm. The water concentrations of total phosphorus (P), total nitrogen (N), ammonium (NH^{4+}), nitrates (NO^{3-}), nitrites (NO^{2-}), calcium (Ca^{2+}), aluminum (Al), copper (Cu), iron (Fe), and manganese (Mn) were identified using a Hanna Hi801 UV–Vis spectrophotometer at 340 to 900 nm range according to the methodologies detailed in Table 2.

Table 2. Methods of physicochemical variables measured.

Variable	Method
Total P	Adaptation EPA (1983) and APHA, AWWA & WEF [57].
Total N	Chromotropic acid method APHA, AWWA & WEF [57].
Ammonia (NH_4^+)	Adaptation Nessler Method [59].
Nitrate (NO_3^-)	Adaptation of the Cadmium reduction method [60].
Nitrite (NO_2^-)	Adaptation of the EPA diazotization method [60].
Calcium (Ca^{2+})	Adaptation of the Oxalate method [61].
Aluminum (Al)	Adaptation of the aluminum method [62].
Copper (Cu)	Adaptation EPA [60].
Iron (Fe)	Adaptation of the TPTZ method [63].
Manganese (Mn)	Adaptation of the PAN method [64].

2.4. Analysis of Zooplankton Diversity

Zooplankton species were identified through a stereomicroscope at 10× magnification; in particular, the species *Daphnia pulex* was classified according to the descriptions of Araya and Zúñiga [65] and Hebert [66]. The presence of other species was also quantified by individual counts for each taxon to determine ecological variables of species richness (S', Taxa m^{-3}), which consists of the number of species found in a sample, total abundance (Ni', individual m^{-3}), which corresponds to the total number of individuals in a sample, and specific abundance (ni', individual m^{-3}), which is the total number of individuals of a particular species [67]. These variables were used to calculate alpha and beta diversity, according to Moreno [40]. Alpha diversity is the local diversity or diversity of a given environment [40]. Here, this ecological variable was measured as the Shannon and Weaver index [68] (H' (nats)). This index is compared with a maximum Shannon ($H'max$), estimated using the values of the natural logarithm of richness (Ln (S')). According to Krebs [67], this index combines total abundance (ni'), species richness (S'), and evenness (J'), where evenness is measured according to Pielou [69]. Beta diversity, which is the ratio of the alpha diversity of different localities or environments, was estimated by the biocenotic similarity of the zooplankton community present in the lakes and measured using Bray–Curtis cluster analysis along with the complete linkage method [40,70].

2.5. Statistical Analysis

The data obtained were checked for normality (Shapiro–Wilk test) and homogeneity of variance (Levene test). Significant differences were analyzed using a parametric ANOVA test (95% significance level) and then compared using Tukey's test (HSD). The significant relationships detected were analyzed using Pearson's correlation analysis. Significance was set at $p \leq 0.05$. Statistical tests were performed using R Foundation for Statistical Computing Version 3.6.3 (R Development Core Team 2009–2018). Using the Factoextra package of R software, a principal component analysis (PCA) was performed, considering the lakes and the stations in the lakes as groups. Models were made by selecting the predictors from a previous model considering all the variables; finally, a simplified model was generated. Then, normality and homogeneity were checked using "nortest and lmtest" packages. Likewise, the "vif" function from the "car" package was used to test collinearity. The model was selected based on its R-adjusted value as well as the Akaike information criterion (AIC).

3. Results

3.1. Anthropogenic Economic Activities Increase the Nutrient and Heavy Metal Concentration in North Patagonian Lakes

The environmental variables obtained in this study are shown in Table 3. Lakes Icalma and Llanquihue have a difference of ±1 °C in water body temperature (ANOVA, Lakes: $F = 894.4$, $p < 0.001$). In general, all sampling sites in Lake Icalma showed a transparency of the water column at a depth of 20 m; the opposite was observed in Lake Llanquihue, where a lower transparency of the water column was observed at all sampling sites (10 m). This lower transparency is related to an increased concentration of total dissolved solids (TDS) in the aquatic environment, registering significantly higher concentrations of TDS in Lake Llanquihue (Lakes, $F = 35465$, $p < 0.001$); site LL_4, which is located in front of Puerto Varas bay, showed significantly higher and different values than all the other sampling sites. Electric conductivity (EC) behaved in the same way as TDS, showing higher values in Llanquihue than in Icalma. Among the sites (Lakes, $F = 6851$; $p < 0.001$), I_3 showed the lowest value in Icalma, and non-significant differences were observed in Llanquihue, while LL_4 showed a higher EC (91.20 µS cm^{-1}) than the other sampled sites.

The water in Lake Llanquihue was more acidic than Lake Icalma, with significantly lower pH values (Lakes, $F = 2185$, $p < 0.001$). The highest dissolved oxygen (DO) value was recorded for the LL_1 site, followed similarly by LL_4 and I_2, then I_1 and I_3, and finally I_4, LL_2, and LL_3, which obtained the lowest values (Lakes, $F = 1005$, $p < 0.001$). Chlorophyll-a (Ch-a) values were significantly higher in Icalma (Lakes, $F = 36.65$, $p < 0.001$) (I_2, I_3, and I_4) than in Llanquihue (LL_2 and LL_4). Its highest average concentration was recorded at site I_2 (6.30 µg L^{-1}), in contrast to sites I_1 (2.14 µg L^{-1}) in Icalma and site LL_2 in Llanquihue (2.68 µg L^{-1}), where the lowest concentrations were obtained.

Table 3. Physicochemical variables measured in situ in the sampling sites of the North Patagonian lakes Icalma and Llanquihue in conjunction with laboratory-measured chlorophyll-a (Ch-a) parameters. Average values ± standard deviation. Lower case letter indicates significant differences ($p \leq 0.05$), including the interaction of site × lake.

Lake		**Icalma**								**Llanquihue**							
Site		**I_1**		**I_2**		**I_3**		**I_4**		**LL_1**		**LL_2**		**LL_3**		**LL_4**	
Secchi Disc	m	20 ^{a}		20 ^{a}		20 ^{a}		20 ^{a}		10 ^{b}		10 ^{b}		10 ^{b}		10 ^{b}	
Temperature	°C	19.7 ^{a}	±0.05	19.8 ^{a}	±0.05	19.7 ^{a}	±0.05	19.7 ^{a}	±0.05	18.8 ^{b}	±0.05	18.7 ^{b}	±0.05	18.8 ^{b}	±0.05	17.8 ^{c}	±0.05
pH		7.19 ^{d}	±0.02	7.71 ^{a}	±0.01	7.43 ^{c}	±0.01	7.59 ^{b}	±0.03	6.02 ^{f}	±0.005	6.05 ^{f}	±0.02	6.28 ^{e}	±0.07	6.04 ^{f}	±0.03
EC	µS cm^{-1}	52.8 ^{c}	±0.86	53.3 ^{c}	±1.03	47.4 ^{d}	±0.14	52.7 ^{c}	±0.09	88.7 ^{b}	±0.05	89.2 ^{b}	±0.05	89.7 ^{b}	±0.08	91.2 ^{a}	±0.36
Ch-a	µg L^{-1}	2.14 ^{e}	±0.13	6.30 ^{a}	±1.01	4.01 ^{b}	±0.40	4.06 ^{b}	±0.04	3.88 ^{bc}	±0.02	2.68 ^{de}	±0.01	3.56 ^{bcd}	±0.39	2.93 ^{cde}	±0.18
TDS	mg L^{-1}	23.6 ^{d}	±0.33	19.8 ^{f}	±0.08	21.7 ^{e}	±0.05	27.6 ^{c}	±0.05	44.7 ^{b}	±0.05	44.7 ^{b}	±0.05	44.8 ^{b}	±0.00	45.7 ^{a}	±0.05
DO	mg L^{-1}	8.55 ^{c}	±0.01	8.78 ^{b}	±0.02	8.47 ^{d}	±0.02	8.27 ^{e}	±0.03	9.11 ^{a}	±0.02	8.22 ^{e}	±0.02	8.17 ^{f}	±0.01	8.78 ^{b}	±0.02
Ca^{2+}	mg L^{-1}	157.25 ^{c}	±0.50	178.75 ^{a}	±0.50	176.75 ^{ab}	±0.50	174.75 ^{b}	±1.50	108.50 ^{e}	±1.00	112.25 ^{d}	±0.50	114.00 ^{d}	±1.63	113.25 ^{d}	±1.26
Total P	mg L^{-1}	0.02 ^{d}	±0.01	0.05 ^{cd}	±0.01	0.04 ^{d}	±0.01	0.04 ^{d}	±0.01	0.09 ^{b}	±0.02	0.07 ^{bc}	±0.01	0.08 ^{b}	±0.01	0.12 ^{a}	±0.01
Total N	mg L^{-1}	0.15 ^{e}	±0.06	0.18 ^{e}	±0.05	0.58 ^{cd}	±0.17	0.25 ^{de}	±0.06	1.93 ^{a}	±0.10	0.70 ^{c}	±0.12	1.40 ^{b}	±0.28	1.83 ^{a}	±0.17
Fe	mg L^{-1}	0.02 ^{a}	±0.002	0.01 ^{b}	±0.001	0.01 ^{b}	±0.002	0.01 ^{c}	±0.001	0.01 ^{b}	±0.002	0.01 ^{c}	±0.001	0.01 ^{c}	±0.001	0.02 ^{a}	±0.001
Al^{3+}	mg L^{-1}	0.00 ^{ab}	±0.01	0.01 ^{ab}	±0.01	0.00 ^{ab}	±0.01	0.00 ^{b}	±0.00	0.01 ^{a}	±0.01	0.00 ^{ab}	±0.01	0.00 ^{ab}	±0.01	0.01 ^{ab}	±0.01
Cu	µg L^{-1}	10.25 ^{cd}	±1.50	4.25 ^{e}	±0.50	14.00 ^{bc}	±2.00	9.50 ^{d}	±1.73	14.00 ^{bc}	±2.00	18.00 ^{b}	±2.00	28.75 ^{a}	±1.50	18.00 ^{b}	±2.00
Mn	µg L^{-1}	5.50 ^{bc}	±0.58	3.00 ^{cd}	±0.00	6.25 ^{b}	±0.50	2.50 ^{d}	±0.58	13.50 ^{a}	±1.73	13.50 ^{a}	±1.73	14.25 ^{a}	±0.96	14.50 ^{a}	±1.73

The water chemical variables associated with anthropization were measured in both lakes, including total phosphorus (total P) (ANOVA, F = 34.68, $p < 0.001$), total nitrogen (total N) (F = 102.4, $p < 0.001$), calcium (Ca^{2+}) (F= 4023, $p < 0.001$), and heavy metals such as iron (Fe) (F = 49.43, $p < 0.001$), aluminum (Al^{3+}) (F = 2.651, $p = 0.03$), copper (Cu) (F = 72.39, $p < 0.001$), and manganese (Mn) (F = 82.81, $p < 0.001$), which are shown in Table 3. We observed that there was a significant difference within lakes. In fact, at the Llanquihue sites, the concentration of total N was 5.6-fold higher than the values obtained by Lake Icalma. In addition, the Cu concentration was 2.0-fold higher, and the Mn concentration was 3.2-fold greater compared to Lake Icalma.

3.2. Zooplankton Diversity Negatively Affected in North Patagonian Lakes under Anthropogenic Economic Activities

A total of three species were recorded in the crustacean zooplankton community of lakes Icalma and Llanquihue (Table 4). Icalma showed the highest specific abundance (ni′) values for zooplankton (except in I_2). According to their ni′, the Daphnia pulex species was recorded as presenting the highest ni′ in the two lakes and within the same lake in the sampling sites (Table 4).

Table 4. Zooplankton diversity in North Patagonian lakes Icalma and Llanquihue. Indicated as species abundance (ni′ m^{-3}); ± standard deviation.

Class	Order	Family	Species	Icalma (ni′ m^{-3})								Llanquihue (ni′ m^{-3})							
				I_1		I_2		I_3		I_4		LL_1		LL_2		LL_3		LL_4	
Crustacea	Cladocera	Daphniidae	*Daphnia pulex* Leydig, 1860	43	±7	10	±3	56	±4	38	±3	4	±4	8	±2	5	±4	0	-
		Bosmidae	*Neobosmina chilensis* (Daday, 1902)	2	±2	0	-	0	-	4	±4	2	±4	1	±1	1	±2	0	-
	Copepoda	Cyclopidae	*Mesocyclops longisetus* (Thiébaud, 1912)	15	±2	6	±2	23	±1	19	±3	4	±1	2	±1	5	±4	4	±2

The community structure of the lakes studied was described according to calculations of alpha and beta diversity indices. Significant differences were found in the total abundance (Ni′), the calculated alpha diversity variables (Figure 3a), between the studied lakes (Lakes, F = 219.7, $p < 0.001$). Species richness (S′) (Figure 3a) showed a total of three taxa in the lakes, with no significant differences, except at site LL_4, which is located in front of Puerto Varas bay, where only the species Mesocyclops longisetus was found, with 4 ind. m^{-3} (±2) (Table 4). No significant differences were found in Shannon's maximum estimated (H′max) and Shannon's diversity calculated (H′) indices (Figure 3b). However, when comparing both indices between lakes, only LL_4 showed significant differences (H′max LL_4, F = 13.53, $p < 0.001$; H′Shannon LL_4, F = 23.53, $p < 0.001$). A higher H′max:H′Shannon ratio was observed at the Llanquihue sites compared to Icalma, where it expressed a high dominance of Daphnia pulex species. By contrast, Lake Icalma showed high diversity and a balance in its community structure (Table 4). The evenness values tendency (J′) showed a decrease in values at Llanquihue compared to Icalma. However, the I_1 site in Icalma was similar to sites LL_2 and LL_3 in Llanquihue, while site LL_1 was similar to I_2–I_4. The lowest J′ values were obtained at LL_4.

Beta diversity measured according to similarity variables (Figure 4) showed two clusters greater than 50% similarity at the sampling sites when looking at the specific abundance values (ni′) of the species found in lakes Icalma and Llanquihue (Table 4), calculated using the alpha diversity indices. These groupings were slightly more than 90% different, separating the community structure of both lakes. However, the community structure of sampling sites I_2 in Icalma and LL_2 in Llanquihue is similar at 76.92%, with both lakes being similar at these two sampling sites. The LL_4 site showed a low similarity with other sites in Llanquihue and Icalma.

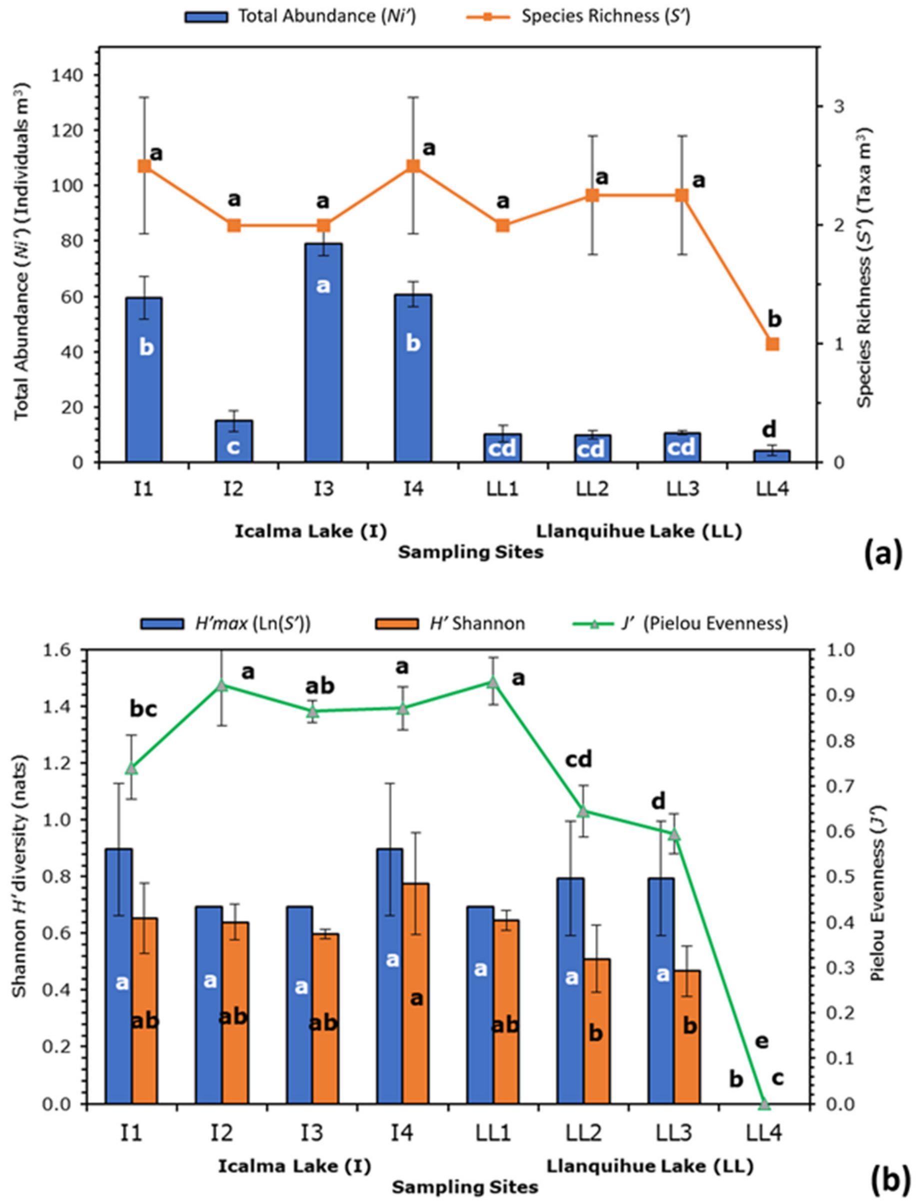

Figure 3. Alpha diversity: Total abundance (Ni′) plus species richness (S′) (**a**) and Shannon species diversity index (H′Shannon and H′max) plus evenness (J′) (**b**), at sampling sites in lakes Icalma (I_1, I_2, I_3, I_4), and Llanquihue (LL_1, LL_2, LL_3, LL_4). Lower letter indicates significant differences ($p \leq 0.05$) including the interaction of site × lake.

3.3. Relationships between Physicochemical and Ecological Variables

The correlation matrix between physicochemical properties and ecological variables in both lakes is shown in Figure 5. In both lakes, the ecological variables were negatively and significantly ($p < 0.05$) correlated with the Mn, total N, total P, TDS, and EC variables (Figure 5). The evenness (J′) was negatively correlated with Mn (R = 0.56), total P (R = 0.65), total N (R = 0.49), Cu (R = 0.47), and EC and TDS (R = 0.55). Ni´ was negatively correlated with Mn (R = 0.68), total N (R = 0.61), total P (R = 0.74), TDS (R = 0.73), and EC (R = 0.83). Total P and N were negatively correlated with S′, Hmax′, and HShannon′. Interestingly, Ca^{2+} was positively correlated with Ni′, HShannon′, and J′. Moreover, temperature was positively correlated with all ecological variables measured. We also found that Cu was positively correlated with Mn, EC, TDS, and total P and N, and that TDS and EC were highly and positively correlated (R = 0.98).

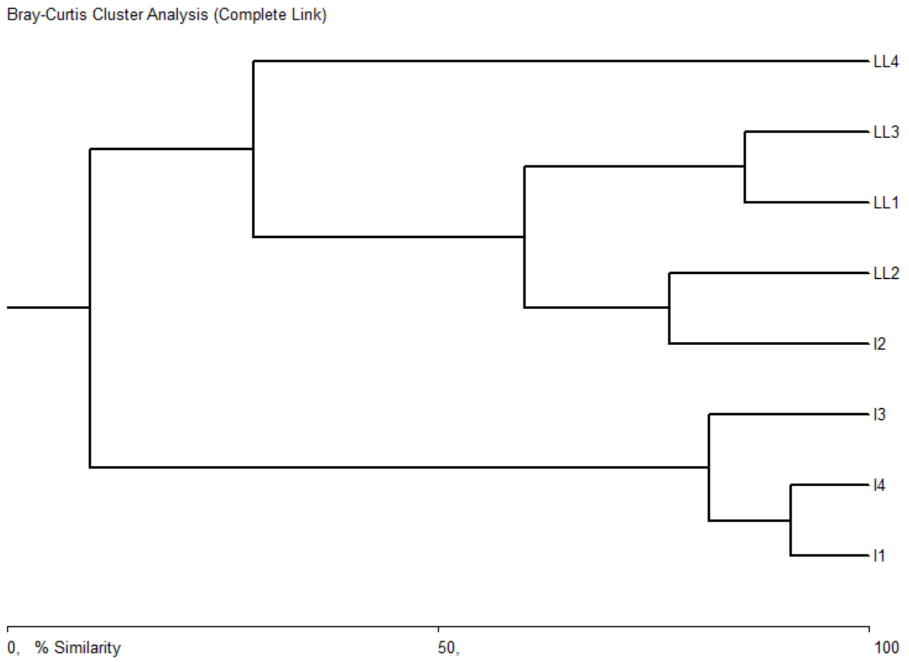

Figure 4. Beta diversity. Cluster analysis of specific abundance for similarity of zooplankton communities at sampling sites in lakes Icalma (I_1, I_2, I_3, I_4) and Llanquihue (LL_1, LL_2, LL_3, LL_4). Bray–Curtis analysis and complete linkage were used as aggregation criteria.

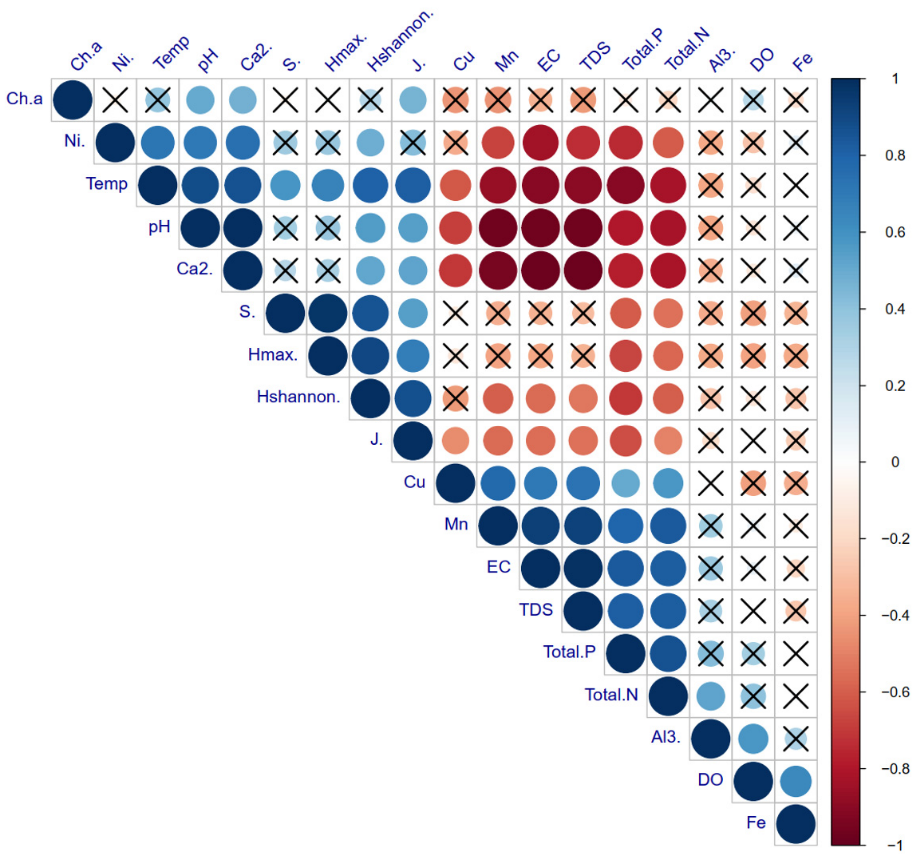

Figure 5. Correlation matrix of the physicochemical and ecological variables at sampling sites in lakes Icalma and Llanquihue. Blue circles denote positive correlation while negative correlations are shown using red circles. Non-crossed circles indicate significant correlations ($p \leq 0.05$) according to Pearson's test.

A principal component analysis (PCA) performed on the physicochemical and ecological variables measured in the North Patagonian lakes Icalma and Llanquihue indicated that the first components explained 75.8% of the total variance (Figure 6). The first dimension explained 56.5% of the variability data and the second dimension explained 17.3% of the variability.

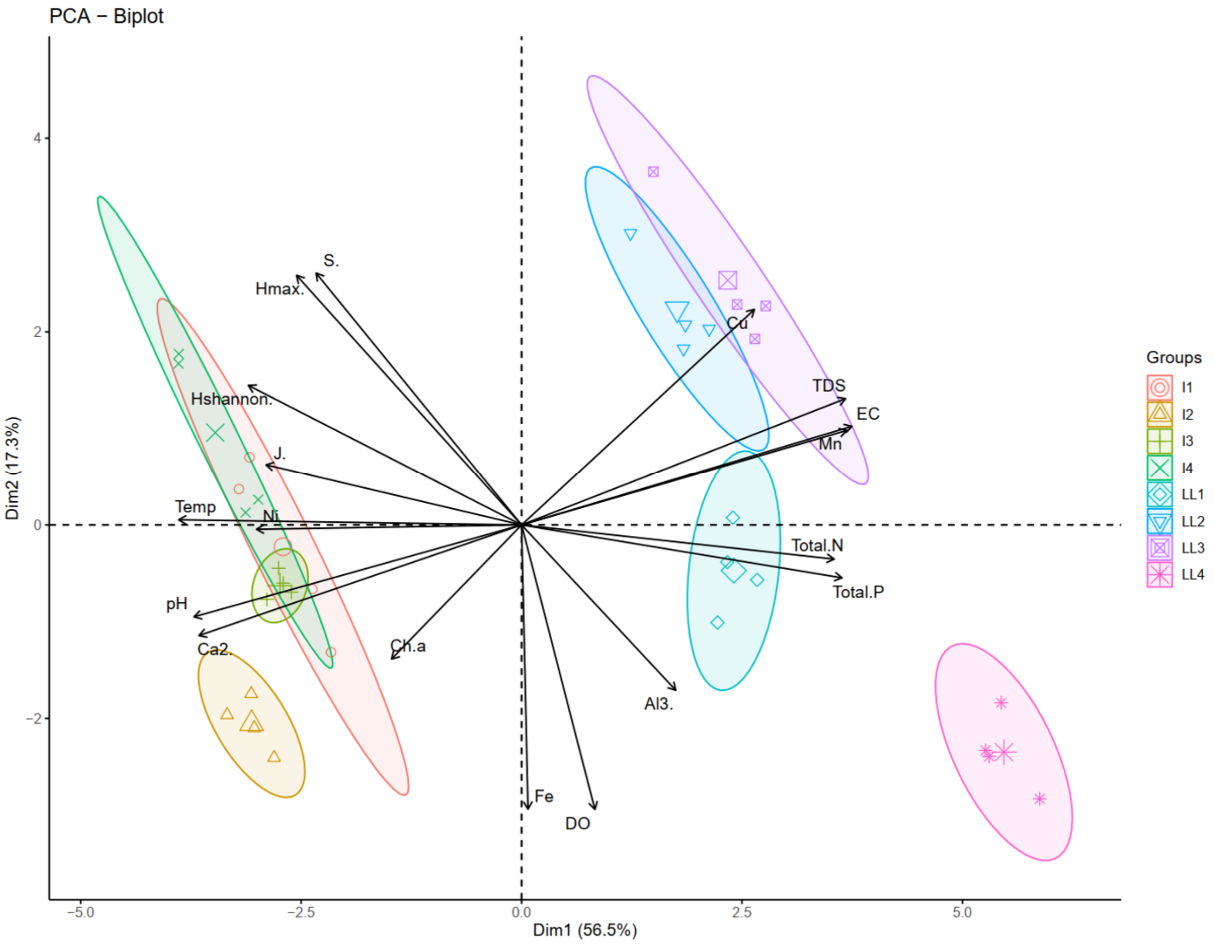

Figure 6. Principal component analysis (PCA) of physicochemical and ecological variables, measured in lakes Icalma and Llanquihue.

The representation of the variables in the PCA factor map showed a spatial separation within the lakes (Figure 6). Icalma followed a positive direction with the variables of temperature (Temp), pH, Ch-a, Ca^{2+}, and ecological variables of total abundance (Ni′), diversity (Hmax′ – Hshannon′), and evenness (J′). On the other hand, Llanquihue followed a positive direction with the variables of total P, total N, Cu, Mn, total dissolved solids (TDS) and conductivity (EC). All the Icalma sites followed a positive direction with the variables temperature (Temp), pH, and ecological variables, where only I_2 was separated from the other sites. In Llanquihue, a separation within sites was observed, wherein LL_4 was highly separated from LL_1, while LL_2 and LL_3 were located at the top, and they followed a negative direction for the environmental variables measured.

In the linear regression model, total N was the explanatory variable with the highest effect on the ecological variables (Table 5). TDS, EC, Cu, and Fe also contributed to the development of these models. In addition, temperature was a significant predictor for the explanation of J′, Hmax′, and HShannon′. Calcium was also an important predictor contributing to the model by explaining the variables Ni′, S′, and Hmax′. The developed models explained the variability of the ecological variables studied by 52 to 95%.

Table 5. Multiple linear regression model showing the predictors and explaining the ecological parameters of both North Patagonian lakes.

Variable	Predictors	R-Adjusted	*p*-Value	AIC
J′	pH (−0.38) *** + Temperature (0.71) *** + Ch-a (0.05) ** + Total N (0.12) * + Cu (−0.01) ** + Fe (−15.0) **	0.93	<0.001	−65.43
Ni′	TDS (4.71) *** + Cu (0.93) *** + EC (−5.51) *** + Ca^{2+} (−0.88) ***	0.95	<0.001	216.9
S′	Fe (−66.2) ** + EC (−0.06) ** + Ca^{2+} (−0.04) ** + Total N (−0.44) *	0.52	<0.001	37.21
Hmax′	Temperature (0.59) *** + Ca^{2+} (−0.01) *** + Total N (−0.15) * + Fe (−16.7) *	0.78	<0.001	−27.04
HShannon′	Temperature (0.62) *** + TDS (0.03) *** + DO (0.17) * + Total N (−0.10) *	0.85	<0.001	−54.34

* *J′* = evenness; *Ni′* = total abundance; *S′* = species richness; *Hmax′* = Shannon maximum diversity; *HShannon′* = Shannon diversity; TDS = total dissolved solids; EC = electric conductivity. The estimate of each predictor in the model is shown in parentheses. *p* values of the predictors are denoted as "***" < 0.01 "**" 0.01 "*" 0.05.

4. Discussion

The temperature of the water column recorded in lakes Icalma and Llanquihue (Table 3) during the sampling period in March (summer season) showed a difference of ±1 °C; however, this temperature difference is tolerated by the aquatic organisms under study [71]. In this study, increases in the concentration of physicochemical variables associated with anthropogenic activities were recorded. The physicochemical properties of the anthropized Lake Llanquihue were consistent with the studies performed by Yang et al. [72], Wu et al. [73], and Yoon et al. [74], which were obtained in rivers and lakes in China and South Korea under pressure from economic activities, demonstrating the anthropogenic impact and evidencing some degree of the eutrophication of the freshwater ecosystem. The pH values from the waters of Icalma reported here were similar to 7.70 reported by Scasso and Campos [75] and 7.40 reported by Alvial et al. [76]. By contrast, pH values in Llanquihue were lower than 8.40 reported by Campos et al. [48]. The pH values of Llanquihue were significantly lower than those obtained in Icalma, which would indicate acidification, evidencing some effect of anthropogenic activities [77]. In addition, we reported an increase in water column transparency (Table 3, Secchi Disc) in Lake Icalma compared to 15 m reported by Scasso and Campos [75]. According to Delegido et al. [78], water column transparency indicates the amount of sunlight penetrating the water column, associating the depth of light penetration with the presence of particles such as total dissolved solids (TDS) or microalgae that can absorb it [58]. Transparency was lower in Llanquihue (10 m) than Icalma, which may indicate the presence of particles such as TDS in the aquatic environment. In general, TDS could be related to the effect of anthropogenic activities located either in different places in a watershed tributary to Llanquihue (Blanco River, Pescado River) or urban areas near the sampling points (Puerto Varas, Puerto Rosales). The TDS in Icalma reported here were lower than 43.80 mg L^{-1} reported by Scasso and Campos [75]. Interestingly, we found that TDS values for Llanquihue were not previously reported. Consequently, our study reports updated data, in which the TDS values are significantly higher than Icalma. In fact, site LL4, which is located in areas in front of the bay city of Puerto Varas, reported the highest values, indicating an urban anthropogenic effect at this sampling point. According to Naseem and Nadeem [79], increases in TDS are greatly associated with urban, industrial, and agricultural activities in watersheds. In fact, it has been reported that Lake Llanquihue is surrounded by urbanization, livestock, forestry, and aquaculture activity areas [4,12,80].

According to Wetzel [77], TDS can be linked to the conductivity (EC) values. Interestingly, our findings showed high and positive correlations between EC and TDS. In fact, we found that, in Icalma, the EC values were lower than 87.50 µS cm^{-1} reported by Scasso and Campos [75], and slightly higher than 47.70 µS cm^{-1} reported by Alvial et al. [76]. In Llanquihue, EC values were lower than 228.70 µS cm^{-1} reported by Campos et al. [48] and higher than 79.9 µS cm^{-1} reported by Geller [81] for the summer period, the same time when sampling was performed in this study. Beyhan and Kaçıkoç [82] reported that

EC values' variability may be associated with the period of the year in which the samples were collected, related to temperature and the presence of different nutrient elements (total N and P) and the dissolved heavy metals in the aquatic environment. We found that Lake Llanquihue obtained higher values of EC compared to Lake Icalma. Accordingly, Figueroa et al. [83] mentioned that aquaculture activities increase conductivity, salinity, and total dissolved solids even 100 m downstream of the outflow point. On the other hand, urban waste from the effluent discharged on Lake Llanquihue, detected in Puerto Varas (Supplementary Material, Table S1, Figures S1 and S2), might also be attributed to an increases in heavy metal concentration and nutrients, promoting the high EC values [84].

Dissolved oxygen (DO) values are reported in Icalma for the first time in our study. We observed that DO values showed differences between and within lakes at each sampling site, being the highest at site LL4 in Llanquihue and I2 in Icalma. The values in Llanquihue were lower than those reported by Campos et al. [48]. Although there are new reports and differences with values reported in previous studies, the DO values in the two lakes showed similar magnitudes (8–9 mg L^{-1}); however, significant differences were found between lakes and sites, with these values being characteristic of oligotrophic lakes in North Patagonia [9,48]. According to the description of EC, it is likewise possible to find different DO values at different times of the year; moreover, when samples were taken in summer, a period of higher temperatures, DO values similar to those obtained in the mesotrophic Lake Eğirdir in Turkey during the summer season were observed [82]. The slight differences found may be due to meteorological factors such as atmospheric pressure, mineral salt, and nutrient contents, as well as the temperature of the water column at the sampling sites in lakes Icalma and Llanquihue [44,48,50,77].

Chlorophyll-a (Ch-a) showed significant differences when comparing values between lakes and within sites. In Icalma, Ch-a ranged from 2.14 to 6.30 μg L^{-1}, being higher than 0.80 μg L^{-1} reported by Soto and Zuñiga [13] and De Los Ríos-Escalante et al. [10]. By contrast, in Llanquihue, Ch-a values ranged from 2.68 to 3.88 μg L^{-1}, higher than 0.5 μg L^{-1} reported by Soto and Zuñiga [13] and lower than 12.70 μg L^{-1} reported by De Los Ríos-Escalante et al. [34]. These differences in Ch-a may be due to the biomass rate of autotrophic organisms that form part of the phytoplankton in the lakes under study, specifically at the point when the samples were taken in March 2020. This biomass may be related to grazing by higher trophic organisms, such as the crustacean zooplankton species recorded [28,77]. However, in Lake Llanquihue, the decrease in Ch-a could be related to the effect of pollution on phytoplankton due to heavy metal toxicity [37]. Accordingly, we observed that ecological variables were modulated by Cu and Fe concentrations. Moreover, Cu input from antifouling paints used in Chilean salmon farms could be 64 tons per production cycle [85]. It has been previously mentioned that a high Cu concentration in marine water would cause severe damages to phytoplankton [86]. Thus, the abundance of zooplanktonic crustacean species might be diminished due to food scarcity from the phytoplanktonic biomass source [41,42]. Furthermore, we found that increases in Ch-a were positively related to increases in evenness (*J*′).

It has been reported that a higher concentration of nutrients (total N and total P) is associated with an increase in Ch-a attributed to phytoplankton biomass, the increase of which over time can produce eutrophication [42]. This study reported a higher amount of nutrients in Llanquihue as compared to Icalma. Moreover, the lowest Ch-a concentration was found, which can be attributed to UV radiation and ionizing radiation from solar flares; together, they have contributed to the weakening of the ozone layer, inhibiting the photosynthetic process [87,88].

The presence of zooplanktonic crustaceans in a low amount of Ch-a may be due to a feeding change. These zooplankton may be feeding from detritus or bacterioplankton suspended in the aquatic environment [32]. Santos et al. [84] mentioned this behavior of zooplankton species, mentioning that total P can be considered to be a better indicator of food availability than Ch-a. North Patagonian lakes have been characterized by their oligotrophy and low diversity of zooplankton species, with a dominance of species of the order Copepoda, such as *Boeckella gracilipes* [15,48]. The physicochemical conditions reported here might have led to changes in the community structure of zooplanktonic crustaceans, particularly in Llanquihue, where a dominance of the cosmopolitan species *Daphnia pulex* was observed as well as a decrease in diversity. Diversity is understood as a concept of specific diversity where richness, abundance, and evenness should be observed, monitoring the stability of the community [67]. This was shown by the Shannon and Weaver index [68], where an observed diversity can be calculated with an expected maximum. The index allows us to compare lakes Icalma and Llanquihue (alpha diversity) as well as the different communities belonging to the two lakes (beta diversity) [40]. This is in addition to the presence and dominance of *Daphnia pulex*, which provides evidence of a change in the zooplanktonic community structure.

Significant differences were found in alpha diversity variables, according to abundance (*Ni′*) and species richness (*S′*) variables, in addition to the application of diversity indices, measured according to Shannon's diversity indices (*H′*), compared to Shannon's estimate of maximum diversity (*H′max*) and Pielou's evenness (*J ´*) of the zooplankton in lakes Icalma and Llanquihue. The zooplankton community in lakes Icalma and Llanquihue included species of the order Cladocera such as *Daphnia pulex* and *Neobosmina chilensis*, as well as species of the order Copepoda such as *Mesocyclops longisetus*. However, the value reported in this study was different from that reported in Icalma by Soto and Zuñiga [13], as they only reported the presence of *N. chilensis*. Furthermore, De Los Ríos-Escalante et al. [10,89] only reported the presence of *D. pulex* and *M. longisetus*, with great dominance of the species *Boeckella gracilipes* (order Copepoda). By contrast, the dominance and presence of *B. gracilipes* was not reported in our study. In addition, an equilibrium of the zooplankton community was found in Icalma, where the total abundance and total species richness were balanced across sampling sites.

In addition, Icalma showed higher species diversity than Llanquihue. Our findings also differed from that reported in Llanquihue by Campos et al. [48], who only found the presence of *M. longisetus*. Furthermore, De Los Ríos and Soto [33] found no species reported in our study in Llanquihue. Subsequently, De Los Ríos-Escalante et al. [44] reported the presence of *D. pulex*, *N. chilensis*, as well as *B. gracilipes* species belonging to the order Copepoda, which they described as dominant in Llanquihue. However, our study found a high dominance of *D. pulex*, supporting a change in the zooplankton structure and diversity in Llanquihue following some degree of anthropic impact, such as the contribution of nutrients from different effluents of urban origin or some human activity. This enables the presence of detritus or bacterioplankton suspended in the aquatic environment—food that *D. pulex* chooses in the face of the decrease in phytoplankton [32].

These changes in zooplankton community structure are related to changes in environmental variables, as reported by Wang et al. [90] and Hamil et al. [91], where they mentioned that changes in alpha diversity variables are highly related to the impact of anthropogenic activities (pollution), explained by the clear separation between lakes Icalma and Llanquihue, being particularly influenced by the variables total P, total N, Mn, Cu, TDS, and CE. Moreover, the evidence of the anthropization effect, due to the increases in some physicochemical variables reported here, were confirmed through the generation of the different models presented in our study. The decrease in the abundance of individuals may be due to increases in total P and/or N, leading to the eutrophication of the freshwater ecosystem as well as to the greater values of Cu and Mn obtained in Llanquihue as compared to Icalma. The higher concentrations observed may be related to the contribution of anthropogenic effluents located in Puerto Varas bay. An effluent previously detected at the time of field sampling in March 2020 (Figure S1) and its chemical characterization showed that EC and TDS, as well as total P and N values, were very high (Table S1 and Figure S2).

Moreover, the values of total N exceed the values allowed by Chilean legislation in Supreme Decree 90 (DS 90) on the Emission Standard for the Regulation of Pollutants Associated with the Discharge of Liquid Waste into Marine and Inland Surface Waters (Table S1). In addition, a higher concentration of heavy metals was observed, where Mn values were close to the maximum value allowed by Chilean legislation DS 90. The effluent was of urban origin and was discharged directly into the bay of Puerto Varas (shown in Figure S1), specifically in the beach sector that different people use for recreational purposes; such activities should not be carried out in the waters of Lake Llanquihue, until an adequate time has elapsed to allow the establishment of a Biomonitoring and Bioremediation plan for the waters of Lake Llanquihue, which will improve the health of this freshwater ecosystem without affecting human health [92,93].

In general, it was suggested by Yang et al., [72] and Yoon et al. [74] that TDS, EC, the concentration of total P and N nutrients, and heavy metals are adequate to assess the anthropogenic effect. Here, the increases in total N, total P, as well as Cu, Fe, and Mn, were in sufficient amounts to decrease the abundance of some zooplankton species, thus affecting aquatic life. This is evidence of how the degree of the anthropization impact observed in Llanquihue may affect the structure and diversity of the zooplankton community supported by the negative correlations between total abundance (*Ni′*), species richness (*S′*), maximum Shannon diversity (*H′max*), Shannon diversity (*H′Shannon*) and evenness (*J′*) with total P and N, Mn, Cu, TDS, and EC.

5. Conclusions

Our study showed significant changes in the abundance, richness, and diversity of crustacean zooplankton species in Llanquihue as compared to Icalma, both belonging to the North Patagonian lakes. These changes were significantly related to the environmental variables measured, highlighting that Llanquihue registered higher levels of total phosphorus and nitrogen as well as some heavy metals, total dissolved solids, and conductivity than Icalma. We found that there was a balance in the structure of species in Icalma, showing a greater diversity. By contrast, our findings indicated decreases in species abundance in the anthropized Lake Llanquihue. Furthermore, the dominance of species was changed from the order Copepoda to the order Cladocera, with the *Daphnia pulex* being the most dominant species compared to other studies.

Even when our study assessed differences between lakes, we observed some differences within lakes which might indicate a contrasting degree of anthropization in the same lake. Thus, Llanquihue is a lake under anthropogenic pressure in recent decades that has seen its trophic status greatly change compared to Lake Icalma. Finally, we can conclude that water quality is highly influenced by zooplankton diversity, which is supported by decreases in such ecological variables such as abundance, richness, Shannon diversity, and evenness due to the high levels of nutrients, heavy metals, total dissolved solids, and conductivity values obtained in the anthropized Llanquihue lake. This study provides fundamental information on the impact of anthropogenic activities on water quality, as well as on aquatic diversity, contributing to the importance of the monitoring of the health of North Patagonian freshwater ecosystems.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/su14106052/s1, Table S1. Physicochemical variables measured in effluent from Puerto Varas bay in Lake Llanquihue. DS 90: Chilean law, Supreme Decree 90. SD: Standard deviation. EC: electric conductivity; TDSs: total dissolved solids; P Total: total phosphorus; N Total: total nitrogen. Data obtained in the same field work where the samples analyzed in this study were taken. Figure S1. Effluent from Puerto Varas bay in Lake Llanquihue. Picture was taken on the same day as field work where the samples analyzed in this study were taken. Figure S2. Nutrients and heavy metal variables measured in effluent from Puerto Varas bay. P Total: total phosphorus; N Total: total nitrogen; NH4: Ammonium; Al: Aluminum; Cu: Copper; Fe: Iron; Mn: Manganese. With data obtained in Table S1 above, a graph is shown to better visualize the fluctuations of the quantities. Data obtained in the same field work where the samples analyzed in this study were taken.

Author Contributions: Conceptualization, J.-A.N., P.P.-G. and J.F.B.; methodology, J.-A.N.; software, J.-A.N. and P.P.-G.; validation, J.G.F. and P.D.L.R.-E.; formal analysis, J.-A.N. and P.P.-G.; investigation, J.-A.N.; resources, J.-A.N. and J.G.F.; data curation, J.-A.N.; writing—original draft preparation, J.-A.N.; writing—review and editing, J.-A.N. and P.P.-G.; visualization, J.-A.N.; supervision, J.G.F.; project administration, J.-A.N. and J.G.F.; funding acquisition, J.-A.N. and J.G.F. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by ANID scholarship, grant number 21180143, and the projects FONDECYT 1180387, 3210228 and MECESUP UCT 0804.

Acknowledgments: Authors are grateful to the Chilean Navy, for their operational support in the fieldwork.

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

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