

Article

Taxonomic and Functional Responses of Species-Poor Riverine Fish Assemblages to the Interplay of Human-Induced Stressors

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Abstract: The effects of human-induced stressors on riverine fish assemblages are still poorly understood, especially in species-poor assemblages such as those of temperate South American rivers. In this study we evaluated the effects of human-induced stressors on the taxonomic and functional facets of fish assemblages of two central-southern Chilean rivers: the Biobío River (flow regulated by multiple dams) and the Valdivia River (free-flowing). The study design considered reference condition, urban polluted, and urban-industrial polluted sites. To evaluate the effects of stressors on fish assemblages we assessed: (i) components of beta diversity; (ii) spatial and temporal patterns of fish structure using a multivariate approach; and (iii) functional diversity (specialization, originality, dispersion, and entropy) using linear models. We found a strong association between taxonomic and functional fish assemblage facets with a predominance of natural processes in the Valdivia River reflected in marked temporal dynamics. In contrast, the Biobío River showed a clear loss of association with seasonal pattern, and both taxonomic and functional facets appeared to respond significantly to pollution zones. Implementation of stricter environmental policies and integrated river basin management are instrumental for conserving species-poor fish assemblages in Chilean temperate rivers characterized by low functional traits redundancy.

Keywords: beta diversity; functional diversity; pollution; functional traits; temperate rivers



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1. Introduction

A variety of human activities associated with river ecosystems worldwide have generated different stressors that negatively affect these ecosystems and their biota [1,2]. These stressors will be superimposed by climate emergency and are expected to cause significant impacts on both short and medium temporal scales. For instance, winter precipitation and associated river discharges are expected to decrease about 40% within three decades in central-southern Chile [3,4]. This discharge decrease, in turn, will exacerbate effects of other stressors (e.g., water pollution) by directly increasing concentrations of pollutants [5]. Furthermore, whereas examples of effective management of freshwater ecosystems that are focused on improvement of water quality in all freshwater ecosystems are available (e.g., European Union policies), in Chile and Latin America environmental public policies are still often inefficient to ensure good ecological status and effective management of

freshwater ecosystems [6]. As a consequence, accelerated deterioration of water and its ecological qualities are currently evidenced in multiple river basins across Chile [5].

Our knowledge of the effects of the interplay of stressors on freshwater biota is still rudimentary, even though some recent studies in European rivers have provided empirical evidence of these effects using examples of fish and macroinvertebrate assemblages (e.g., [7–10]). Until the last few decades, assessment of the effects of individual stressors was often applied. However, human induced stressors have currently incremented in number and intensity and evaluation of individual stressors is frequently not valid [7,11,12]. Industrial residuals, waste-water effluents, flow regulation, and invasive species are the most common human-induced stressors that interact to affect rivers often causing severe changes in their ecological functioning [1,12,13]. Therefore, representative assessment necessitates consideration of at least two different stressors in order to obtain some insights into an integrated ecological response [14]. Furthermore, many studies of the effects of stressors concentrate on sub-individual responses, whereas studies that concentrate on biological assemblages' composition and function are ecologically relevant and can be instrumental to evaluate whether stressor effects are chronic [15].

Fish assemblages in Chilean river systems are highly endemic and species-poor [16–18]. The singularity of these assemblages is caused by geological history with recent glaciation event (18,000 years ago), isolation by geographic barriers as well as short length, steep slopes, and oligotrophic waters of Chilean rivers [16,18,19]. Furthermore, many species that form these unique assemblages are presently highly threatened with more than 80% classified as vulnerable, endangered or critically endangered [20]. A recent study of freshwater fish assemblages in Chilean rivers documents their homogenization and loss of beta diversity due to declines of native species [21]. These changes are mainly driven by human activities [21,22]. Indeed, multiple recent studies have shown that changes in beta diversity can be an excellent indicator of the response of fish assemblages to human activities [22–24]. Next to beta diversity based on taxonomy, functional facets of biodiversity can also be evaluated to assess integrated response of biota to stressors as loss of key functions can help to understand the severity of stressor impacts and their potential consequences [25]. Functional facets of fish assemblages in Chilean rivers and their response to stressors have not been studied (with the exception of one recent study that documented loss of functional stability: [26]). Still, functional diversity facets related to community singularity and complexity (e.g., specialization, originality, entropy) that can give insights into responses of assemblages to stressors have not yet been evaluated [25]. The evaluation of the functional aspects is especially relevant in species-poor assemblages given their low functional redundancy level and associated vulnerability to stressors [27,28]. As such, an integral approach to assess the effects of stressors on fish assemblages should incorporate both taxonomic and functional facets since they deliver complementary information [8,25,29].

The Biobío and Valdivia River basins are located in central-southern Chile and each accommodate a total of 18 fish species [26]. This total number is reflected in a mean number of five to eight species coexisting in local habitats [30]. Both the Biobío and Valdivia River basins are under the influence of a range of anthropogenic activities that are significantly more intensive in the Biobío River basin. As such, the Biobío River accommodates several major hydropower plants, of which three are located in the main stem and significantly alter its natural flow regime [31,32]. Furthermore, the Biobío River receives important quantities of both industrial and domestic pollutants with three operational pulp mill plants and eleven urban centers within the basin [33,34]. It is worth mentioning that pulp mill plants that operate in the Biobío River basin use outdated technologies and as a consequence their effluents contain different organic residuals such as PHAs and phenols [33,35]. The Valdivia River basin is free of major hydropower dams. It accommodates one pulp mill plant located in the Cruces River tributary and it has fewer urban centers (three) compared to the Biobío River basin [36–38].

The present study aimed to assess the effects of the interplay of the three principal human-induced stressors (flow regulation by hydropower, industrial pollution and ur-

ban pollution) on taxonomic and functional diversity of species-poor fish assemblages in Chilean temperate rivers. Specifically, we assessed beta-diversity as it has been shown to be an excellent indicator of stressor induced biodiversity loss [24]. Furthermore, we evaluated the responses of four complementary functional diversity facets (functional specialization, originality, dispersion and entropy) to the interplay of these stressors. These facets were chosen as they have been previously shown to be suitable for species-poor assemblages such as those in central-southern Chilean rivers [8,39]. We expected significant alteration (reflected by both taxonomic and functional indicators) of fish assemblages in the Biobío River due to high intensity pollution stressors and flow regulation that affect it. In contrast, fish assemblage in the Valdivia River was expected to follow more natural dynamics due to lower stressor intensity and absence of anthropogenic flow regulation.

2. Materials and Methods

2.1. Study Area

The study area is located in central-southern Chile in the Biobío and Valdivia River basins (Figure 1). The Biobío River, with a catchment area of 24,260 km², accommodates high fish species richness but is also severely affected by human activities [40]. Eleven cities discharge waste-water to the river and extract water for drinking from it, and three pulp mill industries use it intensely. As a consequence, deterioration of water quality and biodiversity has been registered during last decades (e.g., endocrine disruption, presence of toxic metabolites in fish, etc.) [33,36,41]. Furthermore, the Biobío River is currently fragmented by 18 hydropower dams and is projected for further rapid hydropower development with up to 158 dams in 2050 [31]. Finally, the Biobío River receives diffuse pollution as a consequence of land-use intensification and conversion of native forest to agriculture and forest plantations dominated by *Pinus radiata* and *Eucalyptus* sp. [35,42]. Valdivia River, with a catchment area of 11,468 km², originates from a chain of eight lakes that function as buffer for nutrients and sediments and as a consequence is characterized by ultra-oligotrophic water in upper main stem of the river. The Valdivia River accommodates important touristic activities due to its largely natural riparian zones with a leafy temperate rainforest, clear waters, and scenic landscapes within the basin. The Cruces River is the main tributary of the Valdivia River which originates at the footsteps of the Andes. It does not accommodate headwater lakes and is characterized by oligotrophic water [43]. The Lower Cruces River accommodates extensive wetlands inundated after the 1960 earthquake and tsunami. These wetlands are characterized by high diversity of macrophytes and ferns, and presence of a high number of aquatic birds. Presently the wetland is protected as a Nature Reserve (Carlos Anwandter Nature Sanctuary) and is the first Ramsar site in Chile (established in 1975). There are few industrial activities registered in the basin and main ones are low scale milk production and a pulp mill plant that is currently highly regulated [44]. Valdivia River basin is to date free of hydropower dams in the main stem and accommodates only one dam (48.6 MW) installed in a small tributary (Huanahue River) of one of the headwater lakes (Panguipulli Lake).

2.2. Study Design and Fish Sampling

In order to assess the structure of the fish assemblages and determine its response to multiple stressors, a total of 12 sampling sites were selected (6 in the Biobío River basin characterised by the flow regime highly altered by hydropower development and 6 in the Valdivia River basin with no flow regime alterations). The study design considers two reference condition sites (REF), two sites affected by urban pollution (PU) and two sites affected by urban and industrial pollution (PIU) in each basin (Figure 1). Polluted and reference sites were selected based on stressor distribution within each river network. It was ensured that all sampling sites were characterized by similar geomorphology and similar Strahler order. Fish were sampled in four seasons: spring (SP), summer (SU), autumn (AU) and winter (WI) in order to assess the temporal pattern of assemblage structure in each basin. In addition, at each sampling site, in situ water quality variables (temperature,

pH, dissolved oxygen, conductivity, total dissolved solids) were measured using hand-held devices (Hanna HI-9828 and Hanna HI-98703, Woonsocket, RI, USA). Furthermore, concentrations of nitrite, nitrate, ammonium, phosphate and total hardness were measured using colorimetric test kit VISOCOLOR® (MACHEREY-NAGEL GmbH and Co., KG., Dueren, Germany).

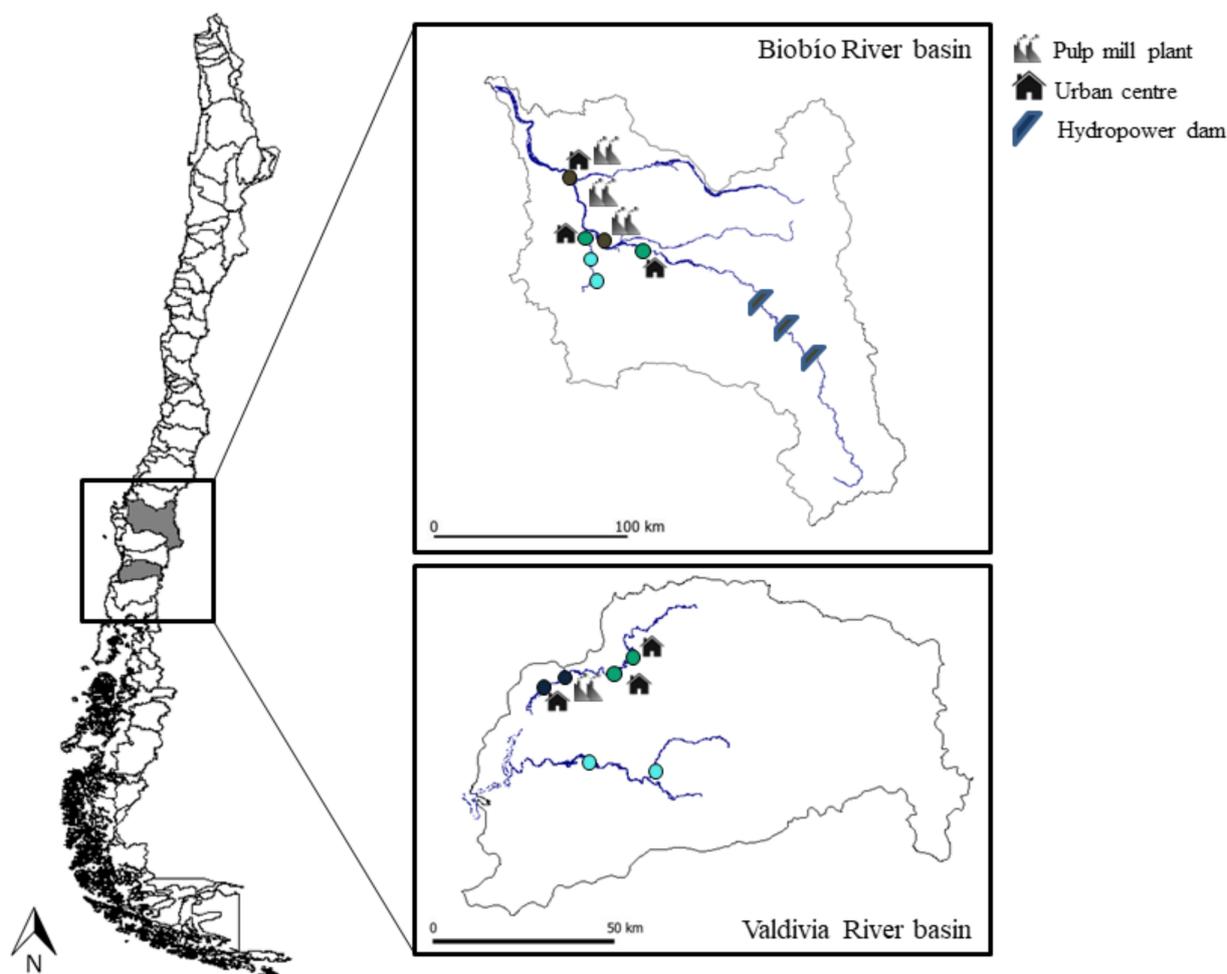


Figure 1. Study area. The Biobío River basin (**top**) and Valdivia River basin (**bottom**) located in the central-southern Chile. Sampling sites are indicated by coloured dots (blue, reference sampling sites; green, urban pollution sites; black, urban and industrial pollution sites).

Fish were sampled by two complementary capture methods: backpack electrofisher (Halltech HT-2000, Guelph, ON, Canada) and beach seine (1.5 m high, 10 m long and 6 mm stretched mesh size). Each electrofishing sample took 30 min of active fishing and covered riffle and pool habitats (electrofishing settings: 250 volts, 50 A). Beach seine samples were collected in littoral/shoreline habitats at each site with three to four hauls at each site depending on littoral habitat availability. Fishing effort did not significantly differ among sites and sampling events, therefore total numbers of fish collected by both methods were pooled for analyses. Fish collected at each site were anaesthetized, identified in situ to species level, counted and released back to their original habitats.

2.3. Beta Diversity Analyses

Beta diversity was estimated using Sørensen and Bray-Curtis indices for presence-absence and abundance matrices, respectively, with betapart package [45]. Beta diversity reflects variation of species composition of biological assemblages and provides information

about processes that drive assemblage structure when turnover and nestedness components are considered [46]. This information is highly relevant for assessment of the effects of stressors as these components reflect antithetic processes: species replacement and species loss [13,45]. Therefore, we computed following specific components of beta diversity: (1) total beta diversity based on Sørensen dissimilarity index (β_{SOR}), (2) beta turnover estimated from Simpson dissimilarity index (β_{SIM}), and (3) beta nestedness calculated by subtraction of turnover effect from the total beta diversity ($\beta_{\text{NES}} = \beta_{\text{SOR}} - \beta_{\text{SIM}}$) [45]. These components of beta diversity were calculated for each basin in order to allow comparisons. Using an analogous reasoning, Bray-Curtis dissimilarity (d_{BC}) is separated into balanced variation ($d_{\text{BC-bal}}$) and abundance gradient ($d_{\text{BC-gra}}$) components [47]. Then, Bray-Curtis dissimilarity is the addition of two antithetic sources of dissimilarity, namely balanced changes in species abundances and unidirectional abundance gradients [47].

Subsequently, we evaluated the hypotheses that variability in spatial and temporal patterns of fish assemblage composition and abundance (pair-wise beta diversity) could be explained by water quality variables (\log_{x+1} transformed) related with human-induced stressors (i.e., conductivity, pH, nitrate). The hypothesis was tested using distance-based linear models (DISTLM; [48,49]). DISTLM performs partitioning of the variation in data matrices similar to regression and it generates probability values by a permutation routine [49]. Probability values were obtained for predictor variables by 9999 permutations. Initially, models were run to identify significant predictors of fish assemblage structure (based on Bray-Curtis resemblance matrix from fourth-root transformed abundance dataset) when fitted individually (marginal test) and then sequentially using BEST selection procedure. Model parsimony was assessed by corrected Akaike's Information Criterion (AICc). The best-fit model was visualized in multidimensional space using dbRDA ordination. These analyses were performed in PRIMER-E software (v.6) [49].

2.4. Estimation of Functional Diversity Indices

Estimation of functional diversity was based on functional traits and fish abundance matrices. Seven eco-morphological and ecological traits were selected to describe different functional roles of species in assemblage structure (Table 1). The traits were selected based on available knowledge about Chilean native fish species. Trait data were extracted from online sources <https://www.fishbase.se> (accessed on 15 January 2021) and <http://especies.mma.gob.cl/> (accessed on 15 January 2021), and published articles [18,50,51].

Functional diversity (FD) indices of fish assemblages were estimated using different dissimilarity algorithms based on the Gower distance matrix computed from trait-pairs of a given species within the total trait pool [25,29,39]. A multidimensional functional space was defined using Principal Coordinates Analysis (PCoA) in order to position traits in space [29]. We used four FD indices that can be calculated from species composition in each sampling site: functional specialization (F_{Spe}), functional originality (F_{Ori}), functional dispersion (F_{Dis}) and functional entropy (F_{Ent}, [39]). The choice of FD indices was based on species-poor system criterion proposed by [39] and applied to empirical data by [8,9]. As species pool within the assemblage differs between the Biobío and Valdivia River basins, indices were calculated separately for each basin.

Table 1. Biological and ecological traits used to estimate functional diversity of the fish assemblage in the Biobío and Valdivia rivers. These traits were selected based on available knowledge about Chilean native fish species. Trait data were extracted from online sources <https://www.fishbase.se> (accessed on 15 January 2021) and <http://especies.mma.gob.cl/> (accessed on 15 January 2021)), and published articles (Habit et al. 2006 [18], Valdovinos et al. 2012 [50], Vila et al. 2015 [51]).

Trait Type	Function	Trait	Categories	Categorical Value
Ordinal	Trophic interaction	Trophic guilds	Detritivore	1
			Omnivore-invertivore	2
			Omnivore-piscivore	3
			Omnivore	4
Ordinal	Habitat use	Vertical position	Benthic	1
			Benthopelagic	2
			Pelagic	3
		Velocity preference	Reophilic	1
			Limnophilic	2
			Eurytopic	3
		Floodplain use	Frequently	1
			Scarcely	2
Ordinal	Life history	Migration	Facultative amphidromous	1
			Anadromous	2
			Facultative catadromous	3
			Non-migratory	4
Continuous	Morphology	Mean length		
		Maximum length		

F_{Spe} was calculated as the abundance-weighted mean distance in the functional space to the average value of all of the species present at the basin scale [52]. It shows high values when the species with the highest abundance has the most extreme traits within the basin species pool. F_{Ori} was estimated as the abundance-weighted mean distance to the closest species within functional space [25]. It reaches high values when species with unique trait combinations have the highest abundances in the community. F_{Dis} was estimated as abundance-weighted deviation of species trait values from the center of the functional space [53]. The center of F_{Dis} presents the highest values when trait abundances are similar [53]. Finally, F_{Ent} was estimated as the abundance-weighted sum of pairwise functional distance among species within the community [54]. F_{Ent} increases when species with the highest abundances are functionally distinct [25].

2.5. The Effects of Stressors on Functional Diversity

Principal component analysis (PCA; the function principal, R package psych) was applied in order to summarize eight water quality variables (log_{x+1} transformed) in form of principal component axes. Resulting two first PC axes (that explained more than 50% of the accumulated variation) were re-named as gradients of human-induced stress based on the stressors that were associated with them (Table 2): ‘water quality’ (PC1) and ‘nutrients’ (PC2). The ‘varimax’ rotation facilitated the interpretation of axes, and the number of axes was selected based on explanatory power [55]. Generalized linear models (GLM, the function glm, car package R) were used to assess the effects of the gradients of human-induced stressors (summarized in PCA axes, Table 2) on functional diversity indices (F_{Spe}, F_{Ori}, F_{Dis}, F_{Ent}). As other human-induced stressor related directly with study design and indirectly with non-included variables, we included pollution zones (REF, PU, PIU) and seasons (natural temporal gradient) in the models. Quasibinomial errors/logit link was used for proportions of each functional index (F_{Spe}, F_{Ori}, F_{Dis} and F_{Ent}), whereas Poisson

errors/log link was used for species abundance data. Model validation was executed by visually inspecting diagnostic plots of residuals. The analysis was executed in R software with car package.

Table 2. Loadings for axes 1 and 2 according to PCA built using water quality variables. Values $\geq |0.65|$ are showed in bold.

	Biobío River		Valdivia River	
	PC1 (39%)	PC2 (33%)	PC1 (38%)	PC2 (35%)
pH	0.6076	−0.2326	0.8375	0.0959
Temperature	0.7225	0.4152	0.8939	0.1486
Conductivity	0.7607	−0.1050	0.6449	−0.2490
Hardness	0.6277	0.0108	0.0454	− 0.6015
Nitrite	0.0970	0.9186	0.2425	0.7647
Nitrate	−0.0948	−0.1542	−0.0858	0.7947
Ammonium	−0.3662	0.6901	−0.0176	−0.0382
Phosphate	0.2669	0.4748	0.1685	0.4762

To determine the relative contribution of each functional trait in the fish assemblage–water quality relationships, we used fourth-corner and RLQ analyses based on the criterion introduced in [56]. RLQ is a multivariate technique that provides ordination scores to summarize links among species distributions across sampling sites, environmental variables, and species traits. This analysis was computed with R package ade4.

3. Results

3.1. Taxonomic Assemblage Structure and Environmental Effects

A total of 21 species was registered in this study, 14 in the Biobío River, and 15 in the Valdivia River. Eight species were shared between the basins. Among these, three were non-native (*Salmo trutta*, *Oncorhynchus mykiss* and *Gambusia holbrooki*) and five were native (*Basilychthys microlepidotus*, *Galaxias maculatus*, *Geotria australis*, *Percichthys trucha*, *Trichomycterus areolatus*) (Table A1). The species registered only in one basin are inside their endemic distribution ranges; in the Biobío River these were *Bullockia maldonadoi*, *Cheirodon galusdae*, *Percilia irwini* and *Percichthys melanops*, and in the Valdivia River, *Diplomystes camposensis*, *Cheirodon kiliani*, *Aplochiton taeniatus* and *Hatcheria macreii*.

Decomposition of beta diversity based on species presence-absence revealed significant differences in fish assemblages structuring between the Biobío and Valdivia River basins. Even though β_{SOR} had a similar range between basins, the Valdivia River was characterized by higher density (number of species registered by site) compared to the Biobío River (this was reflected in peak of gross curves (Figure 2)). Furthermore, Valdivia River is characterized by a strong turnover (β_{SIM}) compared to the Biobío River, which in turn is characterized by marked nestedness (β_{SNE}) (Figure 2). Nevertheless, decomposition of beta diversity based on Bray-Curtis dissimilarity estimated from fish abundance data showed less marked pattern and D_{BC-gra} as well as D_{BC-bal} were characterized by similar range values in both rivers (Figure 2). These results indicate that beta diversity patterns of fish assemblage in these rivers are primarily driven by species composition and less by assemblage structure based on abundances.

DISTLM models and dbRDA ordination revealed significant differences in species abundances among sampling sites and their associations with water quality variables (Figure 3). Indeed, the fish assemblage structure in the Biobío River showed clear separation among pollution zones (Figure 3, left). Specifically, fish assemblages in reference sites were significantly different from those in sites characterized by both urban as well as urban and industrial pollution (pseudo $F = 2.12$; $p = 0.04$). The most different group was strongly influenced by conductivity (pseudo $F = 1.241$; $p = 0.03$) and nitrate concentration (Figure 3, left). A different pattern was observed in fish assemblage structures in the Valdivia River with little separation among pollution zones but marked grouping among seasons with

significant differences between warmer (spring and summer) and colder (autumn and winter) seasons (Figure 3, right). Differentiation among seasons was mostly explained by physical and chemical water quality variables: temperature (pseudo $F = 3.378$; $p = 0.008$) and pH (pseudo $F = 5.130$; $p = 0.001$).

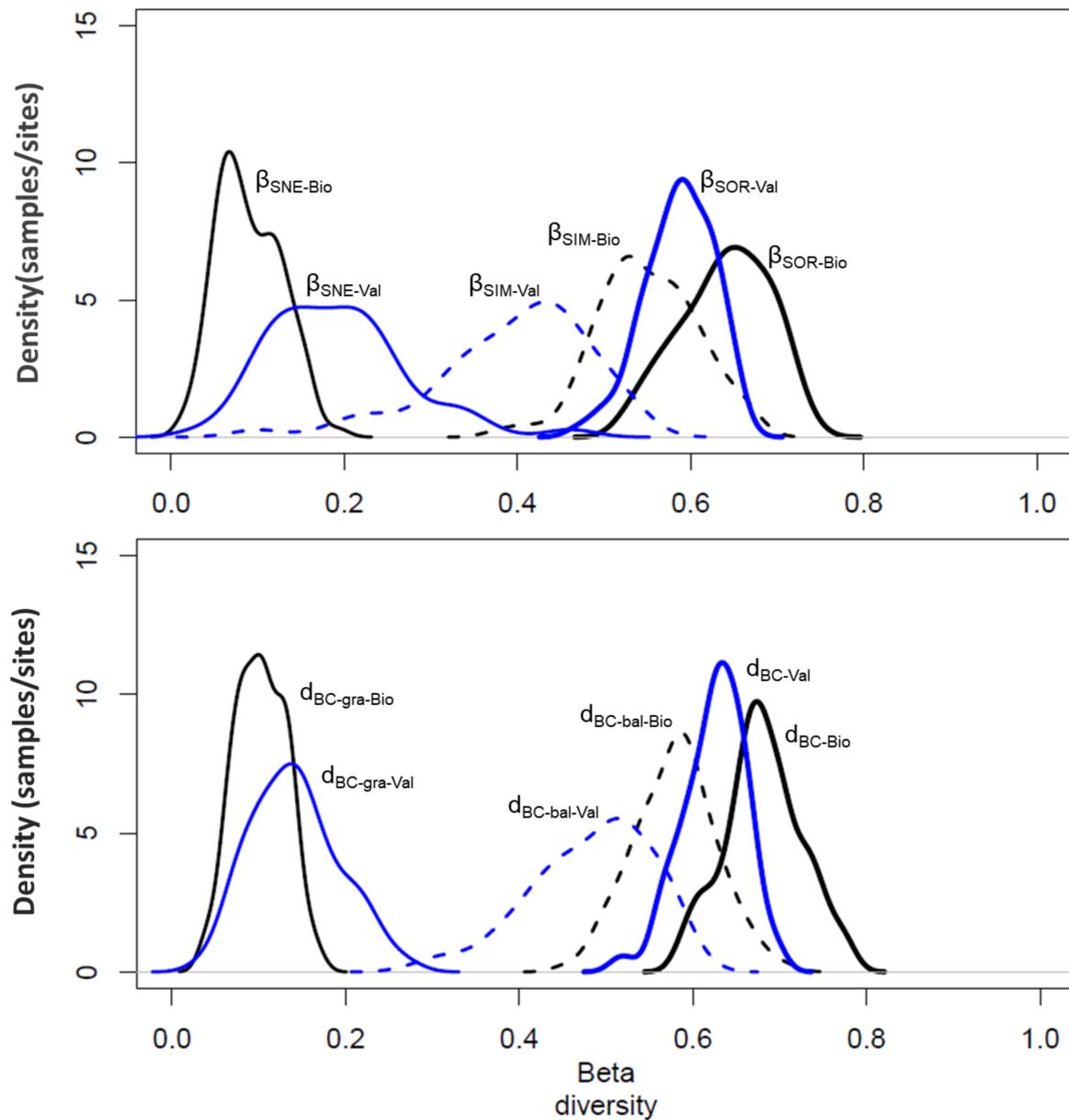


Figure 2. Beta diversity decomposition for the Biobío River (black) and Valdivia River (blue), based on Sørensen dissimilarity (top, species presence-absence matrix) and Bray-Curtis dissimilarity (bottom, species abundance matrix). Sørensen dissimilarity β_{SOR} was partitioned into β_{SIM} (turnover) and β_{SNE} (nestedness) and Bray-Curtis dissimilarity into balanced variation (d_{BC-bal}) and abundance gradient (d_{BC-gra}) components.

3.2. The Effects of Stressors on Functional Diversity

Functional assemblage structure based on traits and abundance of registered species in the study area showed different patterns in each analyzed river (Table 3, Figures 4 and 5). As such, functional dispersion and entropy of the fish assemblage in the Biobío River significantly responded to the pollution zones. Indeed, ANOVA results highlighted that these functional facets were characterized by a higher range of values in the reference

zone compared to polluted zones (Table 3, Figure 4). Different patterns were observed in the Valdivia River where functional diversity indices of the fish assemblage responded to nutrient pollution. Specifically, functional specialization, dispersion, and entropy decreased with increasing concentration of nutrients (PC2) and increased with the increasing of temperature, conductivity, and pH (PC1) which occurs in warmer seasons (Table 2, Figures 4 and 5). Furthermore, in contrast to the Biobío River, the functional specialization, dispersion and entropy of the fish assemblage in the Valdivia River was characterized by significant temporal dynamics with the highest values in summer and spring, and lowest in winter and autumn (Table 2, Figure 5).

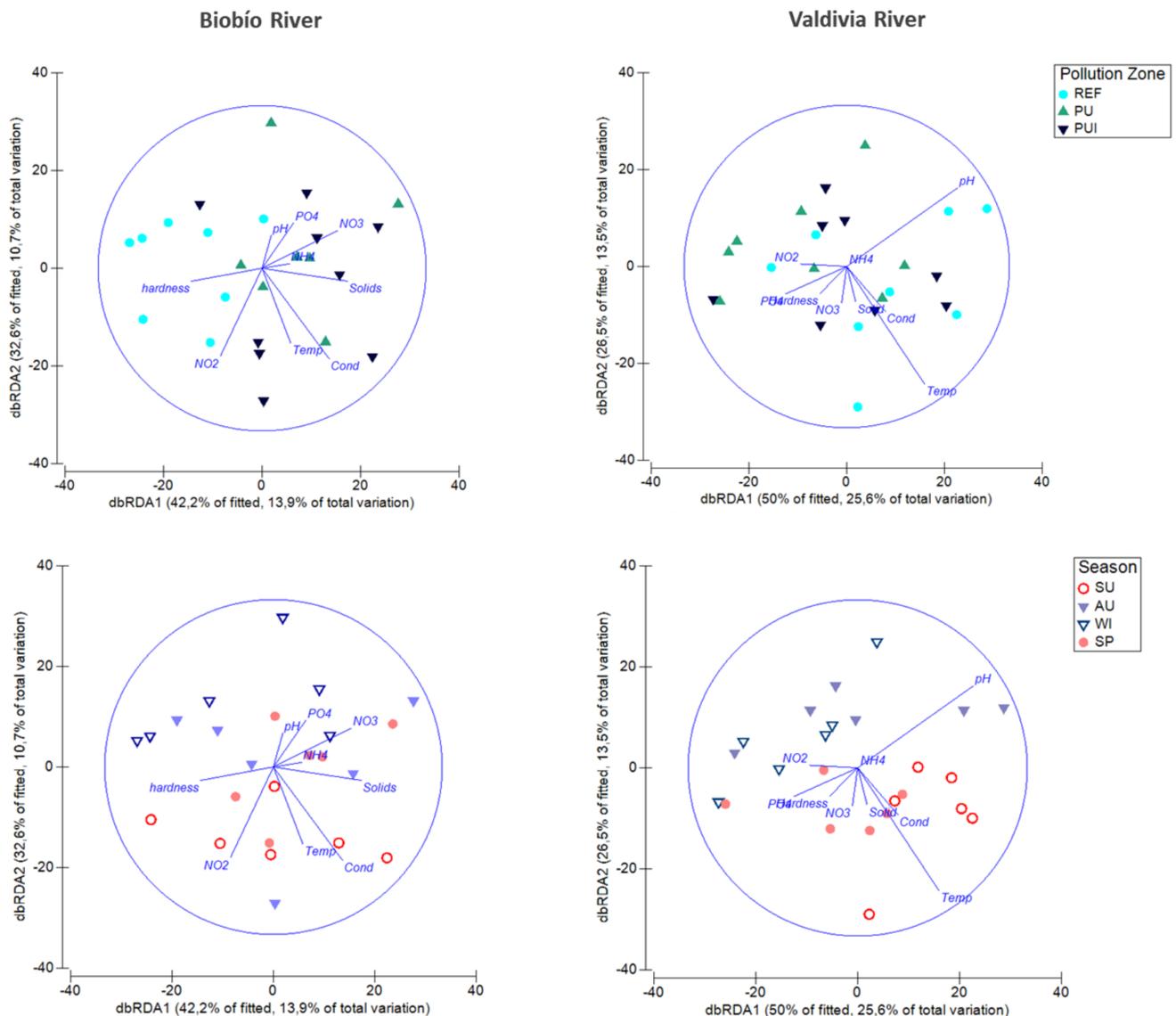


Figure 3. Redundancy analyses (RDA) based on BrayCurtis dissimilarity matrix. Species abundance data were fourth-root transformed. Environmental variables were logx+1 transformed. Graphs show grouping of study sites and their associations with environmental variables (lines) for the Biobío River (**left**) and Valdivia River (**right**), and for pollution levels (**top**) and seasons (**bottom**).

Table 3. Results of ANOVA based on the general linear model (GLM) for functional indices estimated for fish assemblages from the Biobío and Valdivia rivers; functional specialization (FSpe), functional originality (FOri), functional dispersion (FDis) and functional entropy (FEnt), as a function of axes from environmental factors PCA (Varimax). Significant probabilities are showed in bold. Levels of significance are indicated by (*): * $p < 0.01$, ** $p < 0.05$ and *** $p < 0.001$.

	FSpe		FOri		FDis		FEnt	
	Chi sq	<i>p</i>	Chi sq	<i>p</i>	Chi sq	<i>p</i>	Chi sq	<i>p</i>
Biobío								
Physical water quality (PC1)	4.9166	0.0266 *	0.4903	0.4838	2.0049	0.15679	1.9758	0.1598
Zone					6.4841	0.03908 *	7.7944	0.0203 *
Valdivia								
Nutrient pollution (PC2)	5.280	0.02157 *	1.2546	0.2627	3.1996	0.07366	4.5285	0.008486 **
Season	30.343	<0.001 ***	23.2988	<0.001 ***	2.9122	0.40537	11.6996	0.033334 *

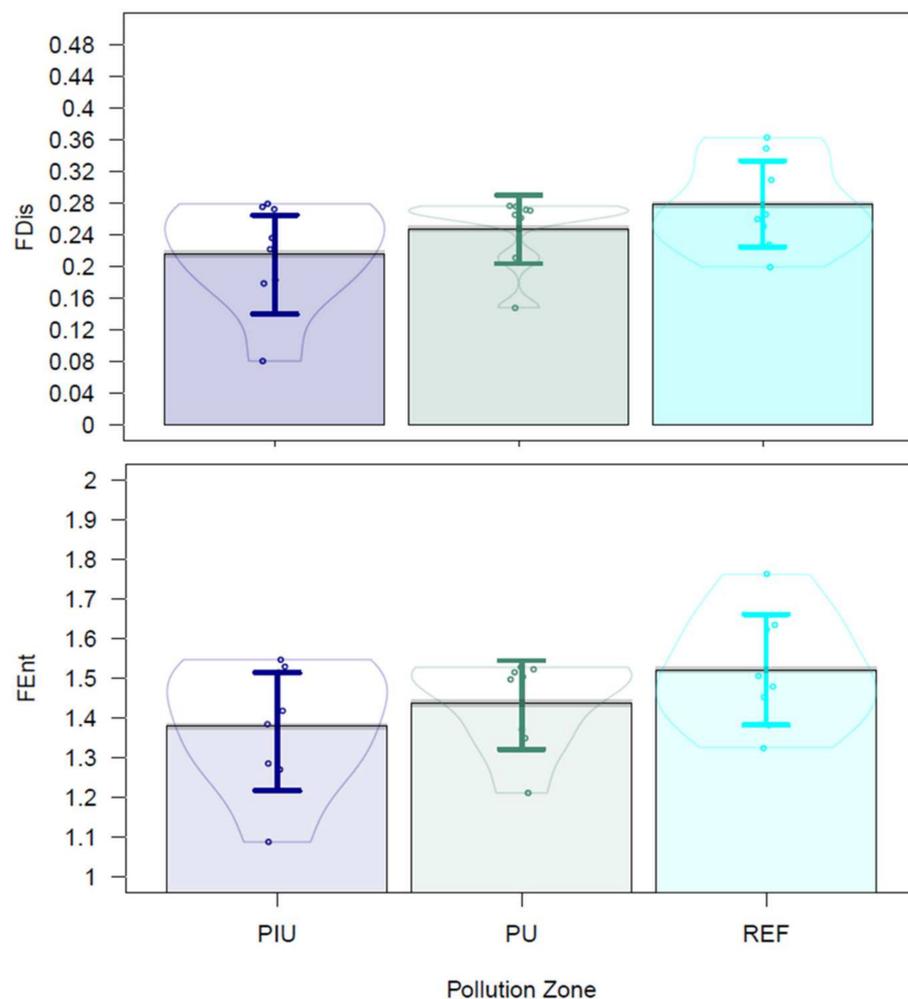


Figure 4. Significant relationships of functional diversity indices with pollution zones in the Biobío River (cyan, reference; green, urban pollution; dark blue, urban and industrial pollution). The bars are means, whiskers 95% CI, dots sample sites, and bean data density.

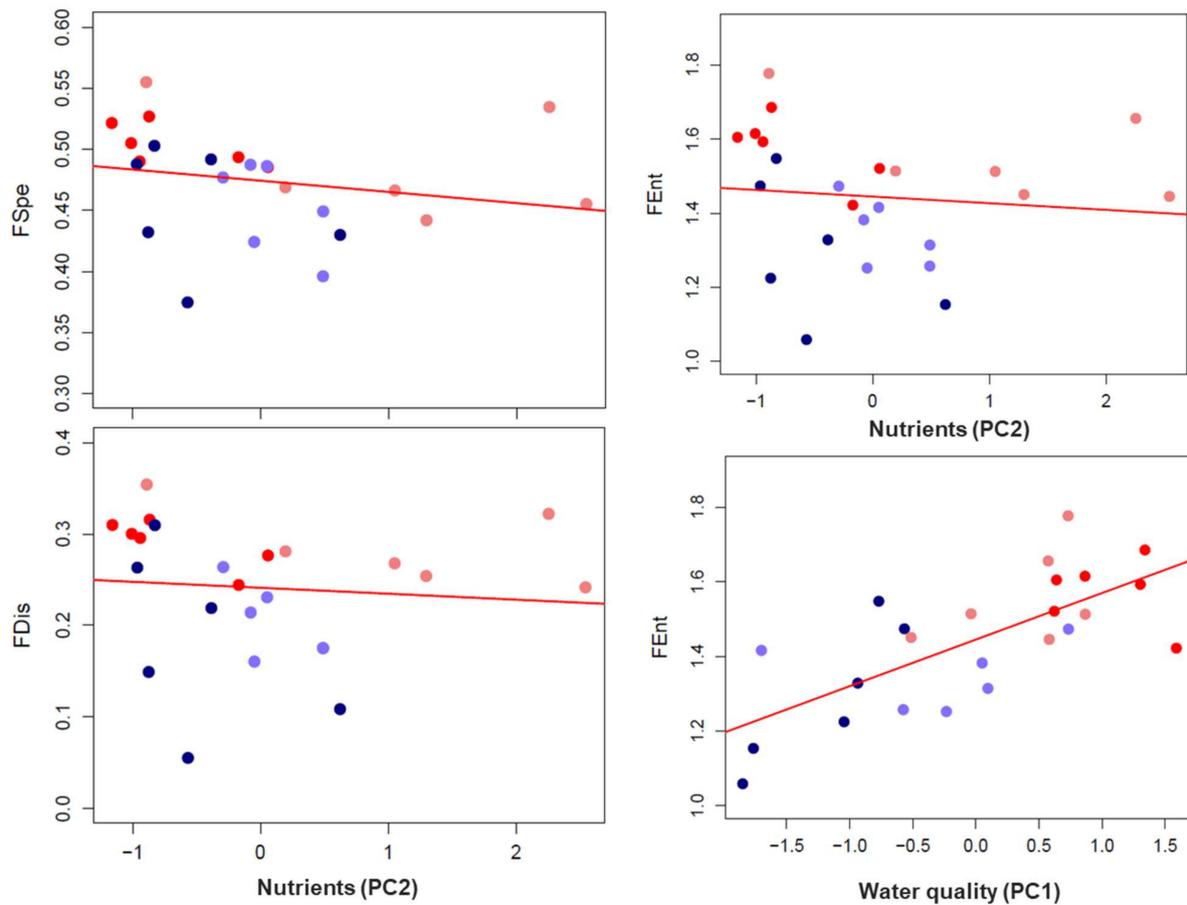


Figure 5. Significant relationships of functional diversity indices with nutrients and water quality (PC1 and PC2), and season (red, summer; violet, autumn; dark blue, winter; Indian red, spring) in the Valdivia River.

The observed decrease of the values of functional indices reflects the loss or depletion of some functional traits. In the Biobío River, the most affected traits were trophic guilds associated with pollution zone, and floodplain use associated with water quality and presence of non-native species (*Australoheros facetus* and *Gambusia holbrooki*, Figure 6). A different pattern was observed in the Valdivia River where the strongest association was observed between migratory life-histories (*Geotria australis* and *Aplochiton taeniatus*) and seasons that reflect natural flow fluctuation (Figure 7). Floodplain use and trophic guilds also appeared as relevant traits that structured the natural seasonal dynamic of the fish assemblage (Figure 7).

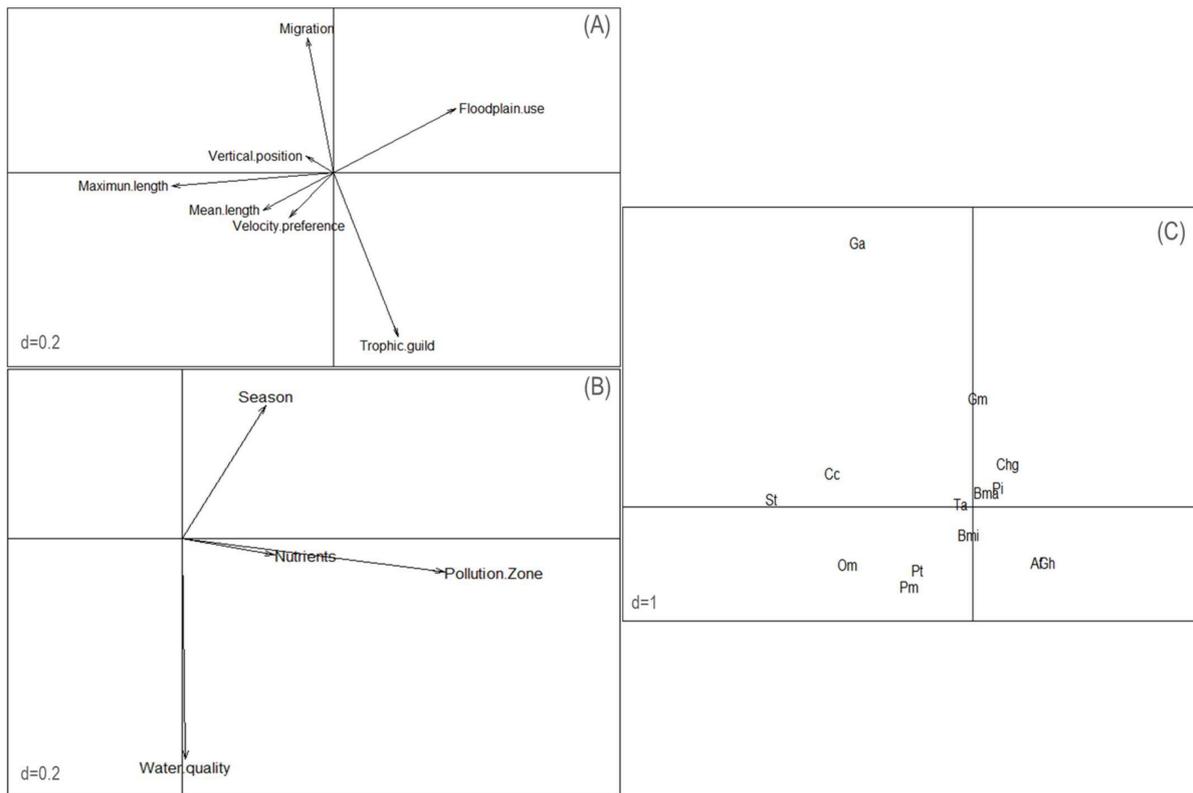


Figure 6. Results of the first two axes of the RLQ analysis for the Biobío River: (A) coefficients for traits (descriptions in Table 1); (B) coefficients for environmental variables; (C) species scores (see Table A1 for species codes). The values of d give the grid size indicate stress data level.

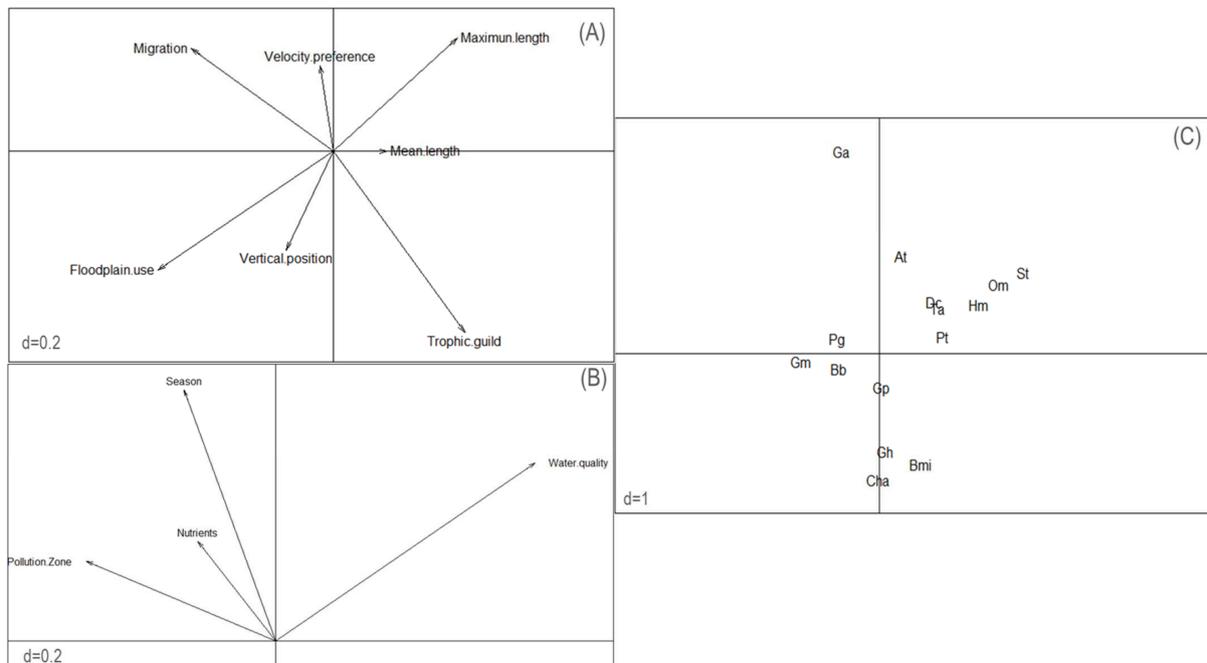


Figure 7. Results of the first two axes of the RLQ analysis for the Valdivia River: (A) coefficients for traits (descriptions in Table 1); (B) coefficients for environmental variables; (C) species scores (see Table A1 for species codes). The values of d give the grid size indicate stress data level.

4. Discussion

This study is the first to assess the effects of human-induced stressors on the functional and taxonomic aspects of fish assemblage structure in South American temperate rivers and represents an important contribution to advancing our understanding of the effects of interplay of stressors on species-poor assemblages. First, we showed that beta diversity structuring significantly differed between the Biobío and Valdivia rivers; the Biobío River showed marked nestedness whereas turnover in the Valdivia River was more significant. Secondly, we showed that the fish assemblage structure and function in the Biobío River was strongly driven by human-induced stressors reflected in water quality alterations, whereas fish assemblage in the Valdivia River was characterized by more natural seasonal dynamics.

The Biobío and Valdivia river basins are characterized by the highest fish species richness and diversity among Chilean river basins [30,57]. However, significant human-induced alterations during the last few decades have strongly influenced composition and structure of fish assemblages in the Biobío River basin and resulted in native fish diversity loss. Ref. [57] reported a significant decrease in fish abundance and diversity as a consequence of human activities such as forestry, pulp mill plants, and hydropower dam operation. Currently, there are 18 hydropower plants operating in the Biobío River basin and were shown to influence pollutant concentration in water column and affect sediments dynamics [34]. Furthermore, these hydropower dams have been shown to significantly obstruct fish movement, affect population genetic diversity, and cause local population extirpations [22,58,59].

Hydropower dams that operate in hydropeaking regime directly affect habitat availability for native benthic and benthic-pelagic species such as *Trychomycterus areolatus* and *Percilia irwini* [60,61]. Severe flow alteration caused by hydropeaking strongly affects the natural temporal dynamic of riverine habitats [61]. Possibly as an effect of this, habitat use traits and specifically floodplain use has emerged among the most affected traits in the Biobío River according to the RLQ results. Lateral habitats and floodplains are essential for reproduction and recruitment of many fish species and often serve as feeding grounds or provide food subsidies [62,63]. Trophic traits also appeared affected, most probably since several macro-benthos species are sensitive to hydropeaking and due to changes in the nutrient concentrations and organic matter accumulation in the substrate [32,64]. Indeed, in the most polluted zones, invertivorous fish such as *Percilia irwini* and *Percichthys trucha* disappeared or diminished notably their abundances in Biobío River. As such, the loss of specialist species contributes to the loss of trophic roles in the most impacted reaches [65]. Furthermore, these declines could have been also influenced indirectly by changes in sediment dynamics downstream of hydropower dams and/or high amount of organic matter from pulp mill residues due to inefficient waste treatment.

In the Valdivia River, the absence of hydropower dams and the presence of only one pulp mill in the middle section of the tributary is reflected in fish assemblage that seems to preserve more natural structure and dynamics. Water column preference traits are linked with season, which reflects the natural seasonal flow fluctuations. In summer, discharge and water velocity decrease, and this decrease is associated with an increase of abundance of pelagic and decrease of benthic species in littoral fish samples [30]. Furthermore, the presence of specialist reophilic and invertivorous species such as *Diplomystes camposensis* in the Valdivia River indicates that diverse traits and functions are still present in the system [21]. In the Biobío River, the sister specialist species *Diplomystes nahuelbutaensis* has not been registered within this study. Species from the *Diplomystes* genus are characterized by high dispersal capacities and low genetic diversity [66–68]. Furthermore, they require highly oxygenated water and a substrate with a low content of sediment and organic matter [69,70]. These characteristics make species of this genus highly sensitive to river network fragmentation and water quality deterioration. Indeed, operations of hydropower dams and water pollution have caused *D. nahuelbutaensis* habitat loss in the Biobío River and a gradual decrease of its abundance that may explain its absence in our samples [71].

These vulnerable species support singular functions in riverine species-poor assemblages that are weakly buffered against functional diversity loss due to low trait redundancy [27].

The results of beta diversity analyses corroborate these findings, as the marked nested assembly pattern found in the Biobío River may reflect a loss of specialist species incorporation to the assemblage as a consequence of human-induced stressors. Indeed, the prevalence of a nested pattern rather than a turnover one has been shown to characterize river biota assemblage influenced by anthropogenic stressors in Mediterranean rivers and in Spain and Chile [13,21]. Such a pattern indicates that new species are poorly incorporated in the assembly process. Indeed, in the Biobío River, this pattern principally reflects invasive and generalist species such as *Australoheros facetus* and *Gambusia holbrooki* that are added to the assemblage in polluted reaches. Furthermore, the addition of these species to assemblages in the most severely affected reaches also explains changes in trophic guilds. Non-native species in degraded systems gain importance in species-poor assemblages, especially if non-native species functional characteristics significantly differ from those of native fish [8,9]. In contrast, Valdivia River fish assemblages show a marked turnover beta diversity pattern most probably driven by natural processes, as human-induced stressors are not significant to remove specialist species in the assembly process [21,26].

This contrasting pattern between the Biobío and Valdivia rivers is also reflected in the results of redundancy analyses. Fish assemblages in the Biobío River are characterized by spatial grouping with a clear differentiation of reference and pollution locations that reflect distinct fish assemblages. Furthermore, no significant differences in fish assemblages were observed between polluted zones, suggesting significant effects of flow regulation at the interplay with pollution at both pollution intensities. In contrast, fish assemblages in the Valdivia River are characterized by differentiation among seasons, that suggests more natural dynamics of a functional structure significantly associated with functional specialization, dispersion, and entropy patterns. Furthermore, functional specialization and entropy also significantly decreased along the nutrient concentration gradient with low specialization of fish assemblages in spring. This pattern may be related to seasonal inundation of floodplain habitats and the associated increase of accumulation and decomposition of organic matter of both natural and anthropogenic origins [72]. In addition, inundated floodplain habitats are used principally by pelagic galaxiids (*Galaxias maculatus*) and silversides (*Basilichthys microlepidotus*), whereas in summer, the river channel narrows and most fish species occupy shoreline habitats to some degree increasing the functional specialization of the sampled littoral fish assemblage.

Alteration of water quality variables such as conductivity and nitrate concentration may have influenced the observed pattern. The effects of nutrient pollution on riverine fish assemblages have been widely studied (e.g., [73–75]). Concentrations registered in Chilean rivers remain low compared to other rivers, but the natural oligotrophic state of southern Chilean rivers makes them highly sensitive to nutrient concentration increase [6]. Already in 2010, a significant (up to six-fold) increase of concentrations of nitrate was registered in the Biobío and Valdivia rivers [76]. These significant increases of nutrient concentrations are expected to continue and affect riverine biota, including fish [6]. The sensitivity of species-poor riverine biota that inhabits these rivers to pollution could have been clearly evidenced in 2004 in the Cruces River (tributary of the Valdivia River) which has undergone ecosystem collapse due to nutrient pollution and water quality decreasing as consequence of inefficient operation of a pulp-mill plant [44]. During the collapse, various taxonomic groups of organisms died massively due to bottom-up effect [37,77]. Currently, an increase in depuration technology standards has allowed for the improvement of the rivers' ecological status, reflected in the composition and functional structure of fish assemblages in the Cruces River (which does not seem to be highly affected by pollution according to our results). However, strict environmental policies should be applied in order to control nutrient pollution increase and hydropower development, as these have significant effects on fish assemblages (as shown with the example of the Biobío River basin).

5. Conclusions

We found a strong association between taxonomic and functional fish assemblage facets with a predominance of natural processes in the Valdivia River, reflected in its marker temporal dynamics. In contrast, the Biobío River showed a clear loss of association with temporal seasonal pattern and, instead, both taxonomic and functional facets of the fish assemblage appeared to respond significantly to pollution zones. High quantity of pollutants in the Biobío River and their significant effects on its species-poor fish assemblages necessitates management actions if ecological health of the river is to be improved. These actions are especially urgent given future climate scenarios and a projected significant decrease of precipitations and river discharges in the region which will further increase pollutant concentrations in river waters, exacerbating the risk of functional traits loss. Therefore, stricter environmental policies should be generated by governmental entities in order to preserve the biodiversity and ecosystem services of central Chilean rivers. These should focus on: (1) obtaining spatially and temporally explicit diagnosis of pollution distribution and its effects on organisms and ecosystem function; (2) decreasing number and concentration of pollutants by technological improvements in pulp-mill industry and urban waste-water treatment; (3) generating strategies that assure conservation of native fish species, especially those with restricted distribution ranges, with a long-term biomonitoring; and (4) the implementation of integrated river basin management in order to plan human activities in balance with natural ecosystem functions to assure water quality and species conservation.

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Appendix A

Table A1. List of species registered in each studied river basin indicating species code, local Chilean common name and family (X, species presence).

Species	Code	Common Name	Family	Origin	Basin	
					Biobio River	Valdivia River
<i>Australoheros facetus</i>	Af	chanchito	Cichlidae	non-native	x	
<i>Aplochiton taeniatus</i>	At	peladilla	Galaxiidae	native		x
<i>Bullockia maldonadoi</i>	Bma	bagrecito	Tricomycetidae	native	x	
<i>Basilichthys microlepidotus</i>	Bmi	pejerrey chileno	Atherinopsinae	native	x	x

Table A1. Cont.

Species	Code	Common Name	Family	Origin	Basin	
					Biobio River	Valdivia River
<i>Brachylaxias bullocki</i>	Bb	puye rojo	Galaxiidae	native		x
<i>Cyprinus carpio</i>	Cc	carpa	Ciprinidae	non-native	x	
<i>Cheirodon kilianii</i>	Cha	pocha	Characidae	native		x
<i>Cheirodon galusdae</i>	Chg	pocha	Characidae	native	x	
<i>Diplomystes camposensis</i>	Dc	tollo	Diplomystidae	native		x
<i>Geotria australis</i>	Ga	lamprea	Geotriidae	native	x	x
<i>Gambusia holbrooki</i>	Gh	pez mosquito	Poeciliidae	non-native	x	x
<i>Galaxias maculatus</i>	Gm	puye	Galaxiidae	native	x	x
<i>Galaxias platei</i>	Gp	puye grande	Galaxiidae	native		x
<i>Hatcheria macreii</i>	Hm	bagre grande	Tricomycetidae	native		x
<i>Onchorhynchus mykiss</i>	Om	trucha arcoiris	Salmonidae	non-native	x	x
<i>Percilia irwini</i>	Pi	carmelita de Concepción	Perciliidae	native	x	
<i>Percilia gillissii</i>	Pg	Carmelita	Perciliidae	native		x
<i>Percichthys melanops</i>	Pm	perca negra	Percichthyidae	native	x	
<i>Percichthys trucha</i>	Pt	perca	Percichthyidae	native	x	x
<i>Salmo trutta</i>	St	trucha café	Ssalmonidae	non-native	x	x
<i>Trichomycterus areolatus</i>	Ta	bagre chico	Tricomycetidae	native	x	x

References

- Strayer, D.L.; Dudgeon, D. Freshwater biodiversity conservation: Recent progress and future challenges. *J. N. Am. Benthol. Soc.* **2010**, *29*, 344–358. [[CrossRef](#)]
- Vörösmarty, C.J.; McIntyre, P.B.; Gessner, M.O.; Dudgeon, D.; Prusevich, A.; Green, P.; Glidden, S.; Bunn, S.E.; Sullivan, C.A.; Reidy Liermann, C.; et al. Global threats to human water security and river biodiversity. *Nature* **2010**, *467*, 555–561. [[CrossRef](#)] [[PubMed](#)]
- Araya-Osses, D.; Casanueva, A.; Román-Figueroa, C.; Uribe, J.M.; Paneque, M. Climate change projections of temperature and precipitation in Chile based on statistical downscaling. *Clim. Dyn.* **2020**, *54*, 4309–4330. [[CrossRef](#)]
- Bozkurt, D.; Rojas, M.; Boisier, J.P.; Valdivieso, J. Projected hydroclimate changes over Andean basins in central Chile from downscaled CMIP5 models under the low and high emission scenarios. *Clim. Chang.* **2018**, *150*, 131–147. [[CrossRef](#)]
- Habit, E.; Górski, K.; Alò, D.; Ascencio, E.; Astorga, A.; Colin, N.; Contador, T.; de los Ríos, P.; Delgado, V.; Dorador, C.; et al. *Biodiversidad de Ecosistemas de Agua Dulce. Mesa Biodiversidad-Comité Científico COP25*; Ministerio de Ciencia, Tecnología, Conocimiento e Innovación: Santiago, Chile, 2019; p. 64.
- Pastén, P.; Vega, A.; Guerra, P.; Pizarro, J.; Lizama, K. Water Quality in Chile: Progress, Challenges and Perspectives. In *Water Quality in the Americas*; IANAS: Ciudad de México, Mexico, 2019; p. 160.
- Schinegger, R.; Pucher, M.; Aschauer, C.; Schmutz, S. Configuration of multiple human stressors and their impacts on fish assemblages in Alpine river basins of Austria. *Sci. Total Environ.* **2018**, *616*, 17–28. [[CrossRef](#)] [[PubMed](#)]
- Colin, N.; Villéger, S.; Wilkes, M.; de Sostoa, A.; Maceda-Veiga, A. Functional diversity measures revealed impacts of non-native species and habitat degradation on species-poor freshwater fish assemblages. *Sci. Total Environ.* **2018**, *625*, 861–871. [[CrossRef](#)] [[PubMed](#)]
- Sánchez-Pérez, A.; Oliva-Paterna, F.J.; Colin, N.; Torralva, M.; Górski, K. Functional response of fish assemblage to multiple stressors in a highly regulated Mediterranean river system. *Sci. Total Environ.* **2020**, *730*, 138989. [[CrossRef](#)] [[PubMed](#)]
- Birk, S.; Chapman, D.; Carvalho, L.; Spears, B.M.; Andersen, H.E.; Argillier, C.; Auer, S.; Baattrup-Pedersen, A.; Banin, L.; Beklioglu, M.; et al. Impacts of multiple stressors on freshwater biota across spatial scales and ecosystems. *Nat. Ecol. Evol.* **2020**, *4*, 1060–1068. [[CrossRef](#)]
- Ormerod, S.J.; Dobson, M.; Hildrew, A.G.; Townsend, C. Multiple stressors in freshwater ecosystems. *Freshw. Biol.* **2010**, *55* (Suppl. S1), 1–4. [[CrossRef](#)]
- Schinegger, R.; Trautwein, C.; Melcher, A.; Schmutz, S. Multiple human pressures and their spatial patterns in European running waters. *Water Environ. J.* **2012**, *26*, 261–273. [[CrossRef](#)]
- Gutiérrez-Cánovas, C.; Millán, A.; Velasco, J.; Vaughan, I.P.; Ormerod, S.J. Contrasting effects of natural and anthropogenic stressors on beta diversity in river organisms. *Glob. Ecol. Biogeogr.* **2013**, *22*, 796–805. [[CrossRef](#)]
- Colin, N.; Maceda-Veiga, A.; Monroy, M.; Ortega-Ribera, M.; Llorente, M.; de Sostoa, A. Trends in biomarkers, biotic indices, and fish population size revealed contrasting long-term effects of recycled water on the ecological status of a Mediterranean river. *Ecotoxicol. Environ. Saf.* **2017**, *145*, 340–348. [[CrossRef](#)] [[PubMed](#)]
- Amiard-Triquet, C.; Cossu-Leguille, C.; Mouneyrac, C. Biomarkers of defense, tolerance, and ecological consequences. In *Ecological Biomarkers: Indicators of Ecotoxicological Effects*; CRC Press: London, UK, 2013; pp. 45–74.

16. Vila, I.; Fuentes, L.; Contreras, M. Peces límnicos de Chile. *Bol. Mus. Nac. Hist. Nat.* **1999**, *48*, 61–75.
17. Dyer, B.S. Revisión sistemática de los Pejerreyes de Chile (Teleostei, Atheriniforme). *Estud. Oceanol* **2000**, *200019*, 99–127.
18. Habit, E.; Dyer, B.; Vila, I. Estado de conocimiento de los peces dulceacuícolas de Chile. *Gayana (Concepc)* **2006**, *70*, 100–113. [[CrossRef](#)]
19. Habit, E.; Piedra, P.; Ruzzante, D.E.; Walde, S.J.; Belk, M.C.; Cussac, V.E.; González, J.; Colin, N. Changes in the distribution of native fishes in response to introduced species and other anthropogenic effects. *Glob. Ecol. Biogeogr.* **2010**, *19*, 697–710. [[CrossRef](#)]
20. MMA. Ministerio del Medio Ambiente. 2021. Available online: <http://especies.mma.gob.cl/CNMWeb/Web/WebCiudadana/Default.aspx> (accessed on 16 January 2021).
21. Castro, S.A.; Rojas, P.; Vila, I.; Habit, E.; Pizarro-Konczak, J.; Abades, S.; Jaksic, F.M. Partitioning β -diversity reveals that invasions and extinctions promote the biotic homogenization of Chilean freshwater fish fauna. *PLoS ONE* **2020**, *15*, e0238767. [[CrossRef](#)]
22. Díaz, G.; Górski, K.; Heino, J.; Arriagada, P.; Link, O.; Habit, E. The longest fragment drives fish beta diversity in fragmented river networks: Implications for river management and conservation. *Sci. Total Environ.* **2021**, *766*, 144323. [[CrossRef](#)]
23. Vellend, M. Do commonly used indices of β -diversity measure species turnover? *J. Veg. Sci.* **2001**, *12*, 545–552. [[CrossRef](#)]
24. Socolar, J.B.; Gilroy, J.J.; Kunin, W.E.; Edwards, D.P. How should beta-diversity inform biodiversity conservation? *Trends Ecol. Evol.* **2016**, *31*, 67–80. [[CrossRef](#)]
25. Mouillot, D.; Graham, N.A.; Villéger, S.; Mason, N.W.; Bellwood, D.R. A functional approach reveals community responses to disturbances. *Trends Ecol. Evol.* **2013**, *28*, 167–177. [[CrossRef](#)] [[PubMed](#)]
26. Rojas, P.; Castro, S.A.; Vila, I.; Jaksic, F.M. Exotic species elicit decoupled responses in functional diversity components of freshwater fish assemblages in Chile. *Ecol. Indic.* **2021**, *133*, 108364. [[CrossRef](#)]
27. Teichert, N.; Lepage, M.; Sagouis, A.; Borja, A.; Chust, G.; Ferreira, M.T.; Argillier, C. Functional redundancy and sensitivity of fish assemblages in European rivers, lakes and estuarine ecosystems. *Sci. Rep.* **2017**, *7*, 1–11. [[CrossRef](#)] [[PubMed](#)]
28. Vogel, A.; Manning, P.; Cadotte, M.W.; Cowles, J.; Isbell, F.; Jousset, A.L.; Wagg, C. Lost in trait space: Species-poor communities are inflexible in properties that drive ecosystem functioning. *Adv. Ecol. Res.* **2019**, *61*, 91–131. [[CrossRef](#)]
29. Villéger, S.; Mason, N.W.; Mouillot, D. New multidimensional functional diversity indices for a multifaceted framework in functional ecology. *Ecology* **2008**, *89*, 2290–2301. [[CrossRef](#)]
30. Colin, N.; Piedra, P.; Habit, E. Variaciones espaciales y temporales de las comunidades ribereñas de peces en un sistema fluvial no intervenido: Río San Pedro, cuenca del río Valdivia (Chile). *Gayana (Concepc)* **2012**, *76*, 24–35. [[CrossRef](#)]
31. Díaz, G.; Arriagada, P.; Górski, K.; Link, O.; Karelavic, B.; Gonzalez, J.; Habit, E. Fragmentation of Chilean Andean rivers: Expected effects of hydropower development. *Rev. Chil. Hist. Nat.* **2019**, *92*, 1–13. [[CrossRef](#)]
32. Elgueta, A.; Górski, K.; Thoms, M.; Fierro, P.; Toledo, B.; Manosalva, A.; Habit, E. Interplay of geomorphology and hydrology drives macroinvertebrate assemblage responses to hydropeaking. *Sci. Total Environ.* **2021**, *768*, 144262. [[CrossRef](#)]
33. Chiang, G.; Munkittrick, K.R.; McMaster, M.E.; Barra, R.; Servos, M. Marco conceptual de Monitoreo Regional de Efectos Acumulativos: Brechas y Desafíos para la Cuenca del río Biobío en el Centro-Sur de Chile. *Gayana (Concepc)* **2014**, *78*, 109–119. [[CrossRef](#)]
34. Alonso, Á.; Figueroa, R.; Castro-Díez, P. Pollution assessment of the Biobío River (Chile): Prioritization of substances of concern under an ecotoxicological approach. *Environ. Manag.* **2017**, *59*, 856–869. [[CrossRef](#)]
35. Orrego, R.; Hewitt, L.M.; McMaster, M.; Chiang, G.; Quiroz, M.; Munkittrick, K.; Gavilán, F.; Barra, R. Assessing wild fish exposure to ligands for sex steroid receptors from pulp and paper mill effluents in the Biobío River Basin, Central Chile. *Ecotoxicol. Environ. Saf.* **2019**, *171*, 256–263. [[CrossRef](#)] [[PubMed](#)]
36. Schaefer, K.; Einax, J.W. Analytical and chemometric characterization of the Cruces River in South Chile. *Environ. Sci. Pollut. Res.* **2010**, *17*, 115–123. [[CrossRef](#)] [[PubMed](#)]
37. Marín, V.H.; Delgado, L.E.; Vila, I.; Tironi, A.; Barrera, V.; Ibáñez, C. Regime shifts of Cruces River wetland ecosystem: Current conditions, future uncertainties. *Lat. Am. J. Aquat. Res.* **2014**, *42*, 160–171. [[CrossRef](#)]
38. Barra, R.O.; Chiang, G.; Saavedra, M.F.; Orrego, R.; Servos, M.R.; Hewitt, L.M.; McMaster, M.; Bahamonde, P.; Tucca, F.; Munkittrick, K.R. Endocrine Disruptor Impacts on Fish From Chile: The Influence of Wastewaters. *Front. Endocrinol.* **2021**, *12*, 208. [[CrossRef](#)] [[PubMed](#)]
39. Maire, E.; Grenouillet, G.; Brosse, S.; Villéger, S. How many dimensions are needed to accurately assess functional diversity? A pragmatic approach for assessing the quality of functional spaces. *Glob. Ecol. Biogeogr.* **2015**, *24*, 728–740. [[CrossRef](#)]
40. Elgueta, A.; Thoms, M.C.; Górski, K.; Díaz, G.; Habit, E. Functional process zones and their fish communities in temperate Andean river networks. *River Res. Appl.* **2019**, *35*, 1702–1711. [[CrossRef](#)]
41. Quiroz-Jara, M.; Casini, S.; Fossi, M.C.; Orrego, R.; Gavilán, J.F.; Barra, R. Integrated Physiological Biomarkers Responses in Wild Fish Exposed to the Anthropogenic Gradient in the Biobío River, South-Central Chile. *Environ. Manag.* **2021**, *67*, 1145–1157. [[CrossRef](#)]
42. Pauchard, A.; Aguayo, M.; Peña, E.; Urrutia, R. Multiple effects of urbanization on the biodiversity of developing countries: The case of a fast-growing metropolitan area (Concepción, Chile). *Biol. Conserv.* **2006**, *127*, 272–281. [[CrossRef](#)]
43. Niemeyer, H.; Cereceda, P. *Hidrografía. Ediciones Geografía de Chile*; Instituto Geográfico Militar: Santiago, Chile, 1984.
44. Mulsow, S.; Grandjean, M. Incompatibility of sulphate compounds and soluble bicarbonate salts in the Río Cruces waters: An answer to the disappearance of *Egeria densa* and black-necked swans in a RAMSAR sanctuary. *Ethics Sci. Environ. Politics* **2006**, *2006*, 5–11. [[CrossRef](#)]

45. Baselga, A. Partitioning the turnover and nestedness components of beta diversity. *Glob. Ecol. Biogeogr.* **2010**, *19*, 134–143. [[CrossRef](#)]
46. Baselga, A.; Orme, C.D.L. betapart: An R package for the study of beta diversity. *Methods Ecol. Evol.* **2012**, *3*, 808–812. [[CrossRef](#)]
47. Baselga, A. Separating the two components of abundance-based dissimilarity: Balanced changes in abundance vs. abundance gradients. *Methods Ecol. Evol.* **2013**, *4*, 552–557. [[CrossRef](#)]
48. McArdle, B.H.; Anderson, M.J. Anderson. Fitting multivariate models to community data: A comment on distance-based redundancy analysis. *Ecology* **2001**, *82*, 290–297. [[CrossRef](#)]
49. Anderson, M.J.; Gorley, R.N.; Clarke, K.R. *PERMANOVA+ for PRIMER: Guide to Software and Statistical Methods*; PRIMER-E: Plymouth, UK, 2008.
50. Valdovinos, C.; Habit, E.; Jara, A.; Piedra, P.; González, J.; Salvo, J. Dinámica espacio-temporal de 13 especies de peces nativos en un ecotono lacustre-fluvial de la Cuenca del Río Valdivia (Chile). *Gayana (Concepc)* **2012**, *76*, 45–58.
51. Vila, I.; Habit, E. Current situation of the fish fauna in the Mediterranean region of Andean river systems in Chile. *FiSHMED Fishes Mediterr. Environ.* **2015**, *2*, 19. [[CrossRef](#)]
52. Bellwood, D.R.; Hughes, T.P.; Hoey, A.S. Sleeping functional group drives coral-reef recovery. *Curr. Biol.* **2006**, *16*, 2434–2439. [[CrossRef](#)]
53. Laliberté, E.; Legendre, P. A distance-based framework for measuring functional diversity from multiple traits. *Ecology* **2010**, *91*, 299–305. [[CrossRef](#)]
54. Ricotta, C.; Szeidl, L. Diversity partitioning of Rao's quadratic entropy. *Theor. Popul. Biol.* **2009**, *76*, 299–302. [[CrossRef](#)]
55. Queen, J.P.; Quinn, G.P.; Keough, M.J. *Experimental Design and Data Analysis for Biologists*; Cambridge University Press: Cambridge, UK, 2002; p. 527.
56. Dray, S.; Choler, P.; Dolédec, S.; Peres-Neto, P.R.; Thuiller, W.; Pavoine, S.; ter Braak, C.J. Combining the fourth-corner and the RLQ methods for assessing trait responses to environmental variation. *Ecology* **2014**, *95*, 14–21. [[CrossRef](#)]
57. Habit, E.; Belk, M.C.; Cary Tuckfield, R.; Parra, O. Response of the fish community to human-induced changes in the Biobío River in Chile. *Freshw. Biol.* **2006**, *51*, 1–11. [[CrossRef](#)]
58. Valenzuela-Aguayo, F.; McCracken, G.R.; Manosalva, A.; Habit, E.; Ruzzante, D.E. Human-induced habitat fragmentation effects on connectivity, diversity, and population persistence of an endemic fish, *Percilia irwini*, in the Biobío River basin (Chile). *Evol. Appl.* **2020**, *13*, 794–807. [[CrossRef](#)] [[PubMed](#)]
59. Vivancos, A.; Górski, K.; Manosalva, A.; Toledo, B.; Reid, M.; Habit, E. Hydrological connectivity drives longitudinal movement of endangered endemic Chilean darter *Percilia irwini* (Eigenmann, 1927). *J. Fish Biol.* **2021**, *98*, 33–43. [[CrossRef](#)] [[PubMed](#)]
60. Habit, E.; Belk, M.C.; Parra, O. Response of the riverine fish community to the construction and operation of a diversion hydropower plant in central Chile. *Aquat. Conserv. Mar. Freshw. Ecosyst.* **2007**, *17*, 37–49. [[CrossRef](#)]
61. García, A.; Jorde, K.; Habit, E.; Caamaño, D.; Parra, O. Downstream environmental effects of dam operations: Changes in habitat quality for native fish species. *River Res. Appl.* **2011**, *27*, 312–327. [[CrossRef](#)]
62. Górski, K.; Winter, H.V.; De Leeuw, J.J.; Minin, A.E.; Nagelkerke, L.A.J. Fish spawning in a large temperate floodplain: The role of flooding and temperature. *Freshw. Biol.* **2010**, *55*, 1509–1519. [[CrossRef](#)]
63. Górski, K.; Collier, K.J.; Hamilton, D.P.; Hicks, B.J. Effects of flow on lateral interactions of fish and shrimps with off-channel habitats in a large river-floodplain system. *Hydrobiologia* **2014**, *729*, 161–174. [[CrossRef](#)]
64. Fierro, P.; Valdovinos, C.; Arismendi, I.; Díaz, G.; Jara-Flores, A.; Habit, E.; Vargas-Chacoff, L. Examining the influence of human stressors on benthic algae, macroinvertebrate, and fish assemblages in Mediterranean streams of Chile. *Sci. Total Environ.* **2019**, *686*, 26–37. [[CrossRef](#)]
65. Maceda-Veiga, A.; Mac Nally, R.; de Sostoa, A. Environmental correlates of food-chain length, mean trophic level and trophic level variance in invaded riverine fish assemblages. *Sci. Total Environ.* **2018**, *644*, 420–429. [[CrossRef](#)]
66. Oyanedel, A.; Habit, E.; Belk, M.C.; Solis-Lufi, K.; Colin, N.; Gonzalez, J.; Jara, A.; Muñoz-Ramírez, C. Movement patterns and home range in *Diplomystes camposensis* (Siluriformes: Diplomystidae), an endemic and threatened species from Chile. *Neotrop. Ichthyol.* **2018**, *16*, e170134. [[CrossRef](#)]
67. Muñoz-Ramírez, C.P.; Unmack, P.J.; Habit, E.; Johnson, J.B.; Cussac, V.E.; Victoriano, P. Phylogeography of the ancient catfish family Diplomystidae: Biogeographic, systematic, and conservation implications. *Mol. Phylogenet. Evol.* **2014**, *73*, 146–160. [[CrossRef](#)]
68. Victoriano, P.F.; Vera, I.; Olmos, V.; Dib, M.; Insunza, B.; Muñoz-Ramírez, C.; Montoya, R.; Jara, A.; Habit, E. Patrones idiosincráticos de diversidad genética de peces nativos del Río San Pedro (Cuenca del Río Valdivia), un sistema de la región glaciada del sur de Chile. *Gayana (Concepc)* **2012**, *76*, 01–09. [[CrossRef](#)]
69. Habit, E. Aspectos de la biología y hábitat de un pez endémico de Chile en peligro de extinción (*Diplomystes nahuelbutaensis* Arratia, 1987). *Interciencia* **2005**, *30*, 8–11.
70. Habit, E.; Jara, A.; Colin, N.; Oyanedel, A.; Victoriano, P.; Gonzalez, J.; Solis-Lufi, K. Threatened fishes of the world: *Diplomystes camposensis* Arratia, 1987 (Diplomystidae). *Environ. Biol. Fishes* **2009**, *84*, 393–394. [[CrossRef](#)]
71. Habit, E.; García, A.; Díaz, G.; Arriagada, P.; Link, O.; Parra, O.; Thoms, M. River science and management issues in Chile: Hydropower development and native fish communities. *River Res. Appl.* **2019**, *35*, 489–499. [[CrossRef](#)]
72. Shrestha, J.; Niklaus, P.A.; Pasquale, N.; Huber, B.; Barnard, R.L.; Frossard, E.; Schleppei, P.; Tockner, K.; Luster, J. Flood pulses control soil nitrogen cycling in a dynamic river floodplain. *Geoderma* **2014**, *228*, 14–24. [[CrossRef](#)]

73. Smith, V.H.; Tilman, G.D.; Nekola, J.C. Eutrophication: Impacts of excess nutrient inputs on freshwater, marine, and terrestrial ecosystems. *Environ. Pollut.* **1999**, *100*, 179–196. [[CrossRef](#)]
74. Camargo, J.A.; Alonso, Á. Ecological and toxicological effects of inorganic nitrogen pollution in aquatic ecosystems: A global assessment. *Environ. Int.* **2006**, *32*, 831–849. [[CrossRef](#)] [[PubMed](#)]
75. Hord, N.G.; Beaver, L.; Axton, E.; Truong, L.; St. Marie, L.; Tanguay, R.; Logan, C.; Choi, J.; Spagnoli, S.; Stevens, J.F. Nitrate and nitrite exposure affect cognitive behavior and oxygen consumption during exercise in zebrafish. *FASEB J.* **2017**, *31* (Suppl. S1), lb277. [[CrossRef](#)]
76. Pizarro, J.; Vergara, P.M.; Rodriguez, J.A.; Sanhueza, P.A.; Castro, S.A. Nutrients dynamics in the main river basins of the centre-southern region of Chile. *J. Hazard. Mater.* **2010**, *175*, 608–613. [[CrossRef](#)]
77. Lagos, N.A.; Paolini, P.; Jaramillo, E.; Lovengreen, C.; Duarte, C.; Contreras, H. Environmental processes, water quality degradation, and decline of waterbird populations in the Rio Cruces wetland, Chile. *Wetlands* **2008**, *28*, 938–950. [[CrossRef](#)]