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Pervasive decline of subtropical aquatic insects over 20 years driven by water transparency, non-native fish and stoichiometric imbalance

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Insect abundance and diversity are declining worldwide. Although recent research found freshwater insect populations to be increasing in some regions, there is a critical lack of data from tropical and subtropical regions. Here, we examine a 20-year monitoring data set of freshwater insects from a subtropical floodplain comprising a diverse suite of rivers, shallow lakes, channels and backwaters. We found a pervasive decline in abundance of all major insect orders (Odonata, Ephemeroptera, Trichoptera, Megaloptera, Coleoptera, Hemiptera and Diptera) and families, regardless of their functional role or body size. Similarly, Chironomidae species richness decreased over the same time period. The main drivers of this pervasive insect decline were increased concurrent invasions of non-native insectivorous fish, water transparency and changes to water stoichiometry (i.e., N:P ratios) overtime. All these drivers represent human impacts caused by reservoir construction. This work sheds light on the importance of long-term studies for deeper understanding of human-induced impacts on aquatic insects. We highlight that extended anthropogenic impact monitoring and mitigation actions are pivotal in maintaining freshwater ecosystem integrity.

Keywords: damming/reservoir construction fish invasion, freshwater ecosystems, human impacts, insect decline, neotropical

1. Introduction

Globally widespread declines in insect populations have garnered much recent scientific and public attention [1,2]. However, nuanced analysis of these global trends revealed complex and divergent patterns among regions, different taxonomic groups and between freshwater and terrestrial insects [3,4]. Whereas freshwater ecosystems include some of the most threatened biota worldwide [5,6], a recent meta-analysis found freshwater insect populations to be increasing, in contrast to declining terrestrial insects [7]. This apparent recovery of freshwater insect populations is possibly driven by more effective policy and improving water quality in some temperate regions. Yet this work has suffered from a significant lack of data from tropical and subtropical regions [4,8,9].

Tropical and subtropical freshwater insects are threatened by multiple stressors [5]. These regions have some of the highest rates of human population growth, increasing resource demands and economic development, globally [10]. Consequently, rapid land-use changes for agricultural expansion and dam building for hydroelectric power and water extraction [11,12] have led to habitat degradation, changing hydrological regimes, disrupted nutrient dynamics and the introduction of non-native species [6]. Although these regions contain the vast majority of global insect diversity [13], the impacts of these threats on tropical and subtropical freshwater insects are poorly understood, due to a paucity of long-term studies.

Here, we examine one of the most comprehensive monitoring data sets of subtropical freshwater insects, spanning 20 years of data. We aimed to determine the long-term changes in species richness of Chironomidae (Diptera) and abundance of major functional feeding groups of insects inhabiting a suite of freshwater habitats from the Upper Paraná basin, including channels, backwaters, shallow lakes and rivers [12]. This system of diverse freshwater habitats, which drains much of south-central South America, has been impacted by the construction of over 150 dams across its tributaries [11,14]. Reservoir construction can impact insect communities from the bottom-up by disrupting hydrological and nutrient dynamics [12,15], and from the top-down by removing natural geological barriers, such as waterfalls, facilitating invasions of insectivorous fish [16]. Thus, we aimed to determine whether environmental factors associated with these changes negatively influence abundances of freshwater insects and species richness of a diverse family Chironomidae. To determine potentially different responses of functional and taxonomic groups to these anthropogenic impacts, we compared temporal changes in chironomid richness and abundances of seven major insect orders and eight insect families, comprising shredders, grazers, gatherers, scrapers, filter feeders and predators of varying body sizes. Taking into account that larger organisms from higher trophic levels (e.g., Odonata, Megaloptera) are among the most sensitive and vulnerable taxonomic groups [17,18], we predicted that their abundances will be more strongly impacted by human-induced changes compared to smaller organisms.

2. Material and Methods

(a) Sampling and data description

We analyzed a 20-year (2000-2019) dataset from a long-term ecological research program (PELD-Sitio PIAP), carried out in the Upper Paraná River Floodplain, Brazil (20º40'–22º50'S; 53º10'–53º24´W). The region is situated within a protected reserve with no agricultural areas in the surroundings. Physicochemical analyses of water did not detect heavy metals or other pollutants in the studied ecosystems (D.A. Moi, unpublished data). We took four annual samples, once during summer, spring, autumn, and winter (except for years

2001, 2003, 2016, 2017, 2018, and 2019, which were sampled twice annually, in summer and winter due to funding constraints), of insects, non-native fish and environmental variables. Samples were collected from 12 independent environments, comprising three rivers, six shallow lakes, two channels and one backwater (Figure S1). All sampling was performed simultaneously at the same sites, following a standard protocol.

Aquatic insect larvae were collected following a standard methodology [19]: three samples were obtained from each environment, including two samples at both sides and one in the center, using a Petersen sampler (0.0345 m^2). The collected insects were identified to order (Coleoptera, Megaloptera, Hemiptera, Trichoptera, Odonata and Ephemeroptera) or family level (Ephemeroptera: Baetidae, Caenidae, Leptophlebiidae; Diptera: Dolichopodidae, Chaoboridae, Ceratopogonidae, Culicidae and Chironomidae) by expert taxonomists. Chironomidae larvae were additionally identified to morphospecies level by an expert taxonomist. We calculated insect abundance (order, family) and Chironomidae species richness per m^2 captured in each environment during each sampling over 20 years. These insect orders and families comprised all key functional feeding groups, including predators, shredders, scrapers, grazers, gatherers and filter feeders, and spanned a wide range of body sizes, from small (e.g., Culicidae, Chaoboridae, Chironomidae) to large organisms (e.g., Megaloptera, Ephemeroptera, Trichoptera).

Time-matched with the insect collections, we took water samples from each aquatic environment to quantify nutrient concentrations (total phosphorus and total nitrogen; $\mu g\ L^{-1}$) and turbidity (NTU). Total nitrogen (N) was analyzed through the persulfate method [20] and determined in a spectrophotometer in the presence of cadmium, using a flow-injection system [21]. Total phosphorus (P) was measured according to Golterman et al. [22]. Turbidity was measured using a turbidimeter (LaMotte, Chestertown, MD, U.S.A). We also measured water level (m) using a fixed water level ruler. All these variables can indicate human-induced disturbance, such as damming (low turbidity and depth) and underlying changes in nutrient dynamics.

Recent studies have reported a decrease of native fish diversity associated with accelerated invasions of the non-native fish over time [23,24]. We sampled these non-native, insectivorous fish in each aquatic environment using two gear types: seines and gillnets. We used two standard gillnets, which were 10-m long, each with 11 mesh sizes (2.4, 3, 4, 5, 6, 7, 8, 10, 12, 14, and 16 cm from knot to knot). The gillnets were stitched and tied together, making a 20 m-long set that was deployed from the margin to the middle of each environment for 24 hours. Simultaneously, a 20 m-long seine net with a mesh size of 0.5 cm was used in the littoral zone of the lakes for 24 hours. We identified the non-native fish to species level using their historical records according to specialized literature [25-27] (electronic supplementary material, Table S1).

(b) Statistical analysis

To evaluate the temporal dynamics of each insect group in each environment, we applied generalized additive mixed effects models (GAMMs) with the Gaussian family, using restricted maximum likelihood (REML) as smoothness selection [28]. We used environment type and the sampling month nested within year as random factors, year as a continuous predictor, and insect abundance (all orders and families), chironomid richness, and environmental variables as response variables. Normality and homoscedasticity were verified using graphical inspections (QQ plots and residual plots). When necessary, we log-transformed the response variables prior to each analysis to achieve normality of the residuals and homogeneity of the variances. The analyses were conducted using the *gamm4* function of the package *gamm4* [28] and the graphs were built using the *stat_smooth(method = 'gam')* function in *ggplot2*.

To determine the main drivers of insect decline, we used a model selection approach. We compared the set of candidate models consisting of every environmental driver individually or in combination (turbidity, nitrogen, phosphorus, water level, as well as abundance and richness of non-native fish) as predictor variables (Table S2), and insect abundance and chironomid richness as response variables. A null model was also included into the model selection (Table S2). We checked the multicollinearity between the environmental drivers by calculating the variance inflation factor (VIF) for each predictor. VIF > 3 indicates possible collinearity but was not present in our data (all relationships had VIF < 2). The set of candidate models was constructed using a linear model and contrasted using corrected Akaike Information Criteria (AICc) and AICc weights (wi) [29]. We considered an evidence ratio ≥ 2 (ΔAICc ≤ 2) to identify the most plausible model, using the function *ICtab* of the *bbmle* package [30] (Table 2). All analyses were performed in R [31]. Data are accessible in [32].

3. Results

All insect orders evaluated, regardless of their body size and functional group, decreased consistently in abundance over the last 20 years (Figure 1, Table 1). Abundance of all Diptera families, including Dolichopodidae, Chaoboridae, Ceratopogonidae, Culicidae and Chironomidae, also decreased over the 20-year period (Table 1, Figure S1). Similar results were observed for Chironomidae species richness (Table 1, Figure S1), and for the three Ephemeroptera families analyzed, namely Baetidae, Caenidae and Leptophlebiidae (Table 1, Figure S2). In contrast, the abundance and richness of insectivorous non-native fish increased over the same time-period (Figure 2, Table 1). While turbidity decreased, nitrogen concentration and N:P ratios of the water increased over time (Figure 2, Table 1). However, water depth and phosphorus concentration did not change over the same 20-year period (Table 1, Figure 2, Figure S3).

The model selection revealed that a combination of increased richness of non-native fish and water N:P ratio, and decreased turbidity, were the key drivers of decline of almost all insect groups (Table 2). Two exceptions included Trichoptera and Ceratopogonidae, which were largely influenced by an increase in N:P ratios and invasions of insectivorous fish (Table 2).

4. Discussion

This study revealed a pervasive decline of aquatic insect abundance (across all studied orders) and chironomid richness over a 20-year period, in a suite of subtropical freshwater ecosystems in the Upper Paraná floodplain. There were similarly strong declines for all taxa, comprising different functional feeding groups, including predators, filter feeders, scrapers, gatherers, grazers, and shredders. These findings, from a major South American waterway, contrast with a recent meta-analysis [7], suggesting a global increase in aquatic insect abundances over time, based primarily on temperate studies. The main drivers of the declines detected here were a combination of decreased turbidity, and increased invasions of non-native insectivorous fish and changes in N:P stoichiometry over time. All these drivers exemplify human impacts caused by reservoir construction [12,23,24].

Decreased turbidity, which translates to increased water transparency, is closely related to sediment and nutrient deposition upstream, trapped by reservoir cascades built in the Upper Paraná basin [12]. In concert with increasing water transparency, the upper Paraná River floodplain underwent massive fish invasion caused by a hydroelectric power plant built downstream, which removed their natural geographical barrier (a set of waterfalls) separating the Lower from the Upper Paraná River [16,23,24]. The increase in non-native predators and water transparency likely strengthened the top-down control of insect prey which, bearing a dark integument, had reduced ability to camouflage. Freshwater transparency is a key factor mediating predator-prey encounter rates [33]. Therefore, increased encounter rates and predation pressure over 20 years must be considered as a potential underlying mechanism explaining the decline of aquatic insects in the Upper Paraná basin.

Changes in water stoichiometry was another important driver of insect decline. It is known that reservoir construction has strong impacts on river flow and nutrient dynamics [34]. In particular, reservoirs increase the N:P ratio of river discharge, largely due to increased in-reservoir N-fixation [15] and decreased P via upstream sedimentation [12]. Although we did not observe temporal shifts in P concentrations in pooled environments, such changes have been reported for several aquatic environments in the Upper Paraná floodplain [12]. These changes result in a stoichiometric imbalance towards increasing N saturation [35] with consequent changes in ecosystem productivity [15,34,36]. This may lead to a change in the phytoplankton composition [36], and likely changed the availability of nutrients to primary producers [37], making them suboptimal resources for primary consumers. Changes in the elemental composition of primary producers can create elemental imbalances between consumers and their resources with negative consequences for energy transfer among trophic levels, including insects. Indeed, increased N:P ratios such as those observed here (N:P >> 16) can cause P-limitations in phytoplankton and periphyton, thus reducing primary productivity in shallow subtropical lakes [35,38]. Thus, the inundation of adjacent lowlands by the Paraná river, connecting it with floodplain environments during the seasonal flood pulse [39], contributes to changing N:P ratios and productivity in the shallow floodplain environments. We show that these hydrodynamics have potential bottom-up cascading effects in the food web, leading to insect decline.

Aquatic insects underpin several key functions and services that freshwater ecosystems provide to tropical and subtropical regions. These include detritus processing and biogeochemical cycling, bioturbation, biological control, and food sources that fuel and stabilize aquatic and terrestrial food webs [40,41]. Therefore, long-term anthropogenic impact monitoring and mitigation strategies are pivotal in maintaining freshwater ecosystem integrity. Here we showed that reservoir constructions resulted in less productive environment for aquatic insects and in habitats with stronger predation by non-native fish. This highlights the importance of more careful planning of reservoir construction and the need for long-term studies to evaluate impacts on aquatic insect abundances and diversity, as well as the drivers of such decline, which are still poorly understood [3]. Our findings from the Upper Paraná floodplain, which is among the biggest floodplains in South America, suggest that aquatic insects from subtropical ecosystems are likely more threatened by human activities than those from temperate regions [7].

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Table 1. Generalized additive mixed effects models (GAMMs) examining the temporal trends on abundance of several insect orders and families, on Chironomidae species richness, and on environmental variables. Year is the fixed effect, whereas environment type and month nested within year are the random factors.

Source of variation	F	P	$R^2_{adjusted}$
Insect orders			
Coleoptera	16.54	<0.001	0.42
Megaloptera	9.43	<0.001	0.31
Hemiptera	10.21	<0.001	0.24
Trichoptera	12.9	<0.001	0.09
Odonata	3.21	0.032	0.09
Ephemeroptera	9.68	0.002	0.11
Diptera families			
Dolichopodidade	21.19	<0.001	0.05
Chaoboridae	9.73	0.002	0.11
Ceratopogonidae	9.45	<0.001	0.19
Culicidae	9.07	<0.001	0.24
Chironomidae (abund.)	17.28	<0.001	0.26
Chironomidae (richness)	10.25	<0.001	0.39
Ephemeroptera families			
Baetidae	72.9	<0.001	0.37
Caenidae	47.02	<0.001	0.38
Leptophlebiidae	64.97	<0.001	0.4
Environmental variables			
Water depth	1.7	0.126	0.02
Turbidity	15.5	<0.001	0.04
Nitrogen concentration	15.61	<0.001	0.3
Phosphorus concentration	1.61	0.3	0.01
N:P ratios	8.69	<0.001	0.18
Invasive fish abundance	29.04	<0.001	0.13
Invasive fish richness	59.84	<0.001	0.11

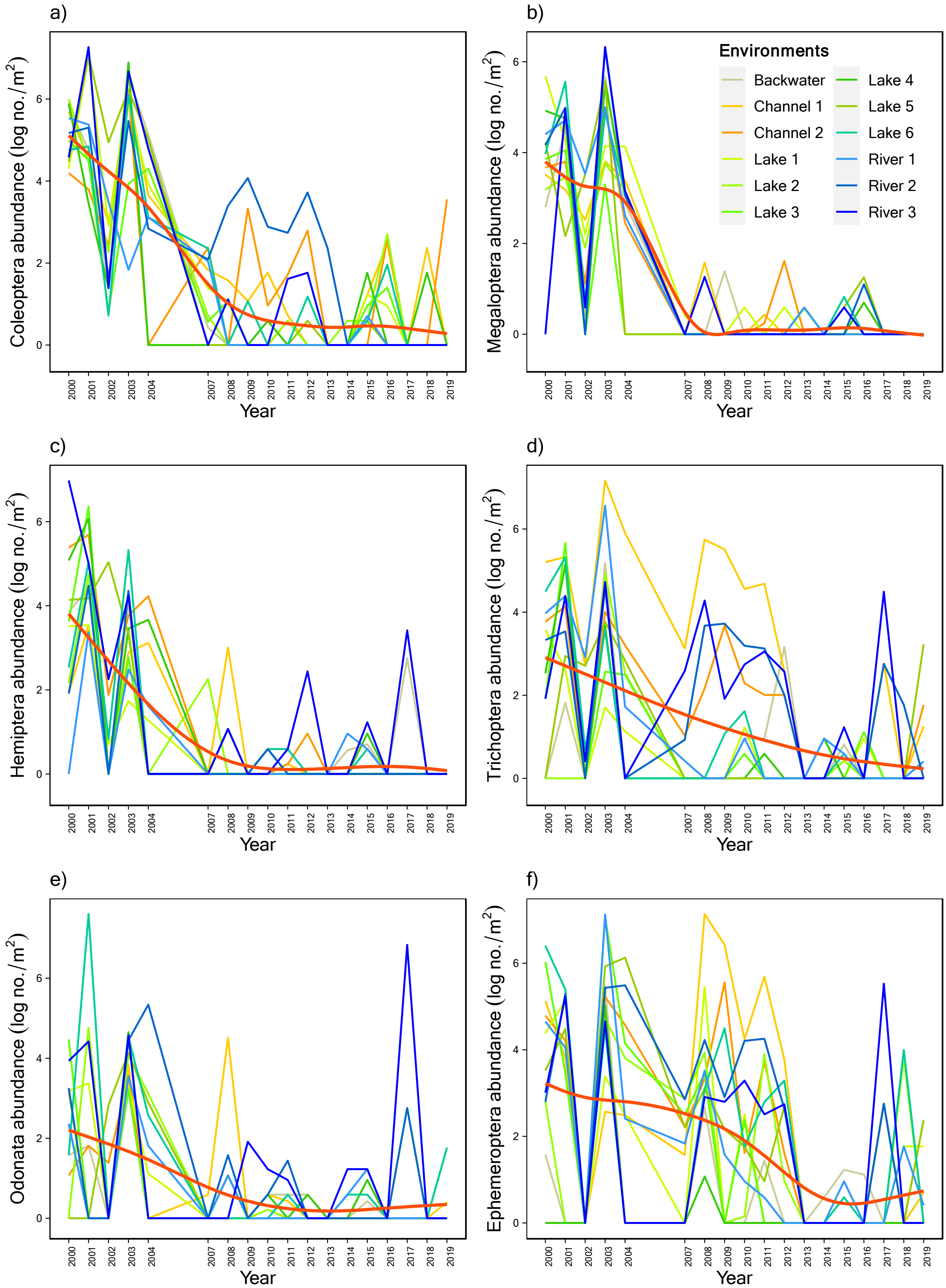
Table 2. Contrasting the impacts of ecological drivers on insect decline. Detailed outcomes of the model selection performed using corrected Akaike Information Criteria (AICc) to assess the different contributions of water depth, turbidity, nitrogen, phosphorus, N:P ratio, invasive fish abundance, invasive fish richness and combinations of these predictors (the full models are presented in the Table S2) on decline of insect orders and Diptera families. Model selection was performed using function '*ICtab*' in '*bbmle*' package. ΔAICc = difference between the model with the lowest score and subsequent models. Only the best subset models (ΔAICc ≤2) are presented.

Response	Models	AICc	ΔAICc	df	Weight
Insect orders					
Coleoptera	(i) Turbidity + Invasive fish richness + N:P ratio	720.7	0	6	0.84
Megaloptera	(i) Turbidity + Invasive fish richness + N:P ratio	650	0	6	0.65
	(ii) Invasive fish richness + N:P ratio	651.2	1.3	5	0.35
Hemiptera	(i) Invasive fish richness + N:P ratio	637.1	0	5	0.72
	(ii) Turbidity + Invasive fish richness + N:P ratio	639.1	1.9	6	0.28
Trichoptera	(i) Invasive fish richness + N:P ratio	649.9	0	5	0.74
Odonata	(i) Invasive fish richness + N:P ratio	629.9	0	5	0.58
	(ii) Turbidity + Invasive fish richness + N:P ratio	630.6	0.6	6	0.42
Ephemeroptera	(i) Turbidity + Invasive fish richness + N:P ratio	751.3	0	6	0.55
	(ii) Invasive fish richness + N:P ratio	752.1	0.7	5	0.38
Diptera families					
Dolichopodidade	(i) Invasive fish richness	533.4	0	3	0.23
	(ii) Invasive fish richness + N:P ratio	533.7	0.2	5	0.203
	(iii) Invasive fish abundance	533.7	0.3	3	0.197
	(iv) Turbidity + Invasive fish richness	534.3	0.8	4	0.151
	(v) Turbidity + Invasive fish richness + N:P ratio	534.3	0.8	6	0.15
Chaoboridae	(i) Invasive fish richness	676.1	0	3	0.301
	(ii) Invasive fish richness + N:P ratio	676.2	0.1	5	0.287
	(iii) Turbidity + Invasive fish richness + N:P ratio	677	0.9	6	0.189
	(iv) Turbidity + Invasive fish richness	677.5	1.4	4	0.15
Ceratopogonidae	(i) Invasive fish richness + N:P ratio	667	0	5	0.64
Culicidae	(i) Turbidity + Invasive fish richness + N:P ratio	619.8	0	6	0.5
	(ii) Invasive fish richness + N:P ratio	619.8	0	5	0.5
Chironomidae (abund.)	(i) Invasive fish richness + N:P ratio	798.4	0	5	0.465
	(ii) Invasive fish richness	799.8	1.4	3	0.236
	(iii) Turbidity + Invasive fish richness + N:P ratio	800.3	1.8	6	0.185
Chironomidae (richness)	(i) Invasive fish richness + N:P ratio	461	0	5	0.503
	(ii) Turbidity + Invasive fish richness + N:P ratio	461.2	0.2	6	0.449
Ephemeroptera families					
Baetidae	(i) Turbidity + Invasive fish richness + N:P ratio	600.5	0	6	0.961
Caenidae	(i) Turbidity + Invasive fish richness + N:P ratio	579.7	0	6	0.97
Leptophlebiidae	(i) Turbidity + Invasive fish richness + N:P ratio	577.7	0	6	0.996

Figure captions

Figure 1. Average abundance of Coleoptera (a), Megaloptera (b), Hemiptera (c), Trichoptera (d), Odonata (e), and Ephemeroptera (f) over 20 years in 12 different environments (backwater, channels, lakes and rivers) in the Paraná floodplain. Solid orange lines and orange shadings are the model fitting (using 'gam' function) and 95% confidence intervals, respectively.

Figure 2. Average water depth (a), turbidity (b), nitrogen concentration (c), nitrogen to phosphorus (N:P) ratio (d), invasive fish abundance (e), and invasive fish richness (f) over 20 years in 12 different environments (backwater, channels, lakes and rivers) in the Paraná floodplain. Legend indicating the environments is presented in the Figure 1b. Solid orange lines and orange shading are the model fitting (using 'gam' function) and 95% confidence intervals, respectively.



a)
Coleoptera abundance (log no./m²)
Year
b)
Megaloptera abundance (log no./m²)
Year
Environments
Backwater
Channel 1
Channel 2
Lake 1
Lake 2
Lake 3
Lake 4
Lake 5
Lake 6
River 1
River 2
River 3
c)
Hemiptera abundance (log no./m²)
Year
d)
Trichoptera abundance (log no./m²)
Year
e)
Odonata abundance (log no./m²)
Year
f)
Ephemeroptera abundance (log no./m²)
Year

Figure 1

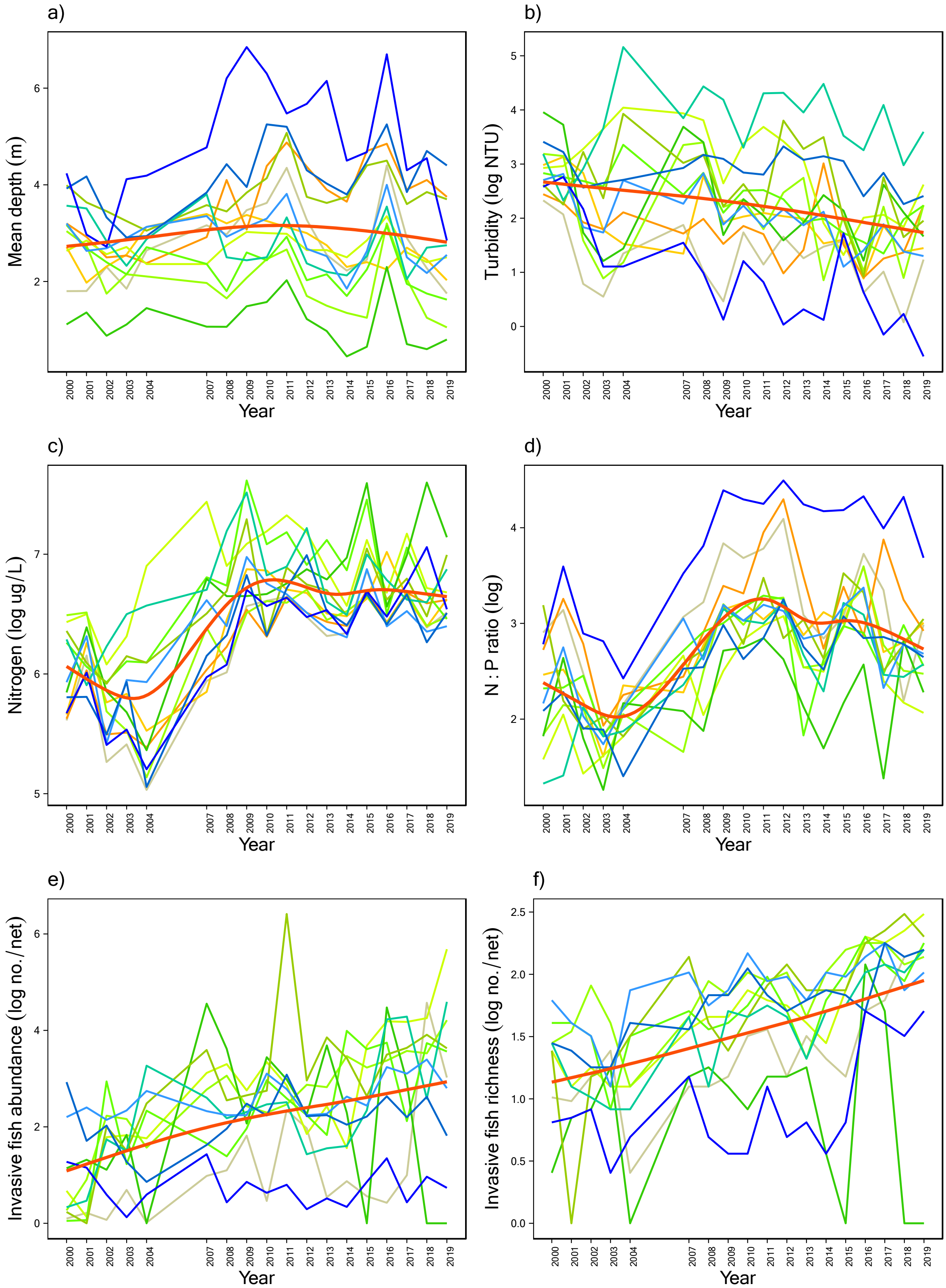

a)
Mean depth (m)
Year
b)
Turbidity (log NTU)
Year
c)
Nitrogen (log ug/L)
Year
d)
N : P ratio (log)
Year
e)
Invasive fish abundance (log no./net)
Year
f)
Invasive fish richness (log no./net)
Year

Figure 2

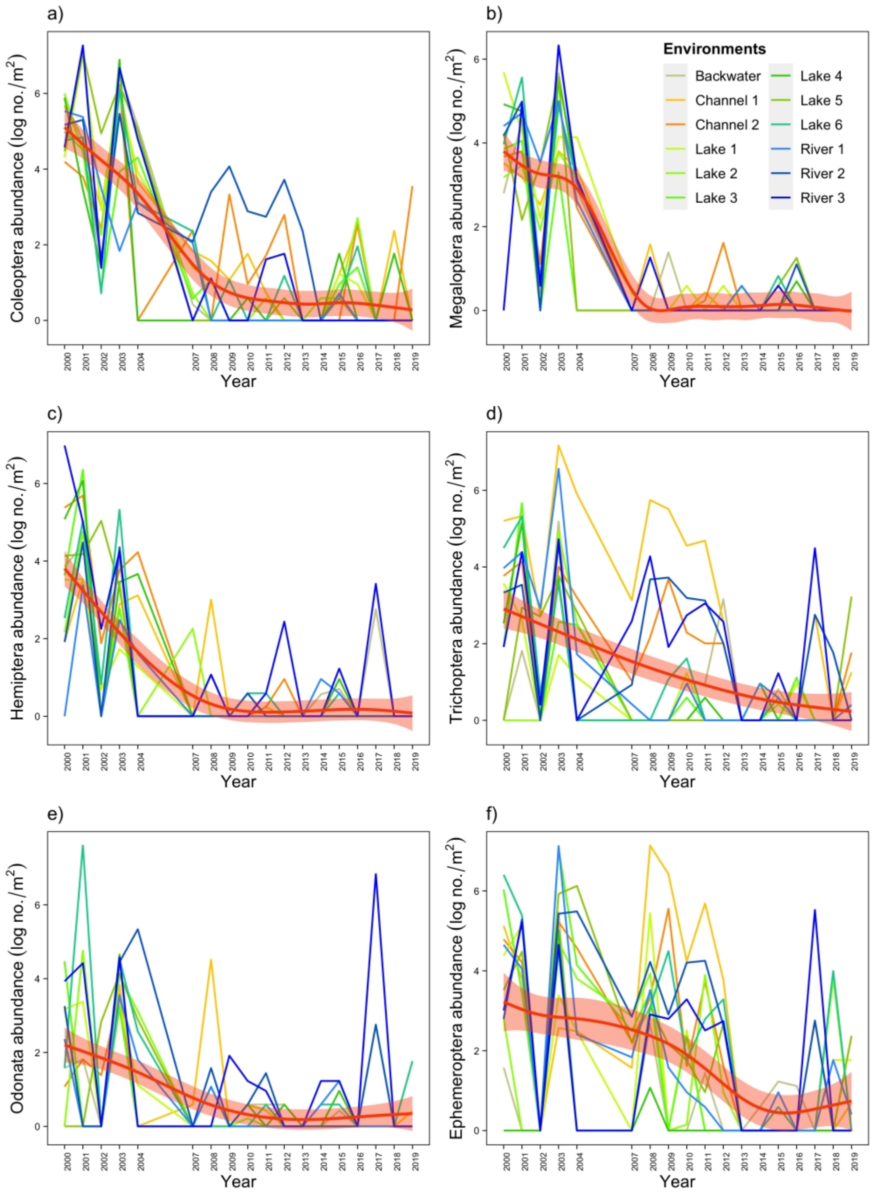


Figure1

916x1249mm (72 x 72 DPI)

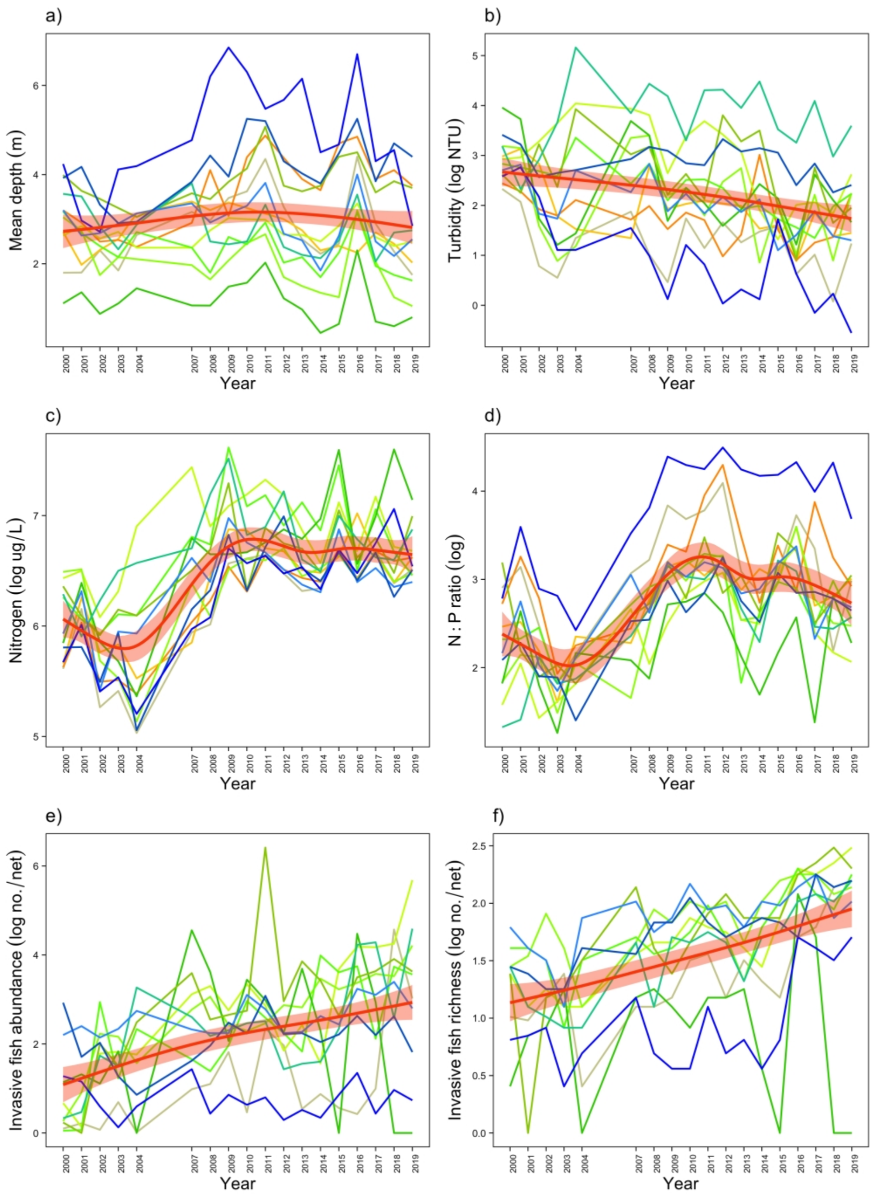


Figure2

916x1249mm (72 x 72 DPI)