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New insights on bioindicator value of Chironomids by using occupancy modelling

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ABSTRACT

The use of bioindicators requires environmental information and a detailed knowledge of the biota to which it is assign a sensitivity value. The objective of this work was assessing the sensitivity of chironomids to several water quality variables. We used multivariate analysis and occupancy modelling to understand the relationship between abundance and occupancy of chironomid taxa and water quality. The study was performance in 43 sites of eight Pampean streams of Argentina during the fall in 2012 and 2015. We determined the association between 21 taxa of the chironomid assemblage and five variables of water quality (i.e., conductivity, dissolved oxygen, 5-days biochemical oxygen demand, concentrations of phosphate and dissolved inorganic nitrogen). We identified taxa associated to low water quality (e.g., *Chironomus calligraphus*) and high-water quality (e.g., *Rheotanytarsus*). In some chironomid taxa their potential as bioindicator increased as the taxonomic level decreased (e.g. Chironominae). However, in other taxa this potential as bioindicator of water quality remains at subfamily level (e.g. Orthocladiinae). The occupancy of *Parachironomus* sp. and *Chironomus calligraphus* increased in the sites with the lowest concentration of dissolved oxygen, while *Rheotanytarsus* sp. and *Onconeura analiae* behaved in an inverse way. *C. calligraphus* and *O. analiae* occupied sites with high values of both, conductivity and 5-days biochemical oxygen demand. *C. calligraphus* was the single species showing a positive relationship between its occupancy and concentration of phosphate. The occupancy of *Allocladius neobilobulatus* and *Cricotopus* sp. were negatively related to the increase of the concentration of nutrients. The description of species sensitivity in terms of occupancy, offers a new methodology to understand how species shows a pattern along abiotic gradient. Further studies about sensitive of local chironomid species to different types of stressors will allow researchers to generate more accurate biotic indices based on these taxa. The water quality of lowland streams would be inferred from the occupancy of chironomid taxa.

1. Introduction

The family Chironomidae, or non-biting midges, is usually the most abundant and species-diverse insect group found in freshwater ecosystems (Ferrington, 2008). More than 6000 chironomids species from all types of biotopes were described, occupying a wide range of niches and exhibiting different feeding behaviors and modes of life (Coffman and Ferrington, 1996; Qi et al., 2018). They are a key component of aquatic food webs and biogeochemical cycles by consuming and recycling organic matter and, therefore, a vital nexus between primary producers and secondary consumers (Pinder, 1986). The family includes sensitive species as well as several species groups with tolerances to environmental gradients, ranging from undisturbed to human-impacted ecosystems (Heino and Paasivirta, 2008; Tang et al., 2010; Roque et al., 2010). Therefore, they have a noteworthy importance to freshwater monitoring, protection, and conservation (Barbosa et al., 2001; Nedeau et al., 2003).

The development of biological indicators for monitoring programs requires environmental information and a detailed knowledge of the biota to which it is assign a sensitivity value. In several European and North American countries where the chironomid fauna is widely known, these insects play a key role as bioindicators (e.g. Lencioni et al., 2012; Lunde & Resh, 2012; Verdonschot et al., 2012). Contrary, the information about chironomids and their environment are still

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scarce in the subtropical region where there is a great taxonomic variability and the identification of larvae require specialists even at generic level (Ferrington, 2008). So far, the available sensitivity information for some taxa are based on either expert knowledge and/or empirical derivation about presence of species in relation to environmental variables through multivariate approaches (Moya et al., 2007; Scheibler et al., 2008; Restello et al., 2014; Cortese et al., 2019). Furthermore, there are few studies that attempt to model species–environment interactions (Luoto, 2011).

Occupancy modelling has become increasingly useful to ecologists because provides a flexible framework to investigate ecological questions and processes such as species distribution modelling and habitat relationships (Bailey et al., 2014; Berkunsky et al., 2015; Cortelezzi et al., 2017). Some salient features of this approach, respect to multivariate techniques or other commonly used methods is: first, the possibility to record histories of absence/ presence (0 or 1 respectively), which implies an advantage in terms of sampling effort. Second, the occupancy modelling allows to estimate the occupancy probability of the site taking into account explicitly for detectability. Third, the power predictive of occupancy modelling allows to extrapolate the results to new situations (i.e.; changes in environmental variables). Fourth, from these models we can infer the sensitivity of the taxa. If the occupancy of a species decrease as the habitat quality decrease (Boyce et al., 2016; Cortelezzi et al., 2017; Cortelezzi et al., 2018), and if we are able to identify the stressors affecting species occupancy, then we will be able to described the sensitivity as a change in the occupancy along the range of water quality variables (Boyce et al., 2016).

The objective of this work was assessing the sensitivity of chironomids to several water quality variables in lowland streams. In this study we: 1) described the association between the chironomid taxa and physical and chemical variables of water quality; and 2) we modelled the occupancy of chironomids species from these variables. Occupancy could be a useful tool to describe the sensitivity of the chironomids along the gradients of each physical and chemical variable.

2. Methods

2.1. Study area

The study area is located in the Southeastern center of Buenos Aires province, in the area occupied by the Tandilia mountain system. Along the northern slope of Tandilia, there are the headwaters of many streams that drain in the NE direction. These streams cross run through the 'Pampa' – a vast grassy plain that covers central Argentina. Despite this area is considered endangered and of maximum priority of conservation since its strong transformation, biological uniqueness, and the absence of protected areas (Bilenca and Miñarro, 2004), the information on the ecological status of these aquatic systems is scarce. The Pampean streams are characterized by the absence of riparian forest vegetation, the lack of a dry season or extreme temperatures and development of dense and rich macrophyte communities (Feijoó et al., 2005).

2.2. Sampling

We conducted surveys in 43 sites of 8 pampean streams. In order to promote independence and avoid seasonal variability, the minimum distance among sites was 4 km and all surveys were conducted during the fall of 2012 and 2015. At each site, we collected three samples of sediment (i.e. 129 sediment samples) with an Ekman grab (100 cm^2). At laboratory, we washed each sample separately over a 500 μm mesh sieve, we separated the chironomids under a stereomicroscope (Olympus SZ40), and we identified them through standard keys according to Epler (2001), Andersen et al. (2013), and Silva et al. (2018).

At each site we recorded: dissolved oxygen [DO] (YSI 52 dissolved oxygen meter), temperature and pH (Hanna HI 8633), and conductivity (Lutron CD-4303). We also collected one sample of water to analyze 5-day biochemical oxygen demand [BOD_5], chemical oxygen demand [COD], and concentrations of phosphate [PPO_4^{-3}], ammonium [NNH^{+4}], nitrate [NNO_3^{-1}], and nitrite [NNO_2^{-1}], (Mackereth et al., 1978; APHA, 1998).

2.3. Data analysis

To explore the relationships between the presence of chironomids and the physical and chemical variables, we used "multivariate analysis" and "occupancy modelling" approaches. We exclude from the analysis those taxa that were detected in less than 10% of the sites (i.e., rare taxa). To reduce the variable dimensions, we explored the structure of covariation of physical and chemical variables, resulting in five independent variables, which are globally used to define the water quality: dissolved oxygen (%DO, range: 14–160; Q1 = 79.9, Q3 = 122.3), conductivity (range: 185–1207 μS/cm; Q1 = 621, Q3 = 807), dissolved inorganic nitrogen (DIN, range: 0.3–13.1 mgN/l; Q1 = 0.77, Q3 = 6.52), 5-day biochemical oxygen demand (BOD_5, range: 0.00–47.00 mgO/l; Q1 = 1, Q3 = 10), and phosphate (PPO_4, range: 0.02–1.29 mgP/l; Q1 = 0.05, Q3 = 0.31). Dissolved inorganic nitrogen (DIN) was calculated by the sum of ammonium, nitrate and nitrite. Temperature, pH, and DQO were not included in the analysis because exhibited a high correlation with the selected water quality variables (Pearson $R > 0.7$, $p < 0.001$). We considered that a value of our physical or chemical variable was high if exceed Q3 (i.e.; quartile 3); and low if it was below Q1.

We used a Detrended Correspondence Analysis (DCA) to evaluate how taxa are distributed along the environmental gradient. We conducted a Canonical Correspondence Analysis (CCA) to quantify the contribution of each physical and chemical variables to the environmental gradient (ter Braak, 1995). The significance of each variable was tested with a Monte Carlo permutation test (999 unrestricted permutations) using the significance level of $p \leq 0.05$. Because the multivariate analysis (i.e. DCA and CCA) require the presence of at least one taxon on each site, we performed the analysis with a subset of 29 sites. Therefore, we exclude all sites where none taxon was found, and/or rare taxa were only found.

The multivariate approach gives us a picture about the relationships of the whole assemblage of chironomids, while the occupancy modelling allows us to individually explore each species in relation to each water quality variable. We used occupancy models to estimate the influence of physical and chemical variables affecting the occupancy of each taxon. Occupancy is usually defined as the probability that the focal taxon occupies, or uses, a sample unit during a specified period of time during which the occupancy state is assumed to be static (Bailey et al., 2014). These models are based in two stochastic processes that occur when a taxon is detected at a site. A site may be either occupied or unoccupied by the taxon; if it is occupied then at each visit there is some probability of detecting the taxon. We built detection history of three simultaneous visits for each site. We evaluated the baseline model for each taxon, in which both detection and occupancy probabilities were assumed to be constant across all sites [denoted as $\psi(.)\ p\ (.)$]. Then, we developed a model set that incorporated site variables through a logit link function. Under the assumption that the occupancy of taxa decreases as the water quality decrease, we expected a negative relationship between occupancy and conductivity, dissolved inorganic nitrogen and phosphate; and a positive relationship between occupancy and dissolved oxygen. We evaluated all potential models with 2–4 parameters (including the intercept and probability of detection) to avoid the occurrence of spurious results, and by maintaining an approximate ratio of data to parameters > 10 ($n = 43$ sites; maximum number of parameters $= n/10$; Burnham and Anderson, 2002). For each model, we calculated the estimates of parameters (β) and their standard errors for the intercept (βo) and each variable. Also, we excluded those models which water quality variables had confidence

Table 1
Record of the frequency of chironomid taxa in 43 sites of eight Pampean streams of northern hillside of Tandilia Mountain System, Argentina.

Subfamily	Tribe	Genus/Species	Frequency (n = 43 sites)
Chironominae	Chironomini	*Chironomus calligraphus* Goeldi	33
		Cryptochironomus Kieffer	9
		Parachironomus Lenz	21
		Polypedilum Kieffer	19
	Tanytarsini	*Tanytarsus* van der Wulp	9
		Paratanytarsus Thienemann & Bause	9
		Rheotanytarsus Thienemann & Bause	21
Orthocladiinae	Corynoneurini	*Corynoneura* Winnertz	9
		Onconeura analiae Donato et al.	23
		Thienemanniella Kieffer	12
	Metriocnemini	*Allocladius neobilobulatus* Paggi	9
		Limnophyes Eaton	7
		Parametriocnemus Goetghebuer	2
	Orthocladiini	*Cricotopus* van der Wulp	21
		Nanocladius Kieffer	5
	Undetermined	Orthocladiinae 1	2
		Orthocladiinae 2	5
Tanypodinae	Pentaneurini	*Ablabesmyia* Johannsen	5
		Labrundinia Fittkau	12
		Larsia Fittkau	2
	Tanypodini	*Tanypus* Meigen	7

interval containing the zero due to lack of effect. Finally, we ranked models using Akaike's Information Criterion (AIC). We kept all models that were better than constant occupancy model [i.e., ψ(.) p(.)] and that were less than two AIC units [$\Delta AIC < 2$] of the best model.

Following Cortelezzi et al. (2017), we analyzed the sensitivity of taxa to each physical and chemical variable included in the top occupancy models. The sensitivity of a taxon is described by phases of occupancy decline, where *resistance* is reflecting the capacity of taxon to hold occupancy as habitat quality decreases, and *tolerance* is the range of the water quality variable for which the occupancy shows the highest decline.

All the analyses were performed using R software. We used Vegan package (Oksanen et al., 2017) to perform canonical analysis and Unmarked package (Fiske and Chandler, 2011) to perform occupancy model. We used VeusZ ® to create graphs.

3. Results

We recorded 21 taxa of chironomids in 129 sediment samples (Table 1). In 72% of sites, we found at least one taxon of Chironomidae (i.e., 31 of 43 sites). The most frequent taxa were *Chironomus calligraphus* (32% of sites), *Rheotanytarsus* sp. (27% of sites), and *Onconeura analiae* (25% of sites). In only one site, we observed the presence of these three taxa together. Eight taxa were rare and excluded from the analysis (i.e. *Tanypus* sp., *Larsia* sp., *Ablabesmyia* sp., Orthocladiinae 1, Orthocladiinae 2, *Limnophyes* sp., *Parametriocnemus* sp., *Nanocladius* sp.).

The length of the first axis of the DCA (i.e., beta diversity or environmental gradient) was 3.88; while the length of the second axis was 0.66. The first two CCA axes accounted for the 79.34% of the constrained variance (k1 = 0.66, k2 = 0.25; Fig. 1). Along the first axis of the CCA, physical and chemical variables explained 74% of the species ordination. Axis 1 was negatively associated with phosphate and conductivity, and positively associated with dissolved oxygen. The axis 2 was positively associated with dissolved inorganic nitrogen and BOD_5. Four water quality variables significantly influenced the chironomid assemblage: phosphate ($F = 3.55$, $p = 0.007$), dissolved inorganic nitrogen ($F = 4.84$, $p = 0.001$), conductivity ($F = 3.05$, $p = 0.004$), and dissolved oxygen ($F = 2.33$, $p = 0.026$).

C. calligraphus was isolated from the rest of the taxa, and positively associated to the highest values of phosphate and conductivity. *Rheotanytarsus* sp. was associated to high concentrations of dissolved oxygen. The remaining species of the subfamily Chironominae and the species of the subfamily Orthocladiinae occupied a mean position in the CCA. *Labrundinia* sp. (the only representative of the subfamily Tanypodinae included in the analysis) was associated to sites with high concentrations of dissolved inorganic nitrogen and dissolved oxygen (Fig. 1).

We modeled the occupancy of 13 taxa. For *Cryptochironomus* sp. none of the models converged, neither the constant. For six taxa (*Corynoneura* sp., *Labrundinia* sp., *Paratanytarsus* sp., *Polypedilum* sp., *Tanytarsus* sp. and *Thienemanniella* sp.) the addition of physical and chemical variables did not improve the constant model. In the remaining six species we obtained a total of 17 models including physical and chemical variables and ranking better than the constant model [ψ(.) p (.)] (Table 2). Twelve of these models included two water quality variables and five only one. Phosphate was the only variable that explained the occupancy of the six species, usually accompanied by another variable. Dissolved oxygen was included as variable in most of the models (13/15) and it explained the occupancy in four of the six species. In those cases where conductivity and dissolved inorganic nitrogen were included as variables in occupancy models, they always were inversely related to occupancy (Table 2).

We describe the occupancy of the six species across the range of each physical and chemical variable (Fig. 2). The occupancy of *Parachironomus* sp. and *C. calligraphus* increased in the sites with the lowest concentration of dissolved oxygen, while *Rheotanytarsus* sp. and *O. analiae* behaved in an inverse way (Fig. 2A). *C. calligraphus* and *O. analiae* occupied sites with high values of both, conductivity and BOD_5 (Fig. 2B, C). *C. calligraphus* was the single species showing a positive relationship between its occupancy and concentration of phosphate. The occupancy of *Allocladius neobilobulatus* and *Cricotopus* sp. were negatively related to the increase of phosphate (Fig. 2D).

4. Discussion

The Chironomid richness reported in this study was the expected for the region (Rodrigues Capítulo et al., 2001; Paggi, 2007). As occurs in most of Pampean streams, the chironomid assemblage was dominated by subfamily Chironominae followed by the subfamily Orthocladiinae (Marchese and Paggi, 2004; Cortelezzi et al., 2011).

4.1. Chironominae subfamily

From the traditional multivariate analysis, and as expected, subfamily Chironominae, as a single taxon, is not a good indicator of water quality. Chironominae occupies a large habitat range, breeding in both oligotrophic and eutrophic environments, rivers and streams of different sizes, and with different levels of pollution (Martinez et al., 2002). The seven taxa of Chironominae were distributed along the CCA axes preventing generalizations about the subfamily.

From CCA results, we observed that *C. calligraphus* was associated to sites with high concentration of phosphate, high conductivity, low percentage of dissolved oxygen, suggesting that this species is a valid indicator of disturbed Pampean streams. The larvae of this species commonly breed in aquatic ecosystems, subjected to high organic nutrient enrichment (Freimuth and Bass, 1994). The physiological and morphological adaptation of the larvae allow *C. calligraphus* to dominate sites with extremely low dissolved oxygen levels, tolerating anoxic waters (Panis et al., 1996, Beneberu et al., 2014). The top occupancy models showed a positive relationship with the concentration of phosphate and BOD_5, and a negative relationship with dissolved oxygen

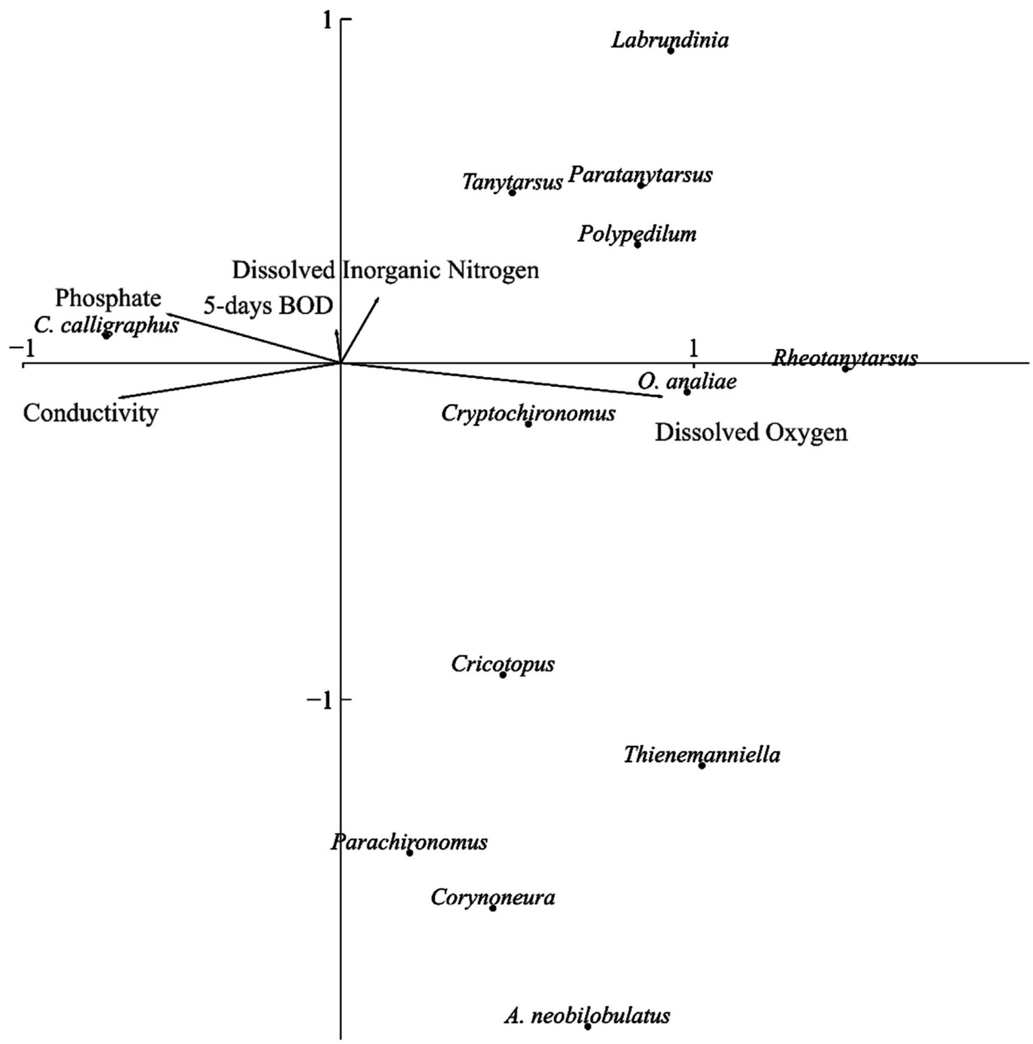

Fig. 1. Canonical correspondence analysis (CCA) biplot of water quality variables and chironomid taxa of Pampean streams, Argentina.

and conductivity. However, the range of resistance phase of *C. calligraphus* to conductivity was wide suggesting it would be a poor indicator of this variable.

Rheotanytarsus is a filtering-collector larva usually reported as tolerant to a wide range of environmental disturbances, including the nutrient enrichment, a common situation in aquatic environments with an agricultural influence (Roque et al., 2000; Gonçalves Junior et al., 2003; Biasi et al., 2013; König and Santos, 2013). The way of getting food of the larvae is favored from a high-velocity current, which is usually associated to high concentration of dissolved oxygen (Sanseverino and Nessimian, 2001; Kikuchi and Uieda, 2005). The abundance of *Rheotanytarsus* was positively associated to high values of dissolved oxygen, low concentration of phosphate and low conductivity, then it could be a good indicator of undisturbed streams (Fig. 1). However, even when the same three water quality variables explained the occupancy in the top models for *Rheotanytarsus*, in all cases the tolerance phase was wide (i.e., decreasing its occupancy along a wide range of the variable). Then, its merely presence would be a poor indicator of the condition of streams.

Larvae of *Parachironomus* occurs in standing and flowing waters of a wide range of environmental conditions, frequently found in fine sediment (Epler, 2001). *Parachironomus* was reported to sites with relatively high conductivity and negatively associated to BOD_5 (Cortese et al., 2019). In our case, the abundance of *Parachironomus* was associated to low concentrations of BOD_5 and dissolved inorganic nitrogen. The occupancy modelling approach included the five water quality variables in the top models for *Parachironomus*. In all cases, the tolerance phase was wide.

Other Chironominae were reported as associated to some environmental conditions. For example, *Tanytarsus* was previously associated to high concentration levels of nitrogen and phosphate in wetlands (Campbell et al., 2009); *Paratanytarsus* is often found in the sediments of oxygen rich lakes (Augenfeld, 1967); and the genus *Polypedilum* includes species tolerant to a broad range of environmental conditions (Silva et al., 2013). In our studied sites of Pampean streams, the abundances of *Tanytarsus, Paratanytarsus*, *Polypedilum* and *Cryptochironomus* were associated to relatively moderate concentrations of dissolved inorganic nitrogen and dissolved oxygen and low concentration of phosphate and low conductivity. However, none of the water quality variables helped us to explain the occupancy of these Chironominae taxa.

4.2. Orthocladiinae subfamily

The ecological requirements of several Orthocladiinae include rivers and streams with high slopes, high levels of oxygen, and the predominance of a hard substrate (Pinder, 1986). Other Orthocladiinae show hygropetric behavior in small standing waters, while several genera semiterrestrial or fully terrestrial (Silva et al., 2018). The Orthocladiinae species inhabit environments of varying water quality statuses (Simião-Ferreira et al., 2009) and this could be attributed to their functional diversity, enabling them to survive in diverse

Table 2

Top performing site occupancy models (ΔAIC < 3) and coefficients of water quality variables (β) for six chironomids taxa (listed in alphabetical order) in Pampean streams, Argentina. DIN = dissolved inorganic nitrogen; PPO_4 = phosphate; DO = dissolved oxygen; BOD_5 = 5-days biochemical oxygen demand.

Taxon	Model	n	*ΔAIC*	*ρ*	Intercept	Conductivity	BOD_5	DIN	DO	PPO_4
Allocladius neobilobulatus	Ψ(*DIN* + *PPO₄*)ρ(.)	4	0.00	0.05 ± 0.72	−6.91 ± 3.49			−2.58 ± 1.78		−7.91 ± 6.15
	Ψ(*DIN*)ρ(.)	3	0.85	0.06 ± 0.72	−3.26 ± 1.47			−2.18 ± 1.78		
	Ψ(.)ρ(.)	3	3.12	0.07 ± 0.71	−2.15 ± 0.55					
Chironomus calligraphus	Ψ(*Conductivity* + *PPO₄*)ρ(.)	4	0.00	0.72 ± 0.36	−0.74 ± 0.63	−2.40 ± 0.94				3.98 ± 1.62
	Ψ(*DO* + *BOD5*)ρ(.)	4	2.48	0.68 ± 0.37	−0.55 ± 0.58		1.89 ± 1.22		−2.12 ± 0.93	
	Ψ(.)ρ(.)	2	21.66	0.68 ± 0.37	−0.67 ± 0.33					
Cricotopus	Ψ(*DIN* + *PPO₄*)ρ(.)	4	0.00	−0.32 ± 0.43	−10.54 ± 4.51			−3.97 ± 1.81		−17.87 ± 8.50
	Ψ(.)ρ(.)	2	15.21	−0.35 ± 0.51	−1.04 ± 0.46					
Onconeura analiae	Ψ(*Conductivity*)ρ(.)	3	0.00	−0.33 ± 0.39	−1.22 ± 0.60	−4.53 ± 2.50				
	Ψ(*Conductivity* + *BOD5*)ρ(.)	4	0.84	−0.32 ± 0.41	−0.67 ± 0.76	−3.82 ± 2.01	1.51 ± 1.23			
	Ψ(*Conductivity* + *DO*)ρ(.)	4	1.55	0.01 ± 0.45	−1.38 ± 0.54	−1.61 ± 1.00			0.69 ± 0.60	
	Ψ(*PPO₄* + *BOD5*)ρ(.)	4	2.64	−0.15 ± 0.42	−1.36 ± 0.67		3.70 ± 1.95			−4.92 ± 2.30
	Ψ(.)ρ(.)	2	8.64	0.03 ± 0.43	−0.89 ± 0.39					
Parachironomus	Ψ(*PPO₄* + *DO*)ρ(.)	4	0.00	0.12 ± 0.47	−1.65 ± 0.56				−1.93 ± 0.82	−2.07 ± 1.01
	Ψ(*DIN*)ρ(.)	3	0.09	0.12 ± 0.47	−1.66 ± 0.60			−1.44 ± 0.74		
	Ψ(*DIN* + *DO*)ρ(.)	4	0.41	0.13 ± 0.47	−1.77 ± 0.65			−1.47 ± 0.75	−0.66 ± 0.52	
	Ψ(*DO* + *BOD5*)ρ(.)	4	1.64	0.16 ± 0.45	−1.58 ± 0.53		−1.50 ± 0.81		−1.26 ± 0.62	
	Ψ(*Conductivity* + *DO*)ρ(.)	4	2.96	0.07 ± 0.50	−1.38 ± 0.48	−0.98 ± 0.58			−1.00 ± 0.56	
	Ψ(.)ρ(.)	2	4.60	0.13 ± 0.47	−1.19 ± 0.40					
Rheotanytarsus	Ψ(*DO*)ρ(.)	3	0.00	−0.09 ± 0.41	−1.02 ± 0.48				1.32 ± 0.59	
	Ψ(*Conductivity* + *DO*)ρ(.)	4	0.63	−0.08 ± 0.41	−1.16 ± 0.52	−0.60 ± 0.56			1.28 ± 0.63	
	Ψ(*PPO₄*)ρ(.)	3	2.66	−0.10 ± 0.42	−1.24 ± 0.72					−1.91 ± 1.54
	Ψ(.)ρ(.)	2	5.61	−10.0 ± 0.42	−0.73 ± 0.39					

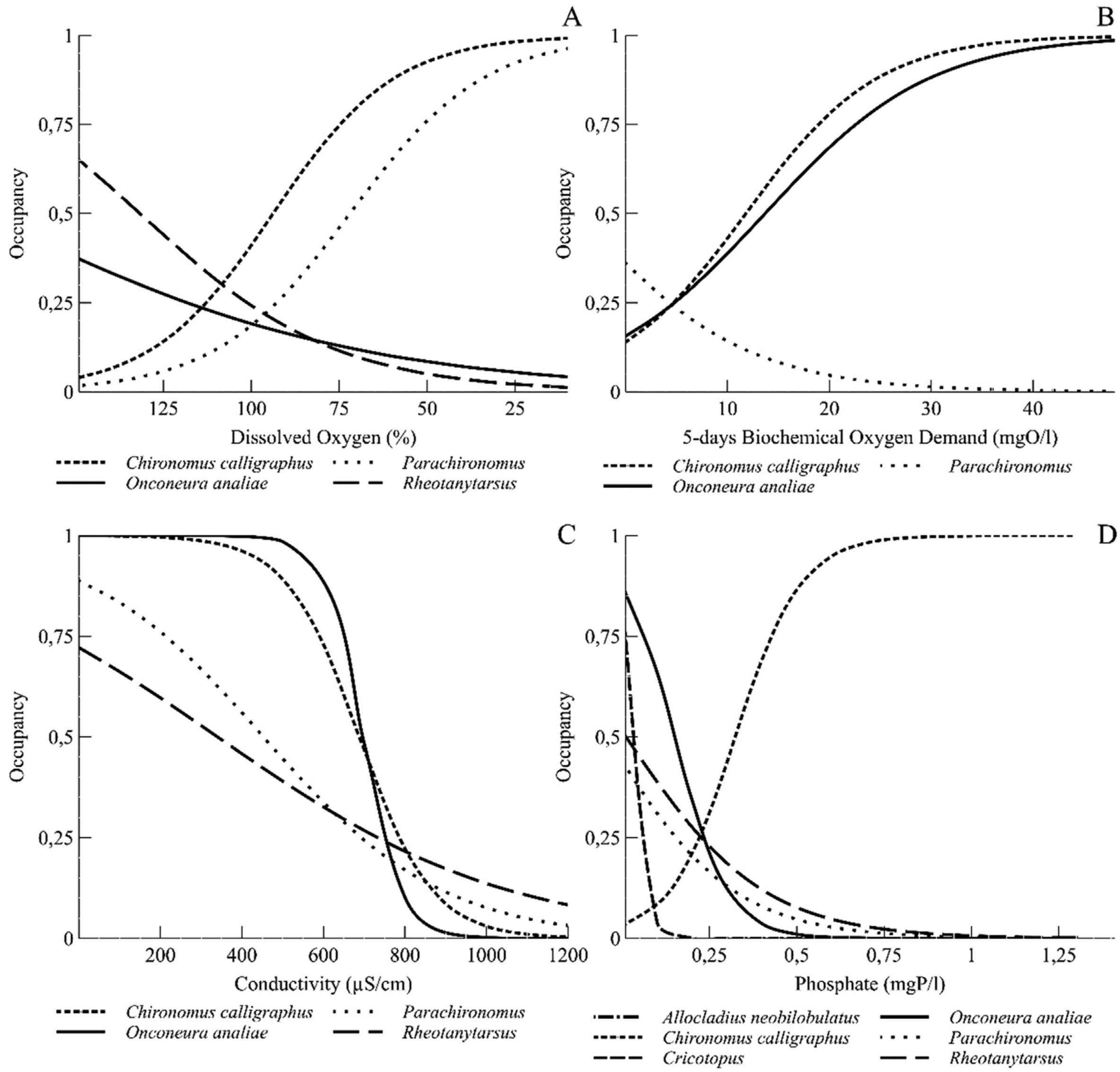

Fig. 2. Occupancy of six taxa of chironomids of Pampean streams along the observed range of four water quality variables: A) Dissolved Oxygen, B) 5-days Biochemical Oxygen Demand, C) Conductivity and D) Concentration of Phosphate.

environmental conditions (Armitage et al., 1995). The orthoclads collected during this study have been reported previously associated to particular environmental conditions. For example, *Cricotopus* is generally tolerant of organic pollution, living mainly associated with macrophytes (Félix dos Anjos and Takeda, 2005); *Allocladius* and *Thienemanniella* species show affinity by rapids with substrates of sand, pebble and rock (Oviedo-Machado and Reinoso-Florez, 2018) being found frequently in interstices, where their small size may allow them to resist high water flow, and *Corynoneura* is globally distributed, occupying a wide variety of habitats (Wiederholm, 1983; Epler, 2001). Orthocladiinae subfamily showed more homogeneous behavior than Chironominae in the space of the CCA. The abundance of Orthocladiinae were associated to relatively low conductivity, low phosphate and nitrogen concentrations, and moderate to high values of dissolved oxygen. This observation would support the use of the subfamily as a biological indicator for Pampean streams.

Larvae of the genus *Onconeura* is usually reported as tolerant to high conductivity and organic pollution (Wilson and Ruse, 2005; Félix dos Anjos and Takeda, 2005). *O. analiae* is an endemic species of Pampean streams, usually reported as associated to high water quality sites (Donato et al., 2012). From the CCA approach, we found the abundance of this species could be a good biological indicator of undisturbed sites. However, the occupancy modelling approach showed that its value as biological indicator would be limited to some water quality variables. *O. analiae* showed a high resistance to conductivity (up to 600 µS/cm), wide tolerance phases for the dissolved oxygen and the BOD_5, and a narrow tolerance phase to phosphate, occupying sites with very low concentrations of this nutrient.

4.3. Tanypodinae subfamily

Tanypodinae species are widely distributed, occupying a diverse array of habitats including small streams and ponds to lakes and bays (Silva et al., 2011). We found four taxa belonging to this subfamily, generally with low frequencies. *Labrundinia* was the only Tanypodinae taxa included in the CCA analysis, and its abundance was associated to high concentration of dissolved inorganic nitrogen and dissolved oxygen. Immatures of *Labrundinia* species are commonly found in a variety of unpolluted freshwater habitats such as streams, rivers, shallow ponds (Silva et al., 2011).

4.4. General remarks

The analysis including both multivariate analysis, and occupancy modelling allowed us a better understanding of the relationship between presence and abundance of taxa and water quality variables. From our results we consider that some taxa of the chironomid assemblage of Pampean streams would be valuable bioindicators of water quality. As occurs in many other regions of the globe, larvae of *C. calligraphus* were dominant in the disturbed sites. Larvae of *O. analiae* and *Rheotanytarsus* would be good indicators of relatively undisturbed sites; while most of the Orthocladiinae were abundant in sites with low concentration of nutrients. Future studies aimed to use chironomid as bioindicators on under-represented freshwater ecosystems of South America, would help assess the ecological thresholds (i.e., the point at which there is an abrupt change in an ecosystem quality) and the extent of human impacts on freshwater environments (Nicacio and Juen, 2015).

A major debate concerning the use of macroinvertebrates in rapid environmental assessment is the level of taxonomic resolution required (Resh, 1994). We found that in some taxa, the potential of chironomids as bioindicator increase as the taxonomic level decrease. Even in the same subfamily, the tolerance to conditions varies by genus. For example, in Chironominae, *C. calligraphus* and *Parachironomus* were generally tolerant to low water quality, while *Rheotanytarsus*, *Paratanytarsus* and *Polypedilum* seems intolerant to low water quality. In the case of the Orthocladiinae, the taxa showed a similar behavior suggesting that in this particular case, the subfamily would be the appropriate taxonomic level for biomonitoring work in Pampean streams. However, the reliability of the higher taxa approach to detect general ecological patterns depends on how species within higher taxa respond to environmental gradients (Siqueira et al., 2009).

Finally, the four explored water quality variables, conductivity, dissolved oxygen, and concentrations of phosphate and dissolved inorganic nitrogen were associated to the abundances of chironomid taxa. The abundance of most chironomid taxa were positively associated to high water quality. Only few chironomid taxa were abundant at lowland streams with low water quality. The description of species sensitivity in terms of occupancy, offers a new methodology to understand how the species shows a pattern along abiotic gradient. Further studies about sensitive of local chironomid species to different types of stressors will allow researchers to generate more accurate biotic indices based on these taxa. The water quality of lowland streams would be inferred from the occupancy of chironomid taxa.

CRediT authorship contribution statement

Agustina Cortelezzi: Conceptualization, Investigation, Resources, Writing - original draft, Writing - review & editing, Visualization, Supervision, Project administration. **María V. Simoy:** Methodology, Software, Formal analysis, Validation, Writing - original draft, Writing - review & editing. **Augusto Siri:** Conceptualization, Investigation, Resources. **Mariano Donato:** Conceptualization, Investigation, Resources. **Rosana E. Cepeda:** Methodology, Formal analysis. **Claudia B. Marinelli:** Methodology, Formal analysis. **Igor Berkunsky:** Conceptualization, Writing - original draft.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecolind.2020.106619.

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