



Spatiotemporal structuring factors in the Chironomidae larvae (Insecta: Diptera) assemblages of an ultraoligotrophic lake from northern Patagonia Andean range: implications for paleolimnological interpretations

Natalia Williams · Diego Añón Suárez · Romina Juncos · Mariano Donato · Sergio Ribeiro Guevara · Andrea Rizzo

Received: 4 April 2019 / Revised: 2 August 2019 / Accepted: 28 September 2019 / Published online: 16 October 2019
© Springer Nature Switzerland AG 2019

Abstract Chironomid larvae assemblages and their seasonal variations were analyzed in several substrate types from Lake Moreno Oeste (Northern Patagonia) in order to understand the spatiotemporal patterns controlling its community. Information on the factors structuring chironomid communities is important to understand their ecological aspects and to improve the interpretation of paleolimnological records based on the analyses of subfossil assemblages. The chironomid community of Lake Moreno Oeste exhibited spatial heterogeneity, with four distinctive groups of taxa associated with different substrate types: (1) macrophyte *Myriophyllum* sp. (mainly represented by

Parapsectrocladius escondido, *Parachironomus* sp. and *Apedilum griseistriatum*); (2) submerged riparian leaves (*Ablabesmyia* sp.); (3) deep sediment (*Cryptochironomus* sp. and *Polypedilum* sp.2.); and (4) littoral and sublittoral sediment (*Riethia truncatocaudata* and *Djalmabatista*). Along the sampling period, significant seasonal changes in chironomid composition were observed in substrates from sublittoral and littoral zones; however, a relatively stable community was recorded in the deeper zone. In Lake Moreno Oeste, the spatiotemporal structure of chironomid larvae assemblages is mainly driven by the substrate type and environmental factors associated with depth, such as organic matter content, sediment granulometric composition, and dissolved oxygen. The results presented here contribute to our understanding of chironomid ecology in North Patagonia and constitute an essential step forward to improve biomonitoring

Handling editor: Jasmine Saros

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s10750-019-04089-w>) contains supplementary material, which is available to authorized users.

N. Williams (✉) · R. Juncos · S. R. Guevara · A. Rizzo
Laboratorio de Análisis por Activación Neutrónica,
Centro Atómico Bariloche, CNEA, Av. Bustillo km 9.5,
8400 Bariloche, Río Negro, Argentina
e-mail: natywilliams86@gmail.com

N. Williams · R. Juncos · A. Rizzo
Centro Científico Tecnológico CONICET, Patagonia
Norte, Buenos Aires, Argentina

D. A. Suárez
Laboratorio de Fotobiología (CRUB-INIBIOMA),
Quintral 1250, 8400 Bariloche, Argentina

M. Donato
Instituto de Limnología “Dr. Raúl A. Ringuelet” (ILPLA,
CONICET, UNLP), Boulevard 120 y 62 s/n, La Plata,
Buenos Aires, Argentina

and paleolimnological studies in lacustrine environments in the region.

Keywords Patagonian lake · Chironomid distribution · Spatial patterns · Seasonal changes · Paleolimnology

Introduction

Chironomidae (Insecta: Diptera) are the most broadly distributed, species-rich and abundant family of benthic macroinvertebrates in freshwater ecosystems. Their larvae are an important component of benthic invertebrate communities (Pinder, 1986; Paggi, 2001). Several species have narrow environmental requirements (e.g., temperature, pH, oxygen concentrations, salinity, productivity, etc.) and are associated with different types of substrates (e.g., organic debris, twigs, and submerged macrophytes) (Pinder, 1986; Lencioni & Rossaro, 2005). These characteristics have made Chironomidae larvae a widely used tool as indicators of lake trophic conditions in biomonitoring studies (Saether, 1979; Rosenberg, 1992). Additionally, given that the chitinous head capsules of the larvae are often well preserved and abundant in lake sediments, they are also used in paleolimnological studies for environmental reconstruction (Walker, 2001). For these reasons, chironomids have proved to be excellent proxy indicators in lake studies throughout the world (Lotter et al., 1997).

Given that the majority of freshwater organisms distribute non-randomly in a lake, spatial pattern analysis is the cornerstone of ecological research and science-based environmental management in lacustrine environments (Árva et al., 2015, 2017). The structure and the spatiotemporal distribution of chironomid assemblages are strongly influenced by several environmental factors (Brown, 2007; Puntí et al., 2009; Árva et al., 2017). For instance, substratum type, in addition to food quality and quantity, has been considered as an important factor influencing the qualitative and quantitative structure of chironomid communities (Reuss et al., 2014). Factors associated with water depth, such as bottom dissolved oxygen (DO) and temperature, have also been found to influence the habitat-scale community structure of chironomid larvae (Zhang et al., 2011). It is therefore

essential to generate information on the environmental factors structuring the chironomid community, both for insights on their ecological dynamics and their use in paleolimnological studies.

Although information about chironomid fauna from the Andean-Patagonian region has been recently expanded, additional work is required to improve our understanding on chironomid assemblage structure and distribution in Patagonia, especially related to their ecology and taxonomy. In this sense, many chironomid species descriptions lack larval stage information because its identification is time-consuming and requires sound taxonomic expertise (Donato et al., 2008; Eggermont et al., 2008; Puntí et al., 2009). In recent years, paleolimnological studies in Patagonia have used chironomids as proxies for understanding climatic variations in the past (Massafiero & Brooks, 2002; Massafiero, 2009; Massafiero & Larocque-Tobler, 2013; Williams et al., 2016, 2019). Additional studies have used chironomids to track the impact of volcanic ash (tephra) depositions in lacustrine ecosystems (Araneda et al., 2007; Williams et al., 2016, 2019) and to investigate the long-term response of subfossil chironomids to environmental disturbances (Massafiero et al., 2017; Montes de Oca et al., 2017). Other studies document the responses of subfossil communities to environmental changes associated with known natural and anthropogenic events or conditions that affected Lake Morenito during the past 100 years (Massafiero et al., 2005) and Lake Moreno Oeste during the past 700 years (Williams et al., 2016).

Ideally, the study of the subfossil chironomid assemblages in a lake ecosystem should be complemented with information on living communities to obtain a more complete and detailed overview for the inference of past changes (van Hardenbroek et al., 2011; Tarrats et al., 2018). However, in South America only a handful of studies have explored the relationship between living and subfossil chironomid assemblages. Some studies have focused on ecological and taxocenosis aspects of chironomids within the macroinvertebrate community in streams and rivers of the Andean mountain range and the Andean Patagonian Plateau (Miserendino, 2001; Paggi & Rodríguez Capítulo, 2002; Miserendino & Pizzolon, 2003; García & Añón Suárez, 2007; Miserendino et al., 2008). Ecological studies on Patagonia have mainly dealt with chironomid distribution (Kaisin, 1989,

Añón Suárez, 1991, 1997, 2002) and compositional changes in response to eutrophication (Mauad et al., 2016). The spatial organization of communities and ecology of Chironomidae in this region, however, has received little attention.

Our current understanding of the ecological processes governing chironomid assemblages stems from studies focused on the littoral areas of lakes, with only a handful including the entirety of the lake's depth and temporal variations in the chironomid community (Rieradevall & Prat, 1999; Tarrats et al., 2017). In this study, we analyzed the spatial and seasonal structure of the Chironomidae larvae assemblages of Lake Moreno Oeste, in order to identify the composition and distribution of this community and the main environmental factors involved. In this context, the objectives of this study are (1) to describe and analyze the structure and composition of the Chironomidae larvae assemblages, considering their seasonal variations, in different substrates from Lake Moreno Oeste, and (2) to evaluate the relationship between environmental factors and the chironomid larvae taxa composition. We hope the new information on the dynamics of this community can contribute to improve the environmental inferences made using chironomid larvae in lakes from this region.

Site description

Lake Moreno Oeste (MO) (41° 03' 33" S; 71° 32' 24" W) is located in the Nahuel Huapi National Park (NHNP; 7100 km²) (Northern Patagonia, Argentina), on the eastern slope of the southern Andes (41° 40' S, 71° to 72° W) (Fig. 1). This lake is the western branch of Lake Moreno (Ribeiro Guevara et al., 2005) and, as most lakes in the NHNP, it is ultraoligotrophic of glacial origin. It is located 758 m above sea level, with an area of 5.22 km² and a maximum depth of 90 m (Buria et al., 2007). It is a warm monomictic lake and stratifies from late spring to early autumn with a thermocline at about 30 m depth. It has an extended euphotic zone (Secchi disk ~ 15 m), dissolved organic carbon concentrations around 0.8 mg L⁻¹, chlorophyll *a* of 1 µg L⁻¹, total phosphorus of 4 µg L⁻¹ and total nitrogen of 140 µg L⁻¹ (Díaz et al., 2007).

Lake MO has a highly irregular coastline, with peninsulas, bays, flooded areas, and gently sloping sandy and gravelly shorelines (Buria et al., 2007; Arcagni et al., 2013). The littoral zone is mostly inhabited by macrophytes, both submerged (*Myriophyllum* sp. and *Nitella* sp.) and emergent (*Schoenoplectus californicus*, *Ranunculus* sp., *Hydrocotyle chamaemorus*, and *Galium* sp.) (Arcagni et al., 2013 and references therein). The flooded areas are surrounded by a dense forest dominated by *Nothofagus dombeyi* (Coihue) and *Austrocedrus chilensis* (Cordilleran cypress) (Queimaliños et al., 1999).

The food web structure of Lake Moreno system was described by Arcagni et al. (2013). The benthic community is dominated by insect larvae (Diptera, Trichoptera, Odonata, Ephemeroptera, and Plecoptera), mollusks (*Chilina* sp. and *Diplodon chilensis*), annelids (Oligochaeta), *Hyaella* sp. amphipods, the anomuran *Aegla* spp., and the crayfish *Samastacus spinifrons*. The fish community is composed by exotic species such as the rainbow trout (*Oncorhynchus mykiss*), brown trout (*Salmo trutta*) and brook trout (*Salvelinus fontinalis*) and the native species creole perch (*Percichthys trucha*), big puyen (*Galaxias platei*), small puyen (*Galaxias maculatus*), velvet catfish (*Diplomystes viedmensis*), and Patagonian silverside (*Odontesthes hatcheri*) (Barriga et al., 2012).

Methods

Substrate sample collection

The sampling was conducted seasonally: April (autumn), August (winter) and November (spring) 2014 and February (summer) 2015. Substrate samples, biological and sedimentary, were collected from littoral (from the shoreline to 6 m deep), sublittoral (6 m deep), and deeper (20, 40, and 90 m deep) zones from bay Llao Llao (LL) in Lake MO (Figs. 1, 2).

In the littoral zone, four substrate types were selected (Fig. 2): (i) submerged riparian leaves, (ii) sediment from rocky bottoms, (iii) submerged macrophyte *Myriophyllum* sp., and (iv) bottom sediment from three vegetated sites dominated by different species (*Myriophyllum* sp., *Nitella* sp., or *S. californicus*).

Sampling was conducted according to the substrate type of each zone (Fig. 2). In the littoral zone, submerged riparian leaves (i) were collected manually from a 531 cm² area. Sediments from the rocky areas (ii) were taken with an Eckman dredge (225 cm³), with prior removal of rocks and stones. Sampling in the *Myriophyllum* sp. (iii) substrate was conducted by manually collecting entire plants, where a plant was considered as a replicate. Sediments from the zone dominated by *Myriophyllum* sp. and *Nitella* sp. (iv) were collected with the Eckman dredge as well. The fraction dominated by *S. californicus* was sampled with two methods: (a) using a plastic syringe, to extract the upper 3 cm corresponding to the water–sediment interface, and (b) with a short plastic corer, to collect the deeper sediment (approx. 10 cm). In the sublittoral and deeper zones, sediment samples were obtained with an Eckman dredge. Three replicates of each substrate type were collected at each sampling season. All samples were stored in plastic labeled bags and were later transported to the laboratory.

Environmental variables

The water physicochemical variables temperature (°C), conductivity (µS/cm), pH, and dissolved oxygen (DO) (%) were measured seasonally along the profile of deeper zones (20, 40 and 90 m) using a multiparameter probe (YSI 6600 V2-2). In the littoral–sublittoral zone, temperature, conductivity, pH, and DO were also seasonally measured using a conductimeter Thermo Orion Model 135A, a pHmeter Corning 360i, and an oxymeter SensION 6.

Laboratory

Chironomid larvae

Chironomid larvae were manually separated from each substrate sample, cleared with KOH 10%, mounted on slides following to Paggi (2001) and identified taxonomically using specialized guides (Wiederholm, 1983; Coffman & Ferrington, 1996; Cranston, 2000; Epler, 2001). For Tanypodinae identification, we followed Rieradevall & Brooks (2001) based on cephalic setation; for morphotypes of Tanytarsini (Chironominae) we followed Massferro et al. (2013) based on the antennal pedestal, pre-mandible teeth, mentum and ventromental plates.

Organic matter content and granulometric composition

From each sample of sedimentary substrate (Fig. 2), a subsample (~ 50 g) was obtained through quartering technique for subsequent laboratory analyzes (Krumbein & Pettijohn, 1938). For organic matter analysis, the subsamples were freeze-dried at – 55°C in a Snijders Scientific lyophilizer until they were completely dry (once a constant mass was reached). The dry subsamples were homogenized, and organic matter (OM) content was estimated using loss on ignition (LOI) at 550°C for 4 h (Heiri et al., 2001). Granulometric composition was determined by sieving in order to separate and quantify the sediment components: (1) silt and clay (grain size ≤ 0.063 mm) (2) fine sand (0.063–0.5 mm) and (3) coarse sand (0.5–2 mm) fractions.

Statistical analyses

In order to analyze the community structure and the species turnover among sites, abundance (ind./m²), diversity index of Shannon–Weaver (H) and taxa richness (TR) were calculated seasonally in each substrate. Relative abundance was obtained as the number of individuals of each taxon divided by the total number of individuals. Rare taxa, defined as those taxa that did not reach a relative abundance of ≥ 2% in at least two samples, were not considered in statistical analysis.

We used a combination of ordination and cluster analyses, as well as analyses of similarity, to characterize and test differences in structure and composition of chironomid assemblages. First, a non-metric multidimensional scaling (NMDS) analysis was performed to explore the spatial (substrate type) and temporal (seasonal) patterns of Chironomid larvae assemblages. This ordination technique is appropriate for ecological data, which often have several zeroes (absence of a species in a sample; Clarke & Warwick, 2001). The Bray–Curtis similarity coefficient was used to quantify the similarity between all pairs of samples (Clarke & Warwick, 2001), computed on the log₁₀ transformed chironomid abundance. The stress value was used as a measure of the goodness of fit of the ordination obtained by the NMDS. Stress values below 0.2 correspond to a reasonable fit for an ordination (Clarke & Warwick, 2001). Then, a

Table 1 Summary of environmental variables (temperature, conductivity, pH and dissolved oxygen) measured seasonally in different zones from Lake Moreno Oeste (MO)

Zone	Temperature (°C)				Conductivity (µs/cm)				pH				Dissolved oxygen (%)			
	A	W	Sp	Su	A	W	Sp	Su	A	W	Sp	Su	A	W	Sp	Su
Littoral-Sublittoral	7.2	6.6	12.9	17.6	31.2	29.9	34.7	40.9	7.1	6.4	7.4	7.4	93.3	95.2	96.1	94
20 m	9.9	6.9	11.4	14.9	42	43	43	43	7.7	7	6.4	6.9	89.1	91	93.9	90.7
40 m	8	6.8	9.35	9.4	42	43	43	43	7.6	7.1	6.5	6.8	84.5	89.4	91	87.9
90 m	6.8	6.7	7.3	7.5	43	43	43	43	7.5	7.2	6.5	6.7	75.4	86.1	82.8	79.2

hierarchical agglomerative cluster analysis based on the \log_{10} transformed chironomid abundances for each substrate type was performed to determine groups with similar taxa composition, using the Bray–Curtis coefficient of similarity (multiplied by 100) based on a similarity matrix and the group-average linking method (Clarke & Warwick, 2001). A one-way analysis of similarities (ANOSIM) was performed to test for the significance of the defined chironomid groups, first considering chironomid assemblages between different substrates and then between sampling seasons within each substrate. Similarity percentage analysis (SIMPER) was used to determine the contribution of each taxa to the observed similarity/dissimilarities within/between each group. SIMPER allows identification of the most important taxa for creating the observed pattern of similarity within a defined group. All these analyses were performed in PRIMER Version 5.2.9 (Clarke & Gorley, 2001).

The association of chironomid taxa with each habitat was determined by the relative preference index (RPI) (Tickner et al., 2000), which was calculated dividing the abundance of each taxon in a determined habitat by the total abundance of each taxon. The RPI can vary between 0 and 1, meaning no preference and strong affinity for a particular habitat, respectively.

To evaluate the relationship between the chironomid taxa and the environmental factors, we considered the chironomid relative abundance and temperature, conductivity, pH, DO and OM content. In order to include the effect of the sediment granulometric composition on the structure of chironomid assemblages, we decided to include the percentage of finer sediment fraction (% silt and clay) in the analysis. The relationships between these environmental factors and the most abundant chironomid taxa were analyzed by a

canonical correspondence analysis (CCA) (Legendre & Legendre, 1998). A detrended correspondence analysis (DCA) was run to test the gradient length of species composition and to determine the appropriate constrained ordination technique (i.e., canonical correspondence analysis, CCA, or redundancy analysis, RDA). Our dataset responded unimodally to environmental gradients ($SD > 2$), so CCA was performed. This analysis was run in CANOCO 4.5 (ter Braak & Šmilauer, 1998).

Results

Environmental factors

Temperature, conductivity, pH and DO data recorded seasonally in the littoral-sublittoral and deeper zones (20, 40 and 90 m) are summarized in Table 1. The highest temperature in Lake MO was recorded in summer, and the lowest in winter. The range of variation was higher in the littoral-sublittoral zone, while more stable in the deeper zone, where temperature remained around 7°C. Conductivity behaves similarly, remaining around 43 µs/cm in the deeper zone, and between 29.9 and 40.9 µs/cm in the littoral-sublittoral zone. In general, pH was stable, not changing significantly from 7 during the sampling period. DO levels were high in the littoral-sublittoral zone (93.3–96%), but were lower in the deeper zone (75.4–86.1%) of the lake.

Sediment from the littoral and sublittoral zones was characterized by high percentages of coarse (up to 81%) and fine sand (up to 96%), and lower proportions of silt and clay (1–30%) (Fig. 3). On the other hand, low percentages of coarse (up to 29%) and fine sand (up to 40%) and higher proportions associated with silt

Table 2 Annual relative abundances of taxa (> 2%) in the different substrate considered in Lake Moreno Oeste (MO)

Substrate	<i>Ablab.</i> sp.	<i>A.</i> <i>vittigera</i>	<i>A.</i> <i>griseis.</i>	<i>Apsect.</i> sp.	<i>Chiron.</i> sp.	<i>Crypt.</i> sp.	<i>D.</i> <i>emb.</i>	<i>Dic.</i> sp.2	<i>Djaln.</i> sp.1	<i>Parach.</i> sp.	<i>P.</i> <i>escond.</i>	<i>Polyp.</i> sp.1	<i>Polyp.</i> sp.2	<i>R.</i> <i>truncat.</i>	<i>T.</i> <i>patag.</i>
Littoral															
Sed. <i>Myriophyllum</i> sp.	1.4	6.6	1.4	24.4	17.4	3.3	–	–	7	0.5	5.6	6.1	3.3	20.2	0.5
Sed. <i>Nitella</i> sp.	6.8	–	0.5	5.3	–	20.5	0.5	0.5	46.3	–	0.5	7.4	2.6	5.8	–
WS Sed. <i>S. californicus</i>	3.6	–	–	–	–	–	–	–	14.3	–	–	–	–	7.1	57.1
Deep sed. <i>S.</i> <i>californicus</i>	–	6.4	–	–	–	12.8	–	–	44.7	–	–	4.3	–	21.3	8.5
Sed. from rocky bottoms	–	–	–	–	–	2.7	–	–	5.4	–	–	2.7	2.7	43.2	29.7
<i>Myriophyllum</i> sp. leaves	1.7	–	23	0.6	–	0.6	0.6	2.9	0.6	16.1	36.8	–	1.7	8	3.4
Riparian vegetation leaves	50	41.7	–	–	–	–	–	–	4.2	–	–	–	–	–	–
Sublittoral															
Sed. 6 m	1.3	0.3	0.6	2.2	1.3	5	6	8.8	5.3	–	–	2.2	0.3	60.8	–
Deep															
Sed. 20 m	1.8	–	–	3.6	–	38.2	–	–	12.7	–	–	7.3	29.1	3.6	–
Sed. 40 m	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Sed. 90 m	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

Ablab. Sp., *Ablabesmyia* sp.; *A. vittigera*, *Alotanypus vittigera*; *A. griseis.*, *Apeditum griseistriatum*; *Apsec.* sp., *Apsectrotanypus* sp.; *Chiron.* sp., *Chironomus* sp.; *Crypt.* sp., *Cryptochironomus* sp.; *D. emb.*, *Dicrotendipes embalsensis*; *Dic.* sp.2, *Dicrotendipes* sp.2; *Djaln.* sp.1, *Djalnabatista* sp.1; *Parach.* sp., *Parachironomus* sp.; *P. escond.*, *Parapsectrocladius escondido*; *Polyp.* sp.1, *Polypeditum* sp.1; *Polyp.* sp.2, *Polypeditum* sp.2; *R. truncat.*, *Riethia truncatocaudata*; *T. patag.*, *Tanytarsus patagonicus*

Table 3 Abundance (in sedimentary substrates and submerged riparian leaves; values are expressed as ind./m², and in *Myriophyllum* sp. as ind./g. of plant), taxa richness (TR) and diversity index of Shannon–Weaver (H) of Chironomidae larvae recorded seasonally in different substrates from Lake Moreno Oeste

Substrate	Abundance						Richness						Diversity					
	A		W		Su		A		W		Su		A		W		Su	
Littoral																		
Sed. <i>Myriophyllum</i> sp.	311	267	1244	1259	8	5	9	9	9	0.72	0.64	0.61	0.52					
Sed. <i>Nitella</i> sp.	104	326	1096	1200	4	6	10	6	10	0.44	0.49	0.61	0.59					
WS Sed. <i>S. californicus</i>	–	–	–	826	–	–	–	–	–	–	–	–	0.31					
Deep sed. <i>S. californicus</i>	826	–	118	905	7	–	1	5	–	0.61	–	–	0.59					
Sed. from rocky bottoms	–	–	–	201	–	–	–	9	–	–	–	–	0.53					
<i>Myriophyllum</i> sp. leaves	6.5	7.81	0.54	8.26	11	4	3	8	3	0.62	0.39	0.24	0.62					
Riparian vegetation leaves	–	–	18.8	132	–	–	–	2	3	–	–	0.3	0.3					
Sublittoral																		
Sed. 6 m	1081	1763	1126	474	3	5	14	7	14	0.23	0.14	0.81	0.31					
Deep																		
Sed. 20 m	326	281	104	74	4	5	5	3	5	0.44	0.63	0.41	0.46					
Sed. 40 m	–	–	–	–	–	–	–	–	–	–	–	–	–					
Sed. 90 m	–	–	–	–	–	–	–	–	–	–	–	–	–					

A Autumn, W Winter, Sp Spring, Su Summer

Table 4 Main chironomid taxa associated with each substrate type and their relative preference index (RPI)

RPI	Sed. 20 m	Sed. 6 m	Sed. <i>Myriophyllum</i> <i>sp.</i>	Sed. <i>Nitella</i> <i>sp.</i>	<i>Myriophyllum</i> <i>sp.</i>	Sed. <i>S.</i> <i>californicus</i>	WS Sed. <i>S.</i> <i>californicus</i>	Sed. Stoned bottom	Riparian leaves
<i>Ablabesmyia</i> sp.	0.03	0.11	0.08	0.35	0.08	0	0.03	0	0.32
<i>Alotanypus vittigera</i>	0	0.03	0.5	0	0	0.11	0	0	0.36
<i>Apedilum griseistriatum</i>	0	0.04	0.06	0.02	0.87	0	0	0	0
<i>Apsectrotanypus</i> sp.	0.03	0.1	0.72	0.14	0.01	0	0	0	0
<i>Chironomus</i> sp.	0	0.1	0.9	0	0	0	0	0	0
<i>Cryptochironomus</i> sp.	0.23	0.17	0.08	0.43	0.01	0.06	0	0.01	0
<i>Dicrotendipes</i> sp.2	0	0.82	0	0.03	0.15	0	0	0	0
<i>Djalmabatista</i> sp.1	0.04	0.11	0.1	0.56	0	0.13	0.02	0.01	0
<i>Parachironomus</i> sp.	0	0	0.03	0	0.96	0	0	0	0
<i>Parapspectrocladius</i> <i>escondido</i>	0	0	0.15	0.01	0.83	0	0	0	0
<i>Polypedilum</i> sp.1	0.1	0.17	0.32	0.34	0	0.05	0	0.02	0
<i>Polypedilum</i> sp.2	0.5	0.03	0.21	0.15	0.1	0	0	0.03	0
<i>Riethia truncatocaudata</i>	0.01	0.66	0.15	0.04	0.05	0.03	0	0.05	0
<i>Tanytarsus patagonicus</i>	0	0	0.03	0	0.16	0.1	0.42	0.29	0

RPI \geq 0.5 are indicated in italics

Table 5 Summary of the main taxa registered in Lake Moreno Oeste, the seasonal period and substrate type in which they are dominant, and their feeding and living habits

Taxa	Subfamily	Season	Main substrate in Lake MO	Feeding habit	Living habit	Paleolimnological insights
<i>Ablabesmyia</i> sp.	Tanypodinae. T. Pentaneurini.	Spring– Summer	Riparian leaves	Predators. Collectors– gatherers (early instars) (1)	Widespread. Lentic littoral (1)	Indicative of <i>littoral habits</i> , generally associated with vegetated and riparian zones (8)
<i>Alotanypus vittigera</i>	Tanypodinae. T. Macropelopiini	Spring– Summer	Littoral zone (Sed. from vegetated zones and riparian leaves)	–	Standing water shallow. In soil contains a high percentage of organic matter (5)	Taxa <i>Alotanypus</i> sp. is generally indicative of warm conditions (10)
<i>Apeditum griseistriatum</i>	Chironominae. T. Chironomini	Summer	<i>Myriophyllum</i> sp.	–	Lentic–Lotic Submerged vegetation (1)	Generally associated with <i>Parachironomus</i> and <i>Cricotopus</i> . Their presence is indicative of littoral/warm conditions (4)
<i>Apsectrotanypus</i> sp.	Tanypodinae. T. Macropelopiini	Spring– Summer	Generalist	Predators. Collectors– gatherers (early instars) (1)	Lotic. Predators collectors in early stages (1)	Indicative of higher temperatures and littoral zones (8)
<i>Chironomus</i> sp.	Chironominae. T. Chironomini	Summer	Bottom sediment <i>Myriophyllum</i> sp.	Collectors– gatherers (a few filterers), shredders– herbivores	Littoral Lotic (1)	Indicative of higher temperatures (7) or warmer seasons
<i>Cryptochironomus</i> sp.	Chironominae. T. Chironomini	Annual	Deep Sediment	Predators– shredders (1)	Lentic littoral Deep zones (1)	Indicative of deeper cooler zones and fine sediment (8)
<i>D. embalsensis</i>	Chironominae. T. Chironomini	Spring– Summer	Sublittoral sediment	Collectors, filterers, scrapers (1)	Lentic littoral (1)	Taxa <i>Dicrotendipes</i> sp. is mainly associated with colder temperatures (7; 8)
<i>Dicrotendipes</i> sp.2	Chironominae. T. Chironomini	Summer	Sublittoral sediment	Collectors, filterers, scrapers (1)	Lentic littoral (1)	Taxa <i>Dicrotendipes</i> sp. is mainly associated with colder temperatures (7; 8)
<i>Djalmabatista</i> sp.1	Tanypodinae. T. Procladiini	Annual	Generalist	Predators (1)	Lotic (1)	Associated with warmer waters , prefer littoral areas inhabited by aquatic vegetation

Table 5 continued

Taxa	Subfamily	Season	Main substrate in Lake MO	Feeding habit	Living habit	Paleolimnological insights
<i>Parachironomus</i> sp.	Chironominae. T. Chironomini	Spring	<i>Myriophyllum</i> sp.	Predators, collectors, some parasites (1)	Lentic littoral (1)	Their presence is indicative of littoral/warm conditions (4)
<i>Parapspectrocladius excondido</i>	Orthocladinae	Autumn–Winter	<i>Myriophyllum</i> sp.	Collectors, scrapers (2)	Aquatic vegetation (2). Shallow environments (6).	Associated with littoral zones and submerged vegetation development (8)
<i>Polypeditum</i> sp.1	Chironominae. T. Chironomini	Annual	Generalist	Shredders, collectors, predators (1)	Lentic. Lotic (2)	Indicative of higher temperatures and littoral habitat (8)
<i>Polypeditum</i> sp.2	Chironominae. T. Chironomini	Annual	Deep sediment	Shredders, collectors, predators (1)	Lentic. Lotic (2)	Indicative of deeper cooler zones and fine sediment (8)
<i>Riethia truncatocaudata</i>	Chironominae. T. Pseudochironomini	Autumn–Winter	Generalist, although dominant in sublittoral zone	Burrowers (3)	Lentic. Lotic. Habitats with high amounts of fine particle organic matter. Temperate environments in Patagonian Chile and Argentina (3).	<i>Riethia</i> sp. is generally associated with profundal areas and cold conditions (9)
<i>Tanytarsus patagonicus</i>	Chironominae. T. Tanytarsini	Spring–Summer	Generalist, but preferably in littoral zones	Filters collectors. Some scrapers (1)	Vascular hydrophytes. Lentic or lotic zones (1)	Generally morphotypes of <i>Tanytarsus</i> re-associated with the littoral zone of the lakes (8) and higher temperatures (7)

1. Coffman and Ferrington (1996); 2. Añón Suárez (1997); 3. Trivinho-Strixino et al. (2009); 4. Donato et al. (2015); 5. Siri et al. (2011); 6. Cranston (2000); 7. Massaferro & Larocque-Tobler (2013); 8. Williams et al. (2016); 9. Montes de Oca et al. (2017); 10. Massaferro et al. (2017)

The bold words indicate the main environmental characteristics to which the taxa are associated

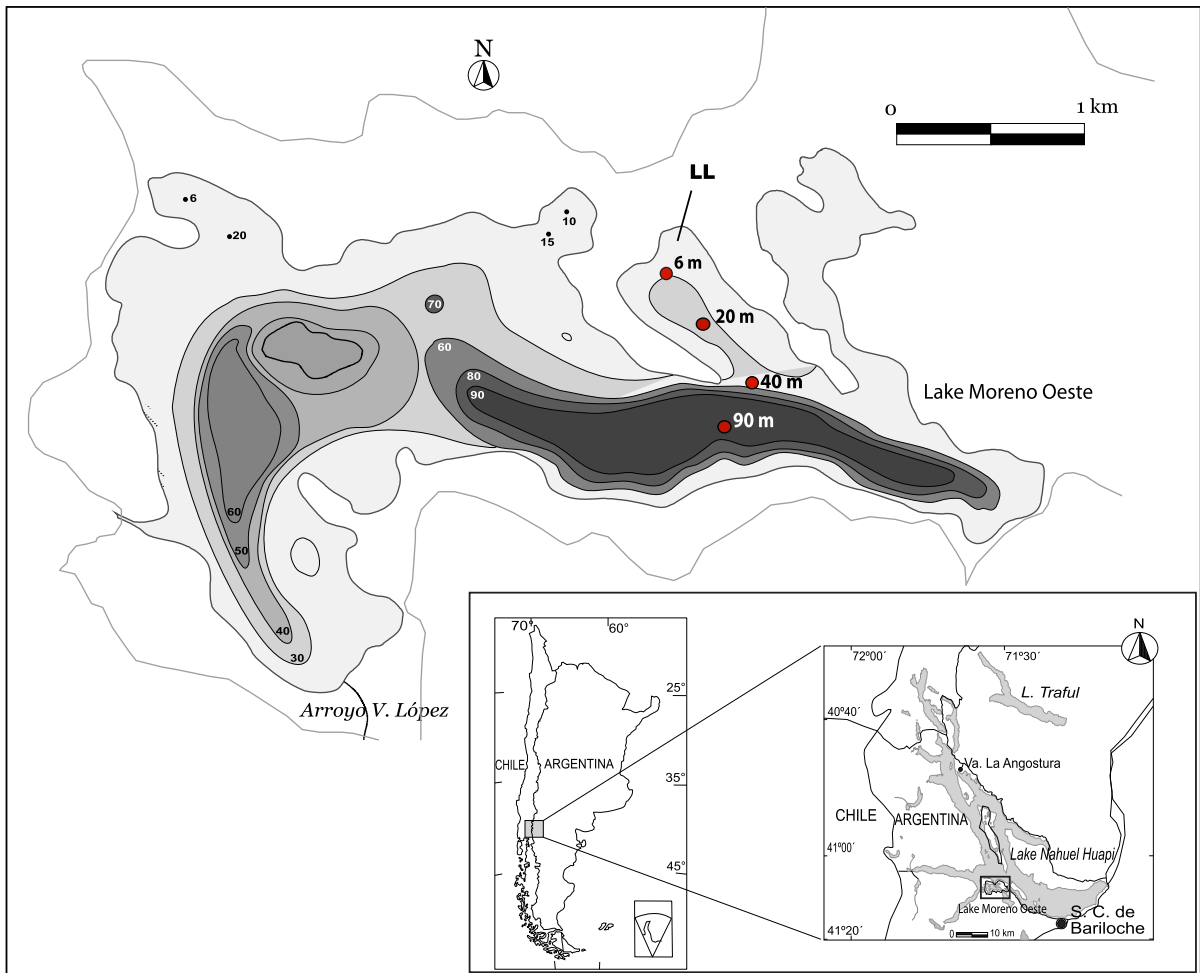


Fig. 1 Study area indicating sampling sites at Lake Moreno Oeste (MO). GP (Guardaparque) and LL (Llao–Llao) indicate sampled bays for littoral substrates, and red points 6, 20, 40, and

90 indicate sampled depths in sublittoral and deeper zones. Extracted and modified from Arcagni et al. (2013)

and clay (39–96%) were recorded in deeper sites (20, 40 and 90 m). Sediment at 90 m depth showed the highest values of fine-grained fraction (Fig. 3).

In general, higher OM values were reported in sediment with higher proportion of silt and clay (Fig. 3). The highest values of OM were observed in sediment from the zones dominated by *Myriophyllum* sp. during spring and summer (19 and 11%, respectively), and the sublittoral zone during summer (18%). Sediments from deeper zones presented OM values between 4 and 12%.

Chironomid community

A total of 1087 chironomid larvae were recorded in Lake MO during the sampling period. We identified twenty-four chironomid taxa from subfamilies Chironominae (64.4%), Tanypodinae (27.4%), Orthoclaadiinae (8%) and Prodiamesinae (0.2%). Subfamily Chironominae was composed by tribes Chironomini (33.2%), Pseudochironomini (26.9%) and Tanytarsini (4.3%).

The most abundant taxa were *Riethia truncatocaudata* (Edwards, 1931) (26.8%), *Djalmabatista* Fittkau sp.1 (14.3%), *Cryptochironomus* Kieffer sp. (8.4%) and *Parapsectrocladius escondido* Cranston and Añón

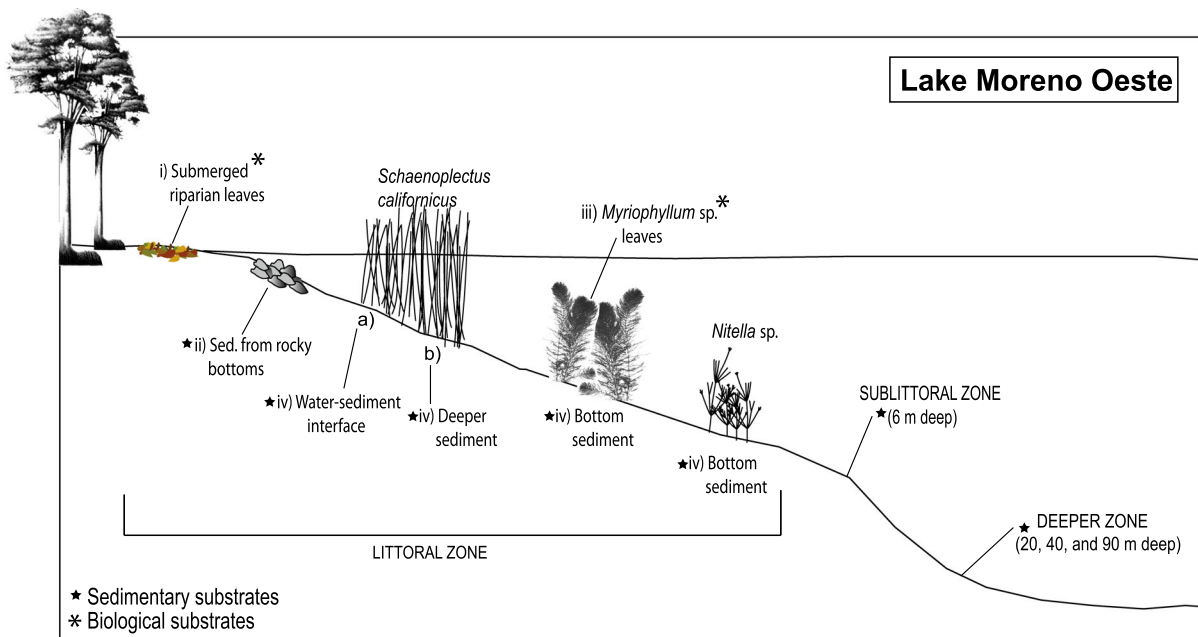


Fig. 2 Diagram of the zones (littoral, sublittoral, and deeper) in Lake Moreno Oeste, and detail of the different substrates (biological and sedimentary) in the littoral zone

Suárez, 2000 (7.1%), followed by *Apsectrotanypus* Fittkau, 1962 sp. (6.6%), *Apedilum griseistriatum* (Edwards) (Donato et al., 2015) (4.2%), *Polypedilum* Kieffer sp.1 (3.8%), *Chironomus* Meigen sp. (3.8%), *Tanytarsus patagonicus* (Reiss, 1972) (3.5%), *Ablabesmyia* Johannsen sp. (3.5%), *Dicrotendipes* Kieffer sp.2 (3.1%), *Polypedilum* sp.2 (3%), *Parachironomus* Lenz sp. (2.7%), *Alotanytus vittigera* (Edwards) (Siri et al., 2011) (2.6%), *Dicrotendipes embalsensis* Paggi, 1978 (1.9%) and *Dicrotendipes* sp.1 (1.5%) (Table 2). Other taxa presented a relative abundance lower than 1%, such as *Tanytarsus* van der Wulp, 1874 sp. (0.8%), *Paracladopelma* Harnisch sp. (0.7%), *Cricotopus* van der Wulp sp. (0.6%), *Djalmabatista* sp.2 (0.3%), *Limnophyes* Eaton, 1875 sp. (0.3%), Tanypodinae ind. 1 (0.2%), *Monodiamesa* Kieffer, 1922 sp. (0.2%) and *Dicrotendipes* sp.3 (0.1%). Representative images of the most abundant taxa are shown in Fig. 4.

The chironomid community presented high values, both in terms of abundance and richness, in the littoral zone, mainly during spring and summer (Table 3). In the sublittoral zone, the highest abundance (1763 ind./m²) was observed in winter and the lowest (474 ind./m²) in summer; the highest richness (14) was registered during spring and the lowest (3) in autumn.

Sediment at 20 m depth showed the highest abundance (326 ind./m²) in autumn and the lowest (74 ind./m²) in summer (Table 3); and richness recorded relatively stable values (between 3 and 5) along the sampling period. No chironomid larvae were observed at 40 and 90 m depth along the entire sampling period. Chironomid diversity was high in the littoral zone, with the highest values of H' at the bottom sediment of *Myriophyllum* sp. during autumn and winter (0.72 and 0.64, respectively). In the sublittoral zone, a remarkably high H' (0.81) was registered during spring, and the lowest values were recorded during autumn and winter (0.23 and 0.14, respectively) (Table 3). Sediments at 20 m depth recorded relatively constant diversity (0.41–0.46) along the sampling period, with the highest value (0.63) recorded in winter.

Spatial and temporal structure of the chironomid larvae assemblages

We found that the chironomid community of lake MO aggregates in three main groups when observing the results of the NMDS: (1) Submerged leaves (SL); (2) *Myriophyllum* sp. (M) and; (3) Sediment at 20 m depth (20). The rest of samples can be grouped as one big

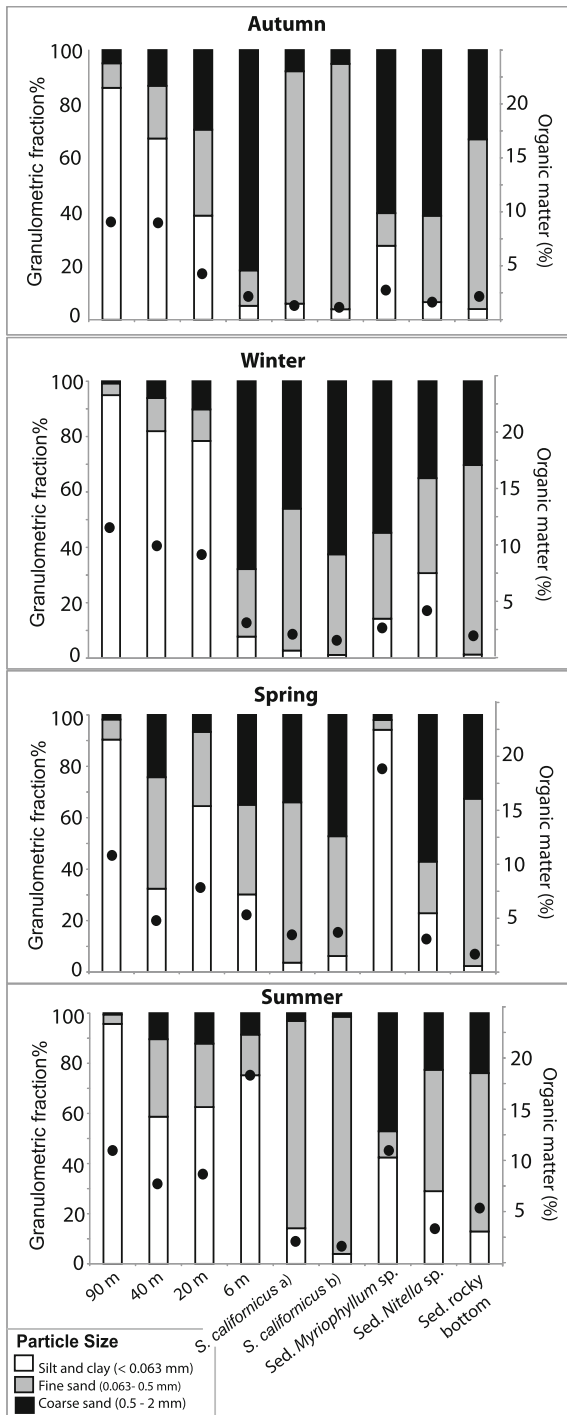


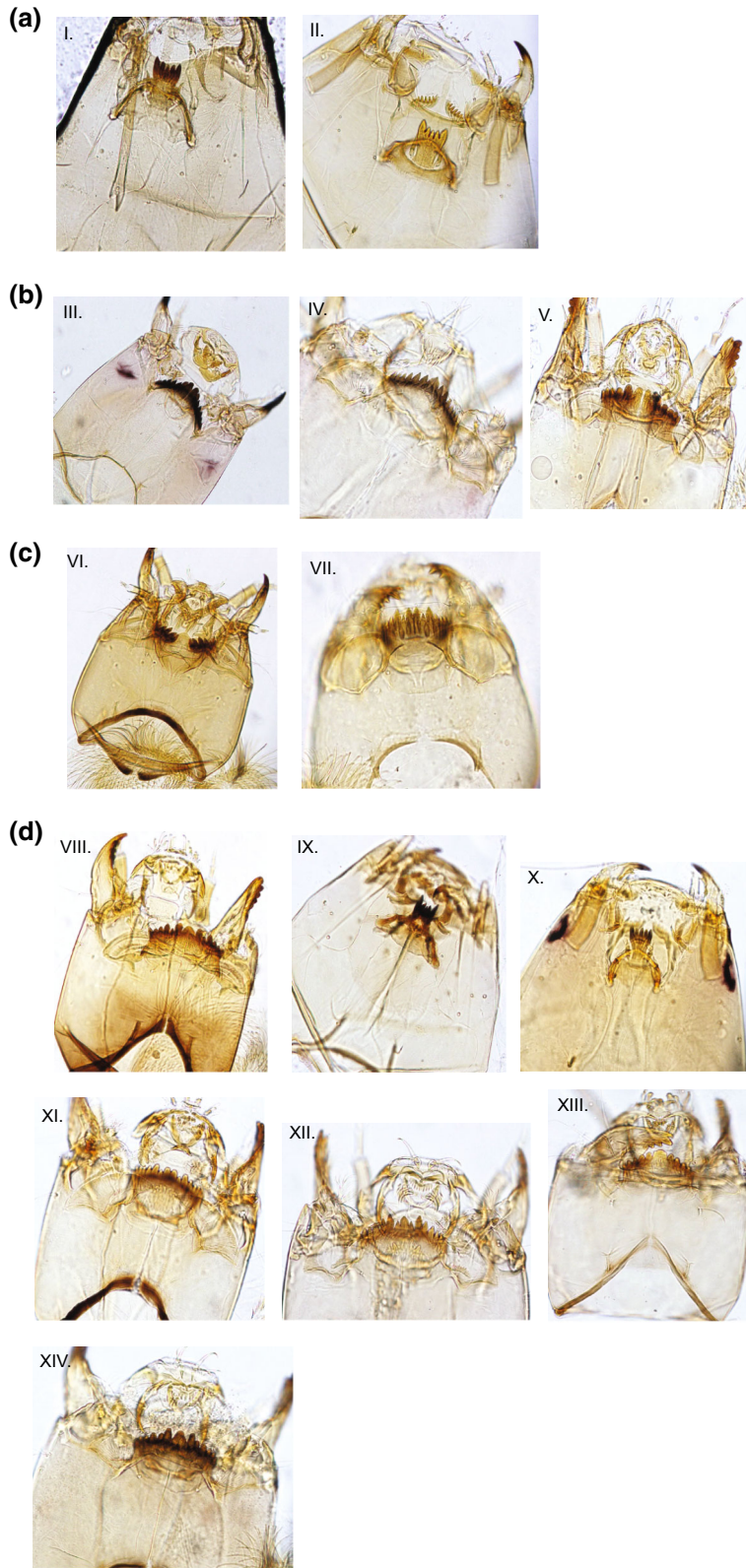
Fig. 3 The bars indicate the composition (%) of particle size (fine sand = 0.063 – 0.5 mm; coarse sand = 0.5 – 2 mm; silt and clay \leq 0.063 mm) and the dots show the organic matter content (%) of sedimentary substrates collected seasonally from Lake Moreno Oeste

and less defined group, conformed by sedimentary substrates from littoral and sublittoral zones (SED), which includes all sediment samples from vegetated zones, rocky bottoms and sublittoral zone (Figs. 2, 5a). One-way ANOSIM validated this grouping (Global $R = 0.598$). SL was separated from the groups M, SED and 20 (R value = 0.85, 0.82 and 0.97, respectively; $P = 0.001$), while group 20 was in turn parted from group M (R value = 0.88; $P = 0.001$) and group SED (R value = 0.32; $P = 0.001$). Finally, SED was statistically different from M (R value = 0.64; $P = 0.001$) (Table I, supplementary material).

NMDS analysis results are in agreement with the results obtained from the cluster analysis, where a depth gradient in the substrates according their chironomid composition is evident (Fig. 5b). Biological substrates (submerged leaves and *Myriophyllum* sp.) are separated from the sedimentary substrates, which are clustered with the most littoral ones (rocky bottoms and sediment from vegetated zone dominated by *S. californicus*) separated from the deeper ones (sed. *Myriophyllum* sp., sed. *Nitella* sp., and sediment from sublittoral and deeper zones).

The averages similarity in the SIMPER analysis for the groups SL, 20, M, and SED were 68.57, 51.9, 38.23 and 31.46%, respectively. In group SL, *Ablabesmyia* sp. (71.43%) and *Alotanypus vittigera* (28.57%) contributed to the 100% of similarity. In group 20, *Cryptochironomus* sp. (47.75%) and *Polypedilum* sp.2 (29.39%) accounted up to 90.5% of the total contributions to the group similarity. The species that contributed the most to the total similarity in group M were *Parapsectrocladius escondido* (30.72%), *Parachironomus* sp. (29.63%) and *Apedilum griseistriatum* (21.65%). Lastly, *Djalmabatista* sp.1 (25.73%), *Riethia truncatocaudata* (26.11%) and *Cryptochironomus* sp. (9.58%) contributed the most of the 85.33% of the total group similarity in group SED (Table II, supplementary material).

Further support for species-substrate association is provided by the RPI analysis (Table 4). For instance, *A. griseistriatum*, *Parachironomus* and *P. escondido* displayed an elevated RPI by *Myriophyllum* sp. (0.87, 0.96 and 0.83, respectively). At 20 m depth, *Polypedilum* sp.2 (0.5) and *Cryptochironomus* sp. (0.23) showed high RPI values. In the sublittoral zone, both *R. truncatocaudata* (0.66) and *Dicrotendipes* sp.2 (0.82) showed high RPI (Table 4).



◀ **Fig. 4** Photomicrographs of chironomid larvae from Lake Moreno Oeste. Dominant taxa on **a** Submerged leaves: I. *Ablabesmyia* sp., II. *Alotanypus vittigera*. **b** *Myriophyllum* sp.: III. *Parapspectrocladius escondido*, IV. *Parachironomus* sp., V. *Apedilum griseistriatum*. **c** Sediment at 20 m depth: VI. *Cryptochironomus* sp., VII. *Polypedilum* sp.2. **d** Sediment littoral and sublittoral: VIII. *Riethia truncatocaudata*, IX. *Djalmabatista* sp.1, X. *Apsectrotanypus* sp., XI. *Polypedilum* sp.1, XII. *Chironomus* sp., XIII. *Tanytarsus patagonicus*, XIV. *Dicotendipes embalsensis*

The chironomid assemblages in each season are presented in Fig. 6. After assessing the results of the NMDS analysis, a one-way ANOSIM was performed for each substrate to test for the significance of the seasonal variations in the assemblage, if any. Chironomid assemblages presented significant seasonal differences in the *Myriophyllum* sp. substrate (group M) ($R = 0.938$; $P = 0.001$), where *P. escondido* was dominant during autumn and winter (51.3 and 60%, respectively). In spring, the most abundant taxa were

Parachironomus sp. (75%) and *Tanytarsus patagonicus* (25%); and in summer were *Apedilum griseistriatum* (47.3%), *Riethia truncatocaudata* (23.6%) and *P. escondido* (10.9%) (Fig. 6). Additionally, sed. *Myriophyllum* sp. ($R = 0.974$; $P = 0.001$), sed. *Nitella* sp. ($R = 0.613$; $P = 0.002$), and sed. 6 m depth ($R = 0.969$; $P = 0.001$) evidence a potential trend of seasonal chironomid assemblage variability (Figs. 5a and 6).

In group 20, although chironomid assemblages were not differentiated by season ($R = 0.299$; $P = 0.039$), some variability in abundance can be described: *Cryptochironomus* sp. was present during all seasons, being dominant in autumn, spring and summer (59.1, 57.1 and 40%, respectively). In winter, *Polypedilum* sp.2 was the most abundant taxa (42.1%) (Fig. 6). In group SL, we only found larvae during summer and spring. In summer, *Ablabesmyia* sp. (52.4%) was the most abundant taxa, followed by

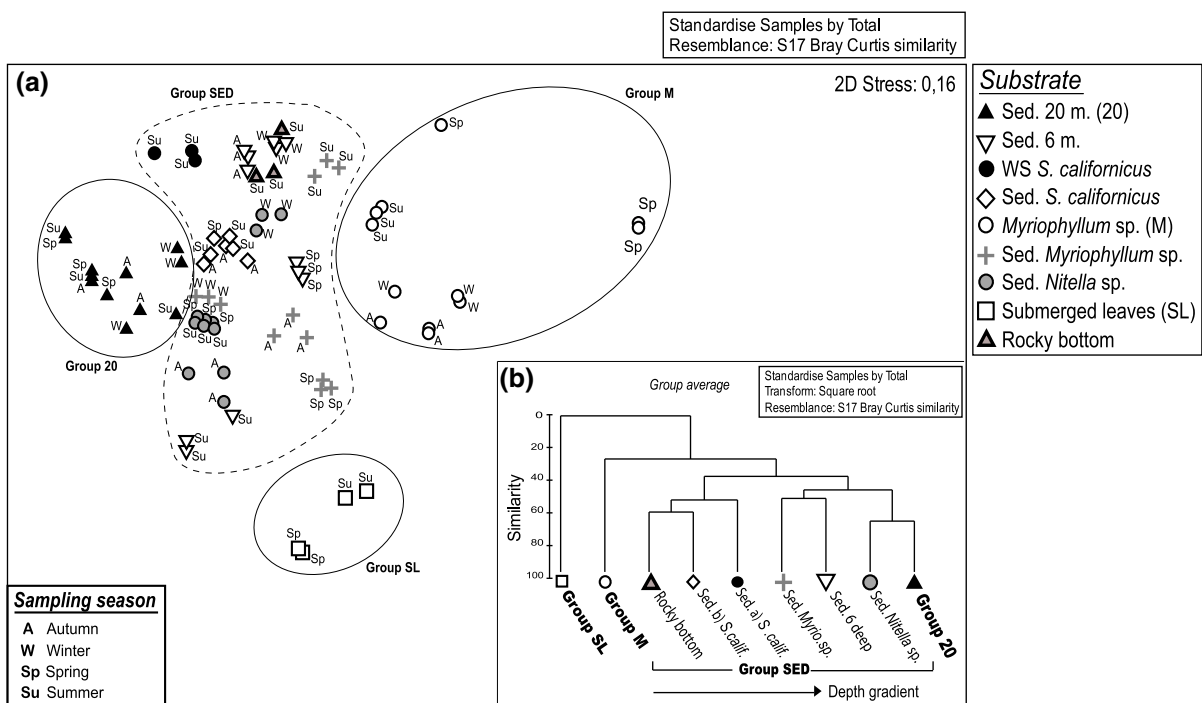


Fig. 5 a Analysis of non-metric multidimensional scaling (NMDS) of chironomid larvae assemblages samples in Lake Moreno Oeste according substrate type. Principal groups of chironomid assemblages (*Myriophyllum* sp.: Group M; submerged leaves: Group SL; sediment at 20 m deep: Group 20; and sediment littoral and sublittoral: Group SED) are exhibited.

The letters A (autumn), W (winter), Sp (spring) and Su (summer) indicate the sample season. **b** Clustering analysis based on the log₁₀ transformed chironomid abundances for each substrate type. Main chironomid groups (M, SL, 20, and SED) and depth gradient are indicated in the inferior margin

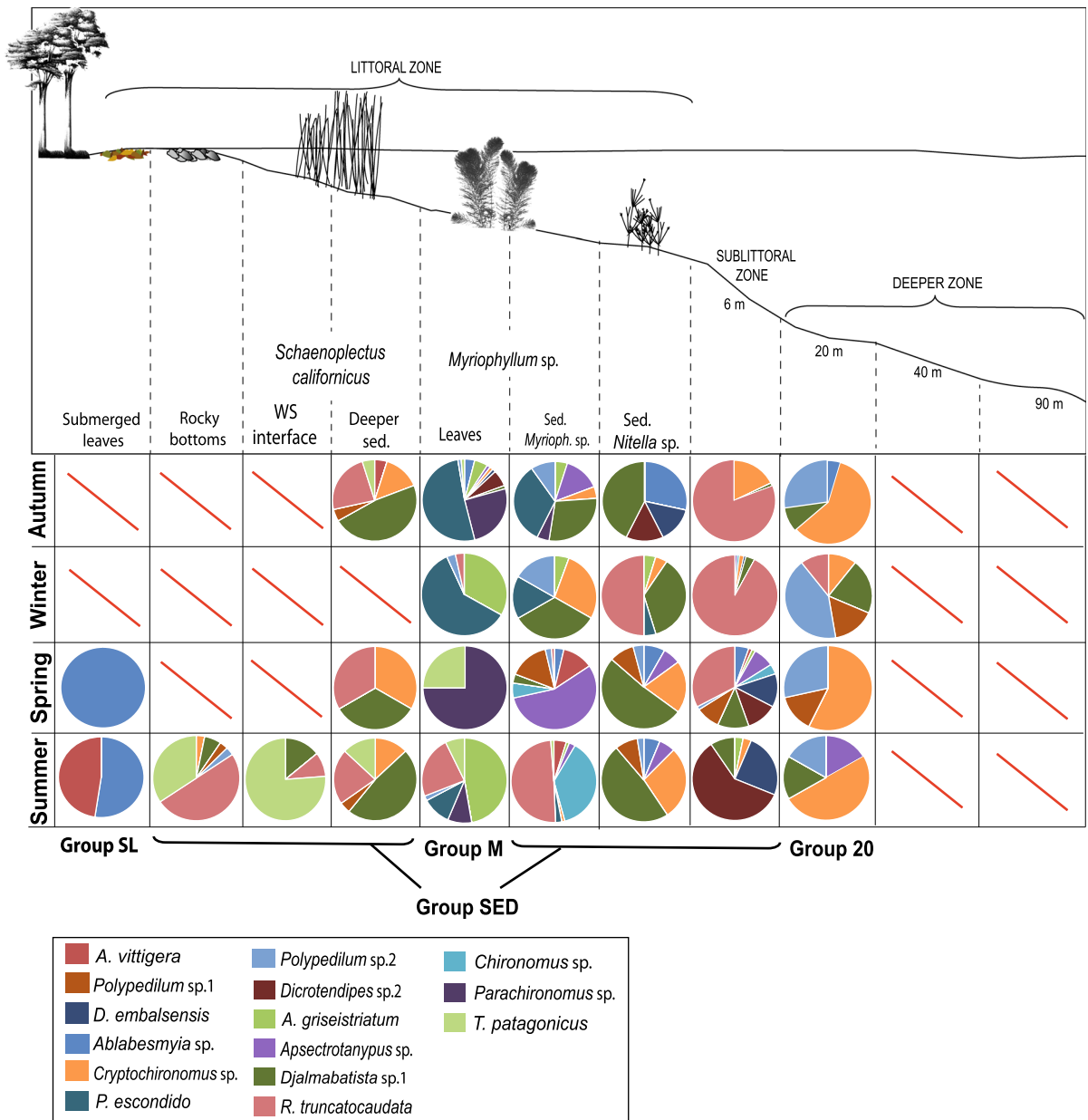


Fig. 6 Relative abundance (%) of chironomid taxa recorded seasonally in the different substrates from Lake Moreno Oeste. Main chironomid groups (M, SL, 20, and SED) are also indicated

Alotanytus vittigera (47.6%). In spring, *Ablabesmyia* sp. (50%) and *Djalmabatista* sp.1 (50%) were the dominant taxa (Fig. 6).

Influence of environmental variables on the chironomid larvae assemblages

The relationship between environmental factors and the most abundant chironomid taxa were determined by examining the two axes of the CCA (Fig. 7). The first axis describes 37% of the relationship between

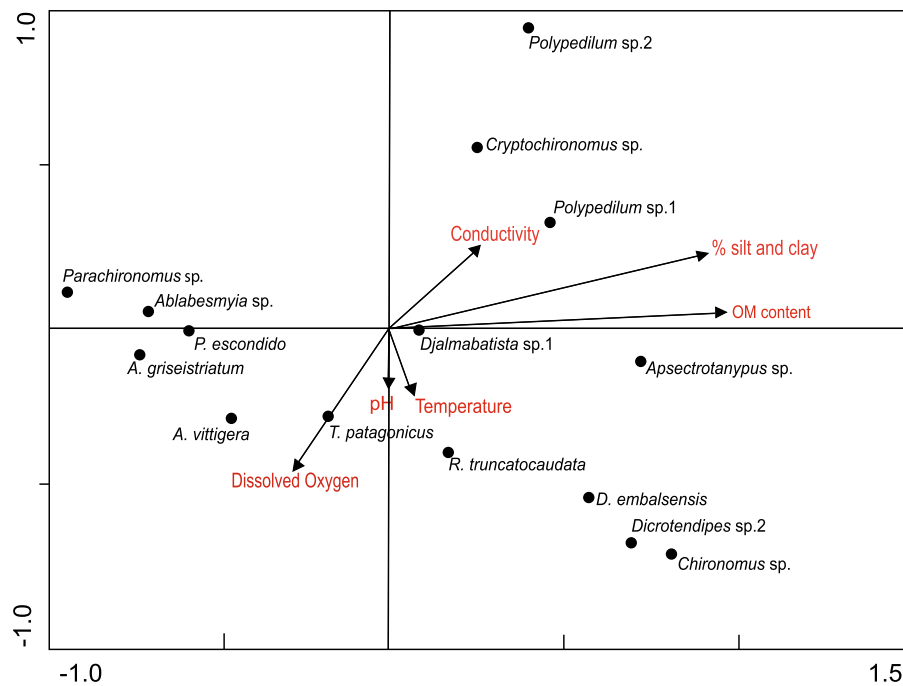


Fig. 7 Relationship between environmental factors (temperature, pH, dissolved oxygen, conductivity, SC granulometric fraction, and organic matter (OM) content) and the main chironomid taxa according to CCA analysis

species and these environmental factors, and the second axis describes 27.7% of said relationship. Looking at the first axis, *Apsectrotanypus* sp. is positively related with OM content and silt and clay granulometric fraction. *P. escondido*, *Parachironomus* sp., *A. griseistriatum*, and *Ablabesmyia* sp., from *Myriophyllum* sp. and submerged leaves substrates are located in the negative area of this first axis. The second axis shows that *Cryptochironomus* sp., *Polypedilum* sp.2 and *Polypedilum* sp.1 are positively related with conductivity and silt and clay granulometric fraction, and negatively related with DO. Conversely, *T. patagonicus* and *R. truncatocaudata* are positively related to pH, temperature and DO (Fig. 7).

Discussion

General description of the actual chironomid community from Lake Moreno Oeste

In Lake MO, chironomid community vary from the shore toward the lake center, with biological substrates

(submerged riparian leaves and *Myriophyllum* sp.) presenting distinctive taxa composition compared with the sediment substrates. In turn, within sedimentary substrates, the most different taxa composition was observed in the deepest sediment where chironomid larvae were recorded (20 m deep) (20 m deep) (Figs. 2, 5, 6). In the deepest section of the lake, the only substrate available for benthic invertebrates consists of soft organic mud. In shallower sections of the lake, particle size of the sediments becomes coarser and habitats are supplemented by macrophytes, leaf litter, and dead wood. Many studies have identified the nature of the substratum as an important factor influencing patterns of chironomid distribution and community structure but, although specific preferences are usually apparent, many species are capable of utilizing a variety of substrata (Pinder, 1986; Armitage et al., 1995; de Mendoza & Catalan, 2010). In this sense, in Lake MO we observed that some chironomid taxa are preferably associated with the macrophyte *Myriophyllum* sp., submerged riparian leaves or deep sediment, and others indistinctly inhabited several substrates from the littoral and sublittoral zones. Representative taxa, their principal

seasonal period and substrate type, and feeding and living habits are summarized in Table 5.

A more diverse and species-rich chironomid community was observed in the littoral substrates from Lake MO in comparison with deeper zones (Table 3; Fig. 6). The littoral zone of lakes is usually characterized by a higher spatial heterogeneity, owing to the more diverse vegetation and sediment composition, which provides a variety of microhabitats, food availability, breeding areas and shelters against predators, increasing the coexistence of a greater number of species in the same area (Begon et al., 1999; Tolonen et al., 2001; Stoffels et al., 2005). Moreover, changes in granulometric composition, related to the annual pattern of precipitation, were seasonally observed in littoral-sublittoral sediment. These runoff cycles produce a higher percentage of coarse particles during humid seasons (autumn and winter) in these zones. All these factors added to physically complex substrate types (wood, leaves, gravel or cobbles, macrophytes, mosses) provide the littoral areas the ability to support a more diverse zoobenthic community than structurally simple substrates (sand or bedrock) of deeper zones (Miserendino, 2001; Lencioni & Rossaro, 2005).

Spatiotemporal structure of chironomid larvae assemblages

Myriophyllum sp. in Lake MO favored the colonization and establishment of certain chironomid taxa (as was observed in the NMDS and clustering analyzes) with determinate characteristics (Group M) (Figs. 5, 6). This community is dominated by different species of collector or scraping habits (feeding both on diatoms and detritus) according the sampling season, such as *P. escondido* (autumn and winter), *Parachironomus* sp. (spring), and *A. griseistriatum* (summer) (Fig. 6). In freshwater ecosystems, macrophytes are known to affect the composition and distribution patterns of aquatic communities because they increase the available substrate area, providing higher habitat complexity, shelter, and breeding area (Ali et al., 2007). Moreover, macrophytes can enhance sedimentation, contribute directly to the particulate organic pool and the development of diatom communities used by invertebrate populations as a food source, or contribute through leaching of dissolved OM (Beaty et al., 2006; Tarkowska-Kukuryk, 2013; Reuss et al.,

2014). Therefore, submerged vegetation may support a dissimilar and relatively small number of chironomid species compared to the surrounding sediments (Eggermont et al., 2008).

Taxa associated with *Myriophyllum* sp. in Lake MO are known to live in association with aquatic plants, feeding on epiphytic diatoms and filamentous algae, or detritus and microorganisms associated with vegetation (Tarkowska-Kukuryk, 2014). For instance, *P. escondido* larvae, as most Orthocladiinae, are adapted to low temperatures and have collector-scraper habits (Lamberti & Moore, 1984; Coffman & Ferrington, 1996) (Table 5). This specie was also recorded in the macrophytes area from a nearby lake, Lake Escondido (Añón Suárez, 1997). On the other hand, *Apedilum* sp. is a taxon associated with the littoral zone of great lakes and has been previously reported in the Patagonia lakes Nahuel Huapi, Gutierrez, Espejo and Futalaufken (Donato et al., 2015). It should be noted that although relative abundance of *Cricotopus* sp. did not exceed 2% during the sampling period, it was only found on sediment from *Myriophyllum* sp. during spring. This evidences its utility as an indicator of vegetated littoral zones in agreement with the information previously reported for this genus (Cattaneo, 1983; Pinder, 1986; Árvá et al., 2015).

Another substrate type containing a distinctive chironomid community in Lake MO was the submerged riparian leaves (Group SL), where larvae were only observed during spring and summer (Figs. 5, 6). The taxa found in this substrate belonged to the Tanypodinae subfamily, *Ablabesmyia* sp. being the most important, followed by *Alotanypus vittigera* in summer (Fig. 6). *Ablabesmyia* sp. was previously recorded in Lake Escondido and, as in Lake MO, it was highly abundant in the littoral zone (Añón Suárez, 1997). This genus can feed on detritus or prey on other invertebrates (Chironomidae larvae and microcrustaceans) and has low diet selectivity, being considered a generalist feeder (Tambelinni et al., 2013). Larvae of Tanypodinae are better adapted to warmer environments and present larval-stage specific changes in their feeding habits (Ashe et al., 1987; Berg, 1995; Coffman & Ferrington, 1996). For example, larvae belonging to early stages (I and II) have collector habits, while latter stages (III and IV) are mainly predators, feeding mainly on cladocerans, copepods, and other chironomids (Añón Suárez, 1997). Based on the small sizes observed in chironomid larvae

collected from submerged leaves in Lake MO (personal observation), we conclude that they belonged to early collector stages of Tanypodinae.

On the basis that most of the chironomid larvae in submerged riparian leaves present collector habits, a possible factor influencing the invertebrate community structure is the availability of food in the form of leaf litter, and its associated microflora. Moreover, riparian leaves and fine roots can act as food traps, contributing to soil organic matter accumulation (Xia et al., 2018). Therefore, during the warmer months in Lake MO, a combination of higher temperatures along with greater food availability (Table 1; Fig. 3) could be factors that improve the establishment and development of early collector larval stages of Tanypodinae in submerged riparian leaves from Lake MO. In the case of predators, the food source would be organisms feeding on leaf litter (Lancaster & Hildrew, 1993). In this way, the presence of plant matter provides chironomids with substrate and specific environmental conditions, while their decomposing parts offer direct (live tissues or detritus) and indirect (bacteria and fungi growing on detritus, for example) food sources (Tokeshi & Pinder, 1985; Papas, 2007; Árvá et al., 2015).

Among chironomid larvae assemblages observed in sedimentary substrates, those groups at the deepest part of the lake inhabited by these organisms (20 m deep) were the most distinctive (Group 20) (Figs. 5, 6). The main environmental factors that influenced these chironomid assemblages are associated with depth, such as organic matter content, sediment granulometric composition, and dissolved oxygen (Fig. 7). Thereby, chironomid community in Lake MO at 20 m depth is characterized by some dominant taxa, such as *Polypedilum* sp.2 and *Cryptochironomus* sp. Because these taxa are mainly considered deep associated taxa, they are positively related with finer sediment (> % silt and clay) and negatively related with O₂ concentration (Fig. 7). While there were no observed significant seasonal changes in the chironomid taxa composition from deeper zones, a notable increase in *Apsectrotanypus* sp. was detected during summer (Fig. 6). Although in Patagonia *Polypedilum* sp. and *P. quinquesetosus* (Edwards, 1931) are mainly associated with warm weather (Donato et al., 2008; Massaferró & Larocque-Tobler, 2013), in Tasmania *P. vespertinus* Skuse and *P. watsoni* Freeman are restricted to cooler lakes (Rees et al., 2008), which

suggest that particular requirements of temperature of *Polypedilum* are species dependent. In Lake MO two species of *Polypedilum* were identified: *Polypedilum* sp.1, associated with littoral habitats and relatively higher temperatures; and *Polypedilum* sp.2, associated with cooler and deeper zones. Moreover, previous works concluded that *Polypedilum* genus includes species tolerant to a wide array of environmental conditions, including temperature (Sanseverino et al., 1998; Tambellini et al., 2013). *Cryptochironomus* is a genus known to be better adapted to sediment composed by fine sand (Higuti & Takeda, 2002). In Lake Bosten (China), it was observed a higher abundance of *Cryptochironomus* sp. in deeper zones, for which it was named as a deep taxon. In Lake MO, *Cryptochironomus* sp. was dominant at 20 m depth, where sediment recorded a higher fraction associated corresponding to fine-grained fraction to silt and clay (< 63 µm) compared with sediment from littoral and sublittoral zones (Figs. 2, 5).

The complete absence of any chironomids at depths greater than 40 m was also observed by other authors (Eggermont et al., 2008). The low larval density in deep sediments is associated with the strong influence of depth on the distribution of chironomid larvae (Lindegaard, 1995; Zhang et al., 2011). Water depth has a mostly indirect influence on chironomid distribution patterns as it is associated with several other environmental factors, such as substrate composition, food availability, hypolimnetic oxygen, wind induced currents, distribution of aquatic macrophytes, and water temperature (Engels et al., 2012). These factors would prevent the establishment and development of larvae (Zhang et al., 2011, 2013; Chen et al., 2014). Temperature itself directly affects the rate of chironomid egg and larval development, being the most important factor in ultraoligotrophic lakes from Patagonia (Massaferró et al., 2014). In this context, it is common to find depth as a significant factor in chironomid training sets, observing a decrease in its abundance as water gets deeper (Jamez et al., 1998; Rieradevall et al., 1999); hence, greater sampling effort in Lake MO could reveal depth associated taxa that are valuable as indicators of deep-water environments.

Finally, in the rest of sedimentary substrates from sublittoral and littoral zones (Group SED) (Figs. 5, 6), some taxa exhibit a considerable flexibility in their mode of feeding habits according to the particular

substrate and different seasons. In this sense, *R. truncatocaudata* and *Djalmabatista* sp.1 were found in several microhabitats from littoral (vegetated and rocky bottoms) and sublittoral zones from Lake MO along the entire sampling year. *Riethia* sp. is a genus endemic of the southern Hemisphere (Wiederholm, 1983) that is known to occur in habitats dominated by fine particle organic matter in both lotic and lentic systems. On the other hand, *Djalmabatista* is a genus mostly associated with sites where the predominant fraction of the sediment is sand (Pressinate et al., 2016). Similar observations were made by Trivinho-Strixino & Strixino (1991), Santos & Henry (2001) and Da Silva et al. (2007). Our results agree with these studies, since sediment collected from littoral and sublittoral zones recorded a greater fraction associated with fine (0.063–0.5 mm) and coarse (0.5–2 mm) sand (Fig. 3).

We failed to detect seasonality in the community structure within the group SED, although a significant seasonal structure in the littoral and sublittoral zones is evident when the sediments are considered separately (Figs. 5, 6). Shallow areas are more susceptible to seasonal changes in environmental factors, such as temperature, conductivity, and DO, than deeper zones (Table 1). These changes, in turn, have a great influence on Chironomidae assemblages through changes in adult emergence (Armitage et al., 1995; Tokeshi, 1995) and habitat conditions, e.g., higher temperature levels can enhance macrophytes production (higher DO values) (Hering et al., 2010). This close relationship between chironomid taxa composition and seasonal changes might explain the results we obtained. Tarrats et al. (2017) proposed this explanation in their study of Lake Enol (Spain), where the temporal changes of littoral chironomid assemblages were mainly driven by seasonal variability. Seasonal changes in temperate regions often have offsetting magnitudes when compared to site dependent variations within a particular lake, which makes it challenging to explore the latter without compensating for the former. A possible solution to this problem would have been to concentrate sampling effort on a particular season so as to eliminate seasonal variance. This could very well be the focus of subsequent research.

We should consider that although we report these results over a year of sampling, different species assemblages may be found at the same site and season in successive years, as response to different climatic

conditions. Moreover, the interaction of several environmental factors makes it difficult to disentangle the effects of single variables on the fauna and to extract those most important for the biota. Habitat selection by chironomids depends on the interaction of numerous abiotic (e.g., water temperature, substrate stability) and biotic (e.g., presence and biomass of macrophytes, predation and competition) factors, which further complicates interpretations (De Haas et al., 2006).

Implications for paleolimnological interpretations

Chironomid taxa observed in this study were compared with paleolimnological records from this region to analyze past interpretations by contrasting with their actual spatiotemporal distribution. Table 5 summarizes the principal paleolimnological inferences conducted in Patagonia based on the most abundant living taxa observed in this lake. Several living taxa observed in Lake MO have also been recorded in previous paleoecological studies from the northern Patagonian Andes (Massaferro et al., 2005, 2017; Montes de Oca et al., 2017; Williams et al., 2019). For instance, of the twenty-nine chironomid taxa identified in a sedimentary sequence of Lake MO comprising the last 700 years (Williams et al., 2016), only twenty-three were observed in this study. Two rare chironomid taxa (*Parakiefferella* sp., *Labrundinia* sp.) and some morphotypes of Tanytarsini were only identified in the subfossil assemblages. On the other hand, by studying the lake's living midge fauna, the chance always exists to encounter additional morphotypes not represented in the subfossil assemblages, hence, improving our knowledge on Lake MO biodiversity. For example, some of the main living taxa (i.e., *Parachironomus* spp) were not abundant in paleolimnological studies. Moreover, this study allowed us to recognize different species within *Polypedilum* and *Dicrotendipes*. Key diagnostic features on the head capsule are generally better preserved on live specimens than on subfossil specimens. They can, therefore, be used to improve larval descriptions and to resolve issues of taxonomic uncertainty typical of subfossil studies. We now have the antennae (which is a key diagnostic head part but has the lowest preservation potential), body parts, or labral elements of all recovered taxa, elucidating some taxonomic relationships.

Several actual taxa recorded in biological substrates (*Myriophyllum* sp. and submerged leaves) were also observed along sequences from Lake MO and Lake Morenito. For example, since AD 1960, it was observed a notable increase in the abundance of *Parapsectrocladius*, *Apedilum* and *Ablabesmyia* in both lakes. These increases were associated with a dam's construction in 1960 that separate Lake Morenito from Lake MO, producing a greater vegetation development due to the expansion of the littoral zone (Massaferro et al., 2005; Williams et al., 2016). Therefore, the association of mentioned taxa with abundant macrophyte vegetation and riparian zones observed in the present study supports the findings of the paleoecological studies of lakes MO and Morenito, considering these three taxa as strong candidates for direct inference of vegetated and riparian zones in the lake.

Another taxon commonly observed in paleolimnological researches from Patagonia is *Cricotopus* (Araneda et al., 2007, 2013; Urrutia et al., 2010; Massaferro & Larocque-Tobler, 2013; Williams et al., 2016, 2019). For instance, in Lake Tonček, a high-altitude lake, *Cricotopus* was the second most abundant taxa along the last millennium, and its peaks of abundance were associated with a greater development of aquatic vegetation (Williams et al., 2019), since this taxon is well adapted to mine or graze on the surfaces (Wiederholm, 1983). In turn, Urrutia et al. (2010) suggest that increases in *Cricotopus*, combined with the chemical parameters of the sediment (e.g., organic matter, carbonates, and total phosphorous all increasing toward the end of the core), suggest that Lake Laja has been more productive during the last ~ 450 years, and particularly over the last ~ 80 years. Likewise, Massaferro & Larocque-Tobler (2013) observed that this genus has a bimodal distribution based on their optimal temperatures, with two abundance peaks between 3–5 and 6–7°C. Given the differences in temperatures of the abundance peaks of this taxa between our study (warm spring temperatures) and that of Massaferro & Larocque-Tobler (2013), its probable that both *Cricotopus* observed in Lake Tonček and MO are different species within genus *Cricotopus*, which have not yet been taxonomically differentiated.

Other taxa have been extensively used in paleolimnological studies as indicators of climate change or temperature regime shifts in Patagonia. In Lake MO,

the major change in chironomid assemblages occurred at ~ AD 1760, which was characterized by a decrease in “cold taxa” (*Polypedilum* sp.2 and *Dicrotendipes*), and an increase in “warm taxa” (*Apsectrotanypus* and *Polypedilum* sp.1) (Williams et al., 2016). This replacement was associated with climatic conditions concurrent with the end of a cold period at ~ AD 1500–1700 and the beginning of drying climate at ~ AD 1740–1900 in northern Patagonia. In living chironomid assemblages from Lake MO, we could differentiate two *Polypedilum* species: *Polypedilum* sp.1 associated with higher temperatures and littoral habitats; and *Polypedilum* sp.2 related with lower temperatures and deeper distribution. The recognition of different species and contrasting ecological requirements within a particular genus made it possible to establish patterns in the subfossil chironomid assemblages and to associate the replacement of taxa with characteristic climatic events. In the present study, not only *Polypedilum* sp.2 was mostly associated with deeper zones and lower temperatures, but *Cryptochironomus* was also dominant at 20 m depth. This fact suggests a strong relationship between both taxa and environmental conditions at deeper zones, including a preference for lower temperatures and finer sediment (with high proportion of silt and clay) poor in plant debris. Regarding *Dicrotendipes*, it is difficult to distinguish between different species within the subfossil chironomid community, since in the present community, several species belonging to this genus were recorded (*D. embalsensis* and *Dicrotendipes* sp.2). Therefore, additional studies are still necessary for a more specific use of *Dicrotendipes* as a paleoindicator of environmental conditions, particularly as indicator of climate change or temperature regime shifts in Patagonia.

In the sequence from Lake MO, the dryer climate period (~ AD 1740–1900) was associated with a trend toward a warmer community indicated by an increase in the relative abundances of *Apsectrotanypus*, *Polypedilum* sp.1, and *Parapsectrocladius escondido*. In the present study, these taxa are associated with macrophytes and vegetated littoral zones. Therefore, the evidence provided here emphasizes the utility of these taxa as palaeoindicators of littoral environments. Other less common taxa (< 0.5%), like *Chironomus*, *Tanytarsini C* and *Tanytarsini 1D*, were also associated with higher temperatures (Massaferro

et al., 2005; Massafiero & Larocque-Tobler, 2013) or warmer seasons in Lake MO (Williams et al., 2016).

Although the taxonomic composition of the living and subfossil chironomid assemblages from Lake MO was similar, a number of differences existed between them. Therefore, it is desirable to use both living and subfossil chironomid fauna of a lake for inferring past environmental and limnological conditions. In Lake MO, living chironomid assemblages present a wide and diverse spatial and temporal distribution, which could be related to different habitat requirements, changes in the emergence patterns, and other ecological traits (dispersion, predation, competence, etc.). The knowledge of the present exemplifies how the understanding of the present spatial and temporal heterogeneity of chironomids can contribute to the interpretation of paleolimnological studies based on these insects.

Conclusion

The results obtained in this study are a contribution to understand the spatiotemporal dynamics of chironomid assemblages in Lake Moreno Oeste, constituting a step forward to improve the interpretation of the past changes of the lake by means of the subfossil chironomid community. As we have shown, chironomid community of Lake MO is in part structured according to the substrate type (biological or sedimentary) and environmental factors associated with depth, such as organic matter content, sediment granulometric composition, and dissolved oxygen. Moreover, we found that chironomid assemblages in substrates from sublittoral and littoral zones are more affected by seasonal environmental variability than in deeper areas where this community is less modified by seasonality. Therefore, these data about the autoecology of recent chironomid larvae provide information to improve explanations of past changes recorded as subfossil remains and biomonitoring studies from Patagonian lakes.

We should consider that it is possible that the yearly pattern we describe might be affected by other climatic factors of larger time scale that might change the chironomid community structure. Moreover, the synergistic nature of environmental factors makes it difficult to assess the effects of single variables on the

fauna, and to distinguish those most important for the biota.

The ecological information on the living chironomids obtained in this study is expected to be valuable in the future understanding of aquatic lake ecosystems in general, and as a stepping stone for the use of contemporary ecological lake studies in the evaluation of paleolimnological records based on analyses of assemblage structure of subfossil chironomid head capsules. Moreover, besides relating ecological data to specific chironomid larval morphotypes, examination of the living assemblages has other significant benefits for palaeoecologically oriented investigations. Key diagnostic features on the head capsule, being better preserved on live specimens than on subfossil specimens, can be also used to improve larval descriptions and to resolve issues of taxonomic uncertainty typical of subfossil studies.

References

- Ali, M. M., A. A. Mageed & M. Heikal, 2007. Importance of aquatic macrophyte for invertebrate diversity in large subtropical reservoir. *Limnologia* 37: 155–169.
- Añón Suárez, D., 1991. Distribución del bentos del lago Escondido (Río Negro, Argentina) con especial énfasis en los quironómidos (Diptera: Chironomidae). *Studies on Neotropical Fauna and Environment* 26: 149–157.
- Añón Suárez, D., 1997. Estructura y dinámica de la taxocenosis Chironomidae (Diptera, Nematocera) de un lago Andino. Tesis doctoral, Universidad Nacional de La Plata, pp. 181.
- Añón Suárez, D., 2002. Life history and secondary production of *Ablabesmyia reissi* (Diptera: Chironomidae) from Lake Escondido, Bariloche, Argentina. *Journal of the North American Benthological Society* 21: 414–429.
- Araneda, A., F. Cruces, L. Torres, S. Bertrand, N. Fagel, H. C. Treutler, L. Chirinos, R. Barra & R. Urrutia, 2007. Changes of sub-fossil chironomids assemblages associated with volcanic deposition in an Andean lake (38°S), Chile. *Revista Chilena de Historia Natural* 80: 141–156.
- Araneda, A., P. Jana, C. Ortega, F. Torrejón, S. Bertrand, P. Vargas, N. Fagel, D. Alvarez, A. Stehr & R. Urrutia, 2013. Changes in sub-fossil chironomid assemblages in two Northern Patagonian lake systems associated with the occurrence of historical fires. *Journal of Limnology* 50: 41–56.
- Arcagni, M., L. M. Campbell, M. A. Arribére, K. Kyser, K. Klassene, R. Casaux, M. L. Miserendino & S. Ribeiro Guevara, 2013. Food web structure in a double-basin ultra-oligotrophic lake in Northwest Patagonia, Argentina, using carbon and nitrogen stable isotopes. *Limnologia* 43: 131–142.

- Armitage, P. D., P. S. Cranston & L. C. V. Pinder, 1995. The Chironomidae: Biology and Ecology of Non-Biting Midges. Chapman & Hall, London: 572.
- Árva, D., M. Tóth, H. Horváth, S. A. Nagy & A. Specziár, 2015. The relative importance of spatial and environmental processes in distribution of benthic chironomid larvae within a large and shallow lake. *Hydrobiologia* 742: 249–266.
- Árva, D., M. Tóth, A. Mozsár & A. Specziár, 2017. The roles of environment, site position, and seasonality in taxonomic and functional organization of chironomid assemblages in a heterogeneous wetland, Kis-Balaton (Hungary). *Hydrobiologia* 787: 353–373.
- Ashie, P., D. A. Murray & F. Reiss, 1987. The zoogeographical distribution of Chironomidae (Insecta: Diptera). *Annales de Limnologie* 23: 27–60.
- Barriga, J. P., M. A. Battini, M. García-Asorey, C. Carrea, P. J. Macchi & V. E. Cussac, 2012. Intraspecific variation in diet, growth, and morphology of landlocked *Galaxias maculatus* during its larval period: the role of food availability and predation risk. *Hydrobiologia* 679: 27–41.
- Beaty, S. R., K. Fortino & A. E. Hershey, 2006. Distribution and growth of benthic macroinvertebrates among different patch types of the littoral zones of two arctic lakes. *Freshwater Biology* 51: 2347–2361.
- Begon, M., Harper, J.L., Townsend, C.R., 1999. *Ecología: individuos, poblaciones y comunidades*. 3^o edición, Ediciones Omega, pp. 1148.
- Berg, M. B., 1995. Larval food and feeding behavior. In Armitage, P., P. S. Cranston & L. V. Pinder (eds), *The Chironomidae: the biology and ecology of non-biting midges*. Chapman and Hall, London: 136–168.
- Brown, B. L., 2007. Habitat heterogeneity and disturbance influence patterns of community temporal variability in a small temperate stream. *Hydrobiologia* 586: 93–106.
- Buria, L., S. J. Walde, M. Battini, P. J. Macchi, M. Alonso, D. E. Ruzzante & V. E. Cussac, 2007. Movement of a South American perch *Percichthys trucha* in a mountain Patagonian lake during spawning and prespawning periods. *Journal of Fish Biology* 70: 215–230.
- Cattaneo, A., 1983. Grazing on epiphytes. *Limnology and Oceanography* 28: 124–132.
- Chen, J., E. Zhang, S. J. Brooks, X. Huang, H. Wang, J. Liu & F. Chen, 2014. Relationships between chironomids and water depth in Bosten Lake, Xinjiang, northwest China. *Journal of Paleolimnology* 51: 313–323.
- Clarke, K. R. & R. N. Gorley, 2001. Primer v5: user manual/tutorial. Primer-E, Plymouth Marine Laboratory.
- Clarke, K. R. & R. M. Warwick, 2001. A further biodiversity index applicable to species lists: variation in taxonomic distinctness. *Marine Ecology Progress Series* 216: 265–278.
- Coffman, W.B., Ferrington, L.C., 1996. Chironomidae. In: Merritt, W., Cummings, K.W. (eds) *An introduction to the Aquatic Insects of North America*. Kendall/Hunt Dubuque, Iowa: 2nd ed., 551–643.
- Cranston, P.S., 2000 with updates. Electronic guide to the Chironomidae of Australian.
- Da Silva, F. L., D. C. Moreir, S. S. Ruiz & G. L. Bochini, 2007. Avaliação da importância da unidade de conservação na preservação da diversidade de Chironomidae (Insecta: Diptera) no córrego Vargem Limpa, Bauru, Estado de São Paulo. *Acta Scientiarum Biological Sciences* 29: 401–405.
- de Mendoza, G. & J. Catalan, 2010. Lake macroinvertebrates and the altitudinal environmental gradient in the Pyrenees. *Hydrobiologia* 648: 51–72.
- De Haas, E. M., C. Wagner, A. A. Koelmans, M. H. S. Kraak & W. Admiraal, 2006. Habitat selection by chironomid larvae: fast growth requires fast food. *Journal of Animal Ecology* 75: 148–155.
- Díaz, M., A. Pedrozo, C. Reynolds & P. Temporetti, 2007. Chemical composition and the nitrogen-regulated trophic state of Patagonian lakes. *Limnologia* 37: 17–27.
- Donato, M., J. Massaferrero & S. J. Brooks, 2008. Chironomid (Chironomidae: Diptera) checklist from Nahuel Huapi National Park, Patagonia, Argentina. *Revista de la Sociedad Entomologica Argentina* 67: 163–170.
- Donato, M., A. Siri, J. Massaferrero & S. J. Brooks, 2015. *Apedilum griseistriatum* comb. Nov., placement of *Chironomus* (Polypedilum) *griseistriatum* (Diptera, Chironomidae). *Iheringia, Série Zoologia* 105: 5–11.
- Eggermont, H., D. Kennedy, S. T. Hasiotis, D. Verschuren & A. Cohen, 2008. Distribution of living larval Chironomidae (Insecta: Diptera) along a depth transect at Kigoma Bay, Lake Tanganyika: implications for palaeoenvironmental reconstruction. *African Entomology* 16: 162–184.
- Engels, S., L. C. Cwynar, A. B. H. Rees & B. N. Shuman, 2012. Chironomid-based water depth reconstructions: an independent evaluation of site-specific and local inference models. *Journal of Paleolimnology* 48: 693–709.
- Epler, J.H., 2001. Identification manual for the larval Chironomidae (Diptera) of North and South Carolina. EPA Region 4 and Human Health and Ecological Division. North Carolina Department of Environment and Natural Resources. Division of Water Quality, pp. 516.
- García, P. E. & D. A. Añón Suárez, 2007. Community structure and phenology of chironomids (Insecta: Chironomidae) in a Patagonian Andean stream. *Limnologia* 37: 109–117.
- Heiri, O., A. F. Lotter & G. Lemcke, 2001. Loss on ignition as a method for estimating organic and carbonate content in sediments: reproducibility and comparability of results. *Journal of Paleolimnology* 25: 101–110.
- Hering, D., A. Haidekker, A. Schmidt-Kloiber, T. Barker, L. Buisson, W. Graf, G. Grenouillet, A. Lorenz, L. Sandin & S. Stendera, 2010. Monitoring the responses of freshwater ecosystems to climate change. In Kernan, M., R. W. Battarbee & B. Moss (eds), *Climate change impacts on freshwater ecosystems*. Wiley-Blackwell Publishing, New York: 84–118.
- Higuti, J. & A. M. Takeda, 2002. Spatial and temporal variation in densities of Chironomid larvae (diptera) in two lagoons and two tributaries of the upper Paraná River Floodplain, Brazil. *Brazilian Journal of Biology* 62: 807–818.
- Jamez, M. R., M. Weatherhead, C. Stanger & E. Graynoth, 1998. Macroinvertebrate distribution in the littoral zone of Lake Coleridge, South Island, New Zealand—effects of habitat stability, wind exposure, and macrophytes. *New Zealand Journal of Marine and Freshwater Research* 32: 287–305.
- Kaisin, F.J., 1989. Dinámica, producción y balance energético del zoobentos en un embalse norpatagónico. Tesis Doctoral, Facultad de Ciencias Exactas y naturales. Universidad de Bs As.

- Krumbein, W. C. & F. J. Pettijohn, 1938. Manual of Sedimentary Petrology. Appelton-Century-crofts Inc, New York: 549.
- Lamberti, G. A. & J. W. Moore, 1984. Aquatic insects as primary consumers. In Resh, V. H. & D. M. Rosenberg (eds), The Ecology of Aquatic Insects. Praeger, New York: 164–165.
- Lancaster, J. & A. G. Hildrew, 1993. Flow refugia and the microdistribution of lotic macroinvertebrates. Journal of the North American Benthological Society 12: 385–393.
- Legendre, P. & L. Legendre, 1998. Numerical Ecology, 2nd ed. Elsevier, Amsterdam.
- Lencioni, V. & B. Rossaro, 2005. Microdistribution of chironomids (Diptera: Chironomidae) in Alpine streams: an autoecological perspective. Hydrobiologia 533: 61–76.
- Lindgaard, C., 1995. Classification of water-bodies and pollution. In Armitage, P. D., P. S. Cranston & L. C. V. Pinder (eds), The Chironomidae: Biology and Ecology of Nonbiting Midges. Chapman and Hall, London: 384–404.
- Lotter, A. F., H. J. B. Birks, W. Hofmann & A. Marchetto, 1997. Modern diatom, cladocera, chironomid, and chrysophyte cyst assemblages as quantitative indicators for the reconstruction of past environmental conditions in the Alps. I. Climate. Journal of Paleolimnology 18: 395–420.
- Mauad, M., A. Siri & M. Donato, 2016. Does type of substratum affect Chironomid larvae assemblage composition? A study in a river catchment in Northern Patagonia, Argentina. Neotropical Entomology 46: 18–28.
- Massaferro, J., 2009. Paleocología: el uso de los quironómidos fósiles (Diptera: Chironomidae) en reconstrucciones paleoambientales durante el Cuaternario en la Patagonia. Revista de la Sociedad Entomológica Argentina 68: 209–217.
- Massaferro, J. & S. Brooks, 2002. Response of chironomids to late quaternary environmental change in the Taitao Peninsula, southern Chile. Journal of Quaternary Science 17: 101–111.
- Massaferro, J. & I. Larocque-Tobler, 2013. Using a newly developed chironomid transfer function for reconstructing mean annual air temperature at Lake Potrok Aike, Patagonia, Argentina. Ecological Indicators 24: 201–210.
- Massaferro, J., S. Ribeiro Guevara, A. Rizzo & M. A. Arribére, 2005. Short-term environmental changes in Lake Morenito (41°S, 71°W, Patagonia, Argentina) from the analysis of sub-fossil chironomids. Aquatic Conservation: Marine and Freshwater Ecosystems 15: 23–30.
- Massaferro, J., C. Ortega, R. Fuentes & A. Araneda, 2013. Guía para la identificación de Tanytarsini subfósiles (Diptera: Chironomidae) de la Patagonia. Ameghiniana 50: 319–334.
- Massaferro, J., I. Larocque-Tobler, S. J. Brooks, M. Vanderdoes, A. Dieffenbacher-Krall & P. Moreno, 2014. Quantifying climate change in Huelmo mire (Chile, Northwestern Patagonia) during the Last Glacial Termination using a newly developed chironomid based temperature model. Palaeogeography, Palaeoclimatology, Palaeoecology 399: 214–224.
- Massaferro, J., A. Correa-Metrio, F. Montes de Oca & M. Mauad, 2017. Contrasting responses of lake ecosystems to environmental disturbance: a paleoecological perspective from northern Patagonia (Argentina). Hydrobiologia 816: 79–89.
- Miserendino, M. L., 2001. Macroinvertebrate assemblages in Andean Patagonian rivers and streams. Hydrobiologia 444: 147–158.
- Miserendino, M. L. & L. A. Pizzolon, 2003. Distribution of macroinvertebrate assemblages in the Azul-Quemquem-treu river basin, Patagonia, Argentina. New Zealand Journal of Marine and Freshwater Research 37: 525–539.
- Miserendino, M. L., C. Brand & C. Y. Di Prinzio, 2008. Assessing urban impacts on water quality, benthic communities and fish in streams of the Andes Mountains, Patagonia (Argentina). Water Air Soil Pollution 194: 91–110.
- Montes de Oca, F., L. Motta, M. S. Plastani, C. Laprida, A. Lami & J. Massaferro, 2017. Reconstructing recent environmental changes using nonbiting midges (Diptera: Chironomidae) in two high mountain lakes from northern Patagonia, Argentina. Journal of Paleolimnology 59: 175–187.
- Paggi, A. C., 2001. Diptera: Chironomidae. In Fernández, H. R. & E. Domínguez (eds), Guía para la determinación de los artrópodos bentónicos sudamericanos. Facultad de Ciencias Naturales e Instituto M, Lillo: 167–193.
- Paggi, A. C. & A. Rodríguez Capítulo, 2002. Chironomid composition from drift and bottom samples in a regulated north-Patagonian river (Rio Limay, Argentina). Verhandlungen des Internationalen Verein Limnologie 28: 1229–1235.
- Papas, P., 2007. Effect of macrophytes on aquatic invertebrates—a literature review. Technical Report Series No. 158, Arthur Rylah Institute for Environmental Research, Melbourne.
- Pinder, L. C. V., 1986. Biology of freshwater Chironomidae. Annual Review of Entomology 31: 1–23.
- Pressinate, S., G. Perbiche-Neves & A. Michiyo Takeda, 2016. The environmental heterogeneity of sediment determines Chironomidae (Insecta: Diptera) distribution in lotic and lentic habitats in a tropical floodplain. Insect Conservation and Diversity 9: 332–341.
- Puntí, T., M. Rieradevall & N. Prat, 2009. Environmental factors, spatial variation, and specific requirements of Chironomidae in Mediterranean reference streams. Journal of the North American Benthological Society 28: 247–265.
- Queimaliños, C. P., B. E. Modenutti & E. Balseiro, 1999. Symbiotic association of the ciliate *Ophrydium naumanni* with *Chlorella* causing a deep chlorophyll *a* maximum in an oligotrophic South Andes lake. Journal of Plankton Research 21: 167–178.
- Rees, A. B. H., L. C. Cwynar & P. S. Cranston, 2008. Midges (Chironomidae, Ceratopogonidae, Chaoboridae) as a temperature proxy: a training set from Tasmania, Australia. Journal of Paleolimnology 40: 1159–1178.
- Reuss, N. S., L. Hamerlik, G. Velle, A. Michelsen, O. Pedersen & K. P. Brodersen, 2014. Microhabitat influence on chironomid community structure and stable isotope signatures in West Greenland lakes. Hydrobiologia 730: 59–77.
- Ribeiro Guevara, S., A. Rizzo, R. Sánchez & M. A. Arribére, 2005. Heavy metal inputs in Northern Patagonia lakes from short sediment core analysis. Journal of Radioanalytical and Nuclear Chemistry 265: 481–493.
- Rieradevall, M. & N. Prat, 1999. Chironomidae from high mountain lakes in Spain and Portugal. In Hoffrichter, O. (ed.), Late 20th Century Research on Chironomidae: An

- Anthology from the 13th International Symposium on Chironomidae. Shaker Verlag, Aachen.
- Rieradevall, M. & S. J. Brooks, 2001. An identification guide to subfossil Tanypodinae larvae (Insecta: Diptera: Chironomidae) based on cephalic setation. *Journal of Paleolimnology* 25: 81–99.
- Rieradevall, M., N. Bonada & N. Prat, 1999. Substrate and depth preferences of macroinvertebrates along a transect in a Pyrenean high mountain lake (Lake Redó, NE Spain). *Limnetica* 17: 127–134.
- Rosenberg, D. M., 1992. Freshwater biomonitoring and chironomidae. *Netherlands Journal of Aquatic Ecology* 26: 101–122.
- Saether, O. A., 1979. Chironomid communities as water quality indicators. *Holarctic Ecology* 2: 65–74.
- Sanseverino, A. M., J. L. Nessimian & A. L. H. Oliveira, 1998. A fauna de Chironomidae (Diptera) em diferentes biótopos aquáticos na Serra do Subaio (Teresópolis, RJ). *Oecologia Brasilienses- Ecologia de insetos aquáticos* 5: 253–263.
- Santos, C. M. & R. Henry, 2001. Composição, distribuição e abundância de Chironomidae (Diptera, Insecta) na Represa de Jurumirim (Rio Paranapanema-SP). *Acta Limnologica Brasiliensia* 13: 99–115.
- Siri, A., M. Donato, G. Orpella & J. Massafiero, 2011. *Alotanypus vittigera* (Edwards) comb. nov.: adult redescription, immature description and a phylogenetic analysis of the genus (Diptera: Chironomidae: Tanypodinae). *Zootaxa* 2795: 46–64.
- Stoffels, R. J., K. R. Clarke & G. P. Closs, 2005. Spatial scale and benthic community organization in the littoral zones of large oligotrophic lakes: potential for cross-scale interactions. *Freshwater Biology* 50: 1131–1145.
- Tambellini, M., C. T. Callil, I. Fantin-Cruz & P. Girard, 2013. Factors structuring the spatial distribution of Chironomidae larvae community in the floodplain of the Northern Pantanal, Brazil. *Acta Limnologica Brasiliensia* 25: 131–139.
- Tarkowska-Kukuryk, M., 2013. Periphytic algae as food source for grazing chironomids in a shallow phytoplankton dominated lake. *Limnologica* 43: 254–264.
- Tarkowska-Kukuryk, M., 2014. Spatial distribution of epiphytic chironomid larvae in a shallow macrophyte-dominated lake: effect of macrophyte species and food resources. *Limnology* 15: 141–153.
- Tarrats, P., M. Cañedo-Argüelles, M. Rieradevall & N. Prat, 2017. Chironomid communities as indicators of local and global changes in an oligotrophic high mountain lake (Enol Lake, Northwestern Spain). *Journal of Limnology* 76: 355–365.
- Tarrats, P., M. Cañedo-Argüelles, M. Rieradevall & N. Prat, 2018. The influence of depth and macrophyte habitat on paleoecological studies using chironomids: Enol Lake (Spain) as a case study. *Journal of Paleolimnology* 60: 97–107.
- ter Braak, C. & P. Šmilauer, 1998. CANOCO Reference Manual and User's Guide to Canoco for Windows: Software for Canonical Community Ordination (version 4). Micro-computer Power, Ithaca, NY: 352.
- Tickner, D., P. D. Armitage, M. A. Bickerton & K. A. Hall, 2000. Assessing stream quality using information on mesohabitat distribution and character. *Aquatic Conservation: Marine and Freshwater Ecosystems* 10: 170–196.
- Tokeshi, P., 1995. Species interactions and community structure. In Armitage, P. D., P. S. Cranston & L. C. V. Pinder (eds), *The Chironomidae: biology and ecology of non-biting midges*. Chapman & Hall, London: 297–335.
- Tokeshi, M. & L. C. V. Pinder, 1985. Microhabitats of stream invertebrates on two submerged macrophytes with contrasting leaf morphology. *Holarctic Ecology* 8: 313–319.
- Tolonen, K. T., H. Hämäläinen, I. J. Holopainen & J. Karjalainen, 2001. Influences of habitat type and environmental variables on littoral macroinvertebrate communities in a large lake system. *Archiv für Hydrobiologie* 152: 39–67.
- Trivinho-Strixino, S. & G. Strixino, 1991. Duas novas espécies de *Nimbecera* Reiss (Diptera, Chironomidae) do Estado de São Paulo, Brasil. *Revista Brasileira de Entomologia* 35: 173–178.
- Urrutia, R., A. Araneda, L. Torres, F. Cruces, C. Vivero, F. Torrejón, R. Barra, N. Fagel & B. Scharf, 2010. Late Holocene environmental changes inferred from diatom, chironomid, and pollen assemblages in an Andean lake in Central Chile, Lake Laja (36°S). *Hydrobiologia* 648: 207–225.
- van Hardenbroek, M., O. Heiri, M. F. Wilhelm & A. F. Lotter, 2011. How representative are subfossil assemblages of Chironomidae and common benthic invertebrates for the living fauna of Lake De Waay, the Netherlands? *Aquatic Sciences* 73: 247–259.
- Walker, I. R., 2001. Midges: Chironomidae and related Diptera. In Smol, J. P., H. J. B. Birks & W. M. Last (eds), *Tracking Environmental Changes Using Lakes Sediments, Vol. 4., Zoological Indicators* Kluwer Academic Publishers, Dordrecht: 43–66.
- Wiederholm, T. (ed.), 1983. Chironomidae of the Holarctic region. Keys and diagnoses. Part I, Larvae. *Entomologica Scandinavica Supplement* 19: 1–457.
- Williams, N., M. Rieradevall, D. Añón Suárez, A. Rizzo, R. Daga, S. Ribeiro Guevara & M. A. Arribére, 2016. Chironomids as indicators of natural and human impacts in a 700-yr record from the northern Patagonian Andes. *Quaternary research* 86: 120–132.
- Williams, N., D. Añón Suárez, M. Rieradevall, A. Rizzo, R. Daga, M. A. Arribére & S. Ribeiro Guevara, 2019. Response of Chironomidae to environmental disturbances in a high mountain lake in Patagonia during the last millennium. *Quaternary Research*. <https://doi.org/10.1017/qua.2019.5>.
- Xia, M., A. F. Talhelm & K. S. Pregitzer, 2018. Long-term simulated atmospheric nitrogen deposition alters leaf and fine root decomposition. *Ecosystems* 21: 1–14.
- Zhang, E., P. Langdon, H. Tang, R. Jones, X. Yang & J. Shen, 2011. Ecological influences affecting the distribution of larval chironomid communities in the lakes on Yunnan Plateau, SW China. *Fundamental and Applied Limnology* 179: 103–113.
- Zhang, E., Y. Cao, P. Langdon, Q. Wang, J. Shen & X. Yang, 2013. Within-lake variability of subfossil chironomid assemblage in a large, deep subtropical lake (Lugu lake, Southwest China). *Journal of Limnology* 72: 117–126.