Ecological Indicators 125 (2021) 107551

Contents lists available at ScienceDirect

Ecological Indicators

journal homepage: www.elsevier.com/locate/ecolind

Comparison of sampling methods for benthic macroinvertebrates in forested wetlands

Francisco Correa-Araneda [a,*,1], Daniela Núñez [b,1], Ma Elisa Díaz [c], Francisco Gómez-Capponi [a], Ricardo Figueroa [d], Jaquelinne Acuña [e], Luz Boyero [f,g], Carlos Esse [a]

[a] *Unidad de Cambio Climático y Medio Ambiente, Instituto Iberoamericano de Desarrollo Sostenible, Universidad Autónoma de Chile, Temuco, Chile*
[b] *Programa de Doctorado en Ciencias mención en Ecología y Biología Evolutiva, Facultad de Ciencias, Universidad de Chile, Chile*
[c] *Departamento de Ciencias Ambientales, Facultad de Recursos Naturales, Universidad Católica de Temuco, Chile*
[d] *Water Quality Bioindicators Laboratory, Faculty of Environmental Sciences and EULA-Center, University of Concepción, P.O. Box 160-C, Concepción, Chile*
[e] *Laboratorio de Ecología Microbiana Aplicada (EMALAB), Departamento de Ciencias Químicas y Recursos Naturales, Universidad de La Frontera, Temuco, Chile*
[f] *Department of Plant Biology and Ecology, Faculty of Science and Technology, University of the Basque Country (UPV/EHU), Leioa, Spain*
[g] *IKERBASQUE, Bilbao, Spain*

ARTICLE INFO

Keywords:
Corer
Hand net
Litterbags
Mediterranean zones
Permanent water regime
Temporary water regime

ABSTRACT

Forest wetlands are biodiversity hotspots that perform functions of vital ecological importance, but they are among the world's most threatened ecosystems. Due to their high diversity of habitats and species, the study of their benthic macroinvertebrate communities is challenging, and there is no consensus on which sampling methods allow a better representation of these communities. Here we compared the performance of 3 sampling methods (hand net, corer and litterbags) in 2 temporary and 3 permanent forested wetlands in southern Chile, which were bimonthly sampled throughout a year, with 108 samples per wetland. Our results indicated that the greatest abundance and diversity were collected with the hand net, followed by the litterbags and the corer. The composition of communities collected by the hand net and litterbags were more similar between them than that of communities collected by the corer (where Chironomidae and Oligochaeta were common). We suggest that the combined use of the hand net and corer could provide a good representation of macroinvertebrate communities in forest wetlands, as they would allow recording most of the diversity, including taxa that are both sensitive and tolerant to stressors.

1. Introduction

Wetlands occupy 21.6% of the global land area (Tootchi et al., 2019) and provide habitat for > 40% of the species of the world (Mitra et al., 2003), being recognized as global biodiversity hotspots (Lavoie et al., 2016). Moreover, they perform other important ecological functions such as flow, water quality and microclimate regulation, nutrient retention and transformation, and sequestration of carbon and contaminants in sediments, also providing multiple recreational services to humans (Correa-Araneda et al., 2011; Welsch et al., 2015; Janse et al., 2019). Despite their value, however, they are among the most threatened ecosystems at the global scale as a result of anthropogenic pressures (Sica et al., 2016). Wetlands and their biological diversity have been severely affected in the last decades (Millennium Ecosystem Assessment, 2005; Davidson, 2014; Díaz et al., 2018), with 35% of their surface area being lost between 1970 and 2015, and a particularly fast decline in Latin America and the Caribbean (Darrah et al., 2019).

Wetland condition assessment and monitoring often includes the measurement of physicochemical and biological variables, the latter including diversity and biotic indices based on several taxonomic groups (bacteria, protozoa, algae, plants, invertebrates, fishes and birds). Among these, benthic macroinvertebrates have been extensively used to evaluate wetland ecological condition (Burton et al., 1999; Findlay et al., 2002; Basset et al., 2004; Alba-Tercedor et al., 2005; Mistri and Munari, 2008), given that communities include a variety of taxa with varied ecological requirements and levels of tolerance to stressors, so their diversity and composition often reflect changes in the ecosystem (Hauer and Resh, 2017).

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* Corresponding author.
E-mail address: francisco.correa@uautonoma.cl (F. Correa-Araneda).
[1] These authors equally contributed to this work.

https://doi.org/10.1016/j.ecolind.2021.107551
Received 1 January 2021; Received in revised form 19 February 2021; Accepted 23 February 2021

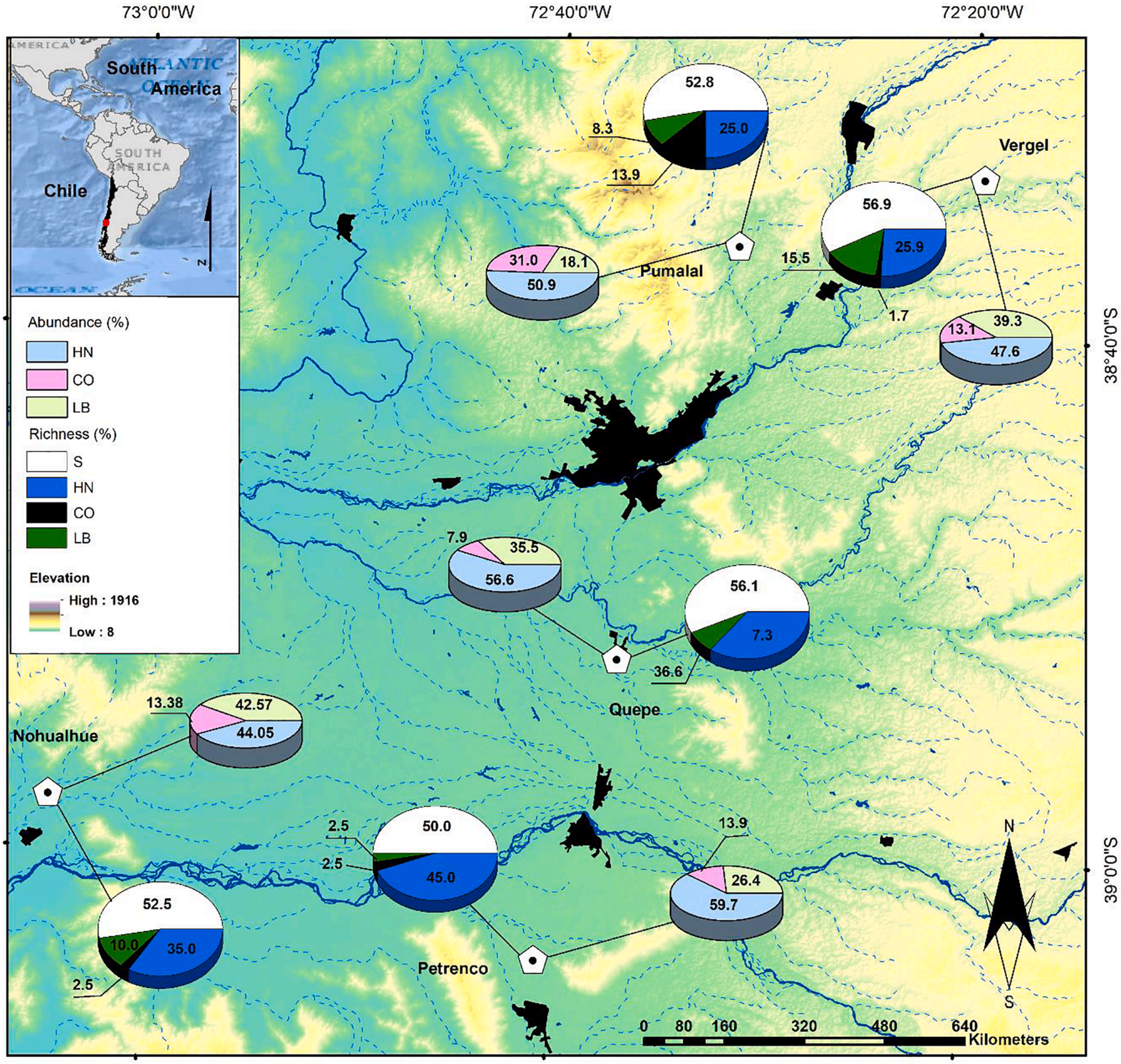

Fig. 1. Location of the study area in Chile. Graphics show percentages of abundance and taxonomic richness in each method and studied wetland. HN = hand net; CO = corer; LB = litterbags; S = shared taxonomic richness.

Table 1
Geographic position (UTM coordinates) and morphometric, hydrological and physicochemical characteristics (mean ± SD) of the studied forested wetlands. For more information on physicochemical variables and their measurement see Correa-Araneda et al. (2017).

	Nohualhue	Petrenco	Quepe	Pumalal	Vergel
Coordinate UTM East (m)	666,663	701,969	706,851	715,536	732,886
Coordinate UTM South (m)	5,685,140	5,663,350	5,694,505	5,723,631	5,728,273
Wetland Surface (Ha)	107	269	346	192	138
Basin Surface (Ha)	2778	3630	1642	3344	2840
Elevation (m a.s.l.)	26	94	95	158	182
Hidroperiod (days)	365	365	365	215	199
Maximum Depht (cm)	83.5	69.7	59.9	51.2	82.4
Minumum Depht (cm)	22.1	30.8	15.7	0	0
Dissolved oxygen (mg/l)	6.6 ± 5.3	4.3 ± 3.0	5.3 ± 3.2	3.5 ± 3.4	6.4 ± 6.0
pH	5.3 ± 2.7	6.1 ± 0.5	6.2 ± 0.6	3.7 ± 2.9	5.1 ± 2.5
Temperature (°C)	7.8 ± 5.0	9.9 ± 3.2	12.2 ± 4.5	5.5 ± 4.5	7.8 ± 4.5
Conductivity (μS/cm)	34.8 ± 22.2	57.7 ± 28.2	80.8 ± 60.9	54.5 ± 59.0	25.1 ± 33.0
Total suspended solids (mg/l)	8.8 ± 3.4	11.0 ± 9.0	13.1 ± 16.3	347.2 ± 786.6	49.8 ± 98.9
Total organic carbon (mg/l)	2.5 ± 3.4	4.8 ± 1.4	3.9 ± 2.4	3.4 ± 4.1	3.2 ± 3.2
DBO$_5$ (mg/l)	1.4 ± 1.0	2.2 ± 1.1	1.3 ± 0.3	2.6 ± 2.8	2.8 ± 4.1
Total phosphorus (mg/l)	0.0 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	0.9 ± 2.0	0.1 ± 0.2
Total nitrogen (mg/l)	0.4 ± 0.1	0.5 ± 0.2	0.7 ± 0.2	1.9 ± 3.5	1.6 ± 1.7
Nitrates (mg/l)	179.6 ± 438.6	0.2 ± 0.3	708.1 ± 806.1	0.3 ± 0.4	2359.6 ± 2742.5

Nevertheless, the study of macroinvertebrates in wetlands is challenging because these ecosystems are highly diverse in terms of habitats and species (Secretaría de la Convención de Ramsar, 2015; Welsch et al., 2015; Baldwin et al., 2018). In particular, the submerged portion of plants creates a highly heterogeneous environment that hosts great diversity of macroinvertebrates and is difficult to sample (Correa-Araneda et al., 2011, 2014, 2017; Gómez-Capponi et al., 2017). Even if different sampling methods have been developed through time (e.g., kick nets, Surber nets, Hess samplers, freezing corers, litterbags and artificial substrates), there is no consensus on which ones are more effective (Ramírez, 2010; Pinna et al., 2013, 2014; Sangiorgio et al., 2014; Correa-Araneda, 2016a; Hauer and Resh, 2017). As not all these methods can be used in all habitats (Rosenberg, 1978; Brooks, 1994; Che Salmah et al., 2007, 2014; Sangiorgio et al., 2014), the choice of sampling method is crucial to quantitatively characterize the diversity of macroinvertebrates inhabiting wetlands (Perán et al., 2001; Correa-Araneda, 2016a) while, at the same time, optimizing collection time and effort (Torralba-Burrial and Ocharan, 2007; Reyes-Morales and Springer, 2014; Ghani et al., 2016).

Forested wetlands are dominated by different plant species in different parts of the world. For example, arboreal species such as red maple (*Acer rubrum* L.), ash (*Fraxinus pennsylvanica*), swamp white oak (*Quercus bicolor*) or bald cypress (*Taxodium distichum*) are dominant in some parts of the USA (Welsch et al., 2015); mangroves (genus *Rhizophora*) and palms are common in tropical and subtropical areas (Gumbricht et al., 2017; Quiceno and Palacio, 2008; Fluet-Chouinard et al., 2015); and wetlands in the Amazonas basin contain more than 22,000 plant species (Infante-Betancour and Rangel-Ch, 2018). In Chile, forested wetlands are dominated by species of the Myrtaceae family (Correa-Araneda et al., 2011; Urrutia and Hauenstein, 2017), which are distributed through the Central, South and Austral regions of the country (29°54′-42°30′ S), from the coast to the pre-mountain areas (Ramírez and San Martín, 2005; Urrutia and Hauenstein, 2017; Urrutia-Estrada et al., 2018).

Here, we characterized the macroinvertebrate community composition, diversity and abundance of 2 temporary and 3 permanent forested wetlands in southern Chile using 3 different sampling methods (hand net, corer and litterbags), with bimonthly sampling campaigns throughout a year. We aimed to determine which of the sampling methods or combination of methods provided a better representation of the community, as a first step to establish a standard methodology that may help improve our knowledge about the ecological status of forested wetlands. We further compiled the published information on macroinvertebrate communities sampled using different methods in a wide variety of freshwater habitats in order to compare our results with those of other studies and provide more information about the usefulness of different sampling methods in a variety of habitats.

2. Methodology

2.1. Study area

The study was carried out in 5 forested wetlands of southern Chile, locally known as 'pitrantos' or 'hualves'. The dominant tree species in the area are the Myrtaceae *Myrceugenia exsucca, Blepharocalyx cruckshanksii, Luma gayana* and *L. chequen,* and the Winteraceae *Drimys winteri*, all of which form a closed, multi-stage tree canopy with heights between 10 and 15 m (Correa-Araneda et al., 2011, 2012; Urrutia and Hauenstein, 2017; Urrutia-Estrada et al., 2018). The altitudinal range is 26 to 182 m a.s.l. (37–40 °S; Fig. 1), wetland surface area ranges from 138 to 346 Ha, and catchment area from 1642 to 3630 Ha (Table 1).

The climate of the study area is Mediterranean, with dry summers with temperatures > 15 °C from November to April, and rainy winters with monthly rainfall > 40 mm from May to September (Sarricolea et al., 2017). Three of the studied wetlands (Nohualhue, Petrenco, and Quepe) have a permanent water regime (16 to 84 cm depth; Table 1), and the other 2 (Pumalal and Vergel) are temporary (0–83 cm depth) and remain dry for 5 months of the year, although they have a permanently saturated substrate (Correa-Araneda et al., 2014). All wetlands correspond to the typology of cold Mediterranean climate with silty loam soil and low slopes (Correa-Araneda et al., 2016b). Their physicochemical characteristics are included in Table 1.

2.2. Macroinvertebrate sampling

Macroinvertebrate samples were collected every 2 months for one year, between April 2011 and April 2012. In each sampling occasion, 6 replicates of each of 3 sampling methods were collected, with a total of 540 samples (108 per wetland). The methods used were the following:

1) A hand net or D-frame dip net of 900 cm^2 opening area and 250 μm mesh. Samples were taken by disturbing the substrate in front of the net using the feet and moving backward, for a 5-minute period per sample, and covering the largest number of habitats possible (water column, substrate, plant roots, etc.) (Correa-Araneda, 2016a).
2) A stainless steel corer with a volume of 400 cm^3, provided with an edge that allowed penetrating different types of substrate (sand, roots, leaf litter, etc.) and a closure system to prevent the loss of

Table 2
List of taxa, number of individuals collected (N) and their relative abundance (%) with respect to the total sample recorded with each of the sampling methods. HN = hand net; CO = corer; LB = litterbags.

Taxon	N	HN	CO	LB
Oligochaeta	5055	12.62	32.67	17.25
Polychaeta	4	0.01	0.06	0.00
Hirudinea	14	0.00	0.00	0.17
Nematoda	181	0.15	0.93	0.86
Tricladida				
Dugesia sp.	142	0.87	0.02	0.47
Gastropoda				
Ancylidae	269	0.93	0.50	2.85
Chilina sp.	13	0.03	0.00	0.06
Littoridina sp.	1017	2.73	0.93	3.75
Physa sp.	26	0.08	0.00	0.03
Biomphalaria sp.	56	0.18	1.02	0.12
Bivalvia				
Sphaeriidae	120	0.42	0.51	0.12
Crustacea				
Hyalella araucana	5347	12.55	4.02	14.91
Heterias exul	10,025	20.85	9.36	23.21
Parastacus pugnax	115	0.72	1.13	0.24
Aegla araucaniensis	10	0.03	0.00	0.06
Collembola	45	0.28	0.15	0.36
Hymenoptera	1	0.01	0.00	0.00
Diptera				
Chironomidae	6930	25.95	27.65	19.71
Culicidae	1303	2.06	0.08	0.34
Limoniidae	15	0.02	0.13	0.03
Ephydridae	18	0.07	0.01	0.05
Ceratopogonidae	83	0.44	1.79	0.26
Simuliidae	30	0.18	0.14	0.00
Tipulidae	59	0.15	0.47	0.31
Psychodidae	41	0.20	0.82	0.02
Empididae	54	0.50	0.49	0.00
Athericidae	12	0.02	0.03	0.07
Coleoptera				
Luchoelmis sp.	833	5.50	4.59	1.71
Cyphon sp.	117	0.57	0.88	0.52
Gyrinidae	6	0.06	0.00	0.00
Hydraenidae	64	0.67	0.05	0.36
Haliplidae	4	0.01	0.24	0.00
Hydrochus stolpi	24	0.09	0.00	0.22
Lancetes sp.	69	0.35	0.05	0.34
Listronotus sp.	14	0.07	0.01	0.00
Staphylinidae	50	0.24	0.09	0.23
Salpingidae	1	0.01	0.00	0.00
Chrysomelidae	1	0.01	0.00	0.00
Coleoptera Indet.	29	0.06	0.15	0.27
Lepidoptera				
Pyralidae	19	0.20	0.04	0.00
Trichoptera				
Smicridea sp.	349	1.21	0.90	1.31
Limnephilidae	58	0.40	0.00	0.06
Leptoceridae	12	0.09	0.00	0.02
Brachysetodes sp.	15	0.09	0.00	0.05
Hydroptilidae	1	0.00	0.00	0.03
Stenopspychidae	1	0.00	0.00	0.01
Polycentropus sp.	3	0.01	0.00	0.01
Megaloptera				
Megaloptera Indet.	19	0.08	0.00	0.00
Protosialis sp.	134	0.71	0.81	0.33
Odonata				
Odonata Indet.	5	0.02	0.00	0.02
Coenagrionidae	98	0.26	0.00	0.18
Libellulidae	29	0.05	0.00	0.12
Lestidae	14	0.12	0.00	0.01
Aeshnidae	10	0.15	0.00	0.00
Calopterygidae	2	0.00	0.00	0.01
Hemiptera				
Veliidae	61	0.37	0.07	0.00
Gerridae	11	0.03	0.00	0.00
Mesoveliidae	3	0.01	0.00	0.00
Notonectidae	1	0.01	0.00	0.00
Corixidae	2	0.01	0.00	0.00
Hebridae	5	0.01	0.00	0.07
Plecoptera				
Antarctoperla michaelseni	100	0.50	0.11	0.22
Perlugoperla personata	14	0.02	0.00	0.03
Perlidae	174	0.07	0.00	0.62
Neonemura barrosi	140	0.09	0.17	1.25
Ephemeroptera				
Murphyella needhami	122	1.05	0.00	0.10
Leptophlebiidae	1019	1.45	0.00	3.64
Nousia crena	44	0.40	0.00	0.06
Caenis sp.	39	0.31	0.00	0.03
Neuroptera				
Osmylidae	8	0.13	0.00	0.00
Chelicerata				
Acari	499	2.45	8.93	2.93
N° Taxa		67	37	53

material. Samples were collected at a distance > 1 m, collecting a sediment core 10 cm deep following the protocol described by Correa-Araneda (2016a) based on Domínguez and Fernández (2009).

3) Litterbags made of plastic mesh (5 mm opening, 15 × 15 cm), containing 6 g of a mixture of dry leaves of the 2 dominant species in the studied wetlands (*B. cruckshanksii* and *M. exsucca*; 3 g each). Replicated litterbags were equidistantly attached 1 m away with a nylon string and submerged for 60 days with a weight attached to both ends of the string to limit their movement (Ramseyer and Marchese, 2009; Gutiérrez-López et al., 2016; Pinna et al., 2017).

All the collected samples were individually stored, labeled and preserved with 95% ethanol. In the laboratory they were sorted for macroinvertebrates, which were counted and identified under a trinocular stereoscopic magnifying glass with a camera (Optika SZM-LED2). Identification was done to the lowest possible taxonomic level using available literature (McCafferty and Provonsha, 1998; Heckman, 2002, 2003; de Barros et al., 2007; Domínguez and Fernández, 2009; Palma, 2013; Rudolph, 2013, 2013b).

2.3. Data analyses

We quantified macroinvertebrate abundance (N), taxon richness (S), Pielou's evenness (J'), and Shannon diversity (H') in each sample and examined differences in these variables among sampling methods using Kruskal-Wallis tests, separately for each wetland, because most of the treatments were not normally distributed. In addition, the data contained outliers and were heteroscedastic. When differences were significant ($p < 0.05$) we made pairwise comparisons with t-Student post hoc tests evaluate. These analyses were carried out in Package agricolae 1.3–3 (De Mendiburu, 2009).

We examined differences in community composition among sampling methods with non-metric multi-dimensional scaling (NMDS) using a Bray-Curtis similarity matrix and a one-way ANOSIM similarity test. Both analyses were performed for all wetlands together and for each wetland individually. We identified the taxa most associated to each sampling methods using SIMPER analysis. These analyses were done using PRIMER v.6 (Clarke and Gorley, 2006).

3. Results

We collected 71 macroinvertebrate taxa across the 5 studied wetlands, with the hand net collecting the highest number of taxa (67 taxa), followed by the litterbags (53) and the corer (37). The most abundant taxonomic groups were the Crustacea (38% of which were collected with the litterbags, 34% with the hand net and 15% with the corer),

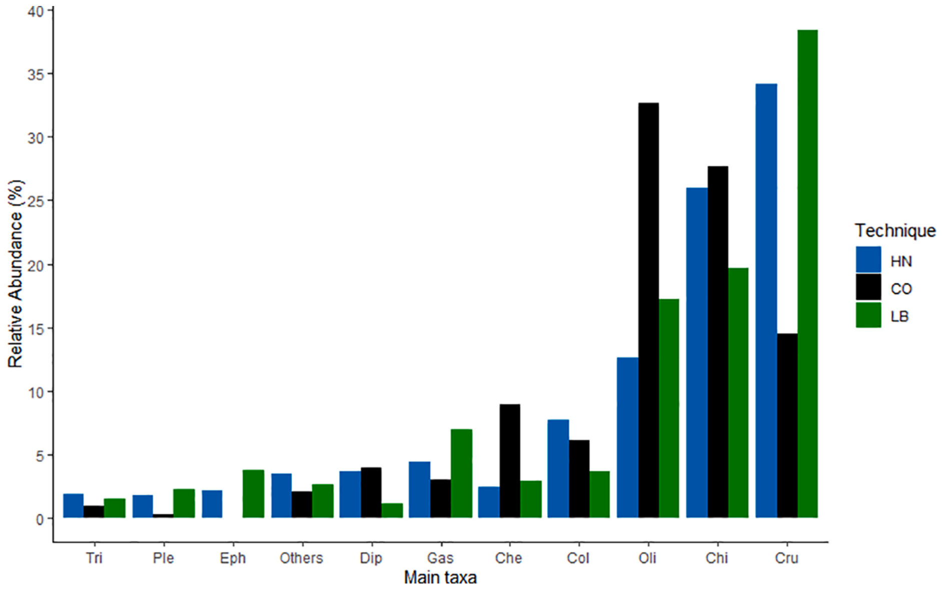

Fig. 2. Relative abundance (%) of the main macroinvertebrate taxonomic groups in relation to the different sampling methods. Tri = Trichoptera, Ple = Plecoptera, Eph = Ephemeroptera, Dip = Diptera, Gas = Gastropoda, Che = Chelicerata, Col = Coleoptera, Oli = Oligochaeta, Chi = Chironomidae, Cru = Crustacea.

Chironomidae (20, 28 and 26%) and Oligochaeta (17, 33 and 13%) (Table 2; Fig. 2). The Hirudinea and Calopterygidae were exclusively collected with the litterbags. The Gyrinidae, Aeshnidae, Osmylidae, Megaloptera, Hymenoptera, 4 Hemiptera and 2 Quelicerata taxa were collected only with the hand net. The corer did not present any exclusive taxa but allowed to collect greater relative abundance of the Oligochaeta, Acari, Limoniidae, Ceratopogonidae, Psychodidae, Haliplidae, *Parastacus pugnax* (Parastacidae) and *Biomphalaria sp.* (Planorbidae). The corer did not collect 6 Trichoptera taxa, Odonata or Ephemeroptera (Appendix A; Appendix B).

Macroinvertebrate abundance was significantly higher in hand net samples than in litterbags samples in 3 wetlands (Petrenco, Quepe and Pumalal) but not in the other 2 (Nohualhue and Vergel). Both hand net and litterbags samples collected higher abundances than corer samples. Both richness and diversity were highest in hand net samples followed by litterbags samples and, finally, corer samples, in all wetlands except in Pumalal, where litterbags and corer samples did not differ. Evenness was higher in hand net and litterbags than in corer samples in 2 wetlands (Quepe and Vergel); higher in litterbags than corer samples in 1 wetland (Pumalal); and showed no differences in the other 2 (Nohualhue, and Petrenco) (Fig. 3; Appendix C).

The NMDS separated hand net and corer samples when all wetlands were examined together, with no clear separation of litterbag samples (Fig. 4A). When each wetland was examined individually, hand net and litterbag samples were grouped together and separated from corer samples (Fig. 4B-F). The ANOSIM found significant differences among sampling methods for all wetlands together (Global $R = 0.200$, $p = 0.001$) and each wetland separately, with better fit for permanent than for temporary wetlands, and paired comparisons were significant in all cases (Table 3).

The SIMPER analysis revealed that the most influential taxa on the differences observed between communities sampled by different methods were *Heterias exul* (Müller, 1892) and *Hyalella araucana* (Grosso and Peralta, 1999) collected mostly with hand net and litterbags, and Chironomidae and Oligochaeta principally collected with corer. These taxa represent more than 67% of the cumulative dissimilarity between the factors in each paired comparison (Tables 2 and 3).

4. Discussion

The goal of this study was to compare the performance of 3 macroinvertebrate sampling methods (hand net, corer and litterbags) in forested wetlands. Given the scarcity of similar studies to ours conducted in forested wetlands, here we compare our results with those of studies conducted in different types of freshwater ecosystems including coastal lagoons, lakes, ponds, rivers and streams (Table 4). We discuss the potential advantages of using each of these methods, individually or in combination, to characterize benthic macroinvertebrate communities.

Our results showed that the hand net recorded the greatest taxonomic richness and Shannon diversity overall, followed by the litterbags and, finally, the corer. The pattern was similar for abundance (although values did no differ between the hand net and the litterbags in 2 of the wetlands) and more variable for evenness. These results are in concordance with those of a study in wetlands of southern Texas, which compared the performance of hand net and corer and found that the former recorded higher richness and abundance (but not higher diversity) (McIntosh et al., 2019). This greater efficiency of the hand net could be related to the variety of habitats that this method can sample, which include shallow and medium-depth waters (<40 cm) and all types of substrate (Correa-Araneda et al., 2017). Thus, the hand net recorded the majority of Crustacea (the most abundant order) found in our study, with 66% of all specimens (compared to 33% with the litterbags and 1% with the corer), and also recorded high numbers of Chironomidae (the second most abundant group). The hand net also registered a greater number of exclusive taxa (i.e., taxa only collected with this method) compared to litterbags (2 exclusive taxa) and corer (no exclusive taxa). The majority of exclusive taxa collected by the hand net were insects (Hemiptera, Coleoptera, Hymenoptera, Megaloptera and Neuroptera).

The litterbags method registered abundance and richness levels lower than those of the hand net, but the taxonomic composition of communities was similar between both methods. However, this method registered 2 exclusive taxa (Hirudinea and Calopterygidae) and greater abundance of gastropods than the other methods. This is relevant because common macroinvertebrate sampling methods often do not reveal the presence of gastropoda (Narr and Krist, 2019). Other authors have claimed that litterbags are an effective method not only to collect macroinvertebrates, but also to recolonize impoverished habitats and increase their biodiversity (Dumeier et al., 2020). In Mediterranean lagoons, litterbags were able to record greater diversity than the corer method, even if both methods together provided a better representation of communities (Pinna et al., 2017).

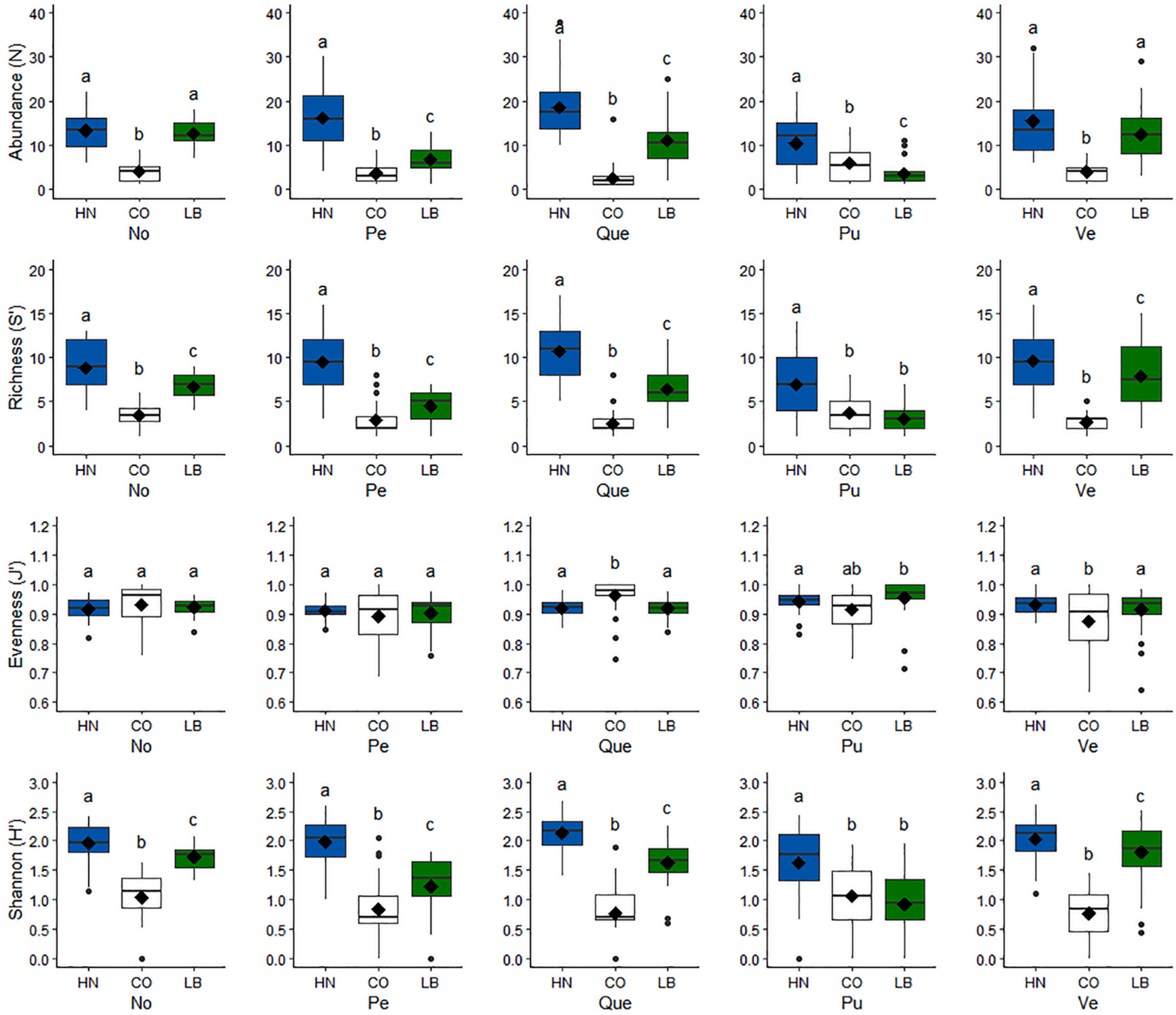

Fig. 3. Macroinvertebrate abundance, taxonomic richness, Shannon diversity and Pielou's evenness for each sampling method (HN = hand net; CO = corer; LB = litterbags) in each studied wetland (No = Nohualhue, Pe = Petrenco, Que = Quepe, Pu = Pumalal, Ve = Vergel). Boxplots show the maximum and minimum values as well as the interquartile ranges (25–75%), with solid lines representing median values. Treatments with the same letter are not significantly different ($p > 0.05$).

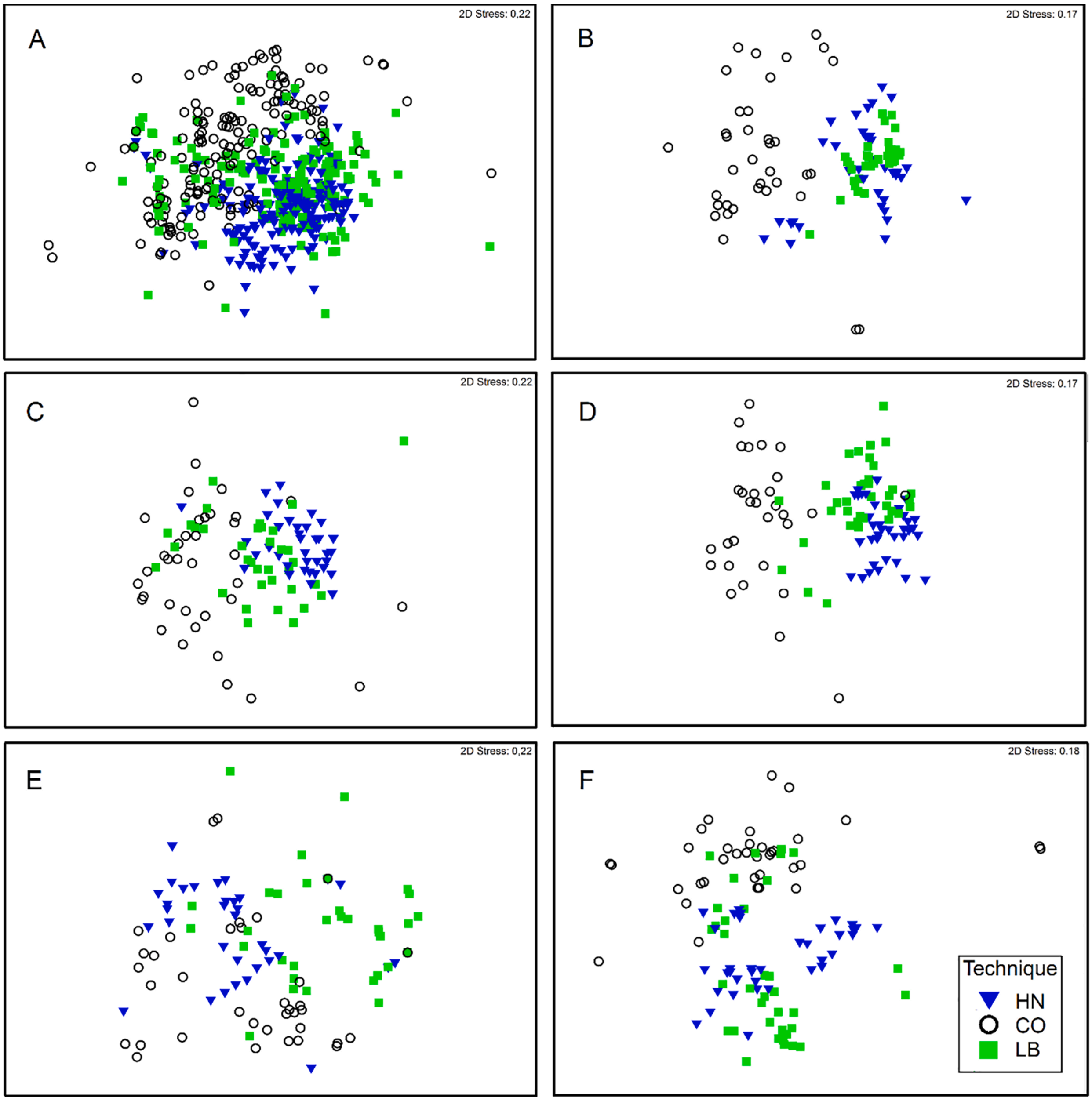

Fig. 4. Non-parametric multidimensional scaling analysis (NMDS) of the similarity matrix of the macroinvertebrate community using sampling method as factor. A = all wetlands; B = Nohualhue; C = Petrenco; D = Quepe; E = Pumalal; F = Vergel.

Table 3
ANOSIM pairwise tests determining differences between sampling methods in each studied wetland (*$p = 0.03$; **$p = 0.001$) and SIMPER analysis identifying the overall contribution of each taxon to the differences recorded between sampling methods.

	AS vs CO	AS vs HN	CO vs HN
Nohualhue	0.67**	0.22**	0.54**
Petrenco	0.21**	0.18**	0.52**
Quepe	0.59**	0.20**	0.70**
Pumalal	0.28**	0.27**	0.19**
Vergel	0.37**	0.04*	0.42**
Heterias exul	19.93	25.04	20.13
Chironomidae	17.12	18.77	20.89
Hyalella araucana	13.10	14.07	12.09
Oligochaeta	21.63	9.57	16.41
Luchoelmis sp.	2.16	3.94	4.75
Littoridina sp.	3.39	3.73	2.75
Leptophlebiidae	3.54	3.51	1.27
Culicidae	–	2.03	2.00
Acari	3.71	1.98	3.24
Ancylidae	2.28	1.50	0.89
Diptera indet.	–	1.49	1.59
Smicridea sp.	1.40	1.42	1.18
Murphyella needhami	–	0.85	0.96
Dugesia sp.	–	0.80	0.69
Protosialis sp.	–	0.68	0.74
Neonemura barrosi	1.05	0.65	–
Parastacus pugnax	–	–	0.72
Nematoda	1.25	–	–

The corer was the method showing the lowest abundances and taxonomic richness in our study. However, it was important because it registered high numbers of Chironomidae and Oligochaeta. The latter was the third most abundant taxonomic group and it was mainly collected through this method. Some authors have also shown that Chironomidae are collected through both hand net and corer (McIntosh et al., 2019), while others have collected them mostly using hand nets, especially in littoral soft sediments (Szekeres et al., 2019). In Mediterranean lagoons, Oligochaeta were mostly recorded using corer (Pinna et al., 2017). Although we did not detect any taxa exclusive to this method, it allowed to collect a greater relative abundance of annelids, Diptera, *Parastacus pugnax* (Crustacea) and *Biomphalaria sp*. (Gastropoda). Pinna et al. (2017) found 6 out of 10 species to be exclusive to the corer method, with *Loripes orbiculatus* (Bivalvia) being the most abundant, and *Melanoides tuberculata* (Gastropoda) was exclusive to this method in wetlands of southern Texas (McIntosh et al., 2019). On the other hand, the corer did not detect 6 out of the 7 Trichoptera found in our study, or any Odonata or Ephemeroptera. Other studies have recorded Hirudinea, Planorbidae (Gastropoda), Oligochaeta, and Chironomidae with the corer, while other have shown that some Trichoptera (*Hydropsyche* spp.) were more efficiently captured with a dredge in coastal areas of the Danube (Szekeres et al., 2019). Furthermore, a recent study in a non-vegetated Mediterranean wetland has reported that when the main goal is to know the taxonomic richness of the communities, 20 samples with a corer or even more could be needed. However, to assess the environmental quality by means of ecological indexes, three samples could be an acceptable benchmark (Saccò et al., 2020).

The clear separation in the composition of communities collected with the corer compared to those collected with the other two methods indicates that the corer could be efficiently used in conjunction with the hand net or litterbags in order to characterize macroinvertebrate communities in wetlands. Pinna et al. (2017) also detected a separation of community composition between litterbags and corer, supporting the use of more than one sampling method to obtain a more complete description of the communities, and other studies have identified the combination of hand net and corer as more to collect a representative community sample in wetlands and rivers (Batzer et al., 2001; Burdett et al., 2015).

Our study suggests that hand net and corer can provide the best representation of macroinvertebrate communities in forested wetlands in terms of abundance, richness, diversity, evenness, and community composition, including taxa that are exclusive to a given type of habitat. This seems to be valid for both permanent and temporary wetlands, as we found no systematic differences between both types of environments. While the hand net collected the greatest abundance and diversity, and more exclusive taxa compared to the litterbags, the corer recorded higher numbers of Chironomidae and Oligochaeta, which were among the most abundant taxa and often indicators of habitat degradation (Ruaro et al., 2020). Importantly, both methods are easy to use compared to the litterbags, which require multiple field trips for deployment and collection, as well as more time-consuming preparation in the laboratory. Our results may contribute to standardize future studies on macroinvertebrate in forested wetlands or similar ecosystems, and thus improve our knowledge about how these unique ecosystems are affected by environmental impacts. This is among the five strategic goals of the Aichi Biodiversity Targets, part of the Strategic Plan for Biodiversity 2011–2020. That seeks to halt ecosystem loss and biodiversity, to conserve, sustainable use of aquatic species, and identify priority areas in their protection at regional and national levels (RCW, 2018).

CRediT authorship contribution statement

Francisco Correa-Araneda: Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing - review & editing, Visualization, Supervision. **Daniela Núñez:** Formal analysis, Investigation, Data curation, Writing - original draft, Visualization, Supervision. **Ma Elisa Díaz:** Investigation, Visualization, Writing - review & editing. **Francisco Gómez-Capponi:** Investigation, Writing - review & editing. **Ricardo Figueroa:** Validation, Resources, Writing - review & editing. **Jaquelinne Acuña:** Writing - review & editing. **Luz Boyero:** Writing - review & editing. **Carlos Esse:** Formal analysis, Writing - review & editing, Visualization.

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.

Table 4

Comparative analysis of the main results and conclusions of this and other studies on macroinvertebrate sampling methods in freshwater environments. MI = macroinvertebrates; SM = sampling methods; S = taxonomic richness; N = abundance; H'= Shannon diversity; J = Pielou's evenness; HN = hand net; CO = corer; LB = litterbags.

Methods	Environment	Results	Conclusions	Reference
LB: Mixture of *B. cruckshanksii* and *M. exsucca* leaves. CO: 400-cm^3 volume, stainless steel. HN: 900-cm area, 250-μm mesh.	5 forested wetlands, mediterranean climate zone, southern Chile 3 permanent and 2 temporary.	LB: 53 taxa, (2 exclusive). *H. exul* and *Hyalella araucana* were most abundant. CO: 37 taxa, (0 exclusive for all FW). Oligochaeta and Chironomidae were most abundant. HN: 67 taxa, (11 exclusive). *Heterias exul* was most abundant. Abundance: Significantly higher through HN in 3 FW, in others, HN not differing from LB. Both ST collect greater N than CO. Evenness: Significantly higher through HN and LB vs. CO in 2 FW. NMDS and ANOSIM: Significant differences between LB + HN vs. CO.	Better community representation of N, S, H', J' and exclusive taxa in FW was collected with HN, followed by LB and finally CO. Because there are 2 MI assemblies whit respect to the SM, we recommend using a combination of them for the complete characterization of permanent as temporary FW. HN is versatile, low cost and easy to homemade. CO collects different assemblies of HN + LB and more N of Chironomidae and Oligochaeta, taxa abundant in aquatic ecosystems, so the CO technique should not be dismissed.	This study
LB: Three different *Phragmites australis* (PH), *Posidonia oceanica* (PO) and equal mixtures of both leaf (PP). CO: Surface of 0.03 m^2. Samples was sieved through a 1.0 mm mesh size.	2 sites in 2 habitats, prairie (PRA) and unvegetated (UNV) located in a Mediterranean coastal lagoon, Italy.	LB: 21 spp. (16 in PH, 12 in PO, 16 in PP; 17 exclusive) *Microdeutopus gryllotalpa* was most abundant. CO: 10 spp. (6 exclusive). *Loripes orbiculatus* was most abundant. 4 spp. overlapped between in the SM. Richness: LB greater in both habitats. NMDS and PERMANOVA: Significant differences among SM.	LB + CO improves the description of MI assemblies, diversity descriptors and ecological indicators, especially in PRA. MI assemblies sampled with LB and CO are different in spp. composition and structure. LB is attractiveness for MI and arthropods prevail in their communities, annelids and mollusks characterized CO samples. Differences linked to the selectivity of SM; epifauna for LB and infauna for CO.	Pinna et al. (2017)
CO: 36-cm diameter. Sieves with mesh sizes of 6 mm, 4 mm, 2 mm, and 500 μm.	27 Prairie pothole permanently or semipermanently flooded depressional wetlands of northcentral Iowa, U.S.A.	6-mm mesh sieve: reduced sample volume by 35% and processing time by 54% relative to the 500-μm mesh sieve. In particulate matter presence, N of MI retained by 2-mm, 4-mm, and 6-mm mesh sieves were reduced by 35, 53, and 56%, relative to the 500-μm mesh sieve. Pearson correlation coefficients: Strong relationships between environmental variables and MI taxa richness with 6-mm mesh sieve and particulate matter occurred in samples.	It is possible to reduce costs of including MI assemblage variables in wetland condition assessment, and still generate data that reflect wetland condition. Use of a 6-mm mesh sieve instead of a conventional 500-μm mesh sieve for wetland condition assessment would reduce invertebrate sample volume and processing time.	Baldwin et al. (2018)
CO: PVC-constructed sediment corer (8 cm diameter). HN: Dip net with a bottom edge of 30 cm and a 243 μm mesh.	3 freshwater wetlands (secondary channels distributaries, oxbow lakes) whit different ecological status, located in Cameron County, Texas, USA.	CO: 44 taxa within 31 families. Chironomidae, Thiaridae, Naididae and Ceratopogonidae were the groups most abundant groups. HN: 58 taxa within 38 families. Chironomidae, Mysidae, Baetidae and Hyalellidae were the groups most abundant groups. Large differences in the composition of functional feeding groups among wetlands. Abundance: Collected with the HN was higher than CO. ANOSIM: Significant differences between the MI communities in the 3 sampling sites.	The two SM collected a different subset of the overall MI assemblage. CO tended to sample slow moving MI with more benthic affinity, and HN sampled more active MI. Despite the sampled assemblage subsets being different, general community metrics obtained from both SM resulted in similar discriminated the study sites. Trophic structure of the MI community is also useful for discriminating among aquatic ecosystems having different developmental stages.	McIntosh et al. (2019)
DWS: Deep-water sampling. KS: Kick and sweep sampling with hand net. MHS: Multihabitat sampling with framed net.	3 main segments based on hydromorphological features: Upper, middle, and lower in Danube river, Europe. 68 sites along 2,500 km of the river and in its main tributaries.	DWS: 140 taxa, average 24 per site, 7 indicators. KS:217 taxa, average 36 per site, 24 indicators. MHS: 252 taxa, average 47 per site, 64 indicators. Rarefaction analysis: MHS and KS collected significantly more taxa than DWS after 8 samples. All methods were complementary to	Sampling littoral vs DWS was influential in assessing assemblies in a very large river. Different depth zones characterized different MI. Each SM contributed to overall taxa richness and species composition. Clear separation of MI according to SM. A detailed estimation of the share of.	Szekeres et al. (2019)

(*continued on next page*)

Table 4 (*continued*)

Methods	Environment	Results	Conclusions	Reference
		exploring the total taxa richness of the river.	microhabitats and sampling of MI in the very edge of the littoral zone is fruitful.	
Gastropod-specific Sampling Protocol (GSSP): Different methods for habitats with varying flow dynamics, substrates and vegetation. Streams:Silty or sandy substrates: flat-bottomed kicknet. Cobble substrates: disturb by picking up rocks from the bottom. Algal mats: flat-bottomed net. Overhanging vegetation: small net or a sieve. Lakes/Ponds: Pick by hand from logs and rocks in the littoral zone. In deeper: nets to collect debris and vegetation.	Streams, lakes and ponds.	Detected more snails than general MI surveys. 5 genera of freshwater gastropods were associated with specific mesohabitat. 2 genera and 2 sub-genera occurred more often in some substrates.	GSSP recorded snails at many more sites and substantial variation in taxonomic richness among sites. Snails from the same family were just as likely to co-occur with snails from other families as with members of their own family. SM used for general MI surveys do not accurately reflect the presence or diversity of freshwater snails. GSSP targeted specific snail-rich habitats (e.g. overhanging vegetation, floating algal mats, submerged macrophytes) that are frequently overlooked by general MI sampling methods because these habitats are difficult to quantify.	Narr and Krist (2019)
LB: Natural substrate exposure (NSE) fixed to aluminum profiles. Standardized 20 × 20 cm OKALON® mesh bags (mesh size 20 mm), containing wooden sticks and a mixture of dried and alder and beach leaves. 6 weeks exposure for colonize.	2 Streams tributaries of the Rhine river in North Rhine-Westphalia, Germany. Sand bottom lowland.	Abundance: > 100,000 MI colonized the LB. Richness: 70 taxa can be considered “introduced” into the recipient stream. EPT taxa richness was greater in spring than in summer. Exclusive taxa: 45 taxa did not occur in the recipient stream before reintroduction.	Active reintroduction based on LB as a tool to increase biodiversity in impoverished streams. Number of positive Fauna Index and EPT taxa per LB differed significant between reintroduction events in summer and spring. Spring is favourable season for a reintroduction when the abundance of MI is maximum.	Dumeier et al. (2020)
CO: Methacrylate cylinder (5.2 cm inner diameter).	Non-vegetated shallow lagoon (rice field), in the Mediterranean region, Albufera Natural Park, Valencia, Spain.	13 Ostracoda and Tubificidae taxa were the most abundant. A curve with an asymptotic trend more marked as from an effort of 20 samples. IMN (Nutritional mode index)-based rarefaction curve revealed a more marked asymptotic tendency.	When the goal is to characterize the taxa richness of the communities, 20 samples or even more could be needed. To assess the environmental quality by means of ecological indexes such as the IMN, three samples could be an acceptable benchmark.	Saccò et al. (2020)

Acknowledgements

Daniela Núñez thanks to CONICYT-PFCHA/Doctorado Nacional/2019-21191862 for financing her postgraduate studies. Thanks to ANID/FONDAP/15130015.

Funding

Fondecyt de Iniciación 11170390.

CHRIAM Centro de Recursos Hídricos para la Agricultura y la Minería.

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecolind.2021.107551.

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