



How invasive macrophytes affect macroinvertebrate assemblages and sampling efficiency: Results from a multinational survey

Benjamin Misteli, Alexandrine Pannard, Frédéric Labat, Lorraine Kengne Fosso, Nompumelelo Catherine Baso, Sarah Faye Harpenslager, Samuel Nkopane Motitsoe, Gabrielle Thiebaut, Christophe Piscart

► To cite this version:

Benjamin Misteli, Alexandrine Pannard, Frédéric Labat, Lorraine Kengne Fosso, Nompumelelo Catherine Baso, et al.. How invasive macrophytes affect macroinvertebrate assemblages and sampling efficiency: Results from a multinational survey. *Limnologica*, 2022, 96, pp.125998. 10.1016/j.limno.2022.125998 . hal-03729123

HAL Id: hal-03729123

<https://hal.science/hal-03729123>

Submitted on 20 Jul 2022

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

How invasive macrophytes affect macroinvertebrate assemblages and sampling efficiency: results from a multinational survey

Benjamin Misteli ^{1*}, Alexandrine Pannard ¹, Frédéric Labat ^{1,2}, Lorraine Kengne Fosso ³,
Nompumelelo Catherine Baso ⁴, Sarah Faye Harpenslager ^{5,6}, Samuel Nkopane Motitsoe ⁷,
Gabrielle Thiebaut ¹ and Christophe Piscart ¹

¹ *Université de Rennes 1, UMR 6553 CNRS ECOBIO, 263 Avenue du Général, 35042 Rennes*

² *Aquabio, 10 Rue Hector Guimard, 63800 Cournon d'Auvergne, France*

³ *University of Yaounde 1, Faculty of Science, Laboratory of Hydrobiology and Environment,
P.O. Box 812, Yaounde, Cameroon*

⁴ *Rhodes University, Centre for Biological Control, Department of Botany, P.O. Box 94, 6139
Grahamstown, South Africa*

⁵ *Department of Community and Ecosystem Ecology, Leibniz-Institute of Freshwater Ecology
and Inland Fisheries, Müggelseedamm 301, 12587 Berlin, Germany*

⁶ *B-WARE Research Centre, Toernooiveld 1, 6525 ED Nijmegen, The Netherlands*

⁷ *Rhodes University, Centre for Biological Control, Department of Zoology and Entomology,
P.O. Box 94, 6139 Grahamstown, South Africa*

* Correspondence: benjamin.misteli@univ-rennes1.fr

Keywords: shallow lakes, aquatic plants, methods, habitat heterogeneity, sampling
efficiency

Abstract

Macrophytes play an important role in the functioning and structuring of aquatic environments but rapid mass development of invasive macrophytes is causing global concerns. Macroinvertebrate richness and abundance are strongly influenced by macrophytes as macrophytes offer habitats and food resources, increase structural heterogeneity, and provide refuges. Meanwhile, the presence of macrophytes affects the efficiency of standard sampling methods for macroinvertebrates. These effects are not well studied but are leading to biased management decisions. To fill in this knowledge gap, we analysed macroinvertebrate communities from four lakes in four countries in Europe and Africa with mass development of invasive macrophytes. We compared macroinvertebrate communities in sediment samples from a plant-free part of the lake with those in sediment and sweep samples taken within macrophyte stands. We showed that taxa richness and density were higher in sediment samples beneath invasive macrophyte stands compared to plant-free habitats. Unique taxa were found in each sample type. Sampling efficiency of each sampling method varies greatly across lakes especially when replication is low. The taxonomic richness of macroinvertebrates within invasive macrophyte stands is often underestimated compared to open water sections with the same number of samples. To reach a high sampling coverage, a higher number of samples is necessary for sampling within invasive macrophytes. Our findings call for the development of a method that allows for comparable sampling within and outside of macrophyte stands. Such method will be the foundation for future research and management of aquatic systems.

Introduction

Macrophytes play a crucial role in the structuring and functioning of freshwater systems. In the last decades, growth and abundance of macrophytes have increased globally due to anthropogenic impacts like eutrophication (Verhofstad et al., 2017). The fact that invasive macrophytes can rapidly inhabit a complete aquatic system by forming dense mats is of great concern (Hussner et al., 2017; Strayer, 2010). The presence of dense macrophyte stands, often hinders or interferes with important socio-economic activities (e.g., boating, swimming, fishing, clogging of hydropower plants). Thus, in recent years, macrophytes have become an important target in water management and are often removed at high costs (Hussner et al., 2017; Thiemer et al., 2021).

Apart from affecting important socio-economic activities, macrophytes also have a strong effect on the ecology of freshwater ecosystems, changing population dynamics of other aquatic organisms and their interactions, including macroinvertebrates (Burks et al., 2006). These effects on macroinvertebrates vary depending on the plant species, life form, structural complexity and density (Ferreiro et al., 2014; Taniguchi et al., 2003; Thomaz et al., 2008; Walker et al., 2013).

The most direct positive effect of macrophytes on macroinvertebrates is the provision of structural complexity and heterogeneity in aquatic systems (Scheffer, 2004; Thomaz et al., 2008). Complex structures have been shown to support higher invertebrate richness and abundance compared to less complex structures. This may be due to complex structures providing more microhabitats and creating new niches for small invertebrates (Labat et al., 2022; Morse et al., 1985; Taniguchi et al., 2003; Thomaz et al., 2008). Alternatively the higher abundance could be linked to reduced predation, as complex structures offer refugia for

invertebrates (Balci and Kennedy, 2003; Dahl and Greenberg, 1997; Sato et al., 2014; Warfe and Barmuta, 2004).

Macrophytes are important primary producers and produce oxygen via photosynthesis. As a result, waterbodies with macrophytes often have increased fluctuations of dissolved oxygen (DO) concentrations (daily and seasonal) than open water sites (Caraco et al., 2006; Frodge et al., 1990), with periods of low DO availability at nights and during warm periods. Increased dark respiration of macrophytes and of the attached biofilm (Żbikowski et al., 2019) as well as reduced oxygen exchange and transportation due to low mixing in macrophytes can lead to hypoxia (Caraco and Cole, 2002; Vilas et al., 2017). Many macroinvertebrate species depend directly on the availability of dissolved oxygen for their survival and low oxygen levels can critically change their diversity, community composition and biomass (Chapman et al., 2004; Dodds and Whiles, 2019; Verberk and Bilton, 2013).

Moreover, macrophytes act as food sources for herbivorous and omnivorous macroinvertebrates. Traditionally, herbivory on macrophytes has been considered insignificant (Lodge, 1991; Mann, 1988; Newman, 1991), but this assumption has been proven wrong. Different studies showed that herbivory on aquatic plants exceeds herbivory in terrestrial systems (Cyr and Face, 1993; Wood et al., 2017). Besides being a direct food source, macrophytes also provide habitats for epiphytic algae which are being grazed at high rates (Cyr and Face, 1993; Wolters et al., 2019). Typical herbivores that can be found living and grazing on macrophytes and epiphytic algae are larvae of aquatic insects such as Lepidoptera, Trichoptera, Diptera, Gastropoda, Oligochaeta and Crustacea (Cattaneo et al., 1998; Dehedin et al., 2013; Jacobsen and Sand-Jensen, 1992; Li et al., 2009).

Macrophytes can have both positive and negative effects on macroinvertebrates, affecting taxonomic richness and abundance compared to plant free sections. Such differences

may not be well-captured by the standard sampling protocols. A great variety of methods are used to sample benthic macroinvertebrates in lentic systems, depending on the waterbody types. In wadable systems, a hand net can be used to sweep through the sediment (García-Criado and Trigo, 2005; Labat et al., 2022; Porst et al., 2016), while in deeper lakes, grab samples and core samples are the standard methods for sampling benthic macroinvertebrates (Blomqvist, 1991; Jónasson, 2004; Ntislidou et al., 2018; Sly, 1969). However, there is a lack of commonly accepted sampling methods and research on how to account for differences in the compositions between sections with and without plants. It can be expected that sediment samples alone are not enough to get a complete profile of macroinvertebrates in macrophyte beds, given that macrophytes offer an additional habitat for macroinvertebrates. Therefore, complementary sampling within the plants should be performed (Indermuehle et al., 2010; Oertli et al., 2005). As macrophytes come in a wide variety of forms, a sweep net is commonly used for sampling within plants as it can be used within all plant types. Methodological studies on macroinvertebrate sampling mainly focus on comparing different sampling methods and their efficiency within macrophytes (Kornijów, 2014; Sychra and Adamek, 2010). Understanding the differences on macroinvertebrates sampling efficiency between macrophyte beds and plant-free sections are essential, especially when macroinvertebrate communities from these two sections are compared. However, research on this topic is scarce.

In this study we compare freshwater macroinvertebrate communities within dense stands of invasive macrophyte to those in plant-free sections in the same lake. Study lakes were situated along a latitudinal gradient, covering Germany, France, Cameroon and South Africa. We also compared sampling efficiency of sediment samples collected in plant-free sections, sediment samples within macrophyte stands and sweep samples collected in macrophytes. This methodological assessment provides essential information for future macroinvertebrate studies

in systems with macrophytes. We hypothesize that: (i) the density and taxonomic richness of macroinvertebrates within invasive macrophyte stands are higher than those in open water sections; and therefore (ii) a higher number of samples is needed in stands of invasive macrophytes compared to plant-free sections to reach the same sample coverage of the macroinvertebrate community. Moreover, (iii) these two abovementioned effects are expected to be smaller in sections with free-floating plants as they offer less complex underwater structures compared to non-floating plants.

Materials and Methods

Study Lakes

In this study, we sampled five invasive macrophyte species in four lakes in Europe and Africa (see Figure 1), all of which differed in their chemical and physical parameters (Table 1). The first lake is the Hartbeespoort Dam in South Africa, invaded by the free-floating macrophyte *Pontederia crassipes* (Mart.) Solms. Since the 1960's the macrophyte infested the lake and covered up to 70% of the full lake. With a mix of mechanical, chemical and biological control measures since the 1990's the cover was reduced to 5% in the summer 2020 (Coetzee et al., 2021). In our second lake in Africa, the Lac municipal d'Ebolowa in Cameroon, we sampled beds of two different macrophytes. First the helophyte *Typha australis* Schumach. & Thonn. and second the free-floating *Pistia stratiotes* L. High nutrient input into the lake is leading to an eutrophication followed by a mass development of macrophytes and sedimentation a typical problem in other lakes in the region (Madjiki et al., 2013). In Europe we sampled Lac de Grand-Lieu in France invaded by the amphibious *Ludwigia grandiflora* subsp. *hexapetala* (Hook. & Arn.) G.L.Nesom & Kartesz and *Ludwigia peploides* subsp. *montevidensis* (Spreng.) P.H.Raven. Lac de Gran-Lieu is an important nature reserve and *Ludwigia spp.* became

139 invasive in the 1970's, threatening the native biodiversity and hindering human activity
140 (Youenn, 2021). The second lake in Europe is the Kemnader See in Germany invaded by
141 submersed *Elodea nuttallii* (Planch.) H. St John. Kemnader See is an important recreational
142 area in a densely populated area frequently used for all kinds of water activities. Since 2001 *E.*
143 *nuttallii* grows in most parts of the lake hindering activities on the lake (Podraza et al., 2008).
144 Our lakes cover a latitudinal gradient from 51°N to 25°S covering tropical (Lac municipal
145 d'Ebolowa), subtropical (Hartbeespoort Dam) and temperate (both European lakes) climates.
146 The lake surfaces range from 13 (Lac municipal d'Ebolowa) to 3700 ha (Lac de Grand-Lieu).

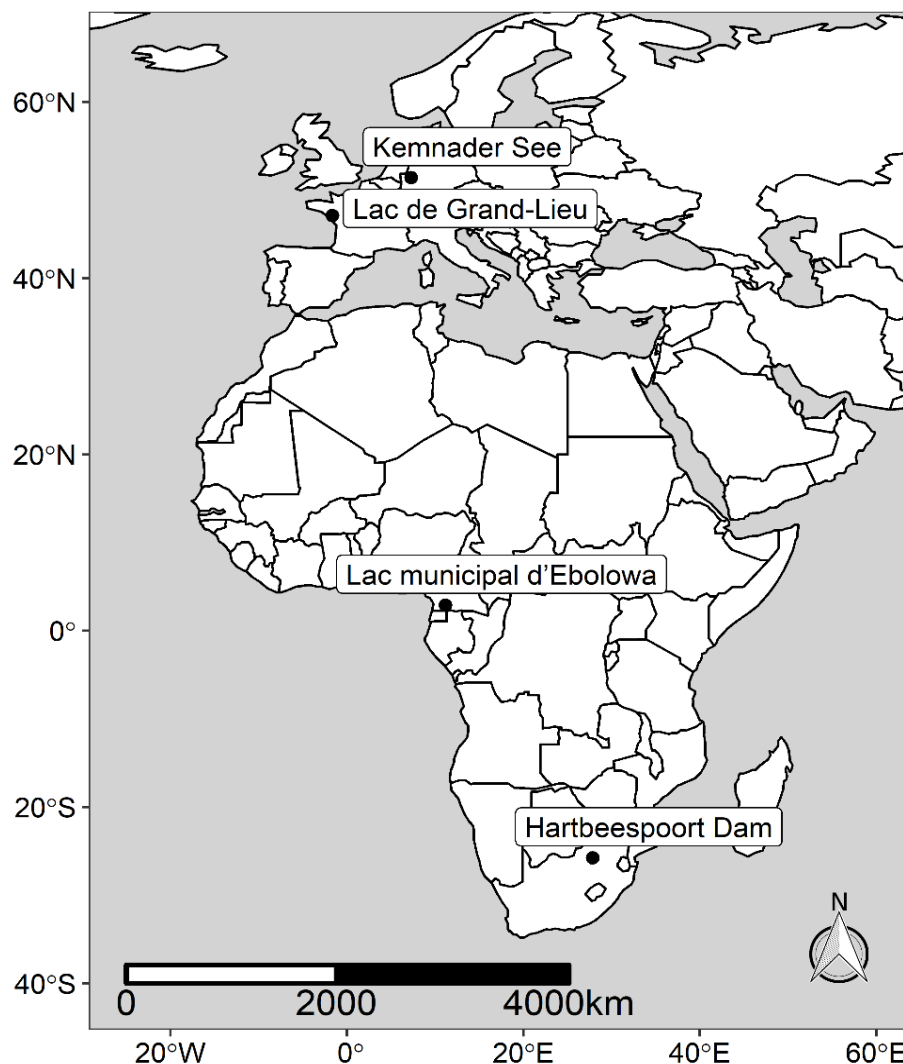


Figure 1: Location of the four lakes: Kemnader See in Germany, Lac de Grand-Lieu in France, Lac municipal d'Ebolowa in Cameroon and Hartbeespoort Dam in South Africa.

147 *Macroinvertebrate Sampling and Processing*

148 In each lake, two similar sections were selected for sampling: one with a dense macrophyte
 149 cover and one open water section without macrophytes. Replicates were distributed within the
 150 sections. Sediment samples were collected in both sections and additional sweep net samples
 151 were taken from vegetated sections to collect macroinvertebrates living within the macrophyte
 152 stands. The sample collection and processing varied slightly between lakes:

153 Table 1. Overview of study lakes characteristics

Lake	Country	Latitude Longitude	Altitude (m MSL.)	Description	Lake Surface (ha)	Depth* (m)	Trophic state	Macrophyte species	Life Form	Fish	Sampling date
Lac municipal d'Ebolowa	Cameroon	2° 55.3349' N 11° 9.4658' E	579	Artificial lake in the city	13	1.5	Hypereutrophic	<i>Typha australis</i> / <i>Pistia stratiotes</i>	helophyte/ free-floating	present	Mar-21
Hartbeespoort Dam	South Africa	25° 44.9572' S 27° 49.9966' E	1168	Reservoir lake	2060	4	Hypereutrophic	<i>Pontederia crassipes</i>	free-floating	present	Jan-20
Lac de Grand- Lieu	France	47° 8.0356' N 1° 40.4613' W	2	Natural shallow lake; Nature Reserve	3700	0.5	Eutrophic	<i>Ludwigia grandiflora</i> / <i>Ludwigia peploides</i>	amphibious	present	Jun-20
Kemnader See	Germany	51° 25.0188' N 7° 15.6079' E	71	Reservoir lake	1250	2,5	Eutrophic	<i>Elodea nuttallii</i>	submersed	present	Jul-20

154 *measured at the location of the sampling
155

- Lac de Grand-Lieu, Hartbeespoort Dam and Kemnader See:

Sediment samples were taken using an Ekman grab sampler sampling an area of 15cm by 15cm. Macroinvertebrates living within macrophyte stands were sampled using a sweep net with 250 µm mesh size. The net was swept harshly through the plants for a duration of 30 seconds and over an area of 1 m². At each lake, we took five sediment samples from the open water section, and ten sediment samples plus ten sweep samples within the macrophytes.

- Lac municipal d'Ebolowa:

The same method was used as described above, apart from using a 400 µm mesh sized sweep net (n=10). Also, this lake contained two different macrophyte types (*T. australis* and *P. stratiotes*), which were sampled separately and named as Ebolowa-Typha and Ebolowa-Pistia hereafter. For both vegetated sections the same open water section is used for comparisons. At both plant-free and the two vegetated sections, ten sediment samples were collected.

All samples were washed using a sieve with the same mesh size as the net used at that lake and were subsequently preserved in 96% ethanol. At the laboratory, macroinvertebrates were handpicked under a dissecting microscope. Macroinvertebrates were counted and identified to the lowest possible taxonomic level according to our expertise. Identification was based on Tachet et al. (2000), Day et al. (2001) and Durand and Lévêque (1981, 1980). For more detailed identification, we used additional references for Trichoptera (Waringer and Graf, 2011), Heteroptera (Savage, 1999), Odonata (Heidemann and Seidenbusch, 2002), Amphipods (Piscart and Bollache, 2012) and Gastropods (Glöer and Meier-Brook, 2003). Because sampled surface areas slightly differ between methods, all invertebrate densities were expressed as

individuals per m², for better comparison.

Statistical Analysis

All statistical analysis was performed in R version 3.6.3 (R Core Team, 2021). Comparisons of density (log+1 transformed) and taxonomic richness between different sample types were made based on Kruskal-Wallis Rank Sum Test (Kassambara, 2020a) and pairwise Wilcox tests from the stats package (R Core Team, 2021). Non parametric tests were chosen due to non-normal distribution of some parameters and *p*-values were adjusted with the Holm-Bonferroni method for multiple testing (Holm, 1979). Macroinvertebrate communities were compared among lakes, macrophyte life forms (free-floating/non-floating) and sample types with permutational multivariate ANOVA (PERMANOVA) using adonis2 function from the vegan package (Oksanen et al., 2019). This analysis was based on a Bray-Curtis dissimilarity matrix (Bray and Curtis, 1957). Venn diagrams, created with ggvenn (Yan, 2021), were used to show overlaps and differences in the macroinvertebrate community by sample type. Because different sampling methods were used, we estimated the total taxonomic richness and computed rarefaction curves, including extrapolations, to compare sampling coverage of the different sample types based on Hill numbers (order $q=0$) (Chao et al., 2014) using the iNEXT package (Hsieh et al., 2020). These diversity estimators allow for an unbiased comparison of effective number of taxa (Chao et al., 2014). Plots were generated using ggplot2 (Wickham, 2016) and ggpubr for boxplots (Kassambara, 2020b).

199 Results

200 *Comparison of taxonomic richness and density among sample types*

201 Taxonomic richness differs among the three sample types (sediment samples in open water,
202 sediment samples in macrophytes and sweep samples in macrophytes) in all lakes except
203 Kemnader See based on the Kruskal-Wallis test results (Figure 2; Table S1). In all samples,
204 but samples from Hartbeespoort Dam, we found significant differences between all sampling
205 type combinations according to the pairwise Wilcox test results (Figure 2). In Hartbeespoort

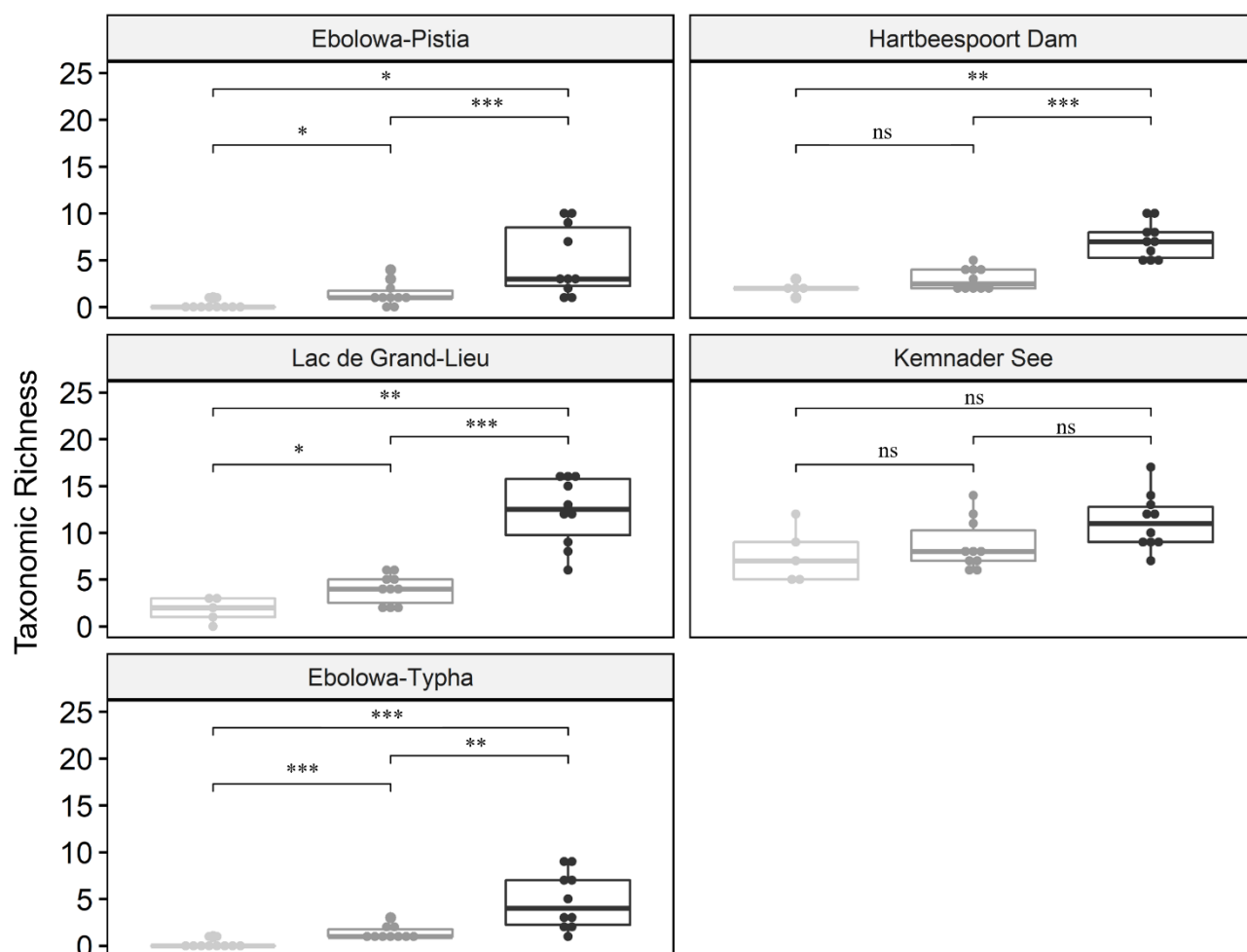


Figure 2: Taxonomic richness of macroinvertebrates recovered from different sampling methods in each macrophyte. Results from pairwise Wilcox tests are shown for all sample type pairs. Significance level is indicated as: ns: p-value > 0.05, *: <0.05, **: <0.01, ***: < 0.001

Dam, there is significantly higher taxonomic richness in sweep samples, but no differences between the two sediment sample types (Figure 2). For all macrophytes the highest estimated taxonomic richness was found in the sweep samples, followed by sediment samples in macrophytes and lowest in sediment samples in the open water (Table S2). Samples from free-floating plants (Ebolowa-Pistia, Hartbeespoort Dam) and non-floating plants (Lac de Grand-Lieu, Kemnader See, Ebolowa-Typha) showed similar patterns.

Macroinvertebrate communities found in each type of samples differed not only in taxonomic richness but also in their density. Figure 3 (and Table S3) shows the density of macroinvertebrates found in different sample types in each macrophyte. Significant differences of macroinvertebrate density among different sample types were found in all country (Table S4). The two types of sediment samples were different in Ebolowa-Pistia, Kemnader See and Ebolowa-Typha. Within plants sediment samples were different from sweep samples in all macrophytes except Ebolowa-Pistia. Open-water sediment samples differed from sweep samples only in Ebolowa-Typha. In all lakes the highest density was found in sediment samples taken within macrophyte stands, except in Hartbeespoort Dam where mean density was highest in sediment samples from the open water area. The lowest density was found either in sediment samples from the open water (Ebolowa-Pistia, Kemnader See, Ebolowa-Typha) or in sweep samples (Hartbeespoort dam, Lac de Grand-Lieu). There were no differences in density between samples collected from within stands of free-floating and non-floating macrophytes.

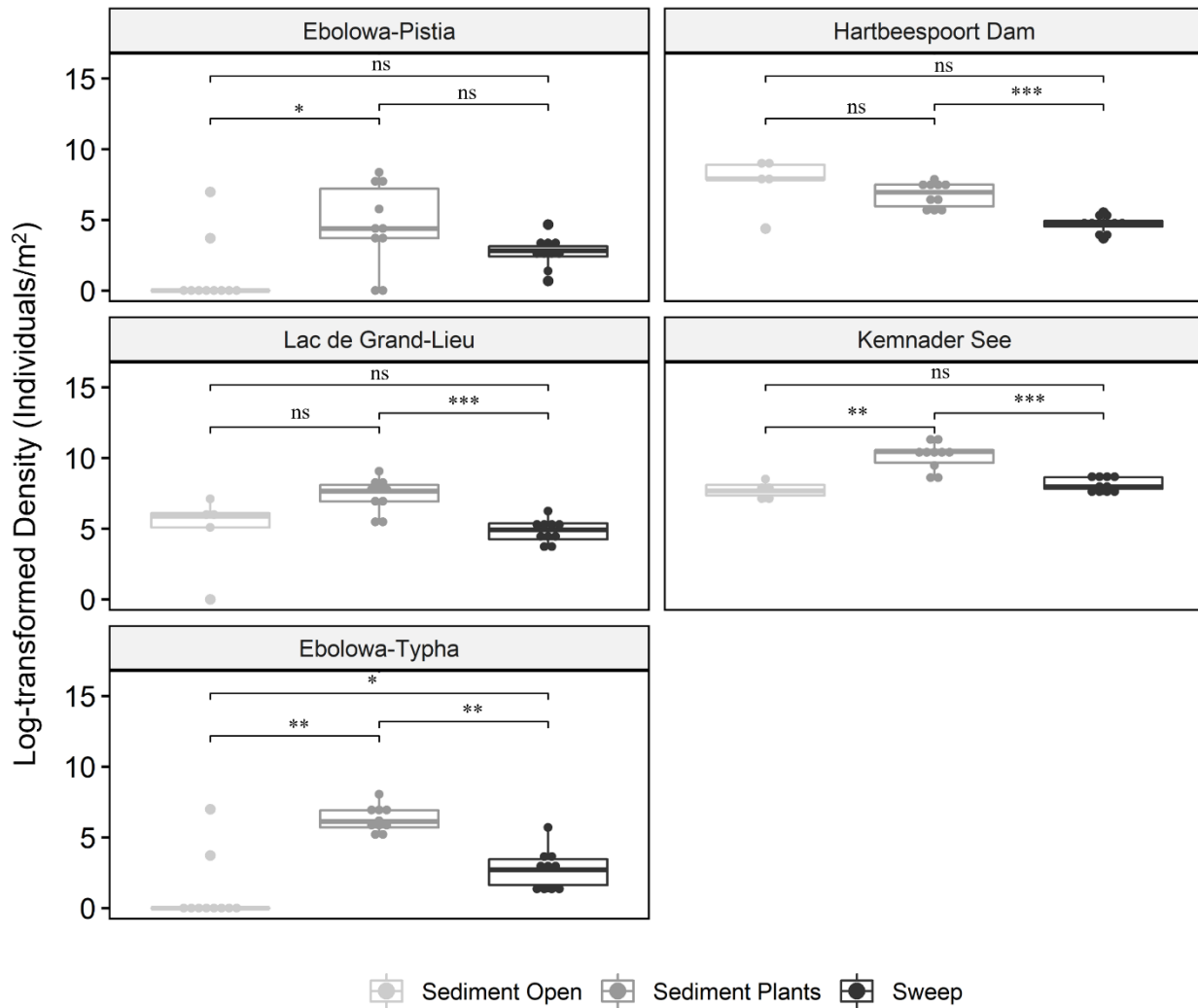


Figure 3: Log-transformed density of macroinvertebrates recovered from different sampling methods in each sampling site. Results from pairwise Wilcoxon tests are shown for all sample type pairs. Significance level is indicated as: ns: p-value > 0.05, *: <0.05, **: <0.01, ***: < 0.001

225 Comparisons of species assemblages

226 Based on the permutational multivariate ANOVA (PERMANOVA) analysis, 33.3% ($F = 35.8$,
 227 $p < 0.001$) of the dissimilarities among macroinvertebrate communities was explained by the
 228 lake (see Table S5). The interaction between lake and sample type accounted for 11.8% ($F =$
 229 6.3 , $p < 0.001$) of the dissimilarities. Further, the sample type accounted for 9.0% ($F = 14.6$,
 230 $p < 0.001$) of the dissimilarities, plant life form for 5.4% ($F = 17.5$, $p < 0.001$) and their

interaction for another 3.1% ($F = 5.0$, $p < 0.001$).

Presence and absence of taxa per sample type

The distribution of macroinvertebrate taxa in the three sample types varies among lakes (Figure 4, Table S6). In general, around 90% of the diversity in all lakes was covered solely by sweep samples (exclusive and shared taxa with other sample types), except in Kemnader See where the sweep samples cover 76% of all taxa. The distribution of species in Kemnader See samples was more even than any other site. In all lakes, we found species exclusive to the sweep samples. In Lac de Grand-Lieu and Kemnader See, some taxa were only present in the sediment samples in open water. Taxa exclusively present in sediment samples within plants were reported in Ebolowa-Pistia, Hartbeespoort Dam, Kemnader See and Ebolowa-Typha.

Common taxa found in all sample types included Oligochaeta (Ebolowa-Pistia, Hartbeespoort Dam, Kemnader See, Lac de Grand-Lieu), amphipods and isopods (Kemnader See), Hydracarina (Acari; Kemnader See), Sphaeriidae (Bivalvia; Lac de Grand-Lieu), Chironomidae (Diptera; Lac de Grand-Lieu, Kemnader See), *Caenis* sp. (Ephemeroptera; Kemnader See), Nematelminthes (Lac de Grand-Lieu) and the gastropods *Stagnicola* sp. (Ebolowa-Pistia, Ebolowa-Typha), *Physella acuta* (Kemnader See), *Gyraulus* sp. (Kemnader See) and *Potamopyrgus antipodarum* (Kemnader See). Several unique species were found within the open water samples: *Corbicula fluminea* (Bivalvia), *Chelicorophium sowinskyi* (Amphipoda), *Limnius* sp. (Coleoptera) and *Acroloxus lacustris* (Gastropoda) in Kemnader See and Ceratopogonidae (Diptera) in Lac de Grand-Lieu. Most taxa found in sediment samples were also found in the sweep samples. The five taxa restricted to sediment samples within plants were the two Diptera genus *Chironomus* sp. (Ebolowa-Pistia) and *Chaoborus* sp. (Hartbeespoort Dam), the two Gastropoda *Potadoma* sp. (Ebolowa-Pistia, Ebolowa-Typha)

254 and *Melania tuberculata* (Ebolowa-Pistia, Ebolowa-Typha) as well as Nematelminthes (Lac
255 de Grand-Lieu). In sweep samples, we found a long list of exclusive taxa from many different

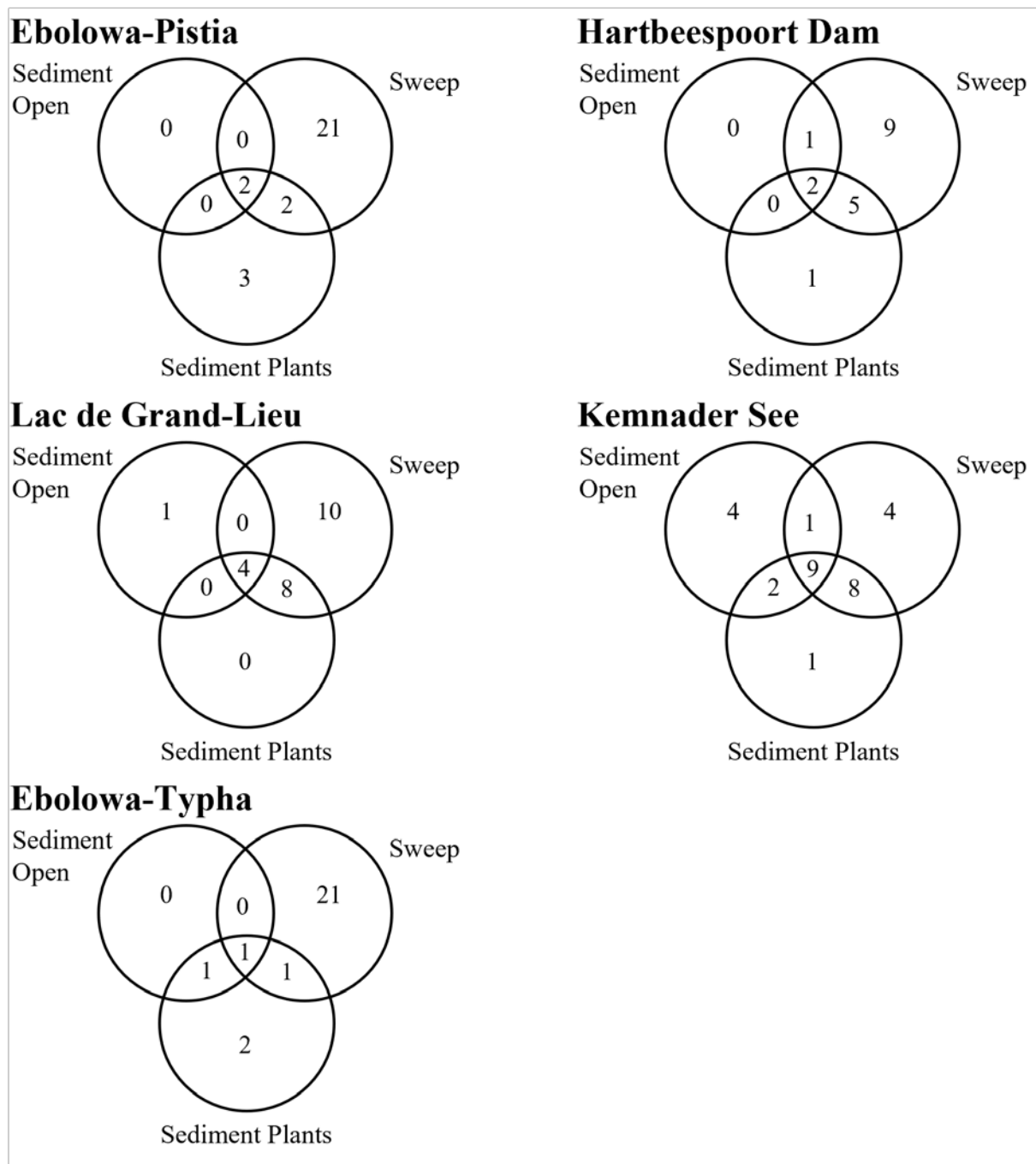


Figure 4: Venn diagrams showing taxa distributions in the three sample types.

256 groups (Table S6).

Macroinvertebrates diversity found in floating plants and in submersed plants differed, although both sampled macrophytes were located in Lac municipal d'Ebolowa and the total number of taxa was close (Figure 5). Ten taxa (Coleoptera: *Curculionidae*, *Cybister lateralimarginalis*, *Dryops* sp., *Hydrophilus* sp.; Gastropoda: *Galba truncatula*, *Gyraulus* sp., *Physa* sp.; Heteroptera: *Ranatra linearis*, *Notonecta* sp.; Arthropoda: *Chaoborus* sp.) only occurred in the sweep samples from the free-floating plants (Ebolowa-Pistia), and nine taxa (Diptera: *Simulini* sp.; Gastropoda: *Planorbis* sp.; Coleoptera: *Dytiscus* sp., *Chaetarthria* sp., Scirtidae; Heteroptera: *Belostoma* sp.; Odonata: *Coenagrion speciosa*, *Platynemis* sp.; Ephemeroptera: *Heptagenia* sp.) were only found in the sweep samples of the non-floating plant (Ebolowa-Typha).

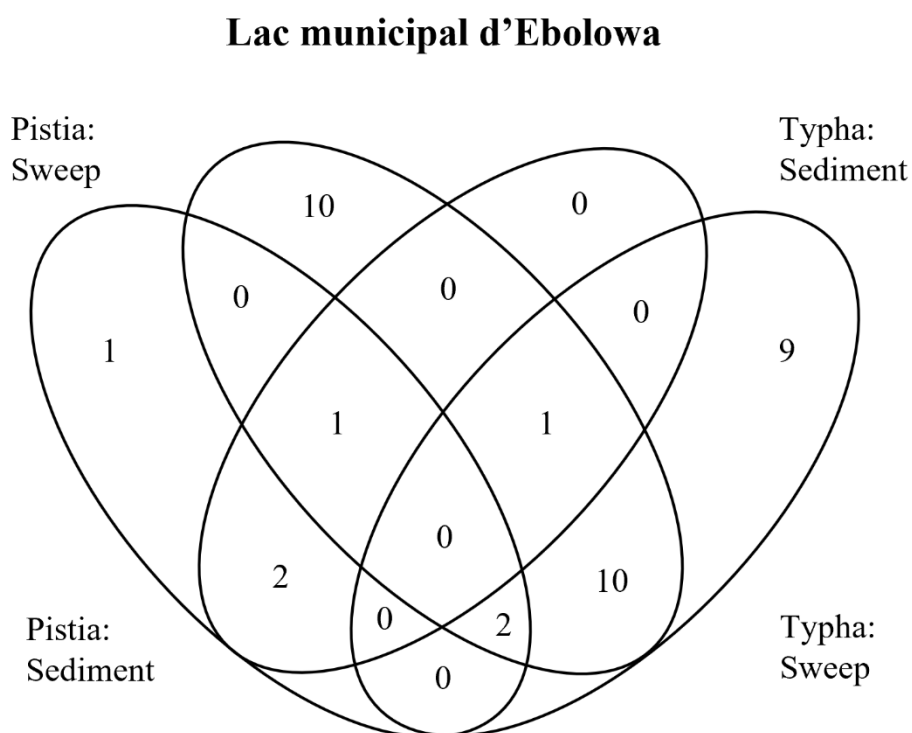


Figure 5: Venn diagram showing the taxa number in samples from within floating plants (Ebolowa-Pistia) and within submersed plants (Ebolowa-Typha) in Cameroon

Differences in sampling coverage by sampling type

Rarefaction curves (Figures 6) showed that reaching a high sample coverage (% of taxa present in the habitat found) requires a high number of samples. For a low number of samples, the curves show considerable differences in the sampling coverage of each sample type. For instance, with a total of five samples, we reach a coverage gap between the lowest and highest sampling coverage by sample type of 5% in Kemnader See, 15% in Hartbeespoort dam, 20% in Lac de Grand-Lieu, 22% in Ebolowa-Typha and 23% in Ebolowa-Pistia. To reach a sample coverage of 95%, based on the extrapolation curves, for open water sediment (Sediment Open) between 5 (Hartbeespoort Dam) and 15 samples (Ebolowa-Pistia), for sediment of the plant zone (Sediment Plants) between 10 (Hartbeespoort dam, Kemnader See) and 26 (Ebolowa-Typha) samples and for within macrophytes (Sweep) between 11 (Lac de Grand-Lieu) and 28 (Ebolowa-Typha) samples are needed. Within lakes, the number of samples which are needed to reach 95% coverage varies by sample type. The differences between number of samples needed per sampling type to reach 95% sample coverage are quite big. The gap between the first sample type to reach 95% coverage to the last one reaches from 11 samples (Ebolowa-Pistia, Kemnader See, Lac de Grand-Lieu) up to 18 samples (Hartbeespoort Dam). Considering the mean number (averaged over all sites) of samples necessary to reach a 95% coverage of the community we need 9.8 sediment sediment samples in open water sections, 17.2 sediment samples in macrophytes and 18.4 sweep samples.

288

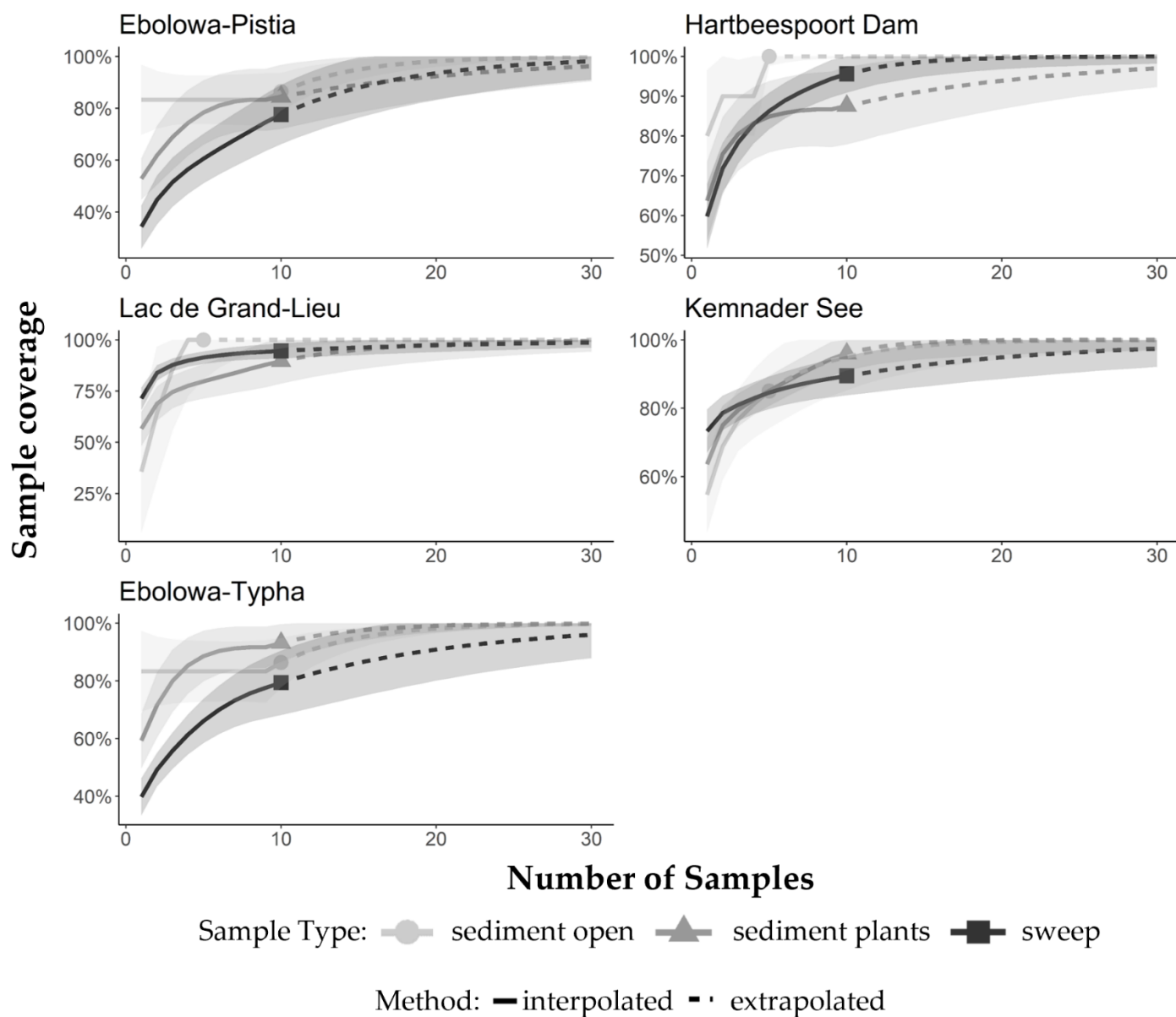


Figure 6: Rarefaction curves showing the sampling coverage depending on the number of samples. Solid line indicates interpolated data and dashed lines extrapolated data. Colour and symbol represent different sample types.

Discussion

This study aims to understand the role of dense mats of macrophytes as a habitat for macroinvertebrates and to provide the first methodical analysis on sampling efficiency of different sampling methods inside and outside of macrophyte stands. The mix of different plant species in four lakes with different characteristics allows us to disentangle global from local effects.

The effect of invasive macrophytes on macroinvertebrate assemblages

Differences in density between sediment samples and sweep samples are biased by the method used, but the density in the two sediment sample types are well comparable. We found a significant increased macroinvertebrate density in Kamnader See, Ebolowa-Pistia and Ebolowa-Typha. Lac de Grand-Lieu showed a trend to a higher density were as in Hartbeespoort Dam the density tends to be lower within the macrophytes. The increase macroinvertebrate density in the sediment in macrophytes can not only be explained by increased habitat heterogeneity by the presence of macrophytes but also by the availability of food sources. Macrophytes offer a direct food source as plant litter is accumulating under macrophyte beds. In addition macrophytes can increase the growth of periphyton on the sediment and plant litter offers an extra surface where periphyton can grow (Smock and Stoneburner, 1980; Vermaire et al., 2013; Wolters et al., 2019). Macrophytes therefore not only offer a food source for Shredders but also for Gatherers and Scrapers on the sediment (Hill, 1985). Sediment samples from Hartbeespoort Dam were the only ones showing a slightly lower macroinvertebrates density in macrophytes. This pattern could be the results of low DO levels within macrophyte stands. Former studies have shown that reduced DO levels lead to decreases of macroinvertebrate density (Casco et al., 2014; Coetzee et al., 2014). The fact that mainly

Oligochaeta and Hirudinea were found in the area also supports the hypothesis as these two taxa are known for their tolerance for low oxygen levels (Glasby et al., 2021). Taxonomic richness in the sediment samples was also higher between the plants compared to the plant-free section. Only in Hartbeespoort Dam and Kemnader See we did not find a significant increase in taxonomic richness. Increased macroinvertebrate density combined with the above described increase in habitat and food resources can be expected to cause this increase in richness. Sweep samples showed a higher taxonomic richness (measured as well as estimated richness) and different taxa than in sediment samples were found. Among the many contrasting characteristics of open water and macrophyte stands, the most fundamental one is structural heterogeneity. The presence of macrophytes offers a complex structure in the water column, providing more diverse habitats and therefore leading to increased macroinvertebrate diversity. This result aligns with findings from earlier studies (Ferreiro et al., 2014; Labat et al., 2022; Scheffer, 2004; Thomaz et al., 2008; Walker et al., 2013). Only in Kemnader See no higher taxa richness was found within the macrophytes with respect to that in open water. Explanations for this could be the production of allelopathic chemicals by *Elodea nuttallii* harming phytoplankton and epiphytic algae in the macrophytes and therefore reducing the benefits of macrophytes (Erhard and Gross, 2006). Another possible explanation could be a higher sediment heterogeneity outside the macrophytes, which we observed during the sampling. Both, higher taxonomic richness and density within macrophytes confirms our first hypothesis.

The impact of different plant types

No difference was found comparing samples from floating and non-floating plants regarding taxa richness. Non-floating macrophytes generate more complex underwater structures than

free floating plants which grow on the water surface and build roots which don't reach down to the sediment (Meerhoff et al., 2003; Thomaz et al., 2008). Hypothesis (iii) can therefore not be confirmed. Even if they are don't grow down to the sediment, plant litter will accumulate under the plant cover. This plant litter only seems to be enough to have comparable effects as the presence of rooted macrophytes. The effects of plant types in our study may be masked by other factors like climate and/or eutrophic levels as they have more profound effects on the aquatic ecosystem across a larger geographic scale (Kosten et al., 2009; Pan et al., 2015). In our study, the two lakes with floating plants (Lac municipal d'Ebolowa, Hartbeespoort Dam) are hypereutrophic and situated in a subtropical or tropical climate, while the lakes with non-floating plants share no common feature. To fully understand the impacts of plant types on macroinvertebrate communities at a large scale, more lakes and macrophytes should be included for further investigations. Nonetheless, the comparisons between samples from within floating and submersed plants in our lake in Cameroon provide insights on the effects of plant types on the macroinvertebrate communities at a local scale. Only 43% of the taxa were found in both plants, while the rest remained exclusive to only one of the two plants. This finding is in line with research from Walker et al. (2013), which showed that different macrophyte species accommodate different macroinvertebrate communities and taxa. Structures provided by macrophytes differ strongly depending on plant life type and species (Thomaz et al., 2008). Aquatic systems with a diverse macrophyte community could be expected to support higher macroinvertebrate diversity than systems dominated by monocultures, as they provide even higher levels of structural heterogeneity (Yofukuji et al., 2021).

Presence and absence of macroinvertebrates in different sample types

Most of the macroinvertebrate taxa found in plants were only found in sweep samples and only

few taxa were unique to sediment samples in our study. The presence of macrophyte offers new and different habitats for macroinvertebrates. Increased habitat heterogeneity from macrophytes offers a niche for a broad variety of invertebrate taxa (Morse et al., 1985; Thomaz et al., 2008). Macroinvertebrate communities were mostly dominated by one or two taxa, although sweep samples also contained several other taxa with intermediate abundance. In most lakes, the same abundant taxa were found in all sample types. However, in samples around macrophytes, especially sweep samples, we found many additional taxa with high abundance known to be herbivorous or/and detritivorous. This includes Gastropoda, Lepidoptera, Ephemeroptera or Isopoda, which feed on plant tissue or the algal biofilm growing on it (Tachet et al., 2000). In addition, a higher density of Coleoptera, Odonata and Hydracarina associated with plants was also detected. All of these taxa are known to be predators to which the increased prey density (macroinvertebrates as well as zooplankton) in macrophytes offers a rich hunting ground (Sagrario et al., 2009). The presence of macrophytes therefore increases the complexity of the macroinvertebrate communities based on the fact that many of those taxa were found in the sweep samples only. Yet, the presence of unique taxa to only one of the three sampling types is reported for all three types of habitats. This finding has important implications for the proper sampling strategy for waterbodies with macrophytes. Sweep samples need to be added to the classical sediment sampling when macrophytes are present as they contain a high diversity exclusive to this sampling type. However, as both sediment samples in open water sections and macrophytes can contain exclusive taxa, sediment samples should be taken in both open water and under macrophyte stands.

The effect of invasive macrophytes on the sampling efficiency

Sampling efficiency differs between sample types. Such gaps are biggest when collecting only

a low number of samples (usually less than 5) which is standard for many research projects and biomonitoring programs. The taxa richness within macrophytes tends to be underestimated compared to open water sections if only a low number of samples are taken. In current macroinvertebrate sampling protocols, such differences in sampling efficiency are overlooked (for example in Oertli et al., 2005; Indermuehle et al., 2010). A project aiming to achieve a complete profile of the macroinvertebrates living in a certain waterbody with macrophytes, requires combined usage of different sampling methods, and the number of samples needs to be high enough to avoid differences in sampling coverage. However, in practice, taking a higher number of samples is difficult to achieve as it usually increases expenses and workload. For research and biomonitoring, the reduction of labor and costs is crucial. However, from a scientific point of view, a higher sample number leads to better comparable results. Finding the right balance between high precision and low labor and costs is a difficult task. Our study shows differences in sampling efficiency with low number of samples. If high number of samples are not possible, a more comprehensive methodical research to develop more comparable sampling strategies is in need to provide a reliable and strong knowledge foundation for making management decision. These findings could be used as part of the guideline for using a commonly accepted method when sampling aquatic systems with macrophytes. Factors like trophic state, climate, system size, plant type and plant species, leading to different patterns among lakes, need to be studied further. Similar findings can also be applied to sampling in rivers inhabited by macrophytes, but such notions remain to be validated by further studies covering more diverse freshwater bodies.

Conclusion

Under increasing anthropogenic pressure, climate change and the increasing spread of invasive

species, aquatic systems are under tremendous pressure and aquatic biodiversity is declining. To reduce the decline of aquatic diversity, good management is the key. All lakes in our study are colonised by an invasive species. Before the invasion of plants, only small parts of the lakes were colonised by macrophytes, and the invasion with macrophytes created new habitats for macroinvertebrates and other organism groups. Even if invasive macrophytes are often considered as problematic and are removed (Hussner et al., 2017; Thiemer et al., 2021), we found positive effects for macroinvertebrates. As long as invasive plants don't repress a more diverse native vegetation, an invasion could be seen as beneficial from a macroinvertebrate perspective. Our findings call for a more flexible management plan for waterbodies with invasive macrophytes, taking these positive effects of invasive macrophytes into account. Moreover, a good management strategy needs to be based on robust scientific data. With this study, we have shown that the presence of macrophytes affects the sampling efficiency of the standard sampling methods, which makes it challenging to reach comparable results of macroinvertebrate sampling. Therefore, further research is needed to tackle this bias.

Acknowledgments

We thank all members from the MadMacs project, especially Jan Köhler, Sabine Hill and Julie Coetzee for organizing the fieldtrips as well as Lena Schulz, Lisa Sanden, Bertrand le Rouzic, Guillaume Bouger and Augustin Soulard for their support during the field campaigns. We thank the Aquabio Team involved in taking and processing some of the samples. A big thank you to Aijuan Liao to her input during the writing process. This project would not have been possible without a good collaboration with the Local Stakeholders National Nature Reserve of the Lake Grand-Lieu, Ruhrverband and the Department of Water and Sanitation South Africa. This study was funded by the French Agence National de Recherche (N° ANR-18-IC4W-0004-06), the South African Water Research Commission (K5/2951), the German Federal Ministry of Education and Research (033WU005), the Research Council of Norway (297202/E10), and the Fundação Araucária in Brazil (N° 186/2019) for funding of MadMacs (Mass development of aquatic macrophytes - causes and consequences of macrophyte removal for ecosystem structure, function, and services) in the framework of the collaborative international consortium of the 2017 call of the Water Challenges for a Changing World Joint Programme Initiative (Water JPI). Additional funding was provided by Aquabio (Saint-Germain-du-Puch, France).

Data Availability Statement

The data presented in this study are available on request from the corresponding author.

Conflicts of Interest

The authors declare no conflict of interest.

Author Contributions

Conceptualization: B.M., G.T., A.P. and C.P.; methodology: B.M., G.T., A.P. and C.P.; validation: L.K.F.; formal analysis: B.M., F.L. and A.P. investigation: B.M., N.C.B., L.K.F. S.F.H. S.N.M. resources: C.P.; data curation: B.M., L.K.F., F.L. and S.F.H.; writing—original draft preparation: B.M.; writing—review and editing: B.M., N.C.B., L.K.F., S.F.H., F.L., S.N.M., G.T., A.P. and C.P.; visualization: B.M.; supervision: G.T., A.P., and C.P.; project administration: G.T.; funding acquisition: G.T. All authors have read and approved the published version of the manuscript.

References

- Balci, P., Kennedy, J.H., 2003. Comparison of Chironomids and Other Macroinvertebrates Associated with *Myriophyllum spicatum* and *Heteranthera dubia*. *Journal of Freshwater Ecology* 18, 235–247. <https://doi.org/10.1080/02705060.2003.9664490>
- Blomqvist, S., 1991. Quantitative sampling of soft-bottom sediments: problems and solutions. *Marine Ecology Progress Series* 72, 295–304.
- Bray, J.R., Curtis, J.T., 1957. An Ordination of the Upland Forest Communities of Southern Wisconsin. *Ecological Monographs* 27, 325–349. <https://doi.org/10.2307/1942268>
- Burks, R.L., Mulderij, G., Gross, E., Jones, I., Jacobsen, L., Jeppesen, E., Van Donk, E., 2006. Center Stage: The Crucial Role of Macrophytes in Regulating Trophic Interactions in Shallow Lake Wetlands, in: Bobbink, R., Beltman, B., Verhoeven, J.T.A., Whigham, D.F. (Eds.), *Wetlands: Functioning, Biodiversity Conservation, and Restoration*. Springer Berlin Heidelberg, Berlin, Heidelberg, pp. 37–59. https://doi.org/10.1007/978-3-540-33189-6_3
- Caraco, N., Cole, J., Findlay, S., Wigand, C., 2006. Vascular Plants as Engineers of Oxygen in Aquatic Systems. *BioScience* 56, 219–225. [https://doi.org/10.1641/0006-3568\(2006\)056\[0219:VPAEOO\]2.0.CO;2](https://doi.org/10.1641/0006-3568(2006)056[0219:VPAEOO]2.0.CO;2)
- Caraco, N.F., Cole, J.J., 2002. Contrasting Impacts of a Native and Alien Macrophyte on Dissolved Oxygen in a Large River. *Ecological Applications* 12, 1496–1509. [https://doi.org/10.1890/1051-0761\(2002\)012\[1496:CIOANA\]2.0.CO;2](https://doi.org/10.1890/1051-0761(2002)012[1496:CIOANA]2.0.CO;2)
- Casco, S.L., Carnevali, R.P., Poi, A.S.G., Neiff, J.J., 2014. The Influence of Water Hyacinth Floating Meadows on Limnological Characteristics in Shallow Subtropical Waters. *American Journal of Plant Sciences* 2014. <https://doi.org/10.4236/ajps.2014.513212>
- Cattaneo, A., Galanti, G., Gentinetta, S., Susana, A., 1998. Epiphytic algae and macroinvertebrates on submerged and floating-leaved macrophytes in an Italian lake. *Freshwater Biology* 39, 725–740. <https://doi.org/10.1046/j.1365-2427.1998.00325.x>

- Chao, A., Gotelli, N.J., Hsieh, T.C., Sande, E.L., Ma, K.H., Colwell, R.K., Ellison, A.M.,
2014. Rarefaction and extrapolation with Hill numbers: a framework for sampling and
estimation in species diversity studies. *Ecological Monographs* 84, 45–67.
- Chapman, L.J., Scnelder, K.R., Apodaca, C., Chapma, C.A., 2004. Respiratory Ecology of
Aquatic Macroinvertebrates between dissolved oxygen and invertebrate. *Biotropica* 36,
572–585. <https://doi.org/10.1111/j.1744-7429.2004.tb00351.x>
- Coetzee, J.A., Bownes, A., Martin, G.D., Miller, B.E., Smith, R., Weyl, P.S.R., Hill, M.P.,
2021. A Review of the Biocontrol Programmes Against Aquatic Weeds in South
Africa. *afen* 29, 935–964. <https://doi.org/10.4001/003.029.0935>
- Coetzee, J.A., Jones, R.W., Hill, M.P., 2014. Water hyacinth, *Eichhornia crassipes*
(Pontederiaceae), reduces benthic macroinvertebrate diversity in a protected
subtropical lake in South Africa. *Biodivers Conserv* 23, 1319–1330.
<https://doi.org/10.1007/s10531-014-0667-9>
- Cyr, H., Face, M.L., 1993. Magnitude and patterns of herbivory in aquatic and terrestrial
ecosystems. *Nature* 361, 148–150. <https://doi.org/10.1038/361148a0>
- Dahl, J., Greenberg, L.A., 1997. Effects of fish predation and habitat type on stream benthic
communities. *Hydrobiologia* 361, 67–76. <https://doi.org/10.1023/A:1003137309726>
- Day, J., Stewart, B. (Eds.), 2001. Guides to the Freshwater Invertebrates of Southern Africa.
Water Research Commission, Pretoria.
- Dehedin, A., Maazouzi, C., Puijalon, S., Marmonier, P., Piscart, C., 2013. The combined
effects of water level reduction and an increase in ammonia concentration on organic
matter processing by key freshwater shredders in alluvial wetlands. *Global Change
Biology* 19, 763–774. <https://doi.org/10.1111/gcb.12084>
- Dodds, W., Whiles, M., 2019. Freshwater Ecology - 3rd Edition, 3rd Edition. ed. Academic
Press, Salt Lake City.
- Durand, J.-R., Lévêque, C., 1981. Flore et faune aquatiques de l’Afrique Sahélo-
soudanienne : tome 2. Insectes et vertébrés. ORSTOM, Paris.
- Durand, J.-R., Lévêque, C., 1980. Flore et faune aquatiques de l’Afrique Sahélo-
soudanienne : tome 1. ORSTOM, Paris.
- Erhard, D., Gross, E.M., 2006. Allelopathic activity of *Elodea canadensis* and *Elodea nuttallii*
against epiphytes and phytoplankton. *Aquatic Botany* 85, 203–211.
<https://doi.org/10.1016/j.aquabot.2006.04.002>
- Ferreiro, N., Feijoó, C., Giorgi, A., Rosso, J., 2014. Macroinvertebrates select complex
macrophytes independently of their body size and fish predation risk in a Pampean
stream. *Hydrobiologia* 740, 191–205. <https://doi.org/10.1007/s10750-014-1953-y>
- Frodge, J.D., Thomas, G.L., Pauley, G.B., 1990. Effects of canopy formation by floating and
submergent aquatic macrophytes on the water quality of two shallow Pacific
Northwest lakes. *Aquatic Botany* 38, 231–248. [https://doi.org/10.1016/0304-3770\(90\)90008-9](https://doi.org/10.1016/0304-3770(90)90008-9)
- García-Criado, F., Trigo, C., 2005. Comparison of several techniques for sampling
macroinvertebrates in different habitats of a North Iberian pond. *Hydrobiologia* 545,
103–115. <https://doi.org/10.1007/s10750-005-2741-5>
- Glasby, C.J., Erséus, C., Martin, P., 2021. Annelids in Extreme Aquatic Environments:
Diversity, Adaptations and Evolution. *Diversity* 13, 98.
<https://doi.org/10.3390/d13020098>
- Glöer, P., Meier-Brook, C., 2003. Süßwassermollusken - Ein Bestimmungsschlüssel für die
Bundesrepublik Deutschland. Deutscher Jugendbund für Naturbeobachtung,
Hamburg.

- Heidemann, H., Seidenbusch, R., 2002. Larves et exuvies des libellules de France et d'Allemagne (sauf de Corse). Société Française d'Odonatologie, Bois-d'Arcy.
- Hill, B.H., 1985. The breakdown of macrophytes in a reservoir wetland. *Aquatic Botany* 21, 23–31. [https://doi.org/10.1016/0304-3770\(85\)90092-0](https://doi.org/10.1016/0304-3770(85)90092-0)
- Holm, S., 1979. A Simple Sequentially Rejective Multiple Test Procedure. *Scandinavian Journal of Statistics* 6, 65–70.
- Hsieh, T.C., Ma, K.H., Chao, A., 2020. iNEXT: Interpolation and Extrapolation for Species Diversity.
- Hussner, A., Stiers, I., Verhofstad, M.J.J.M., Bakker, E.S., Grutters, B.M.C., Haury, J., van Valkenburg, J.L.C.H., Brundu, G., Newman, J., Clayton, J.S., Anderson, L.W.J., Hofstra, D., 2017. Management and control methods of invasive alien freshwater aquatic plants: A review. *Aquatic Botany* 136, 112–137. <https://doi.org/10.1016/j.aquabot.2016.08.002>
- Indermuhle, N., Angélibert, S., Rosset, V., Oertli, B., 2010. The pond biodiversity index “IBEM”: A new tool for the rapid assessment of biodiversity in ponds from Switzerland. Part 2. Method description and examples of application. *Limnetica* 29, 105–120.
- Jacobsen, D., Sand-Jensen, K., 1992. Herbivory of invertebrates on submerged macrophytes from Danish freshwaters. *Freshwater Biology* 28, 301–308. <https://doi.org/10.1111/j.1365-2427.1992.tb00588.x>
- Jónasson, P.M., 2004. The Lakes Handbook, Volume 1: Limnology and Limnetic Ecology | Wiley, in: *The Lakes Handbook Volume 1: Limnology and Limnetic Ecology*. Blackwell Publishing, Victoria.
- Kassambara, A., 2020a. rstatix: Pipe-Friendly Framework for Basic Statistical Tests.
- Kassambara, A., 2020b. ggpubr: “ggplot2” Based Publication Ready Plots.
- Kornijów, R., 2014. A quantitative sampler for collecting invertebrates associated with deep submerged vegetation. *Aquat Ecol* 48, 417–422. <https://doi.org/10.1007/s10452-014-9494-z>
- Kosten, S., Kamarainen, A., Jeppesen, E., Van Nes, E.H., Peeters, E.T.H.M., Mazzeo, N., Sass, L., Hauxwell, J., Hansel-Welch, N., Lauridsen, T.L., Søndergaard, M., Bachmann, R.W., Lacerot, G., Scheffer, M., 2009. Climate-related differences in the dominance of submerged macrophytes in shallow lakes. *Global Change Biology* 15, 2503–2517. <https://doi.org/10.1111/j.1365-2486.2009.01969.x>
- Labat, F., Piscart, C., Thiébaud, G., 2022. Invertebrates in small shallow lakes and ponds: a new sampling method to study the influence of environmental factors on their communities. *Aquat Ecol*. <https://doi.org/10.1007/s10452-021-09939-1>
- Li, K.-Y., Liu, Z.-W., Hu, Y.-H., Yang, H.-W., 2009. Snail herbivory on submerged macrophytes and nutrient release: Implications for macrophyte management. *Ecological Engineering* 35, 1664–1667. <https://doi.org/10.1016/j.ecoleng.2008.05.009>
- Lodge, D.M., 1991. Herbivory on freshwater macrophytes. *Aquatic Botany, Ecology of Submersed Aquatic Macrophytes* 41, 195–224. [https://doi.org/10.1016/0304-3770\(91\)90044-6](https://doi.org/10.1016/0304-3770(91)90044-6)
- Madjiki, A.G., Pial, A.-C., Ndam, N.J.-R., 2013. Caractérisation hydrologique, morphométrique et physicochimique d'un hydro- système urbain : le lac municipal d'Ebolowa (Sud-Cameroun) 13.
- Mann, K.H., 1988. Production and use of detritus in various freshwater, estuarine, and coastal marine ecosystems. *Limnology and Oceanography* 33, 910–930. <https://doi.org/10.4319/lo.1988.33.4part2.0910>

- Meerhoff, M., Mazzeo, N., Moss, B., Rodríguez-Gallego, L., 2003. The structuring role of free-floating versus submerged plants in a subtropical shallow lake. *Aquatic Ecology* 37, 377–391. <https://doi.org/10.1023/B:AECO.0000007041.57843.0b>
- Morse, D.R., Lawton, J.H., Dodson, M.M., Williamson, M.H., 1985. Fractal dimension of vegetation and the distribution of arthropod body lengths. *Nature* 314, 731–733. <https://doi.org/10.1038/314731a0>
- Newman, R.M., 1991. Herbivory and Detritivory on Freshwater Macrophytes by Invertebrates: A Review. *Journal of the North American Benthological Society* 10, 89–114. <https://doi.org/10.2307/1467571>
- Ntislidou, C., Lazaridou, M., Tsiaoussi, V., Bobori, D.C., 2018. A new multimetric macroinvertebrate index for the ecological assessment of Mediterranean lakes. *Ecological Indicators* 93, 1020–1033. <https://doi.org/10.1016/j.ecolind.2018.05.071>
- Oertli, B., Auderset Joye, D., Castella, E., Juge, R., Lehmann, A., Lachavanne, J.-B., 2005. PLOCH: a standardized method for sampling and assessing the biodiversity in ponds. *Aquatic Conserv: Mar. Freshw. Ecosyst.* 15, 665–679. <https://doi.org/10.1002/aqc.744>
- Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., Szoecs, E., Wagner, H., 2019. *vegan: Community Ecology Package*.
- Pan, B.-Z., Wang, H.-Z., Pusch, M.T., Wang, H.-J., 2015. Macroinvertebrate responses to regime shifts caused by eutrophication in subtropical shallow lakes. *Freshwater Science*. <https://doi.org/10.1086/682077>
- Piscart, C., Bollache, L., 2012. *Crustacés amphipodes de surface : gammares d'eau douce., Introduction pratique à la systématique des organismes des eaux continentales de France*. Association Française de Limnologie.
- Podraza, P., Brinkmann, T., Evers, P., Von Felde, D., Frost, U., Klopp, R., Knotte, H., Kühlmann, M., Kuk, M., Lipka, P., Nusch, E.A., Stengert, M., Van der Weyer, K., 2008. Untersuchungen zur Massenentwicklung von Wasserpflanzen in den Ruhrstauseen und Gegenmaßnahmen [Assesment of the mass development of macrophytes in the reservoirs along the Ruhr and countermeasures] (No. 54.173/25-5230). in *istat für Umwelt und Naturschutz, Landwirtschaft und Verbraucherschutz des Landes NRW*.
- Porst, G., Miler, O., Donohue, L., Jurca, T., Pilotto, F., Brauns, M., Solimini, A., Pusch, M., 2016. Efficient sampling methodologies for lake littoral invertebrates in compliance with the European Water Framework Directive. *Hydrobiologia* 767, 207–220. <https://doi.org/10.1007/s10750-015-2500-1>
- R Core Team, 2021. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria.
- Sagrario, G., De Los Angeles, M., Balseiro, E., Ituarte, R., Spivak, E., 2009. Macrophytes as refuge or risky area for zooplankton: a balance set by littoral predacious macroinvertebrates. *Freshw Biol* 54, 1042–1053. <https://doi.org/10.1111/j.1365-2427.2008.02152.x>
- Sato, M., Nishijima, S., Miyashita, T., 2014. Differences in refuge function for prey and tolerance to crayfish among macrophyte species. *Limnology* 15, 27–35. <https://doi.org/10.1007/s10201-013-0410-3>
- Savage, A.A., 1999. *Key to the Larvae of British Corixidae*. Freshwater Biological Association, Far Sawrey.
- Scheffer, M., 2004. *Ecology of Shallow Lakes, Population and Community Biology Series*. Springer Netherlands, Dordrecht. <https://doi.org/10.1007/978-1-4020-3154-0>

- Sly, P.G., 1969. Bottom sediment sampling. 12th Int. Ass. Great Lakes Res. Mtg.; (United States).
- Smock, L.A., Stoneburner, D.L., 1980. The Response of Macroinvertebrates to Aquatic Macrophyte Decomposition. *Oikos* 35, 397–403. <https://doi.org/10.2307/3544656>
- Strayer, D.L., 2010. Alien species in fresh waters: ecological effects, interactions with other stressors, and prospects for the future. *Freshwater Biology* 55, 152–174. <https://doi.org/10.1111/j.1365-2427.2009.02380.x>
- Sychra, J., Adamek, Z., 2010. Sampling Efficiency of Gerking Sampler and Sweep Net in Pond Emergent Littoral Macrophyte Beds – a Pilot Study. *Turk. J. Fish. Aquat. Sci.* 10. <https://doi.org/10.4194/trjfas.2010.0201>
- Tachet, H., Richoux, P., Bournaud, M., Usseglio-Polatera, P., 2000. Invertébrés d’eau douce: Systématique, biologie, écologie. CNRS ÉDITIONS, Paris.
- Taniguchi, H., Nakano, S., Tokeshi, M., 2003. Influences of habitat complexity on the diversity and abundance of epiphytic invertebrates on plants. *Freshwater Biology* 48, 718–728. <https://doi.org/10.1046/j.1365-2427.2003.01047.x>
- Thierner, K., Schneider, S., Demars, B., 2021. Mechanical removal of macrophytes in freshwater ecosystems: Implications for ecosystem structure and function. *Science of The Total Environment* 782, 146671. <https://doi.org/10.1016/j.scitotenv.2021.146671>
- Thomaz, S.M., Dibble, E.D., Evangelista, L.R., Higuti, J., Bini, L.M., 2008. Influence of aquatic macrophyte habitat complexity on invertebrate abundance and richness in tropical lagoons. *Freshwater Biology* 53, 358–367. <https://doi.org/10.1111/j.1365-2427.2007.01898.x>
- Verberk, W.C.E.P., Bilton, D.T., 2013. Respiratory control in aquatic insects dictates their vulnerability to global warming. *Biol Lett* 9, 20130473. <https://doi.org/10.1098/rsbl.2013.0473>
- Verhofstad, M.J.J.M., Alirangues Núñez, M.M., Reichman, E.P., van Donk, E., Lamers, L.P.M., Bakker, E.S., 2017. Mass development of monospecific submerged macrophyte vegetation after the restoration of shallow lakes: Roles of light, sediment nutrient levels, and propagule density. *Aquatic Botany* 141, 29–38. <https://doi.org/10.1016/j.aquabot.2017.04.004>
- Vermaire, J., Greffard, M.-H., Saulnier-Talbot, É., Gregory-Eaves, I., 2013. Changes in submerged macrophyte abundance altered diatom and chironomid assemblages in a shallow lake. *Journal of Paleolimnology* 50. <https://doi.org/10.1007/s10933-013-9737-3>
- Vilas, M.P., Marti, C.L., Adams, M.P., Oldham, C.E., Hipsey, M.R., 2017. Invasive Macrophytes Control the Spatial and Temporal Patterns of Temperature and Dissolved Oxygen in a Shallow Lake: A Proposed Feedback Mechanism of Macrophyte Loss. *Front. Plant Sci.* 8. <https://doi.org/10.3389/fpls.2017.02097>
- Walker, P.D., Wijnhoven, S., van der Velde, G., 2013. Macrophyte presence and growth form influence macroinvertebrate community structure. *Aquatic Botany* 104, 80–87. <https://doi.org/10.1016/j.aquabot.2012.09.003>
- Warfe, D.M., Barmuta, L.A., 2004. Habitat structural complexity mediates the foraging success of multiple predator species. *Oecologia* 141, 171–178. <https://doi.org/10.1007/s00442-004-1644-x>
- Waringer, J., Graf, W., 2011. Atlas der Mitteleuropäischen Köcherfliegenlarven - Atlas of Central European Trichoptera Larvae. Erik Mauch Verlag, Dinkelscherben.
- Wickham, H., 2016. ggplot2: Elegant Graphics for Data Analysis.

- Wolters, J.-W., Reitsema, R.E., Verdonschot, R.C.M., Schoelynck, J., Verdonschot, P.F.M.,
Meire, P., 2019. Macrophyte-specific effects on epiphyton quality and quantity and
resulting effects on grazing macroinvertebrates. *Freshwater Biology* 64, 1131–1142.
<https://doi.org/10.1111/fwb.13290>
- Wood, K.A., O'Hare, M.T., McDonald, C., Searle, K.R., Daunt, F., Stillman, R.A., 2017.
Herbivore regulation of plant abundance in aquatic ecosystems. *Biological Reviews*
92, 1128–1141. <https://doi.org/10.1111/brv.12272>
- Yan, L., 2021. ggvenn: Draw Venn Diagram by “ggplot2.”
- Yofukuji, K.Y., Cardozo, A.L.P., Quirino, B.A., Aleixo, M.H.F., Fugi, R., 2021. Macrophyte
diversity alters invertebrate community and fish diet. *Hydrobiologia* 848, 913–927.
<https://doi.org/10.1007/s10750-020-04501-w>
- Youenn, P., 2021. Campagne d'arrachage des végétaux envahissants sur le bassin versant de
Grand-Lieu - Année 2020 [Invasive plant removal campaign in the Grand-Lieu
watershed - Year 2020]. Syndicat de Bassin versant de Grand Lieu, St-Phibert-de-
Grand-Lieu.
- Żbikowski, J., Simčič, T., Pajk, F., Poznańska-Kakareko, M., Kakareko, T., Kobak, J., 2019.
Respiration rates in shallow lakes of different types: contribution of benthic
microorganisms, macrophytes, plankton and macrozoobenthos. *Hydrobiologia* 828,
117–136. <https://doi.org/10.1007/s10750-018-3807-5>

688 How macrophytes affect macroinvertebrate assemblage and sampling efficiency: results from a multinational survey

689 Supporting Information

690

691 Table S1: Summary of Kruskal-Wallis rank sum test comparing taxonomic richness among different sampling types by country. If the test
692 was significant (p-value < 0.05), pairwise Wilcoxon rank sum tests (p-values, adjusted with Holm-Bonferroni method) were performed. p-
693 values < 0.05 are highlighted in **bold**.

Lake	Kruskal-Wallis	Sediment Open / Sediment Plants	Sediment Plants / Sweep	Sediment Open / Sweep
Ebolowa-Pistia	0.0001	0.0143	0.0006	0.0148
Hartbeespoort Dam	0.0001	0.1246	0.0007	0.0049
Lac de Grand-Lieu	0.0001	0.0345	0.0007	0.0051
Kemnader See	0.053	-	-	-
Ebolowa-Typha	<0.0001	0.0010	0.0028	0.0003

695 Table S2: Estimated taxonomic richness based on Hill numbers (order $q=0$).

Lake	Sediment open	Sediment plants	Sweep 696
Ebolowa-Pistia	3.9	13.4	39.25 697
Hartbeespoort Dam	4.0	14.4	20.4
Lac de Grand-Lieu	3.0	12.4	28.6
Kemnader See	18.8	19.0	32.2
Ebolowa-Typha	3.9	6.9	40.2

698 Table S3. Mean density in individuals/m² (± SE) by site and sampling type

Lake	Sediment Open	Sediment Plants	Sweep Plants
Ebolowa-Pistia	112 ± 108	936 ± 470	24 ± 10
Hartbeespoort Dam	4368 ± 1668	1184 ± 276	118 ± 18
Lac de Grand-Lieu	432 ± 207	2616 ± 797	165 ± 44
Kemnader See	2576 ± 705	35836 ± 8537	3836 ± 605
Ebolowa-Typha	112 ± 108	816 ± 280	43 ± 28

699

700

701 Table S4: Summary of Kruskal-Wallis rank sum test comparing density (log+1 tranformed) among different sampling types by country. If the
702 test was significant (p-value < 0.05), pairwise Wilcoxon rank sum tests (p-values, adjusted with Holm-Bonferroni method) were performed. p-
703 values < 0.05 are highlighted in **bold**.

704

Lake	Kruskal-Wallis	Sediment Open / Sediment Plants	Sediment Plants / Sweep	Sediment Open / Sweep
Ebolowa-Pistia	0.0072	0.032	0.054	0.34
Hartbeespoort Dam	0.0011	0.0858	0.0010	0.0799
Lac de Grand-Lieu	0.0019	0.0799	0.0006	0.3710
Kemnader See	0.0003	0.0013	0.0006	0.2064
Ebolowa-Typha	0.0001	0.0024	0.0013	0.0138

705 Table S5: Results of Permutational multivariate analysis of variance (adonis2 function, vegan package, 999 permutations)

	Df	SumOfSqs	R2	F	Pr(>F)
Plant life form	1	2.159	0.05427	17.4866	0.001
Sample type	2	3.594	0.09036	14.5565	0.001
Lake	3	13.263	0.33342	35.8096	0.001
Plant life form : Sample type	2	1.249	0.03140	5.0591	0.001
Sample type : Lake	6	4.698	0.11812	6.3429	0.001
Residual	120	14.815	0.37243		
Total	134	39.778	1.00000		

Table S6: Presence and absence of taxa in each sample type within each macrophyte. The corresponding order of sample type within each macrophyte is grab open/ grab plants/ sweep samples In each cell, “-” indicates that the taxon is absent in the sample and “0” indicates that it is present.

Group	Taxa	Ebolowa-Pistia	Hartbeespoort Dam	Lac de Grand-Lieu	Kemnader See	Ebolowa-Typha
Ephemeroptera	<i>Baetis</i> sp.	-/-	-/-	-/0/0	-/-	-/-
	<i>Cloeon</i> sp.	-/-	-/-	-/-	-/0/0	-/-
	<i>Caenis</i> sp.	-/-	-/-	-/-	0/0/0	-/-
	<i>Heptagenia</i> sp.	-/-	-/-	-/-	-/-	-/0
Lepidoptera	Pyrilidae	-/-	-/0/0	-/-	-/0/0	-/-
Odonata	<i>Libellula</i> sp.	-/-0	-/-	-/-0	-/-	-/0
	<i>Orthetrum</i> sp.	-/-0	-/-	-/-	-/-	-/0/0
	<i>Coenagrion speciosa</i>	-/-0	-/-	-/-	-/-	-/0
	Coenagrionidae	-/-	-/0	-/-	-/-	-/-
	<i>Ischnura elegans</i>	-/-	-/-	-/0/0	-/-	-/-
	<i>Platynemis pennipes</i>	-/-	-/-	-/-	-/0/0	-/-
	<i>Platynemis</i> sp.	-/-	-/-	-/-	-/-	-/0
Trichoptera	<i>Stactobia moselyi</i>	-/-	-/-	-/-	-/0/0	-/-
	<i>Ecnomus</i> sp.	-/-	-/-	-/-	0/-/0	-/-
Heteroptera	<i>Notonecta glauca</i>	-/-0	-/0	-/0/0	-/-	-/-
	<i>Hydrocyrius</i> sp.	-/-0	-/-	-/-	-/-	-/0
	<i>Ranatra linearis</i>	-/-0	-/-	-/-	-/-	-/-
	<i>Plea pulla</i>	-/-	-/0	-/-	-/-	-/-
	<i>Appasus capensis</i>	-/-	-/0	-/-	-/-	-/-
	Corixinae	-/-	-/-	-/0	-/0/0	-/-
	<i>Belostoma</i> sp.	-/-	-/-	-/-	-/-	-/0
Coleoptera	<i>Amphiops</i> sp.	-/-0	-/-	-/-	-/-	-/0
	<i>Noterus</i> sp.	-/-0	-/-	-/-	-/-	-/0
	<i>Macrolea</i> sp.	-/-0	-/-	-/-	-/-	-/0
	Curculionidae	-/-0	-/-	-/-	-/-	-/-
	<i>Laccophilus</i> sp.	-/-0	-/-	-/-	-/-	-/0
	<i>Cybister lateralimarginalis</i>	-/-0	-/-	-/-	-/-	-/-
	<i>Dryops</i> sp.	-/-0	-/-	-/-	-/-	-/-
	Hydrophilus	-/-0	-/-	-/-	-/-	-/-
	Scirtidae	-/-	-/0/0	-/-	-/-	-/0
	Helodidae	-/-	-/0	-/-	-/-	-/-
	Hydrophilidae	-/-	-/-	-/0	-/-	-/-
	<i>Limnius</i> sp.	-/-	-/-	-/-	0/-	-/-
	<i>Dytiscus</i> sp.	-/-	-/-	-/-	-/-	-/0
	<i>Chaetarthria</i> sp.	-/-	-/-	-/-	-/-	-/0
Megaloptera	<i>Sigara</i> sp.	-/-	-/-	-/0/0	-/-	-/-

Diptera	<i>Chaoborus</i>	-/-/0	-/0/-	-/-/-	-/-/-	-/-/-
	Chironomini	-/0/0	-/-/-	-/-/-	-/-/-	-/-/0
	Syrphidae	-/-/0	-/-/-	-/-/-	-/-/-	-/-/0
	<i>Chironomus</i>	-/0/-	-/-/-	-/-/-	-/-/-	-/-/-
	Chironomidae	-/-/-	-/0/0	0/0/0	0/0/0	-/-/-
	Syrphidae	-/-/-	-/0/0	-/-/-	-/-/-	-/-/-
		Ebolowa-	Hartbeespoort	Lac de	Kemnader	Ebolowa-
Group	Taxa	Pistia	Dam	Grand-	See	Typha
Diptera	Rhagionidae	-/-/-	-/-/0	-/-/-	-/-/-	-/-/-
(Continued)	Ephydriidae	-/-/-	0/-/0	-/-/-	-/-/-	-/-/-
	Psychodidae	-/-/-	-/-/0	-/-/-	-/-/-	-/-/-
	Ceratopogonidae	-/-/-	-/-/-	0/-/-	0/0/-	-/-/-
	Sciomyzidae	-/-/-	-/-/-	-/-/0	-/-/-	-/-/-
	Dolichopodidae	-/-/-	-/-/-	-/0/0	-/-/-	-/-/-
	Stratiomyidae	-/-/-	-/-/-	-/-/-	0/0/-	-/-/-
	Simulini	-/-/-	-/-/-	-/-/-	-/-/-	-/-/0
Decapoda	<i>Procambarus clarkii</i>	-/-/-	-/-/-	-/0/0	-/-/0	-/-/-
Amphipoda	<i>Crangonyx pseudogracilis</i>	-/-/-	-/-/-	-/-/0	-/-/-	-/-/-
	<i>Dikerogammarus villosus</i>	-/-/-	-/-/-	-/-/-	0/0/0	-/-/-
	<i>Chelicorophium sowinskyi</i>	-/-/-	-/-/-	-/-/-	0/-/-	-/-/-
	<i>Gammarus roeselii</i>	-/-/-	-/-/-	-/-/-	-/0/0	-/-/-
Isopoda	<i>Asellus aquaticus</i>	-/-/-	-/-/-	-/-/0	0/0/0	-/-/-
	<i>Proasellus meridionalis</i>	-/-/-	-/-/-	-/-/0	-/-/-	-/-/-
Acari	Hydracarina	-/-/-	-/0/0	-/0/0	0/0/0	-/-/-
Gastropoda	<i>Gyraulus</i> sp.	-/-/0	-/-/-	-/-/-	0/0/0	-/-/-
	<i>Physa</i> sp.	-/-/0	-/-/-	-/-/-	-/-/-	-/-/-
	<i>Stagnicola</i> sp.	0/0/0	-/-/-	-/-/-	-/-/-	0/0/0
	<i>Aplexa hypnorum</i>	-/-/0	-/-/-	-/-/-	-/-/-	-/-/0
	<i>Radix</i> sp.	-/0/0	-/-/-	-/-/-	-/-/-	-/-/0
	<i>Potadoma</i> sp.	-/0/-	-/-/-	-/-/-	-/-/-	-/0/-
	<i>Melania tuberculata</i>	-/0/-	-/-/-	-/-/-	-/-/-	-/0/-
	<i>Galba truncatula</i>	-/-/0	-/-/-	-/-/-	-/-/-	-/-/-
	<i>Oxyloma patentissima</i>	-/-/-	-/-/0	-/-/-	-/-/-	-/-/-
	<i>Physela acuta</i>	-/-/-	-/-/-	-/-/0	0/0/0	-/-/-
	<i>Acroloxus lacustris</i>	-/-/-	-/-/-	-/-/0	0/-/-	-/-/-
	<i>Ferrissia californica</i>	-/-/-	-/-/-	-/0/0	-/-/-	-/-/-
	<i>Menetus dilatus</i>	-/-/-	-/-/-	-/-/0	-/-/-	-/-/-
	<i>Potamopyrgus antipodarum</i>	-/-/-	-/-/-	-/-/-	0/0/0	-/-/-
	<i>Valvata piscinalis</i>	-/-/-	-/-/-	-/-/-	-/0/0	-/-/-
	<i>Radix auricularia</i>	-/-/-	-/-/-	-/-/-	-/-/0	-/-/-
	<i>Planorbis</i> sp.	-/-/-	-/-/-	-/-/-	-/-/-	-/-/0
Bivalvia	Sphaeriidae	-/-/-	-/-/-	0/0/0	-/0/0	-/-/-
	<i>Corbicula fluminea</i>	-/-/-	-/-/-	-/-/-	0/-/-	-/-/-
Oligochaeta	Oligochaeta	0/0/0	0/0/0	0/0/0	0/0/0	0/0/-
Hirudinea	<i>Haementeria costata</i>	-/-/0	-/-/-	-/-/-	-/-/-	-/-/0
	Hirudinea	-/-/-	0/0/0	-/-/-	-/-/-	-/-/-

	Glassipholiidae	-/-/-	-/-/-	-/-/-	-/-/0	-/-/-
Platyhelminthes	Platyhelminthes	-/-/-	-/-/0	-/-/-	-/-/0	-/-/-
Nemathelminthes	Nemathelminthes	-/-/-	-/-/-	0/0/0	-/0/-	-/-/-

716