Supplementary Methods

Emergence patterns of Chironomidae & the Chironomid Pupal Exuviae Technique (CPET).

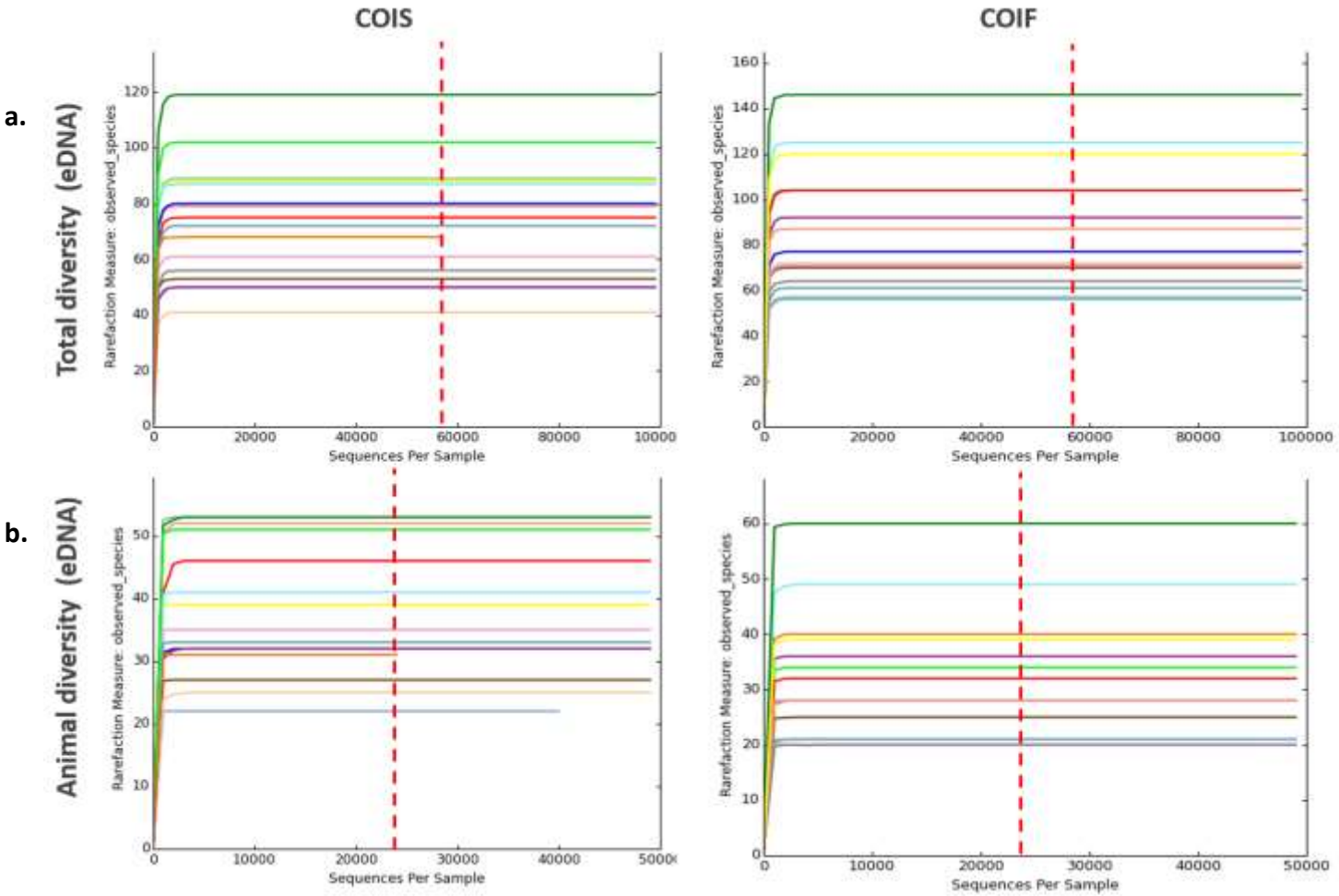
Chironomids exhibit specialised responses to ecological stressors and are acknowledged as one of the most important macroinvertebrate groups for monitoring lake ecosystem health [1]. However, benthic larvae collected with traditional kick-net sampling are notoriously difficult to identify, even by specialists. To overcome these problems lentic Chironomidae biodiversity is assessed via the identification of shed exuviae (skins) of emerging adults that float and accumulate on the leeward edge of lentic ecosystems [1,2]. Exuvial samples therefore offer a unique advantage to simultaneously compare the diversity of recent lentic invertebrate communities and eDNA, and to explore how eDNA is related to ecosystem wide biodiversity. Additionally, using the CPET technique, compared to traditional kick-net sampling, allows for integrated collection of specimens from a wide range of habitats rather than only the profundal zone. The collection and sorting process is fast and the identification of the exuviae is easier than identification of larvae, while the sample collected is also fresh, as the exuviae remain floating for only about 48h [1].

The emergence patterns of Chironomidae are known to differ in different latitudinal zones, due to variations in temperature and photoperiod [3]. In the tropics, the emergence cycles are accelerated, following the lunar cycles, with species emerging all year round. On the contrary, closer to the Arctic, emergence of adults occurs over a limited window over the summer period. Emergence is limited also by surface freezing of the water bodies. For the temperate zones, emergence is higher over the summer but not limited to that time. Species are known to emerge across all seasons, but with less intensity in winter months.

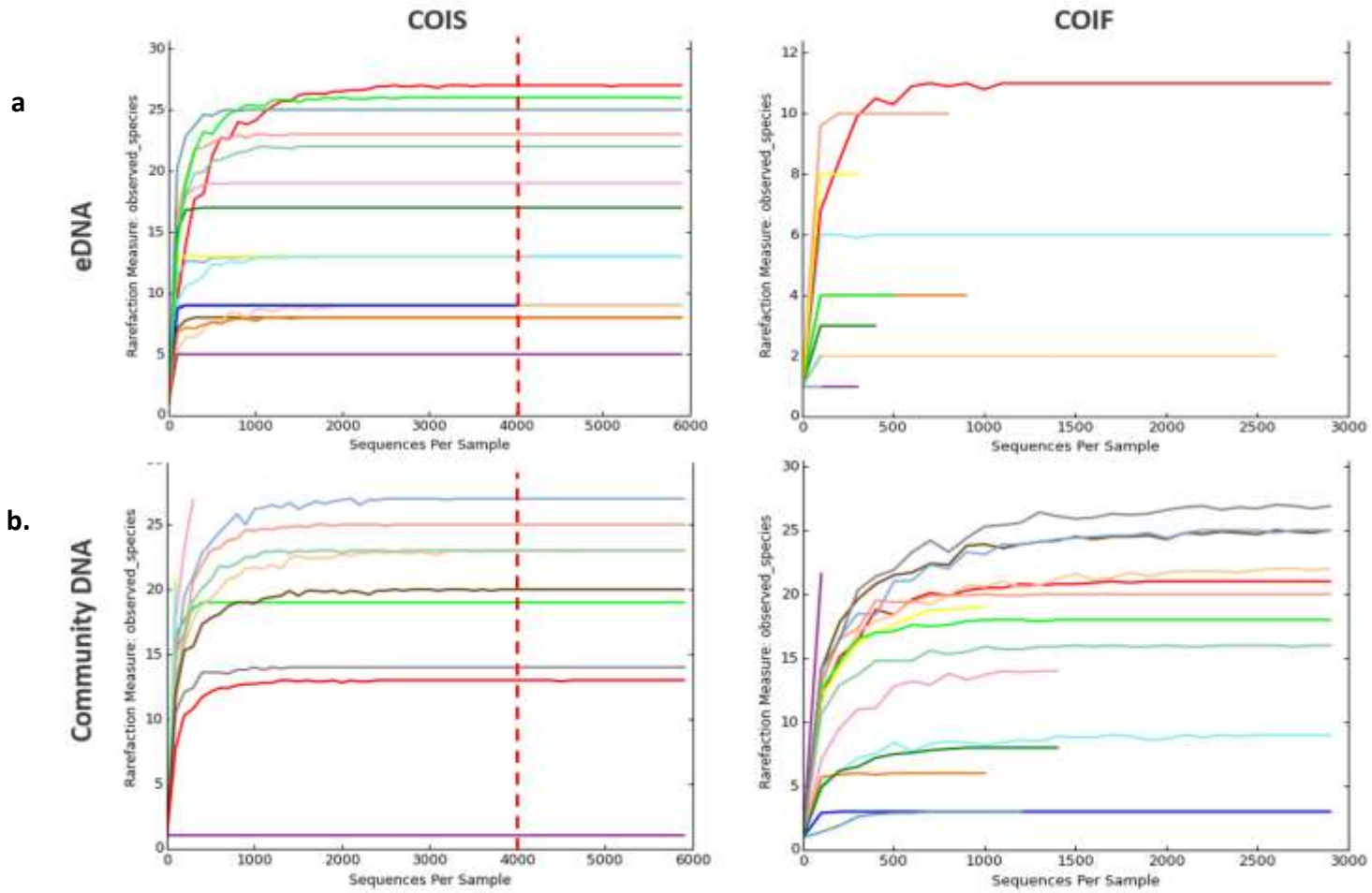
Hence an episodic pattern occurs, with lower emergence over winter, which increases gradually over time.

Testing of capture and extraction protocols for eDNA. Rigorous testing of eDNA capture and extraction protocols was performed prior to commencing the experiment. For testing of filtration methods, two types of filtration membranes at different pore sizes were used: glass fibre at 0.7µm and cellulose nitrate at 0.45µm and 0.2µm. Two volumes of water samples were used at 1L and 2L. Ethanol precipitation and centrifugation, using 15ml water samples was also tested, as well as direct centrifugation of 50ml water samples (no precipitation or filtration). For the latter two, varying centrifugation speeds and centrifugation times were also tested. The extraction protocols included the DNeasy Blood & Tissue kit (QIAGEN), Power Water DNA Isolation kit (MoBio) and Phenol Chloroform extraction protocol (PCI) as per [4] with an added Proteinase K step.
From all the above, the collection of eDNA using 0.45µm cellulose filter membranes (2lt water) coupled with a PCI extraction protocol was considered optimal, due to the following: (1) Higher concentrations of collected DNA as per spectrophotometric quantification (NanoDrop) and quality of DNA from agarose gel visualization. (2) Possibility for collection of larger water sample (2L). (3) Ease of storage of collected samples (filter membrane) until DNA extraction (storage at -80^{o}C). (4) Optimal pore size for collection of smaller DNA molecules (compared to glass fibre 0.7µm) and filtration time efficiency (compared to cellulose 0.2µm). (5) Good performance in PCR amplification of long COI amplicons.

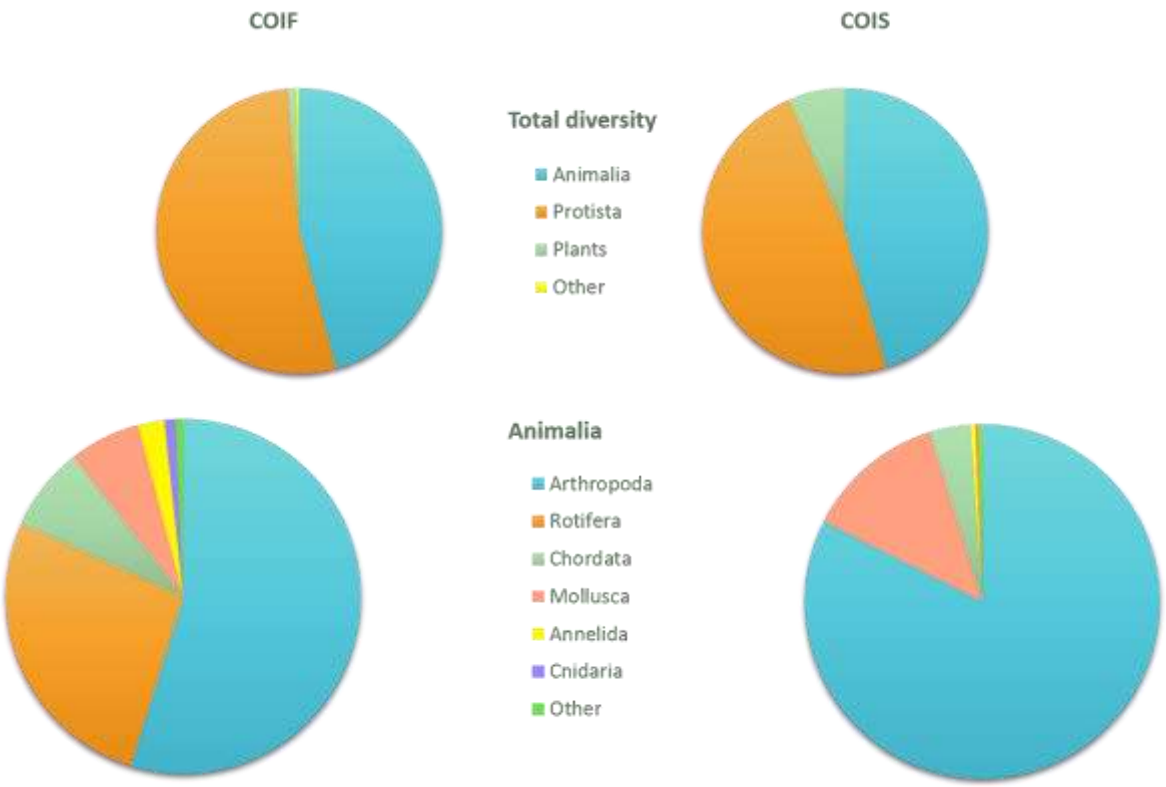
Supplementary Figures



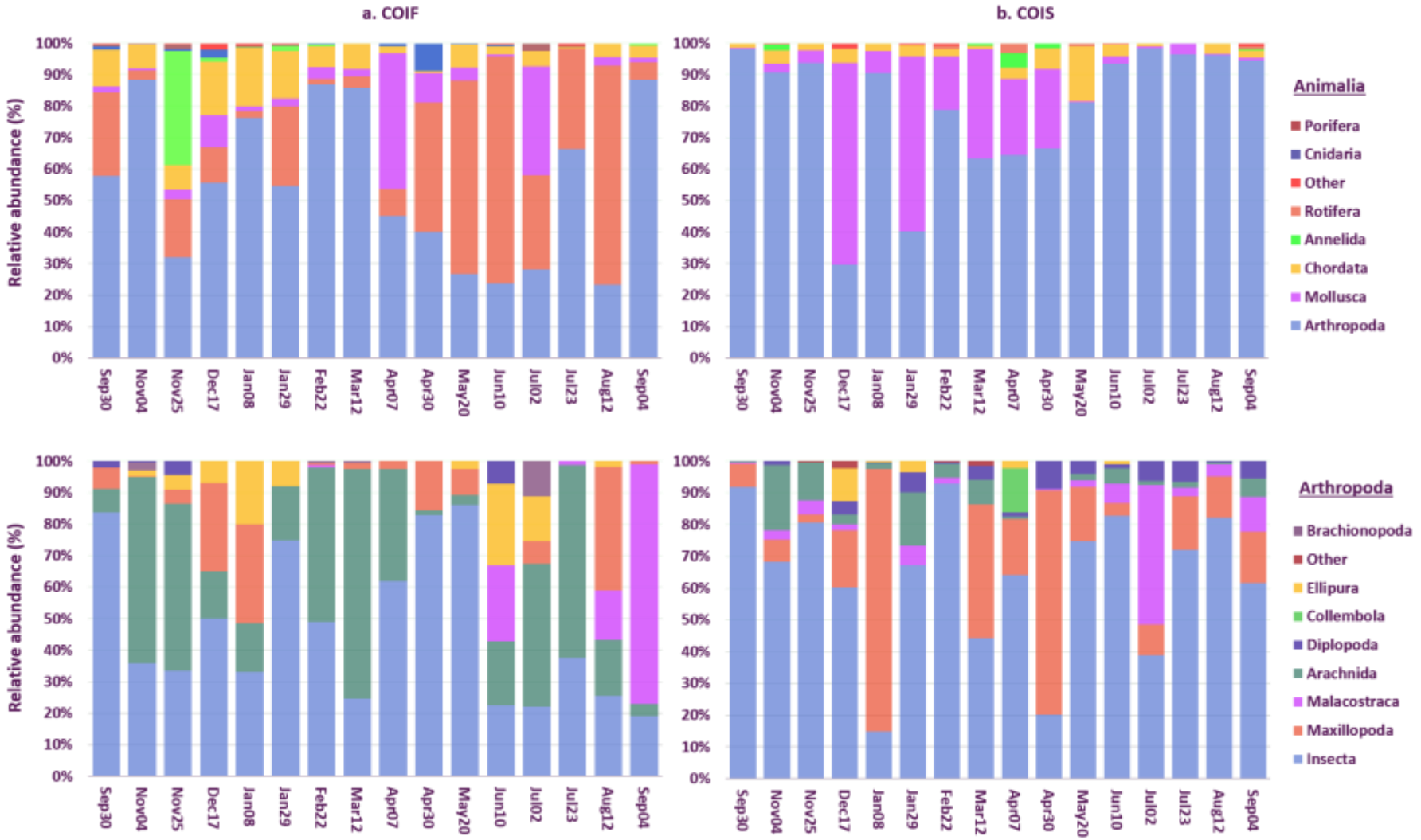
Supplementary Figure 1 | Rarefaction plots. The figure shows (a) total taxa and (b) animal taxa only, based on water extracted eDNA samples only for both amplicons (COIS and COIF). Dashed red lines indicate the rarefaction depth used for analysis (a. total taxa 57,869 reads, b. animal taxa 24,914 reads), x-axis: reads per sample, y-axis: OTU richness (N = 64).



Supplementary Figure 2 | Rarefaction plots. The figure shows Chironomidae identified OTUs, (a.) eDNA samples and (b.) community DNA samples, for both amplicons (COIS and COIF). Dashed red lines indicate the rarefaction depth used for analysis (COIS: 4,000 reads). Due to low coverage of COIF eDNA samples (top), this amplicon was excluded from further analysis. x-axis: reads per sample, y-axis: OTU richness (N = 64).

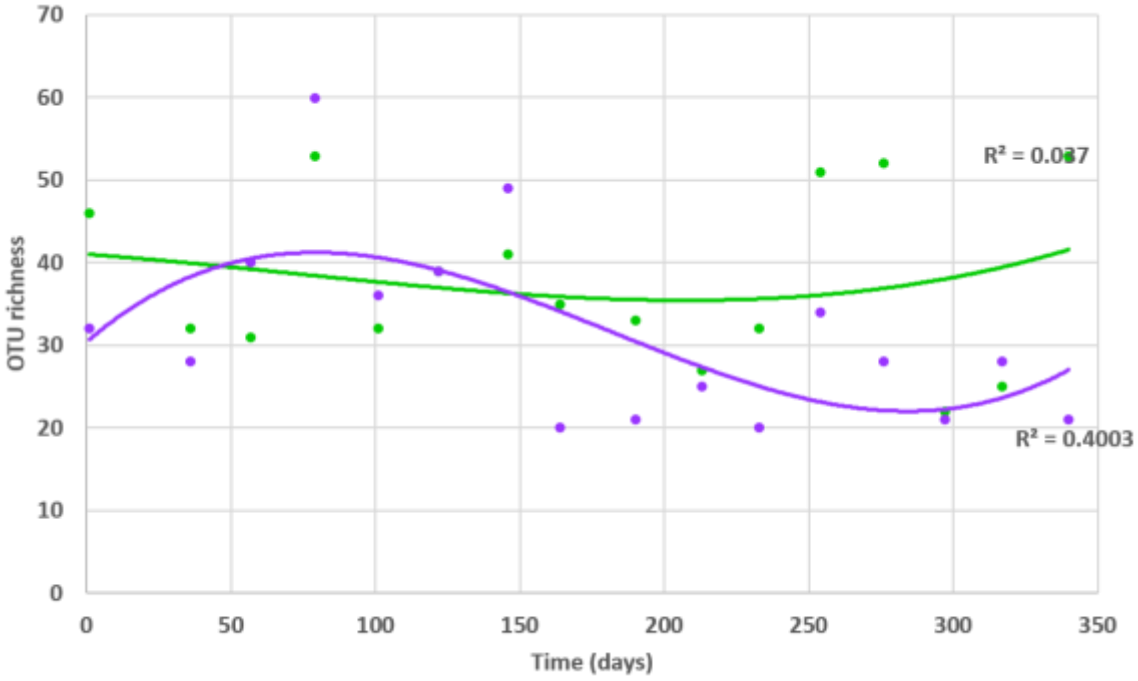


Supplementary Figure 3 | Summary representation of taxa detected. Results shown for eDNA samples for both amplicons (COIF, COIS). Top: Kingdoms, bottom: phylum Animalia.

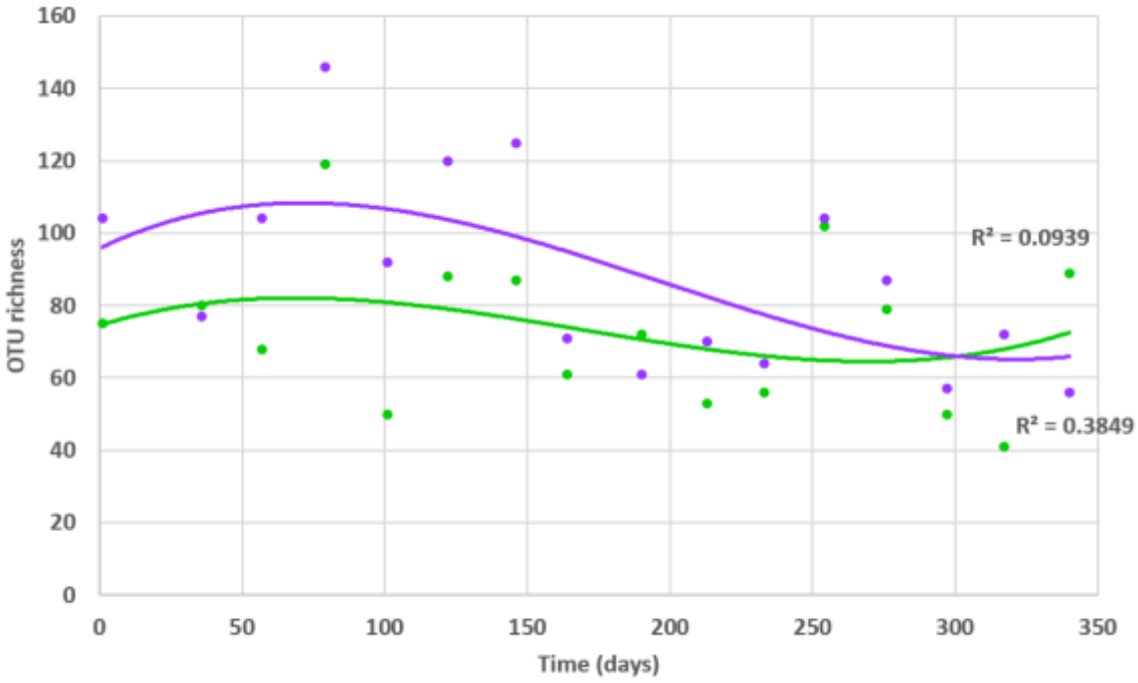


Supplementary Figure 4 | Histogram presenting taxonomic relative abundance for both amplicons. a. COIF, **b**. COIS, for all animal (top) and all arthropod (bottom) taxa in eDNA samples through the year (x-axis: sampling dates). All samples were rarefied at 24,914 read depth.

a

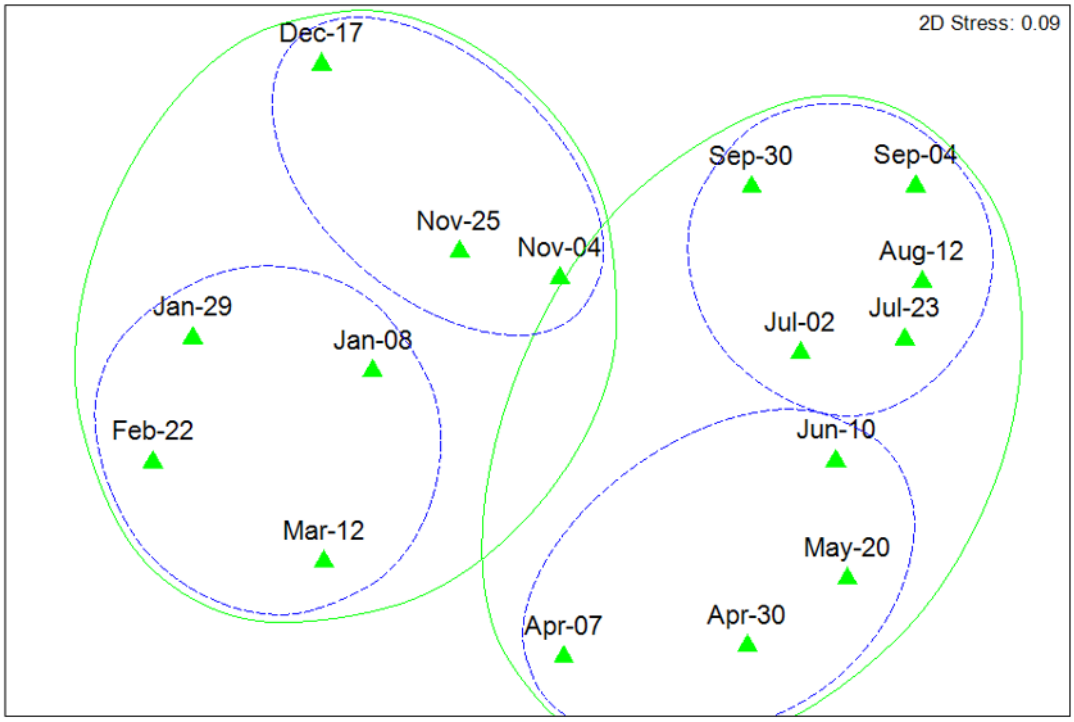


b.

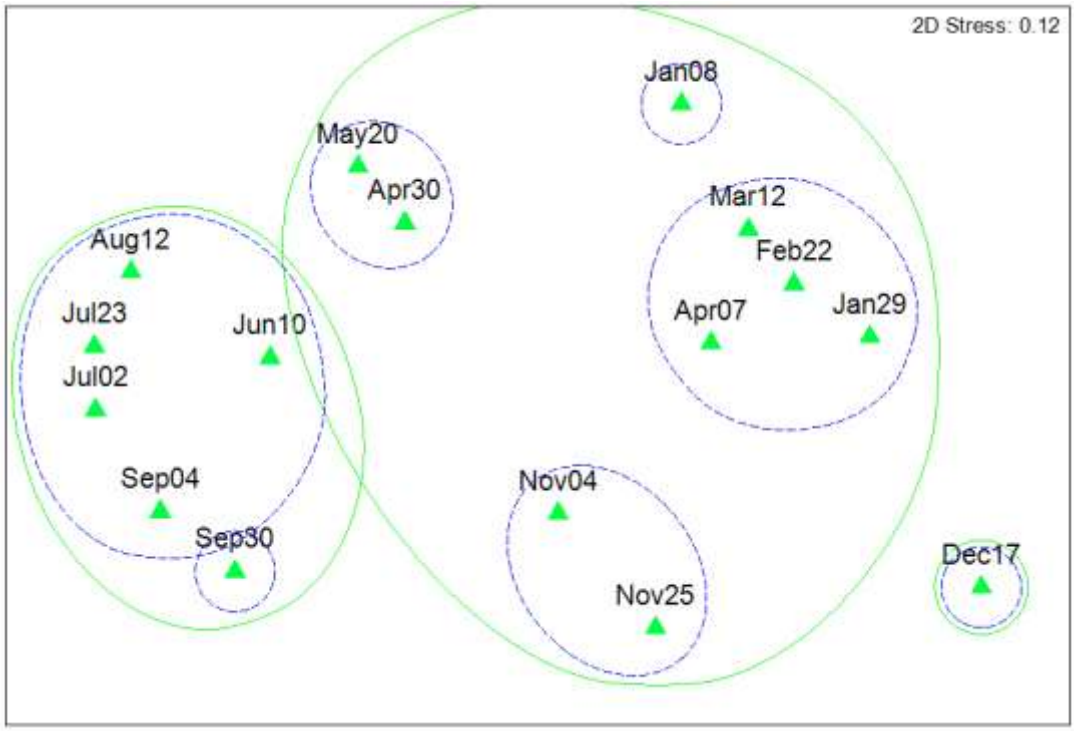


Supplementary Figure 5 | Yearly trends of OTU richness.(a) Animal diversity (b) total diversity, detected by eDNA samples for both COIS (green) and COIF (purple). X-axis: time in days (Sep 30th 2014 - Sep 4 2015), y-axis: OTU richness, (a.) COIS: R^2=0.037, COIF: R^2=0.4003, (b.) COIS: R^2=0.0939, COIF: R^2=0.3849.

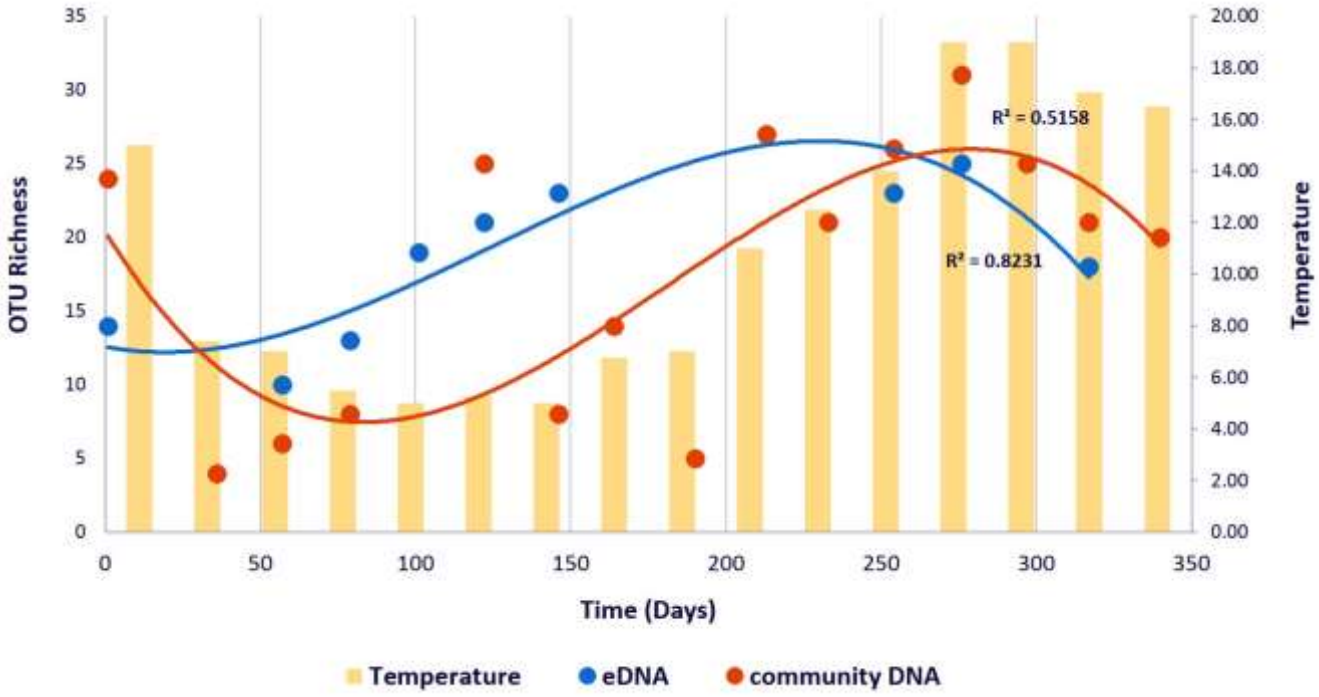
a



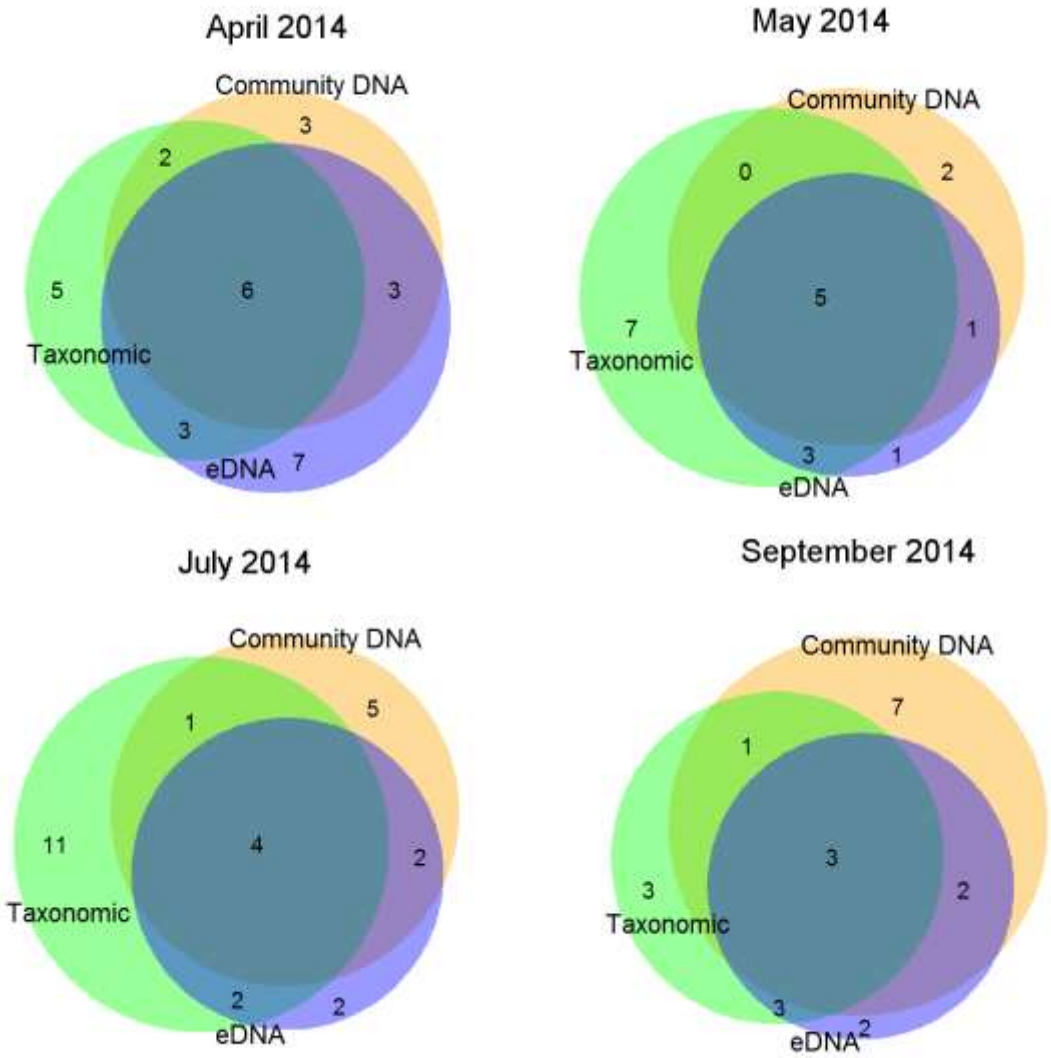
b



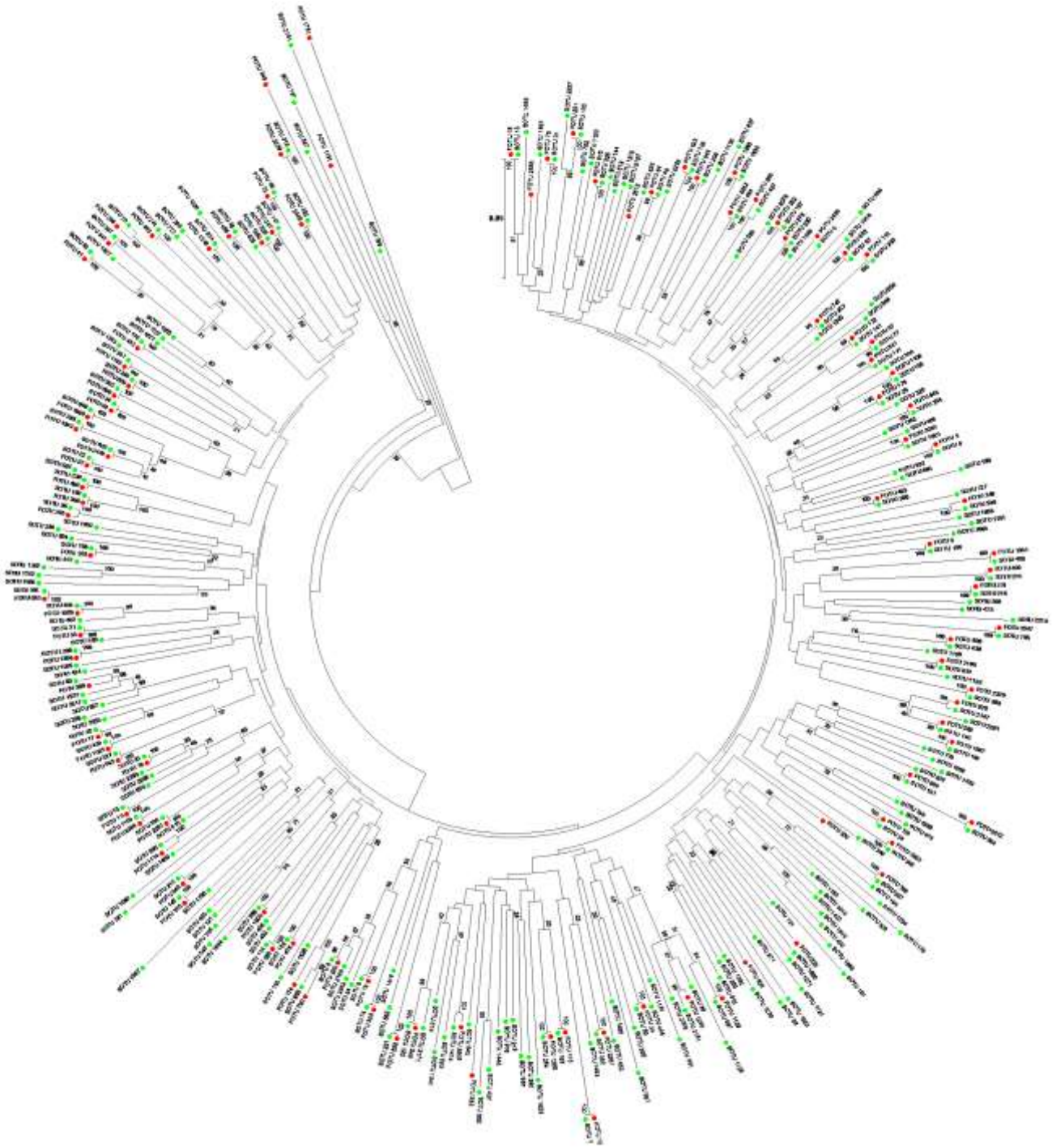
Supplementary Figure 6 | nMDS plots of β-diversity. The Sørensen diversity index was calculated for eDNA samples only. **a**: COIF, **b**: COIS (N = 32). Solid green circles: 30% similarity cut-off (corresponding to "winter" –"summer" groups), dashed blue circles: 40% similarity cut-off (N=32).



Supplementary Figure 7 | OTU richness patterns for Chironomidae OTUs for the COIF amplicon (raw data un-trimmed). Points represent richness values to individual sampling points for eDNA (blue) and community DNA (orange). Best fitted lines from polynomial regressions for eDNA samples (blue) and community DNA (orange), plotted against time (x – axis: Sep. 2013 – Sep 2014), eDNA: R^2=0.823, community DNA: R^2=0.5158, N=24.



Supplementary Figure 8 | Venn diagrams of genera richness (individual time points). Number of Chironomidae genera per sample type (purple: eDNA, orange: community DNA, green: taxonomic data) and the number of genera common between sampling types (overlapped areas). Each plot shows the results for a single time point with the month and year indicated at the top of the respected Venn diagram.



Supplementary Figure 9 | Neighbor-Joining phylogenetic tree. The tree comprises all OTUs identified as Chironomidae prior to abundance filtering, for both amplicons (COIF: red markers (FOTU), COIS : green markers (SOTU)). Distances calculated using the p-distance method 1000 bootstrap replications (N = 351).

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Supplementary Figure 10 | Map of Llyn Padarn, N. Wales (UK). Marked with red the two sites used for sample collection (S1: Site 1, NW: 53.139106, -4.153975, S2: Site 2, SW: 53.122414, -4.126761). Google Earth, August 2016. .

Supplementary Tables

Supplementary Table 1 | Summary table of number of reads obtained per sample. Triplicate PCRs from each time point were pooled and sequenced as one. (EXCOI: exuviae community DNA samples, WCOI: water eDNA samples, COIS; 235bp amplicon, COIF: 658bp amplicon).

#Sample	Sample name	Number of reads		Sample Type	Collection date	Time point
		COIS	COIF			
1	1_EXCOI	159874	383161	Pupal exuviae	30/09/2013	T1
2	2_EXCOI	464	3603	Pupal exuviae	04/11/2013	T2
3	3_EXCOI	442	3808	Pupal exuviae	25/11/2013	T3
4	4_EXCOI	349	2844	Pupal exuviae	17/12/2013	T4
5	5_EXCOI	203602	507	Pupal exuviae	08/01/2014	T5
6	6_EXCOI	387	2406	Pupal exuviae	29/01/2014	T6
7	7_EXCOI	262	365700	Pupal exuviae	22/02/2014	T7
8	8_EXCOI	411	1825	Pupal exuviae	12/03/2014	T8
9	9_EXCOI	475	2755	Pupal exuviae	07/04/2014	T9
10	10_EXCOI	165644	468915	Pupal exuviae	30/04/2014	T10
11	11_EXCOI	139771	363563	Pupal exuviae	20/05/2014	T11
12	12_EXCOI	289842	336948	Pupal exuviae	10/06/2014	T12
13	13_EXCOI	168006	347443	Pupal exuviae	02/07/2014	T13
14	14_EXCOI	343465	15231	Pupal exuviae	23/07/2014	T14
15	15_EXCOI	489950	25608	Pupal exuviae	12/08/2014	T15
16	16_EXCOI	500181	18963	Pupal exuviae	04/09/2014	T16
17	1_WCOI	240086	273799	Water	30/09/2013	T1
18	2_WCOI	189255	260032	Water	04/11/2013	T2
19	3_WCOI	62109	253590	Water	25/11/2013	T3
20	4_WCOI	288282	302474	Water	17/12/2013	T4
21	5_WCOI	261100	346620	Water	08/01/2014	T5
22	6_WCOI	272002	253954	Water	29/01/2014	T6
23	7_WCOI	157903	280711	Water	22/02/2014	T7
24	8_WCOI	253438	263482	Water	12/03/2014	T8
25	9_WCOI	314163	245330	Water	07/04/2014	T9
26	10_WCOI	282801	253024	Water	30/04/2014	T10
27	11_WCOI	224307	154471	Water	20/05/2014	T11
28	12_WCOI	281971	430025	Water	10/06/2014	T12
29	13_WCOI	252773	249347	Water	02/07/2014	T13
30	14_WCOI	276285	285992	Water	23/07/2014	T14
31	15_WCOI	311891	203605	Water	12/08/2014	T15
32	16_WCOI	309147	259862	Water	04/09/2014	T16

Supplementary Table 2 | Positive control contents. Extracts used for preparation of a positive control sample and taxonomic information of the specimens used for extraction

(species level information was not available for some of the specimens). The last two columns show the success of the amplicons in detecting each extract (**v**: detected, **x**: not detected).

Positive Control Contents					Amplicon	
Number	**Extract Code**	**Order**	**Family**	**Species**	**COIF**	**COIS**
1	C_pseudQ24_1	Amphipoda	Crangonyctidae	*Crangonyx pseudogracilis*	v	v
2	G_pulexQ29_2	Amphipoda	Gammaridae	*Gammarus pulex*	v	v
3	G_marinusQ33_2	Coleoptera	Gyrinidae	*Gyrinus marinus*	v	v
4	PA6	Diptera	Chironomidae	*Chironomidae* sp.	v	v
5	PA8	Diptera	Chironomidae	*Chironomidae* sp.	x	v
6	PA16	Diptera	Chironomidae	*Chironomidae* sp.	v	v
7	PA17	Diptera	Chironomidae	*Chironomidae* sp.	v	v
8	SERGPSI6_2	Diptera	Chironomidae	*Sergentia psiloptera*	v	v
9	ABLAMON2	Diptera	Chironomidae	*Ablabesmyia monilis*	v	v
10	CHIRTEN13_1	Diptera	Chironomidae	*Chironomus tentans*	v	v
11	CRYPPSI13_1	Diptera	Chironomidae	*Cryptochironomus psittacinus*	v	v
12	MONOBAT6A	Diptera	Chironomidae	*Monodiamesa bathyphila*	v	v
13	CLATATR10A	Diptera	Chironomidae	*Cladotanytarsus atridorsum*	v	v
14	POLYNUC7B	Diptera	Chironomidae	*Polypedilum nubeculosum*	v	v
15	E_danicaE130	Ephemeroptera	Ephemeridae	*Ephemera danica*	x	v
16	COR1_G18_1	Gastropoda	Lymnaeidae	*Radix* sp.	v	v
17	DEV3_G27_1	Gastropoda	Lymnaeidae	*Radix balthica*	v	v
18	ANG5_G2_1	Gastropoda	Planorbidae	*Ancylus fluviatilis*	v	v
19	A_vortexQ2_2	Gastropoda	Planorbidae	*Anisus vortex*	v	v
20	N_glaucaN10	Hemiptera	Notonectidae	*Notonecta glauca*	v	v
21	A_aquaticus	Isopoda	Asellidae	*Asellus aquaticus*	x	v
22	SCO12_T2_1	Trichoptera	Glossosomatidae	*Agapetus fuscipes*	x	v
23	COR2_T79_1	Trichoptera	Goeridae	*Silo pallipes*	v	v
24	WALE13_T33_1	Trichoptera	Hydropsychidae	*Hydropsyche instabilis*	v	v
25	HE1_T4_1	Trichoptera	Hydroptilidae	*Agraylea sexmaculata*	v	v
26	YO2_T3_1	Trichoptera	Hydroptilidae	*Agraylea multipunctata*	v	v
27	HEA_T37_1	Trichoptera	Hydroptilidae	*Hydroptila vectis*	v	v
28	ANG5_T43_1	Trichoptera	Lepidostomatidae	*Lepidostoma hirtum*	v	v
29	SCOT2_T27_1	Trichoptera	Limnephilidae	*Halesus radiatus*	v	v
30	ANG5_T77_1	Trichoptera	Leptoceridae	*Athripsodes albifrons*	v	v

Supplementary Table 3 | Positive control sequencing results. Summary table of sequencing results obtained from positive control samples for 235bp COIS and 658bp COIF amplicon.

Shown the number of reads, number of OTUs and relative abundance assigned to our target species (target), unidentified OTUs (unknown) and identified OTUs not present in our target species (Non – target).

Positive controls	COIS			COIF		
	reads	%	OTUs	reads	%	OTUs
Target	547569	99.971	33	393068	99.931	29
Unknown	18	0.003	3	246	0.063	16
Non - Target	143	0.026	14	27	0.007	6
Total	547730	100.000	50	393341	100	51

Supplementary Table 4 | Summary of eDNA extracts from filter membranes. Two extractions were performed for each time point which were combined for PCR and sequencing.

Extract Number	Collection date	Extraction date	Site	DNA concentration (ng/µl)	Time point	DNA concentration - Combined (ng/µl)
1	30/09/2013	18/10/2014	1	72.08	**T1**	53
2	30/09/2013	18/10/2014	2	25.34		
3	04/11/2013	18/10/2014	1	34.92	**T2**	37
4	04/11/2013	18/10/2014	2	34.09		
5	25/11/2013	18/10/2014	1	39.03	**T3**	25
6	25/11/2013	18/10/2014	2	11.81		
7	17/12/2013	06/10/2014	1	24.68	**T4**	56
8	17/12/2013	06/10/2014	2	90.58		
9	08/01/2014	06/10/2014	1	45.5	**T5**	46
10	08/01/2014	06/10/2014	2	46.06		
11	29/01/2014	06/10/2014	1	25.73	**T6**	24
12	29/01/2014	06/10/2014	2	21.24		
13	22/02/2014	07/10/2014	1	58.81	**T7**	52
14	22/02/2014	07/10/2014	2	46.87		
15	12/03/2014	06/10/2014	1	36.62	**T8**	36
16	12/03/2014	06/10/2014	2	37.69		
17	07/04/2014	07/10/2014	1	77.77	**T9**	76
18	07/04/2014	07/10/2014	2	75.19		
19	30/04/2014	06/10/2014	1	47.33	**T10**	49
20	30/04/2014	06/10/2014	2	49.72		
21	20/05/2014	18/10/2014	1	80.05	**T11**	68
22	20/05/2014	18/10/2014	2	52.55		
23	10/06/2014	07/10/2014	1	44.49	**T12**	47
24	10/06/2014	07/10/2014	2	50.33		
25	02/07/2014	18/10/2014	1	37.74	**T13**	48
26	02/07/2014	18/10/2014	2	44.94		
27	23/07/2014	18/10/2014	1	66.18	**T14**	62
28	23/07/2014	18/10/2014	2	45.93		
29	12/08/2014	18/10/2014	1	90.28	**T15**	68
30	12/08/2014	18/10/2014	2	35.4		
31	04/09/2014	18/10/2014	1	80.02	**T16**	65
32	04/09/2014	18/10/2014	2	41.33		

Supplementary Table 5 | Summary of DNA extracts from exuviae community samples.

Extract Number	Collection date	Extraction date	DNA concentration (ng/µl)	Time point	Method
1	30/09/2013	23/11/2014	36.98	E1	QIAmp Blood Maxi
2	04/11/2013	19/11/2014	9.81	E2	Qiagen B & T Kit
3	25/11/2013	19/11/2014	6.59	E3	Qiagen B & T Kit
4	17/12/2013	19/11/2014	12.26	E4	Qiagen B & T Kit
5	08/01/2014	19/11/2014	9.57	E5	Qiagen B & T Kit
6	29/01/2014	19/11/2014	9.01	E6	Qiagen B & T Kit
7	22/02/2014	19/11/2014	6.13	E7	Qiagen B & T Kit
8	12/03/2014	19/11/2014	12.15	E8	Qiagen B & T Kit
9	07/04/2014	19/11/2014	16.5	E9	Qiagen B & T Kit
10	30/04/2014	23/11/2014	35.7	E10	QIAmp Blood Maxi
11	20/05/2014	23/11/2014	31.31	E11	QIAmp Blood Maxi
12	10/06/2014	23/11/2014	30.15	E12	QIAmp Blood Maxi
13	02/07/2014	23/11/2014	18.15	E13	QIAmp Blood Maxi
14	23/07/2014	23/11/2014	19.9	E14	QIAmp Blood Maxi
15	12/08/2014	23/11/2014	19.89	E15	QIAmp Blood Maxi
16	04/09/2014	23/11/2014	25.42	E16	QIAmp Blood Maxi

Supplementary Table 6 | Primers used for library preparation. Round 1: forward / reverse universal tail and template specific primer. A multi N region inserted in forward primer to assist cluster formation. Round 2: a forward or reverse Illumina adapter and an i5 or i7 Nextera index with the appropriate universal tail.

Primer pair	Round 1	Direction
LCO1490	**Forward Universal tail** **Template specific primer** ACACTCTTTCCCTACACGACGCTCTTCCGATCT **NNNNN** GGTCAACAAATCATAAAGATATTGG	**Forward**
HC02198	**Reverse Universal tail** **Template specific primer** GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTAAACTTCAGGGTGACCAAAAAATCA	**Reverse**
COI_A_rev	**Reverse Universal tail** **Template specific primer** GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCARAAWCTTATATTATTTATTCGDGG	**Reverse**
	Round 2	
All Forward	**P5 Illumina adapter** **Index 2 (i5)** **Forward Universal tail** 5' AATGATACGGCGACCACCGAGATCTACAC - i5 Index - ACACTCTTTCCCTACACGACGCTC 3'	**Forward**
All Reverse	**P7 Illumina adapter** **Index 1 (i7)** **Reverse Universal tail** 5' CAAGCAGAAGACGGCATACGAGAT - i7 Index - GTGACTGGAGTTCAGACGTGTGCTC 3'	**Reverse**

Supplementary References

1. Wilson, R. & Ruse, L. *A guide to the identification of genera of chironomid pupal exuviae occurring in Britain and Ireland*. (Freshwater Biological Association Publication 13, Ambleside, UK., 2005).

2. Ruse, L. Lake acidification assessed using chironomid pupal exuviae. *Fundam. Appl. Limnol. / Arch. für Hydrobiol.* **178,** 267–286 (2011).

3. Armitage, P. D., Pinder, L. C. & Cranston, P. The Chironomidae: biology and ecology of non-biting midges. (Chapman and Hall, 1995).

4. Renshaw, M. A., Olds, B. P., Jerde, C. L., Mcveigh, M. M. & Lodge, D. M. The room temperature preservation of filtered environmental DNA samples and assimilation into a phenol-chloroform-isoamyl alcohol DNA extraction. *Mol. Ecol. Resour.* **15,** 168–176 (2015).