

Advancing airborne eDNA sampling methods for monitoring diverse terrestrial vertebrate communities

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Running title: Optimised air eDNA sampling for vertebrate surveys

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Supplementary tables

Supplementary Table 1. Inflow and outflow airflow rate for each sampler setup with different filter types (Experiment 1), and combination of power of fan (12 V and 5 V) and filter sizes (small and large filters) (Experiment 3). Airflow measurements were taken using a Testo 405i Smart Probe thermal anemometer (order number: 05601405). Measurements were taken every second for three minutes. The 3-minute average and the standard deviation (SD) of the airflow rates are given in the table.

Fan setup	Filter type	Average supply (Inflow) airflow rate m ³ h ⁻¹ ± SD	Average outlet (Outflow) airflow rate m ³ h ⁻¹ ± SD
12 V auxiliary fan with large filter housing	ISO ePM1 65% (company 1)	0.10 ± 0.11	10.79 ± 0.186
	ISO ePM1 65% (company 2)	1.64 ± 0.089	12.33 ± 0.139
	ISO ePM 2.5 70%	0.65 ± 0.161	10.83 ± 0.349
	ISO ePM10 65%	3.49 ± 0.082	12.27 ± 0.213
12 V auxiliary fan with small filter housing	ISO ePM10 65%	2.46 ± 0.127	12.07 ± 0.189
5 V auxiliary fan with small filter housing	ISO ePM10 65%	1.84 ± 0.109	3.14 ± 0.053
5 V auxiliary fan with large filter housing	ISO ePM10 65%	3.14 ± 0.053	3.78 ± 0.156

Supplementary Table 2. Domesticated taxa and exotic pet taxa detected in each dataset. Number of samples in which each taxa is detected are given in the table. Taxa that are not

26 expected to be found in the sampling area is highlighted in bold font. Average $\pm$ SD of
 27 proportion of sequences in samples are given parenthesis. Potential origin of the taxa is given
 28 in the last column.
 29

Class	Taxa	English name	Experiment 1 (Kalvebod Fælled)	Experiment 2 (Æbelø)	Experiment 3 (Tofte Forest)	Potential origin
Aves	<i>Gallus gallus</i>	Chicken	70 (58.6% $\pm$ 18.2%)	48 (38.3% $\pm$ 30.1%)	73 (19.7% $\pm$ 27.6%)	Domestic
	<i>Meleagris gallopavo</i>	Turkey	30 (2.7% $\pm$ 5.5%)	2 (0.6% $\pm$ 3.7%)		Domestic
	<i>Psittacula</i> sp.	Parakeet	1 (0.04% $\pm$ 0.3%)			Escaped pets
	<i>Pavo cristatus</i>	Indean peafowl	1 (0.07% $\pm$ 0.6%)			Escaped pets
	<i>Cairina moschata</i>	Muscovy duck	1 (0.01% $\pm$ 0.09%)			Domestic
	<i>Lophura nycthemera</i>	Silver pheasant	1 (0.007% $\pm$ 0.06%)			Escaped pets
	<i>Melopsittacus undulatus</i>	Budgerigar	1 (0.004% $\pm$ 0.04%)		1 (0.06% $\pm$ 0.7%)	Escaped pets
	<i>Psittacula krameri</i>	Rose-ringed parakeet		1 (0.02% $\pm$ 0.2%)		Escaped pets
Mammals	<i>Sus scrofa</i>	Pig	71 (62.7% $\pm$ 30.9%)	60 (71.9% $\pm$ 18.7%)	131 (55.1% $\pm$ 38.4%)	Domestic
	<i>Sus spp.</i>	Pig			8 (0.001% $\pm$ 0.006%)	Domestic
	<i>Canis lupus</i>	Dog	56 (13.1% $\pm$ 19.2%)	36 (7.7% $\pm$ 10.7%)	138 (32.7% $\pm$ 38.4%)	Domestic
	<i>Canis spp.</i>	Dog			1 (0.00005% $\pm$ 0.0007%)	Domestic
	<i>Bos taurus</i>	Cattle	53 (6.6% $\pm$ 10.3%)		23 (1.7% $\pm$ 7.8%)	Domestic
	<i>Bos indicus</i>	Zebu		31 (3.3% $\pm$		Misannot

				4.6%)		ated <i>Bos taurus</i> sequences
	<i>Capra hircus</i>	Goat		1 (0.08%±0.58%)		Domestic
	<i>Capra_spp.</i>	Goat			1 (0.2%±2.5%)	Domestic
	<i>Ovis_spp.</i>	Sheep			2 (0.08%±0.8%)	Domestic
	<i>Homo sapiens</i>	Human	64 (12.2%±28.4%)	8 (6.9%±33.8%)	35 (4.7%±17.9%)	Human
	<i>Homo spp.</i>	Human			1 (0.0005%±0.006%)	Human
	<i>Pan troglodytes</i>	Chimpanzee		4 (0.59%±2.9%)	11 (0.5%±2.7%)	Misannotated Human sequences
	<i>Equus caballus</i>	Horse	5 (0.3%±1.5%)	5 (0.18%±0.7%)	2 (0.03%±0.3%)	Domestic
	<i>Felis silvestris</i>	Cat		1 (0.05%±0.38%)		Domestic
	<i>Felis spp.</i>	Cat	2 (0.3%±2.4%)			Domestic
	<i>Loxodonta africana</i>	African Bush elephant	1 (0.04%±0.4%)			Laboratory contamination

Supplementary Table 3. Number of sequences within Operational Taxonomic Units (OTUs) identified as wild birds and mammals in Experiment 1. Due to a fan malfunctioning, one sample belonging to the filter class ISO ePM 2.5 70% was not included in the analyses. Bird identifications are from metabarcoding with the BirT primer set, while mammal identifications are from metabarcoding with the 16Smam primer set. Averages are calculated across samples with detections within the respective taxonomic group.

Filter class	Number	Wild birds			Wild mammals		
		Number samples	Total sequence	Average sequence	Number samples	Total sequence	Average sequence

	analyse d samples	with detection s of wild bird taxa	s within OTUs assigned to wild bird taxa	s per sample of sequence s within OTUs assigned to wild birds (+/- SD)	with detection s of wild mammal taxa	s within OTUs assigned to wild mammal taxa	s per sample of sequence s within OTUs assigned to wild mammal s (+/- SD)
ISO ePM1 65% (LL)	18	17	286,696	16,864 ± 13,744	7	47,762	6,823 ± 3,280
ISO ePM1 65% (FB)	18	18	504,683	28,037 ± 13,775	3	101,387	33,795 ± 17,389
ISO ePM 2.5 70%	17	17	277,011	16,294 ± 6,536	7	51,239	7,319 ± 4,552
ISO ePM1 0 65%	18	18	740,344	41,130 ± 18,746	14	265,597	18,971 ± 15,753
Total	71	70	1,808,73 4	25,839 ± 17,076	31	465,985	29,124 ± 14,354

Supplementary Table 4. Pairwise differences between bird and mammalian taxa richness detected by four different filter types. Significant differences are indicated with bold font.

Primer set	Comparison	Estimate	t.ratio	p
BirT	ISO ePM1 65% (LL) vs. ISO ePM1 65% (FB)	-2.61	-4.346	0.0003
	ISO ePM1 65% (LL) vs. ISO ePM 2.5 70%	-1.04	-1.703	0.3302
	ISO ePM1 65% (LL) vs. ISO ePM10 65%	-5.83	-9.710	<0.0001
	ISO ePM1 65% (FB) vs. ISO ePM 2.5 70%	1.57	2.577	0.0578
	ISO ePM1 65% (FB) vs. ISO ePM10 65%	-3.22	-5.363	<0.0001
	ISO ePM 2.5 70% vs. ISO ePM10 65%	-4.79	-7.858	<0.0001
16S_mam	ISO ePM1 65% (LL) vs. ISO ePM1 65% (FB)	0.0556	0.214	0.9965
	ISO ePM1 65% (LL) vs. ISO ePM 2.5 70%	-0.023	-0.087	0.9998

	ISO ePM1 65% (LL) vs. ISO ePM10 65%	-1.000	-3.850	0.0015
	ISO ePM1 65% (FB) vs. ISO ePM 2.5 70%	-0.078	-0.297	0.9908
	ISO ePM1 65% (FB) vs. ISO ePM10 65%	-1.056	-4.064	0.0007
	ISO ePM 2.5 70% vs. ISO ePM10 65%	-0.977	-3.705	0.0024

Supplementary Table 5. Pairwise differences between beta dispersion (distance to group centroids) of bird and mammalian communities between the four different filter types. Significant differences are indicated with bold font. There was no significant pairwise differences in 16S_mam dataset.

Primer set	Comparison	estimate	t.ratio	p
BirT	ISO ePM1 65% (LL) vs. ISO ePM1 65% (FB)	0.1670	2.705	0.0424
	ISO ePM1 65% (LL) vs. ISO ePM 2.5 70%	0.1468	2.341	0.0994
	ISO ePM1 65% (LL) vs. ISO ePM10 65%	0.2676	4.335	0.0003
	ISO ePM1 65% (FB) vs. ISO ePM 2.5 70%	-0.0202	-0.327	0.9878
	ISO ePM1 65% (FB) vs. ISO ePM10 65%	0.1007	1.655	0.3556
	ISO ePM 2.5 70% vs. ISO ePM10 65%	0.1209	1.958	0.2149

Supplementary Table 6. Number of sequences within Operational Taxonomic Units (OTUs) identified as wild birds and mammals in Experiment 2. Averages are calculated across samples with detections with the respective taxonomic group.

Filter class	Number analysed samples	Wild birds			Wild mammals		
		Number samples with detections of wild bird taxa	Total sequence s within OTUs assigned to wild bird taxa	Average sequence s per sample of sequence s within OTUs assigned to wild birds (+/- SD)	Number samples with detections of wild mammal taxa	Total sequence s within OTUs assigned to wild mammal taxa	Average sequence s per sample of sequence s within OTUs assigned to wild mammals (+/- SD)

Dry-stored filter halves	20	20	679,032	33,951 ± 19,792	18	155,009	8,556 ± 4,788
LB-stored filter halves	20	19	917,337	48,280 ± 57,722	20	582,877	29,143 ± 17,693
LB buffer leftovers	20	8	256,330	32,041 ± 24,616	11	76,449	6,950 ± 4,252
Total	60	47	1,852,699	39,419 ± 40,158	49	813,335	16,598 ± 15,715

Supplementary Table 7. Pairwise differences between bird and mammalian taxa richness detected by different storage methods. Significant differences are indicated with bold font.

Primer set	Comparison	estimate	t.ratio	p
BirT	Dry stored vs. Longmire's buffer stored	6.30	8.765	< 0.0001
	Dry stored vs. Buffer	10.05	13.98	< 0.0001
	Longmire's buffer stored vs. Buffer	3.75	5.218	< 0.0001
16Smam	Dry stored vs. Longmire's buffer stored	0.1	0.399	0.9162
	Dry stored vs. Buffer	1.1	4.389	0.0003
	Longmire's buffer stored vs. Buffer	1.0	3.990	0.0008

Supplementary Table 8. Pairwise PERMANOVA test investigating the community compositional differences between sample storage methods for mammal dataset. There were no significant differences in bird dataset. Significant differences are indicated with bold font.

Primer set	Comparison	F Model	R ²	Adjusted p value
16Smam	Dry stored vs. Longmire's buffer stored	0.8607	0.0288	1.0000
	Dry stored vs. Buffer	10.96	0.2889	0.0003
	Longmire's buffer stored vs. Buffer	26.04	0.4197	0.0003

Supplementary Table 9. Number of sequences within Operational Taxonomic Units (OTUs) identified as wild birds and mammals in Experiment 3. Averages are calculated across samples with detections with the respective taxonomic group.

Filter class	Number analysed samples	Wild birds			Wild mammals		
		Number samples with detections of wild bird taxa	Total sequences within OTUs assigned to wild bird taxa	Average sequences per sample of sequences within OTUs assigned to wild birds (+/- SD)	Number samples with detections of wild mammal taxa	Total sequences within OTUs assigned to wild mammal taxa	Average sequences per sample of sequences within OTUs assigned to wild mammals (+/- SD)
12 V fan with large filter	30	30	1,562,159	52,072 ± 23,248	22	550,421	25,019 ± 19,346
12 V fan with small filter	30	29	777,663	26,816 ± 15,702	13	149,898	11,530 ± 9,084
5 V fan with large filter	30	29	840,253	28 974 ± 20,500	18	426,275	23,681 ± 18,631
5 V fan with small filter	30	11	568,419	24,714 ± 16,015	12	325,171	27,097 ± 31,187
Passive large filter	30	2	981,676	490,838 ± 3,487	3	155,008	51,669 ± 61,821
Passive small filter	30	0	0	0	0	0	0
Total	180	101	4,730,170	64,394 ± 41,859	68	1,606,773	23629 ± 23,671

Supplementary Table 10. Pairwise differences between bird and mammalian taxa richness detected by different air sampler types. Significant differences are indicated with bold font.

Primer set	Comparison	estimate	t.ratio	p
BirT	12V large filter vs. 12V small filter	2.085	3.851	0.0023

	12V large filter vs. 5V small filter	3.918	7.238	< 0.0001
	12V large filter vs. 5V large filter	1.951	3.605	0.0055
	12V large filter vs. large passive filter	5.818	10.75	< 0.0001
	12V large filter vs. small passive filter	6.251	11.55	< 0.0001
	12V small filter vs. 5V small filter	1.833	3.388	0.0112
	12V small filter vs. 5V large filter	-0.133	-0.246	0.9999
	12V small filter vs. large passive filter	3.733	6.898	< 0.0001
	12V small filter vs. small passive filter	4.167	7.700	< 0.0001
	5V small filter vs. 5V large filter	-1.967	-3.634	0.0049
	5V small filter vs. large passive filter	1.900	3.511	0.0075
	5V small filter vs. small passive filter	2.333	4.312	0.0004
	5V large filter vs. large passive filter	3.867	7.144	< 0.0001
	5V large filter vs. small passive filter	4.300	7.947	< 0.0001
	Large passive filter vs. small passive filter	0.433	0.801	0.9670
16Smam	12V large filter vs. 12V small filter	0.600	3.270	0.0163
	12V large filter vs. 5V small filter	0.733	3.997	0.0013
	12V large filter vs. 5V large filter	0.233	1.272	0.7999
	12V large filter vs. large passive filter	0.965	5.256	< 0.0001
	12V large filter vs. small passive filter	1.133	6.177	< 0.0001
	12V small filter vs. 5V small filter	0.133	0.727	0.9783
	12V small filter vs. 5V large filter	-0.367	-2.000	0.3470
	12V small filter vs. large passive filter	0.365	1.988	0.3537
	12V small filter vs. small passive filter	0.533	2.909	0.0467
	5V small filter vs. 5V large filter	-0.500	-2.727	0.0755
	5V small filter vs. large passive filter	0.231	1.261	0.8056
	5V small filter vs. small passive filter	0.400	2.182	0.2521
	5V large filter vs. large passive filter	0.731	3.987	0.0014
	5V large filter vs. small passive filter	0.900	4.908	< 0.0001

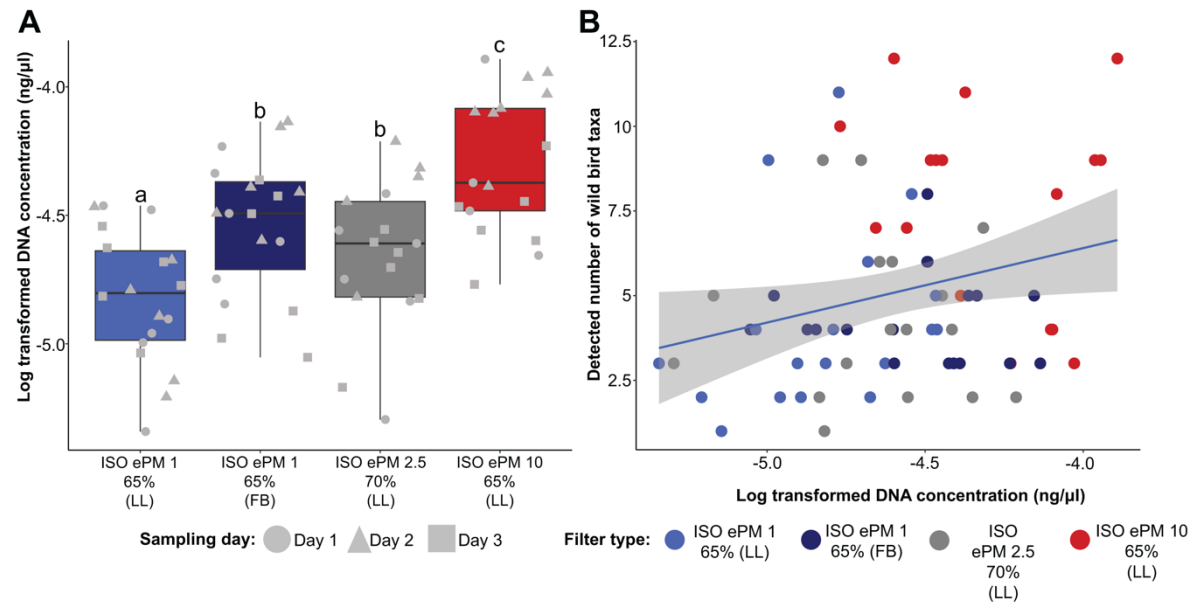
	Large passive filter vs. small passive filter	0.169	0.920	0.9410
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Supplementary Table 11. Amount of template DNA and PCR cycles used for metabarcoding PCRs.

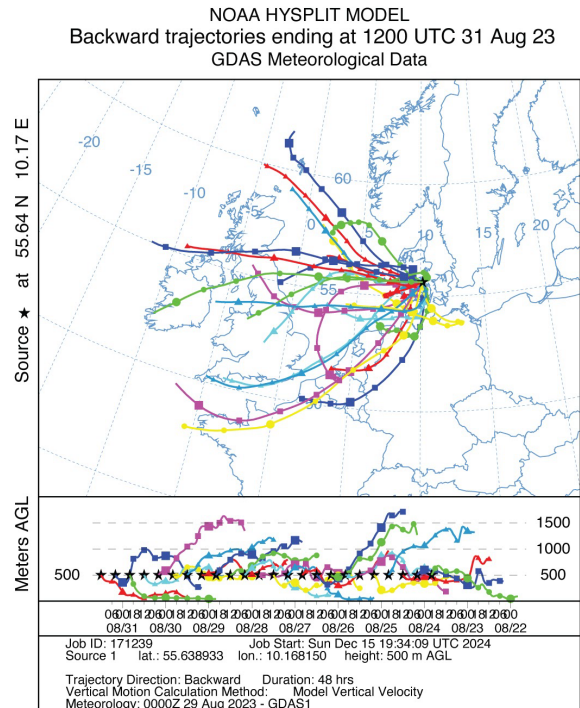
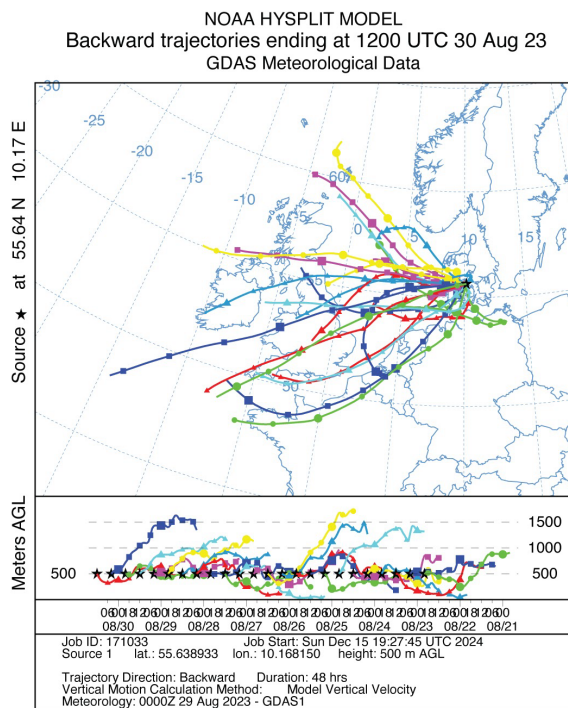
Project	Primer set	Sampler type	Amount of template DNA	Number of PCR cycles
Experiment 1: Air filter type experiment	BirT	12v fan with 6x6cm filters	3 µl	35
	16Smam	12v fan with 6x6cm filters	3 µl	35
Experiment 2: Sample storage experiment	BirT	Dry stored filter half	3 µl	33
		Longmire's buffer stored filter half	3 µl	35
		Buffer extract	3 µl	37
	16Smam	Dry stored filter half	3 µl	35
		Longmire's buffer stored filter half	3 µl	37
		Buffer extract	3 µl	37
Experiment 3: Airflow rate experiment	BirT	12v fan with 6x6cm filters	2 µl	36
		12v fan with 4x4cm filters	2 µl	36
		5v fan with 4x4cm filters	2 µl	36
		5v fan with 6x6cm filters	2 µl	36
		4x4cm passive filters	3 µl	41
		6x6cm passive filters	3 µl	41
	16Smam	12v fan with 6x6cm filters	2 µl	37
		12v fan with 4x4cm filters	2 µl	37
		5v fan with 4x4cm filters	3 µl	37
		5v fan with 6x6cm filters	3 µl	37

		4x4cm passive filters	3 μ l	39
		6x6cm passive filters	3 μ l	39

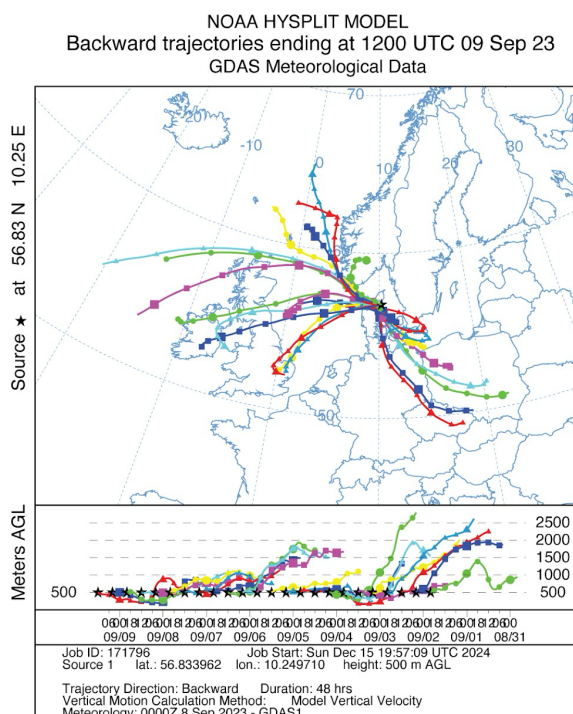
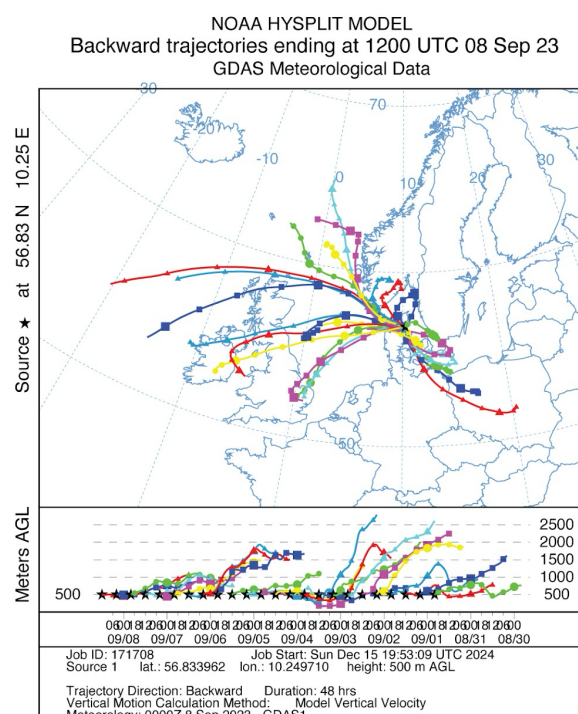
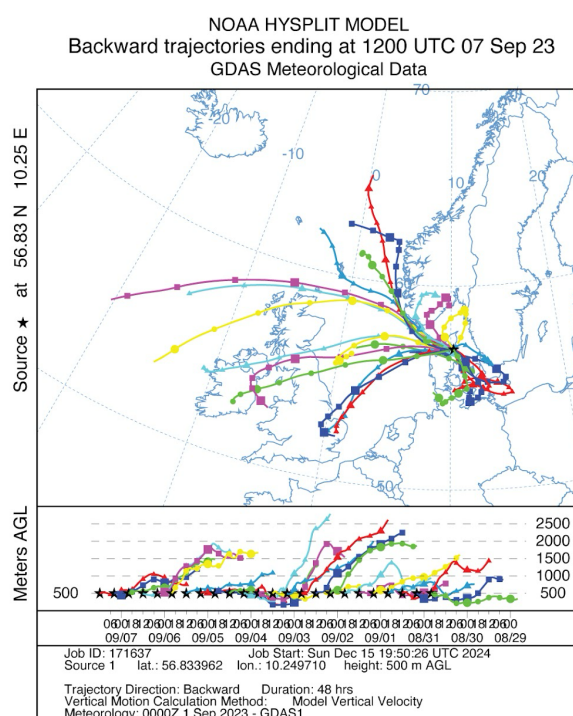
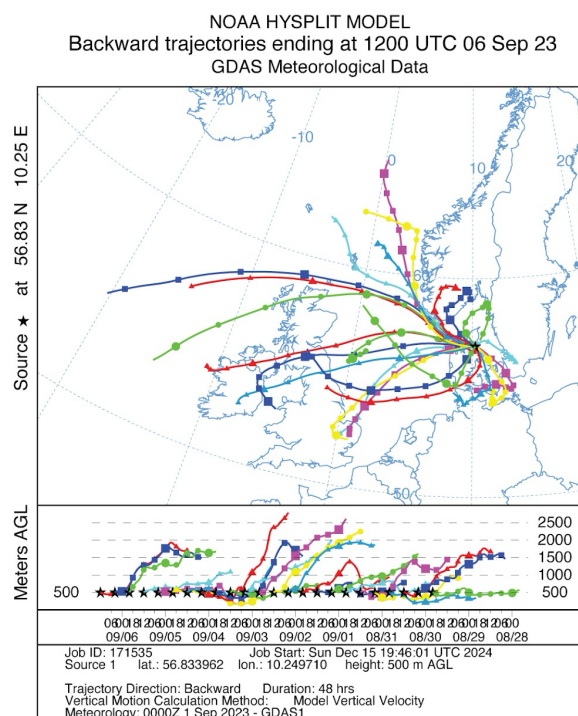
Supplementary figures



Supplementary Figure 1. **A.** Box plot shows the differences in log-transformed bird DNA concentrations accumulated by different filter types based on qPCR results from BirT primers. Letters above box plots indicate the outcome of the post hoc tests. **B.** Association between the log-transformed bird DNA concentrations and the detected number of wild bird taxa in each sample. Gray area around the trend line represents the standard error of the data.



Supplementary Figure 3. Air flow back trajectories on Æbelø (55.638933 N, 10.16815 E) from 30th to 31st August 2023 (Experiment 2).



Supplementary Figure 4. Air flow back trajectories at Tofte skov (56.8339617 N, 10.24971 E) from 6th to 9th September 2023 (Experiment 3).