

*Supporting text and supporting material for:*

## **Holistic monitoring of aquatic and terrestrial vertebrates by camera trapping and aquatic environmental DNA**

### **AUTHORS**

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### **Supporting text with details on methods applied**

#### **Supporting text A:**

For the qPCR (quantitative polymerase chain reaction) setup, we used tagged versions of the MiFish primer set (MiFish-U-F: 5'-GTCGGTAAACTCGTGCCAGC-3' and MiFish-U-R: 5'-CATAGTGGGGTATCTAATCCCAGTTTG-3') that targets a 163-185 bp part of the mitochondrial DNA 12S rRNA gene (Miya al. 2015). Many of the extracted samples looked brownish and dirty.

Discoloration can indicate the presence of humic substances in the extraction, which can inhibit the subsequent PCR procedure (Wilson 1997). Dilution of the extracted DNA material can minimize this inhibition (Jane 2015, Wilcox et al. 2015, McKee 2015, Spear et al. 2015). To determine inhibitors are present in the extractions, we made a dilution series (1:1, 1:8 and 1:32) of three different samples with different degrees of discoloration and performed a qPCR analysis on an Agilent Stratagene Mx3005P machine. This made it possible to check if diluted extraction more easily could amplify the environmental DNA target fragment, and thereby help avoid inhibition in the later PCR metabarcoding setup. Extracted samples were diluted in ddH<sub>2</sub>O. Each qPCR tube was prepared in 25 µL total volume reactions comprising: 1 µL MiFish primer (forward and reverse) (10 mM each), 0.2 µL TaqGold polymerase (5 U/µL), 2 µL dNTPs (2 mM/ dNTP), 2 µL MgCl<sub>2</sub> (25 mM), 2.5 µL AmpliTaq buffer (x10), 0.25 µL BSA (20 mg/mL), 13.05 µL ddH<sub>2</sub>O, 1 µL SYBR green mix [prepared from 1 part SYBR Green (Invitrogen cat. no. S7563), 4 parts ROX reference dye (Invitrogen cat. no. 12223-012), and 2000 parts DMSO (Invitrogen)], and 3 µL diluted DNA-template – i.e. dilution levels being 1:1, 1:8 and 1:32 of original extracted water sample. The thermocycling parameters were set at 95 °C for 10 minutes, followed by 45 cycles of (94 °C in 30 s, 50 °C for 30 s and 72 °C for 1 minute), with collecting of fluorescence at end-point in extension phase. The qPCR with diluted extraction indicated the highest dilution at 1:32 performed best, by amplifying at the earliest cycle of quantification. Based on this, we diluted all the extracted samples into 1:32 for the PCR metabarcoding setup to prevent or minimize inhibition.

### **Supporting text B:**

The PCR for metabarcoding was performed in three replicates per sample. Using the primer set Mamm01 (mamm01\_F:5'-CCGCCCGTCACCCTCCT-3', mamm01\_R: 5'-GTAYRCTTACCWTGTTACGAC-3'), which is optimized for mammals (Taberlet et al., 2018), and the primer set MiFish-U (MiFish-U\_F: 5'-GTCGGTAAACTCGTGCCAGC-3', MiFish-U\_R: 5'-

CATAGTGGGGTATCTAATCCCAGTTTG-3'), which is optimized for bony fishes (Miya et al., 2015). Each PCR was done in a 25  $\mu$ L volume, comprised 13.05  $\mu$ L ddH<sub>2</sub>O, 2.0  $\mu$ L dNTPs (2.0 mM/dNTP), 2.5  $\mu$ L GeneAmp® 10x PCR Gold Buffer, 1.5  $\mu$ L MgCl<sub>2</sub> (25 mM), 0.25 BSA (20 mg/ $\mu$ L), 0.2  $\mu$ L AmpliTaq Gold™ DNA Polymerase (5 U/ $\mu$ L), 1.25  $\mu$ L of each primer (10 mM) (forward and reverse), and 3  $\mu$ L extracted DNA (diluted 1:32). A 1:32 dilution of the extracted DNA was preferred, based on a qPCR analysis testing for inhibition at three different dilution levels (1:1, 1:8, and 1:32). The qPCR showed that the highest dilution level (1:32) yielded the earliest onset of amplification (see supplementary material text A). Thermocycling parameters of the PCR with the MiFish-U primer set were set to an initial 5-minute denaturation at 95°C, followed by 40 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s and extension at 72°C for 90 s, and then a final extension at 72°C for 10 minutes. For the Mamm01 primer set, PCR thermocycling parameters were set to an initial 5-minute denaturation at 95°C, followed by 37 cycles of denaturation at 94°C for 30 s, annealing at 53°C for 30 s and extension at 72°C for 90 s, and then a final extension for 72°C for 10 minutes, as in previous protocols (Sigsgaard et al., 2017). Setup of PCR for metabarcoding is outlined in supplementary table S1-S8.

### **Supporting text C:**

From each of the 12 libraries (2\*3 replicates for MiFish-U and 2\*3 replicates for Mamm01) (supplementary material table S1-S8) we pooled 10  $\mu$ L to a total of 120  $\mu$ L. The 120  $\mu$ L was then purified using the MinElute® PCR purification kit (cat. nos. 28006), following the supplied protocol with an additional centrifuge step for 60 s at 10,000 G after discarding flow-through, and a heat step at 37°C for 10 minutes, before the spin column was added to a new 1.5 mL LoBind Eppendorf tube. We added 20  $\mu$ L EB buffer, heated to 37°C for 10 minutes and centrifuged for 1 minute at 10,000 G, and repeated the addition of 20  $\mu$ L EB buffer and the heat step, giving a total of 40  $\mu$ L per pooled sample.

**Table. S1.** PCR 37 with the Mamm01 primer setup. The yellow plate column number (plt.c No) and plate row number (plt.r No) indicates the individual tag (Tag no) for each sample, the orange color denotes the different pooled samples, except for NegC (negative control) and Mock (positive control), and the blue the three replicates (roman numerals) for each sample. Each sample have had their own tag number, which was the same number (e.g. Tag01) for both forward and reverse primer. ‘Lib.no’ indicates lettercode for pooled libraries A to C. See table S7 for list of tags used for primers. Sample abbreviations (Sampl Abbr) are explained in table S8.

	plt.c No	1	2	3		4	5	6		7	8	9
plt.r No	Sampl Abbr	Tag no	Tag no	Tag no	Sampl Abbr	Tag no	Tag no	Tag no	Sampl Abbr	Tag no	Tag no	Tag no
A	Und2	T01	T01	T01	Skel9	T09	T09	T09	Kat12	T17	T17	T17
B	Und3	T02	T02	T02	Skel12	T10	T10	T10	Str9-17	T18	T18	T18
C	Und5	T03	T03	T03	Kat9-17	T11	T11	T11	Str10-17	T19	T19	T19
D	Und9	T04	T04	T04	Kat10-17	T12	T12	T12	Str1	T20	T20	T20
E	Und12	T05	T05	T05	Kat2	T13	T13	T13	Str2	T21	T21	T21
F	Skel2	T06	T06	T06	Kat3	T14	T14	T14	Str3	T22	T22	T22
G	Skel3	T07	T07	T07	Kat5	T15	T15	T15	Mock	T30	T30	T30
H	Skel5	T08	T08	T08	Kat9	T16	T16	T16	NegC	T31	T31	T31
Repl No		I	II	III		I	II	III		I	II	III
Lib No		A	B	C		A	B	C		A	B	C

**Table. S2.** PCR 38 with the Mamm01 primer setup. The yellow plate column number (plt.c No) and plate row number (plt.r No) indicates the individual tag (Tag no) for each sample, the orange color denotes the different pooled samples, except for NegC (negative control) and Mock (positive control), and the blue the three replicates (roman numerals) for each sample. Each sample have had their own tag number, which was the same number (e.g. Tag01) for both forward and reverse primer. ‘Lib.no’ indicates lettercode for pooled libraries E to F. See table S7 for list of tags used for primers. Sample abbreviations (Sampl Abbr) are explained in table S8.

	plt.c No	1	2	3		4	5	6		7	8	9
plt.r No	Sampl Abbr	Tag no	Tag no	Tag no	Sampl Abbr	Tag no	Tag no	Tag no	Sampl Abbr	Tag no	Tag no	Tag no
A	Str2	T01	T01	T01	Tisin12	T09	T09	T09	Bøs5	T17	T17	T17
B	Str9	T02	T02	T02	Tisout2	T10	T10	T10	Bøs9	T18	T18	T18
C	Str12	T03	T03	T03	Tisout3	T11	T11	T11	Bøs12	T19	T19	T19
D	Mad	T04	T04	T04	Tisout5	T12	T12	T12	NegC	T30	T30	T30
E	Tisin2	T05	T05	T05	Tisout9	T13	T13	T13	Mock	T31	T31	T31
F	Tisin3	T06	T06	T06	Tisout12	T14	T14	T14				
G	Tisin5	T07	T07	T07	Bøs2	T15	T15	T15				
H	Tisin9	T08	T08	T08	Bøs3	T16	T16	T16				
Repl No		I	II	III		I	II	III		I	II	III
Lib No		E	D	F		E	D	F		E	D	F

**Table. S3.** PCR 21 with the MiFish-U primer setup. The yellow plate column number (plt.c No) and plate row number (plt.r No) indicates the individual tag (Tag no) for each sample, the orange color denotes the different pooled samples, except for NegC (negative control) and Mock (positive control), and the blue the three replicates (roman numerals) for each sample. Each sample have had their own tag number, which was the same number (e.g. Tag01) for both forward and reverse primer. ‘Lib.no’ indicates lettercode for pooled libraries G to I. See table S7 for list of tags used for primers. Sample abbreviations (Sampl Abbr) are explained in table S8.

	plt.c No	1	2	3		4	5	6
plt.r No	Sampl Abbr	Tag no	Tag no	Tag no	Sampl Abbr	Tag no	Tag no	Tag no
A	Und9	T45	T45	T45	Str9	T53	T53	T53
B	Und12	T46	T46	T46	Str12	T54	T54	T54
C	Skel9	T47	T47	T47	NegC	T55	T55	T55
D	Skel12	T48	T48	T48	Mock	T56	T56	T56
E	Kat9-17	T49	T49	T49				
F	Kat9	T50	T50	T50				
G	Kat12	T51	T51	T51				
H	Str9-17	T52	T52	T52				
	Repl No	I	II	III		I	II	III
	Lib No	G	H	I		G	H	I

**Table. S4.** PCR 22 with the MiFish-U primer setup. The yellow plate column number (plt.c No) and plate row number (plt.r No) indicates the individual tag (Tag no) for each sample, the orange color denotes the different pooled samples, except for NegC (negative control) and Mock (positive control), and the blue the three replicates (roman numerals) for each sample. Each sample have had their own tag number, which was the same number (e.g. Tag01) for both forward and reverse primer. ‘Lib.no’ indicates lettercode for pooled libraries G to I. See table S7 for list of tags used for primers. Sample abbreviations (Sampl Abbr) are explained in table S8.

	plt.c No	1	2	3		4	5	6
plt.r No	Sampl Abbr	Tag no	Tag no	Tag no	Sampl Abbr	Tag no	Tag no	Tag no
A	Mad9	T45	T45	T45	Mock	T53	T53	T53
B	Tisin9	T46	T46	T46				
C	Tisin12	T47	T47	T47				
D	Tisout9	T48	T48	T48				
E	Tisout12	T49	T49	T49				
F	Bøs9	T50	T50	T50				
G	Bøs12	T51	T51	T51				
H	NegC	T52	T52	T52				
	Repl No	I	II	III		I	II	III
	Lib No	J	K	L		J	K	L

**Table S5.** Measured concentrations on Qubit for all libraries (Lib No) after pooling of samples and MinElute purification in order to get a sufficiently high concentration (ng) to build libraries.

Lib No	Qubit concentration (ng/ $\mu$ L)	Amount of sample ( $\mu$ L) added in order to get 250 ng	EB Buffer added to get final volume of 60 $\mu$ L
A	6.98	35.8	24.2
B	6.68	37.4	22.6
C	7.60	32.9	27.1
D	9.44	26.5	33.5
E	8.54	29.3	30.7
F	9.56	26.2	33.8
G	33.60	14.9	45.1
H	40.80	12.3	47.7
I	32.80	15.2	44.8
J	24.00	20.8	39.2
K	23.80	21.0	39.0
L	24.80	20.2	39.8

**Table S6.** Information needed to pool samples in order to send to the High-throughput DNA Sequencing Centre at the University of Copenhagen. The TapeStation results were used for evaluating the fragment length. Abbreviations used above columns are: 'Lib No' library number; 'TiNm' TruSeq adapter-index name; 'Aseq' Adapter sequence; 'CTS' Conc (ng/ $\mu$ L) TS, TapeStation; 'CQb' Conc (ng/ $\mu$ L), Qubit; 'Psz' Peak size (bp) measured by TapeStation; 'CPk' Conc.of peak (ng/ $\mu$ L), measured by TapeStation; 'VPur' Vol. of purified library ( $\mu$ L) after Qubit and TapeStation; 'Insz' Insert size (bp), incl. primers and tags and adaptor indexed ligated; 'LCo' Lowest conc. (nM); 'c' (Concentration in ng/ $\mu$ l) / (660 g/mol \* average library size in bp) \*  $10^6$ ; 'Ac' Average c (nM); 'V1' A volume between 6  $\mu$ L and volume of purified library; 'V2' Volume ( $\mu$ L) to be used for pooling; 'Psz Ntg' Product size (bp) without tags.

Lib No	TiNm	Aseq	CTS	CQb	Psz	CPk	VPur	Insz	LCo	c	Ac	V1	V2	Psz Ntg
A	AR001	ATCACG(A)	56.5	4.4	530	20.5	27	237	27.5	28.4	29	9	9	97
B	AR003	TTAGGC(A)	228.0	4.5	537	76.5	27	237	27.5	28.5	29	9	9	97
C	AR008	ACTTGA(A)	66.9	5.2	537	13.2	27	237	27.5	33.0	29	9	8	97
D	AR009	GATCAG(A)	76.5	4.3	512	25.4	27	237	27.5	27.7	29	9	9	97
E	AR010	TAGCTT(A)	125.0	4.3	525	95.7	27	237	27.5	27.5	29	9	9	97
F	AR011	GGCTAC(A)	58.2	4.4	497	43.1	27	237	27.5	28.1	29	9	9	97
G	AR002	CGATGT(A)	12.1	5.0	622	6.9	27	358	17.9	21.3	23	9	8	218
H	AR004	TGACCA(A)	11.1	5.0	622	6.9	27	358	17.9	21.2	23	9	8	218
I	AR005	ACAGTG(A)	8.9	4.2	591	5.4	27	358	17.9	17.9	23	9	9	218
J	AR006	GCCAAT(A)	16.4	6.6	582	9.5	27	358	17.9	28.1	23	9	6	218
K	AR007	CAGATC(A)	13.3	5.9	607	8	27	358	17.9	25.1	23	9	6	218
L	AR012	CTTGTA(A)	11.7	5.6	617	7	27	358	17.9	23.6	23	9	7	218

**Table S7.** List of tags and their corresponding nucleotide sequence used for each primer set.

Tag number	Tag nucleotide sequence	Primerset
T01	AACAAC	Mamm01
T02	AACCGA	Mamm01
T03	CCGGAA	Mamm01
T04	AGTGTT	Mamm01
T05	CCGCTG	Mamm01
T06	AACGCG	Mamm01
T07	GGCTAC	Mamm01
T08	TTCTCG	Mamm01
T09	TCACTC	Mamm01
T10	GAACTA	Mamm01
T11	CCGTCC	Mamm01
T12	AAGACA	Mamm01
T13	CGTGCG	Mamm01
T14	GGTAAG	Mamm01
T15	ATAATT	Mamm01
T16	CGTCAC	Mamm01
T17	TTGAGT	Mamm01
T18	AAGCAG	Mamm01
T19	TTGCAA	Mamm01
T20	CACGTA	Mamm01
T21	TAACAT	Mamm01
T22	TGCGTG	Mamm01
T30	GTACAC	Mamm01
T31	AAGTGT	Mamm01
T45	CTATAA	MiFi-U
T46	AATGAA	MiFi-U
T47	CGAATC	MiFi-U
T48	AGAGAC	MiFi-U
T49	TTCGGA	MiFi-U
T50	CGACGT	MiFi-U
T51	CTCATG	MiFi-U
T52	TGTATA	MiFi-U
T53	ACAACC	MiFi-U
T54	TCAGAG	MiFi-U
T55	GTAGTG	MiFi-U
T56	AGCACT	MiFi-U

**Table S8.** Overview of samples. Study sites, sampling date, number of replicates and volume of filtered water. Laboratory work by MM was with the MiFish-U primerset (Miya et al, 2015). Sampling location abbreviations and filtered volume of water sample. Labwork was performed by Anne Marie Rubæk Holm (AMRH) and Malene Månson (MM). Sampling locations are abbreviated as: 'Bøs' Bøstrup; 'Kat' Kattrup; 'Mad' Madesø; 'Skel' Skellingsted; 'Str' Stridsmølle; 'Tisin' Tissø inlet; 'Tisout' Tissø outlet; 'Und' Undløse.

Sampl	Date	No.	Filtered	Labwork
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<b>Abbreviation</b>		<b>replicates</b>	<b>volume (mL)</b>
Bøs	2018.Dec.13	3	3 x 400 AMRH
Bøs	2018.Feb.27	3	3 x 350 MM
Bøs	2018.Mar.28	3	3 x 500 MM
Bøs	2018.May.09	3	3 x 300 MM
Bøs	2018.Sep.04	3	3 x 60 AMRH
Kat	2017.Oct.26	2	2 x 300 MM
Kat	2017.Sep.27	2	2 x 500 AMRH
Kat	2018.Feb.27	3	3 x 200 MM
Kat	2018.Mar.28	3	3 x 350 MM
Kat	2018.May.09	3	3 x 600 MM
Kat	2018.Sep.04	3	3 x 1000 AMRH
Mad	2017.Sep.27	2	2 x 500 AMRH
Skel	2018.Dec.13	3	3 x 400 AMRH
Skel	2018.Feb.27	3	3 x 200 MM
Skel	2018.Mar.28	3	3 x 400 MM
Skel	2018.May.09	3	420/480/480 MM
Skel	2018.Sep.04	3	3 x 600 AMRH
Str	2017.Oct.26	2	2 x 300 MM
Str	2017.Sep.17	2	2 x 500 AMRH
Str	2018.Dec.13	3	3 x 400 AMRH
Str	2018.Dec.13	3	3 x 400 AMRH
Str	2018.Feb.27	3	240/230/205 MM
Str	2018.Jan.31	3	3 x 300 MM
Str	2018.Mar.28	3	3 x 350 MM
Str	2018.May.09	3	480/600/540 MM
Str	2018.Sep.04	3	3 x 600 AMRH
Tisin	2018.Dec.13	3	3 x 400 AMRH
Tisin	2018.Feb.27	2	3 x 200 MM
Tisin	2018.Mar.28	3	500/450/450 MM
Tisin	2018.May.09	3	3 x 500 MM
Tisin	2018.Sep.04	3	3 x 600 AMRH
Tisout	2018.Dec.13	3	3 x 300 AMRH
Tisout	2018.Feb.27	2	3 x 400 MM
Tisout	2018.Mar.28	3	3 x 400 MM
Tisout	2018.May.09	3	3 x 500 MM
Tisout	2018.Sep.04	3	3 x 300 AMRH
Und	2018.Dec.13	3	3 x 600 AMRH
Und	2018.Feb.27	3	3 x 270 MM
Und	2018.Mar.28	3	500/500/450 MM
Und	2018.May.09	3	780/720/720 MM
Und	2018.Sep.04	3	3 x 1000 AMRH

**Supporting text D:**

**Results of mainly local interest.**



Seven fish families were found in total, and at five of the seven sites, all these families were represented. At the two remaining sites, Bøstrup and Tissø inlet, Salmonidae and Cobitidae were absent, respectively. At each of the study sites, the number of fish species detected by eDNA varied between 12 and 19 species, with Katstrup having the largest diversity and Undløse the lowest (Table 1, Suppl. Table S9).

The only detected reptile, grass snake (*Natrix natrix*), was found at Skellingsted. Amphibians were detected at six of the eight study sites. The northern crested newt was detected at Stridsmølle and Undløse, where the smooth newt was detected at Bøstrup and Tissø inlet (Suppl. Table 1).

Mammals were found present at all eight sites via both CT and eDNA and varied in richness from four to 18 species per site. Hedgehog (*Erinaceus europaeus*) and European hare (*Lepus europaeus*) were only detected in Katstrup. None of the three Soricomorpha were detected south of Tissø (neither at Bøstrup or Tissø outlet). Four and six different mammals were detected by eDNA alone at Tissø inlet and Tissø outlet, respectively, as no CT were deployed at these two sites. Six mammal species were detected at Bøstrup with both CT and eDNA (Table 1, Suppl. Table S9). Birds were found present at all eight locations with both CT and eDNA. The number of detected species per site varied from 18 to 47. Katstrup showed the highest diversity of bird species (47 species), while Stridsmølle and Undløse yielded roughly half this richness (23 species at each site). Sites south of Tissø (Bøstrup and Tissø outlet) held the lowest number of bird orders (4 and 5 species, respectively), with Charadriiformes, Columbiformes, Accipitriformes, Coraciiformes, Galliformes, Piciformes, and Strigiformes being absent (Suppl. Table S9, Suppl. Figure 6). At none of the sites were all the 12 orders of birds known from the area represented. Bøstrup, Tissø inlet, and Tissø outlet had 11 to 12 different waterfowl (Anseriformes) at each site. Katstrup had the largest number of passerines (Passeriformes, 25 species).

Northern crested newt and smooth newt were not detected at the same sites (Suppl. Table S9). Smooth newt was only detected at Bøstrup and Tissø inlet, while northern crested newt was detected at Stridsmølle and Undløse. The smooth newt often inhabits meadows, forests, and uncultivated areas, whereas the northern crested newt to a larger degree is dependent on forests and areas with settlements (Fog *et al.*, 2001a, 2001b). Their preferred habitats were confirmed by the eDNA monitored here (Table 1, Suppl. Table S9), but additional research is needed to verify if this is a general trend. Monitoring of these amphibians may turn out to be difficult as the eDNA of stream dwelling salamanders have been shown to be detectable only within few meters of the source animal (Pilliod *et al.* 2014).

The number of mammals at each study site varied a lot, from four (Tissø outlet) to 18 species (Kattrup) (Table 1). The 23 wild mammal species found in total accounts for 68 % of the mammals known from this area from traditional surveys. As the mammals recorded in Åmosen inhabit a wide variety of strata (e.g., fossorial, terrestrial, aquatic, volant or arboreal) the effort needed to efficiently monitor their diversity and distribution need further diversification, as some species was almost never be detected by the CT or eDNA sampling design in our study. The European mole (*Talpa europaea*) are common but fossorial, and six different bats (Vespertilionidae) forage in the evenings outside the hours where eDNA sampling was done, so the absence of these animals from the recorded vertebrates might reflect their ecology and strata they inhabit (Baagøe and Jensen, 2007). Excluding species, that alludes to our monitoring design, raises our diversity record percentage from 68% to 85 %.

The overall detection of birds varied from 18 (Bøstrup) to 47 (Kattrup). The number of birds found was likely caused by the differences in habitat composition i.e. Kattrup having many, tall, old trees. Tissø inlet and outlet had the highest number of waterfowls, and this match these two areas being an oasis for waterfowl species. Several Passerine birds were detected at Kattrup (n = 25) compared to the remaining sites with 2-11 records. Kattrup has forest on one side of the stream with

cover, and a mix of habitat types with more open areas on the other side and makes this habitat diverse for species that require both hiding and foraging places in their habitat (European Commission, 2007; Naturstyrelsen, 2013, 2014).

**Table S9.** Overview of results from camera trapping and eDNA in Åmosen. For each taxon, the number of camera events (CE) (sightings of animal(s) of same species with at least a 30-minute interval), the number of CE of a given taxon per 100 camera days (relative abundance index; RAI) and naïve occupancy (number of sites/samples that are positive to taxon presence divided by the total number of sites/samples) of CT data (six CT sampling sites, and 18 sampling locations in total), together with number of reads and Naïve occupancy from eDNA data (seven sampling sites, and 40 samples in total) is given. DOM = domestic species. Danish Red List status: NT = Near threatened, VU = Vulnerable, EN = Endangered, and CR = Critically endangered (Moeslund et al., 2019). Natura 2000 protection: (N2000) (Naturstyrelsen, 2012). Note that eDNA was not sampled at Bromølle, and CTs were not placed at Tissø inlet and Tissø outlet. Abbreviations in table represents: Camera trapping (CE), environmental DNA (eDNA), relative abundance index (RAI), number of sites (ns), number of locations (nl), number of reads (nr), number of samples (nspl). Sample sites are abbreviated: Bromølle (Bro), Bøstrup (Bøs), Kattrup (Kat), Skellingsted (Ske), Stridsmølle (Str), Tissøinlet (Tisin), Tissøoutlet (Tisou), Undløse (Und).

Class/Family	Taxon	Status	Camera trapping				Environmental DNA											
			CE	RAI	ns	nl	Reads	ns	nspl	Bro	Bøs	Kat	Ske	Str	Tisin	Tisou	Und	
<b>Actinopterygii</b>																		
Anguillidae																		
	<i>Anguilla anguilla</i>	CR					160074	1.0	0.6		x	x	x	x	x	x	x	
Cobitidae																		
	<i>Cobitis taenia</i>	N2000					3667	0.9	0.2		x	x	x	x			x	x
Cyprinidae																		
	<i>Abramis brama</i>						170362	0.9	0.3		x	x	x	x	x		x	
	<i>Alburnus alburnus</i>						95164	0.7	0.2		x	x		x	x		x	
	<i>Carassius auratus</i>						1523	0.3	0.1				x	x				
	<i>Cyprinus carpio</i>						622	0.7	0.2			x	x	x			x	x
	<i>Gobio gobio</i>						148211	0.7	0.6			x	x	x	x			x
	<i>Leucaspius delineatus</i>						198648	0.9	0.4		x	x	x	x	x			x
	<i>Leuciscus idus</i>						556969	1.0	0.5		x	x	x	x	x		x	x
	<i>Rutilus rutilus</i>						2541591	1.0	1.0		x	x	x	x	x		x	x
	<i>Scardinius erythrophthalmus</i>						16458	1.0	0.2		x	x	x	x	x		x	x
	<i>Tinca tinca</i>						193480	0.9	0.4		x	x	x	x	x		x	
Esocidae																		
	<i>Esox lucius</i>		1	0.0	0.2	0.1	305488	1.0	0.8		x	x	x	x	x		x	x

Gasterosteidae														
	<i>Gasterosteus aculeatus</i>		205202	0.7	0.3				x	x	x	x	x	x
	<i>Pungitius pungitius</i>		346094	1.0	0.8				x	x	x	x	x	x
Percidae														
	<i>Gymnocephalus cernua</i>		753260	0.7	0.4				x	x	x	x	x	
	<i>Perca fluviatilis</i>		1776745	1.0	0.9				x	x	x	x	x	x
	<i>Sander lucioperca</i>		12886	0.4	0.1					x		x	x	
Salmonidae														
	<i>Oncorhynchus mykiss</i>		1866	0.6	0.2					x	x	x		x
	<i>Salmo trutta</i>		59469	0.9	0.5					x	x	x	x	x
<b>Amphibia</b>														
Bufonidae														
	<i>Bufo bufo</i>		2679	0.7	0.2				x	x	x	x		x
Ranidae														
	<i>Rana temporaria</i>	NT	344	0.4	0.1						x		x	x
Salamandridae														
	<i>Lissotriton vulgaris</i>		216	0.3	0.1				x			x		
	<i>Triturus cristatus</i>	N2000	4725	0.3	0.1						x			x
<b>Aves</b>														
Accipitridae														
	<i>Accipiter nisus</i>	VU	2	0.0	0.3	0.1			x			x		
	Accipitridae indet,		2	0.0	0.2	0.1					x			
	<i>Buteo buteo</i>		50	0.6	0.5	0.5	54	0.1	0.0	x		x	x	x
Alcedinidae														
	<i>Alcedo atthis</i>	VU	3	0.0	0.2	0.1						x		
Anatidae														
	<i>Anas acuta</i>	EN					16877	0.4	0.1		x	x		x
	<i>Anas clypeata</i>	VU					1321	0.4	0.1				x	x
	<i>Anas crecca</i>	VU	3	0.0	0.2	0.1	17397	0.9	0.3		x	x	x	x
	<i>Anas platyrhynchos</i>		1422	16.2	1.0	0.9	816794	1.0	0.9	x	x	x	x	x
	Anatinae indet,						239	0.1	0.0				x	
	<i>Anser albifrons</i>	NT					3844	0.6	0.2		x		x	x

<i>Anser anser</i>	N2000	45	0.5	0.3	0.3	1989848	0.9	0.7			x	x	x	x	x	x	
<i>Anser indet,</i>						111966	1.0	0.6		x	x	x	x	x	x	x	
<i>Aythya ferina</i>	VU					7481	0.4	0.1		x				x	x		
<i>Aythya fuligula</i>	NT					52391	0.9	0.3		x	x	x		x	x	x	
<i>Branta canadensis</i>						327033	0.7	0.3		x		x	x	x	x		
<i>Bucephala clangula</i>	VU	9	0.1	0.3	0.3						x	x					
<i>Cairina moscata</i>	DOM	1	0.0	0.2	0.1	4968	0.1	0.0	x	x							
<i>Cygnus cygnus</i>	N2000					34090	0.4	0.2						x	x	x	
<i>Cygnus olor</i>						4899	0.4	0.1		x				x		x	
<i>Mareca penelope</i>	CR					388197	0.9	0.5		x	x	x	x	x	x		
<i>Mergellus albellus</i>						18604	0.3	0.1						x	x		
<i>Mergus merganser</i>		1	0.0	0.2	0.1	130	0.1	0.0		x	x						
<i>Tadorna tadorna</i>						91	0.1	0.0		x							
Ardeidae																	
<i>Botaurus stellaris</i>	VU, N2000	2	0.0	0.3	0.1						x			x			
<i>Ardea cinerea</i>		331	3.8	0.9	0.8	59	0.1	0.1	x	x	x	x	x			x	
Certhiidae																	
<i>Certhia brachydactyla</i>		1	0.0	0.2	0.1									x			
Cinclidae																	
<i>Cinclus cinclus</i>	CR	4	0.1	0.3	0.2				x		x						
Columbidae																	
<i>Columba palumbus</i>		642	7.3	0.9	0.8	993	0.4	0.1	x		x	x	x	x		x	
Corvidae																	
<i>Corvidae indet,</i>						2867	0.7	0.1		x	x	x			x	x	
<i>Corvus corax</i>						255	0.3	0.1		x					x		
<i>Corvus cornix</i>		92	1.1	0.2	0.5						x					x	
<i>Corvus corone</i>						2873	0.7	0.2			x	x	x		x	x	
<i>Corvus frugilegus</i>						4021	0.3	0.1			x				x		
<i>Garrulus glandarius</i>		44	0.5	0.3	0.4				x		x						
Emberizidae																	
<i>Emberiza citrinella</i>	VU	1	0.0	0.2	0.1												x
Fringillidae																	
<i>Chloris chloris</i>	NT	4	0.1	0.2	0.1						x						
<i>Coccothraustes coccothraustes</i>		7	0.1	0.2	0.1						x						

<i>Fringilla coelebs</i>		27	0.3	0.2	0.3	230	0.4	0.1				x			x			x
<i>Loxia curvirostra</i>		9	0.1	0.2	0.1							x						
<i>Spinus spinus</i>	NT	1	0.0	0.2	0.1									x				
Laridae																		
<i>Larinae indet,</i>						19035	0.3	0.1						x			x	
Motacillidae																		
<i>Anthus pratensis</i>		1	0.0	0.2	0.1									x				
<i>Motacilla alba</i>		92	1.1	0.5	0.4									x	x			
<i>Motacilla cinerea</i>	VU	26	0.3	0.5	0.3				x	x				x				
<i>Motacilla flava</i>		1	0.0	0.2	0.1				x									
<i>Motacilla indet,</i>		2	0.0	0.2	0.1									x				
Muscicapidae																		
<i>Erithacus rubecula</i>		121	1.4	0.7	0.6				x					x		x		
<i>Phoenicurus phoenicurus</i>		3	0.0	0.3	0.1				x					x				
Paridae																		
<i>Cyanistes caeruleus</i>		3	0.0	0.2	0.2									x				
<i>Parus major</i>		13	0.2	0.5	0.3				x					x		x		
<i>Periparus ater</i>		1	0.0	0.2	0.1									x				
Passeridae																		
<i>Passer montanus</i>		4	0.1	0.2	0.1									x				
Phalacrocoracidae																		
<i>Phalacrocorax carbo</i>		3	0.0	0.3	0.2	33732	0.6	0.2						x		x	x	x
Phasianidae																		
<i>Gallus gallus</i>	DOM	2	0.0	0.2	0.1	376	0.3	0.1	x								x	
<i>Phasianus colchicus</i>		225	2.6	0.5	0.6	3545	0.6	0.1	x			x	x	x				
<i>Meleagris gallopavo</i>	DOM					801	0.1	0.1				x						
Phylloscopidae																		
<i>Phylloscopus trochilus</i>	VU	1	0.0	0.2	0.1				x									
Picidae																		
<i>Dendrocopos major</i>		5	0.1	0.2	0.2									x				
Podicipedidae																		
<i>Podiceps cristatus</i>						3703	0.1	0.1										x
<i>Podiceps grisegena</i>		1	0.0	0.2	0.1									x				
<i>Tachybaptus ruficollis</i>		13	0.2	0.2	0.1	13444	0.7	0.3						x	x	x	x	x

<b>Rallidae</b>																
<i>Fulica atra</i>	VU	71	0.8	0.5	0.4	46088	1.0	0.5		x	x	x	x	x	x	x
<i>Gallinula chloropus</i>	VU	65	0.7	0.9	0.3	177	0.3	0.1	x	x	x	x	x		x	x
<i>Rallus aquaticus</i>		7	0.1	0.2	0.1						x					
<b>Scolopacidae</b>																
<i>Tringa ochropus</i>	EN	3	0.0	0.3	0.1				x		x					
<i>Scolopax rusticola</i>		12	0.1	0.5	0.2	954	0.3	0.1	x		x	x		x		x
<b>Sittidae</b>																
<i>Sitta europaea</i>		9	0.1	0.2	0.2						x					
<b>Strigidae</b>																
<i>Strix aluco</i>		6	0.1	0.3	0.2						x	x				
<b>Sturnidae</b>																
<i>Sturnus vulgaris</i>	VU	4	0.1	0.2	0.1						x					
<b>Sylviidae</b>																
<i>Sylvia atricapilla</i>		2	0.0	0.3	0.1						x		x			
<i>Sylvia communis</i>		1	0.0	0.2	0.1						x					
<b>Troglodytidae</b>																
<i>Troglodytes troglodytes</i>		24	0.3	0.3	0.3						x					x
<b>Turdidae</b>																
<i>Turdus iliacus</i>		7	0.1	0.3	0.2				x		x					
<i>Turdus indet,</i>		178	2.0	0.7	0.8	9921	0.7	0.2	x	x	x	x	x		x	x
<i>Turdus merula</i>		710	8.1	0.7	0.9				x		x		x			x
<i>Turdus philomelos</i>		119	1.4	0.5	0.6	7880	0.4	0.1		x	x	x		x		x
<i>Turdus pilaris</i>		1	0.0	0.2	0.1					x						

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## Mammalia

<b>Bovidae</b>																
<i>Bos taurus</i>	DOM	2	0.0	0.2	0.1	173318	1.0	0.8		x	x	x	x	x	x	x
<i>Ovis aries</i>	DOM					89	0.1	0.0			x					
<b>Canidae</b>																
<i>Canis lupus</i>	DOM	17	0.2	0.5	0.3	20491	1.0	0.3		x	x	x	x	x	x	x
<i>Vulpes vulpes</i>	NT	532	6.1	0.8	0.7	118858	0.7	0.2	x	x	x	x	x	x	x	x
<b>Cervidae</b>																
<i>Capreolus capreolus</i>		1172	13.4	0.5	0.7	95391	0.7	0.4	x		x	x	x		x	x
Cervidae indet,		3	0.0	0.2	0.1						x					



<i>Cervus elaphus</i>		52	0.6	0.2	0.4	17870	0.7	0.3			x	x	x	x	x
<i>Dama dama</i>		3	0.0	0.2	0.1						x				
Cricetidae															
<i>Arvicola amphibious</i>		58	0.7	1.0	0.4				x	x	x	x	x		x
Arvicolinae indet,		4	0.1	0.5	0.2	9889	1.0	0.5	x	x	x	x	x	x	x
<i>Microtus agrestis</i>		2	0.0	0.3	0.1				x			x			
<i>Myodes glareolus</i>		1	0.0	0.2	0.1	43105	0.9	0.3		x	x	x	x	x	x
Equidae															
<i>Equus ferus</i>	DOM					233	0.1	0.1			x				
Erinaceidae															
<i>Erinaceus europaeus</i>		1	0.0	0.2	0.1						x				
Felidae															
<i>Felis catus</i>	DOM	7	0.1	0.3	0.2	16582	0.1	0.1			x	x	x		
Leporidae															
<i>Lepus europaeus</i>		3	0.0	0.2	0.1						x				
Muridae															
<i>Apodemus flavicollis</i>						1302	0.6	0.1				x	x	x	x
<i>Micromys minutus</i>						638	0.3	0.1				x	x		
Muridae indet,		73	0.8	1.0	0.7				x	x	x	x	x		x
<i>Rattus norvegicus</i>		719	8.2	1.0	1.0	20456	0.9	0.3	x	x	x	x	x	x	x
Mustelidae															
<i>Lutra lutra</i>	VU, N2000	35	0.4	0.3	0.3						x	x			
<i>Martes martes</i>	NT, N2000	572	6.5	0.9	0.8				x		x	x	x		x
<i>Meles meles</i>		17	0.2	0.2	0.3						x				
<i>Mustela erminea</i>	NT	15	0.2	0.7	0.3					x	x	x			x
<i>Mustela nivalis</i>	NT	2	0.0	0.3	0.1						x	x			
<i>Mustela putorius</i>	NT	76	0.9	0.7	0.7				x	x	x		x		
Mustelidae indet,		76	0.9	0.8	0.8				x		x	x	x		x
<i>Neovison vison</i>		342	3.9	0.9	0.8				x		x	x	x		x
Sciuridae															
<i>Sciurus vulgaris</i>		234	2.7	0.3	0.3	91	0.1	0.0	x		x				
Soricidae															
<i>Neomys fodiens</i>	NT	14	0.2	0.7	0.3	1413	0.1	0.1	x			x	x		x
<i>Sorex araneus</i>						1692	0.4	0.1					x	x	x

<i>Sorex minutus</i>						339	0.1	0.0			x						
Soricidae indet,		9	0.1	0.7	0.3					x	x	x					x
<i>Myomorpha</i> indet,		191	2.2	0.8	0.7				x		x	x	x				x
Suidae																	
<i>Sus scrofa</i>	DOM					141520	1.0	0.7		x	x	x	x	x	x	x	x
<b>Squamata</b>																	
Colubridae																	
<i>Natrix natrix</i>		2	0.0	0.2	0.1							x					
<b>Total</b>		<b>8674</b>				<b>12166198</b>			<b>36</b>	<b>51</b>	<b>104</b>	<b>66</b>	<b>67</b>	<b>49</b>	<b>49</b>	<b>60</b>	

**Table S10.** Corrected taxa from eDNA samples. Bacteria and other prokaryotes were found but discarded completely. All vertebrates detected by eDNA are explained in this table. The column named ‘Action’ indicates what evaluation was done given the species inferred in the left most column named ‘Given species’. The prefiltering script was applied after sequences had been compared with the NCBI GenBank database using BLAST, but before stacked bar plots were prepared. Notes on species from Månsson (2018) samples from sequencing libraries M-R.

Given species	Action	Reasoning for action
<i>Ablennes hians</i>	Removed	Contamination only found in Månssons samples (2018) (Library M-R). Fish from Qatar
<i>Acanthurus olivaceus</i>	Removed	Prefiltering script connected it with <i>Gasterosteus aculeatus</i> , but only 85 % identical, thereby disregarded. Only found in mock
<i>Alces alces</i>	<i>Cervus elaphus</i>	Corrected for via prefiltering script
<i>Amaurornis phoenicurus</i>	<i>Gallinula chloropus</i>	Corrected for via prefiltering script
<i>Anampses neoguinaicus</i>	<i>Anampses twistii</i>	Closely related genetically, only found in mock samples
<i>Anas chathamica</i>	<i>Anas platyrhynchos</i>	Corrected for via prefiltering script
<i>Anguilla dieffenbachii</i>	<i>Anguilla anguilla</i>	Corrected for via prefiltering script
<i>Anser caerulescens</i>	<i>Anser</i> indet.	Genetically identical to more than one <i>Anser</i> species that are possible in the area
<i>Anser cygnoides</i>	<i>Anser anser</i>	Swan goose has not been spotted in the area for 10 years and is extremely rare in DK. Similar genetics in the 12S gene to the Greylag goose, which is very frequent in the area
<i>Baleana mysticetus</i>	Removed	Contamination only found in Månssons samples (Library M-R).
<i>Bodianus anthiodes</i>	<i>Bodianus axillaris</i>	Closely related genetically, only found in mock samples
<i>Branta sandvicensis</i>	<i>Branta canadensis</i>	Corrected for via prefiltering script
<i>Cephalophus natalensis</i>	Removed	Few reads and only in the mock samples.
<i>Chaerodon selene</i>	Removed	Lab contaminant, only found in mock, under five reads
<i>Chaetodon rafflesii</i>	Removed	Lab contaminant, only found in mock, under five reads
<i>Chanos chanos</i>	Removed	Contamination only found in Månssons samples (Library M-R). Fish from Qatar
<i>Clupea pallasii</i>	Removed	Lab contaminant only found in mock. Under five reads
<i>Corvus brachyrhynchos</i>	<i>Corvidae</i> indet.	Only three NCBI matches for this sequence. One is a subspecies of <i>Corvus corone</i>
<i>Corvus hawaiiensis</i>	<i>Corvus corone</i>	Corrected for via prefiltering script
<i>Corvus splendens</i>	<i>Corvus corax</i>	Genetic match on NCBI GenBank

**Table S11.** Species list of all the vertebrates found in Åmosen, by the means of literature (traditional surveys). Summary of presence of vertebrate taxa fauna found in Nature Park Åmosen through the last ca. 20 years. Data from Atlas over Danske Ferskvandsfisk (Danish Freshwater Fish Atlas) (Carl & Møller 2012) and additional information from the updated atlas database (provided by Henrik Carl and Peter Rask Møller); Dansk Pattedyratlas (Danish Mammal Atlas) (Baagøe & Jensen 2007); Fuglenes Danmark (The birds of Denmark) (Grell 1998) and data from Danish Ornithology Society (DOF) provided by Michael Fink. The national biodiversity portal Arter.dk was also consulted. (†) Presumed locally extinct or not returned. (I) only found in a little spot in the outer edges of Åmosen. (II) Found at 'Flasken' (a border between the stream and the ocean. According to the Danish IUCN Red List: (CR) critically endangered, (EN) endangered, (VU) vulnerable, (NT) near threatened, these statuses is made from the 2019 updated list, note that there can be a difference if the birds are migratory or breeding in the area. The comments also include whether the species is Natura 2000 protected in the area and what directive annex they are protected in if they are (European Commission 2009, Naturstyrelsen 2012).

Family	Species	Common name	Outlier	Comment	eDNA	CT
<b>Agnatha</b>						
Petromyzonti dae	<i>Lampetra fluviatilis</i> †	European river lamprey	Yes	Annex II+V, Natura 2000, latest obs. early 1900s		
	<i>Lampetra planeri</i> †	Brook lamprey	Yes	Annex II, Natura 2000, latest obs. early 1900s		
<b>Actinopterygii</b>						
Anguillidae	<i>Anguilla anguilla</i>	European eel		CR	X	
Cobitidae	<i>Cobitis taenia</i>	Spined loach		Annex II, Natura 2000	X	
Cyprinidae	<i>Abramis brama</i>	Freshwater bream			X	
	<i>Alburnus alburnus</i>	Common bleak			X	
	<i>Carassius auratus</i>	Goldfish		Non-indigoens	X	
	<i>Carassius carassius</i>	Crucian carp				

	<i>Ctenopharyngodon idella</i>	Grass carp		Non-indigoens		
	<b><i>Cyprinus carpio</i></b>	Common carp			X	
	<b><i>Gobio gobio</i></b>	Gudgeon			X	
	<b><i>Leucaspis delineatus</i></b>	Sunbleak			X	
	<b><i>Leuciscus idus</i></b>	Ide			X	
	<b><i>Rutilus rutilus</i></b>	Common roach			X	
	<b><i>Scardinius erythrophthalmus</i></b>	Common rudd			X	
	<b><i>Tinca tinca</i></b>	Tench			X	
Esocidae	<b><i>Esox lucius</i></b>	Northern pike			X	
Gasterosteidae	<b><i>Gasterosteus aculeatus</i></b>	Three-spined stickleback			X	
	<b><i>Pungitius pungitius</i></b>	Nine-spined stickleback			X	
Lotidae	<i>Lota lota</i> <sup>†</sup>	Burbot	Yes	Latest obs. 1927		
Percidae	<b><i>Gymnocephalus cernua</i></b>	Ruffe			X	
	<b><i>Perca fluviatilis</i></b>	European perch			X	
	<b><i>Sander lucioperca</i></b>	Zander			X	
Pleuronectidae	<i>Platichthys flesus</i>	European flounder				
Salmonidae	<b><i>Oncorhynchus mykiss</i></b>	Rainbow trout		Non-indigoens	X	
	<b><i>Salmo trutta</i></b>	Brown trout			X	
Siluridae	<i>Silurus glanis</i>	Wels catfish				
<b>Amphibia</b>						
Bufonidae	<b><i>Bufo bufo</i></b>	Common toad			X	

	<i>Epidalea calamitall</i>	Natterjack toad	Yes	EN, Natura 2000, Annex IV		
Ranidae	<i>Pelophylax esculentus</i>	Edible frog		Natura 2000, Annex V		
	<i>Rana arvalis</i>	Moor frog		Natura 2000, Annex IV		
	<i>Rana temporaria</i>	Common frog		NT	X	
Salamandridae	<i>Lissotriton vulgaris</i>	Smooth newt			X	
	<i>Triturus cristatus</i>	Northern crested newt		Natura 2000, Annex II+IV	X	
<b>Squamata</b>						
Colubridae	<i>Natrix natrix</i>	Grass snake				X
Viperidae	<i>Vipera berus</i>	European viper				
Anguidae	<i>Anguis fragilis</i>	Slowworm				
Lacertidae	<i>Lacerta agilis</i>	Sand lizard		VU, Natura 2000, Annex IV		
	<i>Zootoca vivipara</i>	Viviparous lizard				
<b>Mammalia</b>						
Canidae	<i>Vulpes vulpes</i>	Red fox		NT	X	X
Cervidae	<i>Capreolus capreolus</i>	Roe deer			X	X
	<i>Cervus elaphus</i>	Red deer			X	X
	<i>Cervus nippon</i>	Sika deer		Non-indigoens		
	<i>Dama dama</i>	Fallow deer		Non-indigoens		X
Cricetidae	<i>Arvicola amphibious</i>	European water vole				X
	<i>Microtus agrestis</i>	Field vole				X
	<i>Myodes glareolus</i>	Bank vole			X	X

Erinaceidae	<b><i>Erinaceus europaeus</i></b>	European hedgehog				X
Leporidae	<b><i>Lepus europaeus</i></b>	European hare				X
	<i>Oryctolagus cuniculus</i>	European rabbit	Yes	Relatively rare		
Muridae	<b><i>Apodemus flavicollis</i></b>	Yellow-necked mouse			X	
	<i>Apodemus sylvaticus</i>	Wood mouse		NT		
	<b><i>Micromys minutus</i></b>	Eurasian harvest mouse			X	
	<i>Mus musculus</i>	House mouse		NT		
	<b><i>Rattus norvegicus</i></b>	Brown rat		Invasive	X	X
Mustelidae	<b><i>Lutra lutra</i></b>	Eurasian otter		VU, Natura 2000, Annex II+IV, relatively rare		X
	<i>Martes foina</i>	Beech marten		NT		
	<b><i>Martes martes</i></b>	Pine marten		NT, Natura 2000, Annex V		X
	<b><i>Meles meles</i></b>	European badger				X
	<b><i>Mustela erminea</i></b>	Stoat		NT		X
	<b><i>Mustela nivalis</i></b>	Least weasel		NT, relatively rare		X
	<b><i>Mustela putorius</i></b>	Polecat		NT, Annex V		X
	<b><i>Neovison vison</i></b>	American mink		Invasive species		X
Phocidae	<i>Phoca vitulina</i> <sup>II</sup>	Harbour seal	Yes	Natura 2000, Annex II+V		
Phocoenidae	<i>Phocoena phocoena</i> <sup>II</sup>	Harbour porpoise	Yes	Natura 2000, Annex II+IV		
Sciuridae	<b><i>Sciurus vulgaris</i></b>	Red squirrel			X	X
Soricidae	<b><i>Neomys fodiens</i></b>	Eurasian water		NT	X	X

		shrew				
	<i>Sorex araneus</i>	Common shrew			X	
	<i>Sorex minutus</i>	Eurasian pygmy shrew			X	
Talpidae	<i>Talpa europaea</i>	European mole				
Vespertilionidae	<i>Eptesicus serotinus</i>	Serotine bat		Annex IV		
	<i>Myotis daubentonii</i>	Daubenton's bat		Annex IV		
	<i>Nyctalus noctula</i>	Common noctule		Annex IV		
	<i>Pipistrellus nathusii</i>	Nathusius' pipistrelle		Annex IV		
	<i>Pipistrellus pygmaeus</i>	Soprano pipistrelle		Annex IV		
	<i>Plecotus auritus</i>	Brown long-eared bat		Annex IV		
<b>Aves</b>						
Accipitridae	<i>Accipiter gentilis</i>	Northern goshawk		VU, Annex I		
	<i>Accipiter nisus</i>	Eurasian sparrowhawk		VU, Annex I		X
	<i>Aquila chrysaetos</i>	Golden eagle		CR, Annex I, relatively rare		
	<i>Buteo buteo</i>	Common buzzard			X	X
	<i>Buteo lagopus</i>	Rough-legged buzzard				
	<i>Circus aeruginosus</i>	Western-marsh harrier		Natura 2000, Annex I		



	<i>Circus cyaneus</i>	Hen harrier		Annex I		
	<i>Haliaeetus albicilla</i>	White-tailed eagle		NT, Natura 2000, Annex I		
	<i>Milvus milvus</i>	Red kite		VU, Natura 2000, Annex I		
	<i>Pernis apivorus</i>	European honey buzzard		NT, Annex I		
Acrocephalidae	<i>Acrocephalus arundinaceus</i>	Great reed warbler	Yes	CR, relatively rare		
	<i>Acrocephalus palustris</i>	Marsh warbler				
	<i>Acrocephalus schoenobaenus</i>	Sedge warbler				
	<i>Acrocephalus scirpaceus</i>	Eurasian reed warbler		NT		
	<i>Hippolais icterina</i>	Icterine warbler		VU		
Aegithalidae	<i>Aegithalos caudatus</i>	Long-tailed tit				
Alaudidae	<i>Alauda arvensis</i>	Eurasian skylark		NT, Annex II		
	<i>Lullula arborea</i>	Woodlark	Yes	NT, Annex I		
Alcedinidae	<i>Alcedo atthis</i>	Common kingfisher		VU, Annex I		
Alcidae	<i>Cephus grylle</i> <sup>a</sup>	Black guillemot	Yes			
	<b><i>Anas acuta</i></b>	Pintail		EN, Annex II	X	
	<b><i>Anas clypeata</i></b>	Northern shoveler		VU, Annex II	X	
	<b><i>Anas crecca</i></b>	Eurasian teal		VU, Annex II	X	X
	<b><i>Anas platyrhynchos</i></b>	Mallard		Annex II	X	X
	<i>Anas querquedula</i>	Garganey				

	<i>Anas strepera</i>	Gadwall				
	<b>Anser anser</b>	Greylag goose		Natura 2000, Annex II	X	X
	<b>Anser albifrons</b>	Greater white footed goose		NT, Natura 2000, Annex II	X	
	<i>Anser brachyrhynchus</i>	Pink-footed goose				
	<i>Anser fabalis</i>	Bean goose				
	<b>Aythya ferina</b>	Common pochard		VU	X	
	<b>Aythya fuligula</b>	Tufted duck		NT	X	
	<b>Branta canadensis</b>	Canada goose		Annex II	X	
	<i>Branta leucopsis</i>	Barnacle goose		Annex I		
	<b>Bucephala clangula</b>	Common goldeneye		VU		X
	<i>Cygnus columbianus</i>	Tundra swan		Natura 2000, Relatively rare		
	<b>Cygnus cygnus</b>	Whooper swan		Natura 2000, Annex I	X	
	<b>Cygnus olor</b>	Mute swan		Annex II	X	
	<b>Mareca penelope</b>	Eurasian widgeon		CR	X	
	<b>Mergellus albellus</b>	Smew		Annex I	X	
	<b>Mergus merganser</b>	Common merganser		Annex II	X	X
	<i>Mergus serrator</i>	Red-breasted merganser		Annex II		
	<i>Somateria mollissima</i>	Common eider		NT, Annex II		
	<b>Tadorna tadorna</b>	Common			X	

		shelduck				
Apodidae	<i>Apus apus</i>	Common swift		NT		
Ardeidae	<b><i>Ardea cinerea</i></b>	Grey heron			X	X
	<b><i>Botaurus stellaris</i></b>	Eurasian bittern		VU, Natura 2000, Annex I		X
Caprimulgidae	<i>Caprimulgus europaeus</i>	European nightjar		NT, Annex I, relatively rare		
Certhiidae	<b><i>Certhia brachydactyla</i></b>	Short-toed treecreeper		Relatively rare		X
	<i>Certhia familiaris</i>	Eurasian treecreeper				
Charadriidae	<i>Calidris alba</i> <sup>ll</sup>	Sanderling	Yes			
	<i>Charadrius dubius</i>	Little ringed plover		NT		
	<i>Charadrius hiaticula</i>	Common ringed plover		VU		
	<i>Pluvialis apricaria</i>	European golden plover		CR, Annex II		
	<i>Pluvialis squatarola</i> <sup>ll</sup>	Grey plover	Yes	Annex II		
	<i>Vanellus vanellus</i>	Northern lapwing		VU, Annex II		
Cinclidae	<b><i>Cinclus cinclus</i></b>	White-throated dipper		CR		X
Ciconiidae	<i>Ciconia ciconia</i>	White stork		CR, Annex I, relatively rare		
Columbidae	<i>Columba livia</i> <sup>ll</sup>	Rock dove	Yes	Unknown spread. Annex II		
	<i>Columba oenas</i>	Stock dove		Annex II		
	<b><i>Columba palumbus</i></b>	Common wood pigeon		Annex II	X	X

	<i>Streptopelia decaocto</i>	Eurasian collared dove		NT, Annex II		
	<i>Streptopelia turtur</i>	European turtledove	Yes	EN, Annex II, rare		
Corvidae	<i>Coloeus monedula</i>	Western jackdaw				
	<b><i>Corvus corax</i></b>	Common raven			X	
	<b><i>Corvus cornix</i></b>	Hooded crow				X
	<b><i>Corvus corone</i></b>	Carrion crow	Yes	Annex II	X	
	<b><i>Corvus frugilegus</i></b>	Rook		Annex II	X	
	<b><i>Garrulus glandarius</i></b>	Eurasian jay		Annex II		X
	<i>Nucifraga caryocatactes</i>	Northern nutcracker		Possibly missing in the area, relatively rare		
	<i>Pica pica</i>	Eurasian magpie		Annex II		
Cuculidae	<i>Cuculus canorus</i>	Common cuckoo		NT		
	<i>Emberiza calandra</i>	Corn bunting		NT		
	<b><i>Emberiza citrinella</i></b>	Yellowhammer		VU		X
Emberizidae	<i>Emberiza schoeniclus</i>	Common red bunting		NT		
Falconidae	<i>Falco columbarius</i>	Merlin		Annex I,		
	<i>Falco peregrinus</i>	Peregrine falcon		VU, Annex I, earlier thought to be missing in the area		
	<i>Falco subbuteo</i>	Eurasian hobby		CR, relatively rare		
	<i>Falco tinnunculus</i>	Common kestrel				
Fringillidae	<i>Acanthis flammea</i>	Common redpoll				
	<i>Carduelis carduelis</i>	European				

		goldfinch				
	<i>Carduelis flavirostris</i>	Twite				
	<b><i>Chloris chloris</i></b>	European greenfinch		NT		X
	<i>Coccothraustes coccothraustes</i>	Hawfinch				
	<b><i>Fringilla coelebs</i></b>	Common chaffinch			X	X
	<i>Fringilla montifringilla</i>	Brambling				
	<i>Linaria cannabina</i>	Common linnet				
	<b><i>Loxia curvirostra</i></b>	Red crossbill				X
	<i>Pyrrhula pyrrhula</i>	Eurasian bullfinch				
	<i>Serinus serinus</i>	European serin	Yes	CR, relatively rare		
	<b><i>Spinus spinus</i></b>	European siskin		NT		X
Gruidae	<i>Grus grus</i>	Common crane		Annex I		
Haematopod idae	<i>Haematopus ostralegus</i>	Eurasian oystercatcher		Annex II		
Hirundinidae	<i>Delichon urbicum</i>	Common house martin				
	<i>Hirundo rustica</i>	Barn swallow				
	<i>Riparia riparia</i>	Collared sand martin		NT		
Laniidae	<i>Lanius collurio</i>	Red-backed shrike		Annex I		
Laridae	<i>Chroicocephalus ridibundus</i>	Black-headed gull		EN		
	<i>Larus canus</i>	Mew gull		Annex II		

	<i>Larus fuscus</i> <sup>II</sup>	Lessor black-backed gull	Yes	Annex II		
	<i>Larus marinus</i>	Great black-backed gull		Annex II		
	<i>Hydrocoloeus minutus</i>	Little gull		CR. Thought to be missing in the area. Relatively rare		
	<i>Sterna hirundo</i>	Common tern		NT, Annex I, Natura 2000		
	<i>Sterna paradisaea</i> <sup>a</sup>	Arctic tern	Yes	VU, Annex I		
	<i>Sternula albifrons</i>	Little tern		VU, Natura 2000		
	<i>Thalasseus sandvicensis</i> <sup>II</sup>	Sandwich tern	Yes			
	<i>Locustella fluviatilis</i>	River warbler	Rare			
	<i>Locustella naevia</i>	Common grasshopper warbler				
	<i>Locustella luscinioides</i>	Savi's warbler		CR, relatively rare		
	<b><i>Anthus pratensis</i></b>	Meadow pipit				X
	<i>Anthus trivialis</i>	Tree pipit	Yes			
	<b><i>Motacilla alba</i></b>	White wagtail				X
	<b><i>Motacilla cinerea</i></b>	Grey wagtail		VU		X
	<b><i>Motacilla flava</i></b>	Western yellow wagtail				X
Muscicapidae	<b><i>Erithacus rubecula</i></b>	European robin				X
e	<i>Ficedula hypoleuca</i>	European pied		VU		

		flycatcher				
	<i>Luscinia luscinia</i>	Thrush nightingale		VU		
	<i>Muscicapa striata</i>	Spotted flycatcher				
	<i>Oenanthe oenanthe</i>	Northern wheateater		VU		
	<i>Phoenicurus ochrurus</i>	Black redstart		NT		
	<b>Phoenicurus phoenicurus</b>	Common redstart				X
	<i>Saxicola rubetra</i>	Whinchat				
Oriolidae	<i>Oriolus oriolus</i>	Eurasian golden oriole	Yes	CR, Relatively rare		
Pandionidae	<i>Pandion haliaetus</i>	Osprey		CR, Natura 2000, Annex I		
Panuridae	<i>Panurus biarmicus</i>	Bearded reedling				
Paridae	<b>Cyanistes caeruleus</b>	Eurasian blue tit				X
	<b>Parus major</b>	Great tit				X
	<b>Periparus ater</b>	Coal tit				X
	<i>Poecile palustris</i>	Marsh tit				
Passeridae	<i>Passer domesticus</i>	House sparrow				
	<b>Passer montanus</b>	Eurasian tree sparrow				X
Phalacrocoracidae	<b>Phalacrocorax carbo</b>	Great cormorant			X	X
Phasianidae	<i>Perdix perdix</i>	Grey partridge		VU, Annex II		
	<b>Phasianus colchicus</b>	Common pheasant		Non-indigenous	X	X

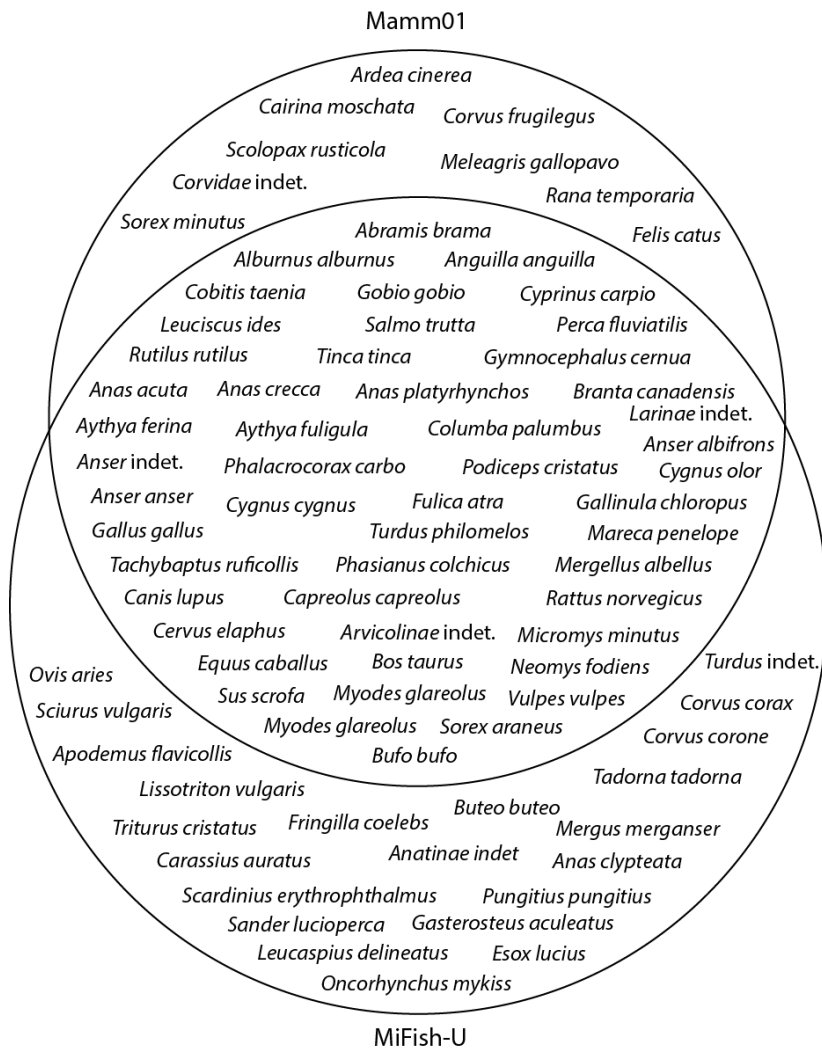
Phylloscopidae	<i>Phylloscopus collybita</i>	Common chiffchaff				
	<i>Phylloscopus sibilatrix</i>	Wood warbler				
	<b><i>Phylloscopus trochilus</i></b>	Willow warbler		VU		X
Picidae	<b><i>Dendrocopos major</i></b>	Great spotted woodpecker				X
	<i>Dryocopus martius</i>	Black woodpecker	Yes	VU, Annex I, relatively rare		
	<i>Dryobates minor</i>	Lesser spotted woodpecker	Yes	EN, relatively rare		
Podicipedidae	<b><i>Podiceps cristatus</i></b>	Great crested grebe			X	
	<b><i>Podiceps grisegena</i></b>	Red-necked grebe				X
	<i>Podiceps nigricollis</i>	Black-necked grebe				
	<b><i>Tachybaptus ruficollis</i></b>	Little grebe			X	X
Prunellidae	<i>Prunella modularis</i>	Dunnock				
Rallidae	<i>Crex crex</i>	Corncrake		VU, Annex I, relatively rare		
	<b><i>Fulica atra</i></b>	Eurasian coot		VU, Annex II	X	X
	<b><i>Gallinula chloropus</i></b>	Common moorhen		VU, Annex II	X	X
	<i>Porzana porzana</i>	Spotted crane		EN, Natura 2000, Annex I, relatively rare		
	<b><i>Rallus aquaticus</i></b>	Water rail		Annex II		X
Recurvirostridae	<i>Recurvirostra</i>	Pied avocet		VU, Annex II		



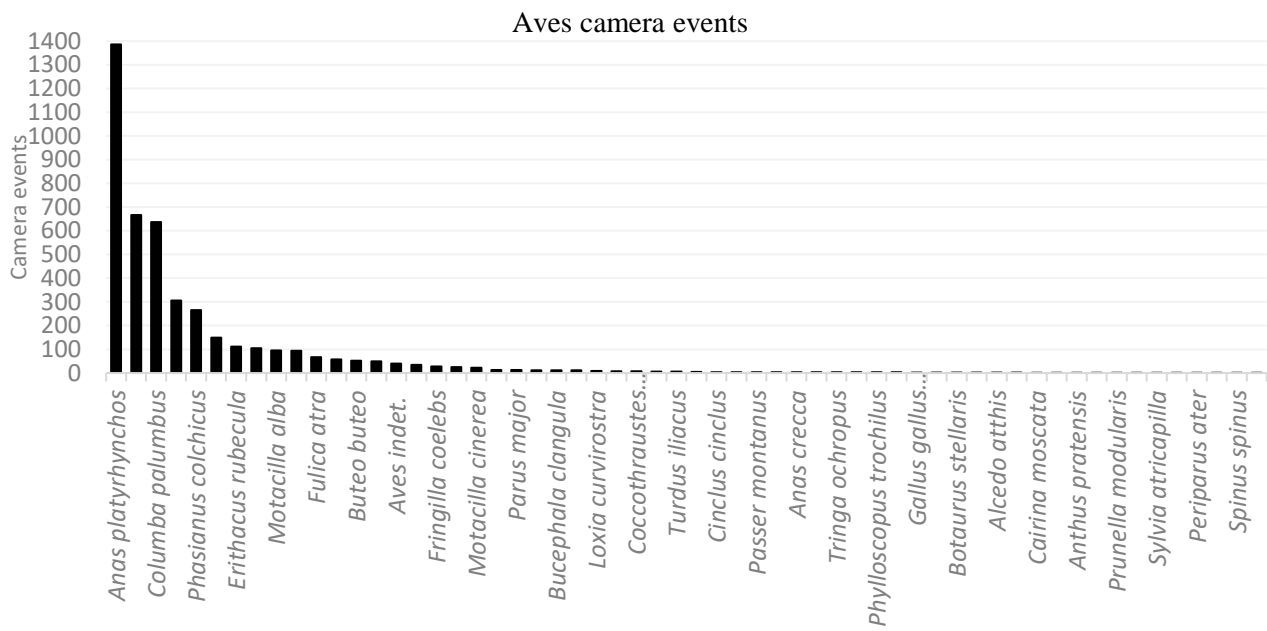
dae	<i>avosetta</i>					
Regulidae	<i>Regulus ignicapilla</i>	Common firecrest		Relatively rare		
	<i>Regulus regulus</i>	Goldcrest				
Remizidae	<i>Remiz pendulinus</i>	Eurasian penduline tit		CR		
Scolopacidae	<i>Actitis hypoleucos</i>	Common sandpiper				
	<i>Calidris alpina</i>	Dunlin		EN		
	<i>Calidris ferruginea</i>	Curlew sandpiper				
	<i>Calidris pugnax</i>	Ruff		EN, Natura 2000		
	<i>Gallinago gallinago</i>	Common snipe		Annex III		
	<i>Limosa lapponica</i>	Bar-tailed godwit		Annex II		
	<i>Limosa limosa</i> <sup>II</sup>	Black-tailed godwit	Yes	VU, Annex II		
	<i>Lymnocyptes minimus</i>	Jack snipe		Annex III, relatively rare		
	<i>Numenius arquata</i>	Eurasian curlew		VU, Annex II		
	<i>Numenius phaeopus</i> <sup>II</sup>	Whimbrel	Yes	Annex II		
	<b><i>Scolopax rusticola</i></b>	Eurasian woodcock		Annex III	X	X
	<i>Tringa glareola</i>	Wood sandpiper		EN, Annex I		
	<i>Tringa nebularia</i> <sup>II</sup>	Common greenshank	Yes	Annex II		
	<b><i>Tringa ochropus</i></b>	Green sandpiper		EN		X
	<i>Tringa totanus</i>	Common redshank		NT, Annex II		

Sittidae	<b><i>Sitta europaea</i></b>	Eurasian nuthatch				X
Strigidae	<i>Asio flammeus</i>	Short-eared owl		CR, Annex I, relatively rare		
	<i>Asio otus</i>	Long-eared owl				
	<b><i>Strix aluco</i></b>					X
Sturnidae	<b><i>Sturnus vulgaris</i></b>	Common starling				X
Sylviidae	<b><i>Sylvia atricapilla</i></b>	Eurasian blackcap				X
	<i>Sylvia borin</i>	Garden warbler				
	<b><i>Sylvia communis</i></b>	Common whitethroat				X
	<i>Sylvia curruca</i>	Lessor whitethroat				
Troglodytidae	<b><i>Troglodytes troglodytes</i></b>	Eurasian wren				X
Turdidae	<b><i>Turdus iliacus</i></b>	Redwing		Annex II		X
	<b><i>Turdus merula</i></b>	Blackbird		Annex II		X
	<b><i>Turdus philomelos</i></b>	Song thrush		Annex II	X	X
	<b><i>Turdus pilaris</i></b>	Fieldfare		Annex II		X
	<i>Turdus torquatus</i>	Ring ouzel	Yes	Relatively rare		
	<i>Turdus viscivorus</i>	Mistle thrush		Annex II		

**Supplementary figures**

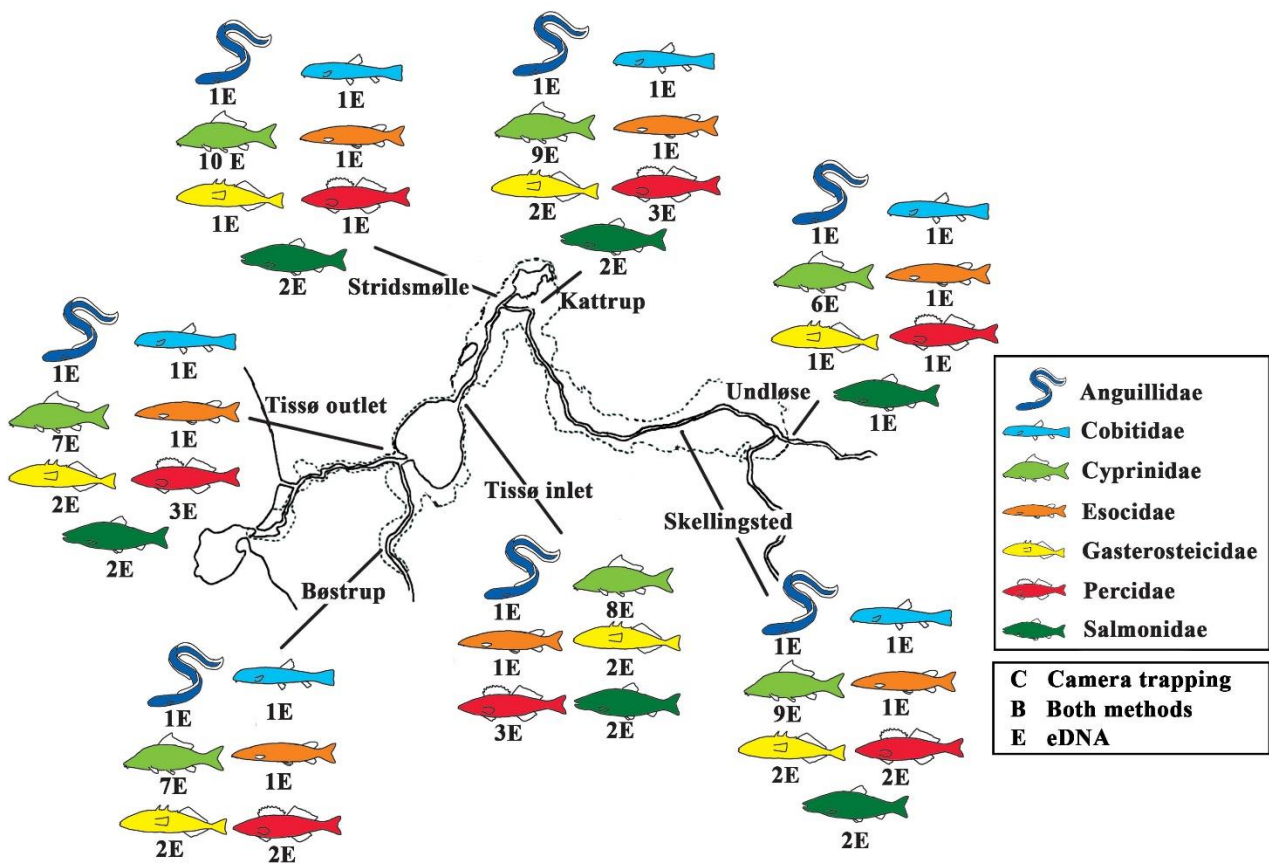


**Supplementary Figure S1. Overview of taxa detected by each primer sets, used in on the water samples collected in Åmosen. Venn diagram showing overlap between the two primer sets: Mamm01 (Taberlet *et al.* 2018a) and MiFish-U (Miya *et al.* 2015). Both primer sets detected 49 taxa, while 10 taxa only were detected with Mamm01 and 23 taxa only detected by MiFish-U.**

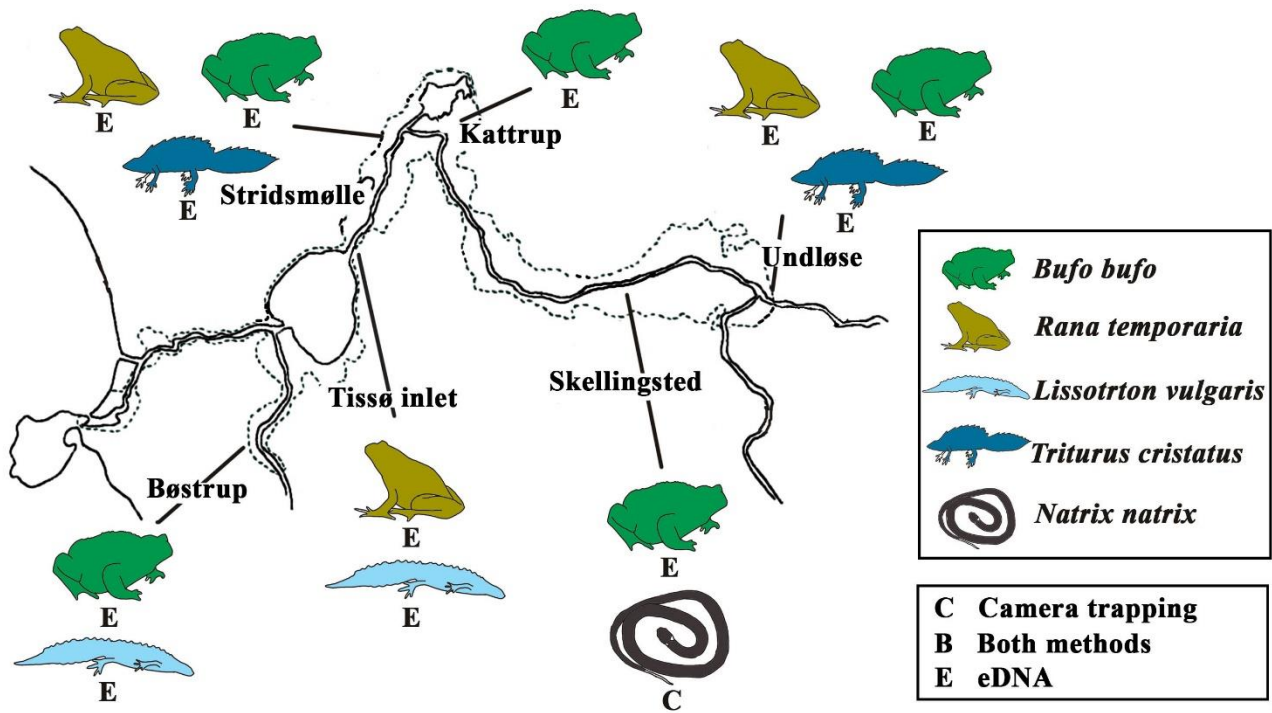


**Supplementary Figure 2.** Camera event (sightings with 30 minutes interval) frequency from all mammal and bird taxa found in Åmosen. A total of 9089 camera events (CE) spread out on 8778 camera days found 87 different taxa. The first graph shows the 29 mammalian taxa found from most frequent to least. The second graph shows the 58 avian taxa, from most frequent to least.





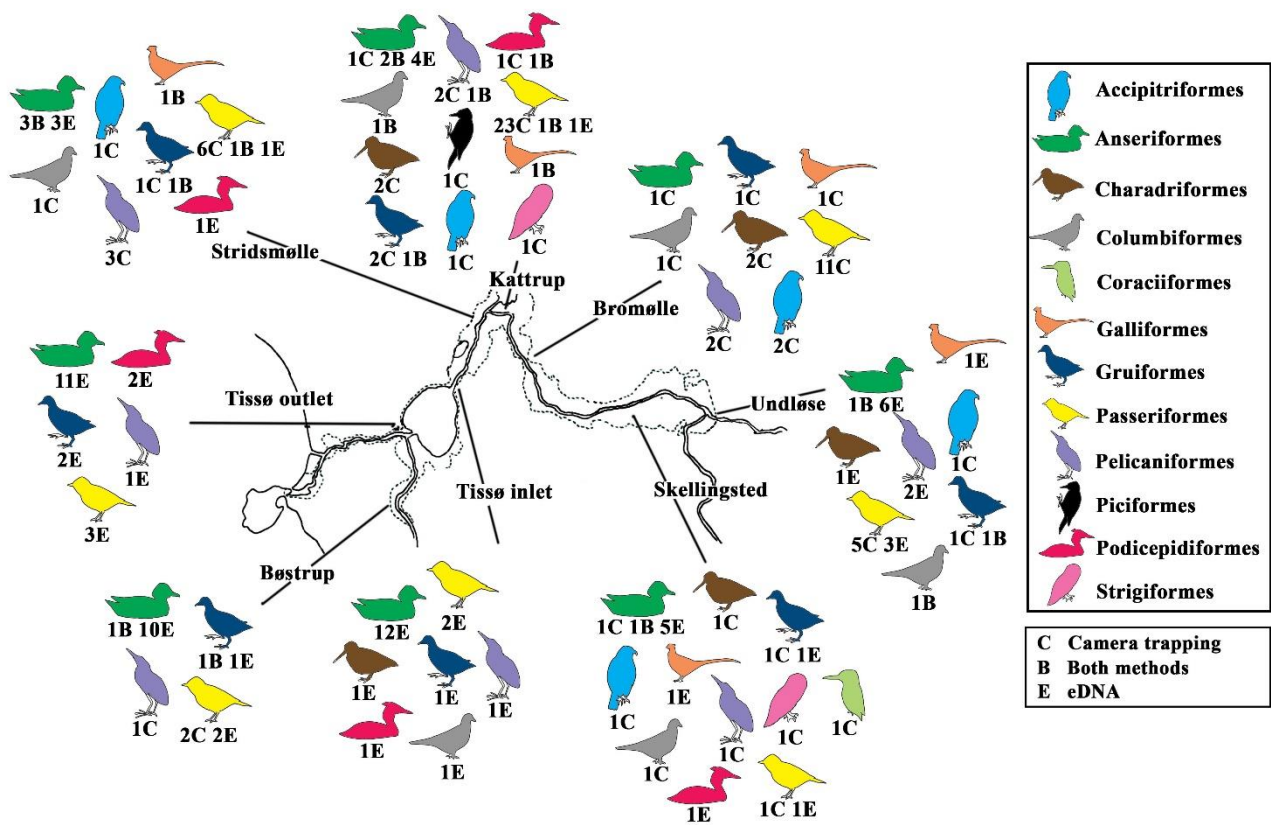
**Suppl. Figure S3. Overview of fish families found in Åmosen.** The qualitative data on fish species found in Åmosen via four years of camera trapping (C), 15 months of eDNA (E), or via both methods (B) sorted into families. Each family has a varying number of maximum detected species in the area: Anguillidae (one species), Cobitidae (one species), Cyprinidae (10 species), Esocidae (one species), Gasterosteidae (two species), Percidae (three species), and Salmonidae (two species) (see Table 2 for species). Illustrations by AMRH. Animals are not to scale.



**Suppl. Figure S4. Overview of amphibians and a squamate found in Åmosen.** The qualitative data on the four amphibians, and one squamate detected via four years of camera trapping (C), 15 months eDNA (E), or via both methods (B) (Table 2). Illustrations by AMRH. Animals are not to scale.



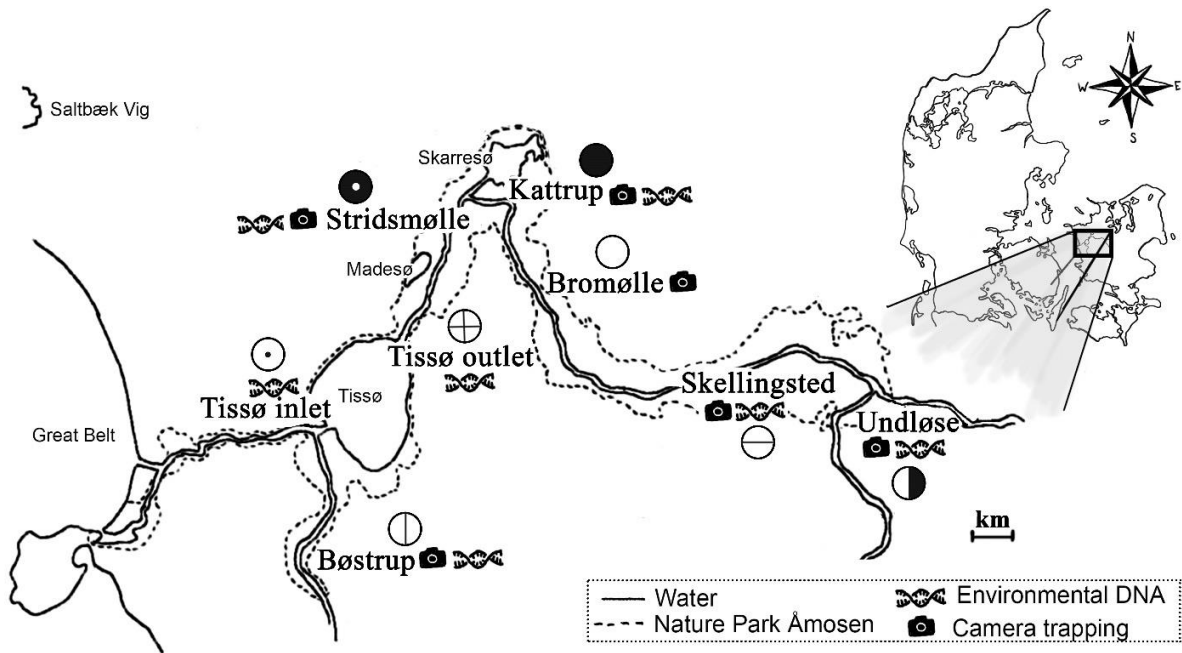




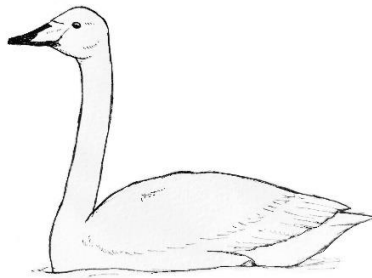
**Suppl. Figure S6 Overview of birds found in Åmosen.** The qualitative data on bird species found in Åmosen via four years of camera trapping (C), 15 months of eDNA (E), or via both methods (B), sorted into orders. Each order has a varying number of maximum detected species in the area:

Accipitriformes (two species), Anseriformes (16 species), Charadriiformes (two species), Columbiformes (one species), Coraciiformes (one species), Galliformes (one species), Gruiformes (three species), Passeriformes (33 species), Pelicaniformes (three species), Piciformes (one species), Podicepidiformes (three species), and Strigiformes (one species) (see Table 2 for species).

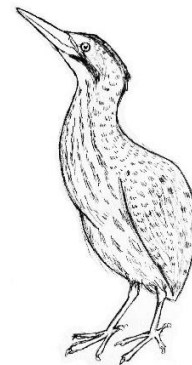
Illustrations by AMRH. Animals are not to scale.



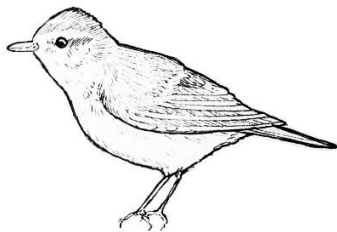
*Accipiter nisus* 📷



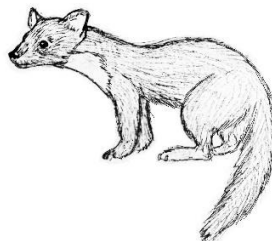
*Cygnus cygnus* 🧬



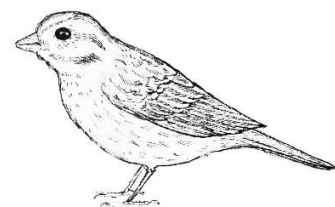
*Botaurus stellaris* 📷



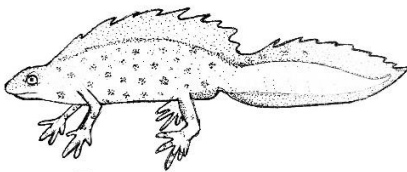
*Phylloscopus trochilus* 📷



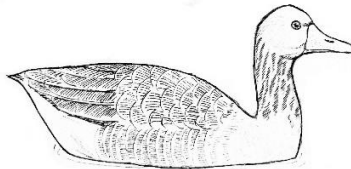
*Martes martes* 📷



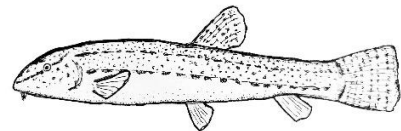
*Emberiza citrinella* 📷



*Triturus cristatus* 🧬

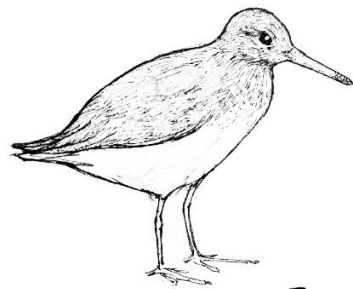


*Anser anser* 📷 🧬

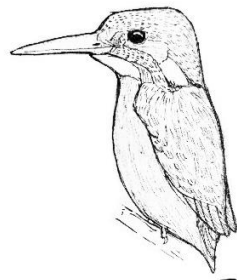


*Cobitis taenia* 🧬

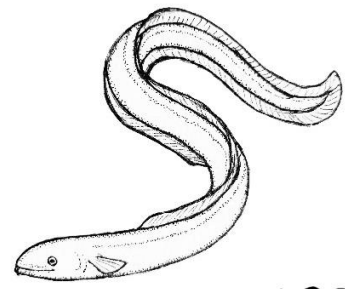




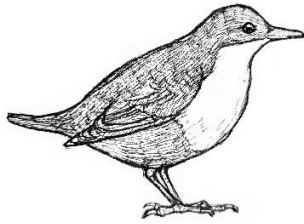
*Tringa ochropus* 📷



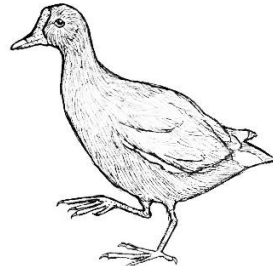
*Alcedo atthis* 📷



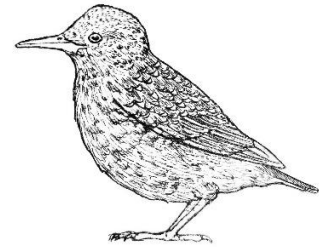
*Anguilla anguilla* 🧬



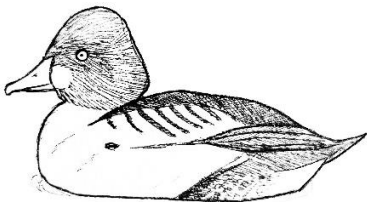
*Cinclus cinclus* 📷



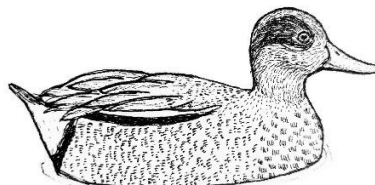
*Gallinula chloropus* 📷 🧬



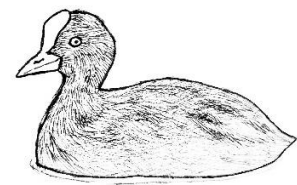
*Sturnus vulgaris* 📷



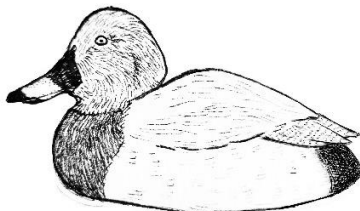
*Bucephala clangula* 📷



*Anas crecca* 📷 🧬



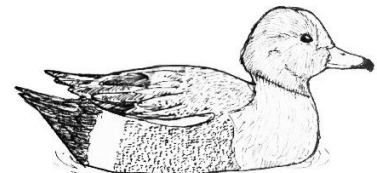
*Fulica atra* 📷 🧬



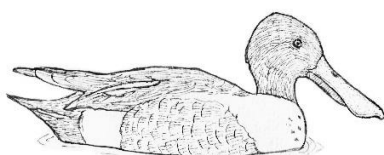
*Aythya ferina* 🧬



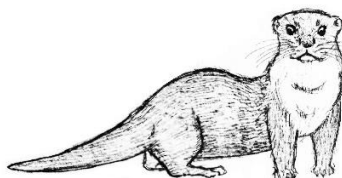
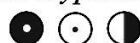
*Anas acuta* 🧬



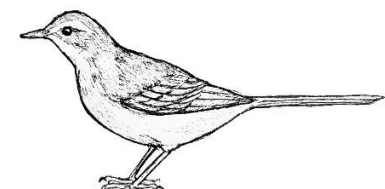
*Mareca penelope* 🧬



*Anas clypeata* 🧬



*Lutra lutra* 📷



*Motacilla cinerea* 📷



**Suppl. Figure S7** The 24 different species protected by Natura 2000 (European Commission, 2009; Naturstyrelsen, 2012) and/or on the Danish Red List (Moeslund *et al.*, 2019) detected with the use of camera trapping and/or eDNA and at which of the eight study sites. Nineteen of the species are

deemed vulnerable, endangered, or critically endangered in Denmark. Furthermore, seven species are extra special for Natura Park Åmosen and have deemed the area Natura 2000 protected. The study sites are shown with circle symbols and the method of detection is indicated by either a camera or a DNA strain, representing camera trapping and eDNA, respectively. All Illustrations by AMRH. Animals are not to scale.

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