

Evaluating metabarcoding to analyse diet composition of species foraging in anthropogenic landscapes using Ion Torrent and Illumina sequencing

M.-A. Forin-Wiart, M.-L. Poulle, S. Piry, F. Cosson, C. Larose and M. Galan.

Supplementary Table S1: Details on the calibration study

Detailed composition of the daily rations distributed during two sessions to the housebound cat, the collected faecal samples and food item detection [not detected (-) or one replicate (one) or two replicates (two)] per food category and HTS platform (Ion PGM and MiSeq runs).

Sessions	Days	Daily composition			Faecal samples	DNA metabarcoding detections						
		Prey items	Pet food	Surplus meat/fish		Prey items		Pet food		Surplus meat/fish		
						Ion PGM	MiSeq	Ion PGM	MiSeq	Ion PGM	MiSeq	
1	1		x									
	2		x									
	3	x	x									
	4	x	x		1	-	-	-	two			
	5	x	x		2	two	two	-	-			
	6	x	x									
	7	x	x		3	two	two	-	-			
	8	x			4	two	two	-	-			
	9		x		5	one	two	-	-			
	10		x									
	11		x		6	-	two	-	one			
	12			x								
	13			x	7			one	two			
	14			x	8			one	two			
	15-1			x	9			-	two			
	15-2			x	10			one	two			
	16			x	11			one	-			
	17			x	12			-	two			
	18			x	13			two	one			
	19			x	14			-	one			
	20			x	15			-	two			
	21			x	16			one	one			
22			x	17			-	-				

Supplementary Table S1 (Continued)

Sessions	Days	Daily composition			Faecal samples	DNA metabarcoding detections					
		Prey items	Pet food	Surplus meat/fish		Prey items		Pet food		Surplus meat/fish	
						Ion PGM	MiSeq	Ion PGM	MiSeq	Ion PGM	MiSeq
2	1		x								
	2		x	x							
	3		x	x	18			-	one	-	-
	4		x	x							
	5		x	x							
	6		x	x	19			-	-	-	-
	7		x	x	20			-	two	-	two/one*
	8		x	x							
	9		x								
	10		x		21			one	two	-	two
	11		x	x							
	12	x	x		22			one	one	one	-
	13	x	x		23			-	-		
	14		x		24	-	two	-	-		
	15		x		25	-	two	-	-		
	16-1		x		26			-	-		
	16-2		x		27			-	one		
	17	x	x		28			-	-		
	18		x		29	-	two	-	-		
	19		x	x	30			-	-		
	20		x		31			-	-	-	-
	21		x		32			-	-		
22-1		x		33			-	-			
22-2		x		34			-	one			

Supplementary Table S1 (Continued)

Sessions	Days	Daily composition			Faecal samples	DNA metabarcoding detections					
		Prey items	Pet food	Surplus meat/fish		Prey items		Pet food		Surplus meat/fish	
						Ion PGM	MiSeq	Ion PGM	MiSeq	Ion PGM	MiSeq
	23-1		x		35			-	-		
	23-2		x		36			-	-		
	24		x		37			one	-		
2	25-1		x		38			-	-		
	25-2		x		39			-	-		
	26		x		40			-	two		
	27		x		41			-	-		

* The one replicate occurrence was associated to surplus raw pig meat while the two replicates occurrence was associated to the surplus raw sheep meat.

Supplementary Table S2: Detailed outputs from Ion PGM and MiSeq platforms during the field study.

Similarity thresholds used to identify variants and observed occurrences of food items in 326 field-collected faecal samples from free-ranging cats. Similarity thresholds and observed occurrences are shown according to the food item and the HTS platform.

Taxa			Ion PGM run						MiSeq run							
Food category	Food sub-category	Food items	Similarity thresholds			Observed occurrence			Similarity thresholds			Observed occurrence				
			95%	98%	100%	in one replicate	in two replicates	Total	95%	98%	100%	in one replicate	in two replicates	Total		
Preys items	small / medium-sized rodents	<i>Apodemus flavicollis</i>			x	2	2	4				x	2	2	4	
		<i>Apodemus sylvaticus</i>			x	0	4	4				x	3	2	5	
		<i>Arvicola terrestris</i>			x	28	47	75				x	23	56	79	
		<i>Micromys minutus</i>			x	0	2	2				x	1	2	3	
		<i>Microtus arvalis</i>			x	38	56	94				x	60	29	89	
		<i>Microtus agrestis</i>			x	7	13	20				x	15	12	27	
		<i>Microtus spp.</i>			x	5	3	8				x	1	1	2	
		<i>Mus musculus</i>			x	1	0	1				x	1	0	1	
		<i>Myodes glareolus</i>			x	7	6	13				x	8	2	10	
		<i>Rattus norvegicus</i>			-	-	-	0	0	0				x	3	2
	<i>Rattus spp.</i>			-	-	-	0	0	0				x	2	3	5
	insectivores	<i>Crocidura leucodon</i>				x	1	0	1	-	-	-	0	0	0	
		<i>Crocidura russula</i>				x	0	2	2				x	6	2	8
		<i>Sorex coronatus</i>				x	0	1	1				x	1	0	1
	wild birds	<i>Carduelis carduelis</i>		-	-	-	0	0	0				x	1	1	2
		<i>Columba livia</i>				x	1	0	1				x	0	1	1
<i>Columba palumbus</i>					x	0	1	1				x	0	1	1	
<i>Delichon urbicum</i>			-	-	-	0	0	0				x	1	0	1	
<i>Falco sp.</i>					x	0	1	1				x	1	1	2	
<i>Hirundo rustica</i>					x	1	0	1				x	0	2	2	
<i>Passer domesticus</i>			-	-	-	0	0	0					x	1	0	1

Supplementary Table S2 (Continued)

Taxa			Ion PGM run						MiSeq run					
Food category	Food sub-category	Food items	Similarity thresholds			Observed occurrence			Similarity thresholds			Observed occurrence		
			95%	98%	100%	in one replicate	in two replicates	Total	95%	98%	100%	in one replicate	in two replicates	Total
Preys items	wild birds	<i>Passer montanus</i>	-	-	-	0	0	0			x	2	0	2
		<i>Passer spp.</i>	-	-	-	0	0	0			x	10	7	17
		<i>Pica pica</i>	-	-	-	0	0	0			x	1	0	1
		<i>Streptopelia decaocto</i>	-	-	-	0	0	0			x	3	0	3
		<i>Sylvia atricapilla</i>	-	-	-	0	0	0		x		1	0	1
		<i>Turdus philomelos</i>			x	0	1	1	-	-	-	0	0	0
		<i>Turdus pilaris</i>			x	1	0	1			x	2	0	2
reptiles		<i>Anguis fragilis</i>	-	-	-	0	0	0			x	1	0	1
		<i>Capreolus capreolus</i>			x	1	1	2		x		1	1	2
game species		<i>Cervus elaphus</i>	-	-	-	0	0	0		x		1	0	1
		Perdicinae*	-	-	-	0	0	0			x	3	3	6
		<i>Oryctolagus cuniculus</i> *			x	3	0	3			x	3	2	5
Human - linked food	anthropogenic food	Acanthomorpha	x			4	0	4		x		2	1	3
		<i>Anser sp.</i>	-	-	-	0	0	0		x		3	0	3
		Anseriformes	-	-	-	0	0	0		x		9	3	12
		<i>Bos frontalis</i>		x		1	0	1	-	-	-	0	0	0
		<i>Bos indicus</i>		x		1	2	3		x		4	0	4
		<i>Bos sp.</i>		x		60	34	94		x		46	40	86
		<i>Bos taurus</i>		x		3	1	4		x		4	1	5
		<i>Cairina moschata</i>		x		17	7	24		x		14	4	18
		<i>Capra sp.</i>		x		0	1	1	x			1	0	1
		Caprinae	x			1	0	1	x			18	5	23
<i>Clupea harengus</i>		x		1	0	1	-	-	-	0	0	0		

Supplementary Table S2 (Continued)

Taxa			Ion PGM run						MiSeq run					
Food category	Food sub-category	Food items	Similarity thresholds			Observed occurrence			Similarity thresholds			Observed occurrence		
			95%	98%	100%	in one replicate	in two replicates	Total	95%	98%	100%	in one replicate	in two replicates	Total
		<i>Clupea sp.</i>	-	-	-	0	0	0		x		1	0	1
		<i>Equus sp.</i>	x			1	0	1	-	-	-	0	0	0
		<i>Esox lucius</i>		x		1	0	1	-	-	-	0	0	0
		<i>Gallus gallus domesticus</i>	-	-	-	0	0	0	x			1	0	1
		<i>Gallus sp.</i>	-	-	-	0	0	0		x		43	34	77
		<i>Katsuwonus pelamis</i>		x		2	0	2	-	-	-	0	0	0
		<i>Meleagris gallopavo</i>		x		4	1	5		x		25	14	39
		<i>Merlangius merlangus</i>		x		1	0	1	-	-	-	0	0	0
Human	anthropogenic	Neognathae - Galloanserae	-	-	-	0	0	0	x			33	21	54
- linked	food	<i>Numida meleagris</i>	-	-	-	0	0	0		x		8	2	10
		<i>Ovis aries</i>		x		2	2	4	x			0	1	1
		<i>Ovis sp.</i>		x		8	0	8		x		18	4	22
		Percomorpha	-	-	-	0	0	0	x			1	0	1
		<i>Salmo salar</i>		x		2	2	4		x		3	1	4
		Suidae	-	-	-	0	0	0		x		8	0	8
		<i>Sus scrofa domesticus</i>		x		8	3	11		x		31	19	50
		<i>Sus sp.</i>	-	-	-	0	0	0		x		15	1	16
		<i>Thunnus sp.</i>	-	-	-	0	0	0		x		1	0	1

* Only available from pet food or human leftovers (rabbit hutches and hunting scraps) for cats in this area.

Supplementary Table S3: Variables used in analyses.

Variables used in the evaluation of HTS platforms efficiency for the food item detection.

Explanatory variables	Type	Description
fPlatform	Categorical	HTS platforms used: (1) Ion PGM; (2) Illumina MiSeq
fFoodItem	Categorical	Food item categories: (1) Human-linked food (HLF); (2) Water vole (WV); (3) Field vole (FV); (4) Common vole (CV); (5) Microtus-like vole (MLV); (6) Murids (harvest mouse, yellow-necked mouse, forest mouse, house mouse, rats <i>Rattus spp.</i> and Norway rats); (7) Bank vole (BV); (8) Birds (Falconiformes, Columbiformes and Passeriformes, BIR); (9) Shrews (<i>Soricidae spp.</i> , SH)
fSample	Categorical	Number code identifying field-collected faecal samples individually
Response variables	Type	Description
Occurrence	Binary	Food item successfully (1) or unsuccessfully (2) detected

Supplementary Table S4: Modelling outputs.

Models explaining the probability of detecting a food item in field-collected faeces of free-ranging cats.

Rank	Model description	K	LL	AIC	ΔAIC
1	fPlatform+ fFoodItem+ fPlatform : fFoodItem	18	-435.0	906.8	0.00
2	fPlatform + fFoodItem	10	-473.6	967.4	60.6
3	fFoodItem	9	-507.1	1032.4	125.6
4	Null	1	-526.0	1054.0	147.2

Supplementary Table S5: List of prey items and surplus products given to the cat during the calibration study

Sessions	Days	Daily composition		
		Prey items	Pet food	Surplus meat/fish
1	1		x	
	2		x	
	3	1 <i>Arvicola terrestris</i>	x	
	4	1 <i>Arvicola terrestris</i> 1 <i>Apodemus sp.</i>	x	
	5	1 <i>Arvicola terrestris</i>	x	
	6	3 <i>Microtus agrestis</i>	x	
	7	1 <i>Apodemus sp.</i> 1 <i>Mus musculus</i>	x	
	8	1 <i>Rattus rattus</i>		
	9		x	
	10		x	
	11		x	
	12		x	
	13		x	
	14		x	
	15-1		x	
	15-2		x	
	16		x	
	17		x	
	18		x	
	19		x	
	20		x	
	21		x	
22		x		
2	1		x	
	2		x	herring
	3		x	chicken
	4		x	cod lamb
	5		x	dogfish pork
	6		x	plaice veal
	7		x	duck beef
	8		x	pork beef

Supplementary Table S5 (continued)

Sessions	Days	Daily composition		
		Prey items	Pet food	Surplus meat/fish
	9		x	
	10		x	
	11		x	duck
	12	1 <i>Taeniopygia guttata</i>	x	
	13	1 <i>Taeniopygia guttata</i>	x	
	14		x	
	15		x	
	16-1		x	
	16-2		x	
	17	1 <i>Mus musculus</i>	x	
	18		x	
2	19		x	wild boar
	20		x	
	21		x	
	22-1		x	
	22-2		x	
	23-1		x	
	23-2			
	24		x	
	25-1		x	
	25-2		x	
	26		x	
	27		x	