

## Supplemental Information for:

### DNA metabarcoding unveils multi-scale trophic variation in a widespread coastal opportunist

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Table S1. Number of pooled full *Crangon crangon* stomachs per sample. Only samples with less than 8 pooled stomachs are shown. \* Samples not used for molecular analysis (< 1000 diet-related reads)

<b>Estuary</b>	<b>Sample</b>	<b>N stomachs</b>
Aveiro	Av_1C	4
Aveiro	Av_2B	7
Eastern Scheldt	ES_2A	5
Eastern Scheldt	ES_2B*	4
Eastern Scheldt	ES_3C	5
Kent	Ke_1C*	5
Kent	Ke_4C	7
Mersey	Me_1C	4
Mersey	Me_2A*	6
Mersey	Me_3B*	7
Western Scheldt	WS_2C	4
Western Scheldt	WS_4C	4

Table S2. Mean ( $\pm$ SD) total length of *Crangon crangon* (>20 mm TL) sampled and dissected for stomach DNA extraction per site. \* Site not used for molecular analysis

Estuary	Site	Sampled		DNA extracted	
		Mean (mm)	SD	Mean (mm)	SD
Aveiro	Av_1	32	5	32	6
Aveiro	Av_2	30	4	30	3
Aveiro	Av_3	31	4	31	5
Eastern Scheldt	ES_1	25	6	27	6
Eastern Scheldt	ES_2	33	7	32	6
Eastern Scheldt	ES_3	30	6	32	6
Eastern Scheldt	ES_4	34	7	34	8
Kent	Ke_1	32	3	31	3
Kent	Ke_2	32	3	32	4
Kent	Ke_3	31	3	32	3
Kent	Ke_4	29	2	29	2
Mersey	Me_1	47	4	48	3
Mersey	Me_2	40	7	42	7
Mersey	Me_3*	40	7		
Mersey	Me_4	39	7	36	5
Mersey	Me_5	41	7	40	6
Mersey	Me_6	42	7	40	4
Minho	Mi_1	41	4	39	3
Minho	Mi_2	39	5	38	4
Minho	Mi_3	35	5	36	3
Western Scheldt	WS_1	32	5	32	4
Western Scheldt	WS_2	26	4	27	4
Western Scheldt	WS_3	36	6	37	6
Western Scheldt	WS_4	44	6	43	7

Table S3. *Purpureocillium lilacinum* sequences detected in *Crangon crangon* stomach samples

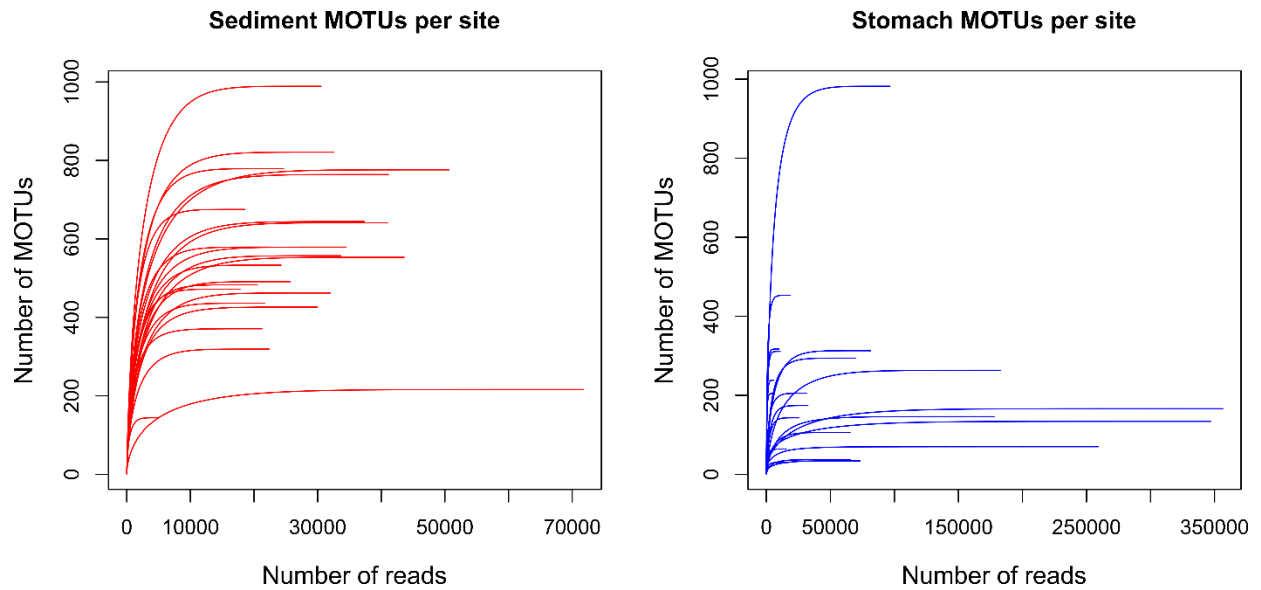
Marker	Sequence	Match	Query cover	E-Value	Identity
COI	TTTATCAGGATTACAAAGTCACAGTGGACCTA GTGTAGATTTAGCAATTTTTGCTTTACACCTTT CAGGGGTAAGTAGTTTATTAGGAGCAATAAA CTTCATAACTACAATCGCTAATATGAGAACAC CAGGAATAAGATTACACAAATTAGCCTTATTC GGGTGAGCTGTAGTTATAACAGCTATCTTATT ATTATTATCATTACCTGTTTTAGCTGGAGGTAT TACAATGGTATTAACAGATAGAAATTTTAACA CTTCATTCTTCGAAGTAGCTGGTGGTGGAGAT CCTATATTATTCCAACACTTATTC	<i>Purpureocillium lilacinum</i>	100%	2e <sup>-156</sup>	99%
ITS	ACTCCCAAACCCACTGTGAACCTTACCTCAGTT GCCTCGGCGGGAACGCCCCGCGCCGCGGCC CCGCGCCGCGCGCCGACCCAGGCGCCCGCCG CAGGGACCCCAAACCTCTCTTGCAATACGCCCA GCGGGCGGAATTTCTCTCTGAGTTGCACAAG CAAAAACAAATGAATCAAACTTTCAACAACG GATCTCTTGGTTCTGGCATCGATGAAGAACGC AGCGAAATGCGATAAGTAATGTGAATTGCAG AATTCAGTGAATCATCGAATCTTTGAACGCAC ATTGCGCCCGCCAGCATTCTGGCGGGCATGCC TGTTGAGCGTCATTTCAACCCTCGAGCCCCC CGGGGGCCTCGGTGTTGGGGGACGGCACACC AGCCGCCCCGAAATGCAGTGCGGACCCCGC CGCAGCCTCCCCTGCGTA	<i>Purpureocillium lilacinum</i> isolate NIOSN_SK56_S7 6	100%	0.0	100%

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Table S4. List of pooled stomach samples removed prior to analysis due to low read depth (< 1000 reads) after removal of predator, parasite and contaminant reads. \* Removal of sediment sample due to lack of site Me\_3 stomach samples included in the final analysis.

<b>Estuary</b>	<b>Sample</b>	<b>Number of reads</b>
Western Scheldt	WS_1B	330
	WS_3B	486
	WS_4A	420
Eastern Scheldt	ES_1A	396
	ES_2B	897
	ES_3A	214
	ES_4A	538
	ES_4C	366
Mersey	Me_2A	364
	Me_3A	179
	Me_3B	486
	Sediment sample Me_3*	27212
Kent	Ke_1C	669
	Ke_2A	189
	Ke_4A	301

Figure S1. Rarefaction curves of the number of MOTUs obtained at increasing numbers of reads in the sediment (A) and stomach (B) samples per site. *Crangon crangon* and *Purpureocillium lilacinum* have been removed from the stomach samples prior to analysis.



# MOLECULAR ECOLOGY

Table S5. Differences in relative mean read abundance of detected phyla in sediment and *Crangon crangon* full stomach samples. Samples are averaged per site (N = 23) and tested with a paired Wilcoxon signed-rank test. \*\*: P < 0.01

Phylum	Mean relative abundance (%)		Wilcoxon signed-rank test		
	Sediment	Full Stomach	W	P	
Annelida	3.43	16.12	225	0.007	**
Arthropoda	5.30	48.56	274	0.000	**
Bacillariophyta	20.47	1.69	0	0.000	**
Chordata	0.08	4.81	208	0.000	**
Cnidaria	0.79	1.08	100	0.259	
Dinoflagellata	6.13	1.55	53	0.008	**
Discosea	10.40	0.20	0	0.000	**
Mollusca	0.16	1.18	192	0.001	**
Oomycota	2.87	0.31	3	0.000	**
Rhodophyta	2.00	1.71	111	0.427	
Unassigned	44.01	18.92	9	0.000	**

Table S6. Pairwise comparisons (Bonferroni-corrected) of square-root transformed Bray-Curtis dissimilarities of *Crangon crangon* pooled stomach contents between estuaries. \* = P < 0.05; \*\* = P < 0.01.

Pairs	F.Model	Df	R <sup>2</sup>	P
Eastern Scheldt vs WScheld	1.684	1,12	0.123	1.000
Eastern Scheldt vs Minho	3.648	1,13	0.219	0.046 *
Eastern Scheldt vs Aveiro	2.948	1,12	0.197	0.460
Eastern Scheldt vs Mersey	2.782	1,17	0.141	0.105
Eastern Scheldt vs Kent	9.698	1,12	0.447	0.010 *
Western Scheldt vs Minho	2.368	1,15	0.136	0.426
Western Scheldt vs Aveiro	1.872	1,14	0.118	0.784
Western Scheldt vs Mersey	1.535	1,19	0.075	1.000
Western Scheldt vs Kent	7.116	1,14	0.337	0.015 *
Minho vs Aveiro	4.914	1,15	0.247	0.015 *
Minho vs Mersey	3.021	1,20	0.131	0.015 *
Minho vs Kent	9.597	1,15	0.390	0.001 **
Aveiro vs Mersey	4.152	1,19	0.179	0.007 **
Aveiro vs Kent	13.336	1,14	0.488	0.004 **
Mersey vs Kent	4.224	1,19	0.182	0.030 *



Table S7. Mean ( $\pm$  SD) Salinity, median grainsize and total organic matter (TOM) per estuary

<b>Estuary</b>	<b>Salinity</b>	<b>Grain Size (<math>\mu\text{m}</math>)</b>	<b>TOM (%)</b>
Aveiro	25.5 ( $\pm$ 8.5)	271 ( $\pm$ 151)	1.32 ( $\pm$ 0.96)
Eastern Scheldt	31 ( $\pm$ 0.2)	294 ( $\pm$ 85)	0.28 ( $\pm$ 0.11)
Kent	0.3 ( $\pm$ 0.1)	76 ( $\pm$ 6)	0.92 ( $\pm$ 0.35)
Mersey	17.6 ( $\pm$ 10.2)	132 ( $\pm$ 90)	2.6 ( $\pm$ 3.47)
Minho	6.5 ( $\pm$ 3.5)	477 ( $\pm$ 181)	0.99 ( $\pm$ 1.15)
Western Scheldt	23.2 ( $\pm$ 5.6)	259 ( $\pm$ 101)	0.81 ( $\pm$ 0.57)