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Diet composition of red-throated divers in the German Bight

1 **Table 1 Main fish prey species of red-throated divers detected in previously published studies using morphological methods (Madsen 1957, Durinck et al. 1994, Guse et**
 2 **al. 2009) and this study using molecular tools listed as FO for the corresponding areas where birds were examined.**

Prey item (Group)	Prey item (Family)	Prey taxa (Genus/species)	Madsen 1957 (North Sea/Inner Danish Waters) n = 173	Durinck et al. 1994 (North Sea) n = 8	Guse et al. 2009 (Baltic Sea) n = 82	This study
Gadiformes	Gadidae	Common Cod (<i>Gadus callarias</i>)	54%	-	-	-
Gadiformes	Gadidae	Whiting (<i>Merlangius merlangus</i>)	-	25%	-	6.9%
Gadiformes	Gadidae	Blue Whiting (<i>Micromesistius poutassou</i>)	-	37.5%	-	-
Gadiformes	Gadidae	Gadoids indet. Common goby (<i>Pomatoschistus microps</i>)	-	50 %	-	31%
Perciformes	Gobiidae	Gobies (<i>Gobius sp.</i>)	-	-	winter 38.2%/spring 10.4%	-
Perciformes	Gobiidae	Gobies (<i>Gobius sp.</i>)	14%	-	winter 41.2%/ spring 20.8%	13.8%
Clupeiformes	Clupeidae	Atlantic herring (<i>Clupea harengus</i>)	12%	87.5%	winter 23.5%/ spring 95.8%	55.2%
Clupeiformes	Clupeidae	European sprat (<i>Sprattus sprattus</i>)	-	75%	winter 14.7%/ spring 27.1%	58.6%
Clupeiformes	Clupeidae	(<i>Clupea sp./Sprattus sp.</i>)	-	37.5%	winter 14.7%/ spring 22.9%	-
Gasterosteiformes	Gasterosteidae	Sticklebacks (<i>Gasterosteus sp.</i>)	11%	62.5%	winter 52.9%/ spring 39.6%	10.3%
Osmeriformes	Osmeridae	Smelt (<i>Osmerus eperlanus</i>)	-	-	winter 44.1%/ spring 4.2%	-
Perciformes	Percidae	Zander (<i>Sander lucioperca</i>)	-	-	winter 91.2%/ spring 10.4%	-
Perciformes	Percidae	European perch (<i>Perca fluviatillis</i>)	-	-	winter 17.6%/ spring 2.1%	-
Perciformes	Percidae	Ruffe (<i>Gymnocephalus cernus</i>)	-	-	winter 38.2%/ spring 20.8%	-
Perciformes	Ammodytidae	Lesser sandeel (<i>Ammodytes tobianus</i>)	< 1%	12.5%	winter 8.8%/ spring 12.5%	31%
Perciformes	Scombridae	Atlantic mackerel (<i>Scomber</i>)	<1%	-	-	55.2%

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Pleuronectiformes	Pleuronectidae	<i>scombrus)</i> Flatfish indet.	5%	37.5%	Winter -/spring 2.1%	51.7%
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3 **Table 2 Timing and sample size of analysed faecal samples of red-throated divers from the German Bight.**
4 **One sample per bird was taken for analysis.**

Sampling year	2015	2015	2016	2016
Time period	March	April	February	March
Sample size (captured birds)	10	6	8	12
Sample size (faecal samples)	10	5	8	11
Positive samples	9	4	8	8
Total of positive samples	13		16	

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9 **Table 3 Sequences of primers used to amplify red-throated diver faecal samples for Next Generation**
 10 **Sequencing. Modifications from original primers (Waap 2015) in bold.**

Target amplification	Gene	Primer name	Sequence (5'-3')	Approximate product size incl. primer sequence (bp)	Annealing temperature (°C)
Fish (Chordata)	mtDNA 16S	FISH2_16S_F	CGAGAAGACCCTDTGRAGC T (20)	~264	58
Fish (Chordata)	mtDNA 16S	modifiedChor d_16S_R1	GCTGTTATCCCTRGRGTAA (19)		
Cephalopod (Molluscs)	mtDNA 16S	Ceph_16S_R	AGGGACGARAAGACCCTAN TGAGC (24)	~244	56
Cephalopod (Molluscs)	mtDNA 16S	Ceph_16S_F	AC S CTGTTAYCCCTATG (17)		
Crustacean (Invertebrate)	mtDNA COI	mICO1int_F	GGWACWGGWTGAACWGTW TAYCCYCC (26)	~332	50
Crustacean (Invertebrate)	mtDNA COI	Nancy_R	CCCGGTAAAATTTAAAATATA AACTTC (26) GTGGA		
Blocking probe	-	-	ACTTAAAAATCAGCAGCCAC CA[SpC3]	-	-

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Diet composition of red-throated divers in the German Bight

28 **Table 4** Detected prey species of red-throated divers with regard to presence (MOTUs) and frequency of occurrence (FO) for each sampling year and the full dataset.

					2015			2016			2015 & 2016		
					MOTUs found in x samples (n=13)	FO (% n = 13)	No of MOTUs (n = 101)	MOTUs found in x samples (n=16)	FO (% n = 16)	No of MOTUs (n = 68)	MOTUs found in x samples (n=29)	FO (% n = 29)	No of MOTUs (n = 169)
Clupeiformes	Clupeidae	<i>Clupea</i>	<i>harengus</i>	Atlantic herring	7	53.8	10	9	56.3	15	16	55.2	25
Clupeiformes	Clupeidae	<i>Sprattus</i>	<i>sprattus</i>	European sprat	9	69.2	15	8	50.0	12	17	58.6	27
Clupeiformes	Clupeidae	<i>Sardina</i>	<i>pilchardus</i>	European pilchard	3	23.1	6	3	18.8	4	6	20.7	10
Clupeiformes	Engraulidae	<i>Engraulis</i>	<i>encrasicolus</i>	European anchovy	1	7.7	1	0	0.0	0	1	3.4	1
Clupeids					9	69.2	32	10	62.5	31	19	65.5	63
Pleuronectiformes	Pleuronectidae	<i>Limanda</i>	<i>sp.</i>	Common dab	8	61.5	8	5	31.3	7	13	44.8	15
Pleuronectiformes	Scophthalmidae	<i>Scophthalmus</i>	<i>maximus</i>	Turbot	1	7.7	2	0	0.0	0	1	3.4	2
Pleuronectiformes	Soleidae	<i>Solea</i>	<i>solea</i>	Common sole	1	7.7	2	1	6.3	1	2	6.9	3
Pleuronectiformes	Pleuronectidae			Right eye flounders	2	15.4	3	1	6.3	1	3	10.3	4
Flatfish					8	61.5	15	7	43.8	10	15	51.7	25
Salmoniformes	Salmonidae	<i>Salmo</i>	<i>trutta</i>	Sea/Brown trout	4	30.8	5	1	6.3	1	5	17.2	6
Gadiformes	Merluccidae	<i>Merluccius</i>	<i>merluccius</i>	European hake	3	23.1	5	1	6.3	2	4	13.8	7
Gadiformes	Gadidae	<i>Pollachius</i>	<i>pollachius</i>	European pollock	2	15.4	2	0	0.0	0	2	6.9	2
Gadiformes	Gadidae	<i>Melanogrammus</i>	<i>aeglefinus</i>	Haddock	2	15.4	4	1	6.3	1	3	10.3	5
Gadiformes	Gadidae	<i>Merlangius</i>	<i>merlangus</i>	Whiting	0	0.0	0	1	12.5	1	1	6.9	1
Gadiformes	Gadidae	<i>Gadus</i>	<i>sp.</i>	Cod	1	7.7	1	0	0.0	0	1	3.4	1
Gadiformes	Gadidae			Codfishes	5	38.5	6	5	31.3	7	10	31.0	13
Gadoids					5	38.5	13	6	37.5	9	11	37.9	22
Gasterosteiformes	Gasterosteidae	<i>Gasterosteus</i>	<i>sp.</i>	Sticklebacks	2	15.4	3	1	6.3	1	3	10.3	4
Perciformes	Moronidae	<i>Dicentrarchus</i>	<i>labrax</i>	European bass	1	7.7	1	0	0.0	0	1	3.4	1
Perciformes	Ammodytidae	<i>Hyperoplus</i>	<i>lanceolatus</i>	Greater sand eel	3	23.1	3	1	6.3	1	4	13.8	4
Perciformes	Ammodytidae	<i>Ammodytes</i>	<i>sp.</i>	Sand eel	6	46.2	8	0	0.0	0	6	20.7	8
Perciformes	Ammodytidae			Sand lance	2	15.4	3	1	6.3	1	3	10.3	4
Ammodytidae					8	61.5	14	1	6.3	2	9	31.0	16
Perciformes	Gobiidae	<i>Pomatoschistus</i>	<i>minutus</i>	Sand goby	2	15.4	2	2	12.5	2	4	13.8	4
Perciformes	Scombridae	<i>Scomber</i>	<i>sombrus</i>	Atlantic mackerel	7	53.8	11	9	56.3	10	16	55.2	21

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29 Appendices

30 **A1: Best blast results for each of the 21 detected taxa and corresponding accession number, the identity**
 31 **with the blast reference sequence, the sequence length and the bitscore from data of both sampling years**
 32 **(2015 and 2016) combined.**

Order	Family	Genus / species	Common name	Accession number	Ident % (blast)	Sequ. length	E-value	Bit-score
Clupeiformes	Clupeidae	<i>Clupea harengus</i>	Atlantic herring	KJ128741	100	210	1.94 E-104	388
Clupeiformes	Clupeidae	<i>Sprattus sprattus</i>	European sprat	KJ128910	100	210	9.04 E-103	388
Clupeiformes	Clupeidae	<i>Sardina pilchardus</i>	European pilchard	FR849599	100	205	1.14 E-101	379
Clupeiformes	Engraulidae	<i>Engraulis encrasicolus</i>	European anchovy	KJ128765	100	211	5.93 E-105	390
Pleuronectiformes	Pleuronectidae	-	Right eye flounders	KU936350	99.1	224	7.49 E-109	403
Pleuronectiformes	Pleuronectidae	<i>Limanda limanda</i>	Common dab	KJ128862	100	224	3.78 E-112	414
Pleuronectiformes	Scophthalmidae	<i>Scophthalmus maximus</i>	Turbot	EU410416	100	217	2.60 E-108	401
Pleuronectiformes	Soleidae	<i>Solea solea</i>	Common sole	KJ128906	99.1	224	7.49 E-109	403
Salmoniformes	Salmonidae	<i>Salmo trutta</i>	Sea/Brown trout	KT633607	100	213	4.25 E-106	394
Gadiformes	Merluccidae	<i>Merluccius merluccius</i>	European hake	KJ128826	100	208	2.49 E-103	385
Gadiformes	Gadidae	<i>Pollachius pollachius</i>	European pollock	FR751400	99.5	208	2.50 E-98	379
Gadiformes	Gadidae	<i>Merlangius merlangus</i>	Whiting	KJ128825	100	208	2.49 E-103	363
Gadiformes	Gadidae	<i>Melanogrammus aeglefinus</i>	Haddock	KJ128822	100	208	2.49 E-103	385
Gadiformes	Gadidae	<i>Gadus sp.</i>	Cod	AP017650	99.52	208	1.16 E-101	379
Gadiformes	Gadidae	-	Codfishes/ True cod	AP017650	99.5	208	2.49 E-103	379

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Gasterosteiformes	Gasterosteidae	<i>Gasterosteus sp.</i>	Stickleback	KJ627974	100	208	1.16 E-101	379
Perciformes	Moronidae	<i>Dicentrarchus labrax</i>	European bass	KJ168065	99.5	211	2.53 E-103	385
Perciformes	Ammodytidae	-	Sand lance	KJ128795	99.1	211	2.53 E-103	379
Perciformes	Ammodytidae	<i>Hyperoplus lanceolatus</i>	Greater sand eel	KJ128795	100	211	2.53 E-103	390
Perciformes	Ammodytidae	<i>Ammodytes sp.</i>	Sand eel	AF315121	100	211	1.18 E-101	390
Perciformes	Gobiidae	<i>Pomatoschistus minutus</i>	Sand goby	KJ128870	100	207	8.89 E-103	383
Perciformes	Scombridae	<i>Scomber scombrus</i>	Atlantic mackerel	KJ128898	100	217	1.21 E-106	396

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