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Diet composition of red-throated divers in the German Bight

1 The diet of red-throated divers (*Gavia stellata*) overwintering in the German Bight (North
2 Sea) analysed using molecular diagnostics

3 * Corresponding author: Birgit.Kleinschmidt@bio.uni-giessen.de

4 *Supplementary material:*

5 *Specific information: Primer design*

6 Primer to amplify fish were modified from Chord_16S_F1/ modified Chord_16S_R1 (Waap
7 et al. unpubl. data) to match the range of potential prey species of red-throated divers
8 occurring in the study site.

9 Primer to amplify cephalopods were modified from modified Ceph_16S_F1/ modified Ceph
10 _16S_R1 (Waap et al. unpubl. data) to match the range of potential prey species of red-
11 throated divers occurring in the study site.

12 *PCR amplification of fish and cephalopod prey DNA from faeces*

13 PCR amplifications of fish and cephalopod prey were performed in single reactions using
14 Multiplex PCR Kits (Qiagen). Each 20 µL PCR reaction volume contained 10 µl Multiplex
15 PCR Master Mix, 1.25 µL of each primer (4µM), 1.25 µL blocking probe (40µM) if present
16 and 4 µL template DNA. For amplification of fish prey DNA, 2 µL of Q solution per sample
17 were added to the reaction mix and for amplification of cephalopod prey DNA 0.2 µL BSA
18 per sample was added to the reaction mix.

19

20 *PCR amplification of crustacean prey DNA from faeces*

21 PCR amplifications of crustacean prey DNA were performed in separate reactions, using
22 Multiplex PCR Kits (Qiagen). Each 20 µl PCR reaction volume contained 10 µl Multiplex
23 PCR Master Mix, 2 µL of each primer (2 µM) and 4 µL template DNA. For amplification of
24 crustacean prey DNA 0.2 µL BSA per sample was added to the reaction mix.

25 *Specific information on bioinformatic analyses*

26 We received the sequences in Illumina 1.8 Phred format + 33 format and for further analysis
27 we processed the data as follows, see also Table 1, 2. First we used Trimmomatic v0.36
28 (Bolger et al. 2014) to trim out low quality sequences and Illumina adapter sequences in the

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29 raw data set. Then we used FLASH v1.2.11 to align paired reads (Magoc et al. 2011), and
30 converted the fastq output to fasta format using FASTX Toolkit v0.0.13.2 (Gordon &
31 Hannon, 2010). Mothur v1.37.1 (Schloss et al. 2009) was used to identify sequence reads with
32 an exact match to the primers & MID-tags, to avoid any spurious results due to sequencing
33 errors. As the sequencing library was prepared using blunt-end ligation, each primer & MID
34 combination was checked in both possible orientations. Sequencing reads matching with
35 primers and MID-tags were then demultiplexed by pulling out the fasta sequence IDs for each
36 sample from the mothur 'groups' file and using this list to extract the corresponding
37 sequences from the mothur 'trim' file, giving a new fasta file with sequences specific to each
38 sample for each gene. The mothur 'trim' sequences file also has the primer and MID-tag
39 sequences removed from each sequence, as just the amplified gene region is wanted for the
40 following clustering step. To condense large numbers of sequences and therefore to define
41 molecular operational taxonomic units (MOTUs) we first we dereplicated the sequence file to
42 remove identical replicates using usearch v7.0.1090 -derep-fulllength, then removed any
43 potential chimeric sequences using usearch-uchime2_denovo, and finally clustered the
44 sequences based on 97% identity into MOTUs (Clare et al. 2016, Elbrecht et al. 2016) using
45 usearch -cluster_fast. Taxonomic information on the sequences was assigned using BLASTN
46 against the nucleotide database and a cut-off of 90% sequence identity and an e value of 1e-
47 10.

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48 Table 1 Working steps, commands and References performed during bioinformatics analysis

| Job to do | Software | Command | Reference |
|--|---------------------|--|---|
| Check and summarise raw data quality | FastQC | fastqc BK-Poolx_R1_trimmed_paired.fq -- outdir=./ fastqc BK- Poolx_R2_trimmed_paired.fq --outdir=. | Andrews S (2010) FastQC: a quality control tool for high throughput sequence data. https://www.bioinformatics.babraham.ac.uk/projects/fastqc/ |
| Step1: Trimming raw data of any sequencing adapters and low quality sequence | Trimmomatic | trimmomatic PE -phred33 BK- Poolx_S5_L001_R1_001.fastq.gz BK- Poolx_S5_L001_R2_001.fastq.gz BK- Poolx_R1_trimmed_paired.fq BK- Poolx_R1_trimmed_unpaired.fq BK- Poolx_R2_trimmed_paired.fq BK- Poolx_R2_trimmed_unpaired.fq \ ILLUMINACLIP:TruSeq3-PE-2.fa:2:30:10 LEADING:3 TRAILING:3 SLIDINGWINDOW:4:20 MINLEN:135 | Bolger AM, Lohse M, Usadel B (2014): Trimmomatic a flexible trimmer for Illumina sequence data. <i>Bioinformatics</i> , 30, 2114-2120. |
| Step2: Aligning paired reads and convert fastq to fasta | Flash FASTX- | flash BK-Poolx_R1_trimmed_paired.fq BK- Poolx_R2_trimmed_paired.fq -M 250 > flash_out fastq_to_fasta -i out.extendedFrag.fastq -Q 33 > | Magoc T & Salzberg SL (2011): FLASH: fast length adjustment of short reads to improve genome assemblies. <i>Bioinformatics</i> , 27 2957-2963. Gordon, A., & Hannon, G. J. (2010). FASTX-Toolkit. Short-reads pre-processing tools. http://hannonlab.cshl.edu/fastx_toolkit/index.html |

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| | Toolkit | BK-Poolx_aligned.fa | |
|--|-----------------------------|---|--|
| Step 3a: Identification and selection for sequences with exact matches to oligos & MIDs used, trimming MIDs and primer sequences | Mothur | <pre> mothur "#trim.seqs(fasta=BK- Poolx_aligned.fa,oligos=oligos_Poolx.txt,checkorient=T)" </pre> | Schloss, P.D., et al., Introducing mothur: Open-source, platform-independent, community-supported software for describing and comparing microbial communities. <i>Appl Environ Microbiol</i> , 2009. 75(23):7537-41. |
| Step3b:_Demultiplex the sequences into a file for each sample | Deplex – custom perl script | <pre> perl deplex_v2.pl SampleList </pre> | |
| | | <pre> while (<INLIST>) { \$lib = \$_; chomp(\$lib); </pre> | |
| | | <pre> \$readids1 = \$lib . "_ids.txt"; \$fa1 = \$lib . ".fasta"; \$readidsa = \$lib . "a_ids.txt"; \$readidsb = \$lib . "b_ids.txt"; \$readids2 = \$lib . "_ab_ids.txt"; </pre> | |
| | | <pre> grep -w \$lib \$indir/BK-Poolx_aligned.groups awk '{print \$1}' > \$outdir/\$readids1" </pre> | |
| | | <pre> perl deplex_v2b.pl SampleListB </pre> | |
| | | <pre> while (<INLIST>) { \$lib = \$_;chomp(\$lib); </pre> | |
| | | <pre> \$readids1 = \$lib . "_ids.txt"; \$fa1 = \$lib . ".fasta"; \$readidsa = \$lib . "a_ids.txt"; \$readidsb = \$lib . "b_ids.txt"; \$readids2 = \$lib . "_ab_ids.txt"; </pre> | |
| <pre> system("cat \$outdir/\$readidsa \$outdir/\$readidsb >> \$outdir/\$readids2"); </pre> | | | |

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| | | |
|---------|--|---|
| | | <pre>perl - ne'.'.if(/^(S+)/){\$c=\$i{\$1}}\$c?print:chomp; \${\$_}=1 if." @ARGV"." \$outdir/\$readids2 \$indir/BK-Poolx_aligned.trim.fasta > \$outdir/\$fa1"</pre> |
| Step 4: | <pre>usearch, custom perl script</pre> | <pre>perl usearchPoolA.pl SampleListB while (<INLIST>) { \$lib = \$_;chomp(\$lib); \$fa = \$lib . ".fasta"; \$usout1 = \$lib . "_rc_uniques.fasta"; \$usout2 = \$lib . "_rc_uniques.out"; \$usout3 = \$lib . "_rc_uniques_results.uchime"; \$usout4 = \$lib . "_chimeras.fasta"; \$usout5 = \$lib . "_nonchimeras.fasta"; \$usout6 = \$lib . "_uchimealns"; \$cent = \$lib . "_centroids.fa"; \$uc = \$lib . "_clusters.uc"; \$cons = \$lib . "_consout.fa"; \$msa = \$lib . "_msa.fa";</pre> |

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| | | | |
|---|----------------------|--|--|
| Step 4: Removal of identical replicates | usearch v7.0.1090 | usearch -derep_fulllength \$indir/\$fa -output \$outdir/\$usout1 -sizeout -minseqlength 187 -minuniquesize 5 -strand both -uc \$outdir/\$usout2 | USEARCH and UCLUST algorithms: Edgar,RC (2010) Search and clustering orders of magnitude faster than BLAST, <i>Bioinformatics</i> 26(19), 2460-2461. doi: 10.1093/bioinformatics/btq461 |
| Step 4: Chimera detection | | usearch -uchime_denovo \$outdir/\$usout1 -uchimeout \$outdir/\$usout3 -uchimealns \$outdir/usout6 -chimeras \$outdir/\$usout4 -nonchimeras \$outdir/\$usout5 | Edgar,RC, Haas,BJ, Clemente,JC, Quince,C, Knight,R (2011) UCHIME improves sensitivity and speed of chimera detection, <i>Bioinformatics</i> doi: 10.1093/bioinformatics/btr381 [PMID 21700674].; UCHIME2 algorithm Edgar, R.C. (2016), UCHIME2: Improved chimera detection for amplicon sequences, http://dx.doi.org/10.1101/074252 . |
| Step 4: Clustering sequences 97%; final dereplication | | usearch -cluster_fast \$outdir/\$usout5 -id 0.97 -centroids \$outdir/\$scent -uc \$outdir/\$suc -sizeout -consout \$outdir/\$cons -msaout \$outdir/\$msa"); | USEARCH and UCLUST algorithms: Edgar,RC (2010) Search and clustering orders of magnitude faster than BLAST, <i>Bioinformatics</i> 26(19), 2460-2461. doi: 10.1093/bioinformatics/btq461 |
| Step 4b: Connecting each sample to the corresponding sequence/MOTU; | | sed 's/^>/>sample_/g' sample_centroids.fa > 146443_centroids_edited.fa cat *_centroids_edited.fa > allsequencesPoolx.fa | |
| Step 4c: Final dereplication | | usearch -derep_fulllength allsequencesPoolx.fa -output allsequencesPoolx_uniques.fasta -sizeout -minseqlength 135 -strand both -uc allsequences_rc_uniques.out | USEARCH and UCLUST algorithms: Edgar,RC (2010) Search and clustering orders of magnitude faster than BLAST, <i>Bioinformatics</i> 26(19), 2460-2461. doi: 10.1093/bioinformatics/btq461 |

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| | | | |
|---------------|--------|--|---|
| Step 5: Blast | blastn | export BLASTDB=/usr/local/extras/Genomics/db/ncbi_ nt/current blastn -query \$outdir/\$scent -db nt -num_threads 4 -evalue 1e-10 -outfmt 6 -perc_identity 90 -out Poolx_blast.txt | Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. Journal of Molecular Biology 215:403-410 |
|---------------|--------|--|---|

49

50 Table 2 Output of Illumina MiSeq sequencing and bioinformatic analysis.

| | PoolA | | PoolB | | Comment |
|---|--------------|-----------|--------------|-----------|------------------------|
| | No sequences | Per cent | No sequences | Per cent | |
| Read pairs MiSeq - paired-end reads | 1162800 | 100% | 916245 | 100% | |
| Step1: Trimming and quality filtering with Average quality score ≥ 20 over a 4-base sliding window, min length 135 bp | | | | | |
| Dropped | 146385 | 12.59% | 105547 | 11.52% | |
| Both surviving | 897,964 | 77.22% | 724,779 | 79% | > 70% % survived |
| For only | 100633 | 8.65% | 72624 | 7.93% | |
| Rev only | 17818 | 1.53% | 13295 | 1.45% | |
| Step2: Aligning both reads | | | | | |
| Total pairs | 897,964 | 100% | 724,779 | 100% | |
| Combined pairs/aligned paired reads | 864,845 | 96.31 | 717,735 | 99.03 | > 90% of pairs aligned |
| Not combined pairs | 33119 | 3.69 | 7044 | 0.97 | |
| Step3: Demultiplexing into a sample specific files and removal of MIDs, primer sequences and sequences without exact match to primer sequence | | | | | |
| Group count_number of sequences assigned to each | 549,782 | 63.6 % of | 421,457 | 58.7 % of | |

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| sample | | combined pairs | | combined pairs | |
|---|---------|--------------------------|---------|--------------------------|--|
| Aligned_groups list of sequence names and their assigned sample-ID | 549,782 | / | 421,457 | / | |
| Aligned_scrap sequences without matching primers | 315,063 | / | 296,278 | / | |
| Aligned_trim sequences with matching primers | 549,782 | 63.6 % of combined pairs | 421,457 | 58.7 % of combined pairs | |
| Demultiplexed sequences in a file for each sample | 549,782 | / | 421,457 | / | |
| Step 4: Removal of sequences with fewer than 5 copies and chimeric sequences, clustering at 97% and final dereplication | | | | | |
| Unique sequences/MOTU/cluster sequence | 392 | 0.05 % of combined pairs | 287 | 0.04 % of combined pairs | |
| Step 5: Blast the representative cluster sequences against NCBI database | | | | | |
| Blast output | 386 | 0.04 % of combined pairs | 195 | 0.03 % of combined pairs | Loss of 1.5 % and 32% respectively due to blast criteria |

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52 Table 3 Quality criteria of MOTUs that were used for taxonomic assignment

| No of MOTUs | q-seq-id | S-seq-id | p-ident | length | e-value | Bit-score |
|-------------|---|----------|---------|--------|-----------|-----------|
| 1 | 146437_M00969_273_000000000-AY5TW_1_1102_14305_3883 | AF315121 | 99 | 211 | 1.18E-101 | 379 |
| 2 | 146437_M00969_273_000000000-AY5TW_1_1102_19526_12384 | AF315121 | 100 | 211 | 2.53E-103 | 385 |
| 3 | 146438_M00969_273_000000000-AY5TW_1_1101_27261_9100 | EF042208 | 100 | 211 | 2.53E-103 | 385 |
| 4 | 146438_M00969_273_000000000-AY5TW_1_1101_13444_19038 | EF042208 | 99 | 211 | 1.18E-101 | 379 |
| 5 | 146439_M00969_273_000000000-AY5TW_1_1102_15025_5592 | AF315121 | 100 | 211 | 5.43E-105 | 390 |
| 6 | 146439_M00969_277_000000000-AYLGV_1_1101_12953_5382 | KJ128795 | 100 | 211 | 2.76E-103 | 385 |
| 7 | 146440_M00969_273_000000000-AY5TW_1_1104_23620_22458 | KJ128795 | 100 | 211 | 2.53E-103 | 385 |
| 8 | 146440_M00969_277_000000000-AYLGV_1_1101_19073_27985 | AF315121 | 99 | 211 | 1.28E-101 | 379 |
| 9 | 146441_M00969_273_000000000-AY5TW_1_1102_7930_5591 | KJ128795 | 100 | 211 | 5.43E-105 | 390 |
| 10 | 146441_M00969_277_000000000-AYLGV_1_1102_15894_4292 | KJ128795 | 99 | 211 | 1.28E-101 | 379 |
| 11 | 146449_M00969_277_000000000-AYLGV_1_1101_12703_21299 | AF315121 | 100 | 211 | 2.76E-103 | 385 |
| 12 | 146450_M00969_273_000000000-AY5TW_1_1103_9519_12161 | AF315121 | 100 | 211 | 2.53E-103 | 385 |
| 13 | 146450_M00969_273_000000000-AY5TW_1_1104_24499_8839 | KJ128795 | 100 | 211 | 2.53E-103 | 385 |
| 14 | 146437_2_M00969_277_000000000-AYLGV_1_1101_18349_9237 | KJ128795 | 100 | 211 | 5.93E-105 | 390 |
| 15 | 146437_M00969_273_000000000-AY5TW_1_1103_20135_15217 | KJ128826 | 100 | 208 | 2.49E-103 | 385 |
| 16 | 146438_M00969_273_000000000-AY5TW_1_1102_19858_13124 | KJ128826 | 99.519 | 208 | 1.16E-101 | 379 |
| 17 | 146438_M00969_273_000000000- | KJ128826 | 99.519 | 208 | 1.16E-101 | 379 |

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| | | | | | | |
|----|--|----------|--------|-----|-----------|-----|
| | AY5TW_1_2103_17482_23139 | | | | | |
| 18 | 146440_M00969_273_000000000- AY5TW_1_1104_19252_3931 | KJ128827 | 100 | 208 | 2.49E-103 | 385 |
| 19 | 146440_M00969_273_000000000- AY5TW_1_1101_8169_10679 | KJ128826 | 100 | 206 | 3.22E-102 | 381 |
| 20 | 146437_M00969_273_000000000- AY5TW_1_1101_9887_10271 | AP017650 | 100 | 208 | 2.49E-103 | 385 |
| 21 | 146437_M00969_273_000000000- AY5TW_1_1101_27425_16396 | KJ128822 | 100 | 208 | 2.49E-103 | 385 |
| 22 | 146437_M00969_273_000000000- AY5TW_1_1103_19670_3702 | KJ128822 | 99.519 | 208 | 1.16E-101 | 379 |
| 23 | 146438_M00969_273_000000000- AY5TW_1_1101_20098_5621 | KJ128822 | 99.519 | 208 | 1.16E-101 | 379 |
| 24 | 146438_M00969_273_000000000- AY5TW_1_1104_6282_12086 | KJ128822 | 98.558 | 208 | 2.50E-98 | 368 |
| 25 | 146438_M00969_273_000000000- AY5TW_1_1104_14160_17658 | AP017650 | 99.519 | 208 | 1.16E-101 | 379 |
| 26 | 146438_M00969_273_000000000- AY5TW_1_1102_15665_10237 | AP017650 | 99.038 | 208 | 5.38E-100 | 374 |
| 27 | 146438_M00969_273_000000000- AY5TW_1_1101_12786_9136 | FR751400 | 98.558 | 208 | 2.50E-98 | 368 |
| 28 | 146440_M00969_273_000000000- AY5TW_1_1106_23026_14975 | FR751400 | 99.519 | 208 | 1.16E-101 | 379 |
| 29 | 146440_M00969_273_000000000- AY5TW_1_1101_16295_9201 | AP017650 | 99.519 | 208 | 1.16E-101 | 379 |
| 30 | 146440_M00969_273_000000000- AY5TW_1_1103_6716_5247 | AP017650 | 99.519 | 208 | 1.16E-101 | 379 |
| 31 | 146444_M00969_273_000000000- AY5TW_1_2102_28368_19489 | AP017650 | 99.519 | 208 | 1.16E-101 | 379 |
| 32 | 146437_2_M00969_277_000000000- AYLGV_1_1102_14038_27379 | AP017650 | 99.038 | 208 | 5.87E-100 | 374 |
| 33 | 146437_M00969_273_000000000- AY5TW_1_1101_24055_10171 | FR849599 | 100 | 205 | 1.14E-101 | 379 |
| 34 | 146437_M00969_273_000000000- AY5TW_1_1101_17362_10139 | KJ128741 | 100 | 210 | 1.94E-104 | 388 |
| 35 | 146437_M00969_273_000000000- | KJ128910 | 99.524 | 210 | 9.04E-103 | 383 |

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|----|--|----------|--------|-----|-----------|-----|
| | AY5TW_1_1102_26108_9370 | | | | | |
| 36 | 146437_M00969_273_000000000- AY5TW_1_1105_9560_15855 | FR849599 | 97.561 | 205 | 2.48E-93 | 351 |
| 37 | 146437_M00969_273_000000000- AY5TW_1_1101_18313_16770 | KC193756 | 100 | 210 | 1.94E-104 | 388 |
| 38 | 146437_M00969_273_000000000- AY5TW_1_1104_20457_22121 | KJ128910 | 99.048 | 210 | 4.21E-101 | 377 |
| 39 | 146438_M00969_273_000000000- AY5TW_1_1101_4468_10820 | KJ128910 | 99.048 | 210 | 4.21E-101 | 377 |
| 40 | 146438_M00969_273_000000000- AY5TW_1_1101_14598_7864 | KJ128740 | 100 | 210 | 1.94E-104 | 388 |
| 41 | 146438_M00969_273_000000000- AY5TW_1_1104_16340_20725 | KJ128741 | 99.524 | 210 | 9.04E-103 | 383 |
| 42 | 146438_M00969_273_000000000- AY5TW_1_1101_15625_23263 | KJ128910 | 99.524 | 210 | 9.04E-103 | 383 |
| 43 | 146438_M00969_273_000000000- AY5TW_1_1101_14475_25427 | DQ912088 | 99.024 | 205 | 2.46E-98 | 368 |
| 44 | 146438_M00969_273_000000000- AY5TW_1_1101_8549_4540 | FR849599 | 99.512 | 205 | 5.29E-100 | 374 |
| 45 | 146439_M00969_273_000000000- AY5TW_1_1101_15967_4762 | FR849561 | 99.048 | 210 | 4.21E-101 | 377 |
| 46 | 146439_M00969_277_000000000- AYLGV_1_1101_11296_2925 | KC193720 | 98.571 | 210 | 2.14E-99 | 372 |
| 47 | 146440_M00969_273_000000000- AY5TW_1_1101_21176_21035 | FR849599 | 100 | 202 | 5.29E-100 | 374 |
| 48 | 146440_M00969_273_000000000- AY5TW_1_1103_14268_9831 | FR849561 | 98.571 | 210 | 1.96E-99 | 372 |
| 49 | 146440_M00969_273_000000000- AY5TW_1_1101_3603_13495 | FR849599 | 99.024 | 205 | 2.46E-98 | 368 |
| 50 | 146442_M00969_277_000000000- AYLGV_1_1101_19724_5258 | KJ128910 | 99.524 | 210 | 9.87E-103 | 383 |
| 51 | 146442_M00969_273_000000000- AY5TW_1_1101_20475_24539 | KJ128740 | 99.048 | 210 | 4.21E-101 | 377 |
| 52 | 146442_M00969_273_000000000- AY5TW_1_1102_15042_17323 | KJ128741 | 99.048 | 210 | 4.21E-101 | 377 |
| 53 | 146444_M00969_273_000000000- | KC193777 | 98.095 | 210 | 9.11E-98 | 366 |

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| | | | | | | |
|----|---|----------|--------|-----|-----------|-----|
| | AY5TW_1_1102_13813_8004 | | | | | |
| 54 | 146444_M00969_273_000000000- AY5TW_1_1101_4546_23469 | KJ128910 | 98.095 | 210 | 9.11E-98 | 366 |
| 55 | 146444_M00969_273_000000000- AY5TW_1_1104_13834_23205 | FR849561 | 97.619 | 210 | 4.24E-96 | 361 |
| 56 | 146450_M00969_273_000000000- AY5TW_1_1101_16275_28455 | KJ128910 | 98.571 | 210 | 1.96E-99 | 372 |
| 57 | 146450_M00969_273_000000000- AY5TW_1_1106_16262_11916 | FR849561 | 99.043 | 209 | 1.51E-100 | 375 |
| 58 | 146450_M00969_273_000000000- AY5TW_1_1101_6461_11968 | KC193768 | 98.571 | 210 | 1.96E-99 | 372 |
| 59 | 146450_M00969_273_000000000- AY5TW_1_1106_22895_25788 | KJ128910 | 98.095 | 210 | 9.11E-98 | 366 |
| 60 | 146452_M00969_277_000000000- AYLGV_1_1101_5272_7177 | KJ128910 | 98.571 | 210 | 7.59E-99 | 370 |
| 61 | 146437_2_M00969_277_000000000- AYLGV_1_1101_18100_2367 | KJ128910 | 100 | 210 | 2.12E-104 | 388 |
| 62 | 146437_2_M00969_277_000000000- AYLGV_1_1101_18439_3172 | KJ128741 | 100 | 210 | 2.12E-104 | 388 |
| 63 | 146437_2_M00969_277_000000000- AYLGV_1_1102_5114_15375 | KJ128910 | 99.048 | 210 | 4.59E-101 | 377 |
| 64 | 146439_M00969_277_000000000- AYLGV_1_1101_14720_9732 | KJ128765 | 100 | 211 | 5.93E-105 | 390 |
| 65 | 146437_M00969_277_000000000- AYLGV_1_1101_17036_4544 | KJ128862 | 100 | 224 | 3.78E-112 | 414 |
| 66 | 146438_M00969_273_000000000- AY5TW_1_1102_15507_15905 | KJ128862 | 99.554 | 224 | 1.61E-110 | 409 |
| 67 | 146439_M00969_277_000000000- AYLGV_1_1101_25155_5000 | KU510499 | 100 | 224 | 3.78E-112 | 414 |
| 68 | 146439_M00969_277_000000000- AYLGV_1_1101_9391_7521 | FJ870412 | 99.107 | 224 | 8.17E-109 | 403 |
| 69 | 146439_M00969_277_000000000- AYLGV_1_1103_6393_5494 | KJ128862 | 99.554 | 224 | 1.76E-110 | 409 |
| 70 | 146440_M00969_273_000000000- AY5TW_1_1101_3584_9022 | KU936350 | 99.107 | 224 | 7.49E-109 | 403 |
| 71 | 146440_M00969_277_000000000- | KJ128862 | 99.554 | 224 | 1.76E-110 | 409 |

Diet composition of red-throated divers in the German Bight

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| | AYLGV_1_1102_22459_12199 | | | | | |
| 72 | 146441_M00969_277_000000000- AYLGV_1_1101_20701_8232 | KJ128862 | 99.554 | 224 | 1.76E-110 | 409 |
| 73 | 146443_M00969_277_000000000- AYLGV_1_1102_5525_24499 | KJ128862 | 98.661 | 224 | 3.8E-107 | 398 |
| 74 | 146445_M00969_277_000000000- AYLGV_1_1101_12011_8918 | KJ128862 | 99.554 | 224 | 1.76E-110 | 409 |
| 75 | 146437_2_M00969_273_000000000- AY5TW_1_1102_24621_21708 | KJ128862 | 100 | 224 | 3.46E-112 | 414 |
| 76 | 146438_M00969_273_000000000- AY5TW_1_1107_19768_20694 | AF122825 | 99.554 | 224 | 1.61E-110 | 409 |
| 77 | 146438_M00969_273_000000000- AY5TW_1_1101_23705_8805 | EU410416 | 100 | 217 | 2.60E-108 | 401 |
| 78 | 146438_M00969_273_000000000- AY5TW_1_1101_9046_9345 | KJ128906 | 99.107 | 224 | 7.49E-109 | 403 |
| 79 | 146438_M00969_273_000000000- AY5TW_1_1106_5566_12823 | EU419747 | 98.198 | 222 | 2.64E-103 | 385 |
| 80 | 146438_M00969_273_000000000- AY5TW_1_1102_14365_5613 | KJ168065 | 99.526 | 211 | 2.53E-103 | 385 |
| 81 | 146438_M00969_273_000000000- AY5TW_1_1101_20270_19169 | KJ128870 | 100 | 207 | 8.89E-103 | 383 |
| 82 | 146437_2_M00969_277_000000000- AYLGV_1_1101_25250_15535 | KJ128871 | 99.034 | 207 | 2.1E-99 | 372 |
| 83 | 146437_M00969_273_000000000- AY5TW_1_1101_15433_7149 | KT633607 | 100 | 213 | 4.25E-106 | 394 |
| 84 | 146438_M00969_273_000000000- AY5TW_1_1102_10784_2931 | KT633607 | 99.531 | 213 | 1.98E-104 | 388 |
| 85 | 146438_M00969_273_000000000- AY5TW_1_1103_11713_13769 | KT633607 | 100 | 212 | 1.53E-105 | 392 |
| 86 | 146439_M00969_273_000000000- AY5TW_1_1101_4637_21155 | KT633607 | 99.531 | 213 | 1.98E-104 | 388 |
| 87 | 146440_M00969_273_000000000- AY5TW_1_1107_19000_23334 | KT633607 | 99.531 | 213 | 1.98E-104 | 388 |
| 88 | 146437_M00969_273_000000000- AY5TW_1_1101_23206_24521 | KJ128898 | 99.539 | 217 | 1.21E-106 | 396 |
| 89 | 146437_M00969_273_000000000- | KJ128898 | 99.539 | 217 | 1.21E-106 | 396 |

Diet composition of red-throated divers in the German Bight

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| | AY5TW_1_1101_19358_7121 | | | | | |
| 90 | 146438_M00969_273_000000000- AY5TW_1_1106_14903_24911 | KJ128898 | 99.078 | 217 | 5.62E-105 | 390 |
| 91 | 146438_M00969_273_000000000- AY5TW_1_1101_23851_8482 | KJ128898 | 99.539 | 217 | 1.21E-106 | 396 |
| 92 | 146439_M00969_273_000000000- AY5TW_1_1102_21124_23388 | KJ128898 | 100 | 217 | 2.60E-108 | 401 |
| 93 | 146440_M00969_273_000000000- AY5TW_1_1101_11208_7721 | KJ128898 | 99.539 | 217 | 4.32E-106 | 394 |
| 94 | 146444_M00969_273_000000000- AY5TW_1_1101_11268_6586 | KJ128898 | 99.539 | 217 | 1.21E-106 | 396 |
| 95 | 146444_M00969_273_000000000- AY5TW_1_1104_19247_28671 | KJ128898 | 99.539 | 217 | 1.21E-106 | 396 |
| 96 | 146445_M00969_273_000000000- AY5TW_1_1108_18728_21966 | KJ128898 | 99.078 | 217 | 5.62E-105 | 390 |
| 97 | 146437_2_M00969_277_000000000- AYLGV_1_1101_10880_6810 | KU510503 | 100 | 217 | 2.83E-108 | 401 |
| 98 | 146437_2_M00969_277_000000000- AYLGV_1_1101_8190_6848 | KU510503 | 99.539 | 217 | 1.32E-106 | 396 |
| 99 | 146437_M00969_273_000000000- AY5TW_1_1101_6839_6659 | KJ627974 | 99.519 | 208 | 1.16E-101 | 379 |
| 100 | 146437_M00969_273_000000000- AY5TW_1_1109_4238_10131 | KJ627974 | 100 | 205 | 1.16E-101 | 379 |
| 101 | 146438_M00969_273_000000000- AY5TW_1_1101_16501_11424 | KJ627974 | 100 | 208 | 2.49E-103 | 385 |
| 102 | 158316_M00969_273_000000000- AY5TW_1_1101_28507_12328 | KC193769 | 100 | 210 | 1.94E-104 | 388 |
| 103 | 158318_M00969_277_000000000- AYLGV_1_1101_15095_4934 | KC193720 | 99.524 | 210 | 9.87E-103 | 383 |
| 104 | 158326_M00969_273_000000000- AY5TW_1_1101_4831_14636 | KC193777 | 99.048 | 210 | 4.21E-101 | 377 |
| 105 | 158326_M00969_273_000000000- AY5TW_1_1103_2468_15724 | KJ128741 | 99.048 | 210 | 4.21E-101 | 377 |
| 106 | 158327_M00969_273_000000000- AY5TW_1_1101_19137_7485 | KC193732 | 100 | 210 | 1.94E-104 | 388 |
| 107 | 158328_M00969_273_000000000- | KJ128741 | 99.524 | 210 | 9.04E-103 | 383 |

Diet composition of red-throated divers in the German Bight

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| | AY5TW_1_1102_3284_15746 | | | | | |
| 108 | 158328_M00969_273_000000000- AY5TW_1_1105_10438_14160 | KJ128910 | 97.619 | 210 | 4.24E-96 | 361 |
| 109 | 158328_M00969_273_000000000- AY5TW_1_1107_16399_4897 | KJ128741 | 99.524 | 210 | 9.04E-103 | 383 |
| 110 | 158329_M00969_273_000000000- AY5TW_1_1106_26173_15504 | KJ128740 | 99.524 | 210 | 9.04E-103 | 383 |
| 111 | 158329_M00969_273_000000000- AY5TW_1_1104_21243_11764 | KC193720 | 98.095 | 210 | 9.11E-98 | 366 |
| 112 | 158331_M00969_273_000000000- AY5TW_1_1104_15579_2627 | KJ128741 | 99.524 | 210 | 9.04E-103 | 383 |
| 113 | 158331_M00969_273_000000000- AY5TW_1_1103_14814_8734 | KC193777 | 97.619 | 210 | 4.24E-96 | 361 |
| 114 | 158332_M00969_273_000000000- AY5TW_1_1103_5388_13732 | KJ128741 | 99.048 | 210 | 4.21E-101 | 377 |
| 115 | 158332_M00969_273_000000000- AY5TW_1_1102_24809_24690 | KJ128740 | 97.619 | 210 | 4.24E-96 | 361 |
| 116 | 158333_M00969_273_000000000- AY5TW_1_1101_23775_21597 | KC193768 | 98.571 | 210 | 1.96E-99 | 372 |
| 117 | 158316_M00969_273_000000000- AY5TW_1_1106_3159_18568 | KJ128910 | 99.524 | 210 | 9.04E-103 | 383 |
| 118 | 158317_M00969_277_000000000- AYLGV_1_1101_9295_7591 | KJ128910 | 99.524 | 210 | 9.87E-103 | 383 |
| 119 | 158326_M00969_273_000000000- AY5TW_1_1103_18938_15756 | KJ128910 | 99.524 | 210 | 9.04E-103 | 383 |
| 120 | 158328_M00969_273_000000000- AY5TW_1_1101_21109_18807 | KJ128910 | 99.524 | 210 | 9.04E-103 | 383 |
| 121 | 158328_M00969_273_000000000- AY5TW_1_1105_3153_13664 | KJ128910 | 98.571 | 210 | 1.96E-99 | 372 |
| 122 | 158329_M00969_273_000000000- AY5TW_1_1103_23245_10923 | KJ128910 | 99.524 | 210 | 9.04E-103 | 383 |
| 123 | 158329_M00969_273_000000000- AY5TW_1_1103_11912_22204 | KJ128910 | 99.524 | 210 | 9.04E-103 | 383 |
| 124 | 158331_M00969_273_000000000- AY5TW_1_1101_13010_19728 | KJ128910 | 99.524 | 210 | 9.04E-103 | 383 |
| 125 | 158332_M00969_273_000000000- | KJ128910 | 99.524 | 210 | 9.04E-103 | 383 |

Diet composition of red-throated divers in the German Bight

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| | AY5TW_1_1105_27871_11343 | | | | | |
| 126 | 158332_M00969_273_000000000- AY5TW_1_1102_23450_20124 | KJ128910 | 99.524 | 210 | 9.04E-103 | 383 |
| 127 | 158333_M00969_273_000000000- AY5TW_1_1107_24795_16622 | KJ128910 | 99.524 | 210 | 9.04E-103 | 383 |
| 128 | 158333_M00969_273_000000000- AY5TW_1_1109_21956_25566 | KJ128910 | 99.524 | 210 | 9.04E-103 | 383 |
| 129 | 158316_M00969_273_000000000- AY5TW_1_1102_17972_18839 | FR849599 | 99.512 | 205 | 5.29E-100 | 374 |
| 130 | 158316_M00969_273_000000000- AY5TW_1_1101_14384_8657 | FR849599 | 99.024 | 205 | 2.46E-98 | 368 |
| 131 | 158327_M00969_273_000000000- AY5TW_1_1101_8872_10503 | FR849599 | 99.512 | 205 | 5.29E-100 | 374 |
| 132 | 158329_M00969_273_000000000- AY5TW_1_1106_5076_21928 | FR849599 | 99.512 | 205 | 5.29E-100 | 374 |
| 133 | 158327_M00969_273_000000000- AY5TW_1_1101_14191_13106 | KJ128827 | 99.038 | 208 | 5.38E-100 | 374 |
| 134 | 158327_M00969_273_000000000- AY5TW_1_1101_18211_28305 | KJ128826 | 99.519 | 208 | 1.16E-101 | 379 |
| 135 | 158333_M00969_273_000000000- AY5TW_1_1102_4609_20805 | KJ128795 | 99.526 | 211 | 2.53E-103 | 385 |
| 136 | 158333_M00969_273_000000000- AY5TW_1_1103_21347_5449 | KJ128795 | 99.052 | 211 | 1.18E-101 | 379 |
| 137 | 158316_M00969_273_000000000- AY5TW_1_1104_8166_5628 | AP017650 | 99.519 | 208 | 1.16E-101 | 379 |
| 138 | 158316_M00969_273_000000000- AY5TW_1_1101_26101_9817 | AP017650 | 99.519 | 208 | 1.16E-101 | 379 |
| 139 | 158318_M00969_277_000000000- AYLGV_1_1101_10887_15643 | AP017650 | 99.519 | 208 | 1.26E-101 | 379 |
| 140 | 158319_M00969_273_000000000- AY5TW_1_1102_15859_8335 | AP017650 | 99.519 | 208 | 1.16E-101 | 379 |
| 141 | 158327_M00969_273_000000000- AY5TW_1_1102_8194_14530 | KJ128822 | 99.519 | 208 | 1.16E-101 | 379 |
| 142 | 158327_M00969_273_000000000- AY5TW_1_1107_25257_10792 | AP017650 | 99.038 | 208 | 5.38E-100 | 374 |
| 143 | 158327_M00969_273_000000000- | AP017650 | 99.519 | 208 | 1.16E-101 | 379 |

Diet composition of red-throated divers in the German Bight

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| | AY5TW_1_1101_20620_19170 | | | | | |
| 144 | 158333_M00969_273_000000000- AY5TW_1_1103_15530_21664 | KJ128825 | 100 | 208 | 2.49E-103 | 385 |
| 145 | 158317_M00969_277_000000000- AYLGV_1_1101_8195_6365 | KJ128862 | 99.554 | 224 | 1.76E-110 | 409 |
| 146 | 158318_M00969_277_000000000- AYLGV_1_1101_10365_8785 | KU510499 | 99.554 | 224 | 1.76E-110 | 409 |
| 147 | 158321_M00969_277_000000000- AYLGV_1_1101_21608_2864 | KJ128862 | 99.554 | 224 | 1.76E-110 | 409 |
| 148 | 158327_M00969_273_000000000- AY5TW_1_1103_21252_10689 | KJ128862 | 100 | 223 | 1.24E-111 | 412 |
| 149 | 158327_M00969_273_000000000- AY5TW_1_1103_5622_6457 | KJ128862 | 97.788 | 226 | 2.12E-104 | 388 |
| 150 | 158327_M00969_273_000000000- AY5TW_1_1102_6728_13312 | KJ128862 | 99.554 | 224 | 1.61E-110 | 409 |
| 151 | 158328_M00969_277_000000000- AYLGV_1_1101_14974_8593 | KJ128862 | 99.554 | 224 | 1.76E-110 | 409 |
| 152 | 158330_M00969_273_000000000- AY5TW_1_1102_18265_2051 | KJ128862 | 99.554 | 224 | 1.61E-110 | 409 |
| 153 | 158322_M00969_277_000000000- AYLGV_1_1101_23960_7813 | KJ128906 | 99.107 | 224 | 8.17E-109 | 403 |
| 154 | 158322_M00969_277_000000000- AYLGV_1_1101_20149_5105 | KJ128906 | 98.661 | 224 | 3.8E-107 | 398 |
| 155 | 158327_M00969_273_000000000- AY5TW_1_1102_9564_24989 | KJ128870 | 99.517 | 207 | 4.14E-101 | 377 |
| 156 | 158329_M00969_273_000000000- AY5TW_1_1102_20113_13469 | KT633607 | 99.531 | 213 | 1.98E-104 | 388 |
| 157 | 158329_M00969_277_000000000- AYLGV_1_1101_17608_18399 | KU510503 | 99.078 | 217 | 6.13E-105 | 390 |
| 158 | 158317_M00969_277_000000000- AYLGV_1_1101_12124_6581 | KU510503 | 99.539 | 217 | 1.32E-106 | 396 |
| 159 | 158318_M00969_277_000000000- AYLGV_1_1101_24645_12079 | KU510503 | 99.078 | 217 | 6.13E-105 | 390 |
| 160 | 158319_M00969_273_000000000- AY5TW_1_1101_4065_16408 | KJ128898 | 99.078 | 217 | 5.62E-105 | 390 |
| 161 | 158319_M00969_273_000000000- | KJ128898 | 99.078 | 217 | 5.62E-105 | 390 |

Diet composition of red-throated divers in the German Bight

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| | AY5TW_1_1101_27338_12669 | | | | | |
| 162 | 158322_M00969_277_000000000- AYLGV_1_1101_10398_4334 | KU510503 | 99.539 | 217 | 1.32E-106 | 396 |
| 163 | 158325_M00969_277_000000000- AYLGV_1_1101_25631_15275 | KU510503 | 98.157 | 217 | 1.33E-101 | 379 |
| 164 | 158326_M00969_273_000000000- AY5TW_1_1101_10267_13492 | KJ128898 | 99.539 | 217 | 1.21E-106 | 396 |
| 165 | 158327_M00969_273_000000000- AY5TW_1_1101_17516_21993 | KJ128898 | 99.539 | 217 | 1.21E-106 | 396 |
| 166 | 158332_M00969_273_000000000- AY5TW_1_1101_25351_7734 | KJ128898 | 99.539 | 217 | 1.21E-106 | 396 |
| 167 | 158329_M00969_277_000000000- AYLGV_1_1101_24101_11074 | KJ627974 | 100 | 208 | 2.71E-103 | 385 |
| 168 | 158329_M00969_277_000000000- AYLGV_1_1101_10726_4988 | KJ128825 | 100 | 208 | 2.71E-103 | 385 |
| 169 | 158334_M00969_277_000000000- AYLGV_1_1101_7624_6843 | KJ128870 | 99.517 | 207 | 4.51E-101 | 377 |

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