DIVER

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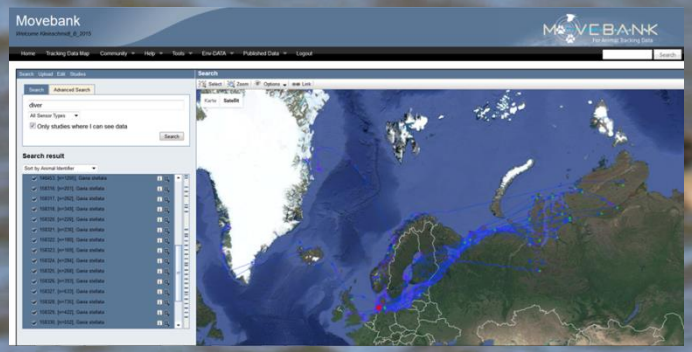

Ecological diet analysis of red-throated divers wintering in the German North Sea based on molecular methods

DHI

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Bio Consult SH

Overview

1. Introduction:

   Background information & project aims

2. Methods

   Molecular tools

3. Preliminary results

4. Summary

   What is done – what needs to be done

Ecological diet analysis of red-throated divers wintering in the German North Sea based on molecular methods_1. Introduction

How important is the North Sea as a wintering habitat for red throated divers & what factors drives them to use the North sea as wintering habitat?

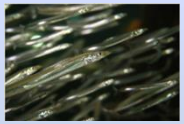
- Many factors like gradients, salinity, water depth, water temperature
- Diet → they need to feed → occurrence of prey species is essential

Knowledge of dietary choices and trophic niches is essential to understand the roles of organisms and species in ecosystems

- Combine tracking data with distribution of prey → spawning times and areas
- the connection between habitat choice of red-throated divers and distribution of prey species

Ecological diet analysis of red-throated divers wintering in the German North Sea based on molecular methods_Introduction

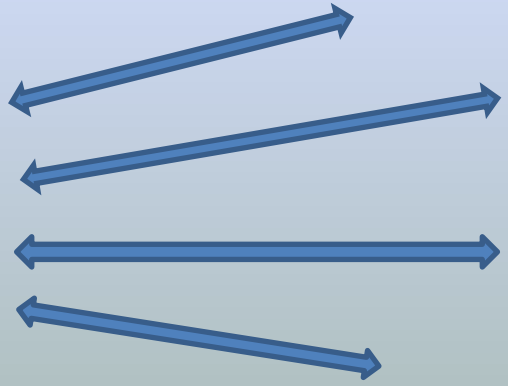
- Habitat choice and movement behaviour of red-throated divers as a migrating species can be influenced by dietary choices



Habitat utilization + staging areas

Home ranges

Diet choice & Distribution of prey



Site fidelity

Migration route + pattern

Knowledge about prey species is an important key to understand habitat conditions

Diet of red throated divers – what is known so far

- Known as diving piscivorous seabirds

- Within breeding grounds main prey consists of fish (e.g. three spined stickleback, Clupeids) & in small amounts of crustaceans and molluscs general and common loon

- Baltic Sea – Pomeranian Bight, Guse et al. 2009:
  - main prey consitst of fish
    - focus on Zander in winter & Atlantic herring in spring

- In the North Sea detailed knowledge about prey species is rare
  - by catched birds were rarely reported
  - birds stay offshore → visual observations or sampling not easy possible

  → But most probable herring, sprat, sandeel & whiting

following information in relation to feeding behaviour are of main interest,

- **Basic information** about prey species composition while wintering in the **North Sea**

→ What **prey groups** are included and in which ratio
(just fish or cephalopods crustaceans)

→ what are the prefered **prey species** within the North Sea

→ **specific** feeders or **opportunistic** feeders

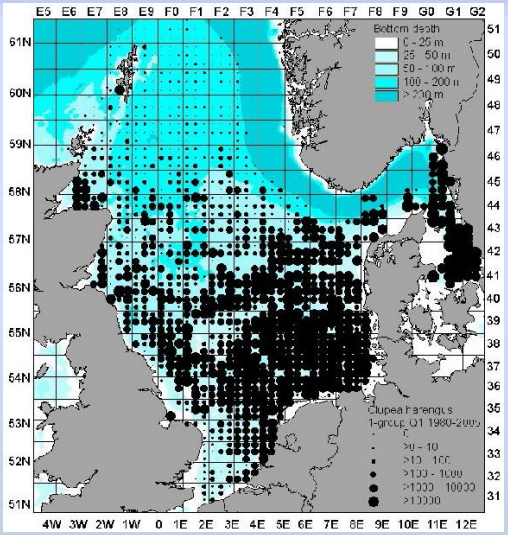
→ Difference in feeding behaviour between **sexes**

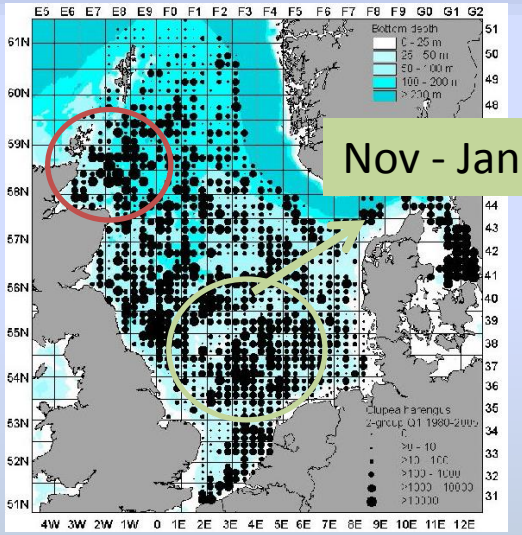
→ Difference in feeding behaviour between **month or year**

- **General comparison between two important wintering sites - North and Baltic Sea**
- **How important is the prey for Habitat choice -** Can the **distribution of red throated divers** be expalined by the **distribution of prey**

DIVER – Project: Ecological diet analysis of red-throated divers wintering in the German North Sea based on molecular methods_Introduction

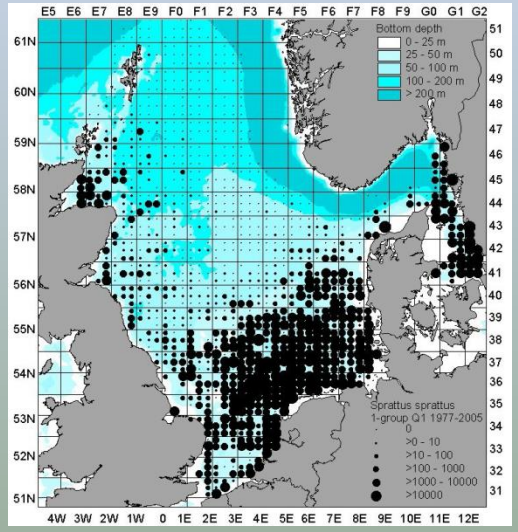
Distribution and spawning areas of potential main prey species based on ICES data

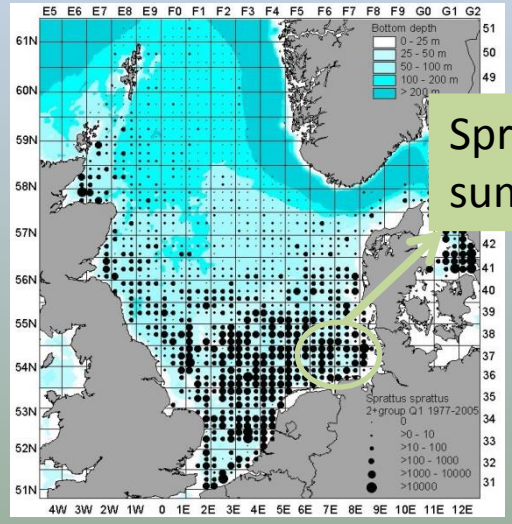




Nov - Jan

Average annual catch rate of **herring** (number per hour fishing) for 1-group and 2-group herring in the quarter 1 IBTS survey, 1977-2005.





Spring - summer

Average annual catch rate of **sprat** (number per hour fishing) for 1-group and 2+ group sprat in the quarter 1 IBTS, 1977 2005.

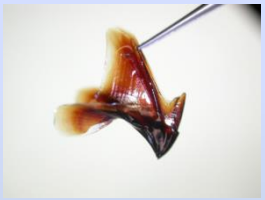
Ecological diet analysis of red-throated divers wintering in the German North Sea based on molecular methods_Methods

2 methods are established and approved to analyse diet composition, such as

- **conventional morphological** analysis of

fish vertebrae, otholiths and cephalopod beaks in gut contents or regurgitates






or

- **molecular tools** - DNA barcoding of gut contents or feacal samples

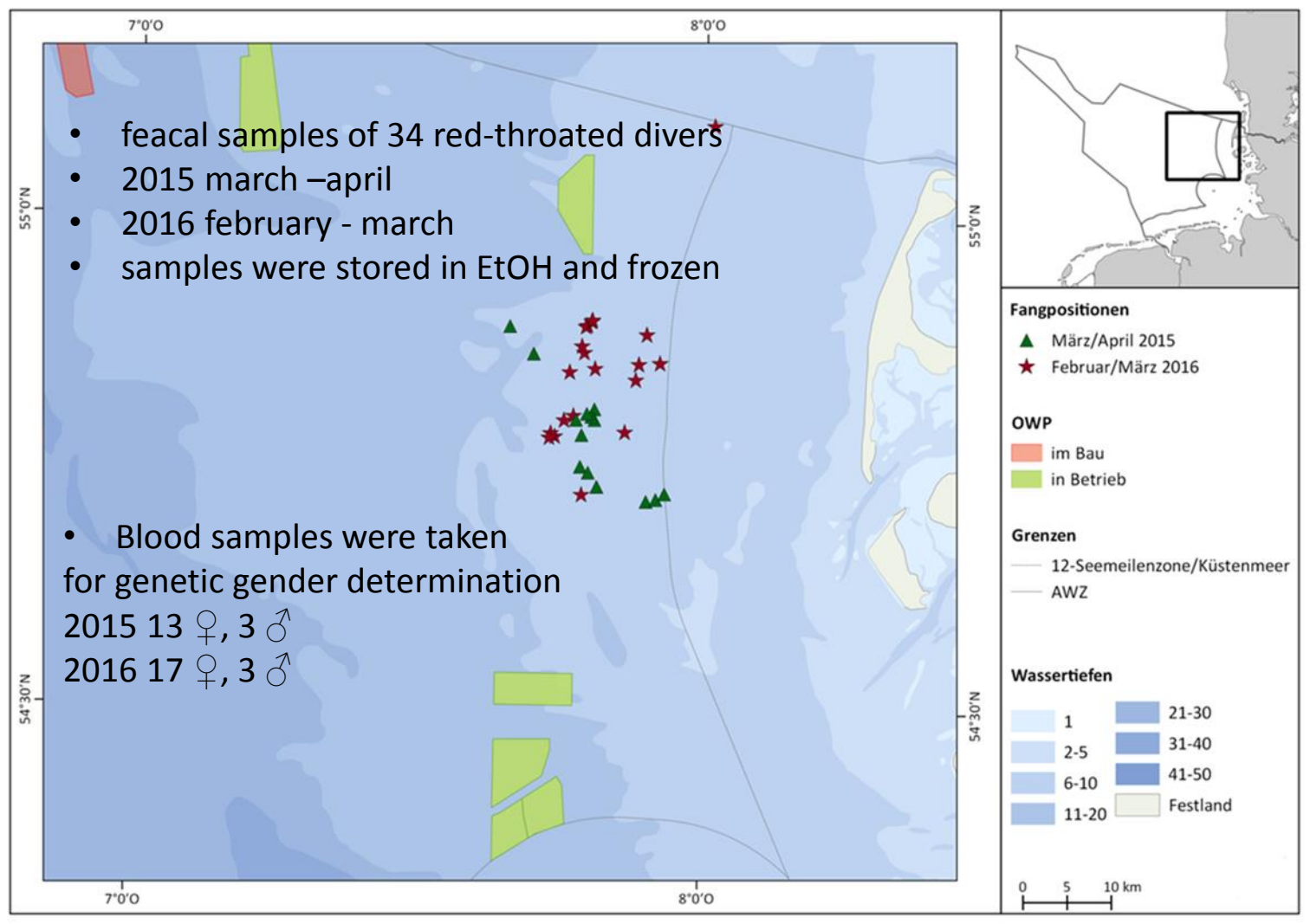
  - DNA Extraction of prey contents from feacal sample,
  - amplification of DNA via PCR
  - sequencing of amplified DNA → Next Generation sequencing

or

- Combination of both

→ In this study **molecular methods using feacals samples** were performed as the most suitable method

Capture positions of 2 field seasons 2015 & 2016



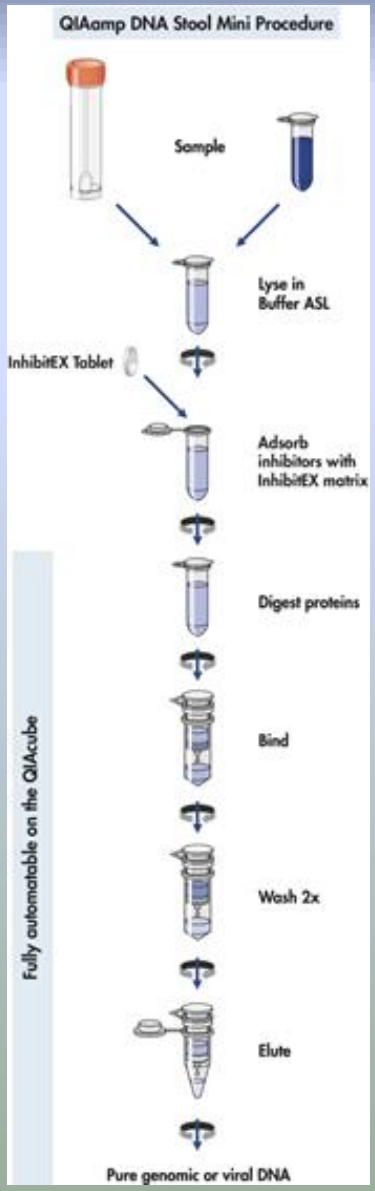
- feacal samples of 34 red-throated divers
- 2015 march –april
- 2016 february - march
- samples were stored in EtOH and frozen

- Blood samples were taken

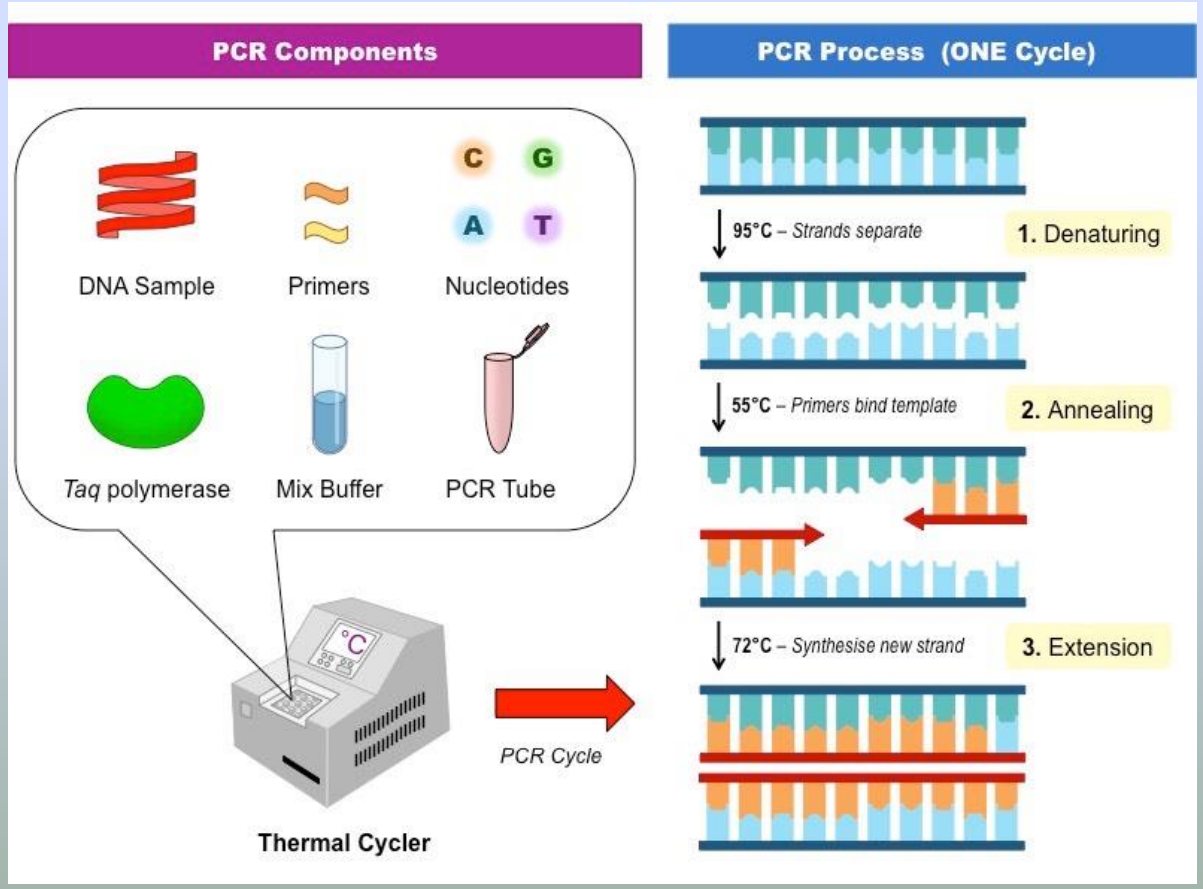
for genetic gender determination
2015 13 ♀, 3 ♂
2016 17 ♀, 3 ♂

Molecular work include

- DNA extraction with Quiagen Dneasy stool kit,



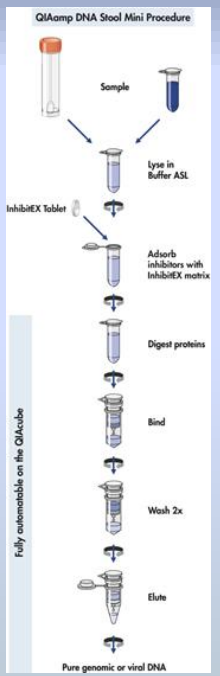
Molecular work include → Polymerase Chain Reaction (PCR)



DIVER – Project: Ecological diet analysis of red-throated divers wintering in the German North Sea based on molecular methods_Methods

Molecular work 1 step include

DNA Extraction Quiagen Dneasy stool kit



Development of 3 universal primer sets for each prey group (fish, cephalopod, crustacean → Deagle et al.; Waap in prep.)

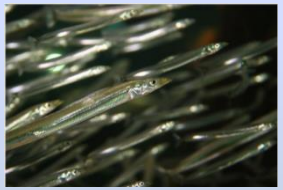
In silico testing of primers by reference to literature of prey and genebank

Lab work → testing primers in the lab = a lot of PCRs

Potential prey species occuring in the North Sea

Fish is the most probable prey group but analysis includes all 3 main prey groups :

→ Fish: 28 potential prey species from 7 order and 15 families
e.g. Clupeidae, Osmeriformes, Gadiformes, Perciformes, Pleuronectiformes, Salmoniformes, Sygnathidae

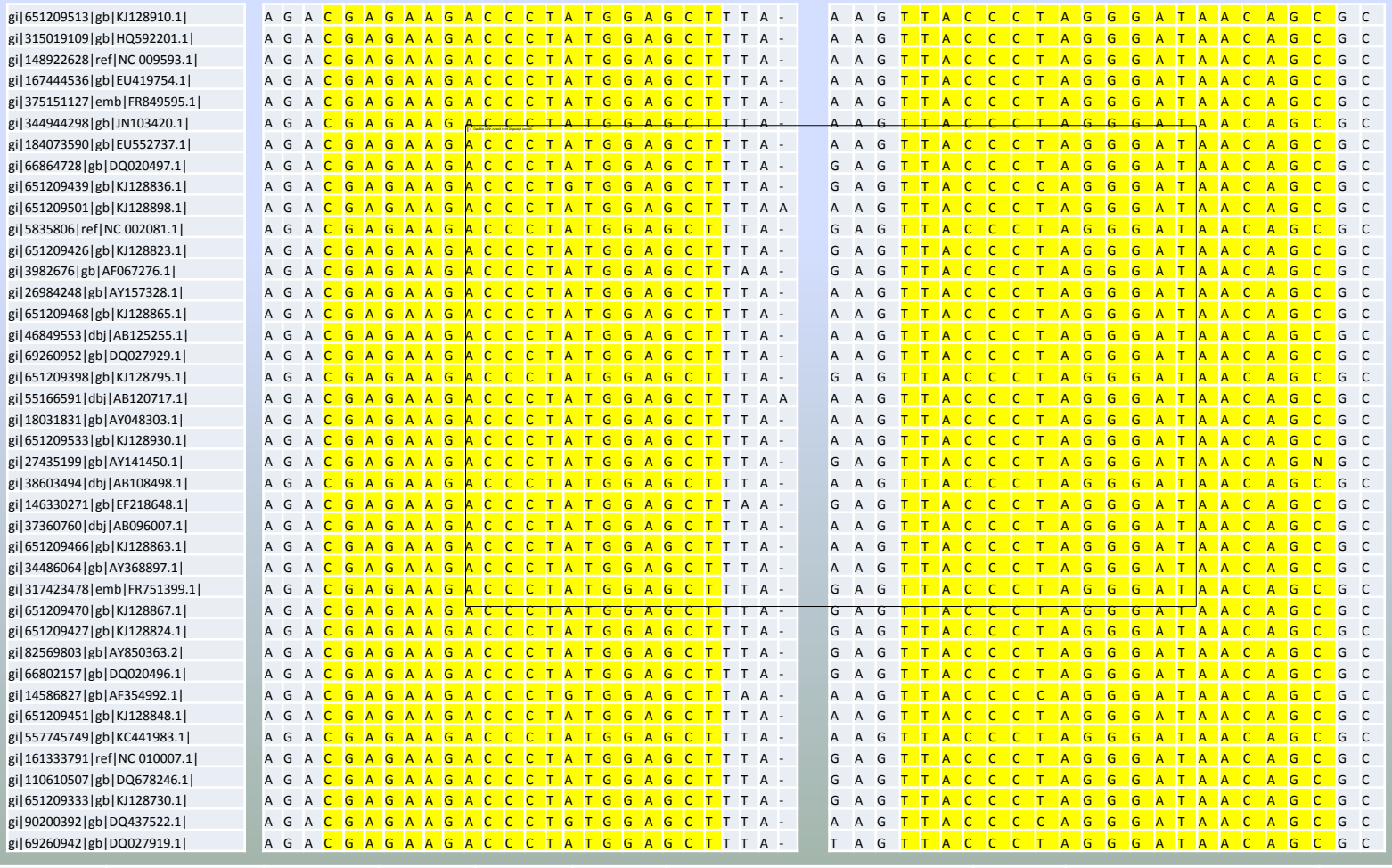


→ Cephalopods: of squid, octopus, cuttlefish 12 species of 5 families
Sepiidae, Sepiolidae, Loliginidae (most abundant), Ommastrephridae, Octopodidae

→ Crustaceans 6 orders and 8 families of shrimp & krill

DIVER – Project: Ecological diet analysis of red-throated divers wintering in the German North Sea based on molecular methods_Methods

Exemplary alignment of 28 fish species and primer binding sites (yellow)



Atlantic Herring Clupea harengus 16S:	gi|315019109|gb|HQ592201.1| Clupea harengus voucher 10001A16S 16S ribosomal RNA gene	mitochondrial 564	Clupeiformes	Clupeidae	Clupea harengus	CGAGAAGACCCTDTGGAG CT	Base 2270 of 16841	TTACCC T AGGGATAACAGC	Base 2534 of 16841	264 bp

Molecular work 2. step include

- to identify each feacal samples

& to identify later the origin of the sample (bird)

- each sample of each bird got a unique combination of MID tagged primers

Bird_Sample ID	Bird_ID	MID For	MID Rev	MID For_Fish	MID Rev_Fish	MID For Ceph	MID Rev Ceph		MID For Invertebr	MID Rev_Invertebr
RTD_S1	146437	_FF1	R1	FISH2_16S_FF1	Fish_16S_R1	Ceph_16S_FF1	Ceph_16S_R1		mICO1int_FF1	Nancy_RR1
RTD_S2	146438	_FF2	R1	FISH2_16S_FF2	Fish_16S_R1	Ceph_16S_FF2	Ceph_16S_R1		mICO1int_FF2	Nancy_RR1
RTD_S3	146439	_FF3	R1	FISH2_16S_FF3	Fish_16S_R1	Ceph_16S_FF3	Ceph_16S_R1		mICO1int_FF3	Nancy_RR1

Tail ID	MID - ID Eurofins	Fish/invert	Primer ID	Name	MID tail	Primer 5' --> 3'		Sequence to order 5' --> 3'
F1	MID-01	Fish_16S	FISH2_16S_F	FISH2_16S_FF1	ACGAGTGCGT	CGAGAAGACCCTDTGRAGCT		ACGAGTGCGT CGAGAAGACCCTDTGRAGCT
F2	MID-02	Fish_16S	FISH2_16S_F	FISH2_16S_FF2	ACGCTCGACA	CGAGAAGACCCTDTGRAGCT		ACGCTCGACA CGAGAAGACCCTDTGRAGCT
F3	MID-03	Fish_16S	FISH2_16S_F	FISH2_16S_FF3	AGACGCACTC	CGAGAAGACCCTDTGRAGCT		AGACGCACTC CGAGAAGACCCTDTGRAGCT
R1	MID-50	Fish_16S	FISH_16S_R	FISH_16S_RR1	ACTAGCAGTA	GCTGTTATCCCTRGRGTAA		ACTAGCAGTAGCTGTTATCCCTRGRGTAA

Molecular work 2. step include

- Adding MID tags (Index adapters or Multiplex identifiers) to primers
- Screening feacal samples → amplification of DNA in the sample
- Pool all amplifications per PCR to 1 equimolar PCR pool
- Pool all PCR pools
- Purify pool
- Sent pool for sequencing

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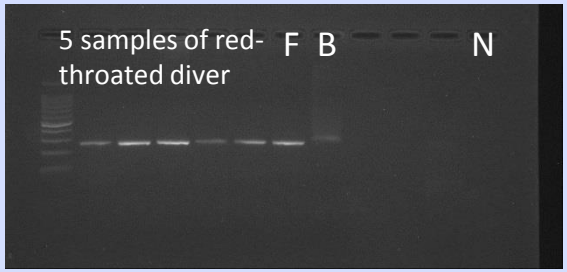
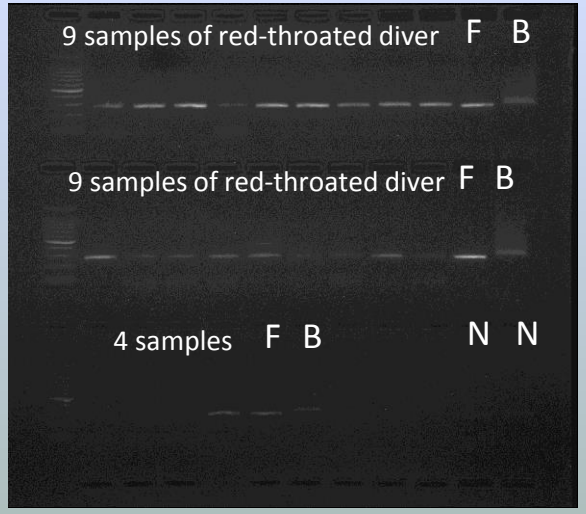
Fish primers



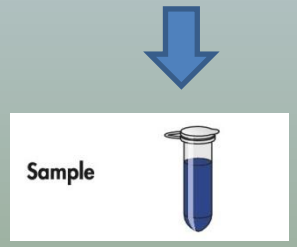
5 samples of red-throated diver + controll + B + N



22 samples of red-throated diver + controll + B + N

7 samples of red-throated diver + controll + B + N

- Positive bands for 34 of 34 samples
- For faint bands more than one pcr were performed & products were pooled together
- DNA concentration varied between 9 ng/µl – > 40 ng/µl
- Samples for each PCR were pooled & purified
- Aliquot of 55 µl and a DNA-concentration 25 ng/µl was sent for sequencing



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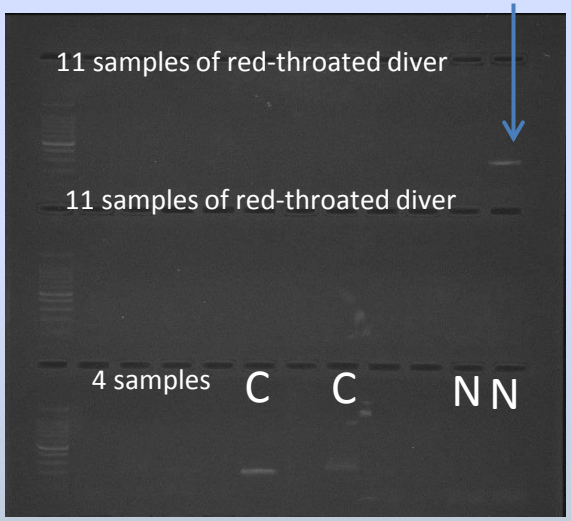
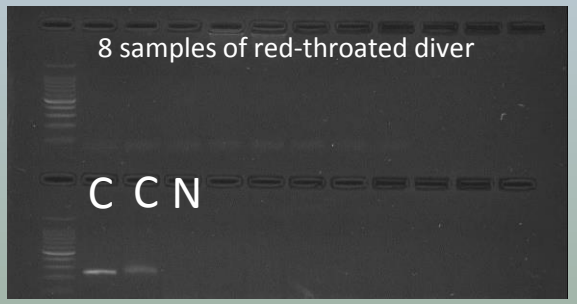
For cephalopods 1 positive sample
red-throated diver Argos ID 158327



26 samples of Red throated diver + controll + negativ



8 samples of Red throated diver + controll + negativ

For crustaceans no positive sample

For my sample size
no evidence that crustaceans are part of the diet &
cephalopods just in very small amounts

DIVER – Project: Ecological diet analysis of red-throated divers wintering in the German North Sea based on molecular methods_results

After Next Generation Sequencing + Bioinformatic analysis

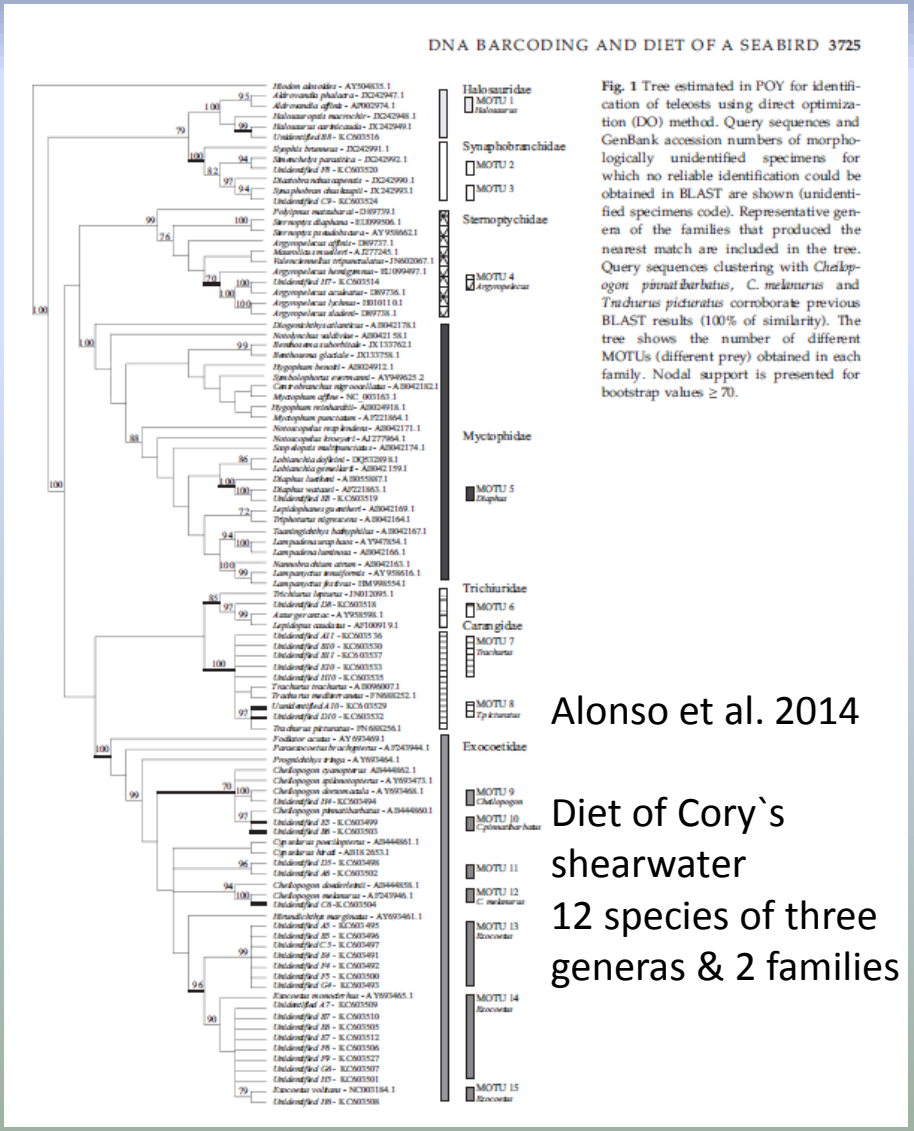


Fig. 1 Tree estimated in POY for identification of teleosts using direct optimization (DO) method. Query sequences and GenBank accession numbers of morphologically unidentified specimens for which no reliable identification could be obtained in BLAST are shown (unidentified specimens code). Representative genera of the families that produced the nearest match are included in the tree. Query sequences clustering with *Cheilopogon pinnatibarbatus*, *C. melanurus* and *Trachurus picturatus* corroborate previous BLAST results (100% of similarity). The tree shows the number of different MOTUs (different prey) obtained in each family. Nodal support is presented for bootstrap values ≥ 70.

Alonso et al. 2014

Diet of Cory`s shearwater
12 species of three generas & 2 families

Summary – state of play

- Extracted DNA-fragments from feacal samples were amplified via Pcr ✓
- All amplicons were prepared and pooled into one sample and sent for ilumina sequencing to read the sequences ✓
- Ilumina sequencing (NGS) and bioinformatic analysis
- When data finally will be available
  - Determine prey species of red throated divers wintering in the North Sea
  - Compare data from North Sea with from the Baltic Sea
  - Analysis of distribution of prey species based on ICES data
  - Test for correlation between distribution of prey and distribution of divers

Thank you!

Stefan Heinänen
Monika Dorsch
Julius Morkūnas
Georg Nehls
Petra Quillfeldt
Ramūnas Žydelis

Claudia Burger
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Thomas Mattern
Jorg Welcker

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Projektträger Jülich Forschungszentrum Jülich

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