

Gavia stellata

Anthropogenic pressure in a marine world:
Distribution, movement, diet and parasite infestation of red-throated
divers *Gavia stellata* in the context of habitat change

Birgit Kleinschmidt

Dissertation presented for achieving the degree of Doctor rerum naturalium

– **Dr. rer. nat.-**

Justus Liebig University Giessen
Faculty of Biology and Chemistry (FB 08)

Dissertation zur Erlangung des Doctor rerum naturalium

– **Dr. rer. nat.-**

Justus Liebig Universität Giessen
Fachbereich Biologie und Chemie (FB 08)

Giessen August 2022

Giessen August 2022



DOCTORAL THESIS / DISSERTATION

Birgit Kleinschmidt (2022) Anthropogenic pressure in a marine world: Distribution, movement, diet and parasite infestation of red-throated divers *Gavia stellata* in the context of habitat change

SUPERVISOR

Prof. Dr. Petra Quillfeldt

DEAN/ DEKAN

Prof. Dr. Thomas Wilke

Department of Animal Ecology and Systematics
Systematics & Biodiversity Group
Faculty 08 of Justus Liebig University Giessen

Institut für Tierökologie und Spezielle Zoologie
AG Spezielle Zoologie und Biodiversitätsforschung
Fachbereich 08 der Justus Liebig Universität Giessen

REVIEWER/ GUTACHTER

Prof. Dr. Petra Quillfeldt

Department of Animal Ecology and Systematics
Behavioural Ecology & Ecophysiology Group
Faculty 08 of Justus Liebig University Giessen

Institut für Tierökologie und Spezielle Zoologie
AG Verhaltensökologie und Ökophysiologie
Fachbereich 08 der Justus Liebig Universität Giessen

Prof. Dr. Volkmar Wolters

Department of Animal Ecology and Systematics
Animal Ecology Group
Faculty 08 of Justus Liebig University Giessen

Institut für Tierökologie und Spezielle Zoologie
AG Tierökologie
Fachbereich 08 der Justus Liebig Universität Giessen

The presented dissertation has been prepared at the Department of Animal Ecology and Systematics of Faculty 08 (Biology and Chemistry) at the Justus Liebig University Giessen.

Die vorgelegte Dissertation wurde am Institut für Tierökologie und Spezielle Zoologie des Fachbereichs 08 (Biologie und Chemie) der Justus Liebig Universität Giessen.

Declaration

I declare that I have completed this dissertation single-handedly without the unauthorised help of a second party and only with the assistance acknowledged therein. I have appropriately acknowledged and cited all text passages that are derived verbatim from or are based on the content of published work of others, and all information relating to verbal communications. I consent to the use of an anti-plagiarism software to check my thesis. I have abided by the principles of good scientific conduct laid down in the charter of the Justus Liebig University of Giessen " Satzung der Justus Liebig Universität Giessen zur Sicherung guter wissenschaftlicher Praxis" in carrying out the investigations described in the dissertation.

Selbstständigkeitserklärung

Hiermit versichere ich, die vorgelegte Arbeit selbstständig und ohne unerlaubte fremde Hilfe und nur mit den Hilfen angefertigt, die ich in der Dissertation angegeben habe. Alle Textstellen, die wörtlich oder sinngemäß aus veröffentlichten Schriften entnommen sind, und alle Angaben, die auf mündlichen Auskünften beruhen, sind als solche kenntlich gemacht. Bei den von mir durchgeführten und in der Dissertation erwähnten Untersuchungen habe ich die Grundsätze guter wissenschaftlicher Praxis, wie sie in der "Satzung der Justus Liebig Universität Giessen zur Sicherung guter wissenschaftlicher Praxis" niedergelegt sind, eingehalten

Giessen, August 2022

Birgit Kleinschmidt



Und die Sterne sind dafür da, dass Du nach ihnen greifst

Turbostaat



CONTENTS

ABSTRACT	I
DEUTSCHE ZUSAMMENFASSUNG	II
 I SYNTHESIS	
1. Rationale	01
2. General Introduction	02
3. Objectives and structure of the thesis	14
4. Chapter outlines	16
5. Concluding remarks	18
6. References	22
 II CHAPTERS	
7. Chapter 1 Kleinschmidt et al. 2019: The diet of red-throated divers (<i>Gavia stellata</i>) overwintering in the German Bight (North Sea) analysed using molecular diagnostics	35
8. Chapter 2 Kleinschmidt et al. 2022: Annual movements of a migratory seabird - the NW European red-throated diver (<i>Gavia stellata</i>) – reveals high individual repeatability but low migratory connectivity	55
9. Chapter 3 Kleinschmidt et al. in preparation: Prevalence of haemosporidian parasites in an arctic breeding seabird species – the NW-European red-throated diver (<i>Gavia stellata</i>)	77
 III APPENDIX	
Supplementary material	96
Curriculum Vitae	99
List of publications and Conference contributions	101
Acknowledgements	103

Abstract

Information on the behaviour and ecology of seabirds provides an important basis for better understanding the effects of changes in their habitats and developing conservation strategies accordingly. The red-throated diver *Gavia stellata* breeds in Arctic regions and is found in coastal marine offshore areas outside the breeding season. German waters contain some important non-breeding areas for this species, with internationally relevant populations such as in the German Bight. Nevertheless, these areas are also characterized by high anthropogenic use, leading to strong responses in this species with avoidance distances of over 10 km and significant changes in distribution patterns. If these responses have long-term effects at the population level is still unclear due to the lack of basic studies on behavioural-ecological aspects. Therefore, this dissertation, aimed at filling the knowledge gaps related to dietary, migratory, and disease ecology.

The cumulative dissertation presented here is structured into three chapters, each of which addresses a behavioural-ecological topic presented in chapters 1, 2, and 3, respectively. Specifically, the diet of red-throated divers in the German Bight was analysed using DNA-metabarcoding on sample sets from two consecutive years (Chapter 1). The results showed an opportunistic piscivorous prey spectrum which, however, included a high proportion of energetic rich species in both years, with clupeids as the most frequented prey items. These findings confirm other studies from e.g. the Baltic Sea. The continuous presence of high-energy prey fish in the diet helps to explain the importance of the German Bight due to the high availability of those fish species. Migratory behaviour and site use throughout the year, was studied using satellite telemetry and analysis of isotopic values from feather samples (Chapter 2). Breeding, moulting, and wintering sites were successfully determined of individuals inhabiting the German Bight. Migratory behaviour was characterized by regular staging stops, probably to refuel energy reserves. Individuals breeding in northern Russia showed low migratory connectivity, indicated by variable site use among individuals. However, between two consecutive years, individual movement patterns showed high repeatability. Furthermore, a correlation between migratory phenology and the location of the breeding area was revealed. A potential stressor, which has not yet been investigated in red-throated divers, could be the infestation of blood parasites or haemosporidia (*Plasmodium*, *Haemoproteus*, *Leucocytozoon*), which can have a negative impact on health (chapter 3). Haemosporidia were detected in red-throated divers using molecular tools, with low prevalence and identification of one new lineage each of *Leucocytozoon* and *Plasmodium*. The low prevalence indicates a rather insignificant impairment.

In summary, the results of the behavioural ecological aspects reported in this dissertation fill previously existing knowledge gaps about a seabird species sensitive to disturbance. The integrative approach of dietary, migratory and disease ecology illustrates that for migratory species, impairments that occur during the annual cycle should be considered cumulatively rather than in isolation. Along their migration route red-throated divers, as well as other migratory species use various staging and stop-over sites and thus are vulnerable to experience a number of impacts, such as gill net fisheries, pollution or ship traffic, that individually, may still be compensable, but collectively might lead to population-level impacts.

Deutsche Zusammenfassung

Informationen über das Verhalten und die Ökologie von Tieren liefern wichtige Grundlagen um die Auswirkungen von Veränderungen in ihrem Lebensräumen besser einordnen und entsprechend Schutzstrategien entwickeln zu können. Der Sterntaucher *Gavia stellata* brütet in arktischen Regionen und ist außerhalb der Brutzeit in küstennahen marinen Gebieten zu finden. Die deutschen Gewässer beinhalten eine Reihe von wichtigen Rastgebieten für diese Art, mit zum Teil international relevanten Beständen, wie unter anderem in der Deutschen Bucht (Nordsee). Diese Gebiete sind gleichwohl auch von hoher anthropogener Nutzung geprägt, was bei dieser Art zu Meide-Distanzen von über 10 km und deutlichen Änderungen der Verteilungsmuster in beeinträchtigten Bereichen führt. Inwieweit sich diese Reaktionen auf Populationsebene langfristig auswirken können ist noch unklar da grundlegende Informationen zu verhaltens-ökologischen Aspekten fehlen. Daher wurden in dieser Dissertation relevante Fragen zu bestehenden Wissenslücken in Bezug auf Ernährungs-, Bewegungs- und Krankheitsökologie untersucht.

Die hier präsentierte kumulative Disseration ist in drei Kapitel gegliedert, welches je ein verhaltens-ökologisches Thema behandelt, entsprechend dargestellt in Kapitel 1, 2 und 3. Konkret wurde das Nahrungsspektrum von Sterntauchern in der Deutschen Bucht mittels DNA-Metabarcoding an Proben aus zwei Jahren untersucht (Kapitel 1). Die Ergebnisse zeigten ein opportunistisches Beutespektrum, welches jedoch in beiden Jahren einen hohen Anteil von energiereichen Arten beinhaltete, mit Heringsartigen als die am meisten frequentierte Beutefischart. Diese Erkenntnisse bestätigen andere Studien aus z.B. der Ostsee. Die kontinuierliche Präsenz von energiereichen Beutefischen in der Nahrung erklärt die Bedeutung der Deutschen Bucht aufgrund der hohen Verfügbarkeit dieser Beutefischarten. Das Migrationsverhalten und die Standortnutzung im Jahresverlauf, wurden anhand von Satellitentelemetrie und der Analyse von Isotopensignaturen aus Federproben untersucht (Kapitel 2). Von den Individuen, die die Deutsche Bucht nutzen, wurden erfolgreich Brut-, Mauser- und Überwinterungsplätze bestimmt. Das Zugverhalten war durch regelmäßige Zwischenstopps gekennzeichnet, wahrscheinlich um Energiereserven aufzutanken. Individuen die in Nordrussland brüteten zeigten eine geringe Migrationskonnektivität, welche sich in einem zwischen den Individuen stark variierendem Verhalten in der Nutzung der Rastgebiete zeigte. Zwischen zwei aufeinanderfolgenden Jahren zeigten die individuellen Bewegungsmuster jedoch eine hohe Wiederholbarkeit. Weiterhin zeigte sich ein Zusammenhang zwischen der Phänologie der Zugbewegungen und dem Standort des Brutgebietes. Ein möglicher Stressor, der bisher in Sterntauchern noch nicht untersucht wurde, ist der Befall von Blutparasiten oder Haemosporidien (*Plasmodium*, *Haemoproteus*, *Leucocytozoon*), welcher sich auf die Gesundheit auswirken kann (Kapitel 3). Haemosporidien konnten anhand von molekularbiologischen Methoden in Sterntauchern mit einer geringen Prävalenz sowie der Identifizierung von jeweils einer neuen *Leucocytozoon* und einer neuen *Plasmodium* Linie nachgewiesen werden. Die geringe Prävalenz deutet auf eine eher insignifikante Beeinträchtigung hin.

Zusammenfassend füllen die Ergebnisse der in dieser Dissertation bearbeiteten verhaltensökologischen Aspekte bis dato bestehende Wissenslücken in Bezug auf eine störungsempfindliche Seevogelart. Damit stellen sie eine verbesserte Bewertungsgrundlage der durch

anthropogene Beeinträchtigungen verursachten Vertreibungseffekte dar. Der hier gezeigte integrative Ansatz von Ernährungs-, Bewegungs- und Krankheitsökologie verdeutlicht dass bei migrierenden Arten Beeinträchtigungen, die im Jahreszyklus auftreten, kumulativ und nicht isoliert betrachtet werden sollten. Entlang ihrer Migrationsroute sind Sterntaucher, wie auch andere Seevogelarten von einer Reihe von Beeinträchtigungen betroffen, welche einzeln betrachtet gegebenenfalls noch ausgleichbar sind, in der Summe aber zu Auswirkungen auf Populationsebene führen können.

Part I

SYNTHESIS

1 RATIONALE

Since recent years, the development of renewable energy became an important source to meet the human energy demand. According to the Renewable Energy Sources Act or EEG (German: Erneuerbare-Energien-Gesetz) renewable energies should cover 65 % of Germany's electricity consumption by 2030 (BMWI 2021). In order to achieve this aim, the expansion of wind power, and in particular offshore wind farms, play an important role (Deutsche Windguard 2021). The expansion of offshore wind farm areas also means environmental or habitat changes in the marine environment (BSH & BMU 2014). From an ecological perspective, offshore wind farms represent anthropogenic structures that can affect marine organisms in several ways if they do not become habituated to them (e.g. Petersen and Malm 2006, Furness et al. 2013, Kelsey et al. 2018). A potential response of some seabird species is avoidance of these structures, which can lead to displacement and habitat loss. Seabird species considered to be vulnerable to displacement include divers, grebes, sea ducks and auks (Kelsey et al. 2018). Concerns have been raised about possible impacts on those bird populations that rely on the North Sea as their permanent or migratory habitat, and information about the individual ecology of the affected species is needed to fully address each aspect of the impact (Dierschke et al. 2006). For migratory species in particular, it is important to assess the extent to which impacts during the period of stay in affected areas may carry-over to affect migratory behaviour or breeding success. In this context, the red-throated diver *Gavia stellata* plays an important role, as this species is considered a key species for decision making offshore wind farm area permissions (e.g. Petersen et al. 2006, 2014, 2011; Dierschke et al. 2012; Mendel et al. 2019). Several studies on the behaviour of this species show great disturbance and displacement effects, as well as avoidance effects (Garthe and Hüppop 2004, Bellebaum et al. 2006, Dierschke et al. 2006, Petersen et al. 2006, 2012, Burger et al. 2019, Mendel et al. 2019, Heinänen et al. 2020, Vilela et al. 2021). In order to understand the interaction between man-made structures and the organisms living in the ecosystem and to keep the potential environmental effects as low as possible, research and the increase of knowledge is essential. In relation to the red-throated diver, research and increased knowledge about ecological aspects such as a species' annual movements, time of stay in the disturbed area, breeding origin, diet selection and potential pre-loads or stressors are needed to understand the cumulative effects between anthropogenic and environmental pressures. Using the red-throated diver as a representative of (several) seabird species, this thesis will investigate the ecology of a disturbance-sensitive species in order to close important knowledge gaps and thus provide conditions to better assess the long-term effects in relation to potential habitat change in an important stationary non-breeding area.

2 GENERAL INTRODUCTION

As marine organisms, the occurrence and distribution of seabirds are directly affected by properties of their oceanic habitat such as water temperature, salinity, or food availability (Ainley 1980). Most seabirds are migratory and show changes in distribution associated with the stage of the annual cycle (Daunt et al. 2014, Reiertsen et al. 2014, Phillips et al. 2017). For migratory species information about abundance, distribution and range maps are essential in conservation planning (Johnston et al. 2020). In this respect, it is important to reveal why animals move, how they move and when and where they move to provide a basis for assessing how they may be affected by external biotic or abiotic factors (Nathan et al. 2008). Migratory strategies can be seen as the assignment of actions (i.e. continued feeding, departure or cessation of migration) and as a response to specific conditions, such as day length, food availability, or wind conditions (Winkler et al. 2014). Therefore, understanding the reason behind migratory movements provides essential information to comprehend how a change in the animals' movements depends on the conditions encountered (Winkler et al. 2014). Behavioural variation among and within individuals (e.g. movement, habitat use or feeding behaviour) affects the use of resources, intra and inter specific competition and niche partitioning and information about these aspects has therefore major implications for our understanding in seabird ecology (Phillips et al. 2017).

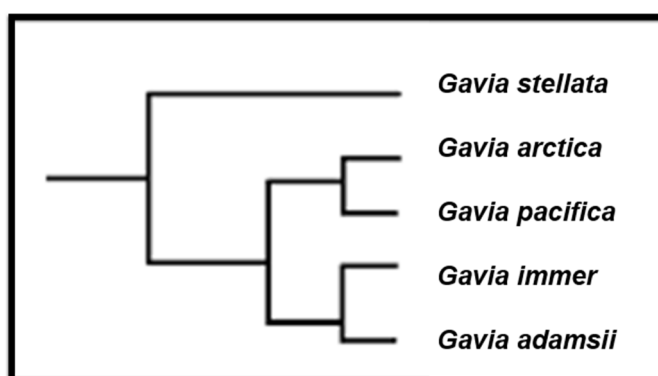
2.1 Definition of seabirds and their role in marine ecosystems

According to Signa et al. (2021) waterbirds are all bird species that live in or around the water and can be divided as:

- Seabirds are all species directly associated with the marine environment, generally divided into pelagic or coastal species
- Shorebirds (or Waders) are all species commonly found along the shorelines of wetlands and coastal systems
- Waterfowl are all species commonly found in wetlands with adaptations to swim (e.g. webbed feet's)

Furness and Monaghan (1987) define seabirds as birds that breed inland or in coastal areas, prefer marine areas during the non-breeding season, feed predominantly in marine waters and are well adapted to the marine environment. They exhibit a wide range of movement patterns ranging from short distanced movements between nesting/roosting sites to feeding sites and seasonal inter-continental migrations (Lundberg and Moberg, 2003, Newton 2007, Signa et al. 2021). In general, seabirds are long-lived species (up to 20 years or more) with late maturity and small clutches where a small change in adult survivorship can have a substantial impact at the population level, because low reproductive rates lead to a slower recovery (Stinen et al 2007). A large proportion of seabirds are piscivorous and in terms of trophic relationships, seabird species can be considered as top predators (i.e. Ainley and Sanger 1979, Schreiber and Burger 2002, Grémillet and Boulinier 2009). Long-distance migrating seabirds create ecological links between otherwise

Red-throated divers belong to the order of Gaviiformes (loons or divers), which is one of the oldest avian orders and represents a monophyletic group with one family, Gaviidae and one genus *Gavia* and five extant species (white billed diver *Gavia adamsii*, great northern diver *Gavia immer*, pacific diver *Gavia pacifica*, black-throated diver *Gavia arctica*, red-throated diver *Gavia stellata*) (Johnsgaard 1987, Boertman 1990, Roselaar et al. 2005, Sprengelmeyer 2014, Moon et al. 2018, Fig. 1). The Gaviidae are named by trivial names as loon in the American area and as diver in the European area, whereas the European trivial name is used in this work.



3

The five diver species vary in some ecological aspects, as well as in body size (Johnsgaard 1987, Gray et al. 2014). They are migratory, piscivorous, long-lived species with a delayed maturity and some of them are reported to be aggressive to con-specifics and con-generics (Johnsgaard 1987, Piper et al. 2008, Gray et al. 2014, Paruk et al. 2014). Divers are suggested to be the most specialised extant foot-propelled diving birds, where the foot propelled diving is considered as an adaptation to forage on rapidly moving prey (Johnsgaard 1987, Boertman 1990, Clifton et al. 2018, Moon et al. 2018). This high adaptation to dive and swim enables high mobility in the water but reduces mobility on land (Johnsgaard 1987, Clifton et al. 2018). Divers are generally classified as waterfowl, but of all divers, the red-throated diver meets the criteria to be defined as a seabird as given by Furness and Monaghan (1987) and Signa et al. (2021). This species is directly associated with the marine environment as it prefers to breed on small lakes or ponds close to the sea and to undertake daily feeding trips to forage in marine waters (Reimchen and Douglas 1984, Eriksson et al. 1990, Rizzolo et al. 2015). Outside the breeding season, red-throated divers spend their time for the most parts exclusively in marine waters, though few exceptions of observation in big inland lakes are reported. The red-throated diver is the smallest of the five diver species and considered to be the most specialised diver species for foot-propelled diving and being the most efficient swimmer (Boertman 1990).

2.3 The red-throated diver *Gavia stellata*, population estimates and distribution

Red-throated divers have a circumpolar distribution with breeding sites in coastal tundra habitats of North America, Europe and Asia (Fig. 2) and a world-wide estimate of 200,000 – 600,000 individuals (Wetland International 2015, BirdLife 2022). Trends of breeding populations are hard to estimate due to the remote and difficult to access breeding sites. Solovyeva et al. (2017) gave an estimate of 0.307 birds/km² in eastern Siberian Russia, but no population trend. In Alaska, long-time data sets showed that the breeding population declined by 53 % in the time period from 1977-1993 and stayed at low levels (Groves et al. 1996, Conant and Groves 2005, Schmutz et al. 2009). Similarly, Erikson (1994) reported that the number of occupied breeding sites in south-west Sweden had decreased by about 50 %.

This thesis focussed on individuals that are associated with the German Bight (eastern North Sea) and related to the European population of red-throated divers (Fig. 3), which equates to 84,200-186,000 mature individuals (Birdlife International 2015, 2022). This population includes breeding regions in Russia (30,000-50,000 pairs), Greenland (5,000-30,000 pairs), Norway (2,000-5,000 pairs), Sweden (1,300-1,900 pairs), Finland (750-1,500 pairs), Great Britain (1,000 – 1,600 individuals), Ireland (6 pairs), Svalbard (500-1,000 pairs) and the Faroe islands (25 pairs, Cramp and Simmons 1977, Birdlife International 2015).

A wintering distribution of about 56,000-72,000 individuals of the European breeding population is associated with temperate waters in the western Baltic Sea, around the British islands, along the Atlantic and North Sea coast of Norway, Denmark, the German Bight, the Netherlands up to Portugal (Cramp and Simmons 1977, Wetlands International 2018, 2022, Fig. 3). Throughout the North Sea an estimate of 48,000

wintering individuals (December – March) has been proposed by Skov et al. (1995). In this context the eastern German Bight presents an internationally important wintering area with recent estimates of 22,000 individuals by Garthe et al. (2015) and 16,330 individuals by Vilela et al. (2021) during the stationary non-breeding season in spring, representing approximately 30 % of the NW-European wintering population using this area. In this area red-throated divers are found in coastal waters characterised by frontal zones that are associated with a local enhancement of nutrients and availability of fish prey, such as clupeids (Skov and Prins 2001). These frontal zones start at the 30 m depth contour when the open North Sea is thermally stratified from autumn to spring (BSH 2019). In general, red-throated divers show a preference for areas with a salinity around 30-32 psu (practical salinity units) and water depth between 20 and 30 m (Heinänen et al. 2019).

How the distribution of red-throated divers in the eastern German Bight is linked with specific breeding regions is unclear. Observations of red-throated divers from sea-watching stations about numbers and utilisation of specific areas in space and time exist, but are only snapshots which do not, or only rarely, indicate where individuals are coming from, going to, or time of stay in respective areas. Ring recoveries from Great Britain could be linked to breeding origins in Shetlands, Greenland, Sweden and Finland (Okill 1994).



Figure 2 World-wide distribution of red-throated divers. In yellow the breeding distribution and in dark blue the wintering distribution and in green areas utilised during passage. Distribution map was taken from Birdlife International (2022).



Figure 3 Distribution range of the North-west European population of red-throated divers taken from Wetlands International (2022)

2.4 Protection status of, potential threats for and stressors of red-throated divers

Red-throated divers are strictly protected, listed in Annex I of the EU Birds Directive, in Annex II of the Bern Convention, in the African Eurasian Waterbird Agreement (AEWA) and are further listed by the HELCOM Convention as a critically endangered species, though as of least concern by the IUCN (Birdlife International 2022).

Similar to other seabird species, red-throated divers are exposed to a number of stressors. Ship traffic can cause disturbance on vulnerable seabird species. A Disturbance Vulnerability Index (DVI) was developed by Fliessbach et al. (2019) and stated red-throated divers as one of the species with highest DVI values, longest escape distances and highest proportions of escaping individuals. Burger et al. (2019) also showed sensitive reactions to disturbance by ship traffic of red-throated divers resulting in higher abundancies in areas less affected by shipping traffic. Fishery, in particular gill net fishery, presents another threat to this species. In the North and Baltic Sea diving seabirds, such as red- and black-throated divers end up frequently as by-catch, dying in fishing nets (Žydelis et al. 2009). Among other seabird species and due to its feeding behaviour, the red-throated diver has been identified as one of the species that suffers potentially significant impacts of gill net mortality (Žydelis et al. 2013). In this context, Morkūnas et al. (2021) described another threat when analysing red-throated divers collected from fishery by-catch. They identified ingested marine debris and found plastic objects in 2.1 % of analysed birds. These incidentally ingested debris/plastics can cause injuries to the digestive tract, absorption of toxins and potential death through starvation (Wright et al. 2013; Tanaka et al. 2013; Zhao et al. 2016).

In addition to plastic exposure, red-throated divers are also exposed to a number of environmental contaminants. Poly- and perfluorinated compounds (PFCs) such as perfluoroalkyl sulfonates (PFSA) and perfluoroalkyl carboxylates (PFCA) are reported to show the highest contamination in piscivorous predators

and were detected in red-throated divers from the German Baltic Sea in the blood, brain, fatty tissue, gall bladder, heart, kidney, liver, lung, muscle and spleen (Rubarth 2011). Mercury was detected in liver, kidney and pectoral muscles in the closely related black-throated diver from the southern Baltic Sea coast, where also red-throated divers occur (Małgorzata Rutkowska et al. 2019). Perfluorinated compounds are suggested to affect detrimental physiology and reproduction of animals (Constantini et al. 2019). Erikson et al. (1992) analysed unhatched eggs from abandoned nests of red-throated divers and related a high content of mercury in these eggs, to the intake of fish in lakes affected by acidification. Mercury concentrations were high enough to expect reproductive impairment. Furthermore, in this context blood mercury levels seem to have immunocompromising effects (Weinandt 2006, Weinandt et al. 2012). In Alaska, high polychlorinated biphenyl (PCB) profiles in red-throated divers were linked to respective wintering grounds in Asia (Schmutz et al. 2009).

Next to direct anthropogenic threats also biological factors are stressors effecting the divers' health. As external parasites leeches, mites and lice, black and bird flies are reported to parasitize divers (Storer 2002). Divers host a number of endoparasites, which include 47 digeneans, 22 cestodes, 14 acanthocephalans and 15 nematode species (Storer 2002). Okulewicz (1989) identified a nematode species (*Contracaecum rudolphii*,) in red-throated divers in Poland that is known to occur in the stomach and intestines of piscivorous birds and is supposed to increase mortality if a massive invasion occurs (Żółtowska et al. 2012). Climate change and warmer temperatures promote the expansion of vector -borne diseases, such as avian malaria, further north (Rochlin et al. 2013, Egizi et al. 2018). Avian malaria or malaria like diseases are caused by intracellular blood parasites of the genera *Plasmodium*, *Haemoproteus* and *Leucocytozoon* of which an infection can cause diverse effects of the physiology i.e. altered food consumption, body weight, mortality. Furthermore, even chronic, or low parasite infections can have effects on host fitness, reproduction and survival (Atkinson et al. 2000, Quillfeldt et al. 2011, Schoenle et al. 2017). These parasites are reported for great northern divers in America (Weinandt 2006, Weinandt et al. 2012, Martinsen et al. 2017), but to date no information about presence or absence of these parasites in red-throated divers has been published.

Offshore wind farms present anthropogenic structures in the marine environment that can cause attraction or avoidance in seabirds. Red-throated divers belong to the group of seabirds that strongly, or nearly completely avoid offshore wind farm areas (Dierschke et al. 2016) with avoidance distances of up to 10 km and more, that lead to a loss of habitat or habitat reduction and subsequent redistribution (Mendel et al. 2019, Heinänen et al. 2020, Vilela et al. 2021). The consequences of not having enough suitable habitats available can be manifold, such as increased inter and intra specific competition, loss of foraging habitats, or increased stress levels which might lead to a possible increased susceptibility to parasites and diseases. Possible consequences at population level make red-throated divers a species group requiring particular consideration with respect to marine spatial planning in Germany and the UK (Busch et al., 2013, Dierschke et al. 2016, 2017). By the end of 2020 more than 20 offshore wind farms consisting of 1,501 turbines with a capacity of

7,770 MW were in operation within German offshore areas (BSH 2020, Deutsche Windguard 2021, Fig. 4). How these numbers and sizes of wind turbines, concerns red-throated divers on population level is not not completely understood as knowledge gaps exist about this species ecology.



Figure 4 Overview of offshore wind farm planning in the German part of North (left side, German Bight) and Baltic (right side) Sea taken from Deutsche Windguard (2022). Each wind mill presents an offshore windfarm, in green already operating windfarms, in red and grey planned wind farms. In dark blue the exclusive economic zone and the dotted line indicates the limit of the 12 nautical mile zone.

2.5 The relevance of integrative ecological studies in relation to anthropogenic pressures

Outside the breeding season most seabirds undergo seasonal migrations that often span large marine ecosystems and include marine areas of different national jurisdictions (Harrison et al. 2018, Alerstam et al. 2019, Strøhm et al 2021). Conservation approaches are therefore only effective if they take into account all annual movements in time and space (Johnston et al. 2020, Strøhm et al 2021). Especially the link between breeding regions and associated wintering or staging/stop-over sites provides important information to link spatially disparate threats and potential carry-over effects with adult survival and breeding success of a species (Strøhm et al. 2021, Buchan et al. 2022). Ecological issues, such as population dynamics, spread of diseases and how to cope with environmental changes are affected by animal movements and space use (Nathan et al. 2008, Spiegel et al. 2017). How migratory birds might respond to long or short-term changes in habitat conditions also depends on the variability in behaviour, ecology, life history and demography (Garthe et al. 1996, Hamer et al. 2002, Weimerskirch 2002, Lewison et al. 2012). A comprehensive knowledge about the ecology and behaviour of the target species is important to conservation biology (Blackwell et al. 2016), as the behaviour affects a species' persistence through a wide range of mechanisms, such as disruption of breeding, dispersal and settlement decisions and foraging strategies (Reed 2002).

In this context and in order to disentangle the effects of environmental change and anthropogenic pressures in the eastern German Bight on red-throated diver populations, it is a prerequisite to know the ecology and potential stressors of this species (Lewison et al. 2012). Therefore, this thesis follows an integrative approach and analyses three behavioural-ecological aspects: dietary-, movement- and disease ecology where no information is available from individuals using the eastern German Bight in order to provide a sound basis for the evaluation of potential impacts. (Fig. 5).

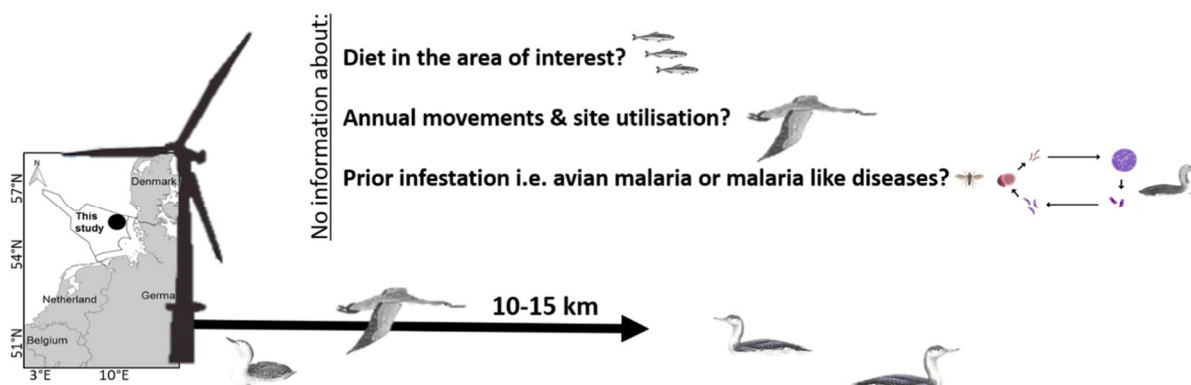


Figure 5 Impact of anthropogenic disturbance on red-throated divers and existing knowledge gaps needed to fully assess the impact on population level modified from Kelsey et al. 2018.

2.5.1 Dietary ecology – identification of prey composition

The analysis of dietary intake and what an animal eats is one of the central questions in animal ecology and provides important background information for understanding the relationships between animals and their environment (Szoboszla et al. 2015, Raubenheimer et al. 2018, Liu et al. 2018, 2021). Understanding how migratory seabirds meet their energy demands at different stages of the annual cycle is fundamental to understand how they cope with changing conditions associated with annual cycles (Dunn et al. 2020). The use of resources, or the level of intra-specific competition is affected by foraging distribution and behaviour and is therefore an important basis for understanding the ecology of seabirds (Phillips et al. 2009, 2017). Distributions and population dynamics are related to feeding ecology and food availability (Newton 1998, 2007, Furness et al. 2006). Nevertheless, knowledge of the diet composition of most seabirds outside the breeding season is limited, because they are then constantly at sea and sampling is difficult (Meier et al. 2017, Phillips et al. 2017). Carry-over effects are defined as the output of processes that influence the success of an individual in the following season (Harrison et al. 2011) and winter habitat quality has been linked with reproductive success in migratory birds (Rushing et al. 2016). A behavioural response such as avoidance reactions towards anthropogenic structures or human activity can result in habitat loss or displacement in altered habitats. Alteration of habitats might result in habitat quality degradation, or in increased intra specific

competition when individuals accumulate in the remaining areas (Burton et al. 2006, Furness 2013, Busch and Garthe 2016). Consequences in terms of a reduced fitness might be linked to an increased energy expenditure by relocating foraging grounds, or a reduced energy acquisition due to altered food availability (Masden 2010, Busch and Garthe 2016). The study of the diet composition in the area of interest represents a basic requirement to assess habitat quality. Consequently, the design of sound conservation and management strategies depend on a detailed knowledge of the diet of a species (Valentini et al. 2009).

Many methods have been established to study the diet of animals including visual observations of foraging behaviour, fatty acid and stable isotope analysis, or morphological analysis of gut contents (Valentini et al. 2009, Oehm et al. 2017). More recent studies include the analysis of consumed species in faecal samples using PCR based methods in combination with next generation sequencing (NGS) and metabarcoding (e.g. Deagle et al. 2007, 2010, Pomanon et al. 2012, Swift et al. 2018). The molecular approach has the advantage to provide a wider prey species spectrum than conventional morphological methods (Deagle 2007, 2010, Thallinger et al. 2016, Oehm et al. 2017) but often does not allow for quantitative analysis of number and size of prey items (Nielson et al. 2018). This high throughput DNA sequencing of faeces is a non-invasive method that has become a popular and accepted tool to study the diet of an animal (Nielson et al. 2018).

Prey composition of red-throated divers has been studied by Guse et al. (2009) on stomach and gut contents of dead birds collected from by-catch from the Baltic Sea using morphological techniques. The study revealed a general opportunistic foraging behaviour and a seasonal switch in consumption of their main prey indicating a preference for clupeid prey fish, if available. For the North Sea two studies are available to report dietary items of non-breeding red-throated divers in this area (Madsen 1957, Durinck et al. 1994), which also morphologically analysed stomach and gut contents of dead birds. Both studies showed that red-throated divers appear to choose energetic rich prey though the older study by Madsen (1957) highlighted gadoid fish as main prey whereas the study by Durinck et al. (1994) indicated clupeid prey fish as the most frequent prey item. Recent dietary information for red-throated divers from the North Sea and in particular the eastern German Bight, is not available so far, but highly important to understand the ecological importance of this area for this species and to place the effects of displacement and habitat shift.

2.5.2 Movement ecology – Annual movements and site utilisation

Understanding migration patterns is an important basis for understanding the behaviour and ecology of a species (Dingle and Drake 2007, Phillips et al. 2017, Spiegel et al. 2017). Migration involves annually repeated seasonal movements between the breeding region and regions utilised during the stationary non-breeding season outside the breeding range. Therefore, the spatial distribution of migratory seabird species changes according to the stage of the annual cycle (Rappole 2013). Migration can be viewed as an adaptation to seasonally fluctuating resources and plays a central role in the spatial dynamics of mobile populations, but

should not be confused in form and function with within-population mixing on a small scale that results from postnatal dispersal and inter-plot movements characteristic of metapopulations (Dingle and Drake 2007). Migratory birds make purposeful movements from breeding to non-breeding areas to take advantage of seasonal peaks in prey abundance, or to avoid adverse weather conditions (Daunt et al. 2014, Reiertsen et al. 2014, Phillips et al. 2017). Two important components of migration are locomotion itself, when a distance is travelled and energy is consumed and fuel deposition, when energy reserves are accumulated and restored (Alerstam and Bäckman 2018). Information about refuelling sites and refuelling behaviour in time and space, or information about migration corridors, wintering areas, or population mix in winter corridors is crucial to identify possible carry-over effects and link explicit threats such as fisheries, oil spills and other pressures, such as offshore wind farms to population dynamics (Weber et al. 1998, Brooke 2004, Gonzáles-Solis et al. 2007, Schmaljohann and Eikenaar 2017). Besides migration, moult and breeding are also considered to be energy demanding events in the yearly cycles of birds (Newton 2011). Environmental change in non-breeding habitats might result in varying energetic costs (i.e. flight expenditure), if birds need to accumulate in undisturbed areas and thus might cause potential fitness consequences that carry-over to affect adult survival, body condition, timing of migration, or breeding success (e.g. Marra et al. 1998, Alves et al. 2013, Catry et al. 2013, Schultner et al. 2014a, Pelletier et al. 2020). The identification of stationary non-breeding seasons and areas is therefore crucial for effective conservation management and measures (Harrison et al. 2011, Rushing et al. 2016). Information about the connectivity of individuals from a shared breeding population or region, how they spread during the stationary non breeding season and mix with individuals from other breeding regions also provides important information to assess how a population might be impacted by environmental change in a specific area (Webster et al. 2002, Finch et al. 2017). Variation among and within individuals is another important category to help better understand seabird ecology (Phillips et al. 2017). Migrants have a spectrum of individual, or population specific strategies from randomness to complete consistency (Wijk et al. 2016, Ruthrauff et al. 2019). Consistent and repeatable behaviour can be advantageous to make the best use of local resources and conditions, but if internal and external cues are annually variable, or environmental conditions change a more flexible strategy may be more beneficial (Wijk et al. 2016, Ruthrauff et al. 2019). An understanding of consistency in timing of movements and the degree of site fidelity is therefore essential to understand the responses of individuals and populations to environmental change and the vulnerability to anthropogenic pressures (McFarlane et al. 2014). In addition, information about how and if annual movements are triggered by the location and environmental conditions of the breeding site helps to better understand the ecology and phenology of annual movements (Gordo 2007, Conklin et al. 2010, Briedis et al. 2016).

A number of various technologies of intrinsic and extrinsic markers are available to study migration. Robinson et al. (2010), as well as Hobson and Norris (2019) showed that each method has its advantages and disadvantages and the appropriate application depends on the research question and feasible conditions.

Intrinsic markers, such as contaminants, parasites, genetics or stable isotopes, require only one capture, but typically have a lower resolution than extrinsic markers. Extrinsic markers, such as banding/markings, radio transmitters, satellite and GPS (Global Positioning Systems) transmitters, passive radar observations, or archival geolocation tags can be applied to a broad range of animals and provide a higher resolution, but require initial capture and recapture. GPS and satellite transmitters usually do not need a recapture, as these transfer the data automatically, but are constrained to animals with a certain minimum weight and might have some effects on behaviour. Tracking technologies enable research questions on evolutionary biology, population ecology and global change to be answered in more detail, e.g., migration links, behaviour, demography, ecology and physiology (Robinson et al. 2010, Bridge et al. 2011, Phillips et al. 2017).

For red-throated divers, detailed information on when and where individuals migrate from a given breeding or wintering area, the linkage between these areas, or where these individuals stage and refuel, or moult is poorly known, as are ecological aspects, such as migratory connectivity, site fidelity and temporal consistency, or the relationship between annual migrations and the location of breeding areas. This information is highly needed to identify breeding populations that could be affected by carry-over effects from stationary non-breeding areas, or to link cumulative effects between multiple utilised areas.

2.5.3 Disease ecology – identification of potential pre-loads in terms of parasite infestation

Wild birds are infested with a broad range of endo-parasites, which can be roughly grouped into gastrointestinal and blood parasites. Although infestations are not always lethal, infections usually impair the physical performance of their hosts, which can lead to deleterious effects (McElroy and de Buron 2014, Kumar et al. 2018). In this context, long-lived migratory species, crossing multiple continents within one annual cycle, might be more exposed to parasite infection than other non-migratory taxa as migratory birds are expected to be exposed to different parasite faunas, whereas resident birds are expected to experience a single parasite fauna (Møller and Erritzøe 1998, Khan et al. 2019). Environmental factors, such as quantity and quality of food resources, levels of local pollution, habitat quality and presence of predators, competitors and/or other parasites can influence the effects of parasites and thus cumulative effects likely play a large role in how parasites influence seabirds at both the individual and population level (Khan et al. 2019).

Blood parasites, such as haemosporidian parasites are vector-borne parasites and the causative agents of avian malaria, or malaria like diseases. These parasites are reported to be rather uncommon in seabirds compared to other bird groups such as land birds and phylogenetic, ecological and life-history parameters are suggested to determine the prevalence of haemosporidian parasites in seabirds (Quillfeldt et al. 2010, 2011). Four primary types of Haemosporidia are identified and each is carried by a different vector. *Plasmodium* species are transmitted to wild birds by mosquitoes (*Culex* and *Aedes* spp.), *Haemoproteus* species by midges (*Culicoides* spp.) and *Leucocytozoon* species by black flies (*Simulium* and *Hippoboscidae* spp.) (Khan et al. 2019). These parasites are widespread from arctic to temperate and tropical regions (Clarke

et al. 2014, Fecchio et al. 2020, Santiago-Alarcon and Marzal 2020). Originally, haemosporidian parasites were thought to be low pathogenic organisms, but in recent years several studies have indicated otherwise and they are now thought to be among the most pathogenic organisms responsible for mortality and population declines (Valkiūnas 2004, Hahn et al. 2018, Palinauskas et al. 2020). Breeding birds have been found to be negatively affected in energetic condition, arrival date and reproductive performance if infected with these parasites and also infected migrating birds showed a later migration timing and reduced energetic condition (Garvin et al. 2003, 2006, De Groote and Rodewald 2010). Though there is ambiguous evidence that a haemosporidian infection affects migration timing, the mechanisms are not clear yet. An effect on physiological capacity in terms of aerobic performance to migrate successfully long distances was excluded by Hahn et al. (2018) and other factors e.g. efficiency of stop-over behaviour like foraging and fuelling are supposed to be considered. Regarding joint negative effects of anthropogenic stress and parasite infections on wild birds not much is known so far. Jiménez-Peñuela (2021) found for non-migratory house sparrows (*Passer domesticus*) that different sources of stress, in this case both urbanisation and parasite infection, can lead to greater deleterious effects. The study suggested that infected sparrows from natural habitats and thus less stressed individuals can promote a greater anti-inflammatory immune response to *Plasmodium* infections that is associated with better food quality.

Prevalence and intensity of haemosporidian infections differ between stages of the annual cycle and have been found to increase from the wintering area through migration to the breeding area (Schrader et al. 2003, Garvin et al. 2004, 2006, De Groote and Rodewald 2010). Transmission strategies of vector-borne parasites differ between year-round transmission, if environmental conditions are favourable, and seasonal transmissions, when the availability of vectors and hosts are tied to specific seasons. Seasonal transmissions often occur in temperate, or even arctic regions, where reproduction of vector borne parasites is timed in order to match the seasonal cycles of highest host susceptibility and vector availability to optimise transmission rates (Antón Pérez-Rodríguez et al. 2015). The transmission of haemosporidian parasites occurs via an heteroxenous life cycle, where haemosporidians alternate between two types of hosts, which are invertebrates (blood sucking dipterans as vectors) and vertebrates (Atkinson et al. 2008, Valkiūnas & Atkinson 2020). Infective stages are essential to complete the life cycle successfully and include gametocytes in vertebrates and sporozoites in invertebrate vectors. Sexual reproduction takes place in the vector, which is considered as the definitive, or final host and vertebrates as the intermediate host, where only asexual multiplication occurs (Valkiūnas & Atkinson 2020).

Haemosporidian parasites can be analysed via microscopy of blood smears, or via molecular tools using PCR techniques. Microscopy of blood smears allows to measure intensity, or parasitaemia, which is the number of parasites of one species that infect a host individual, whereas molecular methods give a higher resolution in taxonomy, but allows only to measure the prevalence, which presents the proportion of

individuals of a sample that are infected and richness, which is the number of parasite species found in an individual host, a group, or a species (Herrera and Nun 2019, Mandal 2021).

To date, no records of the presence or absence of haemosporida are reported for red-throated divers, but this information is important to be considered with regard to the already known stressors and from a cumulative point of view. In great northern divers specific parasitic relationship is documented between these birds and a black fly (*Simulium annulus*) as a potential vector of *Leucocytozoon* (Weinandt et al. 2012). In addition to *Leucocytozoon* infestation, *Plasmodium* has also been detected in Great northern divers in spleen, muscle and kidney, indicating a mixed malaria parasite infection that was associated with mortality (Martinsen et al. 2017).

3 OBJECTIVES AND STRUCTURE OF THE THESIS

This thesis aims to analyse three so far unknown integrative behavioural ecological aspects of red-throated divers in order to provide a sound foundation for comprehensive conservation strategies and prospective management. The results are supposed to increase and improve the scientific knowledge about this species' ecology and therefore fill in knowledge gaps to understand how threats experienced during the non-breeding season might affect this species on population level (Fig. 6). Specifically, three ecological aspects can be termed as research topics, which are:

- Dietary ecology: Document diet composition (Chapter 1)
- Movement ecology: Determining migratory behaviour and site utilisation (Chapter 2)
- Disease ecology: Detection and identification of haemosporidian parasites (Chapter 3)

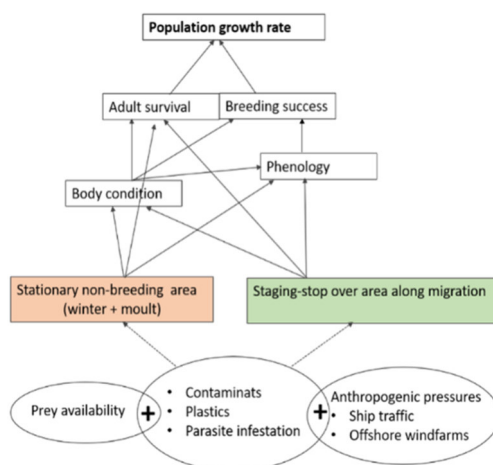


Figure 6 Conceptual links, represented by arrows, between potential threats experienced during migration and in the stationary non-breeding areas and variables that impact population growth rate. Figure modified from Strøm et al. 2021.

This cumulative thesis is structured in three chapters, where each chapter is based on an individual publication focussing on each of the behavioural-ecological aspects and applied methods with the following specific aims:

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

- Apply a non-invasive method to analyse diet composition to provide a detailed overview of dietary items of red-throated divers in the area of interest, the eastern German Bight
- Document the diversity of prey species eaten by the birds in the eastern German Bight when red-throated diver abundance is highest
- Compare data for two consecutive sampling years to determine if the prey species consumed are consistent between years
- Determine whether the abundance and distribution of prey fish correlate with red-throated diver diet and how this may help to explain red-throated diver distribution

Chapter 2. *Annual movements of a migratory seabird - the NW European red-throated diver (Gavia stellata) – reveals high individual repeatability but low migratory connectivity*

- Identify and temporally classify area use, migratory routes and phenology during the annual cycle of individuals captured in the eastern German Bight
- Determine the degree of migratory connectivity in terms of interpopulation mixing in stationary non-breeding areas and population spread of individuals from one shared breeding region
- Characterise fidelity and temporal consistency of site utilisation between consecutive years
- Reveal if the location of breeding areas (longitude/latitude) affects the timing and pattern of annual movements

Chapter 3. *Prevalence of haemosporidian parasites in an arctic breeding seabird species – the NW-European red-throated diver (Gavia stellata)*

- Analyse presence-absence of haemosporidian parasites in terms of detection and identification of these parasites using molecular tools
- Examine the prevalence and richness of haemosporidian parasites in red-throated divers
- Evaluate and discuss possible negative effects of haemosporidian parasites on red-throated divers in relation to anthropogenic pressures

4. CHAPTER OUTLINE

This thesis includes three studies that provide novel information on the behavioural-ecological aspects of the study species, the red-throated diver, in accordance with the main objective and specific aims mentioned above and presented in the chapters accordingly.

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

In chapter 1 I updated and categorised the diet composition of red-throated divers in the eastern German Bight. This area is characterised by increasing offshore wind farm installations which at the same time represents anthropogenic structures that cause avoidance reactions and a distribution shift. As a consequence, red-throated divers are displaced from originally used habitats into alternative habitats and accumulate in certain areas. Redistribution to alternative habitats can also mean changes in food availability. To better understand the effects, I examined the dietary composition of two consecutive years, compared the outcome to the available fish fauna and furthermore contributed to the already available studies on the diet of red-throated divers. Specifically, I documented the diversity of prey species eaten by the birds and whether the prey species spectrum is characterised by a specialised or generalised food choice in this particular area in a time when diver abundance is highest. In this context I revealed how the detected prey species spectrum relates to the availability of prey species. I also compared datasets of two years to see if the behaviour of one year was reflected in the other. I used faeces of 34 individual red-throated divers caught in 2015 and 2016 in the eastern German Bight and DNA metabarcoding as a non-invasive method. Red-throated divers sampled in this study displayed a generalised feeding behaviour with a broad prey spectrum that was dominated by five groups: clupeids, mackerel, gadoids, flatfish and sand lances with clupeids being the most frequently detected prey in both years. The results indicate that pelagic schooling fish that aggregate at frontal zones and have a high energetic value might be favoured. Furthermore, the generalised feeding behaviour and the importance of prey species with a high energetic value were consistent with previous studies. The results indicate some flexibility in food choice as long as suitable prey items with sufficient energetic values are available. If these prey species are not, or less available in changed habitats, displacement effects might lead to a decreased energy intake and subsequently poorer body condition.

Contributions: Lead author, sample collection and bird handling in the field (catching, sampling), data preparation including molecular work (DNA isolation and preparation of NGS libraries) and data analysis (bioinformatic sorting and statistical analyses of sequencing datasets).

Chapter 2: *Annual movements of a migratory seabird - the NW European red-throated diver (Gavia stellata) – reveals high individual repeatability but low migratory connectivity* (Accepted, July 2022)

In Chapter 2 I focussed on annual movements and site utilisation of red-throated divers that were captured and tagged in three consecutive years (2015, 2016, 2017) in the eastern German Bight. Data of 33 individuals

were used to identify breeding regions, migratory movements, migratory phenology and site utilisation within and between years. Additionally stable isotope analyses of feather samples ($\delta^{13}\text{C}$) were used to identify moult locations when no tracking data were available. To better assess the impact of increasing anthropogenic development in an important marine habitat, namely the eastern German Bight, in terms of possible carry-over effects and possible population level effects, I focussed on site utilisation during the stationary non-breeding periods (moult and winter) and applied kernel density estimates to obtain home range sizes during these periods. Furthermore, I analysed three behavioural-ecological aspects, which are the migratory connectivity, repeatability of annual movements and the relation between timing of annual movements and the breeding locations in the high Arctic. Breeding regions of individuals captured in the eastern German Bight were distributed over Greenland, Svalbard, Norway and northern Russia. Autumn migrations contained separate migration steps to moulting and wintering sites. Moult and wintering sites were distributed over the North and Baltic Sea and not all individuals returned to the eastern German Bight during wintering season, but used this site to refuel along migration. Tracking data of individuals from a shared breeding region (northern Russia) indicated a rather low connectivity. Although individuals moved largely along the same route, they dispersed during the stationary non-breeding period with only partly overlapping home ranges. The breeding longitude was related with arrival times at breeding sites with 40 days later arrival at the easternmost than westernmost breeding sites, suggesting a longitudinal gradient. Red-throated divers displayed repeatable movements and site utilisation between years indicating a high site fidelity and some temporal consistency. Implications related to the findings of the study and their significance are discussed. In summary, the data revealed novel and important ecological-behavioural aspects that provide a basis on which future conservation measures can be taken.

Contributions: Lead author, sample collection and bird handling in the field (catching, feather sampling), assistance in bird tagging (implantation of satellite tags), molecular work (sex determination), data and statistical analysis (tracking data, isotope data).

Chapter 3: *Prevalence of haemosporidian parasites in an arctic breeding seabird species – the NW-European red-throated diver (*Gavia stellata*)* (Prepared for submission)

In chapter 3 I analysed a potential stressor that has not yet been investigated for red-throated divers, namely the infestation with blood or haemosporidian parasites. An infestation with haemosporidian parasites in combination with increasing anthropogenic induced stress can be considered as potential pre-load and is, therefore, an important aspect which should be taken into account when evaluating cumulative effects. I used blood samples and molecular tools (nested PCR) to document (i) presence-absence of haemosporidian parasites (*Plasmodium*, *Haemoproteus* and *Leucocytozoon*) and (ii) prevalence and richness of these parasites in NW-European divers (n=45) captured in the eastern German Bight. I found haemosporidian infestation present in NW-European divers with a prevalence of 11 % (n=5) of which each individual was tested

positively for *Leucocytozoon* and one of these individuals showed a double infection of both genera *Plasmodium* and *Leucocytozoon*. A new lineage was found for both *Plasmodium* and *Leucocytozoon*, not documented so far. Prevalence was rather low in NW- European red-throated divers, whereas richness, the number of parasite species found in a host, was comparatively high considering that out of five infested individuals one showed a double infestation. The data obtained in this study describes for the first time the detection of haemosporidian parasites in red-throated divers and provide an important reference that can be used to monitor future parasite distribution. The low prevalence indicates low vulnerability of cumulative effects between anthropogenic pressures and haemosporidian infections, but should nevertheless be considered as an additional stressor, or pre-load in future conservation measures.

Contributions: Lead author, sample collection and bird handling in the field (catching, blood sampling), data preparation including molecular work (DNA isolation and PCR assays), partial data analysis of sequencing datasets resulting from Sanger sequencing

5. CONCLUDING REMARKS

In the present work, three behavioural-ecological aspects were investigated, each contributing to a better understanding of the ecology of the studied species, the red-throated diver. In addition, when combined, the results help to view and identify threats from a cumulative perspective, providing a solid foundation for future conservation strategies.

The first chapter identified the prey items consumed by red-throated divers in an internationally important non-breeding area, the eastern German Bight on a high resolution. Red-throated divers showed to be exclusively piscivorous and foraged on a broad prey spectrum, that contained a consistent presence of energetic rich prey species in two consecutive years, with clupeids as the most frequent species.

If the relative sequence counts generated by Next Generation Sequencing (NGS), reliably reflects the number of prey items consumed remains controversial (Pompanon et al. 2012, Deagle et al. 2013, Liu et al. 2021). Therefore, I cannot say which proportional part, i.e., how much of each prey fish was eaten exactly, but I can state how often the corresponding food fish was present in the sample size on the basis of the frequency of occurrence. Comparing prey consumption with monitoring reports about distributions of consumed prey revealed that some of these prey items reflect general fish availability and suggest red-throated divers to adjust their foraging to some degree in response to availability. The extent to which red-throated divers actually select food organisms, or opportunistically take the best of what is available cannot be conclusively clarified on the basis of these data. In the future, this information in addition with a quantitative analysis of consumed prey items would help to clarify the pattern of foraging behaviour that has emerged so far.

However, the continued presence of energetic rich prey consumed in non-breeding habitats, particularly clupeids demonstrated in this work and in other studies (e.g., Madsen 1957, Durinck et al. 1994, Guse et al.

2009), emphasizes the importance of the availability of these prey items. The eastern German Bight is characterised by a frontal system which attracts clupeid species and has already been suggested to explain red-throated diver abundance in relation to a suitable prey availability in these systems (Skov and Prins 2001, Trueman et al. 2017). My data confirm the consumption of these prey species and therefore highly support the link between red-throated diver abundance and prey availability in these systems. Therefore, predictability of suitable prey in this area might be an important element to explain diver abundance in the eastern part of the North Sea.

In terms of displacement effects, the broad prey spectrum indicates some flexibility to adapt to a changed prey availability when being displaced in altered habitats as long as high-energy species continue to be available. A possible resulting altered availability associated with lower availability of energy-rich food organisms may result in energy requirements not being met and consequences for body condition or staging time. Here the importance of looking at the complete picture becomes apparent, as the migratory data, which were addressed in the second chapter, showed that red-throated divers use many staging sites and seemed to refuel continuously along their migratory routes. Even if the effects in one habitat are only minor and can be compensated, effects in several habitats in total could possibly carry over to affect reproductive success and thus also have an effect on the population level.

Migratory movements and site utilisation along the annual cycle confirmed the eastern German Bight as an important stationary non-breeding site, although red-throated divers tagged in this area returned to varying degrees in the following seasons. Some individuals spent the whole moult and winter period in this area, some moulted there and continued migration to their final wintering destination and other individuals only stopped in this area during migration, indicating a somewhat high turnover rate in this area. Therefore, displacement effects caused by anthropogenic pressures in the eastern German Bight do not affect all individuals to the same extent. Special attention should be paid here to autumn moult, as red-throated divers then change their primary feathers and individuals are less mobile and might be more restricted to certain areas with favourable conditions (Stresemann and Stresemann 1966, Berndt and Drenkhahn 1974, Mendel et al. 2008).

The individual data from breeding regions other than northern Russia did not allow any precise statements due to the small sample size, therefore the statement regarding repeatability and migratory connectivity here apply to long distance migrants from northern Russia. The high site fidelity observed between two years in my studies and in combination with present publications on site fidelity in breeding areas (Okill 1992, Poessel et al. 2020), rather point to a general behavioural pattern of fidelity towards sites. In the context of habitat change and displacement effects an individual with a high site fidelity might be less flexible to voluntarily change sites or adapt more slowly to altered habitats compared to an individual that is used to explore new habitats (Catry et al. 2004, McFarlane et al. 2014, Merkel et al. 2021). Here the opportunistic diet spectrum indicates some flexibility to meet energy needs as long as energetic rich species

are available. Furthermore, the use of multiple core areas suggests some dispersal ability in response to small-scale displacement effects, while large-scale displacement effects may have a stronger influence. Although a congregation outside impacted areas might lead to other effects such as increased intraspecific competition that must also be considered for solitary species, like the red-throated diver. Here it should be taken into account that red-throated are long-lived species with delayed sexual maturity and small clutches and an impact may take some time to become apparent on the population level (Stinen et al. 2007). Considering the effect on population level the data revealed a high variability between individuals from northern Russia and thus a low migratory connectivity i.e., the degree to which individuals from one breeding region migrate and stay together during the non-breeding season (Webster 2002, Finch et al. 2017). If individuals from other regions such as short distance migrants from Norway display a different e.g., higher connectivity cannot be concluded on this data basis. However, the low connectivity and varying staging/stop-over locations, durations and travel times among individuals from northern Russia may indicate some resilience to displacement effects, because only a portion is affected, compared to if all individuals from a region were exposed to the same conditions (Finch et al. 2017). Nevertheless, the majority of individuals from northern Russia followed mainly a similar migratory route, crossing the Baltic Sea to and from breeding sites. From a cumulative perspective, it should be considered that different kind of impairments in several areas along this route could, however, affect several individuals of this breeding region and therefore affect them despite low connectivity as the spatial distribution of threats influence the magnitude of their population scale effects (Buchan et al. 2022). Future studies should consider spatial exposure to all individual threats and impairments to better assess whether threats are interactive or have cumulative effects (i.e., Kennedy et al. 2019, Mahon et al. 2019, Johnston et al. 2020, Buchan et al. 2022).

Winter habitat conditions are shown to influence migratory timing and energetic conditions (Bearhop et al. 2004, González-Prieto and Hobson 2013, Graham et al. 2016, Cohen et al. 2019). Migratory timing and staging behaviour of red-throated divers were correlated with location of breeding sites. At the individual and population level, the timing of spatiotemporal patterns during the annual cycle is critical, as a suboptimal pattern in timing could ultimately affect reproductive success and individual fitness (Marra et al. 1998, Alerstam 2011, McKinnon et al. 2015). In the case of red-throated divers, as a species breeding in arctic regions, a change in migration behaviour, e.g., a change in staging behaviour due to unfavourable winter conditions, such as less availability of energetic rich prey, and thus a change in arrival times, has an even more extreme effect due to a narrowed breeding window in the short arctic summer.

A potential additional stressor that has not yet been taken into account is the infestation with haemosporida, which is addressed in the third chapter. Parasite infections are thought to affect host phenology, movements and survival, but the extent and the mechanisms are not yet clear, as previous studies have found negative, positive, or no response at all (e.g., Hahn et al. 2018, Risely et al. 2018, Emmenberger et al. 2021). Beside blood pathology these parasites also damage various organs due to extensive exo-

erythrocytic development all over the body, which can cause severe diseases (Ilgūnas et al. 2016, Martinsen et al. 2017, Valkiūnas and Iezhova 2017). The identification of the haemosporidian parasites *Leucocytozoon* and *Plasmodium* presented in this thesis revealed that red-throated divers are infested at a low prevalence that can be considered rather insignificant in terms of potential pre-loads or stressors. Also, in combination with higher anthropogenic pressures and with previous results showing a fairly flexible diet spectrum as long as high-energy prey is available and highly variable movements among individuals from a given breeding area (Chapter 1 and 2), the low prevalence does not suggest additional impacts. However, further monitoring of infestation and prevalence should take into account expected future changes, or expansions of the transmission range due to climate change. Here, the data presented in this thesis provide an important first reference, but if additional suitable samples are available, parasitaemia should be analysed to complete the picture.

The studies compiled in this thesis provide important and novel information, contributing to a better general understanding of the behavioural and ecological aspects of the species studied. Furthermore, the findings help to better classify the effects of increasing anthropogenic pressures. However, the results of the present work have also led to follow-up questions that could not be answered within the scope of this thesis. More detailed aspects of foraging activity, migratory connections of individuals from respective other breeding regions and of short-distance migrants, as well as parasitaemia of haemosporida are further important aspects to be considered in order to provide an even more comprehensive basis for future-oriented conservation guidelines.

6. REFERENCES

- Ainley DG (1980) Birds as marine organisms: a review. CalCOFI Rep, 21:48-53
- Alerstam T and Högstedt G (1980) Spring predictability and leap-frog migration. – Ornithol Scand. 11:196–200
- Alerstam T and Bäckman J (2018) Ecology of animal migration. Current Biology, 28(17):968-972
- Alerstam T, Bäckman J, Grönroos J, Olofsson P, Strandberg R (2019) Hypotheses and tracking results about the longest migration: the case of the arctic tern. Ecol Evol 9: 9511–9531
- Alves JA, Gunnarsson TG, Hayhow DB, Appleton GF, Potts PM, Sutherland WJ, Gill JA (2013) Costs, benefits, and fitness consequences of different migratory strategies. Ecology 94:11–17
- Atkinson CT, Dusek RJ, Woods KL, Iko WM (2000) Pathogenicity of avian malaria in experimentally-infected Hawaii Amakihi. Journal of wildlife diseases, 36(2):197-201.
- Atkinson CT (2008) Avian malaria. In: Atkinson CT, Thomas NJ, Hunter DB (Eds) Parasitic diseases of wild birds. John Wiley & Sons
- Bearhop S, Hilton GM, Votier SC, Waldron S (2004) Stable isotope ratios indicate that body condition in migrating passerines is influenced by winter habitat. – Proc. R. Soc. B 271:215–218
- Berndt RK, Drenckhahn D. Vogelwelt Schleswig-Holsteins (1990) 1: Seetaucher bis Flamingo. Wachholtz/Neumünster
- Bellebaum J, Diederichs A, Kube J, Schulz A, Nehls G (2006) Flucht- und Meidedistanzen überwinternder Seetaucher und Meeressäuger gegenüber Schiffen auf See. Ornithologischer Rundbrief Mecklenburg-Vorpommern 45: 86-90.
- BirdLife International (2015) European Red List of Birds. Luxembourg: Office for Official Publications of the European Communities.
- BirdLife International (2022) Species factsheet: *Gavia stellata*. <http://www.birdlife.org>, accessed on 09/05/2022.
- Blackwell BF, DeVault TL, Fernández-Juricic E, Gese EM, Gilbert-Norton L, Breck SW (2016) No single solution: application of behavioural principles in mitigating human–wildlife conflict. Animal Behaviour 120:245-254.
- BMWi 2021 <https://www.bmwi.de/Redaktion/DE/Dossier/erneuerbare-energien>, accessed on 01/04/2022
- Boertmann D (1990). Phylogeny of the divers, family Gaviidae (Aves). Zoological Museum, University of Copenhagen.
- Briedis M, Hahn S, Gustafsson L, Henshaw I, Träff J, Král M, Adamík P (2016) Breeding latitude leads to different temporal but not spatial organization of the annual cycle in a long-distance migrant. Journal of Avian Biology 47(6):743-748
- Bridge ES, Thorup K, Bowlin MS, Chilson PB, Diehl RH, Fléron RW, Hartl P, Kays R, Kelly JF, W. Robinson D, Wikelski M (2011) Technology on the move: recent and forthcoming innovations for tracking migratory birds. BioScience 61(9):689-698.
- Brooke M (2004) Albatrosses and petrels across the world. Oxford, UK: Oxford University Press.
- Buchan C, Franco AM, Catry I, Gamero A, Klvaňová A, Gilroy JJ (2022) Spatially explicit risk mapping reveals direct anthropogenic impacts on migratory birds. Global Ecology and Biogeography. DOI: 10.1111/geb.13551
- Bundesamt für Seeschifffahrt und Hydrographie (BSH) und Bundesministerium für Umwelt, Naturschutz, Nukleare Sicherheit und Verbraucherschutz (BMU) (2014). Ecological Research at the Offshore

- Windfarm alpha ventus – Challenges, Results and Perspectives. Federal Maritime and Hydrographic Agency (BSH), Federal Ministry for the Environment, Nature Conservation and Nuclear Safety (BMU). Springer Spektrum.
- Bundesamt für Seeschifffahrt und Hydrographie (BSH) (Hrsg.) - BSH (2019): Flächenentwicklungsplan 2019 für die deutsche Nord- und Ostsee. Nr. BSH-Nummer 7608, Hamburg (DEU), S: 202.
- Bundesamt für Seeschifffahrt und Hydrographie (BSH) (2020). Environmental Report for the Site Development Plan 2020 for the German Baltic Sea (No. BSH-No. 7608). Hamburg: BSH.
- Burger C, Schubert A, Heinänen S, Dorsch M, Kleinschmidt B, Žydelis R, Morkūnas J, Quillfeldt P, Nehls G (2019) A novel approach for assessing effects of ship traffic on distributions and movements of seabirds. *Journal of environmental management*, 251. <https://doi.org/10.1016/j.jenvman.2019.109511>
- Burton NH, Rehfish MM, Clark NA, Dodd SG (2006) Impacts of sudden winter habitat loss on the body condition and survival of redshank *Tringa totanus*. *Journal of Applied Ecology* 43(3):464-473.
- Busch M, Kannen A, Garthe S, Jessopp M (2013) Consequences of a cumulative perspective on marine environmental impacts: offshore wind farming and seabirds at North Sea scale in context of the EU marine strategy framework directive. *Ocean Coast. Manag.* 71:213–224.
- Busch M and Garthe S (2016) Approaching population thresholds in presence of uncertainty: Assessing displacement of seabirds from offshore wind farms. *Environmental Impact Assessment Review* 56:31-42.
- Catry P, Encarnação V, Araújo A, Fearon P, Fearon A, Armelin M, Delaloye P (2004) Are long-distance migrant passerines faithful to their stopover sites?. *Journal of Avian Biology* 35:170–181.
- Catry P, Dias MP, Phillips RA, Granadeiro JP (2013) Carry-over effects from breeding modulate the annual cycle of a long-distance migrant: An experimental demonstration. *Ecology* 94(6):1230-1235.
- Clark NJ, Clegg SM, Lima MR (2014) A review of global diversity in avian haemosporidians (*Plasmodium* and *Haemoproteus*: Haemosporida): new insights from molecular data. *International journal for parasitology* 44(5):329-338.
- Clifton GT, Carr JA, Biewener AA (2018) Comparative hindlimb myology of foot-propelled swimming birds. *Journal of Anatomy* 232(1):105-123.
- Cohen EB, Rushing CR, Moore FR, Hallworth MT, Hostetler JA, Ramirez MG, Marra PP (2019) The strength of migratory connectivity for birds en route to breeding through the Gulf of Mexico. *Ecography* 42(4):658-669
- Conant B, Groves DJ (2005) Alaska–Yukon Waterfowl Breeding Population Survey, May 15 to June 7, US Fish and Wildlife Service Report, Juneau, AK, pp. 1–36
- Conklin JR, Battley PF, Potter MA, Fox JW (2010) Breeding latitude drives individual schedules in a trans-hemispheric migrant bird. *Nature Communications* 1(1):1-6
- Costantini D, Blévin P, Herzke D, Moe B, Gabrielsen GW, Bustnes JO, Chastel O (2019) Higher plasma oxidative damage and lower plasma antioxidant defences in an Arctic seabird exposed to longer perfluoroalkyl acids. *Environmental research*, 168:278-285
- Daunt F, Reed TE, Newell M, Burthe S, Phillips RA, Lewis S, Wanless S (2014) Longitudinal bio-logging reveals interplay between extrinsic and intrinsic carry-over effects in a long-lived vertebrate. *Ecology* 95:2077–2083
- Deagle BE, Gales NJ, Evans K, Jarman SN, Robinson S, Trebilco R, Hindell MA (2007) Studying seabird diet through genetic analysis of faeces: a case study on macaroni penguins (*Eudyptes chrysolophus*). *PLoS One* 2(9). <https://doi.org/10.1371/journal.pone.0000831>

- Deagle BE, Chiaradia A, McInnes J, Jarman SN (2010) Pyrosequencing faecal DNA to determine diet of little penguins: is what goes in what comes out?. *Conservation Genetics* 11(5):2039-2048.
- Deagle BE, Thomas AC, Shaffer AK, Trites AW, Jarman SN (2013) Quantifying sequence proportions in a DNA-based diet study using Ion Torrent amplicon sequencing: Which counts count? *Mol. Ecol. Resour.* 13:620–633
- DeGroot LW, Rodewald PG (2010) Blood parasites in migrating wood-warblers (Parulidae): effects on refuelling, energetic condition, and migration timing. *Journal of Avian Biology* 41(2):147-153
- Deutsche Windguard (2021): Status des Offshore-Windenergieausbaus in Deutschland Jahr 2021. <http://www.winguard.de>, accessed on 01/04/2022
- Dierschke V, Garthe S, Mendel B (2006) Possible conflicts between offshore wind farms and seabirds in the German sectors of North Sea and Baltic Sea. In: Köller J, Köppel J, Peters W (eds) *Offshore Wind Energy*. Springer, Berlin, Heidelberg . https://doi.org/10.1007/978-3-540-34677-7_10.
- Dierschke V, Exo KM, Mendel B, Garthe S, (2012) Gefährdung von Sterntaucher *Gavia stellata* und Prachtaucher *G. arctica* in Brut-, Zug- und Überwinterungsgebieten—eine Übersicht mit Schwerpunkt auf den deutschen Meeresgebieten. *Vogelwelt* 133:163–194
- Dierschke V, Furness RW, Garthe S (2016) Seabirds and offshore wind farms in European waters: Avoidance and attraction. *Biological Conservation* 202:59-68
- Dierschke V, Furness RW, Gray CE, Petersen IK, Schmutz J, Zydels R, Daunt F (2017) Possible Behavioural, Energetic and Demographic Effects of Displacement of Red-throated Divers. JNCC Report 605, U.K. Joint Nature Conservation Committee, Peterborough
- Dingle H, Drake VA (2007) What is migration?. *Bioscience* 57(2):113-121
- Dunn RE, Wanless S, Daunt F, Harris MP, Green JA (2020) A year in the life of a North Atlantic seabird: behavioural and energetic adjustments during the annual cycle. *Scientific reports* 10(1):1-11.
- Durinck J, Skov H, Danielsen F, Christensen KD (1994) Vinterføden hos Rødstrubet Lom *Gavia stellata* i Skagerrak. *Dansk Ornithol Foren Tidsskr* 88:39–41
- Egizi A, Martinsen ES, Vuong H, Zimmerman KI, Faraji A, Fonseca DM (2018) Using bloodmeal analysis to assess disease risk to wildlife at the new northern limit of a mosquito species. *EcoHealth*, 15(3):543-554
- Ellis JC, Fariña JM, Witman JD, (2006) Nutrient transfer from sea to land: the case of 589 gulls and cormorants in the Gulf of Maine. *J. Anim. Ecol.* 75:565–574
- Emmenegger T, Bensch S, Hahn S, Kishkinev D, Procházka P, Zehntindjiev, P, Bauer S (2021) Effects of blood parasite infections on spatiotemporal migration patterns and activity budgets in a long-distance migratory passerine. *Ecology and evolution* 11(2):753-762.
- Eriksson MOG, Blomqvist D, Hake M, Johansson OC (1990) Parental feeding in the Red-throated Diver *Gavia stellata*. *Ibis* 132:1–13.
- Eriksson MO (1994) Susceptibility to freshwater acidification by two species of loon: Red-throated Loon (*Gavia stellata*) and Arctic Loon (*Gavia arctica*) in southwest Sweden. In: Kerekes JJ (eds) *Aquatic Birds in the Trophic Web of Lakes*. Developments in Hydrobiology, 96. Springer, Dordrecht. https://doi.org/10.1007/978-94-011-1128-7_40.
- Fecchio A, Bell JA, Bosholn M, Vaughan JA, Tkach VV, Lutz HL, Cueto VR, Gorosito CA, González-Acuña D, Stromlund C, Kvasager D, Comiche KJM, Kirchgatter K, Pinho JB, Berv J, Anciães M, Fontana CS, Zyskowski K, Sampaio S, Dispoto JH, Galen SC, Weckstein JD, Nicholas J, Clark NJ (2020) An inverse latitudinal gradient in infection probability and phylogenetic diversity for *Leucocytozoon* blood parasites in New World birds. *Journal of Animal Ecology* 89(2):423-435

- Finch T, Butler SJ, Franco AM, Cresswell W (2017) Low migratory connectivity is common in long-distance migrant birds. *Journal of Animal Ecology* 86(3):662-673
- Fliessbach KL, Borkenhagen K, Guse N, Markones N, Schwemmer P, Garthe S (2019) A ship traffic disturbance vulnerability index for Northwest European seabirds as a tool for marine spatial planning. *Frontiers in Marine Science* 6. <https://doi.org/10.3389/fmars.2019.00192>.
- Furness RW, Monaghan P (1987) *Seabird Ecology*. Blackie, Glasgow.
- Furness RW, Crane JE, Bearhop S, Garthe S, Käkälä A, Käkälä R, Kelly A, Kubetzki U, Votier S, Waldron S (2006) Techniques to link individual migration patterns of seabirds with diet specialization, condition and breeding performance. *Ardea* 94(3):631-638
- Furness RW, Wade HM, Masden EA (2013) Assessing vulnerability of marine bird populations to offshore wind farms. *Journal of environmental management* 119:56-66
- Furness RW, Wade HM, Masden EA (2013) Assessing vulnerability of marine bird populations to offshore wind farms. *Journal of Environmental Management* 119:56-66. DOI: 10.1016/j.jenvman.2013.01.025
- Garthe S, Camphuysen CJK, Furness RW (1996) Amounts of discards by commercial fisheries and their significance as food for seabirds in the North Sea. *Mar Ecol Prog Ser* 136: 1-11
- Garthe S, Hüppop O (2004) Scaling possible adverse effects of marine wind farms on seabirds: developing and applying a vulnerability index. *Journal of applied Ecology* 41(4):724-734
- Garthe S, Schwemmer H, Markones N, Müller S, Schwemmer P (2015) Verbreitung, Jahresdynamik und Bestandsentwicklung der Seetaucher *Gavia spec.* in der Deutschen Bucht (Nordsee). *Vogelwarte* 53:121-138
- Garvin MC, Marra PP, Crain SK (2004) Prevalence of hematozoa in overwintering American Redstarts (*Setophaga ruticilla*): no evidence for local transmission. *Journal of wildlife diseases* 40(1):115-118
- Garvin MC, Szell CC, Moore FR (2006) Blood parasites of nearctic-neotropical migrant passerine birds during spring trans-gulf migration: impact on host body condition. *Journal of Parasitology* 92(5):990-996
- González-Prieto AM, Hobson KA (2013) Environmental conditions on wintering grounds and during migration influence spring nutritional condition and arrival phenology of Neotropical migrants at a northern stopover site. – *J. Ornithol.* 154:1067-1078
- González-Solís J, Croxall JP, Oro D, Ruiz X (2007) Trans-equatorial migration and mixing in the wintering areas of a pelagic seabird. *Frontiers in Ecology and the Environment* 5(6):297-301
- Gordo O (2007) Why are bird migration dates shifting? A review of weather and climate effects on avian migratory phenology. *Climate research* 35(1-2):37-58
- Gray CE, Paruk JD, DeSorbo CR, Savoy LJ, Yates DE, Chickering MD, Gray RB, Taylor KM, Long D, Schoch N, Hanson W, Cooley J, Evers DC (2014) Body mass in Common Loons (*Gavia immer*) strongly associated with migration distance. *Waterbirds* 37(sp1):64-75
- Grémillet D, Boulinier T (2009) Spatial ecology and conservation of seabirds facing global climate change: a review. *Marine Ecology Progress Series* 391:121-137
- Groves DJ, Conant B, King RJ, Hodges JJ, King JG (1996) Status and trends of loon populations summering in Alaska 1971-1993. *Condor* 98:189-195
- Guse N, Garthe S, Schirmeister B (2009) Diet of red-throated divers *Gavia stellata* reflects the seasonal availability of Atlantic herring *Clupea harengus* in the southwestern Baltic Sea. *J Sea Res* 62(4):268-275

- Hahn S, Bauer S, Dimitrov D, Emmenegger T, Ivanova K, Zehtindjiev P, Buttemer WA (2018) Low intensity blood parasite infections do not reduce the aerobic performance of migratory birds. *Proceedings of the Royal Society B: Biological Sciences* 285(1871). <https://doi.org/10.1098/rspb.2017.2307>
- Hamer KC, Schreiber EA, Burger J (2002) Breeding biology, life histories, and life history-environment interactions in seabirds. In: Schreiber EA, Burger J (eds) *Biology of marine birds*. CRC Press, Boca Raton, FL
- Harrison XA, Blount JD, Inger R, Norris DR, Bearhop S (2011) Carry-over effects as drivers of fitness differences in animals. *Journal of Animal Ecology* 80(1):4-18
- Harrison AL, Costa DP, Winship AJ, Benson SR and others (2018) The political biogeography of migratory marine predators. *Nat Ecol Evol* 2:1571–1578
- Heinänen S, Žydelis R, Kleinschmidt B, Dorsch M, Burger C, Morkūnas J, Quillfeldt P, Nehls G (2020) Satellite telemetry and digital aerial surveys show strong displacement of red-throated divers (*Gavia stellata*) from offshore wind farms. *Marine environmental research* 160. <https://doi.org/10.1016/j.marenvres.2020.104989>.
- Herrera J, Nunn CL (2019) Behavioural ecology and infectious disease: implications for conservation of biodiversity. *Philosophical Transactions of the Royal Society B* 374(1781). <https://doi.org/10.1098/rstb.2018.0054>.
- Hill AG, Howe L, Gartrell BD, Alley MR (2010) Prevalence of *Leucocytozoon spp.* in the endangered yellow-eyed penguin *Megadyptes antipodes*. *Parasitology* 137(10):1477-1485
- Hobson KA, Norris DR, Kardynal KJ, Yohannes E (2019) Animal migration: a context for using new techniques and approaches. In: Keith A. Hobson, Leonard I. Wassenaar (Eds) *Tracking Animal Migration with Stable Isotopes* (Second Edition), Academic Press.
- Ilgūnas M, Bukauskaitė D, Palinauskas V, Iezhova TA, Dinopl N, Nedorost N, Weissenbacher-Lang C, Weissenböck H, Valkiūnas G (2016) Mortality and pathology in birds due to Plasmodium (Giovannolaia) homocircumflexum infection, with emphasis on the exoerythrocytic development of avian malaria parasites. *Malaria Journal* 15(1):1-11.
- Jiménez-Peñuela J (2021) Impact of landscape anthropization on pathogen transmission dynamics and wild birds health. PhD Thesis. Universidad de Sevilla, Seville, Spain.
- Johnsgaard P (1987) *Diving birds of North America*. University of Nebraska–Lincoln Libraries
- Johnston A, Auer T, Fink D, Strimas-Mackey M, Iliff M, Rosenberg KV, Brown S, Lancot R, Rodewald AD, Kelling S (2020) Comparing abundance distributions and range maps in spatial conservation planning for migratory species. *Ecological Applications* 30(3). <https://doi.org/10.1002/eap.2058>.
- Khan JS, Provencher JF, Forbes MR, Mallory ML, Lebarbenchon C, McCoy KD (2019) Parasites of seabirds: A survey of effects and ecological implications. *Advances in Marine Biology* 82:1-50.
- Kelsey EC, Felis JJ, Czapanskiy M, Pereksta DM, Adams J (2018) Collision and displacement vulnerability to offshore wind energy infrastructure among marine birds of the Pacific Outer Continental Shelf. *Journal of environmental management* 227:229-247.
- Kennedy CM, Oakleaf JR, Theobald DM, Baruch-Mordo S, Kiesecker J (2019) Managing the middle: A shift in conservation priorities based on the global human modification gradient. *Global Change Biology* 25(3):811–826
- Kolb GS, Ekholm J, Hambäck PA, (2010) Effects of seabird nesting colonies on algae 650 and aquatic invertebrates in coastal waters. *Mar. Ecol. Prog. Ser.* 417:287–300

- Kumar MA, Kumar D, Palanivelu M, Annamalai L, Mathesh K, Singh R, Kumar Sharma A, Dhama K (2018) Pathological and molecular studies of the renal trematode *Paratanaisia bragai* in Indian peafowls (*Pavo cristatus*). *Acta Parasitologica* 63(1):214-219.
- Lewison R, Oro D, Godley BJ, Underhill L, Bearhop S, Wilson RP, Ainley D, Arcos JM, Boersma PD, Borboroglu PG, Boulinier T, Frederiksen M, Genovart M, González-Solís J, Green JA, Grémillet D, Hamer KC, Hilton GM, Hyrenbach KD, Martínez-Abraín A, Montevecchi WA, Phillips RA, Ryan PG, Sagar P, Sydeman WJ, Wanless Watanuki SY, Weimerskirch H, Yorio P (2012) Research priorities for seabirds: improving conservation and management in the 21st century. *Endangered Species Research* 17(2):93-121
- Liu G, Shafer ABA, Hu X, Li L, Ning Y, Gong M, Cui L, Li H, Hu D, Qi L, Tian H, Wang B. (2018) Metabarcoding insights into the spatial and temporal dietary patterns of the threatened Asian Great Bustard (*Otis tarda dybowskii*) with potential implications for diverging migratory strategies. *Ecol. Evol.* 8:1736–1745
- Liu G, Zhang S, Zhao X, Li C, Gong M (2021) Advances and Limitations of Next Generation Sequencing in Animal Diet Analysis. *Genes* 12(12):1-15
- Lovas-Kiss Á, Sánchez MI, Wilkinson DM, Coughlan NE, Alves JA, Green AJ (2019) Shorebirds as important vectors for plant dispersal in Europe. *Ecography* 42 (5):956–967
- Lundberg J, Moberg F (2003) Mobile link organisms and ecosystem functioning: Implications for ecosystem resilience and management. *Ecosystems* 6:87–98
- Madsen FJ (1957) On the food habits of some fish-eating birds in Denmark. Divers, grebes, mergansers, and auks. *Dan Rev Game Biol* 3:19–83
- Mahon CL, Holloway GL, Bayne EM, Toms JD (2019) Additive and interactive cumulative effects on boreal landbirds: Winners and losers in a multi-stressor landscape. *Ecological Applications* 29(5). <https://doi.org/10.1002/eap.1895>
- Rutkowska M, Bajger-Nowak G, Kowalewska D, Bzoma S, Kalisińska E, Namieśnik J, Konieczka P (2019) Methylmercury and total mercury content in soft tissues of two bird species wintering in the Baltic Sea near Gdansk, Poland. *Chemosphere* 219:140-147,
- Mandal FB (2021) Hurdle in taxonomy: A case of malaria parasites and other Haemosporidia. *Open Veterinary Science* 2(1):40-54
- Marra PP, Hobson KA, Holmes RT (1998) Linking winter and summer events in a migratory bird by using stable-carbon isotopes. *Science* 282(5395):1884-1886
- Martinsen ES, Sidor IF, Flint S, Cooley J, Pokras MA (2017) Documentation of malaria parasite (*Plasmodium spp.*) infection and associated mortality in a common loon (*Gavia immer*). *Journal of wildlife diseases* 53(4):859-863
- Masden EA, Haydon DT, Fox AD, Furness RW (2010) Barriers to movement: modelling energetic costs of avoiding marine wind farms amongst breeding seabirds. *Marine Pollution Bulletin* 60(7):1085-1091
- McElroy EJ, De Buron I (2014) Host performance as a target of manipulation by parasites: a meta-analysis. *The Journal of Parasitology* 100(4):399-410
- McFarlane Tranquilla LA, Montevecchi WA, Fifield DA, Hedd A, Gaston AJ, Robertson GJ, Phillips RA (2014) Individual winter movement strategies in two species of murre (*Uria spp.*) in the Northwest Atlantic. *PLoS One*, 9(4). <https://doi.org/10.1371/journal.pone.0090583>.
- McKinnon EA, Stanley CQ, Stutchbury BJM (2015) Carry-over effects of nonbreeding habitat on start-to-finish spring migration performance of a songbird. – *PLoS One* 10. <https://doi.org/10.1371/journal.pone.0141580>.

- Meier RE, Votier SC, Wynn RB, Guilford T, McMinin Grivé M, Rodríguez A, ... Newton J, Maurice L, Chouvelon T, Dessier A, Trueman CN (2017) Tracking, feather moult and stable isotopes reveal foraging behaviour of a critically endangered seabird during the non-breeding season. *Diversity and Distributions* 23(2):130-145
- Mendel B, Sonntag N, Wahl J, Schwemmer P, Dries H, Guse N, Müller S, Garthe S (2008) Artensteckbriefe von See- und Wasservögeln der deutschen Nord- und Ostsee: Verbreitung, Ökologie und Empfindlichkeiten gegenüber Eingriffen in ihrem marinen Lebensraum. *Naturschutz und Biologische Vielfalt* Nr. 59, Bundesamt für Naturschutz/Bonn-Bad Godesberg (DEU).
- Mendel B, Schwemmer P, Peschko V, Müller S, Schwemmer H, Mercker M, Garthe S (2019) Operational offshore wind farms and associated ship traffic cause profound changes in distribution patterns of Loons (*Gavia spp.*). *Journal of environmental management* 231:429-438
- Merkel B, Descamps S, Yoccoz NG, Grémillet D, Daunt F, Erikstad KE, Ezhov AV, Harris MP, Gavrilov M, Lorentsen SH, Reiertsen TK, Steen H, Systad GH, Þórarinnsson PL, Wanless S, Strøm H (2021) Individual migration strategy fidelity but no habitat specialization in two congeneric seabirds. *Journal of Biogeography* 48(2):263-275
- Møller AP, Erritzøe J (1998) Host immune defence and migration in birds. *Evolutionary Ecology* 12(8):945-953
- Moon JI, Park JG, Hur S, Kim YK, Nam DH, Lee DH (2018) Mitochondrial genome of the black-throated loon, *Gavia arctica* (Gaviiformes: Gaviidae): phylogeny and evolutionary history. *Mitochondrial DNA Part B*, 3(2):586-587
- Nathan R, Getz WM, Revilla E, Holyoak M, Kadmon R, Saltz D, Smouse PE (2008) A movement ecology paradigm for unifying organismal movement research. *Proceedings of the National Academy of Sciences* 105(49):19052-19059
- Newton I (1998) Population limitation in birds. Academic press.
- Newton I (2007) Population limitation in birds: the last 100 years. *British birds* 100:518-539
- Newton I (2007) The migration ecology of birds. Elsevier, San Diego (US)
- Newton I (2011) Migration within the annual cycle: species, sex and age differences. *Journal of Ornithology* 152(1):169-185
- Nielsen JM, Clare EL, Hayden B, Brett MT, Kratina P (2018) Diet tracing in ecology: Method comparison and selection. *Methods in Ecology and Evolution* 9(2):278-291
- Oehm J, Thalinger B, Eisenkölbl S, Traugott M (2017) Diet analysis in piscivorous birds: What can the addition of molecular tools offer?. *Ecology and Evolution* 7(6):1984-1995
- Okill JD (1992) Natal dispersal and breeding site fidelity of red-throated Divers *Gavia stellata* in Shetland. *Ringling & Migration* 13(1):57-58
- Okill JD (1994) Ringling recoveries of red-throated divers *Gavia stellata* in Britain and Ireland. *Ringling & Migration* 15(2):107-118
- Okulewicz A (1989) Nematoda of the gastrointestinal system of *Gavia stellata* and *Gavia arctica* (Gaviidae). *Wiadomosci Parazytologiczne* 35(1):35-42
- Palinauskas V, Puente JMDL, Hernández-Soto SR, Marzal A (2020) Experimental parasitology and ecoimmunology: concepts and opportunities in avian haemosporidian studies. In: Santiago-Alarcon D, Marzal A (eds) *Avian Malaria and Related Parasites in the Tropics*. Springer, Cham. https://doi.org/10.1007/978-3-030-51633-8_17

- Paruk JD, Mager JM III, Evers DC (2014) Introduction: an overview of loon research and conservation in North America. *Waterbirds* 37:1–5
- Pelletier D, Seyer Y, Garthe S, Bonnefoi S, Phillips RA, Guillemette M (2020) So far, so good... Similar fitness consequences and overall energetic costs for short and longdistance migrants in a seabird. *PLOS ONE* 15. <https://doi.org/10.1371/journal.pone.0230262>
- Petersen JK, Malm T (2006) Offshore windmill farms: threats to or possibilities for the marine environment. *AMBIO: A Journal of the Human Environment* 35(2):75-80
- Petersen IK, Christensen KC, Kahlert J, Desholm MM, Fox AD (2006) Final results of bird studies at the offshore wind farms at Nysted and Horns Rev, Denmark. National Environmental Research Institute (NERI), Aarhus University/Aarhus (DNK), Commissioned by DONG energy and Vattenfall A/S.
- Petersen IK, Nielsen RD, Mackenzie ML (2014) Post-construction evaluation of bird abundances and distributions in the Horns Rev 2 offshore wind farm area, 2011 and 2012. Aarhus University, DCE – Danish Centre for Environment and Energy/ST. Andrews (UK), Report commissioned by DONG Energy.
- Phillips RA, Bearhop S, McGill RA, Dawson DA (2009) Stable isotopes reveal individual variation in migration strategies and habitat preferences in a suite of seabirds during the nonbreeding period. *Oecologia* 160(4):795-806
- Phillips RA, Lewis S, González-Solís J, Daunt F (2017) Causes and consequences of individual variability and specialization in foraging and migration strategies of seabirds. *Marine Ecology Progress Series* 578:117-150
- Piper WH, Walcott C, Mager JN, Spilker FJ (2008) Fatal battles in common loons: a preliminary analysis, *Anim. Behav.* 75:1109–1115
- Poessel SA, Uher-Koch BD, Pearce JM, Schmutz JA, Harrison AL, Douglas DC, Biela VR, Katzner, TE (2020) Movements and habitat use of loons for assessment of conservation buffer zones in the Arctic Coastal Plain of northern Alaska. *Global Ecology and Conservation* 22. <https://doi.org/10.1016/j.gecco.2020.e00980>.
- Pompanon F, Deagle BE, Symondson WO, Brown DS, Jarman SN, Taberlet P (2012) Who is eating what: diet assessment using next generation sequencing. *Molecular ecology* 21(8):1931-1950
- Quillfeldt P, Martínez J, Hennicke J, Ludynia K, Gladbach A, Masello JF, Riou S, Merino S (2010). Hemsporidian blood parasites in seabirds—a comparative genetic study of species from Antarctic to tropical habitats. *Naturwissenschaften* 97(9):809-817
- Quillfeldt P, Arriero E, Martínez J, Masello JF, Merino S (2011) Prevalence of blood parasites in seabirds-a review. *Frontiers in Zoology*, 8(1):1-11
- Rappole J (2013) The avian migrant. In *The Avian Migrant*. Columbia University Press.
- Raubenheimer D, Simpson SJ (2018) Nutritional ecology and foraging theory. *Curr. Opin. Insect. Sci.* 27:38–45
- Reed JM (2002) Animal behaviour as a tool in conservation biology. In: Aguirre AA, Ostfeld RS, Tabor GM, House C, Pearl MC (Eds) *Conservation medicine. Ecological health in practice*. Pp 145 - 166
- Reiertsen TK, Erikstad KE, Anker-Nilssen T, Barrett RT and others (2014) Prey density in non-breeding areas affects adult survival of black-legged kittiwakes *Rissa tridactyla*. *Mar Ecol Prog Ser* 509:289–302
- Reimchen TE, Douglas S (1984) Feeding schedule and daily food consumption in Red-throated Loons (*Gavia stellata*) over the pre fledging period. *The Auk* 101(3):593-599.

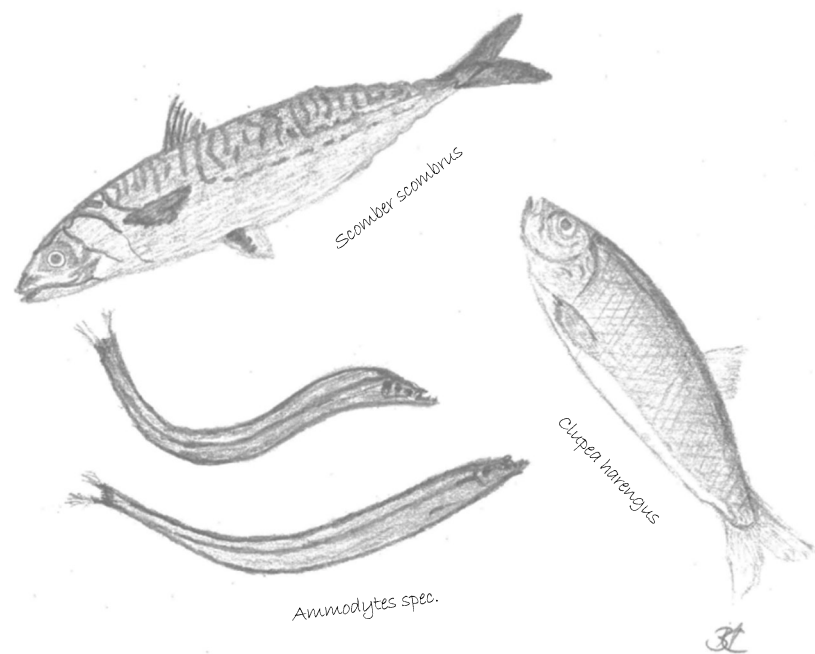
- Risely A, Klaassen M, Hoyer BJ (2018) Migratory animals feel the cost of getting sick: A meta-analysis across species. *Journal of Animal Ecology* 87(1):301-314
- Rizzolo DJ, Schmutz JA, Speakman JR (2015) Fast and efficient: Postnatal growth and energy expenditure in an Arctic-breeding waterbird, the Red-throated Loon (*Gavia stellata*). *The Auk: Ornithological Advances* 132(3):657-670
- Robinson WD, Bowlin MS, Bisson I, Shamoun-Baranes J, Thorup K, Diehl RH, ... Kunz TH, Mabey S, Winkler DW (2010) Integrating concepts and technologies to advance the study of bird migration. *Frontiers in Ecology and the Environment* 8(7):354-361
- Rochlin I, Ninivaggi DV, Hutchinson ML, Farajollahi A (2013) Climate change and range expansion of the Asian tiger mosquito (*Aedes albopictus*) in Northeastern USA: implications for public health practitioners. *PLoS ONE* 8. <https://doi.org/10.1371/journal.pone.0060874>.
- Rubarth J, Dreyer A, Guse N, Einax JW, Ebinghaus R (2011) Perfluorinated compounds in red-throated divers from the German Baltic Sea: new findings from their distribution in 10 different tissues. *Environmental Chemistry* 8(4):419-428
- Ruthrauff DR, Tibbitts TL, Gill Jr RE (2019) Flexible timing of annual movements across consistently used sites by Marbled Godwits breeding in Alaska. *The Auk: Ornithological Advances* 136(1). <https://doi.org/10.1093/auk/uky007>
- Rushing CS, Ryder TB, Marra PP (2016) Quantifying drivers of population dynamics for a migratory bird throughout the annual cycle. *Proceedings of the Royal Society B: Biological Sciences* 283(1823). <https://doi.org/10.1098/rspb.2015.2846>.
- Santiago-Alarcon D, Marzal A (2020) Avian Malaria and Related Parasites in the Tropics. Springer Nature Switzerland AG
- Schmaljohann H, Eikenaar C (2017) How do energy stores and changes in these affect departure decisions by migratory birds? A critical view on stopover ecology studies and some future perspectives. *Journal of Comparative Physiology A* 203(6):411-429
- Schmutz JA, Trust KA, Matz AC (2009) Red-throated loons (*Gavia stellata*) breeding in Alaska, USA, are exposed to PCBs while on their Asian wintering grounds. *Environmental Pollution* 157(8-9): 2386-2393
- Schoenle LA, Kernbach M, Haussmann MF, Bonier F, Moore IT (2017) An experimental test of the physiological consequences of avian malaria infection. *Journal of Animal Ecology* 86(6):1483-1496
- Schrader MS, Walters EL, James FC, Greiner EC (2003) Seasonal prevalence of a haematozoan parasite of red-bellied woodpeckers (*Melanerpes carolinus*) and its association with host condition and overwinter survival. *The Auk* 120(1):130-137
- Schreiber EA, Burger J (2002) Biology of marine birds. CRC Press, Boca Raton, FL
- Schultner J, Moe B, Chastel O, Bech C, Kitaysky AS (2014) Migration and stress during reproduction govern telomere dynamics in a seabird. *Biol Lett* 10. <https://doi.org/10.1098/rsbl.2013.0889>
- Schwemmer H, Markones N, Müller S, Borkenhagen K, Mercker M, Garthe S (2019) Aktuelle Bestandsgröße und -entwicklung des Sterntauchers (*Gavia stellata*) in der deutschen Nordsee. Forschungs- und Technologiezentrum Westküste (FTZ), Büsum, Christian-Albrechts- Universität zu Kiel, Büro für Biostatistik in der Ökologie und Biomedizin (BIONUM), Hamburg, Bericht für das Bundesamt für Seeschifffahrt und Hydrographie und das Bundesamt für Naturschutz.
- Sekercioglu CH (2006) Increasing awareness of avian ecological function. *Trends Ecol. Evol.* 21:464–71

- Signa G, Mazzola A, Vizzini S (2021) Seabird influence on ecological processes in coastal marine ecosystems: An overlooked role? A critical review. *Estuarine, Coastal and Shelf Science* 250. <https://doi.org/10.1016/j.ecss.2020.107164>
- Skov H, Durinck J, Leopold MF, Tasker ML (1995) Important bird areas in the North Sea, including the Channel and the Kattegat. Cambridge: BirdLife International - ISBN 9780903138833.
- Skov H, Prins E (2001) Impact of estuarine fronts on the dispersal of piscivorous birds in the German Bight. *Marine Ecology Progress Series* 214:279–287
- Spiegel O, Leu ST, Bull CM, Sih A (2017) What's your move? Movement as a link between personality and spatial dynamics in animal populations. *Ecology letters* 20(1):3-18
- Sprengelmeyer QD (2014) A phylogenetic reevaluation of the genus *Gavia* (Aves: Gaviiformes) using next-generation sequencing. Master Thesis, Northern Michigan University
- Stienen EW, Van Waeyenberge J, Kuijken ECKHART, Seys J (2007) Trapped within the corridor of the Southern North Sea: the potential impact of offshore wind farms on seabirds. In: de Lucas M, Janss G, Ferrer M (Eds) *Birds and Wind Farms: Risk Assessment and Mitigation*. Madrid: Quercus/Libreria Linneo. Birds and wind farms. Risk assessment and mitigation. 1st ed. Madrid: Quercus, 71-80.
- Stresemann E, Stresemann V (1966) Die Mauser der Vögel. *Journal für Ornithologie* 107:3–448
- Strøm H, Descamps S, Ekker M, Fauchald P, Moe B (2021) Tracking the movements of North Atlantic seabirds: steps towards a better understanding of population dynamics and marine ecosystem conservation. *Marine Ecology Progress Series*, 676:97-116
- Swift JF, Lance RF, Guan X, Britzke ER, Lindsay DL, Edwards CE (2018) Multifaceted DNA metabarcoding: Validation of a noninvasive, next-generation approach to studying bat populations. *Evolutionary Applications*, 11(7):1120-1138
- Szoboszlai AI, Thayer JA, Wood SA, Sydeman WJ, Koehn LE (2015) Forage species in predator diets: Synthesis of data from the California Current. *Ecol. Inform.* 29:45–56
- Tanaka K, Takada H, Yamashita R, Mizukawa K, Fukuwaka MA, Watanuki Y (2013) Accumulation of plastic-derived chemicals in tissues of seabirds ingesting marine plastics. *Mar. Pollut. Bull.* 69(1–2):219–222
- Valentini A, Miquel C, Nawaz MA, Bellemain EVA, Coissac E, Pompanon F, Gielly L, Cruaud C, Nascetti G, Wincker P, Swenson JE, Taberlet P (2009) New perspectives in diet analysis based on DNA barcoding and parallel pyrosequencing: the trnL approach. *Molecular ecology resources* 9(1):51-60
- Valkiūnas G (2004) *Avian malaria parasites and other haemosporidia*. CRC press.
- Valkiūnas G, Iezhova TA (2017) Exo-erythrocytic development of avian malaria and related haemosporidian parasites. *Malaria Journal* 16(1):1-24
- Valkiūnas G, Atkinson CT (2020) Introduction to life cycles, taxonomy, distribution, and basic research techniques. In: Santiago-Alarcon D, Marzal A (eds) *Avian malaria and related parasites in the Tropics*. Springer, Cham. https://doi.org/10.1007/978-3-030-51633-8_2.
- van Wijk RE, Bauer S, Schaub M (2016) Repeatability of individual migration routes, wintering sites, and timing in a long-distance migrant bird. *Ecology and Evolution* 6(24):8679-8685
- Vilela R, Burger C, Diederichs A, Bachl FE, Szostek L, Freund A, Braasch A, Bellebaum J, Beckers B, Piper W, Nehls G (2021) Use of an INLA Latent Gaussian Modeling Approach to Assess Bird Population Changes Due to the Development of Offshore Wind Farms. *Frontiers in Marine Science*. 8:701332. <https://doi.org/10.3389/fmars.2021.701332>.

- Weber TP, Ens BJ, Houston AI (1998) Optimal avian migration: a dynamic model of fuel stores and site use. *Evolutionary Ecology* 12(4):377-401
- Webster MS, Marra PP, Haig SM, Bensch S, Holmes RT (2002) Links between worlds: unraveling migratory connectivity. *Trends in ecology & evolution* 17(2):76-83
- Weimerskirch H (2002) Seabird demography and its relationship with the marine environment. In: Schreiber EA, Burger J (eds) *Biology of marine birds*. CRC Press, Boca Raton, FL.
- Weinandt ML (2006) Conservation implications of common loon (*Gavia immer*) parasites: black flies, haematozoans, and the role of mercury. Thesis, Northern Michigan University.
- Weinandt ML, Meyer M, Strand M, Lindsay AR (2012) Cues used by the black fly, *Simulium annulus*, for attraction to the common loon (*Gavia immer*). *Journal of Vector Ecology* 37(2):359-364
- Wetlands International. 2015. Waterbird Population Estimates. Available at: wpe.wetlands.org. Accessed on 17/09/2015.
- Wetlands International (2022) “Waterbird Populations Portal”. Available at wpp.wetlands.org. Accessed on 09/05/2022.
- Winkler D, Jørgensen C, Both C, Houston AI, McNamara JM, Levey DJ, Partecke J, Fudickar A, Kacelnik A, Roshier D, Piersma T (2014) Cues, strategies, and outcomes: how migrating vertebrates track environmental change. *Movement Ecology* 2(1):1-15
- Wright SL, Thompson RC, Galloway TS (2013) The physical impacts of microplastics on marine organisms: a review. *Environ. Pollut.* 178:483–492
- Yorinks N, Atkinson CT (2000) Effects of malaria on activity budgets of experimentally infected juvenile apapane (*Himatione sanguinea*). *Auk* 117(3):731-738.
- Zhao S, Zhu L, Li D (2016) Microscopic anthropogenic litter in terrestrial birds from Shanghai, China: not only plastics but also natural fibers. *Sci. Total Environ.* 550:1110-1115
- Żółtowska K, Łopieńska-Biernat E, Borowa A, Rokicki J, Dmitryjuk M (2012) Trehalose metabolism enzymes in *Contracaecum rudolphii* (Nematoda). *Russian Journal of Nematology* 20(2):113-118.
- Žydelis R, Bellebaum J, Österblom H, Vetemaa M, Schirmeister B, Stipniece A, Dagys M, van Eerden M, Garthe S (2009) Bycatch in gillnet fisheries—an overlooked threat to waterbird populations. *Biological Conservation* 142(7):1269-1281
- Žydelis R, Small C, French G (2013) The incidental catch of seabirds in gillnet fisheries: a global review. *Biological Conservation* 162:76-88.

Part 2

Chapters



7. CHAPTER 1

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

Birgit Kleinschmidt, Claudia Burger, Monika Dorsch, Georg Nehls, Stefan Heinänen, Julius Morkūnas, Ramūnas Žydelis, Rosemary J. Moorhouse-Gann, Helen Hipperson, William O. C. Symondson, Petra Quillfeldt

Marine Biology (2019) 166(77): 1-18

Key words: Diet composition, DNA Metabarcoding, Next Generation Sequencing, North Sea, Red-throated diver/loon, Site selection



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

Birgit Kleinschmidt^{1,2} · Claudia Burger² · Monika Dorsch² · Georg Nehls² · Stefan Heinänen^{3,8} · Julius Morkūnas⁴ · Ramūnas Žydelis^{3,7} · Rosemary J. Moorhouse-Gann⁵ · Helen Hipperson⁶ · William O. C. Symondson⁵ · Petra Quillfeldt¹

Received: 27 June 2018 / Accepted: 7 May 2019 / Published online: 16 May 2019
 © Springer-Verlag GmbH Germany, part of Springer Nature 2019

Abstract

In Europe, the German Bight is one of the most important non-breeding areas for protected red-throated divers (*Gavia stellata*). It is unclear what attracts the birds to this area, especially as the food composition of seabirds outside the breeding season is notoriously difficult to study. To obtain information on prey species composition of red-throated divers in this area, faecal samples from 34 birds caught alive were analysed using DNA metabarcoding. Prey DNA was detected in 85% of the samples with a mean number of 4.2 ± 0.7 taxa per sample ($n = 29$). Altogether, we found a broad prey spectrum with 19 fish taxa from 13 families dominated by five groups: clupeids, mackerel, gadoids, flatfish and sand lances with clupeids being the most frequently detected prey. Our results indicate that red-throated divers are generalist opportunistic feeders in the German Bight, but pelagic schooling fish that aggregate at frontal zones and have a high energetic value might be favoured. Atlantic mackerel appears to be a more important prey for red-throated divers in this area than previously thought. The precision achievable using metabarcoding has revealed a number of prey species that are consumed by red-throated divers in the German Bight, which helps to explain the selection of this area by divers in winter and spring.

Responsible Editor: V.H. Paiva.

Reviewed by R. Lopes, D. Rizzolo and undisclosed experts.

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s00227-019-3523-3>) contains supplementary material, which is available to authorized users.

✉ Birgit Kleinschmidt
 Birgit.Kleinschmidt@bio.uni-giessen.de

¹ Department of Animal Ecology and Systematics, Justus Liebig University Giessen, 35392 Giessen, Germany

² BioConsult SH, 25813 Husum, Germany

³ DHI, 2970 Hørsholm, Denmark

⁴ Marine Research Institute, Klaipėda University, 92294 Klaipėda, Lithuania

⁵ Cardiff School of Biosciences, Cardiff University, Sir Martin Evans Building, Cardiff CF10 3 AX, UK

⁶ NERC Biomolecular Analysis Facility, Department of Animal and Plant Sciences, University of Sheffield, Sheffield S10 2TN, UK

⁷ Present Address: Ornitela UAB, 03228 Vilnius, Lithuania

⁸ Present Address: Novia University of Applied Sciences, Raseborgsvägen 9, 10600 Ekenäs, Finland

Introduction

Understanding resource utilisation is fundamental for managing wildlife populations. Data on diet composition and feeding strategies are essential for understanding habitat selection and for predicting the ecological consequences of habitat change (Davoren et al. 2003). Predator abundance is often regulated by bottom-up effects of prey abundance (Engelhard et al. 2013). Thus, the availability of prey may affect not only predator distribution and abundance but also foraging strategies (Fauchald et al. 2011; Lynam et al. 2017).

Diet composition of seabirds outside the breeding season, when they remain at sea, is notoriously difficult to study. This is especially true for protected species where only non-invasive methods are applicable. In the past, various techniques have been developed to analyse seabird diet. These include visual observations, morphological identification of regurgitates or gut contents, or biochemical methods such as the analysis of fatty acid and stable isotope concentrations (Barrett et al. 2007; Meier et al. 2017; Quillfeldt et al. 2017; Quinn et al. 2017). A highly efficient alternative approach is to use DNA metabarcoding (Deagle et al. 2005, 2007; Pompanon et al. 2012; Vesterinen et al. 2013; Alonso et al.

2014). This involves amplification of DNA from faecal material and assignment of taxonomical information using next generation sequencing (NGS) and DNA barcode databases.

Our study focused on the prey spectrum of the red-throated diver (*Gavia stellata*), a protected marine bird species, in its wintering and spring staging areas in the German Bight (eastern part of the North Sea). During the non-breeding season about 84,200–186,000 individuals stay in the Baltic Sea, the North Sea and the NE Atlantic (Dierschke et al. 2012; BirdLife International 2018). Around 20% of the NW European wintering population occurs in the German Bight (Garthe et al. 2007; Mendel et al. 2008; Dierschke et al. 2012) classifying it as an internationally important staging area for these birds, especially in spring before migration starts (Garthe et al. 2012, 2015). To date, three

studies have been published on the prey composition of non-breeding red-throated divers in the North Sea and the Baltic Sea, which analysed gut contents using morphological tools (Table 1). However, information is not available from the German Bight (Fig. 1). Red-throated divers feed on a wide range of fish species and, given that the energy content of prey fish varies with size and season, they appear to choose prey of high energetic value (Pedersen and Hislop 2001) such as gadoids (Madsen 1957) or clupeids (Durinck et al. 1994; Guse et al. 2009). Additionally, cephalopods were found in one of these studies (Durinck et al. 1994) in four of eight birds. Small specimens of polychaetes, crustaceans, copepods, bivalves and gastropods were reported in all studies although these were considered to be secondary prey (i.e. prey in the guts of the fish eaten by the divers). The German

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 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) <i>n</i> = 173	Durinck et al. (1994) (North Sea) <i>n</i> = 8	Guse et al. (2009) (Baltic Sea) <i>n</i> = 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.	–	50%	–	31%
Perciformes	Gobiidae	Common goby (<i>Pomatoschistus microps</i>)	–	–	Winter 38.2%/spring 10.4%	–
Perciformes	Gobiidae	Gobies (<i>Gobius</i> sp.)	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</i> sp./ <i>Sprattus</i> sp.)	–	37.5%	Winter 14.7%/spring 22.9%	–
Gasterosteiformes	Gasterosteidae	Sticklebacks (<i>Gasterosteus</i> sp.)	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 lucio-perca</i>)	–	–	Winter 91.2%/spring 10.4%	–
Perciformes	Percidae	European perch (<i>Perca fluviatilis</i>)	–	–	Winter 17.6%/spring 2.1%	–
Perciformes	Percidae	Ruffe (<i>Gymnocephalus cernuus</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 scombrus</i>)	< 1%	–	–	55.2%
Pleuronectiformes	Pleuronectidae	Flatfish indet.	5%	37.5%	Winter –/spring 2.1%	51.7%



Fig. 1 Study site where red-throated divers were captured and sampled in the German North Sea. The German Economical Exclusive Zone (EEZ) and 12 nautical miles are indicated (grey line). Red-throated diver capture positions for both sampling years are summa-

risied as a black dot. Large symbols indicate the locations of previous dietary studies on red-throated diver in adjacent waters; star presents Durinck et al. (1994), triangle presents Madsen (1957), and square presents Guse et al. (2009)

Bight is characterised by an estuarine frontal system, created by the Jutland coastal current (JCC) that is primarily driven by discharges from the Elbe river and other rivers further south (Skov and Prins 2001). Red-throated divers have been shown to concentrate at the productive frontal zone, where prey fish aggregate (Skov and Prins 2001). The area is also suitable for the development of offshore wind farms as it has extensive areas of shallow waters (< 40 m). To date, 17 wind farms have been installed in German North Sea waters. Thus, there is potential overlap between offshore wind farm sites and the preferred habitat of non-breeding red-throated divers (Garthe et al. 2015; Heinänen et al. unpubl data). Red-throated divers have been shown to strongly avoid both shipping traffic and wind farms (Garthe and Hüppop 2004; Bellebaum et al. 2006; Petersen et al. 2006; Dierschke et al.

2006, 2012; Mendel et al. 2019; Heinänen et al. unpubl data; Burger et al. unpubl data). To understand the environmental importance of the German Bight for red-throated divers, to assess the possible impacts arising from displacing divers from substantial parts of their staging areas, and to analyse whether alternative staging areas might be available, it is crucial to understand what resources these birds rely on.

In this study, we had the unique opportunity to collect a small number of faecal samples from red-throated divers captured in the German North Sea in 2015 and 2016 in both winter and spring. We applied DNA metabarcoding as a non-invasive technique to analyse diet composition, and thus to provide a detailed overview of recent meals of these birds in the German Bight. Specifically, we aimed to document the diversity of prey species eaten by the birds

in this particular staging area when red-throated diver abundance is highest. Additionally, we aimed to compare data for two consecutive sampling years to determine if the prey species consumed is consistent between years. By comparing dietary data with published data on local fish distribution, we aimed to determine whether the abundance and distribution of prey fish correlate with red-throated diver diet and how this may help to explain red-throated diver distribution.

Methods

Sample collection and study site

This dietary study was part of a satellite telemetry project on red-throated divers. A total of 36 red-throated divers were captured in March and April 2015, and in February and March 2016 in the German Bight (Fig. 1). Sampling was focused on late winter and spring when red-throated diver abundance is highest in the German Bight (Mendel et al. 2008; Dierschke et al. 2012; Garthe et al. 2015). The capture area was approximately 30 km offshore in water depths of around 20 m, which is approximately in the centre of the staging area for red-throated divers (Fig. 1). Birds were captured from a rigid inflatable boat using a hand net and the “night lighting technique”, where the sea is searched for resting divers with a spot light. If a bird is sighted, it often becomes disoriented by the bright light and can be captured with a net (Whitworth et al. 1997; Ronconi et al. 2010). In 2015, captured birds were kept in boxes for an average time of 18.3 h (min 6.3 h, max 27 h) and in 2016 for an average time of 9.2 h (min 7 h, max 13 h). After release the boxes were searched for scat. The boxes were cleaned and disinfected after every use with bleach (1% hypochlorite solution), water and ethanol (70%) to prevent cross-contamination. During the two field seasons, a total of 34 faecal samples were collected (2015 $n = 15$; 2016 $n = 19$, Table 2). Samples were

preserved in absolute ethanol and stored at -20°C until further analysis.

DNA extraction

Faecal DNA was isolated using the QIAamp DNA Stool Mini Kit (Qiagen) following the manufacturers protocol with the following modifications: (1) the samples were resuspended in the storage ethanol by vortexing before moving 200 μL of the ethanol–scat slurry to a new clean 2-mL Eppendorf tube and centrifuging for 30 s at $4000\times g$ (Deagle et al. 2005); (2) the lysis step was extended by adding 1.4 mL buffer ASL instead of 1.6 mL to each sample and incubating at 70°C for 10 min and then for 1.5 h at room temperature to improve lysis output; (3) the digestion step was extended by adding 20 μL instead of 25 μL proteinase K and incubating samples at 70°C for 30 min prior to an increased incubation time at a lower temperature (56°C for 1.5 h). All remaining steps followed the manufacturer’s instructions except that buffer volumes were cut down to reduce the risk of cross-contamination by minimizing the number of pipetting steps and by reducing the volume of liquid loaded into spin columns and tubes (Deagle et al. 2005). The final elution step used a total elution volume of 100 μL (as recommended by the manufacturer’s protocol), but was divided into two steps with each elution using 50 μL Buffer AE.

Primer design and preparation for sequencing

Three separate PCR primer pairs were used to comprehensively target all the major potential prey species of red-throated divers in this area (Table 3). These prey species are widespread in the North Sea and were informed by previous diet studies on red-throated divers (Table 1; Madsen 1957; Durinck et al. 1994; Guse et al. 2009).

Primers for each prey group were tested *in silico*, using ClustalX 2 (Larkin et al. 2007) and MEGA7 (Kumar et al. 2016). Conserved primer binding sites were tested against a DNA barcode database of barcode-sequences extracted from GenBank. Sequences of 16S DNA of 28 representative fish species from seven orders and 15 families as well as 12 cephalopod species from 5 families were aligned for these tests. For crustaceans, COI barcode sequences of potential prey species from six orders and eight families of shrimp and krill were aligned and tested. Furthermore, primers for each prey group were tested *in vitro* on DNA from tissue samples of corresponding potential prey species occurring in the German Bight (clupeids, perciformes, gadoids, flatfish, octopus, squid, cuttlefish and shrimp) to optimise PCR conditions. Multiplex identifier (MID) tags were added to the primer sequences and used to assign DNA sequences to their respective samples ($n = 34$). MID tags were added to each of the three tested primer sets

Table 2 Timing and sample size of analysed faecal samples of red-throated divers from the German Bight

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	

One sample per bird was taken for analysis

Table 3 Sequences of primers used to amplify red-throated diver faecal samples for next generation 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	CGAGAAGACCCTDTGRAG CT (20)	~ 264	58
Fish (chordata)	mtDNA 16S	modifiedChord_16S_R1	GCTGTTATCCCTRGRGTAA (19)		
Cephalopod (molluscs)	mtDNA 16S	Ceph_16S_R	AGGGACGARAAGACC CTANTGAGC (24)	~ 244	56
Cephalopod (molluscs)	mtDNA 16S	Ceph_16S_F	ACSCGTGTAYCCCTATG (17)		
Crustacean (invertebrate)	mtDNA COI	mICO1int_F	GGWACWGGWTGAACW GTWTAYCCYCC (26)	~ 332	50
Crustacean (invertebrate)	mtDNA COI	Nancy_R	CCCGGTAAAATTAATA TAAACTTC (26)		
Blocking probe	–	–	GTGGA ACTTAAAAATCA GCAGCCACCA[SpC3]	–	–

(fish, cephalopods and crustaceans). For each of the three primer sets, we used 24 forward primers/MID and two reverse primer/MID combinations, and all in vitro testing was performed using primer pairs first without and then with the MID tags to ensure amplification was not affected.

To amplify DNA from fish and cephalopods, we used primers targeting the 16S region originally published by Waap (2015) and modified from Chord_16S_F/Chord_16S_R (Deagle et al. 2009). We further modified the primer sequence to comprehensively match the range of potential prey species (Table 3). To amplify fish DNA, the forward primer has additional CT bases at the 3' end for NGS sequencing to improve the blocking probes (see below), so that the mismatch was not located at the last base pair (Waap, pers comm.). To amplify cephalopod DNA, we modified the forward primer by one base and the 5' end of the reverse primer. Both primer pairs tested positive in silico and in vitro for potential prey of red-throated divers.

To amplify crustacean DNA, a primer combination targeting the cytochrome oxidase I region (COI) was used that was likely to amplify crustaceans and molluscs (Stockdale 2018, Table 3). The forward primer (Leray et al. 2013) was designed to amplify arthropod DNA, including crustaceans and molluscs. The reverse primer (Simon et al. 1994) was also designed to amplify arthropods including crustaceans. The primers tested positive in silico and in vitro for potential prey of red-throated divers and provided a good coverage of our target species and a good coverage with reference sequences available in public databases. This primer set amplified a product size of 332 bp and thus represents a good compromise as it is long enough to provide good taxonomic information and short enough to survive digestion.

Blocking primer

The primers chosen to amplify fish prey were universal chordate primers that could also amplify other chordates, including predator DNA. To prevent the amplification of predator DNA, we developed a blocking probe using a C3 spacer (Table 3; Vestheim and Jarman 2008). However, the blocking probe reduced amplification success and a second amplification of samples was performed excluding the blocking probe. Gel electrophoresis (see below) was used to visually monitor the amplification of predator and prey DNA, assisted by the inclusion of red-throated diver (300 bp) and fish (264 bp) reference samples. This differential in PCR product size allowed for predator amplicons to be easily identified (Fig. 2).

PCR amplification of DNA from faeces

PCR amplifications were performed in single reactions using Multiplex PCR Kits (Qiagen) and a 20 µL PCR reaction volume. Thermal cycling conditions for fish and cephalopod prey were 95 °C for 15 min followed by 45 cycles of: 94 °C for 30 s, a primer specific annealing temperature (Table 3) for 90 s, and 72 °C for 45 s, followed by a final extension at 72 °C for 5 min. Thermal cycling conditions for crustaceans were 95 °C for 15 min followed by 45 cycles of: 94 °C for 30 s, a primer specific annealing temperature (Table 3) for 90 s, and 72 °C for 90 s, followed by a final extension at 72 °C for 15 min.

All PCR products were visualised by gel electrophoresis on 2% agarose gels stained with SYBR®Safe (ThermoFisher Scientific, Paisley, UK) and compared to a standardised 1000-bp ladder. The PCR product concentration in

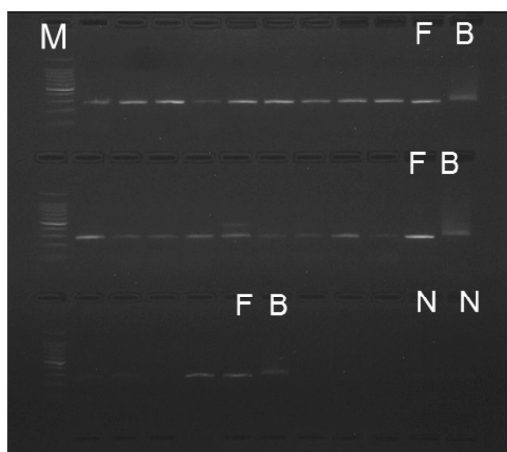


Fig. 2 Agarose gel electrophoresis of 16S mtDNA fragments amplified from faecal samples with fish (chordate) primers. *M* 1000-bp ladder, *F* fish DNA control, *B* red-throated diver DNA control and *N* negative control

successful reactions was quantified with a Qubit fluorometer (Thermo Fisher) and subsequently pooled into two equimolar libraries of individually tagged amplicons (PoolA using a blocking probe and PoolB without a blocking probe). To remove primer dimer, we ran a magnetic clean-up (AMPure). Concentrations of DNA and primer dimer were measured on a tape station (D1000 Screen Tape; Tape Station Analysis Software A.01.05 SR1, Agilent technologies), and a Qubit before and after the magnetic clean-up.

Next generation sequencing

NGS library preparations were performed at the NERC Biomolecular Analysis Facility—Sheffield (NBAF-S), Sheffield, UK, using the NEBNext Ultra DNA Library Prep Kit for Illumina (New England Biolabs, Ipswich, MA). To characterise the diet content of the individually tagged amplicons, the libraries (PoolA and PoolB) were sequenced at the Sheffield Diagnostics Genetics Service (Children's Hospital, Sheffield, UK) using 250-bp paired-end reads on a MiSeq desktop sequencer (Illumina, San Diego, CA).

Bioinformatics

We performed eight steps to transform the raw Illumina sequence data into a list of molecular operational taxonomic units (MOTUs) with assigned taxonomy. These steps included assessing sequence quality, trimming sequences (Bolger et al. 2014), aligning paired reads (Magoc and Salzberg 2011), matching sequences to MID

tags and amplicon primers (Schloss et al. 2009), and demultiplexing sequences into files for each amplicon. We used USEARCH (Edgar 2010) to dereplicate the sequence file, to detect and to remove chimeric sequences and to cluster into MOTUs based on 97% identity. Clustering is an important step in metabarcoding analysis to group similar sequences into distinct taxonomic units, but remains one of the central challenges. If the clustering threshold is too conservative, e.g. 5% sequence divergence, the dietary richness could be underestimated due to a high mean overlap of MOTUs. Conversely, a less conservative decreased threshold, e.g. 2% sequence divergence, could overestimate species richness (Clare et al. 2016). Here, we applied the established clustering threshold of 97% similarity (Edgar 2013, 2016) using the 'cluster_fast' function in USEARCH (Edgar 2010). We applied the BLASTn algorithm (Altschul et al. 1990) to match MOTU sequences to reference sequences in the NCBI GenBank nucleotide database, using a cut-off of 90% minimum sequence identity and a maximum e-value of 0.00001. For detailed information about options, parameters and values, please see Table 1 in the supplementary material.

We subsequently manually performed further filtering steps to produce robust taxonomic assignments. We discarded MOTUs (sequence clusters 97%) that corresponded to contaminants that can occur regularly in faecal samples, such as bacterial, human or predator DNA. MOTUs were retained in a sample only if they contained a minimum of five sequences. Taxonomic assignment was based on the percentage similarity of the query and the reference sequences. Since short fragments are less likely to contain reliable taxonomic information, we only retained sequences with a minimum length of 190 bp and a BLASTn assignment match greater than 98%, following Deagle et al. (2009) and Vesterinen et al. (2013).

Finally, we combined both pools (PoolA with a blocking probe and PoolB without a blocking probe) together for final analyses. To avoid overrepresentation, we excluded prey species of samples from PoolB that were also present in PoolA.

Analysing the blast output

We used MEGAN Community Edition version 6.8.8 to visualise the accession number identifiers on the NCBI taxonomy (Huson et al. 2016). We imported the blast output and used the default LCA parameters to assign a taxon name to each MOTU (Huson et al. 2007). If all retained hits of a MOTU with the same quality criteria (sequence identity, sequence length, e-value) matched the same species, then we have a species-level assignment, otherwise the MOTU was assigned to the lowest shared taxonomic level, e.g. genus or family.

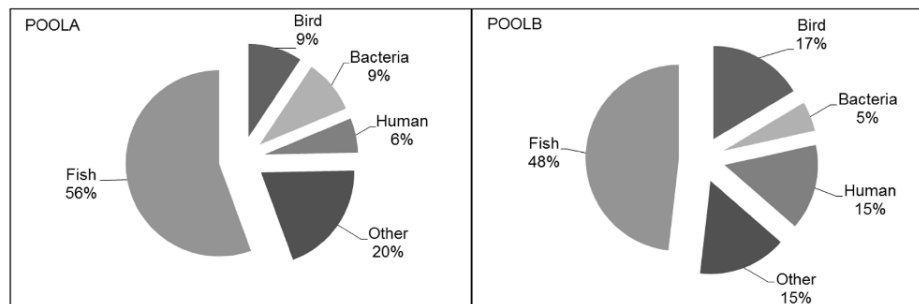


Fig. 3 Proportions of MOTUs in percent for fish, contamination (bacteria, human and others) and for bird assignments amplified with the fish primer set. On the left with the blocking probe (PoolA) and on

the right without the blocking probe (PoolB) for both sampling years (2015 and 2016) combined

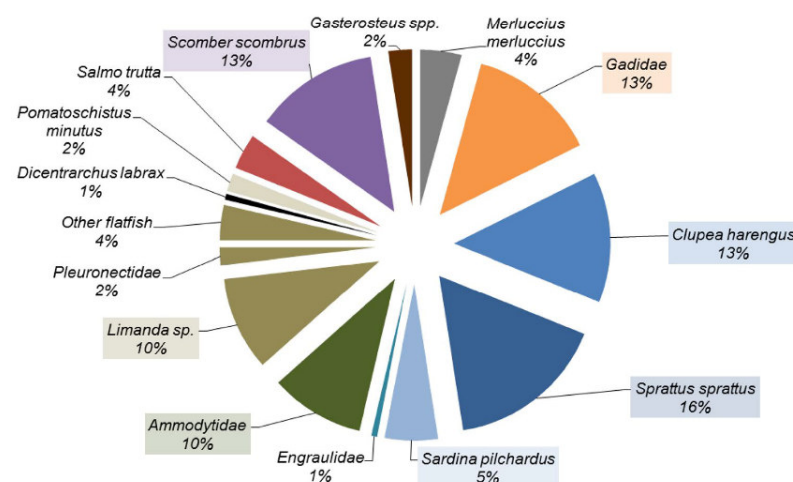
Statistical analysis

We analysed prey range by determining the presence of prey items, their frequency of occurrence (FO) (Barrett et al. 2007, Tollit et al. 2009), and species richness. FO was calculated as $FO = (n/t) \times 100$, where n is the number of samples in which the specific prey item appeared and t is total number of samples containing prey. FO reveals the percentage of sample units in which each prey item occurred (Barrett et al. 2007). The number of MOTUs (defined by 97% clustering threshold, $n = 169$) assigned for each prey taxa was additionally presented as percent occurrence in faecal samples ($n = 29$) to visualise the sequencing output in Fig. 4. No further quantitative analyses were done with these data due to a range of possible biases and as interpretation of sequence proportions generated via high-throughput sequencing requires careful data

analysis (Deagle and Tollit 2007; Pompanon et al. 2012; Deagle et al. 2013, 2018).

Whether or not there is consistency in prey consumption by red-throated divers over time informs our understanding of prey selection in this particular area. We tested this by comparing FO of prey items in 13 samples from 2015 with FO of prey items in 16 samples from 2016. Statistical tests suitable for small sample sizes were performed in Rcmdr (Fox and Bouchet-Valat 2018). We used Pearson's Chi squared test to compare the frequency of occurrence between years for each prey group when sample sizes fulfilled the minimum requirements for this test ($n > 5$). When sample sizes were small ($n < 5$), we implemented the Fisher's exact test for count data. To compare the number of prey detections per sample between sampling years, the T test for independence was used. Small sample sizes precluded further analyses (e.g. comparing seasons) or to use other

Fig. 4 Percent occurrence of MOTUs ($n = 169$) in all faecal samples ($n = 29$) assigned for fish prey: blue—clupeids, orange—gadoids, grey—hake, brown—sticklebacks, violet—mackerels, red—salmonids, beige—sand gobies, black—scabass, grey—light-brown flatfish and green—sand lances. Captions of prey groups with highest proportions ($> 5\%$) are highlighted



statistical tests. Considering the sample size and the temporal scope of faecal DNA sampling, only marked differences were expected to be identified.

Results

Overview of sample quality and prey species found

Neither cephalopods nor crustaceans were detected in the diet, despite successful in vitro PCR amplification using reference tissue samples from potential prey items from the German Bight (octopus, squid, cuttlefish and shrimp samples).

The fish primer set produced more than 800,000 sequences from both pools combined, for specific information on number of sequences during bioinformatics analysis, see Table 2 in supplementary material. Of 34 screened samples 29 samples gave positive PCR amplifications (PoolA: $n=21$; PoolB $n=29$). Both pools had ~50% of MOTUs assigned to prey fish (PoolA=56%; PoolB=48%), plus with other MOTUs being from the predator DNA (red-throated diver) and contaminants such as bacteria and human DNA (Fig. 3). Using the blocking probe, we still amplified predator DNA but the amount of MOTUs assigned to the predator was slightly lower in PoolA (9%) than in PoolB (17%).

After filtering for contaminants, sequence length and mapping to reference sequences, 20 and 24 faecal samples remained for PoolA and B, respectively. After merging both pools, the final sample set consisted of 29 samples (PoolA $n=20$, PoolB $n=9$) which corresponds to 85% of all samples collected (Table 2). Four samples were discarded (PoolB) as they contained only contaminants and predator DNA, and two samples were discarded as the amplicon length criteria were not met (1 \times PoolB, 1 \times PoolA).

Clustering the sequences by 97% similarity to each other and subsequent filtering resulted in 169 MOTUs that were used for further analyses. A list of a representative query sequences of each MOTU and its quality criteria is listed for each prey assignment in Appendix (Table 5) and for all MOTUS in Table 3, supplementary material. For the two sampling periods, 19 taxa from 13 families were identified in 29 faecal samples (Fig. 4, Table 4). In 2015, we detected a slightly higher number of taxa in comparison with 2016 (18 and 13 taxa assigned to species, respectively; Table 4). The prey species spectrum was similar between the 2 years with 12 matching taxa and no significant differences ($\chi^2=1.004$, $p=0.316$). European anchovy (*Engraulis encrasicolus*), turbot (*Scophthalmus maximus*), European pollock (*Pollachius pollachius*), cod (*Gadus* sp.), European bass (*Dicentrarchus labrax*) and sand lances of the genus *Ammodytes* were detected only in 2015, and whiting (*Merlangius merlangus*) only in 2016 (Table 4).

Prey detection

Of the samples where prey were detected, the mean number of taxa found was 4.2 ± 0.7 per sample ($n=29$) with minimum and maximum values of 1 and 16, respectively. There was no significant difference ($t=1.58$, $p=0.135$) between the number of prey items detected in 2015 (mean 5.3) and 2016 (mean 3.1).

Clupeids were the most frequently detected prey group (FO of 65.5%, Table 4). Within this group, Atlantic herring (*Clupea harengus*) and European sprat (*Sprattus sprattus*) occurred most frequently (FO of 55.2% and 58.6%, respectively). No significant differences were found between years for clupeids ($\chi^2=0.030$, $p=0.863$), European sprat ($\chi^2=0.283$, $p=0.595$), or for Atlantic herring ($\chi^2=0.005$, $p=0.945$).

The Atlantic mackerel (*Scomber scombrus*) was the only species of mackerel detected (Table 4), with a total FO of 55.2% and no significant differences between the two sampling years (FO 53.8% in 2015, FO 56.3% in 2016; $\chi^2=0.005$, $p=0.945$).

Flatfish were recorded with a total FO of 51.7% (Table 4) and no significant difference between the two sampling years (61.5% in 2015, 43.8% in 2016; $\chi^2=0.287$, $p=0.592$). Most taxonomic assignments were at the family or genus levels. Righteye flounders (Pleuronectidae) were dominant and where MOTUs were assigned at the species level the common dab (*Limanda limanda*) was the most frequent species detected.

Gadoids (Gadidae) were recorded with a total FO of 37.9% and high similarity between sampling years (38.5% in 2015, 37.5% in 2016; $\chi^2=0.001$, $p=0.972$, Fishers exact test $p=0.976$). Most MOTUs could only be assigned to the family level, but of those assigned to species cod (*Gadus* sp.), European pollock (*Pollachius pollachius*), whiting (*Merlangius merlangus*) and haddock (*Melanogrammus aeglefinus*) were detected at least once. Detections of these species varied between years but sample sizes were too small for statistical tests.

Sand lances had a total FO of 31%, with a similar proportion of greater sand eel (*Hyperoplus lanceolatus*; FO of 13.7%) and sand lances of the genus *Ammodytes* (FO of 20.7%). There were significantly more sand lances detected in 2015 (61.5%) in comparison with 2016 (6.3%; $\chi^2=5.394$, $p=0.020$; Fishers exact test $p=0.026$).

Other prey species infrequently occurred and are detailed in Table 4 and Fig. 4.

Discussion

The aim of this study was to analyse prey species composition in faecal samples from red-throated divers caught in the German Bight, using high-throughput sequencing. In our

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

			2015			2016			2015 and 2016		
			MOTUs found in <i>x</i> samples (n=13)	FO (%) n=13	No of MOTUs (n=101)	MOTUs found in <i>x</i> samples (n=16)	FO (%) n=16	No of MOTUs (n=68)	MOTUs found in <i>x</i> samples (n=29)	FO (%) n=29	No of MOTUs (n=169)
Clupeiformes	Clupeidae	<i>Clupea harengus</i>	7	53.8	10	9	56.3	15	16	55.2	25
		Atlantic her- ring									
Clupeiformes	Clupeidae	<i>Sprattus sprattus</i>	9	69.2	15	8	50.0	12	17	58.6	27
		European sprat									
Clupeiformes	Clupeidae	<i>Sardina pilchardus</i>	3	23.1	6	3	18.8	4	6	20.7	10
		European pilchard									
Clupeiformes	Engraulidae	<i>Engraulis encrasicolus</i>	1	7.7	1	0	0.0	0	1	3.4	1
		European anchovy									
Pleuronecti- formes	Pleuronecti- dae	Clupeids	9	69.2	32	10	62.5	31	19	65.5	63
		Common dab	8	61.5	8	5	31.3	7	13	44.8	15
Pleuronecti- formes	Scophthalmi- dae	<i>Scophthalmus maximus</i>	1	7.7	2	0	0.0	0	1	3.4	2
		Turbot									
Pleuronecti- formes	Soleidae	<i>Solea solea</i>	1	7.7	2	1	6.3	1	2	6.9	3
		Common sole									
Pleuronecti- formes	Pleuronecti- dae	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
Salmoni- formes	Salmonidae	<i>Salmo Trutta</i>	4	30.8	5	1	6.3	1	5	17.2	6
		Sea/brown trout									
Gadiformes	Merlucciidae	<i>Merluccius merluccius</i>	3	23.1	5	1	6.3	2	4	13.8	7
		European hake									
Gadiformes	Gadidae	<i>Pollachius pollachius</i>	2	15.4	2	0	0.0	0	2	6.9	2
		European pollock									
Gadiformes	Gadidae	<i>Melanogrammus aeglefinus</i>	2	15.4	4	1	6.3	1	3	10.3	5
		Haddock									
Gadiformes	Gadidae	<i>Merlangius merlangus</i>	0	0.0	0	1	12.5	1	1	6.9	1
		Whiting									
Gadiformes	Gadidae	<i>Gadus</i> sp.	1	7.7	1	0	0.0	0	1	3.4	1
		Cod									
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

Table 4 (continued)

		2015			2016			2015 and 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)
Gaster-osteiformes	Gaster-osteidae	2	15.4	3	1	6.3	1	3	10.3	4
Perciformes	Moronidae	1	7.7	1	0	0.0	0	1	3.4	1
Perciformes	Ammodyti- dae	3	23.1	3	1	6.3	1	4	13.8	4
Perciformes	Ammodyti- dae	6	46.2	8	0	0.0	0	6	20.7	8
Perciformes	Ammodyti- dae	2	15.4	3	1	6.3	1	3	10.3	4
Perciformes	Gobiidae	2	15.4	2	2	12.5	2	4	13.8	4
Perciformes	Scombridae	7	53.8	11	9	56.3	10	16	55.2	21

Table 5 Best blast results for each of the 21 detected taxa and corresponding accession number, the identity with the blast reference sequence, the sequence length and the bitscore from data of both sampling years (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	1.94E−104	388
Clupeiformes	Clupeidae	<i>Sprattus sprattus</i>	European sprat	KJ128910	100	210	9.04E−103	388
Clupeiformes	Clupeidae	<i>Sardina pilchardus</i>	European pilchard	FR849599	100	205	1.14E−101	379
Clupeiformes	Engraulidae	<i>Engraulis encrasicolus</i>	European anchovy	KJ128765	100	211	5.93E−105	390
Pleuronectiformes	Pleuronectidae	–	Right eye flounders	KU936350	99.1	224	7.49E−109	403
Pleuronectiformes	Pleuronectidae	<i>Limanda limanda</i>	Common dab	KJ128862	100	224	3.78E−112	414
Pleuronectiformes	Scophthalmidae	<i>Scophthalmus maximus</i>	Turbot	EU410416	100	217	2.60E−108	401
Pleuronectiformes	Soleidae	<i>Solea solea</i>	Common sole	KJ128906	99.1	224	7.49E−109	403
Salmoniformes	Salmonidae	<i>Salmo trutta</i>	Sea/brown trout	KT633607	100	213	4.25E−106	394
Gadiformes	Merlucciidae	<i>Merluccius merluccius</i>	European hake	KJ128826	100	208	2.49E−103	385
Gadiformes	Gadidae	<i>Pollachius pollachius</i>	European pollock	FR751400	99.5	208	2.50E−98	379
Gadiformes	Gadidae	<i>Merlangius merlangus</i>	Whiting	KJ128825	100	208	2.49E−103	363
Gadiformes	Gadidae	<i>Melanogrammus aeglefinus</i>	Haddock	KJ128822	100	208	2.49E−103	385
Gadiformes	Gadidae	<i>Gadus</i> sp.	Cod	AP017650	99.52	208	1.16E−101	379
Gadiformes	Gadidae	–	Codfishes/true cod	AP017650	99.5	208	2.49E−103	379
Gasterosteiformes	Gasterosteidae	<i>Gasterosteus</i> sp.	Stickleback	KJ627974	100	208	1.16E−101	379
Perciformes	Moronidae	<i>Dicentrarchus labrax</i>	European bass	KJ168065	99.5	211	2.53E−103	385
Perciformes	Ammodytidae	–	Sand lance	KJ128795	99.1	211	2.53E−103	379
Perciformes	Ammodytidae	<i>Hyperoplus lanceolatus</i>	Greater sand eel	KJ128795	100	211	2.53E−103	390
Perciformes	Ammodytidae	<i>Ammodytes</i> sp.	Sand eel	AF315121	100	211	1.18E−101	390
Perciformes	Gobiidae	<i>Pomatoschistus minutus</i>	Sand goby	KJ128870	100	207	8.89E−103	383
Perciformes	Scombridae	<i>Scomber scombrus</i>	Atlantic mackerel	KJ128898	100	217	1.21E−106	396

dataset, we found an exclusively piscivorous diet, with no evidence of cephalopod or crustacean consumption and a similar prey spectrum between two consecutive sampling years.

Application of high-throughput sequencing to study diver diets

The DNA metabarcoding methodologies utilised in this study have previously been applied in diet studies on other

marine predators (Deagle et al. 2005, 2007; Pompanon et al. 2012). However, this study is the first application of this approach to analyse the diet of red-throated divers in the German Bight or elsewhere. Using reference sequences, we found high taxonomic coverage for both the COI and 16S barcode primers. Because of their commercial importance in the German Bight, many fish species (e.g. Atlantic herring), alongside some cephalopod species, are well studied and the majority of these species appear in the Genbank database (Dickey-Collas et al. 2010; Engelhard et al. 2013).

Sequences were clustered at 97% identity and represented consistent taxonomical units (MOTUs). Some prey species were represented by multiple MOTUs, suggesting that the clustering threshold could have been lower. However, a lower threshold would have increased the risk of clustering two closely related species into a single MOTU and thus reduced taxonomic discrimination. In practice, it is difficult to apply an 'average' threshold when diet is diverse and the prey are likely to have differing evolutionary rates. On balance, we deem the clustering threshold applied as appropriate and this method provided a good estimate of species richness with distinct taxonomic units.

We obtained sufficient sequencing data from 85% of the analysed faecal samples using universal primers. The species richness was higher in 2015 but individual variances may be due to sampling conditions, sample quality and amplification success. The use of a blocking probe proved to be of little advantage, with sufficient prey DNA amplified using both approaches (Fig. 3). The use of a blocking probe reduced the amplification of predator DNA but also amplification success in general since the output of prey-positive samples was higher when the blocking probe was omitted.

The detection rate of prey species can be biased by the method applied. For example, Tollit et al. (2009) found some prey (Ammodontidae, Cottidae and Gadidae) were more reliably detected with morphological tools, whereas other prey (Salmonidae, Pleuronectidae, Elasmobranchii and cephalopods) were only detected with molecular tools. However, the overall results did not dramatically differ. In general, molecular methods have been shown to identify more trophic links (number of taxa identified) with higher rates of taxonomic discrimination in comparison with morphology (e.g. Soininen et al. 2009; Alonso et al. 2014; Berry et al. 2015; Waap et al. 2017). Using molecular methods, we found a similar prey composition to conventional morphological methods applied in previous studies on red-throated diver diet. Using faecal samples coupled with DNA metabarcoding is now an established non-invasive approach for dietary studies. However, it is debatable whether or not this method can provide quantitative (read number) in addition to qualitative (presence and absence) estimates of diet (Deagle and Tollit 2007; Pompanon et al. 2012; Deagle et al. 2013, 2018). In this study, we applied a conservative approach of using only qualitative data. However, if quantitative data are required, we recommend combining DNA metabarcoding and morphological methodologies, where the latter can provide quantitative information as in Alonso et al. (2014) and Waap et al. (2017).

A faecal sample, for most species, will represent an individual's most recent meals. Other methods, including fatty acid composition and stable isotope analyses, can provide information over a longer time frame (Meier et al. 2017). Although our sample size is small, samples were collected

from birds caught in two consecutive years at dispersed intervals encompassing late winter and spring (February–April), when red-throated diver abundance is highest in the German Bight. Thus, this dataset provides dietary information from a time period when this area is particularly attractive to these birds. Wintering home ranges of red-throated divers can cover several connected sites, including sites outside the German Bight, such as the Baltic Sea (Kleinschmidt et al. unpub data). The German Bight also represents an important staging area in spring when some birds have already started migration (Garthe et al. 2015) and the availability of suitable prey types is probably one of the main determinants of habitat quality for these birds. In this context, the time frame over which a faecal sample provides dietary information helps to reflect the situation in the particular area of interest for this study.

Fish availability in the German Bight, red-throated diver diet and comparison to previous studies

Potential prey availability is an important factor affecting habitat choice and diet selection. We searched the species factsheets (ICES 2006a, b), reports and publications (ICES 2008, 2011, 2016, 2017a, b; 2018a, b, c; DFS 2016) to compare fish distribution (a proxy for potential prey availability) with the diet of red-throated divers in our study in addition to previous studies. In our dataset, red-throated divers consumed a wide range of fish prey species consisting of both a pelagic and a benthic component. We found mainly clupeids, mackerels, flatfish, gadoids and sand lances in the diet of red-throated divers but no clear dominance of a single species or species group could be identified. A similarly wide, although slightly different range of prey species was found in previous studies on red-throated diver diet. For example, Madsen (1957) found a broad prey spectrum but the majority of analysed birds (82%) fed exclusively on cod, gobies, sticklebacks and herring with varying intensities. Guse et al. (2009) found 11 species from nine families with clupeids, zander, European smelt, ruffe, lesser sand eel, three-spined stickleback and common goby being dominant species. Similarly, Durinck et al. (1994) identified clupeids and gadoids as the most frequent prey items.

Clupeids, specifically sprat and herring occurred most frequently in both sampling years of our study. These species are typically high in lipid content and energy density (Pedersen and Hislop 2001; Ball et al. 2007). Sprat and juvenile herring are also two of the most abundant pelagic species in the German Bight in spring (ICES 2006a, b), which coincides with our sampling period. The size of available prey fish is also important for prey selection. In general, herring occurs in the North Sea with a size of 20–30 cm but in our sampling period smaller (juvenile) herring with a size <20 cm are the most abundant and widely distributed in

the German Bight and the Kattegat (ICES 2006a; Trueman et al. 2017). Sprat is a pelagic species abundant in frontal areas of the North Sea with a size of < 16 cm (Kanstinger and Peck 2009). We also found European sardine (*Sardina pilchardus*) and European anchovy (*E. encrasicolus*) in the diver diet but less frequently, which is consistent with the distribution of both these clupeid species. They originate from the Mediterranean Sea (Motos et al. 1996) and since 2003 are expanding into the North Sea (Kanstinger and Peck 2009). Like sprat, sardine occurs in frontal areas, whereas anchovy is primarily found in near-shore areas. The distribution of clupeids is in good agreement with red-throated diver distribution, which appears to be attracted by frontal zones (Skov and Prins 2001; Goyert et al. 2016; Heinänen et al. unpubl data). Hence, these areas provide a source of energetically valuable species for red-throated divers. The high detection rate of clupeids is in line with two earlier studies on red-throated diver diet and reinforces their importance as red-throated diver prey (Durinck et al. 1994; Guse et al. 2009).

Atlantic mackerel is widespread throughout the North Sea and is one of the most commonly exploited species (ICES 2011, 2016, 2017a, b). Due to its high energetic value, mackerel is an attractive fish for seabirds (Montevecchi et al. 1984, 1988; Garthe et al. 2014). Overfishing triggered a population collapse in the North Sea in the 1970s but since 2000 the stock has increasing again (ICES 2011; Jansen et al. 2012a, b, 2015; Jansen and Gislason 2013; Jansen 2014; van der Kooij et al. 2016). These changes in mackerel availability may explain why both Madsen (1957) and the current study detected mackerel in the diet, while Durinck et al. (1994) did not. Mackerel appeared in our samples in considerable numbers indicating that it may now be a more important prey than previously thought.

Most flatfish were identified to family level, but of those identified to species level, common dab was the most common in both years. Flatfish have been recorded in low numbers in red-throated diver diet (Madsen 1957; Durinck et al. 1994; Guse et al. 2009), possibly due to their wide-bodied shape making adult flatfish an unfavourable prey item (Reimchen and Douglas 1984; Guse et al. 2009). Dietary studies in the adjacent Wadden Sea have shown that juvenile flatfish are selected as important food items by other water birds such as benthic feeding cormorants (Nehls and Gienapp 1997). The Wadden Sea and adjacent waters are an important nursery ground for several flatfish species (DFS 2016) and juvenile common dab is highly abundant in spring within the German Bight over a wide depth range (Beek et al. 1989; Bolle et al. 1994; Campos et al. 1994; Hufnagl et al. 2013; DFS 2016; ICES 2017a, b). Prey size cannot be deduced from metabarcoding but red-throated divers may be preying on juvenile flatfish. Although flatfish are considered to have a low energy

content (Ball et al. 2007), the probable high encounter rate may explain the high detection rate in our samples.

Gadoids, particularly cod, were described by Madsen (1957) as the most important prey group for red-throated divers in the Kattegat and Belt Sea. In the current study, gadoids were infrequently present in the diet. This is in line with findings of Durinck et al. (1994) from the south-western part of the Skagerrak. Juvenile gadoids (< 20 cm) are more likely than adults to be prey for red-throated divers. Recordings of this size class of gadoids are mostly restricted to the eastern inshore water of the Skagerrak and Kattegat, with low abundances in the German Bight (Munk et al. 1999; Munk 2014; André et al. 2016). Thus, gadoids may be a favoured prey item but low availability at the study site limits feeding on these species.

Sand lances are an important prey for seabirds in general, particularly in the North Sea (Harris and Wanless 1991, 2013; Mendel et al. 2008; ICES 2011; Engelhard et al. 2013; ICES 2016). Sand lances appeared at a high frequency in 2015 but were less common in 2016 in our dataset. This pattern is reflected in commercial catch rates for sand lances in the central and south-eastern North Sea ecoregion (Division 4b–c): average catch rates and a low recruitment in 2015, and low catch rates and high recruitment in 2016 (ICES 2018a, b). Previously, sand lances have been recorded at both high (Guse et al. 2009) and low (Madsen 1957; Durinck et al. 1994) frequencies in red-throated diver diet. These patterns suggest that the frequency of sand lances in the diet is determined by their availability.

Smelt (*Osmerus eperlanus*) was not detected in this study but has been highlighted as an important prey species for red-throated divers in the Baltic Sea (Žydelis 2002; Guse et al. 2009). Smelt occurs in parts of the Wadden Sea with low salinity and close to the coast. Here, it forms dense spawning aggregations in estuaries and anadromous migrations in late winter and early spring (DFS 2016). The German Bight is further away from river mouths, the lack of smelt in our dataset could probably be explained by the low abundance of this species here.

Sea trout (*Salmo trutta*), European hake (*Merluccius merluccius*), sticklebacks (*Gasterosteus* sp.), European bass (*D. labrax*) and sand goby (*Pomatoschistus minutus*) were recorded in our dataset at low frequencies. These species are widely distributed in the North Sea with varying densities. Some, such as gobies, are known to be important prey items for other marine predators (Haelters et al. 2012; Méheust et al. 2015; Andreassen et al. 2017) and were previously recorded as prey items of red-throated divers (Madsen 1957; Durinck et al. 1994; Guse et al. 2009). Sticklebacks were frequently found in all previous studies. However, the current study suggests that these species are of low importance for red-throated divers in the German Bight.

In contrast to our study, Guse et al. (2009) found zander as one of the most important prey items of red-throated divers wintering in the Baltic Sea. This fish species prefers freshwater or brackish habitats and, therefore, is almost absent in the saline waters of the German Bight.

Non-fish prey such as insects, polychaetes, molluscs or crustaceans were detected in small amounts in all previous studies. Cephalopods were detected in a single previous study (Durinck et al. 1994). We found no evidence that non-fish prey were consumed by red-throated divers in the German Bight and thus our results reinforce previous conclusions that these taxa are not an important part of the diet.

In summary, prey species of red-throated divers identified in this study occur in the study area as both adult (e.g. clupeids, sand lances) and juvenile fish (e.g. gadoids, flatfish, mackerels). Thus, the area seems to be a good foraging ground for red-throated divers. There is an overlap between the prey fish of red-throated divers and commercial fish species, such as herring and mackerel (ICES 2011, 2016, 2017a, b). This overlap increases the risk of gill-net mortality, which is a conservation issue in other regions such as the Baltic Sea. In the German Bight, there is a lower potential for such conflicts because trawls are more commonly used to fish as opposed to gill-nets. The oceanographic conditions [sea surface temperature (SST), salinity and chlorophyll a, NAO] were similar between the two sampling years and no important changes in prey community can be expected within such short timeframe, with the exception of the observed fluctuations in sand lance abundance. For this prey group, detections in the diet and reported catch rates (ICES 2018a, b) showed a similar trend. Reasons for this are unclear but sand lance productivity in the North Sea is known to fluctuate. Such fluctuations depend on a combination of several regulating factors including fishing, climate effects, density dependence and food availability (Wright et al. 2017; Lindegren et al. 2018). Although we present data from only two sampling years, the consistent pattern of prey species suggests a relatively stable diet that is likely to reflect the availability of these fish species in the study area. There are long-term increases in sea temperature and species usually associated with warmer waters are expanding their range to include the North Sea. Such species include European sardine and European anchovy (Kanstinger and Peck 2009). The diet of red-throated divers in the German Bight includes these expanding species and also recovering species such as mackerel, indicating that the dietary data may reflect changes in the fish community and some flexibility in prey consumption. However, a larger sample size across a broader temporal scale is required to fully support this conclusion.

The samples analysed here were collected in late winter and early spring, shortly before the migration to the breeding grounds. For non-breeding red-throated divers, little is known about energy expenditure, resource partitioning and

energy requirements during wintering, staging and migration. Schmutz (2014) suggested that marine conditions could affect adult survival of red-throated divers with indications of a higher risk of mortality during the non-breeding season. Red-throated divers are medium-sized birds with weight varying between 1400 g and 2000 g (own observations), and with high wing loading (Storer 1958; Lovvorn and Jones 1994). Despite this, these birds often need to cover long distances to their breeding grounds (<http://www.divertracking.com>; McCloskey et al. 2018), with some individuals travelling as far as 850 km or 1300 km in a single flight (Kleinschmidt et al. unpubl data). Weber and Houston (1997) showed the importance of resting sites for refuelling. Consequently, migration represents periods of high energetic demand and adequate energy reserves seem to be essential. If prey of rich calorific value becomes unavailable due to displacement effects, red-throated divers may fail to balance their energy budgets. In general, these birds winter in temperate marine waters with low ambient temperatures, consequently reliable and sufficient energy intake is likely to be a necessity and influences prey consumption.

Conclusion

Overall, our results demonstrate that the use of faecal samples coupled with DNA metabarcoding and NGS is a valid and appropriate approach to non-invasively study the diet composition of red-throated divers.

Our results provide important dietary data for red-throated divers in the German Bight, which is needed for a good understanding of their habitat preferences during wintering and spring staging. This baseline information can be used to evaluate changes associated with human developments in the offshore environment, changes in oceanography, or population declines. The results for the German Bight complement other dietary studies on red-throated divers that show a somewhat different composition of fish species, reflecting regional differences in fish fauna. Among a generalised prey spectrum, benthic-pelagic schooling fish seem to dominate the diet of red-throated divers (Cramp et al. 2004; Guse et al. 2009). In our study, five species groups are concluded to be major dietary components for red-throated divers in the German Bight. We found clupeids, mackerels, flatfish, and gadoids occurring in substantial proportions in both sampling years, and the frequency of sand lances varied between the two sampling years. Hence, the diet consistently includes some common species with a high nutritional value (Hislop et al. 1991; Ball et al. 2007), indicating the importance of these fish groups as prey items for red-throated divers in the German Bight. Red-throated divers stage in a specific habitat, mostly influenced by frontal zones in coastal areas in the German Bight (Skov and Prins 2001; Heinänen et al.

unpubl data). The preferred feeding at frontal zones may also explain the higher abundance of pelagic fish among the red-throated diver prey, where these species aggregate, while demersal species depend mainly on suitable sediments. Considering the effects of disturbance, displacement or barrier effects arising from anthropogenic activities such as ship traffic and offshore wind farms (Mendel et al. 2019), the broad prey spectrum that we found could indicate resilience of red-throated divers against changes in community composition of available fish or resilience against displacement from suitable habitat. However, if alternative sites of high-quality habitat are not sufficiently available, displacement may result in a decreased energy intake and subsequently poorer body condition. Thus, altered food accessibility as a result of disturbance or displacement could have severe effects on red-throated divers. In general, the availability of some prey species may explain, at least to some extent, the preference of this area as wintering and staging habitat. Further studies could aim to discern whether the birds use this area because of a high abundance of suitable and energy rich prey or if they simply feed on the most abundant prey.

Acknowledgements The DIVER project was supported by the Federal Ministry for Economic Affairs and Energy on the basis of a decision by the German Bundestag (funding ID 0325747 A/B). The library preparation, sequencing steps and bioinformatics training were supported by the Natural Environment Research Council (NERC) Biomolecular Analysis Facility at the University of Sheffield (NBAF-S) and funded by the NERC, UK (funding ID NBAF989). Thomas Grünkorn and Jorg Welcker completed our field team and provided valuable support. Thomas Grünkorn also contributed by ringing of the captured red-throated divers. Many thanks to Silke Waap for providing important advice during primer development, to Isa-Rita Russo for valuable help and advice in the lab and to the Molecular Ecology lab at Cardiff University for support and discussion. Sören Zenner (OS-Energy) allowed flexible organisation and provision of ships during the field seasons, and we thank the ship captains and crews of the MS Madog and MS Arctic Hunter for their reliability and support during fieldwork. Finally, we thank the reviewers for their valuable comments which helped to improve the manuscript.

Compliance with ethical standards

Conflict of interest The authors explicitly declare that they have no conflict of interest.

Ethical approval We herewith assure that the ethical rules as well as the legal requirements for the fieldwork have been met. All filed work (animal capture, sampling and tagging) was conducted under appropriate ethics and approvals; approved by BfN (Federal agency for Nature Conservation, Germany, 05.08.2014) and Ministry of Environment and Food Denmark (Danish—Veterinary and Food Administration 18.12.2014—2014-15-0201-00239).

Appendix

See Table 5.

References

- Alonso H, Granadeiro JP, Waap S, Xavier J, Symondson WO, Ramos JA, Catty P (2014) An holistic ecological analysis of the diet of Cory's shearwaters using prey morphological characters and DNA barcoding. *Mol Ecol* 23(15):3719–3733
- Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. *J Mol Biol* 215:403–410
- André C, Svedäng H, Knutsen H, Dahle G, Jonsson P, Ring AK, Sköld M, Jorde PE (2016) Population structure in Atlantic cod in the eastern North Sea-Skagerrak-Kattegat: early life stage dispersal and adult migration. *BMC Res Notes* 9(1):63
- Andreassen H, Ross SD, Siebert U, Andersen NG, Ronnenberg K, Gilles A (2017) Diet composition and food consumption rate of harbor porpoises (*Phocoena phocoena*) in the western Baltic Sea. *Mar Mamm Sci* 33:1053–1079. <https://doi.org/10.1111/mms.12421>
- Ball JR, Esler D, Schmutz JA (2007) Proximate composition, energetic value, and relative abundance of prey fish from the inshore eastern Bering Sea: implications for piscivorous predators. *Polar Biol* 30(6):699–708
- Barrett RT, Camphuysen K, Anker-Nilssen T, Chardine JW, Furness RW, Garthe S, Veit R (2007) Diet studies of seabirds: a review and recommendations. *ICES J Mar Sci* 64(9):1675–1691
- Beek FV, Rijnsdorp AV, Clerck RD (1989) Monitoring juvenile stocks of flatfish in the Wadden Sea and the coastal areas of the southeastern North Sea. *Helgoländer Meeresuntersuchungen* 43(3):461
- Bellebaum J, Diederichs A, Kube J, Schulz A, Nehls G (2006) Flucht- und Meidedistanzen überwinternder Seetaucher und Meeresenten gegenüber Schiffen auf See. *Ornith. Rundbr. Mecklenburg Vorpommern* 45. Sonderheft 1:86–90
- Berry O, Bulman C, Bunce M, Coghlan M, Murray DC, Ward RD (2015) Comparison of morphological and DNA metabarcoding analyses of diets in exploited marine fishes. *Mar Ecol Prog Ser* 540:167–181
- BirdLife International (2018) Species factsheet: *Gavia stellata*. <http://www.birdlife.org>. Accessed 09 May 2018
- Bolger AM, Lohse M, Usadel B (2014) Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* 30:2114–2120
- Bolle LJ, Dapper R, Witte JJ, Van Der Veer HW (1994) Nursery grounds of dab (*Limanda limanda* L.) in the southern North Sea. *Neth J Sea Res* 32(3–4):299–307
- Fox J, Bouchet-Valat, M (2018) Rcmdr: R commander. R package version 2.4-4
- Campos WL, Kloppmann M, von Westernhagen H (1994) Inferences from the horizontal distribution of dab *Limanda limanda* (L.) and flounder *Platichthys flesus* (L.) larvae in the southeastern North Sea. *Neth J Sea Res* 32(3–4):277–286
- Clare EL, Chain FJ, Littlefair JE, Cristescu ME (2016) The effects of parameter choice on defining molecular operational taxonomic units and resulting ecological analyses of metabarcoding data. *Genome* 59(11):981–990
- Cramp S, Simmons KE, BWPI (2004) Birds of the western palearctic—interactive (DVD-ROM). BirdGuides Ltd, Sheffield
- Davoren GK, Montevecchi WA, Anderson JT (2003) Distributional patterns of a marine bird and its prey: habitat selection based on prey and conspecific behaviour. *Mar Ecol Prog Ser* 256:229–242
- Deagle BE, Tollit DJ (2007) Quantitative analysis of prey DNA in pinniped faeces: potential to estimate diet composition. *Conserv Genet* 8(3):743–747
- Deagle BE, Tollit DJ, Jarman SN, Hindell MA, Trites AW, Gales NJ (2005) Molecular scatology as a tool to study diet: analysis of prey DNA in scats from captive Steller sea lions. *Mol Ecol* 14(6):1831–1842

- Deagle BE, Gales NJ, Evans K, Jarman SN, Robinson S, Trebilco R, Hindell MA (2007) Studying seabird diet through genetic analysis of faeces: a case study on macaroni penguins (*Eudyptes chrysolophus*). *PLoS One* 2(9):e831
- Deagle BE, Kirkwood R, Jarman SN (2009) Analysis of Australian fur seal diet by pyrosequencing prey DNA in faeces. *Mol Ecol* 18(9):2022–2038
- Deagle BE, Thomas AC, Shaffer AK, Trites AW, Jarman SN (2013) Quantifying sequence proportions in a DNA-based diet study using Ion Torrent amplicon sequencing: which counts count. *Mol Ecol Resour* 13(4):620–633
- Deagle BE, Thomas AC, McInnes JC, Clarke LJ, Vesterinen EJ, Clare EL, Kartzinel TR, Eveson JP (2018) Counting with DNA in metabarcoding studies: how should we convert sequence reads to dietary data? *Mol Ecol* 28:391–406
- DFS (2016) Species factsheets for the Wadden Sea fish Strategy. <https://rijkswaddenzee.nl/wp-content/uploads/2016/03/NHP0090-factsheets-DEF-ONLINE-LOW2.pdf>. Accessed 22 June 2018
- Dickey-Collas M, Nash RD, Brunel T, Van Damme CJ, Marshall CT, Payne MR, Hintzen NT (2010) Lessons learned from stock collapse and recovery of North Sea herring: a review. *ICES J Mar Sci* 67(9):1875–1886
- Dierschke V, Garthe S, Mendel B (2006) Possible conflicts between offshore wind farms and seabirds in the German sectors of North Sea and Baltic Sea. In: Köller J, Köppel H, Peters W (eds) (Hrsg): offshore wind energy. Research on environmental impacts. Springer, Berlin, pp 121–143
- Dierschke V, Exo K-M, Mendel B, Garthe S (2012) Gefährdung von Sterntaucher *Gavia stellata* und Prachtaucher *G. arctica* in Brut-, Zug- und Überwinterungsgebieten—eine Übersicht mit Schwerpunkt auf den deutschen Meeresgebieten. *Vogelwelt* 133:163–194
- Durinck J, Skov H, Danielsen F, Christensen KD (1994) Vinterføden hos Rødstrubet Lom *Gavia stellata* i Skagerrak. *Dansk Ornithol Foren Tidsskr* 88:39–41
- Edgar RC (2010) Search and clustering orders of magnitude faster than BLAST. *Bioinformatics* 26(19):2460–2461
- Edgar RC (2013) UPARSE: highly accurate OTU sequences from microbial amplicon reads. *Nat Methods* 10(10):996
- Edgar RC (2016) UNOISE2 improved error-correction for Illumina 16S and ITS amplicon sequencing. *bioRxiv*. 81257
- Engelhard GH, Peck MA, Rindorf A, Smout SC, van Deurs M, Raab K, Brunel T (2013) Forage fish, their fisheries, and their predators: who drives whom. *ICES J Mar Sci* 71(1):90–104
- Fauchald P, Skov H, Skern-Mauritzen M, Johns D, Tveraa T (2011) Wasp-waist interactions in the North Sea ecosystem. *PLoS One* 6(7):e22729
- Garthe S, Hüppop O (2004) Scaline possible adverse effects of marine wind farms on seabirds: developing and applying a vulnerable index. *J Appl Ecol* 41:724–734
- Garthe S, Sonntag N, Schwemmer P, Dierschke V (2007) Estimation of seabird numbers in the German North Sea throughout the annual cycle and their biogeographic importance. *Vogelwelt* 128:163–178
- Garthe S, Markones N, Mendel B, Sonntag N, Krause J (2012) Protected areas for seabirds in German offshore waters: designation, retrospective consideration and current perspectives. *Biol Conserv* 156:126–135
- Garthe S, Guse N, Montevecchi WA, Rail JF, Grégoire F (2014) The daily catch: flight altitude and diving behavior of northern gannets feeding on Atlantic mackerel. *J Sea Res* 1(85):456–462
- Garthe S, Schwemmer H, Markones N, Müller S, Schwemmer P (2015) Distribution, seasonal dynamics and population trend of divers *Gavia spec.* in the German Bight (North Sea). *Vogelwarte* 53:121–138
- Goyert HF, Gardner B, Sollmann R, Veit RR, Gilbert AT, Connelly EE, Williams KA (2016) Predicting the offshore distribution and abundance of marine birds with a hierarchical community distance sampling model. *Ecol Appl* 26(6):1797–1815
- Guse N, Garthe S, Schirmeister B (2009) Diet of red-throated divers *Gavia stellata* reflects the seasonal availability of Atlantic herring *Clupea harengus* in the southwestern Baltic Sea. *J Sea Res* 62(4):268–275
- Haelters J, Kerckhof F, Toussaint E, Jauniaux T, Degraer S (2012) The diet of harbour porpoises bycaught or washed ashore in Belgium, and relationship with relevant data from the strandings database. Royal Belgian Institute of Natural Sciences, Marine Ecosystem Management Unit, Oostende, pp 1–48
- Harris MP, Wanless S (1991) The importance of the lesser sandeel *Ammodytes marinus* in the diet of the shag *Phalacrocorax aris-totilis*. *Ornis Scandinavica* 22(4):375–382
- Hislop JRG, Harris MP, Smith JGM (1991) Variation in the calorific value and total energy content of the lesser sandeel (*Ammodytes marinus*) and other fish preyed on by seabirds. *J Zool* 224(3):501–517
- Hufnagl M, Peck MA, Nash RD, Pohlmann T, Rijnsdorp AD (2013) Changes in potential North Sea spawning grounds of plaice (*Pleuronectes platessa* L.) based on early life stage connectivity to nursery habitats. *J Sea Res* 84:26–39
- Huson DH, Auch AF, Qi J, Schuster SC (2007) MEGAN analysis of metagenomic data. *Genome Res* 17(3):377–386
- Huson DH, Beier S, Flade I, Górka A, El-Hadidi M, Mitra S, Ruscheweyh HJ, Tappu R (2016) MEGAN community edition—interactive exploration and analysis of large-scale microbiome sequencing data. *PLoS Comput Biol* 12(6):e1004957
- ICES (2006a) Species factsheet: Atlantic herring (*Clupea harengus*), an electronic atlas of 15 North Sea fish species. <http://www.ices.dk/marine-data/maps/Pages/ICES-FishMap.aspx>. Accessed 22 June 2018
- ICES (2006b) Species factsheet: European sprat (*Sprattus sprattus*), electronic atlas of 15 North Sea fish species. <http://www.ices.dk/marine-data/maps/Pages/ICES-FishMap.aspx>. Accessed 22 June 2018
- ICES (2008) Report of the ICES Advisory Committee 2008. ICES Advice, 2008. Book 6, North Sea. ISBN 978-87-7482-051-2
- ICES (2011) Report of the working Group on multispecies assessment methods (WGSAM), 10–14 October 2011, Woods Hole, USA, ICES Document CM 2011/SSGSUE:10
- ICES (2016) Greater North Sea Ecoregion-Ecosystem overview. In Report of the ICES Advisory Committee, 2016. ICES Advice 2016. Book 6: section 6.1. pp 1–22
- ICES (2017a) Greater North Sea Ecoregion-Ecosystem—fisheries overview. <https://doi.org/10.17895/ices.pub.3116>
- ICES (2017b) Report of the working group on assessment of demersal stocks in the North Sea and Skagerrak (2017), 26 April–5 May 2017, ICES HQ. ICES CM 2017/ACOM:21
- ICES (2018a) Stock assessment database. Copenhagen, Denmark. ICES. <http://standardgraphs.ices.dk>. Accessed 02 Jan 2019
- ICES (2018b) ICES advice on fishing opportunities, catch and effort Greater North Sea Ecoregion.san.sa.2r. <http://www.ices.dk/sites/pub/Publication%20Reports/Advice/2018/2018/san.sa.2r.pdf>. Accessed 02 Jan 2019
- ICES (2018c) ICES Advice on fishing opportunities, catch and effort Greater North Sea Ecoregion.san.sa.1r. <http://www.ices.dk/sites/pub/Publication%20Reports/Advice/2018/2018/san.sa.1r.pdf>. Accessed 02 Jan 2019
- Jansen T (2014) Pseudocollapse and rebuilding of North Sea mackerel (*Scomber scombrus*). *ICES J Mar Sci* 71:299–307
- Jansen T, Gislason H (2013) Population structure of Atlantic mackerel (*Scomber scombrus*). *PLoS One* 8(5):e64744

- Jansen T, Kristensen K, Payne M, Edwards M, Schrum C, Pitois S (2012a) Long-term retrospective analysis of mackerel spawning in the North Sea: a new time series and modeling approach to CPR data. *PLoS One* 7(6):e38758
- Jansen T, Campbell A, Kelly C, Hátún H, Payne MR (2012b) Migration and fisheries of North East Atlantic mackerel (*Scomber scombrus*) in autumn and winter. *PLoS One* 7(12):e51541
- Jansen T, Kristensen K, Van der Kooij J, Post S, Campbell A, Utne KR, Hatfield EM (2015) Nursery areas and recruitment variation of Northeast Atlantic mackerel (*Scomber scombrus*). *ICES J Mar Sci* 72(6):1779–1789
- Kanstinger P, Peck MA (2009) Co-occurrence of European sardine (*Sardina pilchardus*), anchovy (*Engraulis encrasicolus*) and sprat (*Sprattus sprattus*) larvae in southern North Sea habitats: abundance, distribution and biochemical-based condition. *Scientia Marina* 73(S1):141–152
- Kumar S, Stecher G, Tamura K (2016) MEGA7: molecular evolutionary genetic analysis version 7.0 for bigger datasets. *Mol Biol Evol* 33:1870–1874
- Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, Valentin F, Wallace IM, Wilm A, Lopez R, Thompson JD, Gibson TJ, Higgins DG (2007) Clustal W and clustal X version 2.0. *Bioinformatics* 23:2947–2948
- Leray M, Yang JY, Meyer CP, Mills SC, Agudelo N, Ranwez V, Machida RJ (2013) A new versatile primer set targeting a short fragment of the mitochondrial COI region for metabarcoding metazoan diversity: application for characterizing coral reef fish gut contents. *Front Zool* 10(1):34
- Lindegren M, Van Deurs M, MacKenzie BR, Worsøe Clausen L, Christensen A, Rindorf A (2018) Productivity and recovery of forage fish under climate change and fishing: North Sea sandeel as a case study. *Fish Oceanogr* 27(3):212–221
- Lovvorn JR, Jones DR (1994) Biomechanical conflicts between adaptations for diving and aerial flight in estuarine birds. *Estuaries* 17(1):62
- Lynam PC, Lope M, Möllmann C, Helaouët P, Bayliss-Brown GA, Stenseth NC (2017) Interactions between top-down and bottom-up control in marine food webs. *PNAS* 114:1952–1957
- Madsen FJ (1957) On the food habits of some fish-eating birds in Denmark. Divers, grebes, mergansers, and auks. *Dan Rev Game Biol* 3:19–83
- Magoc T, Salzberg SL (2011) FLASH: fast length adjustment of short reads to improve genome assemblies. *Bioinformatics* 27:2957–2963
- McCloskey SE, Uher-Koch BD, Schmutz JA, Fondell TF (2018) International migration patterns of red-throated loons (*Gavia stellata*) from four breeding populations in Alaska. *PLoS One* 13(1):e0189954
- Méheust E, Alfonsi E, Le Ménec P, Hassani S, Jung JL (2015) DNA barcoding for the identification of soft remains of prey in the stomach contents of grey seals (*Halichoerus grypus*) and harbour porpoises (*Phocoena phocoena*). *Mar Biol Res* 11(4):385–395
- Meier RE, Votier SC, Wynn RB, Guilford T, McMinn Grivé M, Rodríguez A, Newton J, Maurice L, Chouvelon T, Dessier A, Trueman CN (2017) Tracking, feather moult and stable isotopes reveal foraging behaviour of a critically endangered seabird during the non-breeding season. *Divers Distrib* 23(2):130–145
- Mendel B, Sonntag N, Wahl J, Schwemmer P, Dries H, Guse N, Müller S, Garthe S (2008) Artensteckbriefe von See- und Wasservögeln der deutschen Nord- und Ostsee. Bundesamt für Naturschutz, Bonn
- Mendel B, Schwemmer P, Peschko V, Müller S, Schwemmer H, Mercker M, Garthe S (2019) Operational offshore wind farms and associated ship traffic cause profound changes in distribution patterns of Loons (*Gavia* spp.). *J Environ Manag* 231:429–438. <https://doi.org/10.1016/j.jenvman.2018.10.053>
- Montevicchi WA, Ricklefs RE, Kirkham IR, Gabaldon D (1984) Growth energetics of nestling northern gannets (*Sula bassanus*). *Auk* 1:334–341
- Montevicchi WA, Birt VL, Cairns DK (1988) Dietary changes of seabirds associated with local fisheries failures. *Biol Oceanogr* 5(3):153–161. <https://doi.org/10.1080/01965581.1987.10749511>
- Motos L, Uriarte ANDRÉS, Valencia V (1996) The spawning environment of the Bay of Biscay anchovy (*Engraulis encrasicolus*). *Sci Mar* 60(SUPPL 2):237–255
- Munk P (2014) Fish larvae at fronts: horizontal and vertical distributions of gadoid fish larvae across a frontal zone at the Norwegian Trench. *Deep Sea Res Part II* 107:3–14
- Munk P, Larsson PO, Danielssen DS, Moksness E (1999) Variability in frontal zone formation and distribution of gadoid fish larvae at the shelf break in the northeastern North Sea. *Mar Ecol Prog Ser* 177:221–233
- Nehls G, Gienapp P (1997) Prey selection and foraging behaviour of Cormorants *Phalacrocorax carbo* in the Wadden Sea. *Vogelwelt* 118:33–40
- Pedersen J, Hislop JRG (2001) Seasonal variations in the energy density of fishes in the North Sea. *J Fish Biol* 59(2):380–389
- Petersen IK, Christensen TK, Kahlert J, Desholm M, Fox AD (2006) Final results of bird studies at offshore wind farms at Nysted and Horns rev, Denmark. NERI report, commissioned by DONG Energy and Vattenfall A/S, DK. http://www.folkcenter.net/gb/rd/wind-energy/publications_downloads_wind. Accessed 01 Apr 2018
- Pompanon F, Deagle BE, Symondson WO, Brown DS, Jarman SN, Taberlet P (2012) Who is eating what: diet assessment using next generation sequencing. *Mol Ecol* 21(8):1931–1950
- Quillfeldt P, Thorn S, Richter B, Nabte M, Coria N, Masello JF, Massaro M, Neves VC, Libertelli M (2017) Testing the usefulness of hydrogen and compound-specific stable isotope analyses in seabird feathers: a case study in two sympatric Antarctic storm-petrels. *Mar Biol* 164(9):192
- Quinn JT, Hamilton DJ, Hebert CE (2017) Fatty acid composition and concentration of alternative food of Semipalmated Sandpipers (*Calidris pusilla*) in the upper Bay of Fundy, Canada. *Can J Zool* 95(8):565–573
- Reimchen TE, Douglas S (1984) Feeding schedule and daily food consumption in red-throated loons (*Gavia stellata*) over the pre fledging period. *Auk* 101:593–599
- Ronconi RA, Swaim ZT, Lane HA, Hunnewell RW, Westgate AJ, Koopman HN (2010) Modified hoop-net techniques for capturing birds at sea and comparison with other capture methods. *Mar Ornithol* 38(1):23–29
- Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB, Lesniewski RA, Oakley BB, Parks DH, Robinson CJ, Sahl JW (2009) Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Appl Environ Microbiol* 75:7537–7541
- Schmutz JA (2014) Survival of adult red-throated loons (*Gavia stellata*) may be linked to marine conditions. *Waterbirds* 37:118–124
- Simon C, Frati F, Beckenbach A, Crespi B, Liu H, Flook P (1994) Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. *Ann Entomol Soc Am* 87(6):651–701
- Skov H, Prins E (2001) Impact of estuarine fronts on the dispersal of piscivorous birds in the German Bight. *Mar Ecol Prog Ser* 214:279–287
- Soininen EM, Valentini A, Coissac E, Miquel C, Gielly L, Brochmann C, Brysting AK, Sønstebo Ims RA, Yoccoz NG, Taberlet P (2009) Analysing diet of small herbivores: the efficiency of DNA barcoding coupled with high-throughput pyrosequencing

- for deciphering the composition of complex plant mixtures. *Front Zool* 6:16
- Stockdale JE (2018) Using high-throughput sequencing to track habitat use by thrushes exploiting heterogeneous farmland landscapes. Ph.D. Thesis, Cardiff University
- Storer RW (1958) Loons and their wings. *Evolution* 12(2):262–263
- Tollit DJ, Schulze AD, Trites AW, Olesiuk PF, Crockford SJ, Gelatt TS, Miller KM (2009) Development and application of DNA techniques for validating and improving pinniped diet estimates. *Ecol Appl* 19(4):889–905
- Trueman CN, MacKenzie KM, St John Glew K (2017) Stable isotope-based location in a shelf sea setting: accuracy and precision are comparable to light-based location methods. *Methods Ecol Evol* 8(2):232–240
- van der Kooij J, Fässler SMM, Stephens D, Readdy L, Scott BE, Roel BA (2016) Opportunistically recorded acoustic data support Northeast Atlantic mackerel expansion theory. *ICES J Mar Sci* 73(4):1115–1126. <https://doi.org/10.1093/icesjms/fsv243>
- Vesterinen EJ, Lilley T, Laine VN, Wahlberg N (2013) Next Generation Sequencing of fecal DNA reveals the dietary diversity of the widespread insectivorous predator Daubenton's Bat (*Myotis daubentonii*) in southwestern Finland. *PLoS One* 8(11):e82168. <https://doi.org/10.1371/journal.pone.0082168>
- Vestheim H, Jarman SN (2008) Blocking primers to enhance PCR amplification of rare sequences in mixed samples—a case study on prey DNA in Antarctic krill stomachs. *Front Zool* 5(1):12
- Waap S (2015) Trophic relationships among pelagic predators of the deep seas of the Madeira islands. Ph.D. Thesis, Cardiff University
- Waap S, Symondson WOC, Granadeiro JP, Alonso H, Serra-Gonçalves C, Dias MP, Catry P (2017) The diet of a nocturnal pelagic predator, the Bulwer's petrel, across the lunar cycle. *Sci Rep* 7:1384. <https://doi.org/10.1038/s41598-017-01312-3>
- Weber TP, Houston AI (1997) Flight costs, flight range and the stopover ecology of migrating birds. *J Anim Ecol* 66:297–306
- Whitworth DL, Takekawa JY, Carter HR, Mciver WR (1997) A night-lighting technique for at-sea capture of Xantus' Murrelets. *Colon Waterbirds* 20:525–531
- Wright PJ, Orpwood JE, Scott BE (2017) Impact of rising temperature on reproductive investment in a capital breeder: the lesser sandeel. *J Exp Mar Biol Ecol* 486:52–58
- Žydelis R (2002) Habitat selection of waterbirds wintering in Lithuanian coastal zone of the Baltic Sea. Ph.D. thesis. University of Vilnius

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Gavia stellata

3X

8. CHAPTER 2

Annual movements of a migratory seabird - the NW European red-throated diver (*Gavia stellata*) – reveals high individual repeatability but low migratory connectivity

Birgit Kleinschmidt, Claudia Burger, Paco Bustamante Monika Dorsch, Stefan Heinänen, Julius Morkūnas, Ramūnas Žydelis, Georg Nehls, Petra Quillfeldt

Marine Biology (2022) 169:114: 1-19



Annual movements of a migratory seabird—the NW European red-throated diver (*Gavia stellata*)—reveals high individual repeatability but low migratory connectivity

Birgit Kleinschmidt^{1,2} · Claudia Burger² · Paco Bustamante^{7,8} · Monika Dorsch² · Stefan Heinänen^{3,6} · Julius Morkūnas⁴ · Ramūnas Žydelis^{3,5} · Georg Nehls² · Petra Quillfeldt¹

Received: 27 December 2021 / Accepted: 29 July 2022
 © The Author(s) 2022

Abstract

In this study, the annual movements of a seabird species, the red-throated diver (*Gavia stellata*), were investigated in space and time. Between 2015 and 2017, 33 individuals were fitted with satellite transmitters at the German Bight (eastern North Sea). In addition, stable isotope analyses of feathers ($\delta^{13}\text{C}$) were used to identify staging areas during the previous moult. The German Bight is an important area for this species, but is also strongly affected by anthropogenic impacts. To understand how this might affect populations, we aimed to determine the degree of connectivity and site fidelity, and the extent to which seasonal migrations vary among different breeding locations in the high Arctic. Tagged individuals migrated to Greenland ($n=2$), Svalbard ($n=2$), Norway ($n=4$) and northern Russia ($n=25$). Although individuals from a shared breeding region (northern Russia) largely moved along the same route, individuals dispersed to different, separate areas during the non-breeding phase. Kernel density estimates also overlapped only partially, indicating low connectivity. The timing of breeding was correlated with the breeding longitude, with 40 days later arrival at the easternmost than westernmost breeding sites. Repeatability analyses between years revealed a generally high individual site fidelity with respect to spring staging, breeding and moulting sites. In summary, low connectivity and the distribution to different sites suggests some resilience to population decline among subpopulations. However, it should be noted that the majority of individuals breeding in northern Russia migrated along a similar route and that disturbance in areas visited along this route could have a greater impact on this population. In turn, individual site fidelity could indicate low adaptability to environmental changes and could lead to potential carry-over effects. Annual migration data indicate that conservation planning must consider all sites used by such mobile species.

Keywords Red-throated diver · Red-throated loon · Satellite tracking · Stable isotopes · Temporal-spatial pattern · Migratory connectivity · Site fidelity · Anthropogenic pressure

Responsible Editor: T.A. Clay.

✉ Birgit Kleinschmidt
 Birgit.Kleinschmidt@bio.uni-giessen.de

¹ Department of Animal Ecology and Systematics, Justus Liebig University Giessen, 35392 Giessen, Germany

² BioConsult SH, 25813 Husum, Germany

³ DHI, 2970 Hørsholm, Denmark

⁴ Marine Research Institute, Klaipėda University, 92294 Klaipėda, Lithuania

⁵ Present Address: Ornitela UAB, 03228 Vilnius, Lithuania

⁶ Present Address: Novia University of Applied Sciences, Raseborgsvägen 9, 10600 Ekenäs, Finland

⁷ Littoral Environnement et Sociétés (LIENSs), UMR 7266, CNRS-Université de la Rochelle, 2 rue Olympe de Gouges, 17000 La Rochelle, France

⁸ Institut Universitaire de France (IUF), 1 rue Descartes, 75005 Paris, France

Published online: 22 August 2022

Springer

Background

Migratory birds are increasingly affected by environmental changes, disturbances and threats along their migration routes (Wilcove and Wikelski 2008). Therefore, information about annual movements is important for effective conservation and management (Marra et al. 2018; Johnston et al. 2020). Many species, such as seabirds, are long-lived with delayed sexual maturity and low annual reproductive rates (Schreiber and Burger 2001). Survival of adult birds, as well as reproductive success, therefore, influence their population growth rates (Sæther and Bakke 2000). Different migratory strategies, including different winter locations, can influence fitness differently, through variable energy costs and winter habitat conditions (Alves et al. 2013). Altered environmental conditions in the stationary non-breeding periods can affect migratory timing or carry-over to affect survival and reproduction in the breeding areas (Marra et al. 1998; Harrison et al. 2011; Winkler et al. 2014; Rushing et al. 2016). Information about the site use of migratory birds throughout the whole annual cycle and their ability to respond to environmental change is, therefore, important to conserve such long-lived and mobile species (Moore et al. 2005; Runge et al. 2014).

Migratory connectivity, i.e. the degree to which individuals from one breeding or wintering population stay together and use similar sites along the annual cycle, provides essential information to assess the impact of environmental change in habitats along the migratory route (Webster et al. 2002; Martin et al. 2007). The degree of migratory connectivity determines to which extent different breeding populations experience similar non-breeding conditions (Esler 2000; Taylor and Norris 2010; Cresswell 2014; Finch et al. 2017). Migratory connectivity is defined along a continuum from strong connectivity (low interpopulation spread and use of population-specific non-breeding areas) to a low or diffuse connectivity (high interpopulation spread) (Webster et al. 2002; Newton 2008; Finch et al. 2017). In a low connectivity scenario, individuals from a given breeding population may mix with individuals from other breeding regions during the non-breeding season (Finch et al. 2017). Recent studies (i.e. Gilroy et al. 2016) introduced further terms such as migratory diversity which expresses the within-population variability in migratory movements and suggest that migratory diversity may help to facilitate species responses to environmental change.

To understand how migrants might be affected by environmental change in breeding and non-breeding sites, we need to understand the migratory pattern in space and time such as the variation in migration duration, number of staging stops and temporal pattern among the different

migration routes and breeding sites. These baseline measurements are necessary to monitor future shifts in timing or to identify carry-over effects (Gordo 2007; Studds et al. 2008; Duijns et al. 2017). Migratory movements and their timings can be linked with geographic position, resource allocation or climatic variables on the breeding areas (Conklin 2010). Climatic conditions on the wintering grounds can also affect migratory timing, with some species responding to milder winters with earlier arrival on the breeding grounds (Gunnarson and Tómasson 2011). The total duration of migration and thus the arrival time at the destination may also depend on the conditions at stop-over sites, which influence the decision to stay or continue (Weber and Houston 1997; Klinner et al. 2020). Local resource availability and competition at moulting, wintering or stop-over habitats are important for migratory movements (Kokko 1999; Moore 2005; Kölzsch et al. 2016; Fayet et al. 2017). Reduced habitat quality at these locations could result in delayed or extended stays and altered timing of annual movements (Marra et al. 1998).

Individual fidelity towards sites used throughout the year, as well as temporal repeatability, could also be important for predicting the response of migrants to environmental change. Individual behavioural consistency might determine how individuals respond to environmental change and how much populations could be affected by habitat changes and possible carry-over effects (Reed et al. 2009; Dias et al. 2010). Individuals with a high site fidelity may be less flexible to voluntarily change sites and more sensitive to displacement caused by disturbance. Consequently, less flexible individuals might adapt more slowly to a new environment, than an individual that is familiar with multiple sites and can use flexible strategies (Catry et al. 2004; McFarlane et al. 2014; Merkel et al. 2021). Individual site utilisation and movements within and between years are, therefore, important to consider in conservation decisions (Croxall et al. 2005; González-Solís et al. 2007).

In this study, we analysed the migratory behaviour of a seabird species, the red-throated diver (*Gavia stellata*), that is increasingly influenced by human activities in one of their most important winter and spring staging areas in Europe, the German Bight (eastern North Sea) (Garthe et al. 2007, 2015; Dierschke et al. 2012; Burger et al. 2019; Mendel et al. 2019; Heinänen et al. 2020). In this winter population, strong avoidance of offshore wind farm areas was observed (Mendel et al. 2019; Heinänen et al. 2020; Vilela et al. 2021) but, so far, no decline in wintering population numbers of this long-lived species (Vilela et al. 2021). Red-throated divers are listed in Annex II of the Bern Convention, Annex I of the EU Birds Directive and as critically endangered on the HELCOM (Helsinki Commission) convention (BirdLife International 2022). The species is widespread in the Holarctic, with breeding areas in the Arctic tundra regions north of

60° latitude and wintering areas in temperate coastal ocean waters. Breeding populations in Shetland, Sweden, Finland and Greenland have been linked with wintering areas such as the Baltic Sea, Skagerrak, the North Sea and further south to the Bay of Biscay (Cramp and Simmons 1977; Okill 1994; Wetlands International 2019). Ring recoveries suggest that younger birds move further south during winter than older birds (Okill 1994).

Moult is one of the three main energy-demanding events in the annual cycle of birds and usually occurs at a different time from breeding and migration (Newton 2009, 2011). Information about space use in this sensitive period of the year is important for conservation measures, but little is known about the temporal and spatial patterns of moult in red-throated divers. To date, it is known that red-throated divers moult their wing feathers simultaneously, rendering them temporarily flightless. Wing moult takes place in autumn (August to November, Stresemann and Stresemann 1966), in an area that is visited after leaving the breeding area. Recoveries of dead birds washed up on the coast in the North and Baltic Sea have shown that the birds moult their wing feathers in this period and also change from breeding to winter plumage (Berndt and Drenckhahn 1974, Mendel et al. 2008). In the following spring, birds moult their body feathers back into the breeding plumage (Stresemann and Stresemann 1966).

We used satellite telemetry which has been shown to be highly suitable to study migratory movements and spatial-temporal patterns of red-throated divers within and between years (Schmutz 2014; Paruk et al. 2015; Spiegel et al. 2017; McCloskey et al. 2018). Additionally, we used carbon stable isotope analyses of neck feathers in combination with satellite tracking data to infer moulting areas used prior to capture. Stable isotope values of a predator are related to those of its prey and the area where the predator foraged. Stable isotope values of prey vary with its trophic position and geographic region. Nitrogen stable isotope values ($\delta^{15}\text{N}$) increase with trophic position, while carbon stable isotope values ($\delta^{13}\text{C}$) depend more on the carbon uptake by the primary producer and thus differs among habitats (Peterson and Frey 1987; Frey 1988; Hobson 1999; Cherel and Hobson 2007).

We aimed to describe the annual cycle of red-throated divers captured in the eastern German Bight. We focussed on migratory connectivity, how the breeding location influences the temporal pattern of annual movements and on individual site fidelity between years. In particular, we aimed to test the following hypotheses: (1) in accordance with ring recoveries of individuals from Sweden, Britain and other regions in the capture area (Okill 1994; Hemmingson and Eriksson 2002) red-throated divers display a low degree of migratory connectivity, in particular: (1a) individuals from one or more breeding region mix in one non-breeding area

(capture site) and (1b) individuals from one breeding region spread during migration and their stationary non-breeding home ranges do not overlap, (2) the location of the breeding area (longitude/latitude) affects the timing and pattern of annual movements, (3) similar to the high site fidelity to their breeding areas (Okill 1992), individual red-throated divers repeatedly utilise the same areas during their key life history stages between years.

Methods

Fieldwork

We obtained positions of 33 red-throated divers equipped with Argos satellite transmitters (platform transmitter terminals, PTTs) in late winter to early spring (February–April) of 2015 to 2017. Birds were captured in the eastern part of the German Bight (North Sea), approximately 20 km west of the islands of Sylt and Amrum. We captured divers using the night-lighting technique (Whitworth et al. 1997; Ronconi et al. 2010). For a detailed description of tagging, see Burger et al. (2019), Kleinschmidt et al. (2019), Heinänen et al. (2020) and www.divertracking.com. We used implantable PTTs manufactured by Telonics, Inc. (40 units) and Sirtrack, Ltd (5 units). Transmitters were programmed using varying duty cycles with 3 or 4 transmission hours and 12–24 h intervals during winter and 60–68 h intervals between transmissions during the breeding season. Blood samples were taken and stored on Whatman FTA cards (Whatman FTA card technology, Sigma Aldrich) to sex the birds genetically. As the proportion of male birds (6 out of 33) was clearly underrepresented, we did not pursue further analyses regarding sex-specific differences, but combined male and female data for further analyses. A detailed description on the genetic sex determination is provided in the supplementary material (A9).

Data filtering

The tracking data were filtered to reduce noise from location fixes with low or unknown accuracy. Filtering followed the approach in Dorsch et al. (2019), Burger et al. (2019), and Heinänen et al. (2020) and was conducted using the package ‘argosfilter’ (Freitas et al. 2008) in R (R Core Team 2018). First, the `sdfilter` Filter (lat, lon, dtime, lc, vmax = 20) was applied. Then, all locations with unrealistic swimming/flying speeds were removed, using the McConnell algorithm (McConnell et al. 1992), unless the point was located at less than 5 km from the previous location. Second, `ArcGISv.10.1` (ESRI 2012) was used to further inspect the filtered dataset and any remaining obvious outliers, such as unrealistic positions were removed from the dataset. Finally, positions

recorded during the first 2 weeks after the transmitter implantation were excluded from the data set due to possible impacts of capture and surgery on the behaviour during recovery period.

Altogether we received 29,053 satellite transmitter positions. After filtering, 22,744 positions were left for further analyses. Considering the quality of the data, we found that 39.4% ($n=8,962$) of the positions were categorised in location classes 3–1 and 60.6% ($n=13,782$) of positions were assigned to location classes 0–B (Table A2, CLS 2013).

Data collection and definition of terms and seasons

We used the migratory pattern observed in this dataset to define seasons within the annual cycle (Fig. 1, Table 1). The timing of site use varied from individual to individual and from year to year (Fig. 1). Therefore, we decided to define each season by the months in which at least one diver showed activity consistent with that season (i.e. migratory movements, or settlement during breeding, moulting or wintering season, Table 1). After spending some time on inland lakes, presumably for nesting, some red-throated divers moved to adjacent marine waters. Depending on the time period, they spent for nesting, these individuals are likely

failed breeders or non-breeders. We did not consider these as staging periods as long as the diver stayed in the presumed breeding area.

Autumn moult takes place in areas located along the migration route between breeding and wintering areas (Berndt and Drenckhahn 1990; Mendel et al. 2008) and is assumed to involve a stationary period of ≥ 21 days, including a flightless period. Hence, we divided the autumn migration from breeding to moult and from moult to wintering (Table 1).

We defined staging sites along migration routes as areas where an individual diver spent ≥ 5 days. Short stop-overs < 5 days were not considered in separate analyses. This classification of staging and stop-over behaviour followed Warnock (2010).

Analysing tracking data

PTTs were deployed over three separate years so ordinal date (day of the year) was used as the temporal variable allowing comparisons to be made across years.

We used ArcGISv.10.1 (ESRI 2012), QGISv2.18 (QGIS Development Team 2018) and two projections (Lambert Azimuthal Equal Area projection: ETRS89/ETRS-LAEA

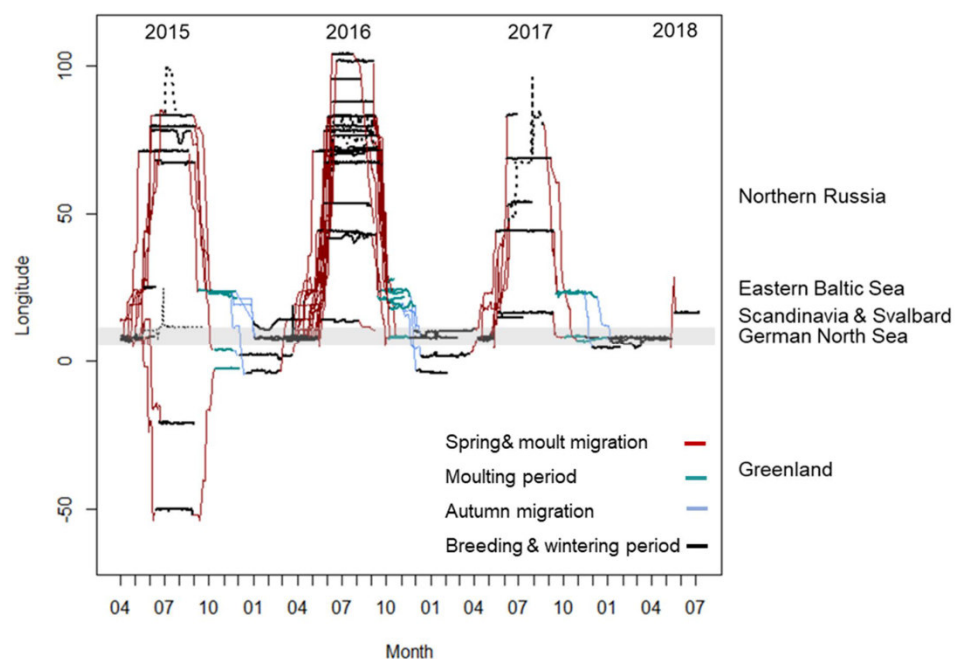


Fig. 1 Longitudinal migration pattern of all tracked red-throated divers ($n=33$) during three consecutive years. Important regions utilised during breeding, moulting and wintering season are indicated on

the right side. Individuals that did not show a clear breeding settlement are indicated with a dotted line

Table 1 Definition of terms and seasons and corresponding data collection used in this dataset

Season	Spring migration	Arrival breeding	Departure breeding	Moult migration	Arrival in potential moult	Departure from potential moult	Autumn migration	Arrival winter	Departure winter
Definition	Trajectory from capture/winter site to breeding site (Directional movements)	1st position after a long-distance flight (> 100 km), min. stay > 31 days	1st position outside the breeding site	Trajectory from breeding site to moulting site (Directional movements)	1st position after a long-distance flight (> 100 km), min. stay > 21 days	1st position outside the moulting site	Trajectory from moulting site to wintering site (Directional movements)	1st position in an area after a long-distance flight (> 100 km), min. stay > 31 days	1st position outside the winter site,
Phenology	March–June	June–September		August–September	September–December		October–January	December–May	
Number of individuals analysed for the 1st year	<i>N</i> = 33	<i>N</i> = 31	<i>N</i> = 19	<i>N</i> = 19	<i>N</i> = 19	<i>N</i> = 13	<i>N</i> = 13	<i>N</i> = 13	<i>N</i> = 10
Number of individuals analysed for the 2nd year	<i>N</i> = 9	<i>N</i> = 7	<i>N</i> = 4	<i>N</i> = 3	<i>N</i> = 3	<i>N</i> = 1	<i>N</i> = 1	<i>N</i> = 1	<i>N</i> = 0

(EPSG: 3035), North Pole Azimuthal Equidistant projection (EPSG: 102016)) to inspect migratory patterns and to quantify migratory movements. We calculated migratory distances between capture and breeding sites by summing the length of all vectors created from point to point of the PTT locations from the first day of departure from a site to first day of arrival at the new site. We did not include movements within staging areas and sites into the distance calculation.

We used R (v 3.6.1) (R Core Team 2018) to analyse and plot longitudinal migratory patterns, home range estimates and repeatability of site use between 2 consecutive years. We used the R package stats4 (R Core Team 2018) to calculate correlations. A Pearson's correlation was run to determine the relationship between spatial (breeding longitude/latitude) and temporal variables (arrival/departure/time of stay). Additionally, we tested if the distance moved between capture area and breeding sites affected the duration of migration and staging behaviour.

For analysing migratory connectivity, we first defined the breeding regions to determine the extent to which individuals from a wintering area head to the same breeding region and use similar migratory routes. We divided breeding regions either by distance (> 700 km), or if separated by a large body of water (as breeding is constrained to land).

Migratory routes were then assigned to the respective breeding region, namely Greenland, Scandinavia (Norway and Svalbard) and northern Russia. We analysed migratory connectivity between the breeding and non-breeding sites by quantifying the number of individuals of which positions were located along the same path using three analytical sections of the migration route (i) from the same starting point (capture site) to the same breeding region (*n* = 33), (ii) from one shared breeding region to their moulting destination (*n* = 19) and (iii) from one shared breeding region to their winter destination (*n* = 13). We analysed migratory connectivity during autumn migration (ii and iii) only for birds breeding in northern Russia (Siberian Arctic) as sample sizes from other regions were too small for further analyses. In addition, we assessed the strength of connectivity by calculating the Mantel correlation coefficient within the R package ade4 (Dray and Dufour 2007; Ambrosini et al. 2009; Trierweiler et al. 2014; Cohen et al. 2018). Statistical significance was determined using 9999 permutations (Trierweiler et al. 2014; Ambrosini et al. 2009). The Mantel correlation coefficient (*r*_M) was calculated between pairwise (orthodromic) distance matrices of (i) individual positions at capture and breeding (*n* = 31 individuals with a fixed breeding position), (ii) breeding and moulting (*n* = 13 individuals breeding in Siberian Russia), and (iii) breeding and winter

($n=9$ individuals breeding in Siberian Russia). Mantel correlation coefficient values range from 0 to 1, and indicate the strength of a population's migratory connectivity. Values ≤ 0.25 suggest no spatial structure, values 0.26 to 0.50 indicate a weak structure, values 0.51 to 0.70 indicate a reasonable structure, and values > 0.71 indicate strong structure (Ambrosini et al. 2009).

We compared home range estimates for an overlapping site use during moult and winter (after the first breeding season) and between individuals from shared and non-shared breeding regions within the same season. We calculated 95% and 50% kernel density contours using the *adehabitatHR* package (Calenge 2011) in R, using $h = "LSCV/h-ref"$ as the smoothing parameter. We used only data from individuals that covered a full season and only one position of the best location class per day to avoid overrepresentation of some intervals. To calculate sizes of home ranges and core areas in km^2 , we converted the area of kernels to UTM units and created a new kernel (95% and 50%) based on the UTM coordinates. The UTM zone was chosen individually depending in which zone the estimated area was located using the WGS1984 datum.

We furthermore used these kernel density estimates to compare consistency in site use and a potential spatial overlap of individual home ranges between 2 consecutive years. When the same time period during winter ($n=4$) and moult ($n=1$) in consecutive years was available, 50% and 95% density contours were calculated. Consistency and flexibility of individual migratory movements, phenology and site utilisation between the 2 years was calculated using an ANOVA-based repeatability index (also called the intra-class correlation coefficient R) as an agreement of measurements between consecutive years (Nakagawa and Schielzeth 2010). The repeatability index offers information about the proportion of the total variation that is reproducible among repeated measurements of the same subject or group (Lessells and Boag 1987). The repeatability index is based on variance components derived from a one-way analysis of variance (ANOVA). This ANOVA-based method is one of the most commonly used methods to calculate repeatability in behavioural and evolutionary biology (Nakagawa and Schielzeth 2010) and has been applied in several studies on shore- and seabirds (Battley 2006; Vardanis et al. 2011; Conklin et al. 2013; Ruthrauff et al. 2019). The F table of an ANOVA, with the individual identities treated as factorial predictors, were used to calculate ANOVA-based repeatability estimates (R_A). The repeatability (R_A) was calculated by the formula introduced by Lessells and Boag (1987), where the mean between individual sum of squares (MS_A), the mean within-individual (residual) sum of squares (MS_W) and the sample size for each individual (2 years' data) are considered. We considered all repeatabilities with 0 as no repeatability, all repeatabilities = 1 as total repeatability, all

repeatabilities < 0.5 as low repeatability and all repeatabilities > 0.5 as medium–high repeatability. The applicability of the method was confirmed in comparison with Linear mixed-effects model (LMM)-based methods (Stoffel et al. 2017), as both methods showed identical results (Appendix A3, Table A1). The utilisation of sites between 2 consecutive years was compared during spring staging ($n=9$ individuals and 13 locations utilised in both years), breeding ($n=7$ individuals) and moulting ($n=3$ individuals by tracking data, $n=19$ individuals by isotope data). We estimated repeatability between 2 consecutive years using Gaussian distributed data of position information, such as longitude/latitude on a small scale or isotope value on a broad scale (only for moult location) and the phenology (arrival/departure) using ordinal dates as the response variable and ID as the explanatory variable of the ANOVA (see Table 1, A1). Additionally, individual tracks were mapped where two seasons of each spring ($n=7$) and autumn migration ($n=3$) were available.

All estimates of averages are provided with standard deviations.

Stable isotope analyses

Stable isotope ratios are used in studies of the foraging ecology of seabirds, because they are proxies for the origin of resources (stable carbon isotope ratios $\delta^{13}C$) and relative trophic levels (stable nitrogen isotope ratios $\delta^{15}N$, e.g. Bedolla-Guzman et al. 2021). Feathers are used for stable isotope analyses, because feather proteins, formed during moult, reflect the stable isotope values of the diet at the time of their synthesis and can thus provide information on distribution and diet at the time of moult (Hobson and Clark 1992; Oppel and Powell 2008). Once grown, feathers are metabolically inert (Hobson 1999; Atkinson et al. 2005) and if potential moulting areas differ in their stable isotope values, it is possible to infer from $\delta^{13}C$ values where the feather was grown (Hobson 1999).

We sampled the white neck feathers that are characteristic for the winter plumage and are grown during the autumn moult area (Stresemann and Stresemann 1966, Berndt and Drenckhahn 1974, Mendel et al. 2008) from all red-throated divers tracked during this project ($n=33$). We used these feathers to determine the area where these feathers are grown and thus, the autumn moulting sites in the season previous to capture on a broad scale using stable isotope analyses. The white neck feathers of red-throated divers are particularly suitable for this purpose as they are easy to distinguish to ensure that this feather sample and its stable isotope values are representative of the autumn moult.

We linked isotope values to moulting regions for birds tracked with satellite transmitters, assuming that birds are faithful to regions between years. We used a sub-sample set of individual data ($n=10$) provided by the satellite

transmitters and determined the moult location of each bird to relate the isotopic information of feathers to geographic regions (North Sea vs. Baltic Sea). Then, we used these values to assign moult locations for the remaining birds tracked with satellite transmitters ($n=9$) and for birds where no information from tracking data was available ($n=14$). The feathers we sampled from satellite tracked birds were grown in the year before and thus tracking data collected in this study did not include the time when the sampled feathers were grown. Therefore, additionally literature of isotope values in the North and Baltic Sea were incorporated to confirm the classification. We revised carbon isotope values from muscle, eggshells and feathers of piscivorous vertebrates whose diets overlap with that of the red-throated divers (Das et al 2004, Céline Mahfouz et al. 2017, Corman et al 2018, Kleinschmidt et al. 2019, St John Glew et al. 2019, Christie 2021, Table 2) to obtain information on differences in carbon stable isotope values between the two seas. Stable isotope values were finally compared (North Sea vs. Baltic Sea) using the Wilks' Lambda test and a One-way MANOVA (Bartlett χ^2) and the package rrcov (Todorov and Filzmoser 2009) to reveal if they statistically differ.

The isotope data were used to assign pre-capture moult locations to all birds tracked in this study ($n=33$). When information about utilised moulting sites after capture was given by the tracking data ($n=19$), it could be compared with the moulting sites assigned by isotope data. Therefore, moulting areas could be identified on a broad scale throughout the year and between years.

Samples were analysed at LIENSs Stable Isotope Facility at the University of La Rochelle. The treatment of feather samples and information about running the stable isotope analyses followed the approach described in Dorsch et al. (2019) and is provided in the supplementary material (A8).

Results

Migratory routes and utilised sites

Breeding destinations of red-throated divers captured in the eastern German Bight ($n=33$) covered the whole breeding range of the NW European wintering population

(65°W–98°E) specified by Wetlands International (2018, 2022). The breeding areas included destinations in Greenland ($n=2$), Svalbard ($n=2$), Norway ($n=4$) and northern Russia ($n=25$) (Fig. 2). Divers from one capture site displayed both, a longitudinal migration (eastern direction to Russia, $n=25$; western direction to Greenland, $n=2$) and a latitudinal migration (central direction to Norway, $n=4$, Svalbard, $n=2$). Consequently, migratory directions are in the following termed as the Greenland direction, the Scandinavian direction (Svalbard and Norway) and the Russian direction. Of birds with a settlement in a breeding site in northern Russia ($n=24$), 79% of breeding positions ($n=19$) were located in the Siberian Arctic (Yamal, Gydan and Taymyr peninsulas and West Siberian Plain) (65°E–98°E) and 21% ($n=5$) in the European part of northern Russia (Kola Peninsula, Kanin Peninsula, Pechora Sea (Tobseida Island) and Novaya Zemlya) (40°E–55°E). The migratory pathways of single individuals from all breeding areas were mixed on the route towards the Scandinavian direction (east Greenland $n=1$, Svalbard $n=2$, Norway $n=4$, and northern Russia $n=1$) and overlapped spatially from 54°N (capture site) till 68/70°N before leading to the final migration direction (Fig. 2).

Along the route to northern Russia ($n=25$), we identified 12 staging sites of which 7 were located within the Baltic Sea. High frequented staging sites were the Skagerrak-Kattegat (7%), the Pomeranian Bight (10%), the Gulf of Bothnia and in particular the Gulf of Riga (24%, $n=9$, Fig. 1, A4). In this context, the following winter showed that the capture area itself (eastern German Bight) served as a staging area (40%, $n=10$) if individuals spent the winter elsewhere (Fig. 4).

In autumn, on the way back from the breeding areas, most red-throated divers followed the directions and pathways they have already used during the spring migration with 77% of red-throated divers performing a step-wise migration with separate moult and winter migrations (Figs. 2 and 6).

All birds moulting in the North Sea according to tracking data had $\delta^{13}\text{C} > -18\text{‰}$, while birds that moulted in the Baltic Sea had $\delta^{13}\text{C} < -18\text{‰}$. This threshold was confirmed by the literature values. We thus applied this classification to all birds tracked through the annual cycle ($n=33$) and assigned moulting regions in the Baltic Sea and the North

Table 2 Isotope values from North and Baltic Sea assigned by published data and data from this study

Organism	Tissue	$\delta^{13}\text{C}$ North Sea	$\delta^{13}\text{C}$ Baltic Sea	References
Harbour porpoise	Muscle	Average – 16.5 ‰	Average – 18.25‰	Das et al 2004
Herring gull eggs	Eggshell	– 16 to (– 19) ‰	– 21‰	Corman et al. 2018
Puffin	Feather	– 15.87 (0.38) to – 16.81 (0.72)	n.a	Glew et al. 2019
Razorbill	Feather	– 16.47 (0.71) to – 16.48 (0.68)	n.a	Glew et al. 2019
Common Guillemot	Feather	– 16.77 (0.91)	n.a	Christie 2021
Red-throated diver	Feather	> – 18 ‰ to (– 15.5)	< – 18 ‰ – (– 22)	Our study

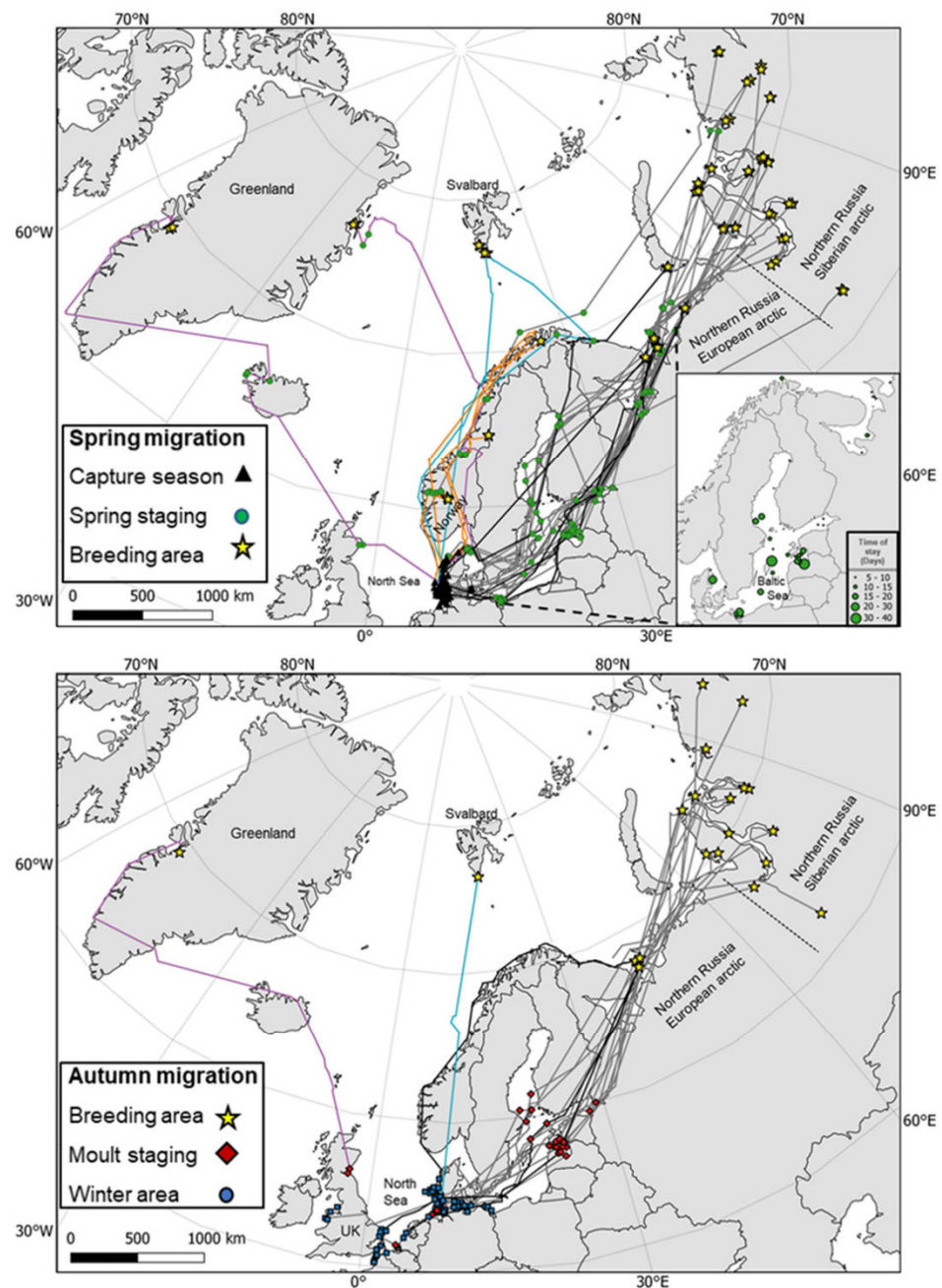


Fig. 2 1st year spring and autumn migration tracks of red-throated divers ($n=31$, $n=19$) from the capture location in the German Bight to their breeding areas (above) and from potential breeding locations to potential moulting locations and to wintering sites (below). Colours of migration tracks indicate different breeding regions (violet

to Greenland; light blue to Svalbard; orange to Norway, black to the eastern arctic of northern Russia; grey to the Siberian arctic of northern Russia). Individual time of stay in spring staging areas along the migration route to Northern Russia for 32 staging stops performed by $n=23$ individuals is visualized as zoom included in the map

Sea to a similar number (51% and 49%, respectively). The isotope values from red-throated divers probably indicated a split between moulting in North Sea and Baltic Sea (Fig. 3). Clusters of SI were significantly different between samples (Wilks' Lambda = 0.084, $\chi^2 = 76.61$, DF = 2.00, $p < 0.001$) that would, based on literature on other species (Table 2), be considered to come from the Baltic Sea or from the North Sea.

Tracking data revealed that wintering sites were distributed in the Baltic Sea, North Sea (eastern German Bight and southern Bight) and Irish Sea with the highest proportion using the eastern German Bight (60%, $n = 10$, Fig. 4) either during the complete season or temporarily.

Migratory connectivity

Starting on the capture site, red-throated divers spread out over a large geographic range for breeding (Figs. 2 and 5). Considering connectivity among individuals from one breeding region, only individuals from northern Russia were included as the sample size for the other regions were too small to show meaningful results. Individuals breeding in northern Russia used mainly routes via the Baltic Sea to migrate to and from breeding sites, with one exception that moved along the Northern Cape (Fig. 2). During spring migration individuals breeding in northern Russia showed no consistent pattern with varying staging stop locations, staging stop durations and travel times (Fig. 2, Table 3). These individuals spread out to several moulting sites (stationary period from September to December > 21 days) in the North and Baltic (38% and 62%, respectively, Figs. 2, 4 and Fig. 5). Within the Baltic Sea, the majority of birds ($n = 67\%$) spent

the potential moulting time in the Gulf of Riga. During winter, 50% of the tracked birds breeding in northern Russia utilised the German Bight, whereas the other 50% distributed elsewhere (25% in the southern Bight, 8% in the Irish Sea and 17% in the Baltic Sea), (Figs. 4 and 5). Kernel density estimation of individuals from one breeding region showed only partly overlapping home ranges but these individuals mixed with individuals from other breeding regions (northern Russia $n = 4$, Svalbard $n = 1$, Fig. 4). The distance between individual areas within the moulting period as well as within the winter period was up to 1000 km. Combination of tracking data ($n = 19$, Figs. 4 and 5) and additional birds determined by stable isotopes ($n = 13$) revealed that individuals that moulted in the Baltic Sea ($n = 15$) migrated all from northern Russia but red-throated divers that moulted in the North Sea ($n = 17$) were composed of individuals from several breeding regions, northern Russia (52.9%), Norway (23.5%), Svalbard (11.8%) and Greenland (11.8%). Migratory patterns varied between individuals with the majority performing a separate moult and autumn migration and a few individuals performing a direct migration (migration to a site that was utilised during moult and winter).

Quantification of migratory connectivity between individuals captured in the German Bight (eastern North Sea) indicated no relation between individuals from one breeding region (i) to moulting or (ii) wintering sites. Calculation of a Mantel correlation coefficient indicate no spatial structure and a low connectivity but gave no significant results: capture site to breeding: $rM = 0.069$, $n = 31$, $p = 0.202$; breeding to moult (Siberian ind.): $rM = 0.135$, $n = 13$, $p = 0.215$; breeding to winter (Siberian ind.): $rM = 0.274$, $n = 9$, $p = 0.103$.

Thus, individuals from northern Russia spread out to several winter sites with no uniform pattern of individuals from this breeding region and various utilisation areas and mix with individuals from other breeding regions (Figs. 4 and 5).

Timing of migration and geographic relations

Migration distances can only be given as minimum estimates assuming straight flight paths between consecutive Argos positions. Referring to Cox (2010) and Rappole (2013), the majority of red-throated divers in this study migrated > 1000 km and can be considered as long-distance migrants (87.9%) and just a small number of birds moved short distances < 1000 km to Norway (12.1%) (Table 3).

Breeding location was significantly correlated with duration of migration (longitude: $r = 0.407$, $n = 29$, $p = 0.027$; latitude: $r = 0.384$, $n = 29$, $p = 0.039$). Overall, individuals breeding at a higher longitude in Russia needed a longer travelling time (Fig. A2a). The longest travel time during spring migration was 65 days and this bird headed to Taymir Peninsula in northern Russia. Departure date (ordinal date-day

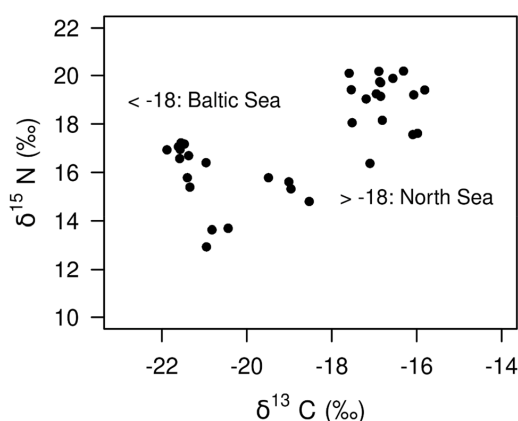


Fig. 3 Stable isotope values of 33 red-throated diver feather samples assigned to the region where they were moulted. Data points show feathers of 16 individuals moulted in the Baltic Sea and 17 individuals that moulted in the North Sea

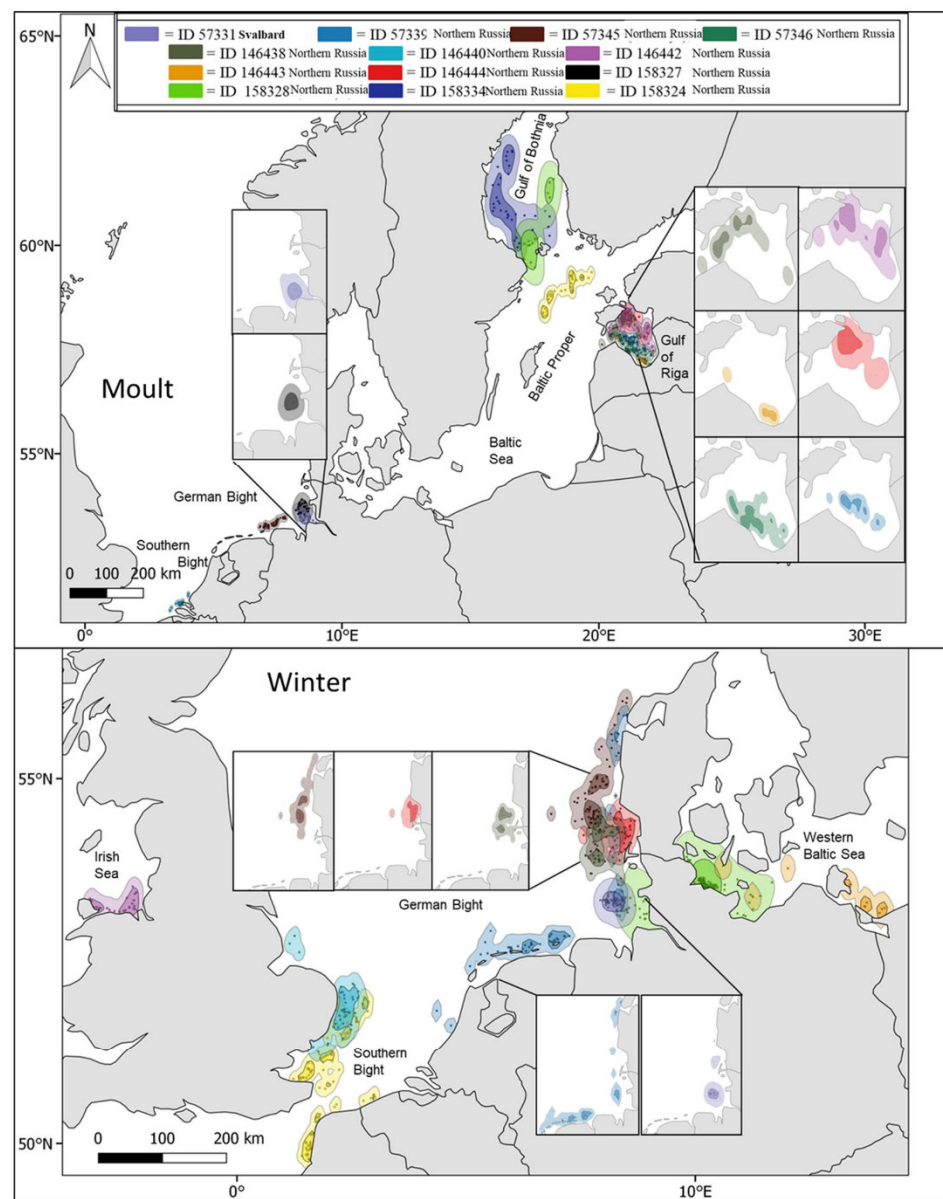


Fig. 4 Areas used by red-throated divers during moult (above) and during winter (below). The legend on top informs about individual colours and corresponding breeding region. Estimated Kernel densities 95% and 50% for utilised areas in Baltic and North Sea during moult season ($n=13$) and in Baltic, North and Irish Sea during winter season ($n=10$) for a time period that lasts from the first date in

the area (arrival) until last date (departure). Individual maps are displayed when birds utilised the same area and their home ranges are not distinguishable, (moult: Eastern German Bight $n=2$, Bay of Riga $n=6$, winter: Eastern German Bight $n=5$). 95% kernel density contours are displayed with 70% transparency and 50% kernel density (core habitat use) with 50% transparency

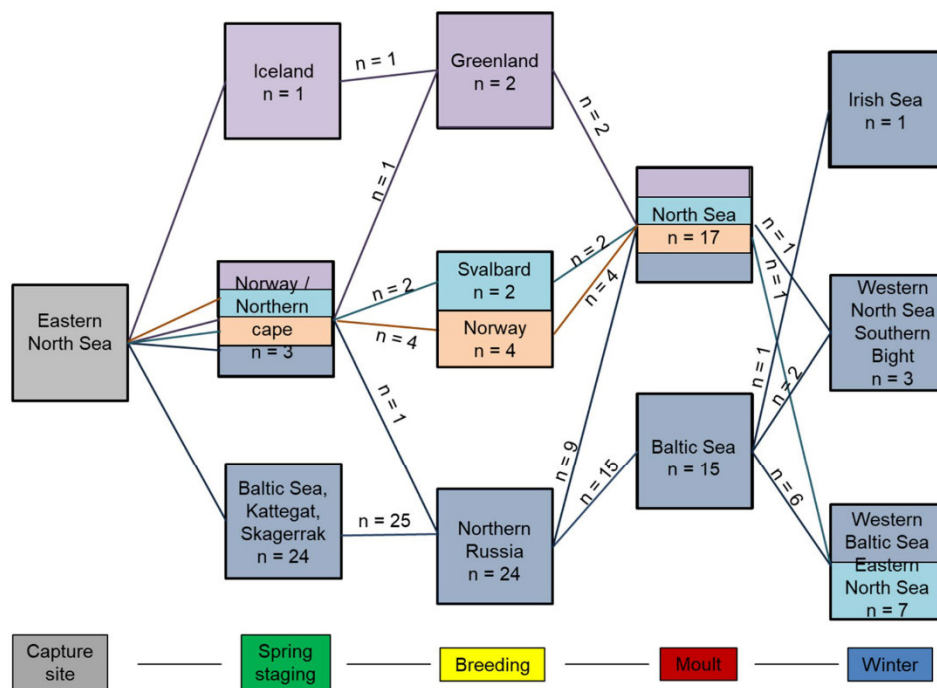


Fig. 5 Population spread and interpopulation mixing of red-throated divers from the NW European winter population starting at the capture site (eastern German Bight) heading to breeding regions and from breeding regions to moulting and winter sites. From capture site to breeding data are based on tracking data, from breeding to moulting data are combined of tracking and additional birds determined by

stable isotopes and from moulting to winter data are based on tracking data. Each breeding region is presented in a specific colour consistent with the division made in Fig. 1 (violet = Greenland, orange = Norway, light blue = Svalbard and dark blue = Northern Russia) and Boxes show number of individuals using this region

of year) from wintering sites was significantly correlated with breeding latitude but not with breeding longitude (longitude: $r=0.335$, $n=9$, $p=0.344$; latitude: $r=0.714$, $n=9$, $p=0.020$). More northerly located breeders departed later from their wintering site than southerly located breeders, suggesting a latitudinal gradient (Fig. A2c). Arrival date to breeding areas (ordinal date-day of year) was significantly correlated with a higher breeding longitude but not with a higher breeding latitude (longitude: $r=0.695$, $n=29$, $p<0.001$; latitude: $r=0.373$, $n=29$, $p=0.078$). More easterly located breeders arrived later at their breeding sites than westerly located breeders with up to 40 days later arrival, suggesting a longitudinal gradient (Fig. A2b). Departure date from breeding sites was neither correlated with breeding longitude ($n=20$, $p=0.941$) nor with breeding latitude ($n=20$, $p=0.285$, Fig. A1b).

We observed no correlation between breeding positions (long/lat) and duration of staging ($n=25$, $p=0.852$, Fig. A1a) but a significant correlation between a longer travelling time and a longer duration of staging ($r=0.643$, $n=27$,

$p<0.001$, Fig. A3) that was correlated with a higher number of staging stops ($r_s=0.468$, $n=27$, $p=0.014$, Fig. A3). Although staging time was positively correlated with travelling time, the distance itself had no effect on either staging time ($n=25$, $p=0.986$, Fig. A1c) or travelling time ($n=31$, $p=0.116$, Fig. A1c).

Repeatability of year-round movements and site utilisation between consecutive years

Not all birds caught in the German Bight in winter and spring returned to this location during the following winter. 32% of the tracked birds used this area for moulting and 54% for wintering. Individuals that did not use this area during moulting or wintering used this area as a staging site or a short stop along migration.

The temporal pattern and repeated site utilisation with regard to spring staging sites, breeding and moulting areas showed high individual consistency between years (Fig. 6, Table A1). Visual inspection of individual

Table 3 Temporal pattern and distances within the first year moved of red-throated divers from different breeding regions during spring and autumn migration (mean \pm SD)

	Breeding region	Migration route	Migration distance (km)	Travel time (days)	Staging stop No (> 5 days)	Duration per staging stop (days)	Breeding: arrival/ departure date \pm days	Moult: arrival/ departure date \pm days	Winter: arrival/ departure date \pm days
Spring	Northern Russia ($n=24$)	Russian direction	4,015 \pm 751	42.30 \pm 12.6	1.5 \pm 0.8	13.21 \pm 744	09.06. \pm 13 ($n=24$) 07.09. \pm 13 ($n=20$)	n.a	n.a
	Norway ($n=4$)	Scandinavian direction	1403 \pm 641	31 \pm 24.8 days	1.3 \pm 1.2	11.2 \pm 975	25.05. \pm 6 ($n=3$) n.a	n.a	n.a
	Greenland ($n=2$)	Greenland direction	4,457 \pm 1213	34.5 \pm 10.6	2 \pm 0	9.75 \pm 303	22.06 \pm 5 ($n=2$) 31.08. \pm 0	n.a	n.a
	Svalbard ($n=2$)	Scandinavian direction	2,303 \pm 301	12.5 \pm 1.4	0.5 \pm 0.5	3 \pm 3	27.05. \pm 2 ($n=2$) 14.09. \pm 0	n.a	n.a
Autumn	Northern Russia ($n=18$)	Russian direction	3,830 \pm 668	97 \pm 23.76	Moult	53.67 \pm 1422	n.a	05.10 \pm 12 ($n=18$) 24.11 \pm 16 ($n=13$)	20.12 \pm 17 ($n=12$) 08.04. \pm 22 ($n=10$)
	Svalbard ($n=1$)	Scandinavian direction	2090.6	22	Moult	43	n.a	07.10. \pm 0 18.11. \pm 0	25.11 \pm 0 08.05. \pm 0

migratory pathways between consecutive years showed similar movements in six of seven individuals, however, one individual (146,444) used different pathways between two spring migrations (Fig. 6). Kernel density estimates from individuals for which tracking data were available from an overlapping period during moult and winter in consecutive years showed that home ranges overlapped or were close, indicating consistent site use in consecutive years (Fig. 6).

Repeatability (Table A1) towards number of individual spring staging stops was moderate between years ($R_A = 0.407$, $F_{8,9} = 2$, $p = 0.161$). The repeatability towards individual spring staging longitudes ($R_A = 0.954$, $F_{12,13} = 42.9$, $p < 0.001$) and spring staging latitudes ($R_A = 0.982$, $F_{12,13} = 107.8$, $p < 0.001$), that appeared in both years, was high. High repeatability's were also found for breeding longitudes ($R_A = 1$, $F_{6,7} = 24,945$, $p < 0.001$), breeding latitudes ($R_A = 1$, $F_{6,7} = 9125$, $p < 0.001$) and moulting locations by isotope analyses ($R_A = 0.785$, $F_{18,19} = 8.278$, $p < 0.001$). Between year repeatability of migratory timing indicated a less consistent pattern for arrival times in spring staging times ($R_A = 0.604$, $F_{12,13} = 4.1$, $p = 0.009$), departure times from spring staging sites ($R_A = 0.751$, $F_{12,13} = 7.0$, $p = 0.001$), arrival times in breeding areas ($R_A = 0.552$, $F_{6,7} = 3.5$, $p = 0.064$), departure times from breeding areas ($R_A = 0.408$, $F_{3,4} = 2.4$, $p = 0.211$) and arrival times in moulting areas ($R_A = 0.876$, $F_{2,3} = 15.11$, $p = 0.027$).

Discussion

Based on tracking data and stable isotope analyses, we obtained a comprehensive dataset on annual movements of NW European red-throated divers that addressed our hypotheses as outlined below. Tracking data lasted for up to 2 consecutive years and thus allowed to assign individual site utilisation within and between years. Stable isotope analyses added information about moulting sites where no tracking data were available. Isotopic values in our study seem to be clearly separable between North and Baltic Sea and in line with the locations determined by the tracking data. Although matching of isotope data and tracking data without temporal overlap may have some uncertainties, published isotopic values from the North and Baltic Sea backed up our assignment of moulting sites. This approach has previously been used successfully by Oppel and Powell (2008) to determine winter locations of eiders (*Somateria spectabilis*). They also used information from satellite tracked birds to isotopically delineate regions and assigned feathers of birds not tracked with satellite transmitters to regions using their stable isotope values.

Do red-throated divers have a low degree of migratory connectivity?

Considering ring recoveries in coastal areas around the North Sea (Okill 1994), we expected a mix of individuals

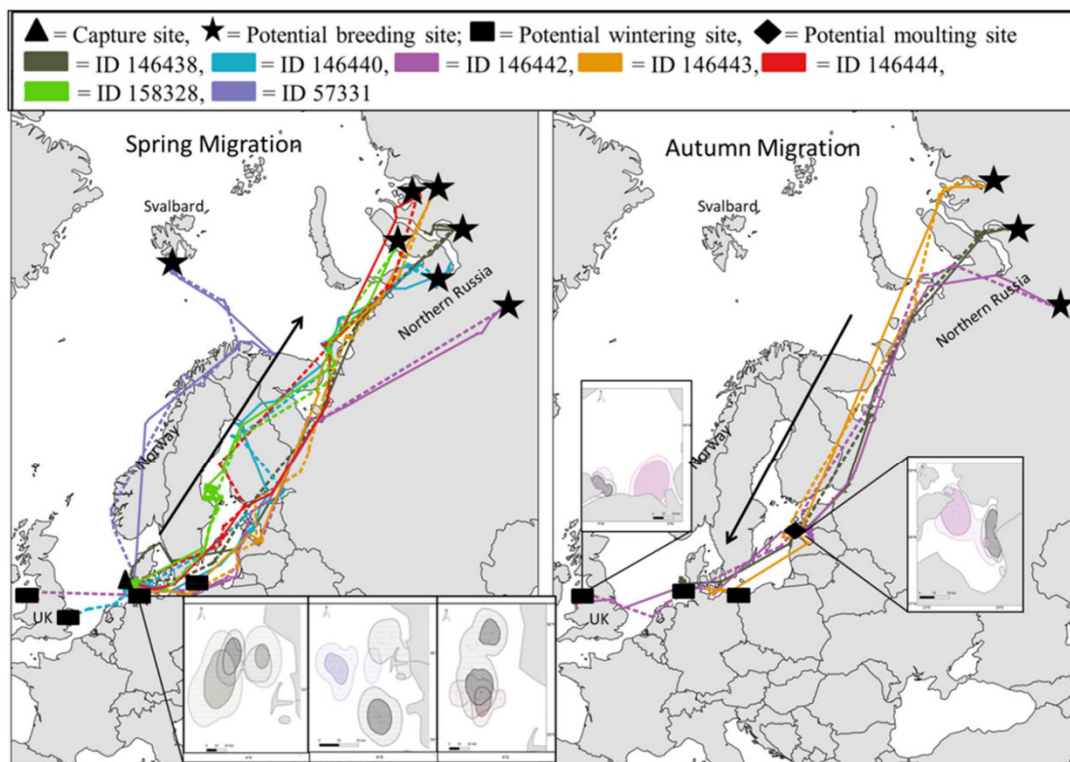


Fig. 6 Migration routes of individuals during two subsequent years (first year=solid line, second year=dotted line, the black arrow indicates the direction of movement). The left side shows individual spring migration tracks marked by colour ($n=7$) from capture site to potential breeding sites in the first year and from wintering site to potential breeding sites in the second year. The right side shows

individual moult migration tracks marked by colour from potential breeding sites to potential moulting sites ($n=3$) and to wintering sites ($n=1$) for two consecutive seasons. Individual home ranges when data transmission allowed for two overlapping time periods during winter and moult in consecutive years are visualized as zoom included in the map

from several breeding regions in this area and thus a low degree of connectivity. We found individuals from four different breeding regions captured in one local area during late winter and early spring. The distances and water bodies between these breeding regions indicates a delimitation of these regions. We captured red-throated divers in one relatively local wintering/spring staging site and not in the breeding area, as most other studies analysing migratory connectivity do. A possible bias could have been that the connectivity would have been overestimated if all birds would have headed from the same winter region to the same breeding region and back. In this case, individuals from a shared breeding region that use other winter regions, would have been missed as they were out of our sample range. In our case, however, we found a relatively high spread from individuals heading to distant breeding regions and of individuals from one breeding region to several non-breeding

regions and therefore this bias is unlikely to affect the results. However, that individuals migrated along their routes to a shared breeding area in northern Russia indicates some degree of connectivity, as most of these individuals moved along the Baltic Sea and used similar staging sites along this route. In another study, McCloskey et al. (2018) tagged red-throated divers in four breeding regions in Alaska. These individuals also followed similar migration routes, indicating some degree of connectivity, but did not display a discrete use of population-specific non-breeding areas. The fact that almost all red-throated divers in this study that bred in northern Russia migrated along the Baltic Sea could also be due to the fact, that they follow an established migration route that is used by various species of waterfowl, the Northeast Atlantic Flyway (BirdLife International 2010), rather than to the fact that they exhibit community-specific patterns (Boere and Straud 2006). On a smaller scale and considering site

utilisation during the stationary non-breeding season (moult and winter), red-throated divers in this study spread to distinct areas. Following the ‘weak–strong’ continuum defined by Webster et al. (2002), this pattern would suggest that red-throated divers displayed a low or diffuse connectivity with individually variable movements and no specific or uniform pattern of individuals from one site or migratory direction. Statistical tests showed no significant correlation between utilised breeding regions, moulting or wintering sites, which supports the low connectivity indicated by Fig. 5. Also, kernel density estimation during moult and winter in the season after capture showed only partly overlapping home ranges between individuals from a given breeding region (Fig. 4). Our results are consistent with the study of Gray (2021) who found low connectivity of red-throated divers in eastern North America, indicating that red-throated divers display a highly individual movement behaviour that is adapted rather to individual qualities and environmental conditions than to community-specific patterns.

Does the location of breeding area (longitude/latitude) affect the timing and spatial pattern of annual movements?

Like other species, red-throated divers seem to follow an endogenous schedule of migration together with the strong phenological gradient along the spring migration route to Arctic breeding areas (Gordo 2007; McNamara et al. 2011; Shariatinajafabadi et al. 2014; Smith et al. 2020). Arrival dates in more westerly located Arctic breeding sites were about 40 days earlier than arrival in more easterly Arctic breeding sites, indicating a longitudinal gradient. We did not find a correlation between departure from breeding sites and breeding location, which could be related to variations in breeding success. Birds from more northern breeding areas departed later from wintering areas, consistent with the pattern of later arrival at breeding areas. The temporal pattern seems to confirm that timing of migration appears to follow environmental conditions (e.g. growing seasons, ice-free conditions and temperatures) in arctic breeding regions, similar to other waterfowl and shorebirds (Schwartz 1998; Shariatinajafabadi et al. 2014; Smith et al. 2020). Migratory movements and breeding events of shorebirds and avian herbivores can be constrained by plant phenology and spring salt marsh productivity (Shariatinajafabadi et al. 2014; Smith et al. 2020). Winkler et al. (2014) stated that migration strategies can be seen as the mapping of actions (e.g. feeding, departure) on cues (e.g. daylength, feeding or wind conditions). Although red-throated divers are piscivorous seabirds and do not directly depend on plant phenology, other factors such as seasonal day length and temperatures could be indicators that lead birds to hit the right time with suitable conditions at breeding sites. In this case, a later arrival time at

more easterly located breeding locations might also explain why a longer travel time (duration of the spring migration) was significantly correlated with a more easterly breeding location, but not with distance travelled (Fig. A1c, A2a). Another factor that should be taken into account here is the individual need to refuel along the route (Weber and Houston 1997). We found a significant correlation between a longer travelling time and a longer duration of staging and a higher number of staging stops (Fig. A30). In this context, as medium-sized birds with weight varying between 1400 and 2000 g (own observations) and high wing loading (Storer 1958; Lovvorn and Jones 1994) using flapping flight, the energy expenditure of divers is relatively high (Pennycuik 1989). To refuel energy reserves, divers, travelling to more distant areas (longitudes) may, therefore, need more and longer staging stops, thus increasing the total travel time. The high energy expenditure though might be counteracted by the use of favourable wind conditions (tailwinds), or very good foraging conditions at staging sites. However, our results are in line with the finding of McCloskey et al. (2018) and Gray (2021) who found red-throated divers to perform long/slow migrations with many stop-overs.

Do individuals faithfully utilise areas during their key life history stages between years?

Information about individual consistency between years helps to understand the capacity to cope with habitat change and selection pressures (Dias et al. 2010; Conklin et al. 2013; McFarlane et al. 2014; Merkel et al. 2020). Based on a high site fidelity observed in breeding areas (Okill 1992; Poessel et al. 2020), we expected a similar high site fidelity for the non-breeding sites. The sample size for individual site utilisation in two consecutive years was rather small with data of ($n=9$) individuals for spring migration and of ($n=7$) individuals for breeding sites. The data set of ($n=3$) individuals tracked for moulting sites could be enhanced by the isotopic data ($n=19$). Although the sample size is rather small, the results of the repeatability analyses of annual migratory movements seem to confirm and extend previous studies on site fidelity (Okill 1992; Poessel et al. 2020).

Similar to the strong winter site fidelity (85%) of common loons/ great-northern divers (*Gavia immer*) shown by Paruk et al. (2015) red-throated divers exhibit a relatively high fidelity towards the different areas visited during the annual cycle: migration routes, staging, breeding and moulting areas, however, with some variation for individuals and temporal pattern. Home ranges estimated by Kernel densities in 2 consecutive years and during the same time period in moult and winter could only be shown on the basis of a few individuals ($n=4$), but revealed that these individuals had either some home range overlap in consecutive seasons, or the home ranges were located in

the same area and close to each other. Home range estimation and calculation of repeatability might indicate that site selection is driven by macro-selection of a larger area with sufficient frequency of opportunistic prey encounters. Once such a profitable area with suitable feeding conditions is found, it is used faithfully from year to year. In this study, we only have 2 years of data and in the context of fidelity and flexibility, it is equally plausible that red-throated divers exhibit fidelity only until a site of use is no longer suitable, in which case the flexibility of the divers would be expressed. Skov and Prins (2001) have shown that the eastern German Bight is known to be particularly attractive to red-throated divers due to the frontal zone and resulting favourable feeding conditions with suitable prey species (Guse et al. 2009; Kleinschmidt et al. 2019). If habitat selection is driven by a macro-selection, it could be that habitat change has an effect on a smaller spatial scale and displacement is a process happening at smaller scales. However, in this context, results from studies on red-throated diver distributions and displacement effects in the eastern German Bight showed that red-throated divers remained in the general area, but shifted their distribution and congregated outside disturbed areas (Mendel et al. 2019; Vilela et al. 2021). Mendel et al. (2019) analysed long-term datasets of aerial and ship based surveys, whereas Vilela et al. (2021) analysed long-term datasets of aerial surveys. However, no population decline was observed (Vilela et al. 2021). Considering this, our data based on the visualised tracks, single home ranges (Fig. 6) and statistical analyses of individual repeatability (Table A1) show an individual consistent site use in consecutive years, considered over a broad scale. Combined with the findings of Mendel et al. (2019) and Vilela et al. (2021), these data suggest that red-throated divers are somewhat flexible to change sites at small scales, but may have limited flexibility to change sites at large scales in response to large-scale habitat loss.

Arrival and departure times were more consistent at non-breeding sites than at breeding sites. The high consistency in arrival times in moulting areas might limit their flexibility in responding to anthropogenic change during that time period when birds are flightless. Similar to other medium-to-large sized diving bird species, divers are expected to perform a synchronous wing moult (Thompson and Kitaysky 2004) rendering them flightless and thus require undisturbed areas during this time. Arctic breeding areas on the other hand are characterised by a short arctic summer and thus a narrow seasonal window where breeding can take place (Klaassen 2003). Therefore, this Arctic breeding bird species may have adapted to a more flexible timing of arrival to match optimal conditions in breeding areas, triggered by colder or warmer winter or spring temperatures.

Conclusions and implication for conservation

In agreement with prior research (e.g., Mendel and Garthe 2010; Dierschke et al. 2012; Mendel et al. 2019), our study confirms the importance of the North Sea, in particular the eastern German Bight, as a wintering area, staging site before spring migration and moulting area for red-throated divers. The consistent use of the Gulf of Riga in our study in spring and autumn confirmed that this area is another important site for red-throated divers migrating from northern Russia and moving to the North Sea and adjacent waters (Berndt and Drenckhahn 1990; Helcom 2013).

Low connectivity might indicate resilience to environmental change on a population level, but the high fidelity towards sites during the stationary non-breeding season indicates a rather high consistency of annual movements which may result in a low individual flexibility. These findings are highly important to be considered for future appropriate conservation measures. All divers in this study were captured in the eastern German Bight but migrated to separate breeding areas and used this area with varying intensity in the following season. The observed low or diffuse connectivity of individuals from one breeding region distributes the effect to only a proportion of individuals from each breeding region. Compared to a high connectivity, where all individuals from one breeding region would experience the same non-breeding conditions over the same time in this area, a higher resilience can be suggested (Newton 2008; Rushing et al. 2016). Interannual movements of red-throated divers on the other hand showed a relatively high individual repeatability and consistent site use. Consistent use of high energetic mobile prey species (Guse et al. 2009; Kleinschmidt et al. 2019) indicates that the occurrence of these prey species seem to be an important habitat criteria. Considering the use of multiple core areas during winter and the dependence of divers on these mobile prey species in dynamic marine habitats, could also indicate some flexibility. Regarding anthropogenic pressures and altered environmental conditions, a poor wintering habitat quality can carry-over to breeding sites and influence reproductive success (Marra et al. 1998; Moore 2005; Harrison et al. 2011; Rushing et al. 2016). The winter population of red-throated divers shows strong avoidance towards the increasing anthropogenic pressure (Garthe et al. 2015; Mendel et al. 2019) but does not decline (Vilela et al. 2021). The low connectivity could counteract a quick population decline by having only small effects on populations of this long-lived species. If the impact always affects only one number or a proportion, but not the entire population, it may take longer for the impact to become apparent. More research on reproductive success in the arctic breeding regions is needed to link population estimates between breeding and non-breeding areas. If a breeding population experiences individually different travel times, caused by

altered conditions in the non-breeding areas, this may result in different arrival times at the breeding site and a possible mismatch (Marra et al. 1998; Moore 2005; Rushing et al. 2016). It should also be noted here that, climate warming can alter ice-free periods in Arctic breeding areas which has the potential to alter the timing of migration (Walther et al. 2002; Catry et al. 2013; Wauchope et al. 2017).

Although anthropogenic pressures in the North Sea appear to be distributed among individuals from multiple populations, when considered cumulatively and taking into account individuals breeding in northern Russia, multiple threats during migration come together, such as gillnet fishing and pollution of the Baltic Sea (Dagys and Žydelis 2002; Rubarth et al. 2011; Žydelis 2013). When it comes to future spatial planning, our data support the finding that all information on species abundance and sites used along the migration route needs to be considered, regardless of whether they are geographically or politically distant (Runge et al. 2014; Johnston et al. 2020).

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s00227-022-04096-x>.

Acknowledgements We thank G. Guillou from the Plateforme Analyses Isotopiques of LIENSs laboratory for running the stable isotope analyses. Thanks are due to the CPER (Contrat de Projet Etat-Région) and the FEDER (Fonds Européen de Développement Régional) for funding the IRMS of LIENSs laboratory. The Institut Universitaire de France (IUF) is acknowledged for its support to PB as a Senior Member. Many thanks to Nadja Küpper and Yvonne Schumm for helpful discussion and support with the statistics. We greatly thank everybody involved in the capture of divers for their support. Thomas Grünkorn and Jorg Welcker completed our field team and supplied valuable support. Thomas Grünkorn also contributed by ringing of the captured red-throated divers. Sören Zenner (OS-Energy) allowed flexible organisation and provision of ships during the field seasons and we thank the ship captains and crews of the MS Madog and MS Arctic Hunter for their reliability and support during fieldwork. Finally, we thank the reviewers for their valuable recommendations.

Author contributions All authors were involved either field work, lab work or in the writing of the manuscript. All authors read and approved the final manuscript.

Funding Open Access funding enabled and organized by Projekt DEAL. The DIVER project is supported by the Federal Ministry for Economic Affairs and Energy on the basis of a decision by the German Bundestag (funding ID 0325747 A/B).

Data availability All data have been deposited in the Movebank repository.

Declarations

Conflict of interest The authors explicitly declare that they have no competing interests.

Ethical approval The ethical rules as well as the legal requirements for the fieldwork have been met. All fieldwork (animal capture, sampling

and tagging) was approved by BfN (Federal Agency for Nature Conservation, Germany, 05.08.2014; and Ministry of Environment and Food Denmark, Danish Veterinary and Food Administration, permit no. 2014-15-0201-00239, issued 18.12.2014).

Consent for publication All co-authors have seen and agreed with the contents of the manuscript for publication.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

References

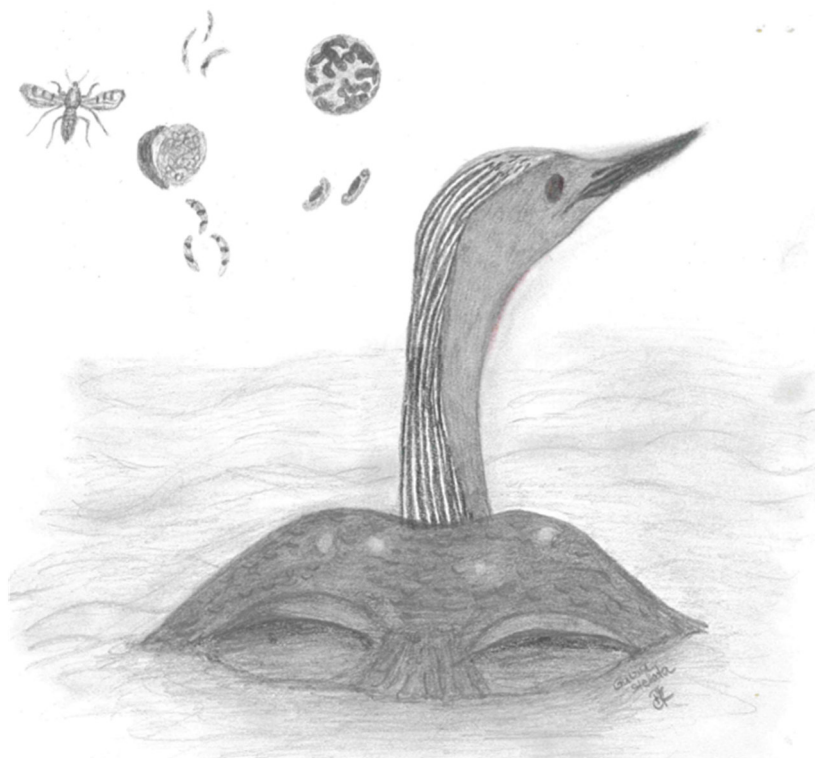
- Alves JA, Gunnarsson TG, Hayhow DB, Appleton GF, Potts PM, Sutherland WJ, Gill JA (2013) Costs, benefits, and fitness consequences of different migratory strategies. *Ecology* 94(1):11–17
- Ambrosini R, Møller AP, Saino N (2009) A quantitative measure of migratory connectivity. *J Theor Biol* 257(2):203–211
- Atkinson PW, Baker AJ, Bevan RM, Clark NA, Cole KB, Gonzalez PM, Newton J, Niles LJ, Robinson RA (2005) Unravelling the migration and moult strategies of a long-distance migrant using stable isotopes: red knot *Calidris canutus* movements in the Americas. *Ibis* 147(4):738–749
- Battley P (2006) Consistent annual schedules in a migratory shorebird. *Biol Lett* 2(4):517–520
- Bedolla-Guzmán Y, Masello JF, Aguirre-Muñoz A, Lavaniegas BE, Voigt CC, Gómez-Gutiérrez J, Sánchez-Velasco L, Robinson CJ, Quillfeldt P (2021) Year-round niche segregation of three sympatric hydrobates storm-petrels from Baja California Peninsula, Mexico, Eastern Pacific. *Mar Ecol Prog Ser* 664:207–225
- Berndt RK, Drenckhahn D (1990) Vogelwelt Schleswig-Holsteins 1: Seetaucher bis Flamingo. (2. korr. Aufl. Auflage). Wachholtz/Neumünster, pp 27–37
- BirdLife International (2010) The flyways concept can help coordinate global efforts to conserve migratory birds. <http://datazone.birdlife.org/sowb/casestudy/the-flyways-concept-can-help-coordinate-global-efforts-to-conserve-migratory-birds>. Accessed 01 Mar 2022
- BirdLife International (2022) Species factsheet: *Gavia stellata*. <http://www.birdlife.org>. Accessed 12 June 2022
- Boere GC, Stroud DA (2006) The flyway concept: what it is and what it isn't. In: Boere GC, Galbraith CA, Stroud DA (eds) Waterbirds around the world. The Stationery Office, Edinburgh, UK, pp 40–47
- Burger C, Schubert A, Heinänen S, Dorsch M, Kleinschmidt B, Žydelis R, Morkūnas J, Quillfeldt P, Nehls G (2019) A novel approach for assessing effects of ship traffic on distributions and movements of seabirds. *J Environ Manag* 251:109511
- Calenge C (2011) Home range estimation in R the adehabitatHR package. Office national de la classe et de la faune sauvage: Saint Benoist, Auffargis, France

- Catry P, Encarnação V, Araújo A, Fearon P, Fearon A, Armelin M, Delaloye P (2004) Are long-distance migrant passerines faithful to their stopover sites? *J Avian Biol* 35(170):181
- Catry P, Dias MP, Phillips RA, Granadeiro JP (2013) Carry-over effects from breeding modulate the annual cycle of a long-distance migrant: an experimental demonstration. *Ecology* 94(6):1230–1235
- Cherel Y, Hobson KA (2007) Geographical variation in carbon stable isotope signatures of marine predators: a tool to investigate their foraging areas in the Southern Ocean. *Mar Ecol Prog Ser* 329:281–287
- Christie AP (2021) Investigating post-breeding moult locations and diets of common guillemots (*Uria aalge*) in the North Sea using stable isotope analyses. *bioRxiv*. <https://doi.org/10.1101/2020.09.01.276857>
- CLS (2013) Argos user's manual. <http://www.argos-system.org/manual>. Accessed 15 June 2016
- Cohen EB, Hostetler JA, Hallworth MT, Rushing CS, Sillett TS, Marra PP (2018) Quantifying the strength of migratory connectivity. *Methods Ecol Evol* 9(3):513–524
- Conklin JR, Battley PF, Potter MA, Fox JW (2010) Breeding latitude drives individual schedules in a trans-hemispheric migrant bird. *Nat Commun* 1(1):1–6
- Conklin JR, Battley PF, Potter MA (2013) Absolute consistency: individual versus population variation in annual-cycle schedules of a long-distance migrant bird. *PLoS ONE* 8(1):e54535. <https://doi.org/10.1371/journal.pone.0054535>
- Corman AM, Schwemmer P, Mercker M, Asmus H, Rüdell H, Klein R, Boner M, Hofem S, Koschorreck J, Garthe S (2018) Decreasing $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in four coastal species at different trophic levels indicate a fundamental food-web shift in the southern North and Baltic seas between 1988 and 2016. *Environ Monit Assess* 190(8):1–12
- Cox GW (2010) Bird migration and global change. Island Press
- Cramp S, Simmons KEL (1977) Birds of the western Palaearctic. Vol. I. Ostrich to ducks. Oxford University Press, Oxford
- Cresswell W (2014) Migratory connectivity of Palaearctic-African migratory birds and their responses to environmental change: the serial residency hypothesis. *Ibis* 156:493–510
- Croxall JP, Silk JRD, Phillips RA, Afanasyev V, Briggs DR (2005) Global circumnavigations: tracking year-round ranges of non-breeding albatrosses. *Science* 307:249–250
- Dagys M, Žydelis R (2002) Bird bycatch in fishing nets in Lithuanian coastal waters in wintering season 2001–2002. *Acta Zool Litu* 12(3):276–282
- Das K, Siebert U, Fontaine M, Jauniaux T, Holsbeek L, Bouqueneau JM (2004) Ecological and pathological factors related to trace metal concentrations in harbour porpoises *Phocoena phocoena* from the North Sea and adjacent areas. *Mar Ecol Prog Ser* 281:283–295
- Dias MP, Granadeiro JP, Phillips RA, Alonso H, Catry P (2010) Breaking the routine: individual Cory's shearwaters shift winter destinations between hemispheres and across ocean basins. *Proc R Soc B* 278:1786–1793. <https://doi.org/10.1098/rspb.2010.2114>
- Dierschke V, Exo KM, Mendel B, Garthe S (2012) Gefährdung von Sterntaucher *Gavia stellata* und Prachtaucher *G. arctica* in Brut-, Zug- und Überwinterungsgebieten—eine Übersicht mit Schwerpunkt auf den deutschen Meeresgebieten. *Vogelwelt* 133:163–194
- Dorsch M, Burger C, Heinänen S, Kleinschmidt B, Morkūnas J, Nehls G, Quillfeldt P, Schubert A, Žydelis R (2019) DIVER – German tracking study of seabirds in areas of planned offshore wind farms at the example of divers. Final report on the joint project DIVER, FKZ 0325747A/B, funded by the federal ministry of economics and energy (BMWi) on the basis of a decision by the German Bundestag. <https://bioconsult-sh.de/en/about-us/documents>. Accessed 14 Mar 2022
- Dray S, Dufour AB (2007) The ade4 package: implementing the duality diagram for ecologists. *J Stat Softw* 22(4):1–20
- Duijns S, Niles LJ, Dey A, Aubry Y, Friis C, Koch S, Anderson AM, Smith PA (2017) Body condition explains migratory performance of a long-distance migrant. *Proc R Soc B*. <https://doi.org/10.1098/rspb.2017.1374>
- Environmental systems research institute (ESRI) (2012) ArcGIS release 10.1. Redlands, CA
- Esler D (2000) Applying metapopulation theory to conservation of migratory birds. *Conserv Biol* 14:366–372
- Fayet AL, Freeman R, Anker-Nilssen T, Diamond A, Erikstad KE, Fiefield D, Kouwenberg AL, Kress S, Mowat S, Perrins CM, Petersen A, Petersen IK, Reiertsen TK, Robertson GJ, Shannon P, Sigurdsson IA, Shoji A, Wanless S, Guilford T (2017) Ocean-wide drivers of migration strategies and their influence on population breeding performance in a declining seabird. *Curr Biol* 27(24):3871–3878
- Finch T, Butler SJ, Franco AM, Cresswell W (2017) Low migratory connectivity is common in long-distance migrant birds. *J Anim Ecol* 86(3):662–673
- Freitas C, Lydersen C, Fedak MA, Kovacs KM (2008) A simple new algorithm to filter marine mammal Argos locations. *Mar Mamm Sci* 24(2):315–325
- Frey B (1988) Food web structure on Georges Bank from stable C, N and S isotopic compositions. *Limnol Oceanogr* 33(5):1182–1190
- Garthe S, Sonntag N, Schwemmer P, Dierschke V (2007) Estimation of seabird numbers in the German North Sea throughout the annual cycle and their biogeographic importance. *Vogelwelt* 128(4):163–178
- Garthe S, Schwemmer H, Markones N, Müller S, Schwemmer P (2015) Distribution, seasonal dynamics and population trend of divers *Gavia* spec. in the German bight (North Sea). *Vogelwarte* 53:121–138
- Gilroy JJ, Gill JA, Butchart SH, Jones VR, Franco AM (2016) Migratory diversity predicts population declines in birds. *Ecol Lett* 19(3):308–317
- Gonzalez-Solis J, Croxall JP, Oro D, Ruiz X (2007) Trans-equatorial migration and mixing in the wintering areas of a pelagic seabird. *Front Ecol Environ* 5:297–301
- Gordo O (2007) Why are bird migration dates shifting? A review of weather and climate effects on avian migratory phenology. *Clim Res* 35(1–2):37–58
- Gray CE (2021) Migration and winter movement ecology of red-throated loons (*Gavia stellata*) in eastern North America. *Dispersions*, The University of Maine
- Gunnarsson TG, Tómasson G (2011) Flexibility in spring arrival of migratory birds at northern latitudes under rapid temperature changes. *Bird Study* 58(1):1–12
- Guse N, Garthe S, Schirmeister B (2009) Diet of red-throated divers *Gavia stellata* reflects the seasonal availability of Atlantic herring *Clupea harengus* in the southwestern Baltic Sea. *J Sea Res* 62(4):268–275
- Harrison XA, Blount JD, Inger R, Norris DR, Bearhop S (2011) Carry-over effects as drivers of fitness differences in animals. *J Anim Ecol* 80(1):4–18
- Heinänen S, Žydelis R, Kleinschmidt B, Dorsch M, Burger C, Morkūnas J, Quillfeldt P, Nehls G (2020) Satellite telemetry and digital aerial surveys show strong displacement of red-throated divers (*Gavia stellata*) from offshore wind farms. *Mar Environ Res*. <https://doi.org/10.1016/j.marenvres.2020.104989>
- HELCOM (2013) Species information sheet: *Gavia stellata*. <http://www.helcom.fi/Red%20List%20Species%20Information%20She>

- et/HELCOM%20Red%20List%20Gavia%20stellata%20(wintering%20population).pdf Accessed 4 Sept 2018
- Hemmingsson E, Eriksson MO (2002) Ringing of red-throated diver *Gavia stellata* and black-throated diver *Gavia arctica* in Sweden. Wetl Int Diver/loon Spec Group News 4:8–13
- Hobson KA (1999) Tracing origins and migration of wildlife using stable isotopes: a review. *Oecologia* 120(3):314–326
- Hobson KA, Clark RG (1992) Assessing avian diets using stable isotopes I: turnover of ^{13}C in tissues. *Condor* 94:181–188
- Johnston A, Auer T, Fink D, Strimas-Mackey M, Iliff M, Rosenberg KV, Brown S, Lancot R, Rodewald AD, Kelling S (2020) Comparing abundance distributions and range maps in spatial conservation planning for migratory species. *Ecol Appl*. <https://doi.org/10.1002/eap.2058>
- Klaassen M (2003) Relationships between migration and breeding strategies in arctic breeding birds. *Avian migration*. Springer, Berlin, Heidelberg, pp 237–249
- Kleinschmidt B, Burger C, Dorsch M, Nehls G, Heinänen S, Morkūnas J, Žydelis R, Quillfeldt P (2019) The diet of red-throated divers (*Gavia stellata*) overwintering in the German bight (North Sea) analysed using molecular diagnostics. *Mar Biol* 166(6):1–18
- Klinner T, Buddemeier J, Bairlein F, Schmaljohann H (2020) Decision-making in migratory birds at stopover: an interplay of energy stores and feeding conditions. *Behav Ecol Sociobiol* 74(1):1–14
- Kokko H (1999) Competition for early arrival in migratory birds. *J Anim Ecol* 68:940–950
- Kölzsch A, Müskens GJ, Kruckenberg H, Glazov P, Weinzierl R, Nolet BA, Wikelski M (2016) Towards a new understanding of migration timing: slower spring than autumn migration in geese reflects different decision rules for stopover use and departure. *Oikos* 125(10):1496–1507
- Lessells CM, Boag PT (1987) Unrepeatable repeatabilities: a common mistake. *Auk* 104(1):116–121
- Lovvorn JR, Jones DR (1994) Biomechanical conflicts between adaptations for diving and aerial flight in estuarine birds. *Estuaries* 17(1):62
- Mahfouz C, Meziane T, Henry F, Abi-Ghanem C, Spitz J, Jauniaux T, Bouveroux T, Gaby K, Amara R (2017) Multi-approach analysis to assess diet of harbour porpoises *Phocoena phocoena* in the southern North Sea. *Mar Ecol Prog Ser* 563:249–259
- Marra PP, Hobson KA, Holmes RT (1998) Linking winter and summer events in a migratory bird by using stable-carbon isotopes. *Science* 282(5395):1884–1886
- Marra P, Cohen E, Harrison AL, Studds C, Webster M (2018) Migratory connectivity. *Conserv Biol Ser-Camb* 14:157
- Martin TG, Chadès I, Arcese P, Marra PP, Possingham PP, Norris DR (2007) Optimal conservation of migratory species. *PLoS ONE* 2:3–7
- McCloskey SE, Uher-Koch BD, Schmutz JA, Fondell TF (2018) International migration patterns of red-throated loons (*Gavia stellata*) from four breeding populations in Alaska. *PLoS ONE*. <https://doi.org/10.1371/journal.pone.0189954>
- McConnell BJ, Chambers C, Fedak MA (1992) Foraging ecology of southern elephant seals in relation to the bathymetry and productivity of the Southern Ocean. *Antarct Sci* 4(4):4393–4398
- McFarlane T, Montevecchi LA, Fifield WA, Hedd DAA, Gaston AJ, Robertson GJ, Phillips RA (2014) Individual winter movement strategies in two species of murre (*Uria* spp.) in the Northwest Atlantic. *PLoS ONE*. <https://doi.org/10.1371/journal.pone.0090583>
- McNamara JM, Barta Z, Klaassen M, Bauer S (2011) Cues and the optimal timing of activities under environmental changes. *Ecol Lett* 14(12):1183–1190
- Mendel B, Garthe S (2010) Kumulative Auswirkungen von Offshore-Windkraftnutzung und Schiffsverkehr am Beispiel der Seetaucher in der Deutschen Bucht. *Coastline Rep* 15:31–44
- Mendel B, Schwemmer P, Peschko V, Müller S, Schwemmer H, Mercker M, Garthe S (2019) Operational offshore wind farms and associated ship traffic cause profound changes in distribution patterns of loons (*Gavia* spp.). *J Environ Manag* 231:429–438
- Mendel B, Sonntag N, Wahl J, Schwemmer P, Dries H, Guse N, Müller S, Garthe S (2008) Artensteckbriefe von See- und Wasservögeln der deutschen Nord- und Ostsee: Verbreitung, Ökologie und Empfindlichkeiten gegenüber Eingriffen in ihrem marinen Lebensraum. *Naturschutz und Biologische Vielfalt* Nr. 59, Bundesamt für Naturschutz, Bonn-Bad Godesberg (DEU)
- Merkel B, Descamps S, Yoccoz NG, Grémillet D, Daunt F, Erikstad KE, Ezhov AV, Harris MP, Gavrilov M, Lorentsen SH, Reierson TK, Steen H, Systad GH, Þórarinnsson PL, Wanless S, Strøm H (2021) Individual migration strategy fidelity but no habitat specialization in two congeneric seabirds. *J Biogeogr* 48(2):263–275
- Moore FR, Woodrey MS, Buler JJ, Woltmann S, Simons TR (2005) Understanding the stopover of migratory birds: a scale dependent approach. In: Ralph C, John R, Terrell D (Eds) *Bird Conservation implementation and integration in the Americas: Proceedings of the third international partners in flight conference*. Pacific Southwest Research Station, USDA Forest Service General Technical Report PSW-191, Albany, Asilomar, California, USA, pp 684–689
- Nakagawa S, Schielzeth H (2010) Repeatability for Gaussian and non-Gaussian data: a practical guide for biologists. *Biol Rev* 85(4):935–956
- Newton I (2008) *The migration ecology of birds*. Academic Press, Oxford, UK
- Newton I (2009) Molt and plumage. *Ring. Migr* 24(3):220–226
- Newton I (2011) Migration within the annual cycle: species, sex and age differences. *J Ornithol* 152(1):169–185
- Okill JD (1992) Natal dispersal and breeding site fidelity of red-throated divers *Gavia stellata* in Shetland. *Ring. Migr* 13(1):57–58
- Okill JD (1994) Ringing recoveries of red-throated divers *Gavia stellata* in Britain and Ireland. *Ring. Migr* 15(2):107–118
- Oppel S, Powell AN (2008) Assigning king eiders to wintering regions in the Bering sea using stable isotopes of feathers and claws. *Mar Ecol Prog Ser* 373:149–156
- Paruk JD, Chickering MD, Long D, Uher-Koch H, East A, Poleschook D, Gumm V, Hanson W, Adams EA, Kovach KA, Evers DC (2015) Winter site fidelity and winter movements in common loons (*Gavia immer*) across North America. *Condor: Ornithol Appl* 117(4):485–493
- Pennycuik CJ (1989) *Bird flight performance: a practical calculation manual*. Oxford Univ. Press, Oxford U.K
- Peterson BJ, Frey B (1987) Stable isotopes in ecosystem studies. *Annu Rev Ecol Syst* 18:293–320
- Poessel SA, Uher-Koch BD, Pearce JM, Schmutz JA, Harrison AL, Douglas DC, Biela VR, Katzner TE (2020) Movements and habitat use of loons for assessment of conservation buffer zones in the Arctic coastal plain of northern Alaska. *Glob Ecol Conserv*. <https://doi.org/10.1016/j.gecco.2020.e00980>
- QGIS Development Team (2018) QGIS geographic information system. Open Source Geospatial Foundation Project. <http://qgis.osgeo.org>
- R Core Team (2018) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>
- Rappole JH (2013) *The avian migrant: the biology of bird migration*. Columbia University Press
- Reed TE, Warzybok P, Wilson AJ, Bradley RW, Wanless S, Sydeman WJ (2009) Timing is everything: flexible phenology and shifting selection in a colonial seabird. *J Anim Ecol* 78:376–387
- Ronconi RA, Swaim ZT, Lane HA, Hunnewell RW, Westgate AJ, Koopman HN (2010) Modified hoop-net techniques for

- capturing birds at sea and comparison with other capture methods. *Mar Ornithol* 38(1):23–29
- Rubarth J, Dreyer A, Guse N, Einax JW, Ebinghaus R (2011) Perfluorinated compounds in red-throated divers from the German Baltic Sea: new findings from their distribution in 10 different tissues. *Environ Chem* 8(4):419–428
- Runge CA, Martin TG, Possingham HP, Willis SG, Fuller RA (2014) Conserving mobile species. *Front Ecol Environ* 12(7):395–402
- Rushing CS, Marra PP, Dudash MR (2016) Winter habitat quality but not long-distance dispersal influences apparent reproductive success in a migratory bird. *Ecology* 97(5):1218–1227
- Ruthrauff DR, Tibbitts TL, Gill RE Jr (2019) Flexible timing of annual movements across consistently used sites by Marbled Godwits breeding in Alaska. *Auk: Ornithol Adv*. <https://doi.org/10.1093/auk/uky007>
- Sæther BE, Bakke Ø (2000) Avian life history variation and contribution of demographic traits to the population growth rate. *Ecology* 81(3):642–653
- Schmutz JA (2014) Survival of adult red-throated loons (*Gavia stellata*) may be linked to marine conditions. *Waterbirds* 37:118–124
- Schreiber EA, Burger J (2001) Breeding biology, life histories, and life history–environment interactions in seabirds. *Biology of marine birds*. CRC Press, pp 235–280
- Schwartz MD (1998) Green-wave phenology. *Nature* 394(6696):839–840
- Shariatinajafabadi M, Wang T, Skidmore AK, Toxopeus AG, Kölzsch A, Nolet BA, Exo KM, Griffin L, Stahl J, Cabot D (2014) Migratory herbivorous waterfowl track satellite-derived green wave index. *PLoS ONE*. <https://doi.org/10.1371/journal.pone.0108331>
- Skov H, Prins E (2001) Impact of estuarine fronts on the dispersal of piscivorous birds in the German bight. *Mar Ecol Prog Ser* 214:279–287
- Smith JA, Regan K, Cooper NW, Johnson L, Olson E, Green A, Tash J, Evers DE, Marra PP (2020) A green wave of saltmarsh productivity predicts the timing of the annual cycle in a long-distance migratory shorebird. *Sci Rep* 10(1):1–13
- Spiegel CS, Berlin AM, Gilbert AT, Gray CO, Montevocchi WA, Stenhouse IJ, Ford SL, Olsen GH, Fiely JL, Savoy L, Goodale MW, Burke CM (2017) Determining fine-scale use and movement patterns of diving bird species in federal waters of the mid-Atlantic United States using satellite telemetry. *OCS Study BOEM*, 69
- St John Glew K, Wanless S, Harris MP, Daunt F, Erikstad KE, Strøm H, Speakman JP, Kärten B, Trueman CN (2019) Sympatric Atlantic puffins and razorbills show contrasting responses to adverse marine conditions during winter foraging within the North Sea. *Mov Ecol* 7(1):1–14
- Stoffel MA, Nakagawa S, Schielzeth H (2017) rptR: repeatability estimation and variance decomposition by generalized linear mixed-effects models. *Methods Ecol Evol* 8(11):1639–1644
- Storer RW (1958) Loons and their wings. *Evolution* 12(2):262–263
- Stresemann E, Stresemann V (1966) Die Mauser der Vögel. *J Ornithol* 107:3–448
- Studds CE, Kyser TK, Marra PP (2008) Natal dispersal driven by environmental conditions interacting across the annual cycle of a migratory songbird. *Proc Natl Acad Sci* 105(8):2929–2933
- Taylor CM, Norris DR (2010) Population dynamics in migratory networks. *Thyroid Res* 3:65–73
- Thompson CW, Kitaysky AS (2004) Polymorphic flightfeather molt sequence in tufted puffins (*Fratercula cirrhata*): a rare phenomenon in birds. *Auk* 121:35–45
- Todorov V, Filzmoser P (2009) An object-oriented framework for robust multivariate analysis. *J Stat Softw* 32(3):1–47
- Trierweiler C, Klassen RHG, Drent RH, Exo KM, Komdeur J, Bairlein F, Koks BJ (2014) Migratory connectivity and population-specific migration routes in a long-distance migratory bird. *Proc R Soc b: Biol Sci*. <https://doi.org/10.1098/rspb.2013.2897>
- Vardanis Y, Klaassen RH, Strandberg R, Alerstam T (2011) Individuality in bird migration: routes and timing. *Biol Lett* 7(4):502–505
- Vilela R, Burger C, Diederichs A, Nehls G, Bachl FE, Szostek L, Freund A, Braasch A, Bellebaum J, Beckers B, Piper W (2021) Use of an INLA latent Gaussian modelling approach to assess bird population changes due to the development of offshore wind farms. *Front Mar Sci* 8(882):1–11
- Walther GR, Post E, Convey P, Menzel A, Parmesan C, Beebee TJ, Fromentin JM, Hoegh-Guldberg O, Bairlein F (2002) Ecological responses to recent climate change. *Nature* 416(6879):389–395
- Warnock N (2010) Stopping vs. staging: the difference between a hop and a jump. *J Avian Biol* 41(6):621–626.57
- Wauchope HS, Shaw JD, Varpe Ø, Lappo EG, Boertmann D, Lantot RB, Fuller RA (2017) Rapid climate-driven loss of breeding habitat for Arctic migratory birds. *Glob Change Biol* 23(3):1085–1094
- Weber TP, Houston AI (1997) Flight costs, flight range and the stopover ecology of migrating birds. *J Anim Ecol* 66(3):297–306
- Webster MS, Marra PP, Haig SM, Bensch S, Holmes RT (2002) Links between worlds: unraveling migratory connectivity. *Trends Ecol Evol* 17(2):76–83
- Wetlands International (2019) Waterbird population estimates. <http://wpe.wetlands.org>. Accessed 23 Apr 2019
- Wetlands International (2022) Waterbird populations portal. <http://wpp.wetlands.org>. Accessed 09 May 2022
- Whitworth DL, Takekawa JY, Carter HR, Mciver WR (1997) A night-lighting technique for at-sea capture of Xantus' Murrelets. *Colon Waterbirds* 20:525–531
- Wilcove DS, Wikelski M (2008) Going, going, gone: is animal migration disappearing. *PLoS Biol*. <https://doi.org/10.1371/journal.pbio.0060188>
- Winkler DW, Jørgensen C, Both C, Houston AI, McNamara JM, Levey DJ, Partecke J, Fudickar A, Kacelnik A, Roshier D, Piersma T (2014) Cues, strategies, and outcomes: how migrating vertebrates track environmental change. *Mov Ecol* 2(2):10
- Žydelis R, Small C, French G (2013) The incidental catch of seabirds in gillnet fisheries: a global review. *Biol Cons* 162:76–88

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Gavia stellata

9. CHAPTER 3

Prevalence of haemosporidian parasites in an arctic breeding seabird species – the NW-European red-throated diver (*Gavia stellata*)

Birgit Kleinschmidt, Claudia Burger, Monika Dorsch, Stefan Heinänen, Julius Morkūnas, Georg Nehls,
Yvonne Schumm, Ramūnas Žydelis, Petra Quillfeldt

Manuscript prepared for submission

Prevalence of haemosporidian parasites in an arctic breeding seabird species – the NW-European red-throated diver (*Gavia stellata*)

Birgit Kleinschmidt^{1,2*}, Claudia Burger², Monika Dorsch², Stefan Heinänen^{3,6}, Julius Morkūnas⁴, Georg Nehls², Schumm YR¹, Ramūnas Žydelis^{3,5} & Petra Quillfeldt¹

1 Department of Animal Ecology and Systematics, Justus Liebig University Giessen, Giessen 35392, Germany

2 BioConsult SH, Husum 25813, Germany

3 DHI, Hørsholm 2970, Denmark

4 Marine Research Institute, Klaipėda University, Klaipėda 92294, Lithuania

5 Ornitela UAB, LT-03228 Vilnius, Lithuania (current address)

6 Novia University of Applied Sciences, Raseborgsvägen 9, 10600 Ekenäs, Finland (current address)

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

Abstract

Haemosporida, vector-transmitted blood parasites, can have various effects on their host and may also exert selection pressures on their hosts. In this study we analyse the presence of haemosporida in a previously unstudied migratory seabird species, the red-throated diver *Gavia stellata*.

Red-throated divers were sampled during the non-breeding period in the eastern German Bight (North Sea). We used molecular methods and tracking data from a related tracking study to reveal (i) if red-throated divers are infected with the haemosporida of the genera *Leucocytozoon*, *Plasmodium* and *Haemoproteus*, (ii) how infection and prevalence are linked with breeding regions of infected individuals.

Divers in this study are assigned to western Palearctic breeding grounds, namely Greenland, Svalbard, Norway and Arctic Russia. We found a prevalence of *Leucocytozoon* of 11% in all birds (n=45) sampled. We found a prevalence of 33% in birds breeding in Norway (one out of three) and a prevalence of 8.3 % in birds breeding in Arctic Russia (two out of 25). Haemosporidian parasites have not been detected in birds from Greenland (n=2) and Svalbard (n=2). For two birds that were infected no breeding regions could be assigned. We found two previously unknown lineages, one each of *Leucocytozoon* and *Plasmodium*. The *Leucocytozoon* lineage was only detected at the breeding site in Norway.

In summary, this study presents the first record of haemosporidian parasites in red-throated divers and reports a new lineage of each, *Plasmodium* and *Leucocytozoon* GAVSTE01 and GAVSTE02, respectively.

Keywords

Red-throated diver/loon, Avian malaria, *Leucocytozoon*, *Plasmodium*

Introduction

Birds are frequently infected with a number of intracellular blood parasites, including Haemosporidia, or hematozoa, of the genera *Plasmodium*, *Haemoproteus* and *Leucocytozoon* (Valkiūnas 2005, Bensch et al. 2009, Clark, et al. 2014, Vanstreels et al. 2014). *Plasmodium*, *Haemoproteus* and *Leucocytozoon* are vector-borne parasites that cause malaria like disease in birds (Scheuerlein and Ricklefs 2004, Wood et al. 2007; Jenkins and Owens 2011). However, there are interspecific differences in the prevalence of haemosporidian parasites and seabirds mostly have low levels or even a complete lack of infections, with only one third of the species showing infections (Quillfeldt et al. 2011). The most common haemosporidian parasites found in seabirds were avian malaria of the genera *Haemoproteus* and *Plasmodium*, each showing infections for 13% of the species studied (Quillfeldt et al. 2011). In Antarctic regions Haemosporida were absent from seabirds (Quillfeldt et al. 2011) whereas in Arctic regions, few records are known so far (Benett et al. 1992, Reed et al. 2018). In the context of climate warming the prevalence and distribution of Haemosporida may change, particular in high latitude areas (Garamszegi 2011, Loiseau et al. 2012, Zamora-Vilchis et al. 2012, Reed et

al. 2018) and some of the vectors are quite common in arctic regions. These are mosquitos (Culicidae) which transfer *Plasmodium*, louse flies (Hippoboscidae) and biting midges (Ceratopogonidae) which transfer *Haemoproteus*, and blackflies (Simuliidae) which transfer *Leucocytozoon* (Atkinson et al. 2009).

In general, haemosporidian parasites are considered low pathogenic in bird populations (Ciloglu et al. 2016), although these parasites have also been associated with mortality events and population declines and extinctions in songbirds and waterbirds (Warner 1968, Herman 1975, Hill et al. 2010). Negative effects on the body condition of their hosts and reduced expression of sexual ornaments, as well as lower reproductive success has been associated with infection (Allander and Bennett 1994, Martinez-Abraín et al. 2004, Marzal et al. 2005, Dunning 2008, Barbosa et al. 2011). Thus, haemosporidian parasites can exert important selection pressure on their hosts through effects on survival and reproduction (Sorci and Møller 1997, Sol D et al. 2003, Møller and Nielsen 2007, Knowles et al. 2010). A co-infection with more than one lineage even increase the risk for a reduced body condition caused by additive cost from single to double infection (Marzal et al. 2008). In this context, Hegeman et al. (2018) found that a haemosporidian infection affects migratory birds' movements and their stop-over ecology.

Our study species, the NW-European red-throated diver/loon *Gavia stellata* is a migratory Arctic-breeding seabird species for which there have been no known studies of haemosporidian parasites, to our knowledge. *Gavia stellata*, colloquial red-throated loon in America or red-throated diver in Europe, has a Holarctic breeding distribution, inhabiting small lochs adjacent to coastal areas. Inland areas are rarely occupied during the breeding season as this species prefers to feed in marine habitats (Reimchen and Douglas 1984). Red-throated divers spend the non-breeding season in the Atlantic Ocean, North- and Baltic Sea and transmission of blood parasite infections via biting insects is thus limited to the breeding season. This seabird species is affected by increasing anthropogenic impacts such as shipping traffic and offshore wind turbines in its non-breeding areas, to which this species reacts very sensitively (Burger et al. 2019, Fliessbach et al. 2019, Mendel et al 2019, Vilela et al. 2020). In this context infestation with haemosporida could have an additional negative effect and information about the presence or absence of these parasites is an important basis when considering cumulative effects. Within the Gaviidae family, an infection with haemosporida is so far only documented for great northern divers (common loons, *Gavia immer*) with infections of *Plasmodium* as well as *Leucocytozoon* (Lindsay unblished, Weinandt 2006, 2012, Martinsen et al. 2017, GenBank record EF077166).

We had the unique chance to take blood samples of 45 red-throated divers captured in an internationally important non-breeding habitat, the eastern German Bight (North Sea) in winter and spring within the framework of the DIVER project (Dorsch et al. 2019, Burger et al. 2019, Kleinschmidt et al. 2019, in prep., Heinänen et al. 2020, www.divertracking.com). The DIVER project is a tracking study and therefore information on the breeding areas in Greenland, Svalbard, Norway and northern Russia of single individuals was available to be linked with haemosporidian parasite infection. The overall objective of the study

presented here is to document infestation of haemosporidian parasites in European red-throated divers. This information can be considered when assessing habitat change and thereby a possibly increased stress level in their European non-breeding habitats. Specifically we aim to (i) present general information about presence/absence of the three haemosporidian species *Haemoproteus*, *Plasmodium* and *Leucocytozoon*, (ii) to study prevalence and (iii) lineage richness among sampled red-throated divers, where prevalence refers to the proportion of individuals that are infected and richness to the number of parasite species/lineages found in an individual host, a group, or a species (Herrera and Nun 2019).

Methods

Sampling and sample preparation

Bird capture and sampling were carried out in accordance with the local legislation. Sampling was conducted in the eastern German Bight (North Sea Germany) about 20 to 30 km west of the island of Amrum during three time periods: March to April 2015, February to March 2016 and March 2017 (see also Kleinschmidt et al. 2019, Burger et al. 2019, Heinänen et al. 2020). A total of 45 red throated divers were captured to be tagged with satellite transmitters within the study area. Birds were captured at night, from a RIB (rigid inflatable boat) using a hand net and the “night lighting technique” (Whitworth et al. 1997, Ronconi et al. 2010). Blood samples of 45 individuals were taken with a sterile needle and stored on FTA cards (Whatman FTA card technology, Sigma Aldrich) for further analysis. In the laboratory, a 2x2 mm piece of the dried blood sample was cut out of the FTA card and the DNA was isolated using an ammonium acetate protocol adapted from Martinez et al. (2015) and purified with NZYGelpure columns (NZYTech, Portugal). The final DNA concentration of the sample was determined with a NanoDrop2000c UV-Vis spectrophotometer (Thermo Fisher Scientific, Wilmington, USA). The extracted DNA was stored frozen until further analysis.

Molecular detection of haemosporidian parasites (Nested PCR and Sanger sequencing)

The blood samples (n=45) were screened for presence-absence of Haemosporidia (*Haemoproteus*, *Plasmodium* and *Leucocytozoon*) using a nested polymerase chain reaction (PCR) targeting a 479 bp region of the cyt b gene (Hellgren et al. 2004). A nested PCR is a two-step PCR and enables simultaneous typing of species from the three most common avian blood parasite genera (*Haemoproteus*, *Plasmodium*, and *Leucocytozoon*). First, an initial PRC step was applied using the primer combination HaemNFI/HaemNR3. Second, a 4 µL aliquot of this PCR product was subsequently used as a template to specifically detect *Haemoproteus* and *Plasmodium* using the primer combination HaemF/HaemR2 or *Leucocytozoon* using the primer combination HaemFL/HaemR2L (Table 1). The three PCR reactions were set each in a 25µl reaction volume that contained 12.5µl 2x Dream Taq PCR Master Mix ready to use solution (Thermo Fisher Scientific, Germany), 4 µl of template DNA, 0.6 µM of each primer (1,65µL of 10 µM) and sterile water. DNA from passerine birds with known infection served as a positive control and deionized water as a

negative control. Cycling conditions followed the protocol given by Hellgren et al (2004) and included an incubation step at 94°C for 3 min, a final extension at 72°C for 10 min and a thermal profile of 30 sec at 94°C, 30 sec at 50°C, and 45 sec at 72°C for 20 cycles in the initial PCR and for 35 cycles in the parasite specific PCR. PCR protocols were carried out on a Biometra TOne Cyclor (Analytik Jena, Germany). All samples were screened two times using the same protocol to back up positive or negative results.

PCR amplicons were visualized using high-resolution capillary gel electrophoresis (QIAxcel Advanced, Qiagen, Switzerland). Samples rendering a clear peak (478/480 bp) during gel electrophoresis were bi-directional Sanger sequenced by Microsynth-Seqlab (Sequence Laboratories Goettingen GmbH, Germany). Forward and reverse sequences were assembled and trimmed in CLC Main Workbench 7.6.4 (CLC Bio, Qiagen, Denmark). Sequences were aligned to reference sequences deposited in MalAvi database (Bensch et al. 2009) and GenBank nucleotide databases using BLASTN 2.3.0+ (Zhang 2000) to identify lineages. If sequences differ by one or more nucleotides in the cyt b fragment (Hellgren et al. 2004, Bensch et al. 2009, Chagas et al. 2017) they were considered as distinct lineages. Lineages that were considered as distinct and had no database records in MalAvi were considered novel and were named according to MalAvi nomenclature.

Table 1 Primer sequences applied to amplify DNA of blood parasites *Haemoproteus*/ *Plasmodium*/ *Leucocytozoon*

Primer	Primer sequence	Target DNA
HaemNFI	5'-CATATATTAAGAGAAITATGGAG-3'	<i>Haemoproteus/Plasmodium/Leucocytozoon</i>
	5'-	
HaemNR3	ATAGAAAGATAAGAAATACCATTC-3'	<i>Haemoproteus/Plasmodium/Leucocytozoon</i>
HaemF	5'-ATGGTGCTTTTCGATATATGCATG-3'	<i>Haemoproteus/Plasmodium</i>
	5'-	
HaemR2	GCATTATCTGGATGTGATAATGGT-3'	<i>Haemoproteus/Plasmodium</i>
HaemFL	5'-ATGGTGTTTTAGATACTTACATT-3'	<i>Leucocytozoon</i>
HaemR2L	5'CATTATCTGGATGAGATAATGGIGC-3'	<i>Leucocytozoon</i>

Phylogenetic analyses

The best matching Malavi lineage and the GenBank hit with the highest similarity (99-100%) were downloaded. The downloaded sequences, the consensus sequences from our own samples, and a sequence from *Babesia* (GenBank record KC754965, Quillfeldt et al. 2014) as the outgroup were aligned in BIOEDIT using the ClustalW multiple alignment tool. A phylogenetic tree was inferred with the reference sequences

and outgroup. The final alignment included 10 nucleotide sequences (one outgroup, four sequences from this study and five reference sequences). The best suitable nucleotide substitution model (HKY) for our alignment was selected using jModelTest 2.1.10 and Bayesian Information Criterion scores. A Bayesian phylogenetic tree was generated with BEAST v1.8.4. Model parameters for this analysis were selected in BEAUTi v1.8.4 with the HKY substitution model, strict clock as clock type and a Yule speciation process as tree prior. The chain length for the Metropolis coupled Markov Chain (MCMC) was set to 25 Mio. generations (burn-in 10%), and one tree was recorded every 1000 generations. Using Tracer v1.6, we verified the trace for convergence. We used TreeAnnotator in BEAST v1.8.4 to generate a maximum clade credibility tree (MCCT). Finally, FigTree v1.4.3 (Rambaut 2007) was used to visualize the final phylogenetic tree. Similarities between sample sequences were calculated in BLAST (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>).

Results

Presence-absence, prevalence and parasite richness

Using molecular methods and a specific nested PCR approach we screened 45 samples for haemosporidian parasites. Samples that were screened positive (n=5) were sequenced successfully and genera as well as lineages could be assigned. Two samples were excluded from the phylogenetic tree reconstruction due to insufficient sequence length. Haemosporidian parasites were present in five individuals resulting in an overall prevalence of 11 % in all screened red-throated divers in this data set. Two haemosporidian parasite species were detected in the sample set (n=45) resulting in a parasite richness of 4.4 %. Of the five infected individuals all were infected with a single *Leucocytozoon* lineage and one of these five individuals showed a heterogenic infection (co-occurrence of different parasite genera) with *Leucocytozoon/Plasmodium*. *Haemoproteus* was not detected in this sample set.

Lineage Diversity

We found a comparatively high lineage diversity in five infected individuals. *Leucocytozoon* was present with three lineages and *Plasmodium* with one lineage (Fig. 1, Table 2).

Table 2 List of positively tested red-throated divers for blood parasite infections, with ID (ARGOS-ID) and parasite genera. Novel lineages are indicated in bold.

ID (ARGOS ID)	Breeding region	Blood Parasite Genus	Lineage
146441	n.a.	<i>Leucocytozoon</i>	CIAE02
146445	n.a.	<i>Leucocytozoon</i>	GAVSTE02
146449	Norway	<i>Leucocytozoon</i>	GAVSTE02
146441	n.a.	<i>Plasmodium</i>	GAVSTE01
158325	Arctic Russia	<i>Leucocytozoon</i>	n.a
57332	Arctic Russia	<i>Leucocytozoon</i>	AMO02

The MalAvi database (MalAvi, Bench et al. 2009) was used to determine if the encountered lineages were previously found in other birds species. One *Plasmodium* lineage GAVSTE01 (n=1) and the most prevalent *Leucocytozoon* lineage GAVSTE02 (n=2) each differed by one nucleotide in the cyt b fragment from lineages reported previously, and were considered as distinct and novel. The novel *Leucocytozoon* lineage GAVSTE02 (GenBank record OP007193) differed by one nucleotide in the cyt b fragment from the lineage GAVIM01 (GenBank No. EF077166), which was detected in the closely related diver species, *Gavia immer* in North America. The novel *Plasmodium* lineage GAVSTE01 (GenBank record OP007192) differed by one nucleotide in the cyt b fragment from a widespread lineage, TURDUS1 (GenBank record JN164734), that was previously found in a variety of European and Asian bird species from the families Fringilidae, Muscicapidae, Turdidae, Motacillidae, Paridae, Sylviidae, Accipitridae, Passeridae, Paridae, Hirundinidae, Sittidae, Scolopacidae and Certhiidae.

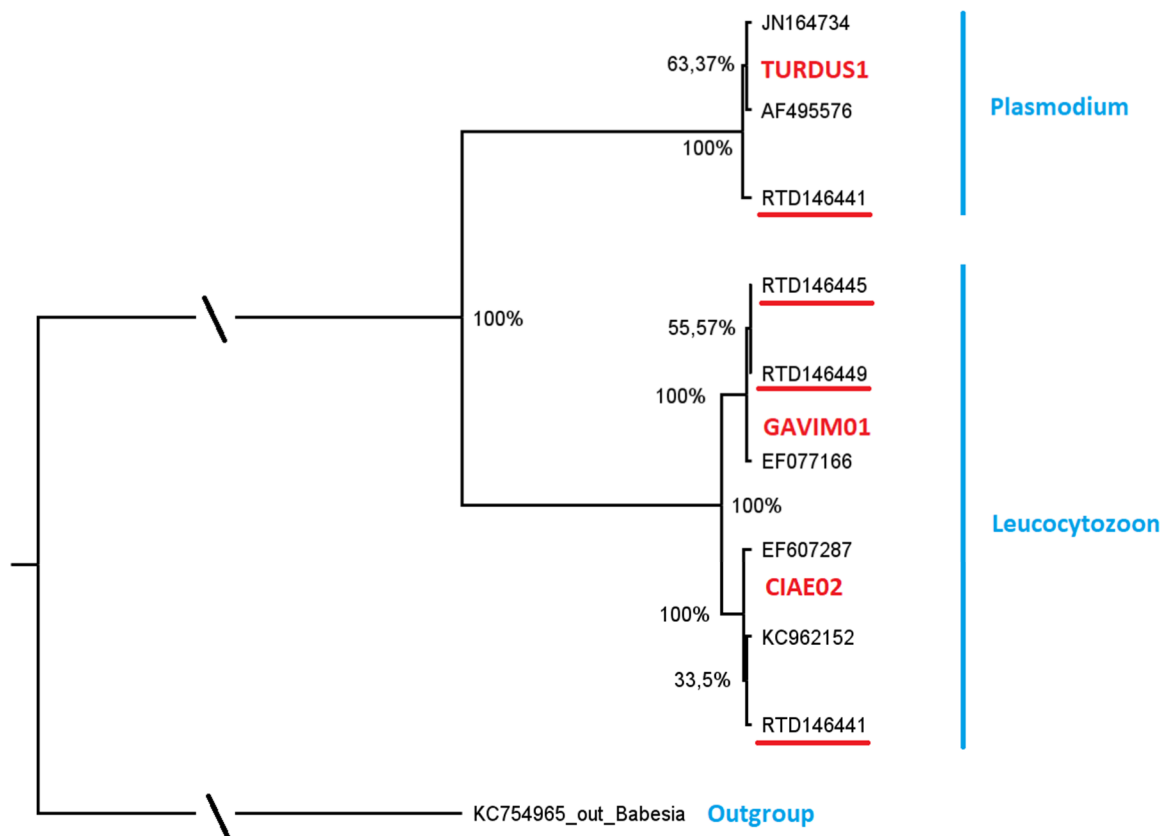


Fig. 1 Molecular phylogenetic analysis by Maximum Likelihood Bayesian Analysis, based on parasite DNA sequences. Posterior probabilities of the nodes are shown. Parasites from Red-throated diver samples are given with bird IDs, and underlined red. Details of the reference sequences (not underlined), including blood parasite and host species identity and code and location, are given in Table 3. MalAvi lineage names are included in red letters, and genus names in cyan.

Table 3 Reference sequences included in the molecular phylogenetic analysis.

Reference	Blood Parasite	MalAvi Lineage	Host and country
JN164734	<i>Plasmodium circumflexum</i>	TURDUS1	<i>Sylvia atricapilla</i> (Spain)
AF495576	<i>Plasmodium circumflexum</i>	TURDUS1	<i>Turdus philomelos</i> (Sweden)
KC962152	<i>Leucocytozoon sp.</i>	CIAE02	<i>Buteo buteo</i> (Turkey)
EF607287	<i>Leucocytozoon sp.</i>	CIAE02	<i>Circus aeruginosus</i> (Germany)
EF077166	<i>Leucocytozoon sp.</i>	GAVIM01	<i>Gavia immer</i> (North America)

The other two *Leucocytozoon* lineages, each found in one individual, had exact matches (100%) from a variety of European and Asian birds. The lineage CIAE02 is documented in Falconiformes, Gruiformes, Charadriiformes, Strigiformes, Piciformes, Ciconiiformes, Cuculiformes, Coraciiformes and the lineage AMO02 in Falconiformes, Columbiformes, Piciformes, Passeriformes. For the one lineage of *Plasmodium* and the three lineages of *Leucocytozoon* this study presents first records in red-throated divers (Table 2).

Prevalence of haemosporidian parasites in connection with breeding regions

Breeding regions of red-throated divers in this data set were identified by additional analysis of tracking data and were located in Greenland, Svalbard, Norway and northern Russia, see Kleinschmidt et al. (in prep.). Breeding regions of two individuals, one (ID 146445) infected with *Leucocytozoon* and one (ID 146441) that showed a heterogenic infection with *Leucocytozoon* and *Plasmodium* could not be assigned due to early mortality of individuals.

Individuals infected with haemosporidian parasites were detected in Norway and northern Russia, (Table 2). In Norway, one in three individuals was infected with the distinct novel *Leucocytozoon* lineage GAVSTE02, resulting in a prevalence of 33% (ID 146449, Table 2). In northern Russia two in 25 birds had each a single infection with *Leucocytozoon* (ID 158325, 57332, Table 2) resulting in a prevalence of 8.3 %. One of these two *Leucocytozoon* infections was assigned to the lineage AEMO02 and a breeding site in Novaya Semla. The *Leucocytozoon* sequence found in the other individual that headed to Taimyr peninsula, had an insufficient sequence length and no lineage could be identified. The birds analysed from breeding areas in Greenland (n=2) and Svalbard (N=2) tested negative.

Discussion

In this study we used molecular methods to detect haemosporidian parasites in a previously unstudied species, the red-throated diver. Although we did not examine the degree of parasitaemia (i.e. the number of infected blood cells), due to the absence of blood smears we could define presence-absence, the prevalence and parasite richness of haemosporidian parasites in red-throated divers.

Presence absence of Haemosporida in red-throated divers

Red-throated divers in our sample set can be assigned to the European breeding population (BirdLife international 2019) and the NW European wintering population (Wetlands International 2019). The sampled birds were captured in the eastern German Bight (North Sea) and originated from Greenland, Svalbard, Norway and Arctic Russia. Of these breeding regions, we detected haemosporida in individuals from Norway and Arctic Russia. No haemosporidian parasites were detected in individuals from Greenland and Svalbard although admittedly sample size of birds from these regions in our sample set was small with two birds for each region, respectively. Not much information is known so far about the presence of avian blood parasite infections in these regions, at least to our knowledge. Investigations in Greenland for gyrfalcons (*Falco rusticolus*) and peregrine falcons (*Falco peregrinus*) showed no presence of haemosporidian parasites (Taft et al. 1999), though potential vectors, such as black flies (*Prosimulium ursinum*) and mosquito (*Aedes impiger*) were sampled. The absence of haemosporidian parasites was linked with temperature limitations and temperatures ranging from 0° - 15° during July and August in Greenland (Taft et al. 1999). In Svalbard little auks were sampled for haemosporidian parasites but these were not detected in sampled birds (Wojczulanis-Jakubas et al. 2010). Martinez et al. (2018) screened samples from snow buntings from Svalbard for haemosporidian parasites but could not detect these parasites either. The absence of haemosporidian parasites in individuals from Svalbard and Greenland in our data set is in line with the general absence of haemosporidian parasites in these high arctic latitudes. From Norway the presence of the haemosporidian parasites *Leucocytozoon* and *Plasmodium* was detected by Hellgren (2005) in bluethroats (*Luscinia svecica*) over a wide geographic area and with various lineages. In other arctic regions the information about the occurrence and distribution is fragmented. Information and positive detections in birds from Alaska has increased over the past years (Ramey et al. 2012, 2021, Oakgrove et al. 2014, Reeves et al. 2015, Smith et al. 2016, Reed et al. 2018). Recent studies investigating the Russian Arctic detected haemosporida in passerine birds in southwestern Yamal (Russian Arctic), with a high prevalence and *Leucocytozoon* being the most prevalent compared to *Haemosproteus* and *Plasmodium* (Yusupova 2022).

Haemosporidian parasite species and lineages diversity detected in red-throated divers

Leucocytozoon was the most abundant haemosporidian parasite in our data set of an arctic breeding seabird species. The more or less exclusively detection of *Leucocytozoon* might be related to the climatic conditions in arctic breeding locations. Oakgrove et al (2014) showed for arctic regions in Alaska also the highest prevalence of *Leucocytozoon* in birds across a latitudinal gradient. *Leucocytozoon* seem to have a higher cold tolerance than other haemosporidian parasites and is able to persist at high elevations and in colder regions (Haas et al. 2012, van Rooyen et al 2013). Furthermore, the actual high number of lineages found in arctic regions in Alaska suggests a potential evolution of tolerant lineages in these arctic regions (Oakgrove et al. 2014). In the Russian Arctic recent studies confirm the high prevalence of *Leucocytozoon* in passerines

(Yusupova 2022) and sea ducks (Vardeh et al unpublished data). Numerous studies suggest the importance of temperature seasonality and vegetation in the patterns of pathogen distribution (Wilson et al. 2002, Yasuoka and Levins 2007, Oakgrove et al. 2014) which might explain *Leucocytozoon* as the most prevalent and genetically diverse of haemosporidian parasites in Arctic regions.

Haemoproteus was absent in our dataset. The absence of *Haemoproteus* might be explained by a possible competitive exclusion between *Leucocytozoon* and *Haemoproteus* that was already suggested by Oakgrove et al. (2014) and Zagalska-Neubauer and Bensch (2016). *Plasmodium* was detected with a distinct novel lineage GAVSTE01 (GenBank record OP007192) in one individual that was co infected also with *Leucocytozoon*. The lower detection rate in our sample set compared to *Leucocytozoon* might be explained by climatic conditions in arctic breeding regions. *Plasmodium* prevalence is thought to be temperature dependent, which is an important factor in explaining variations in *Plasmodium* prevalence (LaPointe et al. 2010).

We found a high lineage diversity for *Leucocytozoon* with three lineages (CIAE02, GAVSTE02, AMO02, GenBank record KC962152, OP007193, LC440381) in our sample set considering five infected individuals and the sample size (n=45). The lineage diversity might be related to the geographical range among the breeding locations of infected individuals and a region specific occurrence of vectors and parasites. Species of *Haemoproteus* and *Leucocytozoon* are described to be more specific and restricted to closely related species or the same family, other than *Plasmodium* which can be considered as more host-generalised and is unlikely coevolved with vector species (Fallis et al. 1974, Atkinson 1986, Waldenström et al., 2002; Beadell et al. 2004, Dimitrov et al. 2010; Kimura et al. 2010, Jenkins and Owens 2011). The distinct novel *Leucocytozoon* lineage GAVSTE02 in our dataset has a 1bp difference from the *Leucocytozoon* lineage GAVIM01 that was previously found in *Gavia immer*, a closely related diver species. The *Leucocytozoon* lineage GAVIM01 is transferred by a highly exclusive relationship between a black fly, *Simulium annulus*, and *Gavia immer* (Adler et al 2004, Weinandt et al. 2006, 2012). Hellgren et al (2008) suggested that the association between blackfly species and host species hinders transmission of parasites between different host-groups. If the novel *Leucocytozoon* lineage displays a similar exclusive relation to a specific vector and red-throated divers cannot be tested with our dataset.

Prevalence of haemosporidian parasites in red-throated divers

Prevalence of all sampled red-throated divers captured in the eastern German Bight (North Sea) during winter and spring and screened in this dataset (n=45) was estimated with 11%. The general low prevalence of seabirds documented in other studies (Quillfeldt et al. 2010, 2011, Khan et al. 2019) therefore also seems to apply for red-throated divers. Red-throated diver breed in arctic coastal inland habitats and outside of this season this species prefers marine offshore habitats. Consequently the transmission of haemosporidian parasites through vectors most likely occurs during summer month which presents the breeding period for

these birds. Even during the breeding season this species prefers coastal locations of nesting sites close to marine habitats (Reimchen and Douglas 1984, Eriksson et al. 1990, Rizzolo et al. 2015) where vector abundance is rather low compared to inland habitats (Mendes et al. 2005). Due to the preference for marine habitats, the transmission risk for blood parasites is restricted to the breeding season. Considering a breeding region specific prevalence and only individuals with a given breeding location we found for individuals breeding in northern Russia a prevalence of 8.3 % and for individuals breeding in Norway a comparatively high prevalence of 33 %. Studies for sea ducks, which show a similar annual routine as red-throated divers showed a similar low prevalence of e.g. adult spectacled eiders in Alaska with 6.5 % (Reed et al. 2018). However, it must also be considered that the prevalence in our study may be underestimated as we sampled red-throated divers during the non-breeding season in winter and spring. Haemosporidium parasites of the genera *Plasmodium*, *Haemoproteus*, and *Leucocytozoon* form persistent stages in their life cycle, which can remain latent in various host tissues and are thus largely protected from the host immune system (Valkiunas 2005). These stages are thought to occur primarily in winter months when vectors are generally unavailable and transmission cannot occur (Neto et al. 2020). Therefore the prevalence in the blood might have been below the detection threshold of our methodology. Breeding origins were not identified for all infected individuals, due to early mortality of some individuals after capture. If these early mortalities were linked to haemosporidian parasite infection and a possible cumulative effect of capture - tagging procedure can neither be ruled out nor proven as we had no chance to recover the dead bodies and to analyse blood smears or organs for an increased parasitaemia. These early mortalities occurred in infected and not infected individuals in similar proportions (infected 8.8%; not infected 11%).

Fitness consequences and importance for interaction with dynamics in wintering areas

Red-throated divers are confronted with increasing anthropogenic activity in their stationary non-breeding habitats that led to displacement effects. Habitat loss and displacement into less suitable foraging habitat can have multiple effects from no effect to an increased stress level, a poorer nutritional condition or intraspecific competition if considerably smaller amount of habitat is available. An increased stress level or poorer body condition might weaken the immune system making the host more vulnerable to an outbreak of the disease. An infection with parasites in general and haemosporidian parasites in particular could cause a downturn/deterioration of the physical condition of a host. Haemosporidian parasites have been shown to have long-term effects on reproductive success, condition and survival as they occur intracellular and affect host metabolism (Marzal et al. 2008). Here the information about a prevalence of haemosporidian parasites provides important information to fully evaluate potential pre-loads. Marzal et al (2008) showed that double infections have generally stronger impacts on life-history traits in birds than single infections. The red-throated diver is a migratory seabirds species. How a haemosporidian infection affects migratory performance is not yet fully understood. In general migratory performance is decreasing with increasing

infection intensity (Risely et al. 2018), though Hahn et al. (2018) showed no effect of a single haemosporidian infection on aerobic performance in relation to metabolic rates and migratory behaviour in either experimentally treated or free living migratory species. Furthermore, no effect on phenotypic changes associated with preparation for migration was observed and thus stayed unaffected by parasitaemia. Whereas Hegeman (2018) considered double infections and revealed a positive correlation between a prolonged stop-over duration and haemosporidian infection. Variation in baseline immune function and haemosporidian infection status was suggested to explain individual variation in stop-over behaviour, including migration speed, migratory success and possible carry-over effects on other annual cycles.

Since haemosporidian infection may have effects on reproductive success, condition, survival, host metabolism, or migratory behaviour, it may be considered a pre-load when evaluating the indirect and cumulative effects of anthropogenic or other stresses that result in an increased risk of suffering from elevated stress levels. Our study presents a first proof of the two haemosporidian parasite genera *Leucocytozoon* and *Plasmodium* in red-throated divers, with a first record of a distinct novel lineage in each of the two parasite genera. Red-throated divers are increasingly affected by impairments in their non-breeding areas and to which they are sensitive. However, in general, the low haemosporidian prevalence suggests a rather low importance of these parasites in this context. If only individuals from Norway are considered, however, the comparatively higher prevalence here indicates a higher sensitivity of individuals from this breeding region.

References

- Adler PH (2004) The Black Flies (Simuliidae) of North America. Cornell University Press in association with Royal Ontario Museum, Ithaca, N.Y.
- Allander K, Bennett GF (1994) Prevalence and intensity of haematozoan infection in a population of Great Tits *Parus major* from Gotland, Sweden. *Journal of Avian Biology* 25:69-74
- Atkinson CT, Greiner EC, Forrester DJ (1986) Pre-Erythrocytic Development and Associated Host Responses to *Haemoproteus meleagridis* (Haemosporina: Haemoproteidae) in Experimentally Infected Domestic Turkeys. *The Journal of protozoology* 33(3): 375-381
- Atkinson CT, Thomas NJ, Hunter DB (2009) Parasitic diseases of wild birds. John Wiley & Sons.
- Barbosa A, Palacios MJ (2011) Health of Antarctic birds: a review of their parasites, pathogens and diseases. *Polar Biology* 32:1095-1115
- Beadell JS, Gering E, Austin J, Dumbacher JP, Peirce MA, Pratt TK, Atkinson CT, Fleischer RC (2004) Prevalence and differential host-specificity of two avian blood parasite genera in the Australo-Papuan region. *Molecular Ecology* 13(12): 3829-3844
- Bennett GF, Montgomere R, Seutin G (1992) Scarcity of haematozoa in birds breeding on the Arctic tundra of North America. *Condor* 94:289-292
- Bensch S, Hellgren O, Pérez-Tris J (2009) MalAvi: a public database of malaria parasites and related haemosporidians in avian hosts based on mitochondrial cytochrome b lineages. *Molecular ecology resources* 9(5):1353-1358

- BirdLife International (2022) Species factsheet: *Gavia stellata*. BirdLife International: The IUCN Red List of Threatened Species 2019. URL: <http://www.birdlife.org>. Accessed 23 12 April June 2022
- Burger, C., Schubert, A., Heinänen, S., Dorsch, M., Kleinschmidt, B., Žydelis, R., Morkūnas, J., Quillfeldt P, Nehls, G. (2019). A novel approach for assessing effects of ship traffic on distributions and movements of seabirds. *Journal of environmental management*, 251, 109511
- Chagas CRF, Valkiunas G, Guimaraes LdO, Monteiro EF, Guida FJV, Simoes RF, Rodrigues PT, Luna EJdA, Kirchgatter K (2017) Diversity and distribution of avian malaria and related haemosporidian parasites in captive birds from a Brazilian megalopolis. *Malaria journal* 16(1):1-20
- Ciloglu A, Yildirim A, Duzlu O, Onder Z, Dogan Z, Inci, A (2016) Investigation of avian haemosporidian parasites from raptor birds in Turkey, with molecular characterisation and microscopic confirmation. *Folia parasitologica* 63:1-8
- Clark NJ, Clegg SM, Lima MR (2014) A review of global diversity in avian haemosporidians (*Plasmodium* and *Haemoproteus*: Haemosporida): new insights from molecular data. *International journal for parasitology* 44(5):329-338
- Dierschke V, Exo KM, Mendel B, Garthe S (2012) Gefährdung von Sterntaucher *Gavia stellata* und Prachtaucher *G. arctica* in Brut-, Zug- und Überwinterungsgebieten – eine Übersicht mit Schwerpunkt auf den deutschen Meeresgebieten. *Vogelwelt* 133:163-194.
- Dimitrov D, Zehindjiev P, Bensch S (2010) Genetic diversity of avian blood parasites in SE Europe: Cytochrome b lineages of the genera *Plasmodium* and *Haemoproteus* (Haemosporida) from Bulgaria. *Acta Parasitologica* 55(3):201-209
- Dunning JB Jr (2008) *Avian Body Masses*. 2 edition. Boca Raton: CRC Press
- Eriksson MOG, Blomqvist D, Hake M, Johansson OC (1990) Parental feeding in the Red-throated Diver *Gavia stellata*. *Ibis* 132:1–13.
- Fallis AM, Desser SS, Khan RA (1974) On species of *Leucocytozoon*. *Advances in Parasitology* 12:1-67
- Fliessbach KL, Borkenhagen K, Guse N, Markones N, Schwemmer P, Garthe S (2019) A ship traffic disturbance vulnerability index for Northwest European seabirds as a tool for marine spatial planning. *Frontiers in Marine Science*. <https://doi.org/10.3389/fmars.2019.00192>
- Garamszegi LZ 2011 Climate change increases the risk of malaria in birds. *Glob Change Biol* 17:1751–1759
- Garthe S, Schwemmer H, Markones N, Müller S, Schwemmer P (2015) Verbreitung, Jahresdynamik und Bestandsentwicklung der Seetaucher *Gavia spec.* in der Deutschen Bucht (Nordsee). *Vogelwarte*, 53:121-138
- Haas M, Lukáč M, Kisková J, Hrehová Z (2012) Occurrence of blood parasites and intensity of infection in *Prunella modularis* in the montane and subalpine zone in the Slovak Carpathians. *Acta Parasitologica* 57(3):221-227
- Hahn S, Bauer S, Dimitrov D, Emmenegger T, Ivanova K, Zehindjiev P, Buttemer WA (2018) Low intensity blood parasite infections do not reduce the aerobic performance of migratory birds. *Proceedings of the Royal Society B: Biological Sciences*. <http://dx.doi.org/10.1098/rspb.2017.2307>
- Hegemann A, Alcalde Abril P, Muheim R, Sjöberg S, Alerstam T, Nilsson JÅ, Hasselquist D (2018) Immune function and blood parasite infections impact stopover ecology in passerine birds. *Oecologia* 188(4):1011-1024
- Heinänen S, Žydelis R, Kleinschmidt B, Dorsch M, Burger C, Morkūnas J, Quillfeldt P, Nehls G (2020) Satellite telemetry and digital aerial surveys show strong displacement of red-throated divers (*Gavia stellata*) from offshore wind farms. *Marine environmental research*. <https://doi.org/10.1016/j.marenvres.2020.104989>

- Hellgren O, Waldenström J, Bensch S (2004) A new PCR assay for simultaneous studies of *Leucocytozoon*, *Plasmodium*, and *Haemoproteus* from avian blood. *Journal of Parasitology* 90(4):797-803
- Hellgren O (2005) The occurrence of haemosporidian parasites in the Fennoscandian bluethroat (*Luscinia svecica*) population. *Journal of Ornithology* 146(1):55-60
- Hellgren O, Bensch S, Malmqvist B (2008) Bird hosts, blood parasites and their vectors—associations uncovered by molecular analyses of blackfly blood meals. *Molecular Ecology* 17(6):1605-1613
- Herman CM, Barrow JH Jr, Tarshis IB (1975) Leucocytozoonosis in Canada Geese at the Seney National Wildlife Refuge. *J Wildl Dis* 11:404-411
- Herrera J, Nunn CL (2019) Behavioural ecology and infectious disease: implications for conservation of biodiversity. *Philosophical Transactions of the Royal Society B* 374. <https://doi.org/10.1098/rstb.2018.0054>
- Hill AG, Howe L, Gartrell BD, Alley MR (2010) Prevalence of *Leucocytozoon spp.* in the endangered yellow-eyed penguin *Megadyptes antipodes*. *Parasitology* 137:1477-1485
- Jenkins T, Owens IP (2011) Biogeography of avian blood parasites (*Leucocytozoon spp.*) in two resident hosts across Europe: phylogeographic structuring or the abundance-occupancy relationship?. *Molecular Ecology* 20(18):3910-3920.
- Khan JS, Provencher JF, Forbes MR, Mallory ML, Lebarbenchon C, McCoy KD (2019) Parasites of seabirds: A survey of effects and ecological implications. *Advances in Marine Biology* 82:1-50
- Kimura M, Darbro JM, Harrington LC (2010) Avian malaria parasites share congeneric mosquito vectors. *Journal of Parasitology* 96(1):144-151
- Kleinschmidt B, Burger C, Dorsch M, Nehls G, Heinänen S, Morkūnas J, Žydelis R, Moorhouse-Gann RJ, Hipperson H, Symondson WOC, Quillfeldt, P (2019) The diet of red-throated divers (*Gavia stellata*) overwintering in the German Bight (North Sea) analysed using molecular diagnostics. *Marine Biology* 166(6):1-18
- Knowles SC, Palinauskas V, Sheldon BC (2010) Chronic malaria infections increase family inequalities and reduce parental fitness: experimental evidence from a wild bird population. *Journal of Evolutionary Biology* 23:557-569
- LaPointe DA, Goff ML, Atkinson CT (2010) Thermal constraints to the sporogonic development and altitudinal distribution of avian malaria *Plasmodium relictum* in Hawai'i. *Journal of Parasitology* 96(2):318-324
- Loiseau C, Harrigan RJ, Cornel AJ, Guers SL, Dodge M, Marzec T, Carlson JS, Seppi B, Sehgal RNM (2012) First evidence and predictions of *Plasmodium* transmission in Alaskan bird populations. *PLoS One* 7. <https://doi.org/10.1371/journal.pone.0044729>
- Martínez -Abraín A, Esparza B, Oro D (2004) Lack of blood parasites in bird species: Does absence of blood parasite vectors explain it all? *Ardeola* 51:225-232
- Martínez J, Vasquez RA, Venegas C, Merino S (2015) Molecular characterisation of haemoparasites in forest birds from Robinson Crusoe Island: is the Austral Thrush a potential threat to endemic birds? *Bird Conservation International* 25:139-152
- Martínez J, Merino S, Badás EP, Almazán L, Moksnes A, Barbosa A (2018) Hemoparasites and immunological parameters in Snow Bunting (*Plectrophenax nivalis*) nestlings. *Polar Biology* 41(9):1855-1866
- Martinsen ES, Sidor IF, Flint S, Cooley J, Pokras MA (2017) Documentation of malaria parasite (*Plasmodium spp.*) infection and associated mortality in a common loon (*Gavia immer*). *Journal of wildlife diseases* 53(4):859-863

- Marzal A, de Lope F, Navarro C, Møller AP (2005) Malarial parasites decrease reproductive success: an experimental study in a passerine bird. *Oecologia* 142:541-545
- Marzal A, Bensch S, Reviriego M, Balbontin J, De Lope F (2008) Effects of malaria double infection in birds: one plus one is not two. *Journal of evolutionary biology* 21(4):979-987
- Mendel B, Schwemmer P, Peschko V, Müller S, Schwemmer H, Mercker M, Garthe S (2019) Operational offshore wind farms and associated ship traffic cause profound changes in distribution patterns of Loons (*Gavia spp.*). *Journal of environmental management* 231:429-438.
- Mendes L, Piersma T, Lecoq M, Spaans B, Ricklefs RE (2005) Disease-limited distributions? Contrasts in the prevalence of avian malaria in shorebird species using marine and freshwater habitats. *Oikos* 109(2):396-404.
- Møller AP, Nielsen JT (2007) Malaria and risk of predation: a comparative study of birds. *Ecology* 88(4):871-881
- Neto JM, Mellinger S, Halupka L, Marzal A, Zehtindjiev P, Westerdahl H (2020) Seasonal dynamics of haemosporidian (Apicomplexa, Haemosporida) parasites in house sparrows *Passer domesticus* at four European sites: comparison between lineages and the importance of screening methods. *International journal for parasitology* 50(6-7):523-532
- Oakgrove KS, Harrigan RJ, Loiseau C, Guers S, Seppi B, Sehgal RN (2014) Distribution, diversity and drivers of blood-borne parasite co-infections in Alaskan bird populations. *International journal for parasitology* 44(10):717-727
- Quillfeldt P, Martínez J, Hennicke J, Ludynia K, Gladbach A, Masello JF, Riou S, Merino S (2010). Hemosporidian blood parasites in seabirds—a comparative genetic study of species from Antarctic to tropical habitats. *Naturwissenschaften* 97(9):809-817
- Quillfeldt P, Arriero E, Martínez J, Masello JF, Merino S (2011) Prevalence of blood parasites in seabirds—a review. *Frontiers in Zoology* 8(1):1-11
- Quillfeldt P, Martinez J, Bugoni L, Mancini PL, Merino S (2014) Blood parasites in noddies and boobies from Brazilian offshore islands—differences between species and influence of nesting habitat. *Parasitology* 141(3):399-410
- Ramey AM, Schmutz JA, Reed JA, Fujita G, Scotton BD, Casler B, Fleskes JP, Konishi K, Uchida K, Yabsley MJ. 2015. Evidence for intercontinental parasite exchange through molecular detection and characterization of haematozoa in northern pintails (*Anas acuta*) sampled throughout the North Pacific Basin. *Int J Parasitol* 4:11–21
- Ramey AM, Buchheit RM, Uher-Koch BD, Reed JA, Pacheco MA, Escalante AA, Schmutz JA (2021) Negligible evidence for detrimental effects of Leucocytozoon infections among Emperor Geese (*Anser canagicus*) breeding on the Yukon-Kuskokwim Delta, Alaska. *International Journal for Parasitology: Parasites and Wildlife* 16:103-112
- Reed JA Sexson MG, Smith MM, Schmutz JA, Ramey AM (2018) Evidence for haemosporidian parasite infections in Spectacled Eiders (*Somateria fischeri*) sampled in Alaska, USA during the breeding season. *Journal of wildlife diseases* 54(4):877-880.
- Reeves AB, Smith MM, Meixell BW, Fleskes JP, Ramey AM. (2015) Genetic diversity and host specificity varies across three genera of blood parasites in ducks of the Pacific Americas Flyway. *PLoS One*. <https://doi.org/10.1371/journal.pone.0116661>
- Reimchen TE, Douglas S (1984) Feeding schedule and daily food consumption in Red-throated Loons (*Gavia stellata*) over the pre fledging period. *The Auk* 101(3):593-599.

- Risely A, Klaassen M, Hoyer BJ (2018) Migratory animals feel the cost of getting sick: a meta-analysis across species. *J Anim Ecol* 87:301–314
- Rizzolo DJ, Schmutz JA, Speakman JR (2015) Fast and efficient: Postnatal growth and energy expenditure in an Arctic-breeding waterbird, the Red-throated Loon (*Gavia stellata*). *The Auk: Ornithological Advances* 132(3):657–670
- Ronconi RA, Swaim ZT, Lane HA, Hunnewell RW, Westgate AJ, Koopman HN (2010) Modified hoop-net techniques for capturing birds at sea and comparison with other capture methods. *Marine Ornithology* 38(1):23–29
- Scheuerlein A, Ricklefs RE (2004) Prevalence of blood parasites in European passeriform birds. *Proceedings of the Royal Society of London Series B: Biological Sciences* 271(1546):1363–1370
- Smith MM, Van Hemert C, Merizon R (2016) Haemosporidian parasite infections in grouse and ptarmigan: Prevalence and genetic diversity of blood parasites in resident Alaskan birds. *Int J Parasitol* 5:229–239
- Sol D, Jovani R, Torres J (2003) Parasite mediated mortality and host immune response explain age-related differences in blood parasitism in birds. *Oecologia* 135(4):542–547
- Sorci G, Møller AP (1997) Comparative evidence for a positive correlation between haematozoan prevalence and mortality in waterfowl. *Journal of Evolutionary Biology* 10(5):731–741
- Taft SJ, Rosenfield RN, Seegar WS, Maechtle TL (1999) Paucity of hematozoa in Peregrine Falcons (*Falco peregrinus*) in West Greenland and coastal Texas. *Journal-Helminthological society Washington* 65:111–113
- Valkiūnas G (2005) *Avian Malaria Parasites and Other Haemosporidia* (1st ed.). CRC Press. <https://doi.org/10.1201/9780203643792>
- Van Rooyen J, Lalubin F, Glazot O, Christe P (2013) Altitudinal variation in haemosporidian parasite distribution in great tit populations. *Parasit & Vectors* 6(1):1–10
- Vanstreels RET, Kolesnikovas CK, Sandri S, Silveira P, Belo NO, Ferreira FC, Epiphanyo S, Steindel M, Braga EM, Catão-Dias JL (2014) Outbreak of avian malaria associated to multiple species of *Plasmodium* in magellanic penguins undergoing rehabilitation in southern Brazil. *Plos one*. <https://doi.org/10.1371/journal.pone.0094994>
- Vardeh S, Schumm YR, Kleinschmidt B, Engelhardt K, Pollet I, Loshchagina J, Glazov P, Kondratyev A, Sokolov A, Sokolov V, Bellebaum J, Kruckenberg H, Eichhorn G, Masello JF and Quillfeldt P (unpublished data) Genetic evidence for blood and gastrointestinal parasites of sea ducks breeding in the Russian Arctic
- Vilela R, Burger C, Diederichs A, Bachl FE, Szostek L, Freund A, Braasch A, Bellebaum J, Beckers B, Piper W, Nehls G (2021) Use of an INLA Latent Gaussian Modeling Approach to Assess Bird Population Changes Due to the Development of Offshore Wind Farms. *Frontiers in Marine Science*. <https://doi.org/10.3389/fmars.2021.701332>
- Waldenström J, Bensch S, Kiboi S, Hasselquist D, Ottosson U (2002) Cross-species infection of blood parasites between resident and migratory songbirds in Africa. *Molecular ecology* 11(8):1545–1554
- Warner RE (1968) The role of introduced diseases in the extinction of the endemic Hawaiian avifauna. *The Condor*, 70(2):101–120
- Weinandt ML (2006) Conservation implications of common loon (*Gavia immer*) parasites: Black flies, haematozoans, and the role of mercury. Master Thesis, Northern Michigan University.
- Weinandt ML, Meyer M, Strand M, Lindsay AR (2012) Cues used by the black fly, *Simulium annulus*, for attraction to the common loon (*Gavia immer*). *Journal of Vector Ecology*, 37(2):359–364

- Wetlands International (2019) Waterbird Population Estimates. [http:// wpe.wetlands.org](http://wpe.wetlands.org). Accessed 23 April 2019
- Whitworth DL, Takekawa JY, Carter HR, Mciver WR (1997) A night-lighting technique for at-sea capture of Xantus' Murrelets. *Colonial Waterbirds* 20:525-531
- Wilson MD, Cheke RA, Fiasse SPJ, Grist S, Osei-Ateweneboana MY, Tetteh-Kumah A, Fiasorgbor GK, Jolliffe FR, Boakye DA, Hougard JM, Yaméogo L, Post RJ (2002) Deforestation and the spatio-temporal distribution of savannah and forest members of the *Simulium damnosum* complex in southern Ghana and south-western Togo. *Transactions of the Royal society of Tropical Medicine and Hygiene* 96(6):632-639
- Wojczulanis-Jakubas K, Svoboda A, Kruszcwicz A, Johnsen A (2010) No evidence of blood parasites in little auks (*Alle alle*) breeding on Svalbard. *Journal of Wildlife Diseases* 46(2):574-578
- Wood MJ, Cosgrove CL, Wilkin TA, Knowles SC, Day KP, Sheldon BC (2007) Within-population variation in prevalence and lineage distribution of avian malaria in blue tits, *Cyanistes caeruleus*. *Molecular Ecology* 16(15):3263-3273
- Yasuoka J, Levins R (2007) Impact of deforestation and agricultural development on anopheline ecology and malaria epidemiology. *The American journal of tropical medicine and hygiene*, 76(3):450-460
- Yusupova D (2022) Avian Haemosporidae parasites in the Russian Arctic. Master Thesis, Ludwig Maximilian University of Munich
- Zagalska-Neubauer M, Bensch S (2016) High prevalence of Leucocytozoon parasites in fresh water breeding gulls. *Journal of Ornithology* 157(2):525-532.
- Zamora-Vilchis I, Williams SE, Johnson CN (2012) Environmental temperature affects prevalence of blood parasites of birds on an elevation gradient: Implications for disease in a warming climate. *PLoS One*. <https://doi.org/10.1371/journal.pone.0039208>
- www.divertracking.com

Part 3

APPENDIX

SUPPLEMENTARY MATERIAL

The following supplementary material is saved on a CD (attached to this thesis). The files are stored in folders sorted and named according to the chapters:

General supplementary information about bird captures

- Table 1: Information on birds, capture date and transmitter performance for all red-throated divers tagged within the DIVER project between 2015 and 2017
- Table 2: Information on data analysis of single birds and the conducted analyses. Grey cells indicate birds for which data were not used for the respective analyses.
- Fig. 1: Capture positions of birds tagged in 2015, 2016 and 2017 and offshore wind farms approved, under construction or in operation at that time.
- Table 3: Transmitter characteristics and duty cycles used in the different deployment years of the project

Chapter 1: Electronic supplementary material

- Specific information: Primer design
- PCR amplification of fish and cephalopod prey DNA from faeces
- PCR amplification of crustacean prey DNA from faeces
- Specific information on bioinformatic analyses
- Table 1 Working steps, commands and References performed during bioinformatics analysis
- Table 2 Output of Illumina MiSeq sequencing and bioinformatic analysis.
- Table 3 Quality criteria of MOTUs that were used for taxonomic assignment

Chapter 2: Electronic supplementary material

- Supplementary information A1: Timing of migration and geographic relations
 - Supplementary Figure A1: Breeding location (longitude/latitude) and temporal patterns of NW European red-throated divers. Figure axes have been transposed for a better visualisation of geographical relations (a) Duration spring staging in relation to breeding longitude ($r_s = 0.0974$ $n = 27$, $p = 0.629$) and latitude ($r = -0.158$ $n = 27$, $p = 0.431$), (b) Departure date from breeding grounds (day 1 = 1 January) (in north-eastern direction from capture site) in relation to breeding longitude ($r_s = -0.169$ $n = 20$, $P = 0.476$) and latitude ($r = 0.251$, $p = 0.285$), (c) Distance spring migration in relation to duration spring staging (r

-
- = 0.297, $n = 31$, $p = 0.111$) and duration spring 10 migration ($r = 0.288$, $n = 31$, $p = 0.117$).
- Supplementary Figure A2: Breeding location (longitude/latitude) and temporal patterns of NW European red-throated divers. Figure axes have been transposed for a better visualisation of geographical relations. (a) Traveling time in spring after capture (duration of spring migration) in relation to breeding longitude (longitude: $r = 0.407$, $n = 29$, $p = 0.027$; 8) and latitude (latitude: $r = 0.384$, $n = 29$, $p = 0.039$), (b) Date of arrival (day 1 = 1 January) on breeding grounds (in north-eastern direction from capture site) in relation to breeding longitude ($r_s = 0.695$, $n = 29$, $p < 0.001$) and latitude ($r = 0.373$, $n = 29$, $p = 0.078$), (c) Date of departure (day 1 = 1 January) from the wintering grounds in relation to breeding longitude ($r = 0.335$, $n = 9$, $p = 0.344$) and latitude ($r = 0.714$, $n = 9$, $p = 0.020$).
 - Supplementary Figure A3: Duration of spring staging of NW European red-throated divers migrating north-west. Above: Duration of spring staging in relation to traveling time in spring after capture (duration of spring migration) ($r = 0.643$, $n = 27$, $p < 0.001$); below: Duration of spring staging in relation to number of utilised fuelling sites (staging sites) ($\rho = 0.468$, $n = 27$, $p = 0.014$).
 - Supplementary information A2: Spring staging stops
 - Supplementary Figure A4: Proportions of 41 spring staging stops (> 5 days) of red-throated divers migrating along the eastern route ($n = 23$) in per cent. Place names are indicated in a central map and labelled by numbers.
 - Supplementary Information A3: Comparative methodological approach to calculate Repeatability
 - Supplementary Figure A5: Estimates (\pm SE) of repeatability (R) of phenology and locations utilised by red-throated divers in successive years. Asteriks indicate significant R values.
 - Supplementary Table A1: Individual repeatability (R, R_A) of red-throated diver phenology and visited locations between two successive years, obtained by two comparative methodical approaches. On the left repeatability results (R) obtained by the LMM based approach and on the right repeatability results (R_A) obtained by the Anova based approach.
 - Supplementary information A4: Data output after applying filter algorithms
 - Supplementary Table A2: : Defined ARGOS position quality classes (CLS 2013) and distribution of received data to the different classes before and after data filtering.
 - Supplementary information A5: Kernel density estimates
-

-
- Supplementary Table A3: List of bird ID (Argos ID), home range size, calculation characteristics, region and time spent when and where home ranges were calculated. Home ranges were calculated using utilisation density estimation (grid=100 cells) and seasons in this analysis includes the full period a bird stayed in the area.
 - Supplementary information A6: Specific Information about treatment of stable isotope samples and running the analysis
 - Supplementary information A7: Genetical sexing of captured birds
 - Supplementary information A8: Additional information Data analysis and Statistics

Supplementary information A8: Additional information Data analysis and Statistics

Chapter 3: Electronic supplementary material

New lineages identified in the 3rd chapter and corresponding requested GenBank records are withheld and will be added officially after the publication is accepted.

- Table 1: Specific information of new lineages submitted to MalAvi
- Table 2: Specific information of sample locations of birds infected with new lineages
- Table 3 List of positively tested red-throated divers for blood parasite infections, with ID (ARGOS-ID), parasite genera and identity match

CURRICULUM VITAE

Der Lebenslauf wurde aus der elektronischen Version der Arbeit entfernt.
The curriculum vitae was removed from the electronic version of the paper.

Der Lebenslauf wurde aus der elektronischen Version der Arbeit entfernt.
The curriculum vitae was removed from the electronic version of the paper.

LIST OF PUBLICATIONS

Kleinschmidt B, Burger C, Bustamante P, Dorsch M, Heinänen S, Morkūnas J, Žydelis R, Nehls G, Quillfeldt P (Accepted July 2022) Annual movements of a migratory seabird - the NW European red-throated diver (*Gavia stellata*) – reveals high individual repeatability but low migratory connectivity. Marine Biology accepted July 2022

Heinänen S, Žydelis R, **Kleinschmidt B**, Dorsch M, Burger C, Morkūnas J, Quillfeldt P, Nehls G. (2020) Satellite telemetry and digital aerial surveys show strong displacement of red-throated divers (*Gavia stellata*) from offshore wind farms. Marine Environmental Research, 104989

Kleinschmidt B, Burger C, Dorsch M, Nehls G, Heinänen S, Morkūnas J, Žydelis R, Moorhouse-Gann RJ, Hipperson H, Symondson WOC, Quillfeldt P (2019) The diet of red-throated divers (*Gavia stellata*) overwintering in the German Bight (North Sea) analysed using molecular diagnostics. Marine Biology 2019 166:77

Burger C, Schubert A, Heinänen S, Dorsch M, **Kleinschmidt B**, Žydelis R, Nehls G (2019) A novel approach for assessing effects of ship traffic on distributions and movements of seabirds. Journal of Environmental Management, 251, 109511.

Kleinschmidt B, Kölsch G (2011) Adopting bacteria in order to adapt to water—how reed beetles colonized the wetlands (Coleoptera, Chrysomelidae, Donaciinae). Insects 2: 540–554.

CONFERENCE CONTRIBUTIONS

Kleinschmidt B, Dorsch M, Žydelis R, Heinänen S, Morkūnas J, Burger, C, Nehls G, Moorhouse-Gann R, Symondsen WOC, Quillfeldt P (2017) Untersuchung des Beutespektrum von auf der Nordsee überwinternden Sterntauchern (*Gavia stellata*) mit Hilfe molekularbiologischer Methoden. Deutsche Ornithologischen Gesellschaft – 150. Jahresversammlung Halle (Saale) (Talk)

Kleinschmidt B, Dorsch M, Žydelis R, Heinänen S, Morkūnas J, Burger C, Nehls G, Quillfeldt P (2017) From effects to impacts: Analysing displacement of red-throated divers in relation to their wintering home ranges. LPO Actes du Seminaire Eolien et Biodiversite – Artigues – pres – Bordeaux (Talk)

Kleinschmidt B, Burger C, Dorsch M, Žydelis R, Heinänen S, Morkūnas J, Nehls G, Moorhouse-Gann R, Symondsen WOC, Quillfeldt P (2017) Diet of red-throated divers at an important wintering site, the German Bight analysed with molecular tools. Youmares 8, Kiel (Talk)

Kleinschmidt B, Dorsch M, Žydelis R, Heinänen S, Morkūnas J, Burger C, Nehls G, Quillfeldt P (2017) Site fidelity and temporal consistency of red-throated divers (*Gavia stellata*) during migration, moult & wintering. British Ornithologists' Union, Annual Conference, University of Warwick, UK (Poster)

Kleinschmidt B, Dorsch M, Žydelis R, Heinänen S, Morkūnas J, Burger C, Nehls G, Quillfeldt P (2015) Red-throated Diver (*Gavia stellata*) habitat use & mobility patterns – revealed by satellite tracking. World Seabird Konferenz, Cape Town, South Africa (Poster)

Kleinschmidt B, Kölsch G (2011) Bacterial endosymbionts of the reed beetles (Coleoptera, Chrysomelidae, Donaciinae) – Molecular proof of the transmission pathway & experiments on the benefit from the symbionts. Deutsche Zoologische Gesellschaft 104. Jahresversammlung, Saarbrücken (Poster)

AKNOWLEDGEMENTS

First of all, I would like to sincerely thank Petra for supervising the work and for warmly welcoming me into the working group. I am grateful to you for many things, of course for the scientific advice, the critical discussions and at the same time to give me the space to develop and implement my own thoughts, but also for the great opportunity to go abroad and expand my experience.

I deeply thank Georg Nehls and Monika Dorsch for bringing me into the project and making this work possible in the first place. Furthermore I want to thank you and Thomas Grünkorn, Claudia Burger, Stefan Heinänen, Julius Morkūnas, Jorg Welcker and Ramūnas Žydelis for your tireless efforts during the field work and to be spontaneously ready whenever the moon or the weather was good for captures.

During my time of stay in Cardiff I met wonderful people which I will always remember fondly. A big thanks goes to William (Bill) Symondson for taking over the supervision during this time and for the fact that I could always discuss questions with him. Rose I thank you for always supporting me patiently with any kind of lab or organisational stuff. I will never forget the coffee 2 am when we pooled our samples or private stuff like hiking or stand up paddling. Many thanks to Helen Hipperson for introducing me into the bioinformatics and for the great advice during the analysis. Mafalda Costa, Isa-Rita Russo and Lollita Amman deserve a lot of thanks for support, discussions or after work pub culture. Rhian and Dave Stanton gave me a home during my time of stay and warmly welcomed me, thank you so much.

Big thanks goes to the behavioural ecology and ecophysiology working group and the people I was happy to meet during the past years. I am so grateful in several ways. Especially I like to thank Daniel, Carina, Justine, Lucie, Melli, Nadja, Sandra, Yvonne and Wiebke for support, discussions and feedback, morning coffees, delicious cakes or simply hugs if needed. This is so much appreciated and not taken for granted. I like to thank Anne for inviting me to the Faroes and patiently searching successfully breeding red-throated divers. I will remember this adventure always fondly. Ingrid, this wonderful Christmas present guided me through the whole publication process. Special thanks to Carina, Melli, Nadja, and Yvonne for offering me “a roof over my head” - back at the times when I was travelling back and forth - you always made me feel like coming home.

Last but not least, I would like to thank my family and the people who have been part of my life since school, regardless of my studies and doctoral thesis, thank you for just being with me during all this time.