Copyright ©

Christian-Albrechts-Universität zu Kiel

Alfred Wegener Institute, Helmholtz Centre for Polar and Marine Research

- Wadden Sea Station Sylt, Germany -

Influence of top predators on the Wadden Sea food web

By **Camille DE LA VEGA**

Doctoral thesis in Marine Ecology

Supervised by Dr. Harald ASMUS

Alfred Wegener Institute, Helmholtz Centre for Polar and Marine Research

– Wadden Sea Station, Sylt, Germany –

Influence of top predators on the Wadden food web

By **Camille DE LA VEGA**

Doctoral thesis in Marine Ecology

Deposited at the Faculty of Mathematics and Natural Sciences
of the Christian-Albrecht University, Kiel

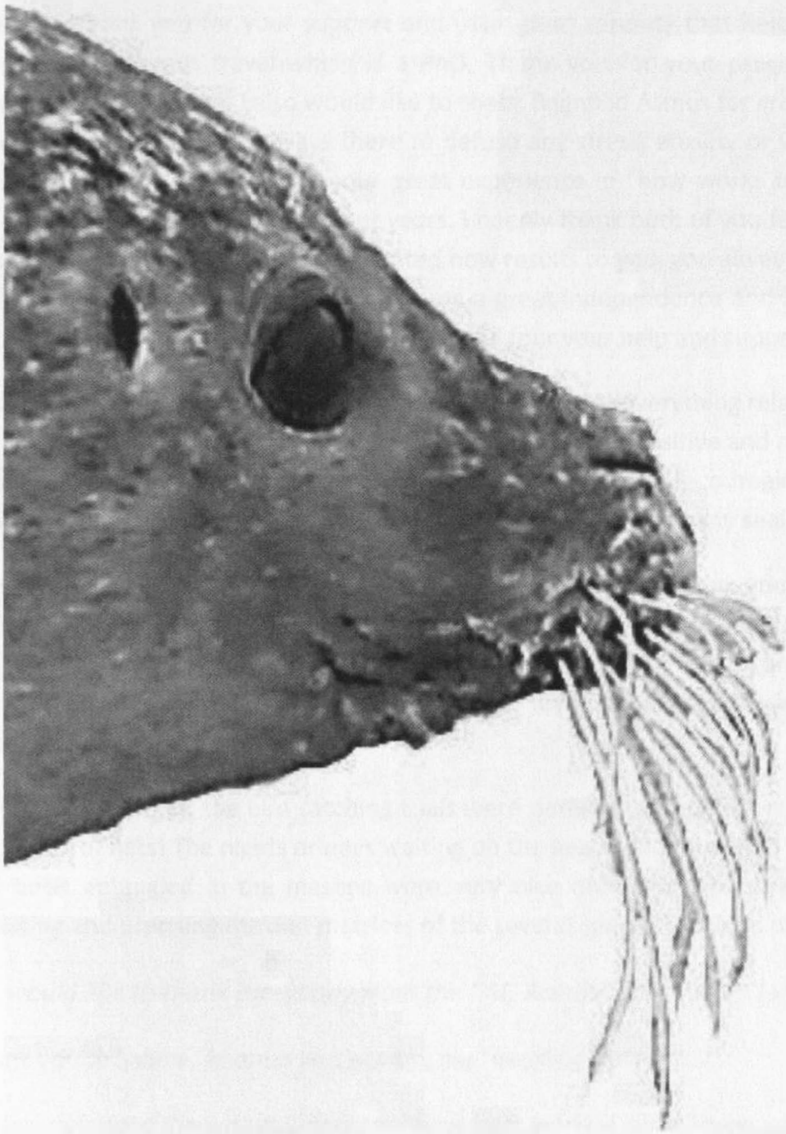
Supervised by

Dr. Harald ASMUS	Main supervisor
Dr. Ragnhild ASMUS	PhD Committee
Dr. Benoit LEBRETON	PhD Committee
Prof. Dr. Ursula SIEBERT	PhD Committee
Dr. Philipp SCHWEMMER	PhD Committee

Defended on 01. 09. **2016**

In front of a jury composed of

Prof. Mojib LATIF	Head of the jury
Dr. Harald ASMUS	Reviewer
Prof. Stefanie ISMAR	Reviewer
Prof. Dan BAIRD	Examiner
Dr. Florence CAURANT	Examiner



Acknowledgements

First of all, I would like to thank the members of my jury for evaluating my work. Thank you to Harald Asmus and Stefanie Ismar for reviewing my thesis. Thank you to Mojib Latif for accepting to be the president of my jury. Thank you, Dan Baird, for coming from so far to attend my defense as examiner and for your precious advice on the model construction and the redaction of the article. Thank you Florence Caurant for accepting to be my second examiner and for the nice exchanges we had in La Rochelle at the first stage of my PhD.

I thank the Alfred Wegener Institute, Helmholtz Centre for Polar and Marine Research for permitting me to realize this work and to the University of Kiel for hosting my PhD.

I warmly thank Harald Asmus, my main supervisor, for giving me the chance to work these four last years at the AWI. Thank you for your support and your great serenity that helped me so much to progress on this long and treacherous travel which is a PhD. Thank you for your precious advice, your comments always positive and supportive. I also would like to thank Ragnhild Asmus for creating such a nice atmosphere in the working group. You were always there to defuse any stress, anxiety or worries I could have during my PhD. Your “feet on the ground” and your great experience in “how works the world of science” made me feel secured and comforted all these four years. I deeply thank both of you for your trust in me. You were always positive and enthusiastic when I presented new results to you, you always supported new ideas and potential collaborations I wanted to try. You gave me a great independence and let me take initiatives while backing me up for every “risks” I took. I´m really grateful four your help and support during these four years.

Ursula Siebert, you were my rock in Büsum helping me for everything related to the marine mammal’s part of my thesis. I warmly thank you for your sphinxian presence, positive and reassuring. Our discussions in Büsum about my plans and perspectives were always productive and encouraging. Thank you also for giving me the chance to participate to one “seals catching session” and assist many seal necropsies in Büsum.

I could never have made all of this without Benoit Lebreton. Thank you for teaching me the stable isotope technics on Sylt (i.e. “boulettes”) and fatty acid methods in La Rochelle where you welcomed me for two months. You were behind me during the entire process, from the sampling to the interpretation of the pile of data, graphs and questions I had at one point on my plate. Our discussions about science were very fruitful and constructive.

Philipp Schwemmer, the bird catching trials were not very successful on Sylt, but I now know how to handle two kinds of nets! The nights or days waiting on the beach with the “bird team” to walk to the net and collect the birds entangled in the meshes were very nice moments. Thank you also for your precious help on updating and precising the diet matrices of the several species included in the food web model.

– I would like to thank everybody from the “AG Asmus” with whom I worked during my PhD –

Thank you to Sabine, Andreas and Martin, my “duckling siblings”…

Sabine, you were the best collaborator I have ever worked with! Thank you for your involvement in my model construction and the many discussions we had when we were lost in the pile of data and assumptions we had to make to construct the network. I really like our little side project that ended so quickly in a beautiful paper! I never thought I will write one day in a scientific publication "we grinded the entire birds with a kitchen cutter"! Thanks also for your involvement in all the events we organized at the institute, like the PhD days and the Christmas concert. We always ended up together folding programs together!

Andreas, thank you for coming with me to collect the eider duck shits, it was great to have help to drive the little AluFun. And you almost all the time found the "shit field" before me! It was also really fun to play piano/drum with you in the library! Next time, we will go to "Røme" with you for a week end and visit with a little Vespa…

Martin, thank you soooo much for involving me in your work during this awesome month on Svalbard. You will stay forever my arctic explorer!

Thank you to the fish monitoring team. Petra, you probably know that, but each time you were gone in holidays, the working group collapsed! Everybody was lost in the lab without you. Thanks for being so helpful and always in a good mood! Birgit, I enjoy very much your stories about the Frisian culture that made me get more in touch with the place where I lived for four years. I thank you both of you for the nice time we had on board during the fish monitoring. These two days per months were very nice moments, when I could be on the sea in every kind of weather to clear my mind! I also thank for that Alfred, the captain of the Mya and Kai; you are the perfect team mixing fishery and science!

– Special thanks to the several colleagues, helpers and advisors from outside the Island –

I warmly thank the dissection team from the Institute for Terrestrial and Aquatic Wildlife Research, Büsum. Special thanks to Connie, Miriam, Eva, Arlena, Abbo. You always welcomed me in Büsum. Thank you for your precious help in collecting the seal´s samples. Thanks also to the team of seal hunters collecting the carcasses on the beach.

Many thanks to the "bird team" from the Research and Technology Centre West Coast, Büsum. Philipp, Dagmar, Leonie and Stephan, thanks for your help on Sylt and your advice on the bird´s diet.

A special thanks to Björn Sörens, the captain of the Theodor Storm and Jochen for providing me the fish species from the North Sea. Thank you for your great collaboration and your enthusiasm in helping me!

I thank all the members of the INFOWEB project for the several productive project meetings. The efficient discussion we had led to constructive decisions about "how to go on". Diana and Victor, our discussion about the link between science, policy and management were very interesting and stimulating for me, and I´m very admiring the work you do. Diana, our participation at the Wadden Sea day 2015, the breakfast with David Thompson and the following road trip to the Netherlands were memorable! Thank you very much for your help and the exchanges we had for the "management related part" in my general discussion. Victor, thank you for welcoming us, the "Sylt team", at the ECSA symposium in London, and for the instructive discussions we had together. Thanks to Sarina, my PhD fellow from the INFOWEB. Your visit on Sylt and the several conferences we attended together were very good moments.

Thanks to the team of the LIENS in the University of La Rochelle that welcomed me for two months in the lab. Hervé, thanks for your support and all the little conversation during the coffee breaks! Our weekly pub quizzes were efficient cheer leaders! Thanks to Gael, the master of the mass spectrometer who run all my samples.

Thanks to Lasse for giving me the seal abundance data from the Sylt-Rømø Bight. We didn´t meet so often, but it was a great feeling to exchange with you by mail knowing that you are on the other side of my studied area, that I enjoyed the view from my office.

A special thanks to Stuart Borrett and David Hines who gave me the R code of the uncertainty function and help to get it work on my big data set. Thank for your availability and your involvement in my work! Thank you for all the mails exchanged and the skype meetings we had to discuss the method and interpret my results. You helped me a lot at the end of my PhD!

Many thanks to TomTom, Valoch, Lolo, Denise and John for correcting the English and proof-reading the chapters of my thesis and the articles I wrote during my PhD.

– Many thanks to the fellows from Sylt, who made the Island a nice place to live –

Dominik, thank you for all the concerts we made together. That was unexpected and so great to meet you and your cello at the institute, my electro-swing brother! Schwör Diga, the disco boy concert in Kiel was memorable! A special thanks to Ondrej, the master of the fire evenings in the dump! Da la bi shi pifko? You were there for all the crazy moments, the “dog un-digging”, the acrobatic rock and roll, the Viking play in Ribbe and so many more. You were one the main pillar of my life on Sylt. Thank you Marieke for all the nice moments, the green tea in the garden, the slack line at lunch break, the sunny afternoon on the beach and the long walks in the dunes to let steam off. Thank you Rene and Anne for closing the Listro for the last time with me and Martin! That was a highlight and a monumental moment of the Syltian life… Thanks for the nice evenings on Sylt and weekends in Husum that make me feel that I start to belong here! Thank you Jonas for being so motivated to play a “trio” with only one clarinet and one piano! I really enjoy our rehearsal sessions. Thanks also for your moral support during the last month of my PhD, and particularly, for considering that esthetic is important! The “little” beers at the harbor were also very useful! Many thanks to Elisabeth for her kindness and our piano/singing sessions; thanks to you I now know that the Ave Maria from Caccini is not from Mozart… Thanks to Julia for her natural joy of life and swimming with me in the noctilucas! Thanks to Nina, the best Disco Fox dancer. Thanks to you, Andreas and Fifi for the nice week end in Neuglobsow. I was very happy to see traces of beavers! Thanks to Ryan, for the Gangnam style performance on the Mya and the super tasty dumplings. Thanks to Kevin my oldest fellow from Sylt. I´m grateful to Karsten for teaching me the bad words in German – my favorite : “Du siest aus wie Wolle Petri!”. Thank you Céline, your little seed of craziness makes you irreplaceable. Thanks to Victor who was there for the last Bambus Claus party. Thank you Essowe for sharing my taste for zouk music! Thanks to Lobsan for his enthusiasm.

Thanks to all of you for the many BBQ evenings in the garden, all the great parties, the afternoons on the beach, the long winter afternoon playing cards, and everything that makes the life easy after work!

Jonas, Sabine, Andreas, Marieke, I can only say: no group has ever been better than the crustacean band…

I also would like to particularly thank Eva Kämke. Thanks for keeping me active in the classical music world! Our piano lessons in Westerland were very important for me. It was great to have the opportunity to play with other students from the music school and I specially thank Lea for being my four hands partner for several concerts. We even were once in the Sylt newspaper! Thanks also to David and Christa Kaplan, as well as Pablo for the great music evening in the Alte Gasthof. Particularly thank you David for playing *Take 5* with me!

– Thanks to my friends –

Esther, my best friend, thank you for being with me at every special and less special moments of my life. Thank for your support during all these years. All the weekends and moments passed together on Sylt, Hamburg, Paris (our general quarter!), La Rochelle, Andernos, Geneva, Budapest are very important to me. And even when it was not possible to be at the same place because of the distance, our "skype aperos" and all the supportive messages of all kinds were there to keep the contact and this was heartening! But who would be surprised… stars have no borders!

JayC, you are my "OEM bro" for so many years and your comments on my work during the few skype we did counted a lot to me! Thanks for your welcome in Roscoff, your spontaneous visit in Andernos, the several weekends spent in Paris and the beautiful sand-castle in Helgoland where we finally had our first conference together! Raphael, thanks for the nice moments in the big city that helped me to "deconnect" from Sylt! And thanks for the cool (every meanings of the word) weekend on Sylt: you almost froze on your bike but you were anyway ready to party in the evening, in music with your trumpet like usual! Thank you Aurélie! You were part of my settlement phase on this new Island! You and Roman, arriving in bath costume at the institute during a course because I had locked you out of the flat by mistake was the best way to introduce me to my boss! :-) Thanks for your friendship which is always as strong as the first year even if we meet only once in a while.

– Thanks to my family –

Thank you p´pa and m´man for your encouragements in everything I do and ideas I have, that make me always go further than I ever imagined. Having your backup permit me to go abroad, fly by my own and discover the world without any worries because I know you´re with me! You gave me the self-confidence and the assurance I have. Your advice is very precious to me. You were always interested to listen and discuss about all the details I told you about my PhD results, colleagues, issues and funny stories from Sylt. You came to visit me up to the North and wanted to meet and see all the people and places I told you about. Our long week end in Berlin before my summer school was super cool! Thanks for the two extraordinary Christmas holidays we passed in Namibia and Angola that cut my long Syltian winters and make me save some sun, heat and colorful memories to stay motivated! Going back to Paris and spending time in Andernos brought me the ease and the calm I needed to go through these four years of PhD.

Thanks to my two sisters for visiting me on Sylt so often! In addition of the times in Andernos, Paris, Edimburg, Riga that would not have been the same without you! Chloée, you´re always on my side, you were all the time there when I called you to complain about my work or to celebrate with me when I had good progress. When you were on Sylt, you were always motivated to go to collect fake birds on the beach or wake up at 7 in the morning to go fishing! Thank you for your involvement in my life and your unconditional support. Our two hiking trip in Cornwall and Scotland were awesome! It´s impossible to tell you all the reasons why I rely on you so much, so thanks for everything my Chlochloni! Vivi, thank you for coming back so often on Sylt, even after the bike tour from Westerland against the wind! ☺ Thanks for the nice New

Year´s Eve in New York! Your good mood and your tranquility, characteristic of a real captain, is the perfect combination for nice boat tour on the basin! You´re my favorite dancing partner because you love dancing on Cabritinha and Zangalewa. I keep a super good memory from our week in Berlin with Valoch. And it was great to exchange our moral support during the last month: you finishing your movie and me finishing my thesis!

I thank all my cousins, uncles and aunts, from Montpellier, Marseille, Hossegor, New Caledonia, Picardie, and Bordeaux for the joyful mess that characterize our meetings and which is my roots! Even if I don´t see you so often, it´s always like "we sow each other yesterday". OuinOuin, thank you for your motivation, enthusiasm and curiosity! You were always interested when I told you about my work and it was great that you came to my presentation at the ECSA symposium in London! You always join me for a visit anywhere I am and the weekends and holidays with you are amazing! Your Elvis performance at Gosch on Sylt was memorable. Thanks for all the nice moments in Southampton, Cornwall, Scotland, Paris, La Rochelle, Andernos and Hossegor, that helped me to relax and stay motivated! Valoch, thank you for your support and your interest in "what I do"! It was very comforting me to call you and skype with you regularly! Thank you mami for the many postcards from Portugal, always so cheering up and encouraging. Thank you Danielle for believing in me for so many years. I kept your four Leaf Clover (= trèfle à quatre feuilles) in my office all these years to give me luck!

– Last but not Least –

Thanks you Martin for supporting me, listening to me and cheering me up any time I felt bored on Sylt! Thank you for your patience and your moral support! You are thoughtful and caring, and sharing the flat with you was the best thing that could happened to me! You made this place homey, and I was happy to come back after work, hearing you practicing your guitar and smelling your good "cuisine"! You almost convinced me that Mac is better than Windows: hopefully I could use your computer for running my HUGE model R function! Having you by my side helped me to get through the long and lonely winter on Sylt. You made me discover the big North – Svalbard, Tromsø, Oslo – and Germany – Berlin, Kiel, Lübeck, Köln, Krefeld, Düsseldorf, the Rhein Valley, the Eifel, Münschen, the Zug Spitze –, and we went together to Rome, Copenhagen, Edinburg and Riga. All these weekends and holidays meant so much to me and were the perfect solution to stay motivated and dynamic when back on Sylt! I´m sure there will be many more. Thanks you for being you.

Table of contents

Part II – Food Web Model 125

Figures

General Introduction

PART I

Chapter 1

Chapter 2

Chapter 3

PART II

Chapter 4

Chapter 5

General Discussion

Tables

PART I

Chapter 1

Chapter 2

Chapter 3

PART II

Chapter 4

Chapter 5

Appendices

PART I

Chapter 2

Chapter 3

PART II

Chapter 5

Summary

The harbor seal (*Phoca vitulina*) is the most abundant marine mammal species in the Wadden Sea which plays an important role for marine mammals in terms of resting, nursing and foraging. Due to their large body size and their high abundance in the Wadden Sea, seals exert a strong pressure of predation on their environment. There are needs to improve the understanding of the trophic behavior of seals in the North Sea and in the Wadden Sea, in order to determine spatio-temporal variations of their foraging activities and to implement better estimations of their diets into food web models.

Trophic markers such as stable isotope and fatty acids have been proven to be a reliable method for the determination of food resources used by marine mammals, and were used in this study to determine the seasonal variation of the diet of the harbor seal from the Sylt-Rømø Bight (Part I). The stable isotopes of carbon give indication about the origin of the food resources and the stable isotopes of nitrogen allow determining the trophic level of the consumers, due to a relatively high step-wise enrichment along the food chain. In the Wadden Sea, harbor seal is a protected species and seal hunting was banned in 1976. Invasive sampling of seals is therefore highly regulated. Thus sampling mostly relies on stranded dead animals, encompassing a high proportion of young-of-the-year due to the high mortality rate during their first year of life. Because the stable isotope composition of young-of-the-year might reflect the lactation and post weaning fast periods, those individuals must be removed from the community diet study. In the Chapter 1, the monthly evolution of the $\delta^{15}N$ and $\delta^{13}C$ values in tissues of young-of-the-years, collected on the coast of the Sylt Island, showed that vibrissae and muscle of individuals older than three to four months and five to six months respectively reflect a prey-based diet. Those individuals, in addition to adult animals collected on the same coast, were therefore used in Chapter 2, to study the seasonal variation of the harbor seal´s diet, in term of foraging location (Wadden Sea *vs.* North Sea) and prey items. The results of Chapter 2, revealed two main seasonal trends in the diet of harbor seals from the Wadden Sea/North Sea. Indeed, harbor seals change seasonally their main feeding location, relying more on coastal (i.e. Sylt-Rømø Bight) food resources in the warm seasons than in the cold seasons, when they migrate to the open North Sea to forage. Furthermore, a shift from a diet more strongly influenced by pelagic prey items in spring to a diet of more influenced by benthic prey items in summer was observed in both locations (Sylt-Rømø Bight and North Sea). This change in the prey items between spring and summer was confirmed by fatty acid analyses presented in Chapter 3. These seasonal variations in both foraging location and prey items are in accordance with the seasonal variation of the prey species biomass and abundance, as shown in detail in Chapter 2.

Abundance and distribution of harbor seals can have a large effect on the structure and the functioning of coastal food webs, and assessing their role in the functioning of ecosystems is a centra issue in ecology and management. Ecosystem-based management has been proclaimed as the solution needed to improve the efficiency of ecosystem management measures, contrary to single species based studies. Ecological Network Analysis (ENA) methodologies were used to assess the seasonal variation of the structure and functioning of the Sylt-Rømø Bight food web, in relation with the presence of top predators (e.g. fish, birds and seals; Part II).

Studies about marine bird and mammal populations are classically based on abundance data, which cannot be directly used to study matter or energy flow within ecosystems. Most of the mass balanced food web models, including ENA are often based on carbon and the flows are therefore expressed in carbon weight per space and time (e.g. mg carbon per m^2 and per day). In Chapter 4, essential relationships between fresh weight and other biomass measures such as carbon content were determined for six of the most abundant bird species in the Wadden Sea (*Calidris canutus*, *Limosa lapponica, Haematopus ostralegus, Chroicocephalus ridibundus, Larus canus, Anas penelope*), and for harbor seal. These conversion factors were then used to include harbor seals and updated biomass of birds in four food web models (one for each season) of the Sylt-Rømø Bight (Chapter 5). The interpretation of the numerous indices and indicators provided by ENA showed that the Sylt-Rømø Bight ecosystem varies seasonally in food web size, stability and resistance in front of external disturbances (Chapter 5). In the warm seasons (i.e. spring and summer), when the biomass of opportunistic predators is high, the system is stable, well developed and resistant. The winter season is characterized by a small and stable system which is sensitive to external perturbations. The system in fall appears to be in an unstable transition state between these two stable periods (i.e. warm seasons and winter), characterized by a high excess of primary production and a large unevenness of flows.

The results from the present study, showed that harbor seals seasonally use the Wadden Sea to forage, and that they probably have a structural role in the system, as the presence of opportunistic carnivorous species seems to increase the stability and resistance of the Wadden Sea ecosystem. The use of ecological network results including top predators, and especially seal species, would improve conservation and management measures in the Wadden Sea.

Zusammenfassung

Der Seehund ist der häufigste Meeressäuger im Wattenmeer, das diesen Tieren als wichtiges Gebiet zur Aufzucht, Ernährung und als Ruhezone dient. Diese Großtiere, die in hoher Anzahl im Wattenmeer vorkommen, üben einen starken Predationsdruck auf ihre Umgebung aus. Es ist daher notwendig, die Ernährungsweise der Seehunde in Nordsee und Wattenmeer besser zu verstehen um die räumlich-zeitliche Variation ihres Freßverhaltens sowie das Beutespektrum dieser Top-Predatoren in die Ökosystemmodelle zu implementieren.

Die Analyse stabiler Isotope und Fettsäuren als trophische Marker hat sich als gute Methode erwiesen, um Nahrungsressourcen von Meeressäugern zu bestimmen. Daher sind diese Marker hier angewendet worden, um die saisonale Variabilität der Nahrung der Seehunde der Sylt-Rømø-Bucht aufzuklären (Teil 1). Das stabile Kohlenstoff-Isotop 13C gibt Hinweise auf die Nahrungsquelle und das stabile 15N Isotop ermöglicht die Bestimmung des trophischen Niveaus des Konsumenten, da es über die Nahrungskette stufenweise angereichert wird. Der Seehund steht im Wattenmeer unter Schutz, daher ist die Seehundsjagd seit 1976 verboten. Probennahmen von Seehunden sind streng reguliert und betreffen überwiegend angetriebene tote Tiere mit einem hohen Anteil von Jährlingen, da die Mortalität im ersten Lebensjahr sehr hoch ist. Da die Isotopenzusammensetzung der Jährlinge stark durch das Säugen und die anschließende Fastenphase nach der Entwöhnung beeinflusst ist, müssen diese sehr jungen Tiere von der Untersuchung der Nahrungsressourcen ausgeschlossen werden. Im Kapitel 1 zeigt die monatliche Entwicklung der δ15N und δ13C Werte in Geweben der Jährlinge von der Sylter Küste, dass Schnurrhaare und Muskelgewebe von Tieren älter als 3-4 Monate beziehungsweise 5-6 Monate zeigen, welche Nahrungsquellen sie genutzt haben. Diese Individuen, die zusätzlich zu den adulten Tieren des gleichen Küstenabschnitts gesammelt wurden werden daher in Kapitel 2 betrachtet, um die saisonale Veränderung in der Seehundnahrung in Hinblick auf Nahrungsgebiet (Wattenmeer oder Nordsee) und -organismen zu untersuchen. Die Ergebnisse aus Kapitel 2 zeigen zwei hauptsächliche saisonale Unterschiede im Nahrungsspektrum der Seehunde des Wattenmeeres und der Nordsee . Die Seehunde ernähren sich tatsächlich je nach Saison in unterschiedlichen Gebieten, indem sie in der wärmeren Zeit des Jahres mehr küstennah in der Sylt- Rømø Bucht fressen, während sie in den kälteren Jahreszeiten in die offenen Nordsee wandern und dort ihre Nahrung suchen. Darüber hinaus wurde eine Verschiebung von mehr pelagischer Nahrung im Frühling zu eher benthischen Beuteorganismen im Sommer beobachtet, sowohl in der Sylt-Rømø Bucht als auch in der Nordsee. Diese Änderung der Nahrungsquellen wurde durch die Fettsäureanalyse bestätigt (Kapitel 3). Die saisonale Veränderung der

Nahrungsgebiete und Futterorganismen stimmt überein mit der saisonalen Variation der Biomasse und Abundanz der Nahrungsorganismen, wie in Kapitel 2 detailliert dargestellt wird.

Die Abundanz und Verbreitung der Seehunde kann große Auswirkung auf die Struktur und Funktion von Nahrungsnetzen in Küstenökosystemen haben, so dass es eine zentrale Aufgabe für Ökologie und Umweltmanagement ist, die Bedeutung für die gesamter Funktion des Ökosystems herauszuarbeiten. Ein Umweltmanagement, das auf der Analyse des Ökosystems basiert wird als Lösung angesehen, um die Effektivität des Management von Ökosystemen zu erhöhen, im Gegensatz zu einem Management, das auf den Studien einzelen Arten beruht. Die Ökologische Netzwerkanalyse (Ecological Network Analysis ENA) wurde angewendet, um die saisonalen Unterschiede in der Struktur und Funktion des Sylt-Rømø-Nahrungsnetzes zu analysieren unter besonderer Berücksichtigung der Top-Predatoren (wie Fische, Vögel und Seehunde; Teil II der Thesis).

Untersuchungen der Populationen von Vögeln und Meeressäugern basieren traditionell auf Abundanzdaten, die nicht direkt für die Bestimmung von Stoff- und Energiefluss im Ökosystem genutzt werden können. Die meisten der massen-balanzierten Nahrungsetzmodelle, wie ENA, sind überwiegent Kohlenstoff-basiert und daher sind die Flüsse in Kohlenstoffeinheiten pro Gebiet und Zeit ausgedrückt (z.B. mg C pro m^2 und Tag). In Kapitel 4 werden essentielle Beziehungen zwischen Frischgewicht und weiteren Biomasse-Einheiten, wie Kohlenstoffgehalt, für den Seehund sowie für die sechs häufigsten Vogelarten im Wattenmeer bestimmt (*Calidris canutus, Limosa lapponica, Haematopus ostralegus, Chroicocephalus ridibundus, Larus canus, Anas penelope*). Diese Umrechnungsfaktoren wurden genutzt, um den Seehund und die aktuellen Biomassewerte für die Vögel in die vier saisonalen Nahrungsnetzmodelle der Sylt-Römö Bucht einzubauen, jeweils eins pro Saison (Kapitel 5). Die Interpretation der verschiedenen Indices und Indikatoren, die aus ENA gewonnen wurden, zeigen, dass das Sylt-Rømø-Ökosystem sich je nach Jahreszeiten einen Trend zu unterschiedliche Größe, Stabilität und Widerstandsfähigkeit gegenüber äußeren Störungen zeigt (Kapitel 5). In den wärmeren Jahreszeiten (Frühling und Sommer) ist die Biomasse opportunistische Räuber groß, das System ist stabil, gut entwickelt und widerstandsfähig. Der Winter ist charakterisiert durch ein kleines, aber stabiles System, das empfindlich gegenüber Störungen von außen ist. Im Herbst ist das System in einem instabilen Übergangszustand zwischen den beiden stabilen Perioden von warmen und kalten Jahreszeiten und ist durch einen hohen Überschuss an Primärproduktion und eine starke Ungleichmäßigkeit (Uneveness) der Flussraten charakterisiert.

Die Ergebnisse zeigen, dass sich die Seehunde saisonal im Wattenmeer ernähren und dass sie höchstwahrscheinlich eine strukturierende Rolle im System ausüben, das durch die Präsens dieser opportunistischen karnivoren Art in seiner Stabilität und Widerstandsfähigkeit gestärkt wird. Die Anwendung von ökologischen Netzwerkanalysen, die Top-Prädatoren berücksichtigen, insbesondere die Seehunde, würde Maßnahmen zum Naturschutz und Management im Wattenmeer verbessern.

Preamble

The Wadden Sea has an important role for marine mammals in terms of resting, nursing and foraging. Marine mammals represent the most prominent members among top predators in the marine environment. Their abundance and distribution can have a large effect on the structure and the functioning of coastal food webs, and assessing their role in the functioning of ecosystems is a central issue in ecology and management.

Harbor seal (*Phoca vitulina*) is the most abundant marine mammal species in the Wadden Sea. Since 1991, a trilateral Seal Agreement has been concluded between Denmark, Germany and the Netherlands, under the umbrella of the Convention on the Conservation of Migratory Species of Wild Animals (Bonn Convention). One of the main goals of this agreement is to achieve and maintain a comprehensive conservation and management of both harbour and grey seal populations in the Wadden Sea through common coordinated measures of the responsible authorities. Assessing the role and the influence of harbor seals (and grey seals) within the Wadden Sea ecosystem is necessary to improve these conservation and management measures.

Ecosystem-based management is considered as the solution needed to improve the efficiency of ecosystem management measures. Ecological Network Analysis (ENA) methodology was developed to assess holistically the complex environmental interactions within an ecosystem and consists of a set of algorithms allowing the structural and functional properties of an ecosystem to be analyzed.

During my PhD, I assessed the seasonal diet of harbor seals from the Wadden Sea *vs*. North Sea to evaluate the role of the Wadden Sea in term of foraging location for harbor seals. I then included the harbor seal compartment in a food web model of the Sylt-Rømø Bight, located in the northern Wadden Sea, to estimate their influence as top predators on the functioning of the ecosystem.

The results are presented in the five following chapters divided in two parts. The first part (Chapters 1 to 3) is dedicated to the assessment of the harbor seal´s diet. The second part (Chapters 4 and 5) focusses on the food web model construction and the interpretation of the ENA results. Before presenting these results, I will first introduce the concept of top down effect in ecosystems and the methods used to study the diet of top predators, the concept of ecosystem based management and more in detail the Ecological Network Analysis, and some generalities about the Wadden Sea ecosystem.

General Introduction

"Oh mer, nul ne connait tes richesses intimes"

"Oh sea, no one knows your most intimate bliss."

Baudelaire

General Introduction

1. Ecosystem based management

1.1. Ecosystem health concept

In the current context of increasingly stressed ecosystems due to anthropogenic activities and global changes (Doney et al. 2012), holistic solutions are crucial to manage and protect those ecosystems (Levin and Lubchenco 2008, Samhouri et al. 2009, Longo et al. 2015). Ecosystem-based management has been proclaimed as the solution needed to improve the efficiency of ecosystem management measures (Pikitch et al. 2004, Levin and Lubchenco 2008, Levin et al. 2009), contrary to single species based studies (McLeod et al. 2005). These management decisions should be based on ecosystem attribute indicators which reflect the aspects of ecosystem structure and function intended by the term ecosystem health (e.g. diversity, energy recycling, resilience) (Samhouri et al. 2009). In theory, a healthy ecosystem has been defined as meeting five criteria: (1) being able to maintain equilibrium within the system (i.e. internal stability), (2) being diverse and complex, (3) being able to cope with external disturbances (i.e. stability and resilience), (4) being a growing and developing system, and (5) being balanced between the compartments (i.e. high evenness of flows) (Costanza et al. 1992, Jørgensen et al. 2010).

Depending on the ecosystem´s “disease” and the management focus, a range of simple to complex indicators can be used for ecosystem health assessment (Samhouri et al. 2009, Jørgensen et al. 2010). For example, these indicators might be (1) the presence or absence of a specific species (e.g. endangered species) (Jørgensen et al. 2010), (2) the status of entire trophic levels (i.e. high abundance of fish being indicator of good water quality) (Jørgensen et al. 2010), (3) the concentration of chemical component or toxins in the blubber stores of coastal resident marine mammals (Bossart 2011), (4) ratios of ecosystem processes such as the production *versus* biomass ratios indicating the development stage of an ecosystem (Odum 1969) and (5) holistic indicators reflecting the resilience, connectivity or recycling magnitude of an ecosystem (Ulanowicz 2004).

1.2. Ecological Network Analysis

In the last decades food web models and ecological networks have become useful tools to represent large scale systems encompassing numerous compartments interacting with each other and responding differently to external stressors in marine (Aarnio et al. 1996, Leguerrier et al. 2007, Ings et al. 2009, Kaufman and Borrett 2010, Fath 2015) and terrestrial systems (Heymans et al. 2002). Results from those models provide significant insight into the fundamental functioning of the ecosystem (Baird et al. 2004, Fath 2015) and are very relevant for marine ecosystem management (Samhouri et al. 2009). Ecological Network Analysis methodology was developed to holistically assess these complex environmental interactions within an ecosystem and consists of a set of algorithms allowing the structural and functional properties of an ecosystem to be analyzed (Ulanowicz and Abarca-Arenas 1997, Ulanowicz 2004, Kaufman and Borrett 2010, Fath 2015). Network analysis has been used for instance to study the structural complexity of the ecosystem, the structure and magnitude of the cycling of energy and material, the efficiency of energy transfer within the system, the rate of energy assimilation and dissipation, the system activity, growth and development and the trophic structure. The analytical methodology is reviewed by Ulanowicz (2004).

To analyze energy flows quantitatively in a food web, it is necessary to define compartments and to measure the interactions between these components. A compartment might be a single species, a genus, a class or a functional group. The analysis of a food web model is based on empirical data, which is obtained by observations or experiments, and depicts a realistic representation of an ecosystem (Ulanowicz 2004). To establish a quantitative food web model, biomass, respiration, consumption, egestion of all compartments, and energy flows between the compartments and export and import of energy or material from adjacent systems are required to be included in the analysis (Fath et al. 2007) (Fig. 1). Such models are often based on carbon and the flows are therefore expressed in carbon weight per space and time (e.g. mg carbon per m^2 and per day) (Baird et al. 2004, Fath et al. 2007).

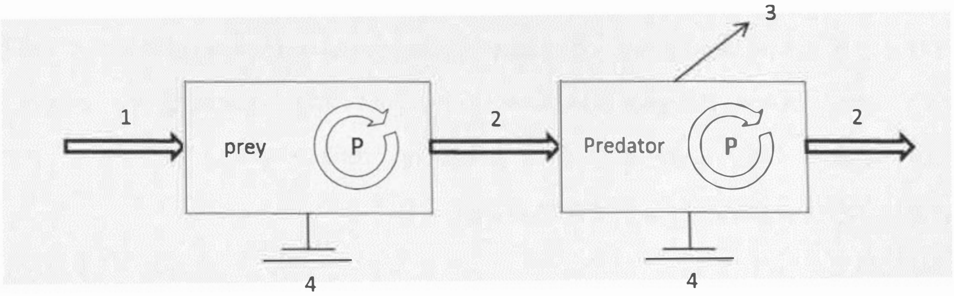


Fig. 1: Possibilities of energy flows in an ecosystem. 1 = hexogen input (e.g. migration of individuals into the system); 2 = exchange between compartments (e.g. predation); 3 = export of material (e.g. migration of individuals from the system); 4 = Export of inorganic material (e.g. Loss of CO2 du to respiration); after Asmus personal communication

The output of ecological network analysis provides many indices and system properties of natural ecosystems. For example, the indices related to the ascendency provides information about the total activity and the organization of a system (Ulanowicz 2004). The Overhead, on the other hand, measures the entropy of the system and represents the redundancy and parallel flows in the internal and exogenous exchanges (Baird et al. 2004). It is a measure of stability, in the sense of resistance in front of external perturbations (Christensen 1995). The sum of these two parameters (i.e. ascendency and overhead) represents the development capacity of the system (Monaco and Ulanowicz 1986, Christensen 1995, Ulanowicz and Abarca-Arenas 1997). The ascendency and the overhead, relatively to the development capacity, are mutually exclusive (Christensen 1995) and a healthy system requires adequate amounts of both (Ulanowicz 2004, Fath 2015). Indeed, if the redundancy is too small, in other words if the system is too organized and the flows highly specialized, the system appears to be rigidly linked and vulnerable to collapse (Ulanowicz 2004). The overhead which plays a role of reserve of parallel flows that can adapt in front of perturbations is then not sufficient to maintain the stability of the system. On the contrary, if the efficiency (i.e. organization) is too small, the system tends to stagnate and cannot develop (Fath 2015). Therefore, a system is sustainable when both ascendency and overhead are in appropriate amount. Fath (2015) developed the Robustness index which represents this balanced tradeoff between efficiency and redundancy (Fig. 2).

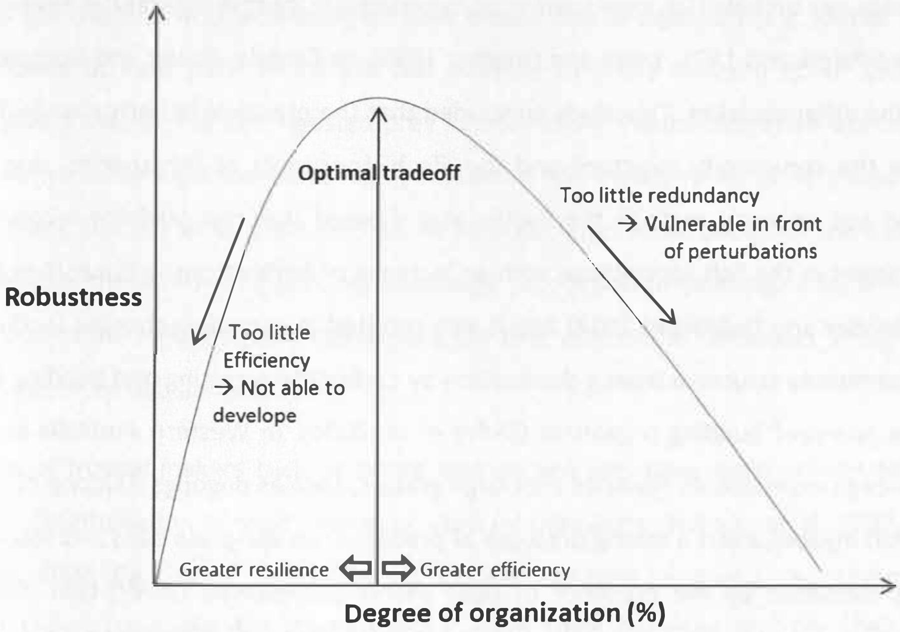


Fig. 2: Theoretical curve representing the Robustness (i.e. ability to adapt in from of perturbations) *versus* the degree of organization (i.e. ascendency/development capacity) in a system. The degree of organization varies from 0 to 100%. After Fath (2015)

More than 20 other indices can be calculated with ecological network analysis and reflect the structure and functioning of an ecosystem describing the cycling structure and magnitude, the trophic structure, the connectivity between the compartments or the flow diversity (Ulanowicz 2004, Borrett and Lau 2014).

2. Top predators in ecosystems

2.1. Top down control and cascading effect

Top predators in marine environment can have a large effect on the structure and the functioning of ecosystems and communities (Estes 1979, Power and Gregoire 1978, Bowen 1997). Large predators are expected to exert a strong influence on smaller-bodied mesoconsumers and the species that they in-turn consume (Heithaus et al. 2008). For example, depletion or loss of top predators leads to a decrease in top-down control. This commonly results in an increase of former prey species and competitors followed by a decrease of the prey of the particular species (Lotze et al. 2005). These large cascading effects have been detected in an increasing number of studies (Bowen 1997, Borer et al. 2005, Frank et al. 2005, Frank et al. 2007). One of the most famous examples is the three level cascade effect observed on the Californian coast where the presence of sea otters (*Enhydra lutris*), predators of the herbivorous large sea urchins (*Strongylocentrotus franciscanus*), permit substantial development of kelp beds (Estes and Palmisano 1974, Estes and Duggins 1995). In Canada, Power and Gregoire (1978) led a study comparing different lakes. This study concluded that the presence of harbor seals (*Phoca vitulina*) was modifying the community structure and the life history traits of fish species, due to predation. Studies carried out on coral reefs in the Pacific also showed that top predator removal by fisheries resulted in changes in the fish assemblage with an increase of herbivorous in opposition to carnivorous species (Friedlander and DeMartini 2002) and it also resulted in cascading changes leading to a shift in the benthic community structure from a domination by carbonate accreting reef building organisms to a domination by non-reef building organisms (Dulvy et al. 2004). In Western Australia in a sub-tropical bay, exclusion-cage experiments revealed that large grazers, such as dugongs (*Dugong dugon*) and green turtles (*Chelonia mydas*), exert a strong pressure of predation on see-grass beds and associated species, which can be mediated by the presence of tiger sharks (*Galeocerdo cuvier*) that hunt these large herbivorous species (Burkholder et al. 2013). The dugongs change their feeding location to avoid their predator and migrate to habitat with low-risk of predation. The presence of tiger sharks therefore initiates a behavior-mediated trophic cascade which influences indirectly lower trophic levels (i.e. see-grass community) (Burkholder et al. 2013).

However, the role of top predators in structuring the ecosystems is still not well constrained (Lesage et al. 2001, Bowen 1997) due to their ecological niches that often exceeds the temporal and spatial scales which are used to define community boundaries (Lesage et al. 2001, Tougaard et al. 2003). Assessing the role of top predators in the functioning of ecosystems is then a central issue in ecology and management (Bowen 1997).

2.2. Marine mammal diet assessment

Marine mammals represent the most prominent members among top predators in the marine environment (Reijnders and Lankester 1990). Understanding their foraging ecology is critical to evaluate how they function within marine ecosystems (Bowen 1997, Iverson et al. 1997) but studies of their feeding ecology face a number of inherent difficulties. First, the consumption of prey items often occurs below the surface, making direct observations impossible (Iverson et al. 1997). Second, top predators are generally very mobile species and their ecological needs often exceed the spatial scales used to define community boundaries (Lesage et al. 2001, Tougaard et al. 2003). Third, classical methods for diet studies such as gut contents and fecal analyses have biases due to digestion (e.g. loss of soft parts and digestion-resistance of hard part) which are not possible to avoid (Iverson et al. 1997) and these methods only give a snapshot of the ingested prey items. Finally, marine mammals are often protected species and invasive sampling is therefore highly regulated and mostly relies on stranded dead animals (Siebert et al. 2006, Lehnert et al. 2007, Siebert et al. 2007, Rijks et al. 2008). In the case of pinniped species for example, these sampling encompass a large proportion of yearlings (i.e., animals less than one year old) due to the high mortality rate during the first year of life (Reijnders 1976, Harding et al. 2005), and are therefore unbalanced.

The use of trophic makers such as stable isotope analyses have been proven to be a reliable method for the determination of food resources used by predators (Hobson et al. 1997, Lesage et al. 2001, Das et al. 2003, Caut et al. 2011). The carbon and nitrogen isotopic composition of consumer tissues reflects their assimilated diet (DeNiro and Epstein 1978, Peterson and Fry 1987). Indeed, the ratio of heavy isotope *versus* light isotope of carbon and nitrogen vary among the primary consumers (France 1995) and can be followed along the food chain in a relatively predictable way: there is an increase in the carbon-13 content ($^{13}C/^{12}C$ ratio) and nitrogen-15 content ($^{15}N/^{14}N$ ratio) in organism tissues due to selective metabolic loss of carbon-12 (^{12}C) and nitrogen-14 (^{14}N) during food assimilation and growth (Peterson and Fry 1987). The stable isotope composition of carbon generally reflects the origin of food resources. It allows a good discrimination between food resources produced in continental areas, those produced in the open ocean, and the ones produced in benthic environments (DeNiro and Epstein 1978, Rau et al. 1983, Hobson et al. 1994) (Fig. 3). The stable isotope composition of nitrogen is commonly used as an indicator of the trophic position of a consumer, due to a relatively high step-wise enrichment (i.e., trophic fractionation factor) between each trophic level (Fig. 3) (Peterson and Fry 1987, Fry 1988, Hobson and Welch 1992).

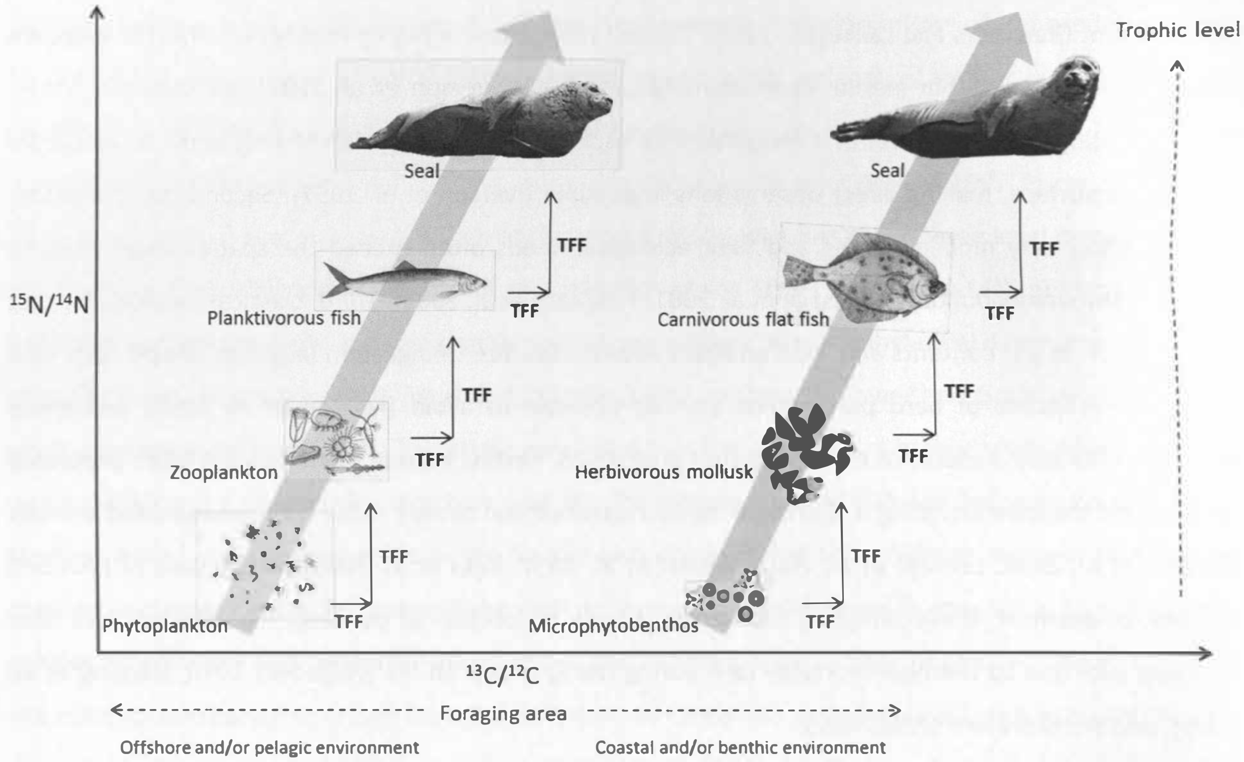


Fig. 3: Schematic figure of the use of stable isotope ratios of carbon and nitrogen in marine environment. Two simplified food chains are illustrated, both supported by different primary producers: oceanic phytoplankton (blue large arrow) and microphytobenthos (orange large arrow). TFF = trophic enrichment factors between each trophic level. After Peterson and Fry (1987), France (1995), Hobson et al. (1997), Hobson (1999)

Isotopic composition reflects the diet of a consumer integrated over a few days (e.g., blood, plasma, and liver) or over a few months (e.g., muscle), depending on the metabolic turnover of the tissue (Hobson 1995, Vander Zanden et al. 2015). The turnover time of muscle tissues is poorly known for large marine mammals. Studies on birds (*Coturnix japonica* and *Corvus brachyrhynchos*) (Hobson and Clark 1992) and small mammals (*Meriones unguiculatus*) (Tieszen et al. 1983) have shown that turnover of muscles has an order of magnitude of a month. Vander Zanden et al. (2015) estimated the isotopic half-life of muscle tissue for a mammal with a body mass of 90 kg to be of about two to three months. On the contrary, mineralized and keratinous tissues, such as vibrissae (Fig. 4), teeth and claws, have the great advantage of preserving a time line of stable isotope deposition during their growth period and therefore allow retrospective diet studies (Hobson 1995, Ferreira et al. 2011, Carroll et al. 2013, Matthews and Ferguson 2015). For example, several studies have revealed that vibrissae provide a powerful way to assess diet and foraging location of marine mammals such as elephant seals (*Mirounga leonine*) (Newland et al. 2011), leopard seals (*Hydrurga leptonyx*) (Hall-Aspland et al. 2005), harp seals (*Pagophilus groenlandicus*) (Hobson et al. 1996) and sea otters (*Enhydra lutris nereis*) (Newsome et al. 2009). Indeed, isotopic composition is archived on a daily basis along a growing vibrissa and remains stable over time (Hirons et al. 2001, Zhao and Schell 2004, Cherel et al. 2009). Vibrissae are thus good recorders of dietary history, giving precise (few days) and long-term (up to one year) information about the food sources used by consumers (Hall-Aspland et al. 2005, Newsome et al. 2009, Newland et al. 2011).

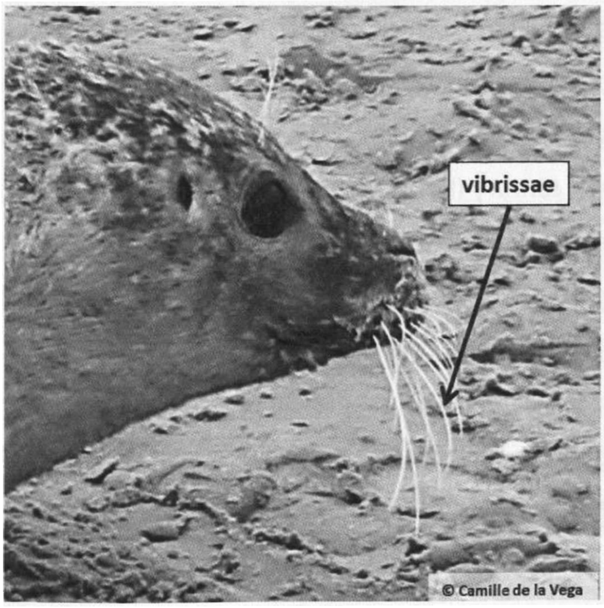


Fig. 4: Harbor seal (*Phoca vitulina*) on a sand bank from the Wadden Sea

Fatty acids can also be used as trophic markers to assess predator-prey interactions. Lipids in marine organisms are characterized by their diversity (> 60 types) and high levels of long-chain polyunsaturated fatty acids which originate in various unicellular algae and seaweeds (Budge et al. 2006, Bowen et al. 2009). Fatty Acids are the largest constituents of lipids and those of carbon chain of 14 or longer are often deposited in animal tissue with minimal modification from the diet (Iverson et al. 1997). Because a limited number of fatty acids can be bio-synthetized by animals (Cook and McMaster 2002), it is possible to distinguish dietary *versus* non-dietary fatty acids (Iverson et al. 2004). Those fatty acids arising only or mostly from the diet (i.e. dietary FAs), also called essential fatty acids (Cook and McMaster 2002), are useful tools to study predator foraging ecology, once fatty acid patterns are characterized in the potential prey items (Iverson et al. 1997, Iverson et al. 2002). The use of fatty acid analysis is relatively new but it has been proved to be a reliable and powerful method to assess the diet of marine predators (Kirsch et al. 1998, Iverson et al. 2004, Nordstrom et al. 2008).

3. The Wadden Sea ecosystem

3.1. An area used by top predators

The present study was conducted in the Wadden Sea (Fig. 5), the largest continuous system of intertidal sand and mudflats in the world (Lotze 2007). This sheltered area, adjacent to the fertile waters of the North Sea extends along the south-eastern margin of the North Sea from the Netherlands to Denmark. The Wadden Sea has been strongly influenced by human activities for centuries (Wolff 2000; Lotze et al. 2006, Lotze et al. 2005). Habitat transformation, overexploitation and pollution in the Wadden Sea led to the loss or severe depletion of most of its top predators (marine mammals, birds and fish) until the early twentieth century (Reise 2005; Erikson et al. 2010; Reijnders et al. 1992; Lotze et al. 2005). In the nineteen seventies, conservation measures were introduced (Hoffman et al. 2011), such as the protection of important breeding, feeding or staging habitats and the prohibition of exploitation and hunting (Lotze et al. 2005, Reijnders and Lankester 1990, Smardon 2009). Since the introduction of these measures several species of birds as well as the grey and harbor seals have recovered (Lotze et al. 2005, Reijnders and Lankester 1990). In 2009, the Dutch and German parts of the Wadden have been declared UNESCO World Heritage Site which was extended to the Danish part of the Wadden Sea in 2014.

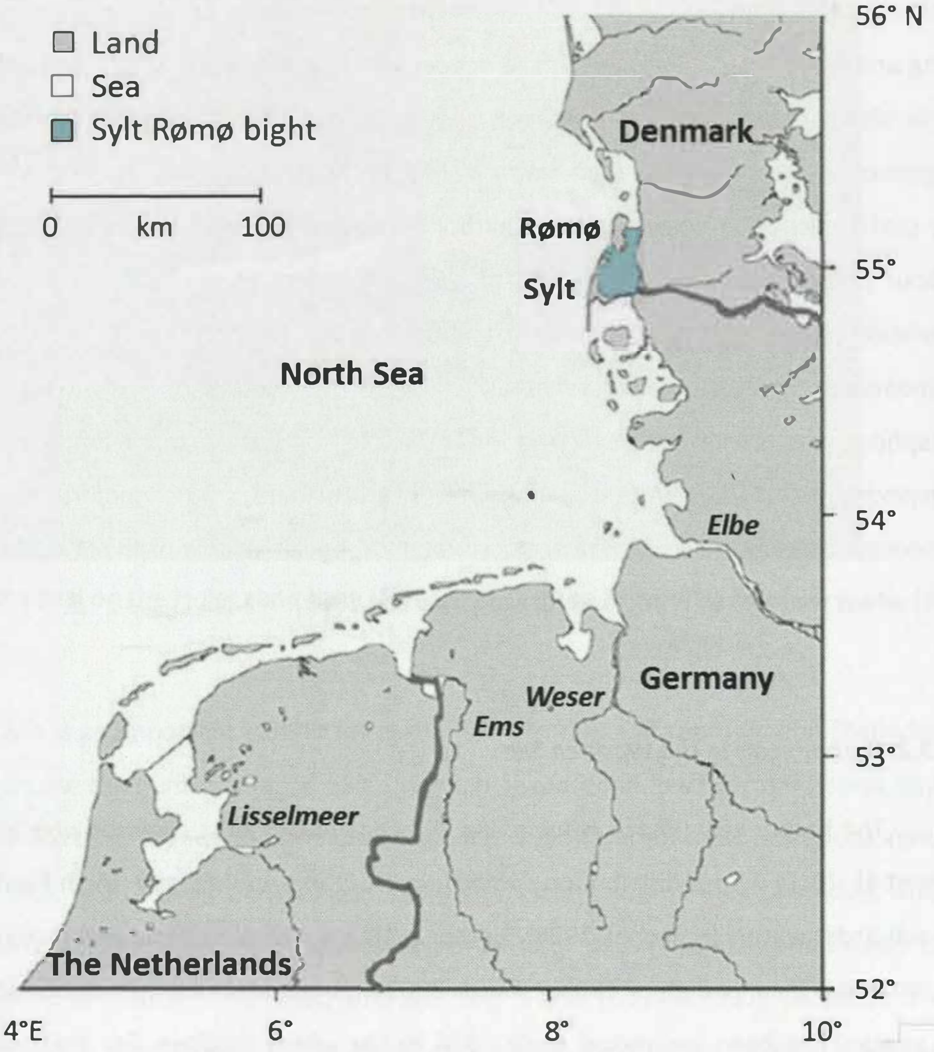


Fig. 5: Map of the Wadden Sea. The names in italics are the three main rivers discharging in the Wadden Sea

The Wadden Sea is of particular importance for several top predator species. For instance, migratory birds use this area as a stopover site in spring and fall on their annual migrations between southern wintering and northern breeding areas (Meltofte et al. 1994, Scheiffarth and Nehls 1997). About 10-12 million birds spend at least a part of their annual life cycle in this area (Scheiffarth and Nehls 1997). Furthermore, the Wadden Sea is an important nursery area for juveniles of several fish species from the North Sea such as *C. harengus, M. merlangus* and *L. limanda,* colonizing the tidal inlets and tidal flats in summer (Daan et al. 1990, Polte and Asmus 2006, Tulp et al. 2008, Baumann et al. 2009). In addition to juveniles, seasonally migrating species such as *Osmerus eperlanus* and *Platichthys* *flesus* are found in the Wadden Sea. Most of these non-resident species migrate into the coastal zone in spring and leave in fall, when they go to deeper waters in the North Sea (Tulp et al. 2008). The Wadden Sea is also a major habitat for several species of marine mammals (Reijnders et al. 2009). Three indigenous species of marine mammals inhabits the Wadden Sea: the harbor seal (*Phoca vitulina*), the grey seal (*Halichoerus grypus*) and the harbor porpoise (*Phocoena phocoena*). Today, the population of harbour porpoises is stable (Gilles et al. 2009, Reijnders et al. 2009, Jansen et al. 2012) and the population of grey and harbour seals is approaching the carrying capacity of the current environment (Reijnders et al. 2010, Reijnders et al. 2009). Other species of seals (*Pagophilus groenlandicus*, *Cystophora cristata*, *Pusa hispida* and *Erignathus barbatus*) from arctic regions and cetacean (*Lagenorhynchus albirostris*, *Lagenorhynchus obliquidens*, *Balaenoptera spp*. and *Megaptera novaeangliae*) from the North Sea are occasional or regular visitors of the Wadden Sea (Reijnders et al. 2009) which they use to breed and forage (Smardon 2009).

3.2. Harbor seals in the Wadden Sea

The harbor seal (*Phoca vitulina;* Fig. 6) is one of the most common phocid species in the world (Berg et al. 2010) whose distribution spreads across North Atlantic and North Pacific in both temperate and sub-arctic waters (Hall et al. 1998). Harbor seals are mid-size phocid and they have a mean length of 170 cm and a mean weigh of 100 kg (Burns 2002). Harbor seals are completely protected and hunting this species has been prohibited since 1976 in the whole Wadden Sea (Reijnders et al. 1995). The Wadden Sea population of harbor seals increases by average of 9.6% per year since the last epizootic in 2002 (Reijnders et al. 2009) and the sliding 5 year average growth rate has decreased since 2008, suggesting that the harbour seal population might approach the carrying capacity of the area (Reijnders et al. 2010, Trilateral Seal Expert Group 2013). In our days, it is, together with harbor porpoise, the most abundant marine mammal species (Liebsch et al. 2006) with 26 576 individuals counted on land in August 2014 (Galatius et al. 2014). It spreads from Denmark to the Netherlands, with 60.7% of its population located along the German coasts (Galatius et al. 2014).

Fig. 6: Harbor seal on the Hojer sand bank (Fig. 7, p. 23) in the Sylt-Rømø Bight

The Wadden Sea is an important habitat for harbor seals in terms of reproduction (Reijnders et al. 2009). Harbour seals use the numerous sand banks regularly exposed at low tide in different bays of the Wadden Sea to give birth, rest and molt (Mees and Rijnders 1994). Harbor seals reproduce annually and, in the Wadden Sea, the birthing period takes place from beginning of May to middle of June (Osinga et al. 2012) followed by a short period of lactation of 24 to 32 days (Muelbert and Bowen 1993, Lang et al. 2005). Pups´ only source of nutrition until weaning is milk (Bowen 1991). Lactation is over when the pups are abruptly weaned and left to begin eating solid food without parental assistance (Bonner 1984, Oftedal et al. 1987). Then, the weaned pups undergo a post weaning fast of 4-6 weeks during which they rely on their blubber storage (Muelbert and Bowen 1993, Muelbert et al. 2003).

Harbor seals also use the Wadden Sea at high tide to forage and feed on the abundant food stock it provides (Smardon 2009, Reijnders et al. 2010). Harbour seals are opportunistic feeders subsisting largely on fish (Mees and Reijnders 1994). One individual consume on average 4 kg of fresh weight per day (Berg et al. 2002, Bjørge et al. 2002). Several studies based on stomach content conducted in the North Sea showed a variation in the dominant species in the seal´s diet depending on the location. Diet studies conducted in the North-Western North Sea (Scotland) found clupeids and sand eels as main prey items with gadoids in secondary importance (Pierce et al. 1991, Thompson et al. 1991, Thompson et al. 1996, Tollit et al. 1997). On the contrary, in the Southern and eastern North Sea (Southwestern North Sea, South East United Kingdom and Denmark), the diet of harbour seals is dominated by gadoids and flat fish with clupeids and sand eel in secondary importance (Härkönen 1987, Härkönen and Heide-Jørgensen 1991, Brown and Pierce 1998, Hall et al. 1998, Das et al. 2003). Along German coasts, in the Schleswig-Holstein area, gadoids (*Gadus morhua* and *Merlangius merlangus*) and flat fish (*Limanda limanda*, *Platichthys flesus* and *Pleuronectes platessa*) are prominent in the seal´s diet with *Ammodytes tobianus* and *Clupea harengus* in secondary importance (Gilles et al. 2008). Thus, harbor seals feed on a large range of prey with the prevalence of some key species, and the contributions to the diet of these prey items vary depending on the area, and probably depending on the prey availability (Tollit et al. 1997, Andersen et al. 2007).

Due to their large body size and their high abundance in the Wadden Sea, seals exert a strong pressure of predation on the environment they live in (Bowen 1997, Reijnders et al. 2010). Even if harbor seals from the Wadden Sea appear to use the North Sea more than previously expected (Tougaard et al. 2003, Reijnders et al. 2005), the pressure of predation they exert on the Wadden Sea food resources remains substantial. Consequently, there are needs to better understand the trophic behavior of seals in the North Sea and in the Wadden Sea, in order to have better estimations of their diets and to determine spatio-temporal variations of their foraging activity. This would permit to include them in food web models and therefore better evaluate their influence on the ecosystem.

3.3. The studied area: the Sylt-Rømø Bight

This study was carried out in the Sylt-Rømø Bight (Fig. 7) (54°52’ - 55°10’ N, 8°20’ - 8°40’ E) which is part of the northern Wadden Sea (Fig. 5). This 404 km^2 semi-enclosed basin is located between the islands of Sylt (Germany) and Rømø (Denmark; Fig. 5). The tidal range inside the Bight is up to 2 m (Martens and Beusekom 2008). The intertidal and subtidal areas cover 134 km^2 and 273 km^2 respectively (Gätje and Reise 1998, Baird et al. 2004). Two causeways connect the islands with the mainland, and prohibit any exchange of water with the adjacent tidal basins. The only connection to the North Sea is a 2.8 km wide deep tidal channel (Lister Deep) between the two islands. Three main tidal gullies (Rømø-Dyb, Høyer-Dyb and Lister-Ley) are connected by the Lister Deep where the maximum depth of 40.5 m below low water level is found (Fig. 7A).

The Sylt-Rømø Bight provides shelter for a stable colony of ≈400 harbor seals on average in summer (2009 to 2015) (Jensen 2015). About 90 newborns in average were counted per year (Jensen 2015). The abundance drastically decreases in winter, with ≈70 animals counted in December 2015 on the sand banks (Jensen, unpublished data). Harbor seals use five sand banks uncovered at low tide as haul out sites. These sandbanks are spread in the whole Bight, with the Jordsand and List sand banks (Fig. 7B) being the most frequented (Jensen 2015). No diet investigation was carried out on harbor seals in the Sylt-Rømø Bight and the use of the Wadden Sea food resources *versus* North Sea food resources is poorly known, although Tougaard et al. (2003) conducted a telemetry study and harbor seals tagged on Rømø had longer foraging trip in winter than in summer.

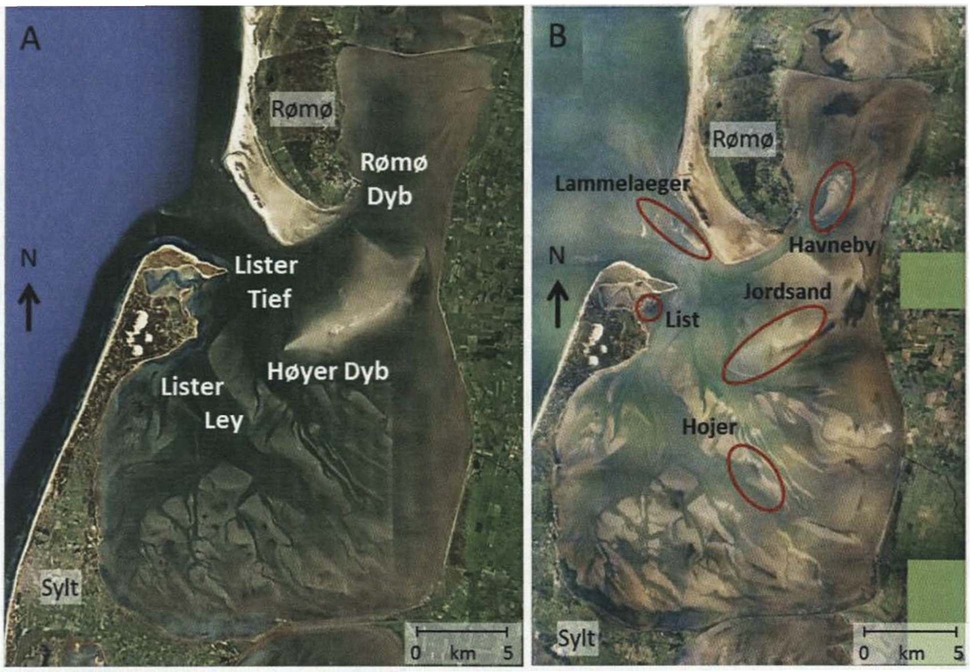


Fig. 7: Aerial pictures of the Sylt-Rømø Bight at A- high tide and B- low tide. The tidal gullies are named on picture A, the sand banks used by seals (red circles and ellipses) are displayed on picture B; Map data ©2016 GeoBasis-DE/BKG (©2009), Google Imagery ©2016 TerraMetrics

The ecosystem of the Sylt-Rømø Bight is well studied and network analysis was used in previous studies to describe the structural and functional properties of its food web (Baird et al. 2004, Baird et al. 2007, Baird et al. 2008, Baird et al. 2011, 2012). Focusses were made on habitat characteristics and properties (Baird et al. 2007, Baird et al. 2011), differences in dynamics of nutrient flows (i.e. carbon, nitrogen and phosphorus) in the food web (Baird et al. 2008, Baird et al. 2011), and potential changes in the ecosystem functioning and structure in the last 15 years due to invasive species (Baird et al. 2012).

Marine mammals were not included in these previous models and the seasonal variation of the structural and functional properties of the Sylt-Rømø Bight system was never evaluated.

4. Aims of the study

The general aim of this study is to evaluate the influence of top predators (i.e. harbor seals) on the Wadden Sea (i.e. Sylt-Rømø Bight) food web. This work is divided in five chapters organized in two parts.

The first part contains the first three chapters (Chapters 1, 2 and 3) and is dedicated to the investigation of the diet of harbor seals from the Sylt-Rømø Bight. The first chapter aims at determining when isotopic compositions of yearling tissues reflect their foraging in the environment in order to use these individuals in community diet studies (i.e. Chapter 2). The second chapter is dedicated to the investigation of the seasonal variation of harbor seal´s diet in relation to prey availability. Stable isotope composition was analyzed in vibrissae for temporal reconstruction and was compared seasonally to prey items from the Sylt-Rømø Bight and from the North Sea, to estimate the proportion coming from one or the other location. The third chapter focusses on the diet composition of harbor seals in spring and summer in the Sylt-Rømø Bight. Fatty acid composition of harbor seal muscles and potential prey items from the Sylt-Rømø Bight was analyzed. The prey species were characterized by specific dietary fatty acids which were then followed seasonally in the seal´s muscles, in order to precise the diet composition of harbor seals.

The second part contains two chapters (Chapters 4 and 5) and focusses on the seasonal variation of the Sylt-Rømø Bight ecosystem structure and functioning, in relation to the presence of top predators. Chapter 4 aims at determining the relationships between fresh weight and various biomass measures (e.g. fresh weight *versus* carbon content) for harbor seals and six of the most abundant bird species in the Wadden Sea, allowing a better estimation of their biomass when they are included in food web models. Chapter 5 is dedicated to the ecological network analysis of the Sylt-Rømø Bight and the seasonal variation of its food web structure and functioning.

5. References

Aarnio, K., E. Bonsdorff, and N. Rosenback. 1996. Food and feeding habits of juvenile flounder *Platichthys flesus* (L.), and turbot *Scophthalmus maximus* L. in the åland archipelago, northern Baltic Sea. Journal of Sea Research **36**:311-320.

Andersen, S. M., J. Teilmann, P. B. Harders, E. H. Hansen, and D. Hjollund. 2007. Diet of harbour seals and great cormorants in Limfjord, Denmark: interspecific competition and interaction with fishery. Ices Journal of Marine Science **64**:1235-1245.

Baird, D., H. Asmus, and R. Asmus. 2004. Energy flow of a boreal intertidal ecosystem, the Sylt-Rømø Bight. Marine Ecology Progress Series **279**:45-61.

Baird, D., H. Asmus, and R. Asmus. 2007. Trophic dynamics of eight intertidal communities of the Sylt-Rømø Bight ecosystem, northern Wadden Sea. Marine Ecology Progress Series **351**:25-41.

Baird, D., H. Asmus, and R. Asmus. 2008. Nutrient dynamics in the Sylt-Rømø Bight ecosystem, German Wadden Sea: An ecological network analysis approach. Estuarine, Coastal and Shelf Science **80**:339-356.

Baird, D., H. Asmus, and R. Asmus. 2011. Carbon, nitrogen and phosphorus dynamics in nine sub-systems of the Sylt-Rømø Bight ecosystem, German Wadden Sea. Estuarine, Coastal and Shelf Science **91**:51-68.

Baird, D., H. Asmus, and R. Asmus. 2012. Effect of invasive species on the structure and function of the Sylt-Rømø Bight ecosystem, northern Wadden Sea, over three time periods. Marine Ecology Progress Series **462**:143-161.

Baumann, H., A. M. Malzahn, R. Voss, and A. Temming. 2009. The German Bight (North Sea) is a nursery area for both locally and externally produced sprat juveniles. Journal of Sea Research **61**:234-243.

Berg, I., T. Haug, and K. T. Nilssen. 2002. Harbour Seal (*Phoca vitulina*) Diet in Vesteralen, North Norway. Sarsia **87**:451-461.

Bjørge, A., T. Bekkby, V. Bakkestuen, and E. Framstad. 2002. Interactions between harbour seals, Phoca vitulina, and fisheries in complex coastal waters explored by combined Geographic Information System (GIS) and energetics modelling. ICES Journal of Marine Science: Journal du Conseil **59**:29-42.

Bonner, W. N. 1984. Lactation strategies in pinnipeds: problems for a marine mammalian group. Symposia of the Zoological Society of London **51**:253-272.

Borer, E. T., E. W. Seabloom, J. B. Shurin, K. E. Anderson, C. A. Blanchette, B. Broitman, S. D. Cooper, and B. S. Halpern. 2005. What determines the strength of a trophic cascade? Ecology **86**:528-537.

Borrett, S. R., and M. K. Lau. 2014. enaR: An r package for Ecosystem Network Analysis. Methods in Ecology and Evolution **5**:1206-1213.

Bossart, G. D. 2011. Marine Mammals as Sentinel Species for Oceans and Human Health. Veterinary Pathology Online **48**:676-690.

Bowen, W. D. 1991. Behavioural ecology of pinniped neonates. Pages 66-127 *in* D. Renouf, editor. The behaviour of pinnipeds. Chapman and Hall, London (UK).

Bowen, W. D. 1997. Role of marine mammals in aquatic ecosystems. Marine Ecology Progress Series **158**:267-274.

Bowen, W. D., A. J. Read, and J. A. Estes. 2009. Feeding Ecology. Pages 217-246 *in* A. R. Hoelzel, editor. Marine mammal biology: an evolutionary approach. John Wiley & Sons.

Brown, E. G., and G. J. Pierce. 1998. Monthly variation in the diet of harbour seals in inshore waters along the southeast Shetland (UK) coastline. Marine Ecology Progress Series **167**:275-289.

Budge, S. M., S. J. Iverson, and H. N. Koopman. 2006. Studying trophic ecology in marine ecosystems using fatty acids: a primer on analysis and interpretation Marine Mammal Science **22**:759-801.

Burkholder, D. A., M. R. Heithaus, J. W. Fourqurean, A. Wirsing, and L. M. Dill. 2013. Patterns of top-down control in a seagrass ecosystem: could a roving apex predator induce a behaviour-mediated trophic cascade? Journal of Animal Ecology **82**:1192-1202.

Burns, J. J. 2002. Harbor seal and spotted seal (*Phoca vitulina* and *Phoca largha*) Pages 552–560 *in* W. F. Perrin, B. Wursig, and J. G. M. Thewissen, editors. Encyclopedia of Marine Mammals, Academic Press.

Carroll, S. S., L. Horstmann-Dehn, and B. L. Norcross. 2013. Diet history of ice seals using stable isotope ratios in claw growth bands. Canadian Journal of Zoology **91**:191-202.

Caut, S., S. Laran, E. Garcia-Hartmann, and K. Das. 2011. Stable isotopes of captive cetaceans (killer whales and bottlenose dolphins). The Journal of Experimental Biology **214**:538-545.

Cherel, Y., L. Kernaléguen, P. Richard, and C. Guinet. 2009. Whisker isotopic signature depicts migration patterns and multi-year intra- and inter-individual foraging strategies in fur seals. Biologie Letters **5**:830-832.

Christensen, V. 1995. Ecosystem maturity—towards quantification. Ecological Modelling **77**:3-32.

Cook, H. W., and C. R. McMaster. 2002. Fatty acid desaturation and chain elongation in eukaryotes. Elsevier: New York, NY, USA.

Costanza, R., B. G. Norton, and B. D. Haskell. 1992. Ecosystem health: new goals for environmental management. Island Press.

Daan, N., P. J. Bromley, J. R. G. Hislop, and N. A. Nielsen. 1990. Ecology of North Sea fish. Netherlands Journal of Sea Research **26**:343-386.

Das, K., G. Lepoint, Y. Leroy, and J. M. Bouquegneau. 2003. Marine mammals from the southern North Sea: feeding ecology data from δ^{13}C and δ^{15}N measurements. Marine Ecology Progress Series **263**:287-298.

DeNiro, M. J., and S. Epstein. 1978. Influence of diet on the distribution of carbon isotopes in animals. Geochimica et Cosmochimica Acta **42**:495-506.

Doney, S. C., M. Ruckelshaus, J. E. Duffy, J. P. Barry, F. Chan, C. A. English, H. M. Galindo, J. M. Grebmeier, A. B. Hollowed, N. Knowlton, J. Polovina, N. N. Rabalais, W. J. Sydeman, and L. D. Talley. 2012. Climate Change Impacts on Marine Ecosystems. Annual Review of Marine Science **4**:11-37.

Dulvy, N. K., R. P. Freckleton, and N. V. C. Polunin. 2004. Coral reef cascades and the indirect effects of predator removal by exploitation. Ecology Letters **7**:410-416.

Estes, J. A., and D. O. Duggins. 1995. Sea Otters and Kelp Forests in Alaska: Generality and Variation in a Community Ecological Paradigm. Ecological Monographs **65**:75-100.

Estes, J. A., and J. F. Palmisano. 1974. Sea Otters: Their Role in Structuring Nearshore Communities. Science **185**:1058-1060.

Fath, B. D. 2015. Quantifying economic and ecological sustainability. Ocean & Coastal Management **108**:13-19.

Fath, B. D., U. M. Scharler, R. E. Ulanowicz, and B. Hannon. 2007. Ecological network analysis: network construction. Ecological Modelling **208**:49-55.

Ferreira, E. O., L. L. Loseto, and S. H. Ferguson. 2011. Assessment of claw growth-layer groups from ringed seals (Pusa hispida) as biomonitors of inter- and intra-annual Hg, δ15N, and δ13C variation. Canadian Journal of Zoology **89**:774-784.

France, R. 1995. Carbon-13 enrichment in benthic compared to planktonic algae: foodweb implications. Marine Ecology Progress Series **124**:307-312.

Frank, K. T., B. Petrie, J. S. Choi, and W. C. Leggett. 2005. Trophic Cascades in a Formerly Cod-Dominated Ecosystem. Science **308**:1621-1623.

Frank, K. T., B. Petrie, and N. L. Shackell. 2007. The ups and downs of trophic control in continental shelf ecosystems. Trends in Ecology & Evolution **22**:236-242.

Friedlander, A. M., and E. E. DeMartini. 2002. Contrasts in density, size, and biomass of reef fishes between the northwestern and the main Hawaiian islands: the effects of fishing down apex predators. Marine Ecology Progress Series **230**:253-264.

Fry, B. 1988. Food web structure on Georges Bank from stable C, N and S isotopic compositions. Limnology and Oceanography **33**:9.

Galatius, A., S. Brasseur, R. Czeck, B. Diederichs, L. F. Jensen, P. Körber, U. Siebert, J. Teilmann, and S. Klöpper. 2014. Aerial surveys of Harbour Seals in the Wadden Sea in 2014 - The highest pup count recorded yet.

Gätje, C., and K. Reise. 1998. Ökosystem Wattenmeer: Austausch-, Transport-und Stoffumwandlungsprozesse. Springer, Berlin.

Gilles, A., H. Andreasen, S. Müller, and U. Siebert. 2008. Nahrungsökölögie von marinen Säugetieren und Seevögeln für das Management von NATURA 2000 Gebieten. Endbericht für das Bundesamt für Naturschutz., Federal Agency for Nature Conservation, Germany.

Hall-Aspland, S. A., T. L. Rogers, and R. B. Canfield. 2005. Stable carbon and nitrogen isotope analysis reveals seasonal variation in the diet of leopard seals. Marine Ecology Progress Series **305**:249-259.

Hall, A. J., J. Watkins, and P. S. Hammond. 1998. Seasonal variation in the diet of harbour seals in the south-western North Sea. Marine Ecology Progress Series **170**:269-281.

Harding, K. C., M. Fujiwara, Y. Axberg, and T. Härkönen. 2005. Mass-dependent energetics and survival in Harbour Seal pups. Functional Ecology **19**:129-135.

Härkönen, T. 1987. Seasonal and regional variations in the feeding habits of the harbour seal, *Phoca vitulina,* in the Skagerrak and the Kattegat. Journal of Zoology **213**:535-543.

Härkönen, T., and M. P. Heide-Jørgensen. 1991. The harbour seal *Phoca vitulina* as a predator in the Skagerrak. Ophelia **34**:191-207.

Heymans, J. J., R. E. Ulanowicz, and C. Bondavalli. 2002. Network analysis of the South Florida Everglades graminoid marshes and comparison with nearby cypress ecosystems. Ecological Modelling **149**:5-23.

Hirons, A. C., D. M. Schell, and D. J. St. Aubin. 2001. Growth rates of vibrissae of harbor seals (*Phoca vitulina*) and Steller sea lions (*Eumetopias jubatus*). Canadian Journal of Zoology **79**:1053-1061.

Hobson, K. A. 1995. Reconstructing Avian Diets Using Stable-Carbon and Nitrogen Isotope Analysis of Egg Components: Patterns of Isotopic Fractionation and Turnover. The Condor **97**:752-762.

Hobson, K. A. 1999. Tracing origins and migration of wildlife using stable isotopes: a review. Oecologia **120**:314-326.

Hobson, K. A., and R. G. Clark. 1992. Assessing Avian Diets Using Stable Isotopes I: Turnover of 13C in Tissues. The Condor **94**:181-188.

Hobson, K. A., J. F. Piatt, and J. Pitocchelli. 1994. Using Stable Isotopes to Determine Seabird Trophic Relationships. Journal of Animal Ecology **63**:786-798.

Hobson, K. A., D. M. Schell, D. Renouf, and E. Noseworthy. 1996. Stable carbon and nitrogen isotopic fractionation between diet and tissues of captive seals: implications for dietary reconstructions involving marine mammals. Canadian Journal of Fisheries and Aquatic Sciences **53**:528-533.

Hobson, K. A., J. L. Sease, R. L. Merrick, and J. F. Piatt. 1997. Investigating trophic relationships of pinnipeds in Alaska and Washington using stable isotope ratios of Nitrogen and Carbon. . Marine Mammal Science **13**:114-132.

Hobson, K. A., and H. E. Welch. 1992. Determination of trophic relationships within a high Arctic marine food web using δ^{13}C and δ^{15}N analysis. Marine Ecology Progress Series **84**:10.

Ings, T. C., J. M. Montoya, J. Bascompte, N. Blüthgen, L. Brown, C. F. Dormann, F. Edwards, D. Figueroa, U. Jacob, and J. I. Jones. 2009. Review: Ecological networks–beyond food webs. Journal of Animal Ecology **78**:253-269.

Iverson, S., K. Frost, and L. Lowry. 1997. Fatty acid signatures reveal fine scale structure of foraging distribution of harbor seals and their prey in Prince William Sound, Alaska. Marine Ecology Progress Series **151**:255-271.

Iverson, S. J., C. Field, W. Don Bowen, and W. Blanchard. 2004. Quantitative fatty acid signature analysis: a new method of estimating predator diets. Ecological Monographs **74**:211-235.

Iverson, S. J., K. J. Frost, and S. L. C. Lang. 2002. Fat content and fatty acid composition of forage fish and invertebrates in Prince William Sound, Alaska: factors contributing to among and within species variability. Marine Ecology Progress Series **241**:161-181.

Jensen, L. 2015. Monitoring of the seal population in the Wadden Sea in 2015 - Monitering af sælbestanden i Vadehavet 2015. Aarhus Universitet, Miljøministeriet, Fiskeri-og Søfartsmuseet Saltvandsakvariet Esbjerg.

Jørgensen, S. E., L. Xu, and R. Costanza. 2010. Handbook of ecological indicators for assessment of ecosystem health. CRC press.

Kaufman, A. G., and S. R. Borrett. 2010. Ecosystem network analysis indicators are generally robust to parameter uncertainty in a phosphorus model of Lake Sidney Lanier, USA. Ecological Modelling **221**:1230-1238.

Kirsch, P. E., S. J. Iverson, W. D. Bowen, S. R. Kerr, and R. G. Ackman. 1998. Dietary effects on the fatty acid signature of whole Atlantic cod (Gadus morhua). Canadian Journal of Fisheries and Aquatic Sciences **55**:1378-1386.

Lang, S. L. C., S. J. Iverson, and W. D. Bowen. 2005. Individual variation in milk composition over lactation in harbour seals (*Phoca vitulina*) and the potential consequences of intermittent attendance. Canadian Journal of Zoology **83**:1525-1531.

Leguerrier, D., D. Degré, and N. Niquil. 2007. Network analysis and inter-ecosystem comparison of two intertidal mudflat food webs (Brouage Mudflat and Aiguillon Cove, SW France). Estuarine, Coastal and Shelf Science **74**:403-418.

Lehnert, K., J. A. Raga, and U. Siebert. 2007. Parasites in harbour seals (*Phoca vitulina*) from the German Wadden Sea between two Phocine Distemper Virus epidemics. Helgoland Marine Research **61**:239-245.

Lesage, V., M. O. Hammill, and K. M. Kovacs. 2001. Marine mammals and the community structure of the Estuary and Gulf of St Lawrence, Canada: evidence from stable isotope analysis. Marine Ecology Progress Series **210**:203-221.

Levin, P. S., M. J. Fogarty, S. A. Murawski, and D. Fluharty. 2009. Integrated ecosystem assessments: developing the scientific basis for ecosystem-based management of the ocean. PLoS biology **7**:23.

Levin, S. A., and J. Lubchenco. 2008. Resilience, robustness, and marine ecosystem-based management. Bioscience **58**:27-32.

Longo, C., S. Hornborg, V. Bartolino, M. Tomczak, L. Ciannelli, S. Libralato, and A. Belgrano. 2015. Role of trophic models and indicators in current marine fisheries management. Marine Ecology Progress Series **538**:257.

Martens, P., and J. E. E. v. Beusekom. 2008. Zooplankton response to a warmer northern Wadden Sea. Helgoland Marine Research **62**:67-75.

Matthews, C. J. D., and S. H. Ferguson. 2015. Weaning age variation in beluga whales (Delphinapterus leucas). Journal of Mammalogy **96**:425-437.

McLeod, K. L., J. Lubchenco, S. Palumbi, and A. A. Rosenberg. 2005. Scientific Consensus Statement on Marine Ecosystem-Based Management. Signed by 221 academic scientists and policy experts with relevant expertise and published by the Communication Partnership for Science and the Sea at http://www.compassonline.org/science/EBM_CMSP/EBMconsensus.

Mees, J., and P. J. H. Reijnders. 1994. The harbour seal, *Phoca vitulina,* in the Oosterschelde: decline and possibilities for recovery. Hydrobiologia **282-283**:547-555.

Meltofte, H., J. Blew, J. Frikke, Rösner Hans-Ulrich, and C. J. Smit. 1994. Numbers and distribution of waterbirds in the Wadden Sea. Results and evaluation of 36 simultaneous counts in the Dutch-German-Danish Wadden Sea 1980-1991. IWRB publication 34. Water Study Group Bulletin 74, Special issue. Common Secretariat for the Cooperation on the Protection of the Wadden Sea.

Monaco, M. E., and R. E. Ulanowicz. 1986. Comparative ecosystem trophic structure of. Marine Ecology Progress Series **1**:9.

Muelbert, M. M. C., and W. D. Bowen. 1993. Duration of lactation and postweaning changes in mass and body composition of harbour seal, *Phoca vitulina*, pups. Canadian Journal of Zoology **71**:1405-1414.

Muelbert, M. M. C., W. D. Bowen, and S. J. Iverson. 2003. Weaning Mass Affects Changes in Body Composition and Food Intake in Harbour Seal Pups during the First Month of Independence. Physiological and Biochemical Zoology **76**:418-427.

Newland, C., I. C. Field, Y. Cherel, C. Guinet, C. J. A. Bradshaw, C. R. McMahon, and M. A. Hindell. 2011. Diet of juvenile southern elephant seals reappraised by stable isotopes in whiskers. Marine Ecology Progress Series **424**:247-258.

Newsome, S. D., M. T. Tinker, D. H. Monson, O. T. Oftedal, K. Ralls, M. M. Staedler, M. L. Fogel, and J. A. Estes. 2009. Using stable isotopes to investigate individual diet specialization in California sea otters (*Enhydra lutris nereis*). Ecology **90**:961-974.

Nordstrom, C., L. Wilson, S. Iverson, and D. Tollit. 2008. Evaluating quantitative fatty acid signature analysis (QFASA) using harbour seals *Phoca vitulina richardsi* in captive feeding studies. Marine Ecology Progress Series **360**:245-263.

Odum, E. P. 1969. The strategy of ecosystem development. Sustainability: Sustainability **164**:58.

Oftedal, O. T., D. I. Boness, and R. A. Tedman. 1987. The Behavior, Physiology, and Anatomy of Lactation in the Pinnipedia. Pages 175-245 *in* H. Genoways, editor. Current Mammalogy. Springer US.

Osinga, N., I. Pen, H. A. Udo de Haes, and P. M. Brakefield. 2012. Evidence for a progressively earlier pupping season of the common seal (*Phoca vitulina*) in the Wadden Sea. Journal of the Marine Biological Association of the United Kingdom **92**:1663-1668.

Peterson, B. J., and B. Fry. 1987. Stable Isotopes in Ecosystem Studies. Annual Review of Ecology and Systematics **18**:293-320.

Pierce, G. J., P. M. Thompson, A. Miller, J. S. W. Diack, D. Miller, and P. R. Boyle. 1991. Seasonal variation in the diet of common seals (*Phoca vitulina*) in the Moray Firth area of Scotland. Journal of Zoology **223**:641-652.

Pikitch, E., C. Santora, E. Babcock, A. Bakun, R. Bonfil, D. Conover, P. Dayton, et al., P. Doukakis, D. Fluharty, and B. Heneman. 2004. Ecosystem-based fishery management. Science(Washington) **305**:346-347.

Polte, P., and H. Asmus. 2006. Intertidal seagrass beds (*Zostera noltii*) as spawning grounds for transient fishes in the Wadden Sea. Marine Ecology-Progress Series **312**:235-243.

Power, G., and J. Gregoire. 1978. Predation by Freshwater Seals on the Fish Community of Lower Seal Lake, Quebec. Journal of the Fisheries Research Board of Canada **35**:844-850.

Rau, G. H., A. J. Mearns, D. R. Young, R. J. Olson, H. A. Schafer, and I. R. Kaplan. 1983. Animal C/C Correlates with Trophic Level in Pelagic Food Webs. Ecology **64**:1314-1318.

Reijnders, P. J. H. 1976. The harbour seal (*phoca vitulina*) population in the Dutch Wadden Sea: Size and composition. Netherlands Journal of Sea Research **10**:223-235.

Reijnders, P. J. H., K. F. Abt, S. M. J. M. Brasseur, K. C. J. Camphuysen, B. Reineking, M. Scheidat, U. Siebert, M. Stede, J. Tougaard, and S. Tougaard. 2005. Marine Mammals. Wadden Sea Ecosystem N°19. pp. 305-318. Wadden Sea Secretariat, Wilhelmshaven, Germany.

Reijnders, P. J. H., S. M. J. M. Brasseur, T. Borchardt, K. Camphuysen, R. Czeck, A. Gilles, L. F. Jensen, M Leopold, K. Lucke, S. Ramdohr, M. Scheidat, U. Siebert, and J. Teilmann. 2009. Marine mammals. Thematic Report N°20. In: Marencic, H. and Vlas, J. de, editors. Quality status report 2009 Wadden Sea Ecosystem N°25. Wadden Sea Secretariat, Trilateral Monitoring and Assessment Group, Wilhelmshaven, Germany.

Reijnders, P. J. H., S. M. J. M. Brasseur, and E. H. W. G. Meesters. 2010. Earlier pupping in harbour seals, *Phoca vitulina.* Biologie Letters.

Reijnders, P. J. H., and K. Lankester. 1990. Status of marine mammals in the North sea. Netherlands Journal of Sea Research **26**:427-435.

Rijks, J. M., J. I. Hoffman, T. Kuiken, A. D. M. E. Osterhaus, and W. Amos. 2008. Heterozygosity and lungworm burden in harbour seals (*Phoca vitulina*). Heredity **100**:587-593.

Samhouri, J., P. Levin, and C. Harvey. 2009. Quantitative Evaluation of Marine Ecosystem Indicator Performance Using Food Web Models. Ecosystems **12**:1283-1298.

Scheiffarth, G., and G. Nehls. 1997. Consumption of benthic fauna by carnivorous birds in the Wadden Sea. Helgoländer Meeresuntersuchungen **51**:373-387.

Siebert, U., A. Gilles, K. Lucke, M. Ludwig, H. Benke, K.-H. Kock, and M. Scheidat. 2006. A decade of harbour porpoise occurrence in German waters—Analyses of aerial surveys, incidental sightings and strandings. Journal of Sea Research **56**:65-80.

Siebert, U., P. Wohlsein, K. Lehnert, and W. Baumgärtner. 2007. Pathological Findings in Harbour Seals (*Phoca vitulina*): 1996–2005. Journal of Comparative Pathology **137**:47-58.

Smardon, R. C. 2009. The Wadden Sea Wetlands: A multi-juridictional Challenge. Pages 21-54 Sustaining the World´s Wetlands - Setting Policy and Resolving Conflicts. springer science and Business Media, College of Environmental Science and Foresty, State University of New York.

Thompson, P. M., B. J. McConnell, D. J. Tollit, A. Mackay, C. Hunter, and P. A. Racey. 1996. Comparative Distribution, Movements and Diet of Harbour and Grey Seals from Moray Firth, N. E. Scotland. Journal of Applied Ecology **33**:1572-1584.

Thompson, P. M., G. J. Pierce, J. R. G. Hislop, D. Miller, and J. S. W. Diack. 1991. Winter Foraging by Common Seals (*Phoca vitulina*) in Relation to Food Availability in the Inner Moray Firth, N.E. Scotland. Journal of Animal Ecology **60**:283-294.

Tieszen, L. L., T. W. Boutton, K. G. Tesdahl, and N. A. Slade. 1983. Fractionation and turnover of stable carbon isotopes in animal tissues: Implications for $\delta^{13}C$ analysis of diet. Oecologia **57**:32-37.

Tollit, D. J., P. M. Thompson, and S. P. R. Greenstreet. 1997. Prey selection by harbour seals, Phoca vitulina, in relation to variations in prey abundance. Canadian Journal of Zoology **75**:1508-1518.

Tougaard, J., I. Ebbesen, S. Tougaard, J. T., and J. Teilmann. 2003. Satellite tracking of harbour seals on Horns Reef. Technical report to Techwise A/S, Biological Papers from the Fisheries and Maritime Museum, Esbjerg.

Tulp, I., L. J. Bolle, and A. D. Rijnsdorp. 2008. Signals from the shallows: In search of common patterns in long-term trends in Dutch estuarine and coastal fish. Journal of Sea Research **60**:54-73.

Ulanowicz, R. E. 2004. Quantitative methods for ecological network analysis. Computational Biology and Chemistry **28**:321-339.

Ulanowicz, R. E., and L. G. Abarca-Arenas. 1997. An informational synthesis of ecosystem structure and function. Ecological Modelling **95**:1-10.

Vander Zanden, M. J., M. K. Clayton, E. K. Moody, C. T. Solomon, and B. C. Weidel. 2015. Stable Isotope Turnover and Half-Life in Animal Tissues: A Literature Synthesis. PLoS ONE **10**:e0116182.

Zhao, L., and D. M. Schell. 2004. Stable isotope ratios in harbor seal *Phoca vitulina* vibrissae: effects of growth patterns on ecological records. Marine Ecology Progress Series **281**:267-273.

PART I

Harbor seal diet

"Tell me what you eat, and I will tell you what you are"

Brillat-Savarin

"At Christmas, I no more desire a rose

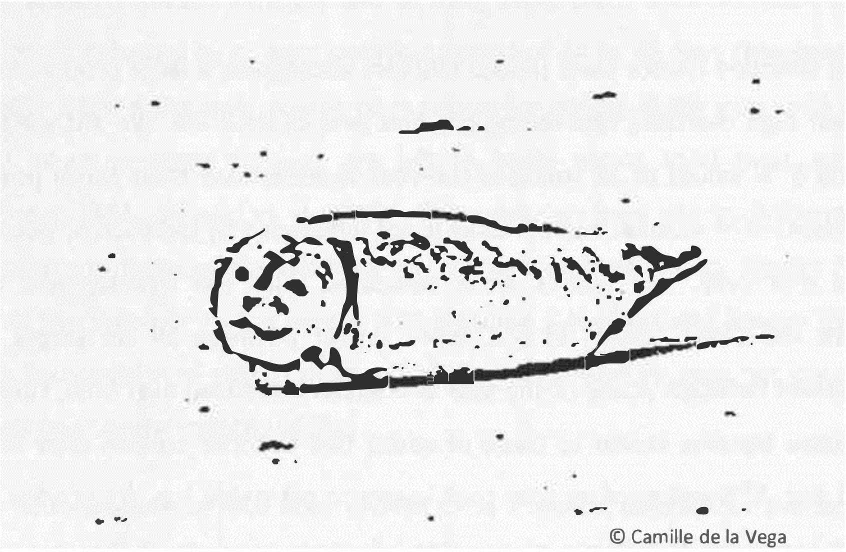
Than wish a snow in May´s newfangled mirth;

But like each thing in season grows"

Shakespeare

Chapter 1

Stable isotope composition and parasitic infections of harbor seal young-of-the-year used as prey-based diet indicators



© Camille de la Vega

Stable isotope composition and parasitic infections of harbor seal young-of-the-year used as prey-based diet indicators

de la Vega Camille[1]*, Lebreton Benoit[2]ɫ, Lehnert Kristina[3]ɫ, Asmus Ragnhild[1], Siebert Ursula[3]
Asmus Harald [1]

Submitted to Marine Mammal Science

[1] Alfred-Wegener-Institut Helmholtz-Zentrum für Polar- und Meeresforschung, Wattenmeerstation Sylt Hafenstr. 43, D-25992 List/ Sylt, Germany
[2] UMR Littoral, Environnement et Sociétés, CNRS - Université de La Rochelle, Institut du Littoral et de l'environnement, 2 rue Olympe de Gouges, 17000 La Rochelle, France
[3] Institut für Terrestrische und Aquatische Wildtierforschung, Stiftung Tierärztliche Hochschule Hannover, Werftstr. 6, 25761 Büsum, Germany
ɫ These authors contributed equally

*Corresponding author: camille.de.la.vega@awi.de

Abstract

Samplings based on stranded harbor seals (*Phoca vitulina*) encompass a large proportion of young-of-the-year, due to their high mortality rate during the first year of their life. We analyzed the temporal variation of $\delta^{13}C$ and $\delta^{15}N$ values of 28 young-of-the-year to determine from which point in time the stable isotope composition of muscles and vibrissae is not influenced by lactation or post-weaning fast, but by prey-based diet only. The results were compared with the development of trophically transmitted parasitic infections. Values of $\delta^{13}C$ were similar between all life stages of seals. The difference of $\delta^{15}N$ values between young-of-the-year and adults decreased over time. Young-of-the-year $\delta^{15}N$ values of vibrissae became similar to those of adults two to three months after birth, therefore reflecting a foraged diet. $\delta^{15}N$ values of muscles took longer to get stable (i.e., four to five months). This time coincided with increased prevalence of parasitic infections in young-of-the-year, indicating that their main food source became fish.

Key words

Phoca vitulina, $\delta^{13}C$ and $\delta^{15}N$, trophically transmitted parasites, lactation, post weaning fast, prey-based diet, Wadden Sea, young-of-the-year

1. Introduction

Harbor seals (*Phoca vitulina*) are top predators subsisting largely on fish (Mees and Reijnders 1994, Brown and Pierce 1998, Hall et al. 1998). They have a strong role in the functioning of coastal food webs (Bowen 1997) and are important indicators of ecosystem health (Reddy et al. 2001, Bossart 2011). In the Wadden Sea, harbor seals are one of the most abundant species of marine mammals (Reijnders et al. 2009). A major issue in studies about trophic ecology of marine mammals is the collection of samples which is mostly opportunistic. In the Wadden Sea, seal hunting was banned in 1976 (Reijnders et al. 1997) and seals are protected under several national and international Conventions, Agreements and Directives (Bonn Convention 1983, Bern Convention 1985, Agreement on the Conservation of Seals in the Wadden Sea 1990, EU Habitats Directive 1992). Invasive sampling of seals is therefore highly regulated and mostly relies on stranded dead animals (Siebert et al. 2006, Lehnert et al. 2007, Siebert et al. 2007, Rijks et al. 2008). Because of the high mortality rate (from 10 to 65%) of seals during their first year of life (Reijnders 1976, Harding et al. 2005), these samplings are unbalanced as they encompass a large proportion of young-of-the-year (*i.e.*, animals less than one year old) whose diet may still be influenced by lactation or weaning fast.

Harbor seals reproduce annually and birthing takes place between early May and early June (Osinga et al. 2012) followed by a short lactation period of 24 to 32 days (Muelbert and Bowen 1993, Lang et al. 2005). Milk is the only source of nutrition for young-of-the-year until an abrupt weaning (Bowen 1991) when young-of-the-year are left to begin eating solid food without any parental assistance (Bonner 1984, Oftedal et al. 1987). The newly weaned young-of-the-year undergo a post weaning fast of two to three weeks and they rely on their blubber energy storage for any daily energy requirements in the first four to five weeks post weaning (Muelbert and Bowen 1993, Muelbert et al. 2003). Isotopic composition of tissues of young-of-the-year therefore does not document a prey based diet during their first several months of life.

Stable isotope analyses have been proven to be a reliable method for the determination of food resources used by marine mammals (Hobson et al. 1997, Lesage et al. 2001, Das et al. 2003, Caut et al. 2011). The isotopic composition of consumer tissues reflects their assimilated diet (DeNiro and Epstein 1978, Peterson and Fry 1987), based on the fact that stable isotopes of carbon give clues about the origin of food resources and that stable isotopes of nitrogen allow determining the trophic level of the consumers, due to a relatively high step-wise enrichment (*i.e.*, trophic fractionation factor) between each trophic level (Rau et al. 1983, Fry 1988). Several studies carried out on diverse marine and terrestrial mammal species showed that the tissues of nursing young-of-the-year are more enriched in ^{15}N than those of their mother, due to their reliance on milk (Bocherens et al. 1995, Hobson and Sease 1998, Jenkins et al. 2001, Polischuk et al. 2001, Newsome et al. 2006). These same studies showed lower δ^{13}C values or no difference of isotopic composition between the tissues of nursing young-of-the-year and those of their mothers. Regarding predator-prey interaction studies, there is a real need to determine when isotopic composition of the young-of-the-year reflects only the isotopic composition of their prey; in other words, to determine until when animals are under the influence of lactation or post weaning fast to not take these individuals into account in community diet studies.

Depending on the tissue, isotopic composition reflects the diet of a consumer integrated over a few days (*e.g.*, blood, plasma, and liver) or a few months (*e.g.*, muscle) in relation to the metabolic turnover of this tissue (Hobson 1995, Vander Zanden et al. 2015). The turnover time of muscle tissues is poorly known for marine mammals. Studies on birds (*Coturnix japonica* and *Corvus brachyrhynchos*) (Hobson and Clark 1992) and small mammals (*Meriones unguiculatus*) (Tieszen et al. 1983) showed that turnover of muscles has an order of magnitude of a month. Vander Zanden et al. (2015) estimated the isotopic half-life of muscle tissue for a mammal with a body mass of 90 kg to be about two to three months. On the contrary, mineralized and keratinous tissues, such as vibrissae, teeth and claws, have the great advantage to preserve a time line of stable isotope deposition during their growth period and therefore allow retrospective diet studies (Hobson 1995, Ferreira et al. 2011, Carroll et al. 2013, Matthews and Ferguson 2015). For example, isotopic composition is archived on a daily basis along a growing vibrissa and remains stable over time (Hirons et al. 2001, Zhao and Schell 2004). Vibrissae are thus good recorders of dietary history, giving precise (few days) and long-term (up to one year) information about the food sources used by consumers (Hall-Aspland et al. 2005, Newsome et al. 2009, Newland et al. 2011).

In parallel to isotopic compositions, marine mammals can be used as bio-indicators for their ecology (Aznar et al. 1994, Marcogliese 2005) and give insights about their foraging activities (Balbuena and Raga 1994, Lafferty et al. 2008). Indeed, parasitic infections are often transmitted trophically (Abollo et al. 1998, Mattiucci and Nascetti 2007). Harbor seals, as ultimate hosts of trophically transmitted endoparasites, are infected from consumption of parasitized invertebrate and fish species (Dailey 1970, Hauksson and Ólafsdóttir 1995, Lehnert et al. 2010). The first exposure of young-of-the-year to trophically transmitted parasite species can then be associated with the shift from post weaning fast to prey-based diet (Lynch et al. 2011).

In this paper, the temporal variation of isotopic compositions of two tissues (*i.e.*, muscle, vibrissae) from young-of-the-year was determined to estimate when the composition of these tissues is not influenced anymore by lactation and post weaning fast. The development of infections from trophically transmitted macro-parasites in the gastro-intestinal and respiratory tracts after weaning and their prevalence over time were studied and used as indicators of the foraging behavior of young-of-the-year, to corroborate the results from the stable isotope analyses. The combination of two tools used for trophic studies (*i.e.*, stable isotopes, parasitic infection) were therefore used to determine when isotopic compositions of muscles and vibrissae reflect the foraging of young-of-the-year in their environment in order to use these individuals in community diet studies.

2. Material and methods

2.1. Sampling of harbor seals

This study was conducted on the Sylt-Rømø Bight (54°52' - 55°10' N, 8°20' - 8°40' E), located in the northern Wadden Sea between the islands of Sylt (Schleswig Holstein, Germany) and Rømø (Denmark). Thirty three harbor seals (twenty eight young-of-the-year and five adults; Table 1, p. 38) were collected along the shore of the Island of Sylt from July 2012 to December 2013 as part of a stranding network established on the German coasts of Schleswig-Holstein (Siebert et al. 2006). Detailed information on the stranding network is provided by Benke et al. (1998). The sample size (n=33) represents ≈10% of the harbor seal population living in the Sylt-Rømø Bight in summer (*i.e.*, ≈400 seals on average) (Jensen 2015). All seals were stranded dead or were killed because of serious illness by authorized seal hunters affiliated to the authorities of Schleswig-Holstein Wadden Sea National Park.

Carcasses were stored in plastic bags at -20°C until necropsies, which were conducted according to the protocol described by Siebert et al. (2007) at the Institute for Terrestrial and Aquatic Wildlife Research of the University of Veterinary Medicine, Hannover Foundation. Individuals were sorted in two age groups according to their standard length (MacLaren 1993): individuals less than 13 months old (*i.e.*, young-of-the-year) and individuals older than 13 months (*i.e.*, adults; Table 1, p. 38). The estimated age of the young-of-the-year (in months) was determined as the number of months between the main birth period (May to June) (Osinga et al. 2012) and the day of collection (Table 1).

Table 1: Date of collection, weight, length, age class and age category of the seals. Young-of-the-year refer to individuals from <1 to 12 months old; adults refer to individuals older than 13 months

Seal #	Collection date	Weight (kg)	Length (cm)	Age class (month)	Age category
1	24-Jun-12	7.6	83	1-2	young-of-the-year
2	30-Jun-12	9.0	87	1-2	young-of-the-year
3	13-Jul-13	9.2	93	2-3	young-of-the-year
4	16-Jul-12	9.4	85	2-3	young-of-the-year
5	20-Jul-12	10.2	90	2-3	young-of-the-year
6	21-Jul-12	11.2	100	2-3	young-of-the-year
7	22-Jul-12	11.4	95	2-3	young-of-the-year
8	19-Aug-12	13.0	97	3-4	young-of-the-year
9	1-Sep-12	12.0	90	4-5	young-of-the-year
10	8-Sep-12	11.6	90	4-5	young-of-the-year
11	10-Sep-12	12.4	101	4-5	young-of-the-year
12	19-Sep-12	11.2	101	4-5	young-of-the-year
13	30-Sep-12	13.0	97	4-5	young-of-the-year
14	30-Sep-12	17.4	100	4-5	young-of-the-year
15	7-Oct-12	12.2	103	5-6	young-of-the-year
16	10-Oct-12	16.4	104	5-6	young-of-the-year
17	13-Oct-12	10.6	86	5-6	young-of-the-year
18	19-Oct-12	15.2	100	5-6	young-of-the-year
19	13-Nov-13	13.0	100	6-7	young-of-the-year
20	6-Dec-12	18.2	109	7-8	young-of-the-year
21	9-Dec-12	20.2	109	7-8	young-of-the-year
22	9-Dec-12	17.6	109	7-8	young-of-the-year
23	10-Dec-12	16.4	112	7-8	young-of-the-year
24	31-Dec-12	15.2	93	7-8	young-of-the-year
25	31-Dec-12	18.0	104	7-8	young-of-the-year
26	31-Dec-12	18.4	98	7-8	young-of-the-year
27	24-Mar-13	19.4	114	10-11	young-of-the-year
28	29-Mar-13	26.6	109	10-11	young-of-the-year
29	29-Jul-12	20.0	125	> 13	adult
30	8-Sep-12	31.4	144	> 13	adult
31	21-Sep-12	17.6	107	> 13	adult
32	24-Apr-13	93.0	169	> 13	adult
33	07-Dec-13	75.8	166	> 13	adult

2.2. Samples collection and preparation for stable isotope analyses

The longest vibrissa of each individual (n=33) was collected in order to cover the longest period of growth. Vibrissae were cleaned using soap in an ultrasonic bath for ten minutes and then rinsed four times in distilled water. Vibrissae were measured, dried and sliced with a sharp cutter in 1 to 2 mm consecutive sections (ranging in mass from 0.8 to 1.5 mg) starting from the proximal end (Cherel et al. 2009). This represented a trade-off between the number of sections (and hence the temporal resolution attainable for the isotopic time series) and the size of the sample (Newland et al. 2011). The number of samples analyzed per vibrissa ranged from 18 to 42 depending on its length.

Muscle tissue was collected on the lower flank of seals (Todd et al. 2010) and samples were kept at -20°C until preparation for analysis. Muscle samples were freeze-dried and ground individually into a fine powder using a ball mill. Lipids were removed from muscle samples using 5 repeated rinses with 2:1 chloroform:methanol to avoid the bias due to the depletion in ^{13}C in lipids relatively to the diet (Tieszen et al. 1983). Samples were then dried at 35°C and ground again. $\delta^{15}N$ analyses were carried out on raw samples in order to avoid any potential bias due to delipidation.

2.3. Stable isotope analyses

Each piece of vibrissae and homogenized powdered samples was precisely weighed (± 1 µg) and was sealed in a tin capsule for stable isotope analyses. Samples were processed on an elemental analyzer (Flash EA 1112, Thermo Scientific, Milan, Italy) coupled to an isotope ratio mass spectrometer (Delta V Advantage with a Conflo IV interface, Thermo Scientific, Bremen, Germany) at the LIENSs stable isotope facility of the University of La Rochelle, France. Results are expressed in the δ notation as deviation from international standards of known composition (Vienna Pee Dee Belemnite and N_2 in air for $\delta^{13}C$ and $\delta^{15}N$ values, respectively) following the formula: $\delta^{13}C$, $\delta^{15}N = [(R_{sample}/R_{standard}) - 1] \times 10^3$, where R is $^{15}N/^{14}N$, $^{13}C/^{12}C$, respectively. Calibration was performed using certified reference materials (USGS-24, IAEA-CH6, -600 for carbon; IAEA-N2, -NO-3, -600 for nitrogen). Analytical precision based on repeated analyses of acetanilide (Thermo Scientific) used as an internal standard was <0.15‰ for carbon and nitrogen.

2.4. Parasite sampling

Macroscopic parasitic infections were determined semi-quantitatively for each seal during necropsy and histopathological examinations. The level of parasitic infection was ranked as following: none = no parasites, mild = mild infection, moderate = moderate infection and severe = severe infection (Siebert et al. 2001, Lehnert et al. 2014). Moderate and severe infections were combined for analysis in this study in order to allow more robust statistic and emphasize global trend. Because parasitic infections in harbor seals most often occur in the respiratory (lungs and bronchi) and digestive tracts (stomach and intestine) (Lehnert et al. 2007), and the species infecting these organs are assumed to be transmitted trophically (Ólafsdóttir and Hauksson 1998, Anderson 2000, Lehnert et al. 2010), their prevalence and level of infection was chosen to be compared to the stable isotope results. Parasite species usually encountered in the investigated organs of harbor seals from the German Wadden Sea are described in Table 2.

Table 2: Parasites species commonly found in the respiratory (bronchi and lung) and gastrointestinal (stomach and intestine) tracts of harbor seals, modified from Lehnert et al. (2007)

		Phylum	Family	Species
Respiratory tract	**Bronchi, pulmonary blood vessels**	Nematoda	Crenosomatidae Filaroididae	*Otostrongylus circumlitus* *Parafilaroides gymnurus*
	Lung	Nematoda	Filaroididae	*Parafilaroides gymnurus*
Gastrointestinal tract	**Stomach**	Nematoda	Anisakidae	*Pseudoterranova decipiens* *Contracaecum osculatum*
	Intestine	Acanthocephala	Polymorphidae	*Corynosoma spp.*

2.5. Data and statistical analyses

2.5.1. Vibrissae

Growth rates used for reconstruction of the temporal variation in isotopic composition of vibrissae were 0.78 mm.d^{-1} from May to September, and 0.075 mm.d^{-1} from October to April (Zhao and Schell 2004). Most of the harbor seals were still alive when beaching and all the carcasses were in good state of preservation, so the day of collection on the beach was considered to be the last day of vibrissae growth. Stable isotope data along the vibrissae were averaged per month (see Table 3 for sample sizes).

The 95% confidence intervals of $\delta^{13}C$ and of $\delta^{15}N$ values were computed on a monthly basis for the young-of-the-year and the adults. The differences of $\delta^{13}C$ and $\delta^{15}N$ values between young-of-the-year and adults are expressed as $\Delta\delta^{13}C$ and $\Delta\delta^{15}N$, respectively. Due to the small sample-size of the adults (Table 3), non-parametric procedures were used to achieve more robust statistics. Wilcoxon tests were applied to compare vibrissae isotopic compositions of young-of-the-year and adults for each month.

Table 3: Number of vibrissae used per month for stable isotope analyses

Month	May	June	July	August	September	October	November	December
Adults	2	3	4	3	3	1	1	2
ng-of-the-year	12	12	14	15	15	9	6	5

2.5.2. Muscle

Non-parametric procedures were used to achieve more robust statistics due to the small sample sizes (sample sizes ≤ 10 per month). Kruskal–Wallis tests were applied on isotopic data of young-of-the-year to test for monthly variations. These tests were followed by multiple pairwise comparisons using the Wilcoxon rank sum test. Only months with more than three individuals (*i.e.*, July, September, October and December) were used for statistics.

2.5.3. Prevalence and level of parasitic infection

The prevalence (*i.e.*, percentage of seals infected by at least one parasite) was computed (Bush et al. 1997). For statistical analyses, lungs and bronchi were combined as respiratory tract, and stomach and intestine were combined as gastrointestinal tract. The distributions of levels of infection (*i.e.*, none, mild and moderate-severe) were compared using Fisher-Snedecor tests. These tests were followed by multiple pairwise comparisons using the Fisher rank test applying the Bonferroni correction.

3. Results

3.1. Stable isotope composition of vibrissae

The differences of $\delta^{15}N$ values between young-of-the-year and adults (*i.e.,* $\Delta\delta^{15}N$) decreased from 3.8‰ in May to 0.2‰ in August, and then these differences remained very low. The $\delta^{15}N$ values were higher in young-of-the-year than in adults in May ($\Delta\delta^{15}N$= 3.8‰; Wilcoxon test, *p*-value: 0.009), in June ($\Delta\delta^{15}N$: 1.7‰; *p*-value: 0.048) and in July ($\Delta\delta^{15}N$: 1.0‰; *p*-value: 0.061; Fig. 1A). Young-of-the-year and adults had similar $\delta^{15}N$ values from August to December (*p*-values: from 0.330 to 0.570). The $\delta^{13}C$ values were higher in young-of-the-year than in adults in May ($\Delta\delta^{13}C$: 1.7‰; Wilcoxon test, *p*-value: 0.009; Fig. 1B) and were similar between young-of-the-year and adults from June to December (all Wilcoxon tests: *p*-values: > 0.500; Fig. 1B).

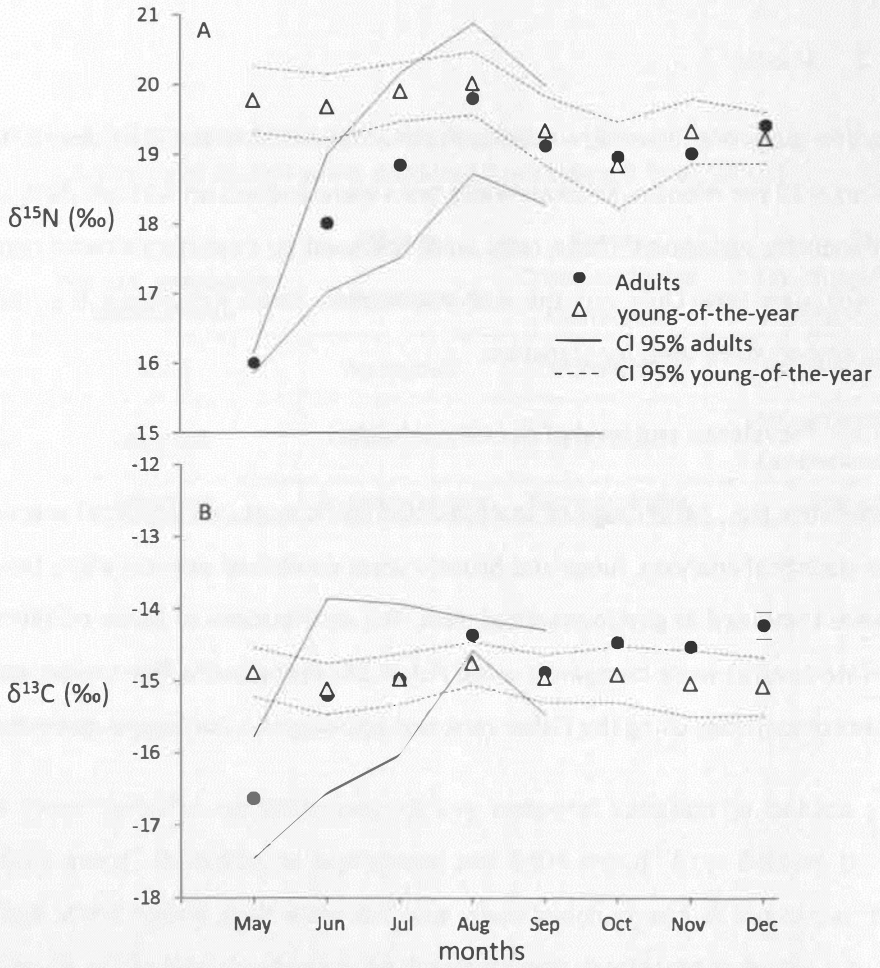


Fig. 1: $\delta^{15}N$ (A) and $\delta^{13}C$ (B) values (mean and 95% confidence interval: 95% CI) of vibrissae from harbor seals (young-of-the-year and adults) per month

3.2. Stable isotope composition of muscles

The $\delta^{15}N$ values of young-of-the-year decreased with the age of the individuals (Kruskal Wallis test, *p*-value = 0.006). The $\delta^{15}N$ values were the highest in June, just after birth (20.3 ± 0.5‰), decreased regularly from July (20.2 ± 0.5‰) to September (18.5 ± 1.3‰; Fig. 2A), and then remained stable after September. Muscles of young-of-the-year had similar $\delta^{15}N$ values in individuals collected from September, October and December (Wilcoxon rank sum tests, *p-values*: from 0.490 to 0.560). Muscle of young-of-the-year had higher $\delta^{15}N$ values in individuals collected in July than in those collected from October to December (Wilcoxon rank sum tests, *p-values:* from 0.034 to 0.078). The $\delta^{13}C$ values of young-of-the-year slightly decreased from June to September (Fig. 2B), although this trend was not significant (Wilcoxon rank sum tests, all *p-values* > 0.130).

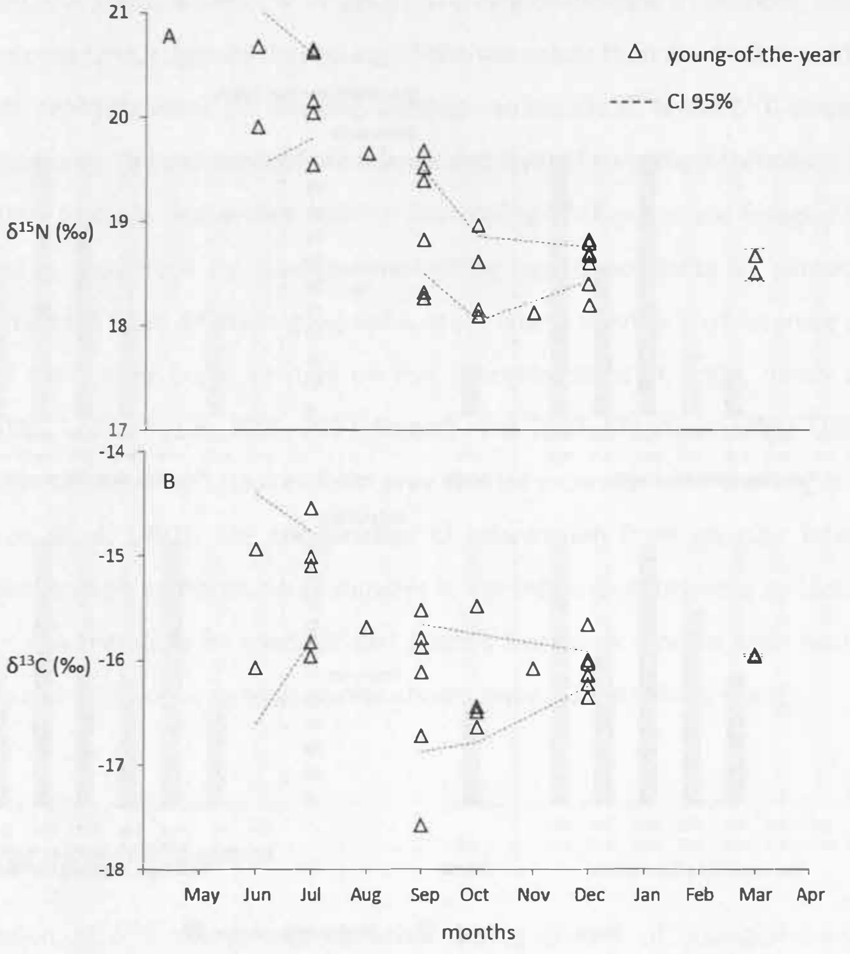


Fig. 2: $\delta^{15}N$ (A) and $\delta^{13}C$ (B) values (individual values and 95% confidence interval: 95% CI) of muscles from harbor seal young-of-the-year per month

3.3. Prevalence and level of parasitic infection

Young-of-the-year from June were not parasitized in the gastrointestinal tract (Fig. 3). We observed low parasite prevalence in July and August (Fig. 3). The prevalence of parasitic infections slightly increased after September although this trend was not significant (Fisher-Snedecor test, *p*-value 0.67). From October to March, 50 to 60% of young-of-the-year were infected in the gastrointestina tract. About 60% of the adults were infected.

Young-of-the-year sampled in June, July and August were not parasitized in the respiratory tracts (Fig. 3). The prevalence and level of parasitic infections in the respiratory tract started to increase significantly in September (Fisher-Snedecor test, *p*-value: 0.008; Fig. 3). Indeed the prevalence of moderate-severe parasitic infections in the respiratory tract was higher in young-of-the-year from September to March compared to those from June to August (Pairwise Fisher tests, p-value=0.006).

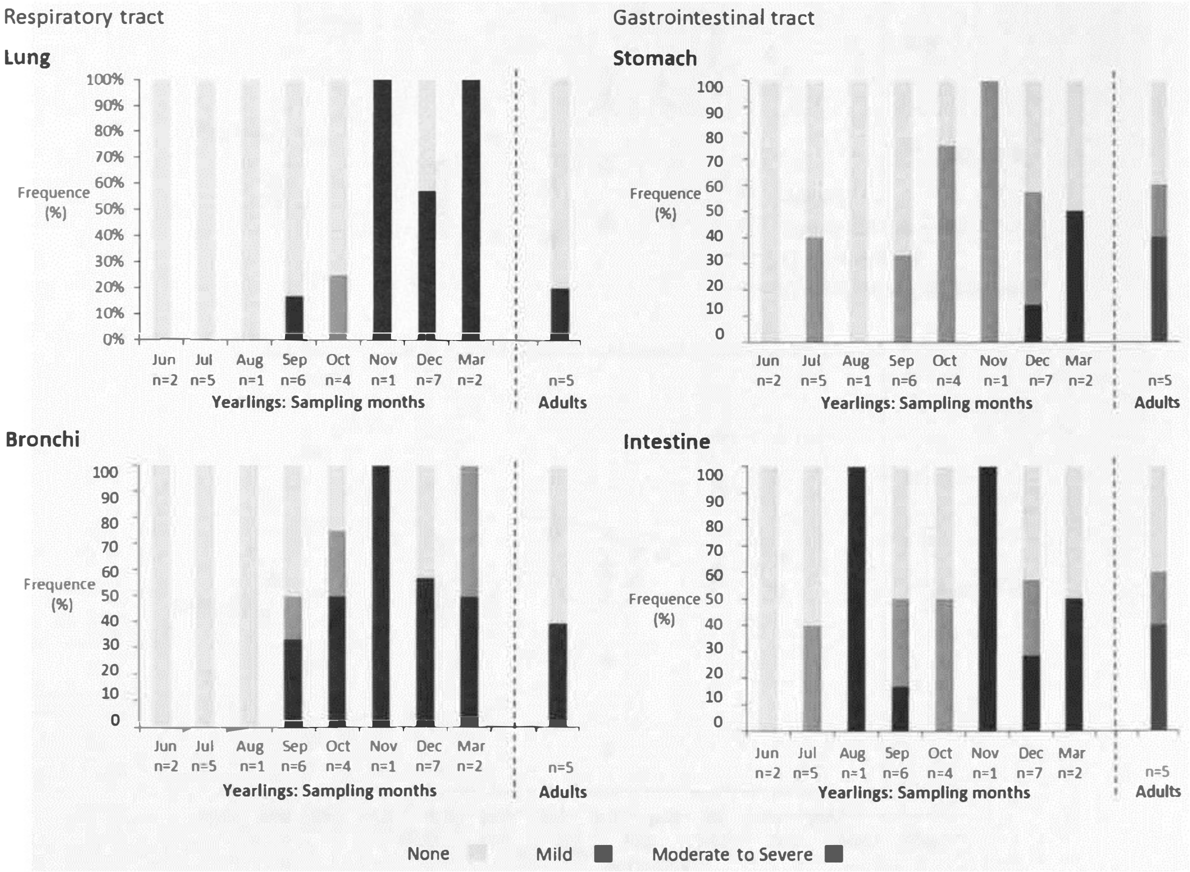


Fig. 3: Prevalence of the different levels of macro-parasitic infection (none, mild, moderate to severe) in the gastrointestinal and respiratory tracts of harbor seals. n indicates sample size

4. Discussion

4.1. When do tissues of young-of-the-year reflect a prey-based diet?

In vibrissae, isotopic compositions of young-of-the-year were similar to those of adults three to four months after birthing (*i.e.*, August), demonstrating that young-of-the-year and adults relied on similar food sources at this time, and that the isotopic composition of the vibrissae of young-of-the-year was then only depicting a diet based on foraging (Jenkins et al. 2001). The beginning of parasitic infection in July in the gastrointestinal tract of young-of-the-year confirms that these individuals start to forage two to three months after birth. Consequently, both δ^{15}N and δ^{13}C values of vibrissae can be used for community diet studies three to four months after birth. Due to a possible remaining influence of lactation, vibrissae from younger individuals should not be used in studies about community trophodynamics.

In muscles, the stabilization of δ^{15}N values in young-of-the-year in October, combined with the stable δ^{13}C values over time, suggests that young-of-the-year older than five to six months (*i.e.*, October) have a stable diet, probably based on foraging, although no muscle δ^{15}N and δ^{13}C values of adults were available for comparison. The increases of prevalence and level of parasitic infections in both respiratory and gastrointestinal tracts in September confirm that young-of-the-year are foraging (Muelbert et al. 2003). This trend is supported by studies investigating age dependency in parasitic infections of pinnipeds and cetaceans from different geographic areas where animals post weaning are described to become infected when they begin to prey on fish (Borgsteede et al. 1991, Smith and Read 1992, Bergeron et al. 1997, Lehnert et al. 2005, 2007, Siebert et al. 2007, Measures 2008). This also underlines that the probability of acquiring parasites from prey species increases with the length of the period of foraging (Bergeron et al. 1997). The combination of information from parasitic infections and δ^{15}N values implies that isotopic composition of muscles is not influenced anymore by lactation in October and that muscles can therefore be used for diet studies five to six months after birth. Muscles from younger individuals should not be used in studies about community trophodynamics.

4.2. Lack of trend in δ^{13}C values

No variation of δ^{13}C values was observed during growth of young-of-the-year, neither in vibrissae nor in muscles, as already observed in other species of marine or terrestrial mammals (Hobson and Sease 1998, Jenkins et al. 2001, Newsome et al. 2006). This can be related to the relatively low trophic fractionation observed for carbon between food sources and consumers (Peterson and Fry 1987, Hobson et al. 1996), leading to no visible difference between nursing young-of-the-year and adults due to the relatively high inter-individual variation of $\delta^{13}C$ values. In addition, the lack of differences in $\delta^{13}C$ values might also be related to several other factors. For example, Hobson and Sease (1998) and Jenkins et al. (2001) observed respectively lower and similar $\delta^{13}C$ values in nursing pups in comparison with adults. These authors related their results with the high proportion of lipids – depleted in ^{13}C (Tieszen and Boutton 1989, Hilderbrand et al. 1996) - in milk, likely leading to lower $\delta^{13}C$ values in milk than in other mother tissues (Jenkins et al. 2001, Polischuk et al. 2001). Furthermore, Newsome et al. (2006) suggested that tissue $\delta^{13}C$ patterns over the nursing period depend on the degree of preferential routing of carbon from milk lipids *versus* proteins to tissue synthesis and Matthews and Ferguson (2015) hypothesized that the absence of nursing effect on $\delta^{13}C$ values in beluga whales reflected the direct incorporation of dietary lipids into the developing blubber layer of calves.

4.3. High $\delta^{15}N$ values and no parasitic infection during lactation

Young-of-the-year under lactation (*i.e.*, from May to June) had higher $\delta^{15}N$ values than individuals predating on fish. This difference is probably due to the reliance on milk. Indeed, due to trophic fractionation, young-of-the-year under lactation are expected to be more enriched in ^{15}N than their mothers (Bocherens et al. 1995, Hobson and Sease 1998, Jenkins et al. 2001, Polischuk et al. 2001, Newsome et al. 2006). Moreover, the young-of-the-year under lactation were not parasitized, confirming that they were not exposed to trophically transmitted parasites and that they exclusively relied on milk (Bowen 1991).

4.4. Transition period from the lactation to a prey-based diet

The gradual decrease of $\Delta\delta^{15}N$ between young-of-the-year and adults in vibrissae from May to August, and of $\delta^{15}N$ values in muscles of young-of-the-year from June to October can be related to several factors. First, the time period which can be investigated based on the isotopic composition of a tissue strongly depends on the turnover of this tissue (Hobson and Clark 1992, Hobson 1995). The relatively long gradual shift of $\delta^{15}N$ values observed in muscles of young-of-the-year (from June to

October) might be partly explained by the relatively low turnover time of the muscle tissue. Therefore, although $\delta^{15}N$ values in young-of-the-year muscle seemingly reflect a prey-based diet in October, these individuals very likely already forage in earlier months, as demonstrated with parasite investigations. The quicker similarity of $\delta^{15}N$ values in vibrissae between young-of-the-year and adults (already observed 3-4 months after birth) compared to the lag needed for stabilization of the $\delta^{15}N$ values in young-of-the-year muscles (observed 5-6 months after birth) is likely related to the short time of integration of isotopic compositions in vibrissae (Cherel et al. 2009).

Second, harbor seal young-of-the-year undergo a post-weaning fast after a short period of lactation (Muelbert and Bowen 1993, Lang et al. 2005). Indeed, although young-of-the-year begin to forage within a few days when weaned, it takes them several weeks to reach a positive energy balance (Muelbert et al. 2003). The relatively higher $\delta^{15}N$ values of young-of-the-year after weaning in comparison to individuals predating on fish might be due to this fasting period of two to three weeks (Muelbert and Bowen 1993, Muelbert et al. 2003). Indeed, fasting may also result in an enrichment in ^{15}N (Hobson et al. 1993), when muscle tissue is catabolized (Polischuk et al. 2001). The same pattern was observed by Hobson and Sease (1998) in northern elephant seals (*Mirounga angustirostri*) which have a parental care strategy similar to harbor seals. The absence of infection in the respiratory tract combined with the low parasite prevalence and infection level in the digestive tract of young-of-the-year from July and August indicates that exposure of young-of-the-year to the infective parasite stages was low. This observation confirms that post weaning young-of-the-year have a low exposure to parasitized prey species, likely because young-of-the-year rely mainly on their blubber energy storage during the first weeks post weaning (Muelbert and Bowen 1993, Muelbert et al. 2003). The findings from this study clearly show that gastrointestinal parasites are the first endoparasites that become established in harbor seals after weaning. The subsequent incidence of lung nematodes and the observed increase in the levels of parasitic infections in the lungs probably reflect shifting prey preferences or a longer somatic migration of larval stages to the respiratory tract.

Finally, the birth period lasts for one month, from early May to early June (Osinga et al. 2012). As a result, from the beginning of June to the beginning of July, the population of young-of-the-year is composed by a mixture of weaned individuals and of young-of-the-year still under lactation in June. This diversity of life stages in June related to the duration of the birth period might also partly explain the gradual decrease of the $\Delta\delta^{15}N$ in vibrissae and the gradual shift of $\delta^{15}N$ in muscles of young-of-the-year.

5. Conclusion

In harbor seals, we estimated the $\delta^{15}N$ values of young-of-the-year to be influenced by the weaning process until three to four months after birth in vibrissae, and five to six months after birth in muscle. Tissues of young-of-the-year older than these respective ages can therefore be used in community diet studies. The lactating young-of-the-year have higher $\delta^{15}N$ values than foraging individuals in vibrissae and in muscle, whereas $\delta^{13}C$ values are similar. The prevalence of parasitic infections in the respiratory and gastrointestinal tracts of young-of-the-year is also a good indicator of the foraging behavior, and can be an important biomarker in ecological studies about marine mammals. It confirmed that harbors seals are under a prey-based diet about three to four months after birth.

The maternal influence on isotopic composition of young-of-the-year depends on the analyzed tissue, in relation with the turnover, and is also related to the duration of the weaning process. Further studies about temporal variations of isotopic compositions in different tissues and species of marine mammals would bring useful information to increase the possibilities of using stable isotope analyses in young-of-the-year for diet investigations. Among these tissues, a particular attention should be paid to inert tissues such as vibrissae, teeth and claws, to have a better temporal resolution of weaning processes and foraging behavior of seals, and marine mammals in a larger extent.

6. Acknowledgements

This study was funded by the German Federal Ministry of Education and Research in the framework of the German-Dutch bilateral Wadden Sea Research Project "The impact of biological invasions on the food web of the Wadden Sea (INFOWEB)" (Förderkennzeichen 03F0636A/B). The collection of carcasses and pathological investigations of seals were partly funded by the Ministry of Energy, Agriculture, Environment and Rural Areas of the Schleswig-Holstein and the authorities for coastal protection, national parks and marine protection of Schleswig-Holstein (LKN). We would like to thank the team of the Institute for Terrestrial and Aquatic Wildlife Research from the University of Veterinary Medicine Hannover Foundation with whom seal dissections were carried out, particularly Eva Wehrmeister, Miriam Hillmanm and Kornelia Schmidt for their assistance in collecting seal samples. We are grateful to the Gaël Guillou for his help at the LIENSs stable isotope facility of the University of La Rochelle and to Petra Kadel for her help at the Wadden Sea Station on Sylt. We also thank the seal hunters from the island of Sylt who collected the seals along the coast of the Island.

7. References

Abollo, E., C. Gestal, A. López, A. F. González, A. Guerra, and S. Pascual. 1998. Squid as trophic bridges for parasite flow within marine ecosystems: the case of *Anisakis simplex* (*Nematoda: Anisakidae*), or when the wrong way can be right. South African Journal of Marine Science **20**:223-232.

Agreement on the Conservation of Seals in the Wadden Sea. 1990. Signed on 16 October 1990 by Denmark, Germany, Netherlands under the Bonn Convention.

Anderson, R. C. 2000. Nematode parasites of vertebrates: their development and transmission, Cabi.

Aznar, F. J., J. A. Balbuena, and J. A. Raga. 1994. Helminth communities of *Pontoporia blainvillei* (*Cetacea: Pontoporiidae*) in Argentinian waters. Canadian Journal of Zoology **72**:702-706.

Balbuena, J. A., and J. A. Raga. 1994. Intestinal helminths as indicators of segregation and social structure of pods of long-finned pilot whales (*Globicephala melas*) off the Faeroe Islands. Canadian Journal of Zoology **72**:443-448.

Benke, H., U. Siebert, R. Lick, B. Bandomir, and R. Weiss. 1998. The current status of harbour porpoises (*Phocoena phocoena*) in German waters. Arch. Fish. Mar. Res. **46**:97–123.

Bergeron, E., J. Huot, and L. N. Measures. 1997. Experimental transmission of *Otostrongylus circumlitus* (Railliet, 1899) (*Metastrongyloidea*: *Crenosomatidae*), a lungworm of seals in eastern arctic Canada. Canadian Journal of Zoology **75**:1364-1371.

Bern Convention. 1985. Council of Europe, CETS No.: 104. Convention on the Conservation of European Wildlife and Natural Habitats. Annex III: Protected fauna species. .

Bocherens, H., M. L. Fogel, N. Tuross, and M. Zeder. 1995. Trophic Structure and Climatic Information From Isotopic Signatures in Pleistocene Cave Fauna of Southern England. Journal of Archaeological Science **22**:327-340.

Bonn Convention. 1983. Convention on the conservation of Migratory species of Wild Animals (Article IV). Annex II: Migratory Species to be the Subject of Agreements.

Bonner, W. N. 1984. Lactation strategies in pinnipeds: problems for a marine mammalian group. Symposia of the Zoological Society of London **51**:253-272.

Borgsteede, F. H. M., H. G. J. Bus, J. A. W. Verplanke, and W. P. J. van Burg. 1991. Endoparasitic helminths of the harbour seal, *Phoca vitulina*, in the Netherlands. Netherlands Journal of Sea Research **28**:247-250.

Bossart, G. D. 2011. Marine Mammals as Sentinel Species for Oceans and Human Health. Veterinary Pathology Online **48**:676-690.

Bowen, W. D. 1991. Behavioural ecology of pinniped neonates. Pages 66-127 *in* D. Renouf, editor. The behaviour of pinnipeds. Chapman and Hall, London (UK).

Bowen, W. D. 1997. Role of marine mammals in aquatic ecosystems. Marine Ecology Progress Series **158**:267-274.

Brown, E. G., and G. J. Pierce. 1998. Monthly variation in the diet of harbour seals in inshore waters along the southeast Shetland (UK) coastline. Marine Ecology Progress Series **167**:275-289.

Bush, A. O., K. D. Lafferty, J. M. Lotz, and A. W. Shostak. 1997. Parasitology Meets Ecology on Its Own Terms: Margolis et al. Revisited. The Journal of Parasitology **83**:575-583.

Carroll, S. S., L. Horstmann-Dehn, and B. L. Norcross. 2013. Diet history of ice seals using stable isotope ratios in claw growth bands. Canadian Journal of Zoology **91**:191-202.

Caut, S., S. Laran, E. Garcia-Hartmann, and K. Das. 2011. Stable isotopes of captive cetaceans (killer whales and bottlenose dolphins). The Journal of Experimental Biology **214**:538-545.

Cherel, Y., L. Kernaléguen, P. Richard, and C. Guinet. 2009. Whisker isotopic signature depicts migration patterns and multi-year intra- and inter-individual foraging strategies in fur seals. Biology Letters **5**:830-832.

Dailey, M. D. 1970. The transmission of *Parafilaroides decorus* (N*ematoda: Metastrongyloidea*) in the California sea lion (*Zalophus californianus*). Proceedings of the Helminthological Society of Washington **31**:215-222.

Das, K., G. Lepoint, Y. Leroy, and J. M. Bouquegneau. 2003. Marine mammals from the southern North Sea: feeding ecology data from δ^{13}C and δ^{15}N measurements. Marine Ecology Progress Series **263**:287-298.

DeNiro, M. J., and S. Epstein. 1978. Influence of diet on the distribution of carbon isotopes in animals Geochimica et Cosmochimica Acta **42**:495-506.

EU Habitats Directive. 1992. Council Directive 92/43/EEC of 21 May 1992 on the conservation of natura habitats and wild fauna and flora - consolidated version 01/01/2007. Annex II: Animal and plant species of community interest whose conservation requires the designation of special areas of conservation. Annex V: Animal and plant species of community interest whose taking in the wild and exploitation my be subject to management measures. .

Ferreira, E. O., L. L. Loseto, and S. H. Ferguson. 2011. Assessment of claw growth-layer groups from ringed seals (Pusa hispida) as biomonitors of inter- and intra-annual Hg, δ15N, and δ13C variation. Canadian Journal of Zoology **89**:774-784.

Fry, B. 1988. Food web structure on Georges Bank from stable C, N and S isotopic compositions Limnology and Oceanography **33**:9.

Hall-Aspland, S. A., T. L. Rogers, and R. B. Canfield. 2005. Stable carbon and nitrogen isotope analysis reveals seasonal variation in the diet of leopard seals. Marine Ecology Progress Series **305**:249-259.

Hall, A. J., J. Watkins, and P. S. Hammond. 1998. Seasonal variation in the diet of harbour seals in the south-western North Sea. Marine Ecology Progress Series **170**:269-281.

Harding, K. C., M. Fujiwara, Y. Axberg, and T. Härkönen. 2005. Mass-dependent energetics and surviva in Harbour Seal pups. Functional Ecology **19**:129-135.

Hauksson, E., and D. Ólafsdóttir. 1995. Grey seal (*Halichoerus grypus Fabr.*), population biology, food and feeding habits, and importance as a final host for the lifecycle of sealworm (*Pseudoterranova decipiens Krabbe*) in Icelandic Waters. Pages 565-572 *in* L. W. Arnoldus Schytte Blix and U. Øyvind, editors. Developments in Marine Biology. Elsevier Science.

Hilderbrand, G. V., S. D. Farley, C. T. Robbins, T. A. Hanley, K. Titus, and C. Servheen. 1996. Use of stable isotopes to determine diets of living and extinct bears. Canadian Journal of Zoology **74**:2080-2088.

Hirons, A. C., D. M. Schell, and D. J. St. Aubin. 2001. Growth rates of vibrissae of harbor seals (*Phoca vitulina*) and Steller sea lions (*Eumetopias jubatus*). Canadian Journal of Zoology **79**:1053-1061.

Hobson, K. A. 1995. Reconstructing Avian Diets Using Stable-Carbon and Nitrogen Isotope Analysis of Egg Components: Patterns of Isotopic Fractionation and Turnover. The Condor **97**:752-762.

Hobson, K. A., R. T. Alisauskas, and R. G. Clark. 1993. Stable-Nitrogen Isotope Enrichment in Avian Tissues Due to Fasting and Nutritional Stress: Implications for Isotopic Analyses of Diet. The Condor **95**:388-394.

Hobson, K. A., and R. G. Clark. 1992. Assessing Avian Diets Using Stable Isotopes I: Turnover of 13C in Tissues. The Condor **94**:181-188.

Hobson, K. A., D. M. Schell, D. Renouf, and E. Noseworthy. 1996. Stable carbon and nitrogen isotopic fractionation between diet and tissues of captive seals: implications for dietary reconstructions involving marine mammals. Canadian Journal of Fisheries and Aquatic Sciences **53**:528-533.

Hobson, K. A., and J. L. Sease. 1998. Stable isotope analyses of tooth annuli reveal temporal dietary records: an example using steller sea lions. Marine Mammal Science **14**:116-129.

Hobson, K. A., J. L. Sease, R. L. Merrick, and J. F. Piatt. 1997. Investigating trophic relationships of pinnipeds in Alaska and Washington using stable isotope ratios of Nitrogen and Carbon. . Marine Mammal Science **13**:114-132.

Jenkins, S., S. Partridge, T. Stephenson, S. Farley, and C. Robbins. 2001. Nitrogen and carbon isotope fractionation between mothers, neonates, and nursing offspring. Oecologia **129**:336-341.

Jensen, L. 2015. Monitoring of the seal population in the Wadden Sea in 2015 - Monitering af sælbestanden i Vadehavet 2015. Aarhus Universitet, Miljøministeriet, Fiskeri-og Søfartsmuseet Saltvandsakvariet Esbjerg.

Lafferty, K. D., S. Allesina, M. Arim, C. J. Briggs, G. De Leo, A. P. Dobson, J. A. Dunne, P. T. J. Johnson, A. M. Kuris, D. J. Marcogliese, N. D. Martinez, J. Memmott, P. A. Marquet, J. P. McLaughlin, E. A. Mordecai, M. Pascual, R. Poulin, and D. W. Thieltges. 2008. Parasites in food webs: the ultimate missing links. Ecology Letters **11**:533-546.

Lang, S. L. C., S. J. Iverson, and W. D. Bowen. 2005. Individual variation in milk composition over lactation in harbour seals (*Phoca vitulina*) and the potential consequences of intermittent attendance. Canadian Journal of Zoology **83**:1525-1531.

Lehnert, K., J. A. Raga, and U. Siebert. 2005. Macroparasites in stranded and bycaught harbour porpoises from German and Norwegian waters. Diseases of Aquatic Organisms **64**:265-269.

Lehnert, K., J. A. Raga, and U. Siebert. 2007. Parasites in harbour seals (*Phoca vitulina*) from the German Wadden Sea between two Phocine Distemper Virus epidemics. Helgoland Marine Research **61**:239-245.

Lehnert, K., H. Seibel, I. Hasselmeier, P. Wohlsein, M. Iversen, N. H. Nielsen, M. P. Heide-Jørgensen, E. Prenger-Berninghoff, and U. Siebert. 2014. Increase in parasite burden and associated pathology in harbour porpoises (*Phocoena phocoena*) in West Greenland. Polar Biology **37**:321-331.

Lehnert, K., G. von Samson-Himmelstjerna, D. Schaudien, C. Bleidorn, P. Wohlsein, and U. Siebert. 2010. Transmission of lungworms of harbour porpoises and harbour seals: Molecular tools determine potential vertebrate intermediate hosts. International Journal for Parasitology **40**:845-853.

Lesage, V., M. O. Hammill, and K. M. Kovacs. 2001. Marine mammals and the community structure of the Estuary and Gulf of St Lawrence, Canada: evidence from stable isotope analysis. Marine Ecology Progress Series **210**:203-221.

Lynch, M., P. J. Duignan, T. Taylor, O. Nielsen, R. Kirkwood, J. Gibbens, and J. P. Y. Arnould. 2011. Epizootiology of Brucella infection in australian fur seals. Journal of Wildlife Diseases **47**:352-363.

MacLaren, I. 1993. Growth in pinnipeds. Biological Reviews **68**:1-79.

Marcogliese, D. J. 2005. Parasites of the superorganism: Are they indicators of ecosystem health? International Journal for Parasitology **35**:705-716.

Matthews, C. J. D., and S. H. Ferguson. 2015. Weaning age variation in beluga whales (Delphinapterus leucas). Journal of Mammalogy **96**:425-437.

Mattiucci, S., and G. Nascetti. 2007. Genetic diversity and infection levels of anisakid nematodes parasitic in fish and marine mammals from Boreal and Austral hemispheres. Veterinary Parasitology **148**:43-57.

Measures, L. N. 2008. Lungworms of Marine Mammals. Pages 279-300 Parasitic Diseases of Wild Mammals. Iowa State University Press.

Mees, J., and P. J. H. Reijnders. 1994. The harbour seal, *Phoca vitulina,* in the Oosterschelde: decline and possibilities for recovery. Hydrobiologia **282-283**:547-555.

Muelbert, M. M. C., and W. D. Bowen. 1993. Duration of lactation and postweaning changes in mass and body composition of harbour seal, *Phoca vitulina*, pups. Canadian Journal of Zoology **71**:1405-1414.

Muelbert, M. M. C., W. D. Bowen, and S. J. Iverson. 2003. Weaning Mass Affects Changes in Bod Composition and Food Intake in Harbour Seal Pups during the First Month of Independence Physiological and Biochemical Zoology **76**:418-427.

Newland, C., I. C. Field, Y. Cherel, C. Guinet, C. J. A. Bradshaw, C. R. McMahon, and M. A. Hindell. 2011. Diet of juvenile southern elephant seals reappraised by stable isotopes in whiskers. Marine Ecology Progress Series **424**:247-258.

Newsome, S. D., P. L. Koch, M. A. Etnier, and D. Aurioles-Gamboa. 2006. Using Carbon and Nitrogen isotope values to investigate maternal strategies in Northeast Pacific Otariids. Marine Mamma Science **22**:556-572.

Newsome, S. D., M. T. Tinker, D. H. Monson, O. T. Oftedal, K. Ralls, M. M. Staedler, M. L. Fogel, and J. A. Estes. 2009. Using stable isotopes to investigate individual diet specialization in California sea otters (*Enhydra lutris nereis*). Ecology **90**:961-974.

Oftedal, O. T., D. l. Boness, and R. A. Tedman. 1987. The Behavior, Physiology, and Anatomy of Lactation in the Pinnipedia. Pages 175-245 *in* H. Genoways, editor. Current Mammalogy. Springer US.

Ólafsdóttir, D., and E. Hauksson. 1998. Anisakid nematodes in the common seal (*Phoca vitulina L.*) in icelandic waters. Sarsia **83**:309-316.

Osinga, N., I. Pen, H. A. Udo de Haes, and P. M. Brakefield. 2012. Evidence for a progressively earlier pupping season of the common seal (*Phoca vitulina*) in the Wadden Sea. Journal of the Marine Biological Association of the United Kingdom **92**:1663-1668.

Peterson, B. J., and B. Fry. 1987. Stable Isotopes in Ecosystem Studies. Annual Review of Ecology and Systematics **18**:293-320.

Polischuk, S. C., K. A. Hobson, and M. A. Ramsay. 2001. Use of stable-carbon and -nitrogen isotopes to assess weaning and fasting in female polar bears and their cubs. Canadian Journal of Zoology **79**:499-511.

Rau, G. H., A. J. Mearns, D. R. Young, R. J. Olson, H. A. Schafer, and I. R. Kaplan. 1983. Animal C/C Correlates with Trophic Level in Pelagic Food Webs. Ecology **64**:1314-1318.

Reddy, L., L. A. Dierauf, and F. M. Gulland. 2001. Marine mammals as sentinels of ocean health. CRC handbook of marine mammal medicine: Health, disease and rehabilitation:3-13.

Reijnders, P. J. H. 1976. The harbour seal (*phoca vitulina*) population in the Dutch Wadden Sea: Size and composition. Netherlands Journal of Sea Research **10**:223-235.

Reijnders, P. J. H., S. M. J. M. Brasseur, T. Borchardt, K. Camphuysen, R. Czeck, A. Gilles, L. F. Jensen, M. Leopold, K. Lucke, S. Ramdohr, M. Scheidat, U. Siebert, and J. Teilmann. 2009. Marine mammals. Thematic Report N°20. In: Marencic, H. and Vlas, J. de, editors. Quality status report 2009. Wadden Sea Ecosystem N°25. Wadden Sea Secretariat, Trilateral Monitoring and Assessment Group, Wilhelmshaven, Germany.

Reijnders, P. J. H., E. H. Ries, S. Tougaard, N. Nørgaard, G. Heidemann, J. Schwarz, E. Vareschi, and I. M. Traut. 1997. Population development of harbour seals *Phoca vitulina* in the Wadden Sea after the 1988 virus epizootic. Journal of Sea Research **38**:161-168.

Rijks, J. M., J. I. Hoffman, T. Kuiken, A. D. M. E. Osterhaus, and W. Amos. 2008. Heterozygosity and lungworm burden in harbour seals (*Phoca vitulina*). Heredity **100**:587-593.

Siebert, U., A. Gilles, K. Lucke, M. Ludwig, H. Benke, K.-H. Kock, and M. Scheidat. 2006. A decade of harbour porpoise occurrence in German waters—Analyses of aerial surveys, incidental sightings and strandings. Journal of Sea Research **56**:65-80.

Siebert, U., P. Wohlsein, K. Lehnert, and W. Baumgärtner. 2007. Pathological Findings in Harbour Seals (*Phoca vitulina*): 1996–2005. Journal of Comparative Pathology **137**:47-58.

Siebert, U., A. Wünschmann, R. Weiss, H. Frank, H. Benke, and K. Frese. 2001. Post-mortem Findings in Harbour Porpoises (*Phocoena phocoena*) from the German North and Baltic Seas. Journal of Comparative Pathology **124**:102-114.

Smith, R. J., and A. J. Read. 1992. Consumption of euphausiids by harbour porpoise (*Phocoena phocoena*) calves in the Bay of Fundy. Canadian Journal of Zoology **70**:1629-1632.

Tieszen, L. L., and T. W. Boutton. 1989. Stable Carbon Isotopes in Terrestrial Ecosystem Research. Pages 167-195 *in* P. W. Rundel, J. R. Ehleringer, and K. A. Nagy, editors. Stable Isotopes in Ecological Research. Springer New York.

Tieszen, L. L., T. W. Boutton, K. G. Tesdahl, and N. A. Slade. 1983. Fractionation and turnover of stable carbon isotopes in animal tissues: Implications for δ^{13}C analysis of diet. Oecologia **57**:32-37.

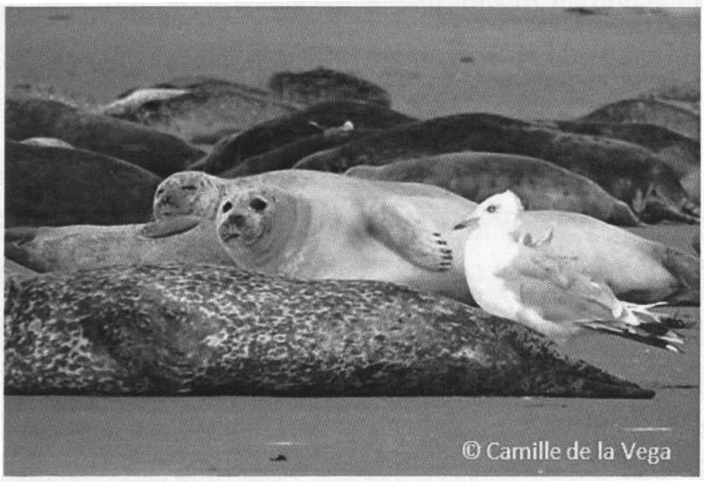
Todd, S. K., B. Holm, D. A. S. Rosen, and D. J. Tollit. 2010. Stable isotope signal homogeneity and differences between and within pinniped muscle and skin. Marine Mammal Science **26**:176-185.

Vander Zanden, M. J., M. K. Clayton, E. K. Moody, C. T. Solomon, and B. C. Weidel. 2015. Stable Isotope Turnover and Half-Life in Animal Tissues: A Literature Synthesis. PLoS ONE **10**:e0116182.

Zhao, L., and D. M. Schell. 2004. Stable isotope ratios in harbor seal *Phoca vitulina* vibrissae: effects of growth patterns on ecological records. Marine Ecology Progress Series **281**:267-273.

Chapter 2

Seasonal variation of harbor seal´s diet from the Wadden Sea in relation to prey availability



Seasonal variation of harbor seal´s diet from the Wadden Sea in relation to prey availability

Camille de la Vega[1]*, Benoit Lebreton[2], Ursula Siebert[3], Gael Guillou[2], Krishna Das[4], Ragnhild Asmus[1], Harald Asmus[1]

PlosOne 11:e0155727 (2016)

[1] Alfred-Wegener-Institut Helmholtz-Zentrum für Polar- und Meeresforschung, Wattenmeerstation Sylt Hafenstr. 43, D-25992 List/ Sylt, Germany
[2] Littoral Envirennement et Sociétés (LIENSs), CNRS-Université de la Rochelle, Bâtiment ILE 2, rue Olympe de Gouges 17 000 La Rochelle, France
[3] Stiftung Tierärztliche Hochschule Hannover Institut für Terrestrische und Aquatische Wildtierforschung Werftstr. 6, 25761 Büsum, Germany
[4] Laboratory of oceanology - MARE center, Université de Liège, Allée de la Chimie 3, Bât. B6c, 4000 Liège (Sart-Tilman), Belgium

*Corresponding author: camille.de.la.vega@awi.de

Abstract

The Wadden Sea has an important role for marine mammals in terms of resting, nursing and foraging. Harbor seal is the most abundant marine mammal species in this area. The use of the food resources of the Wadden Sea by seals is not clear, and previous studies showed that this species can travel kilometers away from their haul-outs to forage in the North Sea. In this study, we analyzed the stable isotopes of vibrissae from 20 dead harbor seals found on the island of Sylt to investigate their diet. The predator´s carbon and nitrogen isotope compositions were compared to the compositions of different potential prey items from the Sylt-Rømø Bight and from the North Sea in order to study seasonal pattern in the diet and in the foraging location. In parallel, seasonal variation of abundance and biomass of the potential prey items from the Sylt-Rømø Bight were studied and compare to their contribution to the seal´s diet. The results revealed a change in the seal´s diet from pelagic sources in spring to a benthic based diet in summer, and an increasing use of the North Sea resources in fall and winter in accordance with the seasonal variation of the availability of prey in the Sylt-Rømø Bight.

Key words

Phoca vitulina; vibrissae; whisker ; $\delta^{13}C$; $\delta^{15}N$; stable isotope; mixing model

1. Introduction

Marine mammals represent the most prominent members among top predators in the marine environment (Reijnders and Lankester 1990). Their abundance and distribution can have a large effect on the structure and the functioning of ecosystems and communities (Power and Gregoire 1978, Estes 1979, Bowen 1997). Assessing the role of top predators in the functioning of ecosystems is then a central issue in ecology and management (Bowen 1997). Nevertheless, the role of top predators in structuring ecosystems is still not well known (Bowen 1997, Lesage et al. 2001) due to their ecological niches often exceeding the temporal and spatial scales which are used to define community boundaries (Lesage et al. 2001, Tougaard et al. 2003).

In the Wadden Sea, harbor seal (*Phoca vitulina*) is, together with harbor porpoise (*Phocoena phocoena*), the most abundant marine mammal species (Reijnders et al. 2009). The conservation measures introduced in the 1970s for marine mammals (Reijnders and Lankester 1990, Lotze et al. 2005, Smardon 2009, Hoffmann et al. 2011), and particularly the protection of harbor seals by the hunting prohibition started in 1976 for the entire Wadden Sea (Reijnders et al. 1997), allowed its population to grow (Reijnders and Lankester 1990, Reijnders et al. 1997, Lotze et al. 2005). Despite two epizootics in 1988 and 2002 which interrupted the upward trend in population growth sharply (Reijnders et al. 2009), the Wadden Sea population of harbor seals increases and might approach the carrying capacity of the area (Reijnders et al. 2010), with 26 576 individuals counted on land in August 2014 (Galatius et al. 2014). Harbor seals´ population spreads from Denmark to the Netherlands, with ~61% of its population located along the German coast (Galatius et al. 2014). The Wadden Sea is an important habitat for harbor seals in terms of reproduction (Reijnders et al. 2009, Osinga et al. 2012, Galatius et al. 2014) and food resources (Smardon 2009). Harbor seals use the numerous sand banks regularly exposed at low tide in different bays of the Wadden Sea to give birth, rest and molt (Drescher 1979). They also use the Wadden Sea at high tide to forage on the abundant food stock it provides (Reijnders et al. 2010).

Harbor seals are opportunistic feeders subsisting largely on fish, although mollusks and crustaceans may sometimes form a significant part of their diet (Behrends 1985, Sievers 1989). Several studies based on seal stomach contents conducted in the North Sea showed a variation in the dominant species in the seal´s diet depending on the location, the main prey species being either gadoids and flat fish (Härkönen 1987, Härkönen and Heide-Jørgensen 1991, Brown and Pierce 1998, Hall et al. 1998), or clupeids and sand eels (Pierce et al. 1991, Thompson et al. 1991, Thompson et al. 1996, Tollit et al.

1997). Along the German coast, in the Schleswig-Holstein area, gadoids (*Gadus morhua* and *Merlangus merlangus*) and flat fish (*Limanda limanda*, *Platichthys flesus* and *Pleuronectes platessa*) are prominent in the seal´s diet with *Ammodytes tobianus* and *Clupea harengus* of secondary importance (Gilles et al. 2008). Thus, harbor seals feed on a wide range of prey with the prevalence of some key species. The contributions of these prey items to the diet vary depending on the area, and likely depending on the prey availability (Tollit et al. 1997, Andersen et al. 2007).

Due to their large body size and their high abundance in the Wadden Sea, seals exert a strong pressure of predation on their environment (Bowen 1997, Reijnders et al. 2010). Even if harbor seals from the Wadden Sea appear to use the North Sea more than previously expected (Tougaard et al. 2003), they might exert a pressure of predation on the Wadden Sea food resources. Consequently, there are needs to improve the understanding of the trophic behavior of seals in the North Sea and in the Wadden Sea, in order to have better estimations of their diets and to determine spatio-temporal variations of their foraging activities. This would allow evaluating their influence on the ecosystems in which they live, in order to improve management plans for conservation of seals and of their food resources.

Stable isotope analysis is a powerful tool for determination of food resources used by marine mammals (Hobson et al. 1997, Lesage et al. 2001, Das et al. 2003, Caut et al. 2011). This method is very complementary to gut content analyses, which have already been carried out on seals from the same area (Behrends 1985, Gilles et al. 2008). Gut content and feces analyses give a snapshot of the ingested prey items whereas the stable isotope composition provides dietary information integrated over few days (e.g., plasma, liver) to few months (e.g., muscle, hair) in function of the differences of metabolic activity (e.g., turnover) or growth rate between the tissues (Tieszen et al. 1983). The stable isotope composition of carbon in predator tissue reflects the origin of food resources: it allows generally a good discrimination between food resources produced in continental areas, those produced in the open ocean and the ones produced in benthic environments (DeNiro and Epstein 1978, Rau et al. 1983, Hobson et al. 1994). The stable isotope composition of nitrogen is commonly used as an indicator of the trophic position of a consumer, thanks to the large trophic fractionation observed for nitrogen between each trophic level (Peterson and Fry 1987, Fry 1988, Hobson and Welch 1992). For the present study, stable isotope analyses were carried out on vibrissae to determine temporal patterns of diet. Indeed, due to daily growth of vibrissae and their metabolic inertia (Hall-Aspland et al. 2005), their isotopic composition reflects the diet at the time of their growth (Cherel et al. 2009). Several studies revealed that vibrissae provide a powerful way to assess diet and foraging location of marine mammals such as elephant seals (*Mirounga leonine)* (Newland et al. 2011), leopard seals (*Hydrurga leptonyx*) (Hall-Aspland et al. 2005), harp seals (*Pagophilus groenlandicus*) (Hobson et al. 1996) and sea otters (*Enhydra lutris nereis*) (Newsome et al. 2009). Zhao and Schell (2004) showed that harbor seal´s vibrissae can archive ecological changes over a long metabolic period. As a result and knowing their growth rate (0.78 $mm.d^{-1}$ from May to September and 0.075 $mm.d^{-1}$ from October to April) (Zhao and Schell 2004) vibrissae segmental isotopic analysis provides an efficient tool for studying foraging ecology of harbor seals giving precise (day) and long term (up to one year) information about the history of their food resources.

The present study aims to first estimate the temporal variation of the diet of harbor seals from the German Wadden Sea using stable isotope analyses, focusing both on the different type of prey items (i.e., trophic groups of prey species) and the different origins of these prey items (North Sea *vs.* Sylt-Rømø Bight). The probability to be part of the seal´s diet is then related to the seasonal patterns of the prey species´ biomass and abundance.

2. Material and Methods

2.1. Ethic Statement

In the Wadden Sea area, harbor seals are protected under the Annex II of the Convention on Migratory Species of Wild Animals, also called Bonn Convention (1983), and particularly since 1991 under the protection of the Trilateral Seal Agreement between Denmark, Germany and the Netherlands (Agreement on the Conservation of Seals in the Wadden Sea 1990). In addition, they are protected under Annex III (protected fauna species) of the Convention on the conservation of European wildlife and natural habitats (Bern Convention 1985). The harbor seal is also listed in the Annexes II and V of the EU Habitats Directive (1992) (consolidated version 2007) on the conservation of natural habitats and of wild fauna and flora. Harbor seals are classified with least concern in the regional red list for Germany (Federal Agency for Nature Conservation and Germany 2009) and in the European red list (International Union for the Conservation of Nature and Natural Resources and IUCN 2012). All seal samples were taken in accordance with these protection measures. Samples were collected as part of a harbor seals stranded network, established on the German coast of Schleswig-Holstein after the 1988/1989 Phocine Distemper Virus epidemic (Benke et al. 1998). All stranded seals were found dead or were killed because of serious illness by authorized seal hunters affiliated to the authorities of Schleswig-Holstein Wadden Sea National Park.

The sampling of prey species from the Sylt-Rømø Bight were part of a monthly fish monitoring supervised by the Alfred Wegener Institute since 2006. No endangered prey species were used in this study. All caught fish, squid and shrimp individuals were measured (length and weight) on board as fast as feasible for biomass and abundance survey, and have been returned to the wild after being held in water. The individuals sampled for stable isotope analyses were rapidly killed and stored in a freezer on board. The individuals of prey species from the North Sea were collected for stable isotope analyses among catches of a professional shrimp trawler from the island of Rømø.

2.2. Study site

The Sylt-Rømø Bight (54°52’ - 55°10’ N, 8°20’ - 8°40’ E) is part of the Wadden Sea, which extends along the south-eastern margin of the North Sea from the Netherlands to Denmark. This 404 km^2 semi-enclosed basin is located between the islands of Sylt (Germany) and Rømø (Denmark; Fig. 1). Two causeways connect the islands with the mainland, and prohibit any exchange of water with the adjacent tidal basins. The only connection to the North Sea is a deep tidal channel between the two islands. The tidal range inside the Bight is up to 2 m (Martens and Beusekom 2008). The Sylt-Rømø Bight provides shelter for a stable colony of ≈ 470 ± 97 harbor seals on average in summer (2009 to 2012) (Jensen 2015). Harbor seals use five sand banks uncovered at low tide as haul out sites. These sandbanks are spread in the whole Bight, with the Jordsand and List sand banks (Fig. 1) being the most frequented (Jensen 2015).

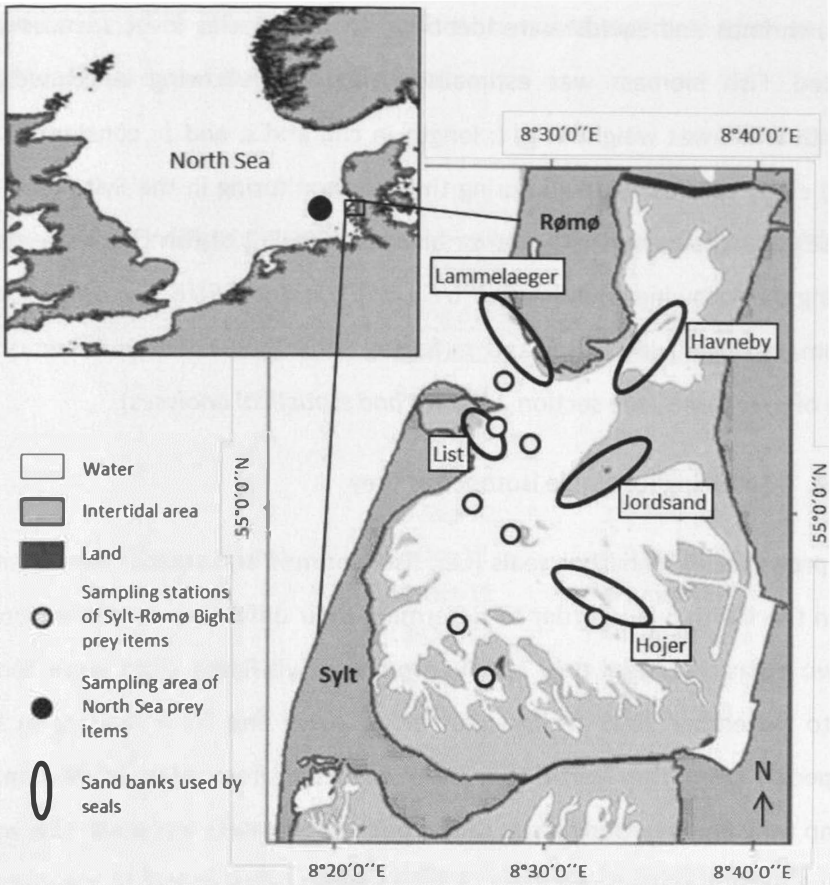


Fig. 1: Location and map of the Sylt-Rømø Bight. Maps created using ArcGIS® 10 Esri software. Sylt-Rømø Bight map data courtesy of the Schleswig-Holstein's Government-Owned Company for Coastal Protection, National Parks and Ocean Protection - National Park Authority, Tönning.

2.3. Prey samples

Fish biomass and stable isotope samples were measured and collected from the catches of fish monitored monthly from 2008 to 2013 in the Sylt-Rømø Bight (Fig. 1).

2.3.1. Sampling for fish biomass and abundance

Sampling for biomass and abundance of the prey species took place monthly from 2010 to 2012 at eight stations in the Sylt-Rømø Bight (Fig. 1) to provide a representative geographical coverage of the area. Two hauls were carried out at every station: one in the water column and one at the bottom, each for 15 minutes at an average speed of approximately 1 m.s^{-1}. Sampling was carried out using a 17 m long mini bottom trawl, also designed to be deployed for pelagic fishing. The mouth of the net was up to 7 m in width and 3 m in height. Mesh size measured 32 mm in the wings, 16 mm in the mid part and 6 mm in the cod end. Fish, shrimps and squids were identified to the species level, measured to the nearest 0.5cm and counted. Fish biomass was estimated using the following length-weight relationship $WW = a \times l^b$, with WW: wet weight in g, l: length in cm, and a and b: constants calculated by for Pockberger (2015) every species sampled during the fish monitoring in the Sylt-Rømø Bight. Catch per unit of effort (CPUE), i.e., the number ($CPUE_n$) or biomass ($CPUE_m$) of fish caught per hour of sampling, was calculated using the following equations: $CPUE_n = \sum n/t$ and $CPUE_m = \sum m/t$, with n: number of individuals,m: biomass of individuals (g) and t: fishing time (hour). The number of individuals was summed by group of prey items (see section 2.5 *Data and statistical analyses*).

2.3.2. Sampling for stable isotopes of prey

Potential prey species of harbor seals (i.e., fish, shrimps and squids) were sampled in the Sylt-Rømø Bight and in the North Sea in order to determine their difference in stable isotope composition between these two areas. Potential prey species from the Sylt-Rømø Bight were sampled seasonally from April 2008 to November 2009 (Kellnreitner et al. 2012) and from January to November 2013. Potential prey species from the North Sea were collected from May to September 2013 by a professional shrimp trawler. The opening size of the net was 5 meters and mesh size was 20 mm. Three individuals from the most abundant size-class of each species were collected, measured to nearest 0.5 cm and then stored at -20°C for further analysis.

2.4. Sampling for stable isotopes of seals

Twenty three harbor seal carcasses in good state of conservation were collected from June 2012 to February 2014 along the coastline of the Sylt Island. This sampling represents about 5% of the population of harbor seals in the Sylt-Rømø Bight during summer (470 individuals on average) and encompasses the totality of stranded adults and most of the stranded young-of-the-year older than 3-4 months collected by the seal´s hunter on the Sylt coast during this period. Necropsies were conducted on the carcasses at the Institute for Terrestrial and Aquatic Wildlife Research (ITAW) of University of Veterinary Medicine Hannover Foundation, according to the protocol described by Siebert et al. (2007). Until necropsy, the carcasses were stored frozen in plastic bags at -20°C. The age (older than 2 years vs. young-of-the-year) was estimated according to the length. The estimated age of the young-of-the-year (in months) was determined as the number of months between the main birth period (May to June) (Osinga et al. 2012) and the day of collection (Table 1).

Table 1: sex, finding date and age of the twenty three sampled harbor seals

seal ID	sex	finding date	age
1	m	29-Jul-12	13-14 months
2	m	8-Sep-12	> 2 year
3	m	21-Sep-12	> 2 year
4	f	30-Sep-12	3-4 months
5	m	30-Sep-12	3-4 months
6	m	7-Oct-12	4-5 months
7	f	10-Oct-12	4-5 months
8	m	13-Oct-12	4-5 months
9	m	19-Oct-12	4-5 months
10	m	6-Dec-12	6-7 months
11	f	9-Dec-12	6-7 months
12	f	9-Dec-12	6-7 months
13	f	10-Dec-12	6-7 months
14	f	31-Dec-12	6-7 months
15	f	31-Dec-12	6-7 months
16	f	31-Dec-12	6-7 months
17	f	24-Mar-13	9-10 months
18	m	29-Mar-13	9-10 months
19	f	24-Apr-13	> 2 year
20	m	12-Jul-13	> 2 year
21	m	13-Nov-13	5-6 months
22	m	13-Nov-13	5-6 months
23	f	11-Feb-14	> 2 year

To evaluate the similarity between vibrissae originating from the same animal, two different vibrissae were collected on seals #1 (adult) and #4 (yearling) (i.e., four vibrissae in total). The R^2 of the linear regression between the two vibrissae from a same individual were calculated to verify the similarity between stable isotope compositions and growth rate. We observed a very good similarity between 2 vibrissae from a same individual for both $\delta^{13}C$ and $\delta^{15}N$ values ($\delta^{13}C$: seal #13: R^2=0.804, seal

#29: R^2= 0.975; $\delta^{15}N$: seal #13: R^2=0.991, seal #29: R^2= 0.944; Appendix 1, p. 85). The longest mystacial vibrissae were sampled for each individual in order to cover the longest period of growth.

2.5. Preparation and analysis of stable isotope samples

Prey samples were freeze-dried and ground individually to a fine powder using a ball mill. Whole eviscerated individuals were analyzed. To avoid the bias due to presence of $CaCO_3$ from fish bones, samples for $\delta^{13}C$ analyses were acidified using 1 $mol.L^{-1}$ hydrochloric acid, then dried at 60°C and ground again (Bunn et al. 1995, Pinnegar and Polunin 1999). $\delta^{15}N$ analyses were carried out on raw samples in order to avoid any potential bias due to acidification.

Harbor seal vibrissae were cleaned using soap in an ultrasonic bath for 10 minutes and then rinsed 4 times in distilled water. Vibrissae were measured, dried and sliced with a sharp cutter in 1 to 2 mm consecutive sections (ranging in mass from 0.8 to 1.5 mg) starting from the proximal end (Cherel et al. 2009). This represented a trade-off between the number of sections (and hence the temporal resolution attainable for the isotopic time series) and the size of the sample (Newland et al. 2011). The number of samples analyzed per vibrissae varied from 18 to 42 depending on its length.

Each piece of vibrissae and homogenized powdered samples of prey were precisely weighed (± 1 µg) and were sealed in a tin capsule for stable isotope analyses. Samples were processed on an elemental analyzer (Vario Microcube, Elementar, Germany) coupled to an isotope ratio mass spectrometer (Isoprime 100, Isoprime, UK). Results are expressed in the δ notation as deviation from international standards (Vienna Pee Dee Belemnite for $\delta^{13}C$ and N_2 in air for $\delta^{15}N$) following the formula: $\delta^{13}C$ or $\delta^{15}N = [(R_{sample}/R_{standard}) - 1] \times 10^3$, where R is $^{13}C/^{12}C$ or $^{15}N/^{14}N$ isotopic ratios. Calibration was performed using certified reference materials (IAEA-C6, IAEA-N2, for nitrogen). Analytical precision based on repeated analyses of glycine (p.a. Merck, Germany) used as an internal standard was <0.15‰ for carbon and nitrogen.

2.6. Data and statistical analyses

2.6.1. Trophic group of prey items

Fish prey species were grouped following three trophic groups (Table 2): planktivorous/piscivorous, benthivorous/piscivorous and benthivorous, as described in Froese and Pauly (2014). The planktivorous/piscivorous group is represented by pelagic species (e.g., *C. harengus*, *A. tobianus*) living in the water column and feeding on zoo- and phyto-plankton and/or small fishes. The benthivorous/piscivorous group comprises benthopelagic species (e.g., *M. merlangus*, *L. limanda*, *Myoxocephalus scorpius*) living partly in the water column but foraging on the seafloor. These species are feeding on crustacean, mollusks and polychaetes, but also on small fishes and cephalopods (Froese and Pauly 2014). The benthivorous group consists mainly of demersal species (e.g., *Pomatoschistus minutus*, *P. platessa*) living on the seafloor and feeding on small crustaceans, mollusks, polychaetes, fish eggs (Froese and Pauly 2014) and, for some groups, on amphipods (Oh et al. 2001). Due to its anadromous behavior, *Osmerus eperlanus* was treated separately than the benthivorous/piscivorous group, although it feeds on shrimps, small crustaceans and small fishes (Froese and Pauly 2014). Only squid species belonging to the genus *Loligo* were found.

Table 2: Groups of species used as prey items in the Sylt-Rømø Bight (for biomass and stable isotope analyses) and in the North Sea (for stable isotope analyses).

Planktivorous/piscivorous	**Benthivorous/piscivorous**	**Strictly benthivorous**
Ammodytes tobianus	*Ciliata mustela* ***	*Agonus cataphractus*
Atherina presbyta *	*Gadus morhua* ***	*Crangon crangon*
Belone belone	*Gasterosteus aculeatus* **	*Pleuronectes platessa*
Clupea harengus	*Limanda limanda*	*Pholis gunnellus* **
Cyclopterus lumpus *	*Merlangius merlangus*	*Pomatoschistus microps*
Hyperoplus lanceolatus	*Myoxocephalus scorpius* **	*Pomatoschistus minutus*
Scomber scomber *	*Platichthys flesus* **	*Solea solea*
Sprattus sprattus	*Spinachia spinachia* **	*Zoarces viviparus*
Trachurus trachurus *	*Syngnathus rostellatus* **	

* species not sampled for stable isotope analysis.

** species only sampled in the Sylt-Rømø Bight for stable isotope analysis.

*** species only sampled in the North Sea for stable isotope analysis.

The seasonal biomass and abundance of trophic groups were similar between the years 2010 2011 and 2012 (Kruskal Wallis rank sum test: Planktivorous/Piscivorous, all p-values > 0.11 for biomass and > 0.10 for abundance; Benthivorous/Piscivorous, all p-values between > 0.33 for biomass and > 0.26 for abundance; Benthivorous group, all p-values > 0.13 for biomass and all p-values > 0.10 for abundance). Therefore, the seasonal biomass and abundance of groups of prey items were averaged per year in order to have a more robust data set representing the seasonal availability of prey for harbor seals.

The stable isotope compositions of trophic groups from the Sylt-Rømø Bight were similar between years of sampling among seasons (Kruskal Wallis rank sum test for $\delta^{13}C$: Planktivorous/Piscivorous, all p-values > 0.19; benthivorous/piscivorous, all p-values > 0.40; Benthivorous group, all p-values > 0.15; *O. eperlanus*, p-value > 0.05; Kruskal Wallis rank sum test for $\delta^{15}N$: Planktivorous/Piscivorous, all p-values > 0.62; benthivorous/piscivorous, all p-values > 0.08; Benthivorous group, all p-values > 0.05; *O. eperlanus*, p-value > 0.70). As a result, the stable isotope compositions of the different trophic groups were averaged on a seasonal basis for the construction of seasonal mixing models (see section 2.7. mixing models).

2.6.2. Trophic fractionation factors

$\delta^{13}C$ and $\delta^{15}N$ values are expressed as means, generally followed by standard deviations. As a net result of isotopic discrimination (i.e., the differential behavior of the stable isotopes during biochemical or physico-chemical reaction), the stable isotopic composition of a consumer is generally different than those of its potential prey. Such difference, called trophic fractionation factor (TFF) is the net result of all fractionations occurring during metabolism and enrichment is generally observed in heavier isotopes of consumer tissues compared to those of its preys. Isotopic composition of prey and predators was compared considering the trophic fractionation factor values in vibrissae from Hobson et al. (1996): TFF $\delta^{13}C$ = 3.2‰ and TFF $\delta^{15}N$ = 2.8‰. Little is known about the variability of TFFs among tissue, species and individuals for marine mammals. For this study, we used 0.8‰ and 0.1‰ as standard deviation for the TFFs of $\delta^{13}C$ and $\delta^{15}N$, respectively, in vibrissae as described in Lesage et al. (1999) for hairs, a keratin tissue comparable to vibrissae.

2.6.3. Temporal reconstruction of vibrissae

Growth rates used for reconstruction of the temporal variation in stable isotope composition of vibrissae were 0.78 mm.d^{-1} from May to September, and 0.075 mm.d^{-1} from October to April (Zhao and

Schell 2004). Most of the harbor seals were still alive when beaching, therefore the day of collection on the beach was considered to be the last day of vibrissae growth. Most of the sampled seals were emaciated and therefore probably starving in the last days of their life. However due to the inertia of this tissue (Hall-Aspland et al. 2005), once grown, the stable isotope composition of vibrissae is not modified with time (Zhao and Schell 2004, Cherel et al. 2009). Nevertheless, to avoid potential bias due to particular feeding behavior or fasting before the death, we removed the sections of vibrissae of potentially starving animals corresponding to the last days of their life from the data set. We thus considered that the vibrissae sections used in this study reflected the stable isotope composition of normally feeding individuals.

In order to exclude the potential influence of lactation and post weaning fast on the stable isotope composition of young-of-the-year (Bocherens et al. 1995, Hobson and Sease 1998, Jenkins et al. 2001, Polischuk et al. 2001, Newsome et al. 2006), we examined the monthly evolution of the $\delta^{15}N$ and $\delta^{13}C$ values of vibrissae sections of young-of-the-year and adults from May (i.e. month of birth of young-of-the-year) to December (Appendix 2, p. 87; Chapter 1). Both $\delta^{15}N$ and $\delta^{13}C$ values of vibrissae sections corresponding to young-of-the-year older than 2-3 months were similar to those of adults (Wilcoxon test, all p-values > 0.1, Appendix 2, p. 87; Chapter 1). Therefore, the sections of the vibrissae of young-of-the-year corresponding to months before September were removed for data analyses and sections of the vibrissae of young-of-the-year corresponding to months from and after September were kept for the analyses in order to use only yearling's vibrissae reflecting the same stable isotope composition as adults.

The temporal moving mean of $\delta^{13}C$ and $\delta^{15}N$ values, taking in account all vibrissae data corresponding to15 days on either side of the central value (30 days in total), was calculated in order to smooth out the short term and inter-individual variability of isotopic composition, and highlight the monthly trends. An example of the data treatment of 4 vibrissae is detailed in Appendix 3, p. 89. Seasonal variation of isotopic composition covering the period from March 2012 to February 2014 was divided into the four following intervals and then studied. Spring: March to May (n=4), summer: June to August (n=5), fall: September to November (n=16) and winter: December to February (n=9).

2.6.4. Statistical analyses

Non-parametric procedures were used to achieve more robust statistics in case of non-independence of data within series (e.g., two seasons along the same vibrissae) or small sample size (sample size ≤ 10). Kruskal–Wallis tests were applied on isotopic data in order to compare the different groups of prey items and to test for seasonal isotopic variations. These tests were followed by multiple pairwise comparisons using the Wilcoxon rank sum test. When data were independent and sample size was ≥ 10 (prey items from the Sylt-Rømø Bight), ANOVA followed by Tukey HSD tests were applied.

2.7. Mixing models

Relative contributions of the different prey trophic groups (isotopic sources) from the Sylt-Rømø Bight and from the North Sea, to the harbor seal diet were estimated by running the SIAR (Stable Isotope Analysis in R) mixing model (Parnell et al. 2010) using $\delta^{13}C$ and $\delta^{15}N$ values. In the model individual harbor seal isotope ratios were used while for prey species, means and standard deviations were entered. Trophic fractionation factor values were 3.2 ± 0.8‰ for $\delta^{13}C$ and 2.8 ± 0.1‰ for $\delta^{15}N$.

Four seasonal mixing models (i.e., spring, summer, fall, winter) were built to study seasonal changes of harbor seals food resources. These models were built using the seasonal mean isotopic values of each vibrissa as predator values (spring: n=4, summer: n=5, fall: n=16, winter: n=9), and the isotopic values per season of the different groups of prey items. For prey items, the yearly average was used when sample size was too small (n < 3; i.e., benthivorous/piscivorous group in winter and *O. eperlanus* in summer for the Sylt-Rømø Bight; planktivorous/piscivorous, benthivorous/piscivorous and benthivorous groups in spring and winter for the North Sea).

The models were run for 500 000 iterations and the first 50 000 iterations were discarded. Credibility intervals (CI) of 0.95, 0.75 and 0.25 were computed. CI is a contiguous interval that contains a specified proportion of the posterior probability (Edwards et al. 1963). For example, if the upper 0.95 CI is A and the lower 0.95 CI is B, the contribution value has 95% chance of lying between A and B.

3. Results

3.1. Seasonal variation of the fish biomass and abundance in the Sylt-Rømø Bight

In the Sylt-Rømø Bight, a strong seasonal pattern was observed in the CPUEm with low values in winter (83 g.h^{-1}) and much higher values in summer 411 g.h^{-1} (Fig. 2A). In all seasons, the CPUE$_m$ were largely dominated by planktivorous/piscivorous species in the Sylt-Rømø Bight (Fig. 2A), ranging from 45 g.h^{-1} (54.2% of the total biomass (TB)) to 321 g.h^{-1} (78.2% of the TB). Second highest CPUE$_m$ is represented by *Loligo spp.* in spring (27 g.h^{-1}, 13.0% of the TB), and is equally spread between benthivorous/piscivorous and benthivorous species in summer (42 g.h^{-1}, 10.0% of the TB and 30 g.h^{-1}, 7.3% of the TB, respectively), fall (26 g.h^{-1}, 15.1% of the TB and 31 g.h^{-1}, 18% of the TB, respectively) and winter (19 g.h^{-1}, 22.9% of the TB and 15 g.h^{-1}, 18.3% of the TB, respectively). The proportion of *O. eperlanus* CPUE$_m$ increased in summer compared to other seasons, but remained still low (15 g.h^{-1}, 4% of the TB; Fig. 2A).

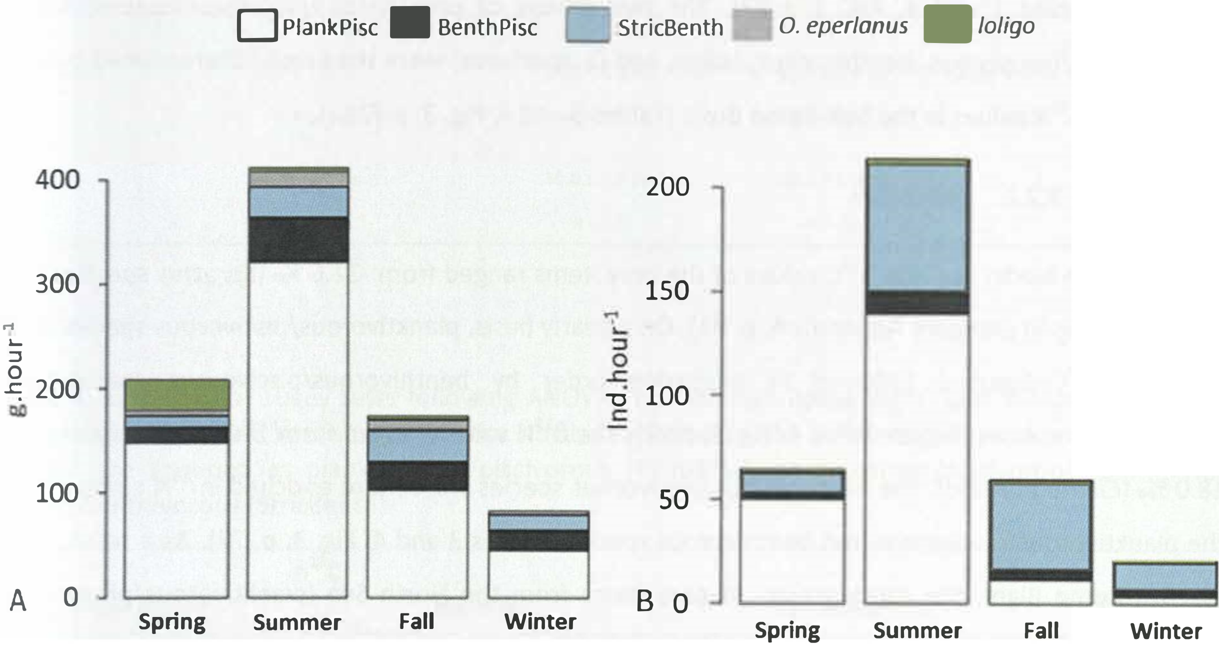


Fig. 2: CPUE$_m$ in g (A) and CPUE$_n$ (B) of the different groups of fish, shrimp and squid species per seasons. Fish species are grouped as planktivorous/piscivorous (PlankPisc), benthivorous/piscivorous (BenthPisc), or strictly benthivorous (StricBenth)

The highest CPUE$_n$ were recorded for planktivorous/piscivorous in spring (50 ind.h^{-1}) and summer (139 ind.h^{-1}; Fig. 2B, p.69) whereas benthivorous species were the most abundant in fall (43 ind.h^{-1}) and winter (14 ind.h^{-1}). The second most abundant groups were benthivorous species in spring and summer, and planktivorous/piscivorous species in fall (Fig. 2B, p.69). In winter, the second most abundant groups were both planktivorous/piscivorous and benthivorous/piscivorous species in equivalent importance (Fig. 2B).

3.2. *Stable isotope composition of prey species*

3.2.1. Sylt-Rømø Bight

In the Sylt-Rømø Bight, $\delta^{13}C$ values of potential prey items ranged from -23.5‰ (*O. eperlanus*) to -11.1‰ (*P. platessa*; Appendix 4, p. 91). On a yearly basis, planktivorous/piscivorous species and *Loligo spp*. were significantly more ^{13}C-depleted than *O. eperlanus*, benthivorous/piscivorous, benthivorous species, and benthivorous species were more ^{13}C-enriched than benthivorous/piscivorous species (Tables 3 and 4, Fig. 3, p.72). $\delta^{15}N$ values of potential prey items ranged from 12.2‰ (*C. harengus*) to 21.1‰ (*M. merlangus*). The benthivorous/piscivorous species and *O. eperlanus* had the highest $\delta^{15}N$ values, followed in decreasing order by benthivorous species, planktivorous/piscivorous species and *Loligo sp* (Tables 3 and 4, Fig. 3, p.72). The five groups of prey items (planktivorous/piscivorous, benthivorous/piscivorous, benthivorous, *Loligo*, and *O. eperlanus*) were then well differentiated by their $\delta^{13}C$ and/or $\delta^{15}N$ values in the Sylt-Rømø Bight (Tables 3 and 4, Fig. 3, p.72).

3.2.2. North Sea

In the North Sea, the $\delta^{13}C$ values of the prey items ranged from -22.6 ‰ (*Sprattus sprattus*) to -14.7 ‰ (*Crangon crangon*; Appendix 4, p. 91). On a yearly basis, planktivorous/piscivorous species were the most ^{13}C-depleted followed in increasing order by benthivorous/piscivorous species and benthivorous species (Tables 3 and 4; Fig. 3, p.72). The $\delta^{15}N$ values ranged from 13.7 ‰ (*P. platessa*) to 18.0 ‰ (*Ciliata mustela*). The benthivorous/piscivorous species were more enriched in ^{15}N compared to the planktivorous/piscivorous and benthivorous species (Tables 3 and 4; Fig. 3, p. 72). As a result, as in the Sylt-Rømø Bight, the three groups of prey items from the North Sea (planktivorous/piscivorous, benthivorous/piscivorous and benthivorous) are well differentiated owing to their isotopic compositions (Fig. 3).

3.2.3. Comparison of prey species between the Sylt-Rømø Bight and North Sea

The comparison between stable isotope composition of prey items in the Sylt-Rømø Bight and the North Sea revealed that prey items from each trophic group were significantly more ^{13}C-depleted in the North Sea than in the Sylt-Rømø Bight (Wilcoxon rank sum tests, p-values: Planktivorous/piscivorous: 0.012, benthivorous/piscivorous: < 0.001, benthivorous: < 0.001; Fig. 3, p. 72). However, the planktivorous/piscivorous group in the Sylt-Rømø Bight had similar stable isotopic composition to the benthivorous and benthivorous/piscivorous groups in the North Sea. Between the Sylt-Rømø Bight and North Sea, no difference of $\delta^{15}N$ values was observed for groups of prey (Wilcoxon rank sum tests, p-values: planktivorous/piscivorous: 0.576, benthivorous/piscivorous: 0.799, benthivorous: 0.383; Fig. 3, p.72).

Table 3: $\delta^{13}C$ and $\delta^{15}N$ values (mean ± standard deviation) of the different groups of prey items in the Sylt-Rømø Bight and the North Sea; n: sample size

	Planktivorous/piscivorous	**Benthivorous/piscivorous**	**Benthivorous**	***Loligo spp.***	***Osmerus eperlanus***
$\delta^{13}C$					
Sylt-Rømø Bight	-18.6 ± 1.3 ‰ n = 141	-17.1 ± 1.6 ‰ n = 118	-15.9 ± 1.7 ‰ n = 177	-19.0 ± 0.7 ‰ n = 15	-16.5 ± 2.8 ‰ n = 20
North Sea	-20.3 ± 1.4 ‰ n = 5	-18.8 ± 1.3 ‰ n = 23	-18.1 ± 1.4 ‰ n = 33		
$\delta^{15}N$					
Sylt-Rømø Bight	16.0 ± 1.0 ‰ n = 141	16.8 ± 1.3 ‰ n = 118	16.4 ± 0.9 ‰ n = 177	14.0 ± 1.0 ‰ n = 15	17.4 ± 1.0 ‰ n = 20
North Sea	15.8 ± 0.5 ‰ n = 5	16.8 ± 0.8 ‰ n = 23	16.2 ± 1.0 ‰ n = 33		

Table 4: Summary of Tukey tests following ANOVAs (for the Sylt-Rømø Bight) and Wilcoxon rank sum tests following Kruskal Wallis tests (for the North Sea) between the different groups of prey items. Fish species are grouped as planktivorous/piscivorous (PlankPisc), benthivorous/piscivorous (BenthPisc), strictly benthivorous (StricBenth)

$\delta^{13}C$				$\delta^{15}N$			
Sylt-Rømø Bight		**North Sea**		**Sylt-Rømø Bight**		**North Sea**	
p-value	**Comparisons of means**	**p-value**	**Comparisons of means**	**p-value**	**Comparisons of means**	**p-value**	**Comparisons of means**
*** < 0.001	PlankPisc < BenthPisc	** 0.008	PlankPisc < StricBenth	*** < 0.001	PlankPisc < BenthPisc	* 0.047	PlankPisc < BenthPisc
*** < 0.001	PlankPisc < StricBenth	º 0.077	PlankPisc < BenthPisc	*** < 0.001	*Loligo spp.* < BenthPisc	º 0.072	StricBenth < BenthPisc
*** < 0.001	PlankPisc < *O. eperlanus*	º 0.086	BenthPisc < StricBenth	º 0.069	StricBenth < BenthPisc		
*** < 0.001	*Loligo spp.* < BenthPisc			*** < 0.001	PlankPisc < *O. eperlanus*		
*** < 0.001	*Loligo spp.* < StricBenth			*** < 0.001	*Loligo spp.* < *O. eperlanus*		
*** < 0.001	*Loligo spp.* < *O. eperlanus*			** 0.002	StrictBenth < *O. eperlanus*		
*** < 0.001	BenthPisc < StricBenth			** 0.002	PlankPisc < StricBenth		
				*** < 0.001	*Loligo spp.* < StrictBenth		
				*** < 0.001	*Loligo spp.* < PlankPisc		

*** α risk < 0.001 ** α risk < 0.01 * α risk < 0.05 º α risk < 0.10

3.3. Stable isotopic composition of vibrissae

On a seasonal basis, the vibrissae were significantly more ^{13}C-depleted in spring (-16.1 ± 0.4 ‰, n=3) than in winter (-14.8 ± 0.5 ‰, n=9), fall (-15.0 ± 0.6 ‰, n=16) and summer (-14.7 ± 0.6 ‰, n=5; Wilcoxon sum rank tests, all p-values < 0.001; Fig. 3). The mean δ^{15}N value of vibrissae was equal to 18.7 ± 1.1 ‰. For δ^{15}N values the same seasonal trend was observed as for δ^{13}C, with significantly lower δ^{15}N values in vibrissae in spring (16.7 ± 1.2 ‰) than in winter (19.2 ± 0.4 ‰), fall (19.1 ± 0.9 ‰) and summer (19.0 ± 0.6 ‰; Wilcoxon rank sum tests, all p-values < 0.001; Fig. 3).

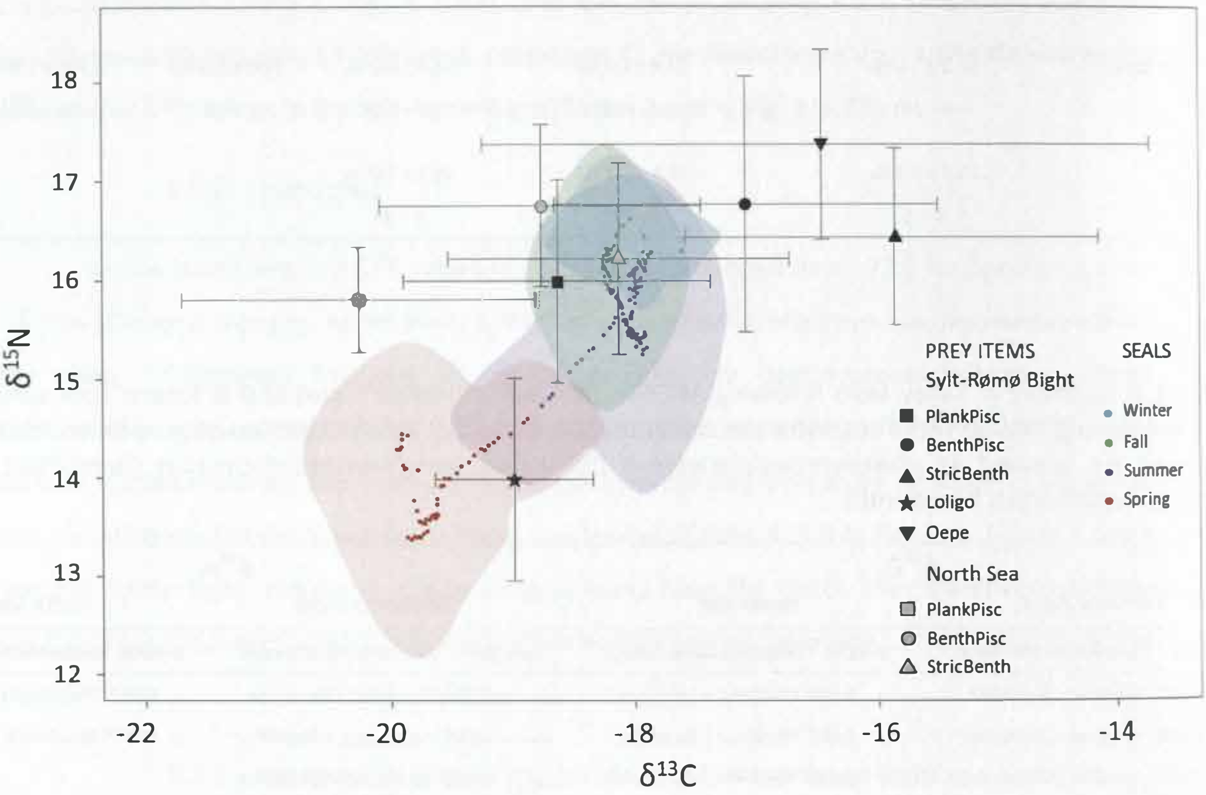


Fig. 3: Mean stable isotope compositions of the groups of prey items (error bars show standard deviations) compared to the moving mean of seal vibrissae per season. The shaded areas represent the isotopic range per season including all standard deviations from each value of the moving mean. Theoretical stable isotope values of prey foraged by seals were computed with TFFs of 3.2 ‰ and 2.8 ‰ for δ^{13}C and δ^{15}N, respectively. Fish species are grouped as planktivorous/piscivorous (PlankPisc), benthivorous/piscivorous (BenthPisc), strictly benthivorous (StricBenth).

3.4. Seasonal variation of the harbor seal´s diet

In every season, the δ^{13}C values of the theoretical prey items were calculated by subtracting the trophic enrichment factor from the vibrissae values and ranged between those of the prey items from the North Sea and the Sylt-Rømø Bight (Fig. 3). In spring, δ^{15}N values of theoretical prey items were much lower than in other seasons and were close to those of the Loligo group (Fig. 3).

At both locations (i.e., Sylt-Rømø Bight and North Sea), planktivorous/piscivorous had a high contribution to the diet in spring (CI 95 from 0% to 26% and from 2% to 31%; Fig. 4, p. 74). In the Sylt-Rømø Bight Loligo spp. had the highest contribution to the diet in spring (CI 95 from 1% to 31%). In summer, benthivorous/piscivorous species (CI 95 from 1% to 28% in the Sylt-Rømø Bight and from 0% to 27% in the North Sea) and benthivorous species (C I95 from 1% to 26% in the Sylt-Rømø Bight and from 0% to 26% in the North Sea) dominated the diet. O. eperlanus had the second highest contribution in the Sylt-Rømø Bight in summer (CI 95 from 3% to 26%; Fig. 4).

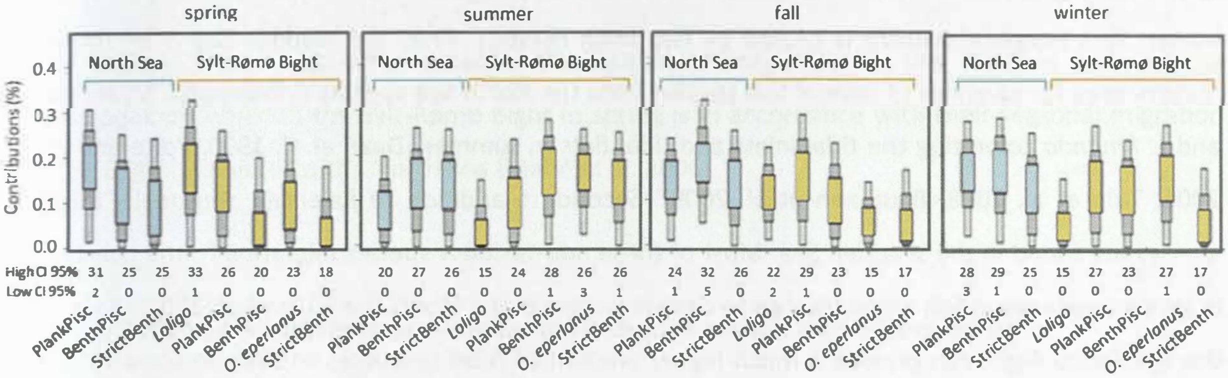


Fig. 4: Contributions per season of the different trophic groups of prey items to diet of seals. Contributions were computed by the SIAR mixing model. Higher and lower values of the 95% credibility intervals (CI) are shown for each trophic group and each season. Fish species are grouped as planktivorous/piscivorous (PlankPisc), benthivorous/piscivorous (BenthPisc) or strictly benthivorous (StricBenth).

In fall and winter, the order of contribution of the group of prey items from the Sylt-Rømø Bight differed from the contribution of these groups from the North Sea (Fig. 4). In the Sylt-Rømø Bight, planktivorous/piscivorous had the highest contribution in fall (CI 95 from 1% to 29%) and in winter (CI 95 from 0% to 27%) together with *O. eperlanus* (from 1% to 27%). In the North Sea, planktivorous/piscivorous, benthivorous/piscivorous and benthivorous had a relatively high contribution with a dominance of benthivorous/piscivorous in fall (CI 95 from 5% to 32%) and a dominance of benthivorous/piscivorous (CI 95 from 1% to 29%) and planktivorous/piscivorous (CI 95 from 3% to 28%) in winter (Fig. 4, p. 73).

4. Discussion

4.1. Large seasonal variation of prey species availability in the Sylt-Rømø Bight

Fish abundance observed in the Sylt-Rømø Bight was dominated by planktivorous/piscivorous and benthivorous species followed by benthivorous/piscivorous species. Biomass was also dominated by planktivorous/piscivorous species in the Sylt- Rømø Bight, mostly *C. harengus* and *A. tobianus*.

The seasonal patterns of biomass and abundance of fish observed in the Sylt-Rømø Bight are in accordance with the life cycle of several species, as already observed in the Wadden Sea and North Sea by other authors (Daan et al. 1990, Polte and Asmus 2006, Tulp et al. 2008, Baumann et al. 2009). In the Sylt-Rømø Bight, biomass and abundance of fish are at their maximum in summer and minimum in winter. This temporal pattern is caused by two main reasons. First, the Wadden Sea is an important nursery area for juveniles of several fish species from the North Sea such as *C. harengus, M. merlangus* and *L. limanda* colonizing the tidal inlets and tidal flats in summer (Daan et al. 1990, Polte and Asmus 2006, Tulp et al. 2008, Baumann et al. 2009). Second, in addition to juveniles, seasonally migrating species are found in the Wadden Sea. Most of these non-resident species migrate into the coastal zone in spring and leave in fall, when they go to deeper waters in the North Sea (Tulp et al. 2008). As a result, the Sylt-Rømø Bight can provide a much higher amount of food resources to seals in summer than in winter.

Some seasonal patterns are observed between the different trophic groups of prey species, which affect their availability to seals. Indeed, planktivorous/piscivorous species (e.g., *C. harengus* and *A. tobianus*) dominate the biomass in the Sylt-Rømø Bight but their abundance is high only in spring and summer. This indicates the presence of small sized individuals in spring and summer in contrast to relatively large individuals in fall and particularly in winter. This observation is in accordance with the high abundance of post larvae of *C. harengus* found in April and May by Dickey-Collas et al. (2009) in the German Bight.

Benthivorous species have the highest abundance in fall and winter, which is mainly due to the high amount of *C. crangon* in these seasons (88% and 80% of the biomass of benthivorous species, respectively). The biomass and abundance of benthivorous species increased in summer in the Sylt-Rømø Bight. This is related to: (1) the recruitment period of *P. minutus* and *P. microps* (del Norte-Campos and Temming 1994) and (2) the settlement of *P. platessa* juveniles in April (Mahé et al. 2006) following offshore spawning in January and February (Daan et al. 1990).

The abundance of benthivorous/piscivorous species (e.g., *M. merlangus*, *L. limanda*) decreased from summer to fall while the total biomass remained stable. This indicates the presence of juveniles from benthivorous/piscivorous species in summer in accordance to the spawning period of *M. merlangus* and *L. limanda* from February to May (Daan et al. 1990). The highest biomass of *M. scorpius* was observed in winter. This might be explained by the spawning from December to February when the adults are mobile and are therefore more easily caught with a trawl net (Luksenburg et al. 2004). *G. morhua* had also its highest biomass in winter which corresponds to the concentration of the juveniles in shallow water during their first winter, as observed along the coasts of Denmark, Germany and the Netherlands (Heessen 1993).

Little is known about the seasonal distribution of *Loligo spp.* in the Wadden Sea, but the biomass peak observed in the Sylt-Rømø Bight in spring is in accordance with their seasonal migration from the English Channel to the North Sea (Mahé et al. 2006).

4.2. Coastal vs. offshore gradient in stable isotope composition of prey items

In the Sylt-Rømø Bight, prey species showed a classical gradient of ^{13}C-enrichment, from planktivorous/piscivorous species (-18.6‰) and *Loligo spp.* (-19.0‰) - revealing, by their low δ^{13}C values, an influence of pelagic food resources (Pierce et al. 1994) - to benthivorous/piscivorous (-17.1‰) and strictly benthivorous (-15.9‰) species being more influenced by benthic food resources (Table3). This gradient is related to the ^{13}C-depletion of planktonic compared to benthic algae (France 1995, Heckey and Hesslein 1995). The presence of small sized pelagic fish and cephalopods in the diet of *M. merlangus* (Daan et al. 1990, Hislop et al. 1991), the main benthivorous/piscivorous species, might explain the lower δ^{13}C values of this group in comparison with benthivorous species (e.g., *Pomatoschistus spp.*, *P. platessa* and *Zoarces viviparus*), feeding only on benthic macrofauna (del Norte-Campos and Temming 1994, Rijnsdorp and Vingerhoed 2001).

The $\delta^{15}N$ values of the prey species in the Sylt-Rømø Bight encompassed a large range, from 12.2 to 21.1‰, demonstrating that the considered species covered several trophic levels (Peterson and Fry 1987). The low $\delta^{15}N$ values of *Loligo spp.* (14.0‰) suggest that these prey species have a lower trophic level than the other groups of potential prey items (from 16.0‰ to 17.4‰), which is in contrast to stomach content observations showing that *Loligo spp.* prey on fish, crustacean, polychaetes and other cephalopods (Pierce et al. 1994). However, the $\delta^{15}N$ values calculated for squids from the Atlantic Ocean (11.31 ± 2.06‰) and from temperate coastal and shelf areas (11.1 ± 2.1‰) by (Navarro et al. 2013), and measured in *Loligo spp.* from the North Sea (12.9‰) (Jennings et al. 2002) are even lower than those from the Sylt-Rømø Bight (14.0‰). These low $\delta^{15}N$ values suggest that trophic enrichment factors in *Loligo spp.* are lower than those in fish, maybe due to different metabolic processes.

In the North Sea, the same benthic *vs.* pelagic gradient was observed for the $\delta^{13}C$ values of benthivorous, benthivorous/piscivorous and planktivorous/piscivorous species as in the Sylt-Rømø Bight and can be explained in a similar way. Although the difference between planktivorous/piscivorous and benthivorous groups was not significant, the $\delta^{15}N$ values followed the same trend as in the Sylt-Rømø Bight, with a ^{15}N-enrichment from planktivorous/piscivorous to benthivorous and to benthivorous/piscivorous.

On a spatial scale, an inshore-offshore pattern was observed between the prey items in the Sylt-Rømø Bight and the ones in the North Sea. The prey species from the North Sea were predominantly influenced by oceanic food resources, while prey species in the Sylt-Rømø Bight were strongly influenced by benthic food resources (Hobson et al. 1994, Hobson 1999, Le Loc'h et al. 2008). A similar inshore-offshore gradient of $\delta^{13}C$ values was observed by Le Loc'h et al. (2008) in the Bay of Biscay.

4.3. Influence of pelagic prey species to the seal’s diet in spring compared to summer

Temporal variations of $\delta^{13}C$ values indicate a shift from a diet more strongly influenced by pelagic prey items in spring to a diet of more benthic prey items in summer (France 1995, Heckey and Hesslein 1995). This change is observed in both locations, the Sylt-Rømø Bight and the North Sea. In spring, the much lower $\delta^{15}N$ values of seals are close to those of *Loligo spp.* As a result, it is very likely that seals forage more intensely on *Loligo spp.* during this season. In spring and summer, a smaller number of individuals were included in the data analysis compared to fall and winter. Indeed, the young-of-the-year were not old enough to forage throughout the year, and their stable isotope composition, which was influenced by lactation and weaning periods, was not included in the data analysis in spring and summer.

Nevertheless, the seasonal variation in the harbor seal's diet observed in spring and summer is in accordance with studies by Brown and Pierce (1998), Hall et al. (1998), Andersen et al. (2007) and Berg et al. (2002) conducted in the southern North Sea. They show a high occurrence of pelagic species in spring (e.g., *C. harengus* and *A. tobianus*) and an increase of gadoids (e.g., *M. merlangus*) and flat fish (e.g., *P. platessa*, *Solea solea*, *P. flesus*) in seals gut contents in summer. This shift can be explained by a change in the availability of fish species (Tollit et al. 1997, Brown and Pierce 1998, Berg et al. 2002). In the Sylt-Rømø Bight, the high contribution of planktivorous/piscivorous and *Loligo* species to seals diet in spring coincides with the highest contribution of these two groups to the fish biomass in the Sylt-Rømø Bight, particularly the seasonal peak of *Loligo spp.*.

4.4. Harbor seals as benthic feeders

Although the biomass and abundance of planktivorous/piscivorous species remain very high in summer, highest contribution of benthivorous species to seals diet are observed in this season, when biomass and abundance of these species show their maximum in the Sylt-Rømø Bight. This confirms the opportunistic behavior of harbor seals foraging on one of the most abundant prey species in the sea, but not necessarily on the most abundant one (Tollit et al. 1997). Furthermore, the higher consumption by seals of benthivorous/piscivorous and benthivorous species when they become more available in summer confirms that harbor seals are primarily benthic feeders (Tougaard et al. 2003). This observation is supported by the results of gut content analysis conducted in the Wadden Sea in Schleswig Holstein, in which flat-fish (benthivorous/piscivorous and benthivorous) and gadoids (benthivorous/piscivorous) were observed as main prey items (Behrends 1985, Sievers 1989, Gilles et al. 2008). Furthermore, Härkönen (1987) showed that along the Danish coast of the North Sea, harbor seals consume the most abundant gadoid (benthivorous/piscivorous) species but do not feed on several other species of fish that are also numerous in this area.

4.5. Higher use of the North Sea resources in fall and winter

In fall and winter, outputs of the SIAR mixing models describe that harbor seals have a diet mostly based on pelagic species in the Sylt-Rømø Bight and/or on benthic species in the North Sea. The very low biomass observed in the Sylt-Rømø Bight during these seasons particularly in winter, suggests that the contributions of Sylt-Rømø Bight food sources were overestimated by the SIAR models. Furthermore, gut content studies of North Sea harbor seals found gadoids (e.g., *M. merlangus, G morhua*) as main prey items in fall and winter (Brown and Pierce 1998, Hall et al. 1998, Berg et al. 2002, Andersen et al. 2007). This is in accordance with the high contribution of benthivorous/piscivorous from the North Sea in fall and winter (5% to 32% and 1% to 29% respectively). Harbor seals might forage more in the North Sea than in the Sylt-Rømø Bight in these seasons. This hypothesis is supported by telemetry studies showing that seals tagged on the Rømø Island show strong seasonal variations in foraging behavior, with significantly longer foraging trips to the North Sea in winter independently of the age or the sex of the animals (Tougaard et al. 2003). Furthermore, Jensen (2015) counted in the Sylt-Rømø Bight about 80% less adult seals in December than in August. This decrease of seal abundance in the bight in winter support the hypothesis that seals might use more of the North Sea food resources in this season. A better knowledge about the stable isotope compositions of prey items from the North Sea and their seasonal and spatial variations would give a better understanding of foraging behavior of seals in the North Sea.

In summary, harbor seals might use the food resources of the Sylt-Rømø Bight and the North Sea in similar amounts in spring and summer with a shift from a pelagic based diet in spring to a benthic based diet in summer in both locations, whereas in fall and winter they probably forage more in the North Sea, seemingly on benthic influenced species.

5. Conclusions

In this study, we observed resource changes and spatial changes. Indeed, a higher influence of pelagic food resources is evident in the harbor seal's diet in spring whereas the diet is dominated by benthic food resources in summer, fall and winter. Furthermore, harbor seals might use more food resources of the Sylt-Rømø Bight in spring and summer compared to fall and winter when the biomass of prey items is relatively low. Thus, the Sylt-Rømø Bight has an important role as a foraging area for harbor seals in addition to its function as a resting and nursery area. The use of the Bight as a foraging area by a large colony of harbor seals might have a seasonal and relatively strong influence on the food web of the Sylt-Rømø Bight, particularly in spring and summer, when the seal abundance and the contribution of Sylt-Rømø Bight food resources to their diet are highest.

These results also highlight the necessity of much more detailed studies about temporal and spatial variations of marine mammal diets. For example, a potential competition of seals with fisheries for commercial species might strongly depend on seasons and location. Vibrissae can be used as very good recorders in marine mammal trophodynamics. Therefore, additional studies on growth rate of vibrissae are needed to precise the correspondence between the vibrissae length and the time scale. Furthermore, the combination of diet studies based on trophic markers such as stable isotopes with telemetry survey would be very valuable for management issues about highly mobile species such as harbor seals.

6. Acknowledgements

We would like to thank captain and crew of the AWI research vessel F. K. Mya, Alfred Resch and Kai von Bohlen, as well as the captain and crew of the SU3 Theodor Storm, from the Rømø Island. Petra Kadel and Birgit Hussel were of great help during field- and lab-work. We are grateful to the team of the Institute for Terrestrial and Aquatic Wildlife Research of University of Veterinary Medicine Hannover Foundation with whom we did the seals dissections, particularly Eva Wehrmeister, Miriam Hillmanm and Kornelia Schmidt for their assistance in collecting seals samples. We thank Cécile Vincent from the Chizé Centre for Biological Studies (UMR 7372 CNRS-University of La Rochelle, France) and Gilles Lepoint from the Laboratory of oceanology (Université de Liège, Belgique) for their valued advices at the beginning of the data treatment. We also thank the seal hunters from the island of Sylt who collected the seals along the coast of the Island.

7. References

Agreement on the Conservation of Seals in the Wadden Sea. 1990. Signed on 16 October 1990 by Denmark, Germany, Netherlands under the Bonn Convention.

Andersen, S. M., J. Teilmann, P. B. Harders, E. H. Hansen, and D. Hjollund. 2007. Diet of harbour seals and great cormorants in Limfjord, Denmark: interspecific competition and interaction with fishery. Ices Journal of Marine Science **64**:1235-1245.

Baumann, H., A. M. Malzahn, R. Voss, and A. Temming. 2009. The German Bight (North Sea) is a nursery area for both locally and externally produced sprat juveniles. Journal of Sea Research **61**:234-243.

Behrends, G. 1985. Zur Nahrungswahl von Seehunden (*Phoca vitulina L.*) im Wattenmeer Schleswig-Holsteins. Zeitschrift für Jagdwissenschaft **31**:3-14.

Benke, H., U. Siebert, R. Lick, B. Bandomir, and R. Weiss. 1998. The current status of harbour porpoises (*Phocoena phocoena*) in German waters. Arch. Fish. Mar. Res. **46**:97–123.

Berg, I., T. Haug, and K. T. Nilssen. 2002. Harbour Seal (*Phoca vitulina*) Diet in Vesteralen, North Norway Sarsia **87**:451-461.

Bern Convention. 1985. Council of Europe, CETS No.: 104. Convention on the Conservation of European Wildlife and Natural Habitats. Annex III: Protected fauna species. .

Bocherens, H., M. L. Fogel, N. Tuross, and M. Zeder. 1995. Trophic Structure and Climatic Information From Isotopic Signatures in Pleistocene Cave Fauna of Southern England. Journal of Archaeological Science **22**:327-340.

Bonn Convention. 1983. Convention on the conservation of Migratory species of Wild Animals (Article IV). Annex II: Migratory Species to be the Subject of Agreements.

Bowen, W. D. 1997. Role of marine mammals in aquatic ecosystems. Marine Ecology Progress Series **158**:267-274.

Brown, E. G., and G. J. Pierce. 1998. Monthly variation in the diet of harbour seals in inshore waters along the southeast Shetland (UK) coastline. Marine Ecology Progress Series **167**:275-289.

Bunn, S. E., N. R. Loneragan, and M. A. Kempster. 1995. Effects of acid washing on stable isotope ratios of C and N in penaid shrimp and seagrass: Implications for food web studies using multiple stable isotopes. Limnology and Oceanography **40**:4.

Caut, S., S. Laran, E. Garcia-Hartmann, and K. Das. 2011. Stable isotopes of captive cetaceans (killer whales and bottlenose dolphins). The Journal of Experimental Biology **214**:538-545.

Cherel, Y., L. Kernaléguen, P. Richard, and C. Guinet. 2009. Whisker isotopic signature depicts migration patterns and multi-year intra- and inter-individual foraging strategies in fur seals. Biologie Letters **5**:830-832.

Daan, N., P. J. Bromley, J. R. G. Hislop, and N. A. Nielsen. 1990. Ecology of North Sea fish. Netherlands Journal of Sea Research **26**:343-386.

Das, K., G. Lepoint, Y. Leroy, and J. M. Bouquegneau. 2003. Marine mammals from the southern North Sea: feeding ecology data from δ^{13}C and δ^{15}N measurements. Marine Ecology Progress Series **263**:287-298.

del Norte-Campos, A. G. C., and A. Temming. 1994. Daily activity, feeding and rations in gobies and brown shrimp in the northern Wadden Sea. Marine Ecology Progress Series **115**:13.

DeNiro, M. J., and S. Epstein. 1978. Influence of diet on the distribution of carbon isotopes in animals. Geochimica et Cosmochimica Acta **42**:495-506.

Dickey-Collas, M., L. Bolle, J. van Beek, and P. Erftemeijer. 2009. Variability in transport of fish eggs and larvae. II. Effects of hydrodynamics on the transport of Downs herring larvae. Marine Ecology Progress Series **390**:183-194.

Drescher, H. E. 1979. Biology, ecology and conservation of harbour seals in the tidelands of Schleswig-Holstein. Beitr. Wildbiol. **1**:1-73.

Edwards, W., H. Lindman, and L. J. Savage. 1963. Bayesian statistical inference for psychological research. Psychological Review **70**:193-242.

Estes, J. A. 1979. Exploitation of Marine Mammals: r-Selection of K-Strategists? Journal of the Fisheries Research Board of Canada **36**:1009-1017.

EU Habitats Directive. 1992. Council Directive 92/43/EEC of 21 May 1992 on the conservation of natural habitats and wild fauna and flora - consolidated version 01/01/2007. Annex II: Animal and plant species of community interest whose conservation requires the designation of special areas of conservation. Annex V: Animal and plant species of community interest whose taking in the wild and exploitation my be subject to management measures. .

Federal Agency for Nature Conservation, and Germany. 2009. Red List of threatened species

France, R. 1995. Carbon-13 enrichment in benthic compared to planktonic algae: foodweb implications. Marine Ecology Progress Series **124**:307-312.

Froese, R., and D. Pauly. 2014. Data Base: FishBase [Internet].

Fry, B. 1988. Food web structure on Georges Bank from stable C, N and S isotopic compositions. Limnology and Oceanography **33**:9.

Galatius, A., S. Brasseur, R. Czeck, B. Diederichs, L. F. Jensen, P. Körber, U. Siebert, J. Teilmann, and S. Klöpper. 2014. Aerial surveys of Harbour Seals in the Wadden Sea in 2014 - The highest pup count recorded yet.

Gilles, A., H. Andreasen, S. Müller, and U. Siebert. 2008. Nahrungsökölögie von marinen Säugetieren und Seevögeln für das Management von NATURA 2000 Gebieten. Endbericht für das Bundesamt für Naturschutz., Federal Agency for Nature Conservation, Germany.

Hall-Aspland, S. A., T. L. Rogers, and R. B. Canfield. 2005. Stable carbon and nitrogen isotope analysis reveals seasonal variation in the diet of leopard seals. Marine Ecology Progress Series **305**:249-259.

Hall, A. J., J. Watkins, and P. S. Hammond. 1998. Seasonal variation in the diet of harbour seals in the south-western North Sea. Marine Ecology Progress Series **170**:269-281.

Härkönen, T. 1987. Seasonal and regional variations in the feeding habits of the harbour seal, *Phoca vitulina,* in the Skagerrak and the Kattegat. Journal of Zoology **213**:535-543.

Härkönen, T., and M. P. Heide-Jørgensen. 1991. The harbour seal *Phoca vitulina* as a predator in the Skagerrak. Ophelia **34**:191-207.

Heckey, R. E., and R. H. Hesslein. 1995. Contributions of Benthic Algae to Lake Food Webs as Revealed by Stable Isotope Analysis. Journal of the North American Benthological Society **14**:631-653.

Heessen, H. J. L. 1993. The Distribution of Cod (*Gadus morhua*) in the North Sea. NAFO Scientific Council Studies **18**:7.

Hislop, J. R. G., A. P. Robb, M. A. Bell, and D. W. Armstrong. 1991. The diet and food consumption of whiting (*Merlangius merlangus*) in the North Sea. ICES Journal of Marine Science: Journal du Conseil **48**:139-156.

Hobson, K. A. 1999. Tracing origins and migration of wildlife using stable isotopes: a review. Oecologia **120**:314-326.

Hobson, K. A., J. F. Piatt, and J. Pitocchelli. 1994. Using Stable Isotopes to Determine Seabird Trophic Relationships. Journal of Animal Ecology **63**:786-798.

Hobson, K. A., D. M. Schell, D. Renouf, and E. Noseworthy. 1996. Stable carbon and nitrogen isotopic fractionation between diet and tissues of captive seals: implications for dietary reconstructions involving marine mammals. Canadian Journal of Fisheries and Aquatic Sciences **53**:528-533.

Hobson, K. A., and J. L. Sease. 1998. Stable isotope analyses of tooth annuli reveal temporal dietary records: an example using steller sea lions. Marine Mammal Science **14**:116-129.

Hobson, K. A., J. L. Sease, R. L. Merrick, and J. F. Piatt. 1997. Investigating trophic relationships of pinnipeds in Alaska and Washington using stable isotope ratios of Nitrogen and Carbon. . Marine Mammal Science **13**:114-132.

Hobson, K. A., and H. E. Welch. 1992. Determination of trophic relationships within a high Arctic marine food web using δ^{13}C and δ^{15}N analysis. Marine Ecology Progress Series **84**:10.

Hoffmann, M., J. L. Belant, J. S. Chanson, N. A. Cox, J. Lamoreux, A. S. L. Rodrigues, J. Schipper, and S. N Stuart. 2011. The changing fates of the world's mammals. Philosophical transactions of the Royal Society B **366**:2598-2610.

International Union for the Conservation of Nature and Natural Resources, and IUCN. 2012. The IUCN Red List of Threatened Species

Jenkins, S., S. Partridge, T. Stephenson, S. Farley, and C. Robbins. 2001. Nitrogen and carbon isotope fractionation between mothers, neonates, and nursing offspring. Oecologia **129**:336-341.

Jennings, S., J. K. Pinnegar, N. V. C. Polunin, and K. J. Warr. 2002. Linking size-based and trophic analyses of benthic community structure. Marine Ecology Progress Series **226**:77-85.

Jensen, L. 2015. Monitoring of the seal population in the Wadden Sea in 2015 - Monitering af sælbestanden i Vadehavet 2015. Aarhus Universitet, Miljøministeriet, Fiskeri-og Søfartsmuseet Saltvandsakvariet Esbjerg.

Kellnreitner, F., M. Pockberger, and H. Asmus. 2012. Seasonal variation of assemblage and feeding guild structure of fish species in a boreal tidal basin. Estuarine, Coastal and Shelf Science **108**:97-108.

Le Loc'h, F., C. Hily, and J. Grall. 2008. Benthic community and food web structure on the continental shelf of the Bay of Biscay (North Eastern Atlantic) revealed by stable isotopes analysis. Journal of Marine Systems **72**:17-34.

Lesage, V., M. O. Hammill, and K. M. Kovacs. 1999. Functional classification of harbor seal (Phoca vitulina) dives using depth profiles, swimming velocity, and an index of foraging success. Canadian Journal of Zoology **77**:74-87.

Lesage, V., M. O. Hammill, and K. M. Kovacs. 2001. Marine mammals and the community structure of the Estuary and Gulf of St Lawrence, Canada: evidence from stable isotope analysis. Marine Ecology Progress Series **210**:203-221.

Lotze, H., K. Reise, B. Worm, J. van Beusekom, M. Busch, A. Ehlers, D. Heinrich, R. Hoffmann, P. Holm, C. Jensen, O. Knottnerus, N. Langhanki, W. Prummel, M. Vollmer, and W. Wolff. 2005. Human transformations of the Wadden Sea ecosystem through time: a synthesis. Helgoland Marine Research **59**:84-95.

Luksenburg, J. A., T. Pedersen, and I. B. Falk-Petersen. 2004. Reproduction of the shorthorn sculpin *Myoxocephalus scorpius* in northern Norway. Journal of Sea Research **51**:157-166.

Mahé, K., J. P. Delpech, and A. Carpentier. 2006. Synthèse bibliographique des principales espèces de Manche orientale et du golfe de Gascogne. Convention Ifremer-Ministère de l'Industrie Centre Manche-mer du Nord. Département Halieutique. Laboratoire Ressources Halieutiques de Boulogne sur mer.

Martens, P., and J. E. E. v. Beusekom. 2008. Zooplankton response to a warmer northern Wadden Sea. Helgoland Marine Research **62**:67-75.

Navarro, J., M. Coll, C. J. Somes, and R. J. Olson. 2013. Trophic niche of squids: Insights from isotopic data in marine systems worldwide. Deep Sea Research Part II: Topical Studies in Oceanography **95**:93-102.

Newland, C., I. C. Field, Y. Cherel, C. Guinet, C. J. A. Bradshaw, C. R. McMahon, and M. A. Hindell. 2011. Diet of juvenile southern elephant seals reappraised by stable isotopes in whiskers. Marine Ecology Progress Series **424**:247-258.

Newsome, S. D., P. L. Koch, M. A. Etnier, and D. Aurioles-Gamboa. 2006. Using Carbon and Nitrogen isotope values to investigate maternal strategies in Northeast Pacific Otariids. Marine Mammal Science **22**:556-572.

Newsome, S. D., M. T. Tinker, D. H. Monson, O. T. Oftedal, K. Ralls, M. M. Staedler, M. L. Fogel, and J. A. Estes. 2009. Using stable isotopes to investigate individual diet specialization in California sea otters (*Enhydra lutris nereis*). Ecology **90**:961-974.

Oh C.-W., R. G. Hartnoll, and R. D. M. Nash. 2001. Feeding ecology of the common shrimp *Crangon crangon* in Port Erin Bay, Isle of Man, Irish Sea. Marine Ecology Progress Series **214**:211-223.

Osinga, N., I. Pen, H. A. Udo de Haes, and P. M. Brakefield. 2012. Evidence for a progressively earlier pupping season of the common seal (*Phoca vitulina*) in the Wadden Sea. Journal of the Marine Biological Association of the United Kingdom **92**:1663-1668.

Parnell, A. C., I. R., S. Bearhop, and A. L. Jackson. 2010. Source partioning using stable isotopes: coping with too much variation. PLoS ONE **5**:1.

Peterson, B. J., and B. Fry. 1987. Stable Isotopes in Ecosystem Studies. Annual Review of Ecology and Systematics **18**:293-320.

Pierce, G. J., P. R. Boyle, L. C. Hastie, and M. B. Santos. 1994. Diets of squid *Loligo forbesi* and *Loligo vulgaris* in the northeast Atlantic. Fisheries Research **21**:149-163.

Pierce, G. J., P. M. Thompson, A. Miller, J. S. W. Diack, D. Miller, and P. R. Boyle. 1991. Seasonal variation in the diet of common seals (*Phoca vitulina*) in the Moray Firth area of Scotland. Journal of Zoology **223**:641-652.

Pinnegar, J., and N. Polunin. 1999. Differential fractionation of $\delta^{13}C$ and $\delta^{15}N$ among fish tissues: implications for the study of trophic interactions. Functional ecology **13**:225-231.

Pockberger, M. 2015. Abiotic and biotic impacts on fish in the Wadden Sea. Evaluating the effect of large scale climate oscillations, local ecosystem characteristics and invasive species. Doctoral dissertation, The Christian Albrecht University of Kiel.

Polischuk, S. C., K. A. Hobson, and M. A. Ramsay. 2001. Use of stable-carbon and -nitrogen isotopes to assess weaning and fasting in female polar bears and their cubs. Canadian Journal of Zoology **79**:499-511.

Polte, P., and H. Asmus. 2006. Intertidal seagrass beds (*Zostera noltii*) as spawning grounds for transient fishes in the Wadden Sea. Marine Ecology-Progress Series **312**:235-243.

Power, G., and J. Gregoire. 1978. Predation by Freshwater Seals on the Fish Community of Lower Seal Lake, Quebec. Journal of the Fisheries Research Board of Canada **35**:844-850.

Rau, G. H., A. J. Mearns, D. R. Young, R. J. Olson, H. A. Schafer, and I. R. Kaplan. 1983. Animal C/C Correlates with Trophic Level in Pelagic Food Webs. Ecology **64**:1314-1318.

Reijnders, P. J. H., S. M. J. M. Brasseur, T. Borchardt, K. Camphuysen, R. Czeck, A. Gilles, L. F. Jensen, M. Leopold, K. Lucke, S. Ramdohr, M. Scheidat, U. Siebert, and J. Teilmann. 2009. Marine mammals. Thematic Report N°20. In: Marencic, H. and Vlas, J. de, editors. Quality status report 2009. Wadden Sea Ecosystem N°25. Wadden Sea Secretariat, Trilateral Monitoring and Assessment Group, Wilhelmshaven, Germany.

Reijnders, P. J. H., S. M. J. M. Brasseur, and E. H. W. G. Meesters. 2010. Earlier pupping in harbour seals, *Phoca vitulina.* Biologie Letters.

Reijnders, P. J. H., and K. Lankester. 1990. Status of marine mammals in the North sea. Netherlands Journal of Sea Research **26**:427-435.

Reijnders, P. J. H., E. H. Ries, S. Tougaard, N. Nørgaard, G. Heidemann, J. Schwarz, E. Vareschi, and I. M. Traut. 1997. Population development of harbour seals *Phoca vitulina* in the Wadden Sea after the 1988 virus epizootic. Journal of Sea Research **38**:161-168.

Rijnsdorp, A. D., and B. Vingerhoed. 2001. Feeding of plaice *Pleuronectes platessa* L. and sole *Solea solea* (L.) in relation to the effects of bottom trawling. Journal of Sea Research **45**:219-229.

Siebert, U., P. Wohlsein, K. Lehnert, and W. Baumgärtner. 2007. Pathological Findings in Harbour Seals (*Phoca vitulina*): 1996–2005. Journal of Comparative Pathology **137**:47-58.

Sievers, U. 1989. Stomach content analysis in the harbour seal (*Phoca vitulina*) from the Schleswig-Holstein Waddensea. Zool. Anz. **222**:249–260.

Smardon, R. C. 2009. The Wadden Sea Wetlands: A multi-juridictional Challenge. Pages 21-54 Sustaining the World´s Wetlands - Setting Policy and Resolving Conflicts. springer science and Business Media, College of Environmental Science and Foresty, State University of New York.

Thompson, P. M., B. J. McConnell, D. J. Tollit, A. Mackay, C. Hunter, and P. A. Racey. 1996. Comparative Distribution, Movements and Diet of Harbour and Grey Seals from Moray Firth, N. E. Scotland Journal of Applied Ecology **33**:1572-1584.

Thompson, P. M., G. J. Pierce, J. R. G. Hislop, D. Miller, and J. S. W. Diack. 1991. Winter Foraging by Common Seals (*Phoca vitulina*) in Relation to Food Availability in the Inner Moray Firth, N.E. Scotland. Journal of Animal Ecology **60**:283-294.

Tieszen, L. L., T. W. Boutton, K. G. Tesdahl, and N. A. Slade. 1983. Fractionation and turnover of stable carbon isotopes in animal tissues: Implications for δ^{13}C analysis of diet. Oecologia **57**:32-37.

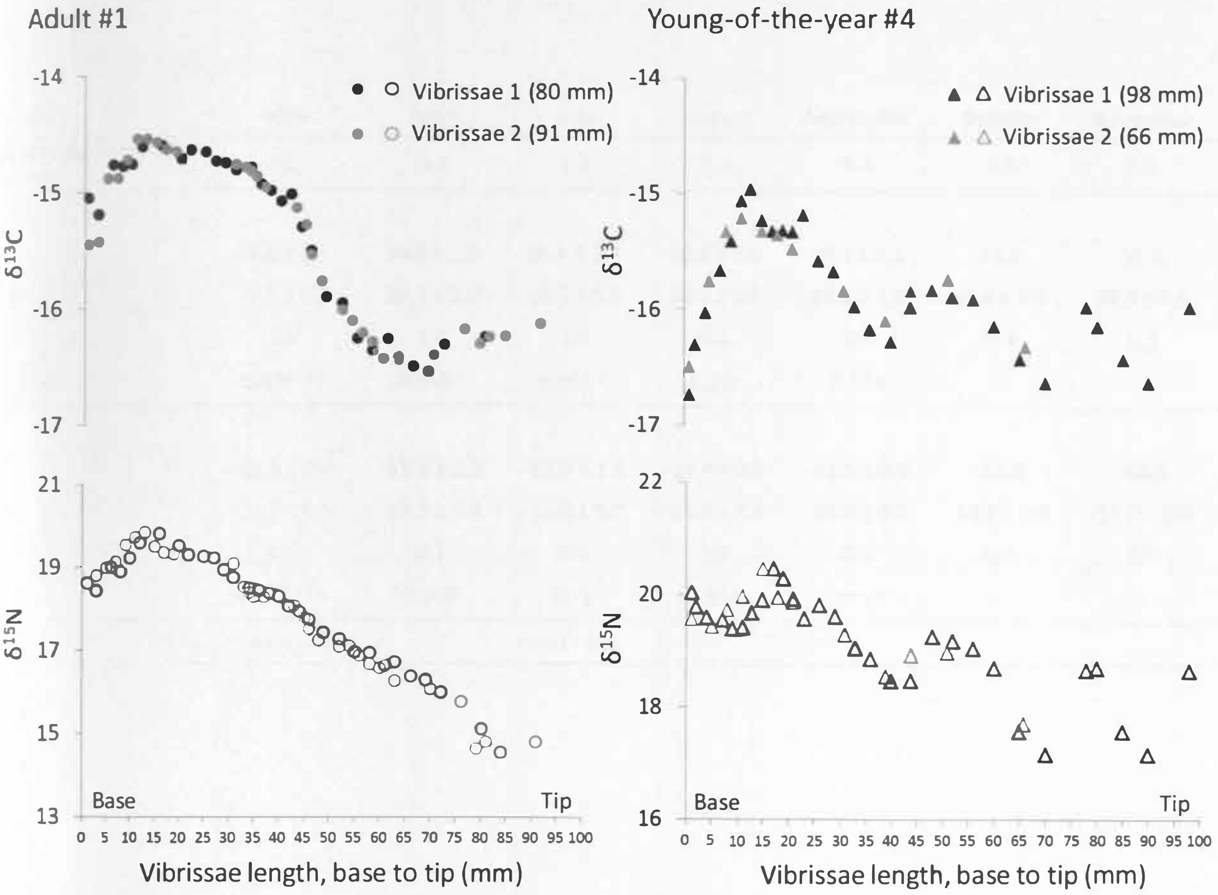
Tollit, D. J., P. M. Thompson, and S. P. R. Greenstreet. 1997. Prey selection by harbour seals, Phoca vitulina, in relation to variations in prey abundance. Canadian Journal of Zoology **75**:1508-1518.

Tougaard, J., I. Ebbesen, S. Tougaard, J. T., and J. Teilmann. 2003. Satellite tracking of harbour seals on Horns Reef. Technical report to Techwise A/S, Biological Papers from the Fisheries and Maritime Museum, Esbjerg.

Tulp, I., L. J. Bolle, and A. D. Rijnsdorp. 2008. Signals from the shallows: In search of common patterns in long-term trends in Dutch estuarine and coastal fish. Journal of Sea Research **60**:54-73.

Zhao, L., and D. M. Schell. 2004. Stable isotope ratios in harbor seal *Phoca vitulina* vibrissae: effects of growth patterns on ecological records. Marine Ecology Progress Series **281**:267-273.

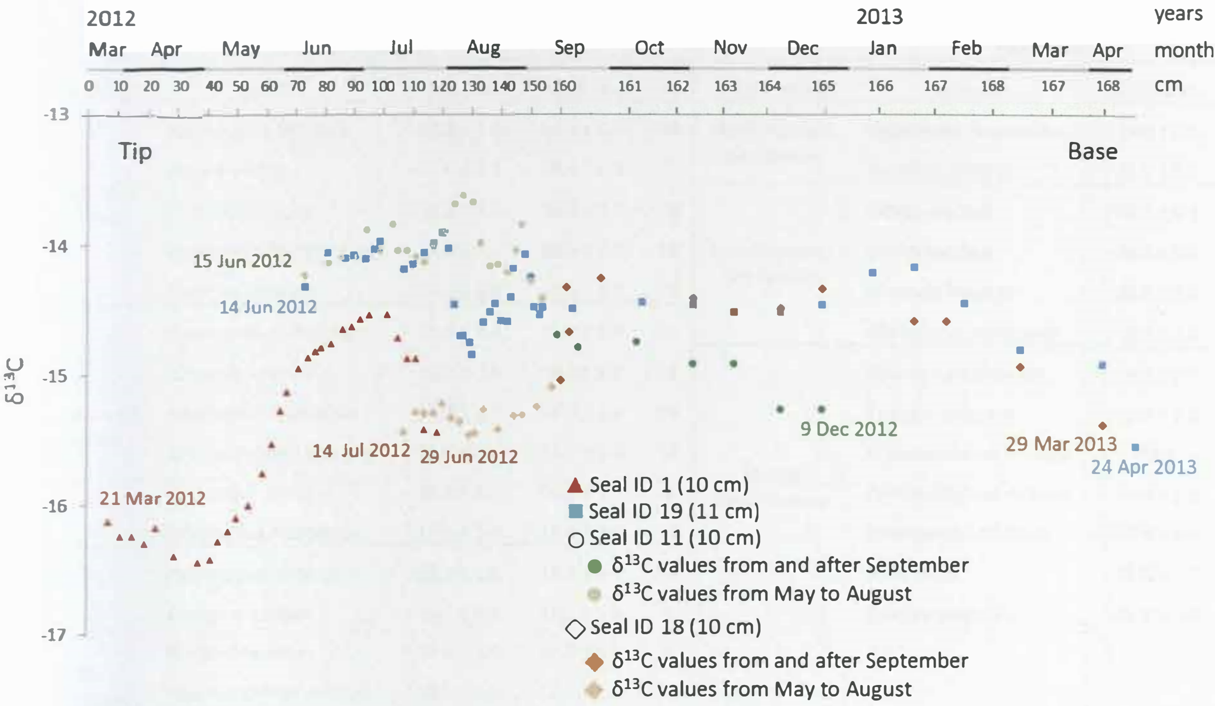
Appendix 1: $\delta^{13}C$ (filled circles and triangles) and $\delta^{15}N$ (open circles and triangles) values along the length of two vibrissae from an adult (circles) and two vibrissae from a young-of-the-year (triangles). The total length (mm) of each vibrissa is expressed in the legend in between parentheses. Information about both individuals is displayed in Table 1, p. 63.



Appendix 2: mean values and standard deviations of harbor seals adults and young-of-the-year, difference between the mean value of young-of-the-year and the mean value of adults ($\Delta\delta^{15}N$ and $\Delta\delta^{13}C$), results of the non-parametric Wilcoxon tests comparing the values of young-of-the-year and adults per month for $\delta^{15}N$ and $\delta^{13}C$. Data in bold, corresponding to vibrissae from adults and section of vibrissae from young-of-the-year from and after September (older than 3-4 months), were used in this study.

Months	May	June	July	August	September	October	November	December
g age of young-of-the-year (months)	<1	0-1	1-2	2-3	3-4	4-5	5-6	6-7
	16.0 ± 0.1	**18.0 ± 1.1**	**18.9 ± 1.4**	**19.8 ± 1.0**	**19.1 ± 0.8**	**19.0**	**19.0**	**19.4 ± 0.1**
-year	19.8 ± 0.8	19.7 ± 0.8	19.9 ± 0.8	20.0 ± 0.9	**19.3 ± 1.0**	**18.8 ± 0.9**	**19.3 ± 0.6**	**19.2 ± 0.4**
	3.8	1.7	1.0	0.2	0.2	0.3	0.3	0.2
t p-value	0.009 **	0.048 *	0.061 º	0.332	0.574	-	-	0.801
	-16.6 ± 0.2	**-15.2 ± 1.2**	**-15.0 ± 1.1**	**-14.4 ± 0.2**	**-14.9 ± 0.5**	**-14.5**	**-14.5**	**-14.2 ± 0.2**
-year	-14.9 ± 0.6	-15.1 ± 0.6	-15.0 ± 0.7	-14.8 ± 0.6	**-15.0 ± 0.7**	**-14.9 ± 0.6**	**-15.0 ± 0.6**	**-15.1 ± 0.5**
	1.7	0.1	0.0	0.4	0.1	0.4	0.5	0.8
t p-value	0.009***	0.978	0.71	0.551	0.813	-	-	0.133
	* α risk < 0.05		º α risk < 0.10					

Appendix 3: Reconstruction of temporal variation of carbon isotopic composition along vibrissae of two adults (seals ID 1 and ID 19; Table 1, p. 63) and two young-of-the-year (seals ID 11 and ID 18; Table 1, p. 63).



All vibrissae were treated in this manner for both Carbon and Nitrogen stable isotope ratios and were included in the moving mean for seasonal variation study.

Correspondence Time/Length: The growth rates used for reconstitution of temporal variation were 0.78 mm.d^{-1} from May to September, and 0.075 mm.d^{-1} from October to April (Zhao and Schell 2004). The time period covered by all vibrissae together goes from March 2012 to April 2013.

Young-of-the-year from May to September: the $\delta^{13}C$ and $\delta^{15}N$ values of young-of-the-year corresponding to the periods of time from May to September were not taking in account in the analyses for the diet study to avoid bias due to the influence of lactation and post weaning fast periods (Personal Data).

Appendix 4: $\delta^{13}C$ and $\delta^{15}N$ values (mean ± standard deviation) of the prey items species from the Sylt-Rømø Bight and the North Sea. n: sample size. These data were collected from April 2008 to November 2009 (Kellnreitner et al. 2012) and from January to November 2013 (present study).

Sylt-Rømø Bight

trophic group	species	d13C (‰)	d15N (‰)	n
planktivorous/ piscivorous	*Ammodytes tobianus*	-19.0 ± 1.2	16.0 ± 0.9	38
	Belone belone	-17.0 ± 1.1	16.5 ± 0.6	7
	Clupea harengus	-18.8 ± 0.4	16.0 ± 0.7	54
	Hyperoplus lanceolatus	-18.4 ± 1.6	16.0 ± 1.3	14
	Sprattus sprattus	-18.3 ± 1.9	15.8 ± 1.1	28
Benthivorous/ piscivorous	*Gasterosteus aculeatus*	-18.9 ± 1.4	16.9 ± 0.6	9
	Limanda limanda	-16.9 ± 0.6	16.6± 1.0	18
	Merlangius merlangus	-17.1 ± 1.9	16.8 ± 1.3	36
	Myoxocephalus scorpius	-15.6 ± 0.6	17.1 ± 1.0	17
	Platichthys flesus	-16.2 ± 1.3	16.2 ± 1.7	8
	Syngnathus rostellatus	-17.6 ± 1.0	16.8 ± 0.6	30
Strictly benthivorous	*Agonus cataphractus*	-15.9 ± 1.6	17.2 ± 0.9	19
	Crangon crangon	-16.1 ± 1.2	16.1 ± 1.3	9
	Pholis gunnellus	-16.6 ± 2.8	17.0 ± 1.0	9
	Pomatoschistus microps	-14.1 ± 1.6	17.0 ± 1.0	21
	Pomatoschistus minutus	-16.9 ± 1.0	16.5 ± 0.4	42
	Pleuronectes platessa	-15.7 ± 1.8	15.8 ± 0.5	61
	Zoarces viviparus	-15.6 ± 1.6	16.7 ± 0.6	16
	loligo sp.	-19.0 ± 0.8	14.0 ± 1.0	15
	Osmerus eperlanus	-16.5 ± 0.9	17.4 ± 0.7	20

North Sea

trophic group	species	d13C (‰)	d15N (‰)	n
planktivorous/ piscivorous	*Hyperoplus lanceolatus*	-19.8 ± 0.9	16.2 ± 0.1	3
	Sprattus sprottus	-21.0 ± 2.2	15.2 ± 0.1	2
Benthivorous/ piscivorous	*Ciliata mustela*	-18.2 ± 0.4	17.6 ± 0.6	2
	Gadus morhua	-18.4 ± 0.5	17.7 ± 0.3	3
	Limanda limanda	-20.0 ± 1.0	15.6 ± 0.2	6
	Merlangius merlangus	-18.4 ± 1.3	16.9 ± 0.4	12
Strictly benthivorous	*Agonus cataphractus*	-16.8 ± 0.1	17.4 ± 0.2	3
	Crangon crangon	-17.3 ± 2.2	16.2 ± 0.7	6
	Pomatoschistus microps	-19.1	15.0	1
	Pomatoschistus minutus	-17.9 ± 0.2	16.3 ± 0.1	5
	Pleuronectes platessa	-19.9 ± 0.9	14.7 ± 0.7	6
	Solea solea	-18.5 ± 0.7	16.4 ± 0.8	3
	Zoarces viviparus	-17.8 ± 0.6	16.8 ± 0.5	9

Chapter 3

Shift between spring and summer in the diet of harbor seals of the Sylt-Rømø Bight assessed with fatty acid analysis


© Camille de la Vega

Shift between spring and summer in the diet of harbor seals of the Sylt-Rømø Bight assessed with fatty acid analysis

Camille de la Vega[1], Benoit Lebreton[2], Ragnhild Asmus[1], Ursula Siebert[3], Harald Asmus[1]

[1] Alfred-Wegener-Institut Helmholtz-Zentrum für Polar- und Meeresforschung, Wattenmeerstation Sylt
Hafenstr. 43, D-25992 List/ Sylt, Germany
[2] UMR Littoral, Environnement et Sociétés (LIENSs), CNRS - Université de la Rochelle, Institut du Littora
et de l'Environnement, 2 rue Olympe de Gouges, 17 000 La Rochelle, France
[3] Stiftung Tierärztliche Hochschule Hannover Institut für Terrestrische und Aquatische Wildtierforschung
Werftstr. 6, 25761 Büsum, Germany

*Corresponding author: camille.de.la.vega@awi.de

Abstract

Knowledge about the foraging ecology of marine mammals is crucial to understand their influence on food webs and to improve ecosystem management measures. But their diet is difficult to investigate with classical methods. Fatty acid composition was recently proved to be an efficient tool to determine predator´s diet. In this study, fatty acid composition of muscles of harbor seals (*Phoca vitulina*) and their potential prey species from the Sylt-Rømø Bight were analyzed to assess seasonal variation of the seal´s diet. Prey species were well characterized by their level in specific dietary fatty acids which showed seasonally variation in the seal´s muscles. The fatty acid composition of the seals suggested a shift in their diet, more influenced by *Clupea harengus* and *Ammodytes tobianus* in spring and by *Pleuronectes platessa* and *Osmerus eperlanus* in summer.

Key words

Fatty acid composition; Wadden Sea; harbor seals, muscle tissue, prey species, diet, seasonal variation

1. Introduction

Harbor seal (*Phoca vitulina*) is one of the most abundant marine mammal species in the Wadden Sea (Reijnders et al. 2009, Siebert et al. 2012) and they subsist largely on fish (Härkönen 1987, Härkönen and Heide-Jørgensen 1991, Brown and Pierce 1998, Hall et al. 1998, Siebert et al. 2012), although mollusks and crustaceans may represent a significant part of their diet (Behrends 1985, Sievers 1989). Although harbor seals can travel more than 100 kilometers away from their haul outs to forage in some seasons (Tougaard et al. 2003, Reijnders et al. 2005), they might exert a relatively strong pressure of predation on the Wadden Sea food resources, particularly in spring and summer (de la Vega et al. 2016) (Chapter 2). Understanding the foraging ecology of harbor seals is critical to evaluate how they function within ecosystems (Bowen 1997, Iverson et al. 1997). Better knowledge about food resources of harbor seals at the species level would allow their influence on the Wadden Sea ecosystem to be more precisely evaluated and conservation and management measures to be improved.

Most pinnipeds are top predators and studies about their feeding ecology face a number of inherent difficulties. First, the consumption of prey items occur below the surface making direct observations impossible (Iverson et al. 1997). Second, top predators are often very mobile species and their ecological needs often exceed the spatial scales used to define community boundaries (Lesage et al. 2001, Tougaard et al. 2003). On a methodological basis, classical methods for diet studies, such as gut contents and fecal analyses have biases due to digestion (e.g. loss of soft parts and digestion-resistance of hard part) which are not possible to avoid (Iverson et al. 1997) and these methods only give a snapshot of the last ingested prey items. Stable isotope analyses have been shown to be useful to identify trophic relationships between prey and predators (Hobson and Welch 1992, Hall-Aspland et al. 2005, Newsome et al. 2006), but this method rarely allows the prey items to be distinguished at a species level and often leads to conclusions based on groups of prey items (e.g. benthic *vs*. pelagic prey) (de la Vega et al. 2016) (Chapter 2).

The use of FA analysis has been proved to be a reliable and powerful method to determine the food sources of marine mammals (Kirsch et al. 2000, Iverson et al. 2004, Nordstrom et al. 2008). Lipids in marine organisms are characterized by their diversity (> 60 types) and high levels of long chain and polyunsaturated fatty acids (PUFAs) which originate in various unicellular and seaweeds (Budge et al. 2006, Bowen et al. 2009). Fatty Acids (FAs) are the largest constituent of lipids and those of carbon chain of 14 or longer are often deposited in animal tissue with minimal modification from the diet (Iverson et al. 1997, Kirsch et al. 2000). Because a limited number of FAs can be bio-synthetized by animals (Cook and McMaster 2002), it is possible to distinguish dietary *versus* non-dietary FAs (Iverson et al. 2004 Those FAs arising only or mostly from the diet (i.e. dietary FAs), also called essential FAs (Cook and McMaster 2002), are useful markers to study predator foraging ecology, once fatty acid patterns are characterized in the potential prey items (Rouvinen and Kiiskinen 1989, Pond et al. 1995, Iverson et a 1997, Kirsch et al. 1998, Raclot et al. 1998, Iverson et al. 2002).

The reflection of the diet by the FA composition is more or less accurate depending on the tissue analyzed (Budge et al. 2006). Blubber is classically used in marine mammal dietary studies (Iverson et al. 1997, Kirsch et al. 1998, Iverson et al. 2004, Budge et al. 2006, Nordstrom et al. 2008). Indeed, adipose tissues contain a high amount of non-structural lipids (i.e. lipids used as energy source), which have a high turnover and therefore mirror changes in the diet of the predator (Budge et al. 2006). On the contrary, muscle tissue contains more structural FAs which have low turnover and would therefore be less influenced by recent dietary lipid intake (Iverson et al. 2004, Budge et al. 2006). However, in some cases, phocid seals rely on lipids as energy source (i.e. non-structural lipids) in muscles (Pearson 2015). Indeed, strong seasonal variations of the blubber thickness can be observed in phocid seals (Bowen et al. 1992, Atkinson 1997). During these fasting periods, lipids are mobilized from the blubber as energy supply to the muscles (Trumble et al. 2010) and energy intake from predation is probably metabolized directly by muscle tissue. Furthermore, it has been shown in Weddell seals (*Leptonychotes weddellii*) that the level of polyunsaturated fatty acids (i.e. dietary FAs) in muscles were consistent with dietary source (Trumble et al. 2010).

The aim of this study is to determine the food resources used by harbor seals originating from the Wadden Sea. In this aim, we first characterized the FA composition of the potential prey items of harbor seals from the Sylt-Rømø Bight and identified the dietary FAs being the most distinguishing factors. Second we determined the FA composition of harbor seal´s muscles on a seasonal basis. Third, we related the seasonal variations of the dietary FAs in the prey species to possible seasonal changes in the seal´s diet.

2. Material and Methods

2.1. Study site

The Sylt-Rømø Bight (54°52' - 55°10' N, 8°20' - 8°40' E) is a semi-enclosed basin from the Wadden Sea, located between the islands of Sylt (Germany) and Rømø (Denmark) (see Fig. 1, p. 61 in Chapter 2). Two causeways connect the islands with the mainland, and prohibit any exchange of water with the adjacent tidal basins. The only connection to the North Sea is a deep tidal channel between the two islands. The Sylt-Rømø Bight provides shelter for a stable colony of ≈ 400 harbor seals on average in summer (2009 to 2012)(Jensen 2015). Harbor seals use five sand banks uncovered at low tide as haul out sites. These sandbanks are spread in the whole Bight, with the Jordsand and List sand banks (see Fig. 1, p. 61 in Chapter 2) being the most frequented (Jensen 2015).

2.2. Sampling of potential prey items

Seven species determined by de la Vega et al. (2016) as potential prey items of harbor seals (Table 1) were sampled from March to October 2013 among the catches of a fish monitoring occurring monthly in the Sylt-Rømø Bight. Details of the fish monitoring can be found in de la Vega et al. (2016) (Chapter 2). Three to ten individuals (Table 1) from the most abundant size-class of each species were collected per season, measured to nearest mm and then stored in aluminum foil at -80°C for further analysis.

Table 1: Number of individuals pooled per aliquot for fatty acid analysis, for each species and each season

Species	Spring (mar/apr)	Summer (jul/sep)	Fall (oct)
Osmerus eperlanus	10	3	-
Ammodytes tobianus	5	10	-
Clupea harengus	6	10	3
Pleuronectes platessa	10	10	10
Pomatoschistus minutus	10	10	10
Merlangius merlangus	-	10	-
Crangon crangon	-	10	10

2.3. Seal sampling

Twelve harbor seals were collected from June 2012 to July 2013 along the coastline of the Sylt Island (Table 2, p. 98). All seals were stranded dead or were killed by a shot to the head by authorized national park rangers because of serious illness. The age of the individuals (young of the year *versus* adults) was estimated according to their standard length (MacLaren 1993). The age of the young of the year (in months) was determined as the number of months between the main birth period (May to June) (Osinga et al. 2012) and the day of collection (Table 2). All individuals were older than five to six months and were assumed to have a prey-based diet (de la Vega et al., submitted). Necropsies were conducted on the carcasses at the Institute for Terrestrial and Aquatic Wildlife Research (ITAW) of University of Veterinary Medicine Hannover Foundation, according to the protocol described by Siebert et al. (2007). Until necropsy, the carcasses were stored frozen in a plastic bag at -20°C during few weeks.

Because most of the sampled individuals were starving before death, we assumed that muscle tissue might contains high level of lipids in the form of energy source (Pearson 2015) and therefore reflect a recent diet (Trumble et al. 2010). Therefore, muscle tissue was sampled on the lower flank (Todd et al. 2010) on each seal for fatty acid analyses. Samples were kept in aluminum foil at -80°C until preparation for analysis.

Table 2: Individual characteristics of seals sampled for fatty acid analysis

ID	Sex	Weight (g)	Length (cm)	Sampled date	Age (month)
seal 1	m	20	125	29 jul. 2012	> 13
seal 2	m	31.4	144	8 sep. 2012	> 13
seal 3	m	17.6	107	21 sep. 2012	> 13
seal 4	f	13	97	30 sep. 2012	4-5
seal 5	m	17.4	100	30 sep. 2012	4-5
seal 6	m	12.2	103	7 oct. 2012	5-6
seal 7	m	15.2	100	19 oct. 2012	5-6
seal 8	f	18	104	31 dec. 2012	7-8
seal 9	f	18.4	98	31 dec. 2012	7-8
seal 10	m	26.6	109	29 mar. 2013	10-11
seal 11	f	19.4	114	24 mar. 2013	10-11
seal 12	m	75,8	166	12 jul. 2013	> 13

2.4. Lipid extraction and FA analysis

All samples were freeze-dried and ground into a fine powder with a ball mill. The samples were stored at -80°C under nitrogen in glass tubes until analyses. Samples of prey items (i.e. fish and shrimp) were prepared per season (i.e. spring, summer and fall) by pooling an equal amount of powder of 3 to 10 individuals. Each seal sample was analyzed individually.

Lipids were extracted, according to Folch et al. (1957), as modified by Iverson (1988). Each sample was extracted 3 times with mixtures of chloroform:methanol (1:2, 2:1 and 4:1, v/v). The samples were split in two phases by adding a volume of 1% NaCl solution. The lower layer containing the lipids was collected and the water removed with dry sodium sulfate. These extracts were stored in the dark at -26°C. Total lipids were then quantified with a flame ionization detector (FID) (Iatroscan TH10 Mk III Iatron Laboratories). Five replicates were measured for each extract. Lipids were trans-methylated by acid catalysis at 60°C for 4 hours in H_2SO_4-methanol (4%, w/v) reagent (Christie, 1984) and toluene (10%, v/v). Known volumes of internal standard (21:0 and 23:0, 1 mg.L^{-1}) were added before trans-methylation to quantify the FA methyl esters (FAMEs). Samples were washed with 10% NaCl solution and extracted with a solution of 80:20 hexane:methyl tert-butyl esther (MTBE) (v:v). FAMEs purification was done in two steps with a HPLC fitted with semi-preparative columns. The first column (100 mm length, 10 mm internal diameter, Puriflash Si-CN 60µm phase) separated the semi-polar compounds like pigments from the non-polar compounds including FAMEs with a polarity gradient from 0.010 to 0.614, based on hexane:dichloromethane:methanol mixtures. The second column (250 mm length, 10 mm internal diameter, Uptosphere Si-CN 5mm phase) separated hydrocarbons, wax or sterol esters from FAMEs with a polarity gradient from 0.010 to 0.121 based on hexane:MTBE:acetone mixtures. The collected FAMEs were quantified by FID as for total lipids. FA compositions were determined using a gas chromatograph (GC-6890N, Agilent Technologies) equipped with an automatic sampler and a J&W DB-23 capillary column (Length: 60 m, Internal diameter: 0.25 mm, film: 0.25 µm). Operating conditions were as follows: injector in split mode (1/20 to 1/40) at 240 °C, 1 mL injected; detector FID at 260 °C; carrier gas: hydrogen in constant flow mode at an average linear velocity of 30 cm.sec^{-1}; linear temperature gradient from 100 to 240°C at 1°C min^{-1}. Identification of FAMES was performed by comparing relative retention times with those of known standard mixtures: 37-FAME Mix, 26-BAME Mix, PUFA-1 and PUFA-3 (Supelco, Sigma Aldrich Chemicals). Equivalent chain lengths (Christie, 1988) were used as an aid in peak localization and identification. Each FAME area was corrected from the corresponding FID response factor (Bannon et al., 1986) and from the difference in weight between the FAME and its corresponding free FA.

2.5. *Data and statistical analyses*

FA results are expressed as the percent of each FA relative to the sum of all identified FAs. Only FAs with proportions higher than 1% in prey item or harbor seal samples were used in the data analysis. FA biomarkers were identified from published literature.

The 15:0, 17:0, 18:1(*n-7*) and 19:1(*n-8*) acids are commonly associated to bacteria markers (Mayzaud et al. 1989, Scribe et al. 1991, Galois et al. 1996, Volkman et al. 1998, Najdek et al. 2002) These FAs were therefore summed and used in the following sections as "bacteria FAs".

The prey species per season were compared on the basis of their FA composition using multivariate principal component analysis (PCA), performed with the R statistic software and the ade4 package (Dray and Dufour 2007). Prior to PCA, the percentage values of the FAs were transformed logarithmically to level out the large numerical differences between FAs (Dalsgaard et al. 2003, Andersen et al. 2004). For trophic relation assessment between prey items and harbor seals, characteristic groups of FAs were assigned to one group or species of prey items, according to the results of the PCA.

These FAs characterizing the prey species were graphically compared to the mean FA seasonal composition of harbor seals. The seasons were defined as followed. Spring: March and April; summer: July and September; fall: October and December.

3. Results

3.1. *FA summary*

Twenty different FAs with relative proportions higher than 1% were identified in prey item and/or harbor seal samples (Appendices 1, p. 116-117 and 2, 120-121). These FAs represented about 90% of both prey species and harbor seal fatty acid composition.

The predominant FAs, in both prey items and harbor seals, were the 16:0 (16.1% in prey species and 13.3% in seals), 18:1(*n-9*) (9,7% in prey species and 17.2% in seals), 22:6(*n-3*) (16.1% in prey species and 5.2% in seals), 20:5(*n-3*) (12.9% in prey species and 4.3% in seals), 18:0 (5.0% in prey species and 10.5% in seals), 20:*4(n-6*) (2.4% in prey species and 8,7% in seals) and 16:1(*n-7*) (4.4% in prey species and 4.0% in seals). These FAs accounted from 57.9% (*Pleuronectes platessa* – fall) to 77.6% (*Osmerus eperlanus* – summer) of the FAs in the prey species, and from 55.6 to 69.3% of the FAs in harbor seals (Appendices 1, p. 116-117 and 2, 120-121).

The bacteria FAs, dominated by the 18:1(*n-7*), represented about 6% and 7.5% of the total FA composition of prey species and harbor seals respectively (Appendices 1 and 2, p. 116-117 and 120-121).

3.2. Prey species

3.2.1.Fatty acid composition of the different prey species

16:0 and 24:1(*n-9*) occurred in high amount in *Clupea harengus* (≈ 17.2% and ≈ 1.4% respectively), *Ammodytes tobianus* (≈ 20.0% and ≈ 1.6% respectively), and *Merlangius merlangus* (19.2% and 2.0% respectively) compared to the other prey species in every season (Appendix 1, p.116-117). Furthermore, 22:6(*n-3*) dominated the fatty acid composition of *C. harengus* in every season (≈20% of total FAs) and 18:0 had the highest amount in *Merlangius merlangus* (9.2 %). 20:4(*n-6*), 22:5(*n-3*), 22:3(*n-3*) and 22:3(*n-4*) occurred in high amount in the benthic species i.e. *P. platessa* (4.1%, 3.1%, 1.2% and 0.7%, respectively), *Pomatoschistus minutus* (3.8%, 3.8%, 1.7% and 0.9%, respectively), and *Crangon crangon* (3.0%, 2.9%, 1.0% and 0.7%, respectively), in comparison to the pelagic species in every season (Appendix 1, p.116-117). Additionally *P. platessa* was characterized by high amount of 20:1(*n-7*) (2.6%) and 20:1(*n-9*) (>1%) in summer and fall and *C. crangon* had the highest level of 20:5(*n-3*) (17.0% in summer and 19% in fall; Appendix 1, p.116-117). The FA composition of *O. eperlanus* was dominated by the 18:1(*n-9*) which represented about 20% of the total FAs (Appendix 1, p.116-117). 20:5(*n-3*) was found in high amount (≈ 13%) in every species, although it was in a higher proportion in *C. crangon* (> 17%; Appendix 1, p.116-117).

3.2.2.Grouping of prey species based on their fatty acid composition

PCA was applied to explore the similarities and dissimilarities in the FA composition of the prey species. The two first axes of the PCA explained 54.2% of the variability in the FA composition (Appendix 3a, p.123) and cluster the species into four groups (Fig. 1, p.103). The gradient along axis 1 separated the species into two groups according to the main distinguishing FAs: Pelagic species, i.e. *C. harengus* and *A. tobianus* were both characterized by high amount of 16:0, and 24:1(*n-9*), and benthic species, i.e. *P. minutus, P. platessa* and *C. crangon* were correlated to 22:5(*n-3*), 22:3(*n-4*), 20:4(*n-6*), 22:5(*n-3*) and bacteria FAs (Fig. 1). The axis 2 separated *A. tobianus* from *C. harengus* which had higher amount of 22:6(*n-3*) and *P. platessa* was distinguished from both *P. minutus* and *C. crangon* by its high amount of 14:0, 16:1(*n-7*), 18:4(*n-3*), 20:1(*n-7*) and 20:1(*n-9*) (Fig. 1).

The variances in the FA composition of *M. merlangus* and *O. eperlanus* were mostly explained by the axes 3 and 4 (Appendix 3b, p.123), representing 12.9 and 10.1% of the total variance, respectively (Appendix 3a, p.123). *M. merlangus* was distinguished on the axis 3 by its high amounts of C18:0 and *O. eperlanus* was characterized on the axis 4 by high amount of C18:1n-9 and C18:2n-6 (Appendix 3b and c, p.123). High amount of C20:5n-3 was related to *C. crangon* in fall on the axis 3 and to *C. harengus* in spring on the axis 4 (Appendix 3b and c, p.123). Except for *C. harengus*, most of the variance of the pre-cited species and FAs were explained by the axes 3 and 4 (Appendix 3b and c, p.123).

As a result, each of the prey items was clearly defined by a characteristic FA composition (Fig. 1 and Appendix 3, p.123). The 15 most contributive of these characteristic FAs (Appendix 3c, p.123) are displayed in Table 3, where they are grouped under the species or a group of species that they are characterizing. This order will be used in the following sections for comparison of seal´s FA composition.

Table 3: FAs characterizing a group or a species of prey items according to the PCA results, listed under the group or species that they are characterizing

<table>
<tr><th>A. tobianus</th><th>C. harengus</th><th>P. platessa</th><th>P. minutus</th><th>C. crangon</th><th>M. merlangus</th><th>O. eperlanus</th></tr>
<tr><td colspan="2">16:0</td><td colspan="3">20:4(n-6)</td><td>18:0</td><td>18:1(n-9)</td></tr>
<tr><td colspan="2">22:1(n-9)</td><td colspan="3">22:3(n-3)</td><td></td><td>18:2(n-6)</td></tr>
<tr><td colspan="2">22:6(n-3)</td><td colspan="3">22:3(n-4)</td><td></td><td></td></tr>
<tr><td colspan="2">24:1(n-9)</td><td colspan="3">22:5(n-3)</td><td></td><td></td></tr>
<tr><td></td><td>20:5(n-3)
(spring)</td><td>14:0</td><td></td><td>20:5(n-3)
(fall)</td><td></td><td></td></tr>
<tr><td></td><td></td><td>16:1(n-7)</td><td></td><td></td><td></td><td></td></tr>
<tr><td></td><td></td><td>18:4(n-3)</td><td></td><td></td><td></td><td></td></tr>
<tr><td></td><td></td><td>20:1(n-9)</td><td></td><td></td><td></td><td></td></tr>
</table>

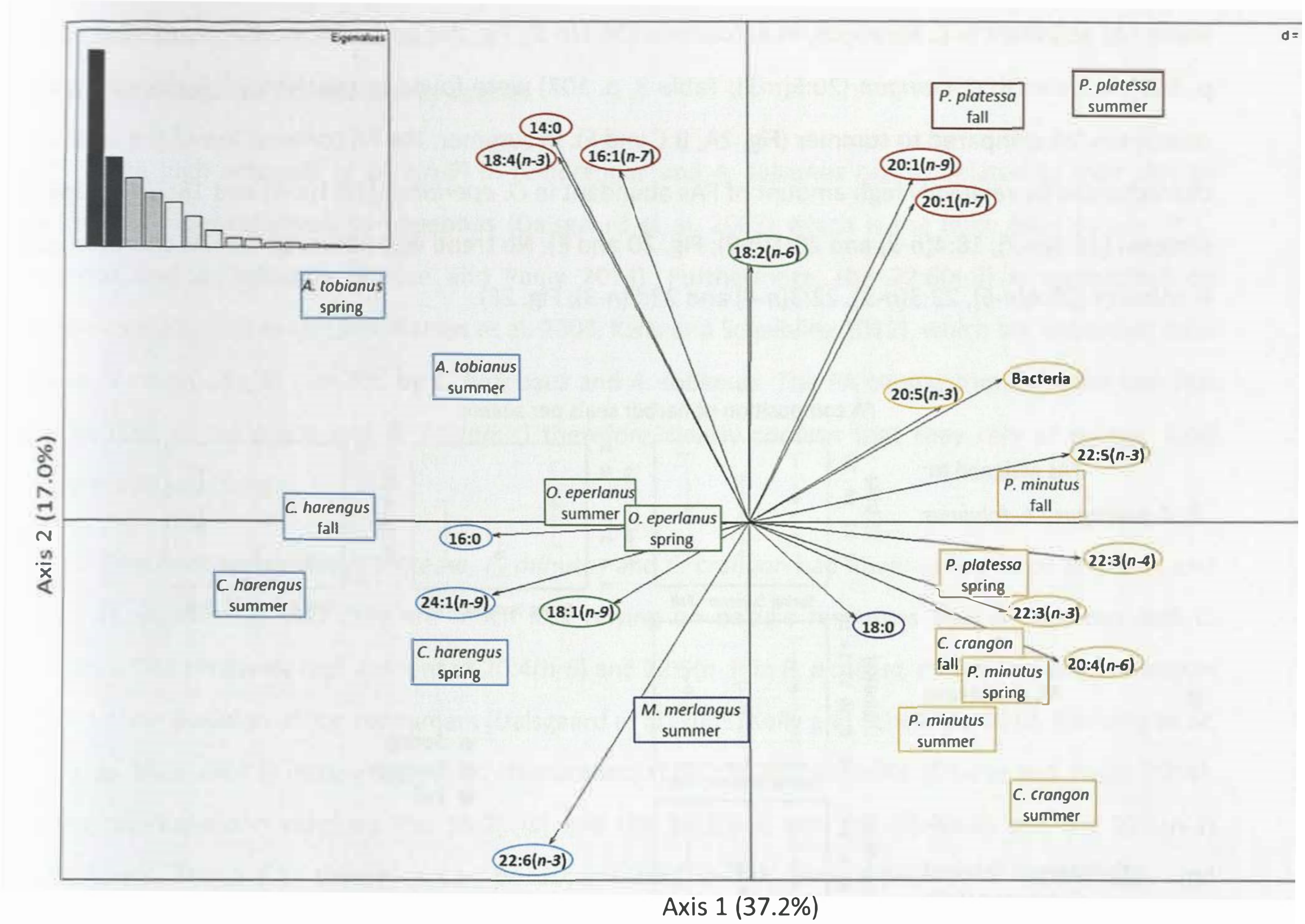


Fig. 1: Principal component analysis plot based on the logarithmic transformation of the FA composition of the prey species. Projection of variables (i.e. FAs) on axes 1 and 2 are represented by the arrows. The percentage of variance explained by each axis is given between parentheses along the axes

3.3. Harbor seals

3.3.1.FA composition of harbor seals

The FA composition of harbor seal´s muscles was dominated by the 18:1(*n-9*) and the 16:0 representing about 17% and 13% of the total proportion of FAs respectively, followed by the 18:0, the 20:4(*n-6*) and the bacteria FAs (each ≈ 10% of the total FAs; Appendix 2, p.120-121). The 20:5(*n-3*), the 22:6(*n-3*) and the 16:1(*n-7*) constituted each about 5% of the total FAs.

3.3.2.Seasonal trend in FA composition of harbor seals

FAs abundant in *C. harengus* (22:6(*n-3*) and 20:5(*n-3*)), *A. tobianus* (22:6(*n-3*)) and *M. merlangus* (18:0; Table 3, p. 102) were in higher amounts in seal tissue in spring than in summer (Fig. 2A, B and C).

Some FAs abundant in *C. harengus*, in *A. tobianus* (24:1(*n-9*); Fig. 2A) and in *M. merlangus* (18:0; Table 3, p. 102), but also in *C. crangon* (20:5(*n-3*); Table 3, p. 102) were found in relatively high amount in seal muscles in fall compared to summer (Fig. 2A, B C and F). In summer, the FA composition of the seals was characterized by relatively high amount of FAs abundant in *O. eperlanus* (18:1(*n-9*) and 18:2(*n-6*)) and *P. platessa* (16:1(*n-7*), 18:4(*n-3*) and 20:1(*n-9*); Fig. 2D and E). No trend was observed for FAs characterizing *P. minutus* (20:4(*n-6*), 22:3(*n-3*), 22:3(*n-4*) and 22:5(*n-3*); Fig. 2F).

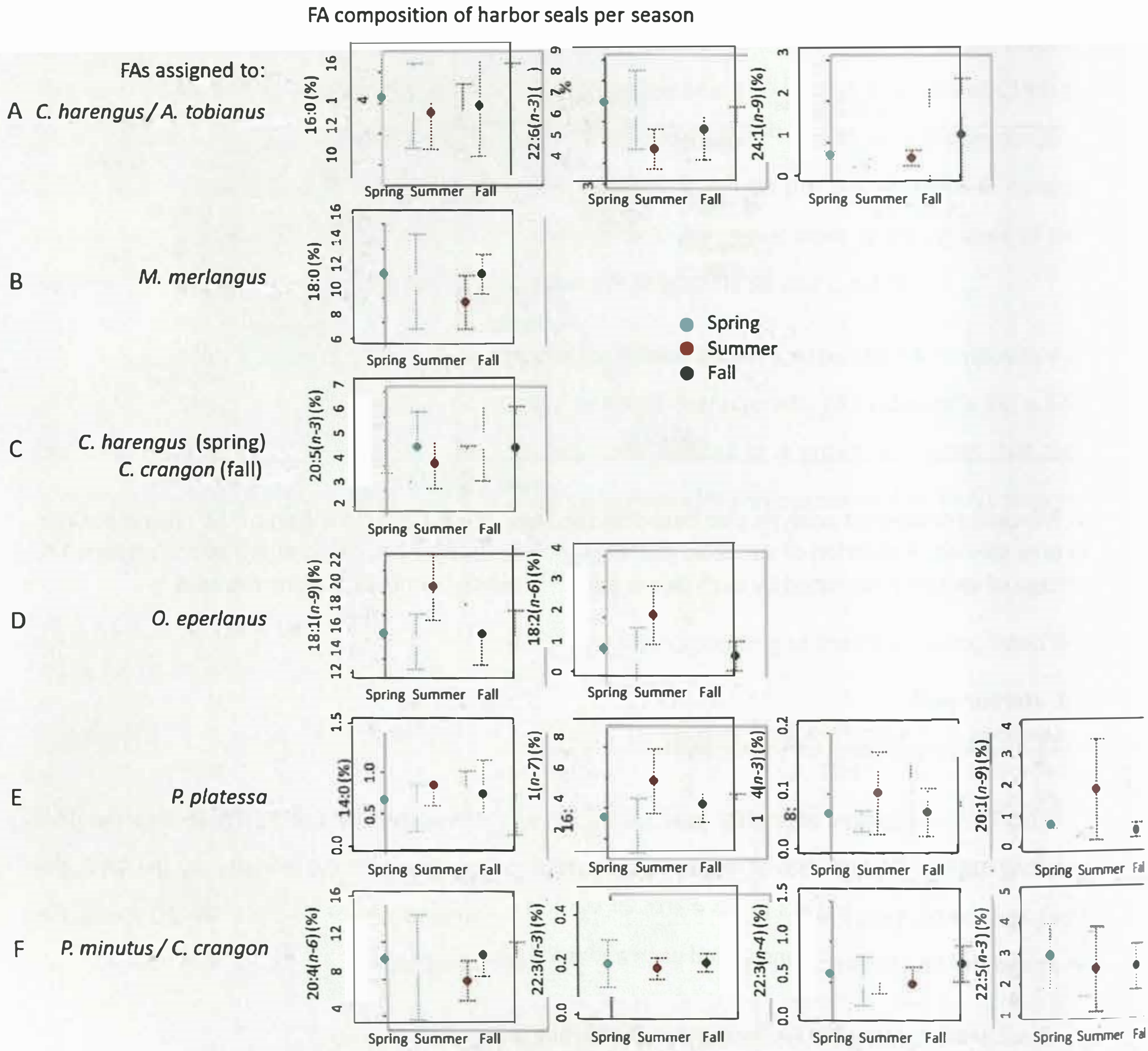


Fig. 2: Seasonal variation (mean and standard deviation) of the relative proportions (%) of FAs in harbor seal´s muscles. FA are grouped per species or group of prey items that they are characterizing: A- *C. harengus* and *A. tobianus*, B- *M. merlangus*, C- *C. harengus* in spring and *C. crangon* in fall, D- *O. eperlanus*, E- *P. platessa* and F- *P. minutus* and *C. crangon*.

4. Discussion

4.1. Characterization of the prey species

The high amounts of 24:1(*n-9*) in *C. harengus* and *A. tobianus* can be related to their diet as 24:1(*n-9*) is bio-synthetized by copepods (Dalsgaard et al. 2003) which is the main food source of *C. harengus* and *A. tobianus* (Froese and Pauly 2014). Furthermore, the 22:6(*n-3*) is synthetized by flagellates (Mayzaud et al. 1989, Ramos et al. 2003, Kelly and Scheibling 2011), which are important food source of copepods, in turn fed by *C. harengus* and *A. tobianus*. The FA composition of these two fish species (i.e., *C. harengus* and *A. tobianus*) therefore clearly confirm that they rely of pelagic food resources all year long.

The benthic species *P. platessa, P. minutus* and *C. crangon* had lower amounts of 24:1(*n-9*) and 22:6(*n-3*), confirming that they are much less relying on pelagic resources that *A. tobianus* and *C. harengus*. The relatively high amount of 20:4(*n-6*) and 22:5(*n-3*) in *P. platessa, P. minutus* and *C. crangon* confirm their position of top consumers (Dalsgaard et al. 2003, Kelly and Scheibling 2011, Monroig et al. 2013), as their diet is mostly based on crustaceans, mollusks and annelids (Froese and Pauly 2014). Indeed, mollusks can elongate the 18:2(*n-6*) and the 18:3(*n-3*) into the 20:4(*n-6*) and the 22:5(*n-3*) respectively. These FAs therefore can be accumulated in fish tissues (Hall et al. 2006, Kelly and Scheibling 2011, Ezgeta-Balić et al. 2012, Monroig et al. 2013), as fish cannot biosynthesize or modify these FAs (Hall et al. 2006, Kelly and Scheibling 2011). Crustaceans also convert the 18:2(*n-6*) and the 18:3(*n-3*) into the 20:4(*n-6*) and 20:5(*n-3*) (Hall et al. 2006, Kelly and Scheibling 2011). The FA composition of *C. crangon* might then be coming from biosynthesis of these FAs by the organisms themselves, from their diet which consists partly of small mollusk species (Froese and Pauly 2014) or from the grazing of benthic diatoms, as diatoms contain high amounts of 20:5(*n*-3)(Lebreton et al. 2011).

The high amounts of 14:0 and 16:1(*n-7*) in *P. platessa* which are synthetized by diatoms (Mayzaud et al. 1989, Galois et al. 1996, Volkman et al. 1998, Kelly and Scheibling 2011) might be explained by their diet based on lungworms and bivalves (Baird et al. 2004). Lugworms rely on benthic diatoms (Baird et al. 2004, Lebreton et al. 2011), as well as bivalves such as small cockles (Kang et al. 1999). In addition, the an important feeding of *P. platessa* on cockles could explain the high amount of 20:1(*n-9*) which can be elongated by bivalves from the 18:1(*n-9*) (Dalsgaard et al. 2003, Ezgeta-Balić et al. 2012).

O. eperlanus FA composition was characterized by a high amount of 18:1(*n-9*) in spring and summer. This might be related to the anadromous behavior of this species and to its annual spawning migration into rivers from February to May (Kottelat and Freyhof 2007). Indeed, 18:1(*n-9*) can be found in high amounts in fresh water chlorophycae (Ahlgren et al. 1992). Furthermore, the characterization of *O. eperlanus* by the 18:2(*n-6*) which is typical of vascular plants (i.e. saltmarshes and terrestrial plants) (Galois et al. 1996, Kharlamenko et al. 2001, Kelly and Scheibling 2011), also suggests an influence of continental food resources on this species. In addition, these two FAs (i.e. C18:1n-9 and C18:2n-6) are indicators of omnivory and carnivory (Dalsgaard et al. 2003, Petursdottir et al. 2008, Ezgeta-Balić et al. 2012) and can be found in high amounts in zooplankton (Zhukova and Kharlamenko 1999, Kharlamenko et al. 2001) which is in accordance with the diet of *O. eperlanus* (Froese and Pauly 2014).

M. merlangus was distinguished from the other species by a high amount of 18:0 which is highly ubiquitous (Lebreton et al. 2011). The lack of particular marker for this species might be due to its reliance on consumers from both pelagic and benthic systems, as *M. merlangus* has a bentho-pelagic behavior. Indeed, *M. merlangus* feed on crustacean, mollusks and polychaetes, but also on small fish feeding in the water column (Froese and Pauly 2014).

Thus, the prey species were well differentiated by their FA composition in accordance with their origin (i.e., benthic *vs.* pelagic, continental *vs.* marine) and/or their diet. Particularly, benthic prey species were distinguished from pelagic prey species by their FA composition.

4.2. Reliability of the method

4.2.1.Which FAs are reliable trophic markers?

In monogastric predators (i.e. non-ruminant) such as marine mammals, the largest contributor to the FA composition is the direct deposition of FAs issued from their diet (Iverson et al. 1997, Iverson et al. 2002, Budge et al. 2006). However, other processes can modify the FA composition between the ingestion and the deposition in tissues (Budge et al. 2006). Mammals are capable of elongating the carbon chain of saturated FAs (SATs) and mono-saturated FAs (MUFAs) by two carbon units (Budge et al. 2006). For example, 16:0 might be modified after consumption to 18:0. The high amount of 18:0 in the seal´s muscles observed in this study might then come partly from elongation process and would therefore not reflect the diet. Another significant modification process is the chain shortening (Budge et al. 2006). For example, mammals can shorten 22:1 and 20:1 isomers coming from prey items, into 18:1

isomers (Budge et al. 2006). Therefore, the high amount of 18:1(*n-9*) in seals observed in this study might come from prey species which are rich in 20:1(*n-9*) and 20:1(*n-7*). However, these two FAs were found in very low amount (≈1%) in comparison to 18:1(*n-9*) (> 10%) in the prey species, suggesting that 18:1(*n-9*) in the seal FA composition comes at least partly from the diet.

FAs in predators can also arise from de novo synthesis (Budge et al. 2006). For example, mammals are capable to biosynthesize 16:0 when the animals are under a low-fat diet (Budge et al. 2006). Again, the percentage of 16:0 was high in the potential prey items (> 16%) and the high amount of this FA in seals is likely coming partly from their diet. A limited number of fatty acids can be biosynthetized by animal (Iverson et al. 2002) and FAs can be distinguished between dietary and non-dietary FAs (Iverson et al. 2004). For instance, 18:2(*n-6*), 20:4(*n-6*), 20:5(*n-3*), 22:6(*n-3*) and 24:1(*n-9*), which were found in high amount in the seal muscles in this study are arising in predator FA composition strictly from the diet (Iverson et al. 2004). On the contrary, the Bacteria FAs are not good indicators of the diet because they can reflect the presence of bacteria in the predator gut flora (Iverson et al. 2004).

4.2.2.Is muscle tissue reliable to study the diet?

The presence of strictly dietary FAs (e.g. 18:2(*n-6*), 20:4(*n-6*), 20:5(*n-3*), 22:6(*n-3*) and 24:1(*n-9*))(Iverson et al. 2004) in harbor seal´s muscles from this study suggest that the FA composition of muscle tissue reflects the seal´s diet, which is in accordance with the results of Trumble et al. (2010) who showed that polyunsaturated FAs level in muscles of Weddell seals were consistent with the level in their prey items.

Some differences were nonetheless observed between pinnipeds blubber, which is classically used for marine mammal diet studies (Iverson et al. 1997), and muscle FA composition (Henderson et al. 1994, Durnoford and Shahidi 2002). Indeed, 14:0, 16:1(*n-7*) and 20:1(*n-9*) which were found in high amounts in *P. platessa* in this study were clearly in higher amount in blubber than in muscles in harbor seals (Durnoford and Shahidi 2002) but were similar between these two tissues in Monk seals (*Monachus monachus)* (Henderson et al. 1994). At the contrary, 16:0, 20:5(*n-3*), 22:6(*n-3*) and 24:1(*n-9*) which were abundant in *C. harengus* and *A. tobianus*, were slightly lower in blubber than in muscle in both harbor and monk seals (Henderson et al. 1994, Durnoford and Shahidi 2002). 18:0 and 20:4(*n-6*) were in very low amount in blubber in comparison with muscle tissue in both previously cited studies (Henderson et al. 1994, Durnoford and Shahidi 2002). This suggests that these FAs in muscles might not reflect the diet and might accumulate in muscle due to other factors than energy supply. 18:1(*n-9*) and

18:2(*n-6*) were found in similar amount in both muscle and blubber with 18:1(*n-9*) dominating the total FA composition in both tissues (Henderson et al. 1994, Durnoford and Shahidi 2002), which was also the case in muscles in this study.

Although the potential differences in the FA composition of muscles and blubber in harbor seals do not allow direct correlation between seal´s muscle and prey species (Iverson et al. 2004), dietary FAs (e.g. 18:2(*n-6*), 20:4(*n-6*), 20:5(*n-3*), 22:6(*n-3*) and 24:1(*n-9*)) observed in this study in muscle tissue likely come from the diet. However, the bad nutritional status of most of the sampled harbor seals might restrain general conclusions to be extrapolated to the whole seal community, and further studies about tissue metabolism and processes leading to FA deposition in muscles should be done to enhance the results of this study. Nevertheless, relative seasonal comparison of seal muscle FA compositions might bring consistent indications about the prey species influencing the seal´s diet.

4.3. *Shift in the seal´s diet between spring, summer and fall*

The FA composition of the seal´s muscles suggests a higher influence of pelagic prey species in their diet in spring than in summer. Indeed, the higher amount of the 22:6(*n-3*) in spring than in summer indicates that *C. harengus*, *A. tobianus* and likely other pelagic species represent a larger part of the seal´s diet in this season. The high contribution of *C. harengus* in the diet of seal in spring is confirmed by the relatively high amount of the 20:5(*n-3*) in this season. This is in accordance with the high abundance of *C. harengus* juveniles in the Wadden Sea bights in spring (Dickey-Collas et al. 2009). As de la Vega et al. (2016) suggested that *Loligo spp*. largely contributes to the diet of seals in spring, it would be of interest to also precise the role of these prey species based on their FA composition.

The 18:2(*n-6*) and 18:1(*n-9*), abundant in *O. eperlanus*, the 18:4(*n-3*), 20:1(*n-9*) and 16:1(*n-7*), abundant in *P. platessa* were in higher amounts in summer in seals than in spring and fall. This suggests that these two prey species have high contributions to the seal´s diet in this season. This is consistent with the settlement of *P. platessa* in the Wadden Sea bight in April (Mahé et al. 2006) and the return of *O. eperlanus* in coastal areas after its annual spawning migration into rivers from February to May (Kottelat and Freyhof 2007), leading to an increase of this species availability in these species in summer.

In fall, the 24:1(*n-9*) abundant in *C. harengus* and *A. tobianus* was in high amount in seals, suggesting that their diet was more influenced by pelagic prey items in this season than in summer. Furthermore, the highest amount of the 20:5(*n-3*) and 22:3(*n-4*) observed in seals indicates that *C. crangon* represents a large part of the seal´s diet in fall. This is in accordance with the extremely high abundance of *C. crangon* in the Sylt Rømø Bight in this season de la Vega et al. (2016).

Therefore, the FA composition of seal suggests a shift in the diet between spring, summer and fall, with individuals more influenced by pelagic prey species in spring and fall, and by benthic prey species in summer. This seasonal variation is in accordance with the findings of the study from de la Vega et al. (2016) conducted in the Sylt Rømø Bight and the studies by Brown and Pierce (1998), Hall et al. (1998), Andersen et al. (2007) and Berg et al. (2002) conducted in the southern North Sea. Indeed, these authors showed a high contribution of pelagic species in spring (e.g., *C. harengus* and *A. tobianus*) and an increase in the contribution of benthic species (e.g. *P. platessa*) in the diet of seal´s in summer.

5. Conclusion

The fatty acid analyses highlighted that harbor seal from the Sylt Rømø Bight are characterized by a seasonal shift of their diet, going from a higher contribution of pelagic species (e.g. *C. harengus* and *A. tobianus*) in spring to a higher contribution of benthic species (e.g. *P. platessa* and *O. eperlanus*) in summer. This study also provided indication of high contribution of *C. crangon* in the seal´s diet in fall. These results confirm the need of more detail studies about temporal variations of harbor seal´s diet, to improve and refine protection management measures. Furthermore, increasing the sampling size of harbor seals and their potential prey species in further studies about fatty acid composition would definitely strengthen the conclusions. Indeed it would improve the characterization of the prey species based on their FA composition and therefore precise their contribution to the diet. A greater number of sampled adult individuals would probably reduce the inter-individual variations and allow stronger conclusions. Furthermore, additional studies comparing blubber and muscle tissues should be carried out to confirm the possibility to use FA composition of muscles in diet studies. This would also allow mixing models such as QFASA to be used for quantitative determination of the seal´s diet.

6. Acknowledgements

This study was funded by the German Federal Ministry of Education and Research in the framework of the German-Dutch bilateral Wadden Sea Research Project "The impact of biological invasions on the food web of the Wadden Sea (INFOWEB)" (Förderkennzeichen 03F0636A/B). The collection of carcasses and pathological investigations of seals were partly funded by the Ministry of Energy, Agriculture, Environment and Rural Areas of the Schleswig-Holstein and the authorities for coastal protection, national parks and marine protection of Schleswig-Holstein (LKN). We would like to thank the team of the Institute for Terrestrial and Aquatic Wildlife Research from the University of Veterinary Medicine Hannover Foundation with whom seal dissections were carried out, particularly Eva Wehrmeister, Miriam Hillmanm and Kornelia Schmidt for their assistance in collecting seal samples. We also thank the seal hunters from the island of Sylt who collected the seals along the coast of the Island. Furthermore, we warmly thank the people from the joint research unit Littoral, Environment and Societies (CNRS - University of La Rochelle) where Camille de la Vega was welcomed two months to do the fatty acid analyses.

7. References

Ahlgren, G., I. B. Gustafsson, and M. Boberg. 1992. Fatty acid content and chemical composition of freshwater microalgae Journal of Phycology **28**:37-50.

Andersen, S. M., C. Lydersen, O. Grahl-Nielsen, and K. M. Kovacs. 2004. Autumn diet of harbour seals (Phoca vitulina) at Prins Karls Forland, Svalbard, assessed via scat and fatty-acid analyses. Canadian Journal of Zoology **82**:1230-1245.

Andersen, S. M., J. Teilmann, P. B. Harders, E. H. Hansen, and D. Hjollund. 2007. Diet of harbour seals and great cormorants in Limfjord, Denmark: interspecific competition and interaction with fishery. Ices Journal of Marine Science **64**:1235-1245.

Atkinson, S. 1997. Reproductive biology of seals. Reviews of Reproduction **2**:175-194.

Baird, D., H. Asmus, and R. Asmus. 2004. Energy flow of a boreal intertidal ecosystem, the Sylt-Rømø Bight. Marine Ecology Progress Series **279**:45-61.

Behrends, G. 1985. Zur Nahrungswahl von Seehunden (*Phoca vitulina L.*) im Wattenmeer Schleswig-Holsteins. Zeitschrift für Jagdwissenschaft **31**:3-14.

Berg, I., T. Haug, and K. T. Nilssen. 2002. Harbour Seal (*Phoca vitulina*) Diet in Vesteralen, North Norway. Sarsia **87**:451-461.

Bowen, W. D. 1997. Role of marine mammals in aquatic ecosystems. Marine Ecology Progress Series **158**:267-274.

Bowen, W. D., O. T. Oftedal, and D. Boness, J. 1992. Mass and energy transfer during lactation in a small phocid, the harbor seal (*Phoca vitulina*). Physiological Zoology **65**:844-866.

Bowen, W. D., A. J. Read, and J. A. Estes. 2009. Feeding Ecology. Pages 217-246 *in* A. R. Hoelzel, editor. Marine mammal biology: an evolutionary approach. John Wiley & Sons.

Brown, E. G., and G. J. Pierce. 1998. Monthly variation in the diet of harbour seals in inshore waters along the southeast Shetland (UK) coastline. Marine Ecology Progress Series **167**:275-289.

Budge, S. M., S. J. Iverson, and H. N. Koopman. 2006. Studying trophic ecology in marine ecosystems using fatty acids: a primer on analysis and interpretation Marine Mammal Science **22**:759-801.

Cook, H. W., and C. R. McMaster. 2002. Fatty acid desaturation and chain elongation in eukaryotes. Elsevier: New York, NY, USA.

Dalsgaard, J., M. St. John, G. Kattner, D. Müller-Navarra, and W. Hagen. 2003. Fatty acid trophic markers in the pelagic marine environment. Pages 225-340 Advances in Marine Biology. Academic Press.

de la Vega, C., B. Lebreton, U. Siebert, G. Guillou, K. Das, R. Asmus, and H. Asmus. 2016. Seasonal Variation of Harbor Seal's Diet from the Wadden Sea in Relation to Prey Availability. PLoS ONE **11**:e0155727.

Dickey-Collas, M., L. Bolle, J. van Beek, and P. Erftemeijer. 2009. Variability in transport of fish eggs and larvae. II. Effects of hydrodynamics on the transport of Downs herring larvae. Marine Ecology Progress Series **390**:183-194.

Dray, S., and A. B. Dufour. 2007. The ade4 package: implementing the duality diagram for ecologists. Journal of Statistical Software **22**:1-20.

Durnoford, E., and F. Shahidi. 2002. Comparison of FA compositions of selected tissues of phocid seals of Eastern Canada using one-way and multivariate techniques. Journal of the American Oil Chemists' Society **79**:1095-1102.

Ezgeta-Balić, D., M. Najdek, M. Peharda, and M. Blažina. 2012. Seasonal fatty acid profile analysis to trace origin of food sources of four commercially important bivalves. Aquaculture **334**:89-100.

Froese, R., and D. Pauly. 2014. Data Base: FishBase [Internet].

Galois, R., P. Richard, and B. Fricourt. 1996. Seasonal variations in suspended particulate matter in the Marennes-Oléron Bay, France, using lipids as biomarkers. Estuarine, Coastal and Shelf Science **43**:335-357.

Hall-Aspland, S. A., T. L. Rogers, and R. B. Canfield. 2005. Stable carbon and nitrogen isotope analysis reveals seasonal variation in the diet of leopard seals. Marine Ecology Progress Series **305**:249-259.

Hall, A. J., J. Watkins, and P. S. Hammond. 1998. Seasonal variation in the diet of harbour seals in the south-western North Sea. Marine Ecology Progress Series **170**:269-281.

Hall, D., S. Y. Lee, and T. Meziane. 2006. Fatty acids as trophic tracers in an experimental estuarine food chain: tracer transfer. Journal of Experimental Marine Biology and Ecology **336**:42-53.

Härkönen, T. 1987. Seasonal and regional variations in the feeding habits of the harbour seal, *Phoca vitulina,* in the Skagerrak and the Kattegat. Journal of Zoology **213**:535-543.

Härkönen, T., and M. P. Heide-Jørgensen. 1991. The harbour seal *Phoca vitulina* as a predator in the Skagerrak. Ophelia **34**:191-207.

Henderson, R. J., N. Kalogeropoulos, and M. N. Alexis. 1994. The lipid composition of selected tissues from a Mediterranean monk seal, *Monachus monachus*. Lipids **29**:577-582.

Hobson, K. A., and H. E. Welch. 1992. Determination of trophic relationships within a high Arctic marine food web using $\delta^{13}C$ and $\delta^{15}N$ analysis. Marine Ecology Progress Series **84**:10.

Iverson, S., K. Frost, and L. Lowry. 1997. Fatty acid signatures reveal fine scale structure of foraging distribution of harbor seals and their prey in Prince William Sound, Alaska. Marine Ecology Progress Series **151**:255-271.

Iverson, S. J., C. Field, W. Don Bowen, and W. Blanchard. 2004. Quantitative fatty acid signature analysis: a new method of estimating predator diets. Ecological Monographs **74**:211-235.

Iverson, S. J., K. J. Frost, and S. L. C. Lang. 2002. Fat content and fatty acid composition of forage fish and invertebrates in Prince William Sound, Alaska: factors contributing to among and within species variability. Marine Ecology Progress Series **241**:161-181.

Jensen, L. 2015. Monitoring of the seal population in the Wadden Sea in 2015 - Monitering af sælbestanden i Vadehavet 2015. Aarhus Universitet, Miljøministeriet, Fiskeri-og Søfartsmuseet Saltvandsakvariet Esbjerg.

Kang, C., P. Sauriau, P. Richard, and G. Blanchard. 1999. Food sources of the infaunal suspension-feeding bivalve Cerastoderma edule in a muddy sandflat of Marennes-OlQon Bay, as determined by analyses of carbon and nitrogen stable isotopes. Marine Ecology Progress Series **187**:147-158.

Kelly, J. R., and R. E. Scheibling. 2011. Fatty acids as dietary tracers in benthic food webs. Marine Ecology Progress Series **446**:1-22.

Kharlamenko, V., S. Kiyashko, A. Imbs, and D. Vyshkvartzev. 2001. Identification of food sources of invertebrates from the seagrass Zostera marina community using carbon and sulfur stable isotope ratio and fatty acid analyses. Marine Ecology Progress Series **220**:103-117.

Kirsch, P. E., S. J. Iverson, and W. D. Bowen. 2000. Effect of a Low-Fat Diet on Body Composition and Blubber Fatty Acids of Captive Juvenile Harp Seals (Phoca groenlandica). Physiological and Biochemical Zoology **73**:45-59.

Kirsch, P. E., S. J. Iverson, W. D. Bowen, S. R. Kerr, and R. G. Ackman. 1998. Dietary effects on the fatty acid signature of whole Atlantic cod (Gadus morhua). Canadian Journal of Fisheries and Aquatic Sciences **55**:1378-1386.

Kottelat, M., and J. Freyhof. 2007. Handbook of European freshwater fishes. Publications Kottelat, Cornol, Switzerland.

Lebreton, B., P. Richard, R. Galois, G. Radenac, C. Pfléger, G. Guillou, F. Mornet, and G. F. Blanchard. 2011. Trophic importance of diatoms in an intertidal Zostera noltii seagrass bed: Evidence from stable isotope and fatty acid analyses. Estuarine, Coastal and Shelf Science **92**:140-153.

Lesage, V., M. O. Hammill, and K. M. Kovacs. 2001. Marine mammals and the community structure of the Estuary and Gulf of St Lawrence, Canada: evidence from stable isotope analysis. Marine Ecology Progress Series **210**:203-221.

MacLaren, I. 1993. Growth in pinnipeds. Biological Reviews **68**:1-79.

Mahé, K., J. P. Delpech, and A. Carpentier. 2006. Synthèse bibliographique des principales espèces de Manche orientale et du golfe de Gascogne. Convention Ifremer-Ministère de l'Industrie Centre Manche-mer du Nord. Département Halieutique. Laboratoire Ressources Halieutiques de Boulogne sur mer.

Mayzaud, P., J. P. Chanut, and R. G. Ackman. 1989. Seasonal changes of the biochemical composition of marine particulate matter with special reference to fatty acids and sterols. Marine ecology progress series. Oldendorf **56**:189-204.

Monroig, Ó., D. R. Tocher, and J. C. Navarro. 2013. Biosynthesis of Polyunsaturated Fatty Acids in Marine Invertebrates: Recent Advances in Molecular Mechanisms. Marine Drugs **11**:3998-4018.

Najdek, M., D. Debobbis, D. Mioković, and I. Ivančić. 2002. Fatty acid and phytoplankton compositions of different types of mucilaginous aggregates in thenorthern Adriatic. Journal of Plankton Research **24**:429-441.

Newsome, S. D., P. L. Koch, M. A. Etnier, and D. Aurioles-Gamboa. 2006. Using Carbon and Nitrogen isotope values to investigate maternal strategies in Northeast Pacific Otariids. Marine Mammal Science **22**:556-572.

Nordstrom, C., L. Wilson, S. Iverson, and D. Tollit. 2008. Evaluating quantitative fatty acid signature analysis (QFASA) using harbour seals *Phoca vitulina richardsi* in captive feeding studies. Marine Ecology Progress Series **360**:245-263.

Osinga, N., I. Pen, H. A. Udo de Haes, and P. M. Brakefield. 2012. Evidence for a progressively earlier pupping season of the common seal (*Phoca vitulina*) in the Wadden Sea. Journal of the Marine Biological Association of the United Kingdom **92**:1663-1668.

Pearson, L. E. 2015. Blubber and Beyond: The Role od Lipids in Thermoregulation and Energy Reserves of Phocid Seals. Alaska Fairbanks.

Petursdottir, H., A. Gislason, S. Falk-Petersen, H. Hop, and J. Svavarsson. 2008. Trophic interactions of the pelagic ecosystem over the Reykjanes Ridge as evaluated by fatty acid and stable isotope analyses. Deep Sea Research Part II: Topical Studies in Oceanography **55**:83-93.

Pond, C. M., C. A. Mattacks, I. Gilmour, M. A. Johnston, C. T. Pillinger, and P. Prestrud. 1995. Chemical and carbon isotopic composition of fatty acids in adipose tissue as indicators of dietary history in wild arctic foxes (A lopex lagopus) on Svalbard. Journal of Zoology **236**:611-623.

Raclot, T., R. Groscolas, and Y. Cherel. 1998. Fatty acid evidence for the importance of myctophid fishes in the diet of king penguins, Aptenodytes patagonicus. Marine Biology **132**:523-533.

Ramos, C., C. Parrish, T. Quibuyen, and T. Abrajano. 2003. Molecular and carbon isotopic variations in lipids in rapidly settling particles during a spring phytoplankton bloom. Organic Geochemistry **34**:195-207.

Reijnders, P. J. H., K. F. Abt, S. M. J. M. Brasseur, K. C. J. Camphuysen, B. Reineking, M. Scheidat, U. Siebert, M. Stede, J. Tougaard, and S. Tougaard. 2005. Marine Mammals. Wadden Sea Ecosystem N°19. pp. 305-318. Wadden Sea Secretariat, Wilhelmshaven, Germany.

Reijnders, P. J. H., S. M. J. M. Brasseur, T. Borchardt, K. Camphuysen, R. Czeck, A. Gilles, L. F. Jensen, M. Leopold, K. Lucke, S. Ramdohr, M. Scheidat, U. Siebert, and J. Teilmann. 2009. Marine mammals. Thematic Report N°20. In: Marencic, H. and Vlas, J. de, editors. Quality status report 2009. Wadden Sea Ecosystem N°25. Wadden Sea Secretariat, Trilateral Monitoring and Assessment Group, Wilhelmshaven, Germany.

Rouvinen, K., and T. Kiiskinen. 1989. Influence of Dietary Fat Source on the Body Fat Composition of Mink (Mustela vison) and Blue Fox (Alopex lagopus). Acta Agriculturae Scandinavica **39**:279-288.

Scribe, P., J. Fillaux, J. Laureillard, V. Denant, and A. Saliot. 1991. Fatty acids as biomarkers of planktonic inputs in the stratified estuary of the Krka River, Adriatic Sea: relationship with pigments. Marine Chemistry **32**:299-312.

Siebert, U., S. Müller, A. Gilles, J. Sundermeyer, and N. U.I. 2012. Species profiles marine mammals Pages 487–542 *in* I. Narberhaus, J. Krause, and U. Bernitt, editors. Threatened Biodiversity in the German North and Baltic Seas – Sensitivities towards Human Activities and the Effects of Climate Change.

Siebert, U., P. Wohlsein, K. Lehnert, and W. Baumgärtner. 2007. Pathological Findings in Harbour Seals (*Phoca vitulina*): 1996–2005. Journal of Comparative Pathology **137**:47-58.

Sievers, U. 1989. Stomach content analysis in the harbour seal (*Phoca vitulina*) from the Schleswig-Holstein Waddensea. Zool. Anz. **222**:249–260.

Todd, S. K., B. Holm, D. A. S. Rosen, and D. J. Tollit. 2010. Stable isotope signal homogeneity and differences between and within pinniped muscle and skin. Marine Mammal Science **26**:176-185.

Tougaard, J., I. Ebbesen, S. Tougaard, J. T., and J. Teilmann. 2003. Satellite tracking of harbour seals on Horns Reef. Technical report to Techwise A/S, Biological Papers from the Fisheries and Maritime Museum, Esbjerg.

Trumble, S. J., S. R. Noren, L. A. Cornick, T. J. Hawke, and S. B. Kanatous. 2010. Age-related differences in skeletal muscle lipid profiles of Weddell seals: clues to developmental changes. Journal of Experimental Biology **213**:1676-1684.

Volkman, J. K., S. M. Barrett, S. I. Blackburn, M. P. Mansour, E. L. Sikes, and F. Gelin. 1998. Microalgal biomarkers: a review of recent research developments. Organic Geochemistry **29**:1163-1179.

Zhukova, N. V., and V. I. Kharlamenko. 1999. Sources of essential fatty acids in the marine microbial loop. Aquatic Microbial Ecology **17**:153-157.

Appendix 1 (p.116 – 117)

Appendix 1: Relative proportions (%) of fatty acids in potential prey species of harbor seals from the Sylt Rømø Bight for the different sampling seasons. Lines in bold correspond to the dominant fatty acids (> 4%). Lines in italic correspond to the fatty acids from bacteria and summed as "bacteria FAs"

	A. tobianus		***C. harengus***			***M. merlangus***	***O. eperlanus***	
FA	spring	summer	spring	summer	fall	summer	spring	summ
14:0	3.6	3.5	1.6	1.9	3.3	0.7	2	2.2
15:0	*0.8*	*0.7*	*0.6*	*0.4*	*0.8*	*0.6*	*0.5*	*0.6*
16:0	**22.2**	**17.9**	**16.5**	**16.7**	**18.3**	**19.2**	**14.5**	**16.9**
16:1(*n-7*)	**9**	**5**	**3.6**	**3.4**	**4.8**	**2**	**7**	**6.3**
16:4(*n-1*)	0.1	0.1	0.3	0.1	0.1	0.3	0.2	0.1
17:0	*0.7*	*0.7*	*0.5*	*0.4*	*0.6*	*1.2*	*0.5*	*0.6*
18:0	**5.2**	**4.8**	**3.6**	**4**	**4.5**	**9.2**	**3.2**	**3.9**
18:1(n-7)	*3.7*	*2.9*	*3.7*	*2.7*	*3.2*	*4.4*	*3.9*	*4.5*
18:1(*n-9*)	**7.8**	**8.9**	**8.5**	**10.9**	**7.8**	**12.3**	**19.6**	**21.2**
18:2(*n-6*)	0.3	0.2	0.1	0.1	0.3	0.6	0.6	0.2
18:4(*n-3*)	1.5	2.3	0.7	1.2	3	0.1	0.3	0.7
19:1(n-8)	*0.1*	*0.1*	*0.1*	*0.1*	*0.1*	*0.3*	*0.2*	*0.3*
20:1(*n-7*)	0.6	1	0.5	0.5	0.3	0.8	0.8	0.5
20:1(*n-9*)	0.7	0.6	0.4	0.4	0.4	0.7	0.4	0.5
20:4(*n-6*)	**0.9**	**1.4**	**1.6**	**0.6**	**1.1**	**2.3**	**2.6**	**1.7**
20:5(*n-3*)	**11.5**	**13.6**	**12.3**	**9.1**	**11.2**	**6.7**	**10.6**	**12**
22:3(*n-3*)	0.4	0.5	0.5	0.3	0.5	0.4	0.6	0.5
22:3(*n-4*)	0.3	0.4	0.2	0.2	0.3	0.7	0.8	0.4
22:5(*n-3*)	0.9	2	1.1	1.1	1.1	1.2	1.7	1.6
22:6(*n-3*)	**13.3**	**19.1**	**23.5**	**19.6**	**24.6**	**13.4**	**16.5**	**15.5**
24:1(*n-9*)	2	1.1	1.3	1.4	1.4	2	1	0.5
bacteria (Sum of 15:0, 17:0, 18:1(*n-7*), 19:1(*n-8*))	5.5	4.7	5.1	3.8	4.8	6.9	5.3	6.2

Appendix 1 continued

	P. minutus			***P. platessa***			***C. crangon***	
FA	spring	summer	fall	spring	summer	fall	summer	fall
14:0	1.1	1.3	1.2	1.3	1.7	3.8	0.8	1
15:0	*0.6*	*0.7*	*1*	*0.8*	*1.2*	*1.3*	*0.8*	*1*
16:0	**12.9**	**15.4**	**13.4**	**12.6**	**14.6**	**13.8**	**16.5**	**15.7**
16:1(*n-7*)	**4.3**	**3.8**	**4.3**	**1.8**	**6.2**	**5.1**	**0.7**	**2.5**
16:4(*n-1*)	0.4	0.1	0.2	0.6	0.1	0.1	0.1	0
17:0	*0.5*	*0.8*	*1*	*0.6*	*1.1*	*1*	*1.3*	*1.4*
18:0	**4.9**	**6.3**	**5.2**	**5**	**4.5**	**5.4**	**6**	**4.2**
18:1(n-7)	*4.2*	*3*	*4.3*	*2.8*	*5.4*	*3.8*	*4.7*	*3.7*
18:1(*n-9*)	**7.4**	**6.2**	**7.9**	**7.3**	**6.9**	**6.7**	**8.4**	**7.4**
18:2(*n-6*)	0.4	0.4	0.3	0.1	0.7	0.4	0	0.1
18:4(*n-3*)	0.1	0.3	0.2	0.4	1.2	2.1	0.2	0.3
19:1(n-8)	*0.2*	*0.2*	*0.4*	*0.4*	*0.6*	*0.4*	*0.4*	*0.4*
20:1(*n-7*)	0.5	0.4	1.1	1	2.6	2.6	1.4	0.3
20:1(*n-9*)	0.6	0.3	0.9	1.1	1.3	1	0.5	0.5
20:4(*n-6*)	**4.3**	**3.4**	**3.7**	**4.6**	**2.7**	**1.8**	**3.9**	**1.9**
20:5(*n-3*)	**11.9**	**12.1**	**12.8**	**14.3**	**15.8**	**14.9**	**17**	**19.8**
22:3(*n-3*)	1	1.1	0.6	0.7	0.8	0.5	0.8	0.5
22:3(*n-4*)	1.7	2	1.3	1.3	1	1.3	1.1	1
22:5(*n-3*)	4.1	4.1	3.2	4.2	3.7	4.4	2.2	3.8
22:6(*n-3*)	**19.2**	**18**	**14.9**	**14.3**	**7.5**	**10.1**	**15.3**	**13.3**
24:1(*n-9*)	0.4	0.5	0.4	1.5	0.3	0.3	0.5	0.1
bacteria (Sum of 15:0, 17:0, 18:1(*n-7*), 19:1(*n-8*))	5.8	5.2	7	4.8	8.9	7.2	7.6	6.8

Appendix 2 (p.120 – 121)

Appendix 2: Relative proportions (%) of fatty acids in harbor seals from the Sylt Rømø Bight. Lines in bold correspond to the dominant fatty acids (>4%). Numbers in italic correspond to the fatty acids from bacteria and summed as "bacteria FAs"

	seal 1	seal 2	seal 3	seal 4	seal 5	seal 6
14:0	0.6	0.7	0.8	0.5	1.2	0.4
15:0	*0.3*	*0.2*	*0.2*	*0.2*	*0.3*	*0.2*
16:0	**13**	**16.1**	**9.7**	**11.7**	**13.5**	**13.3**
16:1(*n-7*)	**2.7**	**4.3**	**5.1**	**5.8**	**8.4**	**2.8**
16:4(*n-1*)	1.1	0.4	0.5	1.2	0.7	0.9
17:0	*0.4*	*0.6*	*0.5*	*0.4*	*0.5*	*0.5*
18:0	**9.2**	**12.7**	**6.8**	**10.5**	**8.9**	**12.1**
18:1(n-7)	*3.9*	*5.4*	*6.4*	*6.6*	*5.8*	*4.6*
18:1(*n-9*)	**19.1**	**21.7**	**23.7**	**16.3**	**17.6**	**14.8**
18:2(*n-6*)	3.3	1.2	1.4	2.2	0.9	0.9
18:4(*n-3*)	0	0	0.2	0.1	0.1	0.1
19:1n-8	*0.6*	*0.4*	*0.8*	*1.1*	*0.7*	*0.8*
20:1(*n-7*)	0.1	0.5	1.1	0.3	0.4	0.3
20:1(*n-9*)	4.3	0.6	2.8	0.8	0.8	0.3
20:4(*n-6*)	**6.1**	**7.8**	**7.6**	**11.3**	**6.2**	**11.7**
20:5(*n-3*)	**3.4**	**2.7**	**3**	**4.5**	**5.2**	**7.2**
22:3(*n-3*)	0.1	0.1	0.2	0.2	0.2	0.3
22:3(*n-4*)	0.2	0.5	0.7	0.5	0.6	0.6
22:5(*n-3*)	1	1.7	3.9	1.7	4	3.2
22:6(*n-3*)	**3.7**	**3.1**	**5.2**	**4.1**	**5.3**	**7.3**
24:1(*n-9*)	0.9	0.5	0.6	0.4	0.7	0.3
bacteria (Sum of 15:0, 17:0, 18:1(*n-7*), 19:1(*n-8*))	5.3	6.9	8.2	8.6	7.6	6.4

Appendix 2 continued

	seal 7	**seal 8**	**seal 9**	**seal 10**	**seal 11**	**seal 12**
C14:0	0.3	0.7	1.2	0.4	0.4	0.9
C15:0	*0.1*	*0.3*	*0.3*	*0.2*	*0.2*	*0.2*
C16:0	**8.7**	**14.6**	**16.4**	**10.8**	**16.1**	**14.7**
C16:1n-7	**3.6**	**3.1**	**5.4**	**4.1**	**0.8**	**3.7**
C16:4n-1	0.8	0.5	0.5	0.9	1.1	0.9
C17:0	*0.4*	*0.6*	*0.6*	*0.4*	*0.6*	*0.3*
C18:0	**9.4**	**13.1**	**10.4**	**10.8**	**15.5**	**7.8**
C18:1n-7	*5.1*	*5.9*	*5.7*	*6.8*	*5.3*	*6.8*
C18:1n-9	**13.6**	**14**	**19.7**	**14.9**	**13.6**	**18.3**
C18:2n-6	1.2	0.1	0.9	1.8	0	0.3
C18:4n-3	0	0	0.1	0	0.1	0.1
C19:1n-8	*1*	*0.6*	*0.6*	*1.1*	*1.2*	*0.9*
C20:1n-7	0.2	0.3	0.6	0.4	0.6	0.4
C20:1n-9	0.3	0.6	0.8	0.7	0.9	0.6
C20:4n-6	**10.6**	**10**	**6.4**	**15.1**	**3.9**	**8.8**
C20:5n-3	**4.3**	**3.6**	**3.8**	**5**	**6.1**	**3.2**
C22:3n-3	0.2	0.2	0.2	0.2	0.1	0.3
C22:3n-4	0.9	0.9	0.5	0.5	0.2	0.8
C22:5n-3	1.8	2	3.1	1.9	2.8	4
C22:6n-3	**5.3**	**3.9**	**4.5**	**4.5**	**8.3**	**7**
C24:1n-9	0.5	3.2	0.7	0.4	0.5	0.6
bacteria (*C15:0, C17:0, C18:1n-7, C19:1n-8*)	**6.8**	**7.7**	**7.6**	**8.8**	**7.6**	**8.4**

Appendix 3: Results of the PCA; a- Repartition of the total inertia between the four first axes; b- Repartition of the inertia between prey species, per axis; c- Repartition of the inertia between FA per axis; in b- and c-, contributions are in % and the signs are the signs of the coordinates; Values in bold represent the largest part of the inertia explained for each species (a) and each FA (b)

	Axis 1	Axis 2	Axis 3	Axis 4
Repartition of the total inertia between axis				
Contribution (%)	37.2	17.0	12.9	10.1
Contr. Cum. (%)	37.2	54.2	67.1	77.2
Repartition of the inertia between prey species per axis				
A. tobianus - spring	**59.9**	17.5	-5.8	2.0
A. tobianus - summer	**40.6**	10.8	0.0	14.6
C. harengus - spring	**57.3**	-12.8	9.7	2.7
C. harengus - summer	**78.7**	-1.8	0.7	1.2
C. harengus - fall	**76.6**	0.0	4.6	0.0
M. merlangus - summer	0.9	-10.0	**-80.3**	-2.3
O. eperlanus - spring	3.7	0.0	3.3	**-67.2**
O. eperlanus - summer	15.8	0.1	11.1	**-20.8**
P. minutus - spring	**-47.4**	-11.7	1.9	-17.0
P. minutus - summer	**-19.9**	-18.8	3.2	-6.7
P. minutus - fall	**-77.7**	0.7	-1.4	-5.9
P. platessa - spring	**-27.6**	-1.3	-3.6	10.4
P. platessa - summer	-40.2	**51.6**	-0.8	-0.1
P. platessa - fall	-21.6	**65.8**	0.1	1.6
C. crangon - summer	**-29.6**	-25.8	-1.1	26.7
C. crangon - fall	-21.2	-6.5	**31.8**	6.8
Repartition of the inertia between FAs per axis				
14:0	29.83	**50.49**	7.33	0.03
16:0	**51.82**	-0.07	-8.32	4.33
16:1(*n-7*)	12.88	**42.59**	4.85	-30.5
18:0	-8.86	-3.27	**-57.69**	3.46
18:1(*n-9*)	14.71	-2.35	-0.37	**-27.76**
18:2(*n-6*)	-0.01	22.68	-10.1	**-54.73**
18:4(*n-3*)	28.16	**45.91**	9.07	6.97
20:1(*n-7*)	-21.36	**35.02**	14.64	2.65
20:1(*n-9*)	-21.01	**40.35**	-18.61	2.51
20:4(*n-6*)	**-74.37**	-6.84	-1.54	-2.77
20:5(*n-3*)	-28.97	4.61	**34.81**	24.6
22:3(*n-3*)	**-51.3**	-2.78	1.63	-4.64
22:3(*n-4*)	**-85.75**	-0.46	-0.14	-2.36
22:5(*n-3*)	**-79.8**	1.61	4.77	0.04
22:6(*n-3*)	**31.71**	**-36.53**	8.13	-0.36
24:1(*n-9*)	**52.08**	-1.85	-25.99	0.00
Bacteria (15:0, 17:0, 19:1(*n-8*), 18:1(*n-7*))	**-49.97**	7.09	-4.40	0.05

CHAPTER 3

Seasonal shift in harbor seal´s diet assessed with fatty acid analyses

PART II

Food Web Model

"Je ne puis pas donner la réalité des faits, je n´en puis présenter que l´ombre."

"I cannot give the reality of the facts; I can only present its shadow."

Stendhal

Chapter 4

Relationships between fresh weight, dry weight, ash free dry weight, carbon and nitrogen content for selected vertebrates

Relationships between fresh weight, dry weight, ash free dry weight, carbon and nitrogen content for selected vertebrates

Sabine Horn[1]*, Camille de la Vega[1]*

Journal of Experimental Marine Biology and Ecology 481 (2016) 41-48

[1] Alfred-Wegener-Institute Helmholtz-Center for Polar- and Marine Research, Wadden Sea Station Sylt
Hafenstrasse 43, D-25992 List/ Sylt, Germany

*Corresponding authors: camille.de.la.vega@awi.de; sabine.horn@awi.de

Abstract

Top predators are relevant indicators of the ecological status of a system and can have a high impact on food webs. But top predators are difficult to include in network analyses because their biomass in ash free dry weight or carbon content is missing. Regression equations were determined for the relationships between fresh weight and dry weight, ash free dry weight, carbon and nitrogen contents respectively for six of the most abundant bird species in the Wadden Sea (*Calidris canutus, Limosa lapponica, Haematopus ostralegus, Chroicocephalus ridibundus*, *Larus canus, Anas penelope*) and harbor seals (*Phoca vitulina*). The relationships for all species were interpreted as linear through the origin. Carbon content *vs.* fresh weight ratios for birds ranged from 0.16 ± 0.01 to 0.22 ± 0.02. Carbon content *vs.* fresh weight ratio was 0.17 ± 0.02 on average for harbor seals. This work highlights that the biomass of top predators was often over- or underestimated in previous studies. The determined conversion factors will be useful for future studies to generate more realistic food web models.

Key words

Sea birds, harbor seals, biomass measures, weight to weight conversion, %C, food web modelling

1. Introduction

In the last decades, food web models and ecological networks have become useful tools to describe the functioning of large and complex ecosystems encompassing numerous compartments interacting with each other and responding differently to external stressors (Ings et al. 2009). In many studies, network analyses have been used to define ecosystem properties. These properties include the ecosystem structural complexity, the structure and magnitude of the cycling of energy and material, the efficiency of energy transfer within the system, the rates of energy assimilation and dissipation, the trophic structure, the system activity, growth and development (Baird et al. 2004). Results from these models provide significant insights into the fundamental functioning of the ecosystem (Baird et al. 2004) and are very relevant for the management of marine ecosystems (Samhouri et al. 2009).

Abundance and distribution of top predators, such as sea birds and marine mammals, can have a large influence on community structures and on the functioning of the ecosystem they live in (Baird et al. 1985, Bowen 1997, Moreira 1997). As a corollary, they are good indicators for ecosystem's health (Furness and Camphuysen 1997, Reddy et al. 2001, Bossart 2011). Therefore, there is an increasing need to include marine birds and mammals in ecosystem models, especially in studies about trophodynamic to have a better understanding of food web functioning, allowing improvement of management plans for conservation.

Studies about marine bird and mammal populations are classically based on abundance data (Reijnders et al. 1997, Brasseur et al. 2013, Markert et al. 2013, Galatius et al. 2014, Mandema et al. 2015), which cannot be directly used to study matter or energy flow within ecosystems (Dumont et al. 1975). These abundance data can be converted to fresh weight values using average individual weight corresponding to the studied species. But the use of fresh tissue might lead to large approximations in the organic matter weight, as body water content can vary between taxa. The fresh weight is therefore a bad proxy for biomass comparison. In ecological studies it is a common practice to use standardized biomass units (e.g. dry weight, ash free dry weight, carbon content) allowing comparison of different species biomass from different locations or periods of time (e.g. seasons, years). Most of the mass balanced food web models such as ECOPATH with ECOSIM (Bradford-Grieve et al. 2003, Leguerrier et al. 2007, Pinkerton et al. 2010) and especially ecological network analyses (Baird et al. 2004, Scharler and Baird 2005, Fath et al. 2007, Baird et al. 2012, Saint-Béat et al. 2013) also rely on these consistent and standardized biomass units (e.g. dry weight, ash free dry weight, carbon content).

Although a large database of conversion factors from fresh weight to standardized biomass units is available for macrobenthic invertebrates (Rumohr et al. 1987, Ricciardi and Bourget 1998a), to our knowledge, no such database exists for marine birds and mammals. As a result, including top predators in ecosystem models is very difficult. It is associated with a high degree of uncertainty and relies on large approximations that might bias the model outputs.

The aim of this study was to determine relationships useful for modeling between fresh weight (FW) and dry weight (DW), FW and ash free dry weight (AFDW), FW and carbon content (CC) and FW and nitrogen content (NC). These relationships were determined for six of the most abundant bird species in the Wadden Sea (Blew et al. 2013) (*Calidris canutus,* Linnaeus, 1758; *Limosa lapponica,* Linnaeus, 1758; *Haematopus ostralegus,* Linnaeus, 1758; *Chroicocephalus ridibundus,* Linnaeus, 1766; *Larus canus,* Linnaeus, 1758; *Anas penelope,* Linnaeus, 1758), and for harbor seal (*Phoca vitulina,* Linnaeus, 1758), one of the most abundant marine mammal species in this area (Reijnders et al. 2009).

2. Material and methods

Carcasses of birds and seals were collected along the shore of the eastern German Wadden Sea, between the coastal city Büsum in the South and the island of Föhr in the North (Fig. 1). Only fresh carcasses which did not show any noticeable signs of starvation or diseases were selected for this study.

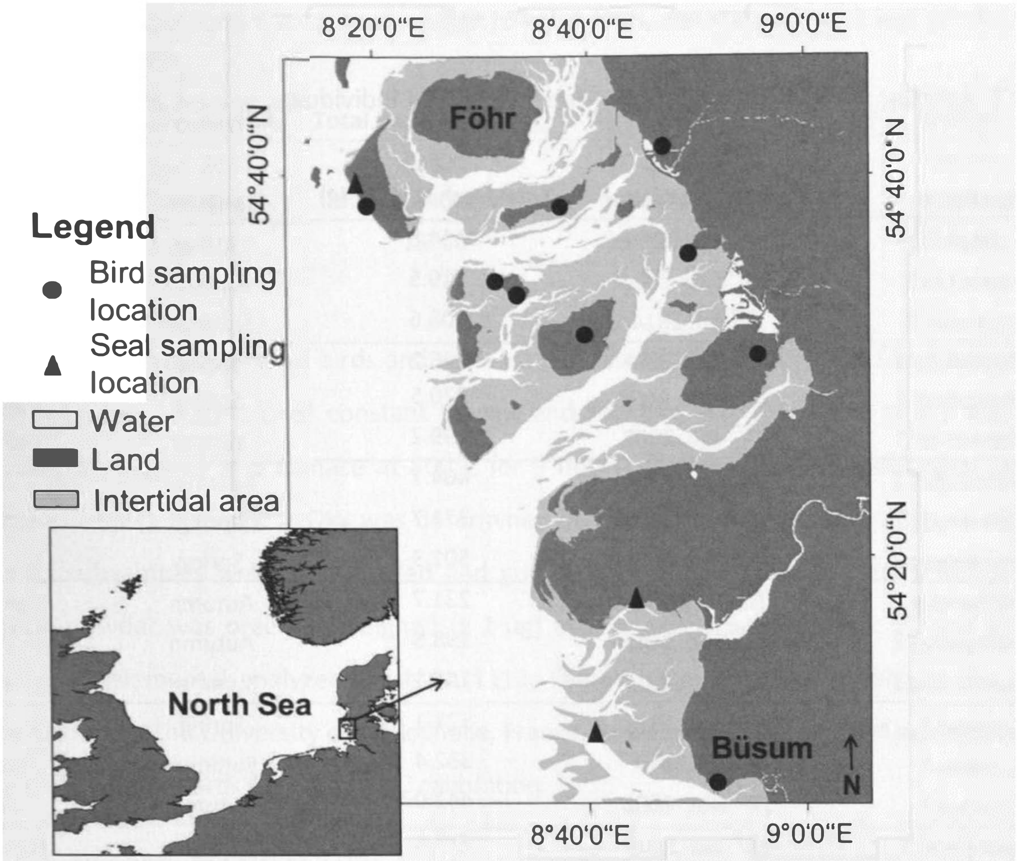


Fig. 1: Location and map of the study area. The circles and triangles refer to the locations where carcasses of birds and seals were respectively found

Seventeen birds from six different species (*C. canutus*, *H. ostralegus*, *L. lapponica*, *C. ridibundus*, *L. canus*, and *A. penelope*) were collected by a network of volunteers. Three individuals were collected for each species, except for *A. penelope* for which only two birds were available. Most individuals died due to collision with lighthouses or cars (Table 1, p.132). Carcasses were stored frozen in plastic bags at -20 °C until preparation for analyses. Each individual was unfrozen and grinded entirely using a kitchen cutter (RCKC-6000, Royal Catering, 750 watts) in order to get a homogenized mixture composed of all the tissues. Four subsamples were collected from each grinded individual: three for determination of fresh weight (FW), dry weight (DW) and ash free dry weight (AFDW), and one for carbon content (CC) and nitrogen content (NC) analyses.

Table 1: Species, date of collection, total fresh weight of individuals, season and cause of death of the birds

Species #	Date of collection	Total fresh Weight (g)	Season	Cause of death
C. canutus 1	4th Apr. 2014	114.8	Spring	Unknown
C. canutus 2	21st Sep. 2014	119.5	Autumn	Unknown
C. canutus 3	7th Jul. 2014	108.6	Summer	Unknown
L. lapponica 1	2nd Apr. 2004	246.2	Spring	Lighthouse collision
L. lapponica 2	20th Mar. 2007	270.5	Spring	Lighthouse collision
L. lapponica 3	25th Jan. 2007	299.2	Winter	Lighthouse collision
H. ostralegus 1	2nd Jun. 2014	464.7	Summer	Unknown
H. ostralegus 2	27th Mar. 2014	371.7	Spring	Unknown
H. ostralegus 3	27th Apr. 2009	501.3	Spring	Unknown
C. ridibundus 1	27th Sep. 2013	231.7	Autumn	Lighthouse collision
C. ridibundus 2	13th Sep. 2013	198.5	Autumn	Unknown
C. ridibundus 3	3rd Jun. 2012	150.1	Summer	Unknown
L. canus 1	6th May. 2013	521.1	Spring	Unknown
L. canus 2	4th Jul. 2014	332.4	Summer	Vehicle collision
L. canus 3	17th Nov. 2006	442.0	Autumn	Vehicle collision
A. penelope 1	15th Jan. 2002	777.5	Winter	Lighthouse collision
A. penelope 2	11th Nov. 2007	795.7	Autumn	Lighthouse collision

Three harbor seals were collected in 2015 (Table 2) as part of the stranding network established along the German coasts of Schleswig-Holstein (Benke et al. 1998, Siebert et al. 2006). Carcasses were stored frozen in plastic bags at -20°C until necropsies, which were carried out according to the protocol described by Siebert et al. (2007), at the Institute for Terrestrial and Aquatic Wildlife Research of the University of Veterinary Medicine, Hannover Foundation. The different tissues were dissected and weighed (± 0.1 g). The contribution of each tissue to the total fresh weight was determined for each individual. Two subsamples were collected from each tissue and each individual: one for determination of FW, DW, AFDW and one for determination of CC and NC.

Table 2: Seal ID, Date of collection, total fresh weight of individuals, age status, length and gender of the three sampled harbor seals

Seal #	Date of collection	Total Fresh Weight (g)	Age status	Length (cm)	Gender
Phoca vitulina 1	3rd Jun. 2015	83800	Adult	180	Female
Phoca vitulina 2	15th Jun. 2015	85400	Adult	173.5	Male
Phoca vitulina 3	1st Aug. 2015	16200	Juvenile	96.5	Female

The FW of each subsample of birds and seals was measured to the nearest 0.1 mg. Subsamples were dried in an oven at 50°C until constant weight and the DW was measured (± 0.1 mg). Each subsample was then burned in a furnace at 500°C for 5 hours, cooled down in a desiccator and ash weight was measured (± 0.1 mg). AFDW was determined by subtracting the ash weight from the DW. For CC and NC, subsamples were freeze-dried and grinded into a fine powder using a ball mill. An amount of each powder was precisely weighed (± 1 µg) and sealed in a tin capsule. CC and NC were measured using an elemental analyzer (Flash EA 1112, Thermo Scientific, Milan, Italy) at the LIENSs stable isotope facility of the University of La Rochelle, France. Acetanilide (Thermo) and peptone (Sigma-Aldrich) were used as standards for CC and NC calibration.

Relationships between FW and DW, AFDW, CC and NC respectively were plotted for bird species and for each seal tissue. These plots were then made for entire seal individuals taking into account the mass proportions of each tissue in FW. Missing data for some tissues were estimated by assuming that the proportion of the weight of missing tissue is the same as in *Phoca vitulina* 1 (Table 6, p. 139).

The regression equations for FW and DW, AFDW, CC and NC respectively were calculated for all individuals of bird species combined, for the seal tissues and for entire seals.

3. Results and discussion

3.1. Birds

3.1.1. Relationships among biomass measures

The regression equations of all measured bird individuals revealed linear relationships that pass through the origin between FW and DW, AFDW, CC and NC respectively (Fig. 2, Table 3) and represented 93% (i.e. FW *versus* CC) to 98% (i.e. FW *vs.* DW) of the variation of the measured data points (i.e. R^2, Table 3). Therefore, these equations allow the use of ratios between the different biomass measures and give confidence to extrapolation to heavier and lighter bird species.

The ratios FW *vs.* DW (FW/DW), FW *vs.* AFDW (FW/AFDW), FW *vs.* CC (FW/CC), FW *vs.* NC (FW/NC), DW *vs.* CC (DW/CC), AFDW *vs.* CC (AFDW/CC) and DW *vs.* NC (DW/NC) were then calculated for each replicate of birds to verify the homogeneity of the mixture.

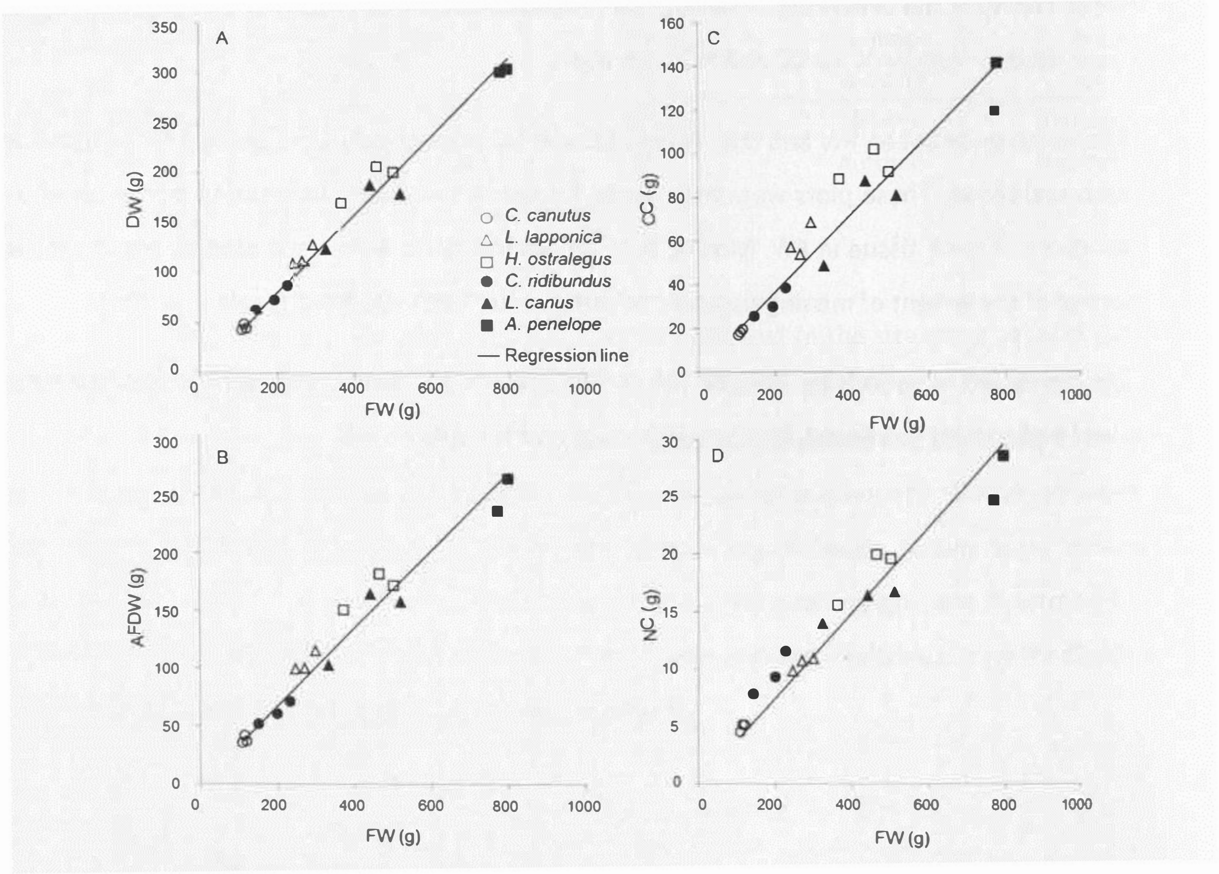


Fig. 2: Relationships between FW and DW, FW and AFDW, FW and CC, FW and NC for all bird species combined. The regression equations are shown in Table 3

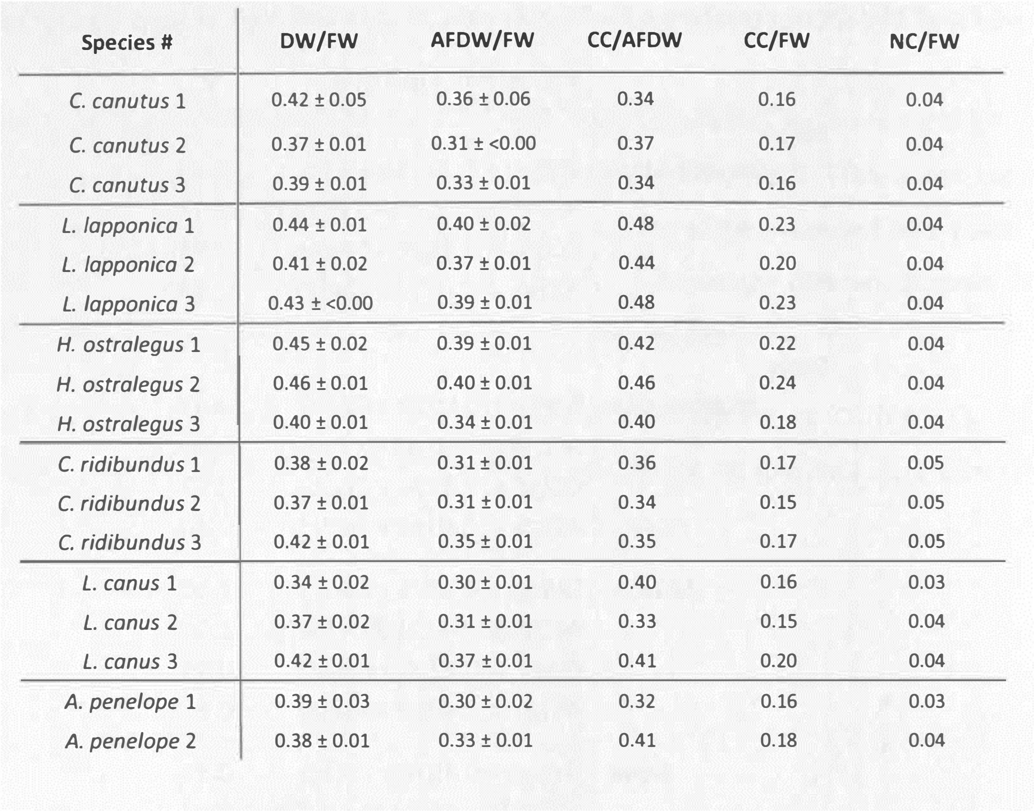
Table 3: Regression equations and R^2 for relationships between FW and DW, FW and AFDW, FW and CC, FW and NC for all bird species combined, for Blubber-skin, Muscle and Bone of seals, and for entire seals

	Regression equation	**R^2**
Birds		
Entire individual	$DW(g) = 0.3953 \times FW(g)$	0.98
	$AFDW(g) = 0.3378 \times FW(g)$	0.97
	$CC(g) = 0.1807 \times FW(g)$	0.93
	$NC(g) = 0.0371 \times FW(g)$	0.95
Seals		
Blubber-skin	$DW(g) = 0.5522 \times FW(g)$	0.97
	$AFDW(g) = 0.538 \times FW(g)$	0.96
	$CC(g) = 0.3274 \times FW(g)$	0.92
	$NC(g) = 0.0291 \times FW(g)$	0.80
Muscle	$DW(g) = 0.2821 \times FW(g)$	1.00
	$AFDW(g) = 0.2699 \times FW(g)$	1.00
	$CC(g) = 0.1295 \times FW(g)$	0.99
	$NC(g) = 0.0391 \times FW(g)$	0.95
Bone	$DW(g) = 0.4576 \times FW(g)$	0.99
	$AFDW(g) = 0.3328 \times FW(g)$	0.97
	$CC(g) = 0.1617 \times FW(g)$	0.95
	$NC(g) = 0.0453 \times FW(g)$	0.87
Entire individual	$DW(g) = 0.3396 \times FW(g)$	1.00
	$AFDW(g) = 0.3029 \times FW(g)$	0.98
	$CC(g) = 0.1617 \times FW(g)$	0.95
	$NC(g) = 0.0453 \times FW(g)$	0.87

3.1.2. Homogeneity of replicates in bird individuals

The intra-individual standard deviations of ratios varied from <0.01 (*L. lapponica* 3) to 0.05 (*C. canutus* 1) for DW/FW and from <0.01 (*C. canutus* 2) to 0.06 (*C. canutus* 1) for AFDW/FW (Table 4, p. 136). The bird mixture was therefore considered to be homogeneous and representative of the whole individual in terms of body tissue composition, thanks to the very small standard deviations between replicates of a same individual. This grinding method is consequently appropriate for biomass estimation studies in birds.

Table 4: DW/FW, AFDW/FW, CC/AFDW, CC/FW and NC/FW ratios for birds; mean per individual ± standard deviation (n=3) is shown for DW/FW and AFDW/FW



Species #	DW/FW	AFDW/FW	CC/AFDW	CC/FW	NC/FW
C. canutus 1	0.42 ± 0.05	0.36 ± 0.06	0.34	0.16	0.04
C. canutus 2	0.37 ± 0.01	0.31 ± <0.00	0.37	0.17	0.04
C. canutus 3	0.39 ± 0.01	0.33 ± 0.01	0.34	0.16	0.04
L. lapponica 1	0.44 ± 0.01	0.40 ± 0.02	0.48	0.23	0.04
L. lapponica 2	0.41 ± 0.02	0.37 ± 0.01	0.44	0.20	0.04
L. lapponica 3	0.43 ± <0.00	0.39 ± 0.01	0.48	0.23	0.04
H. ostralegus 1	0.45 ± 0.02	0.39 ± 0.01	0.42	0.22	0.04
H. ostralegus 2	0.46 ± 0.01	0.40 ± 0.01	0.46	0.24	0.04
H. ostralegus 3	0.40 ± 0.01	0.34 ± 0.01	0.40	0.18	0.04
C. ridibundus 1	0.38 ± 0.02	0.31 ± 0.01	0.36	0.17	0.05
C. ridibundus 2	0.37 ± 0.01	0.31 ± 0.01	0.34	0.15	0.05
C. ridibundus 3	0.42 ± 0.01	0.35 ± 0.01	0.35	0.17	0.05
L. canus 1	0.34 ± 0.02	0.30 ± 0.01	0.40	0.16	0.03
L. canus 2	0.37 ± 0.02	0.31 ± 0.01	0.33	0.15	0.04
L. canus 3	0.42 ± 0.01	0.37 ± 0.01	0.41	0.20	0.04
A. penelope 1	0.39 ± 0.03	0.30 ± 0.02	0.32	0.16	0.03
A. penelope 2	0.38 ± 0.01	0.33 ± 0.01	0.41	0.18	0.04

3.1.3. Conversion factors of bird species

The DW/FW ratios (mean per species ± standard deviation) ranged from 0.38 ± 0.04 (*L. canus*) to 0.44 ± 0.03 (*H. ostralegus*), the AFDW/FW ratios ranged from 0.32 ± 0.01 (*A. penelope*) to 0.38 ± 0.04 (*H. ostralegus*) and the CC/FW rations ranged from 0.16 ± 0.01 (*C. canutus*) to 0.22 ± 0.02 (*L. lapponica*; Table 5). The bird species were then constituted of 16% to 22% of carbon ($gC.100gFW^{-1}$). This is higher than the value of 10% used by Bradford-Grieve et al. (2003) and the value of 4% used by Leguerrier et al. (2007) for sea birds in general (Table 5). These authors probably underestimated the bird biomass in their models. On the contrary, Saint-Béat et al. (2013) and Baird et al. (2004) used a CC/FW ratio of 0.30 (Asmus, personal communication; Table 5), higher than the one measured in this study. As a result, these authors probably overestimated the biomass of birds in their models, and therefore the role of birds in the studied systems. Scharler and Baird (2005) used a CC/AFDW ratio of 0.50 estimated by McLusky (1989), which is in accordance with the CC/AFDW ratios found in this study ranging from 0.49 ± 0.05 (*C. canutus*) to 0.57 ± 0.03 (*L. lapponica*; Table 5).

NC/FW ratios ranged from 0.03 ± <0.01 (*A. penelope*) to 0.05 ± <0.01 (*C. ridibundus*; Table 5). Studying ecosystem and food web structures using nitrogen as proxy is not common yet, although some nitrogen-based models have been constructed (Baird et al. 2011). Nitrogen plays an important role in primary production of marine ecosystems being either accumulated in systems such as seagrass beds (Asmus and Asmus 2000), or being a limiting factor (Vitousek and Howarth 1991). The results of this study of the nitrogen content of top predators will be useful data for the construction of future nitrogen-based ecosystem models.

Table 5: DW/FW, AFDW/FW, CC/FW, CC/DW, CC/AFDW, NC/FW, NC/DW ratios (mean ± standard deviation) for various bird, mammal, macrozoobenthos, and fish taxa. Results from this study are displayed in bold

pecies	DW/FW	AFDW/FW	CC/FW	CC/DW	CC/AFDW	NC/FW	NC/DW	References
C. canutus	**0.39 ± 0.03**	**0.33 ± 0.03**	**0.16 ± <0.01**	**0.41 ± 0.03**	**0.49 ± 0.05**	**0.04 ± <0.01**	**0.11 ± 0.01**	**present study**
L lapponica	**0.43 ± 0.02**	**0.38 ± 0.02**	**0.22 ± 0.02**	**0.52 ± 0.03**	**0.57 ± 0.03**	**0.04 ± <0.01**	**0.09 ± 0.01**	**present study**
H. ostralegus	**0.44 ± 0.03**	**0.38 ± 0.04**	**0.21 ± 0.02**	**0.49 ± 0.03**	**0.56 ± 0.03**	**0.04 ± <0.01**	**0.10 ± <0.01**	**present study**
C.ridibundus	**0.39 ± 0.03**	**0.32 ± 0.02**	**0.16 ± 0.01**	**0.42 ± 0.02**	**0.51 ± 0.03**	**0.05 ± <0.01**	**0.13 ± <0.01**	**present study**
L. canus	**0.38 ± 0.04**	**0.33 ± 0.04**	**0.17 ± 0.03**	**0.44 ± 0.04**	**0.51 ± 0.03**	**0.04 ± 0.01**	**0.10 ± 0.01**	**present study**
A. penelope	**0.39 ± 0.01**	**0.32 ± 0.01**	**0.17 ± 0.02**	**0.44 ± 0.05**	**0.52 ± 0.02**	**0.03 ± <0.01**	**0.09 ± 0.01**	**present study**
All birds	**0.40 ± 0.03**	**0.34 ± 0.04**	**0.18 ± 0.03**	**0.45 ± 0.05**	**0.53 ± 0.04**	**0.04 ± 0.01**	**0.10 ± 0.02**	**present study**
All birds			0.10					Bradford-Grieve et al. (2003)
All birds			0.04					Leguerrier et al. (2007)
All birds			0.30					Baird et al. (2004), Saint-Béat et al. (2013)
All birds					0.50			McLusky (1989), Scharler and Baird (2005)
P. vitulina	**0.34 ± 0.02**	**0.30 ± 0.04**	**0.16 ± 0.02**	**0.47 ± 0.01**	**0.53 ± 0.02**	**0.05 ± <0.01**	**0.10 ± <0.01**	**present study**
seals			0.10					Bradford-Grieve et al. (2003)
seals		0.35	0.15					Pinkerton and Bradford-Grieve (2008)

Table 5 continued: DW/FW, AFDW/FW, CC/FW, CC/DW, CC/AFDW, NC/FW, NC/DW ratios (mean ± standard deviation) for various bird, mammal, macrozoobenthos, and fish taxa. Results from this study are displayed in bold

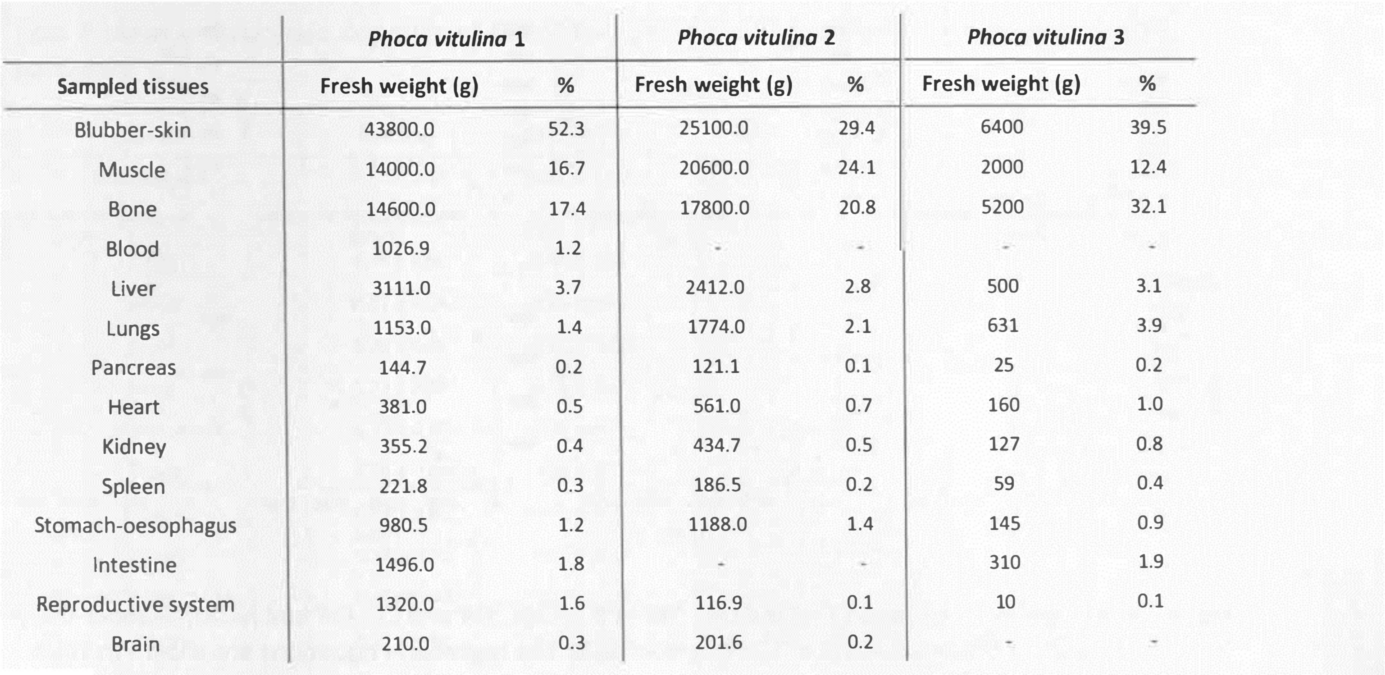
Species	DW/FW	AFDW/FW	CC/FW	CC/DW	CC/AFDW	NC/FW	NC/DW	Referenc
lacrozoobenthos					0.58			Gätje and Reise (1
Polychaeta	0.14			0.38				Cauffope and Heym (2005a)
	0.20	0.16						Ricciardi and Bour (1998b)
	0.18	0.13						Rumohr (1987)
Oligochaeta	0.17							Cauffope and Heym (2005a)
Gastropoda (including shells)	0.09	0.11						Cauffope and Heym (2005a)
		0.09						Rumohr (1987)
Bivalvia (including shells)	0.09	0.06						Cauffope and Heym (2005a)
		0.06						Ricciardi and Bour (1998b)
		0.07						Rumohr (1987)
Crustacea	0.21			0.43				Cauffope and Heym (2005a)
	0.20	0.15						Rumohr (1987)
sh								
Pelagic/Planktivorous (e.g. Clupeids, Sand eel)			0.16					Greenstreet et al. (1 Heath (2007)
Pelagic/Piscivorous (e.g. mackerel species)			0.18					Greenstreet et al. (1 Heath (2007)
Demersal/Piscivorous (e.g. Gadoids)			0.10					Greenstreet et al. (1 Heath (2007)
Demersal/Benthivorous (e.g. flat fish species)			0.11					Greenstreet et al. (1 Heath (2007)
Gadus morua	0.19 ± 0.01	0.14 ± 0.01						Unpublished data fr long term monitor Alfred Wegener Ins Wadden Sea Station
Platichthys flesus	0.19 ± 0.01	0.16 ± 0.01						
Pleuronectes platessa	0.17 ± 0.01	0.13 ± 0.01						
Clupea harengus	0.20 ± 0.05	0.16 ± 0.04						
Ammodytes tobianus	0.21 ± 0.01	0.17 ± 0.01						
errestrial mammals								
Guinea pig	0.37 ± 5.6					0.03 ± 0.4	0.09 ± 1.9	Pace and Rathbun (1
Rat	0.36 ± 0.02					0.04		Pace and Rathbun (1
Rabbit	0.29 ± 0.04					0.03		Pace and Rathbun (1
Dog	0.41 ± <0.01							Pace and Rathbun (1
Cat	0.34					0.03		Pace and Rathbun (1

3.2. Seals

3.2.1. Body composition

Blubber-skin tissue made the highest contribution to the total fresh weight of harbor seals, and represented on average 40.4 ± 11.5% (from 29.4%, *Phoca vitulina* 2 to 52.3%, *Phoca vitulina* 1; Table 6). The next highest contributions to total fresh weight were Bone (23.4 ± 7.7%) and Muscle (17.8 ± 6.0%). All the other tissues represented less than 4% of the total fresh weight (Table 6).

Table 6: Fresh weight of each tissue (g) and contribution of each tissue to total fresh weight (%) for the three sampled harbor seals



	***Phoca vitulina* 1**		***Phoca vitulina* 2**		***Phoca vitulina* 3**	
Sampled tissues	**Fresh weight (g)**	**%**	**Fresh weight (g)**	**%**	**Fresh weight (g)**	**%**
Blubber-skin	43800.0	52.3	25100.0	29.4	6400	39.5
Muscle	14000.0	16.7	20600.0	24.1	2000	12.4
Bone	14600.0	17.4	17800.0	20.8	5200	32.1
Blood	1026.9	1.2	-	-	-	-
Liver	3111.0	3.7	2412.0	2.8	500	3.1
Lungs	1153.0	1.4	1774.0	2.1	631	3.9
Pancreas	144.7	0.2	121.1	0.1	25	0.2
Heart	381.0	0.5	561.0	0.7	160	1.0
Kidney	355.2	0.4	434.7	0.5	127	0.8
Spleen	221.8	0.3	186.5	0.2	59	0.4
Stomach-oesophagus	980.5	1.2	1188.0	1.4	145	0.9
Intestine	1496.0	1.8	-	-	310	1.9
Reproductive system	1320.0	1.6	116.9	0.1	10	0.1
Brain	210.0	0.3	201.6	0.2	-	-

3.2.2. Relationships among biomass measures in seal tissues

The regression equations for each of the tissues revealed linear relationships passing through the origin between FW and DW, AFDW, CC and NC respectively. The relationships between the biomass measures and the regression equations were shown only for the tissues which contribute the most to total fresh weight (Blubber-skin, Muscle and Bone; Fig. 3, p. 140 and Table 3, p. 135). These equations represented a high percentage of the measured data points variation, ranging from 80% (i.e. FW *vs.* NC) to 97% (i.e. FW *vs.* DW) for Blubber-skin, from 95% (i.e. FW *vs.* NC) to 100% (i.e. FW *vs.* DW and AFDW) for Muscle and from 87% (i.e. FW *vs.* NC) to 0.99% (i.e. FW *vs.* DW) for Bone (i.e. R^2, Table 3, p. 135). Therefore, ratios between the different biomass measures for the seal tissues can be used.

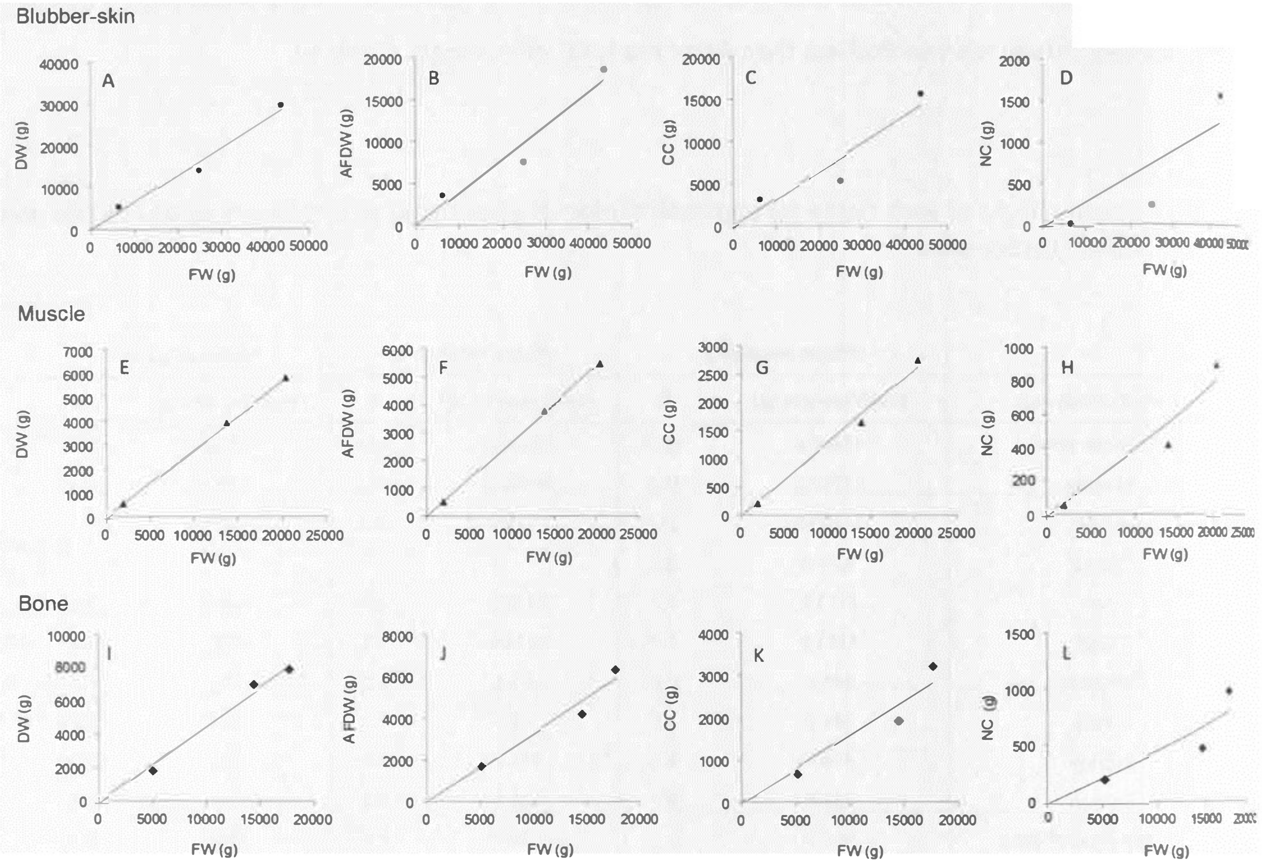
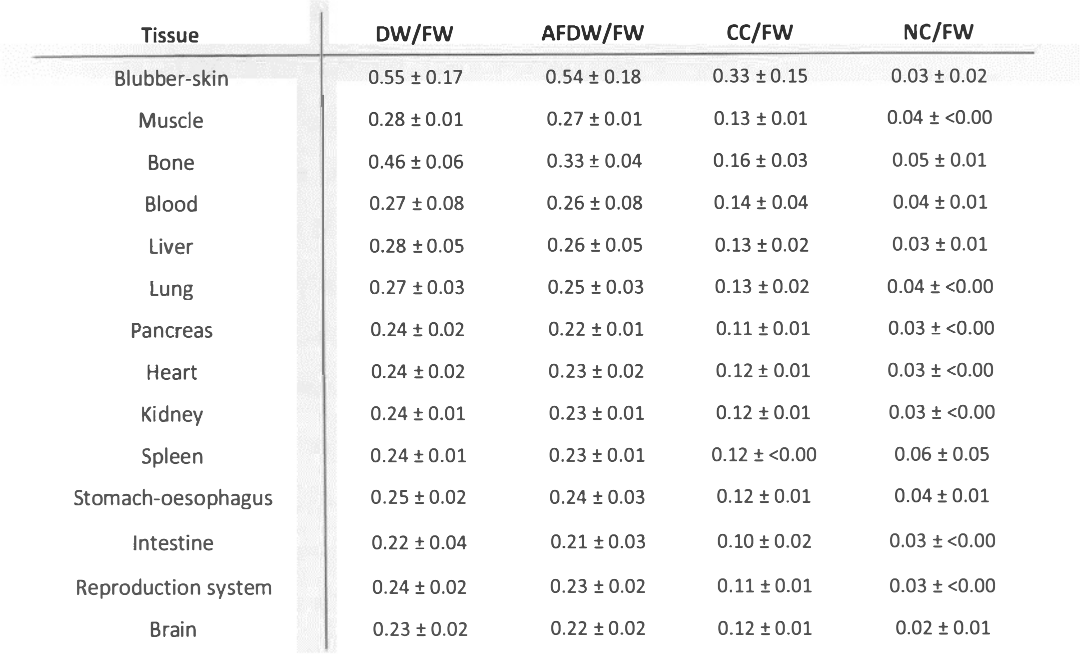


Fig. 3: Relationships for between FW and DW, FW and AFDW, FW and CC, FW and NC for Blubber-skin (A to D), Muscle (E to H), and Bone (I to L) of harbor seals. The regression equations are shown in Table 3, p. 135

3.2.3. Conversion factors of seal tissues

The DW/FW ratios (mean ± standard deviation) of seal tissues ranged from 0.22 ± 0.04 (Intestine) to 0.55 ± 0.17 (Blubber-skin), the AFDW/FW ratios ranged from 0.21 ± 0.03 (Intestine) to 0.54 ± 0.18 (Blubber-skin), the CC/FW ratios ranged from 0.10 ± 0.02 (Intestine) to 0.33 ± 0.15 (Blubber-skin) and the NC/FW ratios ranged from 0.02 ± 0.01 (Brain) to 0.06 ± 0.05 (Spleen; Table 7). Blubber-skin had the highest DW/FW ratio (Table 7), suggesting a low water content. This is consistent with the predominance of hydrophobic lipids in blubber which are stored in low water content (Pearson 2015). The highest AFDW/FW and CC/FW values were also observed in Blubber-skin suggesting a higher organic matter and carbon content than in the other tissues, which can be explained by the large amount of ong chain fatty acids containing 14 to 24 carbons in blubber (Käkelä et al. 1995, Iverson 2009). Brain and Blubber-skin tissues had low NC/FW ratios (0.02 ± 0.01 and 0.03 ± 0.02, respectively), indicating low nitrogen content, which is in accordance with the high lipid content in those two tissues (Henderson et al. 1994). Indeed, most lipids do not contain nitrogen (Mc Mahon et al. 2013). To summarize, fatty tissues, and especially blubber tissue, clearly showed differences in its ratios compared to the other tissues.

Table 7: Mean and standard deviation of DW/FW, AFDW/FW, CC/FW and NC/FW ratios of the different seal tissues



Tissue	DW/FW	AFDW/FW	CC/FW	NC/FW
Blubber-skin	0.55 ± 0.17	0.54 ± 0.18	0.33 ± 0.15	0.03 ± 0.02
Muscle	0.28 ± 0.01	0.27 ± 0.01	0.13 ± 0.01	0.04 ± <0.00
Bone	0.46 ± 0.06	0.33 ± 0.04	0.16 ± 0.03	0.05 ± 0.01
Blood	0.27 ± 0.08	0.26 ± 0.08	0.14 ± 0.04	0.04 ± 0.01
Liver	0.28 ± 0.05	0.26 ± 0.05	0.13 ± 0.02	0.03 ± 0.01
Lung	0.27 ± 0.03	0.25 ± 0.03	0.13 ± 0.02	0.04 ± <0.00
Pancreas	0.24 ± 0.02	0.22 ± 0.01	0.11 ± 0.01	0.03 ± <0.00
Heart	0.24 ± 0.02	0.23 ± 0.02	0.12 ± 0.01	0.03 ± <0.00
Kidney	0.24 ± 0.01	0.23 ± 0.01	0.12 ± 0.01	0.03 ± <0.00
Spleen	0.24 ± 0.01	0.23 ± 0.01	0.12 ± <0.00	0.06 ± 0.05
Stomach-oesophagus	0.25 ± 0.02	0.24 ± 0.03	0.12 ± 0.01	0.04 ± 0.01
Intestine	0.22 ± 0.04	0.21 ± 0.03	0.10 ± 0.02	0.03 ± <0.00
Reproduction system	0.24 ± 0.02	0.23 ± 0.02	0.11 ± 0.01	0.03 ± <0.00
Brain	0.23 ± 0.02	0.22 ± 0.02	0.12 ± 0.01	0.02 ± 0.01

3.2.4. Conversion factor for entire seals

The ratios for entire individuals, calculated taking in account the body composition of each animal, were 0.33, 0.35 and 0.38 for FW/DW, 0.28, 0.32 and 0.36 for FW/AFDW, 0.15, 0.17 and 0.19 for FW/CC and 0.03, 0.04 and 0.04 for FW/NC for *Phoca vitulina* 1, 2 and 3 respectively. The average values for entire seal individuals are displayed in Table 5, p. 137-138. The carbon content of each entire animal found in this study (15%, 17% and 19%) was higher than the value of 10% assumed by Bradford-Grieve et al. (2003) (Table 5, p. 137-138), who probably underestimated the biomass of seals in their model. Pinkerton and Bradford-Grieve (2008) used 15% for carbon content of fresh weight which is in the order of magnitude of the findings from this study (Table 5, p. 137-138).

Using these total ratios, the total DW, total AFDW, total CC and total NC of each entire seal individual were estimated. The relationships between total FW and total DW, total AFDW, total CC and total NC were respectively plotted (Fig. 4) and the corresponding regression equations were computed (Table 3, p. 135). These regression equations showed linear relationships that pass through the origin between total FW and total DW ($R^2 = 0.99$), total AFDW ($R^2 = 0.98$), total CC ($R^2 = 0.99$) and total NC ($R^2 =$ 0.99) respectively (Fig. 4; Table 3, p. 135). This allows the use of ratios as conversion factors for entire seal individuals.

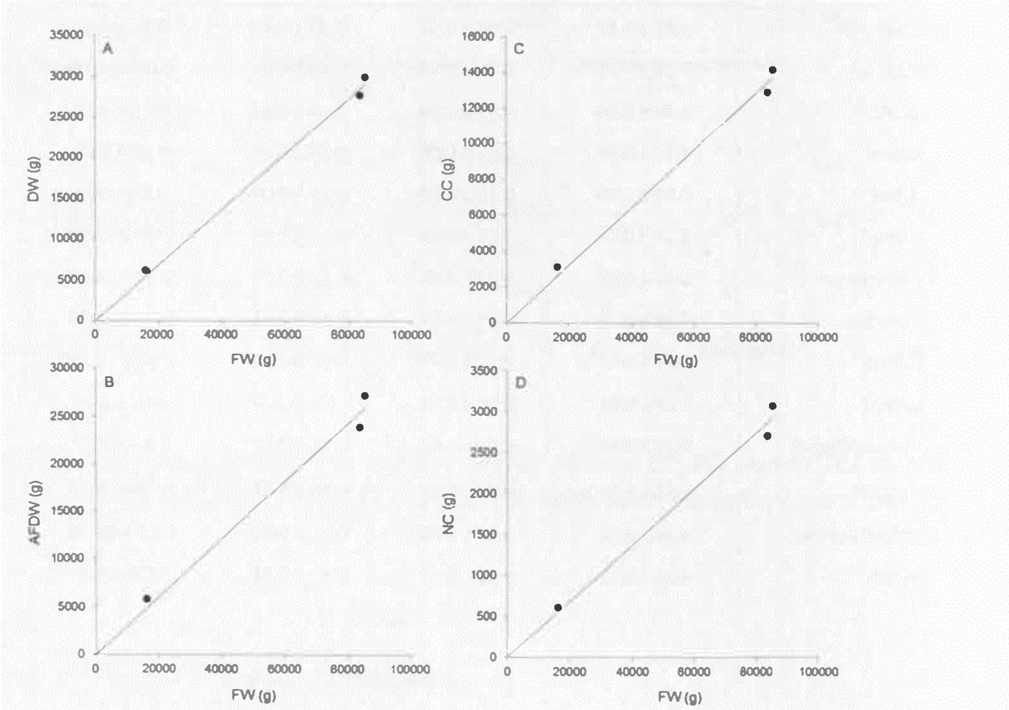


Fig. 4: Relationships between FW and DW, FW and AFDW, FW and CC and FW and NC for entire harbor seal individuals. The regression equations are shown in Table 3, p. 135

However, these total ratios must be applied with caution to other studies. Indeed, fatty tissues (e.g. Blubber-skin) in harbor seals were clearly characterized by specific conversion factors differing from those of other tissues (Table 7, p. 141). This observation implies that variations of the blubber percentage in the body composition would lead to variations of the conversion factors for whole individuals. For pinniped species which undergo huge fasting periods during the reproduction and the molt (Bowen et al. 1992, Atkinson 1997), ratios calculated for each tissue should be preferentially used in relation with the body composition, and particularly the percentage of body fat. The percentage of blubber in phocid seals can be estimated using the following equation determined by Ryg et al. (1990): $\%B = 4.44 + 5693 \times (L \times d) \div FW$ with $\%B$ = % of blubber contribution to total FW, L = the standard length of the seal individual, d = the dorsal blubber thickness and FW = the total FW of the individual.

3.3. Comparison with other taxa

Conversion factors for birds and seals, calculated in this study, were comparable to terrestrial vertebrates (Table 5, p. 137-138). The DW/FW ratios of birds and seals were similar to those measured for terrestrial mammal species (i.e. rodent species and rabbits, Table 5, p. 137-138) (Pace and Rathbun 1945), suggesting similar body water content. On the other hand, DW/FW ratios measured in this study were clearly higher than those measured in macrozoobenthos taxa (Rumohr et al. 1987, Gätje and Reise 1998b, Ricciardi and Bourget 1998a, Cauffopé and Heymans 2005b) and fish species (Greenstreet et al. 1997) (Table 5, p. 137-138), suggesting lower water content in birds and seals. This difference might be related to variations in fat content between the taxa, as fat content is negatively correlated to water content (Friedrich and Hagen 1994). Water content of fish can represent up to 90% of the FW (Dunajski 1980, Friedrich and Hagen 1994) and the typical hydrostatic skeleton of invertebrates (Chapman 1958) also implies high body water content that might also represent up to 90% of the FW (Block 2003). On the contrary, seals have a large proportion of total body weight as fat (Table 6, p. 139), possibly related to their high DW/FW ratio (Table 7, p. 141). Furthermore, the presence of keratinous tissue (e.g. claw, hair, feather) – characterized by low water content (10% to 12%) (Taylor et al. 2004) - in birds and mammals might also be responsible for their higher DW/FW ratios. The CC/FW and CC/DW ratios found in this study were higher than the values measured for polychaetes, crustaceans and fish (Table 5, p. 137-138), but the small number of available values makes comparisons inconclusive. To summarize, the conversion factors from FW to other biomass measures may vary widely among different taxa and global values should therefore be avoided or carefully applied.

4. Conclusion

This study provides new and essential data about the relationships among biomass parameters and weight conversion factors of top predators, allowing a gap to be filled in ecosystem and food web modelling studies. The relationships between fresh weight and other biomass measures are linear and through the origin for birds and seals. The carbon content of sea birds ranged from 16 ± <0.1% to 22 ± 2% of the fresh weight. The mean carbon content of seals was 16 ± 2% of the fresh weight. Blubber tissue of seals had higher DW/FW, AFDW/FW and CC/FW ratios than the other tissues. Further measurements are necessary to cover a larger number of species and investigating the effect of seasonal variation in body fat content on biomass conversion regressions is an important issue to address. This will allow better estimation of the influence and the role of marine birds and mammals on the ecosystems they live in.

5. Acknowledgements

This study was funded by the German Federal Ministry of Education and Research in the frame of the German-Dutch bilateral Wadden Sea Research “The impact of biological invasions on the food web of the Wadden Sea (INFOWEB)” (FKZ 03F0636A/B) and the German joint research project “STopP – From sediment to top predator” (BMBF, FKZ 03F672B). We are thankful to Ragnhild and Harald Asmus for the supervision of the projects. We would like to thank Ursula Siebert and the team of the Institute for Terrestrial and Aquatic Wildlife Research of University of Veterinary Medicine Hannover Foundation who conducted the seals necropsies as well as the seal hunters for the collection of harbor seals on the beach. We would like to thank Philipp Schwemmer and the colleagues and volunteers of the Research and Technology Centre and the Schutzstation Wattenmeer e.V. who collected and provided the bird carcasses. We are grateful to Benoit Lebreton and Gaël Guillou for running the carbon and nitrogen analyses at the LIENSs stable isotope facility of the University of La Rochelle. The collection and necropsies of seals were partly funded by the Agency for Coastal Defense National Park and Marine Conservation, as well as The Ministry of Energy, Agriculture, the Environment and Rural Affairs of the Federal State Schleswig-Holstein, Germany.

6. References

Asmus, H., and R. Asmus. 2000. Material exchange and food web of seagrass beds in the Sylt-Rømø Bight: how significant are community changes at the ecosystem level? Helgoland Marine Research **54**:137-150.

Atkinson, S. 1997. Reproductive biology of seals. Reviews of Reproduction **2**:175-194.

Baird, D., H. Asmus, and R. Asmus. 2004. Energy flow of a boreal intertidal ecosystem, the Sylt-Rømø Bight. Marine Ecology Progress Series **279**:45-61.

Baird, D., H. Asmus, and R. Asmus. 2011. Carbon, nitrogen and phosphorus dynamics in nine sub-systems of the Sylt-Rømø Bight ecosystem, German Wadden Sea. Estuarine, Coastal and Shelf Science **91**:51-68.

Baird, D., H. Asmus, and R. Asmus. 2012. Effect of invasive species on the structure and function of the Sylt-Rømø Bight ecosystem, northern Wadden Sea, over three time periods. Marine Ecology Progress Series **462**:143-161.

Baird, D., P. R. Evans, H. Milne, and P. M. W. 1985. Utilization by shorebirds of benthic invertabrate production in intertidal areas. Oceanogr. Mar. Biol. Ann. Rev. **23**:573-597.

Benke, H., U. Siebert, R. Lick, B. Bandomir, and R. Weiss. 1998. The current status of harbour porpoises (*Phocoena phocoena*) in German waters. Arch. Fish. Mar. Res. **46**:97–123.

Blew, J., K. Günther, B. Hälterlein, R. Kleefstra, K. Laursen, and G. Scheiffarth. 2013. Trends of Migratory and Wintering Waterbirds in the Wadden Sea 1987/1988 - 2010/2011. Wadden Sea Ecosystem No.31. Common Wadden Sea Secretariat, Joint Monitoring Group of Migratory Birds in the Wadden Sea, Wilhelmshaven, Germany.

Block, W. 2003. Water or ice?–the challenge for invertebrate cold survival. Science Progress **86**:77-101.

Bossart, G. D. 2011. Marine Mammals as Sentinel Species for Oceans and Human Health. Veterinary Pathology Online **48**:676-690.

Bowen, W. D. 1997. Role of marine mammals in aquatic ecosystems. Marine Ecology Progress Series **158**:267-274.

Bowen, W. D., O. T. Oftedal, and D. Boness, J. 1992. Mass and energy transfer during lactation in a small phocid, the harbor seal (*Phoca vitulina*). Physiological Zoology **65**:844-866.

Bradford-Grieve, J. M., P. K. Probert, S. D. Nodder, D. Thompson, J. Hall, S. Hanchet, P. Boyd, J. Zeldis, A. N. Baker, H. A. Best, N. Broekhuizen, S. Childerhouse, M. Clark, M. Hadfield, K. Safi, and I. Wilkinson. 2003. Pilot trophic model for subantarctic water over the Southern Plateau, New Zealand: a low biomass, high transfer efficiency system. Journal of Experimental Marine Biology and Ecology **289**:223-262.

Brasseur, S., B. Diederichs, R. Czeck, L. F. Jensen, A. Galatius, S. Ramdohr, U. Siebert, J. Teilmann, P. Körber, and S. Klöpper. 2013. Aerial surveys of Harbour Seals in the Wadden Sea in 2013 - Is the population growth rate slowing down?

Cauffopé, G., and S. J. Heymans. 2005a. Energy contents and conversion factors for sea lion's prey. Fish. Cent. Res. Rep. **13**:225-237.

Cauffopé, G., and S. J. Heymans. 2005b. Energy contents and conversion factors for sea lion's prey. . Fish. Cent. Res. Rep. **13**:225-237.

Chapman, G. 1958. The hydrostatic skeleton in the invertebrates Biological Reviews **33**:338-371.

Dumont, H. J., I. Velde, and S. Dumont. 1975. The dry weight estimate of biomass in a selection of Cladocera, Copepoda and Rotifera from the plankton, periphyton and benthos of continental waters. Oecologia **19**:75-97.

Dunajski, E. 1980. Texture of fish muscle Journal of Texture Studies **10**:301-318.

Fath, B. D., U. M. Scharler, R. E. Ulanowicz, and B. Hannon. 2007. Ecological network analysis: network construction. Ecological Modelling **208**:49-55.

Friedrich, C., and W. Hagen. 1994. Lipid contents of five species of notothenioid fish from high-Antarctic waters and ecological implications. Polar Biology **14**:359-369.

Furness, R. W., and K. Camphuysen. 1997. Seabirds as monitors of the marine environment. ICES Journal of Marine Science: Journal du Conseil **54**:726-737.

Galatius, A., S. Brasseur, R. Czeck, B. Diederichs, L. F. Jensen, P. Körber, U. Siebert, J. Teilmann, and S. Klöpper. 2014. Aerial surveys of Harbour Seals in the Wadden Sea in 2014 - The highest pup count recorded yet.

Gätje, C., and K. Reise. 1998a. Ökosystem Wattenmeer: Austausch-, Transport-und Stoffumwandlungsprozesse. Springer, Berlin.

Gätje, C., and K. Reise. 1998b. Ökosystem Wattenmeer: Austausch-, Transport-und Stoffumwandlungsprozesse. Springer, Berlin.

Greenstreet, S. P. R., A. D. Bryant, N. Broekhuizen, S. J. Hall, and M. R. Heath. 1997. Seasonal variation in the consumption of food by fish in the North Sea and implications for food web dynamics. ICES Journal of Marine Science: Journal du Conseil **54**:243-266.

Heath, M. R. 2007. The consumption of zooplankton by early life stages of fish in the North Sea. ICES Journal of Marine Science: Journal du Conseil **64**:1650-1663.

Henderson, R. J., N. Kalogeropoulos, and M. N. Alexis. 1994. The lipid composition of selected tissues from a Mediterranean monk seal, *Monachus monachus*. Lipids **29**:577-582.

Ings, T. C., J. M. Montoya, J. Bascompte, N. Blüthgen, L. Brown, C. F. Dormann, F. Edwards, D. Figueroa, U. Jacob, and J. I. Jones. 2009. Review: Ecological networks–beyond food webs. Journal of Animal Ecology **78**:253-269.

Iverson, S. J. 2009. Tracing aquatic food webs using fatty acids: from qualitative indicators to quantitative determination. Pages 281-308 Lipids in Aquatic Ecosystems. Springer.

Käkelä, R., R. Ackman, and H. Hyvärinen. 1995. Very long chain polyunsaturated fatty acids in the blubber of ringed seals (*Phoca hispida sp.*) from Lake Saimaa, Lake Ladoga, the Baltic Sea, and Spitsbergen. Lipids **30**:725-731.

Leguerrier, D., D. Degré, and N. Niquil. 2007. Network analysis and inter-ecosystem comparison of two intertidal mudflat food webs (Brouage Mudflat and Aiguillon Cove, SW France). Estuarine, Coastal and Shelf Science **74**:403-418.

Mandema, F. S., J. M. Tinbergen, B. J. Ens, K. Koffijberg, K. S. Dijkema, and J. P. Bakker. 2015. Moderate livestock grazing of salt, and brackish marshes benefits breeding birds along the mainland coast of the Wadden Sea. The Wilson Journal of Ornithology **127**:467-476.

Markert, A., W. Esser, D. Frank, A. Wehrmann, and K.-M. Exo. 2013. Habitat change by the formation of alien Crassostrea-reefs in the Wadden Sea and its role as feeding sites for waterbirds. Estuarine, Coastal and Shelf Science **131**:41-51.

Mc Mahon, K. W., L. L. Hamady, and S. R. Thorrold. 2013. Ocean Ecogeochemistry: A review. Pages 327-374 *in* R. N. Hughes, D. J. Hugues, and I. P. Smith, editors. Oceanography and Marine Biology: an Annual Review. Taylor and Francis Group.

McLusky, D. 1989. The Estuarine Environment. Page 215 The Estuarine Ecosystem. Glasgow and London: Blackie and Son Ltd, New York: Chapman & Hall 1989.

Moreira, F. 1997. The Importance of Shorebirds to Energy Fluxes in a Food Web of a South European Estuary. Estuarine, Coastal and Shelf Science **44**:67-78.

Pace, N., and E. N. Rathbun. 1945. Studies on body composition. 3. The body water and chemically combined nitrogen content in relation to fat content. Journal of Biological Chemistry **158**:685-691.

Pearson, L. E. 2015. Blubber and Beyond: The Role od Lipids in Thermoregulation and Energy Reserves of Phocid Seals. Alaska Fairbanks.

Pinkerton, M., and J. Bradford-Grieve. 2008. Seals: Trophic modelling of the Ross Sea.

Pinkerton, M., J. Bradford-Grieve, and S. Hanchet. 2010. A balanced model of the food web of the Ross Sea, Antarctica. CCAMLR Science **17**:1-31.

Reddy, L., L. A. Dierauf, and F. M. Gulland. 2001. Marine mammals as sentinels of ocean health. CRC handbook of marine mammal medicine: Health, disease and rehabilitation:3-13.

Reijnders, P. J. H., S. M. J. M. Brasseur, T. Borchardt, K. Camphuysen, R. Czeck, A. Gilles, L. F. Jensen, M. Leopold, K. Lucke, S. Ramdohr, M. Scheidat, U. Siebert, and J. Teilmann. 2009. Marine mammals. Thematic Report N°20. In: Marencic, H. and Vlas, J. de, editors. Quality status report 2009. Wadden Sea Ecosystem N°25. Wadden Sea Secretariat, Trilateral Monitoring and Assessment Group, Wilhelmshaven, Germany.

Reijnders, P. J. H., E. H. Ries, S. Tougaard, N. Nørgaard, G. Heidemann, J. Schwarz, E. Vareschi, and I. M. Traut. 1997. Population development of harbour seals *Phoca vitulina* in the Wadden Sea after the 1988 virus epizootic. Journal of Sea Research **38**:161-168.

Ricciardi, A., and E. Bourget. 1998a. Weight-to-weight conversion factors for marine benthic macroinvertebrates. Marine Ecology Progress Series **163**:245-251.

Ricciardi, A., and E. Bourget. 1998b. Weight-to-weight conversion factors for marine benthic macroinvertebrates. Marine Ecology Progress Series.

Rumohr, H., T. Brey, and S. Ankar. 1987. A compilation of biometric conversion factors for benthic invertebrates of the Baltic Sea. Institut für Meereskunde.

Rumohr, H., Brey, T, Ankar, S. 1987. A Compilation of Biometric Conversion Factors for Benthic Invertebrates of the Baltic Sea. The Baltic Marine Biologists Publication **9**:1-56.

Ryg, M., C. Lydersen, N. H. Markussen, T. G. Smith, and N. A. Øritsland. 1990. Estimating the blubber content of phocid seals. Canadian Journal of Fisheries and Aquatic Sciences **47**:1223-1227.

Saint-Béat, B., C. Dupuy, P. Bocher, J. Chalumeau, M. De Crignis, C. Fontaine, K. Guizien, J. Lavaud, S. Lefebvre, and H. Montanié. 2013. Key features of intertidal food webs that support migratory shorebirds.

Samhouri, J., P. Levin, and C. Harvey. 2009. Quantitative Evaluation of Marine Ecosystem Indicator Performance Using Food Web Models. Ecosystems **12**:1283-1298.

Scharler, U. M., and D. Baird. 2005. A comparison of selected ecosystem attributes of three South African estuaries with different freshwater inflow regimes, using network analysis. Journal of Marine Systems **56**:283-308.

Siebert, U., A. Gilles, K. Lucke, M. Ludwig, H. Benke, K.-H. Kock, and M. Scheidat. 2006. A decade of harbour porpoise occurrence in German waters—Analyses of aerial surveys, incidental sightings and strandings. Journal of Sea Research **56**:65-80.

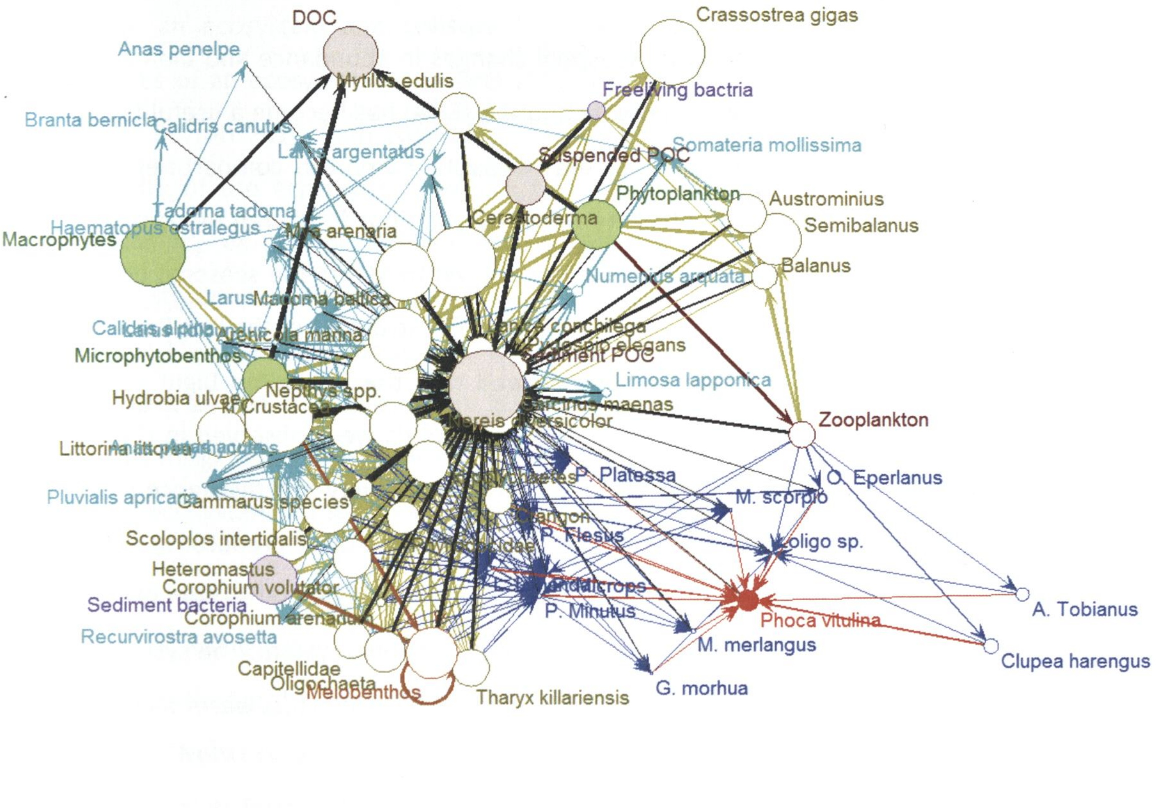
Siebert, U., P. Wohlsein, K. Lehnert, and W. Baumgärtner. 2007. Pathological Findings in Harbour Seals (*Phoca vitulina*): 1996–2005. Journal of Comparative Pathology **137**:47-58.

Taylor, A. M., R. H. C. Bonser, and J. W. Farrent. 2004. The influence of hydration on the tensile and compressive properties of avian keratinous tissues. Journal of Materials Science **39**:939-942.

Vitousek, P., and R. Howarth. 1991. Nitrogen limitation on land and in the sea: How can it occur? Biogeochemistry **13**:87-115.

Chapter 5

Seasonal dynamics and functioning of the Wadden Sea ecosystem food web



Seasonal dynamics and functioning of the Wadden Sea ecosystem food web

Camille de la Vega[1]*, Sabine Horn[1], Dan Baird[2], David Hines[3], Stuart Borret[3,4], Lasse Jensen[5], Philipp Schwemmer[6], Ragnhild Asmus[1], Ursula Siebert[7], Harald Asmus[1]

[1] Alfred-Wegener-Institut Helmholtz-Zentrum für Polar- und Meeresforschung, Wattenmeerstation Sylt Hafenstr. 43, D-25992 List/ Sylt, Germany
[2] Department of Botany and Zoology, Stellenbosch University, 7600 Stellenbosch, South Africa
[3] Department of Biology and Marine Biology, University of North Carolina Wilmington, Wilmington, NC, 28403 USA
[4] Duke Network Analysis Center, Social Science Research Institute, Duke University, Durham, NC, USA
[5] The Fisheries and Maritime Museum, Tarphagevej 2, 6710 Esbjerg V, Denmark.
[6] Christian-Albrechts-Universität zu Kiel, Forschungs- und Technologiezentrum Westküste (FTZ) Büsum, Hafentörn 1, 25761 Büsum, Germany
[7] Stiftung Tierärztliche Hochschule Hannover Institut für Terrestrische und Aquatische Wildtierforschung Werftstr. 6, 25761 Büsum, Germany

*Corresponding author: camille.de.la.vega@awi.de

Abstract

The Wadden Sea undergoes large seasonal changes in abundance and biomass of the multiple components in its food web. Ecological network analysis (ENA) has become a useful tool to describe the functioning of large and complex ecosystems encompassing numerous compartments interacting with each other and responding differently to external stressors. Four food web models were constructed, one for each season, and ENA methodologies were applied to assess the seasonal development of the resultant system properties reflecting on the functioning and structure of the Sylt-Rømø Bight food web. The whole system indicators provided by ENA showed that the Sylt-Rømø Bight ecosystem is stable across seasons and resistant on a yearly basis. Seasonal trends were observed in the indices, showing that there are little seasonal fluctuations in the system size, stability and resistance in front of external disturbances. In the warm seasons, when the biomass of opportunist predator species was high, the system tended to be more stable, well developed and resistant. The winter season was characterized by a stable, but smaller and more sensitive to external perturbations, system. The system in fall appeared to be in a less stable transition state between these two stable periods (i.e. warm seasons and winter).

Key words

Ecological Network Analysis, food web structure, seasonal variation, ecosystem function, Wadden Sea, Sylt-Rømø Bight

1. Introduction

Coastal marine ecosystems are becoming increasingly stressed due to anthropogenic activities and global climate variability (Doney et al. 2012), and undergo continuous changes in rates of production, species abundance, and community diversity. A holistic appreciation of human impacts on natural processes and an assessment of ecosystem function across temporal scales will contribute to the management and protection of coastal ecosystems (Levin and Lubchenco 2008, Samhouri et al. 2009). Ecosystem-based management is considered as the solution needed to improve the efficiency of ecosystem management measures (Pikitch et al. 2004, Levin and Lubchenco 2008, Levin et al. 2009) as opposed to single species based studies (McLeod et al. 2005). The development of ecological analytical methodologies became most useful tools in the assessment of ecosystem function by means of calculating system properties and their comparison over spatial and temporal scales, and to assess the interaction of system components in marine (Aarnio et al. 1996, Leguerrier et al. 2007, Ings et al. 2009, Kaufman and Borrett 2010, Fath 2015) and terrestrial systems (Heymans et al. 2002). Ecological Network Analysis (ENA) methodology was developed to assess holistically the complex environmental interactions within an ecosystem and consists of a set of algorithms allowing the structural and functional properties of an ecosystem to be analyzed (Ulanowicz and Abarca-Arenas 1997, Ulanowicz 2004, Kaufman and Borrett 2010, Fath 2015). Network analysis has been used for instance to study the structural complexity of the ecosystem, the structure and magnitude of the cycling of energy and material, the efficiency of energy transfer within the system, rates of energy assimilation and dissipation, trophic structure, system activity, growth and development (Monaco and Ulanowicz 1986, Baird and Ulanowicz 1989, Monaco and Ulanowicz 1997, Heymans et al. 2002, Baird et al. 2004, Baird et al. 2007, Saint-Béat et al. 2013, Schückel et al. 2015). Results derived from ENA of coastal ecosystems provided significant insight into their fundamental functioning (Baird et al. 2004, Fath 2015) and are relevant for marine ecosystem management (Samhouri et al. 2009).

The Wadden Sea is the largest continuous system of intertidal sand and mudflats in the world (Lotze 2007). The Sylt-Rømø Bight is a 404 km^2 semi-enclosed basin, located in the Northern Wadden Sea. The ecosystem of the Sylt-Rømø Bight is well studied and represents a typical coastal partial system of the Wadden Sea. Network analysis was used in previous studies to describe the structural and functional properties of its food web (Baird et al. 2004, Baird et al. 2007, Baird et al. 2008, Baird et al. 2011, 2012). These studies focused on habitat characteristics and properties (Baird et al. 2007, Baird et al. 2011), differences in the dynamics of nutrient flows (i.e. carbon, nitrogen and phosphorus) in the food web (Baird et al. 2008, Baird et al. 2011), and observed changes in ecosystem function and structure over the last 15 years due to the proliferation of invasive species (Baird et al. 2012). However, these studies were based on yearly averaged models, and the seasonal variation of the structural and functional properties of the Sylt-Rømø Bight system has never been evaluated.

However, the Wadden Sea area in general, and the Sylt-Rømø Bight specifically undergo large seasonal changes in abundance and biomass of the multiple components of the Bight ecosystem (Gätje and Reise 1998), such as the well-known spring and fall blooms of phytoplankton and microphytobenthos has been documented by Asmus (1982), Asmus (1983) and Asmus et al. (1998). Furthermore, due to migration patterns the abundance of birds and their species composition vary substantially between seasons (Blew et al. 2013). Consequently the predation pressure of birds on their prey in the Sylt-Rømø Bight also varies seasonally, with, for example, higher consumption in fall caused by the high abundance of ducks species and in spring due to the presence of a large number of wader species (Scheiffarth and Nehls 1997). The species composition and abundance, as well as the trophic guild structure of the fish assemblage in the Bight also show also large seasonal variation (Kellnreitner et al. 2012). Furthermore, harbor seals are one of the most abundant marine mammal species in the Wadden Sea (Reijnders et al. 2009, Galatius et al. 2014) where they congregate during the reproduction and molting periods in spring and summer (Drescher 1979, Reijnders et al. 2009, Osinga et al. 2012, Jensen 2015). Consequently, the pressure of predation of harbor seals on their prey species in the Sylt-Rømø Bight is higher in the warmer seasons compared to the colder fall and winter periods (de la Vega et al. 2016) (Chapter 2).

In this study, four food web models were constructed, one for each season (i.e. spring, summer, fall and winter) and ENA methodologies were applied to assess the seasonal development of the resultant system properties reflecting on the functioning and structure of the Sylt-Rømø Bight food web. In contrast to previous studies harbor seals and several prey items of seals (e.g. squids, common dab and sand eel) were included into the food web. An "uncertainty analysis" was run on each of the four models to estimate the robustness of the ENA output.

2. Material and Methods

2.1. Study site

The Sylt-Rømø Bight (54°52' - 55°10' N, 8°20' - 8°40' E) is part of the Wadden Sea, which extends along the south-eastern margin of the North Sea from the Netherlands to Denmark. It represents about 4% of the total Wadden Sea area of 10.000 km^2. The Dutch and German part (about 87%) of the Wadden Sea was declared a World Heritage Site by UNESCO in July 2009, and the Danish part followed in June 2014. The Bight is a semi-enclosed tidal basin of 404 km^2 and is located between the islands of Sylt (Germany) and Rømø (Denmark)(see Fig. 1, p. 61 in Chapter 2). About half of the area belongs to Germany, the other half to Denmark. Two causeways connect the islands with the mainland, and thus prevent any exchange of water through the intertidal watersheds with adjacent tidal basins. The only connection to the North Sea is a 2.8 km wide deep tidal channel (Lister Tief) between the two islands of Sylt and Rømø. The tidal range inside the Bight is 2 m (Martens and Beusekom 2008). The average water temperature varies from 2.7°C in winter (Dec-Feb) to 18.3°C in summer (Jun-Aug) with intermediate temperatures in spring (9.0°C; Mar-May) and fall (10.7°C; Sep-Nov). The average salinity ranges from 27.5 in winter to 30.0 in summer with intermediate values in spring (28.2) and fall (28.6). The diurnal tidal prism for the bight is about 550 x 10^6 m^3 , the water residence time in the bight is between 19 and 29 days and 8 to 12% of the Bight waters is exchanged per tidal cycle with the adjacent Wadden Sea through the Lister Tief channel (Baird et al. 2012).

2.2. Models construction

Four network models representing the whole Bight were constructed for each season namely spring (March-May), summer (June-August), fall (September-November) and winter (December-February). Each model consists of 64 living and 3 non-living compartments. Data and information for the construction of the quantified food webs were obtained from the literature as well as from unpublished information deposited at the Alfred Wegener Institute, Wadden Sea Station Sylt, Germany. None of the standing stocks, the diet of the various heterotrophs, or the rates of flow between the components in the Bight were derived from modelling estimates, but were based entirely on empirical data and results obtained from the numerous publications and data sources cited in the text. Carbon (a surrogate for energy) was used as the currency for biomass and flows in the four models. Standing stocks were expressed in mgC.m^{-2} and mgC.m^{-2}.d^{-1} for all fluxes where m = meter, d = day and C = Carbon (Ulanowicz 2004, Fath et al. 2007). The compartments included in the four models are listed in Table 1, p. 154-155.

Table 1: Mean (mgC per m^{-2}) and percentage of variation calculated from the standard deviation of the seasonal standing stock of the compartments in the Sylt-Rømø Bight, sampled years and source of the data

ID	Compartment name	SPRING		SUMMER		FALL		WINTER		sampled years
		Standing stock	% variation	Standing stock	% variation	Standing stock	% variation	Standing stock	% variation	
Living compartments										
1	Phytoplankton	1057.51	52.0	572.47	24.2	912.38	26.9	863.64	17.6	2011-14
2	Microphytobenthos	437.01	52.3	399.89	24.2	546.65	26.9	363.46	17.6	2013-15
3	Macrophytes	2619.92	47.2	4575.57	37.5	3424.39	56.9	25.45	1.0	1989-93
4	Freeliving bactria	9.79	50.0	9.79	50.0	9.79	50.0	9.79	50.0	2010
5	Zooplankton	21.72	24.2	29.56	50.6	13.12	51.9	1.56	51.8	2011
6	*Hydrobia ulvae*	8262.23	50.0	8063.14	50.0	5734.71	50.0	4615.45	50.0	2010
7	*Littorina littorea*	585.32	50.0	571.21	50.0	406.26	50.0	326.97	50.0	
8	*Arenicola marina*	10932.71	50.0	10669.27	50.0	7588.25	50.0	6107.24	50.0	
9	*Scoloplos intertidalis*	581.49	50.0	567.48	50.0	403.61	50.0	324.83	50.0	
10	*Capitellidae*	122.46	50.0	119.51	50.0	85.00	50.0	68.41	50.0	
11	*Oligochaeta*	204.75	50.0	199.82	50.0	142.12	50.0	114.38	50.0	
12	*Heteromastus*	95.99	50.0	93.68	50.0	66.63	50.0	53.62	50.0	
13	*Lanice conchilega*	34.30	50.0	33.47	50.0	23.81	50.0	19.16	50.0	
14	*Nereis diversicolor*	113.79	50.0	111.05	50.0	78.98	50.0	63.57	50.0	
15	*Pygospio elegans*	20.52	50.0	20.02	50.0	14.24	50.0	11.46	50.0	
16	*Corophium arenarium*	3.14	50.0	3.07	50.0	2.18	50.0	1.76	50.0	
17	*Corophium volutator*	159.51	50.0	155.67	50.0	110.72	50.0	89.11	50.0	
18	*Gammarus spp.*	10.37	50.0	10.12	50.0	7.20	50.0	5.79	50.0	
19	*Mytilus edulis*	210.25	50.0	205.18	50.0	145.93	50.0	117.45	50.0	
20	*Crassostrea gigas*	4691.20	50.0	4578.16	50.0	3256.10	50.0	2620.60	50.0	
21	*Semibalanus*	789.96	50.0	770.92	50.0	548.30	50.0	441.29	50.0	
22	*Balanus sp.*	29.65	50.0	28.93	50.0	20.58	50.0	16.56	50.0	
23	*Austrominius*	154.00	50.0	150.29	50.0	106.89	50.0	86.03	50.0	
24	*Cerastoderma*	9933.79	50.0	9694.42	50.0	6894.92	50.0	5549.22	50.0	
25	*Mya arenaria*	1728.08	50.0	1686.44	50.0	1199.44	50.0	965.34	50.0	
26	small polychaetes	256.31	50.0	250.13	50.0	177.90	50.0	143.18	50.0	
27	*Tharyx killariensis*	125.55	50.0	122.52	50.0	87.14	50.0	70.13	50.0	
28	*Macoma baltica*	3129.99	50.0	3054.57	50.0	2172.49	50.0	1748.48	50.0	
29	*Phyllodocidae*	68.28	50.0	66.63	50.0	47.39	50.0	38.14	50.0	

30	Small crustacean	287.63	50.0	280.70	50.0	199.64	50.0	160.68	50.0		
31	*Carcinus maenas*	160.60	50.0	156.73	50.0	111.47	50.0	89.72	50.0		
32	*Cragon crangon*	35.91	50.0	35.05	50.0	24.93	50.0	20.06	50.0		
33	*Nepthys spp.*	482.90	50.0	471.27	50.0	335.18	50.0	269.76	50.0		
34	*P. microps*	0.37	15.1	0.003	80.0	0.002	80.0	0.004	80.0	2010-12	fish monitoring
35	*P. minutus*	0.005	80.0	0.05	80.0	0.02	80.0	0.004	80.0		
36	*P. platessa*	0.003	80.0	0.01	80.0	0.01	80.0	0.004	80.0		
37	*P. flesus*	0.03	80.0	0.04	80.0	0.03	80.0	0.02	80.0		
38	*C. harengus*	1.02	80.0	0.65	59.2	0.26	80.0	0.13	80.0		
39	*M. merlangus*	0.01	80.0	0.04	80.0	0.000001	80.0	0.000001	80.0		
40	*G. morhua*	0.000001	80.0	0.01	80.0	0.02	80.0	0.01	80.0		
41	*M. scorpio*	0.02	80.0	0.01	80.0	0.02	80.0	0.04	80.0		
42	*L. limanda*	0.01	80.0	0.04	80.0	0.03	80.0	0.001	80.0		
43	*A. tobianus*	0.20	80.0	0.51	80.0	0.07	80.0	0.01	80.0		
44	*O. eperlanus*	0.28	42.9	0.06	80.0	0.03	80.0	0.01	80.0		
45	*Loligo sp.*	0.10	80.0	0.01	80.0	0.01	80.0	0.000001	80.0		
46	*Tadorna tadorna*	0.20	93.7	0.15	92.5	2.53	44.2	2.12	32.4	2007-08	National park, administration
47	*Somateria mollissima*	0.27	28.5	1.17	94.8	0.87	50.5	1.34	94.1		
48	*Haematopus ostralegus*	0.19	62.3	0.17	47.8	0.41	27.8	0.56	4.6		
49	*Recurvirostra avosetta*	0.005	61.8	0.01	65.8	0.001	88.6	0.01	72.1		
50	*Pluvialis apricaria*	0.12	19.2	0.04	87.6	0.14	22.0	0.01	91.9		
51	*Calidris canutus*	1.02	57.5	0.05	52.5	0.10	34.3	0.11	65.6		
52	*Calidris alpina*	0.08	32.4	0.03	16.4	0.07	66.0	0.04	55.1		
53	*Limosa lapponica*	0.95	64.3	0.20	42.8	0.32	53.4	0.29	53.2		
54	*Numenius arquata*	0.42	74.6	0.31	55.1	0.42	38.4	0.33	30.5		
55	*Larus ridibundus*	0.02	20.8	0.07	40.3	0.08	47.4	0.02	88.1		
56	*Larus canus*	0.08	13.8	0.13	42.4	0.21	81.4	0.06	22.4		
57	*Larus argentatus*	0.28	37.1	0.33	9.1	0.51	48.0	0.17	58.8		
58	*Anas platyrhynchos*	0.04	20.1	0.09	39.0	0.17	19.6	0.27	26.3		
59	*Anas acuta*	0.003	73.1	0.000001	73.1	0.09	85.7	0.98	60.6		
60	*Anas penelope*	0.02	89.2	0.03	76.1	1.95	76.1	0.16	62.9		
61	*Branta bernicla*	0.32	30.2	0.03	87.2	0.43	76.6	0.16	57.4		
62	Sediment bacteria	591.16	50.0	591.16	50.0	591.16	50.0	591.16	50.0	2010	Baird et al. 2012
63	Meiobenthos	749.64	50.0	749.64	50.0	749.64	50.0	749.64	50.0	2010	Baird et al. 2012
64	*Phoca vitulina*	6.59	32.2	14.94	28.2	6.59	32.2	2.62	30.2	2009-12	Fast Jensen 2015
Non-living compartments											
65	Suspended POC	175.21	50.0	175.21	50.0	175.21	50.0	175.21	50.0	2010	Baird et al. 2012
66	Sediment POC	17971.19	50.0	17971.19	50.0	17971.19	50.0	17971.19	50.0		
67	DOC	1092.24	50.0	1092.24	50.0	1092.24	50.0	1092.24	50.0		

For primary producers (i.e. phytoplankton, microphytobenthos and macrophytes), the gross primary production (GPP) was assumed to be equal to the sum of net primary production (NPP) and respiration (R), and the value of GPP was entered as input in the system (Ulanowicz 2004). Each heterotrophic compartment was defined by a biomass value and a particular energy budget consisting of i.e., production, respiration, consumption and egestion (Fath et al. 2007), in accordance with the energy-balanced equation (1): consumption of food (C) = production (P) + respiration (R) + egestion (E) (Crisp 1971, Fath et al. 2007).

Excess production and egestion by water column organisms (i.e. free-living bacteria and zooplankton) were assumed to remains in suspension as particulate organic carbon (suspPOC). Egestion of meiofauna, macrofauna, fish, squids and birds, and all non-utilized production of these guilds (with the exception of birds) were assumed to become sediment particulate organic carbon (SedPOC). The production of bird compartments which were not consumed was exported from the system. The production of harbor seals and half of their egestion were assumed to be exported from the system, the other half of egestion becoming SedPOC. We assumed that 25% of the NPP of phytoplankton and microphytobenthos, and 2% of the NPP of macrophytes was exuded as Dissolved Organic Carbon (DOC) and is subsequently included in the DOC model compartment (Baird et al. 2012, Valiela 2013). The non-utilized proportion of the standing stock of POC (i.e. suspPOC and sedPOC) and DOC was assumed to be exported from the system.

Each of the seasonal models were assumed to represent steady state conditions based on the concept that the inputs to plants (GPP) and consumption (C) by heterotrophs into each compartment is balanced by outputs (NPP + respiration by plants) and by respiration, secondary production and egestion by heterotrophs (Baird and Ulanowicz 1989, Christensen and Pauly 1992, Ulanowicz 2004, Scharler 2012). Indeed, within the time frame represented by each model (i.e. season), the biomass of each compartment was assumed to be stable over time, as the differences observed between the months in one season were smaller than the differences observed between months from different season.

2.3. Data base

2.3.1. Biomass and energy budget

The standing stocks of all compartments for the four models and their references are presented in Table 1, p. 154-155. The ratios and equations used to determinate the balanced budget parameters of fish, bird and seal species and their reference are given in Appendix 1, p. 185.

The standing stocks of the non-living compartments,(i.e. SuspPOC, SedPOC, and DOC) were taken from the data base used by (Baird et al. 2004) in their food web model of the Sylt-Rømø Bight. The biomass (B), production (P), respiration (R), egestion (E) values and the diet matrices of the living compartments sediment bacteria, free-living bacteria and meiofauna, were taken from Baird et al. (2004). These compartments were considered stable all year long and the same values were used for every season.

The phytoplankton biomass per m^2, net primary production (NPP) and gross primary production (GPP) per m^2 and per day were measured monthly from 2011 to 2014 as part of a phytoplankton monitoring program, while microphytobenthos biomass per m^2 was measured monthly from 2013 to 2015 in the Bight. The phytoplankton and microphytobenthos data are available in the Open Access Data Library "PANGAEA-Data Publisher for Earth & Environmental Science" at the Alfred Wegener Institute for Polar and Marine Research (AWI), Bremerhaven. The biomass in Chlorophyll-a (Chla) per m^{-2} of phytoplankton and microphytobenthos were converted to $C.m^{-2}$ by the equation mg C/l = 50 x µg Chla/l /1000 (Riemann et al. 1989). Values per volume were converted to values per area by dividing it by the mean water depth at mean tidal height and immersion. The seasonal biomass of the macrophytes (*Fucus spp.* and two sea grass species, *Zoltera noltii* and *Zoltera marina*) was taken from Asmus et al. (1998). The dry weight (DW) was converted to carbon content using 38.7% for sea grass species and 36.1% for *Fucus spp.* (Asmus et al. 1998). The NPP and GPP of microphytobenthos and macrophytes were estimated from their respective biomass using the ratios NPP/B and GPP/B used by Baird et al. (2004) for these same two compartments..

The zooplankton abundance was obtained from the Open Access Data Library "PANGAEA-Data Publisher for Earth & Environmental Science" for 2011 (Martens 2012) and averaged per season. The biomass in dry weight (DW) was estimated using the mean DW of each species from Martens and van Beusekom (2008), and the carbon content (CC) was estimated from the DW using CC = DW × 0.299

(Kiørboe 2013). The P, C, E and R values were calculated from the biomass using the P/B, C/B and E/B ratios from Baird et al. (2004) and the energy-balanced equation (1) respectively.

The biomass per season of the macrofauna compartments was estimated by applying the conversion factors - used to convert annuals average to seasonal values - calculated by Beukema (1974) to the annual averages used in Baird et al. (2012). The P, C, E and R values were calculated from the biomass using the P/B, C/B and E/B ratios from Baird et al. (2004) and the energy-balanced equation (1) respectively.

The fish species and *Loligo sp.* biomass was determined using the data from the monthly fish monitoring sampling program in the Sylt-Rømø Bight (de la Vega et al. 2016) (Chapter 2). The abundance and size of the individuals were converted into fresh weight (FW) using length/weight relations established by Pockberger (2015) for the same species in the same area. The FW were converted to DW and in turn in carbon content (CC) using DW/FW and CC/DW ratios of 0.17 and 0.58, respectively (Appendix 1, p. 181) (Remmert 1978). For the fish species included in the study of Baird et al. (2004) (i.e., *Pomatoschistus microps and minutus, Pleuronectes platessa, Platichthys flesus, Clupea harengus, Merlangius merlangus, Gadus morhua, Myoxocephalus scorpio*), the same P/B, C/B and E/B ratios were apply to the updated biomass to calculate the P, C and E values, the R values being calculated using the energy-balanced equation (1). For the newly included fish species (i.e. *Limanda limanda, Ammodytes tobianus, Osmerus eperlanus*) and *Loligo sp*. the estimations of P, R, E and C were based on several publications (Crisp 1971, Duthie 1982, Pihl 1989, Heymans and Baird 2000, Araújo et al. 2005, Maciejewska and Opaliński 2010) and are given in Appendix 1, p. 181.

The bird abundance was monitored along the coast of the Sylt Island in 2007 and 2008 (data provided by Schleswig-Holstein's Government-Owned Company for Coastal Protection, National Parks and Ocean Protection - National Park administration). The mean fresh weight (FW) of species individuals (Bezzel 1985) was applied to the abundance to estimate the total biomass in mg FW. The FW was converted into mgC using the conversion factors measured for several bird species from the Wadden Sea by (Horn and de la Vega 2016) (Appendix 1, p. 181; Chapter 4). The P/B, C/B, E/B ratios (Appendix 1, p. 181) were taken from Baird et al. (2004) and applied to the updated bird biomass to calculate the P, C and E values of the bird species, and R by difference.

Harbor seals (*Phoca vitulina*) biomass was calculated considering the mean fresh weight of 103kg per individual(Atkinson 1997, Bowen et al. 2001). Seal abundance was determined by Jensen

(2015) who counted the harbor seals in the Sylt-Rømø Bight in spring, summer and winter from 2009 to 2015. The abundance in fall was considered the same as in spring, which had an intermediate value between the highest number in summer and the lowest in winter (Table 1, p. 154-155). The FW was converted in carbon content using the conversion factors calculated by (Horn and de la Vega 2016)(Chapter 4) for *Phoca vitulina* in the Wadden Sea (Appendix 1, p. 181). The production of harbor seals was estimated using the number of new born (Jensen 2015) and subsequently reduced 60% due to infant mortality (Reijnders 1976). The consumption was estimated at 4 kg of FW per individual per day (Berg et al. 2002, Bjørge et al. 2002). Assuming that 91.2% of what food is consumed is digested (Ashwell-Erickson and Elsner 1981), the egestion value was estimated as 8.8% of the daily consumption. Values for the energy budget are given in Appendix 1, p. 181.

2.3.2. Diet Matrices

The compartments included in the four models are listed in Table 1. Compartments are linked by trophic fluxes which represent the movement of matter (i.e. carbon) throughout the system; from primary producers and detritus to consumers, or from prey to predator. All of these fluxes are defined by the diet matrix of each heterotroph component, consisting of the percentage of contribution of a prey item to a consumer diet multiplied by the consumption value of this consumer (Ulanowicz 2004).

The diet matrix of each heterotroph compartment was based on several publications and unpublished data; the list of references used to build the diet matrix for each compartment is given in Appendix 2, p. 183. The diet matrix of zooplankton, bacteria, meiofauna and omnivorous macrofauna is given in Appendix 3, p. 185. The diet matrices of carnivorous macrofauna species, fish species and bird species are displayed in Appendix 4 (p.187), Appendix 5 (p.189) and Appendix 6 (p. 192-193) respectively.

The diet of eider ducks (*Somateria molissima*) was adapted from the literature (Appendix 2, p. 183) and updated with unpublished data. Analysis of the fecal material of eider ducks from the Sylt-Rømø Bight revealed a high quantity of crabs and a low amount of blue mussels (Appendix 7, p. 196-197), which is in contradiction with the previous studies on eider duck diet (Nehls et al. 1997, Nehls and Ketzenberg 2002).

Except for Brent goose (*Branta bernicla*) (Nienhuis and Groenendijk 1986, Prop and Deerenberg 1991, Baldwin and Lovvorn 1994, Ganter 2000) and harbor seals (de la Vega et al. 2016) (Chapter 2) which show high seasonal variation in their diet, all diet matrices were assumed to be the same for every season. The seasonal diet matrices of harbor seals are displayed in Appendix 8, p. 199.

For a food web component which does not exclusively rely on the food resource from the modelled area (i.e. Sylt-Rømø Bight) the consumption value was scaled back to represent the proportion of the diet obtained from the Sylt-Rømø Bight. For example, the food resources from the Sylt-Rømø Bight contribute about 30% to the harbor seal diet in spring, with the remaining 70% of food resources coming from the adjacent North Sea (de la Vega et al. 2016) (Chapter 2). The percentage of contribution of the different prey items from the Bight was re-calculated to represent 100% of the diet, and the consumption value of harbor seals in the spring model was considered as 30% of the consumption value from the literature (i.e. 4kgWW.d^{-1}.ind^{-1}; Appendix 1, p. 181). The percentage of the diet coming from the food resources of the Sylt-Rømø Bight for the compartments relying partly on other areas, such as bird species feeding partly on terrestrial or offshore food sources, are given in Appendix 2, p. 183.

Sediment particulate organic carbon (SedPOC) was considered as the energy sources for benthic bacteria. The input into DOC originating only from exudation of algae, served as food sources for free-living bacteria.

2.4. Ecological Network Analyses

2.4.1. Indices

ENA is applied to ecological flow networks to assess them holistically and systematically, while the outputs of ENA provide many indices and system properties of natural ecosystems. The analytical methodology is reviewed by Ulanowicz (2004). We used the package enaR developed for the R statistic software by (Lau et al. 2015) to perform the analyses on the four models. Combinations and ratios of multiple ENA indices might be more informative to quantify ecosystem health and development (Ulanowicz 2004, Kaufman and Borrett 2010) and were used to interpret the ENA output of the four models.

The following index describes the size and activity of the system.

(1) The total system throughput (TST) is the sum of all flows in the system (imports, exports, respiration, and internal flows) (Wulff et al. 1989). It measures the size and activity of the system (Wulff et al. 1989, Heymans et al. 2002, Kaufman and Borrett 2010).

Information-based metrics have been developed to characterize the diversity of flows (i.e. number of interactions and the evenness of flows in a food web) in an ecosystem network (MacArthur 1955, Rutledge et al. 1976, Ulanowicz 1980, 1984, 1986). The total flow diversity (FD), calculated by MacArthur (1955) was partitioned by Rutledge et al. (1976) into the average mutual information (AMI), which corresponds to the organization of the flows inherent in a system (Ulanowicz 2004), and a residual amount of uncertainty (FDresidual). Ulanowicz (1980, 1986) then scaled these flow diversity measures by TST and created the development capacity (DC = FD * TST), the ascendency (A = TST * AMI), and the overhead (ϕ = FDresidual * TST) indices. The development capacity (DC) is the sum of A and ϕ and is demonstrated as the upper limit of A (Monaco and Ulanowicz 1986, Wulff et al. 1989, Christensen 1995, Ulanowicz and Abarca-Arenas 1997). The ascendency provides information about both the size and organization (both growth and development) of a system (Ulanowicz 1986). The Overhead measures the entropy of the system and is represented by the redundancies or parallel flows in the internal and exogenous exchanges (Wulff et al. 1989). A high redundancy reflects on the ability of the system to withstand perturbations (Baird et al. 2004). Although DC, A and ϕ have been used and interpreted on their own, they are sensitive to the structure of the model network and therefore not useful on their own for ecological applications (Mann et al. 1989, Wulff et al. 1989, Kaufman and Borrett 2010). On the contrary, the relative ascendency (RelA = A/DC) and relative overhead (Relϕ = ϕ/DC) are more useful and robust for assessing the condition and development status of an ecosystem and to compare different systems (Baird and Ulanowicz 1989, Wulff et al. 1989, Scharler and Baird 2005, Kaufman and Borrett 2010). These two indices (i.e. RelA and Relϕ) were therefore used in this study to characterize and describe the organization, the ability to cope with external disturbances and the capacity of adaptation of the system.

(2) The relative ascendency (RelA) is the fraction of the development capacity that appears as ordered flows. It is the organized part of DC (Wulff et al. 1989).

(3) The relative overhead (Relϕ) is the fraction of the development capacity that is not organized. A high Relϕ indicates high system resilience and a high capacity to adapt to novel perturbations (Heymans et al. 2002, Ulanowicz 2004). Thus, Relϕ is a measure of stability in the meaning of resistance to external disturbances (Christensen 1995).

RelA and Relϕ are mutually exclusive (Christensen 1995) and a healthy system requires adequate amounts of both ascendency and overhead (Ulanowicz 2004). Goerner et al. (2009) therefore developed an index which represents a balanced tradeoff between efficiency and redundancy.

(4) The Robustness (R) index combines the organization constraint and the redundancy of the system and therefore measures and assesses the necessary configuration for sustainability of the system (Goerner et al. 2009, Fath 2015).

The following index reflects the connectivity of the system which is related to the number of interactions between the compartments.

(5) The effective link density (ELD) also called degree of connectivity of the system, represents the average number of links flowing into or out of a compartment i (Ulanowicz 2004).

The cycling structure and flow size are described by the following indices.

(6) The Finn Cycling Index (FCI) gives the proportion of the flow in the system that is recycled (Finn 1976). It is an indicator of the physical retentiveness of material in the system (Monaco and Ulanowicz 1997, Baird et al. 2011).

(7) The average path length (APL) represents the average number of compartments that a unit of carbon passes through from its entry in the system until it leaves the system (Finn 1976, Wulff et al. 1989). A high value indicates the presence of long cycles. The APL is expected to be higher in systems with high degrees of flow diversity and cycling (Christensen 1995).

(8) The ratio of indirect flows over direct flows (IDF) indicates the relative significance of indirect flow in the system. When it is larger than 1, the indirect flows are more numerous than the direct flows and this implies that indirect effects are dominant (Borrett et al. 2006). It can be related to a greater complexity of the web of interaction.

The trophic structure of the system can be described by the trophic depth of the system and represented by the Lindeman spine. The Lindeman spine transforms each complex network of trophic transfers into a concatenated food chain with discrete trophic levels (Lindeman 1942). It illustrates the amount of carbon that each trophic level receives from the preceding level as the source of energy, as well as the amount leaving it through respiration, export, detritus and the net production passed on to the next higher level. The following indices are related to the trophic structure.

(9) The trophic depth (TD) corresponds to the number of trophic "roles" in the food web and is approximately the effective number of trophic levels in the system (Ulanowicz 2004). A system with a high number of trophic roles is expected to be more specialized.

(10) The system trophic efficiency (TE) which is computed as the logarithmic mean of the trophic efficiency for each level, i.e. the efficiency of transfer from one level to the next (Wulff et al. 1989, Baird et al. 2004).

(11) The detritivory/herbivory ratio (DH) which is considered to be a functional indicator of food web dynamics (Monaco and Ulanowicz 1997, Schückel et al. 2015).

All these indices and ratios of indices were computed for each of the four models in order to compare the characteristics of the system at different seasons.

2.4.2.Uncertainty analyses

The biomass data used for the construction of the models were averaged across several months and years. They therefore contain an uncertainty defined by the percentage of variation which is uniform around the mean and based on the standard deviation, for each compartment biomass (Table 1, p. 154-155). The consumption values (C) for each compartment, were a function of its biomass (B) and were estimated by C/B ratios. Thus, we assumed that the C value of a compartment j (C_j) has the same uncertainty as the biomass of this compartment j, and therefore has the same percentage of variation. The flow matrix from compartment i to compartment j (F_{ij}) in the network were calculated using $F_{ij} = C_j \times a_{ij}$, where a_{ij} is the percentage contribution of compartments i (e.g. prey item) to the diet of compartments j (e.g. predator), given in the diet matrices. Assuming that the variability in a_{ij} is null, we considered that the biomass uncertainty of a compartment j (same as the consumption uncertainty) was a good approximation for the flow uncertainty from compartment i to compartment j (Borrett, personal communication).

To evaluate the robustness of the ENA outputs to these parameter uncertainties, we performed an uncertainty analysis based on a Monte Carlo analysis that determines the variation in a model output given the uncertainties in the model input (Hines et al. 2015). We created 10000 model parametrizations for each of the four seasonal models with a linear inverse modelling approach using the *limSolve* package for R (Soetaert et al. 2015) and a R function developed to facilitate the transition exchange of information from enaR to the *limSolve* package (Hines, personal communication). Each of the plausible models was constrained to be in steady state and to contain parameters with values within the range of uncertainty given for each network flow. For each model realization in the uncertainty analysis, the ENA analysis was performed. This allowed to determine the 95% confidence intervals for each of the ENA indices and to generally estimate the robustness of the model results to the underlying model uncertainty (Hines et al. 2015). The results of the uncertainty analyses are shown as boxplots, presenting the median (black line), the 50% confidence interval (box delimited by the first and third quantile) and the 95% confidence interval (range between the whiskers). No uncertainty analysis was done for the indices related to the trophic structure (i.e. TD, TE, DH and Lindeman spine).

3. Results

3.1. *Seasonal values of the ecosystem indices and properties*

The total production was the highest in fall (3311.0 mgC.m^{-2}.d^{-1}), followed by spring (3174.6 mgC.m^{-2}.d^{-1}), summer (2848 mgC.m^{-2}.d^{-1}) and winter (2150.7 mgC.m^{-2}.d^{-1}; Table 2). The primary production was the highest in fall (2984.6 mgC.m^{-2}.d^{-1}) followed by spring (2682.6 mgC.m^{-2}.d^{-1}), and the smallest in winter (1851.6 mgC.m^{-2}.d^{-1}; Table 2). The secondary production was the highest in spring (492.0 mgC.m^{-2}.d^{-1}) followed by summer (381.0 mgC.m^{-2}.d^{-1}; Table 2). The primary production was dominated by microphytobenthos during every season (from 81.1% in summer to 98.3% in winter) and the contribution of phytoplankton to the total primary production was the highest in spring (17%) and lowest during winter (1.7%; Table 2). The Production/Biomass ratio per day (P/B) of the total system was the lowest in summer (0.042) and the highest in fall (0.060; Table 2).

Table 2: General system attributes of the Sylt-Rømø Bight for 4 seasons. P/B: production/biomass; NPP: net primary production

Attribute	**Fall**	**Winter**	**Spring**	**Summer**
Total system biomass of living compartments (mgC.m-2)	36254.5	26752.6	48710.1	49116.7
Biomass autotroph (mgC.m-2)	4883.4	1252.5	4114.4	5547.9
Biomass heterotroph (mgC.m-2)	31371.1	25500.0	44595.6	43568.8
Total export (mgC.m-2.d-1)	2711.5	1658.4	2416.9	2148.5
Total import (mgC.m-2.d-1)	4870.1	3157.9	4415.0	4652.7
Total system production (mgC.m-2.d-1)	3311.0	2150.7	3174.6	2848.3
Total growth primary production (mgC.m-2.d-1)	4607.3	2870.4	4020.5	3697.2
Total net primary production (mgC.m-2.d-1)	2984.6	1851.6	2682.6	2467.3
Phytoplankton NPP as proportion of the total NPP (%)	6.8	1.7	17.1	16.4
Microphytobenthos NPP as proportion of the total NPP (%)	91.7	98.3	81.6	81.1
Macrophyte NPP as proportion of the total NPP (%)	1.5	0.0	1.3	2.5
Total secondary production (mgC.m-2.d-1)	326.4	299.1	492.0	381.0
NPP efficiency (%)	35.0	34.0	20.0	26.0
P/B (d-1)	0.060	0.047	0.047	0.042
Detrivory/herbivory ratio, DH	1.2	1.4	0.9	1.0

The total system throughput (TST) followed the same trend as the primary production and was the highest in fall (15008.3 mgC.m^{-2}.d^{-1}) and spring (14530.2 mgC.m^{-2}.d 1), with the lowest value in winter (10307.0 mgC.m^{-2}.d^{-1}; Table 3; Fig. 1).

The system had lower RelA values in spring and summer (40.6% and 40.1% respectively) than in fall and winter (44.0% and 43.8% respectively; Table 3 and Fig. 1c). Conversely, the Relϕ was the highest in spring and summer (59.4% and 59.9% respectively) and the lowest in fall and winter (56.0% and 56.2% respectively; Table 3 and Fig. 1c). The robustness index ranged from 0.36 (fall and winter) to 0.37 (spring and summer; Table 3).

The effective link density (ELD) was the highest in spring and summer (2.5) and the lowest in fall and winter (2.2; Table 3 and Fig. 1c).

The system in fall had the lowest values of FCI (4.9%), APL (2.1 compartments) and IDF (0.7) in comparison to winter, spring and summer (Table 3 and Fig. 1b).

Table 3: Global system indices and attributes derived from ecological network analysis of the Sylt-Rømø Bight models for four seasons and the median value of the indices value based on 10000 plausible network parametrization

ENA Indices	**Fall**		**Winter**		**Spring**		**Summe**
Size and Activity	**Initial model**	**Median**	**Initial model**	**Median**	**Initial model**	**Median**	**Initial model**
Total system throughput, TST (mgC.m-2.d-1)	**15008.3**	14989.7	**10307.0**	10313.4	**14530.2**	14520.9	**13309.9**
Capacity of adaptation							
Relative Ascendency, RelA (%)	**44.0**	44.4	**43.8**	44.2	**40.6**	41.2	**40.1**
Relative overhead, Relϕ (%)	**56.0**	55.6	**56.2**	55.8	**59.4**	59.1	**59.9**
Robustness	**0.36**	0.36	**0.36**	0.36	**0.37**	0.37	**0.37**
Connectivity							
Effective link density, ELD (link)	**2.2**	2.1	**2.2**	2.2	**2.5**	2.5	**2.5**
Recycling magnitude and size of flows							
Finn Cycling Index, FCI (%)	**4.9**	5.0	**6.4**	6.5	**6.3**	6.5	**6.9**
Average path length, APL (trophic steps)	**2.1**	2.1	**2.3**	2.3	**2.3**	2.3	**2.3**
Indirect/direct flow, IDF	**0.7**	0.7	**0.9**	0.9	**0.9**	0.9	**1.0**

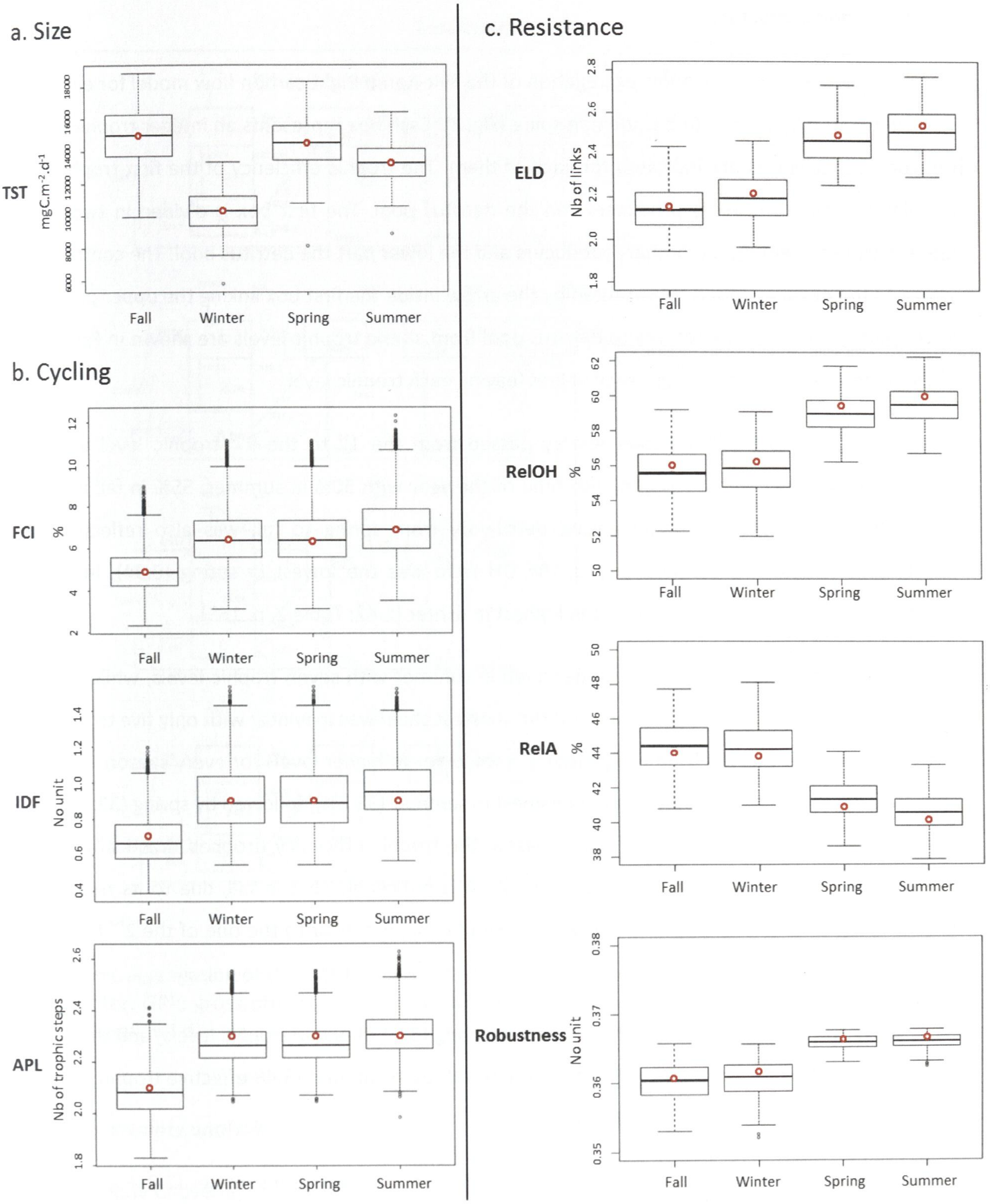


Fig. 1: Whole system indices values per season, based on 10000 plausible network parametrizations. Red circles indicate the values calculated by the initial network parametrization. The median value is shown as a bar in the boxes. The boxes and the whiskers represent the 50%CI and 95%CI respectively

3.2. Trophic structure

The results of the trophic aggregation of the Sylt-Rømø Bight carbon flow model for each season were illustrated by a simplified Lindeman spine (Fig. 2). Each box represents an integer trophic level and the trophic efficiencies are indicated for each of them. The trophic efficiency of the first trophic level is calculated for both primary producers and the detritus pool. The first box is divided in two with the upper part representing the primary producers and the lower part the detritus pool. The contribution by plants to the detritus pool is represented by the arrow inside the first box linking the upper part and the lower part of the box. The returns to detritus pool from all the trophic levels are shown in Fig. 2 as well as the canonical export and respiration values leaving each trophic level.

In spring, 48% of the total energy passed from the 1st to the 2nd trophic level as recycled material. This percentage increased with time of the year with 50% in summer, 55% in fall and 59% in winter (Fig. 2). This trend of increasing detritivory from spring to fall was also reflected by the detritivory/herbivory (DH) ratio. Indeed, the DH ratio was the lowest in spring (0.94), increased in summer (1.02) and fall (1.24) and was the highest in winter (1.42; Table 2, p. 165).

The longest trophic chain was identified in summer with seven trophic levels, while six trophic levels were identified in spring and fall, and the shortest chain was in winter with only five trophic levels. The trophic efficiencies declined progressively from lower to higher levels for every season. The trophic efficiency of the first trophic level was the highest in summer (33.2%), followed by spring (32.6%), winter (30.3%) and fall (23.5%; Fig. 2). For each season, the trophic efficiency dropped drastically at the 3rd trophic level from over 20% to about 3% (Fig. 2). It is noticeable that in fall, due to its relatively low value, the trophic efficiency of the 1st trophic level (23.5%), is similar to the one of the 2nd trophic level (22.4%; Fig. 2).

The trophic depth (TD) was the highest in spring (3.51 effective trophic levels) and the lowest in fall (3.36 effective trophic levels) with intermediate values in summer (3.48 effective trophic levels) and winter (3.46 effective trophic levels; Fig. 2).

The mean trophic efficiency of the system (TE) was the lowest in summer (2.3; Fig. 2), because of the extensive trophic chain in summer when very little energy is transferred between the trophic levels 4 to 7. For the same reason, the shortest chain in winter showed the highest TE (4.4%; Fig. 2). Spring and fall had an intermediate food chain length and TEs (3.2 and 3.1 respectively; Fig. 2).

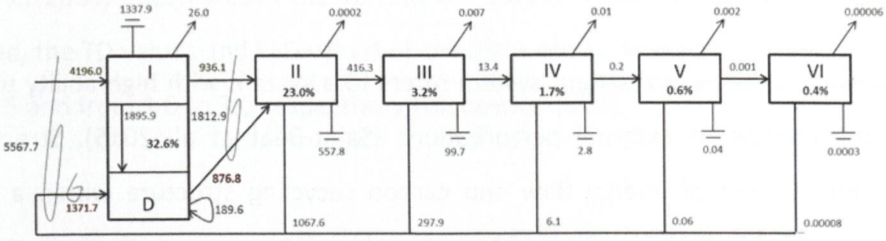
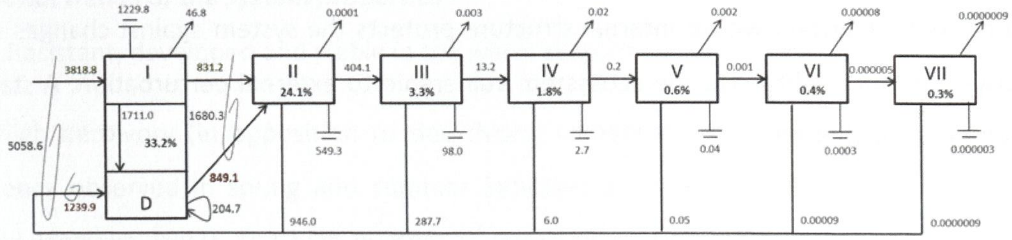
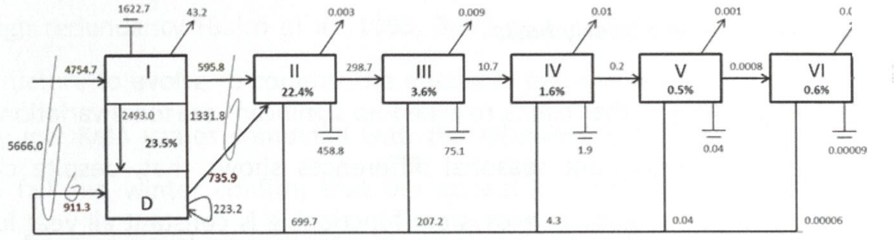
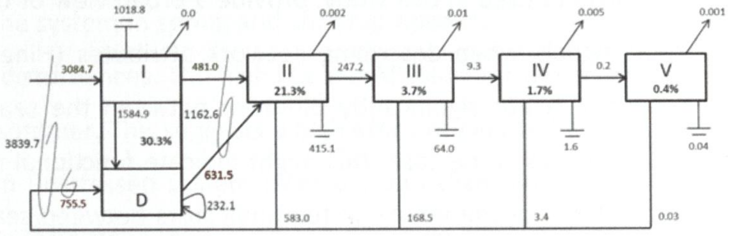
Season	Lindeman spine	TE (%)	TD
spring		3.15	3.36
summer		2.30	3.46
fall		3.11	3.51
winter		4.39	3.48

Fig. 2: Trophic aggregation of the Sylt-Rømø Bight network and trophic efficiency (TE) in spring, summer, fall and winter. GPP: gross primary production; roman numbers: trophic levels; D: detritus pool. Flow rates are in mgC.m^{-2}.d^{-1}

3.3. Uncertainty analysis

The 95% CI overlap for all of the whole system indicators. This indicates that given our level of uncertainty, the whole system indicators do not significantly vary across seasons (Fig. 1, p. 167). Seasonal variations were considered in the following sections as tendencies when the 50%CI were not overlapping.

4. Discussion

4.1. Definitions

In the following sections, a resistant system refers to a system with high ability to maintain its original state in the context of external perturbations (Saint-Béat et al. 2015). Stress refers to a reduction of the organization of energy flow and carbon recycling structure within a food web. A stressed system is expected to have low FCI and low APL values (Monaco and Ulanowicz 1997). A stable system refers to a system whose internal structure protects the system against changes arising within the ecosystem, but might leave the ecosystem vulnerable to external perturbation. A stable system is expected to be unstressed.

4.2. A persistent ecosystem on a yearly basis

Contrary to our expectations, the results revealed no significant seasonal variation in the whole system indicators. This lack of significant seasonal differences shows that, despite clear seasonal changes in species composition, the general ecosystem functioning is constant all year long. The high hierarchical level of whole system indicators used in this study, provide a broad view of the system but can damp out potentially important signals when describing network attributes (Hines and Borrett 2014). For example, the total TST index is not significantly different between the seasons, but the species contributing to TST might vary. If this is the case, this might indicate functional redundancy in the system that might explain the similarity of the whole system indicators between seasons, despite the large seasonal variation in species composition and biomass observed in the Sylt-Rømø Bight. The use of environ analysis would probably help to examine more in details potential seasonal variations in the system functioning. Indeed, environ analysis produces non-overlapping subnetworks, called "environs" that can be summed to recover the original network. Indices calculated for each environ are therefore lower-level indicators which represent the properties of a single node (i.e. node level) or a group of nodes (i.e. neighborhood level) (Hines and Borrett 2014).

On a yearly basis, the system is healthy and sustainable, as all the robustness values (0.36: fall and winter and 0.37: spring and summer) are in the window of vitality defined by Fath (2015) between 0.33 and 0.37. The system had then optimally balanced tradeoffs between A and ϕ (Ulanowicz 2004), and can be defined as a robust system (i.e. high durability of the system integrity and high quantity of perturbation that the system can withstand before changing to another state (Saint-Béat et al. 2015)).

Furthermore, the window of vitality based on the number of trophic roles (i.e. TD) and link density (i.e. ELD) (Ulanowicz 2004) encompasses the TD and ELD values of the Sylt-Rømø Bight ecosystem in every season. Indeed, the TD values and ELD values of the bight are as defined for a robust ecosystem, ranging from 2.0 to 4.5 and from 1.0 to 3.0 respectively (Ulanowicz 2004).

4.3. Seasonal trends of the system properties

4.3.1.Resistant, developed and stable in the warm seasons

The high herbivory (in opposition to detritivory) combined with the high NPP and the high grazing efficiency observed in spring and summer indicates a great diversity of resource utilization (Monaco and Ulanowicz 1997). The high number of interactions and parallel pathways (i.e. high ELD) suggests a high redundancy (Baird et al. 1998, Baird et al. 2004) and therefore a high ability of the ecosystem structure to evolve to counter the effects of external perturbations (Saint-Béat et al. 2015). The relatively low RelA values combined with the relatively high Relϕ values in spring and summer compared to fall and winter confirm that the system in these two seasons has a high capacity of adaptation in front of novel perturbation (Christensen 1995, Heymans et al. 2002, Saint-Béat et al. 2015). Thus, the system in spring and summer appears to be a resistant system with high ability to cope with external disturbances. The high FCI and APL indicate a high cycling magnitude and many long cycles in spring and summer. This suggests a high efficiency in retaining particulate matter within the food web and implies an unstressed system (Monaco and Ulanowicz 1997). The cycling (i.e. FCI and APL) have been shown to increase with maturity (Christensen 1995). The high degree of connectivity between the different compartments (i.e. high ELD), combined with the high recycling magnitude and the many long cycles in spring and summer indicate a well-developed system (Christensen 1995). In spring and summer, the Sylt-Rømø Bight ecosystem is therefore resistant, stable and well developed.

4.3.2. Small, nonresistant but stable system in winter

The low production, low TST value and short food chain with high trophic efficiency (i.e. Lindeman spine) in winter are characteristic of a small and efficient system in size and activity (Heymans et al. 2002). The small connectivity and low redundancy (i.e. low ELD) also reflect a small and simple system, and indicate a low resistance to external perturbation (Christensen 1995, Vasconcellos et al. 1997, Saint-Béat et al. 2015) in winter compared to spring and summer. This is consistent with the low Relϕ suggesting a low capacity of adaptation (Christensen 1995, Heymans et al. 2002). However, the high recycling (i.e. high FCI), the long cycles (i.e. high APL and IDF) in winter compared to fall indicate an unstressed (Monaco and Ulanowicz 1997) system. The Sylt-Rømø Bight ecosystem in winter is stable and efficient, but less resistant to perturbations than in spring and summer, probably due to the simplicity of its web of interactions which tends to decrease ecosystem resistance (Saint-Béat et al. 2015). The winter data used in this study do not reflect ice winter situation with sea-ice cover which might occurs in the Northern Wadden Sea (e.g. 2010, 2011 and 2012), as sampling of fish or counting of seals for examples, cannot be effectuated in such conditions. We might then have underestimated the influence of weather conditions on the food web properties in winter.

4.3.3. Fall season: an unstable transition period

The fall season is characterized by a large and active system (i.e. high production and TST). However, the lower degree of connectivity (i.e. low ELD) indicates a low resistance of the system to external perturbations in fall compared to spring and summer (Saint-Béat et al. 2015). The low capacity of adaptation to disturbance (i.e. low Relϕ) in fall supports the description of the system as nonresistant, compared to spring and summer. The low FCI and APL values, and the high number of direct flows compared to indirect flows (i.e. low IDF) imply that the recycling and flows of carbon occurs over short and fast trophic pathways suggesting a stressed system in this season in comparison with winter, spring and summer (Monaco and Ulanowicz 1997). The high P/B ratio confirmed that the system in fall is stressed (Monaco and Ulanowicz 1997). These observations suggest that the system in fall is less stable than in the other seasons (Saint-Béat et al. 2015). Furthermore, the low recycling (i.e. low FCI, APL, IDF) indicates that the system in fall is at a lower development stage than in spring and summer. The fall season is then characterized by a large, nonresistant, stressed, and therefore unstable system. This system might be in a transition state between two periods both characterized by stable systems having different properties: i.e. the warm seasons characterized by a large, well developed and resistant system (i.e. spring and summer) and the winter season characterized by a small, efficient and nonresistant system.

4.4. Seasonal variability increases resistance

McCann and Rooney (2009) entitled their article *"The more food webs change, the more they stay the same"*. This sentence expresses the idea that temporal variability in the food web dynamics contributes to a greater resistance of the system. Seasonal variability with recurring states at particular periods of time stabilizes food webs (Saint-Béat et al. 2015). For example, Lobry et al. (2008) observed in the Gironde estuary a seasonal shift in the food resources used by consumers inducing an alternation between a system based on primary production pathways in summer and a system based on detrital energy pathways in winter. These regular changes in the structure of the Gironde estuary food web lead to an optimal use of resources and guarantees annual stability in terms of resistance (Saint-Béat et al. 2015). In the Sylt-Rømø Bight, the small seasonal trends in the system functioning observed within a year might increase the resistance of the system on a yearly basis.

4.5. Resistance of the system related to predators

The higher connectivity and number of parallel pathways observed in spring and summer compared to fall and winter might be explained by the increase of predators in the warm seasons (i.e. spring and summer). Indeed, the presence of opportunistic predators (i.e. macrofauna and fish species, and seals) relying on numerous prey species probably increases the number of parallel flows and the pathway length, and therefore the resistance of the system to external disturbances (Baird et al. 2007, Saint-Béat et al. 2015). In the Sylt-Rømø Bight, the heterotrophic production and biomass are the highest in summer and spring. The fish biomass in the Sylt-Rømø Bight is six to eight times higher in spring and summer than in fall and winter and the seal biomass is six times higher in summer than in winter. The biomass of macrofauna predator species such as *Carcinus maenas* or *nephtys hombergii* also drastically increases in the warm seasons compared to fall and winter. Baird and Ulanowicz (1989) observed the same trend in the seasonal variation of the system of the Chesapeake Bay, USA which had longer pathway and better ability to cope with external disturbances in summer than in winter due to the presence of carnivores in the warm season. The changes observed in the Chesapeake system, follow the same trend of results presented here, were linked to the evolution of predator control (Baird and Ulanowicz 1989). The increase of overhead, indicating an increase of adaptation capacity, in the system of the Apalachee Bay, Florida was also related among other factors to the immigration of fish and birds in the system in the warmer months (Baird et al. 1998). In the Brouage Mudflat, South-West France, high redundancy of the flows in summer suggesting a higher capacity of adaptation in this season was also observed in comparison with the winter season (Leguerrier et al. 2007) but no special link with top predator was established.

To the contrary of spring and summer, the system in fall is based on detritus and shows the lowest grazing efficiency, whereas the highest primary production occurred in this season. More than 90% of the primary production was not used in fall. This excess of production leads to an increase of exported material. Although the fall season is a period of decline of total heterotrophic biomass and production, it is also characterized by the highest biomass of coastal birds (three times higher than in summer). These birds do not stay in the system due to their migratory behavior and their production is also exported from the system. High export fluxes might explain partly the relative instability of the system in fall, as the number of interaction between the compartments within the network is then decreased. Furthermore, these coastal bird species rely heavily on few macrofauna species, creating strong links between many predators and few prey species. This unevenness of flows decreases the resistance of the system in front of external perturbations (Baird et al. 2007).

Thus, the presence of higher trophic levels and predator species seem to increase the redundancy and the pathway length and therefore increase the resistance of the system to external perturbation, when those predators have opportunistic feeding behavior.

4.6. Uncertainty analysis

The uncertainty analysis used in this study is a valuable new tool that permits to make more robust inferences from the data. Contrary to most of the previous studies which based the comparison of ecosystem properties on single initial values, the use of uncertainty analysis allow us to determine if two network metrics are significantly different given the observed data uncertainty. The data uncertainty considered in this study was the standard deviation of the component biomass. Uncertainty on the diet matrices may also be measured, for example when assessed with stable isotope mixing models (e.g. harbor seal´s diet; de la Vega et al. (2016); Chapter 2) and can therefore be included in the uncertainty analysis. Thus, the different causes of the flow variability would be taken in account.

5. Conclusion

The Sylt-Rømø Bight ecosystem is stable and resistant on a yearly basis. Despite the large seasonal variation of species composition and biomass, the whole system indicators are constant across seasons. In the warm seasons (i.e. spring and summer) the system tends to be more stable, developed and resistant than in the cold seasons, maybe due to the presence of opportunistic predators which seem to have a structural role in the system. The uncertainty analysis brings useful information that permits to test statistically the differences between two index values. Environ analysis would probably help to explain the lack of significant seasonal variation in the whole system indicators.

6. Acknowledgments

This study was funded by the German Federal Ministry of Education and Research in the framework of the German-Dutch bilateral Wadden Sea Research Project "The impact of biological invasions on the food web of the Wadden Sea (INFOWEB)" (Förderkennzeichen 03F0636A/B).

7. References

Aarnio, K., E. Bonsdorff, and N. Rosenback. 1996. Food and feeding habits of juvenile flounder *Platichthys flesus* (L.), and turbot *Scophthalmus maximus* L. in the åland archipelago, northern Baltic Sea. Journal of Sea Research **36**:311-320.

Araújo, J., S. Mackinson, J. Ellis, and P. Hart. 2005. An Ecopath model of the Western English Channel ecosystem with an exploration of its dynamic properties. Sci. Ser. Tech Rep., CEFAS Lowestoft **125**:45pp.

Ashwell-Erickson, S., and R. Elsner. 1981. The energy cost of free existence for Bering Sea harbor and spotted seals. Pages 869-899 *in* D. Hood and J. Calder, editors. The Eastern Bering Sea Shelf: Oceanography and Resources, Seattle: University of Washington Press.

Asmus, H., R. Asmus, and H. Wolfgang. 1998. Biogenic Exchange and Transformation Processes in the Sylt-Rømø Wadden Sea: An Overview. Pages 215-464 *in* C. Gätje and K. Reise, editors. The Wadden Sea Ecosystem, Exchance, Transport and Transformation Processes. Springer-Verlag Berlin Heidelberg, Germany.

Asmus, R. 1982. Field measurements on seasonal variation of the activity of primary producers on a sandy tidal flat in the northern Wadden Sea. Netherlands Journal of Sea Research **16**:389-402.

Asmus, R. 1983. Benthische und Pelagische Primärproduktion und Nährsalzbilanz. Eine Freilanduntersuchung im Watt der Nordsee. Christian Albrechts Universität, Kiel.

Atkinson, S. 1997. Reproductive biology of seals. Reviews of Reproduction **2**:175-194.

Baird, D., H. Asmus, and R. Asmus. 2004. Energy flow of a boreal intertidal ecosystem, the Sylt-Rømø Bight. Marine Ecology Progress Series **279**:45-61.

Baird, D., H. Asmus, and R. Asmus. 2007. Trophic dynamics of eight intertidal communities of the Sylt-Rømø Bight ecosystem, northern Wadden Sea. Marine Ecology Progress Series **351**:25-41.

Baird, D., H. Asmus, and R. Asmus. 2008. Nutrient dynamics in the Sylt-Rømø Bight ecosystem, German Wadden Sea: An ecological network analysis approach. Estuarine, Coastal and Shelf Science **80**:339-356.

Baird, D., H. Asmus, and R. Asmus. 2011. Carbon, nitrogen and phosphorus dynamics in nine sub-systems of the Sylt-Rømø Bight ecosystem, German Wadden Sea. Estuarine, Coastal and Shelf Science **91**:51-68.

Baird, D., H. Asmus, and R. Asmus. 2012. Effect of invasive species on the structure and function of the Sylt-Rømø Bight ecosystem, northern Wadden Sea, over three time periods. Marine Ecology Progress Series **462**:143-161.

Baird, D., J. Luczkovich, and R. R. Christian. 1998. Assessment of spatial and temporal variability in ecosystem attributes of the St Marks National Wildlife Refuge, Apalachee Bay, Florida. Estuarine, Coastal and Shelf Science **47**:329-349.

Baird, D., and R. E. Ulanowicz. 1989. The seasonal dynamics of the Chesapeake Bay ecosystem. Ecological Monographs **59**:329-364.

Baldwin, J. R., and J. R. Lovvorn. 1994. Habitats and tidal accessibility of the marine foods of dabbling ducks and brant in Boundary Bay, British Columbia. Marine Biology **120**:627-638.

Berg, I., T. Haug, and K. T. Nilssen. 2002. Harbour Seal (*Phoca vitulina*) Diet in Vesteralen, North Norway. Sarsia **87**:451-461.

Beukema, J. 1974. Seasonal changes in the biomass of the macro-benthos of a tidal flat area in the Dutch Wadden Sea. Netherlands Journal of Sea Research **8**:94-107.

Bezzel, E. 1985. Kompendium des Vögel Mitteleuropas: Nonpasseriformes - Nichtsingvögel. AULA-Verlag GmbH, Wiesbaden. Verlag für Wissenschaft und Forschung.

Bjørge, A., T. Bekkby, V. Bakkestuen, and E. Framstad. 2002. Interactions between harbour seals, Phoca vitulina, and fisheries in complex coastal waters explored by combined Geographic Information

System (GIS) and energetics modelling. ICES Journal of Marine Science: Journal du Conseil **59**:29-42.

Blew, J., K. Günther, B. Hälterlein, R. Kleefstra, K. Laursen, and G. Scheiffarth. 2013. Trends of Migratory and Wintering Waterbirds in the Wadden Sea 1987/1988 - 2010/2011. Wadden Sea Ecosystem No.31. Common Wadden Sea Secretariat, Joint Monitoring Group of Migratory Birds in the Wadden Sea, Wilhelmshaven, Germany.

Borrett, S. R., S. J. Whipple, B. C. Patten, and R. R. Christian. 2006. Indirect effects and distributed control in ecosystems: Temporal variation of indirect effects in a seven-compartment model of nitrogen flow in the Neuse River Estuary, USA—Time series analysis. Ecological Modelling **194**:178-188.

Bowen, W. D., S. J. Iverson, D. J. Boness, and O. T. Oftedal. 2001. Foraging effort, food intake and lactation performance depend on maternal mass in a small phocid seal. Functional Ecology **15**:325-334.

Christensen, V. 1995. Ecosystem maturity—towards quantification. Ecological Modelling **77**:3-32.

Crisp, D. 1971. Energy flow measurements. Pages 197-279 *in* A. Holure and A. McIntyre, editors. Methods for the study of marine benthos, International Biological Programme Handbook No 16, Blackwell, Oxford.

de la Vega, C., B. Lebreton, U. Siebert, G. Guillou, K. Das, R. Asmus, and H. Asmus. 2016. Seasonal Variation of Harbor Seal's Diet from the Wadden Sea in Relation to Prey Availability. PLoS ONE **11**:e0155727.

Doney, S. C., M. Ruckelshaus, J. E. Duffy, J. P. Barry, F. Chan, C. A. English, H. M. Galindo, J. M. Grebmeier, A. B. Hollowed, N. Knowlton, J. Polovina, N. N. Rabalais, W. J. Sydeman, and L. D. Talley. 2012. Climate Change Impacts on Marine Ecosystems. Annual Review of Marine Science **4**:11-37.

Drescher, H. E. 1979. Biology, ecology and conservation of harbour seals in the tidelands of Schleswig-Holstein. Beitr. Wildbiol. **1**:1-73.

Duthie, G. G. 1982. The respiratory metabolism of temperature-adapted flatfish at rest and during swimming activity and the use of anaerobic metabolism at moderate swimming speeds. Journal of Experimental Biology **97**:359-373.

Fath, B. D. 2015. Quantifying economic and ecological sustainability. Ocean & Coastal Management **108**:13-19.

Fath, B. D., U. M. Scharler, R. E. Ulanowicz, and B. Hannon. 2007. Ecological network analysis: network construction. Ecological Modelling **208**:49-55.

Finn, J. T. 1976. Measures of ecosystem structure and function derived from analysis of flows. Journal of theoretical Biology **56**:363-380.

Galatius, A., S. Brasseur, R. Czeck, B. Diederichs, L. F. Jensen, P. Körber, U. Siebert, J. Teilmann, and S. Klöpper. 2014. Aerial surveys of Harbour Seals in the Wadden Sea in 2014 - The highest pup count recorded yet.

Ganter, B. 2000. Seagrass (Zostera spp.) as food for brent geese (Branta bernicla): an overview. Helgoland Marine Research **54**:63-70.

Gätje, C., and K. Reise. 1998. Ökosystem Wattenmeer: Austausch-, Transport-und Stoffumwandlungsprozesse. Springer, Berlin.

Goerner, S. J., B. Lietaer, and R. E. Ulanowicz. 2009. Quantifying economic sustainability: Implications for free-enterprise theory, policy and practice. Ecological Economics **69**:76-81.

Heymans, J., and D. Baird. 2000. Network analysis of the northern Benguela ecosystem by means of NETWRK and ECOPATH. Ecological Modelling **131**:97-119.

Heymans, J. J., R. E. Ulanowicz, and C. Bondavalli. 2002. Network analysis of the South Florida Everglades graminoid marshes and comparison with nearby cypress ecosystems. Ecological Modelling **149**:5-23.

Hines, D. E., and S. R. Borrett. 2014. A comparison of network, neighborhood, and node levels of analyses in two models of nitrogen cycling in the Cape Fear River Estuary. Ecological Modelling **293**:210-220.

Hines, D. E., J. A. Lisa, B. Song, C. R. Tobias, and S. R. Borrett. 2015. Estimating the effects of seawater intrusion on an estuarine nitrogen cycle by comparative network analysis. Marine Ecology Progress Series **524**:137-154.

Horn, S., and C. de la Vega. 2016. Relationships between fresh weight, dry weight, ash free dry weight, carbon and nitrogen content for selected vertebrates. Journal of Experimental Marine Biology and Ecology **481**:41-48.

Ings, T. C., J. M. Montoya, J. Bascompte, N. Blüthgen, L. Brown, C. F. Dormann, F. Edwards, D. Figueroa, U. Jacob, and J. I. Jones. 2009. Review: Ecological networks–beyond food webs. Journal of Animal Ecology **78**:253-269.

Jensen, L. 2015. Monitoring of the seal population in the Wadden Sea in 2015 - Monitering af sælbestanden i Vadehavet 2015. Aarhus Universitet, Miljøministeriet, Fiskeri-og Søfartsmuseet Saltvandsakvariet Esbjerg.

Kaufman, A. G., and S. R. Borrett. 2010. Ecosystem network analysis indicators are generally robust to parameter uncertainty in a phosphorus model of Lake Sidney Lanier, USA. Ecological Modelling **221**:1230-1238.

Kellnreitner, F., M. Pockberger, and H. Asmus. 2012. Seasonal variation of assemblage and feeding guild structure of fish species in a boreal tidal basin. Estuarine, Coastal and Shelf Science **108**:97-108.

Kiørboe, T. 2013. Zooplankton body composition. Limnology and Oceanography **58**:1843-1850.

Lau, M. K., S. R. Borrett, D. E. Hines, and P. Singh. 2015. Package enaR.

Leguerrier, D., D. Degré, and N. Niquil. 2007. Network analysis and inter-ecosystem comparison of two intertidal mudflat food webs (Brouage Mudflat and Aiguillon Cove, SW France). Estuarine, Coastal and Shelf Science **74**:403-418.

Levin, P. S., M. J. Fogarty, S. A. Murawski, and D. Fluharty. 2009. Integrated ecosystem assessments: developing the scientific basis for ecosystem-based management of the ocean. PLoS biology **7**:23.

Levin, S. A., and J. Lubchenco. 2008. Resilience, robustness, and marine ecosystem-based management. Bioscience **58**:27-32.

Lindeman, R. L. 1942. The Trophic-Dynamic Aspect of Ecology. Ecology **23**:399-417.

Lobry, J., V. David, S. Pasquaud, M. Lepage, B. Sautour, and E. Rochard. 2008. Diversity and stability of an estuarine trophic network. Marine Ecology Progress Series **358**:13-25.

Lotze, H. K. 2007. Rise and fall of fishing and marine resource use in the Wadden Sea, southern North Sea. Fisheries Research **87**:208-218.

MacArthur, R. 1955. Fluctuations of animal populations and a measure of community stability. Ecology **36**:533-536.

Maciejewska, K., and K. W. Opaliński. 2010. Does ecological energetics lead to trophic competition in juvenile fish? A case study from the Vistula Lagoon (southern Baltic Sea). Marine Pollution Bulletin **61**:178-182.

Mann, K. H., J. G. Field, and F. Wulff. 1989. Network analysis in marine ecology: an assessment. Springer.

Martens, P. 2012. Abundance of zooplankton at times series station List Reede in 2011. Alfred Wegener Institute - Wadden Sea Station Sylt.*in* PANGAEA, editor.

Martens, P., and J. E. E. v. Beusekom. 2008. Zooplankton response to a warmer northern Wadden Sea. Helgoland Marine Research **62**:67-75.

Martens, P., and J. E. van Beusekom. 2008. Zooplankton response to a warmer northern Wadden Sea. Helgoland Marine Research **62**:67-75.

McCann, K. S., and N. Rooney. 2009. The more food webs change, the more they stay the same. Philosophical Transactions of the Royal Society of London B: Biological Sciences **364**:1789-1801.

McLeod, K. L., J. Lubchenco, S. Palumbi, and A. A. Rosenberg. 2005. Scientific Consensus Statement on Marine Ecosystem-Based Management. Signed by 221 academic scientists and policy experts with relevant expertise and published by the Communication Partnership for Science and the Sea at http://www.compassonline.org/science/EBM_CMSP/EBMconsensus.

Monaco, M. E., and R. E. Ulanowicz. 1986. Comparative ecosystem trophic structure of. Marine Ecology Progress Series **1**:9.

Monaco, M. E., and R. E. Ulanowicz. 1997. Comparative ecosystem trophic structure of three U.S. mid-Atlantic estuaries. Marine Ecology Progress Series **161**:239-254.

Nehls, G., I. Hertzler, and G. Scheiffarth. 1997. Stable musselMytilus edulis beds in the Wadden Sea—They're just for the birds. Helgoländer Meeresuntersuchungen **51**:361-372.

Nehls, G., and C. Ketzenberg. 2002. Do common eiders Somateria mollissima exhaust their food resources? A study on natural mussel Mytilus edulis beds in the Wadden Sea. Danish Review of Game Biology **16**:47-61.

Nienhuis, P., and A. Groenendijk. 1986. Consumption of eelgrass (Zostera marina) by birds and invertebrates: an annual budget. Marine Ecology Progress Series **29**.

Osinga, N., I. Pen, H. A. Udo de Haes, and P. M. Brakefield. 2012. Evidence for a progressively earlier pupping season of the common seal (*Phoca vitulina*) in the Wadden Sea. Journal of the Marine Biological Association of the United Kingdom **92**:1663-1668.

Pihl, L. 1989. Abundance, biomass and production of juvenile flatfish in southeastern kattegat. Netherlands Journal of Sea Research **24**:69-81.

Pikitch, E., C. Santora, E. Babcock, A. Bakun, R. Bonfil, D. Conover, P. Dayton, et al., P. Doukakis, D. Fluharty, and B. Heneman. 2004. Ecosystem-based fishery management. Science(Washington) **305**:346-347.

Pockberger, M. 2015. Abiotic and biotic impacts on fish in the Wadden Sea. Evaluating the effect of large scale climate oscillations, local ecosystem characteristics and invasive species. Doctoral dissertation, The Christian Albrecht University of Kiel.

Prop, J., and C. Deerenberg. 1991. Spring staging in Brent Geese Branta bernicla: feeding constraints and the impact of diet on the accumulation of body reserves. Oecologia **87**:19-28.

Reijnders, P. J. H. 1976. The harbour seal (*phoca vitulina*) population in the Dutch Wadden Sea: Size and composition. Netherlands Journal of Sea Research **10**:223-235.

Reijnders, P. J. H., S. M. J. M. Brasseur, T. Borchardt, K. Camphuysen, R. Czeck, A. Gilles, L. F. Jensen, M. Leopold, K. Lucke, S. Ramdohr, M. Scheidat, U. Siebert, and J. Teilmann. 2009. Marine mammals. Thematic Report N°20. In: Marencic, H. and Vlas, J. de, editors. Quality status report 2009. Wadden Sea Ecosystem N°25. Wadden Sea Secretariat, Trilateral Monitoring and Assessment Group, Wilhelmshaven, Germany.

Remmert, H. 1978. Ecosystem. Pages 169-244 Ecology. Springer-Verlag Berlin-Heidelberg, Germany.

Riemann, B., P. Simonsen, and L. Stensgaard. 1989. The carbon and chlorophyll content of phytoplankton from various nutrient regimes. Journal of Plankton Research **11**:1037-1045.

Rutledge, R. W., B. L. Basore, and R. J. Mulholland. 1976. Ecological stability: an information theory viewpoint. Journal of theoretical Biology **57**:355-371.

Saint-Béat, B., D. Baird, H. Asmus, R. Asmus, C. Bacher, S. R. Pacella, G. A. Johnson, V. David, A. F. Vézina, and N. Niquil. 2015. Trophic networks: How do theories link ecosystem structure and functioning to stability properties? A review. Ecological Indicators **52**:458-471.

Saint-Béat, B., C. Dupuy, P. Bocher, J. Chalumeau, M. De Crignis, C. Fontaine, K. Guizien, J. Lavaud, S. Lefebvre, and H. Montanié. 2013. Key features of intertidal food webs that support migratory shorebirds.

Samhouri, J., P. Levin, and C. Harvey. 2009. Quantitative Evaluation of Marine Ecosystem Indicator Performance Using Food Web Models. Ecosystems **12**:1283-1298.

Scharler, U. M., and D. Baird. 2005. A comparison of selected ecosystem attributes of three South African estuaries with different freshwater inflow regimes, using network analysis. Journal of Marine Systems **56**:283-308.

Scheiffarth, G., and G. Nehls. 1997. Consumption of benthic fauna by carnivorous birds in the Wadden Sea. Helgoländer Meeresuntersuchungen **51**:373-387.

Schückel, U., I. Kröncke, and D. Baird. 2015. Linking long-term changes in trophic structure and function of an intertidal macrobenthic system to eutrophication and climate change using ecological network analysis. Marine Ecology Progress Series **536**:25-38.

Soetaert, K., K. Van den Meersche, and D. Van Oevelen. 2015. Package limSolve, solving linear inverse models in R.

Ulanowicz, R. E. 1980. An hypothesis on the development of natural communities. Journal of theoretical Biology **85**:223-245.

Ulanowicz, R. E. 1984. Community measures of marine food networks and their possible applications. Pages 23-47 Flows of energy and materials in marine ecosystems. Springer.

Ulanowicz, R. E. 1986. Growth and development: ecosystems phenomenology. Springer Science & Business Media.

Ulanowicz, R. E. 2004. Quantitative methods for ecological network analysis. Computational Biology and Chemistry **28**:321-339.

Ulanowicz, R. E., and L. G. Abarca-Arenas. 1997. An informational synthesis of ecosystem structure and function. Ecological Modelling **95**:1-10.

Valiela, I. 2013. Marine ecological processes. Springer Science & Business Media.

Vasconcellos, M., S. Mackinson, K. Sloman, and D. Pauly. 1997. The stability of trophic mass-balance models of marine ecosystems: a comparative analysis. Ecological Modelling **100**:125-134.

Wulff, F., J. G. Field, and K. H. Mann. 1989. Network analysis in marine ecology: methods and applications. Springer Science & Business Media.

Appendix 1: Ratios and equations, and their references, used to determir the energy budg of fish, squid, bir and seal species

		Production (reference)		Consumption (ref.)		Respiration (ref.)	Egestion (ref.)		Weight conversion factor (ref.)	
FISH and SQUID										
34	*P. microps*	P/B = 0.010	(Baird et al. 2004)	C/B = 0.082	(Baird et al. 2004)	R = C - E - P (Crisp 1971)	E/B = 0.050	(Baird et al. 2004)		
35	*P. minutus*	P/B = 0.010		C/B = 0.082			E/B = 0.050			
36	*P. platessa*	P/B = 0.007		C/B = 0.055			E/B = 0.033			
37	*P. flesus*	P/B = 0.033		C/B = 0.403			E/B = 0.033			
38	*C. harengus*	P/B = 0.009		C/B = 0.057			E/B = 0.033			
39	*M. merlangus*	P/B = 0.006		C/B = 0.028			E/B = 0.0006			
40	*G. morhua*	P/B = 0.006		C/B = 0.028			E/B = 0.0006			
41	*M. scorpio*	P/B = 0.006		C/B = 0.028			E/B = 0.0006			
42	*L. Limanda*	P/B = 0.070	(Pihl et al. 1989)	C/B = 0.558	(Baird et al. 2004)	log(R in mg O2 per g per h) = 0.772*log(WWg)-0.787 (Duthie 1982)	E = C - R - P	(Crisp 1971)	AFDW = 0.17 x FW CC = 0.58 x AFDW	(Remmert 1978)
43	*A. Tobianus*	P/B = 0.004	(Araujo et al. 2005)	C/B = 0.020	(Araujo et al. 2005)	burried: R/B = 0.00249 ; swimming: R/B = 0.0054 (Araujo et al. 2005)	E = C - R - P	(Crisp 1971)		
44	*O. Eperlanus*	P/B = 0.042	(Maciejewka and Opalinski 2010)	C = E + R + P	(Crisp 1971)	R/B = 0.069 (Maciejewka and Opalinski 2010)	E/B = 0.24	(Maciejewka and Opalinski 2010)		
45	*Loligo sp.*	P/B = 0.025	(Heymans and Baird 2000)	C/B = 0.241	(Heymans and Baird 2000)	R = C - E - P (Crisp 1971)	E = 0.3 x C	(Heymans and Baird 2000)		
BIRD										
46	*T. tadorna*	P/B = 0.003	(Baird et al. 2004)	C/B = 0.121	(Baird et al. 2004)	R = C - E - P (Crisp 1971)	E/B = 0.024	(Baird et al. 2004)	CC = 0.171 x FW	(Horn and de la Vega, 2016; Chapter 4)
47	*S. mollissima*	P/B = 0.003		C/B = 0.136			E/B = 0.027		CC = 0.171 x FW	
48	*H. ostralegus*	P/B = 0.004		C/B = 0.163			E/B = 0.032		CC = 0.201 x FW	
49	*R. avosetta*	P/B = 0.011		C/B = 0.222			E/B = 0.044		CC = 0.198 x FW	
50	*P. apricaria*	P/B = 0.003		C/B = 0.113			E/B = 0.022		CC = 0.198 x FW	
51	*C. canutus*	P/B = 0.004		C/B = 0.252			E/B = 0.052		CC = 0.154 x FW	
52	*C. alpina*	P/B = 0.002		C/B = 0.158			E/B = 0.034		CC = 0.154 x FW	
53	*L. lapponica*	P/B = 0.004		C/B = 0.204			E/B = 0.041		CC = 0.198 x FW	
54	*N. arquata*	P/B = 0.002		C/B = 0.072			E/B = 0.015		CC = 0.198 x FW	
55	*C. ridibundus*	P/B = 0.005		C/B = 0.084			E/B = 0.017		CC = 0.157 x FW	
56	*L. canus*	P/B = 0.005		C/B = 0.050			E/B = 0.010		CC = 0.166 x FW	
57	*L. argentatus*	P/B = 0.003		C/B = 0.044			E/B = 0.010		CC = 0.166 x FW	
58	*A. platyrhynchos*	P/B = 0.001		C/B = 0.034			E/B = 0.013		CC = 0.171 x FW	
59	*A. acuta*	P/B = 0.004		C/B = 0.098			E/B = 0.035		CC = 0.171 x FW	
60	*A. penelope*	P/B = 0.002		P/C = 0.105			E/B = 0.018		CC = 0.171 x FW	
61	*B. bernicla*	P/B = 0.002		P/C = 0.080			E/B = 0.006		CC = 0.171 x FW	
SEAL										
64	*P. Vitulina*	Natality - 60% (Rejnders 1976; Fast Jensen 2015)		4 kgWW.d^{-1}.ind^{-1} (Bowen et al. 2001; Atkinson 1997)		R = C - E - P (Crisp 1971)	E = 0.088 x C (Ashwell-Erikson and Elsner 1981)		CC = 0.149 x FW	(Horn and de la Vega, 2016; Chapter 4)

Appendix 2:

References and sources used for the construction of the dietary matrices of each compartments and proportion of the diet coming from the Sylt-Rømø Bight (i.e. modelled area)

Comp. no	Compartment names	Dietary matrix Sources	Part of the diet provided by the Sylt-Rømø Bight (%)
4	free living bacteria	Baird et al. 2012	100
5	zooplankton	Baird et al. 2012	100
6-33	Macrofauna	Baird et al. 2012 (weighed with updated biomass)	100
34, 35; 38-41	fish *sp.* Included in the model of Baird et al. 2012	Baird et al. 2012 (weighed with updated biomass)	100
36	*P. platessa*	Baird et al. 2012 (weighed with updated biomass)	100
37	*P. flesus*	Baird et al. 2012 (weighed with updated biomass)	100
42	*L. Limanda*	Hinz et al. 2005; Kelreitner et al. 2012 (weighed with updated biomass)	100
43	*A. Tobianus*	Kelreitner et al. 2012 (weighed with updated biomass)	100
44	*O. eperlanus*	Hansson etal. 1997; Kelreitner et al. 2012 (weighed with updated biomass)	100
45	*Loligo sp.*	Guerra et al. 1994; Pierce et al. 1994 (weighed with updated biomass)	100
46	*Tadorna tadorna*	Buxter and Young 2009	91.5
47	*Somateria molissima*	Nehls et al. 1997 + personal measurement	100
48	*Haematopus ostralegus*	Schwemmer et al. 2012	95
49	*Recurvirostra avosetta*	Schwemmer personal communication (weighed with updated biomass)	100
50	*Pluviaris apricaria*	Durell and Kelly 1990; Schwemmer personal communnication (weighed with updated biomass)	100
51	*Calidris canutus*	Piersma et al. 1993	100
52	*Calidris alpina*	Schwemmer et al. Submitted	95
53	*Limosa lapponica*	Scheiffarth et al. 2001	100
54	*Numenius arquata*	Schwemmer et al. 2012	99
55	*Larus ridibundus*	Kubetzki and Garthe 2003; Schwemmer et al. 2011	49
56	*Larus canus*	Schwemmer personal communication.; Kubetzki and Garthe 2003	39
57	*Larus argentatus*	Enners unpublished data	43
58	*Anas plathyrhynchos*	Baird et al. 2012 (weighed with updated biomass)	50
59	*Anas acuta*	Nienhuis and Groenendijk 1986; Meltofte et al. 1994; Baldwin and Lovvorn 1994	75
60	*Anas peneloppe*	Mather and Mongomery 2014; Nienhuis and Groenendijk 1986; Meltofte et al. 1994	50
61	*Branta Bernicla*	Nienhuis and Groenendijk 1986; Baldwin and Lovvorn 1994; Ganter 2000; Prop and Deerenberg 1991	spring: 20 summer: 100 fall: 100 winter: 50
62, 63	sediment bacteria; meiobenthos	Baird et al. 2012	100
64	*Phoca vitulina*	De la Vega et al. 2016	spring: 30 summer: 30 fall: 15 winter: 15

Appendix 3:

Diet matrices of zooplankton, bacteria, meiofauna and omnivorous macrofauna species

		Predator, compartment number												
		Freeliving bactria 4	Zooplankton 5	*Hydrobia ulvae* 6	*Littorina littorea* 7	*Arenicola marina* 8	*Scoloplos intertidalis* 9	*Capitellidae* 10	*Oligochaeta* 11	*Heteromastus* 12	*Lanice conchilega* 13	*Pygospio elegans* 15	*Corophium arenarium* 16	*Corophium volutator* 17
Prey, compartment number			1.00								0.85	0.85		
Phytoplankton	1			0.75	1.00	0.10	0.10						0.75	0.75
Microphytobenthos	2													
Macrophytes	3										0.01	0.01		
Freeliving bactria	4													
Zooplankton	5			0.13		0.45	0.45	0.50	0.50	0.50			0.13	0.13
Sediment bacteria	62													
Meiobenthos	63										0.14	0.14		
Suspended POC	65			0.13		0.45	0.45	0.50	0.50	0.50			0.13	0.13
Sediment POC	66	1.00												

		Mytilus edulis 19	*Crassostrea gigas* 20	*Semibalanus* 21	*Balanus sp.* 22	*Austrominius* 23	*Cerastoderma* 24	*Mya arenaria* 25	*Tharyx killariensis* 27	*Macoma baltica* 28	Small crustacean 30	Sediment bacteria 62	Meiobenthos 63
Phytoplankton	1	0.85	0.85	0.50	0.50	0.50	0.85	0.85		0.43			
Microphytobenthos	2									0.25	0.29		0.25
Macrophytes	3										0.22		
Freeliving bactria	4	0.01	0.01	0.01	0.01	0.01	0.01	0.01					
Zooplankton	5			0.49	0.49	0.49							
Sediment bacteria	62								0.50	0.13	0.24		0.25
Meiobenthos	63												0.13
Suspended POC	65	0.14	0.14				0.14	0.14		0.07			
Sediment POC	66								0.50	0.13	0.24	1.00	0.38

Appendix 4: Diet matrices of predator macrofauna species

rey, compartment umber		**Predator, compartment number**						
		Nereis diversicolor	*Gammarus spp.*	small polychaetes	*Phyllodocidae*	*Carcinus maenas*	*Cragon crangon*	*Nepthys spp.*
		14	18	26	29	31	32	33
hytoplankton	1	1.0E-01		1.1E-01				
icrophytobenthos	2	1.0E-01	1.0E-01	5.0E-02		2.0E-01		
acrophytes	3		4.0E-01					
ydrobia ulvae	6	8.9E-02	8.9E-02	8.9E-02	8.9E-02	8.6E-02	8.9E-02	2.2E-01
ittorina littorea	7	6.3E-03	6.3E-03	6.3E-03	6.3E-03	6.1E-03	6.3E-03	1.6E-02
renicola marina	8	1.2E-01	1.2E-01	1.2E-01	1.2E-01	1.1E-01	1.2E-01	2.9E-01
coloplos intertidalis	9	6.2E-03	6.2E-03	6.2E-03	6.2E-03	6.0E-03	6.2E-03	1.6E-02
apitellidae	10	1.3E-03	1.3E-03	1.3E-03	1.3E-03	1.3E-03	1.3E-03	3.3E-03
Oligochaeta	11	2.2E-03	2.2E-03	2.2E-03	2.2E-03	2.1E-03	2.2E-03	5.5E-03
eteromastus	12	1.0E-03	1.0E-03	1.0E-03	1.0E-03	1.0E-03	1.0E-03	2.6E-03
anice conchilega	13	3.7E-04	3.7E-04	3.7E-04	3.7E-04	3.6E-04	3.7E-04	9.2E-04
ereis diversicolor	14	1.2E-03	1.2E-03	1.2E-03	1.2E-03	1.2E-03	1.2E-03	3.0E-03
Pygospio elegans	15	2.2E-04	2.2E-04	2.2E-04	2.2E-04	2.1E-04	2.2E-04	5.5E-04
Corophium arenarium	16	3.4E-05	3.4E-05	3.4E-05	3.4E-05	3.3E-05	3.4E-05	8.4E-05
Corophium volutator	17	1.7E-03	1.7E-03	1.7E-03	1.7E-03	1.7E-03	1.7E-03	4.3E-03
ammarus spp.	18	1.1E-04	1.1E-04	1.1E-04	1.1E-04	1.1E-04	1.1E-04	2.8E-04
ytilus edulis	19					2.2E-03		
emibalanus	21					8.2E-03		
alanus sp.	22					3.1E-04		
ustrominius	23					1.6E-03		
erastoderma	24	1.1E-01	1.1E-01	1.1E-01	1.1E-01	1.0E-01	1.1E-01	2.7E-01
ya arenaria	25	1.9E-02	1.9E-02	1.9E-02	1.9E-02	1.8E-02	1.9E-02	4.6E-02
mall polychaetes	26	2.7E-03	2.7E-03	2.7E-03	2.7E-03	2.7E-03	2.7E-03	6.9E-03
haryx killariensis	27	1.3E-03	1.3E-03	1.3E-03	1.3E-03	1.3E-03	1.3E-03	3.4E-03
acoma baltica	28	3.4E-02	3.4E-02	3.4E-02	3.4E-02	3.3E-02	3.4E-02	8.4E-02
hyllodocidae	29	7.3E-04	7.3E-04	7.3E-04	7.3E-04	7.1E-04	7.3E-04	1.8E-03
mall crustacean	30	3.1E-03	3.1E-03	3.1E-03	3.1E-03	3.0E-03	3.1E-03	7.7E-03
rcinus maenas	31	1.7E-03	1.7E-03	1.7E-03	1.7E-03	1.7E-03	1.7E-03	4.3E-03
ragon crangon	32	3.8E-04	3.8E-04	3.8E-04	3.8E-04	3.7E-04	3.8E-04	9.6E-04
epthys spp.	33	5.2E-03	5.2E-03	5.2E-03	5.2E-03	5.0E-03	5.2E-03	1.3E-02
ediment bacteria	62	1.0E-01	5.0E-02	2.3E-01			2.0E-01	
eiobenthos	63	1.0E-01		5.0E-02	6.0E-01	4.0E-01	2.0E-01	
uspended POC	65	1.0E-01		3.5E-02				
ediment POC	66	1.0E-01	5.0E-02	1.3E-01			2.0E-01	

Appendix 5:

Diet matrices of fish and squid species

Prey, compartment number		Predator, compartment number											
		P. microps 34	*P. minutus* 35	*P. platessa* 36	*P. Flesus* 37	*C.* harengus 38	*M. merlangus* 39	*G. morhua* 40	*M. scorpio* 41	*L. Limanda* 42	*A. Tobianus* 43	*O. Eperlanus* 44	*Loligo sp.* 45
Zooplankton	5	4.5E-03	4.5E-03			1.0E+00					1.0E+00	5.0E-01	7.0E-02
Hydrobia ulvae	6				3.4E-01					3.4E-01			
Littorina littorea	7												
Arenicola marina	8			4.2E-01	4.5E-01					4.5E-01			
Scoloplos intertidalis	9	1.9E-01	1.9E-01										
Capitellidae	10	4.0E-02	4.0E-02										
Oligochaeta	11	6.7E-02	6.7E-02										
Heteromastus	12	3.1E-02	3.1E-02										
Lanice conchilega	13	1.1E-02	1.1E-02	1.3E-03	1.4E-03					1.4E-03			
Nereis diversicolor	14	3.7E-02	3.7E-02	4.4E-03	4.7E-03					4.7E-03			2.0E-02
Pygospio elegans	15	6.7E-03	6.7E-03	7.9E-04	8.4E-04					8.4E-04			
Corophium arenarium	16	1.0E-03	1.0E-03	1.2E-04	1.3E-04					1.3E-04			
Corophium volutator	17	5.2E-02	5.2E-02	6.1E-03	6.5E-03					6.5E-03			
Gammarus spp.	18	3.4E-03	3.4E-03	4.0E-04	4.3E-04					4.3E-04			
Mytilus edulis	19												
Cerastoderma	24			3.8E-01									
Mya arenaria	25												
small polychaetes	26	8.4E-02	8.4E-02										
Tharyx killariensis	27	4.1E-02	4.1E-02										
Macoma baltica	28			1.2E-01	1.3E-01					1.3E-01			
Phyllodocidae	29	2.2E-02	2.2E-02										
Small crustacean	30	9.4E-02	9.4E-02	1.1E-02	1.2E-02					1.2E-02			
Carcinus maenas	31						7.7E-01	7.7E-01	7.7E-01				
Cragon crangon	32	1.2E-02	1.2E-02	1.4E-03	1.5E-03		1.7E-01	1.7E-01	1.7E-01	1.5E-03		5.0E-01	7.0E-02
Nepthys spp.	33			1.9E-02	2.0E-02					2.0E-02			
P. microps	34						5.3E-02	5.3E-02	5.3E-02				
P. minutus	35						1.1E-04	1.1E-04	1.1E-04				
Pelagic fish	38-40, 43,44												7.7E-01
Loligo sp.	45												7.0E-02
Meiobenthos	63	3.0E-01	3.0E-01	3.5E-02	3.8E-02					3.8E-02			

Appendix 6 (p. 192-193)

Diet matrices of bird species

		Larus ridibundus	*Larus canus*	*Larus argentatus*	*Anas platyrhynchos*	*Anas acuta*	*Anas penelope*	*Branta bernicla*		
		55	56	57	58	59	60	61		
Prey, compartment number								spring	summer-fall	winter
Macrophytes	3				2.50E-01	2.50E-01	5.00E-01		1.00E+00	5.00E-01
Hydrobia ulvae	6	2.50E-02	5.00E-03		1.20E-01	2.79E-01				
Littorina littorea	7									
Arenicola marina	8	6.00E-02	1.00E-02							
Scoloplos intertidalis	9		1.00E-02		3.30E-03	1.90E-02				
Capitellidae	10				3.30E-03	1.90E-02				
Oligochaeta	11				3.30E-03	1.90E-02				
Heteromastus	12	1.00E-02	1.00E-02		3.30E-03	1.90E-02				
Lanice conchilega	13	5.00E-02	1.00E-02		3.30E-03	1.90E-02				
Nereis diversicolor	14	9.00E-02	1.20E-01	5.00E-03	3.30E-03	1.90E-02				
Pygospio elegans	15				3.30E-03	1.90E-02				
Corophium arenarium	16				2.50E-02	3.00E-03				
Corophium volutator	17	1.00E-02	5.00E-03		2.50E-02	3.00E-03				
Gammarus spp.	18									
Mytilus edulis	19		1.00E-02	1.00E-02						
Semibalanus	21									
Balanus sp.	22									
Austrominius	23									
Cerastoderma	24	7.00E-02	5.00E-02	2.00E-01						
Mya arenaria	25	5.00E-03	1.00E-02		8.00E-03	7.00E-03				
small polychaetes	26				3.30E-03	1.90E-02				
Tharyx killariensis	27									
Macoma baltica	28	5.00E-02		1.00E-02	1.90E-02	9.00E-03				
Phyllodocidae	29				3.30E-03	1.90E-02				
Small crustacean	30		2.00E-02		8.00E-03	4.00E-03				
Carcinus maenas	31	2.00E-02	4.00E-02	2.00E-01	6.00E-03	5.00E-03				
Cragon crangon	32	1.00E-01	8.00E-02		6.00E-03					
Nepthys spp.	33		1.00E-02	5.00E-03	3.30E-03	1.90E-02				
Terrestrial		5.10E-01	6.10E-01	5.70E-01	5.00E-01	2.50E-01	5.00E-01	1.00E+00		5.00E-01

		Predator, compartment number								
		Tadorna tadorna 46	*Somateria mollissima* 47	*Haematopus ostralegus* 48	*Recurvirostra avosetta* 49	*Pluvialis apricaria* 50	*Calidris canutus* 51	*Calidris alpina* 52	*Limosa lapponica* 53	*Numenius arquata* 54
Prey, compartment number										
Macrophytes	3	3.00E-02								
Hydrobia ulvae	6	4.75E-01			9.15E-01	3.41E-01	3.84E-01	8.70E-02		
Littorina littorea	7	9.50E-02								
Arenicola marina	8			1.50E-01		9.00E-02			3.30E-02	3.50E-01
Scoloplos intertidalis	9			5.00E-02		1.14E-01			3.82E-01	
Capitellidae	10									
Oligochaeta	11									
Heteromastus	12							5.00E-02		
Lanice conchilega	13							2.00E-02	2.90E-02	5.00E-02
Nereis diversicolor	14	8.00E-02		1.00E-01	1.26E-02	9.51E-02		5.00E-01	2.76E-01	
Pygospio elegans	15							5.00E-02		
Corophium arenarium	16				3.48E-04					
Corophium volutator	17				1.77E-02	6.59E-03				
Gammarus spp.	18				1.15E-03					
Mytilus edulis	19	2.50E-02	6.82E-02	1.00E-01			9.76E-03			
Semibalanus	21		3.84E-04							
Balanus sp.	22		1.44E-05							
Austrominius	23		7.50E-05							
Cerastoderma	24	3.00E-02	3.22E-04	3.50E-01			4.61E-01			1.50E-01
Mya arenaria	25									
small polychaetes	26					1.01E-01				
Tharyx killariensis	27									
Macoma baltica	28	9.00E-02		1.50E-01		1.29E-01	1.45E-01		3.30E-02	5.00E-02
Phyllodocidae	29									
Small crustacean	30	8.00E-02			3.18E-02	1.19E-02		1.43E-01		1.00E-04
Carcinus maenas	31	1.00E-02	9.31E-01		1.78E-02				1.50E-02	2.00E-01
Cragon crangon	32				3.98E-03				1.60E-02	5.00E-02
Nepthys spp.	33			5.00E-02		1.10E-01		1.00E-01	2.16E-01	5.00E-02
Terrestrial		8.50E-02	8.07E-06	5.00E-02				5.00E-02		1.00E-01

		Larus ridibundus	*Larus canus*	*Larus argentatus*	*Anas platyrhynchos*	*Anas acuta*	*Anas penelope*	*Branta bernicla*		
		55	56	57	58	59	60	61		
Prey, compartment number								spring	summer-fall	winter
Macrophytes	3				2.50E-01	2.50E-01	5.00E-01		1.00E+00	5.00E-01
Hydrobia ulvae	6	2.50E-02	5.00E-03		1.20E-01	2.79E-01				
Littorina littorea	7									
Arenicola marina	8	6.00E-02	1.00E-02							
Scoloplos intertidalis	9		1.00E-02		3.30E-03	1.90E-02				
Capitellidae	10				3.30E-03	1.90E-02				
Oligochaeta	11				3.30E-03	1.90E-02				
Heteromastus	12	1.00E-02	1.00E-02		3.30E-03	1.90E-02				
Lanice conchilega	13	5.00E-02	1.00E-02		3.30E-03	1.90E-02				
Nereis diversicolor	14	9.00E-02	1.20E-01	5.00E-03	3.30E-03	1.90E-02				
Pygospio elegans	15				3.30E-03	1.90E-02				
Corophium arenarium	16				2.50E-02	3.00E-03				
Corophium volutator	17	1.00E-02	5.00E-03		2.50E-02	3.00E-03				
Gammarus spp.	18									
Mytilus edulis	19		1.00E-02	1.00E-02						
Semibalanus	21									
Balanus sp.	22									
Austrominius	23									
Cerastoderma	24	7.00E-02	5.00E-02	2.00E-01						
Mya arenaria	25	5.00E-03	1.00E-02		8.00E-03	7.00E-03				
small polychaetes	26				3.30E-03	1.90E-02				
Tharyx killariensis	27									
Macoma baltica	28	5.00E-02		1.00E-02	1.90E-02	9.00E-03				
Phyllodocidae	29				3.30E-03	1.90E-02				
Small crustacean	30		2.00E-02		8.00E-03	4.00E-03				
Carcinus maenas	31	2.00E-02	4.00E-02	2.00E-01	6.00E-03	5.00E-03				
Cragon crangon	32	1.00E-01	8.00E-02		6.00E-03					
Nepthys spp.	33		1.00E-02	5.00E-03	3.30E-03	1.90E-02				
Terrestrial		5.10E-01	6.10E-01	5.70E-01	5.00E-01	2.50E-01	5.00E-01	1.00E+00		5.00E-01

Appendix 7 (p. 196-197)

Appendix 7: Analyses of the fecal material of eider ducks (*Somateria mollissima*) from the Sylt-Rømø Bight

Material and Methods: Feces samples of eider ducks were collected on two locations in the Sylt-Rømø Bight (Fig. 1), where high numbers of resting eider ducks were counted at low tide in 2013 (Kempf 2013) (location 1: 50-250 individuals; location 2: 250-1000 individuals). 87 and 64 samples were collected on a mussel bank (location 1, Fig. 1) and on a sand bank (location 2, Fig. 1) respectively, from May 2014 to July 2014. Collected feces were dried or frozen until they could be analyzed. Feces were mixed and carefully rinsed with water to separate prey fragments, which were then examined under a binocular. Eider duck feces contain a large proportion of indigestible fragments (e.g. mollusk shells and crab claws). Prey remains were identified with reference to prey species determined by Nehls and Ketzenberg (2002). Prey categories, i.e. blue mussel, common cockles, shore crabs, barnacles, sea urchins, to which all types of prey fragments could be dependably assigned, were used for quantitative analyses (Fig. 1 and 2). Relative abundance by volume was visually estimated and scored as followed: 1 (<1%), 2 (1– 10%), 3 (10–50%), 4 (50–90%), or 5 (>90%).

Results: On the mussel bank, 95% of the analyzed feces contained more than 90% of blue mussels, the other 5% contained more than 80% of shore crabs (Fig. 1 and 2). On the sand bank, 94% of the analyzed feces contained more than 90% of shore crabs, the other 6% contained more than 90% of blue mussels (Fig. 1 and 2).

Discussion: Eider ducks from the Sylt-Rømø Bight appeared to feed on blue mussels only when they are in the surrounding of the mussel bank, but feed mainly on shore crabs in the other location. Nehls and Ketzenberg (2002) observed an increase in May and June of shore crabs as prey items for eider ducks feeding on the same mussel banks as the one sampled in this study, but the proportion of crabs in the diet was significantly lower than the one observed in this study on sand banks. The highest number of eider ducks in summer 2013 was counted on the Jordsand and Hojer sand banks (see Fig. 7, p. 23 in the general introduction). This suggests that eider ducks feed mainly on shore crabs in the Sylt-Rømø Bight from May to July. The change of prey availability observed in the area when blue mussel (*Mytilus edulis*) beds have decreased and were partially substituted by beds of the invaded pacific oyster (*Crassostrea gigas*) in the last decades (Diederich 2006, Büttger et al. 2008), is thus reflected in the diet change of the eider from predominant mussels to more readily available crabs.

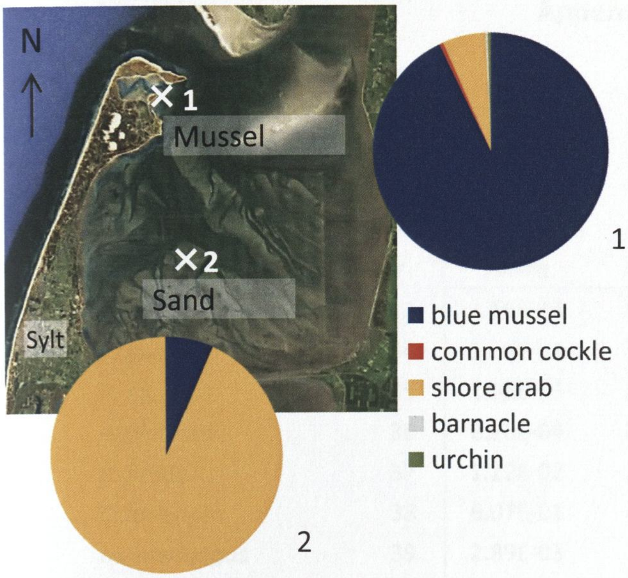


Fig. 1: Location of the sampling sites in the Sylt-Rømø Bight and feces averaged composition (%) for each sampling site; 1: mussel bank, n=87 samples collected on May 22nd, May 28th and June 1st; 2: Sand bank, n=64 samples collected on June 17th and July 5th

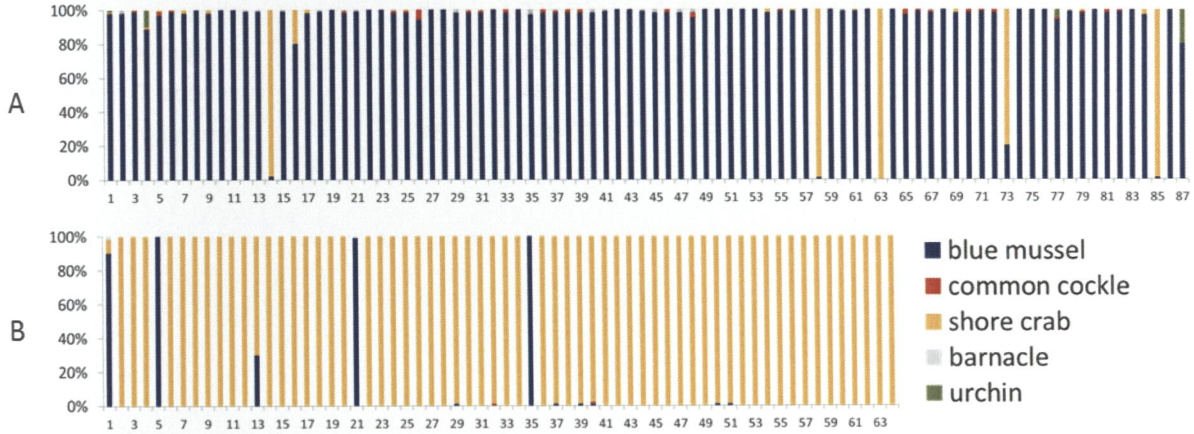


Fig. 2: Feces composition (%) for each sample collected on A the mussel bank and B the sand bank

References:

Büttger, H., H. Asmus, R. Asmus, C. Buschbaum, S. Dittmann, and G. Nehls. 2008. Community dynamics of intertidal soft-bottom mussel beds over two decades. Helgoland Marine Research **62**:23-36.

Diederich, S. 2006. High survival and growth rates of introduced Pacific oysters may cause restrictions on habitat use by native mussels in the Wadden Sea. Journal of Experimental Marine Biology and Ecology **328**:211-227.

Kempf, N. 2013. Eiderenten im Nationalpark Schleswig-Holsteinisches Wattenmeer im Jahr 2013. für den Landesbetrieb für Küstenschutz, Nationalpark und Meeresschutz Schleswig-Holstein.

Nehls, G., and C. Ketzenberg. 2002. Do common eiders Somateria mollissima exhaust their food resources? A study on natural mussel Mytilus edulis beds in the Wadden Sea. Danish Review of Game Biology **16**:47-61.

Appendix 8: Diet matrix of harbor seal (*Phoca vitulina*)

		***Phoca vitulina*, 64**			
Prey, compartment number		**spring**	**summer**	**fall**	**winter**
C. crangon	32	1.55E-03	9.48E-02	3.15E-02	2.27E-01
P. microps	34	1.54E-01	8.00E-04	1.72E-03	2.35E-03
P. minutus	35	1.98E-03	2.06E-02	1.50E-02	2.90E-03
P. platessa	36	8.26E-04	2.06E-03	6.44E-03	2.16E-03
P. flesus	37	1.22E-02	3.08E-02	6.14E-02	6.47E-02
C. harengus	38	6.07E-01	4.34E-01	5.97E-01	5.67E-01
M. merlangus	39	2.89E-03	3.25E-03		2.16E-03
G. morhua	40		8.81E-04	5.73E-04	
M. scorpio	41	2.12E-03	1.20E-03	3.22E-03	5.56E-03
L. limanda	42	1.23E-02	3.49E-02	5.62E-02	2.99E-03
A. tobianus	43	1.16E-01	3.37E-01	1.69E-01	6.19E-02
O. eperlanus	44	1.48E-02	3.52E-02	4.22E-02	6.10E-02
Loligo spp.	45	7.44E-02	4.38E-03	1.52E-02	

General Discussion

> *"Et le surcroit nous vienne en songe, à ton seul nom de mer!"*
>
> *"And may increase come to us in our dream at your single name of Sea!"*
>
> *Saint John Perse*

General Discussion

1. The trophic role of harbor seals in the Sylt-Rømø Bight ecosystem

1.1. Seasonal variation of harbor seal´s diet

Two main seasonal trends were revealed in the diet of harbor seals from the Wadden Sea and the North Sea.

First, the results of this study showed that harbor seal´s main feeding location vary seasonally, relying more on coastal (i.e. Wadden Sea) food resources in the warm seasons than in the cold seasons, when they probably migrate to the open North Sea to forage. This behavior is supported by telemetry studies which showed that seals tagged on the island of Rømø have significantly longer foraging trips to the North Sea in winter than in summer (Tougaard et al. 2003). This trend in the foraging location follows the seasonal abundance of harbor seals and the seasonal variation of prey species availability in the Wadden Sea. Indeed, Jensen (2015) counted about 80% less adult harbor seals in December (≈70 individuals) than in August (≈400 individuals) in the Sylt-Rømø Bight. This result is in accordance with harbor seal´s counts in the Wadden Sea in general which showed higher number of seals on haul outs in the warm season during reproduction and molt periods (Drescher 1979, Reijnders et al. 2009, Osinga et al. 2012, Jensen 2015) than in winter. The seasonal variation of seal abundance in the Wadden Sea therefore supports the findings of this study, showing that harbor seals from the Sylt-Rømø Bight use the Wadden Sea food resources in higher proportion in spring and summer than in fall and winter. Furthermore, the abundance and biomass of prey species in the Wadden Sea are very low in the cold seasons (Daan et al. 1990, Polte and Asmus 2006, Tulp et al. 2008, Baumann et al. 2009), being probably not sufficient to provide food enough to support the whole Wadden Sea population of harbor seals. Therefore, harbor seals migrate to forage outside the Wadden Sea to a higher extent in fall and winter than in spring and summer. Andersen et al. (2007) suggested that the decline of prey availability in Limfjord, Denmark, may have forced harbor seals to make longer foraging trips. This suggests that harbor seals, in accordance with the common observation that their distribution is strongly influenced by the distribution of their prey (Das et al. 2003), adjust their foraging patterns and increase the length and duration of their foraging migrations when necessary.

In addition to its function as a resting and nursery area (Reijnders et al. 2009, Osinga et al. 2012, Galatius et al. 2014), this result clearly shows that the Wadden Sea has also an important seasonal role as a foraging area for harbor seals. Indeed, harbor seals appeared to feed on the food resources from the Wadden Sea in spring and summer, in similar amount than on the food resources from the North Sea.

Second, a shift from a diet more strongly influenced by pelagic prey items in spring to a diet more influenced by benthic prey items in summer was observed in this study. This change was observed in both locations, the Wadden Sea (i.e. Sylt-Rømø Bight) and the North Sea. This is in accordance with studies by Brown and Pierce (1998), Hall et al. (1998), Andersen et al. (2007) and Berg et al. (2002) conducted in the southern North Sea, which showed a high occurrence of pelagic species (e.g. herrings and sand-eels) in spring and an increase of gadoids and flat fish in seals gut contents in summer. In some extent, this change in the prey items can be related to the availability of prey species in the Wadden Sea. Indeed, the high contribution of herring and squids in the harbor seal diet in the Sylt-Rømø Bight corresponded to the high peaks of abundance and biomass of these two prey items in spring. However, despite the remaining high abundance of pelagic species (e.g. herring), harbor seals switch to benthic and demersal prey items when they become available in the Wadden Sea in summer. This confirms that harbor seals are primarily benthic feeders (Tougaard et al. 2003) and that they have an opportunistic behavior, foraging on one of the highly abundant prey species in the sea, but not necessarily on the most abundant one (Tollit et al. 1997). Härkönen (1987) showed that along the Danish coast of the North Sea, harbor seals forage on the most abundant gadoid species but do not feed on several other species of fish that are also numerous in this area. This switch in harbor seals diet to benthic and demersal prey items when they become available is supported by the results of gut content analysis conducted in the Wadden Sea in Schleswig Holstein, in which flat fish and gadoids were observed as main prey items (Behrends 1985, Sievers 1989, Gilles et al. 2008).

This result confirms that harbor seals have preferential prey items but they are adaptable predators and are able to find alternative prey when food conditions change.

1.2. Influence of harbor seals on the food web

Although a direct link between the presence of harbor seals and the variation in the food web functioning observed in this study was identified, tendency of increased stability and greater resistance of the system were related to the presence of predators in general (Fig. 1). Indeed, the presence of opportunistic predators relying on numerous prey species at the top of the food web, such as harbor seals, increases the number of parallel flows and the pathway length, and therefore the resistance of the system to external disturbances (Baird et al. 2007, Saint-Béat et al. 2015).

The seasonal models of the Sylt-Rømø Bight food web showed that, in the warm seasons, the system tends to be well developed, stable and resistant to perturbations, probably due to the presence of predators at the top of the food web, such as fish and seals which are most abundant in spring and summer (Fig. 1) (Kellnreitner et al. 2012, Jensen 2015). On the contrary, the food web in the winter season is stable, but smaller and more sensitive to external perturbations, probably due to the more simple web of interactions which tends to decrease ecosystem resistance (Saint-Béat et al. 2015). The system in fall tends to be in a less stable transition state between these two stable periods (i.e. warm seasons and winter), characterized by a high excess of benthic primary production and a larger unevenness of flows.

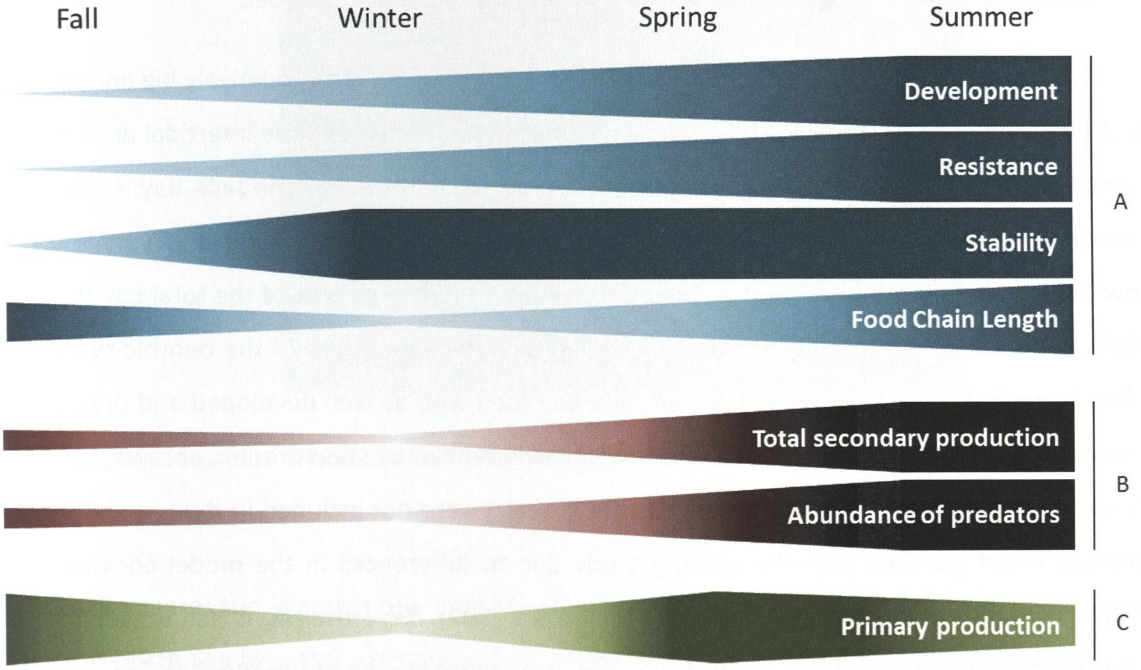


Fig. 1: Summary of the seasonal trends of A- the Sylt-Rømø Bight food web properties, B- the secondary production in the system and C- the primary production in the system.

On a yearly basis, the Sylt-Rømø Bight system is healthy and sustainable. These seasonal trends in the food web properties observed within a year must be seen as small fluctuations in one and the same system. The observed seasonal fluctuations might increase the resistance of the system on a yearly basis. Indeed seasonal variability with recurring states at particular periods of time stabilizes food webs (Saint-Béat et al. 2015) and variability (e.g. temporal) in the food web dynamic enforces the system and makes it more resistant (McCann and Rooney 2009). This suggests that seasonal abundance of top predators, such as harbor seals in the food web has an important role in the structure and functioning of the ecosystem of the Wadden Sea, increasing its resistance in the warm seasons and probably even on a yearly basis. Indeed, opportunistic predators increase the degree of connectivity, the number of interactions and the number of parallel pathways between the food web compartments. They therefore increase the redundancy (Baird et al. 1998, Baird et al. 2004) resulting in a high ability of the food web to cope with external disturbances (Saint-Béat et al. 2015).

2. The food web of the Sylt-Rømø Bight, representative for the entire Wadden Sea?

2.1. Comparison with other food web model studies of the Wadden Sea

Compared to other bays in the Wadden Sea, the Sylt-Rømø Bight is relatively big and deep (Zone 1, Fig. 2). It is connected to the North Sea through a narrow tidal channel, the intertidal area represents only one third of the total bight and its deepest area is 40.5 meter deep. The Jade Bay is another tidal semi-enclosed basin of the Wadden Sea, but contrary to the Sylt-Rømø Bight it is very muddy and shallow (maximum depth of 10 meters). The intertidal area represents 90% of the total bay (Schückel et al. 2015). Schückel et al. (2015) conducted an ecological network analysis of the benthic system of the Jade Bay (Zone 2, Fig. 2). They described the Jade Bay food web as well developed and organized with high amount of recycled material. However, it was characterized by short trophic pathways and a little ability to cope with disturbances. Top predators (e.g. seals) were not included in their model so a direct comparison is not possible with the present study due to differences in the model construction and number of compartments (Abarca-Arenas and Ulanowicz 2002). Nevertheless, it is interesting to notice, that another benthic study conducted in Norderaue area, an open area of the Wadden Sea between the islands Amrum, Föhr, Langeness (Zone 3, Fig. 2), showed low recycling and high herbivory in the system which was well developed and had high resistance to perturbations (Horn, personal communication). This area was dominated by sand flats and was directly in connection with the North Sea. These three studies suggest that depending on the characteristics of the studied part of the Wadden Sea, the ecosystem might function relatively differently. Enclosed and muddy areas seem to have different characteristics from open areas constantly exposed to water exchanges with the North Sea. Extrapolation of the results from the Sylt-Rømø Bight food web model to the entire Wadden Sea should therefore be carefully made.

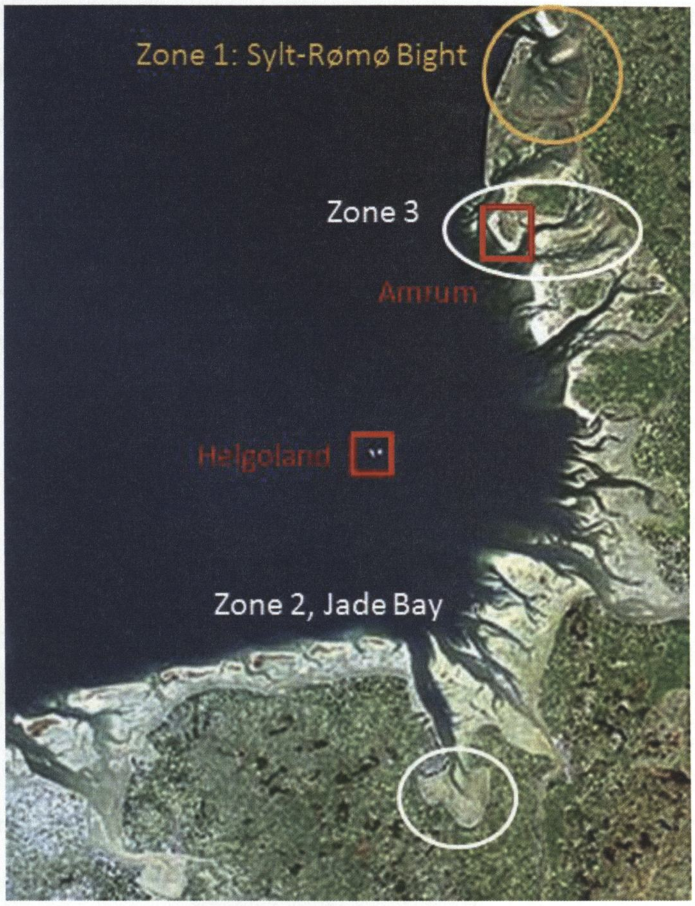


Fig. 2: Satellite image of the Wadden Sea. Modified from http://www.waddensea-secretariat.org/

Furthermore, the Wadden Sea consists of numerous interconnected tidal catchment areas to the adjacent North Sea by water exchanges. Each of the catchment areas or tidal systems might include distinct small-scaled food webs which are probably linked to each other by the pelagic organisms leaving in the water column and the large and mobile predator species using the resources from the different areas. Indeed, birds in the Wadden Sea forage on food sources from various habitats and might shift between the different tidal systems (Laursen et al. 2010). Several fish species, such as *Clupea harengus* and *Ammodytes tobianus*, might travel daily between the Wadden Sea and the North Sea (Gibson et al. 1996). Furthermore, the food requirements of harbor seals are not met within the Wadden Sea and thus exceed its boundaries, as they also use the North Sea for foraging (de la Vega et al. 2016) (Chapter 2). Therefore, the modelled area (i.e. Sylt-Rømø Bight) is smaller than the spatial scale used by several species included in the food web, such as birds, non-resident fish and seals. Studying the functioning of the Wadden Sea at a larger scale, by connecting the numerous sub-webs (i.e. Wadden Sea bights and North Sea) would be relevant. Indeed, the Wadden Sea/North Sea ecosystem might function as the hump-shaped structure defined by McCann and Rooney (2009). Higher trophic levels are expected to couple spatially restricted organisms at the trophic level below them, because predators have generally large mobility and can move between distinct resources from different areas, migrating from a habitat with lower prey density towards one with higher density (McCann and Rooney 2009). These adaptable predatory habits at appropriate times can greatly stabilize systems and make them persistent in time (McCann and Rooney 2009). Including this connectivity between the sub-systems of the Wadden Sea, would improve our overview and our understanding of the Wadden Sea ecosystem functioning.

2.2. Competitive interactions with other predators

Seals are large piscivorous animals and competition for resources with other top predators from the North Sea/Wadden Sea can potentially occur (Thompson et al. 1996, Santos and Pierce 2003, Andersen et al. 2007).

Harbor porpoise (*Phocoena phocoena*) is the predator which is the most similar, in body size, to harbor seals (Santos and Pierce 2003) and it is known to feed on a wide range of pelagic and demersal fish species such as cod (*Gadus morhua*), herring (*Clupea harengus*), sole (*Solea solea*), gobies (*Pomatoschistus* spp.) or dabs (*Limanda limanda*) (Das et al. 2003, Santos and Pierce 2003, Gilles et al. 2008). Both harbor porpoises and harbor seals diets therefore follow a similar pattern, as they exploit the same locally abundant resources (Santos and Pierce 2003). This implies that competition for food resources might occur between these two species. The spatial and seasonal distribution of harbor porpoises in the Wadden Sea is not homogeneous and a hotspot was identified in the north-east of the Wadden Sea, where harbor porpoise densities reach the highest values (Gilles et al. 2009, Reijnders et al. 2009) (Fig. 3). This hotspot seems to play an important role as key foraging area for harbor porpoises (Gilles et al. 2009). In spring and summer, the density of harbor porpoises follows a distinct north-south gradient, with large aggregation of animals in front of the islands of Sylt and Amrum (Fig. 3) (Gilles et al. 2009). In fall, the porpoises are more evenly dispersed throughout the area due to the migration of the animals out of the German Bights in this season (Gilles et al. 2009). These results imply that the foraging location of harbor seals and harbor porpoises overlaps in spring and summer, when they both use the northern German bight and adjacent Wadden Sea tidal basins (e.g. Sylt-Rømø Bight). As for harbor seals, the use of food resources from the Wadden Sea bights by harbor porpoises is probably related to the seasonal prey availability in the area. Including the harbor porpoises in the food web models and particularly in the food web model of the Sylt-Rømø Bight would provide interesting information, and would probably help to better estimate the influence of top predators on the ecosystem.

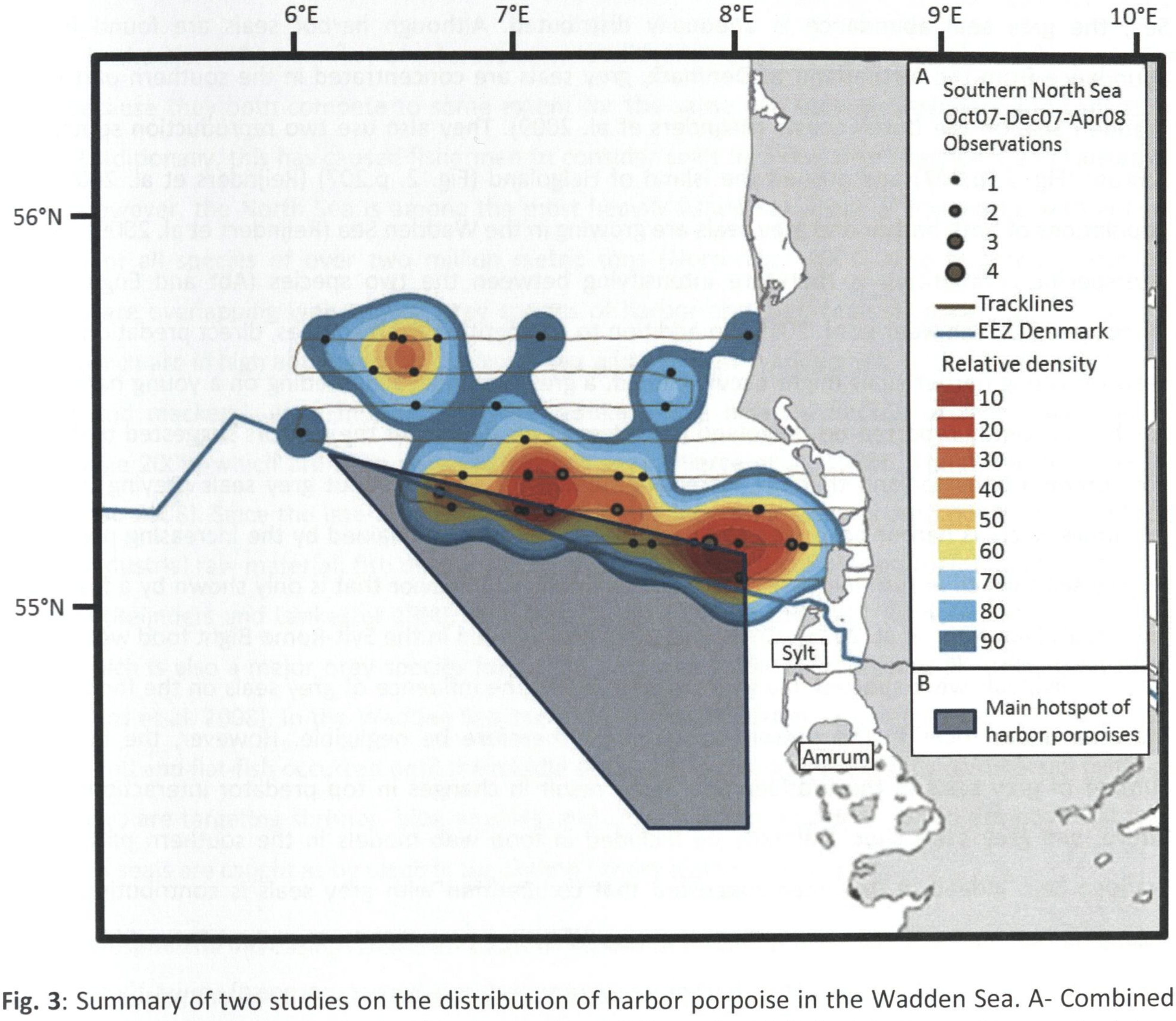


Fig. 3: Summary of two studies on the distribution of harbor porpoise in the Wadden Sea. A- Combined data from three aerial surveys in October, December 2007 and April 2008 with observations and relative density of harbor porpoises. The color scale from blue over yellow and red shows increasing concentration of harbor porpoises. Adapted from Reijnders et al. (2009); B- Approximate area of highest harbor porpoise density values in summer (pooled data from 2002 to 2006). Based on the study of Gilles et al. (2009); EEZ: exclusive economic zone

The diet of harbor seals might also overlap with the diet of grey seals (*Halichoerus grypus*), depending on the location and on the seasons (Pierce et al. 1991a, Hammond et al. 1994, Das et al. 2003). Telemetry studies conducted in Northeast England showed that both grey seals and harbor seals forage in the coastal zone and that their spatial use considerably overlaps (Thompson et al. 1991, McConnell et al. 1999, Sharples et al. 2012). In North East Scotland, a study combining telemetry and feces analyses found dietary and spatial overlap in the foraging of harbor seals and grey seals, although grey seals utilized a wider area and generally travelled further (Thompson et al. 1996). In the Wadden Sea, the grey seal abundance is unequally distributed. Although harbor seals are found in high abundance from the Netherland to Denmark, grey seals are concentrated in the southern part of the Wadden Sea, on the Dutch coasts (Reijnders et al. 2009). They also use two reproduction spots, near Amrum (Fig. 2, p.207) and around the island of Helgoland (Fig. 2, p.207) (Reijnders et al. 2009). The populations of both harbor and grey seals are growing in the Wadden Sea (Reijnders et al. 2009) and the interspecific competition is therefore intensifying between the two species (Abt and Engler 2009, Svensson 2012, van Neer et al. 2015). In addition to competition for resources, direct predation of grey seals on young harbor seals might occur. Indeed, a grey seal individual feeding on a young harbor seal has been recently reported on Helgoland (van Neer et al. 2015). But the authors suggested that this is not common behavior and that the increasing number of reports about grey seals preying on other mammals, such as harbor porpoises and harbor seals, might be explained by the increasing population of grey seals which in turn increases the chance of noticing behavior that is only shown by a few single individuals (van Neer et al. 2015). Grey seals were not included in the Sylt-Rømø Bight food web as only 1 to 5 individuals were spotted in a year (Jensen 2015). The influence of grey seals on the food web of the area under focus in the present study might therefore be negligible. However, the increasing number of grey seals in the Wadden Sea might result in changes in top predator interactions in the future, and grey seals should already be included in food web models in the southern part on the Wadden Sea. Indeed, it has been suggested that competition with grey seals is contributing to the recent harbor seal declines on the English coasts (Sharples et al. 2012). The diet of the different top predators of the Wadden Sea, (e.g. harbor seal, grey seal and harbor porpoise), must therefore be assessed more in details to take into account its spatial, seasonal and inter-annual variations to determine and quantify potential competition between these species. Furthermore, including top predators in food web models will help to estimate the ecological consequences of these competitive interactions among top predators.

3. Advantage of a holistic approach for describing top predators-humans interactions

3.1. Competition between seals and fisheries

Conflict of interest can arise from the potential interactions between marine mammals and fisheries (DeMaster et al. 2001). Indeed, some marine mammals species, such as harbor seals, and fisheries both operate at the top of the food web (Pauly et al. 1998, Kaschner and Pauly 2005) and compete for the same species. It has been shown that on a global scale, competition between marine mammals and fisheries is unlikely to occur, but it might be a potential conflict in the small geographical regions in which marine mammals food consumption overlaps with fisheries (Kaschner and Pauly 2005).

In the North Sea, conflict of interest between fisheries and marine mammals has been on for years because they both compete to some extent for the same fish species (Reijnders and Lankester 1990). Traditionally, this has caused fishermen to consider seals as a nuisance (Reijnders and Lankester 1990). However, the North Sea is among the most heavily fished sea areas of the world, with annual landings of all species of over two million metric tons (Northridge 2009). The targeted species of fisheries are overlapping with several prey species of harbor and grey seals (Reijnders and Lankester 1990), which are in high abundance in the North Sea and adjacent Wadden Sea. In the 1960s and 1970s, herring and mackerel, were intensively fished resulting in a massive decline of these two species (Northridge 2009) which are both potential prey of seals (Pierce et al. 1991b, Thompson et al. 1996, Gilles et al. 2008). Since the late 1990s, industrial fisheries (i.e., their catch is used not for human food but for industrial raw material, fish oil and fish meal) have increased rapidly and their main target is the sand eel (Reijnders and Lankester 1990, McConnell et al. 1999, Yodzis 2001, Furness 2002, Northridge 2009) which is also a major prey species for harbor and grey seals (Thompson et al. 1996, Tollit et al. 1997, Gilles et al. 2008). In the Wadden Sea itself, commercial fisheries targeting clupeids, gadoids and other round and flat-fish occurred until the middle of the 20th century, but the only commercial fisheries in our days are targeting shrimps, blue mussels, and cockles. Plaice and sole, which are potential prey species of seals are caught as by catch in the shrimp fishery (Lotze 2007).

Despite the increasing fishing pressure in the North Sea, both grey and harbor seals population increased during the last decades (Northridge 2009, Reijnders et al. 2009, Galatius et al. 2014). Since the prohibition of seal hunting in the entire Wadden Sea in 1976 (Reijnders et al. 1997), the harbor seal population is growing (Reijnders and Lankester 1990, Reijnders et al. 1997, Lotze et al. 2005). Despite two epizootics in 1988 and 2002 which interrupted the upward trend in population growth sharply

(Reijnders et al. 2009), the number of harbor seals in the Wadden Sea increases and it has reached 26 576 individuals counted on land in August 2014 (Galatius et al. 2014). This suggests that fisheries and seals interactions are complex and not necessarily negative (Yodzis 2001). This observation in the North Sea has been related to two hypotheses.

(1) Indirect effect on abundance

The massive decline in the 1960s and 1970s of Atlantic herring and mackerel which are both predators of sand eels, led to an increase of the sand eel population in the North Sea (Northridge 2009). Thus, the heavy fishing pressure on piscivorous fish species has resulted in an increase in a less commercially valuable species that is a major food source of seals (Fig. 4A). Furness (2002) also concluded that predatory fish abundance, especially mackerel and whiting, may influence sand eel stocks more than industrial fishery in the North Sea.

(2) Indirect effect on size composition

Seals consume generally smaller sized fish (15-20 cm) than the targeted size of fisheries (30cm) (Brown and Pierce 1998, Hall et al. 1998, Andersen et al. 2007). The removal by fisheries of large piscivorous fish feeding on smaller fish might have increase the number of small sized fish, which is advantageous for seals (Northridge 2009) (Fig. 4B).

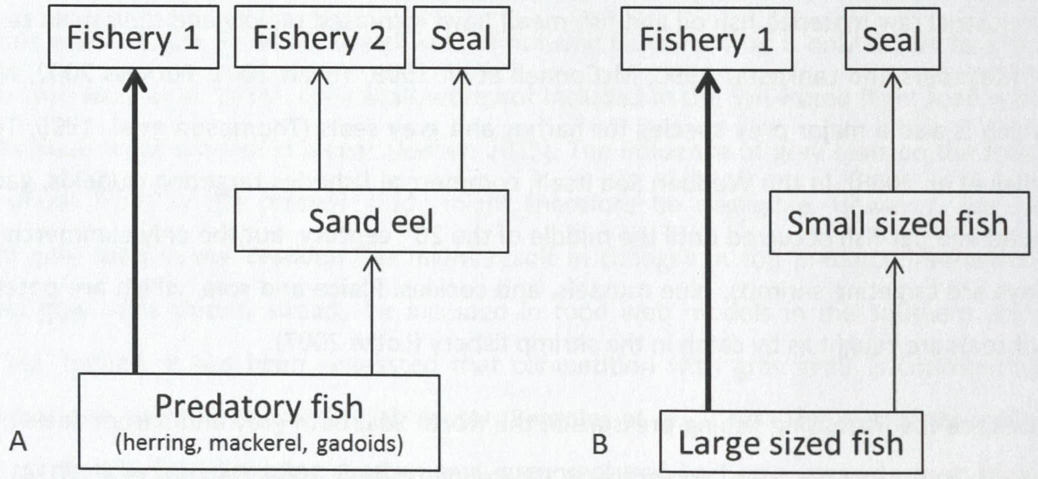


Fig. 4: conceptual scheme of interactions between fisheries, seals and fish in the North Sea. A: The fishery 1, by depleting the stock of predatory fish, boosts the sand eel population, which benefit to the less intense fishery 2 and the seals. B: The fishery 1, by depleting the stock of large sized fish, boosts the number of small sized fish which is advantageous for seals. Inspired by Yodzis (2001) and Northridge (2009)

3.2. Consequences of ecosystem-based versus species-based view for marine mammal Management

The example of the North Sea shows that it is extremely difficult to demonstrate any clear competitive interaction between seals and fisheries, and that the complexities of the marine ecosystem make it very hard to predict the impact of fisheries on the ecosystem (DeMaster et al. 2001, Northridge 2009). This indicates that fisheries function in food webs in the same manner as predators and including human as a component in ecosystem models would be pertinent (Berghöfer et al. 2008, Longo et al. 2015). Assessing qualitatively and quantitatively the ecological role of top predators and the extent of their trophic competition or overlap with fisheries would increase our knowledge about human-nature interactions, and would improve management decisions and conservation measures.

In our days, conservation measures and management in the Wadden Sea are mainly based on monitoring of populations and abundance of single species. Harbor seals are protected under the Annex II of the Convention on Migratory Species of Wild Animals, also called Bonn Convention (1983) and the Annex III (protected fauna species) of the Convention on the conservation of European wildlife and natural habitats (Bern Convention 1985). The harbor seal is also listed in the Annexes II and V of the EU Habitats Directive (1992) (consolidated version 2007) on the conservation of natural habitats and of wild fauna and flora. All these conservations measures focus on species-based management. In addition in the Wadden Sea, a trilateral governance consortium has been developed between Denmark, Germany and the Netherlands since 1991 for the protection of grey and harbor seals (i.e. Trilateral Seal Agreement) (Agreement on the Conservation of Seals in the Wadden Sea 1990). Based on the data base produced by this governance consortium, a management plan is drawn each four years and presented to national decision-makers who adopt the seal management plan (Giebels et al. 2016). Some of the required efforts, objectives and actions formulated in the trilateral management plan (e.g. define criteria for maintaining a favorable conservation status, investigate the habitat requirements of seals in relation to human activities, initiate two trilateral joint projects addressing feeding ecology and habitat requirements of seals), aim to understand and manage seals as an integral part of the broader ecological network. However, such efforts have not been formulated for the actual monitoring yet.

The results of the present study show that, in addition to this species-based monitoring, although it appeared to be relatively efficient to set up species-based management plans, the integration of top predators (e.g. harbor and grey seals) in ecosystem studies would increase our knowledge and understanding about their role in the Wadden Sea. Indeed, the use of ecosystem indicators (e.g. Ecological Network Analysis results) in the management decisions for seal conservation measures would allow taking in account the direct or indirect influence of top predators (e.g. seals) on other species (e.g. competition and predation), and not only the "health" of the top predator species itself. As a corollary, the "health status" of the ecosystem in which they live would influence the harbor seals, providing essential resources in surplus or insufficient amount. For example, abundant prey species (e.g. fish) in the Wadden Sea is likely to have positive influence on the health status of the seal population. Assessing the interactions between top predators (e.g. seals) and the other components of the ecosystem, in other words assessing holistically the complex environmental interactions within an ecosystem, would therefore allow a broader understanding of the factors influencing the status of seal populations in the Wadden Sea. This would improve the decisions about conservation and protection of seals, identifying the potential issues at the base instead of fixing the "symptoms".

Taking an Ecosystem Based Management (EBM) perspective on seal protection in the Wadden Sea would however also provide important evidence to other contested issues. For example, the fishermen complained in 2012 in the media arguing that the decrease in the commercial catchment of flatfish and cod was related to the large number of seals in the German Wadden Sea. They defended that half of the population of seals would be sufficient in the ecosystem and that the other half should be killed (AD 2012). This statement can theoretically even be legitimized by EBM philosophy if informed by principles of sustainable development. Such interpretations, although highly societally contested in the North European Wadden Sea, would allow the commercial use of natural resources as long as the ecosystem resilience is warranted (Giebels, personal communication). To judge upon ecosystem resilience in relation to seal abundance in an evidence-based manner, is problematic and risky when based on single-species monitoring. Only holistic appreciation of human impacts on natural processes and an assessment of ecosystem function across temporal scales will contribute to the management and protection of coastal ecosystems. Those might also help to determine the resistance of the ecosystem to potential changes in the top predator abundance.

4. Conclusion

This study showed that the Wadden Sea plays an important seasonal role as foraging area for harbor seals. Harbor seals use food resources originating from the Wadden Sea in higher amount in spring and summer than in fall and winter, in accordance with the seasonal variation of the abundance and biomass of their prey items. This study also highlighted that, despite the large seasonal variation of species composition, abundance and biomass occurring in the Wadden Sea, the system is relatively stable and sustainable all year long. The presence of top predators in the Wadden Sea might increase the resistance and the stability of the ecosystem. Top predators and particularly opportunistic carnivorous, such as harbor seals, therefore play a structural role in the food web. Top predators should be included in further food web studies to better understand their role in the ecosystem and to improve related conservation measures. Trophic interactions and their consequences on the whole system functioning are complex and difficult to quantify. But more studies should nevertheless focus on holistic approaches and on how ecosystem indicators can be used by management and policy makers.

5. References

Abarca-Arenas, L. G., and R. E. Ulanowicz. 2002. The effects of taxonomic aggregation on network analysis. Ecological Modelling **149**:285-296.

Abt, K., and J. Engler. 2009. Rapid increase of the grey seal (Halichoerus grypus) breeding stock at Helgoland. Helgoland Marine Research **63**:177-180.

AD. 2012. Duitse vissers: schiet zeehonden in Waddenzee af. Article published in the newspaper Algemeen Dagblad, the Netherlands.

Agreement on the Conservation of Seals in the Wadden Sea. 1990. Signed on 16 October 1990 by Denmark, Germany, Netherlands under the Bonn Convention.

Andersen, S. M., J. Teilmann, P. B. Harders, E. H. Hansen, and D. Hjollund. 2007. Diet of harbour seals and great cormorants in Limfjord, Denmark: interspecific competition and interaction with fishery. Ices Journal of Marine Science **64**:1235-1245.

Baird, D., H. Asmus, and R. Asmus. 2004. Energy flow of a boreal intertidal ecosystem, the Sylt-Rømø Bight. Marine Ecology Progress Series **279**:45-61.

Baird, D., H. Asmus, and R. Asmus. 2007. Trophic dynamics of eight intertidal communities of the Sylt-Rømø Bight ecosystem, northern Wadden Sea. Marine Ecology Progress Series **351**:25-41.

Baird, D., J. Luczkovich, and R. R. Christian. 1998. Assessment of spatial and temporal variability in ecosystem attributes of the St Marks National Wildlife Refuge, Apalachee Bay, Florida. Estuarine, Coastal and Shelf Science **47**:329-349.

Baumann, H., A. M. Malzahn, R. Voss, and A. Temming. 2009. The German Bight (North Sea) is a nursery area for both locally and externally produced sprat juveniles. Journal of Sea Research **61**:234-243.

Behrends, G. 1985. Zur Nahrungswahl von Seehunden (*Phoca vitulina L.*) im Wattenmeer Schleswig-Holsteins. Zeitschrift für Jagdwissenschaft **31**:3-14.

Berg, I., T. Haug, and K. T. Nilssen. 2002. Harbour Seal (*Phoca vitulina*) Diet in Vesteralen, North Norway. Sarsia **87**:451-461.

Berghöfer, A., H. Wittmer, and F. Rauschmayer. 2008. Stakeholder participation in ecosystem-based approaches to fisheries management: A synthesis from European research projects. Marine Policy **32**:243-253.

Bern Convention. 1985. Council of Europe, CETS No.: 104. Convention on the Conservation of European Wildlife and Natural Habitats. Annex III: Protected fauna species. .

Bonn Convention. 1983. Convention on the conservation of Migratory species of Wild Animals (Article IV). Annex II: Migratory Species to be the Subject of Agreements.

Brown, E. G., and G. J. Pierce. 1998. Monthly variation in the diet of harbour seals in inshore waters along the southeast Shetland (UK) coastline. Marine Ecology Progress Series **167**:275-289.

Daan, N., P. J. Bromley, J. R. G. Hislop, and N. A. Nielsen. 1990. Ecology of North Sea fish. Netherlands Journal of Sea Research **26**:343-386.

Das, K., G. Lepoint, Y. Leroy, and J. M. Bouquegneau. 2003. Marine mammals from the southern North Sea: feeding ecology data from δ^{13}C and δ^{15}N measurements. Marine Ecology Progress Series **263**:287-298.

de la Vega, C., B. Lebreton, U. Siebert, G. Guillou, K. Das, R. Asmus, and H. Asmus. 2016. Seasonal Variation of Harbor Seal's Diet from the Wadden Sea in Relation to Prey Availability. PLoS ONE **11**:e0155727.

DeMaster, D. P., C. W. Fowler, S. L. Perry, and M. F. Richlen. 2001. Predation and competition: the impact of fisheries on marine-mammal populations over the next one hundred years. Journal of Mammalogy **82**:641-651.

Drescher, H. E. 1979. Biology, ecology and conservation of harbour seals in the tidelands of Schleswig-Holstein. Beitr. Wildbiol. **1**:1-73.

EU Habitats Directive. 1992. Council Directive 92/43/EEC of 21 May 1992 on the conservation of natural habitats and wild fauna and flora - consolidated version 01/01/2007. Annex II: Animal and plant species of community interest whose conservation requires the designation of special areas of conservation. Annex V: Animal and plant species of community interest whose taking in the wild and exploitation my be subject to management measures. .

Furness, R. W. 2002. Management implications of interactions between fisheries and sandeel-dependent seabirds and seals in the North Sea. ICES Journal of Marine Science: Journal du Conseil **59**:261-269.

Galatius, A., S. Brasseur, R. Czeck, B. Diederichs, L. F. Jensen, P. Körber, U. Siebert, J. Teilmann, and S. Klöpper. 2014. Aerial surveys of Harbour Seals in the Wadden Sea in 2014 - The highest pup count recorded yet.

Gibson, R., L. Robb, M. Burrows, and A. Ansell. 1996. Tidal, diel and longer term changes in the distribution of fishes on a Scottish sandy beach. Marine Ecology-Progress Series **130**:1-17.

Giebels, D., A. van Buuren, and J. Edelenbos. 2016. Knowledge governance for ecosystem-based management: Understanding its context-dependency. Environmental Science & Policy **55, Part 3**:424-435.

Gilles, A., H. Andreasen, S. Müller, and U. Siebert. 2008. Nahrungsökölögie von marinen Säugetieren und Seevögeln für das Management von NATURA 2000 Gebieten. Endbericht für das Bundesamt für Naturschutz., Federal Agency for Nature Conservation, Germany.

Gilles, A., M. Scheidat, and U. Siebert. 2009. Seasonal distribution of harbour porpoises and possible interference of offshore wind farms in the German North Sea. Marine Ecology Progress Series **383**:295-307.

Hall, A. J., J. Watkins, and P. S. Hammond. 1998. Seasonal variation in the diet of harbour seals in the south-western North Sea. Marine Ecology Progress Series **170**:269-281.

Hammond, P. S., A. J. Hall, and J. H. Prime. 1994. The Diet of Grey Seals in the Inner and Outer Hebrides. Journal of Applied Ecology **31**:737-746.

Härkönen, T. 1987. Seasonal and regional variations in the feeding habits of the harbour seal, *Phoca vitulina,* in the Skagerrak and the Kattegat. Journal of Zoology **213**:535-543.

Jensen, L. 2015. Monitoring of the seal population in the Wadden Sea in 2015 - Monitering af sælbestanden i Vadehavet 2015. Aarhus Universitet, Miljøministeriet, Fiskeri-og Søfartsmuseet Saltvandsakvariet Esbjerg.

Kaschner, K., and D. Pauly. 2005. Competition between marine mammals and fisheries: food for thought.

Kellnreitner, F., M. Pockberger, and H. Asmus. 2012. Seasonal variation of assemblage and feeding guild structure of fish species in a boreal tidal basin. Estuarine, Coastal and Shelf Science **108**:97-108.

Laursen, K., J. Blew, K. Eskildsen, K. Günther, B. Hälterlein, R. Kleefstra, G. Lüerßen, P. Potel, and S. Schrader. 2010. Migratory Waterbirds in the Wadden Sea 1987- 2008. Wadden Sea Ecosystem No.30. Common Wadden Sea Secretariat, Joint Monitoring Group of Migratory Birds in the Wadden Sea, Wilhelmshaven, Germany.

Longo, C., S. Hornborg, V. Bartolino, M. Tomczak, L. Ciannelli, S. Libralato, and A. Belgrano. 2015. Role of trophic models and indicators in current marine fisheries management. Marine Ecology Progress Series **538**:257.

Lotze, H., K. Reise, B. Worm, J. van Beusekom, M. Busch, A. Ehlers, D. Heinrich, R. Hoffmann, P. Holm, C. Jensen, O. Knottnerus, N. Langhanki, W. Prummel, M. Vollmer, and W. Wolff. 2005. Human transformations of the Wadden Sea ecosystem through time: a synthesis. Helgoland Marine Research **59**:84-95.

Lotze, H. K. 2007. Rise and fall of fishing and marine resource use in the Wadden Sea, southern North Sea. Fisheries Research **87**:208-218.

McCann, K. S., and N. Rooney. 2009. The more food webs change, the more they stay the same. Philosophical Transactions of the Royal Society of London B: Biological Sciences **364**:1789-1801.

McConnell, B. J., M. A. Fedak, P. Lovell, and P. S. Hammond. 1999. Movements and foraging areas of grey seals in the North Sea. Journal of Applied Ecology **36**:573-590.

Northridge, S. 2009. Effect of Fishing Industry. Pages 443-446 *in* W. F. P. Perrin, B. Wursig, and J. G. M. Thewissen, editors. Encyclopedia of Marine Mammals.

Osinga, N., I. Pen, H. A. Udo de Haes, and P. M. Brakefield. 2012. Evidence for a progressively earlier pupping season of the common seal (*Phoca vitulina*) in the Wadden Sea. Journal of the Marine Biological Association of the United Kingdom **92**:1663-1668.

Pauly, D., A. W. Trites, E. Capuli, and V. Christensen. 1998. Diet composition and trophic levels of marine mammals. ICES Journal of Marine Science: Journal du Conseil **55**:467-481.

Pierce, G. J., A. Miller, P. M. Thompson, and J. R. G. Hislop. 1991a. Prey remains in grey seal (Halichoerus grypus) faeces from the Moray Firth, north-east Scotland. Journal of Zoology **224**:337-341.

Pierce, G. J., P. M. Thompson, A. Miller, J. S. W. Diack, D. Miller, and P. R. Boyle. 1991b. Seasonal variation in the diet of common seals (*Phoca vitulina*) in the Moray Firth area of Scotland. Journal of Zoology **223**:641-652.

Polte, P., and H. Asmus. 2006. Intertidal seagrass beds (*Zostera noltii*) as spawning grounds for transient fishes in the Wadden Sea. Marine Ecology-Progress Series **312**:235-243.

Reijnders, P. J. H., S. M. J. M. Brasseur, T. Borchardt, K. Camphuysen, R. Czeck, A. Gilles, L. F. Jensen, M. Leopold, K. Lucke, S. Ramdohr, M. Scheidat, U. Siebert, and J. Teilmann. 2009. Marine mammals. Thematic Report N°20. In: Marencic, H. and Vlas, J. de, editors. Quality status report 2009. Wadden Sea Ecosystem N°25. Wadden Sea Secretariat, Trilateral Monitoring and Assessment Group, Wilhelmshaven, Germany.

Reijnders, P. J. H., and K. Lankester. 1990. Status of marine mammals in the North sea. Netherlands Journal of Sea Research **26**:427-435.

Reijnders, P. J. H., E. H. Ries, S. Tougaard, N. Nørgaard, G. Heidemann, J. Schwarz, E. Vareschi, and I. M. Traut. 1997. Population development of harbour seals *Phoca vitulina* in the Wadden Sea after the 1988 virus epizootic. Journal of Sea Research **38**:161-168.

Saint-Béat, B., D. Baird, H. Asmus, R. Asmus, C. Bacher, S. R. Pacella, G. A. Johnson, V. David, A. F. Vézina, and N. Niquil. 2015. Trophic networks: How do theories link ecosystem structure and functioning to stability properties? A review. Ecological Indicators **52**:458-471.

Santos, M., and G. Pierce. 2003. The diet of harbour porpoise (Phocoena phocoena) in the northeast Atlantic. Oceanography and Marine Biology: an Annual Review **41**:355-390.

Schückel, U., I. Kröncke, and D. Baird. 2015. Linking long-term changes in trophic structure and function of an intertidal macrobenthic system to eutrophication and climate change using ecological network analysis. Marine Ecology Progress Series **536**:25-38.

Sharples, R. J., S. E. Moss, T. A. Patterson, and P. S. Hammond. 2012. Spatial Variation in Foraging Behaviour of a Marine Top Predator (Phoca vitulina) Determined by a Large-Scale Satellite Tagging Program. PLoS ONE **7**:e37216.

Sievers, U. 1989. Stomach content analysis in the harbour seal (*Phoca vitulina*) from the Schleswig-Holstein Waddensea. Zool. Anz. **222**:249–260.

Svensson, C. J. 2012. Seal dynamics on the Swedish west coast: Scenarios of competition as Baltic grey seal intrude on harbour seal territory. Journal of Sea Research **71**:9-13.

Thompson, D., P. Hammond, K. Nicholas, and M. Fedak. 1991. Movements, diving and foraging behaviour of grey seals (Halichoerus grypus). Journal of Zoology **224**:223-232.

Thompson, P. M., B. J. McConnell, D. J. Tollit, A. Mackay, C. Hunter, and P. A. Racey. 1996. Comparative Distribution, Movements and Diet of Harbour and Grey Seals from Moray Firth, N. E. Scotland. Journal of Applied Ecology **33**:1572-1584.

Tollit, D. J., P. M. Thompson, and S. P. R. Greenstreet. 1997. Prey selection by harbour seals, Phoca vitulina, in relation to variations in prey abundance. Canadian Journal of Zoology **75**:1508-1518.

Tougaard, J., I. Ebbesen, S. Tougaard, J. T., and J. Teilmann. 2003. Satellite tracking of harbour seals on Horns Reef. Technical report to Techwise A/S, Biological Papers from the Fisheries and Maritime Museum, Esbjerg.

Tulp, I., L. J. Bolle, and A. D. Rijnsdorp. 2008. Signals from the shallows: In search of common patterns in long-term trends in Dutch estuarine and coastal fish. Journal of Sea Research **60**:54-73.

van Neer, A., L. F. Jensen, and U. Siebert. 2015. Grey seal (Halichoerus grypus) predation on harbour seals (Phoca vitulina) on the island of Helgoland, Germany. Journal of Sea Research **97**:1-4.

Yodzis, P. 2001. Must top predators be culled for the sake of fisheries? Trends in Ecology & Evolution **16**:78-84.

Statement of originality

I, Camille de la Vega, declare that the research reported in this thesis is my original research. The research presented in Chapter 4 entitled "Relationships between fresh weight, dry weight, ash free dry weight, carbon and nitrogen content for selected vertebrates" was done in equal share with Sabine Horn. I effectuated the majority of the work presented in the chapters 1, 2, 3 and 5, with the support of the co-authors listed in each chapter. This thesis has not been submitted for any degree or examination at any other university or institution. This thesis does not contain other persons´ data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons. This thesis does not contain other persons´ writing, unless specifically acknowledge as being sourced from other researchers. Where other written sources have been quoted, their words have been re-written but the general information attributed to them has been referenced and where exact words have been used, their writing has been placed in italics and inside quotation marks, and referenced. This thesis does not contain text, graphics or tables copied and pasted from the Internet, unless specifically acknowledged, and the source being detailed in the thesis and in the References sections.