



PhD thesis

Julius Nielsen

The Greenland shark (*Somniosus microcephalus*)

Diet, tracking and radiocarbon age estimates reveal the world's oldest vertebrate

Academic supervisors

Professor John Fleng Steffensen, Senior Scientist Rasmus Berg Hedeholm, Professor Jørgen Schou Christiansen, Professor Peter Gerald Bushnell and Associate Professor Kim Præbel

Submitted: 30 November 2017

Table of contents

1. PREFACE.....	5
2. ACKNOWLEDGEMENTS	6
3. ENGLISH SUMMARY	8
4. DANSK SAMMENDRAG.....	9
5. KALAALLISUT ESIKKAANEQ	10
6. PROLOGUE	12
7. INTRODUCTION TO THE GREENLAND SHARK.....	15
7.1 Historical interactions with humans	15
7.2 Taxonomy	18
7.3 Distribution	19
7.4 Morphology and sensory organs.....	21
7.5 Body size	23
7.6 Reproduction.....	25
7.7 Migration.....	28
7.8 Diet	29
8. AGE INVESTIGATIONS.....	33
8.1 Age determination techniques on elasmobranchs	33
8.2 The eye lens as biogenic archive	35
8.3 Marine radiocarbon dating	37
8.3.1 Bomb radiocarbon dating.....	38
8.3.2 Pre-bomb radiocarbon dating	41
9. RATIONALE, HYPOTHESIS, AIMS.....	43
9.1 Rationale.....	43

9.2 Hypothesis	43
9.3 Aims	43
10. SUMMARY OF RESULTS.....	44
10.1 Manuscript 1 (MS1) – Feeding ecology.....	44
10.2 Manuscript 2 (MS2) – Tracking	45
10.3 Article 1 (A1) – Age	46
11. DISCUSSION	47
11.1 Rationale.....	47
11.1.1 The Greenland shark chronology	47
11.1.2 The carbon source of the eye lens nucleus Greenland shark chronology	49
11.1.3 Radiocarbon in the northern North Atlantic	50
11.1.4 Conventional use of bomb radiocarbon for age validation	52
11.1.5 Bomb radiocarbon dating of the Greenland shark chronology	52
11.1.6 The Bayesian model on the pre-bomb portion of the Greenland shark chronology.....	53
11.1.7 How to interpret calibrations.....	54
11.1.8 The challenge of reservoir age	54
11.2 The bomb pulse at living depth of the Greenland shark	55
11.2.1 Existing reference chronologies of dietary origin	55
11.2.2 The bomb pulse in deep sea and abyssal food webs	57
11.3 Weaknesses and potential improvements of the study	58
11.3.1 Calibration-associated weaknesses	58
11.3.2 Future improvements	59
12. CONCLUSION.....	62
13. PERSPECTIVES	63
14. REFERENCES	65
15. APPENDIX I	75
16. MANUSCRIPTS.....	76

1. Preface

This thesis has been submitted to the PhD School of The Faculty of Science, University of Copenhagen (Denmark) to obtain the PhD degree. The main part of this work has been carried out at the Marine biological section, Department of Biology, Faculty of Science, University of Copenhagen, Denmark with Professor John Fleng Steffensen as main supervisor. Several co-supervisors have also been involved: Senior Scientist Rasmus Berg Hedeholm from the Greenland Institute of Natural Resources (Greenland), Professor Peter Bushnell from the Indiana University South Bend (USA), Associate Professor Kim Præbel and Professor Jørgen Schou Christiansen from UiT The Arctic University of Norway (Norway).

The thesis consists of a general introduction to the Greenland shark and to marine radiocarbon dating - the age determination technique applied. Two unpublished manuscripts and one published article are included, as well as a discussion meticulously elaborating the theoretical rationale associated with applying radiocarbon dating on an animal living in the North Atlantic deep ocean. The manuscript on feeding ecology is to be submitted to *Polar Biology* and the manuscript on tracking is in preparation as tags are still deployed on sharks. The finding of great longevity of the Greenland shark was published as a report in *Science*, August 2016. In addition to the work presented here, I have been involved in and carried out several spin-off studies from the 310 sharks of which various data has been sampled during my PhD project. A full list of these studies (published and in preparation) is presented in Appendix I.

During my undergraduate and graduate years, I have become extremely fascinated by Greenland and the surrounding ocean. Each summer since 2009, I have worked as a student assistant and cruise leader onboard the RV *Pâmiut* for the Greenland Institute of Natural Resources. Here I encountered whales, birds, fish, crustaceans and squids that fascinated me deeply, and I realized that I wanted to specialize within something that could be studied from this unique platform. Greenland sharks became the topic of my research as a young researcher, but to be honest it could have been any of the intriguing creatures we pulled up from the deep. My future aim as a scientist is to keep doing research that makes it difficult for me to distinguish between work and hobby.

2. Acknowledgements

At first, I want to thank John Fleng Steffensen for giving me the opportunity for doing this Greenland shark project, which initially started as a master project in 2012, and continued with a PhD project in 2014. I am deeply grateful for the flexibility throughout the project for seeking new research directions and establishing new collaborations as the project developed. I also want to thank each of my co-supervisors who, at various times gave me great input via scientific discussions continuously challenging me. Thanks to Peter Bushnell and Jørgen Schou Christiansen for their extra contributions during the process of the Science review as well as all other co-authors to that article especially the “radiocarbon-guys” (Jan Heinemeier and Jesper Olsen from Aarhus University, Denmark and Christopher Bronk Ramsey from Oxford University, United Kingdom). Special thanks to Kim Præbel and Rasmus Berg Hedeholm, who during long stays in Tromsø and Nuuk not only invited me along various trips to experience the unique nature, but also into their homes. Your company has been enjoyed, and cherished friendships established. Jørgen and Rasmus also provided great input to this thesis in past months prior delivery which are much appreciated.

The collaboration with Rasmus and The Greenland Institute of Natural Resources the past five years has formed me as a biologist more than anything else. I am deeply grateful for this. None of the investigations presented in this thesis could have been made without their enormous help and generosity in all aspects of the process. Also thanks to Jacob Hjort Jørgensen and Karl Peter Lange, who at several occasions sampled valuable specimens for my studies. Thanks to Peter Rask Møller for helping with morphological investigations, field assistance and preservation of small Greenland sharks, as well as digging out relevant historical literature from the archives. I am also grateful for the support from Peter Gravlund in a very early stage of the project. Also thanks to Jørgen for inviting me onboard two TUNU expeditions, where valuable sharks were sampled. Thanks to Randi Ingvaldsen from Institute of Marine Research in Norway for letting me join the SI Arctic expedition in Svalbard and sorry for occupying the entire ship for 7 hours when catching 24 sharks on a single long line. Also thanks to Professor emeritus Bjørn Berland from Bergen (Norway) for unpublished material on reproduction of Greenland shark.

A special thanks to crew and scientific staff onboard RVs Pâmiut, Sanna, Avataq, Helmer Hanssen, Johan Ruud, and Porsild. Fieldwork conducted from these platforms has brought me friendships and unique experiences for which I have no words other than ‘thank you’. Also

thanks to Terje Hansen from Andørja Adventures in Norway, who has provided me with numerous unique opportunities associated with my shark research, and also invited me and my family into his home. I am glad to have your friendship. Many people have helped out in the field, with logistics or scientific contributions of various kind. In random order, thanks to: Lorenz Meire, Mia Thorning Christensen, Rasmus Nygaard, Helle Siegstad, Anja Retzel, Nannette Hammeken Arboe, Jørgen Sethsen, Jesper Boje, Henrik Lund, Kaj Sünksen, Jan Yde Poulsen, Bo Hansen, Peter Frandsen, Martin Nielsen, Ole A. Jørgensen, Tanja Hanebrekke, Shripathi Bhat, Apollo Mathiassen, Arve Lynghammer, Egil Ona, Ivan Tatone, Aintzane Santaquiteria, Mats André Hamnli, Thor-Eivind Flakstad, Flemming Heinrich, Rinch Ittu Heinrich, Birgir Sivertsen, Store-Jacob, Ole Jakob Petersen, Thomas K. Kristensen, Kristian Vedel, Marie Kanstrup, Ann Berit V. Jensen, Kirsten Andersen, Steen Honoré, Malene Simon and Sofie Jeremiassen. For those I may have forgot I apologize. I hope you know that I have appreciated your help anyway. The Greenland sharks project and my Phd project was funded by the Danish Council for Independent Research, Den Blå Planet – National Aquarium of Denmark, Greenland Institute of Natural Resources, the Commission of Scientific Investigations in Greenland (KVUG), Carlsberg Foundation, Save Our Seas Foundation, National Geographic Foundation and Danish Centre for Marine Research. I am grateful to each of these funds for their willingness to support a basic biology project like this.

Lastly, and most importantly, thanks to Marie for her endless support and input to each line that has been written in this thesis.

3. English summary

This PhD project has aimed at investigating longevity of the Greenland shark. The largest Greenland sharks measure at least 550 cm, and ever since Poul Marinus Hansen in 1963 presented that a recaptured medium-sized Greenland shark had grown 8 cm in 16 year, longevity of the species has been subject for speculation. Conventional age determination techniques for teleost or elasmobranchs are not applicable on the Greenland shark and its longevity has thus remained a mystery for decades.

Inspired by alternative age estimation techniques applied on other sharks and whales, I have used bomb radiocarbon dating and a Bayesian calibration model to estimate longevity of the Greenland shark. The analyzed tissue stems from the eye lens nucleus – unique material which presumably reflects age 0 of the shark, as it has not undergone metabolic changes during the animal's life. By studying 28 Greenland shark females between 81 cm and 502 cm, I estimate the oldest shark to be between 272 years and 512 years. With an estimated lifespan of at least 272 years, the Greenland shark is the longest living vertebrate animal in the world.

In order to produce these age estimates, it has been necessary to study the carbon source of the eye lens nucleus in more detail. The center of the nucleus consists of proteins and the analyzed tissue stems from the diet of the shark's mother. From feeding ecology and satellite tracking, I have therefore investigated adult females. Sharks of this life stage mainly occupied continental shelf waters in southern Greenland at depths between 200 and 550 m and fed primarily on cod, redfish and seals. From previous investigations of predatory sharks and whales in the north Atlantic, bomb radiocarbon has been widely applied, and I argue that a similar calibration approach is valid to use on the Greenland shark.

The main aim of this thesis is to clarify the biological assumptions behind the radiocarbon dating leading to the age estimates of the Greenland shark. These age estimates rest on classical biological feeding ecology studies, chemical isotope analysis and advanced mathematical modelling. This interdisciplinary approach has been crucial for the success of the project. The thesis also illustrates how a novel cross-combination of techniques can be applied on other marine species difficult to age determine, and how the Greenland shark is unique to the arctic ecosystem. Many aspects of the basic biology of the Greenland shark remain mysterious.

4. Dansk sammendrag

Formålet med dette Ph.d. projekt har været at undersøge, hvor gammel grønlandshajen kan blive. Grønlandshajen kan blive mindst 550 cm lang, og siden Paul Marinus Hansen i 1963 præsenterede genfangstdata fra en mellemstor haj, der over en tidsperiode på 16 år var vokset 8 cm, er der blevet spekuleret over den potentielle alder på de allerstørste grønlandshajer. Eftersom konventionelle aldersbestemmelsesmetoder til benfisk eller bruskfisk ikke er anvendelige på grønlandshajer, er grønlandshajens livslængde forblevet et ubesvaret spørgsmål.

Inspireret af alternative aldersbestemmelsesmetoder, anvendt på andre hajer og hvaler, har jeg i dette projekt brugt bombe kulstof 14-datering og Bayesisk kulstof 14-datering til at estimere grønlandshajens alder. Vævet, der analyseres, stammer fra centrum af hajens øjelinsekerne – et unikt materiale, der ikke har gennemgået stofskifteforandringer gennem dyrets levetid. Jeg har i alt undersøgt 28 grønlandshaj-hunner mellem 81 cm og 502 cm, og estimerer den ældste til at være mellem 272 år og 512 år gammel. Med en alder på mindst 272 år er grønlandshajen verdens længstlevende hvirveldyr.

For at kunne producere disse aldersestimater har det været nødvendigt at undersøge kulstofkilden til det analyserede væv fra øjelinsekernen. Øjelinsekernen består af proteiner, og det analyserede kulstof stammer fra den pågældende hajs moders diæt. Gennem fødeundersøgelser og satellitmærkning har jeg undersøgt netop de voksne grønlandshaj-hunner. Hunnerne opholdte sig hovedsageligt på kontinentalsoklen i Sydgrønland på dybder mellem 200 m og 550 m hvor de primært spiste torsk, sæler og rødfisk. For andre rovdyr i Nordatlanten med lignende diæt og rummelig udbredelse, har man klarlagt hvorledes kulstof 14 målinger kan kalibreres for at kunne omsættes til aldersestimater. Jeg argumenterer for, at en lignende kalibrering kan bruges på grønlandshajer.

Hovedformålet med denne syntese er at klarlægge de biologiske antagelser, som ligger til grund for kulstof 14-dateringen. Aldersestimaterne baserer sig på klassiske biologiske fødestudier kemisk isotopanalyse samt avanceret matematisk modellering. Denne tværfaglige tilgang har været afgørende for projektets succes. Afhandlingen illustrerer også hvordan en ny krydskombination af teknikker kan anvendes på andre marine arter samt at grønlandshajen er et unikt dyr i det arktiske økosystem. Grønlandshajens grundlæggende biologi er til stadighed indhyllet af stor mystik

5. Kalaallisut eqikkaaneq

Ph.d. nngorniutinni matumani siunertaavoq eqalussuit qanoq utoqqaatigilersarnerisa paasisaqarfiginissaat. Eqalussuup qanoq utoqqaatigilersinnaanera Paul Marinus Hansen-ip ukiuni 16-ini eqalussuarnik nalunaaqutsersuilluni pisareqqittarlugillu 1963-milli misissuinermini paasimmaguli ukiuni misissuiffigisamini misissukkami 8 cm-iinnarnik allisimanerat, taamanerniilli paaserusunneqalerpoq eqalussuit anginersuit 550 cm angullugu angissuseqartut qassinillimi ukioqarsimassanerat. Tassami uumasunik imarmiunik saanilinnik nataqqulinnillu utoqqaassusileeriaaseq nalinginnaasoq eqalussuarnut atorneqarsinnaasimanngilaq, taamaalillunilu eqalussuit uummartussusiat ukiorpassuarni paasineqarsinnaasimanani.

Eqalussuarnut allanut arfernillu utoqqaassusileeriaatsit periutsit nalinginnaannginnerusut akuerisaasullu isumassarsiorfigalugit suliniummi matumani kulstof 14 atorlugu kiisalu Bayesisk kulstof 14 atorlugu pisoqaassusiliisarneq nutaamik allanngortillugu periuseqarlunga eqalussuit arnavissat 81 cm-iniit 502 cm-inik takissuseqartut 28-it misileraaffigaakka. Misissuinnini ipiutaasartaq atugara eqalussuup tappiutaata qeqqarpiaaneersuuvoq – timip pisataani immikkooruteqarluinnartoq taamaalilluta misissuinitisinni atorparput, eqalussummi ukuini uumaffigisaani ipiutaasap taassumarpiaap allannguuteqanngivissimanissaa ilimanarmat. Misissuinitisinni eqalussuaq utoqqaanerpaatut ilimagisarput ukiut 272-it 512-illu akornanni utoqqaassuseqanissaa missiliuupparput, taamaalilluni eqalussuaq ukiunik 272-inilluunniit utoqqaassuseqaannaralaruni nunarsuaq tamakkerlugu uummasut nagguallit akornanni uummartunerpaatut oqaatigisariaqarpoq.

Uumasup misissuiffigisap utoqqaassusilerniarnerani ipiutaasap misissukkap suminngaanneersumik kulstoffertaqarnera ilisimaneqartariaqarpoq. Eqalussuup tappiutaata proteinertaqarpoq, taamaattumillu isaata tappiutaata ipiusaartaaniittut proteinit arnaaata nerisarisartagaaneersuusimassapput. Eqalussuit arnavissat nerisarisartagaat kiisalu qaamataasanut nassitsissutilersuinikkut misissorlugillu malinnaaffigaavut. Taakkua misissuinitta nalaani piffissap sivilunerpaartaani Kalaallit Nunaata kujataani immap naqqata imavissuarmut itiseriarnerata killingani itissutsini 200 aamma 550 meterini nassaasaanerupput. Takanani pingaartumik saarullit, puisit suluppaakkallu nerisarinerugunarsimavaat. Atlantikorsuup avannaata imaani kiisortut allat taama nerisaqarnerusartut imaanilu eqalussuartut pissusilersornerusartut kulstof 14-imik periuseq naapertorlugu misissueriaatsinik sanilliussisarlungi utoqqaassusiliiniarnernut atorneqarsinnaanerat paasineqarsimavoq. Uani

allaaserisami uanga tunngavilersorlunga saqqummiuppara, taama saniliussisarlunga periuseqarneq aamma eqalussuit utoqqaassusilerneqarneq atorneqarsinnaammat.

Uumassusilerinermi isummiuteriikkat kulstof 14-ip atorneqartarneranut tunngassuteqartut, kingornagullu eqalussuit utoqqaassusilerneqarneqarneq atorneqartartut paasiuminarsaaviginisaat ilisimatuussutsikkut allaaserisami Ph.D.-nngorniutinni matumani pingaarnertut anguniarpara. Ph.D.-nngorniutinni uumaasusilerinermi tunngaviusumik misissueriaatsiniit, isotopit atorlugit timip sananeqaataanik pisariusumik misissueriaatsit kingornagullu qarasaasiaq atorlugu naatsorsueriaatsinik ineriartortitsinerit pisariusorsuit aqqusaarlugit suliaara ingerlappara. Eqalussuup qanoq utoqqaatigilersinnaanerata paasiniarnerani ilisimatusariaatsinik assigiinngitsunik sammivilinnik iluaquteqarsimanasinnaaneq aaliangiisuulluinnarsimavoq. Suliami matumani aamma takuneqarsinnaavoq imaani uumasoaqatigiiaat utoqqaassusileruminaatsut paasisaqarfiginiarnerini periutsinik assigiinngitsunik atuisariaqarnerup pingaarutaa. Eqalussuup Issittumi uumasoaqatigiiaat ataqatigiiffianni immikkuullarilluinnartumik sulii qulaajaaffigineqanngitsumik tupinnartortalimillu inissisimasup uumasuussusianut tunngassuteqartutigut paasisaqarfiginerunnissaata pisariaqartuunera misissuinerma aamma takutippaa.

6. Prologue

In 1961 at the North Atlantic Fish Marking Symposium at Woods Hole (Massachusetts, USA), the Danish fishery biologist Poul Marinus Hansen presented data from the first tagging experiment of the Greenland shark (*Somniosus microcephalus*) conducted in Greenland waters. His research span from 1936 to 1949 and were conducted in western Greenland with the aim of investigating Greenland shark growth rates. Hansen managed to tag 411 Greenland sharks of which 28 were recaptured (Hansen 1963a). Unfortunately, Hansen never caught any of his tagged sharks himself and almost all of the recaptures were by local fishermen across Greenland (Hansen 1963a). Of the 28 recaptures, most were reported either without associated lengths, with negative growth increments or with increments so big that measurements were considered untrustworthy by Hansen. Therefore, length and associated growth increments are only reported from three animals of unknown sex. One shark measured 285 cm upon capture, and 14 years later the same shark was reported measuring 300 cm; a second shark measured 271 cm and after two years, it measured 272 cm; a third shark caught in 1952 was specifically highlighted by Hansen as it had been measured very reliably upon recapture by the station manager at the small settlement of Prøven in the Upernavik-area, northwestern Greenland. 16 years had passed since it had been tagged in 1936 in Uummannaq Fjord, northwestern Greenland. This animal had grown from 262 cm to 270 cm in the entire tagging period (i.e. 8 cm in 16 years). This was the only recapture measurement in which Hansen had full confidence and he points out that based on this one specimen, it seems that Greenland sharks are slow growing and potentially very long-lived. However, despite Hansen's great effort to investigate growth rate of Greenland shark, his conclusive remarks were that "*the results have been very disappointing*" and "*unfortunately very little progress has been made*" (Hansen 1963a). The idea of long-lived Greenland sharks has survived for half a century, and based on Hansen's data it has often been referred that Greenland sharks were likely to live for more than 100 years (e.g. Fisk et al. 2002, MacNeil et al. 2012). Ages of Greenland sharks have, however, been impossible to investigate due to lack of applicable age determination techniques, and the potential longevity of Greenland sharks has remained an unsolvable mystery.

The main objective for this PhD project has been to study this mystery, as it was hypothesized possible by Professor John Fleng Steffensen from the University of Copenhagen (UCPH) and Professor Jan Heinemeier from Aarhus University (AU) in 2009. Instead of using vertebra tissue (which is applied for age determination of several shark species), the suggested approach was to

analyze tissue from the eye lens. Heinemeier's research group had, in collaboration with Professor Niels Lynnerup from Section of Forensic Pathology (UCPH), demonstrated that radiocarbon levels measured in eye lens proteins of (dead) humans, could be used to estimate their year of birth quite accurately (Lynnerup et al. 2008). Heinemeier and Lynnerup had even been involved in solving a murder case, where forensic investigators wanted to determine time of birth for several dead babies found in a freezer in Germany (Lynnerup et al. 2010). Heinemeier and Steffensen thus expected that eye lenses of Greenland sharks might contain a similar radiocarbon signal, which could allow for age estimation.

The first Greenland shark eye lenses examined by Steffensen and Heinemeier were sampled in 2010 on the TUNU IV Expedition in northeastern Greenland (Christiansen 2012) and revealed that two medium-sized specimens of both sexes were remarkably old. More samples were indeed necessary, but Steffensen faced a challenge task of getting several samples of young, old and medium age Greenland sharks. It was therefore a perfect match when I was presented to these investigations in 2011, during a lecture with Steffensen at UCPH. At that time, I had spent each summer the preceding three years working as student assistant for the Greenland Institute of Natural Resources (GINR) onboard research vessel Pâmiut operating in offshore Greenland shelf waters. Here I had encountered many Greenland sharks and was therefore aware that they were caught as unintended "bycatch" in these annual scientific surveys. Via my master thesis project, collaboration between UCPH and scientists Rasmus Hedeholm and Malene Simon from GINR was established in 2012. From this point on, all sharks caught from GINR's fish surveys were collected for our research project on Greenland shark biology including age. The sampling program quickly expanded to also include specimens caught as bycatch from the commercial trawler Sisimiut and from local fishermen across Greenland as well as a targeted Greenland shark expedition with RV Dana in east Greenland in 2012. The main aim of the master project in 2012/2013 was to investigate Greenland shark eye lenses using two different chemical dating techniques. These were radiocarbon dating and aspartic acid racemization. Radiocarbon dating had previously been applied for age determination of other tissues from several marine animals (Kalish 1993, Campana et al. 2002). Aspartic acid racemization had been applied with success to estimate age of long-lived cetaceans such as bowhead whale (*Balaena mysticetus*), narwhale (*Monodon monoceros*) and fin whale (*Balaenoptera physalus*) (George et al. 1999, Garde et al. 2007, Nielsen et al. 2013) and might also be applicable to Greenland sharks. However, from the master project we learned that aspartic acid racemization was not a reliable technique to apply for age estimates of a cold ectotherm animal (Nielsen 2013). We further learned that the initial

analysis and theoretical rationale associated with radiocarbon dating needed to be more scrutinized, and that we needed a larger dataset to support fundamental assumptions and reduce the observed uncertainties for the preliminary results. Most importantly, we realized that in order to produce valid age estimates from radiocarbon dating we needed information on the carbon source of the analyzed tissue – that is the carbon source of the proteins embedded in the eye lens nucleus.

The carbon source turned out to be pivotal for the Greenland shark age investigations and research concerning this was to shape my entire PhD project.

7. Introduction to the Greenland shark

7.1 Historical interactions with humans

According to Inuit legend, the Greenland shark was created after a woman washing her hair in urine lost the cloth, she had used for drying her hair, into the ocean. The cloth sank to the bottom into the urine pot of the Inuit sea goddess Sedna (in Greenland known as Arnakuagsak). Sedna turned the urinated cloth into Skalugsuak, which was the first Greenland shark to swim in the oceans. Such stories of Greenland shark origin is not flattering compared to those of other marine animals, which according to legends were created from Sedna's fingers which were cut off by her father and after which she drowned. Interestingly, the story of the creation of Greenland sharks somewhat symbolizes the general despise of Greenland sharks among Inuit and fishermen. It is not an appreciated or respected animal. An anthropologic study by Idrobo (2008) investigated Pangnirtung Inuit's (from Baffin Island, Canada) relationship with the Greenland shark and states "*the shark is close to being non-existent in the Inuit oral tradition compared to marine and terrestrial mammals*", and continues "*for most hunters, the sharks are not considered interesting enough to make stories about*" (Idrobo 2008). Another example stems from the Inuit cosmology, where there is no recollection of shamans (or angakkoq) having Greenland sharks as guiding animals helping to communicate with the spiritual world, although most other marine and terrestrial animals have been helpers (Idrobo 2008). The neglect of Greenland sharks in Inuit folklore probably derive from the fact that they are not hunted for human food, but rather considered a pest when destroying fishing equipment and stealing the catch of fish and seals.

Greenland sharks have, however, been utilized in some Inuit societies, where the meat has served as dog food and cutting tools have been made from the razor blade sharp teeth (Fig. 1). Also the liver has been harvested in Greenland, Iceland and Norway due to its high content of oil which has been used to produce lamp oil, vitamin A and machine oil (Jensen 1914, Hansen 1963b). Trade records from Iceland go as far back as 1624 (Jónsson 1994)¹ and in the 17th and 18th

¹ This reference is based on trade with liver from the so-called *hakarl* which is the Icelandic name for Greenland shark and basking shark (combined). Whether this historical fishery mainly was on basking sharks or Greenland sharks is unknown.

century the shark fishery in the North Atlantic expanded and exports to Europe reached as much as 13,000 barrels of liver in 1867 (MacNeil et al. 2012). Trade records from Greenland reveal that from 1890 to 1938, annual Greenland shark landings averaged 44,000 animals (Anon 1942). This number is estimated from the number of barrels traded but due to big variation in liver mass among Greenland sharks (typically ranging from 15-100 kg but also as much as 270-300 kg Nielsen et al in prep1, Hansen 1963b), such estimations of shark landing numbers can only serve as very rough estimates. Nevertheless, there is no doubt that thousands of animals were harvested annually, and that Greenland shark was an important commercial species in Greenland until mid-20th century where the European demand for shark liver ceased completely with the invention of synthetic oils.

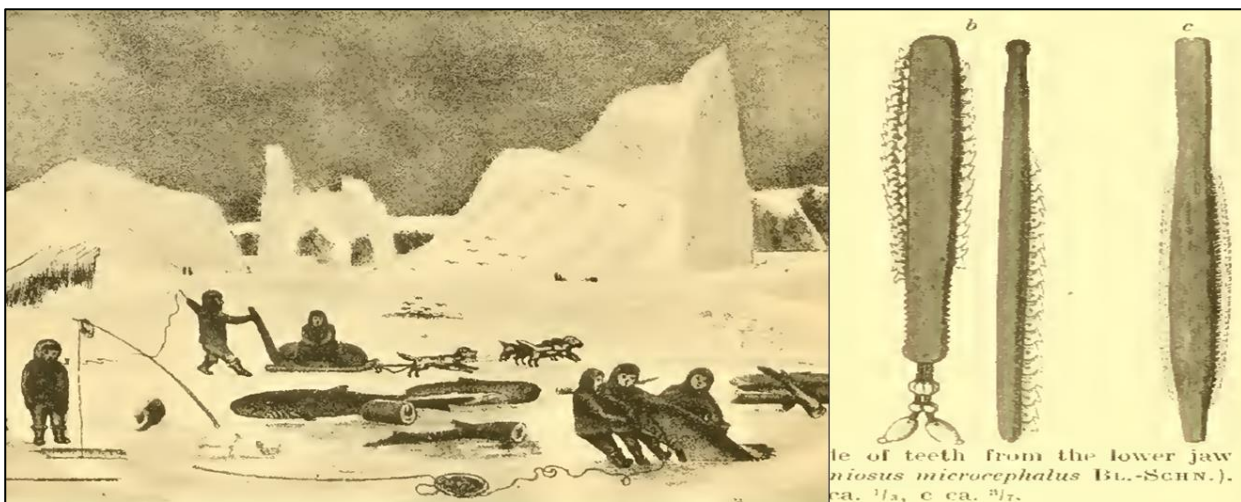


Figure 1. Historically, Greenland sharks have been caught by Inuits and used for dog food and for producing cutting tools from the sharp teeth (picture from Jensen 1914).

The reason why Greenland sharks have not been used for human consumption is a general belief that the meat is poisonous. This belief originates from observations of sledge dogs feeding on large amounts of fresh shark only to become so-called ‘shark drunk’ with symptoms like walking with slow stiff steps, hypersalivation, vomiting, explosive diarrhea, conjunctivitis, muscular twitching, upward and outward turning of the eyes, respiratory distress, tonic and clonic convulsions followed by death (Bøje 1939). These toxic effects have been suggested to be due to high levels of trimethylamine oxide (TMAO) (Anthoni et al. 1991). Interestingly, although levels of TMAO in Greenland sharks are reported as high, they are no higher than in other shark species (Anthoni et al. 1991). Therefore, the explanation why Greenland sharks are considered poisonous, while sharks in general are not, must be that throughout history only Greenland

sharks have occasionally been eaten in large amounts by hungry sledge dogs. If an average human were to consume amounts of Greenland shark meat large enough to experience being



Figure 2. (a) Leo Willy Christiansen in 1939 with a Greenland shark near Ella Ø, NE Greenland caught for feeding his sledge dogs (photo used with permission from Jørgen Schou Christiansen). (b) Bycatch of Greenland shark from commercial trawler in the northern North Atlantic (Photo: Henning Flusund, used with permission).

“shark-drunk”, one person would have to ingest around 20 kg over short period of time (Johansen 2002). When used as dog food in Greenland, the meat is dried, but this is not very commonly used anymore, simply because there are easier alternatives for dog food nowadays (Fig. 2a). Only in Iceland, Greenland shark serve as human food, but like the dog food in Greenland, the meat undergoes some process to detoxify, and the delicacy called *hakarl* is only consumed in relatively small amounts. Another unusual usage of Greenland shark is in the small settlement of Saattut in northwestern Greenland, where slightly ingested prey fishes from the stomach of bycatch Greenland shark are considered a delicacy (J. Nielsen pers. interview with Apollo Mathiassen, local inhabitant).

Nowadays, the greatest interaction between humans and Greenland shark is in arctic demersal commercial fisheries where Greenland sharks have been caught as unintended bycatch for decades. According to fishermen from trawlers in Greenland and Norway, they have all experienced catching 20-30 or even more Greenland sharks in a single trawl haul (pers. comm. with Captain Birgir Sivertsen of RV Pâmiut, Captain John Almestad of RV Helmer Hanssen and Captain Henning Flusund of F/TR Remøy) (Fig. 2b). The installation of sorting grids during 1990s decreased the bycatch problem (GINR unpublished data), and nowadays Greenland sharks are mainly caught as bycatch in longline and trawl fisheries for Greenland halibut (*Reinhardtius hippoglossoides*) (GINR unpublished data). According to Food and Agriculture Organization of the United Nations (FAO) annual bycatch of Greenland shark since the 1980s until present time averages 47 tonnes (SD=19) (FAO 2014). This number is most likely an underestimation.

7.2 Taxonomy

The Greenland shark (order Squaliformes) belongs to the family Somniosidae and the genus *Somniosus* where four more species have been identified. These are: Pacific sleeper shark (*S. pacificus*), little sleeper shark (*S. rostratus*), frog shark (*S. longus*), and southern sleeper shark (*S. antarticus*) (Yano et al. 2004). Little sleeper shark and frog shark represent the subgenus *Rhinoscyrnus* characterized by being much smaller than subgenus *Somniosus*, to which Pacific sleeper shark, southern sleeper shark and Greenland shark belong (*Somniosus* subgenus TL >400 cm vs *Rhinoscyrnus* subgenus TL <150 cm, Yano et al. 2004). The *Somniosus* subgenus further differs from *Rhinoscyrnus* by a range of morphological differences including higher number of tooth rows in lower jaw, hook-like dermal denticles (rather than leaf-shaped), more numerous spiral valves and higher vertebral counts (Yano et al. 2004). Within the two subgenera, species are (partly) separated by distribution area and morphology. For *Rhinoscyrnus*, little

sleeper shark is known from the Mediterranean and the northeastern Atlantic Ocean, whereas frog shark only has been encountered in the western Pacific Ocean, yet they have been considered the same species (Compagno 1984). Morphological differences between these species are associated with size of second dorsal fin and eyes as well as number of tooth rows and spiral valve count (Yano et al. 2004). For *Somniosus*, popular names are implying their respective distribution area as Pacific sleeper shark mainly is associated with the northern Pacific Ocean, Southern sleeper shark with the Southern Ocean and Greenland shark with Greenland waters as well as the remaining northern North Atlantic. Morphological differences between these species includes the distance from snout to first gill opening, placement and height of dorsal fins, as well as number of teeth in lower jaw tooth rows, number of spiral valves and vertebrae. However, no single characteristic is diagnostic within the *Somniosus* subgenus (Yano et al. 2004) making species separation complicated. The number of species and the validity of morphological characteristics to separate these species have been questioned specifically for the *Somniosus* subgenus (Benz et al. 2007). Murray et al. (2008) investigated variation in mitochondrial cytochrome b in the *Somniosus* subgenus and suggested that only two separate species exists, namely Greenland shark and Pacific sleeper shark. Recently, these species have been found to hybridize (Hussey et al. 2014, Walter et al. 2017), which further complicates species separation based on morphological characteristics. All combined, more thorough DNA analysis based on full mitogenomes and nuclear genomes are necessary to properly elucidate the correct number of sleeper shark species worldwide and to determine if morphological differences are usable to distinguish between species or if they are a result of phenotypic plasticity. Currently, all five sleeper shark species are listed on the IUCN Red List of Threatened Species as “Data Deficient” except the Greenland shark which is “Near Threatened” (IUCN 2017).

7.3 Distribution

As the name implies, Greenland sharks are distributed throughout Greenland coastal and offshore waters (Nielsen et al. 2014). Greenland sharks are reported from the Kap Farvel area in the south and the northernmost records are from Wolstenholme Fjord (76°N) in northwestern Greenland and from Peters Bugt (75°N) at Hochstetter Foreland in northeastern Greenland (Yano et al. 2007, Møller et al. 2010, Nielsen et al. 2014, Nielsen et al. in prep2). In Canada, Greenland sharks are reported throughout arctic Canada from Resolute Bay to Jonas Sound south of Ellesmere Island to Ungava Bay in the Hudson Strait (Templeman 1963, Beck & Mansfield 1969, Hussey et al. 2014). Greenland sharks have also been reported from offshore Newfoundland and Gulf of St Lawrence in southern Canada and from Gulf of Maine and Cape

Cod in northeastern USA (Bigelow & Schroeder 1948, Templeman 1963, Harvey-Clark et al. 2005; Campana et al. 2015a). Greenland sharks are also distributed throughout Iceland shelf waters and further across the northern North Atlantic throughout the Barents Sea including Svalbard coastal and offshore waters (McMeans et al. 2010, Fisk et al. 2012, Rusyaev & Orlov 2013). The northernmost report of a Greenland shark is from 82°N (Fisk et al. 2012). Greenland sharks have also been reported from the Russian arctic as far east as the Kara Sea and Laptev Sea (78°N, 133°E) (Chernova et al. 2015). In Norwegian mainland waters, Greenland sharks are reported from multiple deep fjords (Nielsen et al. in prep2) and occasionally also from Faroe Islands (Faroe Marine Research Institute unpublished data, Koefoed 1957). Greenland sharks have also been documented as a relatively normal catch by recreational anglers fishing in Swedish waters of Skagerrak (Nielsen et al. in prep2). From the available data (Fig. 3) it is likely that neither the true northern, eastern, western nor southern limit for Greenland shark distribution is documented. Greenland sharks are likely to be distributed throughout the Arctic and throughout the Arctic Ocean and the deep sea of the North Atlantic.

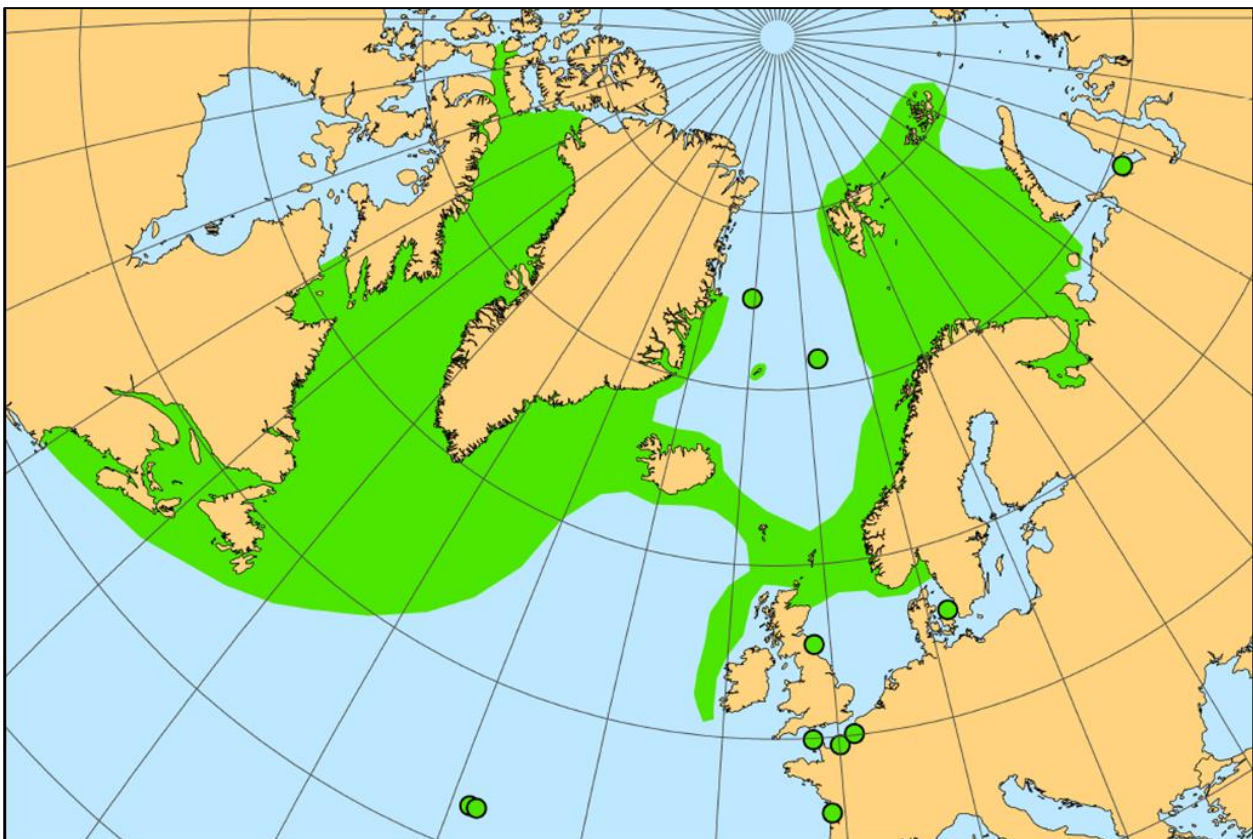


Figure 3. Distribution map of Greenland shark in the northern North Atlantic. Green dots represent punctual observations (Mecklenburg et al. in prep, Pan-Arctic Atlas of Marine Fishes).

7.4 Morphology and sensory organs

The body shape of Greenland shark is cylindrical and elongated with rounded snout. Fins are relatively small and soft as well as the musculature causing the shark to appear flaccid in body structure. The mouth is partly protrusible with labial furrows being visible when open. The gape is characterized by being circular with small pointy teeth in the upper jaw and small razor blade-like teeth in the lower jaw.

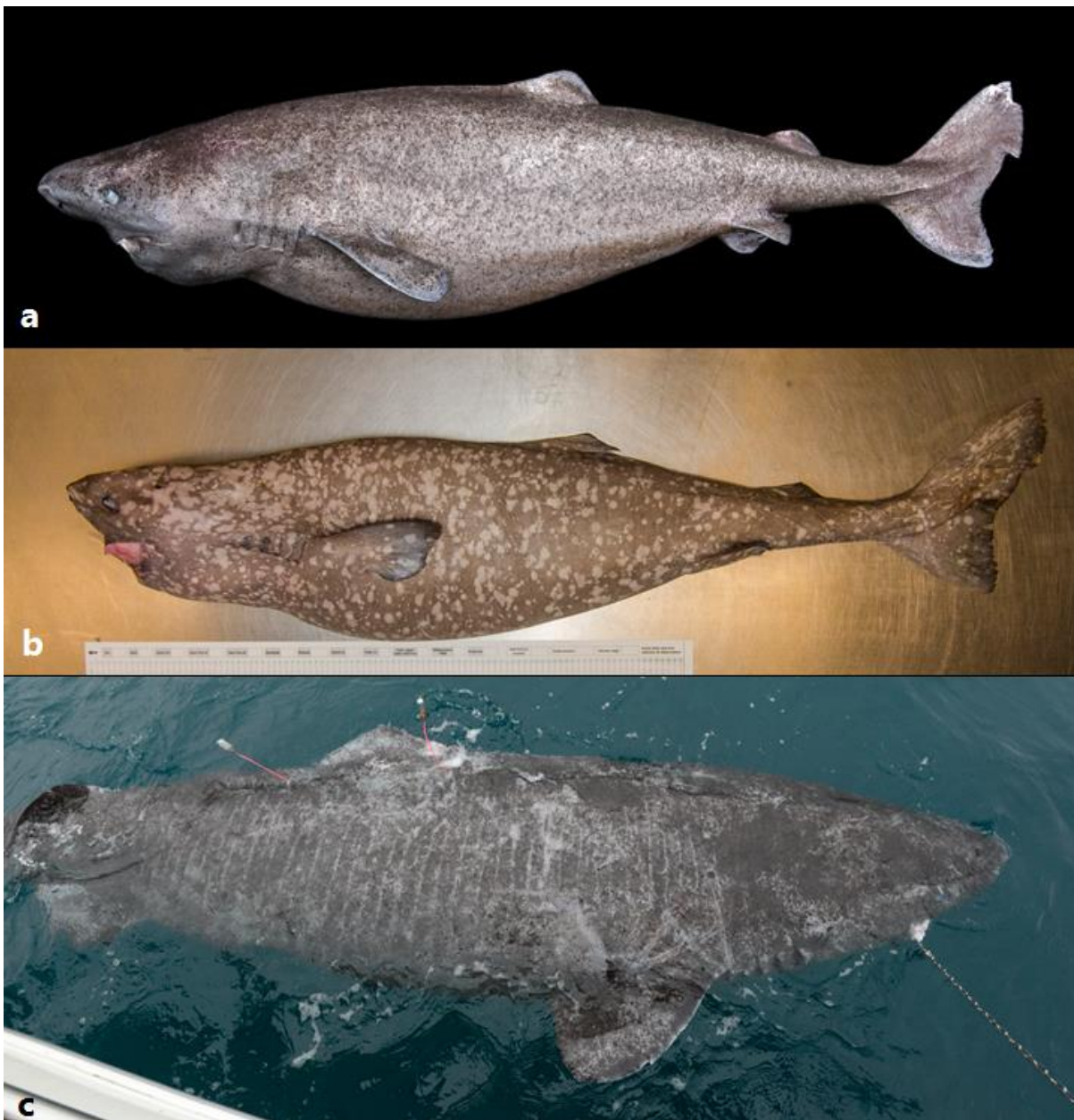


Figure 4. (a): 271 cm male with typical greyish colour from northeast Greenland, TUNU VI. (b): 164 cm male with unusual brownish coloration with multiple light spots from southeast Greenland (this specimen been preserved in the collection of the Natural History Museum of Copenhagen. (c): 410 cm female (tagged with two pop-off satellite tags) with dark grey colour and light transverse bands along body from Andørja, northern Norway (Photo: Julius Nielsen).

The body shape of Greenland shark is cylindrical and elongated with rounded snout (Fig. 4). Fins are relatively small and soft as well as the musculature causing the shark to appear flaccid in body structure. The mouth is partly protrusible with labial furrows being visible when open. The gape is characterized by being circular with small pointy teeth in the upper jaw and small razor blade-like teeth in the lower jaw. All sizes smaller than 400 cm total length (TL) have similar body shape, whereas some specimens longer than 400 cm can be distinctively more dense and plump. For all sizes, pre-dorsal length is typically below 44% of TL (Yano et al. 2004). The body is covered with relatively large dermal denticles except for the tip of the snout and the leading edge of the fins. The front edges of pectoral, pelvic fins and sometimes the pre-dorsal cartilage ridge as well as lower part of caudal fin, are smoothed and white in color. The caudal peduncle has a small keel. Skin coloration is normally light or dark grey with few darker spots or transverse patterns along the body (Fig. 4a-c). For some specimens the color can appear dark almost black or brown. Rarely, pale or white specimens are observed as well as specimens with large black or white spots all over the body (Jensen 1914, Nielsen unpublished data) (Fig. 4b).

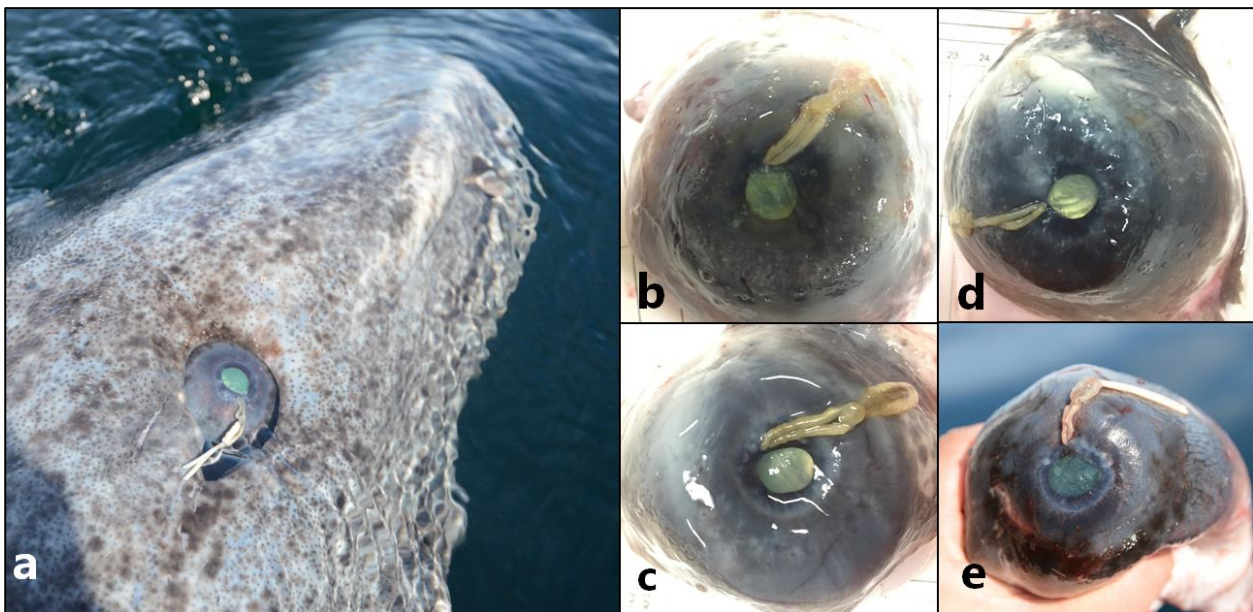


Figure 5. (a): Greenland shark eye in live animals before release. (b-e): Dissected eye globes. For all eyes notice infection of the parasite and transparency of the cornea which appear green in colour due to colour of the retina. Corneal damage is not as severe as often described in the literature (Photo: Julius Nielsen).

The head region for Greenland shark, as for all shark species, is equipped with sensory pores and ampullae Lorenzini widely spread around the snout, the mouth and on top of the head. Sensory pores are smaller than ampullae Lorenzini and run along the lateral line and are likely connected to small vertical chaps of approximately 0.5-1 cm, which have been observed in several specimens (J. Nielsen pers. obs.). The exact function of these chaps is unknown. The nostrals are

large and the olfactory sense is well-developed (Ferrando et al. 2015), and chemoreception via olfactory rosettes is expected to be of main importance for foraging and navigation. The visual capabilities are expected to be limited due to the common infection by the parasitic copepod *Ommatokoita elongata* attaching to the cornea of the shark's eye which (Borucinska et al. 1998) (Fig. 5a). Such infections are also observed with the Pacific sleeper shark (Benz et al. 2002). The parasite typically measures 4-5 cm in length, and 98.9% of 1,505 sharks from Greenland waters were found to be infected in either one or both eyes (Berland 1961). In the St. Lawrence Estuary (Canada), Greenland sharks are reported not to be infected with the parasite, and it has been suggested that Greenland sharks have active vision even if infected by parasites (Harvey-Clark et al. 2005). From personal observations of more than 200 Greenland sharks, it is clear that severe corneal damages due to parasitic infections (as described by Borucinska et al. 1998 and Benz et al. 2002) are not common for Greenland sharks in Greenland or Norwegian waters, although frequency of parasitic infections is similar to that reported by Berland (1961). Greenland shark eyes normally appear green (with and without parasitic infection) and hence light must penetrate through the cornea to retina (which is green in coloration) (Fig. 5b-e). Therefore, Greenland shark eyes must at minimum function as light sensors. Furthermore, the eyes are possibly more important as sensory organs than commonly believed although vision hardly is a central sense in the deep ocean.

7.5 Body size

The largest reliably measured Greenland shark from verified sources is a female of 516 cm fork length (FL) (Campana et al. 2015a), which equals an estimated total length (TL) 550 cm (Nielsen et al. 2014). Male sharks reach a smaller size than females, and the largest male measured is a specimen of 375 cm TL (Nielsen et al. in prep2). The heaviest Greenland shark weighed on a scale was 1,100 kg and measured 447 cm TL (Nielsen et al. 2014).

Normal size ranges of Greenland sharks are difficult to establish as shark sizes vary between geographical regions (Nielsen et al. in prep). For example, sharks from Svalbard and arctic Canada are generally smaller than 350 cm TL (Leclerc et al. 2012, Fisk et al. 2012) whereas the majority of more than 100 sharks encountered in southwestern Greenland are longer than 400 cm TL (Yano et al. 2007, Nielsen et al. 2014, Nielsen et al. in prep2). In the scientific literature, Greenland sharks are often reported to reach potentially 730 cm TL or at least 640 cm (e.g. Compagno 1984, Fisk et al. 2002, MacNeil et al. 2012, Nielsen et al. 2014). However, when scrutinizing the original references, the reports turn out as questionable. The most common

reference for these large sizes is Bigelow & Schroeder (1948) which reads: “It (the Greenland shark) has been said to reach a length of 24 feet (7.3 m), but few, if any, actually grow to so great a size, for the longest of which we find definite record was 21 feet (6.4 m), with specimens of 16 to 18 feet (4.9-5.5 m) unusual”. As stated, the 7.3 m specimen is not considered a valid observation and should not serve as such before being verified from other observations. The “definite record” for the 6.4 m specimen is from the book Fishes of the British Isles (Jenkins 1903) and this report is presented together with associated information on capture location and body mass (May Island in Scotland in 1895, body mass 27.5 cwt equaling 1,397 kg). According to Greenland shark length-weight relationships, a shark of 640 cm TL would have a body mass somewhere between 2,600-2,700 kg (Yano et al. 2007, Nielsen et al. 2014). On the other hand, a basking shark (*Cetorhinus maximus*) of 640 cm would weight approximately 1,300-1,400 kg (Froese & Pauly 2017). Furthermore, basking sharks are common in British coastal waters (Jenkins 1903, Compagno 1984) and although there are very distinct morphological differences between basking shark and Greenland shark, there are also similarities which at first glance could allow for misidentification (Fig. 6).



Figure 6. A basking shark caught as unintended bycatch in Iceland waters. Despite distinct morphological differences between basking sharks and Greenland shark, there are also similarities which could lead to misidentification (Photo: Thorfinnur Petur, www.flickr.com).

Examples of misidentification between Greenland sharks and basking sharks among fishermen are not uncommon in local media from Norway, Iceland and Denmark². I therefore stress that the 122-year-old capture report of a 640 cm Greenland is dubious and very likely to be either a smaller Greenland shark matching with the reported weight, or more likely a 640 cm basking shark with correctly reported weight. Nonetheless, it must also be mentioned that multiple fishermen from longline boats and trawlers, all remember “the one Greenland sharks that must have been more than six meters because it was enormous” (J. Nielsen interview with multiple fishermen throughout the Arctic). Unfortunately, common for all such observations is that length was estimated and no pictures were taken.

7.6 Reproduction

Knowledge on Greenland shark reproductive biology is extremely limited. Only one pregnant Greenland shark female from offshore Faroe Islands has ever been reported (Koefoed 1957). This female bore 10 near-term same-sized fetuses of 37 cm TL revealing that the Greenland shark is live-bearing like other Squaliformes³. Another female from Nordfjord (Norway) was pregnant with one pup measuring 100 cm (Bjerkkan (1957), but this specimen was only inspected by the fishermen catching the shark (not Bjerkkan himself) before the fetus was used as dog food. Birth size of Greenland shark is therefore suggested to be 40-100 cm TL (MacNeil et al. 2012). However, it is unlikely that Greenland sharks produce pups which can vary 150% in length at birth which also corresponds to variation in body mass of approximately 2,000%. Such variation in birth size implies very different reproduction strategies and hence it is an unrealistic range (Nielsen et al. in prep1). The smallest free-swimming Greenland sharks reported measure 41.8 cm, 45 cm, 46 cm, 46.7 and 55 cm (Bigelow & Schroeder 1948, Kukuev & Trunov 2002) (Fig. 7). Hence, the most likely birth size of Greenland shark is approximately in the 40 cm size class (Nielsen et al. in prep1).

² See <https://www.nrk.no/ostlandssendingen/juletorsken-var-hai-1.1328871>. An article from a local norwegian media where a fisherman catches a basking shark but at first glance thinks it is a Greenland shark. The mistake was quickly corrected.

³ Only one pup was inspected by Koefoed and later preserved at Aalesunds Museum (Norway). Unfortunately this specimen has been lost at the museum (J. Nielsen pers. comm. With Aalesund Museum)

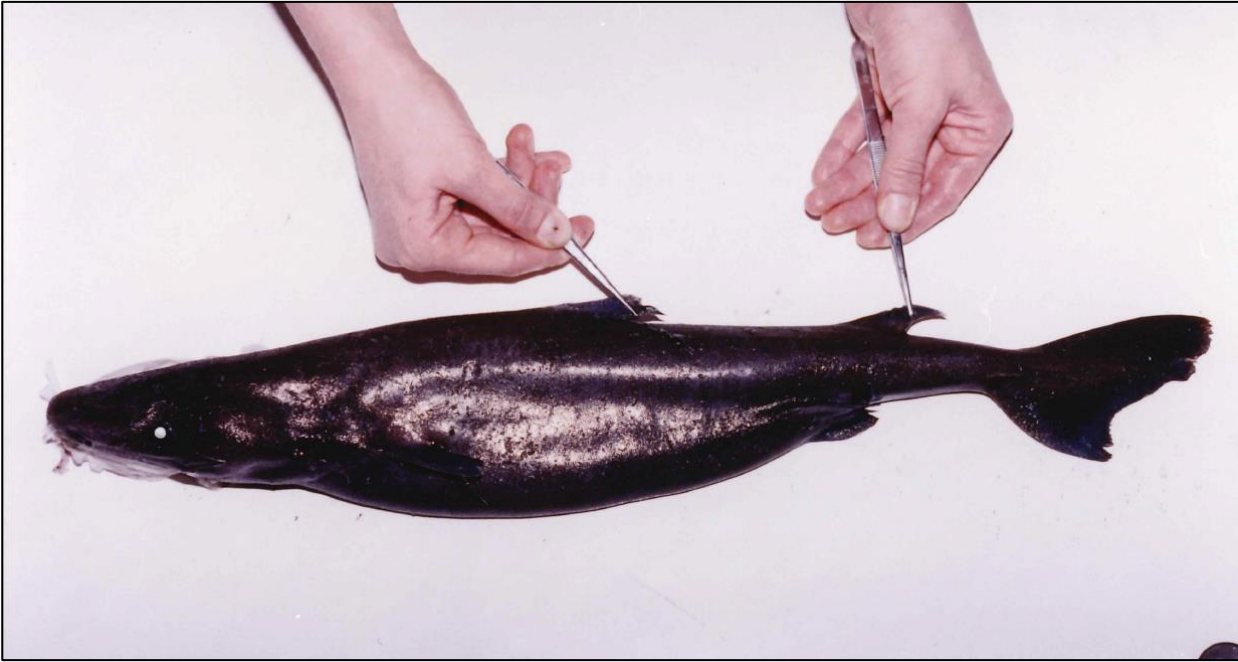


Figure 7. Juvenile Greenland shark from Reykjenes Ridge of 55 cm TL (Photo: Asgeir Gunnarson, used with permission) (Jacobsdóttir unpublished data,).

During sampling for this PhD project, I made multiple observations on sexual maturation of males and females, and unpublished data on ovary mass, liver mass, ova size and size of uterus reveal a TL_{50} for males of 295 cm TL and 398 cm TL for females (Nielsen et al. in prep1). These estimates fit well with Yano et al. (2007) who reported that males reach sexual maturation at approximately 300 cm and females above 400 cm. During the PhD project, females containing as much as 455 (49 kg) and 649 large yolky ova (80 kg) have been observed (Fig. 8). From comparison with closely related species of the family Somniosidae and other families within the order of Squaliformes, it is evident that number of ova on ovaries (ovarian fecundity) and number of pups in the uteri (uterine fecundity) can vary but this variation is in the range of 0-40% (Yano 1995, Clarke et al. 2001, Jones & Ugland 2001, Veríssimo et al. 2003, Figueiredo et al. 2008). Therefore, observations of high ovarian fecundity in Greenland sharks (which also have been reported in the historical literature, see Bjerkan 1957) indirectly suggest that uterine fecundity of Greenland shark females is much higher than 10 pups as observed by Koefoed (1957). Reproductive strategies like oophagy or sibling cannibalism are not likely for a Squaliform shark (which normally only have minimal nutritional input during fetal development, Nielsen et al. in prep1). I therefore hypothesize that uterine fecundity is at least hundreds of pups for the largest females.



Figure 8. (a+b): Greenland shark female of 474 cm found with an ovarian fecundity of 649 ova, which measured 5-6 cm in diameter and had a mass of 80 kg in total (Photo: Julius Nielsen).

7.7 Migration

Previous tracking studies of Greenland sharks from Svalbard, Greenland and Arctic Canada do not include sexual mature females and only three tagged females off Newfoundland have been large enough for potentially being sexual mature. The first of such investigations was conducted by Hansen 1963a and showed that Greenland sharks indeed are capable of migrating over long distances with specimens being recaptured as far as 1,126 km from the tagging location after 7-8 years. However, most of the sharks reported by Hansen remained within a range of 350 km even for as much as 16 years after tagging (Hansen 1963a). Following this, Greenland shark migration behavior has not been studied for decades until Skomal & Benz (2004) tracked six sharks (190-355 cm fork length, FL) from the sea ice for up to 72 hours using ultrasonic telemetry in Baffin Islands (Canada). Interestingly, sharks came as close as 11 m to the surface, and generally remained at shallower depths (<150 m) during night time whereas deeper waters (>150 m) were occupied during day time (Skomal & Benz 2004). Similar diel movement patterns were reported of two sharks (235-270 cm TL) in the St. Lawrence Estuary (Canada) tagged with an acoustic tag and a pop-off satellite archival tag (PSATs) for 47 and 40 days, respectively (Stokesburry et al. 2005). In the same area, a third shark tagged with a PSAT remained at depths 325-350 m without diel swimming patterns (Stokesburry et al. 2005). PSAT investigations on multiple sharks at Svalbard (Norway), Baffin Island (Canada) and off Newfoundland (Canada) all concluded that sharks exhibited no diel depth differences (Fisk et al. 2012, Campana et al. 2015a).

The 14 sharks tagged with PSATs at Svalbard (275-365 cm TL) were monitored for periods between 8-196 days and were found mainly to occupy depths between 50-150 m (Fisk et al. 2012). One specimen was reported as deep as 1,560 m and several sharks were found to ascend to the surface (0-8 m of depth) during the tagging period (Fisk et al. 2012). Migrating distance showed great variation with one shark moving 85 km within 97 days and another as much as 980 km within 59 days (Fisk et al. 2012). However, there was an overall positive relationship between days-at-liberty (DAL) and migration distance (Fisk et al. 2012). A similar positive relationship was observed for 10 sharks tagged with PSATs in the Canadian Arctic (243-325 cm FL) where several animals were found to migrate 1,240-1,615 km for periods of 125-187 days (Campana et al. 2015a). These sharks occupied deeper water masses (300-400 m) than the Svalbard sharks. Four sharks tagged off Newfoundland (376-516 cm FL) were found mainly to

occupy 800-1,100 m of depth and within 148-193 days they migrated 735-1,505 km (Campana et al. 2015a). Pop-off location from these sharks were over abyssal water (>3,000 m depth) evidencing that they must have been swimming in the pelagic⁴ for at considerable amount of time (Campana et al. 2015a). From all tagging studies performed there is no information on Greenland shark group migration or any specific migration patterns. The deepest record from PSATs of a Greenland shark is 1,816 m (Campana et al. 2015a) yet they are capable of occupying even deeper water masses as also suggested by observations of *Somniosus* spp. at 2,200 m and 2,647 m from remote operated vehicles (ROVs) (Herdendorf & Berra 1995, Benz et al. 2007). The deepest record of Greenland shark is 2,909 m from the mid-Atlantic ridge (Porteiro et al. 2017). All combined, tracking studies of Greenland sharks have shown that this species is capable of moving over long distances (>1,000 km) within relatively short time (few months) and that all water masses from deep ocean to the surface are occasionally occupied, yet the sharks predominantly resides in continental shelf waters.

7.8 Diet

Several studies have investigated feeding ecology and ecological role of Greenland shark in Canada, Greenland, Iceland and at Svalbard. Common for analyses based on isotope and fatty acids, is that Greenland shark is placed at high trophic levels deriving most of its carbon from pelagic food webs (McMeans et al. 2010, Hansen et al. 2012, McMeans et al. 2013). Such diet is supported by stomach content analyses as main prey items are found to be relatively large specimens of various demersal or epibenthic fishes as well as pinnipeds (seals) (Yano et al. 2007, McMeans et al. 2010, Leclerc et al. 2012, Nielsen et al. 2014). Species composition does, however, vary between regions. At Svalbard main prey items are Atlantic cod (*Gadus morhua*), ringed seal (*Pusa hispida*), Atlantic wolffish (*Anarhichas lupus*) and haddock (*Melanogrammus aeglefinus*) (Leclerc et al. 2012). In Iceland, the diet is heavily dominated by redfish (*Sebastes* spp.) followed by Atlantic cod, cetaceans and ling (Lotidae) (McMeans et al. 2010). In Greenland, main dietary components are Atlantic cod, Greenland halibut, harp seal (*Pagophilus groenlandicus*) followed by boreoatlantic armhook squid (*Gonatus fabricii*), skates (Rajidae) and spotted wolffish (*Anarhichas minor*) (Yano et al. 2007, Nielsen et al. 2014) (Fig. 9). These

⁴ Depth track of these sharks showed that the sharks did not follow the bottom which at time of release was at a depth of >3,000 m. Crushing depth for PSATs is ~2,000 m.



Figure 9. Prey items found in Greenland shark stomachs. (a) Two juvenile harp seals of 14 and 19 kg. (b) Greenland halibut with hook in its mouth evidencing that this was “stolen” from a long line. (c) Atlantic cod found in one stomach from shark in caught in a school of cod (Photo: Julius Nielsen).

different geographical regions obviously have varying fish fauna and hence varying prey availability for the Greenland shark, which indeed explains some of the variation in prey compositions between studies. However, it is interesting that, for example in western Greenland, redfish had no importance as prey item (Yano et al. 2007, Nielsen et al. 2014) despite having a very high biomass in Greenland shelf waters (ICES 2017). This indicates some level of prey selectivity.



Figure 10. (a) The characteristic circular gape of a Greenland shark (Photo credit: Julius Nielsen). (b) Circular wounds on free-swimming alive beluga whales presumably inflicted by a Greenland shark (picture from MacNeil et al. 2012). (c) Circular pieces of seal skin, blubber, meat and bones from harp seal. Found inside stomach of a Greenland shark (Photo: Julius Nielsen).

While the high trophic position is well established, it is more complicated evaluating whether the Greenland shark mainly feeds as scavenger or as active hunter. Certainly, these sharks are opportunistic and will feed on dead animals whenever possible (Leclerc et al. 2011), but circumstantial evidence suggests Greenland sharks as active hunters too. For example, freshly

ingested entire seals or seal parts have been observed without secondary prey items like scavenging crustaceans (e.g. amphipods and crabs), echinoderms (e.g. brittle stars, sunfish) or hagfish (*Myxine glutinosa*). Such scavenging fauna would be expected in the stomach of the shark if the seal or fish had been eaten as a carcass from the ocean floor. Scavenging fauna is observed but not frequently compared to freshly ingested fish and seals (Leclerc et al. 2012, Nielsen et al. 2014, J. Nielsen pers. obs.). Furthermore, Greenland shark's diet is predominantly composed by epibenthic and demersal fishes and the little importance of more pelagic species, such as redfish, capelin (*Mallotus villosus*), Atlantic herring (*Clupea harengus*), Atlantic mackerel (*Scomber scombrus*) and blue whiting (*Micromesistius poutassou*), further supports the hypothesis of active hunting along the ocean floor rather than random scavenging of food falls. Active predation hypothesis is also supported by two observations of Greenland sharks being caught presumably inside large schools of Atlantic cod (Nielsen et al. 2014). Lastly, although rarely, characteristic circular bite marks on alive and free-swimming beluga whale (*Delphinapterus leucas*) and seals suggest that despite its sluggish swimming behavior, Greenland shark's hunting techniques must allow for catching live prey⁵ (Idrobo & Berkes 2012, MacNeil et al. 2012) (Fig. 10). The designation as opportunistic predator seems appropriate for the Greenland shark.

⁵ The suggestion of Greenland shark is the 'Corkscrew killer' (Lucas & Natanson (2010) is refuted by Gallant (2010) and Bexton et al. (2012). From personal observations of stomach content of +100 Greenland sharks, I have not found corkscrewed seal skin pieces.

8. Age investigations

Age determination of fish is essential for stock assessments and management of commercial fish species, where it is used to describe mortality and growth in age-structured population models (Beverton-Holt 1957). Age determination is also important for non-commercial species, for example to evaluate a species' vulnerability and need for conservational management. The Greenland shark is currently listed as 'Near Threatened' in the IUCN Red List of Threatened Species and as 'Data Deficient' in the Norwegian Red List (IUCN 2017, Henriksen & Hilmo 2015).

8.1 Age determination techniques on elasmobranchs

The reason why no age estimates have been produced for Greenland shark is, that well-established age determination techniques of fishes are not applicable to this particular species (nor to other members of the genus (Kyne & Simpendorfer 2010, Matta et al. 2017)). The age of teleost fishes is normally determined from otoliths – hard calcified structures where yearly growth layers are deposited comparable to growth rings in a tree. Elasmobranchs (i.e. sharks, skates, rays and chimeras) do not have otoliths and it is in general more difficult to investigate age. As for teleosts, age investigations of elasmobranchs rely on identifying hard tissues in which growth layers are deposited. For elasmobranchs such as porbeagle (*Lamna nasus*) and white shark (*Carcharodon carcharias*), age estimates have been produced from growth layers deposited in calcified parts of their cartilage vertebra (Fig. 11a) (Campana et al. 2002, Hamady et al. 2014). Other sharks such as spiny dogfish (*Squalus acanthias*) deposit growth layers in hard dentine-made dorsal fin spines (Campana et al. 2006) or caudal thornes and vomerine tooth plates which have been used to produce age estimates of thorny skate (*Amblyraja radiata*) and spotted ratfish (*Hydrolagus colliei*), respectively (Fig. 11b-d) (Gallagher & Nolan 1999, King & McPhie 2015).

To study Greenland shark longevity, I have used radiocarbon dating of the eye lens nucleus. This is a novel combination of known technique from other studies. The applicability of the eye lens as a biogenic archive of vertebrates has been demonstrated previously (George et al. 1999), and marine radiocarbon dating has been applied for age studies of large predatory fish and whales (Stewart et al. 2006, Hamady et al. 2014). The cross-combination between radiocarbon dating and the eye lens has been applied on humans previously (Lynnerup et al. 2008, Lynnerup et al. 2010) but not previously for marine animals of unknown age.

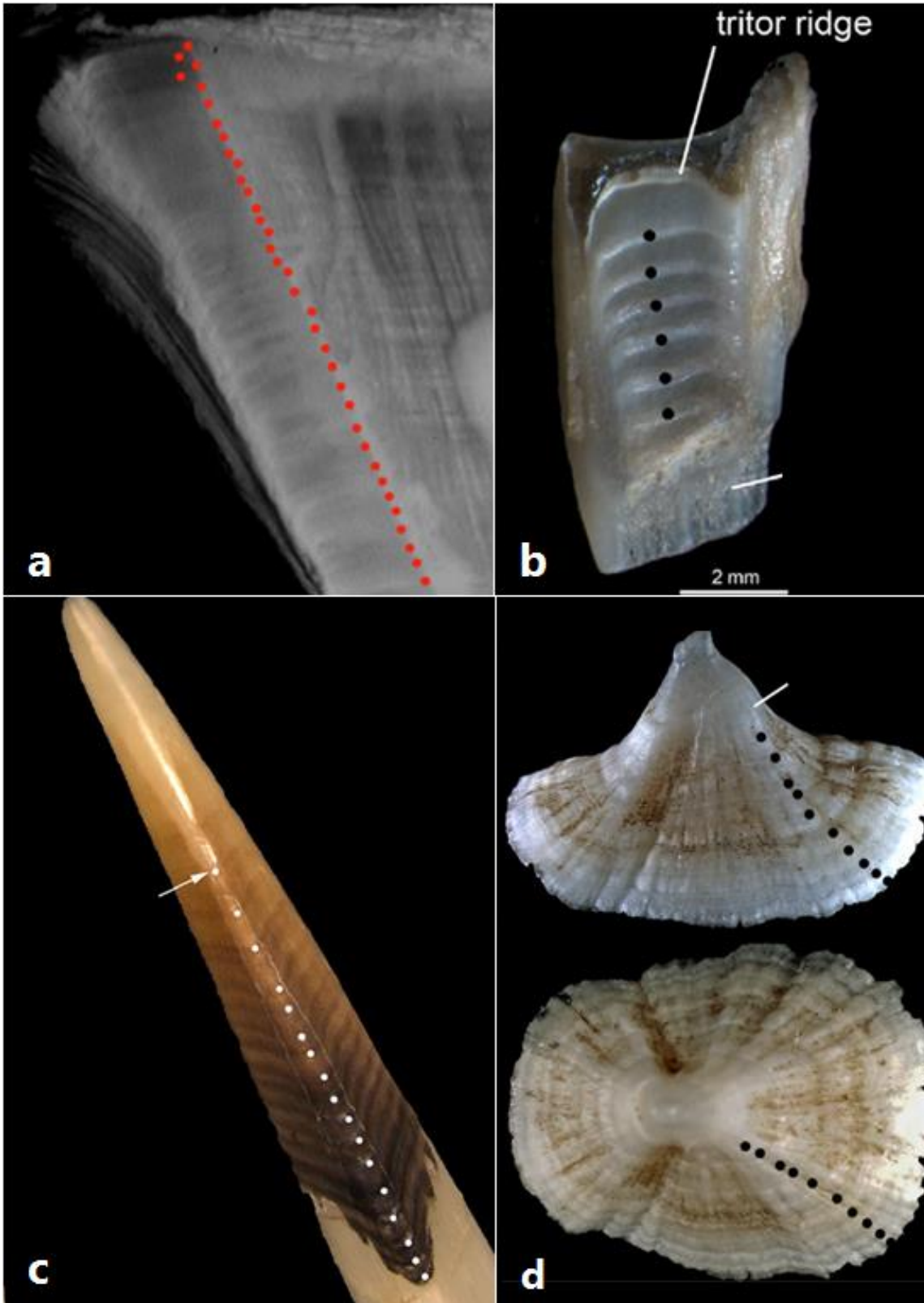


Figure 11. Growth bands in various hard tissues (a) Upper section of vertebra from white shark (Hamady et al. 2014). (b) Vomerine tooth from spotted ratfish (King & McPhie 2015). (c) Dorsal fin spine from north pacific spiny dogfish (*Squalus suckleyi*) (Matta et al. 2017). (d) Caudal thorns from Alaska Skate (*Bathyraja parmifera*) (Matta et al. 2017). Black, white and red dots represents yearly deposited growth bands.

8.2 The eye lens as biogenic archive

Tissue from the eye lens has been applied to study life span of several difficult-to-age species – especially baleen whales which are lacking teeth or other hard structures and hence difficult to age. Normally, the youngest specimens of various baleen whales can be age determined from counting of growth layers in the earplugs (Lockyer 1972). For older individuals, growth bands become indistinguishable and hence difficult to count which is why alternative age determination methods using the eye lens have been developed for whales. The most famous example of this is the bowhead whale which has been estimated to exceed 200 years of age from analysis of the chemical composition of material obtained from the eye lens (George et al. 1999). The same technique has successfully been applied to estimate age of narwhale, fin whale and harbor porpoise (*Phocoena phocoena*) (Garde et al. 2007, Nielsen et al. 2013). These investigations all take advantage of the unique structure of the vertebrate organism's eye lens, which is made up by so-called biological glass - a common feature for all vertebrate organisms with complex eyes (Fig. 12a).

Overall, the eye lens of vertebrates can be divided into three compartments: lens capsule, lens epithelium and lens fiber cells (Cohen 1965). The lens capsule is a transparent soft elastic membrane surrounding the lens, and the lens epithelium is located anteriorly to the lens serving as progenitors of new lens fibers (Cohen 1965, Boulton & Albon 2004). Lens fiber cells are the main compound of the nucleus and are organized in densely packed fiber cells filled with crystalline proteins, which besides being completely transparent (cf. biological glass, Fig. 12a), are also characterized by lacking organelles (Bloemendahl 1977, Wistow and Piatogorsky 1988, Bassnett et al. 2011, Kröger 2013). Mature fibers are therefore metabolically inactive, and the avascular eye lens nucleus is generally considered inert (Bassnet et al. 2011, Lynnerup et al. 2008). New layers of fibers cells produced in the lens epithelium are, however, continuously added during the organism's life and theoretically, the nucleus of an organism can be separated into the adult nucleus, the juvenile nucleus and the embryonic/fetal nucleus depending on life stage of the organism (Taylor et al. 1996). These “different” nuclei are not possible to separate visually, except the embryonic nucleus, which is more homogenous in structure than surrounding layers that are concentrically organized (Fig. 12b-d). Overall, there are two different types of fibers cells: primary fiber cells and secondary fiber cells. The embryonic nucleus is made of primary fibers cells (formed approximately at age 0 during embryonic development) which are arranged in a more homogenous structure than secondary fiber cells, which are added in

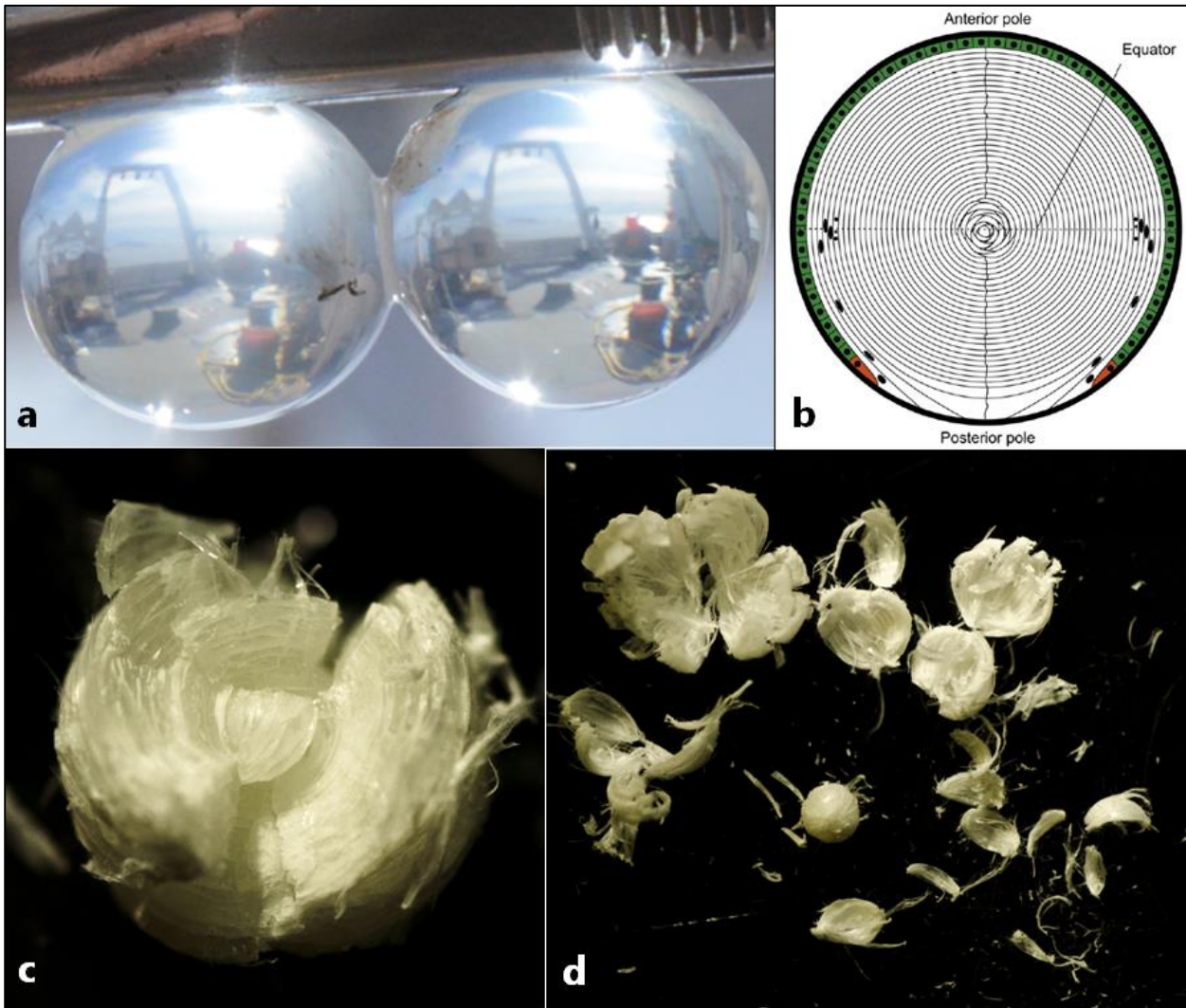


Figure 12. (a) Eye lenses from a Greenland shark immediately after dissection showing the deck of RV Sanna (Photo credit: Julius Nielsen). (b) Structural composition of fish eye lens with embryonic nucleus composed by primary fiber cells whereas outer layers are made of concentrically arranged secondary fiber cells (Kröger 2013). (c) Greenland shark eye lens during dissection becomes milky and non-transparent. The concentric arrangement remains visible (Photo credit: Julius Nielsen). (d) Embryonic nucleus isolated from layers (or flakes) of secondary fiber cells (Photo: Julius Nielsen).

concentric layers only after birth (Taylor et al. 1996, Bassnet et al. 2011, Kröger 2013).

Therefore, the structural composition of the eye lens nucleus very much resembles the structure of an onion, and layers added over the organism's life can be removed under microscope in concentric flakes (J. Nielsen pers. obs., Fig. 12c-d). Because of this, some of the oldest material from the vertebrate organism's body can be isolated in form of crystalline proteins of the embryonic nucleus. It is this tissue, which has been applied in previous age determination of difficult-to-age whales (George et al. 1999). It is also this tissue, which we apply as a biogenic archive in our age study of Greenland shark to create a Greenland shark chronology, which we can calibrate and thus convert into age estimates.

Seasonally deposited growth layers have not been identified in the eye lens nucleus of vertebrates and hence, independent age estimates cannot be obtained from counting of growth layers (i.e. like in a tree or a teleost otolith). Also, eye lens studies of cetaceans have revolved around aspartic acid racemization (George et al. 1999, Garde et al. 2007, Nielsen et al. 2013), a technique which is unsuitable for Greenland sharks as it relies on a constant body temperature and hence also eye temperature – an assumptions which cannot be made for ectotherm fishes such as the Greenland shark.

8.3 Marine radiocarbon dating

Carbon is a fundamental building block of all life on earth and appears naturally in the environment in three different isotopes. These are ^{12}C , ^{13}C and ^{14}C . ^{14}C will be referred to as radiocarbon throughout this text.

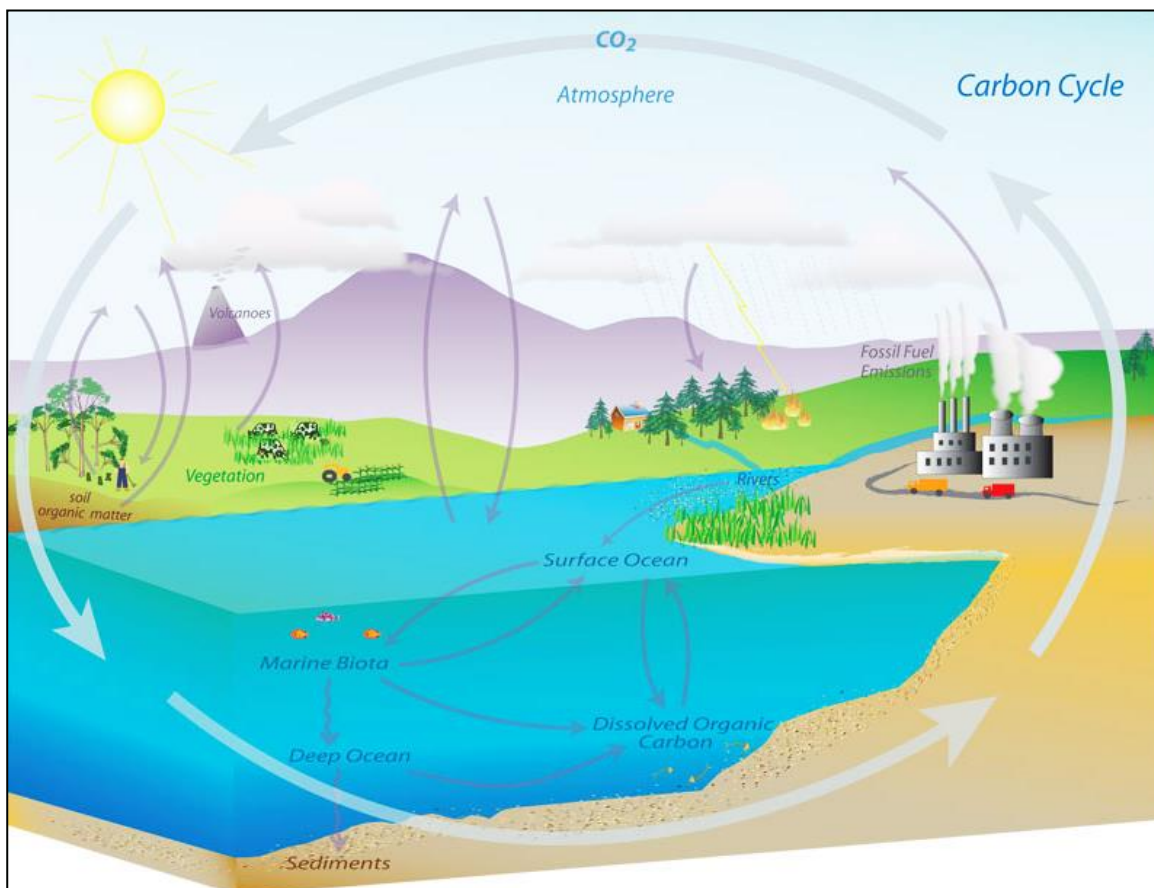


Figure 13. Simplified carbon cycle showing fluxes between atmosphere and ocean. Carbon isotopes are transferred across the atmosphere and the ocean via diffusion, and are incorporated in autotrophic organisms via photosynthesis and subsequently into the food web via heterotrophic transfer. Burning of ^{12}C enriched fossil fuel, volcanic eruptions, cosmic activity and respiration (including testing of atmospheric thermonuclear weapons) are factors affecting the isotopic composition of the environment including radiocarbon (NASA Earth Science Enterprise).

The natural ratio between these isotopes is approximately 100:1:1.1*10⁻¹², and ¹²C and ¹³C differ from radiocarbon as these are stable isotopes, whereas radiocarbon is unstable i.e. radioactive (Merve 1982). Radiocarbon is naturally produced in the stratosphere when cosmic rays cause high energy neutrons to collide with nitrogen-molecules creating an isotope with six protons and eight neutrons – radiocarbon (Anderson 1947). Over time, the radiocarbon isotope will spontaneously decay back into nitrogen and the amount in the atmosphere is therefore a balance between production/input of all the carbon isotopes and decay of radiocarbon (Fig. 13). Carbon isotopes of the atmosphere are spread worldwide as carbon dioxide (CO₂) and in the oceans as dissolved inorganic carbon. Theoretically, all metabolically active tissues of plants and animals mirror each other's isotopic composition as well as the contemporaneous atmosphere (Bowman 1990). This is because any change in isotopic composition of the atmosphere is relatively quickly transferred throughout plants and animals via photosynthesis and heterotrophic transfer. Originally, radiocarbon dating was based on the fact that when an organism died the incorporation of new carbon isotopes also ceased. Based on a combination of the approximate known ratio between the three carbon isotopes back in time (see above), and knowing that radiocarbon's half-life is 5,730 years, it was then possible to calculate an approximate time of death (Libby 1960, Bowman 1990). Radiocarbon dating has, however, evolved to become more accurate and also to be usable on organism of younger age where radioactive decay is of less importance (Bowman 1990; Kalish 1993; Reimer et al. 2013).

8.3.1 Bomb radiocarbon dating

Testing of hydrogen bombs or thermonuclear weapons has provided the opportunity to date young (or modern) carbon-based material. This technique relies on the vast amount of radiocarbon induced from testing of atmospheric thermonuclear weapons in the Pacific region during 1950s. De Vries (1958) initially reported mussels, snails and plants to contain elevated radiocarbon levels in hard calcified and soft body parts which was linked to the (at the time) recent test of thermonuclear bombs in the Pacific region. This was the initial finding of what later was to be established as the world's greatest chemical tagging experiment, in which all living organism on Earth participated. In sample chronologies, the bomb signal appears as a distinct and abrupt increase in radiocarbon which subsequently decreases due to absorption of bomb-induced radiocarbon into the oceans. This signal is referred to as "the bomb pulse" and has been detected in multiple terrestrial and marine chronologies (Fig. 14a-f). In terrestrial environments, the bomb pulse is so well established that single measurements of the bomb

period can be dated very accurately using bomb radiocarbon dating (Fig. 14b) (Lynnerup et al. 2008, Lynnerup et al. 2010).

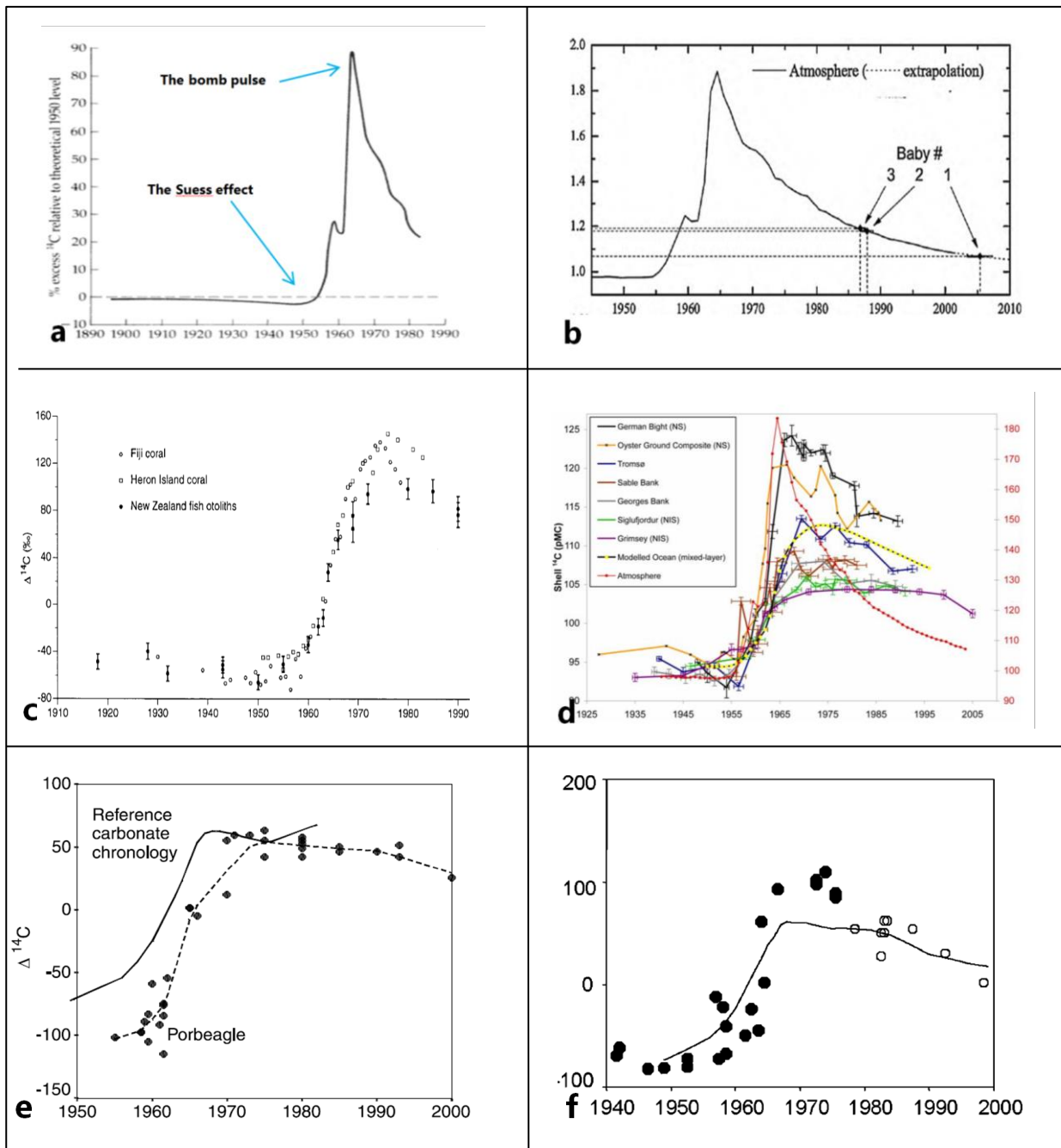


Figure 14. (a) The bomb pulse and the Suess effect in a chronology reflecting atmospheric content of radiocarbon (modified from Bowman 1990). (b) The applicability of the bomb pulse within forensic science where the radiocarbon levels in the eye lens nucleus has been used to learn time of birth of a baby corpse (Lynnerup et al. 2010). (c) Synchronous radiocarbon levels measured in otoliths and a reference chronology from a coral (Kalish 1993). (d) Surface mixed waters across the northern North Atlantic exhibit great variation in bomb pulse behaviour (Scourse et al. 2012). (e+f) The bomb pulse signal incorporated in tissues of metabolic origin in porbeagle and spiny dogfish of the North Atlantic (Campana et al. 2002, Campana et al. 2006).

In the marine environment the applicability of the bomb pulse within age estimation was initially described by Kalish (1993). Kalish demonstrated that radiocarbon levels in the center and oldest part of the otolith matched temporal radiocarbon levels from regional reference chronologies and thereby, age estimates obtained from counting of growth layers in the otolith could be validated (Fig. 14c) (Kalish 1993). It has subsequently been shown that different water masses exhibit great variation in terms of amplitude and duration of the bomb pulse even within little geographical range (Fig. 14d, Scourse et al. 2012). Nonetheless, the applicability of the bomb pulse within age validation of multiple species of sharks, whales and teleosts is well established (Matta et al. 2017). In the North Atlantic the bomb pulse has been detected in chronologies reflecting dissolved inorganic carbon of ambient waters from otoliths of haddock, Atlantic cod, redfish and Greenland halibut (Campana 1997, Kalish et al. 2001, Treble et al. 2008, Campana et al. 2015b) and in shells of ocean quahog (*Arctica islandica*) from across the North Atlantic (Fig. 14d) (Scourse et al. 2012). The bomb pulse has also been identified in chronologies reflecting dietary carbon (i.e. the marine food web) from species like porbeagle (Fig. 14e), white shark, beluga whale, spiny dogfish (Fig. 14f) and thorny skate (Campana et al. 2002, Campana et al. 2006, Stewart et al. 2006, McPhie & Campana 2009, Hamady et al. 2014). Another anthropogenic chemical time mark, the Suess effect, is caused by the emission of ^{13}C depleted carbon into the environment from burning of fossil fuels (Fig. 14a). This phenomenon has been detected in ^{13}C and radiocarbon chronologies which extend to the onset of the Industrial Revolution in mid/late-19th century (Butler et al. 2009, Tans et al. 1979, Schöne et al. 2011, Bowman 1990). Like the bomb pulse, the Suess effect is a chemical time mark, but it is much less established in the marine environment. However, the Suess effect has been suggested detected in a sample chronology from harbor porpoise (*Phocoena phocoena*) (Christensen & Richardson 2008).

It is important to distinguish between reference chronologies which have been made on tissues where carbon is incorporated directly from ambient water (in the form of dissolved inorganic carbon), from those where the carbon source is of dietary origin. The eye lens of Greenland shark is made up by proteins and hence the carbon source of the eye lens is dietary. Therefore, the reference chronologies applied to calibrate our Greenland sharks chronology should also be of dietary origin. The distinction between inorganic and organic carbon source will be discussed in more detail in the Discussion.

8.3.2 Pre-bomb radiocarbon dating

The bomb pulse produced a unique and unmistakable timestamp ideally allowing age determination of organisms dating back to the mid-1950s or at least evaluate if the sample is from pre- or post-bomb time. More long term and less extreme natural variations in radiocarbon levels of the open oceans are described by the International Marine Calibration Curve (Stuvier & Braziunas 1993). The latest version, Marine13 (Fig. 15, Reimer et al. 2013), can be used to age carbon-based material which originates from mixed layer water masses (the upper ~100-200 m of the water column) and pre-dates the bomb pulse. It is generally assumed that changes in pre-bomb radiocarbon concentrations are reflected in the food web with negligible or no delay. This interrelationship occurs because the vast majority of carbon in the marine food web is synthesized in mixed surface waters via photosynthesis and transferred quickly through the food web via heterotrophic organisms. Consequently, the radiocarbon profile of mixed layer waters is reflected in metabolically active tissues of sharks and other fishes, seals and whales with expectedly little delay (Mangerud et al. 2006). The interrelationship between carbon in mixed layer waters and carbon in the marine food web on a long time scale (>100 years) is critical for age estimates from the pre-bomb period.

Prior to the bomb pulse, natural variations in atmospheric radiocarbon levels resulted in temporal fluctuations of marine radiocarbon concentrations characterized by time periods of plateaus and steep changes (Reimer et al. 2013). Consequently, despite the overall pre-bomb trend of radiocarbon concentrations generally declining as one move back in time, converting pre-bomb radiocarbon ages into estimates of calendar age is less accurate and more difficult than for modern samples affected by the bomb pulse. The complexity is partly due, to the fact that the World's oceans are depleted in radiocarbon content relative to the contemporaneous atmosphere leading to an apparent radiocarbon age difference between the two systems which is termed "the marine reservoir age" (R) (Stuvier & Polach 1977; Stuvier & Braziunas 1993). To account for this, the marine calibration curve for the surface mixed layer of the oceans has been constructed (currently Marine13), using the terrestrial radiocarbon calibration curve (IntCal13) as an input parameter to a global ocean-atmosphere diffusion box model (Reimer et al. 2013). Regional offsets in surface ocean ages from the marine calibration curve are expressed as ΔR (Stuvier & Braziunas 1993) and to accurately convert single radiocarbon values to a calendar age, a ΔR value of the water of original photosynthetic carbon fixation must be known (Fig. 15). However, because of the Greenland shark's migratory behavior and lacking information on the distributional range of gravid Greenland sharks during embryonic nucleus formation (see review

on Greenland shark biology in MacNeil et al. 2012), an exact ΔR cannot be associated with each individual measurement. Consequently, individual pre-bomb measurements cannot be converted into age estimates with any accuracy, unless further constraints to the Marine13-based age estimates of pre-bomb sharks is applied (see Discussion and A1).

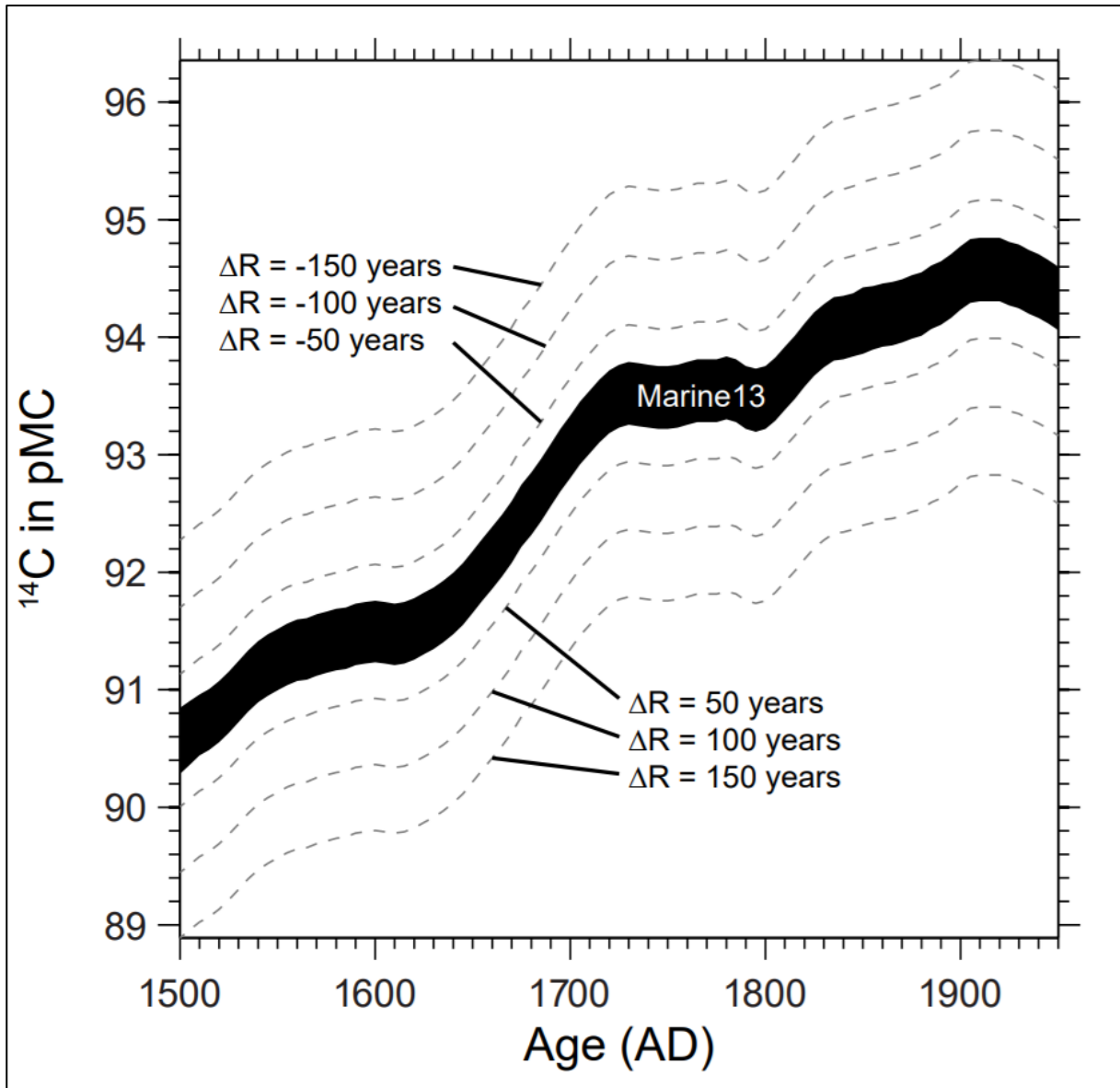


Figure 15. The Marine13 calibration curve spans from mid-1950 and 50,000 years back. Here is a subset from 1500-1950 shown as well as the effect of varying ΔR between waters. This variation is the main reason why it is difficult to convert single measurements from a freely moving animal into age estimates. The applied ΔR for the Bayesian model constructed to estimate the age of pre-bomb sharks is ± 75 years (graph modified by Jesper Olsen from Reimer et al. 2013).

9. Rationale, hypothesis, aims

9.1 Rationale

Greenland sharks longevity has never been investigated and only a single tag-and-release study by P. M. Hansen in early 20th century has addressed its growth rates. This study reported three medium-sized sharks to grow 1 cm, 14 cm and 8 cm over time periods of 2 years, 15 years and 16 years, respectively (Hansen 1963a). This unique observation suggests that Greenland sharks growth rates are extremely slow. With a maximum size of at least 550 cm, the Greenland shark is not only the largest shark native to the Arctic, it is also believed to be exceptionally long-lived. High levels of anthropogenic contaminants suggest that longevity by far exceeds 30 years (Fisk et al. 2002), but due to lack of conventional age determination methods on Greenland shark its longevity has remained an unsolvable mystery for decades.

9.2 Hypothesis

Greenland shark longevity is exceptional and can be quantified using marine radiocarbon dating

9.3 Aims

The ultimate aim of this PhD project is to investigate longevity of the Greenland shark. To do so, we will use two well-established radiocarbon dating methods (bomb radiocarbon dating and a Bayesian calibration) on metabolically inert tissue obtained from proteins of the eye lens nucleus. To justify fundamental assumptions when using these techniques, more basic knowledge on feeding ecology and spatial distribution of Greenland sharks is required.

The specific aims are:

1. To describe feeding ecology of Greenland sharks of various life stages.
2. To investigate the spatial distribution of Greenland sharks in Greenland waters using satellite tags.
3. To use state-of-the-art radiocarbon dating techniques on metabolically inert tissue for investigating longevity of the Greenland shark.

10. Summary of results

10.1 Manuscript 1 (MS1) – Feeding ecology

Ontogenetic dietary shift in the Greenland shark (*Somniosus microcephalus*)

Julius Nielsen, Peter Grønkjær, Peter Bushnell, Jørgen Schou Christiansen, Helene Overgaard, Kim Præbel, John Fleng Steffensen, Rasmus Berg Hedeholm.

To be submitted to Polar Biology.

The aim of this study was to investigate feeding ecology of Greenland sharks. Stomach content and stable isotope $^{15}\delta\text{N}$ levels, from up to 108 specimens ranging in lengths from 81-474 cm, revealed dietary differences between size categories. Greenland sharks smaller than 200 cm (juveniles) were predominantly feeding on lower trophic levels, especially squids. Larger sharks (sub-adults/adults) mainly fed on higher trophic level piscivorous fish and seals. Among the sub-adult/adult size groups, differences in composition of prey were evident at fish species level. Sharks smaller than 400 cm, were feeding on a large variety of fishes including Atlantic cod, Greenland halibut, wolfish, skates and lumpsucker (*Cyclopterus lumpus*). Sharks above 400 cm (i.e. adult females) fed mostly on Atlantic cod and beaked redbfish (*Sebastes mentella*). For all sub-adult and adult shark sizes, harp seal was of great importance. The observed differences in prey composition can for certain prey species be explained by the relative availability in different geographical areas (vertically and horizontally). For redbfish, however, these are abundant throughout Greenland waters in areas where sharks have been caught, and it seems that these are primarily consumed by adult females compared to sharks shorter than 400 cm. This study demonstrated a clear ontogenetic shift in feeding behavior between juvenile and sub-adult/adult Greenland sharks. Furthermore, the different prey preferences across sizes suggest that despite its lethargic appearance, the Greenland shark should be considered an opportunistic predator with the capability to capture fast swimming fishes and smaller marine animals.

10.2 Manuscript 2 (MS2) – Tracking

Spatial and temporal movements of adult Greenland shark females (*Somniosus microcephalus*) in Greenland waters

Julius Nielsen, Jørgen Schou Christiansen, Rasmus Berg Hedeholm, Kim Præbel, John Fleng Steffensen, Peter Bushnell.

The manuscript is in preparation.

The aim of this study was to investigate spatial and temporal movement patterns of adult Greenland shark females in southwestern Greenland. 12 females ranging from 410-455 cm in total length were caught at four different locations. Each shark was tagged with two or three pop-up satellite archival tags (PSATs), programmed to release after 3-13 months. In total, 33 PSATs were deployed and 22 reported usable data. Sharks exhibited varying spatial movements with migrations between 8 km and 2,754 km, and all sharks occupied southern Greenland shelf waters upon tag release. One shark migrated to the Reykjanes Ridge and back to southwest Greenland covering a distance of at least 2,754 km within 402 days in liberty. Temperature tracks, available from all tags, revealed that most sharks exited the cold fjords, in which they were tagged within 3-4 days following capture. Thereafter, sharks entered warmer offshore waters, where they remained throughout the tagging period. Preferred swimming depths and temperatures mainly ranged from 250-550 m and 4.1-5.5° C with the deepest, most shallow, coldest and warmest record being 1,083 m, 13 m, -2.0° C and 9.0° C, respectively. Beyond these general patterns, the study illustrated that: i) sharks tend to congregate in shelf waters off Frederikshåb Isblink in southwestern Greenland; ii) two sharks displayed similar migration in time and space migrating 700 km from Julianehåbsfjord to southeastern Greenland within 3 months. This is indicative of some degree of group migration, which is supported by bycatch information from bottom trawl surveys in the region. In conclusion, although adult Greenland shark females are capable of relatively fast long distance migrations, their preferred habitat is shelf waters of southern Greenland.

10.3 Article 1 (A1) – Age

Eye lens radiocarbon reveals centuries of longevity in the Greenland shark

(Somniosus microcephalus)

Julius Nielsen, Rasmus Berg Hedeholm, Jan Heinemeier, Peter Bushnell, Jørgen Schou Christiansen, Jesper Olsen, Christopher Bronk Ramsey, Richard Brill, Malene Simon, Kirstine Fleng Steffensen, John Fleng Steffensen.

The manuscript was published as a report in Science, 12 August 2016, vol. 353, issue 6300.

The aim of this study was to investigate life span of the Greenland shark - a notoriously slow-growing and difficult-to-age animal. Conventional age determination techniques of elasmobranchs are not applicable on the Greenland shark, and therefore, a novel approach using radiocarbon dating techniques on tissue from the shark's eye lens was applied. Tissue from the center of the eye lens nucleus (i.e. embryonic nucleus) contains the oldest proteins of vertebrates. The chemical composition of these proteins represents age 0 of the animal. We created a Greenland shark chronology, by collecting eye lenses from 28 female Greenland sharks measuring from 81-502 cm. We analyzed the radiocarbon content in the center of the eye lens nucleus. By combining two different techniques of marine radiocarbon dating on this Greenland shark chronology, i.e. bomb radiocarbon dating and a Bayesian calibration model, we found that only three sharks of 220 cm or less (total length) were affected by the bomb pulse. The bomb pulse onset is a time mark of the early 1960s, and the three post-bomb sharks were thus younger than approximately 50 years. On contrary, the remaining 25 larger sharks (258-502 cm) were older than 50 years. For these pre-bomb sharks, a Bayesian calibration model was constructed based on i) biological assumptions on growth patterns, birth size and size at age 50 years; ii) environmental assumptions on local reservoir age (ΔR) ranging between 0 and 150 years. Bayesian age estimates, reported as midpoint and extent of the 95.5% probability range, showed that Greenland shark females become mature at 156 ± 22 years, and that the largest and oldest animal was estimated to be 392 ± 120 years old (502 cm). The age estimates are thus reported as probability ranges which is unconventional for reporting lifespan of animals. Nevertheless, even the lower end of the probability range, which is at least 272 years, places the Greenland shark as the longest living vertebrate known to science.

11. Discussion

In this thesis, I provide estimates of Greenland shark longevity and estimate that it is the longest living vertebrate in the world. The results demonstrate the strength of interdisciplinary research, in this case among biologists, radiocarbon specialists and statisticians. This investigation is not carried out using standard fish ageing determination techniques, but rather a mix of two different radiocarbon dating methods that have not previously been combined in a similar setting. The justification of using these methods lies in biological observations of the shark's feeding ecology and spatial distribution. Our conclusion of exceptional longevity therefore relies on what is known about the biology of Greenland sharks, and what is fair to assume of their biology and physical environment. Scrutinizing these assumptions, which are fundamental to the rationale, is the focus of the following discussion. This is done in three sections: 1) a presentation of fundamental aspects of the rationale, 2) a meticulous elaboration of why timing of the bomb pulse onset in the Greenland shark chronology can be derived from existing reference chronologies and 3) a discussion of the weaknesses of this kind of study, as well as potential improvements which might narrow the longevity estimates of the Greenland shark.

11.1 Rationale

11.1.1 The Greenland shark chronology

Single radiocarbon measurements and absolute values are not suitable for age calibration using marine radiocarbon dating – either samples from before or after the bomb pulse onset. This is a common problem/challenge within radiocarbon dating, and we deal with this challenge in the same way as other studies; by analyzing a chronology. The Greenland shark chronology is created from a series of individual sharks of varying lengths caught around the same point in time (see **A1**). The fact that we analyze a chronology, and not individual measurements, is a central premise of the study. The Greenland shark chronology is constructed by assuming that size and age are positively correlated following a Von Bertalanffy growth curve – which is a fair assumption as this is a relatively common growth pattern for sharks (Hoenig & Gruber 1990). The “chronology assumption” (or length-age assumption) is strongly supported by the trend in radiocarbon levels of the entire chronology (see Fig. 2 in **A1** or Fig. 16). Of the 28 investigated female sharks, 25 were not affected by the bomb pulse (no. 4-28), one was slightly affected (no. 3), and two contained clearly elevated bomb-induced radiocarbon levels in the embryonic nucleus of the

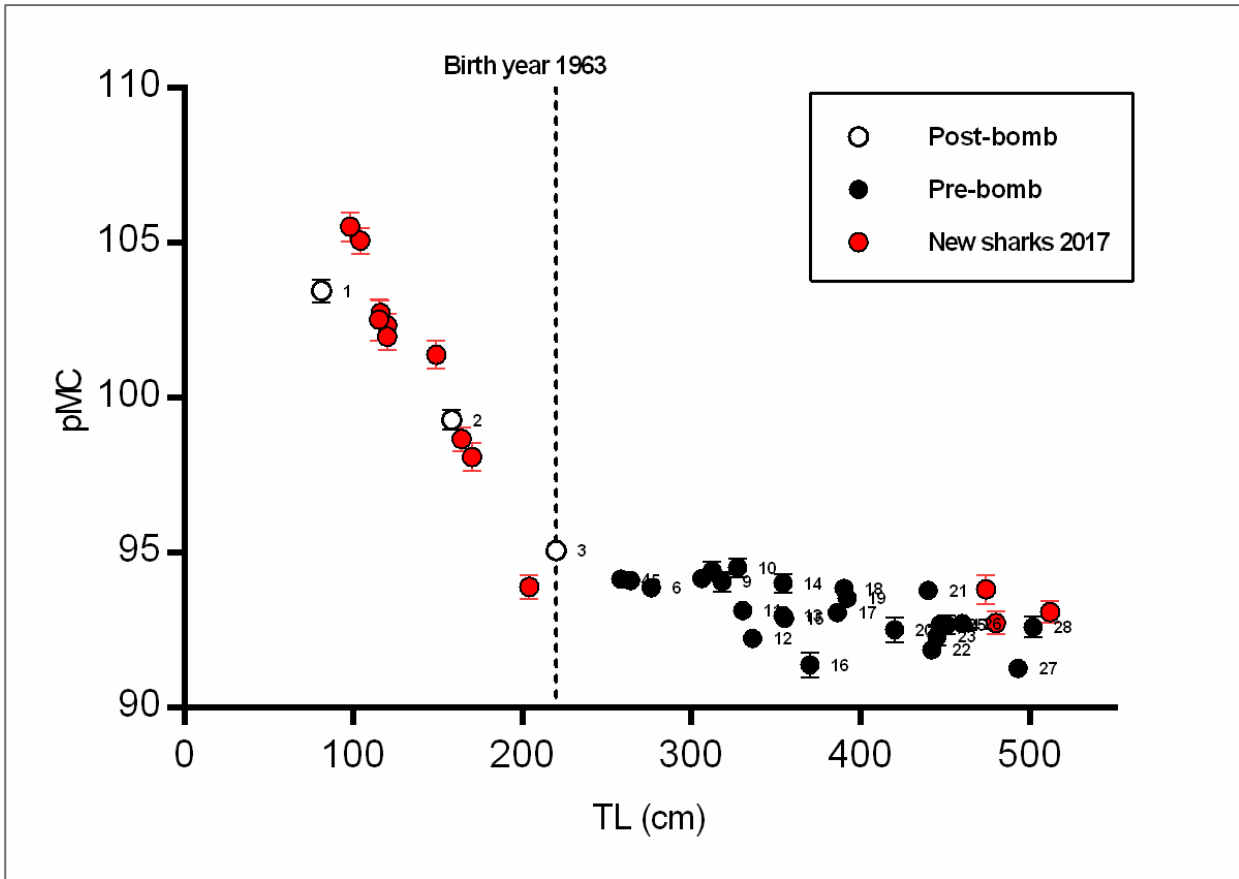


Figure. 16. Radiocarbon in the eye lens nuclei of Greenland sharks. Radiocarbon levels (pMC \pm SD) from 28 females as well 13 new sharks (mixed sex of both pre-and post-bomb origin) analyzed in 2017 (modified from Fig. 2 in A1) (Nielsen et al. unpublished data).

eye lens (no. 1 and 2). In additional support of this are nine Greenland sharks in the size range of 98-170 cm analyzed in 2017 (i.e. after publication of A1), and their radiocarbon levels strongly support the presence of bomb-induced radiocarbon in small juvenile sizes and thus the presence of the bomb pulse in the Greenland sharks chronology (Fig. 16). This strict separation of pre- and post-bomb sharks according to size would only be true if we were indeed evaluating a time series of samples representing a temporal development (i.e. a chronology). Furthermore, a decreasing trend with increasing pre-bomb shark size is to be expected if the sharks are older at larger sizes (Fig. 15). Therefore, the significant negative correlation between size and radiocarbon level for the pre-bomb sharks (see A1), further supports that the Greenland shark chronology is indeed a time series.

11.1.2 The carbon source of the eye lens nucleus Greenland shark chronology

As stated in the Prologue – the carbon source of the Greenland shark eye lens is pivotal for the entire age investigation. The analyzed tissue in the eye lens nucleus consists primarily of protein and hence, the carbon source of the eye lens is of dietary origin. This means that the Greenland shark chronology is an organic carbon chronology, which differs significantly from inorganic carbon chronologies, as the latter is based on radiocarbon from dissolved inorganic carbon of ambient water masses. The dietary origin of the Greenland shark chronology implies that the diet is dictating the chemical composition of the chronology. However, as the tissue analyzed is from the center of the eye lens nucleus (also termed embryonic nucleus), and this is formed during fetal development, it is actually the diet of the shark’s mother which is the carbon source of the Greenland shark chronology - not the shark’s diet itself. The feeding ecology and spatial distribution of adult Greenland shark females is thus of particular interest when calibrating the Greenland shark chronology and converting radiocarbon levels into time estimates.

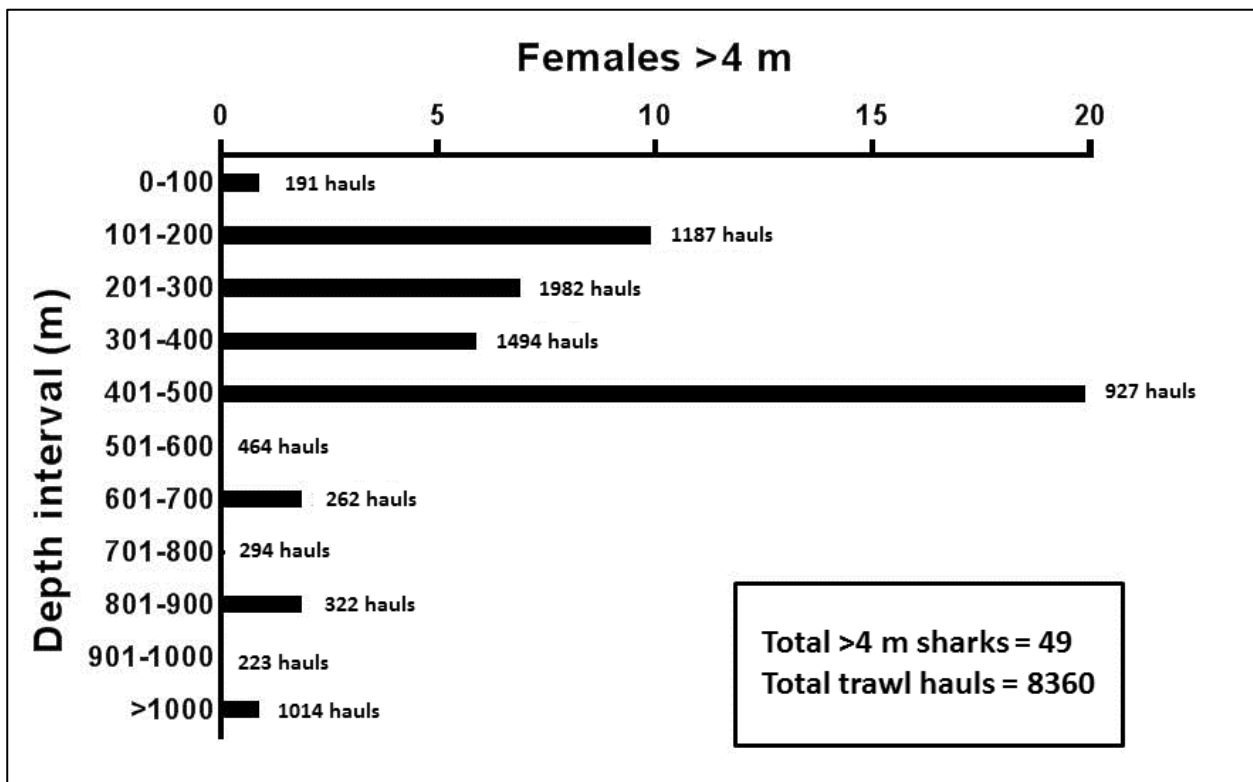


Figure 17: Capture depth of 49 female Greenland sharks longer than 400 cm from 8360 trawl hauls in Greenland waters (GINR unpublished data from yearly trawl surveys between 1995 and 2016, see survey description in Nielsen et al. 2014).

Adult females are longer than 400 cm, and although some feeding ecology studies include a few sharks of this size (Yano et al. 2007, McMeans et al. 2010, Nielsen et al. 2014), none have evaluated adult females separately. In MS1, adult females were found mainly to feed on high

trophic species like Atlantic cod, harp seal, redfish, wolffish (*Anarhichas* spp.), lumpsucker, and skates (Rajidae) – prey items which are relatively common in continental shelf waters across the northern North Atlantic. Such feeding ecology corresponds well with the spatial distribution investigated in MS2, where PSATs revealed that adult females of southwestern Greenland spent most of their time in southern Greenland shelf waters at depths of 200-550 m with infrequent and brief descends below 800 m. Such depth distribution of adult females is strongly supported by unpublished data from annual bottom trawl surveys conducted by Greenland Institute of Natural Resources during the past 20 years, revealing that females longer than 400 cm have been caught all over the continental shelf, but most frequently at depths shallower than 500 m (Fig. 17, see survey description in Nielsen et al. 2014).

However, adult females may also be encountered in continental shelf waters off New Foundland (Canada), Iceland, and mainland of Norway (Nielsen et al. in prep2). Given their pronounced migratory capabilities (Hansen 1963a, Fisk et al. 2012, Campana et al. 2015a, MS2), the carbon source of the Greenland shark chronology must be expected to be of high trophic fish and seals from continental shelf food webs from across subarctic and temperate regions of the northern North Atlantic. Therefore, the calibration (and thus the age estimates) relies on general and common trends of radiocarbon in dietary reference chronologies of these regions – the northern North Atlantic.

11.1.3 Radiocarbon in the northern North Atlantic

Most studies on the “behavior” of radiocarbon in surface mixed waters and marine food webs have been made in the northern North Atlantic. These studies focus on the marine bomb pulse – a chemical time mark, which has been detected in multiple reference chronologies of both inorganic and organic origin (Kalish 1993, Campana et al. 2002). In general, reference chronologies are either constructed from tissues having an inorganic carbon source or a dietary carbon source. Inorganic reference chronologies are made from marine carbonates e.g. in mussel shells or fish otoliths, which are characterized by incorporating radiocarbon directly from ambient waters in the form of $^{14}\text{CO}_2$. Several such chronologies representing surface mixed waters across the northern North Atlantic, reveal that some waters exhibit a rapid response of great magnitude to the bomb pulse, while others exhibit a slower and less distinct reaction (Campana 1997, Kalish et al. 2001, Treble et al. 2008, Campana et al. 2015b, Scource et al. 2012). Such different responses are due to hydrographic differences between water masses and regions. However, it is important to note for these chronologies that the timing of the bomb pulse

onset is almost synchronous for all chronologies. The bomb pulse onset is the point in time (i.e. year) in a chronology, where bomb-induced radiocarbon becomes detectable from the lower levels of naturally occurring radiocarbon; an event different from that of the bomb pulse peak (Fig. 18). Organic reference chronologies (of dietary origin) are available from porbeagle, beluga whale, spiny dogfish, white shark and thorny skate, all from the northern North Atlantic (Campana et al. 2002, Campana et al. 2006, Stewart et al. 2006, McPhie & Campana 2009, Hamady et al. 2014). Like the aforementioned inorganic chronologies, organic chronologies also exhibit great variation in terms of bomb pulse “shape”, as well as an almost synchronous timing of the bomb pulse onset. In organic chronologies representing continental shelf food webs of the northern North Atlantic, the bomb pulse onset is no later than the early 1960s (1963 to be precise, Fig. 1 in A1).

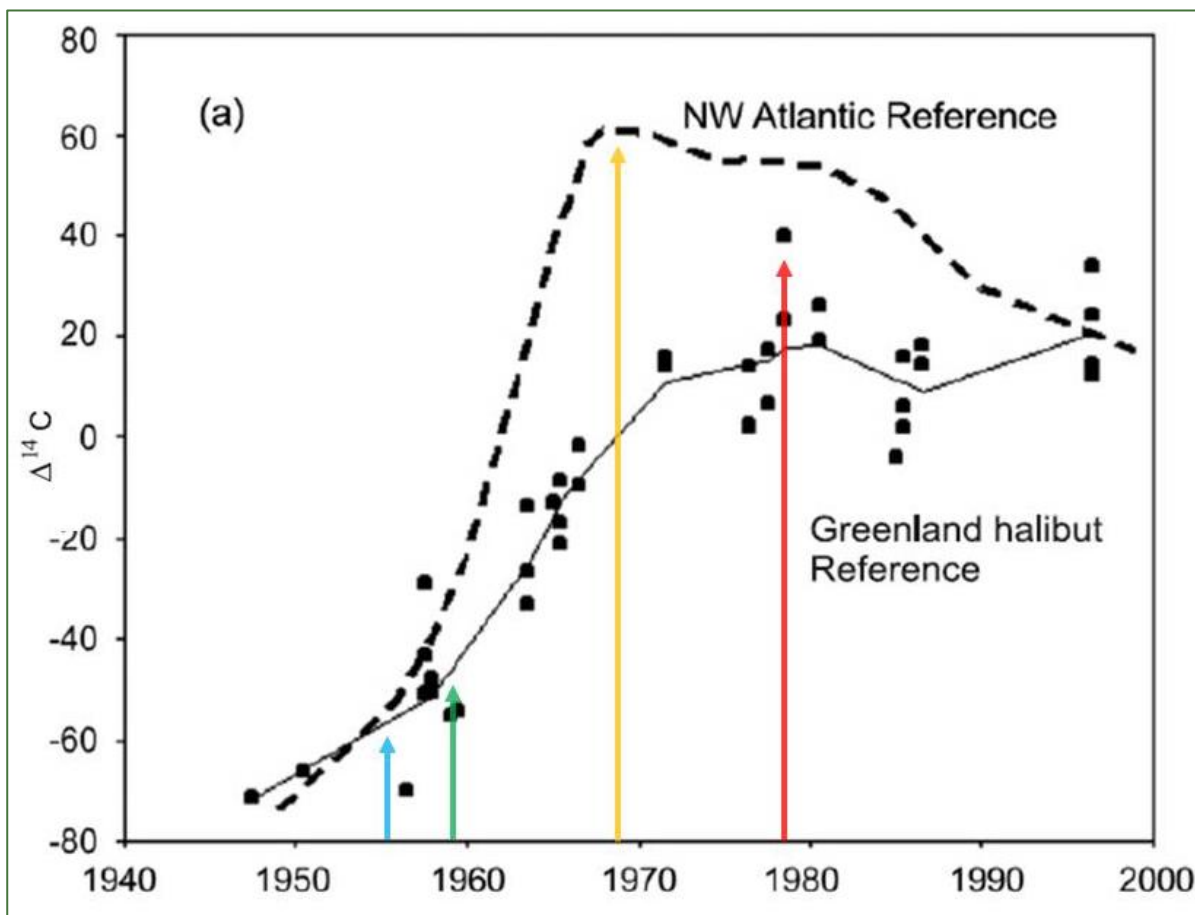


Figure 18: Comparison between the inorganic NW Atlantic Reference chronology (reflecting $^{14}\text{CO}_2$ of surface mixed waters) and the inorganic otolith chronology of Greenland halibut Reference. Green arrow indicates the time of bomb pulse onset for the Greenland halibut reference which is not much different from the blue arrow that represents onset of the bomb pulse in the NW Atlantic Reference. Timing of the peak (i.e. maximum level) for the two chronologies exhibit greater variation: the yellow arrow shows timing of the peak to late 1960s in the NW Atlantic Reference, whereas the Greenland halibut is delayed by another ~10 years as indicated by the red arrow (modified from Treble et al. 2008).

Similarities between inorganic and organic reference chronologies are expected as the pathway of radiocarbon into the food web is via autotrophic organisms incorporating $^{14}\text{CO}_2$ via photosynthesis in surface mixed waters. Radiocarbon is then transferred into higher trophic levels of the food web by heterotrophic organisms. Consequently, the timing of the bomb pulse onset is no more than 0-3 years delayed when compared to inorganic chronologies reflecting surface mixed waters (Fig. 1 in A1). This synchronicity demonstrates well that surface plankton goes directly into the food chain with short turnover in soft metabolically active tissues. It is such soft tissues of high trophic fishes and seals, which dictates the radiocarbon levels in the Greenland shark chronology. Animals that are a part of the same or similar food webs, as porbeagle, white shark, beluga whale, spiny dogfish and thorny ray in the northern North Atlantic, will have incorporated the bomb pulse onset at a similar time - no later than early 1960s.

11.1.4 Conventional use of bomb radiocarbon for age validation

Nowadays, the bomb pulse is a well-established time mark, which has been used for age determination and age validation of multiple marine fishes and whales (Stewart et al. 2006, Hamady et al. 2014, and many more). In these studies, it is the entire portion of the bomb pulse rise which is used as a time mark. The technique is typically applied on animal specimens from museum collections, ideally some which have lived during both the pre- and post-bomb period. A fictive example could be a lamniform shark caught in 1970, which from count of growth rings in the vertebra is estimated to be 20 years old. Radiocarbon levels in each growth ring are then measured creating a chronology spanning from presumably 1970 back to 1950. If the portion of the bomb pulse rise in the chronology was detected in growth layers corresponding to those years, where the rise had been identified in regional reference chronologies (i.e. between late 1950s to early 1960s), the age estimate of 20 years old is validated. However, in case of a mismatch between the investigated chronology and the reference chronology, the age estimate of the animal and the associated method on that specimen or species are questionable. Such mismatches have been observed in multiple studies of sharks and rays (Harry 2017), where the oldest specimens in particular are underestimated due to growth-band-cessation resulting from attenuation of somatic growth (Francis et al. 2007, Hamady et al. 2014, Andrew & Kerr 2015).

11.1.5 Bomb radiocarbon dating of the Greenland shark chronology

The applicability of bomb radiocarbon dating of the Greenland shark chronology differs from the conventional use, as described in previous sections. This is because there are no alternative age estimates of Greenland sharks available and hence no form of validation. Furthermore, for the

investigated sharks, there is only a single sample from each shark, which is associated with age 0 of the shark. Due to the great variation in behavior (or faith) of the bomb pulse across food webs of the northern North Atlantic, we can only identify which sharks have been affected by the bomb pulse (and thus have enriched bomb-induced radiocarbon levels) and which have not been affected. From the findings of MS1 and MS2, it can be justified that adult Greenland shark females are part of a food web highly comparable to that of porbeagle, white shark, beluga whale, spiny dogfish and thorny ray, in terms of dominating prey species composition, prey depth distribution and prey trophic level (see relevant feeding studies in Joyce et al. 2002, Estrada et al. 2006, Heide-Jørgensen & Teilmann 1994 and also Section 11.2.1). Therefore, adult Greenland shark females must also have been affected by the bomb pulse onset from the early 1960s and onwards, meaning that the post-bomb sharks must have been born after the 1960s, while the pre-bomb sharks are born earlier. In A1 only two small sharks were found to contain post-bomb levels but as presented in Fig. 16, more small specimens have been found subsequently to contain post-bomb radiocarbon levels.

11.1.6 The Bayesian model on the pre-bomb portion of the Greenland shark chronology

To estimate the age of the pre-bomb sharks, a Bayesian calibration model was constructed. Bayesian statistics refer to the kind of statistics used when evaluating the match between different probability distributions. In general, because the production of radiocarbon in the atmosphere is not constant over time (predominantly modulated by varying solar activity), and because human burning of fossil fuels during the industrial revolution have significantly changed the atmospheric radiocarbon activity, the resulting calendar-year probability distributions are often multi-modal. This presents an extra challenge when working with radiocarbon from the pre-bomb period, which is generally solved using Bayesian statistical methods allowing for constrained calendar-year probabilities to be produced.

The idea behind the Bayesian model is to combine the likelihood of calibrated range probabilities with prior knowledge on depositional patterns, which in our case is the expected growth pattern. Growth patterns of sharks (and fishes in general) are expressed as a Von Bertalanffy equation (Hoenig & Gruber 1990), in which size-increments are not continuous throughout a life time. A fixed age within a chronology can also be used in a Bayesian model. A parallel example could be, if the age of a certain layer in a sediment core is known due to a signature characteristic from a volcano eruption. In our case, shark no. 3 was included with a fixed age (from 1963 ± 5 years) estimated from the bomb pulse onset (see **A1**). Using the

Marine13 radiocarbon calibration curve, the Bayesian model generates a posterior age probabilities, which are the result of calibrated radiocarbon measurements combined with such prior knowledge (e.g. fixed age and growth pattern). Note that Marine13 is not a reference chronology but rather a calibration curve made from a box diffusion model (Reimer et al. 2013). It must be emphasized that the finding of shark no. 3 being approximately 50 years old is a partial result obtained from ‘bomb radiocarbon dating’, and hence it is independent from the result of pre-bomb radiocarbon dating. This partial result is used as prior knowledge in the effort to produce age estimates of the sharks of the pre-bomb portion of the Greenland shark chronology.

11.1.7 How to interpret calibrations

Age estimation of Greenland shark is achieved by cross-combining two well-established radiocarbon dating techniques applicable for different periods in time. The output (i.e. the age estimates) from these techniques is very different from one another. The bomb dating “only” allows us to evaluate which sharks are older and which sharks are younger than the bomb pulse. The output of the Bayesian model is posterior probability distributions for each pre-bomb shark (Fig. 3 in A1). In A1, I report the 95.5% range of this probability distribution as the final age estimate. Using the oldest shark as an example, the probability range is 392 ± 120 years (Table S2 in A1), which is the midpoint \pm half the range. I could also have written that the oldest shark was 272-512 years old with 95.5% certainty or, that the oldest shark was at least 272 years old. It is essential to understand how these unconventional age estimates differ from normal reports on age in order to understand the Bayesian calibration model and its associated limitations and strengths.

11.1.8 The challenge of reservoir age

Determining the age of marine organisms – especially those as long-lived as the Greenland shark – is subject to uncertainty when using marine radiocarbon dating. Incorporation of radiocarbon in the marine food web can be perceived as sporadic, depleted and sometimes unpredictable. This is, however, to be expected for animals moving freely between water masses with different ΔR (see definition of ΔR in Introduction) and coincident differing ΔR of the ingested prey. By calibrating a chronology, we calibrate a trend which decisively reduces potential challenges due to offsets/depletions between reference chronologies regardless of what might cause these. The absolute level of individual radiocarbon values is therefore, in general, less important both for bomb dating and for the Bayesian model. What is important is how values develop through a chronology (i.e. their relative change over time). This is exemplified by multiple age validation

studies of sharks and whales, where the bomb pulse is identified as a drastic increase in the radiocarbon levels (post-bomb) compared to lower pre-bomb levels (see Stewart et al. 2006, Hamady et al. 2014). The challenge of an unknown ΔR is, however, more acute for the calibration of the pre-bomb portion of the Greenland shark chronology. This is precisely what the Oxcal program and the Bayesian model (see below) are designed to handle. This calibration includes information on local reservoir ages and incorporates the uncertainty into the age output. I used a local reservoir range of 0-150 radiocarbon years (in the model expressed as 75 ± 75 years, mean \pm SD) as this is a reasonable value for open ocean waters in the recent past of temperate and sub-arctic offshore regions of the northern North Atlantic (Scourse et al. 2012). The difference between ΔR of different water masses is exemplified by the different behavior of the bomb pulse detected across the northern North Atlantic (Fig. 3 in Scourse et al. 2012) and is also illustrated for the pre-bomb period in Fig. 15.

11.2 The bomb pulse at living depth of the Greenland shark

The bomb pulse onset is a central time marker in the age analysis of the Greenland shark. First of all, it is important as a fixed time point in the Greenland shark chronology. Secondly, from a theoretical perspective, it demonstrates the coherency between $^{14}\text{CO}_2$ of surface mixed waters and fishes at depths much greater than surface mixed waters (the upper 200 m of the water column). The presence of the bomb pulse in large sharks and whales as well as in abyssal fishes is elaborated below. The following sections thus provide arguments supporting timing of the bomb pulse onset as no later than early 1960s, which have been applied in our calibration of the Greenland shark chronology.

11.2.1 Existing reference chronologies of dietary origin

It might be questioned whether the timing of the bomb pulse onset in chronologies obtained from “surface-swimming and -feeding” species such as the porbeagle or the other species represented in Fig. 1 in A1, is relevant to apply on a deeper swimming Greenland shark. However, this concern would only be relevant if the carbon source of the Greenland shark chronology was inorganic, and hence the transport of radiocarbon from surface mixed water into the Greenland shark chronology was via diffusion and ocean mixing. This is not the case, as the Greenland shark chronology is derived from eye lens proteins which are of dietary origin. Fig. 1 in A1 shows examples of organic reference chronologies from other predatory and piscivorous marine species (i.e. chronologies also based on a dietary carbon source). The Greenland shark overlaps in terms of dietary preferences, as well as distribution to some degree, with these species (i.e.

porbeagle, white shark, beluga whale, and spiny dogfish), which is highly relevant for the theoretical paradigm applied. For example, Joyce et al. (2002) evaluated more than a thousand porbeagle stomachs and found the main prey items to be groundfishes like Atlantic cod, wolffish, unknown flounders (Pleuronectidae) and lumpsucker. Furthermore, long-snouted lancetfish (*Alepisaurus ferox*) – a pelagic predatory fish distributed down to 2 km of depth (Froese & Pauly 2017) – was an important prey item. This diet is in accordance with depth distribution of the porbeagle in the North Atlantic, which tends to occupy the upper 200 m of the water column, but frequently descends to 300, 400 and even 700 m of depth (Saunders et al. 2011). White sharks, which frequently occupy surface waters, have been reported at depths down to 500 m (Boustany et al. 2002). Even though there are no stomach content analysis of white sharks from the northern North Atlantic, their diet is expectedly composed by large predatory fishes and marine mammals from within and below surface mixed waters (Estrada et al. 2006). This diet is similar to adult Greenland shark females, and particularly when taking the seals into consideration. For all Greenland shark stomach content analyses, seals compose a large proportion of the diet (Fisk et al. 2002, Yano et al. 2007, McMeans et al. 2010, Leclerc et al. 2012, Nielsen et al. 2014, MS1). Beluga whales dive as deep as 700 m (Kinze 2001), where they may feed on redfish or Greenland halibut, but perhaps more frequently they descend to depths of 20-350 m and feed on polar cod (*Boreogadus saida*), boreoatlantic armhook squid, arctic cod (*Arctogadus glacialis*) and lantern fish (Myctophidae) (Martin & Smith 1992, Heide-Jørgensen & Teilmann 1994). Such prey items are also important for Greenland halibut (Bowering & Lilly 1992, Hovde et al. 2002) and harp seals which frequently dive to 100-400 m (Folkow et al. 2004, Haug et al. 2004). Furthermore, Spiny dogfish mainly occupy depths between 50-300 m (deepest record = 1,400 m) where they feed on benthic and pelagic fishes (like herring and cod) and crustaceans (Froese & Pauly 2017, Compagno 1984).

All combined, porbeagle, white shark, beluga whale and spiny dogfish are not surface dwelling organisms, but rather occupy and feed on prey from the entire continental shelf. Such fauna is overall highly comparable to the feeding ecology of adult Greenland shark females. We therefore argue that if these predatory sharks and whales incorporated bomb-induced radiocarbon via their diet in the early 1960s, adult Greenland shark females did too. This is indeed fair to expect in an environment as described in MS1 and MS2.

11.2.2 The bomb pulse in deep sea and abyssal food webs

It has been suggested that the adult life of Greenland sharks is spent in deep waters off the continental shelf break which is not similar to the environment as described in MS1 and MS2 (Campana et al. 2015a). This is a possibility as Greenland sharks have been caught in abyssal waters as deep as 2,909 m (Porteiro et al. 2017). It has also been reported that adult females were found at depths below 1,000 m in western Greenland, where they presumably were feeding on Greenland halibut (Yano et al. 2007), which is why it was surprising that we did not find Greenland halibut as an important prey item (see MS1). If the carbon source of the Greenland shark chronology is actually strictly deep ocean or abyssal fishes, and thus dictated by e.g. grenadiers (Macrouridae), slickheads (Alepocephalidae), or Greenland halibut, there would be less reference material available for our calibrations. This is because scientific investigations on behavior of the bomb pulse in deep ocean and abyssal food webs are limited compared to continental shelf food webs and mixed water masses. Nonetheless, a strictly deep sea or abyssal feeding behavior would only have little impact on the calibration, if any at all. This presumption is based on available investigations of radiocarbon in deep ocean and abyssal food webs, which demonstrates that bomb-induced radiocarbon was present in metabolically active muscle tissue from all investigated abyssobenthic and abyssopelagic fishes caught as deep as 2-3 km during the late 1960s and 1970s (Williams et al. 1970, Percy & Stuvier 1983, Williams et al. 1987). This demonstrates that bomb-induced radiocarbon was quickly transferred from surface mixed waters to fishes occupying much greater depths than represented by Fig. 1 in A1. Although the investigations on abyssal fauna were made on fishes sampled in the Pacific Ocean, there are no obvious reasons why a generally similar fast pathway of $^{14}\text{CO}_2$ from surface mixed waters to abyssal fishes should not occur in the northern North Atlantic. The “deepest” carbon chronologies from the northern North Atlantic are made from otoliths of redfish and Greenland halibut (and thereby reflecting $^{14}\text{CO}_2$ in ambient water masses). These chronologies show that the bomb pulse onset penetrated by diffusion and mixing to depths of 400-500 m in the northwestern North Atlantic in the early 1960s (Fig. 18) (Treble et al. 2008, Campana et al. 2015b). Furthermore, the only study (to my knowledge) that has presented data, where the associated discussion mentions the possibility of a delayed bomb signal due to potential feeding on deep ocean prey is Kerr et al. (2006). This study presents an organic chronology from a white shark vertebra in which the bomb pulse onset was found to be late 1970s (Kerr et al. 2006). A subsequent reanalysis showed that the “delayed” bomb pulse was most likely due to biased counting of growth ring layers (Andrews & Kerr 2015) which has been shown to be a common challenge for Lamniform sharks (Francis et al. 2007, Hamady et al. 2014).

There are currently no available data suggesting that the bomb pulse onset should be delayed in food webs of the northern North Atlantic (or anywhere else) beyond what is illustrated in Fig. 1 in A1.

11.3 Weaknesses and potential improvements of the study

Unlike previous studies using the bomb pulse to validate already existing ageing methods, there are no comparable age estimates available for each individual Greenland shark analyzed.

Therefore, regardless of the many previous investigations of the bomb pulse in the North Atlantic, as well as a comparable carbon source for adult Greenland shark females and other predatory fishes and whales, all of this can only serve as justification of the applied approach – not a validation. The lack of validation is the reason for referring to the output of the age investigation as “Estimates” and not “Determinations”.

11.3.1 Calibration-associated weaknesses

There are several weaknesses associated with the approach, which could cause the calibrated and modelled ages to deviate from the true biological ages. First of all, exact age estimates of this study rely on an already established behavior of the bomb pulse in northern North Atlantic surface mixed waters and food webs. It is therefore essential that the scientific literature truly reflects the variation across shelf waters of the northern North Atlantic. It is however possible that a tendency of publishing bias is favoring positive results which in this case means that chronologies might only have been published when in accordance with regional reference chronologies. If such bias is present (and delayed chronologies tend not to be published), that would undermine the usage of the bomb pulse onset and also change the exact age estimates as presented in A1. Note, however, that the sensitivity analysis showed that the overall finding of Greenland sharks having extreme longevity, exceeding centuries, is not particularly sensitive to timing of the bomb pulse onset between 1958 and 1980. Therefore, the overall findings of extreme longevity of Greenland sharks are robust to the assumption timing of the bomb pulse onset in the chronology (see Supplementary Material in A1).

Another potential weaknesses are the multiple assumptions and constraints used by the Bayesian calibration model, including expected shark growth pattern. From a modelling perspective, the growth pattern-input “forces” each shark to be older than the previous smaller shark regardless of radiocarbon level. Such a strict correlation between length and age cannot necessarily be expected,

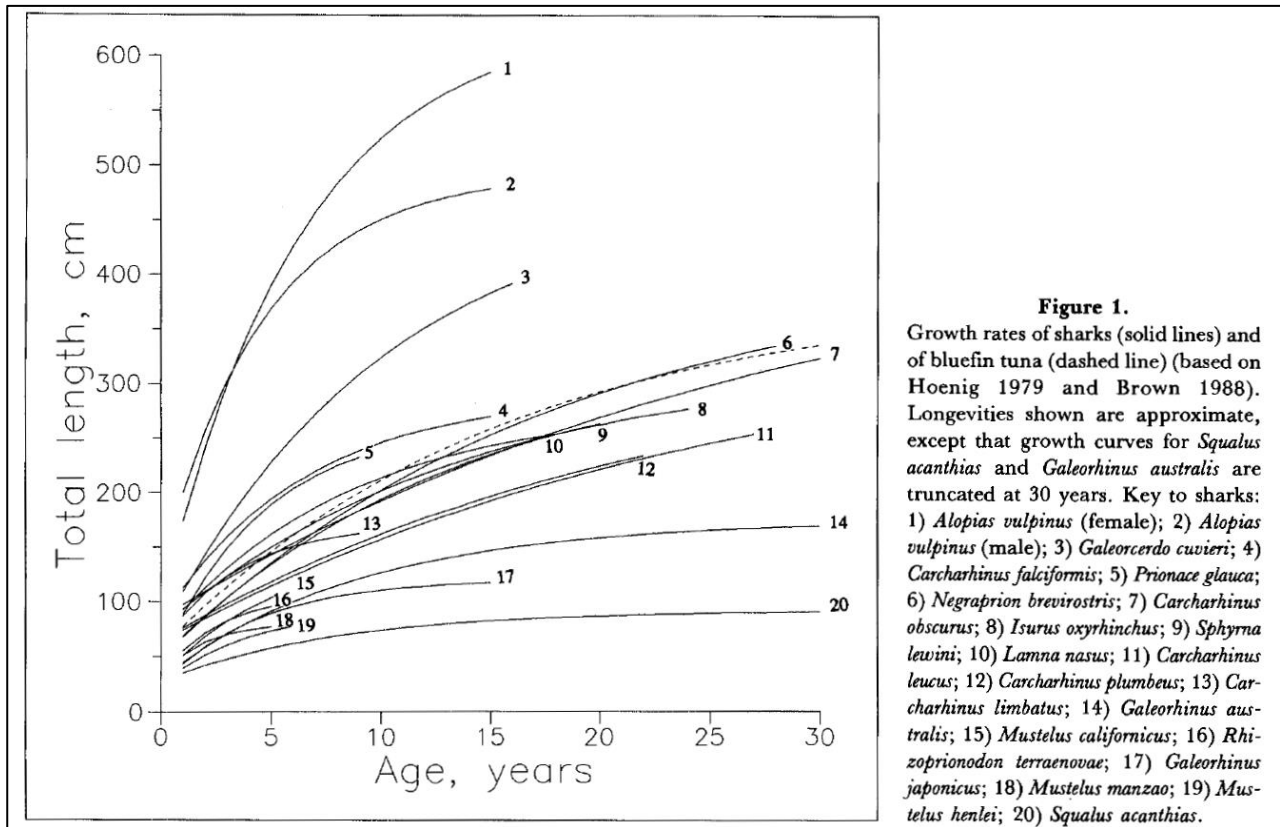


Figure 19. Growth trajectories for different shark species which all can be described by a Von Bertalanffy growth curve or a derivate thereof (from Hoenig and Gruber 1990).

but the Von Bertalanffy growth trajectory is a general growth pattern demonstrated for many species of fish and sharks (Fig. 19). In spite of this, it is fair to calibrate the pre-bomb portion of the chronology using the applied Bayesian model for several reasons: 1) we analyze a chronology and it is implicitly considered a time series of samples; 2) the results indicated good consistency between modeled age ranges and Marine 13, as well as a good internal agreement between individual data points (See A-indices in A1 and Bronk Ramsey 2009); 3) the strict separation between pre- and post-bomb sharks as well as the significant negative correlation between shark size and radiocarbon level suggests that we are evaluating a time series of samples. It must also be emphasized that although model input assumes a strict age-length correlation, the output is not similarly strict. Model output (the posterior probability ranges) indeed overlap for similar sized sharks, and hence our results are flexible allowing for deviations from a strict length-age correlation.

11.3.2 Future improvements

To narrow the age estimates presented in this study, it is necessary to conduct more investigations on the radiocarbon signal in Greenland sharks and in the marine food web.

It must be stressed that although some parts of the Marine13 radiocarbon calibration curve are more diagnostic than others, no time period is nearly as diagnostic as the bomb pulse in the late 1950s/early 1960s. Also, due to an unknown ΔR associated with analyzing a freely moving animal, it is difficult to distinguish between spatial contemporary variations in ΔR and age-related differences for pre-bomb sharks. This is the advantage of analyzing the data as a chronology. This allows for converting the trend of the chronology into age estimates, rather than single values and absolute levels. Great variation for single measurements is to be expected in the pre-bomb period (cf. the light blue probability distribution in Fig. 3 in A1 illustrating how uninformative pre-bomb radiocarbon levels are when calibrated individually). Only by knowing the correct ΔR associated with each sample, the true biological age might have been obtained from the pre-bomb sharks. This is not a realistic scenario, as the exact radiocarbon history of the water from which the analyzed tissue originates is unknown.

Nonetheless, it is possible analyzing more very old sharks larger than 460 cm, could improve age estimates of the oldest animals. This is because large animals may have been born during a more diagnostic part of the Marine13 radiocarbon calibration curve compared to the plateau, characterizing the period from just before the bomb pulse and approximately 250 years back (Fig. 15). It must be stressed, however, that predicting the radiocarbon level in a Greenland shark born during this more time-diagnostic period remains difficult (by diagnostic I mean a time period where the calibration curve does not appear as a plateau). It could, however, be expected that if a large enough dataset was evaluated, more very depleted radiocarbon levels would be encountered (likely <91 pMC). However, such very old specimens do not necessarily have more depleted levels than already observed – once again due to the uncertainty of the ΔR values between waters masses and associated food webs across the northern North Atlantic.

Because of the lethal sampling procedure associated with the eye lens removal and alternative way of confirming the longevity of Greenland sharks would be to 1) establish the eye lens as a chronologically structured biogenic archive, and 2) expand the exploration of the behavior of the marine bomb pulse in more chronologies representing the marine food web. Such chronologies could be made from eye lenses of long- and deep-living redfish, grenadiers, black dogfish or Greenland halibut (age span 30-75 years according to FishBase, Froese & Pauly 2017). Also, measurements on whale eye lenses, which can be aged using alternative techniques (such as aspartic acid racemization), and teeth of seals and whales from museum collections could be usable for such investigation. Lastly, a large-scale tag-and-release study similar to the one

initiated by P. M. Hansen almost 80 years ago, might reveal valuable information of Greenland shark growth rates. Such tagging study should be conducted on a large transatlantic scale with collaboration between scientific institutions in Canada, Greenland, Iceland, Norway, Russia and Denmark allowing for a large number of sharks to be tagged. Perhaps within a 10 to 20-year period, such project could generate usable and valid data for investigating Greenland shark growth rates and longevity.

12. Conclusion

The Greenland shark interacts to a high degree with commercial fisheries throughout the Arctic, yet most aspects of its fundamental biology are poorly elucidated or completely unknown. The main purpose of this thesis was to investigate the life span of the Greenland shark. By combining well-established radiocarbon dating techniques and investigations on feeding ecology and satellite tracking, tissue from the eye lens nucleus was used to produce Greenland shark longevity estimates.

Based on 28 juvenile, sub-adult and adult Greenland shark females, results show that sharks with total length of 258 cm or longer were not affected by the bomb pulse and hence older than 50 years. Smaller sharks were 50 years or younger. A Bayesian calibration model converted radiocarbon levels of the pre-bomb sharks into age estimates. The largest shark was between 272 and 512 years old (reported as 392 ± 120 years in A1). With a maximum age of at least 272 years, the Greenland shark is the longest living vertebrate animal known to science.

Besides demonstrating remarkable longevity, the investigations have revealed several novel aspects of Greenland shark biology. For example, an ontogenetic dietary shift was documented somewhere between 201-250 cm, as smaller sharks mainly fed on squids, while larger sharks mainly fed on fish and seals. Overall, Atlantic cod and harp seal appear as the most important prey items for sharks in Greenland waters. Surprisingly, redfish were found only to be important prey items for adult Greenland shark females. Feeding preferences of adult females matched well with their spatial distribution mainly encompassing southern Greenland shelf waters. The satellite tracking further revealed that movements in southern Greenland were not random and several sharks congregated off Frederikshåb Isblink. Other sharks performed synchronous long-distance-migrations from southwestern to southeastern Greenland. A single adult female shark visited the mid-Atlantic Ridge, which is interesting since the extremely rare neonate Greenland sharks have been reported only from that particular area. I hypothesize that abyssal mountain ridges serve as pupping ground and nursery areas for Greenland sharks.

In conclusion, the results presented in this thesis demonstrate that the Greenland shark is an exceptional animal with extraordinary longevity and life history. This highly migratory and opportunistic predator is likely to have a key role in the arctic marine food web, where each animal may represent centuries of natural history and selection.

13. Perspectives

After my first encounter with a Greenland shark onboard trawler RV Pâmiut in 2010, I quickly learned that these animals are detested by fishermen throughout the Arctic. The reason for this is their status as non-eatable poisonous creatures, which for decades have disturbed commercial long-lines and trawl fisheries, destroying catches and fishing gear. The full magnitude of Greenland shark bycatch is unknown, but it is fair to assume that thousands of animals are caught annually as unintended bycatch by demersal fisheries targeting e.g. Greenland halibut, Atlantic cod and Atlantic halibut (*Hippoglossus hippoglossus*). Given such high bycatch, the abundance of Greenland sharks must be high – at least in some Arctic regions – which is also the impression I got during five years of targeted field work. Greenland sharks do not seem to be endangered. However, it appears that most Arctic regions are dominated by sub-adult non-sexually mature specimens – teenagers basically – whereas adult and juvenile sharks are rarely encountered. I speculate if the current Greenland shark population is skewed towards sub-adult animals. Given the longevity estimates presented in this thesis, it is plausible that the older adult life stages (representing the effective part of the population) have not fully recovered from the intense commercial fishery carried out in the previous century. In that case, the population has not yet rebounded completely. This is, however, difficult to evaluate properly as the natural distribution of juveniles, sub-adults and adult specimens is unknown. From trade records it is though certain that the natural abundance of Greenland sharks in the North Atlantic encompass millions of animals.

The reason for the present day (apparently) high abundance of Greenland sharks is likely due to their enormous suitable habitat, encompassing all waters masses below 10° C from the surface to the abyss. This combined with their migratory behavior, as well as pregnant females potentially having high fecundity and protected abyssal pupping grounds and nursing areas, leads to a resilient population. Nonetheless, due to the general rarity of adult and juvenile sharks, I believe that all management must be precautionary and the most recent categorization of Greenland sharks on the Norwegian Red List of Threatened Species as “Data Deficient” seems appropriate. Therefore, any future initiatives for targeted fisheries and commercial utilization e.g. of their skin or meat is not recommendable anywhere in the Arctic. On the other hand, a trans-Atlantic tag-and-release program should be conducted by scientific institutions in collaboration with recreational anglers. This might be a way of gathering sufficient data for a database of tagged

sharks, which could allow for proper evaluation of growth rates, residency and migration patterns as well investigating the full population structure of Greenland sharks.

Besides finding the Greenland shark as an extremely long-lived animal, the findings of this thesis open up for further research possibilities – on Greenland sharks as well as other marine animals. Chemical composition of the eye lens has previously been applied for age investigation of difficult-to-age cetaceans, but the bomb radiocarbon dating of eye lens tissue of a marine animal has a broader applicability. For example, a combination of aspartic acid racemization (previously been applied on whales) and bomb radiocarbon dating could provide the wanted age validation of e.g. narwhale, bowhead whale and fin whale. These whales are all - like the Greenland shark - estimated to live for +100-200 years based on non-validated mathematical calculations of the chemical composition of the eye lens nucleus (see George et al. 1999, Garde et al. 2007, Nielsen et al. 2013, A1). Validating age estimates of long-lived whales using bomb radiocarbon dating would indirectly improve the accuracy of the Greenland shark age estimates, as would establishment of the bomb pulse onset in chronologies obtained from eye lens nucleus of long-lived deep ocean teleost. In general, any tissue of dietary origin with known-age is usable for elucidating the behavior of the bomb pulse in marine food webs, and would increase its applicability within age validation studies across the marine environment. Suitable animals for such future studies could be redfish and orange roughy (*Hoplostethus atlanticus*) reaching at least 75 years and 149 years, respectively (Campana et al. 1990, Fenton et al. 1991).

The oldest animal in the world is a 507 year old ocean quahog (Butler et al. 2013), whereas the oldest vertebrates is the bowhead whale reaching 211 years (George et al. 1999). Following these are giant tortoises (Testudinidae) which according to anecdotal reports can reach 180-255 years of age. From our study, the Greenland shark thus appears to be the longest living vertebrate. However, in contradiction to e.g. bowhead whales or giant tortoises, Greenland sharks are often caught as unintended bycatch. This bycatch is unavoidable and samples for future scientific projects shark are thus acquirable. This bycatch therefore represents a valuable opportunity for studying e.g. the genetic basis of longevity - a growing research field of which gut microbiomes seem to be of particular relevance (Biagi et al. 2016, Smith et al. 2017). Future projects exploring the hologenome of Greenland sharks could potentially contribute to drive aging research forward.

14. References

- Anderson EC, Libby WF, Weinhouse S, Reid AF, Kirshenbaum AD, Grosse AV (1947). Radiocarbon from cosmic radiation. *Science* 105, 576–577.
- Andrews AH, Kerr LA (2015). Validated age estimates for large white sharks of the northeastern Pacific Ocean: altered perceptions of vertebral growth shed light on complicated bomb $\Delta^{14}\text{C}$ results. *Environmental Biology of Fishes* 98: 971-978.
- Anon (1942). Beretninger vedr. Grønlands Styrelse. Sammendrag af Statistiske Oplysninger om Grønland 1: 647-652 (in Danish).
- Anthoni U, Christophersen C, Gram L, Nielsen NH, Nielsen P (1991). Poisonings from flesh of the Greenland shark *Somniosus microcephalus* may be due to trimethylamine. *Toxicol* 29: 1205-1212
- Bassnett S, Shi Y, Vrensen GFJM (2011). Biological glass: structural determinants of eye lens transparency. *Philosophical Transaction of the Royal Society B* 366: 1250-1264.
- Beck B, Mansfield AW (1969). Observations on the Greenland shark, *Somniosus microcephalus*, in Northern Baffin Island. *Journal of the Fisheries Research Board of Canada* 26:143-145.
- Benz GW, Borucinska JD, Lowry LF, Whiteley HE (2002). Ocular lesions associated with attachment of the copepod *Ommatokoita elongata* (Lernaeopodidae: Siphinostomatoida) to corneas of Pacific sleeper sharks *Somniosus microcephalus* captured off Alaska in Prince William Sound. *Journal of Parasitology* 88, 3: 474-481.
- Benz GW, Hoffmayer ER, Driggers WB, Allen D, Bishop LE, Brown DA (2007). First record of a sleeper shark in the western Gulf of Mexico and comments on taxonomic uncertainty within *Somniosus* (*Somniosus*). *Bulletin of Marine Science* 80, 2: 343-351.
- Berland B (1961). Copepod *Ommatokoita elongata* (Grant) in the eyes of the Greenland shark - a possible cause of mutual dependence. *Nature* 191: 829-830.
- Beverton RJH, Holt SJ (1957). On the Dynamics of Exploited Fish Populations, Fishery Investigations Series II Volume XIX, Ministry of Agriculture, Fisheries and Food.
- Bexton S, Thompson D, Brownlow A, Barley J, Milne R, Bidewell C (2012). Unusual Mortality of Pinnipeds in the United Kingdom Associated with Helical (Corkscrew) Injuries of Anthropogenic Origin. *Aquatic Mammals* 38, 3: 229-240.
- Biagi et al. (2016). Gut microbiota and extreme longevity. *Current Biology* 26:1480-1485.
- Bigelow HB, Schroeder WC (1948). Sharks *In* Fishes of the Western North Atlantic, Part 1 (Tee-Van J, Breder CM, Hildebrand SF, Parr AE, Schroeder WC, eds) pp. 59-546. Yale, CT: Yale University, Sears Foundation for Marine Research.
- Bjerkan P (1957). The reproduction problem of the Greenland Shark, Notes on the Greenland Shark. Fiskeridirektoratets Skrifter, Serie Havundersokelser. Report on Norwegian Fishery and Marine Investigations, XI, 10: 1-7.

- Bloemendal H (1977). The Vertebrate Eye Lens. *Science* 197, 4299: 127-138.
- Borucinska JD, Benz GW, Whiteley HE (1998). Ocular lesions associated with attachment of the parasitic copepod *Ommatokoita elongate* (Gant) to corneas of Greenland sharks, *Somniosus microcephalus* (Block & Schneider). *Journal of Fish Disease* 21: 415-422.
- Bowering WR, Lilly GR (1992). Greenland halibut (*Reinhardtius hippoglossoides*) off southern Labrador and Northeastern Newfoundland (Northwest Atlantic) feed primarily on capelin (*Mallotus villosus*). *Netherlands Journal of Sea Research* 29: 211-222.
- Boulton M, Albon J (2004). Stem cells in the eye. *The International Journal of Biochemistry & Cell Biology* 36: 643-657.
- Boustany AM, Davis SF, Pyl Pe, Anderson SD, Le Boef BJ, Block BA (2002). Satellite tagging: expanded niche for white sharks. *Nature* 415:35-36.
- Bowman S (1990). Interpreting the past, Radiocarbon. British Museum and the University of California: 9-49
- Bronk Ramsey C (2009). Dealing with Outliers and Offsets in Radiocarbon Dating. *Radiocarbon* 51: 1023-1045.
- Butler PG, Scourse JD, Richardson CA, Wanamaker AD, Bryant CL, Bennell JD (2009). Continuous marine radiocarbon reservoir calibration and the C-13 Suess effect in the Irish Sea: Results from the first multi-centennial shell-based marine master chronology. *Earth and Planetary Science Letters* 279: 230-241.
- Butler PG, Wanamaker AD, Scourse JD, Richardson CA, Reynolds DJ (2013). Variability of marine climate on the North Icelandic Shelf in a 1357-year proxy archive based on growth increments in the bivalve *Arctica islandica*. *Palaeogeography, Palaeoclimatology, Palaeoecology* 373: 141–151.
- Bøje O (1939). Toxins in the flesh of the Greenland shark. *Meddelelser om Grønland* 125: 3-16.
- Campana SE, Zwanenburg KCT, Smith JN (1990). $^{210}\text{Pb}/^{226}\text{Ra}$ Determination of longevity in redfish. *Canadian Journal of Fisheries and Aquatic Sciences* 47, 1: 163-165.
- Campana SE (1997). Use of radiocarbon from nuclear fallout as a dated marker in the otoliths of haddock *Melanogrammus aeglefinus*. *Marine Ecology Progress Series* 150: 49-56.
- Campana SE, Natanson LJ, Myklevoll S (2002). Bomb dating and age determination of large pelagic sharks. *Canadian Journal of Fisheries and Aquatic Sciences* 59: 450-455.
- Campana SE, Jones C, McFarlane GA, Myklevoll S (2006). Bomb dating and age validation using the spines of spiny dogfish (*Squalus acanthias*). *Environmental Biology of Fishes* 77: 327-336.
- Campana SE, Fisk AT, Klimley AP (2015a). Movements of Arctic and northwest Atlantic Greenland sharks (*Somniosus microcephalus*) monitored with archival satellite pop-up tags. *Deep-Sea Research Part II* 115: 109-115.

Campana SE, Valentin AE, MacLellan SE, Groot JB (2015b). Image-enhanced burnt otoliths, bomb radiocarbon and the growth dynamics of redfish (*Sebastes mentella* and *S. fasciatus*) off the eastern coast of Canada. *Marine and Freshwater Research* 67, 7: 925-936.

Chernova N, Smirnova E, Raskhozheva E (2015) First record of the Greenland shark *Somniosus microcephalus* (Squaliformes: Somniosidae) in the Siberian Arctic with notes on its distribution and biology. *Journal of Ichthyology* 55:827-835.

Christiansen JS (2012) The TUNU-Programme: Euro-Arctic marine fishes—diversity and adaptation. In: di Prisco G, Verde C (eds) *Adaptation and evolution in marine environments*, vol 1, From pole to pole. Springer, Berlin Heidelberg, pp 35–50. doi:10.1007/978-3-642-27352-0_3.

Christensen JT, Richardson K (2008). Stable isotope evidence of long-term changes in the North Sea food web structure. *Marine Ecology Progress Series* 368: 1-8.

Clarke MW, Connolly PL, Bracken JJ (2001). Aspects of reproduction of the deep water sharks *Centroscymnus coelolepis* and *Centrophorus squamosus* from west of Ireland and Scotland. *Journal of the Marine Biological Association of the United Kingdom* 81: 1019-1029.

Cohen AI (1965). The electron microscope of the normal human eye lens. *Investigative Ophthalmology & Visual Sciences* 4, 4: 433-446.

Compagno LJV (1984). *FAO species catalogue- Sharks of the world. An annotated and illustrated catalogue of shark species known to date.* FAO Fisheries Synopsis 125, 4, pp 655.

De Vries H (1958). Atomic Bomb Effect: Variation of Radiocarbon in Plants, Shells, and Snails in the Past 4 Years. *Science* 128: 250-251.

Estrada JA, Rice AN, Natanson LJ, Skomal GB (2006). Use of isotopic analysis of vertebrae in reconstruction ontogenetic feeding ecology in white sharks. *Ecology* 87, 4: 829-834.

FAO (2014). *Global Capture Production.* FAO FishStat
< <http://www.fao.org/fishery/species/2033/en>> (accessed 8 Nov 2017).

Fenton GE, Short SA, Ritz DA (1991). Age determination of orange roughy, *Hoplostethus atlanticus* (Pisces: Trachichthyidae) using ^{210}Pb : ^{226}Ra disequilibria. *Marine Biology* 109: 197-202.

Ferrando S, Gallus L, Ghigliotti L, Vacchi M, Nielsen J, Christiansen JS, Pisano E (2016). Gross morphology and histology of the olfactory organ of the Greenland shark (*Somniosus microcephalus*). *Polar Biology* 39, 8: 1399-1409.

Figueriredo I, Moura T, Neves A, Gordo LS (2008). Reproductive strategy of leafscale gulper shark *Centrophorus coelolepis* on the Portuguese continental slope. *Journal of Fish Biology*, 73: 206-225.

Fisk AT, Tittlemier SA, Pranscke JL, Norstrom RJ (2002). Using anthropogenic contaminants and stable isotopes to assess the feeding ecology of Greenland sharks. *Ecology* 83, 8: 2162-2172.

- Fisk AT, Lydersen C, Kovacs KM (2012). Archival pop-off tag tracking of Greenland sharks *Somniosus microcephalus* in the High-Arctic waters of Svalbard, Norway. *Marine Ecology Progress Series* 468: 255-265.
- Folkow LP, Nordøy ES, Blix AS (2004). Distribution and diving behaviour of harp seals *Pagophilus groenlandica* from the Greenland Sea stock. *Polar Biology* 27:281-298.
- Francis MP, Campana SE, Jones CM (2007). Age under-estimation in New Zealand porbeagle sharks (*Lamna nasus*): is there an upper limit to ages that can be determined from shark vertebrae? *Marine and Freshwater Research* 58: 10-23.
- Froese R, Pauly D, Editors (2017) FishBase. World Wide Web electronic publication. www.fishbase.org version (11/2017).
- Gallagher M, Nolan CP (1999). A novel method for the estimation of age and growth in rajids using caudal thornes. *Canadian Journal of Fisheries and Aquatic Sciences* 56, 9: 1590-1599.
- Gallant J (2010). Who is the corkscrew killer? < <http://www.geerg.ca/who-is-the-corkscrew-killer.html> > (assessed 23 Nov 2017).
- Garde E, Heide-Jørgensen MP, Hansen SH, Nachman G, Forchhammer MC (2007). Age-specific growth and remarkable longevity in narwhales (*Monodon monoceros*) from West Greenland as estimated by aspartic acid racemization. *Journal of Mammalogy* 88, 1: 49-58.
- George JC, Bada J, Zeh J, Scott L, Brown SE, O'Hara T, Suydam R (1999). Age and growth estimates of bowhead whales (*Balaena mysticetus*) via aspartic acid racemization. *Canadian Journal of Zoology* 77: 571-580.
- Hamady LL, Natanson LJ, Skomal GB, Thorrold SR (2014). Vertebral Bomb Radiocarbon Suggests Extreme Longevity in White Sharks. *Plos One* 9, 1: e84006.
- Hansen PM (1963a). Tagging Experiments with the Greenland shark (*Somniosus microcephalus* (Block and Schneider)) in Subarea 1. International Commission for the Northwest Atlantic Fisheries. Special Publication no. 4: 172-175.
- Hansen PM (1963b). Hager i Grønlandske Farvande. Skrivelse Danmarks Fiskeri- og Havundersøgelse: 83-95 (in Danish).
- Hansen JH, Hedeholm RB, Süksen K, Christensen JT, Grønkjær P (2012). Spatial variability of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) stable isotope ratios in an Arctic marine food web. *Marine Ecology Progress Series* 467: 47-59.
- Haug T, Nilssen KT, Lindblom L (2004). Feeding habits of harp and hooded seal in drift ice waters along the east coast of Greenland in summer and winter. *Polar Research* 23, 1: 35-42.
- Harry AV (2017). Evidence for systemic age underestimation in shark and ray ageing studies. *Fish Fisheries* 2017;00: 1-16.

Harvey-Clark CJ, Gallant JJ, Batt JH (2005). Vision and its relationship to novel behaviour in St. Lawrence River Greenland Sharks, *Somniosus microcephalus*. Canadian Field-Naturalist 119, 3: 355–359.

Heide_Jørgensen MP, Teilmann J (1994), Growth reproduction, age structure and feeding habits of white whales (*Delphinapterus leucas*) in West Greenland waters, Meddelelser om Grønland, Bioscience 39: 195-211.

Henriksen S, Hilmo O, red (2015). Norsk rødliste for arter 2015. Artsdatabanken, Norge (in norwegian).

Herdendorf CE, Berra TM (1995). A Greenland Shark from the wreck of *SS Central America* at 2,200 meters. Transactions of the American Fisheries Society 124, 6: 950-953.

Hoening JM and Gruber SH (1990). Life history Patterns in the Elasmobranchs: Implications for Fisheries Management, page 1-16 in “Elasmobranchs as Living Resources: Advances in the Biology, Ecology, Systematics and the Status of the Fisheries” (Pratt HI, Gruber SH, Taniuchi T, editors). NOAA Technical Report 90.

Hovde SC, OT Albert OT, Nilssen EM (2002). Spatial, seasonal and ontogenetic variation in diet of Northeast Arctic Greenland halibut (*Reinhardtius hippoglossoides*). ICES Journal of Marine Science 59: 421-437.

Hussey NE, Cosandey-Godin A, Walter RP, Hedges KJ, VanGerwen-Toyne M, Barkley AN, Kessel ST, Fisk AT (2014). Juvenile Greenland sharks *Somniosus microcephalus* (Bloch & Schneider, 1801) in the Canadian Arctic. Polar Biology 38, 4: 493-504.

ICES (2017) Report of the North Western Working Group (NWWG). 27 April-4 May 2017, Copenhagen, Denmark. ICES CM 2017/ACOM:08. 642 pp.

Idrobo CJ (2008). The Pingnirtung and the Greenland shark. MSc Thesis, Natural Resources Institute. University of Manitoba, Canada.

Idrobo CJ, Berkes F (2012) Pangnirtung inuit and the Greenland shark: co-producing knowledge of a little discussed species. Human Ecology 40: 405-414.

IUCN (2017). The IUCN Red List of Threatened Species. Version 2017-2. <<http://www.iucnredlist.org/details/60213/0>> (assessed 28 Sep 2017).

Jenkins JT (1903). The Fishes of the British Isles Both Fresh Water and Salt. Frederik Warne & Co., Ltd., London, pp 325.

Jensen AS (1914). The Selachians of Greenland. Zoological Museum of the University Copenhagen. Bianco Lunos Bogtrykkeri: 8-17 (in danish).

Johansen JB (2002). Shark meat shouldn't go to the dogs. Suluk 1: 50-51.

Jones TJ, Ugland KL (2001). Reproduction of female spiny dogfish, *Squalus acanthias*, in the Oslofjord. Fishery Bulletin 99, 4: 685-690.

Jónsson, J (1994). Útgerð og aflabrögð við Ísland 1300-1900. Hafrannsóknir 48, 1-107 (in Icelandic).

Joyce WN, Campana SE, Natanson LJ, Kohler NE, Pratt HL Jr, Jensen CF (2002). Analysis of stomach contents of the porbeagle shark (*Lamna nasus* Bonnaterre) in the northwest Atlantic. ICES Journal of Marine Science 59: 1263-1269.

Kalish JM (1993). Pre- and post-bomb radiocarbon in fish otoliths. Earth and Planetary Science Letters 114: 549-554.

Kalish JM, Nydal R, Nedreaas KH, Burr GS, Eine GL (2001). A time history of pre- and post-bomb radiocarbon in the Barents Sea derived from Arcto-Norwegian cod otoliths. Radiocarbon 43, 2B: 843-855.

Kerr CA, Andrews AH, Caillet GM, Brown TA, Coale KH (2006). Investigations of $D14C$, $d13C$, and $d15N$ in vertebrae of white shark (*Carcharodon carcharias*) from the eastern North Pacific Ocean. Environmental Biology of Fishes 77: 337-353.

King JR, McPhie RP (2015). Preliminary age, growth and maturity estimates of Spotted Ratfish (*Hydrolagus colliei*) in British Columbia. Deep-Sea Research Part II 115: 55-63.

Kinze CC, ed. (2001). Havpattedyr I Nordatlanten – En felthåndbog fra Gads Forlag (Gad). pp. 359. ISBN-13: 9788712034209 (in Danish).

Koefoed E (1957). A uterine Foetus and the Uterus from a Greenland Shark, Notes on the Greenland Shark. Fiskeridirektoratets Skrifter, Serie Havundersokelser (Report on Norwegian Fishery and Marine Investigations), XI, 10: 8-12.

Kukuev EI, Trunov IA (2002). The composition of ichthyofauna of the meso- and bathypelagic zones of the Irminger Current and adjacent waters. Journal of Ichthyology 42, 377-384 (in Russian).

Kröger RHH (2013). Optical plasticity in fish lenses. Progress in Retinal and Eye Research 34: 78-88.

Kyne PM, Simpendorfer CA (2010). “Adaptive Physiology and Conservation” in *Sharks and Their Relatives*, Carrier JC, Musick JA, Heithaus MR, Eds. CRC Press, pp. 37-71.

Leclerc LM, Lydersen C, Haug T, Glover KA, Fisk AT, Kovacs KM (2011). Greenland shark (*Somniosus microcephalus*) scavenge offal from minke (*Balaenoptera acutorostrata*) whaling operations in Svalbard, Norway. Polar Research 30, 7342, DOI: 10.3402/polar.v30i0.734.

Leclerc LM, Lydersen C, Haug T, Bachmann L, Fisk AT, Kovacs KM (2012). A missing piece in the Arctic food web puzzle? Stomach contents of Greenland sharks sampled in Svalbard, Norway. Polar Biology 35: 1197-1208.

Libby WF (1960). Radiocarbon dating. Nobel Lecture, December 12, 1960. Downloaded from: https://www.nobelprize.org/nobel_prizes/chemistry/laureates/1960/libby-lecture.pdf

- Lockyer C (1972). The Age at Sexual Maturity of the Southern Fin Whale (*Balaenoptera Physalus*) Using Annual Layer Counts in the Ear Plug. *ICES Journal of Marine Science* 34, 2: 276-294.
- Lucas ZN, Natanson LJ (2010). Two shark species involved in predation on seals at Sable Island, Nova Scotia, Canada. *Proceedings of the Nova Scotian Institute of Science* 45: 64-88.
- Lynnerup N, Kjeldsen H, Heegaard S, Jacobsen C, Heinemeier J (2008). Radiocarbon Dating of the Human Eye Lens Crystalines Reveal Proteins without Carbon Turnover throughout Life. *PLoS ONE* 3, 1: e1529. doi:10.1371/journal.pone.0001529.
- Lynnerup N, Kjeldsen H, Zwihehoff R, Heegaard S, Jacobsen C, Heinemeier J (2010). Ascertaining year of birth/age at death in forensic cases: A review of conventional methods and methods allowing for absolute chronology. *Forensic Science International* 201: 74-78.
- MacNeil MA, McMeans BC, Hussey NE, Vecsei P, Svavarsson J, Kovacs KM, Lydersen C, Ramsey M, Fisk AT (2012). Biology of the Greenland shark *Somniosus microcephalus*. *Journal of Fish Biology*, 80: 991-1018.
- Mangerud J, Bondevik S, Gulliksen S, Hufthammer AK, Høisaeter T (2006). Marine C-14 reservoir ages for 19th century whales and molluscs from the North Atlantic. *Quaternary Science Reviews* 25: 3228-3245.
- Martin AR, Smith TG (1992). Deep diving in wild, free-ranging Beluga whales, *Delphinapterus leucas*. *Canadian Journal of Fisheries and Aquatic Sciences* 49: 462-466.
- Matta ME, Tribuzio CA, Ebert DA, Goldman KJ, Gburski CM (2017). Chapter six: Age and Growth of Elasmobranchs and Applications to Fisheries Management and Conservation in the Northeast Pacific Ocean. *Advances in Marine Biology* 77: 179-220.
- McMeans BC, Svavarsson J, Dennard, Fisk AT (2010). Diet and resource use among Greenland sharks (*Somniosus microcephalus*) and teleosts sampled in Iceland waters, using $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and mercury. *Canadian Journal of Fishery Aquatic Science* 67: 1428-1438.
- McMeans BC, Arts MT, Lydersen C, Kovacs KM, Hop H, Falk-Petersen S, Fisk AT (2013). The role of Greenland sharks (*Somniosus microcephalus*) in an Arctic ecosystem: assessed via stable isotopes and fatty acids. *Marine Biology* 160: 1223-1238.
- McPhie RP, Campana SE (2009). Bomb dating and age determination of skates (family Rajidae) off the eastern coast of Canada. *ICES Journal of Marine Science* 66: 546-560.
- Merve NJ (1982). Carbon isotopes, photosynthesis and archaeology: Different pathways of photosynthesis cause characteristic changes in carbon isotopes that make possible the study of prehistoric human diets. *American Scientist* 70, 6: 596-606.
- Murray BW, Wang JY, Yang SC, Stevens JD, Fisk AT, Svavarsson J (2008). Mitochondrial cytochrome b variation in sleeper sharks (Squaliformes, Somniosidae). *Marine Biology* 153: 1432-1793.
- Møller PRM, Nielsen JG, Knudsen SW, Poulsen JY, Sünksen K, Jørgensen OA (2010). A checklist of the fish fauna of Greenland waters. *Zootaxa* 2378: 1-84.

- Nielsen, NH, Garde E, Heide-Jørgensen MP, Lockyer CH, Ditlevsen S, Ólafsdóttir D, Hansen SH (2013). Application of a novel method for age estimation of a baleen whale and a porpoise. *Marine Mammal Science*. doi: 10.1111/j.1748-7692.2012.00588.x.
- Nielsen J (2013). Age estimation and feeding ecology of Greenland sharks *Somniosus microcephalus* in Greenland waters. MSc Thesis, Aquatic Science and Technology, Danish Technical University, Denmark.
- Nielsen J, Hedeholm RB, Simon M, Steffensen JF (2014). Distribution and feeding ecology of the Greenland shark (*Somniosus microcephalus*) in Greenland waters. *Polar Biology* 37: 37–46.
- Nielsen et al. (in prep1). Reproduction biology of the Greenland shark (*Somniosus microcephalus*) assessed from novel observations and a review of the existing literature.
- Nielsen et al. (in prep2). Spatial distribution and composition of size-based life stages of Greenland shark *Somniosus microcephalus* across the northern North Atlantic.
- Pearcy WG, Stuvier M (1983). Vertical transport of carbon-14 into deep-sea food webs. *Deep-Sea Research* 30, 4A: 427-440
- Porteiro FM, Sutton TT, Byrkjedal I, Orlov AM, Heino M, Menezes G, Bergstad OA (2017). Fishes of the northern Mid-Atlantic Ridge collected during the MAR-ECO cruise in June-July 2004: an annotated checklist. *Arquipelago Life and Marine Sciences Supplement* 10.
- Reimer PJ, Bard E, Bayliss A, et al. (2013). IntCal13 and Marine13 radiocarbon age calibration curves 0-50,000 years cal BP. *Radiocarbon* 55, 4:1869-1887.
- Rusyaev SM, Orlov AM (2013) Bycatches of the Greenland Shark *Somniosus microcephalus* (Squaliformes, Chondrichthyes) in the Barents Sea and Adjacent Waters under Bottom Trawling Data. *Journal of Ichthyology* 53 1: 111-115.
- Saunders RA, Royer F, Clarke MW (2011). Winter migration and diving behaviour of porbeagle shark, *Lamna nasus*, in the Northeast Atlantic. *ICES Journal of Marine Science* 68: 166-174.
- Schöne BR, Wanamaker AD, Fiebig J, Thebault J, Kreutz K (2011). Annually resolved delta C-13 (shell) chronologies of long-lived bivalve mollusks (*Arctica islandica*) reveal oceanic carbon dynamics in the temperate North Atlantic during recent centuries. *Palaeography, Palaeoclimatology, Palaeoecology* 30, 2: 31-42.
- Scourse JD Wanamaker AD, Weidman C, Heinemeier J, Reimer PJ, Butler PG, Witbaard R, Richardson CA (2012). The marine radiocarbon bomb pulse across the temperate North Atlantic; a compilation of $\Delta^{14}\text{C}$ time histories from *Arctica islandica* growth increments. *Radiocarbon* 54, 2: 165-186.
- Skomal GB, Benz GW (2004). Ultrasonic tracking of Greenland sharks, *Somniosus microcephalus*, under Arctic ice. *Marine Biology* 145: 489-498.

Smith P, Willemsen D, Popkes M, Metge F, Gandiwa E, Reichard M, Valenzano DR (2017). Regulation of Life Span by the Gut Microbiota in The Short-Lived African Turquoise Killifish. *eLife* doi: 10.7554/eLife.27014.

Stewart REA, Campana SE, Jones CM, Stewart BE (2006). Bomb radiocarbon dating calibrates beluga (*Delphinapterus leucas*) age estimates. *Canadian Journal of Zoology* 84: 1840-1852.

Stokesbury MJW, Harvey-Clark C, Gallant J, Block BA, Myers RA (2005). Movement and environmental preferences of Greenland sharks (*Somniosus microcephalus*) electronically tagged in the St. Lawrence Eustuary, Canada. *Marine Biology* 148: 159-165.

Stuvier M, Polach HA (1977). Reporting of 14-C data-discussion. *Radiocarbon* 19: 355-363.

Stuvier M, Braziunas TF (1993). Modeling atmospheric 14C influences and 14C ages of marine samples to 10,000 BC. *Radiocarbon* 35, 1: 137-90.

Tans PP, Dejong AFM, Mook WG (1979). Natural Atmospheric C-14 Variation and the Suess Effect. *Nature* 280, 826-827.

Taylor VL, Al-Ghoul KJ, Lance CW et al (1996). Morphology of the human eye lens. *Investigative Ophthalmology & Visual Sciences* 37: 1396.

Templeman W (1963). Distribution of sharks in the Canadian Atlantic (with special reference to Newfoundland waters). *Bulletin of the Fisheries Research Board of Canada* 140: 36-42.

Treble MA, Campana SE, Wastle RJ, Jones CM, Boje J (2008). Growth analysis and age validation of a deepwater Arctic fish, the Greenland halibut (*Reinhardtius hippoglossoides*). *Canadian Journal of Fisheries and Aquatic Sciences* 65: 1047-1059.

Veríssimo A, Gordo L, Figueiredo (2003). Preproductive biology and embryonic development of *Centroscymnus coelolepis* in Portuguese mainland waters. *ICES Journal of marine Science* 60: 1335-1341.

Walter RP, Roy D, Hussey NE, et al. (2017). Origins of the Greenland shark (*Somniosus microcephalus*): Impacts of ice-olation and introgression. *Ecology and Evolution*; 00: 1–13. DOI: 10.1002/ece3.3325.

Williams PM, McGowan JA, Stuvier M (1970). Bomb carbon-14 in deep ocean sea organisms. *Nature* 227: 375-376.

Williams PM, Druffel ERM, Smith Jr KL (1987). Dietary carbon sources for deep-sea organisms as inferred from their organic radiocarbon activities. *Deep-Sea Research* 34, 2: 253–266.

Wistow GJ, Piatigorsky J (1988). Lens crystallines: the evolution and expression of proteins for a highly specialized tissue. *Annual Review of Biochemistry* 57: 479-504.

Yano K (1995). Reproductive biology of the Black dogfish *Centroscyllium fabricii*, collected from waters off western Greenland. *Journal of Marine Biology* 75: 285-310.

Yano K., Stevens JD, Compagno LJV (2004). A review of the systematics of the sleeper shark genus *Somniosus* with redescrptions of *Somniosus (Somniosus) antarcticus* and *Somniosus (Rhinoscyrnus) longus* (Squaliformes, Somniosidae). *Ichthyological Research* 51: 360-373.

Yano K, Stevens JD, Compagno LJV (2007). Distribution, reproduction and feeding of the Greenland shark *Somniosus (Somniosus) microcephalus*, with notes on two other sleeper sharks, *Somniosus (Somniosus) pacificus* and *Somniosus (Somniosus) antarcticus*. *Journal of Fish Biology* 70: 374-390.

15. Appendix I

Published

Ademollo N, Patrolecco L, Rauseo J, **Nielsen J**, Corsolini (2017). Bioaccumulation of nonylphenols and bisphenol A in the Greenland shark *Somniosus microcephalus* from the Greenland seawaters. *Microchemical Journal* 136: 106-112.

Sanataquiteria A, **Nielsen J**, Klemetsen T, Willasen NP, Præbel K (2017). The complete mitochondrial genome of the long-lived Greenland shark (*Somniosus microcephalus*): characterization and phylogenetic position. *Conservation Genetics Resources* 9, 3: 351-355.

Christiansen JS, Bonsdorff E, Byrkjedal I, Fevolden SE, Karamushko OV, Lynghammar A, Mecklenburg CW, Møller PDR, **Nielsen J**, Nordstrøm MC, Præbel K, Wienerroither RM (2016). Novel biodiversity baselines outpace models of fish distribution in Arctic waters. *The Science of Nature* (2016) 103: 8. <https://doi.org/10.1007/s00114-016-1332-9>.

Constantini D, Smith S, Killen SS, **Nielsen J**, Steffensen JF (2016). The Greenland shark: A new challenge for the oxidative stress theory of ageing? *Comparative Biochemistry and Physiology, Part A* 203: 227-232.

Ferrando S, Gallus L, Ghigliotti C, Vacchi M, **Nielsen J**, Christiansen JS, Pisano E (2015). Gross morphology and histology of the olfactory organ of the Greenland shark *Somniosus microcephalus*. *Polar Biology* 39, 8: 1399-1409.

Nielsen J, Hedeholm RB, Simon M, Steffensen JF (2014). Distribution and feeding ecology of the Greenland shark (*Somniosus microcephalus*) in Greenland waters. *Polar Biology* 37: 37–46.

In preparation

Nielsen J et al. (In prep). Spatial distribution and composition of size-based life stages of Greenland shark *Somniosus microcephalus* across the northern North Atlantic. In preparation.

Nielsen J et al. (In prep) Reproduction biology of the Greenland shark (*Somniosus microcephalus*) assessed from novel observations and a review of the existing literature.

Nielsen J et al. (in prep). The genetic structure of Greenland shark (*Somniosus microcephalus*) in the North Atlantic assessed from restriction site associated DNA markers (RAD) sequencing

Nielsen J et al. (in prep). Satellite tracking of Greenland sharks in Vågsfjorden, northern Norway

McClusky L, **Nielsen J**, Christiansen JS (In prep). Spermatogenesis at icy depths: insights from the testis of the sexually mature Greenland shark.

Ona E, **Nielsen J** (In prep). Acoustic detection and measurements on the Greenland shark (*Somniosus microcephalus*) using multifrequency split beam echo sounders.

Figueiredo F et al. (in prep). DNA barcoding and characterisation of the ectoparasitic fauna of Greenland shark (*Somniosus microcephalus*).

Præbel et al. (in prep). The mitogenomic phylogeny of the extremely long-lived Greenland shark

16. Manuscripts

MS1

1 **Ontogenetic dietary shift in Greenland shark (*Somniosus microcephalus*)**

2 Julius Nielsen^{1,2,3}, Peter Grønkjær⁴, Peter Bushnell⁵, Jørgen Schou Christiansen⁶, Helene Overgaard⁴, Kim
3 Præbel⁷, John Fleng Steffensen¹, Rasmus Hedeholm²

4

5 **Affiliations**

6 1) Marine Biological Section, University of Copenhagen, Strandpromenaden 5, 3000 Helsingør, Denmark.

7 2) Greenland Institute of Natural Resources, Post Office Box 570, Kivioq 2, 3900 Nuuk, Greenland

8 3) Den Blå Planet, National Aquarium Denmark, Jacob Fortlingsvej 1, 2770 Kastrup, Denmark

9 4) Department of Bioscience, Section for Aquatic Biology, Ole Worms Allé 1, 8000 Aarhus C, Denmark

10 5) Department of Biological Sciences, Indiana University South Bend, 1700 Mishawaka Avenue, South Bend,
11 IN, USA.

12 6) Department of Arctic and Marine Biology, UiT The Arctic University of Norway, 9037 Tromsø, Norway

13 7) Norwegian College of Fishery Science, UiT The Arctic University of Norway, 9037 Tromsø Norway

14

15 *Corresponding author: juliusnielsen88@gmail.com

16

17 **Key words:** Stomach content, stable isotopes, Greenland shark, Arctic

18

19 **Abstract**

20 Greenland shark is an abundant key predator in all of the northern North Atlantic. Data on Greenland shark
21 feeding are scarce and based on a narrow size range. Therefore, results differ between studies and any
22 ontogenetic changes in feeding preferences are unknown. Using stomach content and stable isotopes we
23 describe the feeding of juvenile, sub-adult and adult sharks, and relate the feeding pattern to the

24 distribution of Greenland shark. We collected sharks in 2012-2017 from Greenland waters (N=109) and
25 grouped them in nine classes according to size (range 81-474 cm). We estimated the reconstructed biomass
26 of each prey group and estimated prey group importance from the Index of Relative Importance (IRI).
27 'Squids' was the dominant prey group in small sharks (< 200 cm). 'Fish' dominated in larger sharks (> 200
28 cm) and 'Mammal' became increasingly important with size. The stable isotopes showed the same result,
29 with difference in ¹⁵N values being between small and large sharks. In addition to demonstrating an
30 ontogenetic shift in diet, we also show large spatial variation in species-specific prey composition. The
31 study supports that Greenland shark is an active predator capable of catching both live fish and mammal
32 prey.

33

34 **Introduction**

35 During the past 10 years, the Greenland shark (*Somniosus microcephalus*) has been subject to increased
36 scientific focus, and is now recognized as a long-lived, highly migratory and likely abundant key predator in
37 arctic marine food webs (MacNeil et al. 2012, Campana et al. 2015, Nielsen et al. 2016). However, even for
38 the most well-studied aspects Greenland shark biology such as feeding ecology and distribution the
39 conclusions rely on studies based on small samples sizes (<40 sharks). Similar studies on feeding ecology of
40 other (and more easily accessible) shark species are based on datasets of more than 1000 sharks (see
41 Jacobsdóttir 2001, Joyce et al. 2002). The consequences of sparse knowledge on the feeding preferences of
42 Greenland shark is exemplified by conflicting results. Yano et al. (2007) report Greenland halibut
43 (*Reinhardtius hippoglossoides*) as the most important prey in Greenland waters while Atlantic cod (*Gadus*
44 *morhua*) is of little importance. In contrast, Nielsen et al. (2014) demonstrates the opposite pattern in the
45 region. Furthermore, current knowledge on feeding ecology is mostly based on medium-sized sharks (250-
46 350 cm, Fisk et al. 2002, McMeans et al. 2010, Leclerc et al. 2012, McMeans et al. 2013), and studies
47 including both large, medium and/or small Greenland sharks the data has been pooled (Yano et al. 2007,
48 McMeans et al. 2010, Nielsen et al. 2014). Fisk et al. (2002) reports on depleted ¹⁵N levels in two small
49 Greenland sharks compared to 15 larger medium-sized sharks, but besides this study it has not been
50 examined whether Greenland sharks exhibit life-stage related differences in feeding ecology (i.e.
51 ontogenetic dietary shifts). Such shifts are well-documented in other large predatory sharks such as
52 sevengill shark (*Notorynchus cepedianus*), white shark (*Carcharodon carcharias*), and tiger shark
53 (*Galeocerdo cuvier*) (Lowe et al. 1996, Ebert 2002, Estrada et al. 2006). For these sharks, the importance of

54 major prey categories such as invertebrates, fishes and mammals vary in importance during different life
55 stages.

56 The Greenland shark is distributed across the northern North Atlantic (MacNeil et al. 2012) and with a
57 maximum total length of at least 550 cm and body mass well beyond 1000 kg, it is the largest fish in the
58 Arctic, and among the largest carnivore sharks in the world. In some areas, Greenland sharks have
59 repeatedly been encountered at shallow depths (<30 m, Harvey et al. 2005, Eriksen 2011) but they are
60 mostly associated with deep ocean waters (below 200 m, MacNeil et al. 2012). In Greenland waters,
61 Greenland sharks are most often encountered at depths between 400-700 m (Nielsen et al. 2014) but have
62 been observed to occupy depths of at least 1,600 m (Bushnell et al. unpublished data). The deepest record
63 of Greenland shark is from 2,909 m near the mid-Atlantic Ridge (Poteiro et al. 2017).

64 Whether Greenland sharks occupy specific depths during various life stages and this subsequently leads to
65 different life stage specific prey compositions is unknown. It is, however, evident from spatial analyses of
66 data on sizes of Greenland shark between regions across the northern North Atlantic, that life stages
67 (juvenile, sub-adult and adults) are not distributed homogenously (Nielsen et al. in prep1). Combining
68 knowledge on distribution with knowledge on size specific feeding patterns will allow for a more complete
69 understanding of the Greenland shark's role as a key arctic predator. In this study, we investigated the
70 feeding ecology of Greenland sharks of various sizes using detailed snap shots from stomach content and
71 evaluated this against the integrated feeding pattern reflected in the isotopic signature. This will provide
72 mor detailed insights into life history traits of a difficult-to-study species listed as 'Near Threatened' on the
73 IUCN Red List of Threatened Species and as 'Data Deficient' of the Norwegian Red List (Henriksen & Hilmo
74 2015, IUCN 2017).

75

76 **Materials and methods**

77 *Sampling*

78 Greenland sharks were sampled from a range of sampling platforms in 2013-2017 across Greenland waters
79 under the auspices of the internal collaboration project 'Old & Cold - Greenland shark project'. Sampling
80 platforms included the Greenland Institute of Natural Resources (GINR) annual fish survey (RV Pâmiut, RV
81 Sanna), the TUNU IV and V expeditions (RV Helmer Hansen), the commercial trawler Sisimiut and targeted
82 Greenland shark expeditions (RV Porsild, RV Sanna). From these platforms, 78 sharks were caught of which
83 stomach content was evaluated from 73 sharks. Overlap in both sampling area and season allowed us to

84 pool stomach content data from the above-mentioned sharks with another 30 Greenland sharks caught in
85 2012 (reported in Nielsen et al. 2014). The total number of Greenland sharks in this study is therefore 108
86 (Fig. 1). All sharks were measured (total length, cm) and the sex was determined from the presence (males)
87 or absence (females) of claspers at the pelvic fins. Data from males and females were combined for all
88 analysis. To evaluate possible size related feeding differences, sharks were grouped in length bins: <200 cm,
89 201-250 cm, 251-300 cm, 301-350 cm, 351-400 cm and > 401 cm, which from here on will be referred to as
90 the 'shark size groups'..

91 *Stomach content*

92 Stomach content of the sharks was either processed immediately after catch or stored at -20° C and
93 shipped to GINR for later examination. Each stomach was examined as described in Nielsen et al. (2014),
94 with all individual prey items being determined to the lowest possible taxonomical level. Individual prey
95 items were grouped in five different overall prey categories: 'Fish', 'Mammal', 'Squid', 'Crustacean', and
96 'Other'. The 'Other' category included birds and all invertebrates except squids and crustaceans. 'Non-prey
97 items' encompass small rocks/stones, fishing gear, macro algae, metal pieces, fragments of Porifera, small
98 bivalves (<1 cm) and lysianassid amphipods. For these only frequency of occurrence was reported.

99 *Reconstructed biomass*

100 A 'reconstructed biomass' was calculated for all prey items reflecting the original amount consumed by the
101 shark. For each fish prey item reconstructed biomass was calculated from species or genus-specific length-
102 weight relationship acquired from Fishbase (Froese & Pauly 2017). For each mammal prey item, no
103 calculations were made to reconstruct the original biomass. Therefore the reconstructed biomass of each
104 mammal prey item (which is needed for calculation purpose) equaled the measured original wet mass. For
105 each squid prey item, length and reconstructed biomass were calculated from beak size according to
106 Zumhols & Frandsen (2006). For amphipods (Decapoda), crabs (Brachyura), shrimps (Caridae), birds, brittle
107 stars (Ophiuroidea), sea cucumbers (Holothuroidea), sea anemones (Arctinaria), sea urchins (Echinoidea),
108 sun stars (Solasteridea), starfish (Asteroidea) and snails (Gastropoda), the reconstructed biomass was based
109 on wet weight of intact individuals. To provide an overview of the composition of overall prey categories
110 (i.e. 'Fish', 'Mammal', 'Squid', 'Crustacean' and 'Other') across shark size, the proportional reconstructed
111 biomass for each overall prey category was evaluated for the six shark size groups.

112

113

114 *Index of Relative Importance*

115 For sharks size groups with similar proportion between the overall prey categories (sharks <200 cm were
116 different from sharks >200 cm, see Results), an index of relative importance (IRI) was calculated for each
117 prey group (i.e. for sharks <200 cm and >200 cm, separately). IRI was calculated using the numerical
118 importance (%N), the reconstructed biomass (%B) and the frequency of occurrence (%F) as described in
119 Nielsen et al. (2014). IRI was further calculated separately for each shark size group and the importance of
120 various prey, is presented as summarized IRI-scores for overall prey categories and as summarized IRI-
121 scores at prey family level.

122 *Stable isotopes*

123 White muscle tissue taken from above the vertebra in front of the dorsal fin was collected from 38 sharks
124 for stable isotope analysis. Stable nitrogen analyses were performed at Aarhus University as described in
125 Hansen et al. (2012) and expressed in a δ notation as the deviation from international standards in parts
126 per thousand (‰) according to the formula:

$$\delta^{15}\text{N} = \left[\left(\frac{R_{\text{sample}}}{R_{\text{standard}}} \right) - 1 \right] \times 1000$$

127 where R is the corresponding ratio $^{15}\text{N}/^{14}\text{N}$. Standards for $\delta^{15}\text{N}$ were calibrated against atmospheric air.
128 Statistical analysis was carried out in statistical computing program R (R development Core team 2017) and
129 GraphPad Prism 6. Analysis of variance and post-hoc Tukey HSD test were used to analyze variation in $\delta^{15}\text{N}$
130 levels between shark size categories.

131

132 **Results**

133 *Sampling and stomach content*

134 All sampled sharks are listed in Table 1 and catch locations are shown on Fig. 1. The sharks were caught
135 from May to September. Males were between 104 and 372 cm (N=27), females between 81 and 474 cm
136 (N=79) and two sharks were of unknown sex (Fig. 2). In total, 108 sharks were caught, and stomachs were
137 available from 103 of which 14 were empty (Table 1). The stomach content analyses are therefore based on
138 88 non-empty stomachs (81-474 cm, Table 1). The stomach content wet mass ranged between 0.029 and
139 52.0 kg (mean \pm SD:4.7 \pm 8.7 kg) and weighed 509.1 kg combined. Included in this is 3.5 kg which was 'Non-
140 prey items'. Of the remaining 505.6 kg, 96.4% could be assigned to an overall prey category ('Fish',

141 'Mammal', 'Squid', 'Crustacean' or 'Other'), while the remaining stomach content was highly digested
142 biological material. In total, 679 individual prey items were identified representing 57 different prey groups.
143 These prey groups are listed according to family under each overall prey category in Table 2.

144 *Reconstructed biomass and Index of Relative Importance*

145 The total reconstructed biomass for all 679 individual prey items was 624.9 kg. When reconstructed
146 biomass for overall prey categories was evaluated across shark size, 'Squid' was the most important for
147 sharks <200 cm, whereas 'Fish' and 'Mammal' are of major importance for all sharks >200 cm (Fig. 3). For
148 all these larger size groups, 'Fish' constituted approximately 70% of reconstructed biomass, and 'Mammal'
149 became gradually more important with shark size increasing from 10% to 20% (Fig. 3). For sharks <200 cm,
150 armhook squid (*Gonatus* spp.) was present in 100% of investigated stomachs and found to be the single
151 dominating prey (IRI=93.7%, Table 2). For sharks >200 cm, Atlantic cod (IRI=26.6%), unidentified teleost,
152 Greenland halibut, Skates (Rajidae), lumpfish (*Cyclopterus lumpus*), seal (Pinnipedia), armhook squid and
153 spotted wolffish (*Anarhichas minor*) were found as the main prey (Table 2).

154 The 57 identified prey groups represented the following families: Righteye flounders (Pleuronectidae), Cod
155 (Gadidae), Skates (Rajidae), Wolfish (Anarhichadidae), Lump suckers (Cyclopteridae), Sculpins (Cottidae),
156 Eelpouts (Lycodinae), Fatheads (Psychrolutidae), Redfish (Sebastidae), Sleeper shark (Somniosidae), Hagfish
157 (Myxinidae), Mackerels (Scombridae), Grenadier (Macrouridae), Argentine (Argentinidae), Salmon
158 (Salmonidae), Seal, Whale, Bear, Squid, Crab, Shrimp, Bird, Shrimp and Marine sea spider. Summarized IRI
159 for each prey family group is presented for each shark size group in Table 3. Common for groups 251-300
160 cm (N=17), 301-350 cm (N=25), 351-400 cm (N=19) and >400 cm (N=18), was that Cod family (dominated
161 by Atlantic cod), Unidentified teleost, and Seal (dominated by harp seal) had IRI values above five. On the
162 contrary, Righteye flounders, dominated by Greenland halibut, only had IRI above five for the two smallest
163 shark size groups (%IRI of 29.8 and 10.7, respectively), whereas Righteye flounders for the two largest sizes
164 classes had IRI values below 2.5. Redfish (*Sebastes* spp.) were only important in the largest sharks >401 cm
165 (IRI>5) which was also the size group in which skates were of least importance.

166 *Stable isotopes*

167 $\delta^{15}\text{N}$ ranged between 11.8 and 17.2 ‰ (mean \pm SD: 15.2 \pm 1.2‰, N=38) (Fig. 4). There was a clear effect
168 between shark size categories (ANOVA, F=5.81, df=36, P<0.01), with the smallest sharks (<200 cm) having a
169 significantly lower $\delta^{15}\text{N}$ levels compared to the three largest categories (post-hoc Tukey HSD test, P<0.01,
170 Fig. 4). The 251-300 cm category was not significantly different from any of the other categories (post-hoc
171 Tukey HSD test, P>0.1) (Fig. 4). The $\delta^{15}\text{N}$ level for the 201-250 cm category was represented by one

172 measurement only and was not included in the analysis, but the value was similar to that of the larger
173 categories.

174

175 **Discussion**

176 Greenland sharks are presumably born at ~40 cm and can reach a size of at least 550 cm in length. This
177 study describes the prey of Greenland sharks in almost the entire size range. Small Greenland sharks <200
178 cm (juveniles) predominantly feed on prey of lower trophic level compared to larger individuals which is in
179 accordance with Fisk et al. 2002 reporting depleted $\delta^{15}\text{N}$ levels in two juvenile Greenland sharks from
180 Canadian waters. The $\delta^{15}\text{N}$ levels measured in the smallest <200 cm sharks of this study were similar to
181 those of other marine animals having a diet mainly composed by armhook squid (Hooker et al. 2001,
182 Mendes et al. 2007). This strongly supports that armhook squid, which was found as the dominating prey
183 item in the stomach content analysis, indeed is a major prey for the smallest juvenile Greenland sharks. For
184 the larger sub-adult and adult sharks (up to 474 cm), $\delta^{15}\text{N}$ levels indicated that prey of higher trophic origin
185 is the dominating prey. This matches well with a diet dominated by piscivorous groundfishes such as
186 Atlantic cod and Greenland halibut and also seals (Hansen et al. 2012). Hence, both stable isotope and
187 stomach content analysis support an ontogenetic shift in feeding behavior between juvenile and sub-
188 adult/adult Greenland sharks in Greenland waters. This shift appears to take place in the 201-250 cm size
189 category.

190 The advantage of using stable isotope analysis to assess feeding ecology is the integrated and long-term
191 picture of trophic interactions, which cannot be inferred from single samples of stomach content (Hobson
192 & Welch 1992). Stomach content analysis on the other hand, provides a detailed snap shot of recently
193 ingested prey, although with associated caveats; prey might be regurgitated during capture, varying prey
194 types have different digestion rates, as well as a difficulty distinguishing between ingested versus
195 assimilated prey (Wetherbee et al. 2012). Combined or separately, these uncertainties can lead to
196 inaccurate conclusions. In this study, the number of stomachs available was low compared to similar
197 studies on other species, making it important to evaluate findings cautiously. One example is the armhook
198 squid, which in addition to being important for the smallest shark, was also found to be relatively important
199 for sharks >200 cm (IRI=6.8%, Table 2), and very important for sharks 351-400 cm (IRI=27.1%, Table 3).
200 However, for these larger sharks, we are not convinced that IRI-levels accurately reflect the importance of
201 squids as a dietary component. This suspicion is based on: I) the contribution of reconstructed biomass to
202 the squid IRI-score being minimal; II) all squid specimens were much smaller than typically preferred prey

203 sizes (body mass of squids, mean±SD: 125.9±78.7 g) (Nielsen et al. 2014); III) the vast majority of squid
204 observations were of hard-to-digest chitin beaks, which might accumulate over long time periods as well as
205 accumulate in the sharks via its feeding on primary prey items like Greenland halibut and seal; IV) one
206 Greenland shark of 351 cm was found to contain 32 pairs of squid beaks (GS17, Table 1) making this one
207 observation responsible for 29% of all armhook squids encountered in sharks >200 cm. When all of the
208 above is taken into account, we do not think there is reason to consider squids as an important prey item
209 for any other sizes than juvenile Greenland sharks. For juvenile Greenland sharks, however, armhook squids
210 are dominating in terms of prey numerical abundance, frequency of occurrence and reconstructed biomass.

211 Another example of a frequently encountered prey item with high numerical numbers in a few shark
212 individuals, was Atlantic cod, which for sharks >200 cm had the highest IRI-score (%IRI=26.6, Table 2). Two
213 female sharks >400 cm (GS42 and GS43, Table 1) contained as many as 49 and 38 Atlantic cod out of a total
214 of 127 specimens in all shark stomachs combined. The reported IRI level of Atlantic cod of this study could
215 be an overestimate compared to other important prey items such as skates, seal, Greenland halibut,
216 lumpfish and wolffish. However, in contradiction to armhook squid, we stress that Atlantic cod is
217 considered an important prey item for sharks >250 cm, as the Cod category (dominated by Atlantic cod) is
218 of main importance for all sharks size groups 251-300 cm, 301-350 cm, 351-400 cm and >400 cm (Table 3).

219 Although the Cod group was an important overall prey category in sharks larger than 250 cm, our findings
220 also show differences in prey fishes between shark size groups. For example, Righteye flounder (dominated
221 by Greenland halibut, Table 2) was found to be among the most important prey items for sharks between
222 251-300 cm and 301-350 cm, but of little importance in larger sharks. Furthermore, redfish were found only
223 to be of importance in sharks >400 cm, which was surprising since these are highly abundant on the
224 Greenland continental shelf (ICES 2017). For sharks >400 cm, the lack of importance of deep sea fishes
225 such as Greenland halibut, grenadiers or slickheads (Alepocephalidae), could be attributed their preferred
226 swimming depths typically being shallower than 550 m (MS2). As Greenland sharks are considered
227 opportunistic predators (MacNeil et al. 2012) their stomach content will presumably reflect the trophic
228 niche of their primary habitat to some degree. For the >400 cm sharks of this study, it therefore seems that
229 they prefer to occupy (and feed in) waters of the continental shelf and upper part of the continental slope,
230 matching well the vertically and horizontally distributional pattern as described in MS2. The high
231 abundance of redfish in Greenland shelf waters and the general lack of this species in stomachs of sharks
232 <400 cm, further indicates that Greenland sharks are not likely to eat strictly on carcasses. In more detail, if
233 random carcasses were their main prey item, we would expect the fishes with the highest biomass to also
234 be an important food source. This does not seem to be the case and hence, the general absence of redfish

235 in shark stomachs further supports that feeding differences between Greenland sharks of varying size (and
236 life stage) are evident among sub-adult and adult sharks. The greater importance of redfish in large
237 Greenland sharks >400 cm (Table 3), is supported by observations of sharks from Iceland waters (mean
238 length±SE: 415.6±25.2 cm, N=19), having redfish as the main prey item (McMeans et al. 2010).

239 For decades, scientists have emphasized the discrepancy between the lethargic appearance of the
240 Greenland shark and the fast swimming animals found in its stomach, and further speculated if Greenland
241 sharks mainly catch their prey actively or by scavenging (Jensen 1914, Bigelow & Schroeder 1948 Hansen
242 1963). Greenland sharks are indeed slow swimmers (Watanabe et al. 2012) and will feed on carcasses
243 whenever possible (Beck & Mansfield 1969, Leclerc et al. 2011). Yet many studies conclude that Greenland
244 sharks must be capable of catching live seals and fast-swimming fishes despite the lack of any concrete
245 evidence or direct observations (see Leclerc et al. 2012, Lucas & Natanson 2010, MacNeil et al. 2012,
246 Nielsen et al. 2014). To our knowledge, the most compelling circumstantial evidences of active predation
247 are: 1) characteristic circular bite marks on freely swimming beluga whales and seals, presumably caused by
248 Greenland sharks (Fig. 5a+b, MacNeil et al. 2012, Idrobo & Berkes 2012; 2) Capture of two Greenland
249 sharks inside large schools of Atlantic cod, with stomachs full of recently ingested and fully digested
250 specimens (Nielsen et al. 2014). Observations made in this study support that Greenland sharks are capable
251 of inflicting circular bite wounds on large prey (Fig. 5c), whereas smaller prey (relative to the shark) is
252 typically engulfed in one piece. An example of the latter are two freshly ingested seals found in a 470 cm
253 shark (GS174 in Table 1, Fig. 5d). These seals had no external signs of being killed by humans (e.g. bullet
254 holes from failed hunting attempts or fishing nets). Neither did we observe any scavenging fauna in the
255 sharks' stomachs, such as brittle stars, lysianassid amphipods, hagfish, snails or crabs, which could be
256 expected if the seals had been found by the shark as carcasses on the ocean floor (Leclerc et al. 2012,
257 Nielsen et al. 2014). We therefore hypothesize that these two seals were hunted actively by the Greenland
258 shark, although it raises the question; how is a slowly swimming Greenland shark (Watanabe et al. 2012)
259 routinely capable of catching fast swimming fishes, seals and small whales in open and ice-covered waters?
260 Leclerc et al. 2012 suggest predation on sleeping seals in the water column but such still remains to be
261 verified.

262 In conclusion, this study showed a clear ontogenetic shift in feeding behavior of juvenile (<200 cm) and sub-
263 adult/adult Greenland sharks (>200 cm). The Greenland shark should, thus, be considered as an
264 opportunistic predator, occasionally feeding on random carcasses, but with the capability to capture fast
265 swimming fishes and smaller marine mammals.

266

267 **Acknowledgements**

268 We are grateful for the contribution of M. B. Backe throughout the manuscript and for the great effort in
269 the field by crew and student workers onboard research vessels RV Pâmiut, RV Sanna, RV Porsild, and
270 Helmer Hansen. We also thank the Greenland Institute of Natural Resources (GINR), the University of
271 Copenhagen and the TUNU Programme (UiT, the Arctic University of Norway) for ship time. This study is a
272 part of the larger Greenland shark project ('Old and Cold- Biology of the Greenland shark') which was
273 financially supported by the Danish Council for Independent Research, GINR, Den Blå Planet-National
274 Aquarium of Denmark, the Commission of Scientific Investigations in Greenland (KVUG), and Danish Centre
275 for Marine Research.

276

277 **References**

278 Beck B, Mansfield AW (1969). Observations on the Greenland shark, *Somniosus microcephalus*, in Northern
279 Baffin Island. Journal of the Fisheries Research Board of Canada 26:143-145.

280

281 Bigelow HB, Schroeder WC (1948). Sharks *In* Fishes of the Western North Atlantic, Part 1 (Tee-Van J, Breder
282 CM, Hildebrand SF, Parr AE, Schroeder WC, eds) pp. 59-546. Yale, CT: Yale University, Sears Foundation for
283 Marine Research.

284

285 Campana SE, Fisk AT, Klimley AP (2015). Movements of Arctic and northwest Atlantic Greenland sharks
286 (*Somniosus microcephalus*) monitored with archival satellite pop-up tags. Deep-Sea Research Part II 115:
287 109-115.

288

289 Ebert DA (2002). Ontogenetic changes in the diet of the sevengill shark (*Notorynchus cepedianus*). Marine
290 and Freshwater Research 53:517-523.

291

292 Eriksen MW (2011). Hej haj!. Pituffik News, 3: 8-10.

293

294 Estrada JA, Rice AN, Natanson LJ, Skomal GB (2006). Use of isotopic analysis of vertebrae in reconstruction
295 ontogenetic feeding ecology in white sharks. Ecology 87, 4: 829-834.

296

297 Fisk AT, Tittlemier SA, Pranscke JL, Norstrom RJ (2002). Using anthropogenic contaminants and stable
298 isotopes to assess the feeding ecology of Greenland sharks. Ecology 83, 8: 2162-2172.

299

300 Froese R, Pauly D, Editors (2017) FishBase. World Wide Web electronic publication. www.fishbase.org
301 version (11/2017).

302

303 Hansen PM (1963). Hager i Grønlandske Farvande. Skrivelse Danmarks Fiskeri- og Havundersøgelse: 83-95
304 (in Danish).

305

306 Hansen JH, Hedeholm RB, Sünksen K, Christensen JT, Grønkjær P (2012). Spatial variability of carbon ($\delta^{13}\text{C}$)
307 and nitrogen ($\delta^{15}\text{N}$) stable isotope ratios in an Arctic marine food web. Marine Ecology Progress Series 467:
308 47-59.

309

310 Harvey-Clark CJ, Gallant JJ, Batt JH (2005). Vision and its relationship to novel behaviour in St. Lawrence
311 River Greenland Sharks, *Somniosus microcephalus*. Canadian Field-Naturalist 119, 3: 355–359.
312

313 Henriksen S, Hilmo O, red (2015). Norsk rødliste for arter 2015. Artsdatabanken, Norge (in norwegian).
314

315 Hobson KA, Welch HE (1992). Determination of trophic relationships within a high Arctic marine food web
316 using $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ analysis. Marine Ecology Progress Series 84: 9-18.
317

318 Hooker SK, Iverson SJ, Ostrom P, Smith SC (2001). Diet of northern bottlenose whales inferred from fatty-
319 acid and stable-isotope analyses of biopsy samples. Canadian Journal of Zoology 79: 1442-1454.
320

321 ICES (2017) Report of the North Western Working Group (NWWG). 27 April-4 May 2017, Copenhagen,
322 Denmark. ICES CM 2017/ACOM:08. 642 pp.
323

324 Idrobo CJ, Berkes F (2012) Pangnirtung inuit and the Greenland shark: co-producing knowledge of a little
325 discussed species. Human Ecology 40: 405-414.
326

327 IUCN (2017). The IUCN Red List of Threatened Species. Version 2017-2.
328 <http://www.iucnredlist.org/details/0> (assessed 28 Sep 2017).
329

330 Jakobsdóttir KB (2001). Biological aspects of two deep-water squalid sharks: *Centroscyllium fabricii*
331 (Reinhardt, 1825) and *Etmopterus princeps* (Collett, 1904) in Icelandic waters. Fisheries Research 51: 247-
332 265.
333

334 Joyce WN, Campana SE, Natanson LJ, Kohler NE, Pratt HL Jr, Jensen CF (2002). Analysis of stomach contents
335 of the porbeagle shark (*Lamna nasus* Bonnaterre) in the northwest Atlantic. ICES Journal of Marine Science
336 59: 1263-1269.
337

338 Jensen AS (1914). The Selachians of Greenland. Zoological Museum of the University Copenhagen. Bianco
339 Lunos Bogtrykkeri: 8-17 (in danish).
340

341 Leclerc LM, Lydersen C, Haug T, Glover KA, Fisk AT, Kovacs KM (2011). Greenland shark (*Somniosus*
342 *microcephalus*) scavenge offal from minke (*Balaenoptera acutorostrata*) whaling operations in Svalbard,
343 Norway. Polar Research 30, 7342, DOI: 10.3402/polar.v30i0.734.
344

345 Leclerc LM, Lydersen C, Haug T, Bachmann L, Fisk AT, Kovacs KM (2012). A missing piece in the Arctic food
346 web puzzle? Stomach contents of Greenland sharks sampled in Svalbard, Norway. Polar Biology 35: 1197-
347 1208.
348

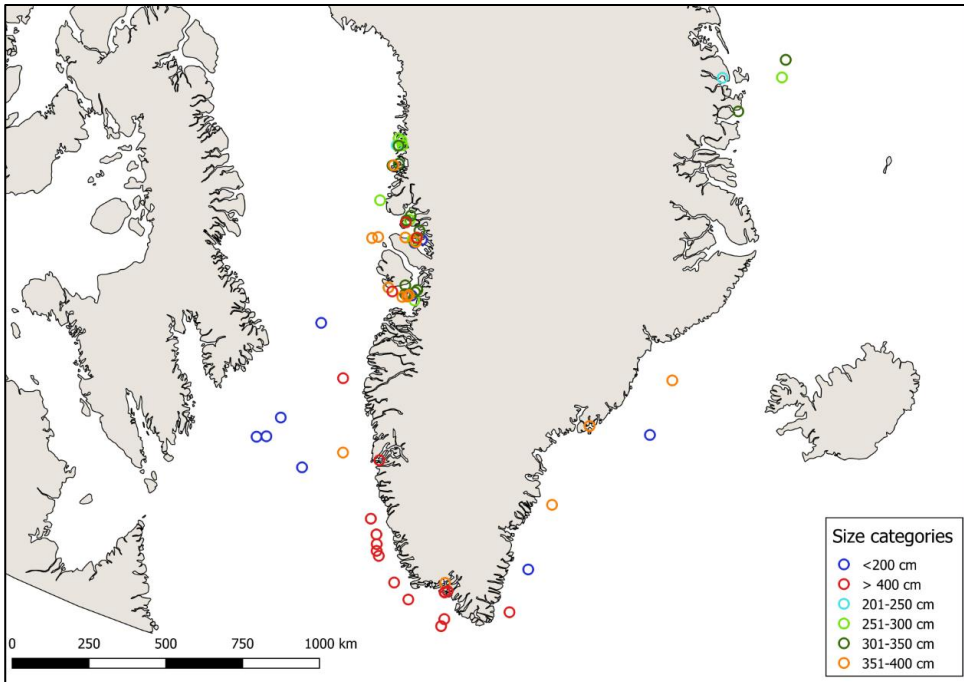
349 Lowe CG, Wetherbee BM, Crow GL, Tester AL (1996). Ontogenetic dietary shifts and feeding behavior of the
350 tiger shark, *Galeocerdo cuvier*, in Hawaiian waters. Environmental Biology of Fishes 47: 203–211.
351

352 Lucas ZN, Natanson LJ (2010). Two shark species involved in predation on seals at Sable Island, Nova
353 Scotia, Canada. Proceedings of the Nova Scotian Institute of Science 45: 64-88.
354

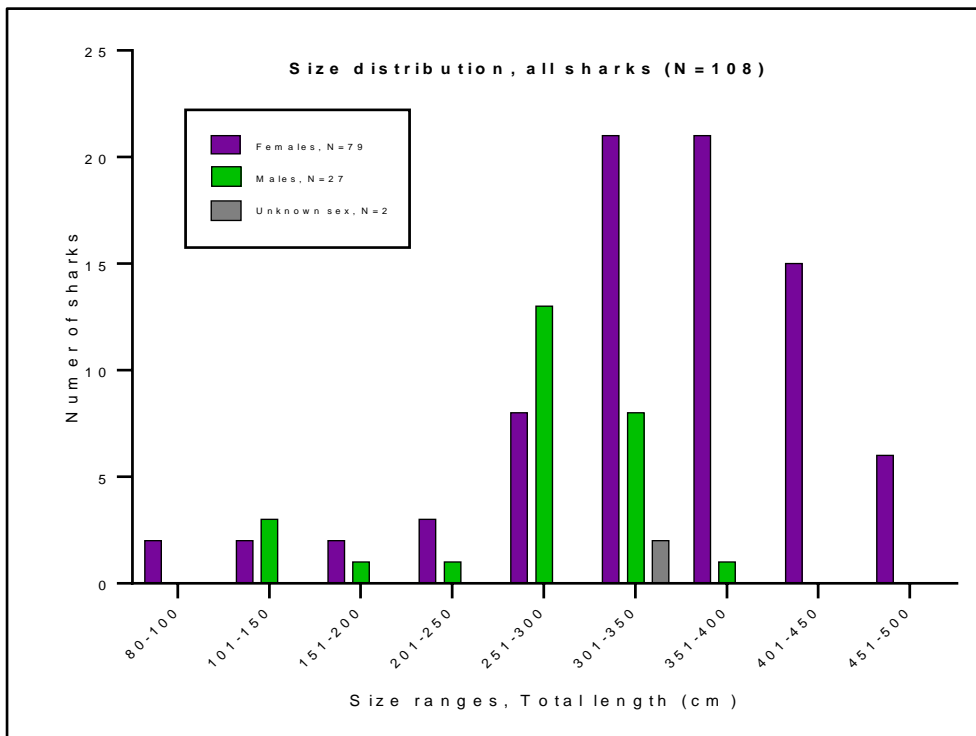
355 MacNeil MA, McMeans BC, Hussey NE, Vecsei P, Svavarsson J, Kovacs KM, Lydersen C, Ramsey M, Fisk AT
356 (2012). Biology of the Greenland shark *Somniosus microcephalus*. Journal of Fish Biology, 80: 991-1018.
357

358 McMeans BC, Svavarsson J, Dennard, Fisk AT (2010). Diet and resource use among Greenland sharks

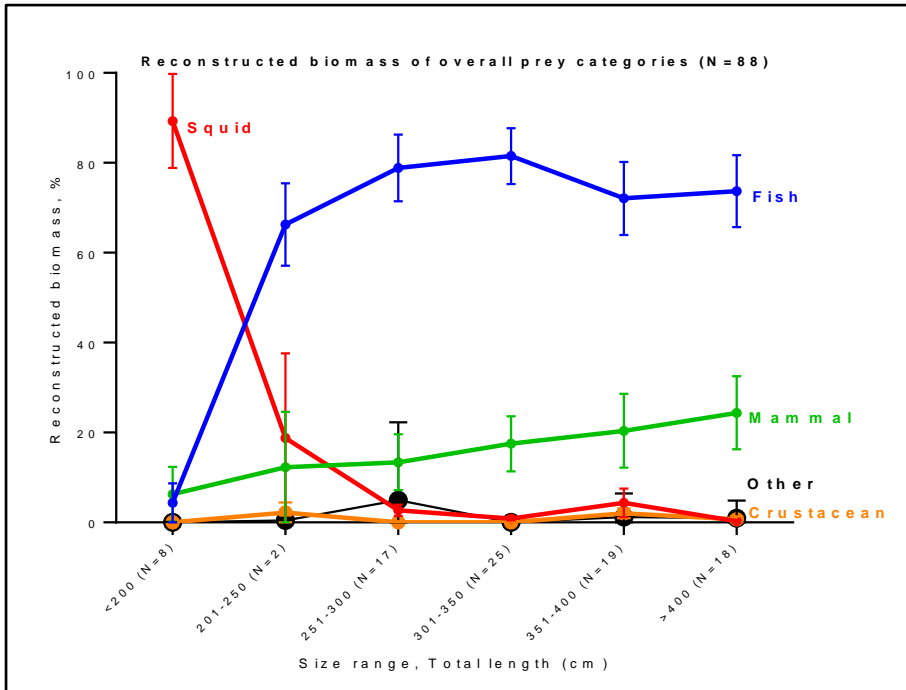
359 (*Somniosus microcephalus*) and teleosts sampled in Iceland waters, using $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and mercury.
360 Canadian Journal of Fishery Aquatic Science 67: 1428-1438.
361
362 McMeans BC, Arts MT, Lydersen C, Kovacs KM, Hop H, Falk-Petersen S, Fisk AT (2013). The role of
363 Greenland sharks (*Somniosus microcephalus*) in an Arctic ecosystem: assessed via stable isotopes and fatty
364 acids. Marine Biology 160: 1223-1238.
365
366 Mendes S, Newton J, Reid RJ, Zuur AF, Pierce GJ (2007). Stable carbon and nitrogen isotope ratio profiling
367 of sperm whale teeth reveals ontogenetic movements and trophic ecology. Oecologia 151: 605-615.
368
369 Nielsen J, Hedeholm RB, Simon M, Steffensen JF (2014). Distribution and feeding ecology of the Greenland
370 shark (*Somniosus microcephalus*) in Greenland waters. Polar Biology 37: 37-46.
371
372 Nielsen J, Hedeholm RB, Heinemeier J, Bushnell PG, Christiansen JS, Olsen J, Ramsey CB, Brill RW, Simon M
373 Steffensen KF, Steffensen JF (2016). Eye lens radiocarbon reveal centuries of longevity in the Greenland
374 shark (*Somniosus microcephalus*). Science 353, 6300: 702-704.
375
376 Nielsen et al. (in preparation1). Spatial distribution and composition of size-based life stages of Greenland
377 shark *Somniosus microcephalus* across the northern North Atlantic.
378
379 Porteiro FM, Sutton TT, Byrkjedal I, Orlov AM, Heino M, Menezes G, Bergstad OA (2017). Fishes of the
380 northern Mid-Atlantic Ridge collected during the MAR-ECO cruise in June-July 2004: an annotated checklist.
381 Arquipelago Life and Marine Sciences Supplement 10.
382
383 Watanabe Y, Lydersen C, Fisk AT, Kovacs KM (2012) The slowest fish: Swim speed and tail beat frequency of
384 Greenland sharks. Journal of Experimental Marine Biology and Ecology 426-427: 5-11.
385
386 Wetherbee BM, Cortés E, Bizzarro JJ (2012). Food Consumption and Feeding Habits *in* Biology of Sharks and
387 Their Relatives. Eds Carrier JC, Musick JA, Heithaus MR. CRC Press. pp 239.
388
389 Yano K, Stevens JD, Compagno LJV (2007). Distribution, reproduction and feeding of the Greenland shark
390 *Somniosus (Somniosus) microcephalus*, with notes on two other sleeper sharks, *Somniosus (Somniosus)*
391 *pacificus* and *Somniosus (Somniosus) antarcticus*. Journal of Fish Biology 70: 374-390.
392
393 Zumholz K, Frandsen RP (2006). New information on the life history of cephalopods off west Greenland.
394 Polar Biology 29: 169-178.
395
396



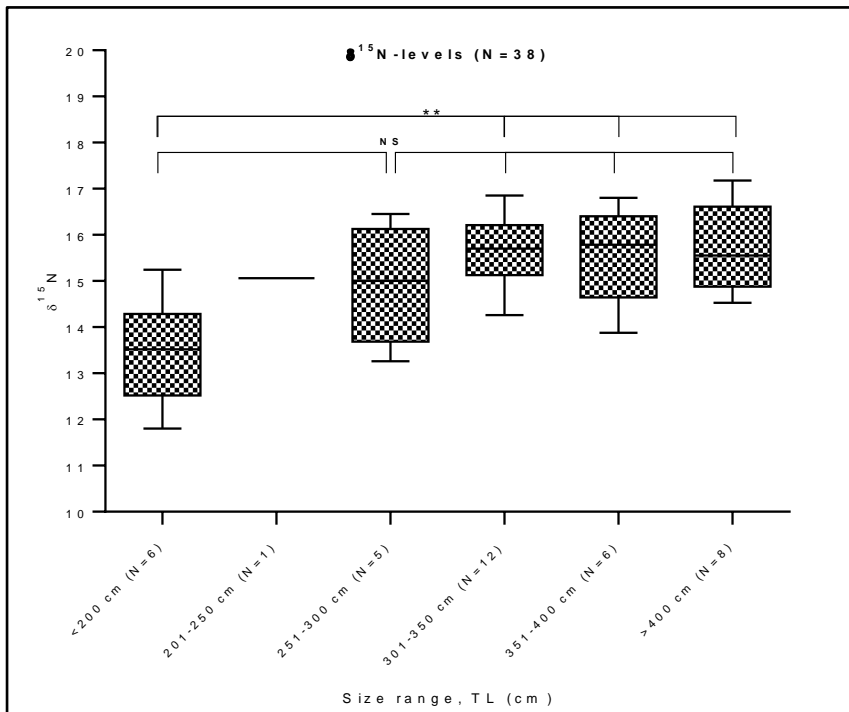
398 **Fig. 1.** Capture positions of all Greenland sharks (N=108) including sharks reported in
 399 Nielsen et al (2014) (N=30). Color indicates shark size.
 400
 401
 402



403 **Fig. 2.** Size distribution by sex of all sharks (N=109).
 404
 405



406
 407 **Fig. 3.** Proportional composition of reconstructed biomass of major prey categories in the different shark size
 408 categories: 'Fish', 'Mammal', 'Squid', 'Crustacean', and 'Other', for the 89 sharks with 'non-empty' stomachs.
 409 Reconstructed biomass of each prey category is plotted as mean±SE and the sample size (N) is given for each size
 410 category.
 411
 412
 413



414
 415 **Fig. 4.** Boxplot (minimum, first quartile, median, third quartile, and maximum) of $\delta^{15}\text{N}$ plotted against size categories.
 416 NS indicate groups where mean values are not significantly different and ** represents significant differences $P<0.01$.
 417



418
 419 **Fig. 5. a:** Greenland shark characteristic circular gape shape (Photo: Julius Nielsen). **b:** circular bite marks on
 420 free swimming beluga whale (picture from MacNeil et al. 2012). **c:** seal chunks of skin, blubber, meat and
 421 ribs from shark stomach (Photo: Julius Nielsen). **d:** juvenile harp seals of 14 kg (top) and 19 kg (bottom)
 422 found in stomach of a 470 cm Greenland shark (Photo: Julius Nielsen).

423
424

Table 1. Information on morphometrics and capture location of all 109 sharks evaluated for either stomach content and/or stable isotope level.

ID	TL (cm)	Sex	Area	Stomach	$\delta^{15}\text{N}$	ID	TL (cm)	Sex	Area	Stomach	$\delta^{15}\text{N}$
GS82	81	F	SW	Yes	13.1	GS45*	322	M	NW	Yes	15.1
GS275	98	F	Canada	Yes	14.0	GS182	322	F	NE	Yes	NA
GS157	104	M	SE	Yes	11.8	GS24*	325	M	NW	Yes	NA
GS276	115	M	Canada	Yes	NA**	GS1*	326	M	SW	Empty	NA
GS99	116	F	NW	Yes	NA	GS123	326	M	NW	Yes	15.8
GS162	120	M	NW	Yes	14.0	GS56	327	F	NW	Yes	NA
GS104	149	F	NW	Yes	15.2	GS122	330	F	NW	NA	16.1
GS52	158	F	SW	Yes	12.8	GS16*	336	F	SE	Empty	NA
GS155	164	M	SE	Empty	NA	GS267	343	F	NW	Yes	NA
GS258	170	F	NW	Empty	NA**	GS21*	345	F	NW	Yes	NA
GS107	204	F	NW	Empty	NA	GS129	345	NA	NW	NA	15.6
GS63	220	F	NE	Yes	NA	GS9*	346	F	SE	Yes	15.3
GS265	230	F	NW	NA	15.1	GS17*	351	F	SE	Yes	NA
GS270	242	M	NW	Yes	NA	GS266	351	F	NW	Yes	NA
GS3*	258	F	NW	Yes	15.8	GS173	352	F	NW	Yes	NA
GS13*	264	F	SE	Yes	13.3	GS41*	354	F	SW	Yes	NA
GS180	266	M	NE	Yes	NA	GS55	354	F	NW	Yes	NA
GS149	267	F	NW	Yes	NA	GS10*	355	F	SE	Yes	14.9
GS181	271	M	NE	Yes	NA	GS23*	357	F	NW	Yes	NA
GS32*	274	M	NW	Yes	NA	GS46*	360	F	SW	Yes	NA
GS19*	276	F	NW	Yes	NA	GS128	363	F	NW	NA	15.7
GS269	278	M	NW	Yes	NA	GS15*	365	F	SE	Yes	NA
GS136	281	M	NW	Yes	15.0	GS6*	370	F	SE	Yes	NA
GS273	282	M	NW	Yes	NA	GS98	370	F	NW	Yes	NA
GS145	284	F	NW	Yes	NA	GS271	370	F	NW	Yes	NA
GS148	284	M	NW	Yes	NA	GS57	372	M	NW	Yes	NA
GS25*	287	F	NW	Empty	NA	GS121	382	F	NW	Yes	16.8
GS11	290	M	SE	NA	14.1	GS126	382	F	NW	Empty	16.3
GS22*	290	M	NW	Empty	NA	GS120	385	F	NW	Yes	15.8
GS166	290	M	NW	Yes	NA	GS14*	386	F	SE	Yes	13.9
GS179	291	M	NE	Yes	NA	GS58	390	F	SE	Yes	NA
GS264	292	M	NW	Yes	NA	GS59	391	F	NW	Yes	NA
GS263	295	M	NW	Yes	NA	GS118	400	F	NW	Empty	NA
GS51*	298	F	NW	Empty	NA	GS268	402	F	NW	Yes	NA
GS85	300	F	NW	Yes	16.5	GS115	410	F	SW	Yes	NA
GS12*	306	F	SE	Yes	14.6	GS130	410	F	NW	Yes	15.3
GS88	306	M	NW	Yes	16.8	GS54	411	F	SW	Yes	NA
GS105	307	M	NW	Yes	NA	GS4*	420	F	SW	Yes	14.5
GS106	307	F	NW	Yes	NA	GS116	425	F	SW	Yes	17.2
GS174	310	F	NW	Yes	NA	GS100	430	F	SW	Yes	NA
GS127	310	F	NW	Yes	16.9	GS178	434	F	SW	Yes	14.8
GS7*	312	F	SE	Yes	14.3	GS301	434	F	SW	Empty	NA
GS44*	312	F	NW	Yes	NA	GS295	440	F	SW	Yes	NA
GS8	313	M	SE	NA	15.2	GS5*	442	F	SW	Yes	NA
GS20*	315	F	NW	Yes	NA	GS53	445	F	SE	Yes	NA
GS262	315	F	NW	Empty	NA	GS2*	447	F	SW	Empty	NA
GS272	316	F	NW	Yes	NA	GS43*	447	F	SW	Yes	NA
GS124	317	F	NW	Yes	NA	GS73	450	F	NW	Empty	NA
GS81	318	F	NW	Yes	NA	GS97	451	F	NW	Yes	NA
GS144	318	M	NW	Yes	16.3	GS114	454	F	SW	Yes	15.2
GS274	318	U	NW	Yes	NA	GS177	454	F	SW	Yes	15.8
GS60	320	F	NE	Yes	NA	GS42*	460	F	SW	Yes	NA
GS183	320	F	NE	Yes	NA	GS175	470	F	SW	Yes	16.0
GS125	321	F	NW	Yes	15.8	GS176	474	F	SW	Yes	16.8

* Sharks from Nielsen et al. (2014)

** Measurements of stable isotope levels are being processed

425 **Table 2.** Index of relative importance (IRI) for sharks <200 cm (N=8) and >200 cm (N=80). For sharks <200
 426 cm, reconstructed biomass is 10.8 kg for 45 prey items. For sharks >200 cm, reconstructed biomass is 614.1
 427 kg for 614 prey items.

Fish	Family	Popular name	% F		% N		% B		%IRI	
			<200 cm	>200 cm	<200 cm	>200 cm	<200 cm	>200 cm	<200 cm	>200 cm
<i>Gadus morhua</i>	Gadidae	Cod		26.3		20.0		19.2		26.6
<i>Gadus</i> sp	Gadidae	Cod		18.8		4.3		4.3		4.2
<i>Micromesistius poutassou</i>	Gadidae	Cod		2.5		3.8		0.7		0.3
<i>Boreogadus saida</i>	Gadidae	Cod		2.5		2.1		0.0		0.1
<i>Gadus ogac</i>	Gadidae	Cod		1.3		0.3		0.4		<0.1
Unidentified teleost				50.0		9.9		3.7		17.6
Rajidae	Rajidae	Skates		36.3		5.8		3.3		8.5
<i>Amblyraja radiata</i>	Rajidae	Skates		8.8		1.7		1.5		0.7
<i>Amblyraja hyperborea</i>	Rajidae	Skates		7.5		0.9		1.5		0.5
Rajidae, egg case	Rajidae	Skates		8.8		1.1		0.0		0.3
<i>Reinhardtius hippoglossoides</i>	Pleuronectidae	Righteye flounders		21.3		6.0		7.0		7.1
<i>Hippoglossoides platessoides</i>	Pleuronectidae	Righteye flounders		2.5		0.8		0.1		0.1
Unknown flounder	Pleuronectidae	Righteye flounders	12.5	1.3	2.2	0.9	0.7	0.2	0.3	<0.1
<i>Cyclopterus lumpus</i>	Cyclopteridae	Lumpsuckers		22.5		4.4		7.4		6.9
<i>Anarhichas minor</i>	Anarhichadidae	Wolffish		13.8		2.7		6.2		3.2
<i>Anarhichas lupus</i>	Anarhichadidae	Wolffish	12.5	3.8	2.2	0.9	1.9	1.0	0.4	0.2
<i>Anarhichas denticulatus</i>	Anarhichadidae	Wolffish		2.5		0.3		1.8		0.1
<i>Anarhicas</i> spp	Anarhichadidae	Wolffish		6.3		0.9		0.6		0.3
<i>Sebastes mentella</i>	Sebastidae	Redfish		7.5		2.7		1.6		0.8
<i>Sebastes marinus</i>	Sebastidae	Redfish		1.3		0.9		0.2		<0.1
<i>Sebeastes</i> spp	Sebastidae	Redfish		5.0		0.9		0.2		0.2
<i>Lycodess</i> spp	Zoaridae	Eelpouts		8.8		2.1		0.4		0.5
<i>Somniosus microcephalus</i>	Somniosidae	Sleeper sharkk		1.3		0.2		2.4		0.1
<i>Cottonculus sadko</i>	Psychrolutidae	Fatheads		2.5		0.3		0.1		<0.1
<i>Cottonculus</i> sp	Psychrolutidae	Fatheads		1.3		0.2		0.1		<0.1
<i>Myoxucephalus scorpius</i>	Psychrolutidae	Sculpins		2.5		0.3		0.1		<0.1
Solmonidae	Solmonida	Salmonids		1.3		0.2		0.3		<0.1
<i>Argentina silus</i>	Argentidae	Argentine		1.3		0.2		0.0		<0.1
<i>Scomber scombrus</i>	Scombridae	Scombrids		1.3		0.2		0.0		<0.1
<i>Macrourus berglax</i>	Macrouridae	Grenadier		1.3		0.2		0.2		<0.1
<i>Myxine glutinosa</i>	Myxiidae	Hagfish		1.3		0.3		0.0		<0.1
Mammal										
<i>Pagophilus groenlandicus</i>	Pinnipeds	Seal		16.3		2.2		14.4		7.0
Unknown seal	Pinnipeds	Seal	25.0	25.0	4.4	3.3	0.1	9.1	0.8	5.9
<i>Erigonathus barbatus</i>	Pinnipeds	Seal	12.5	3.8	2.2	0.5	3.7	2.0	0.5	0.2
Ringsæl	Pinnipeds	Seal		3.8		0.5		1.5		0.2
<i>Cystophora cristata</i>	Pinnipeds	Seal		1.3		0.2		0		<0.1
Unknown whale (blubber)	Cetacea	Whale		2.5		0.3		6.3		0.4
<i>Ursus maritimus</i>	Ursus	Bear		1.3		0.2		0.1		<0.1
Squid										
<i>Gonatus</i> spp	Gonatidae	Armhook squid	100	22.5	80.0	104	53.3	1,2	93.7	6.8
<i>Histioteuthis</i> sp.	Histioteuthidae		12.5		4.4		40.3		3.9	
Crustacean										
<i>Eurythenes gryllus</i>	Decapoda	Amphipod		2.5		2.8		0,0		0.2
<i>Hyas</i> spp	Brachyura	Crab		10,0		2.4		0,1		0.6
<i>Chionoecetes opilio</i>	Brachyura	Crab		2.5		0.3		0,1		<0.01
<i>Lithodes maja</i>	Brachyura	Crab		1.3		0.2		0,0		<0.01
<i>Pandalus borealis</i>	Caridea	Shrimp		1.3		0.2		0,0		<0.01
<i>Pandalus</i> sp	Caridea	Shrimo	12.5	1.3	2.2	0.2	<0.1	0,0	0.2	<0.01
<i>Sclerocrangon boreas</i>	Caridea	Shrimp		1.3		0.2		0,0		<0.01
<i>Pasiphaea</i> sp.	Caridea	Shrimp		1.3		0.5		0,0		<0.01
<i>Colossendeis proboscida</i>	Colossendeidae	Marine sea spider	12.5		2.2		<0.1		0.2	
Other										
Unknown auk		Auk		2.5		0.3		0.2		<0.01
Ophiuroidea		Brittle star		8.8		0.1		0.1		<0.01
Holothuroidea		Sea cucumber		2.5		0.3		6.3		<0.01
Arctinaria		Sea anemone		1.3		0.1		2.0		<0.01
Echinoidea		Sea urchin		1.3		0.2		1.5		<0.01
Solasteridae		Sun star		1.3		0.2		0.2		<0.01
Asteroidea		Starfish		3.8		0.2		0.2		<0.01
Gastropoda		Snail		17.5		0.1		0.0		<0.01

Non-prey items (%F). <200 cm: Amphipods 25. >200 cm: Macro algae 11,3, Bivalvia 5.0, Porifera 3.8, amphipod 26.3, rock 21.3, fishing equipment 8.8, metal pieces 2.5.

Table 3. IRI calculated for each overall prey group and for prey families for each shark size category. Reconstructed biomass and number of prey items within each shark size category is presented together with the most important individual prey groups.

<200, N=8		201-250, N=2		251-300 cm, N=17		301-351 cm, N=25		351-400 cm, N=19		>401 cm, N=18	
Reconstructed biomass	10.8 kg	Reconstructed biomass	2.9 kg	Reconstructed biomass	83.3kg	Reconstructed biomass	153.0 kg	Reconstructed biomass	96.2 kg	Reconstructed biomass	289.1 kg
No. prey items	45	No. prey items	41	No. of prey items	109	No. of prey items	137	No. of prey items	127	No. of prey items	238
Fish (%IRI=0.7)	%IRI	Fish (%IRI=59.6)	%IRI	Fish (%IRI=79.8)	%IRI	Fish (%IRI=88.4)	%IRI	Fish (%IRI=56.0)	%IRI	Fish (%IRI=81.9)	%IRI
Righteye flounders	0.3	Unidentified teleost	20.6	Righteye flounders	29.8	Skate	19,8	Cod	16,7	Cod	56,8
Wolffish	0.4	Sculpin	2.7	Unidentified teleost	21.5	Unidentified teleost	17,8	Unidentified teleost	9,4	Unidentified teleost	9,6
		Eelpout	6.5	Cod	14.8	Lumpsuckers	14,2	Wolffish	8,6	Redfish	5,6
		Cod	17.1	Skates	10.3	Cod	12,4	Skate	8,4	Lumpsucker	3,3
		Skate	12.7	Wolffish	1.9	Wolffish	12,1	Lumpsucker	6,5	Wolffish	3,8
				Lumpsuckers	1.1	Righteye flounder	10,7	Sleeper shark	2,5	Skates	2.0
				Fatheads	0.2	Eelpouts	0,9	Righteye flounders	2,3	Righteye flounder	0,6
				Eelpouts	0.1	Redfish	0,2	Eelpouts	0,9	Salmon	0,1
				Redfish	0.1	Fatheads	0,2	Redfish	0,45	Argentine	<0.1
						Sculpins	0,1	Hagfish	0,23	Grenadier	<0.1
										Mackerel	<0.1
Mammal (%IRI=1.3)		Mammal (%IRI=7.6)		Mammal (%IRI=10.4)		Mammal (%IRI 8.3)		Mammal (%IRI=14.6)		Mammal (%IRI=17.1)	
Seal	1.3	Seal	7.6	Seal	10.2	Seal	8,1	Seal	14.6	Seal	16,7
				Bear	0.2	Whale	0,2			Whale	0,4
Squid (%IRI=97.6)		Squid (%IRI=11.8)		Squid (%IRI=8.6)		Squid (%IRI=2.8)		Squid (%IRI=27.1)		Squid (%IRI=<0.1)	
Armhook squid	93.7	Armhook squid	11.8	Armhook squid	8.6	Armhook squid	2.8	Armhook squid	27.1	Armhook squid	<0.1
Cock-eyed squid	3.9										
Crustacean (%IRI=0.4)		Crustacean (%IRI=14.7)		Crustacean (%IRI=0.9)		Crustacea (%IRI=0.6)		Crustaceans (%IRI=2.2)		Crustaceans (%IRI=0.9)	
Caridae	0.2	Eurythenes gryllys	14.7	Large amphipod	0.8	Crab	0,3	Crab	<u>1,8</u>	Crab	0,9
Colossendeidae	0.2			Crab	0.1	Shrimp	0,3	Shrimp	0,4	Shrimp	<0.1
Other (%IRI=0)		Other (%IRI=6.3)		Other (%IRI=0.2)		Other (%IRI=0)		Other (%IRI=0)		Other (%IRI=<0.1)	
x		Brittle star	3.8	Bird	0.2	x		x		Bird	<0.1
		Snail	2.5								
<u>Main prey items, IRI>5</u>		<u>Main prey items, IRI>5</u>		<u>Main prey items, IRI>5</u>		<u>Main prey items, IRI>5</u>		<u>Main prey items, IRI>5</u>		<u>Main prey items, IRI>5</u>	
Armhook squid		Unidentified teleost, Polar cod, Eurythenes gryllys, Armhook squid, Arctic skate, harp seal, Lycoes sp.		Unidentified teleost, Greenland halibut, Atlantic cod, skates, armhook squid, and harp seal		Unidentified teleost, lumpsucker, spotted wolffish, skate, Greenland halibut, Atlantic cod, harp seal		Armhook squid, Atlantic cod, unidentified teleost, spotted wolffish, skate, lumpsucker, harp seal		Atlantic cod, unidentified teleosts, beaked redfish, harp seal	

MS2

1 **Spatial and temporal movements of adult Greenland shark females**
2 **(*Somniosus microcephalus*) in Greenland waters**

3 Julius Nielsen^{1,2,3*}, John F Steffensen¹, Rasmus Hedeholm², Jørgen Schou Christiansen⁴, Kim Præbel⁵, Peter
4 Bushnell⁶

5
6 **Affiliations**

7 1) Marine Biological Section, University of Copenhagen, Strandpromenaden 5, 3000 Helsingør, Denmark.

8 2) Greenland Institute of Natural Resources, Post Office Box 570, Kivioq 2, 3900 Nuuk, Greenland

9 3) Den Blå Planet, National Aquarium Denmark, Jacob Fortlingsvej 1, 2770 Kastrup, Denmark

10 4) Department of Arctic and Marine Biology, UiT The Arctic University of Norway, 9037 Tromsø, Norway

11 5) Norwegian College of Fishery Science, UiT The Arctic University of Norway, 9037 Tromsø, Norway

12 6) Department of Biological Sciences, Indiana University South Bend, 1700 Mishawaka Avenue, South Bend,
13 IN, USA.

14
15 *Corresponding author: juliusnielsen88@gmail.com

16
17 **Key words:** Tracking, PSAT, Greenland shark, migration

18
19 **Abstract**

20 The Greenland shark (*Somniosus microcephalus*) is an abundant key predator distributed across the
21 northern North Atlantic. Despite being recently identified as being the longest-lived vertebrate on the
22 planet with an estimated longevity exceeding 272 years, other fundamental aspects of its biology remain to
23 be elucidated. Of particular interest in this regard is the reproductive behavior of adult females. In an effort
24 to shed light on the distribution and migratory aspects this life stage, we examined the spatial movements

25 of adult Greenland shark females in southwestern Greenland using pop-up satellite archival transmitting
26 tags (PSATs). 13 females (410-455 cm) were tagged with multiple PSATs (33 in total) programmed to
27 release at intervals ranging from 3-13 months. While five PSATs are still deployed, 22 tags relayed usable
28 data that was included in this analysis. While all sharks were tagged inshore, only one shark appeared to
29 remain there for the duration of the study (150 days), as all other PSATs reported from locations over the
30 offshore shelves of southwest or southeastern Greenland. Interestingly, the shelf water off Frederikshåb
31 Isblink appears to be a “hot spot” for adult females in southwestern Greenland as PSATs from 4 sharks
32 released in this area. While most sharks seemed to stay in the general area of southern Greenland, one
33 shark migrated to the Reykjanes Ridge in the mid-Atlantic, only to return to southwest Greenland 10
34 months later, a total distance of 2,754 km. While at liberty, all sharks tended to occupy similar depths, with
35 means (\pm SD) ranging between 341.6 ± 214.7 m and 455.7 ± 85.9 m (range= 13-1,083m) and mean
36 temperatures ranging between $4.6 \pm 0.6^\circ$ C and $5.1 \pm 1.1^\circ$ C (range = -2.0-9.0° C). When data reported here is
37 combined with historical data in the literature, it appears that the offshore shelf waters of southern
38 Greenland appear to have an important role to play in the life history of Greenland sharks. Exactly what,
39 awaits further studies, particularly on gravid females of the species.

40

41 **Introduction**

42 In recent years, the Greenland shark (*Somniosus microcephalus*) has been subject to increasing scientific
43 focus establishing its position as a long-lived, likely abundant top predator, with the potential to influence
44 trophic dynamics in arctic marine food webs (Leclerc et al. 2012, McMeans et al. 2013, Nielsen et al. 2016).
45 However, despite a relatively frequent bycatch of Greenland shark in commercial fisheries throughout the
46 Arctic (Davis et al. 2013, Rusyaev & Orlov 2013, DFO 2016), and exposure to heavy fishing pressure in the
47 past (Jensen 1914, Anon 1942), many aspects of the basic biology of the Greenland shark remain poorly
48 elucidated. This includes, in particular, the breeding part of the population (i.e. the effective population) of
49 which knowledge on size and distribution is extremely limited, as well as most aspects of their reproductive
50 biology (see review on Greenland shark biology in MacNeil et al. 2012). Greenland sharks are listed as ‘Near
51 Threatened’ on the IUCN Red List of Threatened Species and as ‘Data Deficient’ on the Norwegian Red List
52 (Henriksen & Hilmo 2015, IUCN 2017).

53 Tracking studies have shown that Greenland sharks are highly migratory and occupy a great variety of
54 depths, from surface waters to at least 1,816 m of depth (Hansen 1963, Fisk et al. 2012, Campana et al.
55 2015). Greenland sharks are however capable of occupying much deeper abyssal waters (Herdendorf &

56 Berra 1995, Benz et al. 2007), with the deepest record being 2,909 m (Porteiro et al. 2017). While the
57 distribution area of Greenland shark is reported to encompass the entire northern North Atlantic including
58 the Arctic Ocean, their real distribution probably extends circumpolar and more northward than currently
59 documented (MacNeil et al. 2012, Mecklenburg et al in prep). Based on an evaluation of size composition
60 of Greenland sharks in more than 1000 individuals, from across the northern North Atlantic, great
61 differences in geographical distribution of life stages are evident (Nielsen et al. in prep¹). Most remarkably,
62 adult specimens are rarely encountered in large parts of the North Atlantic. In southwestern Greenland
63 however, the majority of Greenland sharks are females larger than 400 cm in total length (~60% of 119
64 observations, Nielsen et al. in prep1). Yano et al. (2007) reports Greenland shark females to reach sexual
65 maturation larger than 400 cm, and Nielsen et al in (prep2) estimates that 50% of the females are mature
66 at 398 cm. Hence, the majority of sharks in southwestern Greenland are potentially sexual mature, and are
67 referred to as adults. Smaller sub-adults dominate in the more northern regions in Greenland, Arctic
68 Canada and Svalbard (Nielsen et al. in prep1).

69 The objective of this study was to examine spatial movements of adult Greenland shark females in
70 southwestern Greenland using pop-up satellite archival transmitting tags (PSATs). In order to investigate
71 whether this region is continuously important for adult females across seasons, we acquired basic
72 information on preferred depths, temperatures and geographical position over long-term time periods
73 spanning from approximately 3 months to 13 months. Combined, this information provided new insights
74 into fundamental biological aspects of the Greenland shark and elucidated the migratory behavior of a
75 rarely encountered life stage - the adult Greenland shark female.

76

77 **Materials and methods**

78 *Capture*

79 Greenland sharks were caught on benthic long lines during scientific cruises (2015-2017) on RVs Avataq and
80 Sanna (Greenland Institute of Natural Resources) in four different fjords in western Greenland: Ummannaq
81 Fjord in northwestern Greenland, and Grædefjord, Bredefjord and Julianehåbsfjorden in southwestern
82 Greenland (**Fig. 1**). Longlines consisted of two sections - a head (buoy) line and a bottom line. The head line

¹ Data from Nielsen et al. in prep1 is acquired from scientific trawl surveys, the scientific literature and targeted fieldwork during a five year period in context of the Old & Cold Greenland sharks project. The dataset encompasses +1000 Greenland shark from arctic Canada, Greenland, Iceland, Svalbard, Faroe Island, mainland Norway and Denmark.

83 was attached to the weight at one end of the longline, and was as long as the gear deployment depth plus
84 50-100 m. The bottom line (8-10 mm nylon rope) consisted of ganjions attached to the longline with
85 carabiners mounted every 10 m with 70 cm stainless steel chain, and a size 4 Mustad shark hook baited
86 with seal blubber. Two weights (~10-15 kg) anchored each end of the bottom line which consisted of no
87 more than 10 hooks. All lines were deployed between 350 and 500 m of depth and were allowed to soak
88 for 12-18 hours before being recovered.

89 *Tagging*

90 Once the ganjion was removed from the longline, sharks were left in the water to reduce stress, but
91 restrained along the side of a small zodiac using a 10 cm wide tail rope around the caudal peduncle and a
92 barbless hook in the mouth. The sharks were measured (total length, TL, cm), sexed, and a tissue sample
93 for genetic analyses were taken from the caudal fin (~0.5 cm³). All sharks were tagged with floy tags
94 mounted with a FH-69 stainless steel tip attached beneath the shark's cartilage ridge of the first dorsal fin
95 or in the caudal peduncle. Females longer than 400 cm were also tagged with PSATs (Wildlife Computers,
96 Redmond, Washington) if the following criteria were met: sharks were actively moving during handling, skin
97 abrasions were minimal, and no bleeding from the mouth or gill openings. Each tag was attached to the
98 shark using plastic darts (W: 40 mm, L:50 mm) mounted on a 2 mm stainless steel plastic coated tag wire,
99 inserted under the cartilage ridge associated with the dorsal fin.

100 Two different types of PSATs were used in this study: miniPATs and mrPATs (Wildlife Computers, Redmond,
101 Washington). Each adult female was tagged with either two or three PSATs programmed to release after
102 approximately 3 months, 5 months, or 13 months (Table 1). The exact time periods were chosen so tags
103 would release during seasons with minimal sea ice cover. Nine miniPATs and 24 mrPATs were deployed on
104 13 adult Greenland shark females (Shark #1-13, length range 406-455 cm) caught in Grædefjord (N=7),
105 Bredefjord (N=1), Julianehåbsfjord (N=4) and Uummannaqfjord (N=1) (Table 1, Fig. 1). Each shark was
106 tagged with two or three PSATs (two mrPATs; one mrPAT and one miniPAT; or two mrPATs and one
107 miniPAT; Table 1).

108 *Satellite tags*

109 MiniPATs (L 124 mm x Ø 38 mm, weight 60 g) collected data on light, temperature ($\pm 0.1^{\circ}$ C) and depth (± 0.5
110 m) in a pre-defined duty cycle. For long term deployments (~130 days), duty cycles were on for the first
111 three days, and then '1 day on-1 day off' with measurements every 300 second. For short term
112 deployments (~90 days), the duty cycles were 'all days on' with measurements every 150 sec. All data was
113 internally binned in 2 hour intervals. In 2015 and 2016, miniPATs were programmed to release if depth

114 remained constant (± 4 m) for 3 executive days, or if depth exceeded 1,700 m as the crush depth of
115 miniPATs is reached at 2,000 m. For miniPATs deployed in 2017, the critical depth mechanism was turned
116 off. mrPATs (L 121 mm x \varnothing 23 mm, weight 31 g) only collected data on daily minimum and maximum
117 temperature ($\pm 0.5^\circ$ C) for a period of 90-100 days prior to release. The crush depth of mrPATs is 2,000 m
118 and the tags have no 'critical depth' or 'constant depth' release mechanism. For both types of PSATs, once
119 released from the animal, the recorded data is transmitted via Argos satellite network when the
120 antenna/cap of the floating tag is exposed to a non-saline environment (i.e. above the ocean surface).
121 Argos location of the tag (which is considered the approximate position of the shark at the time of the tag
122 release) is provided based on Doppler shift calculations. For the sharks in this study, reconstruction of
123 migration paths using geolocation could not be determined from light-intensity levels as the sharks were
124 mainly swimming deeper than the light measurement capabilities of the tag. Therefore, minimum travelling
125 distance for each shark was calculated as a straight-line distance (not over land) either from capture
126 position or from previous tag pop-up position. The minimum distance travelled is reported in km and
127 $\text{km}\cdot\text{d}^{-1}$ calculated from days at liberty from the last known location.

128

129 **Results**

130 *Reporting tags*

131 Of the 33 deployed PSATs, eight tags failed to report usable data for a variety of reasons including failure to
132 establish contact with the Argos satellite following release (no contact, Table 1); delayed (28 and 97 days)
133 Argos locations (Delayed, Table 1); constant-depth release with an associated mrPAT reporting pop-up
134 position from a nearby beach on scheduled time indicating that Shark #5 died in the tagging period. Three
135 miniPATs (Shark #9-11) released as scheduled but did not establish position until two, two, and eight days
136 following release, respectively (all on 4 August 2017). Data from these tags were included for analysis. In
137 November 2017, three tags still remain deployed on sharks.

138 This analysis is therefore based on reports from 22 PSATs from 12 adult females with transmitted data
139 ranges between 39-63% (miniPATs, N=6) and 25-72% (mrPATs, N=16), respectively (Table 1). Days in liberty
140 for PSATs varied between 86-403 days, and totaled 2,359 tagging days.

141

142

143 *Argos locations*

144 Argos locations were available from 16 mrPATs and 6 miniPATs deployed on 12 sharks and are presented in
145 Fig. 1 together with tagging locations and shortest distance- travelled (SDT) (Table 1). Days at liberty (86-
146 403 days) and associated SDT (8-2,754 km) revealed that daily migrations ranged 0.1-17.5 km per day. One
147 shark was at liberty for 90 days travelling 1,066 km (Shark #8); two sharks were at liberty for 129-135 days
148 travelling 138-155 km (Shark #1 and #3); two shark were at liberty for 150 days and travelled 17-129 km
149 (Shark #6 and #7); five sharks were at liberty for 180 days and travelled 144-963 km (Shark #9-13); two
150 sharks were at liberty for 402-403 days and travelled 27-2,754 km (Shark #2 and #4). SDT was not
151 significantly correlated with days in liberty (Pearson's Correlation test, $r=0.3997$, $P>0.1$). Only Shark #7
152 appeared to spend all its time at liberty inshore as both tags released inside and at the entrance of
153 Grædefjord where the shark was tagged originally (Table 1). All other tags reported from offshore waters
154 8-1,523 km from the original tagging position (Fig. 1). Shark #4 exhibited a distinct migratory behavior, as
155 the first tag released in waters of the Reykjanes Ridge (on the Mid-Atlantic ridge) after 87 days in liberty.
156 315 days later, the shark was had returned to the offshore shelf waters of southwestern Greenland, and
157 had thus covered at least 2,754 km (Fig. 1). All other tags were released in either southwestern or
158 southeastern Greenland shelf waters. Two sharks from Grædefjord (Shark #4 and #6), one from
159 Uummanaqfjord (Shark #8) and one shark from Julianehåbsfjord (Shark #11), all occupied the same area
160 off Frederikshåbs Isblink in southwestern Greenland upon tag release (Fig. 1). For two sharks (Shark #1 and
161 #6) both tags released in this area off Frederikshåb Isblink after 3 and 4.5/5 months in liberty (Table 1).
162 Three sharks from Bredefjord and Julianehåbsfjord (Shark #9, #12, #13) exhibited a distinct northeastward
163 migration covering 629-722 km in the first three months in offshore shelf waters of southeastern Greenland
164 (Fig. 1, Table 1). Another 3 months later these sharks still occupied southeastern Greenland shelf waters
165 with one shark being located further north and two further south (Fig. 1).

166 *Depth and temperature*

167 Comprehensive data from miniPATs allowed us evaluate depth and temperature distribution for six sharks
168 (Sharks #1, #3, #6, and #9-11) (Fig. 2a-f), although no temperature data was transmitted for Shark #6. All
169 sharks generally occupied similar depths with mean depth (\pm SD) ranging between 341.6 \pm 214.7 m and
170 455.7 \pm 85.9 m and mean temperatures ranging between 4.6 \pm 0.6° C and 5.1 \pm 1.1° C (depth range 13-1,083 m,
171 temperature range -0.2-8.0° C, Table 2). Tracks on minimum/maximum temperature the last 3 months of
172 the tagging period of all mrPATs showed that temperature tracks from mrPATs were -2.0-9.0° C (Table2),
173 and generally, for mrPATs and miniPATs overlapping in tagging recording period on the shark, temperature
174 tracks were similar. The most frequently occupied depths were shallower than 650 m (97% of the time

175 between 0-650 m, and 71% of the time between 301-550 m (Fig. 3a). Corresponding water temperatures
176 were above 3.6° C (96% of the time between 3.6-8.0° C, and 83% of the time between 4.1-5.5° C, Fig. 3b). It
177 is evident from the individual daily mean±minimum/maximum levels of temperature and depth (Fig. 2a-f),
178 that temperature was rather constant over large portions of the tagging period, while the occupied depth
179 range could vary several hundreds of meters on a daily basis. For sharks tagged in Grædefjord (Shark #1, #3
180 and #6), daily mean depth was always shallower than 600 m and associated change in depth was typically
181 between 150-250 m (Fig. 2a-c). For sharks tagged in Bredefjord and Julianehåbsfjord (Shark #9, #10, and
182 #11) the daily mean depth varied more and multiple days were spent deeper than 600 m with daily depth
183 changes exceeding 400 m (Fig. 2d-f). Shark# 9 (which migrated to southeastern Greenland from Bredefjord)
184 exhibited the highest daily depth difference of all sharks, which on 13 June 2017 was 897 m (Fig. 2d). On
185 this particular day, Shark #9 covered 9,273 vertical meters (i.e. 4,547 ascending meters, 4,726 descending
186 meters). The same animal also spent the last 5 weeks of the miniPATs tagging period at depths mainly
187 shallower than 200 m (most shallow record was 13 m) which was markedly shallower than for all other
188 tagged sharks (Fig. 2d).

189 The temperature tracks recorded by five miniPATs and five mrPATs (Shark #1, #3, #4, and #7-13) showed
190 that all sharks were released into relative cold waters (below 2.5° C, Fig. 2a-f, Supplementary Fig. 1a, c, d,
191 f-i). In Grædefjord, Shark #7 presumably occupied Grædefjord for 150 days in liberty and experienced
192 temperatures mainly between 2.0-2.5° C (range 0.5-3.5° C) throughout the period of the first tag (90 days).
193 Only the last four days prior the second tag's release, at the entrance of Grædefjord, the shark occupied
194 temperatures up to 4.5° C (Supplementary Fig. 1f). Shark #1, #3 and #4 (also tagged in Grædefjord) only
195 experienced temperatures below 3.0° C for 3-6 days, after which they moved into waters where
196 temperatures constantly were above 3.5° C and generally warmer than 4.5° C until the tags released in
197 offshore water after 3 and 4.5 months (Fig. 2a, c, Supplementary Fig. 1a, c-d²). In Bredefjord and
198 Julianehåbsfjord, similar temperature tracks were evident from all sharks (Shark #9-13) which also occupied
199 water masses below 3.0° C for 3-4 days after release, after which they went into warmer waters (above 3.5°
200 C and generally warmer than 4.0° C) until tag release (Fig. 2d-e, Supplementary Fig. 1h-i). For Sharks #9,
201 #12, and #13, which migrated to southeastern Greenland, temperatures reached 6.0-8.0° C in the last 4-5
202 weeks of the 91 days tagging period and remained (mostly) above 4.0° C for the entire tagging period (Fig.
203 2d, Supplementary Fig. 1h-i). In Uummannaqfjord, Shark #8 mainly experienced temperatures between -
204 0.5-2.5° C for 2 months (range -1.0-3.0° C, Supplementary Fig. 1g) following waters of 4.0-4.5° C for last 3.5
205 weeks prior tag release in offshore waters at Frederikshåbs Isblink in southwestern Greenland.

² Note that miniPATs and mrPATs for Shark #1 and #3 were overlapping in time period where data was recorded. Temperature tracks were similar.

206 Discussion

207 In this study, the aim was to investigate the spatial distribution and depth and temperature preferences of
208 adult Greenland shark females in Greenland using PSATs. We observed that offshore shelf waters in this
209 region, together with southeastern Greenland, are of particular importance for adult females, and also find
210 that the shelf water off Frederikshåb Isblink appears to be a “hot spot” for adult females in southwestern
211 Greenland. Furthermore we revealed that some sharks conducted very similar migration in time and space,
212 and we observe a single shark, which after crossing half the Atlantic Ocean, returned to southwestern
213 Greenland waters. Taken together, our investigations provide novel insights into biology of the Greenland
214 shark, and clearly demonstrate the uniqueness of shelf waters in southern Greenland for this poorly
215 studied life stage.

216 In general, we found adult Greenland shark females to occupy depths much shallower than adult females
217 tracked in southeast Canada, which have been found mainly to occupy waters deeper than 900 m and
218 perform long range pelagic migrations hundreds of kilometers off the continental shelf break (Campana et
219 al. 2015). In our study, only Shark #4 exhibited long distance migration over abyssal water, while the
220 majority of tags released from the remaining sharks were in continental shelf waters. More specifically,
221 nine out of twelve sharks remained in, returned or migrated to southwestern Greenland, which
222 demonstrates that this region is essential for adult females across seasons. Southeast Greenland was also
223 inhabited by three adult females tagged on Southwest Greenland for as much as 3 months, supporting a
224 west-to-east migration occurring in Greenland waters, as also demonstrated by Hansen (1963). Southwest
225 and -east Greenland together with Iceland which have been found to contain a higher proportion of adult
226 females compared to other Arctic regions (Nielsen et al. in prep1). Our tracking results support this distinct
227 size-based distributional pattern of Greenland shark life stages as the adult females remained in these
228 areas over long time periods (spring, summer, late-autumn).

229 Although geolocations are lacking (due to no available sunlight at swimming depth of the sharks), it is
230 possible to deduce information on spatial movements between fjords and offshore water in the tracking
231 period. This was done from data on depths and temperatures combined with the overall thermal structures
232 of the water masses in southern Greenland. Offshore shelf waters are characterized by warmer waters than
233 many fjords (Sutherland et al. 2013, Ribergaard 2014). This was well demonstrated for all sharks caught in
234 Grædefjorden, which were released into relatively cold water masses (below 3.5° C) and carried PSATs that
235 released in offshore and warmer waters (4.0-6.0° C). Only one shark had PSATs that released in inshore
236 waters (Shark #7 in Grædefjorden) and this animal had occupied cold waters throughout the tagging period

237 (except for the last four days, where 4.5° C was occupied). For the remaining sharks from Grædefjorden, all
238 PSATs released in offshore waters and the temperature data from these tags showed that the animals had
239 experienced higher temperatures 3-5 days after tagging. In addition to the increase in water temperatures,
240 the corresponding depth records ranged from 550-600 m, which is deeper than any part of Grædefjord.
241 Similar patterns from temperature and depth records were evident for sharks tagged in Julianehåbsfjord
242 and Bredefjord. Taken together, it seems plausible that most sharks exited the fjords shortly after being
243 released, spending the majority of time in warmer offshore water masses. Such behavior of adult females
244 coincides well with their absence in cold fjords of southeastern Greenland, which was reported during the
245 commercial shark fishery more than 50 years ago, where Berland (1961) mentioned that from observations
246 of 1,505 Greenland sharks, they only encountered large specimens (>3.7 m) in offshore waters. Trawl and
247 longline captures from contemporary times in southeast Greenland support such distinct distributional
248 segregation between in- and offshore waters (Nielsen et al. in prep1). Adult females in southwest
249 Greenland are indeed occasionally occupying inshore waters (note that all sharks in this study were caught
250 in inshore waters), but pop-up positions and temperature tracks suggest that offshore waters are
251 predominantly occupied by adult Greenland shark females throughout southern Greenland.

252 Some offshore waters did however seem more frequently occupied than others, as most tags released near
253 the continental shelf break. More distinctly, we found the shelf water off Frederikshåb Isblink to be
254 particularly important as 4 out of 12 sharks visited this location (Fig. 1). Such a congregation, where sharks
255 from three different tagging locations and from different years, visit the same distinct area has not
256 previously been documented, but data from scientific trawl surveys show that adult females have
257 commonly been encountered here (Nielsen et al. 2014). Another example of novel behavior observed in
258 this study, was Shark #4 which migrated to Reykjanes Ridge during the first 3 months, returning to
259 southwest Greenland 10 months later (Fig. 1), covering a total distance of 2,754 km. Besides demonstrating
260 that Greenland sharks can be highly migratory within a short time span, it is peculiar that this specimen
261 reported from the only area (Reykjanes Ridge), where free-swimming neonate Greenland sharks (~42 cm,
262 46 cm, and 55 cm) have been reported from (Kukuev & Trunov 2002, Nielsen et al. in prep1). Whether this
263 Greenland shark was pregnant upon capture and its migration was related to parturition, is not known, but
264 it seems a plausible scenario. More research is warranted to establish whether Greenland shark pupping
265 grounds could be in abyssal waters, e.g. along the Reykjanes Ridge or other parts of the mid-Atlantic ridge.
266 A final example of novel behavior was sharks #12 and #13 which released tags close to each other after a
267 700 km northeastward migration over 3 months. Another 3 months later, these sharks were however much
268 more dispersed in southeast Greenland (second tag) (Fig. 1). Temperature tracks for the entire tagging
269 period show that these sharks occupied very similar water masses suggesting that they had a similar

270 migration behavior in time and space. There are multiple examples from GINR's trawl survey database, of
271 adult Greenland shark females caught in pairs or more. In more detail, 47.3% of all adult females (N=53, TL
272 range 405-510 cm) and 12.5 % of all smaller sharks (N=40, TL range 99-400 cm) and have been caught in
273 hauls together with one or more sharks (range 2-7 sharks per haul) (GINR unpublished data). Furthermore,
274 release positions from sub-adult Greenland sharks tagged with PSATs off Disko Island, western Greenland,
275 released after three months at liberty only few kilometers from each other but more than 1,000 km from
276 original tagging position (Bushnell et al. unpublished data). All of this combined with our results, strongly
277 suggests that Greenland sharks, and especially adult females, occasionally exhibit group behavior.

278 It is currently unknown why adult Greenland shark females occupy waters in southern Greenland more
279 frequently compared to other more arctic and northern regions. The absence of neonate Greenland sharks
280 in trawl surveys and commercial fisheries in southern Greenland shelf waters strongly suggests that
281 pupping grounds are not found in these waters (or in any other investigated continental shelf waters).
282 Mating is however very likely to take place in southwestern Greenland, as adult females, some with ripe
283 ovaries and bite marks from conspecifics, and adult males with calcified clasper, extruded clasper spur and
284 freely flowing sperm, have been observed to overlap in time and space in this region (Nielsen et al. in
285 prep2). However, due to the production of great amounts of ripe ova (up to at least 80 kg, Nielsen et al. in
286 prep2), great nutritional inputs are needed for adult females. We therefore believe, that the observed
287 behavior of adult female sharks occupying shelf waters is related to foraging. Swimming behavior of the
288 sharks on this study was not characterized by diel depth difference (as observed by Skomal & Benz 2004
289 and Stokesburry et al. 2005, but not by Fisk et al. 2012 and Campana et al. 2015), but rather appeared as
290 more random oscillatory migrations of varying magnitude. On a daily basis, vertical excursions of sharks
291 from Grædefjord typically ranged 150-250 m, whereas sharks from Bredefjord and Julianehåbsfjord
292 typically ranged from 350-500 m in depth. One shark exhibited as much as 896 m in daily depth difference
293 while covering a total of 9,273 vertical meters (Shark #9, 13 June 2017, Fig 2d) suggesting that Greenland
294 sharks might be slow swimmers (Watanabe et al. 2012), but can be very active when searching for prey. For
295 Greenland sharks in this study, which presumably spent the entire tracking period in either inshore fjords or
296 offshore continental shelf waters (except shark #4), it is difficult to evaluate if vertical excursions are due to
297 benthic swimming sharks that follow bottom contours, or if excursions were made into pelagic water
298 masses. As the dominant prey for adult Greenland shark females is composed of benthic and epibenthic
299 fishes (MS1), it suggests that most feeding probably occurs along the ocean floor. Pelagic/shallow
300 swimming was however clearly observed for at least one shark (Shark #9 reaching only 13 m of depth) – a
301 behavior which has been linked to hunting of seals (Skomal & Benz 2004, Fisk et al. 2012, Leclerc et al.
302 2012), another important dietary component of adult Greenland sharks (MS1). We expect that the

303 observed vertical excursions of adult females in this study are a combination of both pelagic and benthic
304 swimming behavior.

305 In conclusion, the study demonstrated that adult female Greenland sharks in Greenland waters mainly
306 occupy continental shelf waters at temperatures above 4.0° C and perform great oscillations at depths
307 between 200-550 m. It is noteworthy that we observed similar migration behavior at the same time to
308 almost the same geographical position for two sharks suggesting that this species might be more social
309 than previously described. One shark also migrated to the only area where neonate Greenland sharks have
310 been observed, only to return to Greenland waters 10 months later, suggesting that Greenland sharks have
311 separated mating, feeding, and pupping areas. Further studies should focus on these two observations as
312 the behavior and reproductive biology of Greenland sharks are the keys to understand the biology and
313 conserve this elusive species.

314

315 **Acknowledgements**

316 We are grateful for the contribution of M. B. Backe throughout the manuscript and for the great effort in
317 the field by crew of RV Sanna and Avataq. Great fisherman, cooking, navigating, fishing skills of Flemming
318 Heinrich in Grædefjorden made the difference. We also thank the Greenland Institute of Natural Resources,
319 University of Copenhagen and UiT The Arctic University of Norway for support with logistics and for
320 providing ship time. This study is a part of the larger Greenland shark project ('Old and Cold- Biology of the
321 Greenland shark') which was financially supported by the Danish Council for Independent Research, GINR,
322 Den Blå Planet-National Aquarium of Denmark, Danish Centre for Marine Research and Save Our Seas
323 Foundation.

324

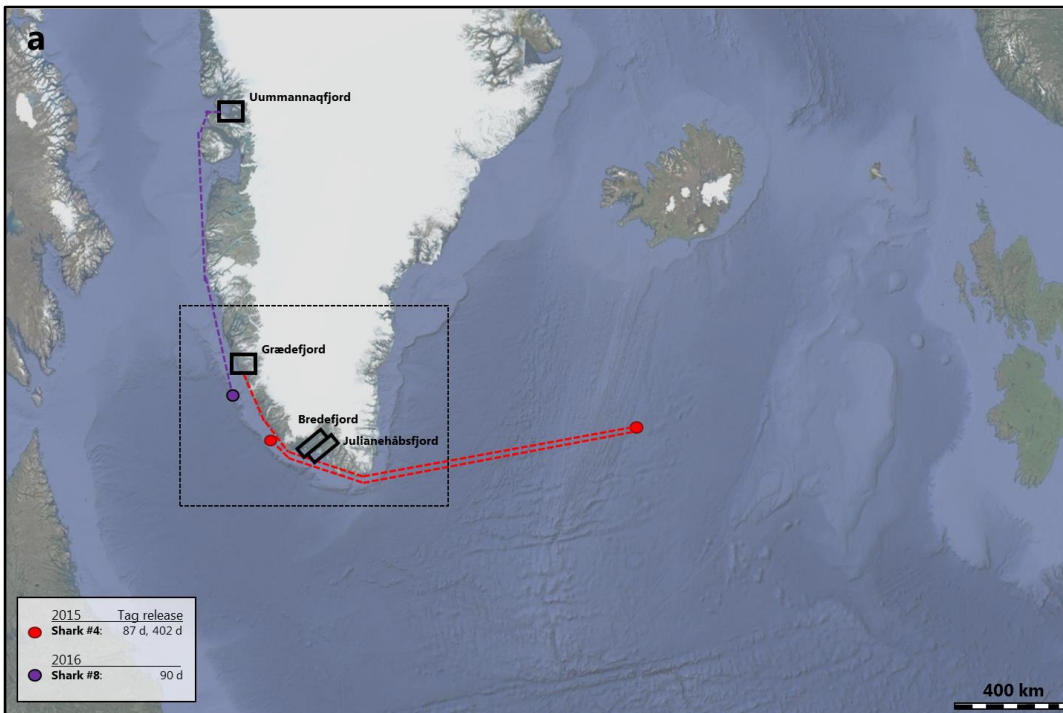
325 **References**

- 326 Anon (1942). Beretninger vedr. Grønlands Styrelse. Sammendrag af Statistiske Oplysninger om Grønland 1:
327 647-652 (in Danish).
328
- 329 Benz GW, Hoffmeyer ER, Driggers WB, Allen D, Bishop LE, Brown PA (2007). First record of a sleeper shark
330 in the western Gulf of Mexico and comments on the taxonomic uncertainty within *Somniosus* (*Somniosus*).
331 Bulletin of Marine Science 80, 2: 343-351.
332
- 333 Berland B (1961). Copepod *Ommatokoita elongate* (Grant) in the eyes of the Greenland shark - a possible
334 cause of mutual dependence. Nature 191: 829-830.
335
- 336 Campana SE, Fisk AT, Klimley AP (2015). Movements of Arctic and northwest Atlantic Greenland sharks
337 (*Somniosus microcephalus*) monitored with archival satellite pop-up tags. Deep-Sea Research II, 115: 109-
338 115.
339
- 340 Davis B, VanderZwaag DL, Consandey-Godin A, Hussey NE, Kessel ST, Worm B (2013). The Conservation of
341 the Greenland Shark (*Somniosus microcephalus*): Setting Scientific, Law, and Policy Coordinates for
342 Avoiding a Species at Risk. Journal of International Wildlife Law & Policy 16: 300-330.
343
- 344 DFO Canada (2016). Greenland Shark Bycatch Estimates in NAFO areas 0A and 0B. Population Ecology
345 Division, Bedford Institute of Oceanography, Dartmouth, NS, Canada.
346
- 347 Fisk AT, Lydersen C, Kovacs KM (2012). Archival pop-off tag tracking of Greenland sharks *Somniosus*
348 *microcephalus* in the High-Arctic waters of Svalbard, Norway. Marine Ecology Progress Series 468: 255-265.
349
- 350 Hansen PM (1963). Tagging Experiments with the Greenland shark (*Somniosus microcephalus* (Block and
351 Schneider)) in Subarea 1. International Commission for the Northwest Atlantic Fisheries. Special Publication
352 no. 4: 172-175.
353
- 354 Herdendorf CE, Berra TM (1995). A Greenland Shark from the wreck of *SS Central America* at 2,200 meters.
355 Transactions of the American Fisheries Society 124, 6: 950-953.
356
- 357 Henriksen S, Hilmo O, red (2015). Norsk rødliste for arter 2015. Artsdatabanken, Norge (in norwegian)
358
- 359 IUCN (2017). The IUCN Red List of Threatened Species. Version 2017-2. <www.iucnredlist.org> (assessed 28
360 Sep 2017).
361
- 362 Jensen AS (1914). The Selachians of Greenland. Zoological Museum of the University Copenhagen. Bianco
363 Lunos Bogtrykkeri: 1-34.
364
- 365 Kukuev EI, Trunov IA (2002). The composition of ichthyofauna of the meso- and bathypelagic zones of the
366 Irminger Current and adjacent waters. Journal of Ichthyology 42, 377-384 (in Russian).
367
- 368 Leclerc LM, Lydersen C, Haug T, Bachmann L, Fisk AT, Kovacs KM (2012). A missing piece in the Arctic food
369 web puzzle? Stomach contents of Greenland sharks sampled in Svalbard, Norway. Polar Biology 35: 1197-
370 1208.
371
372
373

374 MacNeil MA, McMeans BC, Hussey NE, Vecsei P, Svavarsson J, Kovacs KM, Lydersen C, Ramsey M, Fisk AT
375 (2012). Biology of the Greenland shark *Somniosus microcephalus*. *Journal of Fish Biology* 2012 80: 991-
376 1018.
377
378 McMeans BC, Arts MT, Lydersen C, Kovacs KM, Hop H, Falk-Petersen S, Fisk AT (2013). The role of
379 Greenland sharks (*Somniosus microcephalus*) in an Arctic ecosystem: assessed via stable isotopes and fatty
380 acids. *Marine Biology* 160: 1223-1238.
381
382 Nielsen J, Hedeholm RB, Heinemeier J, Bushnell PG, Christiansen JS, Olsen J, Ramsey CB, Brill RW, Simon M
383 Steffensen KF, Steffensen JF (2016). Eye lens radiocarbon reveals centuries of longevity in the Greenland
384 sharks (*Somniosus microcephalus*). *Science* 353, 6300: 702-704.
385
386 Nielsen J, Hedeholm RB, Simon M, Steffensen JF (2014). Distribution and feeding ecology of the Greenland
387 shark (*Somniosus microcephalus*) in Greenland waters. *Polar Biology* 37, 1: 37-46.
388
389 Nielsen et al. (in prep1). Spatial distribution and composition of size-based life stages of Greenland shark
390 *Somniosus microcephalus* across the northern North Atlantic.
391
392 Nielsen et al. (in prep2). Reproduction biology of the Greenland shark (*Somniosus microcephalus*) assessed
393 from novel observations and a review of the existing literature.
394
395 Porteiro FM, Sutton TT, Byrkjedal I, Orlov AM, Heino M, Menezes G, Bergstad OA (2017). Fishes of the
396 northern Mid-Atlantic Ridge collected during the MAR-ECO cruise in June-July 2004: an annotated checklist.
397 Arquipelago Life and Marine Sciences Supplement 10.
398
399 Ribergaard MH (2014) Oceanographic Investigation off West Greenland 2013. Report from Danish
400 Meteorological Insitutte, Centre for Ocean and Ice. pp 1-50.
401
402 Rusyaev SM, Orlov AM (2013). Bycatches of the Greenland Shark *Somniosus microcephalus*
403 (*Squaliformes*, *Chondrichthyes*) in the Barents Sea and the Adjacent Waters under Bottom Trawling Data.
404 *Journal of Ichthyology* 53, 1: 111-115.
405
406 Skomal GB, Benz GW (2004). Ultrasonic tracking of Greenland sharks, *Somniosus microcephalus*,
407 under Arctic ice. *Marine Biology* 145: 489-498.
408
409 Stokesbury MJW, Harvey-Clark C, Gallant J, Block BA, Myers RA (2005). Movement and environmental
410 preferences of Greenland sharks (*Somniosus microcephalus*) electronically tagged in the St. Lawrence
411 Eustuary, Canada. *Marine Biology* 148: 159-165.
412
413 Sutherland DA, Straneo F, Stenson GB, Davidson FJM, Hammill MO, Rosing-Asvid A (2013). Atlantic water
414 variability on the SE Greenland continental shelf and its relationship to SST and bathymetry. *Journal of*
415 *Geophysical Research* 118, doi:10.1029/2012JC008354.
416
417 Watanabe Y, Lydersen C, Fisk AT, Kovacs KM (2012). The slowest fish: Swim speed and tail beat frequency
418 of Greenland sharks. *Journal of Experimental Marine Biology and Ecology* 426-427: 5-11
419
420 Yano K, Stevens JD, Compagno LJV (2007). Distribution, reproduction and feeding of the Greenland shark
421 *Somniosus (Somniosus) microcephalus*, with notes on two other sleeper sharks, *Somniosus (Somniosus)*
422 *pacificus* and *Somniosus (Somniosus) antarcticus*. *Journal of Fish Biology* 70: 374-390.
423

424 **Figures and tables**

425



426

427

428

429

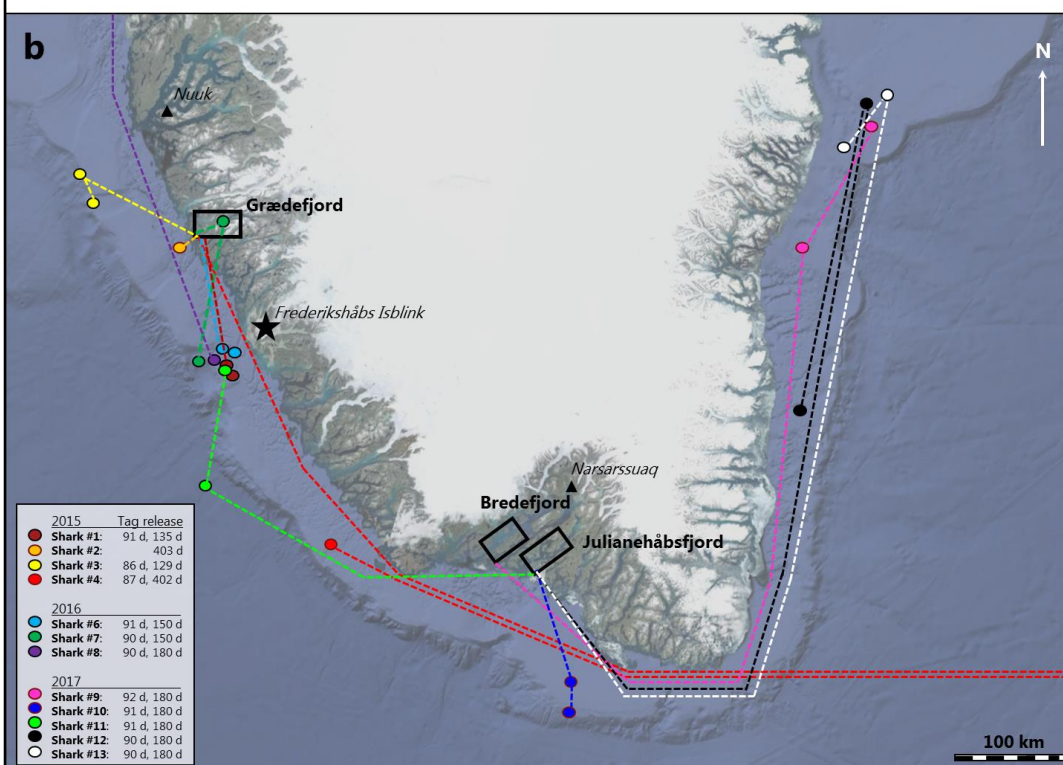
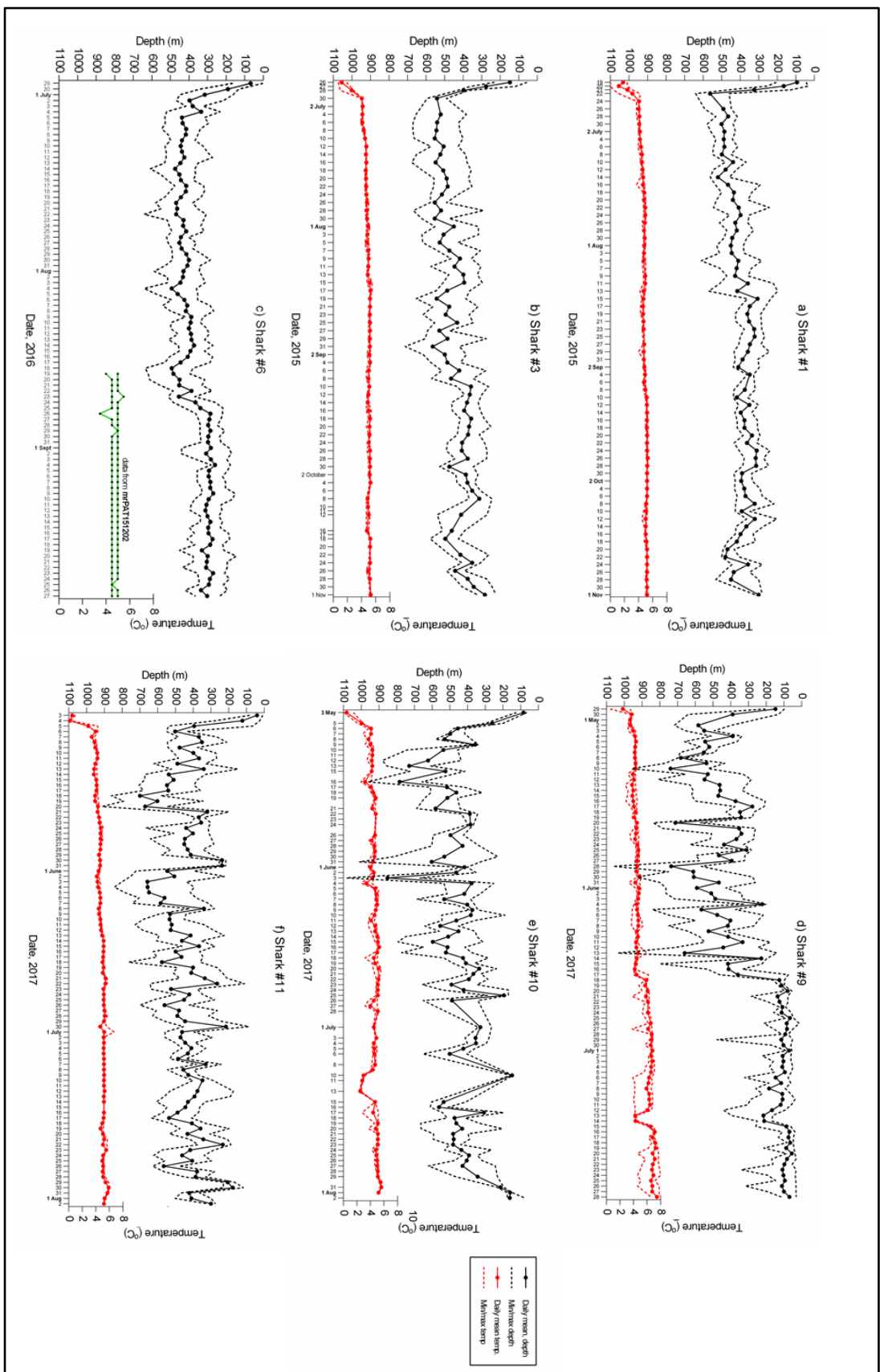


Fig. 1. (a) Map of the three locations in Greenland (Uummannaq Fjord, Grædefjord and Julianehåbsfjord) and long distance migrations of Sharks #4 and #8. (b) Pop-up positions in southern Greenland shelf waters and shortest distance marked for all sharks.



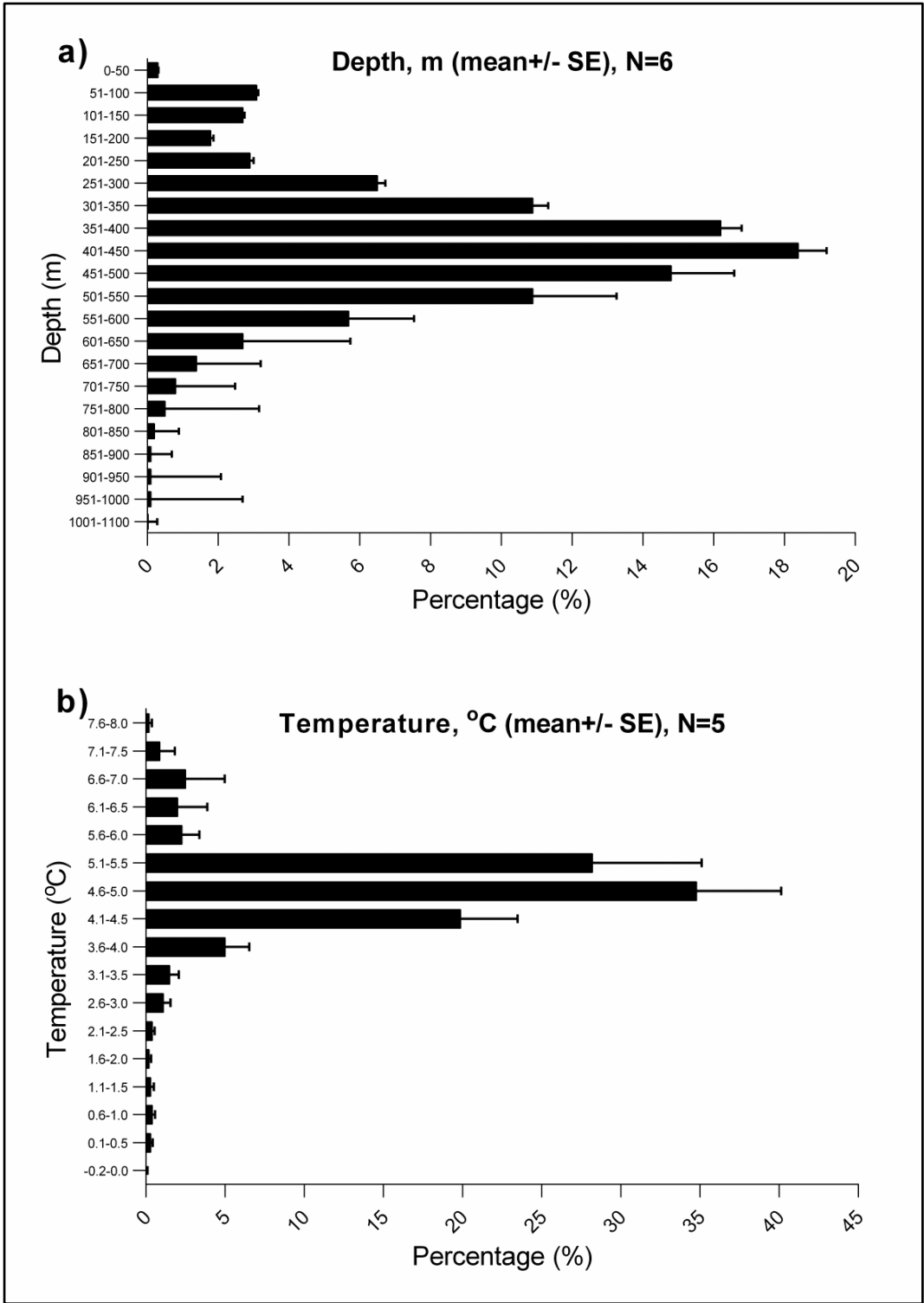
430

431

432

433

Fig. 2. Depth and temperature profiles from sharks tagged with miniPATs (a-f). For shark #6 temperature data from mrPAT (151202) is plotted (green line). Note that tagging periods vary between 3 and 5 months.



434

435

436 **Fig. 3.** Depth (a) and temperature (b) records (mean + 1 SE) for adult Greenland shark females tagged with
 437 miniPATs. Graphs are not correlated.

438

439

Table 1. Tag and release data for Greenland sharks tagged in Greenland shelf waters. DAL=days at liberty. SDT= shortest distance travelled in straight line not crossing land. Capture locations: GRÆ=Grædefjorden, UUM=Uumannaqfjorden, BRE=Bredefjord, JUL=Julianehåbfjorden. Locations are categorized in classes according to accuracy: class 3 (<250 m), class 2 (250-500 m), class 1 (500-1500 m), class 0 (>1,500 m), Class A and B (no accuracy estimate → poor quality position).

Shark	TL, Sex	Location	PSAT / Argos PTT	Date of			Argos position lat;lon (class)	DAL days	SDT Km and km d ⁻¹
				Deployment	Programmed pop-off	Reporting			
Shark #1 (GS168)	420 cm, F	GRÆ	mrPAT/151199	19.06.15	19.09.15	19.09.15	62.294;-50.702 (3)	91	134/1.5
			miniPAT/149843	19.06.15	01.11.15	01.11.15	62.442;-50.677 (3)	135	138/0.9
			miniPAT/151186	19.06.15	01.08.16	29.08.16	Delayed*	-	-
Shark #2 (GS170)	428 cm, F	GRÆ	mrPAT/151201	25.06.15	19.09.15	26.12.15	Delayed*	-	-
			miniPAT/149847	25.06.15	01.11.15	-	No contact	-	-
			mrPAT/151189	25.06.15	01.08.16	01.08.16	63.252;-51.313 (3)	403	27/0.07
Shark #3 (GS171)	410 cm, F	GRÆ	mrPAT/151200	25.06.15	19.09.15	19.09.15	63.721;-53.130 (3)	86	129/1.5
			miniPAT/149844	25.06.15	01.11.15	01.11.15	63.506;-52.895 (3)	129	155/1.2
			mrPAT/151187	25.06.15	01.08.16	-	No contact	-	-
Shark #4 (GS172)	411 cm, F	GRÆ	mrPAT/151198	26.06.15	19.09.15	20.09.15	59.214;-27.983 (3)	87	1,523/17.5
			miniPAT/149846	26.06.15	01.11.15	-	No contact	-	-
			mrPAT/151204	26.06.15	01.06.16	60.944	60.944;-49.176 (3)	402	2,754/6.9
Shark #5 (GS231)	443 cm, F	GRÆ	miniPAT/149848	28.06.16	26.09.16	03.07.15	Premature	5	-
			mrPAT/151196	28.06.16	25.11.15	25.11.16	63.322; -51.147 (A)**	150	-
Shark #6 (GS234)	425 cm, F	GRÆ	miniPAT/158793	29.06.16	27.09.16	28.09.16	62.414;-50.696 (3)	91	121/1.3
			mrPAT/151202	29.06.16	26.11.16	26.11.16	62.395;-50.561 (3)	150	129/0.9
Shark #7 (GS236)	455 cm, F	GRÆ	mrPAT/151192	29.06.16	27.09.16	27.09.16	63.351;-51.045 (3)	90	8/0.1
			mrPAT/151197	29.06.16	26.11.16	26.11.16	63.290;-51.138 (3)	150	17/0.1
Shark #8 (GS250)	425 cm, F	UUM	mrPAT/162825	18.07.16	16.10.16	16.10.16	62.347;-50.533 (3)	90	1,066/11.7
			mrPAT/162826	18.07.16	15.12.16	-	No contact	-	-
Shark #9 (GS280)	410 cm, F	BRE	miniPAT/169369	29.04.17	28.07.17	04.08.17	62.759;-41.147 (3)	92	629/6.9
			mrPAT/162837	29.04.17	26.10.17	26.10.17	63.496;-39.652 (3)	180	742/4.1
			mrPAT/162838	29.04.17	23.06.18	<i>Still out</i>	<i>Still out</i>	<i>Still out</i>	<i>Still out</i>
Shark #10 (GS292)	440 cm, F	JUL	miniPAT/169370	03.05.17	01.08.17	04.08.17	59.763; -45.803 (3)	91	116/1.3
			mrPAT/162833	03.05.17	30.10.17	30.10.17	59.510; -45.186 (3)	180	144/0.8
			mrPAT/162834	03.05.17	27.06.18	<i>Still out</i>	<i>Still out</i>	<i>Still out</i>	<i>Still out</i>
Shark #11 (GS293)	433 cm, F	JUL	miniPAT/169368	03.05.17	01.08.17	04.08.17	61.247;-51.509 (3)	91	308/3.4
			mrPAT/162821	03.05.17	30.10.17	01.11.17	62.271;-50.731 (3)	180	430/2.4
			mrPAT/162822	03.05.17	27.06.18	<i>Still out</i>	<i>Still out</i>	<i>Still out</i>	<i>Still out</i>
Shark #12 (GS294)	430 cm, F	JUL	mrPAT/162835	04.05.17	02.08.17	02.08.17	63.683;-39.614 (3)	90	700/7.8
			mrPAT/162830	04.05.17	31.10.17	31.10.17	61.507;-41.664 (3)	180	963/5.4
Shark #13 (GS298)	410 cm, F	JUL	mrPAT/162839	04.05.17	02.08.17	02.08.17	63.776;-39.251 (3)	90	722/8.0
			mrPAT/162840	04.05.17	31.10.17	31.10.17	63.373;-40.184 (3)	180	787/4.4

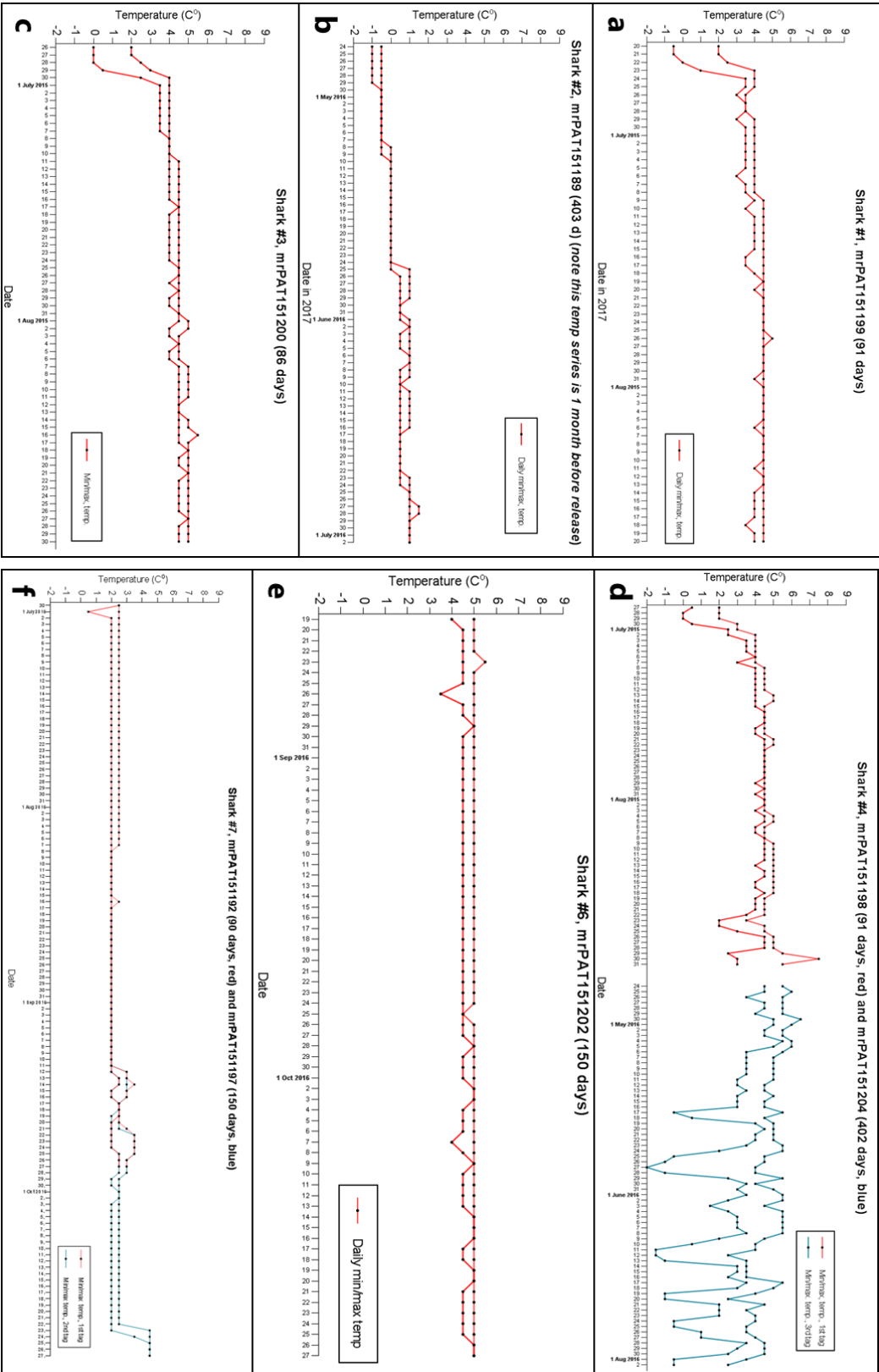
* Tags reporting Argos positions 28/97 days after pop-off → all data excluded for further analysis

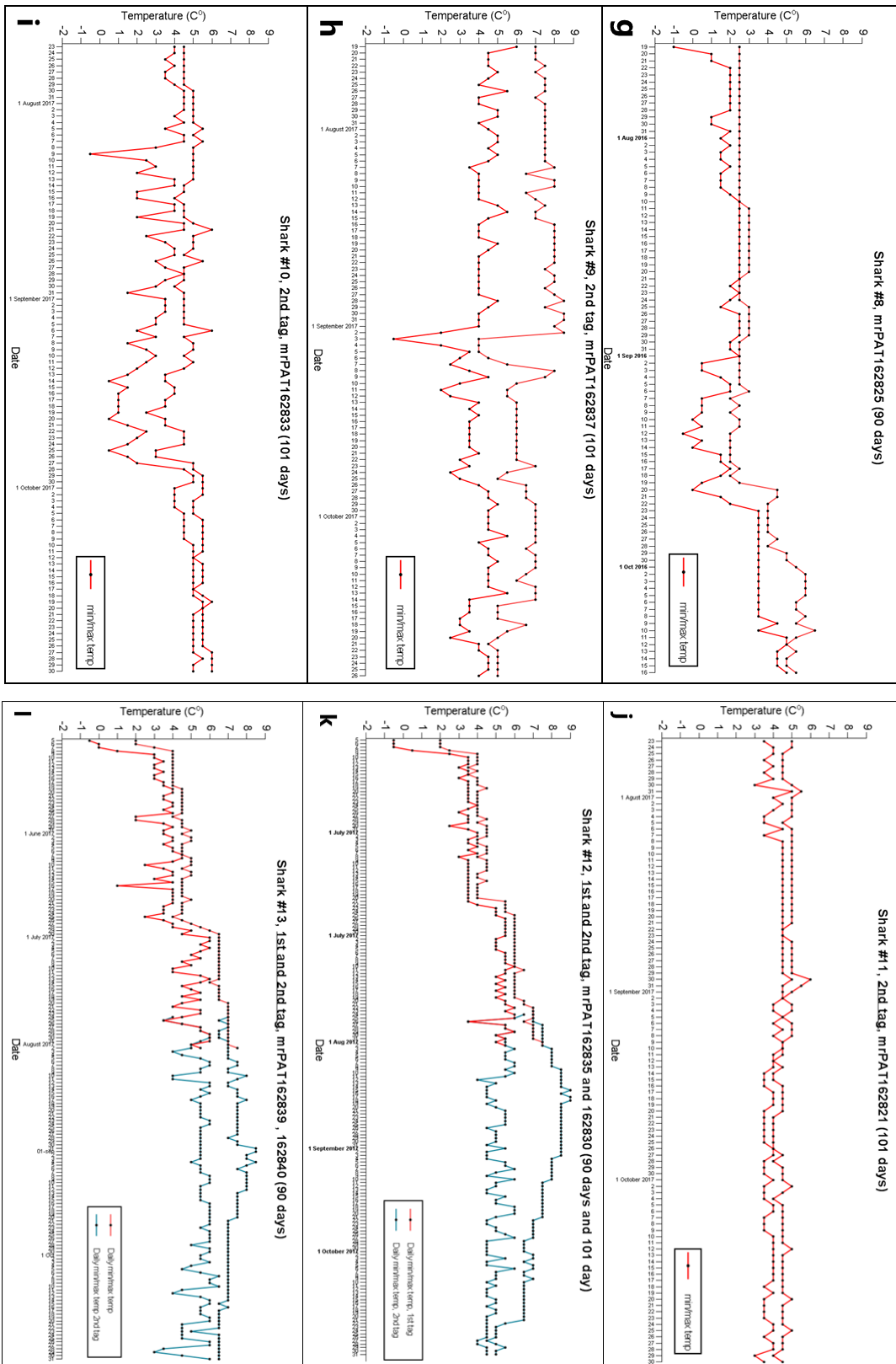
** First PSAT (miniPAT) reporting shortly after release due to constant depth → bad quality position, A, tag probably in the surf (i.e. shark dead) → little temp data transferred → excluded from analysis

Table 2. Temperature and depth (mean±SD, maximum and minimum) from six miniPATs and temperature (maximum and minimum) from 16 mrPATs from 12 Greenland sharks.

Shark		Depth, m			Temp., °C			% data transmitted
		Mean±SD	Min.	Max.	Mean±SD	Min.	Max.	
#1	miniPAT 149843	399.2±80.0	61.5	639.0	4.6±0.8	0	7.2	57
#1	mrPAT 151199					-0.5	5.0	41
#2	mrPAT 151189					-1.0	1.5	78
#3	miniPAT 149844	455.7±85.9	157.5	711.5	4.9±0.4	1.0	5.5	41
#3	mrPAT 151200					0	5.5	48
#4	mrPAT 151198					0	7.5	50
#4	mrPAT 151204					-2.0	6.0	68
#6	miniPAT 158793	375.3±79.9	142.5	640.5	NA	NA	NA	63
#6	mrPAT 151202					3.5	5.5	59
#7	mrPAT 151192					0.5	3.5	53
#7	mrPAT 151197					2.0	4.5	72
#8	mrPAT 162825					-1.0	6.5	20
#9	miniPAT 169369	341.6±214.7	13.5	1061	5.1±1.1	0.6	8.0	53
#9	mrPAT 162837					-0.5	8.5	55
#10	miniPAT 169370	445.4±126.1	76.5	1083	4.6±0.6	2.1	5.7	39
#10	mrPAT 162833					-0.5	6.0	44
#11	miniPAT 169368	441.8±125.0	63	912	4.7±0.7	-0.2	6.7	49
#11	mrPAT 162821					3.5	6.0	19
#12	mrPAT 162835					-0.5	7.5	45
#12	mrPAT 162830					4.0	9.0	64
#13	mrPAT 162839					-0.5	7.5	25
#13	mrPAT 162840					3.0	8.5	28

Supplementary material (MS2)





Supplementary Fig. 1. (a-l) Temperature tracks (daily maximum and minimum) from mrPATs on Sharks #1-4 and #6-13. Note that some tags have overlapping tagging period.

A1

dissociation of this excited state, producing radicals, or by the formation of a diol radical after reaction of an excited-state fatty acid with an adjacent molecule.

Because fatty acid-covered surfaces are ubiquitous, the photochemical production of gas-phase unsaturated and functionalized compounds will affect the local oxidative capacity of the atmosphere and will lead to secondary aerosol formation. This interfacial photochemistry may exert a very large impact, especially if in general the mere presence of a surface layer of a carboxylic acid can trigger this interfacial photochemistry at ocean surfaces, cloud droplets, and the surface of evanescent aerosol particles.

REFERENCES AND NOTES

- C. George, M. Ammann, B. D'Anna, D. J. Donaldson, S. A. Nizkorodov, *Chem. Rev.* **115**, 4218–4258 (2015).
- A. M. Baergen, D. J. Donaldson, *Environ. Sci. Technol.* **47**, 815–820 (2013).
- Y. Dupart *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **109**, 20842–20847 (2012).
- C. Zhu, B. Xiang, L. Zhu, R. Cole, *Chem. Phys. Lett.* **458**, 373–377 (2008).
- S. Enami, M. R. Hoffmann, A. J. Colussi, *J. Phys. Chem. Lett.* **6**, 527–534 (2015).
- M. T. C. Martins-Costa, J. M. Anglada, J. S. Francisco, M. F. Ruiz-Lopez, *J. Am. Chem. Soc.* **134**, 11821–11827 (2012).
- M. T. C. Martins-Costa, F. F. Garcia-Prieto, M. F. Ruiz-Lopez, *Org. Biomol. Chem.* **13**, 1673–1679 (2015).
- K. Mozgawa, B. Mennucci, L. Frediani, *J. Phys. Chem. C* **118**, 4715–4725 (2014).
- R. Vácha, P. Slaviček, M. Mucha, B. J. Finlayson-Pitts, P. Jungwirth, *J. Phys. Chem. A* **108**, 11573–11579 (2004).
- E. C. Griffith, R. J. Rapf, R. K. Shoemaker, B. K. Carpenter, V. Vaida, *J. Am. Chem. Soc.* **136**, 3784–3787 (2014).
- R. Ciuraru *et al.*, *Environ. Sci. Technol.* **49**, 13199–13205 (2015).
- R. Ciuraru *et al.*, *Sci. Rep.* **5**, 12741 (2015).
- H. Fu *et al.*, *J. Am. Chem. Soc.* **137**, 8348–8351 (2015).
- J. D. Coyle, *Chem. Rev.* **78**, 97–123 (1978).
- L. R. Caswell, M. F. Howard, T. M. Onisto, *J. Org. Chem.* **41**, 3312–3316 (1976).
- J. G. Calvert, J. N. Pitts, *Photochemistry* (Wiley, 1966).
- S. Rossignol *et al.*, *Environ. Sci. Technol.* **48**, 3218–3227 (2014).
- A. F. Parsons, *An Introduction to Free Radical Chemistry* (Blackwell Science, 2000).
- J. M. C. Plane *et al.*, "Photochemistry in the sea-surface microlayer" in *The Sea Surface and Global Change*, P. S. Liss, R. A. Duce, Eds. (Cambridge Univ. Press, 1997), pp. 71–93.
- J. R. Kanicky, A. F. Poniatowski, N. R. Mehta, D. O. Shah, *Langmuir* **16**, 172–177 (2000).

ACKNOWLEDGMENTS

This study was supported by the European Research Council (ERC) under the European Union's Seventh Framework Program (FP/2007-2013)/ERC Grant Agreement 290852–AIRSEA. D.J.D. acknowledges ongoing support from the Natural Sciences and Engineering Research Council of Canada. The authors are grateful to P. Mascunan and N. Cristin for the ICP-MS analysis and N. Charbonnel and S. Perrier for the technical support provided by IRCELYON. All the data presented here can be downloaded from the supplementary materials.

SUPPLEMENTARY MATERIALS

www.sciencemag.org/content/353/6300/699/suppl/DC1
Materials and Methods
Figs. S1 to S6
Tables S1 to S3
References (21–26)
Database S1

29 January 2016; accepted 23 June 2016
10.1126/science.aaf3617

LIFE HISTORY

Eye lens radiocarbon reveals centuries of longevity in the Greenland shark (*Somniosus microcephalus*)

Julius Nielsen,^{1,2,3,4,*} Rasmus B. Hedeholm,² Jan Heinemeier,⁵ Peter G. Bushnell,⁶ Jørgen S. Christiansen,⁴ Jesper Olsen,⁵ Christopher Bronk Ramsey,⁷ Richard W. Brill,^{8,9} Malene Simon,¹⁰ Kirstine F. Steffensen,¹ John F. Steffensen¹

The Greenland shark (*Somniosus microcephalus*), an iconic species of the Arctic Seas, grows slowly and reaches >500 centimeters (cm) in total length, suggesting a life span well beyond those of other vertebrates. Radiocarbon dating of eye lens nuclei from 28 female Greenland sharks (81 to 502 cm in total length) revealed a life span of at least 272 years. Only the smallest sharks (220 cm or less) showed signs of the radiocarbon bomb pulse, a time marker of the early 1960s. The age ranges of prebomb sharks (reported as midpoint and extent of the 95.4% probability range) revealed the age at sexual maturity to be at least 156 ± 22 years, and the largest animal (502 cm) to be 392 ± 120 years old. Our results show that the Greenland shark is the longest-lived vertebrate known, and they raise concerns about species conservation.

The Greenland shark (Squaliformes, *Somniosus microcephalus*) is widely distributed in the North Atlantic, with a vertical distribution ranging from the surface to at least 1816-m depth (1, 2). Females outgrow males, and adults typically measure 400 to 500 cm, making this shark species the largest fish native to arctic waters. Because reported annual growth is ≤1 cm (3), their longevity is likely to be exceptional. In general, the biology of the Greenland shark is poorly understood, and longevity and age at first reproduction are completely unknown. The species is categorized as "Data Deficient" in the Norwegian Red List (4).

Conventional growth zone chronologies cannot be used to age Greenland sharks because of their lack of calcified tissues (5). To circumvent this problem, we estimated the age from a chronology obtained from eye lens nuclei by applying radiocarbon dating techniques. In vertebrates,

the eye lens nucleus is composed of metabolically inert crystalline proteins, which in the center (i.e., the embryonic nucleus) is formed during prenatal development (6, 7). This tissue retains proteins synthesized at approximately age 0: a unique feature of the eye lens that has been exploited for other difficult-to-age vertebrates (6, 8, 9).

Our shark chronology was constructed from measurements of isotopes in the eye lens nuclei from 28 female specimens (81 to 502 cm total length, table S1) collected during scientific surveys in Greenland during 2010–2013 (fig. S1) (see supplementary materials). We used radiocarbon (¹⁴C) levels [reported as percent of modern carbon (pMC)] to estimate ages and stable isotopes, ¹³C and ¹⁵N (table S1), to evaluate the carbon source (supplementary materials). Depleted δ¹³C and enriched δ¹⁵N levels established that the embryonic nucleus radiocarbon source was of dietary origin and represents a high trophic level. In other words, isotope signatures are dictated by the diet of the shark's mother, not the sampled animals. We set the terminal date for our analyses to 2012, because samples were collected over a 3-year period. The chronology presumes that size and age are positively correlated.

Since the mid-1950s, bomb-produced radiocarbon from atmospheric tests of thermonuclear weapons has been assimilated in the marine environment, creating a distinct "bomb pulse" in carbon-based chronologies (10). The period of rapid radiocarbon increase is a well-established time stamp for age validation of marine animals (11–14). Radiocarbon chronologies of dietary origin (reflecting the food web) and chronologies reflecting dissolved inorganic radiocarbon of surface mixed and deeper waters, have shown that the timing of the bomb pulse onset (i.e., when

¹Marine Biological Section, University of Copenhagen, Strandpromenaden 5, 3000 Helsingør, Denmark. ²Greenland Institute of Natural Resources, Post Office Box 570, Kivioq 2, 3900 Nuuk, Greenland. ³Den Blå Planet, National Aquarium Denmark, Jacob Fortlingsvej 1, 2770 Kastrup, Denmark. ⁴Department of Arctic and Marine Biology, UiT The Arctic University of Norway, 9037 Tromsø, Norway. ⁵Aarhus AMS Centre, Department of Physics and Astronomy, Aarhus University, Ny Munkegade 120, 8000 Aarhus, Denmark. ⁶Department of Biological Sciences, Indiana University South Bend, 1700 Mishawaka Avenue, South Bend, IN, USA. ⁷Oxford Radiocarbon Accelerator Unit, University of Oxford, Dyson Perrins Building, South Parks Road, Oxford OX1 3QY, UK. ⁸National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Northeast Fisheries Science Center, James J. Howard Marine Sciences Laboratory, 74 Magruder Road, Highlands, NJ 07732, USA. ⁹Virginia Institute of Marine Science, Post Office Box 1346, Gloucester Point, VA 23062, USA. ¹⁰Greenland Climate Research Centre, Greenland Institute of Natural Resources, Post Office Box 570, Kivioq 2, 3900 Nuuk, Greenland. *Corresponding author. Email: julius.nielsen@bio.ku.dk

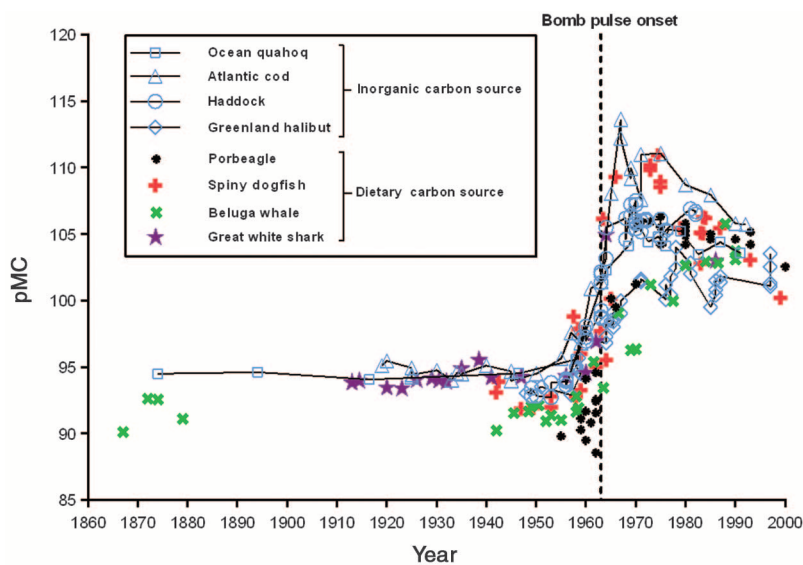


Fig. 1. Radiocarbon chronologies of the North Atlantic Ocean. Radiocarbon levels (pMC) of different origin (inorganic and dietary) over the past 150 years are shown. Open symbols (connected) reflect radiocarbon in marine carbonates (inorganic carbon source) of surface mixed and deeper waters (26, 36–38). Solid symbols reflect radiocarbon in biogenic archives of dietary origin (11, 14, 22, 24). The dashed vertical line indicates the bomb pulse onset in the marine food web in the early 1960s.

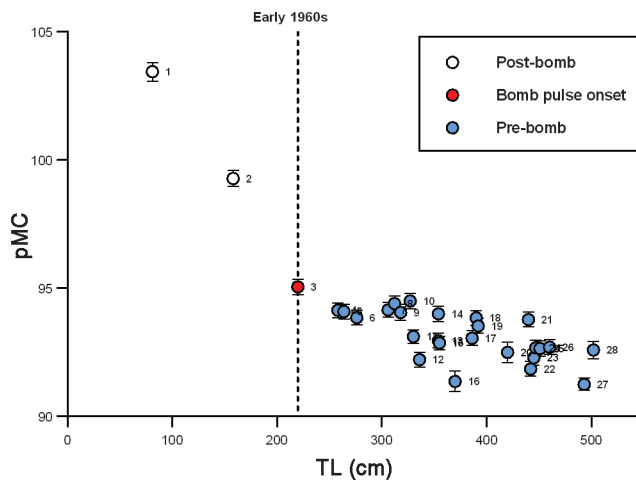


Fig. 2. Radiocarbon in eye lens nuclei of Greenland sharks. Radiocarbon levels (pMC \pm SD, table S1) from 28 females plotted against total length (TL) are shown. Individual animals are identified by the numbers next to the symbols. Nos. 1 and 2 are of postbomb origin, and nos. 4 to 28 are of prebomb origin. We consider shark no. 3 to be from the early 1960s, which is the latest timing of the bomb pulse onset (dashed vertical line).

bomb-produced radiocarbon becomes detectable in a chronology) is synchronous within a few years and no later than early 1960s across the northern North Atlantic (Fig. 1).

Sexually mature females >400 cm have been caught across the Greenland continental shelf at depths between 132 and ~1200 m [(15, 16) and table S1]. Their diet (15–17) and stable isotope signatures (18) (table S1) are comparable to those of other marine top predators such as the porbeagle (*Lamna nasus*), white shark

(*Carcharodon carcharias*), spiny dogfish (*Squalus acanthias*), and beluga whale (*Delphinapterus leucas*) (11, 14, 19–24), for which the bomb pulse onset has been established (Fig. 1). We therefore consider the early 1960s as appropriate for the timing of the bomb pulse onset for the Greenland shark chronology as well.

The two smallest animals (nos. 1 and 2) had the highest radiocarbon levels (>99 pMC), implying that they were indeed affected by the bomb pulse (Fig. 2). However, given the variability

of bomb pulse curves (Fig. 1), no exact age can be assigned to these animals other than that they were born later than the early 1960s. The third animal in the chronology (no. 3, 95.06 pMC), on the other hand, had a radiocarbon level slightly above those of the remaining sharks (nos. 4 to 28, pMC <95), placing its birth year close to the same time as the bomb pulse onset (i.e., early 1960s, Fig. 2). We therefore assign shark no. 3 (total length 220 cm) an age of ~50 years in 2012 and consider the remaining 25 larger animals to be of prebomb origin.

We estimated the age of prebomb sharks based on the Marine13 radiocarbon calibration curve (25), which evaluates carbon-based matter predating the bomb pulse that originates from surface mixed waters. The observed synchronicity of the bomb pulse onset (Fig. 1) supports the presumption that natural temporal changes of prebomb radiocarbon are imprinted in the marine food webs with negligible delay. We contend that the Marine13 curve can contribute to the assessment of the age of prebomb sharks despite the difficulties associated with (i) the low variation in the radiocarbon curve over the past 400 years (25); and (ii) that the degree of radiocarbon depletion in contemporaneous surface mixed waters (local reservoir age deviations, ΔR) differs between regions (26), meaning that the carbon source of the eye lens nucleus reflects food webs of potentially different ΔR levels. Consequently, radiocarbon levels of prebomb animals must be calibrated as a time series under a set of biological and environmental constraints.

We used OxCal (version 4.2) to do this calibration (27). The program uses Bayesian statistics to combine prior knowledge with calibrated age probability distributions to provide posterior age information (28, 29). We constrained age ranges with presumptions about von Bertalanffy growth, size at birth, the age of animal no. 3 deduced from the bomb pulse onset (biological constraints), and plausible ΔR levels from the recent past (environmental constraint). This makes up a Bayesian model that is detailed in the supplementary materials.

Calibrations of single pMC measurements without biological constraints are shown as probability distributions of age with very wide ranges (light blue distributions, Fig. 3). When imposing the model, constrained and narrower age estimates are produced for each prebomb individual, shown as posterior probability distributions of age (dark blue distributions) in Fig. 3 and posterior calibrated age ranges at 95.4% (2σ) probability in table S2. OxCal also calculated agreement indices for each individual shark (A index) and for the calibration model (A_{model}). This allowed us to evaluate the consistency between modeled age ranges and Marine13, as well as the internal agreement between data points of the model (table S2) (30). To test the effect of the fixed age parameter (shark no. 3), a sensitivity analysis was made (supplementary materials and fig. S2), showing that the overall finding of extreme Greenland shark longevity is robust

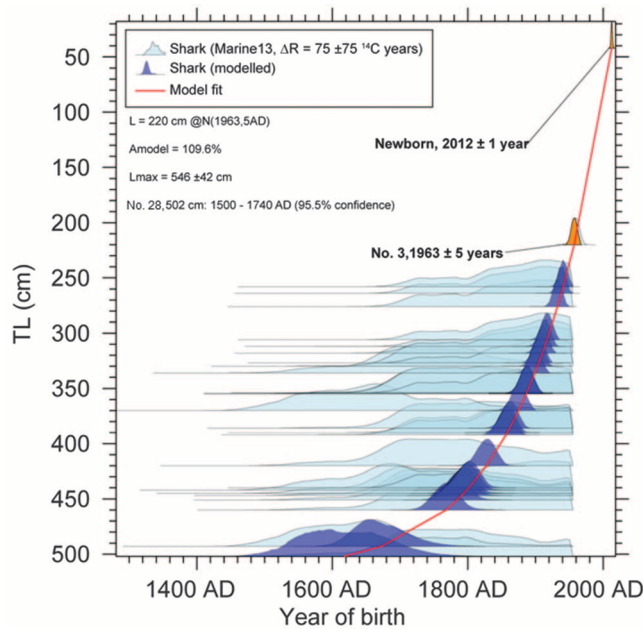


Fig. 3. Bayesian age ranges of prebomb sharks. The estimated year of birth against total length (TL) for prebomb sharks (nos. 4 to 28) is shown. Light blue shows the individual age probability distributions for each shark, and modeled posterior age probability distributions are shown in dark blue. Fixed age distributions (model input) of one newborn shark (42 cm, 2012 ± 1) and of shark no. 3 (220 cm, born in 1963 ± 5) are shown in orange. The red line is the model fit connecting the geometric mean for each posterior age probability distribution. (**Inset**) The model output; i.e., A_{model} , L_{max} , and range of birth year for shark no. 28. Also see the supplementary materials.

regardless of the exact timing of the bomb pulse onset (1958–1980).

The model estimated asymptotical total length to be 546 ± 42 cm (mean \pm SD), a size matching the largest records for Greenland sharks (2), and the age estimates (reported as midpoint and extent of the 95.4% probability range) of the two largest Greenland sharks to be 335 ± 75 years (no. 27, 493 cm) and 392 ± 120 years (no. 28, 502 cm). Moreover, because females are reported to reach sexual maturity at lengths >400 cm (15), the corresponding age would be at least 156 ± 22 years (no. 19, 392 cm) (table S2). A_{model} was 109.6%, demonstrating that samples are in good internal agreement, implying that the age estimates are reliable.

The validity of our Greenland shark age estimates is supported by other lines of evidence. For instance, we found sharks <300 cm to be younger than 100 years (table S2). Such age estimates are indirectly corroborated by their depleted $\delta^{13}\text{C}$ levels (table S1), possibly reflecting the Suess effect, another chemical time mark triggered by emissions of fossil fuels, imprinted in marine food webs since the early 20th century (31, 32). In addition, high levels of accumulated anthropogenic contaminants may suggest that ~ 300 -cm females are older than 50 years (33). Taken together, these findings seem to corroborate an estimated life span of at least 272 years for Greenland sharks attaining more than 500 cm in length.

Our results demonstrate that the Greenland shark is among the longest-lived vertebrate spe-

cies, surpassing even the bowhead whale (*Balaena mysticetus*, estimated longevity of 211 years) (9). The life expectancy of the Greenland shark is exceeded only by that of the ocean quahog (*Arctica islandica*, 507 years) (34). Our estimates strongly suggest a precautionary approach to the conservation of the Greenland shark, because they are common bycatch in arctic and subarctic groundfish fisheries and have been subjected to several recent commercial exploitation initiatives (35).

REFERENCES AND NOTES

- H. B. Bigelow, W. C. Schroeder, in *Fishes of the Western North Atlantic*, A. E. Parr, Ed. (Yale University, New Haven, CT, 1948), pp. 516–523.
- S. E. Campana, A. T. Fisk, A. P. Klimley, *Deep Sea Res. Part II Top. Stud. Oceanogr.* **115**, 109–115 (2015).
- P. M. Hansen, *International Commission for the Northwest Atlantic Fisheries Special Publication* **4**, 172–175 (1963).
- S. Henriksen, O. Hilmo, Eds., *Norsk Rødliste for Arter* (Artsdatabanken, Norge, 2015).
- P. M. Kyne, C. A. Simpendorfer, Adaptive physiology and conservation, in *Sharks and Their Relatives*, J. C. Carrier, J. A. Musick, M. R. Heithaus, Eds. (CRC Press, 2010), pp. 37–71.
- N. Lynnerup, H. Kjeldsen, S. Heegaard, C. Jacobsen, J. Heinemeier, *PLOS ONE* **3**, e1529 (2008).
- S. Bassnett, Y. Shi, G. F. J. M. Vrensen, *Philos. Trans. R. Soc. London Ser. B* **366**, 1250–1264 (2011).
- J. L. Bada, C. D. Vrolijk, S. Brown, E. R. M. Druffel, R. E. M. Hedges, *Geophys. Res. Lett.* **14**, 1065–1067 (1987).
- J. C. George et al., *Can. J. Zool.* **77**, 571–580 (1999).
- H. De Vries, *Science* **128**, 250–251 (1958).
- S. E. Campana, L. J. Natanson, S. Myklevoll, *Can. J. Fish. Aquat. Sci.* **59**, 450–455 (2002).
- J. M. Kalish, *Earth Planet. Sci. Lett.* **114**, 549–554 (1993).

- M. P. Francis, S. E. Campana, C. M. Jones, *Mar. Freshw. Res.* **58**, 10–23 (2007).
- L. L. Hamady, L. J. Natanson, G. B. Skomal, S. R. Thorrold, *PLOS ONE* **9**, e84006 (2014).
- K. Yano, J. D. Stevens, L. J. V. Compagno, *J. Fish Biol.* **70**, 374–390 (2007).
- J. Nielsen, R. B. Hedeholm, M. Simon, J. F. Steffensen, *Polar Biol.* **37**, 37–46 (2014).
- B. C. McMeans, J. Svavarsson, S. Dennard, A. T. Fisk, *Can. J. Fish. Aquat. Sci.* **67**, 1428–1438 (2010).
- J. H. Hansen, R. B. Hedeholm, K. Sünksen, J. T. Christensen, P. Grønkvær, *Mar. Ecol. Prog. Ser.* **467**, 47–59 (2012).
- L. J. V. Compagno, Ed., *FAO Species Catalogue. Vol. 4: Sharks of the World. An Annotated and Illustrated Catalogue of the Shark Species Known to Date. Part 1. Haxanchiformes to Lamniformes* (FAO Fisheries Synopsis, Food and Agriculture Organization of the United Nations, ed. 4, 1984).
- M. P. Heide-Jørgensen, J. Teilman, *Biosci* **39**, 195–212 (1994).
- W. N. Joyce et al., *ICES J. Mar. Sci.* **59**, 1263–1269 (2002).
- S. E. Campana, C. Jones, G. A. McFarlane, S. Myklevoll, *Environ. Biol. Fishes* **77**, 327–336 (2006).
- J. A. Estrada, A. N. Rice, L. J. Natanson, G. B. Skomal, *Ecology* **87**, 829–834 (2006).
- R. E. A. Stewart, S. E. Campana, C. M. Jones, B. E. Stewart, *Can. J. Zool.* **84**, 1840–1852 (2006).
- P. J. Reimer et al., *Radiocarbon* **55**, 1869–1887 (2013).
- J. D. Scourse et al., *Radiocarbon* **54**, 165–186 (2012).
- C. Bronk Ramsey, *Radiocarbon* **37**, 425–430 (1995).
- C. Bronk Ramsey, *Quat. Sci. Rev.* **27**, 42–60 (2008).
- C. Bronk Ramsey, S. Lee, *Radiocarbon* **55**, 720–730 (2013).
- C. Bronk Ramsey, *Radiocarbon* **51**, 1023–1045 (2009).
- J. T. Christensen, K. Richardson, *Mar. Ecol. Prog. Ser.* **368**, 1–8 (2008).
- P. G. Butler et al., *Earth Planet. Sci. Lett.* **279**, 230–241 (2009).
- A. T. Fisk, S. A. Tittlemier, J. L. Pranschke, R. J. Norstrom, *Ecology* **83**, 2162–2172 (2002).
- P. G. Butler, A. D. Wanamaker Jr., J. D. Scourse, C. A. Richardson, D. J. Reynolds, *Palaeogeogr. Palaeoclimatol.* **373**, 141–151 (2013).
- R. B. Stouby, *Eksportkrone for Skiddfisk* (Eksportrådet: The Trade Council, Danish Ministry of Foreign Affairs 2, Copenhagen, Denmark, 2011).
- S. E. Campana, *Mar. Ecol. Prog. Ser.* **150**, 49–56 (1997).
- J. M. Kalish, R. Nydal, K. H. Nedreaas, G. S. Burr, G. L. Eine, *Radiocarbon* **43**, 843–855 (2001).
- M. A. Treble, S. E. Campana, R. J. Wastle, C. N. Jones, J. Boje, *Can. J. Sci. Aquat. Sci.* **65**, 1047–1059 (2008).

ACKNOWLEDGMENTS

We are grateful for the contributions from M. B. Backe throughout the manuscript. We thank the Commission of Scientific Investigations in Greenland (KVUG), Save Our Seas Foundation, National Geographic Foundation, Carlsberg Foundation, Danish Centre for Marine Research, Den Blå Planet–National Aquarium of Denmark, Greenland Institute of Natural Resources (GINR), and the Danish Council for Independent Research for financial support. We thank GINR, the University of Copenhagen and the TUNU Programme (UIT, The Arctic University of Norway) for ship time. We are grateful for the collaboration with K.P. Lange. We thank the crews of the RV *Påmiut*, RV *Dana*, RV *Helmer Hanssen*, RV *Sanna*, and RV *Porsild*. Three anonymous reviewers provided helpful comments and discussion that improved earlier versions of the manuscript.

SUPPLEMENTARY MATERIALS

www.sciencemag.org/content/353/6300/702/suppl/DC1
Material and Methods
Supplementary Text
Figs. S1 and S2
Tables S1 and S2
References (39–50)

29 December 2015; accepted 10 June 2016
10.1126/science.aaf1703



Eye lens radiocarbon reveals centuries of longevity in the Greenland shark (*Somniosus microcephalus*)

Julius Nielsen, Rasmus B. Hedeholm, Jan Heinemeier, Peter G. Bushnell, Jørgen S. Christiansen, Jesper Olsen, Christopher Bronk Ramsey, Richard W. Brill, Malene Simon, Kirstine F. Steffensen and John F. Steffensen (August 12, 2016)
Science **353** (6300), 702-704. [doi: 10.1126/science.aaf1703]

Editor's Summary

This copy is for your personal, non-commercial use only.

- Article Tools** Visit the online version of this article to access the personalization and article tools:
<http://science.sciencemag.org/content/353/6300/702>
- Permissions** Obtain information about reproducing this article:
<http://www.sciencemag.org/about/permissions.dtl>

Science (print ISSN 0036-8075; online ISSN 1095-9203) is published weekly, except the last week in December, by the American Association for the Advancement of Science, 1200 New York Avenue NW, Washington, DC 20005. Copyright 2016 by the American Association for the Advancement of Science; all rights reserved. The title *Science* is a registered trademark of AAAS.



Supplementary Materials for

Eye lens radiocarbon reveals centuries of longevity in the Greenland shark (*Somniosus microcephalus*)

Julius Nielsen,* Rasmus B. Hedeholm, Jan Heinemeier, Peter G. Bushnell, Jørgen S. Christiansen, Jesper Olsen, Christopher Bronk Ramsey, Richard W. Brill, Malene Simon, Kirstine F. Steffensen, John F. Steffensen

*Corresponding author. Email: julius.nielsen@bio.ku.dk

Published 12 August 2016, *Science* **353**, 702 (2016)
DOI: 10.1126/science.aaf1703

This PDF file includes:

- Materials and Methods
- Supplementary Text
- Figs. S1 and S2
- Tables S1 and S2
- References

Materials and Methods

Sampling of sharks and eye lens nuclei

Analyzed sharks were caught from 2010-2013 as unintended bycatch during the Annual Fish Survey of Greenland Institute of Natural Resources, by the commercial fishing fleet and from scientific long lines. All sampling was carried out in accordance with laws and regulations and with authorization from the Government of Greenland (Ministry of Fisheries, Hunting & Agriculture, document number 565466 and 935119). Samples were taken from specimens with lethal injuries caused by conspecifics or fishing equipment. Sharks were euthanized immediately after capture by direct spinal cord transection. Total body length was measured and eye globes were removed and stored at -20°C . The left eye lens was subsequently prepared at the Aarhus AMS Centre (Department of Physics and Astronomy, Aarhus University, Denmark) by isolating the embryonic eye lens nucleus under light microscopy from concentrically arranged layers of secondary fiber cells. A 4-5 mg subsample of the innermost part of the embryonic nucleus was used for isotopic analyses with Accelerator Mass Spectrometry (AMS) and Continuous-Flow Isotope Ratio Mass Spectrometry (CF-EA-IRMS).

Sample preparation and isotope measurements

Embryonic nucleus samples were converted to CO_2 by combustion at 950°C in sealed evacuated quartz ampoules with CuO . A subsample of the resulting CO_2 gas was used for $\delta^{13}\text{C}$ Dual-Inlet analysis on an IsoPrime stable isotope ratio mass spectrometer to a precision of 0.02‰ , while the rest was converted to graphite for AMS ^{14}C measurements (AMS Laboratory, Accium Biosciences, Seattle, WA, USA (41). The

results are reported according to international conventions (42) and ^{14}C content is given as percentage modern carbon (pMC) based on the measured $^{14}\text{C}/^{12}\text{C}$ ratio corrected for the natural isotopic fractionation by normalizing the $\delta^{13}\text{C}$ value to -25‰ VPDB (Vienna Pee Dee Belemnite; $\delta^{13}\text{C}$ calibration standard). The pMC unit is calculated as $100 * F^{14}\text{C}$ (43) and reported as mean pMC \pm SD. ^{14}C measurements are also presented as non-age corrected $\Delta^{14}\text{C}$ values where $\Delta^{14}\text{C} = (\text{pMC}/100 - 1) \times 1000 \text{ ‰}$ (44). Stable isotopes, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, were measured on eye lens nucleus samples weighed into tin cups at the Aarhus AMS Centre by continuous-flow isotope ratio mass spectrometry (Vario Cube elemental analyzer coupled to an IsoPrime stable isotope ratio mass spectrometer). All isotopic measurements are reported as mean \pm SD. The instrument precision is determined by the standard deviation of ~ 16 measurements on the in house standard yielding $\sim 0.2\text{‰}$ for $\delta^{13}\text{C}$ and $0.2 - 0.5\text{‰}$ for $\delta^{15}\text{N}$ for each analysis batch. The in house standard is a commercial gelatin which is calibrated against international IAEA standards. The statistical correlation between TL and $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and pre-bomb ^{14}C levels, were evaluated by Spearman's Rank Correlation Test.

Supplementary Text

Bayesian model design

The biological and environmental constraints of the Bayesian model are: 1) the largest shark with a bomb-induced ^{14}C signature is 49 ± 5 years old (which in the model input is fixed as mean \pm SD), 2) length and age are positively correlated, where length increments decline asymptotically with age as expressed by a Von Bertalanffy growth

curve, 3) size at birth (i.e., age 0) is given by $L_0 = 42$ cm and 4) ΔR can vary according to a normal distribution of 75 ± 75 ^{14}C years (mean \pm SD, $N(75,75)$).

By setting the largest shark with bomb-induced radiocarbon (no. 3 of 220 cm) to be 49 ± 5 years old (i.e. birth year 1963 ± 5 , $N(1963,5)$) we introduce a time range that encompasses the earliest and latest detection of the bomb pulse rise in comparable marine food webs chronologies (Fig. 1) and also the first detection in metabolically active tissues of pelagic deep sea fauna (45, 46). This timing defines a sharp boundary for the successive time sequence of birth dates for the larger sharks which were also presumed to follow an exponential age-length expression:

$$L=L_{max} \cdot [1-\exp(-t/\tau)]$$

equivalent to a traditional Von Bertalanffy growth curve (47). Such growth patterns or derivate thereof have been demonstrated for multiple shark species (48). The sequence starts at the birth dates of the largest (presumed oldest) sharks and ends with a fictive newborn 0 years old shark of 42 cm fixed (i.e. year of birth 2012 ± 1). This size was chosen based on documented near term fetuses of 37 cm (49) and the smallest recorded free-swimming Greenland sharks of 41.8 cm TL (~ 42 cm) (50). The Bayesian statistics of the model assume a strict sequence of birth dates according to shark length. To incorporate the ΔR uncertainty, the model includes a ΔR value which is allowed to vary for individual sharks in the model according to a Gaussian distribution of around 75 ^{14}C years with a 1 sigma of 75 ^{14}C years. This ΔR range is representative for the resent past in

northern North Atlantic surface mixed waters (27). Results of the model output are illustrated in Fig. 3 as full posterior probability distributions for each shark. We present the age range estimates for each pre-bomb shark as 95.4 % (2 sigma) probability (table S2).

Bayesian model function

The Bayesian model was implemented in OxCal (version 4.2) (28-30, 32). In the Bayesian analysis we define a uniform prior for the age of the longest shark t_l and for the time constant τ . Given the imposed constraints (see above), t_l and τ are the only independent parameters in the model. Given these two parameters, the length L_l of the longest shark, and the length at birth L_0 , we can deduce the age t of any animal from its length L using the equation:

$$t = -\tau \ln \left(1 - \left(\frac{L - L_0}{L_l - L_0} \right) \left(1 - \exp \left(\frac{-t_l}{\tau} \right) \right) \right)$$

We sample over all possible values of the two independent parameters (t_l and τ) conditioned on the likelihood from the radiocarbon calibration applied to the radiocarbon measurements on the individual specimens. This gives us a marginal posterior distribution for τ and for the ages of each pre-bomb shark. We have used OxCal to implement this Bayesian model because it is already set up to calculate the likelihood distributions from radiocarbon calibration under such an exponential growth model (equation A44 in 29). The code for implementing this model in OxCal is given below.

The agreement between model and data (A_{model}) are measured using the agreement indices which are a measure of the overlap between the un-modeled and modeled probability distributions provided by Oxcal (51). Generally A_{model} below 60% are considered as poor agreement.

Model priors and likelihoods

Parameter	Explanation
τ	Exponential time-constant for Von Bertalanffy growth curve
t_0	Collection date
t_1	Birth date of longest shark with bomb ^{14}C
t_i	Birth date of shark i , $1 < i < N$
t_N	Birth date of longest shark
d_i	Marine reservoir offset for shark i , $1 < i \leq N$
L_0	The length of sharks at birth (set at 42cm)
L_i	The length of shark i , $1 \leq i \leq N$
$r(t)$	Global marine radiocarbon calibration curve (GMRCC)
$s(t)$	Standard uncertainty in GMRCC
r_i	Radiocarbon date for shark i , $1 < i \leq N$
s_i	Standard uncertainty in radiocarbon date for shark i

The prior for the birth date of the oldest shark is uniform:

$$t_N \sim U(-\infty, t_0)$$

From this parameter the date of birth of all the other sharks can be estimated:

$$t_i = t_0 - \tau \cdot \ln \left(1 - \frac{L_i - L_0}{L_N - L_0} \right) \cdot \left(1 - \exp \left(-\frac{t_i}{\tau} \right) \right)$$

We define a uniform prior for τ :

$$\tau \sim U(0, \infty)$$

The local marine reservoir for each shark is independent and given a normal prior:

$$d_i = N(75, 75^2)$$

Given this and the marine calibration curve the likelihood from radiocarbon calibration is:

$$p(\theta|t_i) = \frac{1}{\sqrt{s_i^2 + s^2(t_i)}} \exp\left(-\frac{(r_i - d_i - r(t_i))^2}{2(s_i^2 + s^2(t_i))}\right)$$

where Θ is the set of variable parameters. This applied to all the sharks ($1 < i \leq N$) except for the youngest shark which has been given a likelihood:

$$p(\theta|t_i) \propto N(AD(1963), 5^2)$$

The collection date is given a prior of:

$$t_0 \propto N(AD(2012), 1^2)$$

The informative independent variables in the model are τ and t_N . The only other independent variables are the marine reservoir offsets for the sharks d_i . MCMC is used to sample over the parameter space defined by $\{\tau, t_N, d_i\}$ using the priors and likelihoods defined above.

Model sensitivity test

Because we cannot verify the exact timing of the bomb pulse onset in the Greenland shark chronology, four additional model runs (scenarios) were conducted to test the model sensitivity of the birth year assigned to the shark with fixed age (no. 3, 220 cm, 49 ± 5 years). The four alternative scenarios are:

- Scenario 1: Shark no. 3 (length of 220 cm) is assumed a birth year of 1975, $N(1975AD, 5)$, corresponding to an age of 37 ± 5 years.
- Scenario 2: Shark no. 4 (length of 258 cm) is assumed a birth year of 1963, $N(1963AD, 5)$, corresponding to an age of 49 ± 5 years. In this scenario shark no. 3 is excluded from the model.

- Scenario 3: Shark no. 4 (length of 258 cm) is assumed a birth year of 1975, $N(1975AD,5)$, corresponding to an age of 37 ± 5 years. In this scenario shark no. 3 is excluded from the model.
- Scenario 4: Shark no. 3 (length of 220 cm) is assumed a uniform prior birth year distribution between 1963 and 2012, $U(1963AD,2012AD)$.

For the model to run these tests adequately the smallest seven sharks (shark nos. 3-10) are assumed to have an uniform prior age distribution, $U(1700AD,1980AD)$. Studies from the Pacific Ocean show that all tissue samples from abyssopelagic and abyssobenthic fishes contained bomb-induced radiocarbon of dietary origin in the 1970s (45, 46, 52). Therefore, we contend that these alternative scenarios represent the most conservative estimates for the timing of the bomb pulse onset in the context of calibrating the Greenland shark chronology.

Model outputs are shown in Fig. S2. It is evident from all scenarios that the estimated age of shark no. 28 and asymptotic length (L_{max}) are robust to changes in fixed age of the youngest sharks. In all four scenarios the A_{model} -values were below 60% (indicating poor agreement between data and model assumptions), and well below that of the model presented in Fig. 3 ($A_{model} = 109.6\%$). Interestingly, scenario 4, where the birth age of shark no. 3 was assigned a weak prior age probability distribution, $U(1963AD,2012AD)$, produced a model output with the highest A_{model} (56 %) and is also most similar to the model presented in Fig. 3. This supports our contention, that the age of shark no. 3 being

~50 years is a valid estimate and hence that the fixed input of birth years between 1958-1968 for this shark in the model presented in Fig. 3 is appropriate.

Oxcal model code

```
Plot()
{
  Curve("Marinel3", "marinel3.14c");
  U_Sequence("Age_vs_Length")
  {
    Tau_Boundary("Tau")
    {
      color="green";
    };
    Delta_R("GS65DR", 75, 75);
    R_Date("10 (GS65, 502 cm)", 617, 30)
    {
      z=502;
      color="blue";
    };
    Delta_R("GS67DR", 75, 75);
    R_Date("16 (GS67 B, 493 cm)", 736, 21)
    {
      z=493;
      color="blue";
    };
    Delta_R("GS42DR", 75, 75);
    R_Date("10, GS42 (460 cm)", 608, 25)
    {
      z=460;
      color="blue";
    };
    Delta_R("GS64DR", 75, 75);
    R_Date("19 (GS64 B, 451 cm)", 612, 27)
    {
      z=451;
      color="blue";
    };
    Delta_R("GS2DR", 75, 75);
    R_Date("15, GS2 (447 cm)", 611, 25)
    {
      z=447;
      color="blue";
    };
  };
};
```

```

Delta_R("GS53DR",75, 75);
R_Date("06 (GS53, 445 cm)",645,27)
{
  z=445;
  color="blue";
};
Delta_R("GS5DR",75, 75);
R_Date("09, GS5 (442 cm)",682,25)
{
  z=442;
  color="blue";
};
Delta_R("GS80DR",75, 75);
R_Date("12 (GS80, 440 cm)",516,25)
{
  z=440;
  color="blue";
};
Delta_R("GS4DR",75, 75);
R_Date("08, GS4 (420 cm)",627,35)
{
  z=420;
  color="blue";
};
Delta_R("GS59DR",75, 75);
R_Date("09 (GS59, 392 cm)",537,25)
{
  z=392;
  color="blue";
};
Delta_R("GS58DR",75, 75);
R_Date("04 (GS58, 390 cm)",510,25)
{
  z=390;
  color="blue";
};
Delta_R("GS14DR",75, 75);
R_Date("07, GS14 (386 cm)",578,25)
{
  z=386;
  color="blue";
};
Date("Typical",U(1600,2000,5))
{
  z=375;
  color="green";
};

```

```

Delta_R("GS6DR",75, 75);
R_Date("13, GS6 (370 cm)",725,35)
{
  z=370;
  color="blue";
};
Delta_R("GS10DR",75, 75);
R_Date("06, GS10 (355 cm)",594,22)
{
  z=355;
  color="blue";
};
Delta_R("GS41DR",75, 75);
R_Date("14 (GS41, 354 cm)",586,25)
{
  z=354.5;
  color="blue";
};
Delta_R("GS55DR",75, 75);
R_Date("15 (GS55, 354 cm)",496,27)
{
  z=354;
  color="blue";
};
Delta_R("GS16DR",75, 75);
R_Date("05, GS16 (336 cm)",651,25)
{
  z=336;
  color="blue";
};
Delta_R("JFS2DR",75, 75);
R_Date("JFS2 (330 cm)",573,22)
{
  z=330;
  color="blue";
};
Delta_R("GS56",75, 75);
R_Date("08 (GS56, 327 cm)",454,26)
{
  z=327;
  color="blue";
};
Delta_R("GS81",75, 75);
R_Date("17 (GS81, 318 cm)",492,28)
{
  z=318;
  color="blue";
};

```

```

};
Delta_R("GS7",75, 75);
R_Date("07 (GS7, 312 cm)",463,26)
{
  z=312;
  color="blue";
};
Delta_R("GS12DR",75, 75);
R_Date("04, GS12 (306 cm)",483,25)
{
  z=306;
  color="blue";
};
Delta_R("GS19DR",75, 75);
R_Date("11, GS19 (276 cm)",509,25)
{
  z=276;
  color="blue";
};
Delta_R("GS13DR",75, 75);
R_Date("03, GS13 (264 cm)",489,25)
{
  z=264;
  color="blue";
};
Delta_R("GS3DR",75, 75);
R_Date("02, GS3 (258 cm)",485,25)
{
  z=258;
  color="blue";
};
Date("Shortest",N(AD(1963),5))
{
  z=220;
  color="green";
};
Boundary("Newborn",N(AD(2012),1))
{
  z=42;
  color="green";
};
};
T=Newborn-Tau;
TT=Newborn-Typical;
};

```

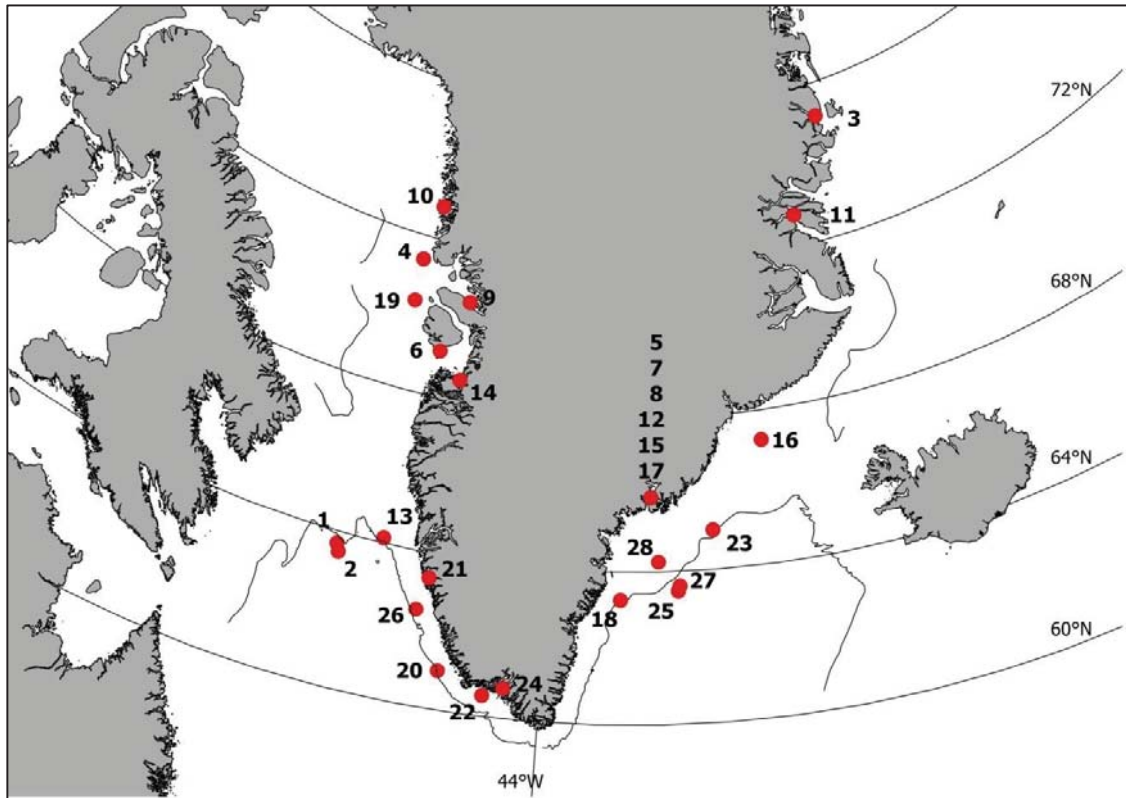


Fig. S1.

Capture positions of Greenland sharks around Greenland. Numbers next to the points identify the individual animals cf. Table S1.

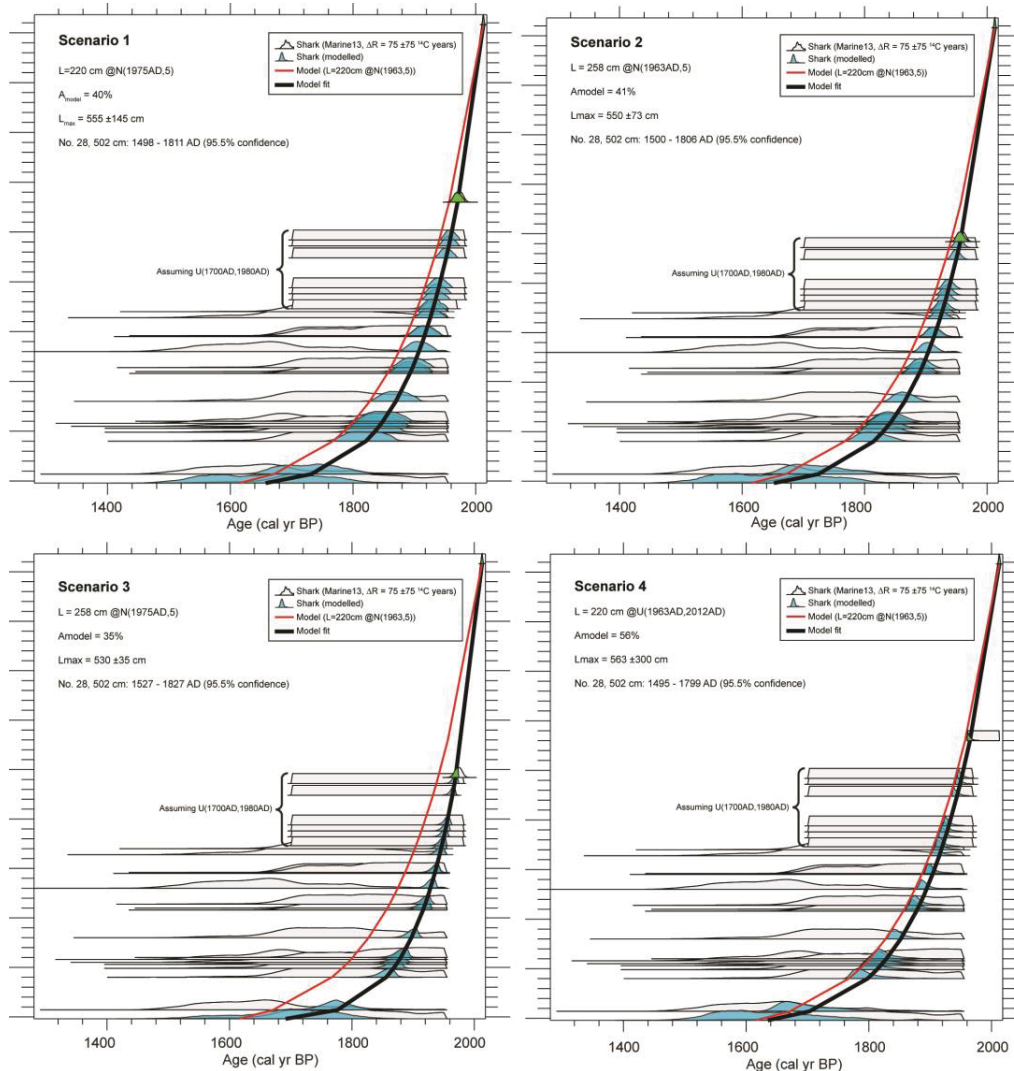


Fig. S2

Sensitivity analysis and Bayesian age ranges. Estimated year of birth against total length (TL, cm) from four different model scenarios. Scenario 1-3 are made with different fixed age of shark no. 3 (220 cm) or no. 4 (258 cm) with birth year either 1963 \pm 5 years or 1975 \pm 5 years, respectively. In scenario 4 the age of shark no. 3 is uniform in years 1963-2012. Light grey shows individual age probability distributions for each shark, whereas modelled posterior age probability distributions are shown in blue. Fixed distribution (model input) of one newborn shark (2012 \pm 1) and the shark with the same age as the bomb pulse onset (37 \pm 5 years or 49 \pm 5 years) are shown in green. The black line is the model fit connecting the geometric mean for each posterior age probability distribution. The red line in each figure represents the similar line for the model presented in Fig. 3. Inserted, the model output i.e. A_{model} , L_{max} , and range of birth year for shark no. 28.

Table S1.

Overview of individual sharks and associated isotope levels. Total body length (TL) and capture depth for each shark with corresponding stable isotopes (reported as $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) and ^{14}C levels in pMC ($\Delta^{14}\text{C}$ are reported for conventional reasons). Sharks no. 1-3 had pMC levels >95 while the remaining individuals had pMC levels between 91.25-94.5 with a significant negative correlation between size and pMC ($t=-4.18$, $df=23$, $P<0.001$, $cor=-0.66$). $\delta^{13}\text{C}$ values ranged between -16.7 ‰ and -13.8 ‰ (mean \pm SD= -14.9 ‰ \pm 0.3, $N=27$) and $\delta^{15}\text{N}$ ranged between 12.0 ‰ and 17.6 ‰ (mean \pm SD= 14.8 \pm 0.2, $N=27$). $\delta^{13}\text{C}$ was positively correlated with TL ($t=3.52$, $df=25$, $P<0.05$, $cor = 0.57$) but not when only evaluated for sharks >300 cm ($t=1.67$, $df=19$, $P=0.11$, $cor=0.36$). There was no significant correlation between $\delta^{15}\text{N}$ and TL ($t=0.49$; $df=25$, $P=0.63$, $cor = 0.10$). AAR-number refers to laboratory identification number at Aarhus AMS Centre, Aarhus University.

No	AAR-ID	TL (cm)	Depth (m)	$\delta^{13}\text{C} \pm \text{SD}$	$\delta^{15}\text{N} \pm \text{SD}$	$\Delta^{14}\text{C}$	pMC \pm SD
1	19177	81	540	-15.9 \pm 0.3	16.0 \pm 0.3	34.4	103.44 \pm 0.37
2	18075	158	1100	-15.5 \pm 0.1	12.0 \pm 0.1	-7.2	99.28 \pm 0.32
3	19179	220	325	-16.2 \pm 0.3	15.2 \pm 0.2	-49.4	95.06 \pm 0.30
4	18076,3	258	175	-15.3 \pm 0.2	14.1 \pm 0.2	-58.6	94.14 \pm 0.29
5	18077	264	380	-15.1 \pm 0.2	13.8 \pm 0.2	-59.1	94.09 \pm 0.29
6	18085	276	205	-15.2 \pm 0.2	14.6 \pm 0.2	-61.4	93.86 \pm 0.29
7	18078	306	394	-15.0 \pm 0.2	16.0 \pm 0.2	-58.4	94.16 \pm 0.29
8	19183	312	350	-14.0 \pm 0.5	13.9 \pm 0.4	-56.0	94.40 \pm 0.30
9	19193	318	990	-16.7 \pm 0.5	17.6 \pm 0.4	-59.4	94.06 \pm 0.32
10	19184	327	296	-15.4 \pm 0.3	13.2 \pm 0.4	-55.0	94.50 \pm 0.30
11	14646	330	500	-	-	-68.8	93.12 \pm 0.27
12	18079	336	596	-14.5 \pm 0.2	13.5 \pm 0.2	-77.8	92.22 \pm 0.29
13	19190	354	492	-15.3 \pm 0.3	13.5 \pm 0.3	-70.4	92.96 \pm 0.29
14	19191	354	407	-14.9 \pm 0.3	14.0 \pm 0.4	-59.9	94.01 \pm 0.31
15	18080,3	355	454	-14.3 \pm 0.2	15.5 \pm 0.2	-71.3	92.87 \pm 0.26
16	18087	370	555	-14.4 \pm 0.2	14.8 \pm 0.2	-86.3	91.37 \pm 0.40
17	18081	386	567	-14.8 \pm 0.2	15.2 \pm 0.2	-69.4	93.06 \pm 0.29
18	19180	390	507	-15.0 \pm 0.3	17.6 \pm 0.4	-61.5	93.85 \pm 0.29
19	19185	391	500	-15.2 \pm 0.5	14.7 \pm 0.4	-64.7	93.53 \pm 0.29
20	18082	420	178	-14.5 \pm 0.2	16.9 \pm 0.2	-75.0	92.50 \pm 0.40
21	19188	440	602	-14.7 \pm 0.3	13.2 \pm 0.3	-62.2	93.78 \pm 0.29
22	18083	442	132	-14.7 \pm 0.2	14.3 \pm 0.2	-81.4	91.86 \pm 0.29
23	19182	445	210	-14.4 \pm 0.3	15.7 \pm 0.3	-77.1	92.29 \pm 0.31
24	18089	447	308	-14.6 \pm 0.2	15.3 \pm 0.2	-73.2	92.68 \pm 0.29
25	19195	451	900	-15.7 \pm 0.3	14.2 \pm 0.3	-73.4	92.66 \pm 0.31
26	18084,3	460	133	-14.6 \pm 0.2	12.7 \pm 0.2	-72.9	92.71 \pm 0.29
27	19192	493	900	-13.8 \pm 0.3	16.0 \pm 0.4	-87.5	91.25 \pm 0.24
28	19186	502	900	-14.5 \pm 0.3	14.7 \pm 0.3	-74.0	92.60 \pm 0.35

Table S2.

Modelled age estimates for pre-bomb sharks. For each shark length (TL), the associated posterior calibrated biological age ranges at 95.4% (2 sigma) probability (reported as mid-point value \pm 1/2 range) are presented together with the associated A index as produced by the Bayesian model. A index values $>$ 60% reflect a good level of consistency between modelled age ranges and Marine13. Three sharks had an A index value $<$ 60%. Although it is not possible to isolate a single reason for this, it is likely to be a combination of variation in local reservoir age combined with deviations from the strict age and length assumption in the model.

No	TL (cm)	Age range (95.4 %)	A index (%)
4	258	71 \pm 12	128.6
5	264	73 \pm 14	130.2
6	276	80 \pm 13	129.6
7	306	96 \pm 15	139.4
8	312	99 \pm 15	143.0
9	318	102 \pm 15	136.4
10	327	108 \pm 16	139.4
11	330	110 \pm 18	99.6
12	336	113 \pm 17	50.0
13	354	126 \pm 19	123.5
14	354	126 \pm 19	100.0
15	355	126 \pm 19	96.0
16	370	137 \pm 20	20.1
17	386	150 \pm 22	111.8
18	390	155 \pm 23	113.2
19	392	156 \pm 22	116.9
20	420	185 \pm 26	108.2
21	440	212 \pm 31	71.9
22	442	215 \pm 33	106.7
23	445	220 \pm 33	125.7
24	447	223 \pm 33	122.1
25	451	229 \pm 33	122.7
26	460	245 \pm 38	121.5
27	493	335 \pm 75	120.0
28	502	392 \pm 120	35.9

References and Notes

1. H. B. Bigelow, W. C. Schroeder, “Sharks” in *Fishes of the Western North Atlantic*, A. E. Parr, Ed. (Yale University, New Haven, CT, 1948), pp. 516–523.
2. S. E. Campana, A. T. Fisk, A. P. Klimley, Movements of Arctic and northwest Atlantic Greenland sharks (*Somniosus microcephalus*) monitored with archival satellite pop-up tags suggest long-range migrations. *Deep Sea Res. Part II Top. Stud. Oceanogr.* **115**, 109–115 (2015). [doi:10.1016/j.dsr2.2013.11.001](https://doi.org/10.1016/j.dsr2.2013.11.001)
3. P. M. Hansen, *International Commission for the Northwest Atlantic Fisheries Special Publication* **4**, 172–175 (1963).
4. S. Henriksen, O. Hilmo, Eds., *Norsk Røddliste for Arter* (Artsdatabanken, Norge, 2015).
5. P. M. Kyne, C. A. Simpendorfer, Adaptive physiology and conservation, in *Sharks and Their Relatives*, J. C. Carrier, J. A. Musick, M. R. Heithaus, Eds. (CRC Press, 2010), pp. 37–71.
6. N. Lynnerup, H. Kjeldsen, S. Heegaard, C. Jacobsen, J. Heinemeier, Radiocarbon dating of the human eye lens crystallines reveal proteins without carbon turnover throughout life. *PLOS ONE* **3**, e1529 (2008). [doi:10.1371/journal.pone.0001529](https://doi.org/10.1371/journal.pone.0001529)
7. S. Bassnett, Y. Shi, G. F. J. M. Vrensen, Biological glass: Structural determinants of eye lens transparency. *Philos. Trans. R. Soc. London Ser. B* **366**, 1250–1264 (2011). [doi:10.1098/rstb.2010.0302](https://doi.org/10.1098/rstb.2010.0302)
8. J. L. Bada, C. D. Vrolijk, S. Brown, E. R. M. Druffel, R. E. M. Hedges, Bomb radiocarbon in metabolically inert tissues from terrestrial and marine mammals. *Geophys. Res. Lett.* **14**, 1065–1067 (1987). [doi:10.1029/GL014i010p01065](https://doi.org/10.1029/GL014i010p01065)
9. J. C. George, J. Bada, J. Zeh, L. Scott, S. E. Brown, T. O’Hara, R. Suydam, Age and growth estimates of bowhead whales (*Balaena mysticetus*) via aspartic acid racemization. *Can. J. Zool.* **77**, 571–580 (1999). [doi:10.1139/cjz-77-4-571](https://doi.org/10.1139/cjz-77-4-571)
10. H. De Vries, Atomic bomb effect: Variation of radiocarbon in plants, shells, and snails in the past 4 years. *Science* **128**, 250–251 (1958). [Medline doi:10.1126/science.128.3318.250](https://doi.org/10.1126/science.128.3318.250)
11. S. E. Campana, L. J. Natanson, S. Myklevoll, Bomb dating and age determination of large pelagic sharks. *Can. J. Fish. Aquat. Sci.* **59**, 450–455 (2002). [doi:10.1139/f02-027](https://doi.org/10.1139/f02-027)
12. J. M. Kalish, Pre- and post-bomb radiocarbon in fish otoliths. *Earth Planet. Sci. Lett.* **114**, 549–554 (1993). [doi:10.1016/0012-821X\(93\)90082-K](https://doi.org/10.1016/0012-821X(93)90082-K)
13. M. P. Francis, S. E. Campana, C. M. Jones, Age under-estimation in New Zealand porbeagle sharks (*Lamna nasus*): Is there an upper limit to ages that can be determined from shark vertebrae? *Mar. Freshw. Res.* **58**, 10–23 (2007). [doi:10.1071/MF06069](https://doi.org/10.1071/MF06069)
14. L. L. Hamady, L. J. Natanson, G. B. Skomal, S. R. Thorrold, Vertebral bomb radiocarbon suggests extreme longevity in white sharks. *PLOS ONE* **9**, e84006 (2014). [doi:10.1371/journal.pone.0084006](https://doi.org/10.1371/journal.pone.0084006)
15. K. Yano, J. D. Stevens, L. J. V. Compagno, Distribution, reproduction and feeding of the Greenland shark *Somniosus (Somniosus) microcephalus*, with notes on two other sleeper

- sharks, *Somniosus (Somniosus) pacificus* and *Somniosus (Somniosus) antarcticus*. *J. Fish Biol.* **70**, 374–390 (2007). [doi:10.1111/j.1095-8649.2007.01308.x](https://doi.org/10.1111/j.1095-8649.2007.01308.x)
16. J. Nielsen, R. B. Hedeholm, M. Simon, J. F. Steffensen, Distribution and feeding ecology of the Greenland shark (*Somniosus microcephalus*) in Greenland waters. *Polar Biol.* **37**, 37–46 (2014). [doi:10.1007/s00300-013-1408-3](https://doi.org/10.1007/s00300-013-1408-3)
 17. B. C. McMeans, J. Svavarsson, S. Dennard, A. T. Fisk, Diet and resource use among Greenland sharks (*Somniosus microcephalus*) and teleosts sampled in Icelandic waters, using $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and mercury. *Can. J. Fish. Aquat. Sci.* **67**, 1428–1438 (2010). [doi:10.1139/F10-072](https://doi.org/10.1139/F10-072)
 18. J. H. Hansen, R. B. Hedeholm, K. Sünksen, J. T. Christensen, P. Grønkjær, Spatial variability of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) stable isotope ratios in an Arctic marine food web. *Mar. Ecol. Prog. Ser.* **467**, 47–59 (2012). [doi:10.3354/meps09945](https://doi.org/10.3354/meps09945)
 19. L. J. V. Compagno, Ed., *FAO Species Catalogue. Vol. 4: Sharks of the World. An Annotated and Illustrated Catalogue of the Shark Species Known to Date. Part 1. Haxanchiformes to Lamniformes* (FAO Fisheries Synopsis, Food and Agriculture Organization of the United Nations, ed. 4, 1984).
 20. M. P. Heide-Jørgensen, J. Teilman, *Biosci* **39**, 195–212 (1994).
 21. W. N. Joyce, S. E. Campana, L. J. Natanson, N. E. Kohler, H. L. Pratt Jr., C. F. Jensen, Analysis of stomach contents of the porbeagle shark (*Lamna nasus* Bonnaterre) in the northwest Atlantic. *ICES J. Mar. Sci.* **59**, 1263–1269 (2002). [doi:10.1006/jmsc.2002.1286](https://doi.org/10.1006/jmsc.2002.1286)
 22. S. E. Campana, C. Jones, G. A. McFarlane, S. Myklevoll, Bomb dating and age validation using the spines of spiny dogfish (*Squalus acanthias*). *Environ. Biol. Fishes* **77**, 327–336 (2006). [doi:10.1007/s10641-006-9107-3](https://doi.org/10.1007/s10641-006-9107-3)
 23. J. A. Estrada, A. N. Rice, L. J. Natanson, G. B. Skomal, Use of isotopic analysis of vertebrae in reconstructing ontogenetic feeding ecology in white sharks. *Ecology* **87**, 829–834 (2006). [Medline doi:10.1890/0012-9658\(2006\)87\[829:UOIAOV\]2.0.CO;2](https://pubmed.ncbi.nlm.nih.gov/16829001/)
 24. R. E. A. Stewart, S. E. Campana, C. M. Jones, B. E. Stewart, Bomb radiocarbon dating calibrates beluga (*Delphinapterus leucas*) age estimates. *Can. J. Zool.* **84**, 1840–1852 (2006). [doi:10.1139/z06-182](https://doi.org/10.1139/z06-182)
 25. P. J. Reimer, E. Bard, A. Bayliss, J. W. Beck, P. G. Blackwell, C. Bronk Ramsey, C. E. Buck, H. Cheng, R. L. Edwards, M. Friedrich, P. M. Grootes, T. P. Guilderson, H. Haflidason, I. Hajdas, C. Hatté, T. J. Heaton, D. L. Hoffman, A. G. Hogg, K. A. Hughen, K. F. Kaiser, B. Kromer, S. W. Manning, M. Niu, R. W. Reimer, D. A. Richards, E. M. Scott, J. R. Southon, R. A. Staff, C. S. M. Turney, J. van der Plicht, IntCal13 and Marine13 radiocarbon age calibration curves 0–50,000 years cal BP. *Radiocarbon* **55**, 1869–1887 (2013). [doi:10.2458/azu_js_rc.55.16947](https://doi.org/10.2458/azu_js_rc.55.16947)
 26. J. D. Scourse, A. Wanamaker Jr., C. Weidman, J. Heinemeier, P. Reimer, P. Butler, R. Witbaard, C. Richardson, The marine radiocarbon bomb pulse across the temperate North Atlantic: A compilation of $\Delta^{14}\text{C}$ time histories from *Arctica islandica* growth increments. *Radiocarbon* **54**, 165–186 (2012). [doi:10.2458/azu_js_rc.v54i2.16026](https://doi.org/10.2458/azu_js_rc.v54i2.16026)

27. C. Bronk Ramsey, *Radiocarbon* **37**, 425–430 (1995).
28. C. Bronk Ramsey, Deposition models for chronological records. *Quat. Sci. Rev.* **27**, 42–60 (2008). [doi:10.1016/j.quascirev.2007.01.019](https://doi.org/10.1016/j.quascirev.2007.01.019)
29. C. Bronk Ramsey, S. Lee, *Radiocarbon* **55**, 720–730 (2013).
30. C. Bronk Ramsey, *Radiocarbon* **51**, 1023–1045 (2009).
31. J. T. Christensen, K. Richardson, Stable isotope evidence of long-term changes in the North Sea food web structure. *Mar. Ecol. Prog. Ser.* **368**, 1–8 (2008). [doi:10.3354/meps07635](https://doi.org/10.3354/meps07635)
32. P. G. Butler, J. D. Scourse, C. A. Richardson, A. D. Wanamaker Jr., C. L. Bryant, J. D. Bennell, Continuous marine radiocarbon reservoir calibration and the ¹³C Suess effect in the Irish Sea: Results from the first multi-centennial shell-based marine master chronology. *Earth Planet. Sci. Lett.* **279**, 230–241 (2009). [doi:10.1016/j.epsl.2008.12.043](https://doi.org/10.1016/j.epsl.2008.12.043)
33. A. T. Fisk, S. A. Tittlemier, J. L. Pranschke, R. J. Norstrom, Using anthropogenic contaminants and stable isotopes to assess the feeding ecology of Greenland sharks. *Ecology* **83**, 2162–2172 (2002). [doi:10.1890/0012-9658\(2002\)083\[2162:UACASI\]2.0.CO;2](https://doi.org/10.1890/0012-9658(2002)083[2162:UACASI]2.0.CO;2)
34. P. G. Butler, A. D. Wanamaker Jr., J. D. Scourse, C. A. Richardson, D. J. Reynolds, Variability of marine climate on the North Icelandic Shelf in a 1357-year proxy archive based on growth increments in the bivalve *Arctica islandica*. *Palaeogeogr. Palaeoclimatol.* **373**, 141–151 (2013). [doi:10.1016/j.palaeo.2012.01.016](https://doi.org/10.1016/j.palaeo.2012.01.016)
35. R. B. Stouby, *Ekspportkroner for Skidtfisk* (Eksportrådet: The Trade Council, Danish Ministry of Foreign Affairs 2, Copenhagen, Denmark, 2011).
36. S. E. Campana, Use of radiocarbon from nuclear fallout as a dated marker in the otoliths of haddock *Melanogrammus aeglefinus*. *Mar. Ecol. Prog. Ser.* **150**, 49–56 (1997). [doi:10.3354/meps150049](https://doi.org/10.3354/meps150049)
37. J. M. Kalish, R. Nydal, K. H. Nedreaas, G. S. Burr, G. L. Eine, *Radiocarbon* **43**, 843–855 (2001).
38. M. A. Treble, S. E. Campana, R. J. Wastle, C. N. Jones, J. Boje, Growth analysis and age validation of a deepwater Arctic fish, the Greenland halibut (*Reinhardtius hippoglossoides*). *Can. J. Sci. Aquat. Sci.* **65**, 1047–1059 (2008). [doi:10.1139/F08-030](https://doi.org/10.1139/F08-030)
39. U. Zoppi, J. Crye, Q. Song, A. Arjomand, *Radiocarbon* **49**, 173–182 (2007).
40. M. Stuiver, H. A. Polach, *Radiocarbon* **19**, 355–363 (1977).
41. P. J. Reimer, T. A. Brown, R. W. Reimer, *Radiocarbon* **46**, 1299–1304 (2004).
42. A. Mahadevan, An analysis of bomb radiocarbon trends in the Pacific. *Mar. Chem.* **73**, 273–290 (2001). [doi:10.1016/S0304-4203\(00\)00113-4](https://doi.org/10.1016/S0304-4203(00)00113-4)
43. P. M. Williams, J. A. McGowan, M. Stuiver, Body carbon-14 in deep sea organisms. *Nature* **227**, 375–376 (1970). [Medline doi:10.1038/227375a0](https://pubmed.ncbi.nlm.nih.gov/1038/227375a0/)
44. P. M. Williams, E. R. M. Druffel, K. L. Smith Jr., Dietary carbon sources for deep-sea organisms as inferred from their organic radiocarbon activities. *Deep-Sea Res.* **34**, 253–266 (1987). [doi:10.1016/0198-0149\(87\)90085-9](https://doi.org/10.1016/0198-0149(87)90085-9)

45. L. V. Bertalanffy, *Hum. Biol.* **10**, 181–213 (1938).
46. J. M. Hoenig, S. H. Gruber, *Elasmobranchs as Living Resources: Advances in the Biology, Ecology, Systematics and the Status of the Fisheries* (NOAA Technical Report 90, National Oceanic and Atmospheric Administration, Honolulu, HI, 1990).
47. E. Kofoed, *Report on Norwegian Fishery and Marine Investigations* **11**, 8–12 (1957).
48. E. I. Kukuev, I. A. Trunov, *J. Ichthyol.* **42**, 377–384 (2002).
49. C. Bronk Ramsey, *Radiocarbon* **51**, 337–360 (2009).
50. W. G. Pearcy, M. Stuiver, Vertical transport of carbon-14 into deep-sea food webs. *Deep-Sea Res.* **30**, 427–440 (1983). [doi:10.1016/0198-0149\(83\)90076-6](https://doi.org/10.1016/0198-0149(83)90076-6)