Title: Eye lens radiocarbon reveals centuries of longevity in Greenland shark (*Somniosus microcephalus*).

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Abstract: Greenland shark (*Somniosus microcephalus*), a species iconic to the Arctic Seas, grows slowly and reach >500 cm total length suggesting a lifespan well beyond those of other vertebrates. Radiocarbon dating of eye lens nuclei from 28 female Greenland shark (81-502 cm in total length) revealed a lifespan of at least 272 years. Only the smallest sharks (≤ 220 cm) showed sign of the radiocarbon bomb pulse, a time marker of the early 1960s. Age ranges of pre-bomb sharks (reported as mid-point ± 1/2 range at 95.4 % probability) 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 Greenland shark is the longest-lived vertebrates known and raise concerns for species conservation.

One Sentence Summary: Radiocarbon dating of Greenland shark eye lenses reveals a lifespan of at least 272 years.

Main Text: Greenland shark (Squaliformes, *Somniosus microcephalus*) is widely distributed in the North Atlantic with a vertical distribution from the surface to at least 1816 m (*1*, *2*). Females outgrow males and adults typically measure 400–500 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 Greenland shark is poorly understood, and age at first reproduction and longevity is 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 shark due to the 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 synthetized approximately at 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 lenses nuclei from 28 female specimens (81–502 cm total length, Table S1) collected during scientific surveys in Greenland during 2010–2013 (Fig. S1) (Supplementary Materials). We used radiocarbon (^{14}C) levels (reported as percent modern carbon, pMC) to estimate ages and stable isotopes, ^{13}C and ^{15}N (Table S1), to evaluate the carbon source (Supplementary Materials). Depleted $\delta^{13}C$ and enriched $\delta^{15}N$ levels established that the embryonic nucleus radiocarbon source is of dietary origin and represent 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 three-year period. The chronology presumes that size and age are positively correlated.

Since the mid-1950s, bomb-induced radiocarbon from atmospheric tests of thermonuclear weapons was 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 bomb-induced 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 that of other marine top predators such as 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 also for the Greenland shark chronology.

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 they were born later than early the 1960s. The third animal in the chronology (no. 3, 95.06 pMC), on the other hand, had a radiocarbon level slightly above the remaining sharks (nos. 4–28, 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 pre-bomb origin.

We estimated the age of pre-bomb sharks based on the Marine13 radiocarbon calibration curve (*25*) which evaluates carbon-based matter pre-dating 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 pre-bomb radiocarbon are imprinted in the marine food webs with negligible delay. We contend that Marine13 can contribute to the assessment of the age of pre-bomb sharks despite the difficulties associated with: (I) the low variation in the radiocarbon curve over the past 400 years (*25*), and (II) 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 pre-bomb 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 on 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 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 pre-bomb individual shown as posterior probability distributions of age (dark blue distributions) in Fig. 3, and posterior calibrated age ranges at 95.4% (2 sigma) 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 modelled 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 Material, Fig. S2) showing that the overall finding of extreme Greenland shark longevity is robust regardless of the exact timing of the bomb pulse onset (1958-1980).

The model estimated asymptotical total length to be 546 ± 42 cm (mean ± SD), a size matching the largest records for Greenland shark (*2*); and the age estimates (reported as mid-point ± 1/2 range at 95.4 % probability) of the two largest Greenland sharks to be 335 ± 75 years (no. 27, 493 cm) and 392 ± 120 years (no. 28, 502 cm). Moreover, as females are reported to reach sexual maturity at lengths >400 cm (*15*), the corresponding age would be at least 156 ± 22 (no. 19, 392 cm) (Table S2). A_{model} was 109.6% demonstrating that samples are in good internal agreement implying that 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 $\delta^{13}C$ levels (Table S1) possibly reflecting the Suess effect, another chemical time mark triggered by emissions of fossil fuels, imprinted in marine food webs since 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 shark attaining more than 500 cm in length.

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

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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, 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 RV Pâmiut, 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:

Material and methods

Supplementary Text

Figs. S1-S2

Tables S1-S2

References (39-50)

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

Fig. 2. **Radiocarbon in eye lens nuclei of Greenland shark.** Radiocarbon levels (pMC ± SD, Table S1) from 28 females plotted against total length (TL, cm). Individual animals are identified by the number next to symbols. Nos. 1–2 are of post-bomb origin and whereas nos. 4–28 are of pre-bomb origin. We consider shark no. 3 to be from early 1960s which is the latest timing of the bomb pulse onset (dashed line).

Fig. 3. **Bayesian age ranges of pre-bomb sharks**. Estimated year of birth against total length (TL, cm) for pre-bomb sharks (nos. 4–28). Light blue shows individual age probability distributions for each shark, whereas modelled posterior age probability distributions are shown in dark blue. Fixed age distributions (model input) of one newborn shark (42 cm, 2012 ± 1) and shark no. 3 (220 cm, 1963 ± 5) are shown in orange. The red line is the model fit connecting the geometric mean for each posterior age probability distribution. Inserted, the model output i.e. A_{model}, L_{max}, and range of birth year for shark no. 28. See also Supplementary Material.