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Peer Review File

The visual system of the longest-living vertebrate, the Greenland shark

Corresponding Author: Dr Lily Fogg

This manuscript has been previously reviewed at another journal. This document only contains information relating to versions considered at Nature Communications.

This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
Having reviewed this interesting manuscript previously and provided extensive feedback, I have focused primarily on assessing the responses to that feedback. I find that the authors have provided detailed responses, valuable explanations, and made appropriate changes to the manuscript that address the reviewers' concerns. I also appreciate the addition of the new experiments on the spectral transmittance of the ocular media and the TUNEL labelling - these strengthen the study, which is already comprehensive.

Minor point: the measurement of transmission, which is the phenomenon of light passing through a medium, is typically referred to as transmittance. Transmissivity is the intrinsic ability of a material to transmit light. Thus, the subheading on line 524 should probably read "Corneal transmittance", and line 530 should read "Transmittance was calculated...".

Line 497 - The subheading should read "In situ hybridization" (which is the technique) rather than "RNA Scope" (which is a specific proprietary technology).

Line 498 - Use italics for "in situ" in keeping with elsewhere in the manuscript.

Reviewer #2

(Remarks to the Author)
This is a resubmission of a paper that I reviewed earlier. The authors have worked hard to streamline and overhaul their earlier paper, and this new version is definitely improved. It still is a bit hyperbolic and some of the methods are still mischaracterized, but overall the paper is in good shape. The takehome message of the paper is basically that this long-lived, cold-water species of shark has a visual system much like that of other vertebrates, and specifically like the visual systems of other sharks. This is an interesting addition to the comparative physiological literature, but not a particularly surprising finding. The paper would best be published in a journal focused on comparative physiology (e.g J. Comp. Physiol. A or J. Exp. Biol.)

Minor comments:

Line 47: "is thought to be the longest-living vertebrate, with an establsihed lifespan . . . " The lifespans of other cold-water vertebrates or cave species are not fully established, and in fact, it is possible that the Pacific sleeper shark, a close relative of the Greenland shark, lives longer.

Line 94: I'm not sure what is meant by "ultramitotomy". I thought the authors might have meant "ultramicroscopy", which they claim to have performed elsewhere in the paper. But in fact, all histological data in this paper is collected using standard light microscopy - I don't see any evidence of scanning or transmission electron microscopy, and in fact, not even confocal microscopy. So perhaps the sentence should read "Histological examination shows that the Greenland shark retina also exhibits a pure-rod structure." Note that, as mentioned in the next sentence, the results show that the retinas of this species

resemble those of other deep-dwelling or nocturnal sharks.

Lines 115-116: If humans lived to 400 years, they could indeed lose 90% of their rods. But humans live at 37 deg C. The sharks live in cold water and are heterotherms, so their body temperature is likely at 10 deg C or lower. Given typical metabolic Q10 values over 2-3, 400 years at 10 deg translates to 25-50 years or so at 37 deg. So the rates of loss are not directly comparable.

Lines 119-120: "In this context, the Greenland shark provides an additional example of long-term preservation . . . . "

Line 144: " and each of the non-visual opsinsopn3, rrh, and va, a pattern which is similar to that of other sharks"

Line 166: Change "Ultrastructural" to "Histological".

Lines 177-178: "is not generalisable in more complex ecological scenarios>" I don't know what is meant here.

Section ending on Line 203: How do the lipid compositions of membranes in Greenland shark rod discs compare to the lipid profiles of other cold-water fishes, and particularly to other sharks? There is a large literature on changes in PUFA composition in animals living in cold temperatures or at high hydrostatic pressures.

Line 206: the spectral sensitivity of the visual pigment formed by the rod opsin"

Lines 220-223: It appears that light transmission in human and shark corneas remains similar throughout life. THe general message continues to be that these sharks are typical vertebrates.

Lines 237-239: Again, Greenland sharks are typical sharks.

Figure 2 caption: Perhaps retitle to "Histology of Greenland shark retinas." Again, there are no ultrastructural data reported in this paper.

Reviewer #3

(Remarks to the Author)
This study by Fogg et al on the visual system of the Greenland shark is a comprehensive investigation of the genomic, transcriptomic, histological and functional aspects of the visual system of a long-lived species inhabiting the deep-sea by a team of well established visual neuroscientists. I have previously reviewed an earlier version of this MS and am pleased that the authors have addressed all of my concerns regarding a more insightful rationale for the study, the interspecific comparison of the gene expression levels and the units used in the presented graphs, their explanation regarding the expression of phototransduction genes in juvenile individuals, the additional experimental data performed and presented using TUNEL to confirm the level of retinal degeneration and the incorporation of additional higher resolution images of the retinal anatomy and finally their revisions pertaining to the unquantified levels of retinal summation. I beleive the MS makes an important contribution to our understanding of phototransduction in a a rare, long-lived species that inhabits the little known habitat of the deep-sea.

It has come to our attention that Human Biological Material (donor cornea) were used in this study, however no information about the ethical oversight, the source of this material or the donor consent is provided in your manuscript. This is a serious issue, and we will not be able to publish your manuscript until we can confirm that all ethical regulations for the use of human donor tissue have been followed.

We apologize for this omission. In the revised version we now added the following information: Human donor eyes were obtained and managed in compliance with the Declaration of Helsinki. De-identified eyeballs from a 50-year-old female donor were obtained from the Willed Body Program at the University of California, Irvine (Irvine, CA) within 12 hours postmortem, in compliance with ethical requirements of the program. The program is registered with the respective state boards to facilitate the distribution of deceased human remains for research purposes. Prior to tissue processing, serological testing was performed on donor blood to exclude samples positive for blood-borne pathogens. Whole eyes were dissected, the anterior segments were further isolated and 5 mm bio punches were taken for further analysis. All samples are preserved in the DSK laboratory. This information has been added to the manuscript at the start of the Methods section.

REVIEWERS' COMMENTS

Reviewer #1 (Remarks to the Author):

Having reviewed this interesting manuscript previously and provided extensive feedback, I have focused primarily on assessing the responses to that feedback. I find that the authors have provided detailed responses, valuable explanations, and made appropriate changes to the manuscript that address the reviewers' concerns. I also appreciate the addition of the new experiments on the spectral transmittance of the ocular media and the TUNEL labelling - these strengthen the study, which is already comprehensive.

We thank this reviewer for their positive comments and for helping us to improve the manuscript. We have implemented all the suggested minor edits.

Minor point: the measurement of transmission, which is the phenomenon of light passing through a medium, is typically referred to as transmittance. Transmissivity is the intrinsic ability of a material to transmit light. Thus, the subheading on line 524 should probably read "Corneal transmittance", and line 530 should read "Transmittance was calculated...".

We have corrected the terminology throughout the text and in Figure 3d.

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Corrected.

Line 498 - Use italics for "in situ" in keeping with elsewhere in the manuscript.

Corrected.

Reviewer #2 (Remarks to the Author):

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We appreciate the careful re-review and have made targeted changes to the text to correct potentially hyperbolic or confusing phrasing, clarify ambiguous concepts, and add requested context/limitations.

We appreciate the reviewer's emphasis on the comparative physiology context, and we agree our analyses situate *S. microcephalus* within broader elasmobranch visual biology. However, our contribution goes beyond establishing similarity to other sharks. Using over 100-year-old specimens, we show that the Greenland shark retina remains preserved at genomic, transcriptomic and cellular levels despite their advanced age. Specifically, rod-specific genes are genomically intact, rod phototransduction genes are robustly expressed, retinal architecture and key cell populations are preserved, and we find no detectable signal of ongoing DNA damage. Together, these data push the known limits of vertebrate organ healthspan. Furthermore, our genomic and transcriptomic evidence starts to get at a putative mechanism - preferential retention and elevated expression of DNA repair factors (ERCC1–XPF) - that may help extend the retinal healthspan over centuries.

Minor comments:

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in fact, it is possible that the Pacific sleeper shark, a close relative of the Greenland shark, lives longer.

We thank the reviewer for this helpful clarification. Indeed, the Pacific sleeper shark (*Somniosus pacificus*) is a close relative of the Greenland shark (*S. microcephalus*) and could plausibly exhibit comparable longevity. However, to the best of our knowledge, no definitive age determinations have yet been published for this species. A pilot study using radiocarbon analysis of eye lens cores from a 3.1 m Pacific sleeper shark suggested that its growth rate, while still extremely slow compared to most fishes, may be approximately twice as fast as that of Greenland sharks (Matta et al., 2024, https://doi.org/10.1007/s00300-024-03247-8). Based on alignment of measured radiocarbon values with the bomb-produced radiocarbon rise period (late 1950s–early 1960s), that individual was estimated to be no more than ~50 years old, compared to an estimated 105 years if the Greenland shark growth curve were applied. These preliminary findings suggest a potentially shorter lifespan for *S. pacificus*, although the authors note that extraction methods were still being refined and that further analyses are underway. Our statement therefore reflects the current body of published work. We agree that there could be other species with greater longevity, but as of now, no confirmed or published age estimates exceeding those for the Greenland shark are available.

Line 94: I'm not sure what is meant by "ultramitotomy". I thought the authors might have meant "ultramicroscopy", which they claim to have performed elsewhere in the paper. But in fact, all histological data in this paper is collected using standard light microscopy - I don't see any evidence of scanning or transmission electron microscopy, and in fact, not even confocal microscopy. So perhaps the sentence should read "Histological examination shows that the Greenland shark retina also exhibits a pure-rod structure." Note that, as mentioned in the next sentence, the results show that the retinas of this species resemble those of other deep-dwelling or nocturnal sharks.

Ultramicrotomy is a histological method for cutting very thin sections from a sample using a diamond or glass knife mounted on an ultramicrotome (as described in the methods - lines 326-329). These thin sections are useful for examining fine structures in the retina at high resolution.

Just to clarify, we used ultramicrotomy to prepare 1-µm semithin sections for light microscopy; confocal microscopy was used for RNAscope and chromatin staining (as described in the methods). We did not perform EM in this study.

To avoid confusion here, we have rephrased the sentence to state: “Using histology, we show that the Greenland shark retina also exhibits a pure-rod structure.”

Lines 115-116: If humans lived to 400 years, they could indeed lose 90% of their rods. But humans live at 37 deg C. The sharks live in cold water and are heterotherms, so their body temperature is likely at 10 deg C or lower. Given typical metabolic Q10 values over 2-3, 400 years at 10 deg translates to 25-50 years or so at 37 deg. So the rates of loss are not directly comparable.

We appreciate the reviewer's point regarding metabolic temperature differences. We agree that cold-bodied physiology would be expected to slow cellular turnover and thus may partially mitigate photoreceptor loss rates relative to endothermic vertebrates. However, our comparison to published human data is intended not as a direct metabolic extrapolation, but to provide a familiar quantitative frame for the scale of retinal preservation observed in *S. microcephalus*. Even accounting for lower metabolic rates at ~0–10 °C, the absence of detectable degeneration in individuals estimated to exceed a century in age still implies extraordinary tissue stability. We have clarified this rationale in the revised text while retaining the comparison for context.

Lines 119-120: "In this context, the Greenland shark provides an additional example of long-term preservation . . . . "

The previous examples given for context were examined at much younger ages than our specimens - maximum 32 years old (elephants) and approximate maximum of 69 years old (tortoises). Our specimens were much older than this - estimated maximum of 134 years old. Furthermore, the paper on tortoises that we cite here (da Silva et al, 2022) did not examine the retina specifically and just looked more generally at mortality and senescence. In light of this, we still believe that the Greenland shark warrants being called a "striking example", rather than an "additional example." We have clarified the context of these studies to make this distinction clearer (lines 120-128).

Line 144: " and each of the non-visual opsinsopn3, rrh, and va, a pattern which is similar to that of other sharks"

We have corrected the wording to match the reviewer's suggestion.

Line 166: Change "Ultrastructural" to "Histological".

We appreciate the reviewer's suggestion. While we did not perform electron microscopy (which is what the term "ultrastructural" often refers to), the chromatin organization, histone modification, and PUFA composition analyses described in this section address subcellular (nuclear) architecture rather than tissue-level histology. To avoid ambiguity, we have replaced the word "ultrastructural" with "subcellular." We have also corrected this in the legend of Figure 2.

Lines 177-178: "is not generalisable in more complex ecological scenarios>" I don't know what is meant here.

We have revised the sentence to avoid confusion. What we mean is that a previous study found that a certain nuclear organisation pattern was associated with being diurnal (or active in bright light conditions) in mammals. There have been some speculations that this could be generalisable across lineages. However, we found the same nuclear organisation in the Greenland shark, which is mostly active in dim light conditions. This suggests that this association between nuclear architecture and diel activity pattern is not generalisable across lineages and ecological contexts. We have tried to make this clearer in the text (lines 180-184).

Section ending on Line 203: How do the lipid compositions of membranes in Greenland shark rod discs compare to the lipid profiles of other cold-water fishes, and particularly to other sharks? There is a large literature on changes in PUFA composition in animals living in cold temperatures or at high hydrostatic pressures.

We thank the reviewer for raising this interesting point. There is indeed substantial literature showing that PUFA content in animal tissues varies with water temperature and habitat depth. However, we have not yet determined the overall PUFA content in the Greenland shark. In contrast, limited research has examined the VLC-PUFA composition of fish retinas. Among cold-water species, the zebrafish (*Danio rerio*) is well studied and exhibits high levels of PUFAs, including VLC-PUFAs, as demonstrated by our group and others (PMID: 38342437; PMID: 33276584). We consider this a key feature of vertebrate retinas, as high PUFA levels are essential for proper rhodopsin function. Therefore, we conclude that the lipid composition of the Greenland shark retina, where approximately 40% of fatty acids are VLC-PUFAs, appears optimal to support rhodopsin activity.

Line 206: the spectral sensitivity of the visual pigment formed by the rod opsin"

We have corrected the wording to match the reviewer’s suggestion.

Lines 220-223: It appears that light transmission in human and shark corneas remains similar throughout life. THe general message continues to be that these sharks are typical vertebrates.

Indeed, our findings indicate that the corneas of Greenland sharks, even when parasitized, are capable of transmitting light effectively, comparable to those of other vertebrates. However, we wish to emphasize that previous studies have suggested that, in this species, factors such as advanced age and the presence of corneal parasites can markedly reduce light transmittance and therefore vision (e.g., PMID: 12099414).

Lines 237-239: Again, Greenland sharks are typical sharks.

In many regards, yes, the Greenland shark is similar to other deep-sea sharks. We highlight these comparisons, where possible, throughout the manuscript. However, this is, in and of itself, quite remarkable. "Typical sharks" do not live to be several hundred years old. Indeed, the maximum lifespan for our eldest comparison species, the whale shark, is approximately 130 years (Perry, C.T., et al., *Marine and Freshwater Research*, 2018), while the Greenland shark is estimated to live for up to 400 years. Thus, the fact that we find that Greenland shark retina remains preserved despite their advanced age is quite extraordinary. This preservation pushes the known limits of vertebrate organ healthspan.

Figure 2 caption: Perhaps retitle to "Histology of Greenland shark retinas." Again, there are no ultrastructural data reported in this paper.

As mentioned in response to a previous comment, we have corrected "ultrastructural" to "subcellular." We feel that this term accurately summarises the chromatin organization, histone modification, and PUFA composition analyses illustrated in this figure.

Reviewer #3 (Remarks to the Author):

This study by Fogg et al on the visual system of the Greenland shark is a comprehensive investigation of the genomic, transcriptomic, histological and functional aspects of the visual system of a long-lived species inhabiting the deep-sea by a team of well established visual neuroscientists. I have previously reviewed an earlier version of this MS and am pleased that the authors have addressed all of my concerns regarding a more insightful rationale for the study, the interspecific comparison of the gene expression levels and the units used in the presented graphs, their explanation regarding the expression of phototransduction genes in juvenile individuals, the additional experimental data performed and presented using TUNEL to confirm the level of retinal degeneration and the incorporation of additional higher resolution images of the retinal anatomy and finally their revisions pertaining to the unquantified levels of retinal summation. I believe the MS makes an important contribution to our understanding of phototransduction in a rare, long-lived species that inhabits the little known habitat of the deep-sea.

We are grateful for the supportive review. We thank the reviewer for their time and consideration and for helping us improve our manuscript.