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CRUISE REPORT

Old, cold and slow, the ecology of Greenland sharks

R/V Dana, Cruise No. Eurofleets+ SEA01_02/2020_GSHARK

Depart: July 31, 2021, Narsarsuaq, Greenland
Return: August 12, 2021 Reykjavik, Iceland

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1 Summary

The research cruise aboard the R/V Dana departed on July 31, 20201 from Narsarsuaq, Greenland (Figure 1) and returned August 12, 2021 to Reykjavik, Iceland. A scientific team of 12 researchers from the Unites States, United Kingdom, France, Sweden and Denmark participated in this Eurofleets+ cruise.

A total of 71 bottom longline sets were deployed between July 30, 2021 and August 7, 2021. In general, 5 to 7 longline sets were deployed daily with soak times ranging between 12-16 hrs (see Table 1). A total of 17,496 hook hours (time gear was actively fishing) resulted in 11 Greenland sharks captured in Southwestern Greenland at depths between 300 and 650m (see Table 2). Three Greenland sharks were released alive; two with multiple satellite tags and acoustic transmitters, and one with an acoustic tag, only. Eight individuals were brought onboard for further study.

For all sharks brought on board, tissue samples were taken to conduct studies on *in-vivo* and *in-vitro* heart function, in-vitro blood and tissue function as it relates to longevity, retina function, reproductive hormone levels, and skin bioluminescence.

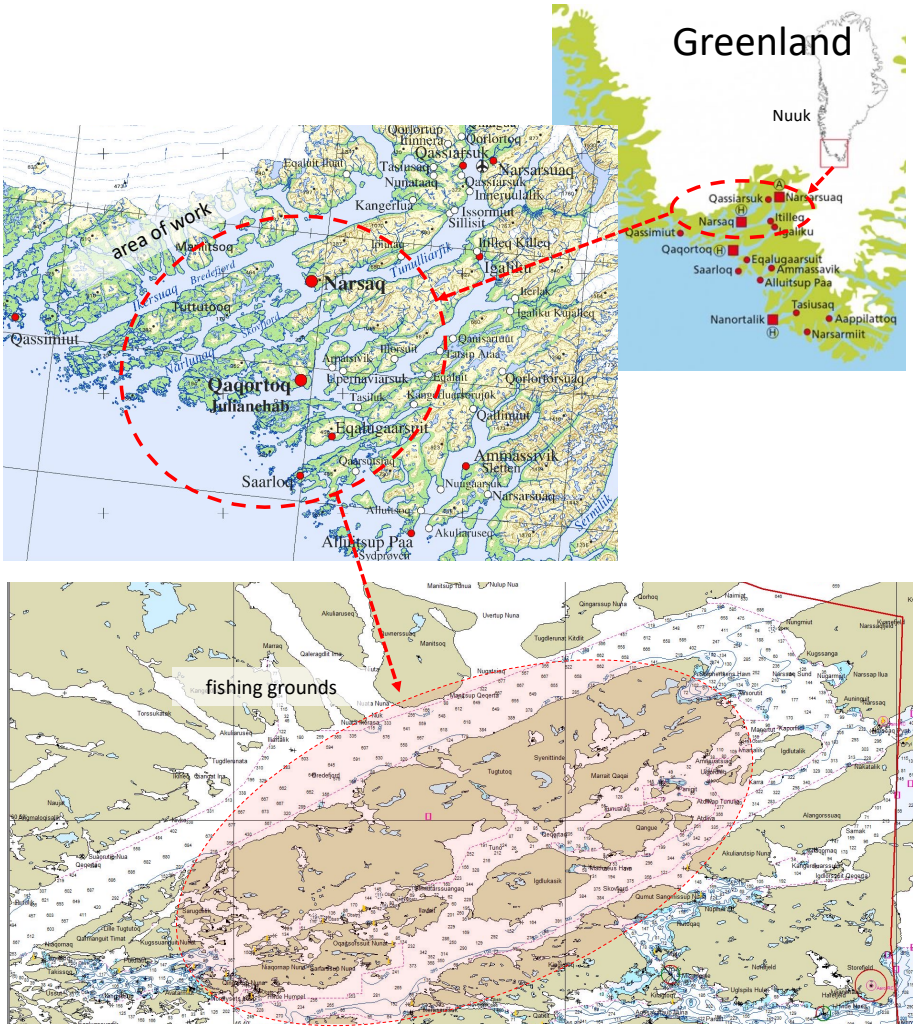

Figure 1. R/V Dana Greenland shark cruise. Station locations for bottom logline gear deployment from July 30, 2021 to August 7,20210. See Appendix 1 for daily cruise tracks.

2 Research Programme/Objectives

The main objectives of the Greenland shark cruise were to:

- Capture a wide size range of Greenland sharks to increase the age determination work based on radiocarbon C^{14} in the nucleus of the eye lens.
- Capture, tag, and release a wide size range of sharks with conventional, archival, and satellite tags to determine migration patterns and possible breeding areas.
- Investigate the metabolic biochemical properties of the swimming muscles in order to estimate maximum swim speeds and their overall swimming capacity.
- Explore the functional, structural, energetic, and molecular properties of the Greenland shark heart to determine the potential consequences of, and mechanisms underlying, cardiac longevity
- Collect tissue samples for DNA analysis for population dynamics studies to increase the genetic library in order to better elucidate population structure and evolutionary relationships between Greenland sharks and other species in the group.
- Collect blood samples for reproductive hormone analysis from mature and immature female, and using ultrasound techniques to non-invasively visualize the reproductive state of large females, and possible of pregnant.
- Determine the visual capabilities of Greenland sharks who are thought to be functionally blind.
- Determine if Greenland shark skin is bioluminescent.
- Measure in vivo cardiac beat frequency and determine the chronotropic effects of parasympathetic blocker and sympathetic agonists.
- Film sharks approaching and eating bait will provide information on swimming kinematics but should provide insights about how they feed.

3 Narrative of the Cruise

The Greenland shark cruise began in Narsarsuaq, Greenland on July 30 and terminated in Reykjavik, Iceland August 12. Seven research ship days were funded by Eurofleets+, and one additional research day by funds from a Danish Research Council to Dr. John Fleng Steffensen. In addition, four transit days (from Greenland to Iceland), were funded by a grant to Dr. Steffensen from the Danish Center for Marine Research.

The basic operations of the research vessel Dana focused around the deployment and retrieval of fishing longlines at depths from 400 to 1200 meters. On several occasion a CTD-profile was obtained from the fishing stations. In general, there was not a 24 h work schedule, and all operations (i.e., deploy and recover longlines) occurred during daytime (normal work hours). Longlines soaked (actively fished) overnight and were recovered in the morning of the following day and redeployed immediately. Work on live animals and or fresh tissues occurred in the afternoon and evening hours and did not require any night time activities from the R/V Dana or its crew.

Gear Deployment. The research vessel Dana reach a predetermined station (at approximately 4pm) in order to quickly deploy the demersal longline gear. Longline hooks (i.e., 10 – 20 hooks, 5 – 10 meters apart) were pre-baited and staged on the aft deck of the ship. Upon the signal from the bridge, the end weight was deployed overboard and the baited line was quickly spooled out to sea. The end of every longline consisted of a buoy line to the surface equipped with a flag, strobe light, and radar

reflector, which facilitated their revery after 12-20 hrs of active fishing time. Each longline set was deployed in approximately 7-10 min, at which time the research vessel moved to the next station (on average 15 min travel time). During transit to the next station the next longline was baited and staged on the aft deck of the vessel. Upon reaching the next station, the deployment procedure was repeated. A total between 5 and seven longline were deployed every day pf the cruise (see Table 2).

Gear Recovery. All terminal buoys were brought alongside the research vessel, and the line attached to the hydraulic winch to begin its removal from the bottom. The duration of the retreival process lasted approximately from 30 to 45 min, unless a shark was captured or the line had to be cleared of any tangles. After compete retrieval the longline was cleaned and stored until its next deployment.

Camera deployment. In addition to setting longlines to catch sharks, at specific stations we deployed a baited camera rig (a metal frame about 70x70x150 cm) with two deep water cameras, and 2 – 4 ultra-bright underwater lights. The camera rig was deployed and set and recovered simultaneously with the longlines.

Tagging of live animals. Three sharks were tagged alive with a traditional spaghetti-ID-tag and in some cases with archival or satellite tags. In order to both decrease the level of stress in the sharks and crew an avoid any additional injury to the sharks, all tagging operations took place from the R/V Dana's small workboats, thus allowing the shark can remain in the water.

On-Board studies: Briefly, eight sharks were lifted from the water to the deck of the research vessel using a steel stretcher and the hydraulic winch. Once abord, shark were quickly euthanized and the tissues used for onboard studies (see section 4)

Day 1- Friday, July 30- Narsarsuaq\Bredefjord-Scientists and crew members landed in Narsarsuaq mid-day and were immediately transferred by charter bus to R\V Dana at the pier. The ship left the dock approximately 2 hours later after loading all the gear and supplies which had arrived by plane. Upon leaving the dock and setting a course for Bredefjord we immediately started hauling equipment up from the hold where they were stored, and began setting them up in the laboratory. We also began working on assembling the long-line fishing gear and thawing bait for deployment in early evening.

In mid-afternoon the first officer assembled all of the scientists in the meeting room for a safety briefing and tour of the ship so we were all aware of the various exits, fire equipment, life vests, survival suits, etc. The dining schedule was also announced; breakfast at 7:30, lunch at 11:30, dinner at 5:30.

After dinner, we baited and set five longlines each with 10 hooks. Gear was allowed to soak overnight before being hauled the following morning.

This was to become the typical schedule; set longlines in the evening, let them soak overnight, and haul them after breakfast. The scientific crew also met every evening around 8PM for a daily debriefing and updates on upcoming plans for fishing, location change, etc. As we became more concerned about the low catch rates, we wanted to get more hooks in the water. . For this reason, in

addition to deploying one or two more longlines, we began rebaiting and resetting the longlines immediately after retrieving them in the morning, letting them soak for the day and then hauling, rebaiting, and resetting them after dinner for the overnight soak as usual.

Appendix 1 shows the navigational charts detailing the general fishing locations each day over the duration of the cruise.

Day 2- Saturday, July 31-Bredefjord- Retrieved longlines after breakfast (no sharks). Made more longlines and deployed five of them that evening; 2 with 10 hooks and 3 with 20 hooks.

Day 3- Sunday, August 1-Bredefjord\Julianehab- Retrieved longlines after breakfast (no sharks). Moved South to Julianehab and set longlines in the late evening after dinner. (Unless noted all deployments are 5 lines each with 20 hooks).

Day 4-Monday, August 2-Julianehab- Sharks On! Three sharks caught. One bitten (dead), but enough left for tissue and organ sampling (GS21-1), one partial carcass (discarded) and one live shark which was brought on board, euthanized, and used for tissue samples and experiments (GS21-2). A CTD was done at 15:40. Five longlines were rebaited and redeployed before dinner.

Day 5-Tuesday, August 3-Julianehab- Seven sharks caught on morning retrieve. Three were tagged with multiple satellite tags and acoustic "pinger" and released (GS21-5, GS21-6, GS 21-9 (pinger only)), three were euthanized and used for tissue samples and experiments (GS21-3, GS21-4, GS21-8), and one brought on board, anesthetized and put in large a holding tank for *in-vivo* heart rate experiments (GS21-7). Longlines were rebaited and redeployed late afternoon.

Day 6-Wednesday, August 4-Julianehab- No sharks on morning retrieve. Rebaited and reset all lines in early afternoon. Deployed baited camera rig as well. Retrieved all lines after dinner (no sharks). Rebaited and reset by 10 PM.

Day 7-Thursday, August 5-Julianahab\Kangerdluar-Ssorujuk- As there were no sharks on the morning retrieve, the decision was made to move to Kangerluar-Ssorujuk a fjord further south (See Appendix 1 for location). Deployed longlines (~13:30), retrieved (no sharks), rebaited and reset after dinner. One additional 20 hook longline and baited camera rig was deployed as well.

Day 8-Friday, August 6, Kangerdluar-Ssorujuk\Julianahab\Skovfjord-No sharks on morning retrieve so moved back to Julianehab where 4 longlines were set at ~13:00. We then moved to Skofjord where 3, 20 hook lines were deployed, as well as 1, 10 hook line just before dinner. We then moved back to Julianahab, pulled (no sharks), rebaited, and redeployed the 4 longlines in evening after arriving back on station,~19:00-. The baited camera rig was deployed as well (20:45).

Day 9-Saturday, August 7, Julianahab\Skofjord\Bredefjord- Retrieved the camera rig and longlines in Julianahab (no sharks) after breakfast and moved to Skofjord to retrieve longlines there (~11:30). One shark caught (GS21-10), brought on-board, euthanized and used for tissues samples

and experiments (14:00). One 20 hook longline, 1 with 40 hooks, and baited camera rig was deployed in Skofjord before we moved back to Bredefjord where 1 longline of 30 hooks was set at ~17:00. Moved back to Skofjord (~19:00) and retrieved and reset 3 longlines (2-20 hook and 1-40 hook).

Day 10-Sunday, August 8, Bredefjord\Skofjord\Julianahab-Attempted to retrieve 30 hook longline in Bredfjord (08:30) but it caught on bottom and parted. Attempts to find remaining line was unsuccessful. Moved back to Skofjord and retrieved camera rig and 3 longlines (~12:15). One shark caught (GS 21-11), brought on board and anesthetized for *in vivo* heart rate experiments. Moved to Julianehab and set the last longlines of the cruise (3-20 hook and 1-40 hook) in late afternoon. Pulled final longlines at 20:30, no sharks.

Day 11-14, Monday-Thursday. August 9-12, Transit to Reykjavik, Iceland- Left vessel the morning of August 12 with most scientists leaving Iceland the following day.

A detailed list of dates, locations, and times of all sets can be found in Section 7`.

4 Preliminary Results

4. 1 Fishing effort during the cruise (Leaders: J. Steffensen, P. Bushnell. Assisted by L. Andersen, D. Bernal)

The scientists and crew boarded R/V Dana in Narsarsuaq, Greenland in the early afternoon of July 30 and steamed to Bredefjord (60° 57.7”N; 46° 13.7”W) where the first sets (5) of ten hooks each were deployed in the early evening. The gear was retrieved in the morning of the following day and reset in the afternoon to soak overnight. While this was the general set and retrieve pattern that was followed, a lack of success catching sharks caused us to eventually increase the number of hooks per set to 20 and increase the number of sets per day by setting more longlines, as well as retrieving and setting them twice per day. The table below summarizes our fishing efforts and results during the cruise.

Table 1.

Number of days fishing	10
Number of longline sets	71
Number of hooks deployed	1410
Average soak time	12.9 hrs (range 3.6—22.6 hrs)
Average depth of deployment	350 m (range 238-681 m)
Total number of shark caught	11 (9 male;2 female)
Mean total length	3.76m (range 3.0 - 4.8 m)

Although we did not catch as many animals as we had hoped, as can be seen from the various scientific result summaries include in this report, our efforts were productive enough to ensure that all scientists were successful in achieving most of what they set out to do on this cruise.

Table 2. Shark morhpmetrics

ID	TL (cm)	mass	sex
GS21-1			
GS21-2	398	935	female
GS21-3	428		female
GS21-4	386	700	female
GS21-5	~360		female
GS21-6	~480		female
GS21-7	346	248	male
GS21-8	305		male
GS21-9	420		female
GS21-10	310		female
GS21-11	325	340	female

4.2 Greenland shark tagging studies (Leaders: J. Steffensen, P. Bushnell. Assisted by L. Andersen, D. Bernal)

Over the last 25 years technological advancements in remote sensing equipment such as satellite tags have allowed scientists to document the horizontal and vertical movements of large fishes around the world. The potential impact of climate change on the Arctic ecosystem has led to a growing interest in trophodynamics and the role that potential apex predators such as the Greenland sharks may play in the structure of Arctic marine food webs. Previous work on Greenland sharks shows that they are highly migratory and occupy a great range of depths from the surface to 1,800.m and can be found in abyssal waters up to 2,909 m. While Greenland sharks are distributed along the entire northern North Atlantic including the Arctic, we wish to obtain a more robust understanding of the spatial and temporal distribution of the Greenland populations in order to understand their ecological significance and better inform fisheries management practices.

One focus of this cruise was the capture, tagging, and release a wide size range of sharks with conventional, archival, and satellite tags to determine migration patterns and possible breeding areas (from tagged large females), which are currently unknown. Over the course of the cruise a total of 11 sharks were caught and used in various studies. An additional 2 carcasses were also recovered but were too damaged to be used. Although most of the sharks were brought on board, three were rereleased after they were measured for length, sexed, equipped with a 3 satellite tags (mrPAT) (2 of the sharks) and\or ultrasonic pingers (all three sharks). Identification spaghetti tags were attached as well. As the three satellite tags on the two sharks were programmed to release in 90, 180, and 270 days, the first set of tags have already released and reported in (Figure 2). .

Figure 2 shows GS21-6 has only moved approximately 100 km from the tagging location, while GS21-5 travelled 800 km in 90 days. If one assumes GS21-5 swam against the 1-1.5 knot prevailing current on the east coast of Greenland then it swam about 10-11 km/day or only 0.13 m/s which for this 4 m shark is only 0.3 BL/s. Very slow. Although data is still coming in, water temperatures encountered by GS21-6 over its short trip ranged between 2.0-6.5 °C. GS21-5 on the other hand, encountered waters as cold as -0.3 °C and as warm as 6.5 °C over its longer journey.

While our catch data will provide a small window into the migratory patterns (capture location) of the sharks, the morphometric information may allow us to determine if Greenland sharks have any size or sex dependent spatial segregation. Any future recapture of a tagged shark will also provide invaluable data on their movement patterns (location of recapture) and potential grow rate (size at recapture) during known period of elapsed time.

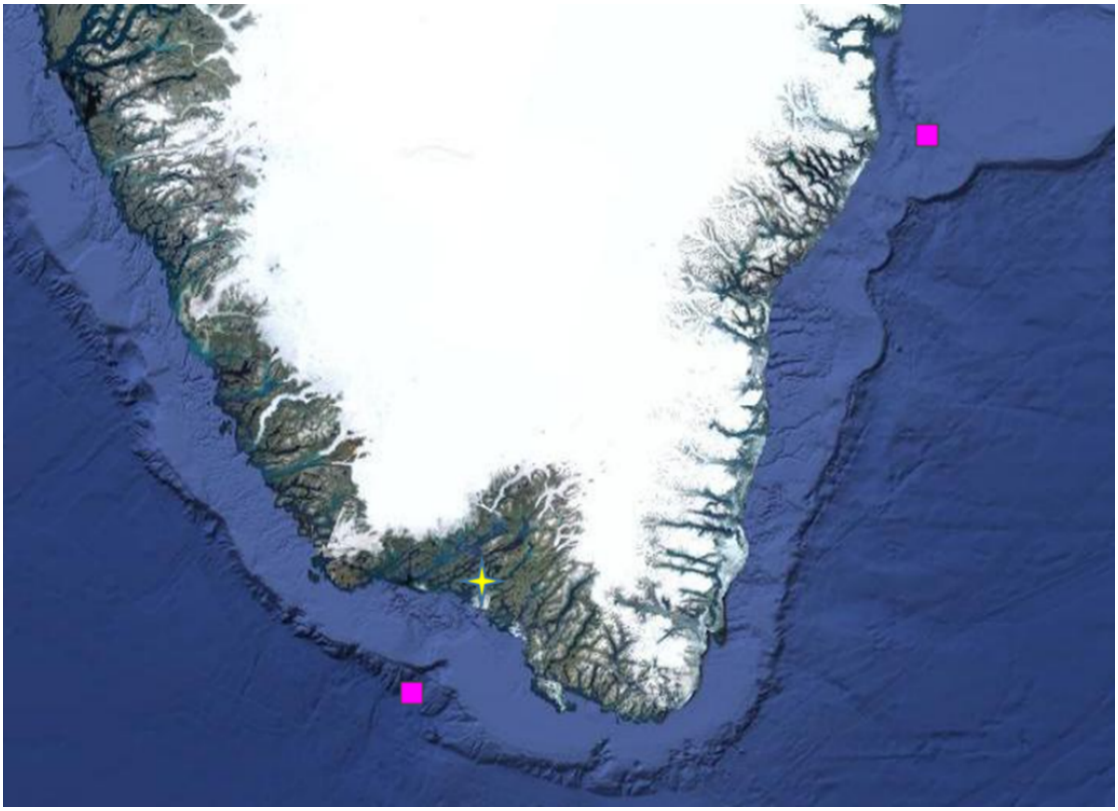

Figure 2. Locations for tag deployment (yellow star) and tag releases (colored boxes).

4.3 In-vivo cardiac responses to temperature in the Greenland shark. (Leaders: D. McKenzie. Assisted by D. Bernal)

The heart is a key organ for the survival and the ecological performance of vertebrate animals. Measuring cardiac activity in the Greenland shark can reveal whether the heart shows specific adaptations to extreme longevity at low temperatures, notably very slow routine heart rates and low maximum heart rates when stimulated. Measuring how temperature, especially warming, influences cardiac activity can also provide direct insight into how temperature might influence whole animal performance. This is interesting to understand how global warming might affect Greenland shark populations and their geographic distributions. The objective of this project was to develop a method to measure heart rate in a living Greenland shark, using an anaesthetized spontaneously ventilating animal captured alive and held in a steel-framed swimming pool on deck (Figure 3). This preparation has been used on various small-bodied fish species, to develop a thermal performance curve (TPC) of heart rate, which has been shown to parallel their TPC for in-vivo cardiorespiratory performance (aerobic metabolic scope).

Figure 3. Greenland sharks were fished and then gently winched, in a sling, into a swimming pool filled with seawater on deck

Sharks (n = 2, GS21-7, male 233 kg; GS21-13, female 240 kg) were captured and placed in a netting sling into a steel-framed swimming pool (2 x 4 m, filled to a water volume of 2.7 m^3) containing aerated seawater and anesthetic (MS-222, 100 mg l^{-1}), until loss of dorso-ventral orientation and cessation of ventilation (Figure 4). Animals were force-ventilated throughout the whole experiment using submersible pumps. Anesthetic concentration was then reduced to 50 mg l^{-1} by increasing seawater volume to 5.4 m^3, which caused ventilation to return to a slow rate (visible contractions of gill slits). ECG leads (plastic-coated stranded steel wire, 0.2 mm, exposed 50 mm at the end) were placed deep into the tissues on either side of the heart and connected to a BIOPAC MR36, running the 'Ectotherm ECG 0.05 to 35 Hz' acquisition program (Figure 4). After 30 min collection of 'routine heart rate', animals were injected with drugs to stimulate maximum heart rate (atropine sulfate and with isoproterenol). In GS21-7, heart rate was then measured over 16h at the prevailing water temperature (7 to 9°C), as a control of the endurance of cardiac activity over time. Drugs were injected at two further intervals (2h and 6h after first dose). In GS21-13, a needle thermistor

Figure 4. Greenland sharks were fished and then gently winched, in a sling, into a swimming pool filled with seawater on deck

was placed in proximity to an ECG lead, to measure core body temperature. After initial drug treatment, water temperature was raised using a heat exchanger in the pool, to raise core body temperature and investigate effects on heart rate. Drugs were injected again at 6h after the first dose.

Both sharks had very low routine heart rates, being 7 beats min^{-1} in GS21-7 at a water temperature of 7.4°C and 5 beats min^{-1} in GS21-13 at a water temperature of 5.5°C and core temperature of 3.7°C (Figure 5). In both sharks, the injections of drugs caused heart rate to increase to a maximum of about 18 beats min^{-1} (Figure 3), then slowly returning to routine over about 2h. In GS21-13, warming of core temperature from 3.7°C caused heart rate to increase up until a steady rate of about 12 beats min^{-1} at 7°C, then to decrease beyond this. At 8°C, heart rate was 4 beats min^{-1} and drug injection caused a transient rise in rate to 12 beats min^{-1} but this rapidly became a slow arrythmia followed by a profound decline in the strength of the ECG signal by 9°C. Both fish were left overnight at the prevailing water temperature (9°C for GS21-7, 13°C for GS21-13) in aerated water.

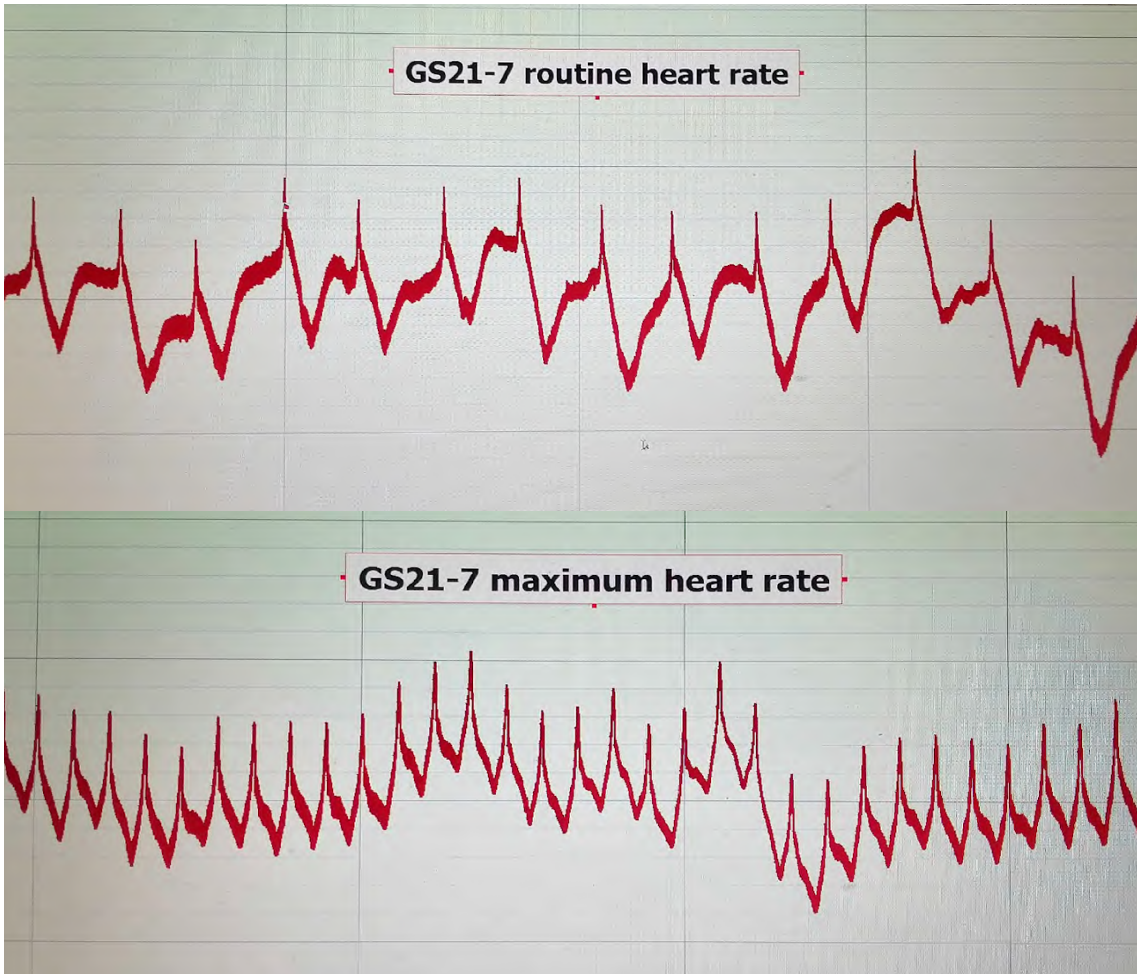

Figure 5 Heart rate of shark GS21-7 (233 kg) in water at 7.3 °C, under routine conditions

After 16h the anaesthetized fish still had slow ECG activity, they were euthanized humanely by spinal transection.

The results demonstrate the feasibility of this experimental approach, notably the endurance of the anaesthetized spontaneously-ventilating preparation. The Greenland shark seems to have the slowest routine and maximum heart rates ever recorded in a fish. The results on GS21-13 may reveal a particular sensitivity to temperature, with a decline in cardiac performance starting at a core temperature of 8°C. Further studies are needed which optimize temperature control in the swimming pool, to measure a TPC for heart rate over the whole natural thermal range of temperatures (-1 to 7°C, based on satellite tag recordings), and to confirm a decline in cardiac performance just beyond this range.

4.4 Whole heart experiments: Greenland Shark Heart Electrical (Leaders: H. Shiels.. Assisted by G. Galli, D. Bernal)

The Greenland shark heart beats very slowly and when observed in vivo through an open chest, or ex vivo when perfused with physiological saline, the contraction pattern across the heart appears erratic. Activation does not propagate from base to apex, or apex to base, which is common in most animals due to their cardiac conduction system. However, even in teleosts, why lack a designated conduction system, activation progresses in an orderly way across the chambers to ensure successful propulsion of blood from the contracting syncytium.

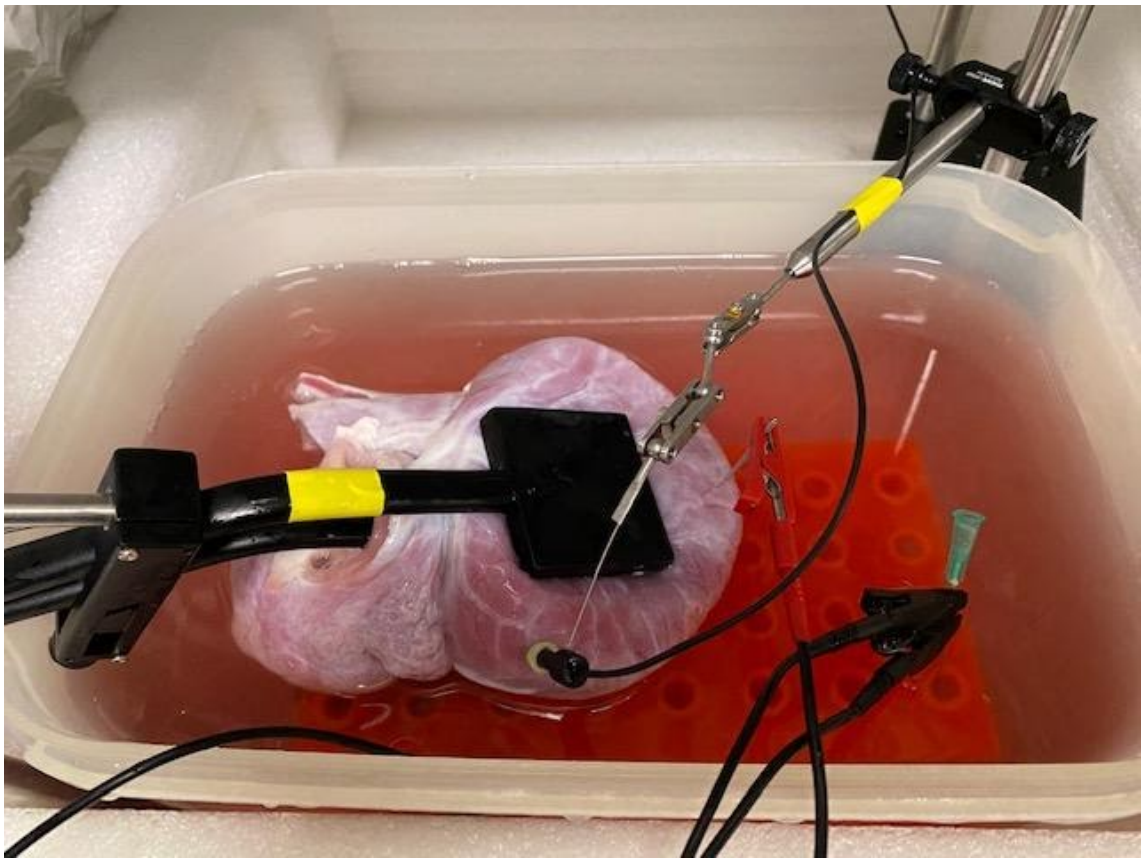

Figure 6. The heart of GS21.3 in the ex vivo mapping preparation. Visible is the epicardial 64 channel Multielectrode Array. The corresponding endocardial array is inside the lumen of the heart. Also visible is the MAP probe measuring from the surface of the ventricle. Finally, the red and black lines of the ECG with the green ground can be seen. Together these systems can assess myocardial exitation, propagation and repolarization.

To understand more about the unusual electrical activation across the surface of the Greenland shark heart, we utilized 2x64 channel multi electrode arrays MEAs) in conjunction with monophasic action potential (MAP) recordings and ECGs, to record the propagation of the excitation way across the surface of the atria, the ventricle and also transmurally across the wall of the 2 chamber simultaneously. Figure 6 shows the perfused preparation with electrical mapping pathways.

During this Greenland Shark expedition 2021, we had access to 3 hearts to characterize their electrical activity. Only 2 of the 3 produced good quality data due to the noise associated with working inside a cold room. Thus, the findings of this study must be considered preliminary.

Due to strong cardiac contractions ex vivo, it was not possible to map atria-ventricular conduction as movement artefacts were too severe. Dropping the calcium in the perfusate from 5 mM to 1 mM reduced movement significantly but not sufficiently for atria-ventricular mapping. However, the reduced movement did allow for transmural mapping of epicardial and endocardial activation patterns simultaneously in each of the atria and the ventricle.

Below are examples of the ECG (Figure 7A) and the MAP (Figure 7B) recorded at 4C from 2 separate ex vivo shark heart preparations. The R-R interval of the ECG is analogous to heart rate and at 4C the ex vivo heart (i.e. with no autonomic tone) was ~8 bpm. The duration of the QT interval (excitation and repolarization of the ventricle) was ~5-6 s which maps well to the duration of the MAP shown in Figure 7B.

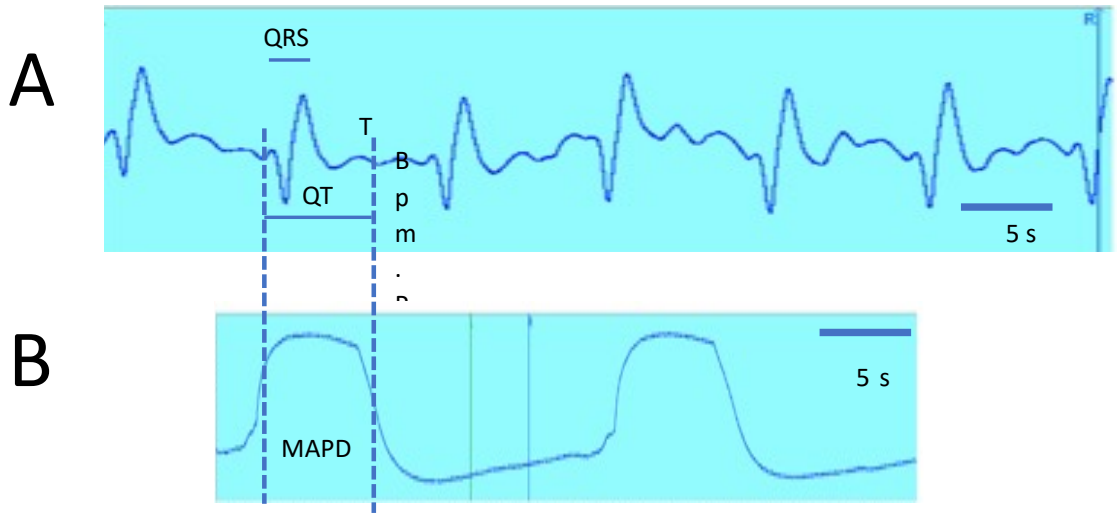

Figure 7. ECGs (A) and MAPs (B) measured from an ex vivo heart preparation at 4C. QRS is ventricular depolarization, t-wave is ventricular repolarization, and p-wave is atrial depolarization. The QT interval of the ECG is analogous to the duration of the ventricular action potential which can be seen by the MAP duration (MAPD).

The activation maps for the endocardium and epicardium are por quality due to movement artefacts but with inclusion of an actomyosin uncoupler (like BDM) we should be able to get very good recordings. Such recordings provide information on the spread of the electrical signal across the heart and may shed light on the chaotic contraction waves previously observed. Ultimately the aim would be to get a steady preparation and then apply pharmacological agents or change abiotic factors like temperature or dissolved gases to understand better how excitation and propagation are controlled/maintained in the heart of the Greenland shark. It will be interesting to see where electrical activation is most impacted by warming. A hypothesis worth testing is that conduction

through the A-V node will be impaired at warm temperatures thus preventing consistent activation of a ventricular QRS complex and causing arrhythmia.

4.5 Cardiac Activation Map Analysis (Leaders: Holly Shiels)

Data collected on 07/08 is much cleaner and more consistent than data collected on 03/08 due the turning off of the fridge during the recording phase. However, both data sets have sufficient data to calculate partial or full activation maps and therefore both can be used to investigate the spread of electrical activity in the GS heart. However, for illustration purposes all figures below are from 07/08.

Firstly, the use of a Low-pass filter (order 2, frequency cut-off 20) allows signals to be viewed, and with further zooming in a nice strong signal can be viewed from both the atria and ventricles.

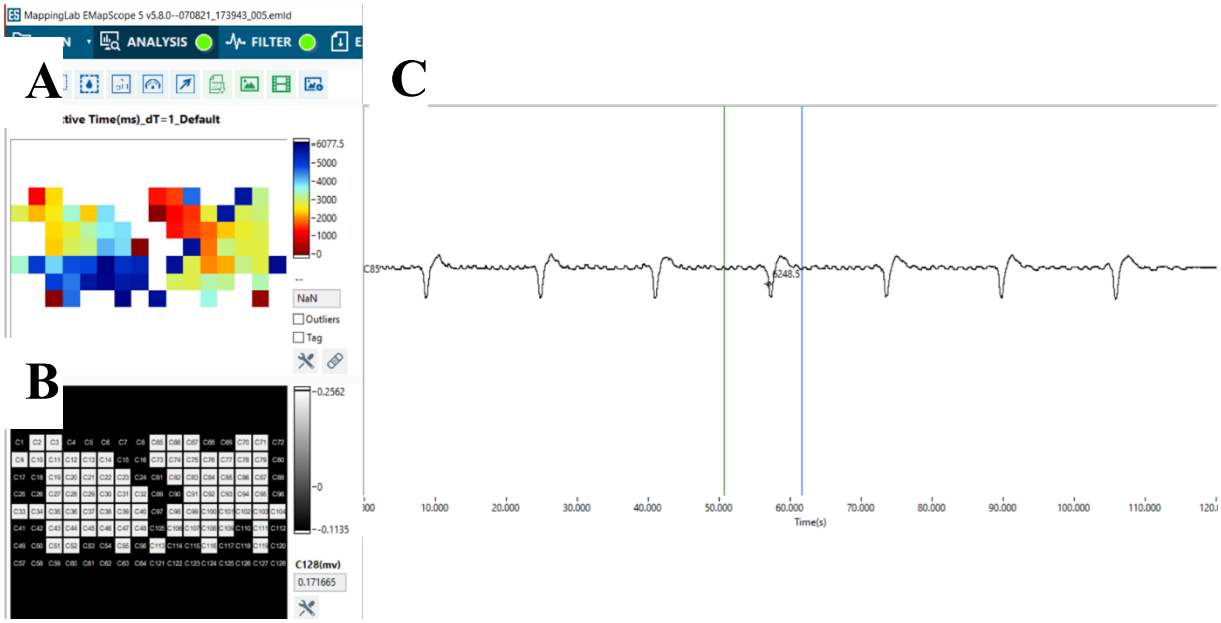

Figure 8. Example MEA recording from GS. A) Activation map calculated for atria automatically by EMapScope software. B) Illustrates the different MEA channels, white = included, black = excluded. C) Atrial signal collected from one of the channels, green and blue line represent the signal used to calculate the activation map.

Figure 8 shows how an activation map is created using the EMapScope software. Once the data has been filtered, the green and blue cursors are used to define one beat, the cursors are placed such that signals from all channels are included. Any channels where the signal is absent, too weak to view or noisy are excluded from the analysis (blacked out channels in Figure 8c). As shown in Figure 8c, channels on the edge of the electrode are most often excluded due to the lack of signal – probably due to lack of sufficient pressure at the edge of the electrodes. An activation map is then created for the available channels (Figure 8a).

The activation map can be filled in using the 'averaging' function, which determines missing signals using data from surrounding channels. Figure 9a and 9b show a smoothed version of the activation map with and without averaging respectively.

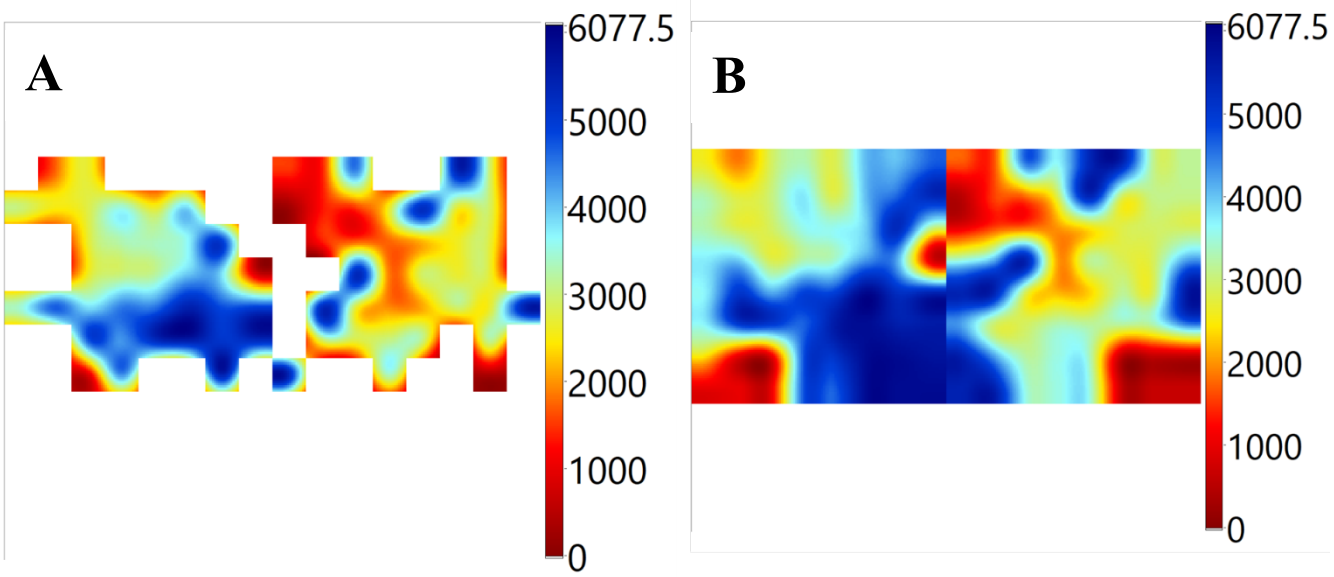

Figure 9: Smoothed atrial activation map from GS**.** A) Original map generated. B) Averaged map where missing signals are filled in using data from surrounding channels. Activation map looks very messy, mainly due to the EMapScope software not identifying peaks/signals correctly – however these can be corrected manually to give a more coherent picture (see ventricle data below).

The same method described above for atria was used on the ventricle to generate ventricular activation maps. Figure 10 shows a representative ventricular trace from two channels. The beat highlighted by the green and blue cursors was used to generate the activation map shown in Figure 11.

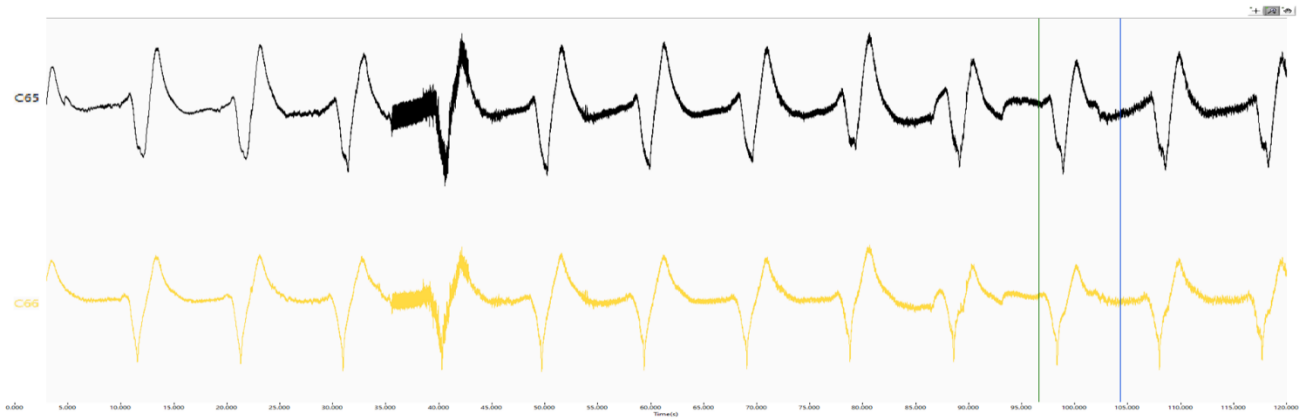

Figure 10: Representative ventricular trace for GS. Data shown from two channels only. Green and blue lines represent the signal used to generate the activation map.

As described above, any channels where a signal was missing can be filled in using the averaging tool, to give a full activation map. Generally, signals from the ventricle were of much better quality than those recorded from the atria, with fewer channels being omitted and therefore generating a more accurate activation map. Furthermore, for the ventricle manual detection of beats was done, hence the activation map looks much better than that of the atria. Better quality data combined with manually picking peaks will give a more accurate activation time.

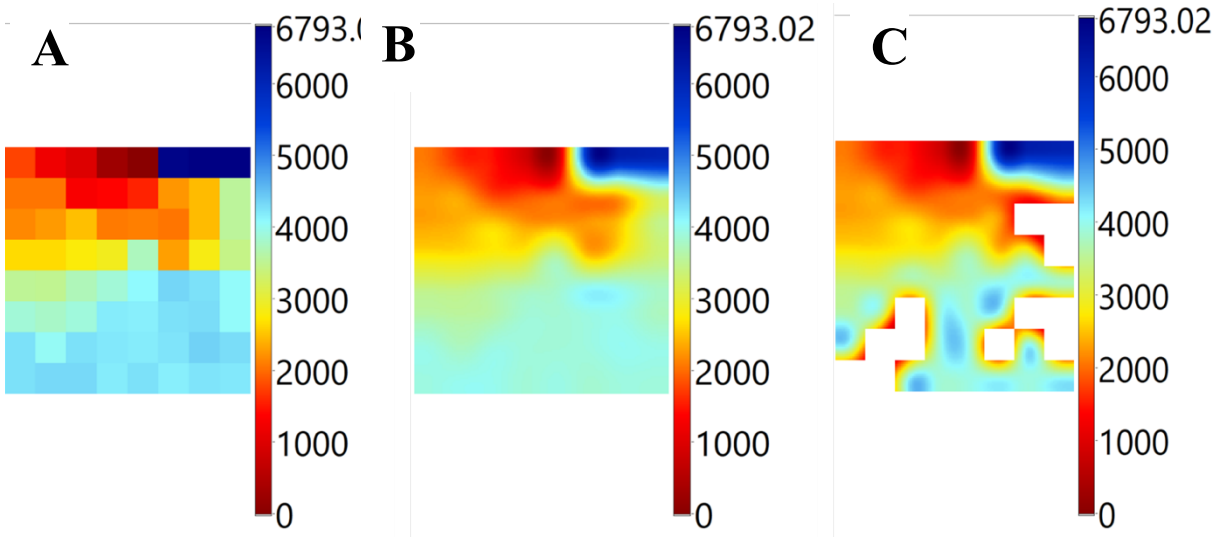

Figure 11: Activation map from ventricle of GS. A) original map with omitted channels filled in using the averaging tool. B) smoothed map representing the same data shown in a. c) smoothed map excluding the omitted channels.

Figure 12a illustrates the ECG collected at the same time as ventricular MAPs. ECGs show only QRS, with no clear P wave. Figure 12 also shows atrial MAP, ventricular MAP and ECG signals together. As shown the ventricular MAP and ECG signals match up with approx. 12 beats during the 120s period i.e. the ventricle is beating once every 10s. Interestingly, the atria are beating at about half this speed, as shown in Figure7c, during the same 120s period only 7 beats occur in the atria i.e. the atria is beating once every 17s.

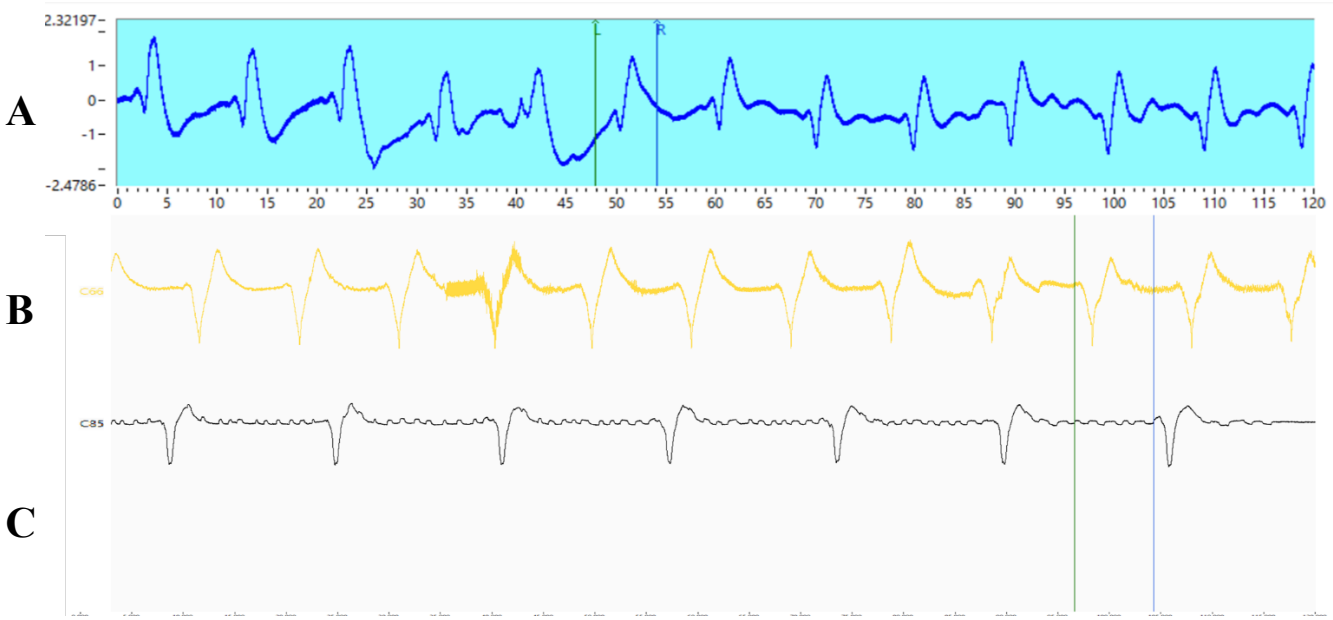

Figure 12: ECG, ventricular MAP and atrial MAP from GS. A) ECG, B) Ventricular signal, c) Atrial signal. Timescale = 120s.

4.6 In vitro Cardiac mitochondria physiology (Leaders: G. Galli, K. Smith)

The extreme longevity of the Greenland shark provides a unique opportunity to identify novel pathways that delay the aging process and prevent the onset of age-related diseases. For humans, one of the major problems associated with aging is the deterioration of cardiac function. The more the heart ages, the higher the risk of heart attack, stroke and other cardiovascular conditions. Numerous studies have shown the rate of cardiac aging depends on the efficiency of the mitochondria to produce energy, suppress reactive oxygen species (ROS) and protect against apoptosis. Therefore, it is likely that the Greenland shark possesses cellular and molecular adaptations to mitigate age-related mitochondrial dysfunction. To this end, the present study aimed to comprehensively characterize mitochondrial function in the Greenland shark heart. Specifically, we had three experimental objectives; 1) measure mitochondrial aerobic capacity and oxygen affinity, 2) investigate ROS production and the ability to tolerate oxidative stress, and 3) assess the sensitivity of the mitochondrial permeability transition pore (MPTP) to apoptotic stress.

Cardiac mitochondria were isolated from the ventricle of 6 Greenland sharks (2 males and 4 females). Mitochondrial oxygen consumption and ROS production (H_20_2) were measured simultaneously with an Oroboros O2k at 5°C. The sensitivity of the MPTP to calcium was studied at room temperature with a spectrophotometric assay that measured mitochondrial calcium retention capacity (CRC).

Mitochondrial aerobic capacity and oxygen kinetics. Isolated cardiac mitochondria from the Greenland shark had a robust and consistent response to substrates and inhibitors (Fig. 13A), and they were very well-coupled, as attested by their RCR values (6-12, Fig. 13B). Interestingly, there

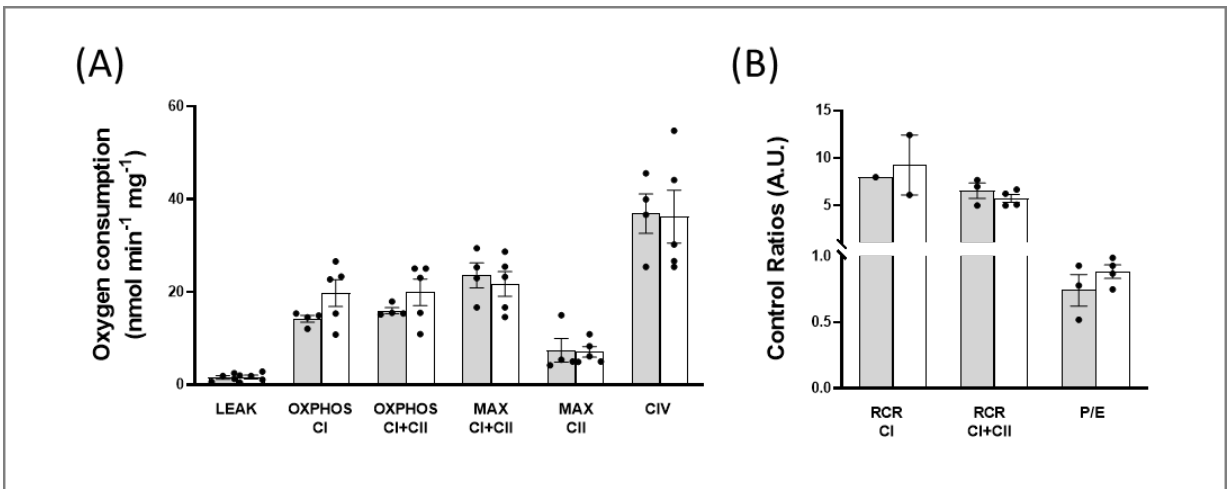

Figure 13. Mitochondrial aerobic capacity (A) and efficiency (B). Two respiratory media were used: (grey bars = mannitol and sucrose; white bars = TMAO and Urea). Aerobic capacity and efficiency were measured in various mitochondrial states: LEAK, oxygen required to offset proton leak; OXPHOS, oxidative phosphorylation with Complex I, or II substrates; MAX, uncoupled respiration with Cl and CII substrates; CIV, respiration through complex IV of the electron transport chain.; RCR, respiratory control media; P/E, fraction of electron transport chain capacity.

was a tendency for aerobic capacity to be higher if the respiration media contained TMAO and urea, rather than mannitol and sucrose (Fig. 13A, OXPHOS; grey vs. white bars).

When we compared these values to other teleosts (salmon, sablefish, lumpfish and icefish), we found that Leak, OXPHOS and RCR values from the Greenland shark were within the range of other teleosts cardiac mitochondria assayed at 5°C (Figure 14).

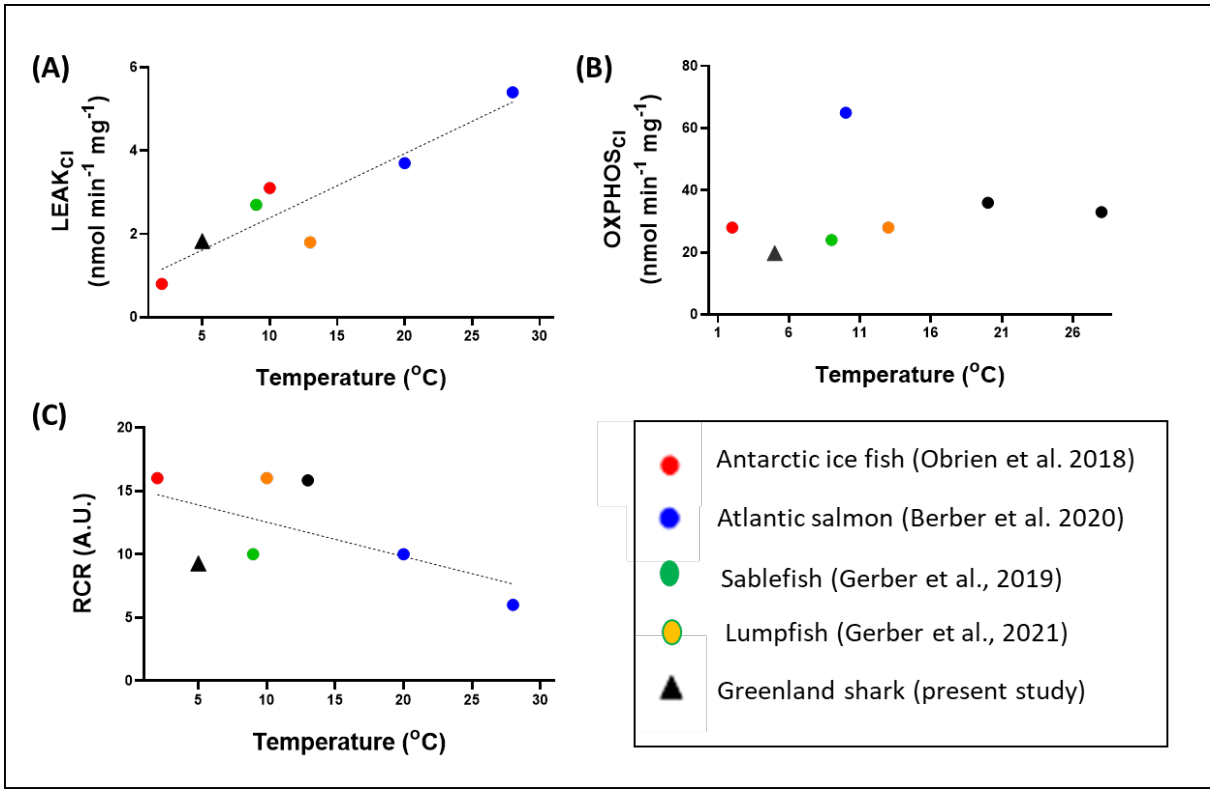

Figure 14. Comparison of teleost cardiac mitochondrial aerobic capacity and efficiency. Values were measured and compared in LEAK state (A), oxygen required to offset proton leak, Oxidative phosphorylation with Complex I (B). The respiratory control ration is also presented (C). Values for Antarctic icefish, Atlantic salmon, lumpfish and sablefish were taken from Obrien et al 2018, and Gerber et al 2019, 2020, 2021

Similar to aerobic capacity, the oxygen affinity of Greenland shark mitochondria were similar to other teleosts, when temperature is taken into account (Km = 0.016, Fig. 15A-B).

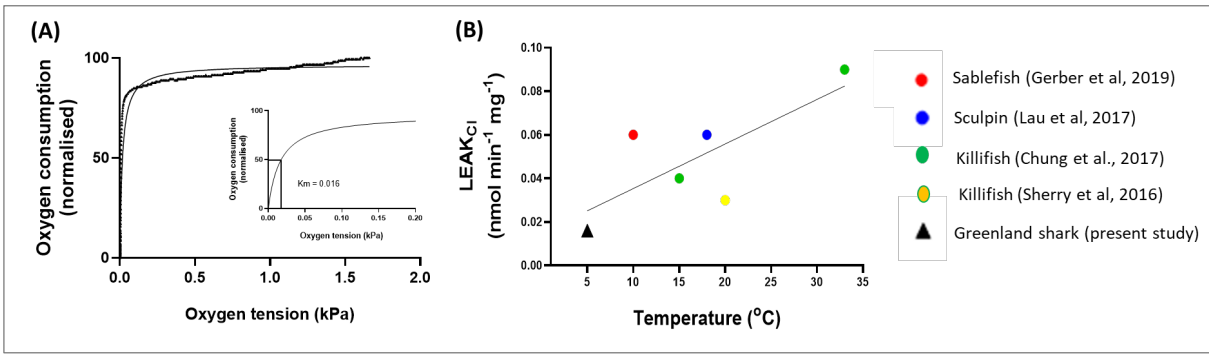

Figure 15. Mitochondrial oxygen affinity, comparisons across teleosts. Mitochondrial affinity in the Greenland shark (A), fell within the range of other teleoss when temperature was taken into account (B).

Mitochondrial ROS production and sensitivity to oxidative stress. We were unable to measure mitochondrial ROS production in the presence of TMAO, as this compound interferes with the H_20_2 probe. However, when TMAO and urea was replaced with equimolar mannitol and sucrose, Greenland shark mitochondria produced low very levels of ROS across all respiratory states (Fig. 16A). Similarly, there was clear evidence that the Greenland shark mitochondria can produce ROS via reverse electron transport (Fig. 14B, succinate levels), but these values were much lower than those found in other vertebrates. Furthermore, when mitochondria were given a bolus injection of H_20_2 to assess their sensitivity to oxidative stress, we found that aerobic capacity was maintained at higher levels when TMAO/Urea was included in the respiration media (Fig. 14C). Taken together, these results suggest the Greenland shark produces very low levels of mitochondrial ROS and that TMAO may protect against oxidative stress.

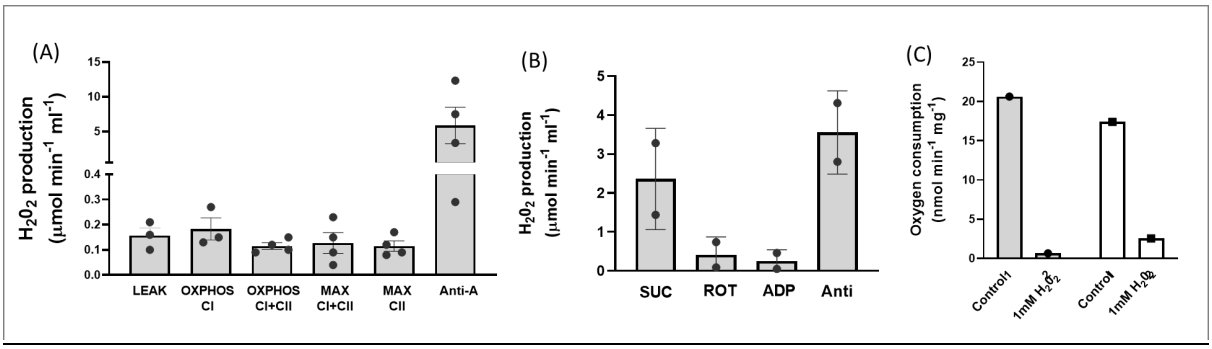

Figure 16. Mitochondrial ROS production and sensitivity to oxidative stress. (A) H2O2 production under various respiratory states: LEAK, oxygen required to offset proton leak; OXPHOS, oxidative phosphorylation with Complex I, or II substrates; MAX, uncoupled respiration with Cl and CII substrates; Anti-A, complex III blocked, (B) H2O2 production under conditions that promote ROS production by reverse electron transport through the complex I, Suc, succinate; Rot, rotenone; ADP, and Anti-a (inhibition of CIII). (C) Oxygen consumption before and after a blous injection of 1mM H2O2. Two respiratory media were used in panel C, grey bars = mannitol and sucrose, white bars = TMAO and Urea.

Mitochondrial MPTP sensitivity. The MPTP is a pore that forms in the inner mitochondrial membrane under pathological conditions (such as calcium or reactive oxygen species overload), and triggers apoptosis. The MPTP is widely implicated in aging, with aged animals showing increased MPTP opening compared to younger animals. Therefore, we investigated how well mitochondria from the Greenland shark could tolerate calcium without opening of the MPTP. Interestingly, mitochondria from the Greenland shark heart were capable of taking up large quantities of calcium at room temperature (Figure 5a), approximately 10-100x more than rat heart (Figure 17b). This could suggest that Greenland sharks have innate protection from conditions that cause MPTP opening, such as oxygen deprivation, which has implications for travelling through hypoxic regions. Further studies in elasmobranchs that inhabit similar water temperatures could help to identify whether this robust calcium uptake contributes to the longevity of the Greenland shark. Interestingly, cyclosporine A (CsA), an inhibitor of the MPTP, had no effect on the amount of calcium taken up by mitochondria. Furthermore, large quantities of calcium uptake were only observed when mitochondria were resuspended in buffer containing sucrose and mannitol, and not when in TMAO and urea.

Our data supports previous work that showed aerobic metabolism of the Greenland shark fell within

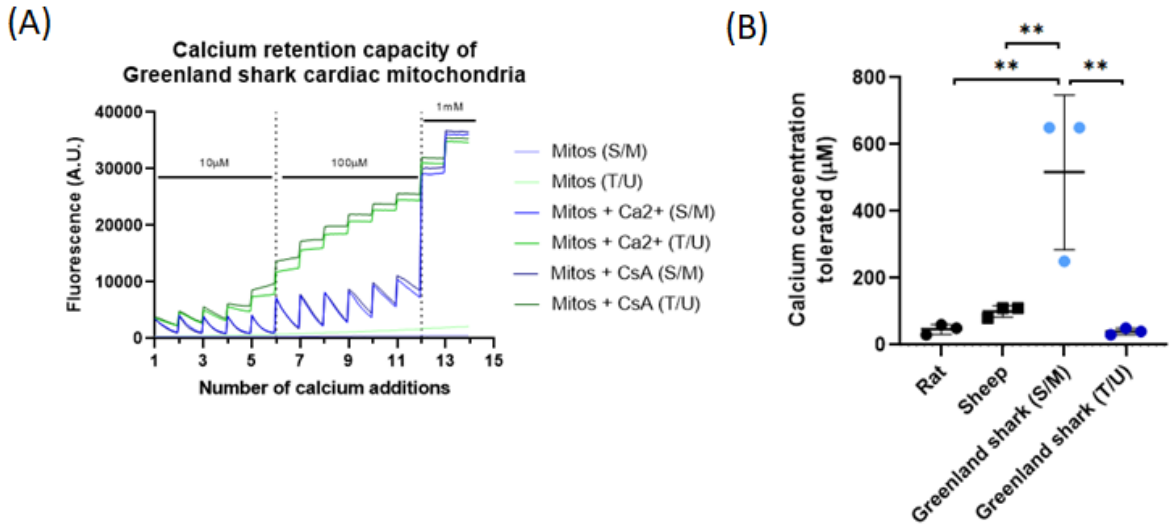

Figure 17 Calcium retention capacity in Greenland sharks mitochondria and comparison to mammals. (A). fluorescence trace from mitochondria in various conditions subject to increasing concentration of calcium, with additions. Increasing from 10uM to 1mM (B) Amount of calcium sequestered by mitochondria of different species before no decrease in fluorescence (= calcium uptake) is observed, ** p>0.01. S/M – sucrose and mannitol buffer, T/U = TMAO and Urea buffer.

values reported for other teleosts. However, we show that mitochondrial ROS production in this species is very low, and it may be protected from oxidative stress by TMAO. Furthermore, our data suggests the Greenland shark mitochondria can sequester huge quantities of calcium, which may protect them from apoptosis. Future studies should be directed at understanding the mechanisms that underlie low ROS production and MPTP insensitivity.

4.7 In vitro blood experiments to determine DNA resiliency to damage? ((Leaders: A. Chelu, D. Brayson. Assisted by D. Bernal)

The Greenland shark is the longest lived vertebrate species known to science. This remarkable longevity begs the questions '*How and why do Greenland sharks live so long?'* Understanding the molecular determinants of this resilience to ageing may offer insights into human ageing. A key determinant of human cell ageing is the accumulation of DNA damage. We have hypothesized that Greenland shark DNA would be less affected by DNA damage with age. Therefore, we sampled and preserved the blood from Greenland sharks for the purpose of using nucleated red blood cells to perform a DNA damage assay called the 'Comet' assay, which will conducted back in the UK as it is sensitive to rocking. In tandem with other experiments, we have performed on tissue sections from Greenland shark hearts, which suggest DNA replication and/or repair mechanisms are highly active, these experiments will enlighten us as to the resilience of Greenland shark blood cells to the accumulation of DNA damage with advancing age.

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Creating the first Greenland Shark cell line. The Greenland shark became an interesting research matter since its lifespan was estimated, making it the longest living vertebrate. During the 2021

expedition on the Dana research vessels we aimed to isolate and culture cells from this animal to create a new and valuable research tool for future research. The creation of a cell line started from 3 different origins: lymphoblasts, skin fibroblasts and dental pulp.

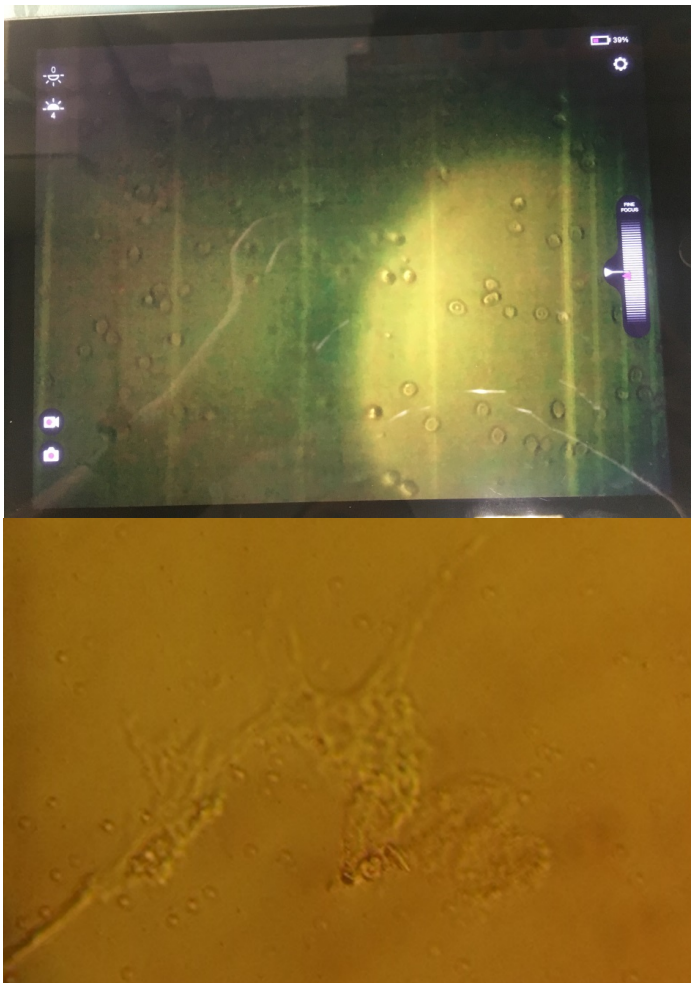

Figure 18. Top) Red Blood cells from Greenland Shark taken with iolight portable fluorescence microscope, Bottom) Greenland shark skin fibroblasts

Isolation was initially performed on skin fibroblasts and lymphoblasts, which were cultured in shark serum. Preliminary data suggests that the dissociation of skin fibroblasts and their survival in vitro was successful (Figure 18B). This encouraging data proves that a cell line derived from Greenland shark skin fibroblasts is possible and upcoming work will focus on establishing this more solidly. Moreover, we expect to generate a cell line from either lymphoblasts and/or dental pulp cells.

Lastly, we have extensively sampled a plethora of tissues from each animal, including the liver, spleen, ovaries, heart, muscle, skin and teeth. These valuable samples will be used to investigate metabolic processes and fibrosis at the University of Manchester. Furthermore, these samples will be shared with other research groups in a collective effort to unravel the mechanisms of ageing in the Greenland shark.

4. 8. Using electroretinography to measure the functional characteristics of the visual system of Greenland shark (Leaders: R. Brill, E. Warerant)

The Recordings of these signals (electroretinograms, ERGs) can be used to quantify the functional characteristics of the visual system. We were able to do this during the Dana cruise using isolated retinal tissue from Greenland shark. An example recording (made at 4°C) is shown below (Figure 19). The ERG of Greenland shark requires approximately 15 seconds to complete, whereas in temperate sharks (at 20-25°C) this occurs within less than 1 second (indicted by the black bar in the figure). Our data clearly imply that Greenland shark would only be able to see objects that are moving very slowly relative to them, such as other Greenland sharks, or objects on the sea floor. Yet Greenland sharks regularly consume prey capable of relatively fast movements (e.g., seals, Greenland halibut, wolffish). How they accomplish this is unknown, but it is very unlikely to be by visual detection, as the very slow responses (ERGs) we recorded imply that fast moving objects would appear to Greenland sharks as a blur, or may simply not be detectable. Although not a perfect analogy, a Greenland shark catching a fast-swimming seal by using its visual system would be equivalent to us using our eyes to snatch a bullet out of the air.

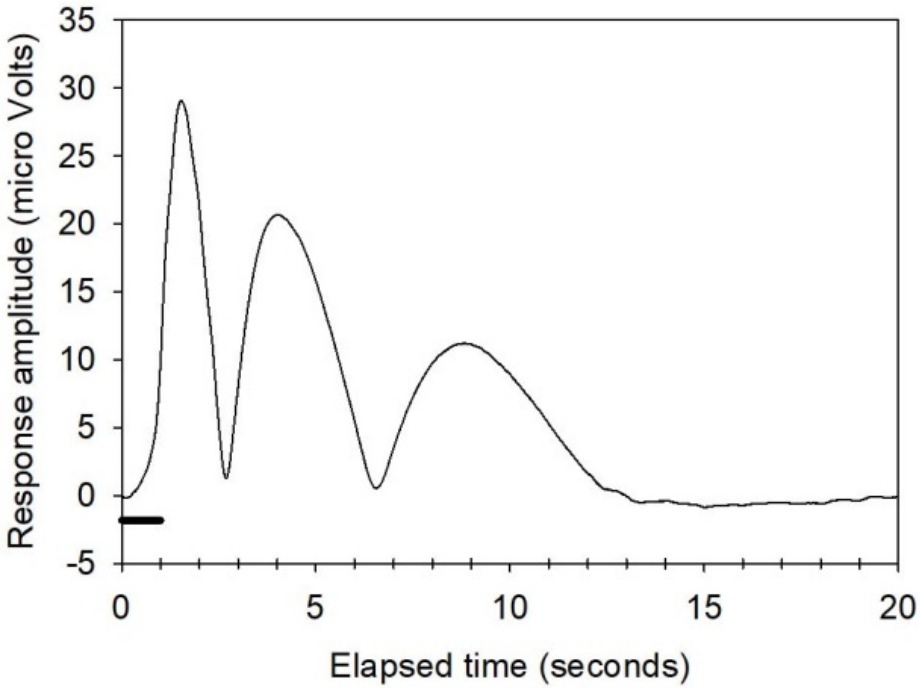

Figure 19 Greenland shark retina response to brief light stimuli.

4.9. Greenland sharks reproductive biology (Leaders: K. Steffensen, P. Bushnell. J. Steffensen, A. Poulsen, H. Jorgensen)

Very little is known about the reproductive state of Greenland sharks so we were very interested in specifically targeting large female sharks in the hope of catching a pregnant female for non-invasive study and release. Although not that many large sharks were caught, we were able to try and

visualize their reproductive state with and Ibex Pro equipped with an HD probe. This was done while the animals were lightly restrained in the water. This method would minimize any stress experienced by the shark and pregnant females would be released with an ID-tag, acoustic tag and satellite tag, to track their future movements. Unfortunately, it turned out that the reproductive organs were too deep below the ventral surface for the probe to properly visualize anything.

The ultrasound system was also used to visualize in vivo heart pumping activity in some sharks that were brought on board. The sharks that were anesthetized and put into the swimming pool for the in vivo cardiac response to temperature experiments yielded very good results that are still being analyzed.. We caught three large females during the cruise that did not come on deck but due to rough sea, we only managed to use the ultrasound on one female. What might have been ripe ova, however, most likely proved to be stomach content. We further managed to make an ultrasound recording of the heart of an anaesthetized animal.

In addition to the ultrasound studies, Dr. Kristine Fleng Steffensen and Dr. Henrik Bang Jorgensen (both veterinarians) took blood samples from the 5 largest females to analyze for progesterone levels. Results indicate that none of them were pregnant or reproductively active as values ranges from <0.6 to 0.9 nmol/l. They also collected other tissue samples that were sent to colleagues at Aarhus University and University of Copenhagen for analysis of the Greenland shark's genome. Tissue sample were also collected and sent to researchers at the Scuola Normale Superiore (University of Pisa) for analysis of ageing in Greenland shark brains.

4.10. Greenland shark feeding (Leaders: J. Steffensen, P. Bushnell. Assisted by L. Andersen)

Over the course trip, we deployed a baited camera rig in 300-400m of water, three times in an effort to photograph a Greenland sharks actively feeding. Despite accruing over 15 hours of footage, we were unsuccessful in attracting a Greenland shark. The recordings are currently being analyzed in order to assemble a species list of what was seen.

4.11 CTD profile (H. shiels, R. Brill. Assisted by D. Bernal)

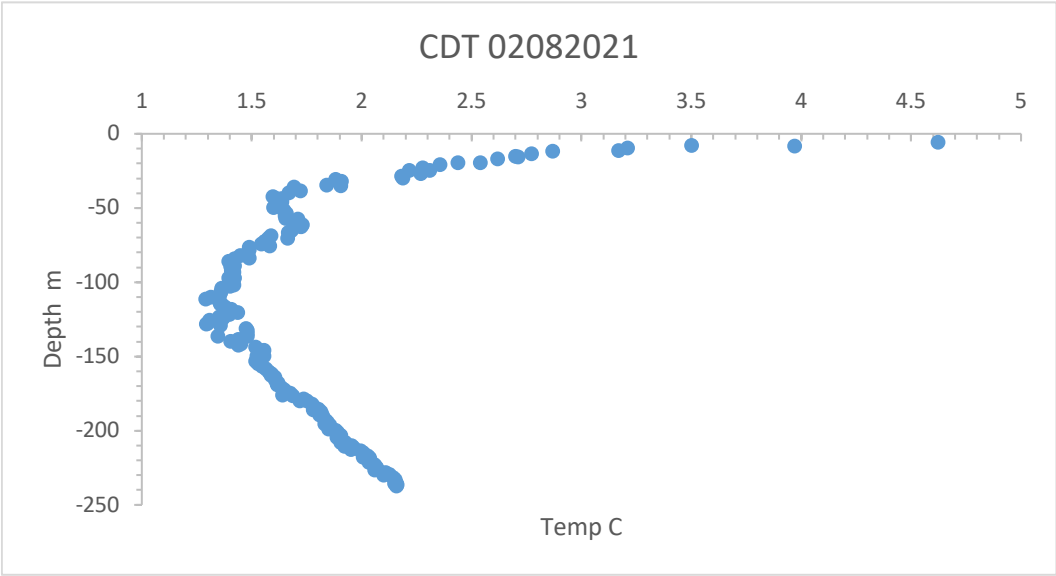

Figure 20 Vertical profile of water column near Station 3.

5 Data and Sample Storage / Availability

All data has been stored by the PI and research team and can be made available upon request.

6 Participants

No.	Name	Early career (Y/N)	Gender	Affiliation	On-board tasks
1	Andersen, Lars-Emil*	Y	M	KU	Fishing, tissue sampling
2	Bernal, Diego*	N	M	UMD	Chief scientist
3	Brayson, Daniel*	Y	M	UCL	Cell culture, tissue sampling
4	Brill, Richard*	N	M	VIMS	Vision
5	Bushnell, Peter*	N	M	IUSB	Fishing, tagging
6	Chelu, Alex*	Y	M	UMan	Cell culture, tissue sampling
7	Galli, Gina*	N	F	UMan	Mitochondrial function
8	Jorgensen, Henrik Bang*	N	M	SHD	Ultrasound, tagging, repro state
9	Koken, Marcel*	N	M	CNRS-L	Bioluminescence
10	McKenzie, David*	N	M	CNRS-M	Cardiac function

11	Poulsen, Amalie Bech*	Y	F	KU	Tissue sampling
12	Shiels, Holly*	N	F	UMan	Cardiac tissue function
13	Smith, Kerri*	Y	F	UMan	Mitochondrial function
14	Steffensen, John Fleng*	N	M	KU	Fishing, tagging, coordination
15	Steffensen, Kirstine Fleng*	N	F	SHD	Ultrasound, tagging, repro state
16	Warrant, Eric^	N	M	LU	Vision

IMPORTANT: Indicate participants funded by EUROFLEETS+, e.g. with an asterisks.

CNRS-L- Centre National de la Recherche Scientifque-LABOCEA
CNRS-M- Centre National de la Recherche Scientifque-Montpellier
IUSB- Indiana University South Bend
KU- København Universitet
LU- Lunds Universitet
SHD- Store Havelse Dyreklinik
UCL- University College London
UMan- University of Manchester
UMD- University of Massachusetts-Dartmouth
VIMS- Virginia Institute of Marine Science

7 Station List

Table 3. Bottom longline gear set details.

Station No.	Date (deployed/ retrieved)	Local time (Greenland)	Lat (N)	Long (W)	Depth (m)	Set ID	Hooks (#)	Gear soak time (h:min)	Effective hook time (h)	Remarks
1	7/30/21 7/31/21	16:00	60 57.6	46 13.7	450	A	10	18:20	183	
2	7/30/21 7/31/21	16:15	60 57.5	47 15.4	450	B	10	16:00	160	
3	7/30/21 7/31/21	18:00	60 57.1	46 17.5	450	F	10	15:46	158	
4	7/30/21 7/31/21	19:00	60 56.7	46 17.7	560	C	10	18:05	181	
5	7/30/21 7/31/21	20:00	60 56.7	46 18.9	630	D	10	16:30	165	
6	7/31/21 8/1/21	20:29	60 57.3	46 23.4	674	A	10	12:01	120	
7	7/31/21 8/1/21	20:47	60 58.5	46 23.8	642	B	20	12:23	248	
8	7/31/21 8/1/21	21:07	60 59.3	46 22.5	674	C	20	13:23	268	
9	7/31/21 8/1/21	21:23	60 59.8	46 21.7	681	D	20	14:07	282	
10	7/31/21 8/1/21	21:42	61 00.6	46 22.4	653	E	10	14:48	148	
11	8/1/21 8/2/21	17:12	60 41.4	46 09.1	280	A	20	15:03	301	GS21-1 bitten
12	8/1/21 8/2/21	17:22	60 41.4	46 05.1	285	B	20	16:38	333	GS21-2, carcasss
13	8/1/21 8/2/21	17:32	60 41.8	46 01.8	245	C	20	17:43	354	
14	8/1/21 8/2/21	17:42	60 42.2	45 59.2	275	D	20	18:03	361	

15	8/1/21 8/2/21	17:52	60 42.9	45 58.5	269	E	20	18:23	368	
16	8/2/21 8/3/21	16:30	60 40.2	46 11.1	322	A	20	16:00	320	GS21-3, GS21-4
17	8/2/21 8/3/21	16:45	60 41.3	46 09.2	256	B	20	17:45	355	GS21-5
18	8/2/21 8/3/21	17:00	60 41.4	46 05.4	285	C	20	18:30	370	GS21-6, GS21-7
19	8/2/21 8/3/21	17:15	60 41.7	46 02.1	245	D	20	8:15	165	GS21-8, GS21-9
20	8/2/21 8/3/21	17:30	60 42.2	45 59.3	278	E	20	9:00	180	
21	8/3/21 8/4/21	16:00	60 41.8	46 01.6	297	A	20	18:10	363	
22	8/3/21 8/4/21	16:15	60 41.6	46 03.6	291	B	20	18:00	360	
23	8/3/21 8/4/21	16:30	60 41.4	46 05.3	251	C	20	16:50	337	
24	8/3/21 8/4/21	16:45	60 41.5	46 06.9	238	D	20	16:03	321	
25	8/3/21 8/4/21	17:00	60 41.4	46 08.8	245	E	20	15:15	305	
26	8/4/21 8/4/21	12:30	60 40.7	46 11.1	304	A	20	6:00	120	Set camera (6 h)
27	8/4/21 8/4/21	12:45	60 40.9	46 09.6	280	B	20	6:30	130	
28	8/4/21 8/4/21	13:00	60 41.4	46 08.6	265	C	20	7:00	140	
29	8/4/21 8/4/21	13:15	60 41.6	46 04.9	297	D	20	7:30	150	

30	8/4/21 8/4/21	13:30	60 42.0	46 01.8	293	E	20	8:00	160	
31	8/4/21 8/5/21	18:35	60 40.7	46 11.1	304	A	20	13:55	278	
32	8/4/21 8/5/21	19:20	60 40.9	46 09.6	280	B	20	13:40	273	
33	8/4/21 8/5/21	20:05	60 41.4	46 08.6	265	C	20	13:25	268	
34	8/4/21 8/5/21	20:50	60 41.6	46 04.9	297	D	20	13:10	263	
35	8/4/21 8/5/21	21:35	60 42.0	46 01.8	293	E	20	12:55	258	
36	8/5/21 8/5/21	13:30	60 35.5	46 01.0	305	A	20	5:00	100	
37	8/5/21 8/5/21	13:45	60 35.1	45 58.9	260	B	20	5:30	110	
38	8/5/21 8/5/21	14:00	60 35.4	45 58.0	374	C	20	6:00	120	
39	8/5/21 8/5/21	14:15	60 35.9	45 56.4	375	D	20	6:05	122	Set camera (13 h)
40	8/5/21 8/5/21	14:30	60 36.3	45 54.9	360	E	20	7:30	150	
41	8/5/21 8/6/21	14:45	60 36.8	45 53.5	338	F	20	17:25	348	
42	8/5/21 8/6/21	22:15	60 36.8	45 53.5	338	G	20	10:15	205	
43	8/5/21 8/6/21	18:45	60 35.5	46 01.0	305	A	20	14:15	285	
44	8/5/21 8/6/21	19:30	60 35.1	45 58.9	260	B	20	14:00	280	

45	8/5/21 8/6/21	20:15	60 35.4	45 58.0	374	C	20	13:45	275	
46	8/5/21 8/6/21	20:35	60 35.9	45 56.4	375	D	20	13:55	278	
47	8/5/21 8/6/21	22:15	60 36.3	45 54.9	360	E	20	13:30	273	
48	8/6/21 8/6/21	13:10	60 57.3	46 23.4	674	A	20	5:05	102	
49	8/6/21 8/6/21	13:15	60 58.5	46 23.8	642	B	20	5:28	109	
50	8/6/21 8/6/21	13:30	60 59.3	46 22.5	674	C	20	5:41	114	
51	8/6/21 8/6/21	13:45	60 59.8	46 21.7	681	D	20	6:10	123	
52	8/6/21 8/7/21	18:30	61 00.6	46 22.4	653	A	20	15:25	308	
53	8/6/21 8/7/21	19:00	60 57.3	46 23.4	674	B	20	14:26	289	
54	8/6/21 8/7/21	20:30	60 58.5	46 23.8	642	C	20	12:30	250	
55	8/6/21 8/7/21	20:35	60 59.3	46 22.5	674	D	20	11:55	238	Set camera (10 h)
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57	8/6/21 8/6/21	15:10	60 47.4	46 16.5	370	F	20	21:50	437	
58	8/6/21 8/6/21	15:17	60 47	46 18.1	375	G	20	22:13	444	
59	8/6/21 8/6/21	15:26	60 46.7	46 19.1	360	H	10	22:34	226	GS21-10
60	8/7/21	14:40	60 50.0	46 10.1	380	A	30	4:20	130	

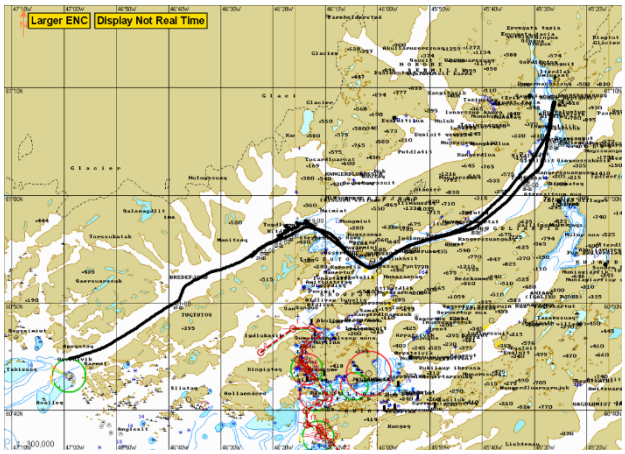
	8/7/21									
61	8/7/21 8/7/21	14:00	60 50.0	46 10.1	380	B	40	6:30	260	
62	8/7/21 8/7/21	14:20	60 50.0	46 10.1	380	C	20	5:15	105	
63	8/7/21 8/8/21	17:00	60 54.5	46 11.5	650		30	19:30	585	Lost gear
64	8/7/21 8/8/21	19:30	60 50.0	46 10.1	380	A	30	16:08	645	GS21-11
65	8/7/21 8/8/21	21:00	60 50.0	46 10.1	380	B	40	16:15	325	5 wolf eels
66	8/7/21 8/8/21	20:00	60 50.0	46 10.1	380	D		19:15	385	Set camera (12 h)
67	8/7/21 8/8/21	19:00	60 50.0	46 10.1	380	C	20	18:45	375	
68	8/7/21 8/8/21	18:00	60 50.0	46 10.1	380	H	20	19:30	585	1 wolf eel
69	8/8/21 8/8/21	16:30	60 57.3	46 23.4	674	A	40	3:35	143	
70	8/8/21 8/8/21	16:40	60 58.5	46 23.8	642	B	20	4:01	80	
71	8/8/21 8/8/21	16:49	60 59.3	46 22.5	674	C	20	4:56	99	

8 Acknowledgements.

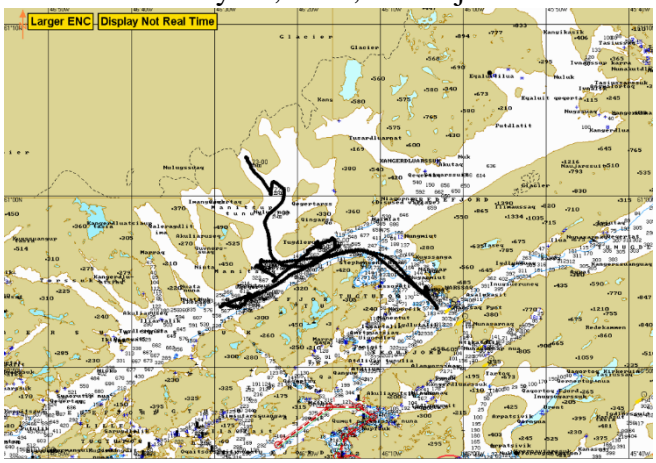
We would like to thanks Eurofleets+ for the funding of the ship time and travel, the Danish Research Councill for the additional ship time, and the Danish Center for Marine Research for the additional transit ship time.

Appendix 1

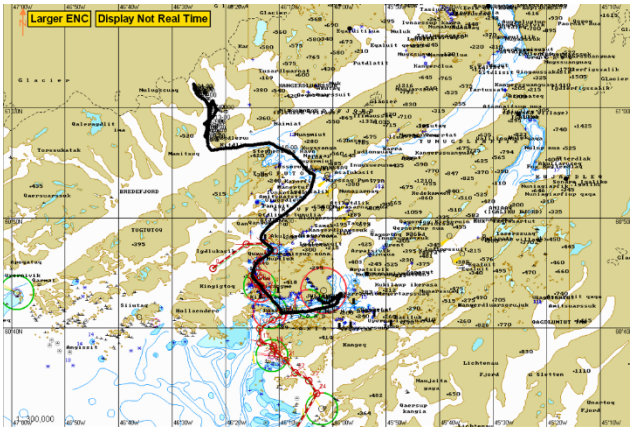
July 30, 2021; Narsarsuaq -Bredefjord



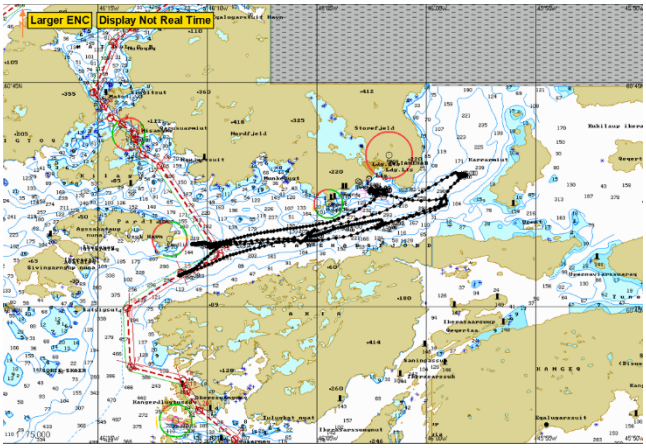
July 31, 2021; Bredefjord



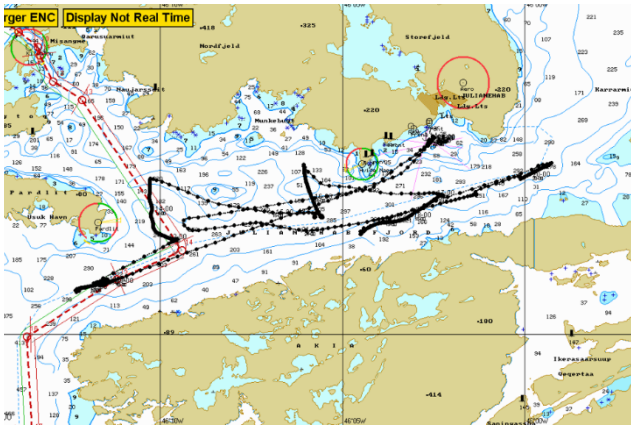
August 1, 2021; Bredefjord-Julianehab



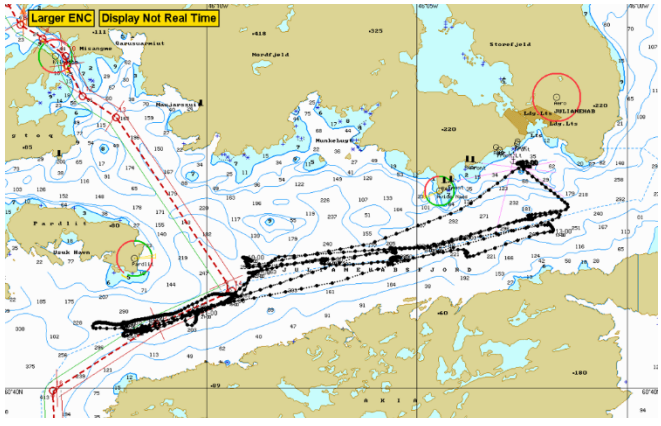
August 2, 2021; Julianehab



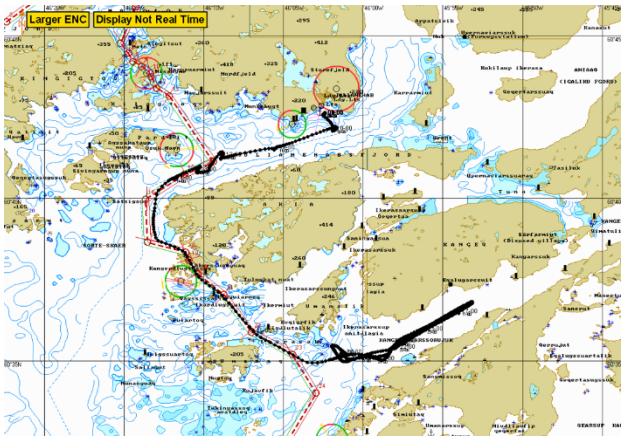
August 3, 2021; Julianehab



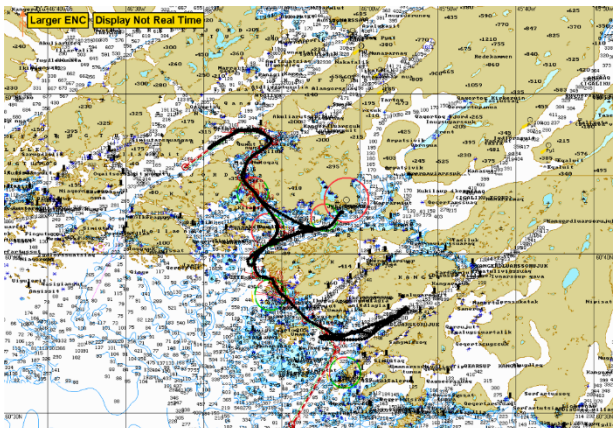
August 4, 2021; Julianehab



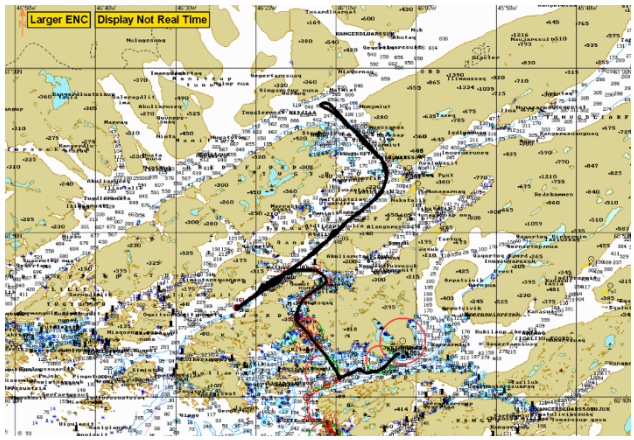
August 5, 2021; Julianehab-Kangerdluar-Ssorujuk



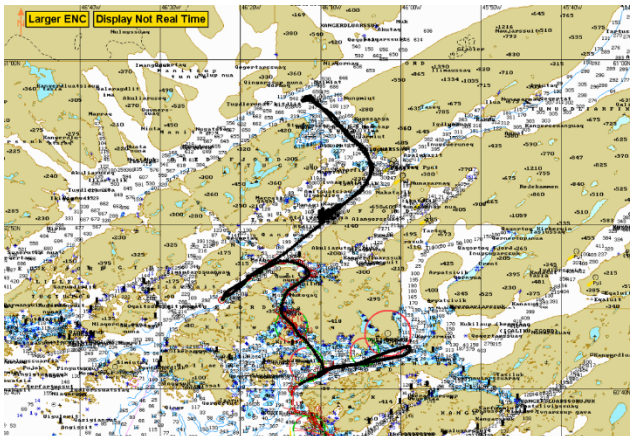
August 6, 2021; -Kangerdluar S.-Julianehab-Skovjord



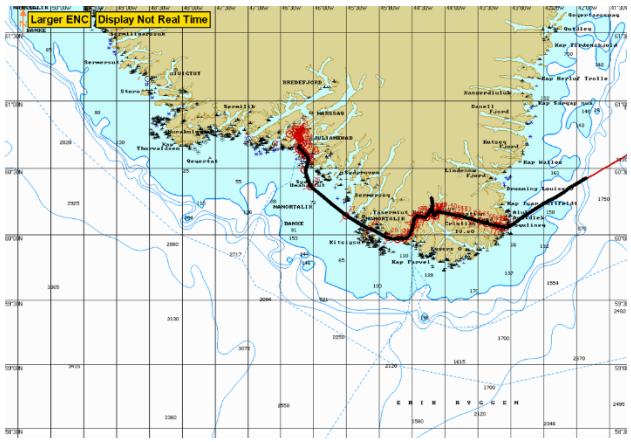
August 7, 2021; Julianehab-Skovfjord-Bredefjord



August 8, 2021; Bredejord-Skovfjord-Julianehab



August 9, 2021; Transit to Iceland



August 10. 2021; Transit to Iceland