Arctic Biology Field Course
Qeqertarsuaq
July 11.th – 22.nd 2022

Arctic Station
University of Copenhagen

Title: Arctic Biology Field Course, Qeqertarsuaq 2022

Published by: Arctic Station, University of Copenhagen, 3953 Qeqertarsuaq, Greenland 2100; Section for Marine Biology, University of Copenhagen, Strandpromenaden 5, Helsingør, Denmark

Publishing year: 2022

Edited by: John Fleng Steffensen, Section for Marine Biology

ISBN: 978-87-89143-27-9

Front page photo: unknown tourist.

Citation: The report can be cited in full:

Arctic Biology Field Course, Qeqertarsuaq 2022. Steffensen J. F. (Ed.), 2022. Arctic Station, University of Copenhagen, p. 1-66.

or in parts:

Authors(s), 2022. Title of paper, In: Arctic Biology Field Course, Qeqertarsuaq 2022. Steffensen J. F. (Ed.), 2022. Arctic Station, University of Copenhagen, pp. x-x.

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Preface and acknowledgements

John Fleng Steffensen (Marine Biological Section, Department of Biology, University of Copenhagen)

The Arctic Biology Field course held in 2022 with 12 students and 4 instructors was successful, but with just about all the problems, anybody can imagine. To mention just a few of the many problems and obstacles: upon arrival in Kangerlussuaq everything was chaotic due to fog along the west coast with many flight cancellations as a result, including a third of our group that got grounded in the airport for two days. The rest of us managed to get to Ilulissat on a flight that was too late to make the connection with the ferry to complete the journey to Qeqertarsuaq, the location of the Arctic Station. Luckily, we know someone at Disko Line who was able to arrange a Targa to bring us that same evening. Two days later the other third of our group, with just minutes notice, were able to board a plane to Ilulissat, but again no connecting ferries were available. Again, Peter Caspersen at Disko Line was amazing, as he picked the group up at the airport, transported them straight to the waiting ferry charter he commanded, and had them on their way to Arctic Station within 30 minutes of their arrival at the airport.

But the problems weren't over yet: The next day one of the instructors tested positive for Covid and went into isolation. Luckily, the rest of us tested negative. That day we caught 5 Greenland sharks and everybody was thrilled as this was their first encounter with these animals. Unfortunately the following day the wind was too strong and waves too high to safely retrieve the longlines so we stayed grounded at the station. Little did we know that despite our best efforts and numerous longline sets, those were the only sharks we were going to catch! Our string of bad luck continued when one of the Porsild crew tested COVID positive the next day, followed the day after by the captain and remaining crew member; no crew, no sailing for the next two days.

Every time we have brought students to the Arctic Station, it has been our custom, to accept the invitation to play a football match against the local soccer club on the beautiful synthetic grass pitch directly in front of the station. Since some of our group were doing experiments we could not field an entire team so the two groups were mixed and teams randomly chosen. The game was fun, and it was probably to our benefit that it wasn't them against us.

Despite more longline sets and retrieves as crew and weather permitted, we were unsuccessful at catching additional sharks. Our inability to provide sharks for the planned experiments forced the students and instructors to devise another set of projects using animals that were available by fishing from the pier. As sculpin (*Myoxocephalus Scorpius)* were easily caught by hook and line from the pier where our equipment for sharks was set up, they were used to conduct the fast escape response and critical thermal maximum experiments.

As the course drew to a close, we still had a camera rig with 3 cameras and 3 longlines deployed which we couldn't leave behind. As the normal crew was still in quarantine, the retired, former Porsild captain, Fari was pressed into service and helped us recover all the equipment on the last day.
Although the course was almost over, apparently our troubles were not. On the day of departure from Qeqertarsuaq 12 of us left in the morning with the remaining 4 scheduled to leave on a high speed ferry in the afternoon. Once they arrived, we had planned to all walk to

the to the incredibly beautiful Ilulissat Icefjord, visit the new Isfjordsecenter, and have dinner together. Apparently, however, high winds and waves forced Disko Line to change the boat for the second group from a high speed Targa (2.5 hr transit) to the regular slow boat (6 hour transit), so the group didn't make it until late in the evening. That's why 4 students are missing from the cover group photo taken at the icefjord.

In summary; this course was designed to introduce students to the challenges of working in the field in the Arctic, how plans and experimental design may change, and how you may have to improvise, think on your feet, and use whatever you have at your disposal. Unfortunately, despite our best efforts to combat misfortune (seemingly at every turn), they really experienced it all! While nothing seemed to go as planned, I believe the students still got a valuable scientific experience, had a chance to explore the local environment in Greenland, and above all had a good time.

The course in 2022 was organized by the Department of Biology, University of Copenhagen and lasted 10 days. All 3 student projects conducted were marine and concerned different aspects of Greenland shark biology.

The selected projects and students were:
1) Range testing of Vemco equipment and tracking of the Greenland shark (*Somniosus microcephalus*):

Aina Rossinyol Fernàndez (nkq414), Gustav Mathias Kronholm (xcb511), Heledd Imogen Smith (jpc520), & Marie Golan (srp286)

2) BRUV study on Greenland sharks and fast escape response on *Myoxocephalus scorpius:*

Colin Moldenhauer (dsl291), Nicoline Nørgaard (fsd271), Terry Pedersen(kfp698), Annika Caroline Reinholdt (knb401).

3) ECG on Greenland shark *Somniosus microcephalus* and critical thermal maximum on shorthorn sculpin *Myoxocephalus scorpius*:

Minik Damgaard Holding (FPR598), Nanna Hamiltorn (CSV718), Kathrine Kock Jonsson (SRX999) and Hannah Juul Michaelsen (HLG495).

The projects were supervised by John Fleng Steffensen, University of Copenhagen; Paolo Domenici, CNR, Italy; David McKenzie, CNRS, France and Peter Bushnell, IUSB, USA.

On behalf of all participants, we thank the staff at the Arctic Station for giving us all the logistical support needed to perform the projects. Particularly we thank the crew on board "Porsild", Jørgen-Peter Lund, Frederik Grønvold, Erik Wille and Eli, as well and the scientific leader Emilie Henningsen and the station manager Kjeld (Akaaraq) Mølgaard.

The Dept of Biology at the Faculty of Science and the Board for the Arctic Station covered the main expenses, and we are grateful for their support. In addition, we gratefully acknowledge important financial support from Elisabeth and Knud Petersens Foundation and Solarfonden.

Helsingør August 16.th 2022

Range testing of Vemco equipment and tracking of the Greenland shark (*Somniosus microcephalus*)

Written by: Aina Rossinyol Fernàndez (nkq414), Gustav Mathias Kronholm (xcb511), Heledd Imogen Smith (jpc520), & Marie Golan (srp286)

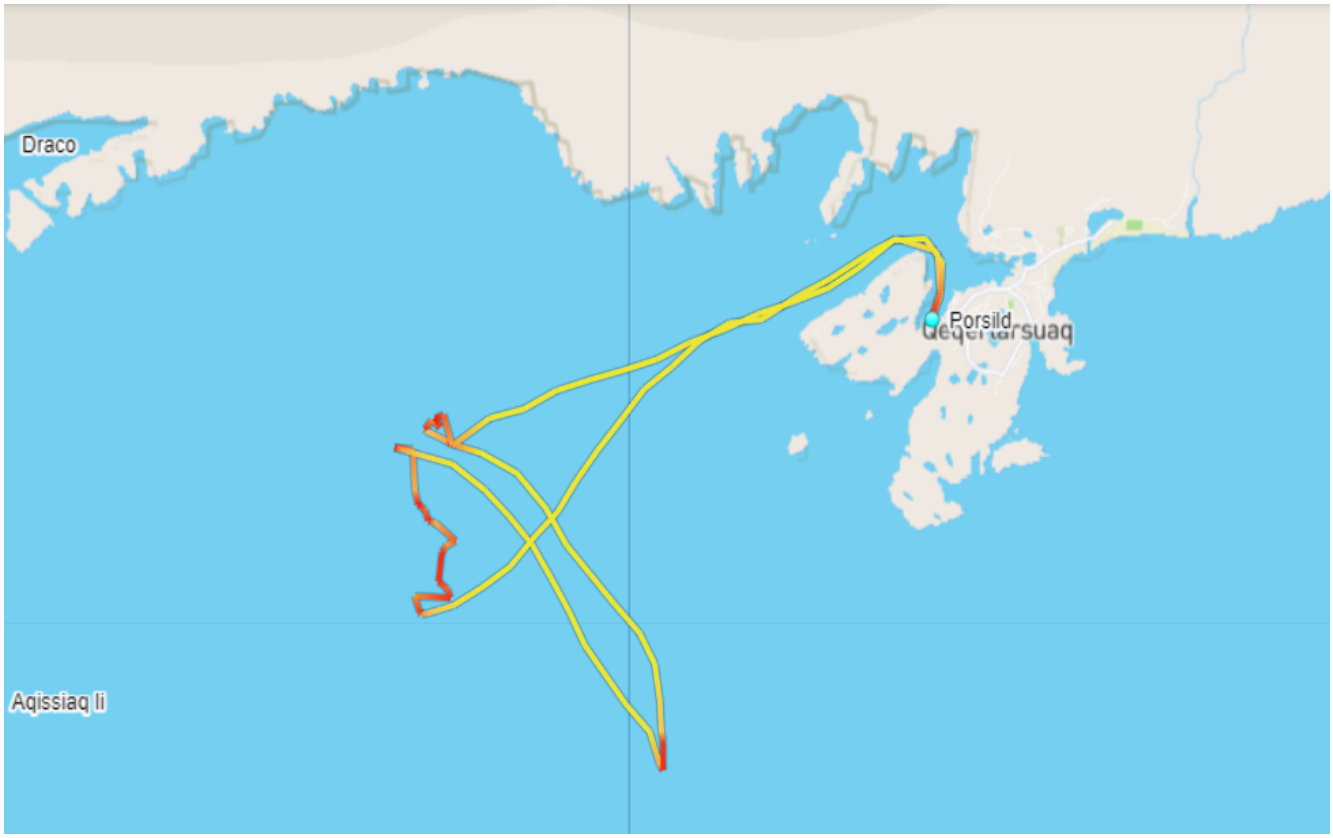

Figure 1. *Sailing route of Porsild on the 14th of July 2022.*

Date: 15.08.2022

Abstract

Passive acoustic telemetry is a common and key method used in studying the ecology of marine animals. The performance and detection range of acoustic telemetry varies greatly depending on environmental and anthropogenic variables. Hence, performing range tests in local conditions at the study site is paramount to ensure adequate study design and correct interpretation of acoustic data. The Greenland shark (*Somniosus microcephalus)*, listed as vulnerable by the IUCN Red List, is an unusually long-lived species, with the oldest recorded individual being 392 ± 120 years old, and is a potential apex predator of Arctic ecosystems. Despite its range being documented across the North Atlantic and Arctic oceans, relatively little is known about the spatial ecology of this species. Range tests were conducted in the waters east of Qeqertarsuaq, north of Disko Bay, West Greenland, an area known for the presence of the species. The aim of this study is to carry out acoustic telemetry range tests, to calculate the maximum effective detection range of the acoustic receivers, under local conditions in order to track the movement of Greenland shark individuals.

1 | Introduction

The Greenland shark (*Somniosus microcephalus*) is a chondrichthyan species that is widely distributed in the North Atlantic and Arctic Oceans. *Somniosus microcephalus* is amongst the largest fish found in the Arctic, with a body length of up to 7 meters. Particularly interesting about this vertebrate is its longevity; some have found that these sharks can live for hundreds of years. Even though it is considered a deep-water benthic-feeding species, its vertical distribution ranges from the surface to around 1800 meters in depth (Nielsen et al., 2016). Despite the importance of a potential apex predator in the Arctic marine ecosystems (MacNeil et al., 2012), the species movement behavior, which is a critical component of the conservation of marine species, is poorly understood.

Passive acoustic telemetry is a common method used to study the spatial ecology and movement behavior of aquatic animals (Reubens et al., 2019). This technique consists of stationary receivers deployed at the bottom of the sea that receives sound waves from an animal tagged with a transmitter (Kessel et al., 2014). Even though it is a costly and laborious method, it

allows to carry out long-term studies with extensive datasets (Mathies et al., 2014). The main limitation of this method is the existence of variables that control the ability of the receivers to detect signals from the transmitters (Reubens et al., 2019). Both natural and anthropogenic variables affect the detectable range of acoustic signals, such as properties of the water column (including temperature, salinity, bubbles and suspended particles), substrate characteristics, weather conditions (wind, water currents) and marine traffic, which leads to noise pollution (Mathies et al., 2014). Accounting that these variables vary, both temporally and spatially, it is essential to carry out detection range tests of acoustic receivers to correctly interpret acoustic data. This makes it possible to calculate the effective detection range of the receivers, defined as the maximum distance in which receivers can detect a tagged animal (Kessel et al., 2014). This detection range can be utilized to maximize the study efficiency by establishing the essential receiver spacing during the receiver deployment design (Kessel et al., 2014).

The aim of this project is to carry out an acoustic telemetry range test to calculate the maximum effective detection range of the receivers and to track the movement of Greenland shark individuals.

2 | Materials and methods

The study was carried out in the east waters of Qeqertarsuaq, north of Disko Bay (West Greenland) (Fig. 2). The area has a depth of between 100–200m, and a water temperature of approximately 2-3°C (Seatemperature.org, July 2022).

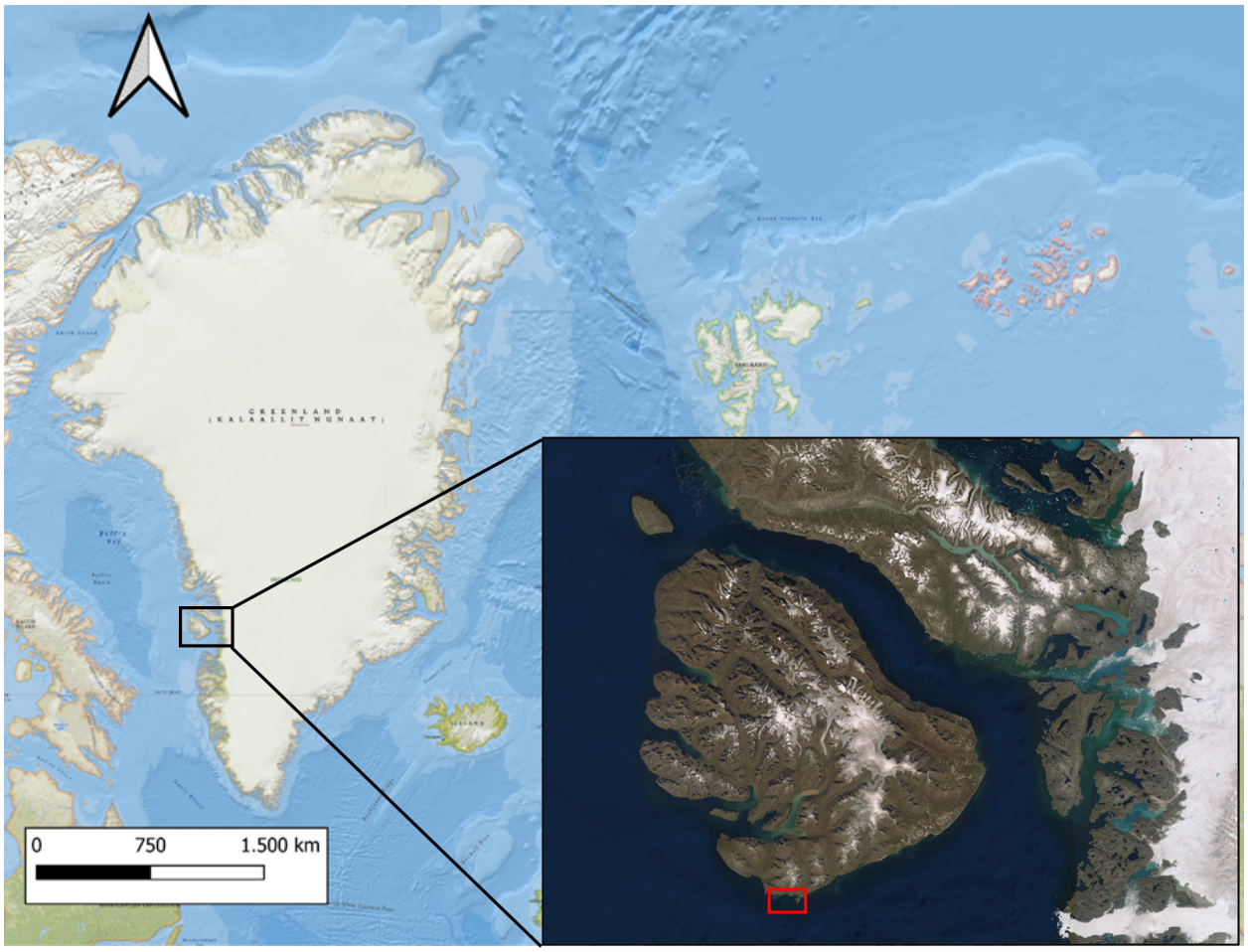

Figure 2. *Location of the study area in the south of Disko Island (red square), in West Greenland*

2.1 | CTD profile

To achieve an understanding of the physical proponents of the water column, a CTD profile was conducted by students at the University of Copenhagen studying plankton biodiversity on a subsequent Arctic Biology Field Course, on 28th July 2022. Measurements were calculated at the permanent measurement station (69°11.172' N, 53°30.506' W) located approximately 2

nautical miles from the location the VR2AR was moored. Therefore, the conditions are assumed to be the same (J. Steffensen, Personal Comm. 2022).

2.2 | Range test

2.2.1 | Conducting the range tests

A total of three range tests were conducted in the vicinity of 69°2172 N, -053°6092W, on the 13th, 16th and 20th of July 2022, using Porsild as a research vessel. To conduct the range test, a VR2AR 69 kHz receiver, a coded 69 kHz transmitter, and a VR100 receiver/transponder with an attached hydrophone were used. The receiver would be lowered into the water at a sufficient depth, and moored to the bottom using two 15kg tire rims as an anchor and a trawl float with a diameter of 26 cm to keep the receiver upright. The position of the receiver would then be noted. Once successfully moored, the VR100 could be used to communicate with the receiver to get the receiver's depth and to make sure the receiver was contactable and moored at a satisfactory angle. Both the transmitter and the hydrophone would be lowered from Porsild into the water, and then the boat would slowly drift away from the receiver. At this point, the VR100 would constantly attempt to connect to the receiver, and after each successful connection, the signal strength, the distance between receiver and VR100, and the time of successful connection would be noted. When connections between the VR100 and receiver no longer could be made, Porsild would continue to drift for 15 minutes, in the case that the receiver still received pings from the transmitter. After 15 minutes without any connections, Porsild would sail back to the receiver, and begin drifting away a second time.

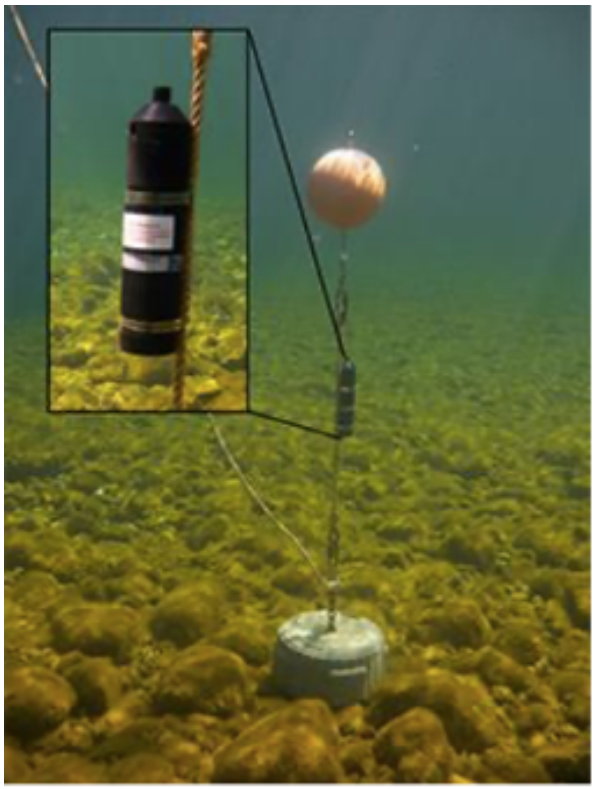

Figure 3. *Indicational setup of the VR2AR receiver rig moored to the seafloor, photograph taken from Polke-Pedersen (2021). An anchor is used to weigh the rig down and a soft surface buoy was attached to the top of the receiver to achieve an upright position.*

2.2.2 | Analyzing data from the range test

Data from the receiver could be offloaded via Bluetooth using the VUElogger software. Additionally, data from the VR100 could be offloaded via USB using the VR100 HS software, but since no range test between the transmitter and the VR100 was conducted, this software was not used. Using www.marinetraffic.com the coordinates for Porsild could be found at all times during the range test, and this was used to calculate the distance between the receiver and the transmitter at the times when a ping was received. By using the coordinates for the receiver and Porsild at the time of a received ping, the distance between them (not considering depth) could be calculated using the Cosine-Haversine formula (Robusto, 1957):

1) $$a = sin^2\left(\frac{\Delta\phi}{2}\right) + cos\phi_1 \cdot cos\phi_2 \cdot sin^2\left(\frac{\Delta\lambda}{2}\right)$$

2) $$d = R \cdot \left(2 \cdot atan2\left(\sqrt{a} \cdot \sqrt{(1-a)}\right)\right)$$

Where ϕ is latitude, λ is longitude, and R is the radius of the earth (6371km). The Cosine-Haversine formula gave the distance between the VR100 and the receiver in a 2D plane, and in order to get the distance in 3D, the Pythagoras equation was used (Kadison, 2002):

3) $$\sqrt{d^2 + b^2} = c$$

Where b is the depth of the receiver.

2.3 | Tracking of Greenland shark

One shark (assumed female due to the size, although it was not possible to confirm this) with a body size of 335cm was tagged with a coded 69 kHz transmitter with ID-13153. A small incision was made in the belly region of the shark, and the transmitter was inserted into the body cavity. The shark was sutured and released immediately. Following the release of the tagged shark, a VR2AR 69 kHz receiver was moored to the bottom of the sea in the same location the shark was released.

3 | Results

3.1 | CTD profile

Temperature and salinity were plotted against depth in a CTD profile.

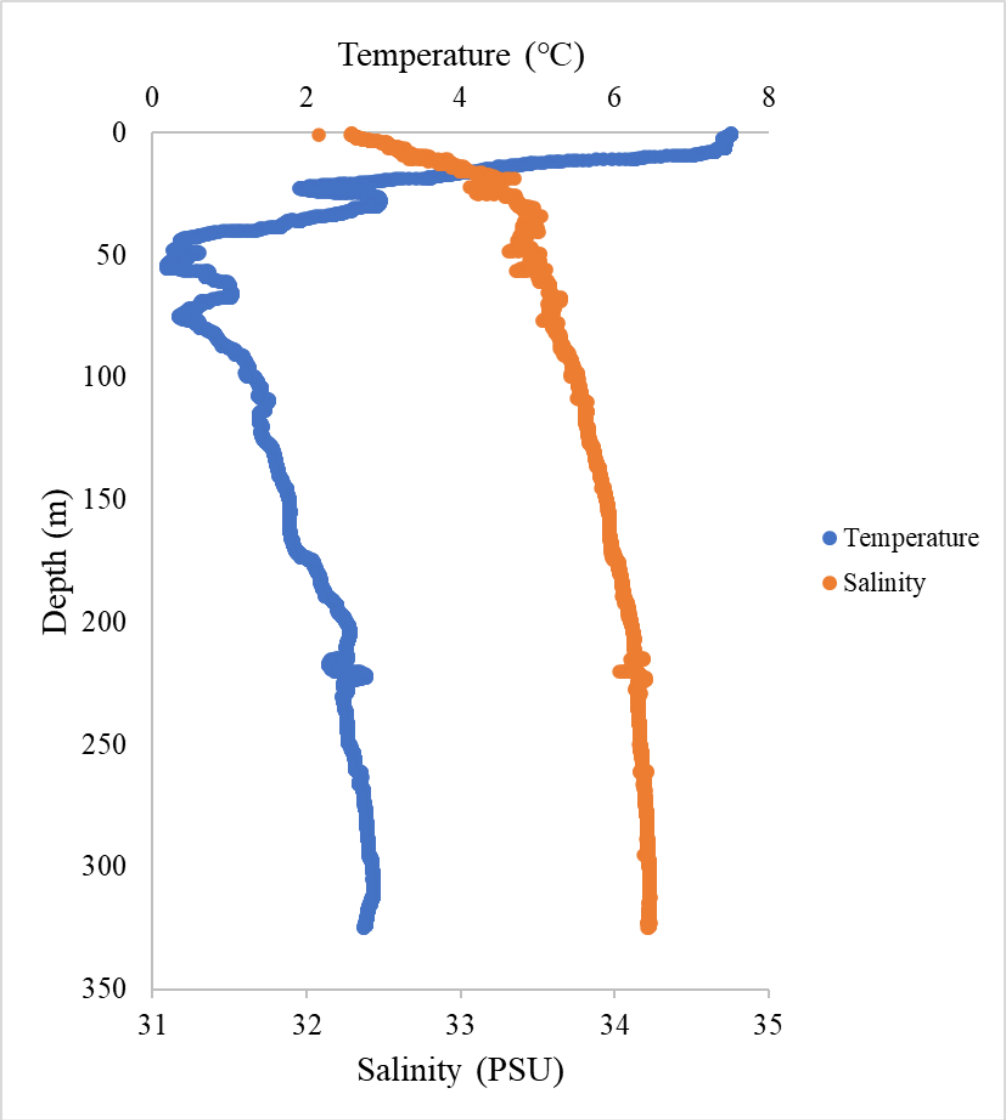

Figure 4. *CTD profile showing temperature and salinity with increasing depth. The profile shows a temperature span of 0.2-7.5℃, a salinity span of 32.1-34.2 PSU, and a maximum depth of 325 meters.*

3.2 | Range test

3.2.1 | Communication between pinger and receiver

All signal strengths between the VR100 and the receiver were plotted against distance.

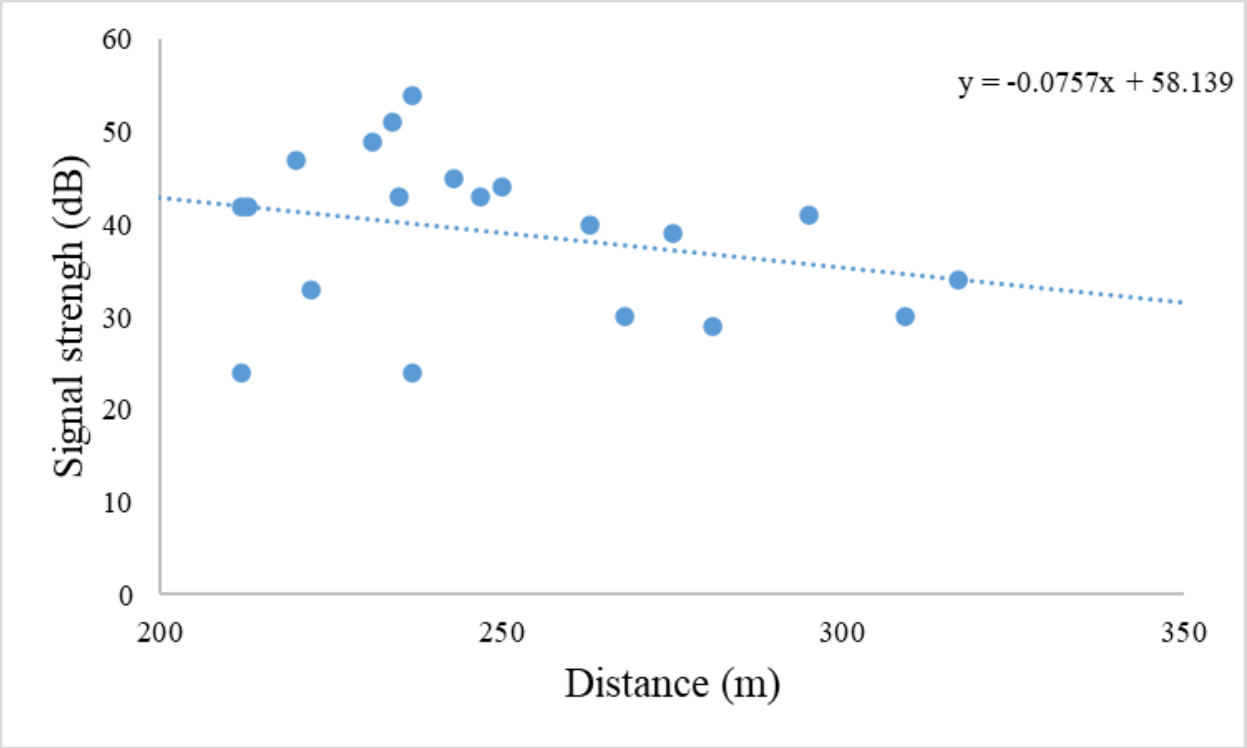

Figure 5. *Signal strength in dB between the VR100 and the receiver on the y-axis, and distance in meters on the x-axis. The trendline is plotted as well.*

The observed trend is a slight decrease in signal strength with increasing distance between the VR100 and the receiver.

The number of successful connections between the VR100 and the receiver min^{-1} was plotted against distance.

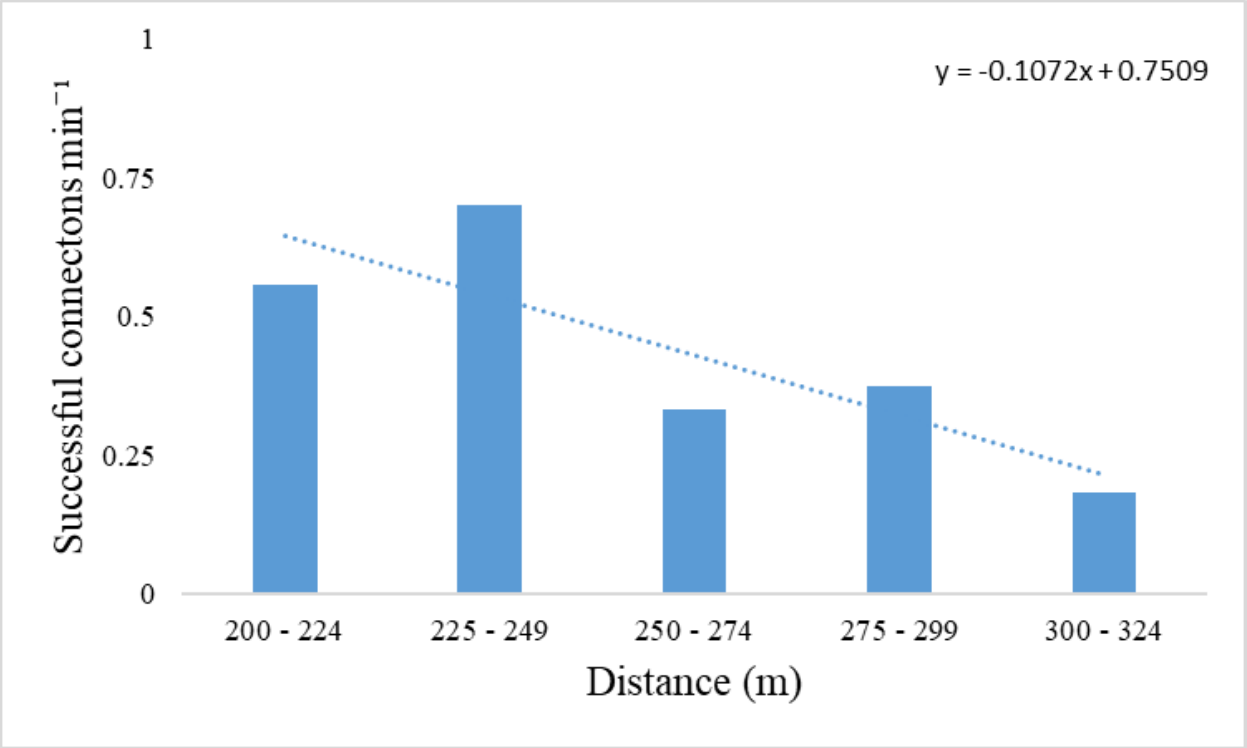

Figure 6*: Successful connections between the VR100 and the receiver min $^{-1}$ on the y-axis, and distance in 25-meter intervals on the x-axis. The trendline is plotted as well.*

The observed trend is a decrease in the number of successful connections min^{-1} with increasing distance between the VR100 and the receiver.

3.2.2 | Communication between VR100 and receiver

The fraction of received pings from the transmitter to the receiver was plotted against distance.

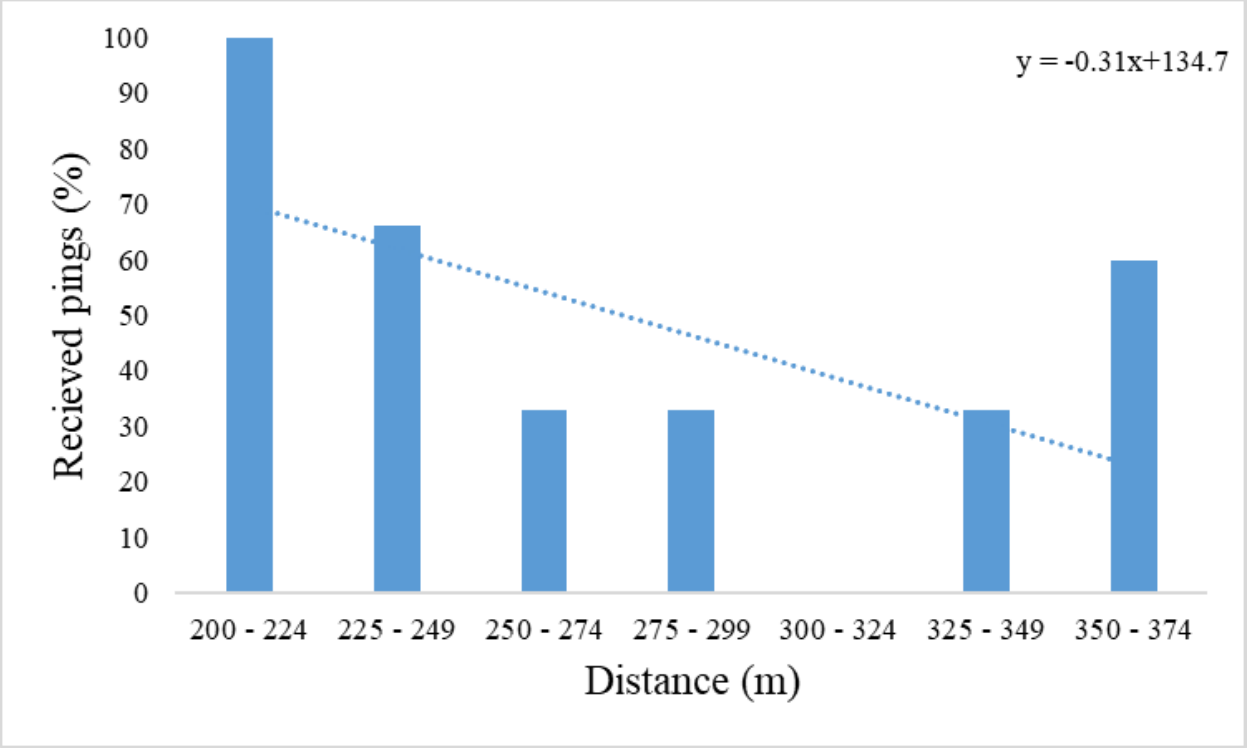

Figure 7. *The fraction of received pings out of all transmitted pings on the y-axis, and distance in 25-meter intervals on the x-axis. The trendline is plotted as well.*

The observed trend is a decrease in the fraction of received pings with increasing distance between the transmitter and the receiver. Based on the data, the 50% frequency mark is 273.2 m, and the 5% frequency mark is 418.4 m.

3.3 | Tracking of Greenland shark

No pings were received from the transmitter that was inserted in the shark.

4 | Discussion

As previously mentioned, many abiotic environmental and anthropogenic factors can influence the detectability of acoustic signals. A similar range test was conducted by Polke-Pedersen

(2021) using the same acoustic telemetry equipment in Øresund, Denmark. This study showed that noise interference from the motor of the boat considerably lowered the detection range between the receiver and the hydrophone (987m) during range testing in Øresund, DK, compared to when the boat's motor was turned off (2361m). Polke-Pedersen's experiment saw VR2AR receivers moored at a depth of 10-13m. In addition, both this study and Polke-Pedersen's (2021) demonstrated the importance of turning off the ship's echolocation while range testing, observing that while the ship's echolocation is active, the receiver is almost unable to receive signals from the coded pingers. Others also mention stratification of the water column as a factor that affects the detection of the signals (Reubens et al., 2019). Although it was possible to detect a slightly decreasing trend in signal strength with increasing distance to the receiver (Fig. 5) as well as the number of successful connections (Fig. 6) between the VR100 and the receiver with increasing distance up to 350m, many of the suggested factors such as motor noise and stratification may have interfered with the signaling, thus minimizing the acoustic range. The relatively short maximum range of approx. 350m found between the VR100 and the receiver when conducting this range test (Fig. 7), in comparison to the max range of 987m found in Polke-Pedersen's study (2021), also indicates that something is interfering with the signaling. However, there are a few differences between the marine environment outside of Qeqertarsuaq and Øresund which may explain the differences in the maximum range found. First is the presence of massive icebergs surrounding Qeqertarsuaq; when chunks of ice break off it generates underwater noise (Glowacki, 2020). The maximum depth the receiver was lowered was also approx. 200m, whereas the receiver was only at a depth between 10 and 13m in Øresund (Polke-Pedersen, 2021).

When looking at the CTD profile of the area outside of Qeqertarsuaq where the range test was conducted on Porsild (Fig. 3) it shows no evidence of a strong halocline, which may otherwise have explained the outcome of the range test. However, it is worth noting that fishing trawlers were seen in the vicinity of the area in which the receiver had been dropped, which may have disrupted the signaling.

It is worth mentioning that the "Close Proximity Detection Interference" (CPDI) phenomenon, which mainly occurs in low ambient noise environments, may also be a factor affecting the detection of the signals of the transmitters. Calm water surfaces may cause reflected

echoes of the pings produced by the transmitter, which, due to the proximity of the receiver, results in signals from the transmitter not being properly decoded (Kessel et al., 2015).

Additionally, the use of a soft surface buoy in the setup of the V2AR2 receiver, as opposed to a solid buoy may have influenced the results. A soft surface buoy was attached to the top of the VR2AR receiver to hold the receiver in an upright position (Fig. 3), however the pressure experienced at this depth crushed the buoy resulting in the receiver lying on its side, as evidenced by measuring the tilt via the VR100 Deck Box. This likely impacted the results as the detection range is known to be affected by receiver tilt (Ruebens et al. 2019).

For further studies, it would be beneficial to conduct research over a longer period, with more tagged individuals. Such a study was performed by Edwards et al. (2022), where a total of 65 sharks were tagged and monitored over six years, using similar equipment as in the present study. Conducting the range test multiple times while also taking the environmental conditions into account would generate more reliable data that could be useful in learning more of the spatial ecology of *S. microcephalus*.

Conclusion

Even though the range test was done under several limitations and therefore considerable uncertainty may account for, it is clear that the performance range of acoustic telemetry is heavily dependent upon environmental conditions. In addition, the differences in yielded results exhibited by the same equipment and methodologies in this study and Polke-Pedersen, demonstrate the importance of performing range tests in local conditions before study implementation to ensure adequate detection rates that are representative of animal movements. Although no data was obtained from the tagged Greenland Shark, hopefully this study can inform future study design in the waters east of Qeqertarsuaq, which appears to be an important habitat for the species. Hence, further research and more precise range tests are required to accurately track the Greenland Shark and therefore effectively manage the species.

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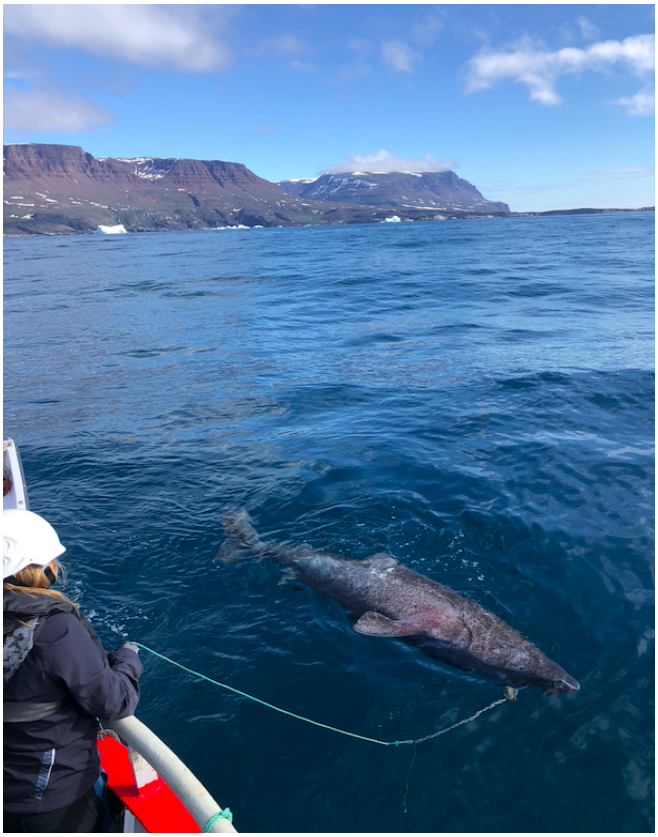
Pictures



BRUV study on Greenland sharks and fast escape response on *Myoxocephalus scorpius*

by Colin Moldenhauer, Nicoline Nørgaard, Terry Pedersen, Annika Caroline Reinholdt

Abstract
The Greenland shark is still very mysterious to science, including its feeding behavior and escape response. This project on the Arctic Field Course 2022 sought to shed some light on these subjects, and hopefully give science more videos of these animals. Unfortunately the course was hit with many hardships that resulted in no caught sharks in the right size for the fast escape experiment. Furthermore the cameras for observing sharks with underwater video also had problems resulting in very few hours of video from the depths, and on the videos that managed to be recorded no Greenland sharks were present. The alternative experiment became a fast escape response on common sculpin at two different temperatures 10.8 °C and 16.8 °C. The elements of the fast escape that was studied was: latency, turning rate, and distance covered at 200 ms. No significance was found between the two temperatures for any of the elements.

Introduction

Fast Escape
Fast escape response is an important part of fish behavior, where the fish reacts within milliseconds(ms) to a predator attack (Dominici and Blake 1997). This behavior helps the fish escape from predators and the time between the stimulus and the fish's response is a good predictor of survival of a predator attack (Schackmann et al, 2021). Fast escape latency has been studied in multiple species of teleosts, but to a lesser extent in elasmobranchs. This response has in experiments been initiated by mechanical stimuli, but light and electromagnetism has also been used (Schakmann et al., 2021). The common sculpin/shorthorn sculpin, *Myoxocephalus scorpius,* fast escape response has previously been studied at 4.5 °C for their escape performance (Jordan et al. 2005). At the writing moment there have yet to be reported escape latency of the common sculpin either in arctic or temperate waters. The temperature could have an impact on the performance as temperature usually increases metabolic activity. With the common sculpin having a wide distribution from temperate areas into the Arctic (AquaMaps 2019), it is possible that its escape performance varies with its geographical origin. There is a possibility that the latency will be shorter as the temperature rises in the oceans.

Fast escape responses of teleosts are typically controlled by specific cells, namely Mauthner cells, which are located in the hindbrain. Mauthner cells receive sensory inputs from visual-, auditory- and mechanosensory cells. The onset of response is determined with video recording of the experiments; (e.g., the first frame of a video where head movement is detected is the fast start moment) (Schakmann et al., 2021). The Mauthner cells have previously been observed in some species of Squaliformes and previously there have not been no studies directly on the fast escape response of Greenland Shark, therefore it would be interesting to see if they have this response both by mechanical- and electrical stimuli. (Bone, 1977; Schakmann et al., 2021). All elasmobranchs have an electro sensory system, ampullary organs named Ampullae of Loronzini, that make them able to detect weak electric

fields, typically within 1 - 8 Hz and with a sensitivity threshold of 5 nV cm^{-1}. The system contains pores/canals, receptor epithelium and sensory nerve fibers. The canals are filled with fluid that makes sure that the electrical stimulus reaches the receptor cells near the afferent nerve fibers. Then the electrical stimulus will be transformed to nerve impulses that reach the brain and give the shark information about the prey, for instance the distance. The Ampullae of Lorenzini are distributed on the entire body of the sharks but concentrate in the head region. T Greenland shark has its ampullae of Lorenzini located near the nose and mouth part (Collin, 2010).

Feeding behavior

The Greenland shark (*Somniosus microcephalus*) is an extremely elusive fish. It has only been captured on camera a few times (Edwards et al., 2019). There are large gaps in our knowledge about *S. microcephalus* in general as the review by Edwards et al. 2019 has summarized. In the Canadian Arctic a baited remote underwater video (BRUV) survey has been used to estimate their relative abundance where individuals were recognized by scars and coloration, independent of data from bycatch (Devine et al., 2018).
In this course Arctic Field Course BRUV is meant to be used for recording their feeding behavior. There is a gap in our knowledge about their feeding habit since active predation and direct observations have not yet been reported. Our knowledge about their diet is mostly derived from stomach contents analysis. Even their feeding frequency is still unknown. It is suggested that *S. microcephalus* can survive a longer period without feeding if their diet contains/includes consumption of energy-dense prey such as seals (Edwards et al., 2019, Yano et al., 2007).

A study of feeding behavior of subadult sixgill sharks (*Hexanchus griseus*) made by McNiel (2016)(McNiel et al., 2016) showed a huge diversity of species consumed by *H. Griseus* and the feeding behavior depends on the situations; *H. griseus* have different positions in the water depending on the location of the prey and the prey species. Some of these methods are possibly utilized by *S. microcephalus* or *S. microcephalus* might have a more similar feeding behavior as other sleeper sharks have.

In a study of *S. microcephalus* feeding behavior on fishing gear and the effect of selective magnetic and repellent-treated (SMART) hooks, they found that *S. microcephalus* utilize a powerful suction feeding mode where they could suck the prey into the mouth from a distance of 25-35 cm (Grant et al., 2018). This behavior has been recorded during the project *Old and cold - biology of the Greenland shark* at Arctic Station, Qeqertarsuaq Greenland, 2018. In this video *S. microcephalus* seems to swim towards the bait, then twists its body before opening the mouth and utilizes suction mode to ingest the bait (http://www.mbl.ku.dk/JFSteffensen/OldAndCold). However, a large amount of arrow worms blocks the view on the shark so that a clear analysis of the video is impossible. How S. microcephalus takes live prey, as stomach contents suggests they do (Nielsen et al., 2019), is still unknown.

The knowledge of feeding behavior known from other shark families and including knowledge from Squaliformes, BRUV recordings would hopefully clarify the exact behavior of *S. microcephalus*. Squaliformes are mainly ram feeders. During ram feeding, a predator attacks its prey with an open mouth and traps the prey within the jaws or engulfs the prey entirely by over-swimming the prey. Squaliformes also utilize other methods in specific

situations where they utilize suction and biting components/methods (McNiel et al., 2016, Grant et al., 2018).

The aim for this project in the Arctic Field Course was to observe the fast escape response of the Greenland Shark (<2,5 m) and its feeding behavior using Baited Remote Underwater Video (BRUV). The goal was to use first electrical stimuli equipment, which we hoped would be sensed by the Ampullae of Lorenzini and afterwards mechanical stimuli. Due to a lack of underwater recordings of foraging Greenland Sharks and because no sharks small enough to fit into a swimming pool on the dock (3 m diameter) were caught, the project was changed. Instead of Greenland shark feeding behavior and fast escape response, we then investigated the fast escape response of common sculpin (*Myoxocephalus scorpius)* at two different temperatures.

Materials and methods

Fast escape

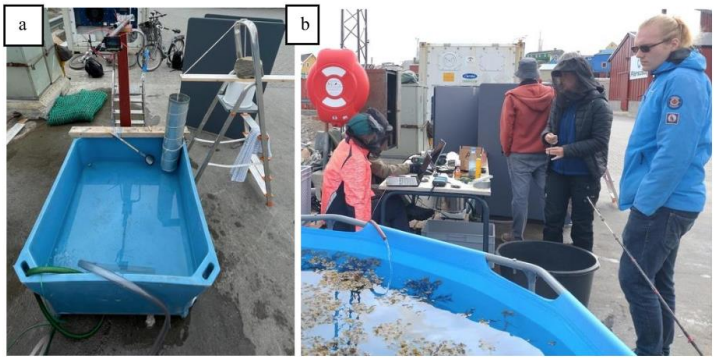
Myoxocephalus scorpius

Animals.

Common sculpin, *Myoxocephalus scorpius,* were caught using a fishing rod at the dock in Qeqertarsuaq, Greenland in July 2022. The fish was then kept in one big pool at the dock. The pool has circular flow-through seawater. The temperature of the pool was approximately 8.8 ° C degrees and diameter of the pool was 3 m.

Setup.

Fast escape experiments were carried out in an insulated fish tub ice box with water at a depth of 12 cm. In one corner a metal tube attached to the walls of the tank acting as concealment to hinder visual stimuli. The tube hung approx. 150 cm above ground level and the opening of the tube was placed approx. 5 cm above water height. Inside the tube was a 50 cl water bottle, filled with saltwater, hung from a string that was attached to a ladder with an opening/closing mechanism. The stimulus was inside the tube to make sure that the individual did not get a visible stimulus. A mirror was placed in an angle to the metal tube so stimulus determination could be accurate for when the bottle broke the surface of the water, see picture 1a-b. An Olympus TG-870 camera was placed approx. 160 cm above ground level in the middle of the tank. The frame rate was set to 240 fps.



Picture 1a-b. Picture 1a-b shows our setup. 1a shows the smaller pool which we used during the trials. We have our metal tube in one corner, where our bottle was attached to a ladder outside the pool, so we had the same height of our stimuli. The camera was filming from the middle of the pool and backwards to the end of the pool, so we were able to see the fish and the stimulus at the same time. We have placed a wooden board at the same end as the metal tube. Here a mirror was tied with tape, so we could see when the stimuli reached the surface. 1b shows how the setup looked when we performed the trials. The smaller pool was shielded so only mechanical stimuli would create a response. At the table there was equipment for measuring oxygen (mg/L) in the water and the temperature (°C). Underneath the table we had our heating system made of flushing water from the pool to our heating pot and back to the pool again.

No fresh seawater supply for the trial tank, but the water got aerated during the trial. We tested if the temperature had an influence on the fish's latency time. Between each animal trial, the tank was emptied, and fresh seawater was filled in. The starting temperature was on average 10.8 °C (± 1,6 °C). Then the water was heated by flushing heated water into the system till we reached on average 16.8 ° C (± 0.5 °C), the water was heated by a makeshift bain-marie: where a tube was coiled into a pot of constantly heated water.

Protocol

The caught fish were not fed for one/two days prior to the experiment. The trial tank was filled with seawater from the acclimatization pool till a water depth of 12 cm was reached. A medium-sized sculpin was then transferred as gently as possible and without air exposure to the trial tank. After a waiting period of 10 min for the fish to destress, the stimulus was applied when the fish was located in appropriate distance to the trial tube. If the fish did not show a clear response, another stimulus was applied. After three failed attempts, the trial was terminated.

After a successful fast escape response, the water was heated. To circulate the water from the pot to the trial-pool, water was siphoned through until the experiment tank water reached an average temperature of 16.8°C. The heating time was on average 19,86 min. After 10 min of acclimation time, the second stimulus was initiated. After the fast escape responses, the fish was measured in length and weight. We had 10 responses out of 20 trials in total.

The response time is calculated based on counted frames recorded by the applied camera at 240 fps. Time zero (t_0) is determined as the frame where the bottle cap touches the water surface, and the time point of response is determined as the first frame in which the fish visibly moves its head.

Videos have been cut down to smaller size so stimuli and escape response together with placement of meter stick is in the used videos. The cut videos are called “Fish # (temp)C short” Due to various programs used for the video length editing by various people the resolution on the videos have deteriorated on some videos possibly causing lower accuracy in video analyzed in Kinovea version 0.8.15 and 0.9.5.

A preliminary trial was set up to test the electrical stimuli equipment, supposed to elicit a response from the Greenland Shark. The apparatus sent out a 5-6V electrical current with a frequency of 6Hz, randomized or constant. This test was made on schooling *Mallotus villosus,* a *Gadus sp.* and sculpins. The M. villosus was caught in a fishing net at the Black Beach near the Arctic Station. There was no response which was also to be expected as Ampullae of Lorenzini are not present in this species. The trial was neither a success nor failure due to this aspect. As no Greenland Sharks small enough to fit into the pool (body length <3m) had been caught, the Greenland Shark’s fast escape response could not be tested. Therefore, the rest of this experiment was carried out with seven individuals of *Myoxocephalus scorpius* where we continued with mechanical stimulus.

The videos were analyzed using Kinovea (versions 0.8.15 and 0.9.5), the latency was determined, head and center of mass identified as shown in the results and the turning rate and distance covered calculated using this software.

Baited Remote Underwater Video-feeding behavior

Somniosus microcephalus

Setup

To survey the feeding behavior of the Greenland shark Baited remote underwater video (BRUV) was set out. The BRUV was placed at approximately 180-200 m depth west of the Arctic Station (AS), Qeqertarsuaq, Greenland. BRUV was used three times.

A GoPro 8 or a Raspberry PI microcomputer was placed on one side of an aluminum frame, while the flashlight was placed at the other end with a skewed angle. A GoPro 3+ was placed in the middle, so that it filmed straight at the bait. The bait (seal meat) was bound with a string to a plastic tube that was attached on top of the aluminum frame, tightened with a hose clamp. The string was tightened to the tube and the bottom part of the aluminum frame, so it would not disappear out of sight. The distance between camera and bait was 70 (± 10) cm. Metal weights were attached to the bottom of the frame to keep it in position. We had smaller weights at the sides, while bigger weights were placed lengthwise. One tire rim was fixed with a robe in the middle of the bottom. The aluminum frame was attached to a buoy that was floating on the surface.

The used cameras were a GoPro 3+, a GoPro 8 and a Raspberry PI microcomputer.
Raspberry PI microcomputer with a camera, external memory of either 250 or 500 Gb hard drive and a 20.000 mAh battery pack, with camera programming by Lars Emil Juel Hansen, housed in a Blue Robotics 4-inch camera house. GoPro 8 with in a 20.000 mAh battery pack, housed in a Blue Robotics 4-inch camera house. GoPro 3 White, black, and white+ in housing from GroupBInc Benthic 2 GpPro UW Housing. The light used is GroupBInc UW light.

Protocol

Two flashlights were available, one for every other day and two different cameras, GoPro 8 and Raspberry PI microcomputer. In addition, two other cameras, GoPro 3 and 3+ were in use. GoPro 3 did not work, so we just used GoPro 3+ and replaced the empty battery with a fully charged battery every day.
We placed the flashlight and one of the cameras as well as the GoPro 3+ approx. halfway up on our frame. We then tightened the bait to both the frame and the tube, so our recordings showed the bait in the center, see picture 2.

Picture 2. This shows our setup for the BRUV. Our bait was placed so it would be in the middle of the cameras. We had a GoPro 3+ in the middle of the frame, between the lamp and the bigger camera, and our GoPro 8 or Raspberry PI on one side and our lamp on the other side. This location of the equipment was a try to avoid too many arrow-worms in front of the camera. These two were placed with an angle towards the bait. The weights are placed at the bottom of the frame. All sides are tired with weights and extra weight was tired with robe in the middle of the bottom.

The same frame was used for each filming opportunity. The camera/power bank and light was demounted and changed each time the frame was set out.

Space on hard disk and memory cards held approx. 16 hours on the Raspberry Pi, 13 hours on GoPro 8 and approx. 3 hours on the GoPro cameras. Practically the GoPro 3 cameras only recorded 45 minutes.

See an overview of the schedule for the experiments at Arctic Station, Qeqertarsuaq, see Field Report in appendix 3.

Statistical methods

Statistical analysis was completed in Microsoft Excel using the f-test to test for variance and the two-sample t-tests assuming equal variance as the variance showed to be equal from the f-test. As the sample size is very small the best test is the t-test.

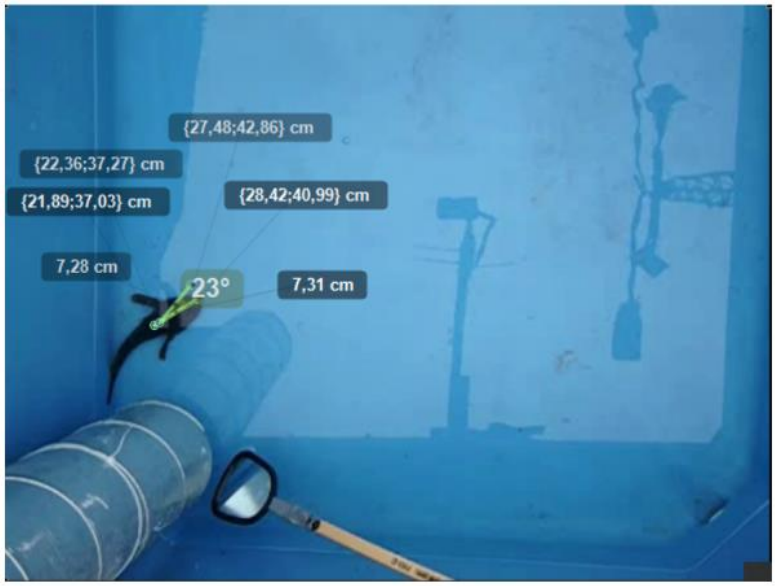
Results

Fast escape response

Out of the seven fish used in the experiment the only fish that did not give a response at either temperature was fish 7. There were no observed responses from fish 4 and 6 at 16.8°C.

Picture 3. We used Kinovea version 0.8.15 for this specific analyze with fish number 2 at 16 °C

We used Kinovea to receive our data of the latency (ms), turning rate (degree/ms) and the distance covered (cm) (how much center of mass has moved). Before analyzing, we needed to calibrate the length of a meter stick, so the future measurements were correct. Then set a coordinate system to receive our data points. The coordinates shown in this picture illustrate how the method of progress is done by multiple steps. See appendix 2 for full guidance and appendix 1 for raw data received from this progress.



Latency, turning rate and distances covered were determined. Then, the significance of observed differences between the two temperatures were analyzed through a t-Test: Two-Sample Assuming Equal Variances. Any observed differences between the temperatures were not significant, latency P=0,21, turning rate P=0,66 and distance covered P=0,37 (see table 2 and appendix 2). The p-values were all above the threshold of significance ($p > 0.05$). The data are visualized in the figures 1 to 3.

Table 1. The full results of the two-sided t-test with equal variance for the latency. The same calculations can be seen for turning rate and distance covered in appendix 2

F-Test Two-Sample for Variances		
	Variable 1	*Variable 2*
Mean	63,19444444	38,54166667
Variance	1162,615741	177,9513889
Observations	6	4
df	5	3
F	6,533333333	
P(F<=f) one-tail	0,07665127	
F Critical one-tail	9,013455168	
variances are equal		

	Latency	
fish	10.8°C	16.8°C
1	45,83	29,17
2	100,00	58,33
3	37,50	33,33
4	33,33	
5	50,00	
6	112,50	33,33
7		
	10.8°C	16.8°C
Average	63,19	38,54
sd	34,10	13,34
Median	47,92	33,33
T.test	0,21	

t-Test: Two-Sample Assuming Equal Variances		
	Variable 1	*Variable 2*
Mean	63,19444444	38,54166667
Variance	1162,615741	177,9513889
Observations	6	4
Pooled Variance	793,3666088	
Hypothesized Mean	0	
df	8	
t Stat	1,355921424	
P(T<=t) one-tail	0,106077378	
t Critical one-tail	1,859548038	
P(T<=t) two-tail	0,212154756	
t Critical two-tail	2,306004135	
discard H1, there is no difference between the means.		

Table 2 Overview of test results from latency, turning rate and distance covered. Including the mean and standard deviation for each experiment temperature.

Test results from fast escape experiment			
	10°C	16°C	Test results
No. sculpin	7	7	
No. Obersved escape responses	6	5	
Latency			
Mean (ms)	63,19	38,54	
SD	34,10	13,34	
t(df)			1,36
P-value			0,21
Turning rate			
Mean (degrees/ms)	1013,36	1163,2	
SD	342,81	525,78	
t(df)			-0,46
P-value			0,66
Distance covered			
Mean (cm)	7,38	5,41	
SD	3,57	2,28	
t(df)			0,95
P-value			0,37

The data suggests that the average turning rate was increased in warmer water, see figure 2. Though the non-significant test results show that it was not significant.

It seems like there is a tendency for colder waters to have a higher covered distance, than

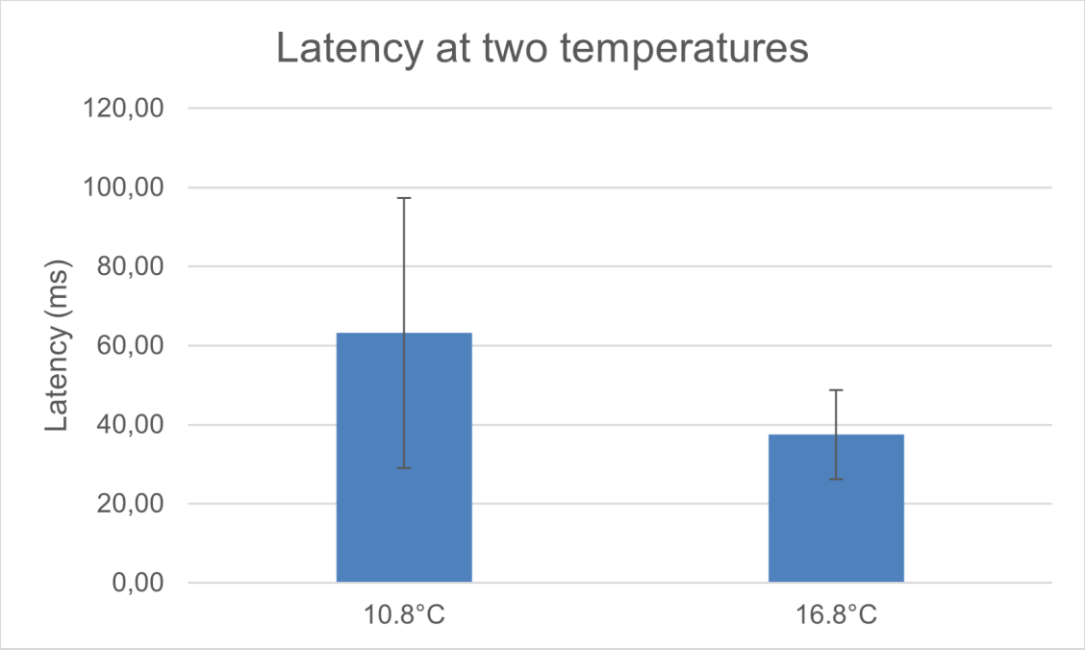


Figure 1. Here we see the average latency at the two different temperatures. At 10.8 ℃ the average latency is 63.19 ms and at 16.8 ℃ the average latency is 38.54 ms. Error bars indicate standard deviation, and the differences are not significant.

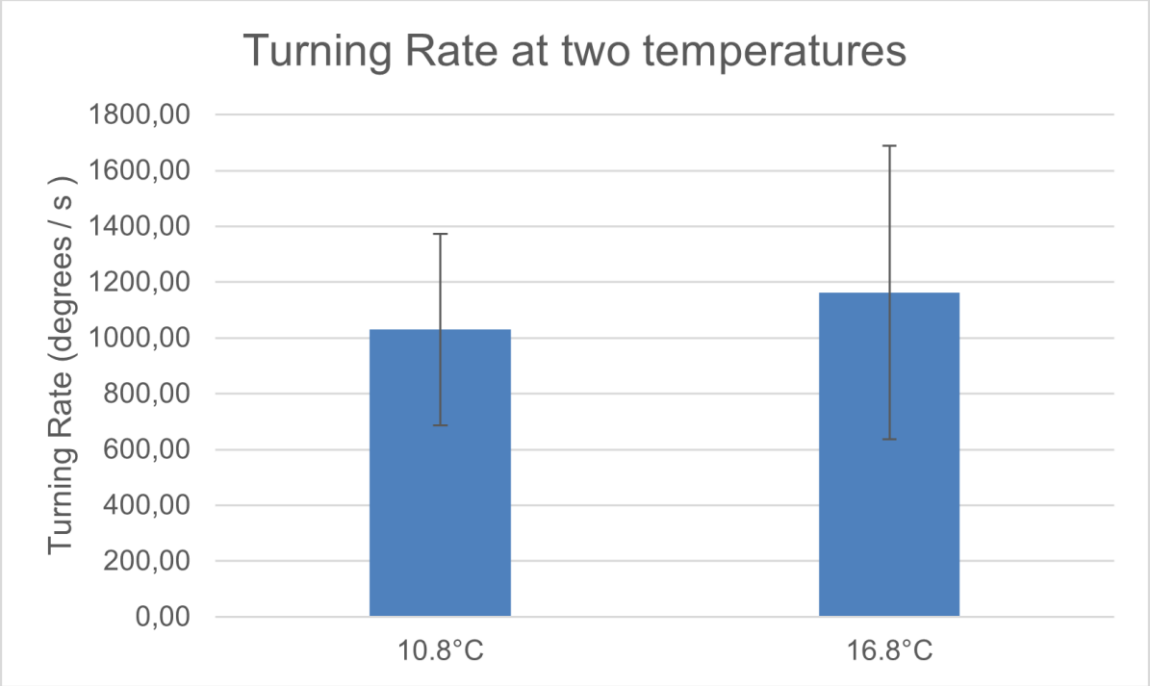


Figure 2. Here we see the average turning rate at two different temperatures. At 10.74 ℃ (10.8 ℃) the average turning rate is 1030.36 degree/s and at 16,86 ℃ (16.8 ℃) the average turning rate is 1163.20 degree/s. Error bars indicate standard deviation and the differences are not significant.

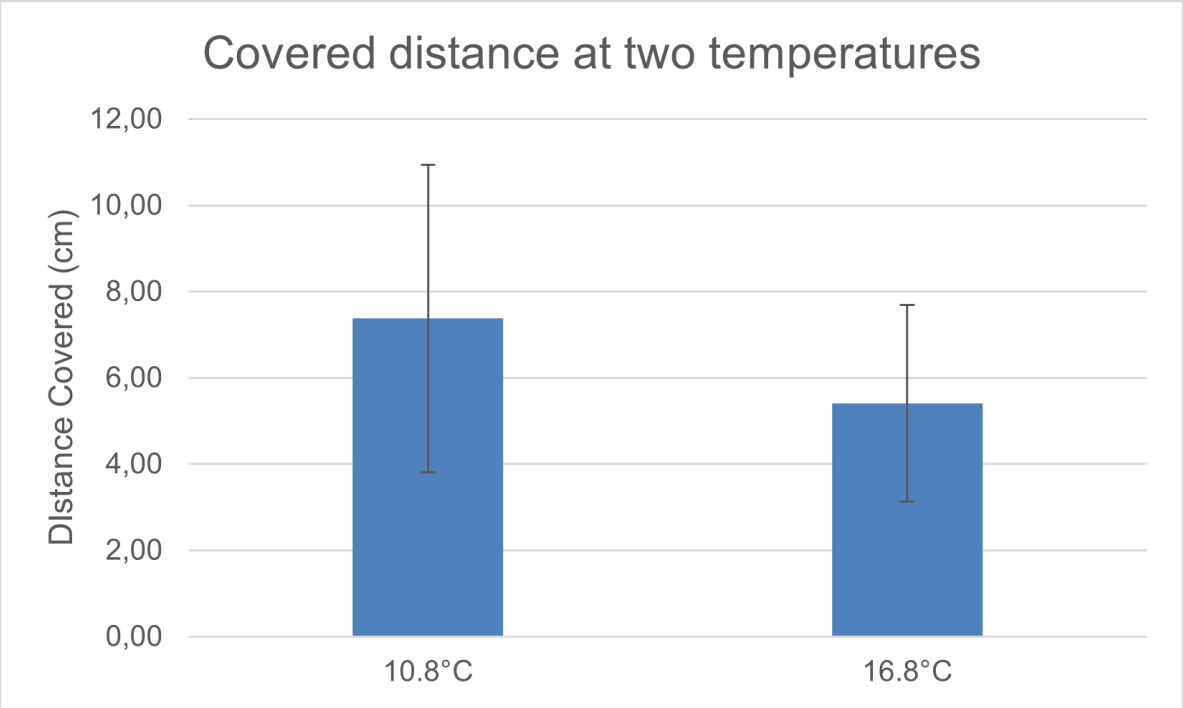


Figure 3. Here we see the average covered distance at two different temperatures. At 10.8 ℃ the average covered distance is 7.38 cm and at 16.8 ℃, it is 5.41 cm. Error bars indicate standard deviation and the differences are not significant.

BRUV and feeding behavior of *Somniosus microcephalus*

The feeding behavior of the Greenland shark has not been uncovered due to the small amount of BRUV recordings as well as technical issues with the equipment and limited time on the water, because of unfavorable weather conditions and shortage of crew on the research vessel Porsild.

In total, more than 26 hours of recording from GoPro 8 and no usable recordings from the Raspberry pi camera are available. GoPro 3+ recorded a small number of videos, but nothing that GoPro 8 had not captured.

Overall, the set-up of the BRUV experiment was good and worked in the field. The quality of the videos from GoPro 8 is excellent and the underwater lamp worked well, especially when it was placed at an angle, so the frame is not disturbed by chaetognatha and plankton attracted by the light.

In the videos there is no sign of Greenland sharks swimming by, but the seabed is full of life and many species of fish and crustaceans are attracted by the set-up and the light. Species such as *Myoxocephalus Scorpius, Pandalus borealis, Gadus morhua, Gadus ogac, Chionoecetes opili*, *Calanus sp.* and *Chaetognatha* are frequently captured by the cameras.

From similar previous studies we know that it is possible to record Greenland sharks at the same depth and temperature and the reason behind the failure of this study is mainly found in the limited amount of BRUV recordings we have. The feeding behavior of the Greenland shark is still a mystery and theories of the feeding strategies as well as position in the food web are made based on stomach content (Nielsen et al., 2019). Therefore it would be revolutionary to capture their feeding on camera.

Discussion

Fast escape response of *Myoxocephalus scorpius*

Given the limited dataset and the insignificance of the observed differences, it is impossible to draw final conclusions regarding our hypothesis that the fast escape response of a common sculpin is faster at higher temperatures, due to higher metabolic activity at increased temperatures. Moreover, several specimens did not show any analysable response to the applied stimuli (Fish 4 and 6 at 16.8°C, Fish 7 at both temperatures). However, even though statistical analyses did not show any significant differences between the fast escape responses of common sculpins at lower and higher temperatures, it is notable that the average values of each investigated parameter indicated a faster escape response at higher temperature (shorter latency and higher turning rate at 16.8°C compared to 10.8°C, Figures 1 to 3). The average latency, for instance, decreased with a factor of around 1.6 from 63.19 ms at 10.8°C to 38.54 ms at 16.8°C (Figure 1). The insignificance of this difference results from a large variance of the obtained values at both temperatures. This large variability of the responses is possibly a result of the relatively uncontrolled experimental conditions at the dock of Qeqertarsuaq. Usually, studies investigating fast escape responses are carried under quiet conditions and with as little disturbance as possible. During our experiments, however, the noise level was elevated and inconsistent so that different fish were possibly impacted to varying degrees by external noise. In addition, due to time pressure, it was not always possible to wait long enough until a fish was in a good position to receive the stimulus. The escape response of fishes is impacted by several factors, including the distance to the stimulus, which was not consistent throughout the different experiments (Schakmann et al., 2021).

The latency of teleosts is expected to lie within 10 - 40 ms (Schakmann et al., 2021). Interestingly the fish investigated in this study only were within this interval at 16.8°C but were far above that at 10.8°C (Figure 1). However, the elevated average at 10.8°C results from two fish with exceptionally high latencies (fish 2 and fish 6). The fish - which showed a response - were mostly within this interval. This shows that more experiments with a larger number of individual fish will be needed to address this question in future studies to avoid that individual outliers have a too pronounced impact on average values. In addition, several parameters which can influence an escape response have not been taken into consideration, such as distance from the stimulus, length/ age of the fish, and feeding status.

There is also the possibility that Type 2 errors have been made as the variance was checked with an f-test, making the test results weaker and therefore the accidental acceptance of the

H0 hypothesis that the difference is not significant. The small data set is the key weakest point of the statistics.

BRUV and feeding behavior:

Unfortunately, no Greenland shark was caught by the cameras and as mentioned earlier, the reason behind the failure might be due to the short number of actual recordings from the water compared to what we had intended. For future studies it would be sufficient to test out the whole set up under water before using it in the field, that might have solved the technical issues with the Raspberry PI camera.
We suspect that the type of bait is an important factor for attracting sharks to the set-up and recommend using fresh seal bait. The location and depth of the placed set-up is assumingly optimal as it is known from literature and local hunters hat Greenland shark scavenger and travels in similar areas.

It is also worth mentioning that more recording is not a guarantee for seeing the sharks on camera. Very little is known about their migration and population biology and time and place is a crucial factor in this kind of experiment. The only time we caught sharks on the long line we caught 5 at the same time which is leading us to believe that the sharks could be traveling in smaller or bigger groups.

We suspect that the feeding behavior of the Greenland sharks is somewhat similar to the more studied Pacific sleeper shark due to their genetic relatedness. But ecological factors are not taken into account and the food source and water temperature may influence the response and strategies for catching food

For success in future BRUV studies we recommend GoPro 8 as the main camera as well as the underwater lamp. The point of view of the camera and the depth is primarily dependent on the light and adding an extra lamp would perhaps give a bigger visible frame. Also, we recommend adding a laser pointer at the seabed to measure the size of the fish swimming by. The bait in this experiment was a mixture of rotten seal head, fresh seal head and pieces of Greenland shark meat. None of which was successful. We recommend only using fresh seal meat as it turned out to be the only form of bait where we had success in catching the sharks by long lines.

Another setup used by McNiel (2016) is more advanced than our BRUV setup (four lights, a bait box and five cameras) and recorded for a longer period (January 2003 - May 2005) and with easier access to change bait etc. (located in shallower waters).

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Appendix

A1: Raw data. We had seven fish/individuals which were studied in two different temperatures, respectively on average 10,74 ° C og 16.86 ° C.

Raw Data

					PHASE 1		
Fish no.	T [°C]	Response	Stimulus X coordinate	Stimulus Y coordinate	Stimulus [frame number]	First movement (frame)	Head X start coordinate
1	10	Y	16,85	38,35	434	445	13,66
1	16	Y	28,13	37,73	2040	2047	50,87
2	10	Y	30,57	26,68	416	440	21,12
2	16	Y	21,43	17,47	432	445	28,42
3	10	Y	30,9	24,83	509	518	33,1
3	16	Y	42,3	31,64	1691	1699	37,45
4	10	Y	27,89	28,77	238	246	23,42
4	16	N	-	-	-	-	-
5	10	Y	18,64	26,1	215	227	23,7
5	16	N	-	-	-	-	-
6	11	Y	23,06	9,27	357	384	51,35
6	17	Y	27,82	15	86	94	25,91
7	11	N					
7	17	N					

	com= center of mass				
Head Y start coordinate	COM X start coordinate	COM Y start coordinate	End Phase 1 (frame)	Head X END1	Head Y END1
58,11	14,53	65,67	460	21,5	61,6
31,06	57,1	30,94	2063	57,76	25,79
38,35	27,79	40,85	459	22,51	44,74
40,99	21,89	37,03	435	27,48	42,86
36,97	30,9	44,41	533	39,17	41,93
47,46	37,78	55,54	1712	44,23	51,34
29,1	21,53	35,72	256	26,26	29,57
-	-	-	-	-	-
39,42	21,57	46,61	243	29,3	44,75
-	-	-	-	-	-
13,59	51,35	13,59	?	?	?
39,71	24,27	30,55	103	27,27	39,55

		END PHASE 1
COM X END1	COM Y END1	Turning Angle
13,95	62,76	90
54,59	30,95	122,7
28,06	40,1	63
22,36	37,27	22
31,45	41,93	75
36,81	53,28	70
22	35,01	24
-	-	-
21,84	44,48	76
-	-	-
?	?	?
23,18	31,64	24

200 ms

48 frames after first mov	Head end 2 X	Head end 2 Y	COM end 2 X	COM end 2 Y	total lenght of move (ms)
493	33,12	57,24	26,4	61,02	200
2086	61,7	24,22	59,1	30,17	200
488	-	-	25,84	42,79	200
493	31,44	41,23	27,02	40,29	200
566	43,86	37,24	38,07	41,66	200
1747	46,17	43,27	43,17	50,37	200
294	29,46	25,45	26,47	30,7	200
#VÆRDI!	-	-	-	-	#VÆRDI!
275	39,42	42,28	28,23	46,34	200
#VÆRDI!	-	-	-	-	#VÆRDI!
432	54,83	13,93	48,28	12,39	200
142	21,82	44,18	25,91	36,27	200

Calculations

Fish no.	T [°C]	Latency ms	Distance from stimulus (cm)	Duration of turn (ms)	not completely sure here turning angle calculated
1	10	45,83	20,02	62,50	267,85
1	16	29,17	23,70	66,67	122,66
2	10	100,00	15,02	79,17	119,55
2	16	54,17	24,54	-41,67	#REFERENCE!
3	10	37,50	12,34	62,50	253,54
3	16	33,33	16,55	54,17	257,70
4	10	33,33	9,42	41,67	#REFERENCE!
4	16	#VÆRDI!	#VÆRDI!	#VÆRDI!	#VÆRDI!
5	10	50,00	14,25	66,67	255,59
5	16	#VÆRDI!	#VÆRDI!	#VÆRDI!	#VÆRDI!
6	11	112,50	28,62	#VÆRDI!	#DIVISION/0!
6	17	33,33	24,78	37,50	162,81
7	11	0,00	0,00	0,00	#DIVISION/0!
7	17	0,00	0,00	0,00	#DIVISION/0!
		0,00	0,00	0,00	#DIVISION/0!
		0,00	0,00	0,00	#DIVISION/0!
		0,00	#VÆRDI!	0,00	#DIVISION/0!
		0,00	0,00	0,00	#DIVISION/0!
		0,00	#REFERENCE!	0,00	#DIVISION/0!
		0,00	0,00	0,00	#DIVISION/0!
		0,00	0,00	0,00	#DIVISION/0!

	for 200ms(48frames)
Turning Rate (degree/s)	Distance covered (cm)
1440,00	12,75
1840,50	2,14
795,79	2,75
-528,00	6,08
1200,00	7,68
1292,31	7,47
576,00	7,04
#VÆRDI!	#VÆRDI!
1140,00	6,67
#VÆRDI!	#VÆRDI!
#VÆRDI!	3,30
640,00	5,95
#DIVISION/0!	0,00
#DIVISION/0!	50,52
#DIVISION/0!	0,00
#DIVISION/0!	0,00
#DIVISION/0!	0,00
#DIVISION/0!	0,00
#DIVISION/0!	0,00
#DIVISION/0!	#REFERENCE!
#DIVISION/0!	0,00

A2: T-test results

F-Test Two-Sample for Variances		
	Variable 1	*Variable 2*
Mean	63,19444444	38,54166667
Variance	1162,615741	177,9513889
Observations	6	4
df	5	3
F	6,533333333	
P(F<=f) one-tail	0,07665127	
F Critical one-tail	9,013455168	
variances are equal		

	Latency	
fish	10.8°C	16.8°C
1	45,83	29,17
2	100,00	58,33
3	37,50	33,33
4	33,33	
5	50,00	
6	112,50	33,33
7		
	10.8°C	16.8°C
Average	63,19	38,54
sd	34,10	13,34
Median	47,92	33,33
T.test	0,21	

t-Test: Two-Sample Assuming Equal Variances		
	Variable 1	*Variable 2*
Mean	63,19444444	38,54166667
Variance	1162,615741	177,9513889
Observations	6	4
Pooled Variance	793,3666088	
Hypothesized Mean	0	
df	8	
t Stat	1,355921424	
P(T<=t) one-tail	0,106077378	
t Critical one-tail	1,859548038	
P(T<=t) two-tail	0,212154756	
t Critical two-tail	2,306004135	
discard H1, there is no difference between the means.		

F-Test Two-Sample for Variances		
	Variable 1	*Variable 2*
Mean	1163,201923	1030,357895
Variance	276448,1887	117517,4825
Observations	4	5
df	3	4
F	2,352400534	
P(F<=f) one-tail	0,213418915	
F Critical one-tail	6,591382116	
varainces equal		

	Turning Rate (degrees / s)	
fish	10.8°C	16.8°C
1	1440,00	1840,50
2	795,79	880,00
3	1200,00	1292,31
4	576,00	
5	1140,00	
6		640,00
7		
Average	1030,36	1163,20
sd	342,80823	525,7834048
median	1140,00	1086,15
t.test	0,659714101	

t-Test: Two-Sample Assuming Equal Variances		
	Variable 1	*Variable 2*
Mean	1030,357895	1163,201923
Variance	117517,4825	276448,1887
Observations	5	4
Pooled Variance	185630,6423	
Hypothesized Mean	0	
df	7	
t Stat	-0,459632729	
P(T<=t) one-tail	0,329857051	
t Critical one-tail	1,894578605	
P(T<=t) two-tail	0,659714101	
t Critical two-tail	2,364624252	

F-Test Two-Sample for Variances		
	Variable 1	*Variable 2*
Mean	7,377345885	5,410108506
Variance	12,74080961	5,216434606
Observations	5	4
df	4	3
F	2,442436372	
P(F<=f) one-tail	0,244547281	
F Critical one-tail	9,117182253	
variances are equal		

	Distance covered (cm)	
fish	10.8°C	16.8°C
1	12,75	2,14
2	2,75	6,08
3	7,68	7,47
4	7,04	
5	6,67	
6		5,95
7		
Average	7,38	5,41
sd	3,569427071	2,283951533
median	7,04	6,01
t.test	0,611103217	

t-Test: Two-Sample Assuming Equal Variances		
	Variable 1	*Variable 2*
Mean	7,377345885	5,410108506
Variance	12,74080961	5,216434606
Observations	5	4
Pooled Variance	9,516077467	
Hypothesized Mean	0	
df	7	
t Stat	0,950651913	
P(T<=t) one-tail	0,186720766	
t Critical one-tail	1,894578605	
P(T<=t) two-tail	0,373441531	
t Critical two-tail	2,364624252	

A3: Guide to analyze frame-by-frame in Kinovea

Guide for analasis of fast escape videos in Kinovea ver. 0.8.15	
1.	calibrate length of meter stick: 24 cm folded, 42 foldet out[line tool-rightclick-calibrate]
2.	set coordinate system for quadrant 1 to fit image [Image-Coordinates system origin]
3.	find aproximate coordinate for stimuli approx. the middle of the hididng tube around the second line/spiral turn
4.	find stimuli start frame# and first frame# of fast start
5.	find coordinates for head and center of mass(COM) at first fast start frame
6.	find end of fase 1: fist starts to glide instead of turning
7.	coordinates for head and COM af fase 1 end
8.	find angle with line tool and angle tool
9.	find fase 2 end frame # and coordinates for head & COM

Field report - Greenland Shark project filming group 11-22th july 2022

Field personnel: Annika Caroline Reinholdt, Nicoline Nørgaard, Colin Moldenhauer, Terry Pedersen, Paolo Domenici, David McKenzie, Lars Emil Juel Andersen and John Fleng Steffensen.

Preparation before field trip (30-05-22): Øresundsakvariet Helsingør

Field personnel: Annika Reinholdt, Nicoline Nørgaard, Colin Moldenhauer, Terry Pedersen, Lars Emil Andersen, John Fleng Steffensen.

As preparation for the project, we have visited Marin biological section Copenhagen University in Helsingør multiple times to test the equipment on live fish in Øresundsakvariet as well as preparing the metal frame for the cameras we have selected to use in the project.

We visited the Marine Biological section in April and May 2022.
In April we tested the Raspberry pi camera which Lars Emil has made as well as the new interface programmed for the camera. Small additions were added to the python interface script.
We also compared the different cameras (GoPro 3, 3+, 8, raspberry with different lenses) width and point of view.
In May we tested the cameras again and decided to use the high-quality raspberry pi with the D-Link lens (blue cap). We have selected the optimal parameter and settings for contrast, brightness, fps, saturation, alpha and sharpness and saved these under “Profile 1”, which can be selected under the interface prior to recording.
The parameter was selected while testing the raspberry pi camera in the red algae and lobster aquarium in Øresundsakvariet in complete darkness to simulate the environment at the seabed at a depth of 180 meters.
We have also decided to use the GoPro 8 camera and two GoPro 3 cameras. So we can switch between different types of camera during the field trip.

Our biggest challenge with cameras at this point is the lifespan of the batteries.

Day 1 (11.07.2022): Travel from Copenhagen to Arctic Station Disko Island.

Field personnel: Annika Reinholdt, Nicoline Nørgaard, Colin Moldenhauer, Terry Pedersen.

Today we travelled from Copenhagen to Qeqertarsuaq Arctic Station. The trip was very long and due to foggy weather, we had to wait in Kangerlussuaq Airport for 8 hours but with a stroke of luck 10 people were finally able to get to Arctic Station in the middle of the night. 5 people (including Terry) were still stuck in Kangerlussuaq.

Day 2 (12.07.2022): Arctic Station, Disko Island.

Field personnel: Annika Reinholdt, Nicoline Nørgaard, Colin Moldenhauer, Terry Pedersen.

Terry is still stuck in Kangerlussuaq. The rest of the group spent the day charging batteries for the underwater lamp, GoPro 8, GoPro 3 and GoPro 3+ as well as making sure that every camera was working correctly. We have also decided not to use the Gopro 3 as the on/off button is causing

problems but we are still charging the battery so we can use it for the GoPro 3+. We have assembled the metal frame for the cameras and added more weight to the frame, making sure that the frame is stable under water.

Day 3 (13.07.2022): Arctic Station, Disko Island – half day trip on Porsild

Field personnel: Annika Reinholdt, Nicoline Nørgaard, Colin Moldenhauer, Terry Pedersen.

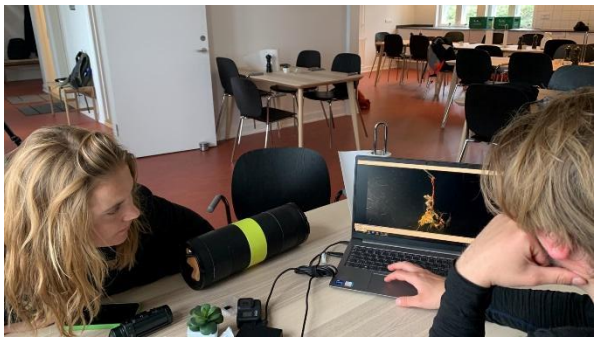
Today was the first day trying out the equipment in the water. We spent half a day on Porsild (2-5:30 pm) and were able to put GoPro 8 and GoPro 3+ on the frame and in the water at a depth of 182 meters. For bait we used a rotten seal head.



Day 4 (14.07.2022): Arctic Station, Disko Island – half day trip on Porsild

Field personnel: Annika Reinholdt, Nicoline Nørgaard, Colin Moldenhauer, Terry Pedersen.

Today we spent a half day at Porsild (9-12 am) and pulled up the frame and collected the GoPro 8 and GoPro 3+. The bait was still on.
We wanted to put raspberry pi in the water but due to technical issues we were not able to. The battery of the GoPro 3+ was not fully charged. So unfortunately, we were not able to put any cameras on the frame today.
When we got back to Arctic Station, we watched the recordings from the GoPro 8 and found that the quality was good, and the recording time was as expected (13+ hours split into 17minutes videos).
We spent the afternoon trying to solve the issues with the raspberry pi. The power bank seems to be broken but luckily Colin has an extra power bank. Also, we found out that some of the electrical connection within the raspberry pi was loose (maybe due to the travel).
We had a zoom meeting with Lars-Emil to fix some issues regarding the python script to the raspberry pi. The path to the directory where the recording is saved needs to be changed manually before every recording session every time.



Day 5 (15.07.2022): Arctic Station, Disko Island.

Field personnel: Annika Reinholdt, Nicoline Nørgaard, Colin Moldenhauer, Terry Pedersen.

Today we were not able to go out on Porsild due to bad weather.
We spent the day preparing for the on-dock pool experiments, where we hope to film the fast escape response of the Greenland shark. We have set up a ladder with a PVC pipe with a camera attached. The setup is not complete yet.

Day 6 (16.07.2022): Arctic Station, Disko Island, full day on Porsild

Field personnel: Annika Reinholdt, Nicoline Nørgaard, Colin Moldenhauer, Terry Pedersen, David McKenzie

Today we spent a full day on Porsild and managed to put raspberry pi and GoPro 3+ in the water at a depth of 186 meter. We turned on the Raspberry pi camera back at Arctic Station at 8:30 am. And turned GoPro 3+ on the boat around 9:30 am. As bait we used the same rotten seal head as well as a fresh seal head. Today was the first and only day we caught Greenland sharks on the long line.

Nicoline stayed home working on the pool experiment set up with David and prepared and charged the camera and lamps for the next trip.

Day 7 (17.07.2022): Arctic Station, Disko Island, half day Porsild, half day Porsild

Field personnel: Annika Reinholdt, Nicoline Nørgaard, Colin Moldenhauer, Terry Pedersen, Paolo Domenici.

Today we spent half a day on Porsild. We changed the cameras on the frame back to GoPro 8 and GoPro 3+ with a new battery pack as well as Paolo's Vaquita diving camera (that can measure temperature and depth). When we got back to the Arctic Station, we found out that the raspberry pi camera stopped the recordings before entering the water and the same for GoPro 3+. This was really bad news, and we came to the conclusion that raspberry pi is too sensitive to work with, and we therefore only will use GoPro 8 in the future.
In the afternoon we tested the mechanical and electrical stimulus on different species of fish (*Mallotus villosus* and *Myoxocephalus scorpius*) caught by the harbor.

Day 8 (18.07.2022): Arctic Station, Disko Island.

Field personnel: Annika Reinholdt, Nicoline Nørgaard, Colin Moldenhauer, Terry Pedersen.

Today we were not able to get out on Porsild, due to the captain was infected with covid-19. We spent the day waiting on news from the captain.

Day 9 (19.07.2022): Arctic Station, Disko Island.

Field personnel: Annika Reinholdt, Nicoline Nørgaard, Colin Moldenhauer, Terry Pedersen. John Fleng Steffensen and Peter Bushnell.

Today we were still not able to go out on Porsild due to Covid-19. But John and Peter managed to get out on the water to collect the video frame. GoPro 8 had 13+ hours of recordings.

Day 10 (20.07.2022): Arctic Station, Disko Island.

Field personnel: Annika Reinholdt, Nicoline Nørgaard, Colin Moldenhauer, Terry Pedersen, David McKenzie, Paolo Domenici.

We spent the day at the dock working on the fast escape response experiments on sculpin, *Myoxocephalus scorpius.* The setup of the experiments had many modifications but at last we got it right.

In the afternoon Nicoline and Annika caught some young boys and girls playing with the sculpins and the experimental set up, mixing all the fish together. This was unfortunate as later experiments now could not be performed correctly.



Day 11 (21.07.2022): Arctic Station, Disko Island, half day on Porsild

Field personnel: Annika Reinholdt, Nicoline Nørgaard, Colin Moldenhauer, Terry Pedersen, David McKenzie.

Today Porsild managed to sail again, and the last long lines were pulled in but with no sharks on. Collin and David spent the day at the dock continuing the fast escape experiments and CTMax.

Day 12 (22.07.2022): Travel from Arctic Station to Ilulissat to Copenhagen.

After 11 days at artic station, we are going home with one night in Ilulissat before flying back to Copenhagen.

Improvements for next time:

- Two GoPro 8 or more if you want to have two cameras recording at the same time, instead of GoPro 3+
- Test setup of Raspberry pi camera as in situ before packing the equipment
- Fresh seal bait (seems to be the only thing working for catching Greenland sharks)
- More power banks (if something goes wrong)
- Two or more flashlights on the frame - but make sure that the light does not interfere with the camera's point of view.
- Test if light has an impact on the sharks were inferred light could be an option

ECG on Greenland shark *Somniosus microcephalus* and critical thermal maximum on shorthorn sculpin *Myoxocephalus scorpius*

Picture taken by Kathrine Kock Jonsson

15. of august 2022

Minik Damgaard Holding (FPR598), Nanna Hamiltorn (CSV718), Kathrine Kock Jonsson (SRX999) and Hannah Juul Michaelsen (HLG495)

Arctic Biology Field course A 2022

Abstract

The Arctic is fundamentally characterised by a cold environment, but effects of climate change are causing the temperatures in the Arctic to rise. Previous and ongoing studies investigating these effects to assess the impact of rising temperatures in the future clearly show the organisms living there are affected. In this study the effects of temperature on the Greenland shark and shorthorn sculpin are examined. The whole project being based on catching a shark under a certain length and the lack thereof, essentially caused a full focus on experiment with the sculpin. Therefore, this report with sections on the Greenland shark experiment has remained theoretical.

The experiment with shorthorn sculpins measured the CT-max of 14 individuals with a rise in temperature at maximum 14.6 ℃. The highest CT-max was measured to be 25.4 ℃, while the lowest was measured to be 21.5 ℃. Data did eventually show that individuals over a certain weight threshold had a harder time to adapt to higher temperatures. The reason for this must be examined further along with a repetition of the experiment to confirm the results. Moreover, an experiment examining the optimal temperature of shorthorn sculpin are also very relevant in connection to this study.

Introduction

Temperature has a major impact on the organisms living in the Arctic, both in the terrestrial, limnic and marine ecosystem. With the rising temperature in the Arctic, organisms are getting more and more challenged to cope with the higher temperatures, especially marine organisms. How they do it is still a mystery being unravelled.

One of the biggest mysteries living in the Arctic is the Greenland shark (*Somniosus microcephalus*) (Edwards et al., 2019).

Greenland sharks, also known as sleeper sharks, inhabit deep waters in the Atlantic and Arctic oceans, where they are believed to largely be scavengers, but may also be an active predator (Edwards et al., 2019). They are known to have a low swimming speed along with a low activity level and have shown no apparent aggressive behaviour (Edwards et al., 2019). Greenland sharks are associated with the possession of K-selected life history traits such as slow growth, relatively low recruitment rates and late maturity, and the lifespan of a Greenland shark has so far been shown to be more than 272 years (Edwards et al., 2019; Nielsen et al., 2016). Due to their habitat, they are adapted to cold temperatures with a thermal range of -1.8 to 7 ℃ (Edwards et al., 2019). Combining their adaptation to low temperatures and their life history traits of slow growth, these sharks may be vulnerable to the rising sea water temperatures regarding the influence on their metabolic scope, the difference between resting

and maximum metabolic rates, and species-specific temperature adaptations (Edwards et al., 2019; Seth et al., 2013).
But the mysteries about the Greenland shark are still numerous and the effect from the higher sea water temperatures is no exception. This mystery is our focus in this study, where we will be measuring the ECG of an anaesthetised Greenland shark under a natural rising of water temperatures. By measuring the ECG, we will be looking at the heart rate along with the body temperature to assess the effect of the rising water temperatures.

Unfortunately, we didn't catch any sharks that had the right size for our setup. The experiment and expected results will therefore only be described theoretically in this paper.
Due to the very unfortunate situation with the Greenland sharks, we had to make a back-up experiment with shorthorn sculpins (*Myoxocephalus scorpius*) instead. This paper will for that reason focus on both experiments.

The shorthorn sculpin is a widespread benthic fish living over a large geographical range from the Eastern coast of North America to the Arctic Ocean (Filatova et al., 2019). This species is an eurythermal fish, which means it has a broad temperature tolerance range (Filatova et al., 2019). It has been shown that there is a difference in the life history parameters between the sculpin populations, where the northern populations are characterized by slower growth rates and late age of sexual maturation compared to the populations in central Europe (Filatova et al., 2019). The shorthorn sculpin is a temperature generalist, meaning it is able to adjust its physiology to different temperatures (Filatova et al., 2019). Theoretically the physiological response of the sculpins would respond better to temperature changes than stenothermic fish species such as Brown trout (*Salmo trutta fario*) (Filatova et al., 2019).
Besides the shorthorn sculpin, two other species of sculpins also inhabit the same area as the caught sculpins. The species are the Arctic staghorn sculpin (*Gymnocanthus tricuspis*) and Arctic sculpin (*Myoxocephalus scorpioides*), who all compete for the same resource in the form as cover (Seth et al., 2013). Therefore, the relative ability to compete for cover would have a great ecological relevance, since the individuals with the largest metabolic scope would have a competitive advantage over the others (Seth et al., 2013).
As known, temperature can have a big influence on the metabolic scope, which is reduced at an upper critical temperature in fishes (Seth et al., 2013). This reduction can lead to a limitation in performance

traits such as locomotion, digestion and even reduced fitness (Seth et al., 2013). The critical thermal maximum, also known as CT-max, is the specific temperature where fish are unable to escape conditions that will ultimately lead to thermal death (Long, J.H., 2011). At this point the individual must use protective mechanisms such as the capacity of anaerobic metabolism, anti-oxidative defence and heat-shock response to try to extend the period of passive tolerance before thermal death (Long, J.H., 2011). It is also known that larger individuals are more affected by the heat stress in line with the exacerbation of oxygen limitation at larger body sizes (Long, J.H., 2011).

In this study we will measure the CT-max of shorthorn sculpins of different sizes. The purpose of this study was to investigate the CT-max and whether a high CT-max might be correlated to a small body weight (below 150 grams) in the shorthorn sculpin. This will further be used to discuss the ability of the shorthorn sculpin to cope with rising water temperatures and hereby the effects of climate change.

This experiment is part of a combined study of shorthorn sculpins. The other part of the study focuses on the fast-escape response under warming seawater temperatures. Some of the individuals of the sculpins were used in both experiments, where they had a long resting period between the experiments. All members in this group participated in both kinds of experiments with main focus on the experiment about the CT-max measurements.

Materials and methods

The ECG on the Greenland shark *Somniosus microcephalus*

The measurement of ECG on anaesthetized Greenland Sharks would be measured under increasing seawater temperature by monitoring its heart rate and its body temperature. The individual was expected to be about 2 m long. However, we did not catch any Greenland sharks in the desired size and as mentioned, the experimental setup and outcome are only outlined theoretically.

Experimental setup

On the harbour of Qeqertarsuaq (Godhavn) a pool was set up with a tarp underneath. The pool was 3 m in diameter and was the decisive factor in the preferred size (2 m) of the Greenland shark individual. Two pumps filled the pool with seawater and the seawater was continuously refreshed as an overflow vent was used. Additionally, two air stones were added to the pool to keep the seawater oxygenated.

The caught Greenland shark would slowly be pulled into the harbour and carried onto the harbour dock in a sling. Before it would be transferred into the prepared pool the stomach contents of the shark must be emptied out, which we would do by letting it slide out while holding it on an angle.

The shark would then be transferred into the pool that would contain a concentration of MS-222 to anaesthetize it. The amount of the anaesthesia that would be dissolved and mixed into the seawater would be determined by calculation of the seawater volume in the pool and the calculated weight of the shark. After the shark would be anaesthetized, tubing would be installed to force-ventilate the gills.

Atropine would be injected into the shark to increase the heart rate by removing vagal cholinergic inhibition along with the injection of Isoproterenol, which causes maximal adrenergic stimulation (Casselman et al., 2012; Ferreira et al., 2014; Mckenzie, n.d.). This ensures that the heart of the shark under the experiment would perform at its maximum heart rate, which therefore also means that the shark would have been euthanized, when the experiment ends, since anything else would not be considered ethically right.

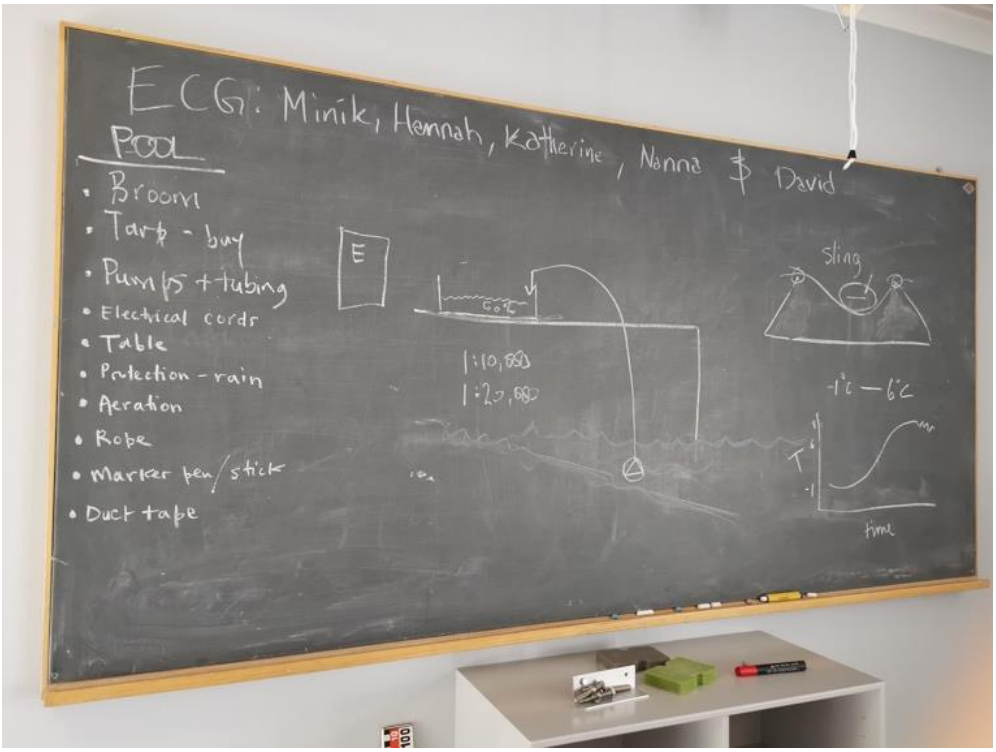


Figure 1: ECG experimental setup illustration by David J. McKenzie

Experimental method

The Greenland shark would be held on its back by an old trawling net cut open and stretched over the pool. This also makes it possible to keep the shark anaesthetised with injection in the big vein of the tail. The shark would be monitored constantly to ensure the anaesthesia would not wear off. The electrons of the ECG would be placed around the heart to monitor the heart rate and thereby the ECG itself.

The ECG would be measured on the shark under increasing temperature conditions, as the Greenland sharks have been caught in seawater temperatures ranging from -1.8 to 7 ℃ (Edwards et al. 2019). However, we do not have control over the temperature, and therefore it will heat up naturally. To measure the body temperature of the shark a temperature probe would be inserted into the shark.

Since we have no control over the temperature, we would not be able to perform a control experiment beforehand. This would of course raise a lot of concerns with the results that we would have acquired in doing the measurements.

Hypothetical effect of temperature on Greenland shark

It has been assessed before that the maximum heart rate can be used to determine the Arrhenius breakpoint temperature in anaesthetized fish, which further can be used to determine the optimal temperature (Casselman et al., 2012; Ferreira et al., 2014). It has also been shown that the maximum heart rate and the associated rate transition temperatures can help estimate the upper thermal tolerance of stenothermic as well as eurythermal fish independent of acclimation temperature (Ferreira et al., 2014).

With our experimental setup, we would have expected to see an increase in the heart rate along with an increase in both body and water temperature. As shown from earlier studies (Casselman et al., 2012; Ferreira et al., 2014), we would have expected the maximum heart rate to indicate the Arrhenius breakpoint temperature and thereby show the upper thermal temperature limit for the Greenland shark. As mentioned, this could further have been used to determine the optimal temperature for the shark (Ferreira et al., 2014).

Due to the physiology of the Greenland shark, we would expect it to be a stenothermic species with a narrow temperature range, but this still needs to be determined experimentally. With this expectation, we would have expected to see the maximum heart rate at a very low temperature around 6-8 ℃ based on precious data obtained (Mckenzie, n.d.).

The setup was very down-to-earth and creative, which was necessary at this point, and a lot of human mistakes could have happened such as incorrect dose of anaesthetic, incorrect dose of atropine and isoproterenol, inserting the temperature probe correctly etc. As mentioned also, many uncertainties would also have been relevant due to no control experiment or no regulation of the temperature.

This experiment will hopefully be carried at another time with the same method. It would be sensible to improve the setup and try to incorporate a way to naturally regulate the temperature and have a control experiment as well.

Critical thermal maximum on the shorthorn sculpins *Myoxocephalus Scorpius*

Due to the situation with no Greenland sharks in the preferred size, we instead made an experiment with shorthorn sculpins and their CT-max. CT-max was determined for 14 individuals of the shorthorn sculpin in two separate experiments, divided in two groups with each seven individuals. The shorthorn sculpin was caught in Qeqertarsuaq (Godhavn) at the harbour area with a fishing rod. After the individuals were captured, they were transferred into a big pool where the fast response group then started doing their own experiments, but afterwards they were kept unhandled for more than 12 hours before initiating the experiment. To keep stress levels down, an amount of fucus was added to the pool. The fucus would create a refuge for the fish in the pool, to counteract raised stress levels that could affect the data.

Figure 2: Photo of the experimental setup.
Photo taken by Nanna Hamiltorn

Figure 3: The heating system, where the seawater circulated in and was indirectly heated.
Photos taken by Kathrine Kock Jonsson

Experimental setup

To determine CT-max for the experiment, individuals were placed in black bucket with an air stone and two plastic tubes connected to a pump. This was done to circulate the seawater between the black bucket and into the heating system. The heating system was constructed of a 15L cooking pot

installed with plastic tubes from an old washing machine that was secured and separated with cable ties and wooden drawls that effectively created an indirect water heater. This homemade setup can also be seen in figure 3. The pot was filled with freshwater and placed on a hot plate as the heating source. Lastly, a thermometer was also added to the black bucket to monitor the temperature.

Materials for the experiment were collected from the Arctic Station and collected from local resources, such as the supermarket and the dump.

Experimental method

Seven individuals were transferred into the experimental setup, when the seawater temperature was 10.8 ℃. This is the temperature chosen because the harbour area seawater was determined to be 10.8℃. The temperature was set to increase by 0.33 ℃/min or 1 ℃/3 min. The behaviour of the individual was observed throughout the experiment to notice any unusual behaviour or losing control of its movement, which would lead to lose of its equilibrium. When an individual lost equilibrium, the temperature was noted, and the weight and length of the individual was measured. Afterwards the individual was transferred to a separate bucket with aerated seawater that was 10.8 ℃. After the experiment ended, they were released into the harbour. The experiment was repeated with seven new individuals of the same species. The transfer method of the fish was very important, since exposure to air would cause elevated cortisol levels for hours. So, we made sure to not expose them to the open air when transferring by slowly lowering the container into the water with the specimen and thereby also avoiding any kind of stress factor.

Results

Table 1.A: Temperature, weight and length measured from fish 1-7 in experiment 1. Start temperature was 10,8 ℃, while the end temperature was 25,4 ℃.

FISH	1	2	3	4	5	6	7
Temperature (℃)	21,5	21,9	23,2	23,3	23,6	24,6	25,4
Weight (g)	131	240	123	209	190	141	159
Length (cm)	23,3	27,5	22,0	26,5	25,5	23,0	24,0

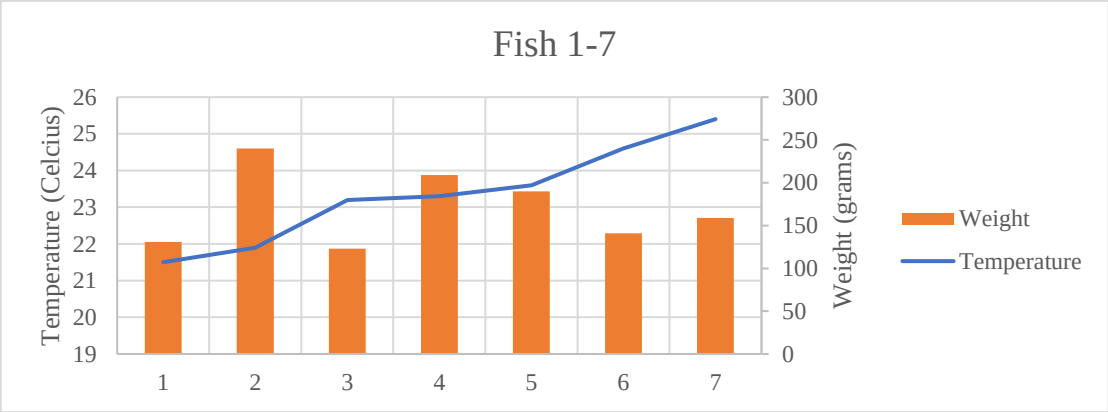


Figure 4: Temperature vs. weight from fish 1-7 in experiment 1. The fish were named by number after their CT-max or the temperature where they lost equilibrium, why fish 7 preserved at the highest temperature (25, 4 ℃) and was the last fish to leave the tub during experiment 1. After experiment 1 had ended, all fish were released into the sea, where they swam straight to the sea bottom. Fish 1-7 is the x-axis, the temperature with the unit ℃ is the y-axis to the left shown as the blue line, while the time with the unit seconds the y-axis to the right right shown as the orange pillars.

Table 1.B: Temperature, weight and length measured from fish 7-14 in experiment 2. The start temperature was 10,8 ℃, while the end temperature was 25,2 ℃. The length of fish 13 was not measured by pure mistake.

FISH	8	9	10	11	12	13	14
Temperature (℃)	23,5	24,4	24,5	24,6	24,6	24,5	25,2
Weight (g)	149	119	160	82	102	95	84
Length (cm)	23,5	22,5	24,0	19,5	23		19,5

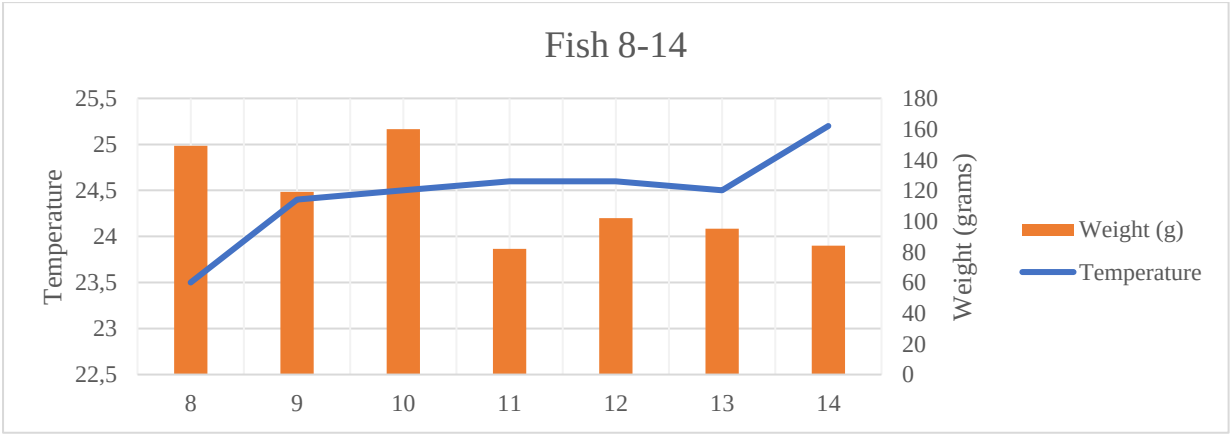


Figure 5: Temperature vs. weight from fish 8-14 in experiment 1. The fish were named by number after their CT-max or the temperature where they lost equilibrium, why fish 14 preserved at the highest temperature (25,2

°C) and was the last fish to leave the tub during experiment 2. After experiment 2 had ended, all fish were released into the sea, where they swam straight to the sea bottom. Fish 8-14 is the x-axis, the temperature with the unit °C is the y-axis to the left shown as the blue line, while the time with the unit seconds the y-axis to the right shown as the orange pillars.

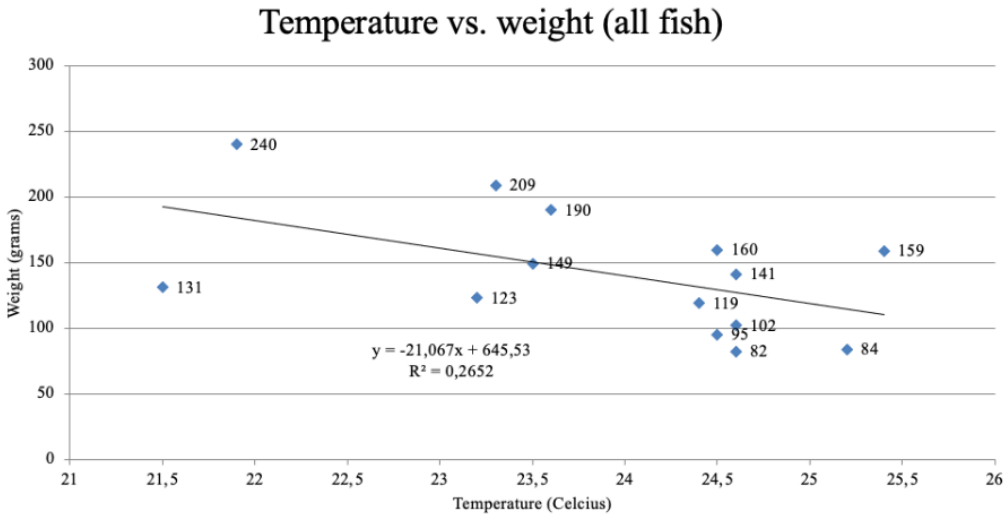


Figure 6: Temperature vs. weight from fish 1-14. Linear regression was performed to investigate the correlation between the maximum temperature and weight, with a R^2 value noted to be 0,2652. Temperature with the unit °C is the x-axis, while the weight with the unit grams is the y-axis.

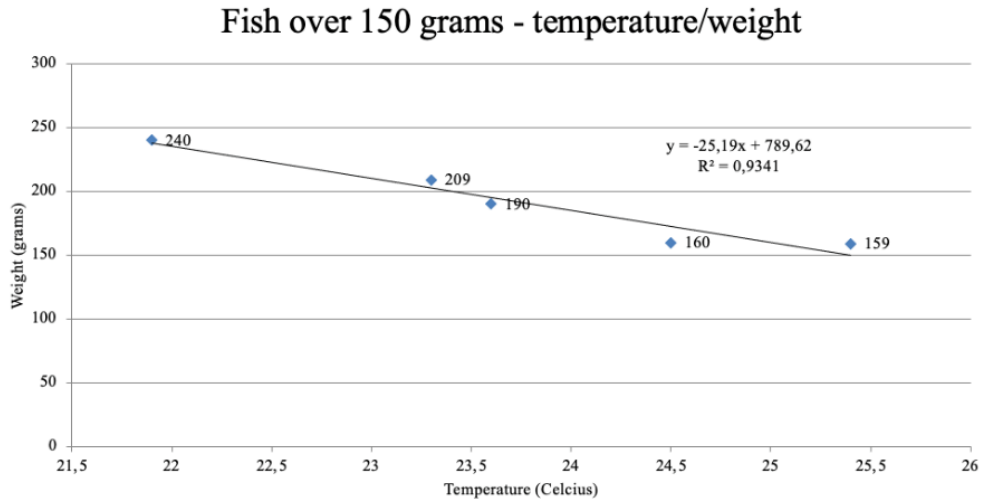


Figure 7: Temperature vs. weight from all fish with a weight over 150 grams. Linear regression was performed to investigate the correlation between the maximum temperature and weight when only fish over 150 grams were considered. The R^2 value was noted to be 0,9341. Temperature with the unit °C is the x-axis, while the weight with the unit grams is the y-axis.

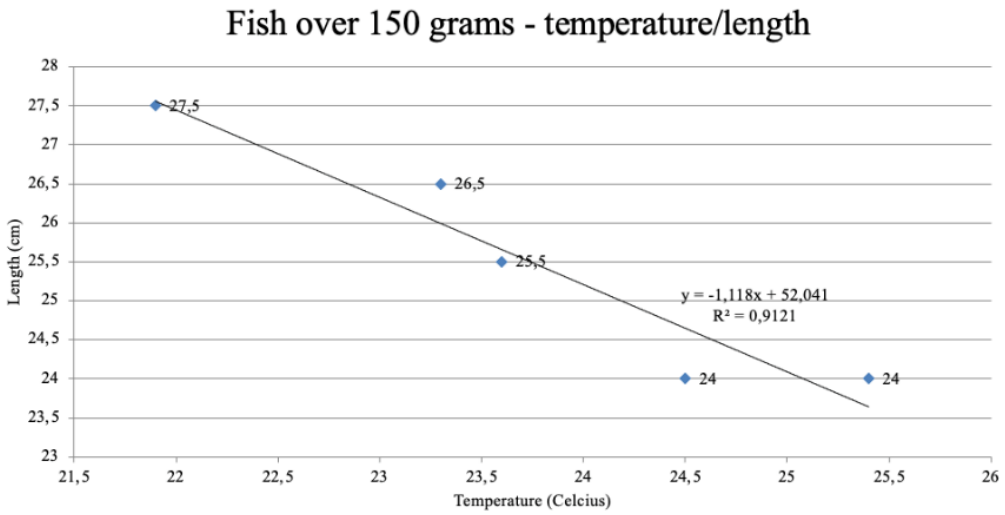


Figure 8: Temperature vs. length from all fish with a weight over 150 grams. Linear regression was performed to investigate the correlation between the maximum temperature and length when only fish over 150 grams were considered. The R^2 value was noted to be 0,9121. Temperature with the unit °C is the x-axis, while the length with the unit centimetres is the y-axis.

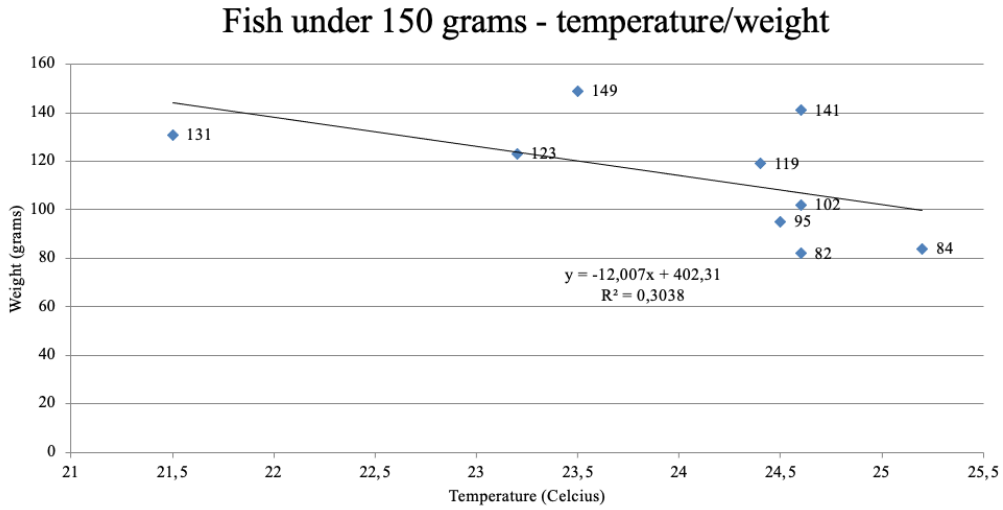


Figure 9: Temperature vs. length from all fish with a weight under 150 grams. Linear regression was performed to investigate the correlation between the maximum temperature and weight when only fish under 150 grams were considered. The R^2 value was noted to be 0,3038. Temperature with the unit °C is the x-axis, while the weight with the unit grams is the y-axis.

Discussion

Effect of temperature on shorthorn sculpin

After finishing experiment 1 and 2, the data was noted in table 1.A and 1.B and the correlation between the temperature, weight and length of the fish was illustrated in figure 4 (experiment 1) and figure 5 (experiment 2). The correlation between the temperature, weight (the length was not included due to missing data on fish 13) of the 14 fish was also illustrated in figure 6 and tested through linear regression that gave a $R^2 = 0.2652$. This R^2-value indicates a low significance between temperature and weight, hence why the data did not seem very promising at first. The results were inconclusive and scattered and showed no significance, in both situations when comparing temperature with either weight or length. When comparing the weight of all 14 fish, illustrated in figure B in the Appendix, a large gap was noticed. So therefore, it was attempted to separate them and found no or very low significance with the fish weighing below 150 g, illustrated in figure 9 with an $R^2 = 0.3038$. But an irrefutable significance between weight and temperature with fish above 150 g was found. This significance is illustrated in figure 7, where a linear regression for weight and temperature has a $R^2 = 0.9341$. This indicates that fish with a weight above 150 g have less ability to adapt to higher temperatures than fish with a weight below 150 g.

In addition, the correlation between the temperature and lengths from the five fish with a weight above 150 g (rough measure with only one decimal) were investigated and showed a similarity in those specific fish as well. This was also tested through linear regression illustrated in figure 8, where a strong significance is shown with a $R^2 = 0.9121$. This could embrace a hypothesis that the fish length as well as weight is some of the defining weakness to temperature adaptation for shorthorn sculpin. This hypothesis can also be supported when comparing the linear regressions shown in figure 7 and figure 9 as the greatest R^2-value, and significance between the maximum temperature and size/weight only can be found for the fish with a weight above 150 g. This indicates that not only does weight matter during the temperature increase in experiment 1 and 2, but also that fish of a greater size were considerably more vulnerable to the warmer temperatures. This would be relevant to examine further.

Eurythermal species and climate change

As an eurythermal fish, the shorthorn sculpin naturally has a great temperature tolerance range, why it, as a species, has a better chance of adapting to the temperature increase in experiment 1 and 2 (Filatova et al., 2019). The average CT-max was calculated to 23.9 °C based on the data from all 14 fish noted in table 1.A and 1.B. When the average CT-max is compared with the start temperature

measured to 10.8 ℃, it illustrates a temperature range of 13.1℃, which indicates a great temperature tolerance. A similar study (Farrell et al., 2013) also did an experiment on shorthorn sculpin at Qeqertarsuaq (Godhavn) during the summer in 2013 and measured the seawater temperatures to a range between 6-8 ℃. This seawater temperature was measured at the shoreline close to the Danish polar marine station, while the seawater temperature of this study was measured in the harbour close to the dock. Due to the warm weather conditions that was experienced during this study the sun could have affected the seawater temperature as the temperature was measured at the water surface. Therefore, the seawater temperature could have been measured to 6-8 ℃ as done in the previous study in 2013, but if the two seawater temperatures are compared, it shows a difference of approximately 2.8 ℃ and signs of a general temperature increase. If this temperature increase were taken into account, the shorthorn sculpins have already acclimated to temperature increase during the last 9 years, which most likely is due to climate change (Farrell et al., 2013). In future studies it would be interesting to do a similar study on shorthorn sculpin over a greater timeline investigating their ability to acclimate to temperature increase. Moreover, an experiment examining the optimal temperature of shorthorn sculpin would also be very relevant in connection to this study.

Experimental difficulties

The experiment had several uncertainties. One of them being human factors as the power to the heating system had to be manually turned on and off to regulate the increasing temperature. Human activity and the fact that the primary part of the experiment was carried out manually also made it difficult to keep the individual fish undisturbed during the experiment. This was due to lack of space at the harbour and lack of walls and other materials shielding the fish from surrounding activity that could be a stress factor. Moreover, four out of 14 sculpins were also used in a fast-escape-response experiment before they were used in this study. Therefore, it is uncertain if the sculpins were still influenced by stress from the previous experiment, which could affect the CT-max of the fish. The four fish included in the fast-escape-response experiment prior to our experiment are noted in Table B in the appendix. Finally, in between the fast-escape-response experiment and the critical thermal maximum experiment, some local children played with the sculpins and mixed up the selected individuals. Therefore, it is uncertain if the sculpins were additionally stressed by the children and how much it might have influenced the data.

It is advisable to improve the setup of this experiment to avoid present uncertainties. It could be improved by shielding the fish better and making sure that they are more undisturbed. It would also be an improvement to make sure not to use the same individuals in two different experiments and thereby avoiding any stress factor from this.

Conclusion

The aim of this study was to investigate increasing temperature influence on the shorthorn sculpins through CT-max experiment on 14 individuals. The data showed that the weight of the individual influenced its ability to cope with the increasing temperature. Individuals over a certain threshold weight could not cope with the increasing temperature, as they lost their equilibrium, and had a lower maximum temperature than individuals at a lower weight. Additionally, the highest CT-max was determined at 25.4 ℃, whereas the lowest temperature was determined to be 21.5 ℃, However, this specific correlation between maximum temperature and weight must be studied further through repetition along with examination of the optimal temperature of shorthorn sculpin.

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Appendix

Table A: The increase in temperature over time. The heating of the seawater in the tub measured over time with a start temperature of 10,8 ℃. This table is correlated to table 2 and illustrates the temperature increase over time during experiment 1. The change in temperature was noted every 3 min after the seawater had been heated up for 15 min. The increase was stable and followed a linear development shown in figure 4, which secured a stable heating process during the experiment. This is apart from one notation mistake after approximately 3-4 min, where 60 sec were notated wrongly and should be replaced with a time between 3-6 min. Table A is illustrated in figure A.

Temperature (℃)	Time (sec)	Time (min)
10,8	0	00.00.00
12,3	42	00.00.42
12,8	180	00.03.00
12,9	60	00.01.00
13,8	380	00.06.20
15,1	600	00.10.00
15,8	900	00.15.00
16,8	1080	00.18.00
17,8	1260	00.21.00
18,8	1440	00.24.00
19,8	1620	00.27.00
20,8	1800	00.30.00
21,8	1980	00.33.00
22,8	2160	00.36.00
23,8	2340	00.39.00
24,8	2520	00.42.00
25,4	2700	00.45.00

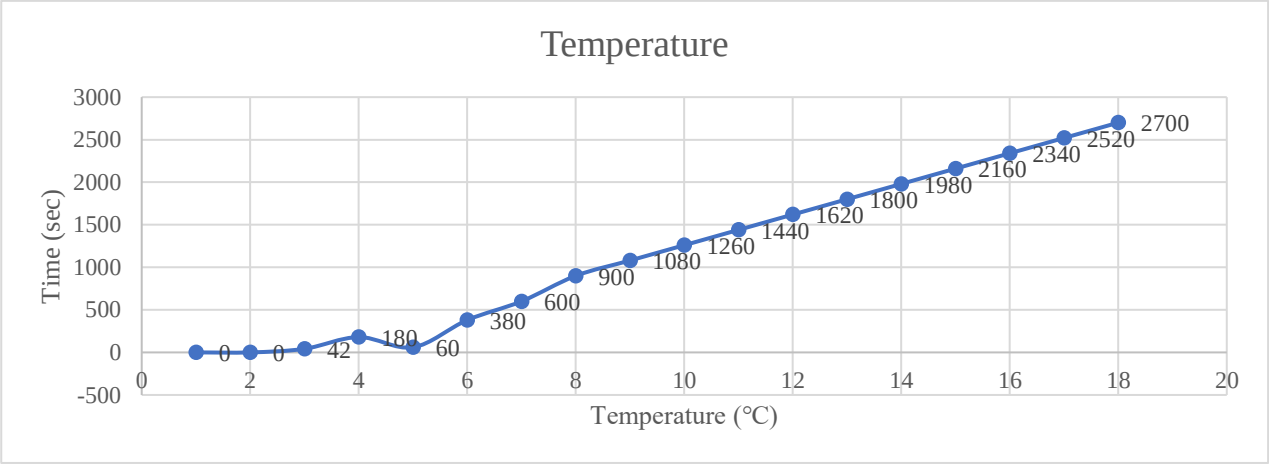

Figure A: The development of the increase in temperature over time. Temperature with the unit °C is the x-axis, while the time with the unit seconds is the y-axis.

Table B: Fish from the "Fast escape response" experiment that matches fish from experiment 1 and experiment 2 (CT-max). 4 of the fish were used in both experiments and are illustrated in this table.

Fast escape response experiment	CT-max experiment (experiment 1 + 2)
2	3
3	1
6	5
7	4

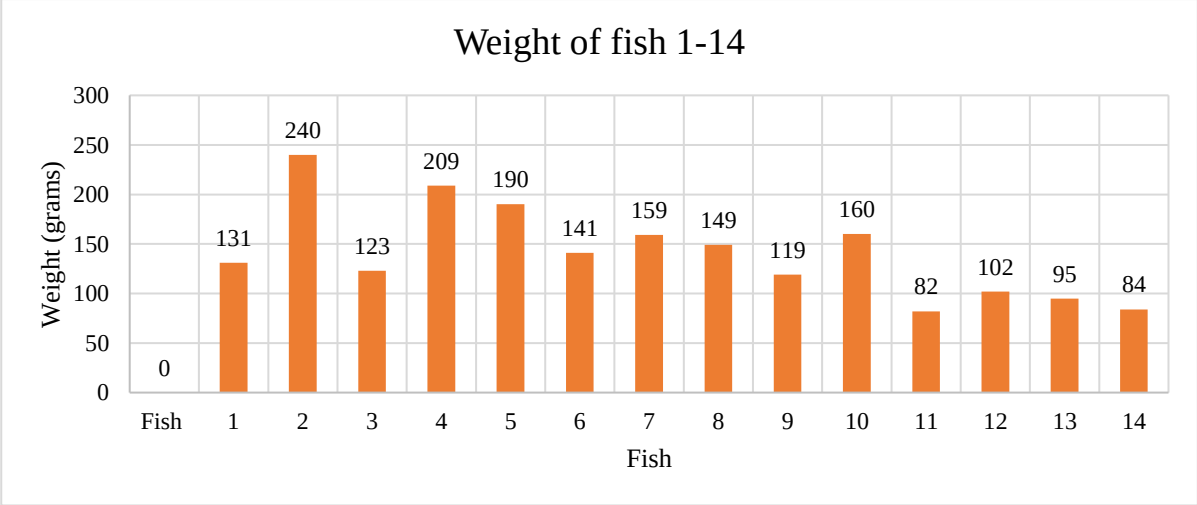

Figure B: The weight of all 14 fish that participated in the experiment. Weight with the unit grams is the x-axis, while the fish is the y-axis.

Arctic biology field course - Diary july 2022

Course A - marine fish physiology

Written by Kathrine Jonsson and Hannah Juul Michaelsen

11th of july

We had all been looking forward to the course and our trip to Greenland when we showed up Monday morning in Kastrup airport. The airport and the check-in at Air-Greenland was packed with people but we all got through and on the plane to Kangerlussuaq airport. We arrived around 09:30 am Greenlandic time and were then divided into two groups to catch a boat to our final destination Qeqertarsuaq. One group of 5 people were going with a plane for Aasiaat and one group of 10 people were going with a plane to Ilulissat, while Paulo would come later. Unfortunately all of the planes were unable to fly due to fog covering the whole west coast and some of the planes were also unable to fly after the fog disappeared due to mechanical issues. Both of our planes got delayed several hours which affected our boat trip to Qeqertarsuaq, so John, Peter and Dave tried to contact the boat crew to make them wait. As that succeeded the situation got worse and both our flights were cancelled which intensified the situation as the possibility of us arriving at Qeqertarsuaq the same night was very small and that created a new problem as there were no hotels available around Kangerlussuaq or in Ilulissat and Aasiaat. After 9 hours the group of 10 people were lucky to get a plane to Ilulissat but the group of 5 was unfortunately unable to get a plane until thursday and stayed behind at Kangerlussuaq. After a lot of arguing with Air Greenland they finally gave the 5 stranded people rooms to sleep in, since they couldn't get them on a flight. The rooms were on the american military base located around 20 minutes walk from the airport, where the food provided by Air Greenland was.

Meanwhile the group of 10 arrived in Ilulissat and John was able to make a deal with a skipper from "Disko line" that could take the group to Qeqertarsuaq with sleeping opportunities at the Arctic field station. The group of 10 arrived at Qeqertarsuaq at midnight.

Hopefully the last 5 people will get on a plane tomorrow!

12th of july

The group of 10 woke up at the Arctic field station after a long night of travelling. In all there are 3 project groups, tracking, ECG and escape-response, but it was only the tracking group that had all members present. John was alone to guide us through the equipment and we had

not got the seal that John had ordered to catch the sharks. Therefore one group went shopping for groceries and dinner, while the other groups unpacked the equipment or went to explore the island and later on all the groups ended up exploring different parts of the island. Luckily John was also able to find three dead seals that hopefully will be used for the long lines tomorrow.

The group of 5 woke to the news that they were forced to check out of their rooms and had to walk back to the airport and get information about their new situation regarding their plane and a new hotel, which all had to be provided by Air Greenland. It was a great struggle since a lot of other people had been trapped under the same conditions in Kangerlussuaq and the group of 5 is unfortunately still at the end of the line when the plane tickets are distributed. The time was used to pressure Air Greenland for earlier flight tickets before Thursday to either Ilulissat or Aasiaat, playing games, and trying to get food from the cafeteria along with 100 other people. There was no free wifi at the airport either, so it was hard to keep contact with the remaining 10 people at the Arctic Station. Around 16 o'clock there were no more flights from or to the airport, so the 5 stranded people finally got a real hotel room at the airport hotel. Finally some good things are happening for the stranded people.
Fingers are crossed for a plane for the stranded and for the sail tomorrow.

13th of july

Today was a great day as the stranded group of 5 finally got a plane for Ilulissat and a boat for Qeqertarsuaq, so they arrived around 13:30 at the Artic Field station. At the same time the remaining 10 people went to the harbour for their first trip on Porsild. The purpose of the trip was to test the satellite tag equipment for the tracking group and place the frame/camera for the escape-response group and the long-lines won't be set before tomorrow. The ECG group had tried to set up their pool at the harbour earlier that day, but had to continue with it tomorrow when all the members and the assigned teacher were there to help. It was a nice and successful trip where all groups tried out their equipment, saw even more of the beautiful nature and got to go on Porsild and meet the crew. John also got another seal for the long lines from a local hunter. When the boat got back, everybody was happy to be reunited with the last 5 people and their arrival was celebrated at dinner with snow crabs.
Tomorrow is finally going to be the first day, where all students have arrived and ready for adventures and experiences.

14th of july

Today, the filming group and tracking group went out on Porsild with Dave and John to set the long lines. The lines were baited with the fresh seal received from the passing hunter yesterday and three lines were set out in total with 20 hooks on each. The camera frame was recovered to collect the first camera that was set out.

The ECG group stayed behind and went to the harbour to set up the pool for the shark. 2 water pumps were fastened on the side dock and connected to tubes that led the water into the pool. Several measurements of the water level and temperature were taken to prepare for the shark that we hopefully will catch tomorrow.

Afterwards, it was just relaxation and having fun at the Arctic station. Some used the time to prepare for tomorrow, some went hiking and some used the time to explore the town a little bit more.

We are all just looking forward to tomorrow, where there hopefully will be a shark or more.

15th of july

Another unlucky day. The weather today was way too windy to go sailing, so everybody had to stay home. We got the final update around 13, where the trip for today was officially cancelled by the captain. We all looked at it positively and went hiking instead. 3 groups went on 3 different hiking routes, where one group went hiking to a nearby waterfall and the red hut on the same route. The 2 other groups started together to go up the mountain behind the Arctic station. 1 group of 4 had to turn around halfway up due to being astray from the path, so they turned around and got back safely. The last group of 4 went all the way to the top of the mountain and visited both a weather station and some glaciers up there.

While the last group was on the mountain, everyone else had got back and started worrying about the others, because suddenly the weather got very foggy and dangerous to hike in. Fortunately they all came back safely and unharmed.

That evening the group that was the assigned food team had made Drømmekage (danish cake), so we were all ready to go with the boat tomorrow.

It is going to be very interesting to check the longlines, because normally they are not out for more than 24 hours and these will have been out for 36 hours, when we will collect them, but let's see. We hope for the best!

16th of july

AMAZING DAY!

The weather was better, so the boat trip was good to go. We all got ready and set off to collect the longlines. After about 45 minutes of sailing, we recovered the camera frame to collect the camera that was set out and set another one out. Afterwards the first longline was reached and surprisingly there were 2 wolffish on it and A SHARK! A massive one! It was so amazing and beautiful. It was an unbelievable experience and all the students were so stunned and amazed.

The shark was too big to bring back to the pool, so it was decided to tag it, but unfortunately it got unhooked and escaped.

With that amazing and surprising experience, we headed for the second longline and here we were even more lucky. 2 WHOLE SHARKS! It was unbelievable! 2 big, alive sharks were on this longline.

Both were too big for the pool, so John wanted to tag them, but one of them got unhooked. The other one managed to get tagged and sent off to the deep again.

The expectations were high, when we went for the last longline, but this one only had a dead shark on it (a shark head). The rest had been eaten by others and that is one of the big problems by leaving the longlines out for too long.

John still got some samples of the brain from the shark head and then the tracking group made an experiment and test for an hour, before we headed back.

Absolute stunning and amazing experience! 5 sharks in total with 2 dead and 3 alive.

Besides the sharks, we also caught 3 wolffish, which we ate for dinner the next 2 days. Very delicious!

All 3 longlines were set again and tomorrow we are going again in the hope of finding more sharks and maybe even a smaller one that fits in the pool.

17th of july

Luckily another day of sailing! Since there is only room for 12 passengers in the boat, 4 people stayed behind, while the rest went out to collect the longlines and hopefully get some sharks.

At the same time the 4 people staying behind had sunday-mood on and took a well-deserved rest day. Some went bird-watching, some stayed home and did some schoolwork and others just relaxed. Around 2 pm the boat came in, but only with bad news. No sharks or anything else on the longline, so they had put out the longlines again with new bait. Unfortunately we didn’t get a seal for bate yesterday as hoped, so we used some leftovers from the wolffish

caught yesterday and also some heads of cod. Hopefully this shift in bait won't affect our possibilities of catching.

The rest of the day was used on some small hikes in the area, some schoolwork or movies in the TV-room. This evening we ate the caught wolffish as fish soup, which was delicious. It is very interesting and exciting to eat locally and own caught food. It makes the experience of this stay more fulfilled in some way.

Tomorrow the longlines are hopefully going to be checked for some sharks, especially small sharks. The ECG-group and the fast escape response group are running out of time, so fingers crossed!

18th of july

Another unlucky day... Just before we all were leaving for the boat, Morten (the station manager) announced that the crewmen of the ship were sick and the boat could not go out today. John was still optimistic, since he knew another skipper, but after getting hold of him he wouldn't go out without his crew and the boat therefore stayed at the dock.

The ECG-group and the fast escape response group had made a backup experiment yesterday evening if something like this would happen. The backup plan was to make experiments with sculpins instead, so today was all about catching and fishing for sculpins down at the dock, while the setup was changed to the new back-up experiments. The fast escape response group was still going to measure fast escape response for the sculpins, but under the influence of warming of the water. The ECG-group was changed to make an experiment about the CT-max of the sculpins.

Due to the scarcity of equipment, the new setup was very creative and innovative with the use of a lot of material from the dumpster at the dock and some equipment from the kitchen at the Arctic station. After a while the setup was done and it worked!

Around 20 sculpins in different sizes were caught and put in the pool, where they stayed until the start of the experiment. The fast escape response experiment had to be done first since some of the fish could potentially die of the CT-max.

While this was ongoing, the tracking group went exploring around town and also went on some hikes nearby. Later in the afternoon some from the other groups also went hiking out to the whale-spot place. No whales, but a beautiful view!

After a dinner with more of the caught wolffish there was time to relax and play games, especially the game Sequence. This game was played a lot up here.

For tomorrow the backup experiments are ready in case that the boat is still not sailing tomorrow, but we hope!

19th of july

Still no sailing. All crew members were still sick, so the boat trip was cancelled again for today. The back-up experiments were then initiated with the fast escape response experiment first. It took some time to find the right routine of the experiment and there was only limited space at the setup, so a shift plan was made between the groups.

Since the fast escape response experiment took a very long time to perform for each individual, we only managed to test 5 fish in total. Depending on the plan for tomorrow, we may be able to test some more tomorrow and also to do the CT-max experiment as well.

While the experiment was ongoing, the rest of the people went for hikes, relaxed at home or did some schoolwork.

After another game-night with Sequence everybody went to bed in hope of getting one last boat trip, since tomorrow is the last day to go out before we have to go home.

20th of july

Last day at Qeqertarsuaq began with the delayed, but lovely news that we could sail today! The crew was finally all feeling better and ready to take us all out. Since it was our last day here, we were only going to retrieve the longlines and not set any new ones out. There wasn't anything on any of the longlines, which could be due to the time the longlines have been out (more than 76 hours). Some of the bait was still on, so maybe there is a preference in bait afterall?

After the retrieving of the longlines the tracking group did a measurement before we went back again. Although there weren't any sharks on the longlines, it was still nice to get a last trip on the boat and experience the amazing nature of the sea.

After the boat got back, the ECG-group and some from the fast-escape response group went down to the dock again and continued the back-up experiments with the CT-max.

Meanwhile John had arranged a local football match with the local senior football team, so the remaining students and teachers went down and participated in the match (teachers doing the cheerleading of course, while the students did the hard work).

Afterwards we all had a splendid and truly amazing dinner with snow crab as starter and greenlandic halibut and whale steaks as main course. It was a perfect way to end a chaotic, but very learning and interesting field trip to Qeqertarsuaq.

Tomorrow is travelday and the first team of 12 will be going already at 6 am with the ferry, while the last 4 people will be going at 17 pm with the ferry.
We hope that everything will go smoothly with the ferries.

21th of july

The first team of 12 got safely to Ilulissat and since they had the whole day there, they all went on a hike together to the Ice Fjord and further around the city until they could check in at the hotel. At the same time they were also looking forward to an arranged dinner at the restaurant of the hotel, which was said to be very delicious.
The last day at the station for the remaining 4 students went by with a visit to the museum, a movie and a last trip to the supermarket for lunch and an ice cream. When it was time for us to get on the boat, another traveller told us that the small boat couldn't sail, so we were all put on a bigger boat that would make the crossing in 6 hours instead of 2. It was a very choppy 6 hours but the farther into the fjord we got the calmer the waves. So the last 1½ hours through the icebergs were very pleasant. We first arrived at 22:30 (thereabouts) so we missed the nice dinner with a view of the icefjord that night. But a visit to the brasserie and a hike to the isefjord viewpoint were still on for the night owls.
After the fancy dinner and the hike for the night owls everybody went to their hotel rooms to pack the last things and get ready for the big travel-day tomorrow.

22th of july

Travel-day! The day where we all go home, hopefully without any complications. Once again we were split in 2 groups with the earlier stranded people in one and John with 9 students in the other group. The first group flew to Kangerlussuaq directly as the first plane from Ilulissat, while the bigger group had to fly to Assiat to middle land before going to Kangerlussuaq. Luckily both groups ended without any complications in Kangerlussuaq and all boarded on the same plane to Copenhagen. After 4 hours and 30 minutes we were finally in Copenhagen again, where we parted ways at the baggage claim.
It has been some amazing and most unbelievable days, and it is almost certain that some of the students will be returning somehow.