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PigghåFRI – Hvordan unngå problemer med pigghå?

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Authors: Pradeep Lal, Antonie Oosterkamp, Mette Espedal Brynildsrud, I-Hao Chen, Naouel Gharbi, Helena Hauss, Robert Lennox, Lotte Svengård Dahlmo, Alan Le Tressoler, Christian Andreas Hansen, Marius Nilsen, Marius Takvam, Neda Gilannejad, Valentina Tronci, Mary-Scarlett Sharp, Simon Menanteau-Ledouble, Patrik Tang

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Correspondence	Pradeep Lal (plal@norceresearch.no) Antonie Oosterkamp (anoo@norceresearch.no)
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Summary	This study has mapped the impact of interaction of spiny dogfish (*Squalus acanthias*) with aquaculture installations in Norway and studied the behavior of spiny dogfish, in laboratory and field studies, in response to sensory cues for possible development of an anti-shark measure for fish farms. Electromagnetic pulse and smell of dead conspecific (skin extract) induce aversive response without inducing chronic stress in spiny dogfish; orca sound has no effect as repellent against spiny dogfish behaviour. This suggests a promising approach in development of technology based on electromagnetic pulse stimulation and aversive chemical cues to keep spiny dogfish away from fish farms.

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1. Summary

English

The spiny dogfish (*Squalus acanthias)* is one of the common shark species found in Norwegian coasts and fjords. Unfortunately, their interaction with aquaculture installation is a financial, welfare and ecological challenge. The fish farmers, predominantly at locations in Southern and Western Norway, report that during the autumn and winter season, spiny dogfish bite through the nets and get in the fish cages. The holes in the cages result in escape of farmed fish. This is both a financial loss and an ecological challenge. The dogfish are usually attracted to dead fish found at the bottom of the cages. Once inside the cage, they also eat and harm the live farmed fish, forming a severe welfare challenge. To prevent this, farmers continuously remove dead fish and must constantly inspect the cages for holes with the help of divers and underwater cameras. To date there is no effective method to prevent spiny dogfish incidents in fish farms. Hence, it is crucial to test and develop active and passive methods to prevent these spiny dogfish incidents in fish farms. In the project we tested active measures that interact with the dogfish sensory system in both laboratory and field trials.

For laboratory trials, spiny dogfish of size 60-85cm were caught and housed in laboratory aquaria (flow through) of size 2m diameter and 85cm water height. These were supplied with sea water at 9 °C; the light intensity was 10 lux at 10cm above the water surface. The experimental tank was equipped with a custom-made low light camera. Electromagnetic (EM) pulses, sound of orca (natural predator) and skin extract from conspecific were used as deterrent stimuli; an extract from mackerel was prepared and used as an attractive stimulus. The animal behavior was recorded prior and after application of stimuli and analyzed for change in locomotive behavior. The spiny dogfish showed change in locomotive behavior (increased/decreased speed) in response to EM, skin extract and food stimuli; however, they showed no change in response to orca sounds. Food stimulus or smell of dead mackerel induced food-seeking behavior- sharks were found probing the odor inlet. Both EM and the conspecific skin extract induced avoidance response- sharks moved away from the source area. Field trials with Orca sound and EM pulses showed comparable results; Orca sound had no effect on the presence of spiny dogfish around the experimental rig while EM showed effect as repellent for spiny dogfish.

The effect of EM on salmon smolt (200-300g) were tested in similar condition. Stress markers such as cortisol, plasma metabolites and stress gene markers were analyzed for both short and long-term stress response. EM pulses caused increased glucose and lactate levels and cortisol (although non-significant) on 0- and 1- day after the treatment; however, there was no change in stress related gene expression. Furthermore, there was no difference in the levels of the analyzed markers (plasma metabolites and gene expression) 2-3 weeks after the treatment. In addition, growth parameters were similar across the treatment and control groups. This suggests that EM treatment had no long-term stress response in salmon smolt.

In sum, we find that both EM and chemicals in skin extract could be used as effective shark deterrent. Both EM and aversive skin extract are effective in inducing flight response under controlled condition. Field trials showed that EM is an effective repellent in natural environment. EM treatment did not show any stimulus induced stress response in sharks and or long-term stress

response in Atlantic salmon smolt. Olfaction/chemical repellent needs further research and development for implementation in aquaculture installations. Further studies are needed to optimize the stimulus conditions for EM against spiny dogfish of varied size and physiological conditions (eg. hunger). The limitation of such method must be assessed by monitoring their long-term effectiveness at an affected fish farm.

Norsk

Pigghå (*Squalus acanthias)* er en av de mest utbredte haiartene i Norge og finnes nær kysten og i fjorder. Pigghåens samhandling med akvakulturanlegg er imidlertid en økonomisk, velferdsmessig og økologisk utfordring. Oppdrettere, for det meste på Sør- og Vestlandet, rapporterer at pigghåen i høst- og vintersesongen biter gjennom nøter og kommer seg inn i merdene. Hullene i nøtene resulterer ofte i rømming av oppdrettsfisk som fører til økonomiske tap og økologiske utfordringer. Pigghå tiltrekkes vanligvis av død fisk som finnes i bunnen av merdene. Engang inn i merdene angriper og spiser pigghå også levende oppdrettsfisk, noe som skaper utfordringer ved fiskevelferd. For å forhindre dette fjerner oppdrettere fortløpende død fisk og kontinuerlig inspiserer merdene for hull ved hjelp av dykkere og undervannskameraer. Til dags dato finnes det ingen effektiv metode for å forhindre isse uønskede hendelser med pigghå i oppdrettsanlegg. Derfor er det avgjørende å teste og utvikle aktive og passive metoder for å forhindre pigghåhendelser i oppdrettsanlegg. I dette prosjektet har vi testet aktive tiltak som påvirker pigghåens sensoriske system i både laboratorie- og feltforsøk.

I laboratorieforsøkene ble pigghå i størrelse 60-85 cm fanget og plassert i laboratorieakvarier (gjennomstrømning) med 2 m diameter og 85 cm vannhøyde. Sjøvannstemperaturen var på 9 °C og lysintensiteten 10 lux ved 10 cm over vannoverflaten. Akvariet brukt i eksperimentene var utstyrt med et spesiallaget lys sensitiv kamera. Pulserende elektromagnetiske felt, lyden av spekkhoggere (naturlig rovdyr) og hudekstrakt fra pigghå ble brukt som avskrekkende stimuli; et ekstrakt fra makrell ble tilberedt og brukt som en attraktiv stimulans. Pigghåens atferd registreres før og etter påføring av stimuli og analyseres for endring i lokomotivatferd. Pigghå viste endring i lokomotivatferd (økt/redusert hastighet) som respons på EM, hudekstrakt og matstimuli; den viste imidlertid ingen endring som respons på lyden av spekkhoggere. Matstimulus eller lukt av død makrell induserte matsøkende atferd – blant annet hos pigghå individer som undersøkte luktinntaket. Både EM og hudekstrakt induserte en unngåelsesrespons - pigghå beveget seg bort fra EM kilden. I Feltforsøk med spekkhogger-lyd og EM-pulser ble det oppnådd lignende resultater; spekkhogger-lyd hadde ingen effekt på tilstedeværelsen av pigghå rundt riggen, mens EM var effektiv i å skremme bort pigghå.
Effekten EM pulsene har på laksesmolt (200-300g) ble testet under lignede forhold. Stressmarkører som kortisol, plasmametabolitter og stressgenmarkører ble analysert for å kartlegge kort og langvarig stressrespons. EM-pulsene forårsaket økte glukose- og laktatnivåer og kortisol (selv om det ikke var signifikant) på 0- og 1-dagers behandling; Det var imidlertid ingen endring i stressrelatert genuttrykk. Videre var det ingen forskjell i nivåer av analyserte markører 2-3 uker etter behandlingen. Dessuten var vekstparametere like på tvers av behandlings- og kontrollgruppene. Dette tyder på at EM-behandling ikke resulterte i en langvarig stressrespons av laksesmolten.

Oppsummert viser resultatene av forsøkene at både EM pulser og hudekstrakt kan brukes som et effektivt haiskremmende tiltakk. Både EM og aversiv hudekstrakt er effektive for å indusere fluktrespons under kontrollerte betingelser. Feltforsøkene viste at EM er et effektivt skremmende tiltak i naturlige omgivelser. EM-behandling forårsaket hverken stimulusindusert stressrespons hos pigghå eller langvarig stressrespons hos atlantisk laksesmolt. De virksomme lukter/kjemikalier trenger videre forskning og utvikling for implementering i akvakulturanlegg. Ytterligere studier er nødvendig for å optimalisere stimulusforholdene for EM mot pigghå av ulik størrelse og fysiologiske forhold (sult). Begrensningen av en slik metode må vurderes ved å overvåke effektiviteten i et berørt oppdrettsanlegg.

2. Introduction

Fish farmers in Norway have reported incidents where large numbers of spiny dogfish bite holes in the nets and get into the cages. Several salmon farms at the Norwegian coast, and especially in Vestland and Rogaland municipalities, face major challenges related to such attacks. The attacks contribute to the escape of fish and incurrence of significant expense to repair the resulting holes and associated damage to cages. The spiny dogfish are attracted by the dead fish at the bottom of the cage, but in addition eat and harm the live salmon. Therefore, this is both a financial and a fish welfare challenge. The farmers have initiated measures to prevent the spiny dogfish from entering the net cages. They remove dead fish almost continuously and constantly inspect the nets with the help of divers to check for holes. To date, no satisfactory methods, techniques, or devices have been identified that reliably can keep dogfish away and out of the fish cages. Shark barriers and deterrents have been developed against specific species of sharks. Hart and Collin (2015) and Espedal (2023) provide an overview of shark repellents; these have been used to keep sharks away from bathing areas and to offer personal protection for swimmers, divers and surfers. Some of these have also been tested to keep sharks away from bait and to avoid bycatch in line and net fishing. However, their effectiveness varies depending on the species and geographical area; none of the measures provide a full deterrence.

PigghåFRI is a contribution to understanding the impact and behavior of spiny dogfish in relation to aquaculture in Norway. It is crucial to map both the experience of fish farmers against spiny dogfish and the past research. Behavior of these sharks in relation to aquaculture needed to be understood so that an effective deterrent can be developed against these sharks without harming them. Furthermore, these anti-shark measures should have no or little impact on farmed fish. PigghåFRI has employed an interdisciplinary research approach (qualitative research methods, animal husbandry, quantitative behavioral neuroscience, endocrinology, machine learning, marine biology and ecology) together with stakeholder involvement (service provider and user group) to address this.

The project is coordinated by NORCE and is led by Senior researcher Pradeep Lal. The project group consisted of Antonie Oosterkamp (Researcher II) and Shaw Bamber (Researcher II). Participants were Mette Espedal Brynildsrud (MSc student NORCE/UiB), I-Hao Chen (PhD Candidate), Naouel Gharbi (Researcher II, Research leader), Neda Gilannejad (Researcher II), Marius Nilsen (Research Assistant), Marius Takvam (Research assistant), Simon Menanteau-Ledouble (Researcher II), Helena Hauss (Research leader, Researcher II), Alan Le Tressoler (Senior Engineer), Christian Andreas Hansen (Senior Engineer), Valentina Tronci (Senior Engineer), Robert Lennox (Researcher I), Lotte Svengård Dahlmo (PhD Candidate), Patrik Tang (Researcher III), and Mary-Scarlett Sharp (Undergraduate Intern student at NORCE). We thank Tom Ole Nilsen (Associate professor, UiB/ Researcher I, NORCE) Frank Midtøy (Senior engineer, UiB) and Heikki Juhani Savolainen (Senior engineer, UiB) for valuable their advice and help with the animal experiments, and Atlanterhavsparken, Ålesund for useful advice on animal husbandry.

The laboratory trials with spiny dogfish were conducted at the University of Bergen, Department of Biological Sciences and the trials with Atlantic salmon were conducted at the Marine Research Centre at Mekjarvik, NORCE. All analysis, except for cortisol measurement, were conducted at

NORCE. Cortisol measurement was performed by Prof. Marnix Gorrisen at Radboud University, Netherland.

The project was financed by FHF, and the reference group in the project consisted of Eirik Matre (Kobbevik og Furuhomen Oppdrett AS-Biologisk sjef), Michael Niesar (Sulefisk AS – Daglig leder), and Robert Aakvik (Emilsen fisk AS – Produksjonsleder sjø).

3. Objectives

The affected fish farms have initiated measures to prevent the spiny dogfish from entering the net; dead fish are regularly removed, and the net are inspected with the help of divers for holes. So far, it has been a challenge to find reliable methods, techniques or devices that keep these predators away from the fish cages. Often the same localities are exposed to incidents over many years. Spiny dogfish breed in Norway during the winter months and the incidents with fish cages usually occur in the period November-March. The spiny dogfish occurs in large shoals and helps itself to dead salmon or wrasse lying in and at the bottom of the net cages, often in connection with delousing of salmonids, as after such treatment high mortality occurs. If there is a strong current in the sea during such treatment, the dead fish is pushed up from the double net at the bottom of the cage. It is thus more accessible to predatory fish from the outside. As the spiny dogfish hunt in flocks, an attack can cause significant damage to the cage. The incidence of such attacks has been far more frequent in the last three years than was previously common, which may indicate that the population of spiny dogfish has increased in Norwegian fjords.

The project has great significance for the aquaculture industry. To illustrate the financial consequences, one of the incidents in 2020 in Western Norway led to the escape of 1,500 salmon of size 1.5 kg after eight spiny dogfish had made it through net into the cage; this corresponds to a financial loss in the order of 100-200 kNOK. With up to 200,000 salmon in a cage, the financial consequences are in the multimillion kroner when the incident is discovered too late. In this study, the affected farms report a normal cost of about 500 kNOK per season associated with repair of damaged nets, surveillance for dogfish, handling dogfish after an incident and loss due to escapees.

Reducing or eliminating spiny dogfish attacks at aquaculture installations leads to a reduced number of holes in the cages and resulting salmon escape incidents. There are direct economic benefits for the industry related to reducing the number of cases of holes in the cages, both through reduced repair costs and reduced salmon escape. Escaped farmed salmon form also a threat to the wild salmon population. Reducing the risk of escape thus also has a positive effect on both the industry's reputation and the health of the wild salmon population. The spiny dogfish has been a red-listed species until 2023; with signs of improving stock size, the Norwegian government has recently again allowed fishing of spiny dogfish. Nonetheless, considering that spiny dogfish is a slow growing species, their stock size is under pressure. Keeping spiny dogfish away from breeding facilities will reduce mortality by preventing them from getting stuck in the nets. The results of the project can also provide useful lessons in general about how attacks by shark species can be avoided.

The overall goal of this project was to document and develop measures to avoid problems with spiny dogfish at fish farms (**main objective**). This project also set out to create a knowledge base on the behaviour of spiny dogfish in relation to aquaculture installations (**subgoal 1**) and to document the measures against spiny dogfish attacks and experience gained from stakeholders (**subgoal 2**). Through a combination of literature search, surveys and interviews of experts and stakeholders, the project has fulfilled in providing a knowledge base on spiny dogfish interaction with aquaculture and possible counter measures (Professional end report to FHF and master's thesis, Espedal, ME, 2023. UiB). The results are openly accessible.

Based on the findings for subgoal 1 and 2, it also carried out testing and documentation of shark deterrents that are considered effective without harming the spiny dogfish (**subgoal 3**). Based on the knowledge gained, a range of candidate anti-shark measures, both active (auditory/Orca sound, olfactory/aversive smell, electromagnetic) and passive (anti-shark nets) were selected for laboratory and field trials. **Laboratory trials** provided crucial and novel behavioural data necessary for field trial design, and for the future development of anti-shark measures. It showed that both aversive smell and electromagnetic pulses are successful in inducing aversive response in spiny dogfish, however, no significant behavioural changes were associated with exposure to orca sound. Laboratory trials also aimed to test the learning ability of spiny dogfish. Unfortunately, this could not be implemented fully; major resources were utilized in establishing the husbandry conditions (as it has not been documented before) and analysis of basic behavioural and endocrine response were prioritized. Our husbandry method achieved near 100% success in maintaining good health and welfare of wild caught spiny dogfish. These findings are also crucial for future research and development of anti-shark measures.

Field trials were consistent with laboratory experiments. Trials with EM based deterrents showed its effectiveness against spiny dogfish. However, absence of spiny dogfish in the fjords during several of the field trials affected the statistical significance of the test result of EM based deterrent; several successful field trials were necessary for obtaining field data valid for diverse groups (sex, size, age, hunger status etc.) of spiny dogfish. Field trials for passive deterrent (net type) could not be conducted as there was absence of spiny dogfish around the test area.

One of the **extended goals** was to test whether the measures have an **impact on the farmed fish** production. Hence, PigghåFRI also carried out testing the effect of shark deterrent on Atlantic salmon. The electromagnetic pulse method was selected for the trial with salmonid as EM based deterrents showed positive outcome and is most market ready.

The PigghåFRI project was originally planned to be implemented between September 2021-September 2023. However, there were several implementation challenges (e.g. required approval for animal experiment-FOTs application, challenges with husbandry, establishing experimental system for a new species not commonly used in laboratory experiment, weather conditions for the field trials and non-sighting/absence of spiny dogfish during the field trials were some of the major challenges) and hence the project period needed to be extended.

In summary, the planned tasks in the project were modified based on the research outcomes, advice from researchers and input from the reference group and availability of spiny dogfish; the project timeline was extended as needed to achieve the objectives. Thus, the project has successfully fulfilled the main objectives with only minor deviations.

4. Project Implementation

4.1. Knowledge base on spiny dogfish behaviour and industry's experience (AP1)

To document the existing methods to deter spiny dogfish from sea-based fish farms, we aimed to first gather the current knowledge on behaviour and habitat of spiny dogfish and the experience of fish farmers with spiny dogfish in Norway. To achieve this, we conducted a literature review, surveys and interviews of national and international experts and fish farmers.

4.1.1. Literature study

Relevant literature about Spiny dogfish (*Squalus acanthias*) and anti-shark measures was retrieved using the databases of ScienceDirect, PubMed and Google Scholar. The search keywords included several combinations of the terms: Spiny dogfish AND/OR Sensory physiology AND/OR Avoidance response AND/OR Shark repellent AND/OR shark proof AND/OR Aquaculture AND/OR Electromagnetic pulse AND/OR Acoustic deterrent AND/OR olfactory deterrent AND/OR visual deterrent. Wildcards had been added where applicable. Inclusion criteria were language (English or Norwegian) and peer reviewed. We considered the first 50 upcoming articles fulfilling the criteria (for the cases the respective search output had that many articles).

4.1.2. Survey and interview national and international experts and fish farmers

Subjects, questionnaire, and procedures: Initial knowledge on possible cause and impact of spiny dogfish was collected from published literature, newspaper and informal communication with fish famers. We applied a qualitative study design combining KIQ (key informant questionnaire interview) and FGD (focused group discussion) (**Appendix 1**). The study was conducted between November 2021 and June 2022 in Norway. Fish farmers, production manager and operation managers, across 9 production areas in Norway were invited to participate through personal emails and through aquaculture cluster organizations. Fish farm of a company located in different production areas are considered as separate organizations. A total of 34 fish farms participated in the study. Participants completed an electronic questionnaire. Participants were also given an option for further contact. A subset of such fish farmers were contacted for further discussion. Interviews were conducted via Teams.

Analysis and interpretation: The study aimed to reach a nuanced and comprehensive exploration of perspectives on an ecological and industrial challenges rather than to compare differences from different study participants. The answers to the questionnaire were designed as categorized answers with the possibility to add additional information. The data is visualized in frequency diagrams. We used an empirical data driven and inductive approach. The response to questionnaire and interviews of fish farmers were discussed with the project team and verified by matching the comments in the questionnaire and notes from the interview.

4.2. Behavioural and physiological response of spiny dogfish to promising shark deterrents in lab (AP3)

The laboratory trials aim to identify sensory cues that are physiologically aversive to spiny dogfish without harming them. Here, we tested orca sounds (natural predator of spiny dogfish), skin extract of conspecific (danger signal) and electromagnetic pulses (saturating electro sensing). In addition, food cue (smell from dead mackerel) is used as attractive control cue.

4.2.1. Authorization

The capture, handling, and all experimental procedures with spiny dogfish were approved by the Norwegian Food Safety Authority (FOTS ID #29768). The research was conducted according to laws and regulations established by the European Union (Directive 2010/63/EU).

4.2.2. Catching spiny dogfish

The sharks were caught on five separate trips in Herdlafjorden (figure). A 35 ft. Westcruiser equipped with a 1000L ISB tank, filled with seawater pumped from 6m depth at the location, was used for temporary housing and transporting the captured sharks. The salinity and temperature in the tank were measured with a Xylem's WTW Cond 3110 conductivity meter (Xylem, Germany). Measured salinity and temperature at the location were 21-30 ppm and 7-9 °C. The spiny dogfish were caught between November 2022 and May 2023.

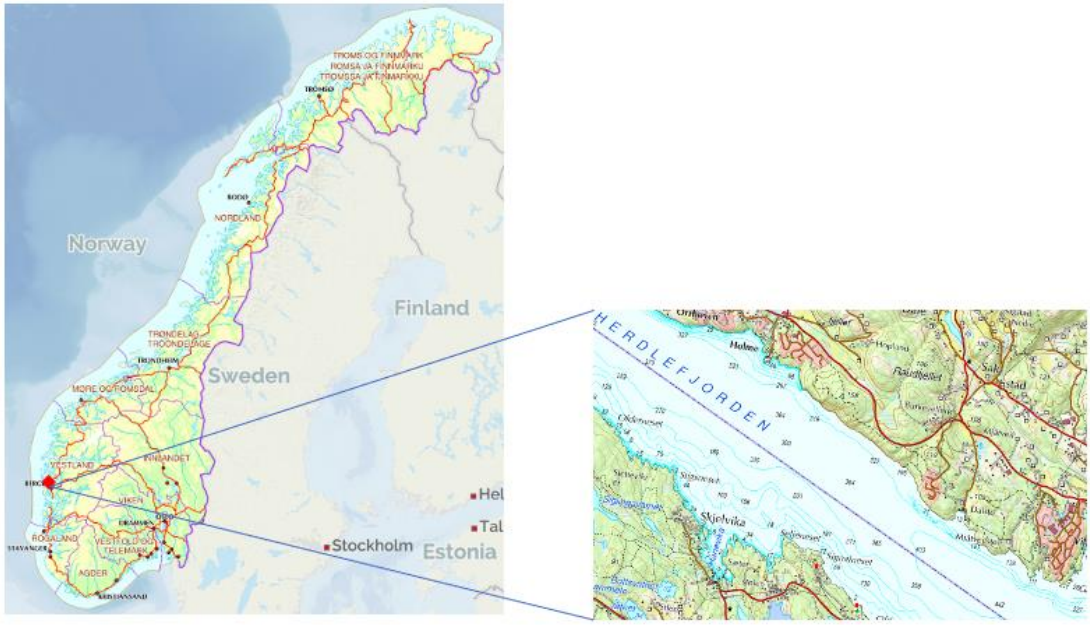


Figure 1 Herdlefjorden in Vestland county where the sharks were captured. Maps gathered from www.norgeskart.no.

Spiny dogfish were caught with fishing rods equipped with a circle hook. A circular hook is used to reduce the likelihood of the fish swallowing the hook and the smaller barb of the circle hook causes less damage when removed. We used fishing rods equipped with lights and luminescent rigs, baited

with herring or mackerel. The bait was lowered to approximately 30-90 m depth. The caught sharks were transferred to the IBC tank with a landing net after the hook was retrieved. The animals were measured with a measuring tape on a stuffed fishing mat. Sharks in the size range 50-85 cm were kept, and the others were released. A roller tank was used to transport the spiny dogfish from the boat to the aquatic facility. A total of 23 sharks were caught, and 22 of them were used to experimental trials. The weight and sex distribution are presented in **Figure 2**.

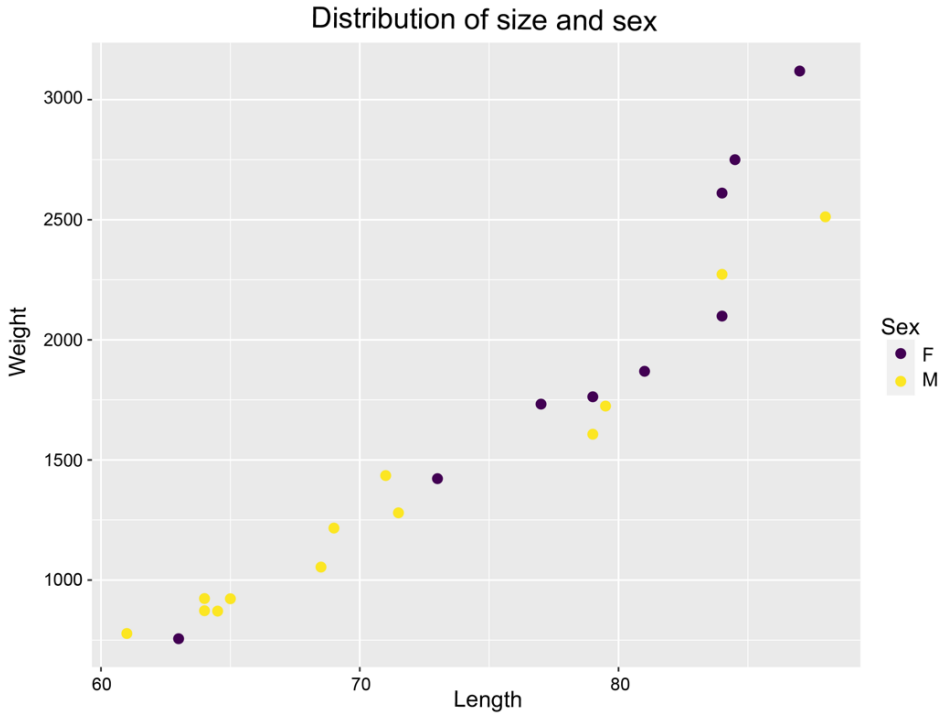


Figure 2 Distribution of the weight, length, and sex of the 22 sharks included in the trials.

4.2.3. Husbandry of Spiny dogfish

The spiny dogfish were divided into two or three individuals in each tank depending on their total length. A minimum of 3x was provided, where sharks > 68 cm in length were kept in pairs and three were kept together if the individual length was < 68 cm. We recorded salinity and temperature of the water from daily measurements. All animals were observed daily. Their well-being was highly prioritized; their general conditions were evaluated by swimming pattern, presence of any visible wounds/damages, appetite, and anomalies in their behavior.

Feeding: Knowledge about their eating habits in captivity is scarce. Based on the input from Aquariums and researchers and response of spiny dogfish to offered food, the feeding conditions and feed were optimized. They were offered pieces of frozen mackerel initially, and after one week of habituation, we started to observe bitemarks on the food. However, their appetite seemed low. Hence, we changed the feed from frozen mackerel to defrosted mackerel. We fed each shark one piece of mackerel every 3rd day. One tank was fed salmon.

Housing tanks: This study was conducted in the aquatic facility of the University of Bergen. The laboratory facility was divided into two rooms: one for the technical setup and tissue sampling and

the other one for housing and behavioral experiments. Three tanks numbered 1, 3, and 4 (the housing tanks) were used for habituation and housing. Tank number 2 was used to conduct the experiments (the experimental tank) (**Figure 3** and **Figure 4**). This tank was modified to optimize recording and stimuli devices and will be explained in detail below. All tanks had the same dimensions of 200 cm in diameter and 111 cm in depth (**Figure 3**) and received full strength seawater. The water inlet was through Wavin pipes (Netherlands) mounted directly over the surface, which created a surface current. Tanks held 2.67 m^3 of seawater with a depth of 85 cm at an average temperature of 9°C and salinity at approximately 33 ppt. Temperature and oxygen levels were connected to an alarm system to provide a secure environment for the animals. Housing tanks were covered by a wooden lid with a hatch and a feeding hole and illuminated by a LED lamp mounted across the diameter parallel to the hatch in the lid. Due to the biological low light condition need of the spiny dogfish, the lights were covered in dark plastic bags, giving a light intensity of 10 lux at the water surface. Additional LED lamps were mounted on the roof of the facility. All lights followed the same day/night cycle of 9:12 L:D. The dusk/dawn simulation lasted for 1.5 hours each.

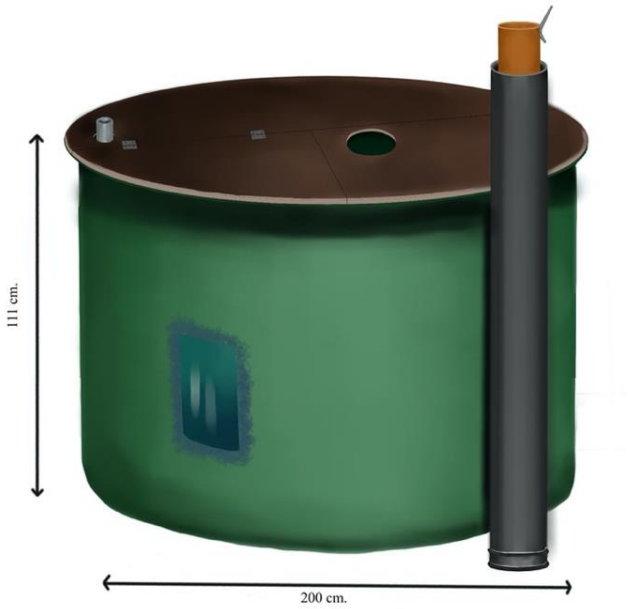


Figure 3 The schematic of the housing tanks. The housing tanks had a dimension of 111 cm height and 200 cm diameter. Water depth was 85cm.

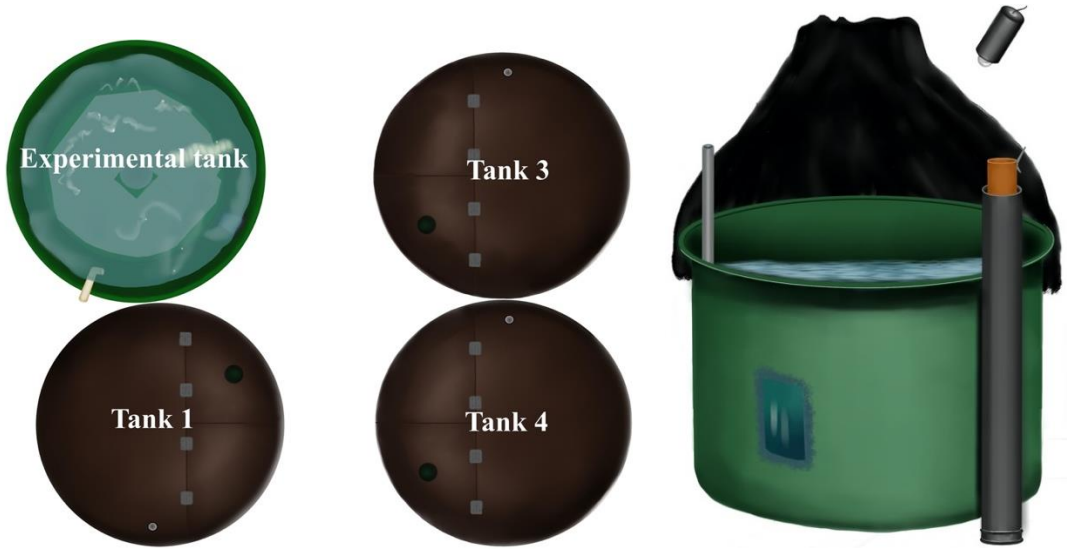


Figure 4 ***Overview of housing tanks and experimental tanks. The experimental tank was lidless and covered with black drapes. The camera was mounted on the ceiling.***

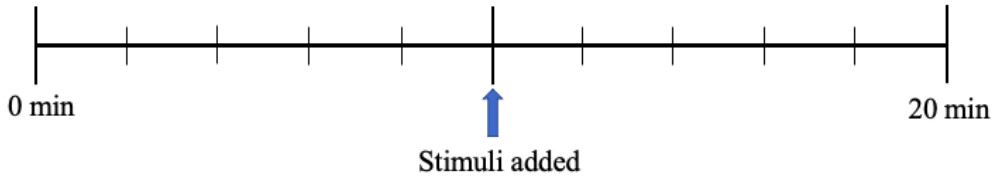
4.2.4. Experimental tank setup

All trials were recorded in the experimental tank with a custom camera mounted to the ceiling that gave a 120° field of view (**Figure 4**). The two initial trials were executed with a 65 cm water depth, and after a camera change resulting in an increase of the field of view the water depth was changed to 82 cm. An LED-light source mounted on the roof of the facility, provided a light intensity of 10 LUX at the water surface. Black drapes were mounted around the tank to shelter the shark from disturbances such as movement, sound, and light. The water outlet pipe was mounted just beneath the water surface. White plastic tiles covered with acrylic plates were added to the bottom of the tank, this improved contrast between the background and the animals.

4.2.5. Behavioral trials

The spiny dogfish were transferred in solitary from the housing tank to the experimental tank one day prior to trials to habituate. The animals were offered food 1-3 days in advance before the trial initiation and did not receive food during experiment period. The trials were conducted over 1-5 consecutive days between 08.00 and 19.00 (GMT+1). After the behavioral trials, the animals were euthanized, and tissue samples were collected.

The trials were conducted under three conditions: Condition 1 included testing of all three stimuli (odour, sound and electromagnetic pulse), the aim of Condition 2 was to observe the effect of pulses with increasing electromagnetic field strength, and the aim of Condition 3 was to observe the behavioral effects of dose dependent response to skin extract, an aversive odor cue. Each trial was recorded with OBS 27.1.3 (Open broadcaster software). Each recording followed the timeline below.



4.2.6. Condition 1 – Effect of auditory, odor and electromagnetic pulse

The initial trials were conducted to evaluate if any of the three stimuli could elicit behavioral changes. Spiny dogfish number 1, 2, 3, 4, 5, 7, 11 and 12 were used.

Sound stimulus: The acoustic stimuli were delivered through a waterproof custom-designed speaker (**Figure 5**). The sound was amplified by a BILTEMA amplifier powered by a 12V PS-5241-03 LITEON power supply (LITEON technology, Taiwan). A 1 Kg rubber-coated weight was mounted underneath to submerge the speaker into the middle of the water column. The speaker was mounted in front of the water outlet 1 hour prior to the initiation of trials and removed post-trials. Due to surface corrosion, the three last trials were conducted with a plastic bag surrounding the speaker, which lowered it closer to the bottom. Recordings of screams and clicks of the North *O. orca* were used as auditory stimuli. The control was a shuffled version of the same recording (**Figure 6**); 0 and 7500 Hz frequency audio file ranging between 0 and 20 000 Hz. Each audio file played for 1 minute.

Figure 5 Illustration of the custom speaker.

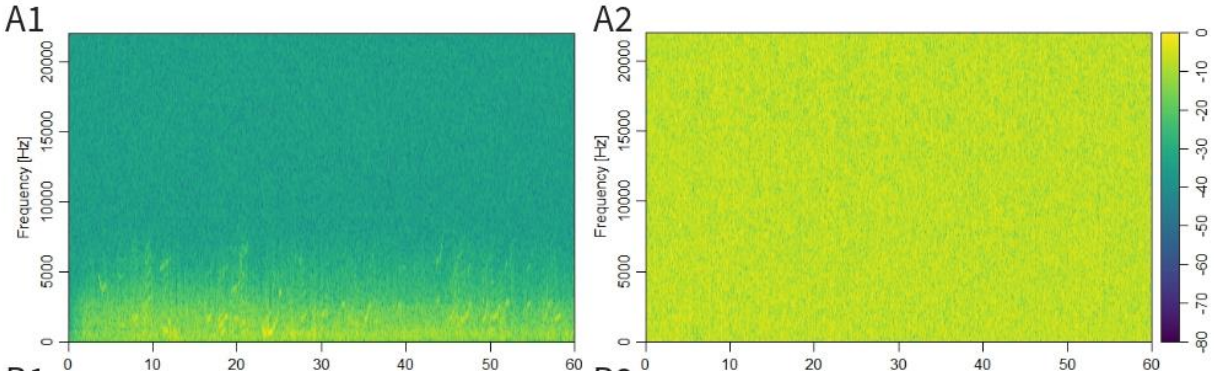


Figure 6 Spectrograms of the four audio files played during audio trials. The lower frequency orca sounds (A1) with a shuffled noise of the same audio (A2). The color bar represents the amplitude in decibel (dB).

Electromagnetic pulse equipment and preparations: The electromagnetic field was created by a self-constructed electrode arrangement (

Figure *7*). Two iron rods acted as electrodes separated by an acrylic plate. Supply leads were attached to the electrodes with alligator clips. This allowed for creating an electrical circuit from a power supply to the electrodes with the seawater being part of the circuit. The device was powered by a 360W BK precision 1687B power supply. An emergency switch was mounted to the electrode circuit to quickly deactivate the current if necessary. The supply had an output voltage capacity of 37 V and a continuous output current capacity of 10 A. A Raspberry Pi computer (Raspberry Pi Foundation, UK) with custom program was used to create pulse patterns of different duration and inter pulse interval. The Raspberry Pi controlled a mosfet relay in the circuit allowing the generation of block pulses. The device was tested in an empty tank filled with seawater before being implemented into the trial. Field strength were measure at different spatial points and an ANSYS Maxwell simulation of the system shows the strength and extension of electromagnetic field in response to a pulse is presented in **Figure 8**. To visualize the electrical impulses in the recordings, the Raspberry Pi also controlled an LED lamp synchronized to the EM pulse; the LED light was mounted on the edge of the tank surrounded by black foam rubber to prevent any light to be visible and affecting animal behavior.

The EM device was mounted by the water outlet with a rope one hour prior to trials for habituation and removed once the trials were finished. Three sets of electromagnetic stimuli of different inter pulse intervals (5ms pulse with inter pulse interval 100ms, 300ms, 600ms) were applied; each shark received a total of nine stimuli.

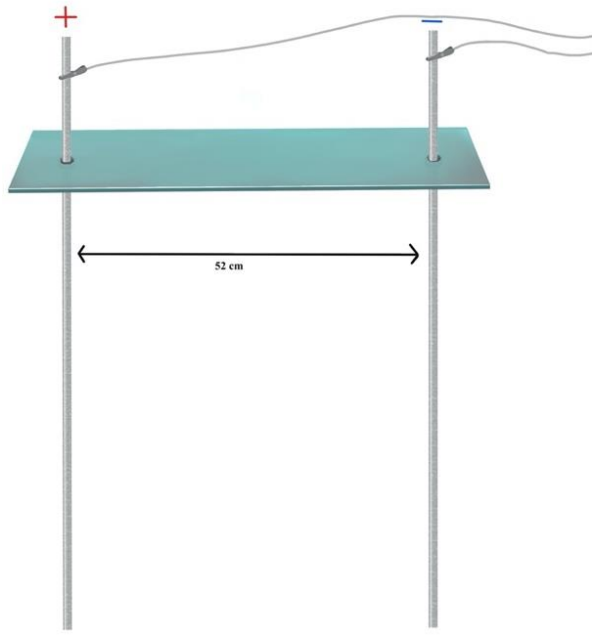


Figure 7 The custom-made electrode used to create the electromagnetic field.

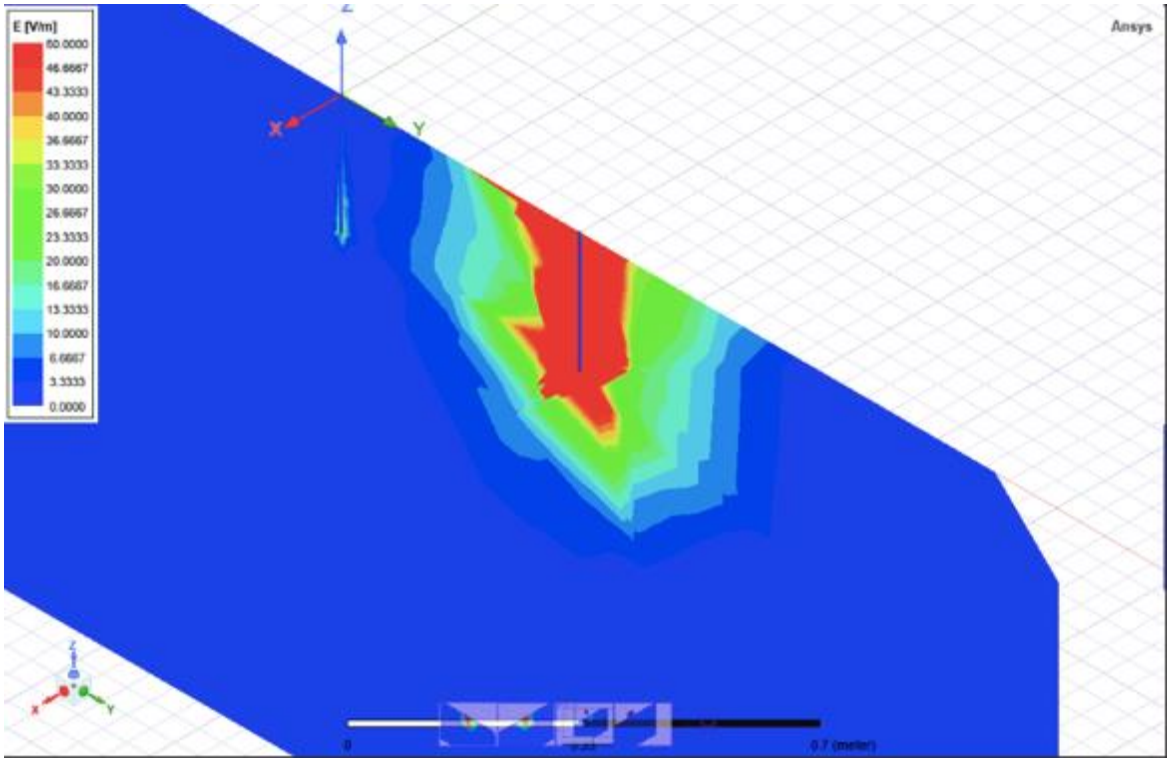


Figure 8 Simulation of the strength of the electromagnetic field. The current is at its strongest close to the electrodes (>50 E[V/m]) and dramatically decreases by the distance of 0.3 m. Sharks experience less than 3.33V/m field at a distance of 0.3 m from the electrode for an input voltage of 10 V.

Odour stimuli: Odor stimuli were applied in a liquid solution through a plastic tube permanently mounted through a pipe equal to the water outlet pipe (Wavin, Netherlands) and placed adjacent to the water outlet pipe. A funnel was mounted on the top of the plastic tube. We applied food odor and skin extract, as well as seawater as control. Food odor was prepared by cutting approximately 33 g. of thawed mackerel into pieces blended in 1000 mL seawater in a 1000 mL glass bottle. The mackerel solution was further diluted with seawater to a 300 mL/1000 mL mixture. The skin extract was prepared with skin collected from earlier euthanized spiny dogfish. Thawed skin extract was minced for 5-10 minutes with a small amount of seawater using a mortar. The solution was evenly distributed into three 50 mL Corning Falcon tubes and further diluted with seawater until a 30 mL solution was obtained. All three Falcon tubes were used as stimuli for the same shark. The seawater control was collected from the same water system supplying the flowthrough system of the tanks. The pipes were flushed with 30L seawater to remove excessive odor in the tube after the trials.

Table 1 The general setup of how the behavioral trials conducted under Condition 1. Variability in the order of trials did occur. Sample size = 8. Stimuli x = the stimuli of interest for tissue sampling.

	Day 1	Day 2	Day 3	Day 4	Day 5
1.	Odor: Control	Odor: Control	Odor: Control	EM: 600ms duration	Stimuli x

2.	Sound: Control	Sound: Control	Odor: Food	EM: 600ms duration	Tissue sampling
3.	Sound: Orca	Sound: Orca	Odor: Food	EM: 600ms duration	
4.	Sound: Control	Sound: Control	Odor: Control	EM: 300ms duration	
5.	Sound: Orca	Sound: Orca	Odor: Skin extract	EM: 300ms duration	
6.	Odor: Control	Odor: Control		EM: 300ms duration	
7.	Odor: Food	Odor: Food		EM: 100ms duration	
8.	Break (2 hours)	Break (2 hours)		EM: 100ms duration	
9.	Sound: Control	Sound: Control		EM: 100ms duration	
10.	Odor: Control	Odor: Control			
11.	Sound: Orca	Sound: Orca			
12.	Sound: Orca	Sound: Orca			
13.	Sound: Control	Sound: Control			
14.	Odor: Skin extract	Odor: Skin extract			

4.2.7. Condition 2 – Investigating the effect of increasing strength of electromagnetic pulse.

In this trial series, the behavioral response to increasing voltage of the electromagnetic field was measured. The same equipment was used as described under Condition 1. The sharks were exposed EM pulses with three different voltage amplitudes 5 V, 10 V, and 20 V with 300ms intervals between subsequent pulses (**Table 2**). Shark 6, 8, 10, 15, 18, 20, and 21 was subjected to Condition 2 trials.

***Table 2** The general setup of how trials under Condition 3 were conducted. Sample size = 7. Stimuli x = the stimuli of interest for tissue sampling.*

Day 1	**Day 2**
EM: 300ms interval, 5V	EM: 300ms interval, 20V
EM: 300ms interval, 10V	EM: 300ms interval, 5V
EM: 300ms interval, 20V	EM: 300ms interval, 10V
EM: 300ms interval, 5 V	EM: 300ms interval, 20V
	EM: 300ms interval, 20V
	Stimuli x
	Tissue sampling

4.2.8. Condition 3 – Investigating the dose dependent behavioral response to skin extract.

We executed specific trials to investigate the behavioral effect of increasing units of skin extract (**Table 3**). We used the same equipment as described for the odor trials in Condition 1, however, the preparation of skin extract was altered. Frozen skin samples were defrosted, measured, and put in a blender (Philips, Amsterdam Netherlands) and blended for 2 minutes. A concentrate equivalent to 7 cm of skin was filled in each falcon tube and placed in the freezer at -35°C.

$$\frac{x\ cm\ of\ skin}{7} = n\ Falcon\ tubes$$

One Falcon tube was defrosted and diluted with seawater until a 175 mL solution was obtained. Three units of skin extract were prepared for three consecutive trials: 25 mL (0.5 U), 50 mL (1 U), and 100 mL (2 U). The solutions were kept on ice until use. The food odor was prepared identically to that of Condition 1. Shark 9, 13, 15, 16, 17, 18, 20, and 21 was subjected to Condition 3 trials.

Table 3 The general setup of trials conducted under Condition 3. Sample size= 8. Stimuli x = the stimuli of interest for tissue sampling.

Day 1	**Day 2**
Odor: Control	Odor: Control
Odor: Food	Odor: Food
Odor: Skin extract 25 mL (0.5 U)	Odor: Skin extract 100 mL (2 U)
Odor: Skin extract 50 mL (1 U)	Odor: Skin extract 50 mL (1 U)
Odor: Skin extract 100 mL (2 U)	Odor: Skin extract 25 mL (0.5 U)
	Stimuli x
	Tissue sampling

4.2.9. Image processing with DeepLabCut

All recordings were uploaded to a Western Digital 44 TB By Book Duo external Hard Drive (WD, United States). To extract the movement and location of the animal on the recordings, DeepLabCut™ software was used. DeepLabCut™ allows markerless tracking; it uses human-annotated labels in a deep-neural network to track and estimate the position of the animal (Mathis et al., 2019; Nath et al., 2019). The tracking was performed in an Ubuntu 22.04.2 LTS operative system, with a 13th Gen Intel® Core™ i9-13900K x 32 processor and graphics from NVIDIA Corporation. Prior to analysis, one video from each day of trials were extracted; the recordings that showed the sharks having the widest range of locomotion were selected and copied into a Seagate Expansion HDD hard drive (Shark # 1-8, 12, 13 and 15). Each recording was duplicated as defined by frames with a processing package from ImageJ, Fiji (Schindelin et al., 2012). The first trials were conducted with 10 fps (frames per second) recordings, while the remaining trials were recorded with 20 fps. Subsequently, the number of frames in the recordings varied between 12 000 (10 fps)

and 24 000 (20 fps). The videos were further utilized to create a deep-neural network to automatize the annotation of all the recordings.

DeepLabCut protocol:

1. **Created a new project** "Training 2.0". This created a config file called "config2.yaml" file.
2. **Set the labels** of choice in the "config2.yaml" file and drew a skeleton (Nath et al., 2019).
    - A total of eight labels were annotated and placed on the most visible body parts on the recordings.
3. **Labeled frames** from a total of 60 videos á 3 minutes. Each video provided 20 frames to annotate. A total of 1200 frames were labeled.
    - The videos were chosen to reflect the most diversity in movement, speed, angle, video quality, and individual variation for the network to familiarize itself with fluctuating images from each video.
    - Tracking accuracy increases with more body parts annotated compared to "specialized" training with only one body part annotated (Mathis et al., 2018).
    - Labeled each frame according to **Figure 9**.

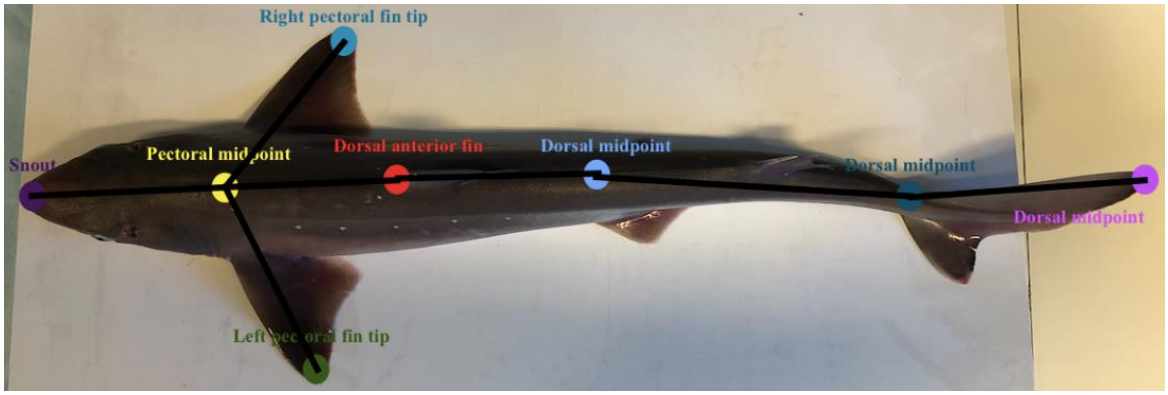


Figure 9 Names and positions of the eight labels. The size and shape of the labels were identical for all annotated frames. The "disc" shape worked as a tool to align the labels on the same location of the body parts for each frame. The snout was labeled by aligning the disc to the snout tip. The pectoral midpoint was labeled by aligning the back of the disc where the cross section from both pectoral fins meets the top of the dorsal area. The Left and the right pectoral fin were labeled by aligning the outside of the disc with the fin tips. The dorsal anterior fin was labeled by placing the disc in the center of the anterior root of the fin. The dorsal midpoint was labeled by centering the disc by cross-section between the dorsal midpoint and ventral root of the pelvic fins. The caudal peduncle was labeled by placing the disc at the thinnest point before the caudal fin. The caudal fin tip was labeled by aligning the back of the disc to the tip of the fin.

4. **Created a training dataset** on shuffle 6, network architecture: resnet_50, and "imgaug" augmentation method. This training dataset was later used to train the network.
5. **Trained the network** for maximum iterations of 400000 (recommendation is >100000) (Nath et al., 2019). The deep-network is now trained based on the training dataset from Step 4. The training took about 1.5 hours.

6. **Evaluated the network** to see if the accuracy of the network was sufficient. This step provided three test heatmaps showing the hotspots for the program to annotate (Nath et al., 2019) (**Figure 11**). This allowed to look for network mistakes in the detection of the animal. The p-cutoff value of Shuffle 6 was 0.6, which displayed all values with a likelihood below 0.6 as uncertain, giving a training error with a p-cutoff of 3.23 and a test error with a p-cutoff of 7.93. The annotations made by me, and the network are illustrated in
7. **Figure *11*.**

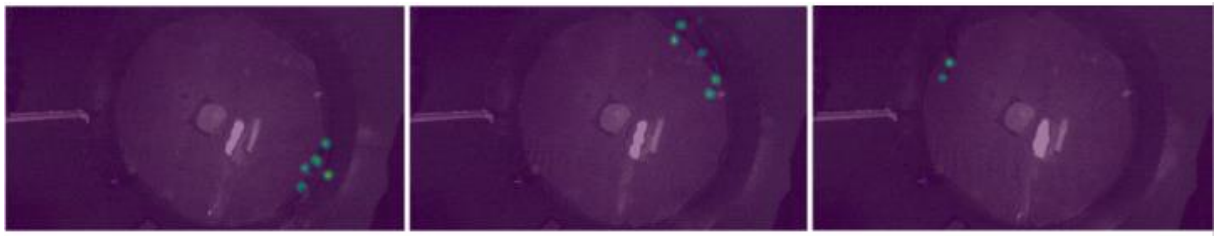


Figure 10 Heatmap generated by DeepLabCut which shows a location of the animal.

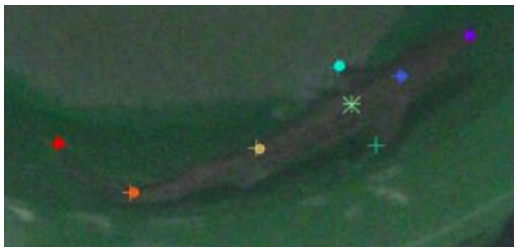


Figure 11 Example of the evaluation of the network used for analysis. The dots (•) represent DeepLabCut´s predictions with a likelihood ≥ 0.6 p - cutoff, the x's (×) for predictions with a likelihood < 0.6 p – cutoff, and the "plus" (+) represent the human annotations (Nath et al., 2019).

8. **Analyzed the videos** using the GUI which generated a csv. file with x and y coordinates and likelihood confidence for each of the eight labels.
    - All videos of each individual shark were selected for analysis, which took between 8-12 hours for 30-40 videos.
9. **Created videos** of the same selected videos from Step 7. This created videos with constant labels showing when the spiny dogfish was visible and drew a skeleton showing the angle of the body (
10. **Figure *12***).

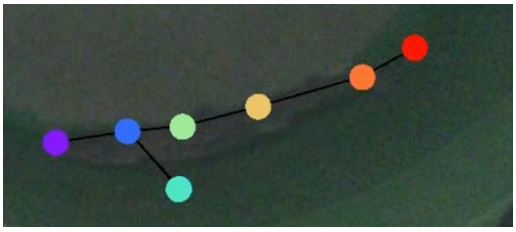


Figure 12 A snapshot from the recordings of Shark 7, displaying the accuracy of labeling in the videos created in Step 8.

11. **Extracted outliers** to improve the labeling results by extracting frames from training videos with high uncertainty. The datasets were further merged, and the network was trained again on Shuffle 5.
    - If outliers were extracted, steps 4-8 were repeated.

All videos that were analyzed were transcribed into a CSV file with a timeframe, x and y coordinates, and the likelihood for each of the eight labels, respectively. The CSV files were stored in the folder to which the video belonged and acted as the foundation for creating videos with tracking.

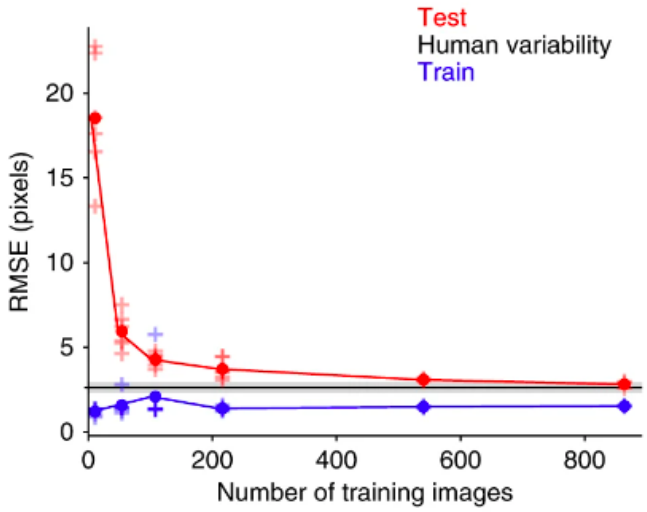


Figure 13 After approximately 200 images for training the deep network, the accuracy of DLC is close to human accuracy. RMSE in pixels are compared between one human labeler, labeling two distinct datasets and the trained network. The figure is reproduced from Mathis et al. (2018).

The accuracy of DeepLabCut

Analyzing the videos with the DLC software saved many hours of observations. The error for using DLC compared to human observations is minimal when comparing the RMSE (root mean square error) (Mathis et al., 2018).

The initial trials were processed with an iteration of 100 000 and without merging the datasets after extracting outlier frames. The training and test error with p-cutoff on Shuffle 5 was 8.08 and 9.9, respectively. The network was trained with an iteration of 400 000 which reduced the train-

and test error on Shuffle 6 with p-cutoff to 3.23 and 7.93, respectively. As the time limit of the project was limited, I evaluated the accuracy of the tracking ability in Shuffle 6 as sufficient and continued to extract the coordinates. Training the network with 4x higher number of iterations increased the processing time for each video drastically. However, the DLC accuracy in detecting the shark from the recordings is shown in

Figure 13Error! Reference source not found..

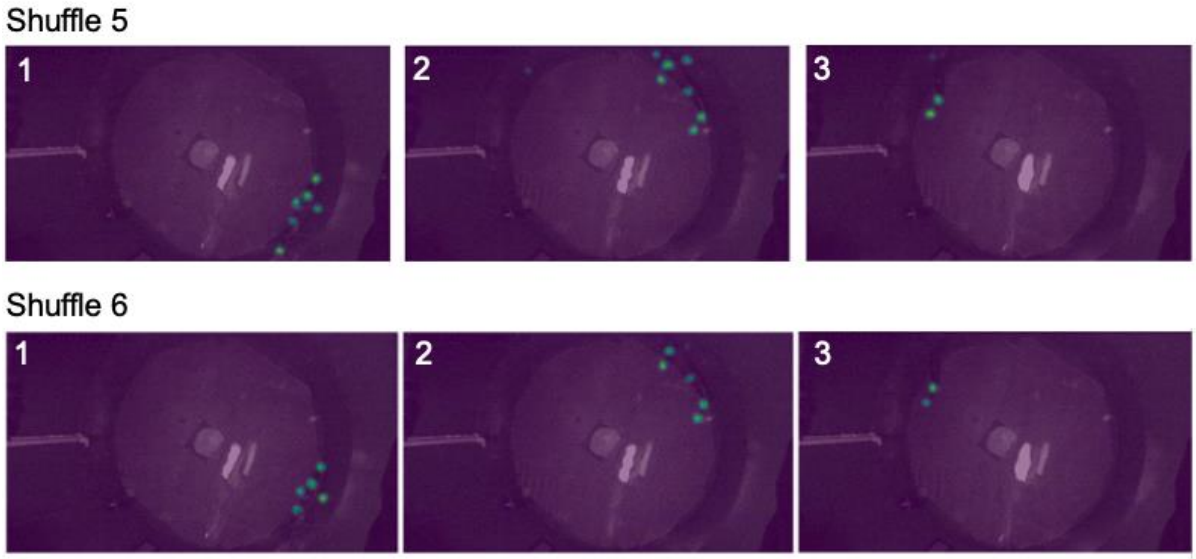


Figure 14 Comparison of the two main shuffles that were considered to analyze the videos. These images are heatmaps reflecting the DLC ability to locate and recognize the eight labels that were used to identify the shark on the recordings. Shuffle 5 depicts some inaccuracy in the picture 2.

4.2.10. Analyzing the locomotion with RStudio

Custom analysis codes in RStudio were used for analysis. With this code, a list with CSV files was introduced to a loop that iterated a series of commands and stored the data in respective files from where the CSV files originated.

1. Each label **(Figure 9**) was assigned to its own matrices ranging from V1 to V8.
2. To increase the precision of the location of the shark in the tank, all rows with coordinates with > 0.6 likelihood exchanged the equivalent row in V1. All rows with likelihood < 0.1 in V1 were removed to eliminate noise.
3. The x- and y- coordinates were smoothened with Simple Moving Average (SMA) and a filtering window of 40 points.
4. The timeframe from before and after stimuli had been introduced was divided into separate data frames. For EM and Audio trials frames 10800-12000 (Before stimuli) and 12000-13200 (After stimuli) were extracted. For odor trials, frames 9600-12000 (Before stimuli) and 12000-14400 (After stimuli) were extracted. This selected interval is based on qualitative behavioral observation during the trial.
5. All distances were converted to a timeframe in seconds and to a numeric value.
6. Calculations of total distances traveled, means, standard deviation, and standard error were calculated and saved into a new data frame.

$$Distance\ travelled = \sqrt{(x_2 - x_1)^2 + (y_2 - y_1)^2}$$

7. All files were saved in their respective folder and used for further analysis and making of figures.

To evaluate quantitative changes in behavior on a large scale, the total distance traveled and the animal's placement in the tank based on the x and y coordinates were used. The total distance was used to evaluate if the treatment from stimuli influenced the speed and movement in the tank.

4.2.11. Physiological studies with serum analysis

The physiological response to food odor, skin extract, and electromagnetic field was evaluated by euthanizing the animals 30-40 minutes after being exposed to the different stimuli treatments. The different treatments are listed in **Table** .

Tissue sampling: After trials were conducted, the animals were euthanized with an overdose of Tricaine mesylate anesthetics (MS 222) mixed with seawater. Blood, gills, skin, spine, and brain samples were collected. When the animal was fully anesthetized and gill motion ceased, the animals were weighed (g), measured (total length in cm), and photographed. Subsequently, we collected blood samples from the dorsal aorta with a VACUETTE® quickshield safety tube holder and a VACUETTE® Multiple Use Drawing Needle 18G x 1 1/2 in a 5 ml VACUETTE® Z Serum Clot Activator Blood Collection Tube (Greiner bio-one, Germany). Blood samples were stored at room temperature and centrifuged within two hours after withdrawal. We used a Beckman Coulter Allegra X-15R Refrigerated Centrifuge, with a frequency of 4000 rpm for 10 minutes to separate clotted blood from the serum. Serum was transferred and aliquoted into 500 μL samples and stored in the freezer at -80 °C. After blood sampling the animal was decapitated. The 2nd gill arch and one olfactory bulb together with half the telencephalon were collected in 25 mL RNA later™ Soln (Invitrogen AM7021, USA) and stored at 4 °C for 24 hours and then transferred to the -80 °C freezer. The rest of the brain and forebrain was stored in 20 mL Formalin (Biopsafe® 3178-200-21 NO, Vedbæk, Denmark) at 4 °C. Skin samples were stored in 3.5 mL 1xPBS (Invitrogen AM9625, USA) and diluted with distilled water to a 1x10 PBS solution, and subsequently frozen. Spines were collected and cleaned for excessive tissue before storing dry.

Table 4 The stimuli that the sharks were exposed to 30-40 minutes prior to euthanasia.

Shark #	Sampled after stimuli	Shark #	EM
1	EM	13	Control
2	Skin extract	14	Food odor
3	Control	15	Skin extract
4	Control	16	Skin extract
5	Skin extract	17	Food odor

6	Food odor	18	Control
7	Food odor	19	EM
8	EM	20	EM
9	Skin extract	21	Skin extract
10	EM	22	
11	Food odor		
12	Control		

Serum analysis: All serum samples were chemically analyzed with a Pentra C400 (HORIBA ABX SAS, Montpellier, France). The samples were defrosted and centrifuged for 5 minutes at 6000 RPM at 4 °C. Clear serum was split into 200µL Pentra sample cups and placed into respective trays. The parameters examined were cholesterol, calcium, glucose, lactate, magnesium, phosphorus, total protein, triglycerides, chloride ion, sodium, potassium, creatin enzyme, lactate dehydrogenase, high-density lipoprotein, and low-density lipoprotein. Samples from Shark 3 and Shark 14 were diluted with distilled water x2 and x5 to read Total protein.

4.2.12. Statistical analysis and visualization

Alterations of the locomotive activity were quantified based on the distances traveled and the shark's position between four squared areas in the tank (**Figure 15**). The behavioral effect of the stimuli before and after treatment was compared with a paired t-test. Similarly, the fold change between all stimuli in Condition 1 and Condition 2 were compared with the paired t-test. Fold changes of total distances traveled in Condition 3 was compared with one-way ANOVA. To detect differences between position counts and further statistical significance between the four squares in the tank, one-way ANOVA and post-hoc analysis without correction for multiple comparisons was used. The quantity of serum metabolites was compared using one-way ANOVA and Dunnets multiple comparisons test.

All behavioral trials under all three conditions were visualized equally. The selection of coordinates is described before. The mean of total distances traveled by each shark in all conditions were color-coded and visualized in pairwise-comparison dot plots. The change of movement and placement in the tank was visualized using binned heatmaps (bin size = 10) created with the ggplot 2 package in RStudio. The heatmaps represented the movement before and after the stimuli, with a third heatmap showing the change of positions. Shark 1 and 2 was excluded from the heatmaps in Condition 1. The total distances were visualized as the mean of the total distance from all trials separated into each individual shark in a bar plot to compare the effect of the stimuli before and after the stimuli were introduced. The distances were visualized as meters. The tank was about 880 pixels in diameter, representing the 200 cm real size, making each pixel roughly 4.4 cm. One meter would therefore be roughly 440 pixels.

The fold change was calculated and visualized as a bar plot to compare the locomotive activity between trials. To quantify the areas of the tank that was favored, especially to locate avoidance, the tank was divided into four quadrants (squares). The position counts were calculated using RStudio and visualized with bar plots as total position counts and the fold change between before and after the stimuli was applied. The used annotations are shown in **Figure 15**.

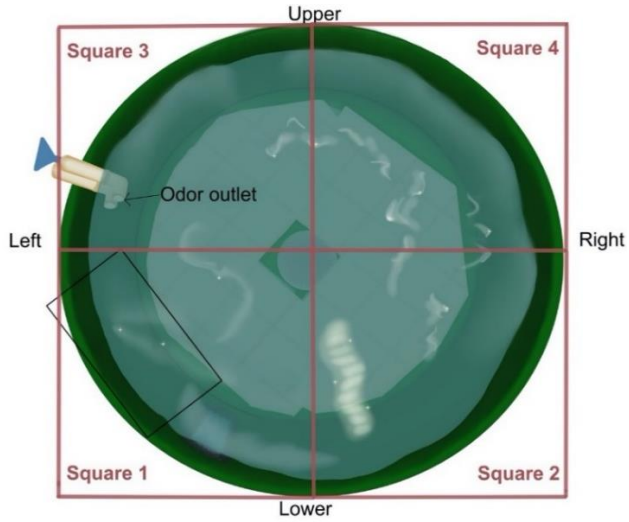


Figure 15 The experimental tank as seen from above. White tiles with plexiglass on the top cover the bottom. The odor was applied from an outlet next to water outlet. The speaker and electrode were placed in the area marked by the black square. The squares used to determine the position of the shark are marked with red lines.

4.3. Field trial of promising anti-shark measures (AP2)

4.3.1. Pulsed electromagnetic field (EM system)

Equipment provided by Salarsafe AS was used for the experimental setup used in the field trials. On a principal level, the system is similar to that used in the laboratory trials but developed to be deployable at fish farm locations for at least the duration of a salmon production cycle, addressing the known challenges of electrode corrosion/lifespan and power usage optimization.

Salarsafe has spent considerable effort on developing electrode materials that can last for the duration of a whole production cycle. It is well known that generating an electromagnetic field between a pair of exposed conducting electrodes causes electrolysis reactions in the seawater surrounding the electrodes. Some of the ions generated have a severe corroding effect on electrode materials, particularly chloride ions. For this reason, stainless steel or nickel electrodes dissolve in matter of days. Other aspects of the electrochemistry of saltwater electrolysis result in scale deposits on the electrodes. These increase the effective electrical surface resistance between electrode and seawater over time, reducing the strength of the electro-magnetic field generated with the same power input. The Salarsafe system is optimized with the aim to avoid these issues and the duration and time distribution of the pulses are optimized to minimize energy use of the equipment (for further information about the technical details of the system, Stian Rennestraum at Salarsafe AS can be contacted). Initially the tests were conducted at similar field strength levels as the laboratory tests. After the first test rounds, Salarsafe upgraded the system for higher field

strengths so that we would be able to determine the threshold values for chasing dogfish in feeding modus on the bait (feeding frenzy). On this system four pairs of electrodes surrounding the bait are controlled, while providing full flexibility on the choice of the pulse patterns and parameters. Unfortunately, we did not get the chance to test in the field with this updated equipment due to absence of dogfish on the last two field trials. This is generally the problem we encountered doing field trials: out of six trips we had a 50% success rate of finding and attracting dogfish at the locations. Field trials are essential as they're the only realistic way of the dogfish to study the behavior in its natural habitat and include group behavior, which might be a significant factor in the deterrence of spiny dogfish.

To conduct the trials, a rig (**Figure 16**, **Figure 17, Figure 18**) was made to 'protect' bait in the form of a bag with bait (mackerel) placed in between the two EM electrodes. The bait and electrodes are in the same plane and the conducting path created momentarily through the seawater during a pulse creates an electro-magnetic field in the radial direction normal to this path. It is our current understanding that this path of ions for the current to flow momentarily from anode to cathode has several similarities with the path lightening takes between a cloud and earth in the atmosphere. The conduction pathway can thus be expected to occur on different locations from anode to cathode and the spatial distribution of EM field strength therefore will vary from pulse to pulse. The rig has mounting points that place two GoPro cameras (Hero 4) at sufficient distance to view the entire area of electrodes, bait, and the area of sea behind it from both directions. The GoPro Hero 4 allows filming with a 170 field of view. Recordings were made at 30 pfs and 1920x1080 resolution. The GoPros are used to record the trials. On the rig there are also observation cameras mounted that allow real time onboard vision during the trials. This is necessary to identify when the dogfish are near the rig and to time the experiments.

The rig is lowered to initially thirty meters depths. Below the rig two ballast weights are hung to keep the rig level. A separate bait bag is lowered to 80-100 meters and moved slowly up and down to attract the sharks who will be expected to be near the bottom. When it is felt that sharks are taking the bait, the bag is slowly taken up to get the sharks to move upwards towards the rig. When there is sufficient shark activity around the rig, registered with the onboard cameras, the experiment is started. A trial contains of at least five minutes of complete shutdown of the EM system, followed by 5 minutes of the EM system switched on.

On the camera boom there are also two Ag+/AgCl electrodes mounted at 20 and 30 cm distance from the electrode plane respectively. By subtracting the two measured potentials using an oscilloscope (Picoscope) and dividing by the distance (10 cm) these measurements provide a good indication of the strength of the electromagnetic field outwards in the normal direction of the plane of the electrodes. This also gives a good idea of the variation of the field over time. In every trial with EM the measurement is used to verify that the EM is functioning as intended.

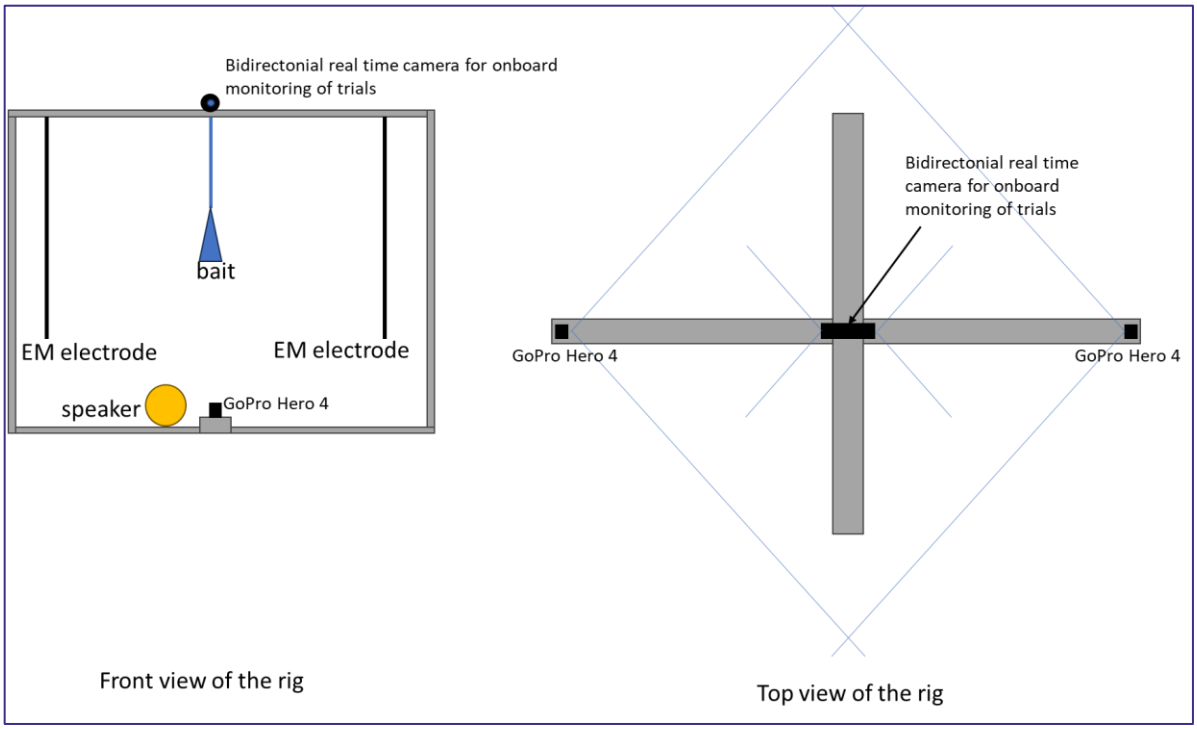


Figure 16 Schematic diagram of test rig with camera locations, field of view and experimental equipment.

Figure 17 Deployment of rig. Showing electrodes, bait bag and top cameras for onboard vision.

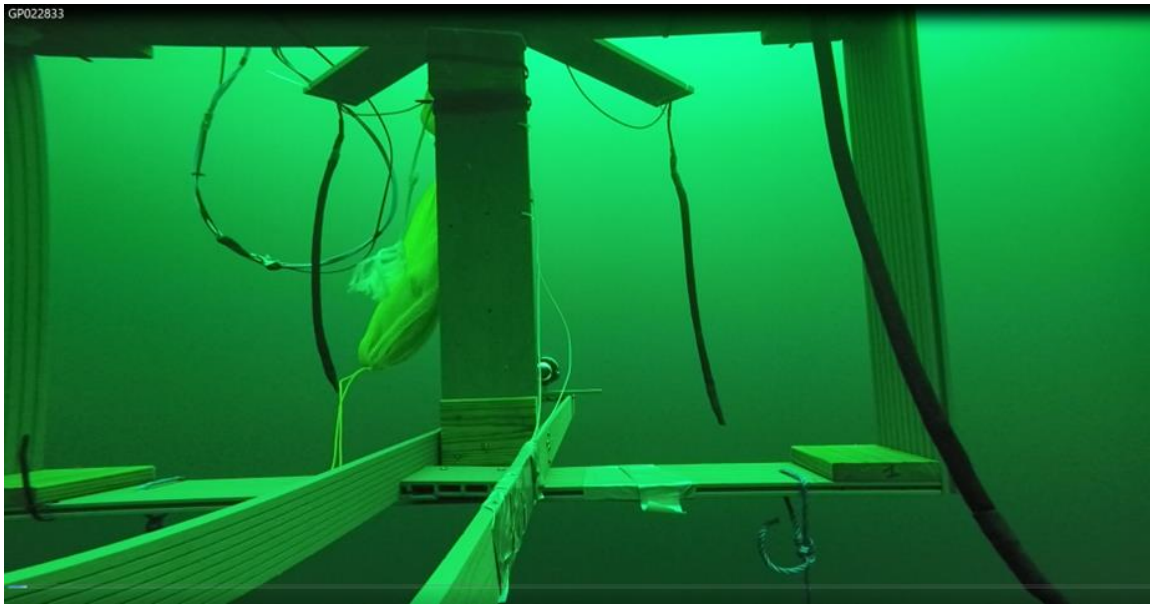


Figure 18 Second configuration of EM system on rig, using four electrodes, pair wise.

The behavior of the sharks around the rig and bait during these two periods is quantified according to the following criteria:

- Passing the rig within the field of the GoPro camera
- Bumping rig visual
- Biting rig
- Approach to rig/bait with mouth closed
- Approach to rig/bait with mouth open
- Bait contact
- Biting bait/feeding
- Slow Retreat from bait/rig
- Fast Retreat from bait/rig
- Startle/Panic

We also took note of the number and sizes of sharks in an event, and, if possible, its sex. During the tests both the observations cameras and the GoPro camera are recording. This makes it possible afterwards to synchronize all the cameras in time. The time synchronized GoPro footage of the tests was then AI enhanced on exposure to improve visibility of the dogfish in low light conditions. The analysis was done by one person noting each event with time for each GoPro camera for each test, playing back the footage at half speed. Each test gives then two sets of events for baseline and test, one for each GoPro camera having views out in opposing directions on the rig and the sea area

behind it. These two sets were compared and used to compose one set, functioning both as an observational error control for rig interaction events and allowing shark passing events at both directions to be counted. A weakness of the analysis method is that this was not done blind; the observer knew which footage is from the baseline and which from the test. The results should therefore be quality controlled by another observer with the tests randomized.

4.3.2. Orca sound

The sound of orcas was taken from an audio cassette with whale song recordings. The recordings were done by Wildland Productions in Canada and released on audio cassette in 1986. The songs from the audio cassette were digitalized in Sonar Professional using a NAD 613 audio tape deck feeding into a Focus Rite Scarlett digital audio interface connected to pc. The digital recordings are as stereo wav audio files with 2822 kbps rate (32-bit depth, 44.1 kHz sample rate).

The recordings were played back underwater using the VLC player on a portable pc. The portable pc is connected to an 100W audio amplifier. The loudspeaker is an inhouse NORCE design and construction. The principle of the speaker is that of soundwave transmission by air through a thin rigid membrane into the seawater. The system uses a standard two-way hifi 4” audio car door speaker (combined woofer/midrange and tweeter). The speaker is inside a stainless-steel cylindrical enclosure, closed and sealed with air at atmospheric pressure. The wall thickness of the enclosure is 0.7 mm. The speaker is connected to the amplifier with heavy gage copper speaker cable. The frequency response of the speaker is 90-20000 Hz with a sound pressure of 87 dB in air. This is the same configuration as used in the laboratory trials.

These field tests were conducted with the same rig as used in the EM tests. The loudspeaker was attached to the rig, the rig lowered to 30 meters and the GoPro cameras used for recording dogfish activity around the rig with bait. In this case the GoPros were mounted centrally on the rig instead of the camera boom but looking in the same opposing principal directions. Again, a five minute baseline was compared to five minutes exposure to the sound stimuli by counting the number of dogfish visible.

4.3.3. Odor stimuli

Application of chemical/odour in field trial faced practical challenges, as odour is easily washed away. Hence, no odour trial was conducted.

4.3.4. Shark-proof netting material

Several netting materials are being sold as shark proof. Based on the input from fish farmers, the project selected HDPE netting material as a candidate for testing. SAPPHIRE CFR nets (Garware Technical Fibres) and standard nylon net (Garware Technical fibres) were obtained for the trial. One GoPro camera was mounted above the rig to see the entire rig and another GoPro camera was mounted from the side to observe any approach of sharks to the rig.

Figure 19 Deployment of rig showing two different netting material: white nylon net and blue SAPPHIRE CFR net. A top mounted GoPro camera is visible here.

4.4. Effect of electromagnetic pulse on physiology and growth of Atlantic salmon *Salmo Salar* (AP4)

4.4.1. Authorization

The handling, and all experimental procedures with Atlantic salmon were approved by the Norwegian Food Safety Authority (FOTS ID #30397). The research was conducted according to laws and regulations established by the European Union (Directive 2010/63/EU).

4.4.2. Experimental conditions

Post-smolt salmon were obtained from iLab and delivered to NORCE's experimental facility in Mekjarvik on November 7, 2023. The fish were distributed to eight 800L tanks (18 fish each). They were maintained at 8.8C (+-0.04) and 24h light in flow through tanks (flow rate XXL/min). Salinity was adjusted from an initial brackish (23.7psu) to fully saline (34.5 +-0.38) over the following ten days. Fish were fed Skretting Nutra Supreme 4 mm pellets twice a day to satiation. Experimental exposure commenced on Nov 22. Swimming behaviour was recorded before, during and after exposure using a GoPro 11 camera mounted centrally over the respective round tank. Fish were euthanized and sampled in groups of four individuals one hour, one day and three weeks post exposure. Length, weight and sex was recorded, and blood sampled from the caudal

4.4.3. Behavioral response to electromagnetic pulse

The electromagnetic field was created as described in 4.2.6 using the custom EM pulse generator (

Figure *7*). Two stainless steel rods acted as electrodes separated by an acrylic plate. To visualize the electrical impulses in the recordings, a LED lamp was connected to the electrode and mounted on the edge of the tank surrounded by black foam rubber to prevent any light to be visible and affecting animal behavior. The EM device was mounted by the water outlet with a rope one hour prior to trials for habituation and removed once the trials were finished. Three different voltages of electromagnetic stimuli were given a maximum of nine times for each tank. Every trial with differing pulse interval was repeated three times with pulse interval of 300 ms and 5, 10 and 10V voltage. A pulse duration of 5 ms was used in all trials.

4.4.4. Stress response

Plasma analysis: The blood samples were centrifuged in an Eppendorf centrifuge 5424 R (Eppendorf AG, Hamburg, Germany) for 5 minutes at 6000 RPM at 4 °C. The plasma samples collected from sampling were analyzed with a Pentra C400 analyzer (HORIBA ABX SAS, Montpellier, France). Clear plasma samples were transferred into 200µL Pentra sample cups and placed into respective trays. The parameters examined were calcium, glucose, lactate, magnesium, phosphorus, total protein, triglycerides, chloride ion, sodium, and potassium.

Cortisol analysis: Plasma cortisol concentrations were quantified by radioimmunoassay as previously reported (Gorissen et al., 2012, Madaro et al., 2016, 2015) using xm210 cortisol antibody (Abcam, Cambridge, UK) and 3H-cortisol (Perkin Elmer, Groningen, The Netherlands).

Acetylcholinesterase assay: For the determination of Acetylcholinesterase (AChE) activity, frozen fish brains were weighed to the nearest 0.1 mg and analysed according to Ellman (1961) using the reagent kit by Ikzus Environment (Italy, Cat-nr. 1418-050-K). Briefly, individual brains were homogenized in 1.5ml of extraction buffer, centrifuged at 10000RCF for 20min at 4C and the supernatant drawn to a new tube. All steps were done on ice. Then 50 µl of sample were mixed with 0.938µl of pre-warmed (23C) reaction buffer, incubated for 15 min and 12µl of acetylcholine substrate added. Absorbance kinetics were determined in a Thermo Multiskan Go spectrophotometer at 412nm for each sample every 30 seconds for 5 min against an experimental blank (sample + reaction buffer with no substrate). From the linear slopes of the corrected absorbance (sample – blank), the weight specific AChE activity was calculated using the extinction coefficient determined from a standard calibration.

Stress marker expression: Total RNA was extracted from whole telencephalon using the NucleoSpin® RNA kit (Macherey-Nagel, Düren, Germany), according to the manufacturer's protocol 5.1: RNA purification from cultured cells and tissue. The samples were lysed in 10 mM TECP in Buffer RA1 followed by homogenization at 5000 rpm for 15 sec using metal beads on the Precellys™ control device (Bertin Technologies, Montigny-le-Bretonneux, France). RNA concentration was determined using Qubit™3 Instrument (Thermo Fisher Scientific, Eugene, Oregon). A random subset of the samples (25%) was selected to assess total RNA integrity. RNA integrity values (RIN) between 8 and 10 on the Agilent 2100 Bioanalyzer using RNA 6000 Nano LabChip® kit (Agilent technologies, Palo Alto, CA, USA) indicated sufficient quality. cDNA synthesis was reverse transcribed using 1000 ng of total RNA input in concert with qScript™ cDNA Synthesis Kit, according to the manufacturer protocol.

For RT-qPCR, gene specific primers (Tang et al., 2022) and SYBR Green Master Mix (Applied Biosystems, Foster City, CA, USA) on the CFX-384 Real-Time PCR detection system platform (Bio-Rad Laboratories, Inc., CA, USA) was used. The reaction mix contained 2.5 µl of cDNA, 0.25 µl of forward primer (10 µmol/l), 0.25 µl of reverse primer (10 µmol/l), 3.25 µl of Nuclease free water and 6.25 µl SYBR Green Master Mix; the final reaction volume was 12.5 µl. Melt-curve analysis of primer-sets was performed to ensure that only a single product was amplified and there was no detectable primer-dimer artefacts or genomic contamination. Primer amplification efficiencies (E) were generated by running a 2-fold dilution series (1:10, 1:20, 1:40, 1:80, 1:160) (pool) in triplicates; an optimal primer efficiency between 1.8 and 2.2 was used. The cDNA samples were assayed at a 1:10 dilution. The primer amplification efficiency (E) was calculated based by the slope of the regression line; log cDNA dilution plotted against the threshold cycle (Ct) using the formula E = 10–1/slope. Relative gene expression, normalized for efficiency, was determined following the method described by Pfaffl (2004), utilizing the endogenous reference genes elongation factor 1a (ef1a) and bACTIN. Each qPCR plate included a duplicate no-template control (NTC) to verify the absence of contamination. Additionally, plate inter-calibration was ensured by running a duplicate pool on each plate.

5. Findings, discussion and conclusion

5.1. Knowledge base on spiny dogfish behaviour and industry's experience

The spiny dogfish (*Squalus acanthias*) is Norway's most common shark species. It is found across the entire Norwegian coast and is mainly a demersal fish. It lives at depths up to 1400 meters, and often appears in shoals of several thousand individuals. Usually, the schools are composed of either, (1) very large mature females, (2) medium size individuals, all mature males or all immature females, or, (3) small immature individuals of both sexes in about equal numbers (Bigelow and Schroeder 1953; McMillan and Morse 1999). Although study on spiny dogfish population in Norway are limited, in general male dogfish were found in shallower water than females of the same size (Albert, Edwards, and Matthiessen 1961). However, large pregnant females migrate to shallower water to bear their young. Juveniles are most abundant in banks and shallow water. Their movement is partly dependent on seasonal temperature variation and studies show their temperature preference between 7 - 13 °C range. Studies show that primary diet of spiny dogfish is fish but they also eat bottom dwelling and swimming invertebrates (Albert, Edwards, and Matthiessen 1961). Although killer whales may feed on dogfish, the natural predators of spiny dogfish in Norwegian water are not known.

Th spiny dogfish is a slow growing species. The female becomes sexually mature at 11-12 years of age. It takes two years for eggs to mature. Breeding takes place from December till February. Gestation period is 23 months and pups are born in November/December. New eggs develop during the females' pregnancy, and one month after she has given birth, she is ready to breed again. Sexual maturity occurs at the age of 5–6 years for males in the Atlantic Ocean. Spiny dogfish appear to maintain a certain level of site association, often within coastal areas, in contrast, offshore units appear highly migratory. Little is known about the ecology, habitat, population size or migratory behaviour for dogfish population in Norway.

Plans and measures to counter act the witnessed spiny dogfish interaction with aqua culture installations, resulting in escape incidents of the produced fish, must take this into account. ICES considers the spiny dogfish stock to be at a historically low level, but recently sees signs of an increase in biomass and recruitment. The precautionary approach is nevertheless used in the absence of a stock index.

5.1.1. Incidents of spiny dogfish in fish farms

Fiskeridirektoratet in 2020 (https://www.fiskeridir.no/Akvakultur/erfaringsbase-romming/erfaringshendelser/Pigghaa-og-fare-for-roemming) reported an article on incidents of spiny dogfish in fish farms. ROV inspection showed a lot of dogfish activity around the lower parts of the netcages. There were holes in the net during Lift UP and spiny dogfish were inside the netcage. The light from the ROV attracted salmon which then pressed onto the net and approx. 20 salmon were squeezed out through the holes. There were several dogfish left inside the netcage, that had to be removed. Prior to the incident, the cage in question was treated for lice. Due to bad weather conditions for next two days, there was no recording of dead fish. There was a

problem with the LiftUp which had stopped due to of the increased quantity of dead fish, and the next day the Lift Up funnel was hoisted up to resolve this agglomeration of dead fish. It is probable that dead fish have slipped into the tip of the net and remained below the LiftUp funnel when LiftUp was subsequently lowered. Dead fish attracted the dogfish.

Spiny dogfish are attracted to dead fish that remain in the net and they can bite holes in the net to eat the dead fish. Such incidents have led to the farmed fish being able to escape, and at the same time for large numbers of dogfish to enter the netcage. In recent years, the Directorate of Fisheries has multiple reports of such incidents with spiny dogfish in Ryfylke in Rogaland.

In areas with dogfish activity, the risk of increased mortality and incorrect placement of the LiftUp / dead fish system must be included in the risk assessments. Routines must be established that ensure proper control, so that the dead fish system is installed and set up correctly and functions as intended. Furthermore, there must be routines and extra care to be taken in the event of increased mortality and/or weather conditions that prevent daily emptying.

Fiskeridirektoratet recommended following measures in response to the incident:

• Perform the lice treatment of fish during the day, as it turns out that the dogfish are more active and aggressive at night.

• Escape of fish in connection with accumulation of dead fish under the LiftUp funnel that attracts predators has been introduced as a new risk factor.

• Turn the net bag at the bottom of the net when exposing LiftUp.

- Introduce a routine for inspection also on the outside of the seine tip when processing fish and exposing LiftUp.
- Mount the net bag under / around the tip of the net to prevent predators from reaching.

Incidence at Stad Municipality: escape of 1500 salmon with an average growth of 1.5 kg after a spiny dogfish bit through the net and entered the netcage at Nordforsk experimental station (https://ilaks.no/piggha-forarsaket-lakseromning-ved-stadt/).

July 2021. Hundreds of dogfish broke into a waiting cage for mackerel (https://www.fiskeribladet.no/fiskeri/hai-spiste-opp-makrellen-var-sikkert-et-hundretall-piggha-som-brot-seg-inn-i-ventemerden/2-1-1037240). Fisherman report that they have had problems with dogfish when fishing for mackerel but have not experienced that the dogfish has broken into mackerel cages. The fishermen experience is that the dogfish activity correlates to the lunar phases, but have the impression that the problem with a dogfish is more frequent now than before and less dependent of lunar phases.

October 2020: On 23 October, Osland Stamfisk reported fish escape from their locality at Søreide in the Høyanger municipality in Western Norway (https://www.firda.no/truleg-piggha-som-sleppte-fri-oppdrettsaure/s/5-15-1099271). There were a series of holes in the net, probably caused by spiny dogfish. The company has recaptured 421 rainbow trout.

October 2017: Rainbow trout escaped after spiny dogfish attacks (https://ilaks.no/regnbogeaure-pa-rommen-etter-piggha-angrep/).

December 2020: spiny dogfish created a problem in a fish cage in Måren in the Sognefjord, at Osland Havbruk (https://www.bt.no/nyheter/lokalt/i/oJe4V/hai-angrep-oppdrettsanlegg). Dogfish were found to have entered one of the cages. The dogfish had gnawed several holes in the net. About 40-50 fish escaped.

December 2020: Mowi experienced spiny dogfish attacks at their site at Vedøya in Namsos (https://ilaks.no/spiste-hull-i-to-noter-hos-mowi/).

The possibility is opted that larger wild fish are attracted to salmon farms due to aggregations of smaller prey fish feeding on the associated waste (predominantly unconsumed feed pellets sinking through the bottom of net cages). The majority of the wild fish that aggregate at salmon farms stay within 25 m of the cages. In Mediterranean open-cage fish farming, where bluefish Pomatomus saltatrix is present, there have been incidents where these bite holes in the net wall to prey on the farmed fish. However, similar problems have not been documented for salmon farming, but spiny dogfish Squalus are thought to have caused escapes because they created holes in the cage during attempts to prey on dead salmon through the lower part of the netcage.

In (Gaitán-Espitia et al. 2017) the stomach contents of over 100 spiny dogfish, collected from bycatch in Chilean waters in the vicinity of salmon pens were analyzed. Over half of the specimens had salmon feed pellets as part of their stomach contents, indicating that spiny dogfish is directly feeding on waste pellets at the pen locations. We are in the process of conducting interviews with fish farmers in Norway to gain better insight into increased risk of attack by spiny dogfish.

5.1.2. Mapping the incidents and impact of spiny dogfish

Geographical distribution of spiny dogfish incidents: To investigate the occurrence and impact of spiny dogfish incidents in fish farms in Norway, we conducted survey-based research. The questionnaire was distributed by email to specific fish farms and aquaculture clusters across Norway. A summary of distribution of affected fish farms and extent of incidents is presented in **Figure 20**. Twenty-four large and small fish farms across all production areas in Norway responded to this questionnaire. Out of the 24 respondents, 10 respondents (42%) reported no incidences of spiny dogfish attacking their fish cage, while 14 respondents (58%) reported that they have had incidents of spiny dogfish attacking fish cages in the last 2-5 years (Figure 21). The affected farms were mostly located in Vestland (Bømlafjorden, Hardange fjorden, Sørfjorden, Sognefjorden, Sunnfjorden, Høgsfjorden, Frøysjøen, Bjorøyosen), Rogaland (Boknafjorden, Vindafjord, Ryfylke, Nedstrandsfjorden, Hjeltefjorden) and Trøndelag (Rørvik, Namsenfjorden, Ånholmen). The reported depth of fjord surrounding the fish farms ranged from 50 m to 400 m with an average depth of 147.1 m. The majority of respondents (10 out of 14, 71%) reported that usually 1-2 cages at a location are attacked in an incident; 14% (2 of 14) reported that over half of all cages gets attacked; 14% (2 of 14) reported that all cages at a location were affected by spiny dogfish. Interestingly, one respondent (7%) reported that none of the cages were damaged; however, this farm still had to take measures to avoid spiny dogfish attacking the cages. Some commented that they have taken measures that limited the number of affected cages.

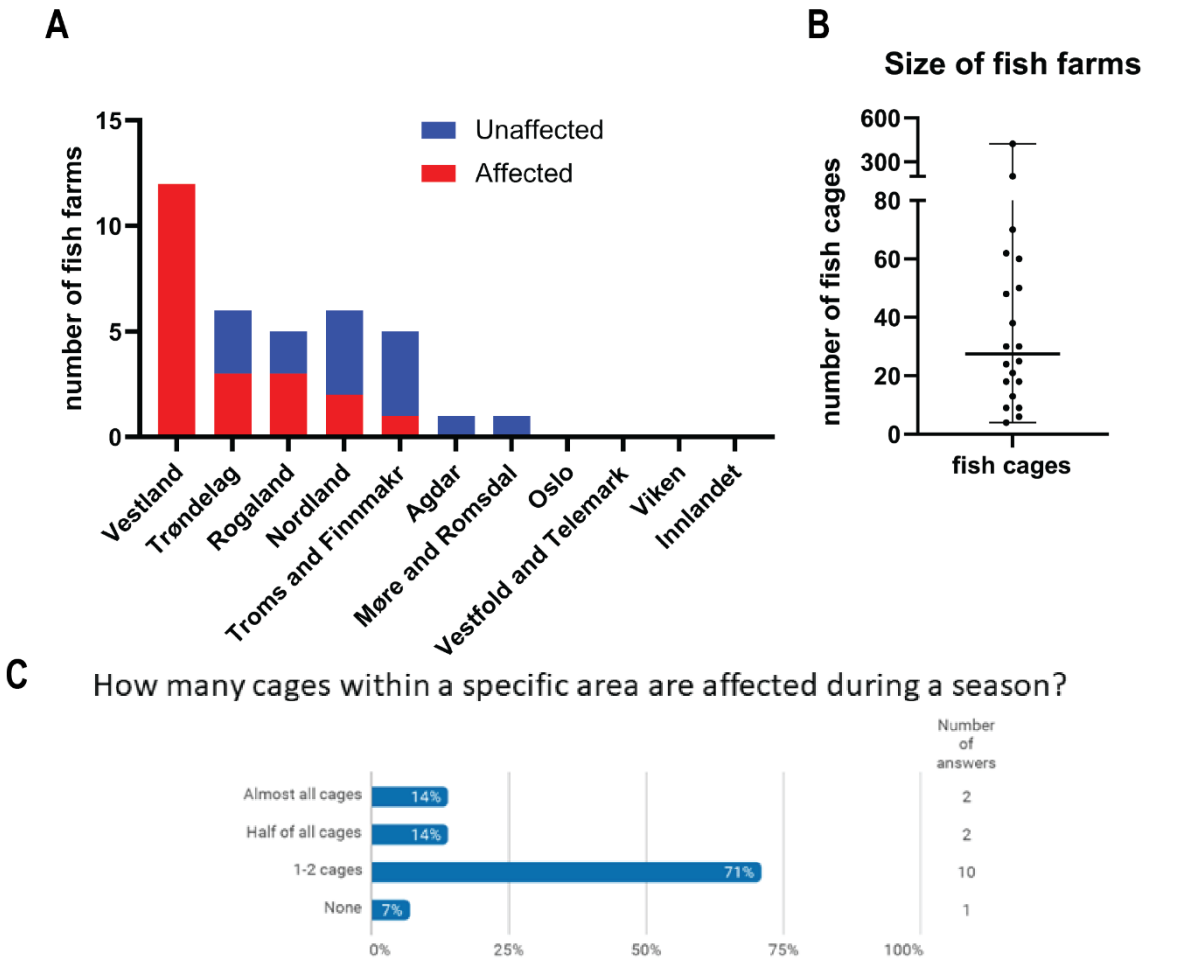


Figure 20 Distribution of fish farms. (A) Affected fish farms are largely located in Vestland, Trøndelag and Nordland regions. (B) Both small and large fish farms responded to the survey (C) Fraction of affected cages at a site during a season.

Economic impact: These incidents are seasonal. Farmers take necessary measures to avoid dogfish incidents and contain the damage caused by these predators; When there is suspicion of dogfish incidence, the cages are inspected with divers or ROV. The self-reported economic cost is reported to range from 50 000 NOK to over a million NOK; over half of the respondents report a normal cost of about 500 kNOK per season and have spent 5-8 mill NOK over last 5 years per affected site. This cost is associated with repair of damaged nets, surveillance for dogfish, handling dogfish after an incident and loss due to escapees.

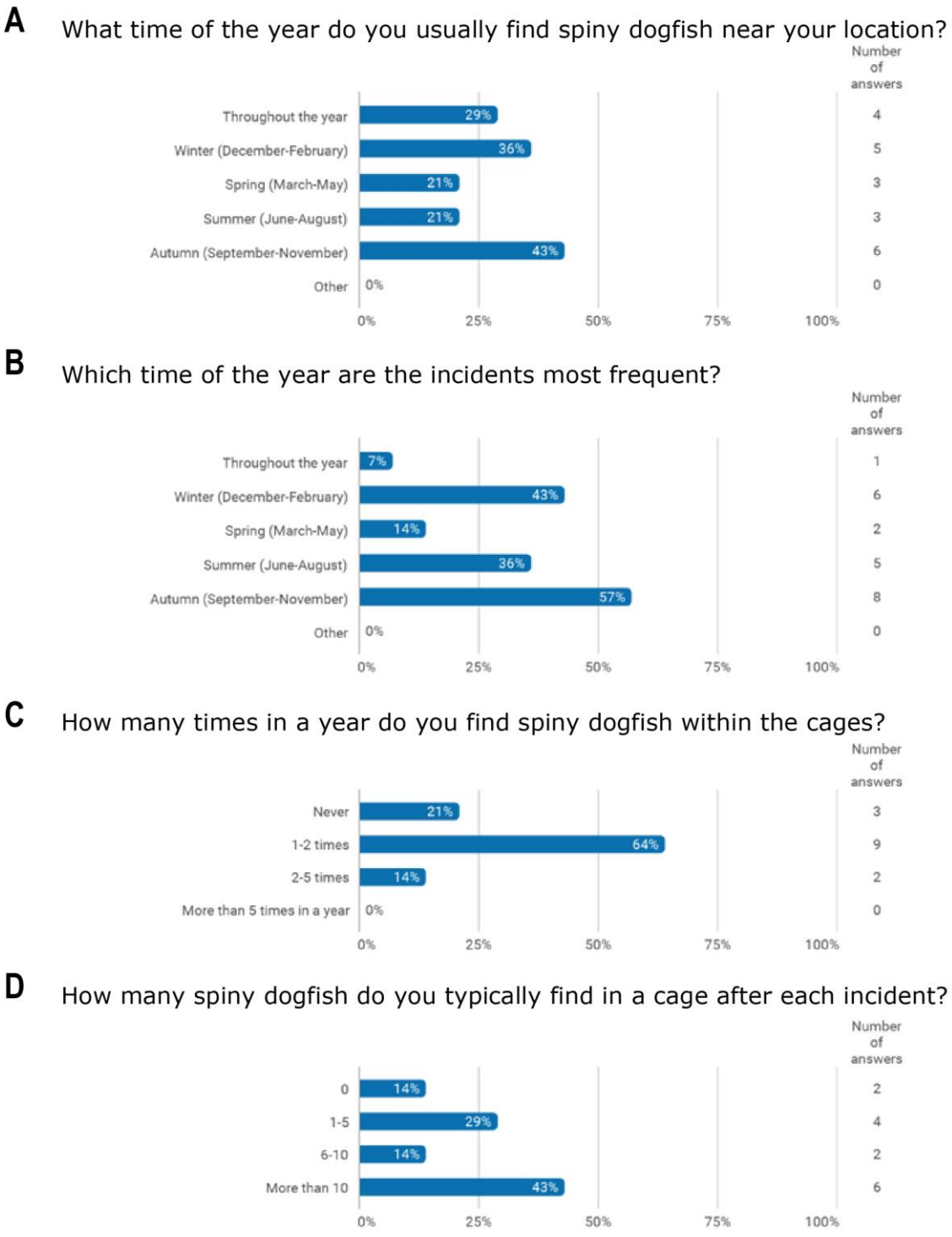


Figure 21 Seasonality of dogfish attack. (A) Sighting of dogfish. (B) Incidents of dogfish in fish farms. (C) Frequency of finding dogfish in cages. (D) Number of dogfish in cages per incident.

Seasonality and characteristic of dogfish incident: The spiny dogfish incidents were reported to be seasonal (**Figure 21**). The spiny dogfish were observed around the fish farm mostly during autumn and winter, however in some sites they have been reported throughout the year (**Figure 21A**). The incidents of dogfish attack follow a similar pattern as their sighting; autumn and winter months present higher risk (**Figure 21B**). Diverse variety of fish species including pollock, haddock, horse mackerel, mackerel, whiting, cod and in some case harbour seals are sighted around the fish cages

during the affected seasons. These sharks in most cases make a hole and get inside the cage (**Figure 21C**); 64% (9 of 14) reported that dogfish are found inside the fish cage 1-2 times a year and 14% (2 of 14) respondent reported that dogfish manage to get inside the cage 2-5 times a year; 21% (3 of 14) respondent said the dogfish never manage to get inside the fish cages (**Figure 21D**).

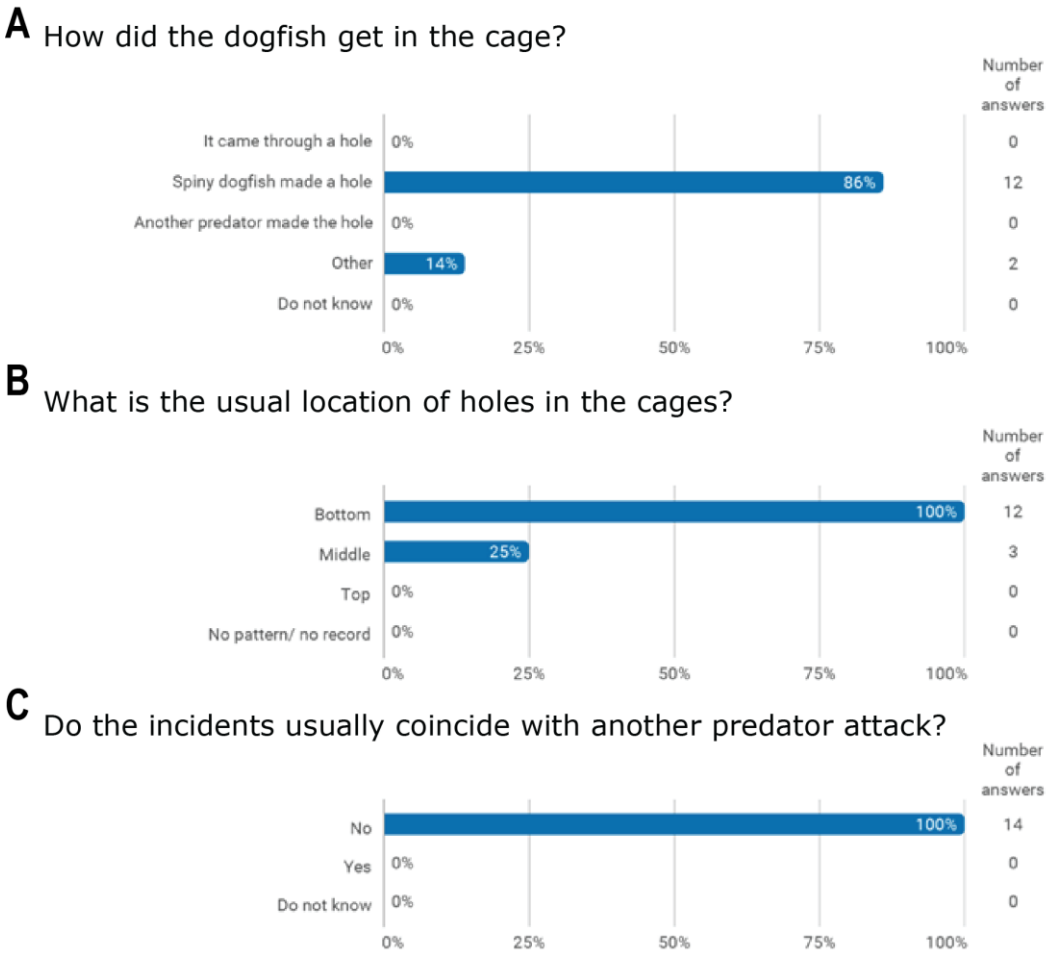


Figure 22 About spiny dogfish and fish cage. (A) How did the dogfish get in the cage. (B) location of the hole. (C) Predator attack.

The reported size for spiny dogfish was 50-120cm with about 70-80cm length being most frequent; the sex distribution is not known. Farmers usually did not perform further investigation on sex or physiological status such as pregnancy in found dogfish. Almost all farmers report that spiny dogfish made a hole to get in the fish cages (12 of 14, 86%) (**Figure 22A**); usually the spiny dogfish incidents do not coincide with another predator (**Figure 22C**). One farmer reported that mackerel sturgeon chasing mackerel when the incidence happens. The usual reported size of the hole in the cage was 10-15cm, but sometime the holes were larger than 25-40cm.The hole were mostly present at the bottom (12 of 12, 100%) and middle (3 of 12, 25%) part of the cage (**Figure 22B**). Almost all respondents used usual nylon net as fish cage material. Age of the netting material varied from 2 months to 5 years. Hence, both new and old cages were affected. Some farmers have started using HDPE nets and other shark safe nets. Although evidence is not apparent, responded report fewer incidents of dogfish biting through the net. Nonetheless, farmers follow similar routine of cleaning dead fish in the cages and surveillance for damage to net.

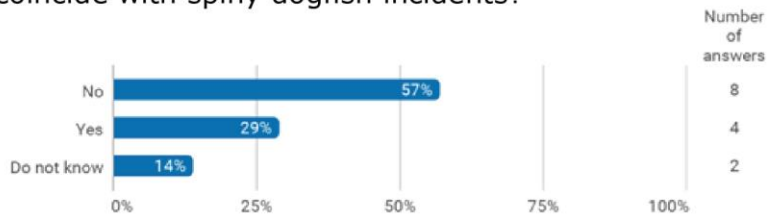
5.1.3. What attracts spiny dogfish to fish farms?

To identify the factors that may attract these sharks to a particular fish farm, we investigated the correlation of dogfish incidents with parameters of farm operation. The majority of respondents (8 of 14, 57%) think that dogfish attack is not related to any farm operation including delousing, and high sea temperature (**Figure 23A**). It did not correlate with transfer of smolts (**Figure 23B**) even though highest mortality of farmed salmon is reported in the first two months of smolt transfer. 11 of 14 respondents (79%) reported that the affected cages had fish of size above 2Kg (**Figure 23C**); 9 of 14 respondents (64%) reported that there were dead fish in the affected cages (**Figure 23D**). The sharks ate the dead fish (6 of 9, 67%) (**Figure 23E**); the dogfish were not aggressive inside the cage and did not attack the live fish (9 of 14 respondent) except in one case where dogfish attacked the live fish (**Figure 23F**).

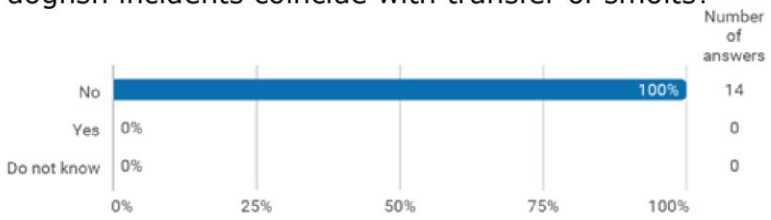
Since delousing operation is associated with stress response and mortality in farmed salmon, we further investigated whether dogfish incidents are directly correlated with delousing. Delousing operation was not seasonal and dependent on the farm site (**Figure 24A**). Majority (10 of 14, 71%) of respondents reported using freshwater treatment as preferred delousing method, however respondents also reported using medicated feed, cleaner fish and thermal method (**Figure 24B, 23E**); there was no relation between delousing method and dogfish incidents. 47% (6 of 14) thought that dogfish incidents correlate with delousing operation; they reported holes in the cage within 7 days of delousing (**Figure 24C-24D**). It is likely that the smell from dead fish during the delousing operation attracts spiny dogfish to a location.

The majority of spiny dogfish incidents happened during autumn and winter (**Figure 24A**), we explored whether light conditions in the farm attract dogfish. Most respondents use anti-maturation lights (12 of 14); submerged light was used by all respondents (12 of 12, 100%). However, the majority (8 of 12, 67%) reported that dogfish incidents do not coincide with turning on anti-maturation lights in the cages.

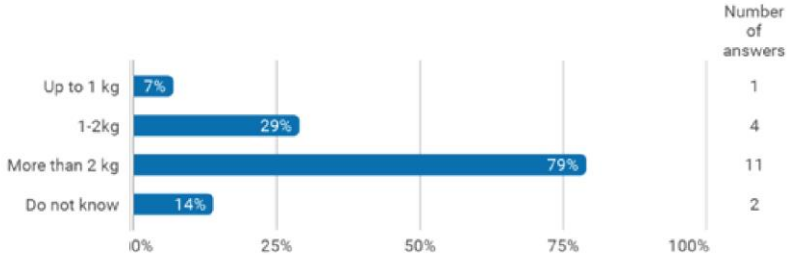
A Are there any operations (e.g. delousing, high sea water temperature etc.) that usually coincide with spiny dogfish incidents?



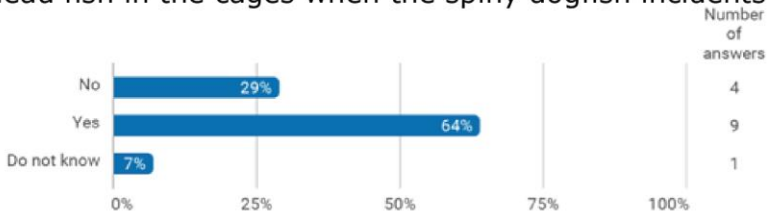
B Do the spiny dogfish incidents coincide with transfer of smolts?



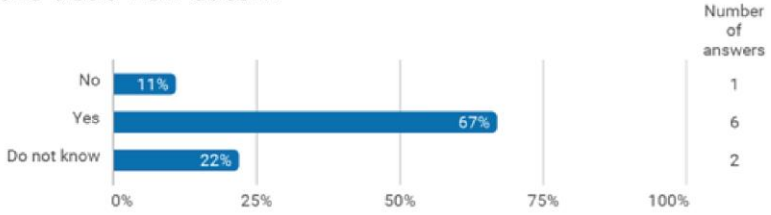
C What is the size of aquaculture fish when the spiny dogfish incidents are most frequent?



D Were there dead fish in the cages when the spiny dogfish incidents happened?



E If yes, were the dead fish eaten?



F Were the live fish attacked?

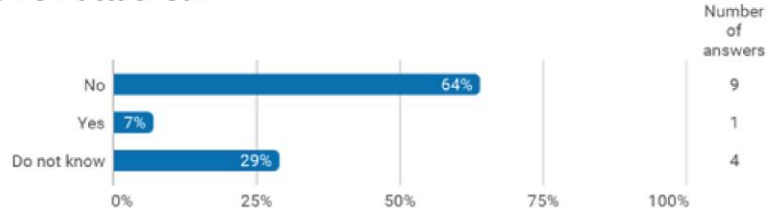
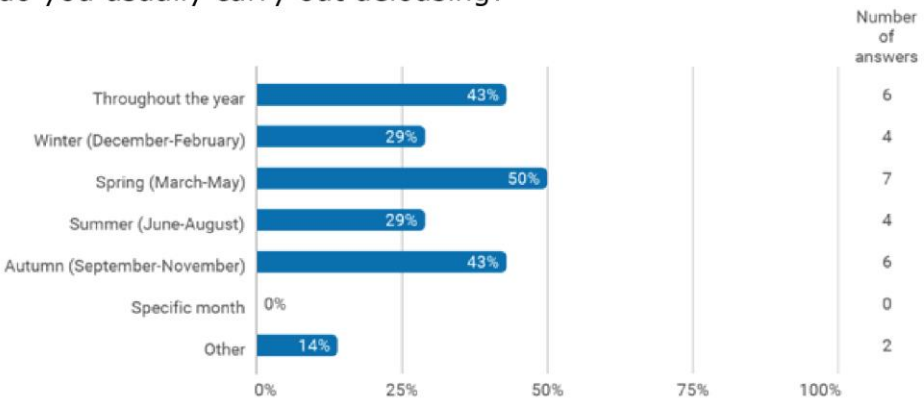
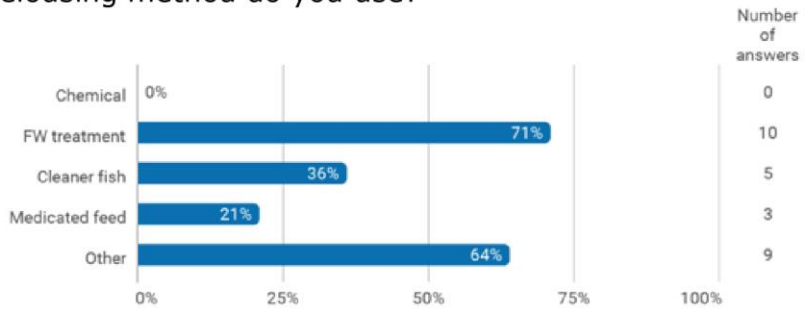


Figure 23 Farm operation and dogfish behaviour. (A) Farm operation vs dogfish incident. (B) Transfer of smolts vs dogfish incident. (C) Size of farmed fish in affected cages. (D) Presence of dead fish. (E) Feeding behaviour of dogfish in the cage. (F) Aggression of dogfish in the fish cage.

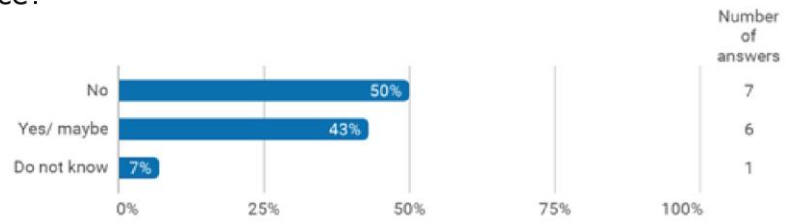
A When do you usually carry out delousing?



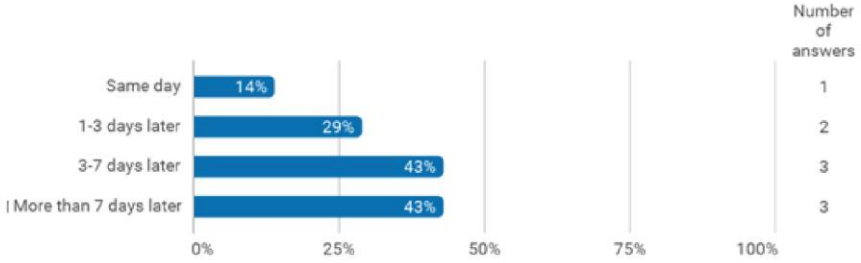
B What type of delousing method do you use?



C Farmers often report dogfish incidents after delousing operations. Do you have similar experience?



D How many days after delousing operations, did you find spiny dogfish inside the cages?



E What type of delousing methods had you used for the affected cages?

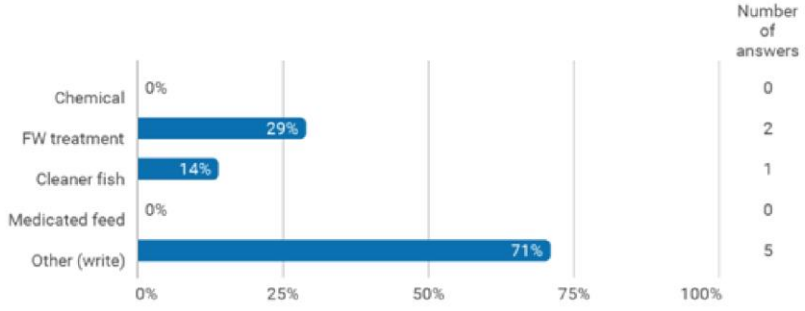


Figure 24 Delousing and dogfish behaviour. (A) Seasonality of delousing. (B) Delousing methods. (C) Opinion on delousing and dogfish incidents. (D) Presence of dogfish after the delousing operation. (E) Delousing methods used in affected cages.

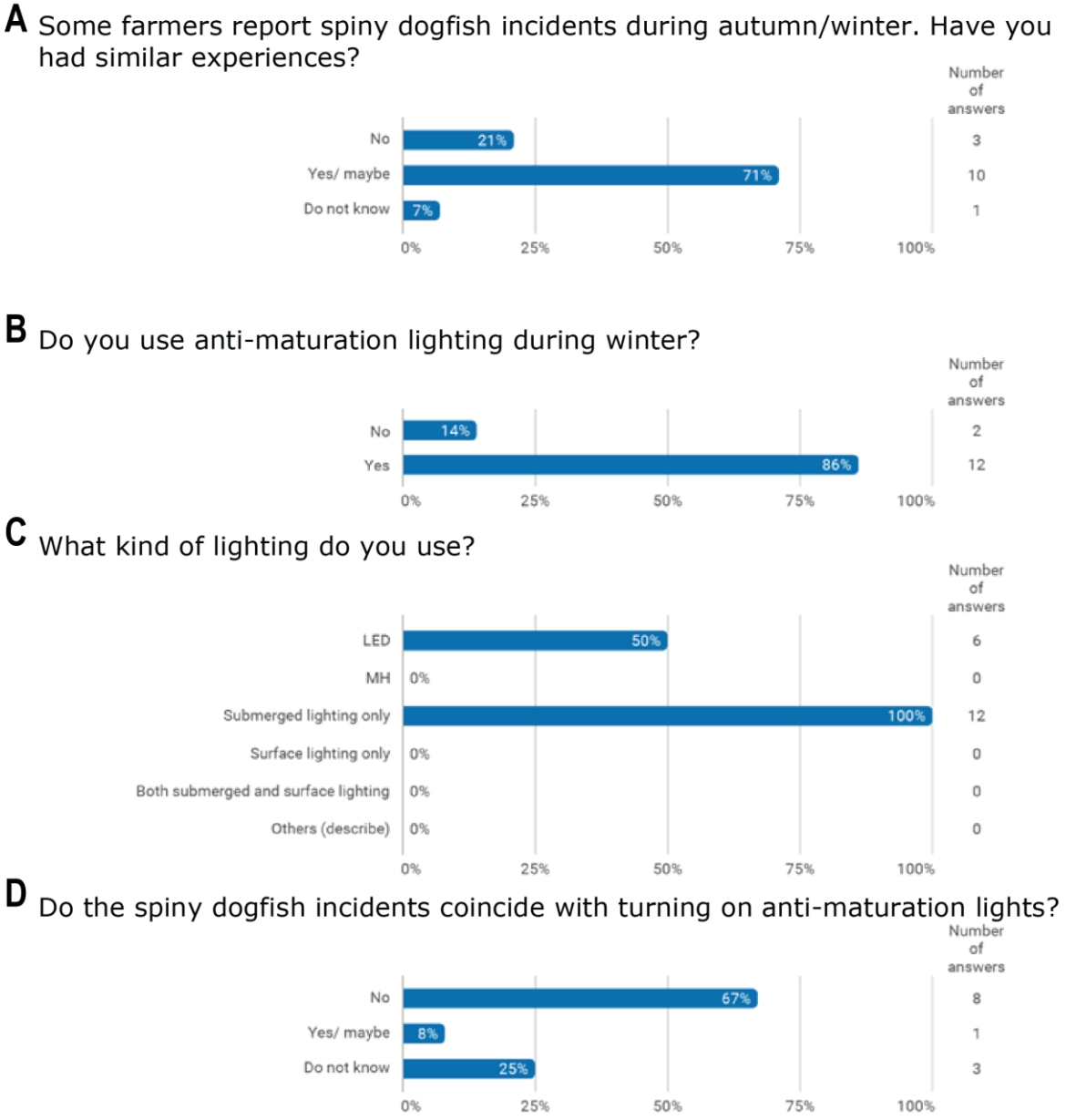


Figure 25 Delousing and dogfish behaviour. (A) Seasonality of delousing. (B) Delousing methods. (C) Opinion on delousing and dogfish incidents. (D) Presence of dogfish after the delousing operation. (E) Delousing methods used in affected cages.

5.1.4. Summary of survey result and recommendations

The spiny dogfish are always around in the fjord. The smell from dead and decaying dead fish in the cages attract these sharks to a particular cage. The shark incidents are rare if dead fish cleaning is well managed; regular removal of dead fish from the cages seems to mitigate dogfish-related issues. Fish farmers regularly remove dead fish. But circumstances such as extreme weather, current, storm and wind conditions and/or technical failure of dead fish cleaning equipment that hamper normal

farm operation can lead to some dead fish being left along the net wall, providing an opportunity for these sharks to feed. Many farms use wrasse as cleaner fish: it takes longer for dead wrasse to sink to the bottom as wrasse has too little mass to sink. The smell from decaying wrasse can travel by currents. This scavenging behavior of spiny dogfish appears to be the main cause of holes in the net. In addition, stressful treatments like de-lousing can lead to heightened dogfish activity and potential net damage. Overall, diligent management of dead fish and regular observation of net damage using ROV, and divers help prevent dogfish-related damage to nets.

The sharks do not attack all the cages. The attack mostly happens at night. The dogfish are attracted to dead fish and are observed mostly with a calm swimming pattern when they are inside the cages. Some say that sharks eat only the dead fish while others report that spiny dogfish also attacks the live farmed fish in the cages. Interestingly, farmers report that spiny dogfish appear to be particularly aggressive during mating season. Facilities situated along the permanent migration route of dogfish, especially those in fjords where they give birth, are more likely to experience encounters. The moon phase also appears to influence their activity; however, it is likely that spiny dogfish behavior could be regulated by natural light rhythm.

Based on the survey and interview of respondents, the following recommendations may help keep spiny dogfish away from fish farms:

- Treatment of fish during the day as spiny dogfish are more active at night
- Periodic inspection of cages after delousing operations
- Improved routines on cleaning dead fish especially during autumn and winter
- Use of shark safe nets such as HDPE and PET and double netting should be considered.

5.2. Sensory induced behavioural response in spiny dogfish

A detail overview on sensory biology and anti-shark measures have been discussed in the thesis (Brynildsrud, M.E. 2023). The following results are reused from the thesis with some modifications.

Here, we aimed to successfully catch and temporarily house wild spiny dogfish to investigate whether stimulatory cues of biological importance would elicit aversive behavior. We tested screams from their natural predator (Orca screams), odor from deceased conspecifics (skin extract) and electromagnetic fields with varying impulse duration and strength. Additionally, we wanted to evoke a contrasting attractive behavioral response with food odor from mackerel. The behavior was observed in real-time for qualitative evaluation and analyzed with a deep-neural network. Additionally, we examined whether the skin extract, electromagnetic field, and food odor would imprint as a physiological response. This was evaluated by analyzing the metabolite composition in blood serum after being subjected to the stimuli.

5.2.1. Husbandry of spiny dogfish

Capturing wild spiny dogfish and then keeping them alive, fed, and in good health throughout the trials were paramount to the project. We captured a total of 22 spiny dogfish, 13 males and 9 females between 61-88 cm. As all sharks survived until euthanization, we could conclude with a near

100% survival rate for Spiny dogfish in captivity in this project. It was necessary to euthanize one shark in Group 1 before the initiation of trials due to an abrupt change in behavior; the shark was swimming in small circles on its side, and the locomotive capability seemed impaired. We observed injuries in some individuals, snout damage was most common and found in 5/22 sharks. In addition, we observed several sharks with lice (unknown species), mostly attached to either of the dorsal fins.

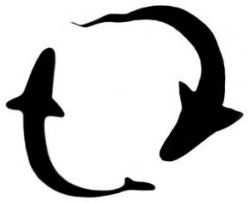
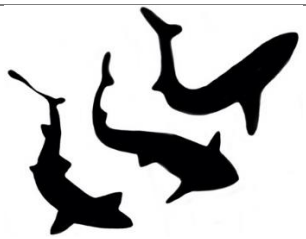
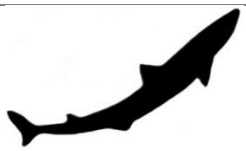
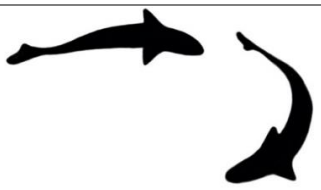
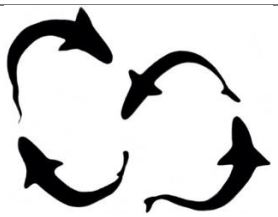
When provided with frozen feed, the sharks showed little to no interest in eating. Some biting on the feed was observed but most of the pieces were left on the bottom of the tank. The appetite was enhanced when they were fed with thawed mackerel every third day. Group 1 started to eat 9 days post-capture, while groups 2, 3, and 4 started to eat 3-4 days post-capture. The most successful feeding regime was implemented through the rest of the trial; one piece of thawed mackerel was offered to each shark in each tank every 3rd or 4th day, and leftover feed was removed after two days. Two sharks in Group 3 Tank 1 were fed salmon, as they did not eat the mackerel.

5.2.2. Qualitative observations of locomotion

The animals performed individual behavioral traits and swimming patterns. Based on real-time observations during experimental trials, the locomotive performances were characterized into nine swimming patterns: normal swimming, circling, hovering, sideways swimming, looping, freezing, resting, C-shaped turn, and foraging (**Table 5.1**). The normal swimming pattern of spiny dogfish was characterized when the shark kept a horizontal position in the water column with a back-and-forth flap of the caudal fin and swimming at moderate speed. Due to the closed confinement in a circular tank, the baseline swimming pattern included soft turns following the edge of the tank with occasional crossings through the center. When performing a turn, the locomotion performance was initiated by a head nudge followed by a contraction of the mediolateral body and a flap of the caudal fin. The placement in the water column was mostly towards the surface or in the middle, with occasional crossings towards the bottom of the tank. Differences in swimming routines between individuals ranged from a stationary “resting” position at the bottom of the tank to continuous movement. A common locomotive performance, hereby termed “hovering”, was observed among all experimental animals **Table 5.1**. The hovering took place at the water surface, mainly at two hotspots: near the wall under the light source, and near the water outlet.

Table 5.1. The observed swimming patterns during the qualitative observations in the behavior trials.

Locomotion performance	Description	
Normal swimming	A horizontal position, anterior and posterior body on the same level in the water. As the caudal fin flaps to one side, their mediolateral part of the same side of the body contract (ipsilateral). Caudal fin flaps back and forth which influence the speed.	

Circling	(1) Moving away from the wall, towards the middle of the tank and back, or (2) swimming in circles only using the middle of the tank. Mostly towards the bottom.	
Hovering	A vertical position, head above water and tail towards the bottom. Shark stays at the same place despite moving. Tail flapping frequency increases. Often include a succeeding change of direction.	
Sideways swimming	Shark swims with a lateral orientation towards one specific side. The white ventrolateral underside of the body can be seen.	
Looping	Swimming towards the bottom and turning vertically towards the surface with the belly up, before circulating down in the water column again. Have earlier been described as a stress response in other shark species.	
Freezing	Momentary detain of movement.	
Resting	The shark displays an abrupt cease in movement and sinks to the bottom, but keeps steady and continues movement of the gills. The duration of this behavior could continue for a couple of seconds to a couple of hours.	
C-shaped turn	A sudden change of direction. Depending on situation and speed of execution of the turn, this locomotion was often observed during a possible escape response or during foraging.	
Foraging	Searching towards the bottom with rapid turns and tranquil motion.	

The behavioral effect of orca sounds

We did not see an aversive response or a clear change of locomotion or behavior when visually observing the shark behaviour during exposure to sound stimuli.

Effect of food odor on behavior

Individual variations in response to food odor were observed. Some sharks elicited a change of behavior between 30 seconds to 1 minute succeeding the application of the odor. They shift from a normal swimming pattern following the tank wall to an abrupt turn towards the plastic tube where the odor was delivered. This response could be followed by foraging behavior. Shark 11 elicited prolonged foraging behavior, similar to that observed during feeding in the housing tanks. However, many food trials showed little to no clear response succeeding the addition of food odor.

Effect of skin extract on behavior

Most sharks elicited a change of locomotion succeeding exposure to skin extract from their conspecifics. Despite individual behavioral responses, the most frequent locomotive change was increased swimming speed and rapid turns. Shark 5 showed a loss of navigational skills and looping behavior. The effect of skin extract was especially remarkable in Shark 2, as this animal elicited frequent "resting" behavior which was abrupted succeeding the addition of odor. In some trials, no significant change of behavior was observed. One of the 0.5 U trials of Condition 3 evoked an extreme behavioral change, as the shark swam seemingly out of control at high speed for about 30 seconds.

Effect of electromagnetic field on behavior – qualitative observations

Change in locomotion was observed succeeding the initiation of electromagnetic stimuli with 600ms, 300ms, and 100ms intervals between electric impulses. Most sharks had an immediate response when the electrical impulses started and returned to their normal swimming pattern shortly after the stimulus was gone. When entering the electromagnetic field, many sharks changed their swimming direction and seemed to avoid being in proximity to the electrodes. The initial response was typically a quick twitch of the head, abrupt or gradual C-turns, increased swimming speed avoiding the electrode area, and change of locomotion. The latter mostly included a change from normal swimming to circling. In general, the sharks had a more abrupt and significant change of locomotion during the first trials compared to the last trials. Most animals kept a distance from the electrodes and the EM-field during stimuli. However, they occasionally passed the rig closely. A general trend seemed to be a change of swimming direction either to the opposite side of the tank or passing from a greater distance when passing the negative electrode. Some of the sharks initiated looping.

5.2.3. Condition 1 – Behavioral effects of audio, odor, and electromagnetic field

A summary of locomotive response (distance travelled) to the audio, odor, and electromagnetic field by all sharks tested in Condition 1 is presented in **Figure 26**. "No stimulus" was recorded each morning before the trial each day. This is reflected in the plot by the cluster of data points and represents the baseline of their movement. However, these recordings were available for only four of the specimens. The general behavioral response was not altered by the sound stimuli (Orca and control). The seawater control, food odor, and skin extract trials elicited a change in locomotive activity. The figure shows a larger variation in distances traveled, in addition to individual behavioral

responses. Likewise, this can be observed in the distances traveled during the electromagnetic trials. The figure reflects the qualitative observations well.

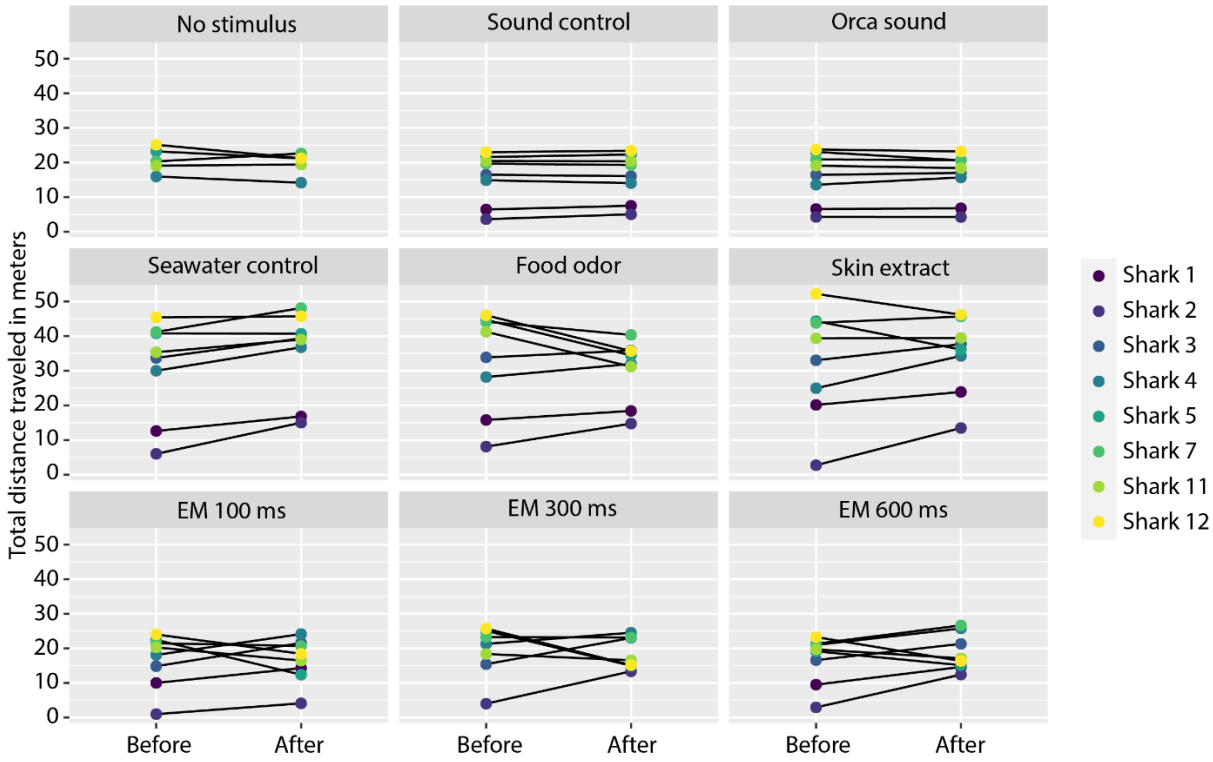


Figure 26 Comparing the mean of total distances traveled in meters before and after the addition of stimuli. No stimulus (n=5), rest of the stimuli (n=8). Each dot represents the average response from one shark to the stimuli.

Effect of Orca sound on spiny dogfish behavior

As illustrated in the before and after heatmap, shown in **Figure 27A**, the sharks mostly swam along the edge of the tank during the sound control and orca sound trials, performing occasional crossings, seemingly unaffected by the auditory stimulus. However, the change in distribution of position heatmap shows that the intensity of activity level following the tank walls was elevated during the sound control compared to the orca sound, where the activity was evenly distributed. The audio trials did not alter the locomotive activity significantly (t-test, $p>0.05$) (**Figure 27B1**). There was no significant difference in the fold change of locomotive activity between the control sound and the orca sound (t-test, $p>0.05$) (**Figure 27B2**).

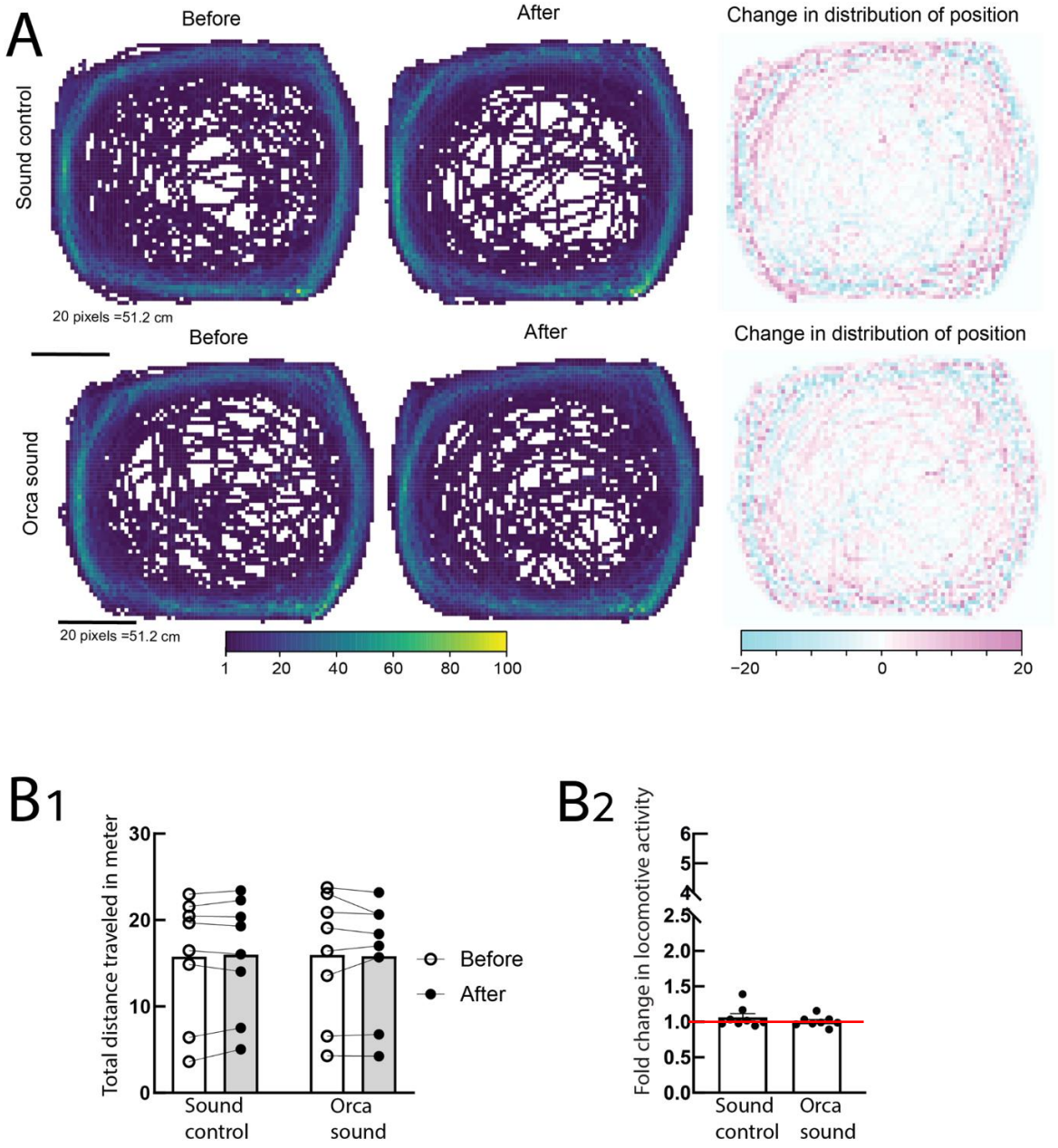


Figure 27 The behavioral change in response to orca sound. (A) Based on the density of data points from the x and y coordinates, the positions of the sharks (n=6) from all trials are combined and visualized in binned heatmaps before and after the stimulus. The color scale of the heatmap translates the movement intensity in the tank: purple represents locations with minimal movement, while green and yellow indicate locations with frequent movement. The white pixels show no registered movement. Data points exceeding 100 counts are oversaturated and can be observed in proximity to high-intensity areas. The scale bar is adequate for all heatmaps, where 20 pixels roughly represent 51,2 cm. of the tank. The change in position frequency is illustrated in a blue and pink color scale heatmap: decreased space use is indicated by blue and increased space use is indicated by pink. (B1) Bar plot with a pairwise comparison of the mean distances traveled before and after sound control and orca sound. Each data point represents the mean distance traveled from all trials executed with one specific shark. (B2) Bar plot with error bar comparing

the fold change from every trial conducted independently from the individual sharks. The red line divides the points >1 which signifies increased distance traveled succeeding stimuli, and points <1 which signifies decreased distances traveled.

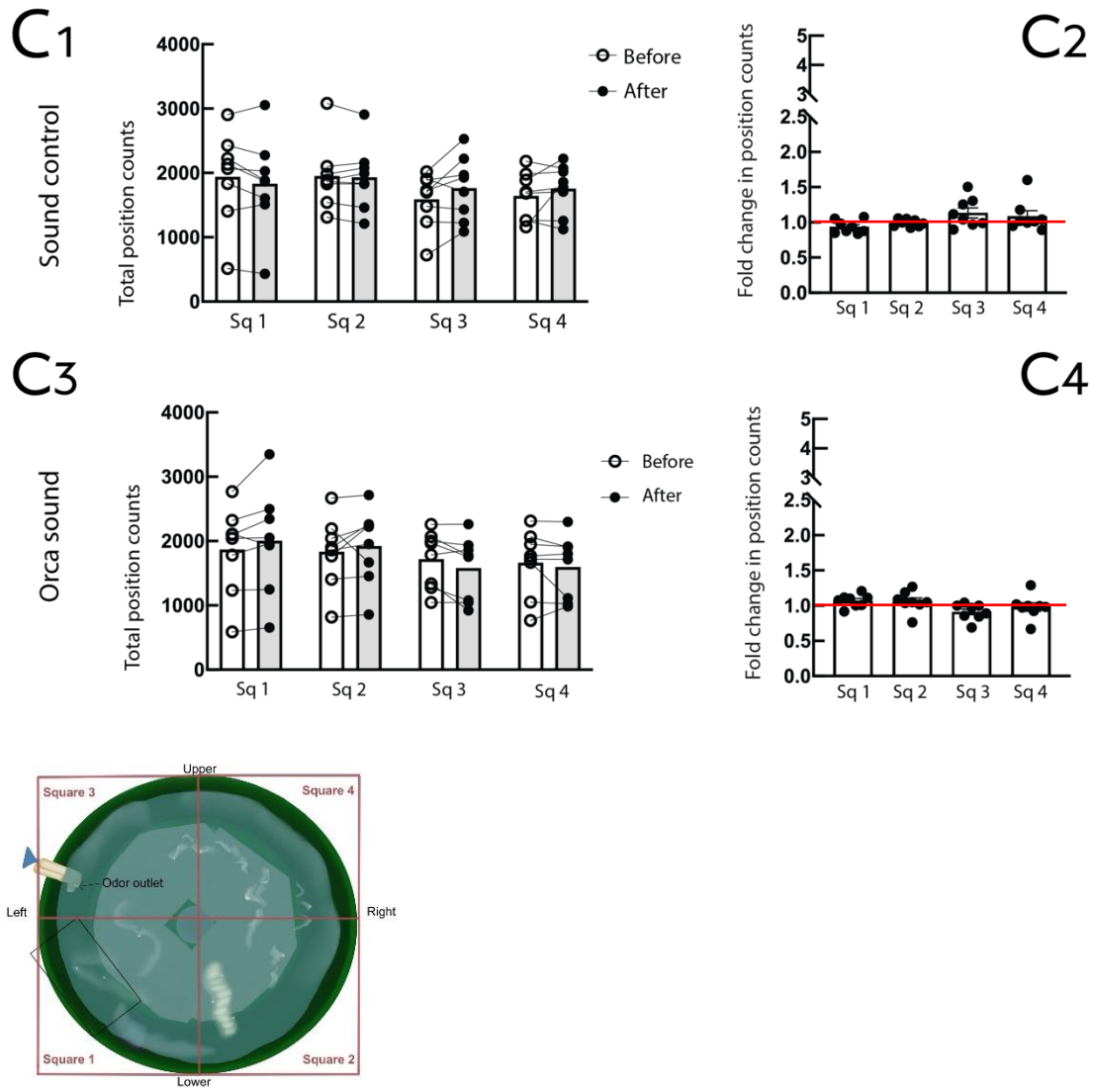


Figure 28 The total position counts from four arenas in the tank: Square 1 (Sq1), Square 2 (Sq 2), Square 3 (Sq 3), and Square 4 (Sq 4). (C1 and C3) Bar plots show the mean position count and compare the total position counts before and after stimuli, where each data point represents the combined location counts of all trials per shark (n=8). (C2 and C4) Bar plots show the mean fold change with each dot representing one shark. The red line indicates the fold change of 1, where the position counts before and after stimuli would be equal. Points above the line indicate increased activity after the onset of the stimuli. Below the line indicates more activity.

Alterations of the position in the tank as an effect of behavioral and locomotive change were studied by extracting the position counts from the four squares illustrated in **Figure 15**. The total position counts illustrated in **Figure 28 C1** and **C3** reflect the scarce change of behavior elicited by the sharks. The fold change was analyzed with one-way ANOVA and Tukey´s post hoc analysis and none of audio

stimuli resulted in a significant change of tank position ($p>0.05$). However, some sharks seem to have favored the upper part of the tank as several points in squares 3 and 4 surpass the red line after the sound control. Contrarily, the lower tank area was favored succeeding the orca sound (**Figure 28 C4**).

Odour trial: Behavior of spiny dogfish in response to food odor and skin extract of conspecific

Both odor stimuli and control affected the movement of the sharks and is visualized in the combined heatmaps (**Figure 29 A**). Prior to the stimuli the sharks mostly circled along the walls of the tank with occasional circling. Post-stimuli all odors stimulated a locomotive shift that resulted in increased space use of the tank. Interestingly, the seawater control seemed to cause the largest and uniform locomotive alteration, leaving nearly all pixels in the after-heatmap colored. The change of distribution heatmap in the seawater control trials appear with a blue outline and a dark pink center, which indicates that there was a shift from swimming in circles to rapid use of the whole tank. The change in distribution of position heatmap from the food odor trial shows the same blue coloration; however, the pink coloration in the lower and left sides of the tank (near the odor outlet) is noticeably darker. This shows that the food odor evoked a behavioral response in which the sharks swam in proximity to where the odor was applied. In the corresponding heatmap representing the skin extract trial, the coloration is to a greater extent evenly spread across and shows a darker pink coloration on the lower left side. In addition, there is a darker pink circle pattern in the lower middle part of the tank. This indicates that the addition of skin extract affected the swimming pattern and placement in the tank. Overall, the animals showed avoidance from the source of odor.

In **Figure 29 B1**, the mean of total distance traveled is compared. There was a significant change in the total distances traveled as an effect of seawater control (t-test, $p<0.05$). Surprisingly, this was the only chemical stimuli evoking a significant change. The food odor and the skin extract did not significantly alter the distances traveled unlike what was observed in the qualitative observations. It is evident from the figure that behavioral alterations did take place, however, the alterations were not homologous as some sharks shortened the traveled distance, while others increased theirs. In **Figure 29 B2,** the fold change is compared and shows similar results. These values were used to investigate the effect of the three stimuli compared to each other. There was a statistically significant change between the seawater trial and food odor (t-test, $p<0.05$), which could indicate that these stimulatory cues triggered diverging behavioral responses. By looking at the fold change in **Figure 29 B2**, these individual responses are clear. Shark 2 changed its behavior to an increasingly active state, which additionally can be observed in **Figure 29**.

Following exposure to seawater control the sharks exhibited increased position counts in square 1, while maintaining their movement in the rest of the tank (**Figure 30 C1 and C2**). Food odor affected the alteration of position counts significantly (*ANOVA*, $p<0.01$) (**Figure 30 C3 and C4**). Tukey´s post-hoc analysis revealed significant changes between Squares 1&3 ($p=0.02$), Squares 1&4 ($p=0.02$), Squares 2&3 ($p=0.04$) and Squares 2&4 ($p=0.03$). This indicates that the sharks favored the upper part of the tank before (Square 3 and 4) and shifted their preference to the lower part (Square 1 and 2) after food odor was applied. As for skin extract, **Figure 30 C5 and C6** show indications of positional alterations as the sharks increased their movement in the lower area of the tank. However, no significant changes were detected.

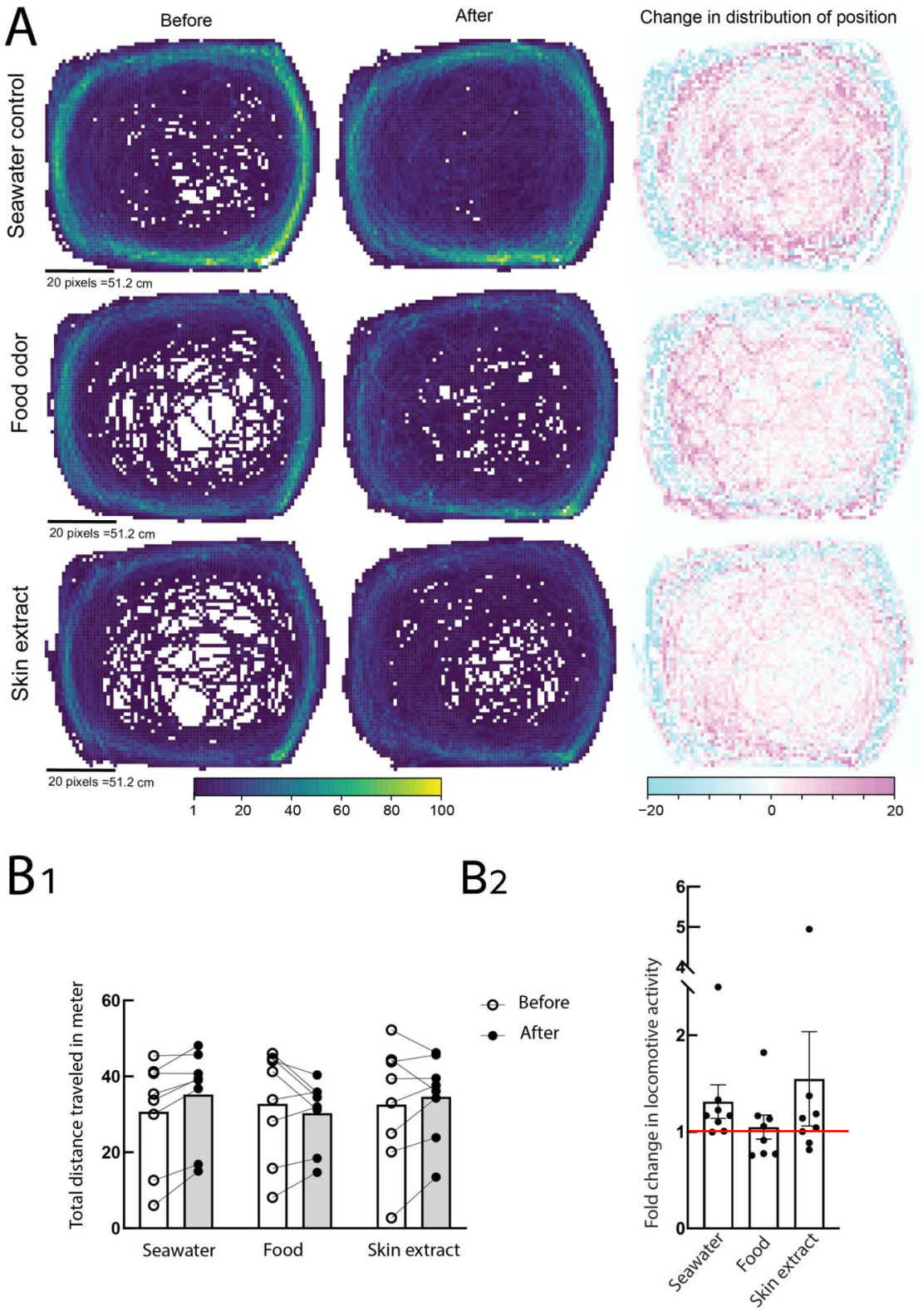
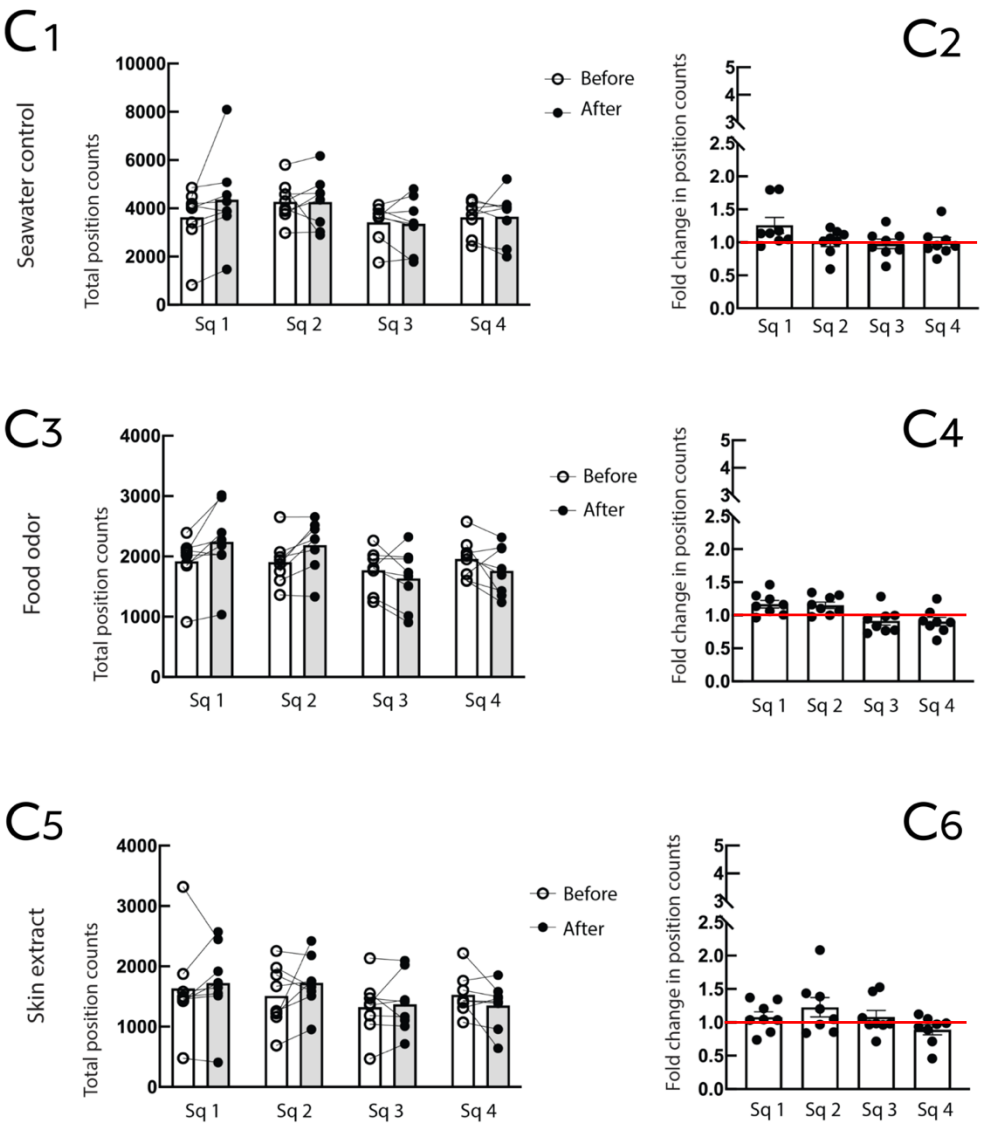


Figure 29 The behavioral change in response to seawater control, food odor, and skin extract, respectively. (A) Based on the density of data points from the x and y coordinates, the positions of the sharks (n=6) from all trials are combined and visualized in binned heatmaps before and after the stimulus. The color scale of the heatmap translates the movement intensity in the tank: purple represents locations with minimal movement, while green and yellow indicate locations with frequent movement. The white pixels show no registered movement. Data points exceeding 100

counts are oversaturated and can be observed in proximity to high-intensity areas. The scale bar is adequate for all heatmaps, where 20 pixels represent roughly 51,2 cm. of the tank. The change in position frequency is illustrated in a blue and pink color scale heatmap: decreased space use is indicated by blue and increased space use is indicated by pink. (B1) Comparing the mean distances traveled before and after seawater control, food odor, and skin extract. Each data point represents the mean distance traveled from all trials executed with one specific shark. (B2) Comparing the fold change from every trial conducted independently from the individual sharks. The red line divides the points >1 which signifies increased distance traveled succeeding stimuli, and points <1 which signifies decreased distances traveled.



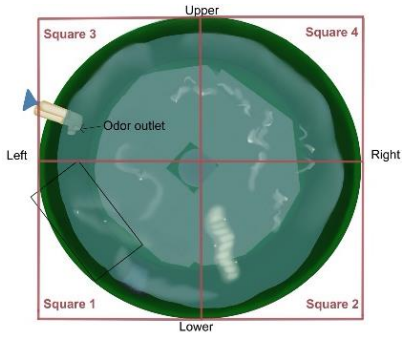


Figure 30 The total position counts from four arenas in the tank: Square 1 (Sq1), Square 2 (Sq 2), Square 3 (Sq 3), and Square 4 (Sq 4). (C1, C3, and C5) The bar plots show the mean position count and compare the total position counts before and after stimuli, where each data point represents one shark and their combined location counts (n=8). (C2, C4, and C6) The bar plots show the mean fold change with each dot representing one shark. The red line indicates the fold change of 1, where the position counts before and after stimuli would be equal. Points above the line indicate increased activity after the onset of the stimuli. Points below the line indicate a decrease in activity. The food odor trial (C4) showed a statistically significant change in placement.

Electromagnetic pulse induced behavior of spiny dogfish.

The impact of the 10 V electromagnetic field with 600ms, 300ms and 100ms pulse intervals affected the movement of the sharks (**Figure 31 A**). Prior to the onset of electromagnetic fields, the trend of swimming along the wall is clear – with occasional center crossings. The “before” heatmaps from all three intervals exhibit a similar pattern with high movement intensity following the tank walls. An alteration of movement is visible in the “after” heatmaps, where larger areas of the tank are being utilized, and the color scale shows less intensity along the walls. This indicates a shift in locomotive activity as an effect of the electromagnetic fields. The 600ms and 300ms “change” heatmap shows a dark pink color on the right side, which indicates that the shark spent more time on this side after the stimuli onset. The behavioral change indicated by the “after” heatmap succeeding 100ms EM field is similar but with darker pink colorations, which indicates that the movement in the upper and lower parts of the tank increased while the electromagnetic field was active. There are no indications shorter pulse intervals increase the locomotive activity.

The means of the total distances succeeding the exposure to the electromagnetic fields (**Figure 31 B1**) were not significantly changed when compared with a paired t-test ($p>0.05$). There was no statistically significant alteration of the locomotive activity between any of the EM trials when comparing the fold change with a paired t-test ($p>0.05$). However, most of the sharks altered their locomotive activity, by either decreasing or increasing their speed. This is reflected by the clear difference between the large spread of data points in the fold change of 100ms, 300ms and 600ms pulse interval compared to the control where the points are clustered (**Figure 31 B2**).

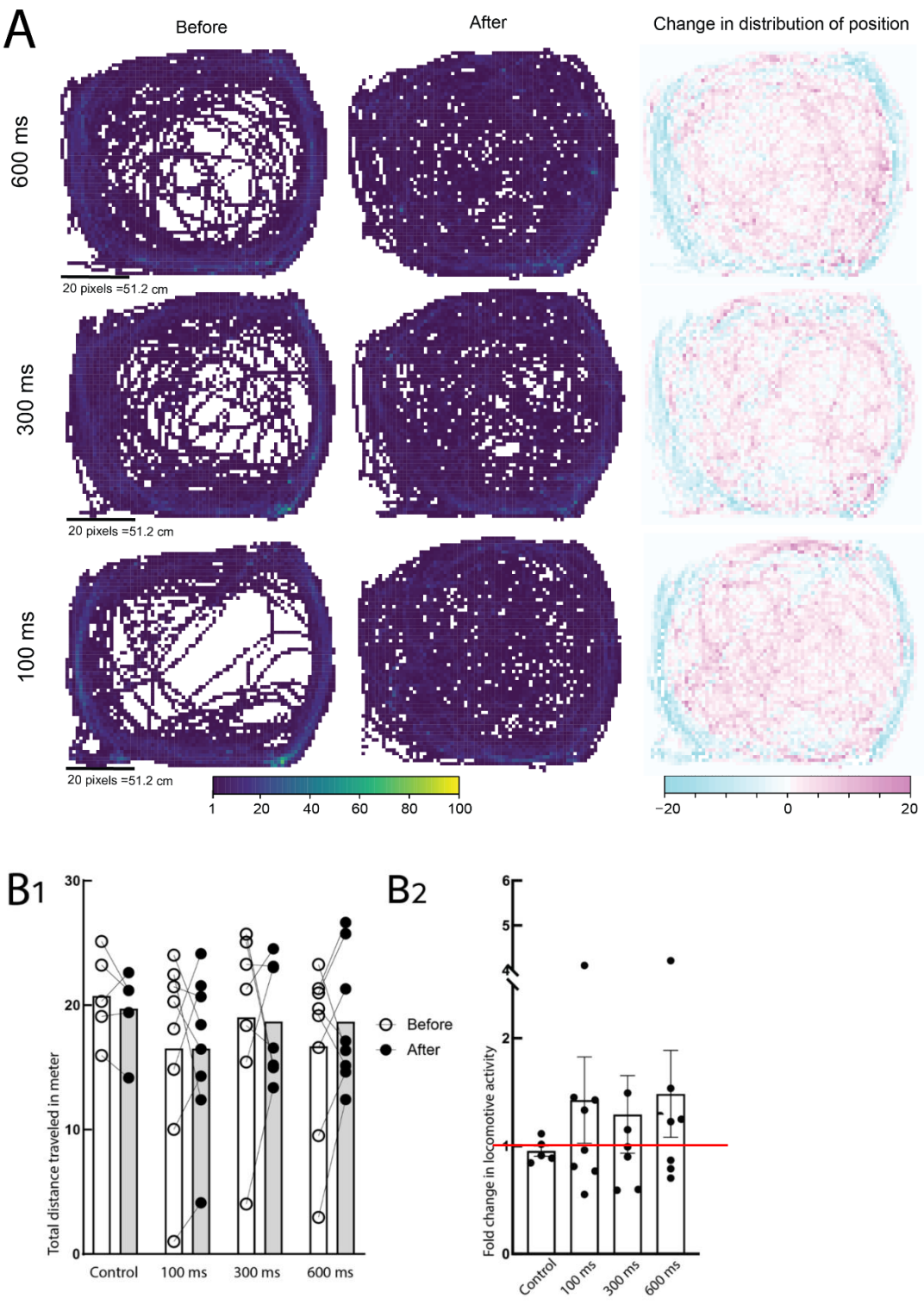
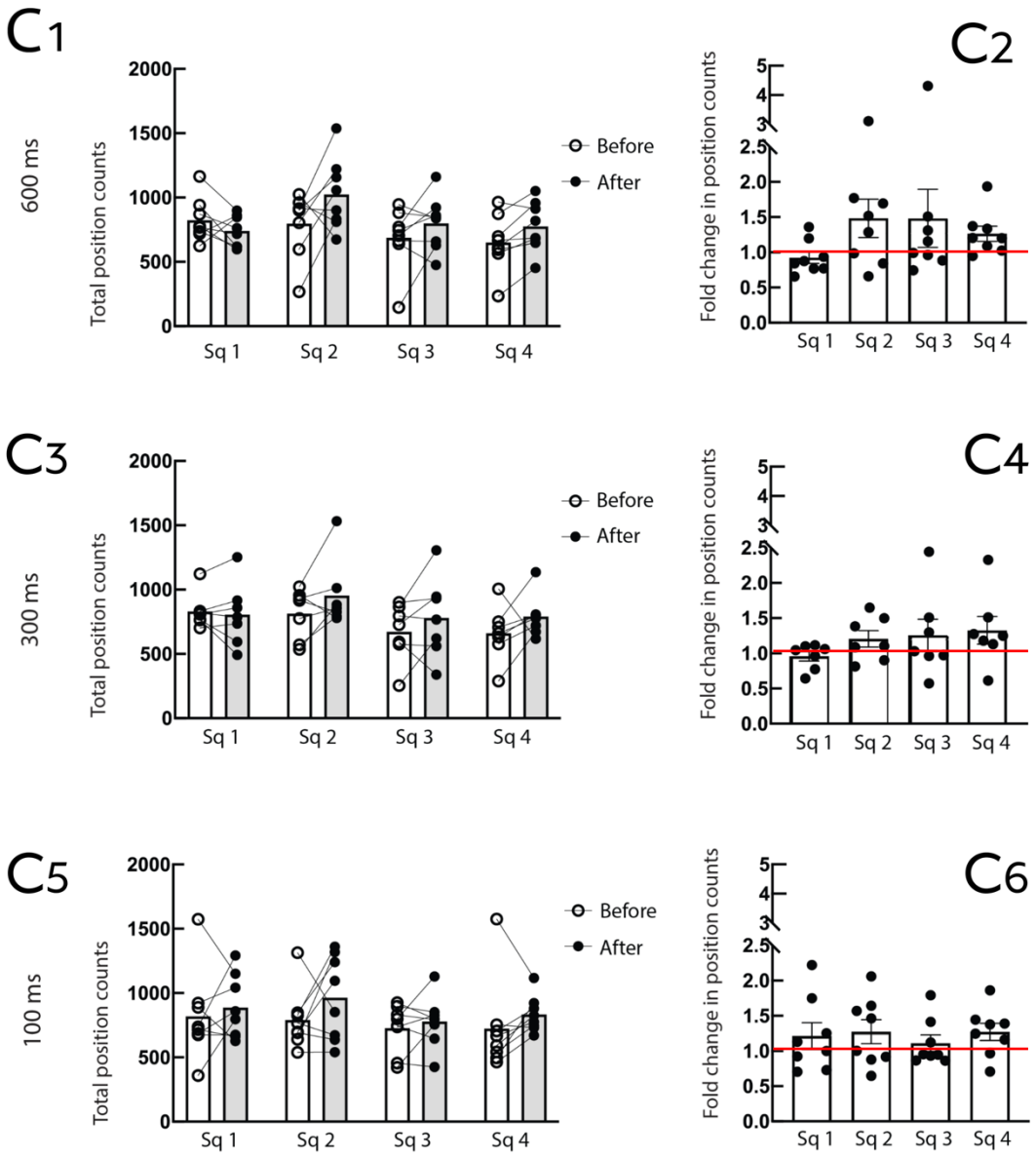


Figure 31 The behavioral change in response to 10 V electromagnetic fields with a 600ms, 300ms and 100ms pulse interval, respectively. (A) Based on the density of data points from the x and y coordinates, the positions of the sharks (n=6) from all trials are combined and visualized in binned heatmaps before and after the stimulus. The color scale of the heatmap translates the movement intensity in the tank: purple represents locations with minimal movement, while green and yellow indicate locations with frequent movement. The white pixels show no registered movement. Data points exceeding 100 counts are oversaturated and can be observed in proximity to high-intensity areas. The scale bar is adequate for all heatmaps, where 20 pixels roughly represent 51,2 cm. of

the tank. The change in position frequency is illustrated in a blue and pink color scale heatmap: decreased space use is indicated by blue and increased space use is indicated by pink. (B1) Comparing the mean distances traveled before and after seawater control (no stimulus), 100ms, 300ms, and 600ms pulse intervals. Each data point represents the mean distance traveled by a single shark from all trials. (n=7) (B2) Comparing the fold change from every trial conducted independently from the individual sharks. Points <1 signifies decreased distances traveled and points >1 signifies increased in locomotive activity.



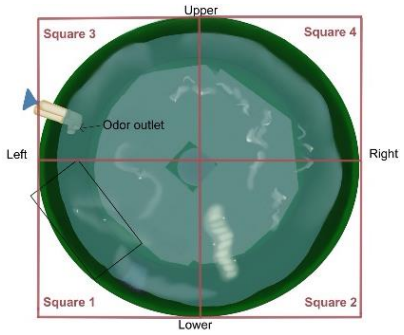


Figure 32 The total position counts from four arenas in the tank: Square 1 (Sq1), Square 2 (Sq 2), Square 3 (Sq 3), and Square 4 (Sq 4). (C1, C3, and C5) The bar plots show the mean position count and compare the total position counts before and after stimuli, where each data point represents the movement of one shark (n=8). (C2, C4, and C6) The bar plots show the mean fold change with each dot representing one shark. The red line indicates the fold change of 1, where the position counts before and after stimuli would be equal. Points above the line indicate increased activity after the onset of the stimuli. Below the line is a decrease in activity.

No significant alterations of the position in the tank succeeding exposure to 10 V electromagnetic fields with 600ms, 300ms, and 100ms pulse intervals were detected according to one-way ANOVA analysis (*ANOVA,* $p>0.05$). Even though no significance could be established, **Figure 32 C1 and C2** show that locomotive alterations succeeding exposure to 600ms pulse intervals took place. 6 out of 8 sharks altered their position moving away from the electrode and into the other arenas. Similar patterns are visible in **Figure 32 C3 and C4**. In common for the 600ms and 300ms interval trials are the increased position counts in all arenas except for square 1 where the electrode was positioned, which indicates that the locomotive activity succeeding stimuli onset escalated. All four squares show elevated position counts after the 100ms pulse interval condition, which suggests that the sharks elevated their activity level in these trials as well (**Figure 32 C5 and C6**).

5.2.4. Condition 2 - Behavioral effects of 5, 10, and 20 V electromagnetic pulse

Condition 2 was executed to evaluate whether three increasing voltages or levels of electromagnetic field strength would affect the behavioral response differently; 5, 10, and 20 V voltage were applied on a total of seven sharks (Shark 6, 8, 10, 15, 18, 20, and 21). We expected to observe larger alterations correlating to higher voltages. Alterations in the locomotive activity were evaluated based on the total distances traveled succeeding the onset of the three strengths of EM fields compared to the absence of stimulus (**Figure 33**). When no stimuli were applied, most sharks maintained the same activity level. The three EM stimuli caused a more noticeable change, as most sharks either increased or decreased the length of their course. In the 5 V trial, all sharks altered their activity level apart from Shark 21. In the 10 V trial, five of the sharks extended their swimming distances, while two shortened their route. During the 20 V trial, all but one shark demonstrated elevated swimming distances, while Shark 18 clearly decreased its activity level. Similar to the results of condition 1, the shark did not elicit a homologous behavioral response, but rather individual behavioral changes.

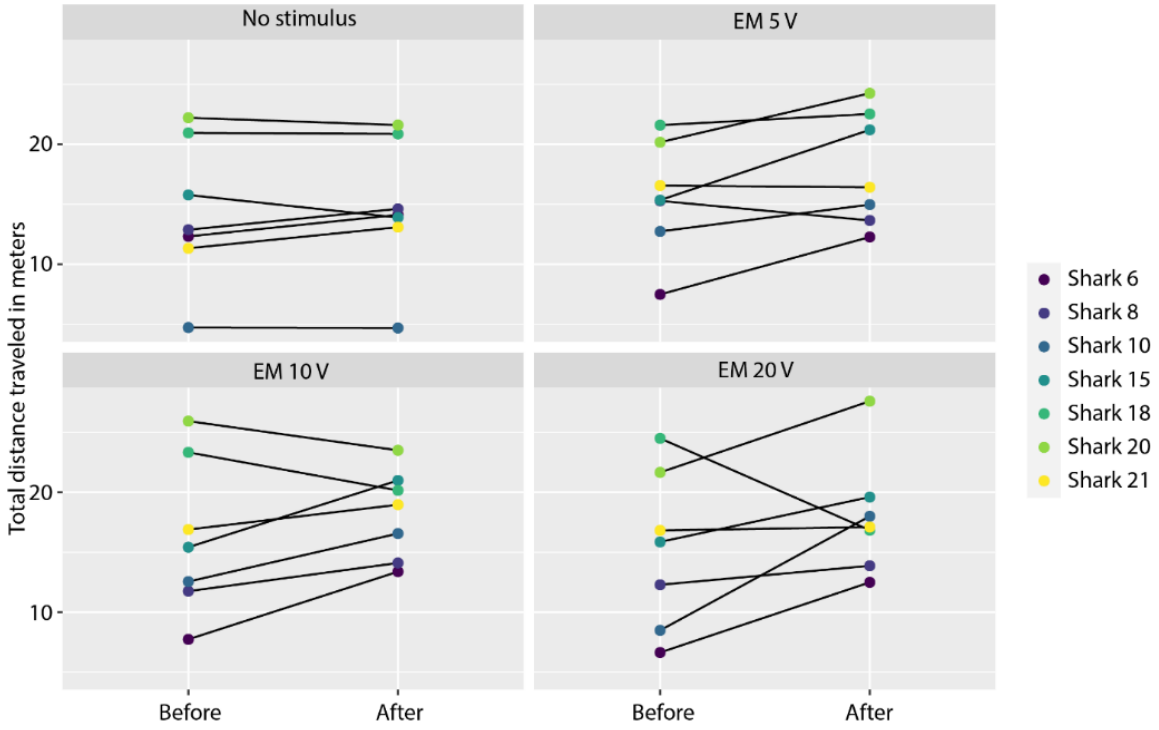
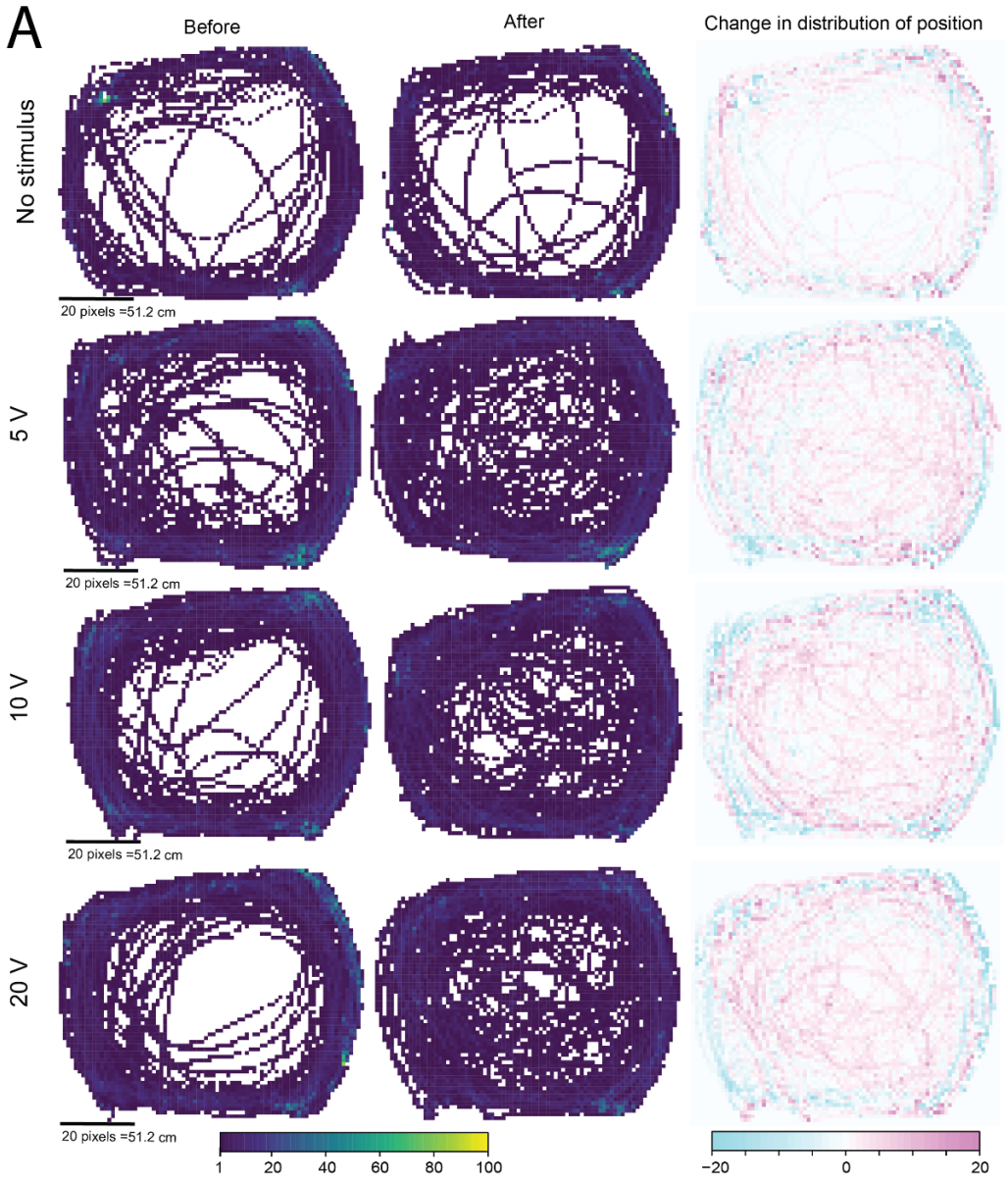


Figure 33 The mean of total distances traveled before and after the addition of stimuli. All stimuli sample size = 7. Each dot represents the average response from one shark to the stimuli.

By comparing the heatmaps of the trials with no stimulus and the EM trials, a clear change in locomotive activity is visible (**Figure 34 A**). When no stimuli were applied, the sharks continued to circle along the tank walls. They demonstrated similar circling behavior prior to the EM trials. During the application of the electromagnetic fields, however, the movement changed as the sharks increased the utilization of the middle part in addition to the walls. These alterations are visible in the heatmaps, which complement the findings from the qualitative real-time studies where increased circling and locomotive alterations were observed. The "change in the distribution of position" heatmaps enhances how the sharks changed their movements, as the blue pixels mostly obtain the outer circle while pink pixels are frequent in the middle of the tank (**Figure 34 A**). It is difficult to determine whether increasing voltages affected the placement in the tank differently from viewing the heatmaps. The 5 V "change in distribution of position" heatmap shows a slight increase of pink coloration on the right side, which corresponds to the area furthest away from the EM field. Contrastingly, the 10 V heatmap shows an increased intensity on the left side of the tank, near the electrode however separated by blue region from the electrode, in addition to the lower right corner. The coloration of the 20 V heatmap indicates that circling increased towards the upper left part of the tank, in addition to movement in the upper and lower right corners but separated by blue region from the electrode.

The alterations of locomotive activity before and after the application of stimuli were not homogenous which demonstrates how the sharks exhibited individual behavior (**Figure 34 B1**). Consequently, no significant change was found (*t-test*, $p<0.05$). To compare the locomotive alteration between the stimuli, the fold changes are presented in **Figure 34 B2**. As the voltage of the electromagnetic fields increases, so does the variance in fold change values. This could indicate an

increasing change in locomotive activity as an effect of increased electromagnetic fields. However, no significant differences were detected between the fold change (*t-test*, p>0.05).



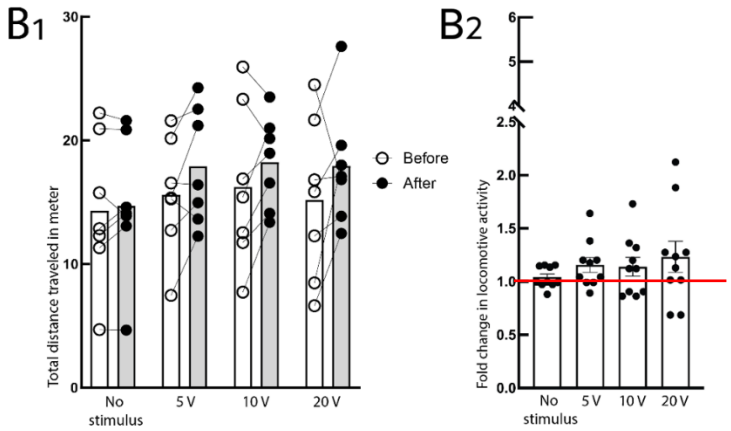


Figure 34 The behavioral change in response to no stimulus, 5 V, 10 V, and 20 V electromagnetic fields with a 300ms impulse interval. (A) Based on the density of data points from the x and y coordinates, the positions of the sharks (n=7) from all trials are combined and visualized in binned heatmaps before and after the stimulus. The color scale of the heatmap translates the movement intensity in the tank: purple represents locations with minimal movement, while green and yellow indicate locations with frequent movement. The white pixels show no registered movement. Data points exceeding 100 counts are oversaturated and can be observed in proximity to high-intensity areas. The scale bar is adequate for all heatmaps, where 20 pixels roughly represent 51,2 cm. of the l tank. The change in position frequency is illustrated in a blue and pink color scale heatmap: decreased space use is indicated by blue and increased space use is indicated by pink. (B1) Comparing the mean distances traveled before and after no stimulus, 5 V, 10 V, and 20 V. Each data point represents the mean distance traveled from one shark from all trials. (B2) Comparing the fold change from every trial conducted independently from the individual sharks. Points >1 signifies increased distance traveled succeeding stimuli. Points <1 signifies decreased distances traveled.

The positional changes succeeding the electromagnetic fields are visualized in **Figure 35**. The bar plots representing the trial where stimulatory cues were absent (**Figure 35 C1 and C2**) depict the baseline position counts during the trials of Condition 2. One-way ANOVA and Tukey´s post hoc analyses were performed to detect significant changes of the position in the tank, but no significant changes were detected (*ANOVA*, $p>0.05$). Despite showing similar results to the baseline, the position count and fold change of the 5 V EM trial (**Figure 35 C3 and C4**) indicates that the sharks slightly favored the use of the right side of the tank after initiation of the electrical pulses. This observation corresponds to the 5 V heatmaps in **Figure 34 A**. The result from 10 V, visualized in **Figure 35 C5 and C6**, is a bit harder to interpret as the diagonally opposite squares 2 and 3 were most frequently occupied by the sharks. However, the mean fold change on square 1 is maintained at the red line which suggests that the sharks still approached the electrode. These movements correspond to the 10 V heatmaps in **Figure 34 A**. In **Figure 35 C7 and C8**, the results from the 20 V trial are visualized. Square 1-3 shows a large spread between the fold change data points compared to the lower voltages and no stimulus. These large variations were also observed in **Figure 34 B2**. Despite not being statistically changed, the results from the 20 V trial indicate large individual behavioral responses. Additionally, all squares show an increase in position counts with square 4 as an exception. However, all sharks except for two visited this tank area more succeeding stimuli onset. Ultimately this indicates a general increase in locomotive activity.

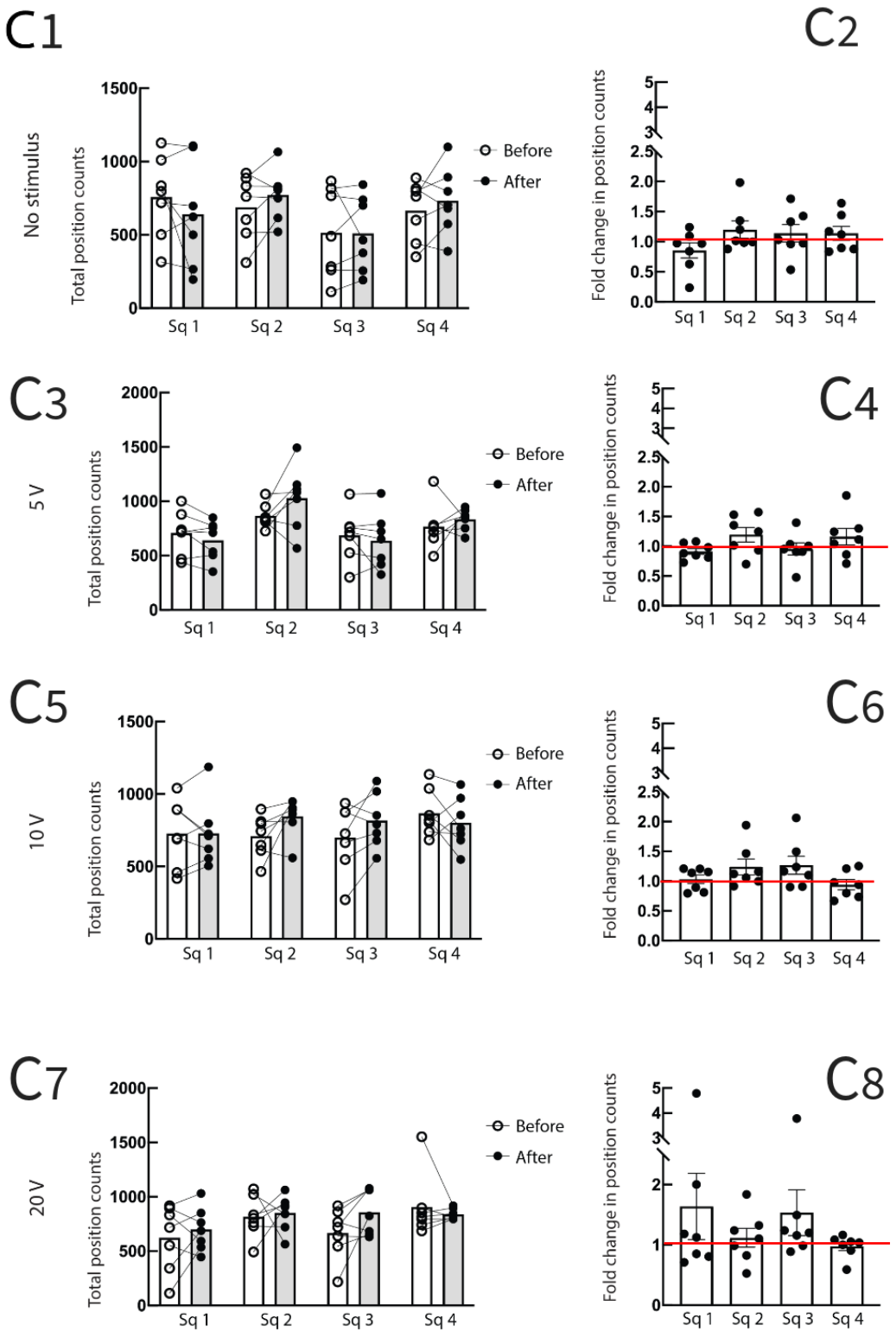


Figure 35 The preferred location of the tank was evaluated by the total position counts from four arenas: Square 1 (Sq1), Square 2 (Sq 2), Square 3 (Sq 3), and Square 4 (Sq 4). (C1, C3, and C5) The bar plots show the mean position count and compare the total position counts before and after stimuli, where each data point represents the combined location counts of all trials per shark (n=8). (C2, C4, and C6) Bar plots showing the mean fold change with each dot representing one shark. The red line indicates the fold change of 1, where the position counts before and after

stimuli would be equal. Points above the line indicate increased activity after the onset of the stimuli. Points below the line represents a decrease in activity.

5.2.5. Condition 3 - Behavioral effects of food odor and dose dependent response to skin extract

Condition 3 was performed to evaluate whether three increasing units of skin extract would affect the behavioral response differently. 0.5, 1, and 2 units of skin extract were inflicted on a total of eight sharks (Shark 9, 13, 15, 16, 17, 18, 20, and 21). We expected to observe larger alterations in locomotive activity with increasing units of skin extract. The same sharks were also exposed to food odor, where we expected the sharks to elicit foraging behavior and attractive response.

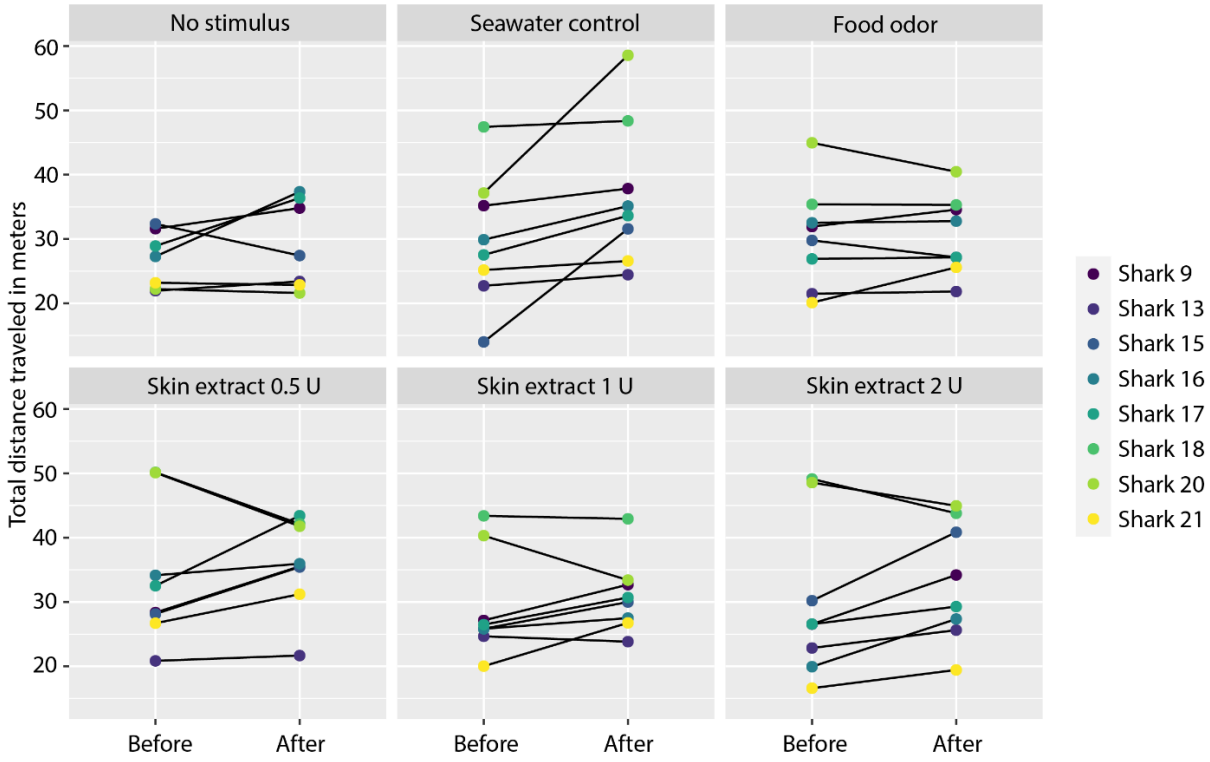
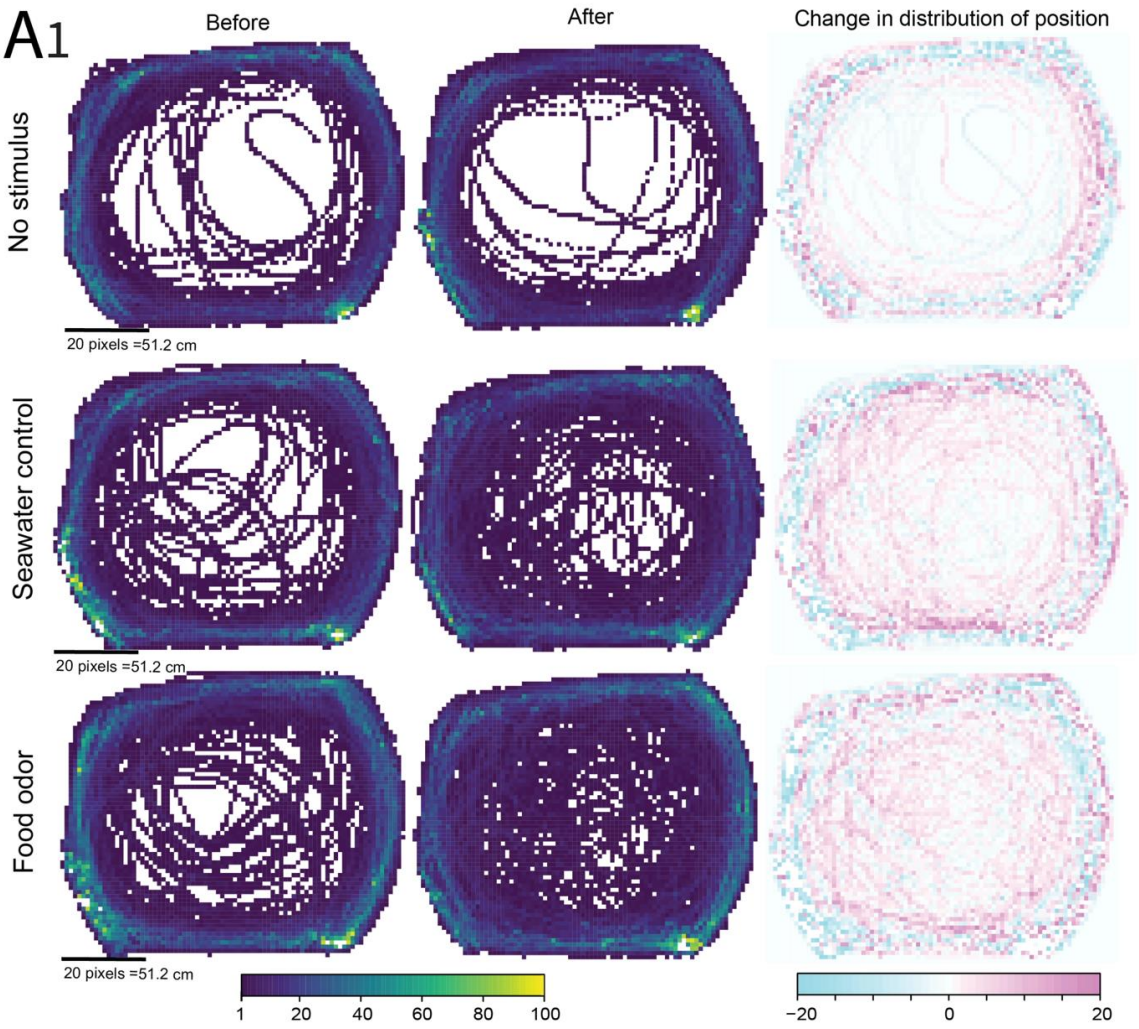


Figure 36 Comparing the mean of total distances traveled before and after the addition of chemical stimuli. Sample size = 8. Each dot represents the average response from one shark to the stimuli.

The mean of total distances traveled by each shark affected by chemical stimuli tested in Condition 3 is visualized in **Figure 36**. The clustering of data points is least prevalent from the trial without any stimulus applied. In the aftermath of odorant application, however, the spread of data points indicates that the individual behavioral response was more comprehensive. The seawater control is the only trial where we can observe a somewhat equal behavioral change, as all sharks increased their locomotive activity. The effect of the food odor seems moderate, as most sharks maintained a similar level of activity. Succeeding the skin extract trials, we can observe individual variations in the behavioral response. In all skin extract trials, the general trend among all sharks was an increase in locomotive activity. Some individuals, like Shark 20, decreased its activity after being encountered with the skin extract odor.


A1
Before
After
Change in distribution of position
No stimulus
Seawater control
Food odor
20 pixels =51.2 cm
20 pixels =51.2 cm
20 pixels =51.2 cm
1
20
40
60
80
100
−20
0
20

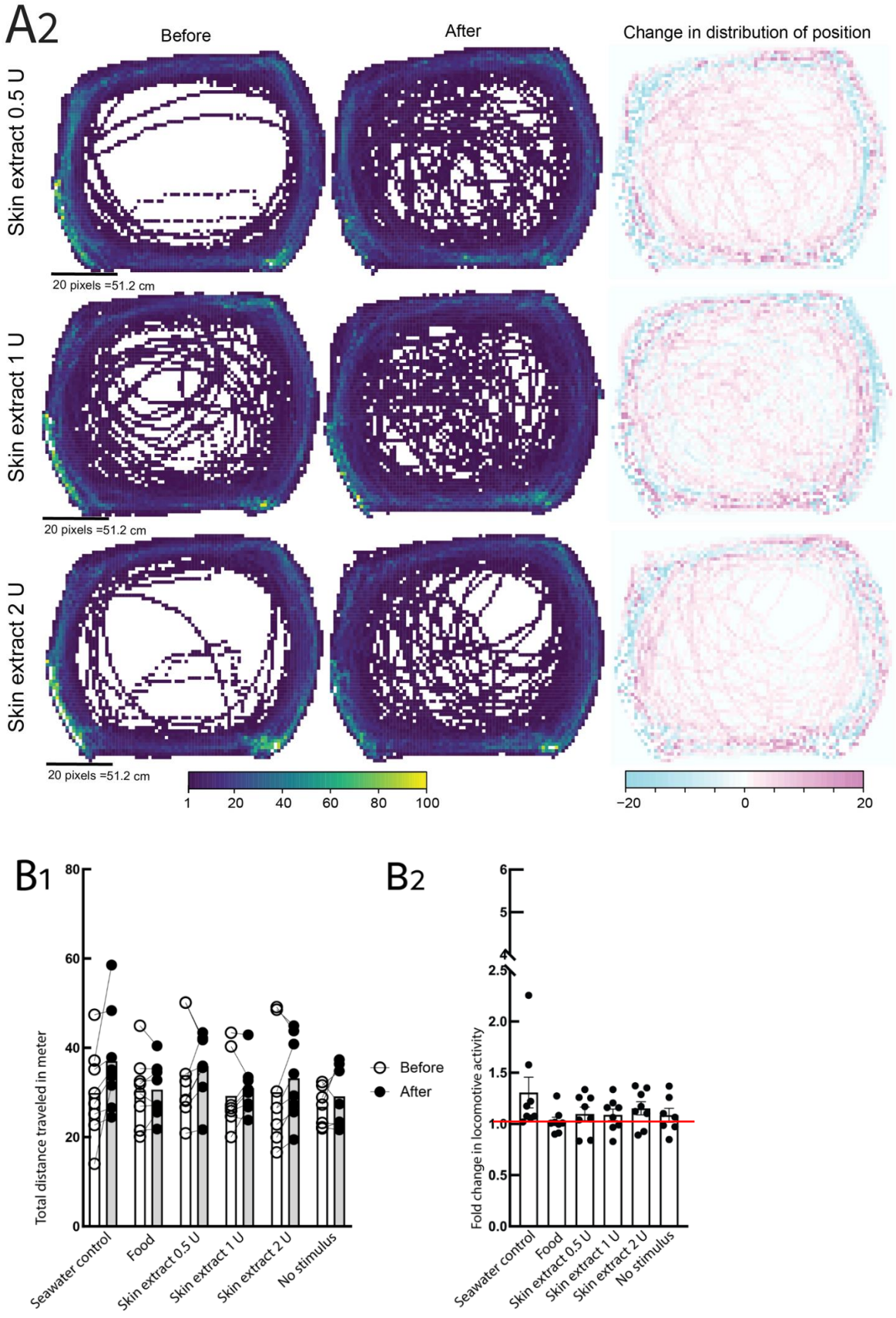
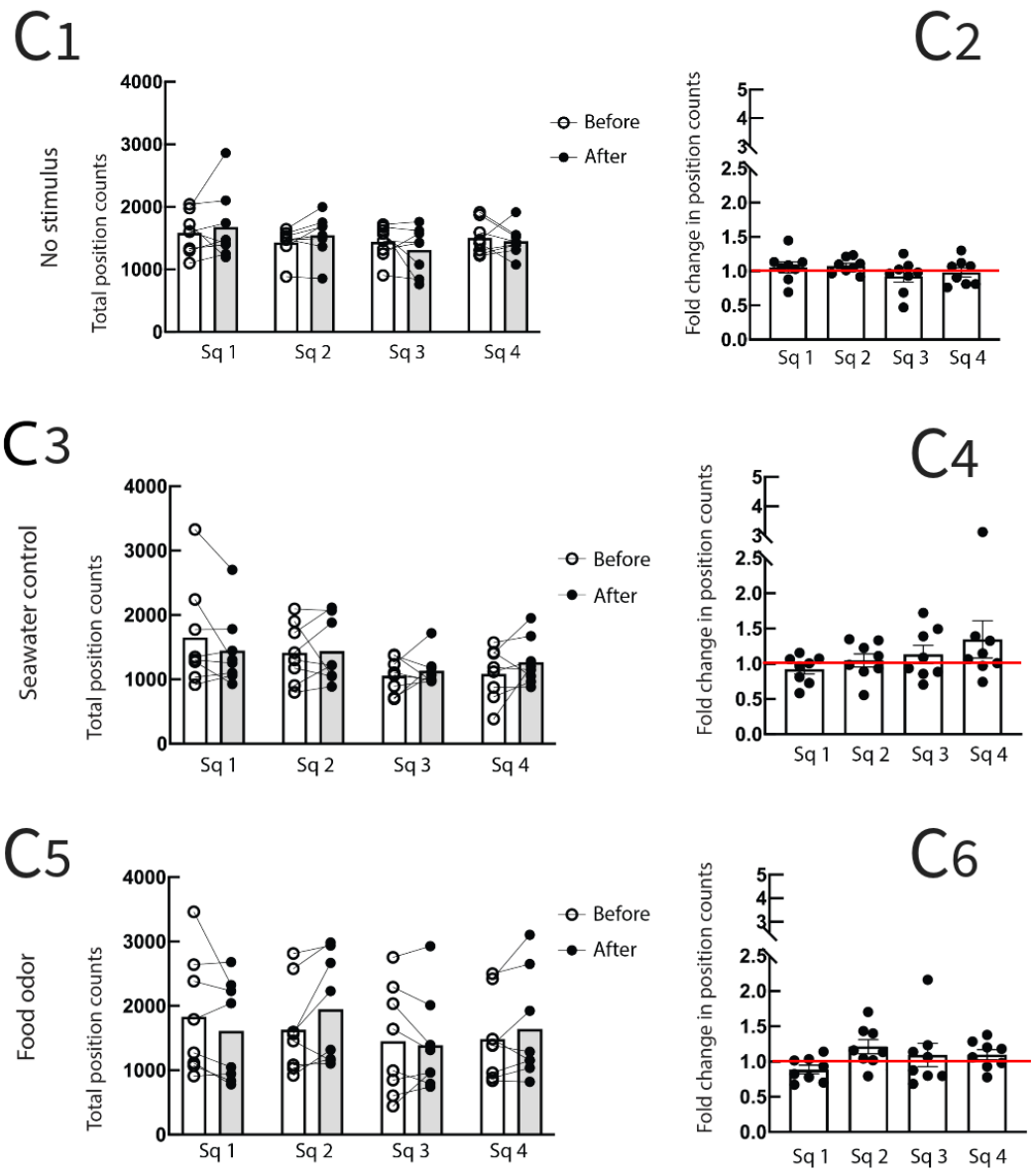


Figure 37 The behavioral change in response to no stimulus, seawater control, food odor, 0.5 U skin extract, 1 U skin extract, and 2 U skin extract. (A1 and A2) Based on the density of data points

from the x and y coordinates, the positions of the sharks (n=8) from all trials are combined and visualized in binned heatmaps before and after the stimulus. The color scale of the heatmap translates the movement intensity in the tank: purple represents locations with minimal movement, while green and yellow indicate locations with frequent movement. The white pixels show no registered movement. Data points exceeding 100 counts are oversaturated and can be observed in proximity to high-intensity areas as empty pixels. The scale bar is adequate for all heatmaps, where 20 pixels roughly represent 51,2 cm. of the tank. The change in position frequency is illustrated in a blue and pink color scale heatmap: decreased space use is indicated by blue and increased space use is indicated by pink. (B1) Comparing the mean distances traveled before and after seawater control, food odor, 0.5 U skin extract, 1 U skin extract, 2 U skin extract, and no stimulus. Each data point represents the mean distance traveled by one shark through all trials. (B2) The fold change from every trial conducted from the individual sharks was compared using ANOVA and Tukey´s post hoc analysis. Points >1 signifies increased distance traveled succeeding stimuli. Points <1 signifies decreased distances traveled.

Alteration of the movement in the tank is present in all the heatmaps where odor was applied, which differs greatly from the heatmaps when no stimulus was present (**Figure 37 A1)**. The seawater control evoked, similarly to the seawater trial in Condition 1, a behavioral change; there was an increase in circling across the middle of the tank. The swimming pattern resembles the pattern seen in the food trial, and in both heatmaps it is difficult to determine if the sharks favored one particular part of the tank. 0.5 U skin extract caused a change of movement, from circling the edge of the tank towards circling the middle. Dark pink coloration is visible on the right side of the tank, in addition to a larger area in the upper left corner. The latter could be the result of increased circling behavior here. The heatmap illustrating the effect of 1 U is harder to interpret, as both darker blue and pink coloration is evenly scattered. The before and after heatmap shows a clear change of locomotion, where the middle of the tank was utilized to a bigger extent after the stimuli. Finally, the behavioral response succeeding the 2 U skin extract trials seems to have been increased circling behavior close to the odor outlet and an inclination of further movement towards the right side of the tank.

Nearly all stimuli, including no stimulus, show an increase in locomotive activity as the total distances traveled increase after the odor stimuli are applied. Only the food odor maintains an indifferent activity level (**Figure 37 B1**). As illustrated in **Figure 36** , a great variety of individual behavior was elicited, and only the seawater control altered the distances traveled unidirectionally with statistical significance (*t-test*, $p<0.05$). The impact of the different stimulatory cues was compared by the fold changes of distances traveled in **Figure 37 B2**. ANOVA analysis did not reveal any significant difference between the stimuli (*ANOVA*, $p>0.05$). The food odor did not notably alter the shark activity levels. Similarly, the skin extract units did not seem to have a great imprint on distances traveled either, despite having impacted the swimming pattern. However, as already observed in **Figure 36 and Figure 37 A1, A2, and B1**, most sharks did alter their swimming pattern and activity level to some extent.


C1
No stimulus
Total position counts
4000
3000
2000
1000
0
Sq 1
Sq 2
Sq 3
Sq 4
Before
After
C2
Fold change in position counts
5
4
3
2.5
2.0
1.5
1.0
0.5
0.0
Sq 1
Sq 2
Sq 3
Sq 4
C3
Seawater control
Total position counts
4000
3000
2000
1000
0
Sq 1
Sq 2
Sq 3
Sq 4
Before
After
C4
Fold change in position counts
5
4
3
2.5
2.0
1.5
1.0
0.5
0.0
Sq 1
Sq 2
Sq 3
Sq 4
C5
Food odor
Total position counts
4000
3000
2000
1000
0
Sq 1
Sq 2
Sq 3
Sq 4
Before
After
C6
Fold change in position counts
5
4
3
2.5
2.0
1.5
1.0
0.5
0.0
Sq 1
Sq 2
Sq 3
Sq 4

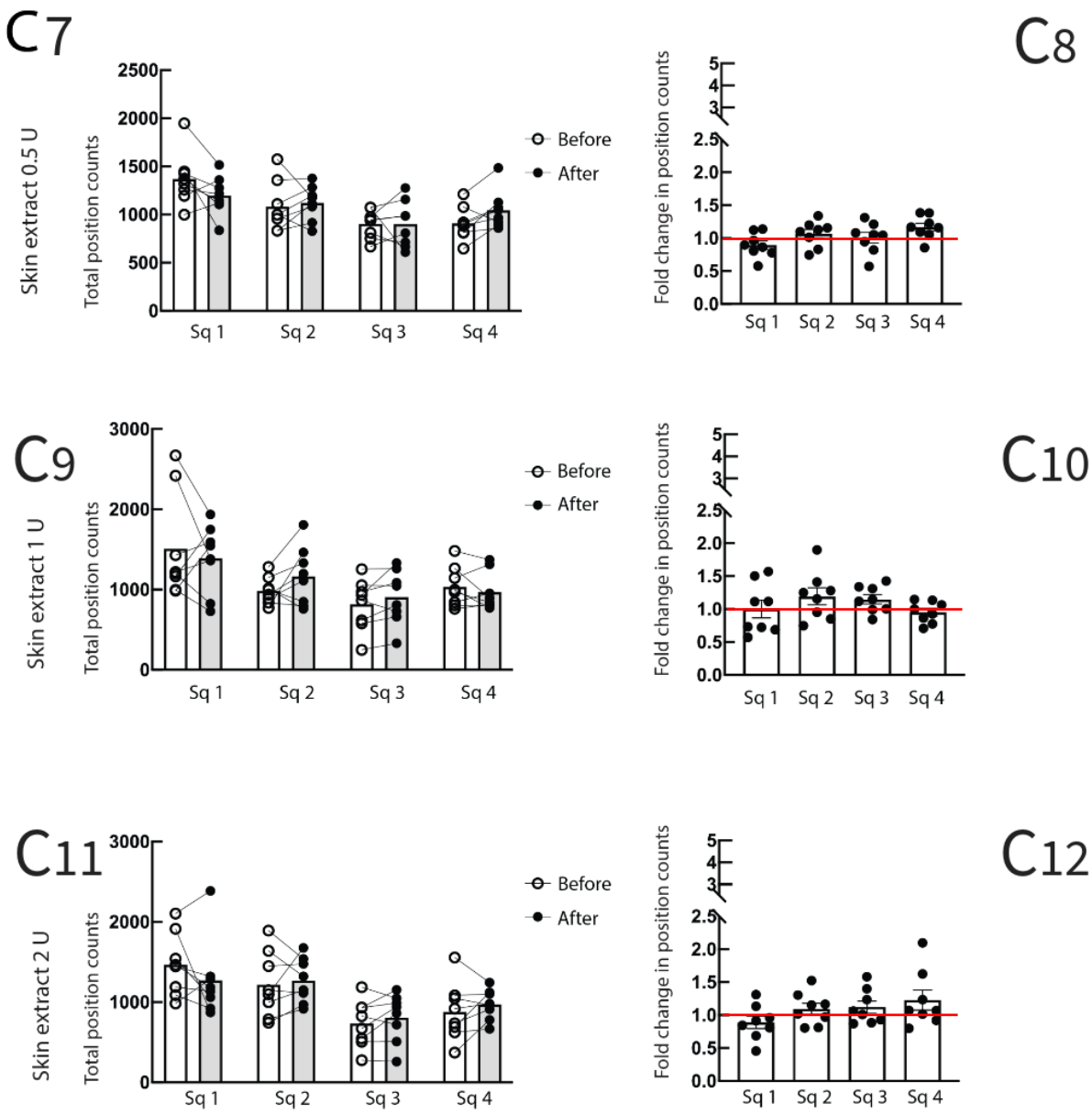


Figure 38 The preferred location of the tank was evaluated by the total position counts from four arenas: Square 1 (Sq1), Square 2 (Sq 2), Square 3 (Sq 3), and Square 4 (Sq 4). (C1, C3, C5, C7, C9, and C11) The bar plots show the mean position count and compare the total position counts before and after stimuli, where each data point represents the combined location counts of all trials per shark (n=8). (C2, C4, C6, C8, C10, and C12) The bar plots show the mean fold change with each dot representing one shark. The red line indicates the fold change of 1, where the position counts before and after stimuli is equal. Points above the line indicate increased activity after the onset of the stimuli. Points below the line indicates a decrease in activity. One-way ANOVA and Tukey´s post hoc analyses were performed to detect significant changes of the position in the tank as a result of stimuli onset. No significant changes were detected with ANOVA. Tukey´s post-hos

analysis showed a significant difference between Square 1 and 4 succeeding the skin extract 0.5 U stimuli (C8).

In the absence of stimuli, the total position counts are not heavily fluctuating (**Figure 38 C1**). The fold change shows that the clustering of points is nearly evenly distributed on both sides of the red line, which indicates that the sharks did not alter their behavior (**Figure 38 C2**). During the seawater control, the total position counts are most abundant in the quadrants representing the right side of the tank (**Figure 38 C3**), and the largest fold changes are represented in the upper area (**Figure 38 C4**). Combined with the heatmap, the sharks elicited a behavioral change, by utilizing larger areas of the tank. The total position counts from the food odor trial suggest that the sharks favored square 2, the bottom right corner of the tank. Especially after the odor was introduced (**Figure 38 C5**). Additionally, this area also shows the largest fold changes. The activity level in square 3 was nearly maintained, while a slight increase appeared in square 4 (**Figure 38 C6**). In the skin extract trial with a 0.5 unit, the total position counts imply that the bottom of the tank (squares 1 and 2) was most frequently visited (**Figure 38 C7**). The fold change shows that in general, the sharks avoided this area to a bigger extent after the addition of the skin extract. Despite not being the most occupied area of the tank based on the position counts, the upper right corner showed the largest fold change > 1 (**Figure 38 C8**). It could therefore be assumed that the sharks altered their spatial distribution to avoid the area of the odor outlet. In addition, a significant difference between Square 1 and 4 in was found with Tukey´s post-hoc analysis ($p<0.05$). These results match the visual trails of the "change" heatmap where the lower and upper right part of the tank show dark pink areas and trails of circling extending into the area of square 3. Similarly, the position counts succeeding the 1-unit skin extract trial shows that most frequently the sharks inhabited the lower part of the tank (squares 1 and 2) (**Figure 38 C9**). Simultaneously, **Figure 38 C10** shows that half of the sharks spent less time in this region succeeding the odor exposure. Square 2 was more frequently visited. A more moderate increase can be observed for square 3. Additionally, square 4 shows a fold change below 1. This could be explained by the pink trails visualized in **Figure 38 A2,** as some of the sharks circled diagonally between the upper left and lower area. The lower area was mostly utilized during the skin extract trials with 2 units according to the total position counts (**Figure 38 C11**). On the contrary, the upper squares (squares 3 and 4) show an increasing fold change exceeding 1 (**Figure 38 C12**) despite the lowest total of position counts.

5.2.6. Changes in serum metabolite in response to sensory cues

To study the effect of sensory stimuli on physiology of the animal, serum samples were collected from 22 spiny dogfish after being exposed to three stimuli treatments and control (no stimulus) treatments. Five (5) sharks acted as a control group. Six (6) sharks were exposed to skin extract from conspecifics. Five (5) sharks were exposed to food odor (mackerel). Six sharks were exposed to a 10 V electromagnetic field. All sharks were euthanized between 30-40 minutes after the stimulus was applied. As the skin extract and electromagnetic field caused the larges locomotive alterations and are believed to cause stressful and aversive behavior, we expected to observe notable differences in metabolite levels affected by stress, such as increased glucose and lactate acid, in addition to increased levels of salts. To unveil additional possible effects on metabolite levels, several metabolites were analyzed.

Table 2. The mean (SD) values of metabolites from serum samples in spiny dogfish from treatment groups; Control, Skin extract, Food odor, and Electro Magnetic stimuli. TP=Total protein, Mg=Magnesium, LA = Lactic acid, Na = Sodium, K = Potassium, Chol = Cholesterol, Tri = Triglycerides, Pho = Phosphorus, Glu = Glucose, Ca = Calcium, Ch = Chloride, Cre = Creatine enzyme, CholH = Cholesterol HDL, Chol LDL = Cholesterol low-density lipoprotein. Only five sharks were analyzed for Chol LDL.

Metabolite	Control (n=5)	Skin extract (n=6)	Food odor (n=5)	EM (n=6)
TP (g/L)	30.13 (33.59)	59.21(32.28)	87.94 (87.12)	40.80 (29.50)
Mg (mmol/L)	1.19 (0.11)	1.14 (0.09)	1.01 (0.072)	0.99 (0.16)
LA (mmol/L)	1.80 (1.30)	1.31 (0.26)	1.06 (0.49)	1.19 (0.82)
Na (mmol/L)	268.3 (6.23)	270.89 (4.13)	267.45 (8.13)	262.93 (5.03)
K (mmol/L)	2.85 (0.66)	2.83(0.61)	3.22 (0.28)	3.08 (0.67)
Chol (mmol/L)	2.28 (0.42)	2.76 (1.09)	2.42 (0.96)	2.80 (0.47)
Tri (mmol/L)	1.24 (0.53)	2.32 (1.68)	3.87 (3.64)	1.94 (0.96)
Pho (mmol/L)	1.64 (0.10)	1.69 (0.21)	1.74 (0.50)	1.57 (0.30)
Glu (mmol/L)	3.56 (0.96)	4.61 (0.47)	4.14 (0.67)	3.41 (0.94)
Ca (mmol/L)	3.73 (0.20)	3.64 (0.27)	3.44 (0.23)	3.51 (0.32)
Ch (mmol/L)	253.78 (7.10)	258.52 (5.29)	256.36 (7.69)	249.55 (6.66)
Cre (µmol/L)	2.25 (1.44)	4.67 (2.87)	3.12 (1.11)	5.08 (2.65)
CholH (mmol/L)	0.09 (0.05)	0.15 (0.05)	0.19 (0.07)	0.14 (0.09)
Chol LDL (mmol/L)	0.47 (0.38)	0.51 (0.14)	-	0.643

The glucose and lactate have been used as proxy for stress response. However, glucose and lactic acid levels in **Figure 38** did not show significant differences between treatment groups (ANOVA and Dunnet's multiple comparison, $p>0.05$). Large variations in the metabolite levels were observed across groups. In example, the total protein, triglycerides, cholesterol, and sodium shows a wide distribution in the food odor treatment. The EM and skin extract show a relatively higher mean value

of creatinine enzyme compared to the food odor and control group. Magnesium showed a statistically significant difference (ANOVA and Dunnets multiple comparisons test, $p<0.05$) between the control group (mean = 1,19 mmol/L) and the electromagnetic field group (mean = 0.99 mmol/L) (**Table 7**). Magnesium has been reported to be involved in stress buffering. Increased serum magnesium level suggests a stress coping in the EM group. No significant difference in serum levels of total protein, lactic acid, sodium, potassium, cholesterol, triglycerides, phosphorus, glucose, calcium, chloride, creatinine enzyme, cholesterol HDL or Cholesterol low-density lipoprotein were found (ANOVA and Dunnet's multiple comparison, $p>0.05$).

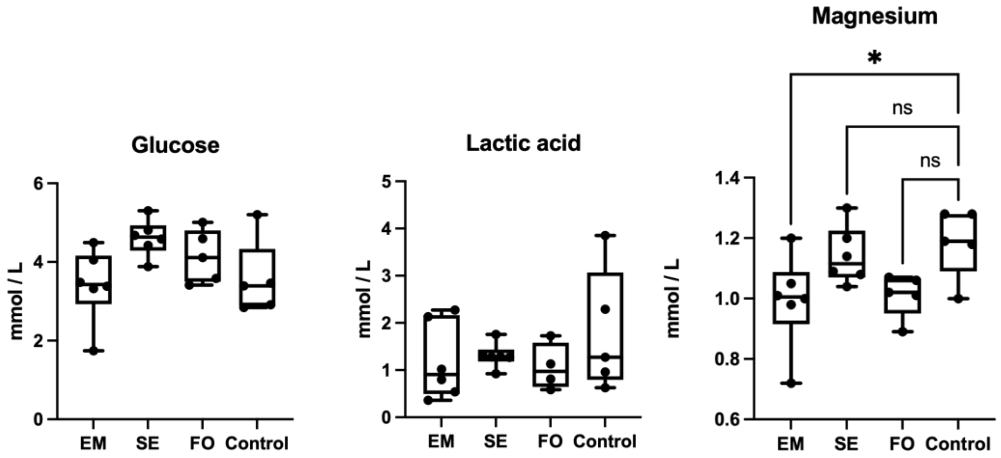


Figure 39 The glucose, lactic acid and magnesium levels from serum samples. Glucose and lactic acid levels were similar across all groups. There was a significant difference between the control and the electromagnetic field treatment group. EM = Electromagnetic field, SE = Skin extract, FO = Food odour.

5.2.7. Discussion on laboratory trials

The objective of this study was to investigate if auditory, chemical, and electromagnetic stimuli of biological importance would evoke aversive or stressed behavior and how these experiences would affect the physiological homeostasis of captive spiny dogfish. The findings contribute to valuable knowledge regarding how the species behave, and how they might be affected by stressful situations, in addition to contribution towards developing a shark deterrent. 23 sharks were captured by line fishing and housed with a near 100% survival rate throughout the project. By real-time qualitative observations, their locomotive performance was described by swimming patterns and styles. These descriptions were further used to describe the change in behavior during the trials. The skin extract and electromagnetic fields of various intervals and voltages did elicit clear behavioral changes in several sharks, however, the individual responses differed. Interestingly, the seawater control also seemed to alter the locomotive activity. Heatmaps, mean distances traveled, and position counts in the tank were successfully extracted from all trials and reflected the observed behavioral alterations. It proved challenging, however, to quantify the observed behavior and to further analyze the data with an appropriate statistical method to distinguish the locomotive activity in response to the stimulatory cues. The serum samples were analyzed for several metabolites, and magnesium ion levels were significantly lower in the sharks subjected to an electromagnetic field compared to the control group.

Whether or not the sharks elicited an aversive response is challenging to determine, as the closed confinement obstructed the animals from escaping the stimuli. By attempting to avoid the stimuli, they would follow the wall and end up with the speaker, odor outlet, or electrode. Researching the behavior of animals in captivity will not necessarily reflect the behavior they will elicit in the wild. Factors such as water parameters, social interactions, competition, predation, foraging, life history traits, fecundity, season of the year, age, and individual behavior are all affecting how animals behave and are difficult to account for in trials such as these. Especially as the spiny dogfish is a schooling species, the lack of conspecifics present during the trials could affect how they responded, or rather how there was a lack of response. However, trials like these are important to establish immediate behavioral and physiological consequences of exposing them to stressful, fearful stimuli or attractive stimuli.

5.3. Field trials of promising anti-shark measures

5.3.1. Electromagnetic pulse induces varying repellent in spiny dogfish

The first field trip was undertaken on the 16th of may 2023 at the Herdlafjord location with the MS Ognøysjefen vessel, a combi boat designed for transporting crew and provisions (**Figure 40**). During this day the test with orca sound were conducted. The plan was also to test the EM system from Salarsafe in the afternoon. Increasingly adverse weather conditions with larger waves made station keeping a challenge and unfortunately the umbilical to the test rig was cut by the propellor, after which the tests had to be abandoned.

Figure 40 MS Ognøysjefen, with captain Erik Eikje, participating in the first two field trips.

The second field trip was undertaken on the 24th of may 2023 at again the Herdlafjord location, with Ognøysjefen. This time the focus was on the EM trials. The test rig was lowered to 30 meters depth, with the Salarsafe system installed. No sharks were observed during this day, but we ran into problems with the cameras (the water tight seals gave away, leading to internal flooding and destruction of the camera electronics). To do the tests we are dependent to monitor the dogfish activity at the rig in real time, so the tests had to be concluded early. New cameras were made with an upgraded camera housing, but the field trials had to be postponed until the autumn: the sharks are deeper in the fjord during summer and hence the remaining field trials needed to be conducted early autumn.

The third and fourth field trip were undertaken on the 6th and 7th of November. This time with MS Solvik (**Figure 41**), a boat specially designed for research purposes and sea bottom soil sampling. This vessel had the advantage of automatic station keeping. Location was the same in Herdlafjorden. On both days successful trials were conducted with the EM system from Salarsafe. Both days we did not manage to bait sharks to the rig before relatively late in the afternoon. This gave a challenge with the light conditions at 30 m depth. The GoPro footage of the last trials ended underexposed, so we used AI postprocessing on these videos to increase the exposure. On the first day, three tests were conducted. The first test was with the rig at 30 m deep, the second and third at 12 m depth, to maintain visibility (late afternoon/sunset ambient light conditions). On the second day, the first test was conducted at 30 m depth and the next three at 12 m depth.

Figure 41 **MS Solvik, with captain Leon Pedersen, participating inn the remaining field trips.**

During each test the following procedure was used:

With bait in a bait bag on a long line, dogfish residing at larger depth (close to seabed) are lured and slowly attracted upward to the rig. The baseline is started when there is significant shark activity visible on the monitoring cameras. The baseline is run for five minutes, after which the EM test is conducted by switching the system on for the next five minutes. The GoPros are recording continuously what is happening on and around the rig.

On both days, EM field strength was measured using Ag+/AgCl electrodes installed on the rig. The voltage difference between the two electrodes placed at 10 cm distance was recorded using an oscilloscope. A typical field strength measurement series of the 6th of November is shown in Fig.6 and on the 7th of November in Fig.2.

Between the first and second series of tests (day 1 versus day 2) different EM supply cable dimensions were used, resulting in differences in the electrical resistance to the electrodes (**Figure 42-43**). On the 6th of November, 50 m long supply cables with AWG8 dimension were used, while on the 7th of November, 40 m long supply cables with AWG14 dimension were used. Because of the higher resistance, the voltage drop over the cables to the electrodes is estimated to be 50 V for the latter compared to 15 V for the first (with 120 V supply @ 40A). The second cable is therefore expected to result in lower field strengths, as witnessed on 7th of November.

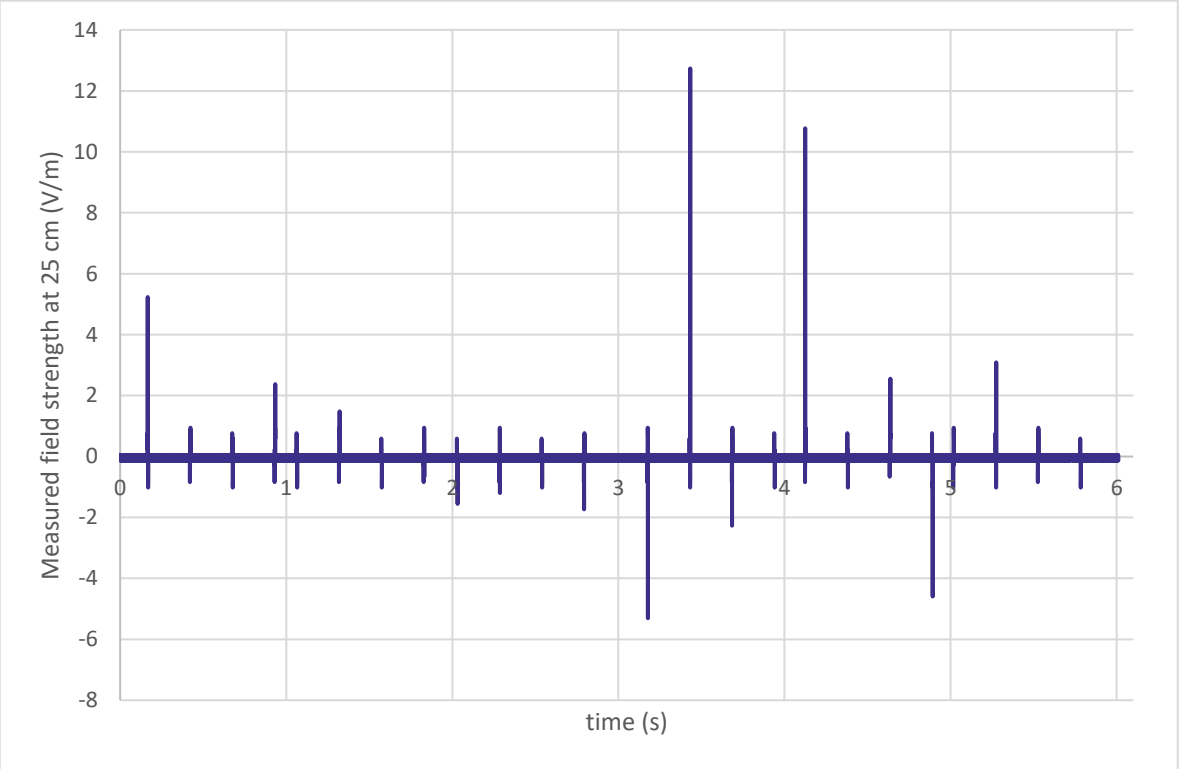


Figure 42 Measured field strength 6th of November 2023

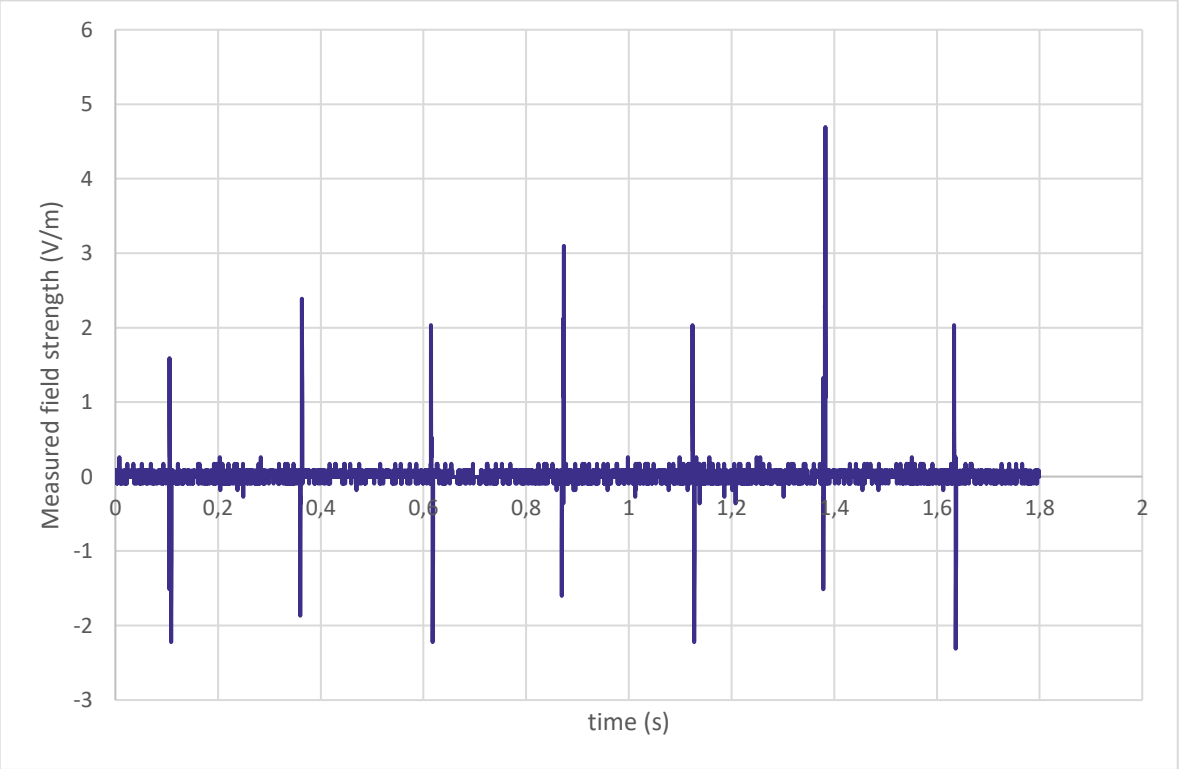


Figure 43 Measured field strength 7th of November 2023

Salarsafe updated their system after these field trials to be able to give an order of magnitude higher EM field strength. The estimated length of the dogfish registered around the rig varies from 80 to 120 cm. According to literature, a field strength of 7 V/m is required for a 75 cm long shark to induce

a response, with the threshold being inverse proportional with the shark length. The assumption was therefore that the Salarsafe system was operating slightly below the minimum assumed field strength levels to provide a 100% consistent flight response. The updated system was designed to give a factor 10 higher field strength and thus the ability to run the system with pulse parameters exceeding by large margin the minimum requirements for deterrence.

New field trial was conducted on the 26th of February 2024. Again, the location was Herdlafjorden with MS Solvik. During this day we did not manage to attract and observe presence of dogfish. The test rig was deployed with the Salarsafe system, but not tests with dogfish could be carried out. This did however give the opportunity to do field strength measurements with the upgraded system (**Figure 44**).

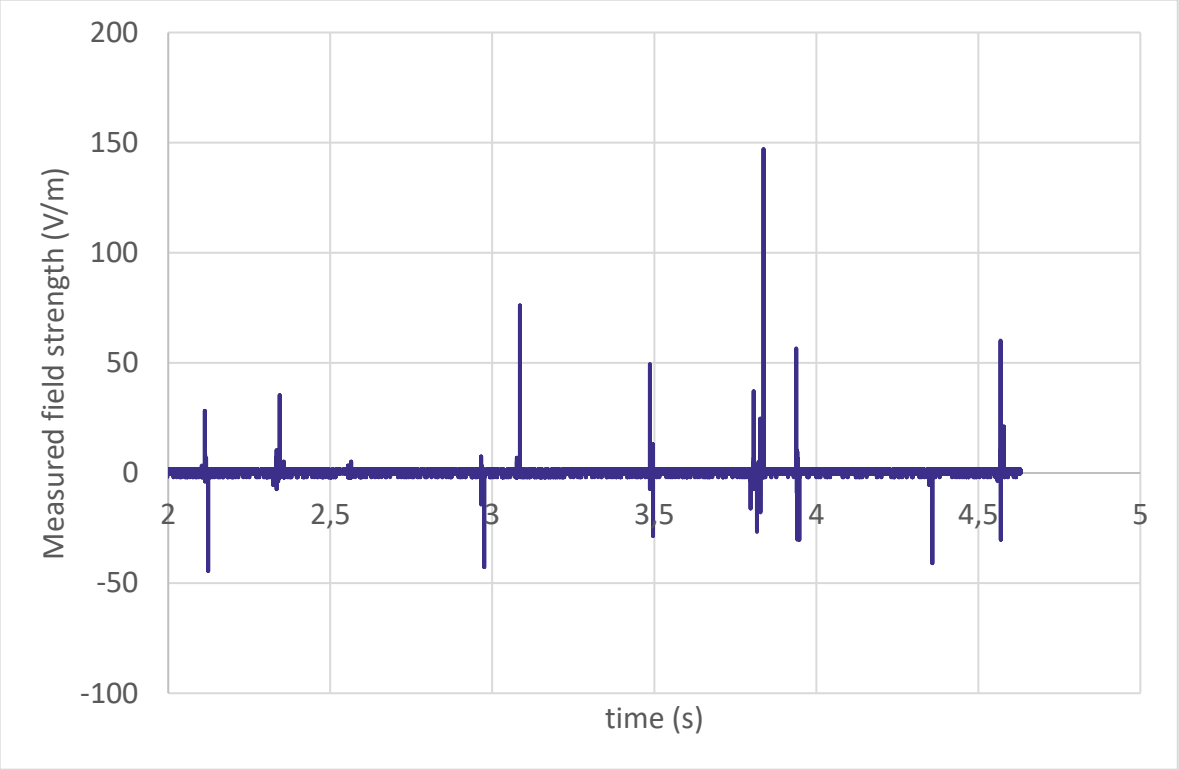


Figure 44 Measured field strength 26th of February 2024

The final field trip was taken on the 3rd of March 2024 with MS Solvik. This time also a separate camera rig was used to explore the seabed. On the Herdlafjorden location, no dogfish were found, only a large sized ling was registered on the seabed camera rig. We tried at two more locations based upon recent dogfish observations. On the last location, Veafjorden, the seabed rig recorded some very young dogfish (30 cm size) but no adults.

On the first test starting 6th of November 15:59, the baseline is accidentally repeated (two sequential five-minute periods). We have included here the data from both baselines. The shark observations in and around the test rig from the GoPro footage for the first test is summarized in **Figure 45**.

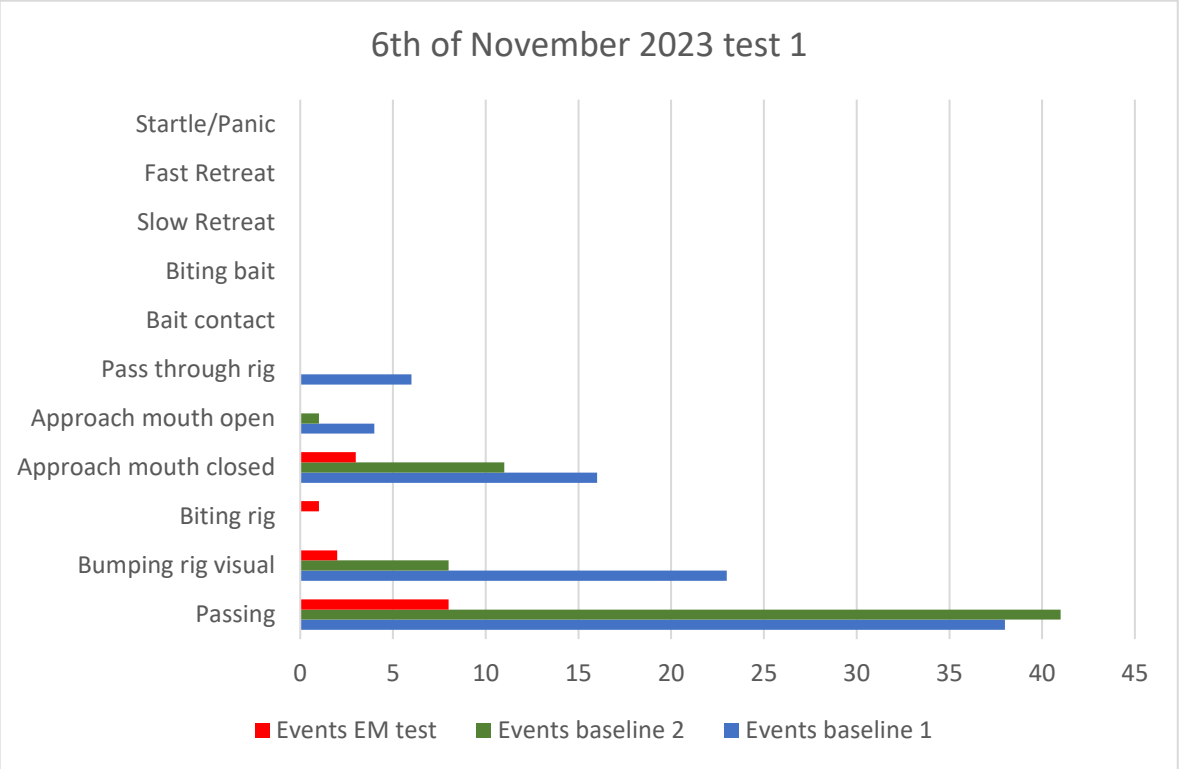


Figure 45 Summary of results from test 1 on 6th of November 2023

We register that during the EM test the number of dogfish observed near the test rig reduces with 80% compared to the baseline. The number of interactions with the test rig reduces with 70- 88%. During this first test there is no bait contact despite numerous approaches. The number of approaches with EM on reduce with 75-85%.

The second test on the 6th of November 2023 starts at 16:30. The observations are shown in Figure 46. The number of dogfish observed near the rig reduces with 67% and the number of rig interactions with 100%. Similar to the first test, there is no bait interaction. There is also no rig interaction with the EM switched on.

The third test on the 6th of November commences at 16:55. At that time the ambient light conditions are insufficient to be able to analyze the video footage, sundown time at that day is at 16:29.

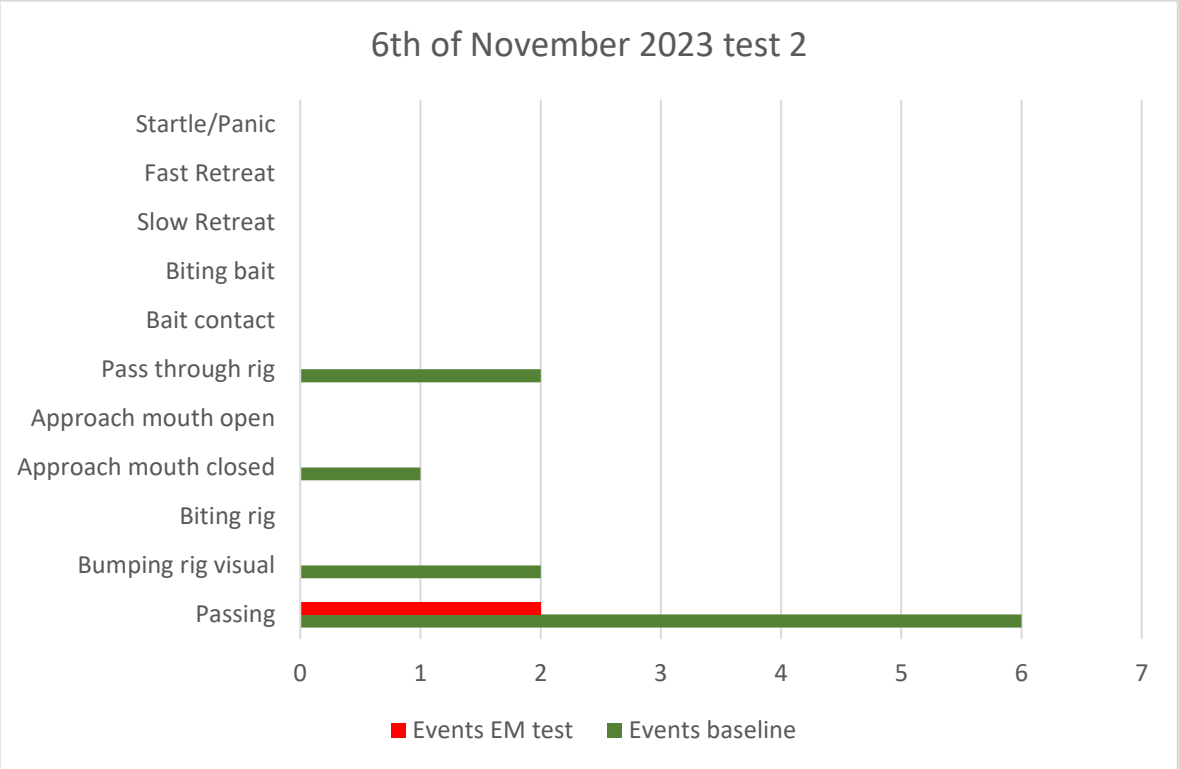


Figure 46 Summary of results from test 2 on 6th of November 2023

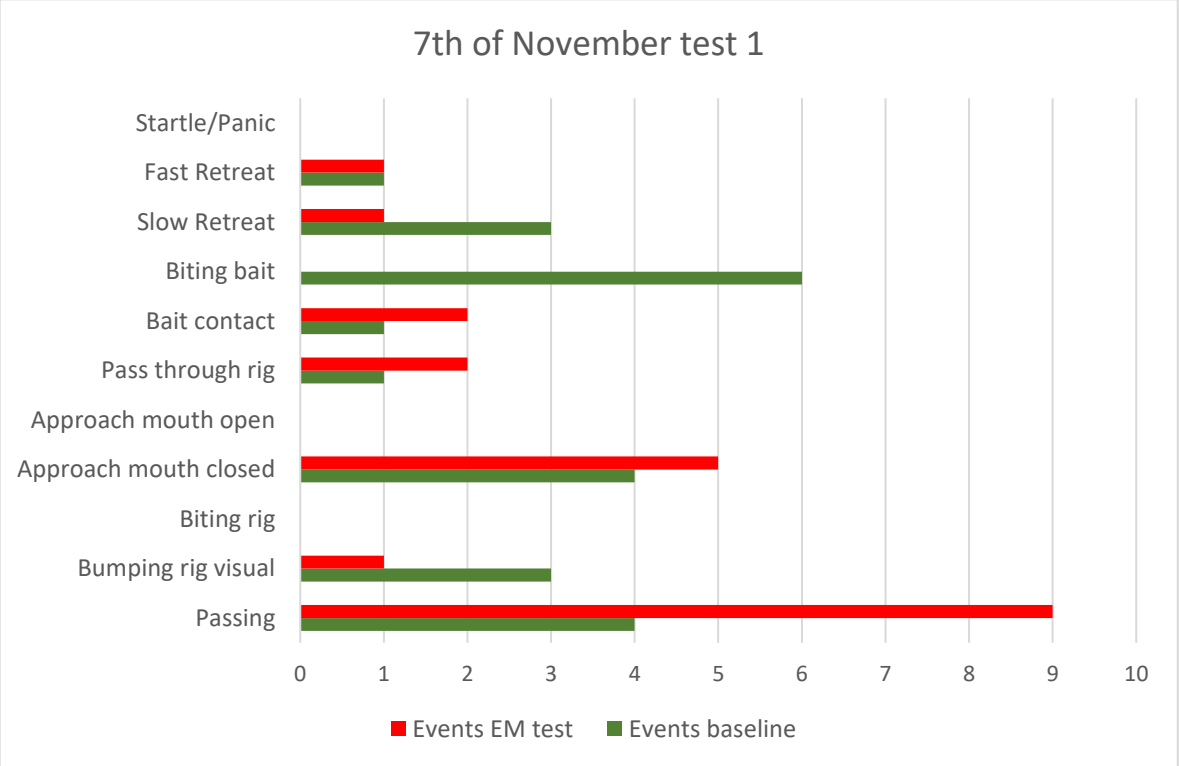


Figure 47 Summary of results from test 1 on 7th of November 2023

The first test on the 7th of November starts at 15:52 at 30 meters depth with observations shown in **Figure 47**. During this test the dogfish interact with the bait on the test rig during the baseline period. From the GoPro video footage, when the EM system is activated, there is actually a bait interaction ongoing, with a subsequent flight response of this dogfish. During the EM we observed

an increase of sharks near the rig, but the number of rig interactions reduces by half. The dogfish have become interested in the bait on the rig during the baseline and keep approaching the rig and bait when the EM is on but are not carrying through their approach on most occasions, and do not bite the bait.

The second test on the 7th of November starts at 16:10, with the rig at 20 meters depth. The observations are shown in **Figure 48**. This time the dogfish are interacting with the bait during the baseline period but not when the EM system is switched on. During the baseline, it is with a high degree of certainty the same dogfish, a smaller female (80 cm length), on the bait. During the EM period the dogfish do not approach the rig but stay at some distance.

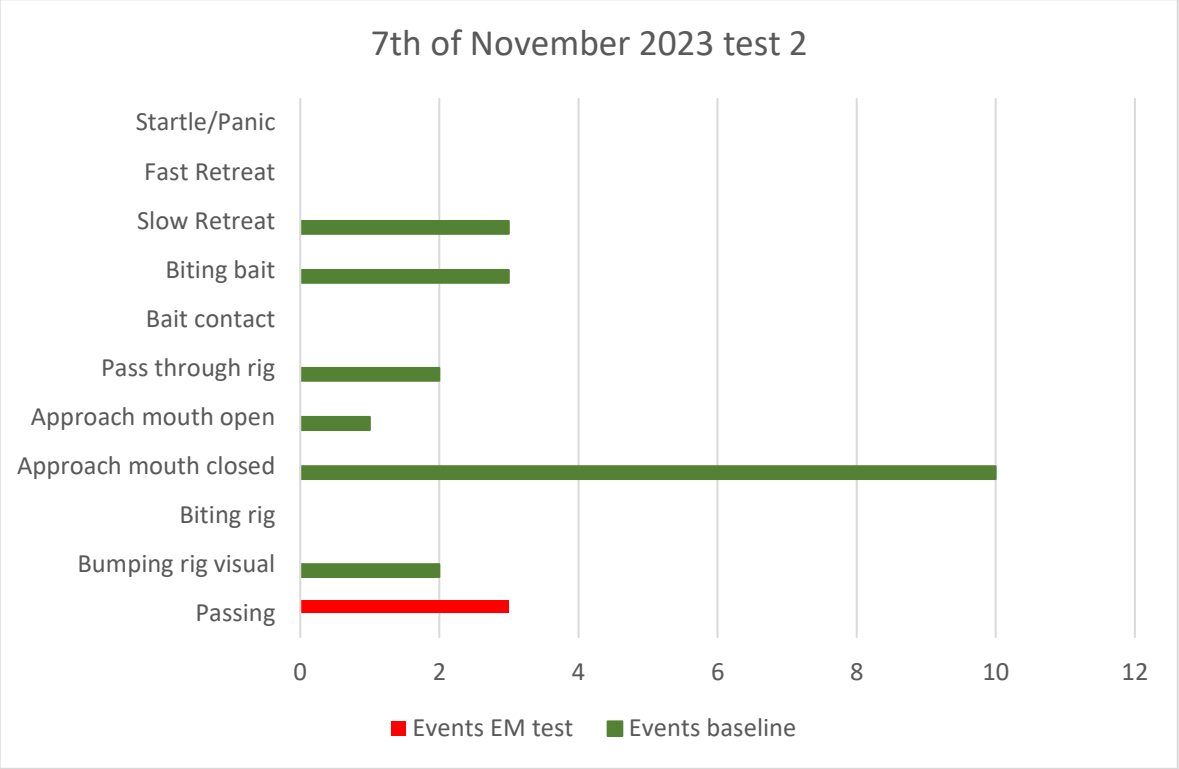


Figure 48 Summary of results from test 2 on 7th of November 2023

The third test on 7th of November starts at 16:21 and shows the same trend as the previous tests. The results are shown in **Figure 49**. Again, there is a dogfish feeding on the bait at the end of the baseline and showing an escape response when the EM system is switched on. The reduced light conditions during this test make it difficult to say with certainty that this is the same shark as in test 2. With the EM system on there are less dogfish near the rig and no rig interactions.

The fourth test on the 7th of November starts at 16:46. During this test the light conditions are very poor. The results are shown in **Figure 50**. The reduction in dogfish presence is reduced by 62% and the rig interaction by 100% with the EM system on compared to the baseline.

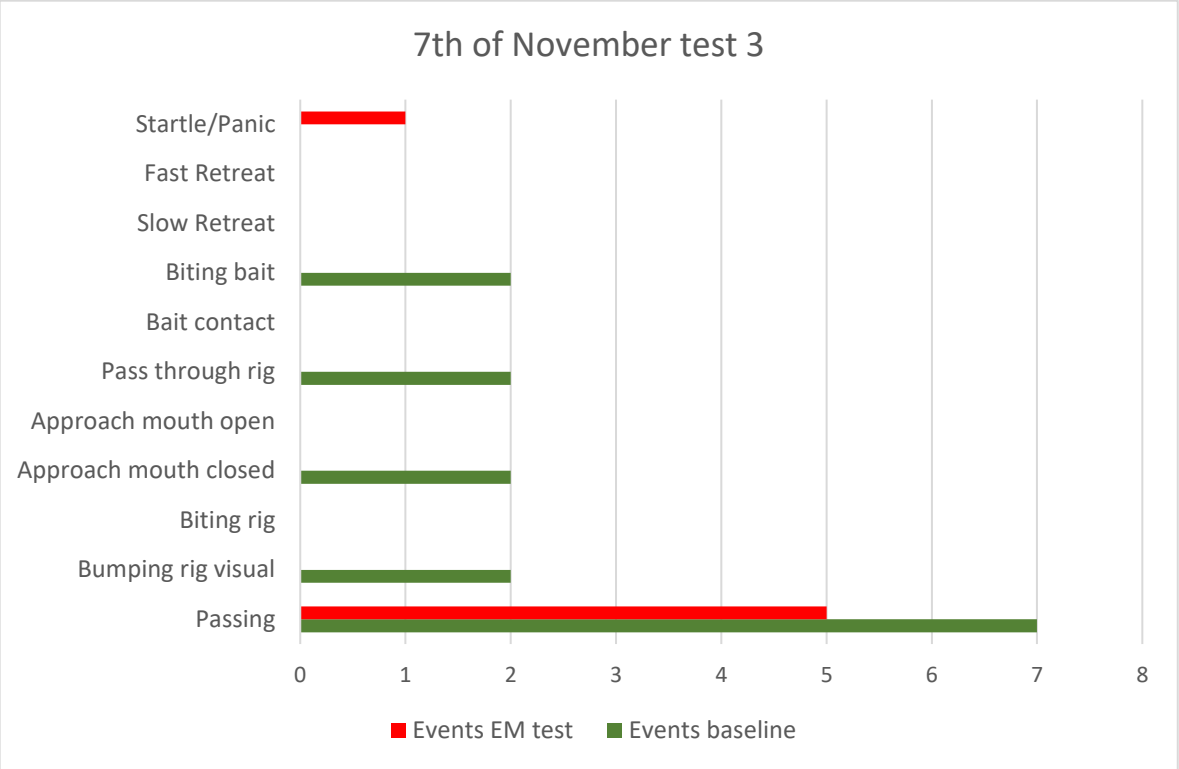


Figure 49 Summary of results from test 3 on the 7th of November 2023

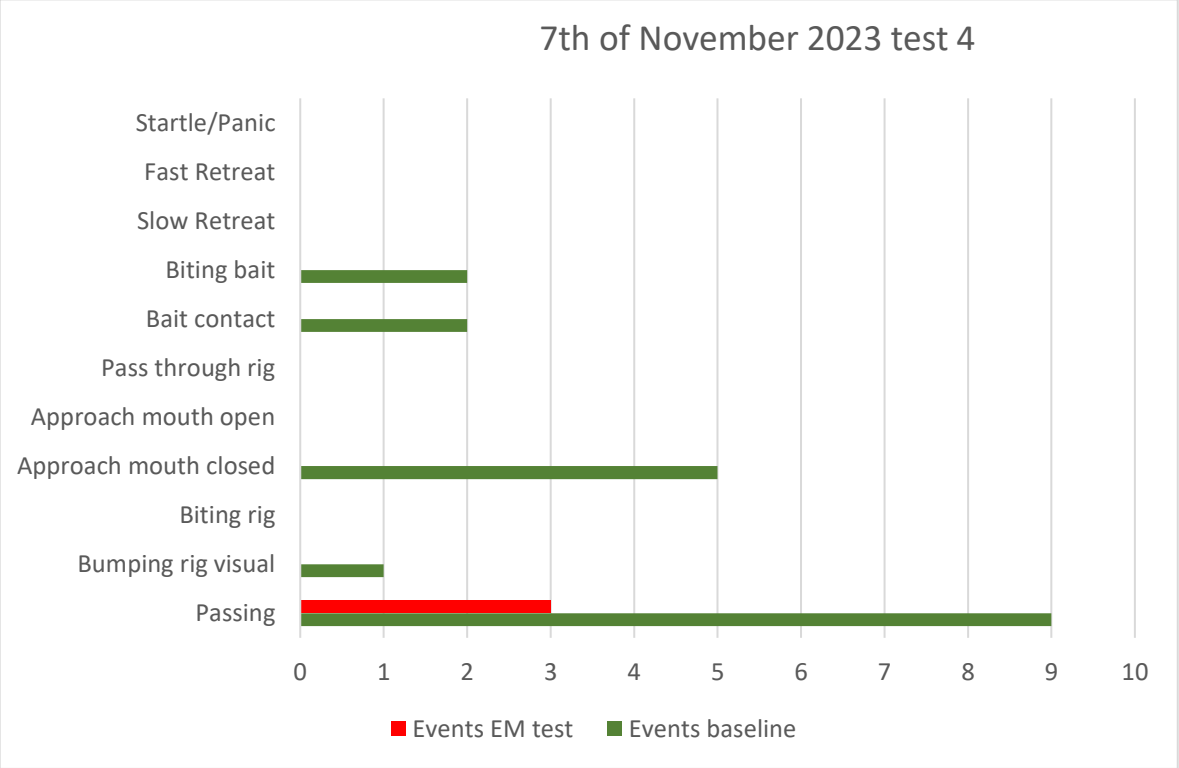


Figure 50 Summary of results from: test 4 on 7th of November 2023

The test observations were summarized as pairs of datasets, one for the five minute baseline period, and one for the EM on period, according to three different parameters. These are:

A: the number of dogfish passing the rig events

B: the number of interaction events with the rig

C: the number of interacting with bait events

Parameter B includes the categories 'Bumping rig visual', 'Biting rig', 'Approach to rig/bait with mouth closed', 'Approach to rig/bait with mouth open', 'Bait contact' and 'Biting bait/feeding'. Parameter C includes the categories 'Bait contact' and 'Biting bait/feeding'. The data sets are shown in **Table 3**.

Table 3. datasets for parameter A (the number of dogfish passing the rig events), B (the number of interaction events with the rig) and C (the number of interacting with bait events).

Parameter	**A**	**A**	**B**	**B**	**C**	**C**
Period	**Baseline**	**EM on**	**Baseline**	**EM on**	**Baseline**	**EM on**
day 1 test1	41	8	22	6	0	0
day 1 test2	6	2	4	0	0	0
day 2 test 1	4	9	13	7	8	1
day 2 test 2	0	2	15	0	3	0
day 2 test 3	7	5	8	0	2	0
day 2 test 4	8	3	8	0	2	0

A Paired Samples t-test was conducted on the datasets to verify against the null hypothesis that there is no difference between the two pairs of data within a 95 % confidence interval. Accepting the null hypothesis implies that the EM has no effect on the measured parameter statistically within the confidence interval of 95%.

For parameter A (the number of dogfish passing the rig events), P(T<=t) one-tail= 0.16. This implies that we need to accept the null hypothesis and that within a 95% confidence interval the EM system has no effect on the number of sharks visibly passing the rig.

For parameter B (the number of interaction events with the rig), P(T<=t) one-tail= 0.003. The null hypothesis can therefore be rejected within a 95% confidence interval, and also within a 99% confidence interval. The tests show thus that within a 99% confidence interval the EM system reduces the number of dogfish interaction events with the rig.

For parameter C (the number of interacting with bait events), P(T<=t) one-tail= 0.04. The null hypothesis can therefore be rejected within a 95% confidence interval. The tests show thus that within a 95% confidence interval the EM system reduces the number of dogfish interactions with bait.

5.3.2. Orca sound did not cause any aversive behaviour.

In total six tests were conducted on the 16th of May 2023 at Herdlafjorden in the middle of the day. These tests are analyzed in a slightly different way. During the trial, there was no approach to bait or biting observed. Hence, we used only the presence or passing of dogfish near the rig as parameter. The average number of dogfish present on the video footage in the test period is determined for the 5 minutes baseline and 5 minutes test period. The results are summarized in **Figure 51**; the result shows that the effect of orca sound is not significant (paired t-test, p> 0.05).

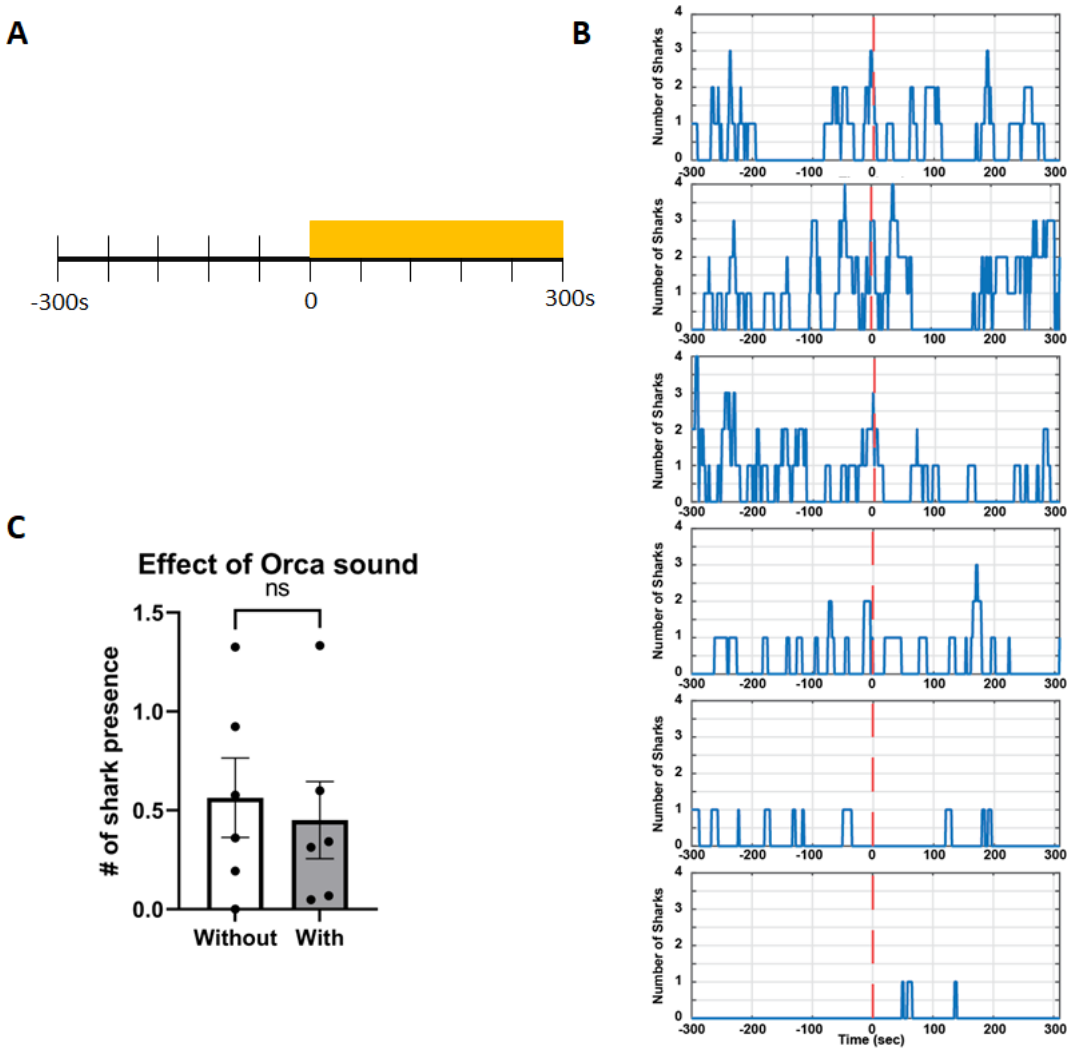


Figure 51 Orca sound has no statistically significant aversive effect. (A) Trial design. A 5 min baseline was recorded before application of Orca sound for 5 min. (B) Number of spiny dogfish around the cages (counted every 10 seconds) for six different trials. (C) Effect of orca sound on presence of spiny dogfish. Values are presented with mean and SE together with scatter of the data.

5.3.3. Challenges with net type trials

Nylon nets provide little protection against spiny dogfish (**Figure 52**). Several attempts were made for testing the effectiveness of different net types without success. In all field trips, no spiny dogfish were observed (5 trial days across 3 months). Efforts were made to locate them using underwater

camera, ROV and also through communications with fisherman, nearby fish farms and researchers conducting tracking of dogfish. However, no successful trial could be conducted.

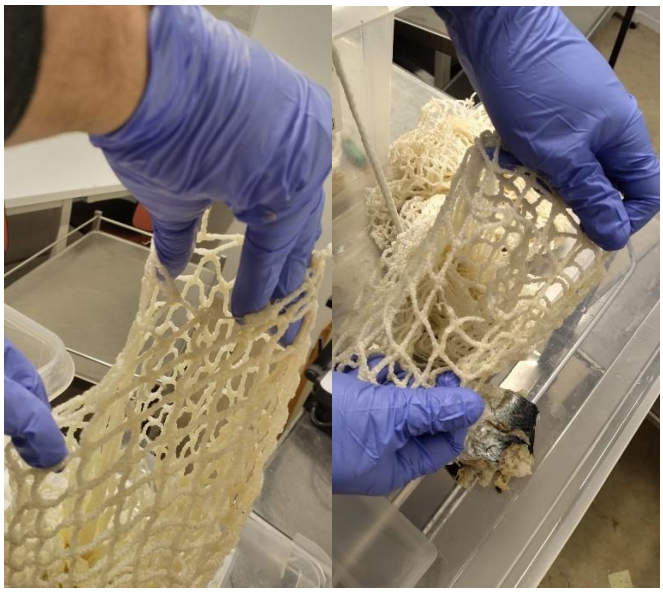


Figure 52 Nylon nets provide little protection against spiny dogfish. A hole of size 10-15cm was created within 2-5 minutes of spiny dogfish engaging with the bait.

5.3.4. Discussion on field trials

The results of the field tests show that there is no significant effect of using sound of orcas (killer whales) as a repellant for dogfish. The field tests with the EM system do indicate a deterrent effect, but only very near, and close up to the rig. The effect of EM on the number of sharks nearby, but at larger distance of the rig is not statistically significant within a 95% confidence interval according to the test results. However, for five out of the six tests conducted the result is also overall reduced numbers of dogfish near the rig with the system active. The EM pulses spatially decay exponentially in the seawater with distance from the electrodes. Electromagnetic wave attenuation in seawater is exponential, and at two meters distance from the electrodes it is expected that the EM field has reduced to below background levels, and thus will not be noticeable for the dogfish. Only when approaching the electrodes, the EM field will become progressively more effective.

The test results do show that the EM system leads to reduced dogfish interaction with the rig itself within in a 99% confidence interval. Test results also do show that the EM system reduces the dogfish interaction with the bait within a 95% confidence interval. In addition, the EM system showed a potential to scare a dogfish of the bait. On two of the tests, the transition from baseline (no EM) to EM test period coincides with a dogfish feeding on the bait. The video footage shows than abortion of the feeding behaviour and the subsequent withdrawal of the dogfish from the rig. These two observations can from a statistical standpoint only be considered anecdotal but do back up the statistically significant test results reported here. This is with a system operating on relative low levels of EM field strength. Unfortunately, we could not test the system with higher EM field strength on dogfish, so we could not with certainty establish the necessary threshold values for the EM field strength necessary to deter dogfish 100% of the time. We did not see that on the second day of testing there any signs of habituation or individual dogfish becoming bolder from test to test,

based on the current analysis of the results. This is however indicative at this stage; more testing needs to be done to determine the longer-term effectiveness as a deterrent.

Field trial for net types could not be conducted. However, several anti-predator nets are currently available, and use of anti-predator net does add additional operations. A comprehensive study on different material should be conducted using a model cage and in collaborations with users of these nets.

5.3.5. Recommendations based on field trials.

The EM system has shown promising results and further field testing should be conducted. The tests should include determining field strength needed to scare off dogfish feeding on the bait as well as more series to determine if there is habituation. The tests should be done with ambient lightning to make it easier to identify individual dogfish.

5.4. Response of Atlantic salmon to electromagnetic pulse

5.4.1. Electromagnetic pulse has no effect on growth of Atlantic salmon.

To test the effect of EM pulse on physiology, growth and stress response of farmed fish, an EM trial was conducted. Atlantic salmon smolt of size 200-300g were used in this study. EM pulse of three different strength (5V, 10V and 20V) were applied three times each. Behaviour of the fish before and after application of EM pulse were recorded. Fish moved away from the source of the EM (not shown here). However, the EM field was present throughout the tank and all fish experienced the EM field irrespective of their location. Fish were sampled 45min after the last stimulus (d0 sampling), 24hours past stimulus (d1 sampling) and 21 days past stimuli (3w sampling). Fish were euthanized with overdose of MS-222 and tissue samples were collected. Both the control and EM group showed similar weight and length throughout the experimental period. The fish showed similar growth parameter after 21 days of treatment suggesting that application of EM has no effect on the growth of Atlantic salmon smolt (**Figure 52**, ANOVA with Tukey post-hoc comparison, $p > 0.05$).

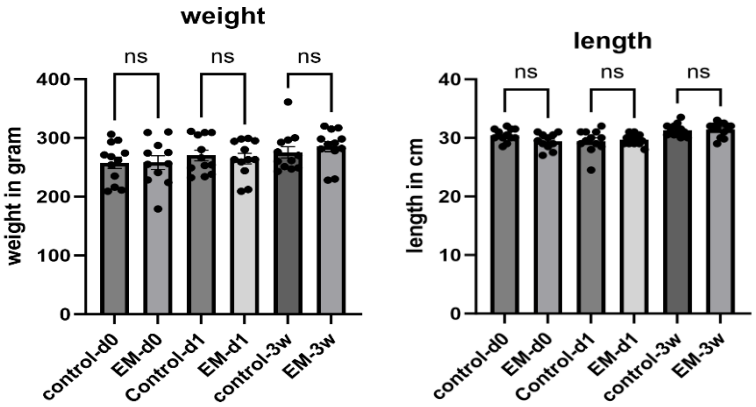


Figure 53 No change in growth parameters (weight and length) was observed between EM and control groups. Values are presented with mean and SE together with scatter of the data.

5.4.2. Electromagnetic pulse does not cause long term stress response.

Plasma cortisol, ions (sodium, chloride, phosphorous, magnesium) and metabolites (triglycerides, gluocose, lactate, cholesterol, total protein) were measured (**Table 4**). Glucose and lactate levels were significantly higher in EM group in d0 and d1 of the sampling (**Figure 54**, ANOVA with Tukey post-hoc comparison, $p < 0.05$). Plasma cortisol was also higher in EM group in d0 and d1 sampling points however the difference was statistically non-significant ($p > 0.05$). At 3 weeks after EM treatment, there was no difference in plasma metabolites and cortisol ($p > 0.05$). Whole telencephalon expression of neural activity (cFos), neural plasticity markers (BDNF, NeuroD) and stress markers (mineralocorticoid receptor MR and glucocorticoid receptors GR1) expression were analyzed. No statistical difference between control and treated groups were found (**Figure 55,** ANOVA with Tukey post-hoc comparison, $p > 0.05$) except for BDNF. BDNF showed increased expression level one day after EM treatment compared to the control group ($p < 0.001$), however the expression of BDNF were similar 3 weeks after EM treatment. Stress markers MR and GR1 showed no statistical change in response to EM throughout the observation period. This suggests that repeated experience of EM pulse cause stress response, however, salmon smolts recover from such stress within three weeks.

Table 4. The mean (SD) values of metabolites from serum samples in salmon smolts in control and EM group on d0, d1 and 3w sampling. TP=Total protein, Mg=Magnesium, LA = Lactic acid, Na = Sodium, Chol = Cholesterol, Tri = Triglycerides, Pho = Phosphorus, Glu = Glucose, Ca = Calcium, Ch = Chloride.

Metabolites	Control-d0 (n=12)	EM-d0 (n=12)	Control-d1 (n=11)	EM-d1 (n=11)	Control-3w (n=12)	EM-3w (n=12)
Na (mmol/L)	159.48 (8.21)	161.70 (9.80)	159.94(12.02)	157.23(9.11)	153.80(3.43)	145.30(8.87)
Chl (mmol/L)	136.45 (6.98)	131.08(11.07)	129.11 (6.94)	129.58(6.33)	123.53(1.51)	135.48(4.93)
Mg (mmol/L)	0.65 (0.11)	0.89 (0.39)	0.72 (0.07)	0.76 (0.11)	0.74 (0.08)	0.71 (0.11)
Ca (mmol/L)	2.64 (0.16)	2.47 (0.14)	2.58 (0.12)	2.40 (0.13)	2.57 (0.13)	2.51 (0.14)
Pho (mmol/L)	2.01(0.33)	2.27 (0.54)	2.48 (0.32)	2.59 (0.60)	2.67 (0.56)	2.26 (0.44)
Glu (mmol/L)	5.01(0.67)	5.71 (0.84)	5.25 (0.57)	5.90 (0.93)	5.64 (0.38)	5.42 (0.71)
LA (mmol/L)	3.40 (0.69)	4.31 (1.18)	4.05 (0.90)	3.49 (0.82)	3.32 (0.48)	2.70 (0.35)
Chol (mmol/L)	7.12 (1.22)	6.88 (1.08)	7.52 (0.72)	6.11 (0.96)	7.25 (1.71)	7.38 (1.53)
Tri(mmol/L)	1.65 (0.42)	1.28 (0.68)	1.33 (0.29)	1.25 (0.43)	2.03 (0.65)	1.63 (0.68)
TP (g/L)	30.74 (4.18)	28.28 (2.55)	30.13 (2.51)	26.03 (2.45)	29.34 (4.24)	32.11 (4.33)

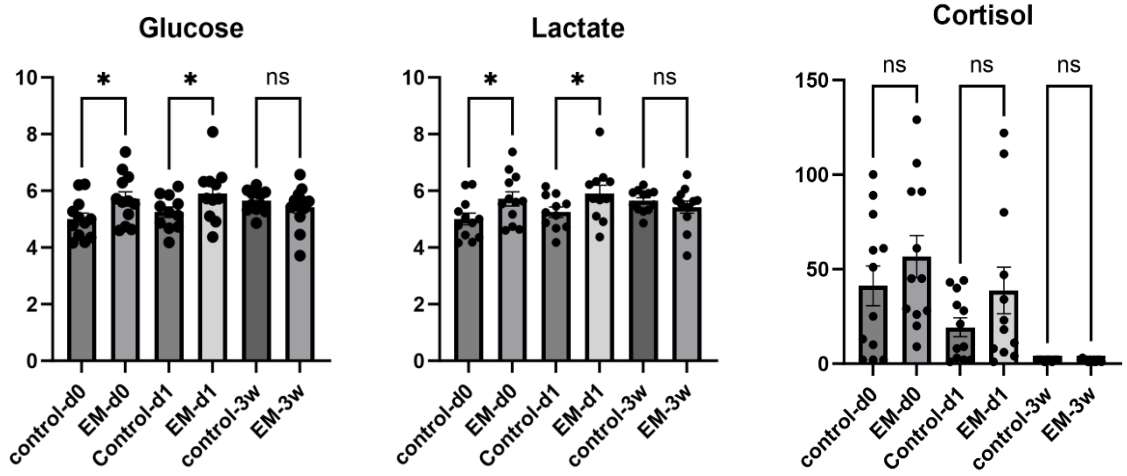


*Figure 54 Glucose, lactate and plasma cortisol in the control and EM treatment groups after one hour, one day, and 21 days. Glucose (mmol/l), Lactate (mmol/l), Cortisol (ng/ml). Values are presented with mean and SE together with scatter of the data. *p<0.05.*

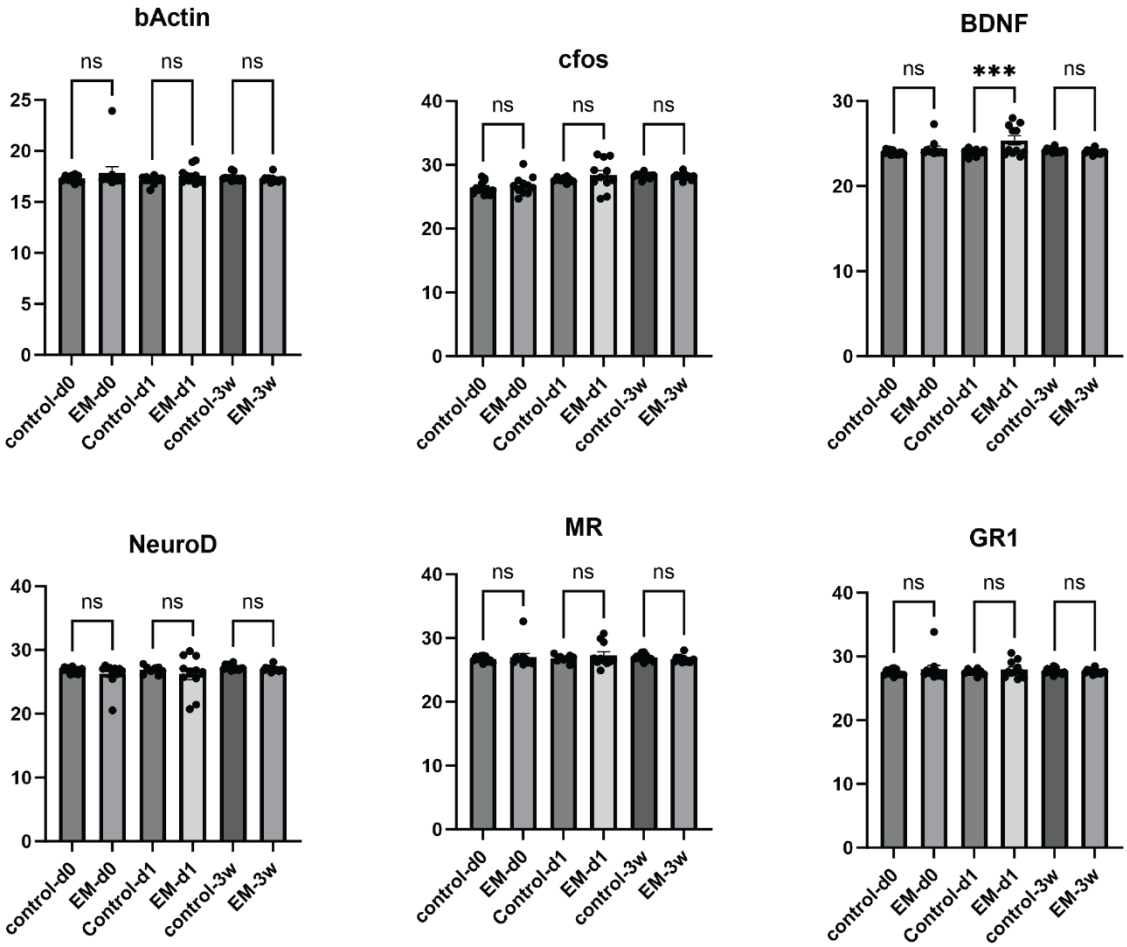


Figure 55 Changes in whole telencephalon expression of reference gene bActin, neural activity marker cfos, neural plasticity markers BDNF (brain derived neurotrohpic factor) and NeuroD

(neurogenic differentiation factor), stress markers MR (mineralocorticoid receptor) and GR1 (glucocorticoid receptors) in the control and EM treatment groups after one hour, one day, and 21 days. Values are presented with mean and SE together with scatter of the data. ***p<0.001.

5.4.3. No long-term impact on Acetylcholinesterase activity.

Acetylcholinesterase (AChE) catalyses the hydrolysis of acetylcholine and thereby terminates the synaptic transmission. Beyond this classical function, AChE has multiple functions in embryogenesis, neuromodulation and stress response. While neurotoxic chemicals such as organophosphate and carbamate pesticides or snake venoms are known AChE inhibitors, the physiological regulation of AChE often leads to increased levels under stress, injuries or inflammation responses (Das, 2012).

AChE activity was significantly lower in the first sampling of the control group (tanks 3, 4, and 8) compared to all other treatment groups (**Figure 56**, ANOVA with Tukey post-hoc comparison P<0.001). When both treatment and tank were included in an ANOVA, both were significant (p<0.0001 and p<0.05, respectively), with only tank 4 being significantly lower than several of the other tanks. Across tanks, there was a tendency to increase AChE activity over the observation period. On day 21 sampling no significance difference in activity was observed.

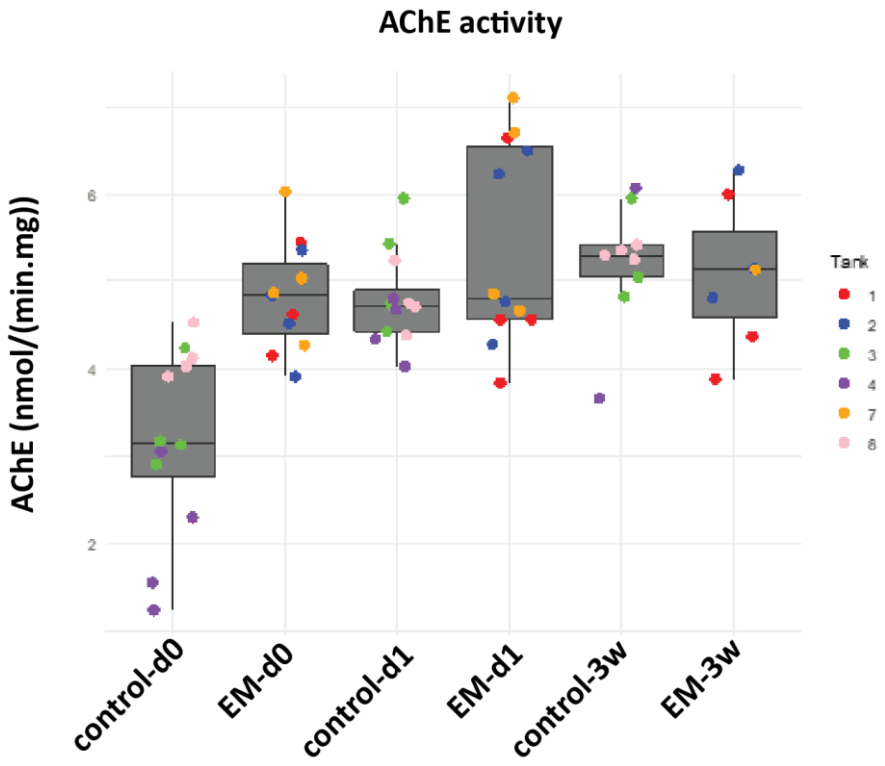


Figure 56 Acetylcholinesterase activity in the control and EM treatment groups after one hour, one day, and 21 days, colored by tank ID.

5.4.4. Recommendations based on the effect of EM on salmonid physiology.

The EM system induce aversion and short-term stress response; however, it has no impact on growth and salmon smolts recover from stress within short duration. Nonetheless, the design of EM based deterrent should be such that the farmed fish experience as little EM stimuli as possible. It is recommended that EM system is outside the cage and EM field does not penetrate the inside of the cage.

5.5. Implementation of research results in the industry

Shark barriers and deterrents have been developed against specific species of sharks; these have been used to keep sharks away from bathing areas, to offer personal protection for swimmers, divers and surfers; some of these have also been tested to keep sharks away from bait and catch in line and net fishing. However, their reported effectiveness varies, depending on the species and geographical area, and none of the measures provide a full deterrence. PigghåFRI has aimed to document anti-shark measures that can be implemented in aquaculture installations.

PigghåFRI finds that both electromagnetic pulse-based deterrent and chemical deterrent (skin extract) are effective in repelling spiny dogfish. SalarSafe, a Norwegian start up, has been designing and developing a shark safe rig based on EM that can be deployed in a fish farm. SalarSafe had provided their EM generator for the field trials. This cooperation provided opportunity to help refine an anti-shark measure for aquaculture installations through a research-based approach. Chemical based deterrent needs further research and development work. Recent findings in fish species (zebrafish) suggest that specific group of chemicals in the skin extract of conspecific induce fear response (Masuda et al. 2024) and it is possible to identify such chemicals. In future, these chemicals can be used as a coating or impregnant for the cage nets. Thus, the nets itself are equipped to scare sharks away during the shark attack. Cooperation with researchers/chemists and net manufacturers will be sought for a future project aiming to develop chemical based shark deterrents.

PigghåFRI had also aimed to test different net material that are currently in use. Unfortunately, due to failure in finding sharks during the field trials, such this test could not be conducted.

Further steps needed to make these results applicable in industry:

1. Chemical based deterrents: The effectiveness of skin extract as a repellant for keeping spiny dogfish away from dead fish in an aquaculture setting must be determined. This includes investigations into which chemical compounds of skin extract induce a fear response with spiny dogfish and what the minimum effective concentrations are. The variation in response between individual dogfish, its effectiveness within a group setting and habituation effects influencing long term effectiveness also needs to be investigated. Trials need also to be conducted to ensure no stress response is induced in salmonids.

2. EM methods: EM methods, like the technology from SalarSafe used in the field tests should be tested in an aquaculture setting for long term effectiveness. This could for example be carried out as a pilot test on a single aquaculture installation where both an aquaculture company and SalarSafe participate with necessary public funding.

5.6. Contribution of research results towards enhanced sustainability

Protecting a vulnerable slow growing species: The spiny dogfish (*Squalus acanthias*) is Norway's most common shark species and is an integral part of fjord ecosystem. The spiny dogfish has long generation time. The female becomes sexually mature at 11-12 years of age while for the male it is 5-6 years of age. It takes two years for eggs to mature. The gestation period is 23 months. New eggs develop during the females' pregnancy and are fertile one month after she has given birth. Norwegian fishing for spiny dogfish has previously been around 1000-5000 tons per year. The species had been placed on the red list of the Species Data Bank and was classified as highly endangered. All commercial and recreational fishing was therefore prohibited in Norwegian waters. ICES considers the spiny dogfish stock to be at a historically low level, but recently sees signs of an increase in biomass and recruitment. The precautionary approach is nevertheless used in the absence of a stock index. Recently, the Norwegian Government has opened commercial fishing of spiny dogfish; this puts them in a vulnerable situation in the future. PigghåFRI has aimed to find a solution that reduces or eliminates spiny dogfish attack to fish farm without harming them. Thus, it will help reduce mortality of spiny dogfish associated with fish farms thereby PigghåFRI contributes to enhanced sustainability of current farming operations.

Reducing escapee incidents associated with spiny dogfish: In a shark incident, the spiny dogfish bite through the net and get in the fish cages. The holes in the cages cause escapees of farmed fish, forming a leading ecological challenge. PigghåFRI project contributes to finding a solution that stops spiny dogfish attacks on fish farms and hence will lead to decreased incidents of predator associated escapee of farmed salmon. Reduced escapee of farmed salmon from fish farms will increase the yield. Moreover, resources needed to catch escaped salmon are also reduced. These in turn will reduce the necessary resources and emissions per kg of salmon produced.

6. Main findings

English

Knowledge base on incidents and behaviour of spiny dogfish:

- Fish farms in southern and western Norway report high incidence of spiny dogfish during autumn and winter periods. Spiny dogfish attack in groups the bottom of fish cages and often attack the same cage multiple times.
- The incidence of spiny dogfish is associated with the presence of dead fish in the fish cages but without apparent significant relation to any other farm operations.
- Removing the dead fish frequently is currently the most effective measure employed at fish farms to avoid dogfish incidents.

Laboratory study of sensory induced behavioural response of spiny dogfish:

- Wild caught spiny dogfish can be kept in captivity with good welfare conditions.
- Electromagnetic pulse and smell of dead conspecific (skin extract) induce fear like response without inducing chronic stress response in spiny dogfish. Orca sound has no effect as repellent against spiny dogfish behaviour.

Field trial of promising anti-shark measures:

- Orca sound has no effect on spiny dogfish behaviour.
- Electromagnetic pulse shows promise as effective repellent but has varying effect; smaller sharks may need higher strength of the EM pulses compared to larger sharks.

Effect of electromagnetic pulse on Atlantic salmon:

- Electromagnetic pulse had no effect on growth. Atlantic salmon shows no sign of chronic stress induced by electromagnetic pulse.

Norsk

Kunnskapsbase om hendelser og adferd hos pigghå:

- Oppdrettsanlegg i Sør- og Vestlandet rapporterer om høy forekomst av pigghå i løpet av høst- og vinterperiodene. Pigghåene angriper i grupper og oppholder seg ofte ved bunnen av merden, og kan angripe ofte samme merd.
- Forekomsten av pigghå er signifikant knyttet til døde fisker i merden, men ingen betydelig sammenheng er funnet med andre aktiviteter/operasjoner på anlegget.
- Fjerning av død fisk er for tiden det mest effektive tiltaket i bruk ved anlegg for å unngå pigghåangrep.

Laboratoriestudie av sensorisk induserte adferdsresponser hos pigghå:

- Villfanget pigghå kan holdes i fangenskap under gode velferdsforhold.
- Orca-lyd har ingen avskrekkende effekt på pigghåadferd.
- Elektromagnetiske impulser og lukten av døde artsfrender (hudekstrakt) induserer en fryktlignende respons, uten å indusere kronisk stressrespons.

Feltforsøk med lovende anti-haitiltak:

- Orca-lyd har ingen effekt på pigghåadferd.
- Elektromagnetiske impulser viser potensiale som effektivt avskrekkende middel, men effekten varierer: Mindre haier trenger høyere EM-styrke enn store haier for å ha full effekt.

Effekt av elektromagnetisk puls på atlantisk laks:

- Elektromagnetiske impulser viste ingen effekt på **vekst**. Heller ikke viste Atlantisk laks tegn til langvarig stress på grunn av elektromagnetiske impulser.

7. Deliverables

Dissemination:

1. Havbruk 2022, Bergen, October 10-21. “Påvirkning og tiltak for å unngå påvirkning av pigghå i akvakulturanlegg". Pradeep Lal, Antonie Oosterkamp and Shaw Bamber.
2. Sharks International 2022, Valencian, October 20-22, “Mapping the interaction of spiny dogfish (*Squalus acanthias*) with aquaculture installations in Norway”. Pradeep Lal, Antonie Oosterkamp and Shaw Bamber.
3. Aquaculture Europe 2023, Vienna, September 18-21, “Antishark measures to avoid interaction of spiny dogfish (*Squalus acanthia*) with aquaculture installations”. Pradeep Lal, Mette Espedal Brynildsrud, Antonie Oosterkamp, Naouel Gharbi, Shaw Bamber.
4. AquaNor 2023, Trondheim, August 22-24, “Antishark measures to avoid interaction of spiny dogfish (*Squalus acanthias*) with aquaculture installations” Pradeep Lal, Mette Espedal Brynildsrud, Antonie Oosterkamp, Naouel Gharbi, Shaw Bamber.
5. NORCE workshop AquaFuture: Facing aquaculture’s challenge in the next decade 2024 Bergen, April 9, Presentation of research results from PigghåFRI.
6. HavExpo 2024, Bergen May 6, “Antishark measures to avoid interaction of spiny dogfish (Squalus acanthias) with aquaculture installations”. Pradeep Lal, Mette Espedal Brynildsrud, Antonie Oosterkamp, Naouel Gharbi, Shaw Bamber.

Publications:

1. “Investigating sensory cue-induced behavioral and physiological responses in Spiny dogfish *Squalus acanthias* for an effective shark deterrent”, Thesis Master of Science in Biology - Aquaculture Biology, University of Bergen. Student-Mette Espedal Brynildsrud.
2. Effect of electromagnetic pulse on behaviour and physiology of post smolt Atlantic salmon *Salmo salar*. (manuscript/results included in the report)
3. Sensory physiology of spiny dogfish in response to auditory, olfactory and electromagnetic pulse in laboratory and field study. (manuscript)

Popular engagement/media coverage

1. Sundnes, Hans Morten. Sårbar og strøm-følsom plageånd, **NorskFisk-Magasin**, september 2022

Reporting to FHF:

1. Minutes from reference group meeting
2. Status reports (7)
3. Faglig sluttrapport som beskrevet (https://www.fhf.no/fhf/slik-arbeider-fhf/):
4. Administrativ sluttrapport: 30.05.2024
5. Administrativ sluttrapport i tråd med FHFs *Retningslinjer for sluttrapportering* (se https://www.fhf.no/prosjekter/prosjektdokumenter/):

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9. Appendix 1- Questionnaire for survey

Questions	Spørsmål	Response	Svar
1. Information on extent of spiny dogfish problem	**1. Informasjon om omfanget av pigghå problem**		
How many fish cages do you have?	Hvor mange merder har du?	number	nummer
Where are the cages located?	Hvor er merdene plassert?	(county/commune/)	(fylke/kommune/)
What is the water depth at/around the location?	Hva er vanndypet på/rundt stedet?	depth (meter)	dybde (meter)
List your most affected sites.	List opp de mest berørte nettstedene.	(county/commune/)	(fylke/kommune/)
How many cages within an area are affected during a season?	Hvor mange merder i et område er berørt i løpet av en sesong?	(a) almost all cages (b) half of all cages (c) 1-2 cages (d) none	(a) nesten alle merdene (b) halvparten av alle merdene (c) 1-2 merder (d) ingen
What are your estimated economic costs due to spiny dogfish?	Hva er dine anslåtte økonomiske kostnader på grunn av pigghå?	_____NOK	_____ NOK
2. Information on sighting of spiny dogfish	**2. Informasjon om observasjon av pigghå**		
What time of the year do you usually find spiny dogfish near your location?	Hvilken tid på året finner du vanligvis pigghå nær din plassering?	(a) never (b) specific season/month (write month) (c) throughout the year	(a) aldri (b) spesifikk sesong/måneder (skriv måned) (c) gjennom året

Which time of the year are the incidents most frequent?	Hvilken tid på året er hendelsene hyppigst?	write month/season _____	skriv måned/sesong _____
How many times in a year do you find spiny dogfish in the cages?	Hvor mange ganger i året finner du pigghå i merdene?	(a) never (b) 1-2 (c) 2-5, (d) more than 5 times in a year	(a) aldri (b) 1-2 ganger (c) 2-5 ganger (d) mer enn 5 ganger i året
What species do you observe in the vicinity of your facility during the most affected season?	Hvilke arter observerer du i nærheten av anlegget ditt i den mest berørte sesongen?	write name of fish _____	skriv navn på fisk _____
3. Information on spiny dogfish and cages associated with the incidences	**3. Informasjon om pigghå og merder assosiert med hendelsene**		
How many spiny dogfish do you typically find in a cage after each incident?	Hvor mange pigghåer finner du vanligvis i et bur etter hver hendelse?	(a) 0 (b) 1-5 (c) 10-20 (d) more than 20	(a) 0 (b) 1-5 (c) 10-20 (d) mer enn 20
What is the size range of spiny dogfish caught inside the cages?	Hva er størrelsesområdet for pigghå fanget inne i merdene?	length (cm) _____ comment	lengde (cm) _____ kommentar
How did the dogfish get in the cages?	Hvordan kom pigghå i burene?	(a) spiny dogfish made a hole (b) another predator made the hole (c) it came through a hole that was scheduled for repair	(a) pigghåer laget et hull (b) et annet rovdyr laget hullet (c) det kom gjennom et hull som var

		(d) do not know	planlagt for reparasjon, (d) vet ikke
What was the size of hole?	Hva var størrelsen på hullet?	diameter (cm) _____	diameter (cm) _____
What was the type of net used in the affected cages?	Hva var nettypen som brukes i de berørte burene?	describe material /specification _____	beskriv materiale /spesifikasjon _____
What was the average age of the net for the affected cages?	Hva var gjennomsnittsalderen for nettet?	age in year/month _____	alder i år/måned _____
What is the usual location of holes in the cages?	Hva er den vanlige plasseringen av hull i merdene?	(a) bottom (b) middle (c) top (d) no pattern/ no record	(a) bunn (b) midten (c) topp (d) ingen mønster/ ingen post
Do you find more males or females (picture) in the caught spiny dogfish?	Finner du flere hanner eller hunner (bilde) i de fangede pigghåene?	(a) mostly male (b) mostly female, (c) almost equal number of male and female (d) did not notice	(a) hovedsakelig hann pigghåer (b) hovedsakelig hunn pigghåer (c) nesten like mange mannlige som kvinnelige (d) la ikke merke til det
When the spiny dogfish were caught and killed/died during handling, did you perform any autopsy?	Utførte du obduksjon da de pigghåene ble fanget og drept/død under håndtering?	(a) no (b) yes	(a) nei (b) ja

If yes, do you often find babies inside the female?	Hvis ja, finner du ofte babyer inne i hunnen?	(a) no (b) yes	(a) nei (b) ja
4. Information on possible cues that attract spiny dogfish	**4. Informasjon om mulige ledetråder som tiltrekker seg pigghå**		
Are there any operations that usually coincident with spiny dogfish incidents?	Er det noen operasjoner som vanligvis er sammenfallende med en pigghåhendelser?	(a) no (b) yes (describe_____)	(a) nei (b) ja (beskriv_____)
What is the size of fish when the spiny dogfish incidents are frequent?	Hva er størrelsen på fisk når den pigghåhendelser er hyppige?	(a) Up to 1 kg, (b) 1-2kg (c) more than 2 kg (d) no specific pattern	(a) Opptil 1 kg (b) 1-2 kg (c) mer enn 2 kg (d) ikke noe spesifikt mønster
Were there dead fish when the incident happened?	Var det død fisk da hendelsen skjedde?	(a) no (b) yes	(a) nei (b) ja
If yes, were the dead fish eaten?	Hvis ja, ble den døde fisken spist?	(a) no (b) yes	(a) nei (b) ja
Were the live fish attacked?	Ble den levende fisken angrepet?	(a) no (b) yes	(a) nei (b) ja
Do the incidents usually coincide with another predator attack?	Er hendelsene vanligvis sammenfallende med et annet rovdyrangrep?	(a) no (b) yes (describe)	(a) nei (b) ja (beskriv)
Farmers often report dogfish incidents after delousing	Fiskeoppdretter rapporterer ofte om pigghåhendelser etter avlusing operasjoner.	(a) no (b) yes	(a) nei (b) ja

operations. Do you have similar experience?	Har du lignende erfaring?		
If yes, how many days after delousing operations, did you find spiny dogfish inside the cages?	Hvis ja, hvor mange dager etter avlusing operasjoner, fant du pigghå inne i merdene?	(a) on the same day, (b) 1-3 days later, (c) 3-7 days later, (d) not applicable	(a) på samme dag, (b) 1-3 dager senere, (c) 3-7 dager senere, (d) ikke aktuelt
When do you usually do delousing?	Når gjør du vanligvis avlusing?	month/season	måned/sesong
What type of delousing method do you use?	Hva slags avlusningsmetode bruker du?	(a) chemical (b) FW treatment (c) cleaner fish (d) medicated feed (e) other _____	(a) kjemisk (b) FW behandling (c) renere fisk (d) medisinert fôr (e) annen ____
What type of delousing methods had you used for the affected cages?	Hva slags avlusningsmetoder hadde du brukt for de berørte burene?	(a) chemical (b) FW treatment (c) cleaner fish (d) medicated feed (e) other _____	(a) kjemisk (b) FW behandling (c) renere fisk (d) medisinert fôr (e) annen ____
Some farmers report spiny dogfish incidents during autumn/winter. Have you had similar experiences?	Noen fiskeoppdretter rapporterer om pigghåhendelser i høsten/vinteren. Har du hatt lignende erfaringer?	(a) no (b) yes	(a) nei (b) ja
Do the spiny dogfish incidents	Samsvarer de pigghåhendelsene	(a) no	(a) nei

coincide with transfer of smolts?	med overføring av smolts?	(b) yes	(b) ja
Do you use anti-maturation lighting during winter?	Bruker du antimodningsbelysning om vinteren?	(a) no (b) yes	(a) nei (b) ja
If yes, do the spiny dogfish incidents coincide with turning on anti-maturation lights?	Hvis ja, sammenfaller de pigghåhendelsene med å slå på modningslamper?	(a) no (b) yes	(a) nei (b) ja
What kind of lighting do you use?	Hva slags belysning bruker du?	(a) LED, (b) MH, (c) submerged lighting only (d) surface lighting only (e) both submerged and surface lighting (f) others (describe)	(a) LED, (b) MH, (c) bare nedsenket belysning (d) bare overflatebelysning (e) både nedsenket og overflatebelysning (f) annen (beskriv)
Do you use any antifouling (e.g. copper coating) on the nets?	Bruker du bunnstoff (f.eks. Kobberbelegg) på merder?	(a) no (b) yes (name the antifouling)	(a) nei (b) ja ()
What are the standard power cables used in the cages (220-360V)?	Hva er standard strømkabler som brukes i burene (220-360V)?	describe	beskriv
Do you have specific comments on what attracts spiny dogfish to the specific cages?	Har du spesifikke kommentarer til hva som tiltrekker pigghå til de spesifikke merdene?	describe	beskriv

5. Information on handling and measures to avoid spiny dogfish	**5. Informasjon om håndtering og tiltak for å unngå pigghå**		
What is your procedure for handling spiny dogfish?	Hva er fremgangsmåten din for håndtering av pigghå?	(a) caught and released (b) when hard damaged, so sacrificed. (c) do not have a preferred method (d) no comments/ No record	(a) fanget og løslatt (b) når hardt skadet, så ofret (c) har ikke en foretrukket metode (d) ingen kommentarer/ Ingen post
Have you taken any anti-shark measures for your cages?	Har du tatt noen tiltak anti-hai for merdene dine?	(a) no, no knowledge/not sure of any anti-shark equipment (b) yes (describe)	(a) nei, ingen kunnskap / ikke sikker på noe anti-hai utstyr (b) ja (beskriv)
If yes, what type of anti-shark measure do you currently use?	Hvis ja, hvilken type tiltak mot hai bruker du for øyeblikket?	(a) stronger/anti-shark net (b) protection shield (c) other _____	(a) sterkere/anti-hai nett (b) beskyttelsesskjold (c) annen _____
Did anti-shark measures help in reducing the frequency of spiny dogfish incidents?	Hjalp anti-hai-tiltak med å redusere hyppigheten av pigghå-hendelser?	(a) no effect (b) some reduction (c) now shark incidence free facility (d) not sure yet	(a) ingen effekt (b) noe reduksjon (c) nå hai forekomstfritt anlegg (d) ikke sikker ennå
If the incidents correlated with delousing, have you changed your delousing method?	Hvis hendelsene korrelerte med avlusing, har du endret avlusningsmetoden?	(a) no (b) yes (name the new method)	(a) nei (b) ja (navngi den nye metoden)

If yes, did the number of shark incidents change?	Hvis ja, endret antall haihendelser seg?	(a) no effect (b) some reduction (c) now shark incidence free facility (d) increased incidents (e) not sure yet	(a) ingen effekt (b) noe reduksjon (c) nå hai forekomstfritt anlegg (d) økte hendelser (e) ikke sikker ennå
Did you change the light conditions?	Har du endret lysforholdene?	(a) no (b) yes	(a) nei (b) ja
If yes, did it help?	Hvis ja, hjalp det?	(a) no effect(b) some reduction (c) now shark incidence free facility (d) increased incidents (e) not sure yet	(a) ingen effekt (b) noe reduksjon (c) nå hai forekomstfritt anlegg (d) økte hendelser (e) ikke sikker ennå
Are you aware of any law/regulations on handling/catching spiny dogfish?	Kjenner du til noen lov/forskrifter for håndtering/fangst av pigghå?	(a) no (b) yes (Comment)	(a) nei (b) ja (Kommentar)
6. Miscellaneous	**6. Andre**		
Do you have and will you be willing to share videos (before vs after) of the spiny dogfish incidents in your fish farm?	Har du og vil du være villig til å dele videoer (før og etter) av de pigghåhendelsene i oppdrettsanlegget ditt?	(a) no (b) yes Not decided yet	(a) nei (b) ja Ikke bestemt enda

We want to invite selected fish farmers for an in-depth interview on their experiences with spiny dogfish. Please provide your contact information if you are willing to take part in this process.	Vi ønsker å invitere utvalgte oppdrettere til et dybdeintervju om deres erfaringer med pigghå. Oppgi kontaktinformasjonen din hvis du er villig til å delta i denne prosessen.	(a) Do not want to be contacted (b) Name. email. phone	(a) Vil ikke bli kontaktet (b) Navn. E -post. Telefon