Investigating sensory cue-induced behavioral and physiological responses in Spiny dogfish (*Squalus acanthias*) for an effective shark deterrent

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SUMMARY

The Spiny Dogfish (*Squalus acanthias*) is a common and important shark species found along the Norwegian coast. However, they are known troublemakers in the aquaculture industry as they bite holes in and enter the sea cages attracted by the smell of dead fish, causing distressful situations for themselves and the farmed fishes including Atlantic salmon (*Salmo Salar*). By utilizing biological stimulatory cues evoking an aversive response, shark-repellant devices could be effective in keeping spiny dogfish away from fish farm facilities. To develop such a device, proper knowledge about their sensory biology, behavior, and physiology is necessary.

This study aimed to successfully capture and house wild spiny dogfish while maintaining good welfare, and further investigate their behavior, in laboratory trials, in response to stimuli of biological importance. We applied a series of sensory cues: the sound of orcas (a natural predator of spiny dogfish), food odor (an extract from mackerel, a natural prey), skin extract of conspecifics and electromagnetic pulse, and then observed the locomotive behavior of the sharks. Their behavioral response was recorded and studied with real-time observations, and the recordings were analyzed with a non-invasive tracking system using a deep-neural network. Tissue samples were collected to research the secretion of stress-indicating substances through blood serum analysis.

We successfully housed spiny dogfish in captivity with a near 100% survival rate. Our findings show that the sound of orcas had no effect on their locomotive activity; both skin extract and electromagnetic pulse induced change in locomotive behavior. These changes were characterized by either sudden increases or decreases in speed, changes in preference for a location in the tank, or general shifts in behavior. Analysis of serum metabolites showed no noticeable stress response suggesting that these cues have no long-term impact on the health of these sharks.

List of species mentioned in the thesis

Ameiurus nebulosus= Catfish
Carcharhinus acronotus = Black nose shark
Carcharhinus brevipinna = Spinner shark
Carcharhinus cautus = Nervous shark
Carcharhinus falciformes = Silky shark
Carcharhinus galapagensis = Galapagos shark
Carcharhinus leucas = Bull sharks
Carcharhinus limbatus = Blacktip shark
Carcharhinus melanopterus = Blacktip reef shark
Carcharhinus perezi = Caribbean reef shark
Carcharhinus plumbeus = Sandbar shark
Carcharias tarus = Sand Tiger sharks
Carcharodon carcharias = Great white shark
Cephaloscyllium laticeps= Australian swell shark
Cephaloscyllium ventriosum = Swell shark
Crocodylus acutus = American crocodile
Danio rerio = Zebrafish
Dipturus nidarosiensis = Black sea skate
Eschrichtius robustus = Grey whale
Galeocerdo cuvier = Tiger shark
Ginglymostoma cirratum = Nurse shark
Heterodontus francisci = Horn shark
Heterodontus portusjackson = Port Jackson shark
Hypanus sabinus = Atlantic stingray
Mustelus canis = Smooth dogfish
Myliobatis californica = Bat ray
Negaprion brevirostris = Lemon shark
Oncorhynchus mykiss = Rainbow trout
Orcinus orca = Killer whale
Pardachirus marmoratus = Red Sea flatfish
Pardachirus pavoninus = Congener peacock sole
Raja eglanteria = Clear nose skate
Salom salar = Atlantic salmon
Scyliorhinus Canicula = Small-spotted catshark
Squalus acanthias = Spiny dogfish
Squalus suckleyi = Pacific spiny dogfish
Sphyrna tiburo = Bonnethead shark
Trachurus symmetricus = Pacific jack mackerel
Triaenodon obesus = Whitetip reef shark
Triakis semifasciata = Leopard shark
Urobatis jamaicensis = Yellow stingray
Urogymnus granulatus = Mangrove whip

1 Introduction

The spiny dogfish (*Squalus acanthias*) is a common shark species inhabiting Norwegian waters. Despite being an important species to Nordic ecosystems, their unfortunate interactions with aquaculture facilities have become an issue of economic and welfare relevance. In search of food, they bite holes in the sea cages of farmed Atlantic salmon (*Salmo salar*) through which they enter. The smell of dead fish at the bottom of the cages can attract large packs of them. This opposes potential welfare issues for both farmed and wild stocks of salmon (Forseth et al., 2017). The outcome of violating the cages could be fatal for the sharks as well as the salmon. To prevent these situations, farmers continuously remove dead fish, inspect their nets with cameras or divers, and enhance their nets (Lal et al., *In preparation*). Throughout history, Spiny dogfish have been caught frequently as bycatch in long-line fisheries and trawling. As a result, research on chemical and electromagnetic repellents, in particular, has been of interest to prevent this and other shark species from biting baited hooks or intertwining in fishing nets (O'Connell et al., 2014a). However, there are currently no effective methods or devices to keep spiny dogfish from attacking the nets.

1.1 *Squalus acanthias*

Squalus acanthias, commonly known as piked dogfish or spiny dogfish, is a medium-sized cartilaginous fish in the elasmobranch subgroup. Elasmobranchs represent 96% of all Chondrichthyes and constitute other sharks, skates, and rays. It is a strong lineage that likely departed from other subgroups evolutionarily 350 million years ago (Yopak et al., 2007). The spiny dogfish belong to the most primitive superorder of sharks, Squalomorphs (Klimley, 2013). Additionally, there are three other suborders: Batoids (skates and rays), Squatinomorphs (angel sharks), and Galemorphs (lamniformes and carcharhiniforms). The spiny dogfish has a slender, stretched body that is counter-shaded with a grey and white-spotted dorsal side and a white ventral side. A characteristic trait is their anterior and posterior dorsal fins, each equipped with a venomous spine that secrete a mild toxin (Evans, 1920). Both spines are preceding the dorsal fins, and the posterior spine is larger than the anterior (Bigelow et al., 1953). They lack an anal fin. The head is quite flat and equipped with round big eyes situated bilaterally. The snout is pointy with nares placed bilaterally on the ventral side, dorsal to the mouth (**Figure 1.1**).

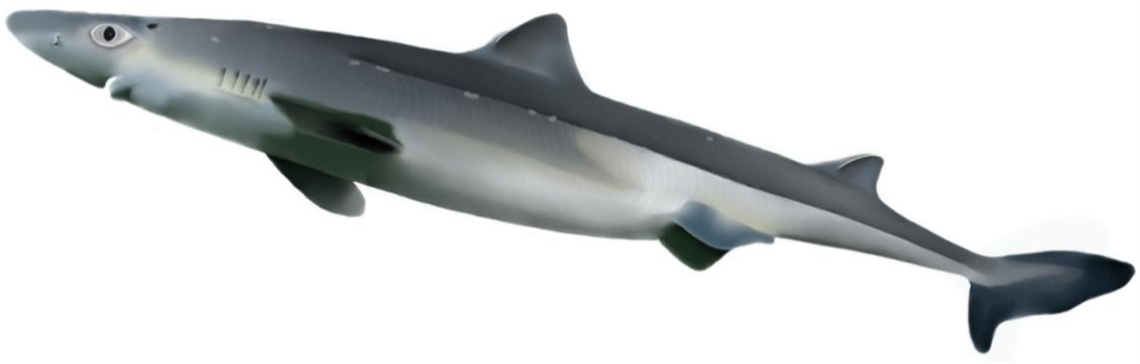


Figure 1.1: *The spiny dogfish. Illustrated by Mette Espedal Brynildsrud.*

The Spiny dogfish are a schooling species. They gather by size prior to maturation, and by size and sex after reaching maturity (Nammack et al., 1985). They inhabit benthopelagic environments in general, but their spatial distribution depends on temporal changes, age, and sex, and can therefore be observed in both coastal and open waters. For example, females tend to seek out warmer temperatures when carrying pups, and mature individuals tend to seek coastal waters (Shepherd et al., 2002). Predominantly they inhabit temporal waters on the continental shelves, among temperatures ranging between 6-13 °C (Compagno, 1984; Shepherd et al., 2002). As opportunistic feeders, their diet includes a wide variety of organisms, but some characteristic prey is associated with age and sex (Stehlik, 2007). Spiny dogfish are sexually dysmorphic as mature females typically grow larger than males. In addition, males have claspers (Stehlik, 2007). Adults can reach approximately 120 cm in length and are estimated to live for 25-40 years; this has been studied by counting growth rings on their posterior dorsal spine (Compagno, 1984; Huse et al., 2018; Jones et al., 2001; Nammack et al., 1985). They reach maturity between 9-16 years, and the female brings forth her pups through nearly two years of pregnancy, giving birth to between 7-20 live pups (Holden et al., 1964; Huse et al., 2018).

1.1.1 North Atlantic distribution

The Spiny dogfish is globally distributed in temporal waters and are common in the western and eastern Atlantic Ocean (**Figure 1.2**), and in the southern and northern Pacific Ocean (Compagno, 1984). Tagging experiments conducted on in the 1950s showed that the Northeast Atlantic spiny dogfish migrated from the coast of Scotland during summer, and inhabit Norwegian waters during winter (Huse et al., 2018; Aasen, 1962). Twenty years later tagging revealed a more southern prevalence. However, because of the inclining catch rate in northern waters, it is assumed they have returned in greater numbers despite the lack of tagging experiments and estimation of current stock size (Huse et al., 2018). The spiny dogfish has long been viewed as a problematic species, as they often are caught as bycatch by fisheries (Mandelman et al., 2006; Stoner et al., 2008). As they gather in schools, they easily get caught in great numbers at a time. Historically they have also been deliberately hunted for their flesh and liver oil. Bycatch and fishing in combination with their long gestation period and late maturation, resulted in a drastic decline in their population from the 1960s to 2005 (Huse et al., 2018). For the last 20 years, the populations have been protected by strict regulations regarding bycatch and prohibition against direct fishing, and slowly their decline has ceased. In 2022 International Council for the Exploration of the Sea (ICES) once more permitted fishing for spiny dogfish in the Atlantic Ocean, effective from January 2023. (ICES, 2022).

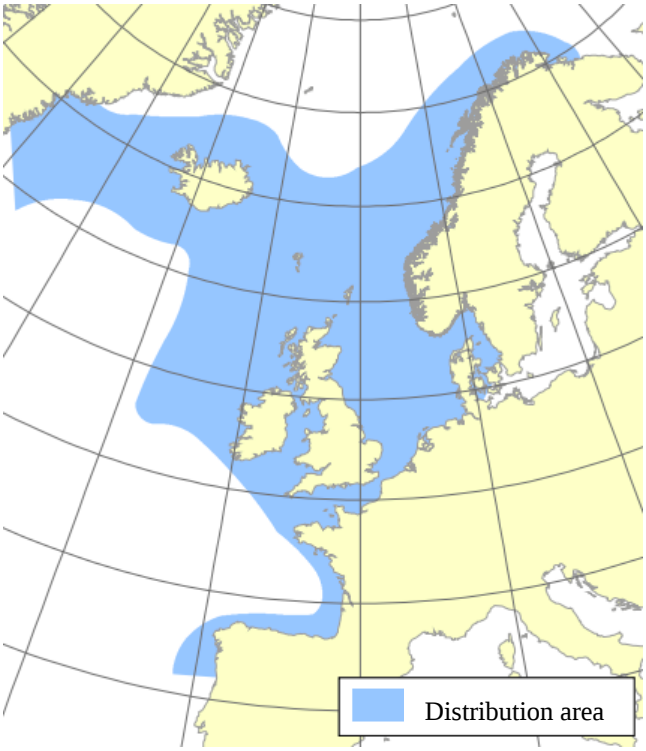


***Figure 1.2**. Distribution of Spiny Dogfish in the North Atlantic region. The map is gathered and modified from http://hdl.handle.net/11250/2562510.*

1.1.2 The spiny dogfish as a problem in the Norwegian aquaculture

Prior to the initiation of trials, PigghåFRI conducted a survey of Norwegian aquaculture farmers to evaluate the extent of the damage caused by the spiny dogfish (Lal et al., *In preparation*). The species is mainly problematic in Vestland, Rogaland, and Trøndelag, regions with occasional disturbances in Troms. Attacks are reported through all seasons; however, they seem to be more frequent during autumn and spring. They enter through autogenic holes mostly in the bottom of the cages, where a few to hundreds of individuals enter depending on school size and the size of the rupture. The attacks happen throughout the whole year but are more frequent during spring and autumn. They are attracted to dead fish. As salmon tend to die more frequently succeeding delousing, farmers report more incidents of attacks a few days after these procedures. Measurements that seem to reduce shark attacks in fish farms are frequent removal of dead fish, predator resistant nets such as HDPE nets mounted especially towards the cage bottom, and double netting of the cage with frequent surveillance for holes in the cage.

1.2 The auditory sense

The auditory sense is developed to perceive sound waves. Sharks have a well-developed hearing organ that enables them to perceive low frequent sounds (>1500 Hz), although their hearing threshold varies between species (Kelly et al., 1975). Sharks have not yet been documented to generate any sound, and their auditory ability has likely been formed by ambient noise (Carrier et al., 2012; Mickle et al., 2021).Elasmobranchs are likely to only detect the particle motion of the soundwaves as they lack the swimbladder that is typical for many bony fishes (Maisey, 2001). Sound travels 4.5 times faster in water compared to air, as water is approximately 830 times denser (Schellart et al., 1992). Sound propagated in water is composed

of two components: the near field is dominated by the particle motion, while the far field is dominated by the sound pressure. Low-frequency sounds have a larger acoustic near-field (Chapuis et al., 2022).

1.2.1 Morphology of the inner ear

The inner ear is the sound-detecting and vestibular organ found in jawed vertebrates (Chapuis et al., 2022). The endolymphatic duct connects the inner ear to the exterior environment and is located dorsal rostral (**Figure 1.3A**). Surrounding this duct is the parietal fossa, a loose tissue that is thought to act as a pathway for sound particle motion (Chapuis et al., 2022; Tester et al., 1972). The particle motion is received in the fenestra ovalis, which leads into the posterior semicircular canal (*psc*) of the inner ear that ultimately terminates at the membranous sac called macula neglecta (*mn*) (Carrier et al., 2012; Tester et al., 1972). Three membranous semicircular canals and three additional membranous sacs mediate hearing; the sacculus, lagena, and utricle (Myrberg Jr, 2001). These are covered by a sensory epithelium with hair cells called macula which is overlayed the otoconia: a gelatinous mass with granules of calcium carbonate embedded (Corwin, 1977; Myrberg Jr, 2001). The hair cells are called stereocilia, which increase in length towards a single longer kinocilium surrounded by support cells (**Figure 1.3 B**) (Corwin, 1981; Myrberg Jr, 2001; Popper et al., 1977). Sound is perceived because the otoconial mass is moving slower compared to the underlying hair cells, causing them to bend and open their ion channels (Carlström, 1963; Carrier et al., 2012; Myrberg Jr, 2001). The spiny dogfish has been found to have incorporated grains of sand in the otoconial mass (Carlström, 1963). Sharks possess two additional patches of sensory epithelia called macula neglecta (*mn*) (Tester et al., 1972). This sensory epithelia lacks the otoconial mass (Tester et al., 1972).

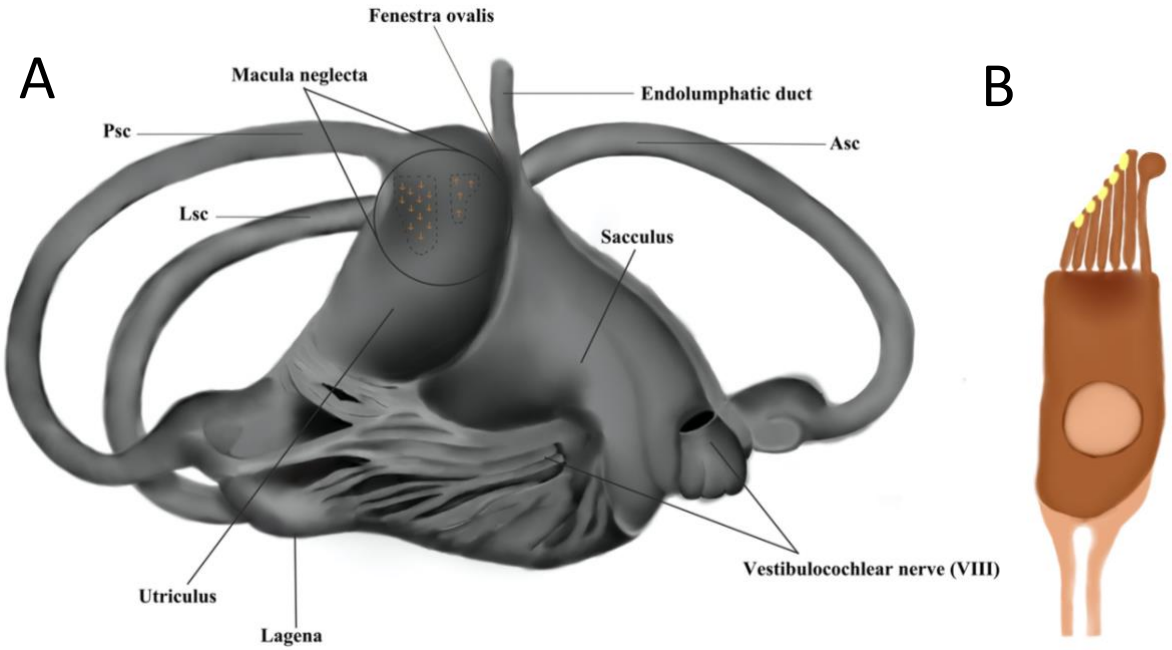


***Figure 1.3**. (A)The inner ear from the medial view of the spiny dogfish. Psc=posterior semicircular canal, Lsc=Lateral semicircular canal, Asc=Anterior semicircular canal. Redrawn from "Anatomy of the shark" by Lionel J. Rosenzweig. Department of Veterinary Biology, University of Minnesota, 1988.(B) The hair cell with stereocilia bending towards a single kinocilium. Illustrated by Mette Espedal Brynildsrud*

1.2.2 Response to auditory stimuli

Auditory thresholds have only been documented in a handful of shark species capable of perceiving frequencies between 10 and 1500 Hz, with a particular sensitivity to frequencies between 40 and 600 Hz (Mickle et al., 2021). Attractive sounds are typically low frequency 25-1000 Hz and irregularly pulsed among epipelagic sharks such as the silky shark (*Carcharhinus falciformes*) (Myrberg Jr et al., 1972). Sounds simulating struggling prey have also been successful in attracting *N. brevitostris* (Banner, 1972). Aversive behavior in gray whales (*Eschrichtius robustus*) has been documented in response to naturally occurring sounds elicited by orcas (*Orcinus orca)* within a range of 500 Hz continuous tone to a sudden increase towards 2000 Hz (Cummings et al., 1971). Orcas also hunt a variety of shark species and their scream is thought to evoke an aversive response in these animals as well (Visser, 2005; Visser et al., 2000). However, Klimely et al. (1979) observed aversive behavior in *N. brevitostris* toward abrupt and loud noises, but no response to orca sounds. Similarly Myrberg Jr et al. (1978) found that audio with abrupt changes in loudness caused several oceanic sharks to withdraw from speakers in field trials. Similar trials have not been conducted with the spiny dogfish, but as a close neighbor in Norwegian fjords, the screams of orcas might spike aversive behavior.

1.2.3 Research on auditory repellents

Previous research on elasmobranch response when being exposed to different sounds has been conducted in laboratory tank trials (Klimely et al., 1979; Nelson, 1965; Ryan et al., 2017) and field trials (Chapuis et al., 2019; Myrberg Jr et al., 1972; Nelson, 1965; Nelson et al., 1972; Ryan et al., 2017). Sharks respond to various sounds and frequencies and have elicited both attractive (Myrberg Jr et al., 1969; Myrberg Jr et al., 1972; Nelson, 1965; Nelson et al., 1972), and aversive responses (Klimely et al., 1979; Ryan et al., 2017). Low-frequency pulsating sounds between 20 and 60 Hz, which were indicated to resemble struggling fish, attracted *G. cuvier, C. falciformis*, and bull sharks (*Carcharhinus leucas*) (Nelson et al., 1963). On the contrary, aversive or startle responses were obtained in field experiments when abrupt changes in sound level were elicited shortly after an attractive sound cue (Myrberg Jr et al., 1978). Similar observations were done in tank experiments with *N. brevirostris* when exposed to 500-4000 Hz noise-band sounds and recorded screams from the killer whale (Klimely et al., 1979). The only commercially available auditory shark repellant has been the Sharkstopper®, which implemented an altered orca sound. Recent research by Chapuis et al. (2019) exposed reef sharks and great white sharks (*Carcharodon carcharias*) to an artificial sound (20-1000Hz) and a mixture of orca sounds in field trials from a baited rig. The reef sharks spent significantly less time in proximity to the rig when the orca sounds played compared to the control, while no significant change was observed when the artificial sounds played. *C. carcharias* spent less time by the rig when artificial sounds played. This study revealed the great inter- and intraspecific differences in behavioral responses to auditory stimuli.

1.3 The electromagnetic senses

The oceans are filled with ions and accompanied by the earth's magnetic field a flow of electrons is induced. Compared to nonconductive air, salt water is highly conductive, and electric fields are frequent in aquatic environments. Cartilaginous fish can detect such signals

with a specialized organ called the ampullae of Lorenzini. They use their perception of weak bioelectric fields to locate and detect prey (Kalmijn, 1982; Tricas, 1982), avoid predators (Kempster et al., 2013), detect conspecifics (Tricas et al., 1995) and possibly navigate the oceans guided by magnetic fields (Keller et al., 2021). Some terrestrial animals, such as the garden warbler (*Sylvia borin*), also possess the ability to navigate by using the geomagnetic field (Kavokin et al., 2014). The sensitivity to electromagnetic fields in elasmobranchs differs between species and ranges between 0.1 nV/cm in the bat ray (*Myliobatis californica*), to 4 nV/cm in the mangrove whip ray (*Urogymnus granulatus*) and black tip reef shark (*Carcharhinus melanopterus*). Spiny dogfish have a minimum threshold to electric stimuli of 0.2 nV/cm (Jordan et al., 2009; Jordan et al., 2011).

Figure 1.4. *The pores distributed ventrally on the rostrum of the Spiny Dogfish. Illustrated by Mette Espedal Brynildsrud.*

1.3.1 Morphology of the electrosensory organ

The Ampullae of Lorenzini are a specialized organ for detection of electric pulses, clustered in pores distributed dorsally and ventrally on the rostrum of sharks and batoids. The latter also having pores distributed on their pectoral fins (Newton et al., 2019). The spiny dogfish have pores surrounding its mouth and head in a beautiful pattern (**Figure 1.4**). From the epidermis, the pore extends into a tubule with several ampullae clustered at the base (**Figure 1.5**) (Tricas et al., 2004). The ampullae consist of smaller structures called alveoli, which are covered by an epithelial layer with primary afferent sensory neurons and support cells (Tricas, 2001). Electron travel from surrounding saltwater through the tubule into the ampullae lumen filled with a resistless hydrogel with similar ionic composition as saltwater (Carrier et al., 2012). The apical surface of the receptor neuron project into the alveoli lumen, and is innervated by

an efferent neuron on the basal end (Tricas et al., 2004). The spatial arrangement and length of the canals seem to be proportional to the sensitivity and the size of the electrosensory field of the shark and to receptor projection to the brain (Newton et al., 2019; Rivera-Vicente et al., 2011).

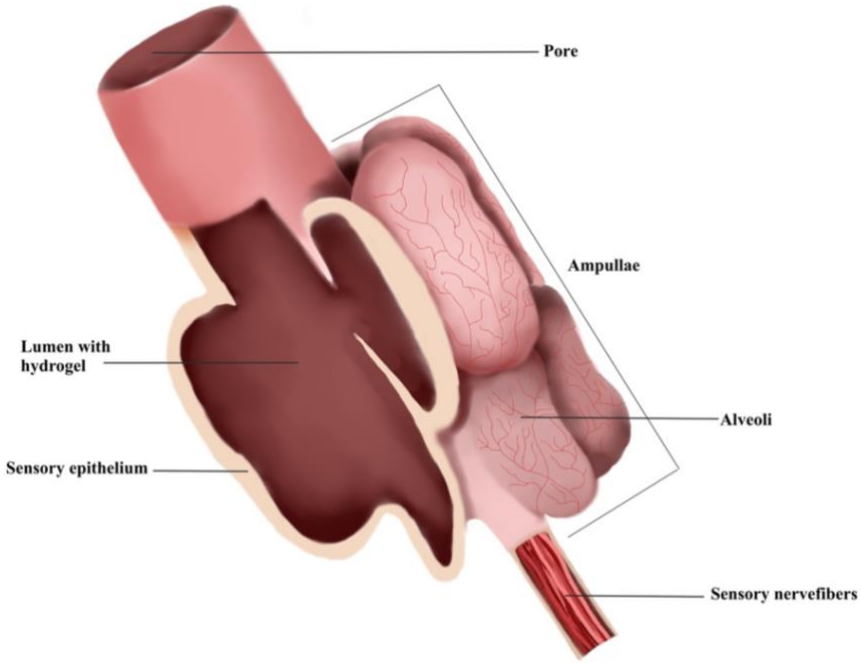


***Figure 1.5**. A single ampullae of Lorenzini from the shovelnose ray (Glaucostegus typus). Redrawn and modified from Wueringer et al. (2008). Illustrated by Mette Espedal Brynildsrud.*

The receptor cells are bottle-shaped and possess a single kinocilium, a hair cell that projects into the lumen (Tricas et al., 2004). The receptor cells are surrounded by support cells, and they are connected by tight junctions to prevent ionic leakage (Tricas, 2001). This ensures an electrically resistant barrier between the lumen and the exterior part of the ampullae (Newton et al., 2019). Upon electric stimuli, the receptor is stimulated by a negative charge and transmits a signal to a ribbon synapse through five efferent nerves at the basal end. Further, the signal travels through the anterior lateral line nerve which terminates in the dorsal octavolateral nucleus (DON) of the medulla oblongata in the hindbrain (Bodznick et al., 1980). From DON, there are pathways descending to the mesencephalon, telencephalon, and cerebellum which have been described in batoids (Bodznick et al., 1984; Schmidt et al., 1987; Tong et al., 1982). The somatotopic organization of ampulla projection to the nervous system has mostly been investigated in rays, but shows an anterior and posterior projection of afferent nerves to the ventral and dorsal part of the DON, respectively (Newton et al., 2019). Pore abundance and projections in the DON can be informative about life history traits and differ between sexes based on specific needs (Newton et al., 2019).

1.3.2 Research on electromagnetic repellants

Electromagnetic fields can cause aversive behavior which has been the fundament of research regarding electrical and magnetic repellants. The first observation from 1917 documented electroreception in blindfolded Catfish (*Ameiurus nebulosus*), actively swimming away from an iron wire (Parker et al., 1917). Several attempts in finding an efficient repellant have been

conducted since, using active electrical repellants producing direct currents and passive electrical repellents with permanent or electropositive metals, creating galvanic currents (Hart et al., 2015; Hurley et al., 1987). The “Shark shield” was the first commercially available deterrent, powered by a battery device that gave 120 V square pulses of 60 ms, and a frequency of 1-2 Hz (Hurley et al., 1987). A subsequent device originated from South African studies, where the power was generated by an electrical wave generator innervating two widely separate electrodes; the “SharkPOD”. As the commercial availability of this product ceased, the same technology was used to develop the presently available device “Scuba 7” and “Freedom 7”. The latter produces exponentially decaying electrical pulses with a 1.2 ms duration and a peak amplitude of about 105 V with an inter-pulse period of 0.6 s (Huveneers et al., 2013).

Electropositive metals which oxidize in reaction to seawater create an electrical field altering the behavior of sharks and have been investigated as a possible deterrent, particularly in relation to fisheries to reduce shark bycatch. The spiny dogfish has been one of the target species in such research, mostly because of their interference with fisheries (Tallack et al., 2009). Metal such as cerium mischmetal was tested in laboratory conditions by Stoner et al. (2008), where Pacific spiny dogfish (*Squalus suckleyi*) elicited aversive behavior towards mischmetal. However, their reluctance in biting the baits was reduced when being deprived of food (Stoner et al., 2008). Similar results were obtained with Atlantic spiny dogfish, by equipping fishing hooks with cerium and lanthanide mischmetal in field studies. However, when being deprived of food for 2-4 days the mischmetal had no effect in reducing bycatch (Tallack et al., 2009). On the contrary, Jordan et al. (2011) observed that the spiny dogfish fed less on baits protected by neodymium.

Permanent magnets are thought to interfere with shark’s ability to detect the earth´s electromagnetic field (Klimley, 1993) and have been tested for repellent effects mainly to prevent shark bycatch (O'Connell et al., 2011; O'Connell et al., 2014a; O’Connell et al., 2010; Rigg et al., 2009; Robbins et al., 2011). Rare-earth magnets like neodymium–iron–boride dissolve in water and create a stronger magnetic field (O'Connell et al., 2014b). Permanent magnets have also shown deviating results in terms of efficiency despite sharks being able to detect magnetic fields (Rigg et al., 2009). Ceramic magnets have significantly repelled *G. cirratum* from baits, and small-spotted catsharks (*Scyliorhinus Canicula*) have avoided baits equipped with neodymium-iron-boron (Smith et al., 2014). However, baits protected by permanent rare earth magnets (neodymium–iron–boride) did not significantly reduce the number of baits eaten by captive the spiny dogfish (Stoner et al., 2008) or wild Galapagos shark (*Carcharhinus galapagensis*) (Robbins et al., 2011). Neodymium permanent magnets also failed to repel Sand Tiger sharks (*Carcharias tarus*) in a recent study (Polpetta et al., 2021). The differing results in electropositive metal- and permanent magnet efficiency might be a result of varying metal functions, interactions between conspecifics or other species, competition, hunger, and different life history traits, which makes it challenging to find an efficient deterrent utilizing electric fields from metals.

1.4 The sense of olfaction

The sense of olfaction is the ability to detect and respond to chemical substances. Sharks are notorious for their sense of smell, which has been investigated since the early experiments of Parker and Sheldon (Parker, 1914; Parker et al., 1913; Sheldon, 1909, 1911). Detection of chemical substances does not solely belong to the olfactory sense, as gustation and the common sense also perceive taste and sensation, respectively, through chemosensory systems (Lundström et al., 2011). Most vertebrates have well-developed olfactory organs but the morphology of olfaction organs differs between species (Poncelet et al., 2020). Terrestrial animals have developed an olfactory system that detects airborne volatile molecules, while aquatic animals are able to detect dissolved water-borne molecules (Freitag et al., 1998). Sharks use their olfactory sense for interspecific communication, detection, location of prey, and interactions with conspecifics (Gardiner et al., 2010; Johnson et al., 1978; Parker, 1914). It has also been hypothesized that olfaction may be important in navigation (Jacobs, 2012; Yopak et al., 2015). The extent to which elasmobranchs rely on their sense of smell is dependent on their habitat and choice of feed. For example, the olfactory organs of some benthopelagic species possess seems to be more well-developed compared to sedentary, and coral reef living species (Schluessel et al., 2008).

The spiny dogfish detects odor through the nares which are situated ventro-lateraly on the snout (**Figure 1.4**). The nares are divided into an incurrent and a medial excurrent nostril, both extending further into the nasal cavity (Theisen et al., 1986). The spiny dogfish has an oval incurrent nostril, with a preceding immersion that allows the water flowing across the opening to enter the nasal cavity. The water moves through the in- and excurrent nostrils unidirectionally in a continuous water flow (Theisen et al., 1986; Tricas et al., 2009). The excurrent nostril is larger, with both an anterior and posterior margin with a depression posterior to the nostril. Both margins act as a basis for nasal flaps (*Nf*) which forms an incomplete bridge separating the nostrils and extending into the nasal cavity as a valve flap (*Vf*) (**Figure 1.6**) (Theisen et al., 1986).

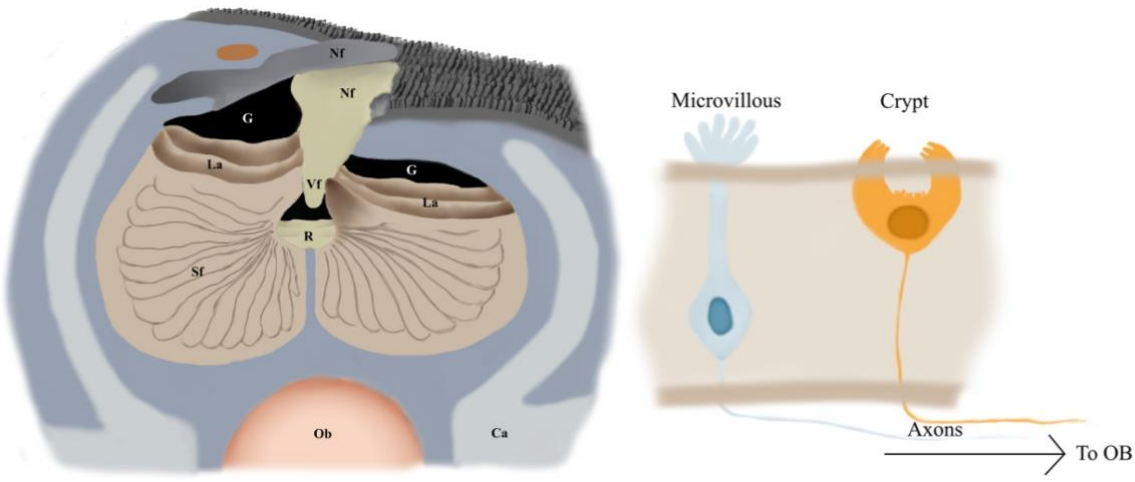


***Figure 1.6**. The olfactory organ of Spiny dogfish, inspired and modified from Theisen et al. (1986). A transverse section of the nasal cavity. Lamellae (La) with secondary folding (Sf) and the transverse raphe (R), nasal flaps (Nf), valve flap (Vf), nasal capsule (Ca), inlet chamber (Ic) of the incurrent nostril, galleries (G) of the excurrent nostril, and the olfactory bulb (Ob). Microvillous and Crypt*

ORNs are distributed in the lamellae, with axons leading to the OB. Illustrated by Mette Espedal Brynildsrud.

1.4.1 Morphology of the olfactory organ

The nasal cavity houses the olfactory organ which is largely occupied by the olfactory rosette which consists of two stacks of lamellae (*La*) separated by the transverse raphe (*R*) and extends towards the walls on each side of the olfactory cavity (**Figure 1.6**) (Theisen et al., 1986). The lamellae consist of the olfactory epithelium (OE), a sensory area that is enlarged by secondary folding (*Sf*). The degree of secondary folding differs between sharks, characterized by ridges and troughs carrying a varying degree of sensory and non-sensory epithelium (Schluessel et al., 2008). For example, the spiny dogfish have sensory epithelia in both the ridges and the troughs of the secondary folding, while the Port Jackson shark (*Heterodontus portusjackson*) only possesses patchy locations (Meredith et al., 2012; Theisen et al., 1986).

The olfactory receptor neurons (ORNs) bind odorant molecules and project signals to the olfactory bulb (OB) in the forebrain via the olfactory peduncle (Carrier et al., 2012). The neurons are bipolar and randomly distributed over the epithelial layer, with olfactory knobs expressing ciliated, microvillous, or crypted structures (Laberge et al., 2001; Schluessel et al., 2008; Theisen et al., 1986). The spiny dogfish only express microvillous receptors, but crypt cells have been reported in other elasmobranchs (**Figure 1.6**) (Meredith et al., 2013). This is where the olfactory receptors (OR) are located. In the vertebrate olfactory organ, ORs belong to the gene superfamily of G-protein coupled receptors which use subunit-coupled pathways to transduce signals, illustrated in **Figure 1.7** (Ferrando et al., 2009; Hansen et al., 2004). The ORN axons synapse with secondary mitral neurons and gather in glomeruli in distinctive layers of the OB (Laberge et al., 2001; Meredith et al., 2013). Further, the mitral cell axons bundle into the olfactory peduncle which connects the OB to the brain (Hamdani et al., 2007; Hodgson, 1978a). The glomeruli layer receives projections from ORNs situated between three and five lamellae anterior in the OE, which indicates a somatotopic organization of odor processing in sharks (Meredith et al., 2013).

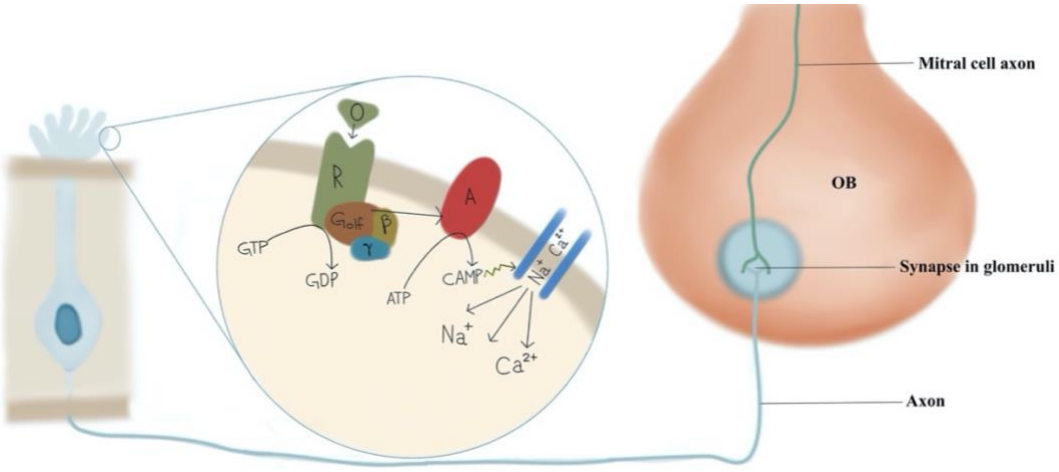


***Figure 1.7**. The cascade effect from odor binding to signaling to the olfactory bulb. The odor molecule (O) binds to the olfactory receptor (R) which is accompanied by a G-protein (G_{olf}) with subunits (β and γ). The G-protein is released and further activates adenylate cyclase III (A). This increases the cAMP concentration in the cell which activates ion-channels leading to depolarization along the neuron. An*

action potential is sent via the OR axon to glomeruli in the OB where it synapses with mitral cells. Illustrated by Mette Espedal Brynildsrud.

ORs are mostly oligospecific, which might be a correlation between ORN morphology, the nature of the odorant receptor and its expressed G-protein, in addition to the receptor distribution in the epithelium (Hansen et al., 2004). This mechanism has mostly been studied in teleosts, and due to similarities between the teleost and elasmobranch olfactory organs the neurological findings in teleost olfactory systems are a good starting point in exploring elasmobranch olfaction (Døving et al., 1980; Hansen et al., 2004; Poncelet et al., 2020; Rolen et al., 2003). The olfactory epithelium of sharks mainly consists of microvillous ORNs, which typically detect amino acids in zebrafish (*Danio rerio*) and rainbow trout (*Oncorhynchus mykiss*) (Lipschitz et al., 2002; Sato et al., 2001). Crypt cells have been shown to respond to amino acids isolated from the Pacific jack mackerel (*Trachurus symmetricus*) (Vielma et al., 2008). Despite their lack of ciliated ORNs, sharks are able to detect bile salts which has led to the suggestion that sharks and skates might possess unknown ORN mechanisms to detect specific odors (Meredith et al., 2012). The detection threshold of specific amino acids has been established in the clear nose skate (*Raja eglanteria*), yellow stingray (*Urobatis jamaicensis*), atlantic stingray (*Hypanus sabinus*), lemon shark (*Negaprion brevirostris*), and the bonnethead shark (*Sphyrna tiburo*). The latter elicited a threshold ranging between 10^{-9} mol^{-1} to $10^{-6.9}$ mol^{-1} in response to the most stimulatory amino acids; alanine, histidine, methionine, phenylalanine, and serine (Meredith et al., 2010). Similar thresholds have also been documented in nurse sharks (*Ginglymostoma cirratum*) and the Black Sea skate (*Dipturus nidarosiensis*), but not in the spiny dogfish to my knowledge (Hodgson, 1978b; Nikonov et al., 1990).

The acute sense of smell in sharks have been suggested to be related to the size of olfactory organ structures such as OB size (Schluessel et al., 2008), number of lamellae and lamellae surface (Meredith et al., 2010), and adaptation to varying habitats (Meredith et al., 2010; Schluessel et al., 2008). However, no correlation between lamella surface and odor detection threshold has been documented (Meredith et al., 2010; Schluessel et al., 2008).

1.4.2 Research on chemical deterrents

The development of chemical repellents was initiated to shield ocean-stranded personnel from shark attacks during WWII. Rotten shark flesh proved to reduce feeding behavior in the smooth dogfish (*Mustelus canis*), and copper acetate was the compound selected for future trials (Burden, 1945; Springer, 1955). Fogelberg (1944) found that copper acetate combined with a dye repelled sharks and reduced interaction with baits in US and Australian experiments in species such as black nose sharks (*Carcharhinus acronotus*), *N. brevirostris*, tiger sharks (*Galeocerdo cuvier*), spinner sharks (*Carcharhinus brevipinna*), sandbar sharks (*Carcharhinus plumbeus*) and nervous sharks (*Carcharhinus cautus*) (Fogelberg, 1944; Hart et al., 2015). However, copper acetate showed a low effect in inhibiting feeding behavior in a later study, and this field of study halted (Hart et al., 2015; Hodgson, 1978b). The discovery of three ichthyotoxic peptides, pardaxin 1-3, which are active principles of the repellent secretion of the Red Sea flatfish (*Pardachirus marmoratus*) (Primor, 1985), once more lit a spark in the field. These compounds caused mouth paralysis and irritation of the gills and pharyngeal cavity of spiny dogfish (Primor, 1985). Escape responses were also documented

by secretions from the congener peacock sole (*Pardachirus pavoninus*) in the whitetip reef shark (*Triaenodon obesus*) (Thompson et al., 1986). The lipid-disrupting characteristics of paradaxin were further utilized to find similar deterrents; sodium dodecyl (SDS) and lithium dodecyl sulfate (LDS) (Zlotkin et al., 1984). SDS did provoke aversive behavior in horn sharks (*Heterodontus francisci*), swell sharks (*Cephaloscyllium ventriosum*), and leopard sharks (*Triakis semifasciata*), but was discarded as repellant due to its toxicity and rapid dilution in water (Smith Jr., 1991; Yadav et al., 2022).

The effect of chemical compounds of biological importance (semiochemicals) was first investigated by Springer (1955), who utilized rotten shark flesh to inhibit the feeding response in captive spiny dogfish. Semiochemicals have been used in other studies, where odor from the naturally occurring predator of *N. brevirostris* was tested by Rasmussen et al. (1992). When encountered with water from their natural predator, the American crocodile (*Crocodylus acutus*), reverse tonic immobility was triggered even at low concentrations. Likewise, tissue from putrefied sharks was effective in inhibiting feeding response in *C. acronotus* and the Caribbean reef shark (*Carcharhinus perezi)* in low concentrations (Stroud et al., 2014). Anti-predator behavior is elicited by many aquatic animals when encountered chemical leakage from the damaged skin of conspecifics which acts as an alarm substance (Ferrari et al., 2010). Studies investigating such responses have been conducted with many bony fishes but are scarce for sharks (Chivers et al., 1998).

1.5 Biological responses to stress

External stressors evoke behavioral changes for an organism to maintain homeostasis, expressed by avoidance of stressful situations of stimuli. Human interpretation of which stimuli act as stressful, and the consequential change of biological function cannot be projected on animals of other species as human behavior average from other animals. To understand how stress is expressed and how biological functions change as an outcome, species-specific studies are required. Alteration of behavior, neuroendocrine, and autonomy responses are all coping mechanisms related to avoiding unpleasant situations (Moberg, 1985). Several factors can assess behavioral response, including qualitative locomotion observations and physiological response. As a part of the behavioral field of studies, biomechanics has become important to interpret the complex relationship between the structure and function of animals (Webb, 1984). The locomotion of spiny dogfish is slow compared to teleosts, as their body has a low posterior center of mass at 33% of their body length (Domenici et al., 2004). The posterior part of the body (from the center of mass to the caudal fin) moves bidirectionally and causes the shark to move forward while they maneuver using the pectoral fins. Quantitative or qualitative analyses of locomotion in spiny dogfish have not been thoroughly researched, except for a pioneering study by Domenici et al. (2004), who investigated their escape locomotion. Behavioral responses can also be evaluated by internal conditions, where the change of concentration in metabolites, electrolytes, hematocrit, and hormones in the blood plasma might disclose a change of nature.

1.5.1 Behavioral studies

Behavior can be descriptive to evaluate how an animal responds to their surroundings and is important to consider when deciding whether to introduce new equipment in their habitats. Aversive behavior allows the animal to remove itself from stressful situations. Quantification of such locomotion can be of help to interpret the level of stress an animal is under. Domenici et al. (2004) obtained an escape response by thrusting a pole toward the body of the Spiny dogfish, describing the locomotive response as a C-shaped turn away from the pole. These turns were categorized as slow and fast responses (Domenici et al., 2004). Field studies testing the effect of aversive cues on shark behavior typically use semi-quantitative methods, such as time spent away from the testing field or type of interactions (Chapuis et al., 2019; Ryan et al., 2017). Sharks in captivity will act differently compared to free-swimming sharks, which enhances the complexity of laboratory behavioral studies. Typical swimming behavior in stressed captive sharks has been described in a review by Charbeneau (2004), which describe constant/rapid swimming with quick maneuvers and slow swimming with elaborated lateral head movements as signs of stress. Additionally, poor navigation, tail below and head above the horizontal plane with occasional head movements above the surface, and tight circles and/or looping might be behavioral stress indicators.

1.5.2 Physiological stress responses

It is important to understand the physiological response in animals to properly determine how they respond to certain stimuli, as homeostatic changes might have negative effects on the animal in the aftermath of a stressful situation. The neuroendocrine and autonomic responses are regulated by the hypothalamus, which commences internal alterations. The neuroendocrine system mainly increases glucose levels and changes the blood supply, while the autonomic response is related to the release of hormones and the typical "fight or flight" response (Moberg, 1985). Measuring the level of corticosteroids in blood plasma has been frequently used as a physiological stress indicator in teleosts (Barton et al., 1991). As a primary response to stress, the so-called stress hormone cortisol has been of special interest in stress-related research towards teleosts (Aluru et al., 2009). In elasmobranchs, cortisol seems to be absent while the corticosteroid α1-hydrocorticosterone seems to be the most abundant (Anderson, 2012; Kime, 1977). Serological changes succeeding gill-net capture have been studied in Australian swell sharks (*Cephaloscyllium laticeps*), *H. portusjacksoni*, *S. tiburo*, the blacktip shark (*Carcharhinus limbatus*), and *C. leucas* (Frick et al., 2009; Manire et al., 2001). Secondary physiological responses are alterations in the acid-base composition of the blood, and changes associated with metabolism (Skomal et al., 2010). Plasma concentrations of lactate, glucose, K, Cl, Mg, Fe, potassium, inorganic phosphate, ALP (alkalic phosphate), calcium, hematocrit, and total protein have been studied in sharks, as well as magnesium, potassium, and chloride (Frick et al., 2009; Manire et al., 2001; Scott, 1921). Elevated levels of glucose after capture and transport of Spiny dogfish have been documented (Mandelman et al., 2006). Elevations of salts such as magnesium, potassium, and chloride could be a consequence of elevated lactate levels, which could cause cellular disruption (Cliff et al., 1984).

Neuronal activation in specific brain regions associated with fear recognition has also been used as a marker for stress response. Expression of Immediate-early genes (IEG) is used as

anatomical markers to locate activated neurons in the brain (Kovács, 2008). Such genes have been necessary to obtain information about neurological pathways and cellular targets in response to a variety of influential factors including stress (Figueiredo et al., 2003; Kovács, 2008). The most common marker is *c-fos*, a gene that expresses the fos protein. Due to the low expression at the baseline level and activation during neural polarization, it has become a popular tool. Some neurons have shown increased *c-fos* activation when receptors are activated, compared to regular spike activation (Luckman et al., 1994). The genetic expression of c-fos is elevated about 30 minutes after stress methods to capture prey like the thresher sharks (*Alopias*) (Yopak et al., 2007).

In this study, we aimed to successfully catch and temporarily house wild spiny dogfish to further investigate whether stimulatory cues of biological importance would elicit aversive behavior in spiny dogfish. Evidently, our results will contribute towards the development of a shark deterrent to inhibit spiny dogfish from attacking aquaculture sea cages. They were subjected to 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.

2 Material and methods

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).

2.2 Catching dogfish

All sharks were caught in five separate trips in Herdlafjorden (**Figure *2.1***). A 35 ft. Westcruiser equipped with a 1000L ISB tank was used for all fishing trips. The tank was used for temporary housing and transporting them to shore. It was filled with seawater pumped from a 6 m depth. We measured the salinity and temperature in the IBC tank with a Xylem's WTW Cond 3110 conductivitymeter (Xylem, Germany). Details in (**Table 2.1**).

Table 2.1. *Date of fishing trips to catch sharks, number of sharks and the conditions in the seawater during capture.*

Date	N Sharks	Salinity average	Temperature average
30th November	2	28.7 ppm	8.65 °C
1st December	4	28.7 ppm	8.65 °C
13th February	5	21.0 ppm	7 °C
27th March	6	31 ppm	9.1 °C
20th of May	6	30 ppm	8.8 °C

We caught all sharks with fishing rods equipped with a circle hook, intended to reduce the likelihood of the fish swallowing the hook. The small barb of the circle hook causes less damage when removed. We used four rods equipped with lights and luminescent rigs baited with herring or mackerel. The bait was lowered to approximately 30-90 m depth. They were brought to the IBC tank with a landing net after the hook was retrieved and the animal was measured with a measuring tape on a stuffed fishing mat. Sharks between 50-85 cm were kept, and the rest was released. We used a roller tank to transport the sharks from the boat to the facility.

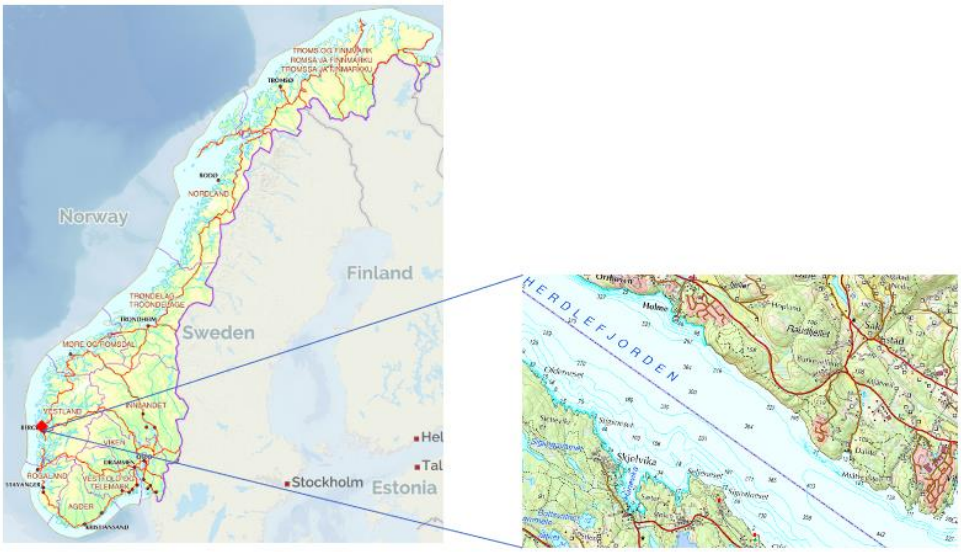


***Figure 2.1**. Herdlefjorden in Vestland county where the sharks were captured. Maps gathered from www.norgeskart.no.*

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 2.2**.

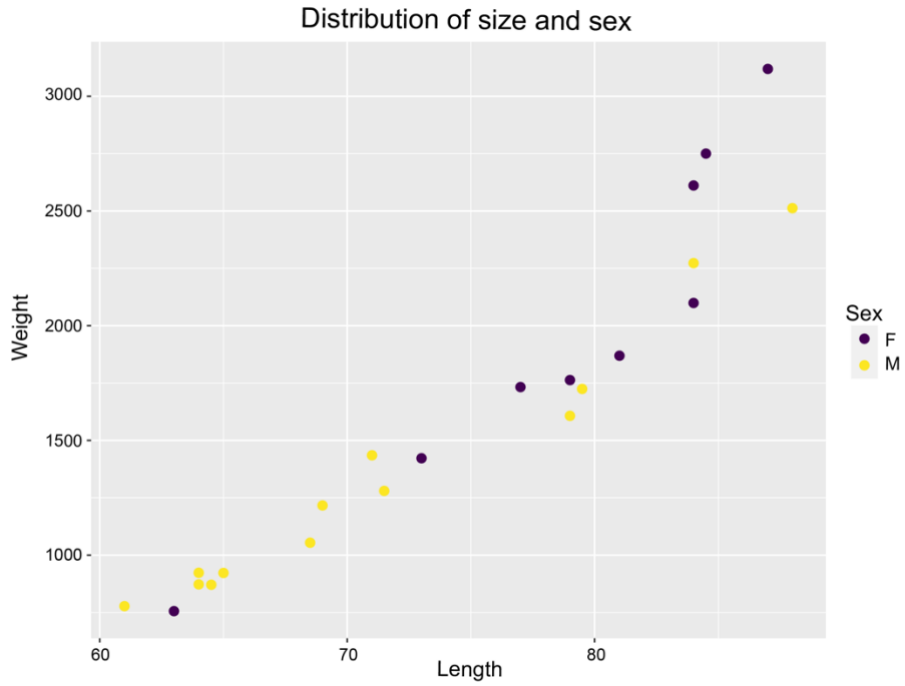


***Figure 2.2.** Distribution of the weight, length, and sex of the 22 sharks included in the trials.*

2.3 Husbandry and housing of Squalus acanthias

The sharks were divided into two or three individuals in each tank depending on their total length. A minimum of 3x was provided, where sharks >68cm 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 by daily measurements which were logged manually. All animals were observed daily, and their well-being was highly prioritized. We evaluated their general condition by swimming pattern, wounds/damages, appetite, and anomalies in their behavior. (APPENDICES 1A-1S).

2.3.1 Feeding routine

Knowledge about their eating habits in captivity is scarce, which we initially solved by continuously offering food. They were offered pieces of frozen mackerel, and after one week of habituation, we started to observe bitemarks on the food. However, their appetite seemed low. We fed each shark one piece of mackerel every 3rd day. One tank was fed salmon.

2.3.2 Housing tanks

This study was conducted in aquatic facility at the University of Bergen located at Marineholmen, in the A unit of Biologen. The laboratory facility was divided into two rooms: one for the technical setup and tissue sampling and one for experimental conduction. Three tanks numbered 1, 3, and 4 are hereby referred to as the housing tanks, were used for habituation and housing. Tank number 2 was used to conduct the experiments and is hereby referred to as the experimental tank (**Figure *2.4***). 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 111cm in depth (**Figure 2.3**) and received full strength seawater from a flowthrough system pumped from 100 m depth in Damsgårdssundet near the laboratory facility. 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 with an average temperature of 9°C and salinity at approximately 33 ppt. Temperature and oxygen levels were connected to an alarm system which provided 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 conditions 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.

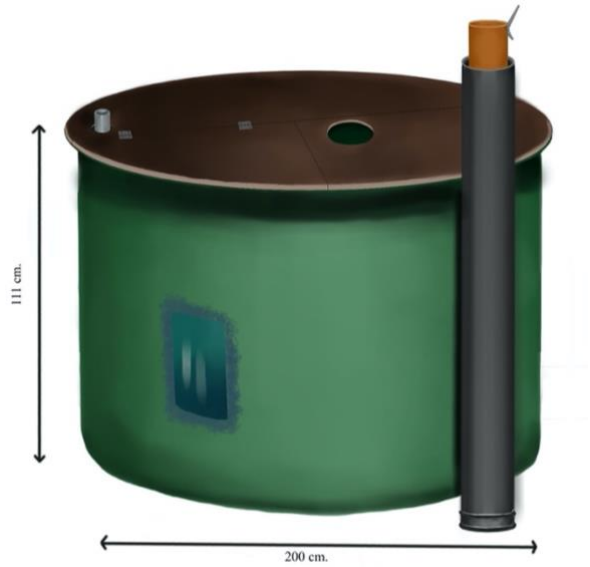


Figure 2.3. *The proportions of the housing tanks. Illustrated by Mette Espedal Brynildsrud*

2.4 Experimental tank setup

All trials were recorded in the experimental tank with a waterproof 3.0 USB camera costume designed by NORCE (Antonie Oostercamp, NORCE technology). It was mounted to the ceiling which gave a 120° field of view. To record as much as possible of the tank the wooden lid was removed. The two initial trials were executed with a 65 cm water depth, and proceeding a camera change increasing the field of view the water depth was changed to 82 cm. The rigidity of the tank was maintained by mounting a 4 tonnes resistant jack strap around the circumference. The light source was a LED-light mounted on the roof of the facility, providing 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. The bottom of the tank was brightened to increase the contrast between the background and the animals, by adding white plastic tiles covered with acrylic plates. The plates were joined by stripes to prevent them from sliding.

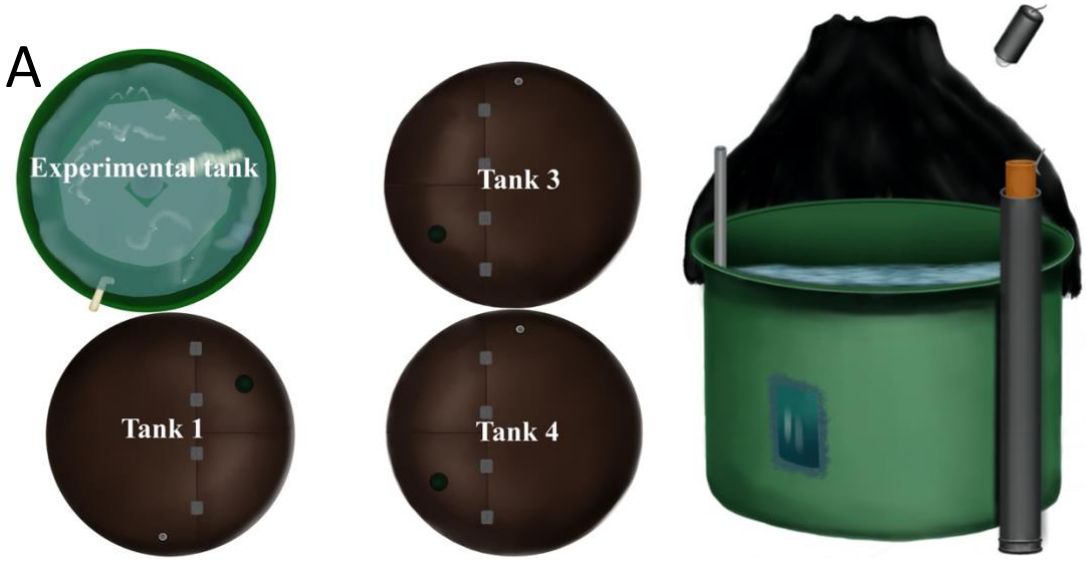
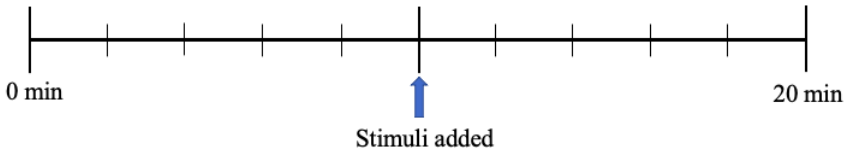


Figure 2.4*. (A) Overview of housing tanks and experimental tank. (B)The experimental tank was lidless and covered with black drapes. The camera was mounted in the ceiling. Illustrated by Mette Espedal Brynildsrud*

2.5 Behavioral trials

All behavioral experiments were conducted between January and June 2023. The sharks 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 of trial initiation and did not receive food during experimental conduction. The trials were conducted over 1-5 consecutive days between 08.00 and 19.00 (GMT+1) and terminated by euthanization and tissue sampling of the shark. The trials were conducted under three conditions: Condition 1 included testing of all three stimuli (**Table 2.1**), the aim of Condition 2 was to observe the effect of increasing electromagnetic field strength (**Table 2.3**), and the aim of Condition 3 was to observe the behavioral effects of food odor and dose dependent response to skin extract (**Table 2.4)** which will be described further below. Each trial was recorded with OBS 27.1.3 (Open broadcaster software) and files were directly stored on a Seagate Expansion HDD hard drive. Each recording followed the timeline below.



2.5.1 Condition 1 – General tests of audio, odor, and electromagnetic field

The initial trials were conducted to evaluate if any of the three stimulatory cues could elicit behavioral changes. Shark 1, 2, 3, 4, 5, 7, 11 and 12 were subjected to the trials of this condition.

Audio trials equipment and preparations

The acoustic stimuli were delivered through a waterproof costume-designed speaker from NORCE design (Antonie Oostercamp, NORCE technology) (**Figure 2.5**). 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 for the animal to habituate and removed post-trials. Due to rust, 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. We tested a lower (**Figure 2.6 A1 & A2**) ranging between 0 and 7500 Hz, and a higher (**Figure 2.6 B1 & B2**) frequency audio file ranging between 0 and 20 000 Hz. Each audio file played for 1 minute.

***Figure 2.5**. The custom speaker. Illustrated by Mette Espedal Brynildsrud*

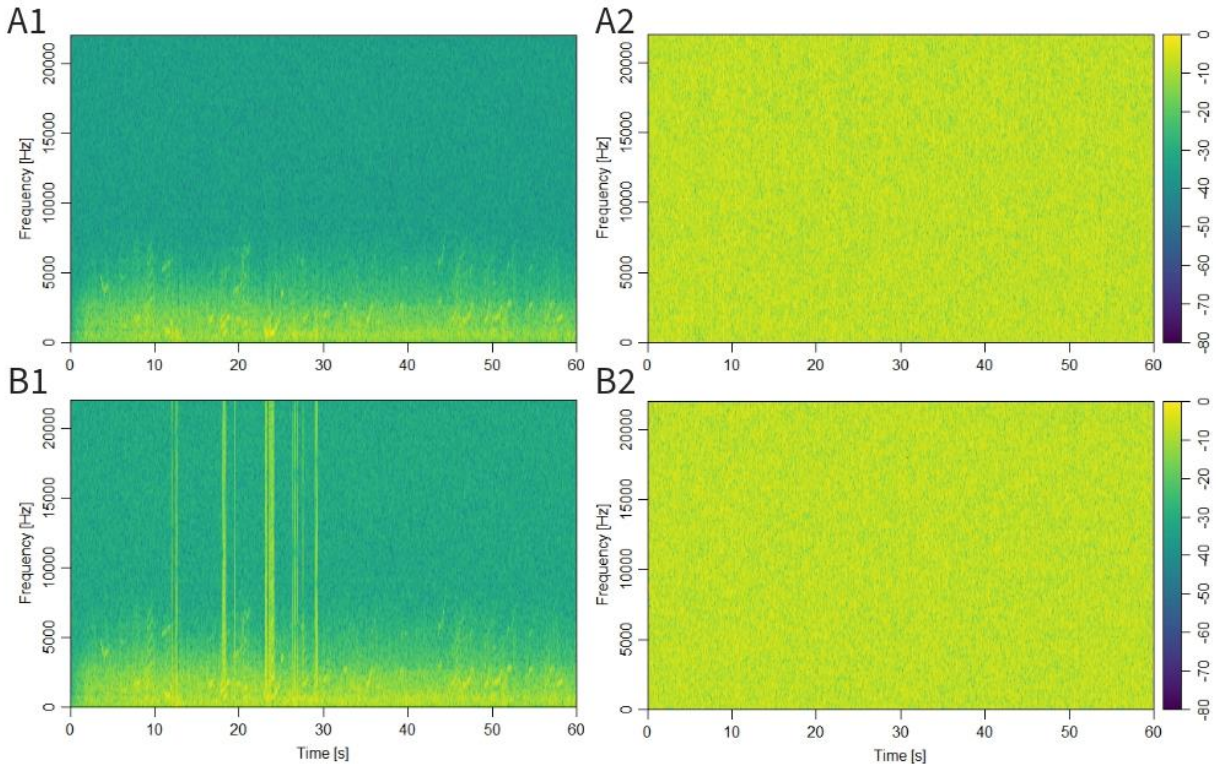


***Figure 2.6.** Spectrograms of the four audio files played during audio trials. The lower frequency orca sounds (A1) with a shuffled noise of the same audio (A2). The higher frequency orca sound (B1) and the compatible shuffled sound (B2). The color bare represents the decibel (dB).*

2.5.2 Electromagnetic field equipment and preparations

The electromagnetic field was created by a self-constructed electrode (**Figure 2.7**). Two iron rods acted as electrodes separated by an acrylic plate. Alligator clips were attached to the electrodes, creating a current between the negative and positive probes.

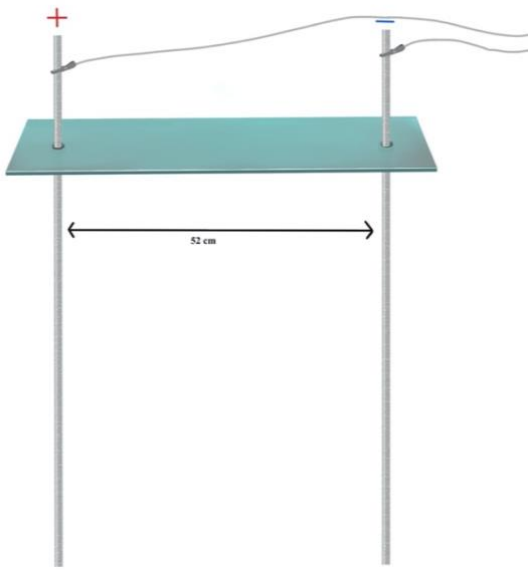


Figure 2.7. *The custom made electrode used to create the electromagnetic field. Illustrated by Mette Espedal Brynildsrud.*

The device was powered by a 360W BK precision 1687B as a power supply. We mounted an emergency switch to the electrode circuit to quickly deactivate the current if necessary. The supply had an output voltage capacity of 37 V and an output current capacity of 10 A. With a Raspberry Pi computer (Raspberry Pi Foundation, UK) we monitored the electromagnetic pulse and duration with a program that could alter the stimuli to our preference (Antonie Oostercamp, NORCE technology). The device was tested in an empty tank filled with seawater before being implemented into trials. Field strength were measure at different spatial points and a simulation showing the strength of electromagnetic field is presented in **Figure 2.8**. 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.

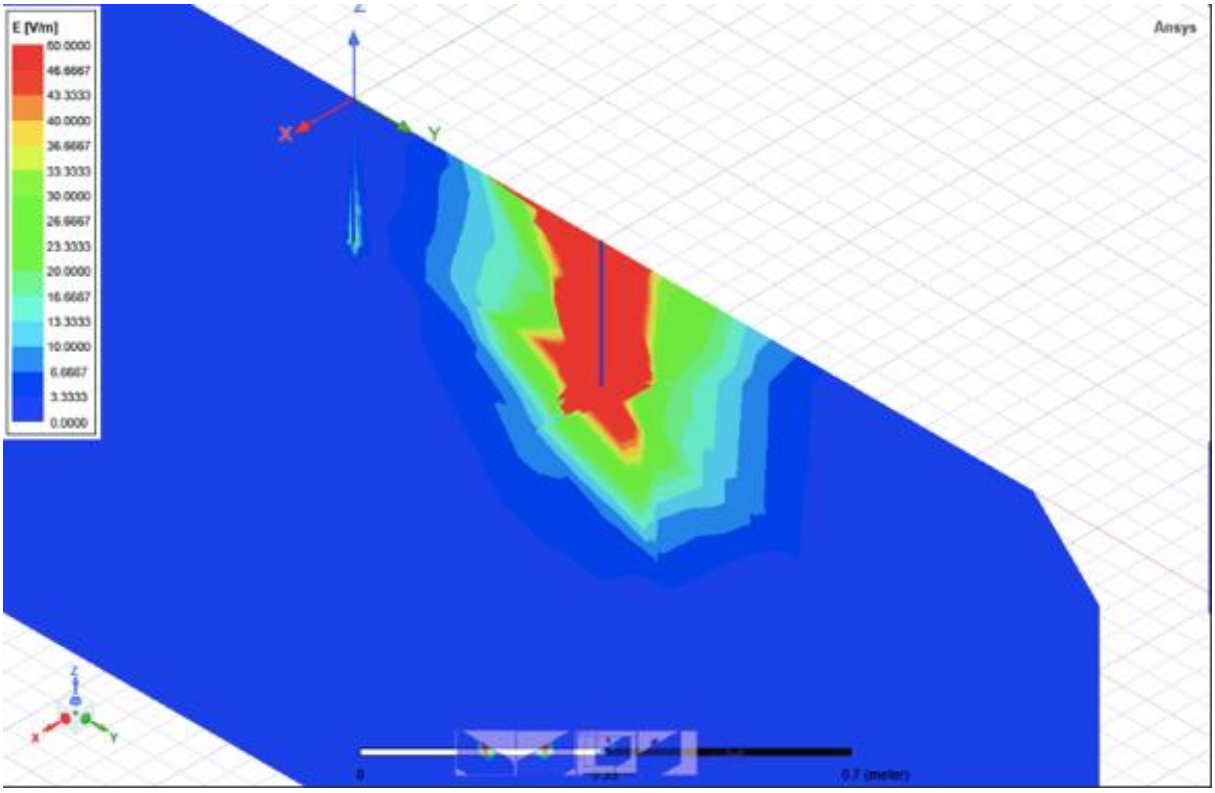


***Figure 2.8**. Simulation of the strength of the electromagnetic field. The current is at its strongest close to the electrodes (50 E[V/m]) and dramatically decreases by the distance of 0.3 m. Sharks experience less than 3.33V/m field at a distance of 0.3m from the electrode (simulation by Antonie Oosterkamp).*

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 intervals of electromagnetic stimuli were given a maximum of nine times for each shark. Every trial with differing pulse interval was repeated three times with pulse interval of 0.6, 0.3 and 0.1 seconds (**Table 2.2**). A pulse duration of 3 ms was used in all trials.

Odor trials equipment and preparations

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 with stripes. A funnel was mounted on the top of the plastic tube (**Figure 2.15**). 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 VWR borosilicate 3.3 glass bottle. The seawater and mackerel solution were further diluted with seawater to a 300 mL/1000 mL mixture. The skin extract was prepared with skin collected from earlier euthanized experimental animals (**explained in 2.7.1 Tissue sampling**). Thawed skin extract was minced in a mortar for 5-10 minutes with a small amount of seawater and evenly distributed into three 50 mL Corning Falcon tubes 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.

Table 2.2. *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: 0.6 s duration	Stimuli x
2.	Sound: Control	Sound: Control	Odor: Food	EM: 0.6 s duration	Tissue sampling
3.	Sound: Orca	Sound: Orca	Odor: Food	EM: 0.6 s duration	
4.	Sound: Control	Sound: Control	Odor: Control	EM: 0.3 s duration	
5.	Sound: Orca	Sound: Orca	Odor: Skin extract	EM: 0.3 s duration	
6.	Odor: Control	Odor: Control		EM: 0.3 s duration	
7.	Odor: Food	Odor: Food		EM: 0.1 s duration	
8.	Break (2 hours)	Break (2 hours)		EM: 0.1 s duration	
9.	Sound: Control	Sound: Control		EM: 0.1 s 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			

2.5.3 Condition 2 – Investigating the effect of three voltages of electromagnetic field

We executed specific behavioral trials in response to increasing voltage of the electromagnetic field. The same equipment was used as described under Condition 1. The sharks were exposed to three different voltages 5 V, 10 V, and 20 V with 0.3 s intervals (**Table 2.3**). Shark 6, 8, 10, 15, 18, 20, and 21 was subjected to Condition 2 trials.

Table 2.3*. 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: 0.3 s interval, 5V	EM: 0.3 s interval, 20V
EM: 0.3 s interval, 10V	EM: 0.3 s interval, 5V
EM: 0.3 s interval, 20V	EM: 0.3 s interval, 10V
EM: 0.3 s interval, 5 V	EM: 0.3 s interval, 20V
	EM: 0.3 s interval, 20V
	Stimuli x
	Tissue sampling

2.5.4 Condition 3 – Investigating the effect of three increasing units of skin extract

We executed specific trials in response to investigate the behavioral effect of increasing units of skin extract. 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, put in a blender (Philips, Amsterdam Netherlands) and blended for 2 minutes. A concentrate equivalent to 7 cm of skin was filled in a 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). To prevent the solutions from degrading they 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 2.4. *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 (0.5U)	Odor: Skin extract 100 mL (2U)
Odor: Skin extract 50 mL (1U)	Odor: Skin extract 50 mL(1U)
Odor: Skin extract 100 mL (2U)	Odor: Skin extract 25 mL (0.5U)
	Stimuli x
	Tissue sampling

2.6 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 I used DeepLabCut™, a software providing a non-invasive tracking system that uses human-annotated labels in a deep-neural network to track and estimate the position of the animal (Mathis et al., 2018; 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, I extracted one video from each day of trials by copying the recordings where sharks elicited the widest range of locomotion into a Seagate Expansion HDD hard drive (Shark # 1-8, 12, 13 and 15). I duplicated each recording 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. I used a graphical user interface (GUI) and the following protocol.

2.6.1 My 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 2.9**.

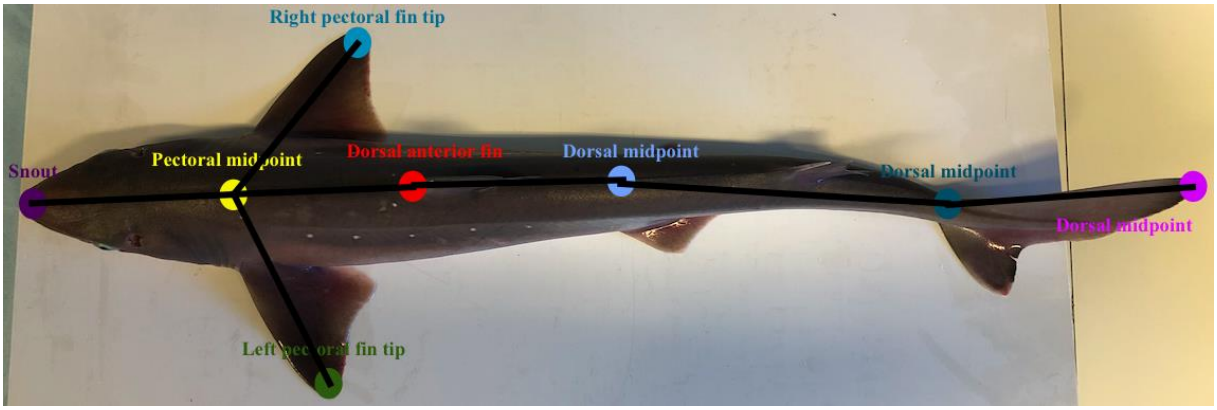


Figure 2.9. *Names and positions of the eight labels. The size and shape of the labels were identical for all annotated frames. The "disc" shape worked as a tool to align the labels on the same location of the body parts for each frame. The snout was labeled by aligning the disc to the snout tip. The 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). This allowed me 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 **Figure 2.11**.

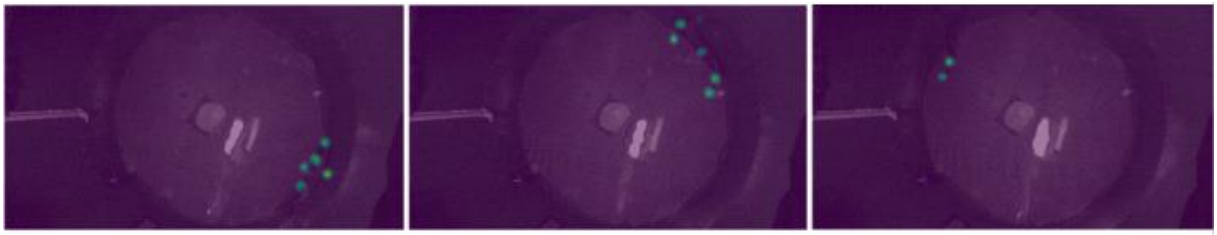


Figure 2.10*. Heatmap generated by DeepLabCut which shows a location of the animal.*

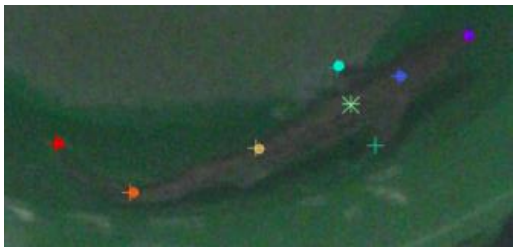


***Figure 2.11**. Example of the evaluation of the network used for analysis. The dots (•) represent DeepLabCut´s predictions with a likelihood ≥ 0.6 – - cutoff, the x's (×) for predictions with a likelihood < 0.6 p – cutoff, and the "plus" (+) represent the human annotations (Nath et al., 2019).*

7. **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.
8. **Created videos** of the same selected videos from Step 7. This created videos with constant labels showing when the shark was visible and drew a skeleton showing the angle of the body (**Figure 2.12**).

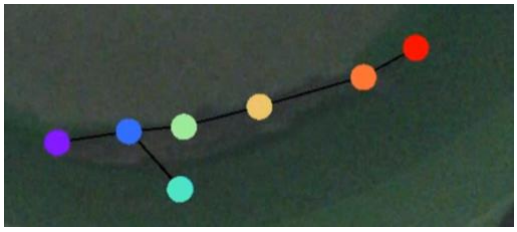


***Figure 2.12**. A snapshot from the recordings of Shark 7, displaying the accuracy of labeling in the videos created in Step 8.*

9. **I 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.

2.6.2 The accuracy of DeepLabCut

Analyzing the videos with the DLC software spared us for 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).

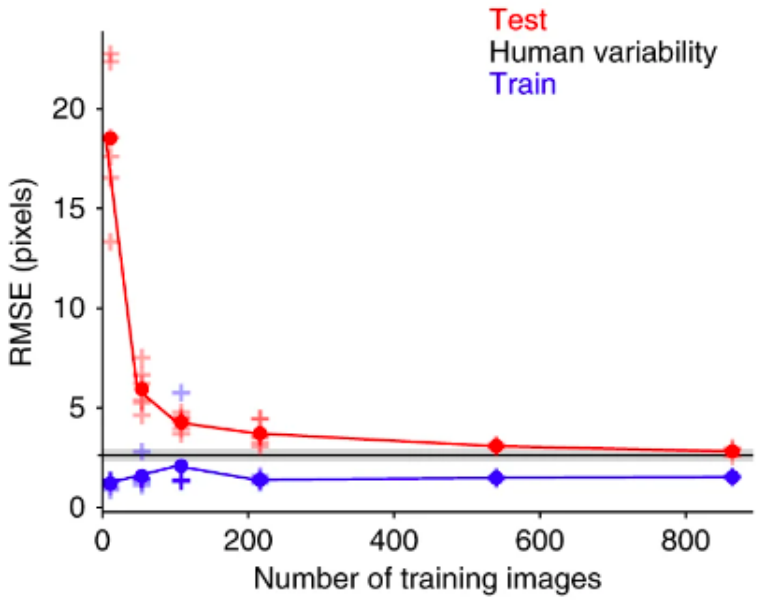


***Figure 2.13**. After approximately 200 images for training the deep network, the accuracy of DLC is close to human accuracy. RMSE in pixels are compared between one human labeler, labeling two distinct datasets and the trained network. The figure is collected from Mathis et al. (2018).*

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 2.14Error! Reference source not found.**.

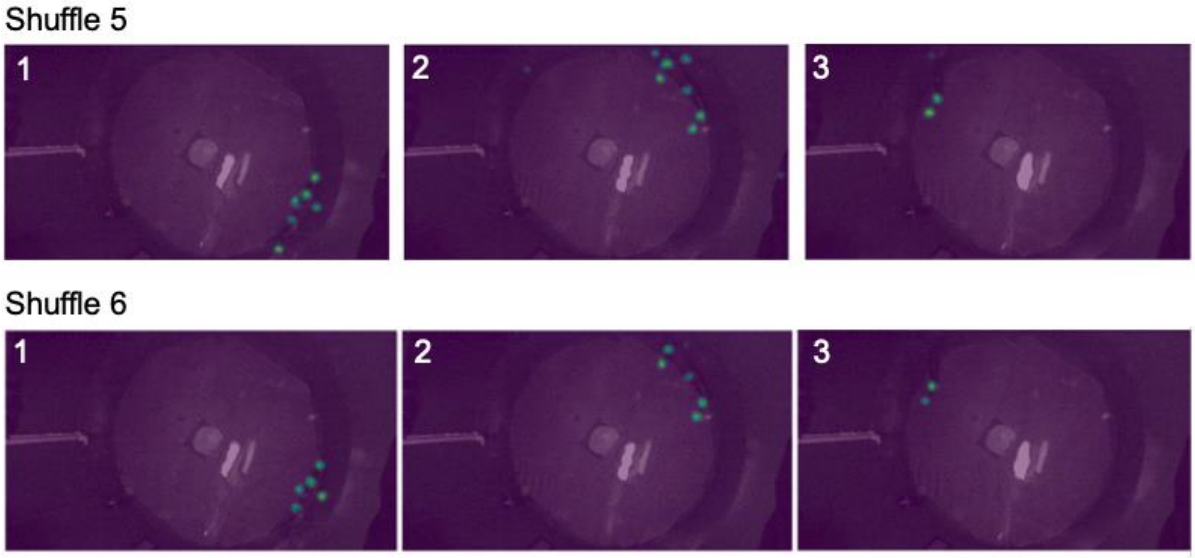


***Figure 2.14**. Comparison of the two main shuffles that were considered to analyze the videos. These images are heatmaps reflecting the DLC ability to locate and recognize the eight labels that were used to identify the shark on the recordings. Shuffle 5 depicts some inaccuracy in the picture 2, that were eliminated by the extraction of*

2.6.3 Analyzing the locomotion with R studio

I designed an automated code in Rstudio to analyze all the coordinates most efficiently. With this code, a list with CSV files to my preference 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 2.9**) 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, I utilized the total distance traveled and the animal's placement in the tank based on the x and y coordinates. The total distance was used to evaluate if the treatment from stimuli influenced the speed and movement in the tank.

2.7 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 2.5**.

2.7.1 Tissue sampling

After trials were conducted, the animals were euthanized with an overdose of Tricaine mesylate anesthetics (MS 222) mixed with seawater. Tissue samples from blood, gills, skin, spines, and brain were collected for serum analysis and gene expression analysis. When the animal was fully anesthetized and gill motion ceased the animal was 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. 500 µL serum was transferred to six Eppendorf 1,5 mL tubes (Eppendorf AG, Hamburg,

Germany) with a 1000 µL Gilson PIPETMAN (Gilson, Middleton, USA) and stored in the freezer at -35 °C. After blood sampling the animal was decapitated before we sampled the 2nd gill arch and one olfactory bulb together with half the telencephalon. These samples were stored in 25 mL RNA later™ Soln (Invitrogen AM7021, USA) and stored at 4 °C for 24 hours and then transferred to the freezer at -35 °C. The rest of the brain and forebrain was stored in 20 mL Formalin (Biopsafe® 3178-200-21 NO, Vedbæk, Denmark) at 4 °C. We collected skin patches which were stored in 3,5 mL 1xPBS (Invitrogen AM9625, USA) diluted with distilled water to a 1x10 PBS solution, and frozen at -35°C. Spines were collected and cleaned for excessive tissue before storing dry at -35 °C.

Table 2.5. *The stimuli that the sharks were exposed to 30-40 minutes prior to euthanization*

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		

2.7.2 Serum analysis

All serum samples collected from sampling were chemically analyzed with a Pentra C400 (HORIBA ABX SAS, Montpellier, France). 500µL from each specimen was analyzed. The samples were defrosted and centrifuged in an Eppendorf centrifuge 5424 R (Eppendorf AG, Hamburg, Germany) 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.

2.7.3 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 2.15**). The behavioral effect of the stimuli before and after treatment was compared with a paired T-test. Similarly, I compared the fold change between all stimuli in Condition 1 and Condition 2 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, I used one-way ANOVA and post-hoc analysis without correction for multiple comparisons. 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 in chapter 2.6.3. 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.

$$Meters\ traveled = \frac{Pixels\ traveled}{440}$$

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 annotations in **Figure 2.15** will be used further.

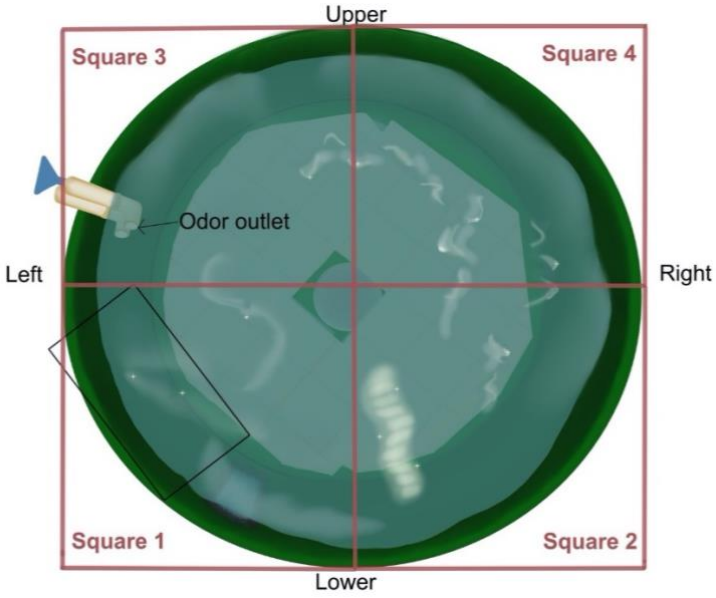


Figure 2.15. *The experimental tank as seen from above. White tiles with plexiglass on the top cover the bottom. The odor was applied from the pipe in the figure. The speaker and electrode were placed in the area marked by the black square. The squares used to determine the position of the shark are marked with red lines. Illustrated by Mette Espedal Brynildsrud.*

3 Results

3.1 Husbandry and housing of spiny dogfish

To perform these trials, we were deeply dependent on managing to locate wild spiny dogfish and succeed in keeping them alive, fed, and in good health throughout the trials. We were able to locate wild populations and bring a total of 22 spiny dogfish, 13 males and 9 females between 61-88 cm to the experimental facility. As all sharks survived until euthanization, we could conclude with a near 100% survival rate for Spiny dogfish in captivity in this project. We saw it 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 severely 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.

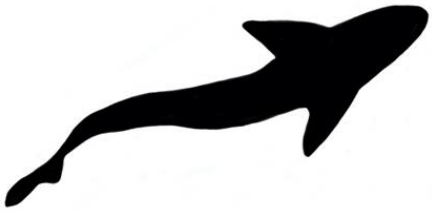
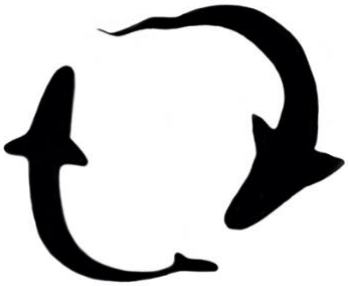
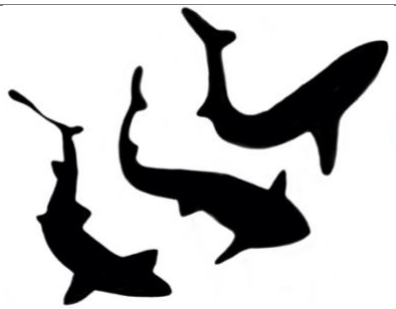
3.1.1 Feeding

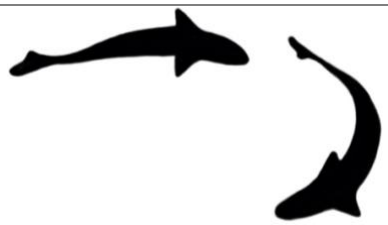
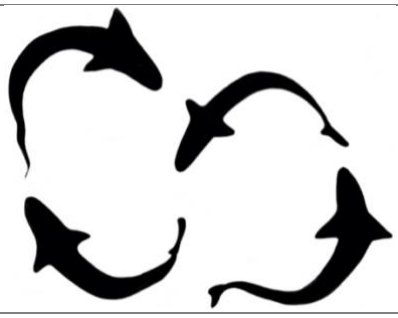
When provided with frozen feed, the sharks showed little to no interest in eating. We did observe some biting on the feed; however, most of the pieces were left in the bottom of the tank. The appetite was enhanced when they were fed with thawed mackerel every third day. Group 1 started to eat 9 days 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 due to being picky eaters.

3.2 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 3.1**). 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. I observed clear differences in swimming routines between individuals, ranging 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 3.1**. 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 3.1**. The observed swimming patterns during the qualitative observations in the behavior trials. Illustrated as seen from above, by Mette Espedal Brynildsrud.*

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

By visual observation the sound stimuli did not seem to elicit an aversive response or a clear change of locomotion or behavior.

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 application, shifting from a normal swimming pattern following the tank 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 in rapid 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 600 ms, 300 ms, and 100 ms 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 (**Figure 2.8**) during stimuli. However, they occasionally passed the rig in 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 sharks initiated looping.

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

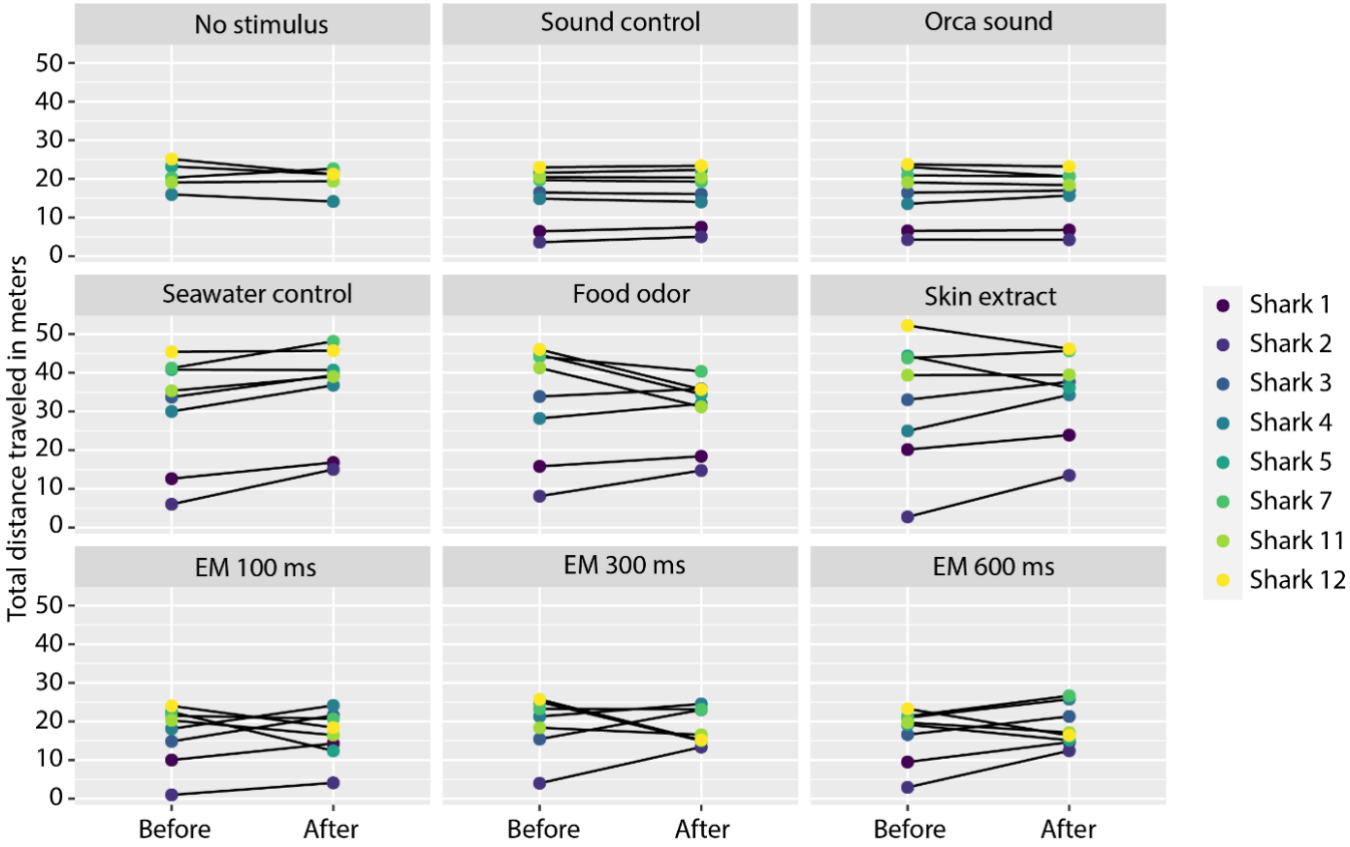


Figure 3.1. *Comparing the mean of total distances traveled in meters before and after the addition of stimuli. No stimulus (n=5), rest of the stimuli (n=8). Each dot represents the average response from one shark to the stimuli.*

Figure 3.1 illustrates how the audio, odor, and electromagnetic field affected the total distance traveled by all the sharks involved in Condition 1. "No stimulus" was recorded each morning before any trial had begun, which left the sharks unaffected by any human interaction. 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 orca sounds or the sound control. The seawater control, food odor, and skin extract trials were more effective in eliciting a change in the 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.

Audio trials

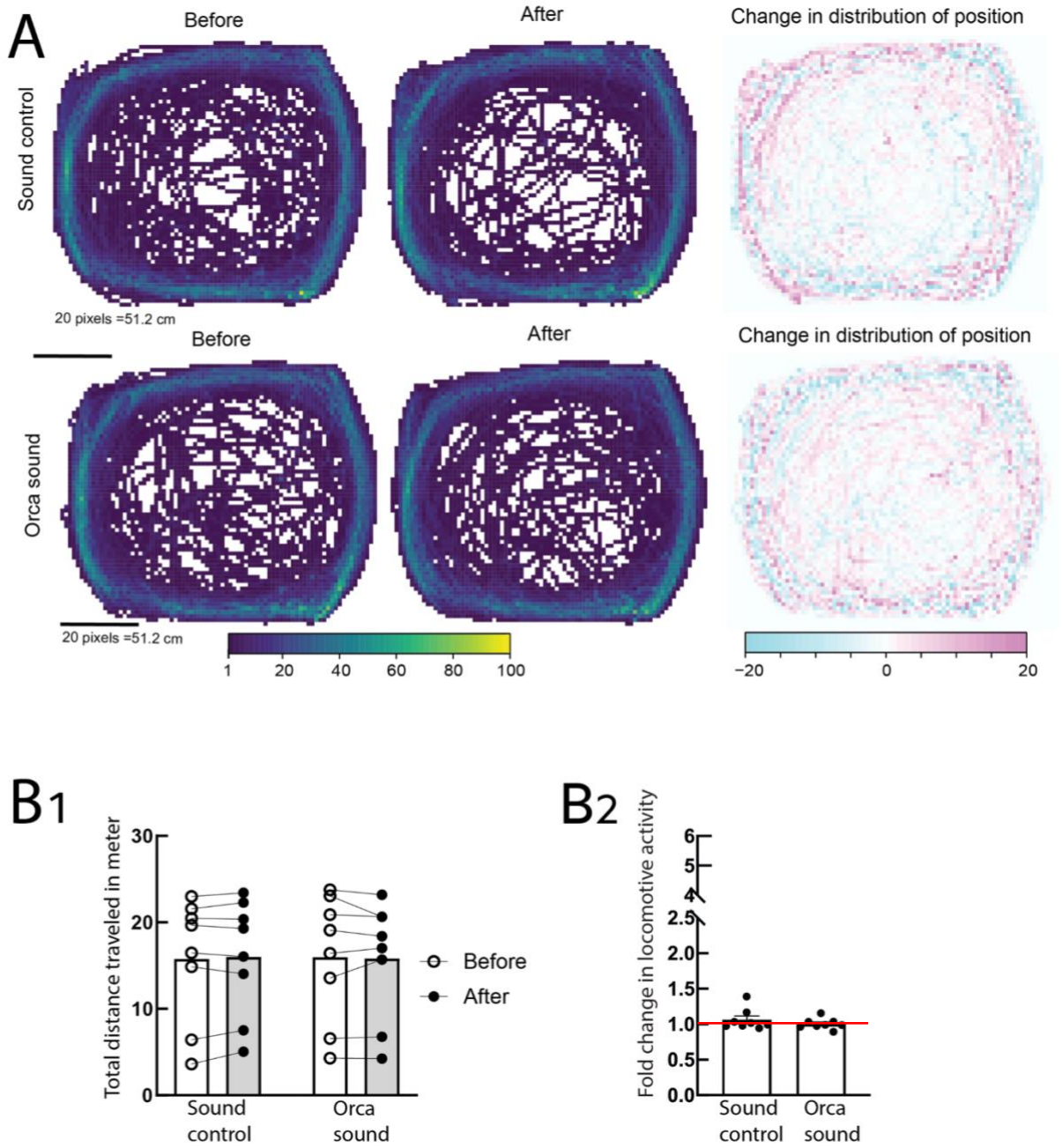


***Figure 3.2**. The behavioral change in response to sound control and orca sound. (**A**) Based on the density of data points from the x and y coordinates, the positions of the sharks (n=6) from all trials are combined and visualized in binned heatmaps before and after the stimulus. The color scale 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: pre-stimuli movement is indicated by blue and post-stimuli movement 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.*

As illustrated in the before and after heatmap (

Figure *3.2* A), the sharks mostly swam along the edge of the tank during 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 *3.2* B1**). 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 *3.2* B2).

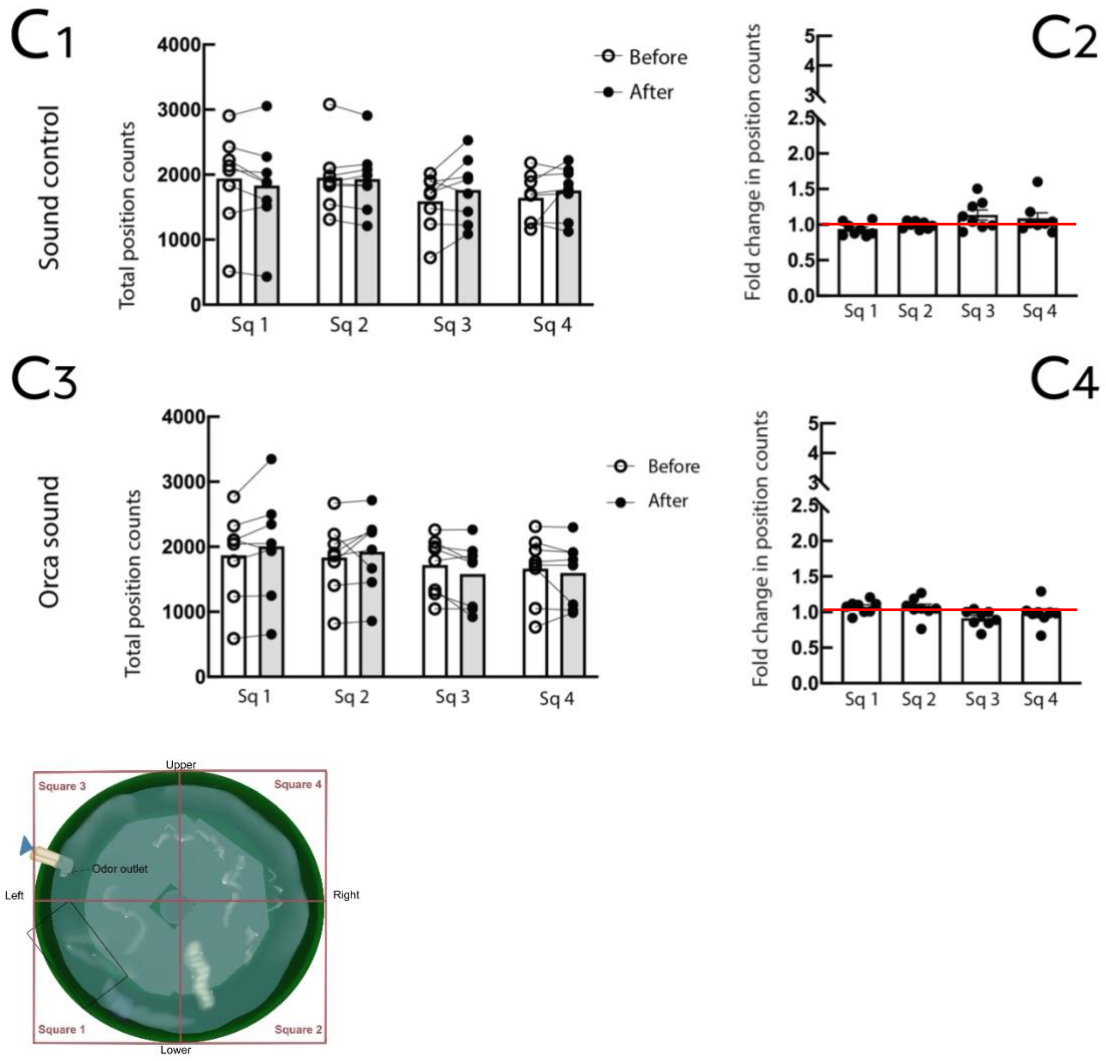


***Figure 3.3**. The total position counts from four arenas in the tank: Square 1 (Sq1), Square 2 (Sq 2), Square 3 (Sq 3), and Square 4 (Sq 4). (**C1 and C3**) Bar plots show the mean position count and compare the total position counts before and after stimuli, where each data point represents the combined 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 2.15**. The total position counts illustrated in **Figure 3.3 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 3.3 C4**).

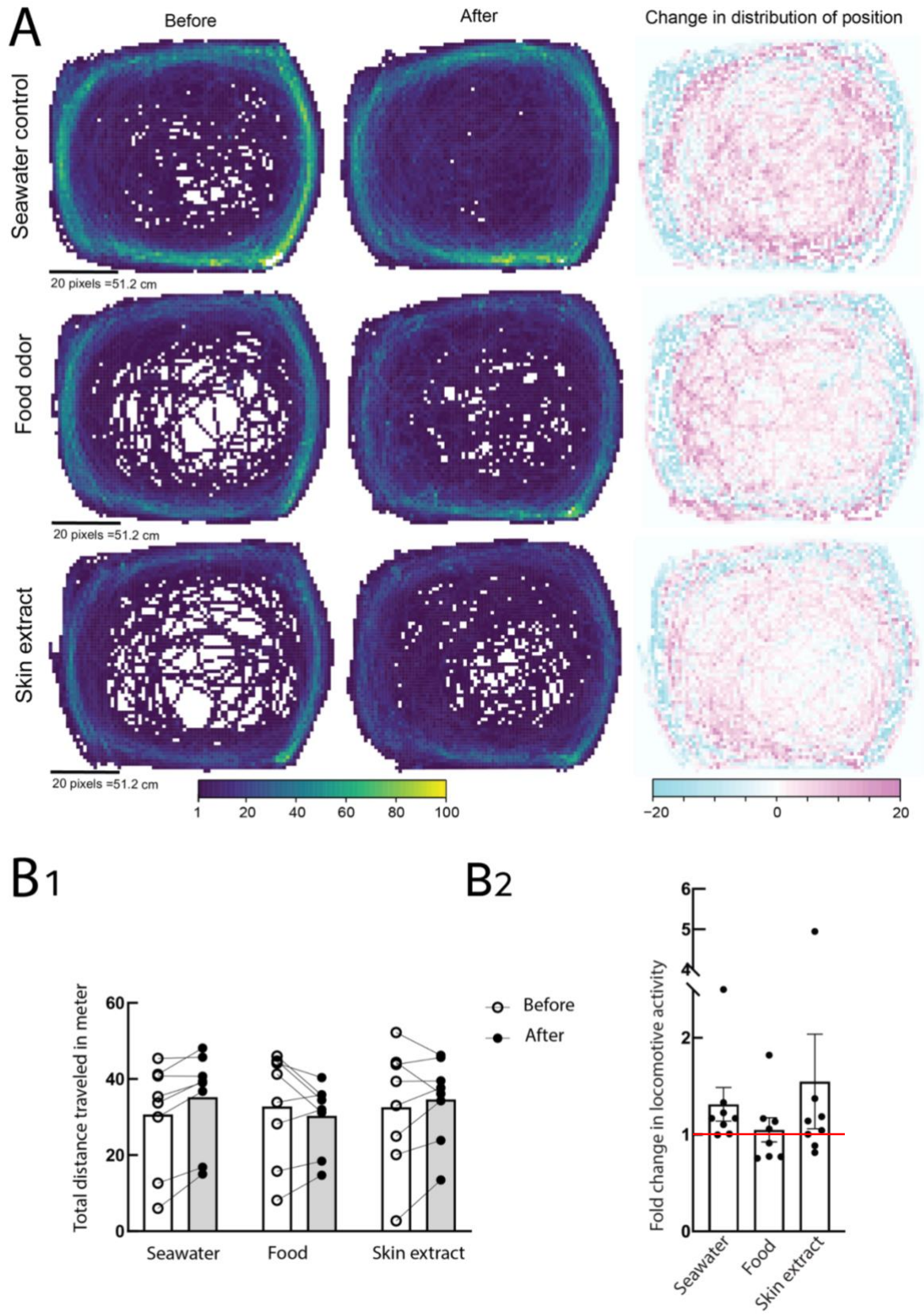


Figure 3.4. *The behavioral change in response to seawater control, food odor, and skin extract, respectively. (**A**) Based on the density of data points from the x and y coordinates, the positions of the sharks (n=6) from all trials are combined and visualized in binned heatmaps before and after the 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: pre-stimuli movement is indicated by blue and post-stimuli movement 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.*

Both odor stimuli and control affected the movement of the sharks and is visualized in the combined heatmaps (
Figure 3.4 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 into a fuller use of the tank. Interestingly, the seawater control seemed to cause the largest 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 *3.4* 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 I 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 *3.4* 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 *3.4* B2, these individual responses are clear. Shark 2, in particular, changed its behavior to an increasingly active state, which additionally can be observed in **Figure *3.1***.

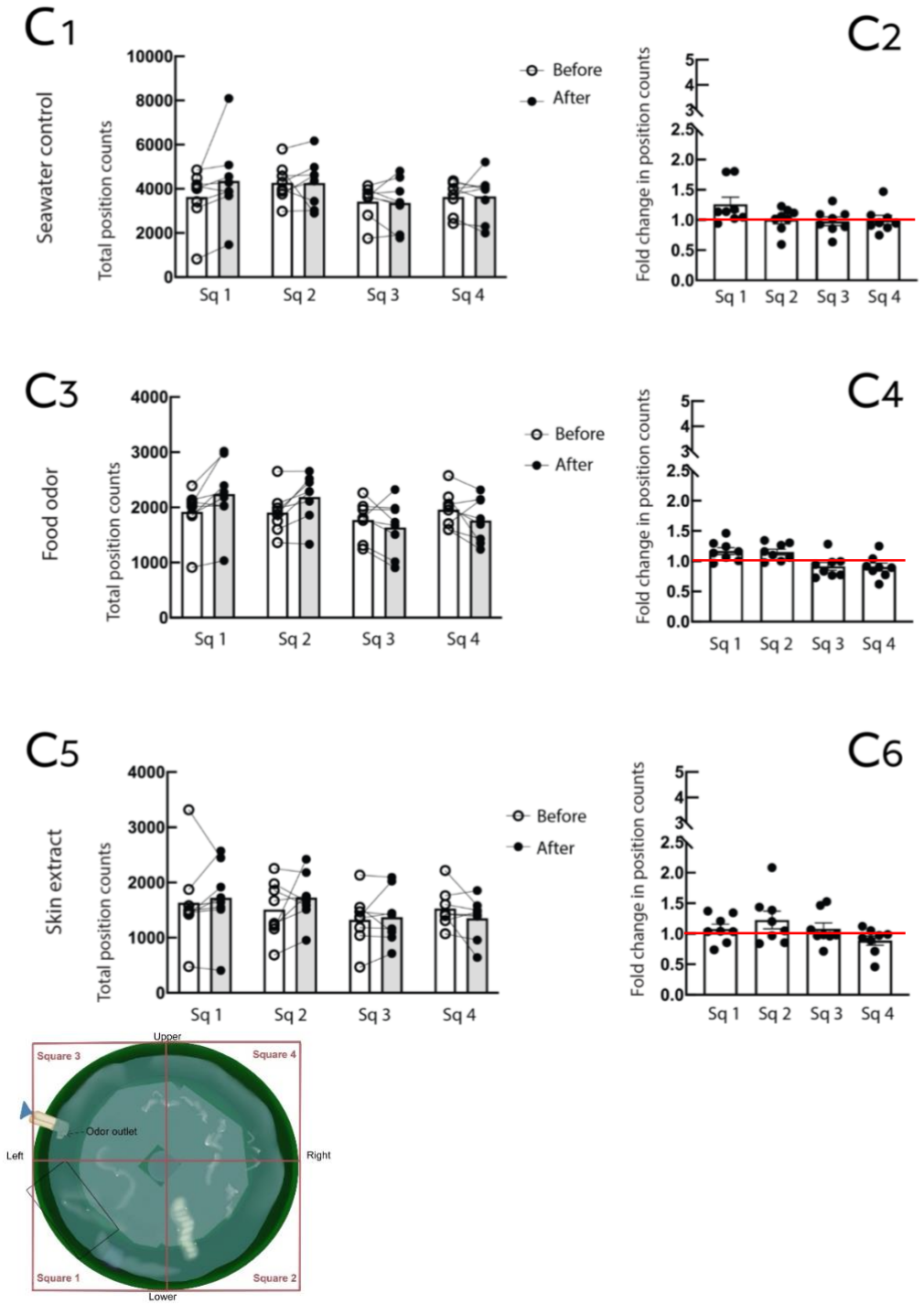
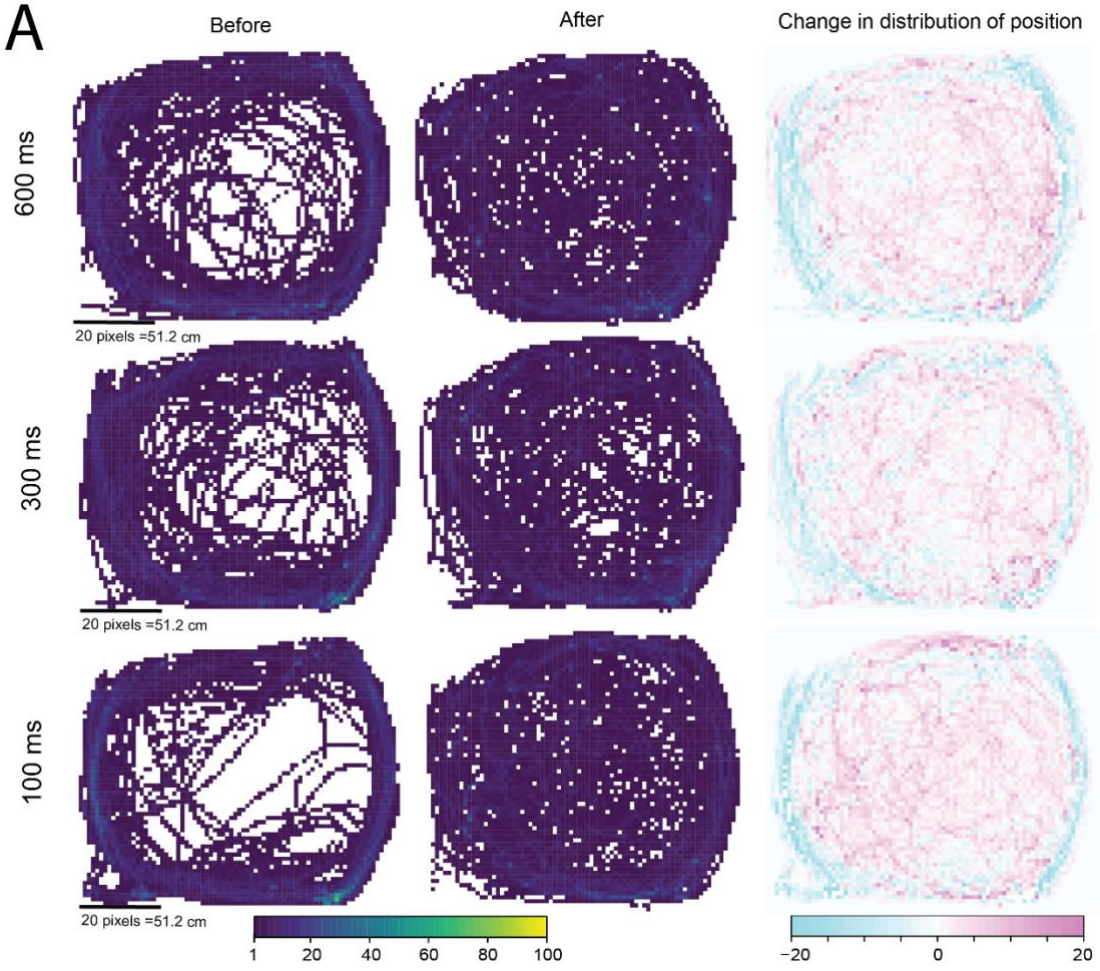


***Figure 3.5**. The total position counts from four arenas in the tank: Square 1 (Sq1), Square 2 (Sq 2), Square 3 (Sq 3), and Square 4 (Sq 4). (**C1, C3, and C5**) The bar plots show the mean position count and compare the total position counts before and after stimuli, where each data point represents 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.*

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 3.5 C1 and C2**). Food odor affected the alteration of position counts significantly (*ANOVA*, $p<0.01$) (**Figure 3.5 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 3.5 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.

Electromagnetic field trials



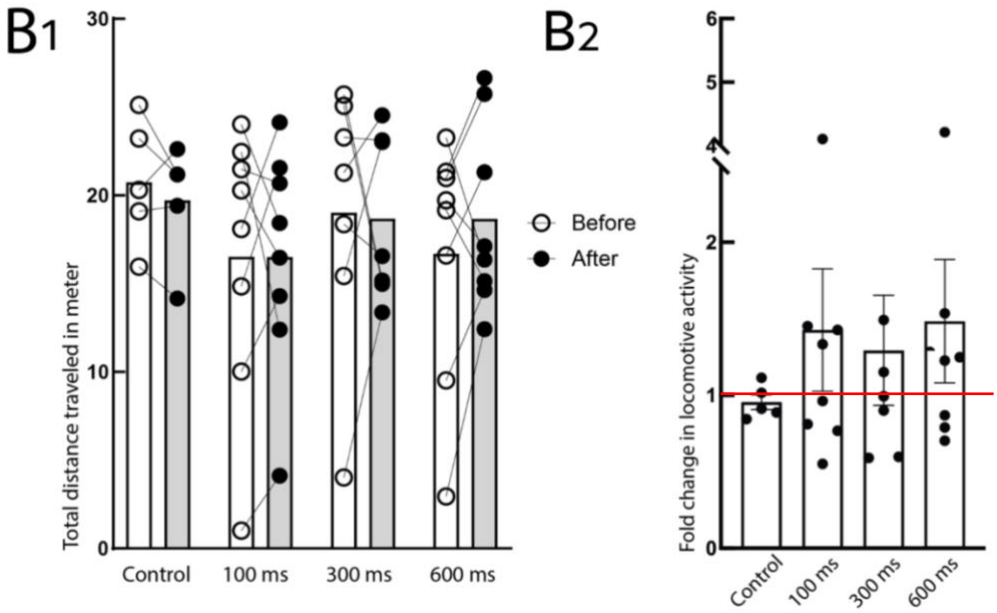


Figure 3.6*. The behavioral change in response to 10 V electromagnetic fields with a 600 ms, 300 ms and 100 ms pulse interval, respectively. (**A**) Based on the density of data points from the x and y coordinates, the positions of the sharks (n=6) from all trials are combined and visualized in binned 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: pre-stimuli movement is indicated by blue and post-stimuli movement is indicated by pink. (**B1**) Comparing the mean distances traveled before and after seawater control (no stimulus), 100 ms, 300 ms, and 600 ms pulse intervals. Each data point represents the mean distance traveled by a single shark from all trials. (**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.*

The impact of the 10 V electromagnetic field with 600, 300, and 100 ms pulse intervals affected the movement of the sharks (**Figure 3.6 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 600 and 300 ms "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 100 ms 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 of rapid pulses increasing the locomotive activity.

The means of total distances succeeding the onset of the electromagnetic fields (**Figure 3.6 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 100, 300 and 600 ms pulse interval compared to the control where the points are clustered (**Figure 3.6 B2**).

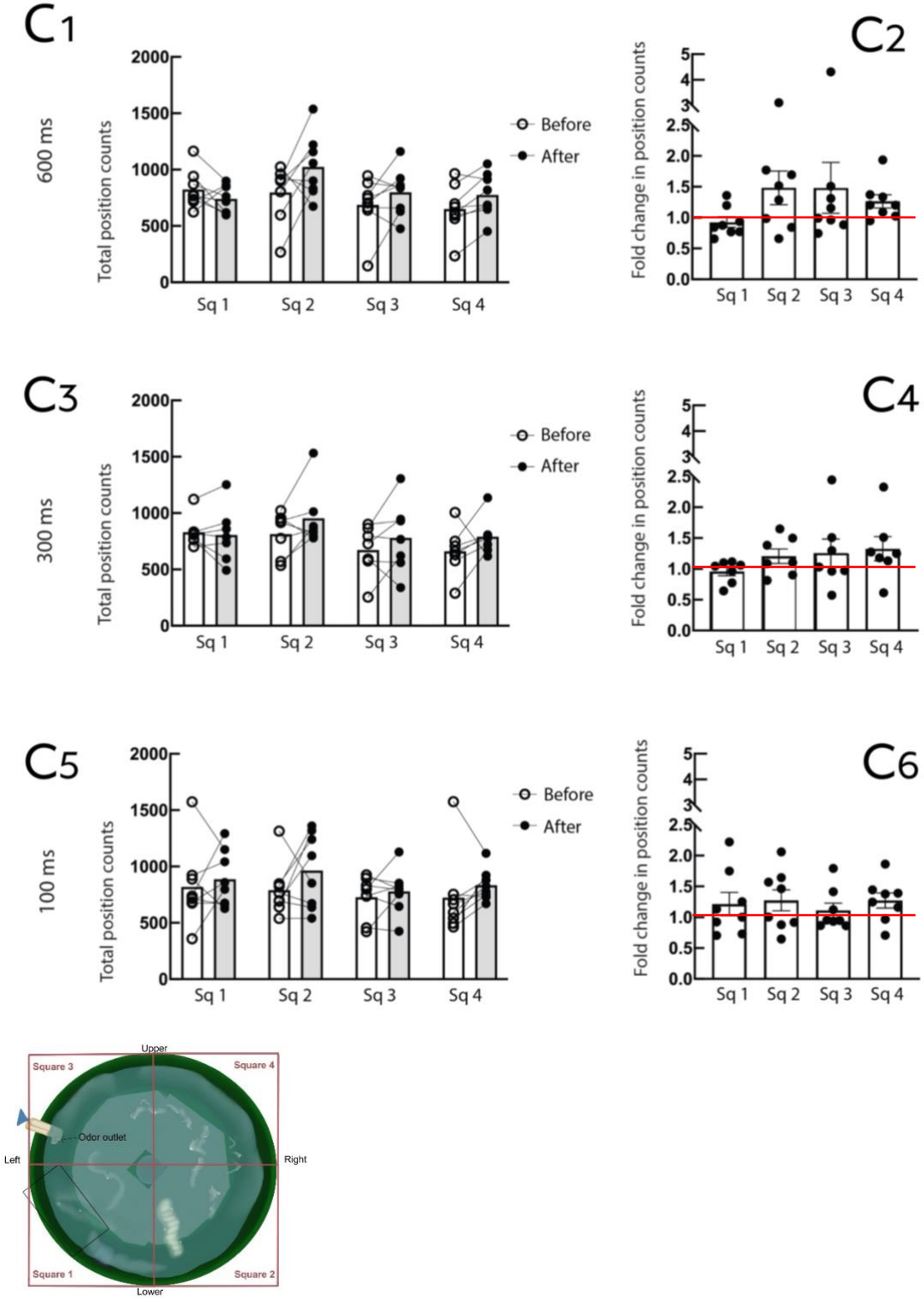


Figure 3.7*. The total position counts from four arenas in the tank: Square 1 (Sq1), Square 2 (Sq 2), Square 3 (Sq 3), and Square 4 (Sq 4).* ***(C1, C3, and C5)*** *The bar plots show the mean position count*

*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 10 V electromagnetic fields with 600ms, 300ms, and 100 ms pulse intervals were detected according to one-way ANOVA analysis (*ANOVA*, p>0.05). Even though no significance could be established, **Figure 3.7 C1 and C2** show that locomotive alterations succeeding 600 ms 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 3.7 C3 and C4**. In common for the 600 and 300 ms 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 100 ms pulse interval condition, which suggests that the sharks elevated their activity level in these trials as well (**Figure 3.7 C5 and C6**).

3.4 Condition 2 - Behavioral effects of 5, 10, and 20 V electromagnetic fields

Condition 2 was executed to evaluate whether three increasing voltages would affect the behavioral response differently. 5, 10, and 20 V were inflicted on a total of seven sharks. Shark 6, 8, 10, 15, 18, 20, and 21. We expected to observe larger alterations apace with larger voltages.

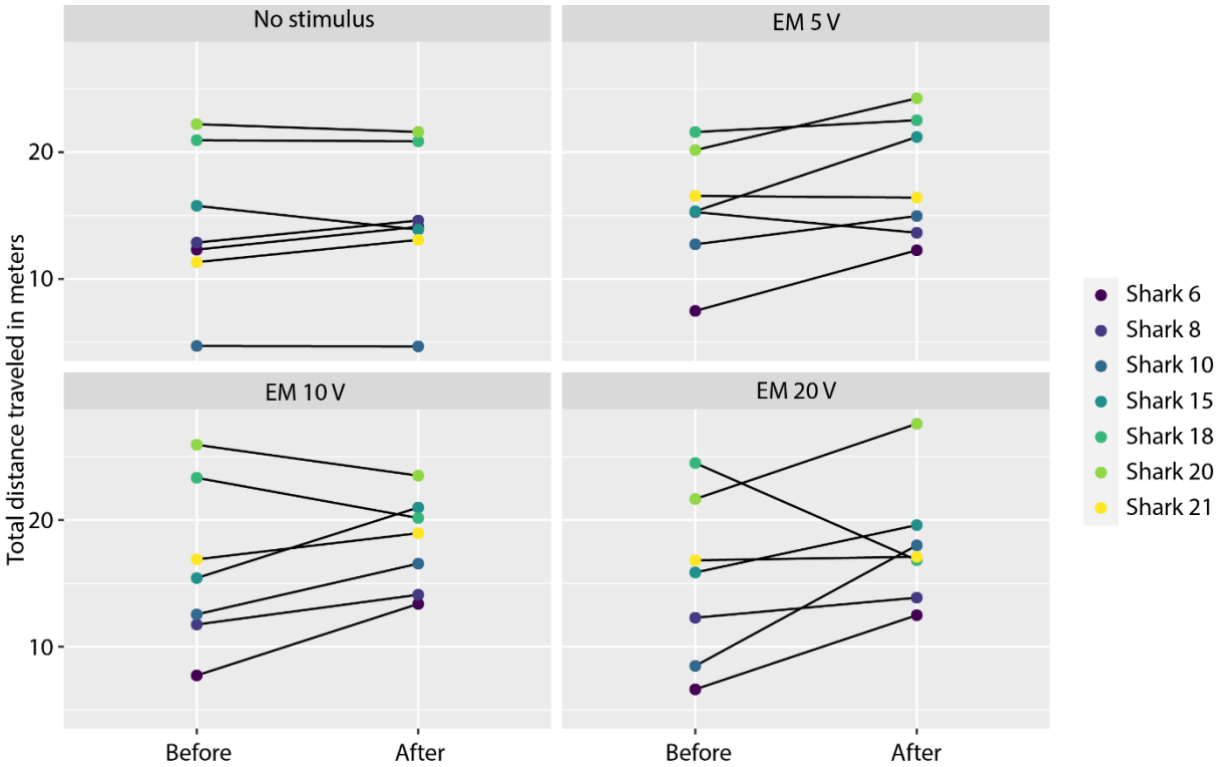
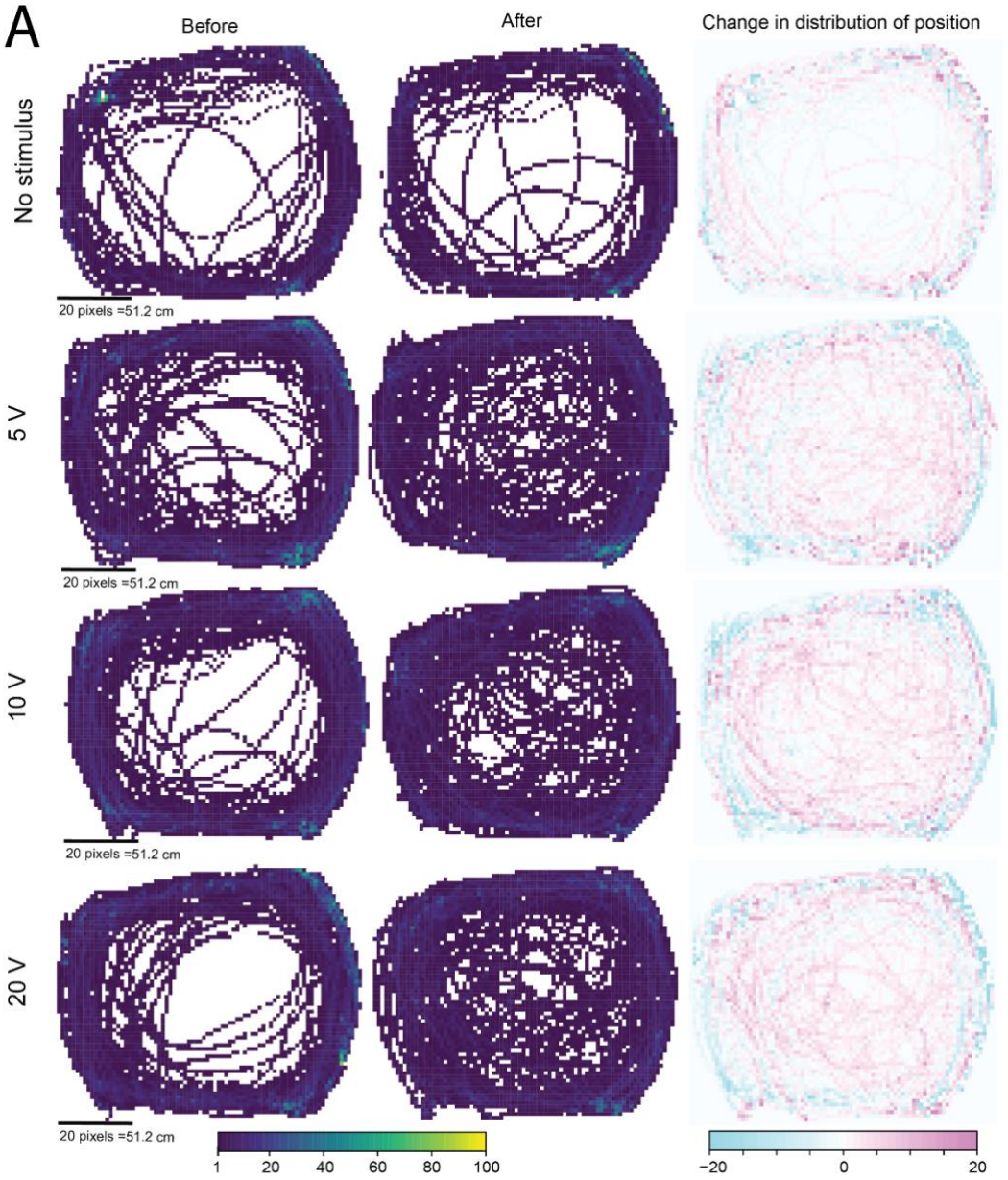


***Figure 3.8**. The mean of total distances traveled before and after the addition of stimuli. All stimuli sample size = 7. Each dot represents the average response from one shark to the stimuli.*

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 3.8**). 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.


A
Before
After
Change in distribution of position
No stimulus
5 V
10 V
20 V
20 pixels =51.2 cm
1
20
40
60
80
100
−20
0
20

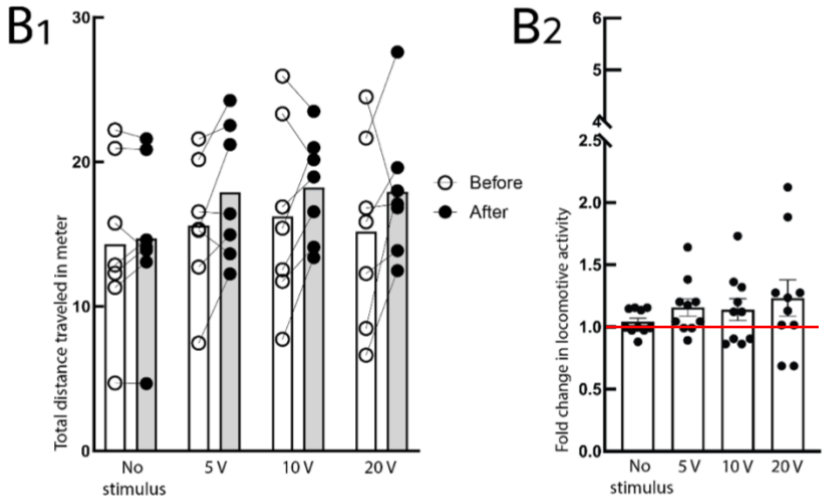

B1
B2
Total distance traveled in meter
Fold change in locomotive activity
Before
After
No stimulus
5 V
10 V
20 V

Figure 3.9*. The behavioral change in response to no stimulus, 5 V, 10 V, and 20 V electromagnetic fields with a 300 ms 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: pre-stimuli movement is indicated by blue and post-stimuli movement 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.*

By comparing the heatmaps of the trials with no stimulus and the EM trials, a clear change in locomotive activity is visible (**Figure 3.9 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. After the onset 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 3.9 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, 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.

The alterations of locomotive activity before and after the application of stimuli were not homogenous which demonstrates how the sharks exhibited individual behavior (**Figure 3.9 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 3.9 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*, $p>0.05$).

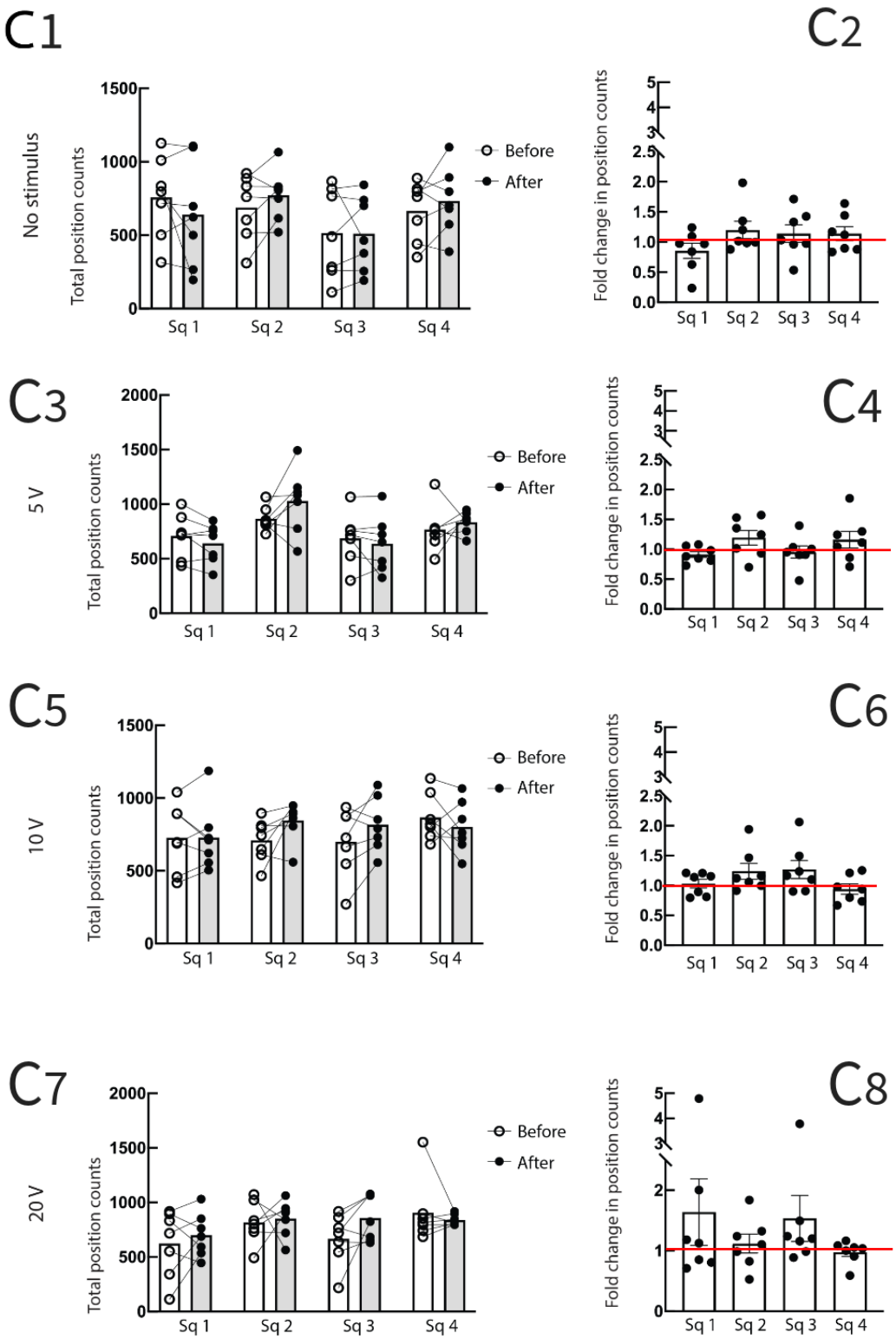


Figure 3.10. *The preferred location of the tank was evaluated by the total position counts from four arenas: Square 1 (Sq1), Square 2 (Sq 2), Square 3 (Sq 3), and Square 4 (Sq 4).* ***(C1, C3, and C5)*** *The bar plots show the mean position count and compare the total position counts before and after 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.*

The positional changes succeeding the electromagnetic fields are visualized in **Figure 3.10**. The bar plots representing the trial where stimulatory cues were absent (**Figure 3.10 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 3.10 C3 and C4**) indicates that the sharks slightly favored the use of the right side of the tank after initiation of the electrical current. This observation corresponds to the 5 V heatmaps in **Figure 3.9 A**. The result from 10 V, visualized in **Figure 3.10 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 3.9 A**. In **Figure 3.10 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 3.9 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.

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

Condition 3 was executed 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.

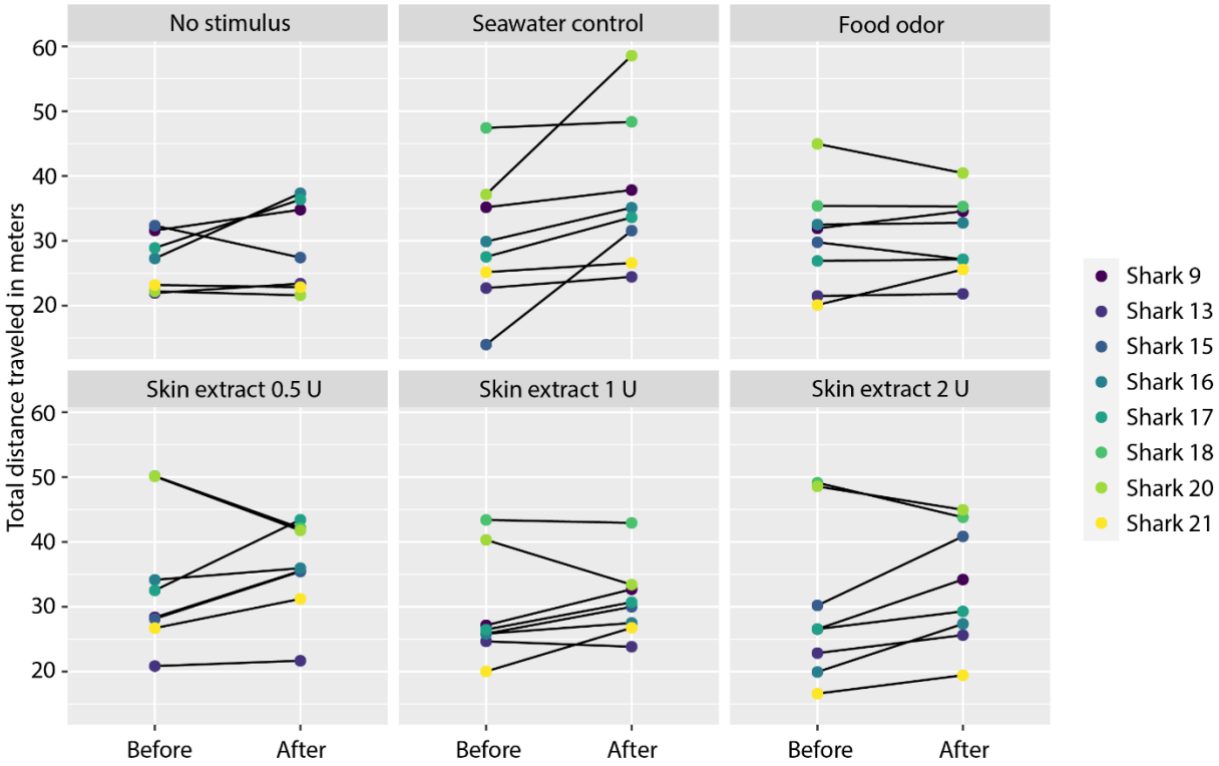
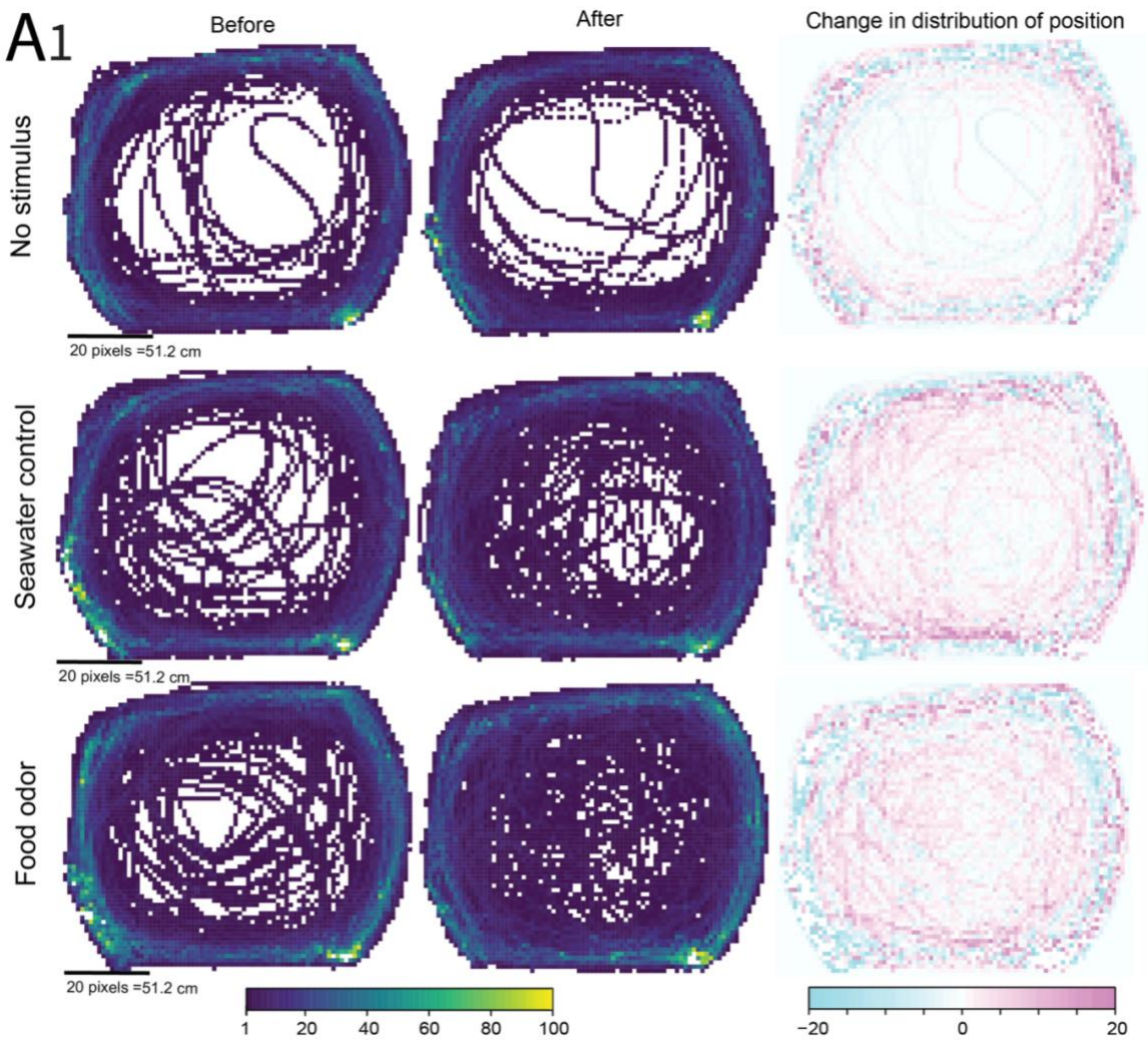


***Figure 3.11**. Comparing the mean of total distances traveled before and after the addition of chemical stimuli. Sample size = 8. Each dot represents the average response from one shark to the stimuli*

The mean of total distances traveled by each shark affected by chemical stimuli tested in Condition 3 is visualized in **Figure 3.11**. 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 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

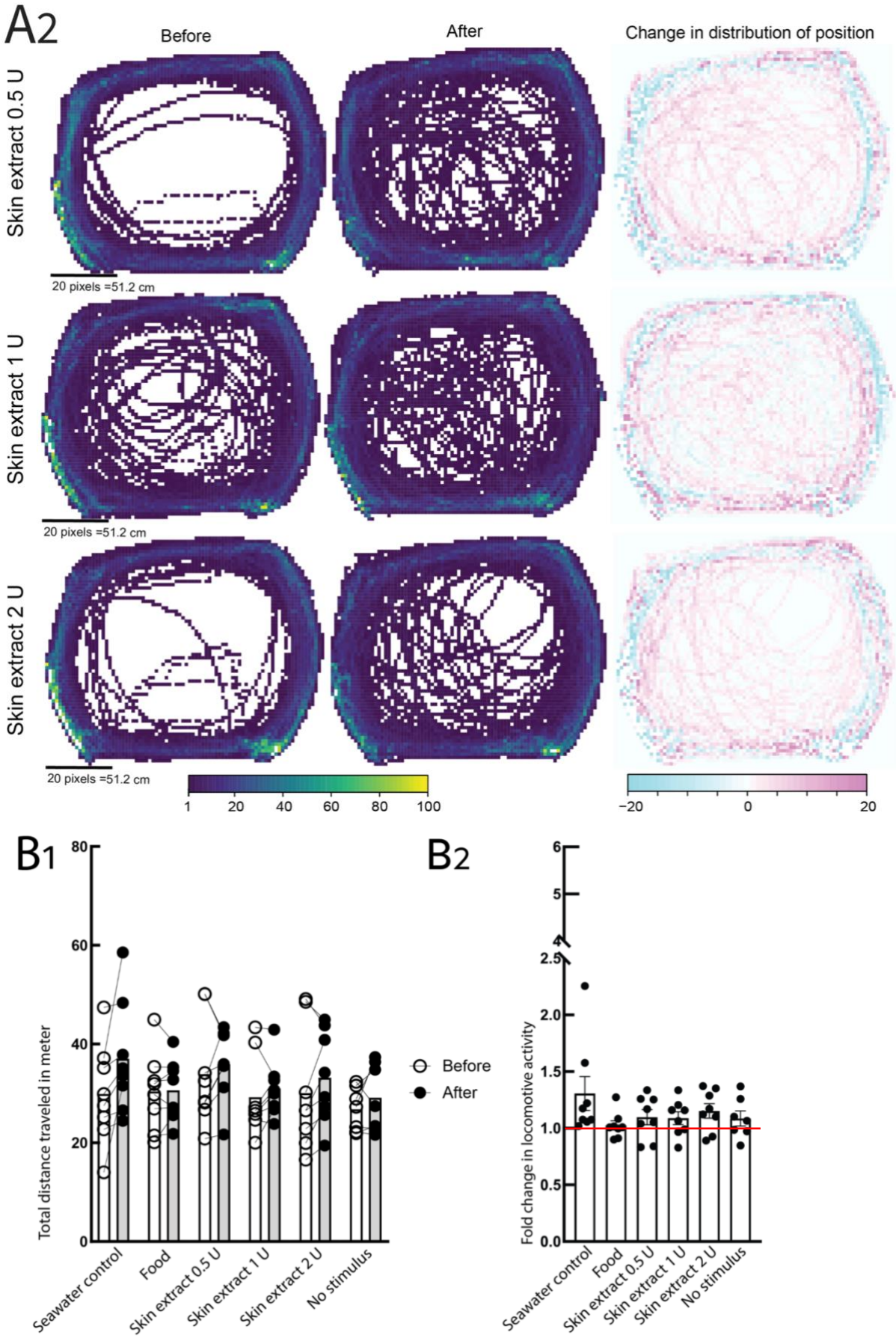
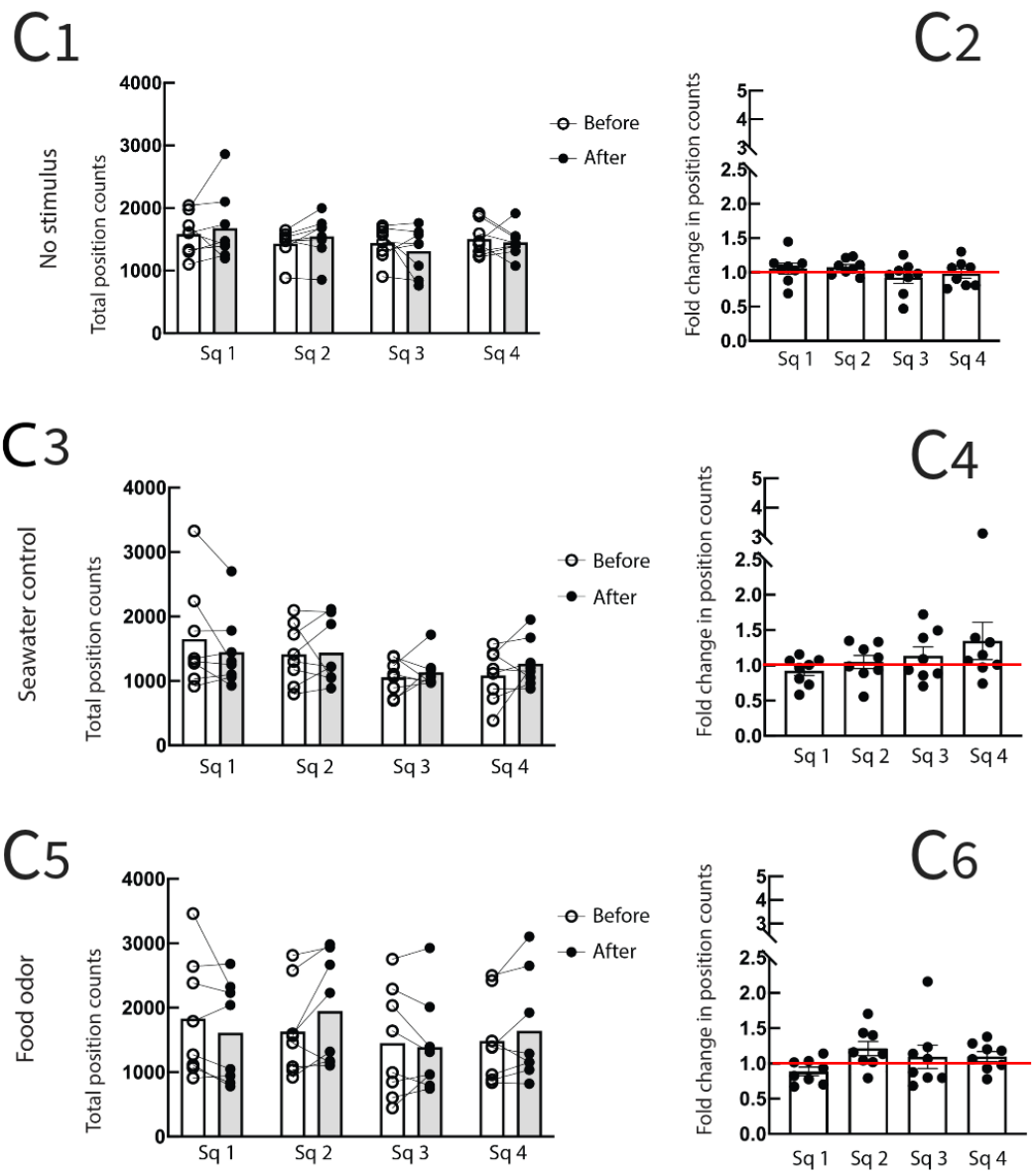


Figure 3.12*. The behavioral change in response to no stimulus, seawater control, food odor, 0.5 U skin extract, 1 U skin extract, and 2 U skin extract.* ***(A1 and A2)*** *Based on the density of data points*

*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: pre-stimuli movement is indicated by blue and post-stimuli movement 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 from where no stimulus was present (**Figure 3.12 A1)**. The seawater control evoked, similarly to the seawater trial in Condition 1, a behavioral change; there was 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 3.12 B1**). As illustrated in **Figure 3.11**, a great variety of individual behavior was elicited, and only the seawater control altered the distances traveled unidirectionally which inflicted a 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 3.12 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 3.11 and Figure 3.12 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

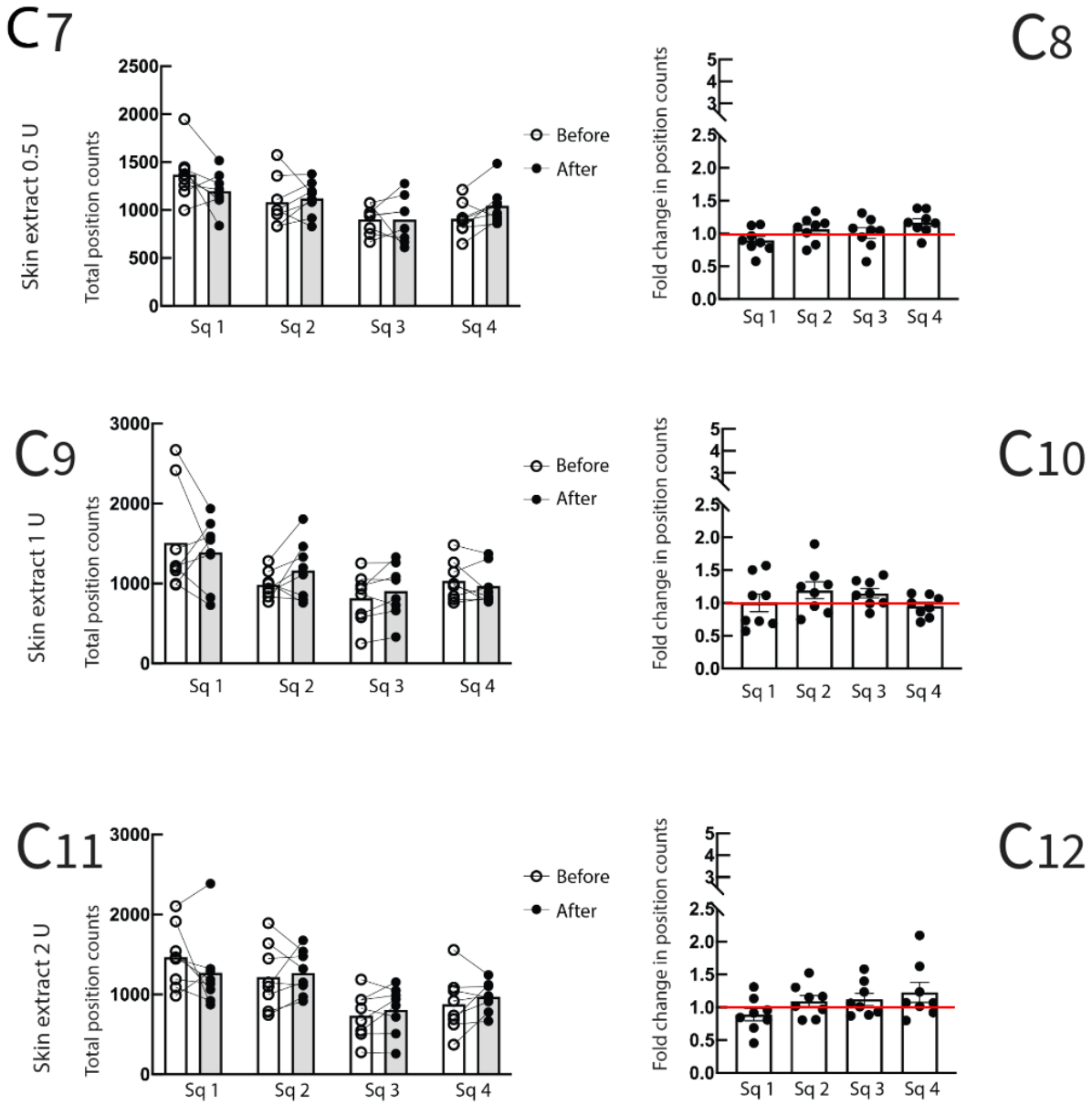


Figure 3.13*. The preferred location of the tank was evaluated by the total position counts from four arenas: Square 1 (Sq1), Square 2 (Sq 2), Square 3 (Sq 3), and Square 4 (Sq 4). (**C1, C3, C5, C7, C9, and C11**) The bar plots show the mean position count and compare the total position counts before 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 3.13 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 3.13 C2**). During the seawater control, the total position counts are most abundant in the quadrants representing the right side of the tank (**Figure 3.13 C3**), and the largest fold changes are represented in the upper area (**Figure 3.13 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 3.13 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 3.13 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 3.13 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 3.13 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 3.13 C9**). Simultaneously, **Figure 3.13 C10** shows that half of the sharks spent less time in this region succeeding the odor. Square 2 were 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 3.12 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 3.13 C11**). On the contrary, the upper squares (squares 3 and 4) show an increasing fold change exceeding 1 (**Figure 3.13 C12**) despite the lowest total of position counts.

3.6 Serum analysis

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=Calsium, 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

A single metabolite significantly differed between the treatment groups. 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)

Figure 3.14). 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 (**Figure 3.15**).

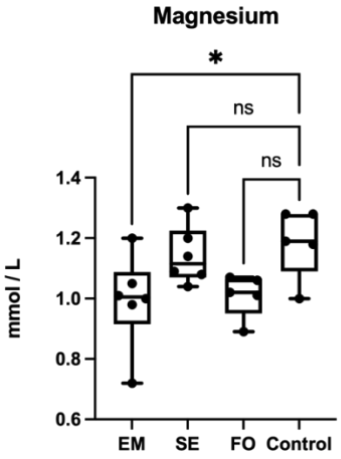


***Figure 3.14**. The magnesium levels from serum samples. There was a significant difference between the control and the electromagnetic field treatment group.*

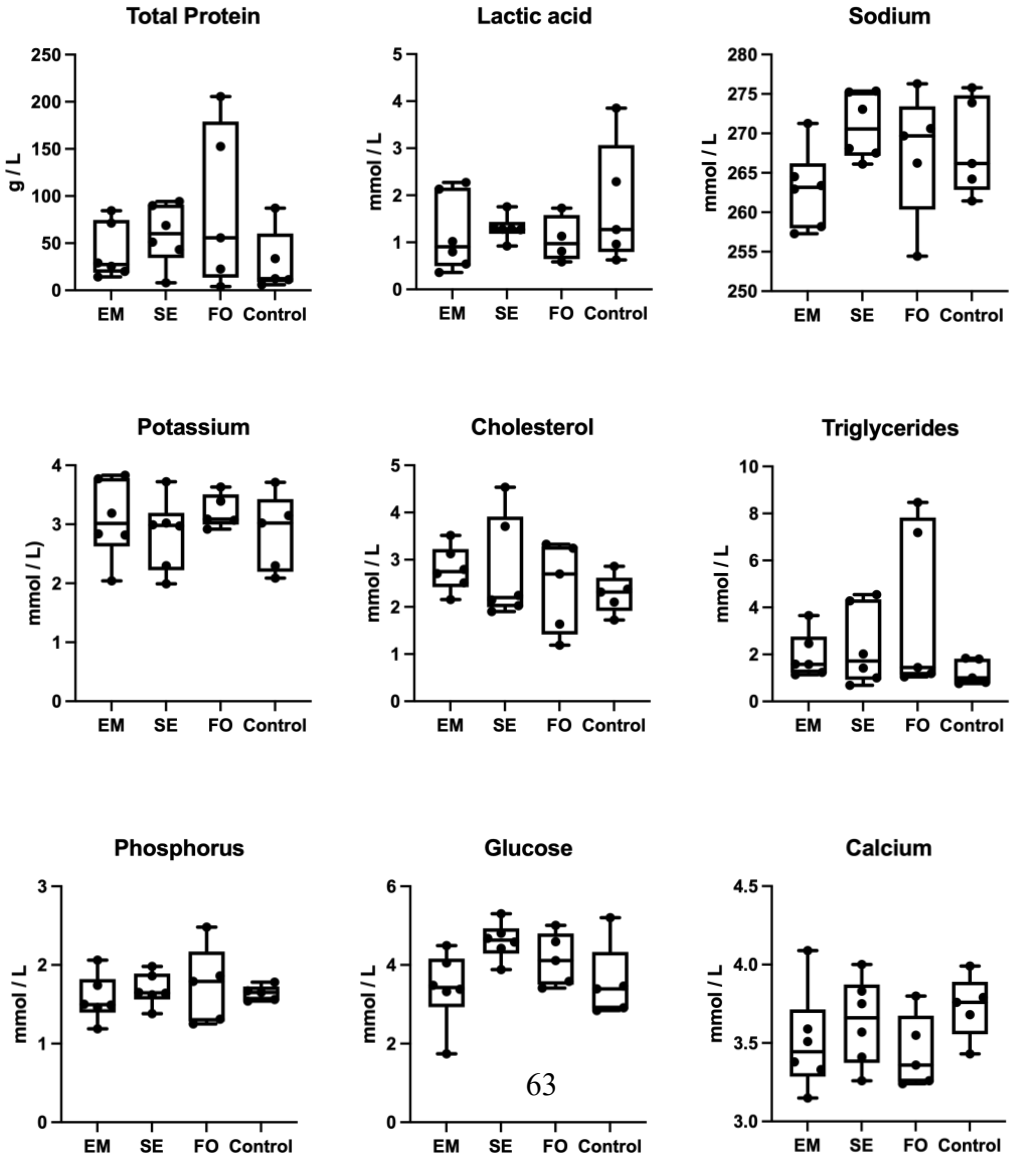


***Figure 3.15**. The metabolite levels in serum samples succeeding four treatment groups. EM = 10 V Electromagnetic field with 0.3 second interval, SE. = Skin extract, FO = Food odor, Control. Notice the varying units of measurement. Significant differences between treatment groups were not found.*

The metabolites in Figure 3.15 did not show significant differences between treatment groups. However, some groups elicited large variations in the metabolite levels. 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.

4 Discussion

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. Our findings will contribute to developing future shark deterrents to prevent these sharks from attacking fish cages. Additionally, the findings of his project will add valuable knowledge regarding how the species behave, and how they might be affected by stressful situations. 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 prior to euthanization compared to the control group.

4.1 Housing and feeding of Spiny dogfish

Spiny dogfish from Norwegian waters has, to our knowledge, not been housed in conditions such as ours before. The first weeks following the capture of the first group of sharks were therefore crucial in determining whether the project could be accomplished. Their well-being was of high priority, and daily observations of their behavior, swimming patterns, wounds, and appetite were conducted. The first sharks to be euthanized showed a change in its swimming pattern of concern, where small circling and seemingly severe loss of navigational skills were apparent. After several hours of observation and no signs of improvement, we concluded that its welfare might have been compromised, and the shark was euthanized.

The spiny dogfish consume food equivalent to 0.56% of their body weight each day (Bangley et al., 2014). The initial week, the sharks were fed every day, but as their appetite seemed low they received food each 3rd day instead. Additionally, the feed was frozen in the beginning. Food in the wild is not frozen and their skepticism towards this feeding strategy was with thawed pieces of mackerel was reasonable. The regime was shifted to thawed mackerel given every third day and after dark. Now the sharks started to eat nearly all food given, and this strategy were implemented throughout most of the trial. One exception was the sharks in tank 3 from group 3. These sharks did not eat salmon, despite showing foraging behavior. Instead of mackerel, we instead fed them salmon fillets which enhanced their appetite promptly. Mackerel was used as bait when we caught the third group of sharks, and negative associations to the smell of it might have caused the loss of appetite for this feed.

4.2 The effect of auditory, olfactory, and electromagnetic stimulatory cues on behavior

Prior to the experiments, we expected the sharks to show behavioral changes such as avoidance from the area of the tank where the stimuli were elicited (**Figure 2.15**). Additionally, we expected alterations of the locomotive activity and swimming pattern. The trials conducted under Condition 1 provided an excellent guideline to whether audio, odor or electromagnetic fields affected the behavior of the sharks. The qualitative observations were conducted by one person, which makes these results subjective. However, the observations were sufficient to describe varying but clear alterations of behavior succeeding the seawater trials, skin extract trials, food odor trials, and electromagnetic (EM) trials. Common responses were increased swimming speed, rapid turns, change of directional swimming, circling along the bottom of the tank, or in general, deviations from the common locomotion of each individual shark. The audio could be excluded for further trials as no behavioral response was elicited among any of the sharks.

4.2.1 Audio trials

Previous research on the sensory biology of elasmobranchs shows them to be susceptible to sounds between 10 and 1500 Hz, and especially sensitive to sounds between 40 and 600 Hz in carcharhinoid sharks and batoids (Mickle et al., 2021). Auditory stimuli have been successful in inducing both attractive and aversive behavioral responses in sharks. While low-frequent pulsating sounds have been successful in attracting carcharhinid sharks, sounds with abrupt level changes have provoked an aversive response (Myrberg Jr et al., 1978; Nelson et al.,

1963). Earlier observations from fishermen establish that loud voices could scare wild individuals from surface waters. Similar strategies have been used against other predators such as seals and sea lions. Use of non-specific sounds not only can impact other marine species but also its effect can be habituated. Hence, a natural aversive sound that has specific effect on sharks is desirable. The screams of orcas have also been successful in triggering an aversive response in reef sharks, but similar experiments have not been conducted with spiny dogfish. However, as the spiny dogfish share their environment with orcas which are known to hunt sharks (Visser, 2005; Visser et al., 2000) the audio of their screams was expected to trigger an escape response.

The sharks were subjected to orca screams with a frequency mainly ranging between 0-7500 Hz, with occasional spikes exceeding 20 000 Hz (**Figure 2.6**). They did not show any signs of an escape response during or after the stimuli and did not alter their locomotive activity notably. These results show that orca sound may not be suitable for use as shark deterrent against spiny dogfish. We did not measure the noise level in the experimental tank, but the sound elicited from the speaker was audible across the wall of the facility which indicates that the orca sound was loud enough to hear.

4.2.2 Odor trials

Sharks can detect thresholds of stimulatory amino acids ranging from 10^{-9} mol^{-1} to $10^{-6.9}$ mol^{-1} in batoids, Carcharhinidae and Sphyrnidae (Meredith et al., 2010). Such thresholds have to this day not been researched in *S. acanthias*, but due to the ancestry between the superorders of Squaliformes and Galeomorphii sharks, these traits can be assumed to reflect the sensory abilities in Squalidae as well (Naylor et al., 2005). Sharks are notorious for their sense of smell, a sense that has been a target in shark repellant research (Fogelberg, 1944; Primor, 1985; Springer, 1955; Thompson et al., 1986; Zlotkin et al., 1984). Chemical compounds derived from rotting shark flesh or irritants from flatfish secretion have proved to repel sharks, but due to toxicity and the necessary quantity needed for proper repelling effect, these substances are still unsuitable as deterrents. Apparently, the only chemical repellant tested on *S. acanthias* is Pardaxin (From the red sea flatfish), which caused paralysis and irritation of the mouth- and gill area (Primor, 1985). Semiochemicals from predators or harmed conspecifics have evoked behavioral avoidance in *N. brevirostris, Carcharhinus acronotus,* and *Carcharhinus perezi* (Schmidt et al., 1987; Stroud et al., 2014). Due to the interspecific differences in social interactions and predator relationships, it is necessary to investigate this phenomenon in other species as well. Therefore, it was interesting to observe the response in spiny dogfish when being exposed to skin extract from conspecifics. On the contrary, it was also interesting to evoke an attractive response with food odor to portray two opposite behavioral responses and distinguish the aversive from the attractive response.

From observations during feeding in housing tanks, the shark would turn sharply towards the piece of food and swim away with it in its mouth. During food trials, the sharks elicited similar behavior, by swimming to the bottom of the tank and circulating for a short while with snake-like movements. In addition, the swimming speed was slower, and the number of sharp turns increased (not quantified here). This is reflected in the heatmaps (**Figure 3.2** and **Figure 3.12**), and the total distance traveled (**Figure 3.1** and **Figure 3.11**) from Condition 1 and

Condition 3. The heatmaps show how the sharks utilized larger areas of the tank, and shortened the distances traveled. However, there was a clear individual variation in behavioral response, perhaps influenced by their amount of stress- and hunger which has been documented as an influential factor in other experimental trials (Tallack et al., 2009). As the spiny dogfish is a schooling species, competition from conspecifics is also probably influential on their foraging behavior and might be a reason for why few of the sharks properly elicited such behavior. Additionally, the environment in the experimental tank differed from the housing tank as the lid, lighting, and flooring were altered. Two sharks were offered mackerel after all trials were conducted but did not show any interest in eating. This could indicate that the sharks were stressed in the experimental tank due to the circumstances – especially as stressful stimuli were conducted in advance making the tank an uncomfortable place and further reducing their appetite.

In response to skin extract, several sharks elicited aversive behavior characterized by abrupt change of direction away from the odor outlet, head shaking, increased swimming speed, and a general alteration of the swimming pattern. Some elicited short-term navigational loss, as they swam into the water and odor pipes. In the heatmaps from skin extract trials in Condition 1, the change of locomotive activity is visible as the sharks shift into closer circling and increase the time spent on the opposite side of where the odor is added (**Figure 3.2** and **Figure 3.12**). In addition, they elicited a slight increase in distances traveled and were more active after the stimuli were added compared to the food odor trial. From the qualitative observations it took about half a minute after the odor was added before the response was visible and decreased shortly after the smell was spread to larger parts of the tank with the current. As the normal swimming pattern was returned shortly after the behavioral alteration, the imprints on the heatmaps might be a bit veiled.

Condition 3 was conducted to observe whether the observed aversive behavior to skin extract has a dose-dependent relationship. We expected the increasing units of skin extract to induce a stronger change in locomotive activity. The heatmaps suggest the opposite as there are fewer colorated pixels in the 2 U “after” heatmap compared to the lesser units (**Figure 3.12**). The fold change, however, indicates a slight increase in locomotive activity in conjunction with the larger units. Some responses were unique, such as shark 9 during a 0.5 U trial, where an extreme response was elicited with rapid swimming speed and a loss of directional control. This behavior could be characterized as an intense aversive response. It resumed a normal swimming pattern after a couple of minutes. Furthermore, the position counts representing the fold change in the four quadrants (**Figure 3.13**) showed a larger expansion with increasing units, which might indicate that larger amounts of skin extract induce stronger alterations of motion. Overall, the animals showed large variability in type and strength of response; further studies are necessary to identify the relationship between this variability to physiological states, such as age, sex, maturation status, hunger state etc.

An interesting result from the odor trials of Condition 1 and Condition 3, is the behavioral response succeeding the seawater trials. This was the only stimulatory cue that induced a homologous behavioral response among all sharks, by significantly increasing their activity

level. Even though this was supposed to act as a control, it appeared to affect the sharks more than expected. A limitation in our odor trials was the single odor outlet used to deliver all the different odors. Despite flushing the tubes after the delivery of food or skin extract odor, odor molecules might still have been present when the seawater trials were conducted. Consequently, the observed behavior was not necessarily a result of the smell we intended to enter the tank. Despite the seawater being from the same water outlet system as the flowthrough system, the sharks with their delicate senses could have detected small changes in temperature or salinity. Another reason for this change in behavior could be caused by odor or fragments from the plastic container in which the seawater was stored during the trials.

Due to the size of the tank and the continuous water flow, it is also plausible that the odor was scattered throughout the whole tank too rapidly to observe the actual response. For example, avoidance behavior was characterized by avoidance of the area where the odor is present, and odor being present in the entire tank might disrupt this response as there would be no place to escape from the smell. Odor from the applicant could also contaminate the odorant solutions. Sharks possess chemoreceptors in their oral cavity, which also could affect their behavioral response.
It is difficult to evaluate if the sharks react to the chemical stimuli due to odor or taste, but the projections to the brain might be of help. In teleosts, the stimulation of taste buds projects to the cranial nerves VII, X, and XI (Finger et al., 1992). The innervation of chemoreceptors from the brain in sharks could be similar and used for further neuroanatomical and functional studies, necessary to identify whether the chemoreception of these odors was perceived by the receptors in the nasal- or oral cavity in spiny dogfish.

4.2.3 Electromagnetic pulse trials

Sharks are sensitive to weak electromagnetic signals which they detect through the ampullae of Lorenzini. These senses are utilized in social, foraging, and possibly navigational interactions and have been one of the primary target senses to utilize in the search for an effective shark repellant. Electromagnetic fields mediated by active or passive devices have been thought to repel sharks of different species due to overstimulation or irritation of these sensitive pores (Kaimmer et al., 2008; Rice, 2008). Various approaches have been conducted with electropositive metals and permanent magnets to keep spiny dogfish from winding up as bycatch in trawls and long-line fishing. These techniques have been successful in eliciting aversive behavior in these sharks but are shown to be insufficient when the animals are hungry (O'Connell et al., 2011; Tallack et al., 2009). In addition, the electropositive metals and magnets quickly dissolve in seawater and lose their effect, and to create magnetic fields over a longer period of time, they require rapid placement (O'Connell et al., 2014b). Creating an EM field from an active source like a battery-powered electrode has been the objective for commercially available gadgets used to protect humans from shark attacks and has mainly been tested on sharks from the Carcharhinidae family. No device has to this date been successful in deterring sharks over a longer period, as hunger and competition repress the fear of the repellant devices (Jordan et al., 2011).

The spiny dogfish has an amazing sensory ability to detect small electromagnetic fields of 0.2 nV/cm (Jordan et al., 2011). This allows the animal to detect the weak electrical cues elicited by prey and conspecifics. During our trials, we expected the sharks to avoid the electromagnetic field, but we were also intrigued to see if they would elicit foraging behavior. The reach of the EM field was approximately 30 cm from the electrodes, according to the simulations in **Figure 2.8**. The sharks were subjected to 9.6 E[V/m] (5 V), 19.2 E[V/m] (10 V), and 38,5 E[V/m] (20 V); in 10V condition, sharks experienced 33.33mV/cm of electromagnetic field at 25cm from the electrode. The qualitative and quantitative observations from the EM trials suggest that the sharks elicited an aversive response when encountering the electromagnetic field or when they were in physical contact with the electrodes. This was displayed by rapid turns, increased swimming speed, and a change of locomotive activity. In addition, on several occasions, they would keep their distance by circling the opposite side of the tank. The immediate response when the stimuli were initiated would be an instant jerk of the head, a short moment of tonic immobility, or a change of direction launched by a C-turn or hovering. As the spiny dogfish can detect electromagnetic cues of 0.2 V/cm, they probably detected our EM stimuli everywhere in the tank. Instant responses were documented also when being furthest away from the electrodes.

During the EM trials of Condition 1, the sharks were subjected to three different intervals of 10 V electromagnetic impulses. Alternation of the pulse intervals seemed to increase the abruptness of the initial reaction, as shorter intervals caused stronger behavioral changes. The electromagnetic fields caused an alteration of the locomotive activity, as illustrated in the heatmaps (**Figure 3.2**). In addition, the distance traveled, and corresponding fold changes show a great variance (**Figure 3.3**). These results indicate how the individual responses differed. Nevertheless, despite the response not being homologous, it is clear that the electromagnetic field had an effect on their behavior and possibly be characterized as aversive.

The purpose of the EM trials of Condition 2 was to evaluate if the strength of the electromagnetic fields influenced the behavioral response. We expected the increasing voltage to cause larger behavioral alterations, as a 5 V EM field (9.6 E[V/m]) could be less stimulatory compared to a 20 V EM field (38,5 E[V/m]). The heatmaps (**Figure 3.9**), similarly to the heatmaps of Condition 1(**Figure 3.2**), show that the movement of the shark was altered succeeding the stimuli onset. Interestingly, the distances traveled seem to increase with the increasing voltages – as well as the fold changes. This indicates that the activity level was increased in general, even though no significant changes were found. The fold changes from the position count in the four squares also indicate that the sharks were more active after the stimuli (**Figure 3.10**). The sharks altered their behavior when introduced to the electromagnetic fields. However, their individual response fluctuated which could be affected by several biological and environmental factors such as age, sex, length of animals, stress level, and tank effects (**Figure 3.8**). Some individuals did not elicit any response to the electromagnetic field and swam past the electrodes in the same manner as if no EM field was present. In some trials, the sharks seemed to be attracted to the electromagnetic field by swimming close to the bottom in sharp C-turns – a behavior resembling the foraging behavior

observed during feeding or food odor trials. Additionally, depending on the sequence of the trials, their ability to learn and to habituate to the EM fields might have affected their response. These observations give a mere conception of how these sharks would respond to these cues in their natural habitat, unaffected by confinement and altered social influence like in these laboratory trials. This reflects the complexity of behavioral quantification but shows a possible promising shark deterrent.

4.2.4 Metabolites in serum samples

Due to the difference in animal behavior across species, it was interesting to evaluate whether a change in locomotion, which could imply a stressful response, would be expressed as a physiological response as elevations or demotions of an array of metabolites in the serum. Elevated levels of glucose, lactate, and salts such as magnesium, calcium, and potassium have been documented in sharks, and therefore we expected to observe alterations in these measurements (Mandelman et al., 2006; Manire et al., 2001). In addition to the physiological indicators of stress mentioned above, cortisol is frequently used as an indicator of a primary stress response (Skomal et al., 2010). However, cortisol is not the main corticosteroid in sharks but rather α1-hydrocorticosterone (Anderson, 2012). I did not investigate the serum levels of α1-hydrocorticosterone, however this could be interesting for future investigation. In this experiment, the sharks were subjected to a 10 V EM field with a 0.3 second pulse interval, skin extract, or food odor 30-40 minutes prior to euthanization. One group acted as control. Frick et al. (2009) observed elevated lactate in blood plasma after sedation in *H. portusjacksoni* and *C. laticeps* but not after confinement, which shows how sedation could evoke a stress response in sharks. Cliff et al. (1984) found elevated potassium, calcium, and magnesium levels in plasma after the capture and transport of dusky sharks (*Carcharhinus obscurus*). Additionally, they found elevated lactate levels which in turn can cause lacacidosis and cellular disruption, indicated by elevated levels of minerals (salts). Lactate and glucose levels in the blood plasma indicate that the animals have been stressed, but also be altered by other factors such as hunger (deRoos et al., 1985). Metabolite levels are difficult to compare across species as age, sex, and life history traits might affect the results (Manire et al., 2001).

As the sharks elicited an aversive response succeeding skin extract and EM trials, elevated levels of glucose, lactate, calcium, and magnesium in the serum were expected. Magnesium was the only metabolite that significantly differed between the control group and the EM-treated group (**Figure 3.14**). An earlier study investigating the physiological stress response in other shark species observed that serum levels of magnesium increased progressively with stress (Cliff et al., 1984). However, the levels of magnesium were significantly *lower* than in the control group. The other metabolites showed large variations in all treatment groups and no significant trend in any of the treatment groups. These results could signify that the sharks, despite showing aversive behavior when subjected to skin extract and electromagnetic fields, were less stressed than we imagined. If so, using these stimulatory cues as shark deterrents could have a low physical impact on the species despite evoking aversive behavior. On the contrary, these results could indicate that the sharks, despite showing what I interpret as

aversive behavior, in fact, were not stressed or acted aversive towards the stimuli. The metabolite levels might also have been affected by the hunger level of the sharks.

The metabolites in the serum samples might have been showing mostly insignificant results as the timing for sampling was customized according to the activation of the stress-related gene c-fos. An upcoming part of Pigghå FRI project is to perform histology and analyses of genes expressed primarily activated about 30 minutes after the induction of stress (Kovács, 1998).

4.2.1 Establishing a behavioral paradigm and analyses

Originally, a modified commercial automated tracking and infra-red (IR) recording system was employed for tracking and recording the animals during experiments, which would instantly transcribe coordinates throughout the entire recordings. This system was specifically chosen because of its ability to record in low light conditions and tracking in real-time, which would be optimal due to the natural light conditions of the spiny dogfish. We wanted to keep the experimental tank as similar as possible to the housing tanks to remove factors that could interfere with our results. We encountered several challenges with this setup, and evidently, the lid had to be removed for the camera to record the whole tank area. Next, we mounted infrared lights over the tank to illuminate the arena for the IR-camera. However, this setup was inefficient in illuminating the water and the animal could not be recorded or tracked. Therefore, the initiation of experiments was postponed until our new (and current) setup was ready to use as described in the method section.

The locomotive activity of captive spiny dogfish (or any species of shark) has not, to my knowledge, been processed with deep learning tools such as DeepLabCut before, and the process of extracting the data was therefore an unpredictable task. Mainly because of how the angle of the camera was tilted and the obstruction of the movement of the shark due to ripples in the water. However, by training the DLC with several recordings differing in quality (10 fps vs 20 fps) and angles (Shark 1 and 2 compared to the rest), nearly all noise such as ripples and water outlet was ignored by the tracking system. Additionally, many hours of manual video processing were spared by using this deep-neural network.

4.2.2 Limitations of the experiment

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.

The low light camera eventually used, provided sufficient recordings for further analyse. The lens used for recording of the two first sharks were too narrow, and to get a proper picture the water column was decreased to 65 cm. Additionally, the recordings were of low quality which disrupted the further analysis. Some recording in the same trials were filmed with 10 fps. Shark 3 was recorded with a 80 cm water column, 20 fps and wider field of view. This provided larger inaccuracy during video analysis, and further data analyses. Consequently, the first two trials were excluded in the making of the heatmap, however they were included in the statistical analyses and plots.

The system was not flawed, and some noises were included in the tracking. Such fluctuations impacted our results, as sudden labels appearing far from the shark would increase the distances traveled. To reduce the error from this, the data was smoothened with Simple Moving Average which might have reduced the larger fluctuations in the behavioral response.

4.3 Conclusion

In this project, we successfully captured and housed spiny dogfish, and managed to keep them alive and in seemingly good health throughout the termination of the experiments. I established a behavioral paradigm to investigate sensory cue-dependent behavior and tested various cues: sound (orca and control sound), smell (food odor and skin extract), and electromagnetic pulse. I tracked sharks using artificial intelligence and was able to quantify the behavior that I observed in real-time while conducting the trials. I found that sound has no obvious effect on behavior response. Food odor induced persistent attraction and searching behavior – which I have defined as foraging behavior. Skin extract and EM pulses induced aversive response, however the animals showed large variability in quality and strength of response, as they elicited individual responses. Serum metabolite analyses showed that the treatments caused no physiological acute stress response. The development of a shark deterrent to use near aquaculture facilities could be possible by utilizing odorants or electromagnetic fields, while also avoiding causing large stress responses that possibly alter the animal's homeostasis. Further field studies are necessary to validate the laboratory findings.

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APPENDIX 1A – Behavioral observations of Shark 1

Stimuli	Time of appliance	Time of stimuli appliance	Comments		
Day 1: 24.01.2023	One hour of habituation to speaker	10.00-10.10 into trial	Resting the whole time	Water level:	65 cm
Seawater (control)	kl. 09.25-09.44	10.00-11.00 into trial		From tank #:	3
Control sound	Kl.10.00-10.20	10.00-11.00 into trial		Days since capture:	53
Test sound	Kl.10.20-10.40	10.00-11.00 into trial		Days since last feed opportunity:	2 days before first trial started
Control sound	Kl.10.42-11.02	10.00-11.00 into trial		Sex:	Male
Test sound	Kl. 11.05-11.25	10.00-11.00 into trial		Tissue from state:	BM trial
Seawater (control)	Kl. 11.27-11.47	10.00-10.10 into trial		Length:	71,5 cm
Food odor	Kl.-11.48-12.08	10.00-10.15 into trial	1/10X odor	Weight:	1280/1290 g
Break (2 hours)	-	-	-	Blood sample:	yes
Control sound	Kl. 14.00-14.20	10.00-11.00 into trial		Gill sample:	yes, 2nd gill arch
Seawater (control)	Kl. 14.21-14.41	10.00-10.12 into trial		Brain sample:	Yes, brain and telencephelon-ob
Test sound	Kl. 14.43-15.03	10.00-11.00 into trial		Both spines (age mapping):	Yes
Test sound	Kl. 15.03-15.23	10.00-11.00 into trial		Skin extract:	
Control sound	Kl. 15.24-15.44	10.00-11.00 into trial		Comments:	Have a scratch on right side of dorsal fin
Skin extract	Kl. 15.45-16.05	10.00-10.12 into trial	Swimming faster, change in locomotion. Swimming more agetated	Photo:	
Day 2: 25.01.2023	One hour of habituation to speaker	10.00-10.10 into trial			
Seawater (control)	Kl. 09.10-09.30	10.00-10.10 into trial			
Control sound	Kl. 09.30-09.50	10.00-11.00 into trial	Seems like no response		
Test sound	Kl. 09.51-10.11	11.15-12.15 into trial	No response		
Control sound	Kl. 10.13-33	10.00-11.00 into trial			
Test sound	Kl. 10.35-10.55	10.00-11.00 into trial	10.45-switch on speaker		
Seawater (control)	Kl.10.56-11.16	10.00-10.10 into trial		Teams meeting, possibly on speaker in tank :) Meeting started at 10.39, started to speak at ca. 10.50	
Food odor	Kl. 11.17-11.37	10.10-10.20 into trial	2/10X odor. Some response	Teams meeting, possibly on speaker in tank :)	
Break (2 hours)	-	-	-	Teams meeting, possibly on speaker in tank :)	
Control sound	Kl. 15.13-15.33 (14.13-14.33 & 13.37-13.57)	10.00-11.00 (11.15-12.15	Swimming around the speaker for several turns. Interesting video.	In failed recordings: Teams meeting, possibly on speaker in tank :)	
Seawater (control)	Kl. 15.34-15.54	10.00-10.10 into trial	Swimming in the middle of the tank much more than earlier		
Test sound	Kl. 15.55-16.15	10.20-11.20 into trial	Swimming close to speaker after sound is finished. Tail is beating more - stress response?	From here on we turn on speaker 15 seconds before sound and turn of 15 seconds after sound is finished	
Test sound	Kl. 16.15-16.35	10.00-11.00 into trial	Swimming close to water outlet.	15 seconds befor/after	
Control sound	Kl. 16.36-16.56	10.00-11.00 into trial	Passes the speaker during audio play	Dusk has started	
Skin extract	Kl. 16.57-17.17	10.00-10.26 into trial	Slightly faster swimmin pattern. Trying to escape in a closed confinement?	Dusk has started	
Day 3: 26.01.2023					
Seawater (control)	Kl. 09.50-10.10	10.00-10.00 into. trial			
Food odor	Kl. 10.11-10.31	10.00-10.20 into trial	2/10X. More active swimming. Swimming across the middle of the tank.		
Food odor	Kl. 13.20-13.40	11.02-11.23 into trial	2/10X.		
Seawater (control)	Kl.15.40-16.00	10.00-10.10 into trial			
Skin extract	Kl. 16.01-16.21	10.00-10.20 into trial	Difference in locomotion. Fast swimming.	Feeding one piece of mackrell	
17.00					
Day 4: 27.01.2023					
EM 0.6 ms duration	Kl. 13.02-13.22	10.00-11.00 into trial	Change in behavior. Touched the positive electrode. Second time passing the electrodes, it avoided the electrodes. at 13.50 it swam close to electrodes again. 10.37 a strong physical reaction		
EM 0.6 ms duration	Kl. 13.23-13.43	10.00-11.00 into trial	Swimming faster. Touch negative electrode		
EM 0.6 ms duration	Kl. 13.44-13.04	10.00-11.00 into trial	Swimming faster. Touch the positive electrode with tail and immediately swims away. Avoiding the electric field. Seems comfortable by the electrodes when the power is turned off.		
EM 0.6 ms duration	Kl. 14.05-14.25	10.00-11.00 into trial	Seems like it is attracted to the electric field when on the opposite side of the tank.		
EM 0.6 ms duration	Kl. 14.26-14.46	10.01-11.01 into trial	05.47, Pradeep was inside and the shark escapes		
EM 0.1 ms duration	Kl. 14.49-15.09	10.00-11.00 into trial	Increasing pulse from 600ms -> 100ms. More immediate response, swimming fast. Definately a stronger reaction compared to 600ms.		
EM 0.1 ms duration	Kl. 15.10-15.30	10.00-11.00 into trial	Swimming faster, immediater response when getting close to the positive electrode		
EM 0.1 ms duration	Kl. 15.31-15.11	10.00-11.00 into trial	Swimming faster, a powerful avoidance behavior when touching the positive electrode		
EM 0.1 ms duration	Kl.16.17-16.37	10.00-11.00 into trial			

APPENDIX 1B – Behavioral observations of Shark 2

Stimuli	Time of applance	Time of stimuli applance	Comments
Day 1: 31.01.23	One hour of habituation to speaker		
Seawater (control)	Kl. 09.12-09.22	10.00-10.10 into trial	Uses more of the tank compared to the first shark
Control sound	Kl. 09.33-09.53	10.00-11.00 into trial	Turn on speaker: 09.00 into trial. Swims around speaker
Test sound	Kl. 09.53-10.13	10.00-11.00 into trial	Turn on speaker: 09.00 into trial. Stays in one place on the right to the tank window
Control sound	Kl. 10.14-10.34	10.01-11.01 into trial	Turn on speaker: 09.16 into trial. Does not swim close to speaker
Test sound	Kl. 10.34-10.54	10.01-11.01 into trial	Turn on speaker: 09.18 into trial. Does not swim close to speaker, stays in one place the entire time. on the right side of the tank window. Goes into "rest"
Seawater (control)	Kl. 10.54-11.14	10.00-10.10 into trial	"Resting" half way into trial
Food odor 2/10X	Kl. 11.15-11.35	10.06-10.30 into trial	
Break (2 hours)	-	-	
Control sound	Kl. 13.35- 13.55	10.00-11.00 into trial	No response. Opened window at 14.00
Seawater (control)	Kl. 13.55-14.15	10.00-10.10 into trial	No response.
Test sound	Kl. 14.15-14.35	10.00-11.00 into trial	Turn on speaker: 09.00 into trial.
Test sound	Kl. 14.36-14.55	10.00-11.00 into trial	Turn on speaker:09.18 into trial
Control sound	Kl. 14.56-14.16	10.00-11.00 into trial	Turn on speaker: 09.00 into trial. No response. "Rest" through whole trial.
Skin extract	Kl. 15.17-15.37	10.00-10.21 into trial	"resting" until skin extract is applied
			Seawater afterwards makes bubbely sound and could elicit the response, but it did not elicit a response in the food odor or control trial.
Day 2: 01.02.23	One hour of habituation to speaker		
Seawater (control)	Kl. 09.31-09.51	10.07-10.17 into trial	
Control sound	Kl. 09.52-10.12	10.00-11.00 into trial	Turn on speaker at 09.00 into trial. No appearant response
Test sound	Kl. 10.12-10.32	11.50-12.50 into trial	Turn on speaker at 11.45 into trial. Using the normal orca track
Control sound	Kl. 10.33-10.53	10.46-11.46 into trial	Turn on speaker at 10.30. "Resting"
Test sound	Kl. 10.53-11.13	10.00-11.00	Turn on speaker at 09.00 into trial. No response. "resting the entire trial"
Seawater (control)	Kl. 11.14-11.34	10.01-10.11	No reaction. Stops resting, starts resting
Food odor 2/10X	Kl. 11.35-11.55	10.00-10.30	Comes close to odor outlet, increased swimming speed. Startles to swim along bottom. No very obvious change in behavior.
Break (2 hours)	-	-	
Control sound	Kl. 13.51-14.11	10.00-11.00 into trial	Turn on speaker at 09.00 into trial. New sound control used
Seawater (control)	Kl. 14.11-14.31	10.00-10.10 into trial	
Test sound	Kl. 14.31-14.51	10.00-11.49 into trial	Turn on speaker at 09.00 into trial. New orca sound used. No response
Test sound	Kl. 14.51-15.11	10.02-11.02 into trial	Turn on speaker at 09.00 into trial. New orca sound used. Response; stopped "resting and swimming around the tank.
Control sound	Kl. 15.12-15.32	10.00-11.00 into trial	No response.
Skin extract	Kl. 15.33-15.53	10.00-10.30 into trial	Increased swimming speed
Day 2: 02.02.23	One hour habituation to electrodes		
EM, 0.6 ms duration	Kl. 11.19-11.39	10.00-11.00 into trial	From a homing behavior right to the window, to a swimming behavior. Passes the electrodes after the trial is finished.
EM, 0.6 ms duration	Kl. 11.39-11.59	10.00-11.00 into trial	Strong swimming response. Swims fast around the tank. Tail beat frequency seems to increase. Seems disoriented, trying to escape.
EM, 0.6 ms duration	Kl. 12.00-12.20	10.00-11.00 into trial	Abrutps homing behavior. Swims close to negative electrode. Increase swimming pattern but swims close to the electrodes. Definite change in behavior but does not as aggetated as last trial. Returning to homing behavior aft
EM, 0.3 ms duration	Kl. 12.21-12.41	10.00-11.00 into trial	Avoids the electrodes. Trouch the negative electrode and quickly escape.
Break			
EM, 0.3 ms duration	Kl. 13.24-13.44	10.00-11.00 into trial	5 seconds after signal is turned on, startes swimming around the tank avoiding the part with the electrodes. Does not swim near any of them.
EM, 0.3 ms duration	Kl. 13.45-14.05	10.00-11.00 into trial	Induce circling behavior. Circling the tank.
EM, 0.1 ms duration	Kl. 14.05-14.25	10.00-11.00 into trial	Reaction after 7 seconds. Starts swimming more actively after stimuli. Goes into "rest" after 1-2 minutes after stimuli is finished. Is the "rest" a response of fear? a freezer?
EM, 0.1 ms duration	Kl. 14.26-14.46	10.00-11.00 into trial	"Resting", too tired to respond, not moving.
Food odor 2/10X	Kl. 14.48-15.08	10.00-10.20 into trial	"Resting". Starts to swim after food odor is added.
Day 4: 03.02.23	One hour habituation to electrodes		
Seawater (control)	Kl. 08.52-09.12	10.11-01.21 into trial	
Food odor 2/10X	Kl. 09.13-09.33	10.00-10.30 into trial	
Seawater (control)	Kl. 11.31-11.51	10.00-10.12 intor trial	from 17.00 Pradeep enter the room to attach electrodes. Swimming fast
EM, 0.1 ms duration	Kl. 11.52-12.12	10.00-11.00	Resting much of the trial. Starts swimming after stimuli is finished. Resting again shortly after trial.
Skin extract	Kl. 13.07-13.27	10.00-10.27	Resting at bottom. Reacts ca. 1 minute after applying odor. Aggitated swimming and swimming around the whole tank. Wants to escape it seems. Moments of freezing then continue to swim.

Water level:	65 cm
From tank #:	4
Days since capture:	61
Day since last feed opportunity:	2 days before first trial started
Sex:	Male
Tissue from state:	Odor- skin extract
Length:	64,5 cm
Weight:	870,9 g
Blood sample:	Yes
Gill sample:	Yes, 2nd gill arch
Brain sample:	Yes
Both spines (age mapping):	Yes
Skin extract:	Yes
Comments:	First shark I captured.
Photo:	

APPENDIX 1C – Behavioral observations of Shark 3

Stimuli	Time of appliance	Time of stimuli appliance	Comments
Day 1: 07.02.23	One hour of habituation to speaker		
Seawater (control)	Kl. 09.32-09.52	10.00-10.13 into trial	Normal swimming
Control sound 2	Kl. 09.52-10.12	10.02-11.02 into trial	Turn on speaker 09.00 into trial
Test sound, sound 2	Kl. 10.12-10.32	10.00-11.00 into trial	Turn on speaker 09.00 into trial. No response
Control sound 2	Kl. 10.33-10.53	10.34-11.34 into trial	
Test sound, sound 2	Kl. 10.53-11.13	10.01-11.01 into trial	No response
Seawater (control)	Kl. 11.14-11.34	10.00-10.10 into trial	
Skin extract	Kl. 11.34-11.54	10.00-10.21 into trial	Increased swimming speed
Break (2 hours)	-	-	
Control sound 2	13.50-14.10	10.00-11.00 into trial	No response
Test sound, sound 2	14.11-14.31	10.03-11.03 into trial	Turn on speaker 09.00 into trial. Turned away twice from speaker
Test sound, sound 2	14.31-14.51	10.00-11.00 into trial	Turn on speaker 09.00 into trial. Did not care.
Seawater (control)	14.52-15.12	10.00-10.10 into trial	Normal swimming
Control sound 2	15.12-15.32	10.00-11.00 into trial	Avoid speaker, circle the light. Moves close to speaker after sound is finished.
Food odor 2/10X	15.42-16.02	10.00-10.21 into trial	Seeks to the bottom. Starts to circle te middle of the tank and towards the light. Resume tank circeling after two minutes.
			Looping behavior towards the end of last recording.
Day 2: 08.02.23	One hour of habituation to speaker		
Seawater (control)	Kl. 09.31-09.51	10.00-10.10 into trial	
Control sound 2	Kl. 09.52-10.12	10.00-11.00 into trial	Turn on speaker at 09.00.
Test sound, sound 2	Kl. 10.12-10.32	10.02-11.02 into trial	Keeps away from speaker. One turn away. Passes it once.
Control sound 2	Kl. 10.33-10.53	10.00-11.00 into trial	Does not seem to mind
Test sound, sound 2	Kl. 10.53-11.13	10.00-11.00 into trial	Did not care about sound. Pass speaker several times
Seawater (control)	Kl. 11.13-11.33	10.00-10.10 into trial	Increased swimming speed. It could see me through the curtains
Food odor 2/10X	Kl. 11.34-11.54	10.00-10.21 into trial	Seems a bit more aggitated
Break (2 hours)	-	-	
Cont rol sound 2	Kl. 13.42-14.02	10.00-11.00 into trial	
Seawater (control)	Kl. 14.03-14.23	10.00-10.10 into trial	Change in swimming pattern. More aggetraded swimming and using the middle of the tank.
Test sound, sound 2	Kl. 14.23-14.43	10.00-11.00 into trial	No clear response
Test sound, sound 2	Kl. 14.44-15.04	10.51-11.51 into trial	No clear response
Control sound 2	Kl. 15.04-15.24	10.00-11.00	No response
Skin extract	Kl. 15.25-15.55	10.00-10.15	No clear response. Avoid the area of there odor is emitted and circles the other side of the tank.
Day 3: 09.02.23	One hour habituation to electrodes		
Seawater control	Kl. 09.49-10.09	10.00-10.10 into trial	
EM 0.6 ms duration	Kl. 11.17-11.37	10.00-11.00 into trial	Does not seem to care. Swim close to the electrodes.
EM 0.6 ms duration	Kl. 11.38-11.58	10.00-11.07 into trial	Swims further around than last trial now.
EM 0.6 ms duration	Kl. 11.58-12.18	10.00-11.00 into trial	Aversive response once, passes the electrodes quite frequently.
EM 0.3 ms duration	Kl. 12.19-12.39	10.00-11.00 into trial	Does not seem to care.
Break			
EM 0.3 ms duration	Kl. 13.24-13.44	10.00-11.00 into trial	Does not seem to care. Swims around the electromagnetic field.
EM 0.3 ms duration	Kl. 13.45-14.05	10.00-11.00 into trial	Did not seem to be affected.
EM 0.1 ms duration	Kl. 14.05-14.25	10.00-11.00 into trial	Avoiding the electromagnetic side og the tank, but does not care very much.
EM 0.1 ms duration	Kl. 14.26-14.46	10.00-11.00 into trial	No response.
EM 0.1 ms duration	Kl. 14.46-15.06	10.00-11.00 into trial	Weak response
Food odor 3/10X	Kl. 15.07-15.27	10.00-10.10 into trial	Swims a bit faster
Day 4: 10.02.23			
Seawater (control)	Kl. 08.38-08.58	10.00-10.10 into trial	Swimming faster within one minute of seawater
Skin extract	Kl. 08.58-09.18	10.00-10.20 into trial	Swimming more aggitaded, but no clear aversive response.
Food odor 3/10X	Kl. 11.09-11.29	10.00-10.20 into trial	

Water level:	
From tank #	1
Days since capture:	82 cm
Day since last feed opportunity:	68
Sex:	3 days before first trial started
Tissue from state:	Female
Length:	Control
Weight:	63
Blood sample:	755.8 g
Gill sample:	Yes
Brain sample:	Yes
Both spines (age mapping):	Yes
Skin extract:	Yes
Comments:	
Photo:	

APPENDIX 1D – Behavioral observations of Shark 4

Stimuli	Time of appliance	Time of stimuli appliance	Comments
Day 1 11.02.23	One hour of habituation to speaker		
Seawater (control)	Kl. 11.42-12.02	10.00-10.10 into trial	
Controll sound 2	Kl. 12.02-12.22	10.02-11.02 into trial	Turn on speaker at 09.00
Test sound, sound 2	Kl. 12.23-12.43	10.06-11.06 into trial	No response
Control sound 2	Kl. 12.43-13.03	10.00-11.00 into trial	
Test sound, sound 2	Kl. 13.04-13.24	10.01-11.01 into trial	No response
Seawater (control)	Kl. 13.24-13.44	10.00-10.10 into trial	
Food odor 3/10X	Kl. 13.45-14.05	10.00-10.20 into trial	
Break (2 hours)			
Control sound 2	Kl. 15.51-16.11	10.00-11.00 into trial	Normal swimming
Test sound, sound 2	Kl. 16.11-16.31	10.00-11.00 into trial	No apparent response
Test sound, sound 2	Kl. 16.32-16.52	10.00-11.00 into trial	No apparent response
Seawater (control)	Kl. 16.52-17.12	10.00-10.0 into trial	
Control sound 2	Kl. 17.13-17.33	10.00-11.00 into trial	
Skin extract	Kl. 17.33-17.53	10.00-10.20 into trial	Increased swimmin speed
Day 2: 12.02.23	One hour of habituation to speaker		
Seawater (control)	Kl. 12.10-12.30	10.00-10.10 into trial	Pipe moved, might induce some stress. Aggetated swimming a couple of minutes after stimuli.
Control sound 2	Kl 12.30-12.50	10.00-11.00 into trial	
Test sound, sound 2	Kl. 12.50-13.10	10.00-11.00 into trial	No apparent response
Control sound 2	Kl. 13.11-13.31	10.00-11.00 into trial	
Test sound, sound 2	Kl. 13.32-13.52	10.00-11.00 into trial	No apparent response
Seawater (control)	Kl. 13.52-14.12	10.00-10.10 into trial	
Food odor 3/10X	Kl. 14.13-14.33	10.00-10.20 into trial	Swimming up to the odor outlet.
Break (2 hours)			
Control sound 2	Kl. 16.21-16.41	10.00-11.00 into trial	
Seawater (control)	Kl. 16.41-17.01	10.00-10.10 into trial	
Test sound, sound 2	Kl. 17.03-17.23	10.00-11.00 into trial	No response
Test sound, sound 2	Kl. 17.23-17.43	10.00-11.00 into trial	No response
Control sound 2	Kl. 17.44-18.04	10.04-11.04 into trial	
Skin extract	Kl. 18.05-18.25	10.00-10.20 into trial	A bit more aggitated swimming
Day 4: 13.02.23			
Seawater (control)	Kl. 13.32-13.52	10.00-10.10 into trial	
Food odor 3/10X	Kl. 13.52-14.12	10.00-10.20 into trial	No clear response
Skin extract	Kl. 15.55-16.15	10.00-10.15 into trial	Circle towards the odor outlet, swimming calmly. Avoids the outlet after one minute and swimming towards the bottom, sideways.
Field work			
Day 6: 15.02.23	One hour habituation to electrodes		
Seawater control	Kl. 08.48-09.08	10.00-10.10 into trial	Increased swimming speed
Food odor 3/10X	Kl. 09.09-09.29	10.00-10.15 into trial	No apparent response
EM 0.6 ms duration	Kl. 11.13-11.33	10.00-11.00 into trial	Increased swimming speed, avoid the side of the tank with electrodes.
EM 0.6 ms duration	Kl. 11.50-12.10	10.00-11.00 into trial	Increased swimming speed. Did not quit the program after trial, but power and emergency switch was of. Hence the continuous blinking.
EM 0.6 ms duration	Kl. 13.04-13.24	10.02-11.00 into trial	Swimming calmer this time.
EM 0.3 ms duration	Kl. 13.24-13.44	10.00-11.00 into trial	Some response, but still calm. Signs of habituation?
EM 0.3 ms duration	Kl. 13.45-14.05	10.00-11.02 into trial	Some response, sudden turning.
EM 0.3 ms duration	Kl. 14.06-14.26	10.00-11.00 into trial	Aggetated swimming
EM 0.1 ms duration	Kl. 14.26-14.46	10.00-11.00 into trial	Headshaking when close to electrode. Definately thinks its unfomfortable being in close proximity.
EM 0.1 ms duration	Kl. 14.49-15.09	10.00-11.00 into trial	Swimming a bit faster.
EM 0.1 ms duration	Kl. 15.10-15.30	10.00-11.00 into trial	No apparent response.

Water level:	82 cm
From tank #	3
Days since capture:	72
Day since last feed opportunity:	3 days before first trial started
Sex:	Male
Tissue from state:	Control
Length:	79 cm
Weight:	1607 g
Blood sample:	
Gill sample:	
Brain sample:	
Both spines (age mapping):	
Skin extract:	
Comments:	Developed a weird swimming pattern including looping, wiggeling and vertical swimming.
Photo:	

APPENDIX 1E – Behavioral observations of Shark 5

Stimuli	Time of appliance	Time of stimuli appliance	Comments			
Day 4: 21.02.23				Water level:		
Seawater (control)	Kl. 09:27-19:47	10.00-10.10 into trial		From tank #:	82 cm	
Controll sound	Kl. 10:04-10:14	10.02-11.02 into trial				3
Test sound	Kl. 10:24-10:44	10.00-11.00 into trial		Days since capture (when transfered to		81
Control sound	Kl. 10:45-11:05	10.00-11.00 into trial		Days since last feed opportunity:	2 days before first trial started	
Test sound	Kl. 11:05-11:25	10.02-11.02 into trial		Sex:	Male	
Seawater (control)	Kl. 11:26-11:46	10.00-10.10 into trial		Tissue from state:	Skin extract	
Food odor	Kl. 11:46-12:06	10.00-10.20 into trial	Swims more towards the bottom	Length:	79,5 cm	
Break (2 hours)				Weight:	1725 g	
Control sound	Kl. 13:47-14:07	10.00-11.00 into trial	No respse	Blood sample:	Yes	
Seawater (control)	Kl. 14:08-14:28	10.00-10.10 into trial		Gill sample:	Yes	
Test sound	Kl. 14:28-14:48	10.00-11.00 into trial	Turns away from the speaker at one point during trial	Brain sample:	Yes	
Control sound	Kl. 14:49-15:09	10.02-11.02 into trial	No apparent reaction	Bath spines (age mapping):	Yes	
Test sound	Kl. 15:09-15:29	10.00-11.00 into trial		Skin extract:	Yes	
Skin extract	Kl. 15:29-15:49	10.02-10.18 into trial	Change in swimmin pattern. Turning a lot, changing directions. Vertical swimming	Comments:	A nice swimming pattern	
				Photo:		
Day 2: 22.02.23						
EM 0.6 ms	Kl. 09:58-10:18	10.00-11.00 into trial	Showing change in behavior after stimuli is added. Swims against bottom of the tank and turn before entering electromagnetic field.			
EM 0.6 ms	Kl. 10:18-10:38	10.00-11.00 into trial	Change in behavior. Swim min towards bottom, quick head turning when close to the electrodes.			
EM 0.6 ms	Kl. 10:40-11:00	10.00-11.00 into trial	Immediate head turning away from the EM, change in swimming behavior. Swims more slowly, short moments of a "freezing" kind of behavior.			
EM 0.3 ms	Kl. 11:00-11:20	11.05-12.05 into trial	Change in behavior, sharp turns away from EM and a slower swimming pattern. Takes some time between the typical "crunches". Normal swimming when stimuli is stopped			
EM 0.3 ms	Kl. 11:21-11:41	10.00-11.00 into trial	Immediate change in behavior when EM is turned on.			
EM 0.3 ms	Kl. 11:41-12:02	10.00-11.00 into trial	Immediate change in behavior. Combination of sharp turns and "searching" behavior towards the bottom of the tank. Return to normal swimming pattern immediately after stimuli is gone.			
Food odor	Kl. 12:02-12:22	10.00-10.20 into trial				
Break (2 hours)						
EM 0.1 ms	Kl. 13:44-14:04	10.00-11.00 into trial	Change in behavior, but does not escape from the electrodes - rather a short immediate head turning.			
EM 0.1 ms	Kl. 14:05-14:25	10.03-11.03 into trial	Seems like he starts to search the bottom of the tank - foraging behavior? Starts to circle the tank immediately after signal is turned off.			
EM 0.1 ms	Kl. 14:25-14:45	10.00-11.00 into trial				
Seawater (control)	Kl. 14:46-15:06	10.13-10.28 into trial				
Skin extract	Kl. 15:06-15:26	10.00-10.15 into trial	He did a flip! Change in behavior, back to normal a couple of minutes after odor is added.			
Day 3: 23.02.23						
Seawater (control)	Kl. 09:47-10:07	13.20-13.30 into trial				
Control sound	Kl. 10:08-10:28	10.00-11.00 into trial				
Test sound	Kl. 10:29-10:49	10.00-11.00 into trial				
Control sound	Kl. 10:49-11:09	10.00-11.00 into trial				
Test sound	Kl. 11:10-11:30	10.00-11.00 into trial				
seawater (control)	Kl. 11:30-11:50	10.00-10.10 into trial				
Food odor 3x10	Kl. 11:50-12:10	10.00-10.20 into trial	Searching behavior towards bottom of the tank (this is where the food usually lies in the housing tanks)			
Break (2 hours)						
Control sound	Kl. 13:42-13:02	10.00-11.00 into trial				
Seawater (control)	Kl. 13:03-14:23	10.00-10.10 into trial				
Test sound	Kl. 14:23-14:43	10.02-11.02 into trial	Did some sharp turns			
Test sound	Kl. 14:43-15:03	10.00-11.00 into trial	No apparent change in swimming pattern			
Control sound	Kl. 15:04-15:24	10.00-11.00 into trial				
Skin extract	Kl. 15:25 -15:45	10.00-10.15 into trial	Disoriented, lose the navigational ability. Starts "hovering" and change the swimming pattern. Definately an aversive/fear response.			
Day 4: 24.02.23						
Food odor 3x10	Kl. 09:30-09:50	10.00-10.21 into trial				
Seawater (control)	Kl. 11:44-12:04	10.00-11.00 into trial				
Skin extract	Kl. 12:44-13:02	10.00-10.20 into trial	A short freezing and turning away from odor. A bit disoriented. Not as strong response as yesterday, but definately change in swimming pattern. Crash into pipe.			
Euthanization						

APPENDIX 1F – Behavioral observations of Shark 6

Stimuli	Time of appliance	Time of stimuli appliance	Comments				
Day 1: 27.02.2023	One hour of habituation to electrode						
EM 0.3 ms duration, 5V	Kl. 10.45-11.05	10.00-11.00 into trial	Head twist at onset of stimuli. Change in behavior			Water level:	82 cm
EM 0.3 ms duration, 5V	Kl. 11.06-11.26	10.00-11.00 into trial	Not as much change in behavior as last trial.	No EM field, clip fell off	Deleted	From tank #	1
EM 0.3 ms duration, 5V	Kl. 11.26-11.46	10.00-11.00 into trial	Instant reaction, head turning away from EM. Hovering a lot on the oppo	No EM field, clip fell off	Deleted	Days since capture:	14
EM 0.3 ms duration, 10 V	Kl. 11.46-12.06	10.00-11.00 into trial	Hovering a lot	No EM field, clip fell off	Deleted	Day since last feed opportunity:	2 days before first trial started
Lunch				No EM field, clip fell off	Deleted		
EM 0.3 ms duration, 10 V	Kl. 12.53-13.13	10.00-11.00 into trial	Does not seem very bothered by the EM.	No EM field, clip fell off	Deleted	Sex:	Male, not mature
EM 0.3 ms duration, 10 V	Kl. 13.14-13.34	10.00-11.00 into trial		No EM field, clip fell off	Deleted	Tissue from state:	Food odor
EM 0.3 ms duration, 20 V	Kl. 13.34-13.54	10.00-11.00 into trial	Does not seem to care.	No EM field, clip fell off	Deleted	Length:	64 cm
EM 0.3 ms duration, 20 V	Kl. 14.13-14.33	11.20-12.20 into trial	Spends the most time at the other side of the tank.	No EM field, clip fell off	Deleted	Weight:	923 g
EM 0.3 ms duration, 20 V	Kl. 15.07-15.27			No EM field, clip fell off	Deleted	Blood sample:	Yes
EM 0.3 ms duration, 10V	Kl. 15.28-15.48	10.00-11.00 into trial	Change in swimming pattern			Gill sample:	Yes
EM 0.3 ms duration, 20V	Kl. 15.49-16.09	Stopped trial	Stopped trial due to "resting"			Brain sample:	Yes
						Both spines (age mapping):	Yes
Day 2: 28.02.2023	One hour of habituation to electrode					Skin extract:	Yes
EM 0.3 ms interval, 20V	Kl. 10.24-10.44	10.00-11.00 into trial	Instant reaction when stimuli is turned on. Change in swimming pattern			Comments:	Lots of food in the stomack. Last fed 5
EM 0.3 ms interval, 5V	Kl. 10.48-11.08	10.00-11.00 into trial	Change in swimming pattern			Photo:	days prior. Two sea lice on dorsal posteior
EM 0.3 ms interval, 10V	Kl. 11.08-11.28	10.00-11.00 into trial	Change in swimming pattern. Head twitch when stimuli is turned on				
EM 0.3 ms interval, 20V	Kl. 11.29-11.49	10.00-11.00 into trial	Change in swimming pattern. Avoidance from electric field.				
EM 0.3 ms interval, 5V	Kl. 11.50-12.10	12.07-13.07 into trial	Some change in swimming pattern. But swims closer to the electrodes.				
EM 0.3 ms interval, 10V	Kl. 12.13-12.33	10.00-11.00 into trial	Quick head shake when stimuli is turned on. Swims close to the electodes. Change in swimming pattern. Crossing the tank towards the floor.				
EM 0.3 ms interval, 20V	Kl. 12.33-12.53	10.30-11.30 into trial	Startle response, it was by the electrode when stimuli was started. Swimming across the tank towards the bottom.				
Food odor	Kl. 13.37-13.57	04.00-04.20 into trial					

APPENDIX 1G – Behavioral observations of Shark 7

Day 1: 02.03.23			
Seawater (control)	Kl. 09.27-09.47	10.00-10.10 into trial	Swimming very fast
Food odor 3/10 X	Kl. 09.48-10.08	10.00-10.20 into trial	Swims across the tank, change in speed. Swimming more slowly and across the floor.
Break (2 hours)			
Seawater (control)	Kl. 11.43-12.03	10.00-10.10 into trial	Change in swimming pattern. Strange - seems aggetated when seawater is added
Skin extract	Kl. 12.03-12.23	10.00-10.15 into trial	Change in swimming pattern, aggetated swimming and crossing the bottom floor. Shark turns. Semmes distressed.
Break (2 hours)			
Seawater (control)	Kl 13.59-14.19	10.10 into trial	Clearly detect addition of the seawater. Maybe some smells from the applicant (me) from the water?
Food odor 3/10 X	Kl. 14.20-14.40	10.00-10.20 into trial	Swimming more towards the bottom
Day 2: 02.03.23			
Seawater (control)	Kl. 08.42-09.02	10.00-10.10 into trial	Change in swimming pattern
Skin extract	Kl. 09.14-34	10.00-10.17 into trial	Change in swimming pattern, disoriented, crashing into pipes, crossing tank.
Break (2 hours)			
Seawater (control)	Kl. 11.04-11.24	10.00-10.10 into trial	Change in swimming speed
Food odor 3/10 X	Kl. 11.24-11.44	10.00-10.20 into trial	Some change in swimming pattern/speed
Break (2 hours)			
Seawater (control)	Kl. 13.27-13.47	10.00-10.10 into trial	Increased swimming speed
Skin extract	Kl. 13.48-14.08	10.00-10.15 into trial	Twisting/twitching, increased swimming speed
Day 3: 06.03.23			
EM 0.6 ms	Kl. 11.50-12.10	10.00-11.00 into trial	Swims fast, change in swimming pattern. A sensitive shark compared to earlier.
EM 0.6 ms	Kl. 12.10-12.30	10.02-11.02 into trial	Change in swimming pattern. Avoiding negative electrode, quick turns when near the electrode.
EM 0.6 ms	Kl. 13.00-13.20	10.00-11.00 into trial	Change in swimming pattern, increased swimming speed.
EM 0.3 ms	Kl. 13.21-13.41	10.00-11.00 into trial	Swims in smaller circles and some looping. Increased swimming pattern.
EM 0.3 ms	Kl. 13.41-14.01	10.00-11.00 into trial	Loss of orientation to some extent (crashing into pipe). Increased swimming speed and head turning in proximity to electrodes.
EM 0.3 ms	Kl. 14.02-14.22	10.00-11.00 into trial	Swims close to electrodes and then avoids it. Seems to habituate to the stimuli?
EM 0.1 ms	Kl. 14.22-14.42	10.00-11.00 into trial	Increased swimming speed
EM 0.1 ms	Kl. 14.42-15.02	10.00-11.00 into trial	Avoiding the field at once, swimming closer before avoiding again. Swimming in smaller circles. Some twitching.
EM 0.1 ms	Kl. 15.03-15.23	10.00-11.00 into trial	By the electrodes when stimuli is given, quick head shake.
Day 4: 07.03.23			
Control sound	Kl. 09.36-09.56	10.00-11.00 into trial	Turns immediately away when turned on, but then it does not care for the rest of the trial
Orca sound new	Kl. 09.56-10.16	10.30-11.30 into trial	No reaction
Control sound	Kl. 10.16-10.36	10.00-11.00 into trial	
Orca sound new	Kl. 10.37-10.57	10.00-11.00 into trial	No reaction
Control sound	Kl. 10.57-11.17	10.02-11.02 into trial	
Orca sound new	Kl. 11.17-11.37	10.03-11.03 into trial	
Control sound	Kl. 11.37-11.57	10.00-11.00 into trial	
Orca sound new	Kl. 11.57-12.17	10.00-11.00 into trial	
Control sound	Kl. 12.18-12.38	10.00-11.00 into trial	Took a turn but pass the speaker normally afterwards. Swims calmly. Does some looping long after the stimuli is gone - nice reference for discussion?
Orca sound new	Kl. 12.38-12.58	10.02-11.02 into trial	
Control sound	Kl. 12.58-12.18	10.01-11.01 into trial	
Orca sound new	Kl. 13.19-13.39	10.00-11.00 into trial	Did it get scared when turned on? Avoids speaker area a bit.
Seawater control	Kl. 13.39-13.59	10.00-10.07 into trial	Swimming faster
Seawater control	Kl. 15.59-14.19	10.00-10.10 into trial	Did not react very much
Food odor 3/10X	Kl. 14.20-14.40	10.00-10.15 into trial	Some swimming across the bottom of the tank

Water level:	82 cm
From tank #	4
Days since capture (when transfered to ex. tank):	16
Day since last feed opportunity:	2 days before first trial started
Sex:	Male
Tissue from state:	Food odor
Length:	65 cm
Weight:	922,5 g
Blood sample:	
Gill sample:	
Brain sample:	
Both spines (age mapping):	
Skin extract:	
Comments:	Seemed distressed by the
Photo:	speaker, only odor and EM trials.

APPENDIX 1H – Behavioral observations of Shark 8

Day 1: 09.03.23			
EM 0.3 ms interval, 5V	Kl. 10:39-10.59	10.00-11.00 into trial (10.01)	Swimmin faster immediately after stimuli is added. Swims across the tank and does some sharp turns.
EM 0.3 ms interval, 10V	Kl. 10.59-11.19	10.00-11.00 into trial	Starts crossing the bottom, does not swim close to the electrodes. Still calm speed. Swims closer to the positive electrode than the negative.
EM 0.3 ms interval, 20V	Kl. 11.20-11.40	10.00-11.00 into trial	Increased wimming speed, starts to cross the floor dwelling towards the bottom. Change direction before enetering the EM field several times, does not swim close to the electrodes.
EM 0.3 ms duration, 5V	Kl. 11.40-12.00	10.00-11.00 into trial	Immediate reaction. Swimmin towards the surface, turning direction of swimming. Some "hovering" on opposite side. Not near negative electrode. Turn away after swimming close.
EM 0.3 ms duration, 10 V	Kl. 12.05-12. 25	11.12-12.12 into trial	Turns away from negative electrode. Avoiding the EM field. Turns away and swims to the other side of the tank.
Lunch			
EM 0.3 ms duration, 20 V	Kl.13.02-13.22	10.00-11.00 into trial	Swimming across the bottom, avoiding the EM field, especially the negative electrode. Swimmin speed is normal, but does n
EM 0.3 ms duration, 5 V	Kl. 13.22-13.42	10.00-11.00 into trial	Avoiding the EM field. Same swimming speed.
Day 3: 10.03.23			
EM 0.3 ms duration, 20V			
EM 0.3 ms duration, 10V	13.56-	10.14-11.14 into trial	

Water level:	
From tank #	82 cm
	4
Days since capture (when transfered to ex. tank):	
Day since last feed opportunity:	2 days before first trial started
Sex:	Male, not mature
Tissue from state:	EM
Length:	61 cm
Weight:	778 g
Blood sample:	Yes
Gill sample:	Yes
Brain sample:	Yes
Both spines (age mapping):	Yes
Skin extract:	Yes
Comments:	
Photo:	

APPENDIX 1I – Behavioral observations of Shark 9

Day 1: 29.03.23			
Seawater (control)	Kl. 09.23-09.43	09.50-10.00 into trial	Swimming more towards the bottom of the tank. Swimming into the pipe - reason for increased damage on snout?
Food odor 3/10 X	Kl. 09.43-10.03	10.00-10.22 into trial	No apparent change in locomotion, check again when stimuli is given on recording.
Break (2 hours)			
Skin extract 0.5 U, 25 mL	Kl. 11.50-12.10	10.00-10.15 into trial	A massive change in locomotion 30 seconds after stimuli is added. Swimming very fast, almost jumping out of the water. Initiated by a sharp turn towards the outlet towards the bottom followed by almost uncontrollable swimming.
Break (2 hours)			
Skin extract 1 U, 50 mL	Kl. 13.50-14.10	10.00-10.20 into trial	Swimming faster but a very much calmer response compared to last trial 2 food
Break (2 hours)			
Skin extract 2 U, 100 mL	Kl. 15.50-16.10	10.00-10.20 into trial	Some change in locomotion but no major reaction
			3 conc. of skin
			2 seawater
Day 2: 30.03.23			
Seawater (control)	Kl. 09.46-10.06	10.00-10.10 into trial	Swimming towards the middle of the tank, bottom
Food odor 3/10 X	Kl. 10.08-10.28	10.00-10.20 into trial	No obvious change in behavior
Break (2 hours)			
Skin extract 0.5 U, 25 mL	Kl. 12.02-12.22	10.00-10.20 into trial	Change of locomotion - swimming towards the bottom and initially staying away from odor outlet
Break (2 hours)			
Skin extract 1 U 50 mL	Kl. 14.03-14.23	10.00-10.20 into trial	Change in locomotion. Swimming faster, increased number of turns and crossing the tank.
Break (2 hours)			
Skin extract 2 U 100 mL	Kl. 15.58-16.18	10.00-10.20 into trial	Low change in locomotion.
Day 3: 31.03.23			
Skin extract 0.5 U, 25 mL	Kl. 13.04-12.22	10.00-10.20 into trial	Change in locomotion; faster pace,some sideways swimming.
Euthanized at ____			

Water level:	82 cm
From tank #:	1
Days since capture (when transfered to ex. tank):	44
Days since last feed opportunity:	5 days before trial start
Sex:	Male, not mature
Tissue from state:	Skin extract
Length:	64 cm
Weight:	873 g
Blood sample:	Yes - white serum
Gill sample:	Yes
Brain sample:	Yes
Both spines (age mapping):	Yes
Skin extract:	Yes
Comments:	Damage on snout, had this since catching but has gotten red since
Photo:	

APPENDIX 1J – Behavioral observations of Shark 10

Stimuli	Time of appliance	Time of stimuli appliance	Comments
Day 1: 03.04.2023	One hour of habituation to electrode		
EM 0.3 ms duration, 5V	Kl. 10.51-11.11	10.00-11.00 into trial	Change in locomotion, but pass the electrode fine the second time.
EM 0.3 ms duration, 10V	Kl. 11.11-11.31	10.00-11.00 into trial	Change in locomotion. Pass the electrode calmlu but not
EM 0.3 ms duration, 20V	Kl. 11.32-11.52	10.00-11.00 into trial	No response. Swimming very strange the entire time - hovering a lot.
Lunch			
EM 0.3 ms duration, 5 V	Kl. 13.11-13.31	10.01-11.04 into trial	No change in locomotion.
EM 0.3 ms duration, 10 V	Kl. 13.31-13.51	10.00-11.00 into trial	No change in locomotion.
Day 2: 04.04.2023			
EM 0.3 ms interval, 20V	Kl. 10.18-10.38	10.00-11.00 into trial	Definite change in locomotion. Swimming faster, does cross the electrodes but does some turning away from them.
EM 0.3 ms interval, 5V	Kl. 10.38-10.58	10.00-11.00 into trial	Change in locomotion. Swimming towards the bottom, staying away from electrodes. This time it has a more rapid swimming pace before the trial compared to other trials
EM 0.3 ms interval, 10V	Kl. 10.59-11.19	10.02-11.02 into trial	Change in locomotion.
EM 0.3 ms interval, 20V	Kl. 13.00-13.20	12.30-13.30 into trial	Change in locomotion. Swims past electrodes but avoid them some when swimming through the EM field

Water level:	82 cm
From tank #	1
Days since capture:	50
Day since last feed opp	2 days before first trial started
Sex:	Male, not mature
Tissue from state:	EM
Length:	68.5
Weight:	1054g
Blood sample:	yes
Gill sample:	yes
Brain sample:	yes
Both spines (age map)	yes
Skin extract:	yes
Comments:	Static swimming, hovering a lot and does not swim around the tank as
Photo:	

APPENDIX 1K – Behavioral observations of Shark 11

Stimuli	Time of appliance	Time of stimuli appliance	Comments
Day 2: 12.04.2023	One hour of habituation to speaker		
Seawater (control)	Kl. 09:19-09:39	10.00-10.20 into trial	Change in locomotion. Swimming towards the bottom and seems to be seeking more.
Control sound new	Kl. 09:40-10:00	10.00-11.00 into trial	Turned on the speaker at 09:03, he does a flip afterwards.
Test sound new	Kl. 10.00-10.20	10.04-11.04 into trial	Change in locomotion. Swimming across the tank shortly after audio is on and seems to avoid speaker. Swims past shortly after though.
Control sound new	Kl. 10.20-10.40	10.02-11.02 into trial	Turned o speaker at 09.00 into trial. No apparent change of locomotion.
Test sound new	Kl. 10.41-11.01	10.00-11.00 into trial	Swimming calmly. Does not seem to mind the orca sound
Food odor	Kl. 11.02-11.22	10.16-10.46 into trial	Beautiful example of foraging behavior. Searching towards the bottom, with sharp turns
Break (2 hours)			
Seawater (control)	Kl. 13.00-13.20	10.00-10.20 into trial	Swimming a bit faster. I stumbleted upon the stair, made some noise when I walked out.
Control sound new	Kl. 13.21-13.41	10.03-11.03 into trial	No apparent reaction
Seawater (control)	Kl. 13.41-14.01	10.00-10.20 into trial	Changes in locomotion
Test sound new	Kl. 14.02-14.22	10.00-11.00 into trial	Some change in locomotion but swims close to the speaker.
Test sound new	Kl. 14.22-14.42	10.01-11.01 into trial	Swimming calmly, does not seem to care.
Control sound	Kl. 14.42-15.02	10.04-11.04 into trial	
Skin extract	Kl. 15.03-15.23	10.00-10.30 into trial	No apparent reaction. Starts to swim faster almost 4 minutes after the stimuli is added?
Day 3: 13.04.2023	One hour of habituation to speaker		
Food odor	Kl. 09.35-09.55	10.00-10.45 into trial	Foraging behavior. Searching towards the bottom for several minutes after odor is added.
Break (2 hours)			
Control sound new	Kl. 12.27-12.47	10.00-11.00 into trial	Keeps swimming normally
Test sound new	Kl. 12.48-13.08	10.00-11.00 into trial	No major change of locomotion. Cuts the outline of the circle while the sound is playing.
Seawater (control)	Kl. 13.09-13.29	10.00-10.20 into trial	Change in locomotion for a short time.
Test sound new	Kl. 13.29-13.49	10.01-11.01 into trial	No change in locomotion.
Control sound new	Kl. 13.49-14.09	10.00-11.00 into trial	
Control sound new	Kl. 14.10-14.30	10.00-11.00 into trial	No change of locomotion
Seawater (control)	Kl. 14.30-14.50	10.00-10.36 into trial	Some change in behavior
Test sound new	Kl. 14.50-15.10	10.01-11.01 into trial	Swimming in the middle of the tank, increased number of turns but keeps a calm swimming speed and behavior. This swimming pattern is different from earlier recordings, but is kept for the whole trial.
Test sound new	Kl. 15.10-15.30	10.01-11.01 into trial	Continuing to swim the same as before, under and after trial. No change in locomotion.
Seawater (control)	Kl. 15.31-15.51	10.00-10.16 into trial	Does some rapid turns after stimuli is added, but low response
Control sound	Kl. 15.51-16.15	10.02-11.02 into trial	No change in locomotion.
Skin extract	Kl. 16.15-16.35	16.15-16.35 into trial	No appearant change in locomotion.
Day 3: 14.04.2023			
Food odor	Kl. 08.06-08.26	10.00-10.30 into trial	Doing a lot of turns and twists. Not the same foraging behavior as yesterday. Keeps this behavior for a couple of minutes
Seawater (control)	Kl. 10.03-10.23	11.00-11.16 into trial	No change in locomotion.
EM 0.6 ms interval	Kl. 10.27-10.47	11.00-12.00 into trial	Change in locomotion, starts to swim towards the bottom, especially close to the device. Increased number of turns.
EM 0.6 ms interval	Kl. 10.47-11.07	10.00-11.00 into trial	Touched the electrode when stimuli turned on. Startle response but crosses the electrode shortly afterwards despite stimuli.
EM 0.6 ms interval	Kl. 11.08-11.28	10.00-11.00 into trial	Change of behvior. Increased turning rate, but swims close to the electrodes.
Seawater (control)	Kl. 11.28-11.48	10.00-10.16 into trial	Some change of locomotion.
Skin extract	Kl. 11.49-12.09	10.00-10.16 into trial	
Lunch.			
EM 0.3 ms interval	Kl. 13.19-13.39	10.02-11.02 into trial	Swimming along the bottom of the tank, touches the positive electrode and swims away but seems calm.
EM 0.3 ms interval	Kl. 13.39-13.59	10.00-11.00 into trial	Strong reaction when the stimuli is turned on. Swims close to the positive electrode twice in a row.
EM 0.3 ms interval	Kl. 14.00-14.20	10.00-11.00 into trial	Strong responses near the device when stimuli is turned on. Gets startled but swims near the electrodes closely after stimuli is turned off.
EM 0.1 ms interval	Kl. 14.20-14.40	10.01-11.01 into trial	Strong responses when initiated. Stays away from the electrodes
EM 0.1 ms interval	Kl. 14.40-15.00	10.00-11.00 into trial	Immediately change direction towards the electrode. Swims towards the bottom of the tank.
EM 0.1 ms interval	Kl. 15.01-15.21	10.00-11.00 into trial	Immediate, but weaker response. Change in locomotion from swimming around the tank towards the wall, to close swimming towards the bottom.
Food odor	Kl. 15.21-15.41	10.03-11.03 into trial	

Approximate age based on spines:	
Water level:	82 cm
From tank #:	4
Days since capture:	11 days
Days since last feed opportunity:	3 days before first trial started
Sex:	Immature female
Tissue from state:	Food odor
Length:	77 cm
Weight:	1732 g
Blood sample:	Yes
Gill sample:	Yes
Brain sample:	Yes
Both spines (age mapping):	Yes
Skin extract:	Yes
Comments:	Swimming very nicely
Photo:	

APPENDIX 1L – Behavioral observations of Shark 12

Stimuli	Time of appliance	Time of stimuli appliance	Comments
Day 1: 17.04.2023	One hour of habituation to speaker		
Seawater (control)	Kl. 10.01-10.21	10.00-10.20 into trial	No apparent change in locomotion.
Control sound new	Kl. 10.22-10.42	10.00-11.00 into trial	No apparent change.
Test sound new	Kl. 10.42-11.02	10.00-11.00 into trial	Some change, suddenly change direction half way into recording.
Test sound new	Kl. 11.02-11.22	10.00-11.00 into trial	No change.
Control sound new	Kl. 11.23-11.43	10.00-11.00 into trial	No apparent change in locomotion.
Seawater (control)	Kl. 11.43-12.03	10.15-10.30 into trial	Change of locomotion
Food odor	Kl. 12.05-12.25	10.00-10.20 into trial	Foraging behavior. Searching along the bottom in snake-like movements, middle of the tank. Continues for several minutes after stimuli is a
Break (2 hours)			
Control sound new	Kl. 13.57-14.17	10.00-11.00 into trial	No apparent change.
Seawater (control)	Kl. 14.39-14.39	10.00-10.15 into trial	Increased number of turns
Test sound new	Kl. 14.40-15.00	10.00-11.00 into trial	No reaction
Test sound new	Kl. 15.00-15.20	10.00-11.00 into trial	No reaction
Control sound	Kl. 15.20-15.40	10.03-11.03 into trial	No reaction
Stirr extract	Kl. 15.41-16.01	10.00-10.15 into trial	Change in locomotion, but does not seem aversive.
Day 2: 19.04.2023	One hour of habituation to speaker		
Control sound new	Kl. 10.01-10.21	10.00-11.00 into trial	No change of locomotion.
Test sound new	Kl. 10.22-10.42	10.00-11.00 into trial	No apparent change of behavior.
Seawater (control)	Kl. 10.43-11.03	10.00-10.16 into trial	Change of locomotion. Swimming towards the bottom and keep swimming lower in the tank.
Test sound new	Kl. 11.04-11.24	10.00-11.00 into trial	No change of locomotion.
Control sound new	Kl. 11.24-11.44	10.00-11.00 into trial	No change of locomotion.
Control sound new	Kl. 11.44-12.04	10.32-11.32 into trial	No change of locomotion.
Food odor	Kl. 12.05-12.25	10.00-10.16 into trial	Foraging behavior. Searching along the bottom of the tank, and towards the walls. This behavior is aslo common when feeding in housing tanks.
Break (2 hours)			
Seawater (control)	Kl. 13.57-14.17	10.00-10.15 into trial.	Foraging behavior. Probably food odor left from previous trial.
Test sound new	Kl. 14.20-14.40	10.00-11.00 into trial	Continue in the same swimming pattern as usual. No response.
Test sound new	Kl. 14.38-15.58	10.00-11.00 into trial	Continue in the same swimming pattern as usual. No response.
Seawater (control)	Kl. 15.04-15.24	10.00-10.15 into trial	Change of locomotion. Swimming towards the bottom and keep swimming lower in the tank.
Control sound	Kl. 15.24-15.44	10.05-11.05 into trial	No reaction
Stirr extract	Kl. 15.45-16.05	10.00-10.20 into trial	Change of swimming pattern. Swimming across the tank. Swims faster several minutes post stimuli.
Day 3: 21.04.2023	One hour of habituation to electrode		
Seawater (control)	Kl. 08.45-09.05	10.30-10.46 into trial	Change of locomotion.
Food odor	Kl. 09.05-09.25	10.00-10.24 into trial	Foraging behavior, stops quickly though.
EM 0.6 ms interval, 10 V	Kl. 10.16-10.36	10.09-11.09 into trial	Head shake when turned on, swift swimming around the tank, but no rea
EM 0.6 ms interval, 10 V	Kl. 10.37-10.57	10.00-11.00 into trial	A small reaction when stimuli is initated, but seem to not care a lot. M
EM 0.6 ms interval, 10 V	Kl. 10.57-11.17	10.00-11.00 into trial	No apparent reaction
EM 0.3 ms interval, 10 V	Kl. 11.17-11.39	10.00-11.00 into trial	No major change of locomotion, swims a bit deeper in the tank, but swi
EM 0.3 ms interval, 10 V	Kl. 11.39-11.59	10.00-11.00 into trial	No reaction.
Seawater (control)	Kl. 12.00-12.20	10.00-11.00 into trial	Forgot to flush properly (flushed with 5 L of seawater, not 20L) after last
Skin extract	Kl. 12.21-12.41	10.00-10.20 into trial	No apparent response, maybe some change of locomotion
Lunch			
EM 0.3 ms interval, 10 V	Kl. 13.09-13.09	10.00-11.00 into trial	Discovered that the electrode was not connected. (
EM 0.1 ms interval, 10 V	Kl. 14.10-14.30	10.03-11.03 into trial	Increased number of turns, change of locomotion.
EM 0.1 ms interval, 10 V	Kl. 14.30-14.50	10.00-11.00 into trial	Change of locomotion. Going towards the bottom, looking like a foraging
EM 0.1 ms interval, 10 V	Kl. 14.50-15.10	10.00-11.00 into trial	Circles the bottom of the tank. Close to the electrodes when stimuli is
EM 0.3 ms interval, 10 V	Kl. 15.11-15.31	10.00-11.00 into trial	Startle response. Swims more slowly and avoid the electric field.
EM 0.3 ms interval, 10 V	Kl. 15.32-15.58	15.30-16.30 into trial	Immediate response. Avoids the electrodes for a while afterwards. Keep
EM 0.3 ms interval, 10 V	Kl. 15.58-16.18	10.00-11.00 into trial	Startle response. Avoiding the electrodes. Avoids the positive electrode
EM 0.6 ms interval, 10 V	Kl. 16.18-16.38	09.58-10.58 into trial	Startle response. Swims calmly. Swims close to the electrode but keeps
EM 0.6 ms interval, 10 V	Kl. 16.39-16.59	10.03-11.03 into trial	Immediate response by light shake of head. Avoiding the EM side of tank
EM 0.6 ms interval, 10 V	Kl. 16.59-17.19	10.01-11.01 into trial	Startle response. Twirls to get away, swimming in circles on the opposite
Food odor	Kl. 17.19-17.39	10.00-10.30 into trial	No apparent response. Can not see any signs of foraging behavior.

Approximate age based on spines:	
Water level:	82 cm
From tank #	4
Days since capture:	16 days
Days since last feed opportunity:	3 days before trial
Tissue from state:	Immature female
Length:	Control
Weight:	73 cm
Blood sample:	1422 g
Gill sample:	Yes
Brain sample:	Yes
Both spines (age mapping):	Yes
Skin extract:	Yes
Comments:	
Photo:	

APPENDIX 1M – Behavioral observations of Shark 13

Day 1: 25.04.23			
Seawater (control)	Kl. 09.51-10.11	10.00-10.15 into trial	No response
Food odor 3/10 X	Kl. 10.12-10.32	10.00-10.20 into trial	No response. Swims very calmly
Break (2 hours)			
Skin extract 0.5 U, 25 mL	Kl. 12.09-12.29	10.00-10.15 into trial	No major response. Does a lot of "hovering" and crashes into the pipe once.
Break (2 hours)			
Skin extract 1 U, 50 mL	Kl. 14.10-14.30	10.00-10.20 into trial	Change of locomotion. Swimmin faster and crossing the tank. Return to normal swimming pattern, ut crash into the pipe at some point.
Break (2 hours)			
Skin extract 2 U 100 mL	Kl. 16.06-16.26	10.00-10.15 into trial	Increased number of turns, but does not elicit a major change in behavior
Day 2: 26.04.23			
Seawater (control)	Kl. 09.40-10.00	10.00-10.10 into trial	A bit change of locomotion
Skin extract 2 U, 100 mL	Kl. 10.01-10.21	10.00-10.15 into trial	A bit of loss of navigation. Crossing the tank but is not very responsive.
Break (2 hours)			
Skin extract 1 U 50 mL	Kl.11.58-12.18	10.00-10.15 into trial	A bit of loss of navigation, crashing into pipes but does not show aversive behavior.
Break (2 hours)			
Skin extract 0.5 U 25 mL	Kl. 14.01-14.21	10.00-10.15 into trial	Not a lot of reaction.
Break (2 hours)			
Food odor 3/10 X	Kl. 16.00-16.20	10.00-10.20 into trial	No Apparent reaction
Day 3: 27.04.23			
EM 0.3 ms, 10 V	Kl. 13.21-13.41	12.03-13.03 into trial	Change of locomotion. Chnage of swimming pattern, swimming close to the bottom

Approximate age based on spines:	
Water level:	82 cm.
From tank #	1
Days since capture (when transfered to ex. tank):	
Day since last feed opportunity:	3 days prior to first trial
Sex:	Mature male
Tissue from state:	EM
Length:	84 cm
Weight:	2273 g
Blood sample:	Yes
Gill sample:	Yes
Brain sample:	Yes
Both spines (age mapping):	Yes
Skin extract:	
Comments:	
Photo:	

APPENDIX 1N – Behavioral observations of Shark 15

Day 1: 02.05.23			
Food odor 3/10 X	Kl. 09:29-09:49	10.00-10.26 into trial	Some small signs of foraging behavior, but no big change of locomotion
Break (2 hours)			
Seawater (control)	Kl. 11:26-11:46	11.00-11.15 into trial	No major change of locomotion
Skin extract 0.5 U, 25 mL	Kl. 11:48-12:08	10.00-10.20 into trial	Swimming a bit faster
Break (2 hours)			
Skin extract 1 U, 50 mL	Kl. 13:58-14:18	10.00-10.20 into trial	Swimming faster and change of locomotion. Swimming towards the bottom and a seems a bit disoriented.
Break (2 hours)			
Skin extract 2 U 100 mL	Kl. 15:54-16:14	10.00-10.20 into trial	Some increase in swimming speed. Starts to change the swimming pattern after a couple of minutes. Seems a bit distressed after the odor has filled the tank.
Day 2: 03.05.23			
Food odor 3/10 X	Kl. 09:26-09:48	10.00-10.20 into trial	Swimming towards the bottom of the tank
Break (2 hours)			
Seawater (control)	Kl. 11:45-12:05	10.00-10.10 into trial	Some change of swimming pattern but no other apparent reaction.
Skin extract 2 U, 100 mL	Kl. 12:05-12:25	10.04-10.19 into trial	Increased swimming speed. Swimming towards bottom
Break (2 hours)			
Skin extract 1 U 50 mL	Kl. 14:10-14:30	10.00-10.20 into trial	Change of swimming pattern
Break (2 hours)			
Skin extract 0.5 U 25 mL	Kl. 16:13-16:33	10.00-10.20 into trial	Change of locomotion. swims into the "cloud of odor" and starts to swim more towards the inner part of the tank
			Flushed x 3
Day 3: 05.05.23			
Food odor 3/10 X, salmon	Kl. 12:38-12:58	10.00-10.10 into trial	Crossing the tank more, not clear foraging behavior but does change swimming pattern.
EM 0.3 ms interval, 5 V	Kl. 13:12-13:32	10.00-11.00 into trial	Swimming fast, avoiding the upper side of the tank and the electrodes. Circles the tank in the same place. Calms down event
EM 0.3 ms interval, 10 V	Kl. 13:33-13:53	10.11-11.11 into trial	Increased swimming speed. Increased number of turns.
EM 0.3 ms interval, 20 V	Kl. 13:53-14:13	10.05-11.05 into trial	Increased swimming speed. Swims close to the bottom. Increased number of turns and is in touch with the negative electrod
EM 0.3 ms interval, 5 V	Kl. 14:13-14:33	10.08-11.08 into trial	Avoiding the electrodes, but calmer response compared to first 5V trial. Swims close to electrode shortly after stimuli is off.
EM 0.3 ms interval, 10 V	Kl. 14:32-14:52	11.20-12.20 into trial	Increased swimming speed, but goes close to the electrode.
EM 0.3 ms interval, 20 V	Kl. 14:55-15:15	10.00-11.00 into trial	Changing swimming pattern, circeling the tank towards the bottom.
Food odor 3/10 X, mackerel	Kl. 14:16-15:36	10.00-10.14 into trial	No apparent response.
EM 0.3 ms interval, 5 V	Kl. 16:08-16:28	10.00-11.00 into trial	Avoiding the electrodes. Instant reaction when stimuli is turned on.
EM 0.3 ms interval, 10 V	Kl. 16:28-16:48	10.00-11.00 into trial	Low response, but some increased swimming speed.
EM 0.3 ms interval, 20 V	Kl. 16:49-17:09	10.00-11.00 into trial	Startle response. Swimming faster and circeling the tank towards the bottom. Avoiding the electromagnetic field.
Food odor 3/10 X, mackerel	Kl. 17:09-17:29	10.03-10.18 into trial	Does not seem to care about the food odor

Approximate age based on spines:	
Water level:	82 cm.
From tank #	3
Days since capture (when transferred to ex. tank)	44
Days since last feed opportunity	3 days prior to first trial
Sex:	Male
Tissue from state:	Food odor
Length:	
Weight:	1216 g
Blood sample:	69 cm
Gill sample:	
Brain sample:	
Photo:	Swimming very calmly

APPENDIX 1O – Behavioral observations of Shark 16

Day 1: 09.05.23			
Food odor 3/10 X Mackerel	Kl. 09.24-09.44	10.01-10.27 into trial	Some change of locomotion. Turns back to where the odor comes. Investigating the tank.
Break (2 hours)			Flushed x3
Seawater (control)	Kl. 11.22-11.42	10.00-10.13 into trial	No apparent change in locomotion.
Skin extract 0.5 U, 25 mL	Kl. 11. 42-12.02	11.15-11.40 into trial	No apparent reaction
Break (2 hours)			
Skin extract 1 U, 50 mL	Kl. 13.49-14.09	10.00-10.27 into trial	Hovering more in the period after application.
Break (2 hours)			
Skin extract 2 U 100 mL			Did not start trial, the shark was swimming a bit on its side so I descided to give it a break until tomorrow. Maybe a response
Day 2: 10.05.23			
Food odor 3/10 X Mackerel	Kl. 09.10-09.30	10.00-10.35 into trial	Change of swimming pattern. Swimming more towards the bottom of the tank. Increased swimming speed. Investigating the
Break (2 hours)			Flushed x3
Seawater (control)	Kl. 11.10-11.30	10.00-10.15 into trial	Change of locomotion. Swimming occationally on the side, looping. 16-20 min into rerroding is interesting.
Skin extract 2 U, 100 mL	Kl. 11.47-12.07	10.00-10.20 into trial	Increased swimming speed. Changed direction of swimming.
Break (2 hours)			
Skin extract 1 U 50 mL	Kl. 13.48-12.08	10.00-10.20 into trial	Change of swimming pattern. Does some sideways swimming and a bit disoriented.
Break (2 hours)			
Skin extract 0.5 U 25 mL	Kl. 15.47-16.07	10.00-10.20 into trial	Change of locomotion. Changing direction and swimming towards the bottom of the tank.
Day 2: 11.05.23			
Seawater control	Kl. 09.49-10.09	10.00-10.20 into trial	
Skin extract 2 U, 100 mL	Kl. 11.00-11.22	11.30-11.45 into trial	Change of locomotion.

Approximate age based on spines:	
Water level:	82 cm.
From tank #	3
Days since capture (when transfered to ex. tank):	42
Days since last feed opportunity:	3 days prior to first trial
Sex:	Mature male
Tissue from state:	Skin extract
Length:	79 cm
Weight:	1435 g
Blood sample:	
Gill sample:	
Brain sample:	
Both spines (age mapping):	
Skin extract:	
Comments:	Swimming a bit strange. Seems a
Photo:	

APPENDIX 1P – Behavioral observations of Shark 17

Day 1: 31.05.23	Trial start	Stimuli added	
Food odor 3/10 X Mackerel	KI. 9:16-9:36	10:00-10:30 into trial	Change of behavior, swimming slower, sharper turns, close to bottom
Break (2 hours)			
Seawater (control)	KI. 11:02-11:22	10:00-10:20 into trial	Avoided tube once seawater was poured in
Skin extract 0.5 U, 25 mL	KI. 11:23-11:43	10:00-10:20 into trial	more aggitated swimming, tail faster, might be disoriented-crashed into pipes.
Break (2 hours)			
Skin extract 1 U, 50 mL	KI. 13:26-13:46	10:00-10:20 into trial	changed directions often, tried to bite/bump pipe, hovers often, cut through middle 3 times, goes slower towards pipe. Hovers before and near pipe-then swims regularly after pipe. Avoids pipe and turns around before getti
Break (2 hours)			
Skin extract 2 U 100 mL	KI.15:45-16:05	10:00-10:30 into trial	making half circles around pipe, faster movement, goes slower around pipe, poking snout into air a lot.

Day 2: 01.06.23	Trial start	Stimuli added	
Food odor 3/10 X Mackerel	KI. 8:42-9:02	10:00-10:30 into trial	Slower movement, less hovering, making full circles, lower in water, cut though middle 3 times
Break (2 hours)			
Seawater (control)	KI. 10:58-11:18	10:00-10:30 into trial	reacted, slightly faster, bumped pipe
Skin extract 2 U, 100 mL	KI. 11:18-11:38	10:00-10:30 into trial	Swims faster and circles pipe, slower at pipes
Break (2 hours)			
Skin extract 1 U 50 mL	KI. 1:18-1:38	10:00-10:30 into trial	Hovers in front of pipe, bumped pipe. (hit pipe right before
Break (2 hours)			
Skin extract 0.5 U 25 mL	KI. 15:18-25:38	10:00-10:30 into trial	gets closer to pipes, hovering near pipes

Day 2: 02.06.23	Trial start	Stimuli added	
Seawater control	KI. 8:37-8:57	10:00-10:30 into trial	no big reaction, just makes a half circle around pipe to look at it.
Skin extract 2 U, 100 mL	KI. 8:58-9:18	10:00-10:30 into trial	slower, hovers at pipes, faster tail movement
Skin extract 1 U, 50 mL	KI. 1:16-1:36	10:00-10:30 into trial	more tail movement, slowly swimmming, swimming around pipe
Euthanize at			

Approximate age based on spines:	10-12 fully mature
Water level:	82 cm.
From tank #	3
Days since capture (when transfered to ex. tank):	11
Day since last feed opportunity:	3 days prior to first trial
Sex:	Female, fully mature.8 eggs
Tissue from state:	Skin extract
Length:	84cm
Weight:	2611g
Blood sample:	
Gill sample:	
Brain sample:	
Both spines (age mapping):	
Skin extract:	
Comments:	Wound on left side of the face, between the eye and spirac
Photo:	

APPENDIX 1Q – Behavioral observations of Shark 18

Day 1: 06.06.23	Trial start	Stimuli added	Observation
EM 0.3 ms interval, 5 V	(voltage incorrect)		
EM 0.3 ms interval, 10 V	Kl. 11:50-12:10	10:00-11:00 into trial	Swimming lower
EM 0.3 ms interval, 20 V	Kl. 12:57-13:17	10:00-11:00 into trial	swimming lower and making small circles on bottom and slightly swam on side. Stopped moving for a second.
EM 0.3 ms interval, 5 V			making small circles at the bottom
EM 0.3 ms interval, 10 V	Kl. 12:36-12:56	10:00-11:00 into trial	swimming lower and smaller circles
EM 0.3 ms interval, 20 V	Kl. 13:28-13:48	10:00-11:00 into trial	swimming on side. stays at bottom of tank and makes small circles, had a small fast swim
Food odor 3/10 X. mackerel	Kl. 13:52-14:12	10:00-10:30 into trial	slower, making half circles, staying near bottom, slows by pipe,
EM 0.3 ms interval, 5 V			
EM 0.3 ms interval, 10 V	Kl. 14:48-15:02	10:00-11:10 into trial	stilled and went to the bottom of the tank and slowly turns on the bottom
EM 0.3 ms interval, 20 V	Kl. 15:08-15:28	10:00-11:00 into trial	gets still and goes to bottom of tank and swims more on its side
food odor 2		10-	
Day 2: 07.06.23	Trial start	Stimuli added	
Seawater (control)	Kl. 9:03-9:23	10:00-10:30 into trial	half circles, swims more on side, swims faster
Skin extract 0.5 U, 25 mL	Kl. 9:23-9:43	10:00-10:30 into trial	faster movement, smaller circles, sharper turns
Break (2 hours)			
Skin extract 1 U, 50 mL	Kl. 11:02-11:22	10:00-10:30 into trial	slower, makes small circles around pipe. swimming more on side
Break (2 hours)			
Skin extract 2 U 100 mL	Kl. 13:18-13:38	10:00-10:30 into trial	Half circles, faster tail movement, hovering near pipe
Break (2 hours)			
Food odor 3/10 X Mackerel	Kl. 15:03-15:23	10:00-10:30 into trial	half circles, hovering near pipe, faster tail movement
Day 3: 08.06.23	Trial start	Stimuli added	
Seawater (control)	Kl. 9:03-9:23	10:00-10:30 into trial	smaller circles, more tail movement
Skin extract 2 U 100 mL	Kl. 9:23-9:43	10:00-10:30 into trial	makes half circles, faster tail movement, slows near pipe
Break (2 hours)			
Skin extract 1 U 50 mL	Kl. 11:17-11:37	10:00-10:30 into trial	half circles, more tail movement, gets closer to pipes and is maki
Break (2 hours)			
Skin extract 0.5 U 25 mL	Kl. 13:14-13:34	10:00-10:30 into trial	stills, faster tail movement, some hafl circles, circles pipe
Break (2 hours)			
Food odor 3/10 X Mackerel	Kl. 15:00-15:20	10:00-10:30 into trial	slows near pipe, makes full circles, hovers by pipe
Day 4: 09.06.23	Trial start	Stimuli added	Observation
EM 0.3 ms interval, 5 V	Kl. 11:54-12:14	10:04-11:04 into trial	No apparent reaction to the stimuli. Continues too swim normally
EM 0.3 ms interval, 5 V	Kl. 12:14-12:24	10:05-11:05 into trial	No apparent reaction to the stimuli. Continues too swim normally
Food odor 3/10 X Mackerel	Kl. 14:17-14:37		

Approximate age based on spines:	
Water level:	82 cm.
From tank #	3
Days since capture (when transfered to ex. tank):	
Day since last feed opportunity:	1 Day before trial
Sex:	Female, mature
Tissue from state:	Food odor
Length:	81 cm
Weight:	1869 g
Blood sample:	
Gill sample:	
Brain sample:	
Both spines (age mapping):	
Skin extract:	
Comments:	
Photo:	

APPENDIX 1R – Behavioral observations of Shark 20

Day 1: 06.06.23	Trial start	Stimuli added	Observation
EM 0.3 ms interval, 5 V	(voltage incorrect)		
EM 0.3 ms interval, 10 V	Kl. 11:50-12:10	10:00-11:00 into trial	Swimming lower
EM 0.3 ms interval, 20 V	Kl. 12:57-13:17	10:00-11:00 into trial	swimming lower and making small circles on bottom and slightly swam on side. Stopped moving for a second.
EM 0.3 ms interval, 5 V			making small circles at the bottom
EM 0.3 ms interval, 10 V	Kl. 12:36-12:56	10:00-11:00 into trial	swimming lower and smaller circles
EM 0.3 ms interval, 20 V	Kl. 13:28-13:48	10:00-11:00 into trial	swimming on side, stays at bottom of tank and makes small circles, had a small fast swim
Food odor 3/10 X, mackerel	Kl. 13:52-14:12	10:00-10:30 into trial	slower, making half circles, staying near bottom, slows by pipe,
EM 0.3 ms interval, 5 V			
EM 0.3 ms interval, 10 V	Kl. 14:48-15:02	10:00-11:10 into trial	stilled and went to the bottom of the tank and slowly turns on the bottom
EM 0.3 ms interval, 20 V	Kl. 15:08-15:28	10:00-11:00 into trial	gets still and goes to bottom of tank and swims more on its side
food odor 2		10-	
Day 2: 07.06.23	**Trial start**	**Stimuli added**	
Seawater (control)	Kl. 9:03-9:23	10:00-10:30 into trial	half circles, swims more on side, swims faster
Skin extract 0.5 U, 25 mL	Kl. 9:23-9:43	10:00-10:30 into trial	faster movement, smaller circles, sharper turns
Break (2 hours)			
Skin extract 1 U, 50 mL	Kl. 11:02-11:22	10:00-10:30 into trial	slower, makes small circles around pipe, swimming more on side
Break (2 hours)			
Skin extract 2 U 100 mL	Kl. 13:18-13:38	10:00-10:30 into trial	Half circles, faster tail movement, hovering near pipe
Break (2 hours)			Break (2 hou
Food odor 3/10 X Mackerel	Kl. 15:03-15:23	10:00-10:30 into trial	half circles, hovering near pipe, faster tail movement
Day 3: 08.06.23	**Trial start**	**Stimuli added**	
Seawater (control)	Kl. 9:03-9:23	10:00-10:30 into trial	smaller circles, more tail movement
Skin extract 2 U, 100 mL	Kl. 9:23-9:43	10:00-10:30 into trial	makes half circles, faster tail movement, slows near pipe
Break (2 hours)			
Skin extract 1 U 50 mL	Kl. 11:17-11:37	10:00-10:30 into trial	half circles, more tail movement, gets closer to pipes and is maki
Break (2 hours)			
Skin extract 0.5 U 25 mL	Kl. 13:14-13:34	10:00-10:30 into trial	stills, faster tail movement, some half circles, circles pipe
Break (2 hours)			
Food odor 3/10 X Mackerel	Kl. 15:00-15:20	10:00-10:30 into trial	slows near pipe, makes full circles, hovers by pipe
Day 4: 09.06.23	**Trial start**	**Stimuli added**	**Observation**
EM 0.3 ms interval, 5 V	Kl. 11.54-12.14	10.04-11.04 into trial	No apparent reaction to the stimuli. Continues too swim normally
EM 0.3 ms interval, 5 V	Kl. 12.14-12.24	10.05-11.05 into trial	No apparent reaction to the stimuli. Continues too swim normally
Food odor 3/10 X Mackerel	Kl. 14.17-14.37		

Approximate age based on spines:	
Water level:	82 cm.
From tank #	3
Days since capture (when transfered to ex. tank):	
Day since last feed opportunity:	1 Day before trial
Sex:	Female, mature
Tissue from state:	Food odor
Length:	81 cm
Weight:	1869 g
Blood sample:	
Gill sample:	
Brain sample:	
Both spines (age mapping):	
Skin extract:	
Comments:	
Photo:	

APPENDIX 1S – Behavioral observations of Shark 21

Day 1: 14.06.23	Trial start	Stimuli added	Observation
Seawater control	Kl. 8:46-9:06	10:00-10:30 into trial	more tail movement, bumps pipe
Food odor 3/10 X, mackerel	Kl. 9:06-9:26	10:00-10:30 into trial	hovering a lot near pipe, more tail movement
Break (2 hours)			
EM 0.3 ms interval, 5 V	Kl. 11:09-11:29	10:00-11:00 into trial	small effect, jolted a tiny bit, swimming slightly faster
EM 0.3 ms interval, 10 V	Kl. 11:29-11:49	10:00-11:00 into trial	freaked out, swam faster, and went ot middle
EM 0.3 ms interval, 20 V	Kl. 11:49-12:09	10:00-11:00 into trial	Went to bottom of tank
EM 0.3 ms interval, 5 V	Kl. 13:17-13:37	10:00-11:00 into trial	swam a little faster
Seawater control	Kl. 15:29-15:49	10:00-10:30 into trial	swam faster, hovering near pipe
Food odor 3/10 X, mackerel	Kl: 15:49-16:09	10:00-10:30 into trial	hovering more, slows near pipe
Break (2 hours)			
EM 0.3 ms interval, 10 V	Kl. 13:37-13:57	10:00-11:00 into trial	avoided electrodes and went to bottom of tank
EM 0.3 ms interval, 20 V	Kl. 13:57-14:17	10:01-11:00 into trial	avoided electrode, and is swimming lower
EM 0.3 ms interval, 5 V	Kl. 14:18-14:38	10:05-11:05 into trial	swims lower, make sharp turns
EM 0.3 ms interval, 10 V	Kl. 14:45-15:05	10:10-11:10 into trial	swam a little faster, slightly slower
EM 0.3 ms interval, 20 V	Kl. 15:07-15:27	10:00-11:00 into trial	swam low in middle

Day 2: 15.06.23	Trial start	Stimuli added	
Seawater (control)			Approximate age based on spines:
Skin extract 0.5 U, 25 mL			Water level:
Break (2 hours)			From tank #
Skin extract 1 U, 50 mL			Days since capture (when transfered to ex. tank):
Break (2 hours)			Day since last feed opportunity:
Skin extract 2 U 100 mL			Sex:
Break (2 hours)			Tissue from state:
Food odor 3/10 X Mackerel			Length:
			Weight:
			Blood sample:
			Gill sample:
			Brain sample:
			Both spines (age mapping):
			Skin extract:
			Comments:
			Photo:

			4
			Maturing female
			82 cm.
			1
			EM
			84 cm
			2099 g
			Yes
			Yes
			Yes
			Yes
			No
			Has not been eating in captivity

Day 3: 16.06.23	Trial start	Stimuli added
Seawater (control)		
Food odor 3/10 X Mackerel		
Skin extract 2 U, 100 mL		
Break (2 hours)		
Skin extract 1 U 50 mL		
Break (2 hours)		
Skin extract 0.5 U 25 mL		
Break (2 hours)		

APPENDIX 2A – CONDITION 1

Distances traveled

*Table 3. The total distances traveled by each shark in each trial. The trials are listed in order of execution. The stimuli tested in each trial is represented in the «Stimuli» column. The «Shark» column shows the target shark. The total distances traveled is represented as movement across pixels. * " rested" during trial.*

Trial	Stimuli	Shark	Tot. distance traveled (before)	Tot. distance traveled (after)
1	Seawater control 10 fps	Shark 1	1004.42	1175.85
2	Seawater control 10 fps	Shark 1	8105.09	7867.08
3	Seawater control 10 fps	Shark 1	4569.99	5529.07
4	Seawater control 10 fps	Shark 1	2290.48	4476.79
5	Seawater control 10 fps	Shark 1	1910.81	4325.74
6	Seawater control	Shark 1	9992.13	11201.68
7	Seawater control	Shark 1	7004.43	10636.57
8	Seawater control	Shark 1	9565.63	13960.51
1	Food odor 10 fps	Shark 1	6378.28	6367.91
2	Food odor 10 fps	Shark 1	2186.27	4891.93
3	Food odor	Shark 1	9267.84	9220.63
4	Food odor	Shark 1	9975.43	11935.82
1	Skin extract 10 fps	Shark 1	5621.66	5982.39
2	Skin extract	Shark 1	9820.46	12349.40
3	Skin extract	Shark 1	11154.04	13207.87
1	Control sound 10 fps	Shark 1	2573.17	2710.74
2	Control sound 10 fps	Shark 1	3921.37	3117.48
3	Control sound 10 fps	Shark 1	1701.39	3494.72
4	Control sound 10 fps	Shark 1	2307.24	2251.65
5	Control sound 10 fps	Shark 1	2180.56	2547.10
6	Control sound 10 fps	Shark 1	1572.10	1264.46
7	Control sound	Shark 1	4177.33	5150.34
8	Control sound	Shark 1	4178.24	5876.12
1	Orca sound 10 fps	Shark 1	2983.10	2723.34
2	Orca sound 10 fps	Shark 1	2781.42	2253.91
3	Orca sound 10 fps	Shark 1	2580.71	2889.22
4	Orca sound 10 fps	Shark 1	2436.10	1437.09
5	Orca sound 10 fps	Shark 1	3002.22	3603.13
6	Orca sound 10 fps	Shark 1	1639.35	2991.65
7	Orca sound	Shark 1	3699.04	2845.04
8	Orca sound	Shark 1	3978.55	5104.12
1	EM 0.6 ms	Shark 1	2548.15	6652.77
2	EM 0.6 ms	Shark 1	3931.24	7647.75
3	EM 0.6 ms	Shark 1	6093.38	4794.98
4	EM 0.6 ms	Shark 1	4302.86	7492.26
5	EM 0.6 ms	Shark 1	4070.82	5591.25
1	EM 0.1 ms	Shark 1	3869.03	6528.00
2	EM 0.1 ms	Shark 1	4547.80	5761.61
3	EM 0.1 ms	Shark 1	5444.12	6212.57
4	EM 0.1 ms	Shark 1	3765.21	6650.74
1	Seawater control	Shark 2	4596.04	12298.05
2	Seawater control	Shark 2	103.18	2691.41
3	Seawater control	Shark 2	2271.85	5881.13
4	Seawater control	Shark 2	8170.14	9802.01
5	Seawater control	Shark 2	81.66	2343.22
6	Seawater control	Shark 2	2945.80	8826.02
7	Seawater control	Shark 2	2177.27	7706.85
8	Seawater control	Shark 2	807.03	3301.03
1	Skin extract	Shark 2	3293.87	6742.90
2	Skin extract*	Shark 2	195.77	7697.22
3	Skin extract*	Shark 2	107.27	3343.28
1	Food odor	Shark 2	1867.39	6328.11
2	Food odor	Shark 2	4146.52	7928.61
3	Food odor	Shark 2	122.88	4030.29
4	Food odor	Shark 2	8120.60	7669.20
1	Control sound	Shark 2	3932.42	2369.61
2	Control sound	Shark 2	2104.00	5916.90
3	Control sound*	Shark 2	34.51	34.17
4	Control sound*	Shark 2	72.43	75.36
5	Control sound	Shark 2	4025.60	2631.03
6	Control sound*	Shark 2	40.33	41.63
7	Control sound	Shark 2	1731.80	4939.94
8	Control sound	Shark 2	807.91	1714.40
1	Orca sound	Shark 2	3184.22	1328.57
2	Orca sound	Shark 2	4065.83	1645.43
3	Orca sound	Shark 2	2615.18	3140.37
4	Orca sound	Shark 2	2825.91	2696.68
5	Orca sound	Shark 2	4072.33	4261.09
6	Orca sound*	Shark 2	42.84	42.03
7	Orca sound*	Shark 2	68.43	66.71
8	Orca sound*	Shark 2	67.52	1740.65
1	EM 0.6 ms	Shark 2	1798.30	5494.07

2	EM 0.6 ms	Shark 2	1192.43	5443.48
3	EM 0.6 ms	Shark 2	897.56	5457.59
1	EM 0.3 ms	Shark 2	2802.69	6205.80
2	EM 0.3 ms	Shark 2	865.45	5845.09
3	EM 0.3 ms	Shark 2	1631.99	5596.69
1	EM 0.1 ms	Shark 2	1224.77	5337.79
2	EM 0.1 ms*	Shark 2	43.07	44.06
3	EM 0.1 ms*	Shark 2	54.16	49.37
1	Seawater control	Shark 3	15093.77	16927.74
2	Seawater control	Shark 3	13565.87	16488.56
3	Seawater control	Shark 3	14800.72	17548.62
4	Seawater control	Shark 3	17359.15	16126.88
5	Seawater control	Shark 3	16203.63	19653.68
6	Seawater control	Shark 3	12235.46	15885.01
7	Seawater control	Shark 3	14774.21	15751.45
8	Seawater control	Shark 3	14650.01	20236.42
1	Skin extract	Shark 3	14119.44	18909.81
2	Skin extract	Shark 3	15637.51	12481.88
3	Skin extract	Shark 3	13862.46	18349.54
1	Food odor	Shark 3	15493.00	12727.95
2	Food odor	Shark 3	16322.94	16444.42
3	Food odor	Shark 3	14696.26	20202.19
4	Food odor	Shark 3	13098.72	13737.09
1	Control sound	Shark 3	6369.98	6262.83
2	Control sound	Shark 3	6379.25	7135.37
3	Control sound	Shark 3	8090.04	8371.28
4	Control sound	Shark 3	7476.78	5588.40
5	Control sound	Shark 3	7706.53	7889.76
6	Control sound	Shark 3	7016.76	8730.74
7	Control sound	Shark 3	7882.24	6087.39
8	Control sound	Shark 3	7033.19	6417.32
1	Orca sound	Shark 3	7612.39	7343.21
2	Orca sound	Shark 3	7713.54	7271.50
3	Orca sound	Shark 3	8594.28	7510.51
4	Orca sound	Shark 3	7340.54	7925.08
5	Orca sound	Shark 3	6283.90	6677.06
6	Orca sound	Shark 3	5243.24	8195.33
7	Orca sound	Shark 3	7223.16	7653.42
8	Orca sound	Shark 3	7771.34	7305.47
1	EM 0.6 ms	Shark 3	8071.37	9614.54
2	EM 0.6 ms	Shark 3	8390.54	10202.63
3	EM 0.6 ms	Shark 3	5448.35	8299.68
1	EM 0.3 ms	Shark 3	7223.42	9921.27
2	EM 0.3 ms	Shark 3	4967.16	9915.03
3	EM 0.3 ms	Shark 3	8172.37	10541.15
1	EM 0.1 ms	Shark 3	8140.37	10411.39
2	EM 0.1 ms	Shark 3	6257.66	9813.82
3	EM 0.1 ms	Shark 3	5190.11	8229.35
1	Seawater control	Shark 4	14590.65	16424.14
2	Seawater control	Shark 4	14946.49	20375.95
1	Seawater control	Shark 4	14014.08	13937.06
2	Seawater control	Shark 4	14602.36	18307.80
3	Seawater control	Shark 4	13992.06	17067.46
4	Seawater control	Shark 4	10121.27	12022.77
5	Seawater control	Shark 4	11101.05	12497.20
6	Seawater control	Shark 4	12284.72	18846.65
1	Skin extract	Shark 4	11765.93	13056.97
2	Skin extract	Shark 4	9804.91	15208.47
3	Skin extract	Shark 4	11393.99	17005.46
1	Food odor	Shark 4	13401.88	14277.44
2	Food odor	Shark 4	12336.59	15054.46
3	Food odor	Shark 4	11939.12	12414.42
4	Food odor	Shark 4	11968.94	14570.26
1	Control sound	Shark 4	7181.91	6059.86
2	Control sound	Shark 4	6886.32	7326.62
3	Control sound	Shark 4	8519.05	6813.64
4	Control sound	Shark 4	7766.77	8813.65
5	Control sound	Shark 4	5450.96	4635.00
6	Control sound	Shark 4	6153.45	6008.40
7	Control sound	Shark 4	5777.74	5984.16
8	Control sound	Shark 4	4581.57	3788.98
1	Orca sound	Shark 4	9105.81	8099.35
2	Orca sound	Shark 4	7168.82	8167.38
3	Orca sound	Shark 4	6337.59	6795.52
4	Orca sound	Shark 4	6387.30	6064.22
5	Orca sound	Shark 4	4662.23	7139.03
6	Orca sound	Shark 4	6501.96	6289.56
7	Orca sound	Shark 4	3967.66	5597.72
8	Orca sound	Shark 4	3642.61	7034.64
1	EM 0.6 ms	Shark 4	8508.54	12311.58
2	EM 0.6 ms	Shark 4	8901.11	10028.88
3	EM 0.6 ms	Shark 4	10286.34	11645.55

1	EM 0.3 ms	Shark 4	9762.26	10430.37
2	EM 0.3 ms	Shark 4	9663.28	11440.23
3	EM 0.3 ms	Shark 4	8665.83	10504.60
1	EM 0.1 ms	Shark 4	8532.56	10988.07
2	EM 0.1 ms	Shark 4	7933.37	11373.54
3	EM 0.1 ms	Shark 4	7425.64	9487.50
1	Morning recording	Shark 4	5923.34	5269.33
2	Morning recording	Shark 4	9213.98	8148.21
3	Morning recording	Shark 4	5923.34	5269.33
1	Seawater control	Shark 5	15533.69	19248.28
2	Seawater control	Shark 5	14333.21	17141.12
3	Seawater control	Shark 5	19668.26	16272.04
4	Seawater control	Shark 5	14392.43	17595.72
5	Seawater control	Shark 5	22451.74	18141.69
6	Seawater control	Shark 5	20481.94	17279.35
7	Seawater control	Shark 5	18300.30	19578.72
8	Seawater control	Shark 5	18588.17	18033.82
1	Skin extract	Shark 5	20984.96	14397.68
2	Skin extract	Shark 5	20624.36	15100.43
3	Skin extract	Shark 5	15417.42	16378.06
4	Skin extract	Shark 5	21062.55	17719.87
1	Food odor	Shark 5	21617.75	16339.59
2	Food odor	Shark 5	17832.91	15171.68
3	Food odor	Shark 5	21742.24	15146.09
4	Food odor	Shark 5	17491.51	13999.44
1	Control sound	Shark 5	9495.77	11763.63
2	Control sound	Shark 5	10006.55	10881.10
3	Control sound	Shark 5	9156.78	11455.21
4	Control sound	Shark 5	11027.06	9060.06
5	Control sound	Shark 5	8552.32	9167.17
6	Control sound	Shark 5	10952.77	8923.53
7	Control sound	Shark 5	6466.18	8987.67
8	Control sound	Shark 5	10270.39	8257.15
1	Orca sound	Shark 5	11251.82	7821.58
2	Orca sound	Shark 5	10736.39	8302.39
3	Orca sound	Shark 5	8165.59	8685.85
4	Orca sound	Shark 5	11506.84	9133.71
5	Orca sound	Shark 5	8154.18	9536.52
6	Orca sound	Shark 5	10280.06	10198.61
7	Orca sound	Shark 5	10511.14	8632.84
8	Orca sound	Shark 5	10559.86	10301.30
1	EM 0.6 ms	Shark 5	8212.96	7669.83
2	EM 0.6 ms	Shark 5	8598.19	6108.71
3	EM 0.6 ms	Shark 5	8475.58	6205.13
1	EM 0.3 ms	Shark 5	11351.91	6534.32
2	EM 0.3 ms	Shark 5	10450.33	5744.66
3	EM 0.3 ms	Shark 5	11295.00	7496.52
1	EM 0.1 ms	Shark 5	9979.60	5838.84
2	EM 0.1 ms	Shark 5	9950.58	4896.96
3	EM 0.1 ms	Shark 5	9707.71	5629.41
1	Morning recording	Shark 5	10858.23	11358.20
2	Morning recording	Shark 5	9332.25	6786.81
3	Morning recording	Shark 5	11139.83	11396.54
4	Morning recording	Shark 5	9534.70	7743.54
1	Seawater control	Shark 7	23084.71	21905.53
2	Seawater control	Shark 7	12526.18	22033.60
3	Seawater control	Shark 7	20480.00	21135.48
4	Seawater control	Shark 7	20347.57	21581.36
5	Seawater control	Shark 7	17665.67	20387.79
6	Seawater control	Shark 7	18801.43	22216.38
7	Seawater control	Shark 7	15112.97	20654.92
8	Seawater control	Shark 7	17104.83	19524.58
1	Skin extract	Shark 7	19074.98	20519.98
2	Skin extract	Shark 7	19724.22	20165.12
3	Skin extract	Shark 7	19059.76	19617.44
1	Food odor	Shark 7	17843.94	16896.04
2	Food odor	Shark 7	20928.62	19271.12
3	Food odor	Shark 7	21394.26	17546.98
4	Food odor	Shark 7	17604.57	17331.85
1	Control sound	Shark 7	8717.72	8490.22
2	Control sound	Shark 7	9412.22	7730.84
3	Control sound	Shark 7	8071.07	8678.82
4	Control sound	Shark 7	8655.64	8366.80
5	Control sound	Shark 7	7952.38	8084.25
6	Control sound	Shark 7	9096.09	9538.93
1	Orca sound	Shark 7	10800.11	11533.84
2	Orca sound	Shark 7	10275.39	8311.79
3	Orca sound	Shark 7	7673.50	8486.16
4	Orca sound	Shark 7	8552.89	7623.38
5	Orca sound	Shark 7	8763.62	8814.27
6	Orca sound	Shark 7	9103.67	9759.94
1	EM 0.6 ms	Shark 7	8366.67	13842.47

2	EM 0.6 ms	Shark 7	12199.65	11014.13
3	EM 0.6 ms	Shark 7	7594.64	10314.75
1	EM 0.3 ms	Shark 7	10983.49	8795.22
2	EM 0.3 ms	Shark 7	10758.55	11486.68
3	EM 0.3 ms	Shark 7	8983.99	10234.86
1	EM 0.1 ms	Shark 7	9849.06	9433.28
2	EM 0.1 ms	Shark 7	10147.34	9396.40
3	EM 0.1 ms	Shark 7	8358.09	8475.69
1	Morning recording	Shark 7	9628.88	9194.91
2	Morning recording	Shark 7	8522.13	11684.76
3	Morning recording	Shark 7	8650.41	8976.03
1	Seawater control	Shark 11	16331.50	16632.94
2	Seawater control	Shark 11	18644.04	17732.41
3	Seawater control	Shark 11	14714.83	16537.87
4	Seawater control	Shark 11	14138.17	16698.20
5	Seawater control	Shark 11	13243.57	14579.13
6	Seawater control	Shark 11	16250.25	17206.08
7	Seawater control	Shark 11	17480.09	18891.81
8	Seawater control	Shark 11	13673.86	19151.03
1	Skin extract	Shark 11	20223.45	19147.53
2	Skin extract	Shark 11	15878.77	16388.26
3	Skin extract	Shark 11	15877.96	16606.08
1	Food odor	Shark 11	19672.65	13093.63
2	Food odor	Shark 11	15866.09	11640.12
3	Food odor	Shark 11	17352.63	13634.55
4	Food odor	Shark 11	19739.36	16578.94
1	Control sound	Shark 11	7570.90	9319.11
2	Control sound	Shark 11	9628.29	8393.53
3	Control sound	Shark 11	10588.17	10090.35
4	Control sound	Shark 11	10821.41	10563.71
5	Control sound	Shark 11	8145.08	8148.86
6	Control sound	Shark 11	8414.53	8403.24
7	Control sound	Shark 11	9298.04	9585.11
8	Control sound	Shark 11	7547.23	7136.24
1	Orca sound	Shark 11	8186.27	8683.29
2	Orca sound	Shark 11	8767.57	6749.85
3	Orca sound	Shark 11	8414.65	8001.21
4	Orca sound	Shark 11	7931.55	7685.18
5	Orca sound	Shark 11	7500.30	7447.36
6	Orca sound	Shark 11	7243.06	6040.34
7	Orca sound	Shark 11	8871.26	9091.10
8	Orca sound	Shark 11	10243.98	11025.13
1	EM 0.6 ms	Shark 11	10895.44	9445.51
2	EM 0.6 ms	Shark 11	6558.49	6355.60
3	EM 0.6 ms	Shark 11	8570.65	6801.03
1	EM 0.3 ms	Shark 11	9573.33	6962.57
2	EM 0.3 ms	Shark 11	7213.65	7007.74
3	EM 0.3 ms	Shark 11	7461.79	7881.54
1	EM 0.1 ms	Shark 11	10219.42	6597.15
2	EM 0.1 ms	Shark 11	10079.12	7443.63
3	EM 0.1 ms	Shark 11	6472.23	7693.56
1	Morning recording	Shark 11	8625.90	9239.08
2	Morning recording	Shark 11	7642.09	7780.53
3	Morning recording	Shark 11	8941.15	8601.27
1	Seawater control	Shark 12	24303.11	25105.74
2	Seawater control	Shark 12	20467.42	19664.43
3	Seawater control	Shark 12	16663.73	19062.23
4	Seawater control	Shark 12	15474.21	18668.08
5	Seawater control	Shark 12	20098.11	18580.77
6	Seawater control	Shark 12	20532.19	20141.16
7	Seawater control	Shark 12	22620.07	17253.18
8	Seawater control	Shark 12	19700.26	22549.73
1	Skin extract	Shark 12	24426.28	21566.16
2	Skin extract	Shark 12	24232.32	19838.69
3	Skin extract	Shark 12	20270.18	19606.56
1	Food odor	Shark 12	20710.85	15721.18
2	Food odor	Shark 12	17273.51	13950.97
3	Food odor	Shark 12	24618.03	15285.31
4	Food odor	Shark 12	18428.57	17915.91
1	Control sound	Shark 12	12910.94	12889.08
2	Control sound	Shark 12	10203.39	10025.98
3	Control sound	Shark 12	8883.60	8996.67
4	Control sound	Shark 12	12131.57	12347.96
5	Control sound	Shark 12	6906.48	7511.49
6	Control sound	Shark 12	9134.92	9199.38
7	Control sound	Shark 12	9095.95	9211.28
8	Control sound	Shark 12	11612.85	12214.62
1	Orca sound	Shark 12	12371.17	10278.56
2	Orca sound	Shark 12	10229.49	10184.08
3	Orca sound	Shark 12	12160.91	12312.33
4	Orca sound	Shark 12	9114.32	9358.76
5	Orca sound	Shark 12	7781.03	7705.12

6	Orca sound	Shark 12	8771.47	8654.84
7	Orca sound	Shark 12	12237.71	12318.70
8	Orca sound	Shark 12	10967.49	10802.72
1	EM 0.6 ms	Shark 12	9053.81	8510.22
2	EM 0.6 ms	Shark 12	9131.47	6953.18
3	EM 0.6 ms	Shark 12	12528.51	6131.20
1	EM 0.3 ms	Shark 12	12421.78	6206.54
2	EM 0.3 ms	Shark 12	12241.24	7357.88
3	EM 0.3 ms	Shark 12	9273.67	6474.25
1	EM 0.1 ms	Shark 12	12606.34	6848.82
2	EM 0.1 ms	Shark 12	10094.67	9368.60
3	EM 0.1 ms	Shark 12	8995.76	8112.54
1	Morning recording	Shark 12	10336.02	10131.44
2	Morning recording	Shark 12	11413.11	6381.78
3	Morning recording	Shark 12	11414.53	11495.82

APPENDIX 2B – CONDITION 1

Mean distances traveled and fold change

Table 4. The mean distances traveled are calculated from the total distances in Table 1. The fold change is calculated by dividing mean value of "After_Stimuli" average by "Before_Stimuli".

Shark	Stimuli	Condition	Mean value	In meters	Fold change
Shark 1	Seawater control	Before Stimuli	5555.37	12.63	1.33
Shark 1	Seawater control	After Stimuli	7396.66	16.81	
Shark 1	Food odor	Before Stimuli	6951.95	15.80	1.17
Shark 1	Food odor	After Stimuli	8104.07	18.42	
Shark 1	Skin extract	Before Stimuli	8865.39	20.15	1.19
Shark 1	Skin extract	After Stimuli	10513.22	23.89	
Shark 1	Control sound	Before Stimuli	2826.42	6.42	1.17
Shark 1	Control sound	After Stimuli	3301.58	7.50	
Shark 1	Orca sound	Before Stimuli	2887.56	6.56	1.03
Shark 1	Orca sound	After Stimuli	2980.94	6.77	
Shark 1	EM 0.6 ms	Before Stimuli	4189.29	9.52	1.54
Shark 1	EM 0.6 ms	After Stimuli	6435.80	14.63	
Shark 1	EM 0.1 ms	Before Stimuli	4406.54	10.01	1.43
Shark 1	EM 0.1 ms	After Stimuli	6288.23	14.29	
Shark 2	Seawater control	Before Stimuli	2644.12	6.01	2.50
Shark 2	Seawater control	After Stimuli	6606.22	15.01	
Shark 2	Skin extract	Before Stimuli	1198.97	2.72	4.94
Shark 2	Skin extract	After Stimuli	5927.80	13.47	
Shark 2	Food odor	Before Stimuli	3564.35	8.10	1.82
Shark 2	Food odor	After Stimuli	6489.06	14.75	
Shark 2	Control sound	Before Stimuli	1593.63	3.62	1.39
Shark 2	Control sound	After Stimuli	2215.38	5.03	
Shark 2	Orca sound	Before Stimuli	1882.47	4.28	0.99
Shark 2	Orca sound	After Stimuli	1865.19	4.24	
Shark 2	EM 0.6 ms	Before Stimuli	1296.10	2.95	4.22
Shark 2	EM 0.6 ms	After Stimuli	5465.05	12.42	
Shark 2	EM 0.3 ms	Before Stimuli	1766.71	4.02	3.33
Shark 2	EM 0.3 ms	After Stimuli	5882.53	13.37	
Shark 2	EM 0.1 ms	Before Stimuli	440.67	1.00	4.11
Shark 2	EM 0.1 ms	After Stimuli	1810.40	4.11	
Shark 3	Seawater control	Before Stimuli	14835.35	33.72	1.17
Shark 3	Seawater control	After Stimuli	17327.30	39.38	
Shark 3	Skin extract	Before Stimuli	14539.80	33.05	1.14
Shark 3	Skin extract	After Stimuli	16580.41	37.68	
Shark 3	Food odor	Before Stimuli	14902.73	33.87	1.06
Shark 3	Food odor	After Stimuli	15777.91	35.86	
Shark 3	Control sound	Before Stimuli	7244.35	16.46	0.97
Shark 3	Control sound	After Stimuli	7060.39	16.05	
Shark 3	Orca sound	Before Stimuli	7222.80	16.42	1.04
Shark 3	Orca sound	After Stimuli	7485.20	17.01	
Shark 3	EM 0.6 ms	Before Stimuli	7303.42	16.60	1.28
Shark 3	EM 0.6 ms	After Stimuli	9372.29	21.30	
Shark 3	EM 0.3 ms	Before Stimuli	6787.65	15.43	1.49
Shark 3	EM 0.3 ms	After Stimuli	10125.82	23.01	
Shark 3	EM 0.1 ms	Before Stimuli	6529.38	14.84	1.45
Shark 3	EM 0.1 ms	After Stimuli	9484.85	21.56	
Shark 4	Seawater control	Before Stimuli	13206.59	30.01	1.23
Shark 4	Seawater control	After Stimuli	16184.88	36.78	
Shark 4	Skin extract	Before Stimuli	10988.27	24.97	1.37
Shark 4	Skin extract	After Stimuli	15090.30	34.30	
Shark 4	Food odor	Before Stimuli	12411.63	28.21	1.13
Shark 4	Food odor	After Stimuli	14079.14	32.00	
Shark 4	Control sound	Before Stimuli	6539.72	14.86	0.94
Shark 4	Control sound	After Stimuli	6178.79	14.04	
Shark 4	Orca sound	Before Stimuli	5971.75	13.57	1.16

Shark 4	Orca sound	After Stimuli	6898.43	15.68	
Shark 4	EM 0.6 ms	Before Stimuli	9231.99	20.98	1.23
Shark 4	EM 0.6 ms	After Stimuli	11328.67	25.75	
Shark 4	EM 0.3 ms	Before Stimuli	9363.79	21.28	1.15
Shark 4	EM 0.3 ms	After Stimuli	10791.73	24.53	
Shark 4	EM 0.1 ms	Before Stimuli	7963.86	18.10	1.33
Shark 4	EM 0.1 ms	After Stimuli	10616.37	24.13	
Shark 4	Morning recording	Before Stimuli	7020.22	15.96	0.89
Shark 4	Morning recording	After Stimuli	6228.96	14.16	
Shark 5	Seawater control	Before Stimuli	17968.72	40.84	1.00
Shark 5	Seawater control	After Stimuli	17911.34	40.71	
Shark 5	Skin extract	Before Stimuli	19522.32	44.37	0.81
Shark 5	Skin extract	After Stimuli	15899.01	36.13	
Shark 5	Food odor	Before Stimuli	19671.10	44.71	0.77
Shark 5	Food odor	After Stimuli	15164.20	34.46	
Shark 5	Control sound	Before Stimuli	9490.98	21.57	1.03
Shark 5	Control sound	After Stimuli	9811.94	22.30	
Shark 5	Orca sound	Before Stimuli	10145.73	23.06	0.89
Shark 5	Orca sound	After Stimuli	9076.60	20.63	
Shark 5	EM 0.6 ms	Before Stimuli	8428.91	19.16	0.79
Shark 5	EM 0.6 ms	After Stimuli	6661.22	15.14	
Shark 5	EM 0.3 ms	Before Stimuli	11032.41	25.07	0.60
Shark 5	EM 0.3 ms	After Stimuli	6591.83	14.98	
Shark 5	EM 0.1 ms	Before Stimuli	9879.30	22.45	0.55
Shark 5	EM 0.1 ms	After Stimuli	5455.07	12.40	
Shark 5	Morning recording	Before Stimuli	10216.25	23.22	0.91
Shark 5	Morning recording	After Stimuli	9321.27	21.18	
Shark 7	Seawater control	Before Stimuli	18140.42	41.23	1.17
Shark 7	Seawater control	After Stimuli	21179.95	48.14	
Shark 7	Skin extract	Before Stimuli	19286.32	43.83	1.04
Shark 7	Skin extract	After Stimuli	20100.84	45.68	
Shark 7	Food odor	Before Stimuli	19442.85	44.19	0.91
Shark 7	Food odor	After Stimuli	17761.49	40.37	
Shark 7	Control sound	Before Stimuli	8650.85	19.66	0.98
Shark 7	Control sound	After Stimuli	8481.64	19.28	
Shark 7	Orca sound	Before Stimuli	9194.86	20.90	0.99
Shark 7	Orca sound	After Stimuli	9088.23	20.66	
Shark 7	EM 0.6 ms	Before Stimuli	9386.99	21.33	1.25
Shark 7	EM 0.6 ms	After Stimuli	11723.78	26.64	
Shark 7	EM 0.3 ms	Before Stimuli	10242.01	23.28	0.99
Shark 7	EM 0.3 ms	After Stimuli	10172.25	23.12	
Shark 7	EM 0.1 ms	Before Stimuli	9451.50	21.48	0.96
Shark 7	EM 0.1 ms	After Stimuli	9101.79	20.69	
Shark 7	Morning recording	Before Stimuli	8933.81	20.30	1.11
Shark 7	Morning recording	After Stimuli	9951.90	22.62	
Shark 11	Seawater control	Before Stimuli	15559.54	35.36	1.10
Shark 11	Seawater control	After Stimuli	17178.68	39.04	
Shark 11	Skin extract	Before Stimuli	17326.73	39.38	1.00
Shark 11	Skin extract	After Stimuli	17380.63	39.50	
Shark 11	Food odor	Before Stimuli	18157.68	41.27	0.76
Shark 11	Food odor	After Stimuli	13736.81	31.22	
Shark 11	Control sound	Before Stimuli	9001.71	20.46	0.99
Shark 11	Control sound	After Stimuli	8955.02	20.35	
Shark 11	Orca sound	Before Stimuli	8394.83	19.08	0.96
Shark 11	Orca sound	After Stimuli	8090.43	18.39	
Shark 11	EM 0.6 ms	Before Stimuli	8674.86	19.72	0.87
Shark 11	EM 0.6 ms	After Stimuli	7534.05	17.12	
Shark 11	EM 0.3 ms	Before Stimuli	8082.92	18.37	0.90
Shark 11	EM 0.3 ms	After Stimuli	7283.95	16.55	
Shark 11	EM 0.1 ms	Before Stimuli	8923.59	20.28	0.81
Shark 11	EM 0.1 ms	After Stimuli	7244.78	16.47	

Shark 11	Morning recording	Before Stimuli	8403.05	19.10	1.02
Shark 11	Morning recording	After Stimuli	8540.29	19.41	
Shark 12	Seawater control	Before Stimuli	19982.39	45.41	1.01
Shark 12	Seawater control	After Stimuli	20128.17	45.75	
Shark 12	Skin extract	Before Stimuli	22976.26	52.22	0.89
Shark 12	Skin extract	After Stimuli	20337.14	46.22	
Shark 12	Food odor	Before Stimuli	20257.74	46.04	0.78
Shark 12	Food odor	After Stimuli	15718.34	35.72	
Shark 12	Control sound	Before Stimuli	10109.96	22.98	1.02
Shark 12	Control sound	After Stimuli	10299.56	23.41	
Shark 12	Orca sound	Before Stimuli	10454.20	23.76	0.98
Shark 12	Orca sound	After Stimuli	10201.89	23.19	
Shark 12	EM 0.6 ms	Before Stimuli	10237.93	23.27	0.70
Shark 12	EM 0.6 ms	After Stimuli	7198.20	16.36	
Shark 12	EM 0.3 ms	Before Stimuli	11312.23	25.71	0.59
Shark 12	EM 0.3 ms	After Stimuli	6679.56	15.18	
Shark 12	EM 0.1 ms	Before Stimuli	10565.59	24.01	0.77
Shark 12	EM 0.1 ms	After Stimuli	8109.99	18.43	
Shark 12	Morning recording	Before Stimuli	11054.55	25.12	0.84
Shark 12	Morning recording	After Stimuli	9336.34	21.22	

APPENDIX 2C – CONDITION 1

Square counts and fold change

Table 5. The position counts in each square from each trial. The trials are listed in the columns T1(Trial 1) to T8(Trial 8). The average from each trial is represented in the "Av." column. The fold change is represented in the F.C column and is calculated by dividing the "After_Stimuli" average by the "Before_Stimuli" average.

Stimuli	Shark	Square	Condtition	T1	T2	T3	T4	T5	T6	T7	T8	Av.	F.C
Sound control	Shark 1	Square 1	Before_Stimuli	32	71	63	60	61	22	206	0	515	
Sound control	Shark 1	Square 1	After_Stimuli	33	106	96	38	40	0	104	14	431	0.837
Sound control	Shark 1	Square 2	Before_Stimuli	196	253	74	140	209	23	352	66	1313	
Sound control	Shark 1	Square 2	After_Stimuli	66	186	149	258	102	41	355	53	1210	0.922
Sound control	Shark 1	Square 3	Before_Stimuli	31	76	16	79	48	28	274	172	724	
Sound control	Shark 1	Square 3	After_Stimuli	97	86	54	25	127	162	273	264	1088	1.503
Sound control	Shark 1	Square 4	Before_Stimuli	159	181	164	41	75	131	117	393	1261	
Sound control	Shark 1	Square 4	After_Stimuli	149	91	117	99	71	127	275	195	1124	0.891
Sound control	Shark 2	Square 1	Before_Stimuli	111	450	163	1068	354	224	420	114	2904	
Sound control	Shark 2	Square 1	After_Stimuli	454	0	232	1022	431	188	243	485	3055	1.052
Sound control	Shark 2	Square 2	Before_Stimuli	274	64	201	14	140	429	135	284	1541	
Sound control	Shark 2	Square 2	After_Stimuli	124	463	207	19	250	118	190	90	1461	0.948
Sound control	Shark 2	Square 3	Before_Stimuli	204	84	605	0	172	272	60	86	1483	
Sound control	Shark 2	Square 3	After_Stimuli	73	175	344	2	88	500	177	74	1433	0.966
Sound control	Shark 2	Square 4	Before_Stimuli	172	72	232	119	148	276	46	96	1161	
Sound control	Shark 2	Square 4	After_Stimuli	93	315	418	158	199	395	228	52	1858	1.6
Sound control	Shark 3	Square 1	Before_Stimuli	435	183	249	196	218	140	212	201	1834	
Sound control	Shark 3	Square 1	After_Stimuli	214	233	191	133	296	299	87	153	1606	0.876
Sound control	Shark 3	Square 2	Before_Stimuli	359	356	160	169	161	349	158	174	1886	
Sound control	Shark 3	Square 2	After_Stimuli	224	337	191	545	154	144	273	123	1991	1.056
Sound control	Shark 3	Square 3	Before_Stimuli	93	265	199	268	265	184	202	226	1702	
Sound control	Shark 3	Square 3	After_Stimuli	338	218	233	192	264	237	384	356	2222	1.306
Sound control	Shark 3	Square 4	Before_Stimuli	133	170	211	213	227	271	257	203	1685	
Sound control	Shark 3	Square 4	After_Stimuli	182	216	266	154	202	210	272	205	1707	1.013
Sound control	Shark 4	Square 1	Before_Stimuli	370	336	223	217	313	459	218	292	2428	
Sound control	Shark 4	Square 1	After_Stimuli	309	380	232	310	397	285	168	195	2276	0.937
Sound control	Shark 4	Square 2	Before_Stimuli	214	444	399	386	541	474	193	432	3083	
Sound control	Shark 4	Square 2	After_Stimuli	218	384	412	418	460	756	98	162	2908	0.943
Sound control	Shark 4	Square 3	Before_Stimuli	265	123	273	282	153	15	365	253	1729	
Sound control	Shark 4	Square 3	After_Stimuli	292	214	241	205	146	57	409	363	1927	1.115
Sound control	Shark 4	Square 4	Before_Stimuli	311	209	306	281	98	159	405	213	1982	
Sound control	Shark 4	Square 4	After_Stimuli	349	129	290	196	118	0	492	443	2017	1.018
Sound control	Shark 5	Square 1	Before_Stimuli	269	205	286	247	354	259	313	202	2135	
Sound control	Shark 5	Square 1	After_Stimuli	294	258	288	161	243	173	172	280	1869	0.875
Sound control	Shark 5	Square 2	Before_Stimuli	230	263	300	259	189	259	207	273	1980	
Sound control	Shark 5	Square 2	After_Stimuli	266	326	249	320	269	291	229	127	2077	1.049
Sound control	Shark 5	Square 3	Before_Stimuli	217	266	334	283	198	283	205	232	2018	
Sound control	Shark 5	Square 3	After_Stimuli	285	274	304	356	299	290	358	361	2527	1.252
Sound control	Shark 5	Square 4	Before_Stimuli	348	323	146	216	275	243	426	205	2182	
Sound control	Shark 5	Square 4	After_Stimuli	220	239	222	245	270	291	334	251	2072	0.95
Sound control	Shark 7	Square 1	Before_Stimuli	173	223	223	265	268	253			1405	
Sound control	Shark 7	Square 1	After_Stimuli	273	326	223	205	273	212			1512	1.076
Sound control	Shark 7	Square 2	Before_Stimuli	278	368	340	249	343	253			1831	
Sound control	Shark 7	Square 2	After_Stimuli	249	259	292	374	334	319			1827	0.998
Sound control	Shark 7	Square 3	Before_Stimuli	245	158	185	195	197	261			1241	
Sound control	Shark 7	Square 3	After_Stimuli	126	230	220	210	230	210			1226	0.988
Sound control	Shark 7	Square 4	Before_Stimuli	329	237	183	153	171	188			1261	
Sound control	Shark 7	Square 4	After_Stimuli	200	165	235	236	206	213			1255	0.995
Sound control	Shark 11	Square 1	Before_Stimuli	279	263	284	271	285	307	262	274	2225	
Sound control	Shark 11	Square 1	After_Stimuli	198	234	240	244	253	242	259	216	1886	0.848
Sound control	Shark 11	Square 2	Before_Stimuli	306	306	294	181	242	217	189	368	2103	
Sound control	Shark 11	Square 2	After_Stimuli	300	339	227	199	310	191	188	404	2158	1.026
Sound control	Shark 11	Square 3	Before_Stimuli	199	201	221	230	302	262	215	275	1905	
Sound control	Shark 11	Square 3	After_Stimuli	246	211	209	200	212	245	208	178	1709	0.897
Sound control	Shark 11	Square 4	Before_Stimuli	178	220	242	296	168	257	301	226	1888	
Sound control	Shark 11	Square 4	After_Stimuli	345	188	287	282	236	319	287	279	2223	1.177

Sound control	Shark 12	Square 1	Before_Stimuli	288	215	282	299	248	231	237	267	2067	
Sound control	Shark 12	Square 1	After_Stimuli	274	268	267	256	230	255	243	234	2027	0.981
Sound control	Shark 12	Square 2	Before_Stimuli	209	271	227	186	269	244	247	212	1865	
Sound control	Shark 12	Square 2	After_Stimuli	207	221	216	214	308	246	240	182	1834	0.983
Sound control	Shark 12	Square 3	Before_Stimuli	231	220	253	235	239	267	256	191	1892	
Sound control	Shark 12	Square 3	After_Stimuli	241	265	251	218	245	259	257	233	1969	1.041
Sound control	Shark 12	Square 4	Before_Stimuli	280	196	182	241	190	185	182	248	1704	
Sound control	Shark 12	Square 4	After_Stimuli	267	167	188	278	228	180	179	287	1774	1.041
Orca sound	Shark 1	Square 1	Before_Stimuli	30	106	78	52	24	34	235	28	587	
Orca sound	Shark 1	Square 1	After_Stimuli	122	62	21	29	105	0	298	17	654	1.114
Orca sound	Shark 1	Square 2	Before_Stimuli	142	276	96	134	77	27	13	53	818	
Orca sound	Shark 1	Square 2	After_Stimuli	203	190	130	227	38	17	0	53	858	1.049
Orca sound	Shark 1	Square 3	Before_Stimuli	137	7	78	139	36	107	249	524	1277	
Orca sound	Shark 1	Square 3	After_Stimuli	48	21	43	86	65	85	163	567	1078	0.844
Orca sound	Shark 1	Square 4	Before_Stimuli	106	74	106	165	28	56	143	86	764	
Orca sound	Shark 1	Square 4	After_Stimuli	84	169	76	43	102	116	322	73	985	1.289
Orca sound	Shark 2	Square 1	Before_Stimuli	592	317	389	553	326	333	46	213	2769	
Orca sound	Shark 2	Square 1	After_Stimuli	139	622	271	596	103	344	334	938	3347	1.209
Orca sound	Shark 2	Square 2	Before_Stimuli	124	170	134	77	190	357	185	530	1767	
Orca sound	Shark 2	Square 2	After_Stimuli	507	63	292	157	602	214	345	59	2239	1.267
Orca sound	Shark 2	Square 3	Before_Stimuli	74	129	113	38	235	154	478	114	1335	
Orca sound	Shark 2	Square 3	After_Stimuli	43	0	66	0	96	267	389	59	920	0.689
Orca sound	Shark 2	Square 4	Before_Stimuli	92	129	67	55	131	357	492	344	1667	
Orca sound	Shark 2	Square 4	After_Stimuli	0	96	127	134	185	376	133	61	1112	0.667
Orca sound	Shark 3	Square 1	Before_Stimuli	247	245	252	217	245	180	264	132	1782	
Orca sound	Shark 3	Square 1	After_Stimuli	243	257	289	267	150	247	247	263	1963	1.102
Orca sound	Shark 3	Square 2	Before_Stimuli	190	174	207	161	349	573	201	335	2190	
Orca sound	Shark 3	Square 2	After_Stimuli	188	174	244	151	409	153	197	152	1668	0.762
Orca sound	Shark 3	Square 3	Before_Stimuli	277	233	213	207	246	142	259	207	1784	
Orca sound	Shark 3	Square 3	After_Stimuli	300	257	117	246	212	251	247	231	1861	1.043
Orca sound	Shark 3	Square 4	Before_Stimuli	238	288	245	222	170	133	230	241	1767	
Orca sound	Shark 3	Square 4	After_Stimuli	237	218	260	214	196	199	256	222	1802	1.02
Orca sound	Shark 4	Square 1	Before_Stimuli	196	179	352	534	280	273	257	249	2320	
Orca sound	Shark 4	Square 1	After_Stimuli	319	472	325	222	284	299	326	252	2499	1.077
Orca sound	Shark 4	Square 2	Before_Stimuli	251	420	315	308	602	378	152	241	2667	
Orca sound	Shark 4	Square 2	After_Stimuli	339	309	326	330	382	524	183	321	2714	1.018
Orca sound	Shark 4	Square 3	Before_Stimuli	318	289	284	258	107	144	278	391	2069	
Orca sound	Shark 4	Square 3	After_Stimuli	218	226	260	283	177	139	218	254	1775	0.858
Orca sound	Shark 4	Square 4	Before_Stimuli	425	308	241	91	65	366	497	320	2313	
Orca sound	Shark 4	Square 4	After_Stimuli	286	176	274	353	304	89	455	362	2299	0.994
Orca sound	Shark 5	Square 1	Before_Stimuli	267	252	220	261	228	389	248	243	2108	
Orca sound	Shark 5	Square 1	After_Stimuli	251	224	291	293	206	236	172	262	1935	0.918
Orca sound	Shark 5	Square 2	Before_Stimuli	240	262	291	257	214	181	220	237	1902	
Orca sound	Shark 5	Square 2	After_Stimuli	302	299	243	215	469	287	180	265	2260	1.188
Orca sound	Shark 5	Square 3	Before_Stimuli	319	286	156	259	367	274	310	286	2257	
Orca sound	Shark 5	Square 3	After_Stimuli	258	309	253	339	193	271	359	279	2261	1.002
Orca sound	Shark 5	Square 4	Before_Stimuli	235	252	465	234	291	171	207	209	2064	
Orca sound	Shark 5	Square 4	After_Stimuli	248	258	230	208	225	236	328	173	1906	0.923
Orca sound	Shark 7	Square 1	Before_Stimuli	297	208	234	293	204				1236	
Orca sound	Shark 7	Square 1	After_Stimuli	238	300	173	290	246				1247	1.009
Orca sound	Shark 7	Square 2	Before_Stimuli	186	325	281	340	274				1406	
Orca sound	Shark 7	Square 2	After_Stimuli	227	253	350	316	310				1456	1.036
Orca sound	Shark 7	Square 3	Before_Stimuli	227	163	241	161	254				1046	
Orca sound	Shark 7	Square 3	After_Stimuli	191	203	153	230	268				1045	0.999
Orca sound	Shark 7	Square 4	Before_Stimuli	205	236	210	204	197				1052	
Orca sound	Shark 7	Square 4	After_Stimuli	247	138	314	124	201				1024	0.973

Orca sound	Shark 11	Square 1	Before_Stimuli	303	276	228	257	253	248	237	296	2098	
Orca sound	Shark 11	Square 1	After_Stimuli	294	286	297	279	328	274	342	244	2344	1.117
Orca sound	Shark 11	Square 2	Before_Stimuli	221	226	262	307	340	219	305	162	2042	
Orca sound	Shark 11	Square 2	After_Stimuli	329	321	267	251	274	224	360	197	2223	1.089
Orca sound	Shark 11	Square 3	Before_Stimuli	284	220	300	215	224	247	239	239	1968	
Orca sound	Shark 11	Square 3	After_Stimuli	150	227	240	293	220	242	157	226	1755	0.892
Orca sound	Shark 11	Square 4	Before_Stimuli	185	299	221	199	239	275	301	229	1948	
Orca sound	Shark 11	Square 4	After_Stimuli	271	207	184	189	218	275	292	279	1915	0.983
Orca sound	Shark 12	Square 1	Before_Stimuli	285	216	305	265	225	264	234	249	2043	
Orca sound	Shark 12	Square 1	After_Stimuli	258	274	256	260	216	277	281	230	2052	1.004
Orca sound	Shark 12	Square 2	Before_Stimuli	199	277	232	255	226	243	193	236	1861	
Orca sound	Shark 12	Square 2	After_Stimuli	248	218	223	243	322	264	212	224	1954	1.05
Orca sound	Shark 12	Square 3	Before_Stimuli	225	233	255	300	282	249	221	234	1999	
Orca sound	Shark 12	Square 3	After_Stimuli	256	261	243	291	228	236	188	233	1936	0.968
Orca sound	Shark 12	Square 4	Before_Stimuli	222	212	250	213	197	165	274	186	1719	
Orca sound	Shark 12	Square 4	After_Stimuli	250	167	283	183	221	168	258	184	1714	0.997
Seawater control	Shark 1	Square 1	Before_Stimuli	27	74	211	202	72	88	72	72	818	
Seawater control	Shark 1	Square 1	After_Stimuli	5	204	159	126	50	362	272	291	1469	1.796
Seawater control	Shark 1	Square 2	Before_Stimuli	63	497	371	14	88	377	1098	469	2977	
Seawater control	Shark 1	Square 2	After_Stimuli	54	557	409	95	119	631	676	478	3019	1.014
Seawater control	Shark 1	Square 3	Before_Stimuli	28	206	40	102	96	669	42	571	1754	
Seawater control	Shark 1	Square 3	After_Stimuli	84	250	132	256	56	460	308	369	1915	1.092
Seawater control	Shark 1	Square 4	Before_Stimuli	27	306	65	239	222	629	766	417	2671	
Seawater control	Shark 1	Square 4	After_Stimuli	16	127	122	85	281	590	498	275	1994	0.747
Seawater control	Shark 2	Square 1	Before_Stimuli	985	262	279	356	839	991	594	180	4486	
Seawater control	Shark 2	Square 1	After_Stimuli	335	1881	579	611	1923	604	308	1852	8093	1.804
Seawater control	Shark 2	Square 2	Before_Stimuli	462	917	1063	737	635	187	162	692	4855	
Seawater control	Shark 2	Square 2	After_Stimuli	742	71	265	448	93	535	359	383	2896	0.596
Seawater control	Shark 2	Square 3	Before_Stimuli	141	98	95	466	366	62	66	1513	2807	
Seawater control	Shark 2	Square 3	After_Stimuli	278	87	339	455	239	89	229	65	1781	0.634
Seawater control	Shark 2	Square 4	Before_Stimuli	113	1124	62	453	561	57	41	16	2427	
Seawater control	Shark 2	Square 4	After_Stimuli	601	245	300	343	63	307	379	55	2293	0.945
Seawater control	Shark 3	Square 1	Before_Stimuli	515	323	415	426	440	328	298	395	3140	
Seawater control	Shark 3	Square 1	After_Stimuli	466	442	476	697	429	447	316	413	3686	1.174
Seawater control	Shark 3	Square 2	Before_Stimuli	409	520	338	536	334	700	530	532	3899	
Seawater control	Shark 3	Square 2	After_Stimuli	399	429	433	722	408	713	771	481	4356	1.117
Seawater control	Shark 3	Square 3	Before_Stimuli	502	444	501	500	479	505	662	364	3957	
Seawater control	Shark 3	Square 3	After_Stimuli	550	466	386	306	443	408	372	460	3391	0.857
Seawater control	Shark 3	Square 4	Before_Stimuli	467	502	462	410	426	443	578	449	3737	
Seawater control	Shark 3	Square 4	After_Stimuli	546	527	515	414	556	387	583	502	4030	1.078
Seawater control	Shark 4	Square 1	Before_Stimuli	524	647	537	812	495	464	896	475	4850	
Seawater control	Shark 4	Square 1	After_Stimuli	516	625	938	627	476	559	553	784	5078	1.047
Seawater control	Shark 4	Square 2	Before_Stimuli	667	669	643	758	896	413	848	906	5800	
Seawater control	Shark 4	Square 2	After_Stimuli	1021	653	468	863	825	814	916	611	6171	1.064
Seawater control	Shark 4	Square 3	Before_Stimuli	580	465	524	303	295	691	415	393	3666	
Seawater control	Shark 4	Square 3	After_Stimuli	367	499	400	384	369	434	308	490	3251	0.887
Seawater control	Shark 4	Square 4	Before_Stimuli	577	577	631	472	610	801	124	572	4364	
Seawater control	Shark 4	Square 4	After_Stimuli	444	569	559	452	604	495	507	343	3973	0.91
Seawater control	Shark 5	Square 1	Before_Stimuli	664	501	462	439	505	453	499	492	4015	
Seawater control	Shark 5	Square 1	After_Stimuli	448	671	538	576	489	685	605	541	4553	1.134
Seawater control	Shark 5	Square 2	Before_Stimuli	457	554	452	329	502	486	487	495	3762	
Seawater control	Shark 5	Square 2	After_Stimuli	531	645	640	535	551	630	573	509	4614	1.226
Seawater control	Shark 5	Square 3	Before_Stimuli	583	458	536	578	567	502	459	464	4147	
Seawater control	Shark 5	Square 3	After_Stimuli	720	519	480	598	598	486	519	601	4521	1.09
Seawater control	Shark 5	Square 4	Before_Stimuli	491	507	577	714	447	471	460	335	4002	
Seawater control	Shark 5	Square 4	After_Stimuli	517	361	505	456	505	368	407	376	3495	0.873

Seawater control	Shark 7	Square 1	Before_Stimuli	483	205	575	382	445	412	436	479	3417	
Seawater control	Shark 7	Square 1	After_Stimuli	513	519	454	570	383	526	467	456	3888	1.138
Seawater control	Shark 7	Square 2	Before_Stimuli	500	143	550	592	504	636	763	605	4293	
Seawater control	Shark 7	Square 2	After_Stimuli	668	697	571	549	636	511	646	701	4979	1.16
Seawater control	Shark 7	Square 3	Before_Stimuli	386	836	504	339	394	274	423	459	3615	
Seawater control	Shark 7	Square 3	After_Stimuli	455	492	449	417	354	417	374	391	3349	0.926
Seawater control	Shark 7	Square 4	Before_Stimuli	482	1100	425	575	468	540	304	378	4272	
Seawater control	Shark 7	Square 4	After_Stimuli	498	458	607	511	535	550	467	433	4059	0.95
Seawater control	Shark 11	Square 1	Before_Stimuli	497	538	596	491	495	530	575	464	4186	
Seawater control	Shark 11	Square 1	After_Stimuli	523	607	484	456	544	573	582	521	4290	1.025
Seawater control	Shark 11	Square 2	Before_Stimuli	594	389	592	607	811	406	552	631	4582	
Seawater control	Shark 11	Square 2	After_Stimuli	478	672	692	604	598	404	541	644	4633	1.011
Seawater control	Shark 11	Square 3	Before_Stimuli	510	450	456	462	343	503	578	480	3782	
Seawater control	Shark 11	Square 3	After_Stimuli	615	489	412	506	387	462	561	459	3891	1.029
Seawater control	Shark 11	Square 4	Before_Stimuli	410	664	451	402	505	551	355	668	4006	
Seawater control	Shark 11	Square 4	After_Stimuli	577	348	541	469	566	624	395	632	4152	1.036
Seawater control	Shark 12	Square 1	Before_Stimuli	548	515	528	530	474	507	496	504	4102	
Seawater control	Shark 12	Square 1	After_Stimuli	532	453	491	531	486	450	455	474	3872	0.944
Seawater control	Shark 12	Square 2	Before_Stimuli	418	496	583	531	501	498	419	529	3975	
Seawater control	Shark 12	Square 2	After_Stimuli	446	593	505	318	271	245	719	337	3434	0.864
Seawater control	Shark 12	Square 3	Before_Stimuli	465	484	477	470	454	457	409	441	3657	
Seawater control	Shark 12	Square 3	After_Stimuli	510	513	560	692	602	795	390	743	4805	1.314
Seawater control	Shark 12	Square 4	Before_Stimuli	569	374	640	339	354	369	527	376	3548	
Seawater control	Shark 12	Square 4	After_Stimuli	545	638	608	707	766	745	621	579	5209	1.468
Food odor	Shark 1	Square 1	Before_Stimuli	221	34	315	340					910	
Food odor	Shark 1	Square 1	After_Stimuli	178	85	480	291					1034	1.136
Food odor	Shark 1	Square 2	Before_Stimuli	311	24	708	320					1363	
Food odor	Shark 1	Square 2	After_Stimuli	271	122	309	628					1330	0.976
Food odor	Shark 1	Square 3	Before_Stimuli	83	195	501	531					1310	
Food odor	Shark 1	Square 3	After_Stimuli	126	65	480	344					1015	0.775
Food odor	Shark 1	Square 4	Before_Stimuli	233	103	594	667					1597	
Food odor	Shark 1	Square 4	After_Stimuli	118	321	517	473					1429	0.895
Food odor	Shark 2	Square 1	Before_Stimuli	548	948	322	244					2062	
Food odor	Shark 2	Square 1	After_Stimuli	1155	383	1198	282					3018	1.464
Food odor	Shark 2	Square 2	Before_Stimuli	350	385	527	339					1601	
Food odor	Shark 2	Square 2	After_Stimuli	330	584	422	522					1858	1.161
Food odor	Shark 2	Square 3	Before_Stimuli	278	87	486	395					1246	
Food odor	Shark 2	Square 3	After_Stimuli	161	530	84	129					904	0.726
Food odor	Shark 2	Square 4	Before_Stimuli	48	92	1066	388					1594	
Food odor	Shark 2	Square 4	After_Stimuli	156	313	345	423					1237	0.776
Food odor	Shark 3	Square 1	Before_Stimuli	431	473	537	436					1877	
Food odor	Shark 3	Square 1	After_Stimuli	508	559	516	597					2180	1.161
Food odor	Shark 3	Square 2	Before_Stimuli	354	341	679	564					1938	
Food odor	Shark 3	Square 2	After_Stimuli	929	567	447	509					2452	1.265
Food odor	Shark 3	Square 3	Before_Stimuli	444	463	462	395					1764	
Food odor	Shark 3	Square 3	After_Stimuli	269	425	602	374					1670	0.947
Food odor	Shark 3	Square 4	Before_Stimuli	452	465	488	603					2008	
Food odor	Shark 3	Square 4	After_Stimuli	297	348	543	502					1690	0.842
Food odor	Shark 4	Square 1	Before_Stimuli	837	502	372	424					2135	
Food odor	Shark 4	Square 1	After_Stimuli	646	577	440	612					2275	1.066
Food odor	Shark 4	Square 2	Before_Stimuli	700	657	716	580					2653	
Food odor	Shark 4	Square 2	After_Stimuli	812	729	608	505					2654	1
Food odor	Shark 4	Square 3	Before_Stimuli	443	473	402	643					1961	
Food odor	Shark 4	Square 3	After_Stimuli	376	376	540	665					1957	0.998
Food odor	Shark 4	Square 4	Before_Stimuli	354	714	798	709					2575	
Food odor	Shark 4	Square 4	After_Stimuli	533	625	665	492					2315	0.899

Food odor	Shark 5	Square 1	Before_Stimuli	531	481	436	400					1848	
Food odor	Shark 5	Square 1	After_Stimuli	506	633	553	702					2394	1.295
Food odor	Shark 5	Square 2	Before_Stimuli	540	524	500	509					2073	
Food odor	Shark 5	Square 2	After_Stimuli	625	724	481	454					2284	1.102
Food odor	Shark 5	Square 3	Before_Stimuli	557	557	520	626					2260	
Food odor	Shark 5	Square 3	After_Stimuli	417	418	440	458					1733	0.767
Food odor	Shark 5	Square 4	Before_Stimuli	490	538	384	643					2055	
Food odor	Shark 5	Square 4	After_Stimuli	514	385	708	538					2145	1.044
Food odor	Shark 7	Square 1	Before_Stimuli	498	606	426	488					2018	
Food odor	Shark 7	Square 1	After_Stimuli	348	785	340	557					2030	1.006
Food odor	Shark 7	Square 2	Before_Stimuli	548	385	412	526					1871	
Food odor	Shark 7	Square 2	After_Stimuli	585	567	758	607					2517	1.345
Food odor	Shark 7	Square 3	Before_Stimuli	524	482	378	424					1808	
Food odor	Shark 7	Square 3	After_Stimuli	460	314	320	414					1508	0.834
Food odor	Shark 7	Square 4	Before_Stimuli	422	529	475	527					1953	
Food odor	Shark 7	Square 4	After_Stimuli	588	327	460	421					1796	0.92
Food odor	Shark 11	Square 1	Before_Stimuli	547	559	599	686					2391	
Food odor	Shark 11	Square 1	After_Stimuli	824	954	645	554					2977	1.245
Food odor	Shark 11	Square 2	Before_Stimuli	449	468	441	397					1755	
Food odor	Shark 11	Square 2	After_Stimuli	302	652	664	670					2288	1.304
Food odor	Shark 11	Square 3	Before_Stimuli	416	522	530	549					2017	
Food odor	Shark 11	Square 3	After_Stimuli	822	276	321	567					1986	0.985
Food odor	Shark 11	Square 4	Before_Stimuli	582	606	504	496					2188	
Food odor	Shark 11	Square 4	After_Stimuli	133	343	477	400					1353	0.618
Food odor	Shark 12	Square 1	Before_Stimuli	566	539	520	472					2097	
Food odor	Shark 12	Square 1	After_Stimuli	661	336	553	470					2020	0.963
Food odor	Shark 12	Square 2	Before_Stimuli	479	501	379	629					1988	
Food odor	Shark 12	Square 2	After_Stimuli	323	355	880	555					2113	1.063
Food odor	Shark 12	Square 3	Before_Stimuli	547	449	424	388					1808	
Food odor	Shark 12	Square 3	After_Stimuli	565	973	290	492					2320	1.283
Food odor	Shark 12	Square 4	Before_Stimuli	383	324	507	492					1706	
Food odor	Shark 12	Square 4	After_Stimuli	738	529	455	411					2133	1.25
Skin extract	Shark 1	Square 1	Before_Stimuli	126	32	320						478	
Skin extract	Shark 1	Square 1	After_Stimuli	100	34	275						409	0.856
Skin extract	Shark 1	Square 2	Before_Stimuli	394	80	214						688	
Skin extract	Shark 1	Square 2	After_Stimuli	241	198	515						954	1.387
Skin extract	Shark 1	Square 3	Before_Stimuli	160	613	268						1041	
Skin extract	Shark 1	Square 3	After_Stimuli	124	441	442						1007	0.967
Skin extract	Shark 1	Square 4	Before_Stimuli	132	336	605						1073	
Skin extract	Shark 1	Square 4	After_Stimuli	125	439	393						957	0.892
Skin extract	Shark 2	Square 1	Before_Stimuli	1973	1134	212						3319	
Skin extract	Shark 2	Square 1	After_Stimuli	296	497	1656						2449	0.738
Skin extract	Shark 2	Square 2	Before_Stimuli	116	207	839						1162	
Skin extract	Shark 2	Square 2	After_Stimuli	1154	855	413						2422	2.084
Skin extract	Shark 2	Square 3	Before_Stimuli	11	122	336						469	
Skin extract	Shark 2	Square 3	After_Stimuli	301	241	174						716	1.527
Skin extract	Shark 2	Square 4	Before_Stimuli	301	87	1014						1402	
Skin extract	Shark 2	Square 4	After_Stimuli	343	299	0						642	0.458
Skin extract	Shark 3	Square 1	Before_Stimuli	362	607	490						1459	
Skin extract	Shark 3	Square 1	After_Stimuli	495	449	570						1514	1.038
Skin extract	Shark 3	Square 2	Before_Stimuli	434	333	456						1223	
Skin extract	Shark 3	Square 2	After_Stimuli	405	897	454						1756	1.436
Skin extract	Shark 3	Square 3	Before_Stimuli	552	519	481						1552	
Skin extract	Shark 3	Square 3	After_Stimuli	504	220	384						1108	0.714
Skin extract	Shark 3	Square 4	Before_Stimuli	490	413	408						1311	
Skin extract	Shark 3	Square 4	After_Stimuli	462	573	435						1470	1.121

Skin extract	Shark 4	Square 1	Before_Stimuli	470	497	464						1431	
Skin extract	Shark 4	Square 1	After_Stimuli	599	636	493						1728	1.208
Skin extract	Shark 4	Square 2	Before_Stimuli	614	512	733						1859	
Skin extract	Shark 4	Square 2	After_Stimuli	377	460	751						1588	0.854
Skin extract	Shark 4	Square 3	Before_Stimuli	579	644	158						1381	
Skin extract	Shark 4	Square 3	After_Stimuli	973	517	535						2025	1.466
Skin extract	Shark 4	Square 4	Before_Stimuli	661	680	876						2217	
Skin extract	Shark 4	Square 4	After_Stimuli	426	714	440						1580	0.713
Skin extract	Shark 5	Square 1	Before_Stimuli	447	445	507	476					1875	
Skin extract	Shark 5	Square 1	After_Stimuli	773	769	536	492					2570	1.371
Skin extract	Shark 5	Square 2	Before_Stimuli	578	655	500	522					2255	
Skin extract	Shark 5	Square 2	After_Stimuli	619	439	579	546					2183	0.968
Skin extract	Shark 5	Square 3	Before_Stimuli	582	564	500	490					2136	
Skin extract	Shark 5	Square 3	After_Stimuli	355	557	568	614					2094	0.98
Skin extract	Shark 5	Square 4	Before_Stimuli	496	488	368	411					1763	
Skin extract	Shark 5	Square 4	After_Stimuli	571	398	486	400					1855	1.052
Skin extract	Shark 7	Square 1	Before_Stimuli	465	505	456						1426	
Skin extract	Shark 7	Square 1	After_Stimuli	685	696	538						1919	1.346
Skin extract	Shark 7	Square 2	Before_Stimuli	833	503	642						1978	
Skin extract	Shark 7	Square 2	After_Stimuli	614	560	483						1657	0.838
Skin extract	Shark 7	Square 3	Before_Stimuli	388	428	370						1186	
Skin extract	Shark 7	Square 3	After_Stimuli	412	400	352						1164	0.981
Skin extract	Shark 7	Square 4	Before_Stimuli	485	603	518						1606	
Skin extract	Shark 7	Square 4	After_Stimuli	394	438	665						1497	0.932
Skin extract	Shark 11	Square 1	Before_Stimuli	540	446	596						1582	
Skin extract	Shark 11	Square 1	After_Stimuli	588	475	585						1648	1.042
Skin extract	Shark 11	Square 2	Before_Stimuli	393	667	610						1670	
Skin extract	Shark 11	Square 2	After_Stimuli	545	556	652						1753	1.05
Skin extract	Shark 11	Square 3	Before_Stimuli	452	491	529						1472	
Skin extract	Shark 11	Square 3	After_Stimuli	471	498	445						1414	0.961
Skin extract	Shark 11	Square 4	Before_Stimuli	548	426	454						1428	
Skin extract	Shark 11	Square 4	After_Stimuli	440	436	513						1389	0.973
Skin extract	Shark 12	Square 1	Before_Stimuli	492	501	504						1497	
Skin extract	Shark 12	Square 1	After_Stimuli	614	450	487						1551	1.036
Skin extract	Shark 12	Square 2	Before_Stimuli	389	385	485						1259	
Skin extract	Shark 12	Square 2	After_Stimuli	492	512	507						1511	1.2
Skin extract	Shark 12	Square 3	Before_Stimuli	439	420	495						1354	
Skin extract	Shark 12	Square 3	After_Stimuli	467	498	481						1446	1.068
Skin extract	Shark 12	Square 4	Before_Stimuli	531	527	372						1430	
Skin extract	Shark 12	Square 4	After_Stimuli	486	524	404						1414	0.989
EM 0.6 ms	Shark 1	Square 1	Before_Stimuli	61	0	288	275					624	
EM 0.6 ms	Shark 1	Square 1	After_Stimuli	124	221	229	274					848	1.359
EM 0.6 ms	Shark 1	Square 2	Before_Stimuli	101	335	380	145					961	
EM 0.6 ms	Shark 1	Square 2	After_Stimuli	182	288	216	124					810	0.843
EM 0.6 ms	Shark 1	Square 3	Before_Stimuli	141	198	162	269					770	
EM 0.6 ms	Shark 1	Square 3	After_Stimuli	344	289	260	268					1161	1.508
EM 0.6 ms	Shark 1	Square 4	Before_Stimuli	241	281	133	43					698	
EM 0.6 ms	Shark 1	Square 4	After_Stimuli	228	216	214	300					958	1.372
EM 0.6 ms	Shark 2	Square 1	Before_Stimuli	438	239	100						777	
EM 0.6 ms	Shark 2	Square 1	After_Stimuli	139	162	297						598	0.77
EM 0.6 ms	Shark 2	Square 2	Before_Stimuli	48	114	105						267	
EM 0.6 ms	Shark 2	Square 2	After_Stimuli	139	417	276						832	3.116
EM 0.6 ms	Shark 2	Square 3	Before_Stimuli	67	31	48						146	
EM 0.6 ms	Shark 2	Square 3	After_Stimuli	409	70	150						629	4.308
EM 0.6 ms	Shark 2	Square 4	Before_Stimuli	94	25	115						234	
EM 0.6 ms	Shark 2	Square 4	After_Stimuli	98	236	119						453	1.936

EM 0.6 ms	Shark 3	Square 1	Before_Stimuli	139	228	352						719	
EM 0.6 ms	Shark 3	Square 1	After_Stimuli	327	311	223						861	1.1975
EM 0.6 ms	Shark 3	Square 2	Before_Stimuli	361	294	369						1024	
EM 0.6 ms	Shark 3	Square 2	After_Stimuli	213	243	218						674	0.6582
EM 0.6 ms	Shark 3	Square 3	Before_Stimuli	331	258	157						746	
EM 0.6 ms	Shark 3	Square 3	After_Stimuli	236	276	349						861	1.1542
EM 0.6 ms	Shark 3	Square 4	Before_Stimuli	238	254	102						594	
EM 0.6 ms	Shark 3	Square 4	After_Stimuli	175	235	237						647	1.0892
EM 0.6 ms	Shark 4	Square 1	Before_Stimuli	462	405	297						1164	
EM 0.6 ms	Shark 4	Square 1	After_Stimuli	198	397	304						899	0.7723
EM 0.6 ms	Shark 4	Square 2	Before_Stimuli	138	420	342						900	
EM 0.6 ms	Shark 4	Square 2	After_Stimuli	308	571	279						1158	1.2867
EM 0.6 ms	Shark 4	Square 3	Before_Stimuli	359	196	390						945	
EM 0.6 ms	Shark 4	Square 3	After_Stimuli	338	125	374						837	0.8857
EM 0.6 ms	Shark 4	Square 4	Before_Stimuli	229	175	162						566	
EM 0.6 ms	Shark 4	Square 4	After_Stimuli	355	104	223						682	1.2049
EM 0.6 ms	Shark 5	Square 1	Before_Stimuli	197	279	292						768	
EM 0.6 ms	Shark 5	Square 1	After_Stimuli	180	316	222						718	0.9349
EM 0.6 ms	Shark 5	Square 2	Before_Stimuli	143	247	208						598	
EM 0.6 ms	Shark 5	Square 2	After_Stimuli	325	331	402						1058	1.7692
EM 0.6 ms	Shark 5	Square 3	Before_Stimuli	261	230	391						882	
EM 0.6 ms	Shark 5	Square 3	After_Stimuli	405	304	138						847	0.9603
EM 0.6 ms	Shark 5	Square 4	Before_Stimuli	439	297	228						964	
EM 0.6 ms	Shark 5	Square 4	After_Stimuli	262	243	408						913	0.9471
EM 0.6 ms	Shark 7	Square 1	Before_Stimuli	428	222	220						870	
EM 0.6 ms	Shark 7	Square 1	After_Stimuli	235	290	237						762	0.8759
EM 0.6 ms	Shark 7	Square 2	Before_Stimuli	258	194	352						804	
EM 0.6 ms	Shark 7	Square 2	After_Stimuli	293	428	499						1220	1.5174
EM 0.6 ms	Shark 7	Square 3	Before_Stimuli	217	199	224						640	
EM 0.6 ms	Shark 7	Square 3	After_Stimuli	249	110	117						476	0.7438
EM 0.6 ms	Shark 7	Square 4	Before_Stimuli	158	245	210						613	
EM 0.6 ms	Shark 7	Square 4	After_Stimuli	318	263	238						819	1.3361
EM 0.6 ms	Shark 11	Square 1	Before_Stimuli	331	263	346						940	
EM 0.6 ms	Shark 11	Square 1	After_Stimuli	215	49	352						616	0.6553
EM 0.6 ms	Shark 11	Square 2	Before_Stimuli	232	459	223						914	
EM 0.6 ms	Shark 11	Square 2	After_Stimuli	265	419	217						901	0.9858
EM 0.6 ms	Shark 11	Square 3	Before_Stimuli	287	150	267						704	
EM 0.6 ms	Shark 11	Square 3	After_Stimuli	352	324	246						922	1.3097
EM 0.6 ms	Shark 11	Square 4	Before_Stimuli	279	311	284						874	
EM 0.6 ms	Shark 11	Square 4	After_Stimuli	315	382	353						1050	1.2014
EM 0.6 ms	Shark 12	Square 1	Before_Stimuli	250	238	248						736	
EM 0.6 ms	Shark 12	Square 1	After_Stimuli	198	189	232						619	0.841
EM 0.6 ms	Shark 12	Square 2	Before_Stimuli	260	414	233						907	
EM 0.6 ms	Shark 12	Square 2	After_Stimuli	268	547	722						1537	1.6946
EM 0.6 ms	Shark 12	Square 3	Before_Stimuli	252	179	233						664	
EM 0.6 ms	Shark 12	Square 3	After_Stimuli	367	217	75						659	0.9925
EM 0.6 ms	Shark 12	Square 4	Before_Stimuli	177	221	270						668	
EM 0.6 ms	Shark 12	Square 4	After_Stimuli	347	218	120						685	1.0254
EM 0.3 ms	Shark 1	Square 1	Before_Stimuli									0	
EM 0.3 ms	Shark 1	Square 1	After_Stimuli									0	#DIV/0!
EM 0.3 ms	Shark 1	Square 2	Before_Stimuli									0	
EM 0.3 ms	Shark 1	Square 2	After_Stimuli									0	#DIV/0!
EM 0.3 ms	Shark 1	Square 3	Before_Stimuli									0	
EM 0.3 ms	Shark 1	Square 3	After_Stimuli									0	#DIV/0!
EM 0.3 ms	Shark 1	Square 4	Before_Stimuli									0	
EM 0.3 ms	Shark 1	Square 4	After_Stimuli									0	#DIV/0!

EM 0.3 ms	Shark 2	Square 1	Before_Stimuli	322	141	239						702	
EM 0.3 ms	Shark 2	Square 1	After_Stimuli	314	263	158						735	1.047
EM 0.3 ms	Shark 2	Square 2	Before_Stimuli	166	49	320						535	
EM 0.3 ms	Shark 2	Square 2	After_Stimuli	281	353	248						882	1.6486
EM 0.3 ms	Shark 2	Square 3	Before_Stimuli	142	59	53						254	
EM 0.3 ms	Shark 2	Square 3	After_Stimuli	111	288	222						621	2.4449
EM 0.3 ms	Shark 2	Square 4	Before_Stimuli	113	96	80						289	
EM 0.3 ms	Shark 2	Square 4	After_Stimuli	317	104	252						673	2.3287
EM 0.3 ms	Shark 3	Square 1	Before_Stimuli	230	250	329						809	
EM 0.3 ms	Shark 3	Square 1	After_Stimuli	333	270	256						859	1.0618
EM 0.3 ms	Shark 3	Square 2	Before_Stimuli	308	435	172						915	
EM 0.3 ms	Shark 3	Square 2	After_Stimuli	241	311	273						825	0.9016
EM 0.3 ms	Shark 3	Square 3	Before_Stimuli	296	177	322						795	
EM 0.3 ms	Shark 3	Square 3	After_Stimuli	272	231	264						767	0.9648
EM 0.3 ms	Shark 3	Square 4	Before_Stimuli	231	148	198						577	
EM 0.3 ms	Shark 3	Square 4	After_Stimuli	209	268	244						721	1.2496
EM 0.3 ms	Shark 4	Square 1	Before_Stimuli	262	388	473						1123	
EM 0.3 ms	Shark 4	Square 1	After_Stimuli	428	365	459						1252	1.1149
EM 0.3 ms	Shark 4	Square 2	Before_Stimuli	245	254	72						571	
EM 0.3 ms	Shark 4	Square 2	After_Stimuli	311	307	173						791	1.3853
EM 0.3 ms	Shark 4	Square 3	Before_Stimuli	317	276	308						901	
EM 0.3 ms	Shark 4	Square 3	After_Stimuli	235	267	428						930	1.0322
EM 0.3 ms	Shark 4	Square 4	Before_Stimuli	377	283	346						1006	
EM 0.3 ms	Shark 4	Square 4	After_Stimuli	219	260	138						617	0.6133
EM 0.3 ms	Shark 5	Square 1	Before_Stimuli	253	268	245						766	
EM 0.3 ms	Shark 5	Square 1	After_Stimuli	190	154	250						594	0.7755
EM 0.3 ms	Shark 5	Square 2	Before_Stimuli	253	261	261						775	
EM 0.3 ms	Shark 5	Square 2	After_Stimuli	348	259	248						855	1.1032
EM 0.3 ms	Shark 5	Square 3	Before_Stimuli	288	264	313						865	
EM 0.3 ms	Shark 5	Square 3	After_Stimuli	400	592	314						1306	1.5098
EM 0.3 ms	Shark 5	Square 4	Before_Stimuli	185	272	203						660	
EM 0.3 ms	Shark 5	Square 4	After_Stimuli	209	181	388						778	1.1788
EM 0.3 ms	Shark 7	Square 1	Before_Stimuli	212	316	290						818	
EM 0.3 ms	Shark 7	Square 1	After_Stimuli	250	277	261						788	0.9633
EM 0.3 ms	Shark 7	Square 2	Before_Stimuli	353	252	325						930	
EM 0.3 ms	Shark 7	Square 2	After_Stimuli	352	333	327						1012	1.0882
EM 0.3 ms	Shark 7	Square 3	Before_Stimuli	185	198	192						575	
EM 0.3 ms	Shark 7	Square 3	After_Stimuli	204	178	178						560	0.9739
EM 0.3 ms	Shark 7	Square 4	Before_Stimuli	250	212	171						633	
EM 0.3 ms	Shark 7	Square 4	After_Stimuli	183	312	312						807	1.2749
EM 0.3 ms	Shark 11	Square 1	Before_Stimuli	298	236	298						832	
EM 0.3 ms	Shark 11	Square 1	After_Stimuli	210	380	325						915	1.0998
EM 0.3 ms	Shark 11	Square 2	Before_Stimuli	193	382	380						955	
EM 0.3 ms	Shark 11	Square 2	After_Stimuli	395	170	214						779	0.8157
EM 0.3 ms	Shark 11	Square 3	Before_Stimuli	275	228	225						728	
EM 0.3 ms	Shark 11	Square 3	After_Stimuli	280	414	251						945	1.2981
EM 0.3 ms	Shark 11	Square 4	Before_Stimuli	271	227	210						708	
EM 0.3 ms	Shark 11	Square 4	After_Stimuli	289	168	343						800	1.1299
EM 0.3 ms	Shark 12	Square 1	Before_Stimuli	262	288	214						764	
EM 0.3 ms	Shark 12	Square 1	After_Stimuli	142	99	252						493	0.6453
EM 0.3 ms	Shark 12	Square 2	Before_Stimuli	256	242	525						1023	
EM 0.3 ms	Shark 12	Square 2	After_Stimuli	600	404	529						1533	1.4985
EM 0.3 ms	Shark 12	Square 3	Before_Stimuli	232	225	135						592	
EM 0.3 ms	Shark 12	Square 3	After_Stimuli	99	181	59						339	0.5726
EM 0.3 ms	Shark 12	Square 4	Before_Stimuli	272	234	245						751	
EM 0.3 ms	Shark 12	Square 4	After_Stimuli	326	476	333						1135	1.5113

EM 0.1 ms	Shark 1	Square 1	Before_Stimuli	114	34	211	0					359	
EM 0.1 ms	Shark 1	Square 1	After_Stimuli	331	110	132	225					798	2.2228
EM 0.1 ms	Shark 1	Square 2	Before_Stimuli	89	177	178	196					640	
EM 0.1 ms	Shark 1	Square 2	After_Stimuli	221	418	392	288					1319	2.0609
EM 0.1 ms	Shark 1	Square 3	Before_Stimuli	482	162	186	96					926	
EM 0.1 ms	Shark 1	Square 3	After_Stimuli	233	187	141	239					800	0.8639
EM 0.1 ms	Shark 1	Square 4	Before_Stimuli	155	510	386	524					1575	
EM 0.1 ms	Shark 1	Square 4	After_Stimuli	219	216	397	285					1117	0.7092
EM 0.1 ms	Shark 2	Square 1	Before_Stimuli	194	378	113						685	
EM 0.1 ms	Shark 2	Square 1	After_Stimuli	84	483	292						859	1.254
EM 0.1 ms	Shark 2	Square 2	Before_Stimuli	53	202	438						693	
EM 0.1 ms	Shark 2	Square 2	After_Stimuli	344	178	115						637	0.9192
EM 0.1 ms	Shark 2	Square 3	Before_Stimuli	24	291	583						898	
EM 0.1 ms	Shark 2	Square 3	After_Stimuli	246	255	351						852	0.9488
EM 0.1 ms	Shark 2	Square 4	Before_Stimuli	98	330	67						495	
EM 0.1 ms	Shark 2	Square 4	After_Stimuli	194	285	443						922	1.8626
EM 0.1 ms	Shark 3	Square 1	Before_Stimuli	292	268	362						922	
EM 0.1 ms	Shark 3	Square 1	After_Stimuli	270	366	406						1042	1.1302
EM 0.1 ms	Shark 3	Square 2	Before_Stimuli	233	562	519						1314	
EM 0.1 ms	Shark 3	Square 2	After_Stimuli	306	203	343						852	0.6484
EM 0.1 ms	Shark 3	Square 3	Before_Stimuli	316	66	40						422	
EM 0.1 ms	Shark 3	Square 3	After_Stimuli	323	257	176						756	1.7915
EM 0.1 ms	Shark 3	Square 4	Before_Stimuli	194	68	202						464	
EM 0.1 ms	Shark 3	Square 4	After_Stimuli	248	206	216						670	1.444
EM 0.1 ms	Shark 4	Square 1	Before_Stimuli	682	354	538						1574	
EM 0.1 ms	Shark 4	Square 1	After_Stimuli	396	393	361						1150	0.7306
EM 0.1 ms	Shark 4	Square 2	Before_Stimuli	189	100	250						539	
EM 0.1 ms	Shark 4	Square 2	After_Stimuli	171	212	158						541	1.0037
EM 0.1 ms	Shark 4	Square 3	Before_Stimuli	322	218	259						799	
EM 0.1 ms	Shark 4	Square 3	After_Stimuli	442	313	374						1129	1.413
EM 0.1 ms	Shark 4	Square 4	Before_Stimuli	0	521	147						668	
EM 0.1 ms	Shark 4	Square 4	After_Stimuli	190	280	306						776	1.1617
EM 0.1 ms	Shark 5	Square 1	Before_Stimuli	229	234	275						738	
EM 0.1 ms	Shark 5	Square 1	After_Stimuli	421	550	321						1292	1.7507
EM 0.1 ms	Shark 5	Square 2	Before_Stimuli	274	282	211						767	
EM 0.1 ms	Shark 5	Square 2	After_Stimuli	132	191	350						673	0.8774
EM 0.1 ms	Shark 5	Square 3	Before_Stimuli	246	247	338						831	
EM 0.1 ms	Shark 5	Square 3	After_Stimuli	371	191	232						794	0.9555
EM 0.1 ms	Shark 5	Square 4	Before_Stimuli	194	169	179						542	
EM 0.1 ms	Shark 5	Square 4	After_Stimuli	274	187	286						747	1.3782
EM 0.1 ms	Shark 7	Square 1	Before_Stimuli	195	279	201						675	
EM 0.1 ms	Shark 7	Square 1	After_Stimuli	256	255	165						676	1.0015
EM 0.1 ms	Shark 7	Square 2	Before_Stimuli	297	287	264						848	
EM 0.1 ms	Shark 7	Square 2	After_Stimuli	346	438	457						1241	1.4634
EM 0.1 ms	Shark 7	Square 3	Before_Stimuli	154	155	147						456	
EM 0.1 ms	Shark 7	Square 3	After_Stimuli	160	117	149						426	0.9342
EM 0.1 ms	Shark 7	Square 4	Before_Stimuli	207	205	342						754	
EM 0.1 ms	Shark 7	Square 4	After_Stimuli	248	275	207						730	0.9682
EM 0.1 ms	Shark 11	Square 1	Before_Stimuli	367	357	165						889	
EM 0.1 ms	Shark 11	Square 1	After_Stimuli	161	218	248						627	0.7053
EM 0.1 ms	Shark 11	Square 2	Before_Stimuli	172	172	484						828	
EM 0.1 ms	Shark 11	Square 2	After_Stimuli	538	430	392						1360	1.6425
EM 0.1 ms	Shark 11	Square 3	Before_Stimuli	253	261	231						745	
EM 0.1 ms	Shark 11	Square 3	After_Stimuli	208	247	190						645	0.8658
EM 0.1 ms	Shark 11	Square 4	Before_Stimuli	232	235	239						706	
EM 0.1 ms	Shark 11	Square 4	After_Stimuli	273	284	323						880	1.2465

EM 0.1 ms	Shark 12	Square 1	Before_Stimuli	238	266	212						716	
EM 0.1 ms	Shark 12	Square 1	After_Stimuli	180	333	149						662	0.9246
EM 0.1 ms	Shark 12	Square 2	Before_Stimuli	210	225	263						698	
EM 0.1 ms	Shark 12	Square 2	After_Stimuli	501	235	360						1096	1.5702
EM 0.1 ms	Shark 12	Square 3	Before_Stimuli	209	269	259						737	
EM 0.1 ms	Shark 12	Square 3	After_Stimuli	181	326	320						827	1.1221
EM 0.1 ms	Shark 12	Square 4	Before_Stimuli	255	158	180						593	
EM 0.1 ms	Shark 12	Square 4	After_Stimuli	203	269	353						825	1.3912
Morning recordin	Shark 4	Square 1	Before_Stimuli	230	233	224						687	
Morning recordin	Shark 4	Square 1	After_Stimuli	229	297	262						788	1.147
Morning recordin	Shark 4	Square 2	Before_Stimuli	434	336	209						979	
Morning recordin	Shark 4	Square 2	After_Stimuli	312	484	628						1424	1.4545
Morning recordin	Shark 4	Square 3	Before_Stimuli	217	227	336						780	
Morning recordin	Shark 4	Square 3	After_Stimuli	196	189	201						586	0.7513
Morning recordin	Shark 4	Square 4	Before_Stimuli	285	336	432						1053	
Morning recordin	Shark 4	Square 4	After_Stimuli	433	187	57						677	0.6429
Morning recordin	Shark 5	Square 1	Before_Stimuli	243	254	248	209					954	
Morning recordin	Shark 5	Square 1	After_Stimuli	307	357	237	270					1171	1.2275
Morning recordin	Shark 5	Square 2	Before_Stimuli	325	315	257	327					1224	
Morning recordin	Shark 5	Square 2	After_Stimuli	265	133	251	226					875	0.7149
Morning recordin	Shark 5	Square 3	Before_Stimuli	238	217	279	228					962	
Morning recordin	Shark 5	Square 3	After_Stimuli	245	330	285	227					1087	1.1299
Morning recordin	Shark 5	Square 4	Before_Stimuli	269	262	197	304					1032	
Morning recordin	Shark 5	Square 4	After_Stimuli	239	225	243	285					992	0.9612
Morning recordin	Shark 7	Square 1	Before_Stimuli	210	233	237						680	
Morning recordin	Shark 7	Square 1	After_Stimuli	233	251	245						729	1.0721
Morning recordin	Shark 7	Square 2	Before_Stimuli	277	218	336						831	
Morning recordin	Shark 7	Square 2	After_Stimuli	266	290	233						789	0.9495
Morning recordin	Shark 7	Square 3	Before_Stimuli	239	307	155						701	
Morning recordin	Shark 7	Square 3	After_Stimuli	264	206	228						698	0.9957
Morning recordin	Shark 7	Square 4	Before_Stimuli	239	182	196						617	
Morning recordin	Shark 7	Square 4	After_Stimuli	184	292	275						751	1.2172
Morning recordin	Shark 11	Square 1	Before_Stimuli	236	297	279						812	
Morning recordin	Shark 11	Square 1	After_Stimuli	321	232	292						845	1.0406
Morning recordin	Shark 11	Square 2	Before_Stimuli	241	348	226						815	
Morning recordin	Shark 11	Square 2	After_Stimuli	190	337	247						774	0.9497
Morning recordin	Shark 11	Square 3	Before_Stimuli	287	205	259						751	
Morning recordin	Shark 11	Square 3	After_Stimuli	305	246	231						782	1.0413
Morning recordin	Shark 11	Square 4	Before_Stimuli	209	231	291						731	
Morning recordin	Shark 11	Square 4	After_Stimuli	144	296	322						762	1.0424
Morning recordin	Shark 12	Square 1	Before_Stimuli	274	251	267						792	
Morning recordin	Shark 12	Square 1	After_Stimuli	223	231	248						702	0.8864
Morning recordin	Shark 12	Square 2	Before_Stimuli	227	186	218						631	
Morning recordin	Shark 12	Square 2	After_Stimuli	255	387	187						829	1.3138
Morning recordin	Shark 12	Square 3	Before_Stimuli	237	213	210						660	
Morning recordin	Shark 12	Square 3	After_Stimuli	203	193	215						611	0.9258
Morning recordin	Shark 12	Square 4	Before_Stimuli	157	264	278						699	
Morning recordin	Shark 12	Square 4	After_Stimuli	199	208	307						714	1.0215

APPENDIX 3A – CONDITION 2

Distances traveled

Table 6. The total distances traveled in each trial by each shark in response to the treatments under Condition 2. The trials are listed in order of execution. The stimuli tested in each trial is represented in the «Stimuli» column. The «Shark» column shows the target shark. The total distance traveled is represented as movement across pixels.

Trial	Stimuli	Shark	Total distances traveled (before)	Total distances traveled (after)
1	EM 5 V	Shark 6	2686.29	6659.7
2	EM 5 V	Shark 6	3423.37	4825.03
3	EM 5 V	Shark 6	3749.18	4697.74
1	EM 10 V	Shark 6	3473.43	6208
2	EM 10 V	Shark 6	2779.77	5904.78
3	EM 10 V	Shark 6	3946.95	5544.84
1	EM 20 V	Shark 6	3425.83	5804.52
2	EM 20 V	Shark 6	2566.76	5488.53
3	EM 20 V	Shark 6	2758.09	5188.47
1	EM 5 V	Shark 8	6343.26	7349.42
2	EM 5 V	Shark 8	6570.37	5060.9
3	EM 5 V	Shark 8	7248.89	5599.16
1	EM 10 V	Shark 8	6119.12	6653.75
2	EM 10 V	Shark 8	4351.07	6054.91
3	EM 10 V	Shark 8	5038.55	5906.28
1	EM 20 V	Shark 8	5528.36	6222.28
2	EM 20 V	Shark 8	4674.84	6402.07
3	EM 20 V	Shark 8	6010.96	5684.09
1	Morning recording	Shark 8	6302.42	6454.13
2	Morning recording	Shark 8	5020.24	6402.39
1	EM 5 V	Shark	2366.12	7875.62
2	EM 5 V	Shark	4004.67	5084.72
3	EM 5 V	Shark	10429.2	6794.31
1	EM 10 V	Shark	6966.37	6725.92
2	EM 10 V	Shark	3851.38	5702.25
3	EM 10 V	Shark	5745.38	9428.93
1	EM 20 V	Shark	3850.71	4867.83
2	EM 20 V	Shark	2033.39	10726.26
3	EM 20 V	Shark	5308.34	8167.46
1	Morning recording	Shark	1947.26	1505.38
2	Morning recording	Shark	2190.04	2604.91
1	EM 5 V	Shark	6923.42	10903.8
2	EM 5 V	Shark	6230.56	7397.76
3	EM 5 V	Shark	7093.7	9688.78
1	EM 10 V	Shark	7089.91	8943.28
2	EM 10 V	Shark	5828.28	9196.83
3	EM 10 V	Shark	7432.35	9568.73
1	EM 20 V	Shark	7253.96	8144.64
2	EM 20 V	Shark	7070.63	8570.19
3	EM 20 V	Shark	6610.61	9159.65
1	Morning recording	Shark	7054.65	6016.43
2	Morning recording	Shark	7906.73	6523.83
3	Morning recording	Shark	5854.76	5793.03
1	EM 5 V	Shark	10410.05	9096.52
2	EM 5 V	Shark	9016.32	11275.84
3	EM 5 V	Shark	9078.18	9361.94
1	EM 10 V	Shark	9169.24	9714.96
2	EM 10 V	Shark	10523.92	10511.13
3	EM 10 V	Shark	11109.66	6385.29
1	EM 20 V	Shark	10221.96	7435.12
2	EM 20 V	Shark	11128.59	6839.1
3	EM 20 V	Shark	10991.92	7947.76
1	Morning recording	Shark	7993.84	7648.77
2	Morning recording	Shark	9236.21	9376.93
3	Morning recording	Shark	10423.19	10512.94
1	EM 5 V	Shark	8347.74	13624.81
2	EM 5 V	Shark	7816.36	9293.28
3	EM 5 V	Shark	10460.92	9099.71
1	EM 10 V	Shark	11192.83	10410.82
2	EM 10 V	Shark	11404.74	10445.74
3	EM 10 V	Shark	11644.81	10171.87
1	EM 20 V	Shark	8297.53	12788.77
2	EM 20 V	Shark	8775.92	11621.78
3	EM 20 V	Shark	11515.79	12023.53
1	Morning recording	Shark	11107.12	10873.54
2	Morning recording	Shark	11677.45	11121.94
3	Morning recording	Shark	6537.81	6520.46
1	EM 5 V	Shark	7254.17	7774.9
2	EM 5 V	Shark	7792.58	6907.88
3	EM 5 V	Shark	6801.66	6984.26
1	EM 10 V	Shark	7116.6	8708.5
2	EM 10 V	Shark	8304.42	7152.33

3	EM 10 V	Shark	6877.53	9169.81
1	EM 20 V	Shark	6676.31	7750.7
2	EM 20 V	Shark	7074.61	7340.1
3	EM 20 V	Shark	8450.08	7480.12
1	Morning recording	Shark	5453	5791.67
2	Morning recording	Shark	5111.61	6674.12
3	Morning recording	Shark	4377.86	4801.96

APPENDIX 3B – CONDITION 2

Mean distances traveled and fold change

Table 7. The mean distances traveled are calculated from the total distances in Table 4. The fold change is calculated using the mean values.

Shark	Stimuli	Condition	Mean value	In meters	Fold change
Shark 6	EM 5 V	Before Stimuli	3286.28	7.47	1.6414173
Shark 6	EM 5 V	After Stimuli	5394.16	12.26	
Shark 6	EM 10 V	Before Stimuli	3400.05	7.73	1.7311138
Shark 6	EM 10 V	After Stimuli	5885.87	13.38	
Shark 6	EM 20 V	Before Stimuli	2916.89	6.63	1.8834555
Shark 6	EM 20 V	After Stimuli	5493.84	12.49	
Shark 6	Morning recording	Before Stimuli	5416.77	12.31	1.1462092
Shark 6	Morning recording	After Stimuli	6208.75	14.11	
Shark 8	EM 5 V	Before Stimuli	6720.84	15.27	0.8932154
Shark 8	EM 5 V	After Stimuli	6003.16	13.64	
Shark 8	EM 10 V	Before Stimuli	5169.58	11.75	1.2002867
Shark 8	EM 10 V	After Stimuli	6204.98	14.10	
Shark 8	EM 20 V	Before Stimuli	5404.72	12.28	1.129164
Shark 8	EM 20 V	After Stimuli	6102.81	13.87	
Shark 8	Morning recording	Before Stimuli	5661.33	12.87	1.1354681
Shark 8	Morning recording	After Stimuli	6428.26	14.61	
Shark 10	EM 5 V	Before Stimuli	5599.99	12.73	1.175873
Shark 10	EM 5 V	After Stimuli	6584.88	14.97	
Shark 10	EM 10 V	Before Stimuli	5521.04	12.55	1.319624
Shark 10	EM 10 V	After Stimuli	7285.70	16.56	
Shark 10	EM 20 V	Before Stimuli	3730.81	8.48	2.1230002
Shark 10	EM 20 V	After Stimuli	7920.52	18.00	
Shark 10	Morning recording	Before Stimuli	2068.65	4.70	0.9934701
Shark 10	Morning recording	After Stimuli	2055.14	4.67	
Shark 15	EM 5 V	Before Stimuli	6749.22	15.34	1.3823982
Shark 15	EM 5 V	After Stimuli	9330.11	21.20	
Shark 15	EM 10 V	Before Stimuli	6783.51	15.42	1.3615774
Shark 15	EM 10 V	After Stimuli	9236.28	20.99	
Shark 15	EM 20 V	Before Stimuli	6978.40	15.86	1.2359316
Shark 15	EM 20 V	After Stimuli	8624.83	19.60	
Shark 15	Morning recording	Before Stimuli	6938.71	15.77	0.8807249
Shark 15	Morning recording	After Stimuli	6111.10	13.89	
Shark 18	EM 5 V	Before Stimuli	9501.52	21.59	1.043142
Shark 18	EM 5 V	After Stimuli	9911.43	22.53	
Shark 18	EM 10 V	Before Stimuli	10267.61	23.34	0.863927
Shark 18	EM 10 V	After Stimuli	8870.46	20.16	
Shark 18	EM 20 V	Before Stimuli	10780.83	24.50	0.6870835
Shark 18	EM 20 V	After Stimuli	7407.33	16.83	
Shark 18	Morning recording	Before Stimuli	9217.75	20.95	0.9958561
Shark 18	Morning recording	After Stimuli	9179.55	20.86	
Shark 20	EM 5 V	Before Stimuli	8875.01	20.17	1.2025459
Shark 20	EM 5 V	After Stimuli	10672.60	24.26	
Shark 20	EM 10 V	Before Stimuli	11414.13	25.94	0.9061409
Shark 20	EM 10 V	After Stimuli	10342.81	23.51	
Shark 20	EM 20 V	Before Stimuli	9529.75	21.66	1.274398
Shark 20	EM 20 V	After Stimuli	12144.69	27.60	
Shark 20	Morning recording	Before Stimuli	9774.13	22.21	0.9724977
Shark 20	Morning recording	After Stimuli	9505.32	21.60	
Shark 21	EM 5 V	Before Stimuli	7282.81	16.55	0.9916987
Shark 21	EM 5 V	After Stimuli	7222.35	16.41	
Shark 21	EM 10 V	Before Stimuli	7432.85	16.89	1.1225236
Shark 21	EM 10 V	After Stimuli	8343.55	18.96	
Shark 21	EM 20 V	Before Stimuli	7400.33	16.82	1.0166628
Shark 21	EM 20 V	After Stimuli	7523.64	17.10	
Shark 21	Morning recording	Before Stimuli	4980.82	11.32	1.1556148
Shark 21	Morning recording	After Stimuli	5755.91	13.08	

APPENDIX 3C – CONDITION 2

Square counts and fold change

Table 8. The position counts in each square from each trial. The trials are listed in the columns T1(trial 1) to T3 (trial 3). The average from each trial is represented in the "Av." column. The Fold change is calculated by dividing the "After_Stimuli" average by the "Before_Stimuli" average. M. rec = morning recording

Stimuli	Shark	Square	Condtition	T1	T2	T3	Av.	Fold change
EM 5 V	Shark 6	Square 1	Before_Stimuli	85	116	234	435	0.8114943
EM 5 V	Shark 6	Square 1	After_Stimuli	74	32	247	353	
EM 5 V	Shark 6	Square 2	Before_Stimuli	62	428	459	949	1.5721812
EM 5 V	Shark 6	Square 2	After_Stimuli	424	549	519	1492	
EM 5 V	Shark 6	Square 3	Before_Stimuli	111	117	72	300	1.3966667
EM 5 V	Shark 6	Square 3	After_Stimuli	42	135	242	419	
EM 5 V	Shark 6	Square 4	Before_Stimuli	627	204	352	1183	0.7075232
EM 5 V	Shark 6	Square 4	After_Stimuli	419	295	123	837	
EM 5 V	Shark 8	Square 1	Before_Stimuli	340	146	251	737	0.7272727
EM 5 V	Shark 8	Square 1	After_Stimuli	110	196	230	536	
EM 5 V	Shark 8	Square 2	Before_Stimuli	374	205	240	819	1.2478632
EM 5 V	Shark 8	Square 2	After_Stimuli	426	190	406	1022	
EM 5 V	Shark 8	Square 3	Before_Stimuli	125	278	279	682	0.4765396
EM 5 V	Shark 8	Square 3	After_Stimuli	190	70	65	325	
EM 5 V	Shark 8	Square 4	Before_Stimuli	105	200	189	494	1.854251
EM 5 V	Shark 8	Square 4	After_Stimuli	304	375	237	916	
EM 5 V	Shark 10	Square 1	Before_Stimuli	168	98	202	468	1.0790598
EM 5 V	Shark 10	Square 1	After_Stimuli	230	152	123	505	
EM 5 V	Shark 10	Square 2	Before_Stimuli	265	412	174	851	1.3537015
EM 5 V	Shark 10	Square 2	After_Stimuli	141	612	399	1152	
EM 5 V	Shark 10	Square 3	Before_Stimuli	377	91	290	758	0.9525066
EM 5 V	Shark 10	Square 3	After_Stimuli	366	166	190	722	
EM 5 V	Shark 10	Square 4	Before_Stimuli	114	360	297	771	0.8599222
EM 5 V	Shark 10	Square 4	After_Stimuli	196	127	340	663	
EM 5 V	Shark 15	Square 1	Before_Stimuli	328	156	241	725	0.9806897
EM 5 V	Shark 15	Square 1	After_Stimuli	391	143	177	711	
EM 5 V	Shark 15	Square 2	Before_Stimuli	309	300	117	726	1.530303
EM 5 V	Shark 15	Square 2	After_Stimuli	445	311	355	1111	
EM 5 V	Shark 15	Square 3	Before_Stimuli	161	110	255	526	0.9087452
EM 5 V	Shark 15	Square 3	After_Stimuli	70	247	161	478	
EM 5 V	Shark 15	Square 4	Before_Stimuli	261	228	290	779	1.114249
EM 5 V	Shark 15	Square 4	After_Stimuli	137	346	385	868	
EM 5 V	Shark 18	Square 1	Before_Stimuli	251	272	193	716	1.0572626
EM 5 V	Shark 18	Square 1	After_Stimuli	238	293	226	757	
EM 5 V	Shark 18	Square 2	Before_Stimuli	324	289	201	814	0.6977887
EM 5 V	Shark 18	Square 2	After_Stimuli	158	275	135	568	
EM 5 V	Shark 18	Square 3	Before_Stimuli	299	314	452	1065	1.0075117
EM 5 V	Shark 18	Square 3	After_Stimuli	413	290	370	1073	
EM 5 V	Shark 18	Square 4	Before_Stimuli	256	206	200	662	1.2975831
EM 5 V	Shark 18	Square 4	After_Stimuli	328	213	318	859	
EM 5 V	Shark 20	Square 1	Before_Stimuli	370	334	297	1001	0.8481518
EM 5 V	Shark 20	Square 1	After_Stimuli	382	218	249	849	
EM 5 V	Shark 20	Square 2	Before_Stimuli	426	324	316	1066	1.0150094
EM 5 V	Shark 20	Square 2	After_Stimuli	285	372	425	1082	
EM 5 V	Shark 20	Square 3	Before_Stimuli	182	287	246	715	0.9132867
EM 5 V	Shark 20	Square 3	After_Stimuli	249	217	187	653	
EM 5 V	Shark 20	Square 4	Before_Stimuli	198	255	309	762	1.2440945
EM 5 V	Shark 20	Square 4	After_Stimuli	237	387	324	948	
EM 5 V	Shark 21	Square 1	Before_Stimuli	399	170	310	879	0.8805461
EM 5 V	Shark 21	Square 1	After_Stimuli	239	261	274	774	
EM 5 V	Shark 21	Square 2	Before_Stimuli	287	260	285	832	0.9326923
EM 5 V	Shark 21	Square 2	After_Stimuli	136	303	337	776	
EM 5 V	Shark 21	Square 3	Before_Stimuli	193	310	263	766	1.035248
EM 5 V	Shark 21	Square 3	After_Stimuli	419	143	231	793	
EM 5 V	Shark 21	Square 4	Before_Stimuli	189	309	223	721	1.0443828
EM 5 V	Shark 21	Square 4	After_Stimuli	266	220	267	753	
EM 10 V	Shark 6	Square 1	Before_Stimuli	172	135	110	417	1.2086331

EM 10 V	Shark 6	Square 1	After_Stimuli	240	166	98	504	
EM 10 V	Shark 6	Square 2	Before_Stimuli	253	399	243	895	1.0592179
EM 10 V	Shark 6	Square 2	After_Stimuli	191	226	531	948	
EM 10 V	Shark 6	Square 3	Before_Stimuli	87	101	82	270	2.062963
EM 10 V	Shark 6	Square 3	After_Stimuli	221	271	65	557	
EM 10 V	Shark 6	Square 4	Before_Stimuli	284	234	618	1136	0.9383803
EM 10 V	Shark 6	Square 4	After_Stimuli	262	405	399	1066	
EM 10 V	Shark 8	Square 1	Before_Stimuli	187	373	136	696	0.8936782
EM 10 V	Shark 8	Square 1	After_Stimuli	198	270	154	622	
EM 10 V	Shark 8	Square 2	Before_Stimuli	149	64	253	466	1.9399142
EM 10 V	Shark 8	Square 2	After_Stimuli	254	318	332	904	
EM 10 V	Shark 8	Square 3	Before_Stimuli	261	153	250	664	1.1009036
EM 10 V	Shark 8	Square 3	After_Stimuli	284	161	286	731	
EM 10 V	Shark 8	Square 4	Before_Stimuli	243	258	238	739	0.9797023
EM 10 V	Shark 8	Square 4	After_Stimuli	273	212	239	724	
EM 10 V	Shark 10	Square 1	Before_Stimuli	266	27	165	458	1.2117904
EM 10 V	Shark 10	Square 1	After_Stimuli	170	113	272	555	
EM 10 V	Shark 10	Square 2	Before_Stimuli	177	217	216	610	0.9163934
EM 10 V	Shark 10	Square 2	After_Stimuli	234	86	239	559	
EM 10 V	Shark 10	Square 3	Before_Stimuli	321	178	228	727	1.4979367
EM 10 V	Shark 10	Square 3	After_Stimuli	247	540	302	1089	
EM 10 V	Shark 10	Square 4	Before_Stimuli	248	507	283	1038	0.734104
EM 10 V	Shark 10	Square 4	After_Stimuli	372	196	194	762	
EM 10 V	Shark 15	Square 1	Before_Stimuli	275	243	171	689	1.1567489
EM 10 V	Shark 15	Square 1	After_Stimuli	224	311	262	797	
EM 10 V	Shark 15	Square 2	Before_Stimuli	337	229	178	744	1.1599462
EM 10 V	Shark 15	Square 2	After_Stimuli	361	211	291	863	
EM 10 V	Shark 15	Square 3	Before_Stimuli	143	206	199	548	1.2390511
EM 10 V	Shark 15	Square 3	After_Stimuli	271	203	205	679	
EM 10 V	Shark 15	Square 4	Before_Stimuli	345	232	276	853	0.7995311
EM 10 V	Shark 15	Square 4	After_Stimuli	223	243	216	682	
EM 10 V	Shark 18	Square 1	Before_Stimuli	322	278	293	893	0.8118701
EM 10 V	Shark 18	Square 1	After_Stimuli	227	280	218	725	
EM 10 V	Shark 18	Square 2	Before_Stimuli	228	311	271	810	0.9962963
EM 10 V	Shark 18	Square 2	After_Stimuli	280	329	198	807	
EM 10 V	Shark 18	Square 3	Before_Stimuli	272	302	300	874	1.1647597
EM 10 V	Shark 18	Square 3	After_Stimuli	283	304	431	1018	
EM 10 V	Shark 18	Square 4	Before_Stimuli	312	281	210	803	1.2104608
EM 10 V	Shark 18	Square 4	After_Stimuli	372	258	342	972	
EM 10 V	Shark 20	Square 1	Before_Stimuli	325	364	351	1040	1.1413462
EM 10 V	Shark 20	Square 1	After_Stimuli	379	453	355	1187	
EM 10 V	Shark 20	Square 2	Before_Stimuli	225	214	208	647	1.4652241
EM 10 V	Shark 20	Square 2	After_Stimuli	379	192	377	948	
EM 10 V	Shark 20	Square 3	Before_Stimuli	307	265	304	876	0.9018265
EM 10 V	Shark 20	Square 3	After_Stimuli	217	366	207	790	
EM 10 V	Shark 20	Square 4	Before_Stimuli	275	282	262	819	0.6691087
EM 10 V	Shark 20	Square 4	After_Stimuli	176	143	229	548	
EM 10 V	Shark 21	Square 1	Before_Stimuli	290	330	272	892	0.7970852
EM 10 V	Shark 21	Square 1	After_Stimuli	244	213	254	711	
EM 10 V	Shark 21	Square 2	Before_Stimuli	266	162	367	795	1.1220126
EM 10 V	Shark 21	Square 2	After_Stimuli	322	349	221	892	
EM 10 V	Shark 21	Square 3	Before_Stimuli	315	329	293	937	0.9103522
EM 10 V	Shark 21	Square 3	After_Stimuli	337	262	254	853	
EM 10 V	Shark 21	Square 4	Before_Stimuli	211	274	198	683	1.2532943
EM 10 V	Shark 21	Square 4	After_Stimuli	201	251	404	856	
EM 20 V	Shark 6	Square 1	Before_Stimuli	112	0	0	112	4.7857143
EM 20 V	Shark 6	Square 1	After_Stimuli	196	175	165	536	
EM 20 V	Shark 6	Square 2	Before_Stimuli	82	434	507	1023	0.8240469
EM 20 V	Shark 6	Square 2	After_Stimuli	313	193	337	843	
EM 20 V	Shark 6	Square 3	Before_Stimuli	87	80	51	218	3.7798165
EM 20 V	Shark 6	Square 3	After_Stimuli	295	235	294	824	
EM 20 V	Shark 6	Square 4	Before_Stimuli	629	466	457	1552	0.5914948
EM 20 V	Shark 6	Square 4	After_Stimuli	293	358	267	918	
EM 20 V	Shark 8	Square 1	Before_Stimuli	200	191	162	553	0.8065099
EM 20 V	Shark 8	Square 1	After_Stimuli	96	250	100	446	
EM 20 V	Shark 8	Square 2	Before_Stimuli	161	153	179	493	1.8377282
EM 20 V	Shark 8	Square 2	After_Stimuli	217	464	225	906	
EM 20 V	Shark 8	Square 3	Before_Stimuli	196	135	308	639	0.9874804

EM 20 V	Shark 8	Square 3	After_Stimuli	196	179	256	631	
EM 20 V	Shark 8	Square 4	Before_Stimuli	265	383	205	853	1.052755
EM 20 V	Shark 8	Square 4	After_Stimuli	406	162	330	898	
EM 20 V	Shark 10	Square 1	Before_Stimuli	0	150	193	343	2.0029155
EM 20 V	Shark 10	Square 1	After_Stimuli	264	229	194	687	
EM 20 V	Shark 10	Square 2	Before_Stimuli	438	0	293	731	0.9863201
EM 20 V	Shark 10	Square 2	After_Stimuli	222	212	287	721	
EM 20 V	Shark 10	Square 3	Before_Stimuli	331	198	239	768	0.8880208
EM 20 V	Shark 10	Square 3	After_Stimuli	145	335	202	682	
EM 20 V	Shark 10	Square 4	Before_Stimuli	325	306	164	795	1.0012579
EM 20 V	Shark 10	Square 4	After_Stimuli	250	297	249	796	
EM 20 V	Shark 15	Square 1	Before_Stimuli	253	230	235	718	1.183844
EM 20 V	Shark 15	Square 1	After_Stimuli	282	284	284	850	
EM 20 V	Shark 15	Square 2	Before_Stimuli	304	127	372	803	1.3237858
EM 20 V	Shark 15	Square 2	After_Stimuli	396	303	364	1063	
EM 20 V	Shark 15	Square 3	Before_Stimuli	150	230	165	545	1.2
EM 20 V	Shark 15	Square 3	After_Stimuli	216	202	236	654	
EM 20 V	Shark 15	Square 4	Before_Stimuli	318	401	178	897	0.9074693
EM 20 V	Shark 15	Square 4	After_Stimuli	221	333	260	814	
EM 20 V	Shark 18	Square 1	Before_Stimuli	305	291	300	896	0.8515625
EM 20 V	Shark 18	Square 1	After_Stimuli	298	380	85	763	
EM 20 V	Shark 18	Square 2	Before_Stimuli	302	270	262	834	1.1043165
EM 20 V	Shark 18	Square 2	After_Stimuli	327	244	350	921	
EM 20 V	Shark 18	Square 3	Before_Stimuli	291	288	284	863	1.2387022
EM 20 V	Shark 18	Square 3	After_Stimuli	346	313	410	1069	
EM 20 V	Shark 18	Square 4	Before_Stimuli	262	202	222	686	1.1632653
EM 20 V	Shark 18	Square 4	After_Stimuli	212	236	350	798	
EM 20 V	Shark 20	Square 1	Before_Stimuli	280	270	367	917	1.1243184
EM 20 V	Shark 20	Square 1	After_Stimuli	337	347	347	1031	
EM 20 V	Shark 20	Square 2	Before_Stimuli	464	393	215	1072	0.5261194
EM 20 V	Shark 20	Square 2	After_Stimuli	176	179	209	564	
EM 20 V	Shark 20	Square 3	Before_Stimuli	209	236	275	720	1.4958333
EM 20 V	Shark 20	Square 3	After_Stimuli	371	384	322	1077	
EM 20 V	Shark 20	Square 4	Before_Stimuli	248	291	276	815	1.0380368
EM 20 V	Shark 20	Square 4	After_Stimuli	300	274	272	846	
EM 20 V	Shark 21	Square 1	Before_Stimuli	238	297	297	832	0.7115385
EM 20 V	Shark 21	Square 1	After_Stimuli	232	152	208	592	
EM 20 V	Shark 21	Square 2	Before_Stimuli	284	271	208	763	1.2385321
EM 20 V	Shark 21	Square 2	After_Stimuli	255	350	340	945	
EM 20 V	Shark 21	Square 3	Before_Stimuli	336	308	273	917	1.1592148
EM 20 V	Shark 21	Square 3	After_Stimuli	384	377	302	1063	
EM 20 V	Shark 21	Square 4	Before_Stimuli	234	204	304	742	1.0876011
EM 20 V	Shark 21	Square 4	After_Stimuli	310	236	261	807	
M. rec	Shark 6	Square 1	Before_Stimuli	184	131		315	0.8412698
M. rec	Shark 6	Square 1	After_Stimuli	87	178		265	
M. rec	Shark 6	Square 2	Before_Stimuli	452	468		920	0.8771739
M. rec	Shark 6	Square 2	After_Stimuli	513	294		807	
M. rec	Shark 6	Square 3	Before_Stimuli	159	103		262	0.9770992
M. rec	Shark 6	Square 3	After_Stimuli	78	178		256	
M. rec	Shark 6	Square 4	Before_Stimuli	169	181		350	1.64
M. rec	Shark 6	Square 4	After_Stimuli	310	264		574	
M. rec	Shark 8	Square 1	Before_Stimuli	384	118		502	1.2410359
M. rec	Shark 8	Square 1	After_Stimuli	330	293		623	
M. rec	Shark 8	Square 2	Before_Stimuli	117	396		513	1.0155945
M. rec	Shark 8	Square 2	After_Stimuli	347	174		521	
M. rec	Shark 8	Square 3	Before_Stimuli	218	65		283	1.3321555
M. rec	Shark 8	Square 3	After_Stimuli	151	226		377	
M. rec	Shark 8	Square 4	Before_Stimuli	135	306		441	0.8798186
M. rec	Shark 8	Square 4	After_Stimuli	160	228		388	
M. rec	Shark 10	Square 1	Before_Stimuli	780	54		834	0.235012
M. rec	Shark 10	Square 1	After_Stimuli	54	142		196	
M. rec	Shark 10	Square 2	Before_Stimuli	157	153		310	1.983871
M. rec	Shark 10	Square 2	After_Stimuli	615	0		615	
M. rec	Shark 10	Square 3	Before_Stimuli	11	101		112	1.7142857
M. rec	Shark 10	Square 3	After_Stimuli	7	185		192	
M. rec	Shark 10	Square 4	Before_Stimuli	121	677		798	1.1190476
M. rec	Shark 10	Square 4	After_Stimuli	318	575		893	
M. rec	Shark 15	Square 1	Before_Stimuli	268	245	284	797	0.6273526

M. rec	Shark 15	Square 1	After_Stimuli	195	108	197	500	
M. rec	Shark 15	Square 2	Before_Stimuli	244	222	367	833	0.997599
M. rec	Shark 15	Square 2	After_Stimuli	118	303	410	831	
M. rec	Shark 15	Square 3	Before_Stimuli	184	179	128	491	1.4256619
M. rec	Shark 15	Square 3	After_Stimuli	258	196	246	700	
M. rec	Shark 15	Square 4	Before_Stimuli	269	297	197	763	1.440367
M. rec	Shark 15	Square 4	After_Stimuli	482	357	260	1099	
M. rec	Shark 18	Square 1	Before_Stimuli	178	247	294	719	0.9694019
M. rec	Shark 18	Square 1	After_Stimuli	120	300	277	697	
M. rec	Shark 18	Square 2	Before_Stimuli	302	360	225	887	1.2006764
M. rec	Shark 18	Square 2	After_Stimuli	486	294	285	1065	
M. rec	Shark 18	Square 3	Before_Stimuli	215	256	296	767	0.9634941
M. rec	Shark 18	Square 3	After_Stimuli	145	302	292	739	
M. rec	Shark 18	Square 4	Before_Stimuli	419	264	205	888	0.8975225
M. rec	Shark 18	Square 4	After_Stimuli	394	201	202	797	
M. rec	Shark 20	Square 1	Before_Stimuli	327	375	309	1011	1.0959446
M. rec	Shark 20	Square 1	After_Stimuli	405	406	297	1108	
M. rec	Shark 20	Square 2	Before_Stimuli	234	214	314	762	0.984252
M. rec	Shark 20	Square 2	After_Stimuli	230	196	324	750	
M. rec	Shark 20	Square 3	Before_Stimuli	268	272	276	816	1.0330882
M. rec	Shark 20	Square 3	After_Stimuli	252	302	289	843	
M. rec	Shark 20	Square 4	Before_Stimuli	303	279	233	815	0.8355828
M. rec	Shark 20	Square 4	After_Stimuli	243	222	216	681	
M. rec	Shark 21	Square 1	Before_Stimuli	333	316	478	1127	0.9751553
M. rec	Shark 21	Square 1	After_Stimuli	432	332	335	1099	
M. rec	Shark 21	Square 2	Before_Stimuli	229	228	147	604	1.3509934
M. rec	Shark 21	Square 2	After_Stimuli	280	288	248	816	
M. rec	Shark 21	Square 3	Before_Stimuli	355	303	209	867	0.5351788
M. rec	Shark 21	Square 3	After_Stimuli	167	110	187	464	
M. rec	Shark 21	Square 4	Before_Stimuli	196	168	237	601	1.171381
M. rec	Shark 21	Square 4	After_Stimuli	197	271	236	704	

APPENDIX 4A – CONDITION 3

Distances traveled

Table 9: The total distances traveled by each shark in each trial. The trials are listed in order of execution. The stimuli tested in each trial is represented in the «Stimuli» column. The «Shark» column shows the target shark. The total distance traveled before and after is represented as movement across pixels.

Trial	Stimuli	Shark	Total distance traveled (before)	Total distance traveled (after)
1	Skin extract 0.5 U	Shark 9	13510.42174	17718.25393
2	Skin extract 0.5 U	Shark 9	10087.8247	13483.54093
3	Skin extract 0.5 U	Shark 9	13830.942	15702.26416
1	Skin extract 1 U	Shark 9	12703.67925	14411.79609
2	Skin extract 1 U	Shark 9	11177.6931	14359.58749
1	Skin extract 2 U	Shark 9	10622.69537	14061.56058
2	Skin extract 2 U	Shark 9	12740.72793	16034.80344
1	Food odor	Shark 9	13160.58818	16686.00573
2	Food odor	Shark 9	14933.84738	13737.46135
1	Seawater control	Shark 9	17678.50954	16689.55804
2	Seawater control	Shark 9	13274.25601	16609.26903
1	Morning recording	Shark 9	12825.42052	12270.18937
2	Morning recording	Shark 9	14895.24711	17216.50659
3	Morning recording	Shark 9	13990.72029	16452.77967
1	Seawater control	Shark 13	9182.724322	9283.98896
2	Seawater control	Shark 13	10809.212	12230.15973
1	Skin extract 0.5 U	Shark 13	8701.074145	9476.176925
2	Skin extract 0.5 U	Shark 13	9643.262173	9597.207281
1	Skin extract 1 U	Shark 13	10206.58751	9885.862163
2	Skin extract 1 U	Shark 13	11498.19562	11087.27397
1	Skin extract 2 U	Shark 13	9310.488085	10873.42577
2	Skin extract 2 U	Shark 13	10796.53258	11675.86822
1	Food odor	Shark 13	8224.510428	8894.363063
2	Food odor	Shark 13	10691.85715	10315.88326
1	Morning recording	Shark 13	9696.081408	9819.696546
2	Morning recording	Shark 13	9623.706438	10773.33121
1	Seawater control	Shark 15	993.4525492	11731.37159
2	Seawater control	Shark 15	11328.24049	16050.45138
1	Skin extract 0.5 U	Shark 15	10576.31335	15299.99596
2	Skin extract 0.5 U	Shark 15	14186.67356	15883.52884
1	Skin extract 1 U	Shark 15	10433.46929	13389.88438
2	Skin extract 1 U	Shark 15	12306.53337	13000.21826
1	Skin extract 2 U	Shark 15	14263.03912	18066.89698
2	Skin extract 2 U	Shark 15	12314.94122	17877.08229
1	Food odor	Shark 15	11643.18608	10492.51081
2	Food odor	Shark 15	16495.89101	11435.34568
3	Food odor	Shark 15	10747.56252	11796.27291
4	Food odor	Shark 15	14516.69883	13059.06876
5	Food odor	Shark 15	12120.10785	12947.48024
1	Morning recording	Shark 15	15055.5027	12764.04878
2	Morning recording	Shark 15	15712.11681	11183.33735
3	Morning recording	Shark 15	11935.75311	12249.20737
1	Seawater control	Shark 16	13722.02043	17661.76967
2	Seawater control	Shark 16	12363.18835	13916.02669
3	Seawater control	Shark 16	13371.57336	14778.2606
1	Skin extract 0.5 U	Shark 16	16916.63775	17212.88045
2	Skin extract 0.5 U	Shark 16	13136.90167	14417.49607
1	Skin extract 1 U	Shark 16	13131.23905	15788.99353
2	Skin extract 1 U	Shark 16	9607.401032	8402.914799
1	Skin extract 2 U	Shark 16	7603.029264	13471.85818
2	Skin extract 2 U	Shark 16	9944.132473	10599.93666
1	Food odor	Shark 16	15389.29401	11248.07457
2	Food odor	Shark 16	13230.95786	17593.48057
1	Morning recording	Shark 16	12714.83956	16406.75877
2	Morning recording	Shark 16	11765.49015	16587.64232
3	Morning recording	Shark 16	11532.12673	16301.86568
1	Seawater control	Shark 17	11767.84927	15265.96966
2	Seawater control	Shark 17	12115.24847	13506.83308
3	Seawater control	Shark 17	12458.76846	15608.06997
1	Skin extract 0.5 U	Shark 17	15998.12719	21727.57277
2	Skin extract 0.5 U	Shark 17	12620.11892	16478.5653
1	Skin extract 1 U	Shark 17	9328.641296	13382.23115
2	Skin extract 1 U	Shark 17	12177.33237	12855.23186

3	Skin extract 1 U	Shark 17	13444.18504	14277.46114
1	Skin extract 2 U	Shark 17	11303.74786	12255.96386
2	Skin extract 2 U	Shark 17	11944.42062	14263.33422
3	Skin extract 2 U	Shark 17	11809.35893	12131.54792
1	Food odor	Shark 17	13105.54925	12566.88987
2	Food odor	Shark 17	10566.64617	11317.04291
1	Morning recording	Shark 17	13987.74691	22837.15952
2	Morning recording	Shark 17	11344.69891	11095.64203
3	Morning recording	Shark 17	12827.36276	14072.64524
1	Seawater control	Shark 18	24641.43418	22555.6755
2	Seawater control	Shark 18	17094.39212	20002.85902
3	Seawater control	Shark 18	22854.13339	18054.39205
1	Skin extract 0.5 U	Shark 18	22854.13339	18054.39205
2	Skin extract 0.5 U	Shark 18	21285.86443	19032.15545
3	Skin extract 1 U	Shark 18	20084.30603	17277.39666
4	Skin extract 1 U	Shark 18	18099.49137	20503.03492
1	Skin extract 2 U	Shark 18	22262.60725	18320.93156
2	Skin extract 2 U	Shark 18	20972.99945	20214.06759
1	Food odor	Shark 18	19038.49214	15457.18887
2	Food odor	Shark 18	22550.58021	18153.29564
3	Food odor	Shark 18	21093.99063	14606.80995
4	Food odor	Shark 18	13340.39629	13559.22864
5	Food odor	Shark 18	17397.63327	15876.70554
1	Morning recording	Shark 18	17452.36007	16568.20923
2	Morning recording	Shark 18	17250.41124	18554.27446
3	Morning recording	Shark 18	21344.94276	20873.58599
1	Seawater control	Shark 20	16182.19512	23581.52801
2	Seawater control	Shark 20	16511.37083	27952.53009
3	Skin extract 0.5 U	Shark 20	23276.87747	16433.30155
4	Skin extract 0.5 U	Shark 20	20820.05493	20328.13699
1	Skin extract 1 U	Shark 20	21225.76194	15064.9222
2	Skin extract 1 U	Shark 20	14248.31997	14327.86673
1	Skin extract 2 U	Shark 20	21728.93214	20593.15285
2	Skin extract 2 U	Shark 20	21005.62769	18964.08467
1	Food odor	Shark 20	25051.57057	18532.78621
2	Food odor	Shark 20	13004.31039	19596.11602
3	Food odor	Shark 20	21285.91258	15273.68586
1	Morning recording	Shark 20	11107.11551	10873.54408
2	Morning recording	Shark 20	11677.44985	11121.94477
3	Morning recording	Shark 20	6537.811758	6520.45627
1	Seawater control	Shark 21	8463.715972	10610.05882
2	Seawater control	Shark 21	13539.36121	10889.95155
3	Seawater control	Shark 21	12610.69957	15445.63462
4	Seawater control	Shark 21	9690.017817	9847.767999
1	Skin extract 0.5 U	Shark 21	8021.375871	11998.30225
2	Skin extract 0.5 U	Shark 21	15478.56926	15467.81292
1	Skin extract 1 U	Shark 21	9288.301338	9783.072568
2	Skin extract 1 U	Shark 21	8330.319803	13756.18246
1	Skin extract 2 U	Shark 21	6704.433405	8687.754008
2	Skin extract 2 U	Shark 21	7901.009561	8417.019751
1	Food odor	Shark 21	8628.539892	8849.653501
2	Food odor	Shark 21	4315.553522	11347.82246
3	Food odor	Shark 21	12518.79238	11421.63224
4	Food odor	Shark 21	9925.063459	13414.60429
1	Morning recording	Shark 21	10297.1324	10984.85726
2	Morning recording	Shark 21	11470.30754	12317.10211
3	Morning recording	Shark 21	8838.39262	6884.842222

APPENDIX 4B – CONDITION 3

Mean distances traveled and fold change

Table 10. The mean distances traveled are calculated from the total distances in Table 7. The fold change by dividing the mean value of "After_Stimuli" by the mean value of"Before_Stimuli".

Shark	Stimuli	Condition	Mean value	Fold change
Shark 9	Skin extract 0.5 U	Before Stimuli	12476.3961	1.25314122
Shark 9	Skin extract 0.5 U	After Stimuli	15634.6863	
Shark 9	Skin extract 1 U	Before Stimuli	11940.6862	1.20476257
Shark 9	Skin extract 1 U	After Stimuli	14385.6918	
Shark 9	Skin extract 2 U	Before Stimuli	11681.7117	1.28818297
Shark 9	Skin extract 2 U	After Stimuli	15048.182	
Shark 9	Food odor	Before Stimuli	14047.2178	1.0829001
Shark 9	Food odor	After Stimuli	15211.7335	
Shark 9	Seawater control	Before Stimuli	15476.3828	1.07579489
Shark 9	Seawater control	After Stimuli	16649.4135	
Shark 9	Morning recording	Before Stimuli	13903.796	1.10136531
Shark 9	Morning recording	After Stimuli	15313.1585	
Shark 13	Seawater control	Before Stimuli	9995.96816	1.07614132
Shark 13	Seawater control	After Stimuli	10757.0743	
Shark 13	Skin extract 0.5 U	Before Stimuli	9172.16816	1.0397424
Shark 13	Skin extract 0.5 U	After Stimuli	9536.6921	
Shark 13	Skin extract 1 U	Before Stimuli	10852.3916	0.96629098
Shark 13	Skin extract 1 U	After Stimuli	10486.5681	
Shark 13	Skin extract 2 U	Before Stimuli	10053.5103	1.12146371
Shark 13	Skin extract 2 U	After Stimuli	11274.647	
Shark 13	Food odor	Before Stimuli	9458.18379	1.01553569
Shark 13	Food odor	After Stimuli	9605.12316	
Shark 13	Morning recording	Before Stimuli	9659.89392	1.06590341
Shark 13	Morning recording	After Stimuli	10296.5139	
Shark 15	Seawater control	Before Stimuli	6160.84652	2.25470825
Shark 15	Seawater control	After Stimuli	13890.9115	
Shark 15	Skin extract 0.5 U	Before Stimuli	12381.4935	1.25927962
Shark 15	Skin extract 0.5 U	After Stimuli	15591.7624	
Shark 15	Skin extract 1 U	Before Stimuli	11370.0013	1.16051449
Shark 15	Skin extract 1 U	After Stimuli	13195.0513	
Shark 15	Skin extract 2 U	Before Stimuli	13288.9902	1.35239694
Shark 15	Skin extract 2 U	After Stimuli	17971.9896	
Shark 15	Food odor	Before Stimuli	13104.6893	0.91159244
Shark 15	Food odor	After Stimuli	11946.1357	
Shark 15	Morning recording	Before Stimuli	14234.4575	0.84762845
Shark 15	Morning recording	After Stimuli	12065.5312	
Shark 16	Seawater control	Before Stimuli	13152.2607	1.1748565
Shark 16	Seawater control	After Stimuli	15452.019	
Shark 16	Skin extract 0.5 U	Before Stimuli	15026.7697	1.0524676
Shark 16	Skin extract 0.5 U	After Stimuli	15815.1883	
Shark 16	Skin extract 1 U	Before Stimuli	11369.32	1.06391184
Shark 16	Skin extract 1 U	After Stimuli	12095.9542	
Shark 16	Skin extract 1 U	Before Stimuli	11369.32	1.06391184
Shark 16	Skin extract 1 U	After Stimuli	12095.9542	
Shark 16	Skin extract 2 U	Before Stimuli	8773.58087	1.3718341
Shark 16	Skin extract 2 U	After Stimuli	12035.8974	
Shark 16	Food odor	Before Stimuli	14310.1259	1.0077324
Shark 16	Food odor	After Stimuli	14420.7776	
Shark 16	Morning recording	Before Stimuli	12004.1521	1.3688671
Shark 16	Morning recording	After Stimuli	16432.0889	
Shark 17	Seawater control	Before Stimuli	12113.9554	1.22120511
Shark 17	Seawater control	After Stimuli	14793.6242	
Shark 17	Skin extract 0.5 U	Before Stimuli	14309.1231	1.33502724
Shark 17	Skin extract 0.5 U	After Stimuli	19103.069	
Shark 17	Skin extract 1 U	Before Stimuli	11650.0529	1.15922003
Shark 17	Skin extract 1 U	After Stimuli	13504.9747	
Shark 17	Skin extract 2 U	Before Stimuli	11685.8425	1.10249778
Shark 17	Skin extract 2 U	After Stimuli	12883.6153	
Shark 17	Food odor	Before Stimuli	11836.0977	1.00894456
Shark 17	Food odor	After Stimuli	11941.9664	
Shark 17	Morning recording	Before Stimuli	12719.9362	1.25801068

Shark 17	Morning recording	After Stimuli	16001.8156	
Shark 18	Seawater control	Before Stimuli	20867.9132	1.01971228
Shark 18	Seawater control	After Stimuli	21279.2673	
Shark 18	Skin extract 0.5 U	Before Stimuli	22069.9989	0.84020275
Shark 18	Skin extract 0.5 U	After Stimuli	18543.2738	
Shark 18	Skin extract 1 U	Before Stimuli	19091.8987	0.9894362
Shark 18	Skin extract 1 U	After Stimuli	18890.2158	
Shark 18	Skin extract 2 U	Before Stimuli	21617.8033	0.89127925
Shark 18	Skin extract 2 U	After Stimuli	19267.4996	
Shark 18	Food odor	Before Stimuli	15570.1821	0.99746076
Shark 18	Food odor	After Stimuli	15530.6457	
Shark 20	Seawater control	Before Stimuli	16346.783	1.57627523
Shark 20	Seawater control	After Stimuli	25767.029	
Shark 20	Skin extract 0.5 U	Before Stimuli	22048.4662	0.8336507
Shark 20	Skin extract 0.5 U	After Stimuli	18380.7193	
Shark 20	Skin extract 1 U	Before Stimuli	17737.041	0.82857081
Shark 20	Skin extract 1 U	After Stimuli	14696.3945	
Shark 20	Skin extract 2 U	Before Stimuli	21367.2799	0.92564982
Shark 20	Skin extract 2 U	After Stimuli	19778.6188	
Shark 20	Food odor	Before Stimuli	19780.5978	0.8999153
Shark 20	Food odor	After Stimuli	17800.8627	
Shark 20	Morning recording	Before Stimuli	9774.1257	0.97249773
Shark 20	Morning recording	After Stimuli	9505.31504	
Shark 21	Seawater control	Before Stimuli	11075.9486	1.05619425
Shark 21	Seawater control	After Stimuli	11698.3532	
Shark 21	Skin extract 0.5 U	Before Stimuli	11749.9726	1.16877359
Shark 21	Skin extract 0.5 U	After Stimuli	13733.0576	
Shark 21	Skin extract 1 U	Before Stimuli	8809.31057	1.33604411
Shark 21	Skin extract 1 U	After Stimuli	11769.6275	
Shark 21	Skin extract 2 U	Before Stimuli	7302.72148	1.17112324
Shark 21	Skin extract 2 U	After Stimuli	8552.38688	
Shark 21	Food odor	Before Stimuli	8846.98731	1.27257198
Shark 21	Food odor	After Stimuli	11258.4281	
Shark 21	Morning recording	Before Stimuli	10201.9442	0.98630879
Shark 21	Morning recording	After Stimuli	10062.2672	

APPENDIX 4C – CONDITION 3

Square counts and fold change

Table 11. The position counts in each square from each trial. The trials are listed in the columns T1 (Trial 1) to T5 (Trial 5). The average position count from each trial is represented in the "Av." column. The fold change is calculated by dividing the "After_Stimuli" average by the "Before_Stimuli" average. M. rec = morning recording. SE. 0.5 U = Skin extract 0.5 U.

Stimuli	Shark	Square	Condtition	T1	T2	T3	T4	T5	Av.	Fold change
Seawater control	Shark 9	Square 1	Before_Stimuli	577	340				917	1.155943
Seawater control	Shark 9	Square 1	After_Stimuli	517	543				1060	
Seawater control	Shark 9	Square 2	Before_Stimuli	475	701				1176	0.890306
Seawater control	Shark 9	Square 2	After_Stimuli	534	513				1047	
Seawater control	Shark 9	Square 3	Before_Stimuli	468	247				715	1.493706
Seawater control	Shark 9	Square 3	After_Stimuli	569	499				1068	
Seawater control	Shark 9	Square 4	Before_Stimuli	406	468				874	1.010297
Seawater control	Shark 9	Square 4	After_Stimuli	450	433				883	
Seawater control	Shark 13	Square 1	Before_Stimuli	366	982				1348	1.071958
Seawater control	Shark 13	Square 1	After_Stimuli	736	709				1445	
Seawater control	Shark 13	Square 2	Before_Stimuli	234	565				799	1.110138
Seawater control	Shark 13	Square 2	After_Stimuli	277	610				887	
Seawater control	Shark 13	Square 3	Before_Stimuli	587	110				697	1.721664
Seawater control	Shark 13	Square 3	After_Stimuli	838	362				1200	
Seawater control	Shark 13	Square 4	Before_Stimuli	990	430				1420	0.740141
Seawater control	Shark 13	Square 4	After_Stimuli	403	648				1051	
Seawater control	Shark 15	Square 1	Before_Stimuli	586	689				1275	0.727059
Seawater control	Shark 15	Square 1	After_Stimuli	470	457				927	
Seawater control	Shark 15	Square 2	Before_Stimuli	1249	653				1902	0.556257
Seawater control	Shark 15	Square 2	After_Stimuli	388	670				1058	
Seawater control	Shark 15	Square 3	Before_Stimuli	518	376				894	1.087248
Seawater control	Shark 15	Square 3	After_Stimuli	544	428				972	
Seawater control	Shark 15	Square 4	Before_Stimuli	47	338				385	3.124675
Seawater control	Shark 15	Square 4	After_Stimuli	647	556				1203	
Seawater control	Shark 16	Square 1	Before_Stimuli	574	744	456			1774	1.005073
Seawater control	Shark 16	Square 1	After_Stimuli	558	550	675			1783	
Seawater control	Shark 16	Square 2	Before_Stimuli	363	594	457			1414	1.330976
Seawater control	Shark 16	Square 2	After_Stimuli	520	770	592			1882	
Seawater control	Shark 16	Square 3	Before_Stimuli	526	396	449			1371	0.857768
Seawater control	Shark 16	Square 3	After_Stimuli	566	302	308			1176	
Seawater control	Shark 16	Square 4	Before_Stimuli	379	295	510			1184	0.96875
Seawater control	Shark 16	Square 4	After_Stimuli	315	477	355			1147	
Seawater control	Shark 17	Square 1	Before_Stimuli	525	672	1043			2240	0.583036
Seawater control	Shark 17	Square 1	After_Stimuli	540	152	614			1306	
Seawater control	Shark 17	Square 2	Before_Stimuli	719	492	514			1725	1.226087
Seawater control	Shark 17	Square 2	After_Stimuli	651	818	646			2115	
Seawater control	Shark 17	Square 3	Before_Stimuli	500	297	268			1065	0.935211
Seawater control	Shark 17	Square 3	After_Stimuli	416	254	326			996	
Seawater control	Shark 17	Square 4	Before_Stimuli	489	583	332			1404	1.388889
Seawater control	Shark 17	Square 4	After_Stimuli	424	861	665			1950	
Seawater control	Shark 18	Square 1	Before_Stimuli	510	523				1033	1.06486
Seawater control	Shark 18	Square 1	After_Stimuli	577	523				1100	
Seawater control	Shark 18	Square 2	Before_Stimuli	571	335				906	1.348786
Seawater control	Shark 18	Square 2	After_Stimuli	554	668				1222	
Seawater control	Shark 18	Square 3	Before_Stimuli	595	786				1381	0.705286
Seawater control	Shark 18	Square 3	After_Stimuli	473	501				974	
Seawater control	Shark 18	Square 4	Before_Stimuli	433	685				1118	1.147585
Seawater control	Shark 18	Square 4	After_Stimuli	646	637				1283	
Seawater control	Shark 20	Square 1	Before_Stimuli	781	519				1300	0.964615
Seawater control	Shark 20	Square 1	After_Stimuli	598	656				1254	
Seawater control	Shark 20	Square 2	Before_Stimuli	677	625				1302	0.935484
Seawater control	Shark 20	Square 2	After_Stimuli	744	474				1218	
Seawater control	Shark 20	Square 3	Before_Stimuli	480	626				1106	0.884268
Seawater control	Shark 20	Square 3	After_Stimuli	454	524				978	
Seawater control	Shark 20	Square 4	Before_Stimuli	337	390				727	1.325997
Seawater control	Shark 20	Square 4	After_Stimuli	443	521				964	
Seawater control	Shark 21	Square 1	Before_Stimuli	931	761	904	732		3328	0.811899
Seawater control	Shark 21	Square 1	After_Stimuli	884	735	632	451		2702	
Seawater control	Shark 21	Square 2	Before_Stimuli	393	546	607	550		2096	0.986164
Seawater control	Shark 21	Square 2	After_Stimuli	615	495	371	586		2067	

Seawater control	Shark 21	Square 3	Before_Stimuli	338	311	189	396		1234	1.39141
Seawater control	Shark 21	Square 3	After_Stimuli	339	382	578	418		1717	
Seawater control	Shark 21	Square 4	Before_Stimuli	407	522	345	297		1571	1.063654
Seawater control	Shark 21	Square 4	After_Stimuli	269	340	430	632		1671	
Food odor	Shark 9	Square 1	Before_Stimuli	393	520				913	1.033954
Food odor	Shark 9	Square 1	After_Stimuli	529	415				944	
Food odor	Shark 9	Square 2	Before_Stimuli	508	527				1035	1.1343
Food odor	Shark 9	Square 2	After_Stimuli	427	747				1174	
Food odor	Shark 9	Square 3	Before_Stimuli	258	346				604	1.231788
Food odor	Shark 9	Square 3	After_Stimuli	402	342				744	
Food odor	Shark 9	Square 4	Before_Stimuli	511	327				838	0.97852
Food odor	Shark 9	Square 4	After_Stimuli	437	383				820	
Food odor	Shark 13	Square 1	Before_Stimuli	391	723				1114	0.702873
Food odor	Shark 13	Square 1	After_Stimuli	281	502				783	
Food odor	Shark 13	Square 2	Before_Stimuli	778	307				1085	1.017512
Food odor	Shark 13	Square 2	After_Stimuli	501	603				1104	
Food odor	Shark 13	Square 3	Before_Stimuli	309	539				848	1.137972
Food odor	Shark 13	Square 3	After_Stimuli	605	360				965	
Food odor	Shark 13	Square 4	Before_Stimuli	770	616				1386	0.930014
Food odor	Shark 13	Square 4	After_Stimuli	677	612				1289	
Food odor	Shark 15	Square 1	Before_Stimuli	423	529	641	373	420	2386	0.934619
Food odor	Shark 15	Square 1	After_Stimuli	492	650	395	422	271	2230	
Food odor	Shark 15	Square 2	Before_Stimuli	723	380	579	368	527	2577	1.157936
Food odor	Shark 15	Square 2	After_Stimuli	726	736	500	470	552	2984	
Food odor	Shark 15	Square 3	Before_Stimuli	489	457	512	534	300	2292	0.876963
Food odor	Shark 15	Square 3	After_Stimuli	471	289	501	494	255	2010	
Food odor	Shark 15	Square 4	Before_Stimuli	441	535	401	558	485	2420	1.283471
Food odor	Shark 15	Square 4	After_Stimuli	498	475	893	736	504	3106	
Food odor	Shark 16	Square 1	Before_Stimuli	593	480				1073	0.779124
Food odor	Shark 16	Square 1	After_Stimuli	386	450				836	
Food odor	Shark 16	Square 2	Before_Stimuli	397	523				920	1.433696
Food odor	Shark 16	Square 2	After_Stimuli	729	590				1319	
Food odor	Shark 16	Square 3	Before_Stimuli	530	464				994	0.796781
Food odor	Shark 16	Square 3	After_Stimuli	389	403				792	
Food odor	Shark 16	Square 4	Before_Stimuli	501	379				880	1.180682
Food odor	Shark 16	Square 4	After_Stimuli	452	587				1039	
Food odor	Shark 17	Square 1	Before_Stimuli	527	743				1270	0.824409
Food odor	Shark 17	Square 1	After_Stimuli	357	690				1047	
Food odor	Shark 17	Square 2	Before_Stimuli	671	785				1456	0.793269
Food odor	Shark 17	Square 2	After_Stimuli	550	605				1155	
Food odor	Shark 17	Square 3	Before_Stimuli	446	0				446	2.161435
Food odor	Shark 17	Square 3	After_Stimuli	470	494				964	
Food odor	Shark 17	Square 4	Before_Stimuli	473	487				960	1.202083
Food odor	Shark 17	Square 4	After_Stimuli	809	345				1154	
Food odor	Shark 18	Square 1	Before_Stimuli	642	546	484	462	507	2641	1.015146
Food odor	Shark 18	Square 1	After_Stimuli	324	715	634	451	557	2681	
Food odor	Shark 18	Square 2	Before_Stimuli	391	531	581	959	352	2814	1.044065
Food odor	Shark 18	Square 2	After_Stimuli	744	538	591	792	273	2938	
Food odor	Shark 18	Square 3	Before_Stimuli	644	615	525	285	685	2754	1.062818
Food odor	Shark 18	Square 3	After_Stimuli	583	490	583	327	944	2927	
Food odor	Shark 18	Square 4	Before_Stimuli	613	412	448	284	745	2502	1.059952
Food odor	Shark 18	Square 4	After_Stimuli	693	531	545	361	522	2652	
Food odor	Shark 20	Square 1	Before_Stimuli	688	539	564			1791	1.139028
Food odor	Shark 20	Square 1	After_Stimuli	595	664	781			2040	
Food odor	Shark 20	Square 2	Before_Stimuli	455	725	416			1596	1.398496
Food odor	Shark 20	Square 2	After_Stimuli	1028	550	654			2232	
Food odor	Shark 20	Square 3	Before_Stimuli	602	600	441			1643	0.799757
Food odor	Shark 20	Square 3	After_Stimuli	407	432	475			1314	
Food odor	Shark 20	Square 4	Before_Stimuli	523	434	529			1486	0.777927
Food odor	Shark 20	Square 4	After_Stimuli	331	489	336			1156	
Food odor	Shark 21	Square 1	Before_Stimuli	684	1449	658	671		3462	0.671577
Food odor	Shark 21	Square 1	After_Stimuli	691	713	546	375		2325	
Food odor	Shark 21	Square 2	Before_Stimuli	540	151	450	425		1566	1.704342
Food odor	Shark 21	Square 2	After_Stimuli	570	651	825	623		2669	
Food odor	Shark 21	Square 3	Before_Stimuli	556	482	498	499		2035	0.685012
Food odor	Shark 21	Square 3	After_Stimuli	330	367	362	335		1394	
Food odor	Shark 21	Square 4	Before_Stimuli	396	97	509	394		1396	1.378223
Food odor	Shark 21	Square 4	After_Stimuli	450	240	417	817		1924	

S.E 0.5 U	Shark 9	Square 1	Before_Stimuli	375	1150	421			1946	0.77852
S.E 0.5 U	Shark 9	Square 1	After_Stimuli	415	750	350			1515	
S.E 0.5 U	Shark 9	Square 2	Before_Stimuli	353	360	645			1358	1.012518
S.E 0.5 U	Shark 9	Square 2	After_Stimuli	558	400	417			1375	
S.E 0.5 U	Shark 9	Square 3	Before_Stimuli	538	198	236			972	1.311728
S.E 0.5 U	Shark 9	Square 3	After_Stimuli	394	275	606			1275	
S.E 0.5 U	Shark 9	Square 4	Before_Stimuli	507	189	515			1211	1.225434
S.E 0.5 U	Shark 9	Square 4	After_Stimuli	556	416	512			1484	
S.E 0.5 U	Shark 13	Square 1	Before_Stimuli	671	589				1260	0.965079
S.E 0.5 U	Shark 13	Square 1	After_Stimuli	579	637				1216	
S.E 0.5 U	Shark 13	Square 2	Before_Stimuli	983	591				1574	0.74587
S.E 0.5 U	Shark 13	Square 2	After_Stimuli	696	478				1174	
S.E 0.5 U	Shark 13	Square 3	Before_Stimuli	200	468				668	1.209581
S.E 0.5 U	Shark 13	Square 3	After_Stimuli	382	426				808	
S.E 0.5 U	Shark 13	Square 4	Before_Stimuli	304	599				903	1.159468
S.E 0.5 U	Shark 13	Square 4	After_Stimuli	468	579				1047	
S.E 0.5 U	Shark 15	Square 1	Before_Stimuli	789	657				1446	0.578147
S.E 0.5 U	Shark 15	Square 1	After_Stimuli	378	458				836	
S.E 0.5 U	Shark 15	Square 2	Before_Stimuli	444	514				958	1.129436
S.E 0.5 U	Shark 15	Square 2	After_Stimuli	553	529				1082	
S.E 0.5 U	Shark 15	Square 3	Before_Stimuli	364	388				752	0.945479
S.E 0.5 U	Shark 15	Square 3	After_Stimuli	369	342				711	
S.E 0.5 U	Shark 15	Square 4	Before_Stimuli	469	415				884	1.091629
S.E 0.5 U	Shark 15	Square 4	After_Stimuli	384	581				965	
S.E 0.5 U	Shark 16	Square 1	Before_Stimuli	534	838				1372	0.805394
S.E 0.5 U	Shark 16	Square 1	After_Stimuli	414	691				1105	
S.E 0.5 U	Shark 16	Square 2	Before_Stimuli	520	478				998	1.199399
S.E 0.5 U	Shark 16	Square 2	After_Stimuli	647	550				1197	
S.E 0.5 U	Shark 16	Square 3	Before_Stimuli	450	362				812	0.820197
S.E 0.5 U	Shark 16	Square 3	After_Stimuli	378	288				666	
S.E 0.5 U	Shark 16	Square 4	Before_Stimuli	411	236				647	1.380216
S.E 0.5 U	Shark 16	Square 4	After_Stimuli	529	364				893	
S.E 0.5 U	Shark 17	Square 1	Before_Stimuli	594	728				1322	0.864599
S.E 0.5 U	Shark 17	Square 1	After_Stimuli	528	615				1143	
S.E 0.5 U	Shark 17	Square 2	Before_Stimuli	469	529				998	0.830661
S.E 0.5 U	Shark 17	Square 2	After_Stimuli	403	426				829	
S.E 0.5 U	Shark 17	Square 3	Before_Stimuli	503	442				945	1.044444
S.E 0.5 U	Shark 17	Square 3	After_Stimuli	437	550				987	
S.E 0.5 U	Shark 17	Square 4	Before_Stimuli	522	396				918	1.166667
S.E 0.5 U	Shark 17	Square 4	After_Stimuli	578	493				1071	
S.E 0.5 U	Shark 18	Square 1	Before_Stimuli	503	495				998	1.123246
S.E 0.5 U	Shark 18	Square 1	After_Stimuli	540	581				1121	
S.E 0.5 U	Shark 18	Square 2	Before_Stimuli	534	576				1110	1.154955
S.E 0.5 U	Shark 18	Square 2	After_Stimuli	628	654				1282	
S.E 0.5 U	Shark 18	Square 3	Before_Stimuli	571	502				1073	1.078285
S.E 0.5 U	Shark 18	Square 3	After_Stimuli	620	537				1157	
S.E 0.5 U	Shark 18	Square 4	Before_Stimuli	402	414				816	1.052696
S.E 0.5 U	Shark 18	Square 4	After_Stimuli	452	407				859	
S.E 0.5 U	Shark 20	Square 1	Before_Stimuli	624	571				1195	1.138075
S.E 0.5 U	Shark 20	Square 1	After_Stimuli	790	570				1360	
S.E 0.5 U	Shark 20	Square 2	Before_Stimuli	453	380				833	1.098439
S.E 0.5 U	Shark 20	Square 2	After_Stimuli	568	347				915	
S.E 0.5 U	Shark 20	Square 3	Before_Stimuli	464	475				939	1.051118
S.E 0.5 U	Shark 20	Square 3	After_Stimuli	516	471				987	
S.E 0.5 U	Shark 20	Square 4	Before_Stimuli	551	527				1078	0.855288
S.E 0.5 U	Shark 20	Square 4	After_Stimuli	349	573				922	
S.E 0.5 U	Shark 21	Square 1	Before_Stimuli	764	661				1425	0.89614
S.E 0.5 U	Shark 21	Square 1	After_Stimuli	762	515				1277	
S.E 0.5 U	Shark 21	Square 2	Before_Stimuli	386	446				832	1.33774
S.E 0.5 U	Shark 21	Square 2	After_Stimuli	588	525				1113	
S.E 0.5 U	Shark 21	Square 3	Before_Stimuli	594	477				1071	0.569561
S.E 0.5 U	Shark 21	Square 3	After_Stimuli	255	355				610	
S.E 0.5 U	Shark 21	Square 4	Before_Stimuli	331	481				812	1.385468
S.E 0.5 U	Shark 21	Square 4	After_Stimuli	505	620				1125	
Skin extract 1 U	Shark 9	Square 1	Before_Stimuli	391	606				997	0.731194
Skin extract 1 U	Shark 9	Square 1	After_Stimuli	409	320				729	
Skin extract 1 U	Shark 9	Square 2	Before_Stimuli	531	406				937	1.279616
Skin extract 1 U	Shark 9	Square 2	After_Stimuli	549	650				1199	

Skin extract 1 U	Shark 9	Square 3	Before_Stimuli	398	219				617	1.311183
Skin extract 1 U	Shark 9	Square 3	After_Stimuli	463	346				809	
Skin extract 1 U	Shark 9	Square 4	Before_Stimuli	421	419				840	1.134524
Skin extract 1 U	Shark 9	Square 4	After_Stimuli	419	534				953	
Skin extract 1 U	Shark 13	Square 1	Before_Stimuli	460	525				985	1.570558
Skin extract 1 U	Shark 13	Square 1	After_Stimuli	610	937				1547	
Skin extract 1 U	Shark 13	Square 2	Before_Stimuli	564	272				836	0.952153
Skin extract 1 U	Shark 13	Square 2	After_Stimuli	494	302				796	
Skin extract 1 U	Shark 13	Square 3	Before_Stimuli	551	503				1054	0.99241
Skin extract 1 U	Shark 13	Square 3	After_Stimuli	636	410				1046	
Skin extract 1 U	Shark 13	Square 4	Before_Stimuli	409	855				1264	0.707278
Skin extract 1 U	Shark 13	Square 4	After_Stimuli	394	500				894	
Skin extract 1 U	Shark 15	Square 1	Before_Stimuli	732	696				1428	1.113445
Skin extract 1 U	Shark 15	Square 1	After_Stimuli	648	942				1590	
Skin extract 1 U	Shark 15	Square 2	Before_Stimuli	470	529				999	0.84985
Skin extract 1 U	Shark 15	Square 2	After_Stimuli	452	397				849	
Skin extract 1 U	Shark 15	Square 3	Before_Stimuli	400	464				864	0.842593
Skin extract 1 U	Shark 15	Square 3	After_Stimuli	363	365				728	
Skin extract 1 U	Shark 15	Square 4	Before_Stimuli	340	422				762	1.064304
Skin extract 1 U	Shark 15	Square 4	After_Stimuli	444	367				811	
Skin extract 1 U	Shark 16	Square 1	Before_Stimuli	619	544				1163	1.503009
Skin extract 1 U	Shark 16	Square 1	After_Stimuli	446	1302				1748	
Skin extract 1 U	Shark 16	Square 2	Before_Stimuli	358	655				1013	0.748272
Skin extract 1 U	Shark 16	Square 2	After_Stimuli	355	403				758	
Skin extract 1 U	Shark 16	Square 3	Before_Stimuli	315	263				578	1.145329
Skin extract 1 U	Shark 16	Square 3	After_Stimuli	443	219				662	
Skin extract 1 U	Shark 16	Square 4	Before_Stimuli	560	412				972	0.864198
Skin extract 1 U	Shark 16	Square 4	After_Stimuli	658	182				840	
Skin extract 1 U	Shark 17	Square 1	Before_Stimuli	1121	955	596			2672	0.724177
Skin extract 1 U	Shark 17	Square 1	After_Stimuli	556	739	640			1935	
Skin extract 1 U	Shark 17	Square 2	Before_Stimuli	335	516	429			1280	1.410156
Skin extract 1 U	Shark 17	Square 2	After_Stimuli	590	631	584			1805	
Skin extract 1 U	Shark 17	Square 3	Before_Stimuli	155	163	616			934	1.426124
Skin extract 1 U	Shark 17	Square 3	After_Stimuli	519	412	401			1332	
Skin extract 1 U	Shark 17	Square 4	Before_Stimuli	418	472	591			1481	0.926401
Skin extract 1 U	Shark 17	Square 4	After_Stimuli	465	355	552			1372	
Skin extract 1 U	Shark 18	Square 1	Before_Stimuli	617	581				1198	0.687813
Skin extract 1 U	Shark 18	Square 1	After_Stimuli	356	468				824	
Skin extract 1 U	Shark 18	Square 2	Before_Stimuli	347	542				889	1.249719
Skin extract 1 U	Shark 18	Square 2	After_Stimuli	477	634				1111	
Skin extract 1 U	Shark 18	Square 3	Before_Stimuli	676	578				1254	1.004785
Skin extract 1 U	Shark 18	Square 3	After_Stimuli	719	541				1260	
Skin extract 1 U	Shark 18	Square 4	Before_Stimuli	656	488				1144	1.147727
Skin extract 1 U	Shark 18	Square 4	After_Stimuli	749	564				1313	
Skin extract 1 U	Shark 20	Square 1	Before_Stimuli	647	571				1218	1.116585
Skin extract 1 U	Shark 20	Square 1	After_Stimuli	731	629				1360	
Skin extract 1 U	Shark 20	Square 2	Before_Stimuli	403	748				1151	1.156386
Skin extract 1 U	Shark 20	Square 2	After_Stimuli	689	642				1331	
Skin extract 1 U	Shark 20	Square 3	Before_Stimuli	459	512				971	1.118435
Skin extract 1 U	Shark 20	Square 3	After_Stimuli	505	581				1086	
Skin extract 1 U	Shark 20	Square 4	Before_Stimuli	563	434				997	0.774323
Skin extract 1 U	Shark 20	Square 4	After_Stimuli	337	435				772	
Skin extract 1 U	Shark 21	Square 1	Before_Stimuli	1127	1291				2418	0.57072
Skin extract 1 U	Shark 21	Square 1	After_Stimuli	404	976				1380	
Skin extract 1 U	Shark 21	Square 2	Before_Stimuli	479	292				771	1.898833
Skin extract 1 U	Shark 21	Square 2	After_Stimuli	948	516				1464	
Skin extract 1 U	Shark 21	Square 3	Before_Stimuli	62	186				248	1.33871
Skin extract 1 U	Shark 21	Square 3	After_Stimuli	139	193				332	
Skin extract 1 U	Shark 21	Square 4	Before_Stimuli	461	343				804	1
Skin extract 1 U	Shark 21	Square 4	After_Stimuli	403	401				804	
Skin extract 2 U	Shark 9	Square 1	Before_Stimuli	549	992				1541	0.687865
Skin extract 2 U	Shark 9	Square 1	After_Stimuli	392	668				1060	
Skin extract 2 U	Shark 9	Square 2	Before_Stimuli	465	278				743	1.304172
Skin extract 2 U	Shark 9	Square 2	After_Stimuli	614	355				969	
Skin extract 2 U	Shark 9	Square 3	Before_Stimuli	232	313				545	1.581651
Skin extract 2 U	Shark 9	Square 3	After_Stimuli	432	430				862	
Skin extract 2 U	Shark 9	Square 4	Before_Stimuli	401	223				624	1.06891
Skin extract 2 U	Shark 9	Square 4	After_Stimuli	356	311				667	

Skin extract 2 U	Shark 13	Square 1	Before_Stimuli	912	177				1089	0.844812
Skin extract 2 U	Shark 13	Square 1	After_Stimuli	756	164				920	
Skin extract 2 U	Shark 13	Square 2	Before_Stimuli	537	911				1448	1.018646
Skin extract 2 U	Shark 13	Square 2	After_Stimuli	706	769				1475	
Skin extract 2 U	Shark 13	Square 3	Before_Stimuli	261	411				672	1.39881
Skin extract 2 U	Shark 13	Square 3	After_Stimuli	318	622				940	
Skin extract 2 U	Shark 13	Square 4	Before_Stimuli	362	686				1048	0.919847
Skin extract 2 U	Shark 13	Square 4	After_Stimuli	394	570				964	
Skin extract 2 U	Shark 15	Square 1	Before_Stimuli	948	515				1463	0.808612
Skin extract 2 U	Shark 15	Square 1	After_Stimuli	756	427				1183	
Skin extract 2 U	Shark 15	Square 2	Before_Stimuli	419	580				999	1.151151
Skin extract 2 U	Shark 15	Square 2	After_Stimuli	542	608				1150	
Skin extract 2 U	Shark 15	Square 3	Before_Stimuli	439	388				827	0.868198
Skin extract 2 U	Shark 15	Square 3	After_Stimuli	299	419				718	
Skin extract 2 U	Shark 15	Square 4	Before_Stimuli	319	372				691	1.623734
Skin extract 2 U	Shark 15	Square 4	After_Stimuli	405	717				1122	
Skin extract 2 U	Shark 16	Square 1	Before_Stimuli	1118	796				1914	0.45559
Skin extract 2 U	Shark 16	Square 1	After_Stimuli	275	597				872	
Skin extract 2 U	Shark 16	Square 2	Before_Stimuli	475	623				1098	1.525501
Skin extract 2 U	Shark 16	Square 2	After_Stimuli	1031	644				1675	
Skin extract 2 U	Shark 16	Square 3	Before_Stimuli	203	302				505	1.007921
Skin extract 2 U	Shark 16	Square 3	After_Stimuli	287	222				509	
Skin extract 2 U	Shark 16	Square 4	Before_Stimuli	123	248				371	2.097035
Skin extract 2 U	Shark 16	Square 4	After_Stimuli	414	364				778	
Skin extract 2 U	Shark 17	Square 1	Before_Stimuli	590	509	1004			2103	1.134094
Skin extract 2 U	Shark 17	Square 1	After_Stimuli	680	669	1036			2385	
Skin extract 2 U	Shark 17	Square 2	Before_Stimuli	632	791	469			1892	0.812896
Skin extract 2 U	Shark 17	Square 2	After_Stimuli	465	618	455			1538	
Skin extract 2 U	Shark 17	Square 3	Before_Stimuli	496	169	269			934	1.234475
Skin extract 2 U	Shark 17	Square 3	After_Stimuli	455	302	396			1153	
Skin extract 2 U	Shark 17	Square 4	Before_Stimuli	487	631	439			1557	0.79833
Skin extract 2 U	Shark 17	Square 4	After_Stimuli	512	470	261			1243	
Skin extract 2 U	Shark 18	Square 1	Before_Stimuli	503	480				983	1.310275
Skin extract 2 U	Shark 18	Square 1	After_Stimuli	598	690				1288	
Skin extract 2 U	Shark 18	Square 2	Before_Stimuli	596	553				1149	0.968668
Skin extract 2 U	Shark 18	Square 2	After_Stimuli	544	569				1113	
Skin extract 2 U	Shark 18	Square 3	Before_Stimuli	589	596				1185	0.885232
Skin extract 2 U	Shark 18	Square 3	After_Stimuli	642	407				1049	
Skin extract 2 U	Shark 18	Square 4	Before_Stimuli	481	438				919	1.068553
Skin extract 2 U	Shark 18	Square 4	After_Stimuli	498	484				982	
Skin extract 2 U	Shark 20	Square 1	Before_Stimuli	600	588				1188	0.956229
Skin extract 2 U	Shark 20	Square 1	After_Stimuli	608	528				1136	
Skin extract 2 U	Shark 20	Square 2	Before_Stimuli	391	394				785	1.165605
Skin extract 2 U	Shark 20	Square 2	After_Stimuli	402	513				915	
Skin extract 2 U	Shark 20	Square 3	Before_Stimuli	452	479				931	1.061224
Skin extract 2 U	Shark 20	Square 3	After_Stimuli	459	529				988	
Skin extract 2 U	Shark 20	Square 4	Before_Stimuli	529	555				1084	1.015683
Skin extract 2 U	Shark 20	Square 4	After_Stimuli	563	538				1101	
Skin extract 2 U	Shark 21	Square 1	Before_Stimuli	1137	310				1447	0.913614
Skin extract 2 U	Shark 21	Square 1	After_Stimuli	738	584				1322	
Skin extract 2 U	Shark 21	Square 2	Before_Stimuli	624	1015				1639	0.805979
Skin extract 2 U	Shark 21	Square 2	After_Stimuli	742	579				1321	
Skin extract 2 U	Shark 21	Square 3	Before_Stimuli	145	132				277	0.931408
Skin extract 2 U	Shark 21	Square 3	After_Stimuli	82	176				258	
Skin extract 2 U	Shark 21	Square 4	Before_Stimuli	234	503				737	1.234735
Skin extract 2 U	Shark 21	Square 4	After_Stimuli	466	444				910	
M. rec	Shark 9	Square 1	Before_Stimuli	395	479	429			1303	1.131236
M. rec	Shark 9	Square 1	After_Stimuli	512	461	501			1474	
M. rec	Shark 9	Square 2	Before_Stimuli	464	518	504			1486	0.9179
M. rec	Shark 9	Square 2	After_Stimuli	307	503	554			1364	
M. rec	Shark 9	Square 3	Before_Stimuli	495	419	404			1318	1.080425
M. rec	Shark 9	Square 3	After_Stimuli	490	514	420			1424	
M. rec	Shark 9	Square 4	Before_Stimuli	457	383	468			1308	1.068043
M. rec	Shark 9	Square 4	After_Stimuli	445	436	516			1397	
M. rec	Shark 13	Square 1	Before_Stimuli	274	826				1100	1.133636
M. rec	Shark 13	Square 1	After_Stimuli	463	784				1247	
M. rec	Shark 13	Square 2	Before_Stimuli	219	666				885	0.966102
M. rec	Shark 13	Square 2	After_Stimuli	350	505				855	

M. rec	Shark 13	Square 3	Before_Stimuli	702	204				906	0.92936
M. rec	Shark 13	Square 3	After_Stimuli	525	317				842	
M. rec	Shark 13	Square 4	Before_Stimuli	920	302				1222	1.072013
M. rec	Shark 13	Square 4	After_Stimuli	895	415				1310	
M. rec	Shark 15	Square 1	Before_Stimuli	484	576	660			1720	0.694186
M. rec	Shark 15	Square 1	After_Stimuli	523	230	441			1194	
M. rec	Shark 15	Square 2	Before_Stimuli	402	373	600			1375	1.235636
M. rec	Shark 15	Square 2	After_Stimuli	464	627	608			1699	
M. rec	Shark 15	Square 3	Before_Stimuli	417	424	411			1252	1.257188
M. rec	Shark 15	Square 3	After_Stimuli	473	532	569			1574	
M. rec	Shark 15	Square 4	Before_Stimuli	549	514	408			1471	1.301156
M. rec	Shark 15	Square 4	After_Stimuli	695	547	672			1914	
M. rec	Shark 16	Square 1	Before_Stimuli	564	525	519			1608	0.881841
M. rec	Shark 16	Square 1	After_Stimuli	503	354	561			1418	
M. rec	Shark 16	Square 2	Before_Stimuli	359	603	615			1577	1.110336
M. rec	Shark 16	Square 2	After_Stimuli	537	697	517			1751	
M. rec	Shark 16	Square 3	Before_Stimuli	567	422	467			1456	0.980769
M. rec	Shark 16	Square 3	After_Stimuli	457	451	520			1428	
M. rec	Shark 16	Square 4	Before_Stimuli	507	432	305			1244	1.122186
M. rec	Shark 16	Square 4	After_Stimuli	456	438	502			1396	
M. rec	Shark 17	Square 1	Before_Stimuli	458	418	725			1601	1.08807
M. rec	Shark 17	Square 1	After_Stimuli	527	569	646			1742	
M. rec	Shark 17	Square 2	Before_Stimuli	439	616	476			1531	1.099282
M. rec	Shark 17	Square 2	After_Stimuli	401	810	472			1683	
M. rec	Shark 17	Square 3	Before_Stimuli	582	515	476			1573	0.68595
M. rec	Shark 17	Square 3	After_Stimuli	481	83	515			1079	
M. rec	Shark 17	Square 4	Before_Stimuli	697	646	530			1873	0.812066
M. rec	Shark 17	Square 4	After_Stimuli	561	532	428			1521	
M. rec	Shark 18	Square 1	Before_Stimuli	353	430	552			1335	1.045693
M. rec	Shark 18	Square 1	After_Stimuli	298	554	544			1396	
M. rec	Shark 18	Square 2	Before_Stimuli	461	667	517			1645	1.213982
M. rec	Shark 18	Square 2	After_Stimuli	820	590	587			1997	
M. rec	Shark 18	Square 3	Before_Stimuli	533	545	600			1678	0.964243
M. rec	Shark 18	Square 3	After_Stimuli	448	629	541			1618	
M. rec	Shark 18	Square 4	Before_Stimuli	798	677	439			1914	0.810345
M. rec	Shark 18	Square 4	After_Stimuli	712	414	425			1551	
M. rec	Shark 20	Square 1	Before_Stimuli	664	709	667			2040	1.029412
M. rec	Shark 20	Square 1	After_Stimuli	766	766	568			2100	
M. rec	Shark 20	Square 2	Before_Stimuli	444	425	607			1476	1.01355
M. rec	Shark 20	Square 2	After_Stimuli	426	391	679			1496	
M. rec	Shark 20	Square 3	Before_Stimuli	568	602	549			1719	1.023851
M. rec	Shark 20	Square 3	After_Stimuli	559	621	580			1760	
M. rec	Shark 20	Square 4	Before_Stimuli	582	555	455			1592	0.906407
M. rec	Shark 20	Square 4	After_Stimuli	506	491	446			1443	
M. rec	Shark 21	Square 1	Before_Stimuli	720	454	805			1979	1.44669
M. rec	Shark 21	Square 1	After_Stimuli	604	951	1308			2863	
M. rec	Shark 21	Square 2	Before_Stimuli	585	475	414			1474	1.048168
M. rec	Shark 21	Square 2	After_Stimuli	631	577	337			1545	
M. rec	Shark 21	Square 3	Before_Stimuli	477	695	454			1626	0.46925
M. rec	Shark 21	Square 3	After_Stimuli	338	237	188			763	
M. rec	Shark 21	Square 4	Before_Stimuli	444	485	488			1417	0.761468
M. rec	Shark 21	Square 4	After_Stimuli	502	333	244			1079	

APPENDIX 5A – CONDITION 1 - STATISTICAL ANALYSES

Comparing the mean distances traveled before and after stimuli

*Table 12. The mean distances traveled before and after stimuli were compared using the paired t-test. Statistical difference was determined by p-values <0.05. * = statistical difference*

Stimuli	P-value
No stimuli	0.393
Sound control	0.420481
Orca sound	0.7777418
Seawater control	0.005128321*
Food odor	0.3642777
Skin extract	0.4197998
EM 100 ms	0.9950018
EM 300 ms	0.9115633
EM 600 ms	0.3592365

Comparing the fold change between trials

*Table 13. The fold change was compared between trials with different stimuli using the paired t-test. Statistical difference was determined by p-values <0.05. * = statistical difference*

Stimuli	P-value
Sound control vs. Orca sound	0.3823338
Seawater vs. food odor	0.00533
Seawater vs. skin extract	0.4815
Food odor vs. skin extract	0.2262
EM 0,6 ms vs EM 0,3 ms	0.3790186
EM 0,6 ms vs EM 0,1 ms	0.3498078
EM 0,3 ms vs EM 0,1 ms	0.6245052
EM 0.6 vs Morning rec	0.9027
EM 0.3 ms vs Morning rec	0.3449865
EM 0.1 ms vs Morning rec	0.6390527

Comparing the fold change between position counts in quadrants

*Table 14. Comparing the fold change of position counts from the quadrants with one-way ANOVA and following post-hoc analysis if ANOVA were significant. For the ANOVA analysis 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1.*

Stimuli	ANOVA followed by Post hoc	P-value
Sound control	ANOVA	0.101
Orca sound	ANOVA	0.0894
Sound control	ANOVA	0.101
Food odor	ANOVA	0.00318 **
	Square 2 - Square 1	0.9979129
	Square 3 - Square 1	0.0277822
	Square 4 - Square 1	0.0217900
	Square 3 - Square 2	0.0417990
	Square 4 - Square 2	0.0330534
	Square 4 - Square 3	0.9995912
Skin extract	ANOVA	0.173
EM 0.1 ms	ANOVA	0.862
EM 0.3 ms	ANOVA	0.433
EM 0.6 ms	ANOVA	0.385

APPENDIX 5B – CONDITION 2 - STATISTICAL ANALYSES

Comparing the mean distances traveled before and after stimuli

Table 15. The mean distances traveled before and after stimuli were compared using the paired t-test. Statistical difference was determined by p-values <0.05.

Stimuli	**P-value**
EM 5 V	0.06832
EM 10 V	0.1881
EM 20 V	0.2351

Comparing the fold change between trials

*Table 16. The fold change was compared between trials with different stimuli using the paired t-test. Statistical difference was determined by p-values <0.05. * = statistical difference*

Stimuli	**P-value**
No stimuli vs. 5 V	0.2235
No stimuli vs. 10 V	0.157
No stimuli vs. 20 V	0.1734
5 V vs. 10 V	0.7599
5 V vs. 20 V	0.3854
10 V vs. 20 V	0.406

Comparing the fold change between position counts in quadrants

*Table 17. Comparing the fold change of position counts from the quadrants with one-way ANOVA and following post-hoc analysis if ANOVA were significant. For the ANOVA analysis 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1.*

Stimuli	Test	P-value
No stimuli	ANOVA	0.277
5 V	ANOVA	0.194
10 V	ANOVA	0.162
20 V	ANOVA	0.479

APPENDIX 5C – CONDITION 3 - STATISTICAL ANALYSES

Comparing the mean distances traveled before and after stimuli

Table 18. The mean distances traveled before and after stimuli were compared using the paired t-test. Statistical difference was determined by p-values <0.05.

Stimuli	**P-value**
No stimuli	0.272
Seawater control	0.03809*
Skin extract 0.5 U	0.4453
Skin extract 1 U	0.2413
Skin extract 2 U	0.1516

Comparing the fold change between trials

*Table 19. The fold change was compared between trials with different stimuli using one-way ANOVA, as the data sets from the trials in condition 3 were unequal and impossible to compare with a paired t-test. For the ANOVA analysis 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1.*

The fold change between all stimuli were analyzed with one-way ANOVA	**p=0.222**

Comparing the fold change between position counts in quadrants

*Table 20. Comparing the fold change of position counts from the quadrants with one-way ANOVA and following post-hoc analysis if ANOVA were significant. For the ANOVA analysis 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1.*

Stimuli	Test	P-value
No stimuli	ANOVA	0.401
Seawater control	ANOVA	0.302
Food odor	ANOVA	0.221
Skin extract 0.5 U	ANOVA	0.0689
	Square 4 vs. Square 1	0.0481164*
Skin extract 1 U	ANOVA	0.323
Skin extract 2 U	ANOVA	0.191

APPENDIX 6A – METABOLITE LEVELS FROM SERUM ANALYSIS

Table 21: The serum metabolite levels from analysis. LDHI FCC was excluded as none of the sharks in the control group were analyzed for this.

Shark number	Stimuli X	TP (Total protein) g/L	Magnesium (mmol/L)	Lactic Acid (mmol/L)	Na (mmol/L)	K (mmol/L)	Cholesterol (mmol/L)	Triglycerides (mmol/L)	Phosphorus (mmol/L)
1	EM	29.09	1	2.273	263.4	2.04	2.155	1.234	1.46
2	Skin extract	7.94	1.3	1.277	268.08	1.99	2.149	1.004	1.86
3	Control	5.98	1.28	1.272	264.22	2.3	2.314	1.001	1.56
4	Control	12.49	1.19	0.962	266.18	2.09	2.375	0.813	1.78
5	Skin extract	43.17	1.14	1.756	266.12	2.3	2.025	0.684	1.62
6	Food odor	22.38	1.02	1.729	276.28	3.39	3.326	1.447	1.31
7	Food odor	3.89	1.07	0.812	270.62	2.92	2.699	1.187	1.25
8	EM	14.26	0.98	2.131	262.93	2.84	2.509	1.14	1.19
9	Skin extract	89.91	1.09	1.268	275.35	2.97	2.243	4.28	1.98
10	EM	20.00	1.2	0.538	257.29	3.19	2.792	1.58	1.49
11	Food odor	55.58	1.01	0.585	266.23	3.07	1.635	1.05	1.79
12	Control	33.59	1.18	0.629	261.44	3.02	2.101	1.81	1.54
13	EM	84.54	1.01	0.796	264.52	3.77	2.706	2.47	2.06
14	Control	11.39	1.28	3.851	275.78	3.15	1.724	0.76	1.65
15	Food odor	152.49	1.06	-	254.43	3.63	1.19	7.19	1.86
16	Skin extract	51.16	1.04	1.286	267.52	3.02	1.9	2.02	1.38
17	Skin extract	94.18	1.2	0.923	273.07	2.99	4.535	4.55	1.64
18	Food odor	205.35	0.89	1.133	269.7	3.09	3.245	8.47	2.48
19	Control	87.19	1	2.288	273.88	3.71	2.862	1.84	1.67
20	EM	71.33	1.05	1.018	271.26	2.82	3.514	3.66	1.74
21	EM	25.57	0.72	0.359	258.2	3.83	3.129	1.58	1.50
22	Skin extract	68.89	1.08	1.331	275.23	3.72	3.708	1.42	1.65

Shark number	Stimuli X	Glucose (mmol/L)	Calcium (mmol/L)	Chloride (mmol/L)	Creatinin enzyme (µmol/L)	Cholesterol HDL(mmol/L)	LDH IFCC (U/L)	Cholesterol LDL (mmol/L)
1	EM	3.32	3.33	251	6	0.16	3.06	0.643
2	Skin extract	5.3	3.75	257.1	2.1	0.21		0.409
3	Control	3.39	3.79	247.9	0.8	0.04		0.205
4	Control	3.46	3.76	250.1	1.7	0.06		0.742
5	Skin extract	3.88	3.26	253.8	7.5	0.1	2.72	0.607
6	Food odor	4.59	3.26	266.1	4.5	0.26		
7	Food odor	4.11	3.36	259.1	2.1	0.24		
8	EM	4.49	3.15	251.2	4.3	0.3	1.19	
9	Skin extract	4.81	3.41	268.3	5.3	0.18		
10	EM	3.48	3.51	244.6	6	0.07		
11	Food odor	5.01	3.55	256	3.3	0.12		
12	Control	5.2	3.43	256.8	2.3	0.14		
13	EM	3.38	3.38	256.4	3.6	0.12		
14	Control	2.91	3.68	264.9	-	0.08		
15	Food odor	3.58	3.24	244.8	1.9	0.12		
16	Skin extract	4.42	3.57	254.1	4.7	0.12		
17	Skin extract	4.68	4	258.8	0.6	0.16		
18	Food odor	3.41	3.8	255.8	3.8	0.23		
19	Control	2.84	3.99	249.2	4.2	0.14		
20	EM	4.05	4.09	255.2	1.4	0.13		
21	EM	1.74	3.59	238.9	9.2	0.06		
22	Skin extract	4.58	3.83	259	7.8	0.1		

APPENDIX 6B –SERUM ANALYSIS - STATISTICAL ANALYSES

Table 22: The results from one-way ANOVA analysis of metabolite levels in blood serum. Only magnesium was subjected to dunnet´s test and found statistical difference between the control and EM treatment groups.

Treatment group	ANOVA , p-value	Dunnett's multiple comparison test, p-value
Total protein	0.2971	
Magnesium	0.0304	0.0331 between control and EM treatment groups.
Lactic Acid	0.5310	
Na	0.1704	
K	0.6602	
Cholesterol	0.6456	
Triglycerides	0.2412	
Phosphorus	0.8298	
Glucose	0.0626	
Calcium	0.3374	
Chloride	0.1533	
Creatinin enzyme	-	
Cholesterol	-	
Cholesterol LDL	-	