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Can baleen whales be safely live-captured for studies of their physiology?

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Cover page picture:

Common minke whale (*Balaenoptera acutorostrata acutorostrata*) entrapped in the basin designed between two islets in the SOST minke hearing project, Vesterålen June 16th, 2021. Photo by Lars Kleivane, LK-ARTS.

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Abstract

Studying baleen whales is challenging and complex, where observation of their habitat, sensory modalities, behavior and physiology, are infrequent and brief. The biochemical and biophysical contribution of mysticetes serve a vital role in maintaining a healthy marine ecosystem, but they are facing anthropogenic threats. Before giving any indications of how these threats affect the baleen whales, it is essential to gain more knowledge of their sensory physiology, migration patterns, and energy expenditure. Previous research on mysticetes has been based on post-mortem investigation, modeling, behavioral analyses, and tagging which are advancing with time. However, some of these studies may need validation, which could be conducted with a mysticete live-capture methodology.

This thesis describes an approach on how to possibly live-capture baleen whales to enable safe studies of their physiology. Therefore, my objectives aimed to 1) assess the methodology of live-capturing and restraining baleen whales by reviewing and evaluating documented attempts and those gained in own fieldwork, 2) discuss what sensory modalities baleen whales may use to navigate around nets in such settings. Furthermore, 3) I have reviewed potential studies that could be conducted on a restrained mysticete, and lastly 4) I discuss animal welfare considerations of mysticete live-capture and experimental studies. A large entrapment was created in Vestfjord, Norway, June 2021. Attempts were made to measure the distance of the baleen whales from the various nets that were designed to be better detected by different sensory methods, including recording hydrophones placed in the entrapment. These results did not give enough statistical power for concluding what sensory apparatus the cetaceans may use in detecting the entrapment set-up. We did succeed in leading baleen whales in between islets and trap them there with nets, but were unable to restrain any of them for direct measurements. The 4-year ongoing SOST minke hearing project has potential to succeed though, and may thereby represent a key to a more detailed insight into the physiology of these huge but vulnerable creatures.

Keywords: minke whale, Balaenoptera acutorostrata, cetacean, baleen whale, mysticete, live-capture, entrapment, evoked potential, animal welfare, logistic handling of whales, physiological study, behavioral study, sensory modalities

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Abbrevations:

- Ballistocardiography: a non-invasive technique that measures the body motion caused by blood ejection throughout each cardiac cycle
- Capture myopathy: a non-infectious condition where damaged muscles can occur from excessive exertion, stress or struggle
- C&R: catch and release
- Electrophysiological study: measures of the electrical activity of neurons
- IUCN: International Union for Conservation of Nature and Natural Resources
- Hp: horse power
- Morphometric: quantitative analysis by measuring size and shape
- Pontoon: a type of raft or a flat boat, having two long floating tubes on each side
- Porpoising: the act of leaping over the water surface in speed
- Purse seine net: fishing technique consisting of a large netting wall surrounding a wide area or a school of fish
- Spyhopping: the act of a cetacean vertically raising its head over the water surface
- SOST: Subcommittee of Ocean Science and Technology
- Stranding: the act of cetaceans arriving on shore on purpose or by accident, alive or dead
- Tail noose: type of boat knot, similar to that of bowline
- Tail stock: the area in front of the tail that connects to the rest of the body
- Translational ecology: research that addresses the sociological, ecological and political settings of environmental issues
- Translational research: research intended to convert experimentally laboratory findings into a medically useful therapy or procedure
- Ultrasonography: a method that examines tissue and organs in an organism using high-energy sound waves

1 Introduction

This thesis is a combination of a literature review and a pilot study on how to capture and restrain baleen whales for experimental studies before releasing them back to the wild. Despite much progress in research technologies, large whales are still under-studied because of the inherent logistical challenges to handle animals of such large size (Christiansen et al., 2014; Goldbogen et al., 2013). Research has illuminated various types of anthropogenic impact that these animals may be under threat from (Thomas et al., 2016). Further data on of their physiology is important for our fundamental understanding of how these animals function and adapt to their life underwater, and also to predict how anthropogenic threats might affect them. Ultimately, this information is necessary to manage populations of baleen whales in a sustainable way (Goldbogen et al., 2013; Kvadsheim et al., 2020, pp. 94-95; Thomas et al., 2016). One ongoing study, which is the primary focus of this thesis, aims to increase our understanding of the effects of anthropogenic noise on baleen whales by measuring their hearing. Whales rely on sound for life important functions, such as communication, navigation and feeding, and these functions might be affected by anthropogenic noise, depending on the hearing ability of the animal (Kvadsheim et al., 2020, p. 13).

Before presenting the objectives of this thesis, a background on baleen whale taxonomy, ecology, and anthropogenic threats will be presented. I will also give an overview where gaps in research exist, as well as information on the project that I participated in.

1.1 Mysticetes’ role in the ocean

1.1.1 Taxonomy; the minke whale

Cetacea is a suborder consisting of large mammals, which have evolved to obligately live in water. Toothed whales (Odontoceti) are one of two parvorders of Cetacea (Wursig et al., 2017, p. xxix). Odontocetes are characterized by having asymmetrical skulls, one blowhole and teeth. The second parvorder, baleen whales (Mysticeti), differ by having symmetrical skulls, two blowholes, and replacement of teeth with a filtration system in the upper jaw that consists of keratinous plates, or baleen (Fahlke & Hampe, 2015; Werth, 2018). There are different feeding techniques within the baleen whales, dividing them into 4 families; right whales (Balaenidae), pygmy right whale (Neobalaenidae), gray whales (Eschrichtiidae), and rorquals (Balaenopteridae) (Bannister, 2018).

My thesis is based on my participation in a project to live-capture minke whales (*Balaenoptera acutorostrata*) for hearing measurements. Minke whales belong to the family rorquals, but here there are two different species and three subspecies recognized to date with further genetical and morphological studies underway (Glover et al., 2010; Perrin et al., 2018; Øien, 2019). Minke whales can be found globally, yet the species is one of the most difficult to spot due to their unobtrusive spouts, low profile swimming behavior, and quick surfacing (Eerkes-Medrano et al., 2021; Murphy, 1995; Øien, 2019). Minke whale vocalizations include a variety of sounds described as clicks, grunts, bio-ducks, ratches and thump trains, but which differ between the minke whale species and subspecies (Beamish & Mitchell, 1973; Dominello et al., 2013; Risch et al., 2014; Risch, Norris, et al., 2019; Winn & Perkins, 1976). There are a couple of distinguishing phenotypes between the different species of minke whales. In the southern hemisphere, the Antarctic minke whales (*Balaenoptera bonaerensis*) have a pattern-colored baleen similar to that of the fin whale (*Balaenoptera physalus physalus*). These Antarctic minke whales do not have a white band pattern on their fore-flippers and are larger than the common minke whales (*Balaenoptera acutorostrata*). Furthermore, there are three subspecies of the common minke whale. In the North Pacific there is *Balaenoptera acutorostrata scammoni*, in the North Atlantic *Balaenoptera acutorostrata acutorostrata*, and lastly, the dwarf minke whales (*unnamed subspecies),* which are found below the equator, have a coloration similar to the northern minke whales (Risch et al., 2019; Øien, 2019).

Balaenoptera acutorostrata acutorostrata is the subject species for this thesis. These minke whales can grow up to 9 meters long and oversummer in the Norwegian northern waters (Haug, 2021; Jonsgård, 1966, p. 119; Øien, 2019). Little is known of their migration, but studies so far show a pronounced sexual segregation in the North West Atlantic population, where the females occur further north and west of Greenland, while the males are found further south and east of Greenland (Eerkes-Medrano et al., 2021; Laidre et al., 2009). The East Atlantic population can be found around the British Isles and Norway (Heide-Joergensen et al., 2001; Macleod et al., 2004; Risch, Wilson, et al., 2019; Tetley et al., 2008). Both females and males of the species are solitary. Adult females are seldom accompanied by young during the summer, and are often pregnant at this time. Calving time is assumed to occur midwinter. Their habitat during winter remains a mystery (Eerkes-Medrano et al., 2021; Heide-Joergensen et al., 2001; Øien, 2019).

1.1.2 Ecology; whales and climate change

Baleen whales can be found in a wide range of oceanic environments, from the deep sea of the open oceans to the shallows of continental shelves and coastal waters. The animals range from cold water temperatures in the Arctic and Antarctic to warmer waters crossing both hemispheres and the tropics. Although being the largest carnivores, they subsist mostly on small organisms, such as small fish, zooplankton, and various types of crustaceans. They may at times themselves be hunted by predators such as (killer whales, *Orcinus orca*), sharks and humans (Bannister, 2018). Baleen whales occupy a role near the top of the food chain and hence contribute to the marine ecosystem's balance. Due to the animals' trophic level position, changes to the population dynamics potentially indicate a change in their environment (Trites, 2001). The whale's impact on its prey populations may also bring some species in conflict with human fisheries, interacting and competing for the same food resources (Demaster et al., 2001).

Protecting cetaceans could serve as a mitigation strategy for climate change (Martin & Barefoot, 2017, p. 10; Roman et al., 2014). These marine mammals naturally trap carbon or stimulate processes that can trap carbon, playing an important role in the ocean ecosystem (Pershing et al., 2010; Roman et al., 2014). The "whale pump" is a hypothesis describing how whales retreat to the surface to relax and defecate after a deep dive, thus releasing fecal plumes. Fecal plumes are rich in nutrients and important for the phytoplankton, for which they function as a fertilizer (Chami et al., 2019). By leaving the nutrients in shallower water it builds up the bottom food web, absorbing carbon dioxide and trapping the carbon (Roman et al., 2014). Throughout an average whales' life, 33 tons of carbon dioxide is thought to be sequestered, equivalent to carbon dioxide sequestered from thousands of trees (Pershing et al., 2010). Marine snow is a continuous "shower" of macroscopic aggregated detritus, previous living organisms and inorganic matter falling from the upper layers of the water column, exporting energy from the light-rich photic zone to the aphotic zone in the deep ocean (Brakstad et al., 2018). When a baleen whale dies, it also sinks to the typically nutrient poor region of the ocean floor. A 40-ton whale may contain ~$2x10^6$g organic carbon, and if sunken to sea floor, equated to one to two centuries of background carbon sinking from the euphotic zone (Smith & Baco, 2003). There is also an estimation of phytoplankton sequestering over 37 gigatons of carbon dioxide each year, equivalent to four amazon forests (Chami et al., 2019). An increase in whale populations could contribute even more to photosynthesis with their “whale pump”, as well as plunging up and down, moving and keeping the phytoplankton in the water surface for a longer reproduction (Lavery et al., 2010).

1.1.3 Whales in the Anthropocene

Some mysticete species have a history of being hunted close to extinction. Different mysticete species stand imperiled to date, but whaling is not an active threat today due to international agreements (e.g., International Whaling Commission) (Clapham et al., 1999; Thomas et al., 2016), and careful and scientifically based management of whale populations amongst nations that still harvest baleen whales (Walløe et al., 1995, pp. v-vi). Other population stressors include climate change, fisheries interactions, anthropogenic noise and marine pollutants (Tulloch et al., 2019; Harris et al., 2018; Reijnders et al., 2009). With various climate shifts, reduced availability could occur due to prey redistribution and population changes, as well as competition from fisheries (Greene et al., 2003; Kovacs et al., 2011). Ship strikes are causing mortality, and noise pollution in general is becoming a major problem (Laist et al., 2001; Thomas et al., 2016). Little is known of the auditory physiology of baleen whales, but research strongly suggests that their acoustic cues, navigation, migration, detection of predators or prey, and intraspecific communication and interactions could be masked by anthropogenic sounds (Hatch et al., 2012; Moore et al., 2012; Rolland et al., 2012; Thomas et al., 2016).

The demand for marine food resources is continuously increasing, and as the sea ice continues to melt, ship traffic and industrialization of fisheries will expand to new areas, potentially creating more noise pollution and by-catch of cetaceans (Miller et al., 2022; Weir & Pierce, 2013). Cetacean by-catch in fishing nets is a global issue to date (Henry et al., 2021; Ingman et al., 2021; Ramp et al., 2021). Marine pollutants such as persistent organic pollutants (POPs) and plastic are other growing concerns (Jambeck et al., 2015). The exposure is not yet known in baleen whales, but the accumulation of POPs in blubber and ingested plastic is a worry since it may give adverse effects regarding their health and reproduction, as found in toothed whales (Zantis et al., 2022). Every year it is estimated that six million metric tons of plastic enter the oceans. Within the next decade, the plastic pollution is predicted to be ten times more than it is today (Jambeck et al., 2015)

Effective management of baleen whale populations requires more knowledge of baleen whale physiology and how it is affected by environmental change. The physiology of animals enable us to understand the needs and limitations of different species, and thereby manage populations and anthropogenic threats to populations better (Harris et al., 2018; Thomas et al., 2016; Tulloch et al., 2019). Some physiological investigations have been previously made on baleen whales (Beamish, 1978; Sumich, 2001; Wahrenbrock et al., 1974), but baleen whale physiology is still little-known because they are difficult to study (Supin et al., 2001).

1.2 The knowledge gaps of mysticetes

There is insufficient information on baleen whales due to logistic challenges in handling and instrumenting these large mammals (Goldbogen et al., 2013; Supin et al., 2001). Most studies have involved post-mortem examinations, modelling, tagging and behavioral observations, but important aspects of the physiology still lack adequate knowledge, which must be assessed to conserve these mammals, as well as understanding their adaptions in the context of evolution and translational research and ecology (Hill et al., 2016, p. 4; Schlesinger, 2010; Tian et al., 2017). Within this study field there are knowledge gaps, meaning there is inadequate information to reach a conclusion for a given question (Robinson et al., 2013), but what are those questions? Speculations about the physiology of baleen whales' are numerous (Fiedler, 2009; Kiszka et al., 2018; Lisney & Collin, 2018). A significant number of physiological studies have been performed on toothed whales and pinnipeds, but the findings cannot always be extrapolated to baleen whales, by assuming that certain body similarities and a common ancestry would result in similar physiology (Hirose et al., 2018; Kanwisher & Ridgway, 1983). For example, the sensory modalities used for information on their surrounding environment are fundamentally different.

1.2.1 Gaps in sensory physiology

Investigations of baleen whale sensory physiology could provide information on the whales' physiological adaptions to the aquatic environment. By investigating their senses (i.e., acoustics, photoreception, mechanosensory, chemosensory, magnetoreception), we could better determine how they navigate during migrations, localize mates, and find and detect prey. In addition, these studies could inform how anthropogenic activity affects them (e.g., masking, acoustic disturbance) such that plans can be made to reduce human impact.

1.2.1.1 Auditory physiology in mysticetes

Previous studies have provided information on how toothed whales navigate, their hearing and vision capabilities, and how they use echolocation (Fraser & Purves, 1960; Kröger & Katzir, 2019; Norris & Prescott, 1961). Their auditory physiology has been well studied, understanding how sound travels through the pad of fat in their lower jaw to their curved ear bones, the tympanic bulla (Norris & Prescott, 1961). Through post-mortem studies of baleen whales, the tympanic bulla and thin basilar membrane containing sensory receptors for hearing within the cochlea are different to those seen in toothed whales (Fraser & Purves, 1960; Ketten, 1994). In addition, the baleen whales have a wax plug in the ear canal and fats around the ossicular chain, but the details of how sound is transmitted to the inner ear or what frequencies they hear are still unknown (Yamato et al., 2012). Knowledge of baleen whale hearing should be a high priority due to the potential for noise pollution to affect their acoustic ecology (Kvadsheim et al., 2020, pp. 94-95). With no empirical measures of hearing in baleen whales (Tubelli et al., 2018), hearing sensitivity and range of hearing are estimated from behavioral reactions to sound, anatomic models, and frequencies of sound production (Houser et al., 2017; Parks et al., 2007; Yamato et al., 2012). Hearing could directly be tested in small mysticetes through auditory evoked potential (AEP) methods (Houser et al., 2017). AEPs are electrophysiological measurements that monitor nerve signals from the inner ear to the brain and can be used to determine whether an animal hears a sound (Supin & Popov, 2007).

1.2.1.2 Vision in mysticetes

Hearing and echolocation have presumably developed in odontocetes to enable environmental imaging in poor ocean visibility. There is a significant amount of literature on odontocete echolocation and some on vision, but little investigation has been performed in baleen whales (Griebel & Peichl, 2003; Kröger & Katzir, 2019; Lisney & Collin, 2018; Supin & Mass, 2018). Whether echolocation exists in mysticetes is disputed but their hearing capabilities are considered essential to survive (Frazer & Mercado, 2000). Studies have suggested a form of colorblindness in the animals (Griebel & Peichl, 2003). However, relatively little is known about mysticete vision, but one way of studying vision in baleen whales could be by electrophysiological experiments. Electroretinogram has already been proceeded on pinnipeds giving an estimate of their vision traits (Hogg et al., 2015), so it could in theory be applicable to a baleen whale as well.

1.2.1.3 Tactile sensory in mysticetes

Pinnipeds are also known for their whiskers for tactile identification of objects as well as a longer-range sense organ, but a hypothesized tactile body hair long range sensory system has yet to be demonstrated in baleen whales (Dehnhardt et al., 2001; Thewissen et al., 2008, p. 105). A mechanosensory system provides tactile information about the environment to the animal through biological stimuli, derived either directly from physical contact, or indirectly from the object's interference with the surrounding medium (e.g., detecting water movements due to movement history of a fish) (Dehnhardt & Mauck, 2008). There has been a consistent view of whale hairs being essential in environmental detection, but documentation and analysis of precise function lacks (Drake et al., 2015). Reichmuth et al. (2022) suggested further in-situ observations of the vibrissae in mysticetes' after proceeding studies on the Antarctic minke whale. A potential investigation of the tactile sensory could perhaps be measured through somatosensory evoked potential (SSEP). SSEP quantifies the rate at which the nervous system transmits and receives sensation-transmitting impulses (Markand, 2020).

1.2.1.4 Chemoreception in mysticetes

The use of smell or taste are other methods for animals to navigate or locate prey, but there is limited quantitative data on these chemosensory organs of cetaceans (Thewissen et al., 2008, p. 98). The olfactory nerve systems of baleen whales have a fully equipped anatomical structure involved for the chemoreception compared to the odontocetes, suggesting that the baleen whales do possess chemoreception used for detecting aggregations of prey (Hirose et al., 2018; Thewissen et al., 2011; Thewissen et al., 2008, p. 102). However, their detection is hypothesized to occur at the surface in air rather than in the water based on studies of the bowhead whale (*Balaena mysticetus*) (Thewissen et al., 2011).

1.2.1.5 Biomagnetism in mysticetes

How baleen whales migrate is being debated, and some research suggests that they use geomagnetic cues (Gerrits & Kastelein, 1990; Walker et al., 1992). Biomagnetite sensors, in which magnetic particles contained in mechanoreceptors adjust position in response to the ambient magnetic field, are one putative sensory mechanism. Magnetoreception is frequently demonstrated by the presence of biomagnetic material in an animal. Migratory animals such as birds and fish contain biomagnite, which basically works as an internal compass triggered by Earth's magnetic field (Hofmann & Wilkens, 2019). Bauer et al. (1985) detected biomagnite in the humpback whale's (*Megaptera novaeangliae*) brain. The magnetic sense is likely one of the least understood sensory systems where additional investigation is required (Hofmann & Wilkens, 2019).

Some fields of research in sensory modalities are poorly enlightened when it comes to large baleen whales, where live entrapment could be used to forward knowledge in this area.

Knowing more on which sensory cues the animals use to migrate would be beneficial for a complete understanding of how these animals’ function.

1.2.2 Migration and Energy

In addition to a knowledge gap in the sensory systems used to navigate, communicate, or find prey under water, there is also not a full understanding of their migration pattern (Eerkes-Medrano et al., 2021; Mate et al., 2007). Some species such as humpback whales and gray whales (*Eschrichtius robustus*) have their migration routes well mapped (Clapham & Mead, 1999; Corkeron & Connor, 1999; Swartz, 2018, p. 425). Species such as the common minke whale have on the other hand still missing data. Due to the animals’ evasive behavior, they are difficult to spot, and tags do not last long enough to produce reliable tracking data (Eerkes-Medrano et al., 2021). An effective long-lasting tag could assist in knowledge of their migration (Víkingsson & Heide-Jørgensen, 2015).

Minke whales’ unobtrusive spouts are peculiar, leaving one to wonder what mechanisms lie behind. Do they have a respiratory heat exchange mechanism similar to that of seals to save energy and water (Folkow et al., 1988; Folkow & Blix, 1987)? What is the energy exposure and metabolic rate of all mysticete species? This would be beneficial knowledge in assessing their food requirements and how much they are disturbed by anthropogenic activity (Christiansen et al., 2014). Investigating their energy expenditure could be done through measurements such as respirometry. Conservation of energy has previously been put forward as a possible hypothesis for the function of sleep (Berger & Phillips, 1995). Sleep has been studied in different species of toothed whales through electrophysiological studies (Lyamin & Siegel, 2019), where this procedure could potentially be further applied to temporarily restrained baleen whales.

Some of these gaps are more urgent than others, but an overall understanding of how these mammals’ function could assist in conserving a healthier ocean ecosystem. Many of these knowledge gaps could possibly be filled if it were possible to live-capture and do physiological research on baleen whales such as the minke whale. However, live-capturing such large animals is associated with several animal welfare issues that must be considered.

1.3 Animal welfare considerations

The reasoning behind the knowledge gaps of mysticetes is primarily due to the logistical challenges of their size and doing research in their habitat, in addition to limitations in studying these animals due to animal welfare ethics. Animal welfare has become a key concept in animal husbandry, where it considers the quality of life of animals. There are different definitions given to the term, that reflect different approaches and underlying values (Fraser, 2003; Fraser et al., 1997). The definitions are based on either the animals' natural behavior, emotions, biological function, or a combination of these factors. Good animal welfare for animal husbandry in Norway is characterized by 1) the animal has its needs covered regarding nutrition and water, 2) the animal has a good physical environment, 3) the animal is healthy and unharmed, 4) the animal is not exposed to stress, fear or pain, and 5) the animal can execute normal behavior (Mattilsynet, 2013). With wild animals, these factors are not as applicable as to captive animals. A normal life for a wild animal includes hunger, where malnutrition could be a common cause of death. Seasons create shifting challenges for the animal to obtain adequate nutrition, where the animals' physiology and behavior aims to accumulate energy stores in forms of body fat (e.g. blubber in whales), to rely on when conditions become more difficult (Mejdell, 2004). Humans' obligation in relation to all animal welfare are controlled by the Norwegian food safety authority (NFSA). One of NFSA's responsibilities is to manage the use of animals in scientific experiments. A guiding principle in this management is the 3Rs; Replacement, Reduction and Refinement (Mattilsynet, 2021). To maintain good animal welfare, replace the animals if possible, minimize the number of animals used as much as possible, and refine the experiment technique (Russell & Burch, 1959).

In this thesis I will try to analyze the animal welfare aspect of capturing, handling, and studying a large baleen whale, and to consider mitigative measures to minimize any negative welfare effects.

1.4 Previous baleen whale entrapments

By looking into past baleen whale entrapments where previous research opportunities have occurred (e.g., respirometry, morphometrics, bioacoustics, telemetry studies) (Henriksen, 2017; Sumich, 2001; Wahrenbrock et al., 1974; Winn et al., 1979), it may be possible to plan how to take advantage of future comparable opportunities, as well as how to design a purposeful mysticete live-capture technique. Another reason these earlier entrapments are significant is to understand how to entrap these mammals without compromising their animal welfare, and to explore what kind of experimental studies could be conducted on the animal. However, literature involving direct studies on restrained large cetaceans are scarce.

Successful attempts of live-capturing small cetaceans are well documented, whereas the first dolphin was held captive in 1860, and the first commercial dolphinarium opened in 1938 with an increasing popularity and development of dolphinariums until the 1960s (Jiang et al., 2008). The background for these first captivities seemingly did not have research as a motivation where different species of dolphins and porpoises were put on public display for profit (Castricano, 2008; Jiang et al., 2008). Over time, most live-captures have occurred on toothed whales likely due to the logistics of their smaller sizes compared to baleen whales, but there have been a few documented attempts of mysticete live-captures as well (Kimura & Nemoto, 1956; Norris & Prescott, 1961; Wahrenbrock et al., 1974). In addition to the previous live-capture attempts, there have been documented incidents on opportunistic use of direct studies on baleen whales during accidental entrapments, where whales have trapped themselves into an area needing human interaction to retrieve back to the ocean (Beamish, 1978, 1979; Edds et al., 1993; Henriksen, 2017; Priddel & Wheeler, 1997, 1998; Reidarson et al., 2001; Walsh et al., 1991; Winn et al., 1979). Accidental whale entrapments can occur from fishing gear, tidal water, rivers and canals, or ice (Geraci et al., 2005, p. 93; James et al., 2021; Sullivan, 2000; Thomas et al., 2016). These are situations where whales occasionally get lost when feeding or navigating, where their mobility becomes hindered. Fishing gear can cause entanglements with ropes and nets or have open- and closing mechanisms trapping the whale in the system. Tidal levels can change drastically over a short time, creating pools or strand the animal. Ice traps are possibly caused by unusual metrological conditions. The wind disperses the ice, allowing the whales to move and forage, but then the ice joins together, closing the open lead and blocking oxygen access when the wind changes direction (Beamish, 1979).

By investigating these reported scientific opportunities and experimental studies previously conducted on baleen whales, it may be possible to use this information to bridge the knowledge gaps in future studies.

1.5 The SOST minke hearing project

In Vesterålen, Norway, June 2021, researchers from Norway, Denmark, and the USA, funded by the US Subcommittee on Ocean Science and Technology (SOST), attempted to live-capture and temporarily restrain minke whales (*Balaenoptera acutorostrata acutorostrata)* in order to allow studies of their auditory physiology. The 4-year ongoing project is in a consortium with the Norwegian Defence Research establishment (FFI), National Marine

Mammal Foundation, US Navy Marine Mammal Program, University of Århus, Kristiansand Zoo, and LK-ARTS Norway.

Substantial records of whale catches have given local knowledge of the minke whales migration route in and out of the fjord Vestfjorden, Norway (L. Kleivane, personal communication, 03.06.21; Kvadsheim et al., 2021). The adolescent males start to appear in the fjord in April and continue out the Summer (Eerkes-Medrano et al., 2021; Jonsgård, 1966, p. 119). In this project the minke whales' migration pattern was diverted into a live-capture trap for the research purpose of measuring hearing in a mysticete. Before giving any indications of how the noise pollution is affecting the whales its essential to know how low frequency they hear. Far too little is known about whale auditory physiology, so the plan was to measure auditory evoked potentials (AEP). AEPs is an electrophysiological measurement, measuring nerve signals from the inner ear to the brain, giving an indication that the animal hears a sound. The technology that would be used to test the whales' hearing is very similar to the method used on human infants. Subcutaneous electrodes would be placed below the skin and into the blubber layer for AEP recordings. By stimulating different frequencies, researchers could then find the lowest sound level the animal can hear. The veterinarians and researchers would then monitor the animals' health throughout the test through observation and blood sampling. Understanding the hearing ability of baleen whales will aid in understanding how to protect these whales from anthropogenic noise. Providing the first direct measurements of hearing in a baleen whale could further be used to guide auditory weighting functions and facilitate techniques for future hearing tests in baleen whales found stranded or in planned captures (Kvadsheim et al., 2021).

2 Research objectives

This thesis aims to discuss a new concept of how baleen whales could possibly be live-captured for subsequent use in various studies of their physiology; to experimentally test which sensory modalities are important to baleen whales and orient themselves around nets; to discuss challenges and animal welfare considerations in the live-capture of baleen whales, as well as the physiological mechanisms that could be studied if entrapment of large cetaceans was successful. For the first two parts of this thesis, the primary focus will be on data collected from the SOST minke hearing project. The four objectives are as follows:

2.1 Review and evaluate capture and release methods for baleen whales, for research purposes.

There have been previous attempts of live-capturing baleen whales for research purposes, where some projects have been closer to succeeding than others. For this thesis, previous attempts are mentioned and evaluated, and will be compared with the SOST minke hearing project. Assessing what factors that are important to consider when planning such a procedure will be elaborated upon, providing illustrations and descriptions of the methodology used in the SOST minke hearing project and with cetological literature found in reference to that matter. With the data analysis on the observations from the fieldwork and previous literature, improvements to the catch method will be suggested and discussed.

2.2 Testing the use of sensory modalities by baleen whales to orient themselves around nets, based on observations from the SOST minke hearing project

By capturing and releasing the minke whales under strict protocol, information can be gathered on what sensory adaptions the minke whale utilize. This may possibly indicate how baleen whales detect and avoid fishing nets, giving knowledge that can be used to design nets reducing by-catch. An experiment was created as an observational side study of the SOST minke hearing project. With an entrapped whale in controlled conditions, modified nets were used to investigate whether the whale uses visual, tactile or acoustic cues to detect nets.

2.3 Discussing research opportunities, given access to trapped and restrained baleen whales.

For the third objective, this thesis aims to include and discuss potential studies that could be conducted on a temporarily captured baleen whale. As a part of the 3Rs, it would be beneficial for the mysticete populations to use as few animals as possible but still obtain conclusive results, and if possible, utilize as many studies in an ethical way on a constrained baleen whale. The literature of previous attempts is scarce, where many of the references in mysticetes' physiology to date are supported on the studies of the gray whales captured in 1965, 1974, and 1997 (Sumich, 2001; Wahrenbrock et al., 1974). A greater number of studies is needed to fill the knowledge gap, research important to responsible management of baleen whale populations.

2.4 Discussing the animal welfare considerations of live-capture studies of baleen whales.

Lastly, potential health risks and side effects can occur in animals during experiments such as live-capture and release. The observational data of the animals during the SOST minke hearing project, and consideration of future potential risks that could occur in the next three trials will be investigated and discussed. Previous attempts found in other literature of relevant capture procedures will be included in addition. Based on the experiences in the SOST minke hearing project and those documented in previous entrapment of large whales, this could promote the 3Rs, further improving the animal welfare of the mammals experimented upon in future captures and accidental entanglements or entrapments.

3 Material and Methods

3.1 Evaluating previously published research on restrained baleen whales

I have reviewed previously published research on restrained baleen whales in context of live-capture and accidental entrapments, where experimental studies have been conducted. Three elements will be primarily investigated and addressed, which could aid future attempts to study baleen whales. For the first objective that focuses on the design of mysticete live-capture, I will investigate and discuss the methodology of how baleen whales can be successfully restrained. For the third objective that discusses potential studies that could be conducted on restrained whales, I will consider what experimental studies that have previously been conducted on restrained whales and analyze other research issues that could be pursued in such a context. For the fourth objective, the discussion of animals welfare issues related to capture and restraint of mysticetes, I will investigate the methodology used on entrapped baleen whales health in terms of animal welfare.

3.2 The SOST minke hearing project

3.2.1 Study area

The minke whales arrive on the coast of Nordland every May. Some animals undergo a detour into Vesterålen, before swimming out along the Lofoten wall on their way up to the Barents Sea for pasture (L. Kleivane, personal communication, 03.06.21; Kvadsheim et al., 2021). This is the location of where the researchers tried to capture the minke whales in June 2021. Studies suggests they swim into the fjord feeding on herring (*Clupea harengus*) in these areas, where prey likely is dictating their occurrence. Those studies are based on the months September to August when the herring migrates from the summer feeding grounds in the north to the Vestfjord (Heide-Joergensen et al., 2001). Perhaps the returning minke whales observed in the autumn, migrate the same route in the spring and summer for other reasons than foraging in Vestfjorden on their way to the feeding grounds. The natural conditions outside Stamsund and Henningsvær are optimal for capturing, since the whales have the tendency to swim close to land and navigate between islets and reefs on its migratory route to the Barents Sea (figure 3.2.1a). The area is also considered relatively quiet. Studying these animals in the spring and summer months are optimal, where there is constant daylight and frequently better weather for scouting at sea level. The idea on how to live-capture a baleen whales has been partially based on a previous attempt that took place in Iceland in 2007 and how the whales have accidentally been trapped in bottom nets, combined with the little knowledge on their migration pattern (Henriksen, 2017; J. Teilmann, personal communication, 27.10.21; L. Kleivane, personal communication, 03.06.21).

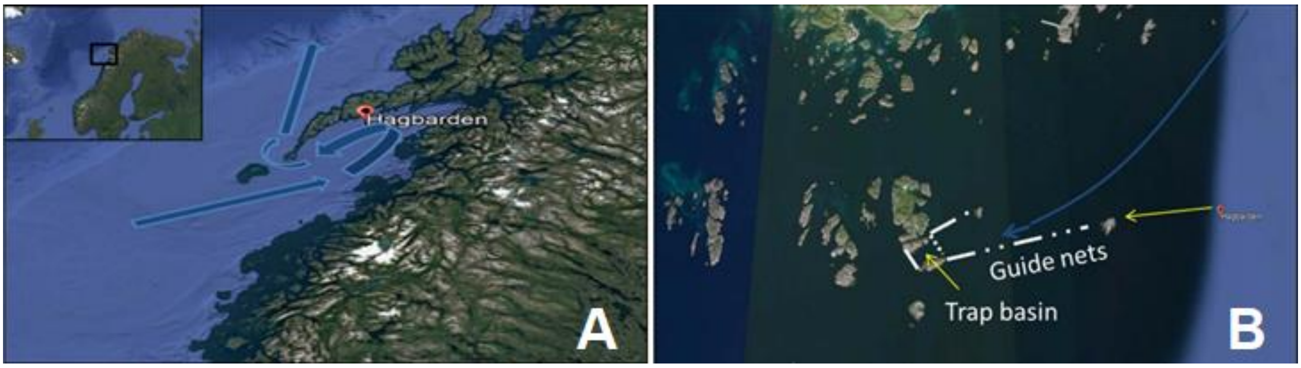


Figure 3.2.1a: Map of the area where the SOST minke hearing project takes place. A: The minke whales follow parts of the Norwegian coastline to their feeding grounds up north. On their way, some whales do a "detour" into Vestfjorden (as shown with the blue arrows). The whales pass the islet Hagbarden since they are forced to migrate along the coast through the Henningsvær peninsula. B: A guiding net which was several kilometers long was placed in this area to divert the migrating whales into the basin placed between two islets (figure from FFI).

The capture method utilized in 2021 with the minke whales was a new approach. To divert the path of these migrating whales, leading them into the basin between two small islands, fine-meshed passive guide nets were used in this project (figure 3.2.1b). When a by-passing minke whale entered the basin between the islets, a part of the net attached along the guiding net was quickly dragged to close the entrapment.

Figure 3.2.1b: The capture location, where nets (dotted lines) were put out to capture minke whales in the SOST minke hearing project. Whales were led to the basin between the islets Kvannholmen and Æsøya, with a guiding net reaching out to a skerry close to Hagbarden. The whale on the map is not proportional in size.

3.2.1.1 The capture facility

The catch and release (C&R) site consisted of a 1100 meter long guiding net and a basin between islets (figure 3.2.1.1a). We had nets drawn between the islets. When the whale swam into the area, one part of the nets was closed creating a basin for the whale to be trapped within. The basin enclosure was estimated to around 1 000 000 cubic meters, approximately 280 meters long, 150 meters wide and an average of 27 meters depth. The basin was divided into different zones to investigate sensory modalities (objective 2.2). These zones reached out 20 meters from the nets and islets creating the basin (figure 3.2.1b). The fish pen connected to the basin was 30 meters in diameter, consisting of a floating ring with a 20-meter-deep net purse connected to the ring.

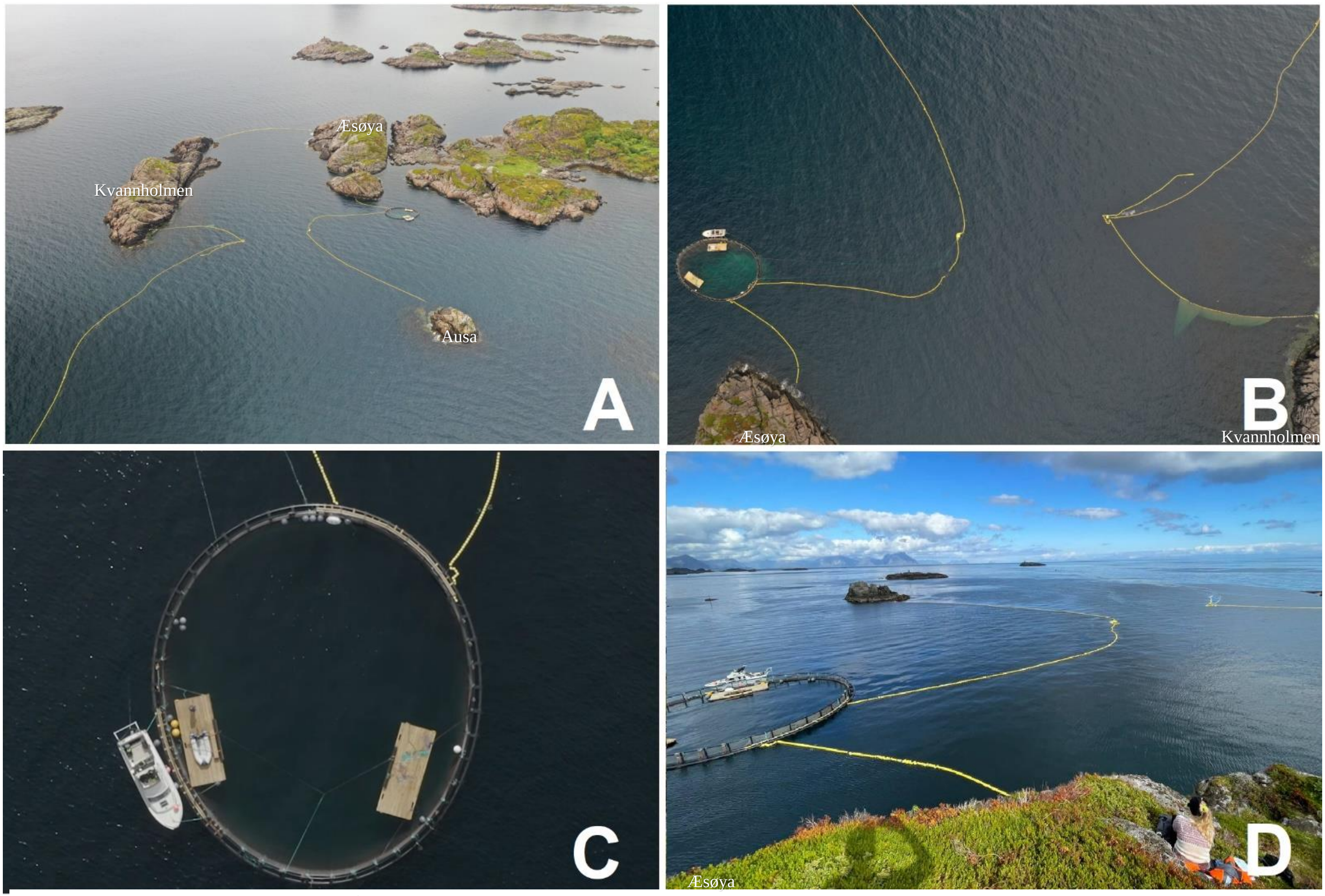


Figure 3.2.1.1a: Drone pictures of the entrapment used during the trial June 2021. A; picture showing the complete set-up, a basin created by the two islets (Kvannholmen on the right and Æsøya on the left). B; picture of the opening to the entrapment where the whale would follow along the guiding net (top right) or net C (top left). The "door" along the guiding net would be closed when the whale was spotted passed the two yellow buoys. C; picture of the complete phase 3 set-up. Rafts were placed on each side, standby for where the whale would be placed in between for AEP measurements. Entrance of the fish pen can be seen on the top part of the picture between the two nets with yellow floaties attached. D; the main lookout post stationed on the edge top of Æsøya, used to observe minke whales in phase 1 (Photos by E. Hofoss & E. V. Naveen, FFI).

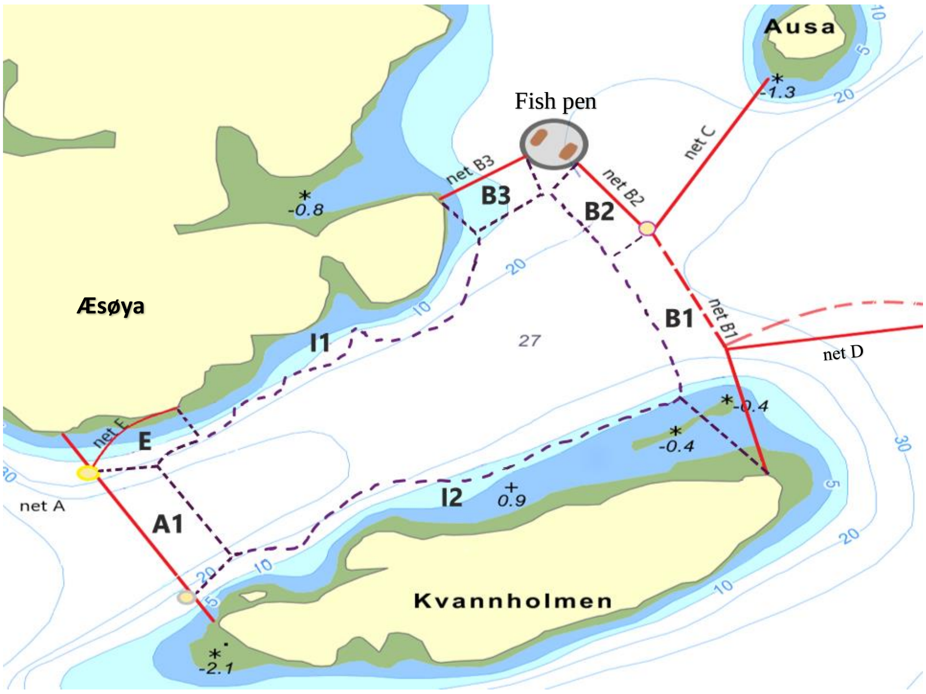


Figure 3.2.1.1b: Map of the basin with the different zones. Zones A1, B1, B2, B3 and E were zones in relation to the net, while zones I1 and I2 were zones in relation to the islets Æsøya and Kvannholmen.

The area had different posts for observing the minke whales under capture attempts. The main post was located on the southeast edge of Æsøya (68°05'56.6"N 13°48'34.6"E), giving a large 180-degree overview reaching several kilometers outward depending on the observer. In addition, this post consisted of a vertical slope giving an optimal view to observe minke whales in the basin. Weather conditions determined the use of the top point of the islets Ausa (68°06'00.9"N 13°48'49.6"E) and Flatskjæret (68°06'04.7"N 13°49'16.2"E) as additional observation posts.

3.2.2 C&R set-up and materials

3.2.2.1 Design of trap

The C&R site was not ready for capture until June 10th, 2021, and the guiding net was incomplete even later, lacking parts of D net attached from buoy C to buoy D (figure 3.2.2a). The plan was to have day and night shifts from the very beginning, but without having the whole team at start date, our observations were based on only day shifts (07:00-19:00, June 16th). In some days of the trial there was difficult weather, where it would be impossible to observe the discrete animals in the choppy water as well managing the different tasks in swells. The procedure of the project was divided into 3 phases (I, II, and III) depending on how far the team would have come with a minke whale in the trap.

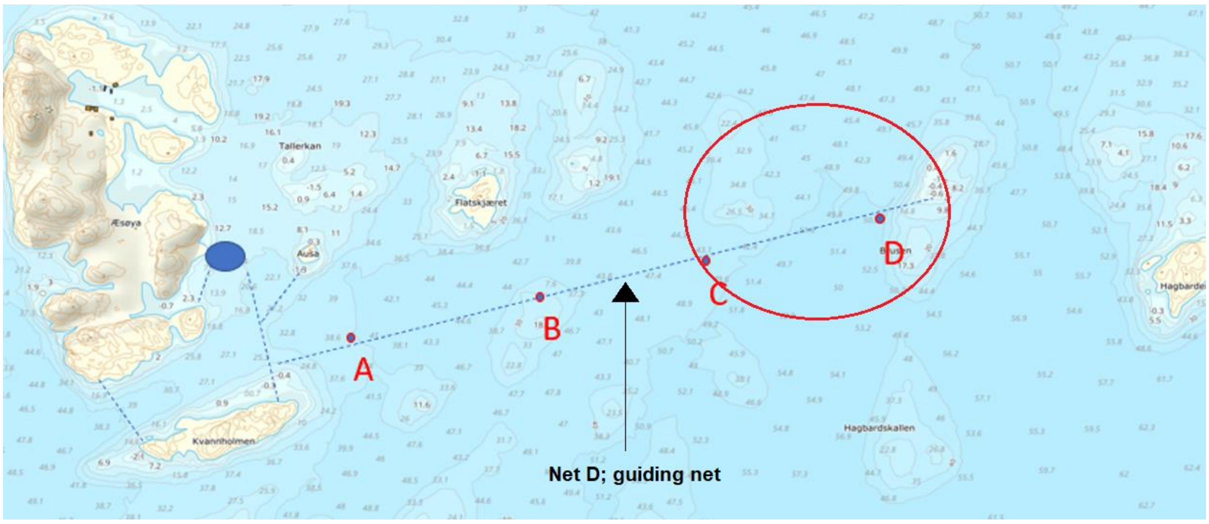


Figure 3.2.2a: The capture location, where nets (dotted lines) were put out to capture minke whales in the SOST minke hearing project. A, B, C, and D are buoys that were attached on the guiding net (net D). However, the net between buoy C and buoy D (encircled in red) was absent for much of the trial, leaving the guiding net much shorter than originally planned.

In "Phase I", also known as the catch phase, the team members were stationed at different posts (the islets Æsøya, Flatskjæret, Ausa, and boats "MOBHUS" and "Zephora") looking for minke whales. "MOBHUS" was a 16 foot long fiber glass man overboard boat with a four-stroke outboard engine (90hp), and "Zephora" was a 29 foot long steel hull Halco offshore vessel, with a 250hp Volvo Penta AQAD engine. Observations were notified to each person on their post through radio, ready to act for the next step to a successful live-capture. The people stationed at the main post on Æsøya were also responsible for noting down the observations of every marine mammal on different sheet papers.

There was an intensive effort to assemble the barrier system to guide the whales into the basin. The involved process required a purse-seine vessel to deploy nets ranging in lengths of 50 meters to 600 meters and depths of 40 meters to 50 meters resting on the bottom (figure 3.2.2b, figure 3.2.2c), where the nets were weighted and stretched to the surface. Over the course of the deployment, nearly 2 kilometers of nets weighting over 20 tons were deployed, many of which were attached and secured directly to land or to 2-ton anchors at the bottom of the seafloor. The work required a purse-seine boat, but also smaller boats that have maneuverability to adjust the nets and tie them off in their positions. Assistance from the local aqua fish company IsQueen was also needed, providing the fish farm and setting it up. In addition to the aqua fisheries having the specialized equipment for the net deployment and the platform placements that were utilized, it was also useful for deployment of various nets in case of any entanglement. This ensured untangled net deployment, settling them straight to the bottom (figure 3.2.2b). The nets belonging to the C&R set-up consisted of different lengths and depths (figure 3.2.2c) (see appendix 7.3 for more details)

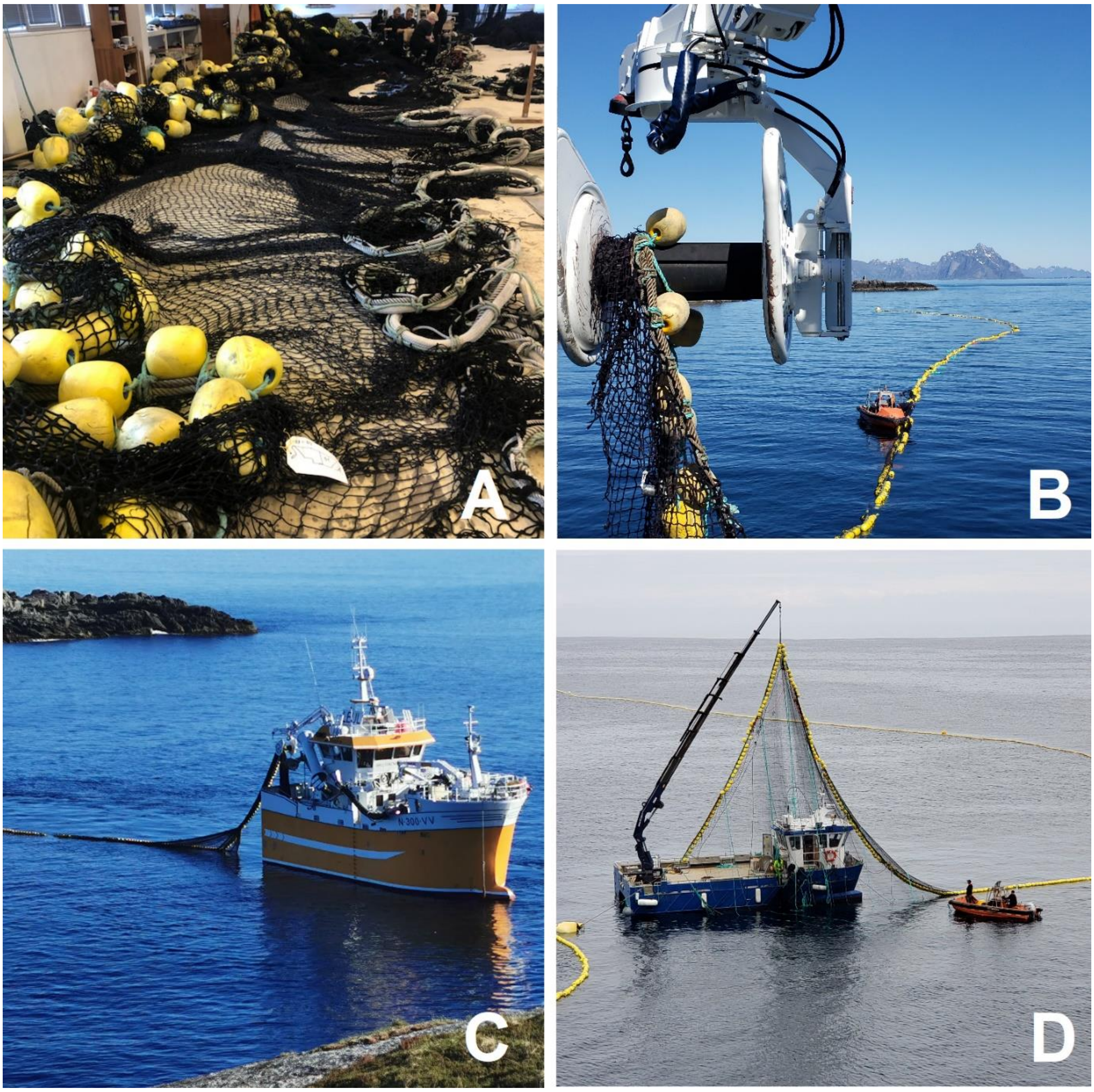


Figure 3.2.2b Deployment of nets in the C&R set-up. A: The barrier nets used for the C&R site set-up. B: Deploying the barrier nets from the purse seine vessel “Ballstadværingen” with help from the smaller boat “MOBHUS”. C: “Ballstadværingen’’ deploying net A from Kvannholmen to Æsøya. D: The aquaculture vessel ‘’Kurt senior’’ from IsQueen’ assisting in straightening the net B2 with the help from the smaller boat “MOBHUS” (Photos by D. Houser & A. V. P. Vinje).

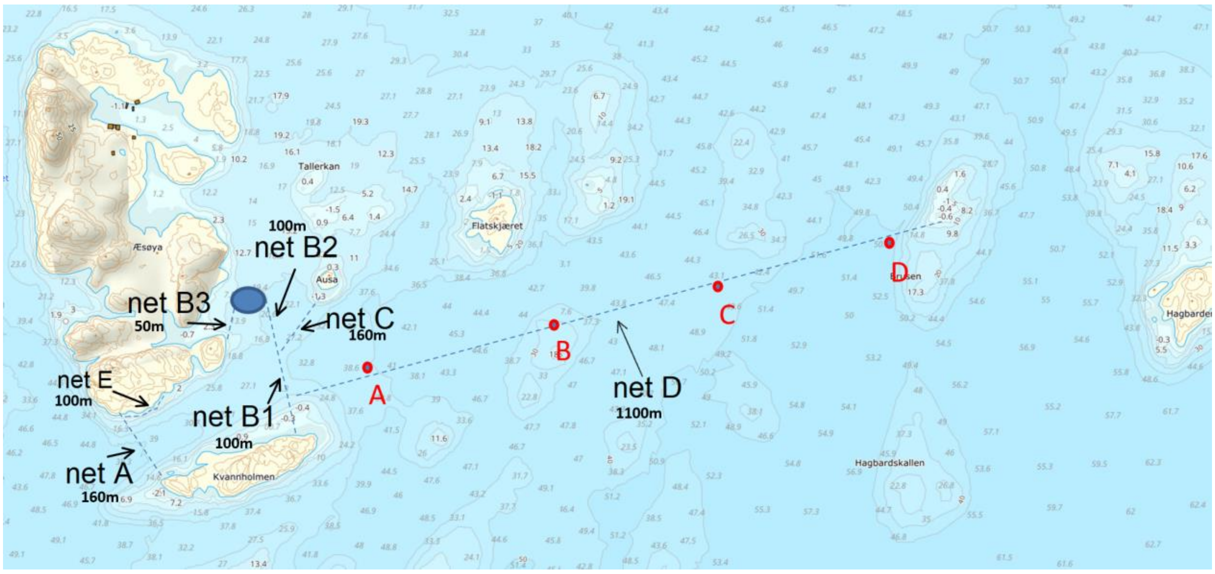


Figure 3.2.2c: Net details of the C&R set-up. The entire area was measured up with echosounder and laser before soldered and detail drawings. This figure shows the simplified profile of the nets. Net A was 160m long reaching to 40m depth, net B1 was 180m long in total (the "door" included being 50m long) and reached to a depth of 30m, net B2 was 100m long reaching to 30m in depth, net B3 was 50m long reaching to 20m in depth, net C was 160m long reaching the depth of 25m, and net D was 1100m long in total reaching to a depth of 45m.

In the catch phase (phase I), the larger boat "Zephora" was docked to the fish farm and utilized as part of the catch process. "Zephora" had a sink line attached to it that went along the seafloor from the "door" (figure 3.2.2d). Net B1 tethered to the guiding net was the "net door". As a whale swam through into the basin, the boat then drove off northwards pulling this net door shut. The net door was then secured by the smaller boat "MOBHUS" (figure 3.2.2d).

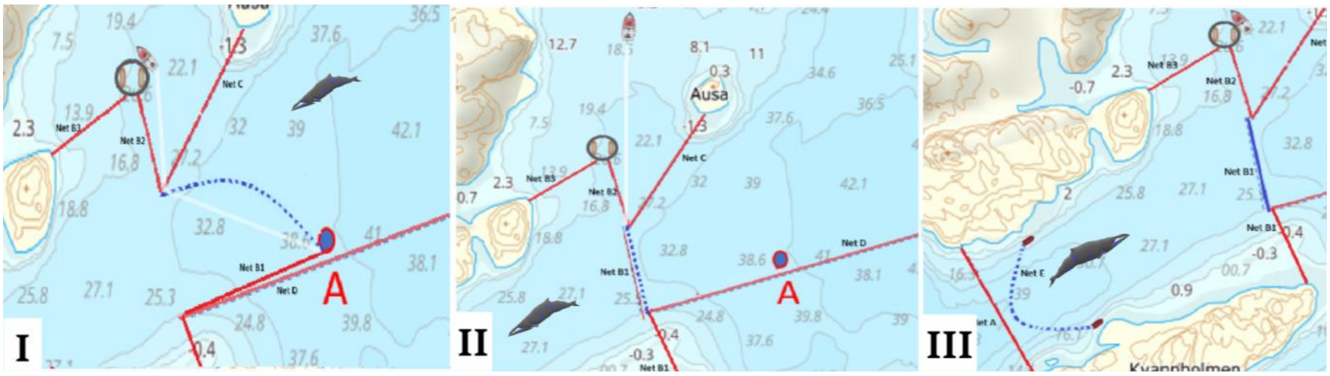
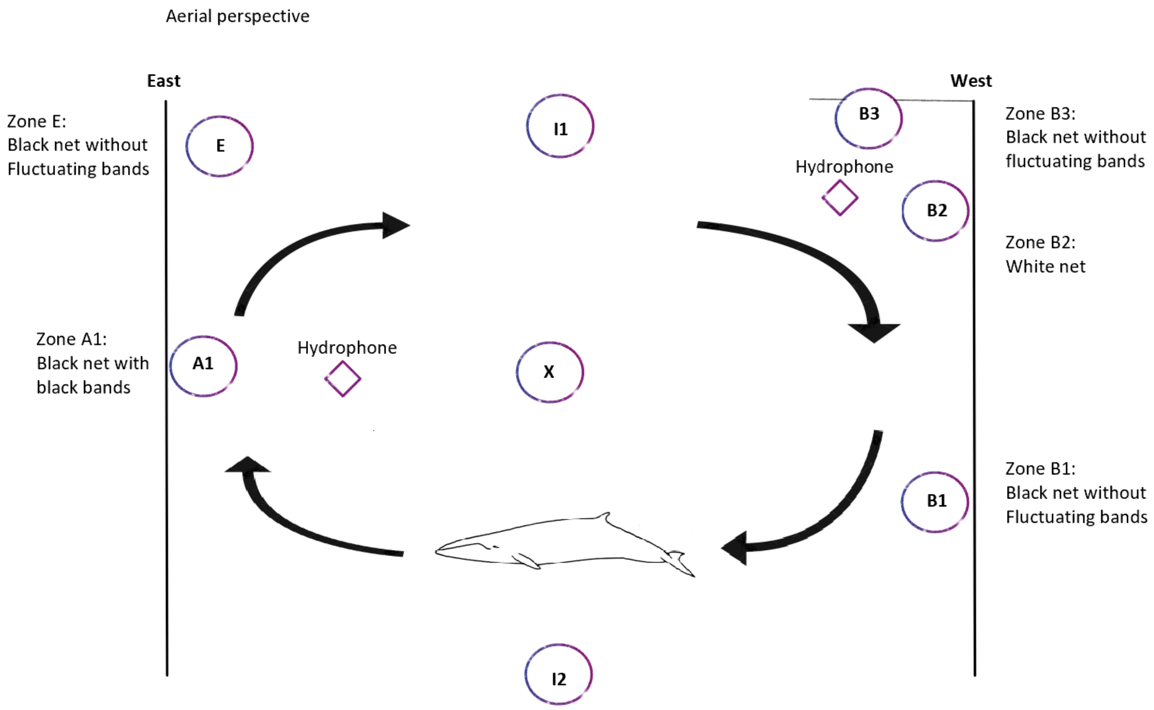


Figure 3.2.2d Schematic drawing of how the entrapment functioned. I; Phase I was the standby ready to catch mode where we had a white rope attached to the door and the boat next to the fish pen. II; in phase II the boats had closed the "door" by steaming north dragging the tethered net to the other side. The whale was then entrapped in the basin and observed. III; phase III would have been to corral the whale to the fish farm with the E net dragged with a boat on each side. The whale is not proportional in size with the rest of the set-up illustrated.

3.2.2.2 Design used to allow studies of sensory cues for net detection and to maximize animal welfare

During “phase II”, the observation phase, the whale was entrapped in the basin. The main goal in this phase was to monitor the whales’ behavior and health (addressing the objective focused on animal welfare (objective 2.4), preparing it for “phase III”. This was also the main phase for the observational side study (this master thesis) to investigate the sensory modalities in the captured baleen whale (addressing objective 2.2). In the side study of the SOST minke hearing project, different nets were modified with coloration contrast and movement bands to examine the visual and tactile responses of the approaching whales, while the bioacoustics were examined through hydrophones placed in the basin. The basin was divided into different zones (figure 3.2.2e). The aim was to use observations on the behavior of whales approaching nets together with acoustic data, to investigate possible use of echolocation or other sensory information used by the animals in orienting themselves along lead nets. The whales’ behavior was studied and compared to the behavior when approaching the unmodified nets. To observe the tactile response, some parts of the net had black fluctuating plastic bands attached to increase the tactile stimuli. Tactile stimuli were hypothesized to only work at close range, and if a moving whale turned away several meters from the net, it was unlikely that the tactile senses were the main modality used to detect the net.



	Tactile stimuli	Visual stimuli	Tactile & Visual stimuli	Acoustic stimuli
Zone A1	+	0	+0	0
Zone B1	0	0	0	0
Zone B2	0	+	0+	0
Zone B3	0	0	0	0
Zone E	0	0	0	0
Zone I1 & I2	+	+	++	+
Zone X	0	0	0	0

Figure 3.2.2e Set-up of the net where the whale was held captive and a hypothesis table categorizing the different areas of the basin to different zones and stimuli. Zone B1, B3 and E is a plain black net reference (0 stimuli), and I1 and I2 is a natural reference (+ stimuli). Zone X has no interaction with the net (0 stimuli) and is only used as a reference point. Zone A1 represents tactile stimuli created by fluctuating bands attached to the net, and zone B2 represents visual stimuli with black-white contrast colored net. All nets have the same acoustic characteristic, but the islets in zones I1 and I2 will represent a stronger acoustic stimulus if the animal uses echolocation. If the whale primarily uses tactile stimuli, we expect more precise orientation towards zone A1, than to the other nets. However, if the whale primarily uses visual senses, we expect more precise orientation towards the B2 zone, than to the other nets. If the whales use a combination of tactile and visual stimuli, we might expect more precise orientation towards both A1, B2, I1 and I2 compared to the rest of the nets. The natural reference (Zone I1 and I2) represents both tactile (i.e., kelp), visual cues (black and white rocks), and a strong acoustic target (solid wall). The use of acoustic senses would be determined by a hydrophone detecting active vocalization.

For the objective investigating sensory cues (objective 2.2), tactile and visual bands were created (figure 3.2.2f). Modifying parts of the basin to investigate changes in the whales' behavior from other areas of the basin was created by attaching lines to different nets. To make the lines sink vertically straight along net B2, a 10mm in diameter white polyester rope was utilized to create 42 nine-meter-long lines with a piece of lead attached at one end. The white lines were placed with a 3-meter distance from each other. The tactile bands consisted of a thinner black string with fluctuating black plastic bands (15mm width) reaching 60-70cm out with a one-meter distance in-between. 42 of these lines were placed with a three-meter distance from each other along net A for zone A1. These lines had a smaller piece of lead attached at one end to make them sink, laying vertically over the nets after knotting it to net A at the surface. Closer to the end of the trial the team relocated the tactile bands along net B1 due to logistics with net E used to corral the whale into the fish pen in case the project would reach phase III. This was also an effort to minimize the factors of an entrapped whale favorizing one end of the basin.

Figure 3.2.2f: Modified rope and line for objective 2.2. The white rope was attached along net B2, while the black line with fluctuating bands were attached along net A.

To proceed with ambient noise recordings to characterize background noise as well as recording of potential vocalization of the trapped whales, two hydrophones; “SoundTrap” 300HF (oceaninstruments New Zealand) and “Loggerhead” LS1X-Al (Loggerhead instruments, the USA), were placed in the basin attached with a buoy and lead. The “SoundTrap” was placed in the northeast part of the basin close to the fish pen (June 11th – June 17th), while the “Loggerhead” was placed close to net A in the west part of the basin during the capture mode of the trial (June 12th -June 24th). Both hydrophones were recording continuously when positioned in the basin. The placements of both hydrophones (i.e., “Loggerhead” placed with a 10-meter distance from net A, and “SoundTrap” placed with a 10-meter distance from net B2) were also used as a visual reference point for determining the distance a minke whale would be interacting with the nets of the basin.

3.2.2.3 The planned restrained procedure

To enable tagging and measurements of the AEP on a minke whale, the minke whale would have to be entrapped in the fish pen. The SOST minke hearing project did not reach this stage on the first trial that took place last year. The intended methodology for this part would have been guiding a whale into the fish pen, where it would be constrained in between two rafts in a hammock (figure 3.2.2.3).

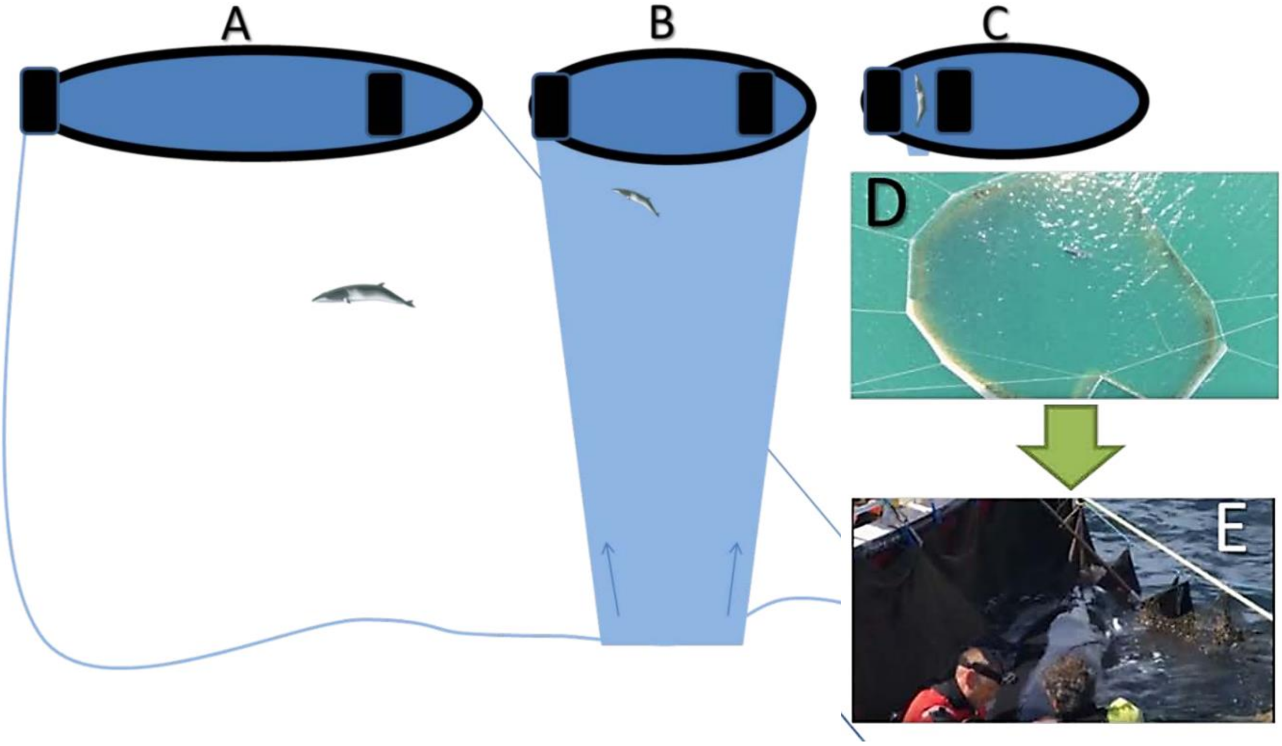


Figure 3.2.2.3. With a modified fish pen (40m in diameter), a minke whale could be temporarily stranded and constrained for AEP measurements and tagged before release. A: The net purse (20m deep) hanging inside the fish pen could be opened by folding it out on the sea floor when a whale entered the fish pen. B: The net would then have been hauled up to close the purse seine net when the whale swam into the fish pen. C: By further hauling up the net, the whale would be to the surface where it would be stranded between two 24^{m2} *(8m long, 3m wide) floats (black rectangles). The floats would be moved towards each other with the whale in-between when the net is lifted upwards. D & E: The "A-B-C" method was developed off the previous minke whales accidentally caught in bottom nets and instrumented with fin-mounted satellite tags in Denmark (J. Teilmann, personal communication, 27.10.21; P. Kvadsheim, personal communication, 29.03.22).*

To proceed with the AEP measures, a whale would be corralled into a modified salmon farm connected to the basin entrapment. In the fish pen the whale would be fully constrained in a net hammock. The needle electrodes used for AEP measurements would result in nothing more than minor discomfort in the animals, being smaller than current accepted procedures for tissue biopsy and tagging. The first attempt of the project that took place last year did however not reach to this point. A total of 12 minke whales are allowed to be examined in the SOST minke hearing project, taken under scientific permit from the NFSA (FOTS ID 19536).

The researchers aimed to capture adolescent minke whales no bigger than 5meters long due to logistics with size and blubber thickness, as well as natural hearing impairment that might deteriorate with age (Kvadsheim et al., 2021).

3.2.3 Collection and analysis of data

3.2.3.1 Phase I; data collection for evaluating the live-capture methodology (objective 2.1) and animal welfare considerations (objective 2.4)

Data was collected for 24 days during the field season in June 2021. Time, position, swimming behavior, number of blows, and orientation for any whales approaching the trap would be noted down in phase I, the catch phase waiting for a whale entering the basin. The hydrophones were also constantly recording sounds in the catch basin, and the sound files were analyzed after the trial. For tracking the whale, the team marked down every surfacing on data sheets with maps and had the orientation of the whale noted as arrows in the cardinal direction they were heading. Orientation was noted in regard to ordinary compass orientation. Their swimming behavior was categorized into 5 different behavioral codes; logging at surface (LAS), calm normal swimming (CNS), fast vigorous swimming (FVS), porpoising (POR), and spyhopping (SPY). Minke whales are known as fast swimmers, where the species have been reported to a swimming speed of 37km/h (Wursig et al., 2017, p. 67). Distinguishing the speed between CNS and FVS was estimated by the observers. Noting the total observations of different directions of all 19 minke whales surfacing in phase I was not always possible to determine due their quick surfacing and sometimes challenging weather conditions. The exact times when the whales were observed surfacing were at times difficult, which unfortunately led to some missing data. The noted time data was used to calculate the respiration rate in the different minke whales observed in phase I.

3.2.3.2 Phase II; data collection for evaluating the live-capture methodology (objective 2.1) and animal welfare considerations (objective 2.4)

In phase II, when there was a whale entrapped in the basin, the same variables were noted as in phase I, but the whales' orientation to the closest net or islet was instead noted down, and its' distance in orientation to the net or islet. The noted time data was used to calculate the respiration rate in the minke whale entrapped in the basin, phase II. The hydrophones were constantly recording in this phase as well, where the sound files were analyzed after the trial. The data of distance to net and sound files from the hydrophones was used for testing the sensory modalities of an entrapped baleen whale (objective 2.2)

3.2.3.3 Analyses of data collected from both phases

All calculations and statistical analysis were completed using Microsoft Excel v.2203 and R Statistical Software (v.4.1.2; R Core Team, 2020). The observation notes from both phases were organized into Excel, were diagrams, plots, and histograms were created, before remaking some of the figures in R. Percentages presented in different settings were done by dividing the number of that variable to the total variable (e.g., percentage of CNS calculated by number of observations of CNS divided with total swimming behavior observation, or percentage of minke whales swimming in on area divided with the total of 19 minke whales). The variables for both phases were time, position, swimming behavior, number of blows, and orientation, but between the phases the variables position and orientation differed slightly. The time variable was essential in order to calculate respiration rate of the whales observed in both phases. An additional variable that was analyzed after the trial rather than during was sound recordings from the two hydrophones placed in the basin.

3.2.3.4 Respiration rate for both phases

To calculate the respiration rate of the minke whales, using the collected time specifications and counted blows of the animal. In phase I the duration time varied between the individuals, and in phase II the counting of blows occurred in intervals; 1 hour observation and 30 minutes break in-between. The respiration rate was calculated as the number of surface blows divided with time (equation 3.2.3).

Equation 3.2.3) Respiration rate

$$\overline{x} = \frac{Number\ of\ respirations}{Number\ of\ minutes}$$

3.2.3.5 Bioacoustics

There were two hydrophones placed in the basin (figure 3.2.2e). All recordings from the hydrophone sound files were transferred to a computer and analyzed through the software program Raven lite 2.0.1. (Cornell Lab of Ornithology, Ithaca, NY, USA) (K. Lisa Yang Center for Conservation Bioacoustics. (2021)). The sound files were visualized in spectrogram and oscillogram (waveform). The spectrogram displayed the relative power spectral density in the sound as a function of time (h.m.s (hour, minute, second), x-axis) and frequency (kHz, y-axis). Power spectral density levels from the sound files were shown by the color of the spectrogram, using the "jet" color map (higher power values were red, and lighter power values blue). The oscillogram displayed the amplitude in sound as a function of time (h.m.s., x-axis) and kilounits (y-axis) (figure 3.2.3.5). Kilounits is a unit of amplitude in the software program, referring to the value of the individual digital samples that together constitute the acoustic recordings. These units are proportional to the sound pressure received by the hydrophones. The sample rates were set to 96 kHz in the “Loggerhead”, and 192kHz in the “SoundTrap”.

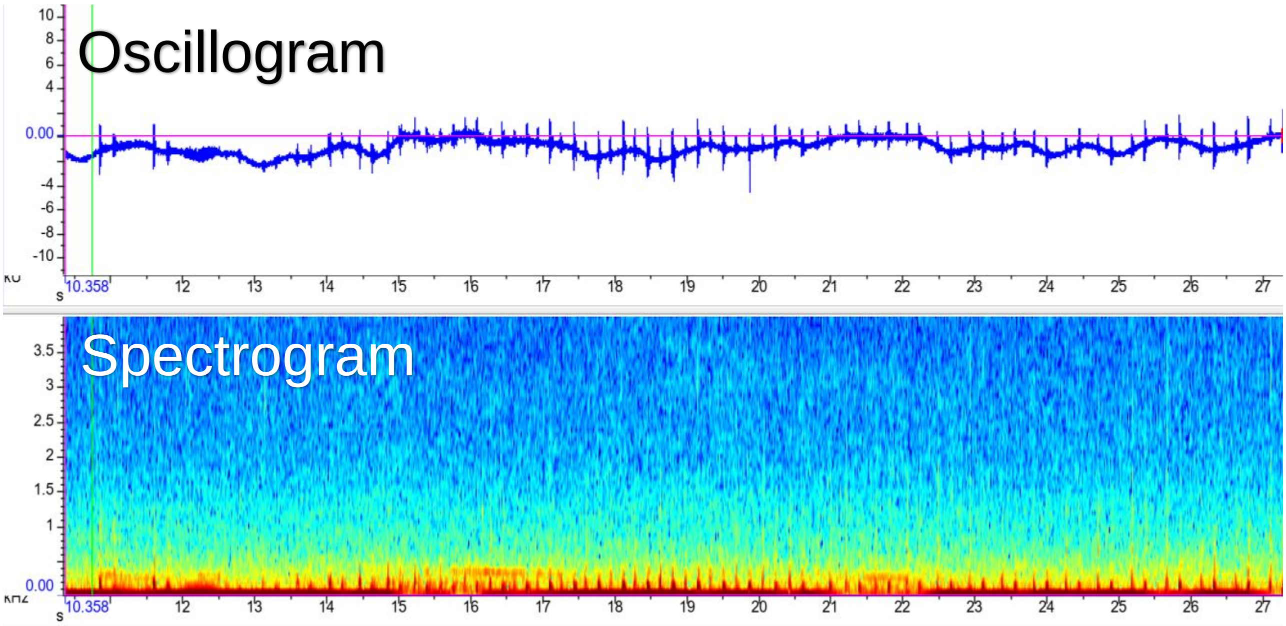


Figure 1.2.3.5: Example figure showing an oscillogram and spectrogram of common minke whale pulses. In the oscillogram the peaks show the amplitude of the minke whale pulses (in kilounits, y-axis) over time (seconds, x-axis). In the spectrogram, the jet color map shows high power values in red peaks (the pulse sounds) along the x-axis (seconds, time) reaching up to ~500Hz (kilohertz along y-axis) (S. C. Martin, personal communication, 11.11.21).

4 Results

4.1 Data collected from the SOST minke hearing project

4.1.1 Observational data from whales approaching entrapment facility (phase I)

In total, 19 minke whales, one humpback whale (*Megaptera novaeangliae*) (figure 4.1.1f), one pod of pilot whales (*Globicephala melas*), one pod of porpoises (*Phocoena phocoena*), 4 observations of grey seals (*Halichoerus grypus*) and 7 observations of pods of killer whales (*Orcinus orca)* were observed during the trial (figure 4.1.1g). The migration routes of the minke whales observed around the C&R site varied (see appendix for the total mapping of all migration routes), where ~21% of the minke whales entered from north between the islets Ausa and Flatskjæret (figure 4.1.1a), while the expected entrance between Flatskjæret and the guiding net had ~26% sightings (figure 4.1.1b) Other first sightings were usually closer at the basin entrance with ~32% (figure 4.1.1c). Results show that ~26% of the minke whales observed swam through the C&R site between the C and D buoys (figure 4.1.1d). Approximately 21% of the minke whales close to the C&R site, were observed between the fish pen and Ausa, where net C might have blocked the animals from entering the basin, making them turn back and avoiding the area (figure 4.1.1d). Three of the minke whales (Ba21_0206, Ba21_0406a and Ba21_0406c), entered the basin before the system was ready for capture (figure 4.1.1e), while one minke whale (Ba21_1106) was judged too big for AEP measurements (figure 4.1.1a).

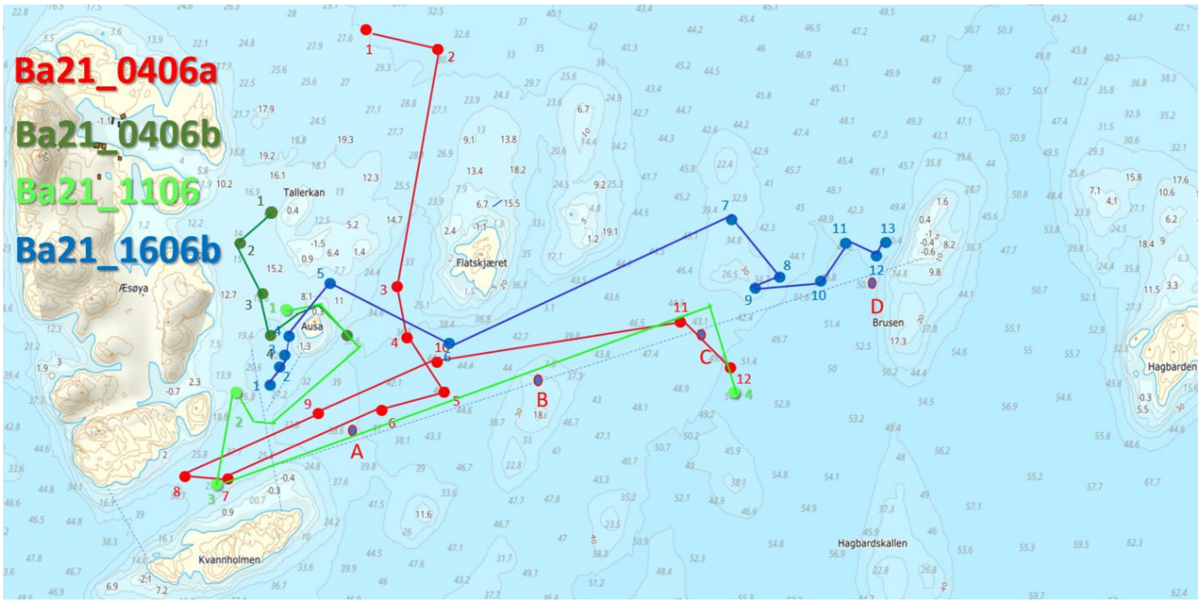


Figure 4.1.1a: ~21% of the minke whales (Ba21_0406a (red), Ba21_0406b (khaki), Ba21_1106 (bright green), and Ba21_1606b (blue)) observed entering the set-up between the islets Ausa and Flatskjæret.

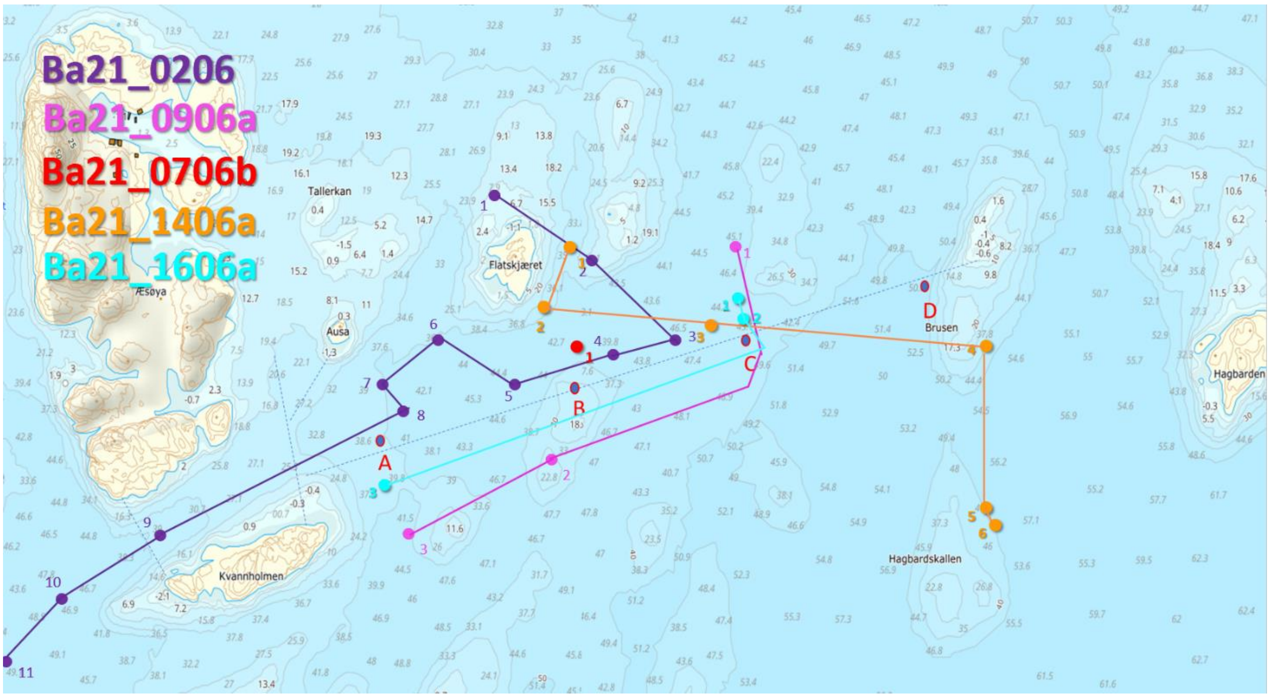


Figure 4.1.1b: ~26% of the minke whales (Ba21_0206(purple), Ba21_0906a (pink), Ba21_0706b (red), Ba21_1606a (orange), and Ba21_1606a (cyan)) observed entering the set-up between the islet Flatskjæret and the guiding net (net D).

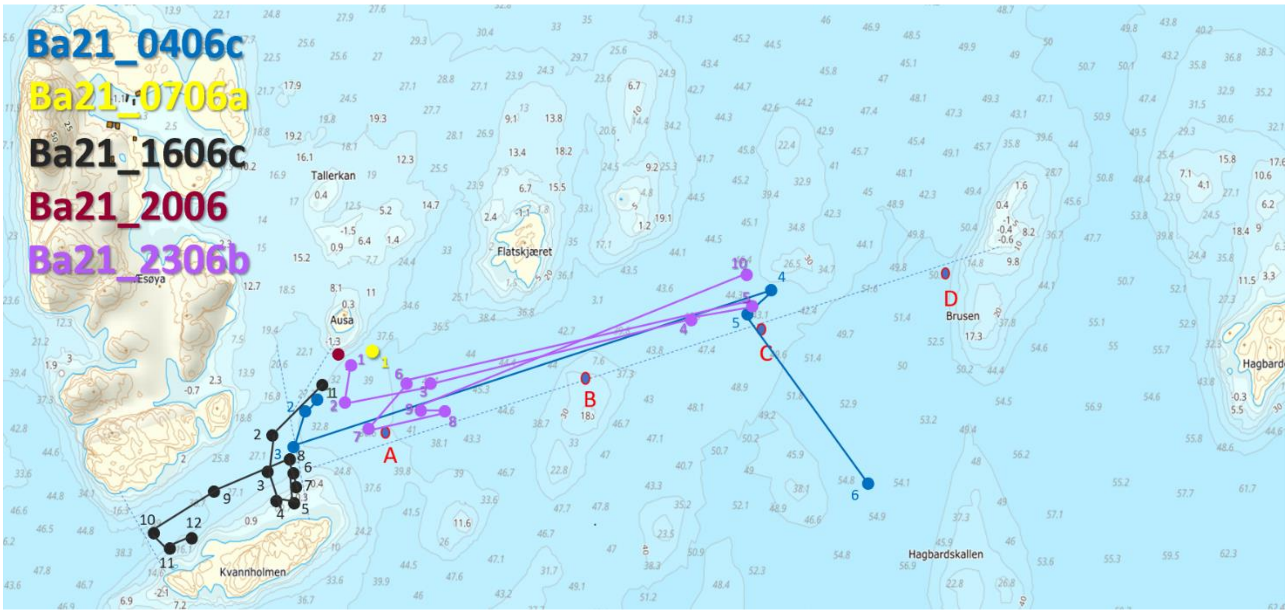


Figure 4.1.1c: ~32% of the minke whales (Ba21_0406c (blue), Ba21_0706a (yellow), Ba21_1606c (black), Ba21_2006 (maroon), Ba21_2306b (purple)) first observations close to the basin entrance.

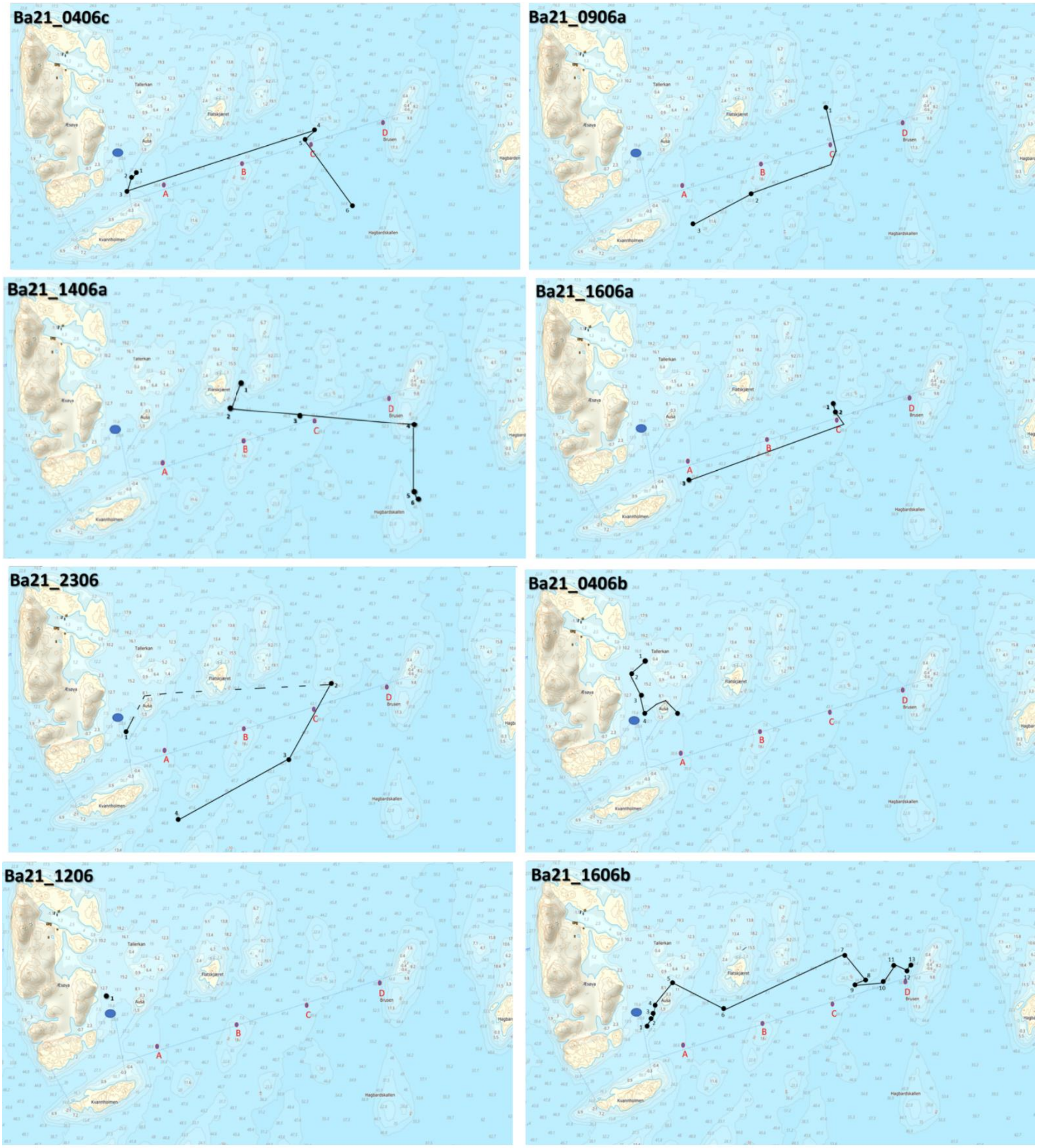


Figure 4.1.1d: Map of migration routes in some of the minke whales during the SOST minke hearing project trial. ~26% of the minke whales observed passing through buoy C and buoy D on the guiding net (Ba21_0406c, Ba21_0906a, Ba21_1406a, Ba21_1606a, and Ba21_2306), and ~21% minke whales first observations towards the area between the fish pen and Ausa (Ba21_2306, Ba21_0406b, Ba21_1206, and Ba21_1606b). The dotted lines shown in Ba21_2306c is due to a less clear pathway from first observation point to second observation point. The whale could have moved north of Ausa and Flatskjæret, or between Ausa and Flatskjæret to point 2 as indicated in the dotted lines shown.

Figure 4.1.1e: One of the three minke whales (Ba21_0406c) that entered the entrapment before it was completed. Ba21_0406 found in the bottom left, on the right there is the boat "MOBHUS" organizing one of the nets in front of the islet Kvannholmen.

Figure 4.1.1f: An adolescent humpback whale in the basin. The whale was first spotted entering the basin and was inside the open entrapment for ~75minutes before it found its way out (Photo by R. A. Ølberg).

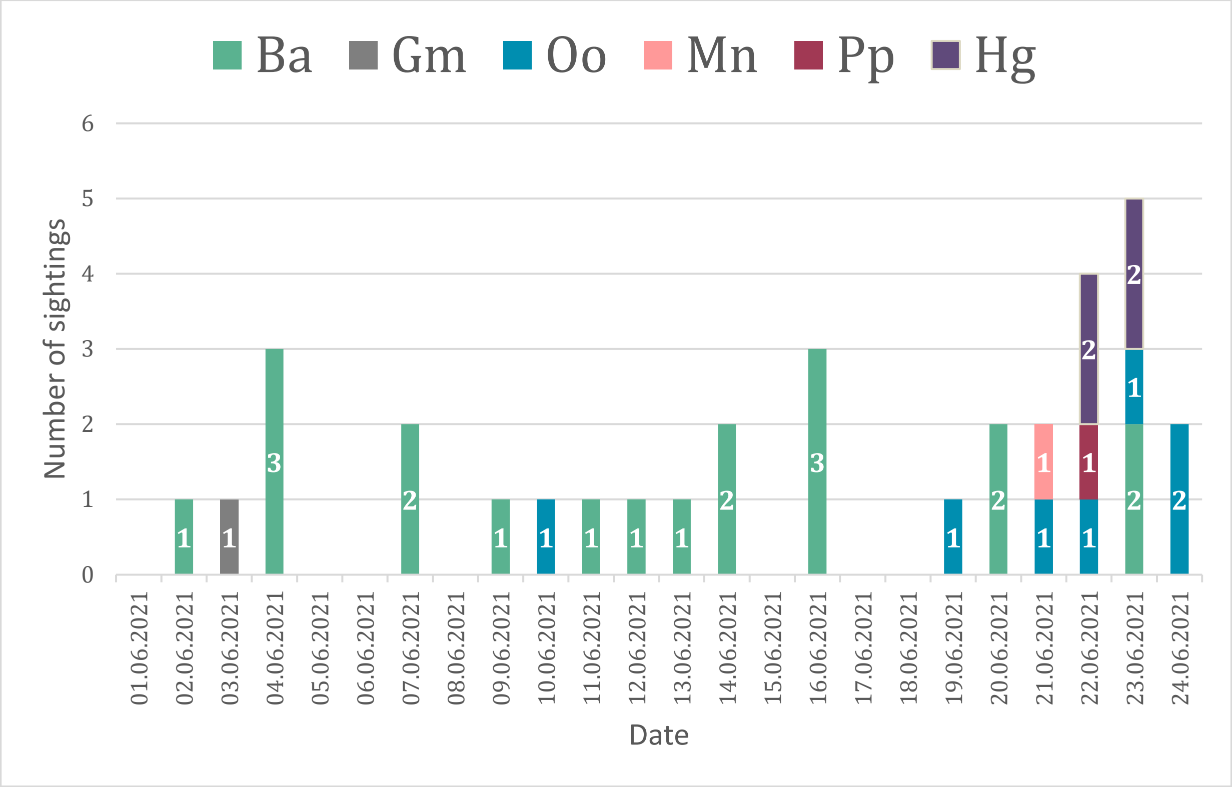


Figure 4.1.1g: Number of sightings of marine mammal species observed during the SOST minke hearing trial. Every registration can be more than one individual observed regardless of species observation. Six different species were observed: Ba; Balaenoptera acutorostrata (minke whale), Gm; Globicephala melas (pilot whale), Oo; Orcinus orca (killer whale), pink; Mn; Megaptera novaeangliaem (humpback whale), Pp; Phocoena phocoena (porpoise), Hg; Halichoerus grypus (grey seal).

In 17 out of the 24 days in the field, there were sightings of marine mammals. Despite the common occurrence of minke whales, there were also several sightings of killer whales. There were usually no sightings of minke whales for hours during the presence of killer whales close to the C&R site. On June 23rd the sightings of minke whales occurred ~02:00 and ~14:00, while the pod of killer whales was observed at ~23:30 (figure 4.1.1g).

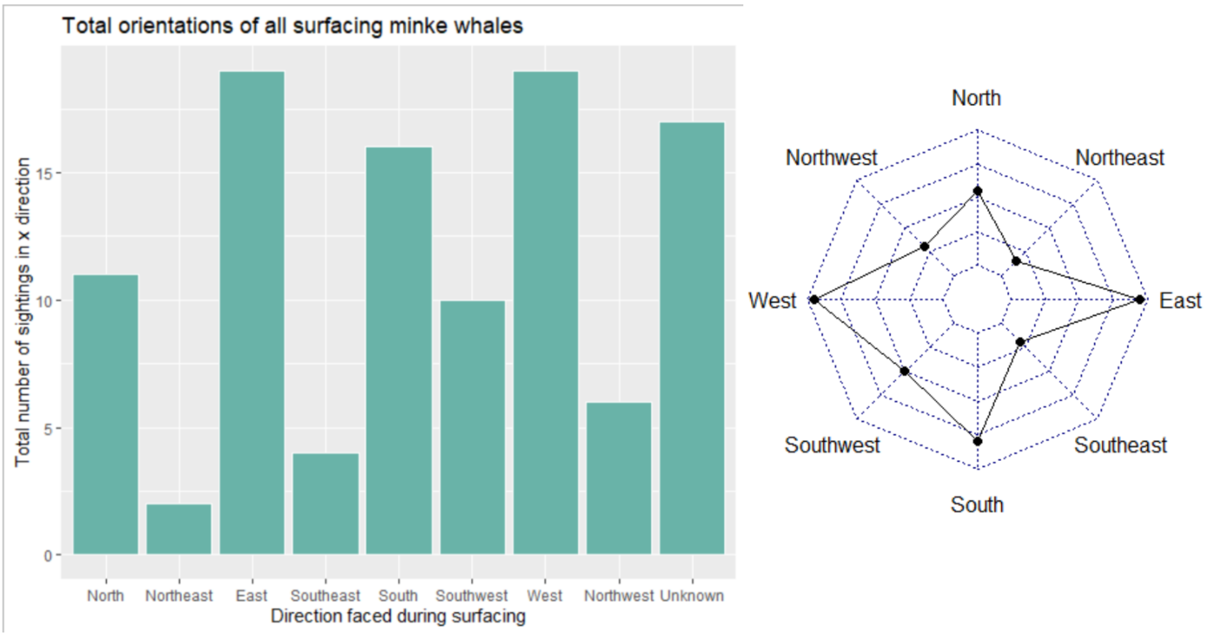


Figure 4.1.1h: Histogram and radar diagram showing the total number of orientations divided to 8 directions as variables when the minke whales were observed surfacing during phase I. Each increment in the radar diagram represents an increase of 5 observations, ranging from 0 to 20 observations.

The majority of directions of all sighted minke whales consisted of an equal amount of surfacing directions heading west and east (figure 4.1.1h). No trend was indentified in the data on cardinal directions of minke whales.

Table 4.1.1: Calculated mean respiration rate of all the minke whales observed during the phase I. Starred values (*) means that data was not collected during the entire observation.

Minke whale ID	**Respiration rate** (breaths min^{-1})	**Observation time** (minutes)
Ba21_0206	0.32	37
Ba21_0406a	0.73*	10*
Ba21_0406b	0.83	6
Ba21_0406c	0.71*	7*
Ba21_0706a	NA	NA
Ba21_0706b	NA	NA
Ba21_0906	1.0	5
Ba21_1106	0.4*	5*
Ba21_1206	NA	NA
Ba21_1306	NA	NA
Ba21_1406a	0.6	10
Ba21_1406b	0.33*	9*
Ba21_1606a	0.67	6
Ba21_1606b	0.57	21
Ba21_1606c	0.68	28
Ba21_2006a	NA	NA
Ba21_2006b	NA	NA
Ba21_2306a	0.4*	5*
Ba21_2306b	1.0	22

In phase I, most of the total swimming behavior of all minke whales observed was noted as calm normal swimming (CNS, ~82%). A few observations were noted as fast vigorous swimming (FVS, ~17%), and one minke whale (Ba21_2006a) was observed spyhopping (SPY, ~0,1%) right next to the C net. Some of the minke whale observations in table 4.1.1 lack a calculated respiration rate due to only spotting the whale surfacing once and then disappearing. Exact time of observation was missing on 5 of the individuals, respiration rate was therefore calculated based only the time stamps accurately noted, ignoring the datapoints without timestamp. Table 4.1.1 only includes the respiration rates measured during phase I; more extensive respiration rates of Ba21_1606c during phase II are included in later calculations.

4.1.2 Observational data from entrapment in inner net facility (phase II)

The minke whale Ba21_1606c was held captive for ~8hours before it escaped. It was first spotted at 17:34 on June 16th, and last seen at 01:55 on June 17th. The “door” was closed 6 minutes after the first spotting. Its size was estimated to be between 4.5-5 meters long, sex unknown. The animal was observed in swimming category CNS on its’ way into the basin, where its’ swimming speed increased (FVS) at 17:47. When data collection for phase II took place at 18:15, the swimming behavior was back to CNS. In total, ~98% of the behavior of Ba21_1606c was noted as CNS, ~0.4% noted as SPY, and ~1% noted as FVS. During the closing of the entrance, the whale was observed at 18:01 swimming submerged parallel to net B2 with an estimate of a 10-meter distance. Most observations of Ba21_1606c were otherwise found close to the net A in zone A1, to the west. No observations were made of the whale close to net B3, the zone between the fish pen and Æsøya (figure 4.1.2a).

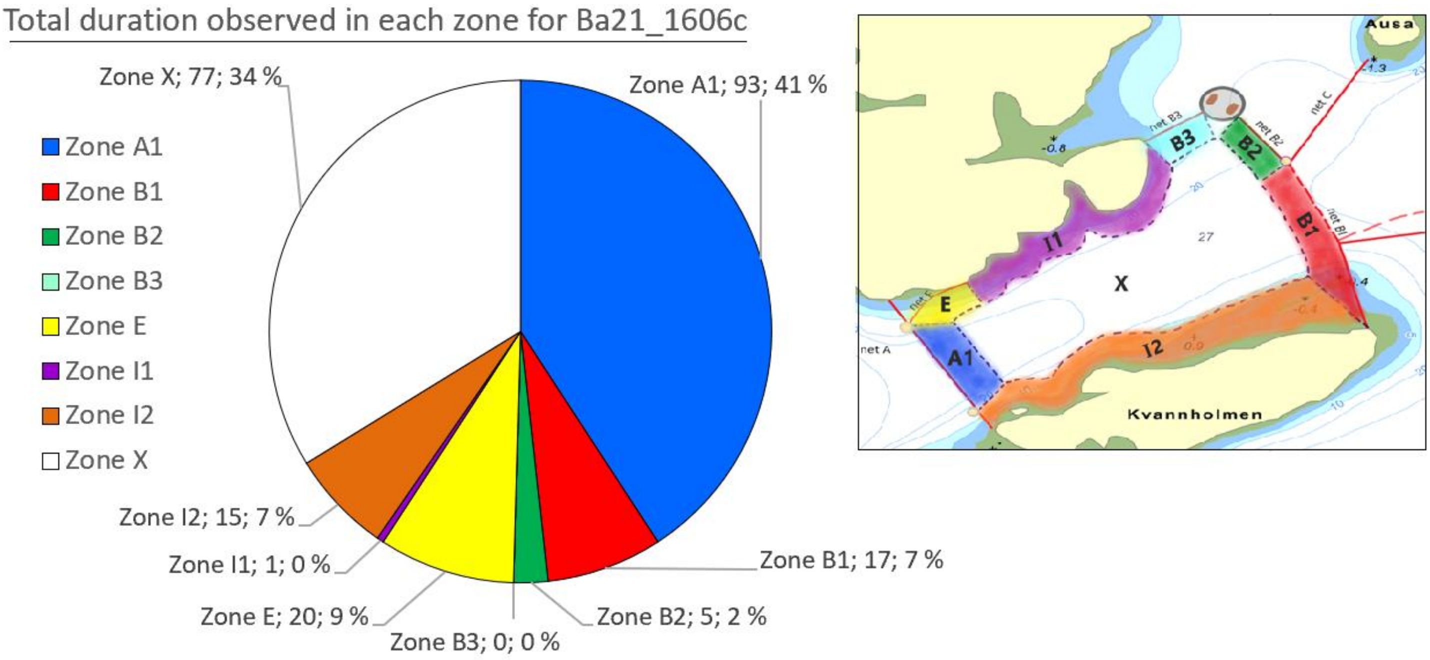


Figure 4.1.2a: The total duration minke whale Ba21_1606c spent in each of the 8 zones. Colored map on the right showing the divided sections of the zones in accordance with the colors in the sector diagram. Number of observations and percentage in each section noted next to each sector of the chart. Each zone (except “X”, being the reference point) reached out 20meters from the nets or islets.

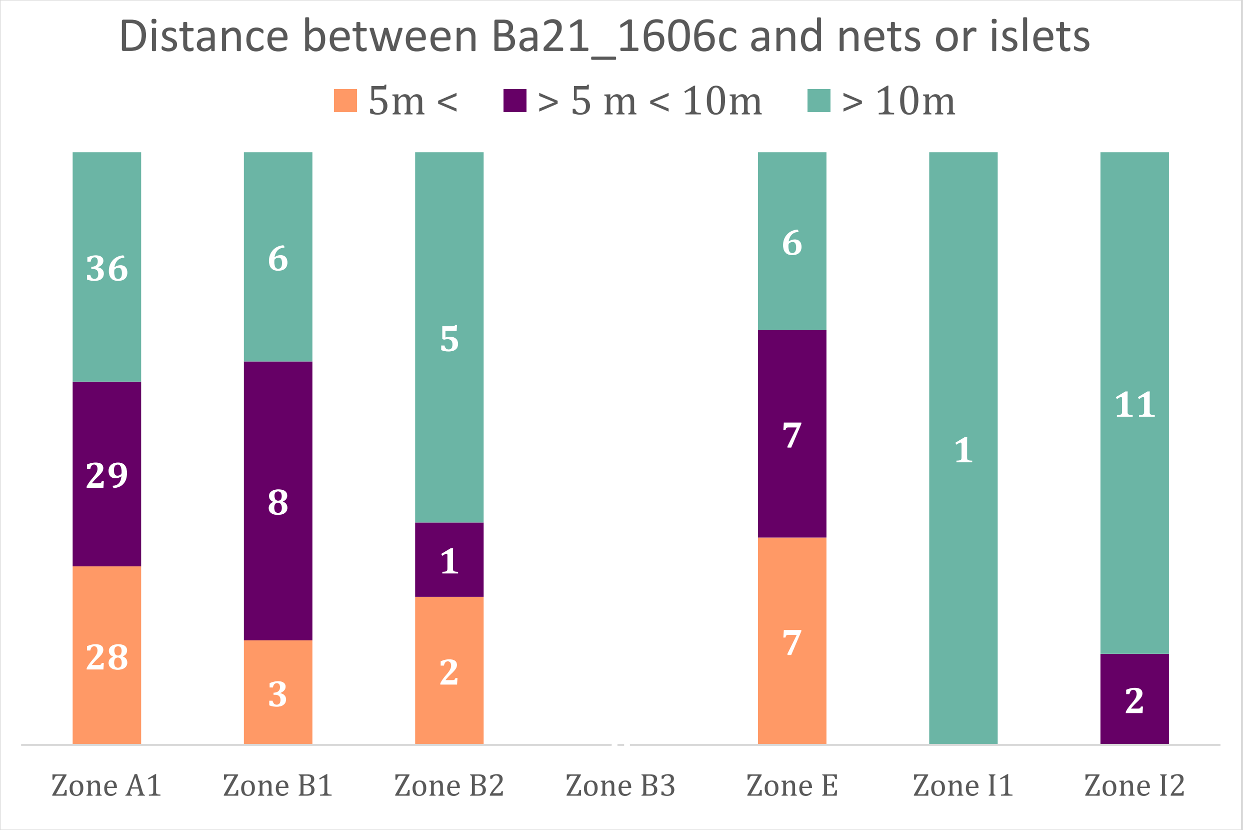


Figure 4.1.2b: The diagram shows the number of sightings of Ba21_1606c in each zone related to net during the entrapment, where each column is a zone, and the colorations defines the distance. Number of sightings in each zone is sorted into 3 distance categories; orange; distance less than 5 meters from the net, purple; a distance including 5m to 10m from the net, green; distance of 10m to 20m from the net.

The hydrophones floats were used as reference points for distance (i.e., “Loggerhead” was placed 10m away from net A, and “SoundTrap” 10m away from net B2). With different observers noting the distances, the positioning accuracy might have slightly differed.

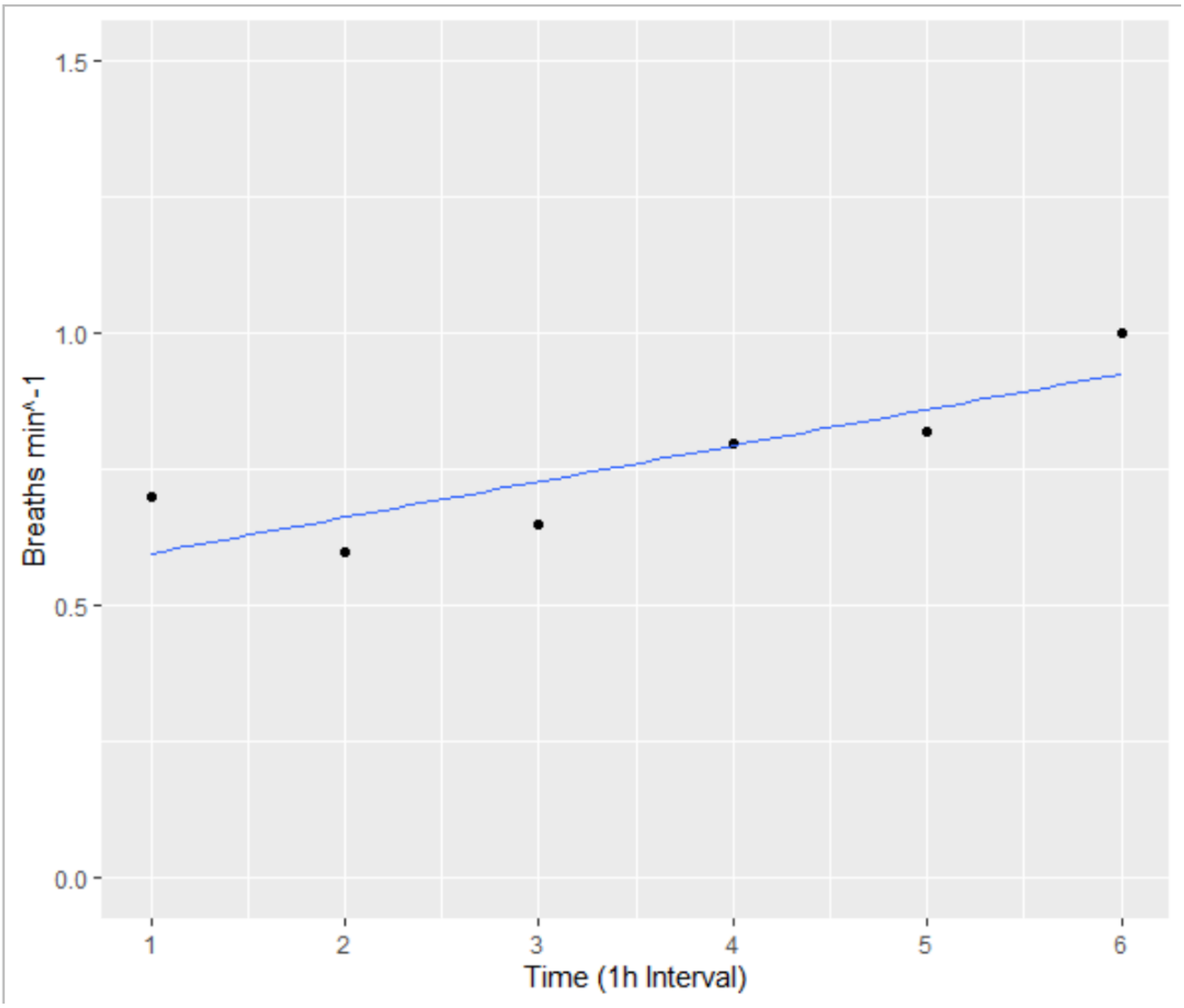


Figure 4.1.2c: Respiration rate of the entrapped minke whale Ba21_1606c. Each point shows the breathing frequency in each hourly interval (1,2,3 etc.). Each of these intervals are expressed as a function of blows per hour, except interval 6 (based on 10 minutes). In between each interval, there was 30 minutes of no data taken into account.

The respiration rate was calculated through hourly averages, where a significant increase of 0.066 breaths min^{-1}hour^{-1} ($p=0.0297$) was observed (figure 4.1.2c). The last observation interval was only based on 10 minutes instead of an hour.

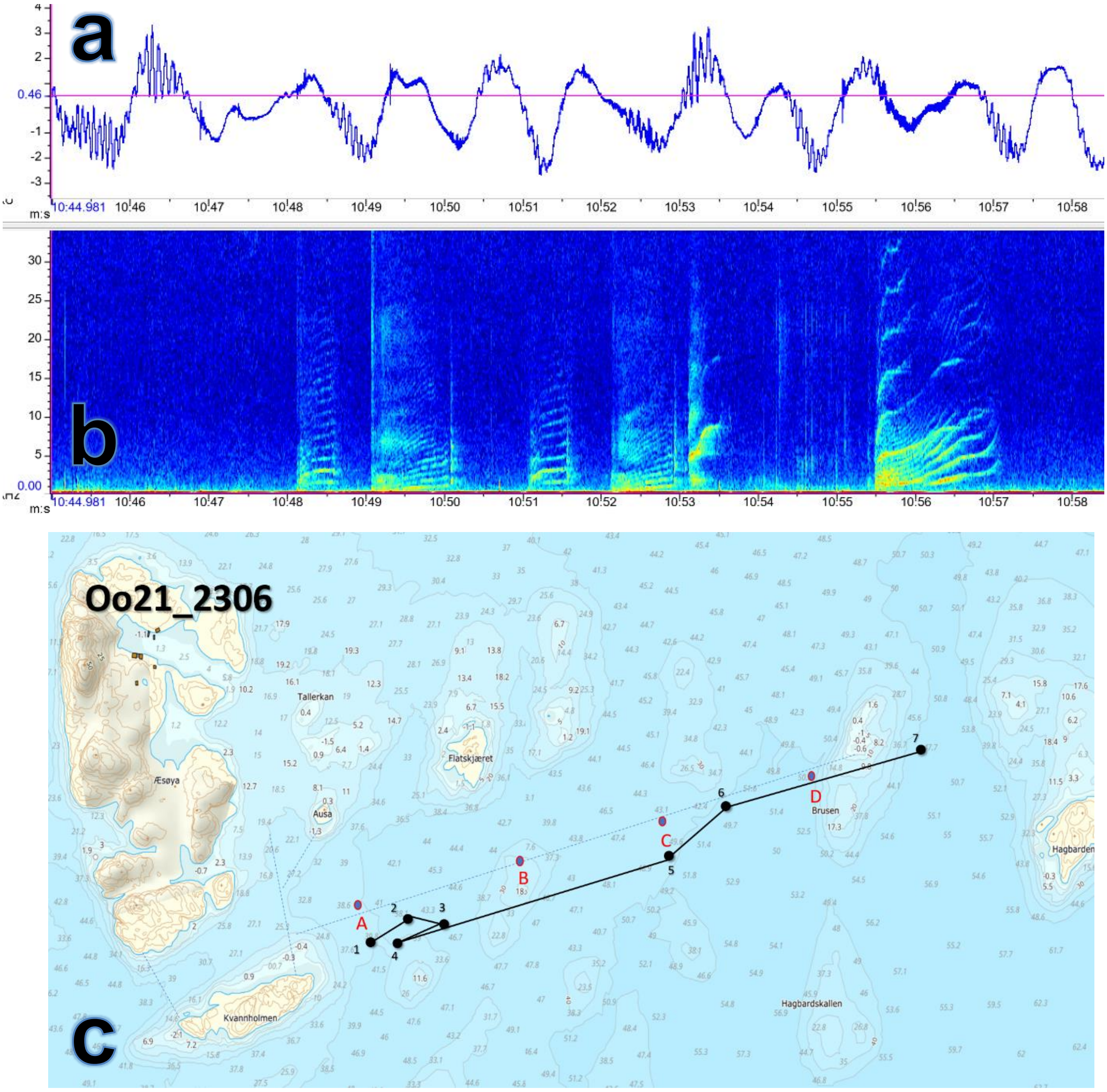


Figure 4.1.2d: Oscillogram and spectrogram of detected sounds produced by a pod of killer whales (Oo21_2306) and their migration route observed. The top part of figure shows a waveform (a) and spectrogram (b) consisting of whistles (time 10:48-10:53, 10.56-10.57) and echolocation clicks (time 10:54-10:55) produced by the killer whale pod (Oo21_2306). Detected vocalization was observed from 23:02-23:58 in the sound file. The pod of orcas was the observed swimming along south of the guiding net from 23:29-23:42 (c).

The data collected from the hydrophones "Loggerhead" and "SoundTrap" showed no signs of vocalization or echolocation produced by the minke whale in the basin or from other minke whales observed close to the entrapment system, nor the humpback whale that also was observed inside the basin for ~75 minutes. The only detectable marine mammal noise found was produced by killer whales (figure 4.1.2d).

5 Discussion

This thesis describes a novel approach aiming to live-capture mysticetes in order to allow studies of their physiology that cannot be studied at distance. The discussion is divided into four parts, according to my four objectives; 5.1) review and evaluate the design of capture and release methods for baleen whales, for research purposes, 5.2) testing the use of sensory modalities by baleen whales to orient themselves around nets, based on observations from the SOST minke hearing project, 5.3) discussing research opportunities, given access to entrapped and restrained baleen whales, and 5.4) discussing the animal welfare considerations of live-capture studies of baleen whales.

5.1 Review and evaluate the design of capture and release methods for baleen whales, for research purposes.

5.1.1 Previous documented catch and release attempts

Important physiological data has been gained in studies of captive baleen whales, but such studies are rare, most likely due to the logistical challenges related to the size of the animals and obtaining complete access to their offshore underwater habitat. Capture in combination with sedation could have reduced the latter challenges and has been attempted before (Wahrenbrock et al., 1974), but as yet with limited success. Here I will summarize some of the findings of previous methodology used to entrap baleen whales and include experimental studies that were conducted on these mammals before further evaluating the capture techniques and comparing them to the approach designed for the SOST minke hearing project.

5.1.1.1 Intentional live-captures

In the late 1950s, there was an unsuccessful attempt to capture a newborn minke whale calf (*Balaenoptera acutorostrata Lacépède,* given the geographical location this was presumably *Balaenoptera acutorostrata scammoni*) for upkeep in captivity in Marineland of the Pacific, Southern California, USA. The crew of the California Department of Fish and Game lassoed the minke whale by the tail and hoisted it onboard the research vessel. With the whale onboard the boat the crew proceeded towards the oceanarium’s pier, but the calf died upon arrival (Norris & Prescott, 1961). However, Mito aquarium in Japan succeeded in obtaining three minke whales (*Balaenoptera acutorostrata Lacépède/ Balaenoptera acutorostrata scammoni*). Over different time periods the whales were captured and held in a 4900m^2 pool with a depth ranging from four to twelve meters. Osiki-ami (a type of fixed fishing net) deployed two kilometers away from the aquarium captured the minke whale. The whale was then wrapped in the nets hanging between two boats, then towed to the pool by another motorboat. The aquarium's first capture occurred in the 1930s, where the whale was kept for three months. In 1954, they obtained a calf that died after 2 weeks, and a year later, a 6-meter long minke whale was kept for 37 days before escaping. During their last minke whale entrapment, swimming behavior was observed and respiration rates were measured (Kimura & Nemoto, 1956).

Two live-captures of gray whales (*Eschrichthius robustus*) were performed by Sea World Inc. and the University of California, in Scammon's Lagoon, Baja California, Mexico (Wahrenbrock et al., 1974). In 1965 the first gray whale "GIGI I" was kept in an oceanarium in San Diego, USA for ~2 months. A number of respiratory and metabolic studies were conducted, but due to the capture technique using a superficial harpoon followed by netting to entrap the whale (Norris & Gentry, 1974), it died from atelectasis (partial or complete lung collapse) and pneumonia caused by the infected wound (Wahrenbrock et al., 1974). Six years later (1971) another gray whale calf, "GIGI II" was caught with a tail noose from a fishing vessel equipped with a bow plank (Norris & Gentry, 1974). "GIGI II" was held captive in larger pools for 363 days in the same oceanarium as the previous whale. A number of studies were conducted on this animal (Curran & Asher, 1974; Duffield, 1974; Evans, 1974; Fish et al., 1974; Gilmartin et al., 1974; Leatherwood, 1974; Mattson, 1974; Medway, 1974; Norris & Gentry, 1974; Ray & Schevill, 1974; Smith & Wahrenbrock, 1974; Wahrenbrock et al., 1974; Zettner, 1974), answering and supporting numerous aspects of baleen whale physiology and behavior.

A more recent attempt of live-capturing baleen whales took place in Iceland in July 2007. A consortium of researchers from Denmark, USA, Iceland, Japan and Russia had a goal to collect data on minke whales' hearing. They used a modified purse seine net designed for herring to entrap minke whales and had a pontoon boat fitted with a stretcher to restrain a whale. The capture net was set out several times after numerous minke whale sightings. Their project was close to succeeding, but the nets were too heavy, taking too long to surround the whale and animals therefore escaped capture attempts. Lighter nets with a larger mesh would have been more time efficient and likely resulted in succeeding a live-capture and hearing measurement of at least one minke whale (J. Teilmann, personal communication, 27.10.21; L. Miller & P. Nacthigall, personal communication, 03.03.22).

5.1.1.2 Accidental entrapments

As previously mentioned in the introduction, there has also been opportunistic experimental use of baleen whales found naturally entrapped. The insight in logistics acquired from these scenarios could benefit in addition to the intentional live-captures, where handling such large animals has been needed to help the mammals return back healthy to the ocean.

For instance, a blue whale (*Balaenoptera musculus*) was temporarily entrapped by ice in southwest coast of Newfoundland, Canada in 1974. The whale was in a reasonable state of health surrounded by 20-50cm thick surface ice. Before the animal escaped the following morning, researchers managed to conduct observations of the whales' behavior, size measurements and acoustic recordings during its entrapment (Beamish, 1979). Humpback whales have also been found entrapped in Newfoundland due to entanglement with fish traps (Winn et al., 1979). Near Branch (Newfoundland, Canada) in 1975, a male humpback whale swam along the net designed for capture of capelin (*Mallotus villosis*), where the doors of the circular trap closed around the whale's tailstock restraining the animals motion. The humpback was temporarily entrapped for two days, during which time behavior was monitored, morphometric and acoustic measurements were taken, as well as tagging the animal before it was released (Beamish, 1979; Winn et al., 1979). The following year a female humpback whale was found entangled near Elliston (Newfoundland, Canada). Disentanglement occurred the day after discovery, but the whale was kept captive for 29 subsequent days in a blindfolded maze experiment with acoustic recordings to test the presence of biological sonar (Beamish, 1978, 1979; Winn et al., 1979).

A stranding of a juvenile Bryde's whale (*Balaenoptera edeni*) occurred off the Gulf Coast of Florida, USA in 1988, where it was rescued and maintained in a 20m diameter and 4m deep pool by Sea World of Florida. The whale's 42 days of rehabilitation consisted of feeding, animal care, behavior observations and acoustic recordings. When the whale reached the state of health to survive, it was released ~190km west offshore Florida with a satellite tracking device attached to the dorsal fin monitored by scientists at the University of Oregon (Edds et al., 1993; Walsh et al., 1991). In 1994, another Bryde's whale was trapped in the Manning River, New South Wales, Australia for 100 days. During the whale's entrapment period, observations of its body condition, diving habits, movement and feeding behavior condition were made. However, in this location the whale's life stood at risk for starvation, ship strike, stranding, entanglement in fishing gear, and exposure to high acidity and low salinity. The

10,3 m long whale was therefore rescued by towing it back to the sea by positioning it on an inflatable pontoon. With the whale on the pontoon blood samples were taken from the central vein of a fluke through venipuncture. Its survival chances appeared to be good after strongly swimming away on release (Priddel & Wheeler, 1997, 1998).

Three years later (1997) a neonatal gray whale (*Eschrichtius robustus*) was rescued and rehabilitated at the animal care facility at SeaWorld of California. The calf named “JJ” was brought to the facility for 436 days after being found beached in Los Angeles. Emergency medical treatment began immediately after the calf’s arrival due to its emaciated and comatose state of health. Several studies such as blood sampling, morphometrics, direct and indirect measures of oxygen extraction, tidal lung volumes, and respiratory rates, were conducted during “JJ”s recovery before its release (Reidarson et al., 2001; Sumich, 2001).

Lastly, in 2017, a northern common minke whale was found entrapped in a fishing net in Denmark. Senior researcher Jonas Teilmann in marine mammal biology of Institute for Bioscience, University of Århus, with his team attached a GPS tag on the whales’ dorsal fin before releasing it from its entanglement. A similar procedure took place in 2002, which has given useful data on the species’ migration (Henriksen, 2017; J. Teilmann, personal communication, 27.10.21).

5.1.2 Results from the SOST minke hearing project and potential improvements to the catch and release site

5.1.2.1 Phase I results, observational data from the catch and release site

Previous experience from the intentional live-capture attempt that took place in Iceland in 2007 (L. Miller & P. Nacthigall, personal communication, 03.03.22), and the opportunistic handling of minke whales found entangled in Denmark in 2002 and 2017 (J. Teilmann, personal communication, 27.10.21), have been used in the planning of capture procedure of the SOST minke hearing project. The C&R site of the SOST minke hearing project might be the largest animal trap in the world. The 2021 summer campaign was a feasibility study to test if animals could be guided with nets and captured between islets. No hearing threshold AEP (auditory evoked potential) measurements could be conducted, but 3 whales did get enclosed in less than a month; one animal was fenced for 8 hours before it escaped, while the other two were too big for the planned AEP measurements. Not reaching to phase III, where the aim was to conduct AEP measurements on a minke whale may illustrate the difficulties in the procedure of live-capture. However, the study area of this project is optimal, where 19 minke whales were observed over 24 days in a location with low background noise (figure 4.1.1g).

Several logistical challenges occurred during the project. It took multiple days to organize and mobilize everything for the first trial. Due to the covid19 pandemic, delivery of various equipment took longer than anticipated and having the C&R site ready to live-capture was delayed by numerous days. With travelling challenges not all researchers arrived in time, causing the set-up and proper attachments of the C&R site to be more time consuming. These administrative and logistical considerations may have interfered with succeeding live-capture. On the other hand, since this was the first year of the project it was necessary to spend time on optimizing the design of the trap. The results from the trial showed several minke whales passing through the C&R site before it was ready for live-capture (figure 4.1.1g). The net between buoy C and buoy D on the guiding net (encircled in red) (figure 3.2.2a) was absent for much of the trial because production of the net was significantly delayed by covid19. Approximately 26% of the observed minke whales escaped the C&R site through this gap (figure 4.1.1d). One could suspect interference due to these delays, thereby giving a low success rate in the results from last year.

Challenging weather was present for some of the days, hindering observations and the handling of nets. Another suspected hinderance to a successful live-capture was the presence of pilot whales and killer whales (figure 4.1.1g, figure 4.1.2d). Killer whales are known for hunting or harassing minke whales and use vocal communication in the water, and the vocalization of pilot whales are similar to that of killer whales (Cosentino, 2015; Ford & Reeves, 2008; Vester et al., 2017), suggesting that these odontocete pods might have scared any minke whales close to the area by their vocal behavior. Several of the sound files from the "Loggerhead" hydrophone consisted of high frequent whistles, calls and clicks produced by killer whales (figure 4.1.2d). The detected time and dates these sounds or visual observations occurred showed that minke whales were not present for hours or days during these periods (figure 4.1.1g).

Interestingly, there was surprisingly little trend found in the directions noted of all the minke whales in phase I, showing an equal number of eastward and westward directions (figure 4.1.1h). Considering the minke whales' typical migration pattern (Kvadsheim et al., 2021), we would assume a higher value in western directions. The lack of trend in direction could suggest that the minke whales is not migrating but feeding in the area. However, the values might be biased, where the observer may have simplified the estimation course of the minke whale to four variables rather than eight (north, west, south, and east, not accounting for those in-between directions) (figure 4.1.1h). The navigating animals between the islets and the observed direction might also not represent the overall migration course. We also hypothesized that most of the minke whales would be first spotted between Flatskjæret and the guiding net. Only ~26% (5 of 19) of the minke whales were spotted entering the C&R site in this area (figure 4.1.1b), while ~21% (4 of 19) of the minke whales entered the C&R site between the islets Ausa and Flatskjæret (figure 4.1.1a). Approximately 32% (6 of 19) of the minke whales were observed closer to the basin entrance (figure 4.1.1c), where three of these individuals (Ba21_0706a, Ba21_2006, Ba21_2306b) were spotted close to Ausa or net C suggesting they may have swum between the islets Ausa and Flatskjæret. Nonetheless, determining the route where these individuals actually came from is not possible from the observations made. Considering the amount of minke whales entering between Ausa and Flatskjæret ~21% and the potential of ~37% if Ba21_0706a, Ba21_2006, Ba21_2306b were included could suggest a higher success in entrapping a minke whale in the basin by having another guiding net attached from Ausa directed north towards Hysskjeran (another islet further north) (appendix 7.5). A counter argument to this suggestion is the possibility of disturbing other marine life (i.e., fish entanglement and bycatch of other marine organisms), as well as having insufficient number of team members to observe this entire area. Net C might also have blocked the animals from entering the basin, where ~21% (4 of 19) of the minke whales were observed between the fish pen and Ausa (figure 4.1.1d). This could suggest removing net C, but then there might be a higher risk of the minke whales not being guided into the basin entrance.

All animals observed seemed relatively calm when approaching the C&R site. Most of the swimming behavior was noted as CNS (~82%), and only some noted as FVS (~17%). Swimming behavior changed from CNS to FVS in three of the minke whales (Ba21_0406a, Ba21_0406c, Ba21_1106) when they entered the basin. The same was observed in Ba21_1606c (the whale in the basin for ~8hours), suggesting increased attentiveness or a more vigilant exploratory behavior. During these timepoints, there were more sound disturbances from the boats “MOBHUS” and “Zephora” in the area, assembling the set-up of the C&R site, and for the procedure of closing the “door”. Previous literature has revealed minke whales increase their swimming rate during noise disturbance, indicating a stress response in the animals (Kvadsheim et al., 2017). However, the respiration rates calculated do not indicate any abnormal values compared to respiration rates calculated by VHF-radio tracked minke whales found in literature (table 4.1.1) (Blix & Folkow, 1995). The mean respiration rates calculated from phase I are based on different short time intervals. Some of the minke whales had a single surfacing. There may therefore not be enough data to fully represent every individual's respiration rate.

5.1.2.2 Phase II results, observational data from the basin

One minke whale (Ba21_1606c) was successfully entrapped for ~8hours but managed to escape between a gap where the "door" was closed. Comparing this to the attempt in Iceland in 2007, one crucial factor is how fast the team must act in enclosing these animals. Minke whales are very fast swimmers and seconds can make a difference in completing an entrapment. Capturing the minke whale Ba21_1606c took 6 minutes, where observers at Æsøya were constantly monitoring the baleen whale's location, making sure it was in the basin. This did succeed last year, but if the "door" could be closed even quicker, that could possibly give better odds for future live-captures. Ba21_0406a entered the basin (figure 4.1.1a) but turned around and escaped back through the door before it was closed. After that we made improvements to the door to close it faster. The observation data collected on the baleen whale is scarce due to the short time of observation, but the data collected has been analyzed to give an indication of the whales' behavior and preference in areas of the basin (figure 4.1.2.a).

During this entrapment, swimming behavior was noted as ~98% CNS, indicating a low stress level in the animal. The mean respiration rate never exceeded 1 breath per minute (figure 4.1.2c), which is normal for minke whales (Blix & Folkow, 1995). In Ba21_1606c which was kept in the basin for ~8hours we did observe a marginal but significant (p-value= 0.0297) increase in respiration rate and a switch to a more vigilant fast swimming behavior. This might indicate increasing stress levels, but the respiration rate was still well within what might be considered normal based on Blix & Folkow,1995. However, the highest mean respiration rate of Ba21_1606c (1 breaths min^{-1}) was based on only ten minutes (rather than an hour) due to the animal's escape. Therefore, the trend could be biased, given an estimated value higher or lower than if that calculation was based on one hour. Assuming there is a correlation between behavior and stress, the stress level in the baleen whales could be interpreted. It is not always the case where other factors may play in, but in this case of handling wild animals it is a reasonable assumption. The respiration rate can be interpreted for other reasons than an expression of oxygen usage, where the minke whale could be surfacing more investigating the area, considering the fact it was observed spyhopping (~0,4% SPY during its entrapment) which is very rare in minke whales (P. Kvadsheim, personal communication, 10.05.22).

We did not reach phase III, where corralling of a minke whale into the fish pen would have been proceeded. There would likely have been challenges moving the E net, dragging it eastwards from each end. We concluded that larger boats with more hp would have been needed to be able to move the net if we reached phase III. During the collection of all nets after the trial, there were difficulties retrieving the nets due to entanglement on the seafloor. Another possibility to corral a baleen whale into the fish pen could have been using acoustic deterrent devices to scare the baleen whale in that direction, but this would likely cause significant distress in the animal and may not be considered ethical. A possible alternative solution for future trials could be leaving the fish pen open. Minke whale Ba21_1606c probed along the nets seemingly looking for ways out. If we leave the fish pen door open, it might swim into it voluntarily, avoiding the possibility of a stressful corralling procedure.
Another consideration for future design of the live-capture would be the repositioning of the fish pen. The minke whale Ba21_1606c showed a clear preference for the A1 area of the basin (figure 4.1.2a). Notably, this was the direction hypothesized all minke whales would have taken, following their typical migration pattern. This could suggest that Ba21_1606c presumably tried to continue its migration but was blocked by net A. Taking that into account, a better solution for reaching phase III would likely be to place the fish pen on the west side of the basin (near A1), due to the amount of time the animal was sighted in that location. This site may not be possible due to the exposure of weather and swells, while the location previously used was more sheltered. Considering the explorative swimming behavior of the minke whale obtained in the basin, leaving the entrance to the net pen open is likely the best solution for luring the animal in there and conduct AEP measurements. If the future trials reach phase III to conduct AEP measurements on a minke whale, a potential improvement to restraining an animal could be inspired from the rescue of the Bryde’s whale in Australia in 1994 (Priddel & Wheeler, 1997). The inflatable pontoon used on the Bryde’s whale may be a better solution than the rafts constructed and placed in the basin. The pontoon might lead to a reduced chance of skin abrasion, but perhaps higher risk of entanglement.

5.1.3 Can baleen whales be safely live-captured?

Experiences gained in entrapment and restrained baleen whales are invaluable when the need arises in rescuing accidentally entrapped baleen whales (Priddel & Wheeler, 1997). The minke whales used for this project and my thesis is one of the smallest baleen whale species, and of least concern in the IUCN Red List of Threatened Species (Cooke, 2018); this makes it an optimal subject species for which the methodology could be applied translationally to other species of baleen whales in the future. The adolescent minke whales would be approximately the same size as previously captured species of odontocetes, such as belugas (*Delphinapterus leucas*) and killer whales. These smaller sized baleen whales would be easier to capture and handle. A humpback whale actually swam into the catch basin and stayed there, behaving calmly with the entrance open before leaving after ~75min (figure 4.1.1f). Based on the experiences gained from the 2021 trial the most important factors of succeeding in mysticete live-capture are efficiency in closing an entrapment quickly, ensuring the nets are enclosing the basin completely to hinder escape, and opening the entrance to the fish pen with people in position ready to close the opening. Furthermore, having the C&R site completely ready for live-capture earlier, preferably from 1st of June would be optimal, considering most minke whales were sighted in the beginning of the month (appendix 7.1).

The methodology used for restraining the animals for direct studies in the SOST minke hearing project is the most promising attempt so far when considering today's animal welfare considerations. Mito aquarium succeeded in capturing 3 minkes in the 1930s and 1950s (Kimura & Nemoto, 1956), but their techniques were questionable from an animal welfare perspective. The live-captures and obtainment of the grey whales in USA in 1965 and 1971 are also somewhat problematic and ethically questionable (Wahrenbrock et al., 1974). These previous mysticete live-captures had smaller pools than that those used in last year's trial (Bruehler et al., 2001; Kimura & Nemoto, 1956). The large basin created by the natural environment is a better solution for the mammals' large size, where documented challenges before have been mobility limitations and the animal outgrowing the pool size (Bruehler et al., 2001). The amount of minke whale sightings observed during 24 days indicate an ideal study area for proceeding an entrapment of these animals. The SOST minke hearing project (2021) was a pilot study to prove the concept of a mysticete live-capture and if direct studies could be safely accomplished. If the C&R site is ready earlier for the next trials, it is more likely to succeed, considering almost half of the mysticete observations were before the C&R site was prepared (June 10th, 2021) (figure 3.2.2a, figure 4.1.1e, figure 4.1.1g). With work shifts around the clock in the Arctic summer, the chances of entrapment are likely to be higher. Minke whales may have been present last year before June 16th during the nighttime, but unfortunately the team was not fully prepared until that date.

With well-documented incidents of baleen whale entrapments, future procedures of studies and live-captures could be validated. Furthermore, a baleen whale entrapped under controlled conditions would give the opportunity to study potential sensory modalities the animals use in a C&R context.

5.2 Testing the use of sensory modalities by baleen whales to orient themselves around nets, based on observations from the SOST minke hearing project

We do not have a good understanding of how baleen whales use different sensory modalities to orientate, navigate and detect prey and objects (Drake et al., 2015; Goldbogen et al., 2017; Thewissen et al., 2011). During the SOST minke hearing project an opportunity occurred to investigate acoustic, visual, and tactile senses by observing the behavior of a baleen whale under controlled conditions in a large entrapment area. Previous literature has suggested that minke whales may be using visual and acoustic cues to avoid nets when researchers simulated shallow water crab and whelk fishing gear with different coloration in a coastal fishing area (Kot B. et al, 2011). However, my experiment differed by giving the minke whales environmental conditions with close interaction with the nets over a longer time period, including investigation of the possibility of a tactile response in the subjects.

5.2.1 Investigation of visual cues in minke whales and other mysticetes

All the minke whales observed in my study during both phases, showed a clear ability to recognize the nets of the C&R site, avoiding direct contact with them, but at the same time moving very close to them. Some of the whales were observed swimming parallel to the nets with different distances. Two of the whales (Ba21_1606c and Ba21_2006a) spyhopped close to the nets, indicating the animals were trying to gather visual information from the surface. It is not clear what level of eyesight and ability to discriminate color baleen whales have (Griebel & Peichl, 2003). Visibility in the ocean can be poor and turbid depending on lighting conditions, including currents making it difficult for marine mammals to see. The experiment by Kot et al. (2012) used different colored singular ropes, held vertically from the seafloor to the surface. Their study results showed that ropes with higher contrast coloration (black and white) are likely easier visually detected by the minke whales (Kot et al., 2012). Since the barrier nets of the C&R site in my project already consisted of thin black meshed net, one area of the basin nets was “colored” with thicker white ropes to investigate possible use of visual cues.

Through behavior analysis in phase II, I was expecting surface observation with a longer distance from net B2 compared to the other basin nets, hypothesizing that the entrapped baleen whale Ba21_1606c would detect that modified part of the net with a stronger color contrast from a further distance (figure 3.2.2e). In the beginning the minke whale was observed swimming submerged parallel with the net B2 at an approximate 10-meter distance. The weather conditions were optimal, with calm sea and sunlight reaching several meters down in the ocean, allowing us to see the submerged baleen whale from the observation post on Æsøya. The visibility in the ocean on that day therefore gave the minke whale perfect condition to use visual cues to navigate. Supporting this theory, most observations of Ba21_1606c’s distance to the net in zone B2 were noted as 10 meters or more (figure 4.1.2b), but it is based on very limited data. Most observations of the minke whale were in the opposite end of the basin (zone A1 and E) (figure 4.1.2a). Its preference for that area is likely due to its hypothesized eastern migration pattern, but may also be swimming towards the sun in the evening, or avoiding the white ropes on net B2. If the minke whale detected the coloration of the white ropes, it may associate it with the coloration of other cetaceans, maybe even as an innate or learnt fear towards killer whales.

Hardly any behavioral, physiological or immunocytochemical data regarding color vision, spectrum sensitivity or cone opsins in baleen whales have been investigated (Griebel & Peichl, 2003). Most mammals possess two types of cone pigments (Hunt et al., 2009), but one of these cone types, the S-cones, are absent in cetaceans (Levenson & Dizon, 2003). A L-cone monochromacy has been suggested in baleen whales similar to that of toothed whales based on deleterious S-opsin genes, indicating an unfavorable adaptation to the blue dominated visual surroundings (i.e., a form of colorblindness) (Griebel & Peichl, 2003; Levenson et al., 2000). The absent S-cones would also have been a better adaptation in perceiving brightness and contrast information (Griebel & Peichl, 2003). In other words, the vision of mysticetes is not thought to be exceptionally good. It is therefore not clear how strong the black and white contrast visual cue of the B2 net are to a minke whale. Black and white colors give both stronger contrasts in the ocean compared to other colors in the spectrum. However, detection of white ropes could be thought to give a stronger stimulus in Ba21_1606c than the unmodified black nets, given a stronger brightness cue, rather than just color cues.

5.2.2 Investigation of tactile cues in minke whales and other mysticetes

There is the possible use of a mechanosensory system as a physiological function. Facial hair is more common in baleen whales than in toothed whales (Slijper, 1976; Yablokov & Klevezal, 1964), and the hairs vary interspecifically within mysticetes (Reichmuth et al., 2022). When other sensory sources are insufficient, baleen whales' sensory hairs are thought to act as displacement detectors and close range receptors (Slijper, 1976; Yablokov & Klevezal, 1964), delivering information to the brain through the mandibular branch of the trigeminal nerve (Pyenson et al., 2012). Whale hairs have the same characteristics as vibrissae; with an exterior connective tissue capsule, a circumferential blood sinus system around the hair shaft, and extensive follicle innervation. This could suggest that the hairs in baleen whales serve as a mechanoreceptive role such as the vibrissae found in pinnipeds (Drake et al., 2015). A recent study on Antarctic minke whales proposed that the distributed rigid sensory hairs on their chin is used to detect prey and interfaces of air and ice (Reichmuth et al., 2022). Histological examinations have revealed that this species have far more sensory hairs than the related species *Balaenoptera acutorostrata acutorostrata*; the subject animal of this thesis (Reichmuth et al., 2022).

In phase II, net A was modified with fluctuating bands during the entrapment of Ba21_1606c. In the ~8hours of observation, the whale showed a distinct preference to the tactile area (zone A1) of the basin (figure 4.1.2a). My hypothesis is that tactile stimuli are important but only works at close range, and that the whale would orient very precisely along the A nets compared to other nets. Figure 4.1.2b did however not show any distinguishing difference in the distance variables in zone A1, and Ba21_1606c's swimming orientation does not seem more precise compared to the other nets. My assumption of the importance of tactile detection could be wrong. Maybe their detection of tactile cues could occur from a longer distance than 4-5 meters, or perhaps the created tactile bands did not give enough stimuli different from the other nets. Nonetheless, there is not enough data to test this hypothesis.

5.2.3 Investigation of auditory physiology in minke whales and other mysticetes

The last sensory system looked upon in mysticetes from the SOST minke hearing project was their auditory system. Sound travels efficiently through water and is likely a reason to why the baleen whales may use hearing, possibly as their primary sensory modality. Studies suggest their auditory physiology allows them to forage, navigate and to communicate in the ocean (Kvadsheim et al., 2020). In cetaceans, acoustic echolocation has two forms: active echolocation and passive echolocation. Active vocalization occurs by generating short broad-spectrum burst-pulses, known as clicks, while passive echolocation involves mapping the surroundings using external noise rather than self-produced sounds (Kritly et al., 2021; Mellinger et al., 2007). Many studies conclude that baleen whales do not echolocate after the findings of Beamish, 1978 who investigated biological sonar in an entrapped humpback whale, but a few papers disagree on the exclusion of the possibility that baleen whales echolocate (Frazer & Mercado, 2000). Most animals with good hearing and the ability to create noise are likely able to use echolocation, where the visual cortex can be stimulated from auditory stimuli (Thaler et al., 2011). I do not expect that mysticetes have echolocation in the same way that odontocetes do. Claiming the animals do not possess echolocation, on the other hand, could be equivalent to saying humans cannot echolocate, which is not true (Thaler et al., 2011). There is a potential gray zone as to how far you can define whether an animal can echolocate or not. Previously, higher frequency sounds have been reported released from a blue whale' anterior section (Beamish, 1979, p. 298). Recordings from humpback whales associated air movement to an underwater source of sound, which appears to be positioned several meters from the front region of the blowholes towards the dorsal fin. These findings corroborate a hypothesis that the mysticetes’ long tapering head works as an acoustic waveguide. Entrapped baleen whales could provide an opportunity to investigate this hypothesis further by evaluating their echolocation ability (e.g., maybe by playing loud sounds to see if a baleen whale’s ability to orient changes) (Beamish, 1979, p. 299).

The data collected from the hydrophones “Loggerhead” and “SoundTrap” showed no signs of vocalization or thereby active echolocation from the minke whales found in the C&R system, nor the humpback whale that also was inside the basin for ~75minutes (figure 4.1.1f). Due to wave movements in the basin, vibrations occurred on the line the hydrophones were attached to, making the analysis of sound files challenging at times. A broad voltage change occurred sporadically in the “Loggerhead”, suggesting a technical problem (self noise). At times, a constant background noise up to 500Hz was detected in the “Loggerhead”, making it difficult to determine if any minke whale noise was present. Previous studies suggest minke whale vocalization ranges from 50Hz to 500Hz (Risch et al., 2019b). However, the amplitude of minke whales vocalizing within 10m of the hydrophone is expected to be easily detectable despite background noise and internal noise in the “Loggerhead”, had they occurred. The quality of the data from the “Loggerhead” was proven by the detection of killer whale sounds (figure 4.1.2d). The “SoundTrap” recorded much lower frequency noise, but still no indication of any vocalization was noted from baleen whales. Unidentified sound sources could be biological, but since the data showed no sound repetition it was unlikely created by a mysticete vocalization. This does not mean the minke whales do not vocalize. They are known to be solitary animals, and generally more quiet than other cetacean species outside of the mating season (Mellinger et al., 2007; Nikolich & Towers, 2020). Perhaps the lack of vocalization of minke whales was because the animal was actively trying to remain quiet due to the presence of killer whales or being entrapped. If the hypothesis of echolocation is ruled out, Figure 4.1.2b suggests Ba21_1606c was using primarily acoustic cues to orientate, considering it was mostly observed with a longer distance from the islets (zone I1 and I2) than the other zones of the basin. In addition, net flow noise could nevertheless be an acoustic indication that allows the minke whale to detect the C&R nets.

5.2.4 Net detection in baleen whales and possible refinements to the experimental design

This thesis objective focused on three sensory modalities, but other sensory systems may also give the baleen whales environmental information (e.g., chemoreception, detecting chemicals of the nets and gear at the surface), and should be a potential future consideration. Furthermore, it could be conceivable that minke whales gather information on their surroundings with visual, tactile and acoustic cues simultaneously. Few observations were made of Ba21_1606c in the islet zones (I1 and I2), where all three senses would be exposed to a stronger stimulus (e.g., white and black rocks, kelp and braking waves give strong visual, tactile and acoustic cues, respectively) (figure 3.2.2e and 4.1.2a). The data collected from these zones was mainly at the distance of 10 meters or more (figure 4.1.2b). This could suggest that the baleen whale was able to detect the islets from a further distance than the nets.

In conclusion, there is not enough data to clarify what sensory modalities were most likely used or were most important in Ba21_1606c. There was no sign of active echolocation, but the animals might use passive acoustic cues. Nor was there enough data from the other minke whales that were observed close to the C&R site during the trial periods. It is important to not generalize mysticete species, since they can differ widely from each other in biology and behavior. Possible refinements for future behavioral studies in vision of entrapped baleen whales could be by utilizing underwater video cameras. Placing video recording instruments in the different zones of basin to further investigate the baleen whales’ orientation at various depths, could give a better indication of how the animals detect nets. Using a secchi-disk to estimate the turbidity in the water could also be considered. Improving a set-up for investigation of tactile sensory could also be by recording with underwater video cameras. Additionally, the plastic bands used to create the tactile bands should have been wider creating a larger tactile stimulus, and potentially in a blue color to minimize visibility. Lastly, to improve the acoustic monitoring by attaching the hydrophones with spring to the surface, would help to avoid the mechanical vibration that was picked up by the hydrophones.

As a pilot study, the methodology could possibly pave the way for future research to investigate these senses with similar methodology to fill some of the knowledge gaps of baleen whales. Perhaps other methodologies, if a baleen whale were to be restrained, could developed in order to further understand the sensory apparatus, beyond what can be done through observational studies.

5.3 Discussing research opportunities, given access to entrapped and restrained baleen whales.

Maintaining large cetaceans, such as baleen whales in dolphinariums, tanks, or laboratory facilities is not optimal, thus the physiological laboratories must be brought to the open ocean. Since certain studies are not possible to measure at range, the SOST minke hearing project brought some of these physiological laboratories to the ocean, using the animals’ natural environment. In this thesis the third objective was to consider and discuss relevant physiological, behavioral, and health assessment studies that could be conducted, given access to captured baleen whales.

5.3.1 Potential methodologies for studying use of sensory modalities in mysticetes

Some methodologies were briefly mentioned in my section on knowledge gaps that will now be further elaborated upon. Investigating sensory modalities in baleen whales could done by measuring their ability to detect different forms of stimuli by measuring evoked potentials. The information gathered from evoked potentials studies occurs through bioelectric responses. Evoked responses can be detected in neurophysiological studies where the conduction of electrical signals to the brain are registered after various sensory stimuli (Creutzfeldt & Kuhnt, 1973) (e.g., touch in the skin (SSEP), visual impressions (VEP), and sound stimuli (AEP)) have been delivered (figure 5.3.1). However, the evoked potentials can only be recorded if a large population of sensors is stimulated at the same time. Regardless upon which form of stimuli, the characteristics of an experimental stimuli must be the same; a sharp gradient and of short duration, consistent, and with an exact controlled rate and intensity (Markand, 2020).

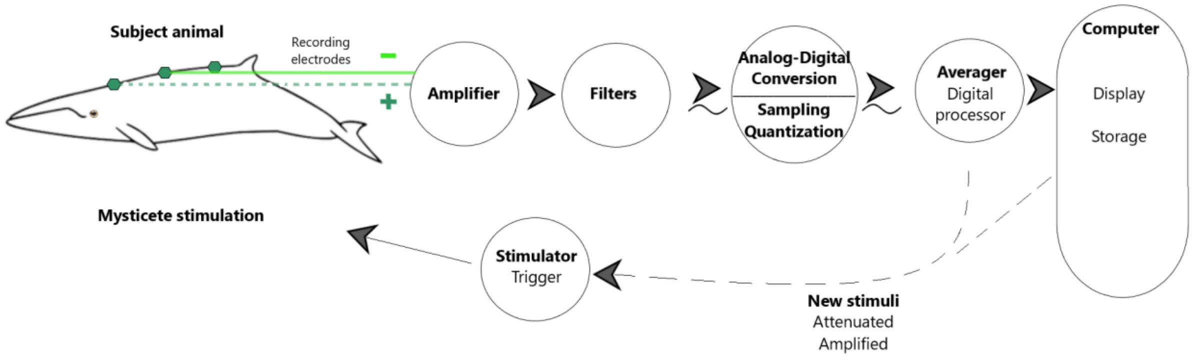


Figure 5.3.1: Simplified diagram of a generalized evoked potential set-up.

General equipment used for evoked potential measurements are stimulators, amplifiers, filter system, analog to digital converter, and a computer to display and store the information (figure 5.3.1). The first step of this methodology set-up is the stimulator. The stimulator triggers a specific form of stimuli in an animal. Depending on what sensory system is being investigated, the trigger could be various levels of visual, acoustic or tactile stimuli. When an animal has received one of these forms of stimuli, electrodes attached on the animal records the animals' nervous traffic triggered by the stimulus (Markand, 2020). The second step of the set-up would be the amplifier. Amplification is necessary since the evoked potential signals through the recording electrodes are very low. The signal is then transported to a filtration system, where a wide range of low frequency and high frequency filters the proper condition of the input signal to the next step, converting analog to digital information. In the analog to digital conversion sampling, the amplitude values are assessed into quantization. Then the quantized values are put into an "averager" to remove any values from irrelevant signals and data processing. Lastly, these values are relayed to a computer, displaying and storing the information of the sensitivity level in the animals' sensory apparatus (Markand, 2020). The stimulus could then be attenuated or amplified from the information in the computer to a new trigger value, before presenting it to the subject animal again, investigating the lowest stimuli value for detection.

The placement of electrodes may vary depending on which sensory apparatus is being investigated. Electrode placements could lie at various positions on the electrical pathway, from the exposed stimuli area to the brain. No instructions on exact electrode placement on mysticetes are available to date. However, there are two common components of evoked potentials, far-field potentials and near-field potentials (Markand, 2020). Measuring hearing with AEP in mysticetes can be done by far-field potentials. This component is easier to utilize in comparison to the other component since the location of electrodes is much less affected by precision and can be placed away from the generator sites. Suggested location for electrodes for AEP measurements are shown in figure 5.3.1. Due to the low brain-to-body mass ratio found in baleen whales, subcutaneous needle electrodes placed below the dermis into the blubber layer are needed for the AEP measurements (Kvadsheim et al., 2021). If testing visual capabilities through VEP or tactile sensitivity through SSEP on the other hand, near-field potential is likely needed (Markand, 2020). This may be challenging to position these electrodes on a mysticete but it is feasible.

Evoked potentials is a frequently used electrophysiological methodology for use on human patients during surgery (Jansen, 2018). This methodology allows researchers to gather information on different forms of stimuli detected from different types of sensory systems from a passive patient. Some of these methodologies have already been similarly conducted on pinnipeds and toothed whales due to their accessibility (Hogg et al., 2015; Li et al., 2011), but if a baleen whale is temporarily restrained, it could be possible to measure on this suborder of cetaceans as well.

5.3.2 Tagging and instrumental attachments

Furthermore, attaching long-lasting recording instruments on a baleen whale could assist in filling some of the earlier mentioned knowledge gaps. Tagging devices have been developed and improved over the last two decades (Cade et al., 2021). Previously, radio tags mainly allowed researchers to study the diving behavior and track the position of an animal around the clock for a short time period (Evans, 1974; Watkins & Schevill, 1977). Earlier there were more challenges with the tagging devices such as short battery life, data reception, storage capacity, invasive or loose attachments, as well as the larger sized tags increasing drag for the animals (Arnemo, 2021; Evans, 1974). Today, tags can be more advanced and include other integrated sensitive instruments. Animal-borne tags (biologgers or physiologgers) have advanced to the point where they can collect data from numerous sensors at high frequencies for extended time periods, making them a valuable tool for behavioral ecologists and physiologists studying wild marine mammals (Holton et al., 2021). The usefulness of tagging marine mammals cannot be overstated, but there are still challenges designing the implementation of these tags, where improvements are continuously underway (Holton et al., 2021; McIntyre, 2014).

Despite the inclusion of many additional types of sensors that widen the biologging tags applicability and utility, the fundamental design from the early radio tracking tags with time-depth recordings remains an essential component. Some of the further integrated instruments that can be found in the tags are cameras, hydrophones, biomedical sampling devices and multi-axial motion sensors (Cade et al., 2021). Cameras could provide visual information on the mammals’ surroundings, and with multi-axial motion sensors potentially determine the fine-scale kinematics of swimming and feeding (Cade et al., 2016; Goldbogen et al., 2017). Hydrophones could be used to quantify acoustic behavior of a tagged baleen whale (Johnson & Tyack, 2003), and biomedical sampling devices could measure the animals gas management during diving (McKnight et al., 2019). The first heart-rate measures conducted on a large mysticete was recently measured through a suction cup ECG tag attached on a blue whale, providing information on its routine heart rate, the dive response, oxygen store management and hemodynamics (Goldbogen et al., 2019).

Going further, a restrained baleen whale would give the possibility to achieve an optimal anchoring of different devices, when an animal can be tagged under controlled forms as opposed to the use of ballistic tags, which often hit and fasten randomly on a cetacean.

5.3.3 Previous captive studies of baleen whale physiology

From the previous documented mysticete live-captures and accidental entrapments, various experiments were conducted on the mammals. From these experiences we know that it is possible to conduct experiments such as respirometry (Wahrenbrock et al., 1974), ballistocardiography (Smith & Wahrenbrock, 1974), ultrasonography (Curran & Asher, 1974), surgical attachment of tags (Evans, 1974), tissue and blood sampling, morphometrics (Reidarson et al., 2001), and bioacoustics (Winn et al., 1979) on a temporarily restrained baleen whale. Because of a baleen whale’s accessibility when entrapped, the handling opportunities would be unparalleled (e.g., the respirometry procedure on the gray whales could assist in an understanding of mysticete metabolism as a whole) compared to any non-captive baleen whale research program. However, more non-invasive methodologies are developing (e.g., data collecting from drones) which can outdate some of these methodologies but may first require validation from direct measures on a restrained baleen whale.

5.4 Discussing the challenges and animal welfare considerations of live-capture studies of baleen whales.

The ethics of choices made by some of the pioneers who caught the first live cetaceans can be questioned, both capture methods as well as husbandry considering their short lifespan after being caught (Jiang et al., 2008). These previous captures were primarily used for human entertainment, but the way humans have treated animals for research and entertainment have luckily changed with changing ethical norms of society and technological development who have offered alternative methods. Human perspective on whales has, in some cultures, evolved to respect, while others have focused on the animals' utilitarian value (Jiang et al., 2008; Sullivan, 2000; Turner, 1990). Thousands of toothed whales have been caught for both public display and research over the last two centuries (Jiang et al., 2008), while the larger suborder, baleen whales, have almost been excluded, likely due to their greater size and the challenging logistics associated with that factor. There are a few documented attempts of live-capturing baleen whales where scientific studies were made, while other studies have taken place when baleen whales were accidentally entrapped. Both scenarios have been scientifically useful, but did these priorities of research in these mammals interfere with the animals' wellbeing?

5.4.1 Animal welfare issues with previous entrapped and restrained mysticetes

Current Norwegian legislation ensures that entrapment of marine mammals for public display in zoos or aquariums, or for research in laboratories is very strictly controlled. Capture and marking of baleen whales create a potentially significant danger and disadvantage for the animal. The necessity of the entrapment and its likely value (in terms of knowledge gained) should be carefully assessed against the burden on the animal subject. It is therefore important that data relevant to animal welfare is collected during the research.

In the previous intentional mysticete live-capture some of the descriptive methodology used to capture the baleen whales could be considered unethical, according to today's standard. First example would be the minke whale calf that was lassoed and hoisted by its tail in the USA in the 1950s, were the animal died shortly after capture (Norris & Prescott, 1961), likely due to those human interventions. In the same time period, other minke whales in Japan were surrounded by nets and towed to the Mito aquarium (Kimura & Nemoto, 1956), possibly causing skin abrasion and stress in the animals. The gray whales captured in the USA in the

1960s and 1970s were held captive in relatively small pools, limiting their mobility. The first of the gray whales (GIGI I) died from the infected wound caused by the capture technique, while the other gray whale (GIGI II) was held for a considerably longer period in captivity before release (Wahrenbrock et al., 1974). I believe that animal welfare considerations were lacking in these examples from over 60 years ago, when the animal welfare culture greatly differed compared to how it is today.

Furthermore, there are some animal welfare considerations to make when an accidental entrapment occurs. The studies of humpback whales found entangled in fishing gear in Canada during the 1970s did not acknowledge the potential increased stress levels in the animals during their experiments, nor were observations made on the individuals after releasing them. These animals were maintained for 2 and 29 days respectively (Winn et al., 1979), which may have caused behavioral, physical and/or physiological stress in the mammals. In addition, the rescue of the entrapped Bryde's whale in Australia (1994) took 100 days before the animal was released back into the ocean (Priddel & Wheeler, 1997). Taking that long for the baleen whale to be relocated raises the question if euthanasia would have been a better solution. However, the incidents of accidental entrapments validate the effectiveness of multiple procedures seldom implemented before; approaches that now can be used with confidence to rescue and proceed with potential studies on baleen whales. Additional knowledge in rescuing baleen whales in situations when intervention is required immediately, such as entanglement or strandings could assist in future rescue operations (Priddel & Wheeler, 1997).

Cetaceans can be generally flexible in their behavioral patterns, where an individual can adapt to different conditions in terms of climate, nutritional composition and even social structure (Sousa et al., 2019). Nevertheless, the mammals urge or motivation for certain types of actions can be very strong (Pirotta et al., 2016). This can be problematic if the animal is deprived of the opportunity to perform such actions, resulting in frustration (Mejdell, 2004). Captivity always places limitations on animal life development and choices. It is therefore not always practically possible to satisfy strongly motivated behavioral needs. For example, during a minke whale entrapment, you cannot allow them to continue on a migration, despite their behavioral urge to do so. Capture and subsequent close contact with humans are also likely to involve a significant stress for a fully grown wild animal; whereas the young and adolescents have not always developed the same level of fear towards humans (Mejdell, 2004). A trapped whale might try to break free and may therefore become entangled in nets, or develop stress-induced deaths, e.g., “capture myopathy” can occur several days after a great deal of stress, even if the animal was physically unharmed (Arnemo et al., 2006). As a result, we mush distinguish between long-lasting captures and short and acute studies such as the SOST minke hearing project.

5.4.2 Animal welfare considerations in potential direct studies

Studies of free-ranging wildlife are challenging, where regulations and principles can often introduce difficulties, especially when working with aquatic mammals. It is difficult to achieve a decent sample size of marine animals with the wanted sex and age. Recaptures can be close to impossible, as well as never having a proper control group (Arnemo, 2021). Regardless of the purpose of capture and tagging, the planned procedures should always be accepted in advance from either NFSA or other relevant animal welfare and ethical assessment committee.

For tagging or attaching instruments on a baleen whale it is important to consider possible technical failures such as GPS, battery life and coverage signal (Arnemo, 2021; Holton et al., 2021). Ensuring a streamlined configuration is essential when attaching objects to the mammals. Tags can affect the animals hydrodynamic shape, increasing resistance in the water as well as increasing the risk of wounds and infections if continuously dragged in the water. In addition, it may be required to recapture the animal to remove tags if they are causing health problems, alternatively the tag may have a drop-off function (Arnemo, 2021). With a restrained baleen whale, the likelihood of infection when attaching an instrument would be less due to the controlled conditions. From a radiological medical point of view, questions have also been raised as to whether radiation from the instrument can affect fertility. If so, it would not be beneficial for the population if juvenile individuals are studied even though they are less susceptible for fear and stress (Balmori, 2016; Mejdell, 2004).

For the different evoked potential studies that could be conducted on a restrained baleen whale, the use of drug immobilization might be necessary. The animal’s level of consciousness would be greatly reduced while it has close contact with people. Therefore, the methodology will not induce fear-related stress, but there instead lies the risk of an adverse reaction to the drug. This is particularly a problem in large animals such as baleen whales where doses are not well defined (Kvadsheim et al., 2021; Moore et al., 2010). Sedation and anesthesia always impose a risk to an animal, which is why it is avoided whenever possible.

5.4.3 Animal welfare considerations in the SOST minke hearing project

Capturing and restraining a minke whale could cause a moderate degree of stress, which has been the focus of negative media attention at the beginning of the 4-year project (Campbell, 2021; Shukman, 2021; Sørgård, 2021; Woodyatt, 2021). However, little direct pain would be involved in the project. Sedation is not anticipated to be required for the hearing test procedures based on similar constraint situations when attaching satellite tags to minke whales (J. Teilmann, personal communication, 27.10.21). Records of how minke whales have responded during bycatch with bottom nets have shown a calm behavior when constrained in this manner, indicating less distress in the animal than previously predicted. Considering the potential risk of entanglement, the results from last years' trial showed that none of the 19 minke whales touched the C&R nets. Scenarios of where direct pain may occur in the future trials would be when proceeding with blood collection for health monitoring and attaching a satellite tag before release. Only mild pain and distress is expected during these procedures. These approaches are frequently applied in the field, whereas the invasive attachment of a satellite tag would also be alleviated with local anesthetic (1.8 ml of lidocaine hydrochloride and epinephrine (1:100,000)) as a refinement to the procedure (Kvadsheim et al., 2019). In addition, there was a quick release system present in the nets that would have been utilized if there was any sign of a baleen whale reaching a defined humane endpoint. Humane endpoints can be referred to as the early indicator of (possible) pain and/or distress in a subject animal that can be used to avoid or limit suffering and/or distress. This may include taking actions such as euthanasia or easing the pain and stress (Hendriksen & Morton, 1999).

A stress response can be difficult to detect in wild animals with any type of human interaction. Cortisol levels are not something that could be measured quickly in the field. This could be studied afterwards through blood samples collected if we reached phase III, as well as monitoring the heart rate looking for abnormalities during that phase. Nevertheless, the mean respiration rate is an indicator that shows if the animal is stressed, where values can go either way, dramatically increasing or decreasing (D. Houser, personal communication, 07.04.22). A potential stress level could have been present in the animals during the SOST minke hearing project (2021), but if so, only over a relatively short period of time. It is unknown how to replace the necessary direct measurements on baleen whales in research, it may be the case that some direct studies are required to validate the indirect studies already completed. Future replacement options are possible after measurements have validated nonintrusive study techniques (e.g., anatomical modeling). In order to manage the entire mysticete population in a humane way, according to NFSA, the benefits of the research outweighs the potential stress in the animals. The research validation from live-captures could ultimately lessen the need for further invasive and/or intrusive experimental studies on wild-captured mysticetes.

5.5 There is no planet B(aleen whale)

The Yangtze River dolphin (*Lipotes vexillifer*), is the first cetacean in this century to go extinct (Turvey et al., 2007; Zhou, 2009). The animal is not just a population, species nor a genus, but an entire family (Lipotidae) of odontocetes that is likely perished. It is dying out from human overuse due to a range of anthropogenic extinction drivers such as boat collisions, bycatch, fishing gear entanglements, and accumulation of various pollutions (Smith et al., 2000). The vaquita (*Phocoena sinus*), Atlantic humpback dolphin (*Sousa teuszii*) and the North Atlantic right whale (*Eubalaena glacialis*) are critically endangered to date (IUCN, 2021) and could possibly be the next victims of extinction.

Mysticetes have an important role in the ocean ecosystem. These species and other animals are facing the challenges of the Anthropocene, where intricate involvement is necessary to shed light on the issues as well as finding solutions to them. Anthropogenic threats are of growing concern in mysticetes as their environment is changing and accumulation of various pollutants in the ocean continue (Jambeck et al., 2015; Kvadsheim et al., 2020; Reijnders et al., 2009). Recent findings of the toxic effects of various contaminants on baleen whales demonstrate that even low concentrations of various pollutants can have a negative health effect on the mammals (Lühmann et al., 2020). Fossi et al. (2014) found a correlation between plastic and POPs in five stranded Mediterranean fin whales (*Balaenoptera physalus physalus*) containing high levels of organochlorines and phthalate metabolite with a hypothesized consumption of over 3500 microplastic items per day. Through scat sampling of some mysticete species, another study found approximately five reputed microplastics per gram of whale scat in New Zealand to date (Zantis et al., 2022).

In the 20th century noise had dramatically increased in the ocean. It is not known how affected baleen whales are by noise pollution and the consequences there of. Researchers know that noise can scare animals away from their habitat, disrupting important biological activities such as feeding, resting and migration (Kvadsheim et al., 2020, pp. 94-95). Observations suggest some cetaceans can be frightened away from grazing areas or be so scared that they swim too fast to the surface and die of increased tissue and blood N_2 levels (a resembling decompression sickness) (Aniceto et al., 2021; Hooker et al., 2012; Kvadsheim et al., 2012). They may also become so stressed that they get stranded, but noise is likely affecting their behavior more. If the burden is great enough, it can result in consequences for cetaceans at the population level (Kvadsheim et al., 2020, pp. 13,93); hypothesizing that a domino effect from noise disturbance creates change in their energy balance when they either increase metabolism avoiding areas of noise disturbance and/or lost feeding opportunities when they stop foraging in response to noise (Gailey et al., 2007; McCauley et al., 2003; Richardson et al., 1998; Thomas et al., 2016). For instance, humpback whales (*Balaenoptera novaeangliae*) have been observed avoiding previous known main feeding grounds due to anthropogenic activity and noise (Sivle et al., 2016).

In order to find solutions to these environmental problems, we need to know enough about the physiology and ecology of the animal. Hearing measurements of a baleen whale would assist in addressing noise pollution by finding out what sounds potentially impacts them (Kvadsheim et al., 2020, pp. 94-95). Noise pollution from a particular noise source is not an issue for a species if they do not hear the frequencies of the noise. Conversely, if the noise pollutions are at lower frequency where the baleen whales are sensitive to the sound, there might be an issue (Kvadsheim et al., 2021). The SOST minke hearing project aims to gather this knowledge, including understanding the vocalization ranges of the baleen whales communication with each other. If research could obtain information on mysticete vision, use of tactile cues or the potential presence of echolocation, fisheries could alter coloration and texture in fishing gear, preventing bycatch of these mammals. With long-lasting tags, and advanced integrated sensory systems included in those attachments, more information on their migration can assist in avoiding future boat collisions. Their energy expenditure could be investigated with tags, alongside biopsies which could inform of potential pollutants, abnormalities in endocrinology, diseases, and genetic identifications (Goldbogen et al., 2013; Holton et al., 2021).

Kenneth et al. (1961) first discovered echolocation in cetaceans by obtaining odontocetes in captivity. Whether captivity was necessary to discover this function can be disputed, but understanding the complexity could be less likely if the studies were only based on research conducted in the wild. I believe science takes many approaches to develop an understanding, but with good documentation of previous experiences and animal welfare considerations, knowledge gaps can be filled. There is no conventional methodology for capturing a baleen whale, where the strategies utilized for last year’s trial was novel. The trial was based on carefully detailed local knowledge of the minke whale migration routes, the bathymetry of the study area, and documented knowledge of the minke whales’ response to nets and being restrained. Moreover, the novel protocol could not have been fully evaluated until the procedure was implemented, where the C&R site was and will be adjusted as experiences and knowledge are gained.

6 Conclusion

Can baleen whales be safely live-captured for studies of their physiology?
Live-capturing and restraining baleen whales is complex, where logistical and various factors such as team efficiency, proper equipment and enclosure settings, in addition to knowledge of baleen whale migration, is crucial to succeed. Reviewing previous experiences with restrained baleen whales, in intentional and accidental entrapments, suggest that the SOST minke hearing project is the most promising attempt to date. An opportunity to investigate the sensory modalities utilized by minke whales was given through an entrapment. However, the results had not enough statistical power to conclude what sensory apparatus was used by an entrapped baleen whale in detecting nets, but the experiment could create opportunities for future investigations. Furthermore, if a baleen whale is restrained in planned future capture attempts, detailed measurements of the sensory apparatus could be conducted through evoked potentials or properly attached advanced instruments to determine which sensory methods are likely utilized for orientation and/or detection of surroundings, conspecifics, prey, and predators. These measurements could assist in validating indirect studies such as biomechanical modelling of the hearing ability, from the smallest to the largest of mysticete species. Mitigative measures to optimize animal welfare is another important factor in handling of these animals. The SOST minke hearing project followed the 3Rs to the greatest extent possible, compared to the previous reviews mentioned in this paper.

More research into the physiology of baleen whales is needed not only to achieve a fundamental understanding of how baleen whales' function and adapt to their marine environment, but also to foresee how anthropogenic threats may influence them, allowing us to manage baleen whale populations in a sustainable manner. The SOST minke whale project did not achieve all of its goals in the first year, but has the potential to pave the way to a plethora of studies that could be performed in the future.

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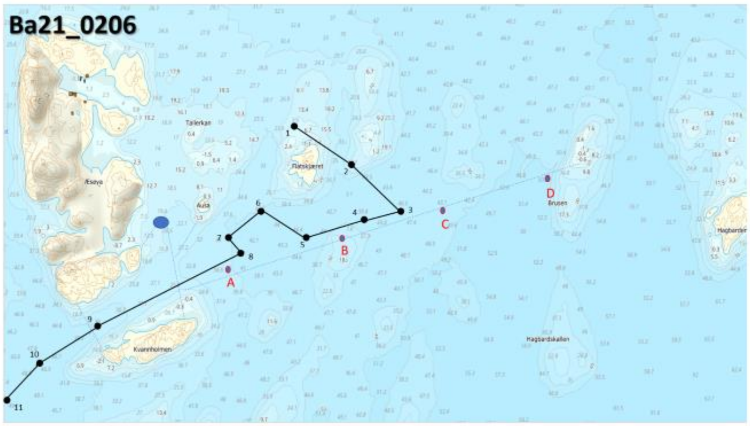
7 Appendices

7.1 Map of the minke whale migration routes in the C&R site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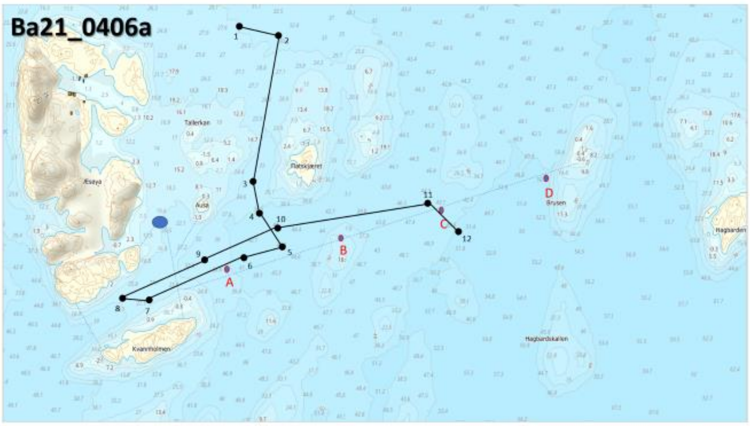
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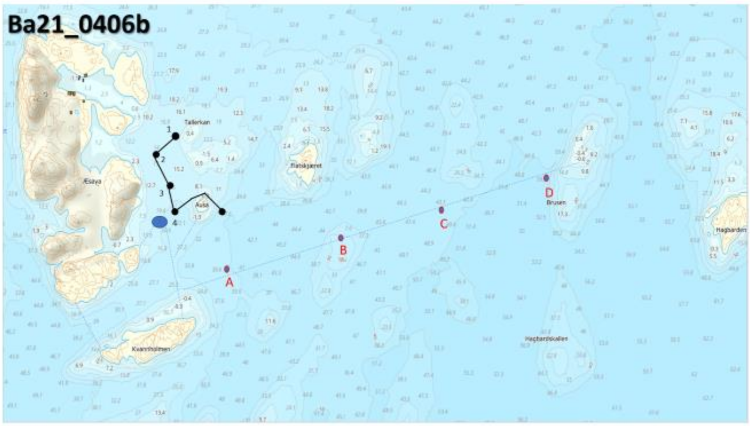
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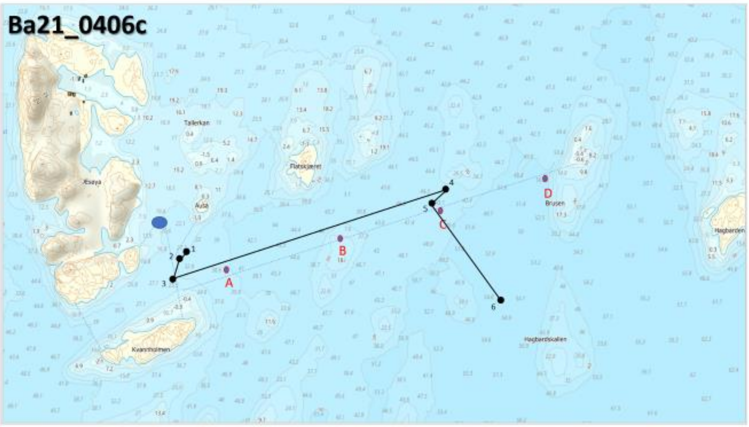
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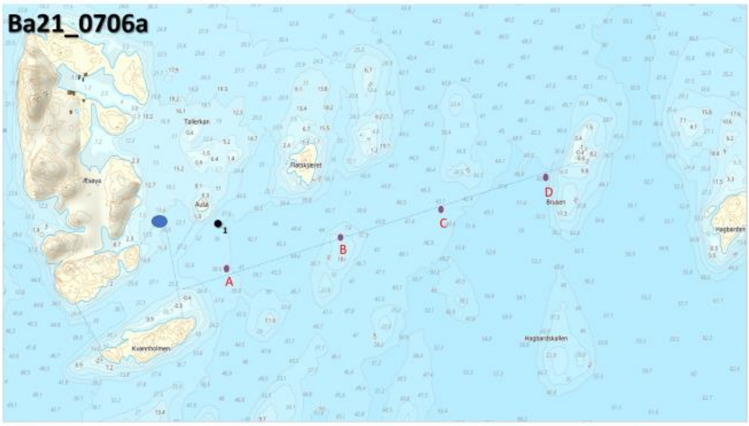
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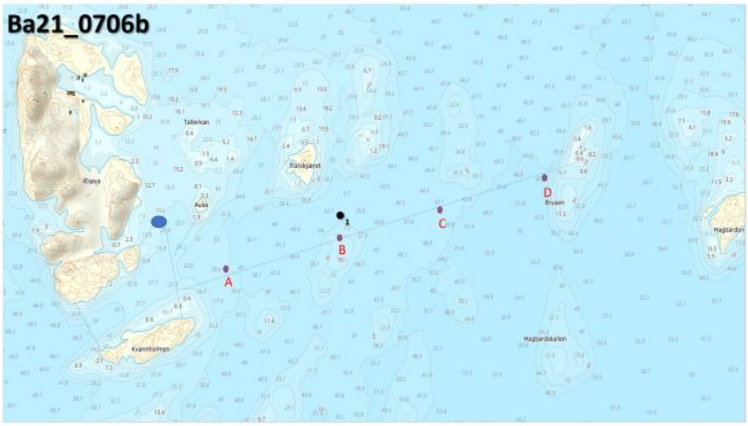
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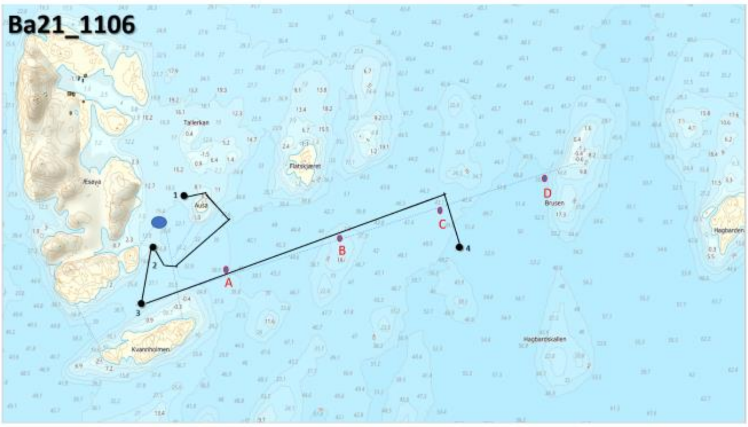
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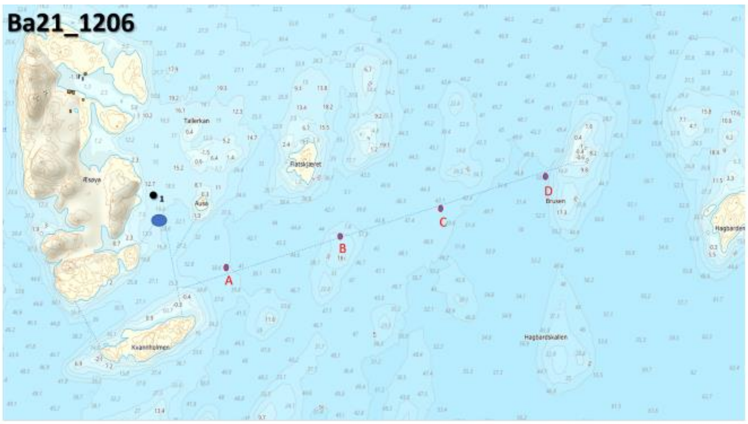
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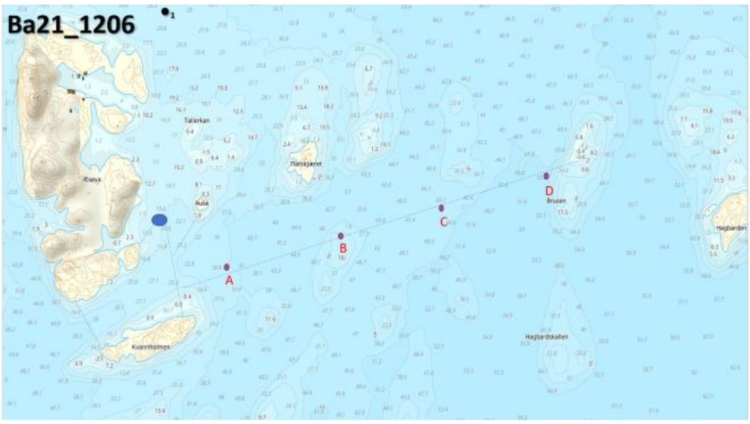
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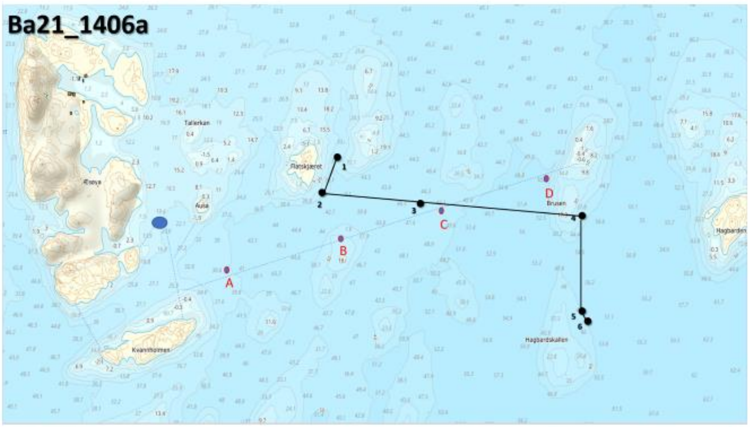
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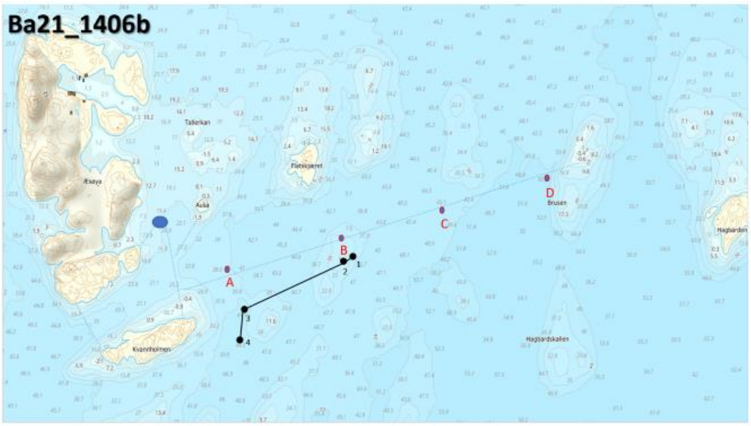
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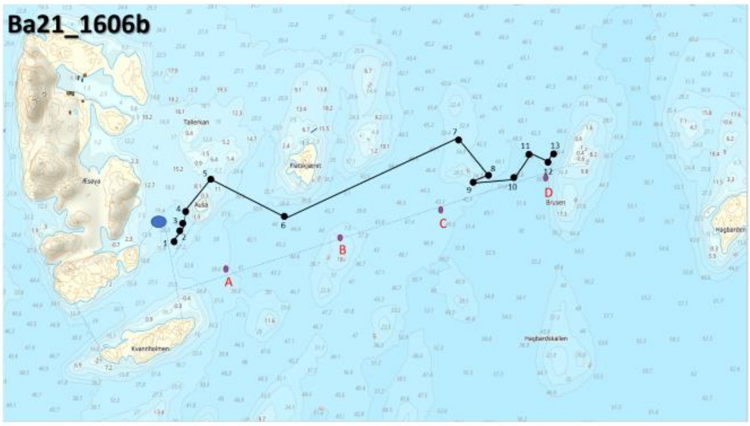
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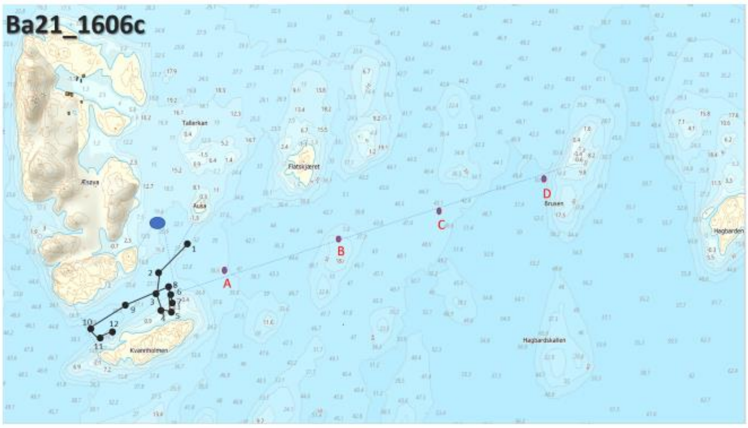
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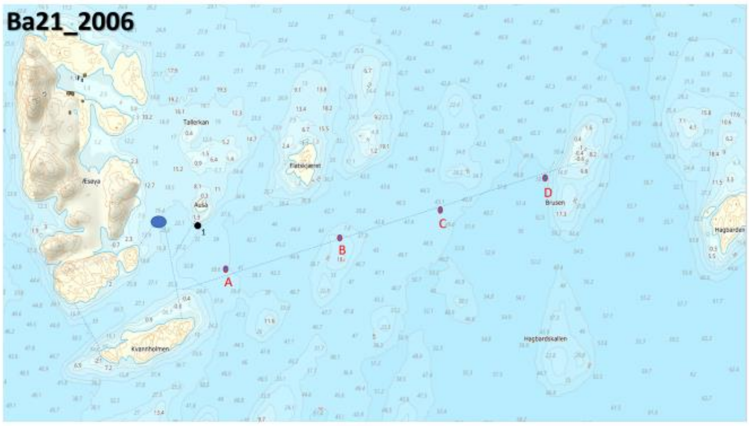
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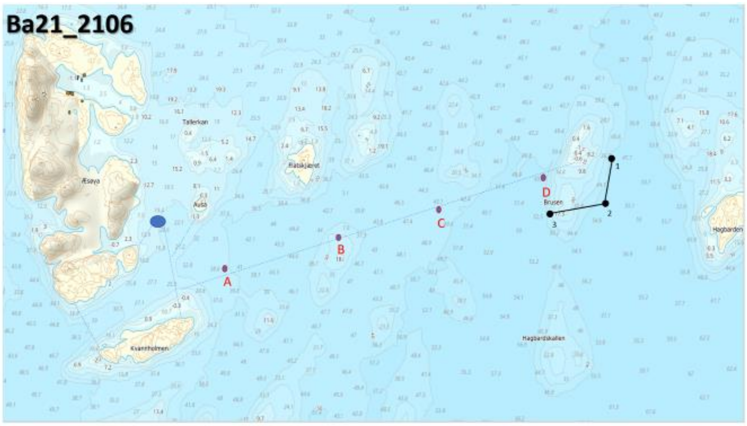
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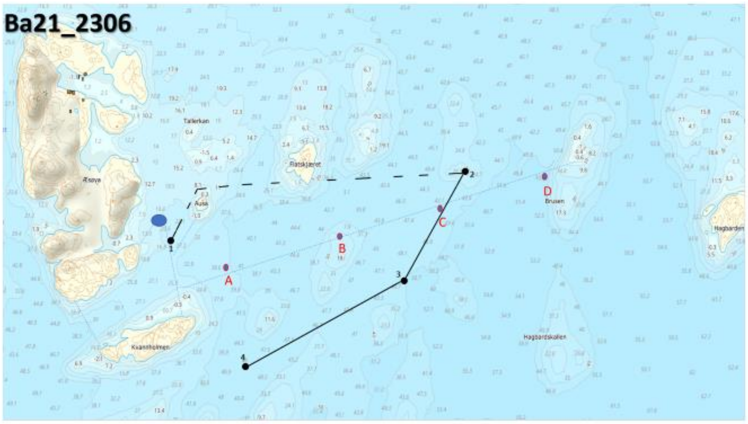
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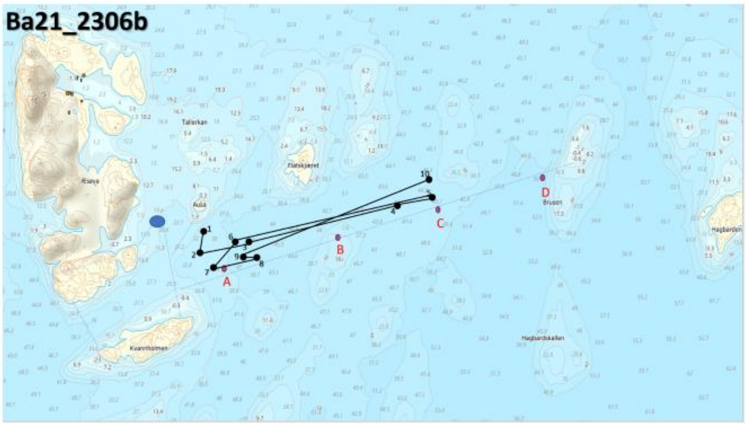
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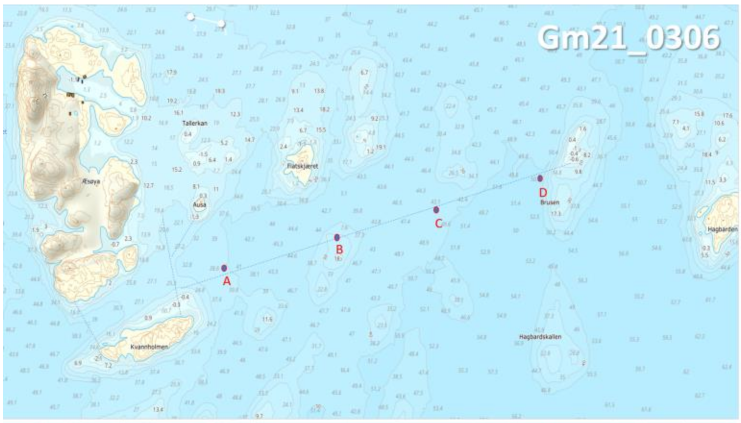
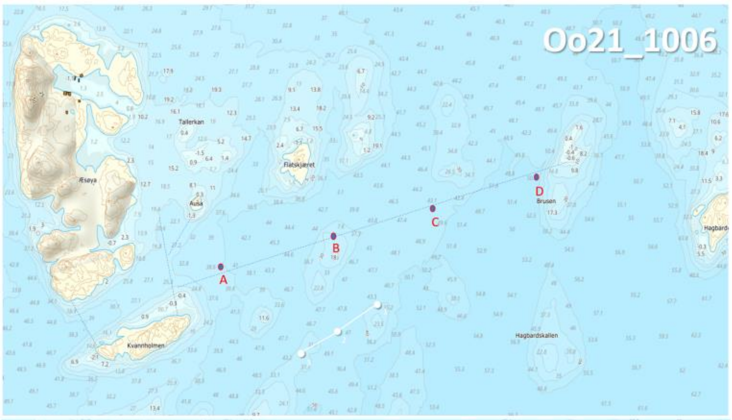
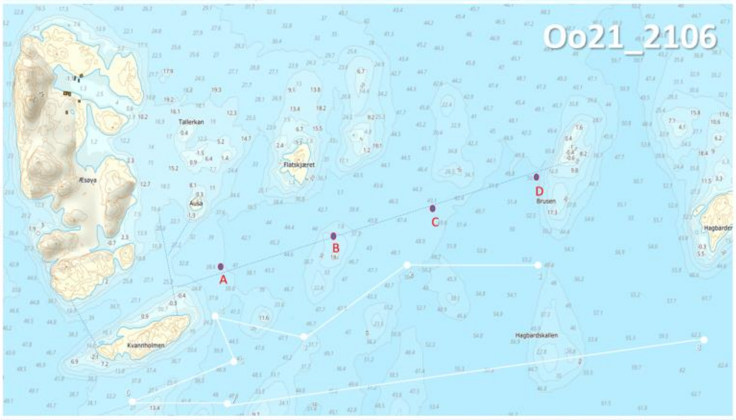
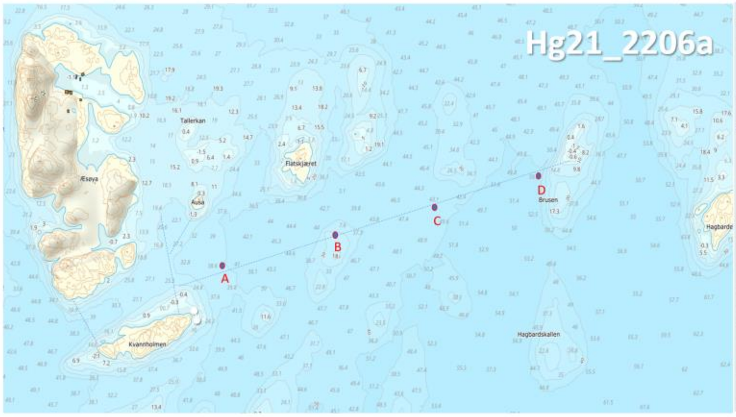
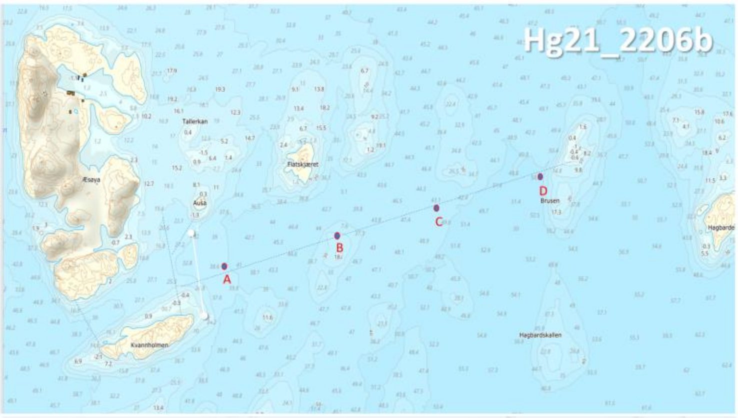
7.2 Map of other marine mammal migration routes in the C&R site









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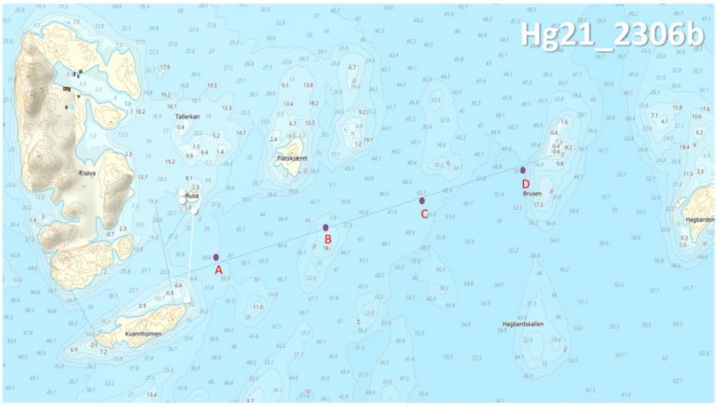
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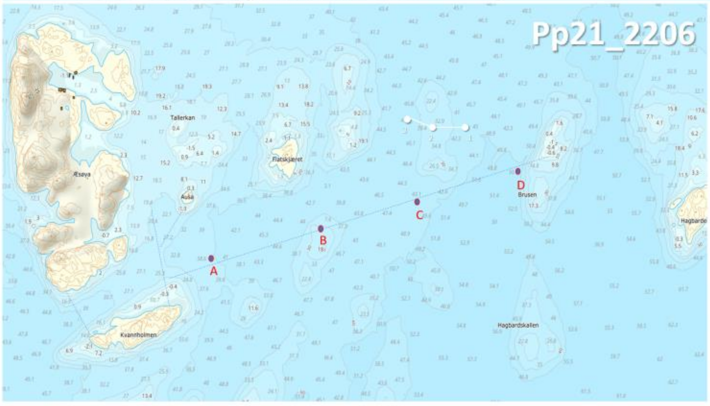
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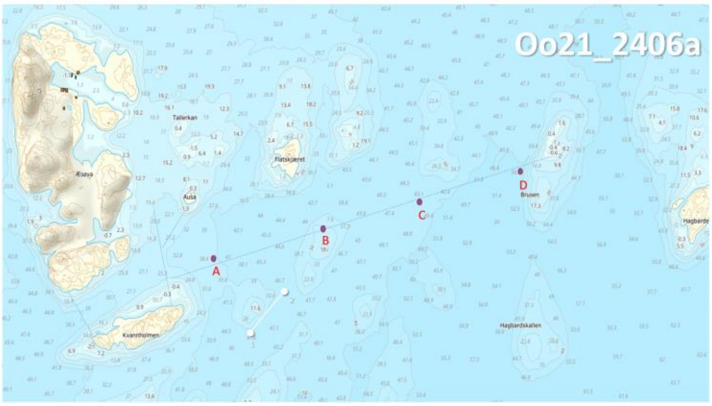
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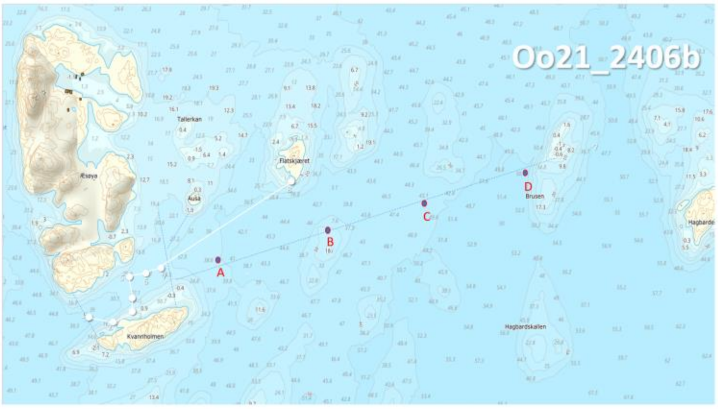
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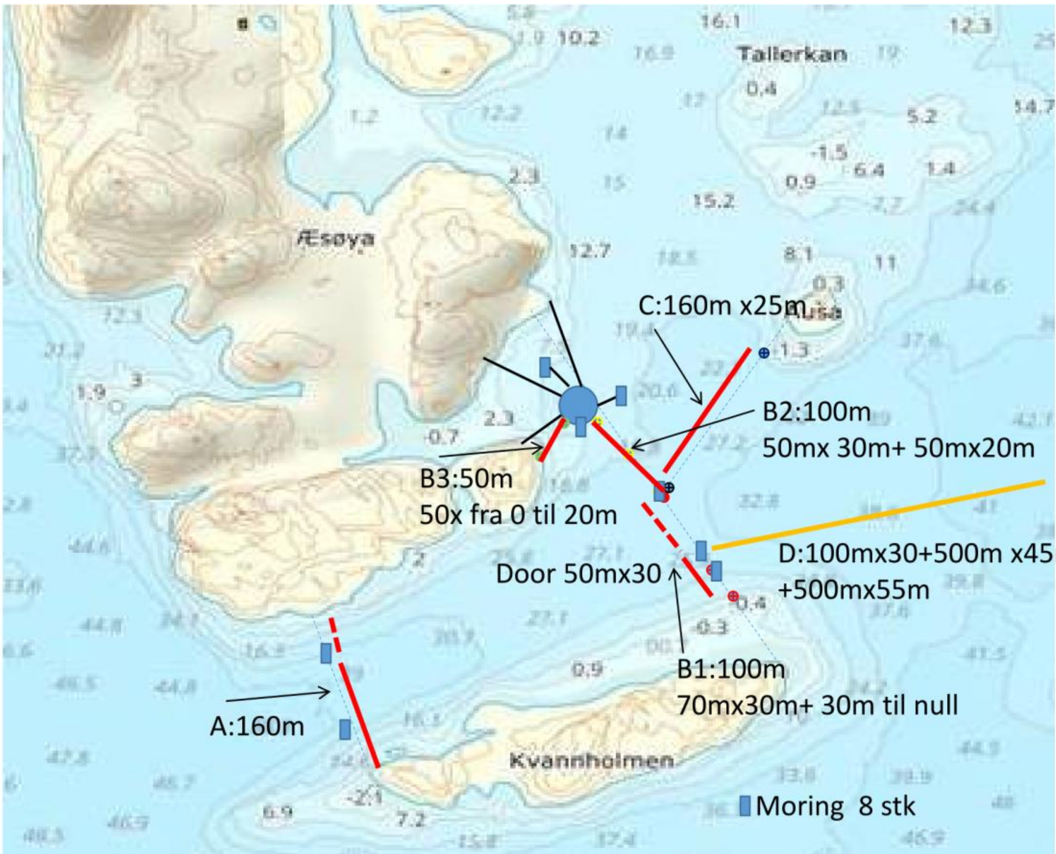

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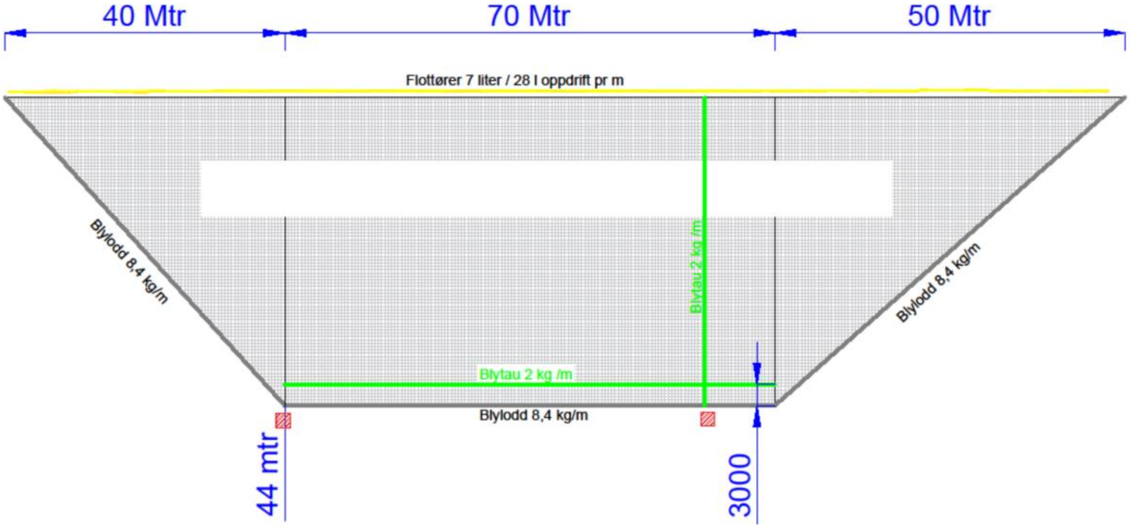

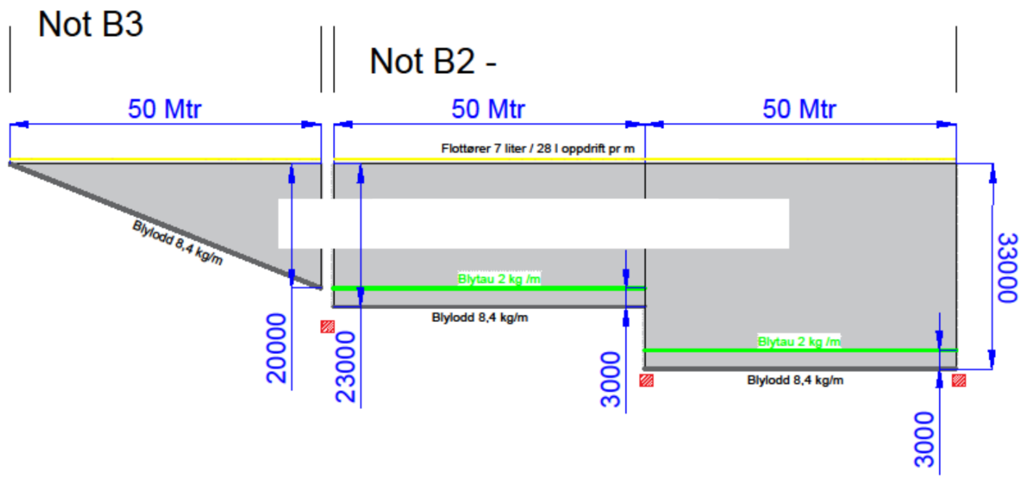
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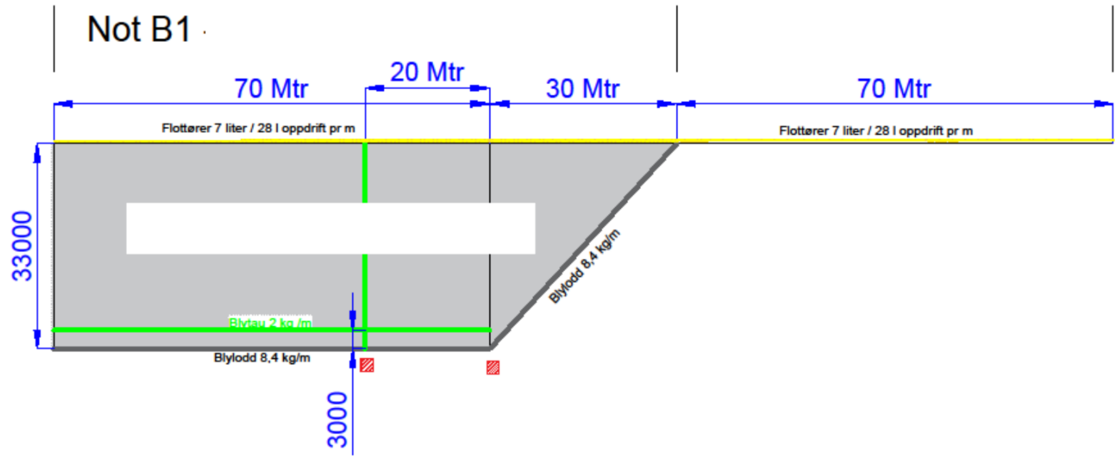
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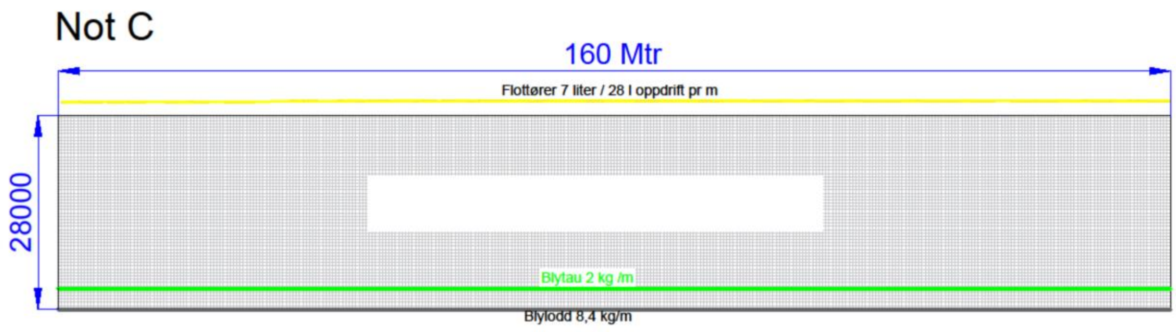
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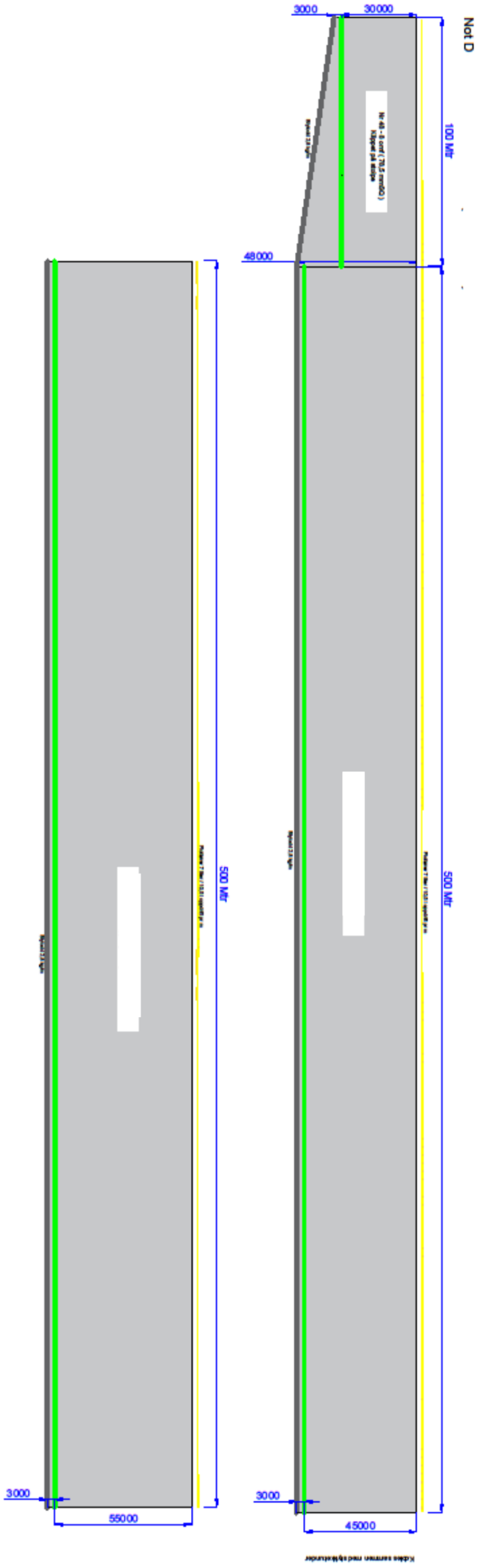
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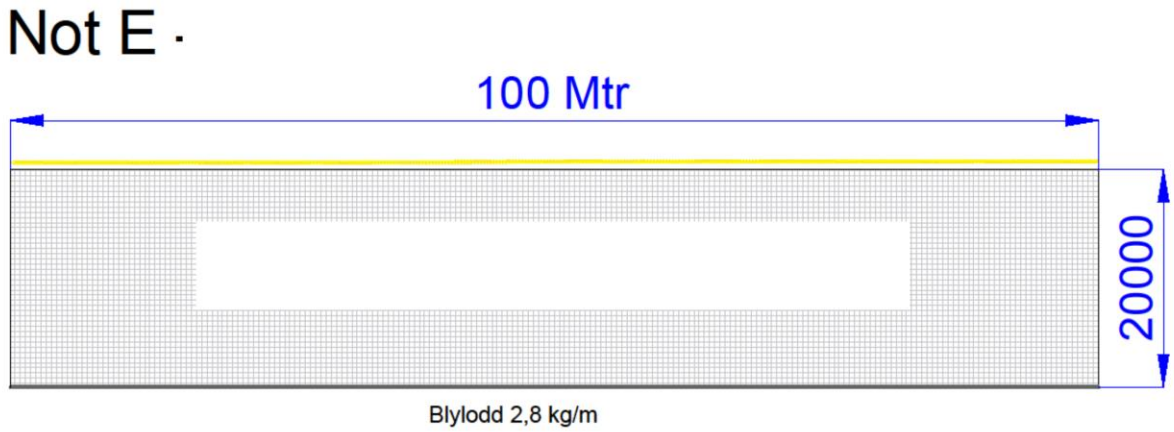

7.4 Approvals and Permits

Pictures used in this thesis are approved by FFI.

A total of 12 minke whales are allowed to be examined in the SOST minke hearing project, taken under scientific permit from the Norwegian food safety authority (FOTS ID 19536) with basis in the regulation concerning the use of animals for scientific purposes § 37, cf. §§ 6 and 12. The general requirements for the animal experimentation in this project are fulfilled, cf. the Regulation §10, §11, §9, and §1.

Permits from the Norwegian Animal Research Authority, the Norwegian Coastal Authorities, and a protocol approved by an Institutional Animal Care and Use Committee (IACUC) (with subsequent approval from the US Navy Bureau of Medicine (BUMED) were/are required for the live-capture and testing of baleen whales in Norwegian waters. Another permit from the Norwegian Coastal Authorities was given for partially obstructing the waterways linked to the C&R site.

7.5 Suggestion of potential extra guiding net

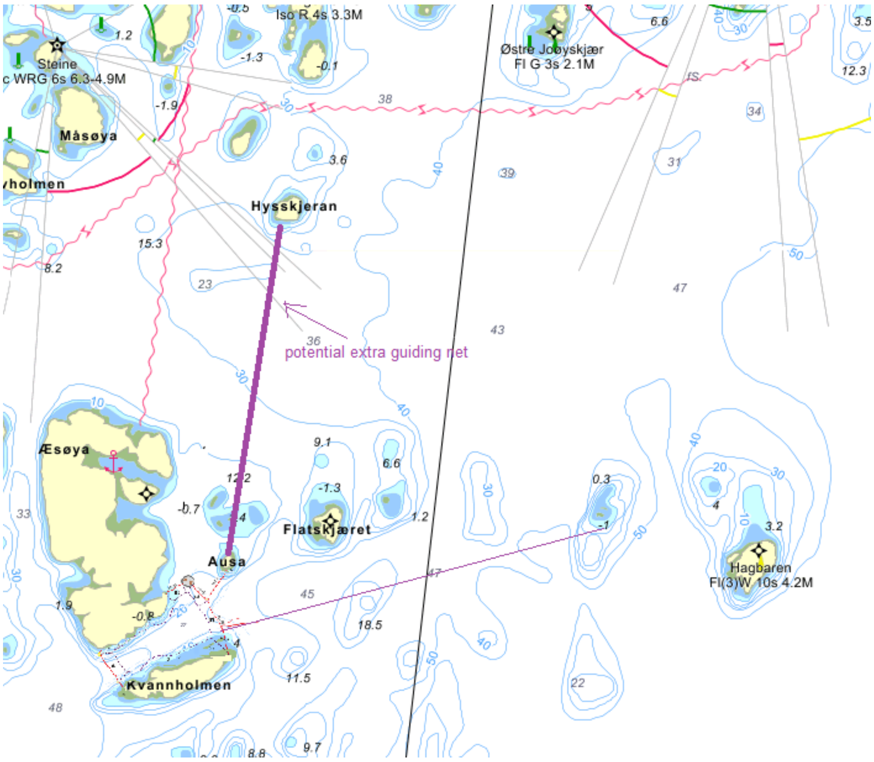

Figure 7.5: Map of the study area of the SOST minke hearing project with a potential extra guiding net. The purple line from islet Ausa northwards to another islet, Hysskjeran.

7.6 Weather conditions during SOST minke hearing project (2021)

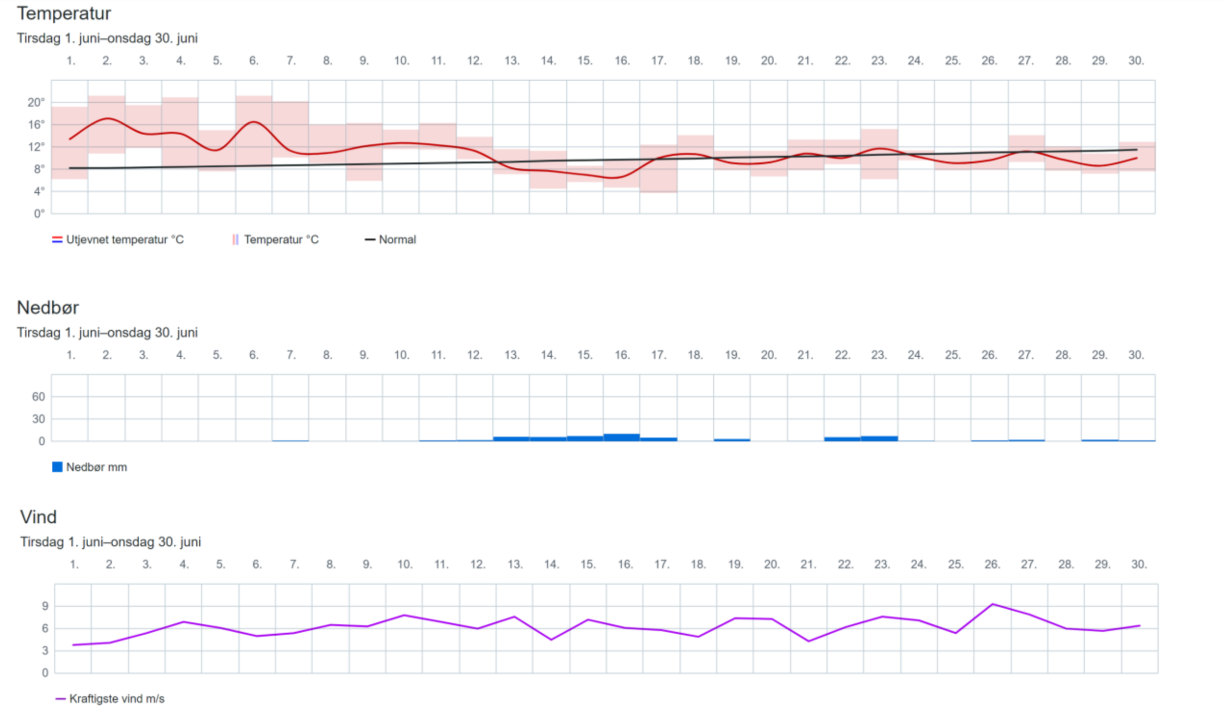

Figure 7.6: Weather conditions during SOST minke hearing project 2021 (1st June- 30th June), showing temperature (top), rain (middle), and wind (bottom) (graph from Yr. Historiske værdata for Æsøya som graf- Juni 2021. Retrieved from https://www.yr.no/nb/historikk/graf/1-276043/Norge/Nordland/Vestv%C3%A5g%C3%B8y/%C3%86s%C3%B8ya?q=2021-06).