Acoustic Monitoring of Marine Seismic Survey Impacts on Fish and Zooplankton in the Northeast Newfoundland Slope Marine Refuge

By

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General Summary

I investigated the impacts of seismic surveying on fish and zooplankton in a coastal and an offshore environment. Sound levels produced by a seismic source (coastal: single vessel deployed airgun; offshore: seismic vessel) were recorded and changes in fish and zooplankton abundance, distribution, and behaviour were monitored using hydroacoustic technology up to a range of 1km in the coastal experiment and 150 km in the offshore environment. For the coastal experiment, both a mooring, deployed to a bottom depth of ~80m, and vessel mounted echosounders used active acoustic technology to collect data during the experiment. The offshore experiment used a mooring equipped with both passive and active acoustic technology deployed at a depth of ~350 m to collect data. This study provided evidence that fish at depths between 50 m and 350 m reacted to offshore seismic surveying within a 62 km horizontal radius. They descended and aggregated deeper in the water column. However, I did not observe any effect on the abundance nor behaviour of zooplankton. There were no significant measurable effects on fish or zooplankton from the single airgun coastal experiment. Mortality rates of zooplankton were also assessed using net sampling and dyeing methods in both coastal and offshore experiments, but I did not detect significant changes in zooplankton mortality. These studies were limited by minor technological issues offshore with acoustic equipment which limited the monitoring of the water column to depth between 50 and 350 meters. The number of zooplankton samples was also limited by inclement weather at the time of sampling and safety restrictions which limited approaching within 5 km of the seismic vessel. Despite these limitations, my findings suggest that the effects of seismic surveying in an offshore subarctic environment are impacting vertical fish distribution but not causing detrimental effects on zooplankton abundance and mortality.

Acknowledgements

I would like to extend a thanks first off to both my advisors, Dr. Maxime Geoffroy and Dr. Corey Morris, as well as my other committee member, Dr. Frederic Cyr. I would not have been able to complete this work without your time, patience and guidance through all of it. Starting a Master's program during a pandemic was not the easiest feat. A lot of online adjustments to begin and a lot of delays when it came to fieldwork could prove difficult at times but it was all worth it and led to this finished product.

I would also like to thank those at the Department of Fisheries and Oceans Canada (DFO) who have been involved in this project and have aided me through field and lab work. A special thanks to Dustin Schornagel, who has been involved in the fieldwork for this project since the beginning. Whether it was small inshore preliminary testing or large offshore sampling, he is to thank for getting all the fieldwork and sampling done for my project. Another thanks to Jacqueline Hanlon for showing me around the DFO lab and helping me analyze the zooplankton samples. I would like to thank all others behind the scenes including the crews on the *Precious Jewel* and the *Atlantic Falcon* fishing vessels that were a part of this project. Even though I did not get to meet everyone, I know this project involved so many different aspects and I am thankful to each and every one involved. Funding was provided through a grant from the Environmental Study Research Fund (ESRF) to Corey Morris at DFO to study the impacts of seismic surveying on marine ecosystems in Newfoundland and Labrador.

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List of Abbreviations

Abbreviation	*Definition*
AI	Aggregation Index
ALTO	Acoustic Long-Term Lander
AMAR	Autonomous Multichannel Acoustic Recorder
AZMP	Atlantic Zone Monitoring Program
CDT	Conductivity, Temperature, Depth
CIL	Cold Intermediate Layer
COM	Centre of Mass
COSEWIC	The Committee on the Status of Endangered Wildlife in Canada
DFO	Department of Fisheries and Oceans
ESRF	Environmental Studies Research Fund
Lpk	Peak Sound Pressure Level
Lrms	Mean-Square Sound Pressure Level
NASC	Nautical Area Scattering Coefficient
PO	Proportion Occupied
SOFAR	Sound Fixing and Ranging Channel
Sv	Backscattering Strength
TS	Target Strength
WBAT	Wideband Autonomous Transceiver

Co-Authorship Statement

The work presented in this thesis is representative of a five-year field study investigating the impacts of seismic surveying on fish and zooplankton in an offshore environment. Chapter 2 was prepared as a stand-alone document with the intent to submit for peer-reviewed publication in a scientific journal following further modifications.

This thesis includes data from both inshore and offshore studies. Inshore studies were designed by Dr. Corey Morris, Dr. Maxime Geoffroy, and myself. The inshore data were collected by myself along with DFO technicians and the crew on the *Shore Dodger.* Offshore data including acoustics and zooplankton sampling were collected by myself and DFO technicians on one survey, and 3 subsequent surveys by DFO technicians and the crews on the *Atlantic Falcon* and the *Precious Jewel*. I completed the zooplankton mortality analyses for both inshore and offshore samples at DFO and MI in St. John's, Newfoundland. The taxonomic sampling analysis was contracted through JASCO Applied Sciences though the Atlantic Zone Monitoring Program (AZMP). I also completed all acoustic data from portable and moored sounders as well as the statistical analysis under the guidance of Dr. Maxime Geoffroy and Dr. Khan Nguyen. I assembled and prepared the manuscript under the guidance and supervision of my committee members, Dr. Maxime Geoffroy, Dr. Corey Morris and Dr. Frederic Cyr. Funding for this project was procured by Dr. Corey Morris through the Environmental Studies Research Fund (ESRF).

Chapter 1: General Introduction

1.1 Northeast Newfoundland Slope Marine Refuge

The Northeast Newfoundland slope marine refuge, established in 2005, is centred at 10.14˚N and 49.12˚W, and consists of 55 353km^2 of federal protected ocean (DFO, 2019). The refuge extends from the continental shelf to the deep-sea and bottom depth varies between 200 – 2500m and serves as a critical habitat for a wide variety of species, including fishes, corals, sponges, and other invertebrates (LGL Limited, 2016). In particular, the refuge provides important spawning and feeding grounds for commercially valuable fish species and also supports deep-sea coral communities that reach hundreds of years old (DFO, 2019).

The primary conservation objective on the Northeast Newfoundland Slope marine refuge is to sustain biodiversity by protecting corals and sponges from ocean trawling (DFO, 2019). Corals and sponges provide habitats for many organisms, including fish (Goldberg, 2013) and are some of the organisms most impacted by ocean trawling, a popular commercial fishing method that is used to catch benthic species (Jones, 1991; Rooper et al. 2011). Ocean trawling involves towing large nets along (benthic) or close (demersal) to the ocean floor to target benthic species, subsequently disturbing the corals and sponges (Jones, 1991; Sainsbury, 1986). By protecting the Northeast Newfoundland Slope Marine Refuge, the Department of Fisheries and Oceans (DFO) can ensure there are no commercial fishing vessels disturbing the bottom of the ocean and also considers mitigation measures for any other human activity permitted inside the refuge (DFO, 2019). Seismic surveying for oil and gas is exempt from these conservation measures (Kuehnemund, 2020). However, seismic activities that may impact conservation goals are subject to mitigation measures and added restrictions. Research conducted as part of this work helps to better inform management decisions to ensure effective conservation benefits of marine refuges in areas with oil and gas exploration.

The Northeast Newfoundland Slope marine refuge covers a small portion of the Orphan Basin, an area that has been the main focus of oil and gas companies for several decades. Oil and gas production has been happening in this area for the past 20 years, and exploration dates back even further (BP Canada, 2018). Currently, there are four oil platforms south of the Orphan Basin in the Jeanne D'Arc Basin: Hibernia (discovery: 1979, production: 1997), Hebron (discovery: 1980, production: 2017), Terra Nova (discovery: 1984, production: 2002) and White Rose (discovery: 1984, production: 2005) (Bangay, 2020). The most recent oil field discovery being the Bay du Nord oil field (discovery: 2013) located in the Flemish Pass Basin, southeast of the Orphan Basin. This field is estimated to start oil production between 2025 and 2028 (Bangay, 2020). While all these platforms are located more than 100km from the marine refuge, expectations of potential oil and gas resources along the Northeast Newfoundland slope have resulted in extensive 3D seismic surveying and highlighted the importance of understanding the potential impacts on marine life inside these refuges (Bangay, 2020, BP Canada, 2018).

1.2 Offshore Zooplankton in the Northwest Atlantic

Zooplankton, small drifting organisms, are the primary food source of planktivorous fish and are responsible for the transfer of energy from primary producers to higher trophic levels (Ismail and Adnan, 2016; Pepin et al., 2011; Lomartire et al., 2021; Richardson, 2008). The abundance and health of zooplankton populations therefore directly influence the productivity and diversity of higher trophic levels. Found in all oceans, zooplankton species range in size from micro to macroscopic, these communities supporting complex food webs making them critical for the function of marine ecosystems and their overall health, and allowing these ecosystems to remain a viable habitat for a variety of organisms (Botterell, 2023, Richardson, 2008). Zooplankton are also a good indicator of ecosystem health as they respond rapidly to any changes in marine environments (Gannon and Stemberger, 1978). The overall health of zooplankton communities can best be assessed by monitoring changes in community composition and biomass over time (Chiba et al., 2018).

Zooplankton are drifting organisms, their small size making them unable to effectively swim against strong ocean currents (Alcaraz and Calbert, 2003). Zooplankton can, however, conduct diurnal vertical movement in the water column, a biological phenomenon known as diel vertical migration (DVM) (Brierley, 2014; Forward, 1988; Hays, 2003; Pinti et al. 2019). These vertical migrations by zooplankton are the largest movement of biomass on earth and generally occurs at dusk, when the zooplankton are migrating to the surface to feed on phytoplankton, and at dawn when they descend to avoid their visual predators (Alcaraz and Calbert, 2003; Brodeur and Pakhomov, 2019; Forward, 1988). Reverse diel vertical migrations have also been noted in some copepods, such as *Pseudocalanus* spp. in Dabob Bay, Washington (Ohman and Frost, 1983).

Zooplankton communities are very diverse and species composition varies depending on geographical location. Species composition also depends heavily on environmental parameters, such as temperature, and can vary over time (Head and Sameoto, 2007). Despite changes in zooplankton community composition, dominant species composition in an area tends to remain consistent over time. In the North Atlantic, calanoid copepods dominate the zooplankton assemblage (Yeh et al., 2020). The most common species in Newfoundland waters are *Calanus finmarchicus, Calanus hyperboreus, Calanus glacialis., Pseudocalanus* spp., and *Metridia longa.* Euphausiids (krill) of the genus *Thysanoessa* and *Meganyctiphanes* are also common, although less abundant than copepods (DFO, 2023a; Head and Sameoto, 2007).

1.3 Groundfish of the Northeast Newfoundland Slope Marine Refuge

The Northeast Newfoundland Slope marine refuge is home to multiple groundfish species that are important culturally and economically to the area. Groundfish consist of fish species that, once mature, dwell near the ocean floor, the most common species in this area being Atlantic cod (*Gadus morhua)*, Acadian and deep sea redfish (*Sebastes mentella* and *Sebastes fasciatus,* respectively*)*, American plaice (*Hippoglossoides platessoides*), and Greenland halibut (*Reinhardtius hippoglossoides*) (DFO, 2019). The groundfish in this area have historically been commercially significant, making the refuge a focal point for conservation and sustainability. Commercial harvesters have been concerned with seismic surveys affecting their catch rates for years, claiming fish are scared away from the area with the elevated noise levels (LGL Limited, 2010).

1.3.1 Atlantic cod (Gadus morhua*)*

Atlantic cod are one of the most widely studied fish and are a prominent piscivorous predator along the entire continental shelf of the North Atlantic Ocean (Mieszkowska, 2009). Atlantic cod have a lifespan up to 25 years, starting as eggs released by females for external fertilization (oviparous) during a short 3 month spawning season (March – May) (DFO, 2021). Once fertilized, eggs hatch into larvae and spend the early months of their life in the top 50m of the water column, moving deeper as they develop (COSEWIC, 2010). As juveniles (1 – 4 years), cod concentrate at bottom depths (demersal) and feed primarily on larger copepods, shifting to larger prey including capelin, herring, and shellfish as they mature (Wienerroither et al., 2011). Mature cod migrate vertically in the water column, but movement has large variation seasonally and is not exclusively a diel vertical migration pattern (Rose, 2009; Pinti et al. 2019). The Committee on the Status of Endangered Wildlife in Canada (COSEWIC) provides advice regarding the status of Canadian species that are at risk for extinction (COSEWIC, 2015). The levels of COSEWIC are as follows: Extinct; species no longer exists, Extirpated; species no longer exists in Canada, Endangered; species is facing imminent extirpation or extinction, Threatened; species is likely to become endangered without intervention, Special Concern, species may become threatened or endangered because of biological characteristics combined with identified threats, Data Deficient; available data on species is insufficient, and Not at Risk; species has been evaluated and faces no current risk of extinction. (COSEWIC, 2015). COSEWIC has classified Atlantic cod in Newfoundland and Labrador as an endangered species since the collapse of the Newfoundland cod fisheries in the early 1990s, the stocks have not regained their once plentiful numbers (COSEWIC, 2010).

Atlantic cod have high ecological significance in many ecosystems offshore of Newfoundland, as well as substantial economic importance in commercial fisheries and for these reasons are widely studied. In the context of this study, cod also hold particular significance as they have been considered a model species for noise research, their hearing capabilities being the second most studied among fishes (Fay and Megela Simmons, 1999; Hawkins and Popper, 2020; McQueen, 2022). There has been extensive research into the bioacoustics of Atlantic cod due to their vocalizations used in courtships and communication (Amorim, 2006), making them an ideal candidate for investigating the effects of elevated noise levels in the ocean (Hawkins and Popper, 2006). Hawkins and Popper (2020) have contributed significantly to the understanding of cod bioacoustics, summarizing their hearing abilities, the structure of the cod ear as well as the role of the swim bladder in cod hearing from previous studies as well as their own. These authors also summarized previous research on the effects of noise on cod, highlighting adverse effects on natural sound detection due to high ambient noise levels as well as horizontal shifts in populations.

Because cod rely on sound for mating purposes, they may be especially vulnerable to disturbance during the spawning season. McQueen et al., (2023), on the contrary, found weak responses from tagged spawning cod when exposed to seismic surveying over a 5-day period. The focus on cod in this study is founded on their importance in the region and history of the stocks in the area, making them an ideal subject for noise-related research.

*1.3.2 Redfish (*Sebastes *spp.); Deep sea redfish (*Sebastes mentella*) and Acadian redfish (*Sebastes fasciatus*)*

Redfish (*Sebastes spp.)* are a type of groundfish on the Northeast Newfoundland Slope, comprised of two similar subspecies; Acadian and deep sea, often visually indistinguishable due to similar external features and therefore managed as one stock (DFO, 2022; Gauthier and Rose, 2002). Female redfish are viviparous, producing eggs that develop inside the female, that are then released as live larvae in the spring (Beyer et al., 2014). Larvae spend their time in surface waters until maturing and settling into deeper waters (Beyer et al., 2014; Planque et al., 2013) 2019). Redfish are considered semi-pelagic fish, with dense schools being found in open ocean, but are also found in deep waters (Planque, 2013). Redfish are known to exhibit a natural diel vertical migration, moving higher in the water column at night and deeper during the day (Gauthier and Rose, 2002). Redfish have one of the longest lifespans among groundfishes, up to 75 years, tripling the average lifespan of the other groundfish species in the area (DFO, 2019; DFO, 2022). The current redfish COSEWIC standing of redfish in Newfoundland is threatened (COSEWIC, 2010), although the stocks have greatly increased over the past years.

Redfish, like Atlantic cod, possess swim bladders, making them easily detectable on echosounders, especially in cases of high-density aggregations (Gauthier and Rose, 2001; Simmonds and MacLennan, 2005). The swim bladder is a buoyancy control organ in these fish, allowing them to control their position in the water column (Gauthier and Rose, 2001; Sand and Hawkins, 1972; Simmonds and MacLennan, 2005). Differentiating swim bladdered fish species, such as Atlantic cod and redfish, with echosounders is difficult, if at all possible, and the acoustic signal is usually groundtruthed with trawl sampling to determine the species detected (Rose, 2009). However, echosounders provide valuable insight into their distribution and density with high temporal and spatial resolutions, which can help to assess their response to seismic activity (Engås et al., 1993, Engås et al.,1996; Simmonds and MacLennan, 2005). Furthermore, the presence of a swim bladder may make these species more vulnerable to the effects of elevated seismic noise in the area (Casper et al., 2012). Previous studies have shown that loud noise from activities like seismic surveying can lead to physiological stress or disorientation (Carroll et al., 2017).

1.3.3. Groundfish Fisheries

The Northeast Newfoundland Slope has been the location of several large scale commercial groundfish fisheries over the past centuries, including cod, halibut, and redfish (DFO, 2019). The largest fishery in this area was the Newfoundland cod fishery, before its collapse in the early 1990s that greatly impacted the province economically (Myers et al. 1993; Schrank, W.E., 2005). In 1992, a moratorium was placed on the cod fisheries in response to the collapse, which was followed by many other closures including, but not limited to, plaice in 1994 and grenadier *(Macrourus berglax)*, haddock (*Melanogrammus aeglefinus)* and redfish in 1997 in an effort to increase population numbers (DFO, 2019). Cod, plaice and haddock moratoria remain in place as of 2024. A stewardship fishery on cod has been in place since 2007, allowing harvesting a limited number of cod with strict regulations contributing to the research into cod populations and their recovery (Simms, 2017).

The sharp decline of these top benthic predators in the ecosystem resulted in lower trophic level populations, including snow crab and shrimp, to increase significantly due to the decrease in predation (Frank et al. 2005). This ecosystem shift in population abundance combined with moratoria, subsequently caused the focus of fisheries in the area to shift heavily to shellfish such as shrimp, lobster and snow crab, that have dominated landings over the past few decades (DFO, 2019; Schrank, 2005). With the introduction of the restrictions, regulations and quotas, groundfish stocks have slowly been rebuilding, but stocks are still not high enough to lift the multi-decade moratoria on these fisheries (DFO, 2021). Even with the help of these regulations and the establishment of a marine protected refuge, additional factors such as climate change and oil and gas exploration remain ongoing concerns for groundfish in the region (Drinkwater, 2005). The impacts of seismic surveying on both groundfish and shellfish fishery resources is not well known in the region.

1.4 Emission and Perception of Sound by Fish and Zooplankton

Sound is a primary sensory input that influences a majority of marine life, including fish (Hawkins and Popper, 2017). Not all fish species emit sounds for communication, however, the mechanisms are quite diverse for those that do. The diversity of mechanisms for sound production is so broad that there is no simple classification (Fine and Parmentier, 2015). Emitting and hearing sounds are both important senses for some groundfish such as Atlantic cod (*Gadus morhua*) (Fine and Parmentier, 2015), these sounds primarily used for mating or combative purposes (Amorim, 2006). Therefore, field experiments incorporating real seismic surveying on commercial fishing is critical to better understand the effects of seismic surveying that are most relevant to regionally important fish species, which include Atlantic cod and redfish in the Northeast Newfoundland slope.

Similar to most other vertebrates, the auditory organs of fish are located within the head, specifically, in the inner ear (Popper et al. 2019). These are the saccule, lagena and utricle which develop only days after hatching (Carroll et al., 2017). All these structures are receptors of acoustic signals, varying in the decibels at which they detect, primarily low frequencies, ranging from 800 – 1000Hz re 1 μPa (Offutt, 1974). Each auditory organ in the ear has its own otolith, a dense area connected to sensory epithelium via the otolithic membrane (Popper and Lu, 2000). Sensory hair cells located on the epithelium of the sacculae, lagena and utricle are the carriers of acoustic information from the ear to the brain (Popper and Lu, 2000). The otolith is the stato-acoustic organ in most fish, meaning it has both auditory and balancing roles in the organism (Lu, 2011). The underlying mechanism relying on relative motion between the otolith and the sensory epithelium that causes the bending of hair cells (Popper and Lu, 2000).

Along with the organs in the inner ear, for some fish the swim bladder has a role in hearing. Due to the gas having a lower density than that of the body, the walls of the swim bladder vibrate resulting in reradiated energy that can produce otolithic motion (Popper and Lu, 2000). Due to these vibrations, the swim bladder has been shown to increase the sensitivity of the hearing organs when connected or located close to the inner ear, the saccule, in particular for Atlantic cod (Offutt, 1973). In this way there is a potential for indirect sound detection if the sound wave is large enough to create a vibration of the swim bladder. Both cod and redfish have physoclistic (independent of the gut) swim bladders that are connected to the otolith via soft tissue (Gauthier and Rose, 2001; Simmonds and MacLennan, 2005). The exact hearing mechanisms in redfish are not as extensively studied as they have been in the widely studied cod, making them a less ideal model species (Hawkins and Popper, 2006). In addition to sound detection, the swim bladder is used for sound production in both cod and redfish. For Atlantic cod, sound is produced via contraction of the sonic muscles attached to the swim bladder wall (Amorim, 2006, Fine and Parmentier, 2015). Low frequency grunts are used by both sexes in combative behaviour, however, only males emit sounds during courtship behaviours (Amorim, 2006). The same frequency grunts are used for both purposes. Even though the same organ is used for sound emittance and buoyancy control, there have been no recordings of a negative overlap between the two functions, in that one has no impact on the other (Fine and Parmentier, 2015).

The lateral line system is another method by which fish can detect their surrounding environment and relies on the particle motion of water molecules (Bleckmann and Zelick, 2009; Simmons and MacLennan, 2005). This system is present in all fish though development and distribution of the system varies across species (Mogdans, 2019). The main sensory structures of the lateral line system are located on the side of the fish's body called neuromasts (Bleckmann and Zelick, 2009; Mogdans, 2019). Neuromasts can be free standing or contained inside fluid filled canals and are sensitive to changes in water pressure or movement caused by displacement of particles (Bleckmann and Zelick, 2009, Mogdans, 2019). Fine hair cells within the neuromasts detect these pressure changes and convert the mechanical stimulus into an electrical signal that is transmitted to the fish's brain (Bleckmann and Zelick, 2009). These signals allow the fish to detect water currents and nearby objects, including obstacles, predators, and prey. This system is more sensitive to low frequency water vibrations and is only effective in detecting disturbances within a few meters of the fish (Bleckmann and Zelick, 2009).

The relevance of sound production in fish is a critical aspect of research in underwater noise and its impact on fish behaviour. Sound generation by fish forms the foundation for studies examining potential masking effects from elevated noise levels and the relationship between noise and fish behaviour (Radford et al., 2014). Fish can hear low frequency noise, often within a limited range between 800 – 1000Hz but varies among species, and are capable of differentiating different amplitudes and frequencies (Offutt, 1973; Popper et al., 2019). Sounds produced within the hearing range of fish can interfere with fish communication creating a masking effect, where there is no signal detection or the signal is difficult to interpret (Clark et al., 2009). For example, frequencies used by scientific instruments such as echosounders, often between 12 and 500kHz, far exceed the hearing range of fish, whereas commercial activities, such as fishing vessels and seismic surveying for oil and gas can generate noise within the same frequency and can disturb the fish (Popper et al., 2019).

Compared to fishes, there is less information available describing the ecological importance of sound for zooplankton. Research has focused more on larger organisms including marine mammals and fishes, however, some research is available describing how zooplankton respond to sound. Studies have shown that zooplankton can detect environmental vibrations and produce behavioural responses to acoustic stimuli (Buksey et al. 2002; Gassie et al., 1993). A more recent study recorded sounds produced by copepods, between 500 and 1200Hz, which were likely generated during an "escape jump" movement to evade predation (Kuhn et al., 2022). Part of this noise threshold falls within the hearing range of fish, as mentioned earlier, suggesting that planktivorous species and life stages might use sounds emitted by plankton as a method of prey detection. Kuhn et al., (2022) suggest the sounds produced by copepods are possibly a by-product of escape behaviours, but could also potentially be communication signals to neighboring organisms to initiate a coordinated escape response. Despite their small size and limited sound production, zooplankton are not to be overlooked when researching noise pollution impacts due to their ecological importance.

1.5 Underwater Acoustics

Sound transmission follows the same principles in water as in the air, however, critical differences arise in sound absorption and distance travelled due to the differences in density and chemical composition of the environments (Fine and Parmentier, 2015). Sound travels approximately five times faster in water than in air, and up to hundreds of kilometers in distance (Webb, P., 2019). As a result, sound is a highly efficient communication tool in the ocean that numerous species rely on. The humpback whale, for example, is capable of communicating over hundreds of kilometers (Mercado and Frazer, 1999). Due to the amplification of sound in water and species reliance on sound, noise pollution holds the potential to cause significant disturbances to underwater ecosystems.

Sound propagation in water is dependent on water salinity, pressure, and temperature (Kleis and Sanchez, 1990; Simmons and MacLennan, 2005). These factors influence the speed and characteristics of sound waves as they travel through underwater environments, and an increase in any of these factors results in an increase in the speed of sound (Webb, 2019). For example, sound traveling in a brackish lagoon will propagate significantly less than sound in the open ocean, where there is higher salinity and pressure to increase the speed of sound. Therefore, understanding the specific properties of the body of water is critical in determining the way sound will propagate. Most studies on the impacts of seismic surveying were conducted in shallower water, over relatively small study areas, which can potentially result in different impacts than in a deep offshore environment. Working in an offshore area with much deeper water over greater distances allows for the assessment of impacts in a real environment impacted by seismic surveying, unlike most inshore studies.

In relatively deep waters (>50 m), as in the Northeast Newfoundland Slope Marine Refuge, sound generally spreads spherically, at a rate of $20\log_{10}R$, (R is the ratio of the distance to where the sound levels are predicted in m to the distance from the source, usually 1m (Wahlberg and Surlykke, 2014). The spherical spreading results in transmission losses, i.e. a decrease in strength and intensity, as the sound energy disperses over an increasing area. In combination with geometrical spreading, scattering and absorption also play a critical role in sound attenuation. Scattering refers to the sound waves encountering objects or irregularities in the water column, resulting in diffraction (bending of the sound waves around obstacles), refraction, (change in direction of sound waves as sound passes through mediums of differing densities), or reflection, (bouncing of the sound waves off objects) (Bass and Clark, 2003; Simmonds and MacLennan, 2005). As sound waves interact with water particles, their intensity decreases through the conversion of acoustic energy into heat energy, a process known as absorption (Bass and Clark, 2003; Simmonds and MacLennan, 2005). Absorption is highly affected by the frequency of the sound waves and temperature of the water; the higher the frequency, the more the molecules collide resulting in more absorption, the opposite is true for temperature, where higher temperatures result in less absorption due to the sound waves traveling at a higher speed.

In a deep offshore environment, significant change in sound speed occurs at the interface of a thermocline layer; where there is an abrupt transition zone between warm surface water and deep, cool water where wind waves and sunlight are not influencing factors (Erbe et al., 2022; Simmonds and MacLennan, 2005). A feature of the Newfoundland shelves is the presence of a cold intermediate layer (CIL) in summer, a water layer formed during winter and resting in between warmer, fresher water above and warmer, saltier water below (Petrie, et al., 1987). The CIL exists between early spring to late fall and is caused by the cold winter mixed waters being insulated from the surface by warming spring surface waters, occurring at depths from 50 – 150 m (Petrie et al., 1987). The speed of sound decreases significantly with decreasing temperature, the minimum speed occurring at the end of the thermocline layer, then the increased pressure causes sound speed to increase again (Webb, 2019). This is one of the guiding principles explaining the SOFAR (Sound fixing and ranging channel) which is how sound can be carried over large distances in the ocean via the 'deep sound channel' (Figure 1) (Nieukirk, S. 2023). Sound waves traveling through the thermocline, primarily influenced by temperature, are bending downward with decreasing speed. Once the sound waves reach the end of the thermocline they are influenced primarily by pressure, resulting in the sound waves being refracted upwards as the sound speed increases. As sound waves propagate through the ocean, they become trapped and bounce at moderate depths (~300 – 1000 m) in this horizontal channel due to the surrounding water layers of higher speed (Webb, 2019). The SOFAR channel allows low frequency sound to propagate over hundreds of kilometers with little attenuation (Nieukirk, S. 2023; Simmonds and MacLennan, 2005; Webb, 2019). Due to propagation and attenuation properties of sound waves, calculating proper absorption coefficients is important when analyzing acoustic data to account for sound attenuation. These physical properties apply to both passive and active acoustic monitoring.

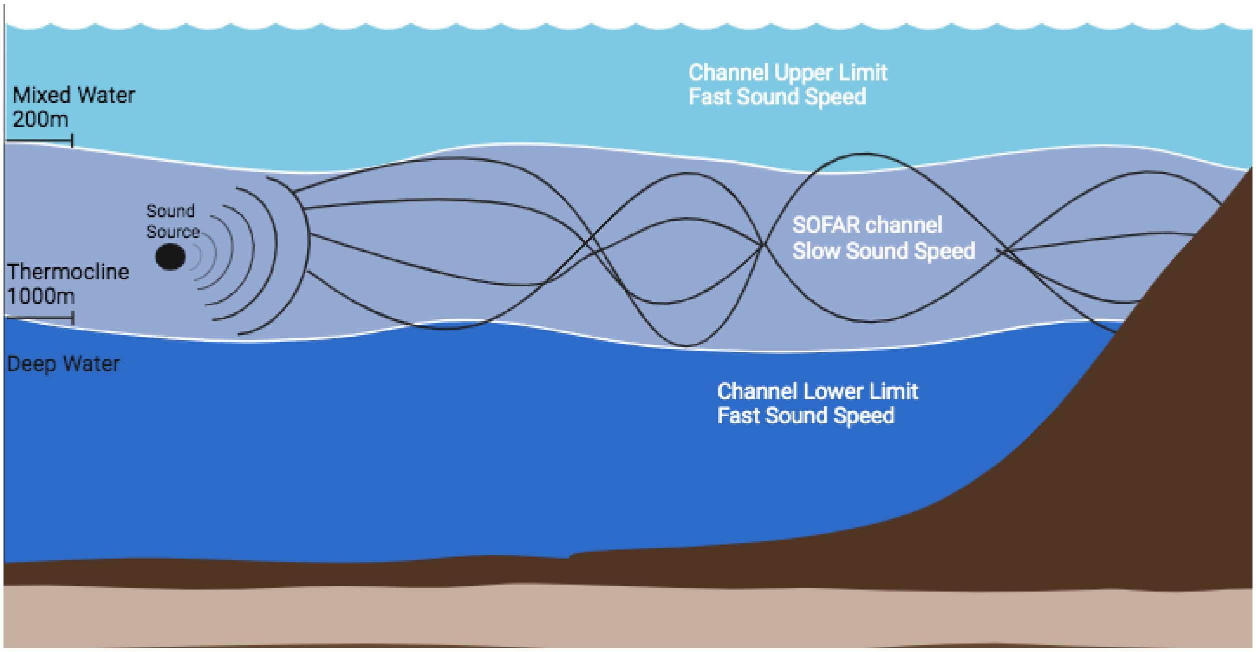

Figure 1. Visualization of the Sound Fixing and Ranging (SOFAR) channel in the ocean. This figure demonstrates how sound can propagate long distances with minimal energy loss due to changes in water pressure and temperature that creates a channel, 'trapping' sound waves in this intermediate layer between warm surface waters and cool deep waters. Created using BioRender (NOAA, 2023)

1.6 Hydroacoustic Ecosystem Monitoring

Hydroacoustic ecosystem monitoring is a widely used technology that involves the use of sound in water to monitor ecosystems over varying periods of time (Pensieri and Bozzano, 2017). Hydroacoustics allow for the study of abundance, behaviour, distribution, biomass, and sound emission of marine organisms in a non-invasive way (Godlewska et al., 2004, Melo et al., 2021). Passive and active are the two types of hydroacoustic monitoring methods (Simmonds and MacLennan, 2005).

1.6.1. Passive Acoustics

Passive hydroacoustic monitoring relies solely on listening and recording sounds produced in an aquatic environment (Simmonds and MacLennan, 2005). Applications of this technology include monitoring of marine mammals that produce vocalizations to communicate, navigate and forage their environment and can also be used to monitor fish populations that produce sound primarily during mating and feeding (Simmonds and MacLennan, 2005). Human activities in the ocean that produce noise including shipping, construction, and seismic exploration, contribute significantly to elevated noise levels. Ambient noise can be monitored using hydrophones (Hildebrand, 2004; Peng et al., 2015). Passive acoustic technology has also facilitated the development and application of active acoustic monitoring (Pensieri, 2017).

1.6.2. Active Acoustics

Active hydro-acoustics is a non-invasive method that can collect real time information and be used to determine overall abundance of fish or zooplankton in the water column, document their vertical distribution and also monitor behavioural changes that may occur (Viehman, 2022). It involves emitting sound waves into the water column and recording the sound waves that bounce off targets in the water column using echosounders or sonars (Simmonds and MacLennan, 2005). Piezoelectric transducers transform bursts of electrical energy into acoustic energy (i.e., a pressure wave), resulting in a 'ping' emitted into the water. The pressure wave propagates through the water until some of the sound energy is reflected back to the transducer when it encounters a sufficiently large "target", resulting in an echo recording that is then converted back to electrical energy and displayed on the echogram (Simmonds and MacLennan, 2005). The difference in time and voltage between the emitted and received pulses allows the sounder to calculate the distance and backscatter of the targets. Echosounders use active acoustics to capture images of the water column in the form of an echogram; a visual output of the acoustic backscatter that the echosounder receives. Active acoustics can be operated at different frequencies based on the intended target detection. Operating at higher frequencies allows for smaller targets to be detected but does not have a long range, whereas operating at a lower frequency allows for the detection of larger targets at longer ranges (Madureira et al., 1993; Simmonds and Maclennan, 2005). When monitoring fish, frequencies between 12 and 200kHz are often used whereas for smaller organisms, such as mesozooplankton, a frequency of >200kHz is standard. All frequencies used in acoustic monitoring are significantly higher than those used by fish, which ensures this monitoring does not disturb or impact the fish (Seri et al., 2023; Simmonds and Maclennan, 2005). Some active acoustic instruments can be moored at a fixed location on the seabed and record the signal of fish and zooplankton at that site over several months (Parra et al., 2019).

Table 1. Comparison of vessel-mounted and bottom-moored hydroacoustic echosounders. The differences between these types of sounders and the pros and cons of each monitoring method are compared to demonstrate the potential applications of different instruments.

	VESSEL-MOUNTED	**BOTTOM MOORED**
Noise	High; electrical vessel interference, wave-hull collisions, weather interference	Limited; background noise and some weather in severe cases
Length	Short-term (days – weeks)	Short- or long-term (months)
Time	Interim	Continuous
Location	Top of water column	Generally at the bottom of the water column
Coverage	High; vessel movement	Low; one area where is it moored
Risk	Low-High; real-time data imaging allows for quick error checking but some errors cannot be fixed	Low-High; less change of errors but no real-time data imaging does not allow for equipment checks after deployment
Disturbance To Environment	Med-High; dependent on vessel, could have some influence on environment. Most disturbance comes from the vessel and not the echosounder	Low; little to no disturbance to environment after deployment, very minimally invasive

1.7 Seismic Surveys

Anthropogenic noise in marine ecosystems has been increasing each decade over the past century with global shipping, marine defense activity and hydrocarbon exploration and production (Hildebrand, 2004; Gisiner, 2016). Seismic surveying is a method of hydrocarbon exploration used extensively by energy companies to locate pockets of fossil fuels under the sea floor, mapping these areas for hydrocarbon exploitation (Figure 2) (Hildebrand, 2004).

Seismic surveys involve the use of ocean liners equipped with airguns that emit high intensity (approximately 200dB re 1uPa on average), low frequency (10 – 300Hz) pressurized air, normally around 2000 psi (Carroll et al., 2017; Hildebrand, 2004; IAGC, 2002). The rapid release of pressurized air creates a bubble underwater and the expansion and contraction of the bubble results in a sound pressure wave (Hildebrand, 2004). The sound pressure waves travel through the water column and into the seafloor, reflecting off geological strata of differing density and are then recorded at the surface by streamers equipped with several hydrophones. The exact depth of the hydrophones varies with the weather and increases with bad conditions to minimize surface noise as much as possible (IAGC, 2002). One seismic vessel can cover up to a couple hundred kilometers in just 24 hours while hauling anywhere between 12 to 48 guns that fire every 6 to 20 seconds, shooting over 14 000 pings of seismic surveying for each 24-hour period (Hildebrand, 2004; Hirst and Rodhouse, 2000). Seismic surveys result in imagery that displays the geology of the sea floor, showing patches of oil and gas that can then be drilled and exploited (Paxton et al., 2017). Deeper seabed imagery can be collected when low frequency (>1000Hz) waves are emitted because sound attenuation is significantly higher at high frequencies (Bass and Clark, 2003; Carroll et al. 2017). A typical survey can collect depth imagery up to 7 km beneath the seafloor and operates between 10 – 300 Hz (Hildebrand, 2004; Gisiner, 2016).

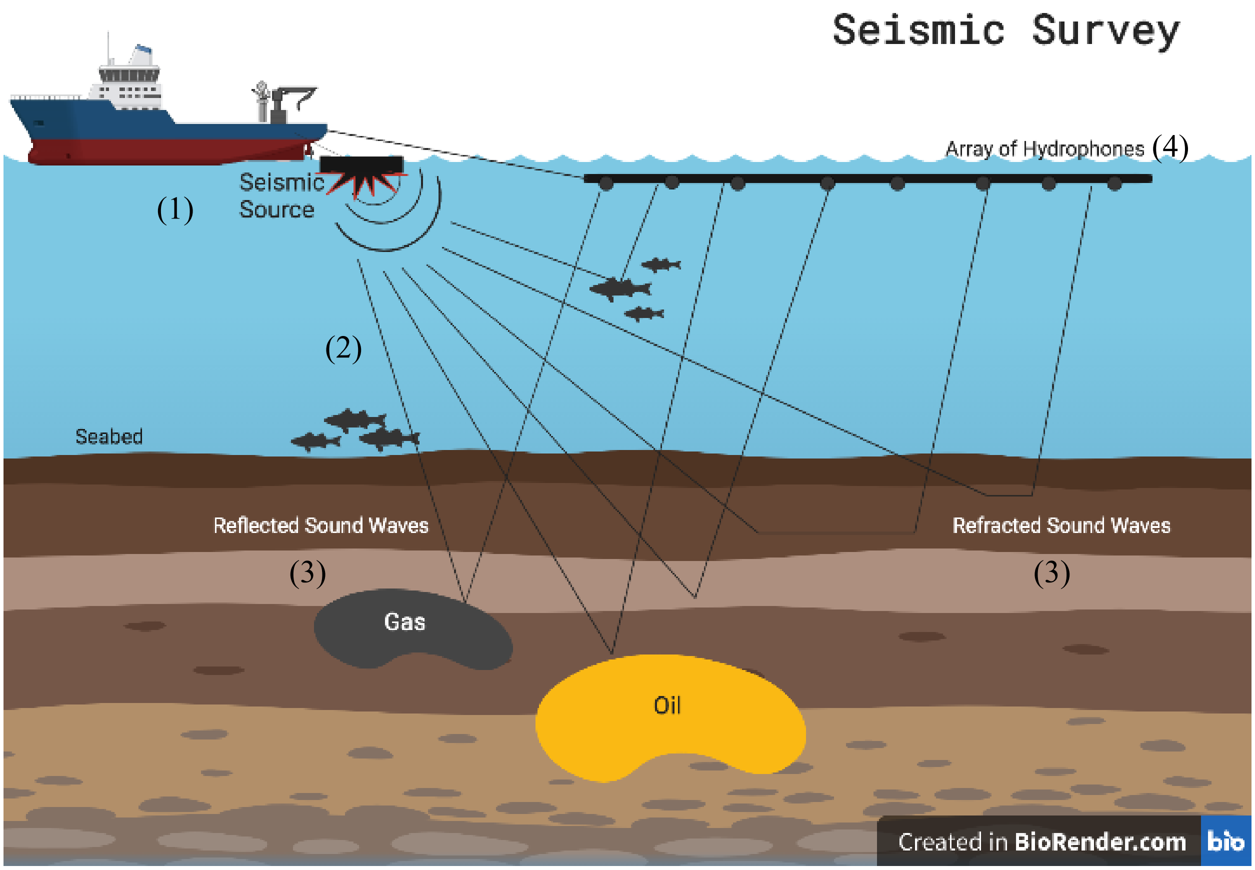

Figure 2. Operational process of a seismic survey. The blasting of acoustic energy occurs at the seismic source (1), the sound waves then propagate through the water column into the seabed (2) where they are reflected or refracted off different subsurface strata (3) back to the surface where the waves are recorded at the surface by an array of hydrophones (4). Created using BioRender.com

The oil and gas exploratory phase consists of three types of seismic surveys offshore: two dimensional (2D) surveys, three dimensional (3D) surveys and four dimensional (4D) surveys (IAGC, 2002). These differing methods allow oil and gas companies to specialize their surveys at different stages of exploration and development of oil fields. A 2D survey involves an ocean liner towing a single cable and is typically designed to map out a large area incorporating long survey lines spaced widely apart (IAGC, 2002). By completing 2D surveys over a large area, a few kilometres in between, a cross-section of the subsurface can give information regarding large scale geological features and potential hydrocarbon stores along the survey line. After these 2D surveys are complete, a 3D survey allows for more specific and detailed mapping of structures identified during the 2D survey (IAGC, 2002). The 3D survey involves multiple closely spaced survey lines parallel to each other allowing for the collection of more data resulting in detailed information on the geological subfloor structures (IAGC, 2002). 4D surveys involve repeating 3D surveys over the same area at different times, creating a time-lapse. This data provides insight into changes in the subfloor structures over time such as fluid movements or depletion of reserves and can optimize production strategies (CAPP, 2016; OGP, 2011)

1.7.1 Seismic Surveying Offshore Newfoundland

Seismic surveying has been ongoing in the Orphan Basin, offshore Newfoundland since 2011. Five 2D surveys were completed from 2011 to 2014, covering 78 759 km (PGS, 2021). Seismic surveying activity increased following the introduction of the Land Tenure system and Statoil's oil and gas discoveries in the Flemish Pass Basin in 2014. With these new discoveries more detailed 3D seismic surveying began in the area in 2015, covering roughly 10 000 km^2 using two vessels combined with two, 2D vessels that covered almost 27 000 km (PGS, 2021). As of 2021, 2D surveying in the Orphan Basin has covered a total of almost 79 000 km and 3D surveying has covered approximately 80 000 km^2 (PGS, 2021). The annual presence of seismic surveying in the Orphan Basin made it an ideal study area to investigate the potential impacts.

1.8 Seismic Survey Impacts

Various studies have been conducted regarding the impacts seismic surveys have on marine life in offshore ecosystems, however, results from these studies have produced contrasting results and do not provide clear understanding of seismic surveying effects on marine organisms due to highly variable environments as well as a vast range of organisms (Table 2). There are growing concerns, particularly from commercial fisheries, on the impacts these surveys have on catch rates

(BP Canada, 2018). There is limited evidence suggesting that seismic surveying causes physiological effects or significant mortality rates of fish populations, however, behavioural effects are commonly reported among conducted studies (Carroll et al., 2017). Seismic surveying could be modifying the behaviours and/or local abundance of fish, zooplankton, or other crustaceans, which could have negative impacts on fisheries (Cote, 2020; Morris et al, 2017). The impacts of seismic surveying activities could be more pronounced in areas where marine species congregate, such as important feeding or mating grounds (Slabbekoorn et al., 2010). Commercial harvesters have been concerned with seismic surveys affecting their catch rates for years, claiming fish are scared away from the area with the elevated noise levels (Engås et al.,1996). The exploration of oil and gas (seismic surveying) being of higher concern than the installation and exploitation (BP Canada, 2018).

Table 2. Summary of previous literature on the effects of seismic surveying on marine fish and invertebrates

Species	Location	Exposure (Db)	Instrument	Distance (M)	Time	Impact (Y/N)	Explain	Source
Lobster	Lab	200	Sleeve gun	3.6 one end	Months	N	Survival rate, Turnover rate (sensory), physical injury	Payne et al. 2007
Lobster	Field	230	Sleeve gun	4 directly under	Days	N	Survival rate, Turnover rate (sensory), physical injury	Payne et al. 2007
Snow Crab	Field	251	Seismic survey	canyon	Months	Y	Catch rates	Morris et al. 2017
Squid	Lab	163 – 169	Airgun	5 – 800	Days	Y	Alarm Responses	Fewtrell et al. 2012
Fish	Lab	163 – 169	Airgun	5 – 800	Days	Y	Alarm Responses	Fewtrell et al. 2012
Fish	Field	191	Airgun		Weeks	Y	Catch rates	Løkkeborg, et al. 2012
Pelagic Fish	Field	~200	Seismic Survey	50 000	Weeks	Y	Abundance and Depth	Slotte et al., 2004
Reef Fish	Field	196	Airgun		Days	N	Alarm Responses	Boeger et al 2006
Reef Fish	Field	230 – 260	Seismic survey	0.7, 6.5, 7.9	Days	Y	Abundance in evening, use of reef	Paxton et al. 2017
Reef Fish	Field	210	Airgun	16	Weeks	N	Abundance	Wardle 2000
Cod	Field	248	Airgun	1800	Weeks	Y	Abundance and Catch rates	Engas et al. 1996
Cod	Field	145	Airgun	40 000	Days	Y	Depth	McQueen et al., 2023
Haddock	Field	248	Airgun	1800	Weeks	Y	Abundance and Catch rates	Engas et al. 1997
Salmon	Lab	210 – 217	Pile Driver	12	Days	Y	Injuries but mild and fish recovered	Casper et al. 2012
Zooplankton	Field	200	Airgun	1200	Days	Y	Abundance and Mortality Rate	McCauley et al. 2017
Zooplankton	Field	180	Airgun	50 – 1200	Days	N	Mortality and Gene Expression	Vereide et al. 2023
Zooplankton	Field	221	Airgun	0 – 25	Days	Y	Mortality and growth	Fields et al. 2019

The reality of a seismic survey involves firing numerous airguns repeatedly over a large area for prolonged periods of time, often spanning months (Hirst and Rodhouse, 2000). The duration may lead to prolonged impacts, perhaps affecting fish or fisheries for extended periods, creating an undesirable overlap between fishing and oil and gas industry activities. If fish disperse in response to seismic surveying, an effect seen in Engås et al. (1996), they may relocate to unfamiliar areas, making it challenging for fishers to locate them. Consequently, this could create a large delay in fishing boats reaching their quotas, and increase fishing effort and fishing footprint on the seabed, as they would be required to spend more time searching for dispersed schools of fish. There are also potential undesirable ecological consequences from the dispersion of fish schools. Significant changes in dispersal of fish populations into new areas can lead to alteration in predator-prey dynamics in both new and previous ecosystems, changing the ecosystem community structure and leading to shifts in ecosystem productivity and stability (Dayton et al. 1995).

Realistic studies on the effect of industry-based seismic surveying activity in natural habitats (*in situ)* are not common because they are considerably more complicated to implement than laboratory studies or controlled field studies. Laboratory and small-scale controlled field studies do not truly replicate industry seismic surveying activities, and therefore many stakeholders are not convinced that the results from such studies are informative regarding the effects of seismic surveying on fishery resources (BP Canada, 2018). However, integrating controlled field experiments into ongoing industry-based seismic surveying is logistically challenging, and even well-developed plans can be ruined by seemingly trivial and unknown factors, such as bad weather, equipment malfunctions, availability of study specimens, or unexpected survey changes. Furthermore, there is limited opportunity for repetition because of the extreme costs associated with seismic surveying and the limited permitted time available to complete surveys (C-NLOPB, 2023). In addition, typically high levels of natural variability in open oceans make it very difficult to measure and detect changes in the response behaviours of fish or zooplankton *in situ* although technology and advancements in the field are rapidly advancing each year (Simmonds and MacLennan, 2005). For animals that are free to disperse, it is challenging to monitor changes in depth, movement, schooling patterns, predator interaction and feeding behaviours, when compared to a laboratory situation where most variables can be controlled, including the environment. Lab experiments are thus more useful in determining physiological impacts from seismic blasting because the environment is controlled and specific physiological parameters, such as heart rate and hormone levels, can be monitored. However, this is much more difficult to implement in a field setting and attributing physiological changes to seismic surveying is complicated when there are multiple other factors (Gordon et al. 2003). It is also difficult to extrapolate results from laboratory experiments because it cannot be determined if the fish will react the same way in a tank as they would in the ocean where they can disperse. A multifaceted approach, including cameras, acoustics, genomics, eDNA catch rates, physiological studies, telemetry and net sampling, to monitor impacts from seismic activities on abundance, dispersion and change in behaviour is a powerful approach to identify specific impacts of seismic surveying in the open ocean (Morris et al., 2017).

1.8.1 Fishing Success, Abundance, and Behavioural Changes

Although the results vary from one study to another, significant reductions in fishing success and changes in abundance of fish as a result of seismic surveying has been documented (Carroll et al. 2017). Engås et al. (1993) found that trawl catches of cod and haddock were reduced by 50% within their entire survey area, reaching a maximum range of approximately 75 km, the highest decline in trawl rates being directly under the seismic blasting where trawl catches were reduced by 70%. Coupled with acoustic monitoring, they determined that the total quantity of cod and haddock near seismic surveying declined by 45% (Engås et al., 1993). Engås et al. (1996) found that cod and haddock trawl catches were reduced by as much as 50% for the five days following seismic surveying, when compared to before firing. A close-range seismic survey exposure experiment, conducted by Paxton et al. (2017), resulted in a decrease in reef fish abundance of 78%. Although these studies have concluded negative impacts on fish abundance, Løkkeborg, et al. (2012) reported redfish and Greenland halibut gillnet catches doubling during seismic surveying. There was a decline, however, in longline catches of 16% and 25% for Greenland halibut and haddock, respectively (Løkkeborg, et al. 2012). The results in this study provide interesting insight into the potential different responses to seismic surveying across species but also demonstrates the importance of accounting for sampling methods. Successful fishing with gillnets relies on swimming activity and movement patterns, therefore, an increase in gillnet catch rates could be due to increased activity from the fish during seismic shooting, where in contrast, longline fishing relies on feeding motivation, suggesting the fish also reduced foraging behaviour (Løkkeborg, et al., 2012).

Fewtrell and McCauley (2012) studied captive reef fish and reported significant alarm responses as per fish swimming closer to the bottom and in tighter schools. Contrasting these results, Boeger et al. (2006) and Meekan et al. (2021) found there were no short-term effects in the former and no short- or long-term effects in the latter in terms of abundance or behaviour of fish when exposed to seismic surveying. Wardle et al. (2000) found no changes in diurnal movement patterns of reef fish but, similar to Fewtrell and McCauley (2012), recorded startle responses in the form of C-starts, where the fish bends in the shape of a C to rapidly change direction, but airgun blasting had little to no effect on the overall day-to-day behaviour and abundance of fish and invertebrates monitored. To summarize, several studies have investigated the effects of seismic surveying on fish and offer differing results across species and study areas using different sampling methods, such as commercial fishing and acoustic monitoring.

1.8.2 Effects on Fish Mortality and Injuries Sustained

A comprehensive review of literature from the Department of Fisheries and Oceans Canada (DFO, 2004) found no documented cases of fish mortality resulting from seismic surveys conducted during field experiments in the ocean, conclusive with Carroll et al. (2017), who in a similar review of literature on seismic surveys found no evidence of fish mortality. In the laboratory it is sometimes possible to generate injuries and mortality when subjects are held in very close proximity to a high-energy noise source, such as an airgun, where the resulting impacts are largely the result of particle motion effects (Carroll et al. 2017).

Halvorsen et al. (2012) used pile driving (similar in nature to seismic surveying) to test for an effect on different fish with varying swim bladder properties; physoclistous (swim bladder independent of the gut), physostomous (swim bladder attached to the gut) and no swim bladder. Fish with no swim bladder did not suffer injuries, whereas both physoclistous and physostomous fishes suffered a variety of injuries, the highest number and most severe impacts were seen in physoclistous fish, such as *Sebastes* spp. Injuries ranged from ruptured swim bladders to partially deflated swim bladders, highly decreasing the chances of survival in these fish.

Although swim bladder injuries are traumatic, fish can recover from these injuries as seen in Ferter et al. (2015) who have studied the impact of barotrauma on Atlantic cod, one of the prominent injuries noted being swim bladder ruptures. This study revealed there were minimal signs of injury after one month and likelihood of survival is high after swim bladder ruptures if there are no other significant injuries and predation is low (Ferter et al. 2015). In slight contrast to Halvorsen et al. (2012), Casper et al. (2012) found injuries to juvenile salmon (physostomous) from pile driving were not lethal and the fish tested in the laboratory had good recovery from injuries they did sustain. Furthermore, this study provided evidence that juvenile salmon can handle much louder pile driving sounds than current guideline standards (Casper et al. 2012). Although a promising study on the potential impacts fish can suffer from pile driving, the above studies were not tested in the wild where fish have many other stress factors that could affect healing time, such as predator defense and feeding (Casper et al. 2012). Based on the conclusions from Hirst and Rodhouse, (2000) and Halvorsen et al. (2012), fish with swim bladders are more prone to physiological damage from seismic surveying. Furthermore, physoclistous fish, such as Atlantic cod, are more susceptible to damage as opposed to physostomous fish such as herring and salmonids (Carroll et al., 2017). All these factors indicate the importance of considering species variability and understanding the physiology of impacted fish in regard to seismic surveying activity, as they play an important role in their vulnerability.

1.8.3 Effects on Invertebrates

Invertebrates have been shown to be less vulnerable to seismic surveying than fish (Carroll et al., 2017), perhaps because they lack the gas-filled organs present in fish and other marine animals (Morris et al. 2017). However, McCauley et al. (2017) concluded that zooplankton are strongly impacted by seismic surveys. McCauley's *in situ* experiment measured an over fifty percent decrease in the abundance of zooplankton after seismic surveying and a three-fold increase in mortality that extended over a 1.2 km range. The effects were measured out to the maximum range of the experiment (1.2 km), while previous research results suggested that the expected impact range to be 10 m (McCauley et al. 2017). Any impacts within a range of only 10 m was thought to be of little ecological importance due to the low impact area and high turnover rate of zooplankton. Considering their results and the duration and spatial footprint of a real seismic survey, McCauley et al. (2017) have suggested that seismic surveys could have a much larger impact on zooplankton than originally proposed and urgently pushed for more studies to assess the impacts of seismic surveys on zooplankton. Two more recent studies by Fields et al. (2019) and Vereide et al. (2023) found little to no effect of seismic surveying on zooplankton mortality or behaviour, contrasting McCauley's findings. Because these varying results were obtained with significant differences in study design and methodology, and that none of these studies assessed the effects on zooplankton abundance and mortality in an *in situ* offshore environment concurrent with a realistic seismic survey, more research is required to better understand effects of seismic surveying on natural zooplankton populations.

1.9 Context of the Study and Research Hypotheses

Environmental Studies Research Fund (ESRF) stakeholders identified critical knowledge gaps and concerns about the effects of seismic surveys on fisheries and marine organisms in Newfoundland and Labrador. Results from studies in other regions led to increased concern about the impacts of seismic surveying on fish and zooplankton mortality and morbidity (Payne et al. 2007). The ESRF has thus been funding research projects under **Assessment of potential risks of seismic surveys to affect groundfish resources** (ESRF, 2018-01S) in collaboration with fisheries and oil and gas companies to better understand potential impacts of one industry on another and to identify better mitigation measures (Morris et al., 2021).

DFO is responsible for contributing to this ESRF-groundfish project and conducted multiple studies between 2018 and 2022 to quantify these impacts and aid in gaining critical knowledge regarding seismic activities to assess the impacts of seismic work on groundfish and zooplankton. Both coastal and offshore testing were conducted with similar protocols in terms of methodology, equipment, data collection and data processing. Offshore studies were completed after coastal pilot studies and provided new evidence on seismic surveying interactions with various offshore groundfish and species of zooplankton. As part of this larger project, the main objectives of this thesis are to assess the impacts of seismic surveying on the abundance and behaviour of groundfish and zooplankton offshore, as well as on zooplankton mortality. Using active acoustic technology, this thesis tests the following hypotheses in chapter 2:

H_1: There is no significant difference in fish and zooplankton abundance based on the proximity to an offshore seismic survey

H_2: There is no significant change in fish and zooplankton behaviours based on the proximity to an offshore seismic survey

H_3: There is no significant difference in zooplankton mortality between areas within the offshore seismic survey and areas outside of the offshore seismic survey

I also test the following hypotheses in Chapter 3:

H_4: There is no significant difference in zooplankton abundance after simulating a 2D seismic survey in a coastal environment

H_5: There is no significant increase in zooplankton mortality after simulating a 2D seismic survey in a coastal environment

This study provides new valuable *in situ* data and knowledge to expand from preliminary seismic survey studies. What mainly sets this study apart from others in the field is the consideration of deeper depths (down to 350 m) and longer time periods (3 months). By investigating greater depths, where sound can propagate further through the water column, as well as longer time periods, when potential acclimation may occur, this study provides a more comprehensive understanding of the noise related impacts of seismic surveying on both groundfish and zooplankton. I also provide suggestions on how to improve future similar studies conducted during seismic surveying.

Chapter 2: Behavioural Response of Groundfish and Zooplankton to Seismic Surveying in the Northwest Atlantic

Abstract

The impacts of seismic surveying on fish and zooplankton were investigated in an offshore environment in Newfoundland, Canada. Seismic surveying was conducted from the Ramform Titan seismic vessel in summer 2021. A seabed moored ALTO lander was equipped with an autonomous multichannel acoustic recorder (AMAR) and a wideband autonomous transceiver (WBAT) to gather both passive and active acoustic data on ambient sound levels, abundance, distribution and behaviour of fish and zooplankton. The distance between the seismic vessel and the moored lander ranged from 0 km to 150 km. Fish between depths of 50 m and 350 m exhibited a response to seismic surveying, descending and concentrating at greater depths when seismic surveying was within 62 km and average sound exposure levels (SEL) was >123 dB re 1μPa. Conversely, no discernible effect on abundance or behavior of zooplankton between 250 m and 350 m depths was measured. Mortality rates of zooplankton were evaluated using net sampling and dyeing techniques, but no significant changes were detected. These findings suggest that the effects of seismic surveying in offshore environments are mainly impacting fish behaviour and impacts were observed at greater distances than previously reported.

2.1 Introduction

Oil and gas industries use offshore seismic surveys to locate pockets of hydrocarbons under the sea floor or to map the sub-bottom in prevision of installing oil rigs. Seismic surveys involve ocean liners firing airguns that emit high intensity, low frequency sound waves across the water column (Paxton et al., 2017). The backscatter of different geological strata is then recorded at the surface by a network of hydrophones towed by the liner. This backscatter provides a map of the bathymetry and geology of the sea floor, including reserves of oil and gas that could eventually be drilled and extracted (Paxton et al., 2017).

Studies addressing the impacts of seismic surveying on marine life have noted potential alarming changes in fish behaviour and abundance whereas others have indicated there is no cause for concern (Carroll et al., 2017), leading to contrasting results and confusion in management and mitigation of commercial fisheries and oil and gas industries. Studies that have been completed are often done over days or weeks, when in comparison, many offshore seismic surveys can last for months (Table 2). These varying results suggest that to understand the effects of seismic surveying in the Newfoundland and Labrador region, more studies over a long-term are needed to examine the impacts of real seismic surveying in offshore environments. While conducting studies in a laboratory setting allows for greater control over variables, conducting research in a real offshore environment allows researchers to obtain accurate data that reflects real-world scenarios. Although implementing such studies may be more challenging, they offer valuable insights into the impacts of seismic surveys under real conditions.

Monitoring fish populations is critical in the sustainable management of fish stocks, ensuring they remain at healthy levels. Conservation of these stocks requires examining all factors that could influence stocks aside from just commercial fishing practices. If seismic surveying is altering fish behaviour and abundance, it could have ecosystem wide effects. Studies have highlighted adverse effects caused by seismic shooting, noting declines in fish catch rates and overall abundance (Engås et al. 1996; Engås et al. ,1993; Paxton et al. 2017), whereas some have noted increased catch rates (Løkkeborg, et al. 2012). It is evident from previous studies that seismic shooting has effects on the behaviour and abundance of fish, the extent and range to which these effects are seen, greatly varies (Carroll et al., 2017). There is an ongoing need for studies in this area to further comprehend the effects seismic surveying.

Seismic surveying could potentially modify the behaviour, distribution and abundance of zooplankton leading to changes throughout the ecosystem caused by a trophic cascade via bottom-up control (Slabbekoorn et al, 2010). Invertebrates like zooplankton have been shown to be less vulnerable to seismic surveying because they lack the gas-filled organs present in fish and other marine animals (Morris et al., 2018). However, McCauley et al. (2017) measured over fifty percent decrease in the abundance of zooplankton after seismic surveying and a three-fold increase in mortality in a 1.2 km range from the airgun in Southern Tasmania. This research suggested that the effects of offshore seismic surveying on zooplankton extend beyond the previously estimated short range of impact McCauley had originally estimated. Two more recent studies (Fields et al., 2019; Vereide et al., 2023) found minimal zooplankton mortality from airgun discharges, however, they used captive zooplankton and did not use acoustic monitoring, which hindered their ability to monitor zooplankton as effectively as McCauley et al. (2017) without acoustics, as the use of acoustics allows for certain behavioural monitoring. Multiple conflicting results render the need for more studies urgent to evaluate the impact of *in situ* offshore seismic surveying on zooplankton.

In summer and fall 2021, I investigated the effects of offshore seismic surveying on the fish and zooplankton communities over the Northeast Newfoundland Slope. Here, I test the hypothesis that seismic activities do not have an impact on the abundance and behaviour of fish and zooplankton communities at the study site. I further provide baseline data and methodological suggestions for future studies on the impacts of seismic surveying.

2.2 Materials and Methods

The effects of a seismic survey in an offshore environment were investigated by using 1) passive hydroacoustics to monitor sound levels in the study area; and 2) active hydroacoustics to monitor the abundance, distribution and behaviour of fish and zooplankton. Zooplankton samples were also collected to investigate zooplankton mortality before and after seismic surveying. This experiment was conducted during the summer and fall of 2021 on the Northeast Newfoundland Slope marine refuge during the Ramform Titan 2021 seismic survey. The scientific survey was conducted from a 65' commercial fishing vessel, the FV *Atlantic Falcon*. Data analysis was completed at the Marine Institute of Memorial University of Newfoundland and Labrador and at the Department of Fisheries and Oceans in St. John's, Newfoundland.

2.2.1 Study Area

The offshore study area was located in the Northwest Atlantic Fisheries Organization (NAFO) division 3K on the Northeast Newfoundland Slope marine refuge, approximately 250 km northeast of St. John's Harbour in Newfoundland, Canada (Figure 3). This area was chosen because it is adjacent to a common fishing area overlapping the location of offshore seismic surveying. The Northeast Newfoundland Slope marine refuge, consisting of approximately 55 000 km^2 and ranging from 200 – 2500 m in depth, is under closure for any unapproved human activities including bottom trawling to protect local corals and sponges (DFO, 2019). Despite this classification as a marine protected area, oil and gas activity is exempt from these conservation measures. The seismic survey area largely overlapped with the marine refuge site (Figure 3). Commercial fishing occurs close to the refuge area, but not inside its boundaries.

The selected offshore study area was divided into three sections: northern control site, marine refuge site and southern site, with a minimum separation of 30 km between each site (Figure 3). The entire study area was approximately 190 km x 96 km with a depth ranging from 250 m to 500 m. The acoustic mooring was located in the marine refuge site, directly under survey lines. The northern site, outside of any survey lines, was selected as a control site for plankton sampling. While samples were also taken in the southern site outside of survey lines, they were restricted by proximity to the seismic vessel and could not sample directly where the vessel passed.

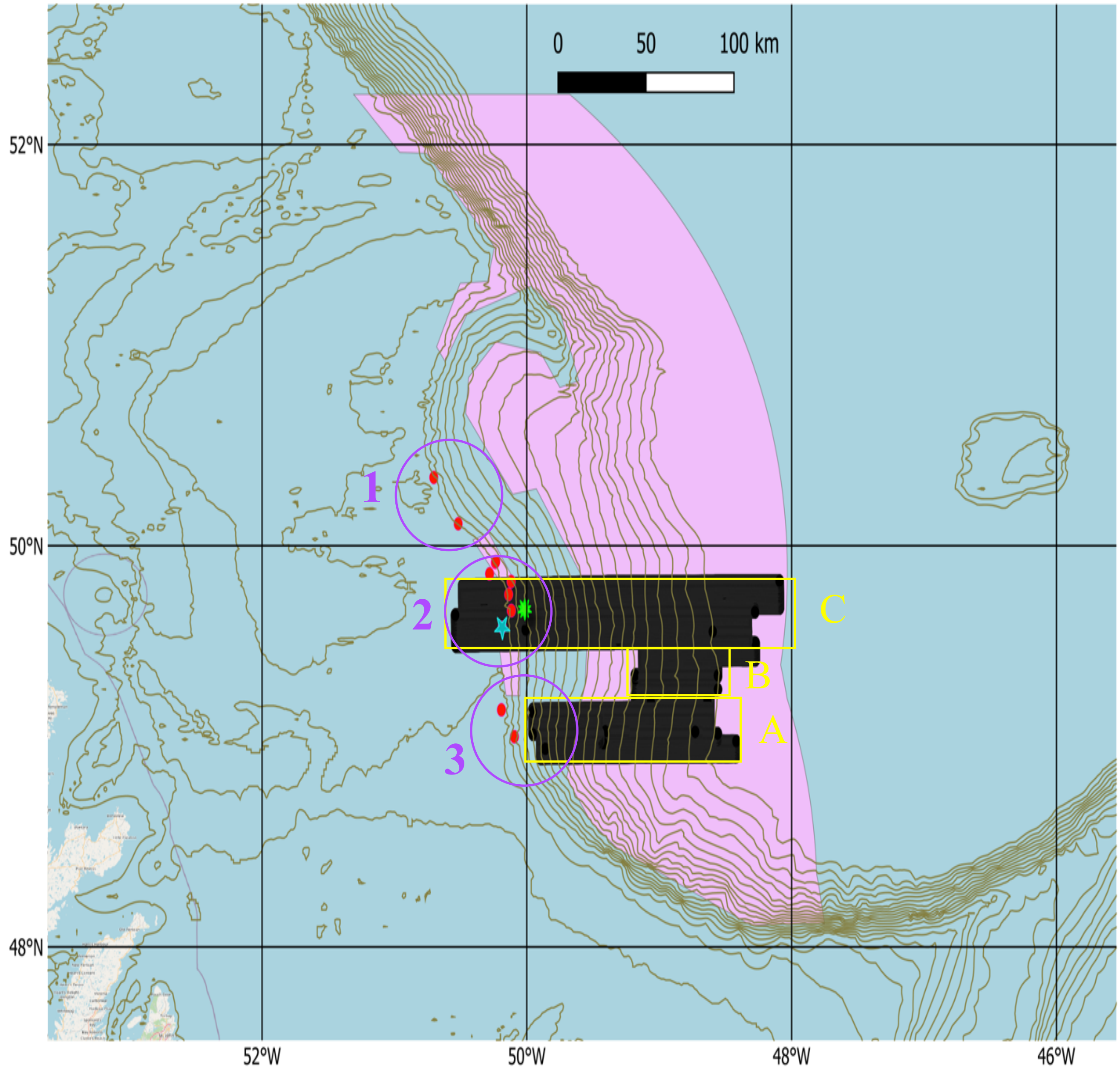

Figure 3. Study site northeast of Newfoundland. The Northeast Newfoundland Slope marine refuge is indicated in pink. The three study sections are indicated by purple circle 1. Northern site 2. Marine refuge site 3. Southern site Survey tracks from the Ramform Titan seismic vessel are indicated in black. The yellow rectangles represent the three racetrack style sections the Ramform Titan completed the survey from zone A to B and then C.. Zooplankton sampling sites are indicated by red points. CTD cast and AZMP zooplankton sample are indicated by green burst and the location of the mooring with the WBAT and AMAR is indicated by the teal star.

2.2.2 Seismic Vessel

Seismic surveying was conducted from the *Ramform Titan* seismic vessel from June 2nd, 2021 at 15:21 to September 3rd, 2021 at 10:53. The seismic vessel moved continuously in a racetrack pattern over the three sections (Figure 3, A,B,C). The vessel was equipped with a 16 x 100 x 8.1km streamer configuration (16 streamers, 100 m separation between streamers, 8.1 km in length) towed at a 20 m nominal depth and a 2x50x4130in^3 source configuration (2 sources, 50m separation between sources, 4130in^3 volume source) towed at a 9 m nominal depth. Each source contained 3 subarrays, comprised of Bolt 1900 LLXT airguns fired at a pressure of 2000 psi every 9 – 15 seconds, totaling 545 991 shots in the study area. When the seismic surveying started, the vessel was located ~150 km to the south east of our acoustic instrument, consistently firing blasts and slowly approaching the acoustic mooring over a period of weeks. The *Ramform Titan* vessel had an average sail time across lines east to west of 12 hours and a projected production rate of roughly 200 km^2/day. The distance between the seismic vessel and the mooring increased and decreased daily as the vessel surveyed from east-west, and slowly progressed northward in approximately 1 km increments, the spacing between adjacent survey lines, as the vessel conducted its survey following a large oval-shaped pattern. The vessel covered approximately 10 000 km^2 over the 2021 survey. Each surveying section (i.e. Northern site, Marine Refuge site and Southern site) was surveyed in an optimized racetrack pattern (Figure 3A, B, C). Racetrack 1 took approximately 28 days to complete, racetrack 2 took 21 days, and racetrack 3 took 53 days.

2.2.3. Hydroacoustics

2.2.3.1. Passive Acoustics

Sound and ambient noise levels were continuously monitored by JASCO's Acoustic Long-Term Observatory (ALTO) lander (Figure 4) moored in the middle of the seismic survey track at 49.59˚N, 50.19˚W at a depth of 347 m (inside racetrack 3). The ALTO measured passive acoustic pressure and particle motion in the water column. The lander consisted of four Geospectrum Technologies Inc. M36 omnidirectional hydrophones each with -200 ± 3 dB re 1V/μPa sensitivity, a JASCO AMAR G4-UD (Autonomous Multichannel Acoustic Recorder Generation 4 Ultra Deep) with a 200dBV dynamic range, three floats, two acoustic releases and one beacon (Mills and Maxner, 2019). The sampling was set at a rate of 32 000 samples per second with a frequency band width of 16kHz.

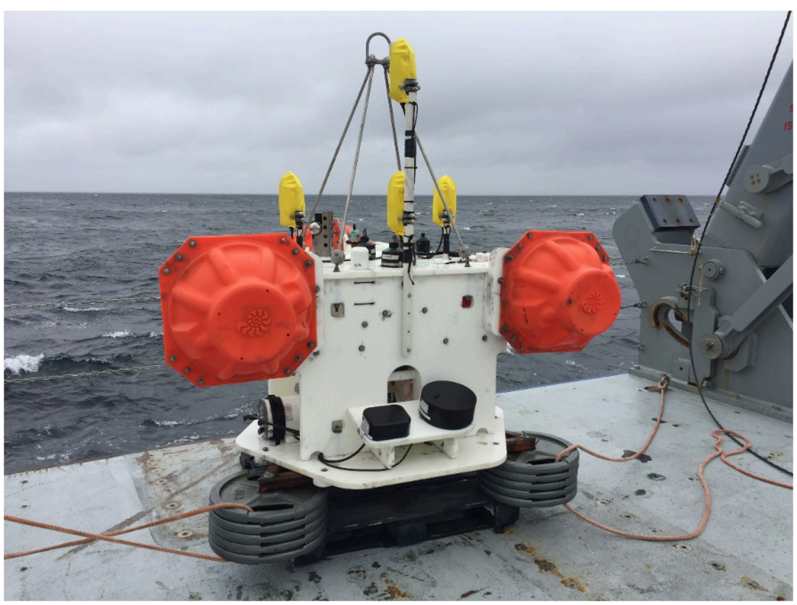

Figure 4. JASCO ALTO Lander Source: Mills and Maxner, 2019

2.2.3.2. Active acoustics

To measure potential change in zooplankton and fish density and behaviour in reaction to the seismic survey, an upward-looking Wideband Autonomous Transceiver (WBAT) was moored from May 29th, 2021 until November 22nd, 2021 at the same site of the ALTO, just above at the seafloor at a depth of 347 m. During this study, the WBAT was set to collect data from June 1st, 2021 until September 6th, 2021. The WBAT was equipped with a split beam 38kHz and a single beam 333kHz transducers, the former frequency used to detect larger organisms including fish and the latter to detect smaller organisms such as zooplankton. The transducers were programmed to use a continuous wave, pulse duration of 1024μs and 2048μs, respectively, and was operated at a rate of 1 minute every 30 minutes at maximum power (500W and 50W, respectively). Calibration of the WBAT was performed prior to fieldwork activities following the standard sphere method (Demer et al. 2015).

To capture the entire water column using active acoustics in an offshore environment with depths exceeding 300 m, sonar equipment would need to be deployed from both the top and the bottom of the water column. A portable vessel-mounted echosounder deployed from a surveying or sampling vessel would allow for the top of the water column to be captured and provide accurate acoustic imaging where the seismic vessel was firing from. I tried to collect such data with a Simrad EK80 portable echosounder, but due to unforeseen equipment malfunctions and logistical challenges including weather restrictions, data were not suitable for analysis. This type of data could have potentially provided acoustic imaging of a 'hole' in the plankton backscatter (indicative of an absence of these creatures after seismic operations) as seen in McCauley (2017). Both vessel-mounted and moored acoustics have their benefits and challenges (see Table 1); ideally both instruments would be implemented to decrease their limitations and increase data collections, however, logistical issues with mounted instruments rendered the data unusable. Despite the limitations, long-term sea floor mooring proved to be the superior method for this study because data could be collected directly beneath the seismic vessel.

2.2.4 Environmental, Fish and Zooplankton Sampling

Conductivity, temperature and depth (CTD) profiles down to 10 m above the bottom depth were completed daily from the FV *Atlantic Falcon* using an EXO1 multiparameter sonde. To calculate the sound speed (c) and absorption coefficients for analyses of the WBAT data, the CTD cast from July 5th, 2021 at 49.68°N, 50.02°W was used. Sound speed and coefficients of absorption at each frequency were calculated and used in acoustic calculations.

To quantify changes in zooplankton mortality, zooplankton sampling was completed at the north site, the marine refuge and the south site before and after the seismic survey using a 60 cm diameter bongo net (Table 3). The bongo net consisted of two nets and cod ends with a 200 µm mesh (i.e. each sample was a duplicate deployed vertically to a depth of 50 m at a speed not exceeding 60 m/min) and then immediately retrieved at a speed of 50 m/min. Sampling was restricted by weather allowances and restricted proximity to the seismic vessel. Before samples were taken on 30/5/2021, 31/5/2021, 30/6/2021, 17/7/2021, 18/7/2021 and 19/7/2021. After samples were only taken on 20/7/2021 due to poor weather and related logistical issues. Due to these restrictions the sampling vessel was not able to obtain samples within 5 km of the seismic survey. Previous studies have exposed zooplankton within meters of seismic, by this standard, zooplankton samples from this study were not exposed to seismic surveying.

Samples from one of the nets were kept alive in 1L plastic jars filled with *in situ* sea water for mortality analyses following the methods in Elliott and Tang (2009). Using a pipette, ~1.5mL of neutral red stock solution prepared prior to the survey was added. Jars were then closed to prevent excess light exposure and let to sit for 15 minutes. After 15 minutes, samples were filtered through a 202 µm sieve and transferred into a small mesh-lined petri dish. Zooplankton samples for mortality analyses were labeled and preserved at -80°C.

Zooplankton samples for taxonomic analyses were collected on July 5th, 2021 by Fisheries and Oceans Canada as part of their Atlantic Zone Monitoring Program (AZMP). The sampling site was located close to the WBAT in the study area at 49.68°N, 50.02°W (station BB-11) (Figure 3). Taxonomic composition and abundance estimates of fish were collected as part of concurrent work on the effects of seismic surveying on fish behaviour using baited camera work and bottom trawling.

Fish taxonomic composition and abundance estimates for each species was assessed using baited cameras deployed throughout the survey area. The baited cameras consisted of a 1080P Mobius ActionCam, Maxi 2.7K with a wide angle view and a 7-hour 250 lumen light source mounted on a commercial snow crab trap allowing for approximately 5 m^2 of seabed visualization. The cameras were baited with 0.5 kg of frozen squid (*Illex* spp.) located 1.5 m from the camera (Nguyen, personal communication). A total of 288 camera deployments were used for the analysis, consisting of 147 in the control site and 141 in the impact site, 73 before seismic surveying and 215 after seismic surveying. Snapshots were extracted from the video recordings at 5-minute intervals and analyzed using DotDotGoose (v.1.5.2) (Nguyen, personal communication)

2.2.5 Analyses

2.2.5.1 Passive Acoustics Analyses

The passive acoustic data from the AMAR were processed by JASCO Applied Science. Ambient noise was measured as sound pressure level in dB re 1µPa and the average (Lrms) pressure levels were averaged over 60-second time intervals at the 16kHz bandwidth. Ambient noise from the AMAR was compared to the location, date and time of seismic shots provided by Petroleum GeoServices, owner and operator of Ramform Titan seismic vessel. The AMAR and seismic shot data were time-synchronized with the exported 30-minute interval WBAT data.

2.2.5.2. Active Acoustic Analyses

The raw active acoustic files from the WBAT were imported into Echoview (Echoview software Pty Ltd v. 13). Acoustic data were cleaned using Echoview's *background noise removal* algorithm (Echoview, 2023) with a signal-to-noise ratio of 10 dB re -1 μPa. WBAT data were then manually scrutinized to remove any noise not removed with the algorithm.

Clean acoustic data at both 38 and 333 kHz were separated into 30-minute intervals and integrated over the water column to measure changes in the abundance and behaviour of all fish and zooplankton in the acoustic beams. Exported values to best describe the abundance and behaviour of organisms comprised 1) integrated nautical area scattering coefficient (NASC), a proxy of total abundance of fish and zooplankton over the water column; 2) centre of mass (COM) to quantify their vertical distribution; 3) proportion occupied (PO) to quantify the biomass dispersion; and 4) aggregation index (AI) to measure their relative aggregation behaviour (Table 3) (Urmy et al. 2012) (Appendix A).

To measure individual behaviour of fish, a single target tracking analysis within the acoustic beam at 38kHz frequency was conducted. The single target tracking analysis was possible at 38kHz due to the calibrated sectional transducer (i.e., split beam). The single target fish tracking algorithm in Echoview allowed for tracking the individual targets across each ping at 38 kHz. These single target tracks allow for individual behaviours to be analyzed including the individual target strength and 3-D orientation of the target i.e., speed and direction (McQuinn and Winger, 2003). To track individual targets over the course of the survey, Echoview's single target tracking (method 2) algorithm was used with the parameters in Table 3:

Table 3. Parameters used in Echoview's single target tracking algorithm for single target tracking analysis

Minimum # of Targets in a Track	8
Minimum # of Pings in a Track	8
Minimum Gap Between Single Targets	3

These parameters were based on Echoview's recommendations. Exported values from the single target tracking analysis comprised 1) Target Strength (TS), which is the strength of the targets echo in dB re $1m^2$; 2) Vertical speed, which is the speed of the target in m/s; 3) Change in depth, which is the variation in depth over the duration of the single track in meters. 2

2.2.5.3. Zooplankton Analyses

Zooplankton samples were analyzed within 10-12 months after collection. Following Elliott and Tang (2009), zooplankton samples were thawed one at a time and analyzed within 60 minutes to avoid samples' colour fading. Ten mL of cold filtered salt water was added to resuspend specimens. Samples were acidified using1mL of 1M HCl then stirred to develop neutral red stain. A subsample of ~300 organisms was then drawn with a pipette and placed in a clean petri dish, those that were living at the time of collection appeared bright pink in colour while specimens that were dead when placed in the red dye appeared light pink or unstained internally. The ratio of dead versus live zooplankton at the moment of sampling was measured and photographed using an Olympus szx 16 stereomicroscope based on the premise that live zooplankton have stained guts. A dark background was used to analyze samples to increase contrast between live and dead specimens.

2.2.5.3. Statistical Analysis

Data visualizations and statistical analyses were performed using R Statistical Software (v.1.3.1.1073).

Distance from seismic activities and ambient noise (passive acoustics) were compared using a segmented model (i.e. hockey stick regression) with the R package 'segmented'. Lrms and Lpk were strongly correlated, and I conducted analyses on Lrms only. Lrms values below 112 dB re 1μ Pa were deemed not representative of ambient noise levels in the water column and were considered outliers and removed from the analysis. The 112 dB threshold was selected based on literature for ambient noise in the region (Carroll et al., 2017; IAGC, 2002) and on the distribution of the points in the scatter plot. . I assumed that values lower than -112 dB likely resulted from occasional masking effects while recording.

Generalized Additive Models (GAMs, *mgcv* package from Wood, 2023) were used to examine the effect of seismic surveying on the marine ecology. Multiple metrics were used to examine the effect of seismic surveying during the period between June and early September 2021, including Nautical Area Scattering Coefficient (NASC), Center Of Mass (COM),Proportion Occupied (PO), and Aggregation Index (AI). These metrics provide, respectively, insight into total biomass abundance in the water column, location of biomass in the water column, proportion of the water column occupied by biomass and an estimate of biomass aggregation in the water column. The GAMs were used because the shape of the relationship between NASC, COM, PO, and AI and the distance from the seismic surveying over time was not known. The candidate model was as follows:

$$equation\ 1\text{: } \log(y) = \alpha + Freq + s(Time) + s(Distance) + \varepsilon$$

where *y* is the dependent variable (NASC, COM, PO, or AI), α is the model intercept, *Freq* is the AMAR frequency (38 kHz and 333 kHz), which is a linear term, and *s* is a thin-plate smoothing-spline function. Different smoothing functions were applied for the dependent variables in the models. *Time* is the day of the year (continuous variable from 152 to 248) calculated using the function *strftime* in R to represent the data collection period from June 1 to September 5, 2021. *Distance* is the interval in km between the AMAR and seismic source (1.75 - > 100 km). Due to the potential non-linear relationships of response variables with time series (day of year) and seismic survey (distance), cubic regression spline smoothers were applied. ε is an error term, incorporating with either gamma, gaussian, or binomial. To avoid over-fitting the models and to obtain temporally (*Time*) and spatially (*Distance)* relevant responses, the maximum number of knots for each of the smoothers was limited to four (k = 4), allowing the smoother to divide the response from each explanatory variable into a maximum of three parts. The best model was selected based on the minimum Akaike information criterion (AIC). Analyses were conducted separately for each response variable.

The GAM was also applied to estimate the diel vertical migration of organism, for which a cyclic cubic regression spline that forces the response to have the same start and endpoint was applied. To test if the TS of fish detected at 38kHz changed over the duration of the study, which would indicate a change in fish size or composition, the GAM was conducted with the daily TS averages as the response variable and time series (day of the year) as the predictor. A similar model was used to further assess the effect of seismic activity distance on fish behaviour represented by Fish Speed and Change in Depth calculated from the TS analysis using the distance as the predictor. The cubic regression spline was applied to smooth the term.

A Generalized Linear Model (GLM) was used to examine the zooplankton mortality that was attributed to the seismic surveying. The model forms:

$$equation\ 2\text{: } \log(M) = \alpha + \beta_1 Trm + \beta_2 Site + \beta_3 Trm * Site + \varepsilon$$

where M the ratio of dead/alive zooplankton as described in 2.2.5.3, α is the model interaction, and β_{1-3} are the regression parameters. *Trm* (Before vs After) and *Site* (Control vs Impact) are the fixed effect factor and ε is the error term. In this Before-After-Control-Impact (BACI) study design, the statistical interaction between spatial (*Site*) and temporal (*Treatment*) fixed effects is included in the model to detect whether the effect of seismic surveying on the mortality ratio is similar over time across the two areas. The GLM model was initially tested and shown that the gamma error distribution was the best fit, having the smallest AIC value.

2.3 Results

2.3.1 Ambient Sound Levels

Ambient sound levels recorded with the AMAR include all ambient noise, such as noise from biological organisms, waves, storms, and other vessels, not just from seismic surveying (Kowarski, 2016). The distance between the seismic vessel and the acoustic mooring ranged from 0 km (directly under seismic shooting) to 150 km (limit of the survey). Distance from seismic activity was significantly inversely correlated to sound levels recorded at the AMAR and explained 78% of the variation in Lrms (Figure 5). Between 0 and 62 km, the average sound level Lrms decreased rapidly as the seismic vessel sailed away from the mooring (173.2-28.0*$\log_{10}$(distance); *p*-value <0.001; Figure 5). Passed that break-out point, between 62 and 150 km, Lrms remained almost constant and only decreased slowly (146.5-13.1*$\log_{10}$(distance); *p*-value <0.001; Figure 5), indicating that the seismic noise was picked-up by the AMAR but contributed little to the ambient noise. Average sound levels Lrms within 62 km of seismic surveying sound levels decreased from

173 to 123 dB, whereas it stayed around 120 dB and only decreased from 123 to 118 dB between 62 and 150 km (Figure 5).

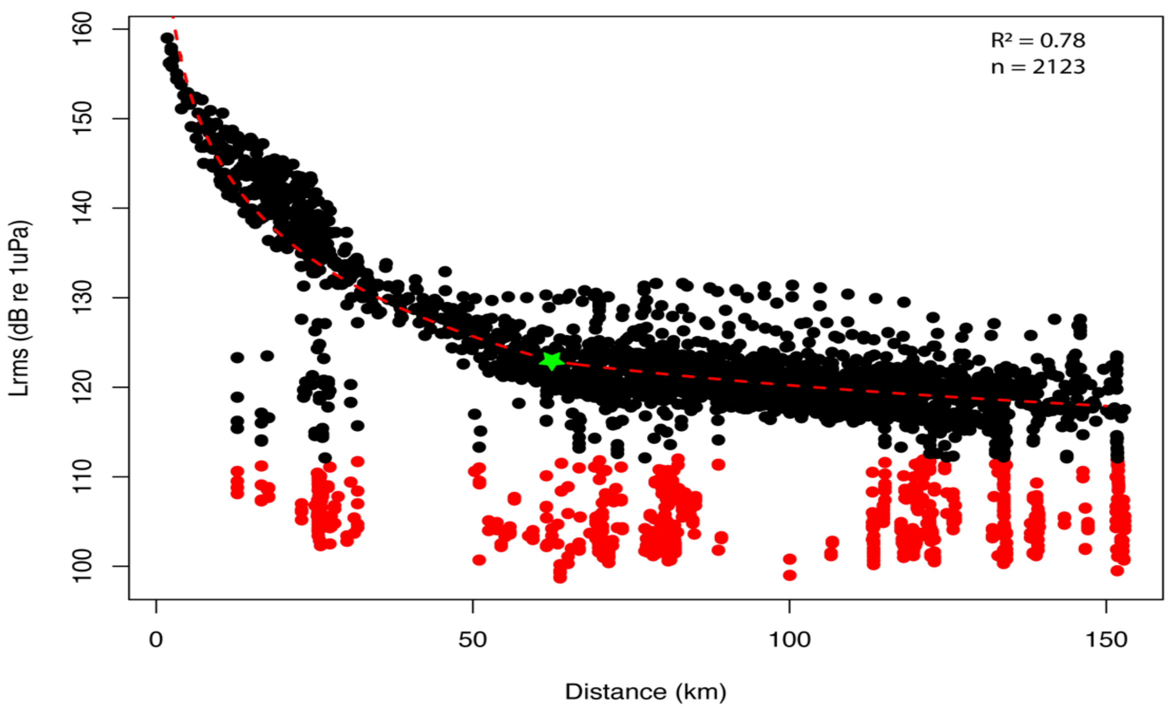

Figure 5. Segmented (i.e. hockey stick) logarithmic regressions between average sound levels recorded from the AMAR (Lrms) and distance from seismic activity. Red dots indicate outliers below -112 dB that were not included in the regression. The green star indicates the break-out point of the segmented regression (62 km).

2.3.2 Effects of Diel Vertical Migrations

Diel vertical migrations (DVM), refer to the vertical movement of fish and zooplankton in the water column on a daily basis, driven by changes in daylight (Pinti et al. 2019). Preliminary visual analysis of echogram data showed clear evidence of DVM on a daily basis (Figure 6). The results in the acoustics showed that the NASC value at 38 kHz had an opposite pattern to 333 kHz, which were significantly higher during nighttime than daytime (F-value= 4.658; p-value= 0.00515; Figure 7). To avoid any confounding effects in NASC, COM, PO, and AI caused by DVM, I kept only daylight values using civil twilight as a threshold. Daylight values were preferred over nondaylight values to remain consistent with concurrent sampling activities, such as CTD casts and zooplankton net sampling, that were conducted during the day.

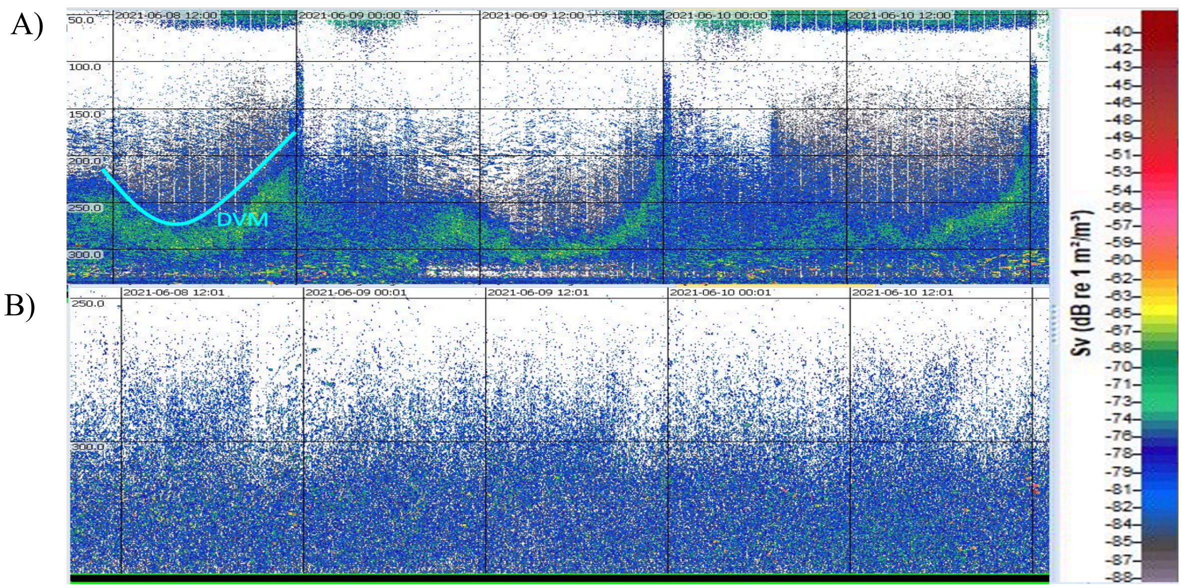

Figure 6. Echograms from June 8th to June 10th, 2021. A) 38kHz, fish dominant echogram with clear diel vertical migration example indicated by the teal line. B) 333kHz echogram.

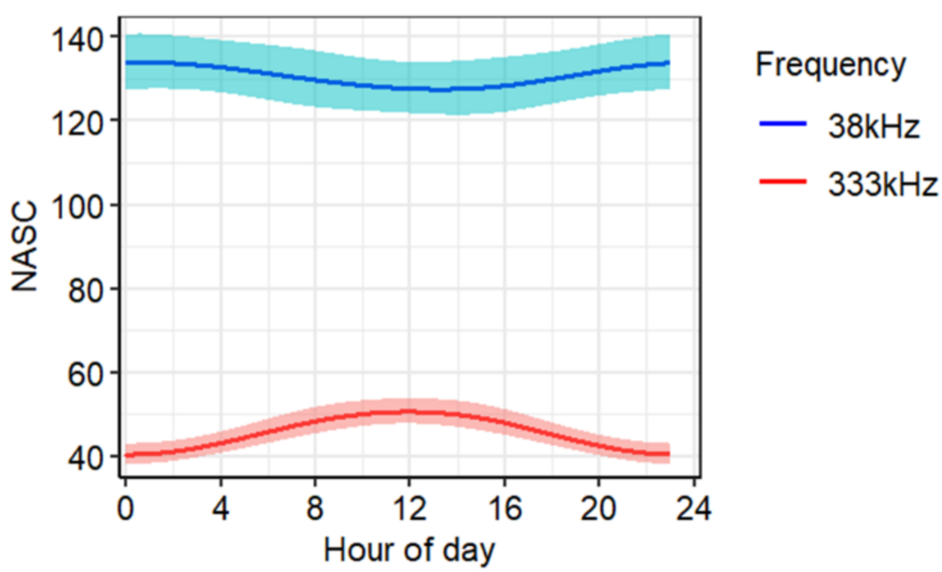

Figure 7. Changes in backscatter (NASC) according to hour of day at 38kHz and 333kHz over the duration of the experiment. Solid lines represent regression lines obtained from GAM (equation 1) and shaded bands represent 95% confidence intervals..

2.3.2 Fish and Zooplankton Response to Seismic Surveying

The NASC varied between 21 m^2/nmi^2 and 688 m^2/nmi^2 (mean of 128 m^2/nmi^2) at 38 kHz. NASC values were significantly higher at 38 kHz than 333 kHz (table 4), which ranged from 12 m^2/nmi^2 to 402 m^2/nmi^2 (mean of 46 m^2/nmi^2) (Figure 8). Values of COM, PO, and AI at 333kHz were significantly higher than at 38 kHz (Figure 8; table 4). The COM ranged between 110 m and 339 m at 38 kHz (mean 240 m) and from 297 m to 344 m at 333kHz (mean of 323 m). The PO ranges from 0.14 to 0.75 (mean of 0.46) at 38 kHz and from 0.3 to 0.7 (mean of 0.49) at 333 kHz. Finally, the AI ranged from 0.0003 m^{-1} to 0.47 m^{-1} (mean of 0.004 m^{-1}) at 38 kHz and from 0.0007 m^{-1} to 1.1 m^{-1} (mean of 0.015 m^{-1}) at 333 kHz.

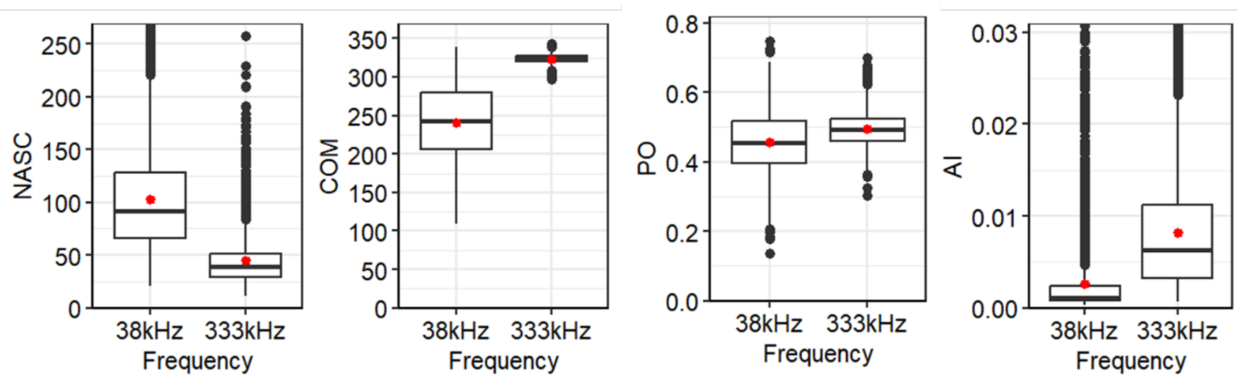

Figure 8. Values of NASC, COM, PO, and AI for 38 kHz and 333 kHz collected during the study period. Red dots represent mean values; Horizontal lines represent median values; The bottom and top of the box indicate the lower (25%) and upper (75%) quartiles, respectively; The ends of the whiskers represent the 1.5 interquartile range, and points depicted as close circles designate outliers.

Table 4. Comparison of average values of NASC, COM, PO and AI between 38 kHz and 333 kHz

Model	Parameter	Estimate	SE	t-value	p-value
NASC	Intercept	3.825	0.013	284.33	< 0.001
	38 kHz	1.025	0.019	53.89	< 0.001
COM	Intercept	5.779	0.003	2212.17	< 0.001
	38 kHz	-0.297	0.004	-80.41	< 0.001
PO	Intercept	-0.705	0.003	-247.9	< 0.001
	38 kHz	-0.080	0.004	-19.98	< 0.001
AI	Intercept	-4.225	0.064	-66.19	< 0.001
	38 kHz	-1.256	0.090	-13.91	< 0.001

All measured metrics (NASC, COM, PO, and AI) were affected by the season, with varying effects at 38 kHz and 333 kHz (Figure 9, Table 5). Changes of NASC and COM were more accentuated at 38 kHz than at 333 kHz and had lowest values in early August, while AI values were lowest in early June for both 38 kHz and 333 kHz (Figure 9, Table 5).

Over the course of the entire survey (June – September), the average NASC values at 38 kHz, a proxy for the abundance of fish, were significantly associated with the distance to the seismic survey. However, the average NASC at 333 kHz, a proxy for zooplankton abundance did not vary with the distance from the seismic survey (p-value = 0.774) (Figure 9, Table 5). The average NASC at 38 kHz decreased by 6% when seismic surveying came within 40 km, then further decreased by 18% when it came within 20 km, compared to the NASC at distances >60km. Indicating that fish abundance between layer between 50 m and 350 m decreased when seismic surveying was closer (Figure 9, Table 5). Overall, the total decrease in NASC at 38 kHz was 24% when seismic surveying was within 62 km compared to when it was >62km.. NASC values at 333 kHz, however, only decreased by a total of 6% when seismic surveying was within 62 km, an insignificant decrease (Figure 9, Table 5).

Along with changes in abundance, differences were also noted in behavioural parameters. Both the centre of mass (COM) and proportion occupied (PO) were significantly correlated with the distance from the seismic survey at 38kHz and 333kHz (Table 5). The COM was approximately 30 m lower in the water column when seismic surveying was within 620 km at 38kHz, and approximately 2 m lower in the water column at 333kHz (Figure 9), indicating fish and zooplankton were deeper when seismic surveying was closer. The 2 m difference in zooplankton, however, was not very relevant ecologically. The PO increased from 46% to 47% of the water column occupied at 38kHz within 62 km but decreased from 50% to 49% at 333kHz, very slight changes (Figure 9) but still significant (table 5). The seismic survey did not affect the aggregation index (AI) at any distance for both 38kHz and 333kHz (Figure 9, Table 5). The overall AI was low at both frequencies (Figure 9), indicating a scattered distribution.

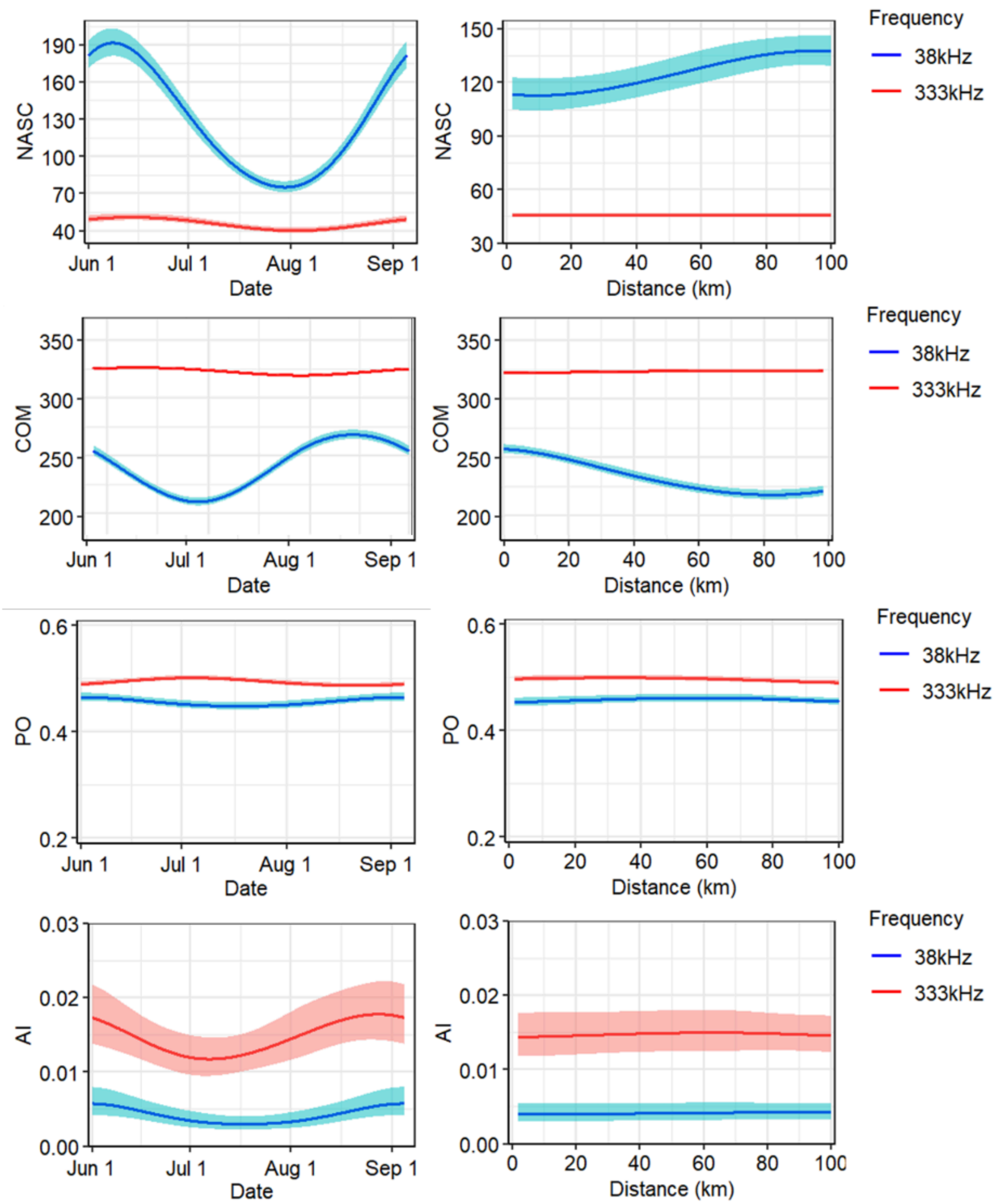

Figure 9. Changes of NASC (m^2/nmi^2), COM (m), PO, and AI (m^{-1}) for 38 kHz and 333 kHz over time series and distances to the seismic survey. Solid lines represent regression lines obtained from GAM and shaded bands represent 95% confidence intervals.

Table 5. The effect of time series and distance to the seismic survey, by using GAMs on the (m^2/nmi^2), COM (m), PO, and AI (m^{-1}).

Model	Frequency (kHz)	Predictor	edf	df	F-value	p-value
NASC	38	Day of year	1.994	2	350.2	< 0.001
		Distance	1.883	2	10.74	< 0.001
	333	Day of year	1.955	2	27.25	< 0.001
		Distance	0.002	2	<0.01	0.774
COM	38	Day of year	1.992	2	336.4	< 0.001
		Distance	1.982	2	126.8	< 0.001
	333	Day of year	1.990	2	272.2	< 0.001
		Distance	1.904	2	18.36	< 0.001
PO	38	Day of year	1.758	2	7.839	0.001
		Distance	1.530	2	3.451	0.011
	333	Day of year	1.875	2	15.05	< 0.001
		Distance	1.780	2	7.321	0.001
AI	38	Day of year	1.940	2	6.067	0.002
		Distance	0.818	2	0.084	0.814
	333	Day of year	1.911	2	4.826	0.006
		Distance	0.805	2	0.136	0.713

The TS was significantly associated with time of the year (F=13.07, p-value < 0.001), which was high in June, July and September and lowest in August (Figure 10). To validate these changes, acoustics results were compared with data from concurrent baited camera work that revealed that Atlantic cod were the most dominant species over the course of the survey but declined in both overall abundance and size from May – September. In contrast, redfish became more abundant over the course of the survey and their abundance increased from May to September. Cod and redfish combined accounted for 90% of fish observed on the baited cameras, confirming they are the dominant signals on our acoustic fish tracking analysis. Changes observed in TS over different months is thus likely resulting from changes in community composition.

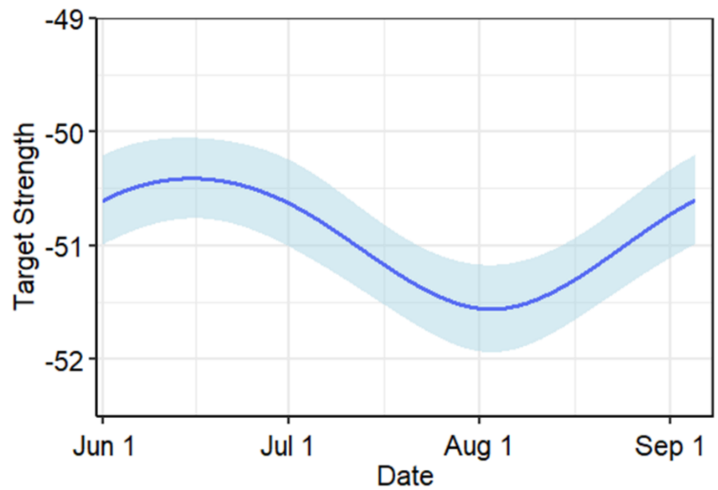

Figure 10. Average target strength values from single target tracking analysis over time series. Solid lines represent regression lines obtained from GAM (equation 1) and shaded bands represent 95% confidence intervals.

A total of 13 461 single target tracks were detected throughout the entire experiment. Changes in vertical fish speed of individual targets measured with the TS analysis were not significantly correlated with distance to seismic surveying (Figure 11A). The average change in depth of individuals fish tracks was also not significantly correlated with distance to seismic surveying (p = 0.392) (Figure 11B).

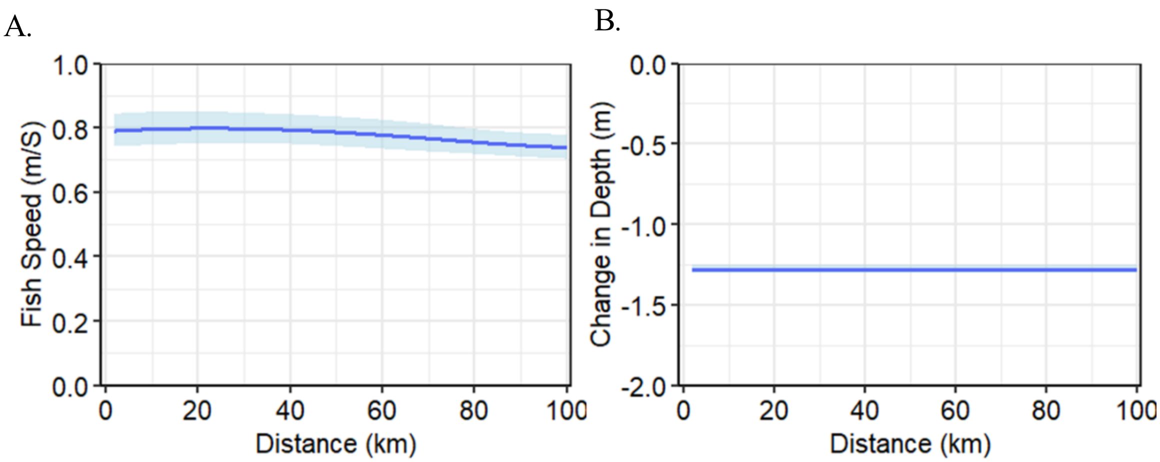

Figure 11. A) Average vertical speed of individual single target (fish) tracks in relation to distance from seismic surveying. B) Average change in depth of individual single target (fish) tracks in relation to distance from seismic surveying

2.3.3. Taxonomic Composition of Fish and Zooplankton on the Northeast Newfoundland Slope Marine Refuge

Taxonomic zooplankton data collected by DFO as part of their Atlantic Zone Monitoring Program (AZMP) (Table 5) indicate that the dominant species near our study site were the copepod *Oithona spp*., which represented 50% of the zooplankton community (Figure 12A). The next most abundant species was *Calanus finmarchicus*, which comprised 11% of the zooplankton community. *Appendicularia* represented 7% of the community.

Fish species composition was assessed as part of the concurrent study using baited cameras. The dominant fish species in the area was Atlantic cod (*Gadus morhua)*, which represented 88% of abundance before seismic surveying and 78% after seismic surveying. The next most abundant fish species was Acadian redfish (*Sebastes fasciatus)*, which represented 2% of the abundance before seismic surveying and 11% after seismic surveying. Other species that were present included roughhead grenadier (*Macrourus berglax)*, thorny skate (*Amblyraja radiata)*, Atlantic wolffish (*Anarhichas lupus)*, summer flounder (*Paralichthys dentatus)*, Atlantic sharp nose shark (*Rhizoprionodon terraenovae*), shortfin squid (*Illex illecebrosus*), turbot (*Scophthalmus maximus*) and American eel (*Anguilla rostrata*) (Figure 12B).

Table 6. Summary of zooplankton sampling and mortality analysis over the course of the offshore experiment

Trip	Date /Time	Station	Latitude (°)	Longitude(°)	BAC	Mortality
1	30/5/2021 9:40	MR	49.04	-50.09	BEFORE	0.05
1	30/5/2021 9:40	MR	49.04	-50.09	BEFORE	0.08
1	30/5/2021 17:47	MR	49.1	-50.19	BEFORE	0.22
1	30/5/2021 17:47	MR	49.18	-50.19	BEFORE	0.11
1	31/5/2021 8:15	MR	49.82	-50.11	CONTROL	0.09
1	31/5/2021 8:15	MR	49.82	-50.11	CONTROL	0.04
1	31/5/2021 14:30	MR	49.85	-50.28	CONTROL	0.05
1	31/5/2021 14:30	MR	49.85	-50.28	CONTROL	0.11
2	30/6/2021 11:06	MR	49.67	-50.11	CONTROL	0.03
3	17/7/2021 15:00	MR	49.67	-50.11	BEFORE	0.12
3	18/7/2021 17:05	MR	49.91	-50.23	BEFORE	0.01
3	18/7/2021 19:43	N	50.10	-50.51	CONTROL	0.11
3	19/7/2021 7:55	N	50.339594	-50.70323	CONTROL	0.01
3	20/7/2021 7:12	MR	49.919513	-50.235212	AFTER	0.01
3	20/7/2021 9:13	MR	49.756432	-50.137618	AFTER	0.15

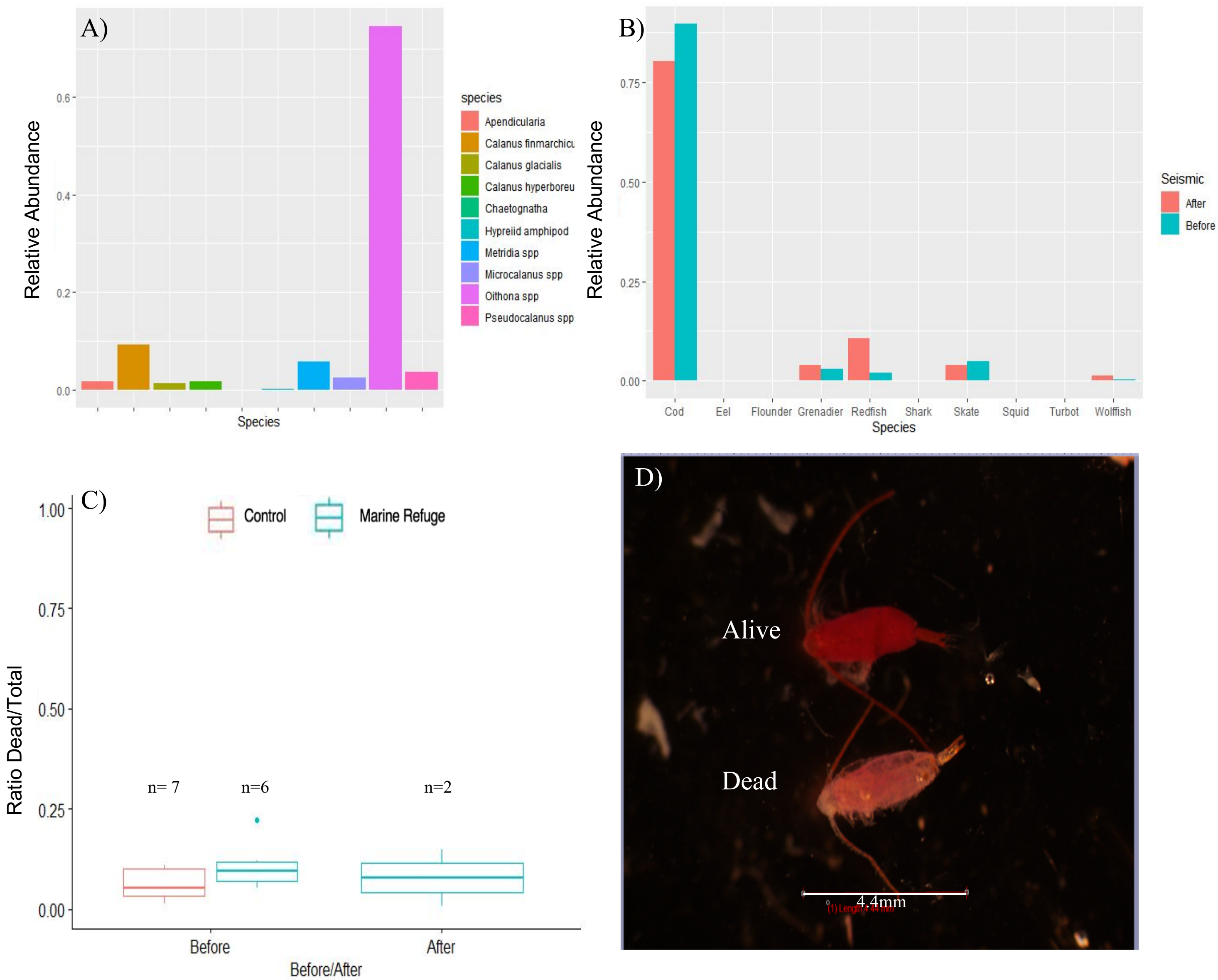

Figure 12. A) Species composition of the main copepod groups sampled on the Northeast Newfoundland Slope Marine Refuge on July 5th, 2021 B) Species abundance of groundfish observed during baited camera deployments over the duration of the survey C) Ratio of dead zooplankton before and after seismic surveying at control and marine refuge locations. D) Examples of alive and dead stained copepods observed under the microscope.

Based on the red-dye experiment on zooplankton, mortality ranged from 1% to 22% between samples and sites. There were no significant differences in zooplankton mortality between sampling locations nor between before and after seismic surveying (Table 6 and Figure 12C). Statistical BACI interactions between study site and seismic treatment were not significant (p-value = 0.68) and thus did not provide evidence that seismic exposure affected the mortality ratio of zooplankton (Table 7).

Table 7. Statistical parameters of the GLM on the effect of seismic surveying on zooplankton mortality

Parameter	Estimate	SE	t-value	p-value
Intercept	15.46	4.90	3.16	0.009
After	0.75	9.48	0.08	0.938
Impact	-4.91	5.65	-0.87	0.404
Before*Impact	-4.64	10.96	-0.42	0.680

2.4 Discussion

2.4.1 Seismic Survey Impacts on Fish and Zooplankton Abundance and Behaviour

The seismic survey had a significantly measurable impact on the depth distribution of fish in the Northeast Newfoundland Slope marine ecosystem. Seismic surveying resulted in a 24% decrease in backscatter and 30 m descent measured at the 38 kHz acoustic frequency when the seismic vessel was within 62 km, exceeding previously hypothesized impact ranges of 5 – 10 km (McCauley et al. 2017). This range was also much larger than measured from previous studies, for example < 1 km on fish and squid startle responses in laboratory, (Fewtrell and McCauley, 2012), 1 km on reef fish abundance in Scotland (Wardle et al. 2000), 1.2 km on zooplankton abundance and mortality in Australia (McCauley et al. 2017), and 8 km on reef fish abundance in the USA (Paxton et al, 2017). This impact range is more comparable to the 75 km impact zone measured for groundfish abundance and catch rates in Norway (Engås et al. 1996). Another similar study was conducted by McQueen et al. (2023), who exposed Atlantic cod to airgun blasts, and measured impacts on their abundance up to 40 km. Similar to our findings, McQueen et al. (2023) observed fish swimming deeper in the vicinity of seismic work, demonstrating an overall weak response.

It was crucial in our experiment to take the entire range (~150 km) into account, because using a range less than 62 km would not have revealed any changes in abundance. Hence, the range of the impacts was larger than initially hypothesized based on previous studies, many of which were conducted in coastal areas. The extended impact range is likely due to the properties and propagation of sound in an offshore environment, where acoustic waves travel further than in coastal environments due to less attenuation and impedance (Bass and Clark, 2003; Hirst and Rodhouse, 2000). This research highlights the value of incorporating realistic seismic surveying conditions into field experiments, including distance, the duration of the seismic surveying period, as well as regional site conditions and impacted species.

Visual analysis of echograms is an informative preliminary analysis method that allows researchers to view obvious changes that may be happening over the duration of an acoustic survey. For example, McCauley et al. (2017) noted a 'hole' that developed in acoustic echograms of zooplankton backscatter. No such visually notable 'hole' was observed on our WBAT echograms during our experiment. However, because our ship-mounted echosounder malfunctioned, we could not survey the top 50 m at lower frequency used to detect fish (38 kHz) and the top 250 m at higher frequency used to detect zooplankton (333 kHz). We can thus not exclude the possibility that such avoidance behaviour or zooplankton mortality could have happened near the surface.

Although significant, the measurable impacts on fish behaviour during this study were not as severe as that reported by some previous studies. They were, however, more severe than other studies that have found no impacts at all. There is great variation among study findings in the field of seismic impact on fish and zooplankton. Here, fish did not appear to leave the impacted area, but rather appeared to change their depth distribution when the vessel was within 62 km and noise levels increased above 123 dB. The centre of mass provided insight into the distribution of biomass in the water column and helped to understand the response of fish when seismic surveying was within 62 km. The backscatter at 38kHz was concentrated approximately 30 m lower in the water column when seismic surveying approached, which suggests that fish responded to seismic noise by descending rather than leaving the area. This would also explain the decrease in backscatter (NASC) at 38 kHz, a proxy for fish abundance. The WBAT transducer was located 1.5 m above the bottom and it is very likely that fish descending closer to the bottom ended up either below the transducer or in the 2-m acoustic blind zone above the transducer. The acoustic blind zone, also known as the acoustic nearfield, is an area where signal interference is too high to collect usable acoustic data, therefore biomass concentrated in this area would also elude acoustic detection (Totland et al. 2009).

Our observations of a descent response by fish is supported by similar acoustic evidence from Slotte et al. (2004) who found that cod and haddock off the coast of Norway moved deeper in the water column and were at an overall lower density during seismic shooting. They also concluded that vertical movement, as opposed to horizontal movement, likely explained the short-term effect of seismic surveying. Importantly, compared to most other studies that investigated shorter durations of seismic exposure, our results show that the response from fish to seismic work in the region do not acclimate to long-term exposures to seismic surveying, i.e. 4 months (Table 3). Indeed, over the whole four months the backscatter was significantly modified when the seismic vessel approached the mooring within 62 km. This is the longest period of study that I am aware of.

Apparently, contrasting our volume backscatter observation, the analysis of individual fish tracks yielded an absence of startle responses or descent behaviour in the monitored fish (i.e. fish tracking analysis). While the overall backscatter diminished, there was no abrupt changes in fish depth or vertical speed. This is likely because the maximum duration of each fish track (i.e. the time a given fish remained in the acoustic beam of the WBAT) was less than one minute. Our results thus suggest that that the descent of fish in the water column was gradual as the seismic vessel approached the mooring, as opposed to a startle induced rapid descent. Furthermore, no startle response was observed with the baited-video camera, which is consistent with our acoustic data.

Some laboratory studies have identified a startle response from fish when seismic guns are operated (Fewtrell and McCauley, 2012; Boeger et al. 2006). Startle responses are often defined as C-starts, schooling behaviour changes, speed changes, and depth changes (Carroll et al. 2017). A similar analysis can be done using acoustics, without the need for video footage. The proportion occupied (PO) and aggregation index (AI) are two metrics that could help indicate a startle response, in this case changes in schooling behaviour, when seismic surveying is near. A lower PO combined with a higher AI would indicate fish are moving in tighter schools, a behaviour noted in Fewtrell and McCauley (2012) when considering behavioural impacts from seismic guns on caged reef fish. Although there was a significant change in PO at both frequencies (38kHz and 333kHz), the difference in total change at both frequencies was a mere 1% between < 62 km compared to > 62 km from seismic surveying. The PO when divided into smaller ranges (0 – 20, 20 – 40, 40 – 60, etc.) fluctuated randomly. It is probable that these random fluctuations and small changes in PO were due to normal daily movements in both fish and zooplankton, rather than behavioural changes caused by seismic surveying. The AI was low at both frequencies as well, indicating aggregation of biomass was not high in this environment. A higher AI could have signaled fish congregating in tighter schools, or zooplankton exhibiting higher density patchiness (Urmy et al. 2012), however, this was not the case based on our data. Overall, based on the indicators used as part of our analyses I did not detect measurable statistically significant differences in either the PO or AI, suggesting minimal or no evidence of startle response or changes in schooling behaviour or aggregation as a result of seismic surveying in this offshore environment.

Individual fish tracking can be used to assess behaviour of fish in an offshore environment (Mei et al. 2022). While startle responses have been recorded in response to seismic surveying (Carroll et al. 2017), the results are highly variable in terms of species, environment and degree of response. Fewtrell and McCauley (2012) found significant alarm responses in caged fish and squid when exposed to seismic surveying but concluded this was not fully applicable to unconfined fish behaviour in a field setting such as ours. Boeger et al. (2006) found similar results in their caged fish, noting a startle response, but also noted acclimation after repeated exposures indicated by less obvious startle responses. McQueen et al. (2023) on the contrary, found no significant behavioural changes in tagged wild spawning Atlantic cod from airguns exposure, demonstrating confinement of fish could lead to significantly different results. Although fish are often 'acclimated' to confinement, the contrasting results in the above studies represents the differences that can arise.

Similarly to our findings, Engås et al. (1996) noted significant decrease in both longline and trawling catch rates of cod and haddock out to the furthest extent of their Norwegian study, approximately 33 km from seismic blasting, and also noted a decrease in abundance of 64% measured with vessel acoustics. Their average trawl catch rates dropped by about 50% for both species and longline catches declined by 45% and 70% inside the survey area, respectively. They inferred that the observed decrease in abundance and catch rates was caused by fish moving away from seismic surveying, however, fish moving closer to the seabed and decreasing foraging behaviours might also explain the observed changes. Engås et al (1996) used similar acoustic technology, however, they opted for a vessel-mounted instrument. This instrument was used in a depth range of 250 – 280 m and had the same or similar limitations in capturing the entire water column as our moored WBAT. Fish shifting deeper in the water column could have been out of the range of the vessel-mounted echosounder, referred to as seabed dead zone, and could potentially explain the observed decrease in abundance (Totland, 2009). In our study area, concomitant baited camera work near the bottom did not measure changes in the abundance of fish, which supports the idea that fish did not excaped the region, contrasting Engås et al (1996) conclusions.

The baited camera showed that Atlantic cod took on average 34 minutes longer to arrive at the baited camera sites during seismic work compared to control measurements. It also took fish approximately 25% longer to consume available bait, suggesting foraging behaviour decreased. These latter observations could explain the decreased longline catch rates measured by Engås et al (1996) because this method of fishing relies on foraging behaviour. In addition, acoustic telemetry studies of several flatfish species, including witch flounder (*Glyptocephalus cynoglossus)*, Greenland halibut, and Atlantic Halibut (*Hippoglossus hippoglossus)*, found that these species exhibited reduced movement when seismic surveying was in close proximity (Corey Morris, DFO, personal communication). Based on my observations, I conclude that fish did not startle or leave an area when seismic surveying activity is in close proximity, but rather moved deeper and perhaps acclimated or adjusted some behaviours to compensate for increased sound levels. A startle response in this type of study is perhaps unrealistic to expect because of the fish acclimating to the gradual increase of sound levels as the ship approaches from a far distance, emphasizing again the difference between lab simulated and real impacts. The potential long-term impact of these behavioural changes due to seismic surveying, however, remain unknown.

Differentiating between species based solely on target strength (TS) measurements is challenging because TS can vary based on species, size and other physical characteristics such as swim bladder presence. TS values are also dependent on depth orientation of the fish, specifically during vertical migrations (Rose, 2009). For this study, there was reliance on concurrent research at the study sites, including trawl and baited video-camera sampling, that identified species composition and relative abundance of the fish community, instead of estimates based on target strength acoustics only. The observed increase in TS in September is thus likely due to the increase in redfish abundance. A larger size fish of a given species has a stronger TS. The increase in TS in September, when Atlantic were smaller, thus cannot explain the increase in TS. However, redfish can have a stronger TS than Atlantic cod and the increase in TS in September is likely due to their higher abundance during that month.

While there was a clear reaction to seismic surveying measured at 38 kHz, the abundance signal at 333 kHz, which was dominated by zooplankton, was not significantly modified by ambient noise nor seismic surveying. Furthermore, there was no difference in the mortality of zooplankton collected before and after the seismic surveying. Ideally, samples would have been taken in close proximity and during the seismic surveying, but safety regulations would not allow for concurrent sampling at the immediate location of seismic surveying. NASC levels at 333kHz in this study showed no changes regardless of seismic proximity, and there was only a small but significant change in COM and PO, of approximately 2 m deeper, which is not ecologically significant. Hence, I conclude that the seismic survey had no biologically relevant impact on zooplankton abundance nor mortality, at depths of 250 to 350 m offshore of Newfoundland. This contrasts the significant reduction and shift in zooplankton abundance seen in the Australian waters by McCauley et al. (2017). It is, however, important to consider the study design and depth at which my study and that of McCauley et al. (2017) were monitoring the response from zooplankton. While I deployed a higher frequency transducer (333kHz versus 120kHz), which allowed me to capture a finer size resolution of targets, the shorter wavelengths do not transmit as far as lower frequencies. This limited my ability to capture data beyond a range of approximately 100 m from the bottom where the instrument was located as opposed to the top 40 m from the surface that was measured by McCauley et al. (2017). McCauley et al. (2017) still reported impacts on zooplankton up to a distance of at least 1200 m, therefore changes at 350 m horizontally or vertically in the water column might be assumed. While our analysis only accounted for changes in zooplankton abundance within 100 m of the seabed, this is an important area where many species of groundfish, such as cod and redfish, feed on zooplankton (DFO, 2019). Although an important depth range of interest, this leaves a gap in my understanding of the seismic impact on zooplankton near the surface and closer to the airgun-blasting. Due to this gap, there are potential changes in zooplankton abundance that I was unable to measure.

The detailed procedure outlined in Elliott and Tang (2009) was followed for mortality analysis of the zooplankton in this study. Zooplankton samples were analyzed 10 – 12 months after collection, which was not recommended by Elliott and Tang (2009), as they hypothesized the plankton would begin to degrade after 3 – 4 months, potentially making counting more difficult. Despite the recommendation, the samples that were stored at -80˚C were analyzed after this period without any obvious degradation of samples or obvious drawbacks. I could distinguish between what were considered unstained dead or stained living plankton when I analyzed the preserved samples based on colour differences (Figure 12D).

My zooplankton mortality analysis was affected by a small sample size due to unforeseen circumstances during offshore sampling trips. There was no significant mortality across sampling locations or before and after seismic, however, the small sample size may have reduced the likelihood of detecting a significant change, if it existed. Although I did not detect a significant increase in mortality caused by zooplankton, it does not contrast some other studies that have ranging conclusions on zooplankton mortality and consequences. For example, Fields et al. (2019) reported limited mortality of bagged zooplankton and a short range (10 m) controlled study. McCauley et al. (2017), however, found a two to threefold increase in mortality at much greater ranges (600m) and concluded 3D seismic surveys could strongly impact zooplankton. Similarly, Vereide et al. (2023) observed a more than threefold increase in overall mortality at varying ranges (50 – 1200 m) using bagged zooplankton but had less concern to detrimental effects of a 3D seismic survey due to the observed immediate exposure mortality rate being lower than the natural mortality rate observed in other studies. When accounting for the wide distribution and turnover of zooplankton, population level effects from seismic surveying seem unlikely. Based on my results, I suggest that challenges exist with respect to understanding the impact of seismic surveying on zooplankton, particularly across highly variable environment conditions.

2.4.2. Ambient Noise Levels

The main natural sources of ambient noise off the coast of Newfoundland are precipitation, wind, and movement of sea ice (Delarue et al. 2018; Pace et al. 2023). These noise sources in some cases become so loud they impede the seismic survey data, making extreme weather conditions including storms, being a restraint for seismic surveying (LGL Limited, 2016). Commercial fishing vessels also contribute to elevated sound levels in marine environments, exceeding normal ambient levels (Daly and White, 2021; Pace et al. 2023). While these vessels are not permitted to fish inside the Northeast Newfoundland slope marine refuge, they can still use commercial fishing methods of deploying pots, gillnets and/or trawling in areas adjacent to the refuge borders and have contributed to increased sound levels in the area (DFO, 2019). During the seismic survey the AMAR recorded noise in the water column ranging from approximately 90 dB re 1μPa to 200dB re 1μPa. The primary source of elevated sound levels can thus be attributed to the seismic vessel, which is supported by the fact that peak sound levels were recorded when the seismic vessel was close to the AMAR. While average sound levels in the area from other vessels and weather conditions ranged between 120-140 dB re 1μPa. Previous literature has recorded noise from seismic surveying up to 220dB (Carroll et al., 2017; IAGC, 2002), which is approximately 20dB higher than the peak noise level recorded in my study. Slightly lower recorded sound levels can be attributed to the AMAR being moored at a 400 m depth on the seabed as opposed to being in much closer proximity to the sound source, which resulted in a decrease in sound intensity with the propagation of the sound through the water column (Webb, 2019). Many previous experiments have been conducted at both shallower depths as well as lesser distances (Table 2). My study thus provides new insight into impacts at greater ranges.

2.4.3. Conclusion

This study demonstrated a significant change in fish behaviour when seismic surveying was within 62 km, where fish moved deeper in the water column. The range over which impacts were observed in this study are appreciably greater than some previous study results, and this is likely because the seismic surveying environment in the Newfoundland and Labrador region includes very deep-water areas that allow noise to transmit greater distances. Lack of startle response, or any rapid swimming movements, suggests a gradual reaction as the seismic vessel was approaching. The repeated reaction over four months indicated that fish did not acclimate to seismic noise. Furthermore, our results indicated no detrimental effects or changes to zooplankton abundance, behaviour or mortality, although more measurements are needed to confirm the absence of impact on zooplankton.

More research in the field of seismic impacts will help to further understand the immediate and long-term effects of seismic surveys. However, although limited, my findings are relevant for oil and gas companies and fisheries alike, as they suggest that, despite a behavioural response of fish to seismic surveying in offshore areas, the impacts of seismic surveys are not as severe as some other studies have previously thought and suggested. By building on this study and investing in research, we can ensure the impacts of seismic surveys are mitigated and controlled to protect and preserve our ecosystems and fish stocks off the coast of Newfoundland and Labrador.

Chapter 3: Impacts of Seismic Surveying Noise on Zooplankton Abundance and Behaviour in a Coastal Environment

Abstract

In this pilot study, I investigated the impacts 2D seismic surveying had on zooplankton in a coastal environment in St. John's, Newfoundland. Seismic blasting was simulated using a vessel-mounted airgun during field trips between November and December 2020. A bottom-moored wideband autonomous transceiver (WBAT) and vessel-mounted EK-80 portable echosounder were used to record active hydroacoustic data on abundance and behaviour of zooplankton. Seismic blasting was simulated by using a vessel-mounted airgun. Plankton net samples were used to identify any significant differences in mortality before and after seismic activity. There were no significant differences found in zooplankton abundance or behaviour nor were there significant changes in zooplankton mortality rates. These data suggest seismic blasting does not impact zooplankton in a coastal environment. However, this pilot project encountered multiple technical issues with both acoustic monitoring as well the seismic airgun blasting. It is important to note that the airgun was not blasting at the desired pressure and the portable echosounder encountered multiple technical issues outside of my control. Despite the challenges with this pilot study, it provided an opportunity to gather some preliminary data as well as to test equipment and methodology for future studies.

3.1 Introduction

Two-dimensional seismic surveys are commonly used in oil and gas exploration, allowing for the general mapping of large offshore subfloor reserves of hydrocarbons (Carroll et al.; 2017; Hildebrand, 2004). This type of survey method differs from 3D and 4D surveys as there is commonly only a single source or array of seismic blasting and a single streamer of hydrophones used (CAPP, 2016; OGP, 2011). Replicating a 3D seismic survey for experimental purposes to study the effect of noise on marine life is difficult because these surveys can be conducted in the same general area for extended periods of time and are costly. In contrast, mimicking a 2D survey with a portable airgun is relatively simple.

Most research conducted on the impacts of seismic surveying consider the impacts of noise over relatively short periods of time, hours or days, and are simulated in a marine environment using single airguns or small groups of airguns deployed from small vessels and a combination of acoustic instruments and sampling techniques to monitor the effects on subjects (Carroll et al., 2017, Fields et al., 2019; McCauley et al. 2017; Vereide et al., 2023). Previous studies including Boeger et al. (2006), Engås et al. (1996) Fewtrell et al. (2012), Fields et al. (2019), Løkkeborg, et al. (2012), and McCauley et al. (2017) used single, or a few, airguns to experimentally simulate the exposure of fish or zooplankton to seismic surveying noise. A similar approach to previous studies was used in this study to assess the impacts of a 2D seismic survey on zooplankton in a coastal marine environment.

Impacts of seismic surveying on zooplankton are far less studied than their fish and marine mammal counterparts. Zooplankton are ecologically highly significant and critical for ecosystems to remain healthy (Botterell, 2023, Richardson, 2008). McCauley et al. (2017) investigated these impacts in a coastal environment off Australia and found startling negative effects on overall abundance and mortality of zooplankton, noting a 2 to 3 fold increase in mortality out to 1 km, the maximum range of their experiment. These extreme results prompted further research on zooplankton, Fields et al. (2019) and Vereide et al. (2023), producing results in the field as well, concluding airgun blasting did not cause significant mortality to cause concern.

The objective of this pilot study was to adapt the experiment approach used by McCauley et al. (2017) in coastal Australia to assess the effects of seismic airgun firing on zooplankton abundance and mortality in the Newfoundland region. This experiment was conducted close to shore where water depths are approximately 180 m, and was independent from commercial seismic activities. The goal was to test field gear, methodology, and data acquisition to provide preliminary results informing similar applications as part of large-scale offshore experiments involving actual commercial seismic surveys. Using a single airgun deployed from a vessel and a combination of plankton net samples, similar to methodology in McCauley et al. (2017) and hull-mounted and moored echosounders, I tested the following hypotheses:

H_1: Abundance of zooplankton will decrease after simulating seismic surveying with an airgun.

H_2: Mortality of zooplankton will increase after simulating seismic surveying with an airgun.

3.2 Materials and Methods

3.2.1 Study area and Design

Station 27 is a location regularly monitored by the Department of Fisheries and Oceans Canada (DFO) and is a part of the Atlantic Zone Monitoring Program (AZMP) that has been operating since 1998. The station is located at 47.55°N, 52.59°W, approximately 7 km outside of St. John’s Harbour in Newfoundland (Figure 13). The depth at this location is approximately 180 meters and environmental information such as wind gusts (km/h), wave height (m), air and water temperature (°C), surface current (Knots) and salinity (PSU) are continuously recorded. This information is available at http://206.162.191.26/sitewebbouee/AZMP-STA27_an.php and is updated every 30 minutes.

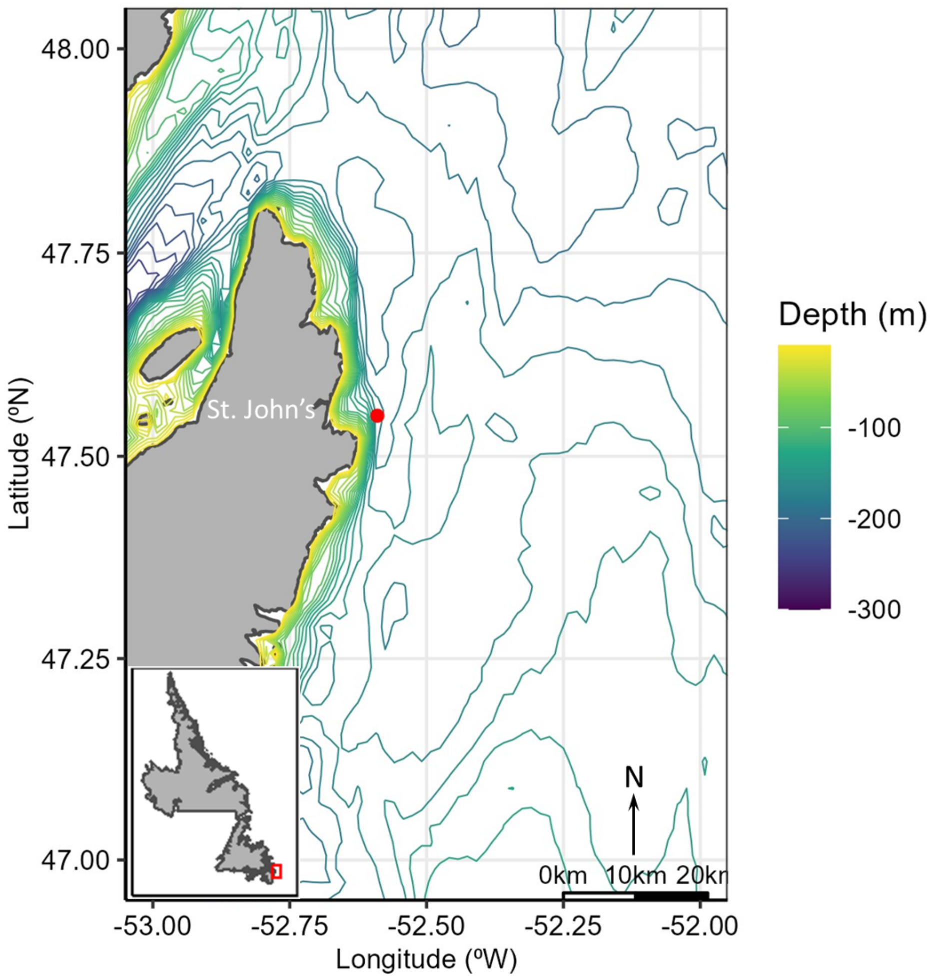

Figure 13. Department of Fisheries and Oceans Canada Station 27 off the coast of St. John's, Newfoundland, located at 47.55°N, 52.59°W and indicated by the red dot.

A total of 6 field trips were conducted between November and December 2020 using *Shore Dodger,* a 35' contracted fishing vessel. Airgun deployment and zooplankton sampling are summarized in Table 5. Several trips experienced technical challenges that limited our data acquisition. I did, however, collect enough data to perform preliminary analysis on airgun effects in this coastal environment. The study design involved using vessel mounted hydroacoustics as well as deployment of moored hydroacoustics 1 km north from the AZMP, where deployment of the airgun would occur. By firing the airgun on top of the moored Wideband Autonomous Transceiver (WBAT) along with the vessel mounted EK80 portable echosounder, I collected data intended to image the entire water column at the time of airgun deployment, and then monitored the surrounding area using the vessel mounted acoustics. The station 27 AZMP monitoring instruments, located 1 km south from the WBAT position and location of airgun shooting, could potentially capture any changes in the water column observed acoustically downstream, as the plankton drift in the current. The dominant current in the area causes drifting organisms to travel south/ southwest through the Avalon Channel (Petrie and Anderson, 1982). Halfway through the field trips, the mooring at station 27 was retrieved for the winter. To account for the change, the blast site was changed to 1 km north of the WBAT to account for currents to drift organisms over the WBAT. Ideally using the vessel acoustics to capture immediate changes from blasting and monitoring the downstream changes with the WBAT.

3.2.2 Acoustic Instruments

The WBAT used in this experiment was moored approximately 1 km upstream from station 27 at 47.56°N, 52.59°W on November 13th, 2020 (Figure 14A). The WBAT was equipped with a split beam 38 kHz and single 333 kHz transducer and had a mission plan set to ping for 12-hour intervals per day with a ping rate of 1 ping per second at 38 kHz and 1 ping per 2.5 seconds at 333 kHz from November 14th, 2020 until December 8th, 2020. The continuous deployment period allowed some flexibility to select preferred field days that were weather dependent, and the ping rate was set to maximize data collection during the experiment to the maximum length of the battery life. Because of different wavelengths, the signal from swim-bladdered fish is usually stronger at a lower frequency of 38 kHz, while the signal from meso- and/or macrozooplankton dominate at a higher frequency of 333 kHz (Simmonds and MacLennan, 2005).

In addition to the moored WBAT, a Simrad EK-80 portable echosounder (EK-80) was mounted to the side of the research vessel using a pole. The EK-80 was equipped with 38kHz and 200kHz transducers that were mounted facing the ocean floor on the vessel and only operated while in the field. The EK-80 was programmed to ping every second to cover the entire mission, including airgun operations. Both the WBAT and portable echosounder were calibrated following the standard sphere methods prior to the experiment (Demer et al. 2015).

Table 8. Technical parameters used for WBAT and EK80 portable echosounders

	WBAT				**EK80**			
Frequency (kHz)	*Power (W)*	*Pulse Duration (µs)*	*Bandwidth*	*Beam Angle (°)*	*Power (W)*	*Pulse Duration (µs)*	*Bandwidth*	*Beam Angle (°)*
38	500	1024	Narrow	18°	500	1024	Narrow	7°
200					50	2048	Narrow	7°
333	50	2048	Narrow	7°				

3.2.3 Acoustic Analyses

Raw acoustic files collected in the field were analyzed using Echoview (v 12.1). Echoview allowed for the visualization of data from both the WBAT and portable echosounder as well as quantifying the acoustic files for statistical analysis. The two acoustic instruments required different algorithms for cleaning the data. Because it was moored on the ocean floor, the WBAT had cleaner raw data as there were no noise introduced by vessel movement, surface bubbles or engine noise from the vessel that can be detected by ship mounted acoustic transducers. The WBAT data were therefore processed using Echoview's *background noise removal* algorithm and manually cleaned (Echoview, 2022). Data from the portable echosounder, however, required the use of multiple noise cleaning algorithms that consisted of *background noise removal;* this filtered out background noise*, attenuated noise removal;* to account for noise created from a cruising ship, *impulse noise removal;* to account for pinging of other acoustic instruments in the area including other ships, *transient noise removal;* to account for other noises such as wave-hull collisions (De Robertis and Higginbottom, 2007). I measured the Nautical Area Scattering Coefficient (NASC in m^2/nmi^2), a proxy for abundance over the whole water column, at 38 and 333 kHz for the WBAT and 38 and 200 kHz for the EK80 (Urmy et al. 2012).

3.2.4 Airgun

For this experiment, one Sercel Mini-G II airgun (Figure 14B) was used as opposed to multiple airguns as in a commercial seismic survey from large ships. The airgun was deployed 8 meters below the surface, positioned horizontally to direct the sound energy downward, and operated at a target pressure of 2000 psi using tanks of nitrogen from a fixed location (Figure 14C). To simulate the timing of a real survey the airgun was fired every 10 seconds until pressure in the tank was depleted, totaling between 20 – 40 shots dependent on pressure source.

Multiple small scuba tanks were used on the first field trip (November 18th, 2023), but the scuba tanks did not contain enough pressure to fire the airgun at full pressure. To get the airgun firing at a higher pressure, large nitrogen tanks were used for subsequent trips. During the third field trip on November 23rd, 2020, the nitrogen tank fired the airgun at the desired 2000 psi for the first 15 shots, then decreasing to 1500 psi – 1250 psi for the next 15 shots and decreasing below 1000 psi for the last 10 shots, totaling 40 shots for one full tank of nitrogen. Visually, there were air bubbles coming from the airgun, indicating that a volume of compressed nitrogen was being lost and that contributed to fewer shots from a tank. The issue was not the tanks used to fire the airgun but an internal airgun malfunction. To ensure the area was impacted by seismic noise, shots were fired at a shorter interval than 10 seconds to compensate for leakage and rapidly decreasing pressure in the tank. No plankton samples were collected due to airgun issues limiting time in the field. During the fourth field trip, multiple tanks of nitrogen were taken to attempt to achieve the desired number of shots at maximum pressure. A total of 30 shots were fired, 25 at 2000 psi and 5 at 1500 psi, while the airgun was still leaking some air bubbles. The fifth field trip on December 7th, 2020 once again used multiple nitrogen tanks, a total of 30 shots, 20 at 2000 psi and 10 at 1500psi. The final field day on December 8th, 2020, all the instruments were retrieved, the airgun was fired one final time, 15 shots at 2000 psi and 15 shots at 1500 psi using one tank of nitrogen.

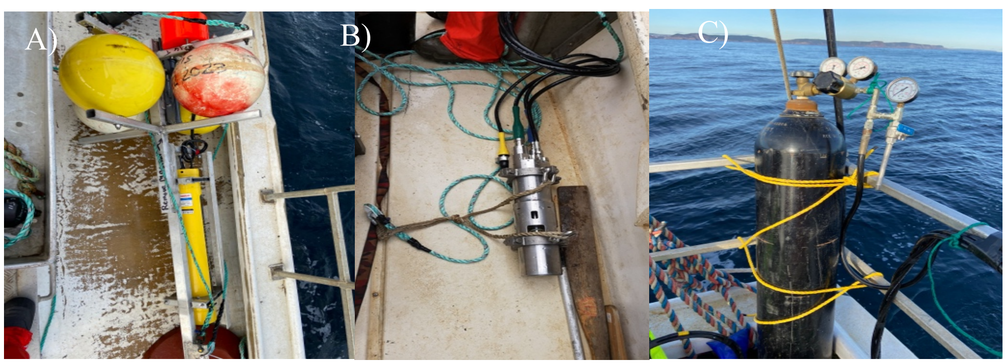

Figure 14. A) WBAT mooring B) Sercel Mini-G II airgun C) Nitrogen tank

Table 9. Summary of airgun operations and zooplankton sampling during station 27 field experiments

Date	**Airgun**					**Sampling**	
	Pressure (psi)	*Depth(m)*	*Interval(sec)*	*# Shots*	*Location*	*Formalin (before/after)*	*Stain (before/after)*
13/11/20	Deployment of Field Gear						
18/11/20	>1000	9	5 – 10	10	47.5631°N, 52.5855°W	8B/8A	0

23/11/20	>1000 –2000	9	2 – 5	40	47.5572°N, 52.5867°W	0	0
4/12/20	1500 – 2000	9	10	30	47.5677°N, 52.5847°W	0B/2A	8B/6A
7/12/20	1500 – 2000	9	10	30	47.5669°N, 52.5867°W	0	0
8/12/20	1500 – 2000	9	10	30	47.5669°N, 52.5867°W	0	0

3.2.5 Zooplankton Sampling

Zooplankton were sampled with vertical bongo net tows before and after airgun operations to assess changes in their mortality rate. The bongo net had a mouth diameter of 1m and a mesh of 200μm. It was deployed to a depth of 50 m at a speed of 40 m/min and then retrieved at 50 m/min. Zooplankton taxonomic composition was assessed through analyses conducted as part of the AZMP program (DFO, 2006; Morris, personal communication). Zooplankton samples were collected on two field trips, November 18th, 2021 and December 4th, 2021.

To assess zooplankton mortality I used a neutral red stain technique as used in the offshore experiment (Chapter 2, Elliott and Tang, 2009). In short, a neutral red stock solution was prepared before field experiments by adding 0.1g of neutral red per 10mL of deionized water. This stock solution was combined with samples in the field by adding 1.5mL of stock to 1L of sample resulting in a concentration of 1:67 000 (Elliott and Tang, 2009). Samples were stained for approximately 15 minutes in dark lighting and at sea temperature; after which stained samples were strained and frozen in a Petri dish for subsequent analysis. Zooplankton samples for taxonomy purposes were filtered through a sieve (200μm) and transferred into a jar with seawater and formaldehyde (37%) and labeled for subsequent analysis. Zooplankton samples were categorized based on location and before/after firing of the airgun and 300 zooplankton were counted per sample.

3.2.6 Laboratory Analyses

Stained samples were analyzed within two months of sampling as per reference (Elliott and Tang, 2009). When the zooplankton samples were collected in the field, they were transferred into a jar containing the neutral red stain. The plankton that were alive at the time of sampling ingested red dye and were stained red internally. Plankton that was not alive at the time of sampling could not ingest the stain and remained colourless/transparent. Samples were acidified using HCl and resuspended in seawater for visualization under a stereomicroscope and the ratio of dead vs alive animals was measured. Taxonomic samples were analyzed by SpryTech Ltd.

3.2.7 Statistical Analyses

NASC values integrated over 30-min intervals were exported from Echoview. A T-test was used to compare mean NASC values on days when the airgun was operated versus when it was not operated. One-way ANOVA was used to compare mean NASC before, during, and after airgun operations on days when it was operated. An ANOVA was used to compare sample means of zooplankton mortality before and after firing the airgun and between sampling locations directly at the blast site and 1km north from the blast site. Metrics were assessed for normality and homogeneity of variance using Shaprio-Wilk and Levene's test respectively. Statistical analyses were completed using R (v. 4.1.3).

3.3 Results

3.3.1 WBAT data

The average NASC at both 38kHz and 333kHz was not significantly different on days when the airgun was operated or not operated (T-test, $p=0.4315$, 0.7419) (Figure 15A,B). During the days when the airgun was operated, NASC values were averaged over 30 minutes and compared to consider effects within a single day (November 18th, November 23rd, December 4th

and December 7th, 2020) (Figure 15). There were no significant difference in NASC values before and after seismic surveying at 38kHz (Figure 16A,B,E,F) (T-test, p=0.2257, 0.8969, 0.3635, 0.589) nor at 333kHz on November 18th, 2020 (Figure 16C) (p=0.1243), December 4th, 2020 (Figure 16G)(p=0.9465) and December 7th, 2020 (Figure 16H) (p=0.0643). There was, however, a significant difference in NASC at 333kHz on November 23rd, 2020 (Figure 16D) (T-test, p=0.004267), when NASC values significantly increased by 37% approximately 6 hours after the seismic surveying (Figure 16B). The WBAT was retrieved prior to airgun firing on December 8th, 2020, so there is no WBAT data from that day.

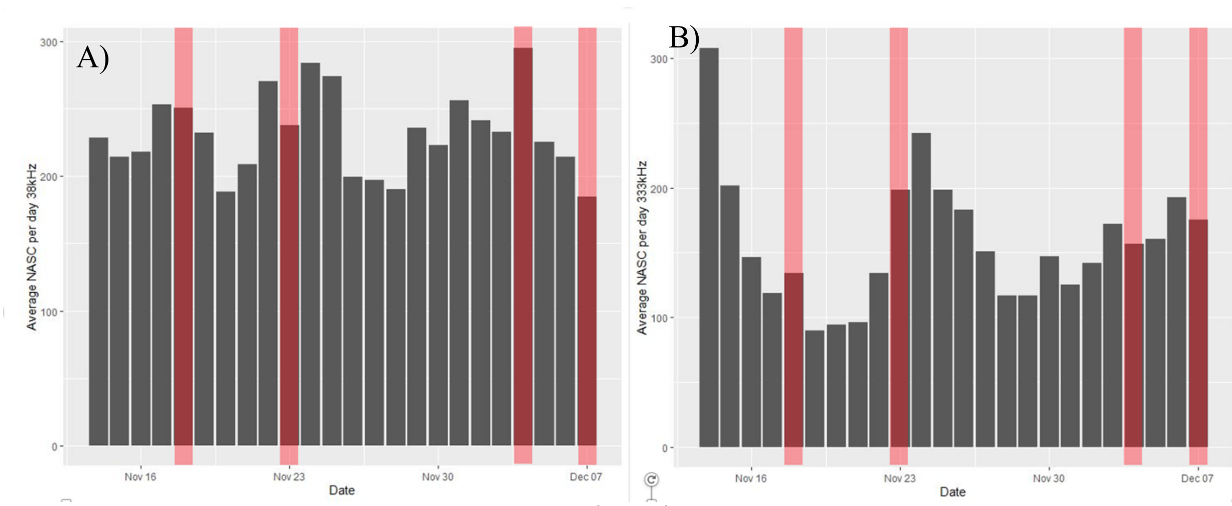

Figure 15. NASC (proxy for abundance in m^2/nmi^2) values averaged per day at 38kHz (A) and 333kHz (B) over the duration of the experiment and measured from the moored WBAT. Days when the seismic gun was operated are highlighted in red.

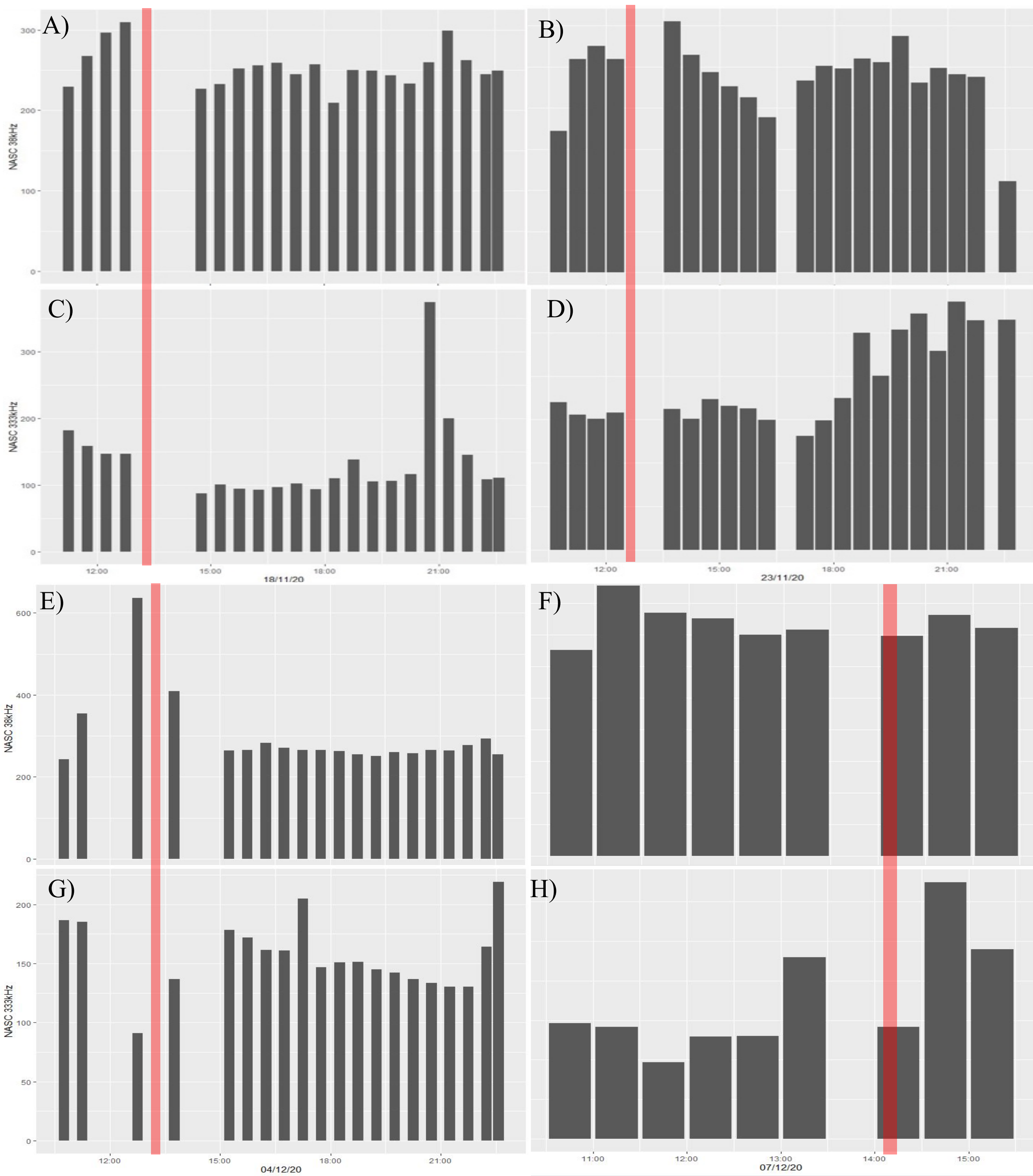

Figure 16. NASC values (in m^2 / nmi^2)averaged per half hour at 38kHz (A,B,E,F) and 333kHz (C,D,G,H) from the moored WBAT when 2D seismic was simulated. Periods when the seismic gun was operated are highlighted in red. A,C) November 18th, 2020 B,D) November, 23rd, 2020 E,G) December 4th, 2020F,H) December 7th, 2020.

3.3.2 EK-80 portable sounder

A before-during-after approach was taken when analyzing the data to measure changes in abundance of zooplankton with the portable echosounder EK-80. The shorter ping rate than the WBAT allowed for smaller time intervals to be analyzed to determine if there were shorter term daily or hourly impacts on abundance and distribution of zooplankton in the water column, in the presence of seismic surveying (Figure 17). There was no significant difference in NASC values between before, during and after firing of the airgun on November 23rd, 2020, December 4th, 2020 or December 7th, 2020 at 38 kHz (Figure 13A,B,E) (ANOVA, p=0.7925, 0.6729, 0.08327) nor 200kHz (Figure 17C,D,G) (ANOVA, p=0.7728, 0.6556, 0.698). On December 8th, 2020, there was a significant difference between before, during and after NASC values at both 38 kHz (Figure 17F) (ANOVA, p=1.793e-11) and 200 kHz (Figure 17H) (ANOVA, p=0.005009), when NASC increased at 38kHz but decreased at 333kHz during seismic surveying (Figure 17D.)

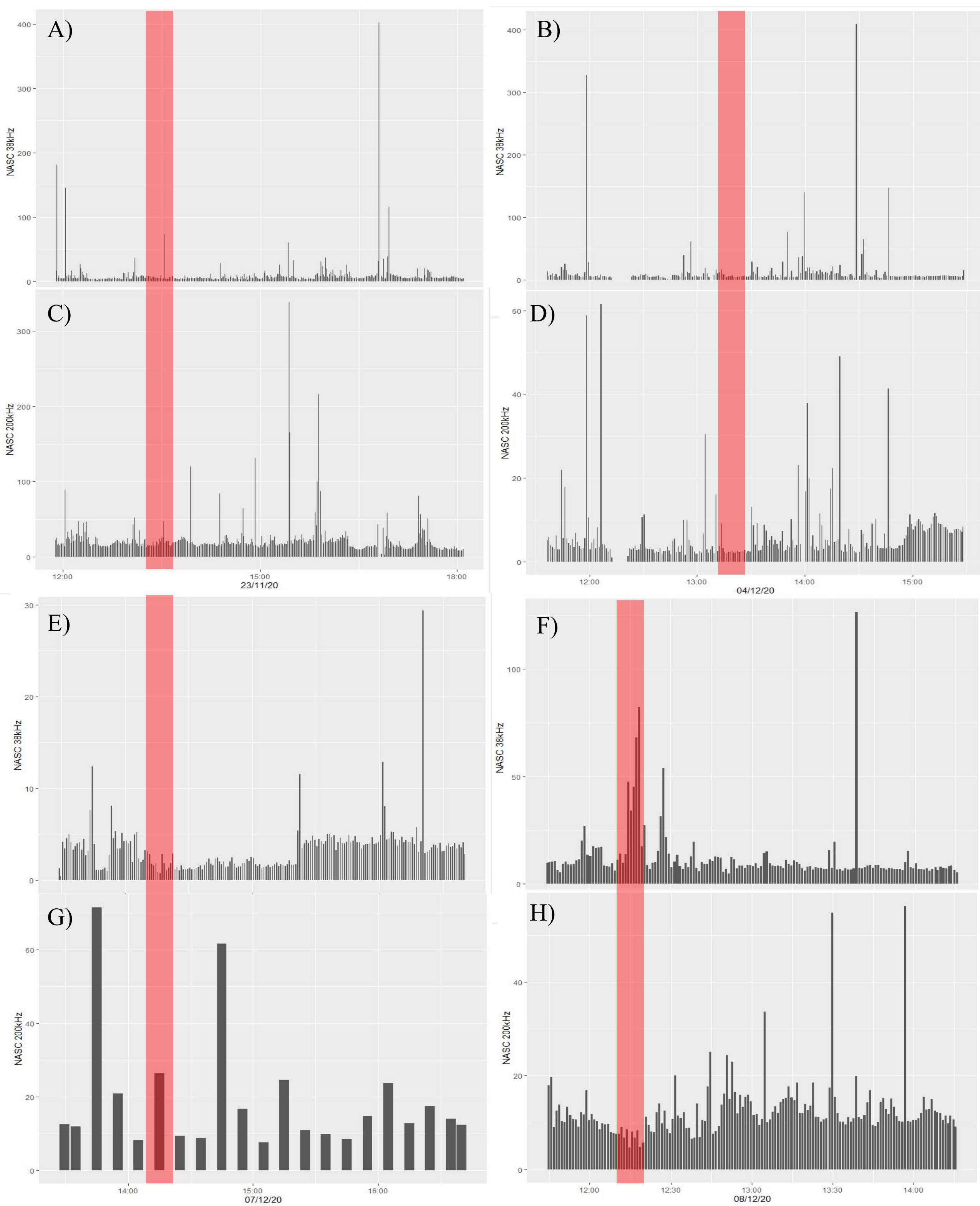

Figure 17. Average NASC values (in m^2/nmi^2) per half hour at 38kHz (A,B,E,F) and 200kHz (C,D,G,H) from the portable echosounder when 2D seismic was simulated. Periods when the seismic gun was operated are highlighted in red. A) November 23rd, 2020 B) December 4th, 2020 C) December 7th, 2020 D) December 8th, 2020.

3.3.3 Zooplankton Analyses

Taxonomic analysis showed that the most abundant copepod was *Oithona spp.,* comprising about 57% of all samples, followed by *Pseudocalanus spp.* at 21% and *Temora longicornis* at 11% (Figure 18D). Using a two-way ANOVA, there was no significant difference in estimated zooplankton mortality between samples collected before and after firing of the airgun (p=0.760) and no significant difference based on location (p=0.222) (Figure 18C).

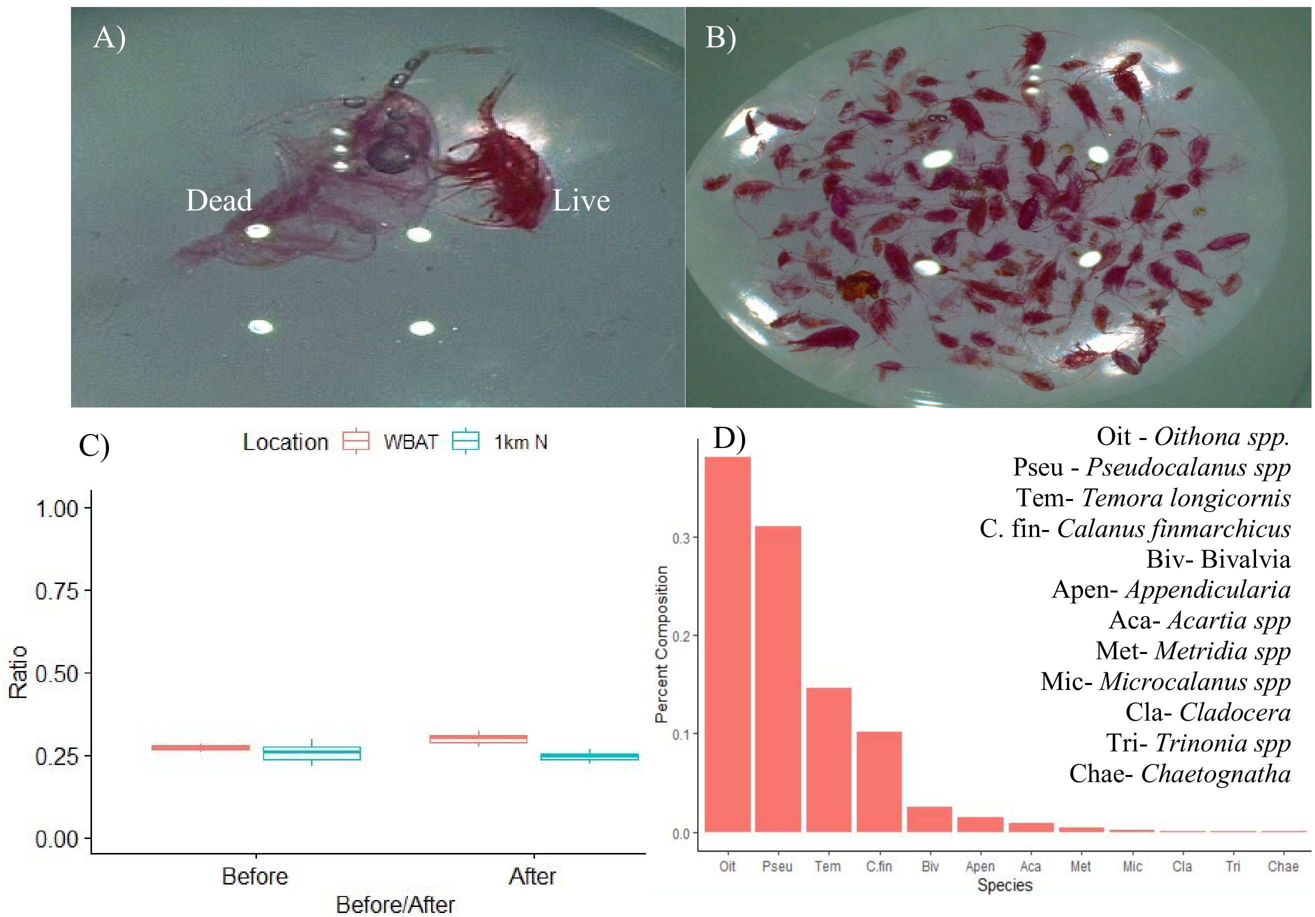

Figure 18. Images of zooplankton samples stained with red neutral. A) Alive and dead individuals from the net tow. B) Sub sample from the net tow. C) Ratio of dead versus total zooplankton counted at the location of the moored WBAT and 1 km north of the mooring, before and after the operation of the seismic gun. D) Species composition of zooplankton sampled at Station 27 on November 26th, 2020.

3.4 Discussion

3.4.2 Limited Seismic Noise Impact on Zooplankton

On November 18th, 2020 and November 23rd, 2020, when the blast site was directly over the WBAT, I expected to measure significant changes in zooplankton abundance and visually capture a hole or halo of avoidance due to the dispersion of zooplankton through the acoustic signal of the WBAT and EK-80, as described by McCauley (2017). Unlike McCauley et al. (2017), NASC values from the EK-80 on these days did not significantly change after firing of the airgun, nor was there any visual change in echograms. The only significant change was measured by the WBAT that captured a significant increase in backscatter at 333kHz 6 hours after the firing of the airgun. This 37% increase could be due to the drifting currents of zooplankton, but it was not seen on any other day of sampling. Zooplankton are known to form relatively high-density aggregations in the water column, resulting in patches that could also explain the variability in zooplankton abundance observed here (Omori and Hamner, 1982).

A change in backscatter was also measured on the EK-80 signal during the last day of sampling when a full seismic blast was achieved. During this trip there was significant change in NASC values recorded on the portable sounder before and after the seismic operations. Unfortunately, there was no WBAT data on this day as the mooring was released prior to firing of the airgun. The NASC values at 38kHz indicated an increase in fish abundance (Figure 17F) when the airgun was firing and at 200kHz, and a small but significant decrease in zooplankton (Figure 17H). Over the subsequent 2 – 3 hours, both fish and zooplankton biomass return to the variable abundance trends seen in previous days. This increase in fish and decrease in zooplankton was not seen on any other days of sampling. The day-to-day variability in zooplankton patchiness and currents can be a significant factor contributing to these patterns in these results. However, the airgun may have factored into these changes as well. More repeated exposures, and less logistical and experimental malfunctions, would have allowed for better indication if these changes in abundance were naturally occurring or influenced by airgun exposure.

Zooplankton net tows were limited for this experiment. For example, stained sampling post exposure to seismic noise occurred only on one day, collecting a total of 14 samples (8 replicates). There was no difference in net tow mortality rate between stations (WBAT and 1 km north of WBAT) nor between samples collected before or after firing the airgun, suggesting that the airgun did not impact the survival rate of zooplankton during this study. McCauley et al. (2017) noted significantly more dead zooplankton after airgun exposure, contradicting the mortality rate in this experiment despite using similar methods for both field experiments. Fields et al. (2019) completed a similar study in 2019 in Norway that assessed *C. finmarchicus* mortality rates during airgun blasting. In that study, zooplankton were contained in bags during the blasting, and placed at the same vertical depth as the airgun (6 m) and at different horizontal distances, ranging from 0.7 –25 m. These distances are in very close proximity to the airgun and since the zooplankton were contained without the ability to disperse, compared to McCauley et al (2017) where plankton were free to disperse and measured through larger net tows. Fields et al. (2019) concluded that at distances over 5m from airgun blasting there was no significant differences in the mortality rates of in zooplankton, compared to control group samples, whereas McCauley et al (2017) concluded there was significant mortality up to the full range of their study, 1.2 km from airgun blasting. Within the 5 m range of airgun blasting as reported in Fields et al. (2019), mortality was never greater than 30% above control samples. Fields et al. (2019) hypothesized that the differing results between these studies could be due to *C. finmarchicus* being more resilient to airgun blasting than other zooplankton species.

Despite the differences in study design, I would expect to see relatively similar results from both studies in terms of zooplankton mortality when using a small-scale airgun blast. Typically, lab studies due to their smaller size scale and more controlled environmental factors, discriminate smaller differences in a measurable way by controlling for known sources of variation versus field studies that incorporate a range of factors that are harder to control. Fields et al (2019) had more controlled sampling by bagging the zooplankton and fixing their position in the water column, but concluded contradicting mortality results than seen in McCauley et al. (2017). The airgun blasting experiment conducted at our coastal Newfoundland research site did not cause measurable mortality rates of zooplankton. However, due to the limited sample size used in our study, our statistical power and confidence is quite low, making it challenging for this research to definitively conclude that there are no impacts.

3.4.1 Limitations of the Study

In some trials the airgun was leaking air and did not pressurize to 2000 psi. In other cases, the airgun reached operating pressure but leaking continued and fewer shots could be generated at full pressure from a single tank. Hence, I identified two main issues with the airgun: 1) not getting up to the pressure required, and 2) not staying at that level for very long because the gas was being wasted due to a gas leak. With multiple scuba tanks I thought I had a pressure problem, so I switched to the large nitrogen tank to see if that was the problem. It was not a tank problem. It was discovered that the leak was caused by a faulty electric solenoid connecting the air-supply to the airgun, which caused a leak. The airgun was deployed after with the new solenoid and there was no air bubbling at the surface and a full pressure blast was achieved.

When the portable echosounder was connected directly to the vessel's power supply, there was a high level of electrical noise rendering the data unusable. The portable echosounder must be connected to a power source to function. A separate external battery was used to power the portable sounder but only enabled approximately 2 hours of data collection without being charged, providing strict time limitations offshore. Subsequent trips used the battery until it was depleted and then plugged in to the vessel, where electrical interference was evident and, in some instances, rendered parts of the data unusable.

3.4.3 Conclusions

The fall 2020 inshore survey saw many complications but was an excellent opportunity to test equipment and methods in the field prior to the spring 2021 offshore field season (Chapter 2). Weather constraints combined with Covid-19 restrictions contributed to issues and delays in trying to complete inshore experiments. Despite minimally significant results found during the Station 27 experiments, there is still a need to monitor and assess the effects of commercial seismic surveys on zooplankton. Repeating this survey in the spring combined with modified field plans and gear would reduce the risks of bad weather and increase the chances of a successful field experiment. Zooplankton staining methods using the bongo nets were successful and were replicated offshore using the same methodology as inshore. The airgun provided the most prominent issues with inshore testing. However, it was not required for offshore experiments as a commercial seismic vessel was used as the seismic source. Lastly, acoustic monitoring from both a vessel mounted and moored echosounders were deemed ideal for collecting the most data on fish and zooplankton abundance and behaviour over the course of the survey.

Chapter 4: Summary & General Conclusion

Oil and gas exploration in marine environments generates significant anthropogenic noise that potentially impacts marine organisms (Hildebrand, 2004; Morris et al., 2017; Williams et al. 2015). To date, limited knowledge exists regarding short-term and long-term impacts of noise on deep offshore environments, due to the large-scale investment of time, money and research required to monitor these ecosystems effectively (Carroll, 2017). This thesis is a cumulative result of almost 5 years of field experiments involving trial and error with acoustic instrumentation deployments and retrievals, zooplankton sampling techniques, and laboratory analyses. Through the extensive collection of acoustic data spanning just over 3 months, the entire length of the survey, I was able to monitor the effects of a long-duration 3D seismic survey *in situ.* I provided important baseline data regarding the *in situ* effects of offshore surveying on fish and zooplankton in an offshore environment, expanding on previous studies. Groundfish on the Northeast Newfoundland Slope have demonstrated resiliency and acclimation to elevated anthropogenic sound levels caused by seismic surveying conducted in this region since the 1960s. Seismic surveying in this area has been ongoing in our study region for over a decade (Pace et al. 2019). During this research, DFO has concurrently investigated the impacts of seismic surveys on groundfish through a project funded by the Environmental Studies Research Fund (ESRF) (Kowarski, 2016). It was not until 2021 that all the methods, technology and instrumentation were fully implemented to complete this large offshore project focusing on groundfish.

During the 2021 survey, acoustic data revealed groundfish in the area exhibited subtle behaviour modifications, such as concentrating lower in the water column and a decrease in overall abundance. The decrease in abundance can be attributed to their descent close to the bottom, as opposed to indicating fish leaving the area, which has been hypothesized in previous studies and is the main concern from commercial fishers. Furthermore, I measured little to no negative effects of seismic surveying on zooplankton. Importantly, new information pertaining to the continued effect of seismic surveying on fish behavior, lasting a period of just over 3 consecutive months, provides an added perspective not possible from shorter seismic studies that might span merely hours or days of exposure to seismic surveying noise. The overall duration of impact is short-term vertical displacement, and I initially hypothesized fish behaviour and abundance. This information can be further used in management and regulation practices that can help to mitigate impacts of offshore seismic surveying activities, including surveying that is allowed inside marine refuges. Further, there is unlikely a significant biological impacts from this change is depth.

The concerns over seismic surveys impacting fish populations from commercial fisheries are justifiable. As I demonstrated, the commercial fishers claim declined catch rates during seismic surveying, which was corroborated by some scientific studies (Carroll et al., 2017). Based on our findings, we suggest that these declines are likely due to fish swimming deeper in the water column, and baited camera work confirmed groundfish reduced their foraging behaviour. This overlap between the interest of oil and gas companies and commercial fisheries demonstrates the need for continued studies in areas undergoing seismic surveying.

Such studies are essential for enhancing our understanding of the impacts and, in turn, enable effective management and mitigation strategies for the coexistence of oil and gas operations, commercial fisheries and marine refuges. Fisheries are limited by seasonal availability of their catch, location of the catch and amount of the catch (DFO, 2023b). Seismic surveys have less limitations as they are only confined to the location allocated by their permits. Despite less limitations than fisheries, the seismic industry is also limited by the weather, which consequently results in an overlap between the fisheries and seismic industry. Management of commercial fisheries and oil and gas companies lies within minimizing the overlap between activities to ensure one is not causing detrimental losses to the other (Andrews et al. 2021). Other potential mitigation strategies between the industries and protection of marine ecosystems and fish stocks include:

1. **Timing**: Offshore exploration and commercial fishing overlap during the same parts of the year offshore Newfoundland. Both companies are restricted by the weather and adverse conditions are a large safety concern. Limiting the timing of these activities in certain areas could reduce this overlap of seasons.
2. **Location**: Zoning out fishing and oil and gas areas is a critical part of minimizing the overlaps between these activities (Andrews et al. 2021). By establishing areas where fishing vessels and oil exploration are exclusive the interactions between the two can be reduced and ensure the safety of the vessels and crew as well help protect important fish stocks.
3. **Communication**: By increasing productive communication between these industries via meetings and lines of communication information can be shared along with potential concerns and ways forward.
4. **Monitoring and Enforcement**: Monitoring and enforcing laws and regulations around these industries and mitigation officers offshore will ensure both parties are following their respective laws and regulations to limit negative overlaps.
5. **Continued Research and Collaboration**: Supporting the research into oil and gas impacts on commercial fisheries will provide more insight into the interactions and how to better manage the two industries using informed decisions and annual adaptations with new research.

Despite the overlap of seismic surveying and fisheries, limiting seismic surveys over short time spans, such as outside the cod spawning season, may be applied as mitigating measures (Bröker, 2019). Geographical and seasonal restrictions have been shown to be effective mitigation measures in Norway (Sivle et al., 2021) Cod are the most vocal during the spawning season, and these vocalizations are coupled with other behavioural patterns and are thus potentially more affected by the elevated sound levels from seismic surveys (McQueen et al., 2022). Cod form tall spawning columns, swimming vertically together as pairs, the females lay eggs while males fertilize them, the vertical motion allowing for maximum fertilization (Rose, 1993). They also form large spawning aggregations, the females concentrating deeper in the water column while males swim above performing courtship behaviours (Rowe and Hutchings, 2003; and Brawn, 1961). Seismic surveying could potentially disrupt male-female interactions, during courtship behaviours including spawning vocalizations. Vocalizations impeded by seismic surveys creates a 'masking' effect where the perception or detection of sounds is impeded by louder sounds (Pine et al. 2020). In contrast, McQueen et al. (2023) investigated the impacts of seismic surveying on cod and found no significant behavioural responses from the spawning fish. This opposes our conclusions, but McQueen et al. 2023 study only exposed spawning fish to 3-hour exposures over five-day periods, significantly shorter than our 4-month study (DFO, 2021; McQueen et al. 2023). McQueen et al., (2022) also investigated if cod leave their spawning grounds during seismic exposure over a 7-day period using telemetry, to which there was no significant displacement. This study supports these latter findings that seismic surveying does not appear to displace fish populations from their grounds. This is beneficial to maintaining healthy ecosystems, ensuring there is no abrupt changes caused by the dispersal of a fish population, as well as to the fisheries who use the same grounds for fishing and do not have to search new grounds for dispersed schools.

Monitoring these effects on long-term during spawning season will be critical in determining if acoustic surveys have a significant impact on spawning fish.

Development of renewable energy sources and the reduction in use of fossil fuels is currently on the forefront of environmental research in Canada and abroad (Government of Canada, 2023). The interest in transitioning to cleaner and more sustainable energy sources is growing on a global scale and combined with global warming concerns, has prompted revaluations of Newfoundland and Labrador's energy strategies (Government of Newfoundland, 2021). Efforts have shifted towards reducing the province's fossil fuel use and diversifying energy sectors by researching other renewable technology opportunities such as wind and tidal energy, while maintaining environmental protection, indigenous input, jobs and industry growth (Government of Newfoundland, 2021). Despite these efforts, transitioning away from a century-long reliance on carbon-based energy sources is not instantaneous. It is evident that fossil fuels will remain necessary for the foreseeable future, resulting in ongoing environmental impacts stemming from their exploration and extraction.

Furthermore, transitioning into these more sustainable energy sources can require the same techniques and technology used in traditional energy methods. For example, seismic surveying is also used to identify and plan offshore wind farms. The surveys provide detailed information on the seafloor and subfloor to map out the bottom-fixed support structures for the wind turbines (Alati et al., 2015). Therefore, as the shift towards renewable energy continues to gain momentum in Newfoundland, it is essential to acknowledge that the techniques used for carbon-based energy exploration, are also critical in planning and developing renewable energy infrastructure.

Offshore oil and gas production in the Newfoundland and Labrador region plays a significant role in the province's economy and energy sectors. The Jeanne D'Arc Basin is home to many offshore oil reserves, attracting major oil companies. Currently there are four offshore oil projects located on the eastern slope of the Grand Banks and north to Orphan Knoll, including; Hibernia, Hebron, Terra Nova and the most recent Bay du Nord (Bangay, 2020). The recent approval of the Bay du Nord oil project was controversial with Canada's clean and renewable energy goals, but the project is lined with conditions including emissions from this project must be net zero by 2050 (Guilbeault, 2022). Yet, the Canada-Newfoundland and Labrador Offshore Petroleum Board (C-NLOPB) actively accepted bids for the 2023 survey season in the Eastern Newfoundland region and southeastern Newfoundland region, totaling 72 225 km^2 and 49 823 km^2, respectively (C-NLOPB, 2023). The C-NLOPB recognizes these areas are important for commercial fisheries and some of the bidding areas overlap again with the Northeast Newfoundland Slope Marine Refuge (C-NLOPB, 2023). The projection indicates the seismic surveying to support oil and gas exploration offshore Newfoundland is thus forecasted to persist in the coming decades. Moreover, 4D seismic surveys are conducted regularly to inform the status of operational oil projects (LGL Limited, 2016).For example, a 4D survey was completed for Hibernia in 2013 and another 4D survey is planned for 2024 (LGL Limited, 2016). It is therefore imperative to conduct research regarding the potential effects on fish and zooplankton to inform necessary mitigation measures.

4.1 Reflections on Methodology

The main objective of this thesis was to investigate the impacts that seismic surveying had on fish and zooplankton abundance in an offshore environment, based on pilot studies that were conducted at DFO's Station 27 in the Fall/Winter of 2020. While monitoring fish offshore was a part of the data collection, it was not anticipated to be the main scope of this thesis. Due to unforeseen circumstances with the zooplankton sampling and vessel-mounted hydroacoustic monitoring at station 27, the analysis of fish abundance and behaviour from the WBAT deployment offshore became an integral part of this study. In addition, the single target fish tracking enhanced my understanding of the fish behaviour offshore in response to seismic surveying. To account for these changes, concurrent DFO projects involving baited cameras and sampling of fish helped to augment this thesis as well to ground truth the acoustic signals to identify species composition. The entire baited camera analysis was not completed as a part of this thesis due to the initial scope of this thesis pertaining to a small coastal study and then further expanding into an offshore study. The baited camera experiments were conducted separately and are expected to comprise a companion publication.

The zooplankton mortality component was one of the most significant challenges encountered during this study. Despite planning for sufficient samples sizes to achieve a high confidence interval, the number of offshore samples collected after seismic surveying was unexpectedly low due to bad weather. This was not due to any fault on the part of the field crew, but rather resulted from time constraints during the survey. Vessels are not permitted to approach within a few kilometers of a seismic survey vessel while it is surveying, as per Canadian regulations, and therefore working in very close proximity is not permitted. Consequently, while the samples collected were close to the survey area, they were not collected as close to the airgun source or as quickly after exposure as was obtained from inshore studies (i.e 50 m) due to these restrictions. The samples were not taken when the seismic vessel was within 10 km leaving a large uncertainty in if samples were an accurate collection of zooplankton mortality when seismic surveying is taking place. In our pilot study for instance, airgun blasting and sampling occurred in the same location, from the same vessel and with very little time in between. Despite the timing and distance differences in sampling, neither the offshore nor the station 27 sampling analyses found a significant change in mortality rates when seismic surveying was conducted. The inshore design was similar to McCauley et al. (2017), but found opposite results from the two to three-fold increase in mortality after airgun blasting they found in coastal Australian waters, largely due to the airgun issues we had.

Similar to the above issues with zooplankton sampling, the sampling vessel was equipped with an EK80 portable echosounder to monitor the top of the water column using a dual 38kHz and 333kHz transducer. This instrument would have provided data on the abundance and behaviour effects seen at the top of the water column, complementing the bottom effects monitored by our WBAT. Unfortunately, during each trip there were detrimental issues with the instrument, including system malfunctions, excess electrical noise, software failures and adverse weather conditions rendering this data unusable. On field trip one the portable sounder was mounted on the side of the vessel with a pole. These data were not usable due to excess noise caused by a combination mounting apparatus, electrical noise, and adverse weather. Although there were some problems with the equipment, the seismic vessel during this trip was also forced to reroute due to fishing vessels in the path. Seismic surveying vessels and fishing vessels do not have authority over one another in the Newfoundland and Labrador offshore, and occasionally industry interactions occur. During this research for example, a fishing vessel deployed fishing gear in the path of a seismic vessel that caused the seismic vessel to stop its intended survey, and in that instance it became logistically beneficial for the seismic vessel to operate in a different area entirely. That incident spoiled several scientific experiments, including acoustic experiments as part of this research, that relied on seismic noise exposure. Because the seismic vessel changed course, the timing of sampling and overlap with scientific equipment did not occur; the seismic blasting reached its closest proximity at 40 km away rather than passing directly over the moorings (Chapter 2).

During offshore trip two, in an effort to reduce noise detected by the ship-mounted echosounder, the mounting apparatus was modified to a keel-mounted transducer to eliminate pole interference and protect the transducer cord. The power source for the portable echosounder was also modified and separated from the vessel's power supply while collecting data. Although electrical noise was reduced, there was still too much noise to gather usable data. During the third trip the portable echosounder itself malfunctioned and was not replaced in time for the fourth trip. This created large gaps in the data, including all data from the third trip and post seismic data on trip four. It was determined that the limited data were not usable for the main component of acoustic analysis for this project. The portable echosounder is an excellent tool for offshore acoustic monitoring, but unfortunately for this project it did not function as planned.

Below are recommendations to operate the portable echosounder offshore:

1. It is essential to mount the echosounder with all cords internally in the boat to reduce the noise. If the cord is dragging too much it creates noise on the echograms.
2. The transducers should be mounted below the boat, attached directly to the hull (or on a fixed "fin") rather than to be mounted externally on the side. This will reduce wave collisions with the transducer and reduce noise.
3. The echosounder should be operated using an external battery. Using the ship's generator adds a layer of noise that can be visible on the echogram.

4.2 Future Work

Future research in this field should strive to build on our data collection and analysis and continue to monitor the effects of seismic surveys. The first recommended component for analysis would be to add the top of the water column in investigations. This would provide insight into whether the abundance and/or behaviour of zooplankton directly beneath the seismic guns are experiencing changes. My second recommendation would be collecting more zooplankton samples for mortality analysis. McCauley et al. (2017) used 6 plankton tows suggesting an appropriate sample size for bongo nets tows. McCauley et al. (2017) also counted between 500 and 1000 plankton per subsample, exceeding the approximate 300 individuals used in Elliott and Tang (2009) and both our offshore and station 27 study. My third recommendation would be extending the monitoring period, for example one month before and one month after the seismic survey. These hydroacoustic monitoring instruments are limited by battery power, ours was sufficient for 4 months, the entire duration of the survey. A stronger battery capable of operating over 6 months would allow for this extended monitoring period. A reduced sampling rate would also allow for an extended monitoring period but would potentially limit data acquisition during critical periods. A stronger battery could increase both length of the monitoring period but also increase acoustic sampling. I was limited to an acoustic sampling rate of every 30 minutes for 1-minute periods, a shorter sampling rate or longer sampling period would increase acoustic data significantly. A more powerful battery would also allow for an increased ping rate which would improve the target tracking analysis. Further, fish could be better monitored by using a hydroacoustic array. This would allow for individuals to be better tracked, giving more concrete evidence of their movements when seismic approaches, as well as help in understanding the horizontal movement of fish which we were unable to monitor during this experiment.

In conclusion, future work should continue to investigate long-term effects from seismic surveying on fish and zooplankton abundance and behaviour in combination with increased sampling using survey ranges > 60 km. Expanding upon this project will be critical in the management and mitigation of commercial fisheries and oil and gas operations as well as to better understand the response of offshore ecosystems to anthropogenic activities.

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Appendices

Appendix A. Equations used by Echoview Algorithms. Where: z=depth; $S_v(z)$=volume backscattering coefficient at depth z; H=water column depth.

Parameter	Units	Formula
Proportion Occupied	-	$P_{occ} = \frac{\int z \mid S_v\,(z) > S_v{}^{Thresh} dz}{H}$
Centre of Mass	m	$CM = \frac{\int z s_v(z) dz}{\int s_v(z) dz}$
Aggregation Index	m^2	$AI = \frac{\int s_v(z)^2 dz}{(\int s_v(z) dz)^2}$
Nautical Area **Scattering Coefficient**	m^2nmi^{-2}	$NASC = 4\pi * 1852^2 * ABC$ $ABC = 10^{\frac{S_v}{10}} * T$