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An Assessment of the Potential Risks of Seismic Surveys to Affect Snow Crab Resources

Évaluation des risques potentiels liés aux relevés sismiques sur les ressources de crabe des neiges

Canada

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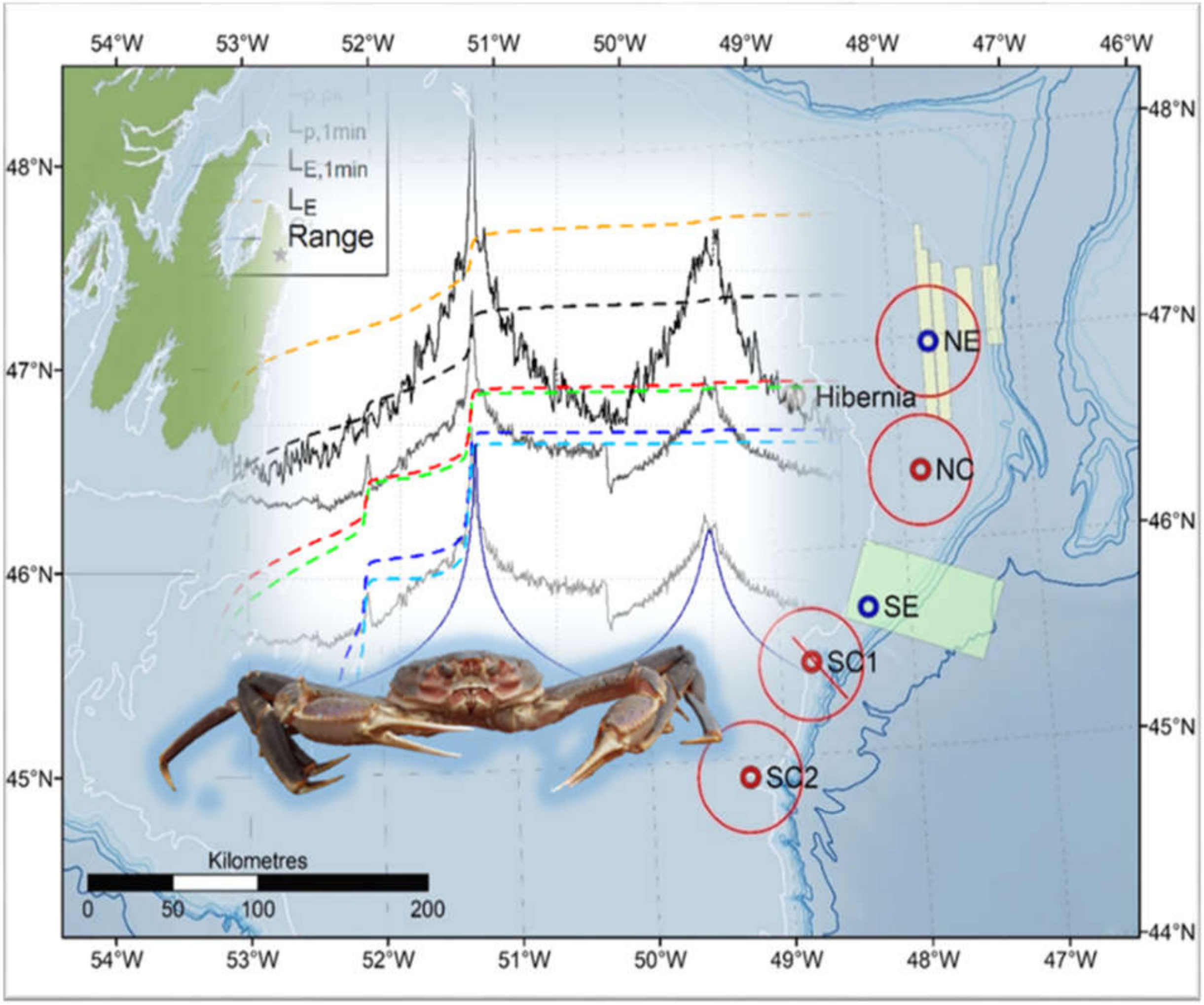
An assessment of the potential risks of seismic surveys to affect Snow Crab resources (ESRF Project 2014-01S)



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Executive Summary

Concerns were expressed by Snow Crab harvesters in the Newfoundland and Labrador region about the potential impacts of seismic oil and gas surveying on catch rates near commercial fishing areas. The impacts of ocean noise are a known societal concern, heightened by significant gaps in ecological understanding of the potential existing and future long-term effects on marine life. This study, funded by the Environmental Studies Research Fund (ESRF), was conducted in collaboration with local stakeholders, with significant participation from the snow crab fishing industry, from the planning stages through to completion. The purpose of this project was to examine effects of seismic exploration on the commercial Snow Crab fishery.

The field experiments were conducted under realistic operational seismic exploration surveying on commercial Snow Crab fishing grounds in offshore areas of Newfoundland and Labrador, and examined effects on Snow Crab catch rates, movement, physiology, and genomic response. The field study was replicated each summer, when both seismic and fishing occurs, over a 4-year period. Sampling was conducted in areas impacted by seismic surveying and other areas that were not impacted by seismic surveying as study controls. Sampling at all locations was conducted before and after seismic surveying for comparison.

This research did not measure consistent statistically significant impacts of seismic oil and gas exploration on commercial Snow Crab. Catch rates were inconsistent, higher in one year and lower in another year for experiments that exposed Snow Crab to extended periods (days-weeks) of seismic exposure (3D surveying), and no difference was detected in catch rate for all short-term (hours-days) exposures (2D surveying) to seismic surveying. The behaviour of Snow Crab exposed to seismic surveying supports the catch rate information; analysis of movement patterns found no significant differences owing to seismic surveying. There was also no evidence of physical damage to internal organs or based on histological examination, which confirmed expectations. Genomic effects of seismic surveying on sound-responsive genes also supported the physiology results, showing inconsistent results from one year to the next and did not show evidence of significant effects. However, environmental variables such as temperature, depth,

time of day, and different locations, had measurable effects on catch rates and the movement of snow crab, thus the analysis was sensitive enough to account for sources of natural variability.

The conclusion from this research is that if seismic surveying impacts commercial snow crab, based on factors considered by our experiments, it is within the range of natural variability. Consistency among several independent measurement metrics used in this study, including measure of catch rate, movement, physiology and genomic response, adds considerable weight-of-evidence support to this conclusion. It should be noted that the Snow Crab fishery only catches large terminally moulted mature male Snow Crab, and this study did not explore potential impacts on juvenile or female snow crab. Findings from this research can help guide future research and decision making in the short, medium, and long term. Results are being used to provide important and accepted science-based management advice to regulators, interested and affected industries, and the general public.

Sommaire

Les pêcheurs de crabes des neiges de la région de Terre-Neuve-et-Labrador ont exprimé des inquiétudes quant aux impacts potentiels des relevés sismiques relatifs au pétrole et au gaz à proximité des zones de pêche commerciale sur les taux de prises. Les impacts du bruit océanique sont une préoccupation connue dans notre société, qui est aggravée par des lacunes importantes dans notre compréhension écologique des effets potentiels actuels et à venir sur la vie marine à long terme. Cette étude, financée par le Fonds pour l'étude de l'environnement (FEE), a été menée en collaboration avec les intervenants locaux et avec la participation considérable de l'industrie de la pêche au crabe des neiges, depuis les étapes de planification jusqu'à son achèvement. Ce projet visait à examiner les effets de la prospection sismique sur la pêche commerciale du crabe des neiges.

Les expériences sur le terrain ont été effectuées dans le cadre de relevés sismiques opérationnels réalistes de prospection sur les lieux de la pêche commerciale du crabe des neiges dans les zones extracôtières de Terre-Neuve-et-Labrador, et ont permis d'examiner les effets sur les taux de prises, les mouvements, la physiologie et la réponse génomique du crabe des neiges. L'étude sur le terrain a été répétée chaque été, au moment où les relevés sismiques et la pêche ont lieu, sur une période de quatre ans. L'échantillonnage a été effectué dans les zones touchées par les relevés sismiques et dans d'autres zones qui n'ont pas été touchées par celles-ci, en guise d'échantillons témoins. Dans toutes les zones, l'échantillonnage a été effectué avant et après la prospection sismique, aux fins de comparaison.

Cette recherche n'a pas mesuré d'incidences statistiquement significatives et cohérentes de la prospection sismique du pétrole et du gaz sur le crabe des neiges commercial. Les taux de prises étaient irréguliers, élevés une année et faibles une autre année, dans le cas des expériences qui ont exposé le crabe des neiges à des périodes prolongées (jours-semaines) aux relevés sismiques (relevés 3D). Aucune différence n'a été détectée dans le taux de prises pour les expositions à court terme (heures-jours) aux relevés sismiques (relevés 2D). Le comportement du crabe des neiges exposé aux relevés sismiques corrobore l'information relative au taux de prise; l'analyse des habitudes de déplacement n'a révélé aucune différence significative attribuable aux relevés

sismiques. Aucun signe de dommages physiques aux organes internes n'a été observé, et il en va de même à la suite d'un examen histologique, ce qui a permis de confirmer les attentes. Les effets génomiques des relevés sismiques sur les gènes sensibles au bruit ont également appuyé les résultats physiologiques, en donnant des résultats incohérents d'une année à l'autre, et aucun signe d'effets notables n'a pu être observé. Cependant, les variables environnementales telles que la température, la profondeur, l'heure de la journée et la diversité des lieux ont eu des effets mesurables sur les taux de prises et les déplacements du crabe des neiges, démontrant que l'analyse était suffisamment sensible pour pouvoir détecter les sources de variabilité naturelle.

Selon la conclusion de cette recherche, si les relevés sismiques ont une incidence sur le crabe des neiges, en fonction des facteurs pris en compte par nos expériences, ces incidences se situent dans la limite de la variabilité naturelle. La cohérence entre plusieurs mesures indépendantes utilisées dans le cadre de cette étude (notamment la mesure des mouvements, de la physiologie, de la réponse génomique et du taux de prises) permet de renforcer considérablement cette conclusion. Il convient de noter que seuls les gros spécimens mâles de crabes des neiges après la mue terminale sont capturés par les pêcheurs de crabes des neiges, et que cette étude n'a pas examiné les répercussions éventuelles sur les jeunes crabes des neiges ou sur les femelles. Les conclusions de ces recherches peuvent aider à orienter les recherches et les décisions à court, à moyen et à long terme. Les résultats sont utilisés pour fournir d'importants conseils de gestion scientifiques et acceptés à l'intention des organismes de réglementation, des industries intéressées et concernées et du grand public.

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I. Introduction

In recent years the Newfoundland and Labrador Snow Crab (*Chionoecetes opilio*) fishery has experienced declines in stock abundance and considerable effort is underway to better understand factors affecting the crab resource. The Snow Crab fishery is currently the highest landed value fishery in the Newfoundland and Labrador region (>$300M per year – see Snow Crab Integrated Fishery Management Plans for details; https://www.dfo-mpo.gc.ca/fisheries-peches/ifmp-gmp/snow-crab-neige/2019/index-eng.html#toc3) and shellfish (American lobster, Snow Crab, northern shrimp) are the highest value fishery sector in eastern Canada (https://www.dfo-mpo.gc.ca/stats/facts-Info-16-eng.htm). Snow Crab harvesters in the Newfoundland and Labrador region are concerned about the potential impacts of seismic oil and gas surveying near commercial fishing areas. The potential effects on Snow Crab were identified as a significant information gap by the Environmental Studies Research Fund (ESRF) in the 2014 call for research proposals, which funded this project. This ESRF project was conducted to address this important information gap related to effects of seismic exploration on the commercial Snow Crab fishery. Specifically, the information gap targeted by this project was the potential impacts of realistic seismic surveying on commercial Snow Crab resources, on important fishing grounds, including effects on crab catch rate, movement, physiology, and genomics.

The Environmental Studies Research Fund has a mandate to fund environmental and social studies pertaining to the manner in which, and the terms and conditions under which, petroleum exploration, development, and production activities should be conducted on Canada's frontier lands. Seismic surveying has been called into question, among other factors including changing environmental conditions and the fishery itself, as potentially contributing to the decline in Snow Crab abundance. Understanding potential impacts of seismic surveying on the Snow Crab fishery, and establishing acceptable mitigation measures are relevant to ESRFs goals and objectives.

Impacts of ocean industrialization including the impacts of noise are a societal concern, heightened by significant gaps in ecological understanding of the potential existing and future long-term effects on marine life. Marine noise levels in the world's oceans doubled in each of five consecutive decades between 1950 and 2000, in large part due to shipping (Andrew et al 2011). The use of seismic airgun arrays to survey for oil and gas deposits under the seabed also propagates sound throughout the world's oceans (Nieukirk et al 2012, Nowacek et al 2015). Scientific reviews have characterized concerns over marine noise (Hawkins 2015; Hildebrand 2009). Most noise related research, and considerable scientific information and regulations, exist to mitigate against sound levels known to have damaging impacts on marine mammals. Far less is known about the effects of noise on other marine animals, such as fish and invertebrates, and there are few (if any) established science-based mitigation measures for marine fish or invertebrates with respect to noise. There are ~140 species of marine mammal compared to ~30,000 species of fish and ~50,000 species of crustacean, therefore the information gap is very large. Moreover, humanity relies heavily on marine fish and invertebrates as a global food source that supports a large commercial fishing industry. While research on marine mammals is important, and indeed these animals are particularly sensitive to noise, improving scientific knowledge to better understand and mitigate or eliminate potential impacts on marine resources and industries is also an important responsibility.

Seismic oil and gas exploration activity is currently necessary to discover hydrocarbon resources, and has been ongoing periodically in Newfoundland and Labrador waters since the 1960's. Until the 1990s this work included broadly spaced seismic survey lines spread over large areas, i.e. 2D surveys. Since 1990, seismic surveying has also included more site-specific surveys that expose smaller areas to seismic noise for longer periods of time, i.e. 3D surveys. Oil and gas interests in Newfoundland and Labrador continue to grow, with some of the largest investments to date occurring in most recent years (https://www.cnlopb.ca/information/development/), therefore, it is likely that seismic surveying will also continue in Newfoundland and Labrador waters in coming years. Commercial fish harvesters in Newfoundland and Labrador are concerned that Snow Crab catch rates are lowered in proximity to seismic surveying in this region, where overlap occurs between industries.

Some Snow Crab harvesters have reported decreases in Snow Crab catch rates that developed over a short time period, within a season, while fishing on the Newfoundland Grand Bank. They are concerned such declines could be linked to seismic exploration activity. However, understanding of the invertebrate auditory system and the impacts of man-made noise is lacking, and scientific information was not available to evaluate the severity of this potential impact. In the absence of known effects, an important starting point is to conduct realistic scientific experiments and observations, including actual industry seismic surveying activities (fishing and oil and gas activities) on commercial snow crab fishing grounds to measure effects.

Some preliminary studies are available on snow crab exposed to seismic sounds in the environment. This includes a pilot field study carried out in coastal Newfoundland with an array of 12 air guns (Christian et al 2003; 2004) as well as a study carried out in offshore Nova Scotia in conjunction with a seismic survey (Courtenay et al 2009). The controlled and realistic experiments conducted as part of this field experiment, examining effects of seismic surveying on commercial Snow Crab resources, have not previously been conducted. The field experiments conducted as part of this ESRF study included realistic operational seismic exploration surveying and commercial fishing activities, replicated each season over a 4 year period, that measured effects on commercial Snow Crab and its fishery. The design was based on recommendations identified in peer-reviewed scientific literature that included a Before-After-Control-Impact (BACI) study design. The project incorporated this design and examined effects on commercial catchability, movement behaviour, physiology, and genomic effects. The collective goal of this research was to provide important and accepted science-based management advice to regulators, interested and affected industries, and the general public. Thus it was important that the project was developed through regularly conduced (twice per year) open and transparent consultations with all interested parties, from the planning stages through to project completion. This report provides an overview of the scientific research conduced. The scientific results are published in scientific peer reviewed literature, open-access copies and online-links to these publications are appended.

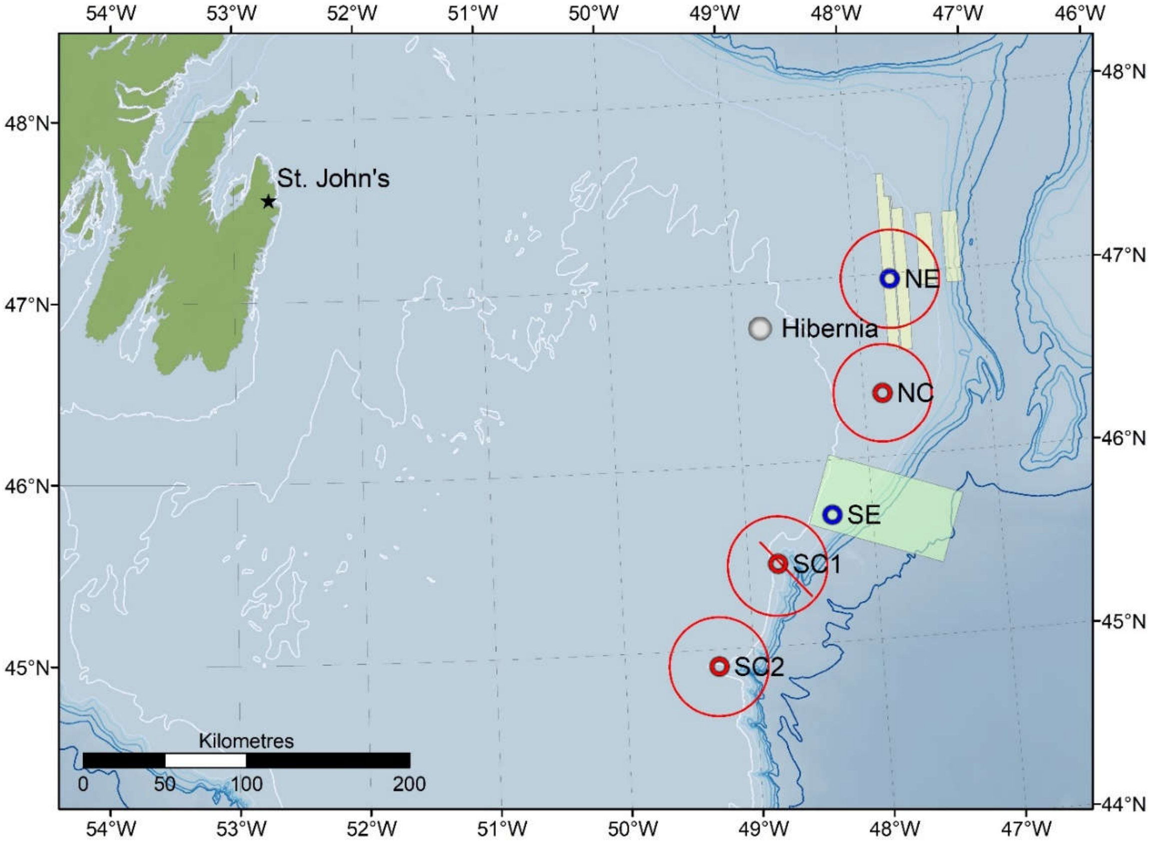

Figure 1a. Map of the ESRF Snow Crab study areas along the Newfoundland continental slope, a prominent commercial Snow Crab fishing area and location of oil and gas exploration / production. The square yellow and green areas represent 3D seismic surveys, and the red line through SC1 is the location of multiple replicated 2D seismic exposures. Commercial Snow Crab fishing as part of this experiment was conducted at each of the small, labeled circle locations. The larger circles are areas where the timing of seismic surveying was regulated as part of this study design.

II. Methods

II.A. Study site selection

This project was conduced as a collaboration among the fishing industry, oil and gas industry, regulators (DFO and CNLOPB), and scientists at DFO and Memorial University of Newfoundland (MUN), and private industry (JACSO Applied Sciences and Oceans Ltd). Harvesters, and representatives of the Fish Food and Allied Workers (FFAW) union that represent most Snow Crab harvesters in the Newfoundland and Labrador region, took part in the initial planning meetings for this project and identified the preferred study location. The location identified by harvesters was particularly important since harvesters made original observations and speculated that commercial Snow Crab might respond rapidly to seismic survey noise, based on their observation of commercial catch rates. To detect this response, commercial Snow Crab harvesters suggested that the study be located on commercial fishing grounds where seismic surveying occurs, and in an area of rapidly changing depth. Therefore the study was located on commercial fishing grounds along the edge of Newfoundland Grand Banks continental shelf, at Carson Canon with a control site at Lilly Canyon, some 350-380 km east of St. John's Newfoundland and Labrador, Canada. An additional study area was selected further north in 2018, to specifically examine effect of 3D seismic surveying on catch rates. The addition of this second study area, which included both a test site and a control site, improved our confidence that location was not affecting our observations. This more northern study location was also an important commercial fishing area, and did not have large changes in depth over short distances.

II.B. Seismic survey exposures

Seismic surveying has taken place in Newfoundland and Labrador waters since the 1960s and has increased in recent years largely through the increase in 3D-type seismic surveying (Figure 2). No other studies in this region, and few other experiments globally if any, have incorporated actual industry-based seismic surveying into replicated over several years and controlled experimental study designs to test for measurable impacts on an invertebrate fishery (or other commercial species). The lack of realism in scientific studies is a gap in understanding the actual impact of seismic surveying on marine resources. As such, the largest breakthrough of this project was the incorporation of actual seismic surveying, including both short duration exposures (a single 2D survey line over 5-8 hours) and longer duration exposures (3D survey lines over several weeks) as part of controlled and replicated field experiments on commercial fishing grounds. Importantly, the exposure of Snow Crab to seismic survey noise was representative of a wide range of seismic oil and gas exploration activities, ranging from realistic minimum (2D) to maximum (3D) levels of exposure, taking place on commercial fishing grounds. These realistic conditions are important in order to provide meaningful management advice for regulatory agencies and marine industries not provided previously.

We incorporated 2D single pass exposures during three field seasons (2015-2017), and we also included 3D survey-noise exposures (several weeks of seismic exposure in the same general area) in two seasons (2017 and 2018). In all cases an operational seismic survey vessel approached from a distance and moved directly over our sampling areas and sound recorders. This approach incorporates the complete range of seismic noise (both intensity and duration of noise) exposure that animals experience in the wild from industry-based seismic surveying. Sampling was also conduced at control sites with no seismic exposure, and sampling was conduced at all sites before (or during) and after seismic surveying. We measured both sound pressure and particle motion on the seabed where the crab are located, and collected baseline noise levels and modelled sound transmission. Experimental testing considered seismic noise impacts that included periods of time that the noise level was above that produced by our typical fishing vessel. Daily sound exposure level, SEL, was the measurement metric used to describe the sound exposure experienced by Snow Crab. This metric is a cumulative measure of the sound that crabs are exposed to in a single 24 hour period, and is a recommended metric in studies such as this. Sound metrics are important in order to compare different studies and to establish operational policy based on observed effects from field studies. For this experiment sound metrics were important to ensure that the sound level at our control areas are low, and similar to or less than the noise levels produced by regular fishing activities (fishing vessel noise). While fishing vessels themselves produce noise, harvester concerns addressed in this study are focused on noise levels greater than that of fishing vessels, produced seismic surveying.

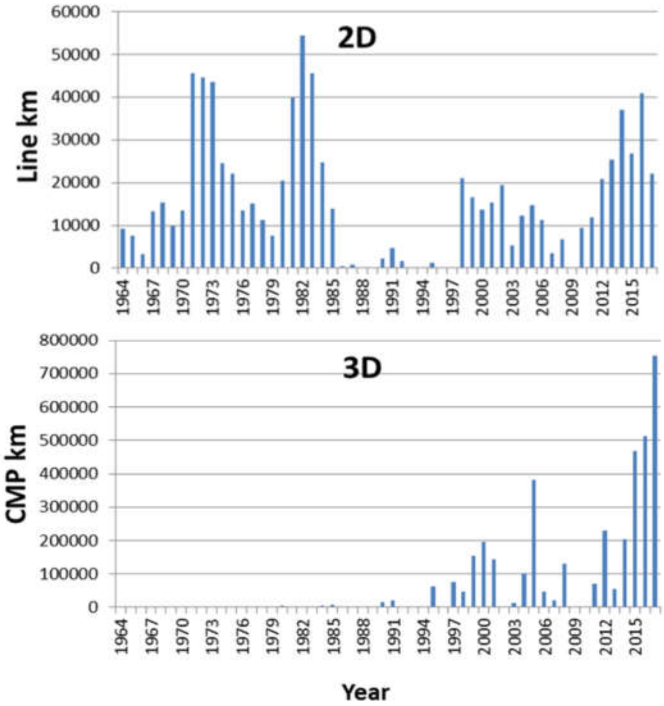

Figure 2. Seismic surveying efforts in Newfoundland and Labrador since 1964, based on the number of lines of seismic (2D) or equivalent common midpoint (CMP) measure (3D). Data was obtained from the CNLOPB website.

II.C. Experimental study design

Having extensive support and involvement from the fishing industry and oil and gas industry, the next challenge was ensuring that our experimental design was sufficient to provide valuable information for industries and regulators. Our offshore field experiment followed a Before-After-Control-Impact (BACI) study design. This approach has been recommended in scientific literature, and is an improvement over studies that do not have control sites. We also conducted separate studies at two different study areas to remove possible effects that could be related to a specific site. We included a control site and experimental site at each study area. As part of the BACI design, we included several measurement metrics as indicators of effect. We measured Snow Crab commercial catch rates, movement, physiology (examining blood / hepatopancreas / muscle), and genomic indicators. Including a multifaceted approach as part of the same BACI study design provided a robust account of potential impacts and overall impact severity.

Laboratory studies complemented our field observations to provide longer term observations and experimental data on mortality, feeding, molting, that we could not observe in the field. For the laboratory work, Snow Crabs were collected from the offshore study areas and brought to Fisheries and Oceans laboratory facilities in St. John's for observation and experimentation. We also collected additional Snow Crab from areas that were not affected by seismic. Snow Crab were held in captivity for at least 2 years. The primary focus of this ESRF study was field research, and while the laboratory work was very valuable, it was conduced primarily to support and validate concerns associated with field work.

II.D. Statistical analysis

A well-established study design enables a strong analytical capability through statistical and mathematical analysis. Typically, when effects from a specific "treatment" are large, and there are no confounding effects from other factors, the interpretation of results comes with a high level of confidence. However, when no statistical differences are detected, either because the effect was small or because sampling had a lot of variability in the data, it is more difficult to detect and determine if a specific treatment had an impact. This is the case in many marine field studies, because the ocean is a dynamic and difficult environment to monitor, where several factors can influence results over large spatial areas, in addition to the experimental treatment. It is possible that an impact could have occurred but went undetected in the data, or alternatively an unknown factor could cause observed differences. In general, this study did not measure obvious or large effects, and in order to reasonable defend our conclusion we relied on a robust study design with replication, and results from multiple study indicators, to inform our interpretation. Data analysis also included simulations, power analysis, post-hoc testing, and several mathematical modelling approaches such as state-space modelling, generalized mixed effects modelling, behavioural changes point analysis and other approaches, to examine trends in the data and to explain observed variation owing to different contributing factors. This included principal co-ordinate analysis and multivariate comparisons. The details of the various mathematical and statistical approaches are described within individual peer-reviewed scientific publications produced from this research project (see appendices) and results section in this report. Importantly, our study design allowed comparison between the relative magnitude of effect from seismic surveying with the range of natural variability in the absence of seismic surveying.

II.E. Project chronology

A total of 4 field seasons were completed as part of this study. Each year, scientific field work consisted of three offshore research trips, each approximately 8 -10 days duration. The primary indicators of effect for this experiment included catch rate, movement behaviour, genomics, and physiology. Catch rate was measured in each field experiment. In 2015 we established and tested only one of two field sites with acoustic telemetry receivers (Carson Canyon). In that year the study targeted catch rate, and conducted limited acoustic telemetry to validate the methodology, to be expanded in future years. This was a go no-go decision point in our project, because there was uncertainty associated with our new and previously untested field methods. Until this work was validated, it was also agreed upon to postpone genomic and physiology components until the following year (2016), when we had a proven study design.

The 2015 study was a breakthrough success, not only did we have logistical success with acoustic telemetry, we were able to incorporate sound exposure from an operational 2D seismic survey and conduct experimental commercial fishing with reasonable catch rates. This enabled the project to collect valuable data during the first year of study, which exceeded expectations. Results were compiled and presented to ESRF and stakeholders in March 2016. During the following 2016 summer field season we conducted the full study design, which included a control and experimental study site, as well as all proposed measurements; catchability, movement, physiology, and genomic sampling and analysis. We also included multiple sound recording instruments on the sea-floor. A 2D seismic exposure was conducted as part of our before-after-control-Impact (BACI) study design, and it replicated the study conducted in 2015. Results were prepared and presented in March 2017 and a peer-review paper was published in the journal Fisheries Research in September 2017. This was a significant and early first-deliverable from this ESRF study.

The field program in 2017 was also a full scale field study that replicated the 2016 initiative, however, 2017 also included sampling and noise measurements from a 3D seismic survey. During 2017 an additional sampling site was added and we investigated catch rate as well as physiology sampling at the site of 3D seismic surveying. Data collected in 2017 indicated a significantly lower catch rate at the 3D seismic survey site. The decreased catch rate occurred while seismic surveying was ongoing, and two of eight physiology indicators were also significantly different both during and after 3D seismic exposure. However, the 3D results from 2017 consisted of only during-after data and lacked suitable control data. In addition, the catch rates were very low and physiology sampling lacked baseline comparisons. These results were presented in March 2018 and it was decided that another field season was needed to validate the important data-measurement collected in 2017, using a more robust dataset enabling a complete BACI design.

During the last field season in 2018 sampling enabled a BACI study design and included sound measurement, commercial catchability metrics, and physiology sampling for only those indicators that showed significant differences in 2017. The 2018 study was also conducted at a new study location, to remove the possibility that the 2017 site conditions might affect sampling variability. In 2018, the study included a control site and additional sampling (4 trips rather than

3) to better resolve temporal differences or an intermediate level of seismic 3D noise exposure. The final field season was completed and results were presented to the ESRF management board and ESRF stakeholders in October and November 2018. During 2019 as data analysis was finalized, the results were presented publicly and to individual interested stakeholders, at a Snow Crab resource assessment meeting held by DFO, and at a DFO Canadian Science advisory Secretariat consultation regarding the Canadian Statement of Practice with respect to seismic surveying. In November 2019 the final results and consultations to date were summarized at a Stakeholders meeting to complete the project.

II.F. Study design considerations

This project addressed many of the design problems reported in scientific literature that have hampered some earlier studies and the state of knowledge regarding effects of seismic surveying. In particular, many of the recommendations identified in a critical review of the potential impacts of marine seismic surveys on fish and invertebrates by Carroll et al (2017) and references within, have been addressed during this project, including; refinement of sound measurement standards, consideration of sound propagation, particle motion measurement, integration of lab and field studies, examining masking, meaningful collaboration among industries affected, publication of data (i.e. genomic gene libraries) and scientific reports, and controlled replicated realistic experiments. We also followed a BACI study design with 2 study areas each having test and control sites, incorporating realistic sound exposures using operational seismic survey vessels and commercial fishing methods, incorporated into a field experiment where commercial fishing and oil and gas explorations activities co-exist. In addition, because of expected high variability associated with sampling marine environments, and a limited ability to conduct many replicates, we included a weight of evidence approach by investigating several different metrics at the same time, including catch rate, behaviour, physiology, and genomic indicators of effect.

General ecological understanding about the effects of noise on marine mammals is further advanced than for fish and invertebrates. Several metrics exist that describe known harmful physiological impacts on marine mammals for a given noise level. Far less is known for invertebrates, and the focus of research is largely targeted towards identifying effects rather than establishing acceptable noise limits. This creates important information gaps when trying to measure, describe, and create noise-related management measures; all of which cannot be addressed in a single study. Therefore, some compromise is needed to prioritize and target specific research questions that can be addressed-well in a single investigation to fill important information gaps. This project focused on commercial Snow Crab, which only includes large mature males, and it does not address other life history stages or female Snow Crab. There are also known trade-offs related to the type of study conducted; on one hand, basic controlled laboratory research is often criticized because it is hard to extrapolate results to the "real world". On the other hand, real world research is expensive, limited in scope, and burdened with natural unexplained and difficult to control variability. Hence, both approaches (laboratory-like and real-world) are needed to make progress in understanding the impacts of marine noise and its relevance to marine management. One means to account for large natural variability is to include many experimental replicates, which is difficult in the case of seismic surveying. Alternatively, this study included multiple lines of evidence to detect effects to compare results and infer impacts. Although the type of research conducted in this study (direct realistic field observations, multifaceted measurement metrics, and companion laboratory studies) is rare, largely because of the high cost and logistical coordination and co-operative complexity among stakeholders, and therefore the results are an important stepping stone for many future studies that will hopefully test and re-examine our conclusions in much greater detail. Therefore this and future work will be an important contribution to our ecological understanding of the impact of seismic surveying noise.

III. Results

The results of this ESRF project are published as several independent manuscripts in peer reviewed scientific literature. Much of the text and figures included here come directly from those open-access publications, published in Fisheries Research and are publically available. The overall results are described briefly here, and the individual manuscripts are included in the appendix of this report, that includes more detailed information (see appendices). These publications build together to provide a comprehensive overview of the results, both complementing each other's findings while continuing to refine the exploration of information to assess potential effects of seismic surveying on commercial Snow Crab resources. Initially we developed and published a paper describing the most appropriate sound metric to evaluate effects on Snow Crab relevant to its fishery (Martin et al 2019; Appendix 1: https://doi.org/10.1121/1.5113578) . Then we explored effects of short-term relatively low exposures from 2D seismic surveying on commercial catch rates Morris et al 2018; Appendix 2: https://doi.org/10.1016/j.fishres.2017.09.012) and then relatively high exposures from 3D seismic surveying on commercial catch rates (Morris et al 2020; Appendix 3: https://doi.org/10.1016/j.fishres.2020.105719). The results did not measure significant impacts consistently or predictably. Catch rates ultimately depend on Snow Crab movement, therefore to examine potential effects in greater detail we explored the effects of seismic surveying on Snow Crab movements using acoustic telemetry monitoring (Cote et al 2020: Appendix 4: https://doi.org/10.1016/j.fishres.2020.105661). Again, the movement information was not conclusive in terms of detecting a clear impact of seismic surveying. It is possible that impacts on animal physiology could affect Snow Crab movement, health, or even survival, thus the fourth study investigated effects of seismic surveying on Snow Crab genomic response (Hall et al 2020; Appendix 5: https://doi.org/10.1016/j.fishres.2020.105794) and physiology These papers look in detail, for the first time, at the genomic response of Snow Crab to noise. Again the results from this research supported the findings of complementary work, and results differed among years with respect to seismic exposure. If effects of seismic surveying exist, they are relatively subtle within the scope of natural variability measured in the absence of seismic surveying. This wide scope of interrelated investigation was needed to rule-out the likelihood of significant adverse impacts of seismic surveying impacts on the commercial Snow Crab catch rates. Replication of studies in several years also helped to build confidence in the interpretation of results. This does not mean that there are not effects of seismic surveying on Snow Crab – it means that we cannot easily measure impacts on large commercial male Snow Crab within the range of natural variability. Furthermore, we did not investigate whether seismic surveys could impact other life history stages or female Snow Crab that are not part of the commercial Snow Crab fishery.

A direct measure of the concerns expressed by Snow Crab harvesters was obtained by examining a realistic situation experimentally, measuring the impact of actual seismic surveying on commercial fishing activities, located on commercial fishing grounds. The challenge for our study was isolating the effect due to seismic from natural variability and assessing the relative contributions of different factors such as differences caused by seismic noise, temperature, depth, location, timing, currents, food availability etc. Our field experiments measured considerable natural variability. To deal with variability, our study went to great lengths to incorporate proper study design, with detailed sampling, controls, replication over several years, extensive statistical and mathematical modeling, simulation analysis, and power analysis. We also incorporated several separate indicators-of-effect measured simultaneously as discussed previously. During seismic exposures we measured catchability, movement behaviour, genomic responses, and physiological effects, to assess the level of impact. Having a broad suite of indicators helped to provide inferences of impacts over a range of potential mechanisms, adding confidence in the interpretation of our results. In addition, we collaborated with a population biologist and considered other factors affecting Snow Crab such as migration (Mullowney et al 2018; https://doi.org/10.1007/s11160-017-9513-y), and environmental conditions and the fishery itself (Mullowney et al 2020; https://doi.org/10.1016/j.fishres.2020.105728). As such the study was comprehensive and robust such that one indicator informed the interpretation of others. For example, catch rates results from crab pots, were compared with analysis of movement information based on the movement of crabs tracked using telemetry. No differences in catch rate were supported by any obvious changes in movement. In addition, genomic results were similar to physiology indicators in both the laboratory and field. In this case, absences in physiological stress corresponded to no or little obvious changes in gene function (this work is ongoing). The results from individual study components are published in reputable and long-standing peer reviewed scientific literature, having undergone anonymous peer review by subject matter experts, ensuring the highest level of confidence available in science.

Our experiment did not consistently measure statistically significant impacts of seismic oil and gas exploration on commercial Snow Crab, with the study metrics employed. We did not find any indication of seismic-related mortality or damage to internal organs based on telemetry tracking or physical or histological examination respectively. Catch rates differed between locations and times of sampling, but differences explained by seismic exposures were not observed in response to 2D seismic exposures, and differences were inconsistent with respect to 3D exposures. For example, catch rates appeared to increase in 2018 in response to long-duration 3D seismic surveying, but decreased in 2017 in response to 3D seismic. Based on opposing observations among years, the data cannot conclude that catch rates are reduced by seismic. However, analysis of trends in commercial catch show high natural variation in catch rates over time and space similar to the scale of effects measured as part of our seismic experiment, indicating that these changes could be explained by natural variability. For 2D seismic survey exposures in particular, natural variability at spatial and temporal scales of our sampling was greater than differences explained by seismic exploration. We did not detect significant changes in movement speed or behaviour in response to seismic noise either, but we did measure different movement patterns that contribute to our understanding of Snow Crab behaviour. There were no consistent physiological impacts measured from biochemistry assay testing, from year to year or with and without seismic. In 2017, as part of our biochemical analysis of crab physiology, we measured a lower level of EROD concentration at the location of 3D surveying; however in 2018 we measured significantly higher EROD concentration at our control area with no change at the exposure site in response to a 3D exposure. Seven other biochemical assays did not show significant differences. Genomic studies identified many sound-responsive genes particularly from lab studies, but when we looked specifically for a response from these “sound-responsive genes” in field studies, or compared results from different years, the same genes did not respond predictably or consistently. Combined, the genomic analysis lends support to physiological observations and the absence of predictable impacts. This study was the first of its kind to examine Snow Crab gene-regulation and we have not fully explored linkages between noise and transcriptomic functions related to seismic surveying. Work is ongoing to study specific functions that particular sound responsive genes might be responsible for. All study components considered, there is consistency in the finding that natural variability between sites and seasons, appear to have greater influence than what we could measure as owing specifically to seismic noise. If indeed seismic noise caused an effect related to our specific indicators, we were not able to measure that effect or it was not consistent.

III.A. Subcomponent 1) Noise Production

Importantly, this realistic study incorporated actual seismic surveying (Figure 2) into our experimental study design. The noise levels experienced by Snow Crab during the experiment are directly representative of the conditions that fish harvesters are concerned about. The same 2D airgun array was used in each of 3 years. We also incorporated noise from a 3D seismic survey, for which the air-gun array was similar to that used for a 2D survey, but the 3D surveys include longer periods of surveying in the same general area. The 3D survey lines in this study were spaced 700 and 800 meters apart in 2018 and 2017 respectively, whereas a 2D survey might consist of lines spaced many kilometers apart and hundreds of kilometres long, therefore operational survey hours in an area are fewer and farther between for 2D surveys. Importantly, seismic air-gun arrays and their operation are representative of seismic surveying operations conducted in the Newfoundland and Labrador region for the last decade, and more.

Figure 2A. Seismic survey vessels used in this study. Top left- 2D seismic survey vessel Atlantic Explorer. Top right and bottom left- 3D seismic survey vessels Ramform Sterling and Ramform Titan. Bottom right is an example of a lead ship that is used to assist seismic vessels while surveying in Newfoundland and Labrador waters.

Figure 2B. Fishing vessels used in this study. Top left to right - Arctic Eagle, Royal Venture, and Executioner. Bottom left to right – Clears Cove Pride, Royal Mariner, and Atlantic Champion. These six commercial Snow Crab fishing vessels and crew were involved in the collection of data used in this study. Commercial harvesters and vessels provided an important perspective and realism, respectively, reflective of the industry.

III.B. Subcomponent 2) Noise measurement

Measuring noise levels was particularly important for this specific study in order to show that our control sites received much less noise than our experimental site. It also helped to better understand particle motion, which is the back-and-forth vibration of water caused by sound as it travels through water. We used sensitive sound recording equipment from JASCO Applied Sciences to record the marine sound scape (Figure 3).

From a management perspective however, measuring and reporting sound exposure level is important to compare against other studies and for applying any future advice for management consideration. A recent paper by Carroll et al (2016) indicated that lack of standardization in terminology and measurements related to sound exposure is one of the main limitations in providing a broad scale assessment of the potential impacts of underwater noise (Hawkins et al 2015). Importantly, this ESRF study defined and published our results in a paper illustrating how Sound Exposure Level (SEL) is a suitable sound metric for our specific research questions pertaining to the Snow Crab fishery (Martin et al. 2029; Appendix 1).

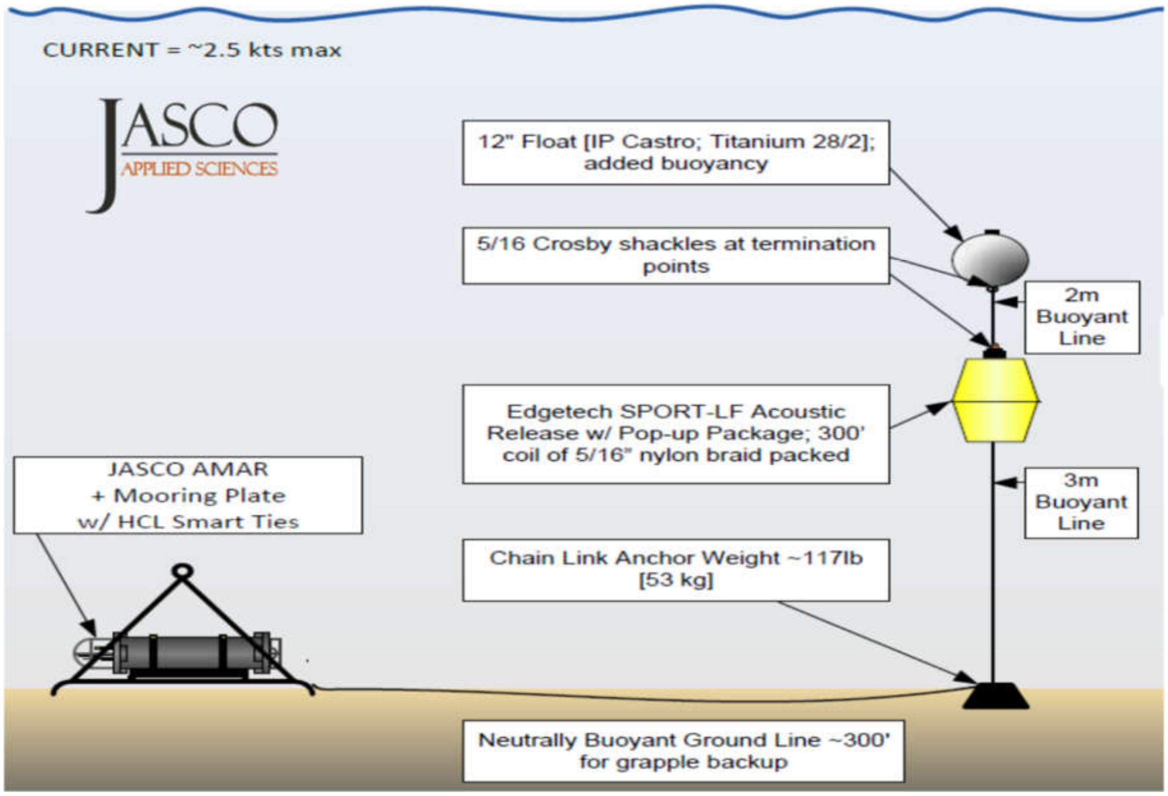

Figure 3. Schematic of a soundscape recorder deployment scenario used to record marine soundscapes in this study.

The daily Sound Exposure Level (SEL) is a sound metric used to assess possible injury to the hearing of marine life. It is a cumulative measure of noise exposure over time and considers a broad frequency range. This makes the daily sound exposure level a natural metric for describing the soundscape, since it relates directly to the regulatory thresholds. We used the daily SEL from nine recording locations including our Snow Crab study, to 1) identify natural soundscapes vs environments affected by human activity; 2) make recommendations on how to collect soundscape data for daily SEL analysis; and 3) demonstrate the use of the daily SEL in soundscape management as an indicator of cumulative effects and masking. We showed that properties of natural soundscapes are: 1) at the daily SEL below 160 dB re 1 $\mu Pa^2 \cdot s$; 2) at least half of the daily SEL is from frequencies above 100 Hz; and 3) the autocorrelation coefficient of the 1-minute SELs remain above 0.6 for time lags of several hours. That means, for most of the time the collected sound level is a good predictor of the future sound level, it is highly auto correlated, and it takes the introduction of a human noise to reduce the "normal" autocorrelation measurement by changing the soundscape. Changes in autocorrelation "stand-out" against a consistently measured natural soundscape, and adds more low frequency energy. When measuring the SEL for regulatory compliance purposes, a 100% duty cycle is recommended because it ensures no periodic sounds are missed. A 100% duty cycle means the instrument is listening all the time, whereas, sometimes a less than 100% duty cycle is used conserve battery power or manage computer memory during long deployments. When less than 100% duty cycles are used, the confidence intervals for the error in the daily SEL are shown to depend on the autocorrelation of the one-minute SELs. The difference between a natural daily SEL and the daily SEL in environments affected by human activity, is an indication of the potential for human sounds to mask biologically important sounds. Noise from human activity can prevent marine life from detecting important natural sounds in the environment, masking, the sounds they might use as part of daily activities, for communicating, foraging, or evading predation for example. We show how the difference between the daily SEL and the temporary threshold shift regulatory limit can be used as a cumulative effects indicator. This indicator is especially important for areas with high continuous sound levels where impulsive sounds may also be added to the environment. Figure 4 illustrates sound recording data with and periods of 3D seismic surveying activity at different distances.

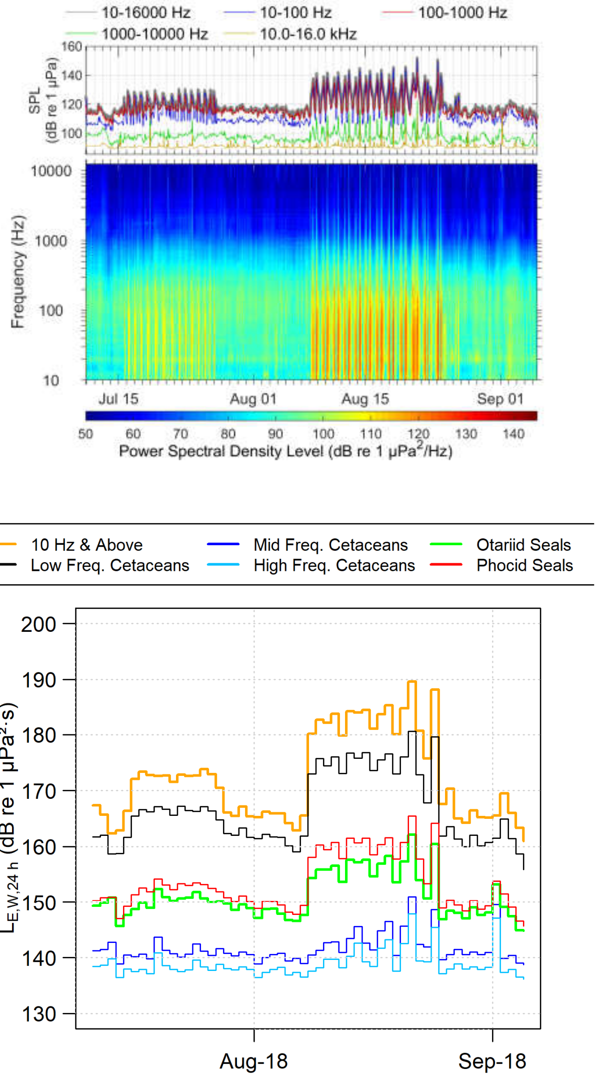

Figure 4A. Sound recording data illustrating sound pressure levels (SPL) at different frequencies (Top), frequencies displayed as a spectrogram (middle), and Daily SEL (Bottom). adapted from Morris et al (2020).. Typically, animals are sensitive to specific frequencies within a specific range but audiograms exist for a very select few of marine species, mostly marine mammal groups that are reported in this figure. We also plot the cumulative SEL for sound at 10 Hz and above. While Snow Crab are likely affected by particle motion, SPL is a good predictor of particle motion at distances greater than 50 meters from the source, however since the components of sound most important for crab are not known we included as wide a range as possible.

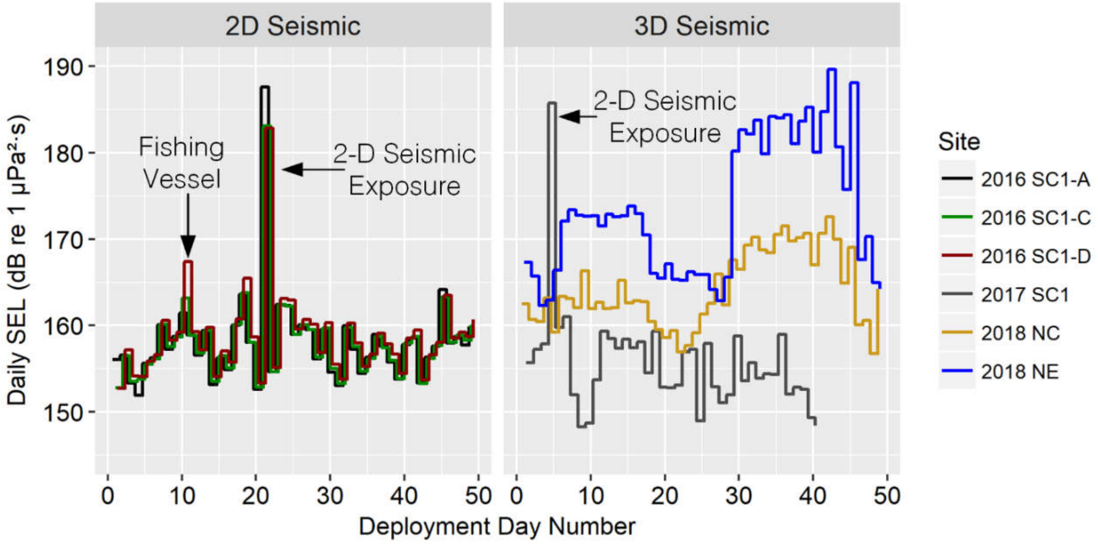

Figure 4B. Dailey SEL during 2D and 3D surveying, adapted from Morris et al (2020). . The left panel also indicates the noise level produced by our commercial fishing vessel while very near the location of a sound recorder.

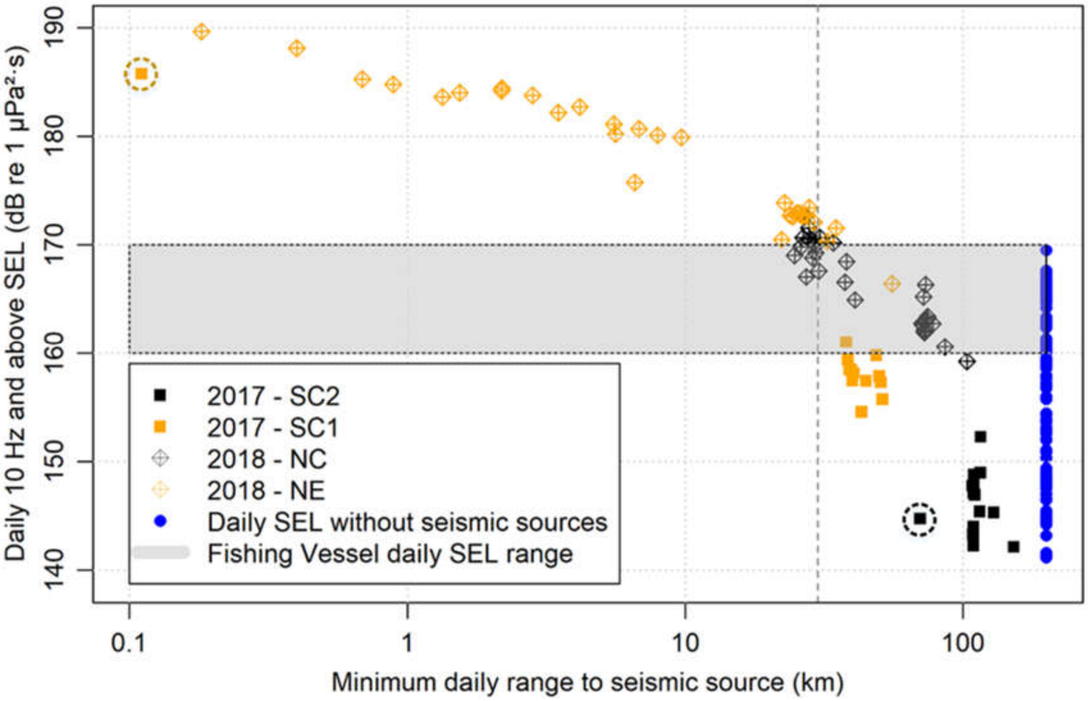

Figure 4C.Daily SEL vs minimum range to the seismic source, adapted from Morris et al (2020). The vertical dashed line is drawn at 30 km range. The gray box indicates the values of daily SEL expected when a fishing vessel is operating near a recorder (0-1 km distances). The dashed circles around one of the 2017 SC1 and SC2 markers indicate the levels associated with the 2D seismic survey. The remainder are associated with the 2017 3D seismic survey. The 2017 SC1 2D data point is lower than the actual daily SEL because the hydrophone was overloaded when the source was directly over the recorder.

Acoustic waves that travel through the ocean cause the water to accelerate back and forth as the sound travels. Far from the sound source, seabed, and sea surface, the acceleration is directly related to the acoustic pressure. Close to sources of sound and near the seabed or sea surface, the acoustic fields have a pressure component and a separate particle motion component. In consequence, several publications in scientific literature point out that it is also important to measure and consider effects of particle motion. Particle motion is another component of marine noise used by marine life in addition to, and possibly separately from sound pressure. Calculations of particle motion from measurement of sound pressure is generally accurate at distance beyond approximately 50 m from the sound source, and certainly at distances of Snow Crab from the seismic air-gun array (depth of approximately 200 meters in this study). While that does not identify separate effects from pressure and particle motion, it helps to take effects of particle motion into account. Our study measured sound energy that transitioned at the water-seabed interface that produced a head wave, which was transmitted through seafloor sediments at a speed faster than sound in water, undetected by individual sound pressure sensors. Snow Crab are equipped with sensory hair cells along their legs to detect vibration in sediments, and could have detected this vibration, but we did not measure change in crab behaviour. There are many unknowns with respect to sound and its effects on marine life. For this study we incorporated the actual noise impact from seismic surveying (which included pressure and particle motion effects), and unlike many other less-realistic studies we are not trying to produce or replicate a noise source experimentally. Finding a head wave associated with marine sediments is an interesting example through which particle motion could affect benthic marine life, however we did not measure any associated changes in Snow Crab behaviour or physiology.

Most fish and invertebrates sense acceleration not pressure. The effects of strong particle motion sound fields from seismic airguns on fish and invertebrates is often raised as a concern during environmental assessments of seismic surveys. To understand this issue the expected behaviour of the acoustic field under a seismic array is derived and demonstrated through sound propagation models. In the summers of 2016 and 2017 we evaluated four methods of measuring the particle motion near the seabed during a seismic exposure study on Snow Crab. We conclude that there are no large amplitude particle motion effects from a seismic impulse that are not measurable using a pressure sensor and thus for our studies pressure measurements are a suitable indicator of exposure to noise. We show that an array of omnidirectional hydrophones is the most flexible measurement solution for pressure and acceleration. Micro-electro-mechanical-system accelerometers are a robust means of making direct acceleration measurements over a wide bandwidth and at high acceleration levels however they are not sensitive enough for ambient measurements.

III.C. Subcomponent 3) Commercial Catchability

Harvesters have a unique perspective on the availability of resources based on catch rates, including subtle changes that affect their industry. Harvesters suggested that potential impacts of seismic surveying could reduce catch rate. Thus, our studies on the impact of realistic seismic surveying on Snow Crab catchability included catch rate as a metric, following a scientific methodology to standardize and replicate our efforts, to measure a response. However, the results from our study did not identify consistent reductions in catch rate owing to seismic surveying. During three initial years of this project we focused on the effects of 2D seismic exploration (Figure 5) and then during the last two years we included longer-duration 3D seismic exploration (Figure 6). Combined these two types of survey activity encompass the realistic range and variability of exposure to seismic noise that Snow Crab are likely to experience from industrial seismic surveying on commercial fishing grounds in this region. No effects of 2D seismic surveying were detected during controlled experiments in 3 different years. Statistical difference in catch rate was observed in response to 3D surveying, but the catch rates were decreased in one year and increased in another; not a predictable decrease in catch rate as hypothesized.

We repeated a Before-After-Control-Impact study over two years to assess the effects of industry scale seismic exposure on catch rates of Snow Crab along the continental slope of the Grand Banks of Newfoundland. Our results did not support the contention that seismic surveying activity negatively affects catch rates in the shorter term (i.e. within days) or longer time frames (weeks). However, significant differences in catch were observed across study areas and years (Figure 7). While the inherent variability of the CPUE data limited the statistical power of this study, our results do suggest that if seismic effects on Snow Crab harvests do exist, they are smaller than natural changes related to spatial and temporal variation.

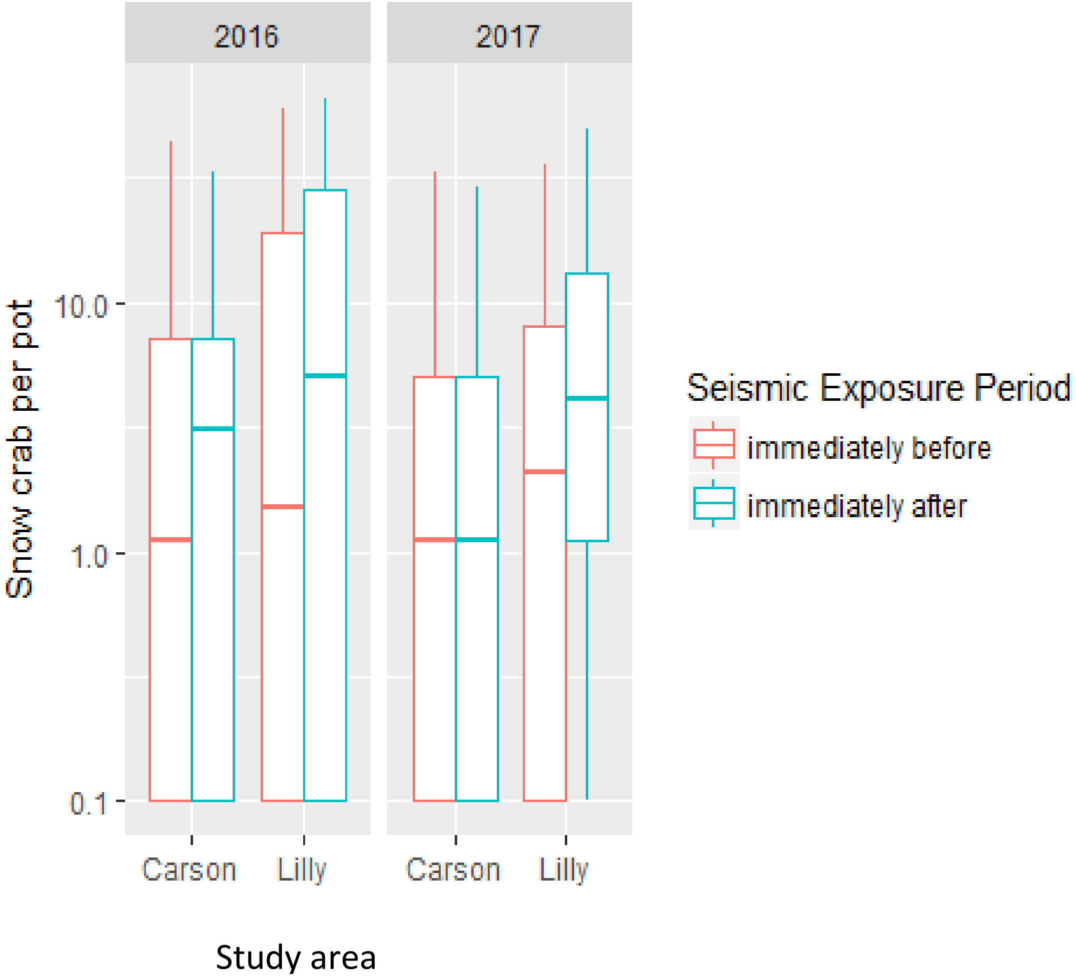

Figure 5. Measured catch rates associated with 2D seismic, without significant differences, adapted from Morris et al (2018). The box plots indicate the median, boxes represent the interquartile range, and "wisker" bars show the distribution of the data 1.5 times the interquartile range.

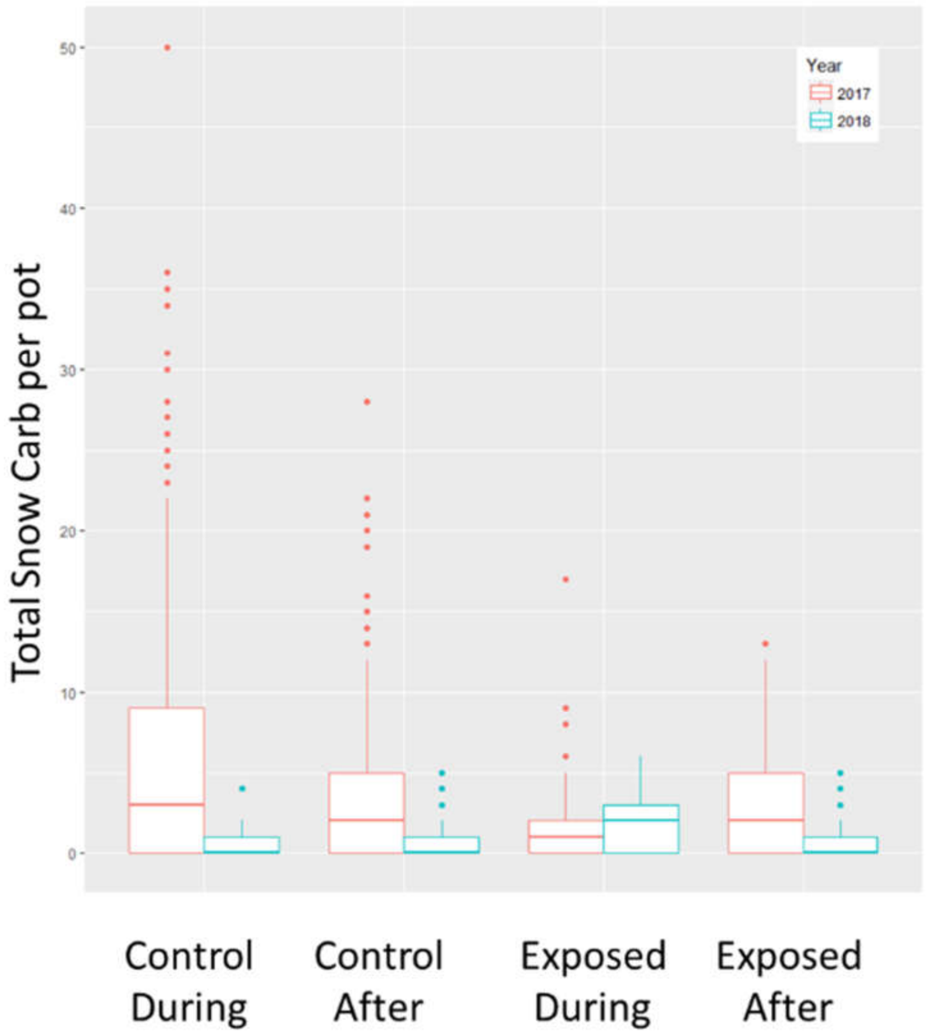

Figure 6.Box plots of the measured catch rates associated with 3D seismic (Exposed During) near Carson Canyon, that measured both relatively increased (Blue bar for 2018 is higher for the "Exposure During" compared to the blue bar at other times) and decreased (Red bar for 2017 is lower for the "Exposed During" period compared to the red bar at other times), catch rates during the seismic survey period, adapted from Morris et al (2020). Single dots are considered statistical outliers, beyond 1.5 times the interquartile range.

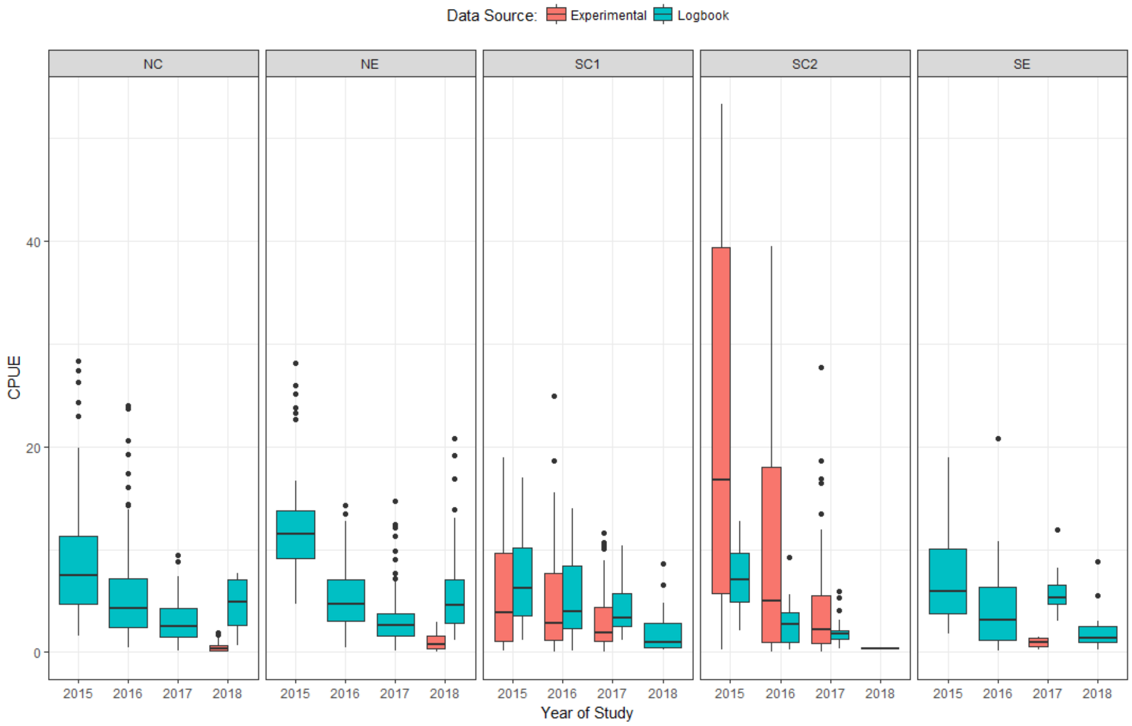

Figure 7. Catch rate data collected during our experimental fishing and Commercial Snow Crab Catch Per Unit Effort data collected from the commercial fishery, adapted from Morris et al (2020). Commercial data was collected by at-sea fishery observers aboard commercial fishing vessel during the commercial fishing season (limited to July) in the vicinity (within 20 km) of our study area near Carson Canyon. Log book data from the fishery shows a marked decline in catch rates over time in our study area.

Regarding the effects of 3D seismic on the Snow Crab fishery, we measured catch rates of Snow Crab exposed to both small (2D) and large (3D) amounts of seismic oil and gas exploration activity, to capture the likely range of noise exposure and to compare with non-impacted areas. A series of comparisons as part of a before-after-control-impact study design measured effects of both 2D (2017) and 3D (2017 and 2018) seismic surveying effects on the catch rate of Snow Crab. Differences in catch rates at locations of 3D seismic survey sites, either before/after or during/after compared with control sites were inconsistent. We measured reduced catch rates in 2017 and increased catch rates in 2018 during the seismic exposure period only. In 2017, where reduced catch rates were observed, there was no significant differences in catch rate detected at the 3D site two weeks after seismic surveying ended and no difference in catch was detected at distances of 30 km or 100 km from survey operations. Interestingly, the 3D results from 2018 showed increased catch rates at the seismic survey location during seismic operations but no differences were measured 30 km away at the 2018 control site, which is opposite the 2017 results. In 2018, catch rates were lower 2 weeks after seismic ended. Our results suggest that seismic exposure does not necessarily reduce Snow Crab catch rates. Maximum exposures from industrial scale surveys can potentially affect catch rates but the impact was not predictable, as catch increased in 2018, and the range of effect appears to be within 30 km and less than 2 weeks duration. The mechanism of a potential impact has not been resolved to explain how changes in catch, either an increase or a decrease, could be explained by seismic surveying. Analysis of commercial catch rates during the commercial fishing season, in the absence of seismic surveying, that investigated changes in catch rate over time and abundance, revealed changes of similar scale, and greater than observed in our catch rate experiment. Natural variability in Snow Crab catch rate over short time scales (two week) and distances (30 km) can have a larger effect then the observed differences observed during our seismic surveying experiments on Snow Crab catch rates.

III.D. Subcomponent 4) Crab movement

A change in behaviour is a possible explanation as to why catch rates might decrease or increase in response to seismic surveying. For example, commercial Snow Crab harvesters speculated that

Snow Crab change their movement patterns in response to seismic surveying, possibly moving to deeper water or stop moving altogether, and are therefore less susceptible to the trap. To investigate crab movement in response to seismic exploration, we tagged and tracked the fine scale (2 meter scale accuracy every few minutes) movement behaviour of several hundred Snow Crab during experiments conducted over 3 years. No information describing the fine-scale movement of Snow Crab in the deep-water areas of Newfoundland and Labrador existed previously. We used acoustic telemetry, Vemco positioning system (VPS), to measure the fine-scale movement of 316 acoustically tagged Snow Crabs, including measures of velocity (Figure 8) and behaviour patterns (Figure 9) and direction (Figure 10). As a result, this new research is an important contribution to Snow Crab biology in addition to understanding the effects of seismic surveying. Our analysis of Snow Crab movements did not detect changes in response to seismic surveying noise. While we did not detect changes related to seismic, we did measure small changes in movement owing to changes in temperature and time of day. This is particularly important because it supports the ability of our methodologies to measure changes when they occur, and suggests that changes in snow crab movements are impacted more by changes in temperature and diurnal effects than seismic surveying noise.

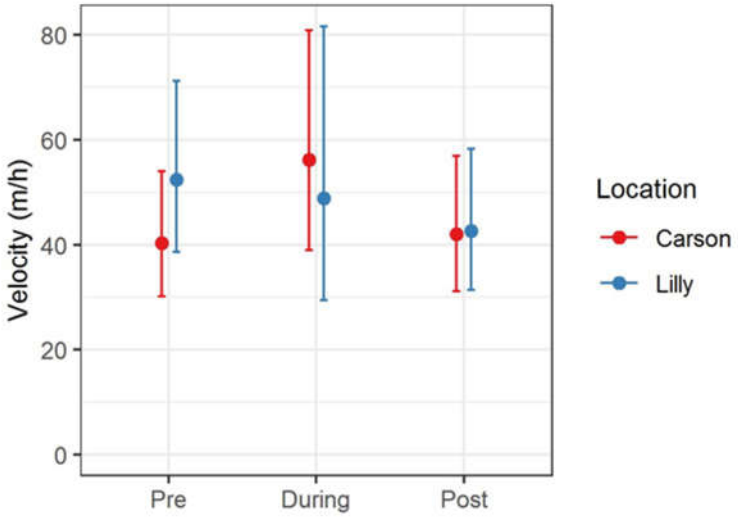

Figure 8.. Model estimates of Snow Crab movement velocity for Before-After-Control-Impact seismic exposure treatments in Carson (exposure) and Lilly (control) canyons adapted from Cote et al (2020). Model estimates standardize the environmental conditions across treatments to 100h post release, at 12pm and a water temperature of 0˚C. Error bars represent 95% confidence intervals.

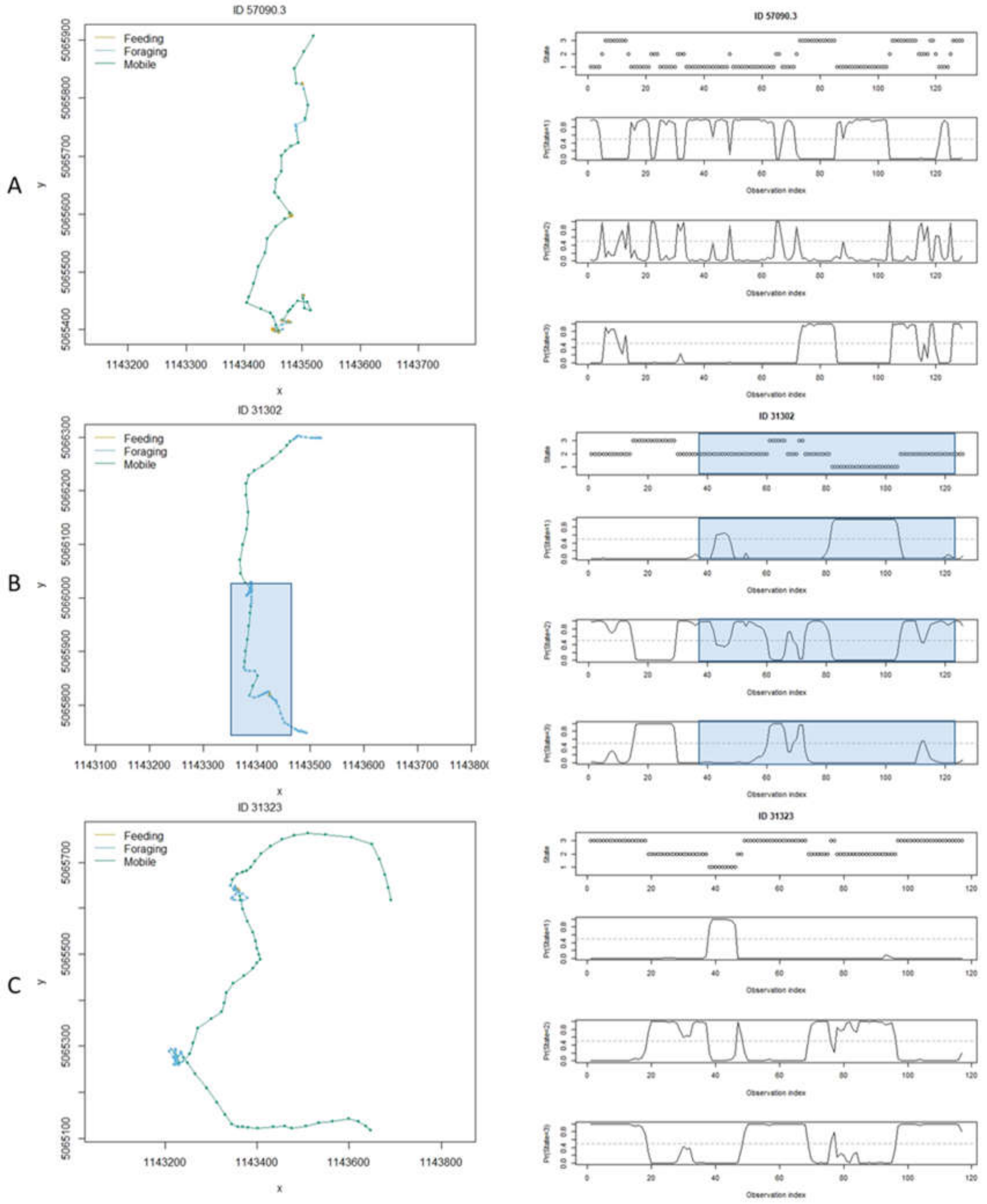

Figure 9:Tracks of three Snow Crab (left panels) with behavior state probabilities (right panels), adapted from Cote et al (2020). Behavior state 1 represents feeding behavior, while states 2 and 3 represent foraging and mobile states respectively. Most probable behavior states are superimposed on the crab tracks. Movement track in panel A occurred entirely during seismic exposure, panel B contains pre, during (shaded blue), and post seismic periods, and panel C contains pre-seismic data only.

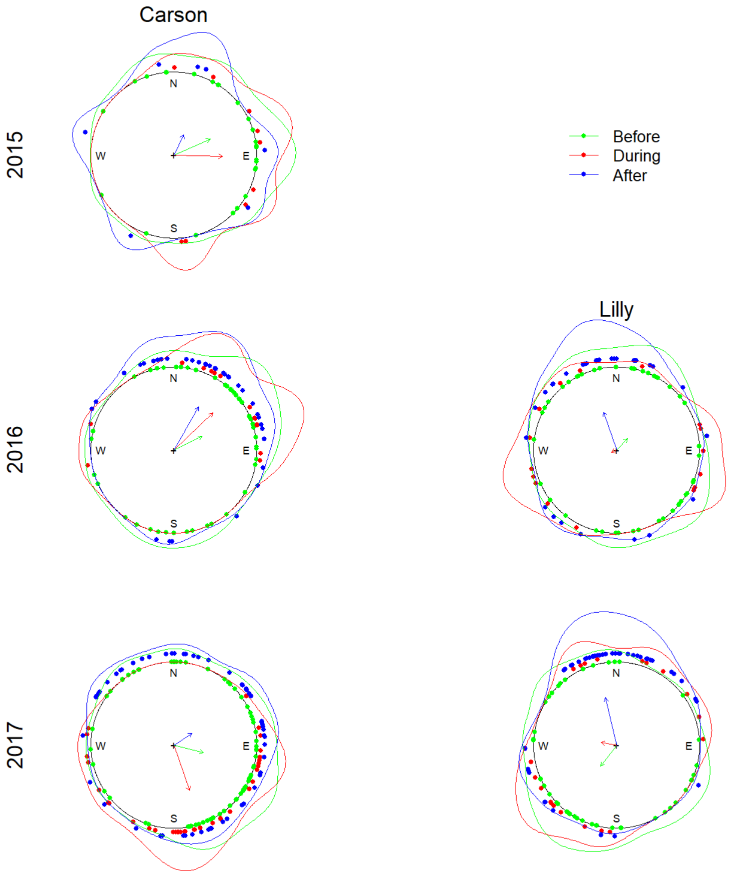

Figure 10: Mean direction of movement for tracked Snow Crab Before, During and After seismic exposure periods at the control (Lilly) and Carson (exposure) canyon sites, adapted from Cote et al (2020). Points represent the mean bearing of individuals, arrows represent the aggregate bearing across individuals, and the length of arrows represents the strength of tendency for a particular time period. Lines external to the plot represent the frequency distribution of bearings for each time period.

State space modelling and Behavioural Change Point Analysis was used to reduce variation and outlier data, before mixed effects modeling was applied to both raw and modelled data, to detect fine scale difference in behaviour in response to seismic noise exposure. An initial flight response within 24-36 hours post release was detected as a faster than average movement speed and provided a valuable interpretation of speed capabilities of Snow Crabs under stress. Comparisons of movement and behaviour with respect to seismic exposures did not identify significant and repeated changes in Snow Crab behaviour. Snow Crab behaviour was measured to be highly variable, with interesting behavioural patterns that include directed linear paths and more constrained localised movements. The noise conditions from seismic surveying experienced in this study, which are representative of industry based 2D surveys, did not have a measurably predictable impact on the Snow Crab behaviours.

III.E. Subcomponent 5) Crab genomics

This ESRF study developed a genomics approach as an assessment tool to evaluate whether specific sound responsive genes were affected by seismic surveying. The available genomics publication (Hall et al. 2020) is publicly available, https://doi.org/10.1016/j.fishres.2020.105794, and these text largely come from that publication. Genomics is a rapidly growing field of research and could be a valuable tool and indicator of environmental impact used in various assessment processes. The genomics-based research used functional genomics techniques [RNA sequencing (RNA-seq)] to characterize the Snow Crab transcriptome and to identify candidate molecular biomarkers of crab response to loud sounds. RNA-seq-based bioinformatics analyses (DESeq2 and edgeR), molecular methodologies (such as Real-time quantitative polymerase chain reaction (qPCR)) and multivariate statistical analyses were then used to elucidate the molecular underpinnings of seismic and chronic noise exposure on the Snow Crab transcriptome (hepatopancreas, hemocytes) in field and laboratory studies, respectively. This research generated transcriptomic sequence data for Snow Crab that was not previously available. It was deposited into a public sequence data repository [NCBI's Sequence Read Archive (SRA)] and represents an important publicly available genetic/genomic resource that future researchers can, and are in fact using, to build on the research conducted herein. Importantly, our study aimed to measure the responsiveness of the Snow Crab hepatopancreas transcriptome and of specific genes to industrial seismic noise exposure (RNA-seq and qPCR for 2016 field collected samples, and qPCR for 2017 field collected samples), and the Snow Crab hepatopancreas and hemocyte transcriptomes and specific genes to chronic noise exposure (RNA-seq and qPCR for lab-based study). Within a given RNA-seq/qPCR study (e.g. 2016 analysis of hepatopancreas transcript expression before and after seismic exposure), we identified (by RNA-seq) and validated (by qPCR) candidate molecular biomarkers of seismic/noise exposure; however, our initial analysis of the 2017 field collected samples (before and after seismic exposure) using qPCR for candidate biomarkers identified in both of the other studies did not appear to consistently (i.e. between years in field-based studies, and between laboratory and field-based studies) detect gene-specific responses owing specifically to the seismic surveying in a predictable way. However, many of the genes that were responsive in a given sound exposure experiment were associated with important aspects of Snow Crab physiology (e.g. molting, immunity, digestion, metabolism and stress-response) which warrants further investigation. Our work is the first of its kind on Snow Crab, and although our initial analysis of the 2017 field sample date has not identified consistent noise-responsive molecular biomarkers, it has built a strong foundation for future research and a more detailed understanding of transcriptomic responses to noise.

For field studies, Snow Crab were collected from Lilly Canyon (LC; control site) and Carson Canyon (treatment site) before 2D seismic exposure (CC), and 18 hours (CC18h) and 3 weeks (CC3w) after 2D seismic exposure. RNA was isolated from the hepatopancreas of 10 crab from each of the four groups and subjected to RNA sequencing (RNA-seq) analyses. A comparison of the transcriptome of individuals from the four groups using both DESeq2 and edgeR analyses identified 1285 and 389 differentially expressed isoforms, respectively. Real-time quantitative polymerase chain reaction (qPCR) assays were designed for a subset (83) of these differentially expressed genes to validate the RNA-seq results. First, transcript levels were measured in hepatopancreas samples from each of the 2 groups in which the transcript was identified as differentially expressed in RNA-seq studies. Transcript levels of twenty-six of these genes were validated ($p < 0.05$), with another seven having differences in expression levels that were borderline significant ($p < 0.1$). In a second qPCR study, levels of these thirty-three transcripts as well as crustacean hyperglycemic hormone (due to high fold-changes with seismic exposure) were then measured in the 40 samples (n=10 per each of the four groups) that had been subjected to RNA-seq analyses. Fourteen transcripts were significantly differentially expressed between the CC and LC sites; at CC, nine were significantly higher expressed after seismic exposure, and eight were trending higher after seismic exposure (Figure 11, 12, 13). These genes are candidate seismic-responsive molecular biomarkers; whether they are seismic-responsive in future studies warrants further investigation.

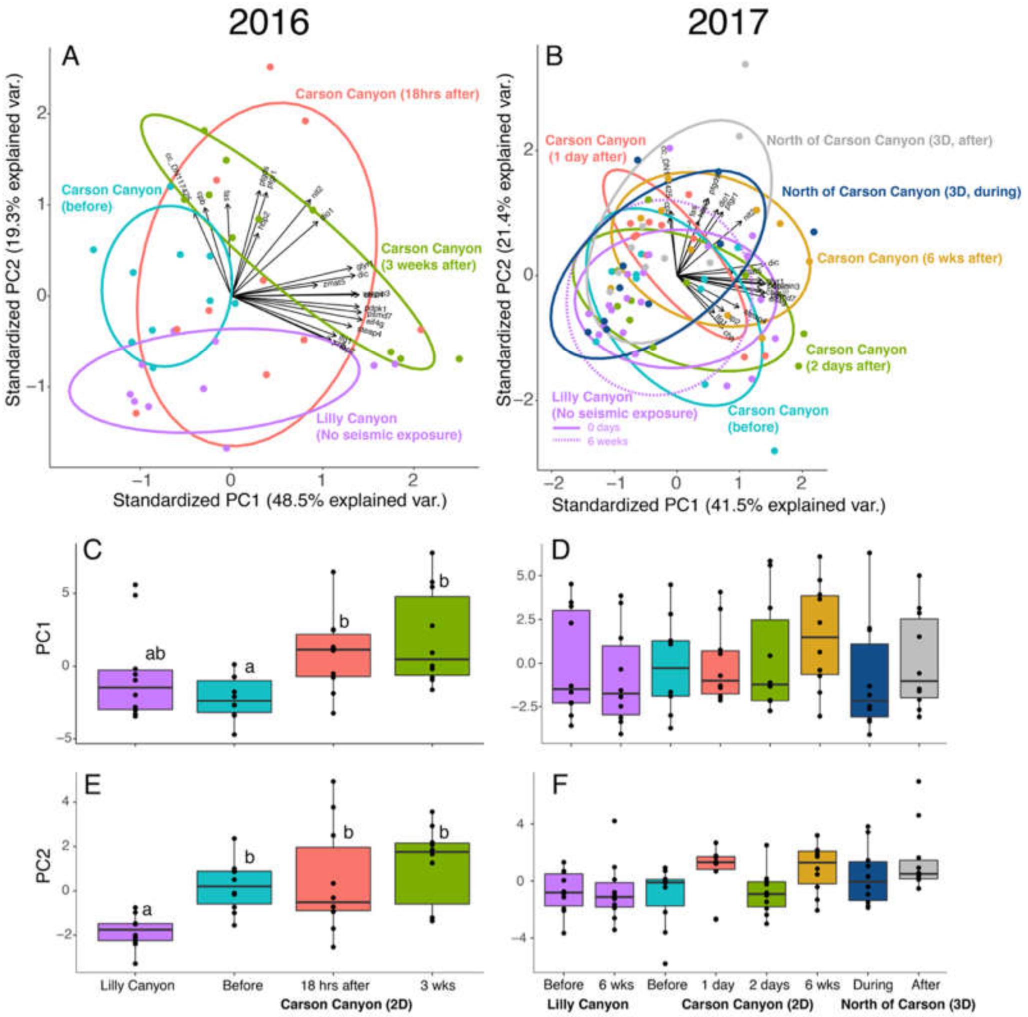

Figure 11. Multivariate analysis (principal component analyses adapted from Hall et al (2020); PCAs) of qPCR-generated expression data for 20 transcripts in the hepatopancreas of Snow Crab subjected to seismic noise and of control Snow Crab in **(A)** 2016 and **(B)** 2017. Length and direction of arrows indicate loading of each transcript on PC axes. Boxplots of PC axis 1 and 2 scores for individual samples in **(C,E)** 2016 and **(D,F)** 2017 data. Different coloured points and boxes represent different sampling groups, and different letters above boxplots indicate significant differences between sampling groups (adjusted *p*-values<0.05; Dunn's post-hoc test for multiple comparisons for Kruskal-Wallis test). For example consider panel C, Lilly Canyon (ab) is not different for any other box, Before (a) is different from 18 hrs after and 3 weeks but no different from Lilly Canyon.

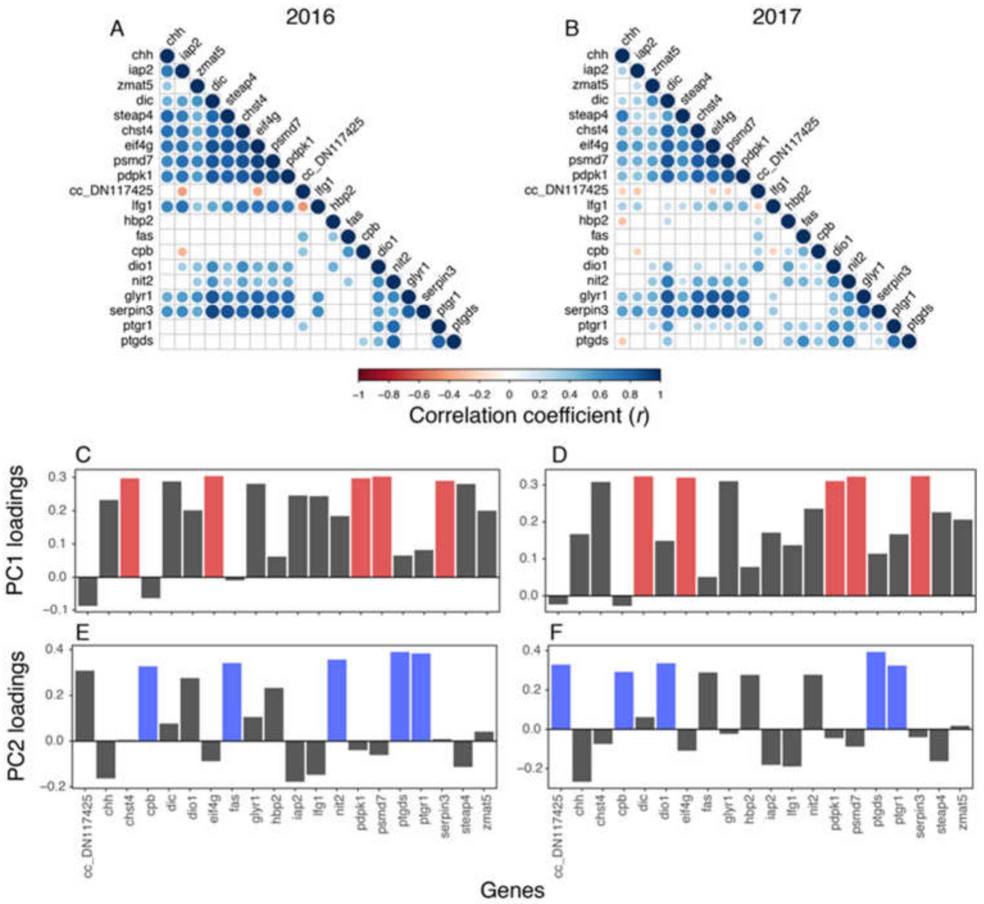

Figure 12. Correlation coefficients (*r*) among 20 transcripts based on expression data from Snow Crab collected in **(A)** 2016 and **(B)** 2017, adapted from Hall et al (2020). Colour and size of circles indicate strength and direction of relationship. Only significant correlations ($p<0.05$) are shown by circles. Loadings of transcripts on principal component (PC) axes **(C,D)** 1 and **(E,F)** 2 for both sampling years (2016 C,E; 2017 D,F). The top five highest loading genes are highlighted in red for PC1 **(C,D)** and in blue for PC2 **(E,F)**.

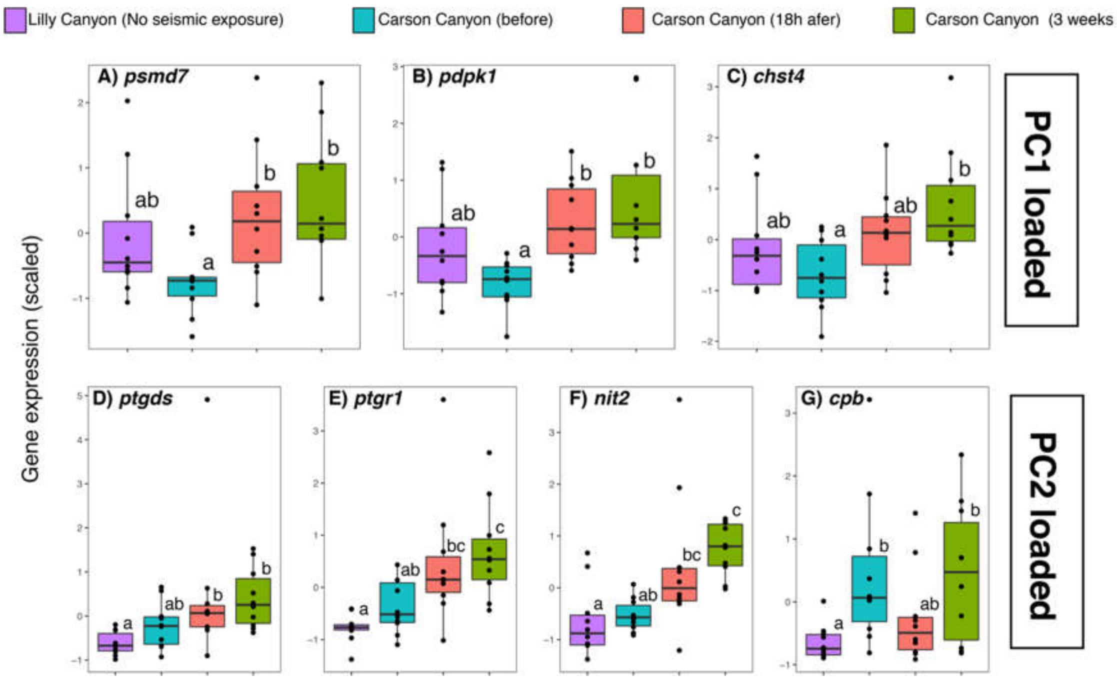

Figure 12a**.** Top loaded transcripts on **(A-C)** PC axes 1 and **(D-G)** 2 that showed significant differences (Kruskal-Wallis test; *p*<0.05) in expression between seismic exposure groups in Snow Crab samples collected in 2016 adapted from Hall et al (2020). Different colours represent different sampling groups, and different letters above boxplots indicate significant differences between sampling groups (adjusted *p*-values<0.05; Dunn's post-hoc test for multiple comparisons). Transcript names are indicated within each panel.

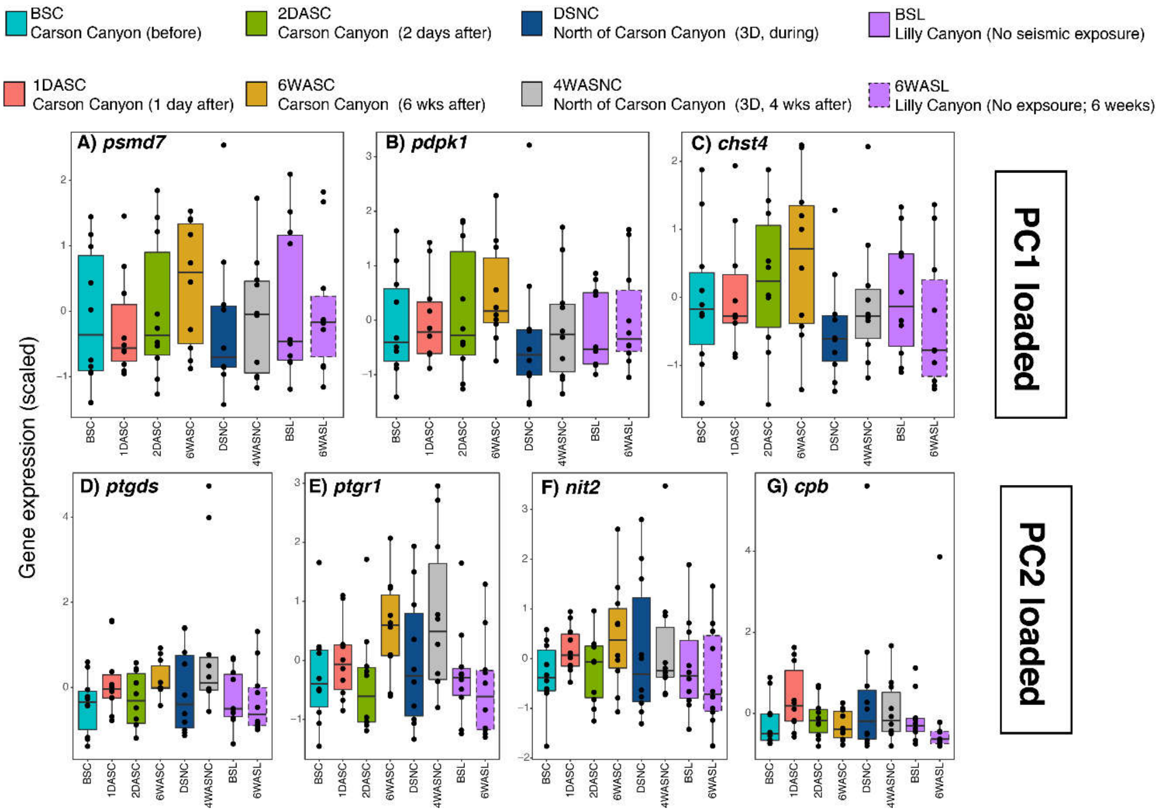

Figure 12b. Expression data for samples collected in 2017 for the same transcripts shown in Figure 12 for 2016, adapted from Hall et al (2020). Boxplots show expression patterns for the top loaded transcripts on **(A-C)** PC axes 1 and **(D-G)** 2 in 2016 that showed significant differences (Kruskal-Wallis test; $p<0.05$). None of the comparisons for 2017 showed significant differences among the eight groups. Transcript names are indicated within each panel.

It has been difficult to resolve effects (using our selected indicators) of noise in the field, and it leads to the question of whether the noise has not caused an effect, or if there is simply too much natural variability to distinguish an effect of the noise. We exposed Snow Crab in the laboratory to unrealistically high levels of noise in a controlled environment to try and identify if indeed we could produce a measurable effect of noise, at all. The purpose of this experiment was to confirm an end-point, which are conditions needed to produce a measurable response. Snow Crab were held in replicate control or experimental tanks at an aquarium facility in the Northwest Atlantic Fisheries Centre, Department of Fisheries and Oceans, St. John's, NL. On alternating days, the experimental tanks were exposed to noise from an underwater speaker which played a recording of a seismic airgun firing every 10 seconds in a continuous loop, over a period of more than 5 months (chronic noise). To investigate the potential impact of chronic noise on immune, digestion, metabolism and stress-response relevant to gene expression, RNA was isolated from the hepatopancreas and hemocytes of 10 crab from control tanks and of 10 crab from experimental tanks, and subjected to RNA sequencing (RNAseq) analyses. In the initial RNAseq analysis, the hepatopancreas and hemocyte transcriptomes were generated separately. A comparison of the transcriptome of individuals from the hepatopancreas control and chronic noise-exposed, and individuals from the hemocytes control and chronic noise-exposed using DESeq2 identified 17 and 11 differentially expressed isoforms with BLASTx hits, respectively. In a second RNAseq analysis, the hepatopancreas and hemocytes transcriptomes were combined and the aforementioned comparisons as well as a comparison of the hepatopancreas and hemocytes control transcriptomes were then performed using DESeq2. Additional differentially expressed transcripts were identified, from which a subset were selected for real-time quantitative polymerase chain reaction (qPCR) analyses. In total, 61 qPCR assays were designed to validate the RNAseq results. cDNA templates for qPCR analyses were synthesized for all individual samples that had been subjected to RNAseq analyses. In the initial qPCR screen, for each of the four groups, the individual cDNAs were pooled and expression levels of these 61 transcripts were then measured in the pools. Expression levels of transcripts that appeared to be differentially expressed in one or both of the intra-tissue pooled comparisons were then analyzed in individual samples. One transcript (regulator of G-protein signaling 2) was significantly ($p < 0.05$) differentially expressed in both tissues; 7 and 5 were either significantly or borderline significantly ($p < 0.1$) differentially expressed in hepatopancreas and hemocytes, respectively.

These genes are candidate noise-responsive molecular biomarkers; whether they are seismic-responsive warrants further investigation.

III.F. Subcomponent 7) Laboratory observations and Physiology.

While this ESRF project was primarily a field study, we conducted some basic laboratory work to help validate and test field observations, and to make observations at time scales not possible in the offshore. We brought Snow Crabs that were exposed to seismic surveying in the offshore back into our laboratory and held them in captivity for more than a year. We also collected crabs from other areas that were not exposed to close-proximity seismic survey noise. This helped to rule out large impacts such as delayed mortality, which was not observed in the field. In the lab we also monitored general Snow Crab health and conducted captive noise exposure experiments in tanks, exposing crabs to extremely loud noise for an extensive (5-6 months) period of time, to help identify starting or end points where impacts could be measured. We conducted the same physiology metrics on laboratory crab as that used to assess crabs sampled in the offshore experiments, as well as the same genomic sampling which informed other studies. In addition we examined specific hearing structures in Snow Crab, the statocyst, to assess potential damage. Snow crab were divided into 4 tanks and two tanks were exposed to intense noise levels (220+ db) for a period of 5 to 6 months. We monitored crab weight, mortality, and feeding during the experiment. We sacrificed the crab at the end of the experiment and examined crab “liver”, “blood”, muscle condition, and “hearing” structures. Examination included bio-chemistry analysis, and histopathology.

We did not detect a significant impact of noise on any of our four indicators; mortality, food consumption, claw muscle weight, or impact on the sensory-hair field inside the Snow Crab statocyst (Table 1). Importantly, this study provides a technical description along with visual aids of the Snow Crab statocyst and its internal sensory-hairs, which is poorly described in scientific literature. While examining the effects of chronic noise exposure is important, the impacts upon Snow Crab remain elusive.

Table 1. Mean ± standard deviation of each of the indicators used to assess effects of seismic recording exposure. The first p-value column shows the comparison between treatment (control

vs exposed). The second p-value column shows two p-values, a) looking at tank effect between exposed tanks and b) tank effect between control tanks.

Indicator	**Control**	**Exposed**	**p-value (treatment)**	**p-value (exposed tanks)**	**p-value (control tanks)**
Mortality	31%	29.5%	0.768		
Food consumption (g) (based on 22 feedings, total food consumed divided by # of crab in tank)	2.31 ± 0.18	2.36 ± 0.17	0.859	a) 0.44	0.26
Group Hair cells	111.39 ± 2.865 (23)	117.16 ± 3.50 (31)	0.23	a) 0.15	0.46
Claw meat:height (g/mm)	0.071 ± 0.0027 (22)	0.074 ± 0.0027 (28)	0.68	a) 0.40	0.46
EROD (pmol/min/mg)	1.03 ± 0.689 (24)	0.968 ± 0.758 (31)	0.76	a) 0.21	0.92
GST (nmol/min/mg)	7.495 ± 2.212 (24)	7.686 ± 1.806 (31)	0.73	a) 0.25	0.86

Phenoloxidase (U/mg protein)	2.379 ± 2.651 (22)	2.209 ± 2.381 (26)	0.70	a) 0.39	0.26
Total Antioxidant (molar Trolox equivalents/mg protein)	0.00281 ± 0.00108 (23)	0.00260 ± 0.00131 (24)	0.89	a) 0.03	0.83
Protein Carbonyl (nmol/mg)	12.616 ± 8.198 (24)	12.022 ± 9.148 (31)	0.465	a) 0.037*	0.792
Protein (mg/ml)	21.156 ± 4.506 (24)	19.479 ± 3.110 (31)	0.11	a) 0.25	0.14

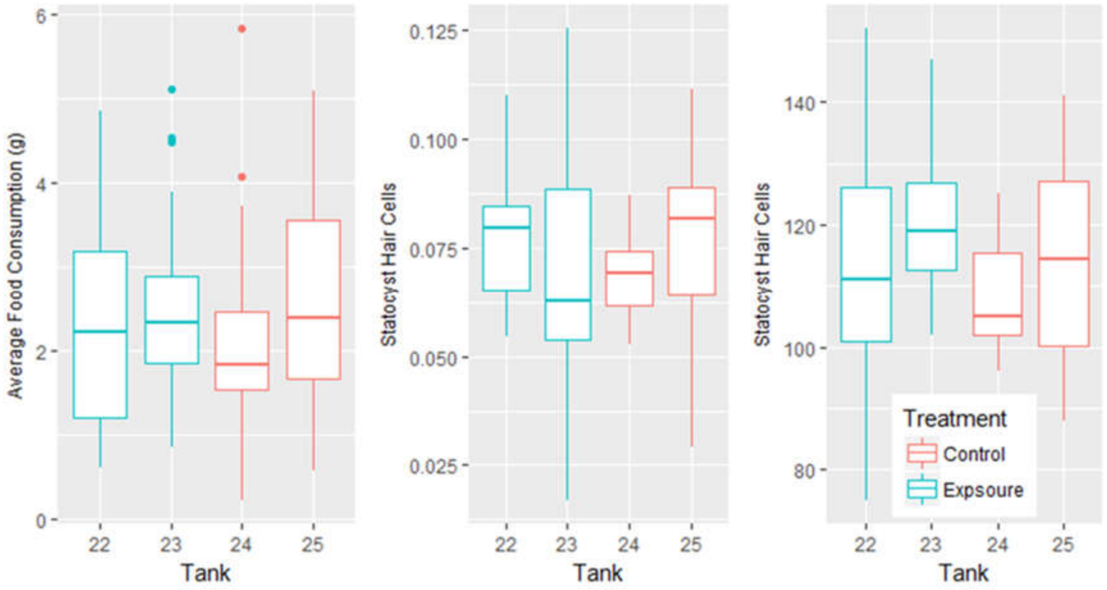

Figure 13. Food consumption (left panel), statocyst group hair cells (middle panel), and claw meat density (right panel) of Exposure and Control treatments, adapted from Hanlon et al (submitted). Horizontal lines represent median values, boxes represent the middle quartiles and whiskers represent 1.5 times the interquartile range. Data beyond the whiskers are represented as individual data points.

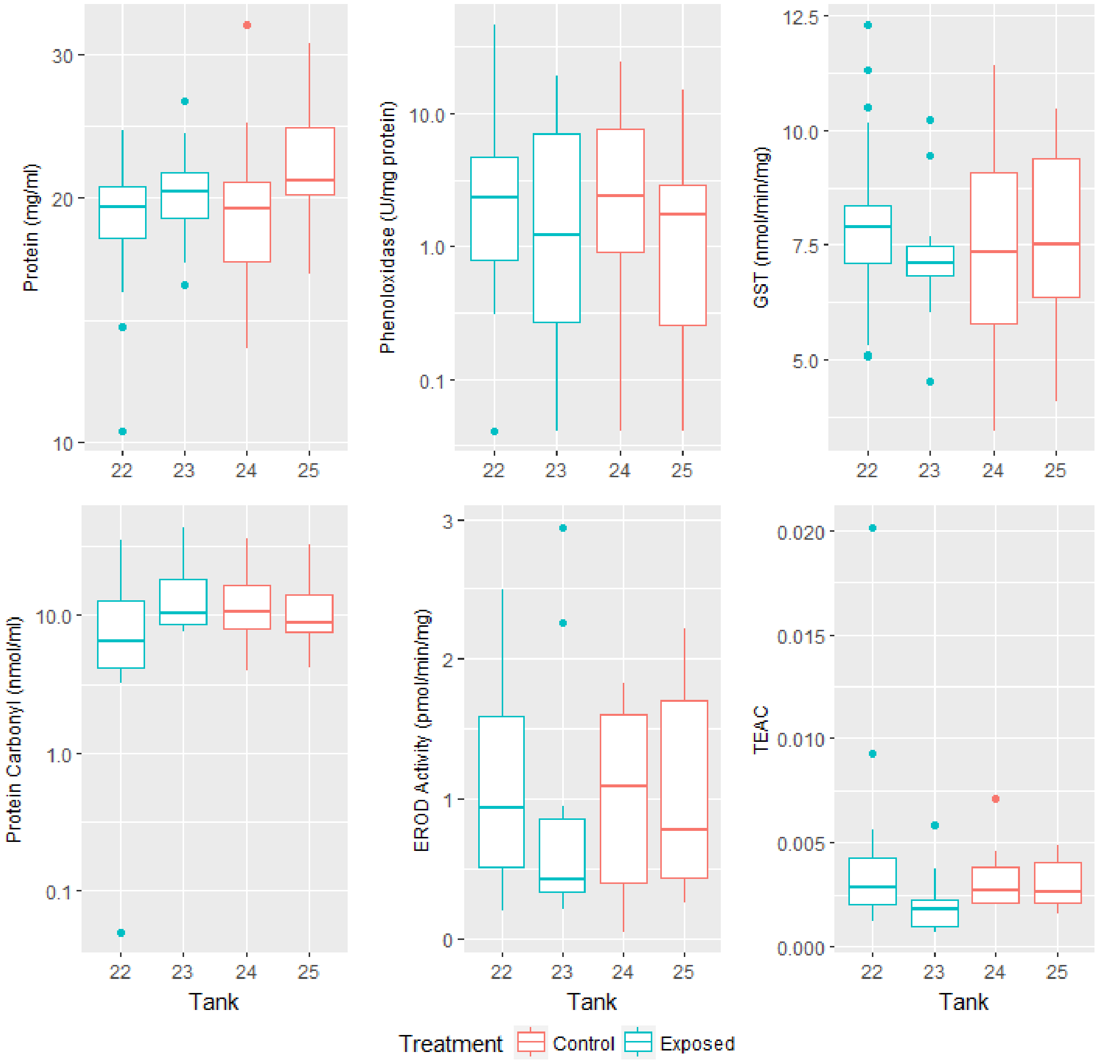

Figure 14. Biochemical responses (protein: top left; protein carbonyl: bottom left; Phenoloxidase: top middle; EROD activity: bottom middle; GST: top right; TEAC: bottom right) of Exposure and Control treatments, adapted from Hanlon et al (submitted). Horizontal lines represent median values, boxes represent the middle quartiles and whiskers represent 1.5 times the interquartile range. Data beyond the whiskers are represented as individual data points.

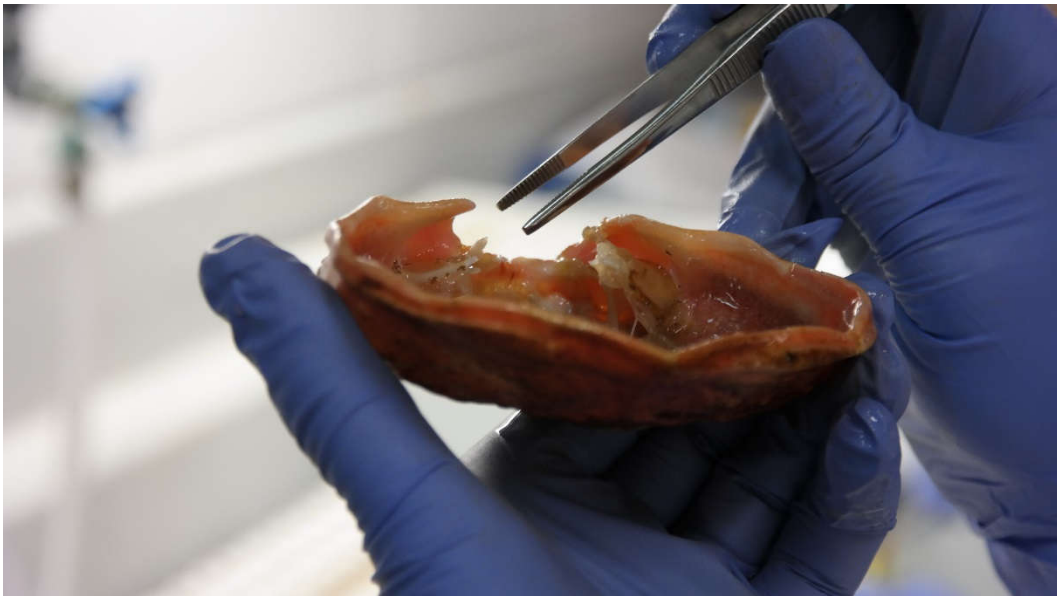

Figure 14. Example of dissecting a Snow Crab to collect tissues for analysis.

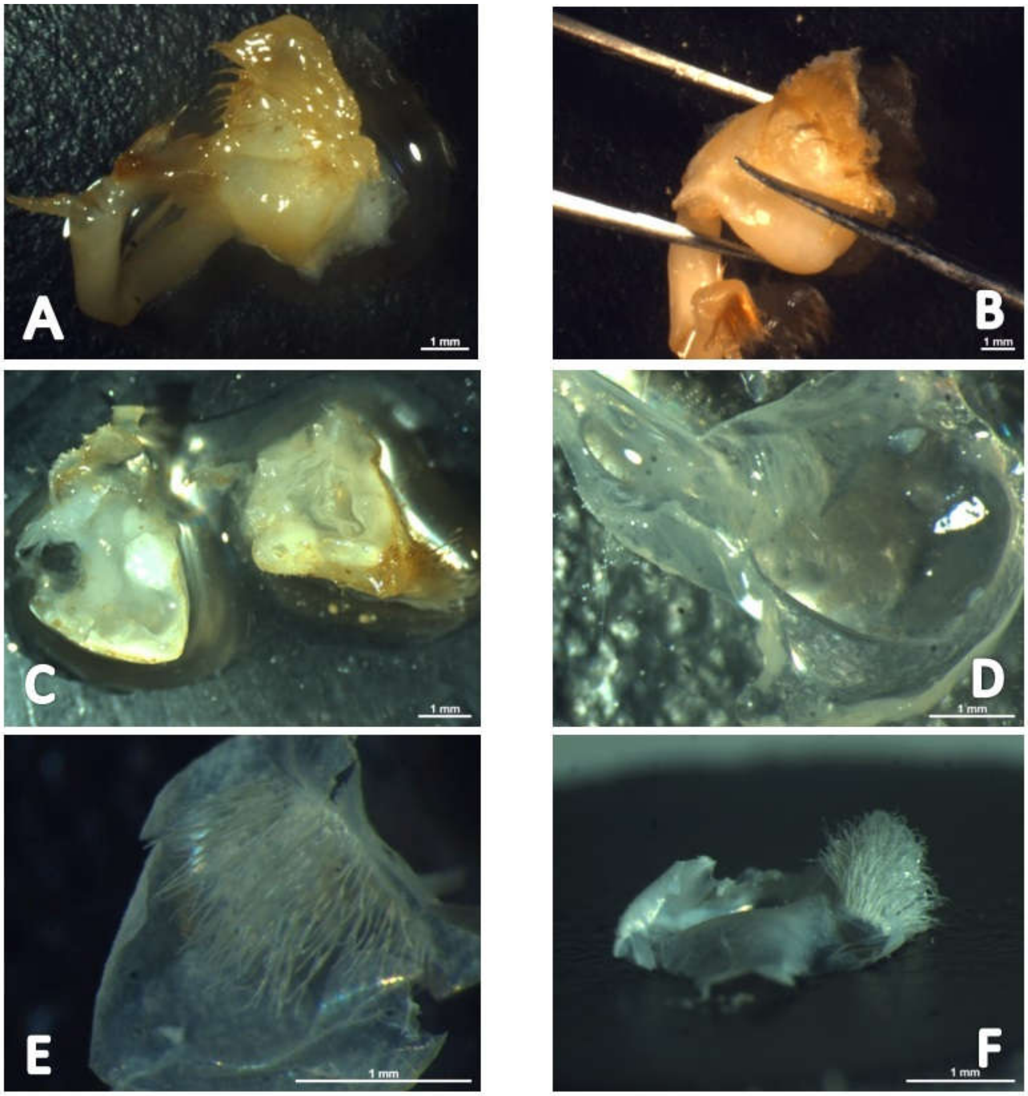

Figure 15. Snow crab statocyst (A), dissecting along the transverse fracture line (B), two halves of the statocysts (C), its inner membrane (D), and group hairs (E) after drying (F).

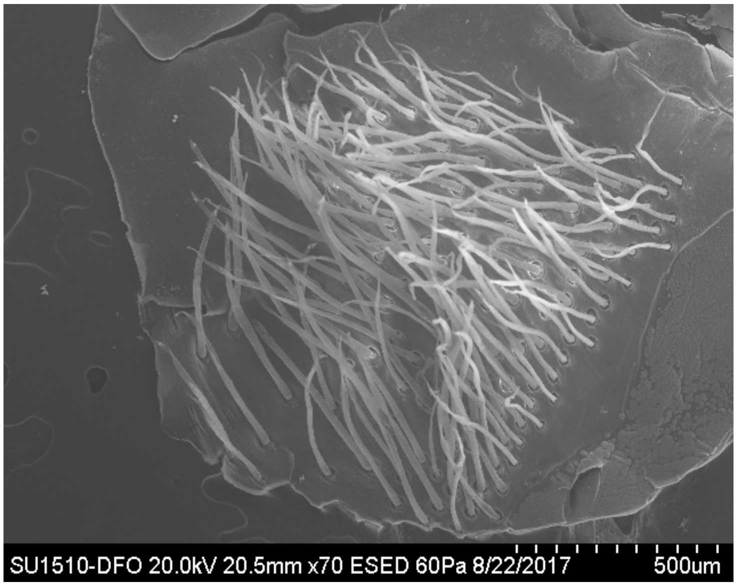

Figure 16. Example of Snow Crab group hairs under scanning electron microscope, to examine potential damage from chronic exposure to noise.

While the focus of results described thus far address behaviour, we also conducted physiological studies to investigate potential sub-lethal but physical impacts that could help to explain any behavioural observations. Physiology studies are commonly used to measure stress and damage caused by various industrial activities and are an important indicator of effect. Results of our physiological testing, including biochemical assay and histology, did not identify significant changes in Snow Crab physiology owing to seismic surveying. During 2017, at the location of a 3D seismic survey our results measured significant differences in 2 of 6 biochemistry indicators; however, the same results were not measured when the study was replicated in a following year. Therefore, we could not conclude that seismic surveying caused a predictable physiological impact, particularly given the large amount of measurement variability associated with the metrics considered. Previous studies have observed biochemical changes in the hemolymph ("blood") of American lobster (*Homarus americanus*) exposed to air gun sound in the Laboratory (Payne et al 2007), while an histological change was noted in the digestive tubules of lobster exposed to a seismic soundtrack (Payne et al 2015). A recent review paper by Edmonds et al (2016) highlights physiological sensitivity to underwater sound among several invertebrates including the Norway lobster (*Nephros norvegicus*).

During year 1 of the project, two sites were studied, Lilly Canyon (control site) and Carson Canyon (experimental site). Samples at Carson Canyon were collected before exposure to seismic, 18 hours, and 3 weeks after exposure. The samples collected at Lilly canyon match those collected at Carson Canyon 18 hours after exposure. During year 2, two new sites where 3D seismic exploration occurred were added to the project, North Carson Canyon 3D before and after sites. Finally, during year 3, two new sites were incorporated into the project and analyses were only carried out on Snow Crabs from these new sites (Harbour Deep control and experimental sites). Biochemical and histological analyses were carried out on samples of Snow Crab hepatopancreas and haemolymph. Biochemical analysis included the measurement of the activity of enzymes involved in phase I of chemical detoxification (ethoxyresorufin-O-deethylase), in phase II of chemical detoxification (glutathione S-transferase), antioxidant enzymes (superoxide dismutase and catalase), markers of lipid, protein and DNA oxidation, factors of the innate immune system (phenol oxidase) as well as protein concentration. Histological analysis included observations and recordings of any tissue abnormalities. Results from year one of the project showed that, generally speaking, no significant differences were observed in any of the biochemical or histological analyses. However, an obvious pattern of increase and decrease in activity/concentration was observed for EROD, GST, SOD and catalase activities as well as in markers of protein and DNA oxidation. In samples from year 2, a significant difference was observed in samples from the 3D seismic exposed crabs with lower EROD activity and higher protein carbonyl concentration as compared to the control site. Latest results from year 3, indicate EROD activity increased significantly after the first sampling period within the control site. No significant differences between control and exposed sites were detected at any of the sampling periods.

IV. Discussion

This study undertook a multifaceted approach, including several unrelated metrics, to offer strong inferences about the effect of seismic surveying on commercial Snow Crab, and did not measure consistent significant impacts. A lack of clearly defined signals in data collected from this field study, for any of the several indicators investigated, suggests that the effects of seismic surveying on commercial Snow Crab in offshore Newfoundland and Labrador waters is not distinguishable from the scope of natural variability observed. The lack of observed changes in catch rate were supported by absence of changes in crab movement behavior in response to seismic. In addition, for both these measurement metrics, the modelling approach used was sensitive to environmental parameters, adding confidence in the power of the applied models to detect an effect if it existed. Furthermore, biochemical indicators, and genomic studies also showed inconsistent results that were variable from one year to the next and among sites, and effects owing specifically to seismic was not discernable. When differences were observed, they did not last longer than 2 weeks and the differences were not detected at control sites located 30 km away.

A strength of this research is that it's based on experimental field-based research using a BACI study design, with replication over several years, and it included realistic field applications of industry based seismic surveying and fishing on commercial grounds. Study realism was a core element of this project, such that the results are directly applicable to both the oil and gas and fishing industries. The study incorporated input from affected industries, from the project design stages through to completion. Had this study not included replication over several years the inferred impacts could be different and wrong, which would have had a tremendous effect on future scientific studies. Furthermore, while we only matched experimental sites with one, or in some instances 2, control sites per experiment the control sites helped to ensure that sampling incorporated natural variability in a realistic way. As a result, the research was designed to produce several rich datasets despite challenges in the Snow Crab fishery related to a declining abundance of Snow Crab. Few, if any, other realistic studies such as this exist globally for fish or invertebrates.

Our study focused on commercial Snow Crab, which are all male and large in size. Future investigations of Snow Crab targeting female crab or different life-history stages (eggs and larvae) could be beneficial.

The tagging results and movement information for Snow Crab using acoustic telemetry is important information for commercial harvesters. Some crab, more than 50 of 250 crabs tagged , were recaptured during commercial fishing operations from 7 months up to 3 years post tagging indicating long-term movement patterns. Harvesters that caught the tagged crab reported that the crabs with tags were in good condition. It indicates that our tagging methods are likely very good, animals retain the tags and the animals appeared to survive in good health after tagging and though multiple exposures to seismic surveying in the region since the crabs were initially released. This information contributed to a paper on the migration of Snow Crab (Mullowney et al 2018) and helps to better understand the biology of Snow Crab.

The short-term telemetry data provided information of crab movement over the temporal scales of a fishing trip. For example, the spacing of crab pots during commercial harvesting has been based on some early assumption of Snow Crab movement between setting and hauling crab pots. Harvesters typically space crab pots about 40 meters apart, which is somewhat consistent throughout the Newfoundland and Labrador Snow Crab fishery. We found that most crab could move that distance within hours. Relevance to the fishery is that we obtained higher catch rates using shorter (ten pots per fleet versus 80 pots per fleet) fishing fleets of crab pots.

Laboratory work was important to rule out mortality and obvious physiological impacts. Cellular level histopathology analysis did not detect any evidence of damage owing to seismic. Furthermore, many tagged Snow Crab were recaptured in the commercial Snow Crab fishery, and the percentage was quite high such that the survival of tagged crabs is likely quite high. This is supported by observations of crabs held in captivity.

Other ESRF Snow Crab studies conducted in the Gulf of St. Laurence suggested that there was a lack of information on the gross physical morphology of Snow Crab statocysts. Because of this data gap, we examined several Snow Crab statocysts to describe the structure and investigate potential impacts of our laboratory experiment on chronic noise. This work helps to fill identified information gaps and also contributes to our understanding of the effects of noise. Our work focused on statistical measurements of group hairs, rather than other sensory hairs in the statocysts, and we suggest that future work focus on these other hair-cell types.

This ESRF project was the first scientific research to start constructing and characterizing gene libraries for Snow Crab, that are now publicly available to interested researchers. Bioinformatics approaches were used to identify functional annotations associations with genes that could be potentially affected by sound. For example, some of the RNA-seq identified genes are known to be associated with physiological stress or immune responses. Physiological indicators can suggest consequence of stress or immune response, and therefore linking indicators at the genomic level with physiological consequences (cellular tissue level), can provide inferences to overall effects. We did not measure physiological or genomic responses in a clearly predictable way, which is encouraging since there are little other contradictory results stemming from the overall project.

V. Deliverables

All proposed field experiments were completed as part of this project, and the results are providing information used in management decision making. This includes the completion of key performance indicators (peer review publication of scientific literature) as it is described in Schedule A of the original project, outlined in a Memorandum of Understanding between DFO and NRCAN.

Scientific detail is provided in separate peer reviewed reports corresponding to separate publications submitted to scientific journals. In addition, results from this project were provided to all stakeholders through a variety of consultations during the project. Results were formally presented at DFO's Snow Crab regional fishery stock assessment meeting in 2019. Results were also presented through DFOs Canadian Science Advisory Secretariat meeting to inform the Canadian statement of practice on seismic surveying. This advice is important to management decision making. Results were also presented at international conferences in Belgium, Australia, Netherlands, United States, and Canada which has generated worldwide interest in our seismic research. Results have been integrated into ongoing academia research including Canada Healthy Ocean Network (II) and has been reported on by various media. The results are incorporated in ongoing Environmental Assessment processes developed by the Seismic Surveying industry in consultation with the fishing industry, and evaluated by the CNLOPB seismic surveying regulator.

In the short term, the fishing industry are benefitting from results observed during this study. In recent years the Snow Crab fishery has declined and quota cuts are an immediate concern for harvesters. The causes of the decline is thought to be associated with environmental changes, as Snow Crab recruitment is reduced during periods of relatively warm water temperatures, that have been observed in this region during several recent years. Moreover, the fishery itself has a large impact on the availability of commercial Snow Crab abundance. The impact of seismic oil and gas exploration appears to have little, if any, negative impact on the catch rate of commercial Snow Crab (large male Snow Crab) or other indicators investigated in this study. This project has directly informed the fishing industry with information annually for the past 4 years, as data became available through industry presentations and consultations. Completion of this study in

2019 is very timely because management decisions for the Snow Crab fishery faces significant challenges, while oil and gas activities continue to develop in the Newfoundland and Labrador region.

In the medium term, results from this work informs CNLOPB and DFO management decisions regarding future environmental assessment processes and potential consideration as part of future land tenure considerations of potential impact and the Canadian standards of practice for seismic surveying. Whether fisheries will be impacted by oil and gas processes is an important part of decision making, which benefits from the best available scientific information. For commercial Snow Crab and its fishery at least, this study indicates that if there are potential effects of seismic, the effects occurred within 30 km of the survey and were not observed 2 weeks after surveying had ended. This information can inform the seismic surveying industry to guide its operations in Newfoundland and Labrador offshore waters in a manner that mitigates potential impacts using the best available information, and helps to ensure appropriate industry interactions with minimal environmental effects.

Future research planning will markedly benefit from the results and challenges identified during this study. Firstly, this study marks a breakthrough in methodologies that demonstrate the possibilities of conducting this type of work with relatively low risk in an offshore environment. As a result, future studies will not be limited by "unproven methodologies". This will allow future studies to focus more effort on dealing with the challenge of natural variability. The largest source of measurement error in this large-scale field study was associated with natural variability. While we encourage future studies to include as many control sites as needed, we also suggest that using multiple lines of evidence is a prudent means to inform decision making. In addition, the availability of baseline data, used to conduct power analysis and simulation modeling to understand natural variability, will help to design future studies to detect impacts during field investigations. While the potential impacts of seismic surveying are clearly important from an economic perspective, commercial fishing enterprises should take some solace in the results provided by this study, which indicate that seismic is probably not having a major impact on large male Snow Crab or their catch rates; at least in comparison to known factors including environmental conditions affecting Snow Crab recruitment and fishing mortality. This study does not dismiss the possibility of seismic surveying effects, and helps to guide future research in other areas.

This was a scientific research project, with a conscious effort to maintain open, transparent, inclusive, and unbiased dialogue with all stakeholders and project participants throughout the study. The primary performance indicators from this study, as per schedule A of the MOU guiding this project, is peer-reviewed primary publications. Peer-reviewed publication of research in credible scientific journals is the highest standard that scientific information can offer. It is, by far, the most valued source of information used by scientists and the scientific community; information provided through the anonymous peer-review process is accepted among scientists as truth unless proven otherwise by research subjected to the same or similar review process. This project has already generated several published manuscripts, and others are in the final stages of review, for peer-review publication. The study was designed such that the results were publishable regardless of the results detecting an impact of seismic or not – both outcomes contribute valuable information. Published information will form the basis of scientifically accepted advice for management decision making authorities, as being the best scientific information available for their consideration. Delivery of scientific advice has already begun, and our findings have been presented, upon request. Several newspaper and radio interviews were conducted that also described the results of this study to the general public. The results were presented at federal review of information pertaining to the state of knowledge informing the Canadian Standards of practice for seismic surveying. Importantly, reporting of results to all stakeholders (fishing industry, oil and gas industry, federal regulators) was conducted at regular meetings, twice per year, throughout the study.

VI. Conclusion

This study did not measure predictable impacts of seismic surveying on commercial Snow Crab catch rates, movement, physiology, or gene-regulation. While statistical analysis showed some differences in some years the results were often different among years. Furthermore, the same conclusions came from multiple lines of information; catch, behaviour, or physiological. We do not dismiss the possibility of seismic impacts generally, and we are quick to point out the low statistical power of sampling associated with some field studies and limitations of low sample replicates (low number of different seismic surveys). Field studies are difficult to control and natural variability is high, making detection of seismic effects difficult to accurately measure. Our sampling did however measure significant differences owing to different sites, timing of sampling, and depth, while not detecting effects specific to seismic surveying. This suggests that if seismic impacts were occurring with the same or similar level of effect then we would have measured them. Furthermore, there is consistency among several independent measurement metrics described, that suggest while impacts are notably difficult to measure, the level of impact from seismic surveying appear to be within the scope of natural variability. Indeed, commercial fishery catch rates are naturally variable, and there are differences in average fishery catch rates from one week to the next or between different fishing areas, that changed as much as or more than the differences we measured in our catch rate experiments among control sites and among before-during-after seismic sampling.

Our study addressed commercial catchability and factors that could affect catch rates, targeting only large terminally moulted mature male Snow Crab. Our research indicates that should effects exist, they might occur at different life history stages or among female crab which we did not examine. Further, we suggest that potential effects undetected by this study are likely to be sub-lethal and subtle in nature relative to the effects of other naturally occurring factors. We do no not suggest that other potential impacts are unimportant or non-existent, rather we hope that our findings will help to guide future research and decision making in the short, medium, and long term.

VII. Acknowledgement

This study involved an team of people including scientists from many disciplines, several different industries, and a range of regulatory managers both federal and provincial. The Environmental Studies Research Fund provided financial support. Dave Taylor's (ESRF East Coast Regional Research Manager) incredible knowledge of the oil and gas industry, the fishing industry, and the people working in these fields, was an enormous contribution towards connecting this project together. Numerous stakeholders were required to work together in order to make this scientific project possible, therefore the project followed a diligent "meeting schedule" to make that happen. Follow-up meetings when requested, which happen often, were conducted with any interested stakeholders and we thank them for their interest and contributions to the success of this project. We thank all stakeholders for their voluntary participation in this project, including; several members of the FFAW that represented crab harvesters in Newfoundland and Labrador, the offshore fishing industry represented by Ocean Choice International (Rick Ellis, Carey Bonnell, and Greg Viscount), the seismic surveying industry (Petroleum GeoServices (Neil Paddy and Jerry Whitney)), the oil and gas industry (Canadian Association of Petroleum Producers), marine regulators responsible for both oil and gas exploration (Canada-Newfoundland and Labrador Petroleum Board) and Fisheries Resources (Fisheries and Oceans Canada), academic interests from Memorial University, as well as the conservation organization Nature NL. Many vessels were used in this project, and we thank the owners and the crews for there invaluable expertise and contributions that ensured this research experiment was as realistic as possible - including actual harvesters and fishing vessels, as well as operational seismic surveying vessel that operated on commercial fishing grounds.

Several people were directly involved in conducting the research-work involved in this project, including Jerry Whitney (PGS), Curtis Pennell (DFO), Dustin Schornagel (DFO), Brianna Newton (DFO), Tiffany Small(DFO), Victoria Han (DFO), Craig Kelley(DFO), Keith Clarke(DFO), Charmaine Duggan(DFO), and several FFAW observers and technicians. Commercial harvesters, including licensed boat owners and their crews, were directly responsible in collecting data used in this experiment; Corey Brothers (Crab Harvester- vessel captain), Robert Daley (Crab Harvester- vessel captain), Walter Drover (Crab Harvester- vessel

captain), Andrew Daley (Crab Harvester- vessel captain), Dennis McCarthy (Carb Harvester-vessel captian) and provided valuable services, experience, and knowledge to this project.

VIII. References and further reading

Andrew, R.K., Howel, B.M., and Mercer, J.A. 2011. Long-time trends in ship traffic noise for four sites off the North American West Coast. Journal of the Acoustical Society of America 129(2): 642-651. https://doi.org/10.1121/1.3518770.

Carroll, A.G., Przeslawski, R., Duncan, A., Gunning, M., Bruce, B. 2017. A critical review of the potential impacts of marine seismic surveys on fish & invertebrates. Mar. Pol. Bul. 114: 9-24. https://doi.org/10.1016/j.marpolbul.2016.11.038.

Christian, J. R.,Mathieu, A.,Thompson, D.H., White, D., Buchanan, R.A. 2003. Effect of seismic energy on Snow Crab (Chionoecetes opilio). Environmental Studies Research Funds Report No. 144, Calgary. 106p.

Christian, J.R., Mathieu, A., Buchanan, R.A. 2004. Chronic effects of seismic energy on Snow Crab (Chionoecetes opilio): Environmental Studies Research Funds Report No. 158 Calgary, 45p.

Cote, D., Morris, C.J., Regular, P.M, Piersiak, M. G. 2020. Effects of 2D Seismic on Snow Crab Movement Behavior. Fisheries Research 230. doi: 10.1016/j.fishres.2020.105661.

Courtenay, S.C., Boudreau, M., Lee, K. 2009. Potential Impacts of Seismic Energy on Snow Crab: an Update to the September 2004 Peer Review. Environmental Studies Research Funds Report No 178. Moncton (181p).

Edmonds, N.J., Firmin, C.J., Goldsmith, D., Faulkner, R. C., Wood, D. T. 2016. A review of crustacean sensitivity to high amplitude underwater noise: data needs for effective risk assessment in relation to UK commercial species. Mar. Pollut Bull ., 108 (2016), pp. 5-11, 10.1016/jmarpolbul.2016.05.006

Hall, J.R., Lehnert, S., Gonzalez, E., Kumar, S., Bradbury, I.R., Hanlon, J.M., Morris, C.J., Rise, M.L. 2020. Snow crab (*Chionoecetes opilio*) hepatopancreas transcrptome: identification and testing of candidate molecular biomarkers of seismic survey impact. Fisheries Research 234, 105794 https://doi.org/10.1016/j.fishres.2020.105794

Hawkins, A. D., Pembroke, A. E., Popper, A.N. 2015. Information gaps in understanding the effects of noise on fishes and invertebrates. Rev. Fish Biol. Fish., 25 (2015), pp. 39-64, 10.1007/s11160-014-9369-3

Hildebrand, J.A., 2009. Anthropogenic and natural sources of ambient noise in the ocean. Mar. Ecol. Prog. Ser. 395:5-20. https://doi.org/10.3354/meps08353

Martin, B.S., Morris, C. J., Broker, K., O'Neill, C. 2019. Sound exposure level as a metric for analyzing and managing underwater soundscapes. Journal of the Acoustical Society of America. https://doi.org/10.1121/1.5113578.

Morris, C.J., Cote, D., Martin, B., Kehler, D., 2018. Effects of 2D seismic on the Snow Crab fishery. Fish. Res. 197: 67–77. https://doi.org/10.1016/j.fishres.2017.09.012 .

Morris CJ, Cote D, Martin SB, Mullowney, D. 2020. Effects of 3D seismic surveying on snow crab fishery. Fisheries Research 232:105719. doi: https://doi.org/10.1016/j.fishres.2020.105719.

Mullowney DRJ, Baker KD, Zabihi-Seissan S, Morris, C.J. 2020. Biological perspectives on complexities of fisheries co-management: A case study of Newfoundland and Labrador snow crab. Fisheries Research 232:105728. doi: https://doi.org/10.1016/j.fishres.2020.105728.

Mullowney, D., Morris, C., Dawe, E., Zagorsky, I., Svetlana, G. 2018. Dynamics of snow crab (Chionoecetes opilio) movement and migration along the Newfoundland and Labrador and Eastern Barents Sea continental shelves. Reviews in Fish Biology and Fisheries. Doi.org/10.1007/s11160-017-9513-y

Nieukirk, S.L., Mellinger, D.K., Moore, S.E., Klinck, K., Dziak, R.P., and Goslin, J. 2012. Sounds from airguns and fin whales recorded in the mid-Atlantic Ocean, 1999–2009. Journal of the Acoustical Society of America 131(2): 1102-1112. https://asa.scitation.org/doi/10.1121/1.3672648.

Nowacek, D.P., Clark, C.W., Mann, D., Miller, P.J.O., Rosenbaum, H.C., Golden, J.S., Jasny, M., Kraska, J., and Southall, B.L. 2015. Marine seismic surveys and ocean noise: Time for coordinated and prudent planning. Frontiers in Ecology and the Environment 13(7): 378-386. https://doi.org/10.1890/130286

Payne, J., Andrews, C., Fancey, L., Cook, A., Christian, J., 2007. Pilot Study on the Effects of Seismic Air Gun Noise on Lobster (Homarus Americanus). Can. Tech. Rep. Fish. Aquat. Sci. 2712: v + 46.

Payne, J.F., Andrews, C.D., Hanlon, J., Lawson, J., Mathieu, A., Wadsworth, B., French, B. 2015. Effects of seismic air gun sounds on lobster (Homarus Americanus): Pilot laboratory studies with (a) a recorded track from a seismic survey and (b) airgun pulse exposure over 5 days. Environmental Studies Research Fund . Government of Canada. Report 197. 38 pages.

IX. Appendices

The appendices included in this report consist of publications that have been produced as a direct result of ESRF research related to the effects of seismic surveying on Snow Crab. These publications are published and available from the publishers' website, and are typically open access.

APPENDIX 1 – Sound Exposure Level as a Metric for Analyzing and Managing Underwater Soundscapes

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Sound exposure level as a metric for analyzing and managing underwater soundscapes[a)]

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The auditory frequency weighted daily sound exposure level (SEL) is used in many jurisdictions to assess possible injury to the hearing of marine life. Therefore, using daily SEL to describe soundscapes would provide baseline information about the environment using the same tools used to measure injury. Here, the daily SEL from 12 recordings with durations of 18–97 days are analyzed to: (1) identify natural soundscapes versus environments affected by human activity, (2) demonstrate how SEL accumulates from different types of sources, (3) show the effects of recorder duty cycling on daily SEL, (4) make recommendations on collecting data for daily SEL analysis, and (5) discuss the use of the daily SEL as an indicator of cumulative effects. The autocorrelation of the one-minute sound exposure is used to help identify soundscapes not affected by human activity. Human sound sources reduce the autocorrelation and add low-frequency energy to the soundscapes. To measure the daily SEL for all marine mammal auditory frequency weighting groups, data should be sampled at 64 kHz or higher, for at least 1 min out of every 30 min. The daily autocorrelation of the one-minute SEL provides a confidence interval for the daily SEL computed with duty-cycled data. © *2019 Acoustical Society of America*. https://doi.org/10.1121/1.5113578

[ANP] Pages: 135–149

I. INTRODUCTION

Mankind's increasing use of the ocean for transportation, food, and energy extraction has led to an increase in marine pollutants, including sound. Studies of these activities demonstrate potentially negative impacts of our activities on marine life (Southall *et al.*, 2019). The effects of sounds on humans and animals can be visualized as a series of four zones or concentric rings of diminishing impact around the sound source (e.g., Fig. 1 in Dooling *et al.*, 2015). In this model, the highest level of impact occurs in zone 1 from exposures that cause physical barotrauma or permanent hearing loss (e.g., Halvorsen *et al.*, 2012a; Casper *et al.*, 2017), followed by temporary hearing loss in zone 2 (see review in Finneran, 2015), then masking of important biological sounds used by animals in zone 3 (Shannon *et al.*, 2016), and finally in zone 4 the sound levels elicit subtle behavioral or physiological stress responses (Rolland *et al.*, 2012).

The zone-view of the effects of noise does not accurately reflect the complexity of auditory injury or impairment and the choices animals make to accept sound exposure for other advantages such as feeding or mating (Ellison *et al.*, 2012). When animals make the choice not to respond to noise, they can stay in an area where very long sound exposures result in auditory injury and impairment, and thus zone 2 may be larger than zone 4 (Hawkins and Popper, 2017). Similarly, behavioral reactions to sound can cause animals to rapidly leave an area, which could result in dangerously rapid depth changes (Jepson *et al.*, 2003; Blix *et al.*, 2013) or entering an area that results in stranding (Cox *et al.*, 2006); in this manner zone 4 becomes zone 1.

As a general rule regulations impose a requirement on human ocean activities to predict the size of zone 1, then ensure that no endangered or threatened animals are within that area (Erbe, 2013). Regulations to reduce masking, disturbance, and behavioral responses are less common but may be applied, for instance, to whale watching boats (e.g., see the Canadian whale watching regulations[1]). As more studies of the effects of sound become available, it will be possible to manage the effects of a wider range of man-made sound to prevent behavioral changes that could affect feeding, navigating, mating, rearing of young, or the harvesting of commercial fish stocks. *The Population Consequences of Acoustic Disturbance* (NRC, 2005) and "Population Consequences of Disturbance" (King *et al.*, 2015) models provide frameworks for understanding the sub-lethal effects of sound on marine populations (Costa *et al.*, 2016).

Managing sound levels requires indicators that relate sound characteristics, including amplitude to effects on marine

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[a)]This paper is part of a special issue on The Effects of Noise on Aquatic Life.

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life. Sound exposure level (SEL), peak sound pressure level, and the sound pressure level are three amplitude metrics typically used to quantify sound in the environment. Early marine sound mitigation regulations were based on keeping sound pressure levels below the level associated with measured injuries to the hearing of marine mammals (NMFS and NOAA, 1995; NOAA, 1998). Evidence has since demonstrated that peak sound pressure level and SEL are better predictors of injury for most groups of marine life (Southall *et al.*, 2007; Popper *et al.*, 2014; Southall *et al.*, 2019). Peak sound pressure level is associated with immediate physiological injury to tissues (Halvorsen *et al.*, 2012b). The sound pressure level varies with the averaging time, which makes it difficult to obtain repeatable values between research teams or methods, especially when analyzing the effects of impulsive sound sources (Madsen, 2005; Hawkins *et al.*, 2014). SEL is associated with fatigue injury through the equal energy hypothesis that states the effects on hearing are the same for the same total energy (Eldredge and Covell, 1958). For example, a sound pressure level of 190 dB re 1 μPa^2 for 1 s or 160 dB re 1 μPa^2 for 1000 s both have a SEL of 190 dB re 1 μPa^2 s and are expected to have the same effect on hearing. The daily SEL metric has an additional advantage over the sound pressure level of an acoustic event in that its duration is precisely defined. It is also simple to compute since it does not depend on detecting when a signal is present.

There are many research results that show the equal energy hypothesis does not represent the complexity of the effects of sound on hearing. It is well established that impulsive sounds affect hearing at lower SELs than continuous sounds (Ward, 1962; Akay, 1978; Finneran, 2015). The temporal pattern of impulses also changes the effects of sound on hearing for the same total SEL. In terrestrial mammals, including humans, 1 pulse per second has significantly greater impact than 10 pulses per second or 1 pulse every 10 s (Danielson *et al.*, 1991; Qiu *et al.*, 2013). Within the American regulations to protect marine life from human sounds, the dependence of hearing effects on sound's temporal patterns are reflected in different equal energy thresholds for continuous and impulsive sounds (Popper *et al.*, 2014; NMFS, 2018). Significant research is still required to understand how sound's characteristics, besides the pressure amplitude and energy, affect marine life. Particularly important are particle motion effects on fish and invertebrates and the temporal patterns of the sound on all marine taxa (Finneran, 2015; Hawkins and Popper, 2017; Houser *et al.*, 2017; Popper and Hawkins, 2018).

The publication of the *Technical Guidance on Assessing the Effects of Anthropogenic Sound on Marine Mammal Hearing* (NMFS, 2016) and a minor revision (NMFS, 2018) have made the auditory frequency weighted SEL, integrated over 24 h, the primary metric for predicting and measuring the effects of human industrial sound on marine life. However, this metric is not well understood—there are few examples of typical SELs or how the SEL depends on movement of sources and receivers, limited information on how to collect data for assessment of daily SEL, or results showing what additional information about the environment can be obtained by analyzing the daily SEL. This study addresses these data gaps through the analysis of 12 long-term data sets that provide examples of natural soundscapes and those affected by human activities. The temporal characteristics of human sound sources and natural environments are addressed in a separate study.

This manuscript is supported by extensive supplemental material[2] that includes: why SEL is a measure of the received energy, how to compute SEL across multiple events, further information on auditory weighting functions, hydrophone and recorder self-noise data, gamma random noise distributions that are similar to typical ocean noise distributions, statistical measures (mean, variance, skewness, kurtosis, gamma fit, and autocorrelation durations) for each data set, and confidence intervals for duty-cycled daily SELs.

II. METHODS

A. Data sets

Twelve data sets from ten recording locations (Fig. 1) were analyzed to provide an indication of the range of daily SEL, show how SEL accumulates from different sources, and provide examples of how different data collection techniques affect daily SEL. All recordings were performed using an AMAR G3 recorder (JASCO Applied Sciences, Dartmouth, NS, Canada) and either M8 or M36 hydrophones (GeoSpectrum Technologies Inc, Dartmouth, NS, Canada) or HTI-99-HF hydrophones (High Tech Inc, Long Beach, MS) (Table I). The data sets were selected to ensure that flow noise and other artefacts did not contribute to the daily SEL.

B. SEL

The acoustic metrics and terminology employed in this analysis follow ISO Standard 18405 (ISO, 2017). The SEL is a representation of sound energy that is defined as 10 dB times the logarithm (base 10) of the sound exposure, which is the integral of the squared sound pressure over some period of time T, normalized by a reference squared pressure p_0^2 and reference time T_0,

$$L_{E,T} = 10\log_{10}\left(\frac{1}{T_0 p_0^2}\int_0^T p^2(t)\,dt\right)\ \text{dB re } 1\ \mu\text{Pa}^2\ \text{s}. \qquad (1)$$

T_0 is normally 1 s and p_0 is 1 μPa, so that the unit of $L_{E,T}$ are dB re 1 μPa^2 s. The daily SEL is 49.4 dB higher than the arithmetic mean of the daily sound pressure level.

There are two pathways by which sound can affect hearing—intense, high amplitude sounds that damage hearing organs, or long-term exposure that causes temporary or permanent threshold shifts. The long-term exposures only affect hearing if the sounds are within an animal's hearing frequency range. Therefore, during SEL analysis recorded sounds are typically filtered by the animal's auditory frequency weighting function before integrating to obtain SEL. Weighted sound exposure and SEL are defined as

$$E_{p,W,T} = \sum_{n=0}^{N}\int_0^{f_s/2} W(f)S_t(f)df\ \text{Pa}^2\ \text{s}, \qquad (2)$$

$$L_{E,W,T} = 10\log_{10}\left(\frac{E_{p,W,T}}{T_0 p_0^2}\right)\ \text{dB re } 1\ \mu\text{Pa}^2\ \text{s}, \qquad (3)$$

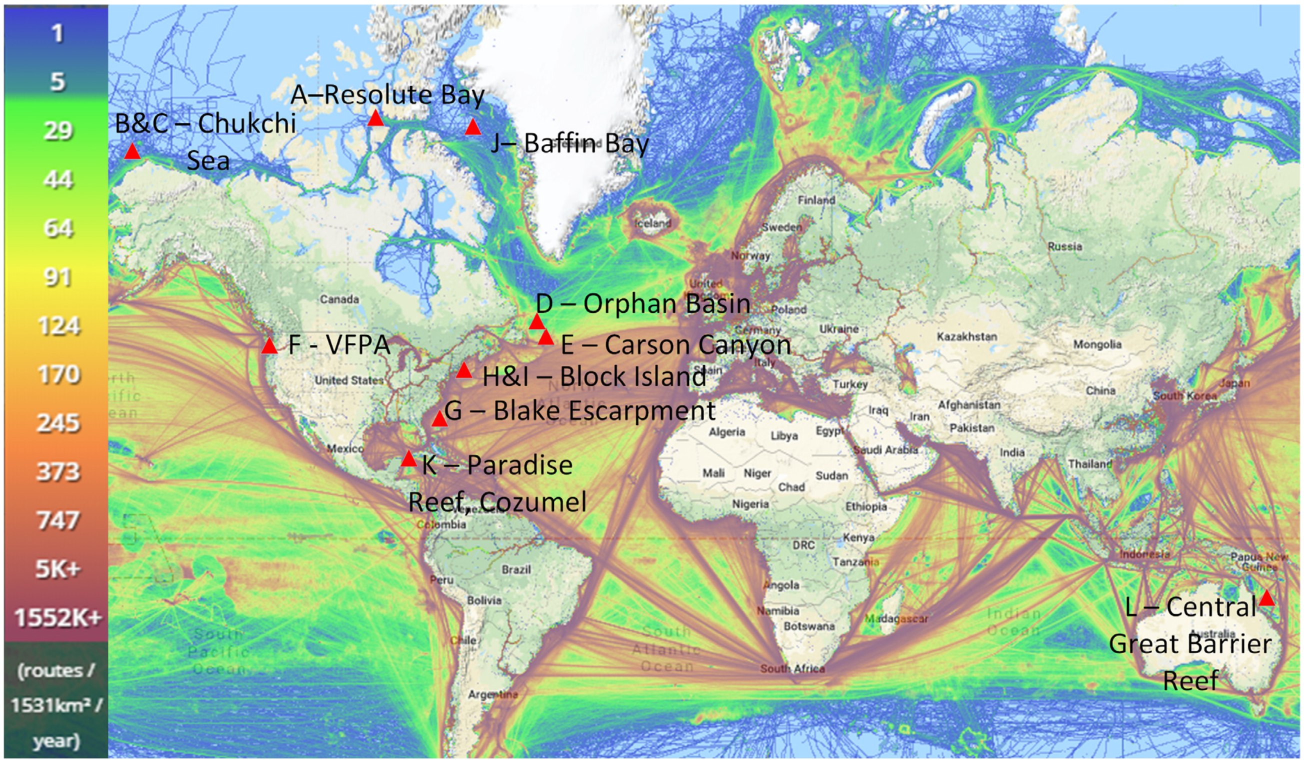


FIG. 1. (Color online) Recording locations whose data were used in this analysis. The underlay of the map is the 2017 marine traffic density (see footnote 3).

where $W(f)$ is the auditory frequency weighting function and $S_t(f)$ is the power spectrum of the pressure time series over a period of t seconds. The total signal duration T is normally divided into N equal sized blocks of duration t whose sound exposures are summed before taking $10\log_{10}$ to convert to the decibel representation.

Auditory frequency weighting functions and auditory injury thresholds have been defined for six groups of marine mammals: low-, high-, and very high-frequency cetaceans, sirenians, as well as otariid and phocid seals in water (Southall *et al.*, 2019; sirenians are not considered here). As a first approximation, the low-frequency auditory weighting function may be thought of as a 100 Hz high pass filter. Similarly, the phocid and otariid functions are ∼4 kHz high pass filters, the high-frequency function is ∼10 kHz high pass filter, and the very high-frequency function is ∼20 kHz high pass filter. For this analysis, the full bandwidth SEL for the recordings is computed starting at the 10 Hz decidecade and is referred to as the "10+ Hz SEL" or the "10 Hz and above SEL." The SEL that is applied under American regulations for marine life other than mammals is the 10+ Hz SEL [Eq. (1)]. Research on the hearing of other marine animal groups is needed to define their auditory frequency weighting functions and exposure thresholds. Equation (3) may be applied to the power spectrum as shown, or it may be applied to the decidecade SEL for an event using the center frequencies of the decidecades to compute the weighting (see the supplemental material[2] or Tougaard and Beedholm, 2019).

C. Determining the effects of duty cycling on SEL

Seven of the data sets used in this analysis were duty-cycled between high and low sample rates (Table I). The high sample rate data were essential for detecting the calls of high- and very high-frequency marine mammals, as well as for computing the weighted SEL for these groups. To estimate the weighted SEL from the duty-cycled data, we first computed the per-minute sound pressure level ($L_{p,1\,\mathrm{min}}$) and per-minute dedidecade sound pressure levels ($L_{p,\mathrm{ddec,1min}}$) for the data from both sample rates. A one-minute duration was chosen since it is the shortest continuous duration used in this analysis (Table I) and a common duration for estimating the sound pressure level (Ainslie *et al.*, 2018). The measured data had missing sound pressure and decidecade sound pressure values due to the duty cycling. These were estimated by linear interpolation of the linear data (i.e., $10^{L_p/(10\,\mathrm{dB})}$) on either side of the missing values. The linear decidecade sound pressures were weighted by the marine mammal auditory frequency weighting functions, then summed to obtain the weighted per-minute sound pressures, and those were summed to obtain the daily sound exposure at each sampling rate,

$$L_{E,W,24h} = 10\log_{10}\left(\sum_{t=0}^{t=1440\,\mathrm{min}} 10^{L_{P,W,t}/10}\right) + 10\log_{10}(60\,\mathrm{s/min}). \tag{4}$$

For each auditory frequency weighting function, the daily SEL was computed from all available data with enough bandwidth. The minimum sample rates were 8000 Hz for 10+ Hz and low-frequency cetacean weightings, 16 000 Hz for otariid and phocid weightings, and 48 000 Hz for high- and very high-frequency cetacean weightings. When data from more than one sample rate were available the data sets were merged in time before interpolating.

TABLE I. Acoustic recordings used in this study. System spectral noise floor values with a superscript "R" indicate that the noise floor limit was from the recorder, and the remainder of the limits are due to the hydrophones.

Location ID	Location name	Primary Sound Sources	Water depth (m)	Latitude (degrees N)	Longitude (degrees E)	Recording dates	Hydrophone type	Hydrophone sensitivity level (dB re 1 V/μPa)	System spectral noise floor (dB re 1 μPa^2/Hz)	Sample rate and duty cycle	10 Hz + daily SEL noise floor (dB re 1 μPa^2)[a]
A	Resolute Bay	Open ocean and small boats (16 Aug–2 Oct); Ice + open water noise (3 Oct–2 Nov)	60	74.65	−94.84	16 Aug–2 Nov 2014	M8E-V35dB	−165	32	2 min at 96 kHz; 2 min sleep	128.2
B	Chukchi Sea, 2014	Open ocean	47	71.34	−163.1	6 Aug–14 Oct 2014	M8E-V35dB	−165	34^{R}	13 min at 16 kHz; 2 min at 375 kHz	135^{R}
C	Chukchi Sea, 2015	Dynamic positioning from semi-submersible drill rig; location is 1 km from Chukchi 2014 site	51	71.19	−163.5	25 Jul–2 Oct 2015	M8E-V35dB	−165	32	64 kHz continuous	126.5
D	Orphan Basin	Open ocean (1 Apr–24 May); seismic airgun survey (25 May–30 Jun)	1282	48.73	−49.38	1 Apr—30 Jun 2016	HTI-99-HF	−163	42	11 min at 8 kHz; 1 min at 250 kHz; 8 min sleep	142
E	Carson Canyon	Open ocean, fishing, seismic airgun survey	120	45.46	−48.79	4 Sept–17 Oct 2016	M36-V35-100	−165	34^{R}	7 min at 16 kHz; 1 min at 375 kHz	135^{R}
F	Vancouver-Fraser Port Authority	Recorded under the port of Vancouver's inbound shipping lane	170	49.05	−123.3	1 Jan–7 Apr 2018	M36-V35-100	−165	32	128 kHz continuous	129.5
G	Blake Escarpment	Open ocean with some shipping	872	29.25	−78.35	15 Mar–9 Jun 2018	M36-V35-100	−165	34 ^{R}	16 min at 8 kHz; 1 min at 250 kHz; 4 min sleep	134 ^{R}
H	Block Island, 850 m from piling	Impact pile driving	26	41.11	−71.52	14 Oct–3 Nov 2015	M8E-V0dB	−200	53 ^{R}	64 kHz continuous	147 ^{R}
I	Block Island, 9100 m from piling	Impact pile driving	42	41.06	−71.45	14 Oct–3 Nov 2015	M8E-V35dB	−165	32	64 kHz continuous	126.5
J	Baffin Bay	Seismic airgun survey except first two days	603	74.16	61.98	30 Jul–30 Sept 2012	M8E-V0dB	−200	56 ^{R}	64 kHz continuous	150 ^{R}
K	Paradise Reef	Coral Reef, 500 m from cruise ship pier; frequented by small tourist dive boats	11	20.47	−86.98	15 Jul–2 Sept 2017	M36-V35-100	−165	34 ^{R}	14 min at 32 kHz; 1 min at 375 kHz	135 ^{R}
L	Central Great Barrier Reef	Coral reef without human sources	18	−18.8	147.5	27 Apr–15 Jul 2013	M8E-V35dB	−164	34^{R}	7 min at 64 kHz; 2 min at 375 kHz; 6 min sleep	135^{R}

[a] Daily 10 Hz and above SEL noise floor is the spectral noise floor + 10 log$_{10}$(86 400 sec/day) + 10 log$_{10}$(recorder bandwidth); see the supplementary material.

The low duty cycle for higher sample rates (Table I) means that the daily SEL for the seals, as well as the high- and veryhigh-frequency cetaceans, were extrapolated from only 4%–10% of a day's data. To estimate the error from this extrapolation, the daily SEL were computed from the continuously sampled data sets (data sets C, F, H, I, and J) with duty cycles simulated by decimating the data to 1 min every 2, 3, 4, 5, 6, 8, 10, 12, 15, or 20 min. The errors were the full bandwidth continuous daily SEL subtracted from the SEL calculated after decimating in time so that a negative value means the subsampled SEL was less than the actual SEL. For each decimation rate, the decimated daily SEL was computed for all starting points of the subsampling, which increased the sample size for estimating the effects of subsampling. For example, the Chukchi Sea 2015 data (data set C) had 67 full daily SEL, 134 daily SEL estimates at the 1:2 decimation rate, and 1340 at 1:20.

When using daily SEL computed from duty-cycled data it is useful to know the range of errors that could result from the duty cycling. Since the duty-cycled SEL were computed by interpolating the available measurements, the accuracy of the daily SEL depends on how well the measurement made at some time T can be predicted from the previous measurement, for example $T - 20$ min for the Orphan Basin data. It was expected that the error would be related to the decimated data's autocorrelation. The error in daily SEL obtained by subsampling each of the continuous data sets was plotted against the first autocorrelation time lag of the subsampled one-minute sound exposures [Eq. (2)] for that day. This corresponds to a lag of 2, 3, 4, 5, 6, 8, 10, 12, 15, or 20 min depending on the subsampling. The resulting distributions were characterized by their mean values and the 95% confidence intervals. We also tested how well the subsampled autocorrelation matched the autocorrelation of the original data. To assess the generality of these results, the same analysis was performed using gamma-distributed random data rather than continuous data sets. The characteristics of the gamma-random data are discussed in the supplemental material.[2] Comparisons were made for the marine mammal auditory frequency weighting function weighted and 10+ Hz daily SEL.

III. RESULTS

A. Daily SEL levels in the data sets

Figure 2 shows the daily SEL for data sets A–L (Fig. 1, Table I). Table II lists the mean daily SEL and standard deviations. The mean high- and very high-frequency auditory frequency weighted SEL were often self-noise limited for the data from Orphan Basin (D) and Baffin Bay (J). Figure 3 provides autocorrelations of the one-minute SEL from the full duration of each recording.

The data sets include natural soundscapes as well as soundscapes with different types of human activity. The Resolute Bay (A) and Chukchi Sea 2014 data (B) are both Arctic recordings in water depths of 50–60 m. In Resolute Bay (A) during open water the daily SEL depends on the passage of small boats. After ice arrives the sound levels drop due to both the ice cover and less wind driven noise. In the Chukchi Sea 2014 during periods of low background sound levels (presumably periods of low winds) the 10+ Hz daily SEL dropped to 140–145 dB re 1 μPa^2 s and increased to 160 dB dB re 1 μPa^2 s during periods of high winds. Since the mean low-frequency cetacean auditory frequency weighted daily SEL were within 2.5 dB below the 10+ Hz SEL, at least half of the sound energy was above 100 Hz in this soundscape. The 10+ Hz, low-frequency, otariid, and phocid weighted SEL were highly auto-correlated for the 26 h shown in Fig. 3, which indicates that a slowly varying process was affecting the sound levels—i.e., wind and wave driven sound. In contrast, ice formation and movement in Resolute Bay data (A) increased the variability in the daily SEL and decorrelated the data within 30 min.

Blake Escarpment (data set I) and Orphan Basin (data set D; Figs. 2 and 3, Table II) had similar mean low-frequency cetacean auditory frequency weighted SEL that were also close to the low-frequency cetacean auditory frequency weighted levels in the Chukchi Sea in 2014. Both had maximum daily 10+ Hz SEL of 160 dB re 1 μPa^2 s, except for three days at Blake Escarpment. At Blake Escarpment, the 10+ Hz SEL were 8.4 dB above the low-frequency cetacean auditory frequency weighted SEL, while in Orphan Basin, prior to the start of seismic surveys on 25 May 2016, the 10+ Hz SEL was only 2.9 dB above the low-frequency cetacean auditory frequency weighted SEL, like the Chukchi Sea in 2014. The autocorrelations of the Orphan Basin data remained high after 26 h, like the results in the Chukchi, whereas the Blake Escarpment autocorrelation dropped below 0.1 within 30 min. The autocorrelation difference indicates that the primary source of sound changes on the scale of 30 min at Blake Escarpment. The differences between the 10+ Hz SEL and low-frequency cetacean auditory frequency weighted SEL were due to energy in the 10–100 Hz frequency band. This is the band with highest energies from heavy shipping (e.g., Wenz, 1962; McDonald *et al.*, 2006; Chapman and Price, 2011), but it may also contain energy from animals (e.g., fishes and large whales), seismic surveys, or flow-induced noise around hydrophones. Manually reviewing the Blake Escarpment data showed that vessels frequently passed the recorder, but there were no other distinct sound sources when no vessels were present.

The Chukchi Sea 2015 exploratory drilling program (data set C) and Vancouver-Fraser Port Authority (data set F) data contain high levels of sound from vessels. The Chukchi Sea 2015 data were 1 km from exploratory oil and gas drilling, and had a 10+ Hz and low-frequency cetacean auditory frequency weighted daily SEL 26 dB higher than the same site in 2014. At 16 km from the drilling activities, the average 10+ Hz daily SEL was 16 dB higher than in 2014 (not shown). Most of the sound was produced by dynamic positioning systems whose energy is above 100 Hz, which can be seen by the small difference between the 10+ Hz and the low-frequency cetacean auditory frequency weighting function SEL. This difference was higher at the 16 km measurement site due to more sound from support vessels instead of the drilling platform. At the Vancouver-Fraser Port Authority the mean 10+ Hz daily SEL was 14 dB above the Chukchi in 2014. The differences decreased with increasing frequency but were still ~7 dB for the high-

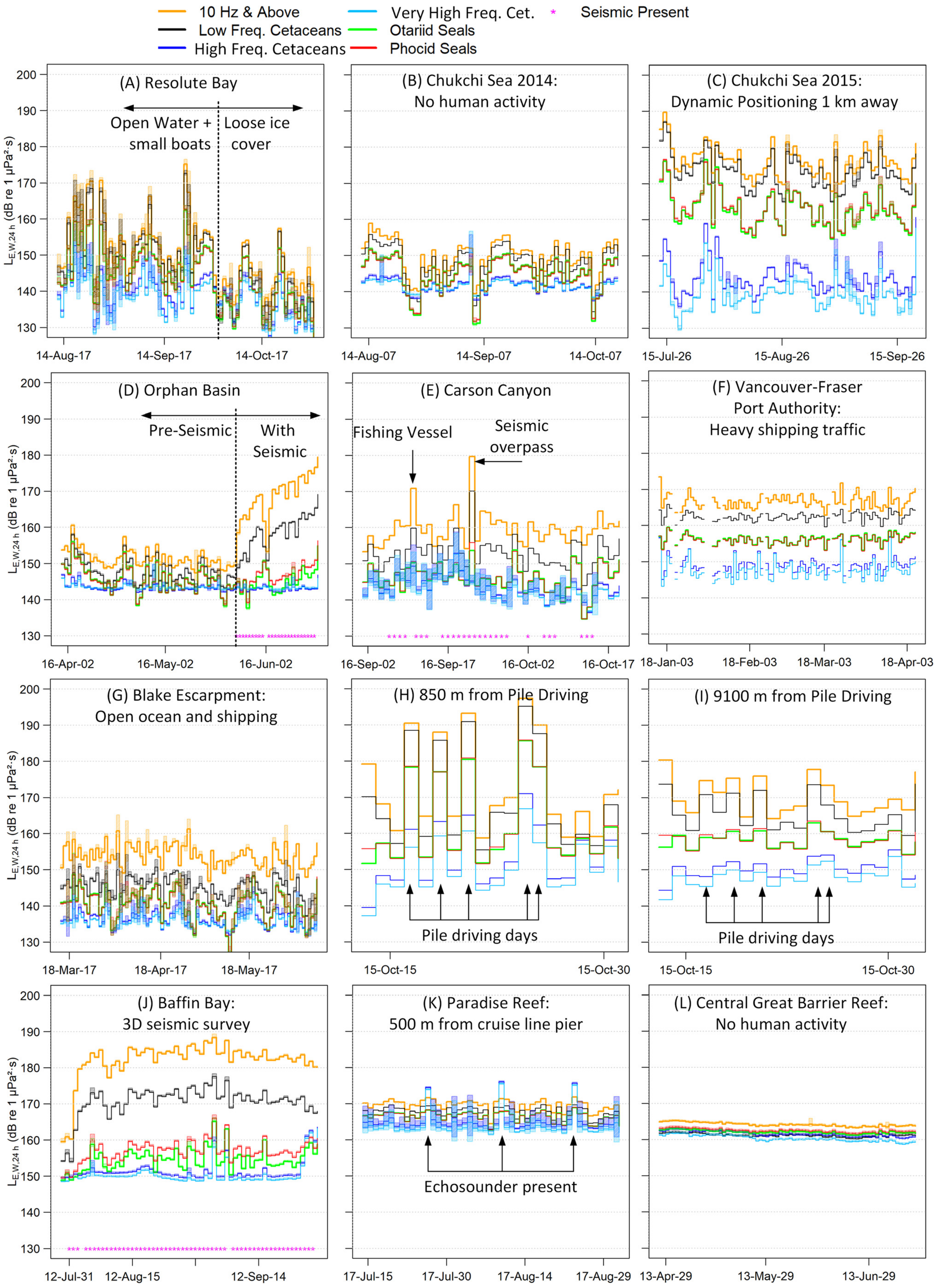


FIG. 2. (Color online) Daily weighted SEL for sites A–L (Table I, Fig. 1) with sound levels shown in Table II. For each figure the 10+ Hz SEL is shown along with the five NMFS (2018) marine mammal auditory frequency weighting functions. For the duty-cycled recordings (data sets A, B, D, E, G, K, and L), the SEL was computed as described in Sec. II C. The 95% confidence interval is shown by the shaded boxes around each days' weighted SELs.

TABLE II. Arithmetic mean daily SEL (dB re 1 μPa^2 s) and standard deviations (gray bracketed text) for data sets A–L (Figs. 1 and 2). The Resolute Bay data (A) has been divided into open-water and small boats (16 Aug–2 Oct) and with ice-cover (3 Oct–2 Nov) periods. The Orphan Basin (D) data have been divided into pre-seismic (1 Apr–24 May) and with-seismic (25 May to 30 Jun) periods.

Location ID	Location name	Data description	10 Hz and above	Low-frequency Cetacean	High-frequency Cetacean	Very high-frequency Cetacean	Phocid seals	Otariid seals
A	Resolute Bay Aug–Sept	Open ocean	162.9 (8.2)	160.5 (8.0)	143.8 (4.9)	141.9 (4.8)	152.6 (6.3)	152.5 (6.3)
A	Resolute Bay Ice Covered (Oct–Nov)	Ice + open water noise	147.2 (5.7)	145.7 (6.7)	138.3 (5.1)	137.2 (4.9)	142.0 (6.1)	142.1 (6.3)
B	Chukchi Sea, 2014	Open ocean	151.8 (4.7)	150.0 (4.9)	142.3 (2.2)	142.1 (1.8)	146.4 (4.8)	146.6 (5.0)
C	Chukchi Sea, 2015	Dynamic positioning from semi-submersible drill rig	178.7 (4.4)	176.2 (4.6)	148.1 (4.4)	143.6 (4.1)	166.5 (4.9)	166.4 (5.1)
D	Orphan Basin—pre-seismic	Open ocean	152.5 (2.6)	149.4 (3.3)	143.5 (1.1)	143.5 (0.6)	147.0 (3.4)	147.3 (3.6)
D	Orphan Basin—with seismic	Seismic survey getting closer to recorder with time	172.2 (6.0)	161.2 (5.0)	142.7 (1.0)	142.8 (0.6)	148.2 (3.3)	146.6 (3.5)
E	Carson Canyon	Open ocean, fishing, seismic airgun survey	159.7 (4.8)	152.4 (4.4)	143.0 (3.0)	142.5 (2.9)	145.9 (3.9)	145.8 (3.8)
F	Vancouver-Fraser Port Authority	Port of Vancouver's inbound shipping lane	167.2 (2.0)	162.6 (1.3)	149.9 (2.3)	148.7 (2.7)	156.3 (1.3)	156.4 (1.3)
G	Blake Escarpment	Open ocean with some shipping	154.8 (3.1)	146.2 (3.1)	137.6 (2.8)	136.5 (2.2)	141.8 (4.1)	141.9 (4.2)
H	Block Island, 850 m from piling	Impact pile driving	187.6 (13.1)	185.4 (13.6)	161.1 (8.4)	157.1 (7.6)	176.0 (11.6)	175.7 (11.7)
I	Block Island, 9100 m from piling	Impact pile driving	172.9 (4.9)	168.0 (4.8)	151.0 (2.6)	148.8 (2.8)	159.1 (2.4)	158.8 (2.7)
J	Baffin Bay	Seismic airgun survey except first two days	183.5 (5.6)	171.8 (4.4)	153.0 (2.8)	152.0 (2.8)	158.0 (3.0)	156.0 (3.2)
K	Paradise Reef	Coral Reef, 500 m from cruise terminal; frequented by small tourist dive boats	169.8 (1.5)	168.0 (1.5)	166.8 (2.8)	165.9 (2.9)	166.7 (1.3)	166.7 (1.3)
L	Central Great Barrier Reef	Coral reef without human sources	164.2 (0.5)	161.2 (0.5)	161.6 (0.7)	160.4 (0.8)	162.5 (0.5)	162.1 (0.5)

and very high-frequency auditory frequency weighted SEL. The Carson Canyon data (data set E) also show the effects of vessel range on SEL. The project's fishing vessel operated for several days within 1–4 km of the recorder, but there was no obvious signal of its presence in the daily SEL results. A different fishing vessel passed directly over the recorder on 11 Sept and generated a daily SEL comparable to the drilling program in the 2015 Chukchi data (data set C) or near the Vancouver transit lanes (data set F).

Seismic surveys (Baffin Bay, Orphan Basin after 25 May, Carson Canyon on 22 Sept; data sets J, D, E, respectively) increased the daily 10+ Hz SEL by 10–40 dB and the low-frequency cetacean auditory frequency weighted SEL increased by 0–30 dB depending on the closest daily range to the vessel. The shortest range recorded to a seismic array was 100 m which occurred in Baffin Bay on 4 Sept 2012 and generated a 10 Hz + daily SEL of 189 dB re 1 μPa^2 s. In Baffin Bay (data set J), the airgun arrays were on average 40 km from the recorder (Martin *et al.*, 2017). At Orphan Basin (data set D; after 25 May), the ranges to the recorder were unknown, but presumed to be longer than 200 km when the survey began, decreasing to ~20 km at the end of recording. In this recording, the 10+ Hz daily SEL values increased 10–30 dB from pre-seismic, and the low-frequency cetacean auditory frequency weighted SEL increased 0–20 dB. The otariid and phocid auditory frequency weighted SEL did not increase due to the seismic pulse energy until several weeks into the survey when the range to the vessel decreased and the high-frequency signal strength increased, similar to the Baffin Bay results (Fig. 2). The change in autocorrelation as a result of the seismic surveys can be seen in monthly plots (Fig. 4).

The daily SEL at Block Island (H and I) were among the highest compared here, likely due to the large amounts of activity associated with the pile driving program (Table II). The average daily SEL at the 9100 m location on days without piling were generally higher than at 850 m. At 9100 m vessels passing the recorder contributed a similar amount to the daily SEL as the pile driving (see also Fig. 6). The impact pile driving increased the daily SEL by 10–25 dB at 850 m from the pile driving compared to the levels at 9100 m. The high- and very high-frequency marine mammal auditory frequency weighted SEL did not increase during pile driving at 9100 m due to the relatively high SEL that had already accumulated from the vessels. The 9100 m location was in 42 m of water and was farther from Block Island, so we presume it received more energy from shipping than the 850 m location.

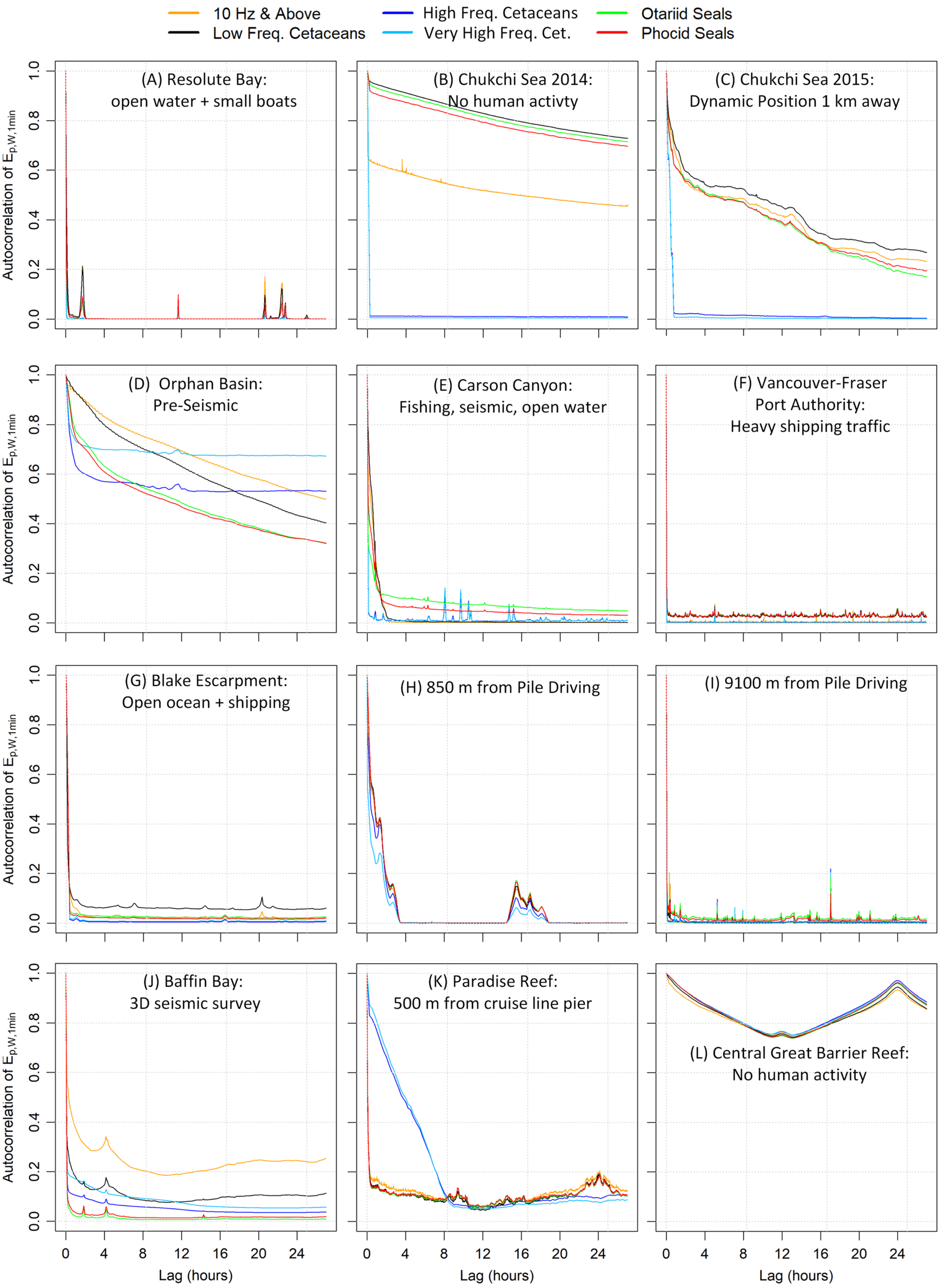


FIG. 3. (Color online) Autocorrelations of the one-minute sound exposure at sites A–L (Table I, Fig. 1). For each plot, the 10+ Hz data are shown along with the five NMFS (2018) marine mammal auditory frequency weighting functions. For data sets that were divided into subsets in Table II, only one subset is included in this figure.

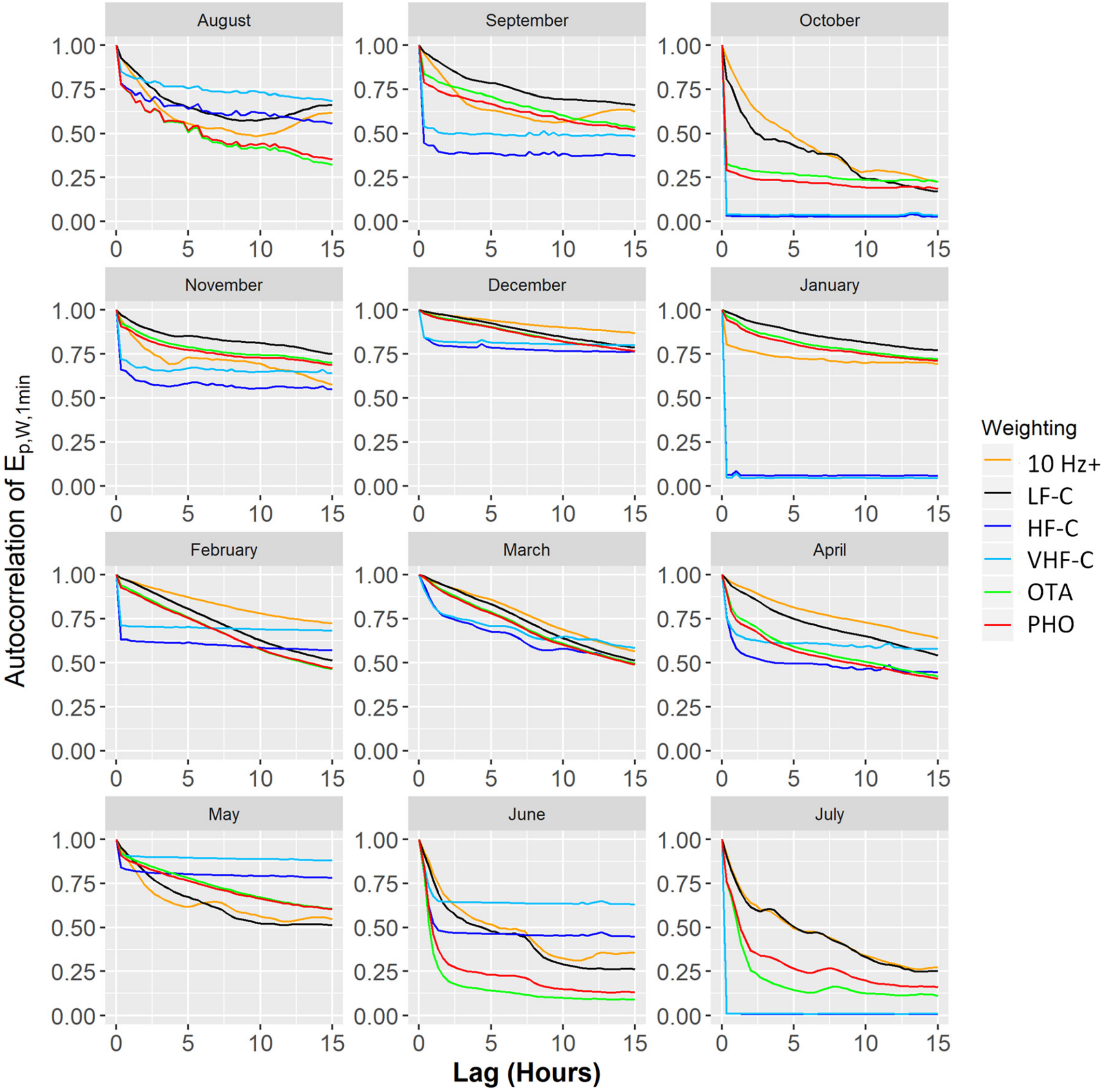


FIG. 4. (Color online) Example of the autocorrelation of each month's one-minute auditory frequency weighted SEL for the Orphan Basin data. (Top left) August 2015, (bottom right) July 2016. August, September, and part of October 2015, as well as part of May, and all of June and July, 2016, had seismic survey activity in the area. The auditory frequency weighting functions shown are 10 Hz+ (10 Hz and above); LF-C, low-frequency cetacean; HF-C, high-frequency cetaceans; VHF-C, very high-frequency cetaceans; PHO, Phocid seals; and OTA, otariid seals.

Coral reef (K and L) soundscapes are substantially different from the other environments measured (Figs. 2 and 3). The daily SEL in these locations was constant with standard deviations less than 1 dB at the Great Barrier Reef and 1–3 dB at Paradise Reef. At both sites there is a peak in the autocorrelation of the one-minute sound exposures at 24 h due to the sonorous activity of many reef animals that are synchronized with the solar cycle. Few anthropogenic sounds were present in the Great Barrier Reef recording (L), which resulted in an autocorrelation of almost 1 after 24 h. The Great Barrier Reef site is the only one where the low-frequency cetacean auditory function weighted SEL was not the highest weighted daily SEL. The Paradise Reef location was ~500 m from a cruise ship pier and frequented by many tourist dive boats. A total of 76 cruise ships visited the port during the recording period with visits typically lasting 10 h from ~08:30–18:30. This human activity elevated the daily SELs by 5–10 dB compared to the Great Barrier Reef, changed the autocorrelation structure, and resulted in the low-frequency cetacean auditory function weighted SEL being the highest weighted SEL on most days. The peak in the autocorrelation structure at 10 h is a result of the vessels entering and leaving port. The peak at 24 h is due to daily patterns in the vessel activity as well as from the soniferous animals on the reef. There is a notable peak in the high- and very high-frequency auditory frequency weighted SEL on 27 July, 10 August, and 24 August, which was caused by a 27 kHz echosounder. The very high-frequency cetacean SEL exceeded the (Southall *et al.*, 2019) permanent threshold shift regulatory limit for continuous noise on those days. The echosounder has been linked to one of the cruise ships that was the only vessel in port on the 27th of July, and her only other port visits were 10 and 24 August. None of the other 16 unique cruise ships that visited the port appeared to have left their echosounders running.

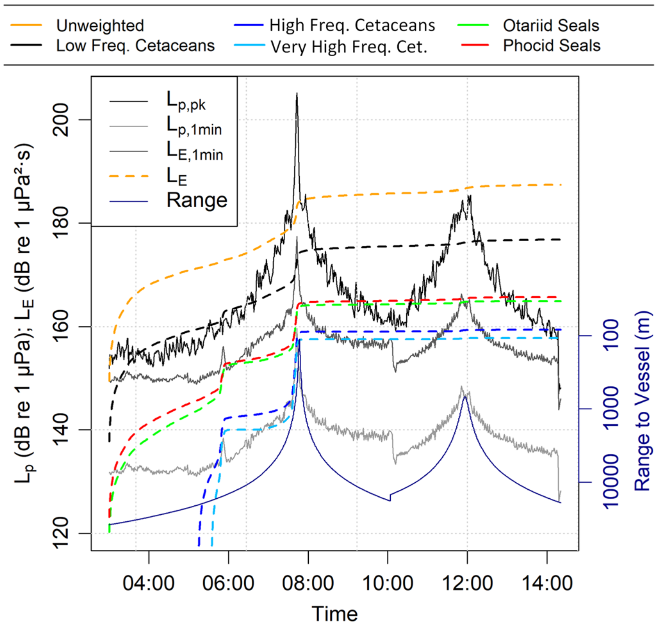


FIG. 5. (Color online) Accumulation of SEL over a 12-h period on 4 Sept 2012 during the overpass of two seismic source vessels in Baffin Bay (M). The 10+ Hz SEL increased from 184.5 dB re 1 μPa^2 s after the first vessel passed at 07:50 to 186.7 dB re 1 μPa^2 s at 12:00 when the second passed. For more on this data set, see Martin *et al.* (2017).

B. Accumulation of SEL

1. Case 1: Vessels and seismic surveys in Baffin Bay

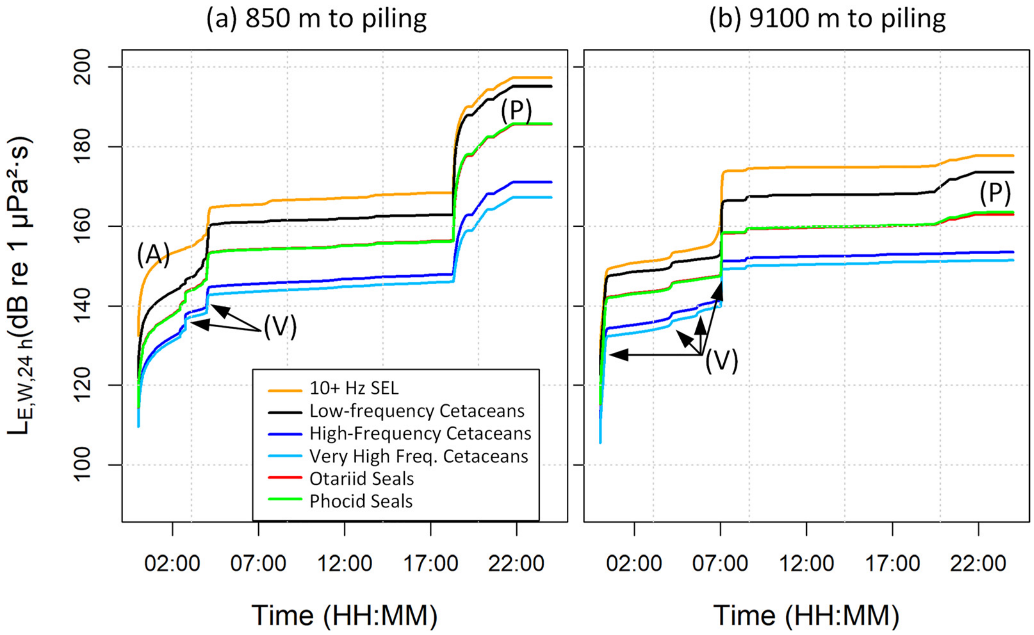
Figure 5 shows 12 h of data from the Baffin Bay data set (J) during which two seismic vessels passed by the recorder. SEL accumulated slowly while the first seismic vessel approached the recorder. At ∼05:45, the seismic support vessel passed near the recorder; its propulsion sounds were the first sounds above the recorder noise floor for the high- and very high-frequency weighted daily SEL. The total SEL increased rapidly in the last kilometer as the per-pulse SEL increased by 20 dB. The remainder of the passage of the first vessel plus the entire passage of the second vessel only increased the 10+ Hz SEL by 2 dB. The weighted SEL increased by smaller amounts. The daily SEL did not increase for the remainder of the 24-h period (not shown).

2. Case 2: Accumulation of SEL near a pile-driving construction site

The daily SEL is the sum of the ambient sound from wind and waves, human activity, and biologic sounds. The daily SEL at a receiver depends on the source level of each source and the attenuation of sounds with distance (Fig. 6). Figure 6 shows the accumulation of SEL on 25 Oct 2015 during pile driving at Block Island (H and I). At a range of 850 m from the piling [Fig. 6(a)], a vessel passed the recorder at ∼04:00, which increased SEL by 5–10 dB. Three bouts of impact piling began at 18:15. The first bout increased the daily SEL by 10–25 dB, depending on the auditory frequency weighting. Between the vessel passage and the start of piling the daily SEL increased slowly, likely due to ambient background sound. The ambient sound did not increase the daily SEL after pile driving. At 9100 m from the pile driving location [Fig. 6(b)], vessel passages at midnight and ∼07:00 were the primary source of daily SEL. The first bout of pile driving did not add enough sound energy to the daily SEL to be discernible. The second and third bouts of pile driving only made a slight increase in the low-frequency cetacean auditory frequency weighted SEL.

C. Effects of duty cycles on SEL

Duty-cycling introduces an error in the daily SEL estimate whose mean value ranges from −1.7 to +1.1 dB. The


(a) 850 m to piling

(b) 9100 m to piling

FIG. 6. (Color online) Comparison of the accumulation of SEL over a 24-h period at two ranges from pile driving on 25 Oct 2015 during construction of the Block Island Wind Farm, USA. The primary source of sound during large changes in SEL are annotated: (A), Ambient; (V), Vessel Passages; (P), impact pile driving. (a) 850 m from the piling location. (b) 9100 m from the piling location.

error in estimated SEL increases with decreasing autocorrelation; the errors are more often underestimates of SEL rather than overestimates. The relationship between autocorrelation (at the first available time lag—i.e., 2, 3, 4, etc., minutes, as described in Sec. II C) and SEL error was not linear and had a large range of error values for any one correlation value. Therefore the 95% confidence intervals were determined empirically from the measured data. To confirm that the behavior of the real data sets was predictable, the results were compared with gamma-distributed random noise. The worst case 95% confidence interval for the error is ± 6 dB, both for the real data and simulations with gamma-distributed random noise (see the supplemental material[2]). As an example of the use of the confidence interval results, the range of SEL error for each day and auditory frequency weighting function were added to Fig. 2 as shaded areas around the expected value.

IV. DISCUSSION

A. Accumulation of SEL from stationary and moving sources and implications for the distance from a source where auditory injury may occur

An important property of SEL from human sound sources is that the relative movement of the source and receiver determines how SEL accumulates, which is clearly shown in Figs. 5 and 6 as well as by the echosounder at Paradise Reef [Fig. 2(K)]. The highest 10 Hz and above daily SEL of 193 dB re μPa^2 s was recorded on 21 Oct 2015 at 850 m from the pile driving. This was 4 dB higher than the maximum seismic daily SEL, even though the seismic vessel passed only 100 m from the recorder (Baffin Bay, 4 Sept 2012). This result underscores how moving sources like seismic and vessels mitigate accumulation of SEL compared to a stationary source like pile driving. A moving biologic receptor would similarly mitigate the accumulation of SEL from stationary sources as well as mobile ones. If we assume that most sensitive biologic receptors will move, even if just over distances of several hundred meters, then the closest point of approach (CPA) to the source will dominate the received SEL (as shown in Figs. 5 and 6 and by Monte Carlo simulations; Gedamke *et al.*, 2011). The duration of CPAs is typically on the order of minutes, and thus integration over a period of an hour will accumulate all of the energy from a moving human source that a biologic receptor would encounter. As noted in Southall *et al.* (2019), further investigation of appropriate SEL integration and rest times is required.

It is known that hearing begins to recover quickly after exposure to loud sounds (Hirsh and Ward, 1952). For example, porpoise recovered from 10 dB of temporary threshold shift (TTS) within an hour (Kastelein *et al.*, 2012). It is therefore reasonable to consider resetting SEL exposure an hour after CPA for moving human sources and/or moving biologic receivers. For continuous sources of sound, such as dynamically positioned oil rigs or sea-floor production facilities, a different approach is required, which is acknowledged in NMFS (2018), although no specific advice is given. For this type of source the distance around the activity where one would expect animals to be affected, and likely excluded, is equal to the area where the average sound level is above the threshold of effective quiet (Ward *et al.*, 1976; Mooney *et al.*, 2009; Kastelein *et al.*, 2017). There is some evidence for this effect in the detections of odontocetes 2 km compared to 20 km from a mobile offshore drilling unit working in 2400 m of water off Nova Scotia (Martin *et al.*, 2019). Further work in understanding effective quiet, hearing recovery, and appropriate accumulation times is required for all marine taxa.

B. Identifying soundscapes dominated by wind and wave sounds

The data sets analyzed here demonstrate a range of effects that our use of the oceans has in changing soundscape experienced by marine life. Recordings such as Chukchi Sea 2014 (B) and Orphan Basin pre-seismic (D) provide a baseline soundscape for the open ocean that is measurably different from the other environments. Similarly, the Great Barrier Reef is a baseline coral reef environment that contrasts with the measurements at Paradise Reef.

From the results we propose the following indicators to identify soundscapes that are unaffected by anthropogenic activity or intense biologic sound production: (1) the daily 10+ Hz SEL is below 160 dB re 1 μPa^2 s, even in high winds; (2) the low-frequency cetacean auditory frequency weighted SEL is within 3 dB of the 10+ Hz SEL (i.e., at least half of the daily SEL is from frequencies above 100 Hz); and (3) the low-frequency cetacean auditory frequency weighted SEL has a correlation coefficient above 0.6 for time lags of at least 3 h when computed with one-minute SEL over periods of at least 1 month. For coral reefs, the proposed indicators are slightly different: (1) the daily 10+ Hz SEL is below 170 dB re 1 μPa^2 s, even in high winds; and (2) the autocorrelations of all auditory frequency weighted one-minute SEL are above 0.75 at 24 h lag when computed using at least 1 month of data. The duration over which the autocorrelations remain high indicates how isolated the soundscape is from variable sound sources, usually of human origin. The details of the low-frequency cetacean auditory frequency weighting are not important for these results—rather the results depend on excluding energy between 10 and 100 Hz—which is the effect of the low-frequency cetacean weighting. We replicated these results by computing SEL using only 100–20 000 Hz decidecades [Eq. (4)].

It is important to understand the properties of the long-term autocorrelation of the one-minute sound exposure as a soundscape indicator. The autocorrelation of the sound exposure is defined as the sum of the sound exposure (E_p) times the delayed version of itself, divided by the summed square,

$$R_{EE}(\tau) = \frac{\sum_{t=0}^{T-1} E_p(t)E_p(t-\tau)}{\sum_{t=0}^{T-1} E_p(t)E_p(t)}. \tag{5}$$

This operation will always have a value of one when τ is zero. When τ is not zero, the autocorrelation measures the change in sound exposure for each value of τ. This operation is susceptible to being dominated by large amplitude values that overwhelm other patterns that may be in the data. For example, the echosounder at Paradise Reef (K) was only present 1 day in 14, yet the high- and very high-frequency weighted sound exposures are above 0.1 for 10 h, the duration that the vessel was in port. This is also a property of the autocorrelation that also makes it useful as a soundscape descriptor—loud sources at random times reduce the autocorrelation and indicates human effects on the acoustic environment. At the same time, it is important to separate the data into periods that are dominated by identifiable sources before assessing the soundscapes during those periods individually. For this reason, we have divided the Orphan Basin data into pre-seismic and with seismic periods, and the Resolute Bay data into open water and ice-covered periods (Table II). In general, consider determining the autocorrelation on a month-by-month basis to look for long-term variability in a soundscape (e.g., Fig. 4).

A few notes on how autocorrelation was used in this analysis are warranted. First, autocorrelation was performed on the sound exposure, rather than the SEL [see Eqs. (2) and (3)]. The choice is essential so that the large range of exposure values can decorrelate the soundscape when sources like ships are present. When SEL is used the correlation coefficient remains near one for all data sets for lags of days. Second, the absolute values of the sound exposure are important, and therefore the data should not be demeaned before performing the autocorrelation. For many other applications of autocorrelation this is not the case. As a result of this choice, autocorrelation coefficient values below zero will not occur. Finally, the data used for autocorrelation must be evenly spaced. For example, the Chukchi Sea 2014 data (B) have 2 min of data at the high sample rate, and 13 min at the low sample rate. All this data, when sorted in time, may be autocorrelated to determine the properties of the 10 Hz and above SEL or the low-frequency cetacean auditory frequency weighted SEL. For the remaining weighted SEL only 1 min of the 2 min of high sample rate data should be used.

C. Selecting hardware and duty cycles for SEL analysis

The data sets analyzed illustrate two considerations when selecting recording equipment and determining the recording configuration: it is possible for the recording system noise floor to be higher than the TTS thresholds for very high-frequency cetaceans, and the recording configuration may not support accurate assessment of the auditory frequency weighted SEL.

The recording system noise floor and sampling rate set the minimum daily SEL that can be measured, which may be higher than the Southall *et al.* (2019) TTS thresholds from non-impulsive sound sources for very high-frequency cetaceans of 153 dB re 1 μPa^2 s and the impulsive threshold of 140 dB re 1 μPa^2 s (see Tables 6 and 7 of Southall *et al.*, 2019). For hydrophone data acquisition systems, the spectral noise floor is the sum of noise from the analog-to-digital converter, hydrophone pre-amplifier, and hydrophone ceramic. Different hydrophone noise floors had a notable effect in the data sets analyzed. The Orphan Basin data (D) were computed from data sampled at 250 000 Hz using HTI-99-HF hydrophones, which resulted in a SEL noise floor of ~142 dB re 1 μPa^2 s (Table I, Fig. 2). The Blake Escarpment data (G) were also recorded at 250 kHz, but with the lower noise GeoSpectrum M36 hydrophone, so that the minimum high-frequency marine mammal daily SEL was ~134 dB re 1 μPa^2 s—which is visible as a lower noise floor in Fig. 2. The Baffin Bay (L) and Block Island 850 m (H) configurations are typical of recordings made near high-intensity human activities such as pile driving and seismic surveys where low sensitivity hydrophones are needed to avoid saturation from the sound source. The low-sensitivity resulted in spectral density noise floor of 53 dB re 1 μPa^2/Hz, which with 64 kHz sampling rate, the noise integrated to a daily minimum SEL of 150 dB re 1 μPa^2 s.

Solutions for the noise floor limit are to reduce the bandwidth analyzed and/or only integrating for the period when the source is present. As discussed above 24 h is the currently recommended duration but should be reconsidered as more data becomes available. With respect to the recording bandwidth, the main sounds of interest for the effects of man-made noise on marine life (pile driving, seismic arrays, vessels, and naval sonar) are all dominated by frequencies below 10 kHz, with some energy reaching 30 kHz and higher at short ranges (Simard *et al.*, 2016; Martin *et al.*, 2017; MacGillivray, 2018). Based on these frequencies and our understanding of the hearing bands of marine mammals, as well as most fishes and invertebrates, recording programs concerned with quantifying SEL should analyze data sampled at ~64 000 Hz. This sampling rate results in a usable frequency band of ~30 kHz, which captures the energy of most sound sources of interest, reaches the 0-dB attenuation range of the very high-frequency cetacean auditory frequency weighting function, and the bandwidth is narrow enough that most recorders and hydrophones will not be self-noise limited. With respect to the daily SEL from human sources, a higher sample rate is only required to study the effects of sources such as echosounders and multibeam sonars. Recording programs whose objectives include detections of odontocete clicks also need to sample faster than 64 000 Hz.

The recording duty cycle is a system configuration parameter that affects the confidence interval of the daily SEL estimates. As the duty cycle decreases the autocorrelation coefficient decreases and the daily SEL error increases—i.e., higher errors at 1 min in 20 min than 1 min in 2 min (see supplemental material[2]). When the duty cycle is less than 1 min in 30 min the decimated autocorrelation does not track the true autocorrelation reliably and SEL should not be computed from such data. When selecting a duty cycle, we recommend recording more often rather for longer periods if daily SEL is a desired output of the project. For example, recording for 1 min every 6 min is much more useful than recording for ten consecutive minutes per hour. This result is also true when determining the presence of mysticete whales using duty-cycled data (Thomisch *et al.*, 2015). The minimum recording duration we recommend is 1 min, however, 30 s would likely provide good data as well. We have also found that when cycling between multiple sample rates, selecting a total duty cycle that is an even number of minutes is preferred as it allows more options when downsampling before autocorrelation (e.g., the Chukchi Sea 2014 data discussed in Sec. IV B). When recording data to measure SEL for regulatory compliance, continuous recording is strongly recommended.

D. Using the daily SEL in soundscape management—Cumulative effects assessment

A goal of many environmental assessments is to understand how a proposed project will add to existing human activity and affect the animals in the area. When estimating the effects of underwater sound from multiple human activities, Ellison *et al.* (2016) provide a method based on summing the SEL from each activity for simulated animals moving through the project area. This operation is difficult for locations with many existing sound sources whose movements and source factors are uncertain. Instead, long-term baseline measurements may be used to determine the existing daily SEL, to which SEL from the proposed activity may be added. It is also possible to use the difference between the daily SEL and accepted sound tolerance levels (e.g., the Southall *et al.*, 2019, TTS thresholds) is such an indicator of how much additional sound may be added to the environment without risk of inducing TTS. This comparison has limitations since it accumulates sound that is likely below the threshold for effective quiet and could, for some recorder configurations, include system noise. It is also limited since it does not account for healing of the hearing system between intermittent exposures and the temporal effects of sound patterns are not accounted for in this approach (or the equal-energy hypothesis in general; Hamernik *et al.*, 2003). Regardless, it is still a useful "first-look" at the capacity of animals in the environment to be exposed to additional sound without hearing injury or impairment. Locations where the sound levels are elevated by continuous sources (e.g., Chukchi Sea 2015, Vancouver-Fraser Port Authority) require special consideration if new impulsive sound sources may be added to the environment. At these locations the continuous sound levels are high enough that low- and high-frequency cetaceans are already past TTS for impulsive sounds before an impulsive source starts. Studies have shown that animals and humans become more susceptible to impulsive sounds when high levels of continuous sound are already present (Henderson and Hamernik, 1986; Ahroon *et al.*, 1993; Kastelein *et al.*, 2015). Examples of these situations include pile driving in a busy harbor, vertical seismic profiling to image newly drilled oil and gas wells, or the narrow beam of an echosounder below a passing ship.

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[1] https://bit.ly/2zx03ye (Last viewed 17 June 2019).

[2] See supplementary material at https://doi.org/10.1121/1.5113578 for material that includes: why SEL is a measure of the received energy, how to compute SEL across multiple events, further information on auditory weighting functions, hydrophone and recorder self-noise data, gamma random noise distributions that are similar to typical of ocean noise distributions, statistical measures (mean, variance, skewness, kurtosis, gamma fit, and autocorrelation durations) for each data set, and confidence intervals for duty-cycled daily SELs.

[3] 2017 marine traffic density provided by www.marinetraffic.com (Last viewed 26 April 2019).

Ahroon, W. A., Hamernik, R. P., and Davis, R. I. (**1993**). "Complex noise exposures: An energy analysis," J. Acoust. Soc. Am. **93**, 997–1006.

Ainslie, M. A., Miksis-Olds, J. L., Martin, S. B., Heaney, K., de Jong, C. A. F., von Benda-Beckmann, A. M., and Lyons, A. P. (**2018**). "ADEON underwater soundscape and modeling metadata standard (version 1.0) [computer program]," Technical Report by JASCO Applied Sciences for ADEON Prime Contract No. M16PC00003.

Akay, A. (**1978**). "A review of impact noise," J. Acoust. Soc. Am. **64**, 977–987.

Blix, A. S., Walløe, L., and Messelt, E. B. (**2013**). "On how whales avoid decompression sickness and why they sometimes strand," J. Explorat. Bio. **216**, 3385–3387.

Casper, B. M., Halvorsen, M. B., Carlson, T. J., and Popper, A. N. (**2017**). "Onset of barotrauma injuries related to number of pile driving strike exposures in hybrid striped bass," J. Acoust. Soc. Am. **141**, 4380–4387.

Chapman, R. N., and Price, A. (**2011**). "Low frequency deep ocean ambient noise trend in the Northeast Pacific Ocean," J. Acoust. Soc. Am. **129**, EL161–EL165.

Costa, D. P., Schwarz, L., Robinson, P., Schick, R. S., Morris, P. A., Condit, R., Crocker, D. E., and Kilpatrick, A. M. (**2016**). "A bioenergetics approach to understanding the population consequences of disturbance: Elephant seals as a model system," in *The Effects of Noise on Aquatic Life II*, edited by A. N. Popper and A. D. Hawkins (Springer, New York), pp. 161–169.

Cox, T. M., Ragen, T. J., Read, A. J., Vos, E., Baird, R. W., Balcomb, K., Barlow, J., Caldwell, J., Cranford, T., and Crum, L. (**2006**). "Understanding the impacts of anthropogenic sound on beaked whales," J. Cetacean Res. Manage. **7**, 177–187.

Danielson, R., Henderson, D., Gratton, M. A., Bianchi, L., and Salvi, R. (**1991**). "The importance of 'temporal pattern' in traumatic impulse noise exposures," J. Acoust. Soc. Am. **90**, 209–218.

Dooling, R. J., Leek, M. R., and Popper, A. N. (**2015**). "Effects of noise on fishes: What we can learn from humans and birds," Integr. Zool. **10**, 29–37.

Eldredge, D. H., and Covell, W. P. (**1958**). "A laboratory method for the study of acoustic trauma," Laryngoscope **68**, 465–477.

Ellison, W. T., Racca, R. G., Clark, C. W., Streever, B., Frankel, A. S., Fleishman, E., Angliss, R., Berger, J., Ketten, D. R., Guerra, M., Leu, M., McKenna, M., Sformo, T., Southall, B. L., Suydam, R., and Thomas, L. (**2016**). "Modeling the aggregated exposure and responses of bowhead whales *Balaena mysticetus* to multiple sources of anthropogenic underwater sound," Endanger. Spec. Res. **30**, 95–108.

Ellison, W. T., Southall, B. L., Clark, C. W., and Frankel, A. S. (**2012**). "A new context-based approach to assess marine mammal behavioral responses to anthropogenic sounds," Conserv. Biol. **26**, 21–28.

Erbe, C. (**2013**). "International regulation of underwater noise," Acoust. Aust. **41**, 12–19.

Finneran, J. J. (**2015**). "Noise-induced hearing loss in marine mammals: A review of temporary threshold shift studies from 1996 to 2015," J. Acoust. Soc. Am. **138**, 1702–1726.

Gedamke, J., Gales, N., and Frydman, S. (**2011**). "Assessing risk of baleen whale hearing loss from seismic surveys: The effect of uncertainty and individual variation," J. Acoust. Soc. Am. **129**, 496–506.

Halvorsen, M. B., Casper, B. M., Matthews, F., Carlson, T. J., and Popper, A. N. (**2012a**). "Effects of exposure to pile-driving sounds on the lake sturgeon, Nile tilapia and hogchoker," Proc. R. Soc. B **279**, 4705–4714.

Halvorsen, M. B., Casper, B. M., Woodley, C. M., Carlson, T. J., and Popper, A. N. (**2012b**). "Threshold for onset of injury in Chinook salmon from exposure to impulsive pile driving sounds," PLoS One **7**, e38968.

Hamernik, R. P., Qiu, W., and Davis, B. (**2003**). "The effects of the amplitude distribution of equal energy exposures on noise-induced hearing loss: The kurtosis metric," J. Acoust. Soc. Am. **114**, 386–395.

Hawkins, A. D., and Popper, A. N. (**2017**). "A sound approach to assessing the impact of underwater noise on marine fishes and invertebrates," ICES J. Mar. Sci. **74**, 635–651.

Hawkins, R. S., Miksis-Olds, J. L., and Smith, C. M. (**2014**). "Variation in low-frequency estimates of sound levels based on different units of analysis," J. Acoust. Soc. Am. **135**, 705–711.

Henderson, D., and Hamernik, R. P. (**1986**). "Impulse noise: Critical review," J. Acoust. Soc. Am. **80**, 569–584.

Hirsh, I. J., and Ward, W. D. (**1952**). "Recovery of the auditory threshold after strong acoustic stimulation," J. Acoust. Soc. Am. **24**, 131–141.

Houser, D. S., Yost, W., Burkard, R., Finneran, J. J., Reichmuth, C., and Mulsow, J. (**2017**). "A review of the history, development and application of auditory weighting functions in humans and marine mammals," J. Acoust. Soc. Am. **141**, 1371–1413.

ISO (**2017**). 18405.2. *Underwater Acoustics—Terminology* (International Organization for Standardization, Geneva, Switzerland).

Jepson, P. D., Arbelo, M., Deaville, R., Patterson, I. A. P., Castro, P., Baker, J. R., Degollada, E., Ross, H. M., Herraez, P., Pocknell, A. M., Rodriguez, F., Howie, F. E., Espinosa, A., Reid, R. J., Jaber, J. R., Martin, V., Cunningham, A. A., and Fernandez, A. (**2003**). "Gas-bubble lesions in stranded cetaceans," Nature **425**, 575–576.

Kastelein, R. A., Gransier, R., Hoek, L., and Olthuis, J. (**2012**). "Temporary threshold shifts and recovery in a harbor porpoise (*Phocoena phocoena*) after octave-band noise at 4 kHz," J. Acoust. Soc. Am. **132**, 3525–3537.

Kastelein, R. A., Gransier, R., Schop, J., and Hoek, L. (**2015**). "Effects of exposure to intermittent and continuous 6–7 kHz sonar sweeps on harbor porpoise (*Phocoena phocoena*) hearing," J. Acoust. Soc. Am. **137**, 1623–1633.

Kastelein, R. A., Helder-Hoek, L., Van de Voorde, S., von Benda-Beckmann, A. M., Lam, F.-P. A., Jansen, E., de Jong, C. A. F., and Ainslie, M. A. (**2017**). "Temporary hearing threshold shift in a harbor porpoise (*Phocoena phocoena*) after exposure to multiple airgun sounds," J. Acoust. Soc. Am. **142**, 2430–2442.

King, S., Schick, R. S., Donovan, C., Booth, C. G., Burgman, M., Thomas, L., and Harwood, J. (**2015**). "An interim framework for assessing the population consequences of disturbance," Methods Ecol. Evol. **6**, 1150–1158.

MacGillivray, A. O. (**2018**). "Underwater noise from pile driving of conductor casing at a deep-water oil platform," J. Acoust. Soc. Am. **143**, 450–459.

Madsen, P. T. (**2005**). "Marine mammals and noise: Problems with root mean square sound pressure levels for transients," J. Acoust. Soc. Am. **117**, 3952–3957.

Martin, S. B., Kowarski, K. A., Maxner, E. E., and Wilson, C. C. (**2019**). "Acoustic monitoring during Scotian Basin Exploration Project: Summer 2018," Technical Report by JASCO Applied Sciences for BP Canada Energy Group ULC.

Martin, S. B., Matthews, M.-N. R., MacDonnell, J. T., and Bröker, K. (**2017**). "Characteristics of seismic survey pulses and the ambient soundscape in Baffin Bay and Melville Bay, West Greenland," J. Acoust. Soc. Am. **142**, 3331–3346.

McDonald, M. A., Hildebrand, J. A., and Wiggins, S. M. (**2006**). "Increases in deep ocean ambient noise in the Northeast Pacific west of San Nicolas Island, California," J. Acoust. Soc. Am. **120**, 711–718.

Mooney, T. A., Nachtigall, P. E., Breese, M., Vlachos, S., and Au, W. W. L. (**2009**). "Predicting temporary threshold shifts in a bottlenose dolphin (*Tursiops truncatus*): The effects of noise level and duration," J. Acoust. Soc. Am. **125**, 1816–1826.

National Marine Fisheries Service (NMFS) (**2016**). "Technical guidance for assessing the effects of anthropogenic sound on marine mammal hearing: Underwater acoustic thresholds for onset of permanent and temporary threshold shifts," NOAA Technical Memorandum NMFS-OPR-55 (U.S. Department of Commerce, National Oceanic and Atmospheric Administration), p. 178.

National Marine Fisheries Service (NMFS) (**2018**). "2018 revision to: Technical guidance for assessing the effects of anthropogenic sound on marine mammal hearing (version 2.0): Underwater thresholds for onset of permanent and temporary threshold shifts," NOAA Technical Memorandum NMFS-OPR-59 (U.S. Department of Commerce, National Oceanic and Atmospheric Administration), p. 167.

National Marine Fisheries Service (US) (NMFS) and National Oceanic and Atmospheric Administration (NOAA) (**1995**). "Small takes of marine mammals incidental to specified activities; offshore seismic activities in southern California: Notice of issuance of an incidental harassment authorization," Fed. Regist. **60**, 53753–53760.

National Oceanic and Atmospheric Administration (U.S.) (NOAA) (**1998**). "Incidental taking of marine mammals; Acoustic harassment," Fed. Regist. **63**, 40103.

National Research Council (NRC) (**2005**). *Marine Mammal Populations and Ocean Noise: Determining When Ocean Noise Causes Biologically Significant Effects* (National Academy Press, Washington, DC).

Popper, A. N., and Hawkins, A. D. (**2018**). "The importance of particle motion to fishes and invertebrates," J. Acoust. Soc. Am. **143**, 470–488.

Popper, A. N., Hawkins, A. D., Fay, R. R., Mann, D. A., Bartol, S., Carlson, T. J., Coombs, S., Ellison, W. T., Gentry, R. L., Halvorsen, M. B., Løkkeborg, S., Rogers, P. H., Southall, B. L., Zeddies, D. G., and Tavolga, W. N. (**2014**). "Sound exposure guidelines for fishes and sea turtles," Technical report, prepared by ANSI-Accredited Standards Committee S3/SC1 and registered with ANSI (ASA Press and Springer).

Qiu, W., Hamernik, R. P., and Davis, R. I. (**2013**). "The value of a kurtosis metric in estimating the hazard to hearing of complex industrial noise exposures," J. Acoust. Soc. Am. **133**, 2856–2866.

Rolland, R. M., Parks, S. E., Hunt, K. E., Castellote, M., Corkeron, P. J., Nowacek, D. P., Wasser, S. K., and Kraus, S. D. (**2012**). "Evidence that ship noise increases stress in right whales," Proc. R. Soc. B **279**, 20112429.

Shannon, G., McKenna, M. F., Angeloni, L. M., Crooks, K. R., Fristrup, K. M., Brown, E., Warner, K. A., Nelson, M. D., White, C., Briggs, J., McFarland, S., and Wittemyer, G. (**2016**). "A synthesis of two decades of research documenting the effects of noise on wildlife," Biol. Rev. **91**, 982–1005.

Simard, Y., Roy, N., Gervaise, C., and Giard, S. (**2016**). "Analysis and modeling of 255 source levels of merchant ships from an acoustic observatory along St. Lawrence Seaway," J. Acoust. Soc. Am. **140**, 2002–2018.

Southall, B. L., Bowles, A. E., Ellison, W. T., Finneran, J. J., Gentry, R. L., Greene, C. R., Jr., Kastak, D., Ketten, D. R., Miller, J. H., Nachtigall, P. E., Richardson, W. J., Thomas, J. A., and Tyack, P. L. (**2007**). "Marine mammal noise exposure criteria: Initial scientific recommendations," Aquat. Mamm. **33**, 411–521.

Southall, B. L., Finneran, J. J., Reichmuth, C., Nachtigall, P. E., Ketten, D. R., Bowles, A. E., Ellison, W. T., Nowacek, D. P., and Tyack, P. L. (**2019**). "Marine mammal noise exposure criteria: Updated scientific recommendations for residual hearing effects," Aquat. Mamm. **45**, 125–232.

Thomisch, K., Boebel, O., Zitterbart, D. P., Samaran, F., Van Parijs, S., and Van Opzeeland, I. (**2015**). "Effects of subsampling of passive acoustic recordings on acoustic metrics," J. Acoust. Soc. Am. **138**, 267–278.

Tougaard, J., and Beedholm, K. (**2019**). "Practical implementation of auditory time and frequency weighting in marine bioacoustics," Appl. Acoust. **145**, 137–143.

Ward, W. D. (**1962**). "Effect of temporal spacing on temporary threshold shift from impulses," J. Acoust. Soc. Am. **34**, 1230–1232.

Ward, W. D., Cushing, E. M., and Burns, E. M. (**1976**). "Effective quiet and moderate TTS: Implications for noise exposure standards," J. Acoust. Soc. Am. **59**, 160–165.

Wenz, G. M. (**1962**). "Acoustic ambient noise in the ocean: Spectra and sources," J. Acoust. Soc. Am. **34**, 1936–1956.

APPENDIX 2 – Effects of 2D Seismic on the Snow Crab Fisheries

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Effects of 2D seismic on the snow crab fishery

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ABSTRACT

Sound is used by a variety of marine taxa for feeding, reproduction, navigation and predator avoidance and therefore alterations to the soundscape from industrial noise have the potential to negatively affect an animal's fitness. Furthermore, responses to industrial noise would also have the potential to negatively influence commercial fishing interests. Unfortunately marine invertebrates are generally underrepresented in the seismic effects literature. Snow crab harvesters in Atlantic Canada contend that seismic noise from widespread hydrocarbon exploration has strong negative effects on catch rates. We repeated a Before-After-Control-Impact study over two years to assess the effects of industry scale seismic exposure on catch rates of snow crab along the continental slope of the Grand Banks of Newfoundland. Our results did not support the contention that seismic activity negatively affects catch rates in shorter term (i.e. within days) or longer time frames (weeks). However, significant differences in catches were observed across study areas and years. While the inherent variability of the CPUE data limited the statistical power of this study, our results do suggest that if seismic effects on snow crab harvests do exist, they are smaller than changes related to natural spatial and temporal variation.

1. Introduction

Sound is a key environmental feature that is used by a wide variety of marine taxa in many life activities such as navigation, foraging, predator avoidance and communication (Carroll et al., 2016; Edmonds et al., 2016). Noise from marine industries (e.g. seismic exploration, ship activities etc.) alters the soundscape (acoustics scene), and the associated effects on organisms and their responses can influence their physiology and fitness. Moreover, anthropogenic noise may have broader consequences, including the potential to influence important ecological processes (e.g. Solan et al., 2016) and commercial fishing interests (Skalski et al., 1992; Løkkeborg and Soldal, 1993; Engås et al., 1996; Slotte et al., 2004).

Marine environments have experienced increases in exposure to industrial noise in recent decades (Slabbekoorn, 2016). Noise has considerable potential to negatively affect marine organisms both physically and behaviourally and the range of potential effects include death, physical and physiological effects, masking of natural sound, and behavioural responses (Hirst and Roadhouse, 2000; Mooney et al., 2010; Edmonds et al., 2016; Hawkins and Popper 2017; McCauley et al., 2017). Measuring and demonstrating disruptions caused as a result of noise, however, has been challenging (Edmonds et al., 2016).

While the science documenting the implications of anthropogenic noise on marine wildlife is expanding, it remains heavily biased to marine mammals and fishes, whereas other ecologically and commercially important taxa like invertebrates are under-represented (Hawkins et al., 2015; Williams et al., 2015; Carroll et al., 2016). Furthermore, the logistical challenges of conducting marine field studies mean that much of what is known is based on lab studies where realism is difficult to achieve (Popper and Hastings 2009; Hawkins and Popper 2017; Slabbekoorn 2016). Field studies typically lack adequate control sites and/or pre-impact conditions and typically fail to quantify the degree of exposure experienced by the study animals (Edmunds et al., 2016). These complexities and related scientific shortcomings make it difficult to resolve/mitigate resource management conflicts.

Such a situation occurs along the shelf and slope marine habitats of Atlantic Canada where active seismic exploration overlaps extensively with an important snow crab fishery. Since the collapse of the groundfish fishery in Atlantic Canada, snow crab has been the highest valued fishery in Newfoundland and Labrador, with a landed value worth in excess of 273 million dollars (CAD) in 2016 (DFA, 2017). Many snow crab harvesters are concerned about seismic exploration and contend that seismic noise has strong negative effects on catch rates (FFAW personal communication; Christian et al., 2003; Mullowney

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et al., 2014); an issue that is likely to become more acute given that the species is currently experiencing unfavourable environmental conditions in many harvesting areas (DFO, 2016).

Two previous studies (Christian et al., 2003; Christian et al., 2004) attempted to assess the effects of seismic activity on snow crab behaviour, physiology, mortality and catchability and found no effects except for delayed development of embryos. Interpretation of these studies (Christian et al., 2003; Courtenay et al., 2009) note however, that they were challenged by equipment failures (Christian et al., 2003), study design limitations, confounding factors (e.g. delays in embryo development may have been caused by differences in water temperature at the study sites rather than seismic) and questions about the relevance of laboratory studies and field manipulations (Courtenay et al., 2009). Consequently, and not surprisingly, the resource conflict remains unresolved. Recent subject reviews of seismic impacts (Courtenay et al., 2009; Carroll et al., 2016; Hawkins and Popper 2017) have suggested potential ways in which study design, metrics, and topics of interest could improve the confidence in conclusions related to the effects of seismic exploration on marine animals. This study attempts to incorporate these recommendations and improve upon snow crab − seismic investigations by 1) using an enhanced study design with a multi-year BACI approach; 2) improving study realism by recreating seismic/fishery interactions using authentic platforms and methods from the respective industries; and 3) measuring exposures of snow crab to seismic-induced pressure and particle motion using recommended exposure metrics.

2. Methods

To ensure study realism, both industry-based snow crab harvesting and seismic surveying industries were consulted during the study design phase to identify an appropriate study area and methodology that aligned with industry standards. The study sites selected during these consultations were Lilly (control site) and Carson (treatment site) canyons − located on the eastern slope of the Grand Banks (Fig. 1). The sites were selected as they serve as important harvesting areas for snow crab and were within areas that were being actively surveyed by commercial seismic vessels. They were also both characterized by bathymetric relief, enabling an evaluation of potential flight responses to deeper water; a snow crab reaction that harvesters believed to occur following exposure to seismic noise.

The selected study sites were separated by a sufficient distance (70 km) such that Lilly Canyon would be unaffected by seismic air-gun exposures at Carson Canyon. Cumulative noise levels at the control site were similar to or less than the noise level generated by fishing vessels. In each year, all seismic operations were prohibited by the Canada-Newfoundland Offshore Petroleum Board within a 70 km radius of each of our sites for a 1 month period before our controlled seismic exposure and for an additional month at the control site only. This period of quiet-time is based on general observations from the fishing industry which indicate that catch rates are affected for days to weeks but not months. These restrictions were implemented consistently across each study site and used to mark beginning and end points for data analysis.

2.1. Snow crab collections

Catch surveys were conducted by industry harvesters across three trips in each of 2015 (Trip 1: Aug 26–28; Trip 2: Sep 13–16; and Trip 3: Oct 9–12; Fig. 1) and 2016 (Trip 1: Sep 2–5; Trip 2: Sep 18–25; and Trip 3: Oct 17–21) using standard industry survey methods, the Fish Food and Allied Workers (FFAW's) Post-Season Snow Crab Pot Survey (Stansbury et al., 2013). Only one vessel was used for all harvesting activities in each of the years. In 2015, seismic exposure occurred between Trip 2 and Trip 3, whereas in 2016, a scheduled seismic exposure occurred during Trip 2 on September 22. The planned exposure in 2016 enabled an equal distribution of trap sets in "Before" and "After" exposure categories ($n_{\text{Carson}} = 20$, $n_{\text{Lilly}} = 10$; Fig. 2) for each sampling area within the trip (Fig. 1). Sampling intensity in 2016 was guided by power analyses that followed collections of 2015 data (see methods below). Sampling areas were restricted to the area bound by the control and test areas (Fig. 2). Within those areas, trap placement was not random but reflected actual commercial fishing practices. Each sampling location was typically sampled using a string of 10 baited commercial crab traps (5.5 inch mesh) spaced at 25 fathom intervals. Coordinates and depth of water were collected for each deployment and strings were soaked for a minimum of 12 h. All snow crab were counted and crab from the third pot in each string was measured by trained sampling personnel from the Observer Program of the FFAW. Only male crabs were caught during commercial fishing activities.

2.2. Seismic exposure

Each year seismic noise was introduced to the Carson Canyon area from the Atlantic Explorer; an industry seismic survey vessel that is typical of those that operate off Atlantic Canada. The exposure lasted for five days in 2015 (September 25 through the 29th; Fig. 3) when an industrial seismic exploration survey was conducted in and near the study area. The closest approach of the vessel to the sound recorders at the treatment site in 2015 was 1465 m. During 2015, more seismic exploration on the Grand Banks was conducted during our study period outside our 70 km radius buffer zones than in 2016. Seismic exposure on September 22nd 2016 at the Carson Canyon experimental site occurred for a duration of 2 h, and the vessel passed within 100 m from the acoustic recorder. Exposure was also conducted while the fishing vessel was on-site, which enabled experimental fishing immediately before and after exposure. In both years, the seismic source was an airgun array with a total volume of 4880 cubic inches, with shots at 10 s intervals, operated at 2000 psi and deployed at 9 m of depth. The seismic source was modeled using the Airgun Array Sound Model (A-ASM, JASCO Applied Sciences, MacGillivray 2006). The horizontal zero-to-peak sound pressure level was 251 dB re 1 μPa @ 1 m and the source sound exposure level was 229 dB re 1 $\mu\text{Pa}^2\cdot\text{s}$ @ 1m. The full recorded sound spectrum for 2016, including natural sources such wind, waves and marine mammals is provided in Fig. 4.

2.3. Acoustic measurements

Acoustic recordings were taken at the treatment and control sites from early September until mid-October in both years to 1) ensure that ambient conditions were quiet relative to seismic surveys and 2) confirm that seismic exploration activity at the treatment site was not greater than fishing vessel noise at the control site. The daily sound exposure level was used to compare the sites because it is believed to best capture the effects of long-term sound exposure on marine life (e.g. Popper et al., 2014, [NMFS] National Marine Fisheries Service 2016). The reported sound exposure level is the arithmetic sum of the sound pressure level in the frequency band of 10–7000 Hz over each 24-h period.

Data were collected using an AMAR acoustic recorder (JASCO Applied Sciences), sampling at 16 kHz. The recorders were located on the seabed (105–115 m deep) on frames that held the hydrophones ~0.6 m above the seafloor. In 2015 a Geospectrum M36-V35-100 hydrophone with sensitivity of −165 dB re 1 V/μPa was used; in 2016 an M36-V0-100 hydrophone with sensitivity of −200 dB re 1 V/μPa was used. In 2016 particle motion was also measured. A Geospectrum M20-101 particle acceleration sensor was suspended 0.5 m above the seabed and a PCB-356B18 micro-electrical-mechanical-system (MEMS) accelerometer was coupled to the seabed mooring plate. The close pass of the seismic vessel in 2015 was not planned before the hydrophones were deployed, and the high levels of received sound caused the hydrophone to reach its maximum signal output when the seismic vessel was 8 km from the recorder while operating over the shallow Grand Banks and

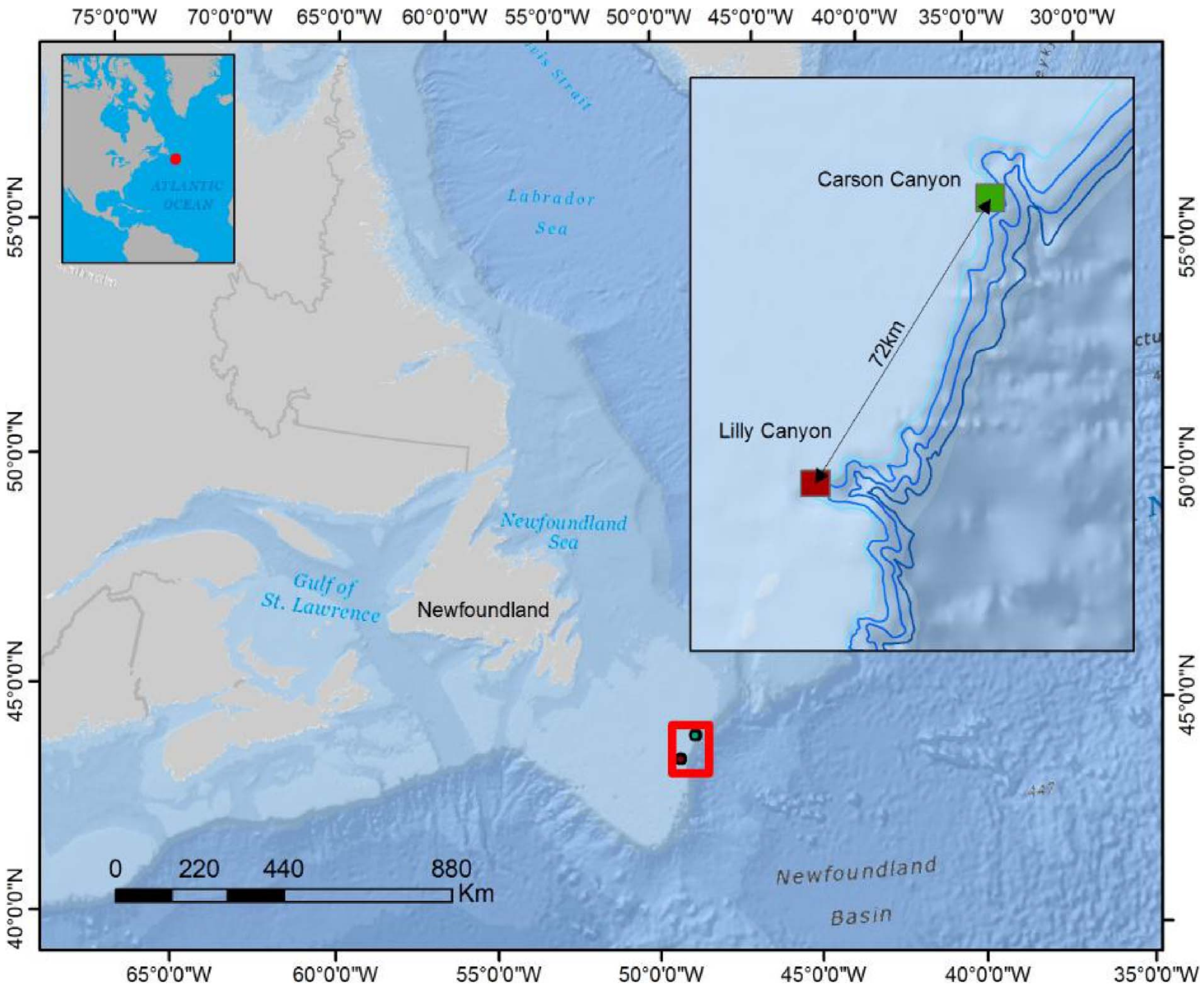

Fig. 1. Geographic location of the control (Lilly Canyon, lower red square) and test (Carson Canyon, upper green square) sites on the slope of the Grand Banks. The configuration of the acoustic receivers is shown in the inset.

16 km while operating over deep water east of the treatment site. Therefore, the reported daily sound exposure levels at the treatment area are less than the actual exposures during the five days of seismic survey. In 2016 only of 16 of the closest seismic pulses (+/− 200 m) caused the M36-V0-100 hydrophone to exceed its maximum levels, which likely reduced the recorded sound exposure level by 3–6 dB.

For comparison, the sound exposure levels measured at the control and treatment sites are compared to the median of the daily sound exposure level measured over the period of 24 August − 13 October 2015 at 45.70N, 51.23W as part of a separate research project. This location, also on the Grand Banks, was not exposed to significant seismic survey or vessel activity during the measurement period.

2.4. Statistical approach

Several previous seismic effect studies have been criticized for lacking adequate controls (Payne et al., 2008; Courtenay et al., 2009; Hawkins and Popper 2017). Consequently, this study employed a multi-year Before-After-Control-Impact (BACI) approach, which safeguarded against Type I errors caused by naturally occurring spatial and temporal variation

The catch data being examined in this document have inherent characteristics that need to be accounted for during statistical analysis. First, count data are bounded by zero and generally do not satisfy the statistical assumptions used in conventional approaches (e.g. ANOVA). Techniques such as generalized linear models can better approximate the underlying data distributions (e.g. Poisson or negative binomial distributions) and should be used in such circumstances (Zuur et al., 2009). Second, the field methods require that the traps are deployed in strings of 10. Since each trap within a string shares a localized sampling area, traps on the same string are not likely to be independent in a statistical sense and this violates the assumptions of most conventional models. Mixed effects models account for such dependence in the model and are recommended over averaging catches within a string (Zuur et al., 2009). Based on these characteristics, a negative binomial generalized linear mixed effects model and associated likelihood ratio tests were selected to analyze the data.

The generalized linear mixed effects model (lme4 extension of R; R Core Team, 2015) used total counts of snow crab within a trap as the response variable, temporal (Before/After Exposure) and spatial (Reference/Treatment) categorical variables as the fixed effects and the string's identity as a random effect. For a BACI study design, the statistical interaction between spatial and temporal fixed effects is also included since to detect a treatment effect we look to see if catch rates responded similarly over time across the two areas.

Graphical examination of the data also indicated that depth may influence catch rates of snow crab. Therefore, to reduce unexplained variance and improve statistical power, depth was also included as a continuous explanatory variable in the statistical model.

In 2016, the planned seismic exposure enabled the evaluation of more immediate effects on catch rates within Trip 2 of 2016. The model structure for that short-term evaluation was as follows:

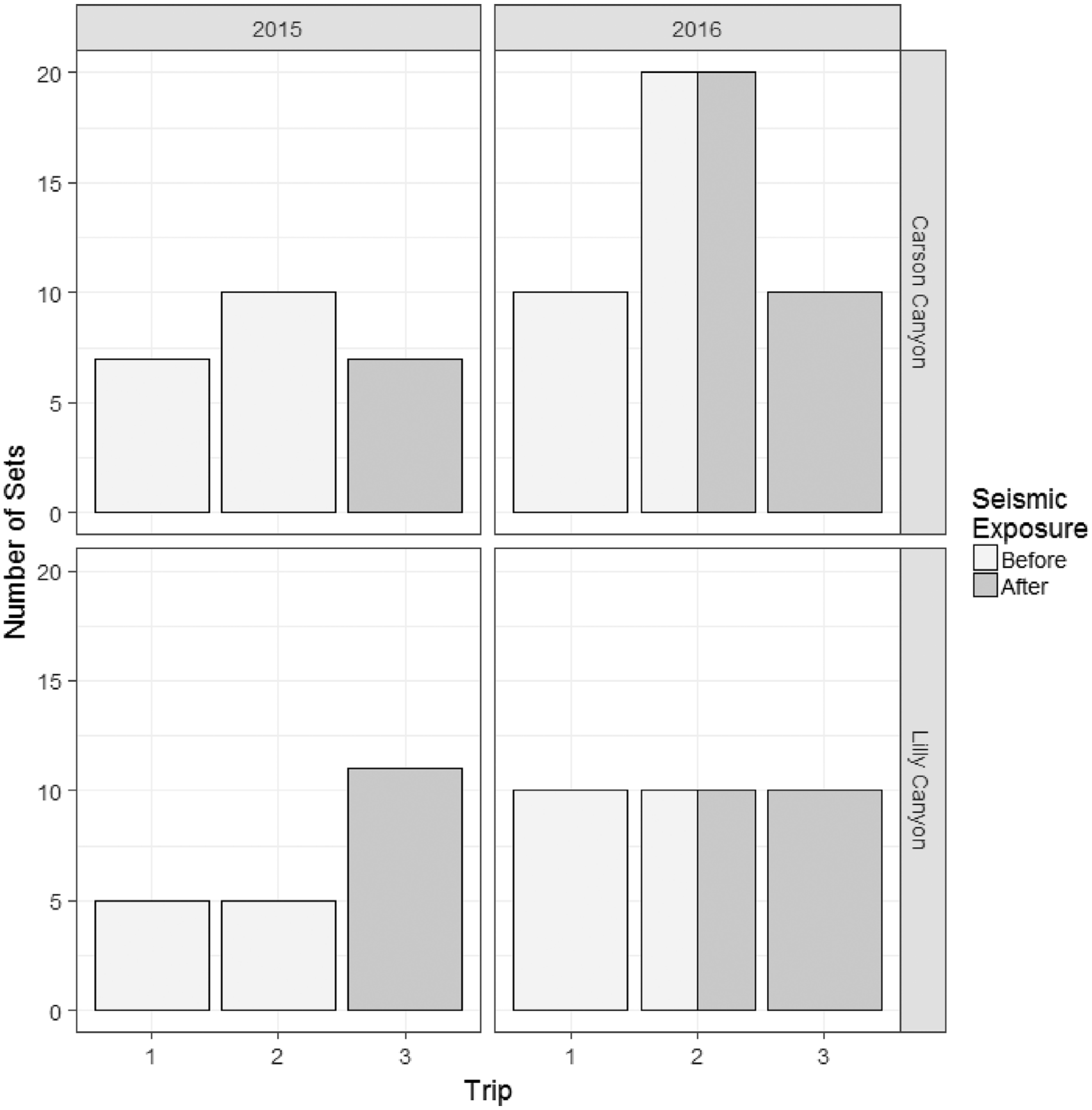

Fig. 2. Sampling effort allocated to the control (Lilly Canyon) and treatment (Carson Canyon) areas before and after exposure to seismic activity in 2015 and 2016. No seismic activity occurred in the control area; shading is provided for comparison purposes only.

Total Snow Crab ~ Study Area*Temporal Period + Depth + (1|String)

For both years, longer term effects of seismic on catch rates were compared (i.e. comparison of Trip 2 "Before" data to Trip 3 "After" data). This evaluation was conducted using the same model as described for short term effects with the exception that a variable was included to account for year effects.

Total Snow Crab ~ Study Area*Temporal Period + Depth + Year + (1|String)

2.5. Power analysis methods

We used power analysis on the 2015 data to guide the 2016 sampling intensity. Statistical power was assessed for a variety of scenarios using a data simulation approach that was informed with pilot data collected at the study site in 2015. Specifically, scenarios that included a variety of sample sizes (6–18 strings) and effect sizes (CPUE reductions of 0–70% of baseline) were assessed by simulating scenario-specific data based on the observed variability in within-string catches and across-string catches during the baseline condition (Trip 2 of Carson Canyon) (Supplementary Fig. 1). The scenarios described above were tested using a more simple design using Before-After or Control-Impact, in which the parameter of interest was either sampling period or site. A final set of scenarios was also conducted using a more complete BACI study design, in which the parameter of interest was the interaction between sites and sampling period. None of the simulations incorporated variance related to depth, since the effect of depth was reflected in the model that was used to generate the input values for the power analysis.

Within a given scenario, 500 simulated data sets were generated; each of which was assessed for significance within a negative binomial generalized linear mixed effects model framework (lme4 extension of R; R Core Team, 2015), where total counts of snow crab were the response variable. In the more simple Before/After simulations, the Before/After condition was the fixed effect and the string was the random effect, whereas the BACI simulations also included a Control-Impact fixed effect and an interaction term. As with the analysis described above, traps within a string were not considered independent, hence the need for a random effect. All "Before", "Before-Impact", "Before-Control" and "After-Control" datasets were generated from the same sampling distribution parameters derived from Trip 2 of Carson Canyon. The "After" or "After-Impact" datasets were similarly created but only after the sampling distribution mean was adjusted to the scenario-specific effect

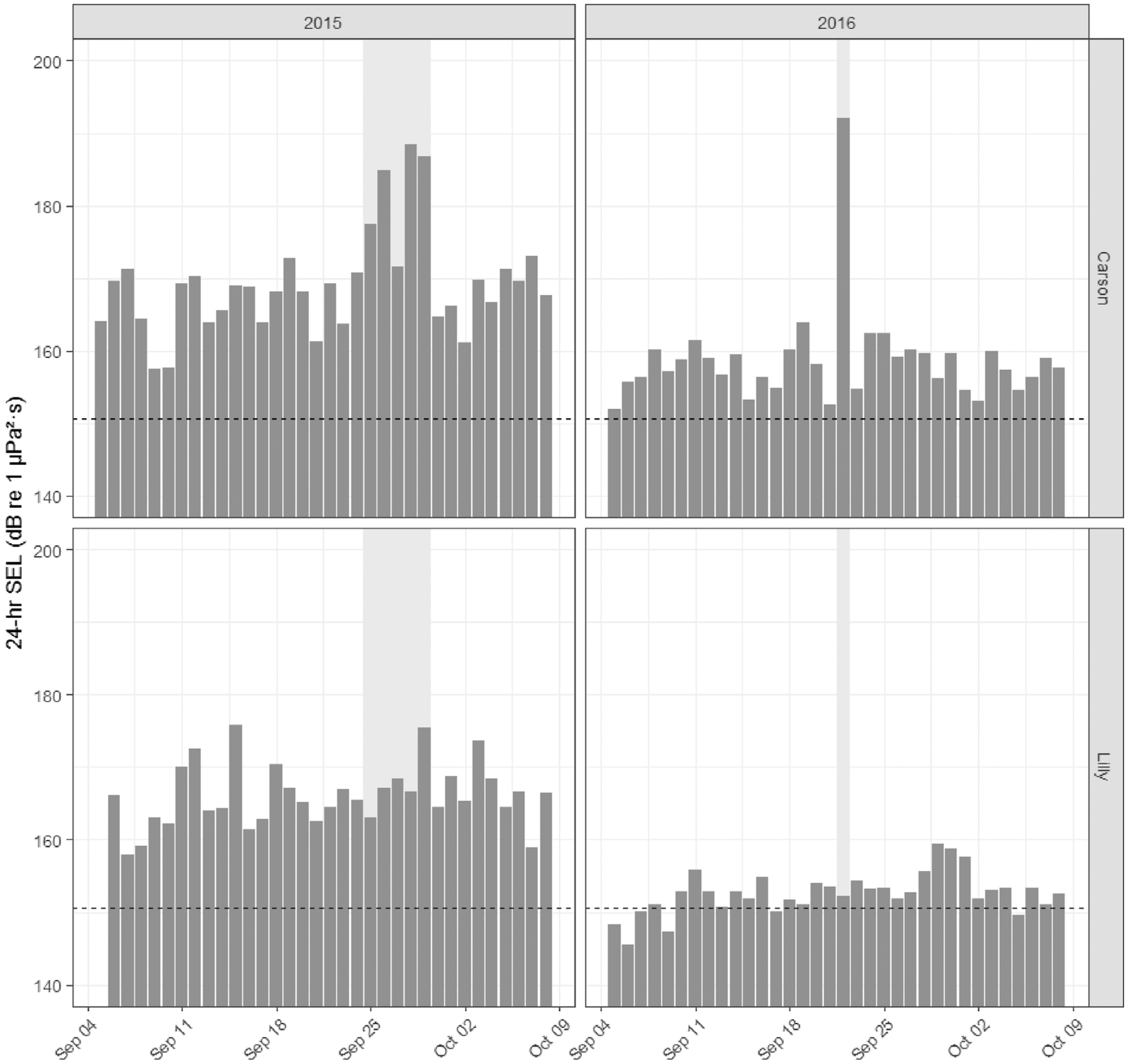

Fig. 3. Daily sound exposure levels measured at the control and treatment sites in 2015 and 2016. The gray-shaded areas are the days that the Atlantic Explorer performed exposures near the treatment site. The dashed line is the median sound level measured at a different Grand Banks location less affected by anthropogenic noise during the same period in 2015. The sound exposure levels for 26 Sep 2015, 28 Sep 2015, 29 Sep 2015 and 22 Sep 2016 are underestimates due to hydrophone saturation.

size. The proportion of significant likelihood ratio test results across these iterations represented the scenario's statistical power.

3. Results

3.1. Sound exposure

The median daily sound exposure level was 13 dB higher in 2015 than 2016 at the control site and 11 dB higher at the treatment site, likely as a result of differences in seismic surveying activities between years (Fig. 3). Despite the louder environment at both sites during 2015, the seismic exposure included as part of this study was approximately 20 dB higher than other recorded sound levels. Sound levels at the control site did not increase while seismic activity occurred at the test site in 2015. Similarly, the sound exposure level at the control site in 2016 did not increase during the 2-h exposure experiment at the treatment site 70 km away. The median of the sound exposures in 2016 were 2 dB higher at the control site than our separate sound measure representing quiet Grand Banks in 2015, indicating that it was representative of offshore Newfoundland. During the periods of time that the commercial fishing vessel, Royal Venture, was working near the sound recorders in 2016 the maximum measured sound exposure levels were typically 155–163 dB (Fig. 3). Thus, the sound exposure level near the seabed due to the seismic vessel was at least 30 dB higher (1000-fold increase) when the seismic vessel passed over the recorders on September 22, 2016 than during a day's fishing.

The particle acceleration and velocities increased during the seismic CPA proportionally to the pressure levels (Fig. 5). No interface waves that would increase particle motion at the seabed (i.e. ground roll) were detected.

3.2. Power analysis

The BACI study design was slightly less powerful approach to detect change than the Before-After approach. The Before-After study design could reliably detect change at the highest sample sizes (n = 18 per group; i.e. the number of samples used in the longer term comparison) when declines reached 50% of baseline while the BACI approach could only do so for declines of 60% or more (n = 18) (Supplementary Fig. 1.). For the smaller sample sizes associated with the short term effects assessment, a BACI design was expected to be able to reliably detect a decline of 70% whereas a Before-After design would be able to detect declines of 60%.

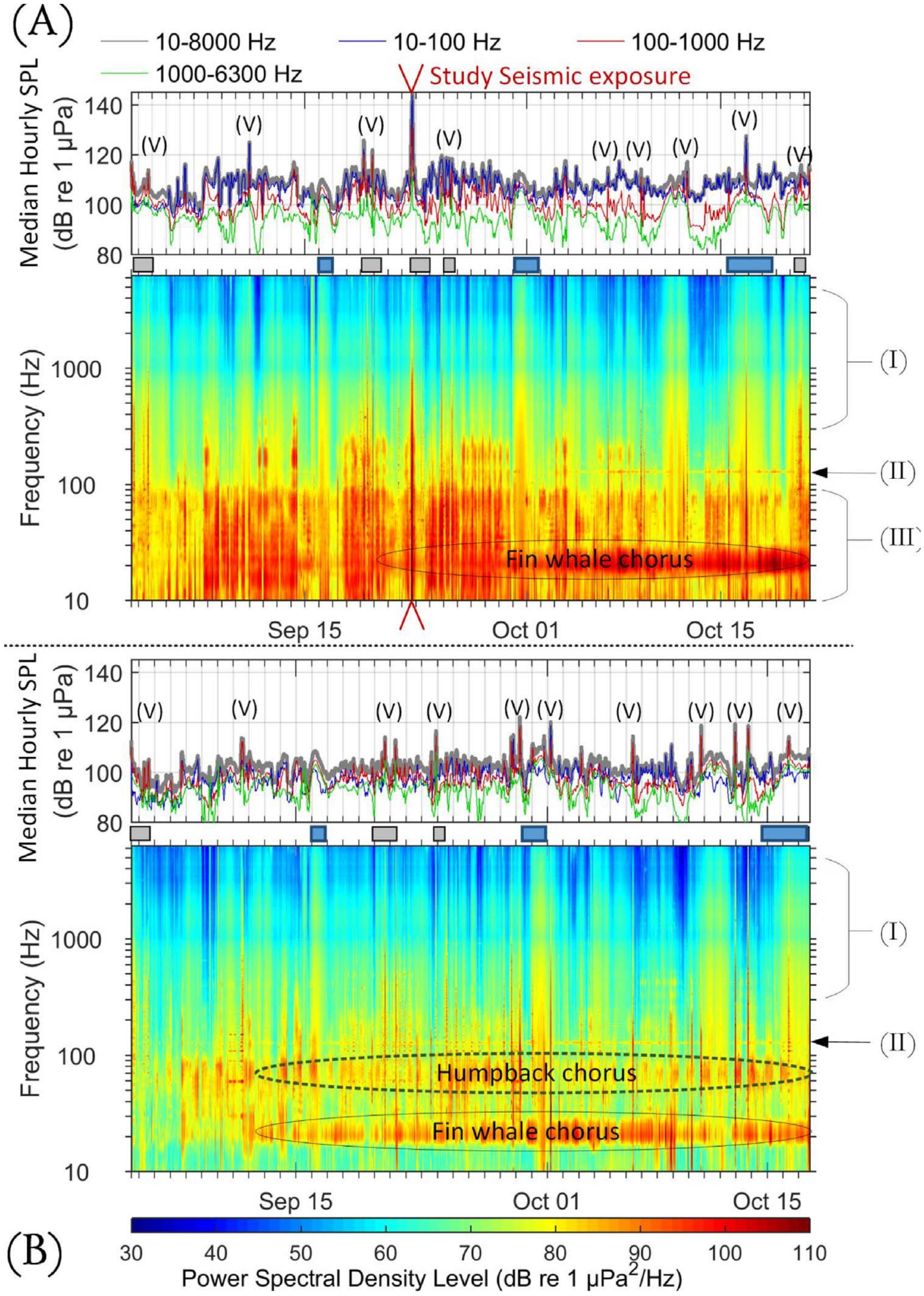

Fig. 4. Acoustic summary at the (A) treatment site (Carson Canyon) and (B) control site (Lilly Canyon) in 2016. For each site the top panel is the median hourly in-band SPL and bottom is the long-term spectral average of the measured sound. Named tropical storms that passed the area are identified by the blue bars (from left-right: Ian, Karl, Nicole). Days when the study's fishing effort occurred within 5 km of the recorders are shown by the gray bars. The study seismic exposure on 22 Sept 2016 at Carson Canyon is framed by the red-arrows. (V) indicatesnotable passages of vessels. Frequency bands with notable sound sources: (I) wind and wave noise; (II) 130 Hz fin whale top note; (III) distant seismic surveys affecting Carson Canyon's sound levels.

3.3. Short term effects of seismic activity on 2016 snow crab catch rates

In Carson Canyon, catch rates dropped from 5.29 ind/pot (range 0–44) to 5.03 ind/pot (range 0–33; a decline of 4.9%), whereas in Lilly Canyon, catch rates increased from 10.2 ind/pot (range 0–60) to 13.9 ind/pot (range 0–65; an increase of 36%) (Fig. 6). BACI interactions between site and exposure category were not significant ($P = 0.838$) and therefore did not provide evidence to suggest that seismic exposure was affecting catch rates. The remaining terms of the model indicate that Lilly Canyon has significantly higher catches ($P < 0.001$) and depth was a significant variable in catch rates ($P < 0.001$; Fig. 7). In light of the increased ability of Before-After comparisons to detect change (described above), the short term effects model was re-run for just Carson Canyon data. The comparison yielded a non-significant result ($P = 0.345$) for the Before-After variable.

3.4. Longer term effects of seismic activity on 2015 and 2016 snow crab catch rates

Combined across both years, average catch rates declined in Carson Canyon by 38%, whereas they increased in Lilly Canyon (control) by 1.7%. However, the BACI interaction term of the model was not significant ($P = 0.450$); indicating that the observed longer term seismic-related differences in catch rates could have occurred by chance. The remaining terms of this model were significant with Lilly Canyon showing elevated catch rates versus Carson Canyon ($P < 0.001$), Before Exposure catch rates (across all sites) were significantly higher than After Exposure ($P = 0.025$), 2016 catch rates were lower than 2015 ($P < 0.001$) and depth remained as a significant predictor variable ($P < 0.001$). As for short term effects, we assessed the more simple Before-After model with only Carson Canyon for Trip 2 and Trip 3 data.

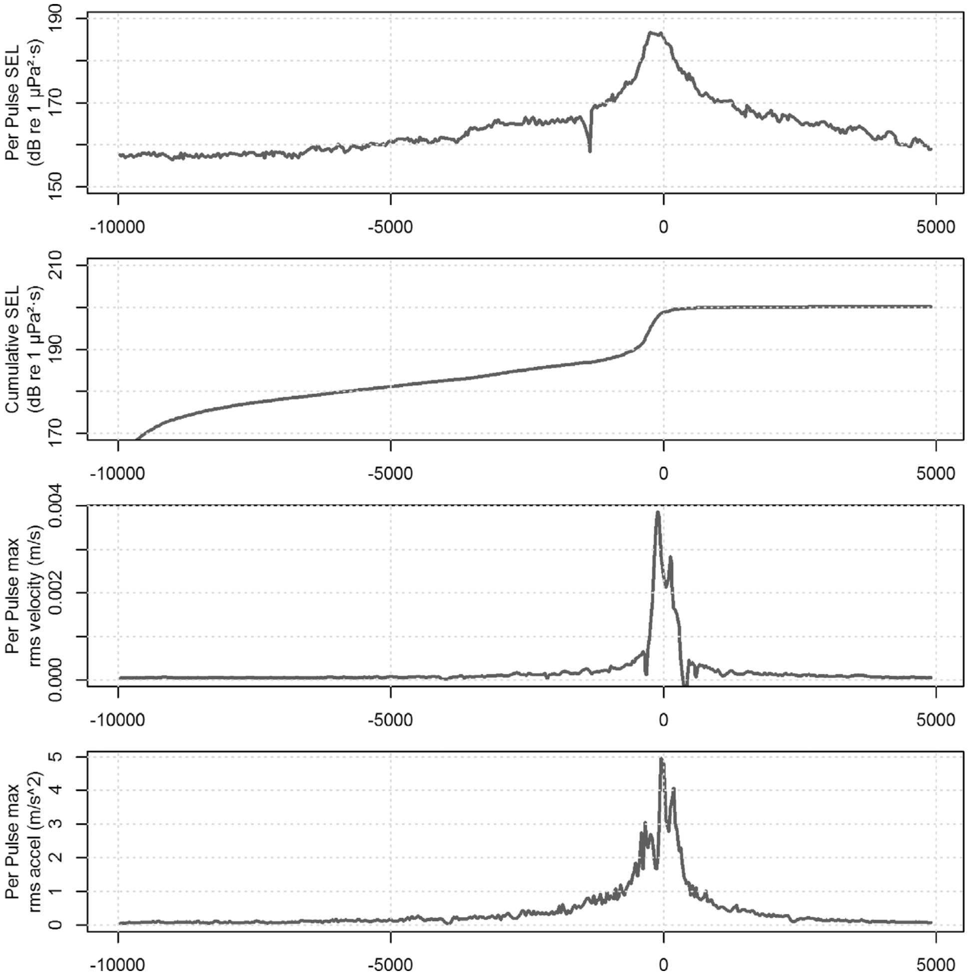

Fig. 5. Sound metrics of the 2D seismic exploration vessel used for this experiment measured near the sea-floor, representing the per-pulse sound exposure of a snow crab directly under the source. The particle acceleration measurements are from the MEMS sensor coupled to the seafloor.

The result was significant ($P = 0.014$).

For interest, we also compared Trip 1 (Before) vs. Trip 3 (After). The results were qualitatively consistent with the initial longer term model in that Carson Canyon catch rates declined by 18% post-exposure, whereas the control site increased by 16% post-exposure. As with the previous comparison, the interaction term was not significant ($P = 0.270$) and the remaining main effects were highly significant (i.e. catches were higher in 2015 at both sites and catches were higher in Lilly Canyon in both years; all $P < 0.001$).

4. Discussion

The experimental manipulations conducted in this study, which featured both spatial and temporal controls, did not support the idea that seismic exploration affects commercial catch rates of snow crab over the shorter (days) or longer (weeks) term examined. If seismic-related declines occurred that were too small for our study design to detect, they were secondary to more important natural spatial (i.e. Control-Impact) and temporal influences (Before-After effects).

Seismic exploration effects on catch rates could be manifested through a variety of mechanisms ranging across death, injury, stress or altering the sensory environment, which influence the individual's physical capacity and/or motivation to enter a trap. Vulnerable taxa only suffer mortality in close proximity to seismic guns (i.e. within meters) (Hawkins and Popper 2017; Slabbekoorn 2016 but see McCauley et al., 2017). The depths that snow crab are commercially harvested put the benthic life stages well beyond the kill zone of seismic arrays (Christian et al., 2003; DFO, 2004) with apparently limited long term impacts on survival. One hundred and twenty snow crab that were captured and transferred to laboratory facilities at the Northwest Atlantic Fisheries Centre from Carson Canyon, after being exposed to seismic noise in the offshore during 2015 were held in captivity for 18 months, and although the total mortality over that time period was 40% (Morris, personal observation June 22, 2017) this is low compared to other mortality rates of snow crab measured in captivity (Siikavuopio et al., 2017).

Sound-related injury and physiological responses can occur farther from the source (Hawkins and Popper 2017). Snow crab and other invertebrates however, are generally considered less vulnerable to noise-related trauma than marine mammals and fishes because they lack gas-filled spaces (Edmonds et al., 2016; MacGregor et al., 2016). Gas-filled morphological features (e.g. swim bladders in fish) are typically the location of barotrauma for noise-exposed animals as sound-generated pressure waves cause rapid motion in these structures and can damage adjacent tissue (Hawkins and Popper, 2017). For example, comparative studies show greater physical trauma to impulsive sound by fishes with swim bladders relative to those that lack that structure (Casper et al., 2016). Instead, invertebrates like snow crab are considered to only be vulnerable to particle motion (Payne et al., 2007; Mooney et al., 2010; Casper et al., 2016; Edmonds et al., 2016; Hawkins and Popper, 2017). Particle motion effects are thought to only be of consequence in close proximity to the sound source (Casper et al., 2016). Accordingly, lab and cage exposures of snow crab (Christian et al., 2003; Courtenay et al., 2009) and American lobster (Payne et al., 2007) to seismic sound did not result in any conclusive physiological effects, beyond some non-lethal signs of organ stress in lobster.

More likely to influence commercial catch rates are behavioural responses to sound (Hirst and Roadhouse, 2000). First, the potential

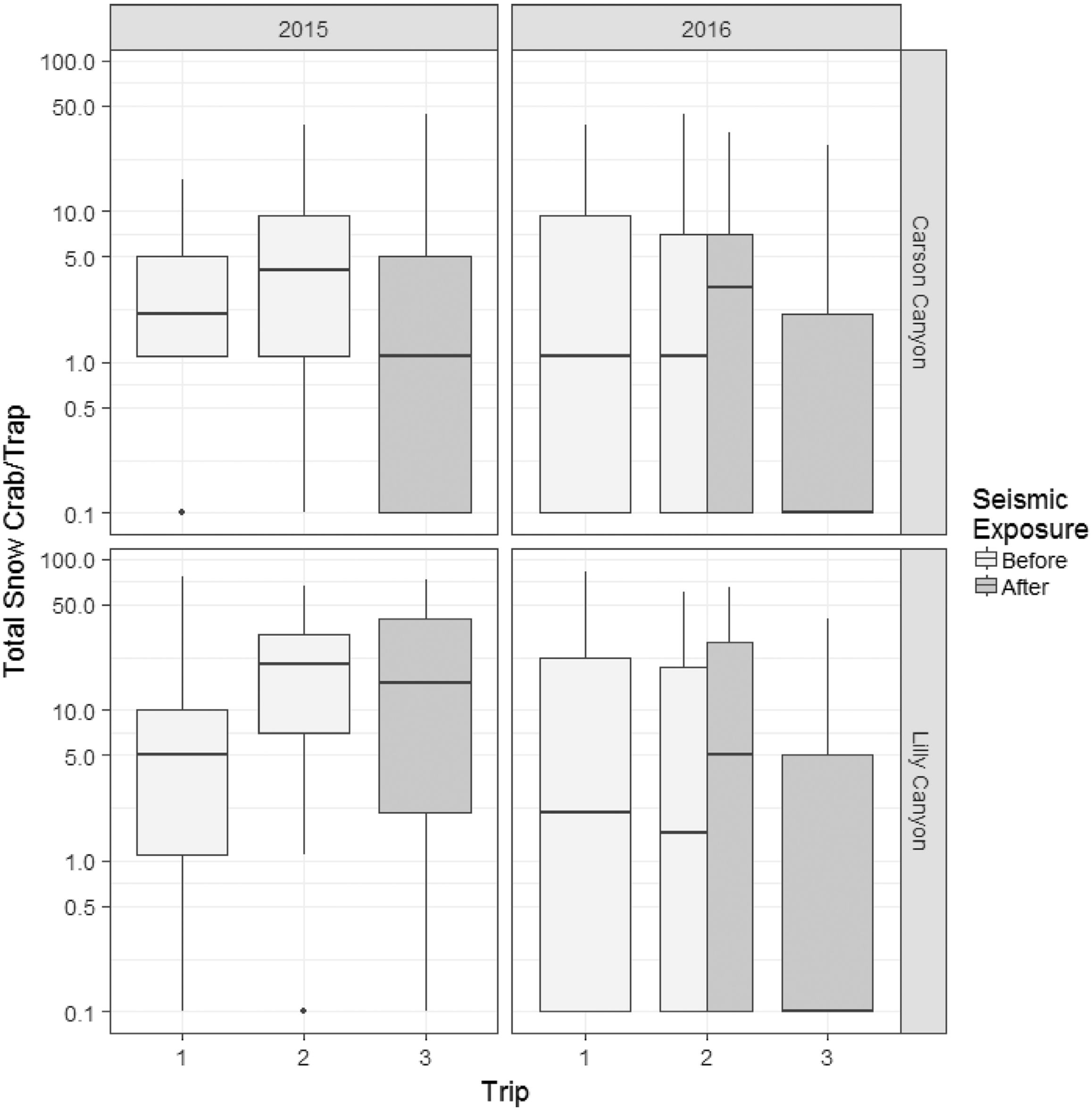

Fig. 6. Snow crab catch per trap in Lilly and Carson canyons on each of three trips. Horizontal lines represent median catch rates, boxes represent the middle quartiles and whiskers represent 1.5 times the interquartile range. Data beyond the whiskers are represented as individual data points.

area of influence is considerably larger (Hirst and Rodhouse 2000; Hawkins and Popper 2017; Slabbekoorn 2016), extending to 10 s of km for some fishes (Engås et al., 1996; Løkkeborg and Soldal,1993); a scale that better corresponds to those of commercial harvests. Second, some marine crustaceans are known to produce and respond to sound (Mooney et al., 2010; Edmonds et al., 2016) and therefore it is likely that the presence of anthropogenic sound could cause behavioural alterations to the likelihood that an individual encounters a trap and is motivated to enter it.

Foreign noises may illicit direct behavioural changes such as antipredator responses, which may trigger cessation or alteration of feeding and/or mating behaviour, and movement away from the perceived threat to less suitable habitats (e.g. Wale et al., 2013a, 2013b; Day et al., 2016). They may also alter the soundscape; reducing the sensitivity at which organisms can perceive and react to their environment. While cage and laboratory exposures did not identify behavioural responses in snow crab (Christian et al., 2003; Christian et al., 2004) or American lobster (Payne et al., 2007), responses have been observed in other crustaceans (Edmonds et al., 2016). Even though crustaceans clearly use and respond to sound (Popper et al., 2001; Mooney et al., 2010), studies suggest that invertebrate commercial catches are usually not affected (Carroll et al., 2016). Snow crab catches actually increased after exposure to seismic sound (Christian et al., 2003), but the authors as well as Courtenay et al., 2009, conceded that the observed differences were likely the result of other phenomena. Certainly, a comparable time interval to Christian et al. (2003) study, between pre and post treatments in our study resulted in significant changes in catch rates even in our control area. Nevertheless, a similar absence of effect has also been observed for crustaceans such as shrimp (Southern Brown, Southern White and Atlantic Seabob, Andriguetto-Filho et al., 2005), rock lobster (Parry and Gason 2006), Norway lobster and mantis shrimp (La Bella et al., 1996) and reflects their reduced sensitivity of these taxa to anthropogenic noise.

Pronounced seismic-induced changes to commercial catches are more common in fishes (Skalski et al., 1992; Løkkeborg and Soldal, 1993; Engås et al., 1996; Løkkeborg et al., 2012; Vold et al., 2012). Observed changes are typically attributed to avoidance responses that occur on both horizontal (Engås et al., 1996; Slotte et al., 2004) and vertical (Skalski et al., 1992; Pearson et al., 1992; Slotte et al., 2004) planes. The change to catch rates depends on gear type, with decreases

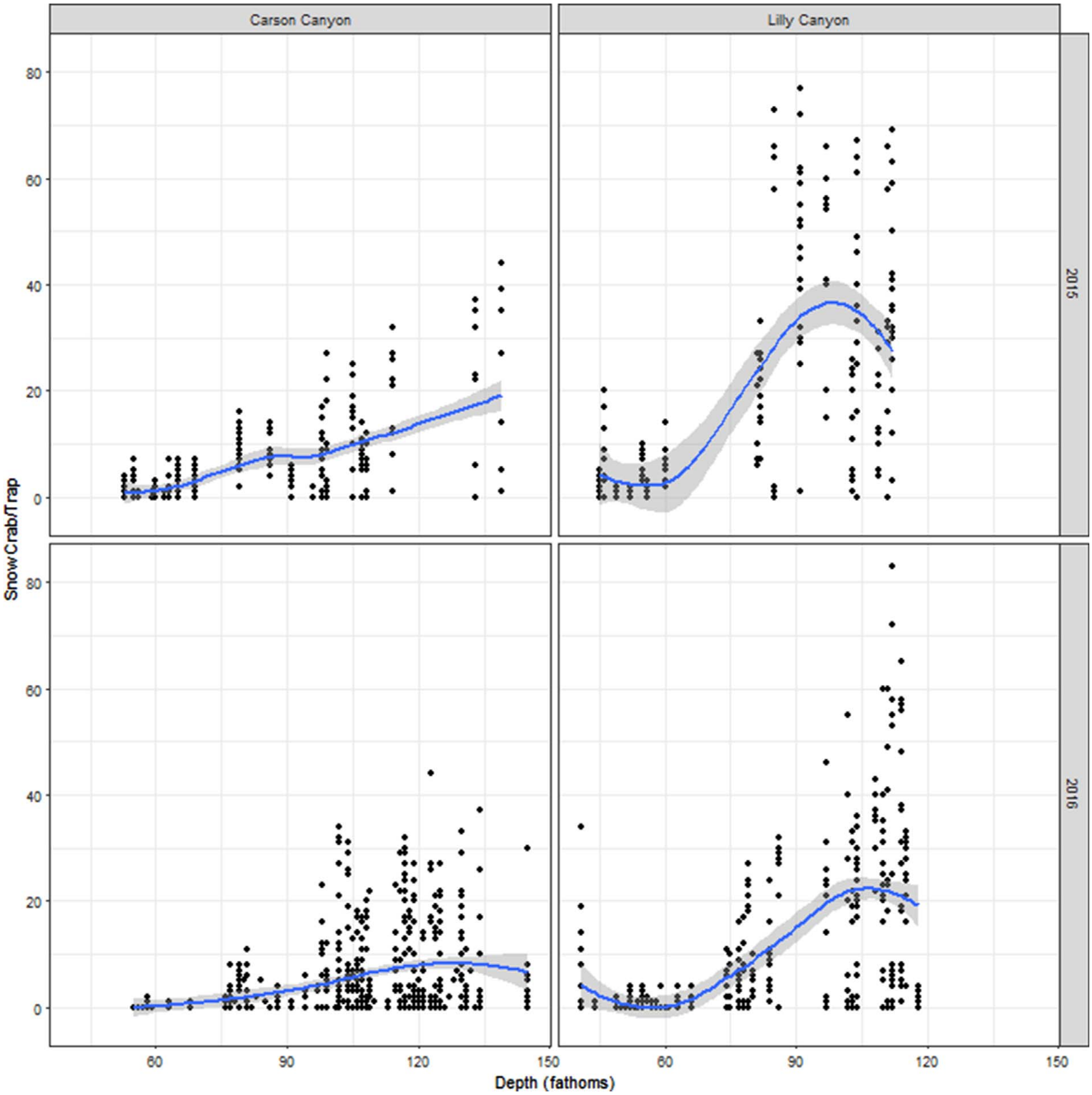

Fig. 7. The effects of depth on catch rates in Carson and Lilly canyons in 2015. A locally weighted smoother and associated 95% confidence interval envelope are superimposed on the data.

often associated with active gear methods (e.g. trawls; Engås et al., 1996) but increases are sometimes noted with passive methods such as gill nets, which initially benefit from the increased movement associated with the avoidance response (Løkkeborg et al., 2012). The avoidance response observed, however is context-dependent (Radford et al., 2016). For example, studies on fish species associated with local habitat features (e.g. sea mounts or reefs) often do not document latitudinal dispersal (Skalski et al., 1992; Wardle et al., 2001) but may still observe changes in depth use and/or a reduced willingness to take baited hooks (Skalski et al., 1992). In contrast, mobile demersal species may show an increased likelihood of horizontal displacement (e.g. Løkkeborg and Soldal, 1993; Løkkeborg et al.,2012). Some fish species may be undeterred by seismic activity when there is sufficient motivation (e.g. good feeding conditions; Peña et al., 2013). The variation of seismic effects, within and among populations, remains relatively unstudied however (Radford et al., 2014).

Studies of seismic effects on marine biota have been plagued by study design issues (Hawkins and Popper 2017); a result of the significant challenge related to isolating seismic effects while still maintaining study designs that provide biologically meaningful results (Slabbekoorn, 2016). Feasibility and experimental control encourage many researchers to use laboratory-based studies even though the resulting studies may have poor acoustic and behavioural validity (Popper and Hastings, 2009; Slabbekoorn, 2016). Similarly, field studies struggle to achieve adequate sample sizes needed to overcome the inherent variability associated with marine environments and consequently suffer from marginal statistical power (Slabbekoorn, 2016; Williams et al., 2015; Payne et al., 2007; Edmonds et al., 2016; e.g. Parry and Gason 2006; Courtenay et al., 2009). Logistical challenges may also cause many researchers to forego the suitable controls needed to effectively test for seismic effects (Hawkins and Popper, 2017) or to design studies opportunistically on existing seismic surveys; a compromise which can detract from the study's goals (Løkkeborg and Soldal, 1993; Parry and Gason, 2006; Vold et al., 2012). In this study, efforts were made to make the manipulations realistic from both a harvester and seismic operations perspective. This study is also a rare case where both spatial and temporal controls were implemented in a field environment. Interestingly, while BACI designs allow for greater confidence in positive (i.e. significant) results, they are not without detractors (e.g. Underwood, 1992). Our power analysis simulations indicate that they come with the penalty of having less statistical power to detect an effect than Before-After designs. Like previous seismic studies on invertebrate commercial catch rates (Parry and Gason 2006; Payne et al., 2007), we remain unable to distinguish small changes in catch rates. However, the use of the BACI design enabled us to place any potential effects in the context of other sources of variation (spatial and temporal); results which were aligned with temporal trends in biomass for the stock (DFO stock assessment). Conclusions regarding more subtle effects of seismic exposure will likely need to be inferred from supporting laboratory studies (Williams et al., 2015; Slabbekoorn, 2016) and complimentary field studies that look for mechanistic evidence of effects (e.g. changes to physiology, genomic expression, movement) that could influence catch rates.

A principal goal of this study was to provide information useful to resolving resource conflicts of two marine industries. To maximize the chance of acceptance of this knowledge and its incorporation to resolving resource conflicts, considerable effort was made to involve the affected industries. These collaborations were initiated at the project planning stages, addressed industry viewpoints, included regulatory support from the Canada-Newfoundland and Labrador Offshore Petroleum Board (C-NLOPB) and Fisheries and Oceans Canada (DFO) and incorporated feedback during the project. We believe that this approach enhanced the relevance and quality of this study and is worth considering for other industry-related studies.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.fishres.2017.09.012.

References

Carroll, A.G., Przeslawski, R., Duncan, A., Gunning, M., Bruce, B., 2016. A critical review of the potential impacts of marine seismic surveys on fish and invertebrates. Mar. Pollut. Bull. 114, 9–24. http://dx.doi.org/10.1016/jmarpolbul.2016.11.038.

Casper, B.M., Carlson, T.J., Halvorsen, M.B., Popper, A.N., 2016. Effects of impulsive pile-Driving exposure on fishes. In: In: Popper, A., Hawkins, A. (Eds.), The Effects of Noise on Aquatic Life II. Advances in Experimental Medicine and Biology 875 Springer, New York, NY.

Christian, J.R., Mathieu, A., Thompson, D.H., White, D., Buchanan, R.A., 2003. Effect of Seismic Energy on Snow Crab (Chionoecetes Opilio). Environmental Research Funds Report No. 144. Calgary. (106p).

Christian, J.R., Mathieu, A., Buchanan, R.A., 2004. Chronic Effects of Seismic Energy on Snow Crab (Chionoecetes Opilio). Environmental Research Funds Report No. 158. Calgary. (25pp).

Editors). In: Courtenay, S.C., Boudreau, M., Lee, K. (Eds.), Potential Impacts of Seismic Energy on Snow Crab: an Update to the September 2004 Peer Review. Environmental Studies Research Funds Report No 178. Moncton (181p).

DFA, 2017. Seafood Industry Year in Review 2016. Department of Fisheries and Land Resources. Prepared by Planning and services Division, Government of Newfoundland and Labrador. 28 pp. http://www.fishaq.gov.nl.ca/publications/pdf/SYIR_2016.pdf (accessed 20.06.17).

DFO, 2004. Potential Impacts of Seismic Energy on Snow Crab Habitat Status Report 2004/003. DFO Can. Sci. Advis. Sec.

DFO, 2016. Assessment of Newfoundland and Labrador (Divisions 2HJ3KLNOP4R) Snow Crab. (DFO Can. Sci. Advis. Sec.Sci.Advis.Rep. 2016/013).

Day, R.D., McCauley, R., Fitzgibbon, Q.P., Semmens, J.M., 2016. Assessing the Impact of Marine Seismic Surveys on Southeast Australian Scallop and Lobster Fisheries. (FRDC Report 2012/008). University of Tasmania, Hobart.

Edmonds, N.J., Firmin, C.J., Goldsmith, D., Faulkner, R.C., Wood, D.T., 2016. A review of crustacean sensitivity to high amplitude underwater noise: data needs for effective risk assessment in relation to UK commercial species. Mar. Pollut Bull . 108, 5–11. http://dx.doi.org/10.1016/jmarpolbul.2016.05.006.

Engås, A., Løkkeborg, S., Ona, E., Soldal, A.V., 1996. Effects of seismic shooting on local abundance and catch rates of cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*). Can. J. Fish. Aquat. Sci 53, 2238–2249.

Hawkins, A.D., Popper, A., 2017. A sound approach to assessing the impact of underwater noise on marine fishes and invertebrates. ICES J. Mar. Sci. 74, 635–651. http://dx.doi.org/10.1093/icesjms/fsw205.

Hawkins, A.D., Pembroke, A.E., Popper, A.N., 2015. Information gaps in understanding the effects of noise on fishes and invertebrates. Rev. Fish Biol. Fish. 25, 39–64. http://dx.doi.org/10.1007/s11160-014-9369-3.

Hirst, A.G., Roadhouse, P.G., 2000. Impact of geophysical seismic surveying on fishing success. Rev. Fish Biol. Fish. 10, 113–118.

Løkkeborg, S., Soldal, A.V., 1993. The influence of seismic exploration with airguns on cod (*Gadus morhua*) behaviour and catch rates. ICES J. Mar. Sci. 196, 62–67.

Løkkeborg, S., Ona, E., Vold, A., Salthaug, A., 2012. Sounds from seismic air guns: gear- and species-specific effects on catch rates and fish distribution. Can. J. Fish. Aquat. Sci. 69, 1278–1291.

La Bella, G., Cannata, S., Froglia, C., Ratti, S., Rivas, G., 1996. First assessment of effects of air-gun seismic shooting on marine resources in the central Adriatic Sea. International Conference on Health, Safety and Environment in Oil and Gas Exploration and Production 227–238.

MacGillivray, A.O., 2006. Acoustic Modelling Study of Seismic Airgun Noise in Queen Charlotte Basin MSc Thesis. University of Victoria, Victoria, BC (98 pp).

MacGregor, P.K., Horn, A.G., Leonard, M.L., Thomsen, F., 2016. Anthropogenic noise and conservation. In: Brumm, H. (Ed.), Animal Communication and Noise, Animal Signals and Communication 2. Springer-Verlag, Berlin, pp. 409–444.

McCauley, R.D., Day, R.D., Swadling, K.M., Fitzgibbon, Q.P., Watson, R.A., Semmens, J.M., 2017. Widely used marine seismic survey air gun operations negatively impact zooplankton. Nature Ecol Evol 1, 095. http://www.ecomarres.com/downloads/seismic.pdf.

Mooney, T.A., Hanlon, R.T., Christensen-Dalsgaard, J., Madsen, P.T., Ketten, D.R., Nachtigall, P.E., 2010. Sound detection by the longfin squid (*Loligo pealeii*) studied with auditory evoked potentials: sensitivity to low-frequency particle motion and not pressure. J. Exp. Biol. 213, 3748–3759.

Mullowney, D.R.J., Dawe, E.G., Colbourne, E.B., Rose, G.A., 2014. A review of factors contributing to the decline of Newfoundland and Labrador snow crab (*Chionoecetes opilio*). Rev. Fish Biol. Fish. 24, 639. http://dx.doi.org/10.1007/s11160-014-9349-7.

Parry, G.D., Gason, A., 2006. The effect of seismic surveys on catch rates of rock lobsters in western Victoria. Australia.Fish. Res. 79, 272–284.

Payne, J.F., Andrews, C.A., Fancey, L.L., Cook, A.L., Christian, J.R., 2007. Pilot study on the effects of seismic air gun noise on lobster (*Homarus americanus*). Can. Tech. Rep. Fish. Aquat. Sci (No. 2712).

Payne, J.F., Andrews, C., Fancey, L., White, D., Christian, J., 2008. Potential Effects of Seismic Energy on Fish and Shellfish: an Update Since 2003. (DFO Can. Sci. Adv Res. Doc.2008/060).

Peña, H., Handegard, N.O., Ona, E., 2013. Feeding herring schools do not react to seismic air gun surveys. ICES J. Mar. Sci. 70, 1174–1180.

Pearson, W.H., Skalski, J.R., Malme, C.I., 1992. Effects of sound from a geophysical survey device on behavior of captive rockfish (*Sebastes* spp.). Can. J. Fish. Aquat. Sci. 49, 1343–1356.

Popper, A.N., Hastings, M.C., 2009. The effects of human-generated sound on fish. Integr. Zool. 4, 43–52.

Popper, A.N., Salmon, M., Horch, K.W., 2001. Acoustic detection and communication by decapod crustaceans. J Comp. Physiol. A 187, 83–89.

R Core Team, 2015. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

Radford, A.N., Kerridge, E., Simpson, S.D., 2014. Acoustic communication in a noisy world: can fish compete with anthropogenic noise? Behav Ecol. 25, 1022–1030. http://dx.doi.org/10.1093/beheco/aru029.

Radford, A.N., Lèbre, L., Lecaillon, G., Nedelec, S.L., Simpson, S.D., 2016. Repeated exposure reduces the response to impulsive noise in European seabass. Glob Change Biol. 22, 3349–3360.

Siikavuopio, S.I., James, P., Olsen, B.R., Evensen, T., Mortensen, A., Olsen, S.H., 2017. Holding wild snow crab Chionoecetes opilio: effects of stocking density and feeding on survival and injury. Aqua. Res. 48, 1590–1595. http://dx.doi.org/10.1111/are.12993.

Skalski, J.R., Pearson, W.H., Malme, C.I., 1992. Effects of sounds from a geophysical survey device on catch-per-unit- effort in a hook-and-line fishery for rockfish (*Sebastes* spp.). Can. J. Fish. Aquat. Sci. 49, 1357–1365.

Slabbekoorn, H., 2016. In: Popper, A.N., Hawkins, A. (Eds.), Aiming for Progress in Understanding Underwater Noise Impact on Fish: Complementary Need for Indoor and Outdoor Studies. In: The Effects of Noise on Aquatic Life II. Springer Science + Business Media, New York, pp. 1057–1065.

Slotte, A., Hansen, K., Dalen, J., Ona, E., 2004. Acoustic mapping of pelagic fish distribution and abundance in relation to a seismic shooting area off the Norwegian west coast. Fish. Res. 67, 143–150.

Solan, M., Hauton, C., Godbold, J.A., Wood, C.L., Leighton, T.G., White, P., 2016. Anthropogenic sources of underwater sound can modify how sediment-dwelling invertebrates mediate ecosystem properties. Sci. Rep . 6, 20540.

Stansbury, D.E., Fiander, D., Maddock-Parsons, D., 2013. Summary of the Industry-DFO Collaborative Post-season Trap Surveys for Snow Crab in Div. 2J3KLOPs4R. http://ffaw.nf.ca/sites/ffaw.nf.ca/files/3PS%20SNOW%20CRAB%20SURVEY%20SUMMARY%202013.pdf/ (accessed 20.06.17).

Underwood, A.J., 1992. Beyond BACI: the detection of environmental impacts on populations in the real, but variable, world. J. Exp. Mar. Biol. Ecol. 161, 145–178.

Vold, A., Løkkeborg, S., Tenningen, M.M., 2012. Using catch statistics to investigate effects of seismic activity on fish catch rates. In: In: Popper, A.N., Hawkins, A. (Eds.), The Effects of Noise on Aquatic Life. Advances in Experimental Medicine and Biology, vol. 730. Springer, New York, NY.P, pp. 411–413.

Wale, M.A., Simpson, S.D., Radford, A.N., 2013a. Noise negatively affects foraging and antipredator behaviour in shore crabs. Anim. Behav. 86, 111–118.

Wale, M.A., Simpson, S.D., Radford, A.N., 2013b. Size-dependent physiological responses of shore crabs to single and repeated playback of ship noise. Biol. Lett. 9, 20121194.

Wardle, C.S., Carter, T.J., Urquhart, G.G., Johnstone, A.D.F., Ziolkowski, A.M., Hampson, G., Mackie, D., 2001. Effects of seismic air guns on marine fish. Cont. Shelf Res. 21, 1005–1027.

Williams, R., Wright, A.J., Ashe, E., Blight, L.K., Bruintjes, R., Canessa, R., Clark, C.W., Cullis-Suzuki, S., Dakin, D.T., Erbe, C., Hammond, P.S., Merchant, N.D., O'Hara, P.D., Purser, J., Radford, A.N., Simpson, S.D., Thomas, L., Wale, M.A., 2015. Impacts of anthropogenic noise on marine life: publication patterns, new discoveries, and future directions in research and management. Ocean Coast. Manag. 115, 17–24.

Zuur, A.F., Ieno, E.N., Walker, N.J., Saveliev, A.A., Smith, G.M., 2009. Mixed Effects Models and Extensions in Ecology. R. Springer, New York.

APPENDIX 3 – Effects of 3D Seismic Surveying on Snow Crab Fisheries

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Effects of 3D seismic surveying on snow crab fishery

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ABSTRACT

Commercial Snow Crab (*Chionoecetes opilio*) harvesters believe marine noise from seismic surveys reduces commercial Snow Crab catch rates. Depending on the type of seismic survey used, animals living in a particular area could be exposed to loud noise (e.g. daily Sound Exposure Level (SEL) > 165 dB re 1 $\mu Pa^2 \cdot s$) for periods ranging from hours (typical 2D survey) to months (detailed 3D survey). This field experiment applied a series of comparisons conducted within a Before-After-Control-Impact study design to investigate the effect of prolonged industrial 3D seismic exposure on the catch rates of Snow Crab over nine weeks in 2017 and five weeks in 2018. Changes in catch rates at 3D seismic surveying sites were inconsistent across years, with reduced catches in 2017 and increased catches in 2018. Catch rates were similar at experimental and control sites within two weeks after exposure, and the potential effect of seismic surveying was not measured at a distance of 30 km. The large variation in catch rates across small temporal and spatial scales coupled with the absence of notable mechanistic responses of Snow Crab in past studies to seismic in associated snow crab movement behavior, gene expression and physiology, we conclude that the observed differences owing to seismic surveying in our study design are likely a result of stochastic processes external to our manipulation.

1. Introduction

Marine industries continue to expand (e.g. shipping, fishing, oil and gas development among many others) and as a result oceans are becoming increasingly noisy (Hildebrand, 2009; Martin et al., 2019). The potential impact of marine noise is a growing concern, particularly for harvesters of commercial species. Unfortunately, there is a general absence of field data to evaluate these concerns, even for valuable invertebrate fisheries (Carroll et al., 2017; Popper and Hawkins, 2019).

Compounding the general lack of information of the effects of seismic surveys on marine species is the issue that the sound exposure associated with seismic surveys is very much context dependent (e.g. depth, bathymetry, bottom type, weather etc.) (Jensen et al., 2011,Matthews and MacGillivray, 2013). Additionally, industry can apply both 2D and 3D survey designs in which the former is typically designed for broad spatial coverage with widely-spaced transects (e.g. Morris et al., 2018) and the latter in a localized area with more closely-spaced transects to achieve high spatial resolution (Caldwell and Dragoset, 2000; Gisiner, 2016). Consequently, despite similar noise sources used in both survey approaches, exposure profiles to biota can be very different across time and space. Specifically, resident or low mobility animals within 3D survey grids will experience more sustained exposures (Hirst and Rodhouse, 2000), which could in turn result in effects that would not occur after exposure to 2D surveys.

In Atlantic Canada, invertebrates such as Snow Crab (Chionoecetes opilio) support the highest valued fisheries (http://www.dfo-mpo.gc.ca/stats/commercial/sea-maritimes-eng.htm). Harvesters in the Newfoundland and Labrador region of Canada believe that noise created during seismic surveying on offshore commercial fishing grounds negatively affects catch rates of Snow Crab despite results of field studies to the contrary (Morris et al., 2018). However, since existing research was evaluated using 2D surveys, there remains a possibility that the longer duration exposures from 3D surveys might illicit different or more pronounced behavioral responses that might affect catch rates. The objective of this study is to examine whether long duration/locally intense 3D seismic surveying alters commercial Snow Crab catch rates.

2. Methods

2.1. Study areas

This study was conducted opportunistically in association with two industry 3D seismic surveys that took place on important commercial Snow Crab fishing grounds along the continental slope edge of the

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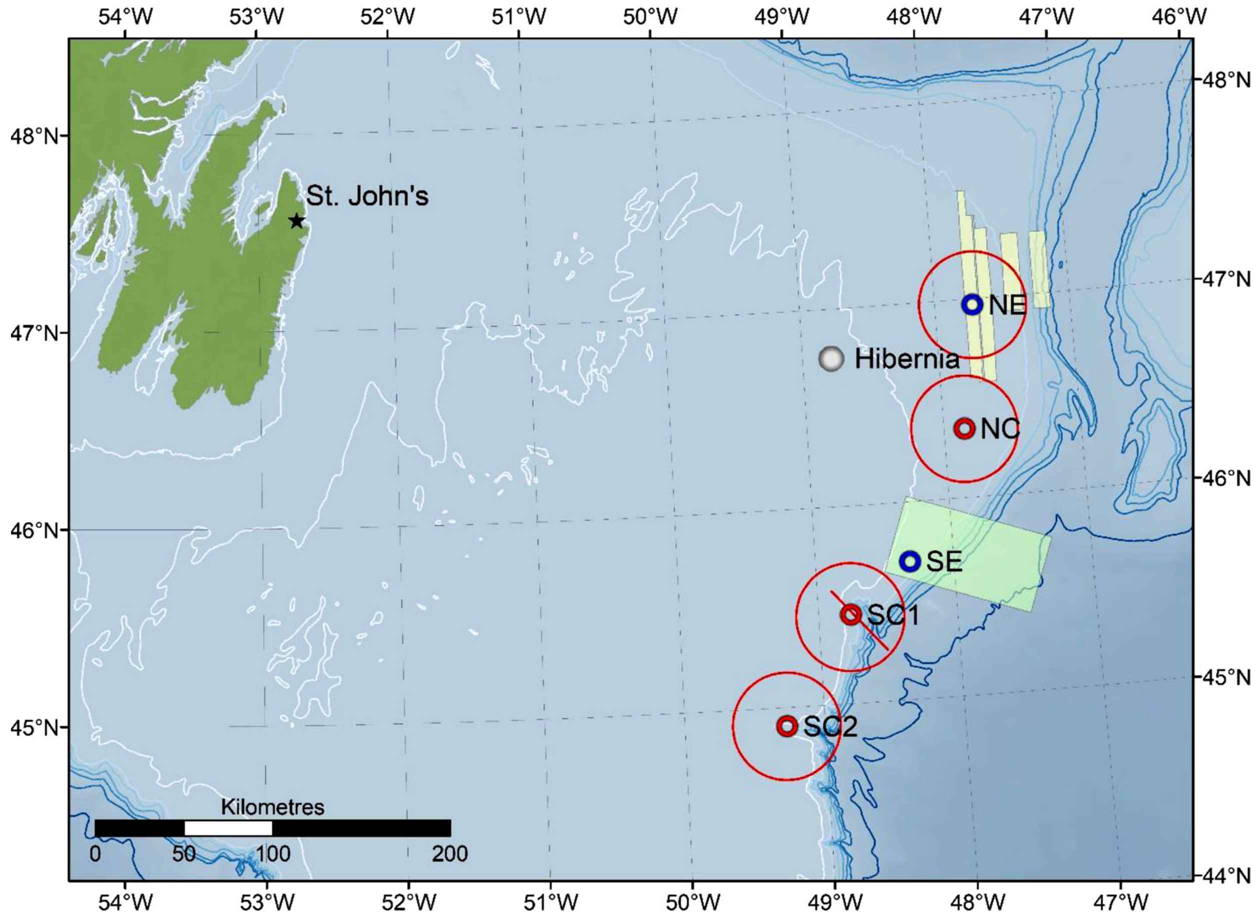

Fig. 1. Locations of the sorthern study area (including the Northern Experimental site (NE), the Northern Control site (NC)), and the southern study area (including the Southern Experimental site (SE) and the Southern Control sites (SC1 and SC2)). SC1 is also the location of the 2D seismic exposure. The 2D seismic survey line is indicated by the line passing across SC1. Red circles (30 km radius) indicate the distance beyond which siesmic noise would attenuate to a level that is less than a fishing vessel. . The 3D survey areas are located at the NE and SE areas, indicated by the light shaded rectangles. The location of the Hibernia oil platform is included for reference (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

Grand Bank off Newfoundland, Canada (Fig. 1). Across this area, large male Snow Crab are known to move tens to hundreds of kilometres and exhibit an ontogenetic movement to deeper water over the course of their lives (Mullowney et al., 2018). Commercial Snow Crab also exhibit different behavioral patterns over shorter time scales, potentially associated with feeding and foraging, however recent research suggests that these short-term movement patterns are not strongly affected by 2D seismic surveying (Cote et al., 2020).

Field experiments were conducted at our southern study area in 2017 and northern study area in 2018. Each area had a control and exposure site, and sites within each area were selected for similar bathymetric and oceanographic conditions. During the study period, water temperatures at our median fishing depth of 155 m was very similar in both 2017 and 2018 for all study sites; with mean temperatures at each site ranging from 0.2–1.2 ˚C (range: -0.4˚C to 2.4˚C) (CMEMS Global Reanalysis, http://navigator.oceansdata.ca, 2020).

The southern study area, assessed in 2017, included three sampling sites; two control sites (SC1 at Carson Canyon and SC2 at Lilly Canyon) and a 3D exposure site (SE $\sim$30 km north of SC1-Carson Canyon). The northern study area was assessed in 2018 and included a site (NE) that was exposed to 3D seismic and a single control site (NC). Control sites in each study year were placed at least 30 km from exposure sites to ensure that daily sound exposure levels from seismic sound did not exceed that of a fishing vessel. This sound threshold was selected based on the assumption that fishing vessel noise is not considered to be detrimental to Snow Crab catch rates by harvesters.

2.2. Seismic noise exposure

The 3D seismic exposures in both 2017 and 2018 were conduced from the *Ramform Titan* (2017) and *Ramform Stirling* (2018); industry vessels operated under similar seismic protocols, and using the same sound source specifications and array configuration. The energy source used by the vessels included a source volume of 4130 in^3, operated at 2000 psi, with a 25 m shot spacing ($\sim$10 s sec) towed at a depth of 7 m. In 2017 the seismic survey was conducted at SE from August 2 to October 4, and progressed from east to west, having parallel vessel transect survey lines spaced 800 m apart. During 2018, the survey at NE occurred from July 8–24 and from August 7–26, and also progressed from east to west, using transect lines spaced at 700 m. The east-west progression used in both surveys meant that sound exposure was intensifying in the experimental exposure areas during the survey.

In addition to 3D exposures, a single vessel pass (2D seismic exposure) was conduced by the seismic survey vessel *Atlantic Explorer* at site SC1 on September 12, 2017 to differentiate potential 2D and 3D exposure effects. This 2D exposure replicated seismic exposures described by Morris et al. (2018) and utilized the same vessel, seismic array (4880 in^3 seismic source array, -10 s shot interval), and vessel path as that study.

2.3. Acoustic measurements and soundscape

Acoustic recordings were taken for the duration of the study using Autonomous Multichannel Acoustic Recorders (AMARs, JASCO Applied

Sciences) located on the seabed. One AMAR was deployed at each study site, with the exception of SE, and were affixed to frames that held the hydrophones −0.6 m above the seafloor. Each AMAR was equipped with Geospectrum M36-V0−100 hydrophones with a nominal sensitivity of −200 dB re 1 V/µPa and sampled at 16 or 32 kHz. The recorder at SC1 in 2017 was directly below the 2D exposure track line (September 12, 2017), whereas the NE recorder in 2018 was 100 m from the track line on August 24, and 400 m from the track line on August 21, 2018. The recorders sampled a range of sound exposures over several weeks as seismic surveying approached from the east and as transect lines were surveyed in northerly and southerly directions. The daily sound exposure level (SEL) was used to quantify sound energy since this metric is generally regarded as the best predictor of hearing threshold shift as a result of long-term sound exposure on marine life (e.g. Popper et al., 2014; NMFS, 2018). The SEL is the arithmetic sum of each second's sound pressure level (SPL) in the frequency band 10−7000 Hz over each 24-h period (UTC). Seismic surveys in this study ranged from 0.1–152 km from a sound recorder and SEL was estimated for all relevant distances. For locally intense exposures to seismic survey sound, the daily SEL is also a relevant metric since the SEL accumulates almost entirely from the local exposure (Martin et al., 2019). Daily SEL also facilitated comparison between the long-term sound exposures over a day from a distant seismic survey and our baseline for normal noise levels - the local operation of a fishing vessel (Morris et al., 2018).

2.4. Snow crab catch rates

Commercial Snow Crab harvesters conducted all fishing operations for this experiment, using typical industry fishing methods and gear (i.e., crab traps). Sampling methods in this study followed that of Morris et al. (2018) who used an established fishing-industry based survey (Stansbury et al., 2013). Sampling included 10–20 long-line type fishing fleets per site, with each fleet consisting of 10 commercial Snow Crab traps along the line (45 m spacing). Each pot was baited with approximately 1.5 kg of squid and set for 12−18 hours. Commercial-sized Snow Crab (> 95 mm carapace width) were counted from all traps.

During 2017, experimental fishing was conducted at the southern area, and included the exposure site (SE) and two control areas (SC1 and SC2). Harvesting in this year started at each site six weeks after 3D seismic surveying was initiated in the region. Therefore no pre-seismic experimental sampling data were available in 2017. Consequently the experimental design was During-After-Control-Impact (DACI). The catch data collected "During" seismic represented crabs that were experiencing seismic exposures that increased as the vessel progressively approached the fishing location; fishing was conducted when the seismic survey was closest to the sampling location. Sampling was conducted again two weeks "After" all seismic exposure ended in the region. Concurrent Snow Crab sampling was also conducted at the southern control sites (SC1 and SC2).

During 2018, experimental fishing was conducted in the northern area. Sampling included four periods; before-seismic (July 5–7), after-distant seismic (15–40 km from sound source; August 4–7), immediately after close-proximity seismic (at least one seismic pass within 5 km each day August 23–26), and two weeks after all seismic surveying ended in the region (September 10−12). Catch data during these time periods were collected at both the exposure (NE) and control (NC) sites.

2.5. Commercial fisheries log book data comparison

A key assumption of BACI designs is that control and impact areas are similar in nature and thus control for environmental variables beyond the experimental manipulation. We evaluated this assumption by examining naturally occurring spatiotemporal variability in commercial catch rates (CPUE) for two Crab Management Areas (CMAs 3LEX and 3N200) in which our seismic experiments were located (see Mullowney et al., 2019 for details). Commercial fishing data did not coincide with our experimental data since the commercial harvesting was completed earlier in the season. To examine variability in commercial catch rates, data on catch and effort were binned to 5-day increments and CPUE medians were plotted for visual assessment. In both CMAs, excluding the fishery start (i.e. days 105−120) and end (i.e. days 195–215) periods, sample sizes of both catch and effort were consistently large ranging from 5−250 t and 1,000–20,000 trap hauls per 5-day time units in each CMA. Synchrony of CPUE trends across CMAs were examined using linear regression models of mean CPUE for each 5-day time bins. This analysis was done for each year.

Sampling in this study is also meant to represent the commercial fishing in the region. We evaluated this by comparing our experimental catch rates with commercial catch rates using fisheries log book data, and tested for differences in catch rate slopes across the two data series (fishery versus experimental) using the following linear mixed model (LMM, lme4 extension, R Core Development Team 2015).

$$\text{CPUEdiff} = \ln(\text{CPUE}) \sim \text{year*source} + \text{site:source} + \text{site} + 1|\text{site:year:source}$$

The model regressed the response variable of natural log-transformed CPUE against the main effects of year, site, and source (logbooks versus experimental) and the interactions of source with both year and site. Catch rate data from July were included in the analysis because it was temporally closest to our experimental sampling period. A random intercept of the interaction of site, year, and source was included. Significance across data series was interpreted by the relative magnitude of the effect size of the year*source interaction and model fit was assessed by visual assessment of the residuals.

2.6. Analysis of seismic effects on catch rates

This study incorporated a similar design, sampling methodology, and statistical analysis as that described by Morris et al. (2018). Generalized linear models using negative binomial error structures were applied to these count data. Mixed effects models and associated likelihood ratio tests were used to meet model assumptions related to sample independence and to assess differences of effect. The generalized linear mixed effects model (lme4 package in R 3.3.3; R Core Development Team 2015) used total counts of Snow Crab within a trap as the response variable, Temporal Period (Before/During/After in 2017 for 2D seismic; During/After Exposure in 2017 for 3D seismic; Before/During Distant Seismic/During Seismic/After Seismic in 2018 for 3D seismic) and Exposure Treatment (Control/Exposure) categorical variables as the fixed effects and the fleet of traps as a random effect. For the 3D seismic experiment in 2017, two control sites were monitored so a second random effect was fitted for the study area to account for potential dependencies of catch rates within the two control sites. Depth was also included as a continuous explanatory variable in the statistical model (Morris et al., 2018).

Thus the following three models were applied:

2.6.1. 2-D experiment

$$\text{Total Snow Crab} \sim \text{Exposure Treatment*Temporal Period} + \text{Depth} + (1|\text{Fleet ID})$$

2.6.2. 3-D experiment

$$\text{Total Snow Crab} \sim \text{Exposure Treatment*Temporal Period} + \text{Depth} + (1|\text{Fleet ID}) + (1|\text{Study Area})$$

2.6.3. 3 -D experiment

$$\text{Total Snow Crab} \sim \text{Exposure Treatment*Temporal Period} + \text{Depth} + (1|\text{Fleet ID})$$

For both years, the statistical interaction between spatial and temporal fixed effects was the key model term to isolate seismic-related

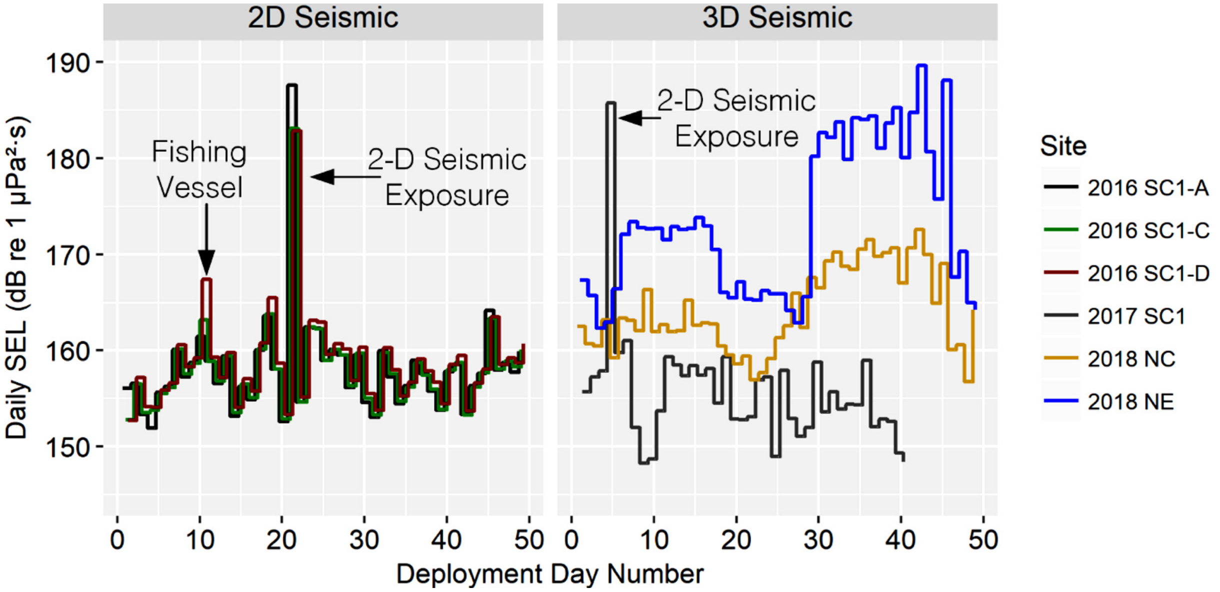

Fig. 2. Comparison of the daily SEL (10-7000 Hz) measured during 2D and 3D seismic surveys. 2D data from 2016 is from Morris et al. (2018) and is included here for comparison. The 2017 SC1 site was exposed to the experimental 2D seismic survey on 12 Sep 17 (day 4 of the 3D Seismic Panel), as well as a 3D seismic survey to the North-East that had a closest point of approach to SC1 of −38 km when the recorders were present (see Fig. 1). No seismic vessels approached closer than 70 km to the 2016 SC1 sites except the 2D experimental exposure on 22 Sept (day 21 in the 2D Seismic panel). A fishing vessel that was not part of the experiment passed almost directly over recorder SC1-D on 12 Sep 16 (day 11 in the 2D Seismic Panel).

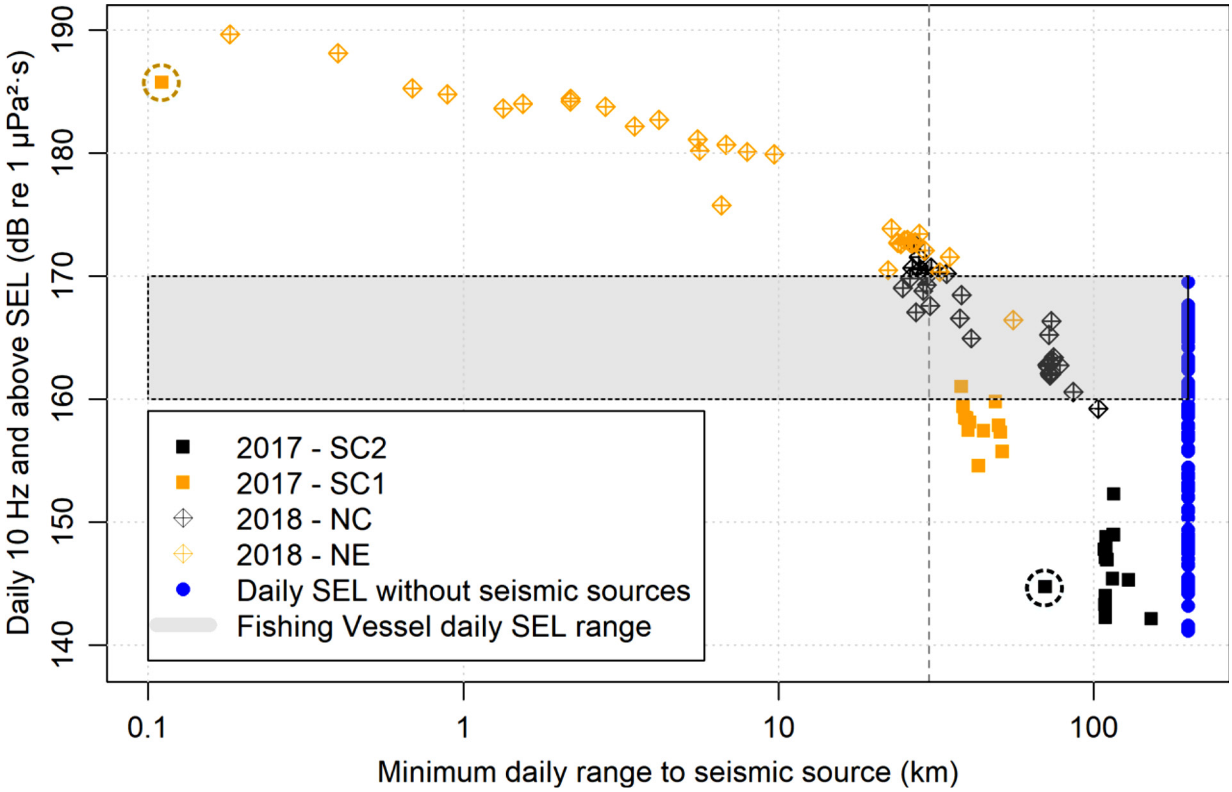

Fig. 3. Daily SEL vs minimum range to the seismic source. The vertical dashed line is drawn at 30 km range. The gray box indicates the values of daily SEL expected at the seabed when a fishing vessel is operating near a recorder (0-1 km distances). The dashed circles around one of the 2017 SC1 and SC2 markers indicate the levels associated with the 2D seismic survey. The remainder are associated with the 2017 3D seismic survey. The 2017 SC1 2D data point is lower than the actual daily SEL because the hydrophone was overloaded when the source was directly over the recorder.

effects within this Before-After-Control-Impact (or During-After-Control-Impact) study design. Accordingly, each year's full model was compared (Chi-square test) to a reduced model that excluded the interaction term, to determine if the model performance was significantly degraded by the exclusion of the interaction term. Model assumptions were evaluated by examining residuals.

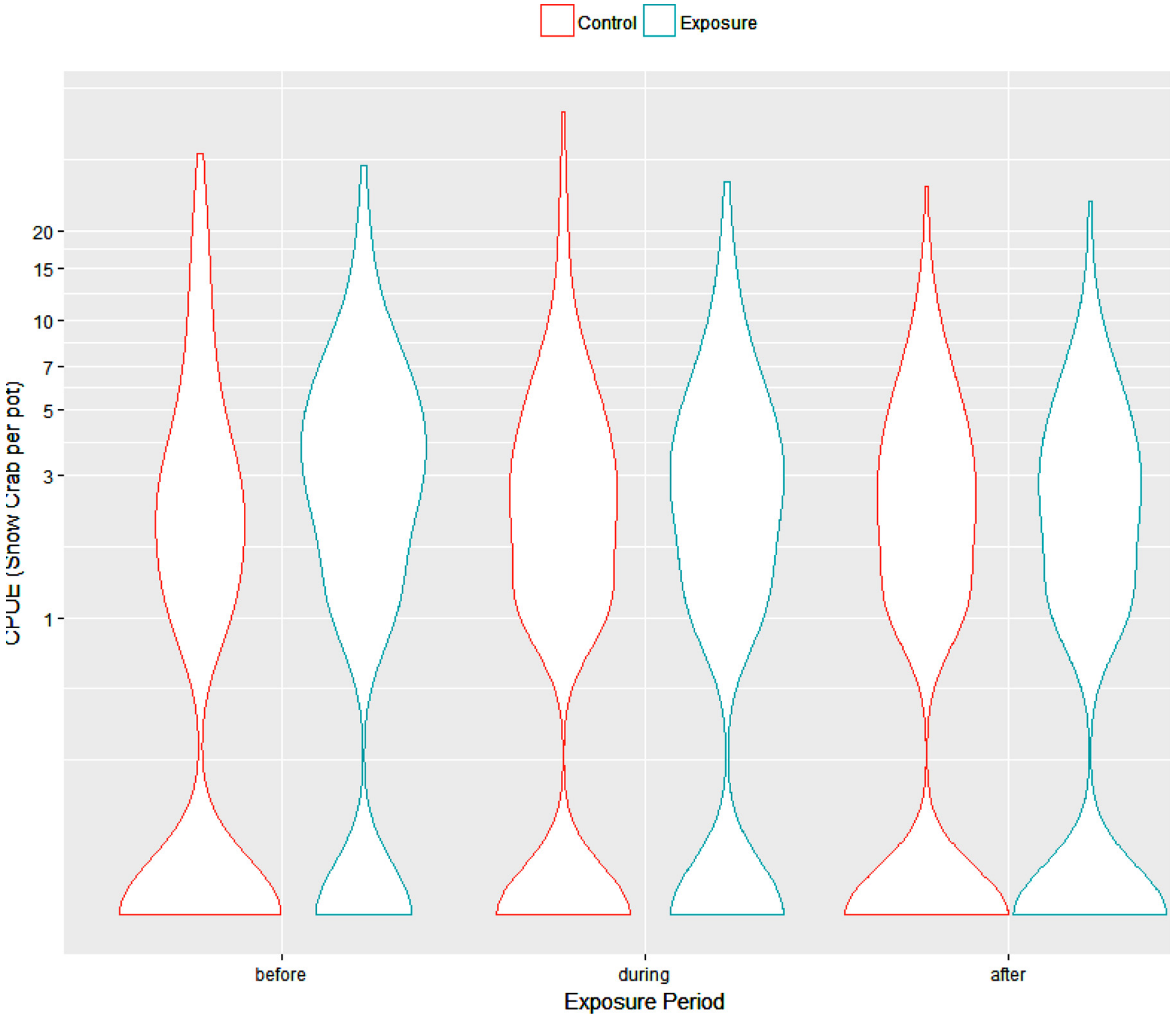

Fig. 4. Catch rates (CPUE) of snow crab Before Seismic, During 2D Seismic and After Seismic at control and exposure sites on the Grand Banks of 2017. Violin plots represent frequency distributions of catch rates across pots. Catch rates did not differ across exposure or temporal treatments.

Table 1
Sampling study design, 3D seismic exposure periods, fishing periods and average catch per pot during 2017 and 2018.

Year	Area	Study design	Site	Seismic period	Fishing dates	Avg. CPUE
2017	Southern	During seismic	Control 1 (SC1)	Sept 12 (2D-7.5 h)	Sept 9 – 10 and 12 – 13	3.8
			Control 2 (SC2)		Sept 7 – 9	6.3
			Experimental (SE)	Aug 2-Oct 4	Sept 14 – 15	1.5
		After seismic	Control 1 (SC1)		Oct 16 – 18	2.9
			Control 2 (SC 2)		Oct 19 – 20	3.8
			Experimental (SE)		Oct 20 – 21	2.9
2018	Northern	Before seismic	Northern Experiment (NE)		Jul 5 – 6	0.6
			Northern Control (NC)		Jul 7 – 8	0.8
		During seismic (15 – 40 km)	Northern Experiment (NE)	Jul 8-Jul 30	Aug 4 – 5	1.5
			Northern Control (NC)		Aug 6 – 7	0.8
		During seismic (0–5 km)	Northern Experiment (NE)	Aug 8 – 24	Aug 25 – 26	1.8
			Northern Control (NC)		Aug 23 – 24	0.5
		After seismic	Northern Experiment (NE)		Sept 11 – 12	0.8
			Northern Control (NC)		Sept 9 – 10	0.6

3. Results

3.1. Sound exposures

Sound levels in the ocean, in the absence of human generated sound, depends largely on the wind speed, which controls wave height and by extension the sound generated by breaking waves (Carey and Evans, 2011). Changes in wind speed resulted in daily SEL in the range of 145 – 165 dB re 1 μPa^2·s that are within − 1 dB of each other when measured in the same area (e.g. sites SC1-A, SC1-C and SC1-D in the 2D Seismic panel of Fig. 2). Human activities add sound sources to the environment that can raise the daily SEL in a location-specific manner;

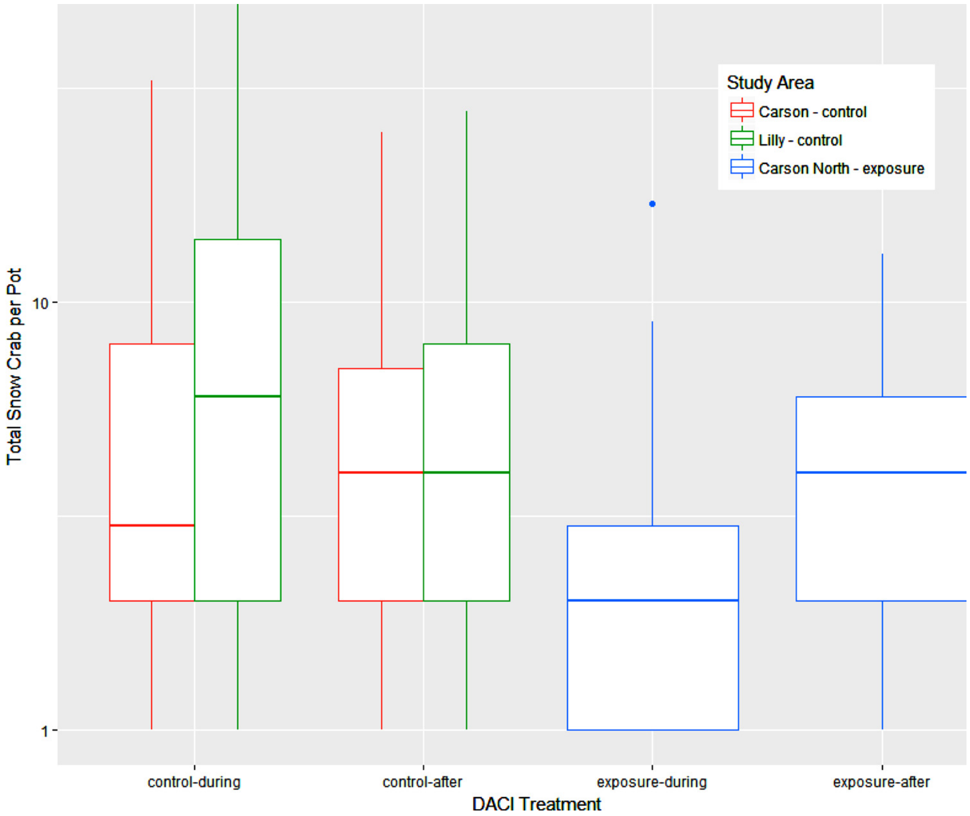

Fig. 5. Catch rates (CPUE) of snow crab during 2017, at the southern area control sites (30 and 100 km away from 3D survey) and 3D seismic survey exposure site. Sampling was conducted during 3D seismic survey exposure period and 2 weeks after the seismic survey was completed.

for example the daily SEL differed by 5 − 8 dB at sound recorder-sites SC1-A, -C and -D depending on whether the seismic source or the fishing vessel passed close to the recorder (for more on the effects of distance to sources on daily SEL see Morris et al. (2018) or Martin et al. (2019)). 2D seismic surveys increased the daily SEL by ∼30 dB in the project area only on the day that the seismic vessel passed over the site. 3D seismic surveys, which remain in an area for weeks to months, increase the daily SEL for the survey period (Fig. 2, 3D Seismic panel). Within the survey period however, the received sound level near a 3D seismic survey increases and decreases as the vessel approaches and then departs an area (e.g. Fig. 4 of Martin et al., 2017). The minimum distance from the survey is the primary determinant of the daily SEL (e.g. NC compared to NE), however, multiple passes in one 24 -h period can also increase the daily SEL compared to a 2D survey.

Daily SEL is very dependant on the distance from the seismic source (Fig. 3). For example, the daily SELs from 2017 at SC1 were ∼10 dB lower than those from 2018 at NC when the seismic source was at the same distance from the recorder (Fig. 3). This was due to the effects of increased attenuation at the 2017 seismic survey location, where the sound from the source travelled upslope from deep water, compared to the 2018 seismic survey conducted on the shelf (Fig. 1; see Jensen et al. (2011) for a discussion of upslope propagation). The use of SEL as the sound metric allowed comparison between the exposure to seismic surveys and a fishing vessel (Fig. 3). At a distance of 30 km from the seismic survey, 40 of 43 daily SEL results associated with the 3D seismic surveys were below the daily SEL of a fishing vessel, and all seismic SEL exposures at a distance of more than 38 km from the survey were below the fishing vessel daily SEL.

3.2. 2017 2-D seismic study

The interaction between exposure treatment and time period was not significant (Chi-square$_{d.f.=2}$ = 3.25; P = 0.197) indicating that 2D seismic exposure did not influence catch rates of Snow Crab (see Fig. 4). Water depth, however was a significant factor in explaining catch rate variability (Chi-square$_{d.f.=1}$ = 88.8; P < 0.001).

3.3. 2017 3-D seismic study

The DACI trial in 2017 revealed a significant interaction between exposure site and exposure period (Chi-square$_{d.f.=1}$ = 6.87; P = 0.009), indicating the temporal response to seismic exposure differed across control and exposure treatments. Specifically, CPUE of Snow Crab was greater two weeks After Seismic surveying exposure than it was During Seismic surveying exposure at the Exposure Site (Table 1; Fig. 5).

3.4. 2018 3-D seismic study

In 2018, the full model that included the interaction term between exposure period and site was significantly better than the reduced model that excluded the interaction term (Chi-square$_{d.f.=3}$ = 17; P = 0.001). The importance of the interaction term signified an apparent effect of seismic. Model results indicate that the catch rates at the control site during 3D exposure did not differ from any other time period (P > 0.05 for all comparisons; Table 1; Fig. 6). In contrast, the catch rates During 3D exposure at the Exposure site was significantly higher than both the Before 3D Seismic (P < 0.001) and After 3D Seismic (P < 0.001) treatments but not the After Distant 3D Seismic treatment (P = 0.145; Table 1, Fig. 6).

3.5. Commercial and experimental catch rates

There was little difference in the CPUE trend over the time series between the commercial fishery and our experimental fishing (Fig. 7). The commercial fishery catch rates declined from a high in 2015,

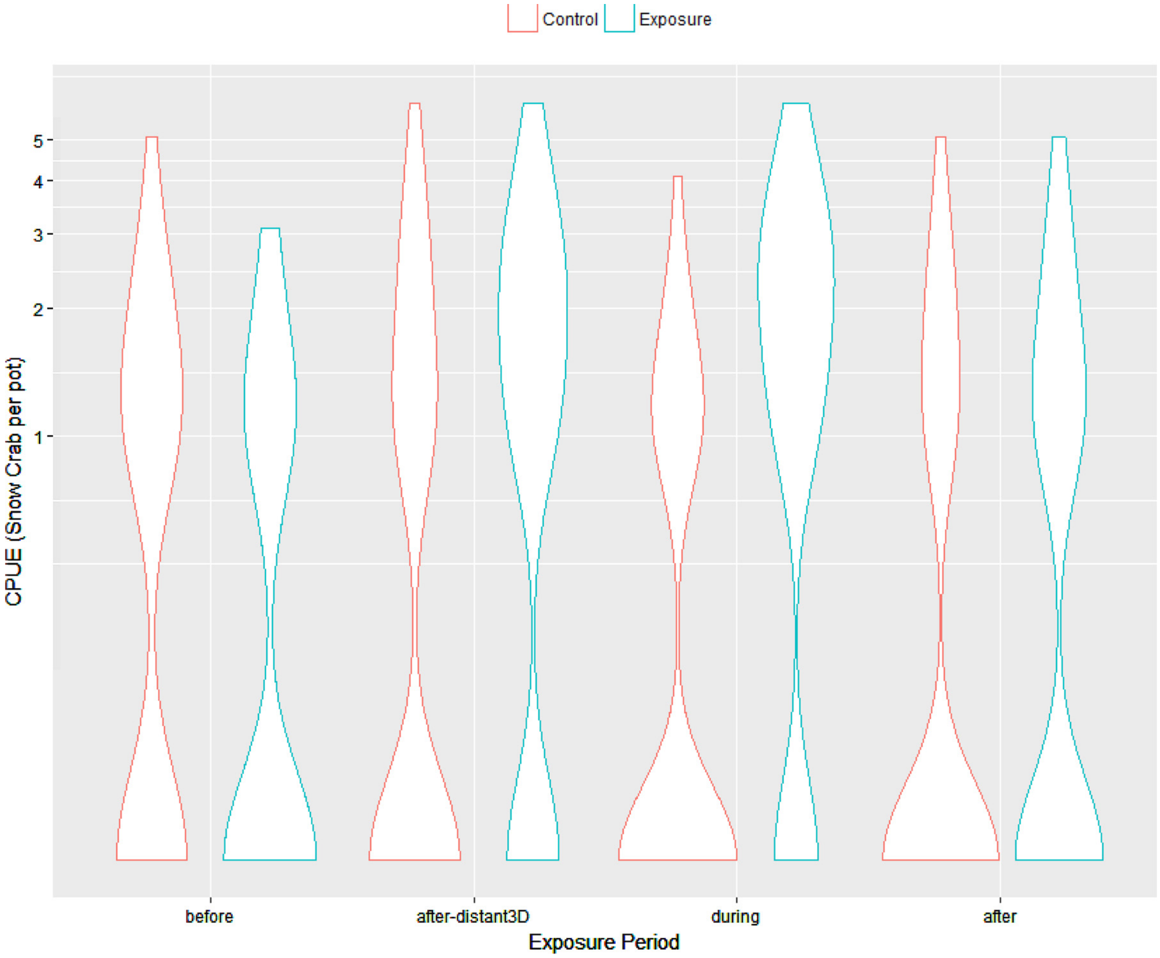

Fig. 6. Catch rates (CPUE) of snow crab during 2018 at the northern areas control site and seismic exposure site. Sampling included Before Seismic, immediately after Distant 3D Seismic, During close proximity 3D Seismic, and 2 weeks After Seismic surveying was completed. Violin plots represent frequency distributions of catch rates across pots. Control Site catch rates did not differ across temporal periods but Exposure Site catch rates During Seismic were significantly higher than Before or After periods.

generally averaging about 5 − 12 kg/trap across sites, to a low in 2017–2018, ranging from about 2 − 5 kg/trap on average. The LMM analysis did not detect a significant effect size of the year:source interaction (t = 0.626), confirming no difference in the slopes of catch rate trends over the time series between the two survey series.

Like the seismic exposure experiments, catch rates in the commercial fishery were variable over short time periods, in different years, and in different fishing areas (Fig. 8). In 2017, we measured a 95 % reduction in catch During seismic compared to two weeks After seismic. In 2018 with pre-seismic data, we measured an increase of 204 % after several weeks of seismic exposure, then a decrease to 43 % of the pre-seismic baseline two weeks after seismic surveying ended. Average commercial snow crab catch rates in the region conducted before any seismic surveying started, in both 2017 and 2018, showed similarly large variation in catch over short time periods (Fig. 8), for example, changing as much as 116 % and 236 % from one five day period to next in 2017 and 2018 respectively. There was also little congruence in temporal trends across commercial fishing areas (Fig. 8).

4. Discussion

Data analysis in this study show that catch rates of Snow Crab were altered upon exposure to 3D seismic, which was in contrast to observations associated with 2D seismic exposure (this paper, Morris et al., 2018). However, the direction of the effect was unpredictable; with lower catch rates observed one year and higher the next. In a multi-year 2D seismic study conducted at the same locations used in our southern study area, Morris et al. (2018) were unable to detect effects of 2D seismic on catch rates of Snow Crab over time periods that ranged from days to weeks. While high variability in catch rates limited statistical power in their study, catch rates were observed to change across time and space suggesting that if effects did exist they were smaller than natural fluctuations.

General responses of invertebrates to seismic exposure vary across studies, ranging from no effect to quite severe impacts (Andriguetto-Filho et al., 2005; Day et al., 2019; Carroll et al., 2017). In part these differing conclusions can result from species-specific sensitivities (Løkkeborg et al., 2012), environmental conditions (Przeslawski et al., 2018), study-design issues (McGaw and Nancollis, 2018; Hawkins and Popper, 2017; Popper and Hawkins, 2018), and poor ecological understanding regarding impacts of noise on invertebrates.

Experimental differences in sound exposures across the years of study do not seem a likely explanation for the divergent trends observed in this study. Indeed, the lower intensity sound exposures in 2017 were associated with the reduced catch rates. Generally however, several weeks of industrial seismic surveying at each of our study areas represents the upper-limits of sound exposure expected from realistic oil and gas exploration, and both surveys incorporated wide variation in sound exposure, that might impact Snow Crab. Alternatively, Snow Crab responses to seismic may be complex and modified by external environmental conditions. Unfortunately, our ability to reconcile seemingly contradictory catch rate results across years is hampered by

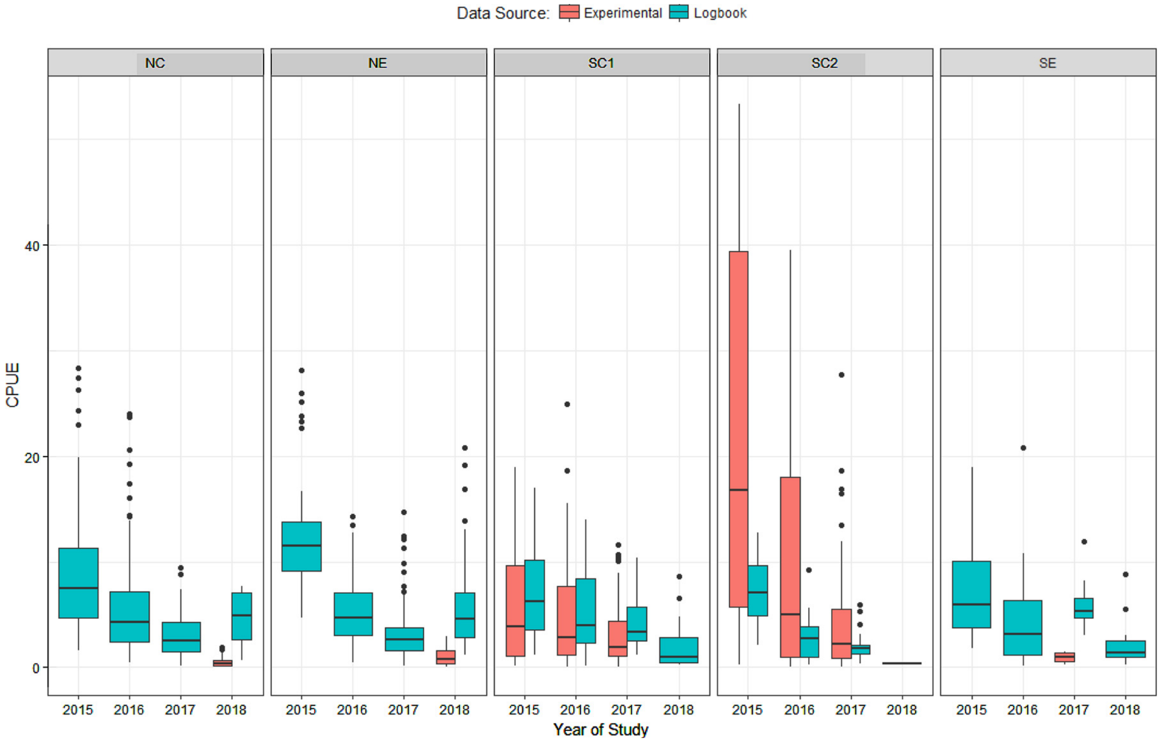

Fig. 7. Experimental and Commercial Snow Crab Catch Per unit Effort data. Commercial data was collected by at-sea fishery observers aboard commercial fishing vessel during the fishing season (July) in the vicinity (within 20 km) of our study area.

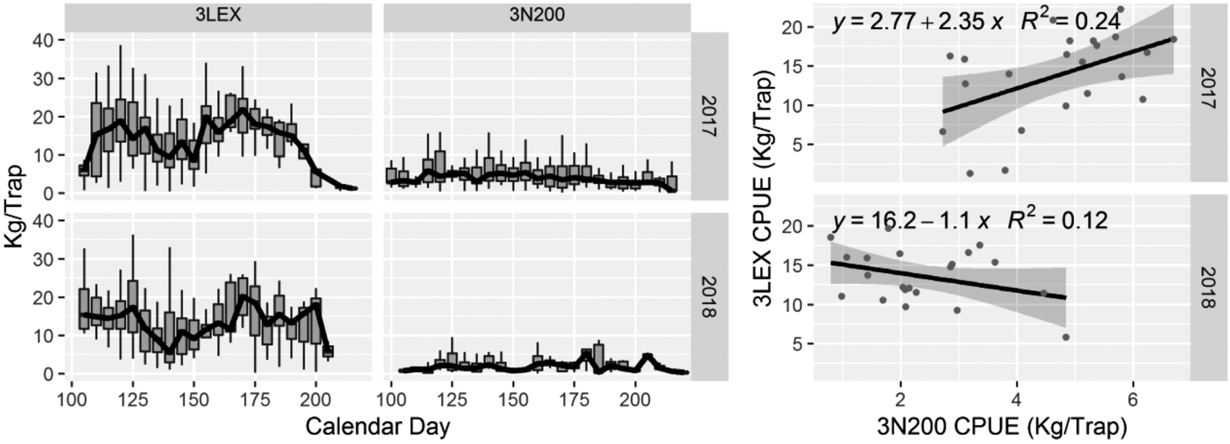

Fig. 8. Commercial Snow Crab catch rates in 2017 and 2018, before seismic surveying, for two management areas in which our experiments were conduced. Median catch rates by the commercial Snow Crab fleet is represented in 5 day intervals, each interval included 5-250 t of caught-crab and 1000-20,000 trap hauls. Areas with high (3LEX) and low (3N200) catch rates are represented.

limited knowledge of Snow Crab behavior. Although this knowledge has increased in recent years, behavior of this species is not well explained by typically important environmental variables such as light, temperature and water velocity (Cote et al., 2019, 2020). Snow Crab fisheries data indicate however, that our study region is in flux with Snow Crab abundance experiencing a pronounced multi-year decline. The stock has shown strong responses to both climatic forcing and chronically heavy exploitation (Mullowney et al., 2014; Mullowney et al., 2019). Total mortality estimates in commercial-size male crab routinely range from 50 to 90% per year in the absence of seismic interference (Baker et al., 2019). While seismic activity has not been implicated as the primary driver in these declines (Mullowney et al., 2014; Mullowney et al., 2020), such conditions leave the possibility of density-mediated responses to seismic exposure. The mechanism behind such a scenario is not obvious, however.

Since we cannot readily explain divergent responses in catch with respect to seismic survey exposure across years, we also accept the possibility that these results may have arose due to external drivers on catch rates (environmental or stochastic) that are unrelated to seismic exposure. While there has been a decline in Snow Crab abundance over time, as we showed in our examination of commercial fishing throughout the season, Snow Crab catch rates can be highly variable in nature, over small spatial and temporal scales similar to that measured in response to seismic exposure in this study. While BACI study designs are considered more robust than simple before-after designs, they still have limitations within such variable study systems, since controlling for environmental factors outside of the experimental manipulation may not be possible (Underwood, 1992). One solution to overcome such issues is to expand the replication of control sites to account for broad level variability (Underwood, 1992). We did this to some degree by including two control sites in 2017 but further replication is likely infeasible across the large spatial scales and given the complex logistics required for research incorporating realistic seismic oil and gas surveys over multiple years. Instead we suggest it is more prudent to utilize multiple lines of evidence in addition to catch; exploring behavioral and physiological mechanisms that might help to explain catchability changes under field conditions, and by supporting such field studies with controlled lab studies.

Like this study, the assessment of 2D seismic surveys on catch rates was challenged by high natural variability in catch rates (Morris et al., 2018). However, multiple lines of evidence were used to support the assessment of 2D seismic effects on Snow Crab, including animal movement behavior (Cote et al., 2020), physiology (Hanlon et al., 2020) and genomics (Hall et al., 2020), that indicated a similar result; i.e. any observed effects were subtle. Some of these results are instructive to the interpretation of our 3D survey results. For example, Snow Crab exposed to 2D surveys did not show strong behavioural responses, particularly when compared to other environmental variables (Cote et al., 2020). Since sound sources used in 2D and 3D surveys are similar, behavior would also not be expected to change after short-term exposure to 3D surveys. Any potential change in behavior would have to arise from the prolonged exposure associated with 3D surveys. However, the prolonged exposures of Snow Crab to seismic noise in the laboratory (Hanlon et al., 2020) did not result in physiological or morphological responses even though the exposures were considered unrealistically high (Hanlon et al., 2020). Collectively, these studies lend support to our supposition that the observed effects of seismic surveying on catch rates were driven by spatiotemporal variation external to the seismic exposures. Nevertheless, we cannot rule out the potential for 3D seismic surveying to affect commercial Snow Crab catch rates. If 3D seismic does indeed have an impact, the effect remains unpredictable, both in magnitude and direction, and occurs at modest temporal (i.e. within a 2 week period) and spatial scales (< 30 km radius).

CRediT authorship contribution statement

Corey J. Morris: Conceptualization, Methodology, Investigation, Validation, Resources, Writing - review & editing, Supervision, Project administration, Funding acquisition. **David Cote:** Investigtaion, Methodology, Formal analysis, Validation, Visualization, Writing - original draft, Writing - review & editing. **S. Bruce Martin:** Conceptualization, Methodology, Formal analysis, Validation, Software, Writing - review & editing. **Darrell Mullowney:** Investigation, Formal analysis, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

[NMFS] National Marine Fisheries Service, 2018. 2018 Revision to: Technical Guidance for Assessing the Effects of Anthropogenic Sound on Marine Mammal Hearing (Version 2.0): Underwater Thresholds for Onset of Permanent and Temporary Threshold Shifts. U.S. Department of Commerce, NOAA. NOAA Technical Memorandum NMFS-OPR-59, pp. 167. https://www.fisheries.noaa.gov/webdam/download/75962998.

Andriguetto-Filho, J.M., Ostrensky, A., Pie, M.R., Silva, U.A., Boeger, W.A., 2005. Evaluating the impact of seismic prospecting on artisanal shrimp fisheries. Cont. Shelf Res. 25, 1720–1727. https://doi.org/10.1016/j.csr.2005.05.003.

Baker, K., Mullowney, D., Pedersen, E., Coffey, W., Cyr, F., Belanger, D., 2019. An assessment of Newfoundland and labrador snow crab (Chionoecetes opilio) in 2018. DFO Can. Sci. Advis. Sec. Res. Doc In press XXXX.

Caldwell, Jack, Dragoset, William, 2000. A brief overview of seismic air-gun arrays". Lead. Edge 19 (8), 898–902. https://doi.org/10.1190/1.1438744.

Carey, W.M., Evans, R.B., 2011. Ocean Ambient Noise. Measurement and Theory. The Underwater Acoustics Series. Springer, New York, pp. 263. https://doi.org/10.1007/978-1-4419-7832-5. ISBN 978-1-4419-7831-8.

Carroll, A.G., Przeslawski, R., Duncan, A., Gunning, M., Bruce, B., 2017. A critical review of the potential impacts of marine seismic surveys on fish & invertebrates. Mar. Pol. Bul. 114, 9–24. https://doi.org/10.1016/j.marpolbul.2016.11.038.

Cote, D., Nicolas, J.-M., Whoriskey, F., Cook, A.M., Broome, J., Regular, P.M., Baker, D., 2019. Characterizing Snow Crab (*Chionoecetes opilio*) movements in the Sydney Bight (Nova Scotia, Canada): a collaborative approach using multiscale acoustic telemetry. Can. J. Fish. Aquat. Sci. 76, 334–346. https://doi.org/10.1139/cjfas-2017-0472.

Cote, D., Morris, C.J., Regular, P.M., Piersiak, M., 2020. Effects of 2D seismic on Snow Crab movement behaviour. This issue. Fish. Res.

Day, R.D., McCauley, R.D., Fitzgibbon, Q.P., Hartmann, K., Semmens, J.M., 2019. Seismic air guns damage rock lobster mechanosensory organs and impair righting reflex. Proc. R. Soc. B. https://doi.org/10.1098/repb.2019.1424.

Gisiner, R.C., 2016. Sound and marine seismic surveys. Acoust. Today 12 (4), 10–18. https://acousticstoday.org/wp-content/uploads/2016/12/Seismic-Surveys.pdf.

Hall, J.R., Lehnert, S.J., Gonzalez, E., Kumar, S., Bradbury, B., Hanlon, J., Morris, C.J., Rise, M.L., 2020. Snow crab (*Chionoecetes opilio*) hepatopancreas transcriptome: identification and testing of candidate molecular biomarkers of seismic survey impact. This Issue. Fish. Res.

Hanlon, J., Morris, C.J., Cote, D., Perez-Casanova, J.C., Xu, J., Han, V., Payne, J.F., 2020. Effect of chronic exposure to a seismic recording on mortality, feeding, condition, statocyst hair cells and selected biochemical indices in snow crab (Chionoecetes opilio). This issue. Fish. Res.

Hawkins, A.D., Popper, A., 2017. A sound approach to assessing the impact of underwater noise on marine fishes and invertebrates. ICES J. Mar. Sci. 74, 635–651. https://doi.org/10.1093/icesjms/fsw205.

Hildebrand, J.A., 2009. Anthropogenic and natural sources of ambient noise in the ocean. Mar. Ecol. Prog. Ser. 395, 5–20. https://doi.org/10.3354/meps08353.

Hirst, A.G., Rodhouse, P.G., 2000. Impacts of geophysical seismic surveying on fishing success. Rev. Fish Biol. Fish. 10, 113–118. https://doi.org/10.1023/A:1008987014736.

Jensen, F.B., Kuperman, W.A., Porter, M.B., Schmidt, H., 2011. Computational Ocean acoustics. AIP Series in Modern Acoustics and Signal Processing, 2nd edition. AIP Press - Springer, New York, pp. 794. https://doi.org/10.1007/978-1-4419-8678-8.

Løkkeborg, S., Ona, E., Vold, A., Salthaug, A., 2012. Sounds from seismic air guns: gear and species-specific effects on catch rates and fish distribution. Can. J. Fish. Aquat. Sci. 69, 1278–1291. https://doi.org/10.1139/f2012-059.

Martin, S.B., Matthews, M.-N.R., MacDonnell, J.T., Bröker, K., 2017. Characteristics of seismic survey pulses and the ambient soundscape in Baffin Bay and Melville Bay, West Greenland. J. Acoust. Soc.Am. 142 (6), 3331–3346. https://doi.org/10.1121/1.5014049.

Martin, B., Morris, C., Broker, K., O'Neill, C., 2019. Sound exposure level as a metric for analyzing and managing underwater soundscapes. J. Acoust. Soc.Am. 146 (1). https://doi.org/10.1121/1.5113578.

Matthews, M.-N.R., MacGillivray, A.O., 2013. Comparing modeled and measured sound levels from a seismic survey in the Canadian Beaufort Sea. Proc. Meet. Acoust. 19 (1), 1–8. https://doi.org/10.1121/1.4800553.

McGaw, I.J., Nancollis, S.J., 2018. Experimental setup influences the cardiovascular responses of decapod crustaceans to environmental change. Can. J. Zool. 96, 1043–1052. https://doi.org/10.1139/cjz-2017-0252.

Morris, C.J., Cote, D., Martin, B., Kehler, D., 2018. Effects of 2D seismic on the snow crab fishery. Fish. Res. 197, 67–77. https://doi.org/10.1016/j.fishres.2017.09.012.

Mullowney, D.R.J., Dawe, E.G., Colbourne, E.B., Rose, G.A., 2014. A review of factors contributing to the decline of Newfoundland and Labrador snow crab (*Chionoecetes opilio*). Rev. Fish Biol. Fish. 24, 639. https://doi.org/10.1007/s11160-014-9349-7.

Mullowney, D., Morris, C.J., Dawe, E., Zagorsky, I., Svetlana, G., 2018. Dynamics of snow crab (Chionoecetes opilio) movement and migration along the Newfoundland and Labrador and Eastern Barents Sea continental shelves. Rev. Fish Biol. Fish. https://doi.org/10.1007/s11160-017-9513-y.

Mullowney, D., Baker, K., Coffey, W., Pedersen, E., Colbourne, E., Koen-Alonso, M., Wells, N., 2019. As assessment of Newfoundland and Labrador Snow Crab (Chionoecetes opilio) in 2017. DFO Can. Sci. Advis. Sec. Res. Doc 2019/003.

Mullowney, D., Baker, K., Petersen, E., 2020. Harvesting Strategies during a forecasted decline in the Newfoundland and Labrador Snow Crab fishery. this issue. Fish. Res This issue.

Popper, A.N., Hawkins, A.D., 2018. The importance of particle motion to fishes and invertebrates Physical aspects of swimbladder function. J. Acoust. Soc.Am. 143 (1), 470–488. https://doi.org/10.1121/1.5021594.

Popper, A.N., Hawkins, A.D., 2019. An overview of fish bioacoustics and the impacts of anthropogenic sounds on fishes. J. Fish Biol. 2019, 1–22. https://doi.org/10.1111/jfb.13948.

Popper, A.N., Hawkins, A.D., Fay, R.R., Mann, D.A., Bartol, S., Carlson, T.J., Coombs, S., Ellison, W.T., Gentry, R.L., et al., 2014. Sound Exposure Guidelines for Fishes and Sea Turtles: a Technical Report Prepared by ANSI-Accredited Standards Committee S3/SC1 and Registered With ANSI. ASA S3/SC1.4 TR-2014. Springer Briefs in Oceanography. ASA Press and Springerhttps://doi.org/10.1007/978-3-319-06659-2.

Przeslawski, R., Huang, Z., Anderson, J., Carroll, A.G., Edmunds, M., Hurt, L., Williams, S., 2018. Multiple field-based methods to assess the potential impacts of seismic surveys on scallops. Mar. Poll. Bull. 129, 750–761. https://doi.org/10.1016/j.marpolbul.2017.10.066.

Stansbury, D.E., Fiander, D., Maddock-Parsons, D., 2013. Summary of the industry-DFO Collaborative Post-season Trap Surveys for Snow Crab in Div. 2J3KLOPs4R. http://ffaw.nf.ca/sites/ffaw.nf.ca/files/3PS%20SNOW%20CRAB%20SURVEY%20SUMMARY%202013.pdf/.

Underwood, A.J., 1992. Beyond BACI: the detection of environmental impacts on populations in the real, but variable, world. J. Exp. Mar. Biol. Ecol. 161 (2), 145–178. https://doi.org/10.1016/0022-0981(92)90094-Q.

APPENDIX 4 – Effects of 2D Seismic on Snow Crab Movement Behavior

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Effects of 2D Seismic on Snow Crab Movement Behavior

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ABSTRACT

Sound is an environmental feature that is used by a variety of marine taxa for feeding, reproduction, navigation and predator avoidance. Consequently, alterations to the soundscape have the potential to alter an individual's behaviour, physiology and ultimately fitness. Furthermore, such responses have the potential to negatively influence commercial fishing interests. In Atlantic Canada, snow crab fishing and hydrocarbon extraction are billion dollar industries. Snow crab harvesters contend that seismic noise from hydrocarbon exploration has strong negative effects on catch rates; an issue that is likely to become more acute given that the species is currently in decline. As part of a collaborative, multi-disciplinary study conducted offshore on the continental slope, positioning telemetry was used from 2015-2017 in a Before-After-Control-Impact (BACI) study design to assess the behavioral responses of snow crab to exposure from industry seismic vessels. While effects of seismic exposure on snow crab movement could not be ruled out completely, effects were at most quite small relative to natural variation. In contrast, snow crab exhibited much clearer responses to handling, temperature and time of day. Overall, our results suggest that seismic effects, specific to the behavior of adult male snow crab, are at most subtle and are not likely to be a prominent threat to the fishery.

1. Introduction

There has been an increasing recognition of importance of sound and the impacts of noise to marine animals (Carroll et al. 2016; Cox et al. 2016; Edmonds et al. 2016; Tifau and Briffa 2016; Putland et al. 2018) and the ecosystems (e.g. Solan et al. 2016) and industries they support (Skalski et al. 1992; Løkkeborg and Soldal 1993; Engås et al. 1996; Slotte et al. 2004; Day et al. 2017; Fitzbibbon et al., 2017). An acute and widespread source of noise pollution is caused by the seismic surveys conducted during petroleum industry exploration activities to identify oil and gas reserves (Vilardo and Barbosa 2018). While studies assessing the effects of seismic surveys on marine biota have been ongoing for decades, few have assessed fish and invertebrate species in ecologically realistic situations while incorporating noise exposure from actual seismic-industry operations (Hawkins et al. 2015; Cox et al. 2016; Tifau and Briffa 2016; Edmonds et al. 2016). Nevertheless, fish and invertebrates have shown some sensitivity to noise pollution; exhibiting responses that are lethal (Day et al. 2017; McCauley et al. 2017), physiological (Payne et al. 2008; Day et al. 2017; Fitzbibbon et al., 2017), behavioral (Day et al. 2017; Bruce et al. 2018), and developmental in nature (Pine et al. 2012; Simpson et al. 2016), and as a result the impacts are difficult to accurately predict. Often, however, field results are mixed, complex and nuanced, frequently differing across species, life-stages and ecological circumstances (Peña et al. 2013; Carroll et al. 2016; Solan et al. 2016; Bruce et al. 2018). Such complexity might be expected given that noise can affect multiple biological systems both directly and indirectly (Kight and Swaddle 2011). It also means that investigations of seismic-effects will often require a multi-faceted approach (Kight and Swaddle 2011; Przeslawski et al., 2018a), in part because ecological reality and controlling sources of external variation are both necessary yet difficult to achieve within a single study (Przeslawski et al. 2018b).

Like many areas of the world, the marine areas off of Newfoundland and Labrador, Canada have been extensively surveyed by seismic vessels that share the marine environment with fish harvesters. The interaction between the two industries is not without contention as fish harvesters have concerns that seismic surveys have negative consequences to the sustainability and profitability of their industry. The most valuable fishery off Newfoundland and Labrador is for snow crab (*Chionoecetes opilio*), with a landed value of more than $325 million in 2017 (see Integrated Fisheries Management Plan for detailed fishery information: https://www.dfo-mpo.gc.ca/fisheries-peches/ifmp-gmp/snow-crab-neige/2019/index-eng.html, accessed online July 15, 2019). The species is currently experiencing severe declines in abundance (Mullowney et al. 2014) over a time that seismic operations are expanding (https://www.cnlopb.ca/information/geoscience/ accessed

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15 July 15, 2019). Catch rate studies (Morris et al. 2018; Morris et al., 2020Morris et al., this issue), using industry seismic and fishing vessels, have been unable to detect consistent or obvious short or medium-term effects of seismic exposure compared to pre-seismic periods and control areas. However, high natural variability in catch rates meant that the potential for more subtle effects on catch rates could not be ruled out. Changes to catch rates could be indirectly manifested as a result of a variety of seismic responses that affect mortality, distribution, movement rates and foraging behavior (Bruce et al. 2018). Moreover, there remains a possibility that snow crab are affected in other ways (e.g. behavioral modification, physiology) that could have longer term effects on catch rates through population level impacts. For these reasons, additional study of potentially direct effects of seismic surveys on snow crab are warranted.

This study uses hyperbolic positioning telemetry within a multi-year BACI approach to evaluate the potential of industry-scale seismic exposure to modify movement behavior of free ranging adult male snow crab. Modified movement behavior might be expected to arise from flight/shelter responses triggered by changes to real or perceived risk and/or altered foraging patterns to accommodate seismic-related physiological responses (e.g. stress, injury, sensory impairment, effects on prey species). These responses may be more sensitive than catch rates because they address some of the principal mechanisms by which catch rates might be affected. While the focus of this study targets changes to snow crab movement behaviour, it was conducted concurrently with harvesting (Morris et al. 2018; Morris et al., this issue), physiological (Hanlon et al., this issue) and genomic studies (Hall et al. this issue).

2. Methods

Effects of seismic exposure on snow crab movement behavior was assessed within the Before-After-Control-Impact (BACI) study design reported in Morris et al. (2018) for the years 2015 (Before-After only) and 2016 and included an additional year of manipulation in 2017 (Morris et al., this issue). Both Test (Carson Canyon) and Control (Lilly Canyon) sites were located in similar depths (Carson: mean = 126 m, range = 111-142 m; Lilly: mean = 131 m, range = 107-162 m) over soft bottom substrates at the edge of the continental shelf of the Grand Banks of Newfoundland, Canada (Fig. 1). Average water temperatures at both sites were below 0 °C but temperatures at the more southerly Lilly Canyon were slightly warmer by an average of ~1.6 °C. The study locations were selected based on consultation with representatives of the snow crab fishing fleet, the Fish Food and Allied Workers Union (FFAW) as well as individual snow crab harvesters, who viewed these sites as important harvesting areas that offered snow crab the opportunity to escape seismic exposure to deeper water. This hypothesized deep-water escape response was believed by some harvesters to be one potential mechanism causing reduced catches. The distance between the sites (~70 km) was such that seismic exposures in Carson Canyon would not be detected above baseline noise at the Control site (Morris et al. 2018).

In each year, all seismic operations were prohibited by the industry regulator within a 70 km radius of each study site for a 1 month period before experimental seismic exposure and for an additional month after exposure at the Control site only. Seismic manipulations were conducted from the Atlantic Explorer, a commercial seismic survey vessel, which deployed a 4880 cu. in. air gun array operated at 2000 psi, 9 m depth and fired at a frequency of 10 s (approximately 25 apart). Seismic exposure was defined as periods when seismic-related noise exceeded vessel-related noise (Morris et al. 2018). The first exposure occurred in 2015 from Sep 25-29 and was followed by single pass exposures on Sep 22, 2016 (2 h) and Sep 12, 2017 (~7.5 h). The use of a commercial survey vessel ensured that particle motion and sound pressure exposures were characteristic of 2D industrial survey exposures. More detailed analysis of the seismic noise used in this study is presented in Morris et al (2018), Morris et al. (this issue), Martin et al (2019), and Martin et al. (this issue).

2.1. Tracking animal movements

Within each study area, animal movements were tracked using a hyperbolic acoustic positioning array consisting of 50 acoustic receivers (VR-2 Vemco/Amirix), spaced at 500 m intervals (Fig. 1). The resulting detection arrays were approximately 4 km^2 in size. Within these, animal positions were estimated at approximately 2 min intervals (± 30 s) when detected on at least three receivers. The exact transmission interval was programmed to change randomly within these limits in order to minimize potential transmission signal collisions and loss of data when multiple animals occupied a similar area. The acoustic receivers were also equipped with an internal thermograph, which logged water temperature on an hourly basis.

Snow crab are sexually dimorphic, with adult males being considerably larger than mature females. Consequently, only mature male crab are targeted and captured in the fishery. Snow crab of the Grand Banks generally move to deeper water as they age, but undertake seasonal up-slope movements in the spring to moult or mate (reviewed in Mullowney et al. 2018). The distance travelled during these migrations can span tens of kilometers and is greater for juveniles than mature males and females (Mullowney et al. 2018). Since habitat preferences and movement behavior of snow crab vary by sex and lifestage, we focused our efforts on adult male snow crab; the demographic which harvesters perceive to be influenced by seismic exposure. Local harvesters set standard commercial traps for adult male snow crab (> 95 mm carapace width) within the respective study areas. Captured individuals were held in sea water of similar temperature to that at the seafloor prior to being tagged with V13 coded acoustic transmitters (Vemco/Amirix; diameter: 13 mm; length: 36 mm, weight in water: 6.0 g, 69 KHz, estimated tag life: 653 d, transmission frequency: 60-180 s). The tags were affixed to the carapace with a water-curing adhesive, a uniquely labelled spaghetti tag (Floy Tag FT-4) and a pipe cleaner; the latter used as a temporary measure to firmly hold the transmitter into the adhesive until it cured (Cote et al., 2019).

Animals were released within six hours of tagging at each study site at a central point within the array. Each tagging group was released to the prescribed location at depth in a cage that was opened using an acoustic release. In total, 201 and 115 snow crab were tagged in Carson and Lilly canyons respectively (Table 1). Before, During, and After exposure periods were matched in time across Control and Test sites.

2.2. Data filtering

Acoustic positioning systems can generate occasional aberrant position estimates, which can introduce error into subsequent movement calculations. We cleaned the tracks of snow crab following the approach used on snow crab in Cote et al. (2019). The method incorporated a one-behaviour first-difference correlated random walk (DCRW) state-space model (Auger-Méthé et al. 2017), except here we fit the model by individual rather than the joint approach used in Cote et al. (2019). This approach accounts for measurement error and accommodates the irregular sampling intervals that characterize these data. The resulting processed tracks were then used in modeling snow crab behaviour.

2.3. Velocity analysis

Changes in snow crab movement velocity could arise from seismic exposure if it influenced the physiology of exposed crabs, altered their ability to forage, or changed their perception of risk. We assessed seismic-related effects to movement velocity, while including potentially confounding environmental variables using the following generalized additive mixed model (GAMM, gamm4 package in R, Wood and Scheipl., 2017):

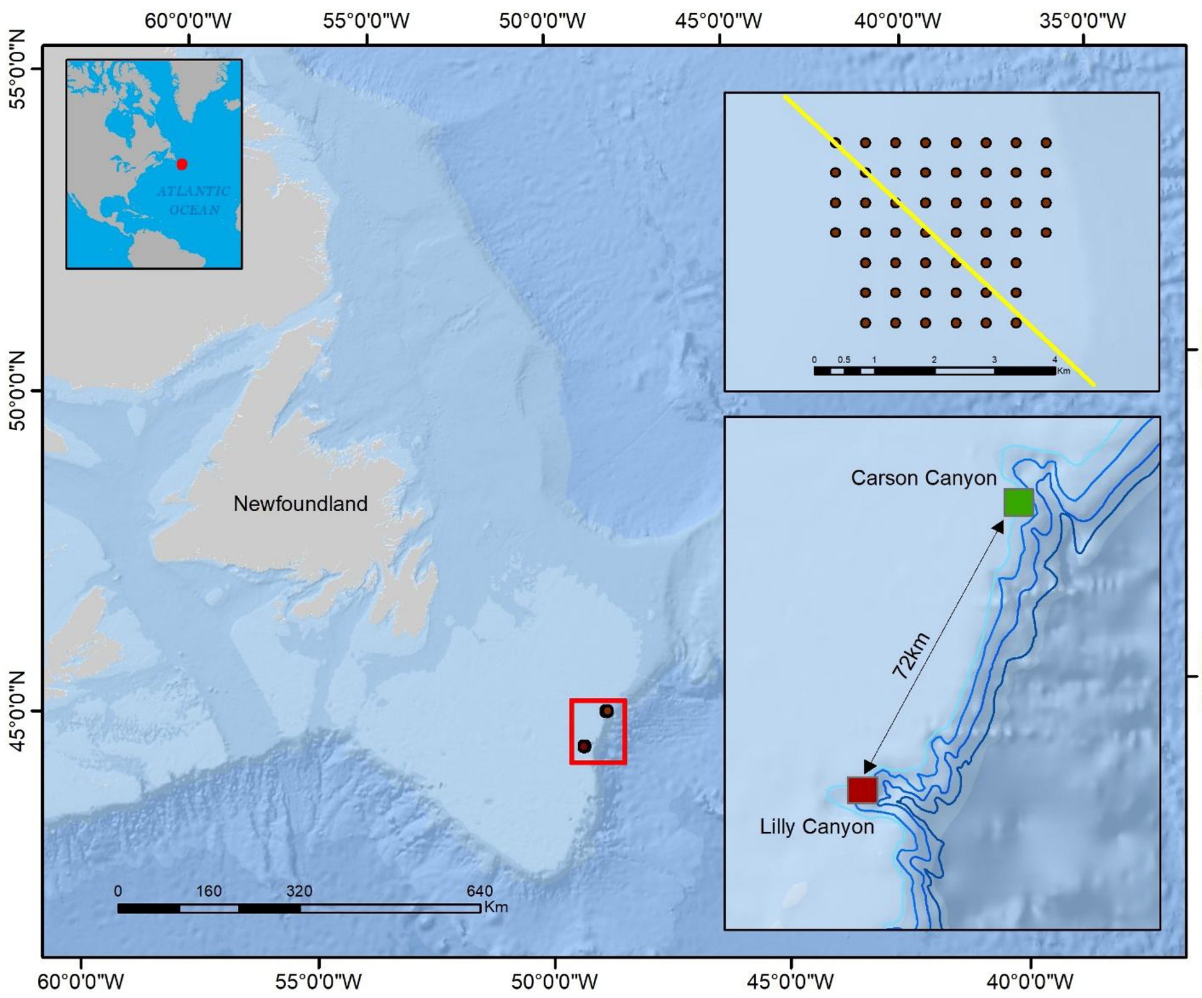

Fig. 1. Location of Carson Canyon (Test) and Lilly Canyon (Control) study sites on the slope of the Grand Banks, NL. Inset on top right shows arrangement of acoustic receivers (red dots) in Carson Canyon and the path of the seismic vessel in 2016 (yellow line). Receiver arrangement for Lilly Canyon is not shown but is similar to Carson Canyon.

$$\text{Velocity} \sim s(\text{TSR}) + s(\text{Temp}) + s(\text{HoD}) + \text{Location}*\text{Exposure Period} + (1 \mid \text{Year/Individual})$$

where velocity was a function of variables that included Time Since Release (TSR), Temperature (Temp), Hour of Day (HoD), and a two way interaction with Location (Control or Test) and Exposure Period (Before, During or After). Due to the potential non-linear relationships of velocity with TSR, Temp and HoD, cubic regression spline smoothers were applied (cyclic cubic regression spline for HoD). The model also incorporated a gamma error structure with a log link. Year (2015, 2016, 2017) and individual, nested within year, were treated as random effects to account for inter-annual differences and accommodate repeated measurements. Despite the use of random effects, diagnostics of early models indicated strong autocorrelation in the residuals. While it was not computationally feasible to account for the temporal autocorrelation within individuals using an AR1 error structure, the lack of independence of the observations was minimized by thinning the data to a six hour interval. The full model, as well as candidate subset models were evaluated using the differences in Akaikie Information Criterion (AIC) scores. Only models with ΔAIC scores within 10 units of the best model were considered to have reasonable support (Burnham and Anderson 2004). Marginal and conditional R^2 values representing variance explained by fixed effects and the entire model, respectively, were also calculated using the r.squaredGLMM function (trigamma output) from the MuMIn package for the mixed models without smoothed variables (Bartoń 2019). These values could not be obtained from gamm4 models with smooth terms as there is no obvious way to sum the variance outputs from these models (Kamil Bartoń, personal communication).

Table 1
Number of individuals and data points (in parentheses) used in velocity analyses across years and field treatments.

Site Type	Location	Year	Tagged Individuals	Exposure Period		
				Before	During	After
Test	Carson Canyon	2015	50	25 (423)	9 (88)	7 (178)
Test	Carson Canyon	2016	74	51 (899)	6 (6)	40 (1622)
Test	Carson Canyon	2017	77	74 (668)	36 (43)	61 (3168)
Control	Lilly Canyon	2016	50	47 (623)	10 (10)	33 (800)
Control	Lilly Canyon	2017	65	48 (131)	23 (23)	51 (1470)

2.4. Displacement direction analysis

Displacement direction analysis was conducted to address harvester concerns that snow crab fled to deeper water when exposed to seismic surveys. The circular nature of bearing data required the use of a Bayesian-based circular ANOVA.

Since we were interested in aggregate direction of displacement, a single bearing was provided from the first and last detection of an individual within each exposure period. These response data were assessed with covariates in the following model:

Bearing ~ Location*Exposure Period*Year

using a projected normal circular GLM (bpnr function in bpnreg R package; Cremers and Klugkist 2018; iterations: 10000, burn-in: 100, lag: 3). Model subsets were compared using DIC scores (Cremers and Klugkist 2018), with particular interest for those that included a Location*Exposure Period interaction, since the importance of that term was most relevant to assessing seismic-related effects. The circular package in R (Pewsey et al. 2013) was used to display the relationship of bearing data to the model covariates.

2.5. Behavior transition analysis

Behavioral transition analysis was performed to assess the impact of seismic activities on movement behaviors of snow crab. Hidden Markov models (HMMs) have recently emerged as a useful form of state-space modelling to identify potentially hidden behavioral processes in time-series animal movement data (Phillips et al. 2015; Bacheler et al. 2019). Selecting the appropriate number of behavioral states is often difficult for HMMs (Pohle et al. 2017), and we considered both two-state and three-state models for our data. For two-state models, behavioral states were defined as Feeding and Mobile, whereas the three-state model behavioral states were defined as Feeding, Foraging, and Mobile, similar to Landry et al. (2018). In the end, the three state model fit our data better. Each respective state had characteristic mean step lengths and turning angles between concurrent detections in which Mobile movements were characterized by long step lengths and high turning angle concentrations, Feeding movements were characterized by short step lengths and dispersed turning angles and with Foraging step length and turning angle parameters lying intermediate.

Prior to model fitting, we filtered our data to meet the requirements of HMMs, which included removing movement tracks with < 100 detections due to issues with model convergence (see also Bacheler et al. 2019). We also split movement tracks if the time elapsed between concurrent detections exceeded 4 h, as large gaps led to unrealistic track interpolations, mostly in instances of individuals leaving our receiver array and returning after extended time durations. Without correction, these gaps between departure and return appeared as a single continuous and steady movement at the edge of the array, rather than as periods in which the animal was beyond detection. All suitable tracks were used to fit a common set of model parameters in our behavior transition models. The final filtering process resulted in 54 movement tracks from 38 individuals. Unfortunately, no animals from our Control location (Lilly Canyon) had movement tracks with sufficient data to fit HMMs, so our behavior transition analyses were restricted solely to individuals in Carson Canyon.

One of the major difficulties associated with HMMs is the choice of appropriate starting parameters that describe mean step length and turning angle concentration for each behavioral state. Failure to choose appropriate parameters may result in model failure in identifying the global maximum of the likelihood function (Michelot et al. 2016). We ran the model fitting procedure 25 times with randomized perturbations of our starting parameters to verify that our chosen values were appropriately estimated (Bacheler et al. 2019). HMMs also require regularized time intervals between detections, so movement tracks for individual crabs were interpolated at 30-min intervals using the R package crawl (Johnson et al. 2008) based on mean values between adjacent detections in our raw data. HMMs were fitted to our data using the R package momentuHMM v1.4.3 (McClintock and Michelot 2018) to investigate the effects of seismic activities on snow crab behavior using the following model covariates:

P(transition) ~ Exposure Period + TSR + HoD + Temp

Where P(transition) indicates the probability of switching between behavioral states, which is dictated by a Markov chain where the probability of an animal occurring in a given state depends on the behavioral state of the animal at the previous detection. Our full model was compared to model subsets using AIC scores, and support for our full model was determined using ΔAIC scores (Anderson and Burnham 2004).

3. Results

Cleaned telemetry tracks were available for 245 snow crab (78% of individuals tagged). Of these, seven animals (1.5% of those released at Lilly Canyon and 3.5% at Carson Canyon) were removed from subsequent analyses since they were suspected of having shed their transmitters or died based on the duration of residence time and the absence of movement. The remaining tags combined for 185,952 position estimates across the three years of study. Similar residence times were observed by tagged individuals within the two arrays, with median values of 9.3 d (range: 1.1-49.8 d) and 8.8 d (range: 1.1-45 d) for Carson and Lilly canyons respectively. The upper bounds of time spent in the array was limited by the removal of the arrays at the end of the study season.

3.1. Velocity analysis

Based on the null model, velocities of snow crab averaged 16.4 m/h (95% confidence interval: 14.0 – 19.3 m/h). Treatment-specific representation of tagged snow crab in the velocity analysis averaged 78% (50-96%). Thinning of the data to address autocorrelation concerns left 10,152 positions for analysis.

Environmental variables were important in influencing the velocity of snow crab (Table 2). The top five models, as determined using ΔAIC values, all included the environmental variables of Time Since Release, Hour of Day and Water Temperature. When environmental variables were looked at in isolation, Time Since Release had the most evidence of being important, followed by Hour of Day and lastly Water Temperature (Table 2). Model predictions indicate that snow crab tend to move at increased speeds at night and in warmer waters (Fig. 2). Elevated movement rates were evident immediately following release and

Table 2
Delta AIC scores for top 5 models and single variable models. HoD = Hour of Day; TSR = Time Since Release; Temp = Temperature.

Variables	ΔAIC
HoD + TSR + Temp + Location*Exposure Period	0[a]
HoD + TSR + Temp + Exposure Period	7.4[a]
HoD + TSR + Temp + Location + Exposure Period	9.0[a]
HoD + TSR + Temp	12.3
HoD + TSR + Temp + Location	14.0
TSR	99.7
Location*Exposure Period	269.6
Exposure Period	305.1
HoD	327.1
Temp	364.9
Location	383.5
Null	386.1

[a] models with reasonable support, based on criteria of Anderson and Burnham (2004).

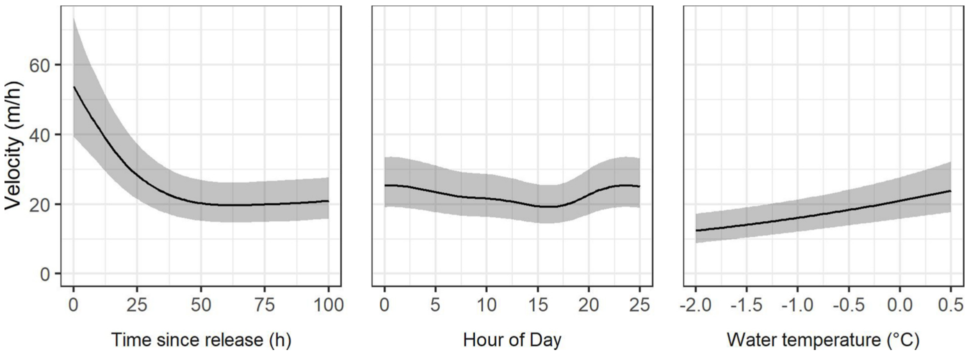

Fig. 2. Model estimates of the effects of Time Since Release, Hour of Day and Water Temperature on snow crab movement velocity from Carson and Lilly canyons 2015-2017. Shaded areas represent 95% confidence intervals. To isolate the effects of these variables, Location and Exposure Period were fixed to "Lilly" and "Before" across all panels and Time Since Release, Hour of Day, and Water Temperature were fixed to 100 h, 12 pm and 0 °C, respectively, across alternate panels.

declined thereafter until stabilizing approximately 60 h later (Fig. 2).

The importance of the Location*Exposure Period interaction term in a BACI study design is reflective of seismic effects on velocity. The best model included the two-way Location*Exposure interaction, however, the second best model, marginally within the ΔAIC cut-off of 10, did not include the Location*Exposure Period interaction. The model including the interaction term alone received significantly less support than the model that only included Time Since Release (ΔAIC of 269.6 versus 99.7, respectively; Table 2), indicating that the effect of seismic exposure is relatively subtle. R^2 values from the Location*Exposure Period model also indicate that the potential effects of seismic exposure accounts for a small portion of the variance (marginal R^2 was 0.9%) relative to random individual variation (conditional R^2 was 34.4%). Predictions from the best model (Fig. 3), while controlling for other environmental variables, indicate the greatest differences in velocity between the study areas occurred in the Before-exposure period (Carson velocities were lower) and that During-exposure velocities were only marginally higher at Carson Canyon compared to Control conditions. Velocities appeared to decline slightly following seismic exposure in Carson Canyon to near Before-exposure and Control levels.

In addition to thinning the data, we also used the gamm function from the mgcv package to fit a GAMM model with an AR1 correlation structure to a subset of the crab telemetry data that had regularized time steps using the crawl package (i.e. the same data used in the HMM analysis below). Because insufficient numbers of snow crab met the data requirements at the Control site, this analysis was limited to crabs tracked in the Carson Canyon (Test) site. Again we used a gamma family error structure with a log link and set individuals as a random effect. The results from this analysis mirrored those from the thinned data and are not discussed further as the data thinning approach let us benefit from the full BACI design.

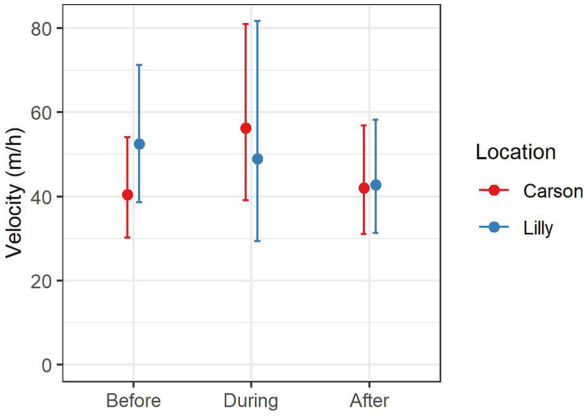

Fig. 3. Model estimates of snow crab movement velocity for Before-After-Control-Impact seismic exposure treatments in Carson (Test) and Lilly (Control) canyons. Model estimates standardize the environmental conditions across treatments to 100 h post-release, at 12 pm and a water temperature of 0 °C. Error bars represent 95% confidence intervals.

Table 3
Estimates of step length and turning angle concentration from hidden Markov models fit to 59 movement tracks from 38 snow crabs in Carson Canyon. Step length and turning angle values were calculated from movement tracks regularized at 30-min time intervals.

	State		
	Feeding	Foraging	Mobile
Step Length (m)	0.84	5.31	22.42
Step Length SD	0.78	3.62	11.56
Angle Concentration	0.46	0.63	0.86

3.2. Behavior transition analysis

We parameterized three behavioral states with the 54 analyzed tracks (Table 3) according to step length and turning angle (Fig. 4). Snow crab transitioned across the three behavior states (Fig. 5), but the likelihood of changing states did not show any obvious response to seismic exposure. Our top three models indicated environmental influences were important, and included Time Since Release (TSR), Hour of Day (HoD), and Water Temperature (Temp)(Table 4). The Exposure Period term, reflecting seismic-related effects, was included in the second and third best models but neither of these had support based on our ΔAIC selection criteria.

3.3. Movement direction

Seismic exposure did not have an important effect on snow crab movement direction as neither the study variables of Location, Exposure Period and Year, or their interactions, improved the DIC scores relative to the null model (Table 5). Direction of movement during seismic exposure appeared more concentrated across individuals at the Test site compared to the Control site, but the direction varied across years (Fig. 6). Average movement direction After exposure appeared to be consistent across sites and years but was not Before and During exposure time periods.

4. Discussion

Seismic effects on snow crab movement behavior were complex and subtle relative to other study factors. For the approaches used in this study, the best models to account for snow crab movement indicated that the magnitude of effects of seismic were at most small (velocity) or were not statistically relevant (behavior states, direction of movement). In comparison, responses to initial handling, temperature and time of day were less subtle, despite a large degree of individual variability. Snow crab are known to display highly variable movement behavior (Cote et al. 2019) and consequently, disentangling a strong effect related to seismic exposure was an expected challenge.

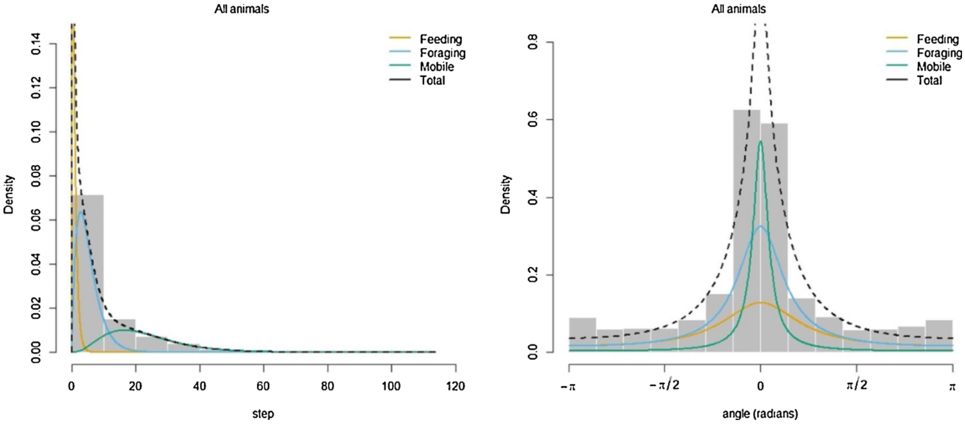

Fig. 4. State-dependent distribution of step lengths (left panel) and turning angles (right panel) for 54 movement tracks from snow crabs in Carson Canyon.

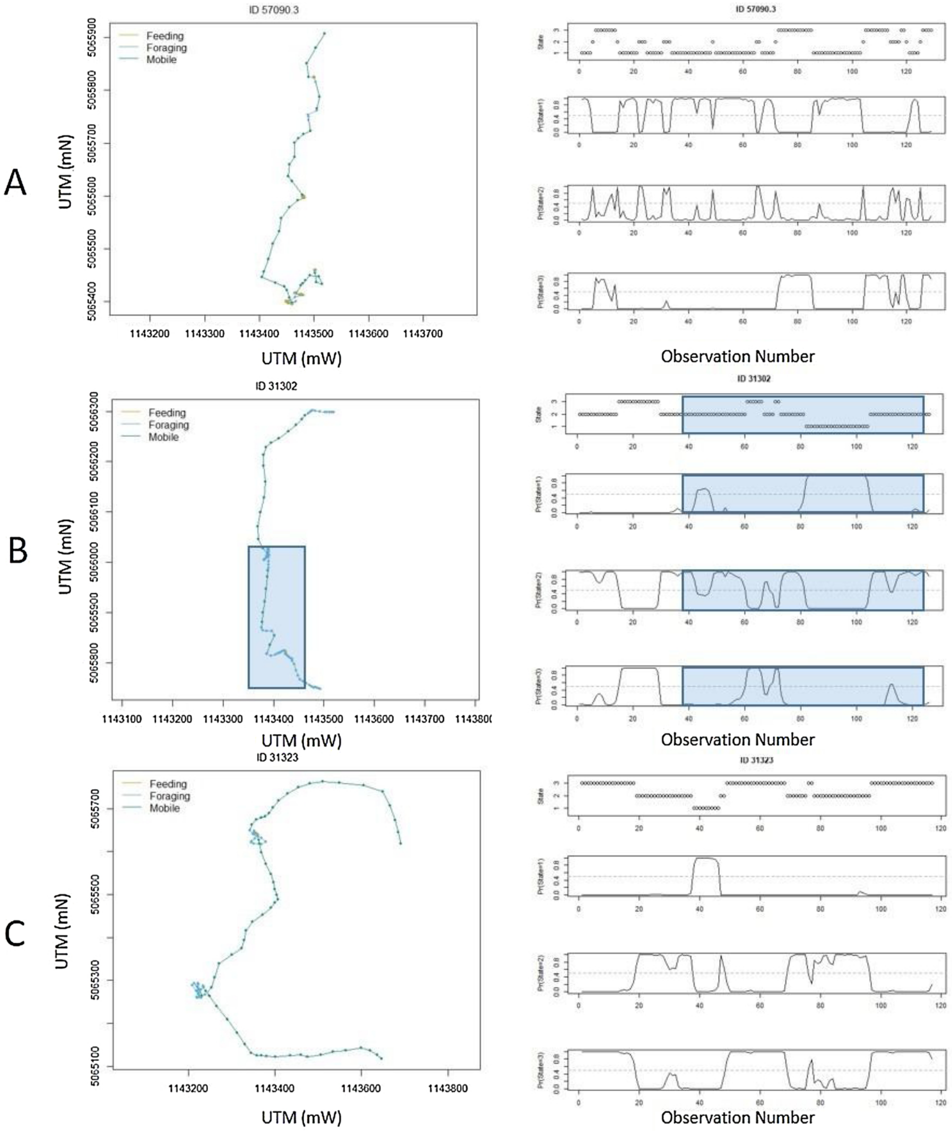

Fig. 5. Tracks of three snow crab (left panels) with behavior state probabilities (right panels). Behavior state 1 represents Feeding behavior, while states 2 and 3 represent Foraging and Mobile states respectively. Most probable behavior states are superimposed on the crab tracks. The movement track in panel A occurred entirely within the During exposure period, whereas panel B contains Before, During (shaded blue), and After exposure periods, and panel C represents a crab for which only After exposure data were available.

Table 4
Behavioral state transition model comparisons using ΔAIC. TSR = Time Since Release, HoD = Hour of Day, Temp = Water Temperature.

Variables	ΔAIC
TSR + HoD + Temp	0
TSR + HoD + Temp + Exposure Period	13.34876
TSR + Exposure Period	24.46662
Exposure Period	25.9447
Temp	31.09017
HoD	33.66875
TSR	36.63346
Null	45.3318

Table 5
ΔDIC scores for models assessing movement direction of snow crab.

Variables	ΔDIC
Null Model	0
Year	0.31
Location	3.68
Exposure Period	6.99
Location * Exposure Period * Year	13.91
Location * Exposure Period	14.80

While detailed hearing studies have not be conducted on snow crab, they are considered less vulnerable to physiological damage from noise since they do not possess gas filled organs that are known to be sensitive to seismic exposure (e.g. swim bladders, etc.; Edmonds et al. 2016; MacGregor et al. 2016). This is supported by laboratory and cage studies which did not show conclusive physiological effects of seismic exposure on invertebrates (Christian et al. 2003; Courtenay et al. 2009, Hanlon et al., 2020Hanlon et al., this issue). Other species of invertebrates have shown sensitivities to anthropogenic sound. For example, Tifau and Briffa (2016) reviewed the effects of anthropogenic sound on crustaceans. Only three described studies were related to seismic exposure, of which only one (Payne et al. 2008) reported effects of increased feeding by exposed American lobster. More recent studies of seismic-exposed taxa have shown that mortality (scallops), immune-response (scallop and southern rock lobster), reflexes (southern rock lobster and scallop) and behavior (scallop) can be altered, in some case over chronic time periods (Day et al. 2016). Studies of other impulsive sound sources on snapping shrimp (Spiga 2016) and Norway lobster (Solan et al. 2016), also documented changes to behaviors such as snapping duration and amplitude, bioirrigation, burying behavior and locomotion.

Many seismic studies have been criticized for having design problems, or not representing realistic environmental and/or seismic conditions (Slabbekoorn, 2016; Hawkins and Popper 2017). Industrial-scale field studies examining seismic effects on unconstrained animals are extremely rare, likely due to the logistics associated with coordinating and funding industry participation and manipulating exposures for scale-appropriate study areas (e.g. seismic surveys restrictions during this experiment were managed across an area of 1,400 km^2). Fewer still have used telemetry to examine fine scale movement responses of free ranging animals to seismic exposure (Przeslawski et al., 2018a) and these have largely been restricted to fish (Wardle et al. 2001; Bruce et al. 2018, but see Christian et al. 2003).

More commonly, seismic surveying effects on behavior have been inferred using commercial catch rates, for which invertebrate species often show no effects (La Bella et al. 1996, Andriguetto-Filho et al., 2005, Parry and Gason 2006, Morris et al., 2018; but see Morris et al. (this issue) who documented opposing changes across two years of study). However, catch rates may not be the most sensitive way to measure impacts from seismic as this metric likely changes in response to a complex suite of behavioral or physiological conditions. Seismic effects on invertebrates have been observed in field studies examining direct responses to behavior, physiology and mortality. For example, Day et al. (2016) and Fitzbibbon et al. (2017) detected changes in haemolymph chemistry in spiny lobster that are associated with immune system performance and nutritional condition, whereas Payne et al. (2008) noted increased feeding in previously seismic-exposed American lobsters. Similarly, Day et al. (2017) detected increased mortality, and altered behavior and physiology in seismic-exposed scallops over time scales of hours to months. In contrast, other studies (Przeslawksi et al. 2018 and Heyward et al. 2018) did not detect changes in physiology or health. Even in studies where seismic effects have been detected, differences can be very small relative to natural variation under field conditions (e.g. Day et al. 2017).

Poor ecological understanding of snow crab limits our ability to predict and understand the pathways of effect of seismic exploration. Despite the commercial importance of snow crab, headway on the fine-scale behavioral ecology has only been made in recent years. Certainly, one characteristic of snow crab movement ecology is the high levels of variation in movement behavior, that are poorly (but significantly) linked to environmental variables related to diel cycles, water temperature and tide (Cote et al. 2019). The observations of this study support these conclusions in that individual-specific tendencies dominated the explained variance and dwarfed population responses to experimental manipulations. The importance of individual effects suggests that the study animals' histories prior to the experiment may be important. For example, motivation to move is likely to be at least partially driven by an animal's energetic state. Since increasing activity can lead to higher mortality via predation (Anholt et al. 2000), motivation to feed is influenced by the trade-off between the need for food and mortality risk (Werner and Gilliam 1984). Snow crab can survive weeks without food and also endure energy intensive stages such as reproduction and molting. Therefore, an individual's behavior during our study could be a reflection of its energetic state before the study. Certainly, a broad disparity in the characteristics of the hepatopancreas (e.g. size, color and consistency), an energy storage organ in crustaceans (Wang et al. 2014), was noted across animals sacrificed in the field for other program elements. Unfortunately, we could not measure hepatopancreas condition for animals that were tagged and released.

Environmental effects of temperature and time of day were variables in the best models for snow crab movement velocity and for behavior state transition. The effects we observed on velocity supported the conclusions found for tagged snow crab in the Cabot Strait (Cote et al. 2019). Snow crab in that study tended to be more active nocturnally. Furthermore, we also observed movement velocity to generally increase with water temperature. In this study the water temperatures were considerably colder, typically ranging from -1.9 to 0.5 °C in contrast to the 0-4 °C temperature range measured in the Cabot Strait. In spite of this, movement velocities from the studies were comparable. This plasticity suggests that typical movement velocities of snow crab sit well within their physiological limits (Cote et al. 2019).

Finally, the importance of Time Since Release as a variable in both movement velocity and behavior state models underscores the need to consider experimental influences on study animals (McGaw and Nancollas 2018); particularly in telemetry studies. Other studies, using tagged snow crab (Kanawa et al. 2014; Cote et al. 2019) noted elevated movement rates following release. One of these (Cote et al. 2019) identified this as a stress response and eliminated data from the first 24 h post-release from their analysis. A similar approach was employed by Bruce et al. (2018) to deal with tagging effects on fish behavior. In this study, the effect of the tagging response was modeled as a continuous response and was shown to be highest immediately after release and declined linearly before stabilizing after approximately 2 days. Aside from controlling for experimental perturbations, the presence of a flight response offers an important positive control of our analytical methods to detect behavior change. Second, the observed flight response offers context that can be useful to interpreting the magnitude of

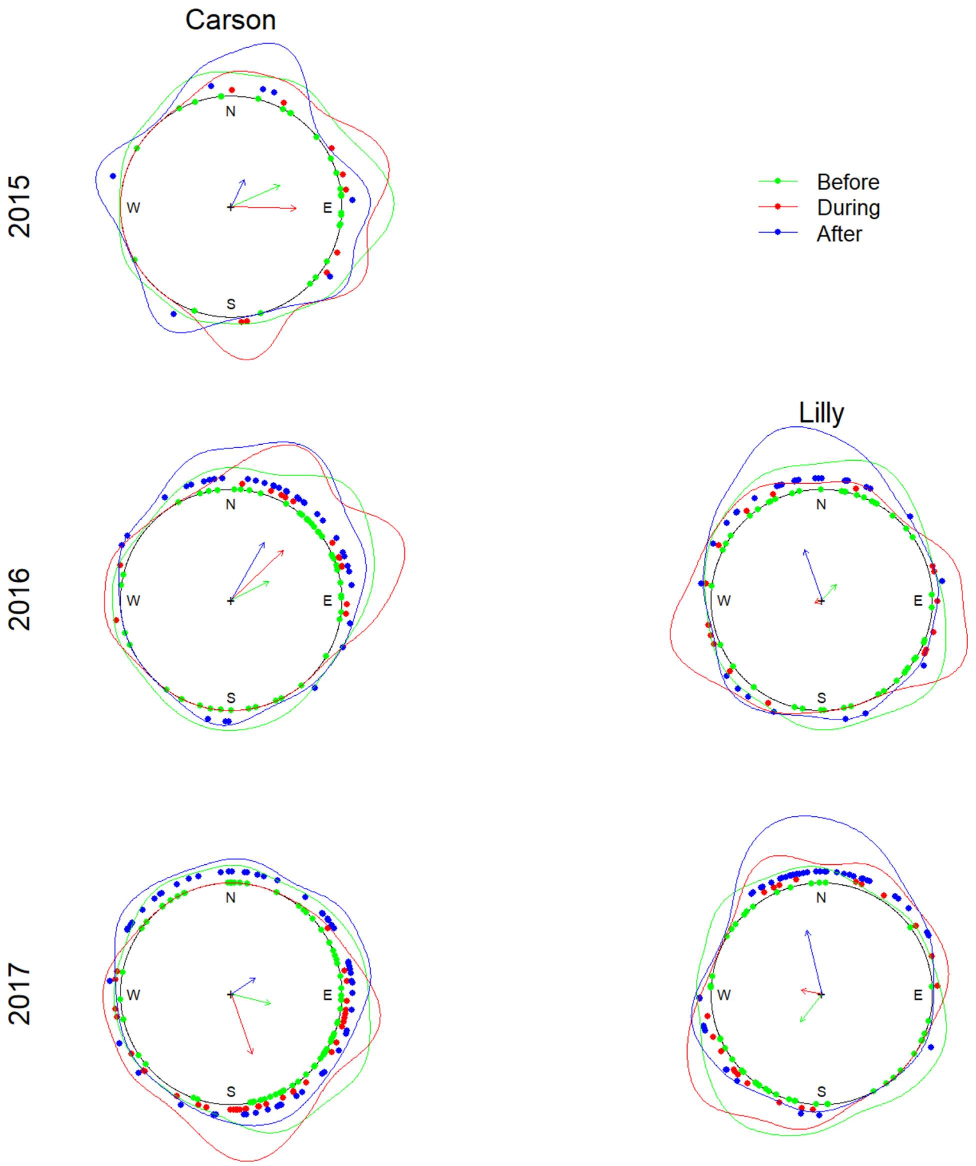

Fig. 6. Mean direction of movement for tracked snow crab Before, During and After seismic exposure periods at Lilly (Control) and Carson (Test) canyons. Points represent the mean bearing of individuals, arrows represent the aggregate bearing across individuals, and the length of arrows represents the strength of tendency for a particular time period. Lines external to the plot represent the frequency distribution of bearings for each time period.

seismic responses on snow crab behavior. Specifically, that handling effects on snow crab are short-lived, and that catch and release effects on snow crab (a conservation measure used on undersized crab) are more pronounced than the seismic exposure response we measured.

Direction of movement exhibited by snow crab varied across years, exposure periods and study locations and did not support the belief of snow crab harvesters that snow crab move to deeper water in response to seismic exposure. Instead snow crab at Carson Canyon typically moved toward the northeast across all Exposure periods (except in 2017 when the population displacement was to the south). Displacement of snow crab in Lilly Canyon also went in a similar direction (north) after seismic exposure but displacement direction was much more variable during other time periods. Other studies (reviewed by Mullowney et al. 2018) indicate that adult male snow crab can range over extensive distances while foraging and during seasonal migrations to and from breeding areas. Our observations of short term displacement direction match those reported by Mullowney et al. (2018), based on recaptures of snow crab 6-10 months post-release from Carson Canyon, that tended to be recaptured 35-60 km northeast along the shelf edge. Snow crab released at Lilly Canyon had a weaker population level displacement direction after several months at large, with most recaptures being caught within less than 20 km of the release site.

Telemetry data can provide extremely rich datasets by which to examine behavior of study animals. However, analysis of such large and temporally-correlated datasets can be daunting. HMMs are great alternatives to more conventional approaches, as they are becoming increasingly accessible and offer ecologists a statistical approach to deal with inherent serial correlation within tracks while also parameterizing potential behavior states (Phillips et al. 2015). The HMM implemented here added another important dimension to our results as a supplement to our velocity analysis, but warrants further development as we were forced to remove significant quantities of our data to meet HMM requirements and this limited our ability to utilize this technique as an independent analysis tool. We expect these methods to become increasingly important in the analysis of acoustic telemetry behavior studies in the future.

Seismic exposure can potentially influence organisms in a multitude of ways over short or extended temporal scales (Fitzbibbon et al., 2017) and this study examines a subset of specific but important ecological aspects of a single life-stage and sex. While only the adult male snow crab are retained commercially, other lifestages and perhaps female crab could be more vulnerable. For example, decapod larvae are influenced by natural sounds (Tifau and Briffa 2016) and juveniles and females may more frequently occupy shallower habitats that are closer to seismic noise sources (Mullowney et al. 2018; Cote et al. 2019). The reduced movement capacity and habitat specificity of smaller sex classes and lifestages may also limit the behavioral responses available to avoid seismic effects. Nevertheless, any effects we observed on movement ecology of adult male snow crab were relatively small compared to individual variation and other experimental factors, suggesting the seismic surveys do not play an obvious role in influencing snow crab movement ecology. Other aspects of potential sensitivity of snow crab to seismic surveys have been examined within this general study design, including mortality, fishery catch rates, and physiological and genomic responses (this issue). These studies collectively have consistent findings with respect to impact of seismic surveying, and support the view that seismic is unlikely to be a prominent threat to adult male snow crab or the fishery they support.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.fishres.2020.105661.

References

Andriguetto-Filho, J.M., Ostrensky, A., Pie, M.R., Silva, U.A., Boeger, W.A., 2005. Evaluating the impact of seismic prospecting on artisanal shrimp fisheries. Cont. Shelf Res. 25, 1720–1727.

Anholt, B.R., Werner, E., Skelly, D.K., 2000. Effect of food and predators on the activity of four larval ranid frogs. Ecology 81, 3509–3521.

Auger-Méthé, M., Albertsen, C.M., Jonsen, I.D., Derocher, A.E., Lidgard, D.C., Studholme, K.R., Bowen, W.D., Crossin, G.T., Flemming, J.M., 2017. Spatiotemporal modelling of marine movement data using Template Model Builder (Tmb). Mar. Ecol. Progr. Ser. 565, 237–249. https://doi.org/10.3354/meps12019.

Bacheler, N.M., Michelot, T., Cheshire, R.T., Shertzer, K.W., 2019. Fine-scale movement patterns and behavioral states of gray triggerfish *Balistes capriscus* determined from acoustic telemetry and hidden Markov models. Fisheries Res. 215, 76–89.

Bartoń, Kamil, 2019. MuMIn: Multi-Model Inference. R package version 1.43.6. https://CRAN.R-project.org/package=MuMIn.

Bruce, B., Bradford, R., Foster, S., Lee, K., Landsdell, M., Cooper, S., Przeslawski, R., 2018. Quantifying fish behavior and commercial catch rates in relation to a marine seismic survey. Mar. Environ. Res. 140, 18–30.

Burnham, K.P., Anderson, D.R., 2004. Multimodel Inference. Understanding AIC and BIC in Model Selection. Sociol Methods Res 33, 261–304.

Carroll, A.G., Przeslawski, R., Duncan, A., Gunning, M., Bruce, B., 2016. A critical review of the potential impacts of marine seismic surveys on fish and invertebrates. Mar. Pollut. Bull. 114, 9–24. https://doi.org/10.1016/jmarpolbul.2016.11.038.

Christian, J.R., Mathieu, A., Thompson, D.H., White, D., Buchanan, R.A., 2003. Effect of Seismic Energy on Snow Crab (*Chionoecetes opilio*)).

Cote, D., Nicolas, J.-M., Whoriskey, F., Cook, A.M., Broome, J., Regular, P.M., Baker, D., 2019. Characterizing snow crab (*Chionoecetes opilio*) movements in the Sydney Bight (Nova Scotia, Canada): a collaborative approach using multiscale acoustic telemetry. Can. J. Fish. Aquat. Sci. 76, 334–346. https://doi.org/10.1139/cjfas-2017-0472.

Cox, K.D., Brennan, L.P., Dudas, S., Juanes, F., 2016. Assessing the effect of aquatic noise on fish behavior and physiology: a meta-analysis approach. Proc. Meet. Acoust. 27.

Courtenay, S.C., Boudreau, M., Lee, K., 2009. Potential Impacts of Seismic Energy on Snow Crab: an Update to the September 2004 Peer Review. Environmental Studies Research Funds Report No 178. Moncton (181p). DFA, 2017. Seafood Industry Year in Review 2016. Department of Fisheries and Land Resources. Prepared by Planning and services Division, Government of Newfoundland and Labrador. pp. 28 pp. (accessed 20.06.17). http://www.fishaq.gov.nl.ca/publications/pdf/SYIR_2016.pdf.

Cremers, J., Klugkist, I., 2018. One Direction? A Tutorial for Circular Data Analysis Using R With Examples in Cognitive Psychology. Front. Psych. 9, 1–13. https://doi.org/10.3389/fpsyg.2018.02040.

Day, R.D., McCauley, R.D., Fitzgibbon, Q.P., Hartmann, K., Semmens, J.M., 2016. Assessing the impact of marine seismic surveys on southeast Australian scallop and lobster fisheries. Fisheries Research & Development Corporation (FRDC), pp. 144 p FRDC Project No 2012/008.

Day, R.D., McCauley, R.D., Fitzgibbon, Q.P., Hartmann, K., Semmens, J.M., 2017. Exposure to seismic air gun signals causes physiological harm and alters behavior in the scallop *Pecten* fumatus. Proc. Nat. Acad. Sci. E8537–E8546. https://doi.org/10.1073/pnas.1700564114.

Edmonds, N.J., Firmin, C.J., Goldsmith, D., Faulkner, R.C., Wood, D.T., 2016. A review of crustacean sensitivity to high amplitude underwater noise: data needs for effective risk assessment in relation to UK commercial species. Mar. Pollut. Bull. 108, 5–11.

Engås, A., Løkkeborg, S., Ona, E., Soldal, A.V., 1996. Effects of seismic shooting on local abundance and catch rates of cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*). Can. J. Fish. Aquat. Sci. 53, 2238–2249.

Fitzbibbon, Q.P., Day, R.D., McCauley, R.D., Simon, C.J., Semmens, J.M., 2017. The impact of seismic air gun exposure on the haemolymph physiology and nutritional condition of spiny lobster, *Jasus edwardsii*. Mar. Poll. Bull. 125, 146–156.

Hall, J.R., Lehnert, S., Gonzalez, E., Bradbury, I., Hanlon, J., Morris, C.J., Rise, M.L. 2020 This issue in review. Snow crab (Chionoecetes opilio) hepatopancreas transcriptome: identification of candidate molecular biomarkers of seismic survey impact. Fisheries Res. XX, XXX-XXX.

Hanlon, J., Morris, C., Payne, J., Xu, J., Cote, D., Han, V. 2020 This issue in review. Effects of chronic noise exposure on captive snow crab. Fisheries Res XX, XXX-XXX.

Hawkins, A.D., Popper, A., 2017. A sound approach to assessing the impact of underwater noise on marine fishes and invertebrates. ICES J. Mar. Sci. 74, 635–651. https://doi.org/10.1093/icesjms/fsw205.

Hawkins, A.D., Pembroke, A.E., Popper, A.N., 2015. Information gaps in understanding the effects of noise on fishes and invertebrates. Rev. Fish. Biol. Fish. 25, 39–64.

Heyward, A.J., Colqhoun, E., Cripps, D., McCorry, M., Stowar, B., Radford, K., Miller, I., Miller, Battershill, C., 2018. No evidence of damage to the soft tissue or skeletal integrity of mesophotic corals exposed to a 3D marine seismic survey. Mar. Poll. Bull. 129, 8–13.

Johnson, D.S., London, J.M., Lea, M.A., Durban, J.W., 2008. Continuous-time correlated random walk model for animal telemetry data. Ecology 89, 1208–1215.

Kight, C.R., Swaddle, J.M., 2011. How and why environmental noise impacts animals: an integrative, mechanistic review. Ecol. Lett. 14, 1052–1061.

La Bella, G., Cannata, S., Froglia, C., Ratti, S., Rivas, G., 1996. First assessment of effects of air-gun seismic shooting on marine resources in the central Adriatic Sea. International Conference on Health, Safety and Environment in Oil and Gas Exploration and Production 227–238.

Landry, J.J., Kessel, S.T., McLean, M.F., Ivanova, S.V., Hussey, N.E., O'Neill, C., Vagle, S., Dick, T.A., Fisk, A.T., 2018. Movement types of an Arctic benthic fish, shorthorn sculpin (*Myoxocephalus scorpius*), during open-water periods in response to biotic and abiotic factors. Can J Fish Aquat Sci 76, 626–635.

Løkkeborg, S., Soldal, A.V., 1993. The influence of seismic exploration with airguns on cod (*Gadus morhua*) behaviour and catch rates. ICES J. Mar. Sci. 196, 62–67.

McCauley, R.D., Day, R.D., Swadling, K.M., Fitzgibbon, Q.P., Watson, R.A., Semmens, J.M., 2017. Widely used marine seismic survey air gun operations negatively impact zooplankton. Nature Ecol Evol. 1, 095. http://www.ecomarres.com/downloads/seismic.pdf.

MacGregor, P.K., Horn, A.G., Leonard, M.L., Thomsen, F., 2016. Anthropogenic noise and conservation. In: Brumm, H. (Ed.), Animal Communication and Noise, Animal Signals and Communication 2. Springer-Verlag, Berlin, pp. 409–444.

Martin, B.S., Morris, C.J. et al. This issue in review. Modeling of Particle Motion from a Seismic Airgun Array and it's Measurement Using Three Commercial Sensors. Fisheries Res. XX, XXX-XXX.

Martin, B.S., Morris, C.J., Broker, K., O'Neill, C., 2019. Sound exposure level as a metric for analyzing and managing underwater soundscapes. Journal of the Acoustical Society of America. https://doi.org/10.1121/1.5113578.

McGaw, I.J., Nancollas, S.J., 2018. Experimental setup influences the cardiovascular responses of decapod crustaceans to environmental change. Can J Zool. 96, 1043–1052.

McClintock, B.T., Michelot, T., 2018. momentuHMM: R package for generalized hidden Markov models of animal movement. Meth Ecol Evol. 9, 1518–1530.

Michelot, T., Langrock, R., Patterson, T.A., 2016. moveHMM: an R package for the statistical modelling of animal movement data using hidden Markov models. Methods in Ecology and Evolution 7 (11), 1308–1315.

Morris, C.J., Cote, D., Martin, B., Kehler, D., 2018. Effects of 2D seismic on the snow crab fishery. Fish Res. 197, 67–77.

Morris, C.J., Cote, D., Martin, B., Mullowney, D. 2020 This issue in review. Effects of 3D seismic surveying on the Snow Crab fishery. Fish Res XX, XXX-XXX.

Mullowney, D.R.J., Dawe, E.G., Colbourne, E.B., Rose, G.A., 2014. A review of factors contributing to the decline of Newfoundland and Labrador snow crab (*Chionoecetes opilio*). Rev. Fish Biol. Fish. 24, 639. https://doi.org/10.1007/s11160-014-9349-7.

Mullowney, D., Morris, C., Dawe, E., Zagorsky, I., Goryanina, S., 2018. Dynamics of snow crab (*Chionoecetes opilio*) movement and migration along the Newfoundland and Labrador and Eastern Barents Sea continental shelves. Rev. Fish Biol. Fish. 28, 435–459. https://doi.org/10.1007/s11160-017-9513-y.

Parry, G.D., Gason, A., 2006. The effect of seismic surveys on catch rates of rock lobsters in western Victoria. Australia. Fish. Res. 79, 272–284.

Payne, J.F., Andrews, C.D., Fancey, L.L., Guiney, J., Cook, A., Christian, J.R., 2008. Are seismic surveys an important risk factor for fish and shellfish? Bioacoustics. 17 (1-3), 262–265. https://doi.org/10.1080/09524622.2008.9753842.

Peña, H., Handegard, N.O., Ona, E., 2013. Feeding herring schools do not react to seismic air gun surveys. ICES J. Mar. Sci. 70, 1174–1180.

Pewsey, A., Neuhäuser, M., Ruxton, G.D., 2013. Circular statistics in R. Oxford University Press, Oxford, UK.

Phillips, J.S., Patterson, T.A., Leroy, B., Pilling, G.M., Nicol, S.J., 2015. Objective classification of latent behavioral states in bio-logging data using multivariate-normal hidden Markov models. Ecol Appl. 25, 1244–1258.

Pine, M.K., Jeffs, A.G., Radford, C.A., 2012. Turbine sound may influence the metamorphosis behaviour of estuarine crab megalopae. PLOS ONE 7, e51790.

Pohle, J., Langrock, R., van Beest, F.M., Schmidt, N.M., 2017. Selecting the number of states in hidden Markov models: pragmatic solutions illustrated using animal movement. J Agri Biol Environ Stat. 22, 270–293.

Przeslawski, R., Brooke, B., Carroll, A.G., Fellows, M., 2018a. An integrated approach to assessing marine seismic impacts: Lessons learnt from the Gippsland Marine Environmental Monitoring project. Ocean Coastal Manag 160, 117–123.

Przeslawski, R., Huang, Z., Anderson, J., Carroll, A.G., Edmonds, M., Hurt, L., Williams, S., 2018b. Multiple field-based methods to assess the potential impacts of seismic surveys on scallops. Mar. Poll. Bull. 129, 750–761.

Putland, R.L., Merchant, N.D., Farcas, A., Radford, C.A., 2018. Vessel noise cuts down communication space for vocalizing fish and marine mammals. Glob. Change Biol. 24, 1708–1721.

Simpson, S.D., Radford, A.N., Holles, S., Ferarri, M.C.O., Chivers, D.P., McCormick, M.I., Meekan, M.G., 2016. Small-boat noise impacts natural settlement behavior of coral reef fish larvae. In: Popper, N.A., Hawkins, A. (Eds.), The Effects of Noise on Aquatic Life II. Springer, New York, pp. 1041–1048.

Skalski, J.R., Pearson, W.H., Malme, C.I., 1992. Effects of sounds from a geophysical survey device on catch-per-unit- effort in a hook-and-line fishery for rockfish (*Sebastes* spp.). Can. J. Fish. Aquat. Sci. 49, 1357–1365.

Slabbekoorn, H., 2016. In: Popper, A.N., Hawkins, A. (Eds.), Aiming for Progress in Understanding Underwater Noise Impact on Fish: Complementary Need for Indoor and Outdoor Studies. The Effects of Noise on Aquatic Life II. Springer Science + Business Media, New York, pp. 1057–1065.

Slotte, A., Hansen, K., Dalen, J., Ona, E., 2004. Acoustic mapping of pelagic fish distribution and abundance in relation to a seismic shooting area off the Norwegian west coast. Fish. Res. 67, 143–150.

Solan, M., Hauton, C., Godbold, J.A., Wood, C.L., Leighton, T.G., White, P., 2016. Anthropogenic sources of underwater sound can modify how sediment-dwelling invertebrates mediate ecosystem properties. Sci. Rep 6, 20540.

Spiga, I., 2016. Acoustic Response to Playback of Pile-Driving Sounds by Snapping Shrimp. In: In: Popper, A., Hawkins, A. (Eds.), The Effects of Noise on Aquatic Life II. Advances in Experimental Medicine and Biology, vol 875 Springer, New York, NY.

Tifau, S., Briffa, M., 2016. Review on behavioral impacts of aquatic noise on crustaceans. Proc. Meetings Acoust 27. https://doi.org/10.1121/2.0000302.

Vilardo, C., Barbosa, A.F., 2018. Can you hear the noise? Environmental licensing of seismic surveys in Brazil faces uncertain future after 18 years protecting biodiversity. Persp. Ecol. Conserv. 16, 54–59.

Wang, W., Wu, X., Liu, Z., Zheng, H., Cheng, Y., 2014. Insights into hepatopancreatic functions for nutrition metabolism and ovarian development in the crab *Portunas trituberculatus*: gene discovery in the comparative transcriptome of different hepatopancreas stages. Plos One 9, e84921.

Wardle, C.S., Carter, T.J., Urquhart, G.G., Johnstone, A.D.F., Ziolkowski, A.M., Hampson, G., Mackie, D., 2001. Effects of seismic air guns on marine fish. Cont. Shelf Res. 21, 1005–1027.

Werner, E.E., Gilliam, J.F., 1984. The ontogenetic niche and species interactions in size-structured populations. Annu Rev Ecol Syst 15, 393–425.

Wood, S., Scheipl, F., 2017. Package 'gamm4'. https://cran.r-project.org/web/packages/gamm4/gamm4.pdf.

APPENDIX 5 – Snow Crab (*Chionoecetes opilio*) Hepatopancreas Transcriptome: Identification and Testing of Candidate Molecular Biomarkers of Seismic Survey Impact

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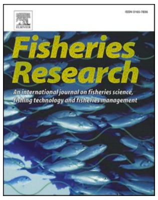

Snow crab (*Chionoecetes opilio*) hepatopancreas transcriptome: Identification and testing of candidate molecular biomarkers of seismic survey impact

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ABSTRACT

The eastern slope of the Grand Banks, Newfoundland and Labrador, Canada, represents an area where active seismic exploration overlaps extensively with an important snow crab (*Chionoecetes opilio*) fishery. Field studies were conducted in 2016 and 2017 to investigate the potential impact of seismic oil and gas surveying exposure on the transcriptome of the snow crab hepatopancreas, which is involved in metabolism and response to environmental stress. In 2016, snow crabs were subjected to 2D seismic noise for 2 h and sampled before (i.e. pre-seismic), and 18 h and 3 weeks after exposure in Carson Canyon (CC). In 2017, the 2D seismic exposure was repeated and samples were collected before and 1 day, 2 days, and 6 weeks after exposure in CC. Additionally, in 2017, snow crabs were subjected to 3D seismic noise for 2 months and sampled during and 6 weeks after exposure at a site north of CC. In both years, snow crabs were also collected from a separate non-seismic exposed site [Lilly Canyon (LC)]. RNA-seq was used to identify candidate seismic-responsive molecular biomarkers in 2016. The quantified transcripts of individuals (i.e. $n = 10$ from each of the four 2016 groups) were compared using two differential expression analysis methods (DESeq2 and edgeR), identifying a total of 1088 and 389 differentially expressed transcripts, respectively. Real-time quantitative polymerase chain reaction (qPCR) assays were designed and run for 83 of these transcripts to confirm RNA-seq results, identifying 9 transcripts with significantly higher expression after seismic exposure at the CC site, and 14 transcripts with significant differential expression between the CC site and the non-seismic LC site. Functional annotations associated with candidate seismic-responsive genes included oxidoreductase activity, apoptotic process, and inflammatory response. To further investigate the performance and utility of candidate biomarkers identified using the 2016 samples, expression levels of 20 selected transcripts were then examined in 2017 samples using both qPCR and multivariate statistical analyses. Principal component analysis of qPCR data separated exposure groups in 2016 but did not result in clear separation of groups in 2017, although some transcripts showed similar trends in both years. This work has built a strong foundation for future research involving the snow crab hepatopancreas transcriptome and its potential responses to environmental stressors including seismic surveying noise.

1. Introduction

Marine industrial activities such as shipping, oil and gas exploration, pile driving, and military sonar use have increased ocean noise levels by about 15 dB, approximately 3 dB (a doubling) each decade from the 1950s to the early 2000s (Andrew et al., 2011; Hildebrand, 2009). Among the various noise sources, seismic surveying by the oil and gas industry now contributes to human-sourced noise throughout the world's oceans (Nieukirk et al., 2012; Nowacek et al., 2015). Seismic surveying has occurred off the east coast of Newfoundland and Labrador

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(NL), Canada, since the 1960s; however, with increasing efforts in this region since the 1990s (https://www.cnlopb.ca/information/geoscience/ accessed July 15, 2019), it has become a growing concern for commercial fish and shellfish harvesters. More specifically, since the mid-1990s, the NL shelf has supported a snow crab fishery, which is not only the world's largest (Mullowney and Dawe, 2009), but also the highest-valued fishery ($325 million CDN in 2017) in NL (https://www.dfo-mpo.gc.ca/fisheries-peches/ifmp-gmp/snow-crab-neige/2019/index-eng.html). This fishery peaked in 2009 but has gradually declined since then (Mullowney et al., 2018a,b). Therefore, assessing if the increase in seismic activity is a contributing factor to the decline in the snow crab fishery in NL is a concern for the fishing industry.

Although the effects of sound on marine life vary depending on the species as well as the type and level of exposure, it has been reported to cause death, permanent or temporary physiological damage, and behavioural and gene expression changes (Ellison and Frankel, 2012; Dooling et al., 2015; Hawkins and Popper, 2017; MacGregor et al., 2016; Andrews et al., 2014). Most animals are not located close enough to a loud sound source to cause immediate death or physical damage (Halvorsen et al., 2012; Hawkins and Popper, 2017); however, there are reported exceptions for marine mammals exposed to naval sonar (D'Amico et al., 2009, Tyack et al., 2011; Deruiter et al., 2013). While much of the work has focused on mammals and fishes (Carroll et al., 2017; Edmunds et al., 2016; Tifau and Briffa, 2016), the impact of sound on invertebrates is not well understood; however, invertebrates detect sound (Pye and Watson, 2004) and are affected by it. Indeed, studies indicate that sound can cause mortality (Day et al., 2017; McCauley et al., 2017), or physiological (Payne et al., 2008; Day et al., 2017; Fitzbibbon et al., 2017) and behavioural (Day et al., 2017) changes, depending on the level of exposure in invertebrates. More specifically, alarm and startle reactions (Fewtrell and McCauley, 2012), metabolic state (Spiga et al., 2016), and feeding patterns (Payne et al., 2007) can be influenced by noise. Therefore, to better measure subtle impacts of seismic surveying on invertebrates, a multi-facetted approach incorporating catch rates as well as various other physiological metrics, including genetic and genomic responses (e.g. transcript expression), is recommended (Kight and Swaddle, 2011).

In this study, we investigated the transcriptomic responses of snow crab hepatopancreas to industry-based seismic surveying on commercial snow crab fishing grounds. Seismic activity has been shown to influence feeding in shellfish including snow crab (Payne et al., 2008), and it is known that the hepatopancreas organ functions in the absorption and storage of nutrients and the production of important enzymes for food digestion (Vogt et al., 1989; Vogt, 1994). In addition to its digestion/absorption associated roles, the crustacean hepatopancreas is also involved in other biological processes including immune responses (Li et al., 2013; Clark et al., 2013) and responses to environmental stressors such as heavy metals (Sun et al., 2016). Therefore, the hepatopancreas is a suitable target organ for transcriptomic studies to measure the impact of seismic surveying (i.e. loud sound) exposure on snow crab physiology and health. To date, this transcriptomics approach has not previously been applied to study seismic survey impacts on crab species; however, this approach has been assayed in other animals (e.g. Andrews et al., 2014; Fields et al., 2019).

We investigated the transcriptomic responses of snow crab to industry-based seismic surveying by using RNA sequencing (RNA-seq) to identify candidate biomarker transcripts that could then be confirmed using real-time quantitative polymerase chain reaction (qPCR). While RNA-seq has previously been used to study other crab species' hepatopancreas transcriptomes [e.g. responses to molting (Huang et al., 2015), cadmium exposure (Sun et al., 2016), disease (Chen et al., 2017), or various diets (Wei et al., 2017; Zhou et al., 2019)], ours is the first transcriptome study on *Chionoecetes opilio* and the first to investigate the potential impact of seismic noise on the crab hepatopancreas transcriptome. To better understand the scope and level of seismic surveying effects on commercial snow crab, the research conducted herein is part of a multi-facetted approach that also included catch rates (Morris et al., 2018; Morris et al., 2020) and foraging behaviour (Cote et al., 2020) analyses. This research aimed to provide scientific information to better manage and mitigate the potentially negative effects of marine industries on snow crab physiology and health.

2. Methods

2.1. *2016 Study design and sampling*

For the 2016 study, samples were collected in conjunction with a recent study examining the effects of seismic surveying on commercial catch rates (Morris et al., 2018), which provides additional information on the study locations, snow crab collection methods, and seismic exposure.

2.1.1. *Geographical location*

This study was conducted along the continental slope of the Newfoundland Grand Banks as depicted in Morris et al., 2020 (see Supplemental File 1 in our study for this map). The sites were selected by commercial snow crab harvesters based on their experience and observations; this study area is an important commercial fishing area and location where seismic effects on snow crab catch rates might be expected to occur. The seismic vessel used as part of this study conducted its experimental exposure at Carson Canyon (CC: 45 27.00 N, 48 43.00 W), which is located ~70 km away from our non-seismic control site at Lilly Canyon (LC: 44 54.00 N, 49 13.50 W). This distance ensured that the non-seismic LC site would be unaffected by seismic air-gun exposures at CC. The cumulative noise levels at the non-seismic LC site were similar to or less than the noise level generated by fishing vessels (Morris et al., 2018); since this study was primarily concerned with the impact of seismic exploration on commercial fishing operations, we accepted the noise level from fishing vessels as a regular part of fishing operations. Both study sites (i.e. CC and LC) are similar in terms of bathymetric relief and environmental conditions, and are part of the same snow crab fishery management regime.

2.1.2. *2D seismic exposure*

The *Atlantic Explorer* [owned and operated by Petroleum GeoServices (PGS)] is the seismic survey vessel that provided the seismic exposure for our experiment. The airgun source array had a total volume of 4880 cubic inches, with shots at 10 s intervals, operated at 2000 psi and was deployed at 9 m depth. Acoustic recording data were collected on the seafloor at a depth of 115 m, within 10 km from where the snow crab were sampled. Sound exposure data were collected using an AMAR acoustic recorder (JASCO Applied Sciences). The modelled horizontal zero-to-peak sound pressure level was 251 dB re μPa deployed at 1 m depth and the source sound exposure level 229 dB re 1 $\mu Pa^2 \cdot s$ deployed at 1 m depth. The exposure lasted 2 h, and the vessel passed within 100 m from our acoustic recorders and within a few hundred meters from where our experimental crab pots were located (see details below). The full recorded sound spectrum for 2016, including natural sources such as wind, waves, and marine mammals is provided in Morris et al., 2018 (see Figure 4 of that article).

2.1.3. *Snow crab collection and sampling*

Snow crab (all male) were captured as part of two (September 18–25, 2016 and October 17–21, 2016) catch surveys that were conducted by industry harvesters using standard industry survey methods as described in the Fish Food and Allied Workers (FFAW's) Post-Season Snow Crab Pot Survey (Stansbury et al., 2013). Seismic exposure occurred on September 22, 2016. Briefly, a single fleet of fishing gear included 10 commercial crab pots each spaced 45 m apart and connected to the same ground-line, having an anchor and surface float at one end to recover the fishing gear. Each pot was baited with 1.3 kg of squid. Pots were set to depths of 160−170 m at commercial fishing locations, the location was recorded, and pots were allowed to soak for a minimum of 12 h before retrieval.

Crab pots were deployed equally before and after exposure at the same locations for both our non-seismic (LC) and seismic (CC) exposure areas. The exact position of crab pots likely varied by a few hundred meters between sets, depending on environmental conditions and vessel operations. At the CC site, snow crab collected immediately before seismic exposure [i.e. pre-seismic Carson Canyon (CC)] were sampled from pots that fished for 12 h and were then recovered within a few hours before seismic exposure began on September 22, 2016. Snow crab collected immediately after seismic exposure were sampled from pots that were set immediately after the seismic exposure ended and were then allowed to fish for 12 h before the pots were recovered on September 23, 2016 [i.e. Carson Canyon 18 h post-seismic exposure (CC18h)]. Non-seismic control site snow crab were collected at Lilly Canyon on September 24, 2016 (LC). Additional snow crabs were collected at CC during the second catch survey on October 20, 2016, approximately 3 weeks after seismic exposure (CC3wk).

To obtain tissues for the functional genomics and molecular analyses conducted herein, at least 30 snow crab per location were immediately removed from the crab pots and placed into a cold seawater holding tank; tank water was maintained at the same temperature as bottom water (1−2 °C). All snow crab sampled were large terminally molted commercial size (>93 mm carapace width) males and were smaller than 120 mm in carapace width. Sampling of individual snow crab was conducted inside an unheated, sheltered deck on the vessel where environmental conditions were cool (~10 °C). Snow crab were dissected on a laboratory-grade work surface (Fisher Scientific). It took approximately 2 min to dissect and preserve tissue samples from each individual snow crab, while the time to collect an entire set of samples was less than 3 h, allowing time to clean dissection tools and surfaces with RNaseZap (Sigma) between individual sampling. For our study, each individual hepatopancreas sample (~100 mg tissue) was added to a 1.5 ml RNase-free tube containing 1 mL of RNAlater RNA Stabilization Solution (Ambion/Thermo Fisher Scientific), kept at 4 °C for 12 h and then flash frozen on dry ice. The frozen samples were transported to the lab and immediately transferred to −80 °C freezers where they were stored until RNA extraction.

2.2. 2017 Study design and sampling

2.2.1. Geographical location and seismic exposure

The 2D seismic exposure at CC occurred September 12, 2017. It utilized the same vessel, seismic array and vessel path as the exposure in 2016 described above and in Morris et al. (2018).

A 3D seismic survey was also conducted in 2017, as part of an industry-based survey located 30 km north of CC (see Morris et al., 2020). The *Ramform Titan*, also owned and operated by PGS, was the seismic survey vessel that provided the 3D seismic exposure for our experiment. The 3D sound source included a volume of 4130 cubic inches, with a 25 m shot spacing (~10 s), operated at 2000 psi, and was deployed at 7 m depth. In the 3D survey, crabs were exposed to seismic surveying from August 2 to October 4, 2017. Seismic surveying progressed from east to west, with parallel vessel transect survey lines spaced 800 m apart (see Morris et al., 2020 for soundscape measurement and exposure details) and passed directly over our sampling location prior to sample collection, ensuring a representative exposure to realistic industry-based seismic surveying.

2.2.2. Snow crab collection and sampling

Snow crab were captured and collected using the same methods as in 2016 (as described above and in Morris et al., 2018). Snow crab adults (n = 30 per group) were collected in eight groups from three sites. Consistent with the previous year, all snow crab sampled were large terminally molted commercial size (>93 mm carapace width) males and were smaller than 120 mm in carapace width. As in 2016, non-seismic LC was the control site and CC was the 2D seismic exposure site, with North of CC (NC) the additional 3D seismic exposure site. At the CC site, crabs were collected before 2D seismic exposure (BSC), and 1 day (1DASC), 2 days (2DASC) and 6 weeks (6WASC) after 2D seismic exposure. At the LC non-seismic site, crabs were collected in a time-matched fashion with the BSC and 6WASC samples, and denoted as BSL and 6WASL, respectively. At the North of CC site, crabs were collected during (September 15; DSNC) and 4 weeks after (October 31; 4WASNC) 3D seismic exposure.

Snow crab were sampled as described above except the individual hepatopancreas samples were each placed in a 1.5 mL RNase-free tube and immediately flash frozen on dry ice (i.e. not stored in RNAlater RNA Stabilization Solution). The frozen samples were transported to the lab and immediately transferred to an −80 °C freezer where they were stored until RNA extraction.

2.3. RNA preparation and integrity assessment

For our study, the goal of the RNA-seq experiment was to identify candidate biomarker transcripts for qPCR confirmation. In the 2016 study, RNA was extracted from the first 25 snow crab hepatopancreas samples from each of the four groups (i.e. LC, CC, CC18h and CC3wk) using TRIzol Reagent (Invitrogen/Thermo Fisher Scientific). The frozen tissues (in RNAlater) were thawed at room temperature, immediately transferred to new 1.5 mL RNase-free tubes and each homogenized in 400 μL of TRIzol using a motorized Kontes RNase-Free Pellet Pestle Grinder (Kimble Chase, Vineland, NJ). In the 2017 study, RNA was extracted from the first 16 snow crab hepatopancreas samples from each of the eight groups (i.e. BSC, 1DASC, 2DASC, 6WASC, BSL, 6WASL, DSNC and 4WASNC) using TRIzol Reagent; however, the frozen tissues were immediately transferred to new 1.5 mL RNase-free tubes and homogenized in TRIzol as above. Thereafter, the RNA extractions from both studies were completed as follows. An additional 400 μL of TRIzol was added, mixed by pipetting, and the homogenates frozen on dry ice and stored at −80 °C. Frozen homogenates were further processed by thawing them on wet ice and then passing them through QIAshredder (QIAGEN, Mississauga, ON) spin columns following the manufacturer's instructions. Next, 200 μl of TRIzol was added to each sample to make a total homogenate volume of approximately 1 mL. The TRIzol total RNA extractions were then completed following the manufacturer's instructions.

The TRIzol-extracted RNA samples had low A260/230 ratios, which interferes with column purification. Therefore, subsamples [50 μg (2016 study); 120 μg (2017 study)] were re-extracted using the phenol-chloroform phase separation method. Briefly, to separate the TRIzol-extracted RNA from organic materials, an equal volume of acid phenol:chloroform:isoamyl alcohol (125:24:1) (pH 4.5) (AM9720; Ambion/Thermo Fisher Scientific) was added to each sample, vortexed for 30 s and centrifuged at 16,100 x g at 4 °C for 20 min. The RNA was then recovered from the aqueous layer from the previous step by precipitation with 0.1 volumes of 3 M sodium acetate (pH 5.5) (AM9740; Ambion/Thermo Fisher Scientific) and 2.5 volumes of anhydrous ethyl alcohol (Commercial Alcohols Inc., Brampton, ON) at −80 °C for 1 h, followed by centrifugation at 16,100 x g at 4 °C for 30 min. To wash the resulting RNA pellet, 1 mL of 75 % ethanol was added and centrifuged at 16,100 x g at 4 °C for 20 min. The ethanol was removed, and the RNA pellet was air-dried at room temperature for 10 min and resuspended in nuclease-free water (Invitrogen/Thermo Fisher Scientific).

The acid phenol:chloroform:isoamyl alcohol extracted RNA samples (45 μg) were then each treated with 6.8 Kunitz units of DNaseI (RNase-Free DNase Set, QIAGEN) with the manufacturer's buffer (1X final concentration) at room temperature for 10 min to degrade any residual genomic DNA. DNase-treated RNA samples were column-purified using the RNeasy Mini Kit (QIAGEN) following the manufacturer's instructions. RNA integrity was verified by 1% agarose gel electrophoresis, and RNA purity was assessed by A260/280 and A260/230 NanoDrop UV spectrophotometry for both the pre-cleaned and the column-purified RNA samples.

In the 2016 study, 10 samples from each of the four groups were selected for molecular (RNA-seq and qPCR) analyses based on RNA integrity and with A260/280 and A260/230 ratios > 2. In the 2017 study, 10 samples from each of the eight groups were selected, using the same criteria, but for qPCR analyses only.

2.4. RNA sequencing (RNA-seq) and analysis

2.4.1. RNA-seq library construction and sequencing

Library construction and sequencing services were performed at the McGill University and Génome Québec Innovation Centre, Montréal, QC, Canada. Prior to library construction, total RNA integrity was further validated using the 2100 Bioanalyzer system (Agilent). Libraries were generated from 250 ng of total RNA. Briefly, mRNA enrichment was performed using the NEBNext Poly(A) Magnetic Isolation Module (New England BioLabs) following the manufacturer's instructions. cDNA was synthesized using the NEBNext RNA First Strand Synthesis Module and NEBNext Ultra Directional RNA Second Strand Synthesis Module (New England BioLabs) following the manufacturer's instructions. Library preparation was then completed using the NEBNext Ultra II DNA Library Prep Kit for Illumina (New England BioLabs) following the manufacturer's instructions. Adapters and PCR primers were purchased from New England BioLabs. Libraries were quantified using the Quant-iT™ PicoGreen® dsDNA Assay Kit (Life Technologies) and the Kapa Illumina GA with Revised Primers-SYBR Fast Universal Kit (Kapa Biosystems) following the manufacturer's instructions. Average fragment size was determined using the LabChip GX (PerkinElmer).

The libraries were normalized and pooled, denatured in 0.05 N NaOH, and then neutralized using HT1 buffer (Illumina). ExAMP (Illumina) was added to the mix following the manufacturer's instructions. The pool was loaded at 200 pM on a cBot (Illumina) and the flowcell was run on a HiSeq 4000 (Illumina) for 2 × 100 cycles (paired-end mode). A phiX library was used as a control and mixed with libraries at the 1% level. The Illumina control software was HCS HD 3.4.0.38 and the real-time analysis program was RTA v. 2.7.7. The program bcl2fastq2 v2.18 was used to demultiplex the samples and generate fastq reads.

2.4.2. De novo transcriptome assembly, contig abundance and annotation

Reads were trimmed from the 3′ end to a minimum Phred score of 30. Illumina sequencing adapters were removed from the reads, and reads < 50 bp were excluded. These trimming and clipping steps were performed using Trimmomatic (Bolger et al., 2014). Reads were assembled into a *de novo* transcriptome using Trinity software (v2.4.0) with default parameters (Haas et al., 2013). Assembled contigs shorter than 200 bp were discarded. Kallisto (v. 0.43.1) (Bray et al., 2016) was used to quantify contig abundance [raw and transcripts per million (tpm) normalized counts]. The *de novo* assembled contigs were annotated using the BLAST (Altschul et al., 1997) program (BLASTx with default parameters was used to search protein databases using a translated nucleotide query) on three major protein databases (nr NCBI non-redundant protein database, SwissProt and UniProt UniRef90 Reference Clusters). An Expect value (i.e. E-value) cutoff of $< 10^{-6}$ was used. A Trinotate annotation pipeline (Bryant et al., 2017) was used for functional annotation including homology search to known sequence data (BLAST+/SwissProt/UniRef90), protein domain identification (HMMER/PFAM), protein signal peptide and transmembrane domain prediction (signalP/tmHMM), and comparison to currently curated annotation databases (EMBL UniProt eggNOG/GO Pathways databases) (Supplemental File 2).

2.4.3. Differential expression analysis

Differential transcript expression between sample groups (i.e. LC, CC, CC18h and CC3wk) was estimated using DESeq2 (Love et al., 2014) and edgeR (Robinson et al., 2009) programs. Two approaches were used here, as the compositional nature of RNA-seq output (i.e. sparsity, transcript counts not being proportional to the sample's total RNA content) represents a well-known challenge to normalization and the use of RNA-seq counts to compare different samples (Quinn et al., 2018). Both methods are included, as both are based on a common – and reasonable – assumption that most of the genes are not differentially expressed between two different conditions, but rely on different normalization approaches, capturing distinct projections of the same biological question. Both methods thus provide a more comprehensive way to observe the difference between conditions. Within each pairwise comparison, only transcripts with a Benjamini-Hochberg adjusted p-value lower than or equal to 0.05 were considered significantly differentially expressed. For differentially expressed transcripts (DET) selected for qPCR (see below), functional annotations [i.e. gene ontology (GO) terms] for molecular function (MF), cellular component (CC), and biological process (BP) were identified and extracted using GeneCards (Stelzer et al., 2016) and UniProtKB (UniProt Consortium, 2019).

2.5. Real-time quantitative polymerase chain reaction (qPCR)

2.5.1. qPCR overview

In the 2016 study, to confirm the results of the RNA-seq analyses, 83 qPCR assays were designed to measure expression levels of transcripts identified as differentially expressed in any one of the six pairwise comparisons (CC18h vs. CC; CC3wk vs. CC; CC3wk vs. CC18h; LC vs. CC; CC18h vs. LC; CC3wk vs. LC). In a preliminary qPCR analysis, levels of these transcripts of interest (TOIs) were measured in 14 samples (i.e. 7 samples from each of the 2 groups in which the TOI was identified as differentially expressed in RNA-seq studies). In a second qPCR analysis, levels of a subset ($n = 34$) of these TOIs were measured in the 40 samples ($n = 10$ per each of the four groups) that had been subjected to RNA-seq analyses. These transcripts were selected as they were either significantly differentially expressed ($p < 0.05$; $n = 26$), showing a non-significant trend ($0.05 < p < 0.10$; $n = 7$), or with high fold changes [*crustacean hyperglycemic hormone* (*chh*)] in the preliminary qPCR analysis.

In the 2017 study, a preliminary qPCR analysis was performed in which levels of the aforementioned 83 transcripts were measured in cDNA pools representing the eight groups in this study. This analysis was performed to help select the transcripts to be assessed in individual 2017 samples. In a second qPCR analysis, expression levels of 23 transcripts (20 of which were assessed in all samples in both the 2016 and 2017 studies) were measured in 80 individual 2017 samples ($n = 10$ per each of the eight groups). Seventeen of these transcripts were selected as they were qPCR confirmed as having either a significant ($p < 0.05$; $n = 9$) (Fig. 1A-I), or a non-significant trend ($0.05 < p < 0.10$; $n = 3$) (Fig. 1J-L) toward higher expression in at least one time point after seismic exposure compared with pre-seismic at the Carson Canyon 2D seismic experimental site in 2016, or agreed with this direction of change ($n = 5$) (Fig. 1M-Q). The application of these candidate seismic-responsive biomarkers to the 2017 samples would determine if expression levels of these transcripts are reproducible across years in the field in response to 2D seismic noise, as well as provide insight into their response to 3D seismic noise, which was not assessed in the 2016 study (i.e. if they are consistent seismic-responsive molecular biomarkers). The additional 6 transcripts (totalling to 23) were selected as they appeared to be differentially expressed in response to seismic noise in the pooled sample screen. Three were either significantly [*cryptocyanin* (*cc*) (DN117425_c1_g1_i3), *carboxypeptidase b* (*cpb*)] or with fold-changes matching the RNA-seq results [*heme binding protein 2* (*hbp2*); $p = 0.217$] higher expressed at the CC experimental site compared with the non-seismic LC site in 2016 (Fig. 2A,L,S, respectively); whereas *lipase 3* (*lip3*), *hemocyanin* (*hcy*) and *sodium dependent nutrient amino acid transporter 1* (*naat1*) had not been assessed in all samples in the 2016 study.

2.5.2. cDNA synthesis and qPCR parameters

First-strand cDNA templates for qPCR were synthesized in 20 μL

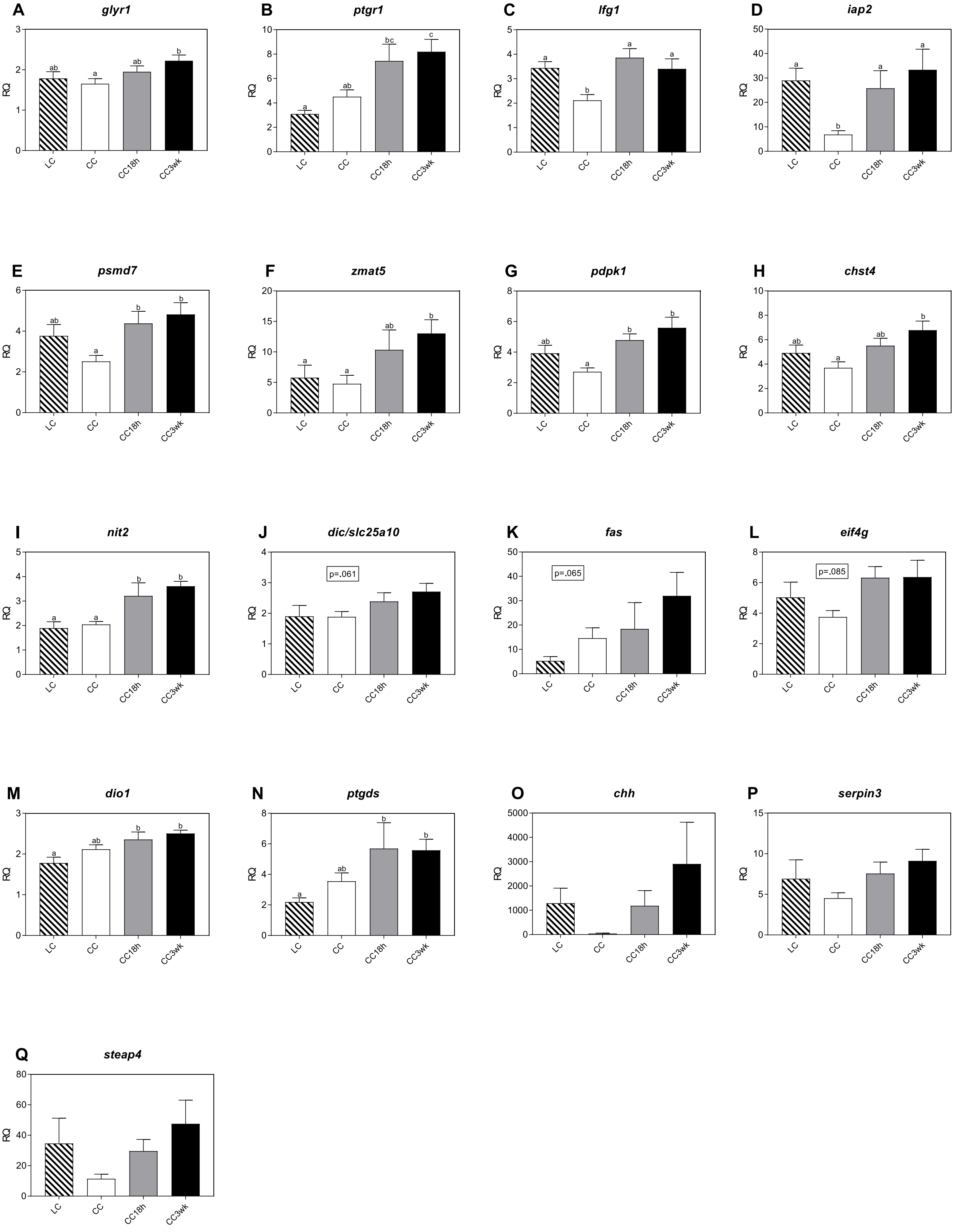

Fig. 1. RNA-seq identified and qPCR confirmed transcripts that were significantly differentially expressed ($p < 0.05$) (A-I), showing a non-significant trend ($0.05 < p < 0.10$) (J-L) or with fold-changes that were higher (M-Q) in the hepatopancreas of crab from Carson Canyon that were exposed to 2D seismic noise compared with pre-seismic control crab from Carson Canyon in the 2016 study. Expression levels of selected transcripts identified as differentially expressed in RNA-seq studies were measured in hepatopancreas samples from crab collected in four groups [Carson Canyon before 2D seismic exposure (CC); Carson Canyon 18 h after 2D seismic exposure (CC18h); Carson Canyon 3 weeks after 2D seismic exposure (CC3wk); Lilly Canyon non-seismic control site 2 days after 2D seismic exposure at Carson Canyon (LC)] using qPCR. Transcript levels are presented as mean ± SE relative quantity (RQ) values (i.e. values for the transcript of interest were normalized to both *rpl32* and *ef1a* transcript levels and were calibrated to the individual with the lowest normalized expression level of that given transcript). Letters indicate Tukey's HSD groupings. In all cases, $n = 10$.

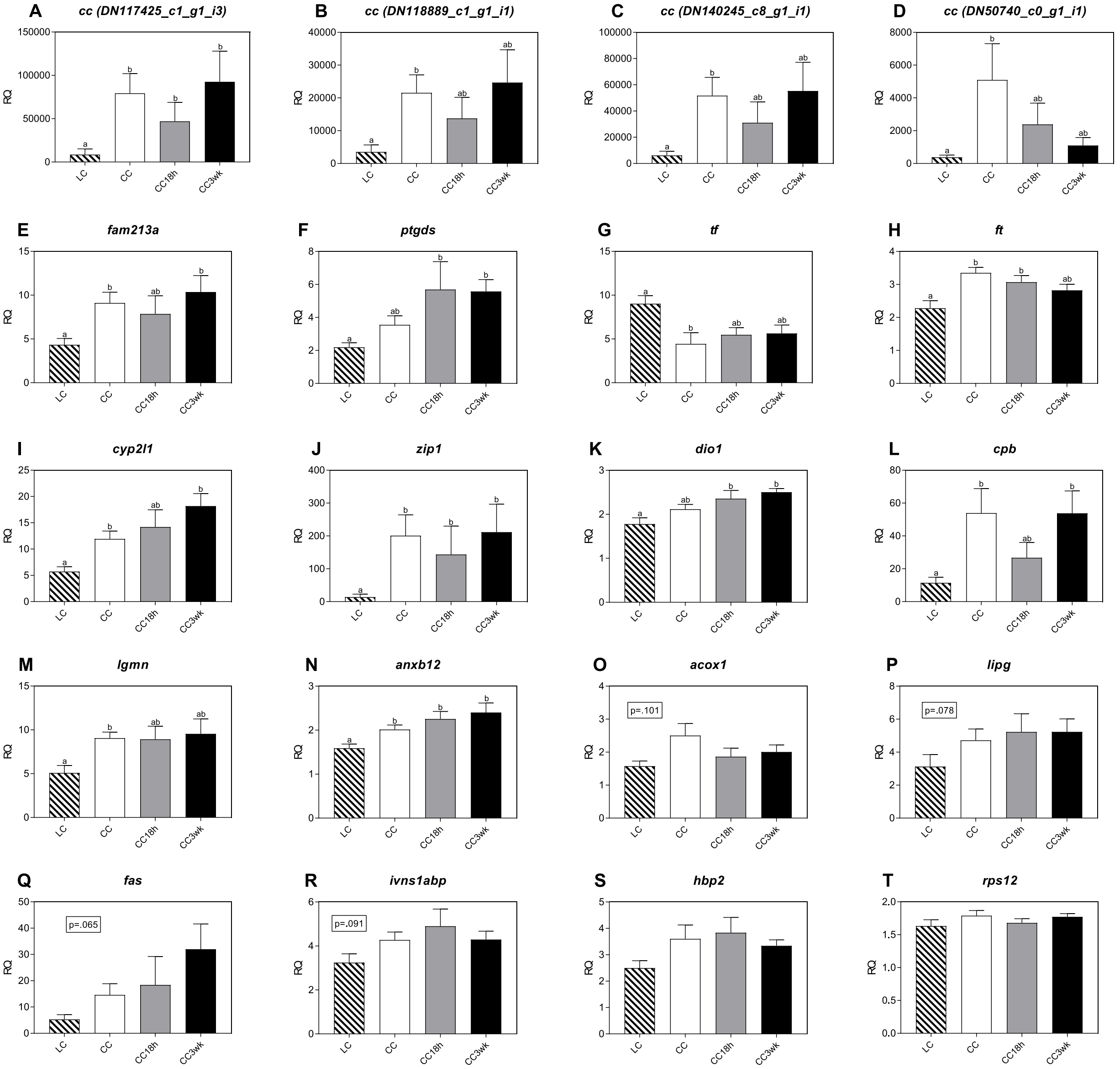

Fig. 2. RNA-seq identified and qPCR confirmed transcripts that were significantly differentially expressed ($p < 0.05$) (A-N), showing a non-significant trend ($0.05 < p < 0.10$) (O-R) or with fold-changes that were higher (S,T) in the hepatopancreas of crab from Carson Canyon compared with Lilly Canyon (non-seismic control site) in the 2016 study. Expression levels of selected transcripts identified as differentially expressed in RNA-seq studies were measured in hepatopancreas samples from crab collected in four groups [Carson Canyon before 2D seismic exposure (CC); Carson Canyon 18 h after 2D seismic exposure (CC18h); Carson Canyon 3 weeks after 2D seismic exposure (CC3wk); Lilly Canyon non-seismic control site 2 days after 2D seismic exposure at Carson Canyon (LC)] using qPCR. Transcript levels are presented as mean ± SE relative quantity (RQ) values (i.e. values for the transcript of interest were normalized to both *rpl32* and *ef1a* transcript levels and were calibrated to the individual with the lowest normalized expression level of that given transcript). Letters indicate Tukey's HSD groupings. In all cases, n = 10.

reactions from 1 μg of DNaseI-treated, column-purified total RNA using random primers (250 ng; Invitrogen/Thermo Fisher Scientific), dNTPs (0.5 mM final concentration; Invitrogen/Thermo Fisher Scientific) and M-MLV reverse transcriptase (200 U; Invitrogen/Thermo Fisher Scientific) with the manufacturer's first strand buffer (1X final concentration) and DTT (10 mM final concentration) at 37 °C for 50 min. To generate the pools for the 2017 study, a subsample of each individual cDNA template (n = 10) from a given group (n = 8) was included in that pool.

PCR amplifications were performed in 13 μL reactions using 1X Power SYBR Green PCR Master Mix (Applied Biosystems/Thermo Fisher Scientific), 50 nM of both the forward and reverse primers, and the indicated cDNA quantity (see below). Amplifications were performed using the ViiA 7 Real Time PCR system (384-well format) (Applied Biosystems/Thermo Fisher Scientific). The real-time analysis program consisted of 1 cycle of 50°C for 2 min, 1 cycle of 95°C for 10 min and 40 cycles of 95 °C for 15 s and 60 °C for 1 min, with fluorescence detection at the end of each 60 °C step, and was followed by dissociation curve analysis.

2.5.3. Primer design and quality assurance testing

To confirm the identities of all transcripts subjected to qPCR analyses and to identify the coding sequences (CDS), a BLASTx 2.7.0+ search of the non-redundant (nr) protein sequences database using a translated nucleotide query was performed between September and October 2017 (Supplemental Table 1). All primers are located in the CDS and in an area that overlapped with that of the best BLASTx-identified sequence. The amplicon size range was between 90–150 bp. Primers were designed using Primer3 (Koressaar and Remm, 2007; Untergasser et al., 2012); however, in the case of the cryptocyanin gene paralogues, they were hand-designed in paralogue-specific areas to ensure specificity. Each primer pair was quality control (QC)-tested to ensure that a single product was amplified (dissociation curve analysis) and that there was no primer-dimer present in the no-template control. Amplicons were electrophoretically separated on 2% agarose gels and compared with a 1 kb plus ladder (Invitrogen/Thermo Fisher Scientific) to verify that the correct size fragment was being amplified. Finally, amplification efficiencies (Pfaffl, 2001) were calculated for cDNA pools representing the two groups in which the TOI had been identified as differentially expressed in RNA-seq studies. Briefly, cDNAs were synthesized (as described in section 2.5.2) for each of the individual RNA samples that had been subjected to RNA-seq analyses. The 10 cDNAs from each group were then pooled and standard curves generated using a 5-point 1:3 dilution series starting with cDNA representing 10 ng of input total RNA. The reported efficiencies (Supplemental Table 2) are an average of the two values with the exception of *chh* and *metalloreductase steap4* (*steap4*) (CC3wk only) and *zinc transporter zip1* (*zip1*) (CC only) due to low expression levels in the other group. These same efficiencies were used in the 2017 study. The sequences, amplicon sizes and efficiencies for all primer pairs used in the qPCR analyses are presented in Supplemental Table 2.

2.5.4. *Endogenous control (normalizer) selection*

Expression levels of the TOIs were normalized to transcript levels of two endogenous controls. To select these endogenous controls, 8 transcripts [*ribosomal protein L10* (*rpl10*), *ribosomal protein 49* (*rpl32*), *40S ribosomal protein S12* (*rps12*), *elongation factor 1-alpha* (*ef1a*), *ras homolog enriched in brain* (*rheb*), *V-type proton ATPase subunit D* (*vpatpd*), *ring finger protein 157* (*rnf157*) and *annexin A4* (*anxa4*)] whose expression levels were stable in the two pools from the primer QC study were analyzed. Briefly, the fluorescence threshold cycle (C_T) values of 24 samples (6 samples from each of the four groups) were measured (in duplicate) for each of these transcripts using cDNA representing 4 ng of input total RNA, and then analyzed using geNorm (Vandesompele et al., 2002). Based on this analysis, *rpl32* (geNorm M = 0.14) and *ef1a* (geNorm M = 0.16) were selected as the two endogenous controls. In the 2017 study, the C_T values of 35 samples (5 samples from each of the 1DASC, 2DASC and DSNC groups; 4 samples from each of the other 5 groups) were measured and analyzed as above for these same transcripts. *Rpl32* (geNorm M = 0.21) and *ef1a* (geNorm M = 0.24) were again selected as the two endogenous control genes.

2.5.5. *Experimental qPCR analyses*

In all four experimental qPCR analyses, cDNA representing 4 ng of input RNA was used as template in the PCRs. On each plate, for every sample, the TOIs and endogenous controls were tested in triplicate and a no-template control was included. In the two studies where expression levels of a given TOI were measured across multiple plates, a plate linker sample (i.e. a sample that was run on all plates in a given study) was also included to ensure there was no plate-to-plate variability. The relative quantity (RQ) of each transcript was determined using the ViiA 7 Software Relative Quantification Study Application (Version 1.2.3) (Applied Biosystems/Life Technologies), with normalization to both *rpl32* and *ef1a* transcript levels, and with amplification efficiencies incorporated. For each TOI, the sample with the lowest normalized expression (mRNA) level was set as the calibrator sample (i.e. assigned an RQ value = 1.0).

2.5.6. *Statistical analysis*

Preliminary qPCR analysis for the 2016 study: In the preliminary qPCR analysis in the 2016 study, as there were some cases where values did not exhibit homogeneity of variance, the Mann-Whitney test was used to assess if there was a significant difference in expression of a given TOI between the 2 groups in which it was identified as being differentially expressed in RNA-seq analyses.

Targeted qPCR analysis for 2016 and 2017 studies: In the second qPCR analysis involving 2016 samples (i.e. in which the TOIs were assessed in individuals from all four groups in the study), and in the individual sample qPCR analysis involving 2017 samples (i.e. in which the TOIs were assessed in individuals from all eight groups in the study), transcript expression data were $\log_{10}$ transformed and one-way ANOVA followed by Tukey's B post-hoc test were used to assess expression levels of a given TOI in the four or eight groups, respectively. In all cases, $p < 0.05$ was considered to be statistically significant. All data are expressed as mean ± standard error (S.E.).

Next, a subset of transcripts (n = 20) from these qPCR analyses that overlapped between 2016 and 2017 studies were subjected to multivariate statistical analyses. For each year, transcript expression data were collapsed into principal components (PCs) using prcomp function in R and plotted using the *ggbiplot* R package (Vu, 2011). Prior to analyses, transcript expression data were standardized (centered and scaled) using the scale function in R. Using PCA, scores on PC axis 1 and 2 among sample groups (four groups in 2016 and eight groups in 2017) were compared using one-way ANOVA in R. Although some data violated assumptions of normality and/or homogeneity of variance for parametric analyses, the ANOVA is generally found to be robust enough to deal with such issues (Underwood, 1981). For significant comparisons, Tukey's post-hoc test was used to test pairwise differences between groups using the function TukeyHSD in R. Next, the contribution of different genes (loadings) on PC1 and PC2 as well as correlations in transcript expression patterns among all genes were examined. For both years, the top 5 genes that loaded the highest on each PC axis were identified.

3. Results

3.1. *Snow crab hepatopancreas reference transcriptome assembly and RNA-seq analyses*

3.1.1. *Transcriptome sequencing and assembly*

RNA-seq libraries were generated for 40 individual snow crab hepatopancreas samples from the 2016 study: 10 each of CC, CC18h, CC3wk and LC. The samples have been deposited in the NCBI BioSample database under accession numbers SAMN11104802-SAMN11104811 (CC), SAMN11104812-SAMN11104821 (CC18h), SAMN11104832-SAMN11104841 (CC3wk), and SAMN11104822-SAMN11104831 (LC). All libraries have been deposited in the NCBI Sequence Read Archive (SRA) under accession number PRJNA526613.

A total of 1.919×10^9 raw reads (100 bases each) were generated from the 4 sample groups (with 10 biological replicate libraries per group); between 4.57×10^8 and 4.95×10^8 raw reads were obtained from each CC, CC18h, CC3wk and LC (see Table 1 for additional details

Table 1

Snow crab hepatopancreas RNA-seq Illumina read quality control.

Sample group	Raw 100 base reads (x 10^6)	Read length (bases)	Reads (100 base) after trimming (x 10^6)	Percentage kept
CC[1]	479	2 × 100	452	94.4%
CC18h[2]	489	2 × 100	464	94.9 %
CC3wk[3]	457	2 × 100	433	94.7%
LC[4]	495	2 × 100	469	94.8 %
TOTAL	1919		1818	94.7%

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[1] CC: Carson Canyon before 2D seismic exposure (10 samples/libraries deposited in NCBI's BioSample database under accession numbers SAMN11104802-SAMN11104811).

[2] CC18h: Carson Canyon 18 h after 2D seismic exposure (10 samples/libraries deposited under accession numbers SAMN11104812-SAMN11104821).

[3] CC3wk: Carson Canyon 3 weeks after 2D seismic exposure (10 samples/libraries deposited under accession numbers SAMN11104832-SAMN11104841).

[4] LC: Lilly Canyon non-seismic site 2 days after 2D seismic exposure at CC site (10 samples/libraries deposited under accession numbers SAMN11104822-SAMN11104831).

on the sample groups). A total of 1.818×10^9 reads remained after trimming low-quality reads, and these quality controlled sequences were used for transcriptome assembly and the identification of candidate differentially expressed transcripts (DETs).

The *de novo* transcriptome assembly of 1.818×10^9 reads yielded 470,453 putative transcripts (lengths from 201 to 19,114 bp; average length: 645 bp) representing 278,540 Trinity genes (Table 2). The total length of all assembled transcripts was 303,412,588 bp, with N50 length of 972 bp and GC content of 45.2 % (Table 2).

3.1.2. Identification of candidate differentially expressed transcripts (DETs)

The number of DETs identified using DESeq2 and edgeR for samples collected from two locations (CC and LC) at different time points (18 h and 3 weeks after seismic exposure for CC only) are summarized in Table 3 (see Supplemental Table 3 for additional information on these DETs). The number of upregulated and downregulated DETs identified using DESeq2 was more than 2.5 and 3 times higher, respectively, when compared with edgeR. The number of candidate seismic-responsive transcripts identified increased with time, i.e. the early time point (18 h) had fewer DETs compared with the later time point (3 weeks). Altogether, a total of 83 upregulated and 109 downregulated DETs were identified in the overlap comparison (i.e. DETs identified by both DESeq2 and edgeR).

3.2. qPCR confirmation of RNA-seq analyses

3.2.1. Transcript selection

A subset (n = 83) of candidate seismic-responsive snow crab hepatopancreas transcripts with significant BLAST hits (and potential functional annotation) was selected for qPCR analyses (Supplemental Tables 1–3). A summary of the BLASTx (performed for primer design), RNA-seq and qPCR analyses for these transcripts is provided in Supplemental Table 1.

3.2.2. qPCR analyses (2016 study)

To confirm the results of the RNA-seq analyses, expression levels of these 83 transcripts were initially measured in hepatopancreas samples from the specific pairwise comparison(s) in which they were identified as differentially expressed. As some of these transcripts were present in multiple pairwise comparisons, 98 analyses were performed [Supplemental Fig. 1a-f; sorted by DEContig ID number (see Supplemental Tables 1 and 3)]. Approximately 73 % of the fold-change values from the qPCR analyses agreed with the direction of change in the RNA-seq analyses; however only ~34 % had p-values < 0.1 (Supplemental Table 1).

Based on these preliminary analyses, expression levels of 34 transcripts that were either significantly differentially expressed ($p < 0.05$; n = 26 transcripts), showing a non-significant trend ($0.05 < p < 0.10$) [(n = 7 transcripts) *carbohydrate sulfotransferase 4* (*chst4*), *eukaryotic translation initiation factor 4 gamma* (*eif4g*), *lifeguard 1* (*lfg1*), *hbp2*, *annexin B12* (*anxb12*), *cc* (DN50740_c0_g1_i1) and *pacifastin heavy chain* (alias *transferrin*) (*tf*)], or with high fold-changes (*chh*), were measured in hepatopancreas samples from all four groups. These data are presented as transcripts found to be either differentially (significant, or non-significant trend) expressed (or which agreed with this change) in crab that were exposed to 2D seismic noise compared with pre-seismic control crab at the CC site (Fig. 1), or in crab sampled at the CC site versus those sampled at the non-seismic LC site (Fig. 2). Three transcripts [*fatty acid synthase* (*fas*), *prostaglandin D synthase* (*ptgds*) and *mitochondrial dicarboxylate carrier* (*dio1*)] are common to Figs. 1 and 2.

At CC, nine transcripts were expressed significantly higher in the hepatopancreas of crab that were exposed to 2D seismic noise (in at least one post-seismic time point group) compared with pre-seismic control crab (Fig. 1A-I); three showed a non-significant trend ($0.05 < p < 0.10$) (Fig. 1J-L), and five had p-values > 0.1 but with fold-changes that were higher post-seismic (Fig. 1M-Q). Functional annotations (i.e. GO terms) associated with candidate seismic-responsive genes that were studied by qPCR include those related to redox homeostasis (e.g. oxidoreductase activity: *glyr1*, *ptgr1*, *dio1*, *steap4*), apoptosis [apoptotic process: *iap2* (alias *birc3*)], immunity (e.g. antigen processing via MHC class I: *psmd7*; neutrophil degranulation: *nit2*), inflammatory response (*chst4*) and prostaglandin metabolic process (*ptgr1* and *ptgds*); for additional information on GO terms associated with genes in Fig. 1, please see Supplemental Table 4.

In the hepatopancreas of crab from the CC experimental site compared with the LC non-seismic control site, 13 transcripts were significantly higher expressed and one, *transferrin* (*tf*), was significantly lower expressed in at least one CC time point group compared with the LC group (Fig. 2A-N); four showed a non-significant trend ($0.05 < p < 0.10$) of higher expression (Fig. 2O-R) and two had p-values > 0.1 but with fold-changes that were higher at CC (Fig. 2S,T). These transcripts include four sequences for cryptocyanin (*cc*), which is involved in molting. When these *cc* translated sequences were aligned with a cryptocyanin 2 protein sequence (ABB59714) from the Dungeness crab (*Metacarcinus magister*) they did not overlap (data not shown); however, expression patterns suggest that the first three could represent the same gene (Fig. 2A-C) and that the fourth may be a paralogue (Fig. 2D). At CC, *cryptocyanin* transcript expression levels were quite variable between individuals (Fig. 2A-D). Functional annotations (i.e. GO terms) associated with genes influenced by geographic location (i.e. differentially expressed between at least one CC group and the LC group) included those related to redox homeostasis [e.g. oxidoreductase activity: *fam213a, cyp2l1, acox1, dio1*; with *dio1* also involved in thyroid hormone generation), iron ion homeostasis (*tf* and *ft*), lipid metabolism (*acox1, fas* and *lipg*), prostaglandin synthesis (*ptdgs*), and immunity (e.g. toll-like receptor signaling pathway: *lgmn*; response to virus: *ivns1abp*); for additional information on GO terms associated with genes in Fig. 2, please see Supplemental Table 4.

3.2.3. qPCR analyses (2017 study)

To provide insight into how the 83 transcripts from the 2016 2D seismic noise study may respond to 2D seismic noise across years in the field as well as to 3D seismic noise (not assessed in 2016), their expression levels were measured in cDNA pools representing the eight groups from the 2017 study. These data are presented in the same order as in Supplemental Fig. 1 (Supplemental Fig. 2).

In a second qPCR analysis, levels of a subset (n = 23 transcripts) of these transcripts were assessed in individual samples from the eight groups in the 2017 study (Fig. 3). Of the 17 transcripts that were either significantly higher expressed, showed a non-significant trend ($0.05 < p < 0.10$) of higher expression after seismic exposure at the CC 2D seismic experimental site in 2016, or agreed with this direction of change, only one (*iap2*) was significantly differentially expressed in the 2017 study but in the opposite direction (i.e. significantly lower expressed in the 4WASNC group compared with the BSL, BSC and 2DASC groups) (Fig. 3D). The two prostaglandin metabolic process-related transcripts (*ptgr1* and *ptgds*) showed a non-significant trend ($0.05 < p < 0.10$) of higher expression with both 2D and 3D seismic exposure with highest

Table 2
Summary statistics of assembled snow crab hepatopancreas transcriptome.

Items	Number
Number of transcripts	470,453
Number of Trinity genes	278,540
Total transcripts length (bp)	303,412,588
Minimum transcript length (bp)	201
Maximum transcript length (bp)	19,114
N50 length (bp)	972
GC content	45.2 %
Number of transcripts < 500 bp	316,642
Number of transcripts > 500 bp	153,811

Table 3

Summary of differentially expressed transcripts/isoforms (DETs) in snow crab hepatopancreas.

DET set	DESeq upreg.[1]	DESeq downreg.[1]	edgeR upreg.[1]	edgeR downreg.[1]	Overlap upreg.[1]	Overlap downreg.[1]	Union of DET sets
CC18h[3] vs. CC[2]	58	39	16	25	10	16	112
CC3wk[4] vs. CC[2]	212	172	77	36	18	36	443
CC3wk[4] vs. CC18h[3]	7	6	17	16	5	3	38
LC[5] vs. CC[2]	223	233	75	61	34	40	518
CC18h[3] vs. LC[5]	12	17	5	13	7	3	37
CC3wk[4] vs. LC[5]	44	65	26	22	9	11	137

[1] Upregulation or downregulation is in the first listed sample group compared with the second listed sample group in a DET set. “Overlap”: DETs in common between DESeq and edgeR analyses. Please see Supplemental Table 3 for additional information on these DET sets.

[2] CC: Carson Canyon before 2D seismic exposure (10 libraries with BioSample database accession numbers SAMN11104802-SAMN11104811).

[3] CC18h: Carson Canyon 18 h after 2D seismic exposure (10 libraries deposited under accession numbers SAMN11104812-SAMN11104821).

[4] CC3wk: Carson Canyon 3 weeks after 2D seismic exposure (10 libraries deposited under accession numbers SAMN11104832-SAMN11104841).

[5] LC: Lilly Canyon non-seismic site 2 days after 2D seismic exposure at CC (10 libraries deposited under accession numbers SAMN11104822-SAMN11104831).

average levels in the 4WASNC group followed by the 6WASC group (Fig. 3B, N). Of the additional six transcripts that appeared to be differentially expressed in response to seismic noise in the pooled sample screen, two were significantly higher expressed; *hbp2* in the 6WASC group compared with the two LC groups (Fig. 3T) and *lip3* in the DSNC group compared with the two LC control groups and the BSC group (Fig. 3U).

3.2.4. *Multivariate analyses in 2016 and 2017*

Principal component analyses (PCAs) were performed on expression data for 20 transcripts that overlapped between the 2016 and 2017 qPCR datasets. For 2016, PCA separated exposure groups along PC axis 1 (ANOVA; $p = 0.013$) and PC axis 2 ($p = 0.0023$) (Fig. 4A,C,E). The first PC axis separated CC samples collected before seismic testing from samples collected after testing at 3 weeks (CC3wk; Tukey post-hoc adjusted $p = 0.01$) (Fig. 4C). The highest loading transcripts on PC1 included *eif4g*, *psmd7*, *chst4*, *pdpk1*, and *serpin3* (Fig. 5C). Expression levels for all of these transcripts were highly and positively correlated (Fig. 5A). Three of these five transcripts (*psmd7*, *chst4*, *pdpk1*) showed significant differences among the groups (p-values < 0.05; Figs. 1,2). For both *pdpk1* and *psmd7*, levels were significantly elevated for samples collected after seismic testing (CC18h and CC3wk) compared with samples collected before (CC) (Fig. 1G,E). *Chst4* levels were significantly elevated 3 weeks after seismic testing (CC3wk) compared with before (CC) (Fig. 1H).

The second PC axis separated the non-seismic LC site from all samples collected at CC (CC, CC18h, and CC3wk) (Tukey's post-hoc adjusted p-values < 0.047). On PC axis 2, transcripts that loaded high were *ptgds*, *ptgr1*, *nit2*, *fas*, and *cpb* (Fig. 4A,E). Expression levels for some of these transcripts were highly correlated (Fig. 5B). Four of these five showed significant differences in levels among the exposure groups ($p < 0.05$; Figs. 1,2). For instance, *ptgds* levels were significantly elevated in samples collected at CC after seismic testing (CC18h and CC3wk) compared with LC (Fig. 1N). The same was true for *ptgr1* and *nit2*, and additional differences were also found for both transcripts, where levels were significantly higher in the CC3wk group compared with CC for *ptgr1* (Fig. 1B) as well as for both time periods after exposure (CC18h, CC3wk) compared with before (CC) for *nit2* (Fig. 1I). *Cpb* levels were significantly higher in the CC and CC3wk groups compared with LC (Fig. 2L).

For 2017, PCA did not reveal significant differences between exposure groups (Fig. 4B). On PC axis 1, no significant differences were found between groups (ANOVA; $p = 0.71$; Fig. 4D). The highest loading transcripts on this PC axis included four of the same five transcripts that loaded high on PC1 in the 2016 data, including *eif4g*, *psmd7*, *pdpk1*, *serpin3*, as well as *dic* (Fig. 5D). Expression levels for all five transcripts were highly and positively correlated (Fig. 5B). Despite similar loadings of these transcripts on PC1 relative to 2016 data, none showed significant differences among exposure groups (Fig. 3). Nonetheless, for these transcripts, levels were generally higher in samples collected after 2D seismic testing at CC in 2016; however, for 2017, transcripts also showed higher values (but not significantly) for some groups after seismic testing (Figs. 1–3).

On PC axis 2, groups from 2017 showed significant differences overall (ANOVA $p = 0.030$; Fig. 4B,F); however, after post-hoc comparisons, no groups were significantly different from each other along PC2 (Tukey's post-hoc test; all adjusted p-values > 0.099). The top five transcripts that loaded high on PC2 were *ptgds*, *ptgr1*, *cpb*, *dio1*, and *cc*_DN117425, and three of these transcripts also loaded the highest on PC2 in 2016 samples (Fig. 5F). Expression levels for many of these transcripts were significantly and positively correlated (Fig. 5B). None of these transcripts showed significant differences in expression among the groups (Fig. 3), except *ptgr1* ($p = 0.043$), although, no comparisons were statistically significant after post-hoc comparison (p-values $>$ 0.11). While expression levels were generally higher after 2D seismic exposure in 2016, transcript levels in 2017 often trended higher (but not statistically significant) after exposure to 2D testing for these same transcripts (Fig. 3).

Given the limited differences found across 2017 samples, we further investigated more subtle differences associated with 2D seismic testing by comparing only samples collected at CC. Using PCA, samples were not separated on the first PC axis, but differences were found on the second PC axis ($p = 0.006$) (Supplemental Fig. 3A-C). On PC2, Tukey's posthoc test revealed that samples collected both before seismic exposure (BSC) and 2 days after seismic exposure (2DASC) differed significantly from samples collected 6 weeks after seismic exposure (6WASC) (Tukey's post-hoc test; adjusted p-values < 0.031) (Supplemental Fig. 3C). Transcripts loading highly on this PC2 axis included *dio1*, *hpb2*, *ptgr1*, *nit2*, and *ptgds*. Using ANOVA, only one of the 20 transcripts showed significant differences in expression among CC samples ($p <$ 0.05). This transcript was *hpb2*, and showed significant difference before seismic testing (BSC) and 6 weeks after (6WASC) (Tukey's post-hoc test; adjusted $p = 0.024$). Other transcripts that approached statistical significance, included *dio1* ($p = 0.096$), *ptgr1* ($p = 0.049$; not significant after post-hoc comparisons), and *ptgds* ($p = 0.065$), which tended to show higher expression levels after seismic activity at CC. In addition, we also examined expression differences for only the samples exposed to 3D seismic testing North of Carson Canyon. PCA did not separate the samples collected during and after 3D seismic exposure along PC axis 1 or 2 (Supplemental Fig. 3D-F) (*t*-test; p-values > 0.26). Nonetheless, we also compared differences for each transcript (*t*-test), and found that only two of the 20 transcripts showed significant differences in levels between samples collected during and after 3D exposure. Expression of *cc*_DN117425 was significantly higher after 3D testing compared with during ($p = 0.036$); whereas, expression of *iap2* was significantly lower after testing compared with during ($p = 0.019$).

4. Discussion

In the current study, we used transcriptomic (RNA-seq) and qPCR analyses to identify and confirm expression levels of candidate

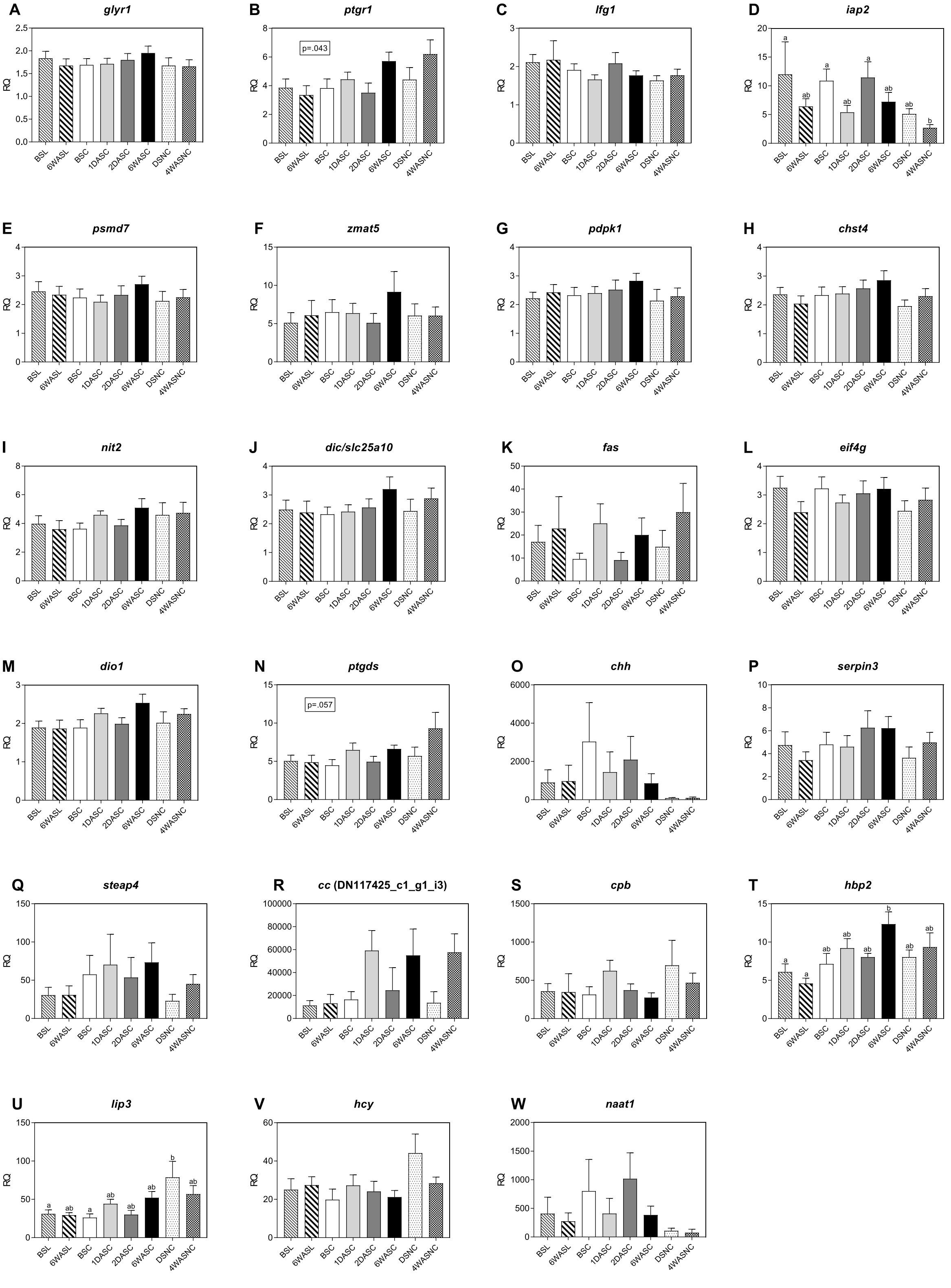

Fig. 3. qPCR analyses of selected transcripts from the 2016 study in the 2017 samples. Expression levels of selected (see *2.5.1 qPCR overview*) transcripts from the 2016 study were measured in hepatopancreas samples from crab collected in 2017 in eight groups. At the Carson Canyon 2D seismic exposure site, crab were collected before (BSC), and 1 day (1DASC), 2 days (2DASC) and 6 weeks (6WASC) after 2D seismic exposure; at the Lilly Canyon non-seismic control site, crab were collected in a time-matched fashion with the BSC and 6WASC samples (BSL, 6WASL); at the North of Carson Canyon 3D seismic exposure site, crab were collected during (DSNC) and 4 weeks after (4WASNC) 3D seismic exposure. Transcripts are ordered based on significance in 2016 [A-Q; see *3.2.2 qPCR analyses (2016 study)*] followed by those which appeared to be seismic-responsive based on the pooled sample screen (R-W; see *3.2.3 qPCR analyses (2017 study)*]. Expression levels are presented as mean ± SE relative quantity (RQ) values (i.e. values for the transcript of interest were normalized to both *rpl32* and *ef1a* transcript levels and were calibrated to the individual with the lowest normalized expression level of that given transcript). Letters indicate Tukey's HSD groupings. In all cases, $n = 10$ and $p < 0.05$ was considered to be statistically significant.

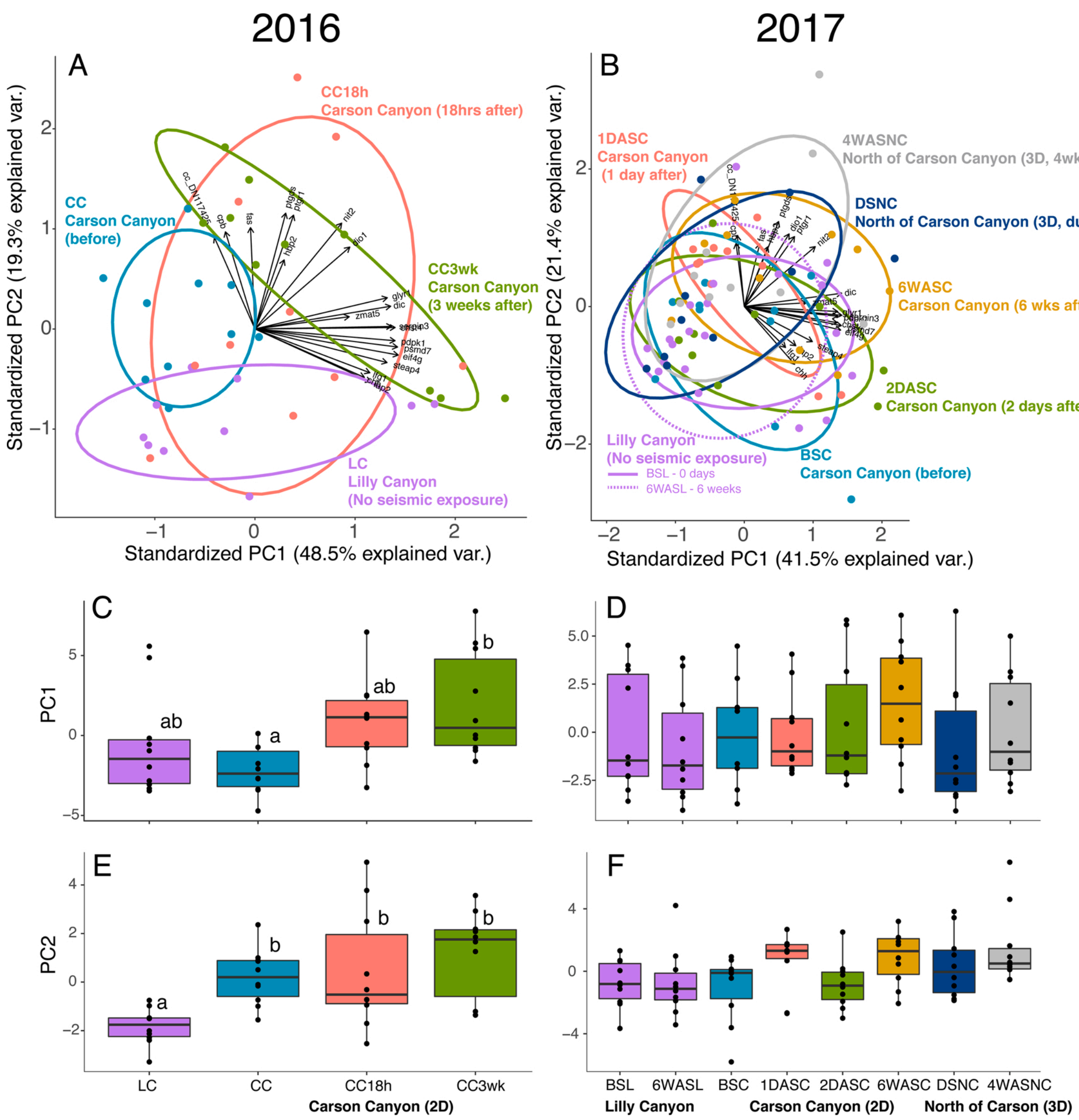

Fig. 4. Multivariate analysis (principal component analyses; PCAs) of qPCR-generated expression data for 20 transcripts in the hepatopancreas of snow crab subjected to seismic noise at Carson Canyon and of control (non-seismic at Lilly Canyon and pre-seismic at Carson Canyon) snow crab in **(A)** 2016 and **(B)** 2017. Length and direction of arrows indicate loading of each transcript on PC axes. Boxplots of PC axis 1 and 2 scores for individual samples in **(C,E)** 2016 and **(D,F)** 2017 data. Different coloured points and boxes represent different sampling groups, and different letters above boxplots indicate significant differences between sampling groups (adjusted p-values < 0.05; Tukey's post-hoc test for multiple comparisons for ANOVA).

molecular biomarkers associated with seismic noise exposure in field-collected snow crab hepatopancreas samples. First, in our 2016 field study, we identified nine transcripts with significantly higher expression after 2D seismic exposure, three additional transcripts that showed a non-significant trend of higher expression ($0.05 < p < 0.10$) and five with p-values > 0.1 but with fold-changes that were higher post-seismic at the Carson Canyon (CC) site (Fig. 1). We also identified 14 transcripts with significant differential expression between the CC and non-seismic Lilly Canyon (LC) sites (Fig. 2). These include transcripts with functional annotations related to oxidation-reduction, immunity, and metabolism.

The majority of transcriptomic studies in wild species often capture transcriptional responses within a given time period (see Alvarez et al., 2015). Our study went beyond a single snapshot by assessing expression levels of the aforementioned transcripts in response to both 2D and 3D seismic exposure in the field the following year where some transcripts showed similar patterns (i.e. increases in transcript expression levels after seismic exposure); however, unlike in 2016, none of these transcripts showed significant increases in expression levels after seismic exposure in 2017. This not only questions their value as biomarkers for assessing the impacts of seismic noise on snow crab but also highlights the potential unpredictable nature of transcriptional responses to anthropogenic disturbance in nature (i.e. in field-based studies).

Although there may be several reasons for the discrepancies in expression levels of these transcripts between years (see below), the results from 2016 still provide a valuable indicator of the potential physiological response of snow crab to seismic noise, which had not been explored to date. Several transcripts including *psmd7*, *chst4*, and *pdpk1* showed significantly higher expression following seismic exposure and were also important for separating treatments in multivariate analysis (PCA) in the 2016 samples, and showed a general trend (although not statistically significant) of higher expression post-2D seismic in 2017 samples. These transcripts are associated with immune function including antigen processing and the inflammatory response, as well as several other biological processes (see Supplemental Table 4). In aquatic organisms, the induction of the immune response is often associated with exposure to chemical pollutants (Luna-Acosta

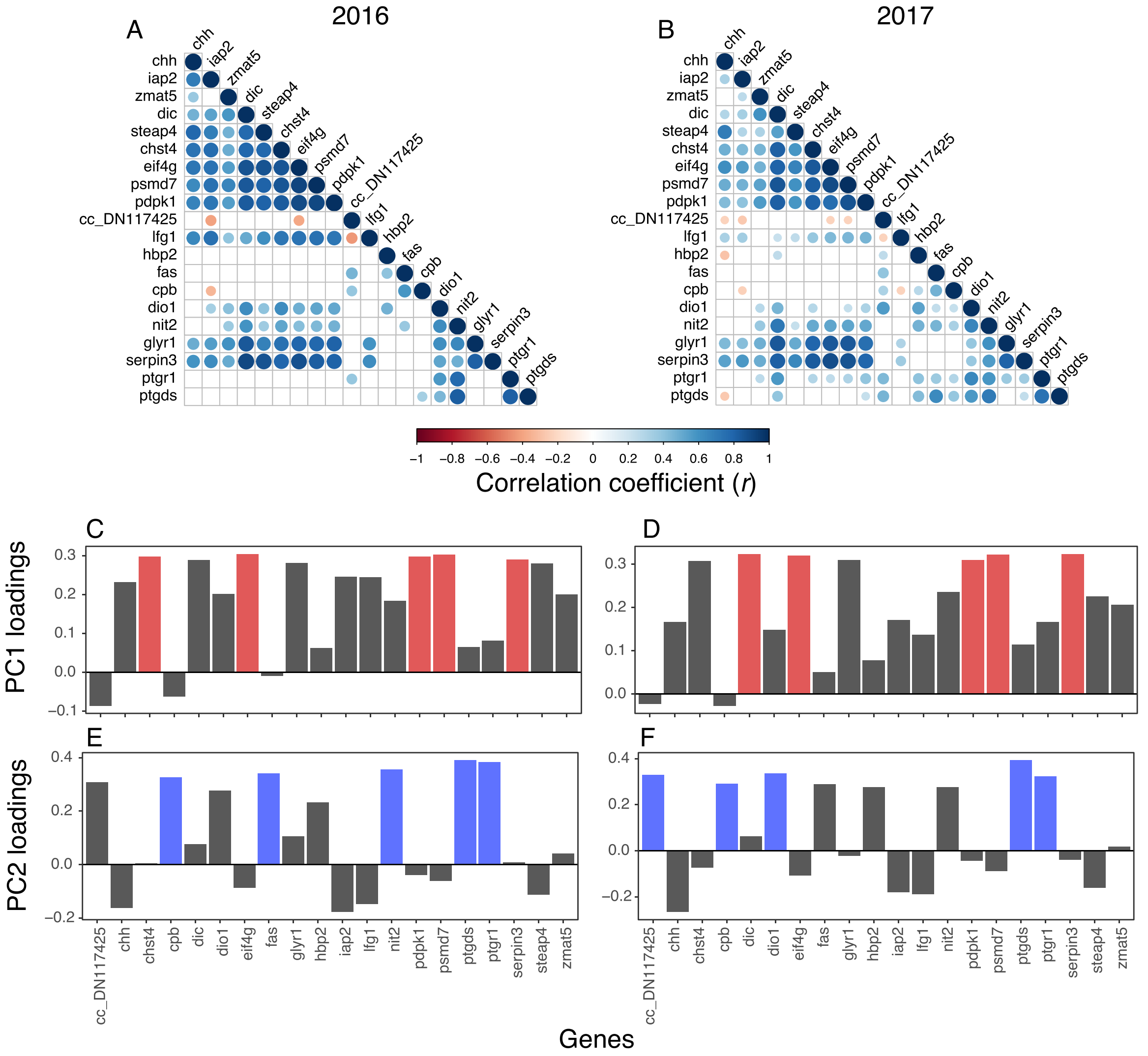

Fig. 5. Correlation coefficients (r) among 20 transcripts based on expression data from snow crab collected in **(A)** 2016 and **(B)** 2017. Colour and size of circles indicate strength and direction of relationship. Only significant correlations ($p < 0.05$) are shown by circles. Loadings of transcripts on principal component (PC) axes **(C,D)** 1 and **(E,F)** 2 for both sampling years (2016 C,E; 2017 D,F). The top five highest loading genes are highlighted in red for PC1 **(C,D)** and in blue for PC2 **(E,F)**.

et al., 2011; Xu et al., 2013), and changes in the immune response have been found in other crustacean species under noise stress (Celi et al., 2014; Filiciotto et al., 2014). For example, in European spiny lobster (*Palinurus elephas*), evidence of immunosuppression was found after exposure to noise (Celi et al., 2014); whereas, in our study, we observed an increase in expression of immune-related transcripts. An enhanced immune response following seismic exposure could suggest a protective role against a stressor, as demonstrated in oyster (*Crassostrea gigas*) following oil spill treatment exposure (Luna-Acosta et al., 2011).

In addition to its immune-relevant functional annotation (e.g. “antigen processing and presentation of exogenous peptide antigen via MHC class I”), *psmd7*, which encodes the 26S proteasome non-ATPase regulatory subunit 7, also has the GO annotations “proteasome complex” and “protein polyubiquitination” (Supplemental Table 4). This transcript was significantly up-regulated at both 18 h and 3 weeks post-seismic compared with pre-seismic CC 2016 samples. The 26S proteasome is responsible for the degradation of ubiquitinated proteins in eukaryotic cells, and plays important roles in many cellular processes including cell cycle, transcription, and stress responses (reviewed by Bard et al., 2018). Proteasome-related transcripts in crab hepatopancreas have previously been shown to respond to environmental stressors, namely toxicants. For example, a recent RNA-seq study of Chinese mitten crab (*Eriocheir sinensis*) hepatopancreas transcriptome response to benzo[α]pyrene (BaP, a highly toxic polycyclic aromatic hydrocarbon) found that several proteasome-related GO terms (e.g. “proteasome-activating ATPase activity” and “proteasome complex”) were significantly enriched in their BaP-responsive gene lists (Yu et al., 2018). Further, in freshwater crab (*Sinopotamon henanense*) hepatopancreas, the proteasome subunit *psmb7* was RNA-seq-identified and qPCR-confirmed to be up-regulated by exposure to the toxic heavy metal cadmium (Cd) (Sun et al., 2016). If exposure to seismic sound, like environmental toxicants PAH and Cd, influences the ubiquitin proteasome pathway (UPP) in crab hepatopancreas, then future seismic-related research should focus on the potential impact of UPP dysregulation on snow crab physiology and health.

In our study, additional transcripts were informative and showed differences between the LC non-seismic control site and exposure groups in CC. Transcripts that were important based on both multi- and univariate analyses included *ptgds*, *ptgr1*, *nit2*, and *cpb*. Collectively, these transcripts play roles in different processes including metabolism, redox homeostasis, and immune function. Dysregulation of transcripts associated with similar functions occurred following seismic exposure in the hearing organ of Atlantic salmon (*Salmo salar*) (Andrews et al., 2014).

Two of the candidate seismic-responsive genes identified using our 2016 field study samples [*ptgds* (encoding Prostaglandin D2 Synthase) and *ptgr1* (encoding Prostaglandin Reductase 1)] are involved in the prostaglandin biosynthesis and metabolism pathway (Supplemental Table 4). Prostaglandins are derived from arachidonic acid, and play roles in several biological processes including inflammation (Fang et al., 2014). Our results showed that both *ptgds* and *ptgr1* had significantly higher expression in CC post-seismic (both 18 h and 3 week) samples compared with LC samples, with *ptgr1* also significantly higher in the 3 week post-seismic compared with the pre-seismic CC hepatopancreas samples (Fig. 1B,N). A Chinese mitten crab Prostaglandin D Synthase encoding gene was previously characterized, and its highest transcript expression is shown to be in hepatopancreas followed by accessory sex gland, testis, and ovaries (Fang et al., 2014). A proteomics study in Chinese mitten crab revealed that Prostaglandin D Synthase was up-regulated in hemocytes after infection with the pathogen *Spiroplasma eriocheiris* (Meng et al., 2014). Further, a proteomics study of porcelain crab (*Petrolishthes cinctipes*) claw muscle tissue response to elevated temperature stress showed that Prostaglandin Reductase was induced during heat shock (Garland et al., 2015). Collectively, these results lead us to hypothesize that dysregulation of the prostaglandin biosynthesis and metabolism pathway in post-seismic 2016 hepatopancreas samples may have been in response to environmental stress and could possibly have immune consequences. While *ptgds* and *ptgr1* are valuable prostaglandin-relevant biomarkers for future studies of snow crab, we did not identify significant differences after seismic exposure for these markers in 2017. Nonetheless, both markers showed elevated expression after seismic activity (Fig. 3B,N).

Another candidate seismic-responsive transcript arising from our 2016 field study, *nit2* (alias: *omega-amidase*), was significantly up-regulated in 18 h post-seismic and 3 weeks post-seismic CC samples compared with both pre-seismic CC and LC samples in the 2016 study (Fig. 1). To our knowledge, *nit2* has not been previously characterized or otherwise studied in crabs. However, in mammals, *nit2* is known to be highly expressed in liver and kidney, and to play important roles in L-glutamine and L-asparagine metabolism, cancer, and rapidly dividing cells (Lin et al., 2007; reviewed by Cooper et al., 2016). While *nit2* transcript expression appeared to be slightly higher at 1 day post-seismic CC and 6 weeks post-seismic CC compared with pre-seismic CC and both LC time point sample groups in the 2017 field study, this up-regulation was not statistically significant (Fig. 3I). Nevertheless, the potential association of seismic noise exposure with elevated transcript expression of *nit2* (a key player in fundamental cellular metabolism) in hepatopancreas (which functions in metabolism and stress-response) in the 2016 field trial underscores the need for continued studies of the impact of seismic exploration on crab physiology.

One point to note is that control samples from both locations (CC and LC) also displayed some differences in expression, as evidenced by their separation in the PCA, even though neither had been subjected to seismic noise at that point of sampling. Although the two sites are located only 70 km apart, there may be several explanations for these differences. While marine species are often expected to be characterized by large connected populations given few physical barriers to gene flow in the ocean, advances in genomic technology often reveal that fine-scale differences can exist between marine populations at small spatial scales (Selkoe et al., 2008; Benestan et al., 2015; Lehnert et al., 2019). Indeed, significant genetic differences are found between some snow crab populations in the Northwest Atlantic; however, populations around NL and other Canadian provinces show weak population structure at neutral genetic markers (Puebla et al., 2008), suggesting that populations studied here are likely genetically similar and highly connected. Differences in transcription between these control sites could thus be attributed to ecological or environmental variation. For example, in red abalone (*Haliotis rufescens*), populations are genetically similar but transcriptomic analyses reveal significant differences at loci associated with genes involved in adaptation to different environmental conditions experienced by the populations (De Wit and Palumbi, 2013). In addition, anthropogenic or ecological conditions at these sites during sampling, such as population density (Gornati et al., 2004), multiple stressors (Altshuler et al., 2015), or prey, predators, and competitor communities (He et al., 2018), could differentially influence the physiological responses of these individuals at the time of collection. Indeed, snow crab catch rates (Morris et al., 2018; Morris et al., 2020) and movement behaviour (Cote et al., 2020), measured at the same times and locations as the sampling for this genomic study, were more affected by sources of natural variation than seismic surveying. It is therefore not surprising that transcriptional differences exist between snow crab populations in nature, and future work focused on common garden studies could help understand the role of genetics and environment on transcriptional responses.

The potential explanations for these differences between the control samples at LC and CC may also explain some differences found between sampling years. In our study, transcripts that showed differential expression following seismic noise in 2016 failed to show consistent and significant patterns in 2017 field samples. It is important to note that in our field study stressors do not occur in isolation, and potential ecological and environmental variability (as discussed above) between years may explain some of the inconsistencies found here. Inconsistencies in a candidate biomarker between years have also been found in other field studies such as those using metallothionein gene expression as an indicator of metal pollution in gudgeons (*Gobio gobio*) (Knapen et al., 2007) and flounder (*Pleuronectes flesus*) (Rotchell et al., 2001). In these studies, environmental variation as well as variation in life stage sampled can explain some discrepancies (Rotchell et al., 2001; Knapen et al., 2007). In our study, an important difference between years was that sampling time points differed. In 2016, differences were detected at 18-h and particularly at 3-weeks post exposure; but in 2017, snow crab were sampled at 1-day, 2-days, and 6-weeks after 2D exposure. It is possible that the physiological response is greatest at a given time period following exposure which is highly detectable at 3-weeks but subsides by 6-weeks, and thus the sampling in 2017 may have missed this critical period in the response. After the Deepwater Horizon oil spill, killifish (*Fundulus grandis*) showed changes in expression over time, where expression differences were greatest during the peak of the oil spill but were reduced 2-months later when most of the oil was no longer present in the area (Whitehead et al., 2012). It is also possible that animals may acclimate (and populations may adapt) to noise disturbance over time (Wright et al., 2007). In a coral reef fish (*Dascyllus trimaculatus*), physiological responses to noise decreased after repeated exposure (Nedelec et al., 2016). Therefore, snow crab in 2017 may be less reactive to the disturbance than in a previous year, especially given that increased noise is becoming commonplace in marine ecosystems (Andrew et al., 2011; Hildebrand, 2009). This could also explain why changes were not observed for these transcripts in snow crab exposed to chronic 3D noise.

Field research sampling in general encompasses sources of natural variability that are difficult to control, including differences in environmental histories or anthropogenic impacts. The abundance of commercial snow crab declined over a large area of the snow crab stock in several recent years (DFO Integrated fisheries management plan, http://www.dfo-mpo.gc.ca/fisheries-peches/ifmp-gmp/snow-crab-neige/2019/index-eng.html; Morris et al., 2020), which could cause density related changes. The decline is thought to be caused by a period of warm ocean conditions several years prior to our study that caused low recruitment, along with continued fishery removals (Mullowney et al., 2014). However, oceanographic conditions during the study period, 2016 and 2017, temperature in particular, were near similar and near normal in our study area (DFO, 2017, 2018). Temperature data from the Ocean Navigator web site (http://navigator.oceansdata.ca) at LC and CC during 2016 and 2017 differed less than 2 °C at our study depth during the middle of September when the study was conducted. Movement of individual snow crab in the study area can occur over many kilometers (Mullowney et al., 2018a,b); therefore, individuals can potentially experience changes in depth and temperature over relatively short distances along the edge of the continental slope that may influence the physiological variability observed. Despite these potential unknown sources of variation, the relative impact of seismic surveying appears within the range of natural variability observed.

Differences between years in transcriptional responses reported here are comparable to the results from behavioural and fishery studies conducted on snow crab at the same time. Our study was part of a multi-facetted approach designed to detect a range of effects due to realistic seismic surveying on commercial snow crab fishing grounds, which also included two catch rate studies (Morris et al., 2018; Morris et al., 2020) and a behavioural analysis study (Cote et al., 2020). All studies had the same realistic industry-based seismic exposures. Many of the findings among the different studies are complementary, in that the impacts of seismic surveying on commercial snow crab were not obvious or consistent. Catch rates were not measurably affected by short-term 2D seismic exposures (Morris et al., 2018), and catch rates both increased (2018) and decreased (2017) in response to longer-duration 3D seismic exposures (Morris et al., 2020). Analysis of movement direction, velocity, and behavioural patterns were not measurably affected by seismic surveying (Cote et al., 2020).

Field-based collection can provide a more realistic setting to examine the effect of a stressor in an ecological context (Alvarez et al., 2015); however, given the differences between years and given the conditions present in the field do not allow for proper control of all environmental influences, we suggest that future work should focus on lab-based studies to first identify reliable biomarkers of seismic stress in snow crab that can then be applied to field-based scenarios. A controlled laboratory setting can provide a more powerful approach to identify biomarkers of noise exposure in snow crab, especially given that the remote marine environment can pose logistical issues for RNA collection and sampling. In addition, common garden experiments would provide the opportunity to evaluate underlying genetic differences in response to seismic noise of snow crab from different locations in the ocean (i.e. population-level differences), as well as evaluate how different environmental factors, such as temperature and salinity, may influence these responses to noise. Laboratory work would also allow the study of acclimation and adaptation to noise over time, as rapid adaptation to anthropogenic stressors is not uncommon in aquatic species (Oziolor et al., 2019). Finally, future work focused on repeated measuring of responses to seismic noise in the field would be valuable. Using large 'omics' datasets across time would provide a powerful approach to identifying seismic-responsive molecular markers that are consistently influenced by noise disturbance. For example, the integration of genomics, epigenomics, transcriptomics, and proteomics could help identify genes and their regulatory pathways and networks involved in the response.

As noise continues to increase in the marine environment, it is important that we understand how animals are impacted by these acute or chronic disturbances. Many studies have shown that marine noise can impact behaviour (Fewtrell and McCauley, 2012), development (De Soto et al., 2013), and physiology (Fitzbibbon et al., 2017). In snow crab, our study finds that transcript expression changes can be detected in response to seismic activity; however, the candidate molecular biomarkers identified here in one field season were not reliable indicators in the next year. The integration of multiple 'omics' approaches could provide a more powerful method to identify consistent pathways involved in the response to seismic exposure rather than individual genes, which may highlight novel pathway biomarkers in the future. Nonetheless, as the cost of large-scale transcriptomic studies decreases, projects using RNA-seq from field collected samples can better harness the power of transcriptomics using powerful sampling designs (Todd et al., 2016) to better understand responses to marine noise under natural conditions.

CRediT authorship contribution statement

Jennifer R. Hall: Methodology, Validation, Formal analysis, Investigation, Resources, Data curation, Writing - original draft, Writing - review & editing, Visualization. **Sarah J. Lehnert:** Formal analysis, Data curation, Writing - original draft, Writing - review & editing, Visualization. **Emmanuel Gonzalez:** Software, Formal analysis, Data curation, Writing - review & editing. **Surendra Kumar:** Formal analysis, Data curation, Writing - review & editing, Visualization. **Jacqueline M. Hanlon:** Methodology, Investigation, Resources, Project administration, Writing - review & editing. **Corey J. Morris:** Conceptualization, Methodology, Investigation, Resources, Supervision, Project administration, Funding acquisition, Writing - original draft, Writing - review & editing. **Matthew L. Rise:** Conceptualization, Investigation, Resources, Writing - original draft, Writing - review & editing, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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References

Altschul, S.F., Madden, T.L., Schäffer, A.A., Zhang, J., Zhang, Z., Miller, W., Lipman, D.J., 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. 25, 3389–3402.

Altshuler, I., McLeod, A.M., Colbourne, J.K., Yan, N.D., Cristescu, M.E., 2015. Synergistic interactions of biotic and abiotic environmental stressors on gene expression. Genome 58, 99–109.

Alvarez, M., Schrey, A.W., Richards, C.L., 2015. Ten years of transcriptomics in wild populations: what have we learned about their ecology and evolution? Mol. Ecol. 24, 710–725.

Andrew, R.K., Howel, B.M., Mercer, J.A., 2011. Long-time trends in ship traffic noise for four sites off the North American West Coast. J. Acoust. Soc. Am. 129, 642–651.

Andrews, C.D., Payne, J.F., Rise, M.L., 2014. Identification of a gene set to evaluate the potential effects of loud sounds from seismic surveys on the ears of fishes: a study with *Salmo salar*. J. Fish Biol. 84, 1793–1819.

Bard, J.A., Goodall, E.A., Greene, E.R., Jonsson, E., Dong, K.C., Martin, A., 2018. Structure and function of the 26S proteasome. Annu. Rev. Biochem. 87, 697–724.

Benestan, L., Gosselin, T., Perrier, C., Sainte-Marie, B., Rochette, R., Bernatchez, L., 2015. RAD genotyping reveals fine-scale genetic structuring and provides powerful population assignment in a widely distributed marine species, the American lobster (*Homarus americanus*). Mol. Ecol. 24, 3299–3315.

Bolger, A.M., Lohse, M., Usadel, B., 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30, 2114–2120.

Bray, N.L., Pimentel, H., Melsted, P., Pachter, L., 2016. Near-optimal probabilistic RNA-seq quantification. Nat. Biotechnol. 34, 525.

Bryant, D.M., Johnson, K., DiTommaso, T., Tickle, T., Couger, M.B., Payzin-Dogru, D., Lee, T.J., Leigh, N.D., Kuo, T., Davis, F.G., Bateman, J., Bryant, S., Guzikowski, A.R., Tsai, S.L., Coyne, S., Ye, W.W., Freeman Jr., R.M., Peshkin, L., Tabin, C.J., Regev, A., Haas, B.J., Whited, J.L., 2017. A tissue-mapped axolotl de novo transcriptome enables identification of limb regeneration factors. Cell Rep. 18, 762–776.

Carroll, A.G., Przeslawski, R., Duncan, A., Gunning, M., Bruce, B., 2017. A critical review of the potential impacts of marine seismic surveys on fish & invertebrates. Mar. Pollut. Bull. 114, 9–24.

Celi, M., Filiciotto, F., Vazzana, M., Arizza, V., Maccarrone, V., Ceraulo, M., Mazzola, S., Buscaino, G., 2014. Shipping noise affecting immune responses of European spiny lobster (*Palinurus elephas*). Can. J. Zool. 93, 113–121.

Chen, X., Wang, J., Yue, W., Liu, J., Wang, C., 2017. Hepatopancreas transcriptome analysis of Chinese mitten crab (*Eriocheir sinensis*) with white hepatopancreas syndrome. Fish Shellfish Immun. 70, 302–307.

Clark, K.F., Acorn, A.R., Greenwood, S.J., 2013. A transcriptomic analysis of American lobster (*Homarus americanus*) immune response during infection with the bumper car parasite *Anophryoides haemophila*. Dev. Comp. Immunol. 40, 112–122.

Cooper, A.J.L., Shurubor, Y.I., Dorai, T., Pinto, J.T., Isakova, E.P., Deryabina, Y.I., Denton, T.T., Krasnikov, B.F., 2016. ω-Amidase: an underappreciated, but important enzyme in L-glutamine and L-asparagine metabolism; relevance to sulfur and nitrogen metabolism, tumor biology and hyperammonemic diseases. Amino Acids 48, 1–20.

Cote, D., Morris, C.J., Regular, P.M., Piersiak, M., 2020. Effects of 2D seismic on Snow Crab movement behaviour. Fish. Res. 230, 105661.

Mullowney, D., Coffey, W., Baker, K., Evans, G., Fiander, D., Colbourne, E., Maddock Parsons, D., Koen-Alonso, M., Wells, N., 2018a. An Assessment of Newfoundland and Labrador Snow Crab (*Chionoecetes Opilio*) in 2016. DFO Can. Sci. Advis. Sec. Res. Doc. 2017/081. Viii + 172 p..

D'Amico, A., Gisiner, R., Ketten, D., Hammock, J., Johnson, C., Tyack, P., Mead, J., 2009. Beaked whale strandings and naval exercises. Aquat. Mamm. 35, 452–472.

Day, R.D., McCauley, R.D., Fitzgibbon, Q.P., Hartmann, K., Semmens, J.M., 2017. Exposure to seismic air gun signals causes physiological harm and alters behavior in the scallop *Pecten fumatus*. Proc. Nat. Acad. Sci. USA 114, E8537–E8546.

De Soto, N.A., Delorme, N., Atkins, J., Howard, S., Williams, J., Johnson, M., 2013. Anthropogenic noise causes body malformations and delays development in marine larvae. Sci. Rep. 3, 2831.

De Wit, P., Palumbi, S.R., 2013. Transcriptome-wide polymorphisms of red abalone (*Haliotis rufescens*) reveal patterns of gene flow and local adaptation. Mol. Ecol. 22, 2884–2897.

Deruiter, S.L., Southall, B.L., Calambokidis, J., Zimmer, W.M., Sadykova, D., Falcone, E. A., Friedlaender, A.S., Joseph, J.E., Moretti, D., Schorr, G.S., Thomas, L., Tyack, P.L., 2013. First direct measurements of behavioural responses by Cuvier's beaked whales to mid-frequency active sonar. Biol. Lett. 9, 1–5.

DFO, 2017. Oceanographic Conditions in the Atlantic Zone in 2016. DFO Can. Sci. Advis. Sec. Sci. Advis. Rep. 2017/031.

DFO, 2018. Oceanographic Conditions in the Atlantic Zone in 2017. DFO Can. Sci. Advis. Sec. Sci. Advis. Rep. 2018/039.

Dooling, R.J., Leek, M.R., Popper, A.N., 2015. Effects of noise on fishes: what we can learn from humans and birds. Integr. Zool. 10, 29–37.

Edmunds, N.J., Firmin, C.J., Goldsmith, D., Faulkner, R.C., Wood, D.T., 2016. A review of crustacean sensitivity to high amplitude underwater noise: data needs for effective risk assessment in relation to UK commercial species. Mar. Pollut. Bull. 108, 5–11.

Ellison, W.T., Frankel, A.S., 2012. A common sense approach to source metrics. In: Popper, A.N., Hawkins, A.D. (Eds.), The Effects of Noise on Aquatic Life. Springer, New York, pp. 433–438.

Fang, D., Yang, Q., Duan, J., Wang, Q., Zhang, M., Zhou, Y., Liu, K., Shi, W., 2014. Characteristic of PGDS potential regulation role on spermatogenesis in the Chinese mitten crab *Eriocheir sinensis*. Gene 543, 244–252.

Fewtrell, J.L., McCauley, R.D., 2012. Impact of air gun noise on the behaviour of marine fish and squid. Mar. Pollut. Bull. 64, 984–993.

Fields, D.F., Handegard, N.O., Dalen, J., Eichner, C., Malde, K., Karlsen, O., Skiftesvik, A. B., Durif, C.M.F., Browman, H.I., 2019. Airgun blasts used in marine seismic surveys have limited effects on mortality, and no sublethal effects on behaviour or gene expression, in the copepod *Calanus finmarchicus*. ICES J. Mar. Sci. 76, 2033–2044.

Filiciotto, F., Vazzana, M., Celi, M., Maccarrone, V., Ceraulo, M., Buffa, G., Di Stefano, V., Mazzola, S., Buscaino, G., 2014. Behavioural and biochemical stress responses of *Palinurus elephas* after exposure to boat noise pollution in tank. Mar. Poll. Bull. 84, 104–114.

Fitzbibbon, Q.P., Day, R.D., McCauley, R.D., Simon, C.J., Semmens, J.M., 2017. The impact of seismic air gun exposure on the haemolymph physiology and nutritional condition of spiny lobster, *Jasus edwardsii*. Mar. Poll. Bull. 125, 146–156.

Garland, M.A., Stillman, J.H., Tomanek, L., 2015. The proteomic response of cheliped myofibril tissue in the eurythermal porcelain crab *Petrolisthes cinctipes* to heat shock following acclimation to daily temperature fluctuations. J. Exp. Biol. 218, 388–403.

Gornati, R., Papis, E., Rimoldi, S., Terova, G., Saroglia, M., Bernardini, G., 2004. Rearing density influences the expression of stress-related genes in sea bass (*Dicentrarchus labrax*, L.). Gene 341, 111–118.

Haas, B.J., Papanicolaou, A., Yassour, M., Grabherr, M., Blood, P.D., Bowden, J., Couger, M.B., Eccles, D., Li, B., Lieber, M., MacManes, M.D., Ott, M., Orvis, J., Pochet, N., Strozzi, F., Weeks, N., Westerman, R., William, T., Dewey, C.N., Henschel, R., LeDuc, R.D., Friedman, N., Regev, A., 2013. De novo transcript sequence reconstruction from RNA-seq using the Trinity platform for reference generation and analysis. Nat. Protoc. 8, 1494.

Halvorsen, M.B., Casper, B.M., Woodley, C.M., Carlson, T.J., Popper, A.N., 2012. Threshold for onset of injury in Chinook salmon from exposure to impulsive pile driving sounds. PLoS One 7, e38968.

Hawkins, A.D., Popper, A.N., 2017. A sound approach to assessing the impact of underwater noise on marine fishes and invertebrates. ICES J. Mar. Sci. 74, 635–651.

He, X., Houde, A.L.S., Neff, B.D., Heath, D.D., 2018. Transcriptome response of Atlantic salmon (*Salmo salar*) to competition with ecologically similar non-native species. Ecol. Evol. 8, 1769–1777.

Hildebrand, J.A., 2009. Anthropogenic and natural sources of ambient noise in the ocean. Mar. Ecol. Prog. Ser. 395, 5–20.

Huang, S., Wang, J., Yue, W., Chen, J., Gaughan, S., Lu, W., Lu, G., Wang, C., 2015. Transcriptomic variation of hepatopancreas reveals the energy metabolism and biological processes associated with molting in Chinese mitten crab, *Eriocheir sinensis*. Sci. Rep. 5, 14015. https://doi.org/10.1038/srep14015.

Kight, C.R., Swaddle, J.M., 2011. How and why environmental noise impacts animals: an integrative, mechanistic review. Ecol. Lett. 14, 1052–1061.

Knapen, D., Reynders, H., Bervoets, L., Verheyen, E., Blust, R., 2007. Metallothionein gene and protein expression as a biomarker for metal pollution in natural gudgeon populations. Aquat. Toxicol. 82, 163–172.

Koressaar, T., Remm, M., 2007. Enhancements and modifications of primer design program Primer3. Bioinformatics 23, 1289–1291.

Lehnert, S.J., DiBacco, C., Van Wyngaarden, M., Jeffery, N.W., Lowen, J.B., Sylvester, E. V.A., Wringe, B.F., Stanley, R.R., Hamilton, L.C., Bradbury, I.R., 2019. Fine-scale temperature-associated genetic structure between inshore and offshore populations of sea scallop (*Placopecten magellanicus*). Heredity 122, 69–80.

Li, X., Cui, Z., Liu, Y., Song, C., Shi, G., 2013. Transcriptome analysis and discovery of genes involved in immune pathways from hepatopancreas of microbial challenged mitten crab *Eriocheir sinensis*. PLoS One 8, e68233.

Lin, C.H., Chung, M.Y., Chen, W.B., Chien, C.H., 2007. Growth inhibitory effect of the human NIT2 gene and its allelic imbalance in cancers. FEBS J. 274, 2946–2956.

Love, M.I., Huber, W., Anders, S., 2014. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol. 15, 550.

Luna-Acosta, A., Kanan, R., Le Floch, S., Huet, V., Pineau, P., Bustamante, P., Thomas-Guyon, H., 2011. Enhanced immunological and detoxification responses in Pacific oysters, *Crassostrea gigas*, exposed to chemically dispersed oil. Water Res. 45, 4103–4118.

MacGregor, P.K., Horn, A.G., Leonard, M.L., Thomsen, F., 2016. Anthropogenic noise and conservation. In: Brumm, H. (Ed.), Animal Communication and Noise, Animal Signals and Communication 2. Springer-Verlag, Berlin, pp. 409–444.

McCauley, R.D., Day, R.D., Swadling, K.M., Fitzgibbon, Q.P., Watson, R.A., Semmens, J. M., 2017. Widely used marine seismic survey air gun operations negatively impact zooplankton. Nat. Ecol. Evol. 1, 0195.

Meng, Q., Hou, L., Zhao, Y., Huang, X., Huang, Y., Xia, S., Gu, W., Wang, W., 2014. iTRAQ-based proteomic study of the effects of *Spiroplasma eriocheiris* on Chinese mitten crab *Eriocheir sinensis* hemocytes. Fish Shellfish Immunol. 40, 182–189.

Morris, C.J., Cote, D., Martin, B., Kehler, D., 2018. Effects of 2D seismic on the snow crab fishery. Fish. Res. 197, 67–77.

Morris, C.J., Cote, D., Martin, B., Mullowney, D., 2020. Effects of 3D seismic surveying on the Snow Crab fishery. Fish. Res. 232, 105719.

Mullowney, D.R., Dawe, E.G., 2009. Development of performance indices for the Newfoundland and Labrador snow crab (*Chionoecetes opilio*) fishery using data from a vessel monitoring system. Fish. Res. 100, 248–254.

Mullowney, D.R.J., Dawe, E.G., Colbourne, E.B., Rose, G.A., 2014. A review of factors contributing to the decline of Newfoundland and Labrador snow crab (*Chionoecetes opilio*). Rev. Fish Biol. Fish. 24, 639.

Mullowney, D., Morris, C., Dawe, E., Zagorsky, I., 2018b. Dynamics of snow crab (*Chionoecetes opilio*) movement and migration along the Newfoundland and Labrador and Eastern Barents Sea continental shelves. Rev. Fish Biol. Fish. 28, 435. https://doi.org/10.1007/s11160-017-9513-y.

Nedelec, S.L., Mills, S.C., Lecchini, D., Nedelec, B., Simpson, S.D., Radford, A.N., 2016. Repeated exposure to noise increases tolerance in a coral reef fish. Environ. Pollut. 216, 428–436.

Nieukirk, S.L., Mellinger, D.K., Moore, S.E., Klinck, K., Dziak, R.P., Goslin, J., 2012. Sounds from airguns and fin whales recorded in the mid-Atlantic Ocean, 1999–2009. J. Acoust. Soc. Am. 131, 1102–1112.

Nowacek, D.P., Clark, C.W., Mann, D., Miller, P.J.O., Rosenbaum, H.C., Golden, J.S., Jasny, M., Kraska, J., Southall, B.L., 2015. Marine seismic surveys and ocean noise: time for coordinated and prudent planning. Front. Ecol. Environ. 13, 378–386.

Oziolor, E.M., Reid, N.M., Yair, S., Lee, K.M., VerPloeg, S.G., Bruns, P.C., Shaw, J.R., Whitehead, A., Matson, C.W., 2019. Adaptive introgression enables evolutionary rescue from extreme environmental pollution. Science 364, 455–457.

Payne, J.F., Andrews, C.A., Fancey, L.L., Cook, A.L., Christian, J.R., 2007. Pilot Study on the Effects of Seismic Air Gun Noise on Lobster (*Homarus Americanus*) in Canadian Technical Report of Fisheries and Aquatic Sciences No. 2712. Science Branch. Fisheries and Oceans Canada, pp. 1–53.

Payne, J.F., Andrews, C.D., Fancey, L.L., Guiney, J., Cook, A., Christian, J.R., 2008. Are seismic surveys an important risk factor for fish and shellfish? Bioacoustics 17 (1–3), 262–265.

Pfaffl, M.W., 2001. A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res. 29, e45.

Puebla, O., Sévigny, J.M., Sainte-Marie, B., Brêthes, J.C., Burmeister, A., Dawe, E.G., Moriyasu, M., 2008. Population genetic structure of the snow crab (*Chionoecetes opilio*) at the Northwest Atlantic scale. Can. J. Fish. Aquat. Sci. 65, 425–436.

Pye, H.J., Watson, W.H.I.I.I., 2004. Sound detection and production in the American lobster, *Homarus americanus*: sensitivity range and behavioral implications. J. Acoust. Soc. Am. 115, 2486-2486.

Quinn, T.P., Erb, I., Richardson, M.F., Crowley, T.M., 2018. Understanding sequencing data as compositions: an outlook and review. Bioinformatics 34, 2870–2878.

Robinson, M.D., McCarthy, D.J., Smyth, G.K., 2009. edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics 26, 139–140.

Rotchell, J.M., Clarke, K.R., Newton, L.C., Bird, D.J., 2001. Hepatic metallothionein as a biomarker for metal contamination: age effects and seasonal variation in European flounders (*Pleuronectes flesus*) from the Severn Estuary and Bristol Channel. Mar. Environ. Res. 52, 151–171.

Selkoe, K.A., Henzler, C.M., Gaines, S.D., 2008. Seascape genetics and the spatial ecology of marine populations. Fish Fish. 9, 363–377.

Spiga, I., Caldwell, G.S., Bruintjes, R., 2016. Influence of pile driving on the clearance rate of the blue mussel, *mytilus edulis* (L.). In: Proceedings of Meetings on Acoustics: Fourth International Conference on the Effects of Noise on Aquatic Life. July 10–16 2016, Volume 27, Dublin, Ireland.

Stansbury, D.E., Fiander, D., Maddock-Parsons, D., 2013. Summary of the industry-DFO Collaborative Post-season Trap Surveys for Snow Crab in Div. 2J3KLOPs4R.

Stelzer, G., Rosen, N., Plaschkes, I., Zimmerman, S., Twik, M., Fishilevich, S., Stein, T.I., Nudel, R., Lieder, I., Mazor, Y., Kaplan, S., 2016. The GeneCards suite: from gene data mining to disease genome sequence analyses. Curr. Protoc. Bioinformatics 54, 1–30.

Sun, M., Li, Y.T., Liu, Y., Lee, S.C., Wang, L., 2016. Transcriptome assembly and expression profiling of molecular responses to cadmium toxicity in hepatopancreas of the freshwater crab *Sinopotamon henanense*. Sci. Rep. 6, 19405.

Tifau, S., Briffa, M., 2016. Review on behavioral impacts of aquatic noise on crustaceans. Proc. Meetings Acoust. 27, 01002.

Todd, E.V., Black, M.A., Gemmell, N.J., 2016. The power and promise of RNA-seq in ecology and evolution. Mol. Ecol. 25, 1224–1241.

Tyack, P.L., Zimmer, W.M.C., Moretti, D., Southall, B.L., Claridge, D.E., Durban, J.W., Clark, C.W., D'Amico, A., DiMarzio, N., Jarvis, S., McCarthy, E., Morrissey, R., Ward, J., Boyd, I.L., 2011. Beaked whales respond to simulated and actual navy sonar. PLoS One 6, e17009.

Underwood, A.J., 1981. Techniques of analysis of variance in experimental marine biology and ecology. Oceanogr. Mar. Biol. 19, 513–605.

UniProt Consortium, 2019. UniProt: a worldwide hub of protein knowledge. Nucleic Acids Res. 47, D506–D515.

Untergasser, A., Cutcutache, I., Koressaar, T., Ye, J., Faircloth, B.C., Remm, M., Rozen, S. G., 2012. Primer3 - new capabilities and interfaces. Nucleic Acids Res. 40, e115.

Vandesompele, J., De Preter, K., Pattyn, F., Poppe, B., Van Roy, N., De Paepe, A., Speleman, F., 2002. Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. Genome Biol. 3 research0034-1.

Vogt, G., 1994. Life-cycle and functional cytology of the hepatopancreatic cells of *Astacus astacus* (Crustacea, Decapoda). Zoomorphology 114, 83–101.

Vogt, G., Stöcker, W., Zwilling, R., 1989. Biosynthesis of Astacus protease, a digestive enzyme from crayfish. Histochemistry 91, 373–381.

Vu, V.Q., 2011. Ggbiplot: a ggplot2 Based Biplot. R Package Version 0.55.

Wei, B., Yang, Z., Wang, J., Chen, A., Shi, Q., Cheng, Y., 2017. Effects of dietary lipids on the hepatopancreas transcriptome of Chinese mitten crab (*Eriocheir sinensis*). PLoS One 12, e0182087.

Whitehead, A., Dubansky, B., Bodinier, C., Garcia, T.I., Miles, S., Pilley, C., Raghunathan, V., Roach, J.L., Walker, N., Walter, R.B., Rice, C.D., 2012. Genomic and physiological footprint of the Deepwater Horizon oil spill on resident marsh fishes. Proc. Nat. Acad. Sci. USA 109, 20298–20302.

Wright, A.J., Soto, N.A., Baldwin, A.L., Bateson, M., Beale, C.M., Clark, C., Deak, T., Edwards, E.F., Fernández, A., Godinho, A., Hatch, L.T., 2007. Anthropogenic noise as a stressor in animals: a multidisciplinary perspective. Int. J. Comp. Psychol. 20.

Xu, H., Shao, X., Zhang, Z., Zou, Y., Wu, X., Yang, L., 2013. Oxidative stress and immune related gene expression following exposure to di-n-butyl phthalate and diethyl phthalate in zebrafish embryos. Ecotox. Environ. Safe. 93, 39–44.

Yu, N., Ding, Q., Li, E., Qin, J.G., Chen, L., Wang, X., 2018. Growth, energy metabolism and transcriptomic responses in Chinese mitten crab (*Eriocheir sinensis*) to benzo [α] pyrene (BaP) toxicity. Aquat. Toxicol. 203, 150–158.

Zhou, Q., Shi, B., Jiao, L., Jin, M., Sun, P., Ding, L., Yuan, Y., 2019. Hepatopancreas and ovarian transcriptome response to different dietary soybean lecithin levels in *Portunus trituberculatus*. Comp. Biochem. Physiol. Part D Genomics Proteomics 31, 100600.