Examining the potential impacts of seismic surveys on Octopus and larval stages of Southern Rock Lobster
PART A: Southern Rock Lobster

Progress Report

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Ryan D Day[1], Quinn P Fitzgibbon[1], Robert D McCauley[2], Jayson M Semmens[1]
[1]Fisheries and Aquaculture Centre, Institute for Marine and Antarctic Studies, University of Tasmania, Hobart, Tasmania 7001
[2]Centre for Marine Science and Technology, Curtin University, Perth, Western Australia 6845

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Inquiries should be directed to:

Researcher Contact Details

Name:	**Professor Jayson Semmens**
Address:	Institute for Marine and Antarctic Studies/University of Tasmania, Private Bag 49, Hobart, TAS 7001. AUSTRALIA
Phone:	+61 (0)3 6226 8275
Fax:	+61 (0)3 6227 8035
Email:	jayson.semmens@utas.edu.au

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Executive Summary

This report details the portion of *FRDC 2019-051: Examining the potential impacts of seismic surveys on Octopus and larval stages of Southern Rock Lobster* focusing on the impacts of exposure to a full-scale seismic survey on the early life stages of the Southern Rock Lobster (*Jasus edwardsii*), undertaken by the University of Tasmania's Institute for Marine and Antarctic Studies in conjunction with Curtin University's Centre for Marine science and Technology.

Lobster Objectives

This study aimed to characterise the impacts of seismic surveys on the puerulus and juvenile life history stage of *J. edwardsii* to determine whether early development and recruitment of this important fishery species might be affected. Specifically, this study was designed to assess i) mortality rates following exposure, ii) impairment of the righting reflex in exposed lobsters and iii) development of exposed lobsters through assessment of progression through the moult cycle.

Lobster Methods

Southern Rock Lobster puerulus (n=16) and post-settlement juveniles (n=56) were collected and transported to the field site off Lakes Entrance, Victoria, where the seismic source vessel MV Geo Coral was conducting a commercial seismic survey. Lobster puerulus were randomly assigned to either Control (not exposed to air gun signals) or E0 (exposed to air gun signals at a nominal range of 0 m from the vessel sail line) treatments. Juveniles were randomly assigned to three treatments, Control (as above), E0 (as above) and E500 (exposed to air gun signals at a nominal range of 500 m from the vessel sail line). Lobsters were contained in weighted mesh oyster baskets on the seabed in 51 m of water in the turning area of the seismic vessel where the air guns were not fired for the control treatment and in 58 m of water at their respective ranges relative to the seismic vessel's planned sail line for the E0 and E500 treatments. Sea noise recorders were placed on the seabed at nominal ranges from the seismic sail line of 0 m (pressure and ground motion sensors), 250 m (pressure and water particle acceleration), 500 m (pressure), 1000 m (pressure) and 2000 m (pressure). Sound exposure histories for each lobster sample site were calculated from the received seismic signals. After the seismic vessel conducted its run with three 2,820 cubic inch sources firing alternatively every 5 s (flip/flop/flap in seismic parlance), lobsters were recovered and tested for dorsoventral righting reflex. Righting is a complex reflex requiring sensory input to mediate neuromuscular coordination and is tested by quantifying the time taken to return to a dorsum-up position after being placed in a ventral side up position. A subsample of lobsters was returned to IMAS' laboratories and held until they had moulted twice (juveniles) or three times (puerulus) to calculate intermoult duration for each lobster. Moulting is a physiologically stressful process that can be delayed in response to external stressors, making intermoult duration an important measurement of physiological condition as well as a marker of growth and development.

Lobster Key findings

Exposure did not result in any elevated mortality for puerulus or juveniles. Immediately after exposure, righting was significantly impaired for all exposure treatments (E0 and E500 for juveniles and E0 for puerulus) compared to their respective controls, indicating that the impact range extended to at least 500 m from the source, the maximum range tested in the present study. After the first moult, there was no significant difference found in righting between juvenile Control and E0 treatments, and for puerulus, small sample size precluded statistical analysis. When these two stages were pooled, the combined E0 treatment was found to be significantly impaired. In the juvenile E500 lobsters, righting was similar to that of Controls, indicating that the lobsters had recovered from prior impairment. After the second moult, juvenile E0 lobsters showed significant impairment compared to controls. When puerulus, which could not be analysed due to small sample size, were pooled with juveniles, the combined E0 treatment was significantly impaired relative to combined Controls. Righting in juvenile E500 lobsters was similar to that of controls, further supporting recovery in this treatment. Impaired righting has previously been

found to correlate with damage to the statocyst, the mechanosensory organ common to many marine invertebrates. The results here from the combined puerulus and juvenile treatments indicated that puerulus and juvenile E0 treatments did not show the capacity for recovery whereas juvenile E500 lobsters recovered from impairment after the first moult, providing evidence of a range threshold for recovery. Intermoult period was significantly increased in E0 juvenile lobsters and appeared to be increased in puerulus, though the latter could not be statistically analysed. Juvenile E500 treatment showed a moderate, non-significant increase in moult duration. Increased intermoult duration suggested impacted development and potentially slowed growth, though the proximate cause was not identified.

Lobster Implications

- Sound exposure levels recorded in this study were similar to those of prior experiments conducted with a single air gun, validating the single air gun approach for future field-based experimental work.
- Air gun signals caused righting impairment to at least 500 m, the maximum range in this study, in lobsters sampled immediately following exposure, a similar result previously reported in adults that corresponded with significant damage to the mechanosensory statocyst organ that provides the sense of balance, body position and movement that are critical for predator avoidance behaviour.
- Impairment resulting from close range exposure (i.e., combined puerulus and juvenile E0 treatments) appeared to be persistent, as previously reported in adult lobsters, whereas lobsters exposed at a more distant range (juvenile E500) showed recovery. This indicates that a range of 500 m may not cause lasting impairment to righting.
- Intermoult duration was significantly increased in E0 juveniles and appeared to be increased in E0 puerulus, indicating the potential for slowed development and growth and physiological stress.

Introduction

Anthropogenic aquatic noise has gained recent widespread recognition as a pollutant capable of harming marine organisms (Hawkins et al. 2015). Seismic surveys, which are used to explore for sub-seafloor oil and gas deposits, have garnered a great deal of research attention. While many factors likely drive this focus, two major contributors are: 1) their widespread use throughout the world's oceans, particularly in coastal areas relied on for fishery production, and 2) the high intensity of the impulsive, low frequency signals they produce (Hildebrand 2009). Historically, the majority of research effort has been directed at the impacts of exposure on marine mammals due to their reliance on sound production and hearing for communication and prey detection, though more recent attention has focussed on understanding impacts on invertebrates (see review by Carroll et al. 2017).

One of the most thoroughly studied marine invertebrate responses to aquatic noise to date is that of the Southern Rock Lobster (*Jasus edwardsii*), which occurs in south-eastern Australia and New Zealand. Rock (or spiny) lobsters from the family Palinuridae comprise some of the most valuable single species fisheries around the globe (Phillips 2006) and are important contributors to socioeconomic value in many countries (Norman-López et al. 2013; Pérez-Ramírez et al. 2016). Given the overlap between the habitats of rock lobsters (and many other seafood fisheries) and areas of interest for seismic exploration, concern has arisen over how this valuable fishery may be impacted by survey activity. To respond to this concern, a series of field-based studies was conducted in which adult *J. edwardsii* were exposed to the signals of a 150 cui capacity air gun as used in seismic arrays. With adult rock lobsters there was no mortality following exposure though there was impact to: 1) the haemocytes (i.e., blood cell analogues) that mediate immune function (Fitzgibbon et al. 2017); 2) the statocyst (Day et al. 2019) the sensory organ that provides a sense of gravity, balance, and movement for a number of marine invertebrates (Buddelman 1992); and 3) to the nutritional condition of exposed lobsters (Fitzgibbon et al. 2017). Lobster eggs exposed to air gun signals did not show any abnormal embryological development, nor any impact on the subsequent hatched larvae (Day et al. 2016). However, the recent finding of increased mortality in zooplankton following exposure to air gun signals (McCauley et al. 2017a) suggests that planktonic, early life stages of marine invertebrates may be more vulnerable than adults or developing embryos.

However, due to the use of a single air gun to emulate a full-scale survey compared to a full-scale array with upwards of 20 air guns and shallow water at the experimental site (10-12 m), these findings have received methodological criticisms over how accurately the exposure emulates "real-world conditions." Although the exposures in these studies were modelled to be the equivalent of a full-scale array passing within several hundred metres of the experimental animals (Day et al. 2019), further data is required to definitively conclude that a single air gun in shallow water can emulate real-world exposure.

In the present study, the effects of seismic signal exposure on *J. edwardsii* were further investigated. To address the methodological issues surrounding the use of a single air gun in shallow water to emulate a survey, experimental work in 51-58 m water depth was conducted in conjunction with a full-scale array employed during a commercial seismic survey. To further the extensive research into the effects of air gun signals on adult lobsters, the present study focused on the impacts on early life-history stage lobsters. Following hatching, palinurid lobsters spend months to years as planktonic phyllosoma, undergoing a series of stages in offshore currents before metamorphosing into the postlarval puerulus stage (Booth & Phillips 1994; George 2005). The non-feeding puerulus is a transitional stage lasting several days, bridging larval and juvenile stages in which the lobster finds a suitable settlement site before metamorphosing into the benthic postpuerulus juvenile form, at which point it is essentially a small, fully developed lobster (Booth & Phillips 1994). These life stages tend to occur in low abundance over a spatially wide distribution (Jeffs & Holland 2000), making them difficult to study and potentially obscuring any impact. To investigate the impacts of air gun signals on these early life stage lobsters, puerulus and juveniles were collected, exposed to the air gun signals of a full-scale array and then assessed for impacts through the quantification of their dorsoventral righting reflex and their post-exposure development. The results provide further insight into the sensitivity of *J. edwardsii* and, more generally, marine invertebrates to aquatic noise and provide crucial new information on the range threshold of exposure and methodological approaches for future studies into the effects of seismic exposure.

Methods

Southern Rock Lobster (*Jasus edwardsii*) puerulus and juveniles were collected from Waubs Bay, Bicheno, Tasmania (Fig. 1). Collectors were based on the "bottlebrush" design described by Mills & Crear (2004) housed in an oyster basket with 5mm mesh (Hexcyl Systems, Ceduna, South Australia). A total of 45 baskets were deployed in 10-12 metres of water during the austral summer and collected after 2 months.

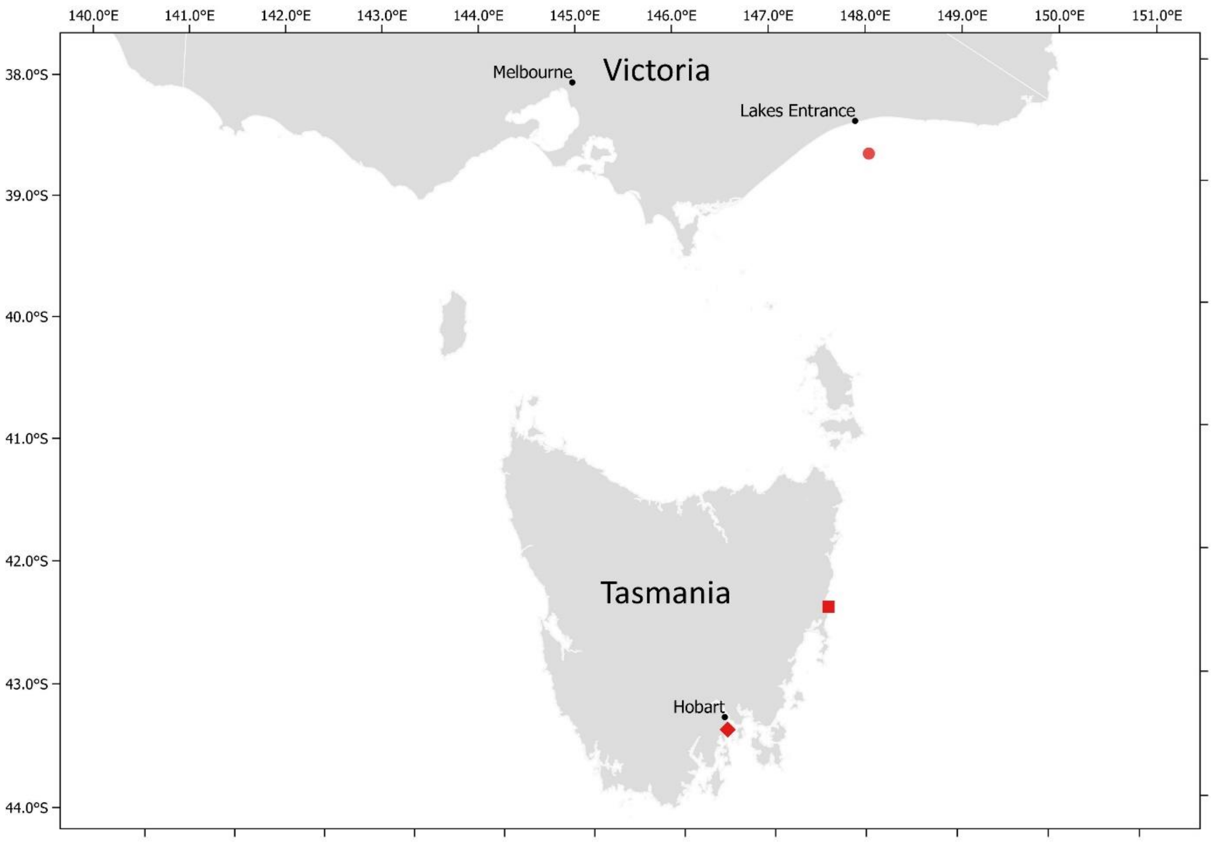

Figure 1. General location of study region showing site where lobsters were collected by the red square on the eastern Tasmanian coast (Waubs Bay, Bicheno) and the experimental location near Lakes Entrance, Victoria (red circle). After exposure, all lobster were transported to Taroona, indicated by the red diamond.

Baskets were wrapped in 1.5 mm mesh bags by divers and floated to the surface for recovery. A 3 m dinghy retrieved the baskets and transferred them to the *FV Dell Richey II*, the 25 m support vessel used hereafter, where they were processed on the deck. Processing consisted of removing each rosette of the bottlebrush to find puerulus or juvenile lobsters, with age class determined according to Lesser (1978). A total of 56 juveniles and 16 puerulus were collected. As they were removed from collectors, they were placed into 1.5 mm oyster mesh bags in replicate groups detailed below. Bags were tagged with unique identification numbers and distributed haphazardly in one of three 60 litre carboys of aerated seawater.

Lobsters were transported to the field site (Fig. 1) approximately 40 nautical miles from Lakes Entrance, Victoria where the *MV Geo Coral* was conducting the Gippsland MC3D seismic survey. During *ca.* 40 h transport and holding, they were contained in 60 litre carboys with strong aeration. Water quality was tested at least 4 times per day for pH, conductivity, dissolved oxygen, and temperature using a Hach HQ40d meter (Hach Australia, Dandenong South, VIC) and for ammonia using a YSI 9500 photometer and ammonia test kit (Xylem Analytics Australia, Hemmant, QLD). Changes of 30% of the carboy seawater were conducted as needed.

At the field site, moorings were constructed to deploy the lobsters, with each mooring comprised of six 3 mm mesh oyster baskets (Hexcyl Systems Australia, Ceduna, SA), each of which represented an experimental replicate. Baskets were lashed to a 12 mm lead-core rope ground line with a 1 kg dive weight attached to the ground line before the first basket, between every second basket and after the last basket to ensure the baskets stayed in contact with the seafloor. The baskets were laid out as: an anchor or sea noise recorder at the distal end, 5 m from this the beginning of the lobster baskets stretching over 25 m of the ground line, weights, then approximately 100 m length of ground line to the mooring end where sacrificial weights were attached via acoustic releases (VR2AR, Innovasea, Bedford, Nova Scotia, Canada) to sub-sea floats. Moorings were deployed in 58 m of water for exposed sites and 51 m at the control site with the oyster baskets on the seabed to represent the post-settlement habit of the life stages used in this study.

Six replicates of juvenile lobsters, each containing 3 individuals, were randomly assigned to moorings deployed: 1) under the chosen seismic line (nominal 0 m range, site E0), 2) 500 m from the seismic line (nominal range, E500) and 3) in the turning zone of the seismic vessel (Control) where the air guns were not in operation. Three puerulus replicates, two comprised of three individuals and one comprised of two individuals, were randomly assigned to the control and 0 m treatments. While the control location did have nearby air gun operations (5.5 km to closest signal) it was the only option for a control site with seismic source vessel overpass and no air guns running as we could not dictate vessel operations. The locations of ends of each mooring are given in Table 1. Moorings have been given designations as listed in Table 1. Moorings were set over 09:00 23-Feb-2020 to 16:00 23-Feb-2020 (UTC+10 hours) and recovered between 08:30 24-Feb-2020 and 15:00 24-Feb-2020 (UTC+10 hours).

After the seismic vessel had passed moorings were recovered in the order they were deployed, so that time in-water was similar for all moorings (20.4 hours for each mooring), and lobsters removed from baskets. Two individuals from each replicate were tested for righting by placing them on their dorsal side in a bin of seawater and timing how long they took to right themselves, defined as when legs from both sides of the body were in contact with the substrate (Day et al. 2019, 2020). Following righting, one individual was fixed in 10% neutral buffered formalin and one in 3.5% glutaraldehyde. The remaining individual from each replicate was returned to the 60-litre carboy used for holding and transported to the aquaculture facility at the Institute for Marine and Antarctic Studies (IMAS) in Taroona, Tasmania. For puerulus, one individual was tested for righting and was then fixed in formalin and the remaining individual from each replicate was returned to the 60-litre carboy for return to IMAS. During the *ca.* 48 h transit, water quality was monitored and maintained as previously described. Upon arrival at IMAS, lobsters were placed individually into 10 litre tanks on a recirculating aquaculture system held at 17-18°C. Lobsters were fed opened mussels *ad libitum* each night and tanks were cleared of remaining mussels and shells each morning. Water quality monitoring of the system was conducted daily using the same methods as during transport.

During holding, lobsters were maintained on a 12:12 light cycle and monitored twice daily for moulting at around 0800 and 1700. Juveniles were held until each lobster had moulted twice, allowing for determination of the intermoult period. Due to the shorter moult cycle of puerulus compared to juveniles (Aiken 1980; Musgrove 2000), a third moult was recorded and two intermoult periods were determined. After the final moult for each lobster, it was tested again for righting and then fixed in 10% neutral buffered formalin.

Table 1: The lobster mooring ends, water depth at deployment and locations. The lobster baskets were located over 5-30 m of the ground line from the mooring end labelled A below on moorings labelled LOBSTER (moorings E0, E500 and Control).

Mooring location	Depth (m)	Latitude & longitude
A E2000 instrument (3641)	58	38º 8.562' S, 148º 10.522' E
E2000 riser	58	38º 8.576' S, 148º 10.436' E
A E1000 instrument (3640)	58	38º 8.049' S, 148º 10.737' E
E1000 riser	58	38º 8.088' S, 148º 10.658' E
A E500 instrument (3639) **LOBSTER**	58	38º 7.785' S, 148º 10.843' E
E500 riser	58	38º 7.804' S, 148º 10.750' E
A E250 anchor	58	38º 7.627' S, 148º 10.935' E
E250 instrument (3638 M20)	58	38º 7.665' S, 148º 10.875' E
E250 riser	58	38º 7.680' S, 148º 10.822' E
A E0 instrument (3637) **LOBSTER**	58	38º 7.537' S, 148º 10.927' E
E0 riser	58	38º 7.557' S, 148º 10.850' E
A Control anchor (no instrument) **LOBSTER**	51	38º 2.246' S, 148º 2.892' E
Control riser	51	38º 2.276' S, 148º 2.851' E

Seismic source

The seismic vessel, the 101 m overall length, 12,812 gross tonne *MV Geo Coral*, towed three 2,820 cubic inch seismic sources at 8 m depth, one 435 m astern the vessel on the vessel sail line and one 100 m either side of this source. Behind the air gun array sources, the *MV Geo Coral* towed 12 x 7.3 km long seismic streamers spaced across 1.3 km, which carried the hydrophones that recorded the seismic signals. Each seismic source was operated alternatively at a median 5 s rate (mean 5.3 s) with a short section of a sail line highlighting consecutive signal locations shown on Figure 2. The navigation officers of the seismic vessel provided the *FV Dell Richey II* with 24 hour 'Lookahead' tables which gave the estimated time and positions for the start and end of seismic lines for the following few days. The seismic lines were approximately 80 nautical mile long, so great circle paths and average vessel speed were used to estimate lobster site locations and times at defined experimental sites and distances perpendicular to the sail line. We were provided with an estimated turn sail line which was used to locate the control lobster site. The top of all mooring gear had to be below 35 m from the sea surface to avoid entanglement with the towed gear. At the cessation of experiments the seismic navigators provided the seismic *.p190 files for all sail lines. These files contain the air gun array central location, fire time, water depth, shot and line numbers.

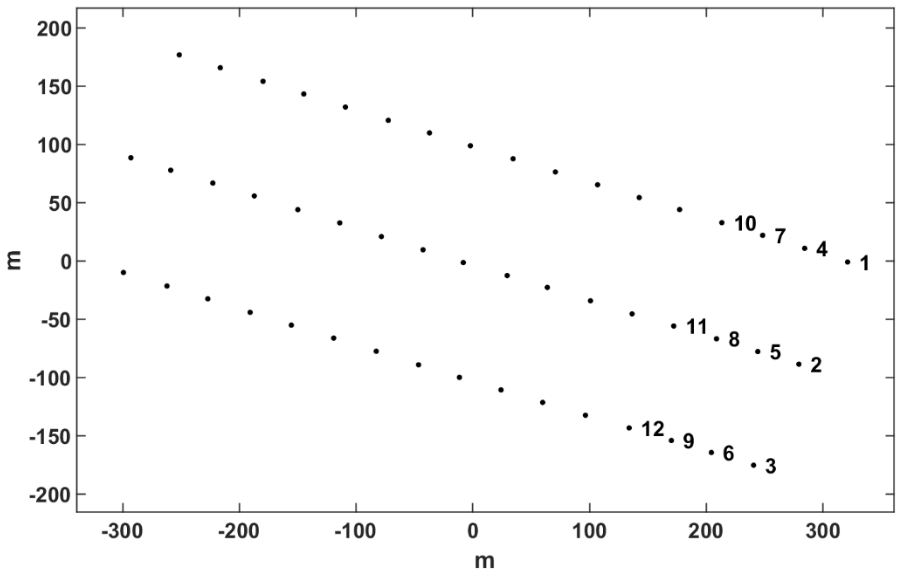

Figure 2. Consecutive air gun signal locations (source centre), with the first 12 signals labelled.

Sea noise recordings

Sea noise recorders were placed at the ends of each experimental lobster mooring plus two instruments were deployed for the long term, with the details of instruments, settings, locations, and samples collected listed in Table 2. Sensors included: sound pressure (all receivers); ground motion (3 axis accelerometers, E0 location only); and particle velocity (GeoSpectrum M20 instrument at E250).

Three types of sea noise recorders were used: Curtin University CMST-DSTO types (McCauley et al. 2017b) with multiple gain settings (E22, E43 & E09); a modified CMST-DSTO instrument which included 3-axis geophones (E02); and a GeoSpectrum M20 particle velocity and sound pressure sensor with a JASCO AMAR recorder. Pre-amplifier and post-gain settings are listed in Table 2. The CMST-DSTO recorders used High Tech HTI U90 or Massa TR 1025-C hydrophones each individually calibrated (nominal sensitivity -197 to -196 dB re 1µPa/V) and laid on the seabed on weighted cables ~3 m from the housing containing electronics. The geophone sensors were *SENSOR Nederland b.v*, types SM-6/U-B 10 Hz in the vertical and SM-6/H-B 10 Hz in the horizontal, mounted in a 30 kg housing aligned flat on the seabed. The CMST-DSTO and geophone pressure sensors were calibrated by injecting and recording white noise of known level (-90 dB re V^2/Hz) in series with the hydrophone. Analysis of the received signal gave gain with frequency, which was used with the hydrophone sensitivity to correct air gun signal waveforms from volts to Pa in the time domain. This was done by selecting the air gun signal, calculating its FFT at a fine (< 0.1 Hz) frequency resolution, applying the appropriate amplitude correction derived from the white noise curve and including the hydrophone sensitivity, for that frequency in the real part of the FFT, and inverting this back to a waveform with an inverse-FFT. The M20 sensors were calibrated using generic gain and phase with frequency curves for the sensors, accounting for the fixed (in hardware) 20 dB sensor gain and 0 dB post gain (AMAR) and accounting for the 5V A-D rail to -1 to 1 V *wav* format in the AMAR. The GeoSpectrum hydrophone signal was converted to Pascal using the white noise injection described above, while the geophone signals used generic calibration amplitude corrections to convert to particle velocity to (ms^{-1}). All except the long term instruments sampled at 10-minute samples with an ~10 s gap before the next sample. The long term instruments sampled 10 minutes of every 15 minutes. All CMST-DSTO instruments sampled at 8 kHz, the geophone instrument sampled at 4 kHz per channel and the GeoSpectrum at 16 kHz per channel.

Table 2: Mooring designations, hardware used, location, in-water sample times, in-water sample numbers and water depth (m). Mooring designations are the Curtin set number and: LE-0 = lobster exposed 0m; LE-500 = lobster exposed 500 m; LE-1000 = lobster exposed 1000 m; LE-2000 = lobster exposed 2000 m; LT = long term; O-0 = octopus exposed 0 m; O-1000 = octopus 1000 m; and O-C = octopus control. Hardware settings are given as electronics, pre-amplifier gain / post gain (dB), sample rate (kHz), and mean sample-length (s) / mean time between start of consecutive samples (s).

Set & designation	Hardware	Latitude & longitude	In-Water samples	Samples	Water depth
3637 LE-0	E02 0/0 8 614/679	38° 7.537' S, 148° 10.927' E	03:58 23-Feb to 23:36 23-Feb	20-124	58
3638 LE-250	M20 20/0 16 599/610	38° 7.665' S, 148° 10.875' E	03:23 23-Feb to 22:52 23-Feb	124-239	58
3639 LE-500	E22 0/0 8 615/655	38° 7.785' S, 148° 10.843' E	02:03 23-Feb to 22:36 23-Feb	9-122	58
3640 LE-1000	E43 20/0 8 616/666	38° 8.049' S, 148° 10.737' E	01:00 23-Feb to 22:06 23-Feb	2-116	58
3641 LE-2000	E09 20/0 8 615/652	38° 8.562' S, 148° 10.522' E	01:00 23-Feb to 21:28 23-Feb	2-115	58
3642 LT	E22 0/0 8 614/898	38° 21.112' S, 147° 45.760' E	02:03 23-Feb to 01:45 16-May	9-7995	54
3643 LT	E43 20/0 8 615/898	38° 21.112' S, 147° 45.760' E	01:00 23-Feb to 01:45 16-May	2-7990	54
3649 O-0	E24 0/0 8 615/652	38° 22.856' S, 147° 43.144' E	02:25 14-May to 22:26 15-May	6-249	50
3650 O-1000	E07 20/0 8 615/654	38° 23.350' S, 147° 42.963 'E	01:04 14-May to 22:28 15-May	13-263	50
3651 O-C	E49 20/0 8 615/654	38° 18.255' S, 147° 35.632' E	04:11 14-May to 03:05 16-May	12-270	41

Statistical analysis

Righting data for the two puerulus treatments was compared only at day 0 post-exposure using a two-tailed Wilcoxon-Mann-Whitney test. Righting data and intermoult period data were not compared for puerulus due to the very low sample numbers at the subsequent sampling points (≤3 individuals). However, righting data from puerulus and juveniles Control and E0 treatments were pooled to facilitate analysis of righting time. These data were compared using a two-tailed Wilcoxon-Mann-Whitney test.

Lobster size (carapace length and weight), righting data and intermoult period data for the three juvenile lobster treatments (Control, E0, E500) were analysed using a Kruskal-Wallis rank sum test due to the small sample size and to mitigate the skewing influence of extreme results. A pairwise Wilcoxon-Mann-Whitney test with Bonferroni correction for multiple comparisons was used for post-hoc comparison between treatments.

All analyses were conducted using R version 3.6.2 (R Foundation for Statistical Computing, 2020).

Air gun signal analysis and units

Air gun signals were extracted from samples using a purpose-built graphical user interface which displayed waveforms (volts) and spectrograms filtered to optimise the air gun signal. A voltage threshold and minimum time separation between air gun shots was used to identify the leading edge of each air gun signal. Extraneous signals or signals occluded by noise sources were removed. The full bandwidth, unfiltered waveform was extracted from approximately 2 s before and 2 s after each identified air gun signal's leading edge. The pressure waveform of each air gun signal was calibrated in the time domain to Pa (above) and a series of signal measurements as defined in McCauley et al. (2003) calculated for each air gun signal. The received signal arrival time was aligned with the source navigation data and a sound speed (1510 ms^{-1}) used to iterate travel times and so align each air gun signal's received time with a fired time. The heading of each air gun array was calculated (noting there were three arrays aligned perpendicular to the vessel sail line so array heading was calculated independently along the tow line of each array) and the horizontal range (using great circle path on a circular earth), slant range (straight line distance between source and receiver through water, assuming constant water depth as per receiver and an 8 m source depth) and the heading of the receiver relative to the array tow direction calculated. An air gun signals' geophone and particle velocity signals were extracted using the time stamps given by the pressure signals. Ground and waterborne motion signals were calibrated to ms^{-1} in the time domain using the generic calibration curves with frequency for each sensor with a low FFT frequency resolution (high number of sample points, to avoid artefacts).

Measurements used here for the sound pressure waveform are sound exposure level (SEL, units of dB re $1\mu Pa^2 \cdot s$) and peak to peak (P-P, units of dB re 1µPa). For ground motion the ground velocity (ms^{-1}) in the x and y horizontal axes and z, vertical axes were returned. These were differentiated to give ground acceleration for each axis (GA in units of ms^{-2}). To represent an air gun signal's ground acceleration a single metric was used, the maximum magnitude of the three axes (x, y, z) vector across the air gun signal. We used the GeoSpectrum M20 particle velocity sensor to measure waterborne particle velocity 47 cm above the seabed again in the x and y horizontal axes and z, vertical axes. The data was treated similarly to the ground motion, velocity was differentiated to acceleration (ms^{-2}) and the maximum magnitude of the air gun signals acceleration used to define an air gun signal's particle acceleration (PA). The units of GA and PA are presented in plots as:

dB re 1µms^{-2} $(20 * log_{10}\left(\frac{GA}{10^{-6}}\right)\ or\ 20 * log_{10}\left(\frac{PA}{10^{-6}}\right))$ Equation 1

as per ISO standard 18405(2017) where the dB reference value is 1µm.

A large number of measurement parameters were calculated and saved for each air gun signal so are available.

For comparison, in previous studies of impacts of a single air gun on scallops and lobster, Day et al. (2016a, 2016b, 2017, 2019, 2020) and Fitzgibbon et al. (2017) similarly measured sound pressure and ground acceleration. At the time of these studies no standards existed for presenting ground acceleration and no commercially available particle velocity sensors were available. Thus, this suite of papers calculated ground acceleration in units of ms^{-2} as here, but presented the dB value as:

$10 * log_{10}\left(\frac{GA}{1}\right)$ Equation 2

where the dB reference value was 1 ms^{-2}.

The conversion value to the ISO 18405(2017) standard is to add 120 dB to the earlier units. To simplify comparison with the earlier work, the decibel values of ground and particle acceleration are presented here with both sets of decibel values, the earlier non-ISO standard work generally in parenthesis.

Data overloads were present in pressure instruments and the GeoSpectrum M20. These were identified firstly by setting flags based on the received maximum or minimum allowable voltage and the instruments A-D rail (± 2.5 V for the CMST-DSTO instruments, ± 0.1 V for the GeoSpectrum M20, with overload thresholds of ±

2.5 V and ± 0.98 V for CMSTO-DSTO and M20 respectively). Signals with a marked overload were removed. The second approach involved setting range thresholds. For an instrument's data overloads the peak-peak values will reach an asymptote and at shorter ranges the signals will not get any higher in level. For each instruments' data these asymptote maximum ranges were found where appropriate (some instruments did not overload) and signals at less than this range were removed. For the low gain instruments the signals at long range will fall into the system electronic noise. This was recognisable as a range at which signal sound exposure levels reached a stable value as the range increased. Note that the noise was removed in calculation of sound exposure level so the calculated SEL within 10 dB of the system electronic noise level is not biased by system noise. For instruments when signals falling into the system electronic noise were evident, signals beyond the range at which the level stabilised were removed (generally 34-60 km except for the geophone measures where it was ~ 24 km).

To calculate sound exposures at experimental lobster sites curves were fitted to each metric of the form:

$$RL = alog_{10}(R) + bR + c \qquad \text{Equation 3}$$

where *RL* is the received level, *a, b,* and *c* are constants derived from the data and *R* is slant range (direct range receiver to air gun in m).

The curves were fitted over range brackets such that the calculated value *c* (or the source level) matched the actual air gun array source level at 50° below the horizontal. An alternative technique for defining trends of received level with range was to calculate statistics of dB values for the appropriate metric in logarithmic range bins, with bin centres and widths defined by one-third octave bands:

$$bc = 1000 * 2^{\left(\frac{N}{3} - 10\right)} \qquad \text{Equation 4}$$

$$bl = \frac{bc}{2^{\frac{1}{6}}} \qquad \text{Equation 5}$$

$$bu = bc * 2^{\frac{1}{6}} \qquad \text{Equation 6}$$

where *bc* is centre of bin (m), *N* is an increasing integer value, *bl* is the lower range limit for that bin-centre, and *bu* is the upper range limit (m). The value N is iterated to include the maximum range to be encountered.

Conventions

- Analysis primarily carried out in the MATLAB (The MathWorks) software environment;
- Unless stated otherwise times displayed are Australian Eastern Standard Time (UTC + 10 hours);
- Tidal predictions for Lakes Entrance (37° 31.8' S, 147° 34.8' E) from software wxtide47.

Results & Discussion

Seismic source levels

Using the air gun array source level model of Duncan (2017) the 2820 cui array source levels were calculated as per Table 13 with the range given through all azimuths (0-360° around the gun tow direction) and the elevation being the angle above the vertical (90° being horizontal). These calculations were made assuming: no surface ghost; a mean gun depth of 8 m; an operating pressure of 13.8 MPa (2000 psi); a sample rate of 4 kHz; and no filters used.

Table 3: Estimated 2820 cui array source levels for different elevations (angle below the horizontal), with the range through all azimuths and the mean for all azimuths.

Elevation (°)	Range, mean P-P (dB re 1µPa)	Range, mean sound exposure level (SEL, dB re 1µPa^2·s)	Range, mean sound pressure level (dB re 1µPa)
90	248-250, 249	227-228, 227	232-233, 232
80	248-250, 249	227-228, 227	232-233, 232
60	248-251, 249	227-228, 228	232-233, 233
50	249-251, 250	228-228, 228	232-233, 233
40	250-251, 250	228-228, 228	233-234, 233
20	252-252, 252	228-229, 229	234-235, 234
0	253-253, 253	229-229, 229	235-235, 235

Lobster seismic exposure

The locations of experimental equipment are shown in Figure 3a. The measured sound exposure levels (SEL) for all lobster instruments are shown by time (Fig. 3b) and logarithmic range (Fig. 3c). The moorings were in the water for a mean of 20.4 hours. The time from completion of the southern line air gun operations to start of the northern line air gun operations was 2.9 hours. All air gun operations are shown on Figure 3c, it was confirmed from the sea noise instruments that no air guns were operated during the turn. The nearest air gun signal for the Control site was 5.5 km to the south so while it was not an air gun free site, it had comparatively low received air gun signals.

The metrics of peak-peak, sound exposure level, ground acceleration (GA, maximum magnitude across an air gun signal) and water particle acceleration (PA, ditto ground acceleration metric) from all instruments set in all experiments are shown in dB units (ISO standard) in Figure 4 and with logarithmic slant range out to 20 km on Figure 5. The curves have little variation with range and little evidence of a beam pattern in the array measures. This simplified estimating sound exposures at each lobster site.

For estimating exposures at each lobster site (0, 500 m and control sites, Table 1) fitted curves were calculated using un-saturated data out to 6 km slant range. Values from these fitted curves were used for slant ranges out to 5,039, 5,039, 20,158 and 6,349 m for peak-peak, sound exposure level, ground acceleration and particle acceleration, respectively. Beyond these ranges a linear interpolation into the curve given by the mean value in logarithmic range bins as per Equations 4-6, were used. The fits and correlation coefficients (r^2) values for the fitted curves calculated over 1m to 6 km for each metric are listed in Table 4. The source levels given by the fitted curves matched the estimated source level of the array for the pressure metrics (the source level estimates for ground and waterborne particle acceleration were not estimated). The cross over range between methods was used as the fitted curve dropped away rapidly below the measured data beyond some range. The cross over ranges were chosen where the two trends (fitted curve Equation 3 and mean level in logarithmic range bins) matched before the fitted curve began to fall away.

To estimate sound exposures the slant distance for all air gun signal generated within 12 hours of the closest point of approach (CPA) to the E0 site (14,739 signals all sites), was calculated for all sites (E0, E250 E500, E1000, E2000 and Control). Lobster site locations were estimated as 5+12.5 m from the mooring instrument end in the mooring lay direction (centre of the line of lobster baskets on the seabed). The respective slant range was then interpolated into the short and long range fitted curves to give estimated exposure. The exposure values are listed in Table 5. Pressure metrics are the same as presented in Day et al. (2016). The ground and particle acceleration metrics follow the ISO standard (Equation 1). For comparison, the non-ISO standard dB units (Equation 2) as presented by Day et al. (2016) also given on Table 35 in parenthesis.

Table 4: Constants for curves (Eqn. 1) fitted to metrics peak-peak, sound exposure level, ground acceleration and particle acceleration. The values a, b and c for Eqn. 1 are listed for each metric along with their r2 value.

	a, *b* & *c* over 1 m - 6 km	r^2
PP	-17.1, -0.002, 250.2	0.918
SEL	-17.1, -0.001, 227.8	0.906
GA	-16.6, -0.000, 178.2	0.811
PA	-16.6, -0.002, 184.9	0.917

A comparison of the estimated sound exposures received here with those measured by Day et al. (2016) during lobster experiments with a 150 cui single air gun are listed in Table 6, noting units for ground acceleration use Equation 2 and water particle acceleration was not available at the time. The maximum exposures experienced at the E0 site here were almost identical to those received in the experimental regime reported by Day et al. (2016), although here the cumulative exposures were greater due to shorter spaced air gun operations in time and space. In addition, the maximum magnitude of ground acceleration reported across all experiments by Day et al. (2016) was 16 dB re $1ms^{-2}$ while here it was 17 dB re $1ms^{-2}$ at the E0 site (units as per Equation 2). In Day et al. (2016) the majority of animals would have received less than the maximum ground acceleration value so again these exposures compare well. Thus, the estimated maximum exposure recorded here indicate that the experimental exposure regime using a single 150 cubic inch air gun as reported in Day et al. (2016a, 2016b, 2019 & 2020) and Fitzgibbon et al. (2017) adequately emulated exposure to a full scale, commercial seismic survey, and that such an approach will be viable for future field-based studies.

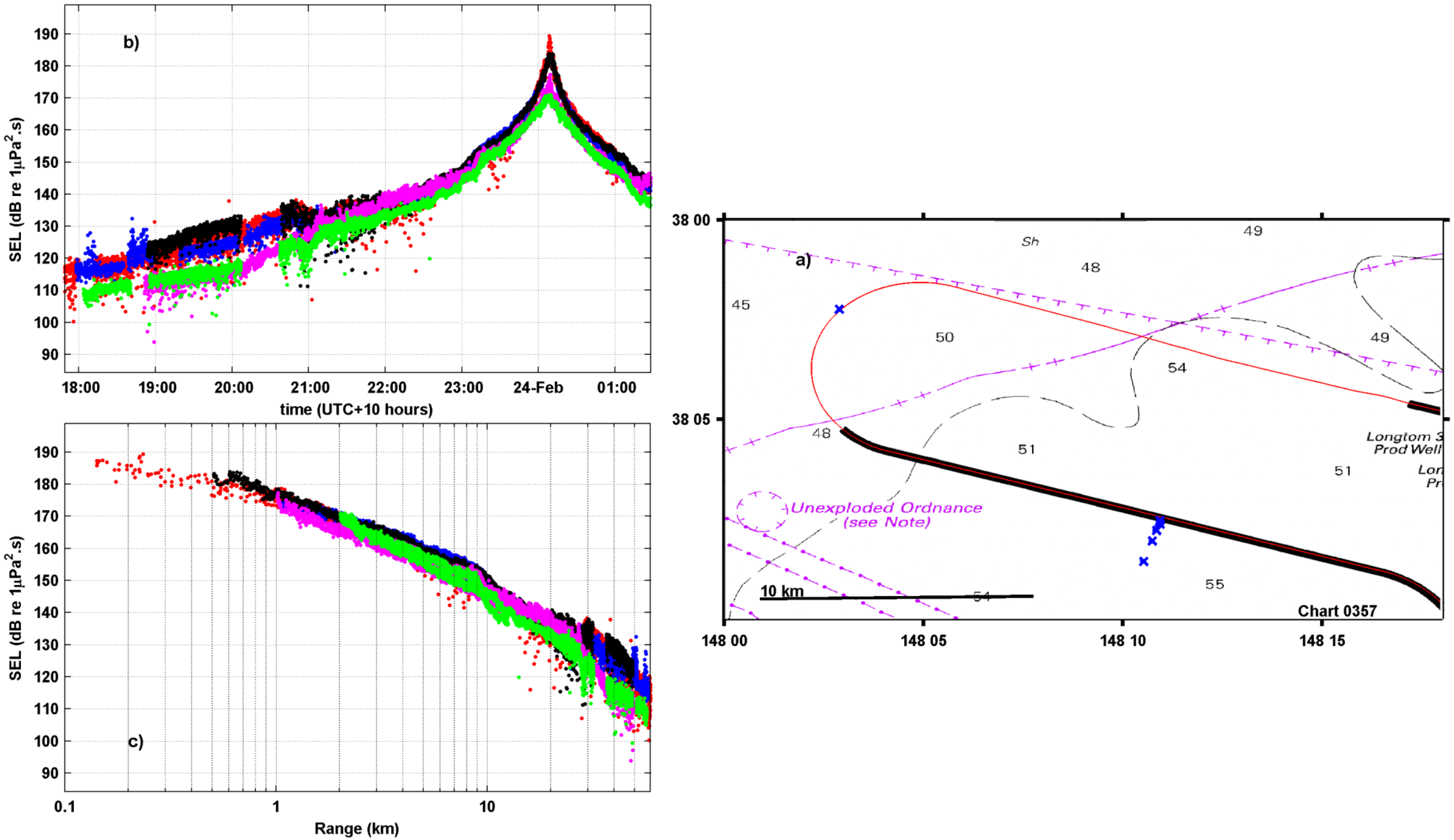

Figure 3. Experimental locations (a) and recorded sound exposure levels with time (b) and logarithmic range (c) for the period up until the end of the southern seismic line. In a), air gun locations are shown by the black dots (may appear as solid black line), the RV Geo Coral track by the red line, and the mooring locations by the blue crosses. The MV Geo Coral completed the southern line from east to west and the northern line west to east. The lower period of seismic operations ended at 01:27, 24-Feb-2020 (UTC+10) and seismic operations began along the northern line at 04:25, 24-Feb-2020 (UTC+10). The colours on b) and c) represent receiver locations: red = E0; blue = E250; black = E500; magenta = E1000; green = E2000.

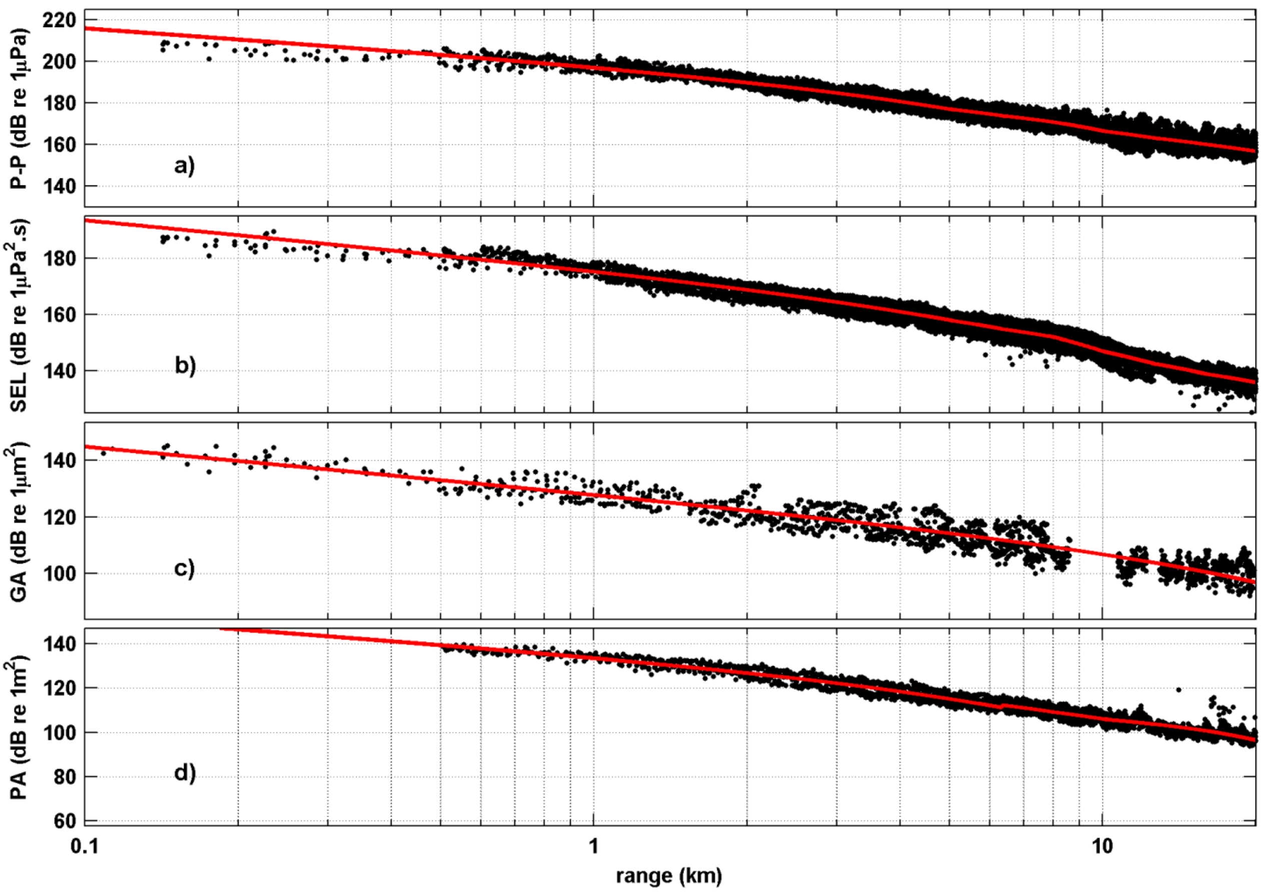

Figure 4. All measurements made of: a) peak-peak pressure; b) sound exposure level (SEL); c) the maximum magnitude of the 3-axis ground acceleration vector across an air gun signal; and d) the maximum magnitude of the 3-axis particle acceleration vector across an air gun signal. The red curves are fitted to each data set and were used for estimating exposures (see text).

Table 5: Exposure histories for each lobster site here. Given are: site noting L = lobster present; minimum slant range for an air gun signal; fired time at range of minimum air gun signal (UTC+10); minimum range to MV Geo Coral; time at MV Geo Coral CPA (UTC+10); maximum peak-peak (dB re 1µPa); number shots within 3 dB of maximum peak-peak; number shots ≥ 200 dB re 1µPa P-P; maximum SEL (dB re 1µPa2·s); number shots within 3 dB of maximum SEL; number shots with SEL ≥ 180 dB re 1µPa2·s; cumulative SEL (dB re 1µPa2·s); maximum magnitude of ground acceleration (GA) vector (dB re 1µms-2); number shots within 3 dB maximum GA; number shots with GA ≥ 130 dB re 1µms-2; maximum magnitude of particle acceleration (PA) vector (dB re 1µms-2); number shots within 3 dB maximum PA; number shots with PA ≥ 130 dB re 1µms-2. The values in parenthesis for ground and particle acceleration are as calculated using Equation 2 for the prior ISO standard, dB values.

site	Min Slant range (m)	Time min shot	Min GC range (m)	Time min GC Range	Mx PP	shots W3 Max-PP	PP > 200	Mx SEL	shots W3 Max-SEL	SEL > 180	CSEL	Mx GA	shots W3 Max-GA	GA > 130 (10)	Mx PA	shots W3 Max-PA	PA> 130 (10)
E0 (L)	64	24-Feb-2020 00:08:57	182	24-Feb-2020 00:05:54	219	3	110	197	3	84	207	148 (17)	3 (3)	117 (26)	155 (20)	3 (5)	231 (76)
E250	205	24-Feb-2020 00:08:29	338	24-Feb-2020 00:05:35	210	13	103	188	15	75	204	140 (11)	16 (41)	109 (7)	146 (14)	15 (30)	227 (61)
E500 (L)	517	24-Feb-2020 00:08:44	657	24-Feb-2020 00:05:48	203	59	49	181	63	12	201	133 (7)	71 (135)	66 (0)	139 (10)	63 (115)	211 (0)
E1000	1033	24-Feb-2020 00:08:44	1170	24-Feb-2020 00:05:48	197	131	0	175	141	0	198	128 (4)	161 (279)	0 (0)	133 (6)	140 (235)	145 (0)
E2000	2028	24-Feb-2020 00:08:57	2171	24-Feb-2020 00:05:48	190	240	0	168	266	0	194	122 (1)	316 (537)	0 (0)	127 (3)	256 (452)	0 (0)
Cont. (L)	5523	24-Feb-2020 01:27:44	48	24-Feb-2020 02:02:58	176	165	0	157	173	0	181	113 (-4)	226 (516)	0 (0)	113 (-3)	216 (340)	0 (0)

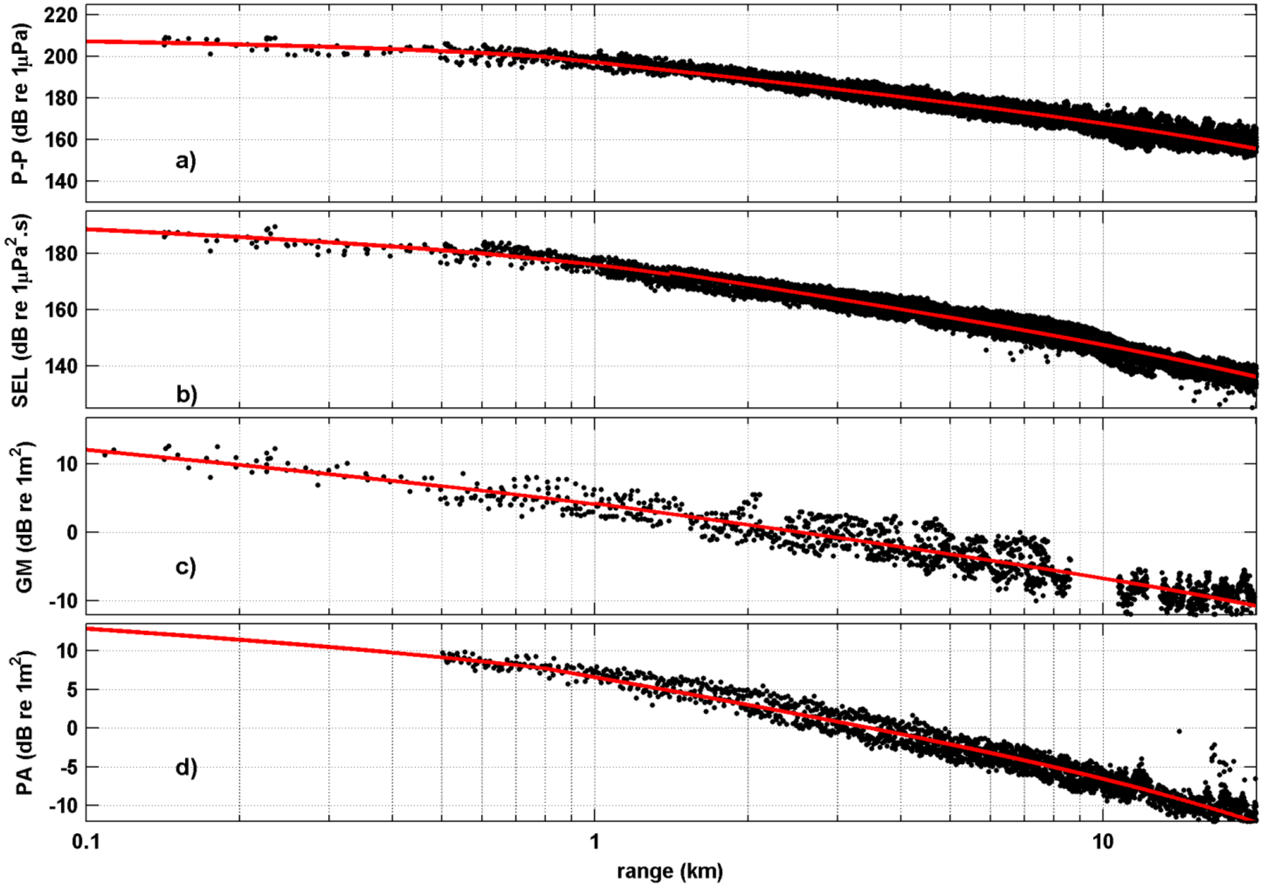

Figure 5. Received air gun levels in decibels with logarithmic slant range (black dots) showing: a) peak to peak; b) sound exposure; c) maximum magnitude of ground acceleration; and d) maximum magnitude of water particle acceleration. The red lines are fitted curves to the data.

Table 6: Comparison of measures made during Day et al. (2016, Table 5 for pressure measures, Table 18 for ground acceleration) lobster experiments with 150 cui air gun and estimated exposures at the E0 site here. Units are given below.

Measure	Day et al. (2016) Table 5	E0 site	E250	E500	E1000
Max PP (dB re 1µPa)	212	219	210	203	197
Within 3 dB Max PP	3	32	13	59	131
PP ≥ 200	38	120	103	49	0
Max SEL (dB re 1µPa2·s)	190	190	188	181	175
Within 3 dB Max SEL	3	7	15	63	141
SEL ≥ 180	25	90	75	12	0
Cum SEL	199	205	204	201	198
Max GA magnitude ground acceleration $(10 * log_{10}\left(\frac{ms^2}{1}\right))$	16	17	11	7	4

Lobster Mortality

Seismic exposure did not affect mortality in either age class of lobster. Upon recovery, one individual each from Control and E0 treatments was found dead. For the control lobster there was evidence of physical trauma or cannibalism, but the cause of death could not be determined for either individual. During holding one juvenile lobster from the E500 site was found dead. Given the low incidence of mortality despite the extensive handling during collection, deployment and recovery at the field site and transport, these results indicate that seismic surveys are unlikely to produce significantly increased mortality in puerulus and juvenile lobsters. These stages have been shown to fare well in aquaculture settings (Hooker et al. 1997; James and Tong 1997) and adult *J. edwardsii* have shown resilience to exposure to seismic signals in regard to mortality (Fitzgibbon et al. 2017).

Lobster Righting Time

In the present study, exposure to a commercial seismic survey had a significant impact on righting reflex ability for puerulus and juveniles when tested immediately after exposure (Table 7). In puerulus, this was demonstrated by a significantly slower righting time in E0 lobsters compared to Controls. For juveniles (Fig. 6), the initial response of E0 and E500 treatments showed a similar increase in righting time over that of Controls, indicating that the range of this impact extended to at least 500 m from the source, the maximum range tested in the present study.

The results for both developmental stages were consistent with previous findings of righting impairment in adult *J. edwardsii* (Day et al. 2019), in which righting impairment was found to correlate to damage to the sensory hairs of the statocyst, the mechanosensory organ shared by lobsters and a variety of marine invertebrates that is responsible for the detection of gravity, body position, spatial movement, and acceleration (Buddelman 1992).

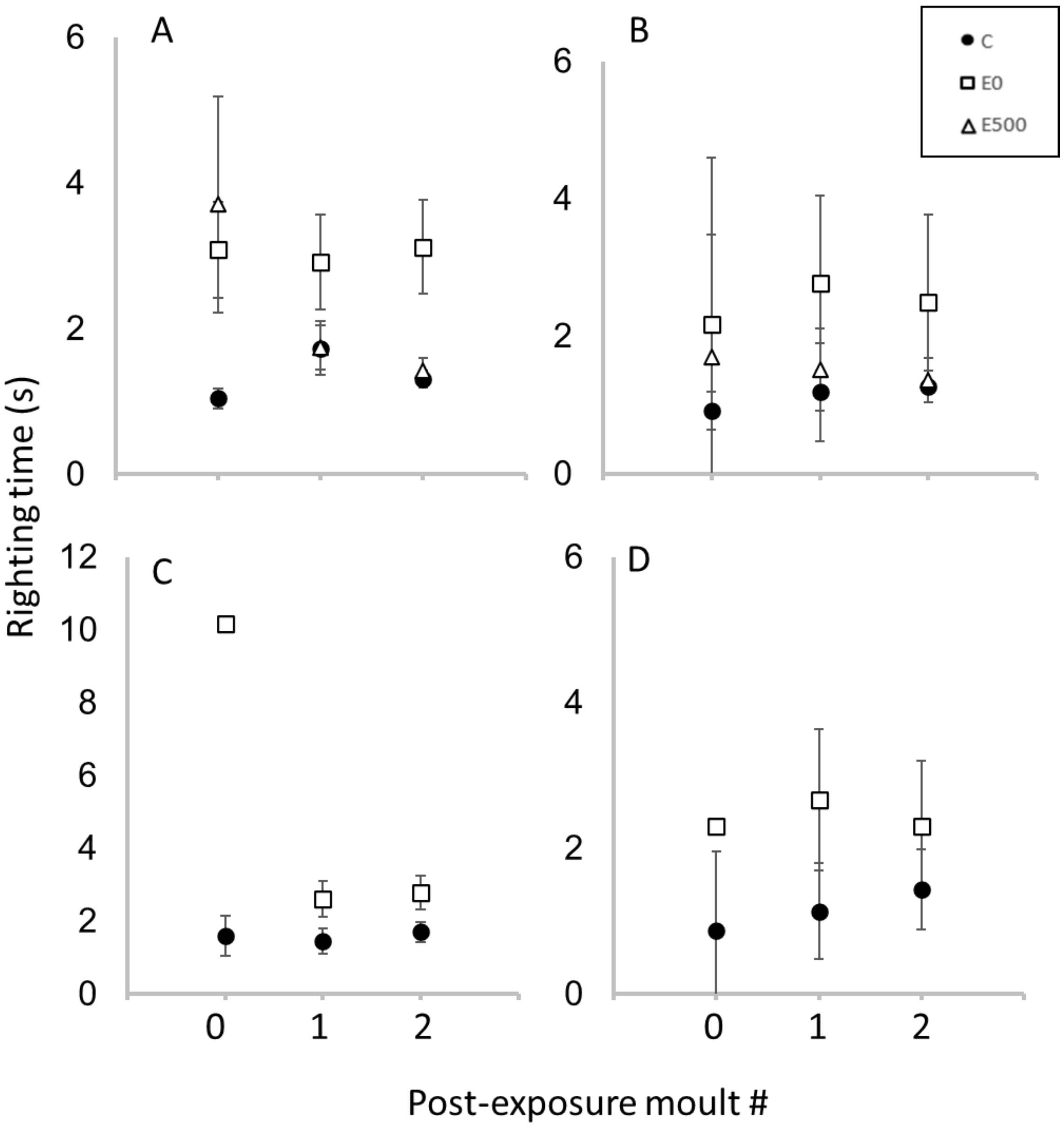

Figure 6. Righting times in Southern Rock Lobster experimental treatments showing A) mean (±SEM) righting time in juveniles, B) median (±95%CI) righting time of juveniles, C) mean (±SEM) righting time of combined juvenile and puerulus and D) median righting time (±95%CI) of combined juvenile and puerulus. Note the differing Y-axis scale in graph C. Error bars are not shown for E0 treatment in graphs C and D due to the introduction of skew to the graph due to extreme values, see Table 7 for values.

The ecological impacts of impaired righting in crustaceans are not well understood. While a righting impairment of only seconds may appear inconsequential and unlikely to lead to lower capacity for predator escape, crustaceans demonstrate predator response behaviours that are measured on the order of milliseconds (Herberholz et al. 2019) that can significantly impact the likelihood of escape (Herberholz et al. 2004). The disruption of a simple reflex, the tail flip, has been attributed to reduced activity, shelter seeking, and predator defence and escape and was suggested to have the potential to undermine more complex behaviours including feeding, locomotion, and intraspecific social behaviours (Brown & Caputi 1983; Vermeer 1986).

The dorsoventral righting reflex is a comparatively complex reflex response and its impairment has been used in a wide variety of animals, ranging from mammals, such as rodents (Wasilczuk et al. (2018) and livestock (Verhoeven et al. 2014), to arboreal dwelling vertebrates and invertebrates (Jusufi et al. 2011) and terrestrial (Harper 2018) and marine (Stoner 2012) invertebrates. Generally, reflexes are valuable proxy measurements of impact due to their automatic and involuntary nature, leading to nearly instantaneous and stereotypical responses that are easy to measure and reproducible (Carlson 2007). In the specific case of the dorsoventral righting reflex, the important consideration is that righting is a complex reflex that involves coordinating input from multiple sensory systems (*e.g.*, statocyst, photoreceptors, chordotonal organs) to mediate neural and muscular control of the abdomen, appendages, and components of the tail (Neil 1993). Thus, impairment of righting reflex is interesting not for characterising an animal's ability to return to a dorsum-up position if turned over in the wild, but for the insight it gives into the sensory and coordination capabilities of said animal. Although the absolute increase in righting time may have appeared to be modest, rising from about 1 second in the juvenile Control treatment to around 3 seconds in Exposed treatments here and, in adults, from ca. 2.5 seconds in Control lobsters to 5-8 seconds in exposed lobsters (Day et al. 2019), these impacts to Exposed lobsters represented an increase of 80-150% over Controls. These results indicate that either the ability to sense gravity in relation to body positioning or the ability to coordinate the movements of the limbs and body posture have been compromised. Such an effect could have more widespread ramifications than simply righting, as lobsters use the sense of gravity provided by the statocyst to orient their body positioning during tail flip mediated swimming escapes (Neil & Newland 1995) and when this sensory input was removed, the abilities to steer swimming direction and to land in an appropriate upright position were lost (Newland & Neil 1990).

Given the important role the statocysts play in processing sensory inputs and mediating reflex and behavioural responses, the indication that damage from exposure to seismic (Day et al. 2019) and environmental (Day et al. 2020) sound sources may be persistent after a lack of recovery following moulting raised concern about the long-term impacts of this damage. However, following the first post-exposure moult, E500 juveniles in this study demonstrated a recovery in righting ability (Table 7) with no statistical difference in righting time relative to the Control treatment following the first or second post-exposure moult. Compared to adults that showed no signs of recovery (Day et al. 2019), this disparity suggests the potential that a threshold range exists, beyond which recovery is possible. Given the modelled exposure equivalent to a full-scale survey at 200-500 m range (Day et al. 2019; Day et al. 2020) for adult lobsters, it is likely that this threshold is somewhere on the order of several hundred meters from the source.

For E0 juvenile lobsters, the results were somewhat equivocal. As previously reported in adult lobsters, there appeared to be persistent impairment of righting, with no observed change in mean or median righting time following moult (Day et al. 2019). However, in this study the slower righting time of post-moult E0 lobsters compared to the Control treatment was only significant after the second post-exposure moult and not after the first moult (Table 7). The limited number of results from the puerulus E0 treatment appeared to support this persistence of impairment, though this difference was not statistically analysed due to low sample numbers. The limited sample size here potentially played a role in confounding the comparison and the potential for a Type II error (*i.e.*, that a real difference between treatments is disregarded due to a nonsignificant statistical result) cannot be ignored considering the significant difference following the second moult. Furthermore, when the Control and E0 treatments for both life stages were pooled to facilitate comparison, the E0 treatment was found to have a significantly slower righting time than Control lobsters at all three assessment points (Table 7). Based on this comparison, it appears likely that three life history stages of *J. edwardsii* (adults, Day et al. 2019; and puerulus and juveniles, this study) demonstrate similar persistent damage following close range exposure to seismic air gun signals.

Table 7: For all measurements, the mean ± standard error of the mean (±SEM) is indicated by the top value, the median (±95%CI) is indicated by the middle value and the sample size is indicated by the bottom value. Statistical results are presented as test statistic (Z for Wilcoxon-Mann-Whitney test or χ2 for Kruskal Wallis test), degrees of freedom (df) and significance level (P). Bolded statistical results indicate a significant difference, with significant pairwise comparisons indicated by differing superscript letters.

Treatment	Carapace length (mm)	Weight (g)	Righting 0 days post-exposure (s)	Righting 1 moult post-exposure (s)	Righting 2 moults post-exposure (s)	Righting 3 moults post-exposure (s)	Intermoult period 1 (days)	Intermoult period 2 (days)
Puerulus Control	10.75 ± 0.45 11.00 ± 0.88 n=8	0.480 ± 0.013 0.471 ± 0.03 n=8	2.8 ± 1.7 0.80 ± 3.33 n=8	0.47 ± 0.01 0.47 ± 0.01 n=2	4.05 ± 0.67 4.05 ± 1.32 n=2	3.11 ± 0.01 3.11 ± 0.02 n=2	16 ± 2 16 ± 4 n=2	18 ± 2 18.5 ± 5 n=2
Puerulus Exposed 0	10.31 ± 0.21 10.60 ± 0.40 n=8	0.411 ± 0.011 0.399 ± 0.02 n=8	28.41 ± 25.28 2.7 ± 49.54 n=8	63.28 ± 71.50 7.13 ± 114.41 n=3	1.99 ± 0.88 1.42 ± 1.41 n=3	2.13 ± 0.36 2.15 ± 0.58 n=3	23 ± 1 23 ± 1 n=3	30 ± 1 30 ± 2 n=3
Wilcoxon-Mann-Whitney Test Statistics	Z = 1.57, P = 0.11	**Z = 2.61** **P = 0.01**	**Z = -1.97,** **P = 0.024**	−	−	−	−	−
Juvenile Control	13.65 ± 0.67 13.40 ± 1.32 n=19	1.145 ± 0.178 0.934 ± 0.348 n=19	1.05 ± 0.14^{a} 0.92 ± 0.27 n=19	1.74 ± 0.37 1.19 ± 0.72 n=7	1.31 ± 0.11^{a} 1.27 ± 0.24 n=7	−	22 ± 2^{a} 22 ± 3 n=7	−
Juvenile Exposed 0	13.92 ± 0.57 14.10 ± 1.12 n=19	1.045 ± 0.130 0.943 ± 0.255 n=19	3.08 ± 0.66^{b} 2.18 ± 1.30 n=19	2.92 ± 0.65 2.73 ± 1.27 n=6	3.12 ± 0.65^{b} 2.41 ± 1.27 n=6	−	36 ± 1^{b} 36 ± 2 n=6	−
Juvenile Exposed 500	13.64 ± 0.54 13.27 ± 1.07 n=18	0.996 ± 0.113 0.970 ± 0.196 n=18	3.71 ± 1.48^{b} 1.71 ± 2.90 n=18	1.74 ± 0.30 1.52 ± 0.60 n=6	1.43 ± 0.16^{a} 1.37 ± 0.32 n=6	−	32 ± 4^{b} 28 ± 8 n=6	−
Kruskal-Wallis Test Statistics	χ^2=0.04, df=2 P = 0.98	χ^2=0.10, df=2 P = 0.95	**χ^2=14.53, df=2** **P < 0.001**	χ2=5.01, df=2 P = 0.08	**χ^2=10.49, df=2** **P = 0.005**	−	**χ2=12.86, df=2** **P = 0.002**	−
Combined Control	−	−	1.61 ± 0.55 0.87 ± 1.08 n=25	1.46 ± 0.34 1.14 ± 0.66 n=9	1.71 ± 0.28 1.44 ± 0.55 n=9	−	−	−
Combined Exposed 0	−	−	10.18 ± 7.09 2.31 ± 13.90 n=25	2.61 ± 0.49 2.78 ± 0.97 n=9	2.79 ± 0.49 2.31 ± 0.90 n=9	−	−	−
Wilcoxon-Mann-Whitney Test Statistics	−	−	**Z=-4.07** **P<0.001**	**Z=-2.74** **P=0.006**	**Z=-2.12** **P=0.034**	−	−	−

N.B. Dashes (−) in Statistic row indicate that data was not analysed due to low sample numbers, dashes in data column indicate that data were not collected for that cohort.

Lobster Intermoult period

Moulting in decapod crustaceans involves a complex interplay between a range of biochemical, physiological, behavioural, and environmental factors (Aiken 1980). As decapods can only grow following the moult of their exoskeleton, the time between moults is indicative of the rate of their development, with a long time between moults potentially indicative of low growth rate (Thomas et al. 2000). Measurements of this time, often referred to as the intermoult duration, have been used to quantify impacts of stressors like toxicity of pharmaceuticals (Gonzalez-Ortegon et al. 2013), thermal stress (Green et al. 2014) and nutritional condition (James & Tong 1997; Oliver & MacDiarmid 2001).

Here, the intermoult period between two successive moults in juveniles was found to differ significantly as E0 and E500 treatments had a similarly increased intermoult duration over that of control lobsters. Puerulus showed a similar pattern over three moults with a marked difference between Control and E0 lobsters and little variation between individuals, although, as before, this could not be statistically analysed due to low sample numbers (Table 7).

Environmental conditions in the present study were tightly controlled in the holding facility, so the mechanism impacting intermoult duration was intrinsic, such as feeding rate, food conversion rate, metabolism, endocrine influence, etc., suggesting a physiological response to seismic survey exposure. Potential causes for this disturbance based on results in adult *J. edwardsii* following exposure to air gun signals include reduced nutritional condition (Fitzgibbon et al. 2017), an immune system response (Fitzgibbon et al. 2017) and a response to damage of the statocyst (Day et al. 2019), the latter of which the righting data presented here offers support for.

Conclusion

The response of the post-settlement puerulus and juvenile life-history stages of the Southern Rock Lobster *Jasus edwardsii* to exposure to a full seismic survey array offered some interesting elaboration of previously reported impacts described in adults exposed to an emulated survey using a single air gun in shallow water. First, the results reported here were consistent with those previously reported, validating the use of a single air gun to emulate exposure to full-scale surveys for field-based studies. Second, these were the first results to give some indication of the range of impact, with exposure affecting juvenile lobsters 500 m from the source, though this requires further investigation as it was the maximum range of the present study due to limited samples available for the experiment. Third, juveniles exposed at a range of 500 m from the source showed the capacity for recovery of the righting reflex, indicating a possible range beyond which permanent impact is unlikely. Fourth, both juveniles and puerulus showed impaired righting immediately following exposure that were similar to that previously observed in adults, which in the latter occurred in conjunction with sensory organ damage following exposure. In adults, this damage was persistent after one moult. Here, E0 lobsters from juveniles and puerulus stages appeared to show a persistent impact, though the lack of statistical power in the experiment confounded the ability to draw clear conclusions. However, when these stages were pooled together, the results indicated a persistent impairment. Finally, juveniles, and potentially puerulus, exposed at 0 m from the source showed an increased intermoult duration indicative of physiological impacts and potentially impaired development or growth. This impairment makes the assessment of the impacts of seismic on lobster phyllosoma a particularly interesting future study, as phyllosoma go through a series of moults over a long pelagic period and characterising their response would provide an understanding of the impacts of seismic exposure across all life history stages of the Southern Rock Lobster, which currently stands as the best studied marine invertebrate in regard to the impacts of seismic exposure.

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